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## Lead optimization of dual tubulin and Hsp27 inhibitors

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### Abstract

Tubulin and heat shock protein 27 (Hsp27) are well-characterized molecular targets for anti-cancer drug development. We previously identified lead compounds that inhibited both Hsp27 and tubulin. These compounds exhibited extensive anti-cancer activities against the proliferation of various human cancer cell lines. In the current study, a systematic ligand based structural optimization led to new analogs that significantly inhibited the growth of a panel of breast cancer cell lines. Furthermore, the most potent compounds were examined with tubulin polymerization assay and Hsp27 chaperone activity assay. The compounds showed potent tubulin polymerization inhibition but no Hsp27 inhibitory effect. The structural optimization dissected the dual activity and improved the selectivity of the compounds for tubulin. The results revealed several structural moieties of the lead compounds that are critical for Hsp27 inhibition. The modification of these structural fragments eliminated Hsp27 inhibition, but did not harm tubulin-targeting effects of the compounds. This result further defined the structure-activity relationship between the tubulin and Hsp27 effects of these compounds.

Keyword: Tubulin, Hsp27, cancer, lead optimization.

### 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.

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** (Figure 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<sub>50</sub> 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 µM to 2.44 µM for SKBR-3 cells, 0.001 µM to 186.0 µM for MCF-7cells, 0.1 µM to 48.1 µM for MDA-MB-231cells, and 0.008 µM to 1.010 µM 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-231cells. 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<sub>50</sub> 0.001-0.004 µM [22]. However N-methylethanesulfonamides about values (6, 14, 16), N-methyl-propylsulfoylamides N-methyl-benzylsulfoylamide (7, 11, 15, 17), (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<sub>50</sub>s of 0.001 µM and 0.003 µM respectively. The general order of potency of various N-methyl alkyl/aryl

sulfonamides for inhibition of SKBR-3 cell growth is methanesulfonamides > ethanesulfonamides > =propylsulfonamides > benzylsulfoylamides > phenylsulfonamides. The anti-proliferative activity of the compounds against SKBR-3 cells seems to be inversely proportional to the size of alkyl/aryl sulfonamide the C moiety. Previous study showed that removal of the N-methyl group in at N-methylmethanesulfonamides significantly impairs their biological activity[22]. Similar phenomenon was observed for most N-methyl alkyl/aryl sulfonamides 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 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-methyl ethanesulfonamide 6. Four acetamide derivatives 30-33 exhibited anti-proliferative activity with IC<sub>50</sub>s in the range of 0.110 µM and 2.44 µM. 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 benzylsulfonamide 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-methyl-benzylsulfonamide **8** and N-methyl-propylsulfonamide **11** with same IC<sub>50</sub>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<sub>50</sub> of 0.015  $\mu$ M and 0.050  $\mu$ M. Unexpectedly, N-methylethanesulfonamide **6** inhibited MCF-7 cell growth with an IC<sub>50</sub> 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<sub>50</sub>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 (Figure 2). In 15 min, compound **10** inhibited tubulin polymerization by 9% at 0.1 $\mu$ M and 56% at 1 $\mu$ M. These inhibitory activities of compounds **10** and **12** are comparable to that of N-methylmethanesulfonamide **5** from the previous study[22].

### 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 Figure 4. Compound 10 may form three major interactions with tubulin. First, 2-methoxyl group in 2,5-dimethoxy benzyl 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 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  $\mu$ M inhibited Hsp27 functions by 27 % [22]. However, both compounds **10** and **12** did not show inhibitory activity against Hsp27 chaperone activity at 10  $\mu$ M, suggesting Hsp27 targeting effect decreased in the new compounds. Substitution of the methanesulfonamide at the C moiety of compound **5** with ethanesulfonamide or benzylsulfonamide 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-, benzyl-sulfonamides showed weaker cell growth inhibition, compared to the corresponding methanesulfonamides. Only N-methylethanesulfonamide **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

corresponding lead compound N-methylmethanesulfonamide **5**. However, both compounds did not show Hsp27 inhibition. The substitution of methanesulfonamide with ethanesulfonamide or benzylsulfonamide 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  $\mu$ M). 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 ( $\delta$ ) 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].

