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ARTICLE TYPE

Synthesis of Phenstatin/*iso*combretastatin-Chalcone Conjugates as Potent Tubulin Polymerization Inhibitors and Mitochondrial Apoptotic Inducers

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A series of phenstatin/*iso*combretastatin-chalcones were synthesized and screened for their cytotoxic activity against various human cancer cell lines. Some representative compounds exhibited significant antiproliferative activity against a panel of sixty human cancer cell lines of the NCI, with GI_{50} values in the range of 0.11 to 19.0 μM. Three compounds (**3b**, **3c** and **3e**) showed broad spectrum of antiproliferative efficacy on most of the cell lines in sub-micromolar range. In addition, all the synthesized compounds (**3a-l** and **4a-l**) displayed moderate to excellent cytotoxicity against breast cancer cells such as MCF-7 and MDA-MB-231 with IC_{50} value in the range of 0.5 to 19.9 μM. Moreover, the tubulin polymerization assay and immunofluoresence analysis results suggest that some of these compounds like **3c** and **3e** exhibited significant inhibitory effect on the tubulin assembly with an IC_{50} value of 0.8 μM and 0.6 μM respectively. Competitive binding assay suggested that these compounds bind at the colchicine-binding site of tubulin. Cell cycle assay revealed that these compounds arrest at the G_2/M phase and leads to apoptotic cell death. Further, this was confirmed by Hoechst 33258 staining, activation of caspase 9, DNA fragmentation, Annexin V-FITC and mitochondrial membrane depolarization. Molecular docking studies indicated that compounds like 3e occupy the colchicine binding site of tubulin.

Introduction

The discovery of natural as well as synthetic compounds that are capable of interfering with the microtubule assembly or disassembly has gained much attention because microtubules constitute an attractive chemotherapeutic target for anticancer drugs.[1-2] Microtubules are present in all eukaryotic cells and play an essential role in the formation of mitotic spindles as well as cell division.[3] The classical microtubule-targeting agents are antimitotic drugs that affect cellular migration, intracellular trafficking and cell secretion.[4] For example, taxanes, vinca alkaloids and the new spindle-specific drugs are inhibitors of Kinesin-5 (aka KSP, Eg5, KIF11), Polo kinase-1 and Aurora kinases and they play a central role in the treatment of a variety of human cancers.[5] More recently, taxanes such as paclitaxel and docetaxel have shown strong activity in treating tumors in breast, ovaries, lung, and other tissues.[6] Vinca alkaloids like vinblastine and vincristine are major drugs for the treatment of leukemia, lymphoma and a variety of other diseases.[7]

Combretastatin A-4 (CA-4, **1a**, Figure 1.) is another microtubule-destabilizing agent, isolated from *Combretum caffrum*,[8] which exhibits promising cytotoxicity towards a wide range of human cancer cell lines, including multidrug-resistant cancer cells[9] which binds to the β-tubulin at the colchicine binding site and significantly inhibits tubulin polymerization.[10] However, the main drawback associated with CA-4 are bioavailability and isomerization of the biologically active Z-configured double bond into the inactive E-configuration during long storage and administration.[11] To overcome this, the ethenyl bridge of the stilbene moiety was replaced by a biologically stable keto group (phenstatin) and bioisosteric replacement of the (Z)-1,2-ethylene group with a 1,1-ethylene bridge of stilbene moiety (*iso*combretastatin) that resulted in the retention of the biological activity with improved physical properties.[12]

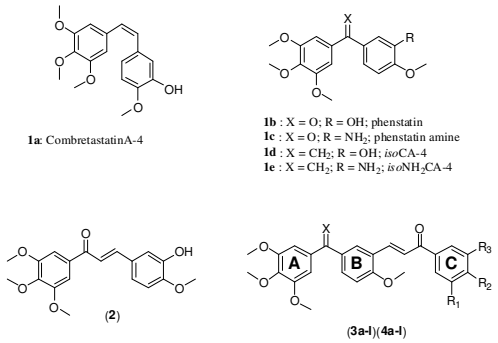


Figure 1. Chemical structures of microtubule targeting agents. CA-4 (**1a**), phenstatin (**1b**) phenstatin amine (**1c**) *iso*combretastatin A-4 (**1d**) *iso*combretastatin A-4 amine (**1e**), chalcone (**2**), phenstatin*iso*combretastatin-chalcone conjugates (**3a-l** and **4a-l**)

Phenstatin (**1b**, Figure 1) and its derivatives reported by the Pettit group, exhibited substantial anticancer and antimitotic activity, comparable to CA-4.[13] In addition, phenstatin and its derivatives were easier to synthesize than the combretastatins (which require control of geometric selectivity) and have greater pharmacological potential due to improved metabolic stability.[14] Moreover, the carbonyl oxygen of phenstatin is capable of forming crucial interactions at the colchicine binding site of the tubulin.[15] Recently, disclosed CA-4 analogues *iso*combretastatin A-4 (*iso*CA-4, **1d**, Figure 1) and its derivatives displayed potent antiproliferative activity against various human cancer cell lines and inhibited tubulin polymerization at micromolar level, and arrested cancer cells in the G_2/M phase of the cell cycle.[16, 12b]

Chalcones (**2**, Figure 1) are naturally occurring compounds that belong to the flavonoid family and have displayed a wide variety of biological activities. Notable anticancer potential of the chalcones involves their capacity to induce apoptosis in a variety of cell types, including breast cancers.[17, 18] Recently, Ducki and coworkers synthesized and developed CA-4 type of chalcones wherein the *cis*-double bond of CA-4 was replaced by an olefin conjugated to a carbonyl group. These chalcone analogs showed potent inhibition of tubulin polymerization and arrested the cell at G_2/M phase of the cell cycle and displayed promising antiproliferative activity.[19] The prodrugs of these chalcones are currently under preclinical evaluation.[20] More recently, a series of resveratrol–chalcone conjugates were shown to possess significant cytotoxic activity against the various human cancer cell lines.[21]

Therefore as described above in view of their potential as anticancer agents, we envisaged to combine the structural features of these three pharmacophores in developing newer more effective cytotoxic compounds. Interestingly, these new molecules exhibited promising cytotoxic activity and prompted us to investigate their role in the proliferation and apoptosis of human breast cancer cells (MCF-7). We also investigated their effect on the proteins that regulate the cell-cycle progression.

Results and Discussion

Chemistry

The 5-iodo salicylic acid (**5**) was converted to aryl iodo ester **6**. The obtained aryl iodo ester **6** is reduced with DIBAL to produce corresponding alcohol (**7**). This alcohol **7** protected with TBDMS to gives tert-butyl (5-iodo-2-methoxyphenoxy)dimethylsilane (**8**). The resulting aryl iodide **8** was reacted with trimethoxy benzaldehyde (**9**) in presence of n-butLi in dry THF to generate benzhydrol derivative **10**. The obtained benzhydrol derivative **10** on oxidation to give ketone **13a** and subsequent witting methylation afforded the olefin **13b**. These compounds **13a** and **13b** were oxidizing with swern method to produce phenstatin 3-aldehyde/ *iso*combretastatin 3-aldehyde (**14a-b**) (Scheme 1).

The Claisen-Schmidt condensation of phenstatin 3-aldehyde/ *iso*combretastatin 3-aldehyde (**14a-b**) with substituted acetophenones (**15a-l**) in the presence of KOH (10%) to produce phenstatin*iso*combretastatin-chalcone conjugates (**3a-l** and **4a-l**) as shown in Scheme 1. All the new compounds synthesized were characterized by spectral analysis.

Biological studies

Antiproliferative activity

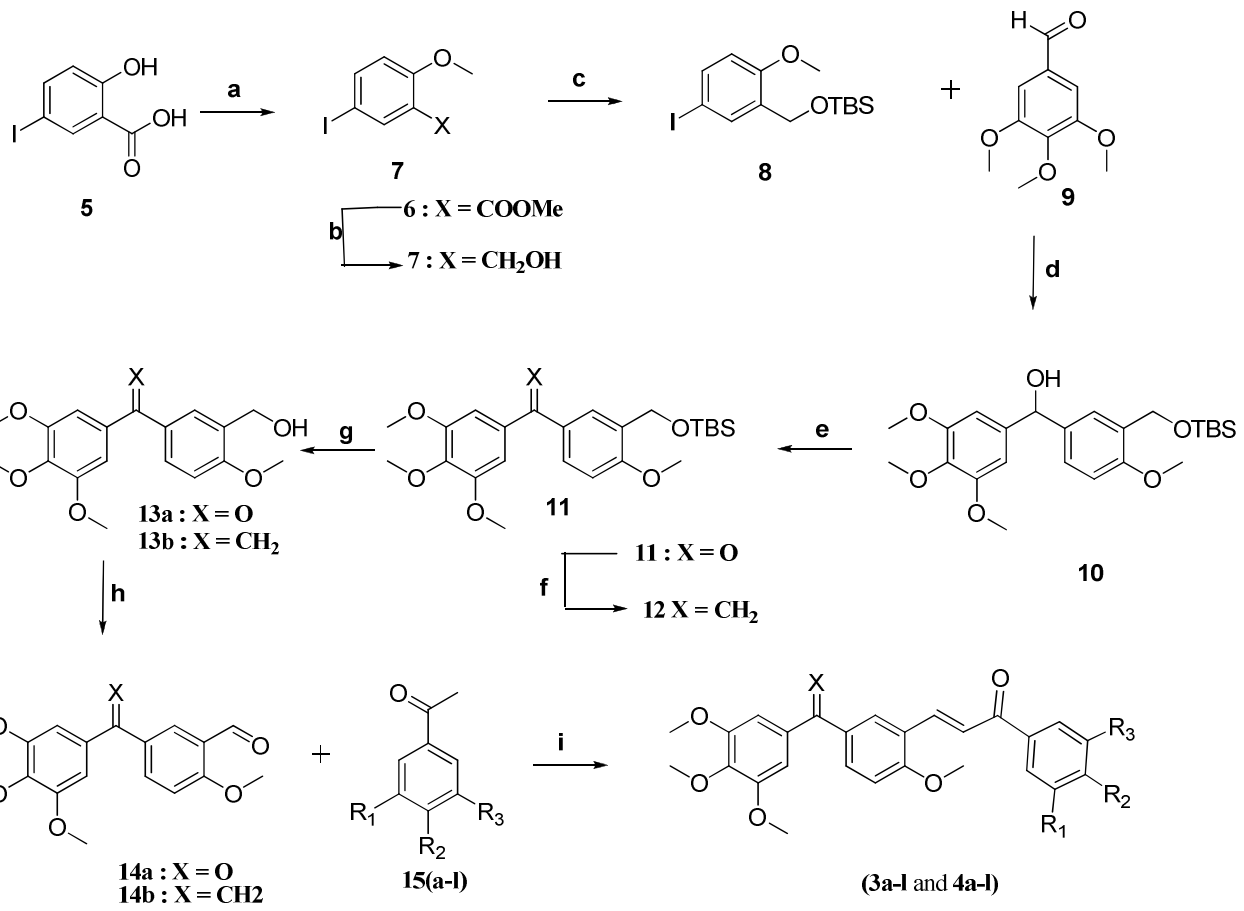
The phenstatin-chalcones (**3a-l**) and *iso*combretstatin-chalcones (**4a-l**) were evaluated for their cytotoxic activity against a panel of 60 human cancer cell lines at the National Cancer Institute (NCI, Bethesda, MD, USA). This panel is organized into nine sub-panels representing leukemia, melanoma, lung, colon, kidney, ovary, breast, prostate, and central nervous system. Among the twenty four compounds, nine compounds **(3a-e**, **3g**, **3j**, **4a** and **4c)** were selected for the preliminary test at a single concentration (10 μM) screen. These nine compounds exhibited significant mean growth inhibitory effect and the results are illustrated in Table 1. Apart from **3a**, other compounds **(3b-e**, **3g**, **3j**, **4a** and **4c)** were further evaluated in the secondary screening at five concentrations with 10-fold dilutions. These eight compounds manifested significant cytotoxic activity with GI_{50} values ranging from 0.11 to 19.0 μM and the results are listed in Table 2.

Particularly, the presence of carbonyl group between A and B rings in **3e** and **3g** shows noticeable cytotoxicity. Compound **3e** having strong electron donating substituents like *meta*-amine and *para*-methoxy groups on the C-ring exhibited a broad spectrum of inhibition with GI_{50} values in nanomolar range against most of the cell lines. More particularly, this conjugate displayed excellent cytotoxic activity with GI_{50} values 350 nM, 350 nM, 370 nM, 110 nM, 380 nM and 330 nM against CCRF-CEM (leukemia), NCI-H522 (non-small cell lung), HCT-15 (colon), UACC-62 (melanoma), NCI/ADR-RES (ovarian), and MCF-7 (breast) cancer cells respectively. In comparison, compound **3g** with electron withdrawing substituent like fluoro at *para* position on C-ring exhibited comparatively less cyotoxicity with GI_{50} value 2.24 μM, 2.02 μM, 2.14 μM, 2.01 μM, 3.69 μM and 2.30 μM respectively against the above cancer cells. This reveals that replacing the electron donating by withdrawing groups on C-ring significantly influences their antiproliferative activities.

However, the presence of an bicyclic ring system on **3c** and **4c** like 3,4-methlenedioxy group on the C-ring resulted in varied cytotoxic activity. Compound **3c** having a carbonyl group between the A and B rings manifested significant antiproliferative activity in leukemia, colon and breast cancer subpanels of NCI. Whereas, compound **4c** having an ethylene bridge in between the rings A and B displayed slightly better antiproliferative efficacy than **3c** against prostate and lung cancer cells.

However, remaining conjugates like **3b**, **3d**, **3j** and **4a** having electron donating substituents like 3,4-dimethoxy, 4-methoxy, 4-hydroxy and 3,4,5-trimethoxy respectively on the C-ring, shows moderate to good cytotoxic activity against most of the cell lines. Specifically, compound **3e** exhibited promising cytotoxicity against leukemia, colon, ovarian and breast cancer cells with submicromolar range (Figure 2, Table 2).

Scheme-1



	R_1	R_2	R_3	X		R_1	R_2	R_3	X
3a	OMe	OMe	OMe	O	**4a**	OMe	OMe	OMe	CH_2
3b	H	OMe	OMe	O	**4b**	H	OMe	OMe	CH_2
3c	H	-OCH_2O-		O	**4c**	H	-OCH_2O-		CH_2
3d	H	OMe	H	O	**4d**	H	OMe	H	CH_2
3e	H	OMe	NH_2	O	**4e**	H	OMe	NH_2	CH_2
3f	H	NH_2	H	O	**4f**	H	NH_2	H	CH_2
3g	H	F	H	O	**4g**	H	F	H	CH_2
3h	H	Cl	H	O	**4h**	H	Cl	H	CH_2
3i	H	Br	H	O	**4i**	H	Br	H	CH_2
3j	H	OH	H	O	**4j**	H	OH	H	CH_2
3k	H	OMe	OH	O	**4k**	H	OMe	OH	CH_2
3l	napthalene			O	**4l**	napthalene			CH_2

Reagents & conditions: (a) DMS, K_2CO_3, Acetone, 60 ºC, 8 h; (b) DIBAL-H (1N), THF, 5 ºC, 3 h; (c) TBDMSCl, imidazole, CH_2Cl_2, 3 h; (d) n-BuLi (1.6N), THF -78 ºC; (e) IBX, DMSO, 10 ºC, 2 h; (f) CH_2PPh_3I, t-ButOK, THF, 5 ºC, 8 h; (g) TBAF (1N), THF, 5 ºC, 6 h; (h) Oxalyl chloride, DMSO, -78 ºC to rt, Et_3N; (i) EtOH, 10% KOH(aq), 6-12 h, rt.

In addition, we have evaluated the antiproliferative activities of the other compounds of this series, apart from the compound screened by the NCI. Interestingly, similar to NCI results, compounds **(3a-l)** harboring carbonyl group in between the A and B rings showed profound cytotoxic activity with an IC_{50} value in the range 0.5 to 19.9 µM against two human breast cancer cells such as MCF-7 and MDA-MB-231 and the results are summarized Table 3. Particularly, compound **3e** which possess an amino group at the *meta* position and methoxy at the *para* position on the C-ring inhibited the growth of breast cancer cells with an IC_{50} value of 0.5 µM (MCF-7) and 1.2 µM (MDA-MB-231). However, substitution of bromo at the *para* position of the C-ring as in **3i** drastically reduced its antiproliferative activity against MDA-MB-231 cells (IC_{50} value - 19.9 µM) as well as MCF-7 cells (IC_{50} value – 15.8 µM). Notably, compounds containing methylene **(4a-l)** instead of carbonyl inhibited cell growth with IC_{50} values in the range 0.7 to 19.7 µM. Compound **4e** that possesses an amino group at the *meta*-position and methoxy at *para* position on the C-ring inhibited the growth of breast cancer cell with IC_{50} value 0.7 µM (MCF-7) and 2.2 µM (MDA-MB-231). Taken together, the compounds that contain *para*-methoxy and *meta*-amino groups on C-ring exhibited substantial cytotoxic effect against most of the cancer cells apart from breast cancer cells. Thus based on these results we considered it worthwhile to investigate the mechanism of action of these conjugates (Figure 2).

