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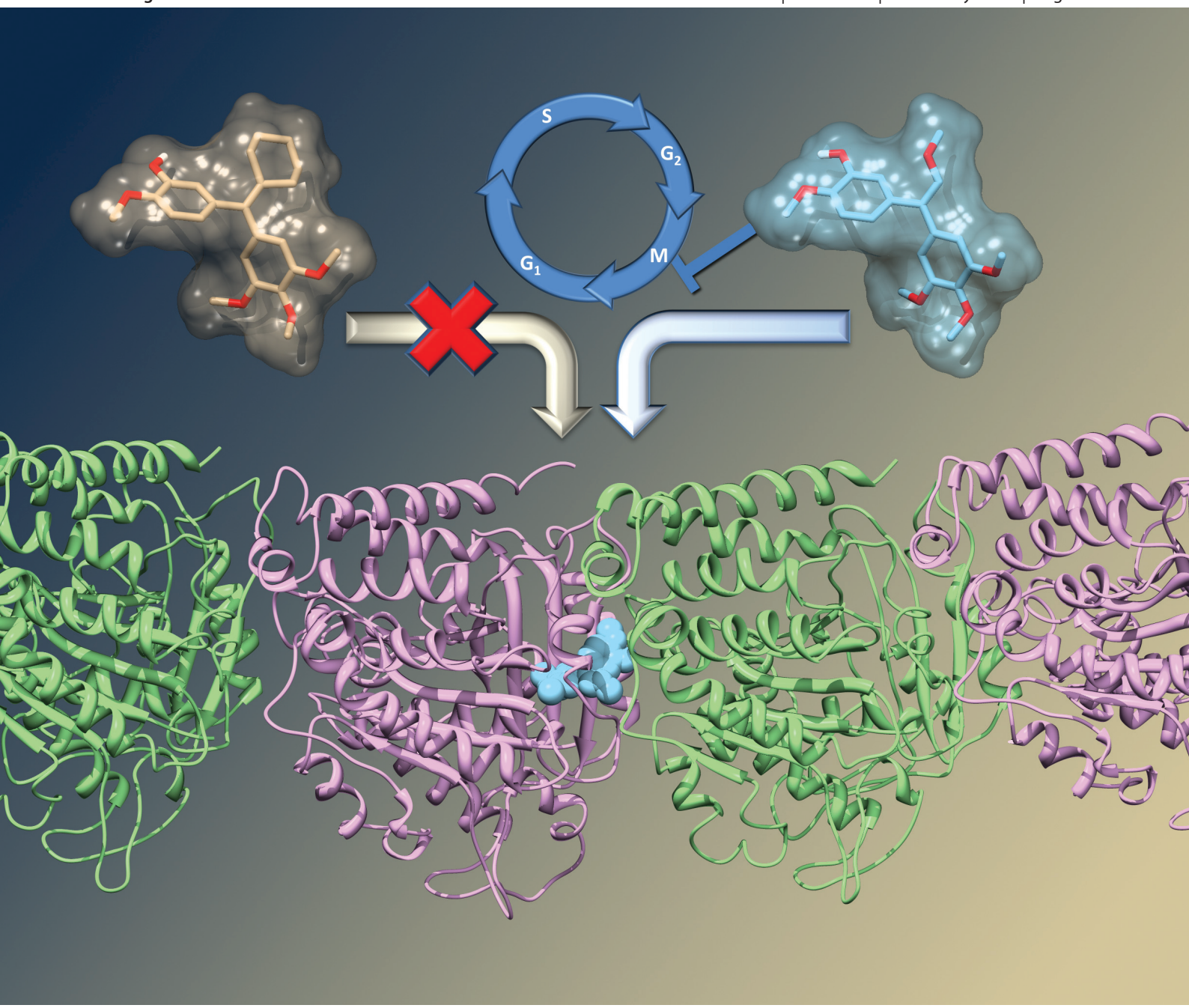

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Synthesis, biological evaluation, and structure–activity relationships of tri- and tetrasubstituted olefins related to isocombretastatin A-4 as new tubulin inhibitors†

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The synthesis and structure–activity relationships associated with a series of 1,1-diarylethylene tubulin polymerization inhibitors **3** and **4** are described. The key step for their preparation involves a palladium-catalyzed coupling of *N*-arylsulfonylhydrazones with aryl halides, thus providing flexible and convergent access to tri- and tetrasubstituted 1,1-diarylolefins **3** and **4** related to isocombretastatin A-4 (isoCA-4). These compounds have been evaluated for tubulin polymerization inhibitory activity as well as for cytotoxic activity. The most potent compounds are 1,1-diaryl-2-methoxyethylenes **4b**, **4d** and **4e** having a trisubstituted double bond. They exhibited good antiproliferative activity against various human cancer cell lines (GI_{50} = 8–80 nM). Compounds **4b** and **4e** strongly inhibited tubulin polymerization with IC_{50} values of 2 and 3 μM, respectively, and induced cell cycle arrest in the G_2/M phase in the K562 cell line. Docking studies in the colchicine binding site of tubulin allowed identification of residues most likely to interact with these inhibitors and explain their potent anti-tubulin activity.

Introduction

Cancer is now one of the most frequent causes of death all over the world.[1] The main reason for this expansion is the ability of cancer cells to develop various types of resistance mechanisms.[2] New antitumor agents have been continuously designed. Among them, natural products such as *Catharanthus* alkaloids (*e.g.*, vincristine, vinblastine),[3] colchicine,[4] and combretastatin A-4 (CA-4)[5] exhibited potent tubulin polymerization inhibitory activity. Disruption of tubulin assembly results in mitotic arrest, and subsequent cell death. CA-4 (Fig. 1) extracted from the South African tree *Combretum caffrum* shows potent cytotoxicity against a wide variety of human cancer cell lines,[6] including those that are multidrug

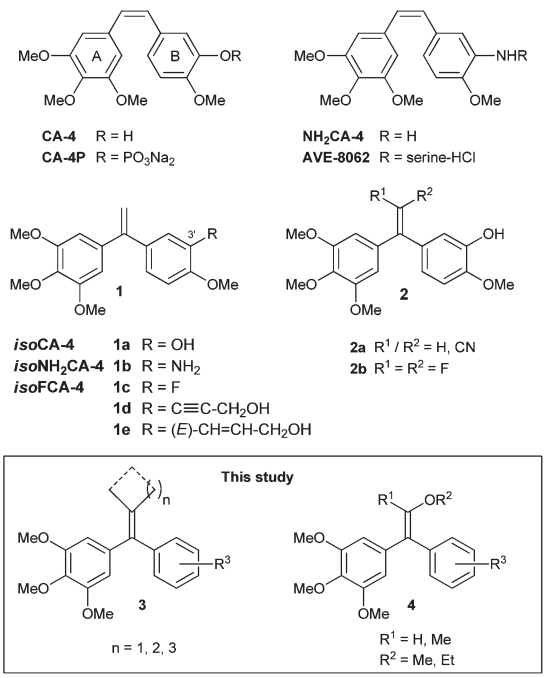

Fig. 1 CA-4, isoCA-4 derivatives, and target structures 3, 4.

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resistant.[7] CA-4P (fosbretabulin) and AVE-8062 (ombrabulin), two water-soluble prodrug derivatives, are examples of clinically relevant vascular disrupting agents (Fig. 1). They have been shown to cause vascular shutdown and reduction in tumor blood flow *in vivo* leading to hemorrhagic necrosis. Both fosbretabulin and ombrabulin are currently undergoing several advanced clinical trials for the treatment of anaplastic thyroid cancer[8] and sarcoma,[9] respectively.

Previous structure–activity relationship (SAR) studies with CA-4 analogues showed the importance of the 3′,4′,5′-trimethoxy substitution pattern on the A-ring, while B-ring structural modifications were tolerated by the target.[10] It is important to note that the *Z* olefinic bridge is able to undergo rapid Z–*E* isomerization under the influence of heat, light, and protic media[10a] and that this olefinic bridge represents a weak point for metabolic stability,[11] resulting in a dramatic loss in antitumor activity. To circumvent the problem of *Z*–*E* isomerization, a substantial range of CA-4 analogues have been designed and synthesized with the objective to replace the olefinic bridge and improve the intrinsic stability as well as the therapeutic index of CA-4.[12] Several reviews outlined this vast array of chemistry focusing on the stabilization of the two aryl rings of CA-4 using one to three atom bridgeheads.[13]

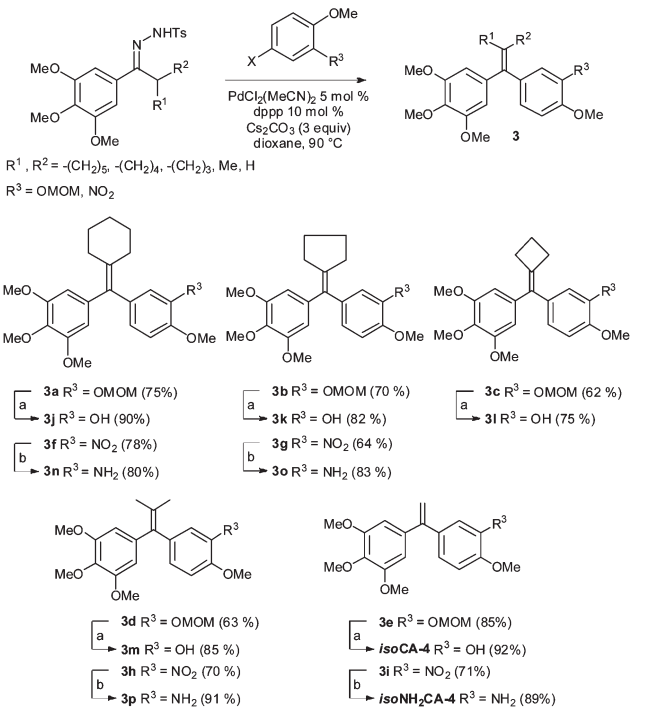
In our efforts to discover non-isomerizable CA-4 analogues,[14] we recently synthesized a series of 1,1-diarylethylene derivatives among which isoCA-4 (**1a**),[15] isoNH$_2$CA-4 (**1b**), and isoFCA-4 (**1c**)[16] have emerged as lead compounds (Fig. 1). They displayed potent antiproliferative activity against various human cancer cell lines with IC$_{50}$ values ranging from 2 to 10 nM, inhibited tubulin polymerization at a micromolar level, and arrested cancer cells in the G$_2$/M phase of the cell cycle. Very recently, replacement of the 3′-hydroxy group of isoCA-4 by a propargylic or (*E*)-allylic alcohol function led us to identify novel B-ring-modified isoCA-4 analogues **1d** and **1e**, respectively, endowed with promising antiproliferative and antimitotic activities.[17] In contrast with the parent natural product (CA-4), isoCA-4 derivatives **1b–e** are easy to synthesize[18] without the need to control the olefin geometry. Bioisosteric replacement was successfully extended to compounds **2a** and **2b** having a tri- or tetra-substituted double bond.[15,19]

