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

Abdallah Hamze,[a] Anne Giraud,[a] Samir Messaoudi,[a] Olivier Provot,[a] Jean-François Peyrat,[a] Jérôme Bignon,[b] Jian-Miao Liu,[b] Joanna Wdzieczak-Bakala,[b] Sylviane Thoret,[b] Joëlle Dubois,[b] Jean-Daniel Brion,[a] and Mouad Alami*[a]

ABSTRACT. *The cytotoxic activity of a series of 23 new isocombretastatin A derivatives with modifications on the B-ring was studied. Several compounds exhibited excellent antiproliferative activity at a nanomolar concentration against a panel of human cancer cell lines. The most cytotoxic compounds, isoFCA4 (**2e**), isoCA4 (**2k**) and isoNH$_2$CA4 (**2s**), 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 in the growth inhibitory activities but displayed similar activities as inhibitors of tubulin polymerization. In addition, they led to the arrest of three cancer cell lines in the G$_2$/M phase of the cell cycle and induced apoptosis. The disrupting in vitro 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 pro-drug modification and development as vascular disrupting agents for treatment of solid tumor cancers.*

Introduction

The formation of microtubules is a dynamic process involved in a variety of cellular process 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 M phase followed by apoptosis. The discovery of natural substances 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 for the treatment of various cancers,[3] these complex drugs suffer from several drawbacks since they are generally difficult to synthesize, they cause neurotoxic side effects in patients,[4] and their clinical potential is now limited by the development of multidrug resistance (MDR).[5] Therefore, the search of 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 substances interfering with the dynamics of tubulin polymerization and depolymerization, combretastatin A-4 (CA4), first isolated from the South African bush willow tree, *Combretum caffrum*,[7] is a promising anticancer drug. CA4 binds to tubulin at the colchicine binding site and is recognized as a very effective inhibitor of tubulin assembly (Figure 1). Moreover, CA4 exhibits strong growth inhibitory activity, at nanomolar concentrations, against a wide variety of human cell lines including multidrug resistant (MDR) positive cancer cell lines.[8] However, the low water solubility of CA4 limits its efficacy *in vivo*. A more-soluble disodium phosphate pro-drug (CA4P) has been developed as the selected lead for human studies.[9] CA4P, and its amino acid derivative AVE-8062[10] have been demonstrated to cause vascular shutdown in established tumors *in vivo*, consistent with an anti-vascular mechanism of action.[6] Currently, CA4-P either as a single agent or in combination therapy is undergoing several advanced clinical trials worldwide for the treatment of age-related macular degeneration (AMD) [9] or anaplastic thyroid cancer.[11]

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 combretastatins. 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 by inserting mainly the *cis*-olefin in a five-membered heterocyclic ring (e.g.; pyrazoles, thiazoles, triazoles, imidazolones).[15]

Our interest in 1,1-diarylethylene unit synthesis,[16] combined with our efforts to discover novel potent tubulin assembly inhibitors, related to CA4,[17] led us to identify a promising class of substances with strong cytotoxic and antimitotic activities, simply by switching the trimethoxyphenyl nucleus from the C(1) to the C(2) position of the ethylene bridge.[18]

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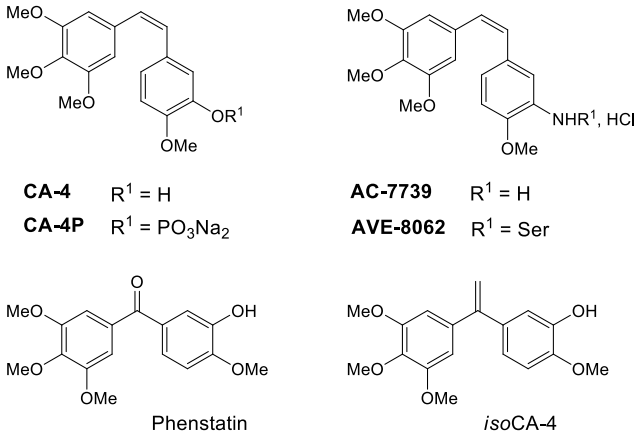


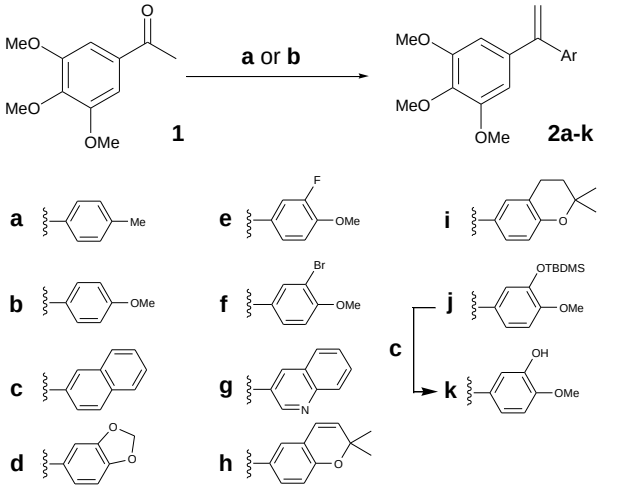
Figure 1. Structure of combretastatin A-4, its synthetic amino-derivative AC-7739, their water soluble pro-drugs CA4P and AVE-8062, phenstatin and *iso*combretastatin A-4.

In contrast to their natural parent 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*CA4 that share a striking structural similarity with phenstatin[19] appears to elicit its tumor cytotoxicity in a fashion similar to CA4, *via* inhibition of tubulin polymerization, which then leads to cell cycle arrest in G_2/M. As the replacement of the 1,2-ethylene bridge by the 1,1-ethylene one resulted in retention of biological activities, our finding encouraged us to use this bioisostere[20] in future structure activity relationships studies. Because the 3,4,5-trimethoxyphenyl nucleus (A-ring) is crucial to obtain relevant cytotoxic and antitubulin responses, we intended to introduce variations in the B-ring that could yield compounds with drug-like properties. Herein we report the synthesis and biological evaluation of a broad range of B-ring-substituted *iso*CA4 analogues, in which the A ring is kept intact. The potencies of newly synthesized compounds to inhibit the growth of cancer cells, to prevent tubulin assembly and to attack the established vessel network were evaluated *in vitro*.

Results and Discussion

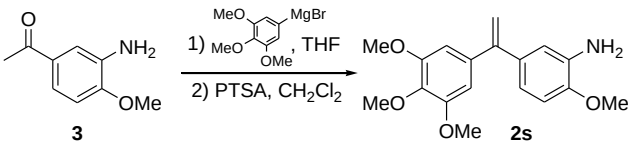
Chemistry.

While the palladium-catalyzed coupling of 3,4,5-trimethoxy-acetophenone *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 of commercially available 3,4,5-trimethoxyacetophenone (**1**) with Grignard reagents in THF furnished the corresponding tertiary alcohols which upon treatment without purification using a catalytic amount of PTSA in CH_2Cl_2 afforded **2a-c** in good overall yields. In a similar way, the synthesis of compounds **2d-j** was realized by treatment of **1** with an aryl lithium species obtained according to a lithium-halogen exchange reaction from the corresponding bromo- or iodo- derivatives. It should be noted that 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* the DMAP elimination of the corresponding mesylate since PTSA was ineffective to produce the expected compound. Finally, desilylation of the TBDMS-ether intermediate **2j** under alkaline conditions led to the formation of *iso*CA4 (**2k**) in excellent yield.

