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Isocombretastatins A: 1,1-Diarylethenes as potent inhibitors of tubulin polymerization and cytotoxic compounds

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ABSTRACT

Isocombretastatins A are 1,1-diarylethene isomers of combretastatins A. We have synthesized the isomers of combretastatin A-4, deoxycombretastatin A-4, 3-amino-deoxycombretastatin A-4 (AVE-8063), naphthylcombretastatin and the *N*-methyl- and *N*-ethyl-5-indolyl analogues of combretastatin A-4. Analogues with a 2,3,4-trimethoxyphenyl ring instead of the 3,4,5-trimethoxyphenyl ring have also been prepared. The isocombretastatins A strongly inhibit tubulin polymerization and are potent cytotoxic compounds, some of them with IC_{50}s in the nanomolar range. This new family of tubulin inhibitors shows higher or comparable potency when compared to phenstatin or combretastatin analogues. These results suggest that one carbon bridges with a geminal diaryl substitution can successfully replace the two carbon bridge of combretastatins and that the carbonyl group of phenstatins is not essential for high potency.

1. Introduction

The eukaryotic microtubule system is composed of α-tubulin and β-tubulin heterodimers arranged head to tail. Microtubules and their dynamics are involved in many essential cell functions, such as development and maintenance of shape, motility and intracellular transport, cell signaling and cell division.[1] Perturbation of tubulin polymerization equilibria can produce metaphase arrest, making microtubules attractive molecular targets for anticancer therapeutics.[2] Most of the antimitotic drugs in clinical use or in clinical development bind at three major binding sites on tubulin: the vinca, taxane, and colchicine sites.[3] The colchicine site at tubulin is very hydrophobic, as shown by the X-ray crystal structures of its complexes with DAMA-colchicine and podophyllotoxin.[4] Many natural and synthetic compounds of diverse origin and structure bind to the colchicine site and modify the polymerization of the protein, and several models have attempted to explain the structure–activity relationships of colchicine site ligands.[5]

Combretastatins are a family of compounds of natural origin which strongly inhibit tubulin polymerization through binding to the colchicine site of tubulin.[6] The most potent combretastatin analogues are substituted *cis*-stilbenes (Fig. 1), such as combretastatin A-1 and combretastatin A-4, whose phosphate prodrugs are currently in clinical development.[7] They have attracted much attention owing to their potent cytotoxic and vascular disrupting activities.[8] The cisoid disposition of the two aromatic rings is essential for the activity, and many attempts to prevent the easy isomerization from cis to trans olefins have been carried out, including the formation of small heterocycles or carbocycles on the bridge,[7] reduction of the number of atoms of the bridge,[9] or formation of macrocycles by linkage of the two aromatic rings.[10]

The phenstatins (Fig. 1) are bisarylketones which display high potency as tubulin polymerization inhibitors.[9] The phenstatins share many of the SARs of combretastatins, such as the importance of a trimethoxyphenyl ring (A) and the need for a non coplanar cisoid disposition of the two aromatic rings. The key structural elements for high activities of inhibitors of tubulin polymerization binding at the colchicine site have been analyzed, and, recently, a common pharmacophore has been proposed based on the consistent structural features and binding interactions found in docking models of structurally diverse colchicine site ligands.[5b,c] According to this pharmacophore, an explanation for the high potency of phenstatins has been put forward: the carbonyl oxygen would hydrogen bond to the backbone N–H group of residues

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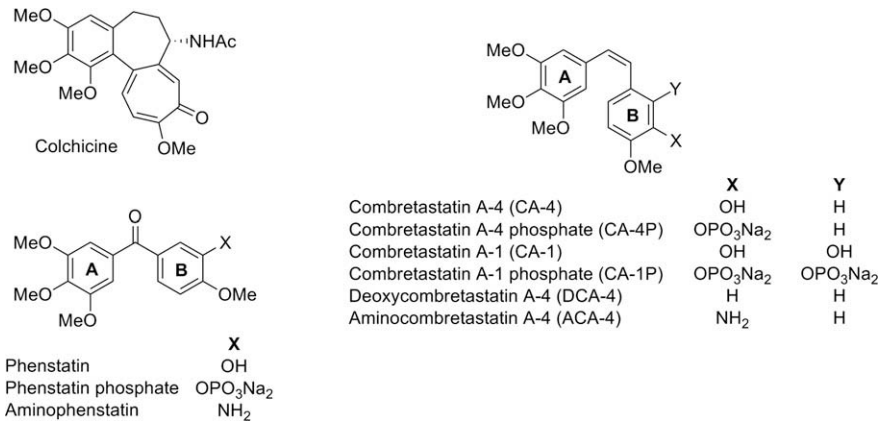


Figure 1. Structures of colchicine, combretastatins and phenstatins.

β248-β249-β250, thus providing one additional hydrogen bond acceptor site (point A3) in phenstatins. Results obtained by the modification of the carbonyl of phenstatins are also in agreement with this model.[11]

Following our research on tubulin polymerization inhibitors based on combretastatins and phenstatins, we have synthesized a new family of substituted 1,1-diarylethenes, which we have called isocombretastatins A[12] because they are structural isomers of well known, highly potent combretastatins A (*Z*-1,2-diarylethenes). In order to fairly compare isocombretastatins A with combretastatins A and phenstatins, we have synthesized and evaluated a series of isocombretastatins with aryl rings that render the combretastatins A and phenstatins more potent.[7,9,11] Thus, compounds with a 3,4,5-trimethoxyphenyl ring combined with either a 3-hydroxy-4-methoxyphenyl ring (CA-4 analogue), a 4-methoxyphenyl ring (deoxyCA-4 analogue), a 3-amino-4-methoxyphenyl ring (AVE-8063 analogue), a 2-naphthyl moiety (naphthylcombretastatin analogue) or a 1-methyl- or 1-ethyl-5-indolyl system have been synthesized, assayed and compared to their combretastatin and phenstatin analogues. Moreover, considering that the lack of the carbonyl oxygen on the bridge of phenstatins (considered to be an important pharmacophoric point by establishing hydrogen bonds) could somewhat release the anchor of the structure at that point and allow for a certain variation in the binding mode, the analogues with a 2,3,4-trimethoxyphenyl ring instead of the 3,4,5-trimethoxyphenyl ring have also been synthesized and evaluated. These later analogues would mimic the colchicine binding mode.

We have found that isocombretastatins display tubulin polymerization inhibitory potencies equal to or better than those of the parent combretastatins. They also have the advantage of not suffering from their *cis–trans* isomerization problems and, in terms of structure–activity relationships, they show that the optimal bridge length is not necessarily two atoms, and that the hydrogen bond acceptor capacity represented by the A3 site of the pharmacophoric model is not essential for high potency. Related substituted 1,1-diarylethenes have been previously shown to inhibit tubulin polymerization (Fig. 2), such as some alkylidenediarylmethanes (ADAMs) originally designed as HIV-RT inhibitors[13] and CC-5079,[14] and recently the first isocombretastatins A have appeared.[15] The series synthesized and assayed in this research enlarge the SAR of colchicines site ligands and expand the scope of isocombretastatins as potential tubulin polymerization inhibitors.

The 1,1-diarylmethanes were synthesized by Wittig reactions of methylenetriphenylphosphorane with appropriately substituted diarylketones. In turn, the phenones were obtained from diarylmethanols by PDC or $KMnO_4$ oxidation, following literature procedures (Scheme 1).[11]

The diarylmethanols were synthesized by reacting the aldehyde of one aromatic moiety with the organolithium or organomagnesium aromatic salts of the other. The organometallic and aromatic aldehyde combination used varied depending on the nature and reactivity of the aromatic systems implied. Thus, for the synthesis of compounds with two phenyl rings, one being 2,3,4-trimethoxyphenyl ring, the *ortho* chelating capability of the methoxy groups was used to generate the *ortho* lithiated species by treatment of 1,2,3-trimethoxybenzene with *n*-butyllithium. On the other hand, the diphenylmethanols with a 3,4,5-trimethoxyphenyl ring were synthesized by reaction of the benzenic aldehydes with the magnesium salt of 3,4,5-trimethoxyphenylbromide, in an attempt to minimize transmetallation reactions to the *ortho* metallated positions (Scheme 2).

Compounds with a naphthalene ring were synthesized by reaction of the naphthyllithium with appropriate aldehydes. Finally, compounds with a *N*-alkyl-5-indolyl moiety were synthesized from the 2,5-dilithium salt, in an effort to prevent transmetallation reactions and to force reaction from the 5-indole position. Functional group modifications at the ketone or olefin states, including phenol protection/deprotection steps and nitro group reductions, led to the desired substitutions on the phenyl rings.

