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New naphthylcombretastatins. Modifications on the ethylene bridge

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Abstract—Compounds with three aromatic systems, carrying a 2-naphthalene and a 3,4,5-trimethoxyphenyl moieties bonded to five-membered, six-membered or fused heterocycles display potent cytotoxic effect and inhibition of tubulin polymerization, in agreement with their structural similarity to combretastatins and their heterocyclic analogues.

1. Introduction

Polymerization of α,β-tubulin dimers is the mechanism by which microtubules are built up. These are in turn the main constituents of the mitotic spindle, which is essential for many cellular processes such as cell division, intracellular transport and maintenance of cell shape, among others.[1] Compounds that modulate the polymerizing activity of tubulin, either stabilizing the microtubules or inhibiting their formation, are interesting as potential anticancer drugs. Several sites have been identified so far on tubulin for ligand binding;[2] the colchicine, *Vinca* alkaloids and Taxol® sites are the best known of them. Combretastatins[3] have shown to be potent inhibitors of tubulin polymerization, displaying cytotoxic[4] and antiangiogenic[5] activities by binding at the colchicine site. The most potent member of this group, combretastatin A-4[6] (CA4, Fig. 1), in the form of its phosphate prodrug[7] (CA4P, Fig. 1), is currently in Phase II clinical trials.

The presence of a 3,4,5-trimethoxy and a 4-methoxy-3-X-substituted phenyl rings (being X=H, OH or NH_2), linked by a *cis*-ethylene bridge, have been considered the essential features for combretastatins to display high potency.[8,9] We have previously shown that a 2-naphthyl moiety can replace either the 3,4,5-trimethoxyphenyl ring **A** or the 3-hydroxy-4-methoxyphenyl ring **B** of combretastatin A-4 without significant loss of potency.[10] Further studies, using different carbocyclic and heterocyclic moieties as replacement for rings **A** or **B**,[11,12] led us to the conclusion that the 2-naphthyl system is a good surrogate for the 3-hydroxy-4-methoxyphenyl (ring **B**) of combretastatins, whereas it produces less potent derivatives when replacing the trimethoxyphenyl system (ring **A**) (Fig. 1).

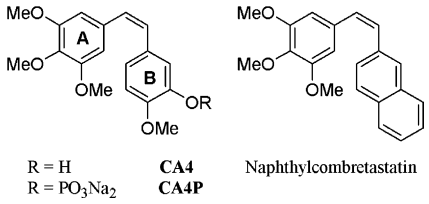

Figure 1. Structure of combretastatin A-4 and naphthylcombretastatin.

In order to enlarge the SAR studies for this type of compounds and to assess the ability of the naphthalene moiety to act as a replacement for the ring **B**, we have extended our investigation to the structure of the bridge linking both aromatic rings. Following our previous work,[13] we have designed new analogues with a heterocycle on the bridge, in order to maintain the *cis*

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disposition of both aromatic systems. The presence of a two-atom bridge as part of a heterocycle in different families of analogues has been reported by other authors,[14] with pyrazole, thiazole, triazole, tetrazole, imidazole, oxazole or pyrazine linking aromatic rings with different substitution patterns.[15–18] The effect of a bicyclic system on the bridge has also been investigated, using benzothiophene,[19,20] benzofuran[21] and indole,[13,21] although the cytotoxicity and inhibition of tubulin polymerization displayed by these derivatives is usually lower than those of similarly substituted monocycle-bridged analogues. In this paper we present the synthesis, cytotoxicity assays and the effect on tubulin polymerization of new analogues carrying the 3,4,5-trimethoxyphenyl and 2-naphthyl moieties on two consecutive carbon atoms of diverse heterocyclic systems, either single or fused rings. Thus, we intend to investigate the influence of steric factors and the type and position of heteroatoms on the biological activity of these analogues.

2. Results and discussion

2.1. Chemistry

The designed compounds have been grouped in two families, depicted in Figure 2: Family **I**, bearing five- or six-membered heterocycles on the bridge, and Family **II**, with fused heterocycles.

The synthesis of derivatives belonging to Family **I** is shown in Scheme 1. Starting from the previously described ketone **1**,[22] the intermediate enaminone **2** was easily obtained by treatment with dimethylformamide dimethylacetal[23] and subsequently transformed into the pyrazole **3**, isoxazole **4** and pyrimidine **5**, following standard methodologies. The corresponding regioisomers were synthesized from ketone **6** using the same procedure, that is, preparation of the enaminone **7** and further transformation into pyrazole **8**, isoxazole **9** and pyrimidine **10**.

SeO_2 oxidation of ketone **6** produced diketone **11**, which was transformed into the pyrazine derivative **12** by treatment with ethylene diamine in acetic acid.