In continuation of our earlier work, and in order to have a better understanding of the SAR, a further panel of compounds **3** and **4** containing the 1,1-diarylethylene moiety were examined as potential tubulin targeting agents. In fact, we reconfigured herein the substitution pattern around the double bond by the preparation of tri- or tetra-substituted olefins **3** and **4**, including those with a cycloalkylidene unit. Our goal was to evaluate the steric and electronic effects of the double bond-substituents on antiproliferative activity. The designed analogues were characterized by the presence of a 3′,4′,5′-trimethoxyphenyl moiety, identical to the A-ring of CA-4, which was considered essential for maximal bioactivity, and examined various substitutions on the B-ring. The synthesized compounds were then evaluated *in vitro* for their capacity to inhibit cancer cell proliferation and to act as potential antimitotic agents.

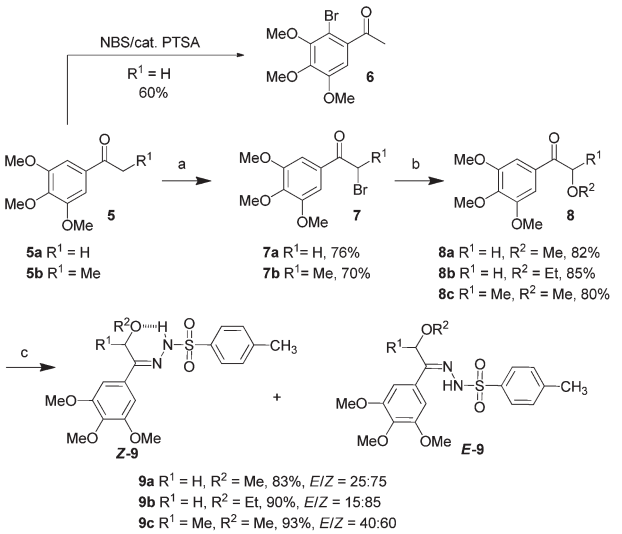
Chemistry

Recently, *N*-tosylhydrazones have emerged as a new type of versatile coupling partner for transition metal-catalyzed cross-coupling reactions and have attracted increasing attention.[20] Our interest in this chemistry[21] led us to evaluate the scope of this reaction for the preparation of the target tri- and tetra-substituted olefins **3** and **4**. As depicted in Scheme 1, the key intermediate *N*-tosylhydrazones derived from the corresponding ketones were coupled with various aryl halides under our previously reported conditions,[21c] using PdCl$_2$(MeCN)$_2$/dppp as the catalytic system and Cs$_2$CO$_3$ as the base. Accordingly, the desired olefins **3a–i** were obtained in good yields (62–85%). The MOM protecting group of compounds **3a–e** was removed using PTSA in EtOH to yield the corresponding phenolic products **3j–m**, as well as isoCA-4. The nitro group of compounds **3f–i** was reduced by iron powder, yielding compounds **3n–p** and isoNH$_2$CA-4.

We have taken advantage of this new synthetic method to prepare the target olefins with general structure **4**. To this end, the synthesis of α-alkoxyarylketones **8** was achieved from 3′,4′,5′-trimethoxyacetophenone derivatives **5** (Scheme 2). Bromination of **5** using the *N*-methylpyrrolidin-2-one hydrotribromide (MPHT) complex,[22] developed in our group, provided selectively α-bromoketones **7** in good yields (70–76%), whereas the use of Pravst's conditions (NBS/cat. PTSA)[23] resulted mainly in the bromination of the aromatic ring leading to **6** (Scheme 2). The required α-alkoxyarylketones **8** were obtained in high yields (80–85%) by treating **7** in MeOH in the presence



Scheme 1 Synthesis of 1,1-diarylcycloalkylidenes: (a) PTSA, EtOH, 60 °C, 2 h; (b) Fe/EtOH/HCl cat. 100 °C, 2 h.



Scheme 2 Synthesis of α-alkoxy *N*-tosylhydrazones **9**: (a) MPHT, PTSA 10 mol%; (b) ROH, Ag_2CO_3, $BF_3{\cdot}Et_2O$, 20 °C; (c) $TsNHNH_2$, MeOH, 20 °C.

of $Ag_2CO_3/BF_3{\cdot}Et_2O$.[24] It should be noted that the use of a solution of sodium methoxide was ineffective (12%).[25] Further reactions of **8** with *p*-toluenesulfonylhydrazide in MeOH[26] furnished *N*-tosylhydrazones **9** as a mixture of *E*/*Z* diastereomers, with a preference for the *Z*-isomer probably due to intramolecular hydrogen bonding, as shown in Scheme 2. Finally, under our optimized coupling conditions,[21c] hydrazones **9a–c** reacted with *p*-methoxy-substituted aryl halides to furnish 1-alkoxy-2,2-diarylethylene derivatives **4** (Table 1).[27] In general, the coupling process was compatible with a variety of substituents (NO_2, NH_2), and enol ethers **4a–j** were obtained in good yields as an *E*/*Z* mixture.[28] As expected, with the sterically hindered tosylhydrazone **9c**, the coupling reaction was also effective providing enol ethers **4i,j** (entries 9 and 10, Table 1).

Biological evaluation

A. *In vitro* cell growth inhibition

All the synthesized compounds were tested in a preliminary cytotoxic assay on a human colon carcinoma (HCT116) cell line using CA-4 and isoCA-4 as reference compounds. Compounds **4** were tested as a mixture of *E* and *Z* isomers.

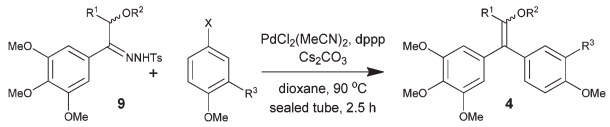
The results reported in Table 2 demonstrated that tetrasubstituted olefins **3**, having the greatest resemblance to CA-4 and NH_2CA-4, displayed only modest antiproliferative activity, which was little affected with ring size variation. The most active analogue in this series was found to be **3l** having a cyclobutylidene unit with a GI_{50} value in HCT116 cells of 250 nM. Compounds having a large cycloalkylidene unit (**3j**, **3k**, **3n**, and **3o**) lead to a loss of antiproliferative activity, suggesting that steric factors account for the loss of activity. It is to note that compound **3m** having an isopropylidene instead of a cyclobutylidene unit (**3l**) retained the antiproliferative activity, whereas its C3′-amino substituted analogue **3p** was over 2-fold less active. A comparison of the substituent effect on the double bond (**4j** *vs.* **3m**) indicated that introduction of an MeO substituent (**4j**) produced cytotoxicity comparable to that of **3m**. The screening was pursued with enol ether derivatives **4a–h** in which the double bond is trisubstituted. The most active compound in this series was found to be **4d** with a GI_{50} value in HCT116 cells of 8 nM. Replacement of the C3′-fluorine atom of **4d** by a C3′–OH or $–NH_2$ substituent gave compounds **4b** and **4e** that were 3- and 10-fold less active, respectively. Finally, the result obtained with compound **4h** *vs.* **4d** clearly revealed that the size of the substituent at the double bond (OEt *vs.* OMe, respectively) plays a critical role in cell growth inhibition (compare also **4f** *vs.* **4b**).

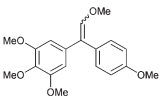
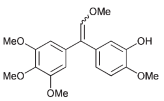
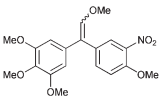
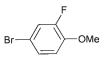
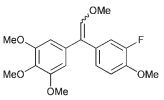
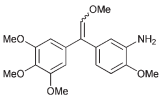
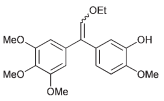
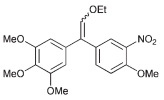
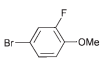
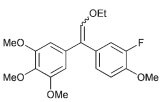
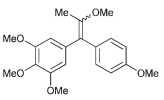
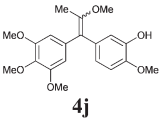
To further characterize the cytotoxicity profiles of these compounds, we investigated the effect of the most active substances **4b**, **4d** and **4e** ($GI_{50} \leq 80$ nM) on the proliferation of two tumor cell lines, myelogenous leukemia (K562) and non-small-cell lung carcinoma human (H1299) (Table 3). The screening results revealed that the selected compounds strongly inhibited the growth of two tumor cell lines with GI_{50} values in the range of 25–60 nM (Table 3).