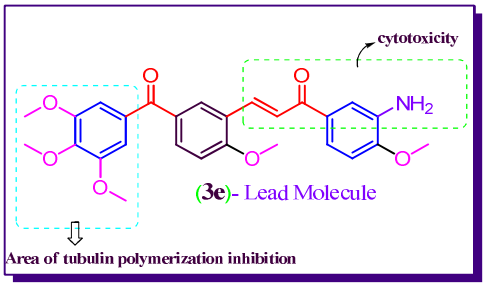


Figure 2. Structure activity relationship of phenstatin\/*iso*combretastatin-chalcone conjugates (**3a-l** and **4a-l**).

Table 1. Single dose screening of the nine selected compounds of phenstatin-chalcones (**3a-l**) and *iso*combretastatin-chalcones (**4a-l**) in NCI-60 cell lines

S.No	Compound	Mean growth percentage
01	**3a**	85.71
02	**3b**	24.41
03	**3c**	8.85
04	**3d**	55.88
05	**3e**	-21.93[b]
06	**3g**	55.77
07	**3j**	22.23
08	**4a**	-8.50[b]
09	**4c**	13.72

[a] Determined at 10 µM: data were obtained from the NCI *in vitro* disease- oriented human tumor cell screen. [b]Negative value indicates cell death.

Table 2. *In vitro* Cytotoxic effect of conjugates **3b, 3c, 3d, 3e, 3j, 4a** and **4c** against a panel of 60 human cancer cell lines

Cancer panel/ cell line	GI_{50} values (µM) [a]							
	3b [b]	**3c** [c]	**3d** [d]	**3e** [e]	**3g** [f]	**3j** [g]	**4a** [h]	**4c** [i]
Leukemia								
CCRF-CEM	0.46	0.58	- [j]	0.35	2.24	0.87	0.35	0.91
HL60(TB)	0.40	2.16	- [k]	1.48	4.80	0.69	2.03	2.67
K-562	0.66	0.37	2.86	0.38	2.34	0.35	0.92	0.78
MOLT-4	0.58	1.61	- [k]	0.61	3.24	0.84	1.61	3.08
RPMI-8226	0.29	1.04	2.54	0.41	2.32	1.56	0.45	1.56
SR	- [k]	0.40	1.08	0.46	2.04	0.43	0.46	0.90
non-small cell lung								
A549/ATCC	- [k]	4.39	- [j]	3.20	16.5	1.87	1.81	3.54
HOP-62	3.41	4.40	11.8	2.03	3.92	21.1	2.27	4.16
HOP-92	1.69	12.0	5.8	2.94	2.70	1.21	1.14	1.78
NCI-H226	- [k]	2.23	4.34	2.14	3.57	2.50	1.41	1.73
NCI-H23	4.13	2.67	- [j]	1.42	6.20	2.93	1.95	3.20
NCIH322M	8.89	4.04	- [j]	1.80	15.4	6.03	2.93	4.73
NCI-H460	2.43	3.09	- [j]	2.13	16.0	2.56	1.44	2.34
NCI-H522	0.68	1.28	2.34	0.35	2.02	2.69	1.10	1.55

Colon								
COLO-205	3.00	3.51	- [j]	1.47	5.18	15.1	2.03	4.15
HCC-2998	4.14	2.09	- [j]	1.27	7.29	1.66	1.84	2.19
HCT-116	0.41	1.65	2.00	0.66	1.75	1.58	0.60	2.01
HCT-15	0.42	1.38	2.54	0.37	2.14	2.15	0.55	1.68
HT29	0.49	1.68	- [j]	0.42	2.44	5.35	0.62	2.98
KM12	1.14	1.67	- [j]	0.55	2.79	2.44	1.16	2.51
SW-620	0.32	0.39	3.07	0.37	2.53	2.51	0.60	1.92
CNS								
SF-268	3.00	2.95	3.51	1.37	3.86	3.98	1.46	3.18
SF-295	5.26	3.43	6.27	2.30	7.21	6.50	2.90	4.14
SF-539	3.08	2.02	- [j]	1.53	1.98	2.72	1.41	2.36
SNB-19	2.70	1.65	4.54	1.06	2.26	4.42	1.39	2.79
SNB-75	3.86	3.11	4.75	1.16	3.18	1.62	0.89	1.64
U251	1.14	1.51	3.11	1.02	1.85	4.58	0.77	2.54
Melanoma								
LOX IMVI	0.55	1.43	2.29	0.74	1.78	1.75	0.58	1.58
MALME-3M	- [k]	2.38	- [j]	1.47	2.76	9.69	1.88	- [j]
M14	2.79	2.09	4.92	0.75	3.39	1.98	1.59	2.05
MDA-MB-435	2.29	2.10	- [j]	1.19	2.88	1.18	0.69	0.60
SK-MEL-2	2.86	2.81	6.04	1.63	9.89	5.01	2.58	4.25
SK-MEL-28	4.59	2.13	- [j]	1.36	2.48	4.75	1.52	2.55
SK-MEL-5	1.63	1.71	- [j]	1.02	4.31	1.46	1.18	1.97
UACC-257	3.19	3.55	- [j]	1.32	12.3	7.77	1.63	6.69
UACC-62	1.14	1.63	2.05	0.11	2.01	1.69	0.49	1.32
Ovarian								
IGROV1	3.08	3.52	8.82	2.07	2.59	9.94	2.07	4.31
OVCAR-3	1.12	1.96	- [j]	0.44	4.83	3.00	1.53	2.98
OVCAR-4	1.95	5.05	- [j]	0.98	4.55	2.73	3.70	3.93
OVCAR-5	3.67	2.64	- [j]	1.33	2.67	11.2	2.07	4.40
OVCAR-8	1.85	2.32	4.61	0.84	3.70	4.48	0.71	2.89
NCI/ADR-RES	0.46	1.46	2.93	0.38	3.69	2.68	0.93	2.77
SK-OV-3	5.76	5.70	- [k]	3.16	17.9	1.63	3.60	5.07
Renal								
786-0	4.71	2.28	7.70	1.54	2.15	1.66	1.68	2.16
A498	2.42	2.43	7.33	2.14	14.0	3.14	1.48	2.72
ACHN	1.32	1.61	3.19	1.08	1.68	5.22	1.26	1.70
CAKI-1	0.82	2.56	- [k]	0.66	2.78	3.07	1.28	3.02
RXF 393	- [j]	1.94	1.47	1.50	7.29	3.57	1.11	1.98
SN12C	1.65	3.07	- [j]	1.62	2.62	2.66	1.87	3.84
TK-10	4.16	3.92	1.40	3.61	4.43	1.18	2.56	4.79
UO-31	5.76	2.04	- [j]	0.76	1.77	3.20	1.07	3.10
Prostate								
PC-3	5.90	4.10	- [j]	1.80	3.30	1.69	1.30	2.91
DU-145	2.21	4.25	- [j]	1.03	7.72	1.88	1.32	5.54
Breast								
MCF7	0.41	0.38	3.05	0.32	2.30	3.16	0.44	0.91
MDA-MB- 231/ATCC	0.65	2.35	3.70	0.71	4.72	4.02	0.99	5.97
HS 578T	11.1	4.56	4.30	1.39	4.48	18.3	1.46	4.65
BT-549	0.90	1.82	2.85	0.82	3.27	2.01	0.89	2.15
T-47D	2.76	- [k]	- [k]	- [k]	3.54	19.0	1.38	2.66
MDA-MB-435	1.25	1.44	3.03	0.33	4.63	2.07	0.70	1.65

[a]Values are reported as GI_{50}, the micro molar concentration of the compound required to cause 50% inhibition of cell growth after an incubation time of 48 h.
[b]NSC 777185; [c]NSC 777187; [d]NSC 777182; [e]NSC 777186; [f]NSC 780196; [g]NSC 780175; [h]NSC 780194; [i]NSC 780198; - [j] = not active; - [k] = not tested.

Table 3. *in vitro* cytotoxicity of phenstatin-chalcone and *iso*-combretastatin-chalcones **(3a-l) (4a-l)**

Compound	IC_{50} (μM)[a]		Compound	IC_{50} (μM)[a]	
	MCF-7[b]	MDA-MB-231[c]		MCF-7[b]	MDA-MB-231[c]
3a	9.5±0.52	7.8±0.25	**4a**	4.2±0.36	8.7±0.25
3b	1.0±0.03	1.8±0.39	**4b**	7.3±0.25	9.3±1.2
3c	0.9±0.08	1.6±0.24	**4c**	7.9±0.54	5.0±0.99
3d	5.4±0.21	7.6±0.91	**4d**	7.7±0.99	10.7±0.54
3e	0.5±0.06	1.2±0.09	**4e**	0.9±0.01	2.2±0.36
3f	3.9±0.12	4.7±0.22	**4f**	10.1±0.57	13.4±0.75
3g	12.7±1.09	10.9±0.65	**4g**	16.7±0.32	19.7±1.1
3h	12.5±1.15	19.9±1.13	**4h**	2.6±0.59	5.2±0.05
3i	15.8±1.14	19.9±1.21	**4i**	16.2±1.2	19.5±+1.19
3j	3.9±0.29	2.6±0.12	**4j**	4.9±0.12	10.5±1.25
3k	5.4±0.35	6.5±0.15	**4k**	10.1±1.12	12.5±1.36
3l	3.1±0.9	3.9±0.12	**4l**	9.1±1.05	12.3±1.25
1b	0.8±0.05	1.5±0.05	**1b**	0.8±0.05	1.5±0.05

[a]Concentration required to inhibition 50% cell growth following 48 h treatment with the tested drug from three different experiments performed in triplicates, [b]MCF-7 and [c]MDA-MB-231-breast cancer cell lines.

Cell cycle analysis

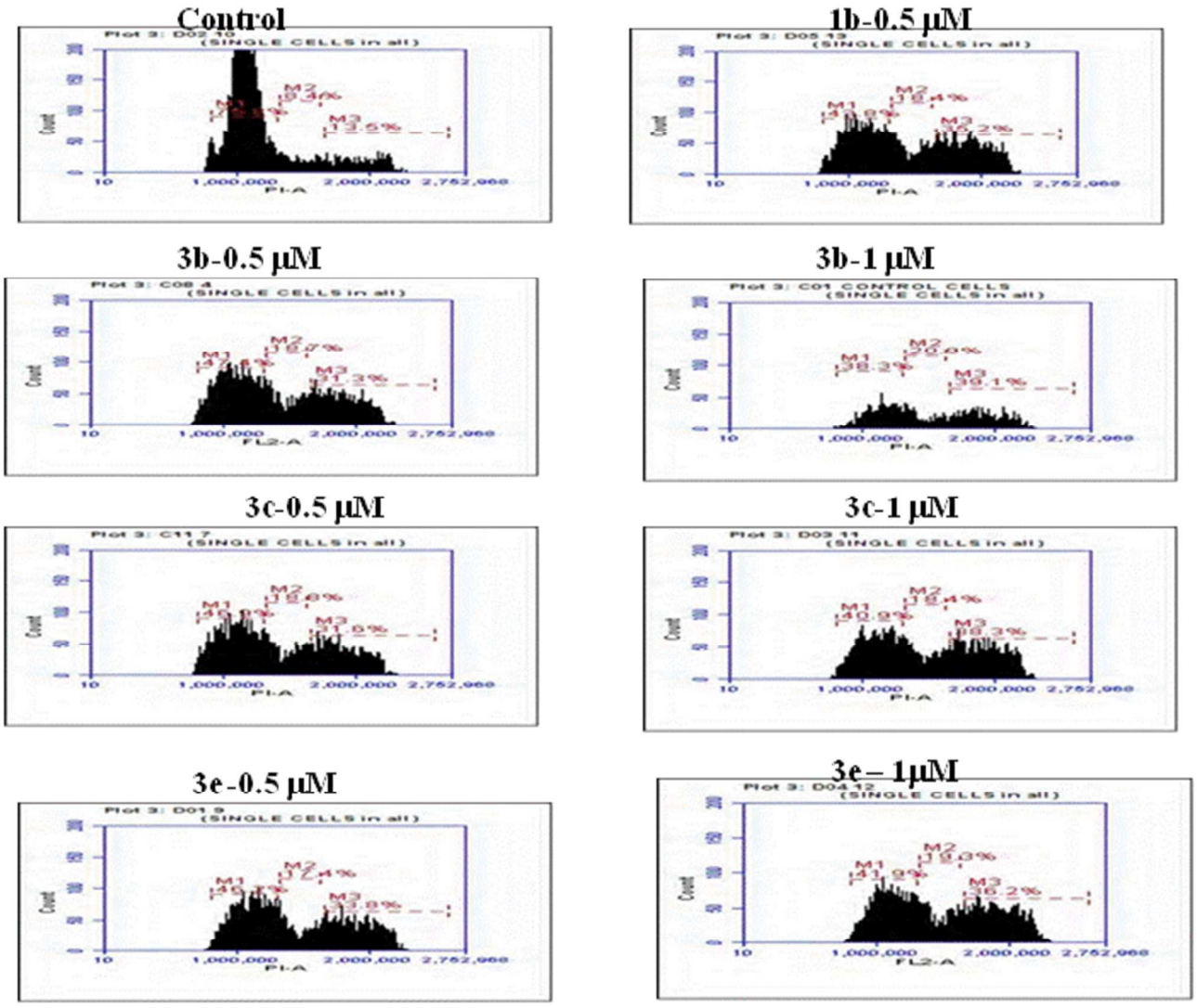


Figure 3. Effects of compounds A-Control, **1b**, **3b**, **3c** and **3e** on DNA content/cell following the treatment of MCF-7 cells at 0.5 µM and 1 µM for 24 h. Cell cycle distribution was analyzed by the standard propidiumiodide procedure as described in Experimental section.

Table 4. Cell-cycle distribution of MCF-7 cells in the presence of **3b**, **3c**, **3e** and **1e** at Conc dependent manner[a]

Compound	G_0/G_1	S- PHASE	G_2/M
Control (DMSO)	75.85	8.45	13.48
3b-0.5 µM	47.40	18.71	31.30
3b-1.0 µM	38.26	20.91	39.06
3c-0.5 µM	46.95	18.78	31.64
3c-1.0 µM	40.90	18.43	38.29
3e-0.5 µM	46.25	17.36	33.78
3e-1.0 µM	41.91	19.29	36.19
1b-0.5 µM	43.79	18.43	35.22

In order to understand the role of some of the potential conjugates like **3b**, **3c** and **3e** in the cell cycle, FACS analysis was performed in breast cancer cells (MCF-7). The cells were treated for 24 h with these compounds (**3b, 3c** and **3e**) at 0.5 µM and 1 µM concentration. It was observed that MCF-7 cells showed 31.30%, 31.64%, 33.78% at 0.5 µM and 39.36 %, 38.39%, 36.18% at 1µM G_2/M cell cycle arrest respectively, whereas the control (untreated cells) exhibited 21% and the positive control phenstatin has showed 35.22% cell cycle arrest at 0.5 µM as illustrated in Figure 3 (Table 4).[22]

Immunohistochemistry of tubulin

In order to substantiate the observed effects of these conjugates on the inhibition of tubulin polymerization to functional microtubules immunohistochemistry studies were carried out to examine the effect of these conjugates like **3b, 3c** and **3e** on cellular microtubules in MCF-7 cancer cells.[23] Thus, MCF-7 cells were treated with **3b, 3c** and **3e** at 1µM concentration for 48 h. In this study, untreated human breast cancer cells displayed the normal distribution of microtubules (Figure 4). However, cells treated with compounds **3b, 3c** and **3e** showed disrupted microtubule organization as seen in Figure 4, thus demonstrating the inhibition of tubulin polymerization.

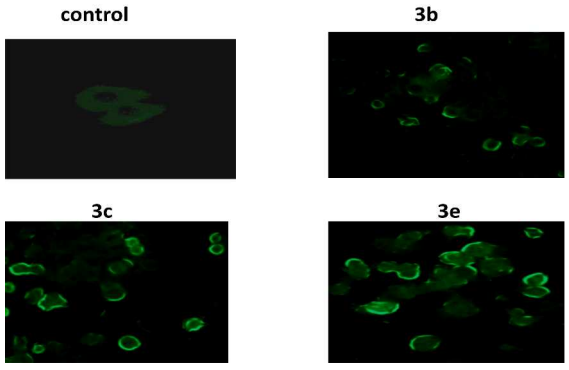


Figure 4. IHC analyses of compounds on the microtubule network: MCF-7 cells were treated with compounds **3b**, **3c** and **3e** at 1 µM concentration for 48 h followed by staining with α-tubulin antibody. Microtubule organization was clearly observed by green color tubulin network like structures in control cells and was found to be disrupted in cells treated with compounds **3b, 3c** and **3e** as positive control.