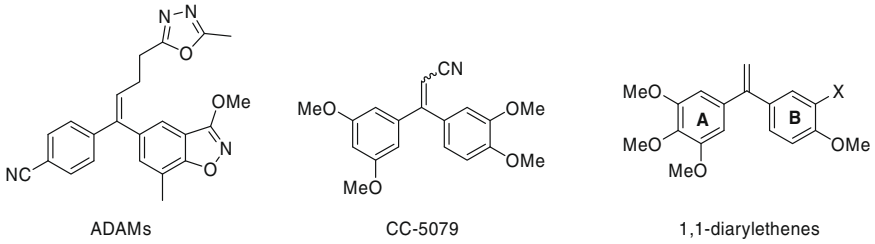
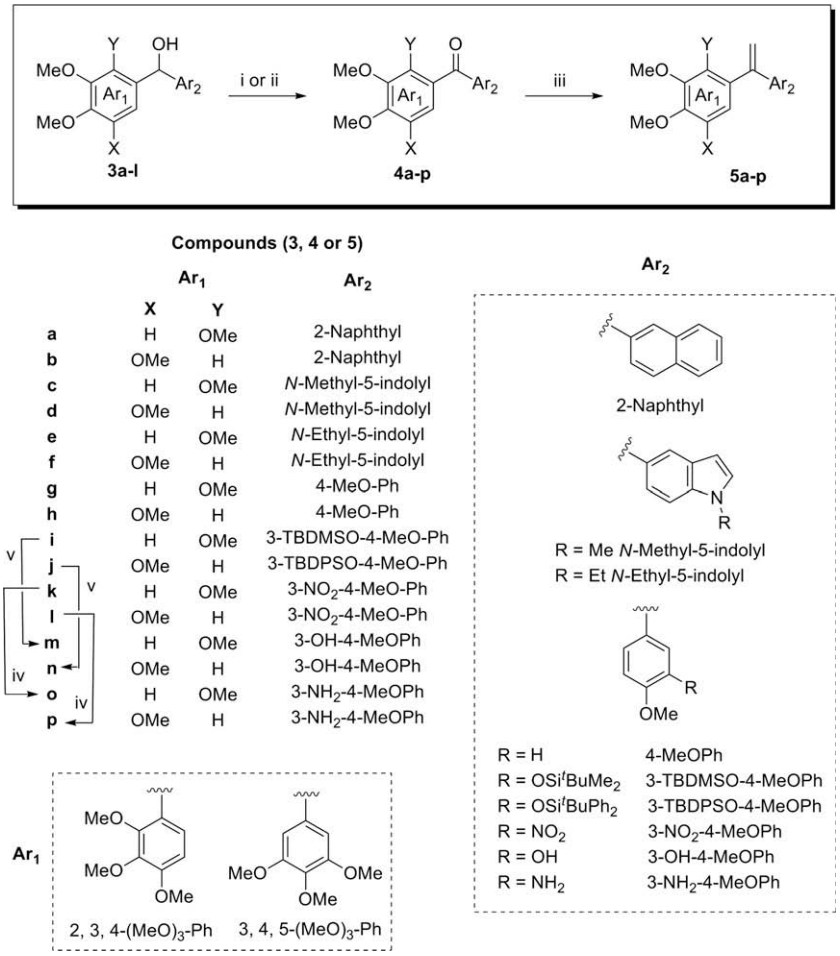


Figure 2. Structures of representative alkylidenediarylmethane inhibitors of tubulin polymerization (ADAMs and CC-5079) and the isocombretastatins A (1,1-diarylethenes).



Scheme 1. Synthesis of diarylketones **4a–p** and isocombretastatins A **5a–p**. Reagents and conditions: (i) $KMnO_4$, $HSO_4^- \cdot Bu_4N^+$, CH_2Cl_2; (ii) PDC, CH_2Cl_2; (iii) methyltriphenylphosponium iodide, THF, −40 °C, *n*BuLi, then ketone; (iv) **4**: Fe, EtOH:AcOH:H_2O, HCl or **5**: Zn, HCl, CH_2Cl_2:AcOH; (v) TBAF, THF.

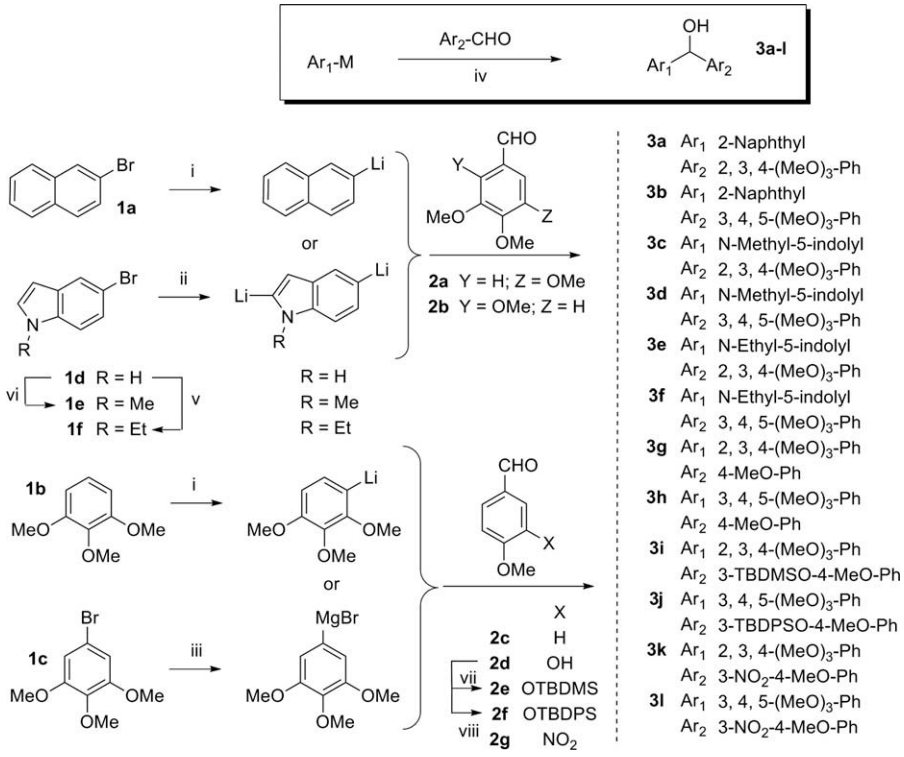
The synthesized compounds were tested at different concentrations in the tubulin polymerization assay and the IC_{50}s were determined as previously described.[11] Our results are shown in Table I, altogether with literature values, with whom they are in good agreement.

Comparison of the IC50 values for all the ketone/1,1-diarylethene pairs with identical aromatic rings (i.e., **4a**–**p** vs **5a**–**p**) reveals that when one compound of a pair behaves somehow in TPI assays, the other usually follows. These results show that the carbonyl oxygen of phenstatins is not essential, and suggest that geometrical factors are possibly more important than the hydrogen bonding acceptor capability for the higher binding affinity of phenstatins than combretastatins. The biggest differences observed are between **4b**–**5b** and between **4e**–**5e**, being in both cases the ketones more potent than the olefins. When the 2,3,4- and 3,4,5-trimethoxyphenyl containing compounds are compared, olefins with bicyclic systems (indole or naphthyl) behave similarly regardless of the substitution of the trimethoxyphenyl ring. On the other hand, olefins containing a phenylic B ring strongly prefer the 3,4,5-trimethoxyphenyl substitution (compare **m** with **n** or o with **p**).[16] With regards to the B aromatic system, the most potent analogues posses either an indole ring or a 3-hydroxy-4-methoxyphenyl or a 3-amino-4-methoxyphenyl ring, in good agreement with previous studies.[17]

We have cross-docked[18] alcohols **3a**–**l**, ketones **4a**–**p**, and olefins **5a**–**p** in a combined podophyllotoxin–colchicine site using the Surflex docking program[19] and AutoDock.[20] Visual examination of the docked poses revealed frequent binding with the trimethoxyphenyl ring in different dispositions than those found for the reference ligands (podophyllotoxin, colchicine, CA-4, and other colchicine site ligands similarly docked, which place their trimethoxyphenyl rings close to those observed in the X-ray structures). Considering the essential role of such a ring in many diverse colchicine—site binding ligands,[7] we decided to give preference to the poses docked at the podophyllotoxin site with a trimethoxyphenyl ring in close proximity to those of colchicine or podophyllotoxin. As previously described, the ketones might hydrogen bond with the carbonyl oxygen atom to a NH group close to the position occupied by the carbonyl oxygen atom of podophyllotoxin. Ketones with a 2,3,4-trimethoxyphenyl ring, probably due to steric hindrance, showed a displaced carbonyl oxygen, thus suggesting an explanation for their lower potency in the ketone series.

The olefins docked in similar dispositions as the corresponding ketones (Fig. 3A). For the olefins, the displacement of the bridge does not result in a hydrogen bond loss and is better tolerated (Fig. 3B), as experimentally seen. In the prediction of a pharmacophore for colchicine site inhibitors, the carbonyl oxygen of phenstatins has been proposed as a hydrogen bond acceptor pharmacophoric point additional to those found in combretastatins. These predictions agree with the results described here. The 3-X-4-methoxyphenyl rings, N-substituted-5-indolyl and the naphthalene units superimpose onto the methylenedioxyphenyl ring of podophyllotoxin (Fig. 3).

The ethyl group of *N*-ethyl-5-indolyl analogues slightly protrudes over the methylenedioxyphenyl ring and displaces the indole moiety. This suggests that the moiety is too large and it



Scheme 2. Synthesis of diarylmethanols **3a–l**. Reagents and conditions: (i) 1 equiv *n*BuLi, THF, −78 °C; (ii) 2 equiv *n*BuLi, THF, −78 °C; (iii) Mg turnings, THF, 0 °C; (iv) aldehydes **2a–g**, 0 °C–rt; (v) NaOH, EtBr, CH_2Cl_2, $HSO_4^- \cdot Bu_4N^+$; (vi) NaOH, MeI, CH_2Cl_2, $HSO_4^- \cdot Bu_4N^+$; (vii) Et_3N, TBDMSCl, THF; (viii) Et_3N, TBDPSCl, THF.

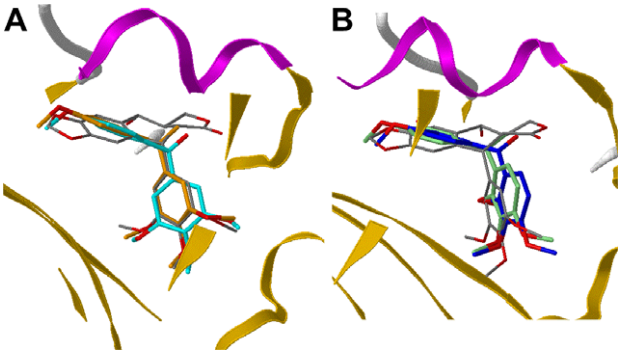


Figure 3. (A) Docked models of **4h** (cyan carbon atoms) and **5h** (orange carbon atoms) showing a similar disposition for isocombretastatins and phenstatins with a 3,4,5-trimethoxyphenyl ring. (B) Docked models of **4m** (blue carbon atoms) and **5m** (pink carbon atoms) showing that the absence of the carbonyl allows for the 2,3,4-trimethoxyphenyl ring to closely resemble that of podophyllotoxin. Podophyllotoxin (gray carbon atoms) is shown in both figures for comparison. The protein backbone is represented as a cartoon.

explains the observed modest potency decrease. Furthermore, for **5e**, this steric hindrance combines with that of the the 2,3,4-trimethoxyphenyl, displacing further the ligand, thus explaining the observed potency loss.