B. Inhibition of tubulin polymerization

To investigate whether the antiproliferative activities of these compounds were related to interaction with tubulin, compounds **3** and **4** were evaluated for their ability to block the assembly of tubulin. Tubulin was purified from sheep brain tissue according to a slight modification of the protocol reported by Guenard and colleagues[29] (Table 2). Compounds **4b** and **4e** were very effective in their ability to inhibit tubulin assembly with IC_{50} values of 2.0 and 3.0 μM, respectively, comparable with those of CA-4 and isoCA-4. Except for **4j** which demonstrated also interesting potency in the inhibition of tubulin assembly (IC_{50} = 7.0 μM), none of the other compounds inhibited tubulin polymerization to a significant degree. The concentrations required to inhibit tubulin polymerization (IC_{50}: 2 μM) are much higher than those required for cytotoxicity (IC_{50} in the nanomolar range). Similar observations have been noticed in many other classes of antimitotic agents, including epithilones,[30] paclitaxel,[31] CA-4 sulfonate analogues.[32] The basis for this difference has been attributed to intracellular retention of the compounds, leading to higher intracellular concentrations.[31] In addition, the tubulin polymerization is represented by a rapid turbidimetric assay that monitors instantaneous effects on polymerization. The difference between long-term continuous treatment in the cell-based assay and instant measurements can theoretically explain such concentration differences.[10a] Furthermore, the effects of selected compounds on cell cycle progression (see *vide infra*) correlated well with their strong antiproliferative activity and inhibition of tubulin polymerization. We observed accumulation of G2–M and apoptotic cells, effects expected for compounds that interact with tubulin. Such effects would strongly suggest that tubulin is a relevant target for our diaryl analogues.

Table 1 Synthesis of 1-alkoxy-2,2-diphenylethylenes **4** by the Pd-catalyzed coupling of *N*-tosylhydrazones **9** with aryl halides



Entry	Hydrazone	ArX	1,1-Diarylethylene	Yield[a] (%)	Ratio *E*/*Z*[b]
1	R^1 = H R^2 = Me		 **4a**	98	60/40
2	R^1 = H R^2 = Me		 **4b**	90[c]	55/45
3	R^1 = H R^2 = Me		 **4c**	61	70/30
4	R^1 = H R^2 = Me		 **4d**	58	44/56
5	R^1 = H R^2 = Me		 **4e**	74	40/60
6	R^1 = H R^2 = Et		 **4f**	65[c]	50/50
7	R^1 = H R^2 = Et		 **4g**	60	80/20
8	R^1 = H R^2 = Et		 **4h**	55	40/60
9	R^1 = Me R^2 = Me		 **4i**	60	50/50
10	R^1 = Me R^2 = Me		 **4j**	64[c]	60/40

[a] Yield of isolated product. [b] *E*/*Z* ratio determined by ^{1}H NMR in the crude mixture reaction. [c] Overall yield obtained after two steps, coupling and deprotection of the OTBS.

It can be noted that compound **4d** which was as active as isoCA-4 with respect to cytotoxicity showed a 10-fold decreased inhibition of tubulin assembly, suggesting that it may exert its cytotoxic effects through other molecular mechanisms.

At this stage, it was of interest to compare the biological activity of the *E*- and *Z*-isomers in the series of compounds **4**, to determine whether greater activity is associated with a particular configuration or whether both isomers have equivalent

Table 2 Effect of the linker on cytotoxic activity against HCT116 cells[a]

Compd	Linker	R^3	GI_{50} (nM) HCT116[b]	ITP IC_{50}[d] (μM)
3j		OH	2500 ± 155	83 ± 16
3n		NH_2	3000 ± 186	nt[c]
3k		OH	2000 ± 124	>100
3o		NH_2	5000 ± 330	nt[c]
3l		OH	250 ± 16	13 ± 2
3m		OH	300 ± 25	55 ± 12
3p		NH_2	700 ± 56	nt[c]
4i		H	1800 ± 100	nt[c]
4j		OH	250 ± 18	7 ± 0.3
4a		H	420 ± 30	>100
4b		OH	24 ± 1.6	2 ± 0.2
4c		NO_2	1700 ± 95	>100
4e		NH_2	80 ± 1.6	3 ± 0.6
4d		F	8 ± 0.2	30 ± 5
4f		OH	230 ± 16	16 ± 1.6
4h		F	240 ± 20	>100
isoCA-4		OH	2 ± 0.2	2 ± 0.3
CA-4		OH	2 ± 0.2	2 ± 0.3

[a] HCT116 human colon carcinoma cells. [b] GI_{50}: compound concentration required to decrease cell growth by 50% following 72 h treatment with the tested drug; values represent the average ± SD of three experiments. [c] nt = not tested. [d] ITP: inhibition of tubulin polymerization; IC_{50}: compound concentration required to decrease the rate of microtubule assembly by 50%; values represent the average ± SD of three experiments.

Table 3 *In vitro* cell growth inhibitory effects of compounds **4b**, **4d–e** and isoCA-4.

	GI_{50}[a] (nM)	
Compd	K562[b]	H1299[b]
4b	32 ± 2	25 ± 1.8
4d	39 ± 2.2	60 ± 4.5
4e	40 ± 2.4	43 ± 2.6
isoCA-4	2.2 ± 0.2	4 ± 0.21

[a] GI_{50}: compound concentration required to decrease cell growth by 50% following 72 h treatment with the tested drug; values represent the average ± SD of three experiments. [b] K562: myelogenous leukemia; H1299: non-small-cell lung carcinoma.

activity. Accordingly, a careful separation of the two isomers of compound **4e** was achieved using the HPLC technique. Interestingly, we found that both isomers *E*-**4e** and *Z*-**4e** showed similar antiproliferative potencies against HCT116 cells with GI_{50} values of 81 and 78 nM, respectively. In addition, *E*-**4e** and *Z*-**4e** inhibited tubulin assembly with mean IC_{50} values of 3.0 μM.

Cell cycle analysis

CA-4 is well-known to block the cell cycle in the G_2/M phase due to microtubule depolymerization and cytoskeleton disruption. The cell growth inhibitory potency of the two most active compounds **4b** and **4e** prompted us to evaluate their effects on the cell cycle by flow cytometry (Fig. 2). The lowest concentration of 5 nM induced no significant distribution of K562 cells through the cell cycle, whereas at a 10-fold higher concentration (50 nM) **4b** and **4e** arrested the majority of cells in the G_2/M phase (73% for **4b**, 90% for **4e**). The observed effects on cell cycle progression correlated well with their strong antiproliferative and antitubulin activities. These results are in agreement with the similar properties previously reported for most of the antimitotic agents.

Molecular modeling

Molecular docking simulations were performed on this series of compounds to examine the interactions of the subject compounds in the colchicine binding site of tubulin. The X-ray crystal structure of tubulin (resolution = 3.58 Å) was obtained from the protein database (PDB ID: 1SA0).[4a]

The docking poses observed for compounds **3j**, **3k**, **3l**, **4b** are depicted in green in Fig. 3, superimposed with isoCA-4 in gray. In the calculated binding mode, the overall orientation of these inhibitors in the colchicine site was found to be similar. In particular, a feature common to all the compounds was the trimethoxyphenyl ring being located in a hydrophobic pocket close to Cys β241.[33] Additionally, Cys β241 may form a hydrogen bond with the oxygen atom of the 4′-OMe in the A-ring of all these molecules. These interactions are consistent with the binding mode of the colchicines site inhibitors studied by Nguyen *et al.*[33] or CA-4 in Brown's work.[34] A hydrogen bond between the 3′-hydroxyl group and the backbone oxygen from

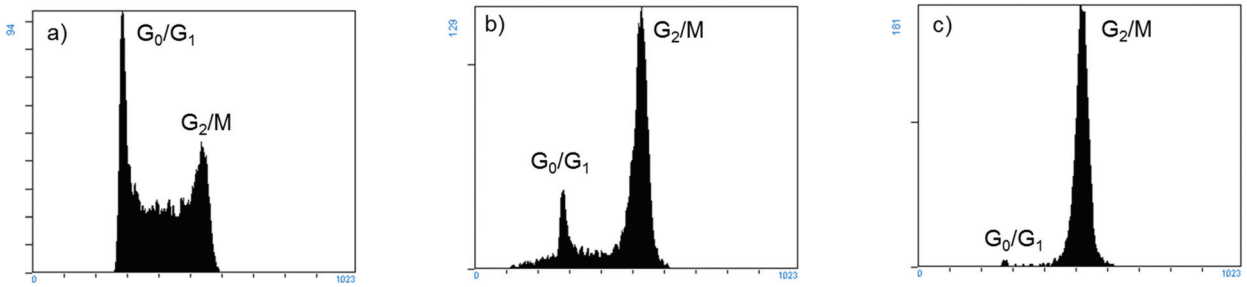


Fig. 2 Effect on cell cycle distribution in K562 as determined by flow cytometry: (a) untreated cells; (b) **4b** at a concentration of 50 nM; (c) **4e** at a concentration of 50 nM.

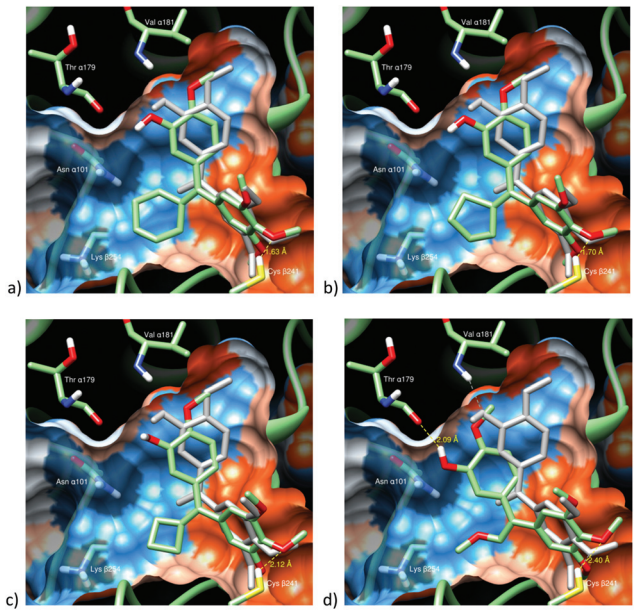


Fig. 3 Putative binding mode of compounds **3j** (a), **3k** (b), **3l** (c) and **4b** (d) in the colchicine binding site. The isoCA-4 pose is overlaid in gray for reference. The solvent accessible surface color code is orange for hydrophobic and blue for polar.