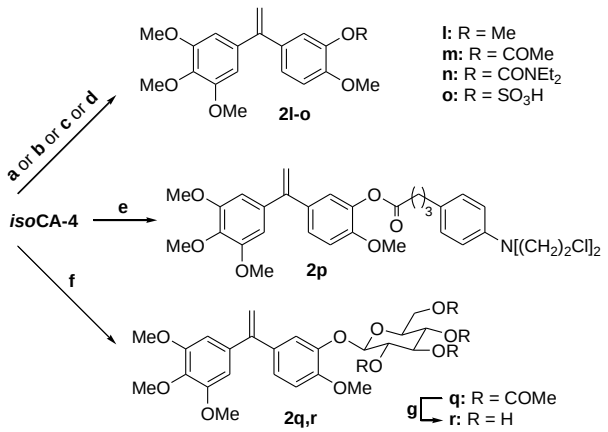


Scheme 1. *Reagents and conditions*: a) (*i*) ArMgBr, THF, -40 °C; (*ii*) PTSA (10 mol%), CH_2Cl_2, 20 °C (**2a**: 44%, **2b**: 54%, **2c**: 81%); b) (*i*) ArLi, hexanes/toluene (1/3), -78 °C; (*ii*) PTSA (10 mol%), CH_2Cl_2, 20 °C (**2d**: 80%, **2e**: 48%, **2f**: 53%, **2h**: 48%, **2i**: 32%, **2j**: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

Among the structural features considered to be interesting, it has been shown that a compound with only a methoxy group in the para position of ring B of CA4 maintains its cytotoxic potential, suggesting that the presence of a free hydroxyl group is not fundamental.[13a] Consequently, as an extension of our SAR efforts with *iso*CA4, a selection of compounds **2l-r** including, esters, carbamates and β-sugar[22] derivatives were prepared. Scheme 2 details the analogous synthesis of 3'-O-substituted *iso*combretastatin analogues from the parent compound *iso*CA4 (**2k**).

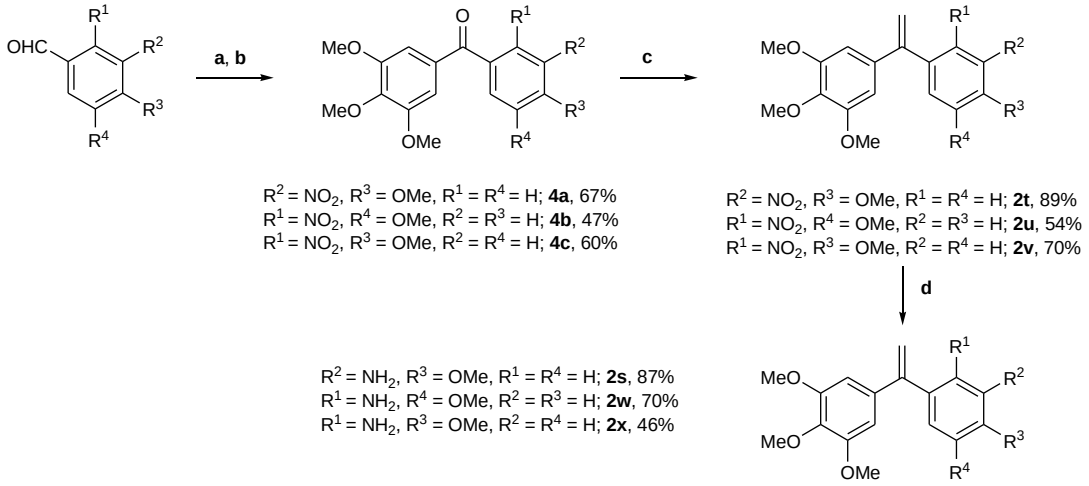


Scheme 3. Synthesis of *iso*NH_2CA4 (**2s**)



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 in the CA4 series of the hydroxyl moiety on the B-ring by an amino group resulted in retention of biological activities,[23] we envisioned to prepare the substance **2s**, which constitutes the simplest isomer of AC-7739. To this end, the coupling reaction between acetophenone **3** and 3,4,5-trimethoxyphenyl magnesium bromide (Scheme 3) led to the tertiary alcohol which upon treatment with a catalytic amount of PTSA provided **2s** in a low 12% yield. To note, 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 have an easier access to a variety of aniline derivatives and their nitro precursors. Scheme 4 outlines the synthetic route followed for the synthesis of these amino substances. The preparation involved a reaction sequence in four steps: (*i*) condensation of the 3,4,5-trimethoxyphenylmagnesium bromide with commercially available appropriate benzaldehyde derivatives, (*ii*) oxidation of the resulting alcohols, (*iii*) Wittig reaction using methyltriphenylphosphonium bromide to afford the corresponding 1,1-diarylethylene **2t-v** derivatives and (*iv*) reduction of the nitro group by Zn/AcOH. Accordingly, the expected amine isomers **2s**, **2w**, **2x** were obtained in good overall yields. Noteworthy, this strategy allowed an easy access to amino*iso*combretastatin analogues **2s**, **2w**, **2x** in the scale of several grams.



Scheme 4.[a] Synthesis of *iso*aminocombretastatin derivatives [a] Reagents: (**a**) 3,4,5-$(MeO)_3C_6H_2MgBr$, 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.

Biological evaluation.

(A) 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*CA4, CA4[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 cancer cell growth-inhibitory activity in a nanomolar range. In particular, the best inhibition results were obtained with compounds **2e**, **2m** and **2s** (7, 8 and 2 nM, respectively). These values were comparable to *iso*CA4 (**2k**, GI_{50} = 2 nM) or CA4 (GI_{50} = 2 nM) and more active than the phenstatin (GI_{50} = 33 nM). A comparison of GI_{50} values exhibited by *iso*CA4 and *iso*aminocombretastatin A-4 (**2s**, *iso*NH_2CA4) revealed that the introduction of an amino group instead of a hydroxyl moiety at the C3'-position on the B-ring, provide a compound with an equal biological efficacy. Additionally, switching the NH_2 group from the C3' to the C2' position retained for **2x** the cellular growth inhibitory activity at a nanomolar range (GI_{50} = 40 nM), while its corresponding nitro precursor, **2v**, showed a decline by a factor of ten. One can note that *iso*CA4 and its corresponding acetate pro-drug **2m**, displayed a similar cell growth inhibition. In contrast, compounds **2n** and **2o** with a carbamate or a sulfonic acid ester functions, respectively, were found to display a weak cytotoxic effect. When *iso*CA4 was attached to chlorambucil[25] *via* an ester linkage, the resulting compound **2p** maintained substantial biological potency (GI_{50} = 25 nM) as compared to other esters and *iso*CA4. It should be noted that the replacement of the hydroxyl at C3'-position by a fluorine atom, gave compound *iso*FCA4 (**2e**) with comparable cytotoxic activity to that of *iso*CA4, while introduction at the C3'-position of a bromine atom (**2f**) resulted in a significant loss of antiproliferative activity to micromolar range.

