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Design, synthesis and biological evaluation of novel stilbene-based antitumor agents

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ABSTRACT

A series of novel stilbene derivatives has been synthesized and studied with the main goal to investigate SAR of the amino compound **1a**, as well as to improve its water solubility, a potentially negative aspect of the molecule that could be a serious obstacle for a pre-clinical development. We have obtained derivatives with good cytotoxic activity, in particular, the derivatives **5c** and **6b** could represent two novel leads for further investigation. Compound **8b**, a morpholino-carbamate derivative, prodrug of **1a**, has a very good solubility in water, and is active in suppressing growth of tumor cells at a concentration of 5000 nM, which is a concentration 100 times higher than the parent stilbene **1a**.

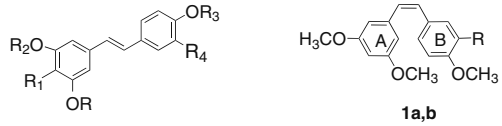
1. Introduction

Stilbene-based compounds are largely present in nature and have become of particular interest to chemists and biologists because of their wide range of biological activities.[1,2] Stilbene itself does not occur in nature, but hydroxylated stilbenes have been found in many medicinal plants.

The hydroxylated stilbene *trans*-resveratrol (*trans*-3,4′,5-trihydroxystilbene, Fig. 1) is a phytoalexin present in grapes and plays a role in the prevention of coronary artery disease associated with red wine consumption.[2–5] Resveratrol has also antioxidant and anti-inflammatory properties and could be a potential chemopreventive[6] and therapeutic agent in cancer.[7] In vitro inhibition of cell proliferation[8] and in vivo anti-neovascularization by resveratrol have been demonstrated.[9]

On the other hand, the *cis*-stilbene motif represents a key structural feature of a broad class of natural and synthetic compounds endowed with an exceptionally strong tubulin polymerization inhibitor activity interfering mainly with microtubule formation at the tubulin colchicine binding site. In this context, the natural *cis*-stilbene combretastatin A-4 (CA-4) (Fig. 1), an antimitotic agent isolated from the bark of the South African tree *Combretum caffrum*, and its 3′-amino derivative (AC-7739) possess a potent and interesting antitumor activity.[10,11] Their mechanism of action is related to tubulin-binding properties that result in rapid tumor endothelial cell damage, neovascular shutdown and subsequent hemorrhagic necrosis.[12]

Our group has recently synthesized a series of derivatives structurally related to both resveratrol and CA-4, in *cis* and *trans* orientations. Several active stilbenes were identified and, among them, *cis*-3,4′,5-trimethoxy-3′-aminostilbene (**1a**) and *cis*-3,4′,5-trimethoxy-3′-hydroxystilbene (**1b**) were found to induce HL60 apoptosis at nanomolar concentrations (IC_{50} = 30 nM).[13] The new stilbenes interfere with microtubule formation at the tubulin colchicine binding site, in a similar manner as CA-4 and AC-7739, inducing apoptosis in HL60 leukemia cells, but not in normal c-kit-positive hematopoietic progenitor cells at similar concentrations.[14] In addition to the effect in leukemia cells, stilbene **1a** is also highly potent



trans-Resveratrol R=R_1=R_2=R_3=R_4=H
Combretastatin A-4 (CA-4) R=R_2=R_3=CH_3,R_1=OCH_3,R_4=OH
AC-7739 R=R_2=R_3=CH_3,R_1=OCH_3,R_4=NH_2

a: R=$NH_3^+Cl^-$
b: R=OH

Figure 1. Natural and synthetic stilbenes.

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against various solid tumor cells and blocks cell cycle progression in G_2-M phase.[13] Stilbene **1a** is tolerated in mice up to 100 mg/kg by intraperitoneal injection without major organ toxicity. In particular, there was no bone marrow toxicity, and the ability of bone marrow engraftment was not affected by stilbene **1a** treatment.[14] Similar to other colchicine site tubulin inhibitors, stilbene **1a** selectively suppresses tumor vascular perfusion without damaging normal vascular perfusion based on a DCE-MRI study.[15] Most importantly, mice treated with five daily injections of **1a** at 25 mg/kg/day did not show any compromise in heart function, indicating that it could be a colchicine site inhibitor with negligible cardiotoxicity.[15]

Here, we have further investigated stilbenes starting from and trying to optimize the most active derivative, *cis*-3,4′,5-trimethoxy-3′-aminostilbene (**1a**). To corroborate our knowledge on the structure–activity relationship (SAR) of **1a**, a primary goal of the investigation was to improve its water solubility, a potentially negative aspect that could be an obstacle for further pre-clinical development. The problem of low water solubility has been a longstanding issue for all colchicine-site tubulin inhibitors, as they should fit into the colchicine-binding pocket that is highly hydrophobic. Indeed, the presence of methoxyl groups would seem to be an essential structural requirement for hydrophobicity and, hence, binding to tubulin. Our first approach to improve water solubility of **1a** was to remove the methoxy group of ring B by converting it, together with the amino group, into benzoheterocycle ring systems, that is, indole and benzoimidazole.[16] To design other novel derivatives, we used computer-based drug design based on the docking geometry and properties of the lead compound **1a** in the tubulin binding site. Our strategy was based on the assumption that the *cis*-alkenyl group is essential to maintain the right distance and position of the two aromatic rings. Keeping this pattern throughout the investigation, our attention was focused mainly on alteration of ring-A (compounds **6a–h**) and ring-B (compounds 4c–**e** and **5c,d**) by their substitution with benzoheterocycles, as well as structural alteration of 3-methoxy group, which was elaborated into a bulkier group.

An alternative approach to improve the water solubility of stilbene **1a** was the development of potential prodrugs **8a–c**.[16] Different water-soluble side chains were added through two different linkages with the free amino group of stilbene **1a**. The side chains used for enhancing water solubility are a morpholine containing group linked by either a carbamate or urea moiety (compounds **8a,b**) and a triethylene glycol (TEG) bound by a carbamate moiety (**8c**).

Here we give full chemical and biological details of all the compounds. Preliminary in vivo activity of compounds **1a** and **8a** has been previously described.[15,16] Evaluation of a primary screening for cytotoxicity on UCI-101 ovarian cancer cells will be described here for all synthesized compounds. For some compounds the data were subsequently confirmed in MDA-MB231 breast cancer cells, MiaPaCa2 pancreatic cancer cells, and SNU423 hepatocellular carcinoma cells. The most active compounds were also tested by in vitro tubulin polymerization inhibition (TPI).

2. Results

2.1. Docking studies: modeling and SAR

To explore the SAR of **1a** and to design new molecules that optimize **1a**, we recently docked the structure of **1a** into the colchicine-binding site of tubulin (Figs. 2 and 3)[14] using the X-ray crystal structure of αβ-tubulin complexed with DAMA–colchicine.[17] The resolution of this structural model is 3.58 Å and therefore may be somewhat limited in accurate fitting. After computational docking with GOLD 3.0,[18] they were rescored with the HINT program[19,20] for examination of the binding characteristics and molecular design. According to the model, compound **1b** has a near perfect overlay with CA-4, but a shift of **1a** was observed relative to the position of CA-4 due to the amino group in **1a** with respect to hydroxyl group in **1b**. Because of the shift of **1a**, the corresponding space occupied by the methoxy group in **1a** becomes larger, allowing an expansion of the methoxy group ($-OCH_3$) into bulkier functional groups such as $-O-CH_2-CH_2OH$ (**6a**) (Fig. 2c), $-O-CH_2-CH_3$ (**6b**), $-O-CH(CH_3)_2$ (**6c**), $-O-CH_2CH{=}CH_2$ (**6e**) and $-CH_2-COOH$ (**6f**). The methoxy group, known to interact with Cys-241, was also replaced with a hydroxy group (**6d**) to dramatically decrease the hydrophobicity. Based on computer modeling, addition of the hydroxy group (**6a**) will form a hydrogen bond with Cys-238 of β-tubulin (not shown), which we hypothesize will improve both the binding affinity and water solubility of the final product.

As previously reported,[16] the computer docking model also predicted that there is enough space in the colchicine-binding pocket to accommodate a bulkier group in the neighborhood of the amino group on ring B of stilbene **1a**. Combining the amino and methoxy groups of the B ring into a 5-atom ring structure, we obtained an indole or a benzoimidazole ring (compounds **4c**–**e**), and moreover the N-methyl indole derivatives (compounds **5c**,**d**) to simulate the methoxy group.

2.2. Chemistry

Stilbenes **4a–l** have been easily prepared as described in Scheme 1, where a Wittig reaction between opportune phosphonium salt **2** and aldehyde **3** was accomplished in THF using sodium hydride as the base.[13] 3-Hydroxy-5-methoxybenzaldehyde was either protected at the OH phenolic moiety as *tert*-butyldimethylsilyl (TBDMS) ether, or variously alkylated, while benzoheterocyclic aldehydes were commercially available.

The *Z* stereoisomers were then separated from the *E* stereoisomers by flash chromatography. The TBDMS ether protecting group of **4b,i** was removed by means of tetrabutylammonium fluoride (TBAF) to give **1b**[13] and **5i** in high yields. The tetrahydropyranyl (THP) protection of **4f** was removed by treatment with *p*-toluenesulfonic acid monohydrate in methanol to give **5f**. The benzoheterocyclic stilbenes **4c,d,l** were methylated at the heterocyclic nitrogen with methyl iodide and potassium hydroxide in a dimethylsulfoxide solution to give **5c,d,l**. Alkaline hydrolysis of the ethyl ester of **4k** gave high yield of the desired carboxylic acid derivative 5k. Finally, nitro compounds **4a,g,h,j**, and **5f,i,k,l** were reduced to amines by means of zinc in acetic acid solution, followed by hydrochloric or oxalic salt formation to give **1a** and **6a–h**. Water soluble carbamates and urea derivatives **8a–c** were simply achieved as previously described[16] and reported in Scheme 2.

