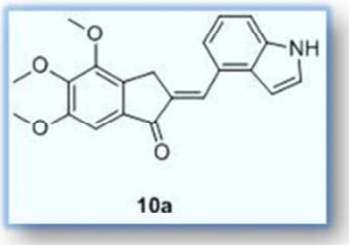
RSC Advances

This article can be cited before page numbers have been issued, to do this please use: J. Chen, J. Yan, J. Hu, Y. Pang, L. huang and X. Li, *RSC Adv.*, 2015, DOI: 10.1039/C5RA14888J.

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

View Article Online
DOI: 10.1039/C5RA14888J



Antiproliferative activity

Cancer cell lines	GI_{50} (μM)
Hela	0.027±0.01
A549	0.026±0.03
Bel-7402	0.035±0.04
PC-3	0.031±0.05
K562	0.031±0.12

Tubulin polymerization inhibition: IC_{50} = 1.99μM

196x105mm (96 x 96 DPI)

View Article Online
DOI: 10.1039/C5RA14888J

Journal Name

ARTICLE

Synthesis, Biological Evaluation and Mechanism Study of Chalcone Analogues as Novel Anti-cancer Agents

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Jie Chen[a], Jun Yan[a], Jinhui Hu[a], Yanqing Pang[a], Ling Huang[a]*, Xingshu Li[a]*

A series of novel chalcone analogues were designed, synthesized and evaluated as antitcancer agents. The results of antiproliferative activity test showed most of most analogues exhibited moderated to very good antiproliferative activities with GI_{50} values in micromol to sub-micromol range. Especially, compounds **10a**, gave 0.026 μM to 0.035 μM of GI_{50} value for five cancer cell line. The mechanism studies including tubulin polymerization inhibition, disruption of microtubule dynamics and cell cycle arrest assay demonstrated that compound **10a** could effectively inhibit in vitro cellular tubulin polymerization, interfere with the mitosis, resulting in a prolonged G2/M cell cycle arrest and ultimately lead to cell apoptosis of cancer cells. Taken together, these results suggested that **10a** may became a promising lead compound for development of new anticancer drugs.

Introduction

Tubulin-microtubule system is an important target for the development of anticancer drugs over the past decades because of its crucial involvement in mitosis.[1-3] Some typical drugs such as taxanes[4] and vinca[5, 6] alkaloids that played a central role in the treatment of diverse human cancers, are well known microtubule-targeting agents.[7, 8]

Combretastatin A-4 (CA-4), a natural cis-stilbene derivative isolated from the bark of the African willow tree Combretum caff rum by Pettit and co-workers in 1982, is a typical tubulin polymerization inhibitor which binds to the colchicine binding site.[9, 10] CA-4P, the disodium phosphate salt of CA-4, exhibited promising results in human clinical anti-tumor trials as a potent vascular disrupting agent.[11] As the *cis* double bond in CA-4 is prone to isomerize to the less bioactive *trans* isomers, to stabilize the conformation, many CA-4 analogues have been developed in recent years.[12] Among them, the replacement of benzene ring by benzoheterocyclic structures has received much attention.[13]

Chalcones, the precursors of flavonoids and isoflavonoids, are abundant in edible plants, and have also been shown to display a diverse array of pharmacological activities.[14] Some chalcon derivatives also exhibited potent activity as microtubule polymerization inhibitor. For example, Ducki incorporated the aryl substitution pattern of CA4 into chalcones and found that some of these compounds exhibited good antimitotic properties.[15] Bu reported the synthesis and evaluation of a series of cytotoxic ortho-aryl chalcones as new scaffold targeting tubulin and mitosis.[16] With millepachine, a chalcone analogue isolated from the Millettia pachycarpa as the model, Chen synthesized and evaluated twenty one dirivatives for their antiproliferative activity and the optimal compound was considered as promising anticancer agent.[17]

Indanones and their derivatives are important bioactive molecules that have been studied to determine their biological activities for the treatment of disease including Alzheimer' s disease[18] and cancer.[19] In our previous work, we reported the evaluation of a series of ortho-(3,4,5-trimethoxybenzoyl)-acetanilides as tubulin polymerization inhibitors.[20] Considering both indoles and indanones exhibited varied pharmacological activities, herein, we designed and evaluated and chalcone analogues derived from indole aldehydes and indanones as inhibitors of tubulin polymerization.

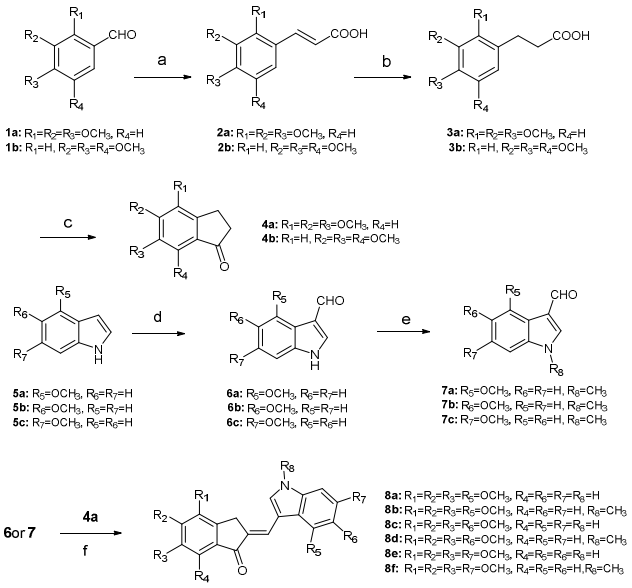
Results and discussion

Chemistry. The synthetic routes for the target compounds are listed in Schemes 1-2. First, the reaction of 2,3,4-trimethoxybenzaldehyde or 3,4,5-trimethoxybenzaldehyde with malonic acid gave propenoic acid derivative **2**,[21] which was hydrogenated in the presence of Pd/C to provide **3**.[20] The cyclization of **4** catalyzed by 1:10 phosphorus pentoxide-methanesulfonic acid afforded the substituted indanones 4 in good yield.[22] On the other hand, 5- (or 6- and 7-)

[a] Address here.
[b] Address here.
[c] Address here.
† Footnotes relating to the title and/or authors should appear here.
Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

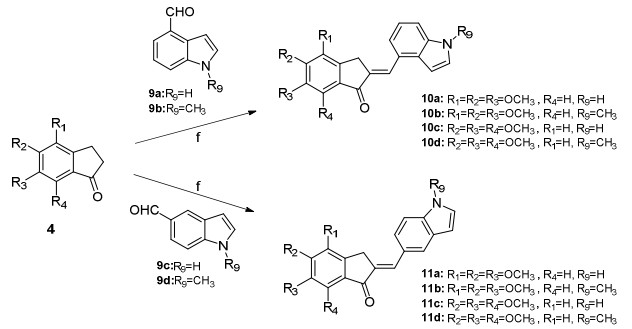
View Article Online
DOI: 10.1039/C5RA14888J

methoxyindole reacted with DMF in the presence of $POCl_3$ to afford indole-3-aldehyde derivatives **6**.[23] Then, reacted with CH_3I to provide the N-methyl products **7**.[24] Finally, target compounds 8 were obtained by the aldol reaction[25] of **6** or **7** with indanone **4a** in good yields (Schemes 1).



