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Synthesis, Biological Evaluation of 1,1-Diarylethylenes as a Novel Class of Antimitotic Agents

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The cytotoxic activities of 23 new *iso*combretastatin A derivatives with modifications on the B-ring were investigated. Several compounds exhibited excellent antiproliferative activity at nanomolar concentrations against a panel of human cancer cell lines. Compounds *iso*FCA-4 (**2e**), *iso*CA-4 (**2k**) and *iso*-NH_2CA-4 (**2s**) were the most cytotoxic, and strongly inhibited tubulin polymerization with IC_{50} values of 4, 2 and 1.5 μM, respectively. These derivatives were found to be 10-fold more active than phenstatin and colchicine with respect to growth inhibition but displayed similar activities as tubulin polymerization inhibitors. In addition, cell cycle arrest in the G_2/M phase and subsequent apoptosis was observed in three cancer cell lines when treated with these compounds. The disruptive effect of **2e**, **2k** and **2s** on the vessel-like structures formed by human umbilical vein endothelial cells (HUVEC) suggest that these compounds may act as vascular disrupting agents. Both compounds **2k** and **2s** have the potential for further prodrug modification and development as vascular disrupting agents for treatment of solid tumors.

Introduction

The formation of microtubules is a dynamic process involved in a variety of cellular processes including cell division, maintenance of cell shape, cell signaling, cell migration and intracellular transport.[1] Microtubules are dynamic hollow structures composed of α- and β-tubulin heterodimers. Because microtubules have crucial roles in the regulation of mitotic spindle formation, the disruption of cellular microtubule dynamics can have quite drastic effects on cell viability, leading to cell-cycle arrest in the M phase followed by apoptosis. The discovery of natural products capable of interfering with the assembly or disassembly of microtubules has attracted much attention because microtubules are recognized as an attractive pharmacological target for anticancer drug discovery.[2] The commonly used drugs belonging to this class of compounds are paclitaxel and vinca alkaloids. Although they have gained wide clinical use in the treatment of various cancers,[3] these complex drugs suffer from several drawbacks since they are generally difficult to synthesize, cause neurotoxic side effects in patients,[4] and their clinical potential is now limited by emerging multidrug resistance (MDR).[5] Therefore, the search for new antimitotic tubulin inhibitors that overcome resistance mechanisms has become a topic of great interest. Recently, it was demonstrated that some tubulin-binding agents also target the vascular system of tumors, inducing morphological changes in the endothelial cells of the tumors blood vessels so as to occlude flow.[6]

Among the large class of natural products interfering with the dynamics of tubulin polymerization and depolymerization, combretastatin A-4 (CA-4), first isolated from the South African bush willow tree, *Combretum caffrum*,[7] is a promising anticancer drug. CA-4 binds to tubulin in the colchicine-binding site and is recognized as a very effective inhibitor of tubulin assembly. Moreover, CA-4 exhibits strong growth inhibition at nanomolar concentrations against a wide variety of human cell lines including multidrug resistant (MDR) cancer cell lines.[8] However, the low water solubility of CA-4 limits its efficacy in vivo. A more-soluble disodium phosphate prodrug (CA-4P) has been developed as the preferred lead for human studies (Figure 1).[9] CA-4P and its amino acid derivative AVE-8062[10] have been demonstrated to cause vascular shutdown in established tumors in vivo, consistent with an antivascular mechanism of action.[6] Currently, CA-4P is undergoing several advanced clinical trials worldwide for the treatment of age-related macular degeneration (AMD)[9] or anaplastic thyroid cancer,[11] either as a single agent or in combination therapy.

Despite their remarkable anticancer activity, these Z-stilbene compounds may be prone to double bond isomerization.[12] The *E*-isomers display dramatically reduced inhibition of cancer cell growth and tubulin assembly.[13] A number of structure–activity relationships (SARs) have been reported for the combre-

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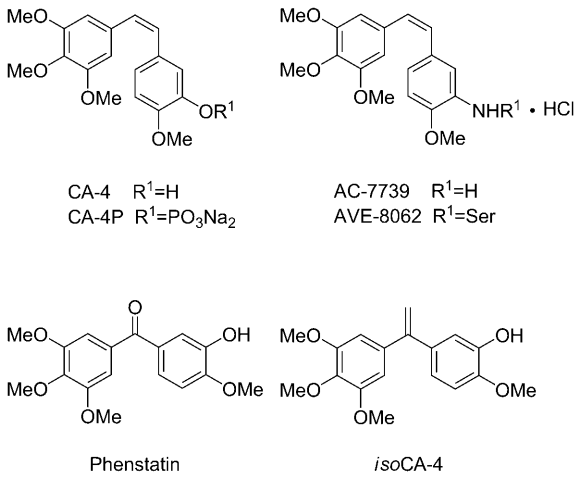


Figure 1. Structures of combretastatin A-4, its synthetic amino derivative AC-7739, their water soluble prodrugs CA-4P and AVE-8062, phenstatin and *iso*-combretastatin A-4.

tastatins. These studies revealed that the 3,4,5-trimethoxyphenyl (TMP) unit as well as the *cis* orientation of the two aromatic rings is a prerequisite for significant biological activity.[14] Therefore, extensive studies have been conducted to prepare various *cis* restricted analogues mainly by inserting the *cis* olefin in a five-membered heterocyclic ring (e.g., pyrazoles, thiazoles, triazoles, imidazolones).[15]

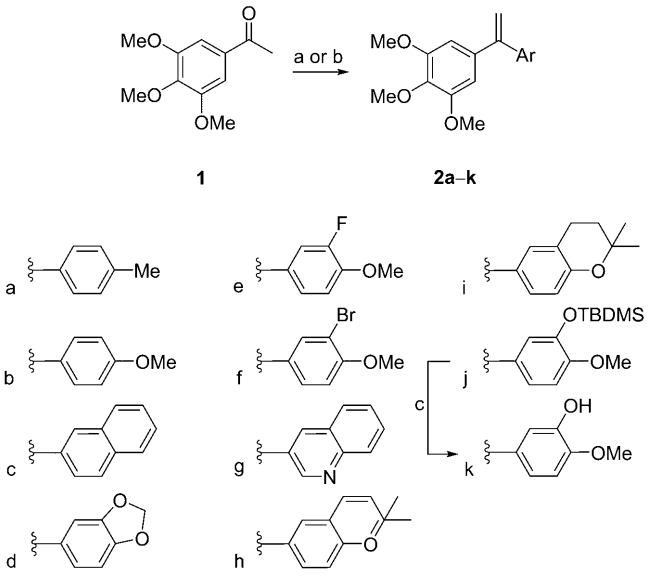
Our interest in the 1,1-diarylethylene unit synthesis,[16] combined with our efforts to discover novel potent tubulin assembly inhibitors related to CA-4,[17] led us to identify a promising class of compounds, with strong cytotoxic and antimitotic activities, simply by switching the TMP moiety from the C(1) to the C(2) position of the ethylene bridge.[18]

In contrast to the parent natural products, combretastatins A-1 to A-6, these synthetic isomers of combretastatins A, named *iso*combretastatins A (*iso*CA), are easy to synthesize without the need to control the olefin geometry, and constitute the simplest isomers of combretastatins A. The most active compound, *iso*CA-4, which shares a striking structural similarity to phenstatin,[19] appears to elicit its tumor cytotoxicity similar to CA-4, via inhibition of tubulin polymerization, which then leads to cell-cycle arrest in G_2/M phase. As replacement of the 1,2-ethylene bridge by 1,1-ethylene resulted in retention of biological activity, our finding encouraged us to use this bioisostere[20] in future SAR studies. Because the TMP moiety (A-ring) is crucial to elicit cytotoxic and antitubulin responses, we intended to introduce variations in the B-ring that could yield compounds with more druglike properties. Herein, we report the synthesis and biological evaluation of a broad range of B-ring-substituted *iso*CA-4 analogues in which the A-ring remains unmodified. These compounds were then evaluated in vitro to assess their tubulin assembly and growth inhibitory activity, and their potential to disrupt established vessel networks in cancer cells.

Results and Discussion

Chemistry

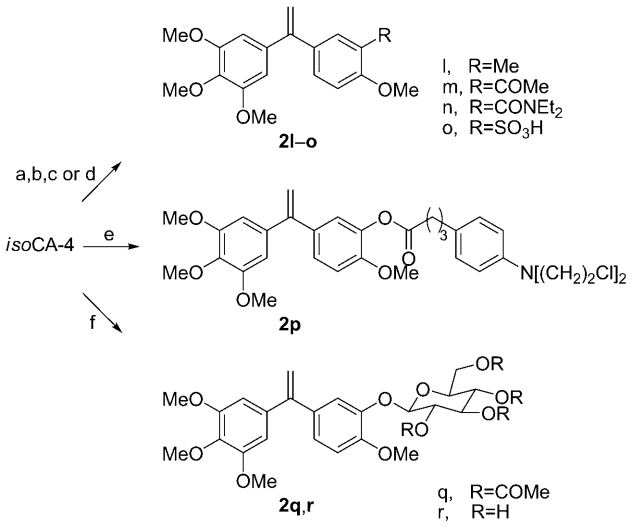
While the palladium-catalyzed coupling of 3,4,5-trimethoxyacetophenone *N*-tosylhydrazone with aryl halides proved to be an efficient procedure for the synthesis of **2**,[18] we examined an alternative synthetic route avoiding the use of palladium catalyst.[21] We envisaged that the terminal double bond in compounds **2** could be generated by dehydration of the corresponding tertiary alcohols. As outlined in Scheme 1, reaction



Scheme 1. *Reagents and conditions*: a) 1. ArMgBr, THF, −40 °C; 2. PTSA (10 mol%), CH_2Cl_2, 20 °C (**2a**: 44%, **2b**: 54%, **2c**: 81%); b) 1. ArLi, hexanes/toluene (1:3), −78 °C; 2. PTSA (10 mol%), CH_2Cl_2, 20 °C (**2d**: 80%, **2e**: 48%, **2f**: 53%, **2h**: 48%, **2i**: 32%, **2j**: 85%); 3. MsCl, DMAP, CH_2Cl_2, (**2g**: 36%); c) K_2CO_3, MeOH, 20 °C, (**2k**: 94%).

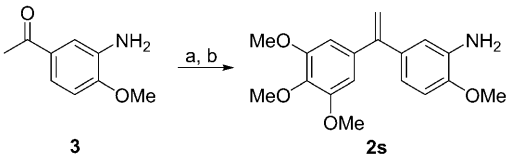
of commercially available 3,4,5-trimethoxyacetophenone (**1**) with Grignard reagents in THF furnished the corresponding tertiary alcohols, which afforded **2a**–**c** in good overall yields upon treatment (without purification) with a catalytic amount of *p*-toluenesulfonic acid (PTSA) in CH_2Cl_2. In a similar way, the synthesis of compounds **2d**–**j** was realized by the treatment of **1** with an aryl lithium species obtained according to a lithium–halogen exchange reaction from the corresponding bromo- or iodo- derivatives. Notably, the condensation reaction should be conducted in a mixture of toluene/hexanes (3:1), as no reaction occurred in THF or Et_2O, presumably due to the enolization of the 3,4,5-trimethoxyacetophenone moiety. Subsequent dehydration of the resulting tertiary alcohols gave the corresponding 1,1-diarylethylene derivatives **2**, except for **2g**. In this case, **2g** was obtained via elimination of the corresponding mesylate in the presence of DMAP since PTSA was ineffective. Finally, desilylation of the TBDMS ether intermediate **2j** under alkaline conditions led to the formation of *iso*CA-4 (**2k**) in excellent yield.

