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B-Ring Modified *iso*Combretastatin A-4 Analogues Endowed with Interesting Anticancer Activities

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A novel class of *iso*combretastatin A-4 analogues with modifications on the 3'-position of the B-ring by replacement with C-substituents was studied. Exploration of the structure-activity relationships of theses analogues led to the identification of several compounds, which exhibited excellent antiproliferative activities at a nanomolar concentration against H1299, MDA-MB 231, HCT116 and K562 cancer cell lines, and inhibited tubulin polymerization similarly to *iso*CA-4. 1,1-Diarylethylenes **8** and **17** having on the 3'-position of the B-ring an (*E*)-propen-3-ol or propyn-3-ol substituent, respectively, proved to be the most active in this series. Both compounds led to the arrest of various cancer cell lines in the G_2/M phase of the cell cycle and strongly induced apoptosis. A docking of molecules **8** and **17** inside the colchicine binding site was performed and indicated that their C-3' substituents guided the B-cycle in a different manner to that observed for *iso*CA-4.

Introduction

Microtubules are hollow tubes consisting of alternating α- and β-tubulin heterodimers found in almost all eukaryotic cells. They are involved in a variety of cellular functions, such as mitosis and cell replication, cell motility, and cell maintenance. Their importance for cellular life, and especially mitosis, makes them one of the prominent targets for the development of anticancer agents, and for the treatment of solid tumors.[1] The dynamic process of assembly and disassembly of microtubules to tubulin is blocked by a variety of natural[2] or synthetic agents[3] that bind to distinct sites, such as binding sites for colchicines, paclitaxel and vinca alkaloids, resulting in mitotic arrest. Paclitaxel, for example, stabilizes microtubules and prevents their disassembly, whereas vincristine interacts with tubulin to inhibit its assembly. However, the problems of complex syntheses, high cytotoxicity associated with undesired side-effects, and lack of efficacy against multidrug resistance cancer cell lines encourage the development of new efficient antimitotic agents of low molecular weight. In this context, combretastatin A-4 (CA-4, **1a**) a natural stilbene, isolated in 1989 by Pettit[4] from the South African tree *combretum caffrum*, is one of most promising and more studied compounds (Figure 1). CA-4 strongly inhibited tumor cell growth, including multidrug resistant (MDR) cancer cell lines.[5] This molecule binds at the colchicine binding site and inhibits tubulin polymerization at a micromolar level, ultimately leading to apoptosis. Moreover, CA-4 also exerts highly selective effects in proliferating endothelial cells inducing irreversible vascular shutdown within solid tumors.[6] Disodium phosphate CA-4P[7] (**1b**) and AVE-8062[8] (**1c**), two water-soluble prodrug derivatives, are currently undergoing several advanced clinical trials for the treatment of age-related macular degeneration,[9] anaplastic thyroid cancer[10] and sarcoma.[11] However, the main problem associated with these stilbenes is the ready isomerization of the (*Z*)-double bond into its (*E*)-inactive form.[12] We have been actively engaged in searching novel tubulin inhibitors[13] and have recently reported the synthesis[14] and the biological evaluation of a new series of 1,1-diarylethylene derivatives with general structure **2**. We demonstrated that the bioisosteric replacement of the (*Z*)-1,2-ethylene by the 1,1-ethylene bridge resulted in retention of biological activities, solving *de facto* the (*Z*)-double bond isomerization problem. Results from these studies enabled identification of *iso*CA-4 (**2a**) as a lead compound, displaying the same activities as the natural product CA-4 (Figure 1).[13a],[13d]

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Further structure-activity relationships (SAR) studies, led us to discover two other substances, *iso*NH_2CA-4 (**2b**) and *iso*FCA-4 (**2c**), which manifest biological activities comparable to that of *iso*CA-4 (Figure 1).[14a]

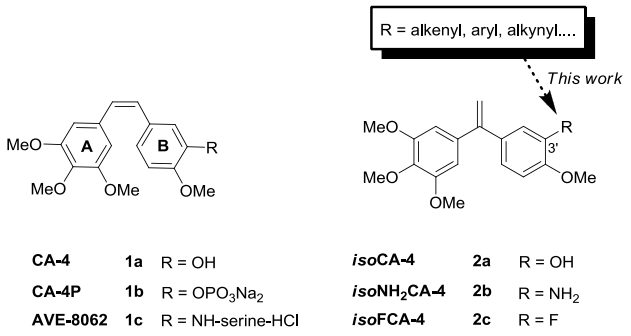


Figure 1. Rational drug design from CA-4 (**1a)** and *iso*CA-4 (**2a)** to 3'C-substituted *iso*CA-4 analogues.

Since the 3'-position of *iso*CA-4 is amenable to bioisosteric modifications, we decided to further explore, the insertion of suitable substituents that could confer interesting pharmacological properties to *iso*CA-4, and to evaluate the steric and electronic effects of these substituents on antiproliferative activity. In this paper, we describe the syntheses and biological activities of novel *iso*CA-4 derivatives bearing an alkenyl-, aryl-, and alkynyl-substituent on the 3'-position of the B-ring. Except in a previous report on a 3-aroyl indazole series where alkyne chains replace the OH group,[15] there are no reports disclosing similar modifications on the B-ring in the CA-4 series (introduction of 3'-Csp^2 and Csp-substituents), probably because isomerization of the (*Z*)-double bond may occur during the chemical manipulations.[16] In view of SAR studies, we have also prepared phenstatin[3c] derivatives **38** and **39** bearing on the 3'-position various alkynol chains. Finally, we synthesized and examined the replacement of the 3'OH-group of *iso*CA-4 with an azide, giving product **34** as a pivotal substrate for the generation of additional analogues **35**-**37** *via* the fashionable click-chemistry reaction. Potencies of newly synthesized compounds were evaluated using a primary cytotoxic screening assay against human colon carcinoma (HCT-116) cell lines. The most interesting derivatives were considered for further investigations, including cell proliferation against various cell lines, inhibition of tubulin polymerization (ITP), cellular cycle analysis, in addition to their ability to induce apoptosis.

Results and Discussion

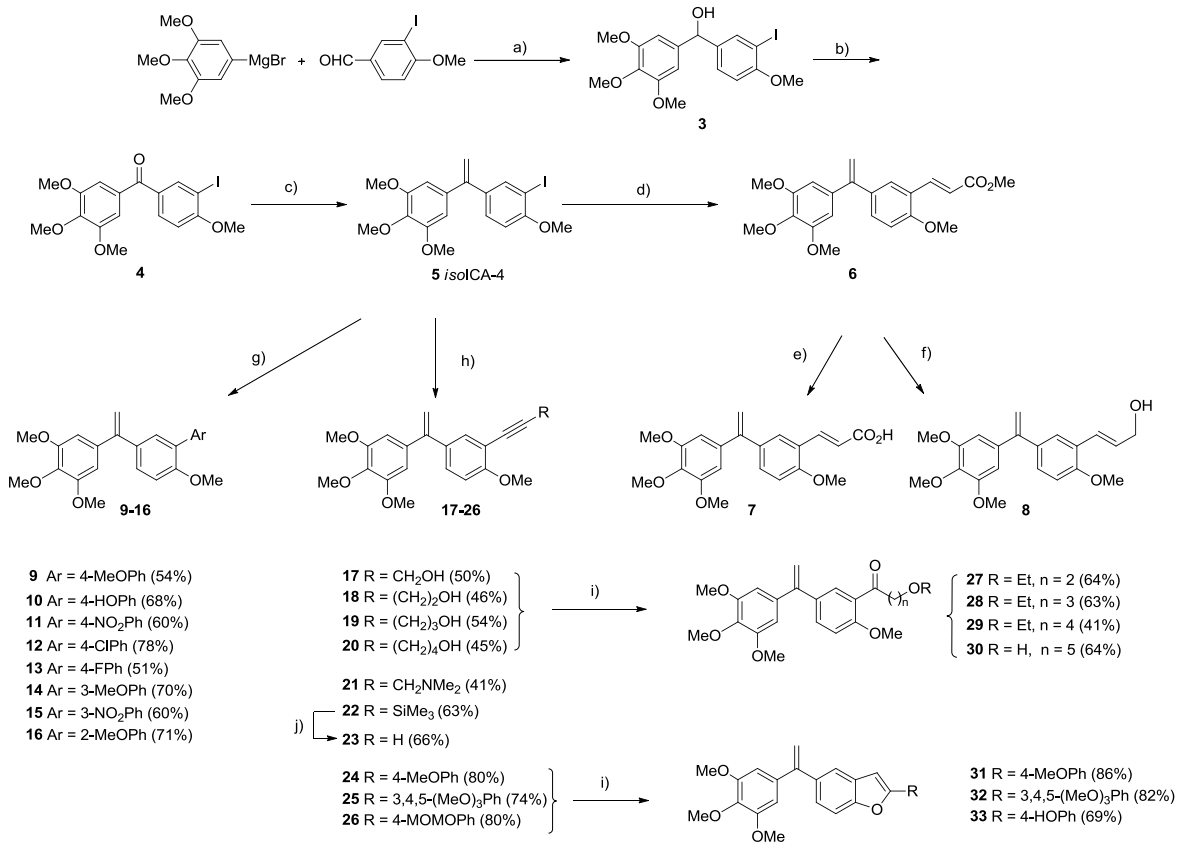
Chemistry

To achieve our goal, we initially focused on the synthesis of diarylethylene derivative **5** having an iodine atom on 3'-position as a valuable substrate in C-C bond-forming reactions through Pd-catalyzed reactions. As shown in Scheme 1, **5** was prepared in a three-step sequence by reaction of 3,4,5-trimethoxyphenylmagnesium bromide with 3-iodo-4-methoxybenzaldehyde in THF. The resulting alcohol **3** was converted to benzophenone **4** by treatment with PCC. Subsequent Wittig reaction of methyltriphenylphosphonium bromide with **4** in THF in the presence of LiHMDS to generate the ylide led to **5** in a good overall yield. With this key intermediate in hand, we subjected the C-I bond to Pd-catalyzed C-C bond formation under typical Heck, Suzuki and Sonogashira reaction conditions. Thus, reaction of **5** with methyl acrylate provided selectively 3'-methyl (*E*)-cinnamate derivative **6**, which underwent saponification under alkaline conditions to afford cinnamic acid **7** in a good yield. To preserve the two carbon-carbon double bonds of methyl cinnamate **6**, the reduction of the ester function was achieved with DIBAH at -78 °C and furnished (*E*)-allylic alcohol **8**. Next, to introduce an aryl substituent, we examined Suzuki couplings between various substituted arylboronic acids and **5**. The reactions proceeded smoothly in the presence of $Pd(PPh_3)_4$ and $NaHCO_3$ in DME-H_2O under reflux to give the corresponding coupling products **9**-**16** in satisfactory yields (51 to 78%).