Scheme 1. The synthesis of target compounds **8**. Reagents and conditions: (a) malonic acid, piperidine, pyridine. (b) Pd/C, H_2, MeOH, 40 ℃. (c) 1:10 phosphorus pentoxide-methanesulfonic acid,50 ℃ .(d)$POCl_3$,DMF,rt. (e)CH_3I,NaH,THF,rt. (f)NaOH,EtOH,rt.

Target compounds **10** and **11** were synthesized with indanones **4**, indole-(4 or 5) -carbaldehyde and their N-methyl derivatives by the same aldol condensation procedure.



Scheme 2. The synthesis of target compounds **10** and **11**.

Biological evaluation

In vitro human cancer cell lines growth inhibition. The antiproliferative activities evaluation of synthesized compounds were performed with five human cancer cell lines, namely, A549 (non-small-cell-lung cancer cell line), HeLa (human epithelial cervical cancer cell line), Bel-7402 (human liver carcinoma cell line), PC-3 (human prostate cancer cell line), and K562 (human gastric cancer cell line) using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay with CA-4 as reference. As the results summerized in table 1, most tested compounds exhibited moderated to very good antiproliferative activities with GI_{50} values in micromol to sub-micromol range. Among them, compound **10a**, the aldol condensation product of 4,5,6-trimethoxy indanone and indole-4-carbaldehyde, gave the best antiproliferative activities in all the five cancer cell lines (GI_{50} = 0.026 μM for A549, 0.027 μM for Hela, 0.035 μM for Bel-7402, 0.031 μM for PC-3, and 0.031 μM for K562). A simple structure–activity relationship analysis showed that the antiproliferative activity was closely related to both the methoxy group position in indanone moiety and the indole carbaldehyde used. Compounds **8a-8f**, the condensation products of 4,5,6-trimethoxy indanone with 4-, 5-, or 6-methoxy-3-indole carbaldehydes (and their N-methyl derivative), gave moderated activities in most cases, besides **8e** and **8f** (products of 6-methoxy-3-indole carbaldehyde and its N-methyl analogue) provided good activities with the GI_{50} range from 0.120 to 1.08 μM. It was interesting that most compounds obtained from 4,5,6-trimethoxy indanone and indole-4-, or indole-5-carbaldehyde exhibited potent antiproliferative activities (**10a**: GI_{50} range from 0.026 to 0.035 μM; **10b**: 0.034 to 0.072 μM; **11a**: 0.034 to 0.072 μM; **11b**: 0.071 to 0.125 μM). However, condensation products of 5,6,7-trimethoxy indanone with indole-4-, or indole-5-carbaldehyde gave very poor activities (**10d**, **11c** and **11d**: more than 10μM of GI_{50} for all five human cancer cell lines; **10c**: GI_{50} range from 1.03 to 2.13 μM).

View Article Online
DOI: 10.1039/C5RA14888J

Journal Name

ARTICLE

Table1. Antiproliferative activity of compounds against five human cancer cell lines[a]

Compounds	GI_{50}[b](μM)				
	A549	Hela	Bel-7402	PC-3	K562
8a	2.46±0.02	2.72±0.56	5.62±0.05	8.63±0.05	6.06±0.15
8b	8.79±0.04	7.90±0.68	8.12±0.05	8.24±0.03	2.88±0.35
8c	2.37±0.16	7.33±0.27	8.09±0.02	8.63±0.02	1.44±0.02
8d	7.93±0.19	11.9±0.20	12.9±0.37	12.4±0.02	8.93±0.25
8e	0.365±0.03	0.612±0.12	0.668±0.03	0.539±0.01	0.615±0.23
8f	0.910±0.02	0.160±0.21	0.250±0.12	1.08±0.03	0.120±0.03
10a	0.026±0.03	0.027±0.01	0.035±0.04	0.031±0.05	0.031±0.12
10b	0.065±0.02	0.072±0.02	0.034±0.02	0.062±0.01	0.039±0.12
10c	1.03±0.15	2.13±0.25	1.59±0.26	1.11±0.28	1.58±0.03
10d	>10	>10	>10	>10	>10
11a	0.099±0.02	0.049±0.03	0.056±0.13	0.066±0.03	0.057±0.04
11b	0.071±0.02	0.087±0.03	0.094±0.02	0.125±0.02	0.089±0.15
11c	>10	>10	>10	>10	>10
11d	>10	>10	>10	>10	>10
CA-4	0.018±0.05	0.0028±0.06	0.012±0.001	0.014±0.004	0.011±0.003

[a] Cell lines were treated with different concentrations of the compounds for 48 h. Cell viability was measured by MTT assay as described in the Experimental Section. [b] GI_{50} values are indicated as the mean±SD (standard error) of three independent experiments.

In vitro Inhibition of Tubulin Polymerization. Among the synthesized compounds, we chosed **10a**, **10b**, **11a** and **11b** for analyzing their ability to block the microtuble assembly because of their good antitumor activity in different cancer cell lines. With the method originally described by Bonne, D. et al with some modification,[26, 27] and CA-4 as the reference compound, the results were obtained as listed in table 2. It could be seen that the tubulin polymerization inhibition (TPI) activities of these compounds were basiclly consisted with their antiproliferative activity. Compound 10a, which gave best antiproliferativ activity in vitro, also displayed most potent TPI activity, with 1.99 μM of the IC_{50}. Comparing with **10a**, **11a** gave relatively lower in vitro antiproliferativ activity, gave weaker TPI activity accordingly. Similarly, their N-methyl derivatives, **10b** and **11b**, also displayed the decrease of their TPI activities. The correlation between the antiproliferative activity and TPI activity indicated that the target compounds indeed target tubulin-microtubule system.

Table 2. Effects of the selected compounds on tubulin polymerization inhibition[a]

Compd	IC_{50}(μM)b
10a	1.99 ± 0.03
10b	5.86±0.05
11a	3.71 ± 0.24
11b	4.36±0.15
CA-4	1.25 ± 0.04

aInhibition of tubulin polymerization. Tubulin was at 20 μM. b IC50 values are indicated as the mean±SD (standard error) of three independent experiments.

In vitro cytotoxicity against human normal cell lines. It is well-known that a main drawback of clinically used anticancer drugs is their same cytotoxicity against human normal cells. Therefore, we determined the selectivity of compound 10a toward three kinds of human normal cells. The HLF (Human embryonic lung fibroblast), BJ (human dermal fibroblasts) and MCF-10A (human normal mammary epithelial cell line) cells were used. The results showed that compound 10a is associated with a relatively low toxicity towards the non-cancerous cell line (Table 3). The selectivity ratio were 217.4 - 328.5-fold, respectivity. These results demonstrate that 10a exhibits lower cytotoxicity towards human normal cells.

Table 3. Antiproliferative activity of compound **10a** against human non-cancerous cells line.

	GI_{50} (μM)[a]	Selectivity ratio[b]
A549	0.027±0.01	
BJ	5.87±0.21	217.4
HLF	8.87±0.33	328.5
MCF-10A	6.54±0.29	242.2

[a] Data are presented as the mean ± SE from the dose-response cures of at least three independent experiments. [b]Selectivity ratio = (IC_{50} human normal cells) / (IC_{50} Hela).

To determine the mechanism (stabilizing agents such as taxanes, or destabilizing agents like such as colchicine)[28] of the target compounds to tubulin, we carried out the microtubule dynamics assays with 1.0, 1.25, 2.50, 5.0, 7.5 and 10 μM of compound **10a**, respectively (Figure 1). The representative raw

View Article Online
DOI: 10.1039/C5RA14888J

data for the polymerization assay of compound **10a** showed that it inhibited tubulin polymerization in a concentration dependent manner, indicating that it belongs to the mircotubule destabilizing agents.