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

**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  $C_{27}H_{33}N_2O_7S [M+H]^+ 529.20$ , found: 529.01

**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 **N-[3-(2,5-dimethoxy-benzyloxy)-4-(phenylsulfonyl-methyl-amino)-phenyl]-4-methoxybenzamide**  (9): <sup>1</sup>H NMR  $\delta$  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  $\delta$  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 **N-[3-(2,5-dimethoxy-benzyloxy)- 4-(ethanesulfonyl-methyl-amino)-phenyl]-4-iodobenzamide (10):** IR v= 1672 (C=O); <sup>1</sup>H NMR  $\delta$  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  $\delta$  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<sub>25</sub>H<sub>28</sub>IN<sub>2</sub>O<sub>6</sub>S [M+H]<sup>+</sup> 611.07, found: 610.88

**N-[3-(2,5-dimethoxy-benzyloxy)- 4-(propanesulfonyl-methyl-amino)-phenyl]-4-Iodobenzamide (11):** <sup>1</sup>H NMR δ 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); <sup>13</sup>C NMR δ 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<sub>26</sub>H<sub>30</sub>IN<sub>2</sub>O<sub>6</sub>S [M+H]<sup>+</sup> 625.09, found 624.87 **N-[3-(2,5-dimethoxy-benzyloxy)-4-(benzylsulfonyl-methyl-amino)-phenyl]-4-iodobenzamide (12):**  IR v= 1645 (C=O); <sup>1</sup>H NMR δ 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); <sup>13</sup>C NMR δ 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<sub>30</sub>H<sub>30</sub>IN<sub>2</sub>O<sub>6</sub>S [M+H]<sup>+</sup> 673.09, found 672.84 **N-[3-(2,5-dimethoxy-benzyloxy)-4-(phenylsulfonyl-methyl-amino)-phenyl]-4-iodobenzamide (13):** <sup>1</sup>H NMR δ 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); <sup>13</sup>C NMR δ 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<sub>29</sub>H<sub>28</sub>IN<sub>2</sub>O<sub>6</sub>S [M+H]<sup>+</sup> 659.07, found: 658.86

**N-[3-(2,5-dimethoxy-benzyloxy)-4-(ethanesulfonyl-methyl-amino)-phenyl]-3,4-dimethoxybenzamid e (14):** <sup>1</sup>H NMR δ 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); <sup>13</sup>C NMR δ 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<sub>27</sub>H<sub>33</sub>N<sub>2</sub>O<sub>8</sub>S [M+H]<sup>+</sup> 545.20, found: 544.98

## N-[3-(2,5-dimethoxy-benzyloxy)-4-(propanesulfonyl-methyl-amino)-phenyl]-3,4-dimethoxybenzami 13

**de** (15): <sup>1</sup>H NMR δ 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); <sup>13</sup>C NMR δ 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<sub>28</sub>H<sub>35</sub>N<sub>2</sub>O<sub>8</sub>S [M+H]<sup>+</sup> 559.20, found: 559.02

N-[3-(2,5-dimethoxy-benzyloxy)- 4-(ethanesulfonyl-methyl-amino)-phenyl]-4-bromobenzamide (16): <sup>1</sup>H NMR δ 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); <sup>13</sup>C NMR δ 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<sub>25</sub>H<sub>28</sub>BrN<sub>2</sub>O<sub>6</sub>S [M+H]<sup>+</sup> 563.09, found: 562.90