2.3. Biological activities

All compounds were first evaluated for their cytotoxicity against UCI-101 ovarian cancer cells. The data were subsequently confirmed for some compounds in MDA-MB231 breast cancer cells, MiaPaCa2 pancreatic cancer cells, and SNU423 hepatocellular carcinoma cells. The most active compounds were also tested by in vitro tubulin polymerization inhibition (TPI).

It is evident at first glance that compound **1a** still remains the most active compound among all the derivatives (IC_{50} = 30 nM). However, structural alterations of the 3-methoxy group allowed compounds **6a–f** of some interest. Cleavage of the methoxy ether to give the corresponding phenolic residue allowed a derivative which biological activity decreases by more than 10 fold compared with **1a** (compound **6d**, IC_{50} = 800 nM). In contrast, the biological activity was maintained when the 3-methoxy group was replaced

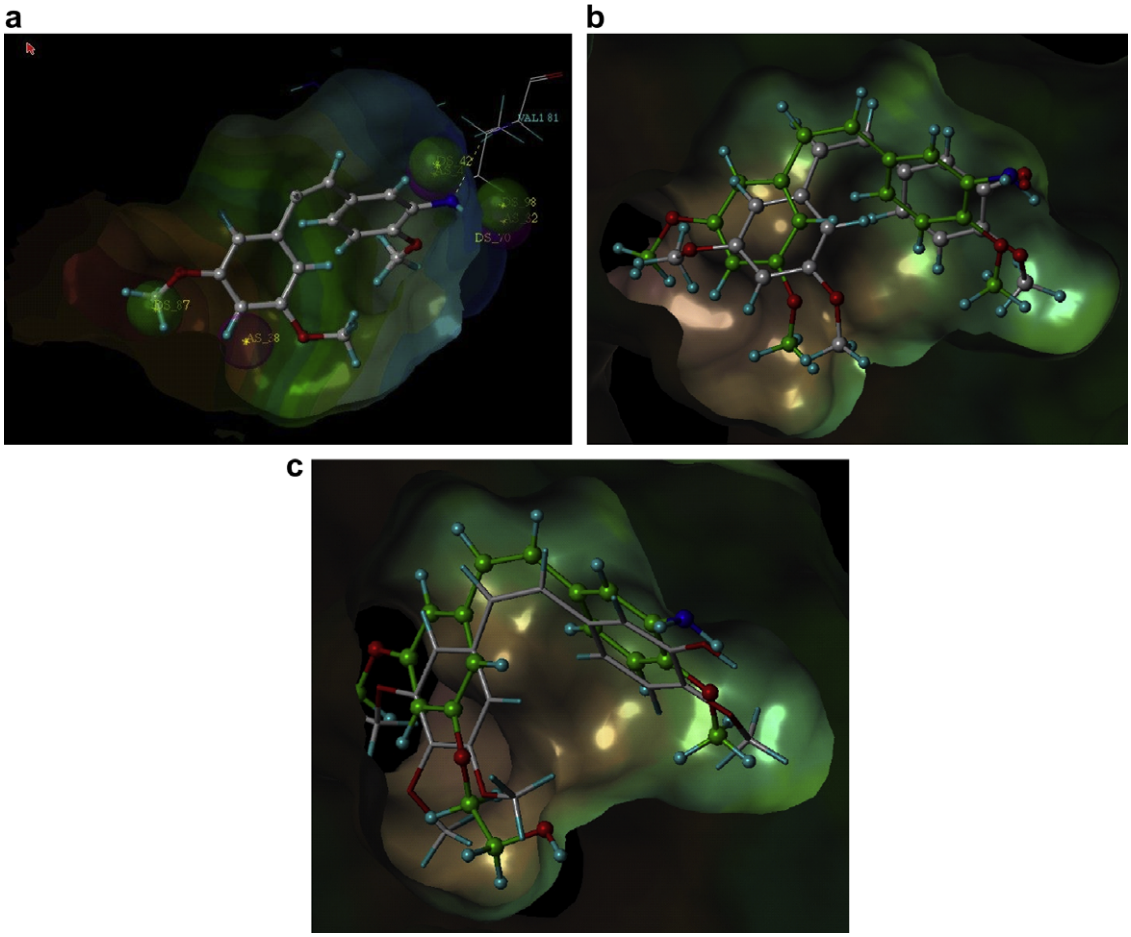


Figure 2. (a–c) Docking of stilbenes into the structure of tubulin. (a) Docking of stilbene **1a** to colchicine-binding pocket of tubulin. The α-tubulin is at right and the β-tubulin is at left side. The carbons of stilbene **1a** are shown in gray, nitrogen in blue, oxygen in red. The background is the surface contour of the colchicine-binding pocket. Blue for positive charge and red for negative charge. The H-bond between the amino group of **1a** and Val181 are shown in dash. (b) Overlay of **1a** (backbone in green) and **1b** (in gray) in the binding pocket. There is a shift of **1a** closer to the β-tubulin at the left side, which opens a space to accommodate a bigger functional group. (c) Molecular modeling of the proposed compound **6a** and alignment with CA4. The carbon of the new molecule is shown in green, whereas the carbon of CA4 is shown in gray. Oxygen is in red; nitrogen in blue, hydrogen in cyan. The extended $-O-CH_2-CH_3OH$ now occupies a space that is opened by the leftward shift of stilbene **1a** compared with CA4, which overlay with **1b**.

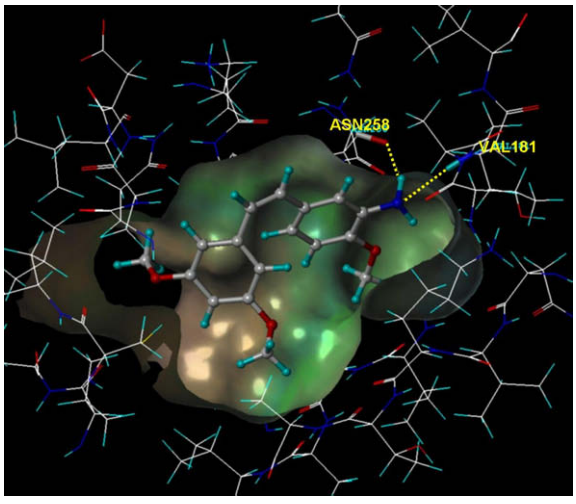
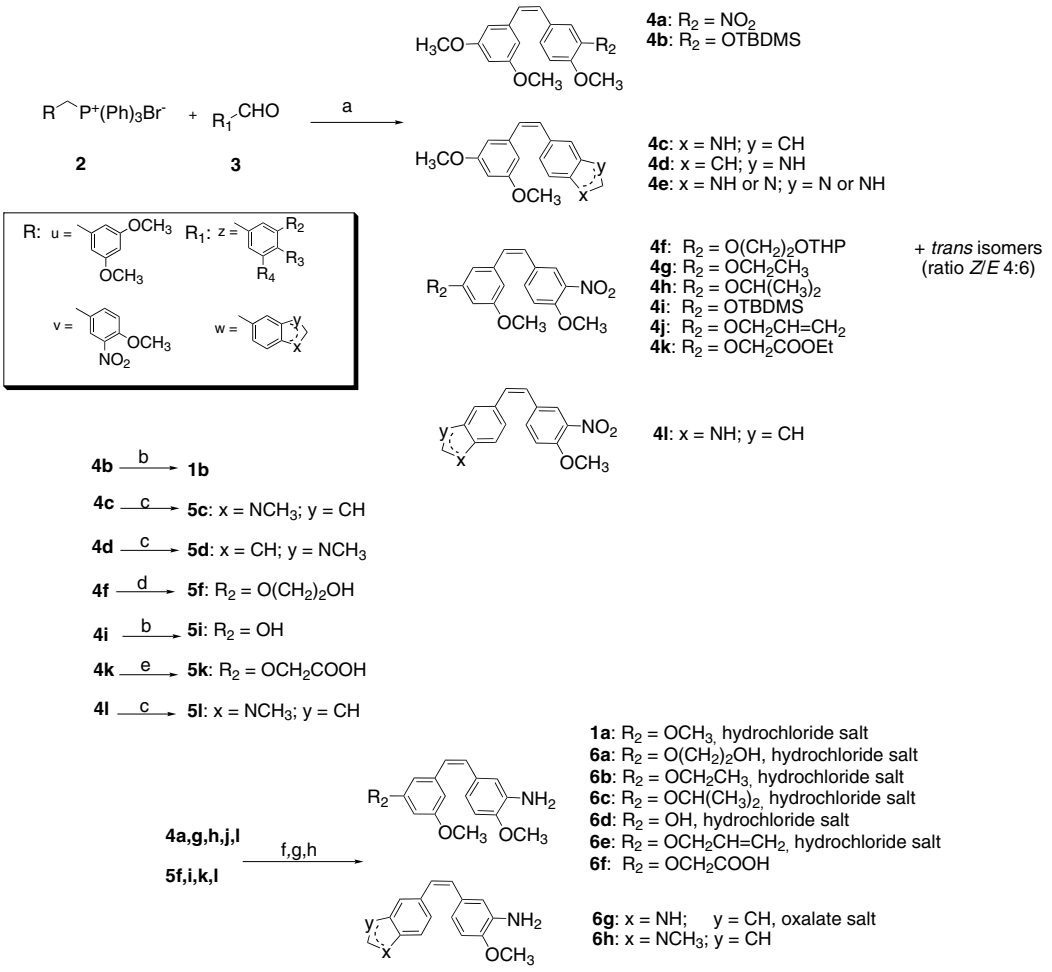


Figure 3. Interaction of stilbene **1a** with tubulin-binding pocket. The hydrogen bond with Val181 and Asn258 is shown by the yellow dash.