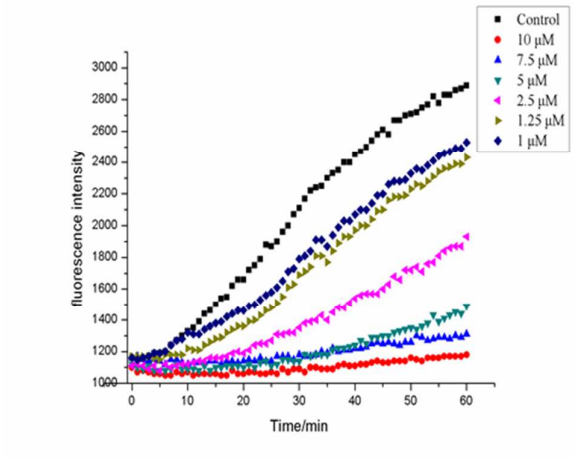


Figure1. Effect of compound **10a** on mircotubule dynamics.

Disruption of Microtubule Dynamics.

To identify the cellular microtubule morphology that may be relevant to the antiproliferative activity of target compounds, we performed the disruption of microtubule dynamics assay with **10a** by tubulin immunofluorescence. As shown in Figure 2, confocal microscopy studies clearly indicated the disruption of microtubule system of Hela cells after treated with different concentration of compound **10a**. Cells in control group was wrapped by uncondensed chromosomes and the regular slim, fibrous microtubules. After the concentration of compound **10a** increased, the microtubule network became disorganized and drastically disrupted at the concentration of 100 nM. The microtubule spindle strongly shrink around the center of the cells, and the irregularity of cytoplasm and chromosomes were also obvious. These abnormal morphology changes of the microtubule suggested that compound **10a** could effectively disrupted the microtubule dynamics.

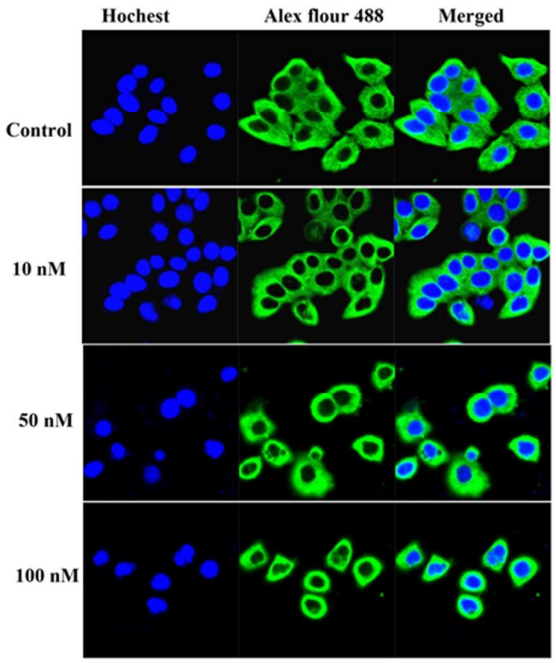


Figure 2. Effects of compound **10a** on mircrotubule dynamics by immunoflurescence. Hela cells were treated with compound **10a** at different concentration (0.01, 0.05, and 0.1μM) or DMSO (0.01%) for 48 h. Nucleuses were stained with Hochest 33342 (blue), and microtubules were stained with mouse anti-α-tubulin conjugated with Alex flour 488 (green). Images were taken using LSM 570 laser confocal microscope.

Cell cycle arrest at G2/M phase.

Studies has indicated that tubulin-destabilizing agents can block the cell cycle at the G2/M phase due to microtubule disruption.[29] To confirm cell cycle arrest effect of **10a**, Hela cells were treated with 10a at different concentrations (0.01, 0.05, and 0.1μM) for 48 h with DMSO (0.01%) as the control group, and then the harvested cells were analysed by flow cytometry. The results exhibited that **10a** led to a significant cell-cycle arrest at G2/M phase, and displayed a dose-dependent manner (Figure 3). As shown in Figure 3, in control group, 10.24% of the cells at G2/M phase, while the percentage of cells arrest at G2/M phase increased to 25.6%, 51.4% and 54.8% when the concentration of compound **10a** at 0.01, 0.05, and 0.1 μM, respectively. Therefore, we draw the conclusion that compound **10a** could arrest cell-cycle progression at the mitotic phase, which is in agreement with most of the antimitotic agents.

View Article Online
DOI: 10.1039/C5RA14888J

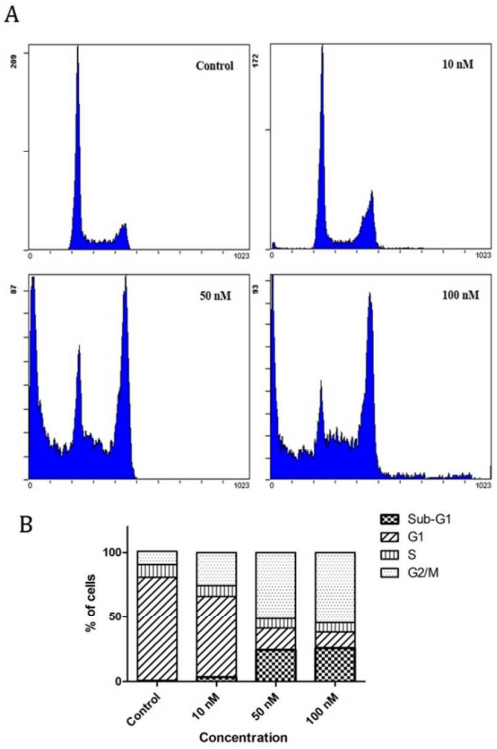


Figure 3. Cell cycle distribution of Hela with or without treatment of compound **10a** at different concentrations(0.01, 0.05, and 0.1 μM) or DMSO (0.01%) for 48 h. The DNA contents of each cell phase were analyzed by flow cytometry. The experiments were performed for three times, and the results of representative experiments were shown.

Cell apoptosis

Considering the obvious sub-G1 peak was observed in Figure 3, we hypothesized compound **10a** may induce cell apoptosis, and then, the flow cytometry was performed to validated our hypothesis. After the Hela cells were treated with compound **10a** at the indicated concentration for 48 h, the harvested cells were stained with Annexin V-FITC and PI to differentiate the early and late apoptosis cells. As shown in Figure 4, the total percentages of early and late apoptosis cells were 18.5%, 57.4%, and 60.9% at the 0.01, 0.05, and 0.1 μM, respectively, which was significantly increased comparing to the control group. These results further proved the compound **10a** could inhibit tubulin polymerization, arrest cell cycle at G2/M phase, and eventally induce cell apoptosis.

