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Diarylmethyloxime and hydrazone derivatives with 5-indolyl moieties as potent inhibitors of tubulin polymerization

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Abstract—We describe the synthesis and biological evaluation of a series of diarylmethyloxime and diarylmethylhydrazone analogues that contain an indole ring and different modifications on the nitrogen of the bridge. Several compounds showed potent tubulin polymerization inhibitory action as well as cytotoxic activity against cancer cell lines. The *N*-methyl-5-indolyl substituted analogues are more potent than ethyl substituted ones. The most potent inhibitors of tubulin polymerization are the diarylketones and the diaryloximes. The cytotoxicity against several cancer cell lines is lower for the oximes than for the ketones. Other substitutions on the imine nitrogen greatly reduce the tubulin inhibitory and/or cytotoxic potencies.

1. Introduction

Microtubules are cylinder-shaped protein polymers composed of α-tubulin and β-tubulin heterodimers arranged head to tail.[1] Many useful anticancer drugs bind to one of at least three distinct binding sites located on the β subunit of tubulin: the colchicine, vinca and taxane domains. Many natural and synthetic products of varied structures bind to the colchicine site of tubulin,[2] thus suggesting a high plasticity of tubulin at the colchicine site, and a common pharmacophore has been proposed for colchicine site ligands.[3,2d] The X-ray crystal structures of tubulin complexes with DAMA-colchicine and podophyllotoxin show very hydrophobic binding pockets, which accommodate to the shape of the binding ligands.[4]

Combretastatins are strong inhibitors of tubulin polymerization,[5] which have attracted much attention owing to their potent cytotoxic and vascular disrupting activities.[6] The phosphate prodrugs of the natural products combretastatin A-1 and combretastatin A-4 are currently in clinical development.[2a,7] Many SAR studies have established the key structural elements responsible for high antitubulin and cytotoxic activities in combretastatins and related compounds: (a) the trimethoxyphenyl ring (A) is seen as an essential element, (b) the bridge may have zero to four atoms and must keep the two aromatic rings in a cisoid disposition, such as that forced by double bonds, small heterocycles or carbocycles and (c) ring B allows more modifications than ring A, while the 4-methoxy substituent is usually conserved the 3-hydroxy is replaceable. More dramatic modifications—that is, the replacement of the B ring by bicyclic systems such as a 2-naphthyl moiety or a 5-indolyl unit—give rise to potent compounds (Fig. 1).[8] These SARs found for combretastatins have been extended to many other structurally related compounds (e.g., phenstatins), with different degrees of fulfillment. In the search for more potent analogues, these findings are relevant to the establishment of broader SAR and for the understanding of the rules that govern the binding to the colchicine site.

A new family of tubulin polymerization inhibitors termed phenstatins has recently attracted much attention, owing to their high potency and improved solubility characteristics.[9,2d] The phenstatins are bisarylketones that share many of the SARs of combretastatins, such as

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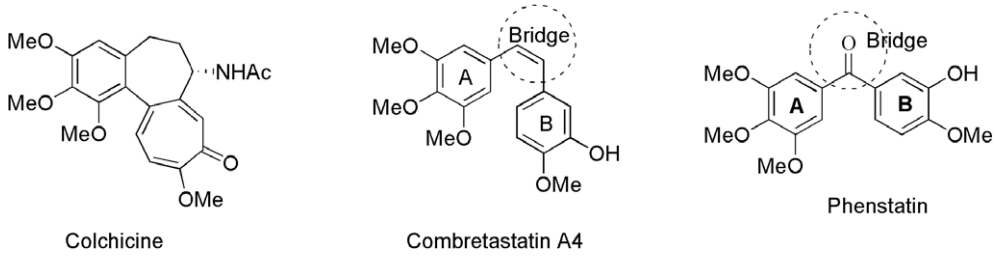


Figure 1. Structure of colchicine, combretastatin A-4 and phenstatin.

the importance of the A ring and the possibility of having a 2-naphthyl B ring[10] or a 5-indolyl ring.[11] However, in some cases the SAR clearly deviates from that of combretastatins thus opening the possibility of new tests of structural modifications.[8,9f,g] There are no reports on phenstatins carrying modifications on the central ketone, other than the hydroxyl and acetoxy derivatives produced as synthetic intermediates. Such modifications may be of interest in the development of new antitumour or vascular disrupting compounds with improved characteristics, a largely explored possibility in combretastatins (Fig. 2).[7,12]

2. Results and discussion

The diarylmethanols were synthesized by condensation of the protected lithioindoles with 3,4,5-trimethoxybenzaldehyde (Scheme 1). The magnesium salts used to synthesize the 2-naphthyl analogues of phenstatins proved unsuccessful since at room temperature the reaction does not occur. Upon heating, the addition occurs mainly through C3 of the indole moiety, often with the formation of double-addition products. Oxidations of the methanols were carried out with $KMnO_4$ and phase transfer catalysis, since CCP or $K_2Cr_2O_7$ did not give the desired ketones in good yields.

Reaction of the ketones with hydroxylamine, hydrazine, and *N*-methylhydrazine led to the corresponding oximes and hydrazones as roughly 1:1 mixtures of the *E* and *Z* isomers, which could not be separated chromatographically. In order to attempt a chromatographic separation and to modify the hydrogen bond donor–acceptor nature of the bridge substitutions, the oximes and hydrazones were acetylated. Thus, *E* and *Z* acetyloximes were separated, but nOe experiments were not conclusive as to the stereochemistry of each isomer. This issue was finally solved by crystallization of one of the isomers of compound **11**, which was shown by X-ray diffraction to have *Z* stereochemistry (Fig. 3). The individual isomeric *Z* oximes (**9*Z*** and **9*E***) were prepared by basic hydrolysis of the acetates, and no interconversion occurred in time not even during column chromatography. The stereochemistry of the remaining compounds was established by spectroscopic comparison with **9** and **11** (Table 1). The main spectroscopic differences between the isomers were observed for the shielding of the two aromatic protons of the 3,4,5-trimethoxyphenyl ring (upfield-shifted in the *Z* isomers) and for the proton at position 6 of the indole ring (opposite trend).

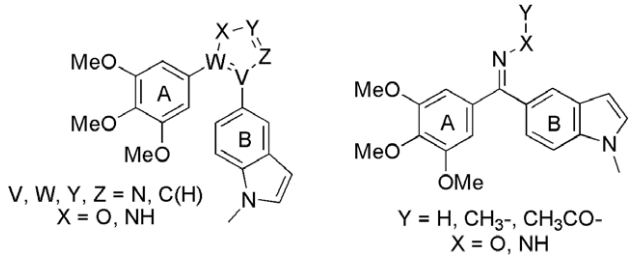
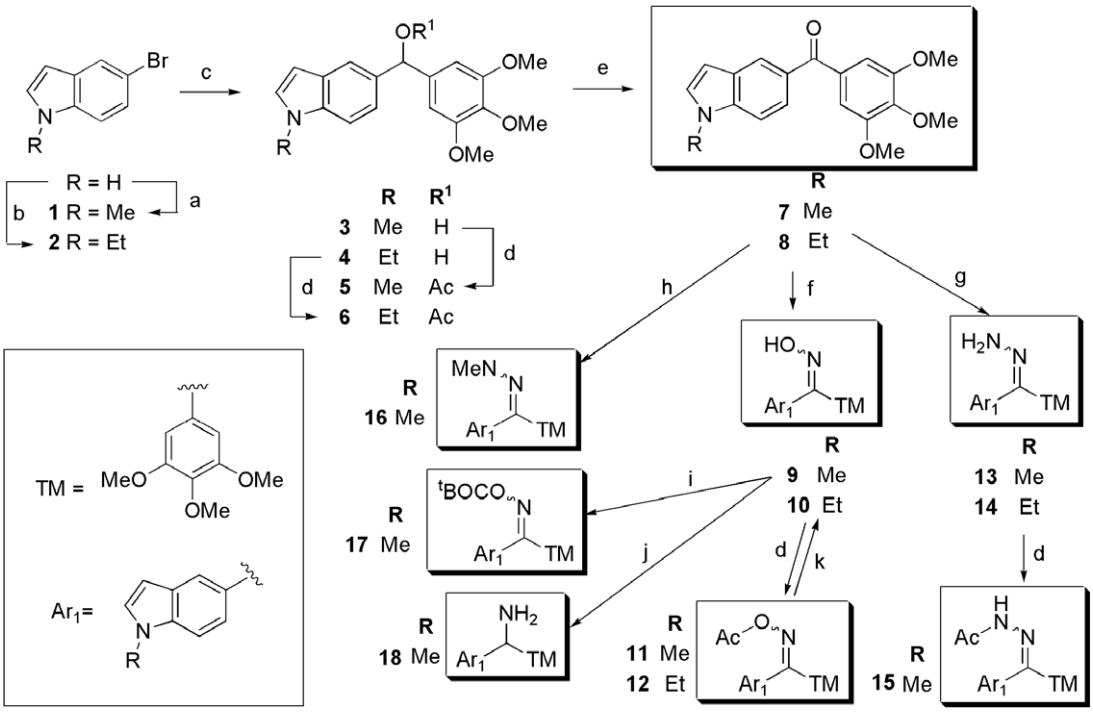