**N-[3-(2,5-dimethoxy-benzyloxy)- 4-(propanesulfonyl-methyl-amino)-phenyl]-4-bromobenzamide** (**17**): <sup>1</sup>H 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*= 8.4Hz), 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); <sup>13</sup>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<sub>26</sub>H<sub>30</sub>BrN<sub>2</sub>O<sub>6</sub>S [M+H]<sup>+</sup> 577.10, found 576.92 **N-[3-(2,5-dimethoxy-benzyloxy)-4-(ethanesulfonyl-amino)-phenyl]-4-methoxybenzamide** (**18**): <sup>1</sup>H NMR δ 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); <sup>13</sup>C NMR δ 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<sub>25</sub>H<sub>29</sub>N<sub>2</sub>O<sub>7</sub>S [M+H]<sup>+</sup>; 501.17, found: 500.96 **N-[3-(2,5-dimethoxy-benzyloxy)-4-(propanesulfonyl-amino)-phenyl]-4-methoxybenzamide (19):** <sup>1</sup>H NMR δ10.137 (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 91H, 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); <sup>13</sup>C NMR δ 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<sub>26</sub>H<sub>31</sub>N<sub>2</sub>O<sub>7</sub>S [M+H]<sup>+</sup> 515.19, found: 514.99

**N-[3-(2,5-dimethoxy-benzyloxy)-4-(benzylsulfonyl-amino)-phenyl]-4-methoxybenzamide** (**20**): <sup>1</sup>H NMR δ 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 = 8.8Hz), 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); <sup>13</sup>C NMR δ 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<sub>30</sub>H<sub>31</sub>N<sub>2</sub>O<sub>7</sub>S [M+H]<sup>+</sup> 563.19, found: 562.97

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); <sup>13</sup>C ΝΜR δ 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 549.17, calculated for  $C_{29}H_{29}N_2O_7S$  $[M+H]^+$ found: 548.95 N-[3-(2,5-dimethoxy-benzyloxy)-4-(ethanesulfonyl-amino)-phenyl]-4-iodobenzamide (22): <sup>1</sup>H NMR δ 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); <sup>13</sup>C NMR δ 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 C<sub>24</sub>H<sub>26</sub>IN<sub>2</sub>O<sub>6</sub>S [M+H]<sup>+</sup>: 597.06, found: 596.85

**N-[3-(2,5-dimethoxy-benzyloxy)-4-(propanesulfonyl-amino)-phenyl]-4-iodobenzamide** (23): <sup>1</sup>H NMR δ 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); <sup>13</sup>C NMR δ 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 C<sub>25</sub>H<sub>28</sub>IN<sub>2</sub>O<sub>6</sub>S [M+H]<sup>+</sup> 611.07, found 610.88 **N-[3-(2,5-dimethoxy-benzyloxy)-4-(benzylsulfonyl-amino)-phenyl]-4-iodobenzamide (24):** <sup>1</sup>H NMR δ 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); <sup>13</sup>C NMR δ 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 C<sub>29</sub>H<sub>28</sub>IN<sub>2</sub>O<sub>6</sub>S [M+H]<sup>+</sup> 659.07, found: 658.84

**N-[3-(2,5-dimethoxy-benzyloxy)-4-(phenylsulfonyl-amino)-phenyl]-4-iodobenzamide (25):** <sup>1</sup>H NMR δ 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); <sup>13</sup>C NMR δ 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 C<sub>28</sub>H<sub>26</sub>IN<sub>2</sub>O<sub>6</sub>S [M+H]<sup>+</sup> 645.06, found 644.83

N-[3-(2,5-dimethoxy-benzyloxy)- 4-(ethanesulfonyl-amino)-phenyl]-3,4-dimethoxybenzamide (26): <sup>1</sup>H NMR δ 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); <sup>13</sup>C NMR δ 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 C<sub>26</sub>H<sub>31</sub>N<sub>2</sub>O<sub>8</sub>S [M+H]<sup>+</sup>: 531.18, found: 530.97