Thr α179 could be observed in the case of 1,1-diaryl-2-methoxyethylene **4b**. However, neither **4b**, nor **3j**, **3k** or **3l** showed the interaction with the Val α181 backbone found with isoCA-4. Interestingly, in the calculated binding mode, the linker moiety interacts with amino acids having polar or charged side chains (Asn α101 or Lys β254), thereby giving rise to unfavorable interactions when the linker is hydrophobic. This hypothesis correlates well with experimental results, since decreasing the size of the nonpolar linker group (*e.g.*, from **3j** to **3l**) or replacing it with a less hydrophobic group (*e.g.*, **4b** with the enol ether linker) was found to be beneficial for activity.

Conclusions

In summary, we have synthesized a series of novel polysubstituted olefins bearing structural similarity to isoCA-4. From a chemical point of view, the preparation of these compounds was particularly simple and was carried out in good yields by a convergent palladium-catalyzed coupling of *N*-tosylhydrazones with aryl halides. With respect to linker modifications, RSA studies showed that trisubstituted olefins **4** were more active than tetrasubstituted derivatives **3**, indicating that the colchicine binding site does not accommodate a bulky lipophilic linker group between the two aromatic rings. The polarity of the linker therefore appears to be crucial for activity, possibly due to this moiety sitting in a hydrophilic subpocket, as hypothesized by molecular modeling. Compounds **4b** and **4e** showed potent inhibitory effects on *in vitro* tubulin polymerization as well as on the growth of a variety of human tumor cell lines. In general, there was a good correlation between the results of the two assays, except for compound **4d** which displayed a good antiproliferative effect but was much less effective at inhibiting tubulin polymerization. In this case, another mechanism of action may be responsible for the activity of this compound. Among the three lead compounds, **4b** was the most potent analogue, with IC_{50} values ranging from 24 to 32 nM. Compound **4b** also had excellent potency as an inhibitor of tubulin polymerization (IC_{50} = 2 μM) in the same range as the values obtained with CA-4 and isoCA-4. On the basis of these excellent properties, this compound is worthy of further *in vitro* and *in vivo* evaluation.

Experimental

Chemistry

Melting points (m.p.) 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), a Bruker AVANCE 300 or a Bruker AVANCE 400 (^{1}H, 400 MHz; ^{13}C, 100 MHz). Chemical shifts δ are given in ppm, and the following abbreviations are used: singlet (s), doublet (d), triplet (t), multiplet (m) and broad singlet (bs). IR spectra were measured on a Bruker Vector 22 spectrophotometer (neat, cm^{-1}). Low resolution mass spectra (*m*/*z*) were recorded on a Bruker Esquire electrospray ionization apparatus. High resolution mass spectra were recorded on a MicrotofQ Bruker Daltonics. Reaction courses and product mixtures were routinely monitored by TLC on silica gel (precoated F254 Merck plates), and compounds were visualized with a UVP Mineralight UVGL-58

lamp (254 nm) and with phosphomolybdic acid/Δ, anisaldehyde/Δ, or vanillin/Δ. Flash chromatography was performed using silica gel 60 (40–63 mm, 230–400 mesh ASTM) at medium pressure (200 mbar). Dioxane, dichloromethane, cyclohexane and tetrahydrofuran were dried using the procedures described in D. Perrin, *Purification of Laboratory Chemicals*, Pergamon Press Ltd, 2nd edn, 1980. Organic extracts were, in general, dried over $MgSO_4$ or Na_2SO_4. All products reported showed ^1H NMR spectra in agreement with the assigned structures.

General procedure for preparation of hydrazones[35]

To a rapidly stirred suspension of *p*-toluenesulfonylhydrazide (5 mmol) in dry methanol (10 mL) was added ketone (5.5 mmol) portionwise. Within 5–10 min the tosylhydrazone began to precipitate as a mixture of *E*- and *Z*-isomers. After approximately 30 min the mixture was cooled to 0 °C and the product removed by filtration, washed with a small quantity of methanol and then dried under vacuum.

9a (*Z*/*E*) 1-(2-Methoxy-1-(3,4,5-trimethoxyphenyl)-ethylidene)-2-tosylhydrazine was obtained as a mixture of two diastereoisomers (*Z*/*E* = 75/25); yield 83%; white solid; m.p.: 118–119 °C; R_f = 0.67 (EtOAc); IR (thin film, neat) ν_{max}/cm^{-1}: 2256, 2178, 2159, 2041, 1997, 1597, 1576, 1509, 1466, 1452, 1414, 1333, 1237, 1187, 1166, 1127, 1081, 1056, 1003, 954, 880, 846, 816, 784, 705, 662; Major *Z* isomer: (*Z*)-1-(2-methoxy-1-(3,4,5-trimethoxyphenyl)ethylidene)-2-tosylhydrazine: ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 9.63 (s, 1H), 7.88 (d, *J* = 8.2 Hz, 2H), 7.30 (d, *J* = 8.2 Hz, 2H), 6.77 (s, 2H), 4.52 (s, 2H), 3.86 (s, 6H), 3.85 (s, 3H), 3.35 (s, 3H), 2.41 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 153.2 (2C), 148.8 (C), 144.1 (C), 139.9 (C), 135.8 (C), 131.3 (C), 129.6 (2CH), 128.1 (2CH), 104.0 (2CH), 69.5 (CH_2), 60.9 (OCH_3), 58.9 (OCH_3), 56.3 ($2OCH_3$), 21.6 (CH_3); Minor *E* isomer (only the most significant resonances are listed): (*E*)-1-(2-methoxy-1-(3,4,5-trimethoxyphenyl)ethylidene)-2-tosylhydrazine: ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.75 (s, 1H), 6.40 (s, 2H), 4.18 (s, 2H); MS (APCI positive, *m*/*z*): 409 $[M + H]^+$; HRMS found (ESI) $(M + Na)^+$ 431.1263 $C_{19}H_{24}N_2O_6NaS$ requires 431.1253.

9b (*Z*/*E*)-1-(2-Ethoxy-1-(3,4,5-trimethoxyphenyl)-ethylidene)-2-tosylhydrazine was obtained as a mixture of two diastereoisomers (*Z*/*E* = 85/15); yield 90%; white solid; m.p.: 111–112 °C; R_f = 0.86 (EtOAc); IR (thin film, neat) ν_{max}/cm^{-1}: 3449, 3332, 2834, 2364, 2208, 2159, 2043, 1769, 1695, 1601, 1580, 1538, 1507, 1462, 1414, 1368, 1335, 1265, 1202, 1168, 1128, 1060, 1030, 1001, 877, 814, 761, 665, 654, 636; Major *Z* isomer: (*Z*)-1-(2-ethoxy-1-(3,4,5-trimethoxyphenyl)ethylidene)-2-tosylhydrazine: ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 9.80 (s, 1H), 7.87 (d, *J* = 8.2 Hz, 2H), 7.29 (d, *J* = 8.2 Hz, 2H), 6.76 (s, 2H), 4.55 (s, 2H), 3.85 (s, 6H), 3.83 (s, 3H), 3.47 (q, *J* = 7.0 Hz, 2H), 2.40 (s, 3H), 1.21 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 153.2 (2C), 149.1 (C), 144.1 (C), 139.9 (C), 135.9 (C), 131.4 (C), 129.6 (2CH), 128.1 (2CH), 104.0 (2CH), 73.7 (CH_2), 67.6 (CH_2), 61.0 (OCH_3), 56.4 ($2OCH_3$), 21.7 (CH_3), 15.2 (CH_3). Minor *E* isomer (only the most significant resonances are listed): (*E*)-1-(2-ethoxy-1-(3,4,5-trimethoxy-phenyl)-ethylidene)-2-tosylhydrazine: ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.80 (d, *J* = 7.0 Hz, 2H), 6.40 (s, 2H), 4.20 (s, 2H), 3.36 (q, *J* = 7.0 Hz, 2H), 2.43 (s, 3H), 1.09 (t, *J* = 7.0 Hz, 3H); MS (APCI positive, *m*/*z*): 423 $[M + H]^+$; HRMS found (ESI) $(M + H)^+$ 423.1593 $C_{20}H_{27}N_2O_6S$ requires 423.1590.

9c (*Z*/*E*)-1-(2-Methoxy-1-(3,4,5-trimethoxyphenyl)propylidene)-2-tosylhydrazine was obtained as a mixture of two diastereoisomers (*Z*/*E* = 60/40); yield 93%; white solid; m.p.: 133–135 °C; R_f = 0.59 (cyclohexane/EtOAc: 5/5); IR (thin film, neat) ν_{max}/cm^{-1}: 3407, 3377, 3315, 3255, 3202, 3150, 2867, 2832, 2702, 2492, 2227, 2192, 2109, 2025, 2002, 1947, 1581, 1506, 1466, 1452, 1415, 1337, 1239, 1169, 1128, 1002, 818, 781, 651, 626; Major *Z* isomer: (*Z*)-1-(2-methoxy-1-(3,4,5-trimethoxyphenyl)propylidene)-2-tosylhydrazine: ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 10.25 (s, 1H), 7.87 (d, *J* = 8.3 Hz, 2H), 7.31 (m, 2H), 6.74 (s, 2H), 4.55 (q, *J* = 6.5 Hz, 1H), 3.86 (s, 6H), 3.85 (s, 3H), 3.15 (s, 3H), 2.40 (s, 3H), 1.47 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 156.2 (C), 153.2 (2C), 144.1 (C), 139.9 (C), 135.9 (C), 131.6 (C), 129.6 (2CH), 128.1 (2CH), 103.9 (2CH), 78.2 (CH), 61.0 (OCH_3), 56.4 ($3OCH_3$), 21.7 (CH_3), 16.9 (CH_3); Minor *E* isomer (only the most significant resonances are listed): (*E*)-1-(2-methoxy-1-(3,4,5-trimethoxyphenyl)propylidene)-2-tosylhydrazine: ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.79 (d, *J* = 8.3 Hz, 2H), 7.61 (s, 1H), 6.28 (s, 2H), 4.04 (q, *J* = 6.5 Hz, 2H), 3.08 (s, 3H), 2.43 (s, 3H), 1.15 (d, *J* = 6.5 Hz, 3H); MS (APCI positive, *m*/*z*): 445 $[M + Na]^+$; HRMS found (ESI) $(M + Na)^+$ 445.1395 $C_{20}H_{26}N_2O_6NaS$ requires 445.1409.