Hydroxyketone **15** is the intermediate compound for the synthesis of oxazole **16**. Naphthalene-2-carbaldehyde underwent reaction with potassium cyanide and *tert*-butyldimethylsilyl chloride. The resulting compound **13** reacted with 3,4,5-trimethoxybenzaldehyde in the presence of LDA to obtain the condensation product **14**, that was deprotected to afford **15**.[24] This was acetylated and reacted with ammonium acetate, yielding oxazole **16**.[17]

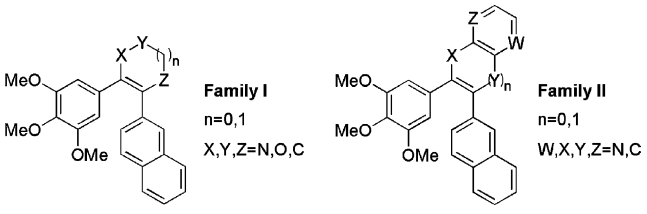


Figure 2. Structure of the designed analogues.

Compounds belonging to Family **II** may be divided into two subgroups: the indole derivatives and the quinoxaline and pyridopyrazine derivatives. Their synthesis is depicted in Schemes 2 and 3. The indole subgroup was synthesized from ketones **1** or **6**, depending on the required regiochemistry, by Fischer indolization followed by the appropriate substitution at the nitrogen atom. The indole analogues (**17** and **20**) and the methyl (**19**), oxiran-2-yl (**18** and **21**) and dimethylamino-2-propanol (**22**) derivatives were thus prepared.

Diketone **11** was used as starting material for the synthesis of fused aromatic systems with two or three nitrogen atoms, also belonging to Family **II** (Scheme 3). Treatment of the diketone **11** with different diamines and catalytic acetic acid, at reflux, afforded the corresponding analogues carrying quinoxaline (**23**), pyridopyrazine (**24** and **25**) and quinoxaline carboxylic acid (**26** and **27**) bridges. The two latter were obtained as a 1:1 mixture of regioisomers.

2.2. Biological evaluation

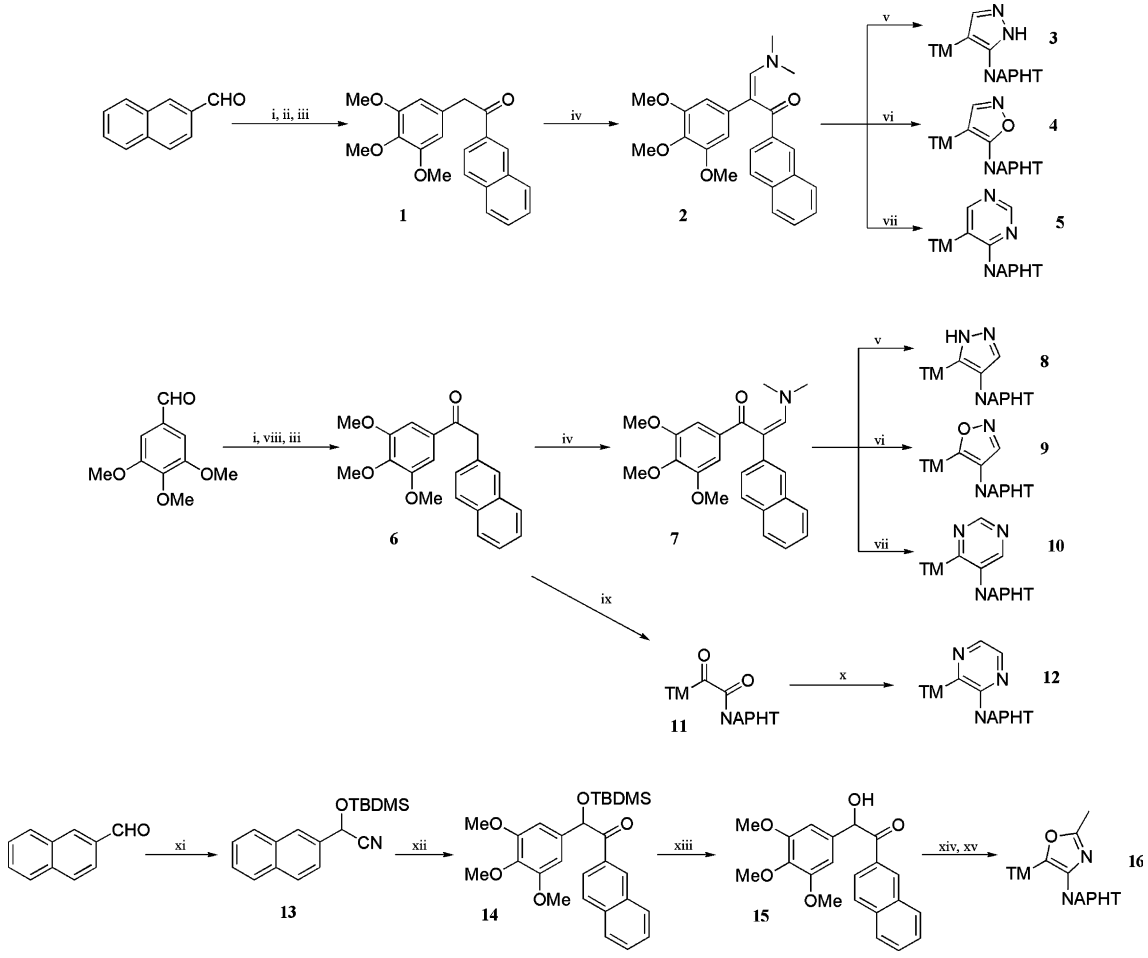
The synthesized compounds were assayed as cytotoxic agents against different tumor cell lines as described.[25] Some of the intermediate compounds, such as enaminones **2** and **7**, were also evaluated, because they can be also considered as combretastatin analogues (diarylethanes). The cytotoxicity results are shown in Table 1.