Table 1. Cytotoxicity of *iso*CA4 analogues against HCT-116 cells (colon carcinoma cells).

Compound	GI_{50} (nM ± SD)[a]	Compound	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*FCA4)	7 ± 1	**2s (*iso*NH_2CA4)**	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*CA4**	2 ± 0.2
2m	8 ± 0.2	**CA4**	2[c] ± 0.1
2n	2800 ± 310	**Phenstatin**	33[c] ± 2.5

[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). [b] NA, non active. [c] The GI_{50} values for CA4 and phenstatin were determined in this study.

It is interesting to note that our lead compounds *iso*FCA4 and *iso*NH_2CA4 with a 1,1-diarylethylene scaffold are as potent than 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 bridge and the *Z*-1,2 ethylene one.[18] As it was reported that the 3-hydroxyl group on the B-ring of CA4 is not essential for potent activity,[13a] we replaced the B-ring of *iso*CA4 with a 4-methoxyphenyl group. As expected, the resulting compound **2b** showed a 20-fold decrease in cytotoxicity compared to that of *iso*CA4. 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*CA4 with a 4-tolyl (**2a**) or 5-benzodioxole (**2d**) rings resulted in an important loss of potency relative to the parent substance *iso*CA4, albeit still cytotoxic (GI_{50} < 0.5 µM). However, the introduction of a heterocyclic moiety such as quinoline to give **2g** led to a decrease of the cytotoxic activity (GI_{50} = 650 nM) against HCT-116 cells. Finally, none of the following compounds **2h**, **2i**, **2l**, **2q** and **2r** exhibited sufficient cytotoxic activities to warrant further biological evaluations.

(B) Inhibition of tubulin polymerization and cytotoxicity for selected compounds

To further characterize the cytotoxicity profile of these compounds, we have investigated the effect of the most active substances **2b**, **2e**, **2m**, **2p**, **2s** and **2x** ($GI_{50} \leq 40$ nM) on the proliferation of a panel 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*CA4 series display similar potencies and showed activities with GI_{50} values in the range of 2-50 nM. These compounds inhibit cell growth at a nanomolar concentration whatever the cancer cell lines used, suggesting the high therapeutic potency of these drugs. Interestingly, substances *iso*FCA4 and *iso*NH_2CA4 bearing on C3'-position a fluorine atom or an amino function respectively show similar cytotoxic potency. The GI_{50} values obtained are comparable to these of CA4 and *iso*CA4 (GI_{50} = 2-8 nM) and significantly lower than the GI_{50} values of 26-41 nM found for colchicine and phenstatin.

To investigate whether the cytotoxic activities of the *iso*CA4 series were related to their interaction with microtubulin system, all compounds presented in Table 2 as well as the reference substances (CA4, phenstatin and colchicine) were evaluated for *in vitro* tubulin polymerization inhibitory activity. The results show that *iso*NH_2CA4 and the fluorinated compound *iso*FCA4 exhibit a similar inhibition of tubulin polymerization as *iso*CA4 and CA4 (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 for **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 where no esterase enzymes are present. A noticeable finding is the high potency of **2b** (IC_{50} = 2.0 µM) indicating that the presence of 3'OH on the B-ring does not play an essential role for strong antitubulin activity as it was previously observed in the CA4 series. [13b]

(C) 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*FCA4, *iso*CA4 and *iso*NH_2CA4 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 drugs at different concentrations. The cell-cycle profiles depicted in Figure 2 show a significant increase in the number of cells arrested at the G_2/M growth stage with increasing concentration (5 to 10 nM) of the studied drugs. The observed effects of *iso*FCA4, *iso*CA4 and *iso*NH_2CA4 on cell cycle progression correlated well with their strong antiproliferative and antitubulin activities. This stays in agreement with the similar properties reported previously for the majority of antimitotic agents. Cell cycle arrest at G2/M is often followed by DNA fragmentation and the morphological features of apoptosis.[28] Therefore, we have investigated the effect of *iso*FCA4, *iso*CA4 and *iso*NH_2CA4 (1, 5, 10 nM) on induction of apoptosis in K562, HCT-116 and MDA-MB-231 cancer cells using a caspases 3 and 7 standard assays.[29] The enzymatic activity of caspases 3 and 7 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 substances.

More interestingly, a spectacular 10- to 15-fold dose-dependent increase in apoptosis was evidenced in K562 leukemic 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 current findings clearly show that, in addition to their antiproliferative and antitubulin effects, the treatment of cancer cells with

compounds *iso*FCA4, *iso*CA4 and *iso*NH_2CA4 activate caspases system leading to programmed cell death.

Table 2. Cytotoxic activity and inhibition of tubulin polymerization of selected compounds

	Cytoxicity GI_{50}, (nM ± SD) [a]							
Compound	HCT116[b]	K562[b]	U87[b]	A549[b]	M435[b]	M231[b]	HUVEC[b]	ITP 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
***iso*FCA4 (2e)**	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
***iso*NH_2CA4 (2s)**	2 ± 0.1	4 ± 0.16	8 ± 0.47	7 ± 0.51	7 ± 0.52	6 ± 0.21	3 ± 0.12	1.5
***iso*CA4**	2 ± 0.2	5 ± 0.19	8 ± 0.36	8 ± 0.7	4.5 ± 0.2	4 ± 0.16	1.5 ± 0.07	2
CA4[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). [b] HCT116, colon carcinoma; K562, myelogenous leukaemia; 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 CA4, colchicin and phenstatin were determined in this study.