Effect of compounds on tubulin polymerization assay

One of the possibilities that these compounds exhibit cytotoxic activity as well as G_2/M cell cycle arrest is by the inhibition of tubulin polymerization[24] as this has been observed in many antimitotic agents such as combretastatins and phenstatin. Hence, it was considered of interest to investigate the tubulin polymerization inhibition capacity of these compounds. As tubulin subunits heterodimerize and self-assemble to form microtubules in a time dependent manner, we investigated the progression of tubulin polymerization by monitoring the increase in fluorescence emission at 420 nm (excitation wavelength is 360 nm) in 384 well plate for 1 h at 37 °C with and without the compounds at 5 µM concentration. These conjugates inhibited tubulin polymerization by 58.4%, 55.4 and 56.8%, respectively, compared to phenstatin and combretastatin A-4 (Figure 5).

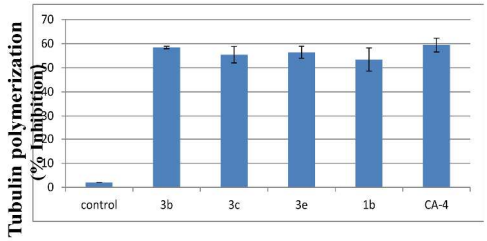


Figure 5. Effect on tubulin polymerization: Tubulin polymerization assay was carried out in a reaction mixture that contained PEM buffer and GTP (1 µM) in the presence or absence of test compounds **3b**, **3c**, **3e**, **1b** and **1a** (CA-4) at 5 µM concentration. The reaction was initiated by the addition of GTP to all the wells. Tubulin polymerization was monitored by the increase in fluorescence at 420 nm (excitation wavelength is 360 nm) was measured for 1 h at 1 min interval in a multimode plate reader (Tecan) at 37 ^{O}C

Table 5. Inhibition of tubulin polymerization (IC_{50}) of compounds **3b, 3c, 3e, 1b** and **1a**.

Compound	Tubulin Polymerization Inhibition $IC_{50} \pm SD$ (µM)
3b	1.3 ± 0.10
3c	0.8 ± 0.02
3e	0.6 ± 0.03
1b	0.7 ± 0.01
1a (CA-4)	1.0 ± 0.05

Effect of conjugates on tubulin polymerization. IC_{50} values for **3b**, **3c**, **3e**, **1b** and **1a** were determined from tubulin polymerization assays.

Furthermore, these three potential compounds (**3b, 3c** and **3e**) were evaluated in an *in vitro* tubulin polymerization assay at different concentrations. These molecules showed potent inhibition of tubulin polymerization with IC_{50} values 1.3 µM, 0.8 µM, and 0.6 µM, respectively, compared to phenstatin (0.7 µM) and combretastatin A-4 (1.0 µM) (Table 5).[25]

Competitive tubulin-binding assay

To confirm the above observations, we investigated the effect of **3b, 3c** and **3e** on tubulin polymerization using an *in vitro* tubulin polymerization assay (Figure 6) and it was observed that they inhibited polymerization of tubulin similar to that of phenstatin. Therefore, we further assessed the ability of these

compounds to compete with the colchicine for binding to tubulin via competitive binding assays. Phenstatin was used as a positive control and paclitaxel as a negative control. As the intrinsic fluorescence of colchicine increases upon binding to the tubulin[26] it was used as an index for competition with colchicine in tubulin binding. Where in paclitaxel did not affect the binding to tubulin as shown in Figure 6. However, the fluorescence of colchicine-tubulin complex was reduced in the presence of these compounds in a dose-dependent manner. These observations indicate that they inhibit the binding of colchicine to the tubulin, thereby suggesting that the compounds (**3b, 3c** and **3e**) bind at the colchicine binding site. In this, F/F_0 where F_0 refers fluorescence of the 5 µM colchicine-tubulin complex, and F represents the fluorescence of a given concentration (5 µM, 10 µM, 15 µM) of compounds **3b**, **3c**, **3e** and taxol competition with the 5 µM colchicine-tubulin complex.

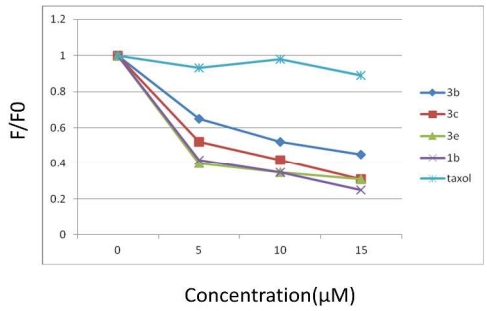


Figure 6. Fluorescence based colchicine competitive binding assay of conjugates **3b, 3c** and **3e** were carried out at various concentrations containing 5 µM of tubulin and colchicine for 60 min at 37 ºC. Phenstatin was used as a positive control where as taxol was used as negative control which binds at taxane site. Fluorescence values are normalized to DMSO (control).

Western blot analysis

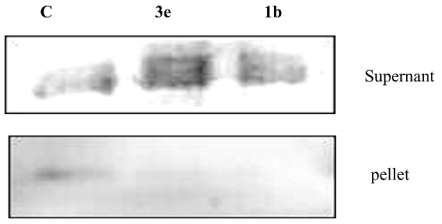


Figure 7. Western blot analysis of tubulin in the soluble and polymerized fractions of MCF-7 cells treated with a 1 µM concentrations of control (**C**), **3e** and **1b**. Ratio of soluble versus polymerized tubulin

The microtubules continuously undergo polymerization and depolymerization which are mediated by α- and β-tubulin, as inhibition of tubulin polymerization disturbs the assembly of microtubules, we analyzed the levels of soluble versus polymerized forms of tubulin in MCF-7 cells. In order to extend the *in vitro* effects of the compounds like **3e** and **1b** on tubulin polymerization to the cellular effects, Western blot analysis of tubulin in MCF-7 were performed by treating the compounds **3e** and **1b** at 1 µM for 48 h. Following this incubation, the medium was removed, cells were washed with PBS, and soluble (containing free tubulin, supernant) and insoluble (containing tubulin from microtubules, pellet) fractions were collected as described in the Experimental Section.[27] Our results indicate that while the tubulin in the supernatant fraction showed an increased level of free tubulin at 3 µM of **3e** and **1b** whereas in the pellet has shown a steady decrease in the polymerized tubulin. Therefore, our results suggest that compound, **3e** and **1b** function as microtubule-destabilizing agents under both *in vitro* and intracellular conditions (Figure 7).

Activation of caspase -9

It is well known that the cell cycle arrest at G_2/M phase is often associated with induction of cellular apoptosis, hence it was considered of interest to examine whether the cytotoxicity of **3b, 3c** and **3e** is by virtue of apoptotic cell death. The MCF-7 cell line lacks endogenous caspase-3, whereas caspase-9 plays an important role in mediating drug-induced apoptosis. Thus the role of caspases was examined in MCF-7 cells treated with conjugates **3b**, **3c** and **3e** at 2 µM for 48 h. Cell lysates were analyzed for active caspase-9 expression levels by a fluorescence-based caspase-9 assay. Up-regulation of caspase-9 was observed for cells treated with these conjugates relative to the control, as shown in Figure 8.[28]

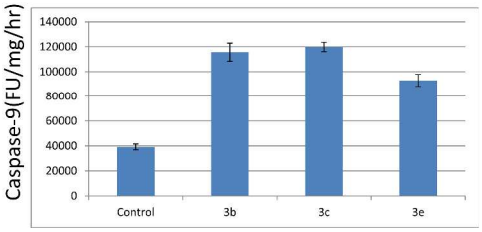


Figure 8. Effect of compounds **3b, 3c** and **3e** on caspase-9 activity: MCF-7 cells were treated for 48 h with 2 µM concentrations of compounds **3b, 3c** and **3e**. Values indicate the mean SD of two different experiments performed in triplicates.

Hoechst Staining

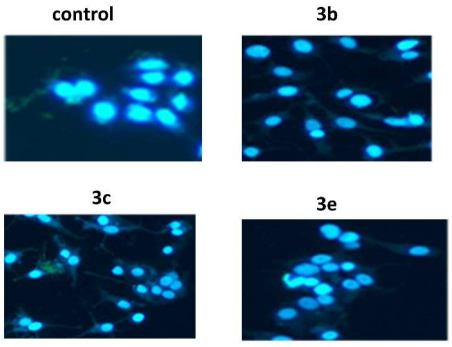


Figure 9. Hoechst staining of the conjugates (**3b, 3c** and **3e**) at 1 µM in breast cancer cells (MCF-7).

Apoptosis is one of the major pathways that leads to the process of cell death. Chromatin condensation and fragmented nuclei are known as the classic characteristics of apoptosis.[29] It was considered of interest to investigate the apoptotic inducing effect of these compounds (**3b, 3c** and **3e**) by Hoechst staining (H33258) method in MCF-7 cancer cell line. Therefore cells were treated with **3b, 3c** and **3e** at 1µM concentrations for 24 h.

Manual field quantification of apoptotic cells based on cytoplasmic condensation, presence of apoptotic bodies, nuclear fragmentation and relative fluorescence of the test compounds (**3b, 3c** and **3e**) indicate cell death by apoptosis (Figure 9).

Effect on mitochondrial depolarization

Mitochondrial membrane potential serves as a marker to estimate the overall function of mitochondria during apoptosis. It is well established that at an early stage, apoptotic stimuli alter the mitochondrial transmembrane potential (Δψmt) and this was monitored by the fluorescence of the dye 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolcarbocyanine (JC-1). It was observed that with the normal cells (high Δψmt), JC-1 displays a red fluorescence (590 nm), which is caused by the spontaneous and local formation of aggregates that are associated with a large shift in the emission. In contrast, when the mitochondrial membrane is depolarized (low Δψmt), JC-1 forms monomers that emit at 530 nm. As shown in Figure 10, compounds **3b**, **3c** and **3e** induced an increase in the proportion of cells to 11.1%, 19.1% and 20.3% respectively with depolarized mitochondria (Figure 10).[30]

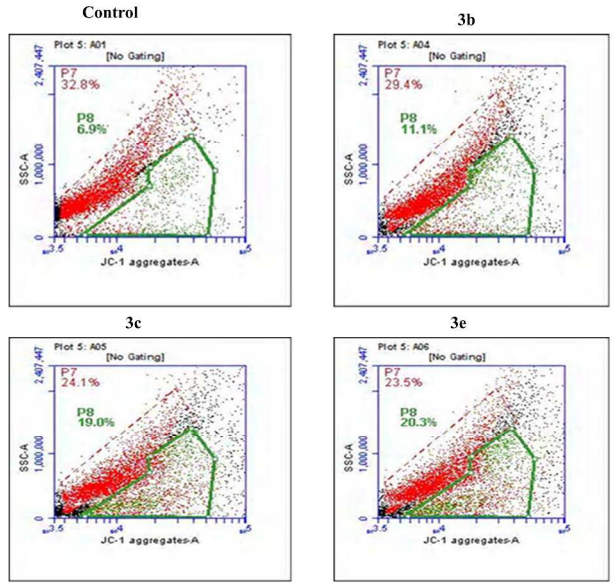


Figure 10. Assessment of Δψmt after treatment of MCF-7 cells with compounds **3b**, **3c** and **3e** Representative histograms of both control cells and cells incubated 48 h in the presence of **3b**, **3c** and **3e** as indicated and stained with the fluorescent probe JC-1 after treatment. The horizontal axis shows fluorescence intensity of the JC-1 monomer, and the vertical axis shows fluorescence of JC-1 aggregates

Annexin V-FITC/Propidium iodide analysis of apoptosis

The apoptotic effect of these conjugates was also evaluated by Annexin V FITC/PI (AV/PI) dual staining assay to examine the occurrence of phosphatidylserine externalization and as well to understand whether it is due to physiological apoptosis or nonspecific necrosis.[31] In this study MCF-7 cells were treated with 3b, 3c and 3e for 48 h at 3 μM concentration to examine the apoptotic effect. It was observed that they showed significant apoptosis against MCF-7 cells (Figure 11). Results indicate that compounds **3b**, **3c** and **3e** showed 28.5%, 21.0% and 23.8% of apoptosis respectively, whereas 10.9% was observed in control (untreated cells). This experiment also suggests that these conjugates have the ability to significantly induce apoptosis in MCF-7 cells.

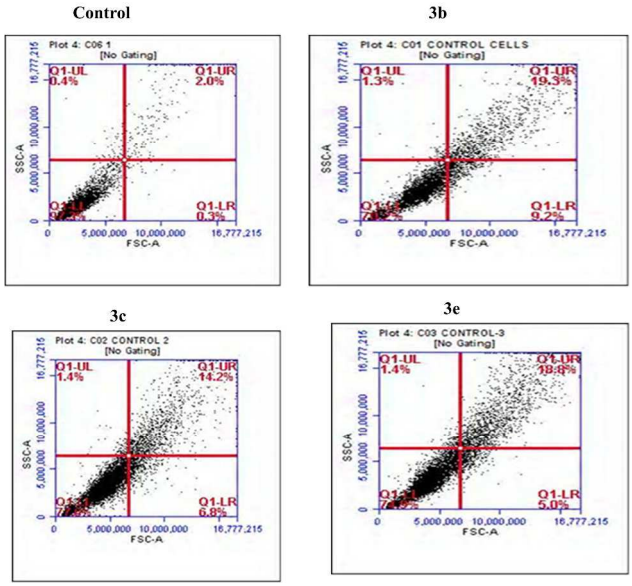


Figure 11. Annexin V-FITC staining. MCF-7 cells were treated with compounds **3b, 3c** and **3e**

DNA fragmentation assay

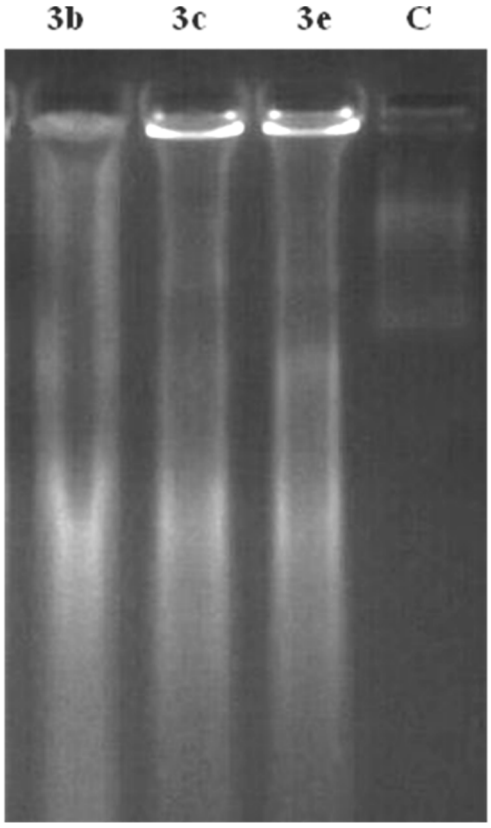


Figure 12. DNA fragmentation of the compounds **3b, 3c** and **3e** in MCF-7 cell line. Wherein C is control (untreated)

Apoptosis was also assessed by electrophoresis of extracted genomic DNA from cells.[32] Endonuclease mediated cleavage of nuclear DNA results in the formation of oligonucleosomal DNA fragment (180–200 base pairs long) a biochemical hallmark of apoptosis in many cell types. DNA laddering assay was performed with MCF-7 cells by treatment of **3b, 3c** and **3e** at 3 µM concentration for 48 h, then the genomic DNA was isolated and electrophoreses was carried out in 1.8% agarose gel. All the tested compounds induce DNA fragmentation and a characteristic ladder pattern was observed in MCF-7 cells, while no laddering was observed in the control cells as shown in the Figure 12.

Molecular modelling studies:

To investigate the possible binding mode on colchicine binding site on tubulin[33] molecular modeling study was performed for the potent molecule **3e** of the series and the coordinates of protein structure of tubulin-colchicine were obtained from the Protein Data Bank (PDB ID 3E22). Docking was accomplished into the colchicine binding sites of tubulin using AutoDock 4.2 software[34] Figure 13 shows that trimethoxybenzoyl (A ring) of 3e gets buried in the hydrophobic pocket by βLeu242, βLeu255, βAla316, βAla250 βVal238, βLeu248 and βLys352 residues located in β-tubulin similar to that of trimethoxyphenyl group of the colchicine. Whereas B and C rings interact with α and β tubulin interface however ring C extends towards the α-tubulin. Methoxyphenyl (B ring) of 3e shows hydrophobic contacts with αVal181, αThr179 and αSer178 residues where the 7-membered ring of the colchicine binds. The amine group of the C ring (4-methoxy 3-amine benzoyl) establishes hydrogen bonding interaction with αGly11, βLys254, βAsn248 and its methoxy group shows hydrogen bonding interaction with αGly11. Whereas the C ring makes hydrophobic contacts with βLeu248. Thus docking investigation suggests that conjugate **3e** interacted with both α-and β-tubulin in the colchicine pocket. Figure 14 shows that proposed binding of conjugate **3e** (green) is very similar to the pose of the colchicine (orange) with trimethoxybenzoyl placed in β-tubulin (pink) chain whereas 4-methoxy 3-amine benzoyl ring placed towards α-tubulin (blue) chain.

