Further Naphthylcombretastatins. An Investigation on the Role of the Naphthalene Moiety

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By synthesis and biological studies of new naphthalene analogues of combretastatins, we have found that the naphthalene is a good surrogate for the isovanillin moiety (3-hydroxy-4-methoxyphenyl) of combretastatin A-4, always generating highly cytotoxic analogues when combined with the 3,4,5-trimethoxyphenyl or related systems. On the other hand, when the naphthalene replaces the 3,4,5-trimethoxyphenyl moiety, the cytotoxic activity is largely decreased. The most cytotoxic naphthalene analogues of combretastatins, which also produce inhibition of tubulin polymerization, exerted their antimitotic effects through microtubule network disruption and subsequent G_2/M arrest of the cell cycle in human cancer cells.

The mitotic spindle, whose formation and activity are required for chromosome segregation and cell division, is constituted by microtubules generated by polymerization of tubulin α,β-dimers.[1] Due to its basic role for mitosis to be completed, the tubulin α,β-dimer is the target for many drugs, which interfere with the dynamic behavior of the microtubules, thus producing cell cycle arrest and ultimately cell death.[2] At least three different sites are distinguished for the spindle poisons to interact with tubulin: the colchicine,[3,4] the vinca alkaloids[5] and the paclitaxel sites.[6,7] Natural and synthetic compounds of varied structures have been described to inhibit the tubulin polymerization by interaction at the colchicine binding site, suggesting a high plasticity of the target protein at this site.[8] The crystal structures of colchicine and podophyllotoxin complexes with tubulin have been published during the revision process of this manuscript.[9]

Combretastatins (Figure 1) are a very interesting class of cytotoxic agents of natural origin, which have received much attention[10] due to their simple structures,[11] their high potency as cytotoxic agents,[12,13] and their antiangiogenic activity.[14,15] The major problem associated with their structure, looking for their application as anticancer agents, is their poor solubility. Efforts directed at the preparation of soluble derivatives[16−19] have led to combretastatin A-4 phosphate prodrug[20] (CA-4P) as a good candidate, which is now in phase I/II clinical trials.

Since they were first described by Pettit in the early eighties,[21] SAR studies have been directed at the elucidation of the structural characteristics required for such small molecules to be cytotoxic.[22−24] The mechanism of action of these compounds is their antimitotic activity, due to the inhibition of tubulin polymerization by interaction at the colchicine binding site. Cytotoxicity and tubulin polymerization inhibition correlate well, combretastatin A-4 (CA-4) being the most potent member of this family as cytotoxic agent, inhibitor of tubulin polymerization, and inhibitor of colchicine binding to the protein.[25] As a result of the SAR studies (Figure 2), which investigated the influence of the structure and substituents on A,B-rings and/or the bridge between them, it was deduced that a 3,4,5-trimethoxyphenyl and a 4-methoxy-3-X-substituted-phenyl system, separated by a two atom bridge and in a cis disposition, are the common structural characteristic for these compounds to be active (X = H, OH, NH_2, and their amino acid, phosphate, or other derivatives for solubilizing purposes). These structural requisites have been attained in derivatives and analogues of diverse types, as those presented in Figure 2. Cis olefins,[10,11,26] sulfonamides,[27−30] and sulfonates,[31,32] amine or amide derivatives,[33−35] ethers,[36] cyclopentanes,[37,38] and heterocycles (pyrazole, thiazole, triazole, or tetrazole;[39] oxazole,[40,41] imidazole, or pyrazole;[40] thiophene;[42,43] furans, furanones, and dioxolanes;[44−46] and indoles[47]) have been used to maintain the spatial arrangement of both aromatic systems, thus resulting in highly potent compounds whenever their substitution pattern is close to that of combretastatin A-4, considered to be the optimal for this family of antimitotic agents.

We recently described the synthesis and evaluation of new analogues of combretastatins carrying a naphthalene moiety, and we called them naphthylcombretastatins (Figure 3). We initially synthesized dihydro derivatives with two aromatic systems bonded through an ethane (e.g. **1** and **2**),[48] a functionalized ethane, or a 2,3-indole bridge,[49,50] which were moderately potent cytotoxic agents. Finally, we prepared analogues **3** and **4** of combretastatin A-4, carrying a naphthalene moiety and a 3,4,5-trimethoxyphenyl or a 3-hydroxy-4-meth-

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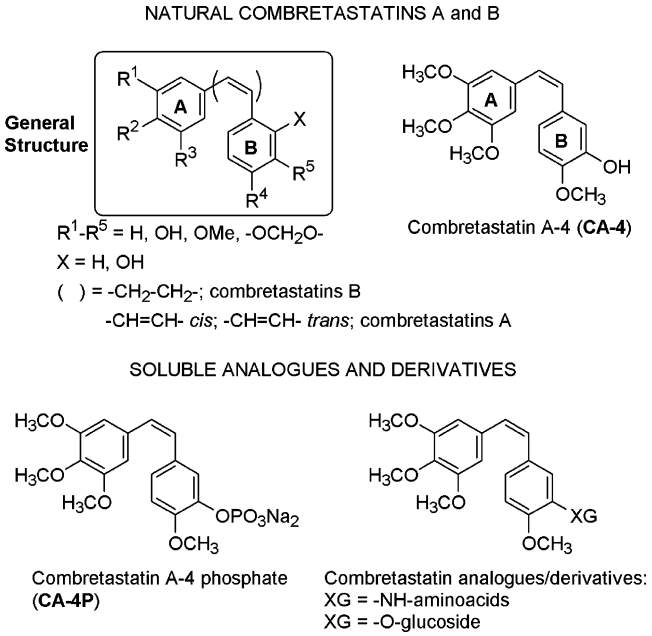


Figure 1. Structure of natural combretastatins and related soluble compounds.

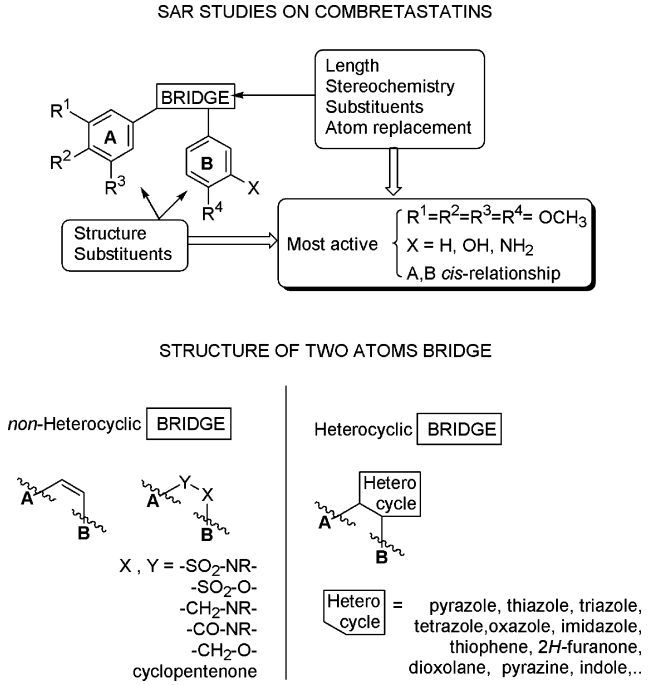


Figure 2. Structural modifications of combretastatins and their analogues for SAR studies.

oxyphenyl moiety in their structures.[51] These compounds showed a very interesting activity, either on the inhibition of cell proliferation, inhibition of tubulin polymerization, cell cycle arrest, or microtubule disruption. After these results, we decided to prepare further naphthalene analogues of combretastatin A-4 in order to know their cytotoxic activity and their inhibitory effect on tubulin polymerization. By preparing and assaying these derivatives, we intended to test our assumption about the capability of the naphthalene moiety in replacing either the 3,4,5-trimethoxyphenyl or the 3-hydroxy-4-methoxyphenyl residues of combretastatin A-4, thus contributing to a better knowledge of the structure−activity relationships among the combretastatins and their analogues. The compounds synthesized and assayed in this paper have a range of mono-, di-, and trisubstituted benzene rings, the naphthalene system, and several monocyclic, bicyclic, and tricyclic heteroaromatic moieties as equivalents of the A,B-rings of combretastatins.

Chemistry

The synthesized compounds were prepared by means of the Wittig methodology (Scheme 1). The appropriate phosphoranes were obtained from commercially available aldehydes (through reduction, bromination, reaction with triphenylphosphine, and treatment with bases) and reacted with the required aldehydes. Mixtures of the *E* and *Z* stereoisomers in variable ratios were produced and separated by chromatography. In general, groupings containing acidic protons were protected during the Wittig reaction (for example, indolyl moieties were protected by phenylsulfonyl groups and phenolic hydroxyls as the ethoxymethyl derivatives) while the amino groups were generated by reduction of the nitro groups present in the starting materials, after the Wittig olefination step.

The isolated products or irresolvable mixtures of stereoisomers were assayed for "in vitro" cytotoxicity and/or inhibition of tubulin polymerization. These compounds include representative structures (a) carrying a naphthyl moiety and substituted benzenes or polycyclic(heterocyclic) systems (**5**−**25**), (b) with a 3,4,5-trimethoxyphenyl instead of the naphthalene (**26**−**35**) for comparative purposes, and, finally, (c) new naphthalene derivatives (**36**−**41**) required to test the conclusions drawn from the activity results of the preceding groups.

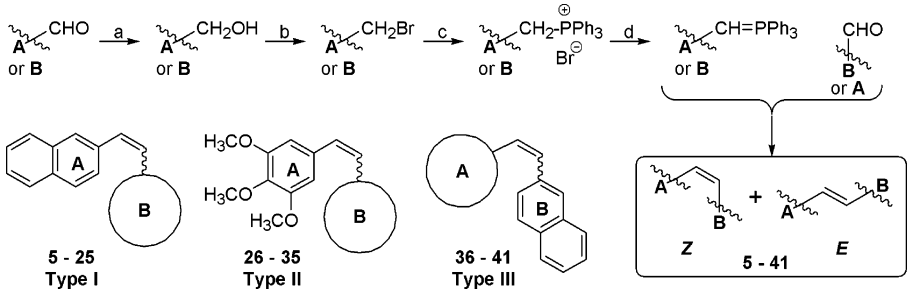
The effect of selected compounds on different phases of the cell cycle was further evaluated by flow cytometry and the effect on the cell microtubular network observed by confocal microscopy.

Cytotoxicity Assays

The synthesized compounds were assayed against several cancer cell lines, including murine P-388, human lung carcinoma A-549, human colon adenocarcinoma HT-29, human colon carcinoma HCT-116, or other neoplastic cell lines and compared with compounds **1**−**4** and CA-4 (Tables 1−3). Initial derivatives with a 2-naphthyl A ring (type I analogues: **5**−**25**) sample B rings spanning from the small furyl to the large 3-fluorenyl (Figure 4). The *E* isomers, compounds **5*E***−**25*E***, were all inactive or display very low cytotoxicity, as expected for *trans*-combretastatins. The lack of cytotoxicity is ascribed to the double bond geometry, irrespective of the degree of similarity of the 2-naphthyl to the A ring and the other system to the B ring of combretastatins.

More significant is the low cytotoxicity of the *Z* isomers (**5*Z***−**25*Z***). None of them is comparable to CA-4 or compounds **3*Z*** and **4*Z***, not even **8**−**10*Z*** and **15*Z***, whose analogues with a trimethoxybenzene instead of the naphthalene are highly potent. This reduction in cytotoxicity (Table 1 and Figure 5a) is especially significant for **8*Z*** (IC_{50}= 1.9 μM) when compared to its trimethoxyphenyl analogue (A ring = 3,4,5-TM, B ring = 4′-MeOPh, $IC_{50} = 2.2 \times 10^{-2}$ to 3.4×10^{-4} μM) and **15*Z***

Scheme 1[a]



[a] Reagents: (a) $NaBH_4$, MeOH, room temperature; (b) PBr_3, ethyl ether, −40 °C, 2−5 h; (c) PPh_3, C_6H_6, room temperature, 24−48 h; (d) BuLi, THF, −15 °C, 30 min; then aldehyde in THF, −15 °C to room temperature.

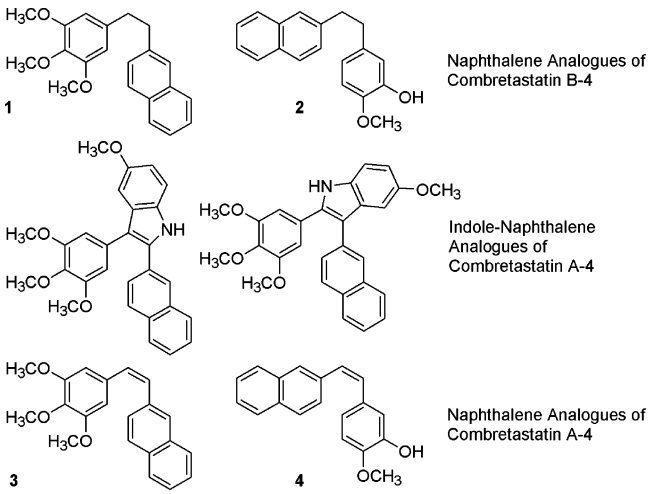


Figure 3. Naphthalene analogues of combretastatins (naphthylcombretastatins).