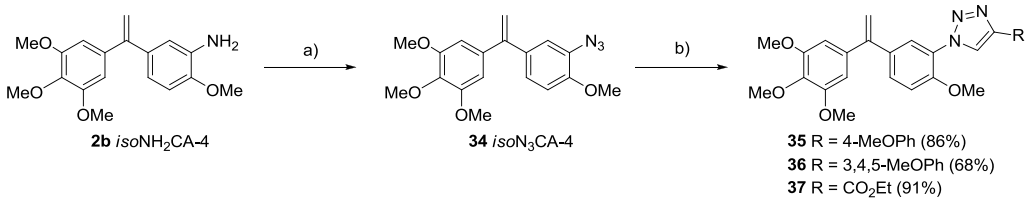
The synthesis of acetylenic *iso*CA-4 analogues was achieved from **5** using standard Sonogashira-Linstrumelle coupling reactions.[17] Accordingly, a small library of alkyne derivatives **17**-**26** was obtained in satisfactory to good yields. In order to evaluate the impact on biological activity of various modifications on the 3'-position, the triple bond of acetylenic *iso*CA-4 analogues provides a focal point for further structural manipulations. Thus, hydration of arylalkynols **17**-**20** was regioselectively achieved in the presence of a catalytic amount of *p*-toluensulfonic acid in boiling EtOH. Under this environmentally metal-free procedure, developed by our group,[18] arylketones **27**-**30** were obtained in good yields. As expected and previously reported by us,[18],[19] aliphatic arylalkynol substrates **17**-**19** having a propyn-3-ol, butyn-4-ol or pentyn-5-ol chain, were subsequently etherified by solvent to ether derivatives **27**-**29**. Under similar conditions,[19] *iso*CA-4 analogues **24**-**26** having an *o*-methoxydiarylalkyne moiety underwent a regioselective 5-*endo*-dig-cyclization[20] to give 2-aryl-substituted benzofurans **31**-**33** as single products in excellent yields.[21]

Finally, the synthesis of *iso*CA-4 analogues bearing a triazole ring at the 3'-position involved the preparation of the azide *iso*N_3CA-4 (**34**), which might also be used as photoaffinity labeling reagent for the colchicine site on *β*-tubulin.[16b],[22] Thus, reaction of *iso*ICA-4 with NaN_3 in the presence of CuI (10 mol%), DMEDA (15 mol%), potassium ascorbate (5 mol%) in DMSO-H_2O at various temperatures led to a mixture of the desired azide **34** together with *iso*NH_2CA-4 in variable ratios, despite extensive experimentation.[23] In order to optimize the yield of *iso*N_3CA-4, we exploited the Sandmeyer diazotization of *iso*NH_2CA-4 (**2b**) in the presence of $NaNO_2$ and NaN_3 under acidic medium. Accordingly, azide **34** was obtained in a good 85% yield. Further Huisgen cycloaddition with various terminal alkynes under standard conditions furnished triazole-substituted *iso*CA-4 analogues **35**-**37** in good to excellent yields (Scheme 2).

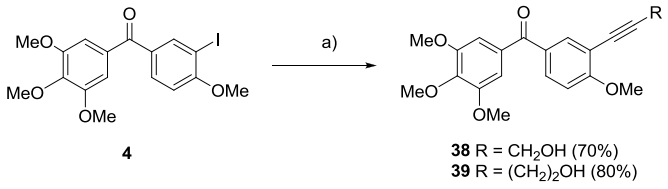
Because of the potent cancer cell line growth inhibition displayed by benzophenone derivatives, named phenstatins, we prepared for SAR studies, two phenstatin analogues **38** and **39** bearing on the 3'-position a propyn-3-ol and butyn-4-ol chain, respectively (Scheme 3).



Scheme 1. Reagents and conditions: a) THF, -78 to 20 °C, 90%; b) PCC, CH_2Cl_2, rt, 85%; c) $MePPh_3Br$, LiHMDS, THF, -78 °C, 90%; d) Methyl acrylate, $PdCl_2(PPh_3)_2$, Et_3N, DMF, 60 °C, 64%; e) NaOH, MeOH, rt, 70%; f) DIBAH, THF, -78 °C, 40%; g) $ArB(OH)_2$, $Pd(PPh_3)_4$, $NaHCO_3$, DME-H_2O, reflux; h) 1-Alkyne, $PdCl_2(PPh_3)_2$, CuI, Et_3N, THF; i) PTSA, EtOH, microwave, 170 °C; j) TBAF, THF, rt.



Scheme 2. Reagents and conditions: a) $NaNO_2$, HCl_{aq}, NaN_3, acetone, 0 °C, 85%; b) 1-Alkyne, $CuSO_4.5H_2O$, potassium ascorbate, *t*BuOH-H_2O, 70 °C.



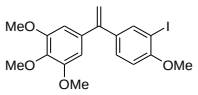
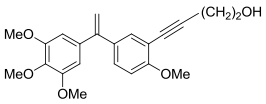
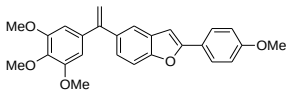
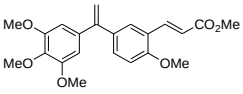
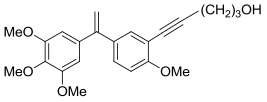
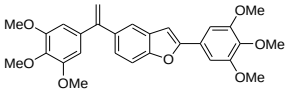
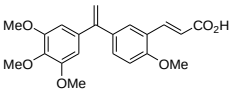
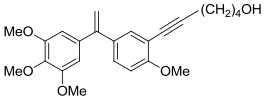
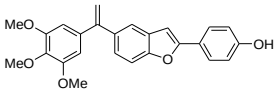
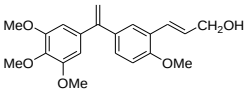
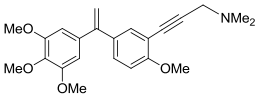
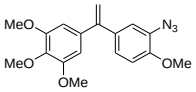
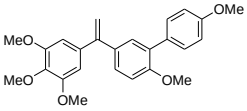
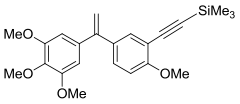
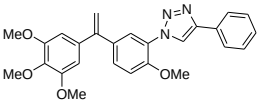
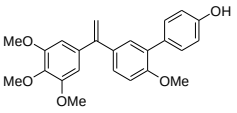
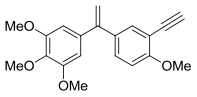
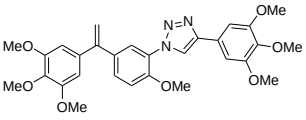
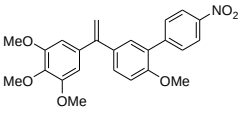
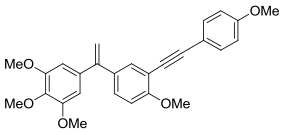
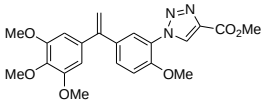
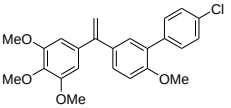
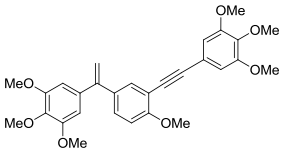
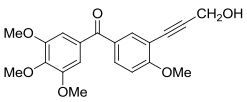
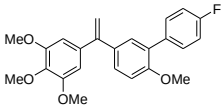
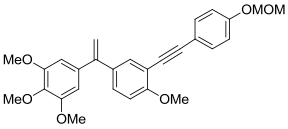
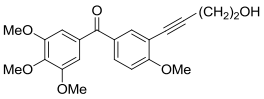
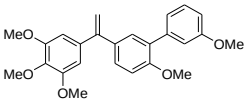
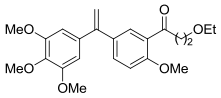
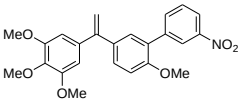
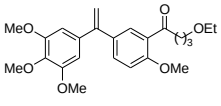
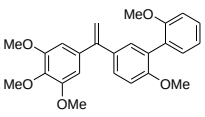
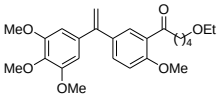
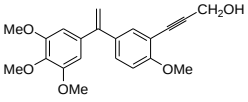
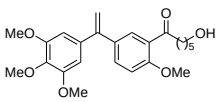
Scheme 3. Reagents and conditions: a) 1-Alkynol, $PdCl_2(PPh_3)_2$, CuI, Et_3N, THF.

Biology.

In vitro cell growth inhibition assay

All the synthesized compounds were tested in a preliminary growth inhibition assay on HCT-116 cells, a human colon carcinoma cell line, using CA-4 and isoCA-4 as reference compounds. The GI_{50} values (the drug concentration that reduced cell growth by 50%) of *iso*CA-4 analogues are listed in Table 1. The results presented in Table 1 clearly reveal that the size of the substituent on the 3'-position plays a critical role in cell growth inhibition. First, **5** was evaluated and showed a promising antiproliferative activity with GI_{50} = 300 nM. Next, 1,1-diarylethylene compounds **6**-**8** bearing 3'-alkenyl substituents on the B-ring exhibited significative antiproliferative activities with GI_{50} values ranging from 45 to 80 nM. Introduction of a series of substituted-benzene rings (**9**-**16**) on the 3'-position resulted in an important decrease in cytotoxicity, probably for steric considerations. Evaluation of acetylenic *iso*CA-4 analogues **17**-**26** was then examined. When small acetylenic chains were introduced onto the 3'-position, the resulting propargyl alcohol **17** and terminal arylalkyne **23** were found to be the most cytotoxic agents against HCT-116 cells (GI_{50} = 30 nM). Only a slight loss of activity was observed when the acetylenic chain was increased by one or two carbons (e.g.; compounds **18** and **19**). However, compounds bearing a longer alkynol chain **20** or having a propargyl amino function **21** resulted in significant reduction in cancer cell growth inhibition, whereas derivatives **24**-**26**, with arylalkyne substituents, were not active.

Table 1. Cytotoxic activity of *iso*CA-4 derivatives against HCT-116 cells.[a]

	Compounds	GI_{50} [nM][b]		Compounds	GI_{50} [nM]		Compounds	GI_{50} [nM]
5		300±28	**18**		70±5	**31**		4500±310
6		80±4	**19**		60±4	**32**		6500±380
7		80±6	**20**		300±31	**33**		2000±1500
8		45±3	**21**		500±42	**34**		72±5
9		300±31	**22**		730±61	**35**		3100±212
10		450±35	**23**		30±1	**36**		750±59
11		300±28	**24**		1500±95	**37**		1800±184
12		NC[c]	**25**		3000±290	**38**		780±61
13		3500±210	**26**		9000±580	**39**		225±15
14		3000±190	**27**		NC[c]	**1a**	**CA-4**	2±0.2[d]
15		750±60	**28**		NC[c]	**2a**	***iso*CA-4**	2±0.1[d]
16		3500±305	**29**		NC[c]			
17		30±2	**30**		NC[c]			

[a] HCT-116 human colon carcinoma. [b] 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). [c] GI_{50} value not calculated owing to the low activity of the compound. [d] The GI_{50} values for CA-4 and *iso*CA-4 were determined in this study.

These observations reveal, from one hand, that steric effects at the 3'-position of the B-ring influence cytotoxic activities and, on the other hand, that C-substitutions are very well tolerated.

Arylketone derivatives **27-30** in which the carbonyl is located at the C-3' are not cytotoxic. Benzofuran-containing *iso*CA-4 analogues **31-33** resulting from the cyclization of compounds **24-26** displayed a weak antiproliferative effect against HCT-116 cells. Triazole derivatives **35**-**37** exhibited only modest antiproliferative activity, whereas their azide precursor *iso*N_3CA-4 (**34**) had a significantly improved cytotoxicity (GI_{50} = 72 nM), supporting the idea that small C- or N-substituents, are welcome on the 3'-position. Finally, for comparison purposes, 3'-alkynol phenstatins (**38** and **39**) and their *iso*combretastatin homologues (**17** and **18**) were evaluated. The cytotoxic activity of **38** and **39** was considerably reduced (from 30 to 780 nM and 70 to 225 nM, respectively), emphasizing the increased therapeutic potency of *iso*combretastatin A-4 derivatives compared to their phenstatin homologues.

To characterize the cytotoxicity profiles of these compounds, we investigated the effect of the most active substances **6**, **7**, **8**, **17**, **18**, **19**, **23** and **34** (GI_{50}< 80 nM) on the proliferation of three other tumor cell lines: H1299 human non-small cell lung carcinoma, K562 myelogenous leukemia cancer cells and MDA-MB231 human hormone-independent breast cancer cells. The screening results revealed that all selected compounds strongly inhibited the growth of all examined tumor cell lines, and this effect did not depend on the cell type (Table 2). It is noteworthy that the GI_{50} values obtained with allylic alcohol **8** were comparable with those of CA-4 and *iso*CA-4 ranging between 2 and 5 nM. Interestingly, other modifications at the 3'-position with small C- substituents on the B-ring led to promising antiproliferative properties, particularly against HCT-116, K562 and H1299 cancer cell lines. Similarly, azide **34** was as active as its C-substituted congeners.

