New Arylthioindoles: Potent Inhibitors of Tubulin Polymerization. 2. Structure−Activity Relationships and Molecular Modeling Studies

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Arylthioindoles (ATIs) that possess a 3-methoxyphenylthio or a 3,5-dimethoxyphenylthio moiety at position 2 of the indole ring were effective tubulin assembly inhibitors, but weak inhibitors of MCF-7 cell growth. ATIs bearing a 3-(3,4,5-trimethoxyphenyl)thio moiety were potent tubulin polymerization inhibitors, with IC_{50}s in the 2.0 (**35**) to 4.5 (**37**) μM range. They also inhibited MCF-7 cell growth at nanomolar concentrations. The 3,4,5-trimethoxy substituted ATIs showed potencies comparable to those of the reference compounds colchicine and combretastatin A-4 in both tubulin assembly and cell growth inhibition assays. Dynamics simulation studies correlate well with the observed experimental data. Furthermore, from careful analysis of the biological and in silico data, we can now hypothesize a basic pharmacophore for this class of compounds.

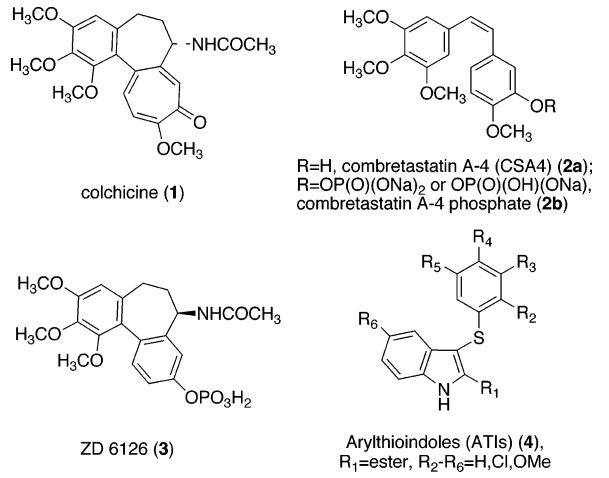
Introduction

Anticancer therapy based on microtubule-targeting agents is receiving growing attention. Tubulin heterodimers, the major component of microtubules, are the molecular target of these agents. Inhibition of tubulin polymerization or interfering with microtubule disassembly disrupts several cellular functions, including cell motility and mitosis.[1−3] The former group of agents includes such compounds as colchicine (**1**), combretastatin A-4 (**2a**; CSA4), and *Catharanthus* alkaloids, such as vinblastine, vincristine, and vinorelbine. The microtubule stabilizing drugs include the clinically important taxoids paclitaxel and docetaxel, as well as more recently discovered natural products, such as the epothilones, discodermolide, and eleutherobin. As a group, drugs that bind to either unpolymerized tubulin or tubulin polymer interfere both with cell division and interphase functions that require a normal microtubule cytoskeleton. Cells treated with these agents invariably undergo apoptosis.[4]

Since clinically useful antitubulin drugs all have problems with toxicity, drug resistance, and bioavailability, there is a continuing effort to find new compounds that might be safer or more effective.[2,5] Several compounds are currently in ongoing clinical trials, for example various epothilones and taxoids, representing the stabilizer class, and the destabilizer combretastatin A-4 phosphate (**2b**), ZD 6126[6] (**3**), the semisynthetic vinca alkaloid vinflunine,[7] the naturally occurring peptide dolastatin 10,[8] the sulfonamide E7010,[9] and T138067/*T*900607.[3]

Several indoles were found to be effective as inhibitors of tubulin assembly.[10−13] We recently discovered arylthioindoles (ATIs) as a new class of potent inhibitors of tubulin assembly and the growth of MCF-7 human breast carcinoma cells[14] (for general formula of ATIs, see structure **4**). The mechanism of action was through binding at the colchicine site on β-tubulin. In our preliminary work, structure−activity relationship (SAR) analysis highlighted four determinant structural requirements: (A) the ester function at position 2 of the indole; (B) the 3-arylthio group; (C) the sulfur atom bridge; (D) the substituent at position 5 of the indole, especially for the inhibition of MCF-7 cell growth.[14] Pursuing our research project, here we describe the synthesis, biological evaluation, and molecular modeling studies of new ATI derivatives (Chart 2).

Chart 1



Chemistry

Synthesis and structures of new ATI derivatives are depicted in Scheme 1 and Table 1, respectively. Compounds **12**, **13**, **14**, **17**, **19**, **28**, **31**−**34**, and **38** were synthesized by treating appropriate indole-2-carboxylates with *N*-arylthiosuccinimides in the presence of boron trifluoride diethyl etherate in anhydrous

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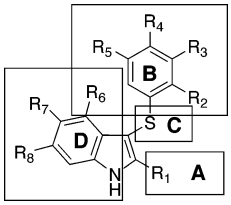
Chart 2. SAR Study of ATIs



CH_2Cl_2, as we previously reported.[15] Compounds **16**, **27**, **36**, and **41** were obtained following a two-step procedure involving addition of 3,4,5-trimethoxylthiophenol to a solution of *N*-chlorosuccinimide (NCS) at −78 °C, and this mixture was treated with the indole-2-carboxylate while the reaction temperature was warmed to 0 °C over 1 h. Compound **25** was prepared by treatment of indole-2-carboxylic acid with 1,1′-(3,5-dimethoxyphenyl)disulfide in the presence of NaH. The crude acid was transformed into the corresponding methyl ester by reaction with trimethylsilyldiazomethane (TMSDM) at room temperature for 30 min. In the case of compounds **37** and **40**, the crude acids were transformed into the corresponding methyl esters by refluxing in methanol in the presence of thionyl chloride for 48 h under a stream of anhydrous argon. This procedure was also used for the preparation of esters **5**−**11** starting from 3-phenylthio-1*H*-indole-2-carboxylic acid[15] and appropriate alcohols. Oxidation of sulfur derivatives **17**, **19**, **21**, **29**, and **38** with 3-chloroperoxybenzoic acid (MCPBA) furnished the corresponding sulfones **18**, **20**, **22**, **30**, and **39**, respectively.

Results and Discussion

Table 2 summarizes the biological data for inhibition of tubulin polymerization, colchicine binding to tubulin (more active compounds only), and growth of MCF-7 human breast carcinoma cells by the indoles **5**−**41** in comparison with data obtained with the reference compounds colchicine (**1**) and CSA4 (**2a**). Biological evaluation of selected ATIs against small cell lung cancer (SCLC) carcinoma cells is also reported.

Previously, we found a 2- to 4-fold increase in the inhibitory effects of ATI derivatives on tubulin polymerization by introducing either a methoxycarbonyl (IC_{50} = 8.2 μM) or an ethoxycarbonyl (IC_{50} = 4.4 μM) function at position 2 of the indole ring of 3-phenylthio-1*H*-indole.[14] We therefore evaluated the effect of additional esters with either a linear or a branched alkoxy chain (compounds **5**−**11**) at this position. Only the isopropyl ester of 3-(phenylthio)-1*H*-indole-2-carboxylic acid (**6**) had any activity (IC_{50} = 18 μM). All other tested compounds were inactive in both the tubulin polymerization and MCF-7 cell assays.