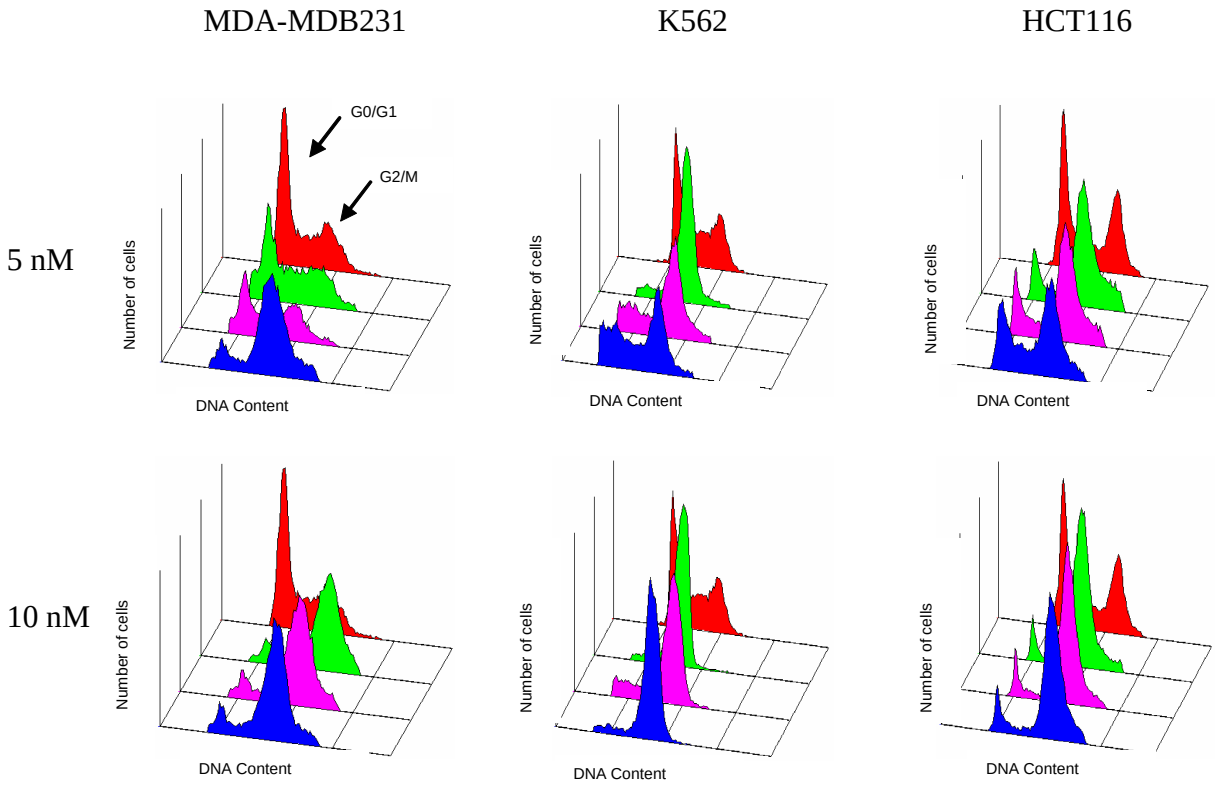


Figure 2. Effect of *iso*FCA4 (green), *iso*CA4 (blue) and *iso*NH_2CA4 (rose) on cell cycle distribution in MDA-MDB-231, K562 and HCT116 cells determined by flow cytometry analysis (DMSO control in red). DNA content was assessed via propidium iodide staining.

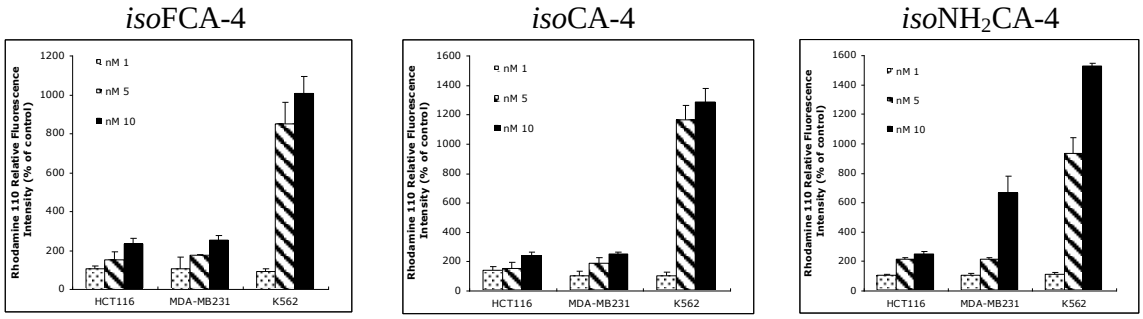


Figure 3. Apoptotic effects of *iso*FCA4, *iso*CA4 and *iso*NH_2CA4 in HCT116, MDA-MDB-231 and K562 cells. The results are expressed in the percentage of apoptotic cells detected following 24 h treatment with *iso*FCA4, *iso*CA4 and *iso*NH_2CA4 at different concentrations.

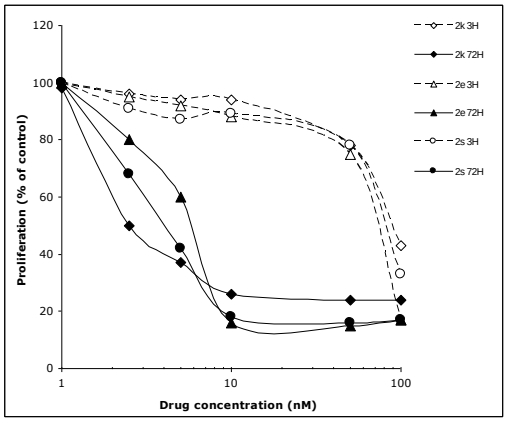


Figure 4. Effect of selected *iso*FCA4 (**2e**), *iso*CA4 (**2k**) and *iso*NH_2CA4 (**2s**), on in vitro endothelial cells (HUVEC) after 3 h and 72 h of treatment.

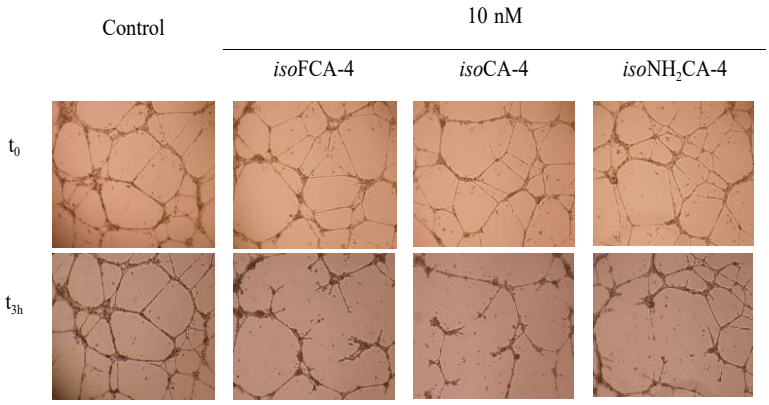


Figure 5. Inhibitory activity of isoFCA4, isoCA4 and isoNH$_2$CA4 on in vitro formed vessel-like structures. IsoFCA4, isoCA4 and isoNH$_2$CA4 or vehicule were added to the vascular tubes formed during 24 h by HUVEC on Matrigel. Images were taken 3 h after addition of the compounds.

(D) Effect on Human Umbilical Vein Endothelial Cells Organization

In order to expand our studies, the effects of our lead compounds *iso*FCA4, *iso*CA4 and *iso*NH_2CA4 on the proliferation of normal endothelial cells (HUVEC) were determined. The results presented in Figure 4 show that after 72 h of incubation, compounds *iso*FCA4, *iso*CA4 and *iso*NH_2CA4 exhibit a similar growth inhibition activity (GI_{50} = 1.5-5 nM) as CA4 (GI_{50} = 2.5 nM). However, no change in the viability of HUVEC cells treated for 3 h with *iso*FCA4, *iso*CA4 and *iso*NH_2CA4 was observed even at the concentration of 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 (Figure 5). To evaluate whether our lead compounds could affect newly formed blood vessels, the *in vitro* assay of tube formation by HUVEC was performed. The addition of *iso*FCA4, *iso*CA4 and *iso*NH_2CA4 for 3 h to formed capillary-like tubes rapidly disrupted the integrity of the network. This effect was evidenced for 10 nM concentration of tested substances 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 use as vascular disrupting agents.