All the analogues belonging to Family **I** (**3**–**5**, **8**–**10**, **12** and **16**), bearing a five- or six-membered heterocycle on the bridge, displayed cytotoxicities (IC_{50}) in the micromolar or submicromolar range. On the other hand, the synthetic intermediates **2** and **7**, without the heterocycle in the bridge to lock the *cis* relationship between both aromatic systems, showed lower cytotoxic potency ($IC_{50} \geqslant 2.5$ μM).

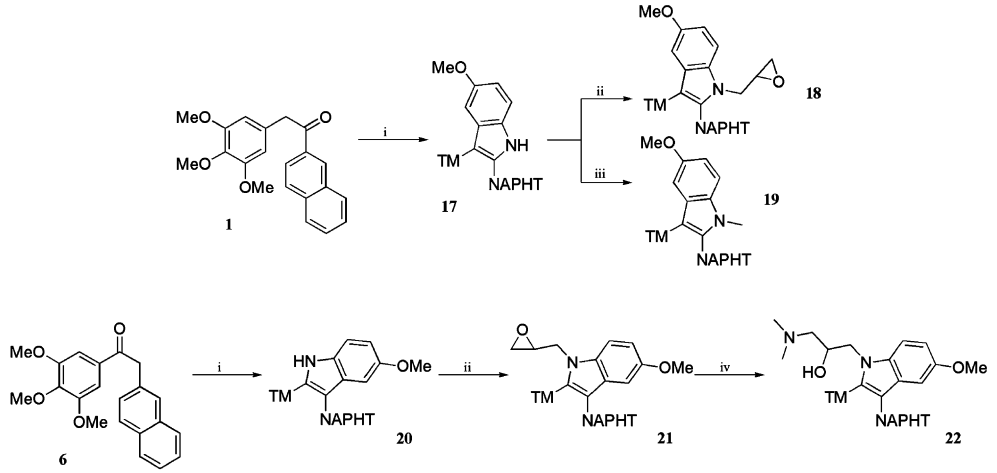
In general, for these single heterocycle derivatives, the five-membered analogues displayed the highest cytotoxicities against the assayed cell lines, while the six-membered analogues, that is, pyrimidines **5** and **10** and pyrazine **12**, were less potent. The pyrazole (**3** and **8**) and isoxazole (**4** and **9**) derivatives elicited the highest cytotoxic potency, in agreement with the results for combretastatin analogues carrying the 3-hydroxy-4-methoxyphenyl system.[15] It can also be noticed that isoxazoles **4** and **9** showed some selectivity against the H 116 cell line (human colon carcinoma).

In general, compounds belonging to Family **II** were less potent as cytotoxic agents than those of Family **I**. However, indole analogues **17** and **20**, as well as the oxiranylmethyl derivative **21**, displayed IC_{50} in the micromolar range. The other derivatives belonging to this group showed rather weak potency, with $IC_{50} \geqslant 5.0$ μM.

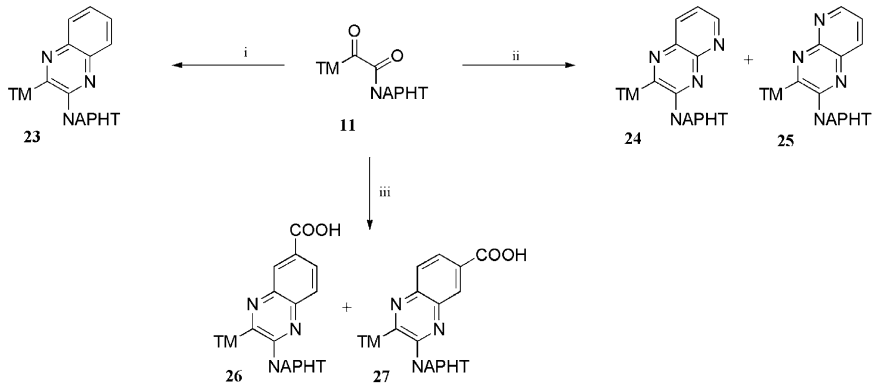
All these analogues were also evaluated as inhibitors of tubulin polymerization (Table 2), as described.[12] The