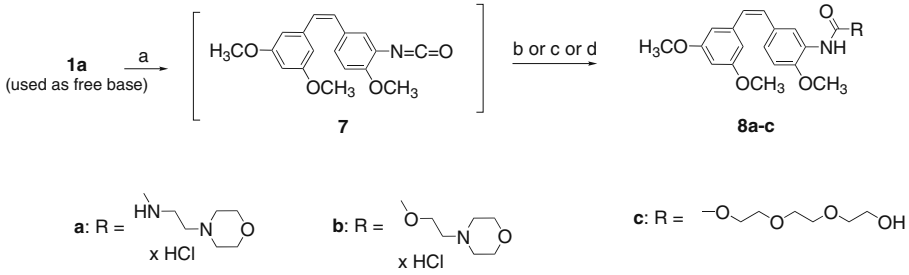
by more bulky substituents, 3-ethoxy or 3-isopropoxy groups, as in compounds **6b** (IC_{50} = 80 nM) and **6c** (IC_{50} = 100 nM), thus suggesting that there is enough space in this region of tubulin to accommodate a large side-chain and that a hydrophobic interaction is crucial. Compound **6a**, bearing a 2-hydroxyethyl group, still maintains biological activity with IC_{50} = 150 nM. However, addition of a carboxy group as in compound **6f** eliminates biological activity, indicating unambiguously that the environment cannot accommodate too much hydrophilicity.

The replacement of the dimethoxyphenyl group (ring A) by an indole ring was also tested. Dramatic loss of activity was observed for compound **6g** which has an IC_{50} 5000 nM. This is not due to lack of space to accommodate the benzoimidazole ring since further addition of a methyl group as in compound **6h** produced an active compound with an IC_{50} = 200 nM. This finding clearly indicates that the hydrophobic environment is essential for the biological activity, confirming the information obtained previously from structural alteration of the 3-methoxy group.

B-ring structural alterations of derivative **1a** also proved interesting. Indole or benzoimidazole derivatives **4c–e** allowed similar activity when tested in UCI-101 ovarian cancer cells. The IC_{50} of these three compounds were 250 nM, 250 nM and 260 nM, respectively (Table 1). In contrast, addition of a methyl group to the nitrogen of indole group of compounds **4c**, improves its biological activity. However, the improvement in activity was not as striking in adding methyl group in compounds **4d**. Compound **5c** has IC_{50} at



Scheme 1. Reagent and conditions: (a) NaH, THF, rt; (b) TBAF, CH_2Cl_2, rt; (c) CH_3I, KOH, DMSO; (d) *p*-toluenesulfonic acid, MeOH; (e) LiOH, MeOH, 50 °C, 24h; (f) from **4a**, **4g–h**, **4j**, **5f** and **5i** (i) Zn, AcOH, rt; (ii) HCl, MeOH; (g) from **5k** adn **5l** (i) Zn, AcOH, rt; (ii) oxalic acid, THF.



Scheme 2. Reagent and conditions: (a) Trichloromethyl chloroformate, dioxane, 60 °C, 3h; (b) (i) 2-morpholino-1-ethylamine; (ii) HCl 0.1 N, MeOH; (c) (i) 2-morpholino-1-ethanol, dioxane; (ii) HCl 0.1 N, MeOH; (d) triethylene glycol, dioxane.

40 nM, and **5d** at 220 nM. It is worth noting that the two regioisomers **5c,d** possess different biological activities, thus indicating a favorable interaction for the methyl group in compound **5c**. Hence we conclude that a hydrophobic environment surrounding the B ring is essential for biological activity. The activity of compound **4c–e**, **5c**, **6a**, and **6d** was also tested in other cell lines including MDA-MB231, MiaPaCa2 pancreatic cancer, and SNU423. Compound **5c** still exhibits potent cytotoxic activity comparable with stilbene **1a**.

Selected compounds were then tested for their activity in blocking tubulin polymerization in vitro. Commercial available pure tubulin without microtubule associated protein was used. The pure tubulin protein contains microtubule-associated protein (MAP) and is less sensitive to inhibition, which explains why the effective concentration is much higher than that for cytotoxic assays. Because the most active compound **1a** is highly potent in blocking tubulin polymerization in vitro at 5 μM, all compounds were thus tested at 5 μM to evaluate their efficacy and compared with **1a**. Paclitaxel (Sigma–Aldrich) was used as positive control and colchicine (Sigma–Aldrich) as negative control. The control curves in Figure 4 reach a plateau after 30 min. Paclitaxel induces a rapid polymerization and reaches plateau at 20 min. The maximal reading is not higher than control due to maximally polymerized tubulin. As described previously, both colchicine and stilbene **1a** suppress tubulin polymerization and **1a** is slightly more potent than colchicine (Fig. 4a).[14] The tubulin inhibitory activities of the

Table 1
Cell growth inhibition (IC_{50}) of various stilbene derivatives compared with colchicine and vincristine in UCI-101 ovarian cancer cells, SNU-423 hepatocellular carcinoma, MDA-MB231 breast cancer and MiaPaCa2 pancreatic cancer cells

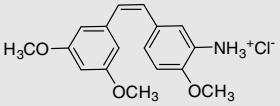
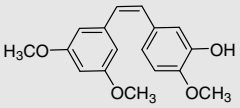
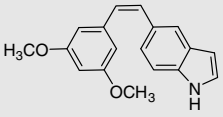
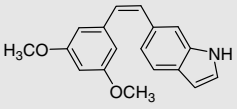
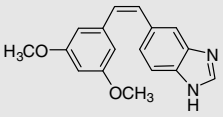
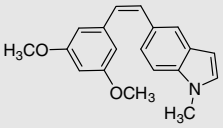
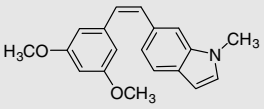
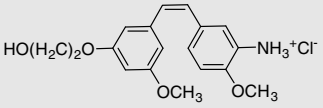
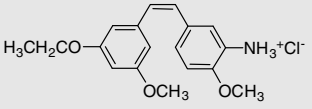
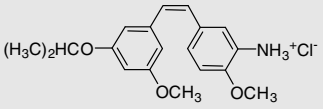
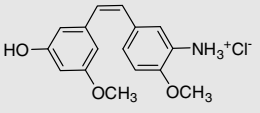
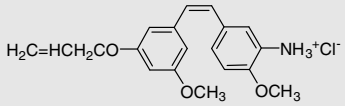
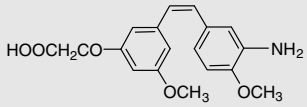
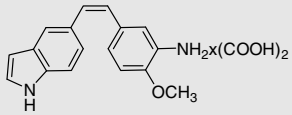
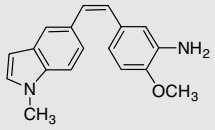
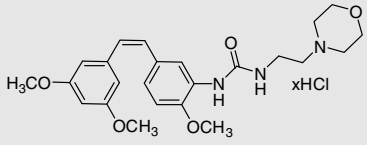
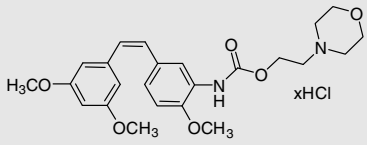
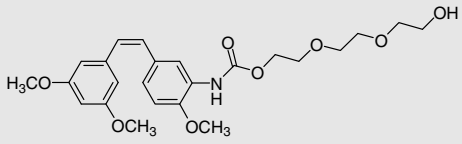
Compound	Structure	Cells IC_{50} (nM)			
		UCI-101	SNU-423	MDA-MB231	MiaPaCa2
Colchicine[a]		30	25	20	25
Vincristine[a]		20	10	15	15
CA-4[a]		2	6	4	8
1a		30	30	30	40
1b		30	15		
4c[b]		250	700	250	200
4d[b]		250	700	250	200
4e[b]		260	1000	800	800
5c[b]		40	30	80	80
5d[b]		220			
6a		150	200	80	180
6b		80			
6c		100			
6d		800	2000	800	700

Table 1 (*continued*)

Compound	Structure	Cells IC_{50} (nM)			
		UCI-101	SNU-423	MDA-MB231	MiaPaCa2
6e		200			
6f		>10,000			
6g		5000			
6h		200			
8a[b]		>10,000			
8b[b]		5000	5000		
8c[b]		>10,000			

[a] Vincristine and colchicine are from Sigma–Aldrich. CA4 has been synthesized in our laboratory.
[b] See also Ref. 16.