The tubulin assembly inhibitory activity of ATIs bearing a single methoxy group on the 3-arylthio moiety was strongly dependent on the position of the methoxy on the phenyl ring. Invariably, within the cohort of compounds synthesized, the greatest activity occurred when the methoxy group was located meta, the least when located para, to the bridging atom (compare **13** with **12** and **14**, **19** with **17** and **21**, **33** with **32** and **34**).

This inhibitory effect was unpredictably affected by substituents at position 5 of the indole ring (compare the ethyl esters **12** and **32** and also the methyl ester **17**, the ethyl esters **13** and **33** and also the methyl ester **19**).

Notably, of the compounds with a single methoxy group on the phenyl ring, potent inhibitory activity against tubulin assembly was observed with compounds **13**, **17**, **19**, and **33**, and all except **17** were reasonably effective inhibitors of MCF-7 cell growth (IC_{50}'s in 0.15 to 0.33 μM range). Thus, for example, methyl 3-[(3-methoxyphenyl)thio]-5-methoxy-1*H*-indole-2-carboxylate (**33**, IC_{50} = 3.1 μM) was as active as colchicine as an inhibitor of tubulin assembly, and methyl 3-[(3-methoxyphenyl)-thio]-5-chloro-1*H*-indole-2-carboxylate (**19**, IC_{50} = 1.8 μM) was 1.8 times more potent than colchicine and slightly superior to CSA4. These potent tubulin assembly inhibitors, however, were more than 10-fold less inhibitory than the reference compounds **1** (colchicine) and **2a** (CSA4) as inhibitors of MCF-7 cell growth.

Introduction of a second methoxy group at position 5 of the arylthio moiety of **19** produced methyl 3-[(3,5-dimethoxyphenyl)thio]-5-chloro-1*H*-indole-2-carboxylate (**25**). Compound **25** differed little from **19** in its inhibitory effect on tubulin assembly, but **25** was almost 10-fold more cytotoxic. Worthy of note, replacement of the 3-(3,5-dimethoxyphenyl)thio group of **25** with a 3-(3,5-dimethylphenyl)thio moiety (**23**) produced an inactive compound. The ethyl ester analogue of **23**, compound **24**, was also essentially inactive.

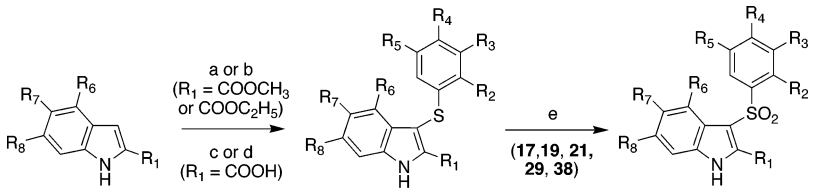
ATI derivatives **15**, **16**, **26**, **27**, **35**, **36**, **37**, **40**, and **41** bear a 3-(3,4,5-trimethoxyphenyl)thio moiety. The first seven were all highly active as inhibitors of tubulin polymerization, with IC_{50} values in the 2.0 (**35**) to 4.5 (**37**) μM range. These values were comparable to those of the reference compounds colchicine (IC_{50} = 3.2 μM) and CSA4 (IC_{50} = 2.2 μM). The 3,4,5-trimethoxy substitution of the phenylthio group was found to be of crucial importance for potent MCF-7 cell growth inhibition. In fact, with the sole exception of the 3,5-dimethoxy derivative **25**, only compounds bearing the 3-(3,4,5-trimethoxyphenyl)thio moiety had IC_{50} values of less than 50 nM against the MCF-7 cells, with the most potent ATI being compound **35** (compare **15** (IC_{50} = 25 nM), **16** (IC_{50} = 40 nM), **26** (IC_{50} = 42 nM), and **35** (IC_{50} = 13 nM)). With the MCF-7 cells, methyl esters were about 2 to 3 times more active than the corresponding ethyl esters (compare **15** with **16**, **26** with **27**, and **35** with **36**). A similar difference was not observed in effects on tubulin assembly. We also obtained some data regarding effects of susbtituents at position 5 of the indole ring. Order of activity, at least for inhibition of cell growth, was H_3CO (**35**) > H (**15**) > Cl (**26**) > NO_2 (**37**), thus suggesting a correlation between electron-donating effect and cytotoxicity.

Changing the position of the methoxy group on the indole from position 5 to 4 or 6 led to loss of activity in inhibiting tubulin assembly (compare **29** with **31** and **28**; all three compounds were inactive against the MCF-7 cells). Similarly, introduction of an additional methoxy group at position 6 of **35** or **36** to give compounds **40** and **41** resulted in a dramatic loss of activity. Finally, as observed previously,[14] oxidation of the sulfur atom to the sulfone always resulted in a large loss of activity (compare **17** with **18**, **19** with **20**, **29** with **30**, and **38** with **39**).

Biological data against the SCLC cells showed good agreement with the data for the MCF-7 cells, although, in this case, compound **25** appeared to be the most active agent in the series.

Due to structural similarly of the ATIs with the Hsp90 inhibitor PU3,[16] we evaluated compound **26** for binding to Hsp90. We found that **26** did not compete with geldanamycin for binding to Hsp90α, and consequently it did not induce the degradation of Her2. As would be expected for an agent with antitubulin activity, however, compound **26** caused MDA-MB-468 breast cancer cells to accumulate in mitotic arrest, as measured with the Tg3 antibody (Tg3 recognizes p-nucleolin, which is highly expressed during mitosis).

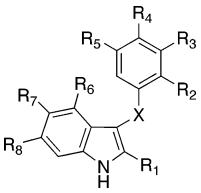
Scheme 1[a]



R_1 = COOMe, COOEt, COO-*n*-Pr, COO-*i*-Pr, COO-*n*-Bu, COO-*i*-Bu, COO-*s*-Bu, COO-*t*-Bu, $COOCH_2Ph$; R_2-R_6 and R_8 = H, OMe; R_7 = H, Cl, OMe.

[a] Reagents and reaction conditions: (a) (**12**, **13**, **14**, **17**, **19**, **28**, **31**−**34** and **38**) *N*-[(R_2−R_5)PhS]succinimide, $BF_3.Et_2O$, anhydrous CH_2Cl_2, r.t., 1.5 h, then 45 °C, 2 h; (b) (**16**, **27**, **36**, and **41**) (i) 3,4,5-$(MeO)_3$PhSH, NCS, CH_2Cl_2, −78 °C to 0 °C, 1 h; (c) (**25**) (i) [3,5-$(MeO)_2PhS]_2$, NaH, anhydrous DMF, 50 °C, overnight, anhydrous argon stream; (ii) TMSDM, CH_3OH/CH_2Cl_2, r.t., 30 min; (d) (**5**−**11**, **37**, and **40**) (i) [(R_2−R_5)$PhS]_2$, NaH, anhydrous DMF, 50 °C, overnight, anhydrous argon stream; (ii), $SOCl_2$, ROH, reflux, 48 h, anhydrous argon stream; (e) (**18**, **20**, **22**, **30**, and **39**) MCPBA (2.5 eq), $CHCl_3$, r.t., 1 h.