Typical procedure for Pd-catalyzed *N*-tosylhydrazones coupling with aryl halides

Tosylhydrazone (1.2 mmol), $PdCl_2(MeCN)_2$ (0.05 mmol, 5 mol%), diphenylphosphinopropane (dppp) (0.1 mmol, 10 mol%), and 5 ml of dioxane were mixed under argon for 5 min at rt. Cs_2CO_3 (3.0 mmol) was then added, the reaction mixture was stirred for 1 min and arylhalide (1.0 mmol) was added. The mixture was stirred at 90 °C until the reaction completed by TLC analysis (3 to 4 h). The crude reaction mixture was allowed to cool to room temperature. EtOAc was added to the mixture, which was filtered through celite. The solvents were evaporated under reduced pressure and the crude residue was purified by flash chromatography on silica gel.

4a (Z/*E*)-1,2,3-Trimethoxy-5-(2-methoxy-1-(4-methoxyphenyl)vinyl)benzene was obtained as a mixture of two diastereoisomers (*Z*/*E* = 40/60); yield 98%; white oil; R_f = 0.38 (cyclohexane/EtOAc: 7/3); IR (thin film, neat) ν_{max}/cm^{-1}: 3473, 3444, 3305, 3244, 3113, 2994, 2942, 2837, 2557, 2338, 2230, 2197, 2165, 2143, 2073, 2032, 2018, 1970, 1944, 1728, 1631, 1606, 1579, 1510, 1464, 1451, 1412, 1376, 1329, 1289, 1254, 1237, 1177, 1154, 1127, 1104, 1035, 1007, 835, 794, 738, 712, 694, 645; Major *E* isomer: (*E*)-1,2,3-trimethoxy-5-(2-methoxy-1-(4-methoxyphenyl)vinyl)benzene: ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.30 (d, *J* = 8.8 Hz, 2H), 6.87 (d, *J* = 8.8 Hz, 2H), 6.54 (s, 1H), 6.50 (s, 2H), 3,78 (s, 3H), 3.74 (s, 9H), 3.72 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 159.1 (C), 154.2 (2C), 146.7 (CH), 137.3 (C), 134.5 (C), 131.7 (2CH), 131.0 (C), 120.6 (C), 114.5 (2CH), 106.8 (2CH), 60.6 (OCH_3), 60.5 ($2OCH_3$), 56.4

(2OCH_3); Minor *Z* isomer (only the most significant resonances are listed): (*Z*)-1,2,3-trimethoxy-5-(2-methoxy-1-(4-methoxyphenyl)vinyl)benzene: ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.15 (m, 2H), 6.64 (s, 2H), 6.47 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 159.5 (C), 153.8 (2C), 146.6 (CH), 134.6 (C), 133.6 (C), 130.1 (2CH), 120.5 (C), 114.0 (2CH), 108.6 (2CH); MS (APCI positive, *m*/*z*): 331 $[M + H]^+$; HRMS found (ESI) $(M + H)^+$ 331.1555 $C_{19}H_{23}O_5$ requires 331.1545.

4b (Z/*E*)-2-Methoxy-5-(2-methoxy-1-(3,4,5-trimethoxyphenyl)vinyl)phenol was obtained as a mixture of two diastereoisomers (*Z*/*E* = 45/55); yield 90%; yellow oil; R_f = 0.19 (cyclohexane/EtOAc: 7/3); IR (thin film, neat) ν_{max}/cm^{-1}: 3473, 3444, 3305, 3244, 3113, 2994, 2942, 2837, 2557, 2338, 2230, 2197, 2165, 2143, 2073, 2032, 2018, 1970, 1944, 1728, 1631, 1606, 1579, 1510, 1464, 1451, 1412, 1376, 1329, 1289, 1254, 1237, 1177, 1154, 1127, 1104, 1035, 1007, 835, 794, 738, 712, 694, 645; Spectroscopic NMR data for the mixture of isomers: ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.10–6.13 (m, 6H), 5.55 (br s, 1H), 3.98–3.62 (m, 15H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 153.1 (2C), 152.8 (2C), 145.7 (CH), 145.6 (C), 145.6 (CH), 145.5 (C), 145.4 (C), 145.1 (C), 137.0 (C), 136.9 (C), 136.4 (C), 133.8 (C), 133.3 (C), 131.0 (C), 121.8 (CH), 120.3 (C), 120.2 (C), 120.0 (CH), 116.2 (CH), 114.5 (CH), 110.6 (CH), 110.3 (CH), 107.4 (2CH), 106.0 (2CH), 60.7 (2OCH_3), 61.0 (2OCH_3), 56.2 (4OCH_3), 56.1 (2OCH_3); MS (APCI positive, *m*/*z*): 347 $[M + H]^+$; HRMS found (ESI) $(M + Na)^+$ 369.1328 $C_{19}H_{22}O_6Na$ requires 369.1314.

4c (Z/*E*)-1-Methoxy-4-(2-methoxy-1-(3,4,5-trimethoxy-phenyl)-vinyl)-2-nitrobenzene was obtained as a mixture of two diastereoisomers (*Z*/*E* = 30/70); yield 61%; white oil; R_f = 0.23 (cyclohexane/EtOAc: 7/3); IR (thin film, neat) ν_{max}/cm^{-1}: 3428, 3405, 3385, 3334, 3191, 2938, 2842, 2315, 2252, 2232, 2167, 2082, 2037, 2004, 1983, 1961, 1940, 1725, 1636, 1616, 1581, 1529, 1504, 1465, 1453, 1413, 1354, 1341, 1282, 1236, 1185, 1154, 1127, 1008, 910, 835, 763, 728, 680, 662, 624; Major *E* isomer: ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.01 (d, *J* = 2.1 Hz, 1H), 7.54 (dd, *J* = 8.2, 2.1 Hz, 1H), 7.01 (d, *J* = 8.2 Hz, 1H), 6.43 (s, 1H), 6.39 (s, 2H), 3.96 (s, 3H), 3.87 (s, 3H), 3.81 (s, 6H), 3.80 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 153.4 (2C), 151.3 (C), 147.1 (CH), 139.6 (C), 137.5 (C), 135.3 (CH), 135.1 (C), 130.4 (C), 126.8 (CH), 118.1 (C), 113.0 (CH), 106.1 (2CH), 61.1 (OCH_3), 61.0 (OCH_3), 56.7 (OCH_3), 56.3 (2OCH_3); Minor *Z* isomer (only the most significant resonances are listed): (*Z*)-1-methoxy-4-(2-methoxy-1-(3,4,5-trimethoxyphenyl)vinyl)-2-nitrobenzene: ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.74 (d, *J* = 2.2 Hz, 1H), 7.35 (dd, *J* = 8.2, 2.2 Hz, 1H), 6.56 (s, 2H), 6.43 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 153.1 (2C), 146.7 (=CH), 134.0 (CH), 126.8 (CH), 118.4 (C), 113.6 (CH), 107.3 (2CH). MS (APCI positive, *m*/*z*): 376 $[M + H]^+$; HRMS found (ESI) $(M + H)^+$ 376.1385 $C_{19}H_{22}NO_7$ requires 376.1396.

4d (*Z*/*E*)-5-(1-(3-Fluoro-4-methoxyphenyl)-2-methoxy-vinyl)-1,2,3-trimethoxybenzene was obtained as a mixture of two diastereoisomers (*Z*/*E* = 56/44); yield 58%; yellow oil; *R* = 0.46 (cyclohexane/EtOAc: 7/3); IR (thin film, neat) ν_{max}/cm^{-1}: 3383, 3283, 3268, 3179, 2940, 2358, 2199, 2166, 2143, 2033, 2017, 1637, 1580, 1516, 1507, 1466, 1452, 1412, 1340, 1270, 1244, 1234, 1127, 1101, 1030, 1007, 924, 761, 731, 680, 663, 635; Spectroscopic NMR data for the mixture of isomers: ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.25 (dd, *J* = 13.0, 2.0 Hz, 1H), 7.09 (m, 1H), 6.91 (m, 1H), 6.41 (s, 2H), 6.39 (s, 1H), 3.89–3.70 (m, 15H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 153.2 (2C), 151.2 (C), 146.2 (CH), 146.0 (C), 137.2 (C), 135.9 (C), 130.8 (C), 125.6 (CH), 119.4 (C), 117.5 (CH), 112.9 (CH), 106.1 (2CH), 61.0 (OCH_3), 60.8 (OCH_3), 56.4 (OCH_3), 56.3 (2OCH_3); Minor *E* isomer (only the most significant resonances are listed): (*E*)-5-(1-(3-fluoro-4-methoxyphenyl)-2-methoxyvinyl)-1,2,3-trimethoxybenzene: ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 6.59 (s, 2H), 6.38 (s, 1H). MS (APCI positive, *m*/*z*): 349 $[M + H]^+$; HRMS found (ESI) $(M + H)^+$ 349.1450 $C_{19}H_{22}O_5F$ requires 349.1451.