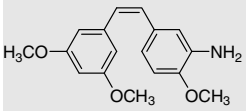
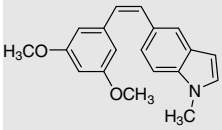
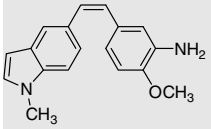
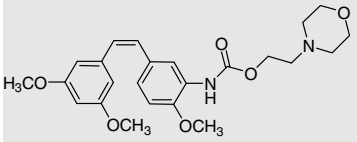
four ring A derivatives showed the order of potency as **6a** > **6d** > **6b** > **6h** (Fig. 4b), which is different from their cytotoxic activity. Especially compound **6d** has the second best tubulin inhibitor activity, but is significantly weaker in cytotoxic activity (IC_{50} at 800–2000 nM in different cancer cell lines) compared with remaining three ring A derivatives **6a**, **6b** and **6h** (IC_{50} at 150 nM, 80 nM, and 200 nM respectively). Similarly, the potency of tubulin inhibitory activity of **6b** is weaker than **6a**, but **6b** is more potent than **6a** in cytotoxic activity.

When selective ring B derivatives were tested for tubulin depolymerization, compound **5c** is the compound that is most active (Fig. 4c). The order of tubulin inhibitory activity (**5c** > **5d** > **4c**) is the same as that in their cytotoxicity, **5c** (IC_{50} = 40 nM) > **5d** (IC_{50} = 220 nM) > **4c** (IC_{50} = 250 nM), which is in contrast to the discrepancy noted in ring A derivatives. Hence, stilbene derivatives could have other mechanisms to induce cell death in addition to tubulin depolymerization. One example is through production of ROS from mitochondrial injury as shown by our other studies.[21]

2.4. Discussion

From these SAR studies, we have obtained novel stilbene derivatives with good cytotoxic activity. While derivatives **5c** and **6b** could represent two novel leads for further investigation, these SAR studies demonstrate unambiguously that the prerequisite for activity of stilbenes to inhibit tubulin polymerization is related to the degree of hydrophobicity of their binding pocket in tubulin. Obviously, this makes it difficult to improve water solubility of these ligands. Indeed, water solubility of the most active compound **5c** is comparable to the starting **1a** as shown in Table 2, and generally, this series of compounds share a low water solubility feature. However, water solubility of this class can be improved in a different way by synthesis of carbamate and urea-linked derivatives of **1a**. The three prodrugs **8a–c**, bearing hydrophilic side chains linked to the amine moiety of **1a**, have very good solubility in water, with **8b** about ten times that of **1a** (see Table 2). They were tested in UCI-101 cells to determine their IC_{50} in vitro. Only **8b** was active in suppressing growth of tumor cells at a concentra-

Table 2
Water solubility at 20 °C

Structure		*x*HCl	*x*(COOH)$_2$
	<1 mg/mL	6 mg/mL (**1a**)	< 1 mg/mL
	<1 mg/mL (**5c**)	–	–
	<1 mg/mL (**6h**)	–	–
	< 1 mg/mL	759 mg/mL (**8b**)	5.9 mg/mL

tion of 5000 nM, which is 170 times higher than the active stilbene **1a**. Both **8a** and **8c** were not able to induce significant inhibition of cell growth even at concentrations at 10,000 nM, indicating that their activity is inferior to **8b**. This could be due to the fact that prodrugs **8a–c** compounds may not be converted into the active form stilbene **1a** under tissue culture conditions, and the intact **8a–c** compounds could never fit into the colchicine-binding pocket due to their bulky side chains. Incubation of the prodrugs with serum for 1 h followed by addition into the culture cells was not able to change the IC_{50}, suggesting that the conversion of prodrugs does not occur within 1 h in circulation (not shown). Because of this finding, further in vivo study was performed on **8a,b**. Compound

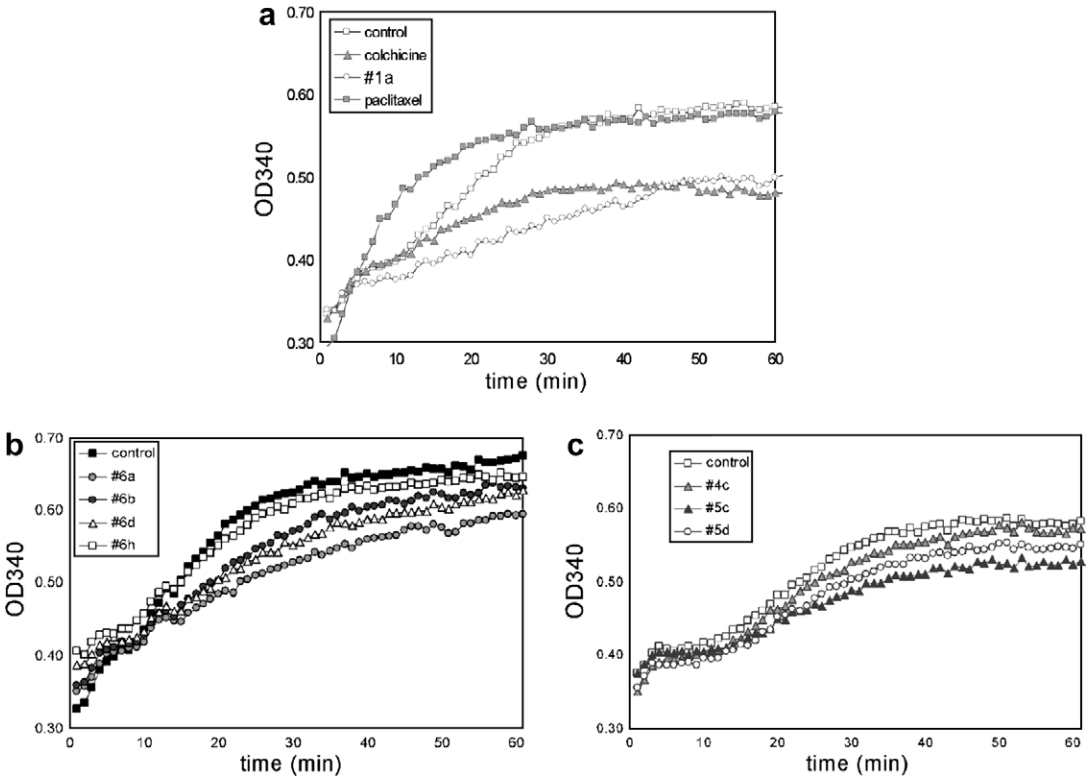


Figure 4. (a–c) In vitro tubulin polymerization study. Commercially available pure tubulin was incubated with 5 μM of various stilbene derivatives for 60 min in 90-well plates at 37 °C. Tubulin polymerization was measured with OD340 nm reading every minute by a microplate reader and plotted against time. Control represents no compounds added (a) paclitaxel, stilbene **1a**, and colchicine (b) compounds **6a**, **6b**, **6d**, and **6h**. (c) compounds **4c**, **5c**, and **5d**.

8a did not induce tumor growth suppression in mouse xenograft models whereas **8b** can suppress tumor growth similar to the active form **1a**.[16]

3. Conclusions

This study reported the development of a series of structural analogs of **1a** that have comparable cytotoxic activity to the lead compound **1a** as well as the ability to inhibit tubulin polymerization. We have shown for the first time that the 3-methoxy group of ring A can be replaced by a bulkier substituent, indicating that there is enough space in this region of tubulin to accommodate large side chains. Such substitution may provide compounds with better cytotoxic activity. SAR studies confirm the role of hydrophobicity into the colchicine-binding pocket of tubulin to select the most active compounds. The slight difference in the trend and effective concentrations between tubulin polymerization and cytotoxicity suggests that stilbene derivatives may have other mechanisms in addition to tubulin depolymerization to induce apoptosis.

Although the goal of improving water solubility of stilbene **1a** was not achieved by substitution of side chains of rings A or B, modification of the amine group of **1a** into a morpholine-carbamate derivative as in compound **8b** did improve water solubility as a prodrug with in vivo activity. In conclusion, this investigation indicates that rational structural alterations of stilbene **1a** could be of great importance in the development of novel anticancer drugs.

4. Experimental

4.1. Chemistry

4.1.1. General methods and materials

Melting points were obtained in open capillary tubes and are uncorrected. Reactions and product mixtures were routinely monitored by thin-layer chromatography (TLC) on Merck silica gel precoated F254 plates. Nuclear magnetic resonance (^{1}H NMR, ^{13}C NMR) spectra were determined using a Bruker AC-200 or with Varian Mercury Plus 400 spectrometer and peak positions are given in parts per million downfield from tetramethylsilane as the internal standard; *J* values are expressed in Hz. Molecular weights of compounds were determined by a mass spectrometer ESI Micromass ZMD-2000, values are expressed as MH^+. Light petroleum ether refers to the 40–60 °C boiling range fractions. Column chromatography was performed with Merck 60–200 mesh silica gel. All drying operations were performed over anhydrous sodium sulfate. Column chromatography (medium pressure) was carried out using the 'flash' technique. Microanalysis of new final synthesized compounds agreed within ±0.4% of calculated values.

4.1.2. General procedure for the synthesis of stilbenes 4a–l and *trans* isomers

To a solution of opportune aldehyde **3** (2 mmol) in 10 mL of anhydrous THF, the appropriate phosphonium salt **2** (2,2 mmol) was added in one portion. The suspension was cooled in an ice bath and NaH was added (50% in mineral suspension, 2.2 mmol, 110 mg). The reaction mixture was then stirred at room temperature for 24 h, filtered on a Celite bed and washed with THF. After solvent evaporation, the residue was solubilized with methylene chloride (15 mL) and washed with water (5 mL) and brine (5 mL), then dried and evaporated again. The residue was purified and the mixture of *Z* and *E* isomers separated by flash chromatography on silica gel.