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

	Cytoxicity GI_{50} (nM)[a]				ITP IC_{50} (μM)[c]
Compound	HCT116[b]	K562[b]	H1299[b]	MDA-MB231[b]	
6	80±4	80±2	70±3	500±28	2.4±0.4
7	80±6	80±2	70±4	500±35	3.1±0.5
8	45±3	2±0.1	5±0.3	5±0.4	2.3±0.2
17	30±2	35±1	25±2	7±0.3	2.0±0.4
18	70±5	90±4	25±2	500±25	3.8±0.3
19	60±4	80±5	70±4	100±16	0.9±0.1
23	30±1	20±3	35±2	30±3	67±9
34 (*iso*N_3CA-4)	72±5	28±2	50±3	30±1	3.4±0.3
1a (CA-4)[d]	2±0.2	4±0.2	5±0.3	3±01	2±0.1
2a (*iso*CA-4)[d]	2±0.1	5±0.2	5±0.1	4±0.1	1.5±0.2

[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, human colon carcinoma; K562, myelogenous leukaemia; H1299, non-small cell lung carcinoma; MDA-MB-231 hormone-independent breast cancer. [c] 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). [d] The GI_{50} and IC_{50} values (cytotoxicity and ITP respectively) for CA-4 and *iso*CA-4 were determined in this study.

Inhibition of tubulin polymerization (ITP)

To investigate whether the antiproliferative activities of these compounds were related to the interaction with the microtubule system, they were evaluated for the inhibition of the assembly of tubulin. This was purified from sheep brains according to a slightly modified Guénard's protocol.[24] With the exception of terminal alkyne **23**, all selected compounds inhibited tubulin polymerization with IC_{50} values of 2 to 4 μM (Table 2). Although the antiproliferative activities of compounds **6**, **7**, **17**, **18**, **19** and **34** were slightly weaker than that of *iso*CA-4, their antitubulin activities are similar to those of the reference molecule. One can note that compound **8**, bearing on the 3'-position an allylic alcohol function was as active as *iso*CA-4, with respect to cytotoxicity and inhibition of tubulin polymerization. The interesting biological results obtained with compounds **8** and **17**, in which the 3'-OH group of *iso*CA-4 is replaced by an (*E*)-propen-3-ol or propyn-3-ol substituent, respectively, demonstrate that structural alteration at this part of *iso*CA-4 offers interesting premises for the design of novel and potent drugs for cancer therapy.

Cell Cycle Analysis and Apoptosis

Tubulin assembly inhibitors often cause alteration of cell cycle parameters, with preferential G_2/M blockade. Therefore, flow cytometry analysis was performed with our lead compounds **8** and **17** on H1299, MDA-MB231, HCT-116 and K562 cells and analyzed 24 h after treatment in the presence of increasing amounts of **8** and **17** (5, 10, and 50 nM).

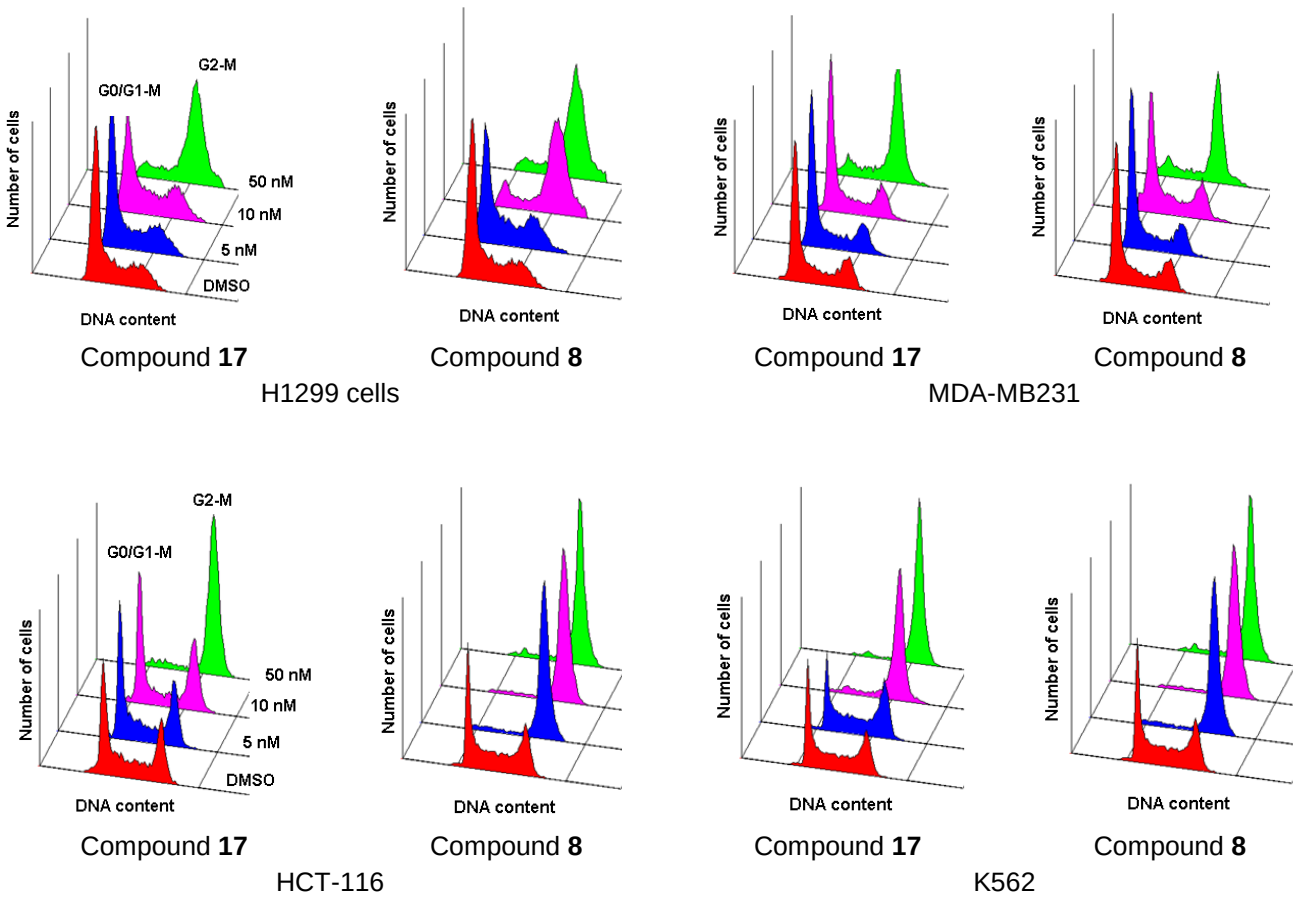
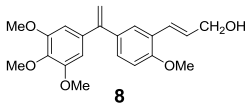


Figure 2. Effect of selected drugs **8** and **17** on cell cycle distribution in various cancer cell lines determined by flow cytometry analysis. DNA content was assessed via propidium iodide staining.

Analysis of Figure 2 revealed that these compounds caused a massive cell accumulation in the G_2/M phase when these drugs were used at a 50 nM concentration. At a lower concentration of 10 nM, compound **8** was still efficient with H1299, HCT116 and K562 cell lines and arrested the majority of cells in G_2/M phase of the cell cycle. It is to note that MDA-MB231 cells were slightly less sensitive to these drugs and their effect on the cellular cycle was noticeable only at a 50 nM concentration. On the contrary, at a low 5 nM concentration, both compounds **8** and **17** accumulated K562 cells at G_2/M. The observed effects of **17** and particularly of **8** on cell cycle progression with all the cancer cell lines correlated well with their strong antiproliferative and antitubulin activities (Tables 1 and 2). This result is in agreement with the similar properties reported previously for the majority of antimitotic agents.Cell-cycle arrest in the G_2/M phase is frequently followed by DNA fragmentation and other morphological features of apoptosis. Therefore, we investigated the effect of compounds **8** and **17** at various concentrations (5, 10, and 50 nM) on the induction of apoptosis in HCT-116, MDA-MB231, and H1299 cancer cells using standard assays for caspases 3 and 7.[25] 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 two studied substances. Figure 3 reveals that allylic alcohol **8** is more prone at inducing apoptosis than its propargylic analogue **17**. One can note that these two apoptotic agents were very efficient against all the tested cancer cell lines with notably, a significative efficiency against H1299 cells at very low concentrations (from 5 to 10 nM). These results clearly indicate that, in addition to their antiproliferative and antitubulin effects, the treatment of cancer cells with these drugs **8** and **17**, activate caspase systems leading to programmed cell death.

To rationalize the binding modes of this new series of *iso*CA-4 analogues inside the tubulin binding site, docking studies were carried out with the most active molecules **6**, **8**, **17** and **18**. For this purpose, the X-ray structure of tubulin cocrystallized with *N*-deacetyl-*N*-(2-mercaptoacetyl)colchicine (DAMA-colchicine, PDB: 1sa0)[26] was used. Taking in consideration the big size of our new tubulin ligands due to their C3'-substituents, we envisioned another binding mode that could accommodate these molecules into the colchicine binding site. Figure 4a illustrates molecules **6**, **8**, **17** and **18** docked in the colchicine-binding site of tubulin, and all of them overlap well. Moreover, the binding of these compounds to tubulin was stabilized by hydrogen bonding interactions with Cys241 (residue numbering derived from the crystal structure). The results of our docking studies, in which the trimethoxyphenyl ring of selected compounds is well positioned in the vicinity of Cys241, are consistent with previous reports concerning DAMA-colchicine[26] and CA-4 analogues[27] that displayed strong antitubulin activities. However, as observed in Figure 4b, compounds **6**, **8**, **17** and **18** showed a binding pose different to the one observed with the co-crystallized DAMA-colchicine as well as *iso*CA-4, and clearly docked in another pocket. On the contrary to previous works where the 3'OH-substituent belonging to CA-4 analogues showed a strong

hydrogen bond with the backbone nitrogen of Val181, this hydrogen bond was completely lost with compounds **6**, **8**, **17** and **18** having an alkenyl or alkynyl substituent on the C3' position.



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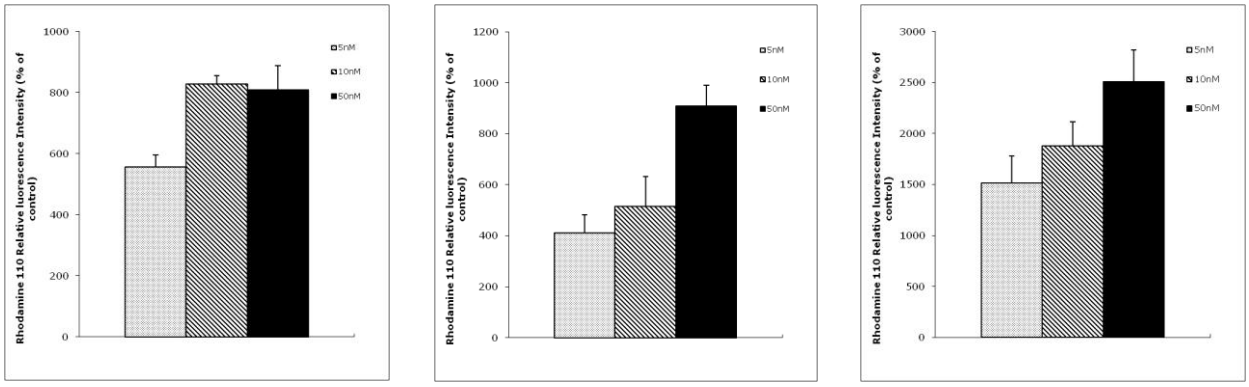
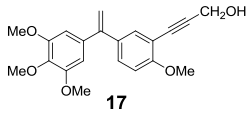


HCT116 **MDA-MB231** **H1299**



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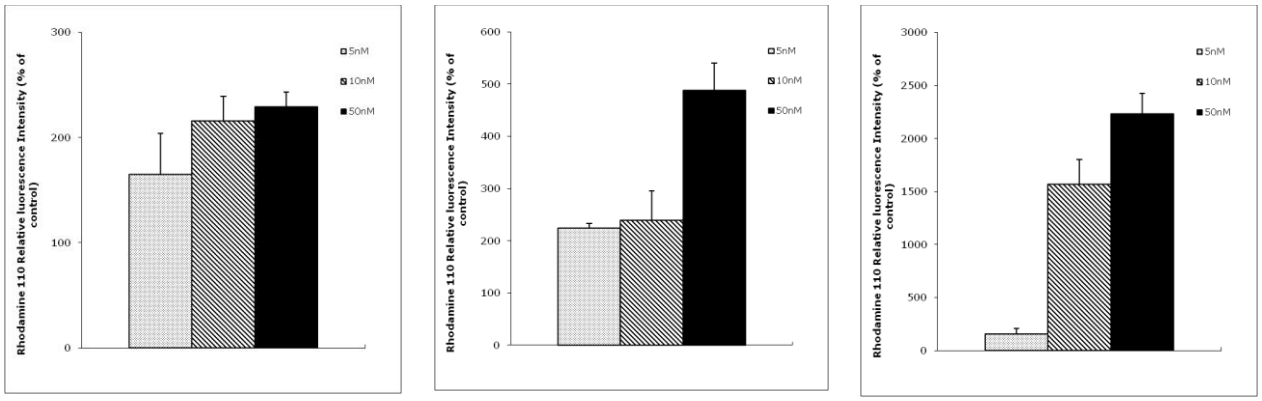