4e (*Z*/*E*)-2-Methoxy-5-(2-methoxy-1-(3,4,5-trimethoxy-phenyl)-vinyl)benzenamine was obtained as a mixture of two diastereoisomers (*Z*/*E* = 60/40); yield 74%; yellow oil; R_f = 0.19 (cyclohexane/EtOAc: 7/3); IR (thin film, neat) ν_{max}/cm^{-1}: 3488, 3449, 3425, 2235, 2163, 2071, 2014, 1995, 1906, 1700, 1578, 1504, 1464, 1449, 1410, 1376, 1246, 1206, 1175, 1149, 1124, 1103, 1050, 1026, 1004, 924, 680, 638, 616; Major *Z* isomer: (*Z*)-2-methoxy-5-(2-methoxy-1-(3,4,5-trimethoxyphenyl)vinyl)benzenamine: ^{1}H NMR (300 MHz, CD_3COCD_3) δ (ppm): 6.79–6.71 (m, 1H), 6.66 (s, 2H), 6.58 (d, *J* = 2.2 Hz, 1H), 6.48 (m, 1H), 6.41 (s, 1H), 4.32 (s, 2H), 3.85–3.67 (m, 15H); ^{13}C NMR (100 MHz, CD_3COCD_3) δ (ppm): 153.7 (2C), 146.9 (C), 146.1 (CH), 138.1 (C), 137.7 (C), 134.8 (C), 134.0 (C), 121.4 (C), 117.8 (CH), 115.2 (CH), 111.0 (CH), 108.7 (2CH), 60.5 (OCH_3), 60.4 (OCH_3), 56.4 (2OCH_3), 55.8 (OCH3); Minor *E* isomer (only the most significant resonances are listed): (*E*)-2-methoxy-5-(2-methoxy-1-(3,4,5-trimethoxyphenyl)vinyl)benzenamine: ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm) 6.50 (s, 2H), 6.46 (s, 1H), 4.26 (s, 2H); MS (APCI positive, *m*/*z*): 346 $[M + H]^+$; HRMS found (ESI) $(M + H)^+$ 346.1655 $C_{19}H_{24}NO_5$ requires 346.1654.

***Z*-4e (*Z*)-2-Methoxy-5-(2-methoxy-1-(3,4,5-trimethoxy-phenyl)-vinyl)benzenamine** was obtained from the *Z*/*E* mixture of **4e**, separation was performed using an HPLC Sunfire C18 column (19 × 150 mm, 5 μm) in isocratic mode, by using a mobile phase containing H_2O/MeOH 45/55; IR (thin film, neat) ν_{max}/cm^{-1}: 3484, 3461, 3412, 3368, 3351, 3334, 3250, 3231, 2932, 2867, 2589, 2364, 2250, 2230, 2164, 2023, 1985, 1954, 1680, 1597, 1579, 1450, 1412, 1248, 1208, 1177, 1149, 1127, 1068, 1027, 1003, 973, 806, 772, 757, 701, 633, 618; ^{1}H NMR (300 MHz, CD_3COCD_3) δ (ppm): 6.74 (d, *J* = 8.2 Hz, 1H), 6.65 (s, 2H), 6.56 (d, *J* = 2.2 Hz, 1H), 6.47 (dd, *J* = 8.2, 2.1 Hz, 1H), 6.40 (s, 1H), 4.31 (s, 2H), 3.81 (s, 3H), 3.73 (s, 3H), 3.72 (s, 9H); ^{13}C NMR (75 MHz, CD_3COCD_3) δ (ppm): 153.7 (2C), 146.9 (C), 146.2 (CH), 138.1 (C), 138.0 (C), 134.8 (C), 134.0 (C), 121.3 (C), 117.8 (CH), 115.2 (CH), 111.0 (CH), 108.7 (2CH), 60.5 (OCH_3), 56.4 (3 OCH_3), 55.8 (OCH_3); MS (APCI positive, *m*/*z*): 346 $[M + H].^+$

***E*-4e (*E*)-2-Methoxy-5-(2-methoxy-1-(3,4,5-trimethoxy-phenyl)-vinyl)aniline** was obtained from the *Z*/*E* mixture of **4e**, separation was performed using an HPLC Sunfire C18 column (19 × 150 mm, 5 μm) in isocratic mode, by using a mobile phase containing H_2O/MeOH 45:/55; IR (thin film, neat) ν_{max}/cm^{-1}: 3396, 3372, 3333, 2186, 2164, 2010, 1635, 1579, 1514, 1503, 1465, 1451, 1410, 1368, 1340, 1286, 1247, 1218, 1150, 1125, 1103,

1028, 1004, 833, 760, 736, 652; ^{1}H NMR (300 MHz, CD_3COCD_3) δ (ppm): 6.74 (s, 2H), 6.64 (dd, J = 8.5, 1.6 Hz, 1H), 6.49 (s, 2H), 6.46 (s, 1H), 3.70–3.82 (m, 15H); ^{13}C NMR (75 MHz, CD_3COCD_3) δ (ppm): 154.0 (C), 146.4 (CH), 137.7 (C), 137.3 (C), 131.5 (C), 121.4 (C), 120.1 (CH), 117.1 (CH), 110.6 (CH), 106.9 (2CH), 60.6 (OCH_3), 60.5 (OCH_3), 56.4 ($2OCH_3$), 55.8 (OCH_3); MS (APCI positive, m/z): 346 $[M + H]^+$.

4f (*Z*/*E*)-5-(2-Ethoxy-1-(3,4,5-trimethoxyphenyl)vinyl)-2-methoxyphenol was obtained as a mixture of two diastereoisomers (Z/*E* = 50/50); yield 65%; yellow oil; R_f = 0.26 (cyclohexane/EtOAc: 5/5); IR (thin film, neat) ν_{max}/cm^{-1}: 2838, 2359, 2154, 2022, 1633, 1580, 1507, 1465, 1412, 1339, 1271, 1249, 1197, 1170, 1126, 1101, 1054, 1029, 1005, 928, 810, 762, 723, 615; (*E*)-5-(2-ethoxy-1-(3,4,5-trimethoxyphenyl)vinyl)-2-methoxyphenol: ^{1}H NMR (400 MHz, CD_3COCD_3) δ (ppm): 6.98 (d, J = 1.8 Hz, 1H), 6.87 (d, J = 8.4 Hz, 1H), 6.82 (dd, J = 8.4, 1.8 Hz, 1H), 6.55 (s, 1H), 6.50 (s, 2H), 3.98 (m, 2H), 3.83–3.72 (m, 12H), 1.31 (m, 3H). Spectroscopic ^{13}C NMR data for the mixture of isomers: ^{13}C NMR (100 MHz, CD_3COCD_3) δ (ppm): 154.1 (2C), 153.7 (2C), 147.2 (C), 147.2 (C), 146.8 (C), 146.7 (C), 145.4 (CH), 145.3 (CH), 138.0 (C), 137.6 (C), 137.5 (C), 134.7 (C), 134.6 (C), 132.0 (C), 122.1 (CH), 120.6 (C), 120.5 (C), 120.3 (CH), 117.7 (CH), 116.1 (CH), 112.3 (CH), 111.8 (CH), 108.6 (2CH), 107.1 (2CH), 69.2 (2 CH_2), 60.6 (OCH_3), 60.5 (OCH_3), 56.4 ($2OCH_3$), 56.4 ($2OCH_3$), 56.3 (OCH_3), 56.2 (OCH_3), 15.8 (CH_3), 15.8 (CH_3); MS (ESI negative, m/z): 359 $[M - H]^-$; HRMS found (ESI) $(M + Na)^+$ 383.1483 $C_{20}H_{24}O_6Na$ requires 383.1471.

4g (Z/*E*)-5-(2-Ethoxy-1-(4-methoxy-3-nitrophenyl)vinyl)-1,2,3-trimethoxybenzene was obtained as a mixture of two diastereoisomers (*Z*/*E* = 20/80); yield 60%; white oil; R_f = 0.33 (cyclohexane/EtOAc: 7/3); IR (thin film, neat) ν_{max}/cm^{-1}: 3494, 3437, 3409, 2839, 2215, 2202, 2161, 2048, 2015, 1633, 1619, 1580, 1528, 1504, 1465, 1451, 1413, 1357, 1339, 1281, 1245, 1236, 1198, 1126, 1105, 1016, 911, 899, 826, 812, 729, 679, 654, 626; Major *E* isomer: (*E*)-5-(2-ethoxy-1-(4-methoxy-3-nitrophenyl)-vinyl)-1,2,3-trimethoxybenzene: ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.04 (d, J = 2.2 Hz, 1H), 7.56 (dd, J = 8.8, 2.2 Hz, 1H), 7.01 (d, J = 8.8 Hz, 1H), 6.48 (s, 1H), 6.39 (s, 2H), 4.02 (q, J = 7.1 Hz, 2H), 3.97–3.78 (m, 12H), 1.36 (t, J = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 153.3 (2C), 151.2 (C), 145.8 (CH), 139.4 (C), 137.3 (C), 135.3 (C), 135.2 (CH), 130.5 (C), 126.7 (CH), 117.8 (C), 112.9 (CH), 106.0 (2CH), 69.4 (CH_2), 61.0 (OCH_3), 56.6 (OCH_3), 56.3 ($2OCH_3$), 15.5 (CH_3); Minor isomer (only the most significant resonances are listed): (*Z*)-5-(2-ethoxy-1-(4-methoxy-3-nitrophenyl)vinyl)-1,2,3-trimethoxybenzene: ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.74 (d, J = 2.3 Hz, 1H), 6.61 (s, 2H); MS (APCI positive, m/z): 390 $[M + H]^+$; HRMS found (ESI) $(M + Na)^+$ 412.1384 $C_{20}H_{23}NO_7Na$ requires 412.1372.