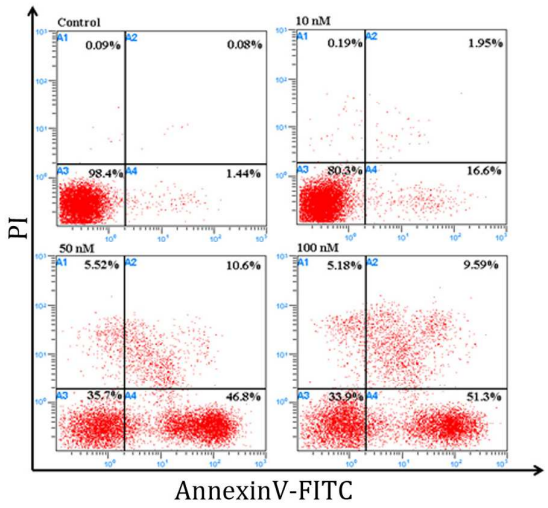


Figure4. Effect of compound **10a** on HeLa cell apoptosis. Cells were treated with compound **10a** (0.01, 0.05, and 0.1 μM) or DMSO (0.01%) for 48 h. The percentages of cells in each stage of cell apoptosis were quantitated by flow cytometry. (upper left quadrant) necrotic cells; (upper right quadrant) late apoptotic cells; (bottom left quadrant) live cells; and (bottom right quadrant) early apoptotic cells. The experiments were performed three times, and representative experiments were shown.

Conclusions

In conclusion, a novel series of new chalcone analogues were synthesized by the aldol condensation of indanones and indole carbaldehydes and evaluated as anticancer agents. The SAR studies showed that the target compounds obtained from 4,5,6-trimethoxy indanone with different indole carbaldehyde usually gave more potent antiproliferative activities than the 5,6,7-trimethoxy indanone derivatives. Most of most analogues exhibited moderated to very good antiproliferative activities with GI50 values in micromol to sub-micromol range. Further mechanism studies showed that the synthesized compounds could effectively inhibit *in vitro* cellular tubulin polymerization, interfere with the mitosis, resulting in a prolonged G_2/M cell cycle arrest and ultimately lead to cell apotosis of cancer cells. Among them, compound **10a** gave the best results *in vivo* antitumor evaluation and the further *in vivo* study of compound **10a** is in progress.

Experimental

^{1}H NMR and ^{13}C NMR spectra were acquired on a Bruker AvanceIII spectrometer with TMS as the internal standard. High resolution mass spectra (HR-MS) were recorded on an Agilent LC–MS 6120 instrument with an ESI mass selective detector in positive ion mode. Melting points were determined on an SRS-OptiMelt automated melting point instrument. The purity of the synthesized compounds was determined by high-performance liquid chromatography (HPLC) with a TC-C18 column (4.6 × 250 mm, 5 μm), an acetonitrile/water or methanol/water mobile phase, and a flow rate of 1.00 mL/min.

General procedure for the preparation of 2

View Article Online
DOI: 10.1039/C5RA14888J

Compounds **2** were prepared in excellent yield (95-100%) by Knoevenagel condensation of an aromatic aldehyde with malonic acid by following a previously reported procedure.[21]

General procedure for the preparation of 3

To a stirred suspension of **2** (9.39g, 39.45mmol) in methanol (20ml), Pd/C (0.50g) was added and the mixture was hydrogenated in 1.2 Mpa of hydrogen pressure for 4h at 50°C. After cooled to room temperature, the mixture was filtered to get the crude product. The crude product was chromatographed on silica gel and eluted with petroleum/ethyl acetate (8:1) as an eluent to afford the proposed compound.

3-(2,3,4-trimethoxyphenyl)propanoic acid(3a)

White solid ,9.1g, yield 96%. mp:78.1℃-79.2℃. R_f= 0.52 (petroleum/ethyl acetate=3:1).^{1}H-NMR(400 MHz, $CDCl_3$) δ 12.03 (s, 1H), 6.53 (s, 1H), 6.86 (s, 1H), 3.72 (s, 3H), 3.77 (s, 3H), 3.65 (s, 3H), 2.77 - 2.81 (t, 2H), 2.49 - 2.54 (t, 2H) .LC-MS(ESI): 239.2 for [M+H]$^-$.

3-(3,4,5-trimethoxyphenyl)propanoic acid(3b)

White solid ,8.9g, yield 94%. mp:98.9℃-100.5℃. R_f= 0.42 (petroleum/ethyl acetate=3:1).^{1}H-NMR (400 MHz, $CDCl_3$) δ 12.05 (s, 1H), 6.42 (s, 1H), 6.39 (s, 1H), 3.70 (s, 3H), 3.72 (s, 3H), 3.75(s, 3H), 2.69 – 2.76 (t, 2H), 2.48 - 2.54 (t, 2H). LC-MS(ESI): 239.1 for [M+H]$^-$.

General procedure for the preparation of 4

Compound **3** (2g, 8.33mmol) was added in small portions to a 30g of rapidly stirred 1:10 phosphorus pentoxide-methanesulfonic acid. The homogeneous reaction mixture was stirred at 50℃ for 1 h.The violet solution was added dropwise to 90 m1 of water and the mixture was stirred rapidly for 5-10 min to ensure the hydrolysis of methanesulfonic anhydride. The mixtue was extracted with ethyl acetate and the organic phase was washed (with solution of sodium bicarbonate and water, succesively), dried (sodium sulfate) and concentrated to give the crude product, which was purified by column chromatography using silica gel and petroleum/ethyl acetate (10:1) as an eluent to afford the proposed compound.

4,5,6-trimethoxy-2,3-dihydro-1H-inden-1-one(4a)

White acicular crystal, 1.44g, yield 78%. mp:87.9℃-88.6℃. R_f= 0.48 (petroleum/ethyl acetate=4:1). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.03 (s, 1H), 3.97 (s, 3H), 3.96 (s, 3H), 3.89 (s, 3H), 3.08 – 3.02 (t, 2H), 2.70 – 2.65 (t, 2H). LC-MS(ESI): 223.1 for [M+H]$^+$.

5,6,7-trimethoxy-2,3-dihydro-1H-inden-1-one (4b)

White squamous crystal, 1.37g, yield 74%.mp:113.9℃-114.7℃. R_f= 0.46 (petroleum/ethyl acetate=4:1). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 6.68 (s, 1H), 4.05 (s, 3H), 3.94 (s, 3H), 3.85 (s, 3H), 3.05 – 3.00 (t, 2H), 2.69 – 2.64 (t, 2H). LC-MS(ESI): 223.1 for [M+H]$^+$.

General procedure for the preparation of 8, 10 and 11

Substituted benzaldehydes **6** and **7** were synthesized according literature method.[23, 24]To a solution of NaOH (180mg, 4.5mmol) in C_2H_5OH (10 mL), **4** (200.0mg, 0.9mmol) was added. After the mixture was stirred for 15 minutes, a substituted benzaldehyde **6**, **7** or **9** (1.02 mmol) in C_2H_5OH (2 mL) was added dropwise to the solution. The reaction mixture was stirred overnight and filtered to yield the crude product. The crude product was chromatographed on silica gel and eluted with petroleum/ethyl acetate (3:1) as an eluent to afford the proposed compound.

(E)-4,5,6-trimethoxy-2-((4-methoxy-1H-indol-3-yl)methylene)-2,3-dihydro-1H-inden-1-one (8a)

Yellow solid, 299.3mg, yield 87.7%. mp:229.3℃-231.7℃. R_f= 0.57 (petroleum/ethyl acetate=1:1). IR(KBr,cm^{-1}) 3151, 3116, 3045, 3010, 2937, 1662, 1598, 1317, 727. ^{1}H NMR (400 MHz, DMSO) δ 8.35 (s, 1H), 7.91 (s, 1H), 7.11 (dd, J = 16.2, 7.7 Hz, 3H), 6.70 (d, J = 7.0 Hz, 1H), 4.00 (s, 3H), 3.97 (s, 3H), 3.88 (s, 3H), 3.86 (s, 3H), 3.83 (s, 2H). ^{13}C NMR (101 MHz, DMSO) δ 191.84, 154.97, 154.33, 150.15, 146.80, 138.00, 134.74, 134.56, 129.31, 128.83, 128.36, 123.89, 116.75, 112.63, 105.91, 101.91, 101.44, 61.13, 60.87, 56.53, 55.76, 30.05. HRMS (ESI) (m/z) $[M+H]^+$ calcd for $C_{22}H_{21}NO_5$, 379.1420; found, 380.1480. Purity: 95.6% (by HPLC).