HCT116 **MDA-MB231** **H1299**

Figure 3. Apoptotic effects of selected compounds **8** and **17** in HCT-116, MDA-MB231 and H1299 cells. Results are expressed as a percentage of apoptotic cells detected following 24 h of treatment with **8** and **17** at different concentrations as indicated

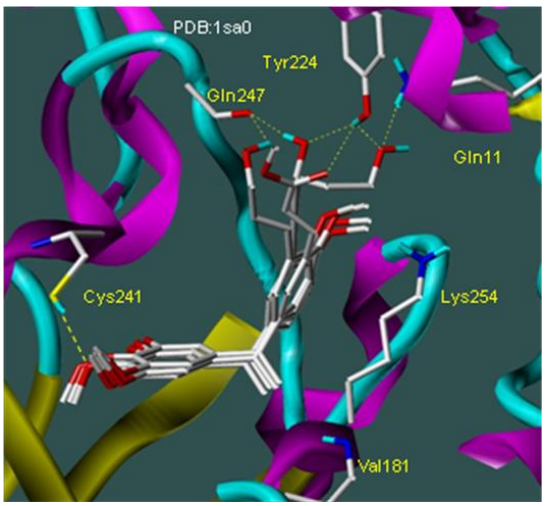


Figure 4a. Superimposition of molecules **6**, **8**, **17** and **18** into the colchicine binding site.

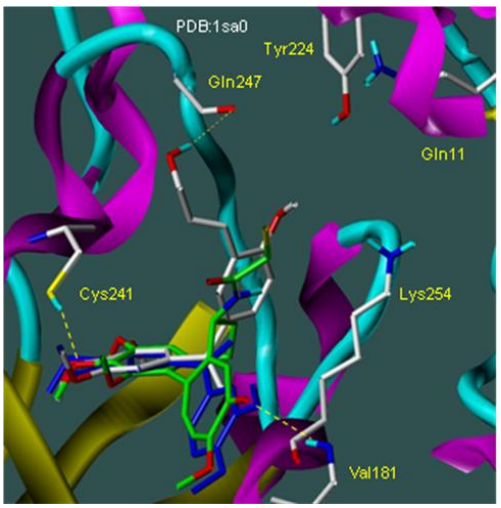
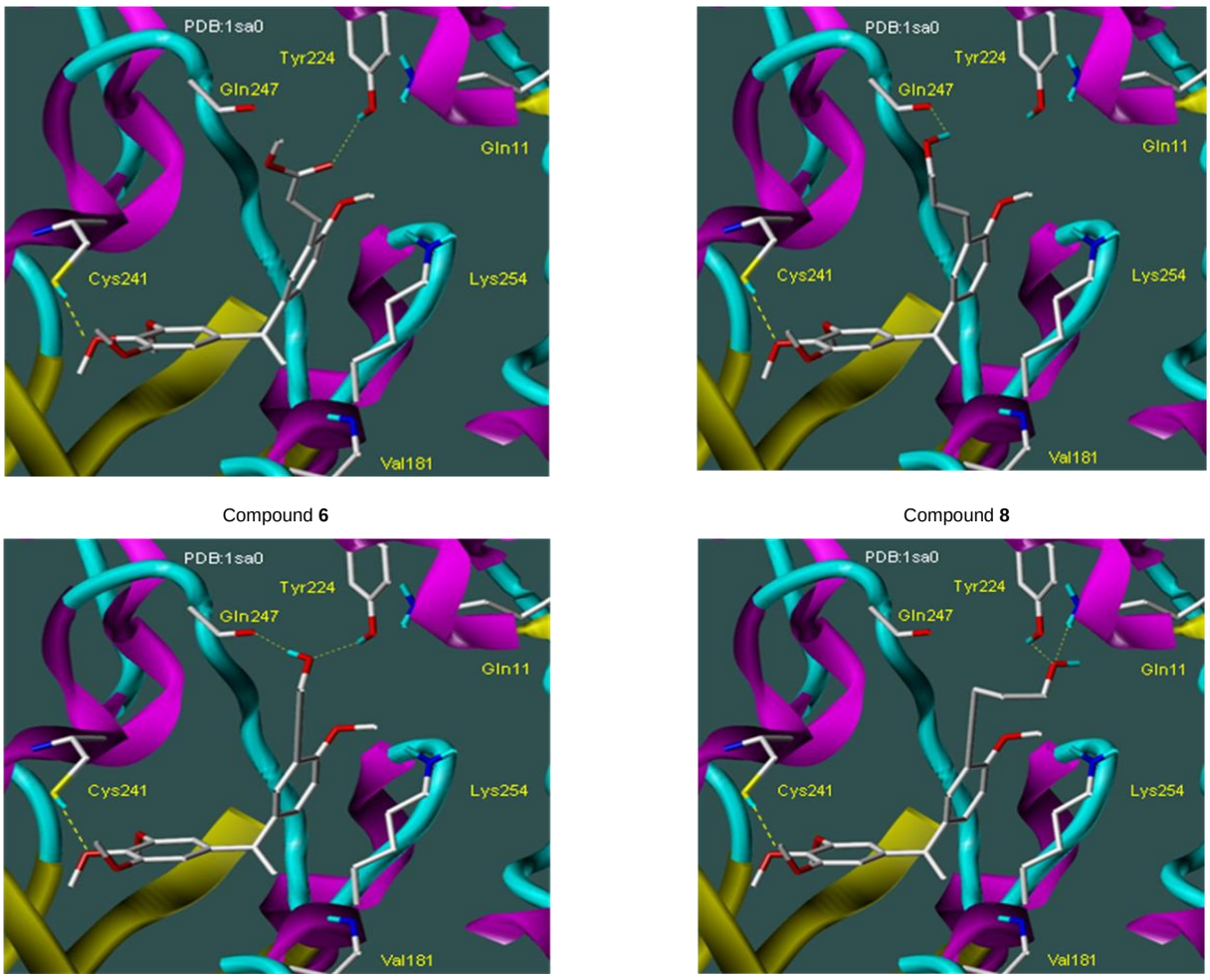


Figure 4b. Superimposition of **8** (silver), DAMA-colchicine (green) and *iso*CA-4 (blue) in the colchicine binding site.

Although the trimethoxyphenyl moiety remains anchored at the same place in proximity of Cys241, a polar cavity formed by Gln247, Tyr224, Gln11 and Lys254 accommodates the C3'-substituents of the B-ring in molecules **6**, **8**, **17** and **18**. In detail, **6** forms a hydrogen bond with Tyr224, and **8** with the backbone carbonyl of Gln247, whereas **17** and **18** form a double hydrogen

bond with Gln247 and Tyr224, and with Tyr224 and Gln11, respectively (Figure 5). Moreover, the 4'-methoxy group of each molecule attached to the B-ring is seen to establish an additional extra hydrogen bond with Lys254 (not displayed in the image). These original binding modes may rationalize the potency observed for our molecules in their inhibition of tubulin polymerization. From all of these considerations, we are planning to rationalize the synthesis of new *iso*CA-4 analogues in order to achieve better interactions with this polar cavity formed by Gln247, Tyr224, Gln11 and Lys254.



Compound **6** Compound **8**

Compound **17** Compound **18**

Figure 5: Presumptive docked pose of **6**, **8**, **17** and **18** in colchicine binding site of tubulin with significant binding residue Cys241, Gln247, Tyr224, Gln11 and Lys254.

Conclusion

In continuing experiments to optimize the *iso*CA-4 skeleton, we have synthesized and biologically evaluated a novel series of 1,1-diaryl compounds in which the 3'-OH moiety of *iso*CA-4 was replaced with various C-substituents (e.g.; aryl, alkenyl, alkynyl,…). From these SAR studies, compounds **8** and **17**, bearing an allylic and a propargylic alcohol function on the C-3' position, respectively, were found to display the most potent antiproliferative activity against a panel of cancer cell lines at a nanomolar level. The investigation of the mechanism of action by which these compounds exert their antiproliferative activity revealed that compounds **8** and **17** were potent inhibitors of tubulin polymerization with IC_{50} of 2 µM. In addition, they affected cell cycle progression of H1299, MDA-MB 231, HCT-116 as well as K562 cancer cells, and resulted in cell cycle arrest in the G_2/M phase. *Iso*CA-4 derivatives **8** and **17** have also been characterized as strong apoptotic agents at low concentrations by inducing cleavage of pro-caspases-3 and 7 in H1299, MDA-MB 231 and HCT-116 cells. These findings enlarge the knowledge of the SAR of the 1,1-diarylethylene derivatives, which are different than those displayed by the related phenstatin compounds. Modeling studies indicate a possible original binding-mode for these inhibitors of tubulin polymerization. It was showed that the binding of these compounds to *β*-tubulin was directed by their C-3' substituents and was stabilized by hydrogen-bonding interactions in a new polar cavity formed with Cys241, Gln247, Tyr224, Gln11 and Lys254. This observation, the easy synthetic access and the excellent biological results obtained with structural changes at C-3' position of *iso*CA-4 demonstrate that modifications at this portion are promising for the design of novel and potent *iso*CA-4 analogues.

Experimental Section

Chemistry

Melting points (m.p.) were recorded on a Büchi B-450 apparatus and were uncorrected. NMR spectra were performed on a Bruker AVANCE 300. Chemical shifts δ are in ppm, and the following abbreviations are used: singlet (s), (br s), broad singlet, doublet (d), triplet (t), multiplet (m). 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). 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 sulfate ($MgSO_4$) or sodium sulfate (Na_2SO_4).

5-(1-(3-Iodo-4-methoxyphenyl)vinyl)-1,2,3-trimethoxybenzene (5): To a THF solution of triphenylmethylphosphonium bromide (334 mg, 0.9 mmol) was slowly added 0.88 mL of a 1.06 M solution of LiHMDS (0.9 mmol) at 0°C. The reaction mixture was stirred at this temperature for 1 h. Then, **4** (200 mg, 0.5 mmol) in THF (20 mL) was added to the ylide solution and the resulting mixture stirred at 0°C for 1 h. The reaction mixture was poured into water (15 mL) and extracted with CH_2Cl_2. The combined extracts were dried ($MgSO_4$) and concentrated. Purification by flash chromatography on silica gel afforded **5** as a pale yellow oil (192 mg, 90 %); R_f = 0.50 (CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 4.07 (s, 6H), 4.13 (s, 3H), 4.15 (s, 3H), 5.60 (m, 2H), 6.70 (s, 2H), 7.03 (dd, *J* = 8.5, 1.7 Hz, 1H), 7.53 (d, *J* = 8.5 Hz, 1H), 8.06 (d, *J* = 1.7 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 56.1 (2C), 56.4, 60.9, 85.7, 105.5 (2C), 113.4, 110.2, 129.5, 135.7, 136.9, 137.8, 139.0, 148.2, 152.9 (2C), 157.7; IR = 2939; 1583, 1490, 1461, 1263, 1126, 732 cm^{-1}; MS (APCI+, m/z): 427 $[M+H]^+$; Anal. Calcd (%) for $C_{18}H_{19}IO_4$: C 50.72, H 4.49, found: C 50.52, H 4.31.