Scheme 1. Reagents and conditions: (i) 1,3-propanedithiol, $BF_3{\cdot}Et_2O$, $CHCl_3$, 0 °C to rt (91%); (ii) BuLi, THF, −78 °C, then 3,4,5-trimethoxybenzyl bromide, THF, −40 °C (93%); (iii) HgO, $BF_3{\cdot}Et_2O$, THF/H_2O (71%); (iv) dimethylformamide dimethylacetal, toluene, reflux (86%); (v) $NH_2NH_2{\cdot}HCl$, EtOH, 4 Å molecular sieves, reflux (81%); (vi) $HONH_2{\cdot}HCl$, Na_2CO_3, AcOH, MeOH/H_2O, reflux (77%); (vii) $NH_4{}^+HCOO^-$, $HCONH_2$, HCOOH, reflux (58%); (viii) BuLi, THF, −78 °C, then 2-bromomethylnaphthalene, THF, −40 °C (97%); (ix) SeO_2, toluene, reflux (98%); (x) ethylenediamine, AcOH, reflux (84%); (xi) KCN, CH_3CN, ZnI_2, TBDMSCl (68%); (xii) LDA, THF, −78 °C, then 3,4,5-trimethoxybenzaldehyde, THF, −78 °C (83%); (xiii) HCl, H_2O/THF, (79%); (xiv) Ac_2O, DMAP, DCM (96%); (xv) $NH_4{}^+AcO^-$, AcOH, reflux (69%). Abbreviations: TM: 3,4,5-trimethoxyphenyl; NAPHT: naphth-2-yl.



Scheme 2. Reagents and conditions: (i) *p*-methoxyphenylhydrazine, EtOH, AcOH, reflux (54%); (ii) NaOH, epichlorohydrine, $Bu_4N^+HSO_4{}^-$, benzene (48%); (iii) NaH, MeI, DMF (75%); (iv) *N*,*N*-dimethylamine, EtOH (90%). Abbreviations: TM: 3,4,5-trimethoxyphenyl; NAPHT: naphth-2-yl.



Scheme 3. Reagents and conditions: (i) *o*-phenylenediamine, AcOH, EtOH, reflux (98%); (ii) 2,3-diaminopyridine, AcOH, reflux (56%); (iii) 3,4-diaminobenzoic acid, AcOH, reflux (67%). Abbreviations: TM: 3,4,5-trimethoxyphenyl; NAPHT: naphth-2-yl.

Table 1. Cytotoxic activity of the assayed compounds, expressed as IC_{50} (μM)

Compound	A-549	H 116
Combretastatin A-4 (CA4)	0.003 ± 0.001	0.003 ± 0.001
Naphthylcombretastatin	0.012 ± 0.005	0.012 ± 0.007
Family ***I***		
2	5.0	2.5
3	0.03 ± 0.01	0.006 ± 0.002
4	1.3 ± 0.4	0.08 ± 0.03
5	2.5	2.5
7	2.5 ± 0.2	0.5 ± 0.1
8	0.037 ± 0.020	0.02 ± 0.01
9	0.165 ± 0.023	0.009 ± 0.002
10	2.5	2.5
12	1.70 ± 0.69	0.54 ± 0.05
16	5.0	2.5
Family ***II***		
17	0.5 ± 0.1	0.5 ± 0.1
18	50	8.3 ± 2.9
19	50	50
20	3.7 ± 0.3	6.8 ± 0.5
21	1.0	1.0
22	3.7 ± 0.3	3.7 ± 0.3
23	5.0	5.0
24 + 25	5.0	5.0
26 + 27	5.0	5.0

Table 2. Inhibition of tubulin polymerization (ITP) of the assayed compounds, expressed as IC_{50} (μM)

Compound	IC_{50} (μM)
2	>>50
3	32
4	13
5	>>50
6	>>50
7	>>50
8	30
9	9
10	>>50
11	>>50
12	ND
15	>>50
16	>>50
17	ND
18	ND
19	>>50
20	>50
21	>>50
22	>>50
23	>>50
24 + 25	ND
26 + 27	ND
CA4	3

ND: not determined.

most potent inhibitors were the pyrazole (**3** and **8**) and isoxazole (**4** and **9**) analogues, thus supporting inhibition of tubulin polymerization as the mechanism of action for their cytotoxic activity. Among them, isoxazoles **4** (IC_{50} = 13 μM) and **9** (IC_{50} = 9 μM) were especially potent, close to the inhibitory potency of naphthylcombretastatin[11] and in the same order of magnitude as CA4 (Fig. 3). Pyrazoles **3** and **8** were less potent and the potency of oxazole **16** was still lower, indicating that the presence of an additional methyl group on the heterocycle is detrimental for the polymerization inhibitory activity. The pyrimidine derivatives **5** and **10** did not inhibit tubulin polymerization, reflecting a negative influence of the increase of the ring size, as it was the case for the cytotoxic activity. The open analogues **2**, **6**, **7** and **15** were not inhibitors either, even though they could be considered closer in structure to naphthylcombretastatin; such an effect is likely due to the absence of a rigid moiety blocking the *syn* disposition between both aromatic systems.