Table 1. Structure of Arythioindoles **5**−**41**



compd	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	X
5	COO-*n*-Pr	H	H	H	H	H	H	H	S
6	COO-*i*-Pr	H	H	H	H	H	H	H	S
7	COO-*n*-Bu	H	H	H	H	H	H	H	S
8	COO-*s*-Bu	H	H	H	H	H	H	H	S
9	COO-*t*-Bu	H	H	H	H	H	H	H	S
10	COO-*i*-Pe	H	H	H	H	H	H	H	S
11	$COOCH_2Ph$	H	H	H	H	H	H	H	S
12	COOEt	OMe	H	H	H	H	H	H	S
13	COOEt	H	OMe	H	H	H	H	H	S
14	COOEt	H	H	OMe	H	H	H	H	S
15[a]	COOMe	H	OMe	OMe	OMe	H	H	H	S
16	COOEt	H	OMe	OMe	OMe	H	H	H	S
17	COOMe	OMe	H	H	H	H	Cl	H	S
18	COOMe	OMe	H	H	H	H	Cl	H	SO_2
19	COOMe	H	OMe	H	H	H	Cl	H	S
20	COOMe	H	OMe	H	H	H	Cl	H	SO_2
21[a]	COOMe	H	H	OMe	H	H	Cl	H	S
22	COOMe	H	H	OMe	H	H	Cl	H	SO_2
23[b]	COOMe	H	Me	H	Me	H	Cl	H	S
24[b]	COOEt	H	Me	H	Me	H	Cl	H	S
25	COOMe	H	OMe	H	OMe	H	Cl	H	S
26[a]	COOMe	H	OMe	OMe	OMe	H	Cl	H	S
27	COOEt	H	OMe	OMe	OMe	H	Cl	H	S
28	COOMe	H	H	H	H	H	H	OMe	S
29[a]	COOMe	H	H	H	H	H	OMe	H	S
30	COOMe	H	H	H	H	H	OMe	H	SO_2
31	COOMe	H	H	H	H	OMe	H	H	S
32	COOEt	OMe	H	H	H	H	OMe	H	S
33	COOEt	H	OMe	H	H	H	OMe	H	S
34	COOEt	H	H	OMe	H	H	OMe	H	S
35[a]	COOMe	H	OMe	OMe	OMe	H	OMe	H	S
36	COOEt	H	OMe	OMe	OMe	H	OMe	H	S
37	COOMe	H	OMe	OMe	OMe	H	NO_2	H	S
38	COOEt	H	H	H	H	H	OMe	OMe	S
39	COOEt	H	H	H	H	H	OMe	OMe	SO_2
40	COOMe	H	OMe	OMe	OMe	H	OMe	OMe	S
41	COOEt	H	OMe	OMe	OMe	H	OMe	OMe	S

[a] Ref 14. [b] Ref 15.

Table 2. Inhibition of Tubulin Polymerization, Growth of MCF-7 Human Breast Carcinoma Cells, Colchicine Binding, and Growth of SCLC Cells by Compounds **5**−**41**

compd	tubulin[a] $IC_{50} \pm SD$ (μM)	MCF-7[b] $IC_{50} \pm SD$ (nM)	inhibition colchicine binding[c] (% ± SD)	SCLC[d] $IC_{50} \pm SD$ (nM)
5	>40	>2.5	-	-
6	18 ± 2	>2.5	-	-
7	>40	>2.5	-	-
8	>40	>2.5	-	-
9	>40	>2.5	-	-
10	>40	>2.5	-	-
11	>40	>2.5	-	-
12	12 ± 2	>2.5	-	-
13	2.9 ± 0.3	150 ± 90	48 ± 7	585 ± 50
14	>40	>2.5	-	>10000
15[e]	2.9 ± 0.1	25 ± 1	74 ± 2	52 ± 3
16	2.9 ± 0.2	40 ± 2	51 ± 3	84 ± 5
17	4.2 ± 0.6	1300 ± 400	34 ± 6	7000 ± 300
18	>40	>2.5	-	-
19	1.8 ± 0.2	330 ± 40	53 ± 4	3200 ± 800
20	31 ± 4	>2.5	-	>10000
21[e]	>40	>2.5	-	>10000
22	>40	>2.5	-	-
23[e]	>40	>2.5	-	-
24[e]	>40	1200 ± 200	-	-
25	2.2 ± 0.2	34 ± 10	82 ± 2	18 ± 1
26[e]	2.5 ± 0.3	42 ± 10	57 ± 2	216 ± 17
27	2.2 ± 0.2	110 ± 20	53 ± 6	93 ± 10
28	>40	>2.5	-	-
29[e]	6.2 ± 0.3	>2.5	30 ± 6	-
30	>40	>2.5	-	-
31	>40	>2.5	-	-
32	16 ± 0.5	350 ± 60	-	2200 ± 200
33	3.1 ± 0.2	280 ± 100	39 ± 3	584 ± 40
34	>40	>2.5	-	>10000
35[e]	2.0 ± 0.2	13 ± 3	90 ± 1	47 ± 2
36	2.4 ± 0.2	46 ± 3	71 ± 2	-
37	4.5 ± 0.1	120 ± 40	32 ± 2	-
38	14 ± 1	>2.5	-	-
39	>40	>2.5	-	-
40	>40	1600 ± 400	-	-
41	22 ± 0.7	1000 ± 200	-	-
Col.[e]	3.2 ± 0.4	13 ± 3	-	-
CSA4[e]	2.2 ± 0.2	17 ± 10	97 ± 0.5	-

[a] Inhibition of tubulin polymerization. [b] Inhibition of growth of MCF-7 human breast carcinoma cells. [c] Inhibition of [^{3}H]colchicine binding. [d] Inhibition of growth of SCLC cells. [e] Data from ref 14.

Molecular Modeling

To investigate the structural basis of the SAR that emerged from the biological results, we carried out docking studies on the entire series of compounds reported in this paper, using the FlexX module included in SYBYL.[17] The results were evaluated using the built in scoring function as well as the functions included in the CScore module, with the aim of finding a good correlation between the experimental results and the in silico predictions. Some docking results were very similar to the proposed binding mode reported recently,[14] but, unfortunately, none of the scoring results correlated with the biological data (data not shown). In particular, the scoring results for the *p*-methoxyphenyl analogues were often similar to or better than the corresponding results for the trimethoxyphenyl compounds.

However, FlexX was able to identify an alternate possible binding conformation for this class of compounds compared

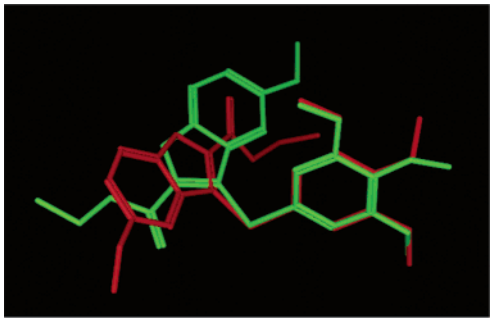


Figure 1. Two possible binding modes of **36**. In green the previously reported conformation (old pose); in red the current proposed conformation (new pose).