It has been shown that a compound with only a methoxy group in the *para* position of ring B of CA-4 retains its cytotoxicity, suggesting that the presence of a free hydroxy group is not fundamental.[13a] Consequently, as an extension of our SAR efforts with *iso*CA-4, a selection of compounds including, esters, carbamates and β-sugar[22] derivatives were prepared (**2l**–**r**). Scheme 2 details the analogous synthesis of 3′-O-substituted *iso*combretastatin analogues from the parent compound *iso*CA-4 (**2k**).



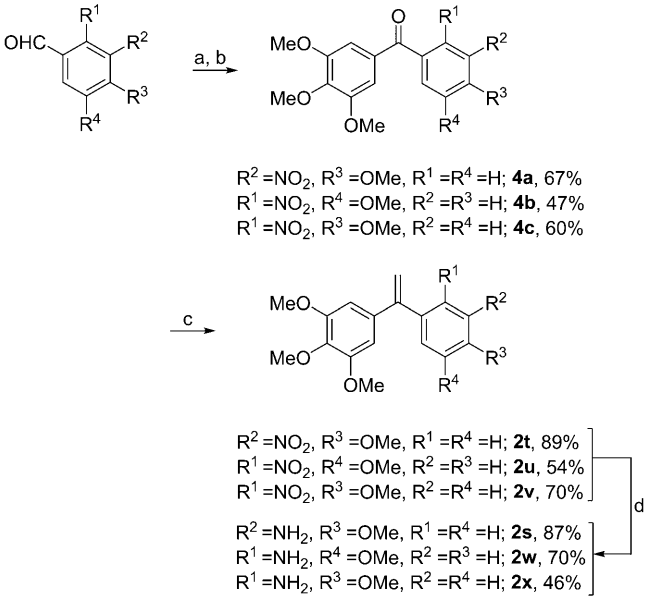
Scheme 2. *Reagents and conditions*: a) Me_2SO_4, K_2CO_3, acetone; b) Ac_2O, Pyr, CH_2Cl_2; c) diethylcarbamoyl chloride, Pyr, CH_2Cl_2; d) SO_3-pyridine complex, Pyr; e) chlorambucil, EDC, DMAP, CH_2Cl_2; f) 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide, KOH, CH_3CN; g) NH_4Cl, MeOH.

As the replacement of the hydroxy moiety on the B-ring by an amino group in the CA-4 series resulted in retention of biological activity,[23] we envisioned compound **2s**, which constitutes the simplest isomer of AC-7739. To this end, the coupling reaction between acetophenone **3** and 3,4,5-trimethoxyphenylmagnesium bromide (Scheme 3) led to the tertiary alcohol,



Scheme 3. Synthesis of *iso*NH_2CA-4 (**2s**). *Reagents and conditions*: a) 3,4,5-trimethoxyphenylmagnesium bromide (3.5 equiv), THF, −40 °C (3 h), then RT (16 h); b) PTSA (cat.), CH_2Cl_2, 30 min, RT.

which upon treatment with a catalytic amount of PTSA provided **2s** in a low 12% yield. The protection of the free amino group into the corresponding pivaloylamino, phthalimide or acetamide derivatives did not significantly improve the yield of the coupling reaction. In view of these results, we adopted a Wittig strategy to easily access a variety of aniline derivatives and their nitro precursors. Scheme 4 outlines the synthetic



Scheme 4. Synthesis of *iso*aminocombretastatin derivatives. *Reagents and conditions*: a) 3,4,5-trimethoxyphenylmagnesium bromide, THF, −78 °C; b) PCC, CH_2Cl_2, 20 °C; c) Ph_3PCH_3Br, LiHMDS, THF, 0 to 20 °C; d) Zn, AcOH, 20 °C.

route followed for the synthesis of these amino substances. The preparation involved a reaction sequence in four steps:

1) Condensation of the 3,4,5-trimethoxyphenylmagnesium bromide with commercially available appropriate benzaldehyde derivatives
2) Oxidation of the resulting alcohols
3) Wittig reaction using methyltriphenylphosphonium bromide to afford the corresponding 1,1-diarylethylene **2t**–**v** derivatives
4) Reduction of the nitro group by Zn/AcOH

Accordingly, the expected amine isomers **2s**, **2w**, **2x** were obtained in good overall yields. Notably, this strategy allowed easy access to amino*iso*combretastatin analogues **2s**, **2w**, **2x** on multiple-gram scale.

Biological evaluation

In vitro Cell Growth Inhibitory Activity

The cytotoxic activity of 23 newly synthesized *iso*combretastatin analogues (**2**) against the human colon carcinoma cell line (HCT-116) was initially evaluated using *iso*CA-4, CA-4[24] and phenstatin as reference compounds. The GI_{50} values corresponding to the concentration of studied compound leading to 50% decrease in HCT-116 cell growth are presented in Table 1.

Several 1,1-diarylethylene candidates retained potent growth inhibitory activity at nanomolar concentrations against cancer cell. In particular, the best inhibition results were obtained with compounds **2e**, **2m** and **2s** (7, 8 and 2 nM, respec-

Table 1. Cytotoxicity of *iso*CA-4 analogues against colon carcinoma cells (HCT-116).

Compd	GI_{50} [nM][a]	Compd	GI_{50} [nM][a]
2a	400±25	**2o**	6000±420
2b	40±3	**2p**	25±2
2c	80±7	**2q**	3000±155
2d	450±60	**2r**	4000±320
2e (*iso*FCA-4)	7±1	**2s** (*iso*NH_2CA-4)	2±0.1
2f	1000±90	**2t**	60±2
2g	650±66	**2u**	4000±250
2h	NA[b]	**2v**	400±33
2i	NA[b]	**2w**	5000±510
2j	180±30	**2x**	40±3
2l	1000±110	*iso*CA-4	2±0.2
2m	8±0.2	CA-4	2±0.1[c]
2n	2800±310	Phenstatin	33±2.5[c]

[a] GI_{50} is the concentration of compound needed to reduce cell growth by 50% following 72 h cell treatment with the tested drug (average of three experiments). Values are give ± standard deviation. [b] NA, not active. [c] The GI_{50} values for CA-4 and phenstatin were determined in this study.

tively). These values were comparable to *iso*CA-4 (**2k**, GI_{50} = 2 nM) or CA-4 (GI_{50} = 2 nM) and more active than phenstatin (GI_{50} = 33 nM). A comparison of GI_{50} values exhibited by *iso*CA-4 and *iso*aminocombretastatin A-4 (**2s**, *iso*NH_2CA-4) revealed that the introduction of an amino group instead of a hydroxy moiety at the C3′-position on the B-ring provide a compound with equal biological efficacy. Additionally, switching the NH_2 group from the C3′ to the C2′ position (**2x**) retained the cellular growth inhibitory activity in the nanomolar range (GI_{50} = 40 nM), while the corresponding nitro precursor (**2v**) showed a factor of ten decrease in activity. Notably, *iso*CA-4 and the corresponding acetate prodrug (**2m**) displayed similar cell growth inhibition. In contrast, compounds **2n** and **2o** with a carbamate or sulfonate functionality, respectively, were found to display weak cytotoxic effects. When *iso*CA-4 was attached to chlorambucil[25] via an ester linkage, the resulting compound **2p** maintained substantial biological potency (GI_{50} = 25 nM) compared with other esters and *iso*CA-4. It should be noted that the replacement of the hydroxy group at the C3′ position in *iso*CA-4 by a fluorine atom (*iso*FCA-4, **2e**) had little effect on the cytotoxic activity, while introduction of a bromine atom in this position (**2f**) resulted in a significant loss of antiproliferative activity (micromolar range).

It is interesting to note that our lead compounds *iso*FCA-4 and *iso*NH_2CA-4, with a 1,1-diarylethylene scaffold, are as potent as their corresponding *Z*-1,2-diarylethylene isomers, C3′-fluorocombretastatin[26] and AC-7739, respectively.[23] These results provide a good example of the bioisosteric equivalence between the 1,1-ethylene and the *Z*-1,2-ethylene bridge.[18] It is known that the 3-hydroxy group on the B-ring of CA-4 is not essential for potent activity,[13a] as such, we replaced the B-ring of *iso*CA-4 with a 4-methoxyphenyl group. As expected, the resulting compound **2b** showed a 20-fold decrease in cytotoxicity compared with *iso*CA-4. A similar cytotoxicity was also observed with compound **2c**, having a 2-naphthyl ring in place of a 4-methoxyphenyl group, indicating that these substituents are bioisosteres.[27] Replacement of the B-ring of *iso*CA-4 with a 4-tolyl (**2a**) or 5-benzodioxole (**2d**) ring system resulted in a significant loss of potency relative to *iso*CA-4, albeit still cytotoxic (GI_{50} < 0.5 μM). However, the introduction of a heterocyclic moiety such as quinoline (**2g**) led to a decrease in cytotoxicity (GI_{50} = 650 nM) against HCT-116 cells. Finally, the following compounds **2h**, **2i**, **2l**, **2q** and **2r** did not exhibit sufficient cytotoxic activities to warrant further biological evaluation.

Inhibition of tubulin polymerization and cytotoxicity for selected compounds

To further characterize the cytotoxicity profiles of these compounds, we investigated the effect of the most active substances **2b**, **2e**, **2m**, **2p**, **2s** and **2x** (GI_{50} ≤ 40 nM) on the proliferation of six tumor cell lines (myelogenous leukemia (K562), human glioblastoma (U87), carcinomic human alveolar basal epithelial (A549), human breast cancer (MDA-MB-435 and MDA-MB-231, hormone-independent breast cancer) and normal primary human umbilical vein endothelial (HUVEC)). As shown in Table 2, all examined compounds of the *iso*CA-4 series displayed similar potencies with GI_{50} values in the range of 2–50 nM. These compounds inhibited cell growth at nanomolar concentrations in all cell lines tested, suggesting their high therapeutic potential as drugs. Interestingly, *iso*FCA-4 and *iso*NH_2CA-4, bearing a fluorine atom or amino group on the C3′-position, respectively, show similar cytotoxic potency. The GI_{50} values obtained are comparable to those of CA-4 and *iso*CA-4 (GI_{50} = 2–8 nM) and significantly lower than the GI_{50} values of colchicine and phenstatin (GI_{50} = 26–41 nM).

To investigate whether the cytotoxic activities of the *iso*CA-4 series were related to their interaction with the microtubulin system, all compounds presented in Table 2, as well as the reference substances (CA-4, phenstatin and colchicine), were evaluated for in vitro tubulin polymerization inhibitory activity. The results show that *iso*NH_2CA-4 and the fluorinated compound *iso*FCA-4 exhibit similar inhibition of tubulin polymerization as *iso*CA-4 and CA-4 (Table 2). When comparing the inhibition of tubulin polymerization versus the cell growth inhibitory effect, we found a good correlation for most of the active compounds, except **2m** and **2p**. It can be assumed that the ester group is hydrolyzed by esterase in the cell. However, this cannot happen in the tubulin polymerization assay, which is a cell-free test and as such no esterase enzymes are present. A noticeable finding is the high potency of **2b** (IC_{50} = 2.0 μM), indicating that the 3′OH on the B-ring is not essential for strong antitubulin activity, as was previously observed in the CA-4 series.[13b]

Cell cycle analysis and apoptosis

Because microtubules as well as microfilaments are essential for cell division, and their disruption can induce G_2/M arrest and apoptosis, the effect of the most active compounds *iso*FCA-4, *iso*CA-4 and *iso*NH_2CA-4 on the cell cycle was measured by flow cytometry. MDA-MDB-231, K562 and HCT116 cancer cell lines were incubated for 24 h with the selected

Table 2. Cytotoxic activity (GI_{50}) and inhibition of tubulin polymerization (ITP) of selected compounds.

