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# Lead optimization of dual tubulin and Hsp27 inhibitors

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## 1. Introduction

Stress factors including chemotherapies can enhance the expression of heat shock proteins (Hsp) in mammalian cells [1–3]. Hsp27, a small Hsp with a molecular weight of 27 kDa, promotes the survival of mammalian cells exposed to anti-cancer agents [4]. The results of several investigations demonstrate that Hsp27 overexpression leads to the resistance of anti-cancer drugs [3,5]. The cellular protective effects of Hsp27 rely on its molecular chaperone functions against apoptotic signals to eliminate anti-cancer agents induced programmed cell death [6–8]. Up-regulation of Hsp27 leads to an over-active chaperone activity, which contributes to the cancer cell survival under chemotherapeutic agents [1,2]. Increased expression of Hsp27 has been detected in a number of cancers such as breast cancer, endometrial cancer and leukemia [1,2,9–13], which supports the hypothesis

that Hsp27 is a good target of novel cancer therapy. Hsp27 inhibitors can decrease the protective function of the protein in cell apoptotic regulation, and enhance the anti-cancer efficacy of chemotherapy [5,14,15]. In fact, several agents diminishing Hsp27 functions exhibited promising anti-tumor activity, and also enhanced the potency of other anti-cancer agents in preclinical models of a variety of tumors expressing high levels of Hsp27 [9,14,15].

Tubulin-containing structures are important for many cellular functions, including chromosome segregation during cell division, intracellular transport, development and maintenance of cell shape, cell motility, and distribution of molecules on cell membrane [16]. Tubulin inhibitors are classical chemotherapeutic agents for cancer treatment. It has been well documented that tubulin inhibitors cause cell cycle arrest and induce apoptosis [17–20]. Several preclinical studies demonstrated that Hsp27 inhibition could potentiate the apoptosis-inducing activity of tubulin inhibitors [21]. Compounds targeting both Hsp27 and tubulin can fuse the two anti-cancer effects into one agent, and create a synergistic activity by itself. These dual tubulin and Hsp27 inhibitors will generate “one stone two birds” effect, and may represent a class of new anti-cancer agents.

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Previously we developed tubulin inhibitor, Hsp27 inhibitor and dual Hsp27 and tubulin inhibitors that exhibited potent anti-cancer activity [22]. In addition, the combination of pure tubulin inhibitor with Hsp27 inhibitor showed a synergistic effect to inhibit cancer cell proliferation [22], suggesting that the compounds with dual molecular targets have advantages over agents with single molecular target. It is desirable to further optimize the dual targeting compounds to generate new analogs with better potency to inhibit both Hsp27 and tubulin, which will lead to more potent anti-cancer agents. In the current study, systematic ligand based optimization resulted in compounds that significantly inhibited breast cancer cell proliferation. Unexpectedly, the Hsp27 inhibitory activity of the compounds was diminished during the lead optimization. The new derivatives became pure tubulin inhibitors. The results revealed several key structural elements critical for Hsp27 inhibition, which provides important structural characteristics for future optimization to improve Hsp27 inhibition.

## 2. Results and discussions

### 2.1. Lead optimization and summarization of the structure–activity relationship (SAR)

Previously, we have shown that structural modification of the lead compound **1** (Fig. 1) by substitution of 2,5-dimethylbenzyl moiety with 2,5-dimethoxybenzyl group dramatically increased the inhibition of cell proliferative activity. Several new derivatives inhibited the proliferation of various cancer cell lines with  $IC_{50}$  values at subnanomolar level [22]. Additionally, replacing methyl sulfonamide group in these compounds with trifluoromethyl sulfonamide group significantly impaired their anti-cancer activity, suggesting that the trifluoromethyl sulfonamide group is not well tolerated. Since no other alternatives to methyl sulfonamide group were investigated in the previous study, we wonder if a bulkier or more hydrophobic group, such as propyl, phenyl, or benzyl at this position, could be tolerated. Therefore, in this study we kept the B moiety as 2,5-dimethoxybenzyl group, and systematically changed the C moiety to several different sulfonamide groups including ethyl-, propyl-, phenyl-, and benzyl-sulfonamide. The acetamide group, which is smaller than the methyl sulfonamide group and shows different electronic distribution, was also evaluated. Previous studies revealed that the N-methyl group at the D position is important for the anti-proliferative activity of methyl sulfonamides. Removal of N-methyl group significantly decreased their cell growth inhibitory potency [22]. In this study, we further confirmed this structure–activity relationship on this moiety with this series of compounds. Some analogs were designed as free –NH moiety and some were designed as –Methyl NH moiety. For all the new derivatives, moiety A was remained as 4-methoxyphenyl, 3,4-dimethoxyphenyl, 4-bromophenyl, and 4-iodophenyl group. The

previous evaluation of 39 different aryl groups at the A position revealed that these four substituted aryl groups were superior to other ones [22].

All these new compounds were synthesized according to the previous method (Scheme 1) [22]. They were examined for potency and selectivity on the growth inhibition of four different breast cancer cells lines including SKBR-3, MCF-7, MDA-MB-231, and MDA-MB-468. SKBR-3 cell is human epidermal growth factor receptor 2 (HER2) positive and estrogen receptor (ER) negative, while MCF-7 cell is HER2 negative and ER positive. Both MDA-MB-231 and MDA-MB-468 cells are HER2 and ER negative. The results are summarized in Table 1. The  $IC_{50}$ s of cell growth inhibition of the compounds ranges from 0.001  $\mu$ M to 2.44  $\mu$ M for SKBR-3 cells, 0.001  $\mu$ M to 186.0  $\mu$ M for MCF-7 cells, 0.1  $\mu$ M to 48.1  $\mu$ M for MDA-MB-231 cells, and 0.008  $\mu$ M to 1.010  $\mu$ M for MDA-MB-468 cells. Most compounds exhibited more potent growth inhibition to SKBR-3 and MDA-MB-468 cells than MCF-7 and MDA-MB-231 cells. For SKBR-3 cells, SAR analysis indicated that the sulfonamide group at the C moiety of these compounds is critical for the biological activity. The previously synthesized compounds N-methylmethanesulfonamide **2–5** showed potent anti-proliferative activity for SKBR-3 cells with  $IC_{50}$  values about 0.001–0.004  $\mu$ M [22]. However N-methylethanesulfonamides (**6, 14, 16**), N-methyl-propylsulfonylamides (**7, 11, 15, 17**), N-methyl-benzylsulfonylamide (**8**), and N-methyl-phenylsulfonamides (**9, 13**) showed decreased activity compared to the corresponding N-methylmethanesulfonamide (**2–5**). Only N-methylethanesulfonamide **10** and N-methylbenzylsulfonamide **12** maintained the potent SKBR-3 cell growth inhibition activity with  $IC_{50}$ s of 0.001  $\mu$ M and 0.003  $\mu$ M respectively. The general order of potency of various N-methyl alkyl/arylsulfonamides for inhibition of SKBR-3 cell growth is methanesulfonamides > ethanesulfonamides > = propylsulfonamides > benzylsulfonylamides > phenylsulfonamides. The anti-proliferative activity of the compounds against SKBR-3 cells seems to be inversely proportional to the size of alkyl/aryl sulfonamide at the C moiety. Previous study showed that removal of the N-methyl group in N-methylmethanesulfonamides significantly impairs their biological activity [22]. Similar phenomenon was observed for most N-methyl alkyl/arylsulfonamides evaluated in the current study. Removal of N-methyl group in N-methylethanesulfonamides (**10, 14, 16**), N-methyl-propylsulfonamides (**7, 11, 15, 17**), N-methyl-benzylsulfonamide (**8, 12**), and N-methyl-phenylsulfonamides (**9, 13**), generated the corresponding ethanesulfonamides (**22, 26, 28**), propylsulfonamides (**19, 23, 27, 29**), benzylsulfonamide (**20, 24**), and phenylsulfonamides (**21, 25**) with 2–43 fold loss in potency. The results confirmed that the free –NH moiety of these compounds decrease the anti-cancer activity. Free-NH can act as a hydrogen bond donor or acceptor, whereas methyl-NR can only act as hydrogen bond acceptor. The hydrogen bond formation on this moiety may be critical for the biological activity