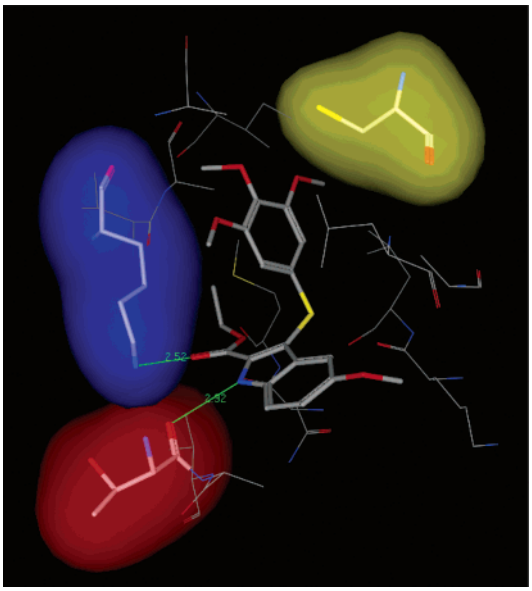


Figure 2. Proposed binding mode for **36**. Lys352 in blue, Thr179 in red, Cys241 in yellow.

with the one reported previously (Figure 1). In this new pose, the indole moiety still forms a hydrogen bond with Thr179 (residue number based on the crystal structure used), but now the ester group is positioned deep in the binding pocket. In addition, the carbonyl of the ATI ester group forms another hydrogen bond with Lys352 (Figure 2).

In comparing these two possible binding modes, the scoring functions employed were not able to clearly indicate which would be the preferred conformation. To solve this problem, we carried out molecular dynamics simulations (MD) on the tubulin−ligand complexes to evaluate the potential energy of the binding of the two different poses of **36** in the colchicine binding site. The calculations were performed using the MOE (Molecular Operating Environment)[18] software package, allowing free movement of the residues within a 15 Å distance of the ligand, keeping the rest of the protein fixed. This site was soaked in water, and the simulation was run in the NVT (number of particles, volume, and temperature of the system are kept constant during the simulation) environment, for a total of 600 ps at 300 K. The states generated during the first 100 ps were not considered in evaluating the results.

The interaction potential energy values calculated by the dynamics simulations revealed a difference of almost 20 kcal/mol between the two conformations (average values: −64.7 kcal/mol for the new pose, −44.9 kcal/mol for the old pose), suggesting that **36** would bind with the indole moiety oriented as in Figure 2.

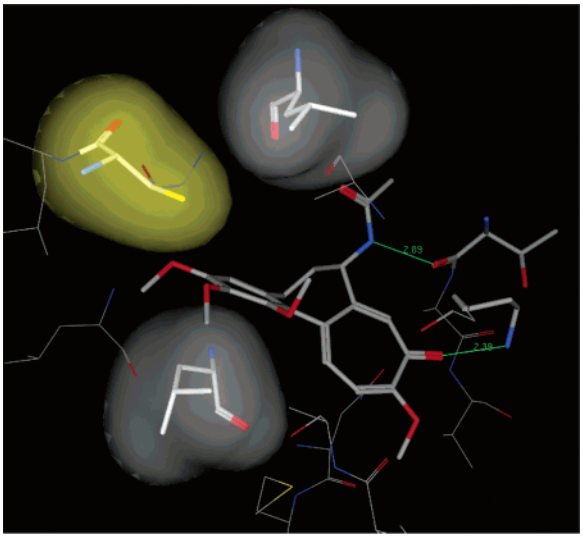


Figure 3. Proposed binding mode for colchicine. Leu248 and Leu255 in gray, Cys241 in yellow, Thr179 and Lys352 in stick representation.

These results encouraged us to extend our molecular dynamics studies to other compounds and, in particular, to the *p*-, *m*-, and *o*-methoxyphenyl analogues (**32**, **33**, **34**). We also performed the simulation under the same conditions on colchicine to have a better understanding of its binding mode with tubulin. For the latter study, we modified the structure of the *N*-deacetyl-*N*-(2-mercaptoacetyl)colchicine cocrystallized with tubulin, and the resulting complex was energy minimized before the molecular dynamics experiment was performed.

Colchicine established one hydrogen bond between the carbonyl group of ring C and Lys352 and another between the NH group and Thr179 (Figure 3), the same residues involved in the binding of **36** (Figure 2). During the simulation, the amide moiety changed its orientation, losing the hydrogen bond with Thr179 and forming another one between the amide carbonyl group and the hydroxyl group of Ser178. The trimethoxy ring A remains placed in a hydrophobic pocket where, in particular, two leucine residues (Leu248 and Leu255) interact strongly with the aromatic ring. Cys241, a key residue for the binding and biological activity of many colchicine analogues,[19] is in close proximity to the C-3 methoxy group of ring A. The average value of the interaction potential energy for colchicine was −71.6 kcal/mol.

The para-substituted arylthioindole **34**, on the other hand, does not establish a hydrogen bond with Thr179 (Figure 4). The interaction with Lys352 remains stable during the simulation time, while the aromatic ring does not establish any contact with Cys241. The energy value obtained for binding, −49.8 kcal/mol, is in agreement with the poor activity observed in the biological experiments. This is ∼15 kcal/mol higher than the value obtained for **36**, indicating a reduced binding affinity for **34** relative to **36**.

The results obtained for the ortho- and meta-substituted arylthioindoles (**32** and **33**) also correlated well with the experimental data. Both compounds established hydrogen bonds with Thr179 and Lys352 through the indole ring. The phenyl ring of compound **33** assumes a similar orientation as the trimethoxy ring of **36**, but, in the case of compound **32**, the *o*-methoxy aryl moiety is almost orthogonal to the corresponding aromatic ring of **36** (Figure not shown). The energy values obtained for **32** and **33** are in agreement with these observations and with the experimental results (Table 3).

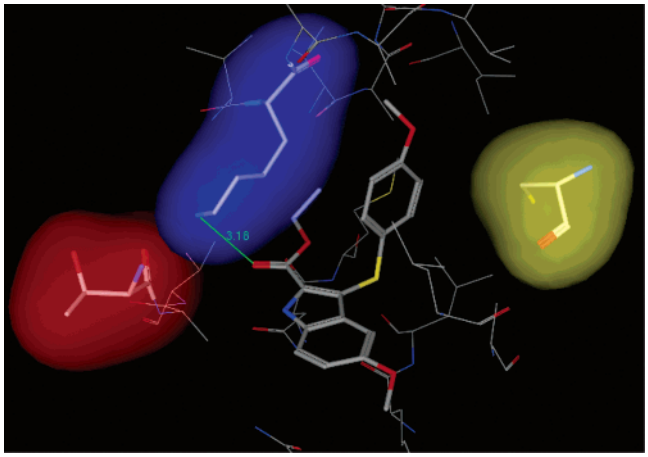


Figure 4. Proposed binding mode for **34**. Lys352 in blue, Thr179 in red, Cys241 in yellow.

Table 3. Comparison between Average Potential Energy of Interaction (U_{ab}) and Inhibition of Tubulin Polymerization for Compounds **32**, **33**, **34**, and **36**

compd	U_{ab} (kcal/mol)	tubulin $IC_{50} \pm$ SD (μM)
32	−57.6	16 ± 0.5
33	−60.1	3.1 ± 0.2
34	−49.8	>40
36	−64.7	2.4 ± 0.2

Conclusions

ATI derivatives that possess a 3-methoxyphenylthio or a 3,5-dimethoxyphenylthio moiety at position 2 of the indole ring were potent tubulin inhibitors but weak inhibitors of MCF-7 cell growth. On the other hand, ATIs bearing the 3-(3,4,5-trimethoxyphenyl)thio moiety were not only potent tubulin inhibitors, with potencies comparable to those of the reference compounds colchicine (**1**) and CSA4 (**2a**), but also potent inhibitors of cell growth. Conversely, the 4-methoxyarylthio substitution was detrimental for inhibition of both tubulin polymerization and MCF-7 cell growth.