The synthesized compounds were assayed for their capacity to inhibit cancer cells proliferation by the XTT method using HL-60 human leukemia, A-549 human lung carcinoma, HeLa human cervix epitheloid carcinoma, and HT-29 human colon adenocarcinoma. The results were compared with those of CA-4 and doxorubicin (Table 1). Olefins **5d**, **5n** and **5p** displayed nanomolar potencies against several cell lines, and other ketones and olefins showed low-tenths nanomolar potencies. HeLa was the most sensitive cell line whereas HL-60, A-549 and HT-29 were more resistant to the compounds. This activity profile is different from that shown by CA-4, which displays similar potencies against HeLa, HL-60 and A-549, with only HT-29 showing a lower sensitivity. Olefins are more potent than the ketones, but, as indicated for TPI, their potency profiles are similar and parallel their tubulin inhibitory potency, suggesting tubulin as a likely target. When compared with combretastatins with identical substitution patterns, phenstatin analogues often display more potent inhibition of tubulin polymerization and less potent cytotoxicity. Isocombretastatins A also suffer this effect, but less so than the phenstatins.

2. Conclusions

Isocombretastatins A, a new family of 1,1-diarylethene analogues of combretastatins and phenstatins, have been synthesized and assayed as tubulin polymerization inhibitors and cytotoxic agents against several human cancer cell lines. The described compounds are more potent than the parent phenones, suggesting that the carbonyl oxygen is not essential for high potency, as had previously been suggested by a pharmacophore model proposed for the colchicine site at tubulin. The isocombretastatins A show a modest decrease in cytotoxic potency with respect to the parent combretastains, but do not suffer of potential isomerization to inactive *trans* isomers. The 1,1-diarylethene analogues constitute thus a new family of colchicine site ligands with improved characteristics.

3. Experimental

3.1. Chemistry. Materials and methods

Reagents were used as purchased without further purification. Solvents (THF, hexanes) were dried and freshly distilled before use according to procedures reported in the literature. Chromatographic separations were performed on silica gel columns by flash

Table 1
Tubulin polymerization inhibitory activity and cytotoxicity IC_{50}'s (micromolar, μM) for **3a–f**, phenstatins **4a–p** and isocombretastatins **5a–p**

Compound				%TPI	TPI	Cytotoxicity IC_{50} (μM)			
	Ar_1	X, Y	Ar_2	(concn, μM)	IC_{50} (μM)	HL-60	A-549	HeLa	HT-29
CA4[7,9,11]	—	—	—	99 (20)	3[9,11] [1–4]	0.002	0.003	0.003	0.032
Doxorubicin	—	—	—			0.027	0.29	0.19	0.24
3a[11b,9h]	2-Naphthyl	H, OH	2,3,4-TM	0 (20)	—	—	>10	>10	>10
3b[11b,9h]	2-Naphthyl	H, OH	3,4,5-TM	0 (40)	—	—	—	—	—
3c	*N*-Methyl-5-indolyl	H, OH	2,3,4-TM	9 (20)	—	—	>10	2.5	—
3d[11a]	*N*-Methyl-5-indolyl	H, OH	3,4,5-TM	86 (20)	18	—	—	—	—
3e	*N*-Ethyl-5-indolyl	H, OH	2,3,4-TM	0 (20)	—	>10	>10	<1.0	>10
3f[11a]	*N*-Ethyl-5-indolyl	H, OH	3,4,5-TM	41 (20)	—	<1.0	>10	<1.0	>10
4a[11b,9h]	2-Naphthyl	–O–	2,3,4-TM	4 (20)	—	3.3	>10	1.7	>10
4b[11b,9h]	2-Naphthyl	–O–	3,4,5-TM	100 (40)	1.1	—	0.25	0.044	—
4c	*N*-Methyl-5-indolyl	–O–	2,3,4-TM	40 (20)	—	—	0.5	0.03	0.03
4d[11a]	*N*-Methyl-5-indolyl	–O–	3,4,5-TM	98 (40)	7.9	—	0.12	0.035	—
4e	*N*-Ethyl-5-indolyl	–O–	2,3,4-TM	47 (20)	5.6	>1.0	>10	0.45	> 1.0
4f[17]	*N*-Ethyl-5-indolyl	–O–	3,4,5-TM	95 (20)	5.7	—	0.14	0.016	0.034
4g	4-MeOPh	–O–	2,3,4-TM	7 (20)	—	2.00	>10	1.8	3.1
4h[9a,9b,9d]	4-MeOPh	–O–	3,4,5-TM	20 (20)	—	0.27	3.10	0.037	0.29
4l[9e]	3-NO_2-4-MeOPh	–O–	3,4,5-TM	12 (20)	—	1.8	>10	2.5	2.9
4n[9b]	3-OH-4-MeOPh	–O–	3,4,5-TM	94 (20)	2.5 [0.4]	0.031	0.29	0.03	1.80 [0.56]
4p[9e]	3-NH_2-4-MeOPh	–O–	3,4,5-TM	81 (5)	1.5 [0.3]	0.03	0.057	0.03	0.032 [0.033]
5[a]	2-Naphthyl	–CH_2–	2,3,4-TM	11 (20)	—	—	—	0.2	>10
5b[15]	2-Naphthyl	–CH_2–	3,4,5-TM	82 (20)	15	0.91	0.36	0.3	0.25
5c	*N*-Methyl-5-indolyl	–CH_2–	2,3,4-TM	97 (20)	4.7	—	0.6	0.24	—
5d	*N*-Methyl-5-indolyl	–CH_2–	3,4,5-TM	96 (20)	0.7		0.035	0.03	0.008
5e	*N*-Ethyl-5-indolyl	–CH_2–	2,3,4-TM	34 (20)	—		>10	>1.0	>1.0
5f	*N*-Ethyl-5-indolyl	–CH_2–	3,4,5-TM	83 (20)	6	—		0.017	0.035
5g	4-MeOPh	–CH_2–	2,3,4-TM	16 (20)	—	0.48	>10	0.3	3.2
5h[15]	4-MeOPh	–CH_2–	3,4,5-TM	52 (20)	25.7	0.3	2.0	0.29	0.31
5k	3-NO_2-4-MeOPh	–CH_2–	2,3,4-TM	12 (20)	—	2.60	>10	0.032	2.90
5l[15]	3-NO_2-4-MeOPh	–CH_2–	3,4,5-TM	61 (20)	12.2	0.032	0.026	0.30	0.06
5m	3-OH-4-MeOPh	–CH_2–	2,3,4-TM	86 (20)	12.7	0.24	0.27	0.035	0.31
5n[15]	3-OH-4-MeOPh	–CH_2–	3, 4, 5-TM	86 (20)	0.8	0.0028	0.028	0.0029	0.44
5o	3-NH_2-4-MeOPh	–CH_2–	2,3,4-TM	32 (20)	—	0.23	2.70	0.06	0.33
5p[15]	3-NH_2-4-MeOPh	–CH_2–	3,4,5-TM	86 (20)	2.2	0.029	0.031	0.0032	0.041

Selected literature values are given under brackets. TM = trimethoxyphenyl ring.

(Kieselgel 40, 0.040–0.063; Merck) or gravity column (Kieselgel 60, 0.063–0.200 mm; Merck) chromatography. TLC was performed on precoated silica gel polyester plates (0.25 mm thickness) with UV 254 fluorescent indicator (Polychrom SI F_{254}). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AC 200-SY spectrometer at 200/50 MHz or on a Bruker SY spectrometer at 400/100 MHz. Chemical shifts (δ) are given in ppm downfield from tetramethylsilane as internal standard, and coupling constants (J values) are in Hertz. GC–MS analyses were carried out on a Hewlett–Packard 5890 Series II apparatus (70 eV). For FABHRMS analyses, a VG-TS250 apparatus (70 eV) was used. A Helios-α UV-320 from Thermo-Spectronic was used for UV experiments and absorption spectra. HPLC analysis were run on an HP-1100 device from Agilent Technologies or a Delta 600 device from Waters instruments, using X-Terra® MS C18 5 μm (4.6 × 150 mm), X-Terra® MS C8 5 μm (4.6 × 150 mm), and X-Terra® MS Phenyl 5 μm (4.6 × 150 mm) columns with water–acetonitrile or water–methanol gradients. Every final compound was analyzed on at least three different column-solvent system combinations.

3.1.1. Synthesis of the benzhydrol derivatives starting from 3,4,5-trimethoxyphenyl bromide (Method A)

3,4,5-Trimethoxyphenylmagnesium bromide (1.0 M) prepared from 3,4,5-trimethoxyphenyl bromide and magnesium turnings (activated with iodine) in anhydrous tetrahydrofuran was slowly added to the corresponding aldehydes in tetrahydrofuran (100 mL/mmol of aldehyde) at 0 °C. The reaction mixture was warmed up to room temperature, and stirring was continued for another 30 min. A saturated NH_4Cl solution was slowly added at 0 °C, and the mixture was extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuum. The crudes were used without purification.

3.1.2. Synthesis of the benzhydrol derivatives starting from 1,2,3-trimethoxybenzene (Method B)

1,2,3-Trimethoxybenzene (3.0 M in dry THF) were reacted with 0.3 mol/mol of *n*BuLi (1.6 M in hexanes) at 0 °C. The reaction was stirred for 1 h and then, 1 mol of the aldehydes in THF (100 mL/mmol of aldehyde) per mol of *n*BuLi were added. The reaction mixture was warmed up to room temperature and stirring was continued overnight. A saturated NH_4Cl solution was slowly added and the mixture was extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuum. Unreacted 1,2,3-trimethoxybenzene was removed by column chromatography, using hexanes as eluent and the crudes were used without further purification.