Compd	GI_{50} [nM][a]							ITP
	HCT116[b]	K562[b]	U87[b]	A549[b]	M435[b]	M231[b]	HUVEC[b]	IC_{50} [μM][b]
2b	40±3	18±0.9	28±1.9	20±1.8	25±1.81	10±0.1	38±2.5	2
2e (*iso*FCA-4)	7±1	4±0.2	7±0.42	7±0.3	8±0.6	3±0.12	5±0.2	4
2x	40±3	20±1.3	58±3.8	45±3.1	36±2.7	26±1.9	ND[c]	6
2m	8±0.2	5±0.3	8±0.5	15±1.2	10±0.9	3±0.14	18±1.2	57
2p	25±2	30±1.7	30±2.5	42±3.6	40±3.1	24±1.7	ND[c]	75
2s (*iso*NH_2CA-4)	2±0.1	4±0.16	8±0.47	7±0.51	7±0.52	6±0.21	3±0.12	1.5
*iso*CA-4	2±0.2	5±0.19	8±0.36	8±0.7	4.5±0.2	4±0.16	1.5±0.07	2
CA-4[d]	2±0.1	3.6±0.21	3±0.25	7±0.45	3±0.15	3.5±0.1	2.5±0.1	1
Colchicine[d]	32±5	29±1.9	ND[c]	ND[c]	ND[c]	26±1.8	30±0.1	2
Phenstatin[d]	33±2.5	41±2.3	ND[c]	ND[c]	ND[c]	28±2	38±2.8	2

[a] GI_{50} is the concentration of compound needed to reduce cell growth by 50% following 72 h cell treatment with the tested drug (average of three experiments). Values are give ± standard deviation. [b] HCT116, colon carcinoma; K562, myelogenous leukemia; U87, glioblastome; A549, carcinomic alveolar basal epithelial; MDA-MB-435, breast cancer and MDA-MB-231 hormone-independent breast cancer; ITP, Inhibition of Tubulin Polymerization; IC_{50} is the concentration of compound required to inhibit 50% of the rate of microtubule assembly (average of three experiments). [c] ND, not determined. [d] The GI_{50} and IC_{50} values (cytotoxicity and ITP respectively) for CA-4, colchicin and phenstatin were determined in this study.

drugs at different concentrations. The cell-cycle profiles depicted in Figure 2 show a significant increase in the number of cells arrested in the G_2/M growth phase with increasing concentration (5–10 nM) of the tested drugs. The observed effects of *iso*FCA-4, *iso*CA-4 and *iso*NH_2CA-4 on cell-cycle progression correlated well with their strong antiproliferative and antitubulin activities. This is in agreement with the similar properties reported previously for the majority of antimitotic agents. Cell-cycle arrest in the G2/M phase is often followed by DNA fragmentation and other morphological features of apoptosis.[28] Therefore, we investigated the effect of *iso*FCA-4, *iso*CA-4 and *iso*NH_2CA-4 (1, 5, 10 nM) on induction of apoptosis in K562, HCT-116 and MDA-MB-231 cancer cells using caspases 3 and 7 standard assays.[29] The enzymatic activity of caspases 3 and 7

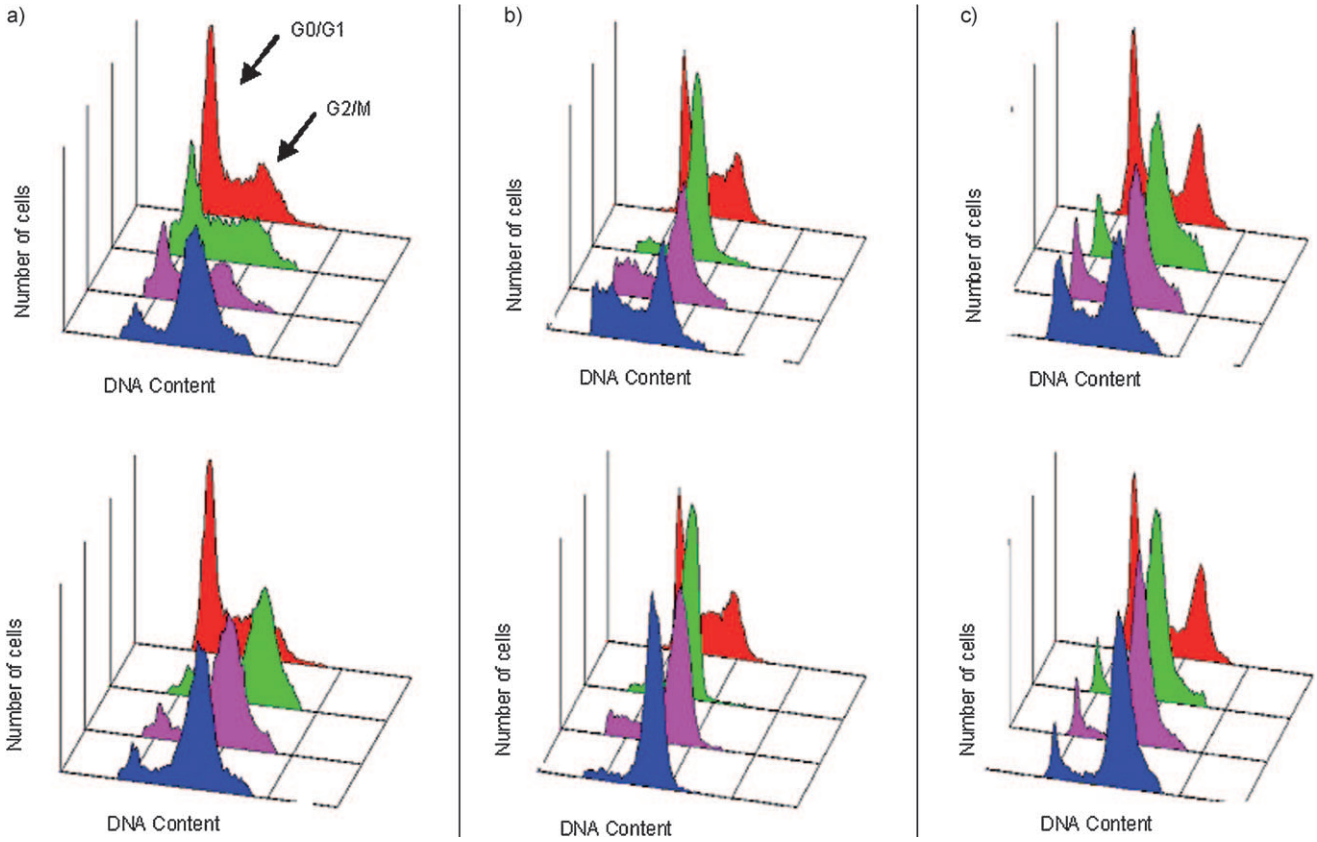


Figure 2. Effect of *iso*FCA-4 (green), *iso*CA-4 (blue) and *iso*NH_2CA-4 (pink) on cell-cycle distribution in a) MDA-MDB-231, b) K562 and c) HCT116 cells at 5 nM (top) and 10 nM (bottom), as determined by flow cytometry analysis (DMSO control in red). DNA content was assessed via propidium iodide staining.

was measured by monitoring the cleavage of the fluorogenic substrate Z-DEVD-R110 in cancer cells. The results presented in Figure 3 show a significant dose-dependent increase in proteolytic activity of both examined caspases in the cells treated for 24 h with the three studied compounds.

More interestingly, a spectacular 10- to 15-fold dose-dependent increase in apoptosis was evidenced in K562 leukemia cells previously described as being resistant to apoptosis induction by a variety of agents, including diphtheria toxin, camptothecin, cytarabine, etoposide, paclitaxel, staurosporine and antifas antibodies.[30] These results clearly show that, in addition to their antiproliferative and antitubulin effects, the treatment of cancer cells with *iso*FCA-4, *iso*CA-4 and *iso*NH_2CA-4 activate caspases system leading to programmed cell death.

Effect on human umbilical vein endothelial cell organization

In order to expand our studies, the effects of our lead compounds, *iso*FCA-4, *iso*CA-4 and *iso*NH_2CA-4, on the proliferation of normal endothelial cells (HUVEC) were determined. The results presented in Figure 4 show that after 72 h of incubation, *iso*FCA-4, *iso*CA-4 and *iso*NH_2CA-4 exhibit a similar growth inhibition activity (GI_{50} = 1.5–5 nM) as CA-4 (GI_{50} = 2.5 nM). However, no change in the viability of HUVEC cells treated for 3 h with *iso*FCA-4, *iso*CA-4 and *iso*NH_2CA-4 was observed even at 10 nM.

The ability of endothelial cells to form tubular structures when plated on a matrigel matrix allows the observation of three-dimensional organization of endothelial cells and offers an in vitro model of angiogenesis.[31] When seeded on matrigel, flattened endothelial cells aggregate to form a reticular vascular network of capillary-like vessels. To evaluate whether our lead compounds could affect newly formed blood vessels, the in vitro assay of tube formation by HUVEC was performed (Figure 5). The addition of *iso*FCA-4, *iso*CA-4 and *iso*NH_2CA-4 and subsequent incubation for 3 h led to the rapid disruption of the capillary-like tubes and the integrity of the entire network. This effect observed at a 10 nM concentration of test compound, which was shown previously to be not toxic for HUVEC after 3 h of treatment. Altogether, our results suggest that these substances might be lead compounds for the development of vascular disrupting agents.

Conclusions

We have shown that 1,1-diarylethylenes, of the general structure **2**, are potent antiproliferative agents. The compounds described herein are structurally simpler than those of the CA-4 series, chemically stable (no isomerization) and easily accessible synthetically. Three representative substances, *iso*FCA-4, *iso*CA-4 and *iso*NH_2CA-4, have emerged as lead compounds. They displayed antiproliferative activity with GI_{50} values ranging from 2 to 10 nM against different human cancer cell lines. Flow cytometric analysis indicated that these drugs act as antimitotic agents and arrest the cell cycle in the G_2/M phase. Moreover, we showed that our lead compounds disrupt newly formed vascular tubes in vitro after only 3 h post-treatment.