The results obtained from the dynamics simulation correlate well with the observed experimental data. The agreement between the SAR findings and the modeling studies increase our confidence in the binding model we have obtained, and these results may enable us to design more selective and potent analogues.

Experimental Section

Chemistry. Melting points (mp) were determined on a Büchi 510 apparatus and are uncorrected. Infrared spectra (IR) were run on a SpectrumOne FT spectrophotometer. Band position and absorption ranges are given in cm^{-1}. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on Bruker 200 and 400 MHz FT spectrometers in the indicated solvent. Chemical shifts are expressed in δ units (ppm) from tetramethylsilane. Column chromatography was performed on columns packed with alumina from Merck (70−230 mesh) or silica gel from Merck (70−230 mesh). Aluminum oxide TLC cards from Fluka (aluminum oxide precoated aluminum cards with fluorescent indicator at 254 nm) and silica gel TLC cards from Fluka (silica gel precoated aluminum cards with fluorescent indicator at 254 nm) were used for thin-layer chromatography (TLC). Developed plates were visualized by a Spectroline ENF 260C/F UV apparatus. Organic solutions were dried over anhydrous sodium sulfate. Concentration and evaporation of the solvent after reaction or extraction was carried out on a Büchi Rotavapor rotary evaporator operating at reduced pressure. Elemental analyses were found within ±0.4% of the theoretical values.

Method A. General Procedure for the Synthesis of Compounds 12, 13, 14, 17, 19, 28, 31−34, and 38. Example. Ethyl 3-[(2-Methoxyphenyl)thio]-1*H*-indole-2-carboxylate (12). Boron trifluoride diethyl etherate (0.067 g, 0.06 mL, 0.00047 mol) was added to a mixture of ethyl 1*H*-indole-2-carboxylate (0.53 g, 0.0028 mol), *N*-(2-methoxyphenylthio)succinimide (0.57 g, 0.0024 mol), and anhydrous dichloromethane (20 mL) under a dry argon atmosphere. After stirring at room temperature for 2 h, boron trifluoride diethyl etherate (0.27 g, 0.24 mL, 0.002 mol) was added, and the reaction was heated at 45 °C for 2 h. After cooling, the reaction mixture was diluted with chloroform and brine while being shaken. The organic layer was separated, washed with a saturated solution of sodium hydrogen carbonate and with brine, and dried. The solvent was evaporated to give a residue that was purified by silica gel column chromatography (ethyl acetate−*n*-hexane 1:3 as eluent) to afford **12,** yield 26%, mp 164−166 °C (from ethanol). Anal. Calcd. ($C_{18}H_{17}NO_3S$ (327.40)) C, H, N, S.

Ethyl 3-[(3-methoxyphenyl)thio]-1*H*-indole-2-carboxylate (13) was prepared as **12** using *N*-(3-methoxyphenylthio)succinimide. Yield 23%, mp 101−104 °C (from ethanol).[20]

Ethyl 3-[(4-methoxyphenyl)thio]-1*H*-indole-2-carboxylate (14) was synthesized as **12** using *N*-(4-methoxyphenylthio)succinimide. Yield 15%, mp 118−120 °C (from ethanol). Anal. Calcd. ($C_{18}H_{17}NO_3S$ (327.40)) C, H, N, S.

Methyl 5-chloro-3-[(2-methoxyphenyl)thio]-1*H*-indole-2-carboxylate (17) was synthesized as **12** using methyl 5-chloro-1*H*-indole-2-carboxylate and *N*-(2-methoxyphenylthio)succinimide. Yield 17%, mp 222−226 °C (from ethanol). Anal. Calcd. ($C_{17}H_{14}ClNO_3S$ (347.82)) C, H, Cl, N, S.

Methyl 5-chloro-3-[(3-methoxyphenyl)thio]-1*H*-indole-2-carboxylate (19) was synthesized as **12** using methyl 5-chloro-1*H*-indole-2-carboxylate and *N*-(3-methoxyphenylthio)succinimide. Yield 17%, mp 160−163 °C (from ethanol). Anal. Calcd. ($C_{17}H_{14}ClNO_3S$ (347.82)) C, H, Cl, N, S.

Methyl 6-methoxy-3-(phenylthio)-1*H*-indole-2-carboxylate (28) was synthesized as **12** using methyl 6-methoxy-1*H*-indole-2-carboxylate and *N*-(phenylthio)succinimide. Yield 5%, mp 120−124 °C (from ethanol). Anal. Calcd. ($C_{17}H_{15}NO_3S$ (313.38)) C, H, N, S.

Methyl 4-methoxy-3-(phenylthio)-1*H*-indole-2-carboxylate (31) was synthesized as **12**, using methyl 4-methoxy-1*H*-indole-2-carboxylate and *N*-(phenylthio)succinimide. Yield 40%, mp 115−118 °C (from ethanol). Anal. Calcd. ($C_{17}H_{15}NO_3S$ (313.38)) C, H, N, S.

Ethyl 5-methoxy-3-[(2-methoxyphenyl)thio]-1*H*-indole-2-carboxylate (32) was synthesized as **12** using ethyl 5-methoxy-1*H*-indole-2-carboxylate and *N*-(2-methoxyphenylthio)succinimide. Yield 35%, mp 158 °C (from ethanol). Anal. Calcd. ($C_{19}H_{19}NO_4S$ (357.43)) C, H, N, S.

Ethyl 5-methoxy-3-[(3-methoxyphenyl)thio]-1*H*-indole-2-carboxylate (33) was synthesized as **12** using ethyl 5-methoxy-1*H*-indole-2-carboxylate and *N*-(3-methoxyphenylthio)succinimide. Yield 31%, mp 104−107 °C (from ethanol). Anal. Calcd. ($C_{19}H_{19}NO_4S$ (357.43)) C, H, N, S.

Ethyl 5-methoxy-3-[(4-methoxyphenyl)thio]-1*H*-indole-2-carboxylate (34) was synthesized as **12** using ethyl 5-methoxy-1*H*-indole-2-carboxylate and *N*-(4-methoxyphenylthio)succinimide. Yield 54%, mp 140−145 °C (from ethanol). Anal. Calcd. ($C_{19}H_{19}NO_4S$ (357.43)) C, H, N, S.

Ethyl 5,6-dimethoxy-3-(phenylthio)-1*H*-indole-2-carboxylate (38) was synthesized as **12** using ethyl 5,6-dimethoxy-1*H*-indole-2-carboxylate and *N*-(phenylthio)succinimide. Yield 35%, mp 137−139 °C (from ethanol). Anal. Calcd. ($C_{19}H_{19}NO_4S$ (357.42)) C, H, N, S.