3.1.3. Synthesis of the benzhydrol derivatives starting from 2-bromonaphthalene (Method C)

2-bromonaphthalene (1.0 M in dry THF) was reacted with 1 mol/mol of *n*BuLi (1.6 M in hexanes) at −78 °C. The reaction was stirred for 1 h and then, 1 mol of the corresponding aldehydes in THF (100 mL/mmol of aldehyde) per mol of *n*BuLi were added. The reaction mixture was warmed up to room temperature and stirring was continued overnight. A saturated NH_4Cl solution was slowly added and the mixture was extracted with CH_2Cl_2. The

combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuum. The crudes were used without purification.

3.1.4. Synthesis of the benzhydrol derivatives starting from N-substituted-5-bromo-1*H*-indoles (Method D)

The 5-bromoindoles (1.0 M in dry THF) were reacted with 2 mol/mol of *n*BuLi (1.6 M in hexanes) at −78 °C. The reaction was stirred for 1 h and then a THF solution of 1 mol of aldehyde per mol of the bromoindole (100 mL/mmol of aldehyde) was added. The reaction mixture was warmed up to room temperature and stirring was continued overnight. A saturated NH_4Cl solution was slowly added and the mixture was extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuum. The crudes were used without purification.

3.1.5. Oxidations with pyridinium dichromate (Method E)

Pyridinium dichromate (PDC, 1.5 moles per mol of benzhydrol) was added at 0 °C to a 1.0 M stirred solution of benzhydrol and 4 Å molecular sieves in dichloromethane. After 24 h, the reaction mixtures were filtered through pads of Celite. The filtrates were concentrated in vacuum, and the residues were purified by flash chromatography to obtain the desired substituted *p*-methoxybenzophenones.

3.1.6. Oxidations with potassium permanganate (Method F)

A 0.2 M solution of the diarylmethanol in CH_2Cl_2 (40–50 mL) was reacted with $KMnO_4$ (1 mmol per mmol of alcohol) in the presence of *n*-$Bu_4N^+HSO_4^-$ (about 1% w/w). The reaction mixture was stirred for 8–12 h at room temperature and then filtered through silica gel, using dichloromethane and ethyl acetate as eluents. The organic solvent was then evaporated.

3.1.7. Wittig reactions. Synthesis of the olefin derivatives (Method G)

The methyltriphenylphosphonium iodide (0.1 M) was suspended in dry THF and cooled to −78 °C under Ar. *N*-butyl lithium (1.6 M in hexanes, 0.75 moles per mol of phosphonium salt) was added dropwise, and the resulting yellow solution was stirred for 1 h. Then a 0.1 M solution of the benzophenone (0.25 moles per mol of phosphonium salt) in THF was added and warmed to room temperature. Once completed the reaction mixtures were cooled to 0 °C, stopped with saturated NH_4Cl solution and extracted with dichloromethane. The organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuum. The residue was purified by flash chromatography to obtain the desired olefins.

3.1.8. Protection of the indole nitrogen (Method H)

2 mmol of finely ground NaOH per mmol of indole derivative and a 10% w/w (with respect to indole derivative) of tetrabutylammonium hydrogenosulfate were added to a 0.25 M solution of the 5-bromo-1*H*-indole in 50 mL of dry CH_2Cl_2. After stirring for 1 h at room temperature under an Argon atmosphere, 2–3 mmol of the alkylating agent (MeI or EtBr) was added, and the reaction was refluxed until completion (as determined by TLC) for 48–144 h. The reaction mixture was then washed with brine and the organic layers were dried, filtered, and rotary-evaporated.

3.1.9. Deprotection of siloxy groups (Method I)

To a stirred 0.05 M solution of the protected phenol in dry THF was added TBAF (1.0 M in THF, 3 moles per mol) and stirred for 1 h under Ar. The reaction mixture was poured into water and extracted with ether, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuum. The residues were purified by flash chromatography.

3.1.10. Synthesis of diarylmethanols 3

3.1.10.1. (1-Methyl-1*H*-indol-5-yl)(2,3,4-trimethoxyphenyl)methanol (3c). Following general method D, **1e** (500 mg, 2.4 mmol) in dry THF (20 mL), 1.6 M *n*BuLi in hexanes (3.3 mL, 5.2 mmol) and 2,3,4-trimethoxybenzaldehyde (564 mg, 2.9 mmol) were reacted to obtain alcohol **3c** (550 mg, 71%), purified by flash chromatography using hexanes/ethyl acetate 7:3 as eluent. ^{1}H NMR δ 3.65 (3H, s, 2′-OCH_3), 3.76 (3H, s, *N*-CH_3), 3.85 (3H, s, 3′- or 4′-OCH_3), 3.87 (3H, s, 3′- or 4′-OCH_3), 6.11 (1H, br s, C*H*–OH), 6.46 (1H, d, J = 3.3, H_3), 6.65 (1H, d, J = 8.4, $H_{5'}$), 7.04 (1H, d, J = 3.3, H_2), 7.07 (1H, d, J = 8.4, $H_{6'}$), 7.27 (2H, m, H_6, H_7), 7.64 (1H, br s, H_4). ^{13}C NMR δ 32.9 (CH_3), 56.1 (CH_3), 60.9 (×2) (CH_3), 72.5 (CH), 101.2 (CH), 107.1 (CH), 109.1 (CH), 118.9 (CH), 120.9 (CH), 122.2 (CH), 128.4 (C), 129.3 (CH), 131.0 (C), 135.3 (C), 136.2 (C), 142.2 (C), 151.3 (C), 153.1 (C). IR (cm^{-1}) 1513, 1600, 3425.

3.1.10.2. (1-Ethyl-1*H*-indol-5-yl)(2,3,4-trimethoxyphenyl)methanol (3e). 11 mL (17.6 mmol) of 1.6 M *n*BuLi in hexanes was reacted with 1.8 g (8 mmol) of **1f** in 40 mL of dry THF and with 1.88 g (9.6 mmol) of 2,3,4-trimethoxybenzaldehyde dissolved in 10 mL of dry THF to yield, after flash chromatography using hexane/ethyl acetate 6:4 as eluent, 1.4 g (51%) of **3e**. ^{1}H NMR δ 1.46 (3H, t, J = 7.0, CH_3), 3.68 (3H, s, 2′-OCH_3), 3.86 (3H, s, 3′- or 4′-OCH_3), 3.87 (3H, s, 3′- or 4′-OCH_3), 4.12 (2H, q, J = 7.0, *N*-CH_2), 6.11 (1H, br s, C*H*–OH), 6.11 (1H, d, J = 3.3, H_3), 6.66 (1H, d, J = 8.5, $H_{5'}$), 7.04 (1H, d, J = 3.3, H_2), 7.08 (1H, d, J = 8.5, $H_{6'}$), 7.23 (1H, dd, J = 8.3; 1.3, H_6), 7.32 (1H, d, J = 8.3, H_7), 7.62 (1H, br s, H_4). ^{13}C NMR δ 15.5 (CH_3), 41.1 (CH_2), 56.0 (CH_3), 60.8 (×2) (CH_3), 72.6 (CH), 101.3 (CH), 107.1 (CH), 109.2 (CH), 119.0 (CH), 120.7 (CH), 122.2 (CH), 127.4 (CH), 128.6 (C), 130.9 (C), 135.1 (×2) (C), 142.2 (C), 151.3 (C), 153.1 (C). IR (KBr): 1599, 3458 cm^{-1}. HRMS m/z calcd for $C_{20}H_{23}NO_4$ (+Na): 364.1519, found 364.1515.

3.1.10.3. (4-Methoxyphenyl)(2,3,4-trimethoxyphenyl)methanol (3g). Following general method B, 7.44 mL (11.9 mmol) of 1.6 M *n*BuLi in hexanes was reacted with 2.0 g (11.9 mmol) of **1b** in 10 mL of dry THF and with 1.62 g (11.9 mmol) of *p*-methoxybenzaldehyde dissolved in 10 mL of dry THF to yield 3.62 g, that were used without further purification. ^{1}H NMR δ 3.67 (3H, s, 2-OCH_3), 3.80 (3H, s, 4′-OCH_3), 3.85 (6H, s, 3,4-OCH_3), 5.89 (1H, d, J = 5, C*H*–OH), 6.64 (1H, d, J = 8.6, H_5), 6.86 (2H, d, J = 8.6, $H_{3'}$, $H_{5'}$), 6.97 (1H, d, J = 8.6, H_6), 7.28 (2H, d, J = 8.6, $H_{2'}$, $H_{6'}$). ^{13}C NMR δ 55.2 (CH_3), 56.0 (CH_3), 60.4 (CH_3), 60.7 (CH_3), 71.6 (CH), 107.0 (CH), 113.6 (×2) (CH), 122.0 (CH), 127.7 (×2) (CH), 130.2 (C), 136.4 (C), 142.1 (C), 151.1 (C), 153.2 (C), 158.7 (C). IR (film): 1096, 1248, 1602, 3472 cm^{-1}.

3.1.10.4. (3-*tert*Butyldimethylsiloxy-4-methoxyphenyl)(2,3,4-trimethoxyphenyl)methanol (3i). Following general method B, 18.75 mL (30.0 mmol) of 1.6 M *n*BuLi in hexanes was reacted with 5.5 g (32.7 mmol) of **1b** in 20 mL of dry THF and with 2.88 g (10.1 mmol) of **2e** dissolved in 20 mL of dry THF to yield 7.54 g. The reaction was flash chromatographied using hexanes/ethyl acetate 9:1 to yield 3.22 g (69%) of **3i**. ^{1}H NMR δ 0.12 (6H, s, $SiCH_3$), 0.97 (9H, s, *tert*Bu), 3.65 (3H, s, 2-OCH_3), 3.78 (3H, s, OCH_3), 3.85 (3H, s, OCH_3), 3.87 (3H, s, OCH_3), 5.82 (1H, d, J = 6.2, C*H*–OH), 6.64 (1H, d, J = 8.6, H_5), 6.79 (1H, d, J = 8.6, $H_{5'}$), 6.86 (1H, dd, J = 8.6; 2.2, $H_{6'}$), 6.88 (1H, d, J = 2.2, $H_{2'}$), 6.94 (1H, d, J = 8.6, $H_{6'}$). ^{13}C NMR δ −4.6 (×2) (CH_3), 18.5 (C), 25,8 (×3) (CH_3), 55.5 (CH_3), 56.0 (CH_3), 60.7 (×2) (CH_3), 71.7 (CH), 106.9 (CH), 111.7 (CH), 119.5 (CH), 119.7 (CH), 122.1 (CH), 129.2 (C), 130.3 (C), 137.0 (C), 144.7 (C), 150.1 (C), 151.5 (C), 153.2 (C).