(*E*)-Methyl 3-(2-methoxy-5- (1- (3,4,5-trimethoxyphenyl) vinyl) phenyl)acrylate (6): To a solution of **5** (0.5 mmol) in DMF (1.5 mL) were slowly added at room temperature, $PdCl_2(PPh_3)_2$ (0.02 mmol), Et_3N (0.3 mL), and methylacrylate (0.4 mL, 4.7 mmol). The resulting mixture was stirred at 60°C for 72 h. After cooling, 40 mL of EtOAc were added to the crude mixture which was washed with a saturated NH_4Cl solution. After extraction with CH_2Cl_2, the combined extracts were dried ($MgSO_4$) and concentrated. Purification by flash chromatography on silica gel afforded **6** as a colorless oil (123 mg, 64 %). R_f = 0.18 (CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 3.79 (s, 3H), 3.81 (s, 6H), 3.89 (s, 3H), 3.92 (s, 3H), 5.37 (m, 2H), 6.52 (d, *J* = 16.1 Hz, 1H), 6.53 (s, 2H), 6.89 (d, *J* = 8.6 Hz, 1H), 7.34 (dd, *J* = 8.6, *J* = 2.2 Hz, 1H), 7.51 (d, *J* = 2.2 Hz, 1H), 7.97 (d, *J* = 16.1 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 51.6, 56.1 (2C), 60.9, 105.5 (2C), 110.8, 113.1, 118.7, 123.0, 128.8, 131.3, 133.8, 137.0, 137.9, 140.1, 149.0, 152.9 (2C), 158.1, 167.8, (one C missing); IR: 2944, 1838, 1714, 1633, 1500, 1251, 1169, 1125 cm^{-1}. MS (APCI+, m/z): 385 $[M+H]^+$. Anal. Calcd for $C_{22}H_{24}O_6$: C 68.74, H 6.29, found C 68.66, H 6.21.

(*E*)-3-(2-Methoxy-5-(1-(3,4,5-trimethoxyphenyl)vinyl)phenyl) acrylic acid (7): Compound **6** (0.1 mmol) in MeOH (2 mL) was stirred with 0.12 mL of a NaOH (3N) solution for 3 h under reflux. After concentration *in vacuo*, the crude was washed with a saturated NH_4Cl solution and extracted with CH_2Cl_2. The combined organic extracts were dried ($MgSO_4$) and concentrated. Purification by flash chromatography on silica gel afforded **7** as a pale yellow oil (26 mg, 70 %); R_f = 0.15 (CH_2Cl_2 / EtOAc: 9/1); ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 3.81 (s, 6H), 3.90 (s, 3H), 3.91 (s, 3H), 5.38 (m, 2H), 6.52 (d, *J* = 16.0 Hz, 1H), 6.54 (s, 2H), 6.90 (d, *J* = 8.5 Hz, 1H), 7.35 (dd, *J* = 8.5, 2.0 Hz, 1H), 7.52 (d, *J* = 2.0 Hz, 1H), 7.98 (d, *J* = 16.0 Hz, 1H), OH not seen; ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 55.6, 56.2 (2C), 60.9, 105.6 (2C), 110.9, 113.2, 118.7, 123.0, 128.9, 131.4, 133.8, 137.1, 140.2, 149.1, 152.9 (3C), 158.1, 167.9; IR: 2953, 1658, 1635, 1502, 1241, 1122 cm^{-1}. MS (APCI+, m/z): 371 $[M+H]^+$. Anal. Calcd for $C_{21}H_{22}O_6$ C 68.10, H 5.99, found C 67.89, 5.75;

(*E*)-3-(2-Methoxy-5-(1-(3,4,5-trimethoxyphenyl)vinyl)phenyl) prop-2-en-1-ol (8): To a solution of **6** (254 mg, 0.664 mmol) in THF was slowly added at -78°C, 0.89 mL of a 1.5M solution of DIBAH in CH_2Cl_2. After 30 min of stirring, the solution was poured into a saturated sodium tartrate in the presence of 0.1 mL of EtOAc. The mixture was stirred for 13 h at room temperature and extracted with Et_2O. The combined organic extracts were dried ($MgSO_4$) and concentrated. Purification by flash chromatography on silica gel afforded **8** as a pale yellow oil (95 mg, 40 %); R_f = 0.38 (Cyclohexane / EtOAc 5 / 5); ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 3.80 (s, 6H), 3.86 (s, 3H), 3.88 (s, 3H), 4.31 (d, *J* = 5.8 Hz, 2H), 5.31 (m, 1H), 5.40 (m, 1H), 6.36 (dt, *J* = 16.0 Hz, 5.8 Hz, 1H), 6.55 (s, 2H), 6.83 (d, *J* = 8.5 Hz, 1H), 6.91 (d, *J* = 16.0 Hz, 1H), 7.21 (dd, *J* = 8.5, 1.4 Hz, 1H), 7.44 (d, *J* =1.4 Hz, 1H), OH not seen; ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 55.4, 56.0 (2C), 60.8, 64.0, 105.5 (2C), 110.3, 112.6 156.5, 125.2, 125.8, 126.9, 128.6, 129.6, 133.6, 137.3,137.6, 149.4, 152.7 (2C); IR: 2943, 2368, 1580, 1500, 1462, 1412, 1345, 1245, 1126, 1007, 845 cm^{-1}. MS (APCI+, m/z): 357 $[M+H]^+$. Anal. Calcd for $C_{21}H_{24}O_5$ C 70.77, H 6.79, found C 70.50, H 6.69.

General procedure for Suzuki couplings: To a solution of ***5*** (1 mmol) in DME (2 mL) were added at room temperature, 2.5 mmol of the required boronic acid, $NaHCO_3$ (5 mmol), 0.4 mL of H_2O and $Pd(PPh_3)_4$ (0.06 mmol). The mixture was stirred for 18 h under reflux. After cooling, the solution was poured into a saturated NH_4Cl solution (15 mL) and extracted with CH_2Cl_2. The combined extracts were dried ($MgSO_4$) and concentrated. Purification by flash chromatography on silica gel afforded compounds **9-16**.

2,4'-Dimethoxy-5-[1-(3,4,5-trimethoxyphenyl)vinyl]biphenyl (9): Colorless oil; Yield (219 mg, 54 %); R_f = 0.47 (CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 3.84-3.86 (m, 9H), 3.87 (s, 3H), 3.88 (s, 3H), 5.38 (m, 2H), 6.60 (s, 2H), 6.92-6.97 (m, 3H), 7.27-7.30 (m, 1H), 7.33 (d, *J* = 2.4 Hz, 1H), 7.46 (d, *J* = 9.0 Hz, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 55.3, 55.7, 56.2 (2C), 60.9, 105.2, 105.7 (2C), 110.8, 112.7, 112.5 (2C), 133.8, 137.5 (2C), 128.1, 129.9, 130.6 (2C), 149.5, 152.9 (3C), 156.3, 158.8; IR: 2937, 2836, 1648, 1494, 1412, 1331, 1265, 1244, 1177, 1124 cm^{-1}; MS (APCI+, m/z): 409 $[M+H]^+$; Anal. Calcd for $C_{25}H_{26}O_5$: C 73.87, H 6.45 found C 73.50, H 6.29.

2'-Methoxy-5'-[1-(3,4,5-trimethoxyphenyl)vinyl]biphenyl-4-ol (10): Brown oil; Yield (251. mg, 68 %); R_f = 0.33 (Cyclohexane/EtOAc 7/3); ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 3.82 (s, 6H), 3.83 (s, 3H), 3.87 (s, 3H), 4.93 (s, 1H), 5.37 (m, 2H), 6.58 (s, 2H), 6.86 (d, *J* = 8.7 Hz, 2H), 6.92 (d, *J* = 8.5 Hz, 1H), 7.28-7.31 (m, 2H), 7.40 (d, *J* = 8.7 Hz, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 55.7, 56.2 (2C), 60.9, 105.7 (2C), 110.8, 112.7, 115.0 (2C), 128.1, 129.9, 130.5, 130.6, 130.8 (2C), 133.8, 137.5, 149.5, 152.8 (3C), 154.9, 156.3; IR: 3428, 2938, 1580, 1502, 1236, 1126 cm^{-1}; MS (APCI+, m/z): 393 $[M+H]^+$; Anal. Calcd for $C_{25}H_{26}O_5$: C 73.87, H 6.45 found C 73.61, H 6.28.

2-Methoxy-4'-nitro-5-[1-(3,4,5-trimethoxyphenyl)vinyl]-biphenyl (11): Yellow oil; Yield (236 mg, 56 %); R_f = 0.25 (CH_2Cl_2 / Cyclohexane 5 / 5) ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 3.76 (s, 6H), 3.80 (s, 3H), 3.81 (s, 3H), 5.32 (m, 2H), 6.51 (s, 2H), 6.91 (d, *J* = 8.3 Hz, 1H), 7.19 (s, 1H), 7.29 (m, 1H), 7.62 (d, *J* = 8.0 Hz, 2H), 8.18 (d, *J* = 8.0 Hz, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 55.7, 56.2 (2C), 60.9, 105.7 (2C), 111.1, 113.2, 123.3 (2C), 128.0, 130.0, 130.4 (3C), 134.2, 137.1, 145.2, 149.1 (2C), 153.0 (3C), 156.2; IR : 2934, 1731, 1579, 1343, 1235, 1126 cm^{-1}; MS (APCI+, m/z): 422 $[M+H]^+$; Anal. Calcd for $C_{24}H_{23}NO_6$: C 68.40, H 5.50, N 3.32, found C 68.21, H 5.32, N 3.21.

4'-Chloro-2-methoxy-5-[1-(3,4,5-trimethoxyphenyl)vinyl]-biphenyl (12): Colorless oil; Yield (320 mg, 78 %); R_f = 0.67 (Cyclohexane / EtOAc 7 / 3); ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 3.78 (6H), 3.81 (s, 3H),

3.88 (s, 3H), 5.37 (m, 2H), 6.56 (s, 2H), 6.94 (m, 1H), 7.27-7.38 (m, 4H), 7.45 (d, *J* = 8.4 Hz, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 55.7, 56.2 (2C), 60.9, 105.7 (2C), 110.8, 112.9, 128.2 (2C), 128.9, 129.1, 130.5, 130.8 (2C), 133.0, 133.9, 136.7, 149.3, 137.3, 152.9 (3C), 156.2; IR: 2952, 1589, 1510, 1465, 1421, 1125, 732 cm^{-1}; MS (APCI+, m/z): 433, 435 $[M+Na]^+$; Anal. Calcd for $C_{24}H_{23}ClO_4$: C 70.15, H 5.64, found C 69.99, H 5.51.

4'-Fluoro-2-methoxy-5-[1-(3,4,5-trimethoxyphenyl)vinyl]-biphenyl (13): Colorless oil; Yield (201 mg, 51 %); R_f = 0.41 (Cyclohexane / EtOAc 8 / 2); ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 3.82-3.88 (m, 12H), 5.38 (m, 2H), 6.59 (s, 2H), 6.95 (d, *J* = 9.0 Hz, 1H), 7.06-7.11 (m, 2H), 7.29-7.32 (m, 2H), 7.46-7.51 (m, 2H); ^{19}F NMR ($CDCl_3$, 188 MHz, decoupled): δ = -115.4; ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 55.7, 56.2 (2C), 60.9, 105.7 (2C), 110.8, 112.8, 114.8, 115.0, 128.6, 129.3, 130.6, 131.0, 131.1, 133.9, 134.2, 137.4, 149.4 (2C), 152.9 (3C) 156.2; IR: 1583, 1509, 1463, 1414, 1333, 1265, 1128, 732 cm^{-1}; MS (APCI+, m/z): 397 $[M+2H]^+$; Anal. Calcd for $C_{24}H_{23}FO_4$: C 73.08, H 5.88, found C 72.88, H 5.71.

2,3'-Dimethoxy-5-[1-(3,4,5-trimethoxyphenyl)vinyl]biphenyl (14): Yellow oil; Yield (284 mg, 70 %); R_f = 0.60 (Cyclohexane / EtOAc 7 / 3); ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 3.77-3.79 (m, 12H), 3.83 (s, 3H), 5.33 (m, 2H), 6.54 (s, 2H), 6.81-7.03 (m, 1H), 7.05 (d, *J* = 6.6 Hz, 1H), 7.03-7.06 (m, 2H), 7.20-7.31 (m, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 55.2, 55.7, 56.1 (2C), 60.9, 105.7 (2C), 110.8, 112.4, 112.7, 115.4, 122.0, 128.5, 128.9, 130.1, 130.7, 133.7, 137.4, 137.8, 139.7, 149.4, 152.9 (2C), 156.3, 159.2; IR: 2939, 2835, 1578, 1503, 1343, 1235, 1124 cm^{-1}; MS (APCI+, m/z): 407 $[M+H]^+$; Anal. Calcd for $C_{25}H_{26}O_5$: C 73.87, H 6.45, found D 73.52, H 6.20.