Figure 2. Representative structures of potent combretastatins modified on the bridge (left) and suggested analogous modifications on related phenstatins (right).

The synthesized compounds were tested at a single concentration (20–40 μM) in the tubulin polymerization assay. The IC_{50} was determined for those compounds exhibiting an inhibition over 50%. In most cases, the *N*-methyl derivatives were more potent than the corresponding ethyl derivatives. With respect to bridge substitution, the ketones (**7** and **8**) and the oximes (**9** and **10**) were the most potent analogues. Both the ketones and the oximes were less potent than phenstatin itself, although oxime **9**, a mixture of the *Z* and *E* isomers, was almost as potent as phenstatin. The low potency shown by isomer **9*Z*** suggests that isomer **9*E*** must be similar in potency to phenstatin. Replacement of the 3-hydroxy-4-methoxyphenyl ring by the *N*-methyl-5-indolyl ring in combretastatins,[8] combretastatin analogues,[13] and phenstatins[11c,d] causes slight changes in potency, similar to those reported here. Neither the alcohols (**3** and **4**), the hydrazones (**13**, **14** and **16**), or any of the acylated derivatives (**5**, **6**, **11**, **12**, **15** and **17**) were seen to be potent inhibitors of tubulin polymerization.

The synthesized compounds were assayed following a previously described procedure[8] against several cancer cell lines, including HeLa human cervix epitheloid carcinoma, A-549 human lung carcinoma, HL-60 human leukemic, and HT-29 human colon adenocarcinoma. The results were compared with those of CA4 (Table 2). The most sensitive cell line was HeLa whereas the other cell lines were more resistant to the compounds. Often, but not always, phenstatin analogues displayed lower cytotoxic potencies than their combretastatin counterparts, even though they were more potent inhibitors of tubulin polymerization. The same reduction in cytotoxicity with respect to tubulin inhibitory activity has also been reported for the replacement of the 3-hydroxy-4-



Scheme 1. Synthesis of compounds **1**–**18**. Reagents and conditions: (a) NaOH, MeI, CH_2Cl_2, HSO_4^- Bu_4N^+; (b) NaOH, EtBr, CH_2Cl_2, HSO_4^- Bu_4N^+; (c) *n*-BuLi, THF, −40 °C, 3,4,5-trimethoxybenzaldehyde; (d) Ac_2O, pyr; (e) $KMnO_4$, HSO_4^- Bu_4N^+, CH_2Cl_2; (f) $NH_2OH{\cdot}HCl$, MeOH reflux, AcOH; (g) $NH_2NH_2{\cdot}HCl$, MeOH reflux, AcOH; (h) $NH_2NHMe{\cdot}HCl$, MeOH reflux, AcOH; (i) $(^tBOC)_2O$, dioxane/water, Na_2CO_3; (j) $NaBH_4$, MeOH; (k) NaOH, MeOH.

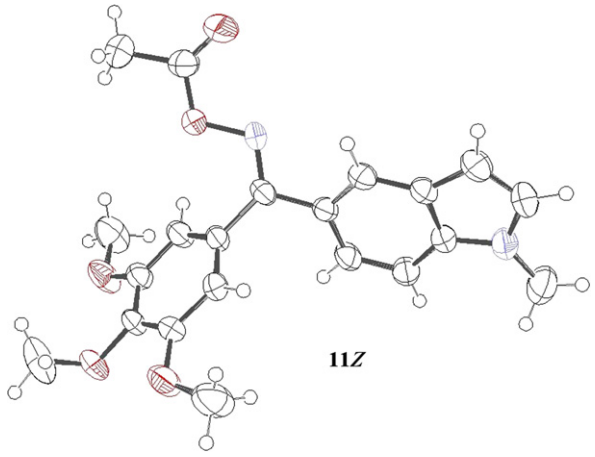


Figure 3. Ortep drawing of the X-ray crystal structure of compound **11*Z***.

Table 1. Chemical shifts of the relevant protons for the establishment of the stereochemistry of compounds **9**–**17** by comparison with those of **9** and **11** (italics)

C6–H δ (ppm)		Compound	C2′, C6′–H δ (ppm)	
E	*Z*		*E*	*Z*
7.27	*7.56*	***9***	*6.74*	*6.65*
7.31	7.56	**10**	6.76	6.68
7.22	*7.65*	***11***	*6.81*	*6.55*
7.20	7.63	**12**	6.81	6.55
7.10	7.68	**13**	6.78	6.52
7.04	7.63	**14**	6.76	6.51
7.02	7.75	**15**	6.85	6.43
7.08	7.70	**16**	6.78	6.51
7.25	7.64	**17**	6.83	6.59

methoxyphenyl ring by the *N*-methyl-5-indolyl ring.[8,11c,d,13] In our case the ketones were unaffected by any of these unfavorable aspects and showed cytotoxic activities comparable to those of CA4, despite their being slightly less potent tubulin polymerization inhibitors. On the other hand, the oximes underwent a significant decrease in cytotoxic potency. The lower cytotoxicity of the oximes as compared with their parent ketones indicates that they are not hydrolyzed under the assay conditions, in conformity with the hydrolysis kinetics of ketoprophen oximes, designed as prodrugs.[14] The lack of activity of the acylated oxime analogues makes them suitable starting points for the design of new (double) prodrugs of phenstatin analogues with improved solubility, pharmacokinetics, or tumor-targeting properties.

The synthesized compounds were cross-docked[15] in a combined podophyllotoxin–colchicine site using the Surflex docking program.[16] The preferred poses fit well into both sites (that of the podophyllotoxin complex and that of the colchicine complex), many ligands binding in both sites with minor energy differences between the poses. However, visual inspection of the docked poses at the colchicine site revealed that the binding site for the trimethoxyphenyl ring is often different from that of colchicine, in sharp contrast with its essential role in many diverse colchicine-site binding ligands.[7] We therefore decided to give preference to the poses docked at the podophyllotoxin site. Most of these poses placed the

Table 2. Activity results for the synthesized compounds

Compound	%TPI	IC_{50} TPI	HeLa	HL-60	A-549	HT-29
CA4[8]	99 (20)	3 (1–4)[7,10]	0.003	0.002	0.003	0.032
Phenstatin	97 (20)	2.5	0.03	0.03	0.3	1.8
3	86 (20)	18	>1.0	>1.0	>10	>10
4	42 (20)	—	>1.0	>1.0	>10	>10
5	41 (20)	—	0.0064	0.26	>10	0.24
6	0 (20)	—	>1.0	>1.0	>10	>10
7	98 (40)	7.9	0.034	—	0.12	—
8	95 (20)	5.7	0.016	—	0.14	0.034
9[a]	82 (30)	1.8	0.36	0.33	>10	0.11
9*Z*	23 (20)	—	—	—	—	—
10	87 (20)	5.9	>1.0	>1.0	>10	>1.0
11*Z*	41 (20)	—	—	—	—	>10
11*E*	38 (20)	—	—	—	—	>10
12*Z*	54 (20)	—	>10	>10	>10	>1.0
12*E*	26 (20)	—	>1.0	>1.0	>10	>1.0
13	35 (30)	—	—	—	—	>10
14	7 (30)	—	>1.0	>1.0	>10	>1.0
15	14 (30)	—	2.3	—	>10	>1.0
16	20 (20)	—	>1.0	>1.0	>1.0	>1.0
17*Z*	17 (20)	—	>1.0	>1.0	>1.0	>1.0
17*E*	10 (20)	—	>1.0	>1.0	>10	>1.0

Tubulin polymerization inhibitory activity expressed as the percentage of polymerization of the sample with respect to the control (%TPI) at a single micromolar concentration (under parenthesis) or as the IC_{50} (μM) for the most potent derivatives (IC_{50} TPI). Cytotoxicity against 4 cell lines expressed as the IC_{50} (μM). Literature IC_{50}(TPI) values for CA4 are given under parenthesis for comparison.