(IC_{50} = 0.36−36 μM) vs the amino−combretastatin analogue (A ring = 3,4,5-TM, B ring = 3′-NH_2-4′-MeOPh, $IC_{50} = 5.1 \times 10^{-3}$ μM), in good agreement with a potency decrease higher than 1 order of magnitude of **4*Z*** vs CA-4.

As mentioned before, in order to complete the comparison of the cytotoxic potency of compounds **5**−**25** with their trimethoxyphenyl analogues, type II compounds **26**−**35** (Figure 6) were synthesized and assayed (Table 2). Additionally, these type II derivatives allowed us to search for plausible replacements for the 3′-hydroxy-4′-methoxyphenyl (IsoV) B ring of CA-4 other than the 2-naphthyl. Taking into account our previous success with the 2-naphthyl, we sought its replacement by heterocycles (Figure 6), the most conservative choice being the indole ring. Several functional groups were also introduced on the nitrogen, in the search for a wider range of hydrogen bonding capabilities, hydrophobicity, and sizes (to this end, also carbazoles were considered as a ring extension). Although there are results that need a particular discussion (**28*Z***, **35*E***), several generalizations can be inferred from these data.

Again, when the 5-indolyl series (or derivatives containing the tricyclic carbazole system) belonging to type I (Table 1) and type II (Table 2) are compared (**21**−**23** and **25** vs **32**−**35**), the naphthalene-containing ones are always inactive but some of the trimethoxyphenyl ones retain high activity (e.g. **33*Z***, IC_{50} = 0.03−0.4 μM vs **22*Z***, IC_{50} = 18.6 μM; Figure 5b). Summarizing, the absence of the trimethoxyphenyl ring reduces the cytotoxicity and the 2-naphthyl moiety is a much better replacement for the B ring than for the A ring.

Parent compound **3*Z*** was the most potent of the series, followed by the 5-indolyl (**33*Z***). The substituents on the indolic nitrogen of **33*Z*** were poorly tolerated and led to inactive compounds (**32*Z*** and the carbazole **35*Z***). The compounds of the 3-indolyl series (**29**−**31**) are of similar potencies, irrespective of the nitrogen substitution (H, Me, Ac), and much less so than unsubstituted 5-indolyl **33*Z***. The two indole series behave quite distinctly, despite the fact that, in both cases, one benzene ring of the naphthalene is replaced by a pyrrole. This different behavior might be explained by the different orientation of their respective fused rings, the unsubstituted 5-indolyl more resembling isovanillin. The low potency of the carbazole derivative (**35*Z***) would suggest that no further room is available for the B ring in these ligands.

A noticeable finding is the high potency of compound **28*Z***, carrying the substitution pattern 3′-amino-4′-dimethylaminophenyl, previously unchecked in combretastatin analogues, as the B ring. Although the size of the 3′-amino-4′-dimethylaminophenyl is comparable to the naphthalene or indole size, the potency of **28*Z*** is even higher than that of combretastatin A-4 or the related 3′-amino analogue. On the other hand, compounds **34*E*** and **35*E***, showing cytotoxicities comparable to those of many combretastatin analogues, cannot be related to these antitubulin derivatives due to their divergent geometry.

Once the analogy between the bicyclic systems 2-naphthyl and 5-indolyl to the B ring of combretastatins (IsoV for CA-4) was observed, it was expected that compounds carrying one of these systems as the B ring would be highly potent cytotoxic derivatives and also tubulin polymerization inhibitors when benzene derivatives close to the TM (3,4,5-trimethoxyphenyl) were used as the A ring. With these ideas in mind, we prepared several analogues **36**−**41**, carrying 3,4,5-trisubstituted benzenes as A ring equivalents and the 2-naphthyl moiety as the B ring, these compounds being grouped as type III analogues (Figure 7 and Table 3). As expected, the *E* isomers are non-cytotoxic in the micromolar range. Although some of the *Z* isomers had low cytotoxicity or were non-cytotoxic, they were the less similar to the model 3,4,5-trimethoxyphenyl A ring, as for example the 4-methoxy-3,5-diamino(dinitro) derivatives **39*Z***−**40*Z*** and the 4-dimethylamino-3,5-dinitro derivative **41*Z***. When two methoxy substituents are maintained at positions 3 and 4, the activity varies with

Table 1. Effect of Compounds of Type I (IC_{50} [μM]) against a Panel of Tumor Cell Lines

	Z-isomer - Type I B=	P-388	A-549	HT -29	MEL -28	H116		*E*-isomer - Type I B=	P-388	A-549	HT -29	MEL -28	H116
5*Z*	3-ethoxymethoxy-4-methoxyphenyl	2.99	2.99	2.99	2.99	2.99	**5*E***	3-ethoxymethoxy-4-methoxyphenyl	2.99	2.99	> 5	2.99	> 5
6*Z*	4-acetoxyphenyl	--	--	--	--	--	**6*E***	4-acetoxyphenyl	> 5	> 5	> 5	> 5	> 5
7*Z*	4-hydroxyphenyl	--	--	--	--	--	**7*E***	4-hydroxyphenyl	4.06	4.06	4.06	4.06	4.06
8*Z*	4-methoxyphenyl	1.92	1.92	1.92	1.92	1.92	**8*E***	4-methoxyphenyl	> 5	> 5	> 5	> 5	> 5
9*Z*	3-fluoro-4-methoxyphenyl	0.45	0.45	0.45	0.45	0.45	**9*E***	3-fluoro-4-methoxyphenyl	4.49	> 5	> 5	> 5	> 5
10*Z*	4-trifluorometoxyphenyl	3.50	3.50	3.50	3.50	3.50	**10*E***	4-trifluorometoxyphenyl	> 5	> 5	> 5	> 5	> 5
11*Z*	4-tolyl	> 5	> 5	> 5	> 5	> 5	**11*E***	4-tolyl	> 5	> 5	> 5	> 5	> 5
12*Z*	4-trifluoromethylphenyl	> 5	> 5	> 5	> 5	> 5	**12*E***	4-trifluoromethylphenyl	> 5	> 5	> 5	> 5	> 5
13*Z*	4-dimethylaminophenyl	3.66	3.66	3.66	3.66	3.66	**13*E***	4-dimethylaminophenyl	3.66	3.66	3.66	3.66	3.66
14*Z*	3-nitro-4-methoxyphenyl	> 20	> 20	3.28	> 20	> 20	**14*E***	3-nitro-4-methoxyphenyl	> 5	> 5	> 5	> 5	> 5
15*Z*	3-amino-4-methoxyphenyl	1.82	> 20	1.82	> 20	0.36	**15*E***	3-amino-4-methoxyphenyl	> 5	1.82	> 5	> 5	1.82
16*Z*	3-acetylamino-4-methoxyphenyl	> 5	1.58	> 5	> 5	> 5	**16*E***	3-acetylamino-4-methoxyphenyl	> 5	> 5	> 5	> 5	> 5
17*Z*	3-diethylamino-4-methoxyphenyl	--	--	--	--	--	**17*E***	3-diethylamino-4-methoxyphenyl	> 5	> 5	> 5	> 5	> 5
18*Z*	2-furyl	> 5	> 5	> 5	> 5	> 5	**18*E***	2-furyl	> 5	> 5	> 5	> 5	> 5
19*Z*	3-furyl	> 5	> 5	> 5	> 5	> 5	**19*E***	3-furyl	> 5	> 5	> 5	> 5	> 5
20*Z*	2-naphthyl	3.57	3.57	3.57	3.57	5.00	**20*E***	2-naphthyl	--	--	--	--	--
21*Z*	N-benzenesulphonyl-5-indolyl	> 5	> 5	> 5	> 5	> 5	**21*E***	N-benzenesulphonyl-5-indolyl	> 5	> 5	> 5	> 5	> 5
22*Z*	5-indolyl	> 5	> 5	> 5	> 5	> 5	**22*E***	5-indolyl	--	--	--	--	--
23*Z*	N-methyl-5-indolyl	> 5	> 5	> 5	> 5	> 5	**23*E***	N-methyl-5-indolyl	> 5	3.53	> 5	> 5	> 5
24*Z*	3-fluorenyl	> 5	> 5	> 5	> 5	> 5	**24*E***	3-fluorenyl	> 5	> 5	> 5	> 5	> 5
25*Z*	N-ethyl-3-carbazolyl	> 5	> 5	> 5	> 5	> 5	**25*E***	N-ethyl-3-carbazolyl	> 5	> 5	> 5	> 5	> 5
1		0.31	0.31	0.31	0.31	0.31							
2		3.59	3.59	8.98	3.59	3.59							
3*Z*		0.02	0.02	0.02	0.02	> 5	**3*E***		0.39	0.39	0.39	0.39	0.39
4*Z*		0.04	0.04	3.62	0.04	3.62	**4*E***		3.62	3.62	3.62	3.62	3.62
CA-4		0.003	0.003	0.032	0.003	0.016							

Table 2. Effect of Compounds of Type II (IC_{50} [μM]) against a Panel of Tumor Cell Lines

	Z-isomer - Type II B=		P-388	A-549	HT -29	MEL -28 or *HeLa*	H116 or *HL-60*		*E*-isomer - Type II B=		P-388	A-549	HT -29	MEL -28 or *HeLa*	H116 or *HL-60*
26*Z*+26*E*	5-methyl-2-furyl		1.82	3.65	3.65	3.65	3.65	**26*E***	5-methyl-2-furyl		3.65	3.65	3.65	3.65	3.65
27*Z*	4-dimethylamino-3-nitrophenyl		> 5	> 5	> 5	> 5	> 5	**27*E***	4-dimethylamino-3-nitrophenyl		> 5	> 5	> 5	> 5	> 5
28*Z*	3-amino-4-dimethylaminophenyl	x	--	0.004	0.004	*0.003*	*0.003*	**28*E***	3-amino-4-dimethylaminophenyl		1.52	1.52	0.30	1.52	1.52
29*Z*	N-acetyl-3-indolyl		2.85	2.85	2.85	2.85	2.85	**29*E***	N-acetyl-3-indolyl		> 5	> 5	> 5	> 5	> 5
30*Z*	3-indolyl		3.23	3.23	3.23	3.23	3.23	**30*E***	3-indolyl		3.23	3.23	3.23	3.23	3.23
31*Z*	N-methyl-3-indolyl		3.09	3.09	3.09	3.09	3.09	**31*E***	N-methyl-3-indolyl		3.09	3.09	3.09	3.09	3.09
32*Z*	N-benzenesulphonyl-5-indolyl		> 5	> 5	> 5	> 5	> 5	**32*E***	N-benzenesulphonyl-5-indolyl		> 5	> 5	> 5	> 5	> 5
33*Z*	5-indolyl	x	--	0.39	0.035	*0.034*	*0.035*	**33*E***	5-indolyl	x	--	0.47	0.28	*0.25*	*0.38*
34*Z*	N-methyl-5-indolyl	x	--	0.47	0.36	*0.25*	*0.33*	**34*E***	N-methyl-5-indolyl	x	--	0.034	0.046	*0.024*	*0.027*
35*Z*	N-ethyl-3-carbazolyl	x	--	>10	>10	*3.1*	*>10*	**35*E***	N-ethyl-3-carbazolyl	x	--	0.53	0.45	*0.33*	*0.34*
1			0.31	0.31	0.31	0.31	0.31								
2			3.59	3.59	8.98	3.59	3.59								
3*Z*			0.02	0.02	0.02	0.02	> 5	**3*E***			0.39	0.39	0.39	0.39	0.39
4*Z*			0.04	0.04	3.62	0.04	3.62	**4*E***			3.62	3.62	3.62	3.62	3.62
CA-4			0.003	0.003	0.032	0.003	0.016								

x: analyzed by the XTT procedure.

the structure of the substituent at position 5, but its IC_{50} is in the submicromolar range when this substituent is the nitro group (see compound **36*Z*** in Table 3). These results were not surprising, as whenever there is a TM or a close structure as the equivalent of the A ring and the IsoV or related structure as the B ring, maintained in a cis relationship, highly cytotoxic and tubulin polymerization inhibitors are produced. This result is in agreement with the assumption that the 2-naphthyl moiety is a surrogate for the IsoV moiety when the B ring of combretastatins is replaced, because highly potent derivatives were produced under these circumstances.