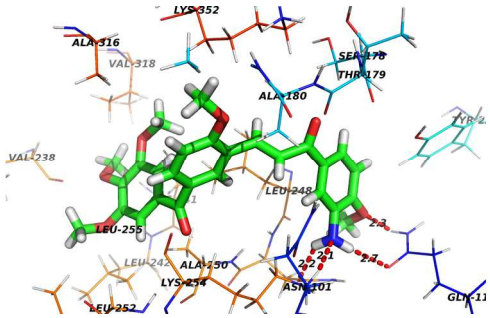


Figure 13. Interaction of the compound **3e** with colchicines binding site of tubulin. The probable hydrogen bonds found were shown in red color. This figure has been generated using the software PYMOL from the tubulin-colchicine crystal structure.

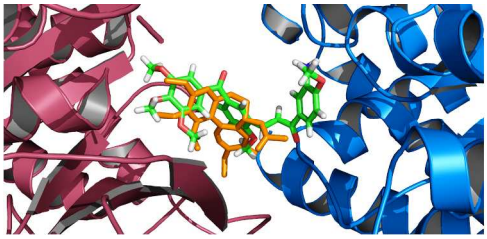


Figure 14. Compound **3e** (green) and colchicine (orange) share the same binding site on tubulin, β-chain shown in pink colour and α-chain shown in blue color.

Conclusion

In summary, a new class of phenstatin/*iso*combretastatin-chalcones (**3a-l** and **4a-l**) were designed and synthesized via Claisen-Schmidt condensation and they were investigated for their cytotoxic activity against various human cancer cell lines. Some investigated compounds **(3b-e)**, **3g**, **3j**, **4a** and **4c** exhibited significant antiproliferative activity against a panel of sixty human cancer cell lines of the NCI, with GI_{50} values in the range of 0.11 to 18.3 µM. Three compounds (**3b, 3c** and **3e**) showed broad spectrum of antiproliferative efficacy on most of the cell lines with sub-micromolar range. In addition, all the synthesized compounds (**3a-l** and **4a-l**) displayed moderate to excellent cytotoxicity against human breast cancer cell lines such as MCF-7 and MDA-MB-231 with IC_{50} value in the range of 0.5 to 19.9 µM. The tubulin polymerization assay and immunofluoresence analysis results suggest that these compounds (**3b**, **3c** and **3e)** exhibit strong inhibitory effect on the tubulin assembly with an IC_{50} value of 1.3 µM, 0.8 µM and 0.6 µM respectively, similar to that of phenstatin (**1b**). Cell cycle assay revealed that these compounds arrest at G_2/M phase of the cell cycle, thereby leading to apoptotic cell death. Occurrence of apoptotic cell death was confirmed by Hoechst 33258 staining, activation of caspase 9, DNA fragmentation, Annexin V-FITC and mitochondrial membrane depolarization**.** The competitive binding assay and docking studies indicate that these conjugates effectively bind at colchicine binding site of the tubulin. Therefore, it may be concluded that phenstatin/*iso*combretastatin-chalcones were potent inhibitors of tubulin and apoptotic inducers that are also amenable for further structural modifications in the discovery and development of effective chemotherapeutic agents.

Experimental Section

I. Chemistry

All chemicals and reagents were obtained from Sigma–Aldrich, Lancaster (Alfa Aesar, Johnson Matthey Company) or Spectrochem Pvt. Ltd. and were used without further purification. Reactions were monitored by TLC performed on silica gel coated glass plates containing 60 GF254 with visualization achieved by UV light or iodine indicator. Column chromatography was performed with Merck 60–120 mesh silica gel. ^1H and ^{13}C spectra were recorded on Bruker UXNMR/ XWIN-NMR (300 MHz) or Inova Varian-VXR-unity (400, 500 MHz) instruments. Chemical shifts (δ) are reported in ppm downfield from an internal TMS standard. ESI spectra were recorded on a Micro mass Quattro LC using ESI+ software with capillary voltage 3.98 kV and ESI

mode positive ion trap detector. High-resolution mass spectra (HRMS) were recorded on a QSTAR XL Hybrid MS–MS mass spectrometer. Melting points were determined with an Electro thermal melting point apparatus, and are uncorrected.

Preparation of methyl 5-iodo-2-methoxybenzoate (6)

To a solution of 5-Iodosalicylic acid (**5**, 5.0 g, 0.019 mol) in dry acetone (30 ml), was added anhydrous K_2CO_3 (7.85 g, 0.056 mol), dimethyl sulphate (7.16 g, 0.056 mol) at O °C. The reaction mixture was stirred at reflux temperature for 6 h. The reaction was monitored by TLC using Ethylacetate:hexane (1:19). After completion of the reaction as indicated by the TLC, after completion of the reaction as indicated by the TLC, K_2CO_3 was removed by filtration and the solvent was concentrated under the vacuum, diluted with water and extracted with ethyl acetate. The combined organic phases were dried (Na_2SO_4) and evaporated under vacuum and the residue was purified by column chromatography (40% EtOAc-hexane) to afford compound **6** as white solid (5.1 g, 92 %); mp: 47-50 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 8.06 (d, *J* = 2.28 Hz, 1H), 7.72 (dd, *J* = 2.28 Hz, 8.85 Hz, 1H), 6.73 (d, *J* = 8.85 Hz, 1H), 3.88 (s, 3H), 3.87 (s, 3H); ESI-MS: m/z 292 $[M+1]^+$.

Preparation of (5-iodo-2-methoxyphenyl)methanol (7)

To a solution of methyl 5-iodo-2-methoxybenzoate (**6,** 5.0 g, 0.017 mol) in dry CH_2Cl_2 (50 ml) was added drop wise DIBAL (34 mL, 1.0M in hexane, 0.034 mol) at 0 °C. The reaction mixture was stirred at room temperature for 2 h. The reaction was monitored by TLC using ethyl acetate-hexane (4:6). After completion of the reaction as indicated by the TLC, Saturated ammonium chloride solution was added to the reaction mixture. Salts were removed by filtration and the solvent was dried (Na_2SO_4) and evaporated under vacuum and it was taken as such for the next step without further purification.

Preparation of tert-butyl (5-iodo-2-methoxybenzyloxy)dimethylsilane (8)

To a solution of (5-iodo-2-methoxyphenyl)methanol (**7**, 4.0 g, 0.015 mol) in dry CH_2Cl_2(30 ml) was added imidazole (1.16 g, 0.017 mol) and TBDMSCl (2.56 g, 0.017 mol) at 0 °C. The reaction mixture was stirred at room temperature for 3 h. The reaction was monitored by TLC using ethyl acetate-hexane (1:10). After completion of the reaction as indicated by the TLC, The water was added to the reaction mixture and separates the organic layer. The solvent was dried (Na_2SO_4) and evaporated under vacuum and the residue was purified by column chromatography (5% concentrated under the vacuum, the combined organic phases EtOAc-hexane) to afford compound **8** as colourless oil (5.2 g, 91%); bp: 287-289 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 7.72 (d, *J* = 2.26 Hz, 1H), 7.48 (dd, *J* = 2.26 Hz, 8.31 Hz, 1H), 6.56 (d, *J* = 8.31 Hz, 1H), 4.68 (s, 2H), 3.78 (s, 3H), 0.95 (s, 9H), 0.11 (s, 6H); ESI-MS: m/z 379 $[M+1]^+$.

Preparation of (3-((tert-butyldimethylsilyloxy)methyl)-4-methoxyphenyl)(3,4,5-trimethoxyphenyl)methanol (10)

To a solution of tert-butyl(5-iodo-2-methoxybenzyloxy)dimethylsilane (**8**, 4.0 g, 0.011 mol) in dry THF (20 ml) was added dropwise n-BuLi (7.5 mL, 1.6 Min hexane, 0.012 mol) at -78 °C. After 1 h, a solution of 3,4,5 trimethoxybenzaldehyde (**9**, 1.96 g, 0.01 mol) in dry THF (4 mL) was added drop wise at -78 °C. After 1 h stirring at rt, The reaction was monitored by TLC using ethyl acetate-hexane (4:6). After completion of the reaction as indicated by the TLC, Saturated ammonium chloride solution (10 mL) was added to the reaction mixture. The reaction mass solvent was evaporated under the vacuum, diluted with water and extracted with ethyl acetate. The combined organic layers were washed with 1 N HCl and then brine water (20 mL), dried over Na_2SO_4, filtered, and concentrated in vacuum. Purification of the residue by column chromatography on silica gel (hexane/EtOAc, 7:3) afforded the desired product **10** as a white colour solid (5.8 g, 63%); mp: 41-43 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 7.41 (d, *J* = 1.98 Hz, 1H), 7.25 (dd, *J* = 1.98 Hz, 8.39 Hz, 1H), 6.79 (d, *J* = 8.39 Hz, 1H), 6.62 (s, 2H), 5.75 (s, 1H), 4.72 (s, 2H), 3.82 (s, 6H), 3.81 (s, 3H), 3.80 (s, 3H), 0.91 (s, 9H), 0.05(d, *J* = 1.67 Hz, 6H); ESI-MS: m/z 449 $[M+1]^+$.

Preparation of (3-((tert-butyldimethylsilyloxy)methyl)-4-methoxyphenyl)(3,4,5-trimethoxyphenyl)methanone (11)

To a solution of (3-((tert-butyldimethylsilyloxy)methyl)-4-methoxyphenyl)(3,4,5-trimethoxyphenyl)methanol (**10**, 1.0 g, 2.2 mmol) in dry DMSO (5 ml) was added, a solution of 2-iodoxy-benzoic acid (IBX) (0.686 g, 2.45 mmol) in dimethyl sulfoxide (DMSO) (10 mL) at 10-15 °C. The reaction mixture was stirred at room temperature for 2 h. The reaction was monitored by TLC using ethyl acetate-hexane (1:4). After completion of the reaction as indicated by the TLC, Appropriate amount water was added to the reaction mixture and filtered through the celite bead. The aqueous layer was extracted with ethyl acetate. The organic layer was washed with water, brine, dried over anhydrous Na_2SO_4, and evaporated by using vacuum to get crude compounds. The residue was purified by column chromatography (10% concentrated under the vacuum, the combined organic phases EtOAc-hexane) to afford compound **11** as light yellow solid (820 mg, 82%); mp: 48-49 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 7.97 (d, *J* = 2.3 Hz, 1H), 7.80 (dd, *J* = 2.3 Hz, 8.3 Hz, 1H), 7.02 (s, 2H), 6.90 (d, *J* = 8.3 Hz, 1H), 4.76 (s, 2H), 3.92 (s, 3H), 3.91 (s, 3H), 3.87 (s, 6H), 0.90 (s, 9H), 0.09 (s, 6H); ESI-MS: m/z 447 $[M+1]^+$.

Preparation of tert-butyl(2-methoxy-5-(1-(3,4,5-trimethoxyphenyl)vinyl)benzyloxy) dimethylsilane (12)

A solution of PPh_3CH_3Br (725.8 mg, 2.02 mmol) in dry THF (15 mL) was added, potassium *tert*-butoxide (188.4 mg, 1.68 mmol) at 10-15 °C under an argon atmosphere. The yellow colour mixture was stirred for 4 h. The (3-((tert-butyldimethylsilyloxy)methyl)-4-methoxyphenyl)(3,4,5-trimethoxyphenyl)methanone (**11**, 500 mg, 1.12 mmol) in THF was added drop wise at 10-15 °C and reaction mixture was allowed to stirred for another 3 h. The reaction was monitored by TLC using ethyl acetate-hexane (1:5). After completion of the reaction as indicated by the TLC, Appropriate amount of saturated ammonium chloride solution was added to the reaction mixture and concentrate it. The aqueous layer was extracted with ethyl acetate. The organic layer was washed with water, dried over anhydrous Na_2SO_4, and evaporated by using vacuum to get crude compounds. The residue was purified by column chromatography (10% concentrated under the vacuum, the combined organic phases EtOAc-hexane) to afford compound **12** as light yellow solid (400 mg, 80%); mp: 60-63 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 7.48 (d, *J* = 2.26 Hz, 1H), 7.21 (dd, *J*

= 2.26 Hz, 8.49 Hz, 1H), 6.78 (d, *J* = 8.49 Hz, 1H), 6.64 (s, 2H), 5.39 (d, *J* = 1.13 Hz, 1H), 5.28 (d, *J* = 1.32 Hz, 1H), 4.74 (s, 2H), 3.85 (s, 3H), 3.84 (s, 3H), 3.81 (s, 6H), 0.88 (s, 9H), 0.07 (s, 6H); ESI-MS: m/z 445 $[M+1]^+$.

Preparation of (3-(hydroxymethyl)-4-methoxyphenyl)(3,4,5-trimethoxyphenyl)methanone (13a)

To a solution of (3-((tert-butyldimethylsilyloxy)methyl)-4-methoxyphenyl)(3,4,5-trimethoxyphenyl)methanone (**11**, 800 mg, 1.79 mmol) in dry THF (10 ml) was added, a solution of tetra butyl ammonium fluoride (2.15 mL, 1.0 M in THF, 2.15 mmol) at 10-15 °C. The reaction mixture was stirred at room temperature for 3 h. The reaction was monitored by TLC using ethyl acetate-hexane (4:6). After completion of the reaction as indicated by the TLC, Appropriate amount of saturated ammonium chloride solution was added to the reaction mixture and concentrate it. The aqueous layer was extracted with ethyl acetate. The organic layer was washed with water, dried over anhydrous Na_2SO_4, and evaporated by using vacuum to get crude compounds. The residue was purified by column chromatography (30% concentrated under the vacuum, the combined organic phases EtOAc-hexane) to afford compound **13a** as light yellow solid (450 mg, 76%); mp: 45-47 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 7.91 (d, *J* = 2.26 Hz, 1H), 7.77 (dd, *J* = 2.26 Hz, 8.31 Hz, 1H), 6.97 (s, 2H), 6.85 (d, *J* = 8.31 Hz, 1H), 4.71 (s, 2H), 3.89 (s, 3H), 3.86 (s, 3H), 3.82 (s, 6H); ESI-MS: m/z 333$[M+1]^+$.

Preparation of (2-methoxy-5-(1-(3,4,5-trimethoxyphenyl)vinyl)phenyl)methanol (13b)

This compound was prepared according to the method described for compound **13a**, employing tert-butyl(2-methoxy-5-(1-(3,4,5-trimethoxyphenyl)vinyl)benzyloxy)dimethyl silane (**12**, 500 mg, 1.124 mmol) to obtain the pure product **13b** as light yellow solid (300 mg, 81%); mp: 59-61 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 7.32 (d, *J* = 2.26 Hz, 1H), 7.23 (d, *J* = 2.26 Hz, 1H), 6.86 (d, *J* = 8.49 Hz, 1H), 6.54 (s, 2H), 5.35 (dd, *J* = 1.13 Hz, 12.8 Hz, 2H), 4.69 (s, 2H), 3.89 (s, 3H), 3.88 (s, 3H), 3.81 (s, 6H); ESI-MS: m/z 331 $[M+1]^+$.

Preparation of 2-methoxy-5-(3,4,5-trimethoxybenzoyl)benzaldehyde (14a)

A solution of DMSO (0.258 g, 3.3 mmol) in dry CH_2Cl_2(3 mL) was added drop wise to a magnetically stirred solution of oxalyl chloride (209 mg, 1.65 mmol) in dry CH_2Cl_2(5.0 mL) kept at -78 °C under an argon atmosphere. The mixture was stirred for another 15 min. The CH_2Cl_2 of (3-(hydroxymethyl)-4-methoxyphenyl)(3,4,5-trimethoxyphenyl)methanone (**13a**, 500 mg, 1.5 mmol) was added drop wise and reaction mixture was allowed to stirred for 3 h. Triethylamine (607 mg, 6.0 mmol) was added drop wise over 5 min, and the stirred solution was allowed to warm to room temperature. The appropriate amount of water was added to reaction mixture and organic layer was separated and the aqueous phase extracted with CH_2Cl_2 (3 ×20 mL). The combined organic layer were washed with brine (10 mL), dried over anhydrous Na_2SO_4 and evaporated by using vacuum to get crude compound. The residue was purified by column chromatography (30% concentrated under the vacuum, the combined organic phases EtOAc-hexane) to afford compound **14a** as light yellow solid (420 mg, 84%); mp: 74-76 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 10.49 (s, 1H), 8.29 (d, *J* = 2.26 Hz, 1H), 8.13 (dd, *J* = 2.26 Hz, 8.87 Hz, 1H), 7.15 (d, *J* = 8.87 Hz, 1H), 7.01 (s, 2H), 4.05 (s, 3H), 3.95 (s, 3H), 3.87 (s, 6H); ESI-MS: m/z 331 $[M+1]^+$.