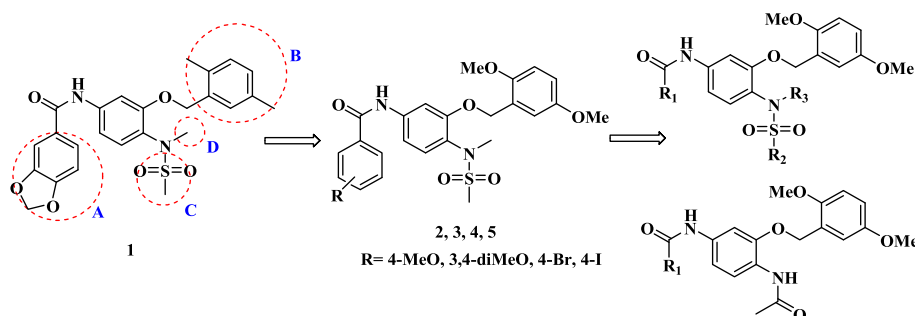
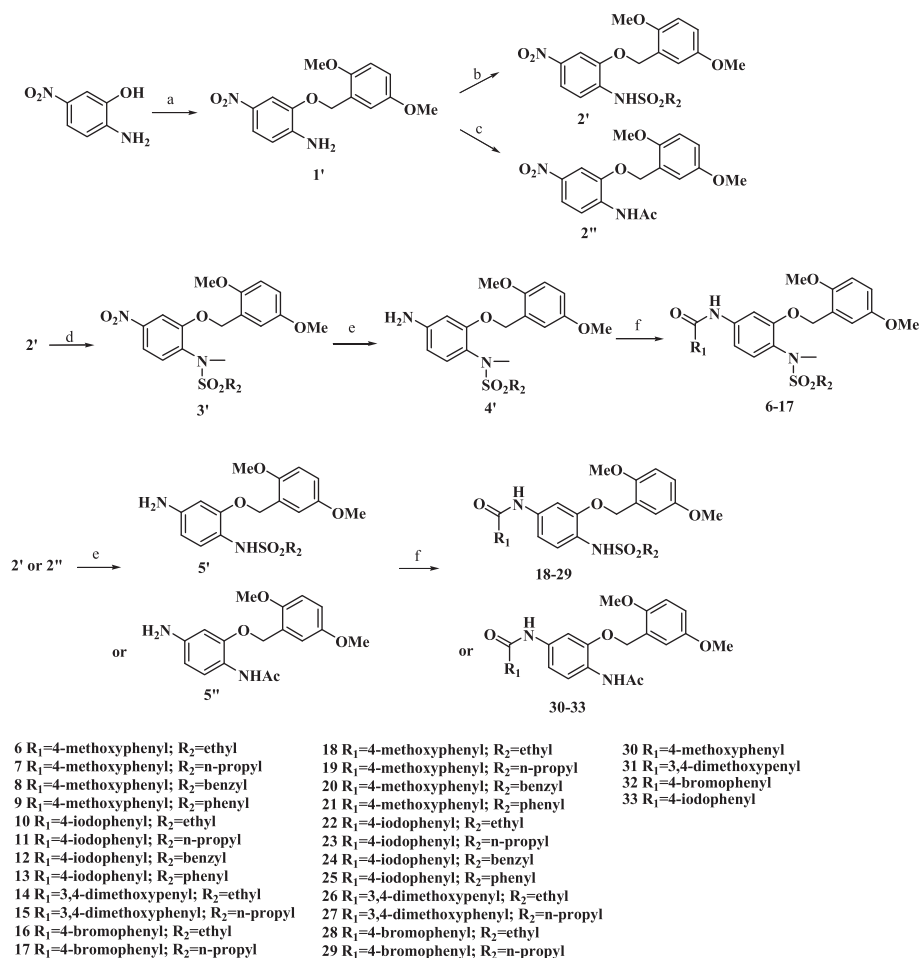


Fig. 1. Ligand based optimization of compound 1.



**Scheme 1.** Reagents and conditions: (a) 2,5-dimethoxybenzyl chloride,  $K_2CO_3$ , DMF; (b)  $R_2SO_2Cl$ , NaH, DMF (c) Acetyl chloride,  $K_2CO_3$ , 1,4-dioxane; (d) MeI, NaH, DMF; (e)  $FeCl_3$ , Zn, DMF/ $H_2O$ ; (f)  $R_1COCl$ ,  $K_2CO_3$ , 1,4-dioxane.

of the corresponding compounds. A sole hydrogen bond acceptor on this moiety benefits the anti-cancer activity based on the cell proliferation assay results. Ethanesulfonamide **18** is exceptionally as potent as N-methylethanesulfonamide **6**. Four acetamide derivatives **30–33** exhibited anti-proliferative activity with  $IC_{50}$ s in the range of 0.110  $\mu M$  and 2.44  $\mu M$ . All of them are much less potent than corresponding N-methyl-alkyl/arylsulfonamides (**6–17**) and alkyl/arylsulfonamides (**18–23**, **26–29**) with the exception of acetamide **33**. This compound is as potent as the corresponding benzyisulfonamide **24** and 3-fold more potent than phenylsulfonamide **25**. The results suggest that sulfonamide group is preferred over acetamide group for the anti-cancer activity.

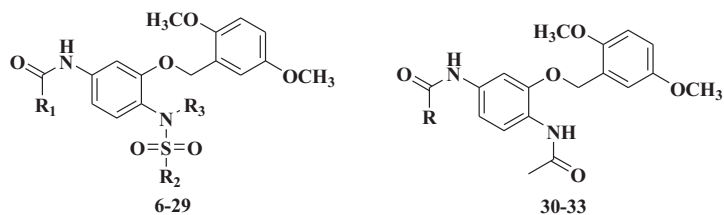
A similar trend was observed in the SAR studies for MDA-MB-468, MDA-MB-231, and MCF-7 cells. For the inhibition of MDA-MB-468 cell growth, the most potent compounds were N-methylbenzylsulfonamide **8** and N-methyl-propylsulfonamide **11** with same  $IC_{50}$ s of 0.008  $\mu M$ . Two potent compounds for growth inhibition of MCF-7 cells were N-methylethanesulfonamide **10** and propylsulfonamide **23** with  $IC_{50}$  of 0.015  $\mu M$  and 0.050  $\mu M$ . Unexpectedly, N-methylethanesulfonamide **6** inhibited MCF-7 cell growth with an  $IC_{50}$  of 0.001  $\mu M$ , which is superior to all other compounds. The result suggested that the compound may interfere with some unknown cell growth pathways to block cell proliferation besides the identified tubulin and Hsp27 pathways. Most of the other compounds showed activity at submicromolar levels for

MCF-7 cells, and are even much less potent for inhibition of MDA-MB-231 cell growth.

## 2.2. Inhibition of tubulin polymerization

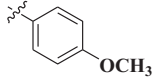
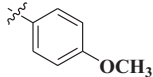
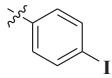
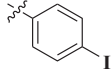
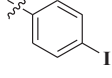
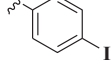
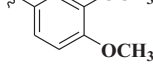
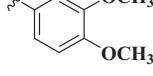
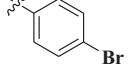
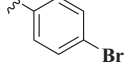
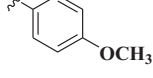
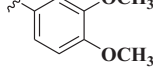
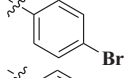
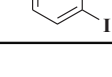
Previous studies revealed that 2,5-dimethoxybenzyl group can enhance the tubulin polymerization inhibitory potency of the compounds, thereby substantially improve their anti-proliferative activity. In the current study, this critical group was remained and various alkyl/arylsulfonamides were synthesized and evaluated by substitution of other moieties. Among these new analogs, N-methylethanesulfonamide **10** and N-methylbenzylsulfonamide **12** displayed potent cytotoxicity against SKBR-3 cells with  $IC_{50}$ s of 0.001  $\mu M$  and 0.003  $\mu M$ , respectively. This potency is comparable to that of the corresponding N-methylmethanesulfonamide **5**. However, the two compounds have different sulfonamide moieties. Therefore, it is necessary to elucidate whether the compounds act through similar anti-cancer mechanisms compared to the lead compounds. First, their tubulin inhibitory activity was determined with tubulin polymerization assay. Both compounds **10** and **12** showed dose-dependent inhibition of tubulin polymerization (Fig. 2). In 15 min, compound **10** inhibited tubulin polymerization by 9% at 0.1  $\mu M$  and 59% at 1  $\mu M$ . Compound **12** displayed a similar tubulin polymerization inhibitory activity by 5% at 0.1  $\mu M$  and 56% at 1  $\mu M$ . These inhibitory activities of compounds **10** and **12** are

**Table 1**  
SAR of alternate A and B moiety methanesulfonamide.



Entry	R <sub>1</sub> (R)	R <sub>2</sub>	R <sub>3</sub>	IC <sub>50</sub> (μM)			
				SKBR-3	MCF-7	MDA-MB-231	MDA-MB-468
6		CH <sub>2</sub> CH <sub>3</sub>	CH <sub>3</sub>	0.021 ± 0.004	0.0008 ± 0.0006	2.03 ± 1.12	0.014 ± 0.005
7		n-C <sub>3</sub> H <sub>7</sub>	CH <sub>3</sub>	0.026 ± 0.02	0.074 ± 0.042	0.42 ± 0.29	0.057 ± 0.021
8		PhCH <sub>2</sub>	CH <sub>3</sub>	0.06 ± 0.02	0.23 ± 0.09	14.2 ± 8.81	0.008 ± 0.004
9		Ph	CH <sub>3</sub>	0.06 ± 0.03	1.25 ± 0.59	0.87 ± 0.51	0.08 ± 0.02
10		CH <sub>2</sub> CH <sub>3</sub>	CH <sub>3</sub>	0.001 ± 0.0002	0.016 ± 0.01	0.125 ± 0.07	0.012 ± 0.005
11		n-C <sub>3</sub> H <sub>7</sub>	CH <sub>3</sub>	0.007 ± 0.003	0.14 ± 0.06	17.9 ± 12.2	0.008 ± 0.003
12		PhCH <sub>2</sub>	CH <sub>3</sub>	0.003 ± 0.001	0.12 ± 0.06	2.25 ± 1.51	0.012 ± 0.005
13		Ph	CH <sub>3</sub>	0.05 ± 0.02	2.88 ± 1.72	0.8 ± 0.44	0.05 ± 0.01
14		CH <sub>2</sub> CH <sub>3</sub>	CH <sub>3</sub>	0.056 ± 0.021	0.097 ± 0.06	0.1 ± 0.06	0.088 ± 0.059
15		n-C <sub>3</sub> H <sub>7</sub>	CH <sub>3</sub>	0.05 ± 0.02	1.27 ± 0.69	0.69 ± 0.41	0.07 ± 0.02
16		CH <sub>2</sub> CH <sub>3</sub>	CH <sub>3</sub>	0.012 ± 0.005	0.16 ± 0.1	0.5 ± 0.31	0.056 ± 0.022
17		n-C <sub>3</sub> H <sub>7</sub>	CH <sub>3</sub>	0.019 ± 0.01	0.06 ± 0.03	21.7 ± 14.5	0.072 ± 0.032
18		CH <sub>2</sub> CH <sub>3</sub>	H	0.019 ± 0.008	11.9 ± 6.64	0.16 ± 0.09	0.048 ± 0.017
19		n-C <sub>3</sub> H <sub>7</sub>	H	0.071 ± 0.021	0.28 ± 0.17	4.6 ± 2.4	0.025 ± 0.007