Tubulin Polymerization Inhibition

After these results we checked the ability of the most potent of the synthesized compounds in inhibiting the polymerization of tubulin. To this purpose we selected representative derivatives of each type of analogue in Tables 1−3, including the model naphthyl derivatives **3*Z*** and **4*Z***, previously described (Table 4). The experiments were carried out with bovine brain tubulin, isolated as described in the literature[52] with small modifications by Dumortier et al.[53] The polymerization was followed by measuring the absorbance in the UV at 450 nm. The results for combretastatin A-4 (CA-4), taken as reference and model in these compounds, showed IC_{50} = 3 μM in the inhibition of the tubulin polymerization, in agreement with the described values for CA-4 in this assay. According to our assumption on the similarity between the 2-naphthyl moiety and the IsoV moiety (3-hydroxy-4-methoxyphenyl), derivative **3*Z*** showed an inhibition of tubulin polymerization of the same order of magnitude, only 3 times lower, than that exhibited by CA-4. This was also corroborated by the inhibition of tubulin polymerization IC_{50} = 13 μM displayed by the 2-naphthyl derivative **36*Z***, which also contains this moiety as a B ring equivalent and the 3,4-dimethoxy-5-nitrophenyl system as a close equivalent of the A ring (TM). Lower inhibition was found for the

Table 3. Effect of Compounds of Type III (IC_{50} [μM]) against a Panel of Tumor Cell Lines

	Z-isomer - Type III A=		P-388	A-549	HT-29	MEL-28 or *HeLa*	H116 or *HL-60*		*E*-isomer - Type III A=		P-388	A-549	HT-29	MEL-28 or *HeLa*	H116 or *HL-60*
36*Z*	3,4-dimethoxy-5-nitrophenyl	x	--	0.59	0.42	*0.11*	*0.34*	**36*E***	3,4-dimethoxy-5-nitrophenyl	x	--	>10	>10	*3.2*	*>10*
37*Z*	5-amino-3,4-dimethoxyphenyl		1.64	1.64	0.33	1.64	0.33	**37*E***	5-amino-3,4-dimethoxyphenyl	x	--	2.15	6.0	*0.46*	*3.15*
38*Z*	5-acetylamino-3,4-dimethoxyphenyl		> 5	> 5	> 5	> 5	> 5	**38*E***	5-acetylamino-3,4-dimethoxyphenyl		> 5	> 5	> 5	> 5	2.88
39*Z*	3,5-dinitro-4-methoxyphenyl		> 5	> 5	> 5	> 5	> 5	**39*E***	3,5-dinitro-4-methoxyphenyl		> 5	> 5	> 5	> 5	> 5
40*Z*	3,5-diamino-4-methoxyphenyl		> 5	> 5	> 5	> 5	> 5	**40*E***	3,5-diamino-4-methoxyphenyl		> 5	> 5	> 5	> 5	> 5
41*Z*	4-dimethylamino-3,5-dinitrophenyl		> 5	> 5	2.75	> 5	2.75	**41*E***	4-dimethylamino-3,5-dinitrophenyl		> 5	> 5	> 5	> 5	> 5
1			0.31	0.31	0.31	0.31	0.31								
2			3.59	3.59	8.98	3.59	3.59								
3*Z*			0.02	0.02	0.02	0.02	> 5	**3*E***			0.39	0.39	0.39	0.39	0.39
4*Z*			0.04	0.04	3.62	0.04	3.62	**4*E***			3.62	3.62	3.62	3.62	3.62
CA-4			0.003	0.003	0.032	0.003	0.016								

x: analyzed by the XTT procedure.

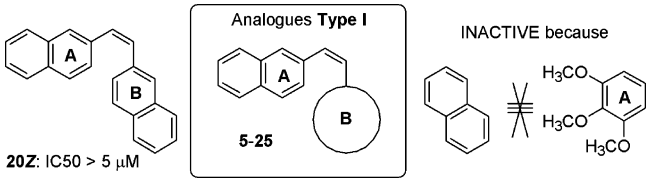


Figure 4.

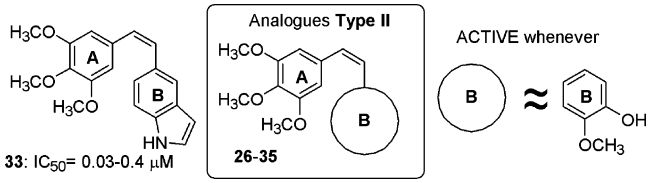


Figure 6.

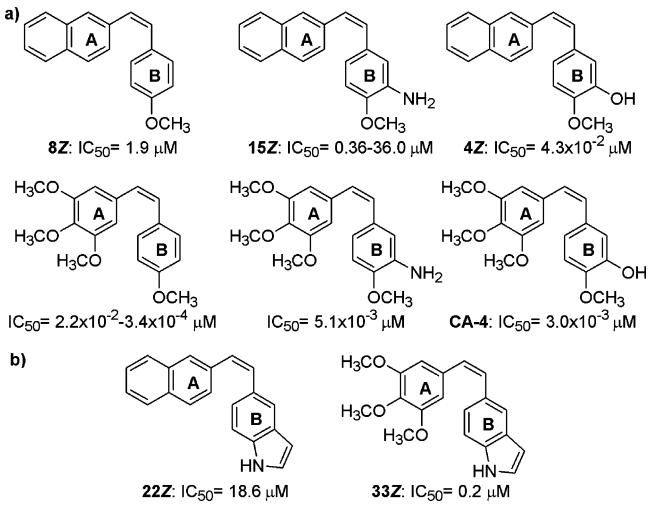


Figure 5. (a) Comparison of analogues type I with described 3,4,5-trimethoxyphenyl containing derivatives. (b) Comparison of representative compounds of type I and type II series.

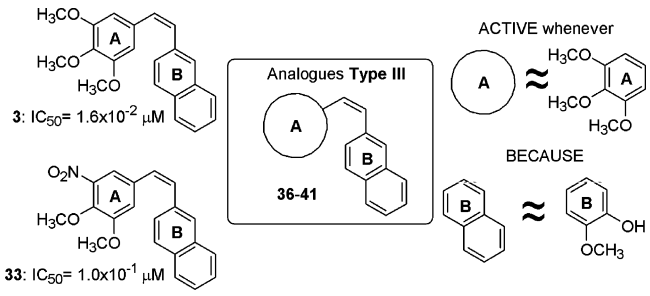


Figure 7.

also cytotoxic derivative **33*Z***, containing a 5-indolyl moiety instead of the 2-naphthyl moiety. All these facts are also in agreement with the lack of effect on tubulin polymerization shown by the most cytotoxic among compounds of type I, the analogues **8*Z*** and **9*Z***, whose IC_{50} > 30 μM is far from that exhibited by the described analogue in the combretastatin family (A ring = 3,4,5-TM, B ring = 4′-MeOPh),[10−12] which has IC_{50} = 2.2 μM. We also tested the most potent among the *E* isomers, **33*E*−37*E***, but as expected they lack any significant effect on tubulin polymerization, supporting the idea of other different targets for these compounds.

It is remarkable that compound **34*Z*** is the most potent in this assay but it is not the most cytotoxic of these analogues. A special case is that of the inactive compound **35*Z***, which is among the more effective as tubulin polymerization inhibitor of the tested compounds. In this case, the lack of effect on cells (cytotoxicity or G_2/M arrest, see next section) can be attributed to a limited penetration into the cell or any other mechanism limiting the accessibility of this molecule, carrying a large carbazole moiety, to the cellular tubulin.

The most cytotoxic derivative, **28*Z***, has no effect on tubulin polymerization at concentrations below 30 μM. This fact does not parallel the usual behavior of other combretastatin analogues, always displaying a strong inhibitory effect on tubulin polymerization whenever they are cytotoxic in the submicromolar range.

Table 4. Inhibition of in Vitro Tubulin Polymerization (ITP) and Biological Effects Shown by Selected Compounds of Types I−III[a]

compd	ITP IC_{50} (μM)	HeLa G_2/M arrest (24 h) 10^{-7} M	HeLa G_2/M arrest (24 h) 10^{-6} M	HeLa G_2/M arrest (24 h) 10^{-5} M	A-549 G_2/M arrest (24 h) 10^{-7} M	A-549 G_2/M arrest (24 h) 10^{-6} M	A-549 G_2/M arrest (24 h) 10^{-5} M	HT-29 G_2/M arrest (24 h) 10^{-7} M	HT-29 G_2/M arrest (24 h) 10^{-6} M	HT-29 G_2/M arrest (24 h) 10^{-5} M	HeLa microtubule network 10^{-6} M
3*Z*	10	+	+	+	+	+	+	+	+	+	disruption
4*Z*	16										
8*Z*	≫30										
9*Z*	≫30										
28*Z*	≫30	+	+	+	+	+	+	+	+	+	disruption
33*Z*	25	+	+	+	+	+	+	+	+	+	disruption
33*E*	≫30	−	+/−	+	−	+	+	−	+	+	mild disruption
34*Z*	2	+/−	+	+	−	+	+	−	+	+	disruption
34*E*	30	+	+	+	+	+	+	+	+	+	disruption
35*Z*	6	−	−	−/+	−	−	−	−	−	−	no disruption
35*E*	≫30	+/−	+	+	−	+	+	+	+	+	disruption
36*Z*	13	−	+	+	−	+/−	+	−	+	+	disruption
36*E*	≫40	−	−	+	−	−	+/−	−	+/−	+	no disruption
37*E*	≫40	−	+/−	+	−	−	+	−	+/−	+	mild disruption
CA-4	3	+	+	+	+	+	+	+	+	+	disruption

[a] For details see Experimental Section. The effect on cell cycle at 24 h for different cell lines was assessed by fluorescence flow cytometry. The effect of the indicated compounds (10^{-6} M) on the cell microtubule network was assessed in HeLa cells by confocal microscopy. Percentage of cells at the G_2/M phase: +, >60%; +/−, 40−60%; −, no effect.

Effects on Cell Cycle and Cellular Microtubules

To gain further insight into the mode of action of these compounds, the most cytotoxic ones were further assayed (Table 4) for their effects on cell cycle (by flow cytometry; Figure 8) and cellular microtubules (by confocal microscopy). All of these combretastatin analogues but the potent tubulin polymerization inhibitor **35*Z*** were able to arrest cells at G_2/M at different concentrations (Table 4), and this G_2/M arrest was correlated with disruption of the cellular microtubule network. When comparing the effects on intact cells (cell cycle arrest and microtubule network integrity) of some selected derivatives versus the in vitro inhibition of tubulin polymerization, we found good correlation for most of the assayed compounds (Table 4). However, this correlation was not observed for some compounds. Thus, compounds that inhibited tubulin polymerization in vitro were efficient in arresting cells at G_2/M, but some compounds that were poorly efficient in inhibiting tubulin polymerization resulted as potent inducers of cell cycle arrest, in particular compound **28*Z***. This suggests that these particular cytotoxic compounds exert their actions on microtubules and cell cycle arrest through processes that do not involve a direct interaction with the tubulin. Alternatively, these compounds can be envisaged to interact with additional microtubule-associated proteins to exert their microtubule disrupting effect. However, both cellular and isolated protein approaches to assess microtubule depolymerization differ in a number of important issues, including tubulin concentration present in the cell and in the inhibition of tubulin polymerization assay, and the presence of distinct microtubule-associated proteins. In addition, regulatory proteins present in the cellular studies can be absent in the tubulin polymerization assay. As previously suggested, the absence of effect on cell cycle observed for compound **35*Z*** can be explained in terms of a poor cellular uptake.

As shown in Figure 8, compound **28*Z***, at low concentration (10^{-7} M), induced a practically complete cell cycle arrest at G_2/M in both human cancer HeLa and A549 cells. Over 90% of these cells were also arrested at G_2/M following treatment with a 10^{-7} M concentration of either CA-4 or **3*Z*** (Figure 8 and data not shown).

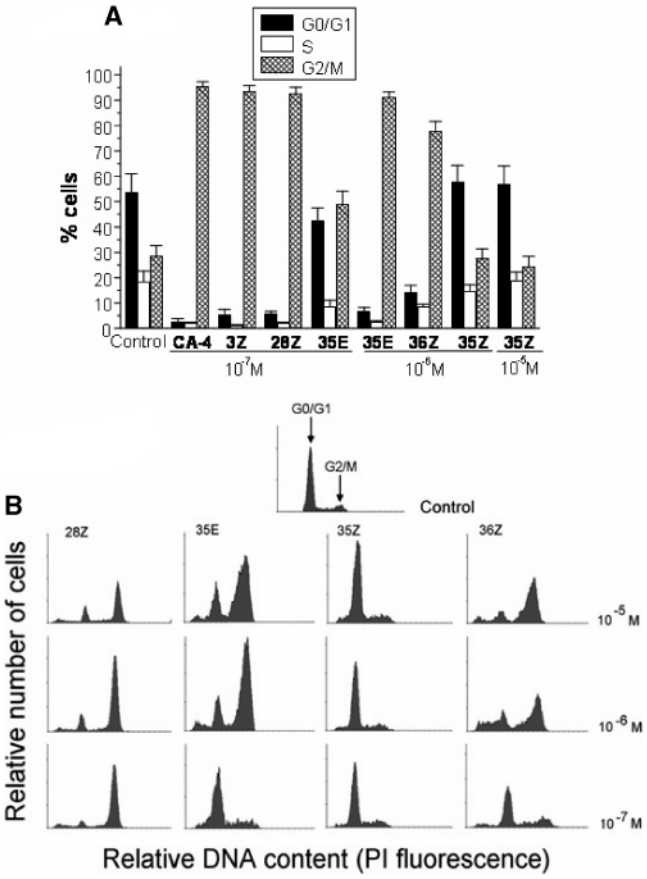


Figure 8. Dose−response of the effects of compounds CA-4, **3*Z***, **28*Z***, **35*E***, **35*Z***, and **36*Z*** on cell cycle in HeLa (A) and A549 (B) cells. Cells were incubated with different concentrations of the indicated compounds for 24 h, and their DNA content was analyzed by fluorescence flow cytometry. The positions of the G_0/G_1 and G_2/M peaks are indicated by arrows (B), and the proportion of cells in each phase of the cell cycle (A) was quantified by flow cytometry. The experiment shown in B was representative of three performed ones. Data in A are shown as means of three independent experiments ± SD.