[a] 50% mixture of *E*/*Z* isomers.

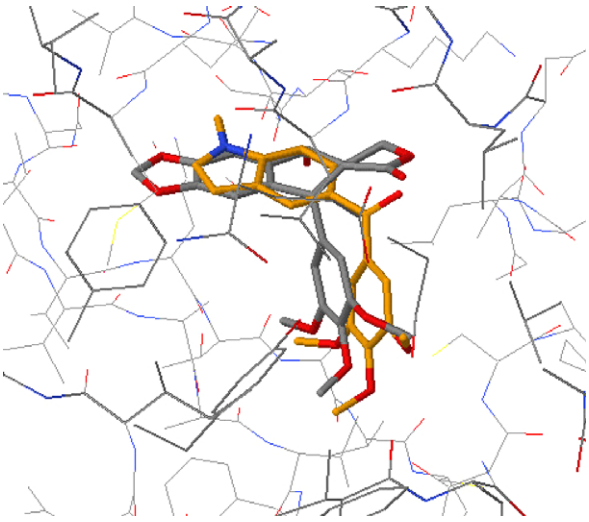


Figure 4. Superimposition of the lowest-energy docked pose of compound **8** (in wireframe, carbons colored orange) over podophyllotoxin at its binding site at tubulin (protein is represented as a wireframe and podophyllotoxin as thicker lines).

trimethoxyphenyl ring in close proximity to the one in podophyllotoxin, and the resulting ranking of the poses agreed more closely with the experimental TPI results. These results are in good concordance with the fact that the phenstatins resemble the structure of podophyllotoxin (a bisarylmethane) more closely than that of colchicine (a phenyltropolone). The ketones and the oximes hydrogen bond with the backbone NH group of residue β248 and place the carbonyl oxygen atom (or iminic nitrogen) close to the position occupied by the carbonyl oxygen atom of podophyllotoxin, explaining hence the difficulty of replacing it by more bulky substituents (such as the acyl derivatives). In the prediction of a pharmacophore for colchicine site inhibitors,[3] 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 *N*-methyl-5-indolyl unit superimposes onto the methylenedioxyphenyl ring of podophyllotoxin (Fig. 4). The ethyl group of *N*-ethyl-5-indolyl analogues slightly protudes over the methylenedioxyphenyl ring and displaces the indole moiety. This suggests that the moiety is too large and it explains the observed modest potency decrease.

3. Conclusions

Several bridge modifications have been introduced for the first time in a new family of phenstatin analogues, and the oximes have been shown to maintain tubulin inhibitory activity. These results indicate that the oximes of phenstatins and their derivatives are potentially good starting points for the design of new cytotoxic compounds with improved characteristics. This functionalization of the bridge of phenstatins also opens up the possibility to prepare new derivatives able to act as prodrugs. The *N*-methyl-5-indolyl has been confirmed as a good surrogate for the 3-hydroxy-4-methoxyphenyl ring of phenstatin.

4. Experimental

4.1. Chemistry

4.1.1. Materials and methods. Reagents were used as purchased without further purification. Solvents

(THF, DMF, CH_2Cl_2, benzene) were dried and freshly distilled before use according to procedures reported in the literature. Chromatographic separations were performed on silica gel columns by flash (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}). Melting points were determined on a Büchi 510 apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AC200 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-R 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 Phenilic 5 μm (4.6 × 150 mm) columns with water–acetonitrile or water–methanol gradients. Combretastatin A4 and phenstatin were synthesized following literature protocols.[8,9]

4.1.2. Protection of the indole nitrogen. Two millimole 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.

4.1.3. Synthesis of diaryl methanols. Three to 35 mL (2.2 mmol per mmol of *N*-alkyl-2-bromoindole) of 1.6 M *n*-BuLi in hexanes was added to a stirred suspension of 2.4–45.2 mmol of the N-alkylated-5-bromo-1*H*-indol in dry THF (20–60 mL) at −78 °C under an Argon atmosphere. After 1–2 h, 1.2 mmol of the aldehyde per mmol of the bromoindole was slowly added to the resulting yellowish solution, allowing the reaction to slowly reach room temperature. After 12–24 h, the mixture was poured over ice and extracted with CH_2Cl_2. The organic layers were dried over anhydrous Na_2SO_4, filtered, and evaporated under vacuum.

4.1.4. Acetylation reactions. A 10 mol excess of acetic anhydride was added to a solution of the hydroxyl derivative in pyridine. After 2–4 h, the reaction was poured onto EtOAc, washed with 2 N HCl, 5% $NaHCO_3$ and brine, and the organic layers were dried over anhydrous Na_2SO_4, filtered, and evaporated under vacuum.

4.1.5. Oxidation of diarylmethanols to diarylketones. One millimole of $KMnO_4$ per mmol of alcohol and a 1% w/w of n-Bu_4N^+ HSO_4^- were added to a 0.15–0.25 M solution of the alcohol in CH_2Cl_2, and the reaction was allowed to proceed until completion (TLC) for 8–12 h. The reaction crude was filtered through silica, using CH_2Cl_2 and ethyl acetate as eluants, and the organic layers evaporated under vacuum to yield the diarylketones.

4.1.6. Oxime preparation. 1.2–15.5 mmol of $NH_2OH{\cdot}HCl$ (10 mmol/mmol of ketone) and 2–4 droplets of pyridine were added to a 0.02–0.06 M solution of the corresponding ketone in 10–25 mL of MeOH, and the reaction was refluxed for 12–48 h. The reaction was cooled, the solvent was evaporated off, and the residue was re-dissolved in CH_2Cl_2, and washed with brine, and the organic layers were dried, filtered, and evaporated.

4.1.7. Hydrazone synthesis. To a 0.02–0.06 M solution of the ketone in methanol were added 10 mmol of the corresponding hydrazine per mmol of ketone and 2–4 droplets of acetic acid. The reaction was refluxed for 48 h, with the addition of 10 mmol of hydrazone per mmol of ketone every 12 h. The crude product was evaporated in vacuum, re-dissolved in CH_2Cl_2 and washed with brine, and the organic layers were dried over anhydrous Na_2SO_4, filtered and evaporated in vacuum.

4.1.8. Synthesis of key intermediates and analogs.

4.1.8.1. 1-Methyl-5-bromo-1*H*-indole (1). Following the general method, 2.5 g of 5-bromo-1*H*-indole and 2.3 mL of MeI yielded 2.3 g (86%) of **1**. ^{1}H NMR δ_H (ppm) (400 MHz): 3.77 (s, 3H), 6.42 (d, J = 3.1 Hz, 1H), 7.05 (d, J = 3.1 Hz, 1H), 7.19 (d, J = 8.8 Hz, 1H), 7.30 (dd, J_1 = 8.8 Hz, J_2 = 1.7 Hz, 1H), 7.76 (d, J = 1.7 Hz, 1H).