**Table 1** (continued)

Entry	R <sub>1</sub> (R)	R <sub>2</sub>	R <sub>3</sub>	IC <sub>50</sub> (μM)			
				SKBR-3	MCF-7	MDA-MB-231	MDA-MB-468
20		PhCH <sub>2</sub>	H	0.19 ± 0.09	1.29 ± 0.7	1.83 ± 1.01	0.09 ± 0.04
21		Ph	H	0.31 ± 0.11	16.7 ± 7.44	12.4 ± 5.9	0.72 ± 0.23
22		CH <sub>2</sub> CH <sub>3</sub>	H	0.026 ± 0.005	0.44 ± 0.12	19.8 ± 13.1	0.073 ± 0.029
23		n-C <sub>3</sub> H <sub>7</sub>	H	0.047 ± 0.023	0.05 ± 0.03	1.84 ± 0.73	0.019 ± 0.007
24		PhCH <sub>2</sub>	H	0.12 ± 0.04	1.69 ± 0.91	1.78 ± 0.86	0.11 ± 0.03
25		Ph	H	0.34 ± 0.17	39.6 ± 21.7	5.74 ± 3.07	0.18 ± 0.06
26		CH <sub>2</sub> CH <sub>3</sub>	H	0.108 ± 0.048	2.48 ± 1.55	0.53 ± 0.29	0.061 ± 0.019
27		n-C <sub>3</sub> H <sub>7</sub>	H	0.096 ± 0.037	0.29 ± 0.16	5.66 ± 2.02	0.45 ± 0.14
28		CH <sub>2</sub> CH <sub>3</sub>	H	0.085 ± 0.031	2.51 ± 1.5	2.31 ± 1.29	0.042 ± 0.014
29		n-C <sub>3</sub> H <sub>7</sub>	H	0.074 ± 0.025	0.36 ± 0.21	20.6 ± 11.4	0.24 ± 0.09
30				0.38 ± 0.13	1.17 ± 0.49	19.0 ± 6.25	0.25 ± 0.08
31				1.24 ± 0.32	1.81 ± 0.7	31.7 ± 8.65	0.99 ± 0.25
32				2.44 ± 1.53	186.0 ± 99.3	48.1 ± 23.6	1.01 ± 0.44
33				0.11 ± 0.02	0.31 ± 0.17	3.65 ± 1.87	0.29 ± 0.04

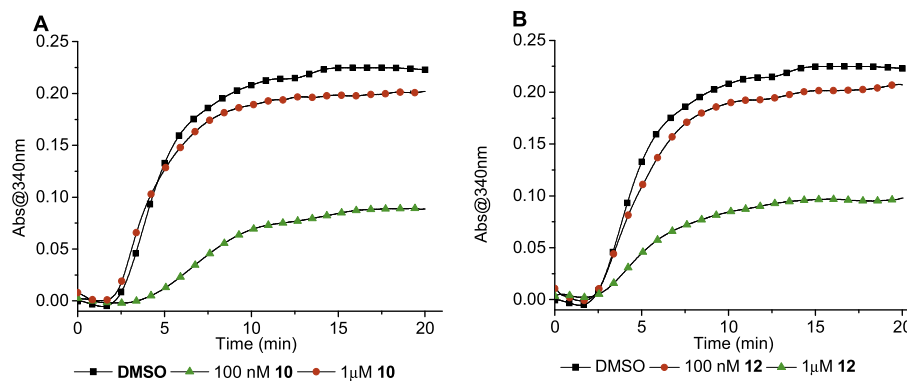
comparable to that of N-methylmethanesulfonamide **5** from the previous study [22] (see Fig. 3).

### 2.3. Tubulin docking studies

In the previous study, lead compound **1** was found to be a colchicine site binder on tubulin [23]. We anticipate the new derivatives **10** and **12** should also target the colchicine-binding site in

tubulin, due to their structural similarity to compound **1**. To further elucidate the interaction between tubulin and compounds **10** and **12**, we performed a docking study of these two compounds into the colchicine-binding site in tubulin. The results are shown in Fig. 4. Compound **10** may form three major interactions with tubulin. First, 2-methoxyl group in 2,5-dimethoxybenzyl moiety may form a hydrogen bond with the side chain of Ser 178. There may be some polar contact between the sulfonamide group and side chains such





**Fig. 2.** Tubulin polymerization in the presence of different concentrations of compounds **10** and **12**.

as Glu 183 and Asn 101. Additionally, the carbonyl group of benzoyl amide moiety may form a hydrogen bond with the main chain of Ala 250. The docking investigation showed that compound **10** has strong binding affinity to the colchicine-binding site of tubulin. Compared to compound **10**, compound **12** showed a different binding mode in the colchicine-binding site with sulfonamide moiety and benzoyl amide moiety switched. The benzyl group in the sulfonamide moiety can extend into a small hydrophobic pocket defined by Tyr 202, Ile 378, Leu 242, and Leu 255. A hydrogen bond may be formed between the carbonyl group of benzoyl amide moiety and the side chain of Asn 258. The 2,5-dimethoxybenzyl moiety in compound **12** slightly shifted away from the side chain of Ser 178, and the possible hydrogen bond between them was forfeited. However, a hydrophobic interaction may form between 2,5-dimethoxybenzyl moiety and Lys 352. The estimated free energies for compound **10** and **12** for binding at the colchicine site were determined to be  $-7.57$  and  $-7.86$  kcal/mol, respectively. Their similar binding energy in the colchicine-binding site may account for their similar inhibition to tubulin polymerization.

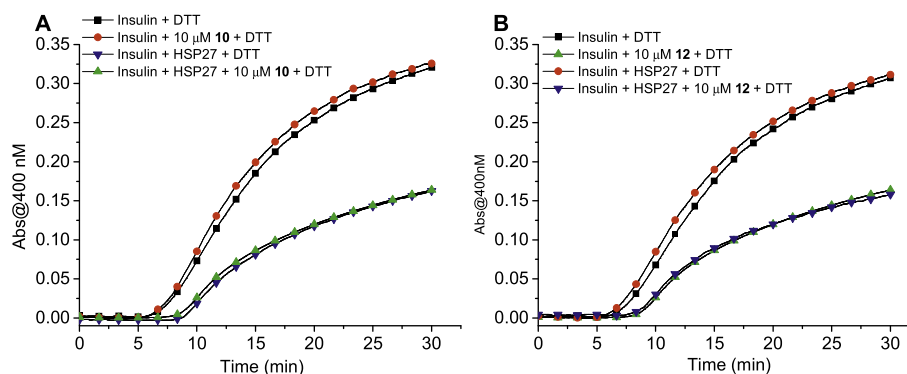
#### 2.4. Hsp27 targeting effect investigation

Previously, lead compound **5** inhibited both Hsp27 and tubulin [22]. The newly synthesized derivatives **10** and **12** inhibited tubulin and showed potent anti-proliferative activity. Whether they also target Hsp27 is still unknown. In order to further elucidate their anti-cancer mechanism, the capabilities of both compounds to modulate the *in vitro* chaperone function of Hsp27 were evaluated. By monitoring dithiothreitol (DTT)-induced insulin aggregation in

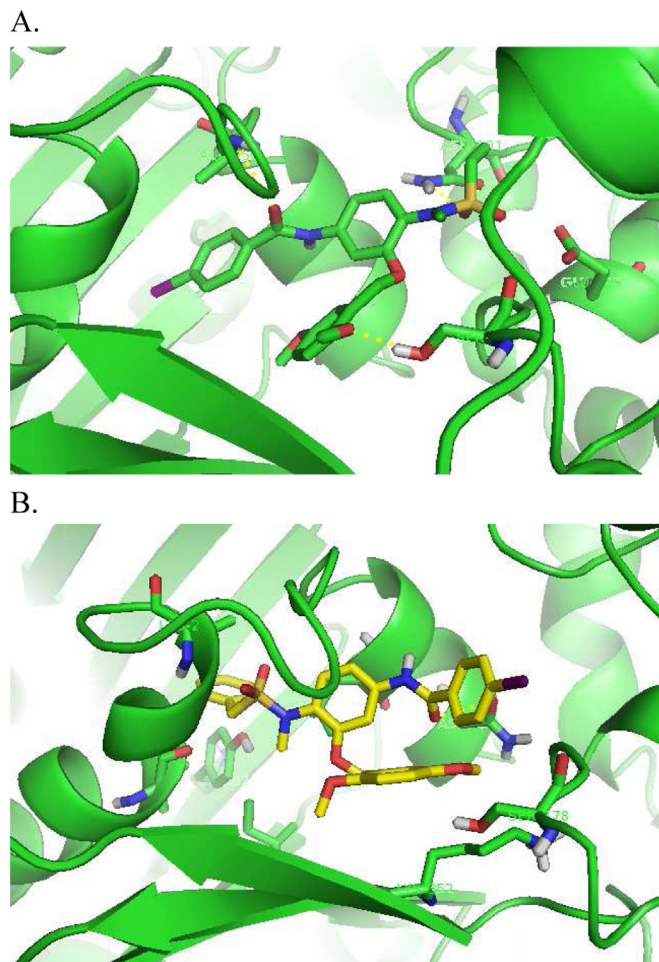
the presence of Hsp27, with or without the compounds, their Hsp27 inhibition can be examined. In this *in vitro* chaperone activity assays, Hsp27 exhibited potent inhibition of DTT-induced insulin aggregation. Previous study showed that the corresponding N-methylmethanesulfonamide **5** at  $10 \mu\text{M}$  inhibited Hsp27 functions by 27% [22]. However, both compounds **10** and **12** did not show inhibitory activity against Hsp27 chaperone activity at  $10 \mu\text{M}$ , suggesting Hsp27 targeting effect decreased in the new compounds. Substitution of the methanesulfonamide at the C moiety of compound **5** with ethanesulfonamide or benzyisulfonamide is detrimental for its Hsp27 targeting effect. However, this modification did not affect tubulin targeting effects. The results suggest that smaller sulfonamide moiety is preferred for Hsp27 inhibition.