Higher concentrations were required to achieve similar percentages of G_2/M arrest with other compounds, such as **35*E*** and **36*Z***, while **35*Z*** resulted as inactive even at a 10^{-5} M dose (Figure 8).

Conclusion

We have demonstrated that the 2-naphthalene is a good surrogate for the isovanillin moiety (4-methoxy-3-hydroxyphenyl) in the combretastatins, generating cytotoxic compounds and inhibitors of tubulin polymerization, but it is not able to replace the 3,4,5-trimethoxyphenyl moiety in searches for this kind of active compound. There are a number of remarkable chemical and biological properties of the naphthyl moiety, including (1) easier manipulation during the synthetic processes, not requiring protection of phenolic groups; (2) the extended aromatic surface, which may facilitate the interaction with hydrophobic pockets in the target biomolecule; and (3) the potent cytotoxic activity against a number of human tumor cells, mainly due to microtubule disruption, displayed by the synthesized analogues in this paper. The data herein reported point to the 2-naphthyl residue as a very interesting substructure to be included in new analogues of antimitotic agents designed to bind at the colchicine site. The 5-indolyl moiety can also be considered as a replacing residue to this purpose. Interestingly, we have reported here the synthesis of a new highly cytotoxic analogue, **28*Z***, which shows some remarkable antimitotic features but, unlike other structurally similar analogues, fails to inhibit in vitro tubulin polymerization.

Experimental Section

Chemistry. Materials and Methods. Reagents were used as purchased without further purification. Solvents (THF, DMF, CH_2Cl_2, benzene) were dried and freshly distilled before use according to literature procedures. Chromatographic separations were performed on silica gel columns by flash (Kieselgel 40, 0.040−0.063; Merck) or gravity column (Kieselgel 60, 0.063−0.200 mm; Merck) chromatography. TLC was performed on precoated silica gel polyester plates (0.25 mm thickness) with fluorescent indicator UV 254 (Polychrom SI F_{254}). Melting points were determined on a Buchi 510 apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker WP 200-SY spectrometer at 200/50 MHz or on a Bruker SY spectrometer at 400/100 MHz. Chemical shifts (δ) are given in ppm downfield from tetramethylsilane as internal standard, and coupling constants (J values) are in Hertz. GC−MS analyses were carried out in a Hewlett-Packard 5890 Serie II apparatus (70 eV). For FABHRMS analyses, a VG-TS250 apparatus (70 eV) was used. A Helios-α UV-320 from Thermo-Spectronic was used for UV experiments and absorption spectra. HPLC analysis were run on HP-1100 from Agilent Technologies or Delta 600 from Waters instruments, using X-Terra MS C_{18} 5μm (4.6 × 150 mm) and X-Terra MS C_8 5μm (4.6 × 150 mm) columns with water−acetonitrile gradients.

General Synthetic Procedures. Substrate concentrations are expresed in molar concentration (M), whereas the ratio of reagents used in moles/mole are expresed as equivalents (equiv), unless otherwise stated.

Synthesis of Bromides. Commercial aldehydes were used as starting materials and reduced to the corresponding alcohols by $NaBH_4$ treatment in MeOH at room temperature. Without further purification, a solution of phosphorus tribromide (1.1 equiv) in ether was added to a cool (−40 °C) solution of the alcohol in the same solvent under Ar. The mixture was stirred for 2 to 5 h, poured onto chilly water and extracted with ether. The ethereal layer was washed once with water followed by brine, dried over Na_2SO_4, and evaporated to dryness to afford the crude bromide.

Synthesis of Phosphonium Bromides. A solution of the freshly prepared bromide (0.9 M) and triphenylphosphine (1.1 equiv) in anhydrous benzene was stirred for 24 to 48 h at room temperature. The resulting precipitate was collected by filtration, washed, and dried under vacuum to provide the corresponding arylmethyltriphenylphosphonium salt.

Wittig Reactions. The required phosphonium bromide (0.08 M) was suspended in dry THF and cooled to −15 °C under Ar. *n*-Butyllithium (1.6 M in hexane, 1.1 equiv) was added dropwise, and the resulting solution was stirred at this temperature for 30 min, turning from deep-red to yellow-red. Then a solution of the aldehyde (0.9 equiv) in THF was added and warmed to room temperature. Once completed, the reaction mixture was cooled to 0 °C, some drops of water were added, and the mixture was extracted with dichloromethane. The organic layers were washed with brine, dried over Na_2SO_4, and concentrated under vacuum. The residue was chromatographed on silica gel to afford the *E* and *Z* isomers in variable yields.

Protection of Alcohols as Ethoxymethylethers. To 0.01−0.05 mol of the hydroxyaldehyde in 40 mL of dry dichloromethane were added 1.45 equiv of EOMCl and 1.48 equiv of DIPEA, and the mixture was stirred and heated at reflux for 17 h. The reaction mixture was treated with a 2 M solution of HCl and extracted with dichloromethane. Then organic layers were washed with a 2 N solution of NaOH and extracted again with dichloromethane. The organic layers were washed with brine, dried over Na_2SO_4, and concentrated under vacuum.

Acetylation of Indoles. To a solution of the indole derivative (0.04 M) in dry toluene at 50°C were added sodium hydride 80% in mineral oil (4.1 equiv) and, after an hour, acetic anhydride (2.47 equiv). The mixture was stirred for 7 days, poured onto a 5% solution of $NaHCO_3$, and extracted with dichloromethane. The organic layers were washed with brine, dried over Na_2SO_4, and concentrated under vacuum. The residue was purified by crystallization from ethyl acetate.

Protection of Indoles as Sulfonamides. To a solution of the indole derivative (0.07 M) in dry dichloromethane were added sodium hydroxide (2.57 equiv) and $Bu_4N^+HSO_4$ (0.008 equiv). After an hour, benzenosulfonyl chloride (1.07 equiv) was slowly added. The mixture was allowed to react for 24 h, and then it was extracted with dichloromethane. The organic layers were washed with brine, dried over Na_2SO_4, and concentrated under vacuum. The residue was purified by crystallization from ethyl acetate.

Deprotection of the Sulfonyl Group from Indoles. To a solution of the 1-sulfonylindole derivative (0.05 M) in dry THF was added tetrabutylammonium fluoride (TBAF, 2 equiv). The brown solution was refluxed for 15 h under Ar, then it was extracted with dichloromethane, and the organic layers were washed with brine, dried over Na_2SO_4, and concentrated under vacuum. The residue was purified by chromatography.

Nitrations. To the required starting aldehyde (1 equiv) in 98% H_2SO_4 was added concentrated HNO_3 (4 equiv). The reaction mixture was stirred for 7 days at −20 °C, and then it was poured onto chilly water, filtered, and concentrated under vacuum. The residue was purified by crystallization from AcOEt/CH_2Cl_2.

Reduction of Nitro Groups. To the nitro derivative (0.01 M) in glacial AcOH was added metallic Zn (10 equiv). The mixture was stirred for 6 days at room temperature. The residue was filtered through Celite and extracted with dichloromethane. By preparative chromatography the amino compound was isolated.

Structure Determination. The structures of the synthesized products were determined by spectroscopic means. The ^{1}H NMR spectra allowed us to easily establish the stereochemistry of the double bond, by the mutual shielding observed on the protons of both aromatic rings for the *Z* isomers in comparison with the corresponding *E* isomers. Furthermore, the two olefinic protons usually appear as an AB system (approximately 12 Hz) below 7.00 ppm for the *Z* isomers, whereas they are most frequently observed as a two protons singlet above 7.00 ppm in the *E* isomers.

3*Z*, (*Z*)-2-[2-(3,4,5-Trimethoxyphenyl)vinyl]naphthalene. Following the general method, by reaction of 2-naph-

thalenecarbaldehyde and triphenyl(3,4,5-trimethoxybenzylidene)phosphorane, compound **3*Z*** was isolated in 45% yield. ^{1}H NMR δ_H (ppm) (400 MHz): 3.59 (s, 6H), 3.84 (s, 3H), 6.53 (s, 2H), 6.59 (d, J = 12.0, 1H), 6.74 (d, J = 12.0, 1H), 7.3−7.4 (m, 3H), 7.68 (d, J = 8.8, 1H), 7.7−7.8 (m, 2H), 7.78 (bs, 1H). HRMS ($C_{21}H_{20}O_3$): calcd 320.1412, found 320.1518.

3*E*, (*E*)-2-[2-(3,4,5-Trimethoxyphenyl)vinyl]naphthalene. From the same reaction, compound **3*E*** was obtained in 50% yield. Mp: 142 °C (Hex/EtOAc). ^{1}H NMR δ_H (ppm): 3.91 (s, 3H), 3.96 (s, 6H), 6.82 (s, 2H), 7.19 (s, 2H), 7.4−7.9 (m, 7H). MS: *m/z* 320 (M^+, 100), 305 (63).

4*Z*, (*Z*)-2-Methoxy-5-[2-(naphth-2-yl)vinyl]phenol. By deprotection of the ethoxymethylether **5*Z*** with HCl in methanol, **4*Z*** was obtained in 68% yield. ^{1}H NMR δ_H (ppm): 3.85 (s, 3H), 6.56 (d, J = 12.1, 1H), 6.66 (d, J = 12.1, 1H), 6.68 (d, J = 8.4, 1H), 6.78 (dd, J_1 = 1.8, J_2 = 8.4, 1H), 6.88 (d, J = 1.8, 1H), 7.3−7.4 (m, 3H), 7.65 (d, J = 8.4, 1H), 7.7−7.8 (m, 2H), 7.76 (bs, 1H). HRMS ($C_{19}H_{16}O_2$): calcd 276.1150, found 276.1190.

4*E*, (*E*)-2-Methoxy-5-[2-(naphth-2-yl)vinyl]phenol. By deprotection of the ethoxymethylether **5*E*** with HCl in methanol, **4*E*** was obtained in 67% yield. Mp: 140°C (Hex/EtOAc). ^{1}H NMR δ_H (ppm): 3.90 (s, 3H), 6.86 (d, J = 8.0, 1H), 7.01 (dd, J_1 = 2.2, J_2 = 8.0, 1H), 7.16 (s, 2H), 7.21 (d, J = 2.2, 1H), 7.3−7.4 (m, 3H), 7.71 (d, J = 8.0, 1H), 7.7−7.8 (m, 2H), 7.83 (bs, 1H). MS: *m/z* 276 (M^+, 100), 215 (52).

5*Z*, (*Z*)-2-[2-(3-Ethoxymethoxy-4-methoxyphenyl)vinyl]-naphthalene. Following the general method, by reaction of 3-ethoxymethoxy-4-methoxybenzaldehyde (prepared by protection of 3-hydroxy-4-methoxybenzaldehyde with ethoxymethyl chloride) and triphenyl(2-naphthylmethylidene)phosphorane, compound **5*Z*** was isolated in 57% yield. ^{1}H NMR δ_H (ppm): 1.02 (t, J = 7.0, 3H), 3.57 (c, J = 7.0, 2H), 3.84 (s, 3H), 5.10 (s, 2H), 6.60 (d, J = 12.4, 1H), 6.68 (d, J = 12.4, 1H), 6.72 (d, J = 8.0, 1H), 6.91 (dd, J_1 = 8.0, J_2 = 2.2, 1H), 7.10 (d, J = 2.2, 1H), 7.4−7.5 (m, 3H), 7.71 (d, J = 8.8, 1H), 7.7−7.8 (m, 2H), 7.81 (bs, 1H). MS: *m/z* 334 (M^+, 100), 59 (73).

5*E*, (*E*)-2-[2-(3-Ethoxymethoxy-4-methoxyphenyl)vinyl]-naphthalene. From the same reaction, compound **5*E*** was obtained in 43% yield. Mp: 123°C (Hex/EtOAc). ^{1}H NMR δ_H (ppm): 1.25 (t, J = 7.0, 3H), 3.81 (c, J = 7.0, 2H), 3.88 (s, 3H), 5.34 (s, 2H), 6.72 (d, J = 8.0, 1H), 7.16 (dd, J_1 = 2.2, J_2 = 8.0, 1H), 7.14 (s, 2H), 7.4−7.5 (m, 3H), 7.75 (d, J = 8.0, 1H), 7.8−7.8 (m, 2H), 7.84 (bs, 1H). MS: *m/z* 334 (M^+, 100), 59 (86).