4h (*Z*/*E*)-4-(2-Ethoxy-1-(3,4,5-trimethoxyphenyl)vinyl)-2-fluoro-1-methoxybenzene was obtained as a mixture of two diastereoisomers (*Z*/*E* = 60/40); yield 55%; white oil; R_f = 0.54 (cyclohexane/EtOAc: 7/3); IR (thin film, neat) ν_{max}/cm^{-1}: 3318, 2209, 2191, 2153, 2108, 1988, 1957, 1634, 1578, 1516, 1504, 1466, 1411, 1338, 1303, 1269, 1237, 1197, 1170, 1125, 1097, 1006, 924, 841, 813, 760, 724, 612; Major *Z* isomer: (*Z*)-4-(2-ethoxy-1-(3,4,5-trimethoxyphenyl)vinyl)-2-fluoro-1-methoxybenzene: ^{1}H NMR (400 MHz, CD_3COCD_3) δ (ppm): 7.25 (dd, J = 13.5, 2.0 Hz, 1H), 7.14–6.95 (m, 2H), 6.59 (s, 1H), 6.51 (s, 2H), 4.03 (q, J = 7.0 Hz, 2H), 3.88–3.76 (m, 12H), 1.32 (t, J = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, CD_3COCD_3) δ (ppm): 154.5 (2C), 153.0 (C), 147.1 (C), 146.3 (CH), 139.2 (C), 132.5 (C), 137.0 (C), 126.7 (CH), 119.8 (C), 118.1 (CH), 114.4 (CH), 108.1 (2CH), 69.6 (CH_2), 60.8 (OCH_3), 57.0 ($2OCH_3$), 56.8 (OCH_3), 15.8 (CH_3); Minor isomer (only the most significant resonances are listed): (*E*)-4-(2-ethoxy-1-(3,4,5-trimethoxyphenyl)vinyl)-2-fluoro-1-methoxybenzene: ^{1}H NMR (400 MHz, CD_3COCD_3) δ (ppm): 6.70 (s, 2H), 1.32 (t, J = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, CD_3COCD_3) δ (ppm): 146.1 (CH), 134.2 (C), 125.1 (CH), 115.0 (CH), 109.4 (2CH), 69.6 (CH_2), 15.8 (CH_3); MS (APCI positive, m/z): 385 $[M + H]^+$; HRMS found (ESI) $(M + Na)^+$ 385.1419 $C_{20}H_{23}O_5FNa$ requires 385.1427.

4i (*Z*/*E*)-1,2,3-Trimethoxy-5-(2-methoxy-1-(4-methoxy-phenyl)-prop-1-enyl)benzene was obtained as a mixture of two diastereoisomers (*Z*/*E* = 50/50); yield 60%; yellow oil; R_f = 0.45 (cyclohexane/EtOAc: 5/5); IR (thin film, neat) ν_{max}/cm^{-1}: 3201, 2936, 2835, 2364, 2171, 1606, 1579, 1507, 1464, 1448, 1410, 1345, 1290, 1267, 1233, 1172, 1124, 1063, 1035, 1007, 975, 838, 807, 787, 762, 720, 689, 662; Spectroscopic NMR data for the mixture of isomers: ^{1}H NMR (400 MHz, CD_3COCD_3) δ (ppm): δ 7.20 (d, J = 8.9 Hz, 2H), 7.08 (d, J = 8.6 Hz, 2H), 6.89 (d, J = 8.6 Hz, 2H), 6.79 (d, J = 8.9 Hz, 2H), 6.54 (s, 2H), 6.43 (s, 2H), 3.82–3.65 (m, 24H), 3.60 (s, 3H), 3.57 (s, 3H), 1.93 (s, 3H), 1.91 (s, 3H); ^{13}C NMR (100 MHz, CD_3COCD_3) δ (ppm): 159.2 (C), 158.6 (C), 154.2 (2C), 153.5 (2C), 151.2 (C), 150.7 (C), 138.8 (C), 138.0 (C), 137.6 (C), 137.3 (C), 135.1 (C), 133.6 (C), 132.3 (2CH), 131.5 (2CH), 122.1 (2C), 114.4 (2CH), 113.7 (2CH), 109.0 (2CH), 108.7 (2CH), 60.6 (OCH_3), 60.5 (OCH_3), 56.5 ($2OCH_3$), 56.4 ($2OCH_3$), 56.2 (OCH_3), 56.0 (OCH_3), 55.5 (OCH_3), 55.4 (OCH_3), 16.3 (CH_3), 16.1 (CH_3); MS (APCI positive, m/z): 367 $[M + H]^+$; HRMS found (ESI) $(M + Na)^+$ 367.1532 $C_{20}H_{24}O_5Na$ requires 367.1521.

4j (*Z*/*E*)-2-Methoxy-5-(2-methoxy-1-(3,4,5-trimethoxy-phenyl)-prop-1-enyl)phenol was obtained as a mixture of two diastereoisomers (*Z*/*E* = 40/60); yield 64%; yellow oil; R_f = 0.32 (cyclohexane/EtOAc: 7/3); IR (thin film, neat) ν_{max}/cm^{-1}: 3477, 3342, 2836, 2142, 2034, 2016, 1701, 1579, 1504, 1465, 1450, 1409, 1345, 1281, 1252, 1232, 1167, 1124, 1027, 1001, 929, 880, 826, 813, 761, 721, 664, 636; Major *E* isomer: ^{1}H NMR (400 MHz, CD_3COCD_3) δ (ppm): 7.46 (s, 1OH), 6.84 (d, J = 2.0 Hz, 1H), 6.79 (d, J = 8.4 Hz, 1H), 6.70 (dd, J = 8.4, 2.0 Hz, 1H), 6.43 (s, 2H), 3.80 (s, 3H), 3.77 (s, 6H), 3.73 (s, 3H), 3.57 (s, 3H), 1.91 (s, 3H); ^{13}C NMR (100 MHz, CD_3COCD_3) δ (ppm): 154.1 (2C), 150.7 (C), 146.4 (C), 138.9 (C), 137.9 (C), 134.4 (C), 122.4 (C), 121.9 (CH), 117.6 (CH), 111.6 (CH), 108.9 (2CH), 60.6 (OCH_3), 56.5 ($2OCH_3$), 56.2 (OCH_3), 56.1 (OCH_3), 16.4 (CH_3); Minor *Z* isomer (only the most significant resonances are listed): (*Z*)-2-methoxy-5-(2-methoxy-1-(3,4,5-trimethoxy-phenyl)-prop-1-enyl)phenol: ^{1}H NMR (400 MHz, CD_3COCD_3) δ (ppm): 6.55 (s, 2H), 1.93 (s, 3H); ^{13}C NMR (100 MHz, CD_3COCD_3) δ (ppm): 153.5 (2C), 122.3 (C), 118.2 (CH), 112.1 (CH), 108.7 (2CH), 16.2 (CH_3); MS (ESI positive, m/z): 383 $[M + Na]^+$;

HRMS found (ESI) $(M + Na)^+$ 383.1467 $C_{20}H_{24}O_6Na$ requires 383.1471.

Typical procedure for cleavage of MOM protecting group

A solution of protected alcohol (1 equiv.) in 14 mL of EtOH and 2 mL of DCM were added to *p*-toluene sulfonic acid (6 equiv.). The temperature was raised to 60 °C for 2.5 h and then the mixture was hydrolysed after cooling to rt. The aqueous phase was extracted with ethyl acetate. The organic phase was dried over $MgSO_4$ then concentrated. The crude residue was then purified on silica gel to give the desired phenol product.

3j 5-(Cyclohexylidene(3,4,5-trimethoxyphenyl)methyl)-2-methoxyphenol: yield 90%; colorless oil; R_f = 0.18 (cyclohexane/EtOAc: 8/2); IR (thin film, neat) ν_{max}/cm^{-1}: 3430, 2923, 1579, 1504, 1448, 1409, 1342, 1282, 1226, 1169, 1123, 1011, 910, 799, 760, 728, 646; ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 6.76 (d, J = 8.2 Hz, 1H), 6.73 (d, J = 1.7 Hz, 1H), 6.62 (dd, J = 8.2, 1.7 Hz, 1H), 6.32 (s, 2H), 5.55 (brs, 1H, OH), 3.85 (s, 3H), 3.82 (s, 3H), 3.78 (s, 6H), 2.32–2.12 (m, 4H), 1.70–1.50 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 152.8 (2C), 145.1 (C), 145.0 (C), 139.0 (2C), 136.5 (C), 136.3 (C), 134.2 (C), 121.2 (CH), 115.9 (CH), 110.0 (CH), 106.7 (2CH), 60.8 (OCH_3), 56.0 ($2OCH_3$), 55.8 (OCH_3), 32.8 (CH_2), 32.5 (CH_2), 28.9 (CH_2), 28.8 (CH_2), 26.9 (CH_2); MS (APCI positive, m/z, %): 385.2 $[M + H]^+$; HRMS found (ESI) $(M + Na)^+$ 407.1818 $C_{23}H_{28}O_5Na$ requires 407.1829.

3k 5-(Cyclopentylidene(3,4,5-trimethoxyphenyl)methyl)-2-methoxyphenol: yield 82%; colorless oil; R_f = 0.19 (cyclohexane/EtOAc: 8/2); IR (thin film, neat) ν_{max}/cm^{-1}: 3463, 2945, 1579, 1504, 1451, 1409, 1344, 1276, 1232, 1121, 1009, 911, 799, 760, 730, 647; ^1H NMR (200 MHz, $CDCl_3$) δ (ppm): 6.81 (d, J = 2.2 Hz, 1H), 6.76 (s, 1H), 6.68 (dd, J = 8.3, 2.0 Hz, 1H), 6.39 (s, 2H), 5.59 (s, 1H), 3.86 (s, 3H), 3.84 (s, 3H), 3.78 (s, 6H), 2.52–2.22 (m, 4H), 1.79–1.58 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 152.7 (2C), 144.9 (C), 144.8 (C), 142.8 (C), 139.3 (C), 136.5 (C), 136.2 (C), 132.5 (C), 120.7 (CH), 115.3 (CH), 110.1 (CH), 106.3 (2CH), 60.8 (OCH_3), 56.0 ($2OCH_3$), 55.8 (OCH_3), 33.4 (CH_2), 33.2 (CH_2), 26.9 (CH_2), 26.8 (CH_2); MS (APCI positive, m/z): 371.3 $[M + H]^+$; HRMS found (ESI) $(M + H)^+$ 371.1842 $C_{22}H_{27}O_5$ requires 371.1853.