4.1.8.2. 1-Ethyl-5-bromo-1*H*-indole (2). Following the general method, 2.5 g of 5-bromo-1*H*-indole and 1.9 mL of EtBr yielded 1.9 g (55%) of **2**. ^{1}H NMR δ_H (ppm) (400 MHz): 1.46 (t, J = 7.5 Hz, 3H), 4.15 (q, J = 7.5 Hz, 2H), 6.44 (d, J = 3.1 Hz, 1H), 7.12 (d, J = 3.1 Hz, 1H), 7,21 (d, J = 8.8 Hz, 1H), 7.29 (dd, J_1 = 8.8 Hz, J_2 = 1.8, 1H); 7.76 (d, J = 1.8 Hz, 1H).

4.1.8.3. (1-Methyl-1*H*-indol-5-yl)(3,4,5-trimethoxyphenyl)methanol (3). 34.7 mL (55.2 mmol) of 1.6 M *n*-BuLi in hexanes was reacted with 5.3 g (25.2 mmol) of **1** in 40 mL of dry THF and with 6.5 g (33.5 mmol) of 3,4,5-trimethoxybenzaldehyde dissolved in 20 mL of dry THF to yield, after treatment according to the general procedure, 6.5 g of a white solid, which after column chromatography gave 4 g (50%) of **3**. Mp: 125–127 °C (CH_2Cl_2/Hex). IR (KBr): 3386, 1591, 1125, 734 cm^{-1}. ^{1}H NMR δ_H (ppm) (400 MHz): 3.77 (s, 3H), 3.81 (s, 6H), 3.83 (s, 3H), 5.88 (br s, 1H), 6.47 (d, J = 3.3 Hz, 1H), 6.67 (s, 2H), 7.06 (d, J = 3.3 Hz, 1H), 7.22 (d, J = 8.6 Hz, 1H), 7.29 (br d, J = 8.6 Hz, 1H), 7.62 (br s, 1H). ^{13}C NMR δ_C (ppm) (100 MHz): 33.0 (CH_3), 56.1 (CH_3) (2), 60.9 (CH_3), 76.9 (CH), 101.2 (CH), 103.5 (CH) (2), 109.5 (CH), 119.2 (CH), 120.7 (CH), 128.4 (C), 129.5 (CH), 135.1 (C), 136.4 (C), 140.4 (C) (2),

153.2 (C) (2). HRMS ($C_{19}H_{21}$ NO_4 + Na): calcd 350.1363, found 350.1321.

4.1.8.4. (1-Ethyl-1*H*-indol-5-yl)(3,4,5-trimethoxyphenyl)methanol (4). 32 mL (51 mmol) of 1.6 M *n*-BuLi in hexanes was reacted with 5.2 g (23.2 mmol) of **2** in 40 mL of dry THF and with 5.5 g (27.8 mmol) of 3,4,5-trimethoxybenzaldehyde dissolved in 20 mL of dry THF to yield, after treatment according to the general procedure, 6.2 g of a white solid, which after column chromatography gave 3.5 g (44%) of **4**. Mp: 114–116 °C (Hex/CH_2Cl_2). IR (KBr): 3457, 1592, 1505 cm^{-1}. ^1H NMR δ_H (ppm) (400 MHz): 1.46 (t, J = 7.1 Hz, 3H), 3.83 (s, 9H), 4.17 (q, J = 7.1 Hz, 2H), 5.89 (br d, J = 3.3 Hz, 1H), 6.48 (d, J = 3.0 Hz, 1H), 6.68 (s, 2H), 7.13 (d, J = 3.0 Hz, 1H), 7.21 (dd, J_1 = 8.4 Hz, J_2 = 1.8 Hz, 1H), 7.32 (d, J = 8.4 Hz, 1H), 7.62 (br s, 1H). ^{13}C NMR δ_C (ppm) (100 MHz): 15.5 (CH_3), 41.1 (CH_2), 56.2 (CH_3) (2), 60.8 (CH_3), 77.1 (CH), 101.4 (CH), 103.8 (CH) (2), 109.5 (CH), 119.3 (CH), 120.6 (CH), 127.6 (CH), 128.6 (C), 135.1 (C), 135.4 (C), 140.4 (C) (2), 153.2 (C) (2). HRMS ($C_{20}H_{23}NO_4$ + Na): calcd 364.1519, found 364.1511. HPLC: 9.72 (C_{18}), 9.34 (C_8), 9.14 (Phen).

4.1.8.5. (1-Methyl-1*H*-indol-5-yl)(3,4,5-trimethoxyphenyl)methylacetate (5). 600 μL of acetic anhydride was added to a solution of 200 mg of **3** in 600 μL of pyridine. After 2 h, 160 mg of **5** (70%), was obtained as a colourless oil. IR (film): 1739, 1592 cm^{-1}. ^1H NMR δ_H (ppm) (400 MHz): 2.18 (s, 3H), 3.76 (s, 3H), 3.83 (s, 6H), 3.85 (s, 3H), 6.48 (d, J = 3.2 Hz, 1H), 6.64 (s, 2H), 7.00 (s, 1H), 7.06 (d, J = 3.2 Hz, 1H), 7.22 (br d, J = 8.6 Hz, 1H), 7.30 (d, J = 8.6 Hz, 1H), 7.62 (br s, 1H). ^{13}C NMR δ_C (ppm) (100 MHz): 21.5 (CH_3), 32.9 (CH_3), 56.2 (CH_3) (2), 60.8 (CH_3), 77.9 (CH), 101.4 (CH), 104.3 (CH) (2), 109.4 (CH), 120.0 (CH), 121.2 (CH), 128.4 (C), 129.6 (CH), 131.1 (C), 136.4 (C), 136.7 (C), 137.5 (C), 153.3 (C) (2), 170.2 (C). HRMS ($C_{21}H_{23}NO_5$ + Na): calcd 392.1468, found 392.1493. HPLC: 11.13 (C_{18}), 10.68 (C_8), 10.37 (Phen).

4.1.8.6. (1-Ethyl-1*H*-indol-5-yl)(3,4,5-trimethoxyphenyl)methylacetate (6). 600 μL of acetic anhydride was added to a solution of 200 mg of **4** in 600 μL of pyridine. After 2 h, 195 mg of **5** (86%) was obtained as a colourless oil. IR (film): 1739, 1592, 1507 cm^{-1}. ^1H NMR δ_H (ppm) (400 MHz): 1.45 (t, J = 7.4 Hz, 3H), 2.20 (s, 3H), 3.83 (s, 9H), 4.15 (q, J = 7.4 Hz, 2H), 6.50 (d, J = 3.3 Hz, 1H), 6.67 (s, 2H), 7.01 (s, 1H), 7.13 (d, J = 3.3 Hz, 1H), 7.22 (dd, J_1 = 8.4 Hz, J_2 = 1.8 Hz, 1H), 7.34 (d, J = 8.4 Hz, 1H), 7.63 (br s, 1H). ^{13}C NMR δ_C (ppm) (100 MHz): 15.5 (CH_3), 21.4 (CH_3), 41.1 (CH_2), 56.2 (CH_3) (2), 60.8 (CH_3), 77.8 (CH), 101.5 (CH), 104.6 (CH) (2), 109.4 (CH), 120.1 (CH), 121.1 (CH), 127.8 (CH), 128.6 (C), 131.1 (C), 135.5 (C), 136.7 (C), 153.3 (C) (2), 170.1 (C). HRMS ($C_{22}H_{25}NO_5$ + Na): calcd 406.1625, found 406.1623. HPLC: 11.85 (C_{18}), 11.30 (C_8), 10.89 (Phen).