Preparation of 2-methoxy-5-(1-(3,4,5-trimethoxyphenyl)vinyl)benzaldehyde (14b)

This compound was prepared according to the method described for compound **14a**, employing (2-methoxy-5-(1-(3,4,5-trimethoxyphenyl)vinyl)phenyl)methanol (**13b**, 500 mg, 1.51 mmol)to obtain the pure product **14b** as light yellow solid (419 mg, 84%); mp: 89-92 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 10.48 (s, 1H), 7.88 (d, *J* = 2.26 Hz, 1H), 7.52 (dd, *J* = 2.26 Hz, 8.49 Hz, 1H), 6.98 (d, *J* = 8.49 Hz, 1H), 6.50 (s, 2H), 5.41 (dd, *J* = 0.92 Hz, 14.3 Hz, 2H), 3.96 (s, 3H), 3.88 (s, 3H), 3.81 (s, 6H); ESI-MS: m/z 329 $[M+1]^+$.

(*E*)-3-(2-methoxy-5-(3,4,5-trimethoxybenzoyl)phenyl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (3a)

a 10% aqueous solution of KOH (5 mL) was added to a stirred solution of 2-methoxy-5-(3,4,5-trimethoxybenzoyl)benzaldehyde (**14a**, 100 mg, 0.303 mmol) and 3,4,5-trimethoxyphenyl acetophenone (**15a**, 63.6 mg, 0.303 mmol) in ethanol (20 mL). The reaction mixture was stirred at room temperature 27 °C for 6 h and was monitored by TLC. After completion of the reaction the solvent was evaporated under vacuum, then the residue was dissolved in EtOAc/H_2O. The organic layer was washed with brine and evaporated. This was further purified by column chromatography using EtOAc/Hex (1:1) as a solvent system to obtain the pure product as light yellow colour solid (120 mg, 76% yield); mp: 181-183 °C; IR (KBr): ῡmax/cm^{-1} - 2945, 2837, 1653, 1587, 1504, 1465, 1441, 1415, 1336, 1287, 1262, 1237, 1192, 1129, 1035, 1020, 989, 938, 862, 841, 811, 797, 764, 747, 551; ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 8.19 (d, *J* = 1.88 Hz, 1H), 8.13 (d, *J* = 15.8 Hz, 1H), 7.87 (dd, *J* = 1.88 Hz, 8.67 Hz, 1H), 7.58 (d, J = 15.8 Hz, 1H), 7.28 (s, 2H), 7.04 (s, 2H), 7.03 (d, J = 8.69 Hz, 1H), 4.02 (s, 3H), 3.98 (s, 9H), 3.95 (s, 3H), 3.89 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 194.2, 188.6, 161.5, 152.7, 153.1, 149.0, 137.9, 133.7, 132.7, 130.7, 130.1, 124.1, 123.3, 123.0, 110.6, 110.4, 109.8, 107.3, 60.8, 56.2, 56.0, 55.9; ESI-MS: m/z 545 $[M+Na]^+$; HRMS (ESI m/z) for $C_{29}H_{30}O_9Na$ calcd: 545.17820, found: 545.17746 $[M+ Na]^+$.

(*E*)-1-(3,4-dimethoxyphenyl)-3-(2-methoxy-5-(3,4,5-trimethoxybenzoyl)phenyl)prop-2-en-1-one (3b)

This compound was prepared according to the method described for compound **3a**, employing 2-methoxy-5-(3,4,5-trimethoxybenzoyl)benzaldehyde (**14a**, 100 mg, 0.303 mmol) and 3,4-dimethoxy acetophenone (**15b**, 54.5 mg, 0.303 mmol) to obtain the pure product **3b** as a yellow solid (122 mg, 82 % yield); mp: 178-180 °C; IR (KBr): $\bar{\upsilon}_{max}$/cm^{-1}- 3080, 2998, 2938, 2835, 2608, 2029, 1655, 1578, 1515, 1459, 1412, 1330, 1261, 1129, 1020, 857, 827, 767, 746, 700, 671, 634, 601, 545; ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 8.19 (d, *J* = 1.88 Hz, 1H), 8.13 (d, *J* = 15.8 Hz, 1H), 7.87 (dd, *J* = 1.88 Hz, 8.67 Hz, 1H), 7.70-7.63 (m, 3H), 7.05 (s, 2H), 7.01 (d, *J* = 8.87 Hz, 1H), 6.93 (d, *J* = 8.31 Hz, 1H), 4.02 (s, 3H), 3.98 (s, 6H), 3.95 (s, 3H), 3.89 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 194.2, 188.6, 161.5, 152.7, 153.1, 149.0, 137.9, 133.7, 132.7, 130.7, 130.1, 124.1, 123.3, 123.0, 110.6, 110.4, 109.8, 107.3, 60.8, 56.2, 56.0, 55.9; ESI-MS: m/z 515 $[M+Na]^+$; HRMS (ESI m/z) for $C_{28}H_{28}O_8Na$ calcd: 515.16764, found: 515.16695 $[M+Na]^+$.

(*E*)-1-(benzo[d][1,3]dioxol-5-yl)-3-(2-methoxy-5-(3,4,5-trimethoxybenzoyl)phenyl)prop-2-en-1-one (3c)

This compound was prepared according to the method described for compound **3a**, employing 2-methoxy-5-(3,4,5-trimethoxybenzoyl)benzaldehyde (**14a**, 100 mg, 0.303 mmol) and 1-(benzo[d][1,3]dioxol-5-yl)ethanone (**15c**, 49.7 mg, 0.303 mmol) to obtain the pure product **3c** as a yellow solid (115 mg, 80 % yield); mp: 181-179 °C; IR (KBr): $\bar{\upsilon}_{max}/cm^{-1}$- 2945, 2837, 1653, 1587, 1504, 1465, 1441, 1415, 1336, 1287, 1262, 1237, 1192, 1129, 1035, 1020, 989, 938, 862, 841, 811, 797, 764, 747, 551; ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm): 8.16 (d, *J* = 2.13 Hz, 1H), 8.10 (d, *J* = 15.8 Hz, 1H), 7.86 (dd, *J* = 2.13 Hz, 8.54 Hz, 1H), 7.66 (dd, *J* = 1.67 Hz, 8.24 Hz, 1H), 7.62 (d, *J* = 15.8 Hz, 1H), 7.53 (d, *J* = 1.67 Hz, 1H), 7.04 (s, 2H), 7.01 (d, *J* = 8.54 Hz, 1H), 6.90 (d, *J* = 8.24 Hz, 1H), 6.07 (s, 2H), 4.02 (s, 3H), 3.95 (s, 3H), 3.89 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 193.4, 187.5, 160.9, 151.1, 147.6, 141.0, 137.6, 133.7, 132.1, 132.1, 130.2, 129.5, 124.1, 122.8, 123.2, 110.2, 107.5, 107.2, 106.6, 101.2, 60.1, 55.6, 55.4; ESI-MS: m/z 499 $[M+Na]^+$; HRMS (ESI m/z) for $C_{27}H_{24}O_8Na$ calcd: 499.13634, found: 499.13549 $[M+Na]^+$.

(*E*)-3-(2-methoxy-5-(3,4,5-trimethoxybenzoyl)phenyl)-1-(4-methoxyphenyl)prop-2-en-1-one (3d)

This compound was prepared according to the method described for compound **3a**, employing 2-methoxy-5-(3,4,5-trimethoxybenzoyl)benzaldehyde (**14a**, 100 mg, 0.303 mmol) and 4-methoxy acetophenone (**15d**, 45.5 mg, 0.303 mmol) to obtain the pure product **3d** as a yellow solid (110 mg, 79 % yield); mp: 166-164 °C; IR (KBr): $\bar{\upsilon}_{max}/cm^{-1}$- 3003, 2941, 2837, 1660, 1646, 1606, 1589, 1505, 1457, 1411, 1338, 1308, 1288, 1268, 1255, 1223, 1168, 1130, 1020, 989, 977, 867, 839, 805, 764, 748, 666, 642, 615, 549, 508; ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm): 8.17 (d, *J* = 1.88 Hz, 1H), 8.12 (d, *J* = 15.8 Hz, 1H), 8.02 (d, *J* = 8.7 Hz, 2H), 7.84 (dd, *J* = 2.07 Hz, 6.6 Hz, 1H), 7.66 (d, *J* = 15.8 Hz, 1H), 7.04-7.03 (m, 2H), 7.00 (d, *J* = 9.06 Hz, 3H), 4.01 (s, 3H), 3.95 (s, 3H), 3.89 (s, 9H); ^{13}C NMR (CDCl3, 75 MHz) δ (ppm):192.2, 186.7, 161.9, 160.0, 158.1, 151.3, 140.1, 136.3, 132.3, 131.2, 129.3, 129.2, 128.6, 122.3, 122.0, 112.4, 109.4, 105.9, 59.1, 54.7, 54.1; ESI-MS: m/z 463 [M+1]+; HRMS (ESI m/z) for $C_{27}H_{26}O_7$ calcd: 463.1756, found: 463.1752 $[M+1]^+$.

(*E*)-1-(3-amino-4-methoxyphenyl)-3-(2-methoxy-5-(3,4,5-trimethoxybenzoyl)phenyl) prop-2-en-1-one (3e)

This compound was prepared according to the method described for compound **3a**, employing 2-methoxy-5-(3,4,5-trimethoxybenzoyl)benzaldehyde (**14a**, 100 mg, 0.303 mmol) and 4-methoxy 3-amino acetophenone (**15e**, 50.4 mg, 0.303 mmol) to obtain the pure product **3e** as a yellow solid (105 mg, 73 % yield): mp: 152-154 °C; IR (KBr): $\bar{\upsilon}_{max}/cm^{-1}$- 3416, 2932, 2839, 1654, 1597, 1578, 1506, 1462, 1442, 1414, 1335, 1293, 1277, 1259, 1229, 1192, 1131, 1020, 990, 863, 840, 817, 796, 763, 751, 556; ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm): 8.18 (s, 1H), 8.12 (d, *J* = 15.7 Hz, 1H), 7.86 (d, *J* = 8.55 Hz, 1H), 7.64 (d, *J* = 15.5 Hz, 1H), 7.49 (d, *J* = 8.39 Hz, 1H), 7.44 (s, 1H), 7.05 (s, 2H), 7.01 (d, *J* = 8.69 Hz, 1H), 6.85 (d, *J* = 8.39 Hz, 1H), 4.01 (s, 3H), 3.95 (s, 3H), 3.94 (s, 3H), 3.89 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 194.3, 189.0, 161.5, 152.8, 151.2, 137.4, 136.3, 133.5, 132.8, 131.2, 130.1, 130.6, 124.2, 123.6, 120.4, 114.2, 110.4, 109.1, 107.4, 60.8, 56.2, 55.8, 55.5; ESI-MS: m/z 478 [M+1]+; HRMS (ESI m/z) for $C_{27}H_{28}NO_7$ calcd: 478.18603, found: 478.18372 [M+1]+.

(*E*)-1-(4-aminophenyl)-3-(2-methoxy-5-(3,4,5-trimethoxybenzoyl)phenyl)prop-2-en-1-one (3f)

This compound was prepared according to the method described for compound **3a**, employing 2-methoxy-5-(3,4,5-trimethoxybenzoyl)benzaldehyde (**14a**, 100 mg, 0.303 mmol) and 4-amino acetophenone (**15f**, 41.3 mg, 0.303 mmol) to obtain the pure product **3f** as a yellow solid (105 mg, 78% yield); mp: 140-143 °C; IR (KBr): $\bar{\upsilon}_{max}/cm^{-1}$- 3466, 3366, 3234, 2938, 2839, 1649, 1604, 1582, 1561, 1503, 1464, 1414, 1334, 1286, 1260, 1234, 1216, 1172, 1126, 1016, 1004, 985, 864, 834, 802, 768, 751, 559; ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm):8.17 (s, 1H), 8.11 (d, *J* = 15.8 Hz, 1H), 7.91 (d, *J* = 8.24 Hz, 2H), 7.83 (d, *J* = 8.54 Hz, 1H), 7.67 (d, *J* = 15.7 Hz, 1H), 7.05 (s, 2H), 7.02 (d, *J* = 8.54 Hz, 1H), 6.69 (d, *J* = 8.39 Hz, 2H), 4.27 (bp, 2H), 4.00 (s, 3H), 3.95 (s, 3H), 3.89 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 194.3, 188.1, 161.5, 152.7, 151.2, 137.1, 133.5, 132.8, 131.0, 130.6, 130.1, 128.2, 124.3, 123.7, 113.5, 110.4, 107.3, 60.8, 55.8, 56.2; ESI-MS: m/z 448 [M+1]+; HRMS (ESI m/z) for $C_{26}H_{26}NO_6$calcd: 448.17546, found: 448.17355 [M+1]+.

(*E*)-1-(4-fluorophenyl)-3-(2-methoxy-5-(3,4,5-trimethoxybenzoyl)phenyl)prop-2-en-1-one (3g)

This compound was prepared according to the method described for compound **3a**, employing 2-methoxy-5-(3,4,5-trimethoxybenzoyl)benzaldehyde (**14a**, 100 mg, 0.303 mmol) and 4-fluoro acetophenone (**15g**, 41.8 mg, 0.303 mmol) to obtain the pure product **3g** as a white solid (99 mg, 73% yield); mp: 114-116 °C; IR (KBr): $\bar{\upsilon}_{max}/cm^{-1}$- 3607, 2942, 2836, 2624, 1665, 1600, 1582, 1504, 1410, 1333, 1287, 1258, 1230, 1214, 1171, 1157, 1130, 1007, 978, 868, 834, 763, 747, 703, 665; ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm): 8.18 (d, *J* = 2.13 Hz, 1H), 8.13 (d, *J* = 15.8 Hz, 1H), 7.96 (d, *J* = 8.54 Hz, 2H), 7.86 (dd, *J* =2.14 Hz, 8.69 Hz, 1H), 7.61 (d, *J* = 15.8 Hz, 1H), 7.47 (d, *J* = 8.54 Hz, 2H), 7.04 (s, 2H), 7.01 (s, 1H), 4.02 (s, 3H), 3.95 (s, 3H), 3.89 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 194.3, 189.1, 161.7, 152.9, 139.2, 134.4, 134.1, 132.8, 131.1, 130.3, 123.9, 123.3, 115.8,115.6, 110.5, 107.4, 107.3, 61.0, 56.3, 56.0; ESI-MS: m/z 451 $[M+1]^+$; HRMS (ESI m/z) for $C_{26}H_{24}FO_6$ calcd: 451.15514, found: 451.15293 $[M+1]^+$.

(*E*)-1-(4-chlorophenyl)-3-(2-methoxy-5-(3,4,5-trimethoxybenzoyl)phenyl)prop-2-en-1-one (3h)

This compound was prepared according to the method described for compound **3a**, employing 2-methoxy-5-(3,4,5-trimethoxybenzoyl)benzaldehyde (**14a**, 100 mg, 0.303 mmol) and 4-chloro acetophenone (**15h**, 46.8 mg, 0.303 mmol) to obtain the pure product **3h** as a white solid (125 mg, 88% yield); mp: 170-172 °C; IR (KBr): $\bar{\upsilon}_{max}/cm^{-1}$- 2942, 2836, 1667, 1642, 1596, 1504, 1454, 1413, 1334, 1287, 1262, 1234, 1214, 1129, 1090, 1010, 865, 830, 803, 769, 745, 665, 641, 562, 527; ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm): 8.17 (d, *J* = 2.14 Hz, 1H), 8.13 (d, *J* = 15.8 Hz, 1H), 8.08-8.03 (m, 2H), 7.87 (dd, *J* = 2.14 Hz, 8.69 Hz, 1H), 7.63 (d, *J* = 15.8 Hz, 1H), 7.18 (t, *J* = 8.69 Hz, 14.8 Hz, 2H), 7.04 (s, 2H), 7.02 (d, *J* = 8.39 Hz, 1H), 4.02 (s, 3H), 3.95 (s, 3H), 3.89 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 193.2, 188.3, 161.1, 152.7, 141.1, 138.7, 138.3, 135.8, 133.6, 132.1, 130.3, 129.6, 129.5, 128.3, 123.1, 122.6, 110.3, 106.8, 60.1, 55.6,

55.1; ESI-MS: m/z 467 [M+1]+; HRMS (ESI m/z) for $C_{26}H_{24}ClO_6$ calcd: 467.1261, found: 467.1245 $[M+1]^+$.