### 3. Conclusion

We synthesized various sulfonamide derivatives and acetamide derivatives based on the previously reported compounds **2–5** [22]. The 2,5-dimethoxybenzyl group, which had been demonstrated to be important for the anti-proliferative activity of these compounds, was maintained for all the new compounds. The methanesulfonamide group at the C moiety was changed to an acetamide group or a diversity of alkyl/aryl sulfonamide groups. The SAR study revealed that most ethyl-, propyl-, phenyl-, benzylsulfonamides showed weaker cell growth inhibition, compared to the corresponding methanesulfonamides. Only N-methyl-ethanesulfonamide **10** and N-methylbenzylsulfonamide **12** maintained similar potency. Further mechanism investigation indicated that compounds **10** and **12** are potent inhibitors of tubulin polymerization. Their tubulin inhibitory activities are comparable to the



**Fig. 3.** Hsp27 chaperone activity for the protection of DTT induced insulin aggregation in the presence of different concentrations of compounds **10** and **12**. The kinetic of the DTT reduction-induced insulin aggregation was monitored in the absence of Hsp27 or in the presence of Hsp27, with or without compounds.



**Fig. 4.** The molecular docking simulation of the interaction of tubulin with compounds **10** and **12**. Proposed binding mode of compound **10** (A) and **12** (B) at the colchicine binding site. The tubulin polypeptide backbones are shown as ribbons. Only important active site amino acids around compounds are shown for clarity.

corresponding lead compound N-methylmethanesulfonamide **5**. However, both compounds did not show Hsp27 inhibition. The substitution of methanesulfonamide with ethanesulfonamide or benzyloxysulfonamide significantly impaired the Hsp27 inhibitory effects. The molecular docking simulation suggested that compounds **10** and **12** may adopt different binding modes to be accommodated in the colchicine binding site of tubulin. Future study will focus on discerning the structural fragments that are important for Hsp27 inhibition, and develop new anti-cancer agents with better potency to target both tubulin and Hsp27.

## 4. Experimental section

### 4.1. Chemistry

Chemicals were commercially available and used as received without further purification. Moisture sensitive reactions were carried out under a dry argon atmosphere in flame-dried glassware. Thin-layer chromatography was performed on silica gel TLC plates with fluorescence indicator 254 nm (Fluka). Flash column chromatography was performed using silica gel 60Å (BDH, 40–63 μM). Mass spectra were obtained on the ABI QStar Electrospray mass spectrometer at Cleveland State University MS facility Center. All the NMR spectra were recorded on a Bruker 400 MHz (<sup>13</sup>C NMR at

100 MHz) using DMSO-*d*<sub>6</sub> as solvent. Chemical shifts (δ) for <sup>1</sup>H NMR spectra were reported in parts per million to residual solvent protons. The IR spectra were obtained on a Bruker ALPHA FT-IR spectrometer with ATR module.

All the compounds were prepared according to the previously published procedures [22].

#### 4.1.1. *N*-[3-(2,5-dimethoxy-benzyloxy)-4-(ethanesulfonyl-methyl-amino)-phenyl]-4-methoxybenzamide (**6**)

<sup>1</sup>H NMR δ 10.200 (1H, s), 7.972 (2H, d, *J* = 8.8 Hz), 7.732 (1H, d, *J* = 2.4 Hz), 7.415 (1H, dd, *J* = 2.4, 8.4 Hz), 7.250 (1H, d, *J* = 8.8 Hz), 7.156 (1H, d, *J* = 2.8 Hz), 7.081 (2H, d, *J* = 9.2 Hz), 7.001 (1H, d, *J* = 9.2 Hz), 6.905 (1H, dd, *J* = 2.8, 8.8 Hz), 5.083 (2H, s), 3.848 (3H, s), 3.785 (3H, s), 3.717 (3H, s), 3.136 (3H, s), 3.056 (2H, q, *J* = 7.6 Hz), 1.114 (3H, t, *J* = 7.6 Hz); <sup>13</sup>C NMR δ 165.43, 162.52, 155.62, 153.57, 151.37, 140.94, 131.79, 130.10, 127.20, 125.53, 124.70, 115.45, 114.39, 114.12, 112.68, 112.28, 105.58, 65.45, 56.27, 55.93, 55.88, 46.09, 38.36, 8.34; ESI-MS calculated for C<sub>26</sub>H<sub>31</sub>N<sub>2</sub>O<sub>7</sub>S [M+H]<sup>+</sup> 515.19, found: 514.98.

#### 4.1.2. *N*-[3-(2,5-dimethoxy-benzyloxy)-4-(propanesulfonyl-methyl-amino)-phenyl]-4-methoxybenzamide (**7**)

<sup>1</sup>H NMR δ 10.200 (1H, s), 7.972 (2H, d, *J* = 9.2 Hz), 7.732 (1H, d, *J* = 2 Hz), 7.416 (1H, dd, *J* = 2, 8.4 Hz), 7.246 (1H, d, *J* = 8.4 Hz), 7.148 (1H, d, *J* = 3.2 Hz), 7.081 (2H, d, *J* = 9.2 Hz), 7.006 (1H, d, *J* = 9.2 Hz), 6.908 (1H, dd, *J* = 3.2, 9.2 Hz), 5.080 (2H, s), 3.847 (3H, s), 3.787 (3H, s), 3.715 (3H, s), 3.134 (3H, s), 2.984 (2H, m), 1.624 (2H, m), 0.812 (3H, t, *J* = 7.2 Hz); <sup>13</sup>C NMR δ 165.43, 162.52, 155.61, 153.59, 151.37, 140.91, 131.79, 130.10, 127.20, 125.55, 124.77, 115.51, 114.31, 114.12, 112.69, 112.36, 105.63, 65.45, 56.32, 55.93, 55.87, 53.09, 38.15, 17.21, 13.13; ESI-MS calculated for C<sub>27</sub>H<sub>33</sub>N<sub>2</sub>O<sub>7</sub>S [M+H]<sup>+</sup> 529.20, found: 529.01.

#### 4.1.3. *N*-[3-(2,5-dimethoxy-benzyloxy)-4-(benzylsulfonyl-methyl-amino)-phenyl]-4-methoxybenzamide (**8**)

<sup>1</sup>H NMR δ 10.191 (1H, s), 7.971 (2H, d, *J* = 8.8 Hz), 7.722 (1H, d, *J* = 2.4 Hz), 7.354 (6H, m), 7.204 (1H, d, *J* = 2.8 Hz), 7.081 (2H, d, *J* = 9.2 Hz), 6.991 (1H, d, *J* = 8.8 Hz), 6.961 (1H, d, *J* = 9.2 Hz), 6.867 (1H, dd, *J* = 2.8, 8.8 Hz), 5.115 (2H, s), 4.415 (2H, s), 3.848 (3H, s), 3.741 (3H, s), 3.680 (3H, s), 3.074 (3H, s); <sup>13</sup>C NMR δ 165.43, 162.53, 155.70, 153.64, 151.21, 140.89, 131.40, 131.34, 130.38, 130.10, 128.74, 128.53, 127.20, 125.61, 125.03, 115.08, 114.43, 114.12, 112.63, 112.28, 105.59, 65.45, 57.21, 56.29, 55.93, 55.84, 38.46; ESI-MS calculated for C<sub>31</sub>H<sub>33</sub>N<sub>2</sub>O<sub>7</sub>S [M+H]<sup>+</sup> 577.20, found 576.98.

#### 4.1.4. *N*-[3-(2,5-dimethoxy-benzyloxy)-4-(phenylsulfonyl-methyl-amino)-phenyl]-4-methoxybenzamide (**9**)

<sup>1</sup>H NMR δ 10.192 (1H, s), 7.958 (2H, d, *J* = 8.8 Hz), 7.624 (3H, m), 7.539 (1H, m), 7.441 (3H, m), 7.074 (3H, m), 6.946 (1H, d, *J* = 8.8 Hz), 6.834 (2H, m), 4.802 (2H, s), 3.845 (3H, s), 3.769 (3H, s), 3.714 (3H, s), 3.146 (3H, s); <sup>13</sup>C NMR δ 165.46, 162.52, 155.65, 153.54, 150.67, 141.07, 139.40, 133.01, 131.05, 130.09, 129.33, 127.45, 127.20, 125.61, 124.38, 114.31, 114.11, 113.67, 112.51, 112.01, 105.66, 65.00, 56.21, 55.92, 55.84, 38.24; ESI-MS calculated for C<sub>30</sub>H<sub>31</sub>N<sub>2</sub>O<sub>7</sub>S [M+H]<sup>+</sup> 563.19, found: 562.96.