Conclusion

We have shown that 1,1-diarylethylenes of general structure **2** are potent antiproliferative agents. The compounds described in this report are structurally simpler than those of the CA4 series, chemically stable (no isomerization), and easily accessible. Three representative substances *iso*FCA4, *iso*CA4 and *iso*NH_2CA4 have emerged as lead compounds. They displayed antiproliferative activity with an GI_{50} values ranging from 2 to 10 nM against different human cancer cell lines. Flow cytometric analysis indicated that these drugs act as antimitotics and arrest the cell cycle in the G_2/M phase. Moreover, we showed that our lead compounds have spectacular disrupting *in vitro* effects on newly formed vascular tubes after 3 h of treatment. These results suggest that *iso*FCA4, *iso*CA4 and *iso*NH_2CA4 might be lead compounds for use as vascular disrupting agents and promising candidates for *in vivo* evaluation. Both compounds *iso*CA4 and *iso*NH_2CA4, have the potential for further pro-drug modification and development as vascular disrupting agents for treatment of solid tumor cancers 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). Unless otherwise stated, $CDCl_3$ was used as solvent. Chemical shifts δ are in ppm, and the following abbreviations are used: singlet (s), doublet (d), triplet (t), multiplet (m) and broad singlet (bs). Elemental analyses (C, H, N) were performed with a Perkin-Elmer 240 analyzer at the microanalyses Service of the Faculty of Pharmacy at Châtenay-Malabry (France) and were within 0.4% of the theorical values otherwise stated. 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 magnesium sulphate ($MgSO_4$) or sodium sulphate (Na_2SO_4).

Synthesis of 2a-c

2,6-Dimethoxy[4-(4-methylbenzene)vinyl]anisole (2a)
To a solution of 3,4,5-trimethoxyacetophenone (420 mg; 2 mmol) in THF (10 mL) was added at -40°C under an argon atmosphere, 6 mL of a 1M solution of *para*-tolylmagnesium bromide in THF (6 mmol). The mixture was stirred for 3 h at this temperature and further 16 h at room temperature. A saturated NH_4Cl solution (10 mL) was slowly added to the mixture to hydrolyze the adduct and the mixture was extracted with Et_2O (10 mL x 3). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The crude mixture was next dissolved in CH_2Cl_2 (10 mL), mixed with some crystals of PTSA and stirred for 3 h at room temperature. The solution was washed with a saturated NaCl solution (20 mL) and extracted with CH_2Cl_2 (10 mL x 2). The organic layers were combined, dried over $MgSO_4$, and concentrated. The residue was further purified by flash chromatography to yield **2a** (307 mg; 54 %). R_f (cyclohexane/EtOAc : 6/4) = 0.75. ^{1}H RMN: (δ ppm, CD_3COCD_3, 300 MHz): 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 (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 (2), one C not detected. IR (cm^{-1}): 2936, 1737, 1578, 1504, 1451, 1409, 1346, 1233, 1182, 1123, 1009. 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)
Compound **2b** was prepared as for **2a** from 3,4,5-trimethoxyacetophenone (420 mg; 2 mmol) and (4-methoxyphenyl)magnesium bromide to afford the title compound (384 mg; 64 %). R_f (cyclohexane/EtOAc : 6/4) = 0.60. ^{1}H RMN: (δ ppm, CD_3COCD_3, 300 MHz): 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 (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. IR (cm^{-1}): 1579, 1507, 1454, 1411, 1346, 1299, 1233, 1174, 1122, 1030, 1004. 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)
Compound **2c** was prepared as for **2a** from 3,4,5-trimethoxyacetophenone (420 mg; 2 mmol) and (2-naphthylmagnesium bromide to afford the title compound (518 mg; 81 %). R_f (CH_2Cl_2/EtOAc : 9/1) = 0.80. mp 89°C. ^{1}H RMN: (δ ppm, CD_3COCD_3, 300 MHz): 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 (m, 4H). ^{13}C RMN: (□ ppm, CD_3COCD_3, 75 MHz): 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 (2). IR (cm^{-1}): 2936, 1578, 1503, 1451, 1412, 1352, 1331, 1237, 1182, 1122, 1003. Anal. ($C_{21}H_{20}O_3$) C, H. Calcd for $C_{21}H_{20}O_3$: C 78.74, H 6.29, found: C 78.64, H 6.20.

Synthesis of 2d-2f, 2h-j

5-[1-(3,4,5-trimethoxyphenyl)vinyl]benzo[1,3]dioxole (2d)
To a -78°C solution of 5-iodobenzo[*d*][1,3]dioxole (124 mg; 0.5 mmol) in hexanes (15 mL) was slowly added *via* syringe, 625 µL (1 mmol) of a 1.6 M solution of *t*BuLi in pentane under nitrogen. After stirring for 45 min. at -78°C, 105 mg (0.5 mmol) of 3,4,5-trimethoxyacetophenone in toluene (5 mL) was added to the solution which was warmed to room temperature, and stirring was continued for 12 h. A saturated NH_4Cl solution (10 mL) was slowly added to the mixture to hydrolyze the adduct and the mixture was extracted with Et_2O (10 mL x 3). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The crude was next dissolved in CH_2Cl_2 (10 mL), mixed with some crystals of PTSA and stirred for 3 h at room temperature. The solution was washed with a saturated NaCl solution (20 mL) and extracted with CH_2Cl_2 (10 mL x 2). The organic layers were combined, dried over $MgSO_4$, concentrated and the crude mixture was treated as for **2a-c** to afford the title compound **2d (**30 mg; 19 %). R_f (CH_2Cl_2/Cyclohexane) = 0.82. ^{1}H RMN: (□ ppm, CD_3Cl_3, 300 MHz): 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 (m, 2H). ^{13}C RMN: (□ ppm, CD_3Cl_3, 75 MHz): 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. IR (cm^{-1}): 2939, 2835,1699, 1578, 1503, 1488, 1463, 1450, 1410, 1340, 1234, 1184, 1161, 1124, 1036, 1006, 936, 907, 866, 844, 814, 783, 733, 702. MS (ESI+, m/z, %): 337 $(M+Na)^+$, 100. 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*FCA4, 2e)
Compound **2e** was 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 the title compound (76 mg; 48 %). R_f (Cyclohexane/EtOAc : 7/3) = 0.52. mp 64-66 °C. ^{1}H NMR: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 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 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 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. ^{19}F NMR: (δ ppm, CD_3Cl_3, 188 MHz): -136.0. IR (cm^{-1}): 3086, 3011, 2939, 2835, 1619, 1576, 1518, 1504, 1462, 1439, 1310, 1205, 1117, 1085, 949, 899, 876. MS (ESI^+) *m/z* (%): 341 $[M+Na]^+$, 100. 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)
Compound **2f** was 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 the title compound (101 mg; 53 %). R_f (Cyclohexane/EtOAc : 7/3) = 0.46. ^{1}H RMN: (δ ppm, CD_3Cl_3, 300 MHz): 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 (m, 2H). ^{13}C RMN: (□ ppm, CD_3Cl_3, 75 MHz): 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. IR (cm^{-1}): 2936, 2835, 1579, 1504, 1485, 1461, 1411, 1336, 1287, 1257, 1231, 1181, 1122. MS (ESI+, m/z, %): 403 $(M+Na)^+$, 100. 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)