(*E*)-1-(4-bromophenyl)-3-(2-methoxy-5-(3,4,5-trimethoxybenzoyl)phenyl)prop-2-en-1-one (3i)

This compound was prepared according to the method described for compound **3a**, employing 2-methoxy-5-(3,4,5-trimethoxybenzoyl)benzaldehyde (**14a**, 100 mg, 0.303 mmol) and 4-bromo acetophenone (**15i**, 60.3 mg, 0.0303 mmol) to obtain the pure product **3i** as a white solid (129 mg, 84 % yield): mp: 128-130 °C; IR (KBr): $\bar{\upsilon}_{max}/cm^{-1}$- 3002, 2924, 2853, 1660, 1646, 1606, 1589, 1505, 1457, 1411, 1338, 1308, 1288, 1268, 1255,1223, 1168, 1130, 1020, 989, 977, 867, 839, 805, 772, 763, 666, 642, 615, 549, 507; ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm): 8.17 (d, *J* = 2.14 Hz, 1H), 8.10 (d, *J* = 15.8 Hz, 1H), 8.04 (dd, *J* = 1.98 Hz, 8.85 Hz, 2H), 7.85 (dd, *J* = 2.14 Hz, 8.69 Hz, 1H), 7.66 (d, *J* = 15.8 Hz, 1H), 7.04 (s, 2H), 7.01 (d, *J* = 8.69 Hz, 1H), 6.98 (dd, *J* = 1.98 Hz, 8.85 Hz, 2H), 4.02 (s, 3H), 3.95 (s, 3H), 3.89 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 194.1, 188.6, 163.2, 161.5, 152.7, 141.6, 137.9, 133.6, 132.7, 130.7, 130.1, 124.0, 123.4, 113.6, 110.4, 107.8, 107.2, 107.1, 63.8, 55.8, 55.1; ESI-MS: m/z 533 [M+Na]+; HRMS (ESI m/z) for $C_{26}H_{23}BrO_6$ calcd: 533.0575, found: 533.0552 [M+Na]+ and 535.0466 ^{81}[M+Na]+

(*E*)-1-(4-hydroxyphenyl)-3-(2-methoxy-5-(3,4,5-trimethoxybenzoyl)phenyl)prop-2-en-1-one (3j)

This compound was prepared according to the method described for compound **3a**, employing 2-methoxy-5-(3,4,5-trimethoxybenzoyl)benzaldehyde (**14a**, 100 mg, 0.303 mmol) and 1-(4-((tert-butyldimethylsilyl)oxy)phenyl)ethanone (**15j**, 81.6 mg, 0.333 mmol) to obtain the pure product **3j** as a white solid (88 mg, 65 % yield); mp: 152-154 °C; IR (KBr): $\bar{\upsilon}_{max}/cm^{-1}$- 3419, 2927, 1651, 1606, 1579, 1504, 1457, 1411, 1335, 1284, 1260, 1223, 1166, 1129, 1018, 984, 839, 763, 749; ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 8.29 (m, 2H), 7.69 (d, *J* = 8.31 Hz, 2H), 7.83-7.69 (m, 2H), 7.19 (d, *J* = 8.68 Hz, 1H), 7.03 (s, 2H), 6.87 (d, *J* = 8.49 Hz, 2H), 3.85 (s, 6H), 3.83 (s, 3H), 3.35 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 191.8, 186.1, 160.7, 159.7, 151.1, 144.9, 135.5, 132.1, 131.06, 129.3, 128.7, 128.4, 122.2, 121.9, 113.8, 113.6, 109.7, 105.7, 58.8, 54.5; ESI-MS: m/z 471 [M+Na]+; HRMS (ESI m/z) for $C_{26}H_{24}O_7Na$ calcd: 471.14142, found: 471.14145 [M+Na]+.

(*E*)-1-(3-hydroxy-4-methoxyphenyl)-3-(2-methoxy-5-(3,4,5-trimethoxybenzoyl)phenyl) prop-2-en-1-one (3k)

This compound was prepared according to the method described for compound **3a**, employing 2-methoxy-5-(3,4,5-trimethoxybenzoyl)benzaldehyde (**14a**, 100 mg, 0.303 mmol) and 1-(3-((tert-butyldimethylsilyl)oxy)-4-methoxyphenyl)ethanone (**15k**, 91.6 mg, 0.333 mmol) to obtain the pure product **3k** as a white solid (89 mg, 62 % yield); mp: 184-186 °C; IR (KBr): $\bar{\upsilon}_{max}/cm^{-1}$- 3429, 2934, 2839, 1651, 1580, 1504, 1455, 1412, 1334, 1261, 1230, 1170, 1125, 1018, 860, 836, 764, 749; ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm): 8.16 (d, *J* = 1.98 Hz, 1H), 8.09 (d, *J* = 15.7 Hz, 1H), 7.85 (dd, *J* = 1.98 Hz, 8.56 Hz, 1H), 7.65-7.62 (m, 3H), 7.05 (s, 2H), 7.01 (d, *J* = 8.54 Hz, 1H), 6.93 (d, *J* = 8.85 Hz, 1H), 5.82 (s, 1H), 4.01 (s, 3H), 3.97 (s, 3H), 3.95 (s, 3H), 3.89 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 194.2, 188.9, 161.6, 152.8, 150.6, 145.5, 141.8, 138.2, 133.7, 132.8, 131.7, 130.9, 130.2, 124.1, 123.5, 122.1, 114.6, 110.5, 109.9, 107.4, 60.9, 56.2, 56.0, 55.9; ESI-MS: m/z 501 $[M+Na]^+$; HRMS (ESI m/z) for $C_{27}H_{26}O_8Na$ calcd: 501.15199, found: 501.15244 $[M+Na]^+$.

(*E*)-3-(2-methoxy-5-(3,4,5-trimethoxybenzoyl)phenyl)-1-(naphthalen-2-yl)prop-2-en-1-one (3l)

This compound was prepared according to the method described for compound **3a**, employing 2-methoxy-5-(3,4,5-trimethoxybenzoyl)benzaldehyde (**14a**, 100 mg, 0.303 mmol) and 1-(naphthalen-2-yl)ethanone (**15l**, 51.6 mg, 0.303 mmol) to obtain the pure product **3l** as a white solid (130 mg, 89 % yield): mp: 145-148 °C; IR (KBr): $\bar{\upsilon}_{max}/cm^{-1}$- 3058, 2973, 2941, 2839, 1664, 1638, 1582, 1503, 1465, 1412, 1335, 1287, 1262, 1232, 1184, 1125, 1010, 984, 893, 854, 828, 753, 672, 644, 562, 478; ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 8.56 (s, 1H), 8.25 (d, *J* = 2.26 Hz, 1H), 8.18 (d, *J* = 15.8 Hz, 1H), 8.10 (d, *J* = 1.51 Hz, 8.31 Hz, 1H), 8.00 (d, *J* = 8.31 Hz, 1H), 7.96-7.85 (m, 3H), 7.79 (d, *J* = 15.8 Hz, 1H), 7.63-7.53 (m, 2H), 7.06 (s, 2H), 7.02 (d, *J* = 9.1 Hz, 1H), 4.03 (s, 3H), 3.96 (s, 3H), 3.90 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 194.3, 190.3, 161.6, 152.8, 141.7, 138.7, 135.4, 133.9, 132.8, 130.8, 130.3, 130.0, 129.4, 128.4, 127.7, 126.7, 124.4, 124.1, 123.7, 110.5, 107.4, 60.9, 56.2, 55.9; ESI-MS: m/z 505 $[M+Na]^+$; HRMS (ESI m/z) for $C_{30}H_{26}O_6Na$ calcd: 505.1627, found: 505.1643 $[M+Na]^+$.

(*E*)-3-(2-methoxy-5-(1-(3,4,5-trimethoxyphenyl)vinyl)phenyl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (4a)

This compound was prepared according to the method described for compound **3a**, employing 2-methoxy-5-(1-(3,4,5-trimethoxyphenyl)vinyl)benzaldehyde (**14b**, 100 mg, 0.305 mmol) and 3,4,5-trimethoxy acetophenone (**15a**, 64.05 mg, 0.305 mmol) to obtain the pure product **4a** as a white solid (130 mg, 82% yield); mp: 160-162 °C; IR (KBr): $\bar{\upsilon}_{max}/cm^{-1}$- 3064, 2968, 2938, 2836, 1726, 1659, 1581, 1502, 1460, 1411, 1339, 1281, 1260, 1229, 1188, 1159, 1123, 1068, 1023, 998, 980, 963, 922, 881, 851, 819, 779, 752, 725, 713; ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm): 8.05 (d, *J* = 15.8 Hz, 1H), 7.63 (d, *J* = 1.52 Hz, 1H), 7.53 (d, *J* = 15.8 Hz, 1H), 7.38 (dd, *J* = 1.83 Hz, 8.54 Hz, 1H), 7.26 (d, *J* = 3.05 Hz, 2H), 6.94 (d, *J* = 8.69 Hz, 1H), 6.56 (s, 2H), 5.40 (d, *J* = 9.01 Hz, 2H), 3.95 (s, 3H), 3.94 (s, 9H), 3.89 (s, 3H), 3.82 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 189.7, 152.7, 152.5, 142.9, 140.8, 136.6, 137.0, 134.6, 133.6, 133.2, 132.6, 130.1, 129.2, 107.6, 106.2, 105.7, 104.4, 103.3, 60.7, 60.6, 56.0, 55.7, 55.5; ESI-MS: m/z 521 [M+1]+; HRMS (ESI m/z) for $C_{30}H_{33}O_8$ calcd: 521.21699, found: 521.21657 [M+1]+.

(*E*)-1-(3,4-dimethoxyphenyl)-3-(2-methoxy-5-(1-(3,4,5-trimethoxyphenyl)vinyl)phenyl) prop-2-en-1-one (4b)

This compound was prepared according to the method described for compound **3a**, employing 2-methoxy-5-(1-(3,4,5-trimethoxyphenyl)vinyl)benzaldehyde (**14b**, 100 mg, 0.305 mmol) and 3,4-dimethoxyacetophenone (**15b**, 54.9 mg, 0.305 mmol) to obtain the pure product **4b** as a white solid (126 mg, 84 % yield); mp: 145-147 °C; IR (KBr): $\bar{\upsilon}_{max}/cm^{-1}$- 2958, 2837, 1725, 1654, 1597, 1580, 1513, 1462, 1415, 1343, 1268, 1162, 1127, 1023, 893, 845, 820, 767, 708, 551; ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm): 8.08 (d, *J* = 15.8 Hz, 1H), 7.67-7.61 (m, 4H), 7.36 (dd, *J* = 8.39 Hz, 1.98 Hz, 1H), 6.93 (dd, *J* = 2.59 Hz, 8.69 Hz, 2H), 6.57 (s, 2H), 5.42 (d, *J* = 7.63 Hz, 2H), 3.96 (s, 6H), 3.95 (s, 3H), 3.89 (s, 3H), 3.82 (s. 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 188.1, 157.8, 152.5, 148.4, 138.5, 137.1, 136.3,

133.1, 130.9, 128.1, 122.8, 122.5, 122.1, 112.6, 110.4, 110.2, 109.7, 104.9, 60.3, 55.4, 55.3, 55.2; ESI-MS: m/z 491 [M+1]+; HRMS (ESI m/z) for $C_{29}H_{31}O_7$ calcd: 491.2069, found: 491.2032 $[M+1]^+$.

(*E*)-1-(benzo[d][1,3]dioxol-5-yl)-3-(2-methoxy-5-(1-(3,4,5-trimethoxyphenyl)vinyl) phenyl)prop-2-en-1-one (4c)

This compound was prepared according to the method described for compound **3a**, employing 2-methoxy-5-(1-(3,4,5-trimethoxyphenyl)vinyl)benzaldehyde (**14b**, 100 mg, 0.305 mmol) and 1-(benzo[d][1,3]dioxol-5-yl)ethanone (**15c**, 50.1 mg, 0.305 mmol) to obtain the pure product **4c** as a white solid (125 mg, 87 % yield); mp: 153-155 °C; IR (KBr): $\bar{\upsilon}_{max}/cm^{-1}$- 3087, 2934, 2842, 2355, 1652, 1605, 1582, 1502, 1443, 1410, 1343, 1323,1288, 1246, 1191, 1128, 1038, 1024, 1001, 937, 910, 890, 851, 833, 807, 784, 722; ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 8.06 (d, *J* = 15.8 Hz, 1H), 7.65-7.63 (m, 2H), 7.56 (d, *J* = 15.8 Hz, 1H), 7.52 (d, *J* = 1.32 Hz, 1H), 7.35 (dd, *J* = 2.07 Hz, 8.49 Hz, 1H),m 6.90 (t, *J* = 8.49 Hz, 16.6 Hz, 2H), 6.56 (s, 2H), 6.05 (s, 2H), 5.41 (d, *J* = 3.02 Hz, 2H), 3.95 (s, 3H), 3.89 (s, 3H), 3.82 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 188.7, 158.7, 152.9, 149.0, 139.6, 137.0, 133.1, 131.4, 129.1, 124.6, 123.6, 122.8, 113.1, 110.8, 108.4, 107.8, 105.5, 101.7, 60.8, 56.1, 55.6; ESI-MS: m/z 475 [M+1]+; HRMS (ESI m/z) for $C_{28}H_{27}O_7$ calcd: 475.17513, found: 475.17477 [M+1]+.

(*E*)-3-(2-methoxy-5-(1-(3,4,5-trimethoxyphenyl)vinyl)phenyl)-1-(4-methoxyphenyl)prop-2-en-1-one (4d)

This compound was prepared according to the method described for compound **3a**, employing 2-methoxy-5-(1-(3,4,5-trimethoxyphenyl)vinyl)benzaldehyde (**14b**, 100 mg, 0.305 mmol) and 4-methoxyacetophenone (**15d**, 45.8 mg, 0.305 mmol) to obtain the pure product **4d** as a white solid (130 mg, 93 % yield); mp: 142-146 °C; IR (KBr): $\bar{\upsilon}_{max}/cm^{-1}$- 3002, 2964, 2937, 2835, 1721, 1654, 1603, 1593, 1577, 1505, 1455, 1412, 1347, 1321, 1287, 1252, 1233, 1217, 1169, 1126, 1032, 1008, 984, 844, 827, 610; ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm): 8.06 (d, *J* = 15.8 Hz, 1H), 8.02 (d, *J* = 8.85 Hz, 2H), 7.64-7.60 (m, 2H), 7.34 (dd, *J* = 2.29 Hz, 8.54 Hz, 1H), 6.97 (d, *J* = 8.85 Hz, 2H), 6.91 (d, *J* = 8.85 Hz, 1H), 6.56 (s, 2H), 5.40 (d, *J* = 7.78 Hz, 2H), 3.95 (s, 3H), 3.89 (s, 3H), 3.88 (s, 3H), 3.82 (s, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm): 189.2, 163.2, 158.4, 156.6, 152.8, 149.0, 139.3, 137.0, 133.8, 131.3, 130.7, 130.3, 129.0, 127.6, 123.7, 122.9, 113.7, 113.0, 112.3, 110.8, 109.1, 105.4, 60.8, 56.1, 56.0, 55.6; ESI-MS: m/z 461 [M+1]+; HRMS (ESI m/z) for $C_{28}H_{29}O_6$ calcd: 461.19587, found: 461.19495 [M+1]+.

(*E*)-1-(3-amino-4-methoxyphenyl)-3-(2-methoxy-5-(1-(3,4,5-trimethoxyphenyl)vinyl) phenyl)prop-2-en-1-one (4e)

This compound was prepared according to the method described for compound **3a**, employing 2-methoxy-5-(1-(3,4,5-trimethoxyphenyl)vinyl)benzaldehyde (**14b**, 100 mg, 0.305 mmol) and 4-methoxy 3-aminoacetophenone (**15e**, 50.6 mg, 0.305 mmol) to obtain the pure product **4e** as a yellow colour solid (106 mg, 73 % yield); mp: 120-122 °C; IR (KBr): $\bar{\upsilon}_{max}/cm^{-1}$- 3358, 2934, 2837, 1654, 1579, 1504, 1440, 1410, 1346, 1318, 1290, 1248, 1171, 1125, 1020, 895, 822, 709; ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm): 8.06 (d, *J* = 15.8 Hz, 1H), 7.63 (d, *J* = 2.26 Hz, 1H), 7.59 (d, *J* = 15.8 Hz, 1H), 7.46 (dd, *J* = 1.51 Hz, 8.31 Hz, 1H), 7.42 (d, *J* = 1.51 Hz, 1H), 7.33 (dd, *J* = 2.26 Hz, 8.31 Hz, 1H), 6.91 (d, *J* = 8.31 Hz, 1H), 6.82 (d, *J* = 8.31 Hz, 1H), 6.56 (s, 2H), 5.40 (d, *J* = 4.53 Hz, 2H), 3.94 (s, 3H), 3.93 (s, 3H), 3.89 (s, 3H), 3.82 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 189.5, 158.4, 152.9, 151.1, 149.1, 138.8, 137.8, 137.1, 136.3, 133.7, 131.6, 131.2, 128.8, 123.8, 123.1, 120.4, 114.4, 113.1, 110.8, 109.2, 105.5, 60.8, 56.1, 55.6; ESI-MS: m/z 476 [M+1]+; HRMS (ESI m/z) for $C_{28}H_{30}NO_6$calcd: 476.20676, found: 476.20639 [M+1]+.