4.1.2.1. (*Z*)-1-[2-(3,5-Dimethoxyphenyl)-ethenyl]-4-methoxy-3-nitrobenzene (4a). Eluent: ethyl acetate–light petroleum 2:8. Yield 39%. ^{1}H NMR ($CDCl_3$) δ: 3.69 (s, 6H), 3.92 (s, 3H), 6.34–6.38 (m, 3H), 6.45 (d, *J* = 12.2, 1H), 6.59 (d, *J* = 12.2, 1H), 6.91 (d, *J* = 8.4, 1H), 7.38 (dd, *J* = 8.4, *J* = 2.0, 1H), 7.76 (d, *J* = 2.0, 1H).

4.1.2.2. (*Z*)-5-[2-(3,5-Dimethoxyphenyl)-ethenyl]-1*H*-indole (4c). Eluent: Diethyl ether–light petroleum 3:7; Yield 27%. ^{1}H NMR ($CDCl_3$) δ: 3.61 (s, 6H), 6.31 (t, *J* = 2.4, 1H), 6.43–6.49 (m, 3H), 6.73 (d, *J* = 12.2, 1H), 7.13–7.18 (m, 3H), 7.24 (d, *J* = 8.8 1H), 7.60 (s, 1H), 8.10 (br, 1H). ^{13}C NMR δ: 55.2, 99.7, 102.9, 106.8, 110.6, 121.5, 123.5, 124.5, 127.9, 128.1, 128.8, 132.0, 135.1, 139.8, 160.5. Anal. Calcd for $C_{18}H_{17}NO_2$: C, 77.40; H, 6.13; N, 5.01. Found: C, 77.15; H, 6.13; N, 5.00.

4.1.2.3. (*Z*)-6-[2-(3,5-Dimethoxyphenyl)-ethenyl]-1*H*-indole (4d). Eluent: Diethyl ether–light petroleum 3:7; Yield 29%. ^{1}H NMR ($CDCl_3$) δ: 3.61 (s, 6H), 6.31 (t, *J* = 2.4, 1H), 6.47–6.51 (m, 4H), 6.72 (d, *J* = 12.4, 1H), 7.08 (dd, *J* = 1.6, *J* = 8.0 1H), 7.17–7.18 (m, 1H), 7.32 (s, 1H), 7.49 (d, *J* = 8.0 1H), 8.05 (br, 1H). ^{13}C NMR δ: 55.3, 99.8, 102.7, 106.8, 111.4, 120.3, 121.6, 124.9, 127.3, 128.7, 131.8, 139.9, 160.6. Anal. Calcd for $C_{18}H_{17}NO_2$: C, 77.40; H, 6.13; N, 5.01. Found: C, 77.24; H, 6.12; N, 4.99.

4.1.2.4. (*Z*)-5-[2-(3,5-Dimethoxyphenyl)-ethenyl]-1*H*-benzoimidazole (4e). Eluent: Diethyl ether–light petroleum 3:7; Yield 25% ^{1}H NMR ($CDCl_3$) δ: 3.04 (br, 1H), 3.61 (s, 6H), 6.31 (t, *J* = 2.2, 1H), 6.41 (d, *J* = 2.2, 2H), 6.54 (d, *J* = 12.2, 1H), 6.73 (d, *J* = 12.2, 1H), 7.25–7.29 (m, 1H), 7.53–7.56 (m, 2H), 8.20 (s, 1H). Anal. Calcd for $C_{17}H_{16}N_2O_2$: C, 72.84; H, 5.75; N, 9.99. Found: C, 72.66; H, 5.76; N, 9.95.

4.1.2.5. (*Z*)-3-[2-(4-Methoxy-3-nitrophenyl)-ethenyl]-5-methoxy-1-(2*H*-tetrahydropyranyloxyethoxy)-benzene (4f). Eluent: ethyl acetate–light petroleum 3:7; Oil; Yield 35%. ^{1}H NMR ($CDCl_3$) δ: 1.53–160 (m, 4H), 1.70–175 (m, 1H), 1.78–1.84 (m, 1H), 3.48–3.55 (m, 1H), 3.69 (s, 3H), 3.72–3.78 (m, 1H), 3.85–3.91 (m, 1H), 3.97 (s, 3H), 3.99–4.02 (m, 3H), 4.66–4.68 (m, 1H), 6.37–6.40 (m, 3H), 6.45 (d, *J* = 12.0, 1H), 6.59 (d, *J* = 12.0, 1H), 6.91 (d, *J* = 8.4, 1H), 7.42 (dd, *J* = 2.0, *J* = 8.4, 1H), 7.76 (d, *J* = 2.4, 1H). *m/z* 430.3 MH^+.

4.1.2.6. (*Z*) 3-Ethoxy-1-[2-(4-methoxy-3-nitrophenyl)-ethenyl]-5-methoxybenzene (4g). Eluent: ethyl acetate–light petroleum 2:3. Oil; Yield 43%. ^{1}H NMR ($CDCl_3$) δ: 1.35 (t, *J* = 7.0 3H), 3.69 (s, 3H), 3.90 (q, *J* = 7.0, 2H), 3.92 (s, 3H), 6.35–6.38 (m, 3H), 6.45 (d, *J* = 11.8, 1H), 6.60 (d, *J* = 11.8, 1H), 6.91 (d, *J* = 8.8, 1H), 7.40 (dd, *J* = 2.2, *J* = 8.8 1H), 7.76 (d, *J* = 2.4, 1H). ^{13}C 14.9, 55.4, 56.4, 63.6, 100.9, 106.6, 107.1, 113.1, 126.1, 127.5, 129.7, 131.5, 134.7, 138.4, 151.7, 160.3, 160.9. *m/z* 330.2 MH^+.

4.1.2.7. (*Z*)-1-[2-(4-Methoxy-3-nitrophenyl)-ethenyl]-5-methoxy-3-(1-methylethoxy)benzene (4h). Eluent: ethyl acetate–light petroleum 1:4. Oil; Yield 33%. ^{1}H NMR ($CDCl_3$) δ: 1.24–1.26 (m, 6H), 3.69 (s, 3H), 3.92 (s, 3H), 4.35–4.40 (m, 1H), 6.33–6.35 (m, 3H), 6.46 (d, *J* = 12.0, 1H), 6.60 (d, *J* = 12.0, 1H), 6.91 (d, *J* = 8.8, 1H), 7.40 (dd, *J* = 2.0, *J* = 8.8 1H), 7.76 (d, *J* = 2.0, 1H). ^{13}C 22.1, 56.3, 56.6, 70.1, 101.9, 106.5, 108.3, 113.2, 125.2, 127.1, 129.8, 131.6, 134.7, 138.4, 151.8, 159.2, 160.9. *m/z* 344.1 MH^+.

4.1.2.8. (*Z*)-1-(1,1-Dimethylethyl)dimethylsilanoxy-3-[2-(4-methoxy-3-nitrophenyl)-ethenyl]-5-methoxybenzene (4i). Eluent: ethyl acetate–light petroleum 1:9; Oil; Yield 40%. ^{1}H NMR ($CDCl_3$) δ: 0.2 (s, 6H), 0.9 (s, 9H), 3.69 (s, 3H), 3.88 (s, 3H), 6.32–6.39 (m, 2H), 6.44–6.55 (m, 1H), 6.60 (d, *J* = 12.0, 1H), 6.66 (d, *J* = 12.0, 1H), 6.91 (d, *J* = 8.8, 1H), 7.40 (dd, *J* = 2.2, *J* = 8.8, 1H), 7.75 (d, *J* = 2.2, 1H). *m/z* 416.2 MH^+.

4.1.2.9. (*Z*)-1-[2-(4-Methoxy-3-nitrophenyl)-ethenyl]-5-methoxy-3-(2-propenoxy)benzene (4j). Eluent: ethyl acetate–light petroleum 1:4. Oil; Yield 45%. ^{1}H NMR ($CDCl_3$) δ: 3.69 (s, 3H), 3.92 (s, 3H), 4.13 (dt, *J* = 1.4, *J* = 5.2, 2H), 5.23 (dq, *J* = 1.2, *J* = 10.6, 1H), 5.34 (dq, *J* = 1.4, *J* = 18.0, 1H), 5.89–6.08 (m, 1H), 6.38 (s, 3H), 6.46 (d, *J* = 12.2, 1H), 6.59 (d, *J* = 12.2, 1H), 6.91 (d, *J* = 8.8, 1H), 7.40 (dd, *J* = 2.2, *J* = 8.8 1H), 7.75 (d, *J* = 2.2, 1H). *m/z* 342.1 MH^+.

4.1.2.10. (*Z*)-Ethyl 3-[2-(4-methoxy-3-nitrophenyl)-vinyl]-5-methoxyphenoxyacetate (4k). Eluent: ethyl acetate–light petroleum 1:4; Oil; Yield 35%. ^{1}H NMR ($CDCl_3$) δ: 1.23 (t, *J* = 7.2, 3H), 3.69 (s, 3H), 3.93 (s, 3H), 4.21 (t, *J* = 7.2, 2H), 4.49 (s, 2H), 6.33–6.35 (m, 1H), 6.39 (t, *J* = 2.2, 1H), 6.40–6.42 (m, 1H), 6.46 (d, *J* = 12.4, 1H), 6.59 (d, *J* = 12.4, 1H), 6.91 (d, *J* = 8.8, 1H), 7.06 (dd, *J* = 2.2, *J* = 8.8 1H), 7.74 (d, *J* = 2.2, 1H). *m/z* 388.2 MH^+.