2-Methoxy-3'-nitro-5-[1-(3,4,5-trimethoxyphenyl)vinyl]-biphenyl (15): Yellow oil; Yield (60 %); R_f = 0.19 (Cyclohexane / CH_2Cl_2 5 / 5); ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 3.83 (s, 6H), 3.87 (s, 3H), 3.88 (s, 3H), 5.40 (m, 2H), 6.59 (s, 2H), 6.99 (d, *J* = 7.9 Hz, 1H), 7.36 (m, 2H), 7.56 (t, *J* = 7.9 Hz, 1H), 7.82-7.86 (m, 1H), 8.15-8.19 (m, 1H), 8.41 (t, *J* = 1.9 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 55.7, 56.2 (2C), 60.9, 105.7, 111.0 (2C), 113.2, 121.9, 124.6, 127.8, 128.9, 129.8, 130.4, 134.2, 135.6, 137.1, 140.0, 148.1, 149.1, 153.0 (3C),156.2; IR: 2939, 1578, 1528, 1503, 1348, 1234, 1126 cm^{-1}; MS (APCI+, m/z): 422 $[M+H]^+$; Anal. Calcd for $C_{24}H_{23}NO_6$: C 68.40, H 5.50, N 3.32, found C 68.26, H 5.35, N 3.20.

2,2'-Dimethoxy-5-(1-(3,4,5-trimethoxyphenyl)vinyl)-1,1'-biphenyl (16): Pale yellow oil; Yield (288 mg, 71 %); R_f = 0.60 (Cyclohexane / EtOAc 7 / 3); ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 3.74 (s, 3H), 3.77 (s, 3H), 3.80 (s, 6H), 3.84 (s, 3H), 5.33 (m, 2H), 6.58 (s, 2H), 6.89-6.99 (m, 3H), 7.30-7.19 (m, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 55.7, 55.8, 56.2 (2C), 60.9, 105.8 (2C), 110.6, 111.2, 112.5, 120.4, 127.4, 127.6, 128.4, 128.7, 131.4 (2C), 133.1, 137.6, 149.5, 152.8 (3C), 156.9, 157.0; IR: 2935, 1578, 1503, 1461, 1237, 1124, 1029 cm^{-1}; MS (APCI+, m/z): 407 $[M+H]^+$; Anal. Calcd for $C_{25}H_{26}O_5$ C 73.87, H 6.45, found C 73.54; H 6.21.

General procedure for the preparation of alkynes 17-22 and 24-26: To a THF solution (25 mL) of **5** (1 mmol) was added NEt_3 (1.2 mL), $PdCl_2(PPh_3)_2$ (0.05 mmol), CuI (0.1 mol). Then, the required alkyne (3 to 6 mmol) in THF (25 mL) was added to the mixture and stirred at 60°C for 16 h. After cooling, 40 mL of EtOAc was added to the crude mixture which was washed with a saturated NH_4Cl solution. After extraction with CH_2Cl_2, the combined extracts were dried ($MgSO_4$) and concentrated. Purification by flash chromatography on silica gel afforded alkynes.

3-(2-Methoxy-5-(1-(3,4,5-trimethoxyphenyl)vinyl)phenyl)prop-2-yn-1-ol (17): Brown oil; Yield (177 mg, 50 %); R_f = 0.23 (CH_2Cl_2 / EtOAc 9 / 1); ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 3.75 (s, 6H), 3.81 (s, 3H), 3.84 (s, 3H), 4.46 (s, 2H), 5.28 (m, 2H), 6.45 (s, 2H), 6.78 (d, *J* = 8.5 Hz, 1H), 7.24 (m, 1H), 7.37 (d, *J* = 2.3 Hz, 1H), OH not seen; ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 51.8, 55.9, 56.1 (2C), 60.9, 81.8, 91.0, 105.6 (2C), 110.3, 111.4, 113.2, 129.9, 133.5, 133.8, 137.1, 148.8, 152.9 (3C), 159.7; IR: 3379, 2935, 1582, 1500, 1456, 1412, 1124, 1022 cm^{-1}. MS (APCI+, m/z): 355 $[M+H]^+$; Anal. Calcd for $C_{21}H_{22}O_5$: C71.17, H 6.26, found C 71.00, H 6.12.

4-(2-Methoxy-5-(1-(3,4,5-trimethoxyphenyl)vinyl)phenyl)but-3-yn-1-ol (18): Yellow oil; Yield (169 mg, 46 %); R_f = 0.20 (CH_2Cl_2 / EtOAc 6 / 4); ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 2.42 (t, *J* = 6.3 Hz, 2H), 2.64 (t, *J* = 6.3 Hz, 2H), 3.73 (s, 6H), 3.79 (s, 3H), 3.81 (s, 3H), 5.26 (m, 2H), 6.44 (s, 2H), 6.75 (d, *J* = 8.6 Hz, 1H), 7.15 (dd, *J* = 8.6, 2.2 Hz, 1H), 7.32 (d, *J* = 2.2 Hz, 1H), OH not seen; ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 24.1, 55.9, 56.1 (2C), 60.8, 60.9, 78.6, 91.0, 105.6 (2C), 110.2, 112.2, 113.1, 133.1, 133.7, 137.2, 137.8, 148.9, 152.9 (3C), 159.7; IR: 3442, 936, 1579, 1499, 1461, 1411, 1342, 1123 cm^{-1}; MS (APCI+, m/z): 369 $[M+H]^+$; Anal. Calcd for $C_{22}H_{24}O_5$: C 71.72, H 6.57, found 71.49, 6.33.

5-(2-Methoxy-5-(1-(3,4,5-trimethoxyphenyl)vinyl)phenyl)pent-4-yn-1-ol (19): Yellow oil; Yield (206 mg, 54 %); R_f = 0.20 (CH_2Cl_2 / EtOAc 6 / 4); ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 1.88 (q, *J* = 6.8 Hz, 2H), 2.60 (t, *J* = 6.8 Hz, 2H), 3.81 (m, 8H), 3.88 (s, 3H), 3.89 (s, 3H), 5.34 (m, 2H), 6.52 (s, 2H), 6.81 (d, *J* = 8.6 Hz, 1H), 7.22 (dd, *J* = 8.6, 2.3 Hz, 1H), 7.39 (d, *J* = 2.3 Hz, 1H), OH not seen; ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 16.8, 31.3, 55.9, 56.15 (2C), 60.9, 62.3, 77.3, 93.9, 105.6 (2C), 110.1, 112.5, 113.0, 129.1, 133.1, 133.7, 137.2, 149.0, 152.9 (3C), 159.7; IR: 3532, 2935, 1579, 1501, 1462, 141, 1344, 1271, 1237, 1126 cm^{-1}; MS (APCI+, m/z): 383 $[M+H]^+$; Anal. Calcd for $C_{23}H_{26}O_5$: C 72.23, H 6.85, found C 72.01, H 6.57.

6-(2-Methoxy-5-(1-(3,4,5-trimethoxyphenyl)vinyl)phenyl)hex-5-yn-1-ol (20): Yellow oil; Yield (178 mg, 45 %). R_f = 0.17 (CH_2Cl_2 / EtOAc 6 / 4). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 1.73 (m, 4H), 2.51 (t, *J* = 6.5 Hz, 2H), 3.71 (t, *J* = 5.5 Hz, 2H), 3.80 (s, 6H), 3.87 (s, 3H), 3.89 (s, 3H), 5.34 (m, 2H), 6.52 (s, 2H), 6.81 (d, *J* = 8.6 Hz, 1H), 7.20 (dd, *J* = 8.6, 2.2 Hz, 1H), 7.39 (d, *J* = 2.2 Hz, 1H), OH not seen; ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 19.5, 24.9, 31.9, 55.9, 56.1 (2C), 60.9, 62.3, 76.8, 94.4, 105.6 (2C), 110.2, 112.7, 112.9, 129.0, 133.3, 133.7, 137.2, 149.0, 152.9 (3C), 159.6; IR: 3423, 2937, 1579, 1499, 1461, 1410, 1124, 1025, 1005 cm^{-1}. MS (APCI+, m/z): 397 $[M+H]^+$. Anal. Calcd for $C_{24}H_{28}O_5$: C 72.70, H 7.12, found C 72.48, H 6.99.

3-(2-Methoxy-5-(1-(3,4,5-trimethoxyphenyl)vinyl)phenyl)-N,N-dimethylprop-2-yn-1-amine (21): Brown oil; Yield (156 mg, 41 %); R_f = 0.11 (Cyclohexane / EtOAc 1 / 9); ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 2.31 (s, 3H), 2.38 (s, 3H), 3.51 (s, 2H), 3.78 (s, 6H), 3.86 (s, 3H), 3.88 (s, 3H), 5.85 (m, 2H), 6.53 (s, 2H), 6.82 (d, *J* = 8.5 Hz, 1H), 7.24 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.46 (d, *J* = 2.1 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 44.1, 44.2, 48.8, 55.9, 56.1 (2C), 60.9, 81.3, 88.9, 105.6 (2C), 110.2, 112.1, 113.0, 129.4, 133.4, 133.6, 137.1, 148.9, 152.9 (3C), 159.8; IR: 2939, 1579, 1500, 1461, 1410, 1344, 1126 cm^{-1}; MS (APCI+, m/z): 382 $[M+H]^+$; Anal. Calcd for $C_{23}H_{27}NO_4$: C 72.42, H 7.13, N 3.67, found C 72.19, 7.06, N 3.59.

(2-(2-Methoxy-5-(1-(3,4,5-trimethoxyphenyl)vinyl)phenyl) ethynyl) trimethylsilane (22): Brown oil; Yield (249 mg, 63 %); R_f = 0.35 (Cyclohexane / EtOAc 8 / 2); ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 0.26 (s, 9H), 3.81 (s, 6H), 3.88 (s, 3H), 3.90 (s, 3H), 5.30 (m, 1H), 5.40 (m, 1H), 6.52 (s, 2H), 6.81 (d, *J* = 8.7 Hz, 1H), 7.21-7.28 (m, 1H), 7.48 (d, *J* = 2.3 Hz,1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 0.1 (3C), 55.9 (2C), 56.0, 56.1, 60.9, 100.9, 105.6 (2C), 110.3, 112.4, 113.1, 130.0, 132.8,

133.9, 137.1, 137.1, 148.8, 152.2 (2C),160.0; MS (APCI+, m/z): 397 $[M+H]^+$. Anal. Calcd for $C_{23}H_{28}O_4Si$: C 69.66, H 7.12, found C 69.51, H 7.00.

5-(1-(3-Ethynyl-4-methoxyphenyl)vinyl)-1,2,3-trimethoxybenzene (23): To a solution of **22** (1 mmol) in THF (6 mL) was added at 0°C a 1M solution of TBAF (2 mL). After 12 h of stirring at room temperature, the solution was extracted with Et_2O and the combined extracts were dried ($MgSO_4$) and concentrated. Purification by flash chromatography on silica gel afforded **23** as a brown oil (214 mg, 66 %); R_f = 0.14 (Cyclohexane / EtOAc 8 / 2); ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 3.30 (s, 1H), 3.82 (s, 6H), 3.88 (s, 3H), 3.93 (s, 3H), 5.30 (m, 1H), 5.40 (m, 1H), 6.52 (s, 2H), 6.86 (d, *J* = 8.7 Hz, 1H), 7.30 (dd, *J* = 8.7, 2.3 Hz, 1H), 7.46 (d, *J* = 2.3 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 55.9, 56.1 (2C), 60.9, 79.9, 81.1, 105.6 (2C), 110.3, 110.9, 113.2, 130.2, 160.3, 133.7, 133.8, 137.0, 152.9 (2C), 148.7 (one C missing); IR: 3300, 2936, 1579, 1497, 1415, 1341, 1272, 1125, 1002, 897, 839 cm^{-1}. MS (APCI+, m/z): 325 $[M+H]^+$. Anal. Calcd for $C_{20}H_{20}O_4$: C 74.06, H 6.21, found C 73.79, H 6.17.