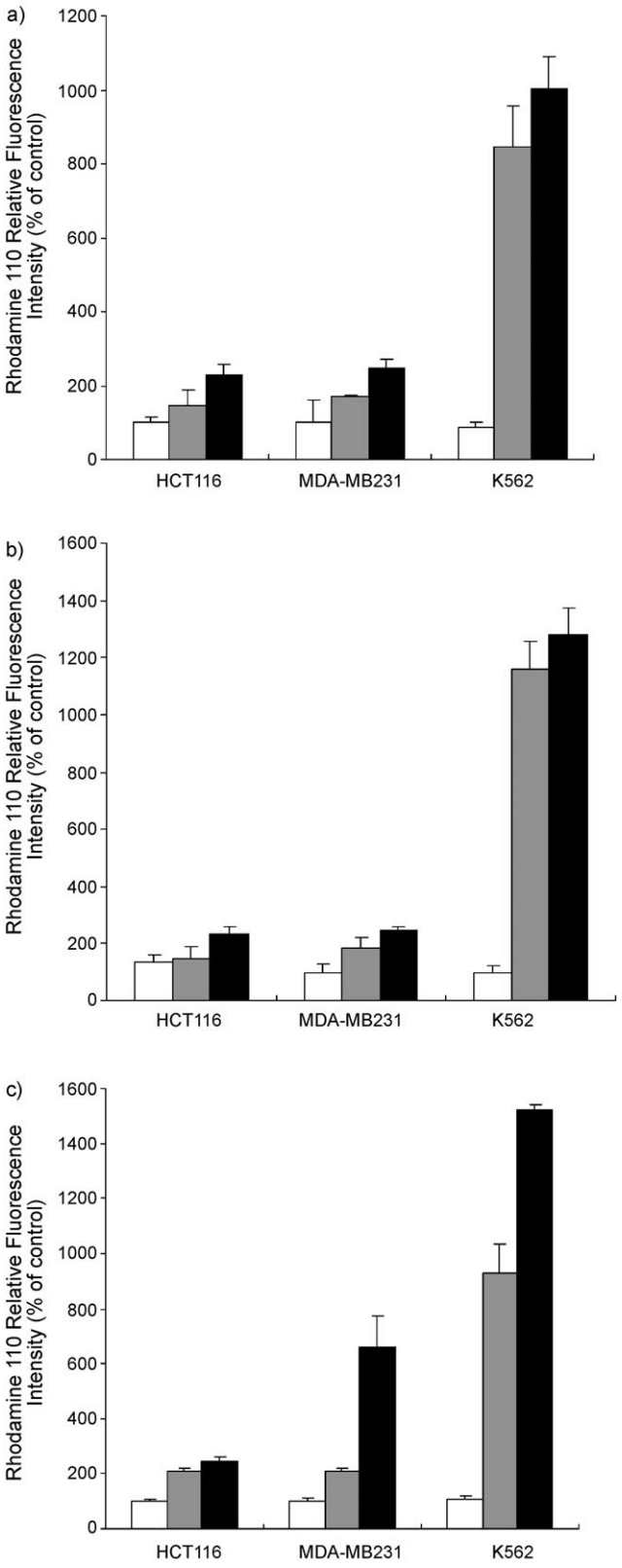


Figure 3. Apoptotic effects of *iso*FCA-4, *iso*CA-4 and *iso*NH_2CA-4 in HCT116, MDA-MDB-231 and K562 cells. The results are expressed in the percentage of apoptotic cells detected following 24 h treatment with *iso*FCA-4, *iso*CA-4 and *iso*NH_2CA-4 at different concentrations (1 nM, □; 5 nM, ■; 10 nM, ■).

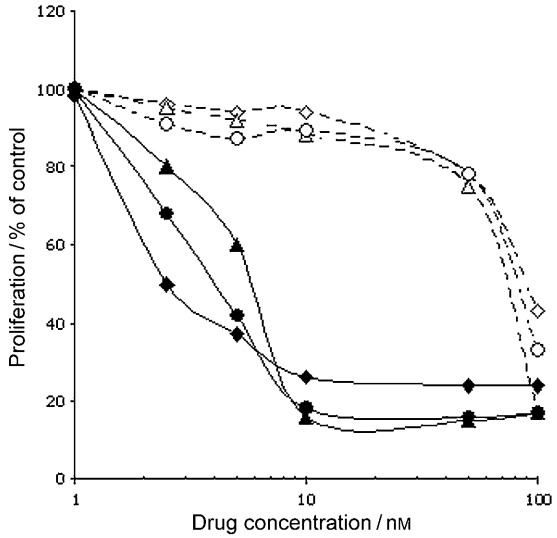


Figure 4. Effect of tested compounds on in vitro endothelial cells (HUVEC) after 3 h and 72 h of treatment: *iso*FCA-4 (**2e**; 3 h, △; 72 h, ▲), *iso*CA-4 (**2k**; 3 h, ◇; 72 h, ◆) and *iso*NH_2CA-4 (**2s**; 3 h, ○; 72 h, ●).

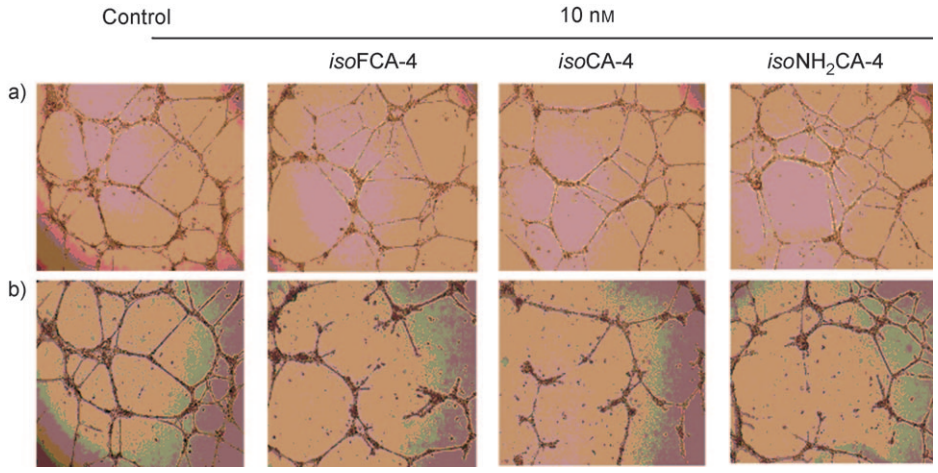


Figure 5. Inhibitory activity of *iso*FCA-4, *iso*CA-4 and *iso*NH_2CA-4 in vitro. These compounds disruption of vessel-like structures when added to the vascula (human umbilical vein endothelial cells (HUVEC)). Images shown were taken at a) $t=0$ and b) $t=3$ h.

These results suggest that *iso*FCA-4, *iso*CA-4 and *iso*NH_2CA-4 might be lead compounds for the development of novel vascular disrupting agents, and they themselves are promising candidates for in vivo evaluation. Both compounds *iso*CA-4 and *iso*NH_2CA-4 have the potential for further prodrug modification and development as vascular disrupting agents for treatment of solid tumors and ophthalmological diseases.

Experimental Section

Chemistry

Melting points (mp) were recorded on a Büchi B-450 apparatus and were uncorrected. NMR spectra were performed on a Bruker AMX 200 (^{1}H, 200 MHz; ^{13}C, 50 MHz), Bruker AVANCE 300 or Bruker AVANCE 400 (^{1}H, 400 MHz; ^{13}C, 100 MHz). Chemical shifts δ are given in ppm, and the following abbreviations are used: singlet (s), doublet (d), triplet (t), multiplet (m) and broad singlet (bs). IR spectra were measured on a Bruker Vector 22 spectrophotometer (neat, cm^{-1}). Elemental analysis (C, H, N) was performed with a Perkin–Elmer 240 analyzer at the Microanalyses Service of the Faculty of Pharmacy at Châtenay-Malabry (France) and data were within 0.4% of the theoretical values. Mass spectra were obtained using a Bruker Esquire electrospray ionization apparatus. Thin-layer chromatography was performed on silica gel 60 plates with a fluorescent indicator and visualized under a UVP Mineralight UVGL-58 lamp (254 nm) and with a 7% solution of phosphomolybdic acid in ethanol. Flash chromatography was performed using silica gel 60 (40–63 μm, 230–400 mesh ASTM) at medium pressure (200 mbar). All solvents were distilled and stored over 4 Å molecular sieves before use. All reagents were obtained from commercial suppliers unless otherwise stated. Organic extracts were, in general, dried over $MgSO_4$ or Na_2SO_4.

Synthesis of 2a–c

2,6-Dimethoxy[4-(4-methylbenzene)vinyl]anisole (2a): A solution of 3,4,5-trimethoxyacetophenone (420 mg; 2 mmol) in THF (10 mL) at −40 °C under Ar was treated with a solution of (*para*-tolyl)magnesium bromide in THF (6 mL, 1 M). The mixture was stirred at −40 °C for 3 h, then warmed to RT and stirred for a further 16 h. Saturated aq NH_4Cl (10 mL) was slowly added to the mixture to hydrolyze the adduct and the mixture was extracted with Et_2O (3×10 mL). The combined organic extracts were dried ($MgSO_4$), filtered and concentrated. The crude was redissolved in CH_2Cl_2 (10 mL), mixed with some crystals of *p*-toluenesulfonic acid (PTSA) and stirred for 3 h at RT. The solution was washed with a saturated aq NaCl (20 mL) and extracted with CH_2Cl_2 (2× 10 mL). The organic layers were combined, dried ($MgSO_4$), filtered and concentrated. The residue was further purified by flash chromatography (cyclohexane/EtOAc, 7:3) to yield **2a** (307 mg; 54%); $R_f=0.75$ (cyclohexane/EtOAc, 6:4); ^{1}H NMR (300 MHz, CD_3COCD_3): $\delta=2.33$ (s, 3H, CH_3), 3,75 (s, 3H, OCH_3), 3.76 (s, 6H, OCH_3), 5.38 (d, 1H, $J=1.2$ Hz), 5.40 (d, 1H, $J=$ 1.2 Hz), 6.59 (s, 2H), 7.22–7.25 ppm (m, 4H); ^{13}C NMR (100 MHz, CD_3COCD_3): 21.1, 56.5, 60.6 (2), 106.9 (2), 113.5, 128.9 (2), 127.9 (2), 138.0, 138.4, 139.3, 151.0, 154.1 ppm (2), one C not detected; IR (neat): $\tilde{\nu}=2936$, 1737, 1578, 1504, 1451, 1409, 1346, 1233, 1182, 1123, 1009 cm^{-1}; Anal. calcd for $C_{18}H_{20}O_3$: C 76.03, H 7.09, found: C 75.74, H 6.99.

2,6-Dimethoxy[4-(4-methoxybenzene)vinyl]anisole (2b): Prepared as for **2a** from 3,4,5-trimethoxyacetophenone (420 mg; 2 mmol) and (4-methoxyphenyl)magnesium bromide to afford **2b** (384 mg; 64%); $R_f=0.60$ (cyclohexane/EtOAc, 6:4); ^{1}H NMR (300 MHz, CD_3COCD_3): $\delta=3.75$ (s, 3H, OCH_3), 3.78 (s, 6H, OCH_3), 3.82 (s, 3H, OCH_3), 5.34 (m, 2H, CH_2), 6.60 (s, 2H), 6.92 (d, 2H, $J=$

8.7 Hz), 7.29 ppm (d, 2H, $J=8.7$ Hz); ^{13}C NMR (75 MHz, CD_3COCD_3): 55.5, 56.4 (2), 60.5, 106.8 (2), 112.7, 114.4 (2), 130.1 (2), 134.4, 138.2 (2), 150.6, 154.1 (2), 160.5 ppm; IR (neat): $\tilde{\nu}=1579$, 1507, 1454, 1411, 1346, 1299, 1233, 1174, 1122, 1030, 1004 cm^{-1}; Anal. calcd for $C_{18}H_{20}O_4$: C 71.98, H 6.71, found: C 71.85, H 6.66.