(E)-4,5,6-trimethoxy-2-((4-methoxy-1-methyl-1H-indol-3-yl)methylene)-2,3-dihydro-1H-inden-1-one(8b)

Yellow solid, 274.6mg, yield 77.6%.mp:145.1℃-146.9℃. R_f= 0.55 (petroleum/ethyl acetate=1:1).IR(KBr,cm^{-1}) 3070, 3037, 2920, 1679, 1610, 1523, 1342, 710.^{1}H NMR (400 MHz, $CDCl_3$) δ 8.58 (s, 1H), 7.46 (s, 1H), 7.21 (d, J = 10.7 Hz, 2H), 6.97 (d, J = 8.2 Hz, 1H), 6.66 (d, J = 7.8 Hz, 1H), 4.04 (d, J = 3.0 Hz, 6H), 3.98 (s, 3H), 3.94 (s, 3H), 3.88 (s, 3H), 3.72 (s, 2H).^{13}C NMR (101 MHz, $CDCl_3$) δ 192.78, 155.40, 154.18, 149.70, 146.70, 138.43, 134.95, 134.54, 130.10, 129.47, 128.76, 123.77, 117.48, 113.02, 102.79, 101.47, 101.42, 61.11, 60.82, 56.28, 55.36, 33.66, 29.90. HRMS (ESI) (m/z) $[M+H]^+$ calcd for $C_{23}H_{23}NO_5$, 393.1576; found, 394.1629. Purity: 98.1% (by HPLC).

(E)-4,5,6-trimethoxy-2-((5-methoxy-1H-indol-3-yl)methylene)-2,3-dihydro-1H-inden-1-one(8c)

Yellow solid, 272.3mg, yield 79.8%. mp:216.5℃-218.7℃. R_f=0.61 (petroleum/ethyl acetate=1:1).IR (KBr,cm^{-1})3131, 3047, 3012, 2939, 1670, 1600, 1503, 1315, 810. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70 (s, 1H), 8.03 (s, 1H), 7.70 (s, 1H), 7.38 – 7.31 (m, 2H), 7.21 (s, 1H), 6.94 (d, J = 8.7 Hz, 1H), 4.04 (s, 3H), 3.98 (s, 3H), 3.94 (s, 3H), 3.92 (s, 3H), 3.79 (s, 2H). ^{13}C NMR (101 MHz, DMSO) δ 191.82, 155.14, 154.33, 150.12, 146.82, 134.73, 134.50, 131.30, 129.62, 129.01, 128.68, 125.46, 113.42, 113.32, 111.95, 101.48, 100.18, 61.11, 60.85, 56.49, 55.82, 30.02. HRMS (ESI) (m/z) $[M+H]^+$ calcd for $C_{22}H_{21}NO_5$, 379.1420; found,380.1480 . Purity: 98.2% (by HPLC).

(E)-4,5,6-trimethoxy-2-((5-methoxy-1-methyl-1H-indol-3-yl)methylene)-2,3-dihydro-1H-inden-1-one(8d)

Yellow solid, 296.2mg, yield 83.7%. mp:197.5℃ - 199.2℃.

View Article Online
DOI: 10.1039/C5RA14888J

R_f=0.53(petroleum/ethyl acetate=1:1). IR(KBr,cm^{-1}) 3037, 2970, 2892, 1670, 1610, 1525, 1314, 1147, 731. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (s, 1H), 7.52 (s, 1H), 7.38 (d, J = 2.3 Hz, 1H), 7.25 (d, J = 9.0 Hz, 1H), 7.21 (s, 1H), 6.96 (dd, J = 8.8, 2.4 Hz, 1H), 4.05 (s, 3H), 3.99 (s, 3H), 3.95 (s, 3H), 3.92 (s, 3H), 3.89 (s, 3H), 3.75 (d, J = 1.7 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 192.86, 155.42, 154.29, 149.72, 146.90, 134.83, 134.54, 131.90, 131.14, 129.21, 129.04, 125.03, 113.71, 111.88, 110.65, 101.44, 100.27, 61.13, 60.83, 56.29, 55.91, 33.67, 29.80. HRMS (ESI) (m/z) $[M+H]^+$ calcd for $C_{23}H_{23}NO_5$, 393.1576; found,394.1635. Purity: 95.1% (by HPLC).

(E)-4,5,6-trimethoxy-2-((6-methoxy-1H-indol-3-yl)methylene)-2,3-dihydro-1H-inden-1-one(8e)

Yellow solid, 277.1mg, yield 81.2%. mp:209.1℃-211.7℃. R_f= 0.51 (petroleum/ethyl acetate=1:1). IR (KBr,cm^{-1}) 3160, 3058, 3014, 2988, 1672, 1600, 1498, 1340, 1127, 723. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.57 (s, 1H), 8.00 (s, 1H), 7.81 (d, J = 8.5 Hz, 1H), 7.61 (d, J = 2.4 Hz, 1H), 7.20 (s, 1H), 6.93 (dd, J = 10.6, 2.0 Hz, 2H), 4.03 (s, 3H), 3.98 (s, 3H), 3.93 (s, 3H), 3.86 (s, 3H), 3.77 (d, J = 1.7 Hz, 2H). ^{13}C NMR (101 MHz, DMSO) δ 191.82, 156.85, 154.35, 150.14, 146.88, 137.27, 134.66, 134.52, 129.62, 128.24, 125.22, 122.00, 119.37, 112.01, 111.12, 101.51, 95.52, 61.13, 60.87, 56.52, 55.68, 30.00. HRMS (ESI) (m/z) $[M+H]^+$ calcd for $C_{22}H_{21}NO_5$, 379.1420; found,380.1482. Purity: 99.3% (by HPLC).

(E)-4,5,6-trimethoxy-2-((6-methoxy-1-methyl-1H-indol-3-yl)methylene)-2,3-dihydro-1H-inden-1-one(8f)

Yellow solid, 319.2mg, yield 90.2%.mp:165.5℃-167.6℃. R_f= 0.57 (petroleum/ethyl acetate=1:1). IR(KBr,cm-1) 3074, 2940, 1662, 1604, 1527, 1345, 1090, 730.^{1}H NMR(400 MHz, $CDCl_3$) δ 7.93 (s, 1H), 7.78 (d, J = 8.7 Hz, 1H), 7.37 (s, 1H), 7.17 (s, 1H), 6.91 (d, J = 8.6 Hz, 1H), 6.74 (s, 1H), 4.04 (s, 3H), 3.98 (s, 3H), 3.93 (s, 3H), 3.86 (s, 3H), 3.82 (s, 3H), 3.65 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 192.88, 157.20, 154.02, 149.54, 146.86, 137.41, 134.52, 129.97, 124.92, 122.61, 119.63, 112.06, 110.65, 101.27, 93.16, 61.10, 60.79, 58.52, 56.26, 55.63, 33.85, 29.56, 18.90. HRMS (ESI) (m/z) $[M+H]_+$ calcd for $C_{23}H_{23}NO_5$, 393.1576; found,394.1628. Purity: 95.9% (by HPLC).