Family **II** compounds did not significantly inhibit tubulin polymerization at concentrations up to 50 μM. This is in agreement with their low cytotoxic potency and reflects the fact that a cycle larger than unsubstituted five-membered ring on the bridge promotes loss of potency.

These results are in agreement with the general trend observed for the combretastatins and other related derivatives carrying two aromatic moieties. High cytotoxicity is usually paralleled by a potent inhibitory effect on tubulin polymerization and vice versa. Potency decreased for both activities due to the presence of substituents on the pentagonal heterocycles,[15,17] as observed for **16**. Derivatives bearing fused rings on the bridge, as indoles **18**–**22**, although they maintain a cyto-

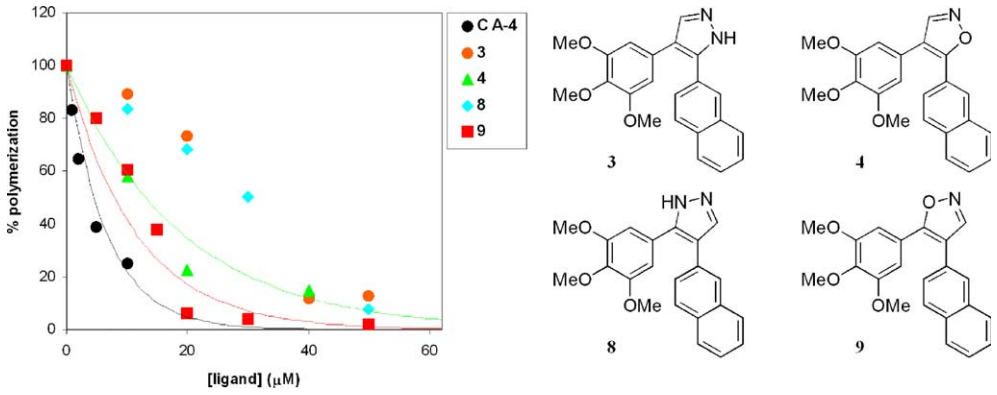


Figure 3. Inhibition of tubulin polymerization (as microtubule protein, MTP), expressed as percentage of polymerization for noninhibited protein. MTP concentration is 1.5 mg/mL.

toxic effect, show a decrease of their IC_{50} to the micromolar range, as it is also reported in the literature for related compounds.[13,21] Finally, the five-membered heterocyclic derivatives produce active compounds, with some variation in their potencies as cytotoxic and inhibitors of tubulin polymerization agents depending on the particular heterocycle. Such variability is clear for the structurally close derivatives **3**, **4**, **8** and **9**, being **4** less potent than the others in the cytotoxicity assays but not in tubulin polymerization inhibition. Pyrazoles **3** and **8** were less potent as inhibitors of tubulin polymerization than isoxazoles **4** and **9**, whereas such relationship is not so clear for the cytotoxic potency.

Most interesting is the proven ability of the 2-naphthyl moiety to replace the 3-hydroxy-4-methoxyphenyl ring and other aromatic rings of the combretastatins and their analogues. This fact, previously described by us[11] and also found in other single cases,[18,26] is now systematically observed.

3. Conclusions

We have designed and synthesized a new family of naphthalene analogues of combretastatins, bearing five-membered, six-membered or fused heterocycles on the bridge in order to lock the *syn* disposition between the aromatic systems. These compounds have been evaluated as cytotoxic agents and inhibitors of tubulin polymerization, showing a good correlation between both activities, thus supporting this mechanism of action. The most potent analogues were those with a five-membered heterocycle on the bridge, which maintain a proper arrangement of both aromatic moieties, while the remaining derivatives, that is, substituted five-membered rings, six-membered rings, fused bicyclic systems and open derivatives, were much less potent.