#### 4.1.5. *N*-[3-(2,5-dimethoxy-benzyloxy)-4-(ethanesulfonyl-methyl-amino)-phenyl]-4-iodobenzamide (**10**)

IR ν = 1672 (C=O); <sup>1</sup>H NMR δ 10.398 (1H, s), 7.943 (2H, d, *J* = 8.4 Hz), 7.754 (2H, d, *J* = 8.4 Hz), 7.703 (1H, d, *J* = 2 Hz), 7.410 (1H, dd, *J* = 2, 8.8 Hz), 7.267 (1H, d, *J* = 8.8 Hz), 7.148 (1H, d, *J* = 3.2 Hz), 6.998 (1H, d, *J* = 9.2 Hz), 6.904 (1H, dd, *J* = 3.2, 8.8 Hz), 5.083 (2H, s), 3.781 (3H, s), 3.714 (3H, s), 3.135 (3H, s), 3.059 (2H, q, *J* = 7.2 Hz), 1.114 (3H, t, *J* = 7.2 Hz); <sup>13</sup>C NMR δ 165.63, 155.64, 153.49, 151.40, 140.32, 137.83, 134.48, 131.82, 130.03, 125.39, 125.20, 115.50,



114.46, 112.96, 112.34, 105.83, 99.80, 65.49, 56.27, 55.86, 46.15, 38.30, 8.26; ESI-MS calculated for  $C_{25}H_{28}IN_2O_6S$   $[M+H]^+$  611.07, found: 610.88.

4.1.6. *N*-[3-(2,5-dimethoxy-benzyloxy)-4-(propanesulfonyl-methyl-amino)-phenyl]-4-iodobenzamide (**11**)

$^1H$  NMR  $\delta$  10.399 (1H, s), 7.944 (2H, d,  $J = 8.8$  Hz), 7.756 (2H, d,  $J = 8.8$  Hz), 7.704 (1H, d,  $J = 2.4$  Hz), 7.412 (1H, dd,  $J = 2, 8.4$  Hz), 7.264 (1H, d,  $J = 8.8$  Hz), 7.140 (1H, d, 3.2 Hz), 7.004 (1H, d,  $J = 8.8$  Hz), 6.907 (1H, dd,  $J = 2.8, 8.8$  Hz), 5.080 (2H, s), 3.784 (3H, s), 3.712 (3H, s), 3.133 (3H, s), 2.985 (2H, m), 1.623 (2H, m), 0.812 (3H, t,  $J = 7.6$  Hz);  $^{13}C$  NMR  $\delta$  165.35, 155.64, 153.59, 151.36, 140.47, 137.78, 134.54, 131.86, 130.07, 125.50, 125.18, 115.50, 114.33, 112.84, 112.38, 105.78, 99.94, 65.49, 56.33, 55.88, 53.11, 38.14, 17.21, 13.13; ESI-MS calculated for  $C_{26}H_{30}IN_2O_6S$   $[M+H]^+$  625.09, found 624.87.

4.1.7. *N*-[3-(2,5-dimethoxy-benzyloxy)-4-(benzylsulfonyl-methyl-amino)-phenyl]-4-iodobenzamide (**12**)

IR  $\nu = 1645$  (C=O);  $^1H$  NMR  $\delta$  10.391 (1H, s), 7.944 (2H, d,  $J = 8.4$  Hz), 7.754 (2H, d,  $J = 8.4$  Hz), 7.692 (1H, d,  $J = 2.4$  Hz), 7.353 (6H, m), 7.196 (1H, d,  $J = 3.2$  Hz), 7.006 (1H, d,  $J = 8.4$  Hz), 6.959 (1H, d,  $J = 9.2$  Hz), 6.866 (1H, dd,  $J = 2.8, 8.8$  Hz), 5.114 (2H, s), 4.418 (2H, s), 3.738 (3H, s), 3.677 (3H, s), 3.072 (3H, s);  $^{13}C$  NMR  $\delta$  165.35, 155.72, 153.64, 151.20, 140.45, 137.79, 134.54, 131.40, 130.36, 130.07, 128.74, 128.54, 125.56, 125.43, 115.05, 114.43, 112.77, 112.29, 105.73, 99.95, 65.48, 57.22, 56.30, 55.84, 38.45; ESI-MS calculated for  $C_{30}H_{30}IN_2O_6S$   $[M+H]^+$  673.09, found 672.84.

4.1.8. *N*-[3-(2,5-dimethoxy-benzyloxy)-4-(phenylsulfonyl-methyl-amino)-phenyl]-4-iodobenzamide (**13**)

$^1H$  NMR  $\delta$  10.390 (1H, s), 7.937 (2H, d,  $J = 8.4$  Hz), 7.741 (2H, d,  $J = 8.4$  Hz), 7.619 (3H, m), 7.541 (1H, m), 7.431 (3H, m), 7.094 (1H, d,  $J = 8.8$  Hz), 6.945 (1H, d,  $J = 8.8$  Hz), 6.833 (2H, m), 4.801 (2H, s), 3.765 (3H, s), 3.712 (3H, s), 3.146 (3H, s);  $^{13}C$  NMR  $\delta$  165.38, 155.67, 153.53, 150.66, 140.64, 139.36, 137.77, 134.54, 133.04, 131.15, 130.07, 129.35, 127.45, 125.56, 124.77, 114.31, 113.69, 112.64, 112.03, 105.79, 99.95, 65.05, 56.22, 55.85, 38.22; ESI-MS calculated for  $C_{29}H_{28}IN_2O_6S$   $[M+H]^+$  659.07, found: 658.86.

4.1.9. *N*-[3-(2,5-dimethoxy-benzyloxy)-4-(ethanesulfonyl-methyl-amino)-phenyl]-3,4-dimethoxybenzamide (**14**)

$^1H$  NMR  $\delta$  10.191 (1H, s), 7.736 (1H, d,  $J = 2.4$  Hz), 7.643 (1H, dd,  $J = 2.4, 8.4$  Hz), 7.534 (1H, d,  $J = 2$  Hz), 7.398 (1H, dd,  $J = 2, 8.4$  Hz), 7.259 (1H, d,  $J = 8.4$  Hz), 7.159 (1H, d,  $J = 3.2$  Hz), 7.104 (1H, d,  $J = 8.4$  Hz), 7.002 (1H, d,  $J = 9.2$  Hz), 6.907 (1H, dd,  $J = 2.8, 8.8$  Hz), 5.086 (2H, s), 3.852 (3H, s), 3.847 (3H, s), 3.785 (3H, s), 3.719 (3H, s), 3.138 (3H, s), 3.059 (2H, q,  $J = 7.6$  Hz), 1.115 (3H, t,  $J = 7.6$  Hz);  $^{13}C$  NMR  $\delta$  165.47, 155.63, 153.58, 152.32, 151.38, 148.87, 140.88, 131.79, 127.24, 125.53, 124.77, 121.54, 115.47, 114.41, 112.80, 112.29, 111.69, 111.42, 105.69, 65.47, 56.19, 56.16, 55.88, 46.10, 38.36, 8.34; ESI-MS calculated for  $C_{27}H_{33}N_2O_8S$   $[M+H]^+$  545.20, found: 544.98.

4.1.10. *N*-[3-(2,5-dimethoxy-benzyloxy)-4-(propanesulfonyl-methyl-amino)-phenyl]-3,4-dimethoxybenzamide (**15**)

$^1H$  NMR  $\delta$  10.192 (1H, s), 7.735 (1H, d,  $J = 2$  Hz), 7.644 (1H, dd,  $J = 2, 8.4$  Hz), 7.534 (1H, d,  $J = 2$  Hz), 7.398 (1H, dd,  $J = 2, 8.4$  Hz), 7.256 (1H, d,  $J = 8.8$  Hz), 7.150 (1H, d,  $J = 2.8$  Hz), 7.104 (1H, d,  $J = 8.4$  Hz), 7.007 (1H, d, 8.8 Hz), 6.910 (1H, dd,  $J = 3.2, 8.8$  Hz), 5.083 (1H, s), 3.582 (3H, s), 3.847 (3H, s), 3.787 (3H, s), 3.717 (3H, s), 3.136 (3H, s), 2.979 (2H, m), 1.623 (2H, m), 0.811 (3H, t,  $J = 7.2$  Hz);  $^{13}C$  NMR  $\delta$  165.46, 155.62, 153.60, 152.31, 151.37, 148.87, 140.85, 131.77, 127.23, 125.54, 124.88, 121.53, 115.53, 114.34, 112.80, 112.39, 111.69, 111.41, 105.73, 65.47, 56.33, 56.20, 56.10, 55.88, 53.10, 38.15, 17.21, 13.13; ESI-MS calculated for  $C_{28}H_{35}N_2O_8S$   $[M+H]^+$  559.20, found: 559.02.

4.1.11. *N*-[3-(2,5-dimethoxy-benzyloxy)-4-(ethanesulfonyl-methyl-amino)-phenyl]-4-bromobenzamide (**16**)

$^1H$  NMR  $\delta$  10.422 (1H, s), 7.921 (2H, d,  $J = 8.8$  Hz), 7.773 (2H, d,  $J = 8.8$  Hz), 7.706 (1H, d,  $J = 2$  Hz), 7.412 (1H, dd,  $J = 2.4, 8.8$  Hz), 7.272 (1H, d,  $J = 8.8$  Hz), 7.149 (1H, d,  $J = 3.2$  Hz), 7.000 (1H, d,  $J = 9.2$  Hz), 6.905 (1H, dd,  $J = 3.2, 9.2$  Hz), 5.086 (2H, s), 3.782 (3H, s), 3.714 (3H, s), 3.137 (3H, s), 3.062 (2H, q,  $J = 7.6$  Hz), 1.116 (3H, t,  $J = 7.6$  Hz);  $^{13}C$  NMR  $\delta$  165.09, 155.65, 153.58, 151.37, 140.49, 134.24, 131.92, 130.27, 126.00, 125.49, 125.14, 115.43, 114.40, 112.83, 112.29, 105.74, 65.49, 56.28, 55.88, 46.11, 38.34, 8.34; ESI-MS calculated for  $C_{25}H_{28}BrN_2O_6S$   $[M+H]^+$  563.09, found: 562.90.