6*E*, (*E*)-Acetic Acid 4-[2-(Naphth-2-yl)vinyl]phenyl Ester. Following the general method, by reaction of 4-acetoxybenzaldehyde and triphenyl(2-naphthylmethylidene)phosphorane, compound **6*E*** was obtained as the sole reaction product and isolated in 53% yield. ^{1}H NMR δ_H (ppm): 2.32 (s, 3H), 7.10 (d, J = 8.8, 2H), 7.22 (s, 2H), 7.46 (d, J = 8.8, 2H), 7.4−7.5 (m, 3H), 7.56 (d, J = 8.4, 1H), 7.7−7.8 (m, 2H), 7.85 (bs, 1H). MS: *m/z* 288 (M^+, 22), 246 (100).

7*E*, (*E*)-4-[2-(Naphth-2-yl)vinyl]phenol. By methanolysis of **6*E*** with KOH in MeOH, compound **7*E*** was obtained in 50% yield. Mp: 95 °C (Hex/EtOAc). ^{1}H NMR δ_H (ppm): 4.78 (s, 1H), 6.68 (d, J = 8.4, 1H), 6.9−7.2 (m, 3H), 7.31 (s, 2H), 7.2−7.4 (m, 4H), 7.67 (m, 3H). MS: *m/z* 246 (M^+, 100).

8*Z*, (*Z*)-2-[2-(4-Methoxyphenyl)vinyl]naphthalene. Following the general method, by reaction of 2-naphthalenecarbaldehyde and triphenyl(4-methoxybenzylidene)phosphorane, compound **8*Z*** was isolated in 45% yield. Mp: 84°C (Hex/EtOAc). ^{1}H NMR δ_H (ppm): 3.67 (s, 3H), 6.55 (d, J = 12.4, 1H), 6.63 (d, J = 12.4, 1H), 6.68 (d, J = 8.8, 2H), 7.17 (d, J = 8.8, 2H), 7.32−7.40 (m, 3H), 7.61 (d, J = 8.8, 1H), 7.6−7.7 (m, 2H), 7.71 (bs, 1H). HRMS ($C_{19}H_{16}O$): calcd 260.1201, found 260.1289.

8*E*, (*E*)-2-[2-(4-Methoxyphenyl)vinyl]naphthalene. From the same reaction, compound **8*E*** was obtained in 21% yield. Mp: 142 °C (Hex/EtOAc). ^{1}H NMR δ_H (ppm): 3.84 (s, 3H), 6.93 (d, J = 8.8, 2H), 7.13 (d, J = 16.4, 1H), 7.22 (d, J = 16.4, 1H), 7.4−7.5 (m, 3H), 7.50 (d, J = 8.8, 2H), 7.7−7.8 (m, 3H), 7.83 (bs, 1H). MS: *m/z* 260 (M^+, 100).

9*Z*, (*Z*)-2-[2-(3-Fluoro-4-methoxyphenyl)vinyl]naphthalene. Following the general method, by reaction of 3-fluoro-4-methoxybenzaldehyde and triphenyl(2-naphthylmethylidene)-phosphorane, compound **9*Z*** was isolated in 32% yield. Mp: 151 °C (Hex/EtOAc). ^{1}H NMR δ_H (ppm): 3.81 (s, 3H), 6.52 (d, J = 12.1, 1H), 6.69 (d, J = 12.4, 1H), 6.74 (d, J = 8.4, 1H), 6.95 (d, J_1 = 2.2, 1H), 7.35 (dd, J_1 = 2.2, J_2 = 8.4, 1H), 7.4−7.5 (m, 3H), 7.66 (d, J = 8.8, 1H), 7.6−7.8 (m, 2H), 7.72 (bs, 1H). HRMS ($C_{19}H_{15}FO$): calcd 278.1107, found 278.0000.

9*E*, (*E*)-2-[2-(3-Fluoro-4-methoxyphenyl)vinyl]naphthalene. From the same reaction, compound **9*E*** was obtained in 20% yield. ^{1}H NMR δ_H (ppm): 3.92 (s, 3H), 6.96 (d, J = 8.8, 1H), 7.13 (s, 2H), 7.25 (d, J = 2.2, 1H), 7.34 (dd, J_1 = 2.2, J_2 = 8.8, 1H), 7.4−7.5 (m, 3H), 7.72 (d, J = 8.4, 1H), 7.7−7.8 (m, 2H), 7.79 (bs, 1H). MS: *m/z* 278 (M^+, 100).

10*Z*, (*Z*)-2-[2-(4-Trifluoromethoxyphenyl)vinyl]naphthalene. Following the general method, by reaction of 2-naphthalenecarbaldehyde and triphenyl(4-trifluoromethoxybenzylidene)phosphorane, compound **10*Z*** was isolated in 22% yield. ^{1}H NMR δ_H (ppm): 6.62 (d, J = 12.1, 1H), 6.80 (d, J = 12.1, 1H), 7.2−7.3 (m, 3H), 7.32 (d, J = 8.4, 2H), 7.57 (d, J = 8.8, 1H), 7.67 (d, J = 8.4, 2H), 7.7−7.8 (m, 2H), 7.71 (bs, 1H). HRMS ($C_{19}H_{13}F_3O$): calcd 314.0918, found 314.0957.

10*E*, (*E*)-2-[2-(4-Trifluoromethoxyphenyl)vinyl]naphthalene. From the same reaction, compound **10*E*** was obtained in 24% yield. Mp: 90 °C (Hex/EtOAc). ^{1}H NMR δ_H (ppm): 7.05 (d, J = 8.4, 2H), 7.26 (s, 2H), 7.5−7.6 (m, 3H), 7.60 (d, J = 8.8, 1H), 7.77 (d, J = 8.4, 2H), 7.7−7.8 (m, 2H), 7.87 (bs, 1H). MS: *m/z* 314 (M^+, 100), 229 (48).

11*Z*, (*Z*)-2-(2-(4-Tolyl)vinyl)naphthalene. Following the general method, by reaction of 2-naphthalenecarbaldehyde and triphenyl(4-methylbenzylidene)phosphorane, compound **11*Z*** was isolated in 25% yield. Mp: 102 °C (Hex/EtOAc). ^{1}H NMR δ_H (ppm): 2.30 (s, 3H), 6.62 (d, J = 12.4, 1H), 6.71 (d, J = 12.1, 1H), 7.00 (d, J = 8.8, 2H), 7.17 (d, J = 8.8, 2H), 7.39−7.44 (m, 3H), 7.64 (d, J = 8.8, 1H), 7.66−7.70 (m, 2H), 7.73 (bs, 1H). HRMS ($C_{19}H_{16}$): calcd 244.1252, found 244.1263.

11*E*, (*E*)-2-(2-(4-Tolyl)vinyl)naphthalene. From the same reaction, compound **11*E*** was obtained in 73% yield. Mp: 112 °C (Hex/EtOAc). ^{1}H NMR δ_H (ppm): 2.37 (s, 3H), 7.19 (d, J = 8.0, 2H), 7.23 (d, J = 8.0, 2H), 7.22 (s, 2H), 7.4−7.5 (m, 3H), 7.73 (d, J = 8.4, 1H), 7.7−7.8 (m, 2H), 7.84 (bs, 1H). MS: *m/z* 244 (M^+, 100), 229 (76).

12*Z*, (*Z*)-2-[2-(4-Trifluoromethylphenyl)vinyl]naphthalene. Following the general method, by reaction of 2-naphthalenecarbaldehyde and triphenyl(4-trifluromethylbenzylidene)phosphorane, compound **12*Z*** was isolated in 40% yield. Mp: 98 °C (Hex/EtOAc). ^{1}H NMR δ_H (ppm): 6.67 (d, J = 12.0, 1H), 6.88 (d, J = 12.0, 1H), 7.32 (d, J = 8.4, 2H), 7.77 (d, J = 8.4, 2H), 7.3−7.5 (m, 3H), 7.7−7.9 (m, 2H), 7.85 (d, J = 8.4, 1H), 7.85 (s, 1H). HRMS ($C_{19}H_{13}F_3$): calcd 298.0969, found 298.0973.

12*E*, (*E*)-2-[2-(4-Trifluoromethylphenyl)vinyl]naphthalene. From the same reaction, compound **12*E*** was obtained in 52% yield. Mp: 150 °C (Hex/EtOAc). ^{1}H NMR δ_H (ppm): 7.22 (d, J = 16.4, 1H), 7.36 (d, J = 16.4, 1H), 7.4−7.5 (m, 5H), 7.73 (d, J = 8.4, 2H), 7.74 (d, J = 8.8, 1H), 7.7−8.0 (m, 2H), 8.33 (s, 1H). MS: *m/z* 298 (M^+, 100), 228 (57)

13*Z*, (*Z*)-*N*,*N*-Dimethyl-4-[2-(naphth-2-yl)vinyl]aniline. Following the general method, by reaction of 4-dimethylaminobenzaldehyde and triphenyl(2-naphthylmethylidene)phosphorane, compound **13*Z*** was isolated in 56% yield. ^{1}H NMR δ_H (ppm): 2.86 (s, 6H), 6.51 (d, J = 8.8, 2H), 6.54 (s, 2H), 7.18 (d, J = 8.8, 2H), 7.3−7.40 (m, 3H), 7.45 (d, J = 8.4, 1H), 7.6−7.8 (m, 2H), 7.75 (bs, 1H). HRMS ($C_{20}H_{19}N$): calcd 273.1517, found 273.1477.

13*E*, (*E*)-*N*,*N*-Dimethyl-4-[2-(naphth-2-yl)vinyl]aniline. From the same reaction, compound **13*E*** was obtained in 44% yield. Mp: 95 °C (Hex/EtOAc). ^{1}H NMR δ_H (ppm): 2.99 (s, 6H), 6.74 (d, J = 8.8, 2H), 7.06 (d, J = 16.0, 1H), 7.19 (d, J = 16.0, 1H), 7.46 (d, J = 8.8, 2H), 7.3−7.5 (m, 3H), 7.71 (d, J = 8.8, 1H), 7.7−7.8 (m, 2H), 7.81 (bs, 1H). MS: *m/z* 273 (M^+, 100).

14*Z*, (*Z*)-2-[2-(4-Methoxy-3-nitrophenyl)vinyl]naphthalene. Following the general method, by reaction of 4-methoxy-3-nitrobenzaldehyde (obtained by nitration of 4-methoxybenzaldehyde) and triphenyl(2-naphthylmethylidene)phosphorane,

compound **14*Z*** was isolated in 41% yield. Mp: 192 °C (CH_2Cl_2/Hex). ^{1}H NMR δ_H (ppm): 3.90 (s, 3H), 6.57 (d, J = 12.1, 1H), 6.81 (d, J = 8.8, 1H), 6.83 (d, J = 12.1, 1H), 7.26 (dd, J_1 = 1.5, J_2 = 8.8, 1H), 7.33 (d, J = 1.5, 1H), 7.40 (dd, J_1 = 2,2, J_2 = 8.8, 1H), 7.4−7.6 (m, 2H), 7.6−7.9 (m, 3H), 7.79 (d, J = 2.2, 1H). Anal. ($C_{19}H_{15}N0_3$) 74.41% C, 5.13% H, 4.64% N.

14*E*, (*E*)-2-[2-(4-Methoxy-3-nitrophenyl)vinyl]naphthalene. From the same reaction, compound **14*E*** was obtained in 30% yield. Mp: 166°C (CH_2Cl_2/Hex). ^{1}H NMR δ_H (ppm): 3.99 (s, 3H), 7.11 (d, J = 8.6, 1H), 7.13 (d, J = 16.5, 1H), 7.24 (d, J = 16.5, 1H), 7.4−7.6 (m, 2H), 7.69 (dd, J_1 = 2.2, J_2 = 8.6, 1H), 7.8−7.9 (m, 5H), 8.06 (d, J = 2.2, 1H). HRMS ($C_{19}H_{15}NO_3$): calcd 305.1051, found 305.1038.

15*Z*, (*Z*)-2-[2-(3-Amino-4-methoxyphenyl)vinyl]naphthalene. By Zn in AcOH reduction of **14*Z***, the title compound was obtained in 89% yield. Mp: 183°C (Acetone/EtOAc). ^{1}H NMR δ_H (ppm): 3.71 (s, 3H), 4.70 (s, 2H), 6.41 (d, J = 12.2, 1H), 6.60 (d, J = 12.2, 1H), 6.85 (d, J = 8.8, 1H), 7.12 (dd, J_1 = 2.0, J_2 = 8.8, 1H), 7.0−7.3 (m, 4H), 7.4−7.6 (m, 4H). HRMS ($C_{19}H_{17}NO$): calcd 276.1391, found 276.1388.

15*E*, (*E*)-2-[2-(3-Amino-4-methoxyphenyl)vinyl]naphthalene. By Zn in AcOH reduction of **14*Z***, the title compound was obtained 94% yield. Mp: 232 °C (Acetone/EtOAc). ^{1}H NMR δ_H (ppm): 3.99 (s, 3H), 7.11 (d, J = 8.8, 1H), 7.14 (d, J = 16.5, 1H), 7.24 (d, J = 16.5, 1H), 7.47 (m, 1H), 7.48 (dd, J_1 = 1.6, J_2 = 8.8, 1H), 7.4−7.6 (m, 2H), 7.71 (dd, J_1 = 2.2, J_2 = 8.6, 1H), 7.8−7.9 (m, 5H), 8.06 (d, J = 2.2, 1H). HRMS ($C_{19}H_{17}NO$): calcd 275.1310, found 275.1322.