1,2,3-Trimethoxy-5-(1-(4-methoxy-3-(2-(4-methoxyphenyl)ethynyl) phenyl)vinyl)benzene (24): Brown solid; Yield (344, 80 %); m.p.: 105 °C; R_f = 0.35 (CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 3.78 (s, 3H), 3.81 (s, 6H), 3.88 (s, 3H), 3.91 (s, 3H), 5.36 (m, 2H), 6.55 (s, 2H), 6.84 (m, 3H), 7.25 (dd, *J* = 8.8, 2.0 Hz, 1H), 7.52-7.46 (m, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 55.1, 55.8, 56.0 (2), 60.7, 84.1, 93.4, 105.5 (2C), 110.2, 112.4, 113.1, 113.8 (2C), 115.4, 129.24, 132.9 (3C), 133.6, 137.1, 148.8, 152.8 (3C), 159.4 (2C); IR: 2939, 2838, 1510, 1462, 1246, 1126, 906 cm^{-1}. MS (APCI+, m/z): 431 $[M+H]^+$. Anal. Calcd for $C_{27}H_{26}O_5$: C 75.33, H 6.09, found C 75.04, H 5.81.

1,2,3-Trimethoxy-5-(2-(2-methoxy-5-(1-(3,4,5-trimethoxyphenyl) vinyl)phenyl)ethynyl)benzene (25): Brown oil; Yield (345 mg, 74 %); R_f = 0.30 (Cyclohexane / EtOAc 7/3); ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 3.78 (s, 3H), 3.80 (s, 3H), 3.81 (s, 6H), 3.84 (s, 3H), 3.88 (s, 3H), 3.91 (s, 3H), 5.36 (m, 2H), 6.55 (s, 2H), 7.25 (s, 2H), 7.30 (d, *J* = 8.7 Hz, 1H), 7.48 (dd, *J* = 8.7, 2.0 Hz, 1H), 7.51 (d, 1H, *J* = 2.0 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 56.0, 56.2 (4C), 60.9 (2C), 84.6, 93.5, 105.7 (2C), 109.1 (2C), 110.4, 112.1, 113.1, 118.4, 129.7, 133.3, 133.8, 137.2, 148.9, 152.9 (3C), 153.0 (3C), 159.6; IR: 2935, 1514, 1452, 1126; 725 cm^{-1}; MS (APCI+, m/z): 491 $[M+H]^+$; Anal. Calcd for $C_{29}H_{30}O_7$: C 71.00, H 6.16, found C 70.79, H 6.02.

1-(2-(2-Methoxy-5-(1-(3,4,5-trimethoxyphenyl)vinyl)phenyl) ethynyl)-4-(methoxymethoxy) benzene (26): Brown oil; Yield (349 mg, 80 %). R_f = 0.45 (Cyclohexane / EtOAc 7 / 3); ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 3.39 (s, 3H), 3.73 (s, 6H), 3.80 (s, 3H), 3.84 (s, 3H), 5.10 (s, 2H), 5.29 (m, 2H), 6.47 (s, 2H), 6.78 (d, *J* = 8.4 Hz, 1H), 6.91 (d, *J* = 8.4 Hz, 2H), 7.16-7.20 (m, 1H), 7.39-7.43 (m, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 56.0, 56.1 (2C), 56.3, 60.9, 84.4, 93.4, 94.3, 105.6 (2C), 110.3, 112.4, 113.1, 116.1 (2C), 116.7, 129.5, 133.1 (2C), 133.2, 133.7, 137.2, 149.0, 152.9 (3C), 157.2, 159.6; IR: 2936, 1579, 1508, 1461, 1411, 1341, 1279, 1233, 1079, 907 cm^{-1}; MS (APCI+, m/z): 461 $[M+H]^+$; Anal. Calcd for $C_{28}H_{28}O_6$: C 73.03, H 6.13, C 72.88, H 5.99.

General protocol for Hydration of alkynes 17-20 and synthesis of 31-33: To an Emrys Optimizer 0.5-2 mL pyrex reaction vessel were added alkyne (1 mmol) and PTSA (0.1 mmol) in EtOH (1 mL). The reaction vessel was then placed in the Emrys Optimizer and exposed to microwave irradiation according to the following specifications: temperature: 170°C, time (30 min.), fixed hold time: on, sample absorption: high, pre-stirring: 60s. After cooling to room temperature, H_2O (3 mL) was added and the mixture was extracted with EtOAc (3 x 2 mL). Organic layers were dried, concentrated and the crude mixture was purified by column chromatography on silica gel.

3-Ethoxy-1-(2-methoxy-5-(1-(3,4,5-trimethoxyphenyl)vinyl) phenyl)propan-1-one (27): Colorless oil; Yield (256 mg, 64 %); R_f = 0.45 (CH_2Cl_2 / EtOAc 95 / 5); ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 1.18 (t, *J* = 7.0 Hz, 3H), 3.29 (t, *J* = 6.7 Hz, 2H), 3.51 (q, *J* = 7.0 Hz, 2H), 3.79-3.83 (m, 8H), 3.87 (s, 3H), 3.92 (s, 3H), 5.37 (m, 2H), 6.51 (s, 2H), 6.92 (d, *J* = 8.6 Hz, 1H), 7.41 (dd, *J*= 2.4, 8.6 Hz, 1H), 7.75 (d, *J* = 2.4 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 15.1, 44.1, 55.7, 56.1 (2C), 60.9, 65.8, 66.4, 105.6 (2C), 111.2, 113.4, 128.0, 130.0, 133.2, 137.0, 137.9, 148.8, 152.9 (3C), 158.3, 200.3; IR: 2934, 1673, 1579, 1497, 1461, 1409, 1124, 1009 cm^{-1}; MS (APCI+, m/z): 401 $[M+H]^+$; Anal. Calcd for $C_{23}H_{28}O_6$: C 68.98, H 7.05, found C 68.58, H 6.79.

4-Ethoxy-1-(2-methoxy-5-(1-(3,4,5-trimethoxyphenyl)vinyl) phenyl)butan-1-one (28): Colorless oil; Yield (270 mg, 63 %); R_f = 0.15 (Cyclohexane / EtOAc 8 / 2); ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 1.16 (t, *J* = 7.0 Hz, 3H), 1.96 (q, *J* = 7.0 Hz, 2H), 3.05 (t, *J* = 7.2 Hz, 2H), 3.42-3.49 (m, 4H), 3.79 (s, 6H), 7.69 (d, *J* = 2.3 Hz, 1H), 3.86 (s, 3H), 3.90 (s, 3H), 5.36 (m, 2H), 6.51 (s, 2H), 6.90 (d, *J* = 8.6 Hz, 1H), 7.38 (dd, *J* = 8.6, 2.3 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 15.1, 25.5, 40.4, 55.6, 56.1 (2C), 60.8, 66.0, 69.8, 105.6 (2C), 111.2, 113.3, 128.4, 129.7, 132.8, 133.7, 136.9, 137.9, 148.8, 152.9 (2C), 158.0, 202.4; IR: 2934, 2857, 1673, 1600, 1578, 1496, 1462, 1409, 1344, 1235, 1008 cm^{-1}; MS (APCI+, m/z): 415 $[M+H]^+$; Anal. Calcd for $C_{24}H_{30}O_6$: C 69.54, H 7.30, found C 69.48, H 7.25.

5-Ethoxy-1-(2-methoxy-5-[1-(3,4,5-trimethoxyphenyl)vinyl] phenyl)pentan-1-one (29): Yellow oil; Yield (175 mg, 41 %); R_f = 0.53 (Cyclohexane/EtOAc: 6/4);^{1}H NMR ($CDCl_3$, 300 MHz): δ = 1.18 (t, *J* = 7.2 Hz, 3H), 1.72-1.81 (m, 2H), 3.00 (t, *J* = 6.9 Hz, 2H), 3.41-3.49 (m, 4H), 3.80 (m, 8H), 3.87 (s, 3H), 3.91 (s, 3H), 5.37 (m, 2H), 6.51 (s, 2H), 6.91 (d, *J* = 8.4 Hz, 1H), 7.39 (dd, *J* = 2.4, 8.4 Hz, 1H), 7.68 (d, *J* =2.4 Hz, 1H), ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 15.2, 21.1, 29.4, 43.5, 55.6, 56.1 (2), 60.9, 66.1, 70.4, 105.6 (2), 111.2, 113.4, 128.5, 129.7, 132.8, 133.8, 137.0, 148.8, 152.9 (3), 158.0, 202.9; IR: 2934, 1674, 1578, 1498, 1462, 1409, 1345, 1236, 1125 cm^{-1}; MS (APCI+, m/z): 429 $[M+H]^+$; Anal. Calcd for $C_{25}H_{32}O_6$: C 70.07, H 7.53, found C 69.87, H 7.39.

6-Hydroxy-1-(2-methoxy-5-(1-(3,4,5-trimethoxyphenyl)vinyl) phenyl)hexan-1-one (30) Colorless oil; Yield (283 mg, 64 %); R_f = 0.13 (Cyclohexane / EtOAc 6 / 4); ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 1.39-1.47 (m, 2H), 1.56-1.65 (m, 4H), 2.99 (t, *J* = 6,9 Hz, 2H), 3.65 (t, *J* = 6.6 Hz, 2H), 3.80 (s, 6H), 3.87 (s, 3H), 3.92 (s, 3H), 5.38 (m, 2H), 6.51 (s, 2H), 6.91 (d, *J* = 8,7 Hz, 1H), 7.40 (dd, *J* = 8.7, 2.4 Hz, 1H), 7.69 (d, *J* = 2.4 Hz, 1H), OH not seen;^{13}C NMR ($CDCl_3$, 75 MHz): δ = 23.9, 25.4, 32.5, 43.6, 55.7, 56.2 (2C), 62.7, 60.9, 105.6 (2C), 111.2, 113.4, 128.5, 129.7, 132.9, 133.8, 137.0, 148.8, 152.9 (3C), 158.0, 203.0; IR : 3375, 2932, 1672, 1579, 1497, 1462, 1236, 1179 cm^{-1}; MS (APCI+, m/z): 415 $[M+H]^+$; Anal. Calcd for $C_{24}H_{30}O_6$ C,69.54 H 7.30, found C 69.47, H 7.19.

2-(4-Methoxyphenyl)-5-(1-(3,4,5-trimethoxyphenyl)vinyl) benzo-furan (31): Colorless oil; Yield (358 mg, 86 %); R_f = 0.27 (Cyclohexane / EtOAc 8 / 2); ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 3.81 (s, 6H), 3.86 (s, 3H), 3.90 (s, 3H), 5.44 (m, 2H), 6.60 (s, 2H), 6.86 (s, 1H), 6.98 (d, *J* = 8.8 Hz, 2H), 7.27 (dd, *J* = 8.5, 1.8 Hz, 1H), 7.50 (d, *J* = 8.5 Hz, 1H), 7.54 (s, 1H), 7.80 (d, *J* = 8.8 Hz, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 55.3, 56.1 (2C), 60.9, 99.7, 105.7 (2C), 110.4, 114.3 (2C), 120.4, 123.2, 124.5, 126.4, 129.4, 136.4, 137.8, 150.3, 152.9 (3C), 154.5, 156.7, 160.1; IR: 2937, 2835, 1613, 1578, 1504, 1249, 1124, 1024, 1006 cm^{-1}. MS (APCI+, m/z): 417 $[M+H]^+$; Anal. Calcd for $C_{24}H_{24}O_5$: C 74.98, H 5.81, found C 74.69, H 5.54.

2-(3,4,5-Trimethoxyphenyl)-5-(1-(3,4,5-trimethoxyphenyl)vinyl) benzofuran (32): Colorless oil; Yield (390 mg, 82 %); R_f = 0.15 (CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 3.81 (s, 6H), 3.89 (s, 3H), 3.90 (s, 3H), 3.96 (s, 6H), 5.44 (m, 2H), 6.59 (s, 2H), 6.93 (s, 1H), 7.09 (s, 2H), 7.28-7.31 (m, 1H), 7.47 (d, *J* = 8.7 Hz, 1H), 7.54-7.55 (m, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 56.1 (2C), 56.3 (2C), 60.9, 61.0, 101.1, 102.2 (2C), 105.7 (2C), 110.6, 113.5, 120.6, 125.0, 125.9, 129.2, 136.6, 137.8, 150.2, 152.9 (3C), 153.6 (3C), 154.6, 156.4; IR : 2937, 1571, 1500, 1463, 1412, 1342, 1236, 1122, 726 cm^{-1}; MS (APCI+, m/z): 477 $[M+H]^+$; Anal. Calcd for $C_{28}H_{28}O_7$: C 70.57, H 5.92, found C 70.49, H 5.84.