### N-[3-(2,5-dimethoxy-benzyloxy)-4-(propanesulfonyl-amino)-phenyl]-3,4-dimethoxybenzamide (27): 17

<sup>1</sup>H NMR δ 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); <sup>13</sup>C NMR δ 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 C<sub>27</sub>H<sub>33</sub>N<sub>2</sub>O<sub>8</sub>S [M+H]<sup>+</sup> 545.20, found: 544.98

**N-[3-(2,5-dimethoxy-benzyloxy)-4-(ethanesulfonyl-amino)-phenyl]-4-bromobenzamide** (28): <sup>1</sup>H NMR δ 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); <sup>13</sup>C NMR δ 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 C<sub>24</sub>H<sub>26</sub>BrN<sub>2</sub>O<sub>6</sub>S [M+H]<sup>+</sup>: 549.07, found: 548.86

**N-[3-(2,5-dimethoxy-benzyloxy)-4-(propanesulfonyl-amino)-phenyl]-4-bromobenzamide** (**29**): <sup>1</sup>H NMR δ 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); <sup>13</sup>C NMR δ 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 C<sub>25</sub>H<sub>28</sub>BrN<sub>2</sub>O<sub>6</sub>S [M+H]<sup>+</sup> 563.09, found: 562.91 **N-[3-(2,5-dimethoxy-benzyloxy)-4-acetamido-phenyl]-4-methoxybenzamide (30):** <sup>1</sup>H NMR δ 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); <sup>13</sup>C NMR δ 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  $C_{25}H_{27}N_2O_6$  [M+H]<sup>+</sup> 451.19, found: 450.99

**N-[3-(2,5-dimethoxy-benzyloxy)-4-acetamido-phenyl]-3,4-dimethoxybenzamide** (**31**): <sup>1</sup>H NMR δ 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 z), 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); <sup>13</sup>C NMR δ 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 C<sub>26</sub>H<sub>29</sub>N<sub>2</sub>O<sub>7</sub> [M+H]<sup>+</sup> 481.20, found 481.01

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

<sup>1</sup>H NMR δ 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 δ 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

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

<sup>1</sup>H NMR δ 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 δ 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>IN<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  $\leq 0.1\%$ ) 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 µL 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 µL/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 5min, 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 0.02mg/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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## 7. Supporting Information

HPLC analysis for compounds 6–33; IR spectra for compounds 10 and 12; <sup>1</sup>H NMR and <sup>13</sup>C NMR for compounds 6-33

**Abbreviations:** heat shock protein 27 (Hsp27), structure-activity relationship (SAR), human epidermal growth factor receptor 2 (Her2), fetal bovine serum (FBS), dithiothreitol (DTT).