(*E*)-1-(4-aminophenyl)-3-(2-methoxy-5-(1-(3,4,5-trimethoxyphenyl)vinyl)phenyl)prop-2-en-1-one (4f)

This compound was prepared according to the method described for compound **3a**, employing 2-methoxy-5-(1-(3,4,5-trimethoxyphenyl)vinyl)benzaldehyde (**14b**, 100 mg, 0.305 mmol) and 4-amino acetophenone (**15f**, 41.5 mg, 0.305 mmol) to obtain the pure product **4f** as a yellow colour solid (100 mg, 74 % yield); mp: 115-117 °C; IR (KBr): $\bar{\upsilon}_{max}/cm^{-1}$- 3355, 2924, 1603, 1341, 1248, 1172, 1126, 1021; ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 8.03 (d, *J* = 15.8 Hz, 1H), 7.89 (d, *J* = 7.55 Hz, 2H), 7.63-7.58 (m, 2H), 7.31 (d, *J* = 8.31 Hz, 1H), 6.91 (d, *J* = 8.31 Hz, 1H), 6.66 (d, *J* = 7.55 Hz, 2H), 6.56 (d, 2H), 5.39 (d, *J* = 3.02 Hz, 2H), 4.02 (bp, 2H), 3.93 (s, 3H), 3.89 (s, 3H), 3.82 (s, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm): 188.5, 158.3, 152.8, 151.1, 149.1, 138.4, 137.7, 137.0, 133.7, 131.1, 130.9, 128.8, 128.5, 129.9, 123.1, 113.7, 113.0, 110.7, 105.4, 60.8, 56.6, 56.1; ESI-MS: m/z 446 [M+1]+; HRMS (ESI m/z) for $C_{27}H_{28}NO_5$calcd: 446.19620, found: 446.19521 [M+1]+.

(*E*)-1-(4-fluorophenyl)-3-(2-methoxy-5-(1-(3,4,5-trimethoxyphenyl)vinyl)phenyl)prop-2-en-1-one (4g)

This compound was prepared according to the method described for compound **3a**, employing 2-methoxy-5-(1-(3,4,5-trimethoxyphenyl)vinyl)benzaldehyde (**14b**, 100 mg, 0.305 mmol) and 4-fluoro acetophenone (**15g**, 42.1 mg, 0.305 mmol) to obtain the pure product **4g** as a white colour solid (98 mg, 72% yield); mp: 120-122 °C; IR (KBr): $\bar{\upsilon}_{max}/cm^{-1}$-3001, 2912, 1687, 1601, 1555, 1526, 1496, 1428, 1345, 1303, 1229, 1187, 1162, 1089, 998, 979; ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 8.09 (d, *J* = 15.8 Hz, 1H), 8.06-8.02 (m, 2H), 7.63 (d, *J* = 2.26 Hz, 1H), 7.59 (d, *J* = 15.8 Hz. 1H), 7.36 (dd, *J* = 2.26 Hz, 8.31 Hz, 1H), 7.20-7.13 (m, 2H), 6.91 (d, *J* = 9.06 Hz, 1H), 6.55 (s, 2H), 5.40 (d, *J* = 1.51 Hz, 2H), 3.96 (s, 3H), 3.89 (s, 3H), 3.82 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 189.9, 161.7, 152.8, 152.7, 141.8, 139.2, 134.2, 133.1, 132.7, 131.1, 131.0, 123.8, 123.2, 115.7, 110.5, 107.4, 60.9, 56.2, 55.9; ESI-MS: m/z 449 [M+1]+; HRMS (ESI m/z) for $C_{27}H_{26}FO_5$ calcd: 449.17588, found: 449.17591 [M+1]+ .

(*E*)-1-(4-chlorophenyl)-3-(2-methoxy-5-(1-(3,4,5-trimethoxyphenyl)vinyl)phenyl)prop-2-en-1-one (4h)

This compound was prepared according to the method described for compound **3a**, employing 2-methoxy-5-(1-(3,4,5-trimethoxyphenyl)vinyl)benzaldehyde (**14b**, 100 mg, 0.305 mmol) and 4-chloro acetophenone (**15h**, 47.1 mg, 0.305 mmol) to obtain the pure product **4h** as a white colour solid (125 mg, 88% yield); mp: 150-152 °C; IR (KBr): $\bar{\upsilon}_{max}/cm^{-1}$- 2994, 2958, 2935, 2838, 1676, 1659, 1596, 1582, 1493, 1458, 1412, 1398, 1347, 1329, 1290, 1266, 1248, 1234, 1208, 1189, 1172, 1129, 1029, 1088, 1011, 987, 966, 914, 857, 966, 914, 857, 842, 825, 782, 769, 720; ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 8.08 (d, *J* = 15.8 Hz, 1H), 7.93 (d, *J* = 8.31 Hz, 2H), 7.63 (d, *J* = 2.26 Hz, 1H), 7.57 (d, *J* = 15.8 Hz, 1H), 7.45 (d, *J* = 8.31 Hz, 2H), 7.35 (dd, *J*

= 2.26 Hz, 8.31 Hz, 1H), 6.94 (d, *J* = 9.06 Hz, 1H), 6.58 (s, 2H), 5.41 (d, *J* = 1.51 Hz, 2H), 3.95 (s, 3H), 3.89 (s, 3H), 3.82 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 189.7, 158.5, 152.7, 148.9, 140.7, 138.9, 137.8, 136.9, 136.6, 133.9, 131.8, 129.8, 129.1, 128.7, 127.5, 122.5, 110.8, 113.2, 105.4, , 60.8, 56.0, 55.6; ESI-MS: m/z 465 [M+1]+ and m/z 467 [M+3]$^+$; HRMS (ESI m/z) for $C_{27}H_{26}ClO_5$ calcd: 465.14633, found: 465.14624 [M+1]+ and $C_{27}H_{26}{}^{37}ClO_5$ calcd: 467.14338, found: 467.14339 [M+3]+.

(*E*)-1-(4-bromophenyl)-3-(2-methoxy-5-(1-(3,4,5-trimethoxyphenyl)vinyl)phenyl)prop-2-en-1-one (4i)

This compound was prepared according to the method described for compound **3a**, employing 2-methoxy-5-(1-(3,4,5-trimethoxyphenyl)vinyl)benzaldehyde (**14b**, 100 mg, 0.305 mmol) and 4-bromoacetophenone (**15i**, 60.7 mg, 0.305 mmol) to obtain the pure product **4i** as a white colour solid (130 mg, 84% yield); mp: 132-134 °C; IR (KBr): $\bar{\upsilon}_{max}$/cm^{-1}-2998, 2842, 1689, 1586, 1468, 1416, 1377, 1266, 1234, 1162, 1155, 1029, 1088, 976, 966, 914; ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 8.08 (d, *J* = 15.8 Hz, 1H), 7.86 (d, *J* = 8.54 Hz, 2H), 7.64-7.62 (m, 3H), 7.55 (d, *J* = 15.8 Hz, 1H), 7.35 (dd, *J* = 2.28 Hz, 8.54 Hz, 1H), 6.91 (d, *J* = 8.69 Hz, 1H), 6.55 (s, 2H), 5.40 (dd, *J* = 1.07 Hz, 3.66 Hz, 2H), 3.95 (s, 3H), 3.89 (s, 3H), 3.82 (s, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm):189.9, 158.6, 152.9, 152.7, 148.9, 140.8, 137.9, 137.1, 136.9, 133.9, 131.2, 130.2, 130.0, 129.5, 129.2, 127.6, 122.6, 113.2, 110.9, 105.5, 60.8, 56.1, 55.1; ESI-MS: m/z 509 [M+1]+ and m/z 511 [M+3]+; HRMS (ESI m/z) for $C_{27}H_{26}BrO_5$ calcd: 509.09581, found: 509.09567 [M+1]+ and $C_{27}H_{26}{}^{81}BrO_5$ calcd: 511.09377, found: 511.09375.

(*E*)-1-(4-hydroxyphenyl)-3-(2-methoxy-5-(1-(3,4,5-trimethoxyphenyl)vinyl)phenyl)prop-2-en-1-one (4j)

This compound was prepared according to the method described for compound **3a**, employing 2-methoxy-5-(1-(3,4,5-trimethoxyphenyl)vinyl)benzaldehyde (**14b**, 100 mg, 0.305 mmol) and 1-(4-((tert-butyldimethylsilyl)oxy)phenyl)ethanone (**15j**, 82.3 mg, 0.336 mmol) to obtain the pure product **4j** as a white colour solid (95 mg, 70% yield); mp: 142-144 °C; IR (KBr): $\bar{\upsilon}_{max}$/cm^{-1}-2999, 2989, 2888, 1659, 1601, 1549, 1524, 1434, 1389, 1222, 1089, 875; ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm): 8.11 (d, *J* = 15.7 Hz, 1H), 7.97 (d, *J* = 7.78 Hz, 2H), 7.63 (d, *J* = 10.9 Hz, 2H), 7.35 (d, *J* = 7.78 Hz, 1H), 6.96-6.90 (m, 3H), 6.56 (s, 2H), 5.40 (d, *J* = 4.42 Hz, 2H), 3.92 (s, 3H), 3.89 (s, 3H), 3.81 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 190.0, 161.3, 158.5, 152.8, 149.0, 139.8, 137.6, 137.1, 133.7, 131.6, 131.1, 130.4, 128.9, 123.5, 122.8, 115.5, 113.2, 110.8, 105.4, 60.8, 56.0, 55.9; ESI-MS: m/z 447 [M+1]$^+$; HRMS (ESI m/z) for $C_{27}H_{27}O_6$ calcd: 447.1807, found: 447.1817 [M+1]$^+$.

(*E*)-1-(3-hydroxy-4-methoxyphenyl)-3-(2-methoxy-5-(1-(3,4,5-trimethoxyphenyl)vinyl) phenyl)prop-2-en-1-one (4k)

This compound was prepared according to the method described for compound **3a**, employing 2-methoxy-5-(1-(3,4,5-trimethoxyphenyl)vinyl)benzaldehyde (**14b**, 100 mg, 0.305 mmol)and 1-(3-((tert-butyldimethylsilyl)oxy)-4-methoxyphenyl)ethanone (**15k**, 92.4 mg, 0.335 mmol) to obtain the pure product **4k** as a white colour solid (96 mg, 66 % yield); mp: 148-146 °C; IR (KBr): $\bar{\upsilon}_{max}$/cm^{-1}-3388, 2929, 1669, 1579, 1508, 1459, 1412, 1330, 1273, 1170, 1125, 1019, 821, 764; ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm): 8.07 (d, *J* = 15.8 Hz, 1H), 7.63-7.61 (m, 3H), 7.59 (d, *J* = 15.8 Hz, 1H), 7.35 (dd, *J* = 2.07 Hz, 8.49 Hz, 1H), 6.95-6.90 (m, 2H), 6.56 (s, 2H), 5.70 (s, 1H), 5.40 (d, *J* = 4.15 Hz, 2H), 3.97 (s, 3H), 3.94 (s, 3H), 3.89 (s, 3H), 3.82 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 189.2, 152.8, 150.3, 148.9, 145.3, 139.4, 137.0, 133.7, 131.9, 131.4, 129.0, 122.8, 121.9, 114.6, 113.1, 110.8, 109.8, 105.4, 60.8, 56.0, 55.6; ESI-MS: m/z 477 [M+1]$^+$; HRMS (ESI m/z) for $C_{28}H_{29}O_7$ calcd: 477.1913, found: 477.1874 [M+1]$^+$.

(*E*)-3-(2-methoxy-5-(1-(3,4,5-trimethoxyphenyl)vinyl)phenyl)-1-(naphthalen-2-yl)prop-2-en-1-one (4l)

This compound was prepared according to the method described for compound **3a**, employing 2-methoxy-5-(1-(3,4,5-trimethoxyphenyl)vinyl)benzaldehyde (**14b**, 100 mg, 0.305 mmol) and 1-(naphthalen-2-yl)ethanone (**15l**, 51.9 mg, 0.305 mmol) to obtain the pure product **4l** as a white colour solid (128 mg, 87 % yield); mp: 126-128 °C; IR (KBr): $\bar{\upsilon}_{max}$/cm^{-1}- 2998, 2960, 2935, 2829, 1653, 1628, 1594, 1578, 1506, 1494, 1459, 1413, 1346, 1327, 1291, 1266, 1251, 1236, 1211, 1184, 1124, 1025, 1009, 985, 966, 910, 895, 851, 825, 813, 784, 769, 762, 709, 627, 599, 556, ; ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 8.52 (s, 1H), 8.16 (d, *J* = 15.8 Hz, 1H), 8.08 (dd, *J* = 1.51 Hz, 8.31 Hz, 1H), 8.01-7.88 (m, 3H), 7.76 (d, *J* = 15.8 Hz, 1H), 7.70 (d, *J* = 1.51 Hz, 1H), 7.63-7.53 (m, 2H), 7.36 (dd, *J* = 2.26 Hz, 9.06 Hz, 1H), 6.92 (d, *J* = 8.31 Hz, 1H), 6.58 (s, 2H), 5.42 (d, *J* = 5.28 Hz, 2H), 3.96 (s, 3H), 3.89 (s, 3H), 3.83 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 190.9, 152.9, 149.0, 140.2, 137.8, 136.9, 135.9, 135.7, 135.3, 133.8, 132.5, 131.6, 129.8, 129.4, 128.4, 127.7, 126.6, 124.5, 123.6, 113.2, 110.8, 105.4, 60.9, 56.1, 55.7; ESI-MS: m/z 481 [M+1]+; HRMS (ESI m/z) for $C_{31}H_{29}O_5$ calcd: 481.20095, found: 481.20049 [M+1]+.

II. Biology

Cytotoxic activity

The cytotoxicity activity of the compounds was determined using MTT assay. 1×10^4 cells/well were seeded in 200 µl DMEM, supplemented with 10% FBS in each well 96wellmicrocultureplates and incubated for 24 h at 37 °C in a CO_2 incubator. Compounds, diluted to the desired concentrations in culture medium, were added to the wells with respective vehicle control. After 48 h of incubation, 10 µL of MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) (5 mg/mL) was added to each well and the plates were further incubated for 4 h. Then the supernatant from each well was carefully removed; formazon crystals were dissolved in 100 µL of DMSO and absorbance at 540 nm wavelengths was recorded.

Cell cycle analysis

Flow cytometric analysis (FACS) was performed to evaluate the distribution of the cells through the cell cycle phases. Human breast cancer cells (MCF-7) were incubated with compounds (**3b**, **3c** and **3e**) at 0.5 µM and 1 µM concentrations for 48 h. Untreated and treated cells were harvested, washed with PBS, fixed in ice-cold 70% ethanol and stained with propidium iodide (Sigma Aldrich). Cell cycle was performed by flow cytometry (Becton Dickinson FACS Caliber) as earlier described.

In Vitro Tubilin polymerization assay

A fluorescence based in vitro tubulin polymerization assay was performed according to the manufacturer's protocol (BK011, Cytoskeleton,Inc.). Briefly, the reaction mixture in a total volume of 10 µL contained PEM buffer, GTP (1 µM) in the presence or

absence of test compounds (final concentration of 5 μM). Tubulin polymerizationwas followed by a time dependent increase in fluorescence due to the incorporation of a fluorescence reporter into microtubules as polymerization proceeds. Fluorescence emission at 420 nm (excitation wavelength is 360 nm) was measured by usinga Varioscan multimode plate reader (Thermo scientific Inc.). phenstatin was used as positive control in each assay. The IC_{50} value was defined as the drug concentration required inhibiting 50% of tubulin assembly compared to control. The reaction mixture for these experiments include: tubulin (3 mg/ml) in PEM buffer,GTP (1 μM), in the presence or absence of test compounds at various concentrations. Polymerization was monitored by increase in the Fluorescence as mentioned above at 37 °C.

Immunohistochemistry

MCF-7 cells were seeded on glass cover slips, incubated for 48 h in the presence or absence of test compounds **3b**, **3c** and **3e** at 1 μM concentration. Following the termination of incubation, cells were fixed with 3% paraformaldehyde, 0.02% glutaraldehyde in PBS and permeabilized by dipping the cells in 100% methanol followed by overnight incubation at 4 °C. Later, cover slips were blocked with 1% BSA in phosphate buffered saline for 1 h followed by incubation with a primary anti tubulin (mouse monoclonal) antibody and FITC conjugated secondary mouse anti IgG antibody. Photographs were taken using the fluorescence microscope, equipped with FITC settings and the pictures were analyzed for the integrity of microtubule network. In parallel experiments, (1 μM) was used as positive control for analyzing microtubule integrity.