4-(5-(1-(3,4,5-Trimethoxyphenyl)vinyl)benzofuran-2-yl)phenol (33): Pale white oil; Yield (277 mg, 69 %); R_f = 0.10 (Cyclohexane / EtOAc 8 / 2); ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 3.81 (s, 6H), 3.90 (s, 3H), 5.44 (d, *J* = 7.8 Hz, 2H), 5.60 (s, 1H), 6.60 (s, 2H), 6.85 (s, 1H), 6.92 (d, *J* = 8.4 Hz, 2H), 7.24-7.28 (m, 1H), 7.45 (d, *J* = 8.4 Hz, 1H), 7.53 (d, *J* = 1.8 Hz, 1H), 7.75 (d, *J* = 8.4 Hz, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 56.1 (2C), 61.0, 99.7, 105.7 (2C), 110.5, 113.4, 115.8 (2C), 120.4, 123.3, 124.5, 126.6 (2C), 129.4, 156.7, 136.4, 137.9, 150.3, 152.8 (3C), 154.5, 156.3; IR: 3609, 2928, 1581, 1264, 1127, 732, 703 cm^{-1}. MS (APCI+, m/z): 403 $[M+H]^+$, 441 $[M+K]^+$. Anal. Calcd for $C_{25}H_{22}O_5$: C 74.51, H 5.51, found C 74.17, H 5.12.

2-(2-Azidoethynyl)-1-methoxy-4-(1-(3,4,5-trimethoxyphenyl)vinyl) benzene *iso*N_3 CA-4 (34): At 0°C, to a solution of *iso*NH_2CA-4 **2b** (1 g, 3.17 mmol) in acetone (50 mL) was added HCl (0.2 N, 50 mL). After 20 min of stirring, $NaNO_2$ (12.68 mmol) was added to the mixture for 20 min followed by the addition of NaN_3 (40 mmol). After 15 min of stirring, the resulting mixture was poured onto Et_2O (50 mL) and water (30 mL). After extraction, the combined extracts were dried ($MgSO_4$) and concentrated . Purification by flash chromatography on silica gel afforded ***iso*N_3 CA-4 34** as a yellow solid: Yield (917 mg, 85 %); m.p.: 83-85 °C; R_f = 0.56 (Cyclohexane / EtOAc 7 / 3); ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 3.82 (s, 6H), 3.88 (s, 3H), 3.90 (s, 3H), 5,30 (m, 1H), 5.40 (m, 1H), 6.52 (s, 2H), 6.85 (d, *J* = 8.4 Hz, 1H), 7.04 (d, *J* = 2.1 Hz, 1H), 7.07 (dd, *J* = 8.4, 2.1 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 56.2, 56.3 (2C), 61.07, 105.8 (2C), 111.7, 113.6, 120.3, 125.8, 132.1, 134.8, 137.1, 148.4, 149.0, 151.8, 153.1 (2C); IR: 2124, 1577, 1503, 1413, 1341, 1240, 1120, 1003, 880, 628 cm^{-1}. MS (ESI+, m/z): 364 $[M+Na]^+$; Anal. Calcd for $C_{20}H_{19}N_3O_4$: C 63.33, H 5.61, N 12.31, found C 63.00, H 5.43, N 12.08.

Synthesis of triazoles 35-37: 34 (1 mmol) and the corresponding alkyne (1 mmol) were mixed in *n*-BuOH/H_2O (1/1, 10 mL) in a sealed tube in the presence of $CuSO_4$ - 5 H_2O (0.01 mmol) and potassium ascorbate (0.1 mmol). The mixture was then stirred for 13 h at 70°C. After cooling, the crude was poured onto water (16 mL) at 0°C and the precipitate was filtered and washed with water. Purification by flash chromatography on silica gel afforded **35-37**.

1-(2-Methoxy-5-(1-(3,4,5-trimethoxyphenyl)vinyl)phenyl)-4-(4-methoxyphenyl)-1*H*-1,2,3-triazole (35): Pale yellow solid; m.p.: 125-127 °C; Yield (381 mg, 86 %); R_f = 0.49 (Cyclohexane / EtOAc 5 / 5); ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 3.81 (s, 6H), 3.82 (s, 3H), 3.87 (s, 3H), 3.91 (s, 3H), 5.40 (m, 1H), 5.45 (m, 1H), 6.56 (s, 2H), 6.96 (d, *J* = 8.5 Hz, 2H), 7.05 (d, *J* = 8.6 Hz, 1H), 7.40 (dd, *J* = 8.6, 2.2 Hz, 1H), 7.82 (d, *J* = 8.5 Hz, 2H), 7.86 (d, *J* = 2.2 Hz, 1H), 8.23 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 55.2, 56.0 (2C), 60.7, 105.6 (2C), 111.8, 113.8, 114.1 (2C), 120.9, 123.2, 125.1, 126.0, 127.0 (2C), 129.6, 134.5, 136.5, 137.9, 147.0, 148.2, 150.8, 152.9 (2C), 159.5, one C missing; IR: 2937, 1580, 1497, 1461, 1412, 1248, 1174, 1125, 1025, 907, 726 cm^{-1}; MS (APCI+, m/z): 474 $[M+H]^+$; Anal. Calcd for $C_{26}H_{25}N_3O_4$: C 70.41, H 5.68, N 9.47, found, C 70.05, H 5.43, N 9.28.

1-(2-Methoxy-5-(1-(3,4,5-trimethoxyphenyl)vinyl)phenyl)-4-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole (36): Pale yellow solid; m.p.: 157-159 °C; Yield (363 mg, 68 %); R_f = 0.26 (Cyclohexane / EtOAc 5 / 5); ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 3.81 (s, 6H), 3.86 (s, 3H), 3.87 (s, 3H), 3.93 (s, 9H), 5.40 (m, 1H), 5.45 (m, 1H), 6.56 (s, 2H), 7.06 (d, *J* = 8.6 Hz, 1H), 7.12 (s, 2H), 7.41 (d, *J* = 8.6 Hz, 1H), 7.83 (s, 1H), 8.25 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 56.1 (2C), 56.2 (3C), 60.8 (2C), 103.1 (2C), 105.6 (2C), 111.9, 113.9, 121.5, 125.3, 125.9, 126.1, 129.8, 134.6, 136.5, 138.0, 138.1, 147.1, 148.2, 150.9, 152.9 (2C), 153.6 (2C); IR: 1585, 1499, 1463, 1234, 1122, 1011, 834 cm^{-1}; MS (APCI+, m/z): 534 $[M+H]^+$; Anal. Calcd for $C_{29}H_{31}N_3O_7$: C 65.28, H 5.86, N 7.88, found C 65.07, H 5.62, N 7.64.

1-(2-Methoxy-5-(1-(3,4,5-trimethoxyphenyl)vinyl)phenyl)-4-phenyl-1*H*-1,2,3-triazole (37): White solid. m.p.: 113-115 °C; Yield (387 mg, 91 %); R_f = 0.30 (Cyclohexane / EtOAc 5 / 5); ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 3.81 (s, 6H), 3.87 (s, 3H), 3.93 (s, 3H), 3.98 (s, 3H), 5.42 (m, 1H), 5.45 (m, 1H), 6.54 (s, 2H), 7.06 (d, *J* = 8.7 Hz, 1H), 7.42 (dd, *J* = 8.7, 2.2 Hz, 1H), 7.88 (d, *J* = 2.2 Hz, 1H), 8.66 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 52.1, 56.1 (3C), 60.8, 105.6 (2C), 111.9, 114.2, 125.0, 125.1, 129.6, 130.4, 134.7, 136.4, 138.1, 139.5, 148.1, 150.5, 152.9 (2C), 161.2; IR: 2949, 1749, 1345, 1233, 1182, 1035, 766 cm^{-1}; MS (APCI+, m/z): 443 $[M+H]^+$; Anal. Calcd for $C_{22}H_{23}N_3O_6$: C 62.11, H 5.45, N 9.88, found C 61.89, H 5.26, N 9.56.

Synthesis of phenstatin derivatives 38 and 39: These compounds were prepared as for alkynes **17** and **18** from (3-iodo-4-methoxyphenyl)(3,4,5-trimethoxyphenyl)methanone:

(3-(3-Hydroxyprop-1-ynyl)-4-methoxyphenyl)(3,4,5-trimethoxy phenyl)methanone (38): Brown solid; m.p.: 137-139 °C; Yield (249 mg, 70 %); R_f = 0.30 (Cyclohexane / EtOAc 5 / 5); ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 3.88 (s, 6H), 3.94 (s, 3H), 3.97 (s, 3H), 4.54 (d, *J* = 3.3 Hz, 2H), 6.96 (d, *J* = 8.7 Hz, 1H), 7.00 (s, 2H), 7.82 (dd, *J* = 8.7, 2.1 Hz, 1H), 7.91 (d, *J* = 2.1 Hz, 1H), OH not seen; ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 51.7, 56.1, 56.3 (2C), 60.9, 80.8, 92.1, 107.4 (2C), 110.1, 111.8, 130.1, 132.3, 132.8, 136.0, 152.8 (2C), 163.0, 193.9, one C missing; IR: 3401, 1632, 1568, 1504, 1415, 1336, 1274, 1241, 1127, 1016, 760 cm^{-1}; MS (APCI+, m/z): 357 $[M+H]^+$; Anal. Calcd for $C_{20}H_{20}O_6$: C 67.41, H 5.66, found C 67.39, H 5.63.

(3-(4-Hydroxybut-1-ynyl)-4-methoxyphenyl)(3,4,5-trimethoxy phenyl)methanone (39): Brown solid; m.p.: 131-133 °C; Yield (285 mg, 80 %); R_f = 0.20 (Cyclohexane / EtOAc 5 / 5); ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 2.75 (t, *J* = 6.1 Hz, 2H), 3.84 (t, *J* = 6.1 Hz, 2H), 3.88 (s, 6H), 3.94 (s, 3H), 3.97 (s, 3H), 6.95 (d, *J* = 8.6 Hz, 1H), 7.01 (s, 2H), 7.79 (dd, *J* = 8.6, 2.1 Hz, 1H), 7.89 (d, *J* = 2.1 Hz, 1H), OH not seen; ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 24.3, 56.4, 56.5 (3C), 61.0, 90.7, 92.0, 107.7 (2C), 110.1, 112.8, 130.3, 132.0, 133.1, 135.7, 153.1 (2C), 163.3, 194.2, one C missing; IR: 2366, 1582, 1500, 1414, 1333, 1272, 1127 cm^{-1}; MS (APCI+, m/z): 371 $[M+H]^+$; Anal. Calcd for $C_{21}H_{22}O_6$: C 68.10, H 5.99, found C 68.01, H 5.87.

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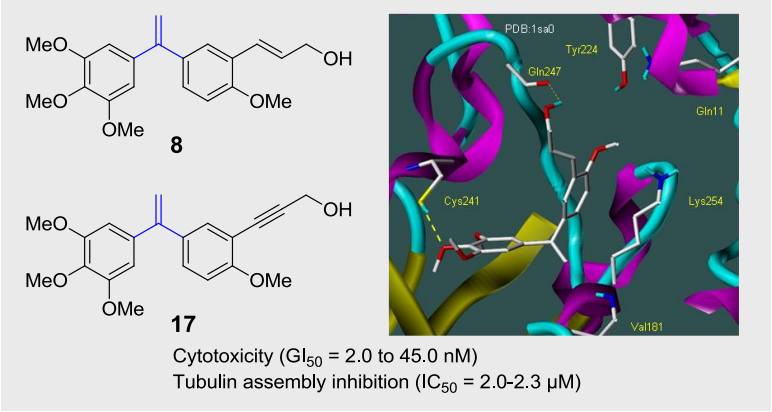

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