### References

- [1] H.J. Arts, H. Hollema, W. Lemstra, P.H. Willemse, E.G. De Vries, H.H. Kampinga, A.G. Van der Zee, Heat-shock-protein-27 (hsp27) expression in ovarian carcinoma: relation in response to chemotherapy and prognosis, Int. J. Cancer. 84 (1999) 234-238.
- [2] C. Garrido, P. Mehlen, A. Fromentin, A. Hammann, M. Assem, A.P. Arrigo, B. Chauffert, Inconstant association between 27-kDa heat-shock protein (Hsp27) content and doxorubicin resistance in human colon cancer cells. The doxorubicin-protecting effect of Hsp27, Eur. J. Biochem. 237 (1996) 653-659.
- [3] H.S. Hsu, J.H. Lin, W.C. Huang, T.W. Hsu, K. Su, S.H. Chiou, Y.T. Tsai, S.C. Hung, Chemoresistance of lung cancer stemlike cells depends on activation of Hsp27, Cancer. 117 (2011) 1516-1528.
- [4] C. Garrido, M. Brunet, C. Didelot, Y. Zermati, E. Schmitt, G. Kroemer, Heat shock proteins 27 and 70: anti-apoptotic proteins with tumorigenic properties, Cell. Cycle. 5 (2006) 2592-2601.
- [5] M. Kamada, A. So, M. Muramaki, P. Rocchi, E. Beraldi, M. Gleave, Hsp27 knockdown using nucleotide-based therapies inhibit tumor growth and enhance chemotherapy in human bladder cancer cells, Mol. Cancer. Ther. 6 (2007) 299-308.
- [6] C. Garrido, E. Solary, A role of HSPs in apoptosis through "protein triage"? Cell Death Differ. 10 (2003) 619-620.
- [7] C. Garrido, J.M. Bruey, A. Fromentin, A. Hammann, A.P. Arrigo, E. Solary, HSP27 inhibits cytochrome c-dependent activation of procaspase-9, FASEB J. 13 (1999) 2061-2070.
- [8] C.G. Concannon, A.M. Gorman, A. Samali, On the role of Hsp27 in regulating apoptosis, Apoptosis.8 (2003) 61-70.
- [9] V. Baylot, C. Andrieu, M. Katsogiannou, D. Taieb, S. Garcia, S. Giusiano, J. Acunzo, J. Iovanna, M. Gleave, C. Garrido, P. Rocchi, OGX-427 inhibits tumor progression and enhances gemcitabine chemotherapy in pancreatic cancer, Cell. Death Dis. 2 (2011) e221.
- [10] G.O. Elpek, S. Karaveli, T. Simsek, N. Keles, N.H. Aksoy, Expression of heat-shock proteins hsp27, hsp70 and hsp90 in malignant epithelial tumour of the ovaries, APMIS. 111 (2003) 523-530.

- [11] S.H. Kang, K.W. Kang, K.H. Kim, B. Kwon, S.K. Kim, H.Y. Lee, S.Y. Kong, E.S. Lee, S.G. Jang, B.C. Yoo, Upregulated HSP27 in human breast cancer cells reduces Herceptin susceptibility by increasing Her2 protein stability, BMC Cancer. 8 (2008) 286-2407-8-286.
- [12] R. Langer, K. Ott, K. Specht, K. Becker, F. Lordick, M. Burian, K. Herrmann, A. Schrattenholz, M.A. Cahill, M. Schwaiger, H. Hofler, H.J. Wester, Protein expression profiling in esophageal adenocarcinoma patients indicates association of heat-shock protein 27 expression and chemotherapy response, Clin. Cancer Res. 14 (2008) 8279-8287.
- [13] M. Sherman, G. Multhoff, Heat shock proteins in cancer, Ann. N. Y. Acad. Sci. 1113 (2007) 192-201.
- [14] J.C. Heinrich, A. Tuukkanen, M. Schroeder, T. Fahrig, R. Fahrig, RP101 (brivudine) binds to heat shock protein HSP27 (HSPB1) and enhances survival in animals and pancreatic cancer patients, J. Cancer Res. Clin. Oncol. 137 (2011) 1349-1361.
- [15] D. Chauhan, G. Li, R. Shringarpure, K. Podar, Y. Ohtake, T. Hideshima, K.C. Anderson, Blockade of Hsp27 overcomes Bortezomib/proteasome inhibitor PS-341 resistance in lymphoma cells, Cancer Res. 63 (2003) 6174-6177.
- [16] R. Heald, E. Nogales, Microtubule dynamics, J. Cell. Sci. 115 (2002) 3-4.
- [17] H.J. Ahn, Y.S. Kim, J.U. Kim, S.M. Han, J.W. Shin, H.O. Yang, Mechanism of taxol-induced apoptosis in human SKOV3 ovarian carcinoma cells, J. Cell. Biochem. 91 (2004) 1043-1052.
- [18] G.C. Das, D. Holiday, R. Gallardo, C. Haas, Taxol-induced cell cycle arrest and apoptosis: dose-response relationship in lung cancer cells of different wild-type p53 status and under isogenic condition, Cancer Lett. 165 (2001) 147-153.
- [19] V.M. Pushkarev, D.V. Starenki, V.A. Saenko, H. Namba, J. Kurebayashi, M.D. Tronko, S. Yamashita, Molecular mechanisms of the effects of low concentrations of taxol in anaplastic thyroid cancer cells, Endocrinology. 145 (2004) 3143-3152.
- [20] V.M. Pushkarev, D.V. Starenki, V.A. Saenko, S. Yamashita, O.I. Kovzun, I.D. Popadiuk, V.V.