16*Z*, (*Z*)-*N*-{2-Methoxy-5-[2-(naphth-2-yl)vinyl]phenyl}acetamide. By treatment of **15*Z*** with an excess of acetic anhydride, the title compound was obtained in 73% yield. ^{1}H NMR δ_H (ppm): 2.20 (s, 3H), 3.80 (s, 3H), 6.59 (d, J = 8.4, 1H), 6.60 (d, J = 12.0, 1H), 6.66 (d, J = 12.0, 1H), 6.92 (dd, J_1 = 2,2, J_2 = 8,4, 1H), 8.30 (d, J = 2.2, 1H), 7.3−7.8 (m, 7H). HRMS ($C_{19}H_{17}NO$): calcd 317.1416, found 317.1388.

16*E*, (*E*)-*N*-{2-Methoxy-5-[2-(naphth-2-yl)vinyl]phenyl}acetamide. By treatment of **15*E*** with an excess of acetic anhydride, the title compound was obtained in 76% yield. ^{1}H NMR δ_H (ppm): 2.22 (s, 3H), 3.91 (s, 3H), 6.88 (d, J = 8.8, 1H), 7.19 (s, 2H), 7.4−7.5 (m, 3H), 7.6−7.9 (m, 5H), 8.70 (d, J = 2.2, 1H). HRMS ($C_{19}H_{17}NO$): calcd 317.1416, found 317.1458.

17*E*, (*E*)-*N*,*N*-Diethyl[2-methoxy-5-[2-(naphth-2-yl)vinyl]aniline. Compound **15*E*** (0.2 mmol) in 1 mL of MeOH (containing one drop of HCl) was treated with 98 μL of acetaldehyde and $NaCNBH_3$ (0.6 mmol) for 30 min at room remperature. The reaction mixture was then poured onto water and extracted with chloroform. After the usual workup, compound **17*E*** was obtained in 40% yield. ^{1}H NMR δ_H (ppm): 0.80 (c, J = 7.0, 6H), 2.90 (t, J = 7.0, 4H), 3.80 (s, 3H), 6.60 (d, J = 16.0, 1H), 6.68 (d, J = 16.0, 1H), 6.73 (d, J = 8.0, 1H), 6.87 (d, J = 2.0, 1H), 6.92 (dd, J_1 = 2.0, J_2 = 8.0, 1H), 7.3−7.5 (m, 3H), 7.6−7.8 (m, 4H). HRMS ($C_{23}H_{25}NO$): calcd 331.1936, found 331.1948.

18*Z*, (*Z*)-2-[2-(Naphth-2-yl)vinyl]furan. Following the general method, by reaction of 2-furylcarbaldehyde and triphenyl(2-naphthylmethylidene)phosphorane, compound **18*Z*** was isolated in 21% yield. ^{1}H NMR δ_H (ppm): 6.30 (d, J = 3.3, 1H), 6.40 (dd, J_1 = 1.8, J_2 = 3.3, 1H), 6.42 (d, J = 12.8, 1H), 6.63 (d, J = 12.8, 1H), 7.48 (d, J = 1.8, 1H), 7.4−7.5 (m, 3H), 7.57 (d, J = 8.8, 1H), 7.7−7.8 (m, 2H), 7.90 (s, 1H). MS: *m/z* 220 (M^+, 100), 191 (78).

18*E*, (*E*)-2-[2-(Naphth-2-yl)vinyl]furan. From the same reaction, compound **18*E*** was obtained in 36% yield. ^{1}H NMR δ_H (ppm): 6.38 (d, J = 3.3, 1H), 6.43 (dd, J_1 = 1.8, J_2 = 3.3, 1H), 7.00 (d, J = 16.1, 1H), 7.20 (d, J = 16.1, 1H), 7.41 (d, J = 1.8, 1H), 7.4−7.5 (m, 3H), 7.63 (d, J = 8.8, 1H), 7.7−7.8 (m, 2H), 7.81 (bs, 1H). MS: *m/z* 220 (M^+, 100), 191 (73).

19*Z*, (*Z*)-3-[2-(Naphth-2-yl)vinyl]furan. Following the general method, by reaction of 3-furylcarbaldehyde and triphenyl(2-naphthylmethylidene)phosphorane, compound **19*Z*** was isolated in 22% yield. ^{1}H NMR δ_H (ppm): 6.14 (d, J = 3.3, 1H), 6.46 (d, J = 12.0, 1H), 6.69 (d, J = 12.0, 1H), 7.21 (dd, J_1 = 1.8, J_2 = 3.3, 1H), 7.40 (d, J = 1.8, 1H), 7.4−7.5 (m, 3H), 7.77 (d, J = 8.8, 1H), 7.75−7.84 (m, 2H), 7.80 (bs, 1H). MS: *m/z* 220 (M^+, 80), 191 (100).

19*E*, (*E*)-3-[2-(Naphth-2-yl)vinyl]furan. From the same reaction, compound **19*E*** was obtained in 69% yield. Mp: 118 °C (Hex/EtOAc). ^{1}H NMR δ_H (ppm): 6.70 (s, 1H), 6.96 (d, J = 16.1, 1H), 7.11 (d, J = 16.1, 1H), 7.24 (s, 1H), 7.38 (s, 1H), 7.2−7.8 (m, 7H). MS: *m/z* 220 (M^+, 75), 191 (100).

20*Z*, (*Z*)-2-[2-(Naphth-2-yl)vinyl]naphthalene. Following the general method, by reaction of 2-naphthalenecarbaldehyde and triphenyl(2-naphthylmethylidene)phosphorane, compound **20*Z*** was isolated in 43% yield. ^{1}H NMR δ_H (ppm): 6.82 (s, 2H), 7.3−7.4 (m, 6H), 7.5−7.7 (m, 8H). HRMS ($C_{22}H_{26}$): calcd 280.1252, found 280.1283.

21*Z*, (*Z*)-1-Phenylsulfonyl-5-[2-(2-naphthyl)vinyl]-1*H*-indol. Following the general method, by reaction of 1-phenylsulfonyl-1*H*-indole-5-carbaldehyde (obtained by the general method for *N*-sulfonylation of indoles) and triphenyl(2-naphthylmethylidene)phosphorane, compound **21*Z*** was isolated in 5% yield. ^{1}H NMR δ_H (ppm): 6.53 (d, J = 3.6, 1H), 6.67 (s, 2H), 7.1−8.0 (m, 16H). HRMS ($C_{26}H_{19}NO_2S$): calcd 409.1137, found 409.1151.

21*E*, (*E*)-1-Phenylsulfonyl-5-[2-(2-naphthyl)vinyl]-1*H*-indole. From the same reaction, compound **21*E*** was obtained in 80% yield. Mp: 173°C. ^{1}H NMR δ_H (ppm): 6.68 (d, J = 3.6, 1H), 7.27 (m, 2H), 7.4−8.1 (m, 16H). HRMS ($C_{26}H_{19}NO_2S$): calcd 409.1137, found 409.1194.

22*Z*, (*Z*)-5-[2-(2-Naphthyl)vinyl]-1*H*-indole. By the standard treatment of **21*Z*** with TBAF, 21% yield of compound **22*Z*** was obtained afted purification. ^{1}H NMR δ_H (ppm): 6.40 (m, 1H), 6.66 (d, J = 12.4, 1H), 6.80 (d, J = 12.4, 1H), 7.00 (m, 1H), 7.2−7.9 (m, 10H). HRMS ($C_{20}H_{15}$ N): calcd 269.1205, found 269.1233.

23*Z*, (*Z*)-1-Methyl-5-[2-(2-naphthyl)vinyl]-1*H*-indol. Following the general method, by reaction of 1-methyl-1*H*-indolecarbaldehyde and triphenyl(2-naphthylmethylidene)-phosphorane, compound **23*Z*** was isolated in 38% yield. ^{1}H NMR δ_H (ppm): 3.76 (s, 3H), 6.40 (d, J = 2.9, 1H), 6.86 (d, J = 12.0, 1H), 6.70 (d, J = 12.0, 1H), 7.04 (d, J = 2.9, 1H), 7.13 (d, J = 8.8, 1H), 7,18 (dd, J_1 = 8.8, J_2 = 1.5, 1H), 7.62 (d, J = 8.4, 2H), 7.3−7.9 (m, 6H). HRMS ($C_{21}H_{17}N$): calcd 283.1361, found 283.1392.

23*E*, (*E*)-1-Methyl-5-[2-(2-naphthyl)vinyl]-1*H*-indol. From the same reaction, compound **23*E*** was obtained in 13% yield. Mp: 210 °C (Hex/EtOAc). ^{1}H NMR δ_H (ppm): 3.82 (s, 3H), 6.51 (d, J = 3.3, 1H), 7.06 (d, J = 3.3, 1H), 7.20 (d, 1H), 7.1−7.4 (m, 11H). HRMS ($C_{21}H_{17}N$): calcd 283.1361, found 283.1328.

24*Z*, (*Z*)-3-[2-(2-Naphthyl)vinyl]-9*H*-fluorene. Following the general method, by reaction of 9*H*-fluorene-3-carbaldehyde and triphenyl(2-naphthylmethylidene)phosphorane, compound **24*Z*** was isolated in 2% yield. Mp: 115°C. ^{1}H NMR δ_H (ppm): 3.80 (s, 2H), 6.77 (s, 2H), 6.85 (s, 1H), 7.2−7.9 (m, 13H). HRMS ($C_{25}H_{18}$): calcd 318.1409, found 318.1426.

24*E*, (*E*)-3-[2-(2-Naphthyl)vinyl]-9*H*-fluorene. From the same reaction, compound **24*E*** was obtained in 56% yield. Mp: 260 °C. ^{1}H NMR δ_H (ppm): 3.95 (s, 2H), 7.32 (s, 2H), 7.3−7.9 (m, 14H). HRMS ($C_{25}H_{18}$): calcd 318.1409, found 318.1454.

25*Z*, (*Z*)-9-Ethyl-3-[2-(2-naphthyl)vinyl]-9*H*-carbazole. Following the general method, by reaction of 9-ethyl-9*H*-carbazole-3-carbaldehyde and triphenyl(2-naphthylmethylidene)phosphorane, compound **25*Z*** was isolated in 9% yield. ^{1}H NMR δ_H (ppm): 1.46 (t, J = 7.0, 3H), 4.33 (c, J = 7.0, 2H), 6.83 (d, J = 12.0, 1H), 7.00 (d, J = 12.0, 1H), 7.2−8.0 (m, 12H), 8.0 (d, J = 7.4, 1H), 8.2 (s, 1H). HRMS ($C_{20}H_{21}NO_3$): calcd 347.1674, found 347.1881.

25*E*, (*E*)-9-Ethyl-3-[2-(2-naphthyl)vinyl]-9*H*-carbazole. From the same reaction, compound **25*E*** was obtained in 16% yield. Mp: 180 °C (Hex/EtOAc). ^{1}H NMR δ_H (ppm): 1.45 (t, J = 7.3, 3H), 4.37 (c, J = 7.3, 2H), 7.2−8.0 (m, 14H), 8.15 (d, J = 7.6, 1H), 8.3 (d, J = 1.5, 1H). HRMS ($C_{26}H_{21}N$): calcd 347.1674, found 347.1671.

26*Z* + 26*E*, 2-Methyl-5-[2-(3,4,5-trimethoxyphenyl)vinyl]furan. Following the general method, by reaction of 5-methylfuryl-2-carbaldehyde and triphenyl(3,4,5-trimethoxybenzylidene)phosphorane, compounds **26*Z* + 26*E*** were ob-

tained in 82% yield as a nonseparable mixture (*Z*/*E* 1:2). ^{1}H NMR data for **26*Z*** (from the spectrum of the mixture) δ_H (ppm): 2.28 (s, 3H), 3.85 (s, 6H), 3.88 (s, 3H), 5.96 (d, J = 3.3, 1H), 6.23 (d, J = 3.3, 1H), 6.28 (s, 2H), 6.70 (s, 2H). MS of the mixture: *m/z* 274 (M^+, 100), 259 (71).

26*E*, (*E*)-2-Methyl-5-[2-(3,4,5-trimethoxyphenyl)vinyl]-furan. From the mixture a small amount of pure **26*E*** was obtained by crystallization. Mp: 90 °C (Hex/EtOAc). ^{1}H NMR δ_H (ppm): 2.35 (s, 3H), 3.86 (s, 3H), 3.89 (s, 6H), 6.02 (d, J = 3.3, 1H), 6.23 (d, J = 3.3, 1H), 6.68 (s, 2H), 6.73 (d, J = 16.0, 1H), 6.89 (d, J = 16.0, 1H). MS: *m/z* 274 (M^+, 100), 259 (64).