3.1.10.5. (3-*tert*Butyldiphenylsiloxy-4-methoxyphenyl)(3,4,5-trimethoxyphenyl)methanol (3j). Following method A, 2.0 g (8.1 mmol) of **1c** was added to a suspension of 304 mg (12.15 mmol) of magnesium and 5 mg of iodine in 2.5 mL of dry THF and the resulting white suspension was added onto a solution of 1.0 g (2.56 mmol) of **2f** dissolved in 3 mL of dry THF to yield, after 1 h at 0 °C, 2.93 g of crude, which was purified by flash chromatography using ethyl acetate/hexanes 1:1 as eluent to yield 1.34 g (94%) of **3j**. ^{1}H NMR δ 1.09 (9H, s, *tert*Bu), 3.60 (3H, s, 4′-OCH_3), 3.74 (6H, s, 3,5-OCH_3), 3.82 (3H, s, 4-OCH_3), 5.49 (1H, d, J = 3.6, *CH–OH*), 6.41 (2H, s, H_2, H_6), 6.66 (1H, d, J = 2.2, $H_{2'}$), 6.74 (1H, d, J = 8.2, $H_{5'}$), 6.85 (1H, *m*, $H_{6'}$), 7.30 (6H, m, Ph), 7.66 (4H, m, Ph). ^{13}C NMR δ 14.2 (C), 26.7 (×3) (CH_3), 55.4 (CH_3), 55.8 (×2) (CH_3), 60.8 (CH_3), 75.5 (CH), 103.2 (×2) (CH), 118.8 (CH), 119.8 (CH), 122.1 (CH), 127.5 (4×) (CH), 129.7 (×2) (CH), 133.5 (×2) (C), 135.4 (4×) (CH), 136.6 (C), 140.0 (C), 144.9 (C), 150.0 (C), 152.9 (×2) (C), 153.4 (C).

3.1.10.6. (4-Methoxy-3-nitrophenyl)(2,3,4-trimethoxyphenyl) methanol (3k). Following general method B, 7.2 mL (11.5 mmol) of 1.6 M *n*BuLi in hexanes was reacted with 2.0 g (11.9 mmol) of **1b** in 10 mL of dry THF and with 2.08 g (11.5 mmol) of 4-methoxy-3-nitro-benzaldehyde (**2g**) dissolved in 10 mL of dry THF to yield 2.53 g. The reaction was flash chromatographied using hexanes/ethyl acetate 9:1 to yield 1.27 g (32%) of **3k**. ^{1}H NMR δ 3.73 (3H, s, 2-OCH_3), 3.85 (3H, s, 3- or 4-OCH_3), 3.87 (3H, s, 3- or 4-OCH_3), 3.95 (3H, s, 4′-OCH_3), 5.88 (1H, d, J = 6.2, C*H*–OH), 6.65 (1H, d, J = 8.4, H_5), 6.93 (1H, d, J = 8.4, H_6), 7.04 (1H, d, J = 8.2, $H_{5'}$), 7.55 (1H, dd, J = 8.2; 2.2, $H_{6'}$), 7.87 (1H, d, J = 2.2, $H_{2'}$). ^{13}C NMR δ 55.6 (CH_3), 56.1 (CH_3), 60.7 (CH_3), 60.8 (CH_3), 70.4 (CH), 107.2 (CH), 113.2 (CH), 121.8 (CH), 123.9 (CH), 129.0 (C), 132.3 (CH), 137.0 (C), 139.1 (C), 142.0 (C), 150.9 (C), 151.8 (C), 153.6 (C).

3.1.11. Synthesis of diarylketones 4

3.1.11.1. (1-Methyl-1*H*-indol-5-yl)(2,3,4-trimethoxyphenyl) methanone (4c). 2.0 g (6.2 mmol) of **3c** was oxidized with 980 mg (6.2 mmol) of $KMnO_4$ and 20 mg of Bu_4NHSO_4, following general procedure F, to yield, after 8 h, 1.8 g of **4c** (89%) as a white solid. ^{1}H NMR δ 3.77 (3H, s, 2′-OCH_3), 3.83 (3H, s, NCH_3), 3.92 (3H, s, 3′- or 4′-OCH_3), 3.93 (3H, s, 3′- or 4′-OCH_3), 6.55 (1H, d, J = 3.3, H_3), 6.73 (1H, d, J = 8.6, $H_{5'}$), 7.10 (1H, d, J = 3.3, H_2), 7.11 (1H, d, J = 8.6, $H_{6'}$), 7.35 (1H, d, J = 8.6, H_7), 7.85 (1H, dd, J = 8.6; 1.6, H_6), 8.10 (1H, d, J = 1.6, H_4). ^{13}C NMR δ 33.0 (CH_3), 56.1 (CH_3), 61.0 (CH_3), 61.8 (CH_3), 103.1 (CH), 106.8 (CH), 108.9 (CH), 123.4 (CH), 124.4 (CH), 125.4 (CH), 127.8 (×2) (C), 130.4 (CH), 139.3 (C), 139.9 (C), 142.2 (C), 152.4 (C), 155.5 (C), 195.7 (C). Mp 104–106 °C (CH_2Cl_2/Hex). IR (KBr): 1651, 1580 cm^{-1}. HRMS *m*/*z* calcd for $C_{19}H_{19}NO_4$ (+Na) 348.1206, found 348.1205.

3.1.11.2. (1-Ethyl-1*H*-indol-5-yl)(2,3,4-trimethoxyphenyl)methanone (4e). 2.3 g (6.8 mmol) of **3e** was oxidized with 1.1 g (6.8 mmol) of $KMnO_4$ and 23 mg of Bu_4NHSO_4, following general procedure F, to yield, after 8 h, 2.1 g of **4e** (91%). ^{1}H NMR δ 1.45 (3H, t, J = 7.3, CH_3), 3.77 (3H, s, 2′-OCH_3), 3.90 (6H, s, 3′,4′-OCH_3), 4.17 (2H, *q*, J = 7.3, CH_2N), 6.53 (1H, d; J = 3.2, H_3), 6.70 (1H, d, J = 8.8, $H_{5'}$), 7.08 (1H, d, J = 8.8, $H_{6'}$), 7.14 (1H, d, J = 3.2, H_2), 7.35 (1H, d, J = 8.8, H_7), 7.83 (1H, dd, J = 8.8, 1.8, H_6), 8.06 (1H, d, J = 1.8, H_4). ^{13}C NMR δ 15.4 (CH_3), 41.3 (CH_2), 56.2 (CH_3), 61.0 (CH_3), 61.8 (CH_3), 103.3 (CH), 106.8 (CH), 109.0 (CH), 123.3 (CH), 124.4 (CH), 125.6 (CH), 128.0 (C), 128.6 (CH), 130.1 (C), 138.4 (×2) (C), 142.2 (C), 152.4 (C), 155.4 (C), 195.2 (C). IR (KBr): 1597, 1651 cm^{-1}. HRMS *m*/*z* calcd for $C_{20}H_{21}NO_4$ (+Na) 362.1363, found 362.1376.

3.1.11.3. (4-Methoxyphenyl)(2,3,4-trimethoxyphenyl)methanone (4g). 3.62 g (11.9 mmol) of **3g** was oxidized with 3.76 g (24.0 mmol) of $KMnO_4$ and 20 mg of Bu_4NHSO_4, according to the general procedure F, to yield, after 8 h, 3.0 g, that were flash chromatographied to yield 604 mg of **4g** (17%). Mp 101–103 °C (Hex/CH_2Cl_2). ^{1}H NMR δ 3.65 (3H, s, OCH_3), 3.71 (3H, s, OCH_3), 3.77 (6H, s, OCH_3), 6.60 (1H, d, J = 8.6, H_5), 6.79 (2H, d, J = 9.0, $H_{3'}$, $H_{5'}$), 6.94 (1H, d, J = 8.6, H_6), 7.67 (2H, d, J = 9.0, $H_{2'}$, $H_{6'}$). ^{13}C NMR δ 55.4 (CH_3), 56.0 (CH_3), 60.8 (CH_3), 61.7 (CH_3), 106.8 (CH), 113.4 (×2) (CH), 124.5 (CH), 126.7 (C), 130.9 (C), 132.2 (×2) (CH), 142.0 (C), 152.2 (C), 155.7 (C), 163.4 (C), 193.9 (C). IR (KBr): 1025, 1596, 1651 cm^{-1}.

3.1.11.4. (4-Methoxy-3-nitrophenyl)(2,3,4-trimethoxyphenyl)methanone (4k). 567 mg (1.62 mmol) of **3k** was oxidized with 1.28 g (8.1 mmol) of $KMnO_4$ and 20 mg of Bu_4NHSO_4, according to the general procedure F, to yield, after 8 h, 220 mg (39%) that were used without further purification. ^{1}H NMR δ 3.71 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 4.00 (3H, s, OCH_3), 6.72 (1H, d, J = 8.6, H_5), 7.10 (1H, d, J = 8.6, H_6), 7.12 (1H, d, J = 8.6, $H_{5'}$), 8.00 (1H, dd, J = 8.6; 2.2, $H_{6'}$), 8.21 (1H, d, J = 2.2, $H_{2'}$). ^{13}C NMR δ 56.2 (CH_3), 56.9 (CH_3), 61.1 (CH_3), 61.9 (CH_3), 107.2 (CH), 113.1 (CH), 125.2 (CH), 127.6 (CH), 130.6 (C), 135.6 (CH), 139.0 (C), 142.0 (C), 151.0 (C), 152.5 (C), 156.0 (C), 156.8 (C), 192.2 (C).