Pushkarev, M.D. Tronko, Effects of low and high concentrations of antitumour drug taxol in anaplastic thyroid cancer cells, Exp. Oncol. 31 (2009) 16-21.

- [21] Y. Matsui, B.A. Hadaschik, L. Fazli, R.J. Andersen, M.E. Gleave, A.I. So, Intravesical combination treatment with antisense oligonucleotides targeting heat shock protein-27 and HTI-286 as a novel strategy for high-grade bladder cancer, Mol. Cancer. Ther. 8 (2009) 2402-2411.
- [22] B. Zhong, S. Chennamaneni, R. Lama, X. Yi, W.J. Geldenhuys, J.J. Pink, A. Dowlati, Y. Xu, A. Zhou, B. Su, Synthesis and Anticancer Mechanism Investigation of Dual Hsp27 and Tubulin Inhibitors, J. Med. Chem. 56 (2013) 5306-5320.
- [23] X. Yi, B. Zhong, K.M. Smith, W.J. Geldenhuys, Y. Feng, J.J. Pink, A. Dowlati, Y. Xu, A. Zhou, B. Su, Identification of a class of novel tubulin inhibitors, J. Med. Chem. 55 (2012) 3425-3435.

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Figure 2. Tubulin polymerization in the presence of different concentrations of compounds 10 and

**Figure 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.





# **Figure 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.



	H <sub>3</sub> CO H O R <sub>1</sub> O CH <sub>3</sub> O CH <sub>3</sub>						-
	$\mathbf{O} = \mathbf{S} = \mathbf{O}$			··· NH			
	R <sub>2</sub> 6-29			30-33			
			R <sub>3</sub>		IC <sub>50</sub> (µM)		
Entry	R <sub>1</sub> (R)	R <sub>2</sub>		SKBR-3	MCF-7	MDA-MB-23 1	MDA-MB-468
6	och3	CH <sub>2</sub> CH <sub>3</sub>	CH <sub>3</sub>	$0.021 \pm 0.004$	0.0008 ± 0.0006	2.03 ± 1.12	$0.014\pm0.005$
7	ocH3	n-C <sub>3</sub> H <sub>7</sub>	CH <sub>3</sub>	$0.026\pm0.02$	0.074 ±0.042	$0.42\pm0.29$	$0.057\pm0.021$
8	och3	PhCH <sub>2</sub>	CH <sub>3</sub>	$0.06 \pm 0.02$	0.23 ± 0.09	$14.2 \pm 8.81$	$0.008\pm0.004$
9	och3	Ph	CH <sub>3</sub>	0.06 ± 0.03	$1.25 \pm 0.59$	$0.87\pm0.51$	$0.08\pm0.02$
10	I	CH <sub>2</sub> CH <sub>3</sub>	CH <sub>3</sub>	0.001 ± 0.0002	$0.016\pm0.01$	$0.125 \pm 0.07$	$0.012\pm0.005$
11	I	n-C <sub>3</sub> H <sub>7</sub>	CH <sub>3</sub>	$0.007 \pm 0.003$	$0.14\pm0.06$	17.9 ± 12.2	$0.008\pm0.003$
12	I	PhCH <sub>2</sub>	CH <sub>3</sub>	$0.003 \pm 0.001$	$0.12 \pm 0.06$	$2.25 \pm 1.51$	$0.012\pm0.005$
13	I	Ph	CH <sub>3</sub>	$0.05\pm0.02$	2.88 ± 1.72	$0.8 \pm 0.44$	$0.05\pm0.01$
14	oCH <sub>3</sub>	CH <sub>2</sub> CH <sub>3</sub>	CH <sub>3</sub>	$0.056\pm0.021$	$0.097 \pm 0.06$	$0.1 \pm 0.06$	$0.088\pm0.059$
15	oCH <sub>3</sub>	n-C <sub>3</sub> H <sub>7</sub>	CH <sub>3</sub>	$0.05\pm0.02$	$1.27\pm0.69$	$0.69 \pm 0.41$	$0.07 \pm 0.02$
16	prof Br	CH <sub>2</sub> CH <sub>3</sub>	CH <sub>3</sub>	$0.012\pm0.005$	$0.16\pm0.1$	$0.5\pm0.31$	$0.056\pm0.022$