2-[1-(3,4,5-Trimethoxyphenyl)vinyl]naphtalene (2c): Prepared as for **2a** from 3,4,5-trimethoxyacetophenone (420 mg; 2 mmol) and (2-naphthyl)magnesium bromide to afford **2c** (518 mg; 81%); $R_f=0.80$ (CH_2Cl_2/EtOAc, 9:1); mp: 89 °C; ^{1}H NMR (300 MHz, CD_3COCD_3): $\delta=3.77$ (s, 9H, OCH_3), 5.54–5.64 (m, 2H, CH_2), 6.67 (s, 2H), 7.50–7.55 (m, 3H), 7.87–7.91 ppm (m, 4H); ^{13}C NMR (75 MHz, CD_3COCD_3): $\delta=56.5$, 60.7 (2), 106.9 (2), 115.0, 127.1, 128.0, 128.5, 128.6, 129.1, 129.5, 130.5, 134.1, 134.4, 137.8, 139.6 (2), 151.1, 154.2 ppm (2); IR (neat): $\tilde{\nu}=2936$, 1578, 1503, 1451, 1412, 1352, 1331, 1237, 1182, 1122, 1003 cm^{-1}; Anal. calcd for $C_{21}H_{20}O_3$: C 78.74, H 6.29, found: C 78.64, H 6.20.

Synthesis of 2d–f, 2h–j

5-[1-(3,4,5-Trimethoxyphenyl)vinyl]benzo[1,3]dioxole (2d): A solution of *t*BuLi in pentane (625 μL, 1 mmol, 1.6 M) under N_2 was added to a solution of 5-iodobenzo[*d*][1,3]dioxole (124 mg; 0.5 mmol) in hexanes (15 mL) at −78 °C via syringe. After stirring for 45 min at −78 °C, 3,4,5-trimethoxyacetophenone (105 mg, 0.5 mmol) in toluene (5 mL) was added and the solution was warmed to RT and stirred for a further 12 h. Saturated aq NH_4Cl (10 mL) was slowly added to the mixture to hydrolyze the adduct and the mixture was extracted with Et_2O (3×10 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated. The crude was redissolved in CH_2Cl_2 (10 mL), mixed with some crystals of PTSA and stirred for 3 h at RT. The solution was washed with saturated aq NaCl (20 mL) and extracted with CH_2Cl_2 (2×10 mL). The organic layers were combined, dried ($MgSO_4$), filtered and concentrated. The crude mixture was treated as for **2a–c** to afford **2d** (30 mg; 19%); $R_f=0.82$ (CH_2Cl_2/cyclohexane); ^{1}H NMR (300 MHz, CD_3Cl_3): $\delta=3.72$ (s, 6H, OCH_3), 3.78 (s, 3H, OCH_3), 5.21 (d, 1H, $J=1.5$ Hz), 5.25 (d, 1H, $J=1.5$ Hz), 5.86 (s, 2H, OCH_2O), 6.46 (s, 2H), 6.67 (d, 1H, $J=8.7$ Hz), 6.72–6.76 ppm (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=56.1$ (3), 60.8, 105.7 (2), 107.9, 108.6, 122.0, 101.1, 112.9, 135.4, 137.3, 137.9, 147.3, 147.5, 149.6, 152.8 ppm; IR (neat): $\tilde{\nu}=$ 2939, 2835,1699, 1578, 1503, 1488, 1463, 1450, 1410, 1340, 1234, 1184, 1161, 1124, 1036, 1006, 936, 907, 866, 844, 814, 783, 733, 702 cm^{-1}; MS (ESI+): *m/z* (%): 337 (100) $[M+Na]^+$; Anal. calcd for $C_{18}H_{18}O_5$: C 68.78, H 5.77, found: C 68.68, H 5.72.

5-(1-(3-Fluoro-4-methoxyphenyl)vinyl)-1,2,3-trimethoxybenzene (*iso*FCA-4, 2e): Prepared as for **2d** from 3,4,5-trimethoxyacetophenone (105 mg; 0.5 mmol) and 2-fluoro-4-iodoanisole (126 mg; 0.5 mmol) to afford **2e** (76 mg; 48%); $R_f=0.52$ (cyclohexane/EtOAc, 7:3); mp: 64–66 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=3.82$ (s, 6H, OCH_3), 3.88 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 5.35 (d, 1H, $J=1.5$ Hz), 5.38 (d, 1H, $J=1.5$ Hz), 6.58 (s, 2H), 6.95 (m, 1H), 7.05–7.19 ppm (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=56.1$ (2), 56.3, 60.9, 105.7 (2), 112.9, 113.4, 115.9 (d, $J=19$ Hz), 124.0, 134.4 (d, $J=6.2$ Hz), 136.9, 138.0, 147.4 (d, $J=10.5$ Hz), 148.7, 150.4, 152.9, 153.6 ppm; ^{19}F NMR (188 MHz, $CDCl_3$): $\delta=-136.0$; IR (neat): $\tilde{\nu}=$ 3086, 3011, 2939, 2835, 1619, 1576, 1518, 1504, 1462, 1439, 1310, 1205, 1117, 1085, 949, 899, 876 cm^{-1}; MS (ESI+): *m/z* (%): 341 (100) $[M+Na]^+$; Anal. calcd for $C_{18}H_{19}FO_4$: C 67.91, H 6.02, found: C 67.80, H 5.94.

5-(1-(3-Bromo-4-methoxyphenyl)vinyl)-1,2,3-trimethoxybenzene (2f): Prepared as for **2d** from 3,4,5-trimethoxyacetophenone (105 mg; 0.5 mmol) and 2-bromo-4-iodoanisole (156 mg; 0.5 mmol) to afford **2f** (101 mg; 53%); $R_f=0.46$ (cyclohexane/EtOAc, 7:3); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=3.65$ (s, 3H, OCH_3), 3.78 (s, 6H, OCH_3), 3.85 (s, 3H, OCH_3), 5.30 (s, 1H), 5.70 (s, 1H), 6.50 (s, 2H), 6.80 (d, 1H, $J=8.7$ Hz), 7.36–7.46 ppm (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=55.9$, 56.2 (2), 60.9, 103.9 (2), 112.8, 112.9, 115.8, 131.7, 132.9, 133.7, 136.3, 137.9, 145.7, 152.9 (2), 156.3 ppm; IR (neat): $\tilde{\nu}=2936$, 2835, 1579, 1504, 1485, 1461, 1411, 1336, 1287, 1257, 1231, 1181, 1122 cm^{-1}; MS (ESI+): *m/z* (%): 403 (100) $[M+Na]^+$; Anal. calcd for $C_{18}H_{19}BrO_4$: C 57.01, H 5.05, found: C 56.78, H 4.90.

2,2-Dimethyl-6-[1-(3,4,5-trimethoxyphenyl)vinyl]-*2H*-chromene (2h): Prepared as for **2d** from 3,4,5-trimethoxyacetophenone (105 mg; 0.5 mmol) and 6-iodo-2,2-dimethyl-2*H*-chromene (143 mg; 0.5 mmol) to afford **2h** (85 mg; 48%); $R_f=0.60$ (cyclohexane/EtOAc, 7:3); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=1.43$ (s, 6H, OCH_3), 3.82 (s, 6H, OCH_3), 3.88 (s, 3H, OCH_3), 5.29 (d, 1H, $J=1.2$ Hz), 5.36 (d, 1H, $J=1.2$ Hz), 5.62 (d, 1H, $J=10.0$ Hz), 6.30 (d, 1H, $J=10.0$ Hz), 6.65 (s, 2H), 6,73 (d, 1H, $J=8.4$ Hz), 6,88 (d, 1H, $J=2.4$ Hz), 7.11 ppm (dd, 1H, $J=8.4$ Hz, $J=2.4$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=28.1$ (2), 56.2 (2), 60.9, 76.4, 105.8 (2), 112.6, 115.9, 120.8, 122.2, 126.1, 129.1, 130.9, 133.7, 137.5, 137.8, 146.9 (2), 152.9 ppm (2); IR (neat): $\tilde{\nu}=2973$, 2935, 2834, 1578, 1504, 1489, 1451, 1410, 1365, 1343, 1265, 1235, 1122, 1005 cm^{-1}; Anal. calcd for $C_{22}H_{24}O_4$: C 74.98, H 6.86, found: C 74.86, H 6.74.

2,2-Dimethyl-6-[1-(3,4,5-trimethoxyphenyl)vinyl]-*2H*-chroman (2i): Prepared as for **2d** from 3,4,5-trimethoxyacetophenone (105 mg; 0.5 mmol) and 6-iodo-2,2-dimethyl-3,4-dihydro-2*H*-chromene (144 mg; 0.5 mmol) to afford **2i** (57 mg; 32%); $R_f=0.56$ (cyclohexane/EtOAc, 7:3); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=1.35$ (s, 6H, CH_3), 1.88 (t, 2H, $J=6.6$ Hz), 2.76 (t, 2H, $J=6.6$ Hz), 3.82 (s, 6H, OCH_3), 3.88 (s, 3H, OCH_3), 5.26 (d, 1H, $J=1.2$ Hz), 5.35 (d, 1H, $J=1.2$ Hz), 6,57 (s, 2H), 6.74 (d, 1H, $J=8.1$ Hz), 7.08 (s, 1H), 7.09 ppm (d, 1H, $J=8.1$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=22.5$, 26.9 (2), 32.8, 58.2 (2), 60.9, 74.5, 105.7 (2), 112.0, 116.8, 120.5, 127.3, 129.1, 137.8, 149.8, 152.8 (2), 154.0 ppm; IR (neat): $\tilde{\nu}=2973$, 2937, 1579, 1496, 1451, 1410, 1384, 1346, 1260, 1124 cm^{-1}; Anal. calcd for $C_{22}H_{26}O_4$: C 74.55, H 7.39, found: C 74.50, H 7.36.

***tert*-Butyl(2-methoxy-5-(1-(3,4,5-trimethoxyphenyl)vinyl)phenoxy)dimethylsilane (2j)**: Prepared as for **2d** from 3,4,5-trimethoxyacetophenone (105 mg; 0.5 mmol) and *tert*-butyl(5-iodo-2-methoxyphenoxy)dimethylsilane (182 mg; 0.5 mmol) to afford **2j** (118 mg; 55%); $R_f=0.51$ (cyclohexane/EtOAc, 8:2); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=0.15$ (s, 6H, $SiCH_3$), 0.98 (s, 9H, CCH_3), 3.75 (s, 3H, OCH_3), 3.78 (s, 6H, OCH_3), 3.85 (s, 3H, OCH_3), 5.33 (d, 1H, $J=1.2$ Hz), 5.34 (d, 1H, $J=1.2$ Hz), 6.60 (s, 2H), 6.83 (t, 1H, $J=1.2$ Hz), 6.96 ppm (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=-3.8$; 19.0, 26.1, 55.8, 56.4 (2), 60.6, 106.8 (2), 112.6, 112.7, 118.8, 121.4, 122.6, 134.9, 138.1, 146.0, 150.5, 151.8, 154.1 ppm (2); IR (neat): $\tilde{\nu}=3417$, 2937, 2837, 1579, 1506, 1460, 1411, 1346, 1281, 1254, 1124, 1005 cm^{-1}; Anal. calcd for $C_{24}H_{34}O_5Si$: C 66.94, H 7.96, found: C 66.85, H 7.92.