3.1.12. Synthesis of olefins 5

3.1.12.1. 2-[1-(2,3,4-Trimethoxyphenyl)vinyl]naphthalene (5a). 60 mg of **5a** (75% isolated yield after flash chromatography using hexanes/EtOAc 95:5) were obtained by method G from 80 mg (0.25 mmol) of **4a** with 439 mg (2.2 mmol) of $CH_3P^+Ph_3{\cdot}I^-$ and 1.1 mL (1.7 mmol) of *n*BuLi (1.6 M in hexanes) in 15 mL of dry THF. ^{1}H NMR δ 3.55 (3H, s, 2′-OCH_3), 3.92 (3H, s, 3′- or 4′-OCH_3), 3.93 (3H, s, 3′- or 4′-OCH_3), 5.45 (1H, d, J = 1.5, CH_2), 5.80 (1H, d, J = 1.5, CH_2), 6.74 (1H, d, J = 8.6, $H_{5'}$), 7.06 (1H, d, J = 8.6, $H_{6'}$), 7.44–7.61 (3H, m, H_3, H_6, H_7), 7.75 (1H, br s, H_1), 7.7–7.9 (3H, m, H_4, H_5, H_8). ^{13}C NMR δ 56.1 (CH_3), 60.7 (CH_3), 60.9 (CH_3), 107.2 (CH), 116.0 (CH_2), 125.1 (CH), 125.4 (CH), 125.7 (CH), 125.9 (CH), 126.1 (CH), 127.7 (×2) (CH), 128.3 (CH), 129.1 (C), 133.0 (C), 133.4 (C), 139.4 (C), 142.5 (C), 147.0 (C), 151.9 (C), 153.8 (C). IR (film): 1594, cm^{-1}. HRMS *m*/*z* calcd for $C_{21}H_{20}O_3$ (+Na) 343.1305, found 343.1330.

3.1.12.2. 2-[1-(3,4,5-Trimethoxyphenyl)vinyl]naphthalene (5b). 80 mg of **5b** (55% isolated yield after flash chromatography using hexanes/EtOAc 7:3) were obtained by method G from 150 mg (0.46 mmol) of **4b** with 941 mg (2.33 mmol) of $CH_3P^+Ph_3{\cdot}I^-$ and 1.2 mL (1.86 mmol) of *n*BuLi (1.6 M in hexanes) in 15 mL of dry THF. ^{1}H NMR δ 3.80 (6H, s, 3′,5′-OCH_3), 3.90 (3H, s, 4′-OCH_3), 5.52 (1H, br s, CH_2), 5.58 (1H, br s, CH_2), 6.60 (2H, s, $H_{2'}$, $H_{6'}$), 7.54–7.62 (3H, m, H_3, H_6, H_7), 7.82 (1H, br s, H_1), 7.7–7.9 (3H, m, H_4, H_5, H_8). ^{13}C NMR δ 56.2 (×2) (CH_3), 61.0 (CH_3), 105.8 (×2) (CH), 114.6 (CH_2), 126.2 (CH), 126.3 (CH), 126.5 (CH), 127.4 (CH), 127.7 (×2) (CH), 128.3 (CH), 133.1 (C), 133.4 (C), 137.3 (C), 137.9 (C), 138.7 (C), 150.2 (C), 153.0 (2) (C). Mp 91–93 °C (CH_2Cl_2/Hex). IR (KBr): 1551, cm^{-1}. HRMS *m*/*z* calcd for $C_{21}H_{20}O_3$ (+Na) 343.1305, found 343.1309.

3.1.12.3. 1-Methyl-5-[1-(2,3,4-trimethoxyphenyl)vinyl]-1*H*-indole (5c). 110 mg of **5c** (55% isolated yield after flash chromatography using hexanes/EtOAc 8:2) were obtained by method G from 200 mg (0.6 mmol) of **4c** with 1.25 g (3.1 mmol) of $CH_3P^+Ph_3{\cdot}I^-$ and 1.5 mL (2.5 mmol) of *n*BuLi (1.6 M in hexanes) in 15 mL of dry THF. ^{1}H NMR δ 3.55 (3H, s, 2′-OCH_3), 3.78 (3H, s, NCH_3), 3.90 (3H, s, 3′- or 4′-OCH_3), 3.92 (3H, s, 3′- or 4′-OCH_3), 5.27 (1H, d, J = 1.8), 5.66 (1H, d, J = 1.8), 6.45 (1H, d, J = 3.3, H_3), 6.71 (1H, d, J = 8.6, $H_{6'}$), 7.02 (1H, d, J = 8.6, $H_{5'}$), 7.03 (1H, d, J = 3.3, H_2), 7.23–7.31 (2H, *m*, H_6, H_7), 7.55 (1H, br s, H_4). ^{13}C NMR δ 32.9 (CH_3), 56.1 (CH_3), 60.7 (CH_3), 60.9 (CH_3), 101.4 (CH), 106.9 (CH), 108.7 (CH), 113.6 (CH_2), 119.3 (CH), 121.0 (CH), 125.4 (CH), 127.7 (C),

128.4 (C), 129.2 (CH), 130.2 (C), 133.6 (C), 136.5 (C), 147.7 (C), 151.9 (C), 153.4 (C). IR (film): 1493, 1595 cm^{-1}. HRMS *m*/*z* calcd for $C_{20}H_{21}NO_3$ (+Na) 346.1414, found 346.1399.

3.1.12.4. 1-Methyl-5-[1-(3,4,5-trimethoxyphenyl)vinyl]-1*H*-indole (5d). 120 mg of **5d** (53% isolated yield after flash chromatography using hexanes/EtOAc 6:4) were obtained by method G from 288 mg (0.7 mmol) of **4d** with 850 mg (2.1 mmol) of $CH_3P^+Ph_3{\cdot}I^-$ and 1.1 mL (1.7 mmol) of *n*BuLi (1.6 M in hexanes) in 15 mL of dry THF. ^{1}H NMR δ 3.80 (3H, s, N- or -OCH_3), 3.82 (6H, s, 3′,5′-OCH_3), 3.93 (3H, s, N- or -OCH_3), 5.41 (1H, s, CH_2), 5.47 (1H, s, CH_2), 6.50 (1H, d, *J* = 3.2, H_3), 6.66 (2H, s, $H_{2'}$, $H_{6'}$), 7.07 (1H, d, *J* = 3.2, H_2), 7.30 (2H, *m*, H_6, H_7), 7.64 (1H, br s, H_4). ^{13}C NMR δ 33.0 (CH_3), 56.2 (×2) (CH_3), 60.1 (CH_3), 101.4 (CH), 106.2 (×2) (CH), 108.9 (CH), 112.5 (CH_2), 120.9 (CH), 122.5 (CH), 128.4 (C), 129.4 (CH), 132.8 (C), 136.6 (C), 137.8 (C), 138.5 (C), 151.2 (C), 152.9 (×2) (C). IR (film): 1582 cm^{-1}. HRMS *m*/*z* calcd for $C_{20}H_{21}NO_3$ (+Na) 346.1414, found 346.1430.

3.1.12.5. 1-Ethyl-5-[1-(2,3,4-trimethoxyphenyl)vinyl]-1*H*-indole (5e). 60 mg of **5e** (40% isolated yield after flash chromatography using hexanes/EtOAc 8:2) were obtained by method G from 150 mg (0.4 mmol) of **4e** with 888 mg (2.1 mmol) of $CH_3P^+Ph_3{\cdot}I^-$ and 1.1 mL (1.7 mmol) of *n*BuLi (1.6 M in hexanes) in 25 mL of dry THF. ^{1}H NMR δ 1.47 (3H, t, *J* = 7.3, CH_3), 3.55 (3H, s, 2′-OCH_3), 3.88 (3H, s, 3′- or 4′-OCH_3), 3.91 (3H, s, 3′- or 4′-OCH_3), 4.16 (2H, *q*, *J* = 7.3, CH_2*N*), 5.24 (1H, d, *J* = 1.8, CH_2), 5.64 (1H, d, *J* = 1.8, CH_2), 6.44 (1H, d, *J* = 3.1, H_3), 6.70 (1H, d, *J* = 8.4, $H_{5'}$), 7.00 (1H, d, *J* = 8.4, $H_{6'}$), 7.09 (1H, d, *J* = 3.1, H_2), 7.27 (2H, *m*, H_5, H_6), 7.53 (1H, br s, H_4). ^{13}C NMR δ 15.7 (CH_3), 41.0 (CH_2), 56.1 (CH_3), 60.7 (×2) (CH_3), 101.6 (CH), 107.0 (CH), 108.8 (CH), 113.5 (CH_2), 119.4 (CH), 120.9 (CH), 125.4 (CH), 127.3 (CH), 128.6 (C), 130.2 (C), 133.5 (C), 135.6 (C), 147.7 (×2) (C), 151.9 (C), 153.4 (C). IR (film): 1595 cm^{-1}. HRMS *m*/*z* calcd for $C_{21}H_{23}NO_3$ (+Na) 360.1570, found 360.1565.

3.1.12.6. 1-Ethyl-5-[1-(3,4,5-trimethoxyphenyl)vinyl]-1*H*-indole (5f). 77 mg of **5f** (52% isolated yield after flash chromatography using hexanes/EtOAc 8:2) were obtained by method G from 150 mg (0.4 mmol) of **4f** with 880 mg (2.2 mmol) of $CH_3P^+Ph_3{\cdot}I^-$ and 1.1 mL (1.7 mmol) of *n*BuLi (1.6 M in hexanes) in 15 mL of dry THF. ^{1}H NMR δ 1.50 (3H, t, *J* = 7.3, CH_3), 3.82 (6H, s, 3′,5′-OCH_3), 3.91 (3H, s, 4′-OCH_3), 4.19 (2H, *q*, *J* = 7.3, CH_2*N*), 5.37 (1H, d, *J* = 1.5, CH_2), 5.45 (1H, d, *J* = 1.5, CH_2), 6.50 (1H, d, *J* = 3.3, H_3), 6.62 (2H, s, $H_{2'}$, $H_{6'}$), 7.14 (1H, d, *J* = 3.3, H_2), 7.24 (1H, dd, *J* = 8.8; 1.4, H_6), 7.32 (1H, d, *J* = 8.8, H_7), 7.65 (1H, br s, H_4). ^{13}C NMR δ 15.5 (CH_3), 41.1 (CH_2), 56.2 (×2) (CH_3), 60.9 (CH_3), 101.6 (CH), 106.2 (×2) (CH), 108.8 (CH), 112.3 (CH_2), 121.0 (CH), 122.3 (CH), 127.5 (CH), 128.0 (C), 128.6 (C), 129.1 (C), 132.7 (C), 138.4 (C), 151.2 (C), 152.9 (×2) (C). IR (film): 1504, 1580 cm^{-1}. HRMS *m*/*z* calcd for $C_{21}H_{23}NO_3$ (+Na) 360.1570, found 360.1560.