3l 5-(Cyclobutylidene(3,4,5-trimethoxyphenyl)methyl)-2-methoxyphenol: yield 75%; colorless oil; R_f = 0.23 (cyclohexane/EtOAc: 8/2); IR (thin film, neat) ν_{max}/cm^{-1}: 3463, 2945, 1579, 1504, 1451, 1409, 1344, 1276, 1232, 1121, 1009, 911, 799, 760, 730, 647; ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 6.83 (d, J = 1.9 Hz, 1H), 6.71–6.68 (m, 2H), 6.43 (s, 2H), 5.51 (s, 1H), 3.85 (s, 3H), 3.83 (s, 3H), 3.66 (s, 6H), 2.97–2.77 (m, 4H), 2.10–1.94 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 152.8 (2C), 145.5 (C), 145.3 (C), 140.1 (C), 137.3 (C), 136.3 (C), 134.1 (C), 132.1 (C), 120.4 (CH), 114.5 (CH), 110.5 (CH), 105.5 (2CH), 61.0 (OCH_3), 56.1 (OCH_3), 56.0 ($2OCH_3$), 39.4 (CH_2), 39.0 (CH_2), 22.1 (CH_2); MS (ESI negative, m/z): 355.4 $[M - H]^-$; HRMS found (ESI) $(M + H)^+$ 357.1690 $C_{21}H_{25}O_5$ requires 357.1697.

3m 2-Methoxy-5-(2-methyl-1-(3,4,5-trimethoxyphenyl)prop-1-en-1-yl)phenol: yield 85%; colorless oil; R_f = 0.32 (cyclohexane/EtOAc: 7/3); IR (thin film, neat) ν_{max}/cm^{-1}: 3458, 2928, 1580, 1505, 1452, 1409, 1342, 1282, 1252, 1233, 1124, 1009, 911, 811, 760, 731, 668; ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): δ 6.78 (d, J = 8.2 Hz, 1H), 6.74 (d, J = 2 Hz, 1H), 6.64 (dd, J = 8.2, 2 Hz, 1H), 6.33 (s, 2H), 5.53 (s, 1H), 3.88 (s, 3H), 3.83 (s, 3H), 3.79 (s, 6H), 1.80 (s, 3H), 1.78 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 152.8 (2C), 145.03 (C), 145.0 (C), 139.3 (C), 136.8 (C), 136.5 (C), 136.4 (C), 130.8 (C), 121.5 (CH), 116.1 (CH), 110.2 (CH), 106.9 (2CH), 61.0 (OCH_3), 56.2 ($2OCH_3$), 56.0 (OCH_3), 22.8 (CH_3), 22.6 (CH_3); SM (APCI positive, m/z): 345.2 $[M + H]^+$; HRMS found (ESI) $(M + H)^+$ 345.1688 $C_{20}H_{25}O_5$ requires 345.1697.

Typical procedure for reduction of nitro-compounds into amines[36]

Iron powder (5.55 g, 99.3 mmol, 10 equiv.) and concd hydrochloric acid (*ca*. 50 mg) were added to a solution of nitro-compound (9.93 mmol) in EtOH (30 mL) and water (7.5 mL), and the mixture was heated to reflux for 90 min. EtOAc (150 mL) was added to the mixture, and it was dried with $MgSO_4$. After filtration and evaporation of the solvent, the residue was purified by chromatography to give the amino product.

3n 5-(Cyclohexylidene(3,4,5-trimethoxyphenyl)methyl)-2-methoxyaniline: yield 80%; colorless oil; *Rf* = 0.2 (cyclohexane/EtOAc: 8/2); IR (thin film, neat) ν_{max}/cm^{-1}: 3383, 2927, 2834, 1579, 1504, 1463, 1409, 1342, 1250, 1223, 1172, 1125, 1010, 911, 730, 647; ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 6.70 (d, J = 8.0 Hz, 1H), 6.58–6.44 (m, 2H), 6.32 (s, 2H), 3.83 (s, 6H), 3.79 (s, 6H), 2.36–2.14 (m, 4H), 1.73–1.49 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 152.7 (2C), 146.0 (C), 139.3 (C), 138.6 (C), 136.3 (C), 135.7 (C), 135.3 (C), 134.5 (C), 120.0 (CH), 116.8 (CH), 109.9 (CH), 106.8 (2CH), 61.0 (OCH_3), 56.2 ($2OCH_3$), 55.5 (OCH_3), 32.8 (CH_2), 32.6 (CH_2), 28.9 (CH_2), 28.8 (CH_2), 27.0 (CH_2). MS (APCI): 384.3 $[M + H]^+$; HRMS found (ESI) $(M + Na)^+$ 384.2167 $C_{23}H_{28}O_5Na$ requires 384.2175.

3o 5-(Cyclopentylidene(3,4,5-trimethoxyphenyl)methyl)-2-methoxyaniline: yield 83%; yellow oil; R_f = 0.18 (cyclohexane/EtOAc: 8/2); IR (thin film, neat) ν_{max}/cm^{-1}: 2940, 1579, 1504, 1462, 1409, 1343, 1230, 1180, 1123, 1008, 910, 800, 758, 647; ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 6.72 (d, J = 8.7 Hz, 1H), 6.63–6.58 (m, 2H), 6.39 (s, 2H), 3.83 (s, 6H), 3.78 (s, 6H), 2.51–2.17 (m, 4H), 1.72–1.55 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 152.8 (2C), 146.1 (C), 142.5 (C), 139.6 (C), 136.1 (C), 134.7 (C), 132.9 (C), 130.0 (C), 120.0 (CH), 116.5 (CH), 110.0 (CH), 106.4 (2CH), 60.9 (OCH_3), 56.1 ($2OCH_3$), 55.5 (OCH_3), 33.5 ($2CH_2$), 27.0 ($2CH_2$); MS (ESI): 370.4 $[M + H]^+$; HRMS found (ESI) $(M + H)^+$ 370.2005 $C_{22}H_{28}NO_4$ requires 370.2013.

3p 2-Methoxy-5-(2-methyl-1-(3,4,5-trimethoxyphenyl)prop-1-en-1-yl)aniline: yield 91%; yellow oil; R_f = 0.18 (cyclohexane/EtOAc: 8/2); IR (thin film, neat) ν_{max}/cm^{-1}: 3373, 2935, 2167, 2047, 1977, 1579, 1505, 1462, 1408, 1341, 1252, 1224, 1171, 1124, 1009, 827, 760, 726, 664; ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 6.71 (d, J = 8.0 Hz, 1H), 6.57–6.48 (m, 2H), 6.34 (s, 2H), 3.83 (s, 6H), 3.79 (s, 6H), 1.80 (s, 3H), 1.77 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 152.7 (2C), 146.0 (C), 139.5 (C), 137.1 (C), 136.3 (C), 136.0 (C), 135.4 (C), 130.3 (C), 120.1 (CH), 116.7 (CH), 109.9 (CH), 107.0 (2CH), 61.0 (OCH_3), 56.2

(2OCH_3), 55.5 (OCH_3), 22.8 (CH_3), 22.7 (CH_3). MS (APCI): 344.3 $[M + H]^+$; HRMS found (ESI) $(M + H)^+$ 344.1859 $C_{20}H_{26}NO_4$ requires 344.1856.

Biolology

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 H1299 cells were grown in Dulbecco minimal essential medium (DMEM) containing 4.5 g L^{-1} glucose supplemented with 10% FCS and 1% glutamine. Human K562 leukemia and HCT116 colorectal carcinoma cells were grown in RPMI 1640 containing 10% FCS and 1% glutamine. 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 (5×10^3 cells per 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 (λ_{ex} = 560 nm, λ_{em} = 590 nm) using a Victor microtiter plate fluorimeter (Perkin-Elmer, USA). The GI_{50} 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. The GI_{50} values for all compounds were compared to the GI_{50} of CA-4 and isoCA-4 and measured the same day under the same conditions.

Tubulin binding assay

Sheep brain tubulin was purified according to the method of Shelanski[37] 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^{-1}). Tubulin assembly was monitored by fluorescence according to the reported procedure[38] using DAPI as a fluorescent molecule. Assays were realized on 96-well plates prepared with a Biomek NKMC and a Biomek 3000 from Beckman Coulter and read at 37 °C on a Wallac Victor fluorimeter from Perkin Elmer. The IC_{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 the compound. The IC_{50} values for all compounds were compared to the IC_{50} of CA-4 and isoCA-4 and measured the same day under the same conditions.

Cell cycle analysis

Exponentially growing cancer cells K562 were incubated with compounds **4b** and **4e** at a concentration of 50 nM or DMSO for 24 h. Cell-cycle profiles were determined by flow cytometry on an FC500 flow cytometer (Beckman-Coulter, France) as described previously.[39]

Molecular modeling

Coordinates for compounds **3j**, **3k**, **3l**, and **4b** were generated using CORINA v3.44 software.[40] Molecules were then docked in the colchicine binding site between chains C and D from PDB structure 1SA0 using GOLD v5.1 software.[41] Conformational space exploration was oriented preferably towards the solutions that exhibited (1) a hydrogen bond between a methoxy group from the A-ring and the Cys β241 side chain and (2) a hydrogen bond between (a) an oxygen atom from the B-ring and the backbone polar hydrogen atom from Val α181 or (b) the hydrogen atom from the hydroxyl group and the backbone oxygen from Thr α179. For all these molecules, the highest scoring poses were the ones where the gem-diarylalkene scaffold orientation matched the most orientation reported for isoCA-4 in a previous work.[15] The ligands were extracted from the solutions, and subjected to an energy minimization at the B3LYP/6-31G* level. Little geometry change was observed, suggesting that calculated bound conformations are located in accessible areas of the corresponding free ligand conformational spaces (see ESI† for details). Structures of complexes were exported for further examination and depiction with Chimera v1.6.1 software,[42] including hydrogen bonds, close contact analysis and representation of the solvent-accessible surface colored according to polarity.

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Notes and references

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