4.1.8.7. (1-Methyl-1*H*-indol-5-yl)(3,4,5-trimethoxyphenyl)methanone (7)[11d]. 3.1 g (9.5 mmol) of **3** was oxidized according to the general procedure to yield, after 8 h, 2.5 g of **7** (77%) as a white solid. Mp: 124.5–125.5 °C (CH_2Cl_2/Hex). IR (KBr): 1649, 1582, 820, 761 cm^{-1}. ^1H NMR δ_H (ppm) (400 MHz): 3.75 (s, 3H), 3.81 (s, 6H), 3.90 (s, 3H), 6.53 (d, J = 3.3 Hz, 1H), 7.04 (s, 2H), 7.08 (d, J = 3.3 Hz, 1H), 7.32 (d, J = 8.8 Hz, 1H), 7.74 (br d, J = 8.8 Hz, 1H), 8.09 (br s, 1H). ^{13}C NMR δ_C (ppm) (100 MHz): 33.0 (CH_3), 56.3 (CH_3) (2), 60.9 (CH_3), 102.9 (CH), 107.6 (CH) (2), 109.1 (CH), 123.7 (CH), 125.0 (CH), 127.7 (C), 129.3 (C), 130.6 (CH), 134.3 (C), 138.9 (C), 141.3 (C), 152.8 (C) (2), 196.3 (C). HRMS ($C_{19}H_{19}NO_4$ + Na): calcd 348.1206, found 348.1185.

4.1.8.8. (1-Ethyl-1*H*-indol-5-yl)(3,4,5-trimethoxyphenyl)methanone (8)[11d]. 2.0 g (5.9 mmol) of **4** was oxidized according to the general procedure to yield, after 8 h, 1.4 g of **8** (70%) as a white solid. Mp: 101–103 °C (Hex/CH_2Cl_2). IR (KBr): 1645, 1582 cm^{-1}. ^1H NMR δ_H (ppm) (400 MHz): 1.51 (t, J = 7.3 Hz, 3H), 3.88 (s, 6H), 3.94 (s, 3H), 4.23 (q, J = 7.3 Hz, 2H), 6.61 (d, J = 3.1 Hz, 1H), 7.08 (s, 2H), 7.21 (d, J = 3.1 Hz, 1H), 7.42 (d, J = 8.8 Hz, 1H), 7.79 (dd, J_1 = 8.8 Hz, J_2 = 1.5 Hz, 1H), 8,14 (d, J = 1.5 Hz, 1H). ^{13}C NMR δ_C (ppm) (100 MHz): 15.4 (CH_3), 41.2 (CH_2), 56.3 (CH_3) (2), 60.9 (CH_3), 103.0 (CH), 107.8 (CH) (2), 109.1 (CH), 123.6 (CH), 125.1 (CH), 127.9 (C), 128.7 (CH), 129.3 (C), 134.3 (C), 138.0 (C), 141.5 (C), 152.8 (C) (2), 196.1 (C). HRMS ($C_{20}H_{21}NO_4$ + Na): calcd 362.1363, found 362.1351. HPLC: 11.63 (C_{18}), 10.90 (C_8), 10.59 (Phen).

4.1.8.9. (*E*/*Z*)-(1-Methyl-1*H*-indol-5-yl)(3,4,5-trimethoxyphenyl)methanone oxime (9). Following the general procedure, 90 mg (0.28 mmol) of **7** yielded 86 mg (90%) of **9** as a 45(*Z*):55(*E*) mixture. IR (film): 3256, 1582 cm^{-1}. ^1H NMR δ_H (ppm) (400 MHz): 3.74 (s, 3H, *E*), 3.76 (s, 6H, *E*), 3.81 (s, 3H, *Z*), 3.85 (s, 6H, *Z*), 3.86 (s, 3H, *E*), 3.93 (s, 3H, *Z*), 6.41 (d, J = 2.9 Hz, 1H, *E*), 6.48 (d, J = 3.1 Hz, 1H, *Z*), 6.65 (s, 2H, *Z*), 6.74 (s, 2H, *E*), 7.00 (d, J = 2.9 Hz, 1H, *E*), 7.06 (d, J = 3.1 Hz, 1H, *Z*), 7.27 (d, J = 8.8 Hz, 1H, *E*), 7.30 (d, J = 8.8 Hz, 1H, *Z*), 7.31 (m, 1H, *E*), 7.55 (br d, J = 8.8 Hz, 1H, *Z*), 7.60 (br s, 1H, *Z*), 7.72 (br s, 1H, *E*). ^{13}C NMR δ_C (ppm) (100 MHz): 33.0 (CH_3, *E*), 33.1 (CH_3, *Z*), 56.2 (CH_3, *Z* + *E*) (2), 60.9 (CH_3, *Z* + *E*), 101.8 (CH, *E*), 101.9 (CH, *Z*), 105.9 (CH, *E*) (2), 106.7 (CH, *Z*) (2), 108.9 (CH, *E*), 109.2 (CH, *Z*), 121.2 (CH, *Z*), 121.6 (CH, *Z* + *E*), 122.7 (CH, *E*), 123.2 (CH, *Z*), 123.5 (C, *Z* + *E*), 124.0 (CH, *E*), 128.0 (C, *E*), 128.1 (C, *Z*), 129.7 (CH, *Z*), 133.2 (C, *Z* + *E*), 136.9 (C, *E*), 137.4 (C, *Z*), 138.2 (C, *Z*), 139.1 (C, *E*), 153.0 (C, *E*) (2), 153.1 (C, *Z* + *E*) (2), 158.4 (C, *Z* + *E*). HRMS ($C_{19}H_{20}N_2O_4$ + Na): calcd 363.1315, found 363.1365. HPLC: 9.41 and 9.59 (C_{18}), 9.05 and 9.20 (C_8), 9.00 and 9.12 (Phen).

4.1.8.10. (*E*/*Z*)-(1-Ethyl-1*H*-indol-5-yl)(3,4,5-trimethoxyphenyl)methanone oxime (10). Following the general procedure, 60 mg (0.2 mmol) of **8** yielded 54 mg (85%) of **10** as a 1:1 mixture of the *E* and *Z* isomers. IR (film): 3421, 1582 cm^{-1}. ^1H NMR δ_H (ppm) (400 MHz): 1.46 (t, J = 7.3 Hz, 1H, *E*), 1.50 (t, J = 7.3 Hz, 1H, *Z*), 4.17 (q, J = 7.3 Hz, 1H, *E*), 4.21 (q, J = 7.3 Hz, 1H, *Z*), 3.75 (s, 6H, *Z*), 3.82 (s, 6H, *E*), 3.88 (s, 3H, *Z*), 3.95 (s, 6H, *E*), 6.47 (d, J = 3.3 Hz, 1H, *Z*), 6.53 (d, J = 3.3 Hz, 1H, *E*), 6.68 (s, 2H, *Z*), 6.76 (s, 2H, *E*), 7.12 (d, J = 3.3 Hz, 1H,

Z), 7.17 (d, *J* = 3.3 Hz, 1H, *E*), 7.30 (d, *J* = 8.9 Hz, 1H, *Z*), 7.31 (dd, J_1 = 8.6 Hz, J_2 = 1.4 Hz, 1H, *E*), 7.41 (d, *J* = 8.6 Hz, 1H, *E*), 7.51 (dd, J_1 = 8.9 Hz, J_2 = 1.8 Hz, 1H, *Z*), 7.62 (d, *J* = 1.4 Hz, 1H, *Z*), 7.76 (d, *J* = 1.8 Hz, 1H, *E*). ^{13}C NMR δ_C (ppm) (100 MHz): 15.6 (CH_3) (2), 41.2 (CH_2) (2), 56.3 (CH_3) (4), 61.0 (CH_3) (2), 102.0 (CH), 102.1 (CH), 105.8 (CH) (2), 106.6 (CH) (2), 109.0 (CH), 109.4 (CH), 121.1 (CH), 121.9 (CH), 123.0 (CH) (2), 127.8 (C), 128.1 (CH), 128.3 (CH), 128.3 (C), 129.0 (C), 133.0 (C), 136.0 (C), 136.5 (C), 138.2 (C), 139.2 (C), 142.4 (C), 142.8 (C), 153.0 (C) (2), 153.1 (C) (2), 158.9 (C) (2). HRMS (C_{20} $H_{22}N_2O_4$ + Na): calcd 377.1472, found 377.1474. HPLC: 10.25 and 10.40 (C_{18}), 9.85 and 9.97 (C_8), 9.67 and 9.80 (Phen).

4.1.8.11. (*E*/*Z*)-(1-Methyl-1*H*-indol-5-yl)(3,4,5-trimethoxyphenyl)methanone *O*-acetyloxime (11). 400 mg of oxime **9** was acetylated to yield 400 mg of a mixture of acetyloximes, which were separated by column chromatography, giving 172 mg (38%) of **11***Z* and 125 mg (28%) of **11***E*.