Compound **2h** was 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 the title compound (85 mg; 48 %). R_f (Cyclohexane/EtOAc : 7/3) = 0.60. ^{1}H RMN: (δ ppm, CD_3Cl_3, 300 MHz): 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 (dd, 1H, *J* = 8.4 Hz, *J* = 2.4 Hz). ^{13}C RMN: (δ ppm, CD_3Cl_3, 75 MHz): 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 (2). IR (cm^{-1}): 2973, 2935, 2834, 1578, 1504, 1489, 1451, 1410, 1365, 1343, 1265, 1235, 1122, 1005. 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)

Compound **2i** was prepared as for **2d** from 3,4,5-trimethoxyacetophenone (105 mg; 0.5 mmol) and 6-iodo-2,2-dimethyl-3,4-dihydro-2H-chromene (144 mg; 0.5 mmol) to afford the title compound (57 mg; 32 %). R_f (Cyclohexane/EtOAc : 7/3) = 0.56. ^{1}H RMN: (δ ppm, CD_3Cl_3, 300 MHz): 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 (d, 1H, *J* = 8.1 Hz). ^{13}C RMN: (δ ppm, CD_3Cl_3, 75 MHz): 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. IR (cm^{-1}): 2973, 2937, 1579, 1496, 1451, 1410, 1384, 1346, 1260, 1124. 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)**

Compound **2j** was 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 the title compound (118 mg; 55 %). R_f (cyclohexane/EtOAc : 8/2) = 0.51. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 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 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = -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 (2). IR (cm^{-1}): 3417, 2937, 2837, 1579, 1506, 1460, 1411, 1346, 1281, 1254, 1124, 1005. 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*CA4, 2k)

To a solution of **2j** (73 mg; 0.17 mmol) in MeOH (10 mL) was added K_2CO_3 (34.5 mg; 0.25 mmol) and the mixture was stirred for 12 h at room temperature. The solution was washed with a saturated NaCl Solution (10 mL) and extracted with EtOAc (2 x 10 mL). The organic layers were combined, dried over $MgSO_4$, and concentrated. The residue was further purified by flash chromatography to afford the title compound **2k** (51 mg; 94%). R_f (cyclohexane/EtOAc : 8/2) = 0.21. mp 112 °C. ^{1}H NMR (δ ppm, 300 MHz, $CDCl_3$): δ = 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 (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 (2). IR (cm^{-1}): 3417, 2937, 2837, 1579, 1506, 1460, 1411, 1346, 1281, 1254, 1005. MS (ESI^+) *m/z* (%): 339 $[M+Na]^+$, 100. 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)

To a -100°C solution of 3-bromoquinoline (104 mg; 0.5 mmol) in Et_2O (15 mL) was slowly added *via* syringe, 625 μL (1 mmol) of a 1.6 M solution of *t*BuLi in pentane under nitrogen. After stirring for 45 min. at -78°C, 105 mg (0.5 mmol) of 3,4,5-trimethoxyacetophenone in toluene (5 mL) was added to the solution which was warmed to room temperature, and stirring was continued for 12 h. A saturated NH_4Cl solution (10 mL) was slowly added to the mixture to hydrolyze the adduct and the mixture was extracted with Et_2O (10 mL x 3). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The crude mixture and 4-(dimethylamino)pyridine DMAP (2.0 mmol) was next dissolved in CH_2Cl_2 (10 mL). Methanesulfonyl chloride (190mL, 2.45 mmol) was added via syringe. The mixture was stirred at room temperature for 1 h and poured into a saturated solution of sodium chloride. The two layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3 x 10 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated. The residue was next dissolved in CH_2Cl_2 (8 mL) and DBU (14 mmol) was added to the mixture which was refluxed for 3 h. After cooling, the mixture was poured into water, extracted with CH_2Cl_2 (3 x 8 mL). The organic layers were combined, dried over $MgSO_4$, and concentrated. The residue was further purified by flash chromatography to yield the title compound **2g** (58 mg; 36 %). R_f (Cyclohexane/EtOAc : 7/3) = 0.36. ^{1}H RMN: (δ ppm, CD_3Cl_3, 300 MHz): 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 (d, 1H, *J* = 2.1 Hz). ^{13}C RMN: (δ ppm, CD_3Cl_3, 75 MHz): 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. IR (cm^{-1}): 2927, 1730, 1575, 1503, 1464, 1447, 1410, 1368, 1347, 1324, 1283, 1177, 1002, 976, 957, 917, 862, 840. MS (ESI+, m/z, %): 341 $(M+Na)^+$, 100. 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)

To a solution of *Iso*CA4 (50 mg; 0.158 mmol) in acetone (5 mL) were added K_2CO_3 (62 mg; 0.632 mmol) and Me_2SO_4 (80 mg; 0.632 mmol). After stirring at room temperature for 12 h, the mixture was poured into H_2O (15 mL) and EtOAc (15 mL). The separated aqueous phase was extracted with EtOAc (3 x 15 mL). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated under reduced pressure to afford the title compound **2l** (42 mg; 80 %). R_f (CH_2Cl_2) = 0.63. ^{1}H RMN: (δ ppm, CD_3Cl_3, 300 MHz): 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 (m, 2H). ^{13}C RMN: (δ ppm, $CDCl_3$, 75 MHz): 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. IR (cm^{-1}): 2998, 2936, 2835,1730, 1679, 1579, 1506, 1452, 1411, 1330, 1248, 1235, 1221, 1173, 1122, 1025, 1005, 951, 889, 857, 845, 815, 766, 734. MS (ESI+, m/z, %): 353 $(M+Na)^+$, 100. 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)

Acetic anhydride (42 μL; 0.442 mmol) was added dropwise to a magnetically stirred solution of *iso*CA4 (31.5 mg; 0.316 mmol), pyridine (53 μL), DMAP (2 mg; 0.016 mmol) in CH_2Cl_2 (1 mL) maintained at 0°C. Stirring was continued for 1 h at this temperature, and H_2O (3 mL) was added to the reaction mixture. After extraction with CH_2Cl_2 (3 x 3 mL), the combined organic layers were dried over $MgSO_4$, and concentrated. The residue was further purified by flash chromatography to yield the desired compound (74 mg; 65 %). R_f (Cyclohexane/EtOAc: 7/3) = 0.44. ^{1}H RMN: (δ ppm, $CDCl_3$, 300 MHz): 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 (dd, 1H, *J* = 8.4 Hz, *J* = 2.1 Hz). ^{13}C RMN: (δ ppm, $CDCl_3$, 75 MHz): 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. IR (cm^{-1}): 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. MS (ESI+, m/z, %): 381 $(M+Na)^+$, 100. 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)