Method B. General Procedure for the Synthesis of Compounds 16, 27, 36, and 41. Example. Ethyl 3-[(3,4,5-Trimethoxyphenyl)thio)]-1*H*-indole-2-carboxylate (16). 3,4,5-Trimethoxybenzenethiol[21] (1.87 g, 0.0093 mol) was added to a solution of NCS (1.26 g, 0.0094 mol) in anhydrous dichloromethane (74 mL) at −78 °C. The reaction was warmed to 0 °C over 15 min, and then a solution of ethyl 1*H*-indole-2-carboxylate (1.5 g, 0.008 mol) in the

same solvent (15 mL) was added. The reaction mixture was stirred at 0 °C for 1 h and concentrated under reduced pressure. The residue was suspended in water (150 mL) and extracted with ethyl acetate, and the organic layer was washed with brine and dried. Removal of the solvent furnished a crude product that was purified by silica gel column chromatography (ethyl acetate−*n*-hexane 1:1 as eluent) to give **16**, yield 1%, mp 90−95 °C (from ethanol). Anal. Calcd. ($C_{20}H_{21}NO_5S$ (387.46)) C, H, N, S.

Ethyl 5-chloro-3-[(3,4,5-trimethoxyphenyl)thio)]-1*H*-indole-2-carboxylate (27) was synthesized as **16** using ethyl 5-chloro-1*H*-indole-2-carboxylate. Yield 6%, mp 110−120 °C (from ethanol). Anal. Calcd. ($C_{20}H_{20}ClNO_5S$ (421.90)) C, H, Cl, N, S.

Ethyl 5-methoxy-3-[(3,4,5-trimethoxyphenyl)thio]-1*H*-indole-2-carboxylate (36) was synthesized as **16** using ethyl 5-methoxy-1*H*-indole-2-carboxylate. Yield 4%, mp 123−128 °C (from ethanol). Anal. Calcd. ($C_{21}H_{23}NO_6S$ (417.48)) C, H, N, S.

Ethyl 5,6-dimethoxy-3-[(3,4,5-trimethoxyphenyl)thio]-1*H*-indole-2-carboxylate (41) was synthesized as **16** using ethyl 5,6-dimethoxy-1*H*-indole-2-carboxylate. Yield 10%, mp 140−146 °C (from ethanol). Anal. Calcd. ($C_{22}H_{25}NO_7S$ (447.51)) C, H, N, S.

Method C. Synthesis of Methyl 5-Chloro-3-[(3,5-dimethoxyphenyl)thio)]-1*H*-indole-2-carboxylate (25). 5-Chloro-1*H*-indole-2-carboxylic acid (0.16 g, 0.0008 mol) was added by portions to a mixture of sodium hydride (60% dispersion in mineral oil, 0.094 g, 0.0023 mol) in anhydrous DMF (4 mL) under a nitrogen stream at 0 °C. After 15 min, 1,1′-(3,5-dimethoxyphenyl)disulfide (0.35 g, 0.001 mol) was added portionwise, then the reaction was heated at 50 °C overnight under a nitrogen atmosphere. After cooling, the mixture was poured onto crushed ice, made acidic with 2 N HCl and extracted with ethyl acetate. The organic layer was separated, washed with brine, and dried. Removal of the solvent gave a residue that was triturated with cyclohexane and filtered to give 5-chloro-3-[(3,5-dimethoxyphenyl)thio)]-1*H*-indole-2-carboxylic acid, which was used as the crude product. The acid was dissolved in dichloromethane (12 mL) and methanol (3 mL) and treated with TMSDM (1.02 mL of a 2 N solution in hexane, 0.002 mol) while stirring at room temperature for 30 min. After reduction to a small volume, the suspension was treated with ethyl acetate (10 mL × 5), and the solvent was evaporated. The crude product was purified by silica gel column chromatography (ethyl acetate−*n*-hexane 1:5 as eluent) to give **25**, yield 10%, mp 160−165 °C (from ethanol). Anal. Calcd. ($C_{18}H_{16}ClNO_4S$ (377.84)) C, H, Cl, N, S.

Method D. General Procedure for the Synthesis of Compounds 5−11, 37, and 40. **Propyl 3-(Phenylthio)-1*H*-indole-2-carboxylate (5).** Methyl 1*H*-indole-2-carboxylate was obtained by esterification of the corresponding acid using TMSDM, yield 88%, mp 145−148 °C (from ethanol). Lit.,[22] mp 149−151 °C (from ethyl acetate−*n*-hexane). Methyl 3-(phenylthio)-1*H*-indole-2-carboxylate was prepared by reaction of methyl 1*H*-indole-2-carboxylate with *N*-(phenylthio)succinimide in the presence of boron trifluoride diethyl etherate as above. The crude residue was purified by silica gel column chromatography (ethyl acetate−*n*-hexane 1:3 as eluent), yield 55%, mp 175−180 °C (from ethanol). Lit.,[23] mp 178−180 °C (from diethyl ether−*n*-hexane). Lithium hydroxide monohydrate (8.46 g, 0.20 mol) was added to a suspension of methyl 3-(phenylthio)-1*H*-indole-2-carboxylate (18.52 g, 0.065 mol) in THF (250 mL) and water (250 mL). The mixture was stirred at room-temperature overnight. After reduction to a small volume, it was made acidic (pH = 2) with 1 N HCl and extracted with ethyl acetate. The organic layer was washed with brine, dried, and evaporated to afford pure 3-(phenylthio)-1*H*-indole-2-carboxylic acid, yield 94%, mp 161−162 °C (from glacial acetic acid). Lit.,[23] 160−162 °C (from glacial acetic acid). To a cooled suspension of 3-(phenylthio)-1*H*-indole-2-carboxylic acid (1.00 g, 0.0037 mol) in 1-propanol (6.50 mL) was added dropwise thionyl chloride (0.33 mL) under an anhydrous argon atmosphere. The reaction mixture was heated at 70 °C for 5 h. After cooling, the suspension was filtered to afford **5**, yield 52%, mp 132−135 °C (from ethanol). Anal. Calcd. ($C_{18}H_{17}NO_2S$ (311.40)) C, H, N, S.

Isopropyl 3-(phenylthio)-1*H*-indole-2-carboxylate (6) was synthesized as **5** using *iso*-propyl alcohol. Yield 50%, mp 146 °C (from ethanol). Anal. Calcd. ($C_{18}H_{17}NO_2S$ (311.40)) C, H, N, S.

***n*-Butyl 3-(phenylthio)-1*H*-indole-2-carboxylate (7)** was synthesized as **5** using *n*-butyl alcohol. Yield 25%, mp 138 °C (from ethanol). Anal. Calcd. ($C_{19}H_{19}NO_2S$ (325.43)) C, H, N, S.

***sec*-Butyl 3-(phenylthio)-1*H*-indole-2-carboxylate (8)** was synthesized as **5** using *sec*-butyl alcohol. Yield 20%, mp 145 °C (from ethanol). Anal. Calcd. ($C_{19}H_{19}NO_2S$ (325.43)) C, H, N, S.

***tert*-Butyl 3-(phenylthio)-1*H*-indole-2-carboxylate (9)** was synthesized as **5** using *tert*-butyl alcohol. Yield 12%, mp 143−147 °C (from ethanol). Anal. Calcd. ($C_{19}H_{19}NO_2S$ (325.43)) C, H, N, S.

***iso*-Pentyl 3-(phenylthio)-1*H*-indole-2-carboxylate (10)** was synthesized as **5** using *iso*-pentyl alcohol. Yield 21%, mp 124−128 °C (from ethanol). Anal. Calcd. ($C_{20}H_{21}NO_2S$ (339.46)) C, H, N, S.