(E)-2-((1H-indol-4-yl)methylene)-4,5,6-trimethoxy-2,3-dihydro-1H-inden-1-one(10a)

Yellow solid, 260.2mg, yield 82.8%.mp:179.8℃-181.9℃. R_f= 0.39 (petroleum/ethyl acetate=2:1). IR(KBr,cm^{-1}) 3288, 3120, 2998, 1662, 1602, 1509, 1127, 710. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.43 (s, 1H), 8.18 (s, 1H), 7.61 (d, J = 7.5 Hz, 1H), 7.47 (d, J = 8.0 Hz, 1H), 7.32 (dd, J = 12.4, 5.0 Hz, 2H), 7.23 (s, 1H), 6.93 (s, 1H), 4.03 (s, 3H)4.01 (d, J = 1.8 Hz,2H), 3.99 (s, 3H), 3.94 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 193.57, 154.40, 149.84, 147.53, 136.26, 136.11, 134.50, 133.88, 130.89, 129.72, 127.20, 125.36, 122.06, 120.62, 112.85, 101.65, 101.29, 61.17, 60.81, 56.31, 29.58. HRMS (ESI) (m/z) $[M+H]^+$ calcd for $C_{21}H_{19}NO_4$, 349.1314; found, 372.1192. Purity: 99.6% (by HPLC).

(E)-2-((1H-indol-4-yl)methylene)-5,6,7-trimethoxy-2,3-dihydro-1H-inden-1-one(10c)

Yellow solid, 281.9mg, yield 89.7%.mp:139.3℃-142.5℃. R_f= 0.43 (petroleum/ethyl acetate=2:1). IR(KBr,cm^{-1}) 3274, 3118, 3029, 2996, 1675, 1602, 1571, 1108, 697. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.41 (s, 1H), 8.13 (s, 1H), 7.52 (d, J = 7.5 Hz, 1H), 7.47 (d, J = 8.1 Hz, 1H), 7.36 – 7.34 (m, 1H), 7.31 (d, J = 7.8 Hz, 1H), 6.93 (d, J = 2.2 Hz, 1H), 6.81 (s, 1H), 4.17 (s, 3H), 4.04 (d, J = 1.5 Hz, 2H), 3.99 (s, 3H), 3.92 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 191.28, 159.56, 152.44, 148.02, 141.06, 136.15, 135.18, 129.57, 129.50, 127.33, 125.44, 124.38, 121.85, 120.19, 112.66, 103.63, 101.09, 62.25, 61.55, 56.36, 32.86. HRMS (ESI) (m/z) $[M+H]^+$ calcd for $C_{21}H_{19}NO_4$, 349.1314; found, 350.1367. Purity: 97.7% (by HPLC).

(E)-4,5,6-trimethoxy-2-((1-methyl-1H-indol-4-yl)methylene)-2,3-dihydro-1H-inden-1-one(10b)

Yellow solid, 282.7mg, yield 86.5%. mp:168.0℃-170.5℃. R_f= 0.39 (petroleum/ethyl acetate=2:1). IR(KBr,cm-1) 3091, 3066, 2978, 1689, 1621, 1510, 1120, 709.^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (d, J= 1.8 Hz, 1H), 7.60 (d, J= 7.2 Hz, 1H),7.39 (d, J= 8.1 Hz, 1H), 7.35 (d, J= 7.5 Hz, 1H), 7.23 (s, 1H), 7.17 (d, J= 3.1 Hz,1H), 6.87 – 6.84 (m, 1H), 4.02 (s, 3H), 3.99 (s, 3H), 3.94 (s, 3H), 3.84 (s, 3H), 3.72(d, J= 1.9 Hz, 2H) ^{13}C NMR (101 MHz, $CDCl_3$) δ 193.41, 154.37, 149.82, 147.46, 136.95, 136.18, 134.46, 133.89, 130.70, 130.25, 129.88, 127.25, 121.62, 120.20, 110.91, 101.59, 99.65, 61.16, 60.79, 56.31, 33.00, 29.57. HRMS (ESI) (m/z) $[M+H]^+$ calcd for $C_{22}H_{21}NO_4$, 363.1471; found, 364.1527. Purity: 98.9% (by HPLC).

(E)-5,6,7-trimethoxy-2-((1-methyl-1H-indol-4-yl)methylene)-2,3-dihydro-1H-inden-1-one(10d)

Yellow solid, 289.9mg, yield 88.7%. mp:169.5℃-171.3℃. R_f= 0.42 (petroleum/ethyl acetate=2:1). IR(KBr,cm^{-1}) 3097, 2930, 1683, 1621, 1602, 1471, 1320, 1114, 706. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (t, J = 2.0 Hz, 1H), 7.49 (d, J = 7.3 Hz, 1H), 7.37 (d, J = 8.1 Hz, 1H), 7.31 (d, J = 7.6 Hz, 1H), 7.16 (d, J = 3.2 Hz, 1H), 6.83 (dd, J = 3.1, 0.6 Hz, 1H), 6.78 (s, 1H), 4.14 (s, 3H), 4.01 (d, J = 1.6 Hz, 2H), 3.97 (s, 3H), 3.90 (s, 3H), 3.84 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 191.06, 159.47, 152.49, 147.91, 141.07, 136.94, 135.28, 130.08, 129.78, 129.25, 127.57, 124.43, 121.50, 119.81, 110.54, 103.56, 99.69, 62.27, 61.55, 56.34, 33.03, 32.86. HRMS (ESI) (m/z) $[M+H]^+$ calcd for $C_{22}H_{21}NO_4$, 363.1471; found, 364.1524. Purity: 98.1% (by HPLC).

(E)-2-((1H-indol-5-yl)methylene)-4,5,6-trimethoxy-2,3-dihydro-1H-inden-1-one(11a)

Yellow solid, 249.5mg, yield 79.4%. mp:211.4℃-213.3℃. R_f= 0.38 (petroleum/ethyl acetate=2:1). IR(KBr,cm^{-1}) 3237, 3035, 2940, 1677, 1618, 1591, 1481, 710.^{1}H NMR (400 MHz, $CDCl_3$) δ 8.42 (s, 1H), 8.03 (s, 1H), 7.81 (s, 1H), 7.58(d, J= 8.4 Hz, 1H), 7.48 (d, J= 8.2 Hz, 1H), 7.26 (s, 1H), 7.22 (s, 1H), 6.67(s, 1H), 4.05 (s, 3H), 4.02 (s, 2H), 3.99 (s, 3H), 3.93 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 193.69, 154.38, 149.84, 147.33, 136.58, 136.03, 135.81, 133.98, 131.82, 128.44, 127.41, 125.51, 125.19, 124.48, 111.63, 103.63, 101.60, 61.16, 60.85, 56.30,

View Article Online
DOI: 10.1039/C5RA14888J

29.43. HRMS (ESI) (m/z) $[M+H]^+$ calcd for $C_{21}H_{19}NO_4$, 349.1314; found, 372.1190. Purity: 95.8% (by HPLC).

(E)-2-((1H-indol-5-yl)methylene)-5,6,7-trimethoxy-2,3-dihydro-1H-inden-1-one(11c)