4.1.2.11. (*Z*)-5-[2-(4-Methoxy-3-nitrophenyl)-ethenyl]-1*H*-indole (4l). Eluent: ethyl acetate–light petroleum 1:4; Yield 30%. ^{1}H NMR ($CDCl_3$) δ: 3.91 (s, 3H), 6.41 (d, *J* = 12.2, 1H), 6.47–6.50 (m, 1H), 6.79 (d, *J* = 12.2, 1H), 6.85 (d, *J* = 8.8, 1H), 7.06 (dd, *J* = 8.4, *J* = 1.6, 1H), 7.19–7.22 (m, 1H), 7.24–7.28 (m, 1H), 7.42 (dd, *J* = 8.8, *J* = 2.0, 1H), 7.53 (s, 1H), 7.76 (d, *J* = 2.0, 1H) 8.14 (br, 1H). *m/z* 295.1 MH^+.

4.1.3. (*Z*)-2-{3-Methoxy-5-[2-(4-methoxy-3-nitro-phenyl)-ethenyl]phenoxy}ethanol (5f)

To solution of **4f** (0.5 mmol, 214 mg) in 20 mL of methanol, was added catalytic amount (about 10 mg) of *p*-toluenesulfonic acid and the mixture was stirred at room temperature for 2 h. The solution was concentrated in vacuo and the residue dissolved in ethyl acetate, washed with 5% sodium bicarbonate, brine (5 mL each), dried and evaporated. The residue was purified by flash chromatography on silica gel (40% ethyl acetate/light petroleum) to afford the expected compound. Yield 90% (194 mg). ^{1}H NMR ($CDCl_3$) δ: 3.70 (s, 3H), 3.90–3.93 (m, 5H), 3.95–3.97 (m, 2H), 6.38–6.40 (m, 3H), 6.46 (d, *J* = 12.4, 1H), 6.60 (d, *J* = 12.4, 1H), 6.92 (d, *J* = 8.8, 1H), 7.40 (dd, , *J* = 2.0, *J* = 8.8, 1H), 7.75 (d, *J* = 2.0, 1H). *m/z* 346.2 MH^+.

4.1.4. (*Z*)-3-Methoxy-5-[2-(4-methoxy-3-nitrophenyl)-ethenyl]phenol (5i)

The residue **4i** (3 mmol) was dissolved in methylene chloride (10 mL) and tetrabutylammonium fluoride (6 mmol, 3 equiv) was added. After 1 h at room temperature, the solution was diluted with methylene chloride (5 mL), washed with water (3 × 5 mL) and brine (5 mL), then dried. After concentration, the residue was purified by flash chromatography (ethyl acetate–light petroleum 1:4) on silica gel. Yield 90%. ^{1}H NMR ($CDCl_3$) δ: 3.69 (s, 3H), 3.92 (s, 3H), 4.95 (br, 1H), 6.28–6.31 (m, 2H), 6.35–6.37 (m, 1H), 6.45 (d, *J* = 12.2, 1H), 6.57 (d, *J* = 12.2, 1H), 6.91 (d, *J* = 8.8, 1H), 7.40 (dd, *J* = 2.2, *J* = 8.8, 1H), 7.75 (d, *J* = 2.2, 1H). *m/z* 302.0 MH^+.

4.1.5. (*Z*)-3-Methoxy-5-[2-(4-methoxy-3-nitrophenyl)-ethenyl]phenoxyacetic acid (5k)

A mixture of ester **4k** (1 mmol, 360 mg), methanol (10 mL), water (6 mL), and lithium hydroxide (1.5 mmol, 36 mg) was allowed to stand at 50–60 °C for 24 h. The solution was concentrated in vacuo to remove methanol, and the remaining aqueous solution was extracted with diethyl ether to separate trace amounts of unreacted ester. The aqueous solution was acidified with 1 M hydrochloric acid and extracted with three portions of ethyl acetate (10 mL each). The combined organic extracts were washed with brine (5 mL) and dried. Removal of the solvent under reduced pressure affords a residue, which was chromatographed on silica gel, 40% ethyl acetate–light petroleum. Yield 92%. ^{1}H NMR ($CDCl_3$) δ: 3.71 (s, 3H), 3.93 (s, 3H), 4.54 (s, 2H), 6.29–6.31 (m, 1H), 6.40 (t, *J* = 2.2, 1H), 6.44 (s, 1H), 6.48 (d, *J* = 12.0, 1H), 6.69 (d, *J* = 12.0, 1H), 6.94 (d, *J* = 8.8, 1H), 7.37 (dd, *J* = 2.6, *J* = 8.8 1H), 7.71 (d, *J* = 2.6, 1H), 11.45 (br, 1H). *m/z* 360.2 MH^+.

4.1.6. General procedure for preparation of *N*-methyl derivatives 5c,d and 5l

A suspension of potassium hydroxide (2 mmol, 112 mg) in dimethyl sulfoxide (5 mL) was stirred for 5 min and then the opportune stilbene **4c,d,l** (1 mmol) and iodomethane (1.2 mmol, 75 μL) were added. The mixture was stirred at room temperature for 30 min, poured into water/ice and extracted with ethyl acetate (2 × 15 mL). The combined organic extracts were washed with brine (5 mL) and dried. Removal of the solvent under reduced pressure gave a residue which was chromatographed on silica gel (20% ethyl acetate–light petroleum).

4.1.6.1. (*Z*)-5-[2-(3,5-Dimethoxyphenyl)-ethenyl]-1-methylindole (5c). Oil, 274 mg. Yield 93%. ^{1}H NMR ($CDCl_3$) δ: 3.63 (s, 6H), 3.76 (s, 3H), 6.32 (t, *J* = 2.2, 1H), 6.40–6.43 (m, 2H), 6.46–6.52 (m, 2H), 7.73 (d, *J* = 12.2, 1H), 7.0 (d, *J* = 2.2, 1H), 7.17–7.19 (m, 2H), 7.58–7.59 (m, 1H). ^{13}C NMR δ: 33.0, 55.4, 99.7, 101.3, 104.7, 106.7, 108.8, 121.7, 123.0, 127.8, 129.1, 132.0, 139.9, 160.5. Anal. Calcd for $C_{19}H_{19}NO_2$: C, 77.79; H, 6.53; N, 4.77. Found: C, 77.95; H, 6.50; N, 4.76.

4.1.6.2. (*Z*)-6-[2-(3,5-Dimethoxyphenyl)-ethenyl]-1-methylindole (5d). Oil, 270 mg. Yield 90% ^{1}H NMR ($CDCl_3$) δ: 3.62 (s, 6H), 3.68 (s, 3H), 6.33 (t, *J* = 2.2, 1H), 6.41 (d, *J* = 3.2, 1H), 6.45 (d, *J* = 3.2, 1H), 6.50–6.54 (m, 3H), 6.74 (d, *J* = 12.0, 1H), 7.02 (d, *J* = 3.2, 1H), 7.09 (d, *J* = 8.4, 1H), 7.46 (d, *J* = 8.4, 1H). ^{13}C NMR δ: 32.7, 55.3, 99.8, 100.0, 101.0, 106.8, 109.8, 120.3, 121.0, 128.6, 129.5, 131.9, 139.9, 160.6. Anal. Calcd for $C_{19}H_{19}NO_2$: C, 77.79; H, 6.53; N, 4.77. Found: C, 77.93; H, 6.55; N, 4.76.

4.1.6.3. (*Z*)-5-[(4-Methoxy-3-nitrophenyl)-ethenyl]-1-methylindole (5l). Oil. Yield 81%. ^{1}H NMR ($CDCl_3$) δ: 3.77 (s, 3H), 3.90 (s, 3H), 6.37–6.46 (m, 2H), 6.79 (d, *J* = 12.4, 1H), 6.85 (d, *J* = 8.8, 1H), 7.03 (d, *J* = 3.2, 1H), 7.10 (d, *J* = 1.4, 1H), 7.16 (s, 1H), 7.42 (dd, *J* = 2.2, *J* = 8.8, 1H), 7.50 (s, 1H), 7.8 (d, *J* = 2.2, 1H). *m/z* 309.1 MH^+.

4.1.7. General procedure for the reduction of nitro groups

To solutions of the appropriate nitrostilbenes **4a,g,h,j, 5f,i,k,l** (1 mmol) in acetic acid (15 mL) was added zinc powder (100 mmol, 6.5 g). The suspension was stirred for 2 h at room temperature. The reaction mixture was filtered over Celite and concentrated. The crude material was dissolved in ethyl acetate (15 mL) and washed with sodium bicarbonate 5% (5 mL), brine (5 mL), dried, and concentrated to afford the crude amino compound which was used for the next salification step without any further purification. Only the crude residue obtained of **5k** and **5l** were purified by chromatography (chloroform–methanol 9.5:0.5) on silica gel to give **6f** and **6h** as free base.

4.1.7.1. (*Z*)-3-[2-(3-Amino-4-methoxyphenyl)-ethenyl]-5-methoxyphenoxyacetic acid (6f). Yield 85%. ^{1}H NMR (DMSO) δ: 3.60 (s, 6H), 3.73 (s, 3H), 4.43 (s, 2H), 6.29 (t, *J* = 2.0, 1H), 6.37 (d, *J* = 12.6, 1H), 6.41–6.50 (m, 4H), 6.63 (d, *J* = 1.8, 1H), 6.71 (d, *J* = 8.4, 1H). Anal. Calcd for $C_{18}H_{19}NO_5$: C, 65.64; H, 5.81; N, 4.25. Found: C, 65.55; H, 5.80; N, 4.24.