Table 1. SAR of alternate A and B moiety methanesulfonamide

17	<sup>r</sup> <sup>r<sup>s</sup></sup> Br	n-C <sub>3</sub> H <sub>7</sub>	CH <sub>3</sub>	$0.019\pm0.01$	$0.06\pm0.03$	21.7 ± 14.5	$0.072 \pm 0.032$
18	ocH3	CH <sub>2</sub> CH <sub>3</sub>	Н	$0.019 \pm 0.008$	$11.9\pm6.64$	$0.16\pm0.09$	$0.048\pm0.017$
19	och3	n-C <sub>3</sub> H <sub>7</sub>	Н	$0.071\pm0.021$	$0.28\pm0.17$	4.6 ± 2.4	$0.025 \pm 0.007$
20	och3	PhCH <sub>2</sub>	Н	$0.19\pm0.09$	$1.29\pm0.7$	$1.83 \pm 1.01$	$0.09\pm0.04$
21	ocH3	Ph	Н	$0.31\pm0.11$	16.7 ± 7.44	12.4 ± 5.9	$0.72\pm0.23$
22	,,,,,,I	CH <sub>2</sub> CH <sub>3</sub>	Н	$0.026\pm0.005$	$0.44 \pm 0.12$	19.8 ± 13.1	$0.073 \pm 0.029$
23	, de la compara de	n-C <sub>3</sub> H <sub>7</sub>	Н	$0.047 \pm 0.023$	$0.05 \pm 0.03$	$1.84\pm0.73$	$0.019\pm0.007$
24	, Art I	PhCH <sub>2</sub>	Н	$0.12 \pm 0.04$	$1.69\pm0.91$	$1.78\pm0.86$	$0.11\pm0.03$
25	Prof.	Ph	н	$0.34\pm0.17$	$39.6\pm21.7$	$5.74\pm3.07$	$0.18\pm0.06$
26	oCH3	CH <sub>2</sub> CH <sub>3</sub>	Н	$0.108 \pm 0.048$	$2.48 \pm 1.55$	$0.53\pm0.29$	$0.061 \pm 0.019$
27	oCH3	n-C <sub>3</sub> H <sub>7</sub>	н	$0.096\pm0.037$	$0.29\pm0.16$	$5.66\pm2.02$	$0.45\pm0.14$
28	br Br	CH <sub>2</sub> CH <sub>3</sub>	Н	$0.085\pm0.031$	$2.51 \pm 1.5$	2.31 ± 1.29	$0.042 \pm 0.014$
29	bir Br	n-C <sub>3</sub> H <sub>7</sub>	Н	$0.074\pm0.025$	$0.36 \pm 0.21$	$20.6 \pm 11.4$	$0.24\pm0.09$
30	of the other			$0.38\pm0.13$	$1.17 \pm 0.49$	$19.0\pm6.25$	$0.25\pm0.08$
31	oCH3			$1.24\pm0.32$	$1.81\pm0.7$	$31.7\pm8.65$	$0.99\pm0.25$

32	<sup>r,r,r</sup> Br	$2.44 \pm 1.53$	$186.0\pm99.3$	$48.1\pm23.6$	$1.01\pm0.44$
33	r <sup>2</sup> r <sup>2</sup>	$0.11 \pm 0.02$	$0.31\pm0.17$	$3.65 \pm 1.87$	$0.29\pm0.04$