4. Experimental

4.1. Chemistry

IR spectra were recorded on a Nicolet Impact 410 instrument, as a thin film unless otherwise stated. Melting points were determined on a Buchi 510 apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker AC 200 (200 MHz) or Bruker Advance 400 DRX (400 MHz). Chemical shift values (δ) are given in ppm relative to TMS as internal standard, in $CDCl_3$ as solvent unless otherwise indicated. Mass spectra were recorder on a Hewlett–Packard 5890 Series II CG/MS and HRMS spectra were obtained on a VG TS-250 spectrometer, using in both cases EI as ionization mode. TLC analysis was performed on SDS precoated silica gel 60 F254 plates, 0.25 mm thick; spots were visualized with 254 and 336 nm UV light and phosphomolybdic acid spray. Preparative TLC chromatography was performed on Merck Si F254 precoated plates, 1 mm thick. Column chromatography was performed with Merck 60 (0.063–0.200 or 0.040–0.063 mm) silica gel. HLPC analysis was run on HP-1100 from Agilent Technologies or Delta 600 form Waters instruments, using X-Terra® MS C_{18} 5 μm (4.6 × 150 mm) and X-Terra® MS C_8 5 μm (4.6 × 150 mm) columns with water/acetonitrile gradients. Preparative HPLC was performed on a Waters instrument, fitted with a Waters Delta 600 quaternary pump and a Waters 2996 photodiode array detector; a Waters X-Terra® Prep MS C_{18} column (5 μm, 10 × 150 mm) was used and the eluent was a gradient of water/acetonitrile. Elemental analyses were run on a Perkin–Elmer 2400 CHN apparatus.

Anhydrous ethanol was kept over 4 Å molecular sieves. Commercial toluene was distilled over CaH_2, under argon, prior to use. Commercial THF was distilled over Na, under argon, prior to use. Commercial benzene was dried over sodium. Commercial DMF was distilled from CaH_2 and stored over 4 Å molecular sieves.

4.1.1. 3-Dimethylamino-2-(3,4,5-trimethoxyphenyl)-1-(naphth-2-yl)propenone (2). *N*,*N*-Dimethyl formamide dimethylacetal (80 μL, 6.9 mmol) was added to a suspension of ketone **1** (153 mg, 0.46 mmol), previously obtained from naphthalene-2-carbaldehyde,[22] in toluene (12 mL). The resulting mixture is refluxed for 24 h and then concentrated under vacuum. The obtained residue was purified by insolubilization from diethyl ether, yielding 155 mg (86%) of enaminone **2** as an oil. ^{1}H NMR δ 2.77 (6H, s, N–CH_3), 3.80 (6H, s, 3-OCH_3 and 5-OCH_3), 3.85 (3H, s, 4-OCH_3), 6.47 (2H, s,

Ar–H), 7.31 (1H, s, CH=N), 7.47–7.52 3H, m, Ar–H), 7.60 (1H, d, $J = 8.8$ Hz, Ar–H) 7.77–7.83 (2H, m, Ar–H), 7.98 (1H, s, Ar–H). ^{13}C NMR δ 42.8 (×2) (CH_3), 55.5 (×2) (CH_3), 60.3 (CH_3), 108.9 (×2) (CH), 111.8 (C), 125.6 (CH), 125.8 (CH), 126.3 (CH), 126.9 (CH), 127.1 (CH), 127.8 (CH), 128.1 (CH), 131.9 (C), 132.2 (C), 133.2 (C), 136.1 (C), 138.8 (C), 152.1 (CH), 153.9 (×2) (C), 194.2 (C). HRMS *m/z* found 391.1752; calcd for $C_{24}H_{25}NO_4$ *m/z* 391.1784.

4.1.2. 4-(3,4,5-Trimethoxyphenyl)-5-(naphth-2-yl)-1*H*-pyrazole (3). A mixture of hydrazine hydrochloride (16 mg, 0.23 mmol) and the enaminone **2** (60 mg, 0.15 mmol) in anhydrous ethanol (10 mL) was refluxed for 24 h. The solvent was then removed, water was added and the aqueous phase extracted with CH_2Cl_2 (×3). The organic layer was dried over Na_2SO_4 and the solvent evaporated under vacuum. The remaining residue was purified by preparative TLC (hexane–ethyl acetate 3:1 with 10% Et_3N) and subsequent insolubilization from hexane/diethyl ether to obtain 18 mg (33%) of pyrazole **3** as an oil. ^{1}H NMR δ 3.60 (6H, s, 3-OCH_3 and 5-OCH_3), 3.87 (3H, s, 4-OCH_3), 6.75 (2H, s, Ar–H), 7.26 (1H, s, pyr–H), 7.40–7.47 (3H, m, Ar–H), 7.76 (1H, d, $J = 8.4$ Hz, Ar–H), 7.79–7.82 (2H, m, Ar–H), 7.85 (1H, br s, Ar–H). ^{13}C NMR δ 55.9 (×2) (CH_3), 60.9 (CH_3), 105.7 (×2) (CH), 120.3 (C), 125.7 (C), 126.0 (CH), 126.2 (CH), 126.4 (×2) (CH), 127.0 (CH), 127.7 (CH), 128.1 (×2) (CH), 128.4 (C), 128.6 (C), 133.0 (C), 133.2 (C), 137.1 (C), 153.2 (×2) (C). HRMS *m/z* found 360.1483; calcd for $C_{22}H_{20}N_2O_3$ *m/z* 360.1474.