2-Methoxy-5-(1-(3,4,5-trimethoxyphenyl)vinyl)phenol (*iso*CA-4, 2k): A solution of **2j** (73 mg; 0.17 mmol) in MeOH (10 mL) was treated with K_2CO_3 (34.5 mg; 0.25 mmol) and the mixture was stirred for 12 h at RT. The solution was washed with saturated aq NaCl (10 mL) and extracted with EtOAc (2×10 mL). The organic layers were combined, dried ($MgSO_4$), filtered and concentrated. The residue was further purified by flash chromatography (cyclohexane/EtOAc, 8:2) to afford **2k** (51 mg; 94%); $R_f=0.21$ (cyclohexane/EtOAc, 8:2); mp: 112 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=3.81$ (s, 6H, OCH_3), 3.87 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 5.30 (d, 1H, $J=1.5$ Hz), 5.37 (d, 1H, $J=1.5$ Hz), 5.60 (bs, 1H, OH), 6.55 (s, 2H), 6.82

(m, 2H), 6.97 ppm (d, 1H, $J=2.1$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$): 55.9, 56.1 (2), 60.9, 105.8 (2), 110.1, 112.8, 114.4, 120.2, 134.4, 134.7, 137.8, 145.2, 148.4, 149.5, 152.8 ppm (2); IR (neat): $\tilde{\nu}=3417$, 2937, 2837, 1579, 1506, 1460, 1411, 1346, 1281, 1254, 1005 cm^{-1}; MS (ESI+): *m/z* (%): 339 (100) $[M+Na]^+$; Anal. calcd for $C_{18}H_{20}O_5$: C 68.34, H 6.37, found: C 68.25, H 6.33.

3-[1-(3,4,5-Trimethoxyphenyl)vinyl]quinoline (2g): A solution of *t*BuLi in pentane (625 μL, 1 mmol, 1.6 M) under N_2 was added slowly via syringe to a solution of 3-bromoquinoline (104 mg; 0.5 mmol) in Et_2O (15 mL) at −100 °C was slowly added via syringe. After stirring for 45 min at −78 °C, 3,4,5-trimethoxyacetophenone (105 mg, 0.5 mmol) in toluene (5 mL) was added and the solution was warmed to RT, and stirred for a further 12 h. Saturated aq NH_4Cl (10 mL) was slowly added to hydrolyze the adduct and the mixture was extracted with Et_2O (3×10 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated. A solution of the crude and 4-(dimethylamino)pyridine (DMAP; 2.0 mmol) in CH_2Cl_2 (10 mL) was then treated with MsCl (190 mL, 2.45 mmol) via syringe, and the reaction stirred at RT for 1 h. The mixture was poured into saturated aq NaCl, separated and the aqueous layer was extracted with CH_2Cl_2 (3×10 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated. The residue was redissolved in CH_2Cl_2 (8 mL) and DBU (14 mmol) was added. The mixture was refluxed for 3 h, cooled poured into water. The mixture was extracted with CH_2Cl_2 (3×8 mL) and the combined organic layers were dried ($MgSO_4$), filtered and concentrated. The residue was further purified by flash chromatography (cyclohexane/EtOAc, 8:2) to yield **2g** (58 mg; 36%); $R_f=0.36$ (cyclohexane/EtOAc, 7:3); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=3.77$ (s, 6H, OCH_3), 3.80 (s, 3H, OCH_3), 5.52 (s, 2H, CH_2), 6.51 (s, 2H), 7.41–7.50 (t, 1H, $J=6.9$ Hz), 7.60–7.64 (t, 1H, $J=6.9$ Hz), 7.72 (d, 1H, $J=6.9$ Hz), 7.96–8.08 (m, 2H), 8.88 ppm (d, 1H, $J=2.1$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=55.2$ (2), 59.9, 104.5 (2), 114.8, 126.0, 126.7, 127.0, 128.1, 128.6, 133.1, 133.7, 135.2, 137.3, 146.1, 146.6, 149.5 (2), 152.2 ppm; IR (neat): $\tilde{\nu}=2927$, 1730, 1575, 1503, 1464, 1447, 1410, 1368, 1347, 1324, 1283, 1177, 1002, 976, 957, 917, 862, 840 cm^{-1}; MS (ESI+): *m/z* (%): 341 (100) $[M+Na]^+$; Anal. calcd for $C_{20}H_{19}NO_3$: C 74.75, H 5.96, N 4.36, found: C 74.61, H 5.90, N 4.29.

2,6-Dimethoxy[4-(3,4-dimethoxybenzene)vinyl]anisole (2l): A solution of *iso*CA-4 (50 mg; 0.158 mmol) in acetone (5 mL) was treated with K_2CO_3 (62 mg; 0.632 mmol) and Me_2SO_4 (80 mg; 0.632 mmol)and stirred at RT for 12 h. The mixture was poured into EtOAc/H_2O (1:1, 30 mL) and separated. The aqueous phase was extracted with EtOAc (3×15 mL) and the combined organic extracts were dried ($MgSO_4$), filtered and concentrated in vacuo to afford **2l** (42 mg; 80%); $R_f=0.63$ (CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=3.80$ (s, 6H, OCH_3), 3.84 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 5.33 (d, 1H, $J=1.5$ Hz), 5.36 (d, 1H, $J=1.5$ Hz), 6.56 (s, 2H), 6.83 (d, 1H, $J=8.4$ Hz), 6.88–6.92 ppm (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=55.9$ (3), 56.0, 56.1, 56.3, 60.9, 105.7, 110.8, 111.5, 112.7, 121.0, 132.5, 134.0, 137.3, 137.9, 142.7, 148.5, 148.9, 149.7, 152.8, 153.0 ppm; IR (neat): $\tilde{\nu}=2998$, 2936, 2835,1730, 1679, 1579, 1506, 1452, 1411, 1330, 1248, 1235, 1221, 1173, 1122, 1025, 1005, 951, 889, 857, 845, 815, 766, 734 cm^{-1}; MS (ESI+): *m/z* (%): 353 (100) $[M+Na]^+$; Anal. calcd for $C_{19}H_{22}O_5$: C 69.09, H 6.71, found: C 68.85, H 6.56.

Acetic acid 2-methoxy-5-[1-(3,4,5-trimethoxyphenyl)vinyl]phenyl ester (2m): Ac_2O (42 μL; 0.442 mmol) was added dropwise to a stirred solution of *iso*CA-4 (31.5 mg; 0.316 mmol), pyridine (53 μL) and DMAP (2 mg; 0.016 mmol) in CH_2Cl_2 (1 mL) maintained at 0 °C and stirring was continued for 1 h at this temperature. Water (3 mL) was added to the reaction mixture and then extracted with CH_2Cl_2 (3×3 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated. The residue was further purified by flash chromatography (cyclohexane/EtOAc, 8:2) to yield **2m** (74 mg; 65%); $R_f=0.44$ (cyclohexane/EtOAc, 7:3); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=2.28$ (s, 3H, CH_3), 3.74 (s, 6H, OCH_3), 3.78 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 5.26 (d, 1H, $J=1.5$ Hz), 5,31 (d, 1H, $J=1.5$ Hz), 6.48 (s, 2H), 6.86 (d, 1H, $J=8.7$ Hz), 6.97 (d, 1H, $J=2.1$ Hz), 7.16 ppm (dd, 1H, $J=8.4$ Hz, $J=2.1$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=20.7$, 55.9, 56.1 (2), 60.9, 105.6 (2), 111.9, 122.8, 126.6, 113.1, 134.0, 137.0, 137.8, 139.3, 148.7, 150.8, 152.9, 169.0 ppm; IR (neat): $\tilde{\nu}=2937$, 2839, 1766, 1680, 1580, 1506, 1455, 1411, 1346, 1330, 1304, 1267, 1234, 1207, 1194, 1175, 1121, 1006, 958, 936, 897, 844, 818, 777, 731, 718 cm^{-1}; MS (ESI+): *m/z* (%): 381 (100) $[M+Na]^+$; Anal. calcd for $C_{20}H_{22}O_6$: C 67.03, H 6.19, found: C 66.88, H 6.06.

Diethyl carbamic acid 2-methoxy-5-[1-(3,4,5-trimethoxyphenyl)-vinyl]phenyl ester (2n): A solution of *iso*CA-4 (31.5 mg; 0.316 mmol) in CH_2Cl_2 (2 mL) was successively treated with pyridine (54 μL) and diethylcarbamoyl chloride (86 mg; 0.632 mmol) and stirred for 12 h at RT. Saturated aq $NaHCO_3$ (5 mL) was added and the reaction was extracted with EtOAc (3×8 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated. The residue was further purified by flash chromatography (CH_2Cl_2) to yield **2n** (57 mg; 50%); $R_f=0.15$ (CH_2Cl_2); mp: 148 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=1.11$–1.20 (m, 6H, CH_3), 3.28–3.39 (m, 4H, CH_2), 3.75 (s, 6H, OCH_3), 3.77 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 5.25 (d, 1H, $J=0.9$ Hz), 5.32 (d, 1H, $J=1.2$ Hz), 6.50 (s, 2H), 6.82 (d, 1H, $J=8.4$ Hz), 7.05–7.10 ppm (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=$ 13.4, 14.0, 42.0, 42.3, 55.9, 56.1 (2), 60.9, 105.6 (2), 111.8, 112.9, 123.3, 126.0, 133.8, 137.2, 137.7, 140.2, 148.9, 151.5, 152.8, 154.0 ppm; IR (neat): $\tilde{\nu}=2937$, 2839, 1766, 1680, 1580, 1506, 1455, 1411, 1346, 1330, 1304, 1267, 1234, 1207, 1194, 1175, 1121, 1006, 958, 936, 897, 844, 818, 777, 731, 718 cm^{-1}; MS (ESI+): *m/z* (%): 438 (100) $[M+Na]^+$; Anal. calcd for $C_{23}H_{29}NO_6$: C 66.19, H 7.04, N 3.37, found: C 74.61, H 6.80, N 3.21.

Sulfuric acid mono-{2-methoxy-5-[3,4,5-trimethoxyphenyl)vinyl]-phenyl} ester (2o): A solution of *iso*CA-4 (246 mg; 0.78 mmol) in pyridine (1 mL) was treated with SO_3–pyridine complex (75 mg; 0.47 mmol)and stirred for 24 h at RT. The mixture was hydrolyzed with H_2O (0.5 mL), concentrated in vacuo and purified by flash chromatography (CH_2Cl_2/MeOH, 8:2) to afford **2o** (247 mg; 80%); $R_f=0.37$ (CH_2Cl_2/MeOH, 7:3); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=3.59$ (s, 3H, OCH_3), 3.71 (s, 6H, OCH_3), 3.82 (s, 3H, OCH_3), 5.22 (s, 1H), 5.30 (s, 1H), 6.47 (s, 2H), 6.66 (d, 1H, $J=8.7$ Hz), 6.95 (dd, 1H, $J=8.7$ Hz, $J=1.8$ Hz), 7.54 ppm (d, 1H, $J=1.8$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=56.1$ (2), 56.4, 60.8, 105.8 (2), 112.1, 113.4, 121.9, 125.7, 134.0, 136.8, 137.9, 140.3, 148.4, 150.3, 152.8 ppm; IR (neat): $\tilde{\nu}=2937$, 2839, 1766, 1680, 1580, 1506, 1455, 1411, 1346, 1330, 1304, 1267, 1234, 1207, 1194, 1175, 1121, 1006, 958, 936, 897, 844, 818, 777, 731, 718 cm^{-1}; MS (ESI−): *m/z* (%): 395 (100) $[M-H]^-$; Anal. calcd for $C_{18}H_{20}O_8S$: C 54.54, H 5.06, found: C 54.44, H 5.00.