4.1.7.2. (*Z*)-2-Methoxy-5-[2-(1-methyl-1*H*-indol-5-yl)-ethenyl]-phenylamine (6h). Yield 88%. ^{1}H NMR ($CDCl_3$) δ: 3.76 (s, 3H), 3.82 (s, 3H), 6.40 (d, *J* = 12.2, 1H), 6.41 (d, *J* = 3.2, 1H), 6.61 (d, *J* = 12.2, 1H), 6.65–6.67 (m, 2H), 6.71 (m, 1H), 7.00 (d, *J* = 3.2, 1H), 7.17 (m, 2H), 7.57 (s, 1H). Anal. Calcd for $C_{18}H_{18}N_2O$: C, 77.67; H, 6.52; N, 10.06. Found: C, 77.58; H, 6.52; N, 10.04.

4.1.8. General procedure for the synthesis of hydrochloride salts 1a and 6a–e

The crude compound obtained from reduction of the nitro derivative was dissolved in ethyl acetate and a slight excess of 0.1 N aq hydrochloride acid solution was added at 0 °C. The mixture was stirred at room temperature about 30 min. The solvent was evaporated in vacuo and the solid residue was washed twice with few mL of diethyl ether to give the desired hydrochloride salt.

4.1.8.1. (*Z*)-5-[3,5-Dimethoxyphenyl)-ethenyl]-2-methoxybenzenamine hydrochloride (1a). Recrystallized from methanol/diethyl ether. Mp 150–153 °C: Yield 85%. ^{1}H NMR (CD_3OD) δ: 3.65 (s, 6H), 3.95 (s, 3H), 6.35–6.36 (m, 3H), 6.54 (d, J = 12.0, 1H), 6.59 (d, J = 12.0, 1H), 7.12 (d, J = 8.4, 1H), 7.24 (d, J = 2.0, 1H), 7.34 (dd, J = 2.0, J = 8.4, 1H). ^{13}C NMR δ: 55.6, 56.8, 100.5, 107.7, 113.1, 120.3, 125.1, 129.4, 131.9, 132.2, 140.0, 153.1, 162.3. Anal. Calcd for $C_{17}H_{20}ClNO_3$: C, 63.45; H, 6.26; Cl, 11.02; N, 4.35. Found: C, 63.42; H, 6.25; Cl, 11.05; N, 4.34.

4.1.8.2. (*Z*)-2-{3-[2-(3-Amino-4-methoxyphenyl)-ethenyl]-5-methoxyphenoxy}ethanol, hydrochloride (6a). Recrystallized from methanol/diethyl ether. Mp 157–159 °C. Yield 81%. ^{1}H NMR (CD_3OD) δ: 3.67 (s, 3H), 3.80–3.82 (m, 2H), 3.88–3.90 (m, 2H), 3.96 (s, 3H), 6.38––6.40 (m, 3H), 6.54 (d, J = 12.4, 1H), 6.60 (d, J = 12.4, 1H), 7.12 (d, J = 8.4, 1H), 7.25 (d, J = 2.4, 1H), 7.35 (dd, J = 2.4, J = 8.4, 1H), 7.76 (d, J = 2.4, 1H). Anal. Calcd for $C_{18}H_{22}ClNO_4$: C, 61.45; H, 6.30; Cl, 10.80; N, 3.98. Found: C, 61.55; H, 6.30; Cl, 10.77; N, 3.97.

4.1.8.3. (*Z*)-5-[2-(3-Ethoxy-5-methoxyphenyl)-ethenyl]-2-methoxyphenylamine, hydrochloride (6b). Recrystallized from methanol/diethyl ether. Mp 127–131 °C. Yield 82%. ^{1}H NMR (CD_3OD) δ: 1.30 (t, J = 7.2, 3H), 3.66 (s, 3H), 3.88 (q, J = 7.2, 2H), 3.96 (s, 3H), 6.33–6.35 (m, 3H), 6.54 (d, J = 12.0, 1H), 6.60 (d, J = 12.0, 1H), 7.12 (d, J = 8.8, 1H), 7.23 (d, J = 2.2, 1H), 7.33 (dd, J = 2.2, J = 8.8 1H). ^{13}C NMR δ: 15.2, 55.7, 56.9, 64.6, 101.2, 107.6, 108.1, 113.2, 125.1, 129.5, 132.0, 132.1, 140.2, 153.1, 161.7, 162.4. Anal. Calcd for $C_{18}H_{22}ClNO_3$: C, 64.38; H, 6.60; Cl, 10.56; N, 4.17. Found: C, 64.16; H, 6.62; Cl, 10.54; N, 4.17.

4.1.8.4. (*Z*)-5-{2-[3-(1-methylethoxy)-5-methoxyphenyl]-ethenyl}-2-methoxyphenylamine, hydrochloride (6c). Recrystallized from methanol/diethyl ether. Mp 120–122 °C. Yield 78%. ^{1}H NMR (CD_3OD) δ: 1.20 (d, J = 6.2, 6H), 3.65 (s, 3H), 3.95 (s, 3H), 4.35–4.48 (m, 1H), 6.31–6.35 (m, 3H), 6.53 (d, J = 12.2, 1H), 6.60 (d, J = 12.2, 1H), 7.11 (d, J = 8.6, 1H), 7.22 (d, J = 2.0, 1H), 7.33 (dd, J = 2.0, J = 8.6 1H). ^{13}C NMR δ: 22.5, 55.8, 57.1, 71.2, 102.7, 107.9, 109.8, 112.5, 113.4, 125.3, 129.5, 132.3, 133.3, 140.3, 153.2, 160.1, 162.6. Anal. Calcd for $C_{19}H_{24}ClNO_3$: C, 65.23; H, 6.91; Cl, 10.13; N, 4.00. Found: C, 65.10; H, 6.93; Cl, 10.16; N, 3.98.

4.1.8.5.(*Z*)-3-[2-(3-Amino-4-methoxyphenyl)-ethenyl]-5-methoxyphenol, hydrochloride (6d). Recrystallized from methanol/diethyl ether. Mp 158–161 °C. Yield 75%. ^{1}H NMR (CD_3OD) δ: 3.63 (s, 3H), 3.95 (s, 3H), 6.23–6.26 (m, 3H), 6.49 (d, J = 12.2, 1H), 6.56 (d, J = 12.2, 1H), 7.10 (d, J = 8.2, 1H), 7.24 (d, J = 2.2, 1H), 7.34 (dd, J = 8.2, J = 2.2, 1H). Anal. Calcd for $C_{16}H_{18}ClNO_3$: C, 62.44; H, 5.89; Cl, 11.52; N, 4.55. Found: C, 62.52; H, 5.90; Cl, 11.49; N, 4.53.

4.1.8.6. (*Z*)-5-{2-[3-(2-Propenyl)oxy-5-methoxyphenyl]-ethenyl}-2-methoxyphenylamine, hydrochloride (6e). Recrystallized from methanol/diethyl ether. Mp 122–125 °C; Yield 70%. ^{1}H NMR (CD_3OD) δ: 3.65 (s, 3H), 3.96 (s, 3H), 4.40 (dt, J = 1.6, J = 5.2, 2H), 5.18 (dq, J = 1.4, J = 10.4, 1H), 5.34 (dq, J = 1.4, J = 17.2, 1H), 5.88–6.07 (m, 1H), 6.37 (s, 3H), 6.53 (d, J = 12.2, 1H), 6.60 (d, J = 12.2, 1H), 7.11 (d, J = 8.4, 1H), 7.25 (d, J = 2.0, 1H), 7.34 (dd, J = 2.0, J = 8.4 1H). ^{13}C NMR δ: 55.9, 57.1, 70.0, 101.6, 108.1, 108.7, 113.4, 117.6, 125.4, 129.6, 132.1, 132.5, 135.0, 140.3, 153.3, 161.5, 162.6. Anal. Calcd for $C_{19}H_{22}ClNO_3$: C, 65.61; H, 6.38; Cl, 10.19; N, 4.03. Found: C, 65.40; H, 6.39; Cl, 10.17; N, 4.02.

4.1.9. (*Z*)-5-[2-(1*H*-Indol-5-yl)-ethenyl]-2-methoxyphenylamine, oxalate salt (6g)

The compound obtained in previous reduction of nitro group was dissolved in tetrahydrofuran (5 mL) and mixed with a solution of oxalic acid dihydrate (1 mmol, 126 mg) in tetrahydrofuran (5 mL). The oxalate salt **6g** separates as colorless solid that is filtered, washed with 15 mL of THF (160 mg).

Recrystalized from methanol/diethyl ether. Mp 112–115 °C. Yield 70%. ^{1}H NMR (CD_3OD) δ: 3.59 (s, 3H), 6.32 (dd, J = 1.0, J = 2.2, 1H), 6.41 (d, J = 12.2, 1H), 6.60 (s, 1H), 6.61 (d, J = 12.2, 1H), 6.96–7.02 (m, 3H), 7.15–7.16 (m, 1H), 7.18–7.21 (m, 1H), 7.23–7.25 (m, 1H), 7.44 (s, 1H). Anal. Calcd for $C_{19}H_{18}N_2O_5$: C, 64.40; H, 5.12; N, 7.91. Found: C, 64.15; H, 5.14; N, 7.88.

4.1.10. Synthesis of isocyanate intermediate (7)

To a mixture of amino stilbene **1a**, free base (1.8 mmol, 519 mg) solubilized in dry dioxane (15 mL), trichloromethyl chloroformate (0.84 mmol, 105 μL), was added in one portion. The mixture was heated at 60 °C for 2 h. After cooling down to room temperature the mixture was concentrated in vacuo and the crude residue was used for the next reaction without any purification.