Competitive tubulin-binding assay

For colchicine competitive binding assay, tubulin was coincubated with indicated concentrations of MPSP-001 and taxol at 37 °C for 1 h. Then colchicine was added to a final concentration of 5 μmol/L. Fluorescence was determined using a Hitachi F-2500 spectro fluorometer (Tokyo, Japan) at excitation wavelengths of 365 nm and emission wave lengths of 435 nm. Blank values (buffer alone) as background were subtracted from all samples. Then the inhibition rate (IR) was calculated as follows: $IR=F/F_0$ where F_0 is the fluorescence of the 5 μmol/L colchicine-tubulin complex, and F is the fluorescence of a given concentration of phenstatin or taxol (12.5μmol/L, 25 μmol/L, 50 μmol/L and 100 μmol/L) competition with the 5 μmol/L colchicine-tubulin complex. taxol, not binding in the colchicine-site of tubulin, was added as a negative control.

Analysis of soluble versus polymerized tubulin in cells:

Cells were plated in 24-well dishes, grown to 60-80% confluency, and treated with compounds of 3e and 1a at 1 μM concentration were used as positive controls .Cells were incubated with drug for 48 h, later the media was removed, cells were rinsed in 1X PBS at 22 °C, harvested at the same temperature in lysis buffer containing 0.1 M Pipes, 1 mM EGTA, 1mM MgSO4, 30% glycerol, 5% DMSO, 5 mM GTP, 0.125% NP-40, and protease inhibitors, including aprotinin [200 units/mL], pH 6.9 and then centrifuged at 15000*g* at 22 °C for 30 min in an SorvallLegendmicro 21R model temperature controlled centrifuge (Thermo scientific), to separate polymerized (P) from soluble (S) tubulin. Pellets of polymerized "P" tubulin were resuspended in a volume of lysis buffer equal to the soluble "S" fraction, and resolved in 7% SDS/PAGE as described earlier. After transfer to NC membrane immunoblotting was performed with mouse anti α-tubulin antibody [DMIA, Sigma, St. Louis, MO], followed by an FITC-conjugated secondary antibody (Sigma). The blot was imaged using Phosphor imager (Fugifilm, Japan). Quantitative analysis of the soluble and polymer fractions was done by densitometry using Gene-box (Syngene).

Hoechst staining

Cells were seeded at a density of 10,000 cells over 18-mm cover slips and incubated for 24 h. After incubation, cells were treated with the compounds **3b**, **3c** and **3e** at 1 μM concentration for 24 h. Hoechst 33258 (Sigma Aldrich) was added to the cells at a concentration of 0.5 mg/mL and incubated for 30 min at 37 °C. Later, cells were washed with phosphate buffered saline (PBS). Cells from each cover slip were captured from randomly selected fields under fluorescent microscope (Leica, Germany) to qualitatively determine the proportion of viable and apoptotic cells based on their relative fluorescence and nuclear fragmentation.

Caspase 9 activity

To determine the caspase-9 activity of **3b**, **3c** and **3e** for detection of apoptosis in breast cancer cell line (MCF-7), the commercially available apoptosis detection kit (Sigma-Caspase 9 Assaykit, Fluorometric) was used. MCF-7 cells were treated with compounds**3b**, **3c** and **3e** at 2 μM concentration for 48 h. Here the substrate used is Ac-LEHD-AFC to the cell lysate and incubation was carried out at 37 °C for 1 h. Readings were taken at excitation wavelength 400 nm and emission wavelength 505 nm.

DNA fragmentation analysis

Cells were seeded (1×10^6) in six well plates and incubated for 24 h. After incubation, cells were treated with compounds **3b**, **3c** and **3e** at 3 μM concentrations for 48 h. After 48 h of drug treatment cells were collected and centrifuged at 2500 rpm for 5 min at 4 °C. Pellet was collected and washed with Phosphate buffered saline (PBS), added 100 μl of Lysis buffer, centrifuged at 3000 rpm for 5 min at 4 °C and collected supernant. And add 10 μl of 10% SDS and 10 μl of (50 mg/mL) RNase-A and incubated for 2 h at 56 °C. After that 10 μl of Proteinase K (25 mg/mL) was added and incubated at 37 °C for 2 h. After incubation, 65 μl of 10 M Ammonium acetate and 500 μl of ice cold ethanol was added and mixed well. And this sample was incubated at 80 °C for 1 h. After that samples were centrifuged at 12000 rpm for 20 min at 4 °C and washed with 80% ethanol followed by air dried for 10 min at room temperature. Dissolved pellet in 50 μLof TE buffer. After that, DNA laddering was determined by 2% agarose gel electrophoresis.

Mesurement of Mitochondrial Membrane Potential

The mitochondrial membrane potential was measured with the lipophilic cationic dye JC-1 (Molecular Probes), as described. Cultures were treated with the test drugs for 48 h. After drug treatment the cells were incubated with JC-1 dye for 20 min at 37 °C. After incubation cultures were used for the measuring mitochondrial membrane potiential (ΔΨm), according to the manufacturer's instructions.The shift of the membrane potential1 was measured by flow cytometry using (Molecular Probes) or JC-1 (Molecular Probes), as previously described.

Flow cytometric evaluation of apoptosis

MCF-7 (1×10^6) were seeded in six-well plates and allowed to grow overnight. The medium was then replaced with complete medium containing 3 µM concentration of compounds, **3b**, **3c** and **3e** for 48 h along with vehicle alone (0.001% DMSO) as control. After 48 h of drug treatment, cells from the supernatant and adherent monolayer cells were harvested by trypsinization, washed with PBS at 3000 rpm. Then the cells (1×10^6) were stained with Annexin V-FITC and propidium iodide using the Annexin-V-PI apoptosis detection kit (Invitrogen). Flow cytometry was performed using a FACScan (Becton Dickinson) equipped with a single 488-nm argon laser as described earlier .Annexin V-FITC was analyzed using excitation and emission settings of 488 nm and 535 nm (FL-1 channel); PI, 488 nm and 610 nm (FL-2 channel). Debris and clumps were gated out using forward and orthogonal light scatter.

Molecular modelling experimental procedure:

All the compounds under study and reference compounds 3D structures were built and optimized using Gaussian 03W. These optimized 3D structures were utilized for Docking. All the compounds were Docked using AutoDockTools software package. The co crystallized structure of colchicine site tubulin downloaded from the PDB data bank (http:// www.rcsb.org/pdb/index.html; PDB code: 3E22).

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Supplementary data

^{1}H NMR and ^{13}C NMR spectra of all phenstatin/*iso*combretastatin-chalcones (**3a-l** and **4a-l**) showed in supporting information.

Notes and References

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1. R. Romagnoli, P. G. Baraldi, M. D. Carrion, O. Cruz-Lopez, C. L. Cara, G. Basso, G. Viola, M. Khedr, J. Balzarini, S. Mahboobi, A. Sellmer, A. Brancale, E. Hamel, *J med chem***.** 2009, **52**, 7, 5551-5555.
2. P. Singh, K. Rathinasamy, R. Mohan, D. Panda, *IUBMB Life*. 2008, **60**, 6, 368-75.
3. A. Jordan, J. A. Hadfield, N. J. Lawrence, A.T. McGown, *Med Res Rev*. 1998, **18**, 259-96.
4. J. J. Field, A. Kanakkanthar, J. H. Miller, *Bioorg Med Chem*. 2014**, 22,** 5050-9.
5. J. Shi, Y. Zhou, H. C. Huang, T. J. Mitchison, *Cancer Res*. 2011, **71**(13), 4518-26.
6. R. N. Hansen, S. D. Ramsey, D. Lalla, A. Masaquel, T. Kamath, M. Brammer, S. A. Hurvitz, S. D. Sullivan, *Springerplus*. 2014, **3**, 259.
7. (a) R. Crazzolara, A. Cisterne, M. Thien, J. Hewson, R. Baraz, K. F. Bradstock, L. J. Bendall, *Blood*. 2009**, 113**(14), 3297-306. (b) S. O. Brien, G. Schiller, J. Lister, L. Damon, S. Goldberg, W. Aulitzky, D. Ben-Yehuda, W. Stock, S. Coutre, D. Douer, L. T. Heffner, M. Larson, K. Seiter, S. Smith, S. Assouline, P. Kuriakose, L. Maness, A. Nagler, J. Rowe, M. Schaich, O. Shpilberg, K. Yee, G. Schmieder, J. A. Silverman, D. Thomas, S. R. Deitcher, H. Kantarjian, *J Clin Oncol*. 2013, **31**(6), 676-83.
8. (a) G. R. Pettit, G. M. Cragg, D. L. Herald, J. M. Schmidt, P. Lohavanijaya, *Can.J. Chem*. 1982, **60**, 1374-1376. (b) G. R. Pettit, S. B. Singh, M. R. Boyd, E. Hamel, R. K. Pettit, J. M. Schmidt, F. Hogan, *J. Med. Chem*. 1995, **38**, 1666-1672.
9. (a) A. T. McGown, B. W. Fox, *Cancer Chemother. Pharmacol*. 1990, **26**, 79. (b) C. Shustik, W. Dalton, P. Gros, *Mol. Aspects Med*. 1995, **16**, 1. (c) O. Fardel, V. Lecureur, A. Guillouzo, *Gen. Pharmacol*. 1996, **27**, 1283.
10. J. A. Woods, J. A. Hadfield, G. R. Pettit, B. W.; Fox, A. T. McGown, *Br. J. Cancer* 1995, *71*, 705-711.
11. (a) K. Ohsumi, R. Nakagawa, Y. Fukuda, T. Hatanaka, Y. Morinaga, Y. Nihei, K. Ohishi, Y. Suga, Y. Akiyama, T. Tsuji, *J. Med. Chem*. 1998, **41**, 3022. (b) G. R. Pettit, B. E. Toki, D. L. Herald, M. R. Boyd, E. Hamel, R. K. Pettit, *J. Med.Chem.* 1999, **42**, 1459-1465. (c) G. R. Pettit, M. R. Rhodes. D. L. Herald, D. J. Chaplin, M. R. L. Stratford, E. Hamel, R. K. Pettit, J. C. Chapuis, D. Oliva, *Anti-Cancer Drug Des.* 1998, **13**, 981-993.
12. (a) M. Alami, J. D. Brion, O. Provot, J. F. Peyrat, S. Messaoudi, A. Hamze, A. Giraud, J. Bignon, J. Bakala, J. M. Liu, *PCT WO2008/122620A1*, 2008. (b) S. Messaoudi, B. Treguier, A. Hamze, O. Provot, J. F. Peyrat, J. R. D. Losada, J. M. Liu, J. Bignon, J. Wdzieczak-Bakala, S. Thoret, J. Dubois, J.-D. Brion, M. Alami, *J. Med. Chem*. 2009, **52**, 4538 –4542. (c) C. Mousset, A. Giraud, O. Provot, A. Hamze, J. Bignon, J. M. Liu, S. Thoret, J. Dubois, J. D. Brion, M. Alami, *Bioorg. Med. Chem. Lett*. 2008, **18**, 3266 – 3271. (d) A. Hamze, A. Giraud, S. Messaoudi, O. Provot, J. F. Peyrat, J. Bignon, J. M. Liu, J. Wdzieczak-Bakala, S. Thoret, J. Dubois, J.-D. Brion, M. Alami, *Chem Med Chem* 2009, **4**, 1912 –1924. (e) B. Trguier, A. Hamze, O. Provot, J. D. Brion, M. Alami, *Tetrahedron Lett*. 2009, **50**, 6549 – 6552.
13. G. R. Pettit, B. Toki, *PCT/US1999/000475* 2005.
14. G. R. Pettit, P. M. Grealish, D. L. Herald, M. R. Boyd, E. Hamel, R. K. Pettit, *J. Med. Chem***.** 2000, **43**, 2731-2737.
15. G. R. Pettit, J. W. Lippert, D. L. Herald, *J. Org. Chem*. 2000, **65**, 7438-7444. (c) G. R. Pettit, B. Toki, D. L. Herald, P. Verdier-Pinard, M. R. Boyd, E. Hamel, R. K. Pettit, *J. Med. Chem*. 1998, **41**, 1688-1695.
16. A. Giraud, O. Provot, A. Hamze, J. D. Brion, M. Alami, *Tetrahedron Lett.*, 2008, **49**, 1107 –1110.
17. M. L. Go, X. Wu, X. L. Liu, *Curr. Med. Chem*. 2005, **12**, 483–499. (chalcons)
18. (a) A. C. Claude, C. L. Jean, T. Patrick, P. Christelle, H. Gerard, J. C. Albert, L. D. Jean, *Anticancer Res***.** 2001, **21**, 3949– 3956. (b) T. Takahashi, N. Takasuka, M. Iigo, M. Baba, H. Nishino, H. Tsuda, T. Okuyama, *Cancer Sci*. 2004, **95**, 448– 453.
19. L. B. Salum, W. F. Altei, L. D. Chiaradia, M. N. Cordeiro, R. R. Canevarolo, C. P. Melo, E. Winter, B. Mattei, H. N. Daghestani, M. C. Santos-Silva, T. B. Creczynski-Pasa, R. A. Yunes, J. A. Yunes, A. D. Andricopulo, B. W. Day, R. J. Nunes, Vogt, A. *Eur J Med Chem*. 2013, **63**, 501-10.
20. S. Sale, R. G. Tunstall, K. C. Ruparelia, P. C. Butler, G. A. Potter, W. P. Steward, A. J. Gescher, *Invest New Drugs*. 2006, **24**, 459-64.
21. D. Kumar, K. K. Raj, S. V. Malhotrab, D. S. Rawat, *Med. Chem. Commun*., 2014, **5**, 528–535.
22. P. S. Frisa, J. W. Jacobberger, *PLoS One*, 2009, **4**, e7064.
23. A. Kamal, Y. V. V. Srikanth, T. B. Shaik, M. N. A. Khan, M. Ashraf, M.K. Reddy, K.A .Kumar, S. V. Kalivendi, *Med. Chem. Commun*., 2011, **2**, 819.
24. M. A. Reddy, N. Jain, D. Yada, C. Kishore, J. R. Vangala, R. P. Surendra, A. Anthony, S. V. Kalivendi, B. Sreedhar, *J. Med. Chem.* 2011, **54**, 6751–6760.
25. (a) A. Kamal, G. B. Kumar, S. Polepalli, A. B. Shaik, V. S. Reddy, M. K. Reddy, Ch. R. Reddy, R. Mahesh, J. S. Kapure, N. Jain, *ChemMedChem*. 2014, **9**, 2565-79; (b) A. Kamal, M. K. Reddy, T. B. Shaik, Rajender, Y. V. V. Srikanth, V. S. Reddy, G. B. Kumar, S. V. Kalivendi, *Eur. J. Med. Chem.* 2012, **50**, 9–17.

26. C. Zhang, N. Yang, C. H. Yang, H. S. Ding, C. Luo, Y. Zhang, *et al*. *PLoSOne*, 2009, **4**, e4881.
27. (a) A. M. Minotti, S. B. Barlow, F. Cabral, *J. Biol. Chem.* 1991, **266**, 3987 – 3994. (b) N. Jain, D. Yada, T. B. Shaik, G. Vasantha, P. S. Reddy, S. V. Kalivendi, B. Sreedhar, *Chem Med Chem,* 2011, **6**, 859 –868.
28. P. Costantini, J. M. Bruey, M. Castedo, D. Metivier, M. Loeffler, S. A. Susin, L. vagnan, N. Zamzami, C. Garrido, G. Kroemer, *Cell Death Differ.* 2002, **9**, 82.
29. E. Pasquier, M. Kavallaris, *IUBMB Life*. 2008, **60**, 165.
30. R. Romagnoli, P. G. Baraldi, O. Cruz-Lopez, C. Lopez-Cara, M. D. Carrion, A. Brancale, E. Hamel, L. Chen, R. Bortolozzi, G. Basso, G. Viola, *J. Med. Chem.*, 2010, **53**, 4248−4258.
31. (a) L. J. Browne, C. Gude, H. Rodriguez, R. E. Steele, A. Bhatnager, *J. Med. Chem.,* 1991, **34**, 725–736. (b) N. M. Weir, K. Selvendiran, V. K. Kutala, L. Tong, S. Vishwanath, M. Rajaram, S. Tridandapani, S. Anant, P. Kuppusamy, *Cancer Biol. Ther.* 2007, **6**, 178–184.
32. S. M. Konstantinov, M. R. Berger, *Cancer Lett.,* 1999, **144**, 153-60.
33. R. B. Ravelli, B. Gigant, P. A. Curmi, I. Jourdain, S. Lachkar, A. Sobel, M. Knossow, *Nature*, 2004, **428**, 198 –202.
34. G. M. Morris, R. Huey, W. Lindstrom, M. F. Sanner, R. K. Belew, D. S. Goodsell, A. J. Olson, *J. Comput. Chem.* 2009, **30**, 2785.