*4.1.8.11.1. (Z)-(1-Methyl-1H-indol-5-yl)(3,4,5-trimethoxyphenyl)methanone O-acetyloxime (**11Z**).* IR (film): 1762, 1583 cm^{-1}. ^{1}H NMR δ_H (ppm) (400 MHz): 2.14 (s, 3H), 3.82 (s, 9H), 3.95 (s, 3H), 6.49 (d, *J* = 3.2 Hz, 1H), 6.55 (s, 2H), 7.07 (d, *J* = 3.2 Hz, 1H), 7.31 (d, *J* = 8.8 Hz, 1H), 7.65 (dd, J_1 = 8.8 Hz, J_2 = 1.4 Hz, 1H), 7.73 (d, *J* = 1.4 Hz, 1H). ^{13}C NMR δ_C (ppm) (100 MHz): 19.9 (CH_3), 33.1 (CH_3), 56.3 (CH_3) (2), 61.0 (CH_3), 102.3 (CH), 106.3 (CH) (2), 109.3 (CH), 122.2 (CH), 123.4 (CH), 125.8 (C), 128.1 (C), 128.8 (C), 130.0 (CH), 130.0 (C), 138.2 (C), 153.0 (C) (2), 165.9 (C), 169.2 (C). HRMS ($C_{21}H_{22}N_2$ O_5 + Na): calcd 405.1421, found 405.1402.

*4.1.8.11.2. (E)-(1-Methyl-1H-indol-5-yl)(3,4,5-trimethoxyphenyl)methanone O-acetyloxime (**11E**).* IR (film): 1762, 1583 cm^{-1}. ^{1}H NMR δ_H (ppm) (400 MHz): 2.10 (s, 3H), 3.78 (s, 6H), 3.86 (s, 3H), 3.88 (s, 3H), 6.54 (d, *J* = 3.3 Hz, 1H), 6.81 (s, 2H), 7.14 (d, *J* = 3.3 Hz, 1H), 7.22 (dd, J_1 = 8.4 Hz, J_2 = 1.4 Hz, 1H), 7.37 (d, *J* = 8.4 Hz, 1H), 7.64 (d, *J* = 1.4 Hz, 1H). ^{13}C NMR δ_C (ppm) (100 MHz): 19.8 (CH_3), 33.0 (CH_3), 56.3 (CH_3) (2), 61.0 (CH_3), 102.0 (CH), 107.0 (CH) (2), 108.8 (CH), 122.9 (CH), 123.0 (CH), 123.1 (C), 127.8 (C), 130.0 (CH), 130.8 (C), 131.4 (C), 137.2 (C), 153.0 (C) (2), 166.2 (C), 169.1 (C). HRMS ($C_{21}H_{22}N_2O_5$ + Na): calcd 405.1421, found 405.1409.

4.1.8.12. (*E*/*Z*)-(1-Ethyl-1*H*-indol-5-yl)(3,4,5-trimethoxyphenyl)methanone *O*-acetyloxime (12). 300 mg (0.9 mmol) of oxime **10** was acetylated to yield 310 mg of a 45(*Z*):55(*E*) mixture of acetyloximes, which were separated by column chromatography, giving 130 mg (39%) of **12***Z* and 115 mg (35%) of **12***E*.

*4.1.8.12.1. (Z)-(1-Ethyl-1H-indol-5-yl)(3,4,5-trimethoxyphenyl)methanone O-acetyloxime (**12Z**).* IR (film): 1762, 1574 cm^{-1}. ^{1}H NMR δ_H (ppm) (400 MHz): 1.47 (t, *J* = 7.3 Hz, 3H), 2.13 (s, 3H), 3.81 (s, 6H), 3.94 (s, 3H), 4.18 (q, *J* = 7.3 Hz, 2H), 6.49 (d, *J* = 3.2 Hz, 1H), 6.55 (s, 2H), 7.14 (d, *J* = 3.2 Hz, 1H), 7.32 (d, *J* = 8.7 Hz, 1H), 7.63 (dd, J_1 = 8.7 Hz, J_2 = 1.8 Hz, 1H), 7.72 (d, *J* = 1.8 Hz, 1H). ^{13}C NMR δ_C (ppm) (100 MHz): 15.5 (CH_3), 19.9 (CH_3), 41.2 (CH_2), 56.3 (CH_3) (2), 61.0 (CH_3), 102.4 (CH), 106.3 (CH) (2), 109.3 (CH), 122.0 (CH), 123.6 (CH), 125.7 (C), 128.1 (CH), 128.8 (C) (2), 137.2 (C), 142.8 (C), 153.0 (C) (2), 165.9 (C), 169.2 (C). HRMS ($C_{22}H_{24}N_2O_5$ + Na): calcd 419.1577, found 419.1567.

*4.1.8.12.2. (E)-(1-Ethyl-1H-indol-5-yl)(3,4,5-trimethoxyphenyl)methanone O-acetyloxime (**12E**).* IR (film): 1762, 1574 cm^{-1}. ^{1}H NMR δ_H (ppm) (400 MHz): 1.52 (t, *J* = 7.3 Hz, 3H), 2.11 (s, 3H), 3.78 (s, 6H), 3.88 (s, 3H), 4.23 (q, *J* = 7.3 Hz, 2H), 6.54 (d, *J* = 3.3 Hz, 1H), 6.81 (s, 2H), 7.20 (d, *J* = 3.3 Hz, 1H), 7.20 (dd, J_1 = 8.4 Hz, J_2 = 1.5 Hz, 1H), 7.39 (d, *J* = 8.8 Hz, 1H), 7.64 (d, *J* = 1.5 Hz, 1H). ^{13}C NMR δ_C (ppm) (100 MHz): 15.5 (CH_3), 19.9 (CH_3), 41.2 (CH_2), 56.4 (CH_3) (2), 61.0 (CH_3), 102.1 (CH), 107.1 (CH) (2), 108.8 (CH), 123.0 (CH) (2), 123.0 (C), 128.0 (C), 128.1 (CH), 131.5 (C), 136.3 (C), 141.1 (C), 153.0 (C) (2), 166.1 (C), 169.2 (C). HRMS ($C_{22}H_{24}N_2O_5$ + Na): calcd 419.1577, found 419.1564.

4.1.8.13. 5-[(*E*/*Z*)-hydrazono(3,4,5-trimethoxyphenyl)methyl]-1-methyl-1*H*-indole (13). Following the general procedure, 120 mg (0.4 mmol) of **7** yielded 110 mg (88%) of **13** as a 40(*Z*):60(*E*) mixture. IR (film): 3396, 1733, 1584 cm^{-1}. ^{1}H NMR δ_H (ppm) (400 MHz): 3.74 (s, 9H, *Z*), 3.82 (s, 3H, *Z*), 3.84 (s, 9H, *E*), 3.93 (s, 3H, *E*), 6.42 (d, *J* = 2.9 Hz, 1H, *Z*), 6.52 (s, 2H, *Z*), 6.53 (d, *J* = 2.9 Hz, 1H, *E*), 6.78 (s, 2H, *E*), 7.01 (d, *J* = 2.9 Hz, 1H, *Z*), 7.10 (d, *J* = 2.9 Hz, 1H, *E*), 7.10 (br d, *J* = 8.4 Hz, 1H, *E*), 7.27 (d, *J* = 8.4 Hz, 1H, *E*), 7.46 (d, *J* = 8.8 Hz, 1H, *Z*), 7.66 (br s, 1H, *Z*), 7.68 (br d, *J* = 8.8 Hz, 1H, *Z*), 7.70 (br s, 1H, *E*). ^{13}C NMR δ_C (ppm) (100 MHz): 33.0 (CH_3, *Z* + *E*), 56.2 (CH_3, *Z* + *E*) (2), 60.9 (CH_3, *Z* + *E*), 101.5 (CH, *Z*), 101.8 (CH, *E*), 104.2 (CH, *Z*) (2), 105.6 (CH, *E*) (2), 109.2 (CH, *E*), 110.3 (CH, *Z*), 120.2 (CH, *Z*), 120.3 (CH, *E*), 121.5 (CH, *E*), 122.0 (CH, *Z*), 123.2 (C, *Z* + *E*), 128.0 (C, *Z*), 128.8 (C, *E*), 129.4 (CH, *Z*), 129.8 (CH, *E*), 129.0 (C, *E*), 130.1 (C, *Z*), 135.1 (C, *Z* + *E*), 136.7 (C, *Z* + *E*), 138.3 (C, *Z*), 140.7 (C, *E*), 150.4 (C, *Z*), 151.0 (C, *E*), 152.9 (C, *Z*) (2), 154.2 (C, *Z*) (2). HRMS ($C_{19}H_{21}N_3O_3$ + Na): calcd 362.1475, found 362.1461. HPLC: 9.12 and 9.39 (C_{18}), 9.73 and 9.93 (C_8), 8.76 and 8.95 (Phen).