4.1.3. 4-(3,4,5-Trimethoxyphenyl)-5-(naphth-2-yl)isoxazole (4). Hydroxylamine hydrochloride (27 mg, 0.40 mmol) and Na_2CO_3 (55 mg, 0.52 mmol) were added to a solution of the enaminone **2** (140 mg, 0.36 mmol) in MeOH/H_2O 2:1 (15 mL). The mixture was acidified with acetic acid to pH 4–5 and subsequently refluxed for 20 h. Next, the pH was raised up to 8 by adding NH_4OH and the mixture was extracted with CH_2Cl_2 (×3). The combined organic layers were dried over anhydrous Na_2SO_4 and the solvent removed under reduced pressure to yield 100 mg (77%) of isoxazole **4** as a solid, which was purified by crystallization from hexane-diethyl ether. Mp: 138 °C (hexane–diethyl ether). ^{1}H NMR δ 3.75 (6H, s, 3-OCH_3 and 5-OCH_3), 3.91 (3H, s, 4-OCH_3), 6.64 (2H, s, Ar–H), 7.47–7.54 (3H, m, Ar–H), 7.69 (1H, d, $J = 8.8$ Hz, Ar–H); 7.81–7.87 (2H, m, Ar–H), 8.26 (1H, br s, Ar–H), 8.40 (1H, s, Ar–H). ^{13}C NMR δ 56.2 (×2) (CH_3), 61.0 (CH_3), 106.0 (×2) (CH), 116.4 (C), 124.1 (CH), 124.9 (C), 125.5 (C), 126.8 (CH), 127.3 (CH), 127.4 (CH), 127.8 (CH), 128.5 (CH), 128.6 (CH), 133.0 (C), 133.7 (C), 138.1 (C), 151.8 (CH), 153.7 (×2) (C), 163.8 (C). HRMS *m/z* found 361.1307; calcd for $C_{22}H_{19}NO_4$ *m/z* 361.1314. Anal. Calcd for $C_{22}H_{19}NO_4$: C, 73.12; H, 5.30; N, 3.88. Found: C, 73.17; H, 5.45; N, 3.76.

4.1.4. 5-(3,4,5-Trimethoxyphenyl)-4-(naphth-2-yl)pyrimidine (5). A mixture of enaminone **2** (729 mg, 1.84 mmol), ammonium formate (1.17 g, 18.4 mmol), formamide (187 μL, 5.07 mmol) and formic acid (187 μL, 4.61 mmol) was heated at 165 °C until water evaporation ceased. Next, it was kept at 180 °C for 8 h and at room temperature for further 72 h. Then, water was added and the resulting mixture was extracted with CH_2Cl_2 (×3). The combined organic layers were dried over anhydrous Na_2SO_4 and the solvent removed under vacuum to yield a crude product (561 mg), which was purified by HPLC, obtaining 79 mg (8%) of **5** as a solid. ^{1}H NMR δ 3.48 (6H, s, 3-OCH_3 and 5-OCH_3), 3.82 (3H, s, 4-OCH_3), 6.76 (2H, s, Ar–H), 7.51–7.55 (3H, m, Ar–H), 7.78–7.82 (2H, m, Ar–H), 7.80 (1H, d $J = 8.4$ Hz, Ar–H), 7.87 (1H, s, Ar–H), 8.82 (1H, s, Ar–H), 9.28 (1H, s, Ar–H). ^{13}C NMR δ 55.7 (×2) (CH_3), 60.9 (CH_3), 107.4 (×2) (CH), 126.8 (×2) (CH), 127.1 (CH), 127.7 (×2) (CH), 128.0 (CH), 128.4 (CH), 132.1 (C), 132.6 (C), 133.0 (C), 133.4 (C), 134.2 (C), 152.8 (×3) (C), 157.4 (CH), 158.5 (CH), 162.7 (C). MS *m/z* 372. Anal. Calcd for $C_{23}H_{20}N_2O_3$: C, 74.18; H, 5.41; N, 7.52. Found: C, 74.20; H, 5.36; N, 7.33.

4.1.5. 3-Dimethylamino-1-(3,4,5-trimethoxyphenyl)-2-(naphth-2-yl)propenone (7). From ketone **6** (457 mg, 1.85 mmol, previously obtained from naphthalene-2-carbaldehyde[22]), enaminone **7** was prepared (48 mg, 67%) by the procedure previously described. It was obtained as an oil by insolubilization from diethyl ether. ^{1}H NMR δ 2.77 (3H, s, N–CH_3), 3.64 (6H, s, 3-OCH_3 and 5-OCH_3), 3.79 (3H, s, 4-OCH_3), 6.71 (2H, s, Ar–H), 7.27 (1H, s, CH–N), 7.28–7.45 (3H, m, Ar–H), 7.62 (1H, d, $J = 8.4$ Hz, Ar–H), 7.72–7.84 (2H, m, Ar–H), 7.77 (1H, br s, Ar–H). ^{13}C NMR δ 43.7 (×2) (CH_3), 55.9 (×2) (CH_3), 60.7 (CH_3), 106.7 (×2) (CH), 111.1 (C), 125.6 (CH), 126.0 (CH), 127.1 (CH), 127.5 (CH), 127.6 (CH), 130.2 (CH), 130.5 (CH), 131.9 (C), 133.0 (C), 135.4 (C), 136.5 (C), 139.1 (C), 153.6 (×2) (C), 152.2 (CH), 193.3 (C). HRMS *m/z* found 391.1752; calcd for $C_{24}H_{25}NO_4$ *m/z* 391.1784.