Benzyl 3-(phenylthio)-1*H*-indole-2-carboxylate (11) was synthesized as **5** using benzyl alcohol. Yield 20%, mp 135−138 °C (from ethanol). Anal. Calcd. ($C_{22}H_{17}NO_2S$ (359.45)) C, H, N, S.

Methyl 5-nitro-3-[(3,4,5-trimethoxyphenyl)thio]-1*H*-indole-2-carboxylate (37) was synthesized as **5** using 5-nitro-1*H*-indole-2-carboxylic acid and 1,1′-(3,4,5-trimethoxyphenyl)disulfide. Transformation of 5-nitro-3-[(3,4,5-trimethoxyphenyl)thio]-1*H*-indole-2-carboxylic acid into **37** was achieved by heating at reflux in methanol in the presence of thionyl chloride. The crude product was purified by silica gel column chromatography (ethyl acetate−*n*-hexane 1:2 as eluent), yield 3%, mp 194−198 °C (from ethanol). Anal. Calcd. ($C_{19}H_{18}N_2O_7S$ (418.43)) C, H, N, S.

Methyl 5,6-dimethoxy-3-[(3,4,5-trimethoxyphenyl)thio]-1*H*-indole-2-carboxylate (40) was synthesized as **5** using 5,6-dimethoxy-1*H*-indole-2-carboxylic acid. Yield 8%, mp 164−168 °C (from ethanol). Anal. Calcd. ($C_{21}H_{23}NO_7S$ (433.48)) C, H, N, S.

Methyl 5-chloro-3-[(2-methoxyphenyl)sulfonyl]-1*H*-indole-2-carboxylate (18). MCPMA (0.16 g, 0.0009 mol) was added to an ice-cold solution of **17** (0.13 g, 0.0004 mol) in chloroform (6 mL). The reaction mixture was stirred at room temperature for 2 h, poured on crushed ice, and extracted with chloroform. The organic solution was shaken with a saturated solution of sodium hydrogen carbonate and with brine and dried. After concentration to a small volume, the solution was purified by silica gel column chromatography (ethyl acetate−*n*-hexane 1:3 as eluent) to afford **18**, yield 50%, mp 250 °C (from ethanol). Anal. Calcd. ($C_{17}H_{14}ClNO_5S$ (379.82)) C, H, Cl, N, S.

Methyl 5-chloro-3-[(3-methoxyphenyl)sulfonyl]-1*H*-indole-2-carboxylate (20) was synthesized as **18**, starting from **19**. Yield 92%, mp 170−173 °C (from ethanol). Anal. Calcd. ($C_{17}H_{14}ClNO_5S$ (379.82)) C, H, Cl, N, S.

Methyl 5-chloro-3-[(4-methoxyphenyl)sulfonyl]-1*H*-indole-2-carboxylate (22) was synthesized as **18**, starting from **21**. Yield 29%, mp 230−232 °C (from ethanol). Anal. Calcd. ($C_{17}H_{14}ClNO_5S$ (379.82)) C, H, Cl, N, S.

Methyl 5-methoxy-3-(phenylsulfonyl)-1*H*-indole-2-carboxylate (30) was synthesized as **18**, starting from **29**. Yield 28%, mp 166−179 °C (from ethanol). Anal. Calcd. ($C_{17}H_{15}NO_5S$ (345.37)) C, H, N, S.

Ethyl 5,6-dimethoxy-3-(phenylsulfonyl)-1*H*-indole-2-carboxylate (39). Was synthesized as **18**, starting from **38**. Yield 47%, mp 264−273 °C (from ethanol). Anal. Calcd. ($C_{19}H_{19}NO_6S$ (389.42)) C, H, N, S.

***N*-(2-Methoxyphenylthio)succinimide.** 2-Methoxythiophenol (2.80 g, 0.02 mol) was added dropwise to an ice-cold mixture of NCS (3.34 g, 0.025 mol) and anhydrous dichloromethane (30 mL) under an argon atmosphere. After 1 h, additional NCS (0.4 g, 0.003 mol) was added, and the reaction mixture was stirred for 2.5 h. Triethylamine (2.83 g, 3.9 mL, 0.028 mol) was added while stirring for 15 min, and then dichloromethane (20 mL) and 1 N HCl (10 mL) were added. After shaking, the organic layer was dried, concentrated to a small volume, and passed through a Celite column. After evaporation of the solvent, the residue was triturated with diethyl ether to give *N*-(2-methoxyphenylthio)succinimide, yield

60%, mp 165−168 °C, (from ethanol), Lit.,[24] mp 173 °C. Anal. Calcd. ($C_{11}H_{11}NO_3S$ (237.27)).

***N*-(3-Methoxyphenylthio)succinimide** was synthesized as *N*-(2-methoxyphenylthio)succinimide using 3-methoxythiophenol. Yield 60%, mp 147−149 °C, Lit.,[25] mp 149−150 °C.

***N*-(4-Methoxyphenylthio)succinimide** was synthesized as *N*-(2-methoxyphenylthio)succinimide using 4-methoxythiophenol. Yield 60%, mp 135−140 °C, Lit.[26] mp 100−106 °C. Anal. Calcd. ($C_{11}H_{11}NO_3S$ (237.27)).

1,1′-(3,5-Dimethoxyphenyl)disulfide. A mixture of 3,5-dimethoxyaniline (5.00 g, 0.033 mol), concentrated HCl (5.9 mL), and crushed ice (8 g) was prepared and cooled to −5 °C. To this mixture was added dropwise a solution of sodium nitrite (2.25 g, 0.033 mol) in water (12 mL) over 45 min. The reaction mixture was stirred at 0 °C for an additional 15 min, at which time it was added to a solution of potassium ethyl xantate (10.46 g, 0.065 mol) in water (31 mL) at 65 °C. After the mixture was stirred for 30 min at 65 °C, it was extracted with ethyl acetate. The organic layer was washed with brine, dried, and evaporated to give 3,5-dimethoxybenzenethiol (2.86 g, yield 51%), which was used without further purification. A solution of iodine (6.10 g, 0.024 mol) in ethanol 96% (15 mL) was added dropwise to a solution of 3,5-dimethoxybenzenethiol (2.86 g, 0.017 mol) in the same solvent (5 mL) until a persistent color was observed. After stirring for 10 min, the reaction mixture was extracted with ethyl acetate. The organic layer was washed with a saturated solution of sodium thiosulfate and with brine and dried. Evaporation of the solvent gave 1,1′-(3,5-dimethoxyphenyl)-disulfide, yield 52%, which was used without purification.

Biology. Tubulin Assembly. The reaction mixtures contained 0.8 M monosodium glutamate (pH 6.6 with HCl in 2 M stock solution), 10 μM tubulin, and varying concentrations of drug. Following a 15 min preincubation at 30 °C, samples were chilled on ice, GTP to 0.4 mM was added, and turbidity development was followed at 350 nm in a temperature controlled recording spectrophotometer for 20 min at 30 °C. Extent of reaction was measured. Full experimental details were previously reported.[27]

Colchicine Binding Assay. The reaction mixtures contained 1.0 μM tubulin, 5.0 μM [^{3}H]colchicine, and 5.0 μM inhibitor and were incubated 10 min at 37 °C. Complete details were described previously.[28]

MCF-7 Cell Growth. The above paper[28] can also be referenced for methodology of MCF-7 cell growth.