Yellow solid, 284.1mg, yield 90.4%. mp:129.1℃-131.3℃. R_f= 0.44 (petroleum/ethyl acetate=2:1). IR(KBr,cm^{-1}) 3345, 3266, 3010, 2980, 1675, 1606, 1592, 1471, 1128, 690. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45 (s, 1H), 7.99 (s, 1H), 7.75 (s, 1H), 7.54 (dd, J = 8.5, 1.4 Hz, 1H), 7.48 (d, J = 8.5 Hz, 1H), 7.31 – 7.29 (m, 1H), 6.83 (s, 1H), 6.67 – 6.62 (m, 1H), 4.15 (s, 3H), 4.05 (d, J = 1.2 Hz, 2H), 4.00 (s, 3H), 3.92 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 191.38, 159.34, 152.38, 147.81, 141.06, 136.39, 134.22, 132.50, 128.39, 127.56, 125.55, 125.05, 124.44, 123.97, 111.60, 103.61, 103.40, 62.24, 61.54, 56.33, 32.80. HRMS (ESI) (m/z) $[M+H]^+$ calcd for $C_{21}H_{19}NO_4$, 349.1314; found, 350.1375. Purity: 98.5% (by HPLC).

(E)-4,5,6-trimethoxy-2-((1-methyl-1H-indol-5-yl)methylene)-2,3-dihydro-1H-inden-1-one(11b)

Yellow solid, 265.7mg, yield 81.3%. mp:138.1℃-140.6℃. R_f= 0.37 (petroleum/ethyl acetate=2:1). IR(KBr,cm^{-1}) 3089, 3026, 1677, 1619, 1591, 1481, 1340, 1120, 708. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (s, 1H), 7.80 (s, 1H), 7.60 (dd, J = 8.6, 1.5 Hz, 1H), 7.39 (d, J = 8.6 Hz, 1H), 7.22 (s, 1H), 7.11 (d, J = 3.1 Hz, 1H), 6.60 (dd, J = 3.1, 0.6 Hz, 1H), 4.05 (s, 3H), 4.01 (d, J = 1.8 Hz, 2H), 3.99 (s, 3H), 3.94 (s, 3H), 3.83 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 193.49, 154.31, 149.80, 147.21, 137.33, 135.92, 135.74, 133.99, 131.60, 130.15, 128.92, 126.84, 124.75, 124.60, 109.71, 102.19, 101.50, 61.14, 60.82, 56.27, 32.95, 29.42. HRMS (ESI) (m/z) $[M+H]^+$ calcd for $C_{22}H_{21}NO_4$, 363.1471; found, 364.1530. Purity: 95.9% (by HPLC).

(E)-5,6,7-trimethoxy-2-((1-methyl-1H-indol-5-yl)methylene)-2,3-dihydro-1H-inden-1-one(11d)

Yellow solid, 305.3mg, yield 93.4%. mp:156.1℃-158.8℃. R_f= 0.56 (petroleum/ethyl acetate=1:1). IR(KBr,cm^{-1}) 3039, 3008, 2920, 1683, 1591, 1484, 1160, 714. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (s, 1H), 7.80 (s, 1H), 7.60 (dd, J = 8.6, 1.5 Hz, 1H), 7.39 (d, J = 8.6 Hz, 1H), 7.22 (s, 1H), 7.11 (d, J = 3.1 Hz, 1H), 6.60 (dd, J = 3.1, 0.6 Hz, 1H), 4.05 (s, 3H), 4.01 (d, J = 1.8 Hz, 2H), 3.99 (s, 3H), 3.94 (s, 3H), 3.83 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 191.23, 159.24, 152.35, 147.74, 141.01, 137.12, 134.12, 132.37, 130.11, 128.88, 127.06, 124.59, 124.43, 124.16, 109.64, 103.58, 102.01, 62.26, 61.53, 56.32, 32.98, 32.77. HRMS (ESI) (m/z) $[M+H]^+$ calcd for $C_{22}H_{21}NO_4$, 363.1471; found, 364.1525. Purity: 97.3% (by HPLC).

Biology

Cell lines and culture. The human cancer cell lines (A549, Hela, Bel-7402, PC-3, K562) used in this study were grown in DMEM containing 10% (v/v) heat-inactivated FBS, 100 units/mL penicillin, and 100 μg/mL streptomycin. The cells were cultivated at 37 °C under a 5% CO_2 and 90% relative humidity (RH) atmosphere.

MTT assay. The antiproliferative activity of the target compounds towards the human cancer cell lines were examined by MTT assay. Briefly, cells were harvested in the logarithmic phase, and plated into the 96-well plates (5×10^3 cells per well) for 24 h, and then, different concentrations of the test compounds were added for 48 h in three replicates. Afterward, 20 μL of 5 mg/mL MTT (Sigma) was added into each well and incubated for another 4 h. Finally, the suspension was discarded and 150 μL of DMSO was added to dissolve the dark blue crystals (formazan). The absorbance at 570nm was measured using a multifunction mircoplate reader (Moleculardevices, Flex Station 3). All experiments were repeated at least three times. The GI_{50} values and SD values were calculated using Grap Pad Prism version 5.0 and Excel software, respectively.

Tublin polymerization assay in vitro. Tublin polymerization assay was monitored by an increase in fluorescence intensity. Purified brain tubulin polymerization kit was purchased from Cytoskeleton (BK110P, Denver, CO). All of the experiments were performed under the guidance of the manufacturer's instruction. The final buffer concentration for tubulin polymerization contained 80.0 mM piperazine-N, N'-bis(2-ethanesulfonic acid) sequisodium salt (pH 6.9), 2.0 mM MgCl2, 0.5 mM EGTA, 1 mM GTP, and 10.2% glycerol. Firstly, 5 μL of tested compounds were added in 96 well, and then warmed to 37 °C for 1 min. The reaction was initiated by the addition of 55 μL of the tubulin reaction mix. Then the fluorescence intensity enhancement were recorded over a 60 minute period at 37 °C (emission wavelength is 410 nm and excitation wavelength is 340 nm) using a multifunction mircoplate reader (Moleculardevices, Flex Station 3). All experiments were repeated at least three times. The IC_{50} values and SD values were calculated using Grap Pad Prism version 5.0 and Excel software, respectively.

Immunofluorescence Microscopy. Hela cells were plated in a confocal culture-dish at 3×10^4 cells/dish, cultured for 24 h and then were incubated with indicated concentrations of compound **10a** for another 48 h. The cells were briefly washed with PBS, fixed with 4 % pre-warmed (37 °C) paraformaldehyde for 15 min, and then were permeabilized with 0.5% Triton X-100 for another 15 min and finally blocked with 10% goat serum for 30 min. The bolcked cells was incubated with FITC-conjugated mouse anti-tubulin antibody (Sigma) at 4 °C overnight. The following day, cells were washed with PBS for three times and incubated with goat anti-mouse IgG/Alexa-Fluor 488 (Invitrogen, USA) for 1 h. The nuclei of cells were labeled with Hochest 33342 (Sigma). After washing, 1 mL PBS was added and the samples were immediately visualized on a Zeiss LSM 570 laser scanning confocal microscope.