4-{4-[Bis-(2-chloroethyl)amino]phenyl}-butyric acid 2-methoxy-5-[1-(3,4,5-trimethoxyphenyl)vinyl]phenyl ester (2p): A solution of *iso*CA-4 (31.5 mg; 0.316 mmol) in CH_2Cl_2 (5 mL) was successively treated with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI; 72 mg; 0.376 mmol), DMAP (42 mg; 0.347 mmol) and chlorambucil (106 mg; 0.347 mmol) and stirred for 1 h at RT. Saturated aq $NaHCO_3$ (3 mL) was added and the mixture was extracted with EtOAc (3×3 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated. The residue was further purified by flash chromatography (cyclohexane/EtOAc, 8:2) to yield **2p** (127 mg; 70%); $R_f=0.42$ (cyclohexane/EtOAc, 7:3); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=1.98$–2.10 (m, 2H), 2.59 (t, 2H, $J=7.5$ Hz, CH_2CO), 2.67 (t, 2H,

J=7.2 Hz, CH_2N), 3.60–3.75 (m, 8H), 3.82 (s, 6H, OCH_3), 3.86 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 5.35 (d, 1H, J=1.0 Hz), 5.40 (d, 1H, J=1.0 Hz), 6.56 (s, 2H), 6.68 (d, 2H, J=8.7 Hz), 6.94 (d, 1H, J=8.7 Hz), 7.03 (d, 1H, J=2.4 Hz), 7.12 (d, 2H, J=8.7 Hz), 7.25 ppm (dd, 1H, J=8.7 Hz, J=2.1 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ=26.9, 33.3, 33.9, 40.3, 53.9, 55.9, 56.2 (2), 60.9, 105.7, 111.9, 112.8, 113.1, 122.8, 126.5, 129.8, 131.4, 134.0, 137.0, 139.3, 143.9, 148.8, 150.9, 152.9 ppm; IR (neat): $\tilde{\nu}$=2934, 2839,1759, 1614, 1579, 1509, 1454, 1411, 1389, 1347, 1303, 1269, 1236, 1177, 1122, 1026, 1005, 958, 908, 845, 816, 770, 729 cm^{-1}; MS (ESI+): *m/z* (%): 624 (100) $[M+Na]^+$; Anal. calcd for $C_{32}H_{37}Cl_2NO_6$: C 63.79, H 6.19, N 2.32, found: C 63.68, H 6.19, N 2.22.

Acetic acid 3,4,5-triacetoxy-6-{2-methoxy-5-[1-(3,4,5-trimethoxyphenyl)vinyl]phenoxy}tetrahydropyran-2-yl methyl ester (2q): 2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl bromide (260 mg; 0.632 mmol) in CH_3CN was slowly added to a stirred solution of *iso*CA-4 (31.5 mg; 0.316 mmol) in CH_3CN (2 mL) containing aq KOH (1.15 mL, 1 N). After stirring for 12 h at RT, the mixture was hydrolyzed with aq HCl (5 mL, 1 N) and extracted with EtOAc (3×5 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated. The residue was further purified by flash chromatography (cyclohexane/EtOAc, 5:5) to yield **2q** (102 mg; 50%); R_f=0.80 (EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$): δ=2.01 (s, 3H, CH_3CO), 2.02 (s, 6H, CH_3CO), 2.05 (s, 3H, CH_3CO), 3.80 (s, 6H, OCH_3), 3.83 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 4.04–4.16 (m, 2H), 4.24 (dd, 1H, J=12.0 Hz, J=5.1 Hz), 4.99 (m, 1H), 5.13 (m, 1H), 5.24–5.30 (m, 2H), 5.31 (s, 1H), 5.35 (s, 1H), 6.52 (s, 2H), 6.83 (d, 1H, J=8.4 Hz), 7.00 (dd, 1H, J=8.4 Hz, J=2.1 Hz), 7.18 ppm (d, 1H, J=2.4 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ=20.6, 56.1, 56.2 (2), 60.9, 61.9, 69.8, 71.3, 72.0, 72.6, 100.8, 105.7, 112.1, 112.9, 119.8, 124.6, 134.1, 137.0, 131.4, 137.9, 145.9, 149.2, 150.4, 152.9, 169.3, 169.4, 170.2, 190.5 ppm; IR (neat): $\tilde{\nu}$=2939, 2840,1749, 1606, 1578, 1508, 1452, 1412, 1367, 1345, 1216, 1206, 1179, 1125, 1065, 1035, 956, 904, 845, 818, 780, 725, 702 cm^{-1}; MS (ESI+): *m/z* (%): 669.7 (100) $[M+Na]^+$; Anal. calcd for $C_{32}H_{38}O_{14}$: C 59.44, H 5.92, found: C 59.30, H 5.84.

2-Hydroxymethyl-6-{2-methoxy-5-[1-(3,4,5-trimethoxyphenyl)-vinyl]phenoxy}tetrahydropyran-3,4,5-triol (2r): A solution of **2q** (50.4 mg; 0.078 mmol) in dry MeOH (2 mL) was treated with aq NH_4Cl (8 mL, 28%) and stirred for 2 h at 60 °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 The mixture was hydrolyzed with aq HCl (5 mL, 1 N) and extracted with EtOAc (3×10 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated. The residue was further purified by flash chromatography (CH_2Cl_2/MeOH, 9:1) to yield the desired compound (34 mg; 90%); R_f=0.12 (CH_2Cl_2/MeOH, 8:2); mp: 154–157 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ=3.10–3.50 (m, 5H), 3.52 (d, 1H, J=11.7 Hz), 3.68 (s, 6H, OCH_3), 3.73 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 4.48 (s, 1H), 4.86 (m, 1H), 4.94 (s, 1H), 5.03 (s, 1H), 5,18 (s, 1H), 5.34 (m, 1H), 5.45 (s, 1H), 6.55 (s, 2H), 6.86 (dd, 1H, J=7.8 Hz, J=1.5 Hz), 6.96 (d, 1H, J=8.4 Hz), 7.12 ppm (d, 1H, J=1.5 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ=55.6, 55.8, (2), 60.0, 60.4, 69.5, 73.1, 76.8, 77.2, 100.4, 105.5, 112.0, 113.1, 115.1, 121.5, 133.0, 136.7, 137.2, 146.2, 148.5, 148.9, 152.5 ppm; IR (neat): $\tilde{\nu}$=3464, 3277, 2924, 2853,1741, 1650, 1578, 1506, 1463, 1425, 1411, 1377, 1340, 1319, 1250, 1233, 1211, 1179, 1154, 1124, 1088, 1050, 1040, 1015, 996, 955, 919, 893, 860, 843, 816, 778, 725 cm^{-1}; MS (ESI+): *m/z* (%): 501 (100) $[M+Na]^+$; Anal. calcd for $C_{24}H_{30}O_{10}$: C 60.24, H 6.32, found: C 60.10, H 6.16.

Synthesis of 4a–c

(4-Methoxy-3-nitrophenyl)-(3,4,5-trimethoxyphenyl)methanone (4a): A solution of 4-methoxy-3-nitroacetobenzaldehyde (2.54 g, 14 mmol) in THF (18 mL) was treated slowly at −78 °C with 3,4,5-trimethoxybenzaldehyde (28 mL; 19.6 mmol, 0.7 N) and stirred for 1 h at RT until the disappearance of starting material (TLC). The reaction was hydrolyzed at 0 °C with saturated aq NH_4Cl (20 mL) and extracted with EtOAc (3×15 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated. The crude alcohol was redissolved in CH_2Cl_2 (30 mL) and pyridinium chlorochromate (PCC; 8.62 g; 40 mmol) was added in portions over 2 h (t=0, 15 mmol; t=1 h, 15 mmol; t=2 h, 10 mmol). The solution was stirred overnight at RT, then filtered over SiO_2 and concentrated in vacuo. The residue was further purified by flash chromatography (cyclohexane/EtOAc, 5:5) to yield **4a** (3.25 g; 67%); R_f=0.44 (cyclohexane/EtOAc, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ=3.88 (s, 6H, OCH_3), 3.94 (s, 3H, OCH_3), 4.06 (s, 3H, OCH_3), 7.00 (s, 2H), 7.20 (d, 1H, J=8.8 Hz), 8.06 (dd, 1H, J=8.8 Hz, J=2.2 Hz), 8.32 ppm (d, 1H, J=2.2 Hz).

(5-Methoxy-2-nitrophenyl)-(3,4,5-trimethoxyphenyl)methanone (4b): Prepared as for **4a** from 5-methoxy-2-nitroacetobenzaldehyde (2.54 g, 14 mmol) to afford **4b** (2.28 g; 47%); R_f=0.54 (cyclohexane/EtOAc, 6:4); mp: 156 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ=3.81 (s, 6H, OCH_3), 3.91 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 6.88 (d, 1H, J=2.9 Hz), 6.99 (s, 2H), 7.08 (dd, 1H, J=9.3 Hz, J=2.9 Hz), 8.22 ppm (d, 1H, J=9.3 Hz).

(4-Methoxy-2-nitrophenyl)-(3,4,5-trimethoxyphenyl)methanone (4c): Prepared as for **4a** from 4-methoxy-2-nitroacetobenzaldehyde (2.54 g, 14 mmol) to afford **4c** (2.91 g; 47%); R_f=0.52 (cyclohexane/EtOAc, 6:4); mp: 165 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ=3.81 (s, 6H, OCH_3), 3.91 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 6.98 (s, 2H), 7.24 (dd, 1H, J=8.4 Hz, J=2.7 Hz), 7.43 (d, 1H, J=8.4 Hz), 7.62 ppm (d, 1H, J=2.7 Hz).

Synthesis of 2t–v

2,6-Dimethoxy[4-(4-methoxy-3-nitrobenzene)vinyl]anisole (2t): A solution of methyltriphenylphosphonium bromide (1.07 g; 3 mmol) in THF (10 mL) was cooled to 0 °C and slowly treated with LiHMDS in THF (3 mL, 3 mmol, 1 N). The yellow ylide solution was stirred at 0 °C for 1 h, then slowly treated with a solution of **4a** (520.5 mg; 1.5 mmol) in THF (10 mL) via syringe. The resulting mixture was allowed to warm to RT and stirred for a further 1 h. The solution was poured in H_2O (10 mL) and extracted with CH_2Cl_2 (2×10 mL). The organic layers were combined, dried ($MgSO_4$), filtered and concentrated. The residue was further purified by flash chromatography (cyclohexane/EtOAc, 8:2) to yield **2t** (460 mg; 89%); R_f=0.33 (cyclohexane/EtOAc, 7:3); ^{1}H NMR (300 MHz, $CDCl_3$): δ=3.82 (s, 6H, OCH_3), 3.88 (s, 3H, OCH_3), 3.98 (s, 3H, OCH_3), 5.44 (s, 2H), 6.50 (s, 2H), 7.05 (d, 1H, J=8.7 Hz), 7.52 (dd, 1H, J=2.0 Hz, J=8.7 Hz), 7.87 ppm (d, 1H, J=2.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ=56.2 (2C), 56.6, 60.9, 105.5 (2C), 113.2, 114.6, 125.1, 133.7, 133.9, 136.1, 138.2, 139.5, 147.6, 152.4, 153.1 ppm (2C); IR (neat): $\tilde{\nu}$=2939, 1619, 1579, 1529, 1504, 1469, 1412, 1354, 1275, 1239, 1184, 1119, 1016, 996, 954, 895 cm^{-1}; MS (APCI): *m/z* (%): 346 (100) $[M+H]^+$; Anal. calcd for $C_{18}H_{19}NO_6$: C 62.60, H 5.55, N 4.06, found: C 62.33, H 5.40, N 3.98.