SCLC Cell Growth. Experiments were carried out following a previously reported methodology.[29]

Hsp90 Competition Assay. Experiments were carried out as previously reported.[30] Briefly: Fluorescence polarization measurements were performed on an Analyst AD instrument (Molecular Devices, Sunnyvale, CA). Measurements were taken in black 96-well microtiter plates (Corning #3650). The assay buffer contained 20 mM potassium HEPES pH 7.3, 50 mM KCl, 5 mM $MgCl_2$, 20 mM Na_2MoO_4, and 0.01% NP40. Before each use, 0.1 mg/mL bovine gamma globulin (Panvera Corporation, Madison, WI) and 2 mM dithiothreitol (Fisher Biotech, Fair Lawn, NJ) were added to the buffer. GM-BODIPY (fluorescently labeled geldanamycin) was synthesized as previously reported[31] and was dissolved in dimethyl sulfoxide to form a 10 μM solution. Recombinant Hsp90α was purchased from Stressgen Bioreagents (cat. No. SPP-776), (Victoria, BC, Canada). For the competition studies, each 96-well contained 5 nM fluorescent GM-BODIPY, 30 nM Hsp90α and potential inhibitor (initial stock in dimethyl sulfoxide) in a final volume of 100 μL. The plate was left on a shaker at 4 °C for 24 h, and the fluorescence polarization values in mP were recorded. EC_{50} values were determined as the competitor concentrations at which 50% of the fluorescent GM-BODIPY was displaced.

Her2 Assay. The SKBr3 breast cancer cells were plated in black, clear-bottom microtiter plates (Corning #3603) at 3000 cells/well in growth medium (100 μL) and allowed to attach for 24 h at 37 °C and in a 5% CO_2 atmosphere. Growth medium (100 μL) with drug or vehicle (dimethyl sulfoxide) was carefully added to the wells, and the plates were placed at 37 °C in the 5% CO_2 atmosphere. Following a 24 h incubation with drugs, wells were washed with ice-cold Tris buffered saline containing 0.1% Tween 20 (TBST) (200 μL). A house vacuum source attached to an eight-channel aspirator was used to remove the liquid from the plates. Methanol (100 μL at −20 °C) was added to each well, and the plate was placed at 4 °C for 10 min. The methanol was removed by washing with TBST (2 × 200 μL). After further incubation at r.t. for 2 h with SuperBlock (Pierce 37535) (200 μL), anti-Her-2 (c-erbB-2) antibody (Zymed Laboratories #28−004) (100 μL, 1:200 in SuperBlock) was placed in each well. The plate was incubated overnight at 4 °C. For control wells, 1:200 dilution of a normal rabbit IgG (Santa Cruz #SC-2027) in Superblock was used. Each well was washed with TBST (2 × 200 μL) and incubated at r.t. for 2 h with an anti-rabbit HRP-linked antibody (Sigma, A-0545) (100 μL, 1:2000 in SuperBlock). Unreacted antibody was removed by washing with TBST (3 × 200 μL), and the ECL Western blotting reagent (Amersham #RPN2106) (100 μL) was added. The plate was immediately read in an Analyst AD plate reader (Molecular Devices). Each well was scanned for 0.1 s. Readings from wells containing only control IgG and the corresponding HRP-linked secondary antibody were set as background and subtracted from all measured values. Luminescence readings from drug-treated cells versus untreated cells were quantified and plotted against drug concentration to give the EC_{50} values as the concentration of drug that caused 50% decrease in luminescence.

Antimitotic Assay. Black, clear-bottom microtiter 96-well plates (Corning Costar #3603) were used to accommodate experimental cultures. MDA-MB-468 cells were seeded in each well at 8000 cells per well in growth medium (100 μL) and allowed to attach overnight at 37 °C in a 5% CO_2 atmosphere. Growth medium (100 μL) with drug or vehicle (dimethyl sulfoxide) was gently added to the wells, and the plates were incubated at 37 °C in a 5% CO_2 atmosphere for 24 h. Wells were washed with ice-cold TBST (2 × 200 μL). A house vacuum source attached to an eight-channel aspirator was used to remove the liquid from the 96-well plates. Ice-cold methanol (100 μL) was added to each well, and the plate was placed at 4 °C for 5 min. Methanol was removed by suction, and plates were washed with ice-cold TBST (2 × 200 μL). Plates were further incubated with SuperBlock blocking buffer (Pierce #37535) (200 μL) for 2 h at r.t. The Tg-3 antibody (gift of Dr. Davies, Albert Einstein College of Medicine) diluted 1:200 in SuperBlock was placed in each well (100 μL), except the control column that was treated with control antibody (Mouse IgM, NeoMarkers, NC-1030-P). After 72 h, wells were washed with ice-cold TBST (2 × 200 μL). The secondary antibody (goat Anti-Mouse IgM, Southern Biotech #1020−05) was placed in each well at 1:2000 dilution in SuperBlock and incubated on a shaker at r.t. for 2 h. Unreacted antibody was removed by washing the plates with ice-cold TBST (3 × 200 μL) for 5 min on a shaker. The ECL Western Blotting Detection Reagents 1 and 2 in 1:1 mixture (100 μL) was placed in each well, and the plates were read immediately in an Analyst AD plate reader (Molecular Devices). Luminescence readings were imported into SOFTmax PRO 4.3.1. Antimitotic activity was defined as a concentration dependent increase in luminescence readings in compound-treated wells as compared to vehicle-treated wells. The antibody (Tg-3), originally described as a marker of Alzheimer's disease, is highly specific for mitotic cells, Tg-3 immunofluorescence being >50-fold more intense in mitotic cells than in interphase cells.

Molecular Modeling. All molecular modeling studies were performed on a RM Innovator with Pentium IV 2.8 GHz processor, running Linux Fedora Core 3 using Molecular Operating Environment (MOE) 2004.03[17] and the FlexX module in SYBYL 7.0.[16] The tubulin structure was downloaded from the PDB data bank (http://www.rcsb.org/pdb/index.html PDB code: 1SA0).[32] All the minimizations were performed with MOE until RMSD gradient of 0.05 kcal mol^{-1} Å^{-1} was reached with the MMFF94x force field. The partial charges were automatically calculated. Docking experiments were carried out using the FlexX docking program of SYBYL 7.0 using the default settings. The output of FlexX docking was visualized in MOE, and the scoring.svl script[33] was used to identify interaction types between ligand and protein. Molecular dynamics

was performed with MOE using the NVT environment for 600 ps and constant temperature of 300 K using the MMFF94x force field with a time step of 2 fs. Residues within 15 Å of the ligand were allowed to move freely, keeping the rest of the protein fixed. The binding site was soaked in a water sphere of 25 Å radius from the sulfur atom of the ligand, and the total charge of the system included in the water droplet did not require any adjustment. The water molecules were energy minimized keeping the coordinates of the protein−ligand complex fixed before the MD simulation. A distance restraint of 25 Å with a weight of 100 between the oxygen atoms of the water molecules and the sulfur atom of the ligand was also applied.

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Supporting Information Available: ^{1}H NMR and IR spectral data, and elemental analyses of new compounds **5**−**12**, **14**, **16**−**20**, **22**, **25**, **27**, **28**, **30**−**34**, and **36**−**41** are available free of charge via the Internet at http://pubs.acs.org.

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