27*Z*, (*Z*)-*N*,*N*-Dimethyl-2-nitro-4-[2-(3,4,5-trimethoxyphenyl)vinyl]aniline. Following the general method, by reaction of 4-dimethylamino-3-nitrobenzaldehyde and triphenyl(3,4,5-trimethoxybenzylidene)phosphorane, compound **27*Z*** was isolated in 41% yield. Mp: 98 °C (CH_2Cl_2/ether). ^{1}H NMR δ_H (ppm): 2.87 (s, 6H), 3.72 (s, 6H), 3.85 (s, 3H), 6.38 (d, J = 12.4, 1H), 6.48 (d, J = 12.4, 1H), 6.49 (s, 2H), 6.84 (d, J = 8.8, 1H), 7.30 (dd, J_1 = 8.8, J_2 = 2.2, 1H), 7.73 (d, J = 2.2, 1H). HRMS ($C_{19}H_{22}N_2O_5$): calcd 358.1529, found 358.1577.

27*E*, (*E*)-*N*,*N*-Dimethyl-2-nitro-4-[2-(3,4,5-trimethoxyphenyl)vinyl]aniline. From the same reaction, compound **27*E*** was obtained in 42% yield. Mp: 75 °C (CH_2Cl_2/ether). ^{1}H NMR δ_H (ppm): 2.90 (s, 6H), 3.86 (s, 3H), 3.90 (s, 6H), 6.70 (s, 2H), 6.90 (s, 2H), 6.98 (d, J = 8.8, 1H), 7.53 (dd, J_1 = 2.2, J_2 = 8.8, 1H), 7.89 (d, J = 2.2, 1H). HRMS ($C_{19}H_{22}N_2O_5$): calcd 358.1529, found 358.1553.

28*Z*, (*Z*)-*N*,*N*-Dimethyl-2-amino-4-[2-(3,4,5-trimethoxyphenyl)vinyl]aniline. By Zn in AcOH reduction of **27*Z***, compound **28*Z*** was isolated in 67% yield. ^{1}H NMR δ_H (ppm): 2.63 (s, 6H), 3.68 (s, 6H), 3,84 (s, 3H), 6.38 (d, J = 12.1, 1H), 6.51 (s, 2H), 6.53 (d, J = 12.1, 1H), 6.60−6.75 (m, 2H), 6.91 (d, J = 2.9, 1H). HRMS ($C_{19}H_{24}N_2O_3$): calcd 328.1787, found 328.1763.

28*E*, (*E*)-*N*,*N*-Dimethyl-2-amino-4-[2-(3,4,5-trimethoxyphenyl)vinyl]aniline. By the same reduction procedure, from the nitro derivative **27*E***, compound **28*E*** was obtained in 80% yield. Mp: 200 °C (EtOH/HCl). ^{1}H NMR δ_H (ppm): 2.67 (s, 6H), 3.90 (s, 6H), 3.91 (s, 3H), 6.70 (s, 2H), 6.8−7.0 (m, 5H). HRMS ($C_{19}H_{24}N_2O_3$): calcd 328.1787, found 328.1743.

29*Z*, (*Z*)-1-{3-[2-(3,4,5-Trimethoxyphenyl)vinyl]-1*H*-indol-1-yl}ethanone. Following the general method, by reaction of 1-acetyl-1*H*-indole-3-carbaldehyde and triphenyl(3,4,5-trimethoxybenzylidene)phosphorane, compound **29*Z*** was isolated in 17% yield. ^{1}H NMR δ_H (ppm): 2.52 (s, 3H), 3.58 (s, 6H), 3.82 (s, 3H), 6.55 (s, 2H), 6.60 (d, J = 12.0, 1H), 6.71 (d, J = 12.0 1H), 7.33 (s, 1H), 7.1−7.5 (m, 4H). HRMS ($C_{21}H_{21}NO_4$): calcd 351.1471, found 351.1470.

29*E*, (*E*)-1-{3-[2-(3,4,5-Trimethoxyphenyl)vinyl]-1*H*-indol-1-yl}ethanone. From the same reaction, compound **29*E*** was obtained in 56% yield. ^{1}H NMR δ_H (ppm): 2.66 (s, 3H), 3.88 (s, 3H), 3.93 (s, 6H), 6.76 (s, 2H), 7.15 (s, 2H), 7.55 (s, 1H), 7.1−7.5 (m, 4H). MS: *m/z* 351 (M^+, 100), 294 (73).

30*Z*, (*Z*)-3-[2-(3,4,5-Trimethoxyphenyl)vinyl]-1*H*-indole. By treatment of compound **29*Z*** with KOH in MeOH (10%), deacetylated derivative **30*Z*** was obtained in 18% yield. ^{1}H NMR δ_H (ppm): 3.62 (s, 6H), 3.84 (s, 3H), 6.51 (d, J = 12.6, 1H), 6.62 (s, 2H), 6.73 (d, J = 12.6, 1H), 7.0−7.5 (m, 5H). HRMS ($C_{19}H_{19}NO_3$): calcd 320.1412, found 320.1455.

30*E*, (*E*)-3-[2-(3,4,5-Trimethoxyphenyl)vinyl]-1*H*-indole. By the same treatment, from **29*E***, compound **30*E*** was obtained in 97% yield. Mp: 156 °C (ether). ^{1}H NMR δ_H (ppm): 3.88 (s, 3H), 3.94 (s, 6H), 6.75 (s, 2H), 7.08 (d, J = 16.4, 1H), 7.22 (d, J = 16.4, 1H), 7.2−7.5 (m, 5H). MS: *m/z* 309 (M^+, 100), 294 (56).

31*Z*, (*Z*)-1-Methyl-3-[2-(3,4,5-trimethoxyphenyl)vinyl]-1*H*-indole. Following the general method, by reaction of 1-methyl-1*H*-indole-3-carbaldehyde and triphenyl(3,4,5-trimethoxybenzylidene)phosphorane, compound **31*Z*** was isolated in 16% yield. Mp: 142°C (Hex/EtOAc). ^{1}H NMR δ_H (ppm): 3.65 (s, 6H), 3.71 (s, 3H), 3.85 (s, 3H), 6.43 (d, J = 12.0, 1H), 6.65 (s, 2H), 6.73 (d, J = 12.0, 1H), 7.0−7.5 (m, 5H). HRMS ($C_{20}H_{21}NO_3$): calcd 323.1521, found 323.1499.

31*E*, (*E*)-1-Methyl-3-[2-(3,4,5-trimethoxyphenyl)vinyl]-1*H*-indole. From the same reaction, compound **31*E*** was obtained in 52% yield. ^{1}H NMR δ_H (ppm): 3.78 (s, 3H), 3.87 (s, 3H), 3.92 (s, 6H), 6.73 (s, 2H), 7.02 (d, J = 16.4, 1H), 7.20 (d, J = 16.4, 1H), 7.1−7.5 (m, 5H). MS: *m/z* 323 (M^+, 100), 308 (85).

32*Z*, (*Z*)-1-Phenylsulfonyl-5-[2-(3,4,5-trimethoxyphenyl)-vinyl]-1*H*-indole. Following the general method, by reaction of 1-phenylsulfonyl-1*H*-indole-5-carbaldehyde and triphenyl-(3,4,5-trimethoxybenzylidene)phosphorane, compound **32*Z*** was isolated in 18% yield. ^{1}H NMR δ_H (ppm): 3.45 (s, 6H), 3.80 (s, 3H), 6.39 (s, 2H), 6.48 (d, J = 12.1, 1H), 6.55 (d, J = 3.6, 1H), 6.61 (d, J = 12.1, 1H), 7.2−7.5 (m, 5H), 7.52 (d, J = 3.6, 1H), 7.7−7.9 (m, 3H). HRMS ($C_{25}H_{23}NO_5S$): calcd 449.1297, found 449.1346.

32*E*, (*E*)-1-Phenylsulfonyl-5-[2-(3,4,5-trimethoxyphenyl)vinyl]-1*H*-indole. From the same reaction, compound **24*E*** was obtained in 13% yield. ^{1}H NMR δ_H (ppm): 3.87 (s, 3H), 3.91 (s, 6H), 6.66 (d, J = 3.8, 1H), 6.73 (s, 2H), 6.98 (d, J = 16.0, 1H), 7.08 (d, J = 16.0, 1H), 7.4−7.5 (m, 4H), 7.56 (d, J = 3.8, 1H), 7.63 (d, J = 1.6, 1H), 7.8−8.1 (m, 3H). HRMS ($C_{25}H_{23}NO_5S$): calcd 449.1297, found 449.1288.

33*Z*, (*Z*)-5-[2-(3,4,5-Trimethoxyphenyl)vinyl]-1*H*-indole. By TBAF treatment of **32*Z*** the indole derivative **33*Z*** was obtained in 24% yield. ^{1}H NMR δ_H (ppm): 3.62 (s, 6H), 3.84 (s, 3H), 6.41 (d, J = 12.0, 1H), 6.48 (m, 1H), 6.56 (s, 2H), 6.71 (d, J = 12.0, 1H), 7.18 (m, 1H), 7.20 (dd, J_1 = 1.5, J_2 = 8.0, 1H), 7.26 (d, J = 8.0, 1H), 7.27(d, 1H), 8.19 (s, 1H). HRMS ($C_{19}H_{19}NO_3$): calcd 309.1365, found 309.1387.

33*E*, (*E*)-5-[2-(3,4,5-Trimethoxyphenyl)vinyl]-1*H*-indole. By TBAF treatment of **32*E***, the indole derivative **33*E*** was obtained in 32% yield. ^{1}H NMR δ_H (ppm): 3.84 (s, 3H), 3.87 (s, 6H), 6.56 (bs, 1H), 6.76 (s, 2H), 7.01 (d, J = 16.0, 1H), 7.16 (d, J = 16.0, 1H), 7.23 (m, 2H), 7.40 (m, 2H), 7.76 (bs, 1H). HRMS ($C_{19}H_{19}NO_3$): calcd 309.1365, found 309.1358.

34*Z*, (*Z*)-1-Methyl-5-[2-(3,4,5-trimethoxyphenyl)vinyl]-1*H*-indole. Following the general method, by reaction of 1-methyl-1*H*-indole-5-carbaldehyde and triphenyl(3,4,5-trimethoxybenzylidene)phosphorane, compound **34*Z*** was isolated in 21% yield. ^{1}H NMR δ_H (ppm): 3.63 (s, 6H), 3.76 (s, 3H), 3.84 (s, 3H), 6.40 (d, J = 2.9, 1H), 6.43 (d, J = 12.0, 1H), 6.58 (s, 2H), 6.70 (d, J = 12.0, 1H), 7.00 (d, J = 2.9, 1H), 7.19 (m, 2H), 7.60 (bs, 1H). HRMS ($C_{20}H_{21}NO_3$): calcd 323.1521, found 323.1562.

34*E*, (*E*)-1-Methyl-5-[2-(3,4,5-trimethoxyphenyl)vinyl]-1*H*-indole. From the same reaction, compound **34*E*** was obtained in 39% yield. Mp: 110 °C (ether). ^{1}H NMR δ_H (ppm): 3.81 (s, 3H), 3.87 (s, 3H), 3.93 (s, 6H), 6.49 (d, J = 3.3, 1H), 6.76 (s, 2H), 7.00 (d, J = 16.4, 1H), 7.05 (d, J = 3.3, 1H), 7.16 (d, J = 16.4, 1H), 7.31 (d, J = 8.8, 1H), 7.47 (dd, J_1 = 8.8, J_2 = 1.6, 1H), 7.74 (d, J = 1.6, 1H). HRMS ($C_{20}H_{21}NO_3$): calcd 323.1521, found 323.1489.

35*Z*, (*Z*)-9-Ethyl-3-[2-(3,4,5-trimethoxyphenyl)vinyl]-9*H*-carbazole. Following the general method, by reaction of 9-ethyl-9*H*-carbazole-3-carbaldehyde and triphenyl(3,4,5-trimethoxybenzylidene)phosphorane, compound **35*Z*** was isolated in 60% yield. ^{1}H NMR δ_H (ppm): 1.34 (t, J = 7.0, 3H), 3.62 (s, 6H), 3.86 (s, 3H), 4.32 (c, J = 7.0, 2H), 6.50 (d, J = 12.0, 1H), 6.60 (s, 2H), 6.78 (d, J = 12.0, 1H), 7.0−8.2 (m, 7H). MS: *m/z* 387 (M^+, 100), 372 (71).

35*E*, (*E*)-9-Ethyl-3-[2-(3,4,5-trimethoxyphenyl)vinyl]-9*H*-carbazole. From the same reaction, compound **35*E*** was obtained in 35% yield. ^{1}H NMR δ_H (ppm): 1.45 (t, J = 7.0, 3H), 3.90 (s, 3H), 3.98 (s, 6H), 4.36 (c, J = 7.0, 2H), 6.80 (s, 2H), 7.08 (d, J = 16.0, 1H), 7.23 (d, J = 16.0, 1H), 7.2−8.3 (m, 7H). MS: *m/z* 387 (M^+, 100), 372 (68).