4.1.8.14. 5-[(*E*/*Z*)-hydrazono(3,4,5-trimethoxyphenyl)methyl]-1-ethyl-1*H*-indole (14). Following the general procedure, 80 mg (0.2 mmol) of **8** yielded 52 mg (61%) of **14** as a 1(*Z*):2(*E*) mixture. IR (film): 3285, 1669, 1583 cm^{-1}. ^{1}H NMR δ_H (ppm) (400 MHz): 1.43 (t, *J* = 7.3 Hz, 3H, *Z*), 1.50 (t, *J* = 7.3 Hz, 3H, *E*), 3.73 (s, 6H, *E*), 3.81 (s, 3H, *E*), 3.82 (s, 6H, *Z*), 3.93 (s, 3H, *Z*), 4.14 (q, *J* = 7.3 Hz, 2H, *Z*), 4.18 (q, *J* = 7.3 Hz, 2H, *E*), 6.40 (d, *J* = 3.3 Hz, 1H, *Z*), 6.52 (d, *J* = 3.3 Hz, 1H, *E*), 6.51 (s, 2H, *Z*), 6.76 (s, 2H, *E*), 7.04 (dd, J_1 = 8.8 Hz, J_2 = 1.8 Hz, 1H, *E*), 7.06 (d, *J* = 3.3 Hz, 1H, *Z*), 7.19 (d, *J* = 3.3 Hz, 1H, *E*), 7.63 (dd, J_1 = 8.8 Hz, J_2 = 1.8 Hz, 1H, *Z*), 7.28 (d, *J* = 8.8 Hz, 1H, *Z*), 7.46 (d, *J* = 8.8 Hz, 1H, *E*), 7.48 (d, *J* = 1.8 Hz, 1H, *Z*), 7.54 (d, *J* = 1.8 Hz, 1H, *E*). ^{13}C NMR δ_C (ppm) (100 MHz): 15.5 (CH_3, *Z* + *E*), 20.9 (CH_3, *Z* + *E*), 41.2 (CH_2, *Z* + *E*), 56.2 (CH_3, *Z* + *E*) (2), 60.9 (CH_3, *Z* + *E*), 101.6 (CH, *Z*), 101.9 (CH, *E*), 104.3 (CH, *Z*) (2), 105.5 (CH, *E*) (2), 109.3 (CH, *E*), 110.4 (CH, *Z*), 120.0 (CH, *E*), 120.4

(CH, *E*), 121.9 (CH, *Z*), 122.1 (CH, *Z*), 127.6 (C, *Z*), 128.0 (CH, *E*), 128.0 (C, *E*), 128.3 (CH, *Z*), 128.3 (C, *E*) (2), 129.3 (C, *Z*), 129.9 (C, *Z*), 135.1 (C, *E*), 135.8 (C, *Z*), 144.2 (C, *E*), 150.6 (C, *Z*), 151.3 (C, *E*) (2), 152.9 (C, *Z*) (2), 154.1 (C, *Z* + *E*). HRMS ($C_{20}H_{23}N_3O_3$ + Na): calcd 376.1632, found 376.1642. HPLC: 10.28 (C_{18}), 9.76 (C_8), 9.68 (Phen).

4.1.8.15. *N′*-[(1*E*/*Z*)-(1-Methyl-1*H*-indol-5-yl)(3,4,5-trimethoxyphenyl)methylene]acetohydrazide (15). 100 mg (0.3 mmol) of **13** gave after acetylation, 75 mg of a 40:60 mixture of the *E* and *Z* isomers (82%). IR (film): 3317, 1674, 1585 cm^{-1}. ^1H NMR δ_H (ppm) (400 MHz): 2.44 (s, 3H, *Z*), 2.47 (s, 3H, *E*), 3.76 (s, 3H, *E* + *Z*), 3.81 (s, 3H, *Z*), 3.83 (s, 6H, *Z*), 3.87 (s, 6H, *E*), 3.94 (s, 3H, *E*), 6.43 (s, 2H, *Z*), 6.46 (d, *J* = 3.0 Hz, 1H, *Z*), 6.53 (d, *J* = 3.0 Hz, 1H, *E*), 6.85 (s, 2H, *E*), 7.02 (br d, *J* = 8.4 Hz, 1H, *E*), 7.05 (d, *J* = 3.0 Hz, 1H, *Z*), 7.17 (d, *J* = 3.0 Hz, 1H, *E*), 7.31 (d, *J* = 8.4 Hz, 1H, *Z*), 7.46 (d, *J* = 8.4 Hz, 1H, *E*), 7.50 (br s, 1H, *E*), 7.59 (br s, 1H, *Z*), 7.75 (br d, *J* = 8.4 Hz, 1H, *Z*), 8.40 (br s, 1H, *Z*), 8.50 (br s, 1H, *E*). ^{13}C NMR δ_C (ppm) (100 MHz): 20.7 (CH_3, *Z* + *E*), 33.1 (CH_3, *Z* + *E*), 56.2 (CH_3, *Z* + *E*) (2), 61.0 (CH_3, *Z* + *E*), 101.7 (CH, *Z*), 102.1 (CH, *E*), 105.2 (CH, *Z* + *E*) (2), 109.3 (CH, *E*), 110.5 (CH, *Z*), 120.4 (CH, *Z*), 121.3 (CH, *Z* + *E*), 121.8 (CH, *E*), 127.8 (C, *E*), 128.2 (C, *Z*), 128.4 (C, *E*), 129.0 (C, *Z*), 129.8 (CH, *E*), 130.4 (CH, *Z*), 133.5 (C, *Z*), 137.1 (C, *E*), 137.5 (C, *E*), 139.8 (C, *Z*), 151.7 (C, *Z*) (2), 153.0 (C, *E*) (2), 154.4 (C, *Z* + *E*), 172.6 (C, *Z*), 172.9 (C, *E*). HRMS ($C_{21}H_{23}N_3O_4$ + Na): calcd 404.1581, found 404.1590. HPLC: 9.53 (C_{18}), 9.04 and 9.22 (C_8), 9.02 and 9.19 (Phen).