4.1.12. *N*-[3-(2,5-dimethoxy-benzyloxy)-4-(propanesulfonyl-methyl-amino)-phenyl]-4-bromobenzamide (**17**)

$^1H$  NMR  $\delta$  10.421 (1H, s), 7.923 (2H, d,  $J = 8.8$  Hz), 7.773 (2H, d,  $J = 8.8$  Hz), 7.708 (1H, d,  $J = 1.6$  Hz), 7.414 (1H, dd,  $J = 2, 8.8$  Hz), 7.270 (1H, d,  $J = 8.4$  Hz), 7.142 (1H, d,  $J = 3.2$  Hz), 7.005 (1H, d,  $J = 8.8$  Hz), 6.908 (1H, dd,  $J = 2.8, 8.8$  Hz), 5.084 (2H, s), 3.785 (3H, s), 3.714 (3H, s), 3.136 (3H, s), 2.987 (2H, m), 1.626 (2H, m), 0.814 (3H, t,  $J = 7.2$  Hz);  $^{13}C$  NMR  $\delta$  165.09, 155.64, 153.59, 151.36, 140.46, 134.24, 131.92, 130.27, 126.00, 125.50, 125.21, 115.50, 114.33, 112.85, 112.38, 105.79, 65.49, 56.33, 55.87, 53.11, 38.13, 17.21, 13.13; ESI-MS calculated for  $C_{26}H_{30}BrN_2O_6S$   $[M+H]^+$  577.10, found 576.92.

4.1.13. *N*-[3-(2,5-dimethoxy-benzyloxy)-4-(ethanesulfonyl-amino)-phenyl]-4-methoxybenzamide (**18**)

$^1H$  NMR  $\delta$  10.136 (1H, s), 8.916 (1H, s), 7.959 (2H, d,  $J = 8.8$  Hz), 7.680 (1H, d,  $J = 2$  Hz), 7.384 (1H, dd,  $J = 2, 8.4$  Hz), 7.256 (1H, d,  $J = 3.2$  Hz), 7.215 (1H, d,  $J = 8.4$  Hz), 7.069 (2H, d,  $J = 8.8$  Hz), 6.984 (1H, d,  $J = 8.8$  Hz), 6.887 (1H, dd,  $J = 3.2, 8.8$  Hz), 5.061 (2H, s), 3.842 (3H, s), 3.792 (3H, s), 3.724 (3H, s), 2.956 (2H, q,  $J = 7.2$  Hz), 1.128 (3H, t,  $J = 7.2$  Hz);  $^{13}C$  NMR  $\delta$  165.31, 162.44, 153.65, 152.90, 151.16, 139.10, 130.04, 128.08, 127.30, 125.72, 121.26, 115.58, 114.09, 113.96, 112.84, 112.09, 105.63, 65.49, 56.29, 55.93, 55.91, 46.75, 8.46; ESI-MS calculated for  $C_{25}H_{29}N_2O_7S$   $[M+H]^+$ : 501.17, found: 500.96.

4.1.14. *N*-[3-(2,5-dimethoxy-benzyloxy)-4-(propanesulfonyl-amino)-phenyl]-4-methoxybenzamide (**19**)

$^1H$  NMR  $\delta$  10.137 (1H, s), 8.941 (1H, s), 7.960 (2H, d,  $J = 9.2$  Hz), 7.678 (1H, d,  $J = 2$  Hz), 7.390 (1H, dd,  $J = 2, 8.8$  Hz), 7.270 (1H, d,  $J = 3.2$  Hz), 7.213 (1H, d,  $J = 8.8$  Hz), 7.070 (2H, d,  $J = 9.2$  Hz), 6.986 (1H, d,  $J = 9.2$  Hz), 6.886 (1H, dd,  $J = 3.2, 9.2$  Hz), 5.068 (2H, s), 3.843 (3H, s), 3.797 (3H, s), 3.728 (3H, s), 2.918 (2H, m), 1.654 (2H, m), 0.790 (3H, t,  $J = 7.2$  Hz);  $^{13}C$  NMR  $\delta$  165.31, 162.44, 153.70, 152.82, 151.07, 139.04, 130.04, 127.96, 127.30, 125.80, 121.34, 115.47, 114.09, 113.82, 112.88, 112.17, 105.70, 65.42, 56.37, 55.95, 55.92, 54.12, 17.33, 13.01; ESI-MS calculated for  $C_{26}H_{31}N_2O_7S$   $[M+H]^+$  515.19, found: 514.99.

4.1.15. *N*-[3-(2,5-dimethoxy-benzyloxy)-4-(benzylsulfonyl-amino)-phenyl]-4-methoxybenzamide (**20**)

$^1H$  NMR  $\delta$  10.149 (1H, s), 8.993 (1H, s), 7.970 (2H, d,  $J = 8.8$  Hz), 7.709 (1H, d,  $J = 2$  Hz), 7.384 (1H, dd,  $J = 2, 8.4$  Hz), 7.304 (6H, m), 7.150 (1H, d,  $J = 8.8$  Hz), 7.075 (2H, d,  $J = 8.8$  Hz), 6.949 (1H, d,  $J = 9.2$  Hz), 6.865 (1H, dd,  $J = 2.8, 8.8$  Hz), 5.103 (2H, s), 4.309 (2H, s), 3.846 (3H, s), 3.740 (3H, s), 3.697 (3H, s);  $^{13}C$  NMR  $\delta$  165.32, 162.45, 153.67, 152.79, 151.16, 139.01, 131.29, 130.33, 130.05, 128.69, 128.46, 127.62, 127.31, 125.73, 121.38, 115.54, 114.10, 112.83, 112.14, 105.69, 65.55, 58.68, 56.29, 55.92; ESI-MS calculated for  $C_{30}H_{31}N_2O_7S$   $[M+H]^+$  563.19, found: 562.97.

4.1.16. *N*-[3-(2,5-dimethoxy-benzyloxy)-4-(phenylsulfonyl-amino)-phenyl]-4-methoxybenzamide (**21**)

$^1H$  NMR  $\delta$  10.082 (1H, s), 9.551 (1H, s), 7.925 (2H, d,  $J = 8.8$  Hz), 7.611 (2H, m), 7.473 (2H, m), 7.345 (3H, m), 7.155 (1H, d,  $J = 8.4$  Hz),

7.099 (1H, d,  $J = 3.2$  Hz), 7.051 (2H, d,  $J = 9.2$  Hz), 6.943 (1H, d,  $J = 8.8$  Hz), 6.853 (1H, dd,  $J = 3.2, 8.8$  Hz), 4.692 (2H, s), 3.833 (3H, s), 3.775 (3H, s), 3.758 (3H, s);  $^{13}\text{C}$  NMR  $\delta$  165.30, 162.43, 153.72, 152.75, 150.48, 140.95, 139.15, 132.73, 130.02, 129.08, 127.41, 127.27, 127.00, 125.96, 120.82, 114.83, 114.07, 113.12, 112.78, 111.76, 105.65, 65.14, 56.25, 56.00, 55.89; ESI-MS calculated for  $\text{C}_{29}\text{H}_{29}\text{N}_2\text{O}_7\text{S}$   $[\text{M}+\text{H}]^+$  549.17, found: 548.95.

4.1.17. *N*-[3-(2,5-dimethoxy-benzyloxy)-4-(ethanesulfonyl-amino)-phenyl]-4-iodobenzamide (**22**)

$^1\text{H}$  NMR  $\delta$  10.336 (1H, s), 8.944 (1H, s), 7.931 (2H, d,  $J = 8.8$  Hz), 7.744 (2H, d,  $J = 8.4$  Hz), 7.657 (1H, d,  $J = 2.4$  Hz), 7.383 (1H, dd,  $J = 2, 8.4$  Hz), 7.245 (2H, m), 6.982 (1H, d,  $J = 8.8$  Hz), 6.886 (1H, dd,  $J = 3.2, 9.2$  Hz), 5.063 (2H, s), 3.789 (3H, s), 3.721 (3H, s), 2.962 (2H, q,  $J = 7.2$  Hz), 1.131 (3H, t,  $J = 7.2$  Hz);  $^{13}\text{C}$  NMR  $\delta$  165.22, 153.64, 152.85, 151.15, 138.62, 137.75, 134.63, 130.05, 128.02, 125.67, 121.69, 115.56, 113.96, 112.97, 112.10, 105.74, 99.81, 65.52, 56.30, 55.93, 46.78, 8.46; ESI-MS calculated for  $\text{C}_{24}\text{H}_{26}\text{IN}_2\text{O}_6\text{S}$   $[\text{M}+\text{H}]^+$ : 597.06, found: 596.85.

4.1.18. *N*-[3-(2,5-dimethoxy-benzyloxy)-4-(propanesulfonyl-amino)-phenyl]-4-iodobenzamide (**23**)

$^1\text{H}$  NMR  $\delta$  10.335 (1H, s), 8.972 (1H, s), 7.931 (2H, d,  $J = 8.8$  Hz), 7.745 (2H, d,  $J = 8.8$  Hz), 7.655 (1H, d,  $J = 2.4$  Hz), 7.389 (1H, dd,  $J = 2, 8.4$  Hz), 7.262 (1H, d,  $J = 3.2$  Hz), 7.231 (1H, d,  $J = 8.4$  Hz), 6.984 (1H, d,  $J = 9.2$  Hz), 6.885 (1H, dd,  $J = 3.2, 9.2$  Hz), 5.070 (2H, s), 3.793 (3H, s), 3.725 (3H, s), 2.924 (2H, m), 1.655 (2H, m), 0.792 (3H, t,  $J = 7.6$  Hz);  $^{13}\text{C}$  NMR  $\delta$  165.22, 153.69, 152.77, 151.06, 138.57, 137.75, 134.63, 130.05, 127.89, 125.74, 121.77, 115.45, 113.83, 113.00, 112.16, 105.80, 99.81, 65.46, 56.36, 55.95, 54.14, 17.33, 13.01; ESI-MS calculated for  $\text{C}_{25}\text{H}_{28}\text{IN}_2\text{O}_6\text{S}$   $[\text{M}+\text{H}]^+$  611.07, found 610.88.