Cell Cycle analysis. For flow cytometric analysis of DNA content, Hela cells were plated in 6-well plates (2×10^5 cells/well) and incubated in the presence or absence of compound **10a** at indicated concentrations for 48 h. Then, the cells were harvested by centrifugation and fixed with ice-cold

View Article Online
DOI: 10.1039/C5RA14888J

70% ethanol overnight. After removing the ethanol by centrifugation, the cells were washed with cold 10% PBS, and then treated with RNAse A (100 μg/mL, Beyotime) at 37 °C for 30 min and DNA staining solution PI (Sigma) at 4 °C for 15 min. The DNA contents of 10,000 events were harvested by flow cytometer (Beckman Coulter, Epics XL) at 488 nm. The percentages of cells in different phases of cell cycle were analyzed by EXPO32 ADC analysis software.

Apoptosis Analysis. Hela cells were plated in 6-well plates (2×10^5 cells/well) and incubated in the presence or absence of compound **10a** at indicated concentrations for 48 h to induce cell apoptosis. The percentages of apoptotic cells were stained with Annexin-V-FITC and PI (Annexin-V-FITC Apoptosis Detection Kit, Beyotime) according to the manufacturer's instructions with some modification. Briefly, both treated and untreated cells were harvested and incubated with 5 μL of Annexin-V-FITC in binding buffer (10 mM HEPES, 140 mM NaCl, and 2.5 mM CaCl2 at Ph 7.4) at room temperature for 15 min. Then the PI solution was then added dropwise to the mixture at cold temperature for 10 min. Almost 10,000 events were collected for each sample and analyzed by flow cytometer (Beckman Coulter, Epics XL). The percentage of cells undergoing apoptosis was calculated using EXPO32 ADC Analysis software.

Acknowledgements

We thank the National Natural Science Foundation of China (No. 21302235, 20972198), Guangdong Natural Science Foundation (2014A030313124) and the Fundamental Research Funds for the Central Universities (15ykpy04) for financial support of this study.

Notes and references

[a] School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou, 510006, China
* For L. Huang: Tel.: +086-20-3994-3051; Fax: +086-20-3994-3051; e-mail: huangl72@mail.sysu.edu.cn; For X. Li.: Tel.: +086-20-3994-3050; Fax: +086-20-3994-3050; e-mail: lixsh@mail.sysu.edu.cn
Electronic Supplementary Information (ESI) available: NMR spectrums and HPLC chromatograms of target compounds. See DOI: 10.1039/b000000x/

1. M. A. Jordan and L. Wilson, *Nat. Rev. Cancer*, 2004, **4**, 253-265.
2. A. E. Prota, K. Bargsten, D. Zurwerra, J. J. Field, J. F. Diaz, K. H. Altmann and M. O. Steinmetz, *Science (New York, N.Y.)*, 2013, **339**, 587-590.
3. M. A. Jordan, Curr. Med. Chem. Anti-cancer Agents, 2002, **2**, 1-17.
4. M. Abal, J. M. Andreu and I. Barasoain, *Curr. Cancer Drug Targets*, 2003, **3**, 193-203.
5. T. E. Hutson, R. Ganapathi, P. Elson, T. Mekhail, T. Olencki, G. T. Budd and R. M. Bukowski, *Invest. New Drugs*, 2004, **22**, 277-284.
6. K. Carlson and A. J. Ocean, *Clin. Breast Cancer*, 2011, **11**, 73-81.
7. M. Kavallaris, N. M. Verrills and B. T. Hill, *Drug Resist. Updates*, 2001, **4**, 392-401.
8. C. Ballatore, K. R. Brunden, D. M. Huryn, J. Q. Trojanowski, V. M. Lee and A. B. Smith, 3rd, *J. Med. Chem.*, 2012, **55**, 8979-8996.
9. C. M. Lin, H. H. Ho, G. R. Pettit and E. Hamel, *Biochemistry*, 1989, **28**, 6984-6991.
10. G. R. Pettit, S. B. Singh, E. Hamel, C. M. Lin, D. S. Alberts and D. Garcia-Kendall, *Experientia*, 1989, **45**, 209-211.
11. D. W. Siemann, D. J. Chaplin and P. A. Walicke, *Expert. Opin. Investig. Drugs*, 2009, **18**, 189-197.
12. G. R. Pettit, B. E. Toki, D. L. Herald, M. R. Boyd, E. Hamel, R. K. Pettit and J. C. Chapuis, *J. Med. Chem.*, 1999, **42**, 1459-1465.
13. A. Brancale and R. Silvestri, *Med. Res. Rev.*, 2007, **27**, 209-238.
14. Z. Nowakowska, *Eur. J. Med. Chem.*, 2007, **42**, 125-137.
15. S. Ducki, D. Rennison, M. Woo, A. Kendall, J. F. D. Chabert, A. T. McGown and N. J. Lawrence, *Bioorg. Med. Chem.*, 2009, **17**, 7698-7710.
16. C. Zhu, Y. Zuo, R. Wang, B. Liang, X. Yue, G. Wen, N. Shang, L. Huang, Y. Chen, J. Du and X. Bu, *J. Med. Chem.*, 2014, **57**, 6364-6382.
17. Z. Yang, W. Wu, J. Wang, L. Liu, L. Li, J. Yang, G. Wang, D. Cao, R. Zhang, M. Tang, J. Wen, J. Zhu, W. Xiang, F. Wang, L. Ma, M. Xiang, J. You and L. Chen, *J. Med. Chem.*, 2014, **57**, 7977-7989.
18. S. Rizzo, M. Bartolini, L. Ceccarini, L. Piazzi, S. Gobbi, A. Cavalli, M. Recanatini, V. Andrisano and A. Rampa, *Bioorg. Med. Chem.*, 2010, **18**, 1749-1760.
19. A. P. Prakasham, A. K. Saxena, S. Luqman, D. Chanda, T. Kaur, A. Gupta, D. K. Yadav, C. S. Chanotiya, K. Shanker, F. Khan and A. S. Negi, *Bioorg. Med. Chem.*, 2012, **20**, 3049-3057.
20. F. C. Meng, F. Mao, W. J. Shan, F. Qin, L. Huang and X. S. Li, *Bioorg. Med. Chem. Lett.*, 2012, **22**, 4462-4466.
21. J. R. Peterson, M. E. Russell and I. B. Surjasasmita, *J. Chem. Eng. Data*, 1988, **33**, 534-537.
22. P. E. Eaton, G. R. Carlson and J. T. Lee, *J. Org. Chem.*, 1973, **38**, 4071-4073.
23. C. J. Trabbic, J. H. Overmeyer, E. M. Alexander, E. J. Crissman, H. M. Kvale, M. A. Smith, P. W. Erhardt and W. A. Maltese, *J. Med. Chem.*, 2015, **58**, 2489-2512.
24. M. S. Pedras and A. Abdoli, *Bioorg Med Chem*, 2013, **21**, 4541-4549.
25. R. Bansal, G. Narang, C. Zimmer and R. W. Hartmann, *Med. Chem. Res.*, 2010, **20**, 661-669.
26. D. Bonne, C. Heuséle, C. Simon and D. Pantaloni, *J. Biol. Chem.*, 1985, **260**, 2819-2825.
27. J. F. A. Diaz, Jose Manuel, *Biochemistry* 1993, **32**, 2747-2755.
28. R. K. K. Gill, Ramandeep; Kaur, Gurneet; Rawal, Ravindra K.; Shah, Anamik K.; Bariwal, Jitender., *Curr. Org. Chem.* , 2014, vol. 57, pp. 2462-2512.
29. P. R. A. Clarke, L. A. ,*Trends. Cell Biol.*, 2009, **19**, 89-98.