3.1.12.7. 1-[1-(4-Methoxyphenyl)vinyl]-2,3,4-trimethoxybenzene (5g). 27 mg of **5g** (20% isolated yield after flash chromatography using hexanes/EtOAc 9:1) were obtained by method G from 137 mg (0.45 mmol) of **4g** with 731 mg (1.8 mmol) of $CH_3P^+Ph_3{\cdot}I^-$ and 0.85 mL (1.36 mmol) of *n*BuLi (1.6 M in hexanes) in 7 mL of dry THF. ^{1}H NMR δ 3.55 (3H, s, 2′-OCH_3), 3.80 (3H, s, 3′-, 4′ or 4-OCH_3), 3.86 (3H, s, 3′-, 4′ or 4-OCH_3), 3.89 (3H, s, 3′-, 4′ or 4-OCH_3), 5.18 (1H, d, *J* = 1.4, CH_2), 5.54 (1H, d, *J* = 1.4, CH_2), 6.67 (1H, d, *J* = 8.6, H_5), 6.83 (2H, d, *J* = 8.8, $H_{3'}$, $H_{5'}$), 6.95 (1H, d, *J* = 8.6, H_6), 7.25 (2H, d, *J* = 8.8, H_3, H_5). ^{13}C NMR δ 55.3 (CH_3), 56.0 (CH_3), 60.7 (CH_3), 60.9 (CH_3), 106.9 (CH), 113.5 (×2) (CH), 113.7 (CH_2), 125.2 (CH), 127.8 (×2) (CH), 129.4 (C), 134.5 (C), 142.1 (C), 146.3 (C), 151.7 (C), 153.5 (C), 159.2 (C). IR (film): 1500, 1602 cm^{-1}.

3.1.12.8. 1-[1-(4-Methoxyphenyl)vinyl]-3,4,5-trimethoxybenzene (5h). The Wittig reaction (method G) produced 28 mg (25% isolated yield) of **5h**. ^{1}H NMR δ 3.81 (6H, s, 2 × OCH_3), 3.83 (3H, s, OCH_3), 3.88 (3H, s, OCH_3), 5.32 (1H, s, CH_2), 5.37 (1H, s, CH_2), 6.56 (2H, s, $H_{2'}$, $H_{6'}$), 6.87 (2H, d, *J* = 8.2, H_2, H_6), 7.30 (2H, d, *J* = 8.2, H_3, H_5). ^{13}C NMR δ 55.3 (CH_3), 56.1 (×2) (CH_3), 61.0 (CH_3), 105.6 (×2) (CH), 112.6 (CH_2), 113.5 (×2) (CH), 129.5 (×2) (CH), 133.7 (C), 137.6 (C), 149.6 (C), 152.9 (2) (C), 159.4 (C), 1 not observed. HRMS *m*/*z* calcd for $C_{18}H_{20}O_4$ (+Na) 323.1254, found 323.1271.

3.1.12.9. 1-[1-(3-*tert*Butyldimethylsiloxy-4-methoxyphenyl)vinyl]-2,3,4-trimethoxybenzene (5i). 480 mg of **5i** (48% isolated yield after flash chromatography using hexanes/EtOAc 9:1) were obtained by method G from 1.00 g (2.31 mmol) of **4i** with 3.7 g (9.2 mmol) of $CH_3P^+Ph_3{\cdot}I^-$ and 4.31 mL (6.90 mmol) of *n*BuLi (1.6 M in hexanes) in 40 mL of dry THF. ^{1}H NMR δ 0.13 (6H, s, $SiCH_3$), 0.98 (9H, s, *tert*Bu), 3.54 (3H, s, OCH_3), 3.79 (3H, s, OCH_3), 3.85 (3H, s, OCH_3), 3.89 (3H, s, OCH_3), 5.17 (1H, d, *J* = 1.4, CH_2), 5.51 (1H, d, *J* = 1.4, CH_2), 6.67 (1H, d, *J* = 8.6, H_5), 6.73 (1H, d, *J* = 8.6, H_6), 6.80 (1H, dd, *J* = 8.6; 2.2, $H_{6'}$), 6.89 (1H, d, *J* = 2.2, $H_{2'}$), 6.93 (1H, d, *J* = 8.6, $H_{5'}$). ^{13}C NMR δ −4.5 (×2) (CH_3), 18.5 (C), 25.8 (×3) (CH_3), 55.5 (CH_3), 56.0 (CH_3), 60.7 (CH_3), 60.8 (CH_3), 106.9 (CH), 111.4 (CH), 113.7 (CH_2), 119.4 (CH), 120.3 (CH), 125.2 (CH), 132.2 (C), 134.9 (C), 142.3 (C), 144.6 (C), 146.4 (C), 150.6 (C), 151.6 (C), 153.5 (C).

3.1.12.10. 1-[1-(3-*tert*Butyldiphenylsiloxy-4-methoxyphenyl)vinyl]-3,4,5-trimethoxybenzene (5j). 177 mg of **5j** (57% isolated yield after flash chromatography using hexanes/EtOAc 9:1) were obtained by method G from 310 mg (0.56 mmol) of **4j** with 653 mg (1.62 mmol) of $CH_3P^+Ph_3{\cdot}I^-$ and 0.84 mL (1.35 mmol) of *n*BuLi (1.6 M in hexanes) in 7 mL of dry THF. ^{1}H NMR δ 1.14 (9H, s, *tert*-Bu), 3.62 (3H, s, 4- or 4′-OCH_3), 3.74 (6H, s, 3′,5′-OCH_3), 3.89 (3H, s, 4- or 4′-OCH_3), 5.10 (1H, d, *J* = 1.4, CH_2), 5.15 (1H, d, *J* = 1.4, CH_2), 6.42 (2H, s, H_2, H_6), 6.72 (1H, d, *J* = 1.8, $H_{2'}$), 6.73 (1H, d, *J* = 8.2, $H_{5'}$), 6.85 (1H, dd, *J* = 8.2, 1.8, $H_{6'}$), 7.35 (6H, m, Ph), 7.70 (4H, m, Ph). ^{13}C NMR δ 19.8 (C), 26.8 (×3) (CH_3), 55.5 (CH_3), 56.0 (×2) (CH_3), 61.0 (CH_3), 105.5 (×2) (CH), 111.7 (CH), 112.4 (CH_2), 120.2 (CH), 121.4 (CH), 127.5 (×4) (CH), 129.7 (×2) (CH), 133.5 (×2) (C), 133.7 (×2) (C), 135.6 (×4) (CH), 137.5 (C), 144.7 (C), 149.5 (C), 150.5 (C), 152.7 (×2) (C).

3.1.12.11. 1-[1-(3-Nitro-4-methoxyphenyl)vinyl]-2,3,4-trimethoxybenzene (5k). 58 mg of **5k** (27% isolated yield after flash chromatography using hexanes/EtOAc 9:1) were obtained by method G from 220 mg (0.56 mmol) of **4k** with 1.02 g (2.54 mmol) of $CH_3P^+Ph_3{\cdot}I^-$ and 1.38 mL (2.22 mmol) of *n*BuLi (1.6 M in hexanes) in 7 mL of dry THF. ^{1}H NMR δ 3.55 (3H, s, 2′-OCH_3), 3.84 (3H, s, 3′- or 4′-OCH_3), 3.89 (3H, s, 3′- or 4′-O CH_3), 3.90 (3H, s, 4-OCH_3), 5.30 (1H, s, CH_2), 5.58 (1H, s, CH_2), 6.68 (1H, d, *J* = 8.4, H_5), 6.94 (1H, d, *J* = 8.4, H_6), 7.00 (1H, d, *J* = 8.8, $H_{5'}$), 7.47 (1H, dd, *J* = 8.8, 2.2, $H_{6'}$), 7.78 (1H, d, *J* = 2.2, $H_{2'}$). ^{13}C NMR δ 56.1 (CH_3), 56.6 (CH_3), 60.7 (CH_3), 61.0 (CH_3), 107.3 (CH), 113.1 (CH), 115.9 (CH_2), 123.5 (CH), 125.2 (CH), 127.7 (C), 132.1 (CH), 134.6 (C), 139.3 (C), 142.4 (C), 144.6 (C), 151.5 (C), 152.2 (C), 154.1 (C). IR (film): 1531, 1598 cm^{-1}.

3.1.12.12. 1-[1-(3-Nitro-4-methoxyphenyl)vinyl]-3,4,5-trimethoxybenzene (5l). 114 mg of compound **5l** were obtained in 58% yield by Wittig reaction (method G). ^{1}H NMR δ 3.79 (6H, s, 2 × OCH_3), 3.85 (3H, s, OCH_3), 3.95 (3H, s, OCH_3), 5.41 (2H, s, CH_2), 6.48 (2H, s, $H_{2'}$, $H_{6'}$), 7.04 (1H, d, *J* = 8.6, H_5), 7.50 (1H, dd; *J* = 8.6, 2.3, H_6), 7.83 (1H, d, *J* = 2.3, H_2). ^{13}C NMR δ 56.2 (×2) (CH_3), 56.6 (CH_3), 60.9 (CH_3), 105.5 (×2) (CH), 113.3 (CH), 114.7 (CH_2), 125.1 (CH), 133.8 (CH), 136.2 (C), 139.3 (C), 142,3 (C),

147.1 (C), 147.6 (C), 152.5 (C), 153.1 (×2) (C). HRMS *m*/*z* calcd for $C_{18}H_{19}NO_6$ (+Na): 368.1105, found 368.1124.