4.1.19. *N*-[3-(2,5-dimethoxy-benzyloxy)-4-(benzylsulfonyl-amino)-phenyl]-4-iodobenzamide (**24**)

$^1\text{H}$  NMR  $\delta$  10.349 (1H, s), 9.018 (1H, s), 7.937 (2H, d,  $J = 8.4$  Hz), 7.755 (2H, d,  $J = 8.4$  Hz), 7.684 (1H, d,  $J = 2$  Hz), 7.382 (1H, dd,  $J = 2, 8.4$  Hz), 7.304 (6H, m), 7.164 (1H, d,  $J = 8.4$  Hz), 6.948 (1H, d,  $J = 9.2$  Hz), 6.864 (1H, dd,  $J = 2.8, 9.2$  Hz), 5.104 (2H, s), 4.317 (2H, s), 3.738 (3H, s), 3.694 (3H, s);  $^{13}\text{C}$  NMR  $\delta$  165.22, 153.67, 152.71, 151.16, 138.51, 137.76, 134.65, 131.29, 130.30, 130.05, 128.70, 128.47, 127.50, 125.68, 121.82, 115.53, 114.07, 112.96, 112.14, 105.81, 99.82, 65.59, 58.70, 56.30, 55.91; ESI-MS calculated for  $\text{C}_{29}\text{H}_{28}\text{IN}_2\text{O}_6\text{S}$   $[\text{M}+\text{H}]^+$  659.07, found: 658.84.

4.1.20. *N*-[3-(2,5-dimethoxy-benzyloxy)-4-(phenylsulfonyl-amino)-phenyl]-4-iodobenzamide (**25**)

$^1\text{H}$  NMR  $\delta$  10.278 (1H, s), 9.579 (1H, s), 7.911 (2H, d,  $J = 8.4$  Hz), 7.709 (2H, d,  $J = 8.8$  Hz), 7.614 (2H, m), 7.464 (2H, m), 7.339 (3H, m), 7.173 (1H, d,  $J = 8.8$  Hz), 7.086 (1H, d,  $J = 3.2$  Hz), 6.941 (1H, d,  $J = 9.2$  Hz), 6.852 (1H, dd,  $J = 3.2, 9.2$  Hz), 4.696 (2H, s), 3.770 (3H, s), 3.753 (3H, s);  $^{13}\text{C}$  NMR  $\delta$  165.20, 153.71, 152.70, 150.48, 140.92, 138.68, 137.72, 134.61, 132.76, 130.02, 129.11, 127.35, 127.00, 125.90, 121.23, 114.82, 113.14, 112.89, 111.78, 105.75, 99.80, 65.17, 56.27, 56.00; ESI-MS calculated for  $\text{C}_{28}\text{H}_{26}\text{IN}_2\text{O}_6\text{S}$   $[\text{M}+\text{H}]^+$  645.06, found 644.83.

4.1.21. *N*-[3-(2,5-dimethoxy-benzyloxy)-4-(ethanesulfonyl-amino)-phenyl]-3,4-dimethoxybenzamide (**26**)

$^1\text{H}$  NMR  $\delta$  10.129 (1H, s), 8.922 (1H, s), 7.682 (1H, d,  $J = 2.4$  Hz), 7.628 (1H, dd,  $J = 2.4, 8.4$  Hz), 7.528 (1H, d,  $J = 2.4$  Hz), 7.366 (1H, dd,  $J = 2, 8.4$  Hz), 7.260 (1H, d,  $J = 2.8$  Hz), 7.227 (1H, d,  $J = 8.8$  Hz), 7.092 (1H, d,  $J = 8.4$  Hz), 6.985 (1H, d,  $J = 9.2$  Hz), 6.889 (1H, dd,  $J = 3.2, 8.8$  Hz), 5.065 (2H, s), 3.847 (3H, s), 3.842 (3H, s), 3.792 (3H, s), 3.727 (3H, s), 2.962 (2H, q,  $J = 7.2$  Hz), 1.131 (3H, t,  $J = 7.2$  Hz);  $^{13}\text{C}$  NMR  $\delta$  165.36, 153.64, 152.91, 152.22, 151.16, 148.83, 139.03, 128.08,

127.33, 125.70, 121.48, 121.32, 115.61, 113.95, 112.99, 112.08, 111.63, 111.39, 105.73, 65.49, 56.28, 56.17, 56.15, 55.92, 46.76, 8.46; ESI-MS calculated for  $\text{C}_{26}\text{H}_{31}\text{N}_2\text{O}_8\text{S}$   $[\text{M}+\text{H}]^+$ : 531.18, found: 530.97.

4.1.22. *N*-[3-(2,5-dimethoxy-benzyloxy)-4-(propanesulfonyl-amino)-phenyl]-3,4-dimethoxybenzamide (**27**)

$^1\text{H}$  NMR  $\delta$  10.128 (1H, s), 8.950 (1H, s), 7.678 (1H, d,  $J = 2.4$  Hz), 7.627 (1H, dd,  $J = 2, 8.4$  Hz), 7.526 (1H, d,  $J = 2$  Hz), 7.370 (1H, dd,  $J = 2, 8.8$  Hz), 7.273 (1H, d,  $J = 2.8$  Hz), 7.223 (1H, d,  $J = 8.8$  Hz), 7.092 (1H, d,  $J = 8.8$  Hz), 6.987 (1H, d,  $J = 9.2$  Hz), 6.888 (1H, d,  $J = 2.8, 9.2$  Hz), 5.071 (2H, s), 3.847 (3H, s), 3.842 (3H, s), 3.797 (3H, s), 3.730 (3H, s), 2.922 (2H, m), 1.654 (2H, m), 0.790 (3H, t,  $J = 7.2$  Hz);  $^{13}\text{C}$  NMR  $\delta$  165.35, 153.70, 152.82, 152.22, 151.07, 148.83, 138.97, 127.94, 127.33, 125.78, 121.47, 121.41, 115.50, 113.82, 113.02, 112.15, 111.63, 111.40, 105.81, 65.44, 56.35, 56.17, 56.15, 55.94, 54.13, 17.34, 13.00; ESI-MS calculated for  $\text{C}_{27}\text{H}_{33}\text{N}_2\text{O}_8\text{S}$   $[\text{M}+\text{H}]^+$  545.20, found: 544.98.

4.1.23. *N*-[3-(2,5-dimethoxy-benzyloxy)-4-(ethanesulfonyl-amino)-phenyl]-4-bromobenzamide (**28**)

$^1\text{H}$  NMR  $\delta$  10.358 (1H, s), 8.947 (1H, s), 7.909 (2H, d,  $J = 8.8$  Hz), 7.760 (2H, d,  $J = 8.8$  Hz), 7.658 (1H, d,  $J = 2$  Hz), 7.384 (1H, dd,  $J = 2, 8.4$  Hz), 7.248 (2H, m), 6.983 (1H, d,  $J = 8.8$  Hz), 6.886 (1H, dd,  $J = 2.8, 8.8$  Hz), 5.065 (2H, s), 3.789 (3H, s), 3.721 (3H, s), 2.963 (2H, q,  $J = 7.2$  Hz), 1.131 (3H, t,  $J = 7.2$ );  $^{13}\text{C}$  NMR  $\delta$  164.95, 153.64, 152.85, 151.15, 138.61, 134.33, 131.88, 130.24, 128.05, 125.89, 125.66, 121.71, 115.55, 113.94, 112.97, 112.08, 105.74, 65.51, 56.28, 55.92, 46.78, 8.47; ESI-MS calculated for  $\text{C}_{24}\text{H}_{26}\text{BrN}_2\text{O}_6\text{S}$   $[\text{M}+\text{H}]^+$ : 549.07, found: 548.86.

4.1.24. *N*-[3-(2,5-dimethoxy-benzyloxy)-4-(propanesulfonyl-amino)-phenyl]-4-bromobenzamide (**29**)

$^1\text{H}$  NMR  $\delta$  10.356 (1H, s), 8.973 (1H, s), 7.909 (2H, d,  $J = 8.8$  Hz), 7.760 (2H, d,  $J = 8.8$  Hz), 7.652 (1H, s), 7.388 (1H, d,  $J = 8.4$  Hz), 7.262 (1H, d,  $J = 3.2$  Hz), 7.234 (1H, d,  $J = 8.4$  Hz), 6.985 (1H, d,  $J = 8.8$  Hz), 6.885 (1H, dd,  $J = 2.8, 8.8$  Hz), 5.071 (2H, s), 3.794 (3H, s), 3.725 (3H, s), 2.924 (2H, m), 1.655 (2H, m), 0.793 (3H, t,  $J = 7.6$  Hz);  $^{13}\text{C}$  NMR  $\delta$  164.95, 153.69, 152.78, 151.04, 138.58, 134.33, 131.88, 130.24, 127.95, 125.90, 125.73, 121.78, 115.45, 113.77, 112.99, 112.11, 105.78, 65.44, 56.33, 55.93, 54.13, 17.35, 13.01; ESI-MS calculated for  $\text{C}_{25}\text{H}_{28}\text{BrN}_2\text{O}_6\text{S}$   $[\text{M}+\text{H}]^+$  563.09, found: 562.91.