4.1.8.16. 5-[(*E*/*Z*)-Methylhydrazono(3,4,5-trimethoxyphenyl)methyl]-1-methyl-1*H*-indole (16). Following the general procedure, 190 mg (0.6 mmol) of **7** yielded 170 mg (90%) of **16** as a 1(*Z*):2(*E*) mixture. IR (film): 3100, 1579, 1504 cm^{-1}. ^1H NMR δ_H (ppm) (400 MHz): 3.00 (s, 3H, *E*), 3.04 (s, 3H, *Z*), 3.76 (s, 6H, *E*), 3.78 (s, 3H, *Z*), 3.84 (s, 6H, *E*), 3.85 (s, 6H, *Z*), 3.94 (s, 3H, *Z*), 6.41 (d, *J* = 3.3 Hz, 1H, *Z*), 6.51 (s, 2H, *Z*), 6.53 (d, *J* = 3.3 Hz, 1H, *E*), 6.78 (s, 2H, *E*), 7.00 (d, *J* = 3.3 Hz, 1H, *Z*), 7.08 (dd, J_1 = 8.4 Hz, J_2 = 1.5 Hz, 1H, *E*), 7.14 (d, *J* = 3.3 Hz, 1H, *E*), 7.27 (d, *J* = 8.4 Hz, 1H, *Z*), 7.45 (d, *J* = 8.4 Hz, 1H, *E*), 7.49 (d, *J* = 1.5 Hz, 1H, *Z*), 7.54 (d, *J* = 1.5 Hz, 1H, *E*), 7.70 (dd, J_1 = 8.4 Hz, J_2 = 1.5 Hz, 1H, *Z*). ^{13}C NMR δ_C (ppm) (100 MHz): 33.0 (CH_3, *Z* + *E*), 38.0 (CH_3, *Z* + *E*), 56.2 (CH_3, *Z* + *E*) (2), 61.0 (CH_3, *Z* + *E*), 101.5 (CH, *Z*), 101.7 (CH, *E*), 103.9 (CH, *Z*) (2), 105.7 (CH, *E*) (2), 109.1 (CH, *E*), 110.3 (CH, *Z*), 119.9 (CH, *E*), 121.5 (CH, *Z*), 120.1 (CH, *E*), 122.3 (CH, *Z*), 124.0 (C, *Z* + *E*), 128.1 (C, *E*), 128.8 (C, *Z*) 129.3 (CH, *E*), 129.8 (CH, *Z*), 130.0 (C, *Z*), 130.3 (C, *E*), 135.3 (C, *Z* + *E*), 136.6 (C, *Z* + *E*), 146.7 (C, *Z*), 147.5 (C, *E*), 152.9 (C, *Z*) (2), 154.1 (C, *E*) (2).

4.1.8.17. *O-tert*-Butoxycarbonyl-(2*E*/*Z*)-2-[(1-methyl-1*H*-indol-5-yl)(3,4,5-trimethoxyphenyl) methanone]oxime (17). 200 mg (0.6 mmol) of **9** was dissolved in a 1:1 mixture of dioxane:water, and 127 mg of ditertbutyldicarbonate (0.6 mmol) and 300 mg of Na_2CO_3 were subsequently added and the mixture was stirred at room temperature for 48 h. The crude was diluted in CH_2Cl_2, was washed with brine, and the organic layers were dried, evaporated and chromatographied to yield 63 mg (28%) of **17***Z* and 57 mg of **17***E* (20%).

*4.1.8.17.1. O-tert-Butoxycarbonyl-(2Z)-2-[(1-methyl-1H-indol-5-yl)(3,4,5-trimethoxyphenyl) methanone]oxime (**17***Z)*.IR (film): 1769, 1575 cm^{-1}. ^1H NMR δ_H (ppm) (400 MHz): 1.52 (s, 9H), 3.81 (s, 9H), 3.94 (s, 3H), 6.48 (d, *J* = 3.2 Hz, 1H), 6.59 (s, 2H), 7.06 (d, *J* = 3.2 Hz, 1H), 7.30 (d, *J* = 8.8 Hz, 1H), 7.64 (br d, *J* = 8.8 Hz, 1H), 7.73 (br s, 1H). ^{13}C NMR δ_C (ppm) (100 MHz): 27.9 (CH_3) (3), 30.0 (CH_3), 56.2 (CH_3) (2), 61.0 (CH_3), 83.3 (C), 102.2 (CH), 106.6 (CH) (2), 109.2 (CH), 122.2 (CH), 123.3 (CH), 125.9 (C), 128.1 (C), 128.5 (C), 129.8 (CH), 138.1 (C), 140.2 (C), 153.0 (C) (2), 153.1 (C), 165.0 (C). HRMS ($C_{24}H_{28}N_2O_6$ + Na): calcd 463.1840, found 463.1846.

*4.1.8.17.2. O-tert-Butoxycarbonyl-(2E)-2-[(1-methyl-1H-indol-5-yl)(3,4,5-trimethoxyphenyl) methanone]-oxime (**17***E)*.IR (film): 2923, 1769, 1575 cm^{-1}. ^1H NMR δ_H (ppm) (400 MHz): 1.50 (s, 9H), 3.77 (s, 6H), 3.81 (s, 3H), 3.88 (s, 3H), 6.53 (d, *J* = 3.3 Hz, 1H), 6.83 (s, 2H), 7.11 (d, *J* = 3.3 Hz, 1H), 7.25 (br d, *J* = 8.8 Hz, 1H), 7.36 (d, *J* = 8.8 Hz, 1H), 7.67 (br s, 1H). ^{13}C NMR δ_C (ppm) (100 MHz): 27.9 (CH_3) (3), 33.0 (CH_3), 56.3 (CH_3) (2), 61.0 (CH_3), 83.3 (C), 101.9 (CH), 106.9 (CH) (2), 108.9 (CH), 122.9 (CH), 123.2 (CH), 127.8 (C), 129.8 (C), 131.6 (C), 129.8 (CH), 137.2 (C), 140.2 (C), 152.7 (C), 152.9 (C) (2), 164.8 (C). HRMS ($C_{24}H_{28}N_2O_6$ + Na): calcd 463.1840, found 463.1844.

4.1.8.18. (1-Methyl-1*H*-indol-5-yl)(3,4,5-trimethoxyphenyl)methylamine (18). 30 mg (0.1 mmol) of **11** in 10 mL of MeOH was stirred for 2 h under H_2 (1 atm) in the presence of 0.1 mmol of Pd/C. The reaction crude was then filtered through silica and the solvent was evaporated to yield 15 mg (52%) of **18**, which readily decomposes. ^1H NMR δ_H (ppm) (400 MHz): 3.75 (s, 3H), 3.79 (s, 6H), 3.80 (s, 3H), 6.44 (d, *J* = 3.2 Hz, 1H), 6.66 (s, 2H), 6.78 (br s, 1H), 7.04 (d, *J* = 3.2 Hz, 1H), 7.07 (br d, *J* = 8.2 Hz, 1H), 7.26 (d, *J* = 8.2 Hz, 1H), 7.60 (br s, 1H).

4.2. Tubulin isolation

Calf brain microtubule protein (MTP) was purified by two cycles of temperature-dependent assembly/disassembly, according to the method of Shelanski,[17] modified as described in the literature.[18] The MTP solution was stored at −80 °C. Protein concentrations were determined by the method of Bradford,[19] using BSA as standard. Six different MTP preparations were used in the tubulin assembly assays.

4.3. Tubulin assembly

In vitro tubulin self-assembly was monitored turbidimetrically at 450 nm, using a thermostated Thermospectronic Helios α spectrophotometer fitted with a Peltier 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.[20] 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.

4.4. 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 (3,3′-[4-(phenylaminocarbonyl)-2,3-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 of XTT labelling reagent and PMS (*N*-methylphenazinium methylsulfate) electron coupling reagent was added to each well at an amount of 50 μL. 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.

4.5. Molecular modeling

The compounds were docked into the colchicine site of tubulin following a described protocol.[21] The X-ray structures of the tubulin complexes with podophyllotoxin and DAMA-colchicine were retrieved from the protein data bank,[22] 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.[23] Initially the backbone was restrained, and then it was set free. The relaxed structures were superimposed and the tubulin–podophyllotoxin complex was shifted 30 Å along the X axes, prior to combining the two protein complexes in a single file.[24] The combined tubulin sites and the podophyllotoxin and colchicine ligands were used to generate a combined protomol with the Surflex docking program.[16] The synthesized compounds together with roughly 300 combretastatins and phenstatin analogues were manually constructed in silico[25] and docked into the combined sites (cross-docking), in an attempt to better reproduce the receptor flexibility by using different configurations of the protein.[15] Additionally, the test set of ACD compounds used in the Surflex validation were equally docked.[16] These compounds were considered a negative control group: that is, lacking biological activity. The combined results were analyzed by receiver operating characteristics (ROCs)[26]; 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.[27]

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Supplementary data

Elemental analysis and NMR data for compounds **3**–**18**. CCDC 675444 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via http://www.ccdc.cam.ac.uk/data_request/cif. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2008.04.054.

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