To a solution of *iso*CA4 (31.5 mg; 0.316 mmol) in CH_2Cl_2 (2 mL) were added successively, pyridine (54 µL) and diethylcarbamoyl chloride (86 mg; 0.632 mmol). Stirring was continued for 12 h at room temperature, and a saturated $NaHCO_3$ solution (5 mL) was added to the reaction mixture. After extraction with EtOAc (3 x 8 mL), the combined organic layers were dried over $MgSO_4$, and concentrated. The residue was further purified by flash chromatography to yield the title compound (57 mg; 50 %). R_f (CH_2Cl_2) = 0.15. mp 148°C. ^{1}H RMN: (δ ppm, $CDCl_3$, 300 MHz): 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 (m, 2H). ^{13}C RMN: (δ ppm, $CDCl_3$, 75 MHz): 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. IR (cm^{-1}): 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. MS (ESI+, m/z, %): 438 $(M+Na)^+$, 100. 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)

To a solution of *iso*CA4 (246 mg; 0.78 mmol) in pyridine (1 mL) was added SO_3-pyridine complex (75 mg; 0.47 mmol). After stirring for 24 h at room temperature, the mixture was hydrolyzed with H_2O (0.5 mL). After concentration under reduced pressure, the residue was purified by flash chromatography to afford the title compound **2o** (247 mg; 80 %. R_f (CH_2Cl_2/MeOH: 7/3) = 0.37. ^{1}H RMN: (δ ppm, $CDCl_3$, 300 MHz): 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 (d, 1H, *J* = 1.8 Hz). ^{13}C RMN: (δ ppm, $CDCl_3$, 75 MHz): 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 IR (cm^{-1}): 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. MS (ESI-, m/z, %): 395 $(M-H)^+$, 100. 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)

To a solution of *iso*CA4 (31.5 mg; 0.316 mmol) in CH_2Cl_2 (5 mL) were added successively, (72 mg; 0.376 mmol) of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI), DMAP (42 mg; 0.347 mmol) and Chlorambucil (106 mg; 0.347 mmol). Stirring was continued for 1 h at room temperature, and a saturated $NaHCO_3$ solution (3 mL) was added to the reaction mixture. After extraction with EtOAc (3 x 3 mL), the combined organic layers were dried over $MgSO_4$, and concentrated. The residue was further purified by flash chromatography to yield the desired compound (127 mg; 70 %. R_f (cyclohexane/EtOAc: 7/3) = 0.42. ^{1}H RMN: (δ ppm, $CDCl_3$, 300 MHz): 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 (dd, 1H, *J* = 8.7 Hz, *J* = 2.1 Hz). ^{13}C RMN: (δ ppm, $CDCl_3$, 75 MHz): 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. IR (cm^{-1}): 2934, 2839,1759, 1614, 1579, 1509, 1454, 1411, 1389, 1347, 1303, 1269, 1236, 1177, 1122, 1026, 1005, 958, 908, 845, 816, 770, 729. MS (ESI+, m/z, %): 624 $(M+Na)^+$, 100. 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-trimetho xyphenyl)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*CA4 (31.5 mg; 0.316 mmol) in CH_3CN (2 mL) containing KOH 1N (1.15 mL). After stirring for 12 h at room temperature, the mixture was hydrolyzed with HCl 1N (5 mL). After extraction with EtOAc (3 x 5 mL), the combined organic layers were dried over $MgSO_4$, and concentrated. The residue was further purified by flash chromatography to yield the desired compound (102 mg; 50 %. R_f (EtOAc) = 0.80. ^{1}H RMN: (δ ppm, $CDCl_3$, 300 MHz): 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 (d, 1H, *J* = 2.4 Hz). ^{13}C RMN: (δ ppm, $CDCl_3$, 75 MHz): 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. IR (cm^{-1}): 2939, 2840,1749, 1606, 1578, 1508, 1452, 1412, 1367, 1345, 1216, 1206, 1179, 1125, 1065, 1035, 956, 904, 845, 818, 780, 725, 702. MS (ESI+, m/z, %): 669.7 $(M+Na)^+$, 100. 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)

To a solution of **2q** (50.4 mg; 0.078 mmol) in dry MeOH (2 mL) was added a 28% NH_4Cl solution (8 mL). After stirring for 2 h at 60°C, the mixture was hydrolyzed with HCl 1N (5 mL). After extraction with EtOAc (3 x 10 mL), the combined organic layers were dried over $MgSO_4$, and concentrated. The residue was further purified by flash chromatography to yield the desired compound (34 mg; 90 %. R_f (CH_2Cl_2/MeOH: 8/2) = 0.12. mp 154-157°C. ^{1}H RMN: (δ ppm, $CDCl_3$, 300 MHz): 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 (d, 1H, *J* = 1.5 Hz). ^{13}C RMN: (δ ppm, $CDCl_3$, 75 MHz): 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. IR (cm^{-1}): 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. MS (ESI+, m/z, %): 501 $(M+Na)^+$, 100. Calcd for $C_{24}H_{30}O_{10}$: C 60.24, H 6.32, found: C 60.10, H 6.16.

Synthesis of 4a-4c

(4-Methoxy-3-nitrophenyl)-(3,4,5-trimethoxyphenyl)methanone (4a)

To a THF (18 mL) solution of 4-methoxy-3-nitroacetobenzaldehyde (2.54 g, 14 mmol) was added slowly at -78°C, a 0.7 N solution of 3,4,5-trimethoxybenzaldehyde (28 mL; 19.6 mmol). The reaction mixture was stirred for 1 h at room temperature until the disappearance of starting material, as judged by TLC. Then, the reaction was hydrolyzed at 0°C with a saturated NH_4Cl solution (20 mL) and extracted with EtOAc (3 x 15 mL). The combined organic layers were dried with $MgSO_4$ and evaporated to dryness. The crude alcohol was then dissolved in CH_2Cl_2 (30 mL) and pyridinium chlorochromate PCC (8.62 g; 40 mmol) was added by portions (15 mmol then 15 mmol after 1 h and 10 mmol after 2 h). The solution was stirred for a night at room temperature and filtered over SiO_2, and the solvent was removed in vacuo. The residue was further purified by flash chromatography to yield the desired compound (3.25 g; 67%). R_f (cyclohexane/EtOAc: 1/1) = 0.44. ^{1}H RMN: (δ

ppm, $CDCl_3$, 300 MHz): 3.88 (s, 6 H, 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 (d, 1H, *J* = 2.2 Hz).

(5-Methoxy-2-nitrophenyl)-(3,4,5-trimethoxyphenyl)methanone (4b)

Compound **4b** was prepared as for **4a** from 5-methoxy-2-nitroacetobenzaldehyde (2.54 g, 14 mmol) to afford the title compound **4b** (2.28g; 47%). R_f (cyclohexane/EtOAc: 6/4) = 0.54. mp 156°C. ^{1}H RMN: (δ ppm, $CDCl_3$, 300 MHz): 3.81 (s, 6 H, 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 (d, 1H, *J* = 9.3 Hz).