4.1.11. (*Z*)-1-{5-[2-(3,5-Dimethoxyphenyl)-ethenyl]-2-methoxyphenyl}-3-(2-morpholin-4-yl-ethyl)-urea hydrochloride salt (8a)

To a solution of crude isocyanate **7** (0.61 mmol, 200 mg) solubilized in dry dioxane (5 mL) 4-(2-aminoethyl) morpholine, (0.67 mmol, 88 mg) was added portionwise. The mixture was heated at 60 °C for 12 h. After cooling down to room temperature, the mixture was concentrated in vacuo and the crude residue was purified by flash chromatography (3% methanol/dichloromethane) on silica gel to afford 105 mg of the expected title morpholine derivative. The compound (0.5 mmol, 220 mg) was dissolved in ethyl acetate and a slight excess of 0.1 N hydrochloride solution was added at 0 °C. The mixture was stirred about 30 min at room temperature. The solvent was evaporated in vacuo and the solid residue was washed twice with few mL of diethyl ether to give 205 mg of title compound. Yield 70%. ^{1}H NMR ($CDCl_3$) δ: 2.83–3.02 (m, 2H), 3.15–3.30 (m, 2H), 3.46–3.66 (m, 8H), 3.67–3.78 (m, 2H), 3.81 (s, 3H), 3.94–4.10 (m, 2H), 4.16–4.35 (m, 2H), 6.26 (t, J = 2.4, 1H), 6.40 (d, J = 12.6, 1H), 6.42 (d, J = 2.4, 2H), 6.52 (d, J = 12.6, 1H), 6.63, 6.67 (m, 1H), 6.90 (dd, J = 1.6, J = 8.4, 1H), 7.39 (s, 1H), 8.0 (s, 1H). ^{13}C NMR δ: 36.0, 53.8, 55.8, 56.5, 60.2, 65.2, 100.8, 108.0, 111.4, 121.8, 125.1, 129.3, 130.4, 131.4, 131.7, 140.8, 149.5, 159.4, 162.2. Anal. Calcd for $C_{24}H_{32}ClN_3O_5$: C, 60.31; H, 6.75; Cl, 7.42; N, 8.79. Found: C, 60.18; H, 6.74; Cl, 7.40; N, 5.80.

4.1.12. (*Z*)-{5-[2-(3,5-Dimethoxyphenyl)-ethenyl]-2-methoxyphenyl}carbamic acid 2-morpholin-4-yl-ethyl ester, hydrochloride (8b)

To a solution of the crude isocyanate **7** (0.59 mmol, 180 mg) solubilized in dry dioxane (5 mL) the 4-(2-hydroxylethyl)morpholine, (0.6 mmol, 74 μL) was added portionwise. The mixture was heated at 60 °C for 12 h. After cooling down to room temperature the mixture was concentrated in vacuo and the crude residue was purified by flash chromatography (3% methanol/dichloromethane) on silica gel to afford 117 mg of the expected desired carbamate. The compound (0.5 mmol, 220 mg) was dissolved in tetrahydrofuran (THF, 7 mL) and mixed with 0.1 N hydrochloride acid solution (a slight excess) in THF (5 mL). The hydrochloride salt **8b** separates as a colorless solid that was filtrated and washed with 10 mL of THF to give 212 mg. Yield 75%. Mp 152–154 °C. ^{1}H NMR ($CDCl_3$) δ: 2.86–

3.0 (m, 2H), 3.28–3.35 (m, 2H), 3.49–3.55 (m, 2H), 3.67 (s, 6H), 3.82 (s, 3H), 3.92–4.06 (m, 2H), 4.28–4.44 (m, 2H), 4.67–4.72 (m, 2H), 6.30 (t, J = 2.2, 1H), 6.41 (d, J = 2.2, 2H), 6.45 (d, J = 12.0, 1H), 6.54 (d, J = 12.0, 1H), 6.70 (d, J = 8.2, 1H), 6.97 (dd, J = 2.2, J = 8.2, 1H), 7.42 (s, 1H), 7.92 (s, 1H), 13.45 (br, 1H). ^{13}C NMR δ: 52.5, 55.3, 55.8, 56.6, 58.5, 63.6, 99.7, 106.7, 109.9, 119.4, 124.3, 126.5, 129.3, 129.9, 130.2, 139.3, 147.2, 152.1, 160.5. Anal. Calcd for $C_{24}H_{31}ClN_2O_6$: C, 60.18; H, 6.52; Cl, 7.40; N, 5.85. Found: C, 60.05; H, 6.53; Cl, 7.42; N, 5.84.

4.1.13. (*Z*)-{5-[2-(3,5-Dimethoxyphenyl)-ethenyl]-2-methoxyphenyl}carbamic acid 2-[2-(2-hydroxyethoxy) ethoxy]ethyl ester (8c)

To solution of triethylene glycol (TEG, 5 mmol, 0.67 mL) in dry dioxane (5 mL) the crude isocyanate **7** (0.49 mmol, 160 mg) dissolved in dry dioxane (3 mL) was added in one portion. The reaction mixture was heated at 60 °C for 48 h. After cooling down to room temperature, the mixture was concentrated in vacuo and the residue was purified by flash chromatography (3% methanol/dichloromethane) on silica gel to afford the expected compound **8c**. Oil, 200 mg; Yield 89%. ^{1}H NMR ($CDCl_3$) δ: 2.55 br, (1H), 3.61–3.76 (m, 16H), 3.81 (s, 3H), 4.29–4.34 (m, 2H), 6.30 (t, J = 2.2, 1H), 6.43 (d, J = 12.0, 1H), 6.44 (d, J = 2.2, 2H), 6.53 (d, J = 12.0, 1H), 6.56 (d, J = 8.4, 1H), 6.92 (d, J = 2.2, J = 8.4, 1H), 7.93 (s, 1H), 8.02 (d, J = 2.2, 1H). ^{13}C NMR δ: 55.3, 55.8, 61.8, 64.0, 69.5, 70.4, 70.6, 72.5, 99.8, 106.7, 109.7, 119.3, 123.5, 127.3, 129.1, 130.1, 130.5, 139.4, 146.9, 153.2, 160.5. Anal. Calcd for $C_{24}H_{31}NO_8$: C, 62.46; H, 6.77; N, 3.04. Found: C, 62.67; H, 6.79; N, 3.05.

4.2. Computational docking of stilbenes to tubulin

Using the X-ray crystal structure of αβ-tubulin complexed with DAMA–colchicine (PDB code 1SA0),[17] which has a resolution of 3.58 Å, we characterized the binding by docking and scoring of the analogues of stilbene **1a** and **1b** in the colchicine-binding pocket of the tubulin dimer. Both α and β subunits were retained, while the stathmin-like domain and the C and D subunits described in the original structural data[22] were removed. Hydrogen atoms were added and optimized, that is, all protein side-chain and backbone atoms were kept fixed, using the Tripos force field, as incorporated in Sybyl 7.1 with 10,000 steps of Fletcher-Powell optimization until an energy gradient of 0.005 kcal-Å/mol was reached. The binding models for stilbene **1a** and other derivatives were constructed using the model of CA-4[23] as a template and reference ligand in the binding site. Computational docking was carried out using GOLD 3.0.[18] Docking models were then rescored with the HINT program[19,20] as described previously.[17] Those models with high HINT score were selected for characterizing the binding site. Furthermore, new putative ligands were docked and scored with the same methodology as part of the process in choosing new analogs to be synthesized.

4.3. Cell lines

MDA-MB231 breast cancer cells, MiaPaCa2 pancreatic cancer cells, and SNU423 hepatocellular carcinoma cells are from ATCC. Cells were grown in DMEM supplemented with 10% fetal bovine serum and penicillium/streptomycin/glutamine in 5% CO_2. UCI-101 ovarian cells were obtained from Dr. Thai Cao, University of Utah,[24] and were grown in Iscove's Modified Dulbecco's medium (IMDM) with the same supplements as above.

4.4. Cell death analysis

To determine tumor growth suppression and IC_{50} of different compounds, cells were grown in 96-well plates and treated with 0, 0.01, 0.03, 0.1, 0.3, 1.0, and 3.0 μM for 48 h before harvested for Alamar blue™ staining. In this staining, 1/10 volume of Alamar Blue™ solution was added to each well and optical density (OD) at 570 and 600 nm was determined by a microplate reader. The percentage of growth inhibition was calculated according to the manufacturer's formula as follows: $[(117216 \times A_{570}) - (80586 \times A_{600})]/[(117216 \times A^{\circ}_{570}) - (80586 \times A^{\circ}_{600})] \times 100$. In this formula, A_{570} is the absorbance of the treated samples at 570 nm; A_{600} is the absorbance of the treated samples at 600 nm; A°_{570} is the absorbance of the untreated samples at 570 nm; and A°_{600} is the absorbance of the untreated samples at 600 nm. The two constants, 117216 and 80586, are the extinction coefficients of Alamar Blue™ at 570 and 600 nm respectively. Each concentration has been used during three different experimet.

4.5. Tubulin polymerization assay

The tubulin polymerization assay kit was purchased from Cytoskeleton (Denver, CO). Tubulin (>99% pure) was mixed with general tubulin buffer (GTB, 80 mM PIPES pH 6.9, 2 mM $MgCl_2$, 0.5 mM EGTA, and 1 mM GTP) in a 96-well plate at 37 °C. Absorbance at 340 nm was measured every 1 min for 60 min by SpectraMAX 250 (Molecular Devices, Sunnyville, CA). Analysis of the results was performed by SoftMAX Pro version 1.2.0 from the same company. Same studies were repeated three times and the representative results are shown.

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