36*Z*, (*Z*)-2-[2-(3,4-Dimethoxy-5-nitrophenyl)vinyl]naphthalene. Following the general method, by reaction of 3,4-dimethoxy-5-nitrobenzaldehyde and triphenyl(2-naphthylmethylidene)phosphorane, compound **36*Z*** was isolated in 26% yield. ^{1}H NMR δ_H (ppm): 3.44 (s, 3H), 3.94 (s, 3H), 6.56 (d, J = 12.0, 1H), 6.87 (d, J = 12.0, 1H), 6.95 (d, J = 2.2, 1H), 7.34 (dd, J_1 = 8.6, J_2 = 1.6, 1H), 7.4−7.6 (m, 3H), 7.6−7.9 (m, 4H). HRMS ($C_{20}H_{17}NO_4$): calcd 335.1157, found 335.1201.

36*E*, (*E*)-2-[2-(3,4-Dimethoxy-5-nitrophenyl)vinyl]naphthalene. From the same reaction, compound **36*E*** was obtained in 52% yield. Mp: 165 °C (CH_2Cl_2/Hex). ^1H NMR δ_H (ppm): 4.01 (s, 6H), 7.13 (d, J = 16.6, 1H), 7.26 (d, J = 16.6, 1H), 7.4−7.6 (m, 3H), 7.52 (d, J = 1.1, 1H), 7.72 (dd, J_1 = 1.4, J_2 = 8.5, 1H), 7.8−8.0 (m, 4H). HRMS ($C_{20}H_{17}NO_4$): calcd 335.1157, found 335.1117.

37*Z*, (*Z*)-2,3-Dimethoxy-5-[2-(naphth-2-yl)vinyl]aniline. By Zn in AcOH reduction of **36*Z***, the title compound was obtained in 85% yield. ^1H NMR δ_H (ppm): 3.53 (s, 3H), 3.81 (s, 3H), 6.58 (d, J = 1.2, 1H), 6.63 (d, J = 12.0, 1H), 6.73 (d, J = 12.0, 1H), 7.20 (d, J = 1.2, 1H), 7.3−7.5 (m, 3H), 7.6−7.8 (m, 4H). HRMS ($C_{20}H_{19}NO_4$): calcd 305.1416, found 305.1317.

37*E*, (*E*)-2,3-Dimethoxy-5-[2-(naphth-2-yl)vinyl]aniline. By Zn in AcOH reduction of **36*E***, the title compound was obtained in 93% yield. ^1H NMR δ_H (ppm): 3.85 (s, 3H), 3.92 (s, 3H), 6.57 (d, J = 2.0, 1H), 6.62 (d, J = 2.0, 1H), 7.14 (d, J = 7.6, 1H), 7.15 (bs, 2H), 7.5−7.6 (m, 2H), 7.71 (dd, J_1 = 1.6, J_2 = 7.5, 1H), 7.8−8.0 (m, 3H). HRMS ($C_{20}H_{19}NO_4$): calcd 305.1416, found 305.1419.

38*Z*, (*Z*)-*N*-{2,3-Dimethoxy-5-[2-(naphth-2-yl)vinyl]phenyl}acetamide. By treatment of **37*Z*** with acetic anhydride, the title compound was obtained in 80% yield. ^1H NMR δ_H (ppm): 2.22 (s, 3H), 3.53 (s, 3H), 3.85 (s, 3H), 6.58 (d, J = 1.2, 1H), 6.63 (d, J = 12.0, 1H), 6.73 (d, J = 12.0, 1H), 7.20 (d, J = 1.2, 1H), 7.3−7.5 (m, 3H), 7.6−7.9 (m, 4H), 7.96 (s, 1H). HRMS ($C_{22}H_{21}NO_3$): calcd 347.1521, found 347.1566.

38*E*, (*E*)-*N*-{2,3-Dimethoxy-5-[2-(naphth-2-yl)vinyl]phenyl}acetamide. By treatment of **37*E*** with acetic anhydride, the title compound was obtained in 83% yield. ^1H NMR δ_H (ppm): 2.25 (s, 3H), 3.92 (s, 3H), 3.95 (s, 3H), 6.88 (d, J = 1.8, 1H), 7.2−7.3 (m, 3H), 7.4−7.6 (m, 3H), 7.6−7.9 (m, 4H), 8.27 (s, 1H). HRMS ($C_{22}H_{21}NO_3$): calcd 347.1521, found 347.1534.

39*Z*, (*Z*)-2-[2-(4-Methoxy-3,5-dinitrophenyl)vinyl]naphthalene. Following the general method, by reaction of 4-methoxy-3,5-dinitrobenzaldehyde (obtained by nitration of 4-methoxy-3-nitrobenzaldehyde) and triphenyl(2-naphthylmethylidene)-phosphorane, compound **39*Z*** was isolated in 12% yield. ^1H NMR δ_H (ppm): 4.10 (s, 3H), 6.56 (d, J = 12.1, 1H), 7.03 (d, J = 12.1, 1H), 7.2−7.8 (m,7H), 7.90 (s, 2H). HRMS ($C_{19}H_{14}N_2O_5$): calcd 350.0903, found 350.0906.

39*E*, (*E*)-2-[2-(4-Methoxy-3,5-dinitrophenyl)vinyl]naphthalene. From the same reaction, compound **39*E*** was obtained in 19% yield. Mp: 158 °C (CH_2Cl_2/Hex). ^1H NMR δ_H (ppm): 4.09 (s, 3H), 7.15 (d, J = 16.1, 1H), 7.36 (d, J = 16.1, 1H), 7.52 (m, 2H), 7.71 (d, J = 8.6, 1H), 7.8−7.9 (m, 4H), 8.18 (s, 2H). HRMS ($C_{19}H_{14}N_2O_5$): calcd 350.0903, found 350.0937.

40*Z*, (*Z*)-2-[2-(3,5-Diamino-4-methoxyphenyl)vinyl]naphthalene. By Zn in AcOH reduction of **39*Z***, the title compound was obtained in 32% yield. ^1H NMR δ_H (ppm): 3.76 (s, 3H), 6.14 (s, 2H), 6.47 (d, J = 12.4, 1H), 6.63 (d, J = 12.4, 1H), 7.4−7.9 (m, 7H). HRMS ($C_{19}H_{19}N_2O_5$): calcd 290.1419, found 290.1447.

40*E*, (*E*)-2-[2-(3,5-Diamino-4-methoxyphenyl)vinyl]naphthalene. By Zn in AcOH reduction of **39*E***, the title compound was obtained in 30% yield. ^1H NMR δ_H (ppm): 3.79 (s, 3H), 6.43 (s, 2H), 7.00 (d, J = 16.1, 1H), 7.11 (d, J = 16.1, 1H), 7.4−7.9 (m, 7H). HRMS ($C_{19}H_{19}N_2O_5$): calcd 290.1419, found 290.1445.

41*Z*, (*Z*)-2-[2-(4-Dimethylamino-3,5-dinitrophenyl)vinyl]naphthalene. Following the general method, by reaction of 4-dimethylamino-3,5-dinitrobenzaldehyde (obtained by nitration of 4-dimethylaminobenzaldehyde) and triphenyl(2-naphthylmethylidene)phosphorane, compound **41*Z*** was isolated in 36% yield. ^1H NMR δ_H (ppm): 3.67 (s, 6H), 6.65 (d, J = 12.0, 1H), 7.20 (d, J = 12.0, 1H), 7.30 (d, J = 9.2, 1H), 7.52 (m, 2H), 7.82 (m, 4H), 8.18 (s, 2H). HRMS ($C_{20}H_{17}N_3O_4$): calcd 363.1219, found 363.1222.

41*E*, (*E*)-2-[2-(4-Dimethylamino-3,5-dinitrophenyl)vinyl]naphthalene. From the same reaction, compound **41*E*** was obtained in 36% yield. Mp: 218 °C (Hex/EtOAc). ^1H NMR δ_H (ppm): 3.73 (s, 6H), 7.26 (d, J = 16.0, 1H), 7.56 (d, J = 16.0, 1H), 7.48 (s, 1H), 7.5−7.6 (m, 2H), 7.75 (d, J = 8.5, 1H), 7.8−7.9 (m, 3H), 8.45 (s, 2H). HRMS ($C_{20}H_{17}N_3O_4$): calcd 363.1219, found 363.1197.

Tubulin Isolation. Calf brain microtubule protein (MTP) was purified by two cycles of temperature-dependent assembly/disassembly, according to the method of Shelanski[52] modified as described in the literature.[53] The MTP solution was stored at −80 °C. Protein concentration was determined by the method of Bradford,[54] using BSA as standard.

Tubulin Assembly. The in vitro self-assembly of tubulin was monitored turbidimetrically using a thermostated Thermospectronic Helios α spectrophotometer fitted with a Peltier temperature controller and a circulating water carrousel system. The increase in turbidity was followed at 450 nm instead of 350 nm in order to avoid light absorption by the ligands. Each turbidimetry measurement was carried out simultaneously for a batch of six cuvettes, a control (i.e., with no ligand) always being included in the batch. Three different MTP preparations were used in these assays.

The assayed ligands were previously dissolved in DMSO and the solutions stored at −20 °C.

Cuvettes contained 1.5 mg/mL MTP in 0.1 M MES buffer, 1 mM EGTA, 1 mM $MgCl_2$, 1 mM β-ME, 1.5 mM GTP, pH 6.7, and the measured ligand concentration. The maximum amount of DMSO in the assayed cuvettes was 4%, which is reported not to interfere with the assembly process.[55]

The samples were preincubated for 30 min at 20 °C in order to allow binding of the ligand, and they were subsequently cooled on ice for 10 min. The cuvettes were then placed in the spectrophotometer at 4 °C. The assembly process was initiated by shifting of the temperature to 37 °C.

IC_{50} was calculated as the concentration of drug causing 50% inhibition of polymerization after 20 min incubation and was determined graphically. At least two independent experiments (or more when required for the most potent inhibitors) with different MTP preparations were carried out for every tested compound.

Cell Growth Inhibition Assays. These assays were carried out according to the colorimetric method of Skehan.[56] The concentration of protein was deduced from the sulforhodamine B bound to the protein, extracted with 10 mM unbuffered Tris base [tris(hydroxymethyl)aminomethane] for determination of optical density.

XTT Procedure. Exponentially growing HeLa, HT-29, or A-549 cells were seeded at 1.5×10^3, 3×10^3, or 5×10^3 (100 μL) cells/well, respectively, in 96-well flat-bottomed microtiter plates, and incubated at 37 °C in a humidified atmosphere of 5% CO_2/95% air for 24 h to let the cells attach to the plates. HL-60 cells were seeded at 5×10^3 (100 μL) cells per well. Then cells were incubated with different concentrations of the assayed compound at 37 °C under the 5% CO_2/95% air atmosphere for 72 h. Cell proliferation was quantified by using the XTT (3,3′-[4-(phenylaminocarbonyl)-2,3-tetrazolium]-bis-(4-methoxy-6-nitro)benzene sulfonic acid sodium salt hydrate) cell proliferation kit (Roche Molecular Biochemicals, Mannheim, Germany) according to the manufacturer's instructions. Briefly, a freshly prepared mixture solution of XTT labeling reagent and PMS (*N*-methylphenazinium methylsulfate) electron coupling reagent was added to each well in the amount of 50 μL. The resulting mixtures were further incubated for 4 h in a humidified atmosphere (37 °C, 5% CO_2), and the absorbance of the generated formazan product was measured with a microtiter plate reader at a test wavelength of 490 nm and a reference wavelength of 655 nm. IC_{50} (50% inhibitory concentration) was then calculated as the drug concentration causing 50% inhibition in cell proliferation.

Cell Cycle Analysis. The effects of the assayed compounds on the distinct phases of the cell cycle were analyzed by flow cytometry as previously described.[57] Briefly, cells (5×10^5) were centrifuged and fixed overnight in 70% ethanol at 4 °C. Then, cells were washed 3 times with phosphate-buffered saline (PBS), incubated for 1 h with 1 mg/mL RNase A and 20 μg/mL propidium iodide at room temperature, and analyzed with a Becton Dickinson FACSCalibur flow cytometer. The

induction of apoptosis was monitored as the appearance of the sub-G_1 peak (hypodiploidy) in cell cycle analysis.

Confocal Microscopy. Cells were grown on poly-L-lysine-coated coverslips, and after drug treatment coverslips were washed three times with HPEM buffer (25 mM HEPES, 60 mM PIPES, 10 mM EGTA, 3 mM $MgCl_2$, pH 6.6) and fixed with 4% paraformaldehyde in HPEM buffer for 15 min. Then cells were permeabilized with 0.5% Triton X-100 in HPEM buffer for 90 s and washed with HPEM. Coverslips were incubated with Ab-1 anti-tubulin monoclonal antibody (diluted 1:200 in PBS) (Oncogene Research Products) for 45 min, washed with PBS, and then incubated with CY2-conjugated antimouse antibody (diluted 1:100 in PBS) (Jackson-ImmunoResearch) for 45 min. Then, microtubules were analyzed by confocal microscopy using a ZeissLSM 310 laser scan confocal microscope. A drop of SlowFade Light Antifading reagent (Molecular Probes) was added to preserve fluorescence. Negative controls were prepared either by omitting the primary antibody or by using an irrelevant antibody, showing no fluorescence staining of the samples.

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Supporting Information Available: Table of elemental analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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