4.1.25. *N*-[3-(2,5-dimethoxy-benzyloxy)-4-(acetamido-phenyl)-4-methoxybenzamide (**30**)

$^1\text{H}$  NMR  $\delta$  10.063 (1H, s), 9.171 (1H, s), 7.950 (2H, d,  $J = 8.8$  Hz), 7.677 (1H, d,  $J = 8.4$  Hz), 7.623 (1H, s), 7.355 (1H, d,  $J = 8.8$  Hz), 7.141 (1H, d,  $J = 2.8$  Hz), 7.058 (2H, d,  $J = 8.8$  Hz), 6.981 (1H, d,  $J = 9.2$  Hz), 6.859 (1H, dd,  $J = 2.8, 8.8$  Hz), 5.097 (2H, s), 3.838 (3H, s), 3.805 (3H, s), 3.718 (3H, s), 2.070 (3H, s);  $^{13}\text{C}$  NMR  $\delta$  168.63, 165.15, 162.36, 153.68, 150.90, 149.91, 136.92, 129.98, 127.40, 126.16, 123.99, 123.61, 114.50, 114.05, 113.66, 112.74, 112.24, 105.88, 65.44, 56.39, 55.90, 55.82, 24.06; ESI-MS calculated for  $\text{C}_{25}\text{H}_{27}\text{N}_2\text{O}_6$   $[\text{M}+\text{H}]^+$  451.19, found: 450.99.

4.1.26. *N*-[3-(2,5-dimethoxy-benzyloxy)-4-(acetamido-phenyl)-3,4-dimethoxybenzamide (**31**)

$^1\text{H}$  NMR  $\delta$  10.056 (1H, s), 9.172 (1H, s), 7.690 (1H, d,  $J = 8.8$  Hz), 7.606 (2H, m), 7.522 (1H, d,  $J = 1.6$  Hz), 7.327 (1H, d,  $J = 9.2$  Hz), 7.142 (1H, d,  $J = 3.2$  Hz), 7.080 (1H, d,  $J = 8.4$  Hz), 6.982 (1H, d,  $J = 8.8$  Hz), 6.861 (1H, dd,  $J = 2.8, 8.8$  Hz), 5.099 (2H, s), 3.842 (3H, s), 3.837 (3H, s), 3.804 (3H, s), 3.720 (3H, s), 2.072 (3H, s);  $^{13}\text{C}$  NMR  $\delta$  168.65, 165.21, 153.68, 152.13, 150.91, 149.90, 148.81, 136.85, 127.45, 126.16, 123.96, 123.70, 121.42, 114.55, 113.65, 112.92, 112.21, 111.61, 111.38, 106.03, 65.48, 56.36, 56.14, 55.80, 24.07; ESI-MS calculated for  $\text{C}_{26}\text{H}_{29}\text{N}_2\text{O}_7$   $[\text{M}+\text{H}]^+$  481.20, found 481.01.

#### 4.1.27. *N*-[3-(2,5-dimethoxy-benzyloxy)-4-acetamido-phenyl]-4-bromobenzamide (**32**)

<sup>1</sup>H NMR  $\delta$  10.286 (1H, s), 9.192 (1H, s), 7.901 (2H, d,  $J = 8.4$  Hz), 7.748 (2H, d,  $J = 8.4$  Hz), 7.710 (1H, d,  $J = 8.8$  Hz), 7.600 (1H, d,  $J = 2$  Hz), 7.357 (1H, d,  $J = 8.4$  Hz), 7.133 (1H, d,  $J = 2.8$  Hz), 6.981 (1H, d,  $J = 9.2$  Hz), 6.860 (1H, dd,  $J = 3.2, 8.8$  Hz), 5.102 (2H, s), 3.803 (3H, s), 3.715 (3H, s), 2.075 (3H, s); <sup>13</sup>C NMR  $\delta$  168.65, 164.77, 153.67, 150.91, 149.85, 136.45, 134.43, 131.84, 130.20, 126.10, 125.76, 123.95, 114.51, 113.67, 112.86, 112.25, 105.96, 99.99, 65.47, 56.39, 55.82, 24.08; ESI-MS calculated for C<sub>24</sub>H<sub>24</sub>BrN<sub>2</sub>O<sub>5</sub> [M+H]<sup>+</sup> 499.09, found: 498.89.

#### 4.1.28. *N*-[3-(2,5-dimethoxy-benzyloxy)-4-acetamido-phenyl]-4-iodobenzamide (**33**)

<sup>1</sup>H NMR  $\delta$  10.263 (1H, s), 9.188 (1H, s), 7.918 (2H, d,  $J = 8.8$  Hz), 7.730 (3H, m), 7.599 (1H, d,  $J = 2$  Hz), 7.253 (1H, dd,  $J = 2, 8.8$  Hz), 7.131 (1H, d,  $J = 3.2$  Hz), 6.979 (1H, d,  $J = 8.8$  Hz), 6.858 (1H, dd,  $J = 3.2, 9.2$  Hz), 5.099 (2H, s), 3.801 (3H, s), 3.714 (3H, s), 2.073 (3H, s); <sup>13</sup>C NMR  $\delta$  168.66, 165.04, 153.67, 150.91, 149.86, 137.71, 136.45, 134.73, 130.02, 126.11, 123.99, 123.95, 114.51, 113.68, 112.86, 112.25, 105.95, 99.66, 65.47, 56.40, 55.82, 24.08; ESI-MS calculated for C<sub>24</sub>H<sub>24</sub>I<sub>2</sub>N<sub>2</sub>O<sub>5</sub> [M+H]<sup>+</sup> 547.07, found 546.86.

### 4.2. Biological studies

#### 4.2.1. Cell culture

Four breast cancer cell lines including SKBR-3, MCF-7, MDA-MB-231, and MDA-MB-468 were obtained from ATCC (Rockville, MD). The cells were maintained in RPMI1640 medium supplemented with 10% fetal bovine serum (FBS), 2 mmol/l L-Glutamine, 1 mmol/l sodium pyruvate, 100 U/ml penicillin–streptomycin. FBS was heat inactivated for 30 min in a 56 °C water bath before use. Cell cultures were grown at 37 °C, in a humidified atmosphere of 5% CO<sub>2</sub> in a VWR CO<sub>2</sub> incubator (Bridgeport NJ).

#### 4.2.2. Cell viability analysis

The effects of the new derivatives on the viability of four breast cancer cell lines were assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide assay in six replicates. Cells were grown in RPMI1640 medium in 96-well, flat-bottomed plates for 24 h, and were exposed to various concentrations of the compounds dissolved in DMSO (final concentration 0.1%) in media for 48 h. Controls received DMSO vehicle at a concentration equal to that in drug-treated cells. The medium was removed, replaced by 200  $\mu$ L of 0.5 mg/ml of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide in fresh media, and cells were incubated in the CO<sub>2</sub> incubator at 37 °C for 2 h. Supernatants were removed from the wells, and the reduced 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide dye was solubilized in 200  $\mu$ L/well DMSO. Absorbance at 570 nm was determined on a plate reader. Statistical and graphical information was determined using GraphPad Prism software (GraphPad Software Incorporated) and Microsoft Excel (Microsoft Corporation). IC<sub>50</sub> values were determined using nonlinear regression analysis.

#### 4.2.3. Tubulin polymerization assay

100  $\mu$ L microtubule-associated protein-rich tubulin (2 mg/ml, bovine brain, Cytoskeleton) in buffer containing 80 mM PIPES (pH 6.9), 2 mM MgCl<sub>2</sub>, 0.5 mM EGTA, and 5% glycerol was mixed with DMSO (as control) or various concentrations of **10** and **12** in DMSO and incubated at 37 °C. 1  $\mu$ L of 100 mM GTP was added to the mixture to initiate the tubulin polymerization. The absorbance was monitored at 340 nm over 20 min using a Varian Cary 50 series spectrophotometer.

#### 4.2.4. Hsp27 chaperone activity assay

A mixture of 0.24 mg/ml human recombinant insulin (Life Technologies), 0.02 mg/ml Hsp27 (Cell Sciences), and compounds **10** and **12** in 98  $\mu$ L sodium phosphate buffer, pH 7.4, was incubated at 37 °C for 5 min, whereupon 2  $\mu$ L of 1 M DTT in the same assay buffer was added to initiate the insulin aggregation. The absorbance was monitored at 400 nm over 30 min using a Varian Cary 50 series spectrophotometer. A mixture of 0.24 mg/ml insulin in the absence or presence of 0.02 mg/ml Hsp27 with DMSO was used as control.

### 5. Molecular docking simulation

Molecular docking of compounds **10** and **12** into the crystal structure of tubulin was performed using software AUTODOCK 4. The X-ray crystal structures of tubulin with PDB code 1SA0 from Protein Data Bank was used for docking simulation of compounds into the colchicine binding site. To prepare tubulin for autodock, all hydrogens were added and the ligand with identifier CN2700 in 1SA0 was deleted. Compound **10** and **12** were drawn and energy minimized with MM2 force field using Chem3D Ultra 10.0 (Cambridge Soft Corp., US). Autogrid was used to pre-calculate the grid maps of the binding energy between tubulin and compounds. A grid box size of 44  $\times$  46  $\times$  42 points in x, y and z directions was built and the grid center was located in x = 116.909, y = 89.688, z = 7.094. For running autodock, Lamarckian genetic algorithm was chosen as the search method. Genetic algorithm parameters were set as default. The top docked conformation for each compound was retained for analysis.

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### Abbreviations

Hsp27	heat shock protein 27
SAR	structure–activity relationship
Her2	human epidermal growth factor receptor 2
FBS	fetal bovine serum
DTT	dithiothreitol

### Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.ejmech.2014.04.038>.

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