2,6-Dimethoxy[4-(5-methoxy-2-nitrobenzene)vinyl]anisole (2u): Prepared as for **2t** from **4b** to afford **2u** (279 mg; 54%); R_f=0.47 (cyclohexane/EtOAc, 6:4); mp: 99 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ=3.77 (s, 6H, OCH_3), 3.83 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 6.45 (s,

2H, CH_2), 6.91 (d, 1H, $J=3.0$ Hz), 6.96 (dd, 1H, $J=9.0$ Hz, $J=3.0$ Hz), 8.05 ppm (d, 1H, $J=9.0$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=$ 55.9, 56.1 (2), 60.8, 104.0 (2), 113.3, 114.4, 117.5, 127.1, 134.7, 138.3, 139.6, 141.6, 147.0, 153.0 (2), 163.0 ppm; IR (neat): $\tilde{\nu}=2939$, 2838, 1576, 1507, 1461, 1414, 1339, 1294, 1235, 1184, 1125, 1063, 1027 cm^{-1}; MS (ESI+): *m/z* (%): 368 (100) $[M+Na]^+$; Anal. calcd for $C_{18}H_{19}NO_6$: C 62.60, H 5.55, N 4.06, found: C 62.50, H 5.50, N 4.01.

2,6-Dimethoxy[4-(4-methoxy-2-nitrobenzene)vinyl]anisole (2v): Prepared as for **2t** from **4c** to afford **2v** (362 mg; 70%); $R_f=0.50$ (cyclohexane/EtOAc, 6:4); mp 123 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=$ 3.77 (s, 6H), 3.83 (s, 3H), 3.89 (s, 3H), 6.44 (s, 2H, CH_2), 7.14 (dd, 1H, $J=8.4$ Hz, $J=2.7$ Hz), 7.34 (d, 1H, $J=8.4$ Hz), 7.42 ppm (d, 1H, $J=2.7$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=$55.8, 56.1 (2), 60.8, 104.1 (2), 109.1, 114.8, 118.9, 128.7, 133.2, 135.1, 138.2, 146.0 (2), 149.4 (2), 153.0 (2), 159.5 ppm; IR (neat): $\tilde{\nu}=2937$, 2838, 1619, 1579, 1528, 1504, 1461, 1412, 1343, 1300, 1266, 1234, 1184, 1123, 1064, 1029, 1005 cm^{-1}; MS (ESI+): *m/z* (%): 368 (100) $[M+Na]^+$; Anal. calcd for $C_{18}H_{19}NO_6$: C 62.60, H 5.55, N 4.06, found: C 62.56, H 5.50, N 4.00.

Synthesis of 2s, 2w, 2x

2-Methoxy-5-(1-(3,4,5-trimethoxyphenyl)vinyl)aniline (*iso*NH_2CA-4, 2s): Compound **2t** (86 mg; 0.25 mmol) and Zn (powder 98%, dust <10 μM; 98 mg, 1.5 mmol) were dissolved in glacial AcOH (5 mL) and the solution stirred for 1 h at RT. The solvent was removed in vacuo, the resulting granular residue was redissolved in EtOAc (15 mL) and the mixture was filtered through a pad of celite. The filtrate was washed with H_2O (10 mL), dried ($MgSO_4$), filtered and concentrated. The residue was purified by flash chromatography (cyclohexane/EtOAc, 7:3) to yield **2s** (68.5 mg; 87%); $R_f=$ 0.37 (cyclohexane/EtOAc, 6:4); ^{1}H NMR (300 MHz, CD_3COCD_3): $\delta=$ 3.80 (s, 3H, OCH_3), 3.64 (s, 6H, OCH_3), 3.70 (s, 3H, OCH_3), 4.38 (s, 2H, NH_2), 5.26 (d, 1H, $J=1.6$ Hz), 5.29 (d, 1H, $J=1.6$ Hz), 6.61 (s, 2H), 6.61 (dd, 1H, $J=8,4$ Hz, $J=2.2$ Hz), 6.71 (d, 1H, $J=2.2$ Hz), 6.79 ppm (d, 1H, $J=8.4$ Hz); ^{13}C NMR (75 MHz, CD_3COCD_3): $\delta=$ 55.8, 58.3 (2), 60.7, 106.9 (2), 110.7, 112.1, 114.9, 117.9, 134.9, 138.0, 138.5, 139.0, 147.8, 151.4, 153.9 ppm (2); IR (neat): $\tilde{\nu}=3371$, 2937, 2835, 1579, 1513, 1462, 1411, 1346, 1296, 1255, 1235, 1221, 1179, 1125, 1027, 1006 cm^{-1}; Anal. calcd for $C_{18}H_{21}NO_4$: C 68.55, H 6.71, N 4.44, found: C 68.38, H 6.60, N 4.32.

4-Methoxy-2-[1-(3,4,5-trimethoxyphenyl)vinyl]aniline (2w): Prepared as for **2s** from the reduction of **2u** to afford **2w** (55 mg; 70%); $R_f=0.39$ (cyclohexane/EtOAc, 5:5); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=3.77$ (s, 3H), 3.80 (s, 6H, OCH_3), 3.85 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 5.32 (d, 1H, $J=1.5$ Hz), 5.71 (d, 1H, $J=1.5$ Hz), 6.59 (s, 2H), 6.66 (d, 1H, $J=8.4$ Hz), 6.72–6.79 ppm (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=$55.9, 56.6 (2), 60.6, 105.4 (2), 115.4, 115.6, 116.6, 117.2, 128.3, 136.3, 139.6 (2), 140.0 (2), 148.7, 152.7, 154.3 ppm (2); IR (neat): $\tilde{\nu}=3440$, 3360, 2938, 2832, 1578, 1498, 1462, 1410, 1340, 1280, 1234, 1177, 1121, 1038, 1004 cm^{-1}; MS (ESI+): *m/z* (%): 338 (100) $[M+Na]^+$; Anal. calcd for $C_{18}H_{21}NO_4$: C 68.55, H 6.71, N 4.44, found: C 68.44, H 6.62, N 4.35.

5-Methoxy-2-[1-(3,4,5-trimethoxyphenyl)vinyl]aniline (2x): Prepared as for **2s** from the reduction of **2u** to afford **2x** (36 mg; 46%); $R_f=0.21$ (cyclohexane/EtOAc, 7:3); mp: 148 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=3.79$ (s, 3H, OCH_3), 3.80 (s, 6H, OCH_3), 3.85 (s, 3H, OCH_3), 5.28 (d, 1H, $J=1.5$ Hz), 5.65 (d, 1H, $J=1.5$ Hz), 6.24 (d, 1H, $J=2.7$ Hz), 6,59 (s, 2H), 7.43 (dd, 1H, $J=8.4$ Hz, $J=2.7$ Hz), 7.03 ppm (d, 1H, $J=8.4$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=55.1$, 56.1 (2), 60.8, 101.0, 103.9, 104.1 (2), 115.3, 120.1, 131.8, 136.1, 138.2, 145.2 (2), 147.0 (2), 153.2 (2), 160.4 ppm; IR (neat): $\tilde{\nu}=3472$, 3374, 2937, 2835, 1608, 1576, 1503, 1410, 1342, 1234, 1207, 1123, 1027, 1004 cm^{-1}; MS (ESI+): *m/z* (%): 338 (100) $[M+Na]^+$; Anal. calcd for $C_{18}H_{21}NO_4$: C 68.55, H 6.71, N 4.44, found: C 68.37, H 6.57, N 4.30.

Biology

Cell culture and proliferation assay: Cancer cell lines were obtained from the American type Culture Collection (Rockville, USA) and were cultured according to the supplier's instructions. Briefly, A549 lung carcinoma, MDA-MB-231 and MDA-MB-435 cells were grown in Dulbecco minimal essential medium (DMEM) containing 4.5 g L^{-1} glucose supplemented with 10% FCS and 1% glutamine. Human K562 leukemia and HCT116 colorectal carcinoma cells were grown in RPMI 1640 containing 10% FCS and 1% glutamine. Human umbilical vein endothelial cells (HUVECs) were obtained from Clonetics (Lonza, USA) and cultured according to the supplier's instructions. Briefly, HUVECs from three to six passages were subcultured to confluency onto 0.2% gelatincoated tissue culture flasks in endothelial cell growth medium (EGM2) containing growth factors and 2% FCS. All cell lines were maintained at 37 °C in a humidified atmosphere containing 5% CO_2. Cell viability was assessed using Promega CellTiter-Blue reagent according to the manufacturer's instructions. Cells were seeded in 96-well plates (5×103 cells per well) containing 50 μL growth medium. After 24 h of culture, the cells were supplemented with 50 μL of the test compound dissolved in DMSO (less than 0.1% in each preparation). After 72 h of incubation, 20 μL of resazurin was added for 2 h before recording fluorescence ($\lambda_{ex}=560$ nm, $\lambda_{em}=590$ nm) using a Victor microtiter plate fluorimeter (Perkin–Elmer, USA). The IC_{50} value corresponds to the concentration of test compound that caused a decrease of 50% in fluorescence of drug treated cells compared with untreated cells. Experiments were performed in triplicate.

Tubulin binding assay: Sheep brain tubulin was purified according to the method of Shelanski[32] by two cycles of assembly–disassembly and then dissolved in the assembly buffer containing 0.1 M MES, 0.5 mM $MgCl_2$, 1 mM EGTA and 1 mM GTP (pH 6.6) to give a tubulin concentration of ~2–3 mg mL^{-1}. Tubulin assembly was monitored and recorded continuously by turbidimetry at 350 nm in a UV spectrophotometer equipped with a thermostatted cell at 37 °C. The GI_{50} value of each compound was determined as the concentration at which the maximum assembly rate of tubulin was decreased by 50% compared to the rate in the absence of compound. The GI_{50} values for all compounds were compared to the GI_{50} of CA-4, colchicine and phenstatin and measured the same day under the same conditions.

Cell cycle analysis: Exponentially growing cancer cells (K562, HCT116, MDA-MB-231) were incubated with tested compound or DMSO for 24 h. Cell-cycle profiles were determined by flow cytometry on a FC500 flow cytometer (Beckman-Coulter, France) as described previously.[33]

Apoptosis assay: Apoptosis was measured by the Apo-one homogeneous caspase 3/7 assay (Promega Co., USA) according to the manufacturer's recommendations. Briefly, cells were subcultured on a 96-well plate with 5×10^4 cells per well in 100 μL medium. After 24 h incubation, the medium in the 96-well plate was discarded and replaced with medium containing different concentrations of *iso*FCA-4, *iso*CA-4 and *iso*NH_2CA-4 (1, 5, and 10 nM) or 0.1% DMSO (negative control). The treated cells were incubated for 24 h, each well then received 100 μL of a mixture of caspase substrate and Apo-one caspase 3/7 buffer. After 1 h incubation, the

fluorescence of sample was measured using a Victor microtiter plate fluorimeter (Perkin–Elmer, USA) at 527 nm.

Cord disruption assay: HUVECs (2×10^4 cells per well) were plated in 96-well plates on a thick layer of matrigel (Becton Dickinson; 10 mg mL^{-1}, 60 μL per well) and allowed to align for 24 h. Test compounds (*iso*FCA-4, *iso*CA-4, *iso*NH_2CA-4) or vehicle were added to the formed cords and left. Images were taken 3 h and 72 h after the addition of compounds.

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