3.1.12.13. 5-[1-(2,3,4-Trimethoxyphenyl)vinyl]-2-methoxyphenol (5m). Following method I, 0.56 mL of 1.0 M TBAF solution in THF was added to 80 mg (0.19 mmol) of **5i** dissolved in 2 mL of dry THF at room temperature under Ar atmosphere. After 1 h, the solution was poured onto water and extracted with ether. The organic layers were washed with brine, dried over anhydrous Na_2SO_4 and evaporated to yield 57 mg (95%) of **5m**. ^{1}H NMR δ 3.57 (3H, s, 2′-OCH_3), 3.85 (3H, s, OCH_3), 3.86 (3H, s, OCH_3), 3.88 (3H, s, OCH_3), 5.18 (1H, d, J = 1.6, CH_2), 5.55 (1H, d, J = 1.6, CH_2), 6.66 (1H, d, J = 8.6, $H_{5'}$), 6.72 (1H, d, J = 8.2, H_3), 6.96 (1H, br d, J = 8.2, H_4), 6.92 (1H, d, J = 8.6, $H_{6'}$), 6.96 (1H, br s, H_6). ^{13}C NMR δ 56.0 (CH_3), 56.1 (CH_3), 60.8 (CH_3), 60.9 (CH_3), 107.0 (CH), 110.1 (CH), 113.0 (CH), 114.1 (CH_2), 118.7 (CH), 125.2 (CH), 129.2 (C), 135.4 (C), 142.3 (C), 145.2 (×2) (C), 146.2 (×2) (C), 153.5 (C). IR (film): 1593, 3415 cm^{-1}.

3.1.12.14. 5-[1-(3,4,5-Trimethoxyphenyl)vinyl]-2-methoxyphenol (5n). Following the general procedures A, E, G and I, **5n** was obtained in 57% yield. ^{1}H NMR δ 3.81 (6H, s, 2 × OCH_3), 3.87 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 5.30 (1H, d, J = 1.4, CH_2), 5.37 (1H, d, J = 1.4, CH_2), 6.55 (2H, s, $H_{2'}$, $H_{6'}$), 6,80 (1H, d, J = 8.2, H_3), 6.85 (1H, dd, J = 8.2, 1.8, H_4), 6.97 (1H, d, J = 1.8, H_6). ^{13}C NMR δ 56.0 (×3) (CH_3), 61.0 (CH_3), 105.7 (×2) (CH), 110.2 (CH), 113.0 (CH_2), 114.5 (CH), 120.0 (CH), 127.7 (C), 134.7 (C), 137.5 (C), 145.2 (C), 146.5 (C), 149.6 (C), 152.8 (×2) (C). HRMS *m*/*z* calcd for $C_{18}H_{20}O_5$ (+Na) 339.1203, found 339.1219.

3.1.12.15. 5-[1-(2,3,4-Trimethoxyphenyl)vinyl]-2-methoxyaniline (5o). 100 mg (1.54 mmol) of Zinc turnings was added to a stirred suspension of 57 mg (0.17 mmol) of **5k** in 10 mL of CH_2Cl_2 and 2 mL of glacial acetic acid under Ar. After 12 h, the reaction mixture was filtered through Celite. The filtrate was concentrated under vacuum to obtain 52 mg (97%) of compound **5o**.^{1}H NMR δ 3.56 (3H, s, 2′-OCH_3), 3.82 (3H, s, OCH_3), 3.85 (3H, s, OCH_3), 3.87 (3H, s, OCH_3), 5.15 (1H, s, CH_2), 5.55 (1H, s, CH_2), 6.66 (4H, m, H_4, H_6, $H_{5'}$, $H_{6'}$), 6.92 (1H, d, J = 8.2, H_3). ^{13}C NMR δ 55.6 (CH_3), 56.0 (CH_3), 60.8 (CH_3), 60.9 (CH_3), 106.8 (CH), 109.9 (CH), 113.7 (CH_2), 114.2 (CH), 118.0 (CH), 125.3 (CH), 129.5 (C), 134.8 (C), 142.3 (C), 146.5 (×2) (C), 147.5 (C), 151.7 (C), 153.4 (C). IR (film): 1596, 3367 cm^{-1}.

3.1.12.16. 5-[1-(3,4,5-Trimethoxyphenyl)vinyl]-2-methoxyaniline (5p). To a stirred suspension of **5l** in CH_2Cl_2:glacial acetic acid 1:5 (0.1 M) under Ar was added 10 equiv of Zinc turnings. After 12 h, the reaction mixture was filtered through Celite. The filtrate was concentrated under vacuum to obtain 94 mg of compound **5p** in quantitative yield. ^{1}H NMR δ 3.82 (6H, s, 2 × OCH_3), 3.87 (6H, s, 2 × OCH_3), 5.27 (1H, d, J = 1.2, CH_2), 5.34 (1H, d, J = 1.2, CH_2), 6.56 (2H, s, $H_{2'}$, $H_{6'}$), 6.74 (3H, s, H_3, H_4, H_6). ^{13}C NMR δ 55.6 (CH_3), 56.2 (×2) (CH_3), 61.0 (CH_3), 105.7 (×2) (CH), 109.9 (CH), 112.4 (CH_2), 115.3 (CH), 119.0 (CH), 134.1 (C), 135.4 (C), 137.6 (C), 137.8 (C), 147.5 (C), 150.0 (C), 152.8 (×2) (C). HRMS *m*/*z* calcd for $C_{18}H_{21}NO_4$ (+Na): 338.1363, found 338.1379.

3.2. Tubulin isolation

Calf brain microtubule protein (MTP) was purified by two cycles of temperature-dependent assembly/disassembly, according to the method of Shelanski,[21] modified as described in the literature.[22] The MTP solution was stored at −80 °C. Protein concentrations were determined by the method of Bradford,[23] using BSA as standard.

3.3. Tubulin assembly

In vitro tubulin self-assembly was monitored turbidimetrically at 450 nm, using a thermostated Thermo-Spectronic Helios α spectrophotometer fitted with a temperature controller and a circulating water carrousel system. The ligands were dissolved in DMSO and stored at −20 °C. The amount of DMSO in the assays was 4%, which has been reported not to interfere with the assembly process.[24] The increase in turbidity was followed simultaneously in a batch of six cuvettes (containing 1.0 mg/mL MTP in 0.1 M MES buffer, 1 mM EGTA, 1 mM $MgCl_2$, 1 mM, β-ME, 1.5 mM GTP, pH 6.7, and the measured ligand concentration), with a control (i.e., with no ligand) always being included.

The samples were preincubated for 30 min at 20 °C in order to allow binding of the ligand, and were cooled on ice for 10 min. The cuvettes were then placed in the spectrophotometer at 4 °C. The assembly process was initiated by a shift in the temperature to 37 °C. The IC_{50} was calculated as the concentration of drug causing 50% inhibition of polymerization after 20 min of incubation and was determined graphically. At least two independent experiments (or more when required for the most potent inhibitors) with different MTP preparations were carried out for each compound tested.

3.4. XTT procedure

100 μL of exponentially growing HeLa (1.5×10^3 cells/well), HT-29 (3×10^3 cells/well), or A-549 (5×10^3 cells/well) cells were seeded in 96-well flat-bottomed microtiter plates, and incubated at 37 °C in a humidified atmosphere of 5% CO_2/95% air for 24 h to allow the cells attach to the plates. HL-60 cells were seeded at 3×10^3 (100 μL) cells per well. Then, cells were incubated with different concentrations of the assayed compound at 37 °C under the 5% CO_2/95% air atmosphere for 72 h. Cell proliferation was quantified using the XTT (3′-[1-(phenylamino)carbonyl]-3,4-tetrazolium-bis(4-methoxy-6-nitro)benzene sulfonic acid sodium salt hydrate) cell proliferation kit (Roche Molecular Biochemicals, Mannheim, Germany) following the manufacturer's instructions. Briefly, a freshly prepared mixture solution (50 μL) of XTT labeling reagent and PMS (*N*-methyldibenzopyrazine methyl sulfate) electron coupling reagent was added to each well. The resulting mixtures were further incubated for 4 h in a humidified atmosphere (37 °C, 5% CO_2), and the absorbance of the formazan product generated was measured with a microtiter plate reader at a test wavelength of 490 nm and a reference wavelength of 655 nm. The IC_{50} (50% inhibitory concentration) was then calculated as the drug concentration causing a 50% inhibition of cell proliferation. Data are shown as mean values of three independent experiments performed in triplicate.

3.5. Molecular modeling

The compounds were docked into the colchicine site of tubulin following a described protocol.[25] The X-ray structures of the tubulin complexes with podophyllotoxin and DAMA-colchicine were retrieved from the protein data bank,[26] while chains C, D and E and the corresponding hetero-groups were removed by hand. The pdb files were energy-minimized and subjected to molecular dynamics simulations at 300 K.[27] We initially restrained the backbone, and then it was set free. The relaxed structures were superimposed and a combined site was generated by shifting the tubulin-podophyllotoxin complex 30 Å along the X axes. The combined tubulin sites and the podophyllotoxin and DAMA-colchicine ligands were used to generate a combined protomol with the Surflex docking program.[19] The individual sites were also used in separate docking experiments with AutoDock 3.[20] The synthesised compounds together with roughly 300 combretastatin and phenst-

atin analogues were manually constructed in silico[28] and docked into the combined sites (cross-docking), in an attempt to better reproduce the receptor flexibility by using different configurations of the protein.[18] Additionally, the test set of ACD compounds used in the Surflex validation were equally docked. These compounds were considered a negative control group: that is, lacking biological activity. The combined results were analyzed by receiver operating characteristics (ROCs);[29] the ACD compounds and the analogues of the colchicine site with published TPI worse than 20 μM were considered inactive. The enrichment factors achieved were similar to those described for similar systems. The structures of the best scored complexes in the colchicine and podophyllotoxin sites were inspected visually and compared with the TPI results.[30]

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