(4-Methoxy-2-nitrophenyl)-(3,4,5-trimethoxyphenyl)methanone (4c)

Compound **4c** was prepared as for **4a** from 4-methoxy-2-nitroacetobenzaldehyde (2.54 g, 14 mmol) to afford the title compound **4c** (2.91 g; 47%). Yield: 60%. R_f (cyclohexane/EtOAc: 6/4) = 0.52. mp 165°C. ^{1}H RMN: (δ ppm, $CDCl_3$, 300 MHz): 3.81 (s, 6 H, 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 (d, 1H, *J* = 2.7 Hz).

Synthesis of 2t-v

2,6-Dimethoxy[4-(4-methoxy-3-nitrobenzene)vinyl]anisole (2t)

To a 0°C cooled solution of methyltriphenylphosphonium bromide (1.07 g; 3 mmol) in THF (10 mL) was added slowly 3 mL of a 1N THF solution of LiHMDS (3 mmol). The yellow ylide solution was stirred at 0 °C for 1 h, then **4a** (520.5 mg; 1.5 mmol) in 10 mL of THF was slowly added *via* syringe. The resulting mixture was allowed to warm to room temperature and stirred further for 1 h. The solution was poured in H_2O (10 mL) and extracted with CH_2Cl_2 (2 x 10 mL). The organic layers were combined, dried over $MgSO_4$, and concentrated. The residue was further purified by flash chromatography to yield the desired compound **2t** (460 mg; 89 %). R_f (cyclohexane/EtOAc: 7/3) = 0.33. ^{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 (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 (2C). IR (cm^{-1}): 2939, 1619, 1579, 1529, 1504, 1469, 1412, 1354, 1275, 1239, 1184, 1119, 1016, 996, 954, 895. MS (APCI) *m/z* (%): 346 $[M+H]^+$, 100. 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)

Compound **2u** was prepared as for **2t** from **4b** to afford the title compound (279 mg; 54 %). R_f (cyclohexane/EtOAc: 6/4) = 0.47. mp 99°C. ^{1}H RMN: (δ ppm, $CDCl_3$, 300 MHz): 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 (d, 1H, *J* = 9.0 Hz). ^{13}C RMN: (δ ppm, $CDCl_3$, 75 MHz): 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. IR (cm^{-1}): 2939, 2838, 1576, 1507, 1461, 1414, 1339, 1294, 1235, 1184, 1125, 1063, 1027. MS (ESI+, m/z, %): 368 $(M+Na)^+$, 100. 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)

Compound **2v** was prepared as for **2t** from **4c** to afford the title compound (362 mg; 70 %). R_f (cyclohexane/EtOAc: 6/4) = 0.50. mp 123°C. ^{1}H RMN: (δ ppm, $CDCl_3$, 300 MHz): 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 (d, 1H, *J* = 2.7 Hz).^{13}C RMN: (δ ppm, $CDCl_3$, 75 MHz): 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. IR (cm^{-1}): 2937, 2838, 1619, 1579, 1528, 1504, 1461, 1412, 1343, 1300, 1266, 1234, 1184, 1123, 1064, 1029, 1005. MS (ESI+, m/z, %): 368 $(M+Na)^+$, 100. 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_2CA4, 2s)

Compound **2t** (86 mg; 0.25 mmol) and 98 mg of Zn (powder 98%, dust< 10 µM (1.5 mmol)) were dissolved in glacial AcOH (5 mL). The reaction mixture was then stirred for 1 h at room temperature. 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 water (10 mL) and the organic layer was dried over $MgSO_4$, and concentrated. The residue was further purified by flash chromatography to yield the desired compound (68.5 mg; 87%). R_f (Cyclohexane/EtOAc : 6/4) = 0.37. ^{1}H NMR (300 MHz, CD_3COCD_3): δ = 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 (d, 1H, *J* = 8.4 Hz). ^{13}C NMR^{1}H NMR (75 MHz, CD_3COCD_3): δ = 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 (2). IR (cm^{-1}): 3371, 2937, 2835, 1579, 1513, 1462, 1411, 1346, 1296, 1255, 1235, 1221, 1179, 1125, 1027, 1006. 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)

Compound **2w** was prepared as for **2s** from the reduction of **2u** to afford the title compound (55 mg; 70 %). R_f (cyclohexane/EtOAc: 5/5) = 0.39. ^{1}H RMN: (δ ppm, $CDCl_3$, 300 MHz): 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 (m, 2H). ^{13}C RMN: (δ ppm, $CDCl_3$, 75 MHz): 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 (2). IR (cm^{-1}): 3440, 3360, 2938, 2832, 1578, 1498, 1462, 1410, 1340, 1280, 1234, 1177, 1121, 1038, 1004. MS (ESI+, m/z, %): 338 $(M+Na)^+$, 100. 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)

Compound **2x** was prepared as for **2s** from the reduction of **2u** to afford the title compound (36 mg; 46 %). R_f (cyclohexane/EtOAc: 7/3) = 0.21. mp 148°C. ^{1}H RMN: (δ ppm, $CDCl_3$, 300 MHz): 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 (d, 1H, *J* = 8.4 Hz). ^{13}C RMN: (δ ppm, $CDCl_3$, 75 MHz): 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. IR (cm^{-1}): 3472, 3374, 2937, 2835, 1608, 1576, 1503, 1410, 1342, 1234, 1207, 1123, 1027, 1004. MS (ESI+, m/z, %): 338 $(M+Na)^+$, 100. 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, MD) and were cultured according to the supplier's instructions. Briefly, A549 lung carcinoma, MDA-MB-231, MDA-MB-435 cells were grown in Dulbecco minimal essential medium (DMEM) containing 4.5 g/L 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, Walkersville, MD, USA) and cultured according to the supplier's instructions. Briefly, HUVECs from three to six passages were subcultured to

confluence 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 TM reagent according to the manufacturer's instructions. Cells were seeded in 96-well plates (5 × 103 cells/well) containing 50 µL growth medium. After 24 h of culture, the cells were supplemented with 50 µL of the tested 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 (λex = 560 nm, λem = 590 nm) using a Victor microtiter plate fluorimeter (Perkin-Elmer,USA). The IC50 corresponds to the concentration of the tested 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 (the concentration of tubulin was about 2-3 mg/mL). 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 which decreased the maximum assembly rate of tubulin 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 CA4, 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, WI) according to the manufacturer's recommendations. Briefly, cells were subcultured on a 96-well plate with 5 × 10^4 cells/well in 100 µL medium. After 24 h of incubation, the medium in the 96-well plate was discarded and replaced with medium containing different concentrations of *iso*FCA4, *iso*CA4 and *iso*NH_2CA4 (1, 5, and 10 nM) or 0.1% DMSO (as 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 of 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. *Iso*FCA4, *iso*CA4, *iso*NH_2CA4 or vehicle were added to the formed cords and left for 3 h. Images were taken 3 h after the addition of compounds.

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