Scheme 1. Reagents and conditions: (a)2,5-dimethoxybenzyl chloride, K<sub>2</sub>CO<sub>3</sub>, DMF; (b) R<sub>2</sub>SO<sub>2</sub>Cl, NaH,

DMF (c) Acetyl chloride, K<sub>2</sub>CO<sub>3</sub>, 1,4-dioxane; (d) MeI, NaH, DMF; (e) FeCl<sub>3</sub>, Zn, DMF/H<sub>2</sub>O; (f)

R<sub>1</sub>COCl, K<sub>2</sub>CO<sub>3</sub>, 1,4-dioxane

- Systematic ligand based structural optimization of dual tubulin and Hsp27 inhibitor led to new analogs.
- The inhibition on the proliferation of four panels of breast cancer cells of the derivatives was determined.
- The lead optimization dissected the dual activity and improved the selectivity of the compounds for tubulin.
- > Several structural moieties of the lead compounds were revealed to be critical for Hsp27inhibition.

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## Supporting information

### **Lead optimization of dual tubulin and Hsp27 inhibitors** Bo Zhong<sup>a</sup>, Rati Lama<sup>a</sup>, Bibo Li<sup>bc</sup>\*, Bin Su<sup>ac</sup>\*

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1. HPLC analysis of the purity of the compounds. Reversed-phase HPLC analysis of compounds 6-33,

were conducted on Beckman HPLC system with AutoSampler. The chromatographic separation was

performed on a C18 column (2.0 mm × 150 mm, 5 µm) from Phenomenex (Torrance, CA, USA). Two

mobile phases were employed for isocratic elution with a flow rate of 0.2 mL/min. The injection volume

was 20  $\mu$ L and the UV detector was set up at 256 and 290 nm.

Table S1. HPLC analysis of the purity of compounds 6-33

Comnd	H <sub>2</sub> O/CH <sub>3</sub> OH (20%	/80%)	H <sub>2</sub> O/CH <sub>3</sub> CN (25%/75%)		
Compa	Retention time/mins	Purity	Retention time/mins	Purity	
6	4.47	98.59%	4.03	99.31%	
7	5.15	97.42%	4.63	97.71%	
8	5.82	98.81%	5.20	98.87%	
9	5.87	98.22%	5.27	98.92%	
10	7.07	97.47%	6.17	98.44%	
11	8.47	99.12%	7.18	96.46%	
12	10.30	96.29%	8.28	97.53%	
13	9.78	97.13%	8.43	97.79%	
14	3.88	97.77%	3.62	98.88%	
15	4.40	98.19%	4.07	98.75%	
16	6.60	96.00%	5.67	96.54%	
17	7.80	97.71%	6.55	97.79%	
18	3.72	97.93%	3.53	99.02%	
19	4.20	99.05%	3.95	98.78%	
20	4.60	98.86%	4.38	97.48%	
21	4.30	97.72%	4.22	98.95%	
22	5.62	98.90%	4.55	98.43%	
23	6.62	96.73%	5.92	98.12%	
## ACCEPTED MANUSCRIPT

24	7.92	98.17%	6.85	96.61%
25	6.82	98.70%	6.42	97.43%
26	3.27	98.34%	3.18	98.97%
27	3.63	98.45%	3.50	98.96%
28	5.28	99.50%	4.73	98.16
29	6.17	97.34%	5.47	98.31%
30	3.62	98.41%	3.07	98.77%
31	3.20	94.87%	2.83	97.55%
32	5.13	98.74%	3.97	97.81%
33	5.43	97.52%	4.20	92.89%

## 2. IR spectra of compounds ${\bf 10}$ and ${\bf 12}$





IR spectrum of compound 12



3. <sup>1</sup>H NMR and <sup>13</sup>C NMR of compounds **6-33** 





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