4.1.6. 5-(3,4,5-Trimethoxyphenyl)-4-(naphth-2-yl)-1*H*-pyrazole (8). From enaminone **7** (60 mg, 0.15 mmol), pyrazole **8** was prepared (37 mg, 79%) by the procedure previously described. It was obtained as a solid that was further purified by crystallization from hexane–ethyl acetate. Mp: 78 °C (hexane–ethyl acetate). ^{1}H NMR δ 3.65 (6H, s, 3-OCH_3 and 5-OCH_3), 3.87 (3H, s, 4-OCH_3), 6.55 (2H, s, Ar–H), 7.26 (1H, s, Ar–H), 7.45–7.50 (3H, m, Ar–H), 7.56 (1H, d, $J = 8.8$ Hz, Ar–H), 7.81–7.85 (2H, m, Ar–H), 8.02 (1H, br s, Ar–H). ^{13}C NMR δ 55.9 (×2) (CH_3), 60.9 (CH_3), 105.3 (×2) (CH), 124.4 (C), 125.8 (CH), 126.2 (CH), 126.2 (C), 126.8 (CH), 126.9 (CH), 127.2 (CH), 127.6 (CH), 127.7 (CH), 127.9 (CH), 130.5 (C), 132.2 (×2) (C), 133.5 (C), 138.1 (C), 153.3 (×2) (C). HRMS *m/z* found 360.1476; calcd for $C_{22}H_{20}N_2O_3$ *m/z* 360.1474. Anal. Calcd for $C_{22}H_{20}N_2O_3$: C, 73.32; H, 5.59; N, 7.77. Found: C, 73.30; H, 5.50; N, 7.58.

4.1.7. 5-(3,4,5-Trimethoxyphenyl)-4-(naphth-2-yl)isoxazole (9). Compound **9** (29 mg, 61%, oil) was synthesized from enaminone **7** (50 mg, 0.13 mmol), following the procedure previously described. ^{1}H NMR δ 3.64 (6H, s, 3-OCH_3 and 5-OCH_3), 3.88 (3H, s, 4-OCH_3), 6.92 (2H, s, Ar–H), 7,49–7.54 (3H, m, Ar–H), 7.82–7.89

(2H, m, Ar–H), 7.87 (1H, d, $J = 8.8$ Hz, Ar–H), 7.94 (1H, br s, Ar–H), 8.44 (1H, s, Ar–H). ^{13}C NMR δ 55.9 (×2) (CH_3), 60.9 (CH_3), 104.5 (×2) (CH), 115.8 (C), 122.7 (C), 126.6 (CH), 126.7 (CH), 127.4 (CH), 127.4 (C), 127.5 (CH), 127.8 (×2) (CH), 128.5 (CH), 132.8 (C), 133.4 (C), 139.7 (C), 152.0 (CH), 153.4 (×2) (C), 163.9 (C). HRMS *m/z* found 361.1296; calcd for $C_{22}H_{19}NO_4$ *m/z* 361.1314.

4.1.8. 4-(3,4,5-Trimethoxyphenyl)-5-(naphth-2-yl)pyrimidine (10). From enaminone **7** (93 mg, 0.24 mmol), following the procedure previously described, pyrimidine **10** (7 mg, 8%) was prepared and isolated as a solid after purification by preparative HPLC of the crude product (53 mg). ^{1}H NMR δ 3.60 (6H, s, 3-OCH_3 and 5-OCH_3), 3.86 (3H, s, 4-OCH_3), 6.43 (2H, s, Ar–H), 7.42–7.53 (3H, m, Ar–H), 7.72 (1H, d, $J = 8.8$ Hz, Ar–H), 7.78–7.83 (2H, m, Ar–H), 8.14 (1H, s, Ar–H), 8.81 (1H, s, Ar–H), 9.30 (1H, s, Ar–H). ^{13}C NMR δ 56.1 (×2) (CH_3), 61.0 (CH_3), 106.8 (×2) (CH), 126.4 (×2) (CH), 127.2 (CH), 127.6 (×2) (CH), 128.7 (CH), 130.0 (CH), 132.9 (C), 133.5 (C), 134.5 (C), 134.7 (C), 136.7 (C), 153.5 (×2) (C), 153.7 (C), 157.5 (CH), 158.0 (CH), 163.3 (C). MS *m/z* 372. Anal. Calcd for $C_{23}H_{20}N_2O_3$: C, 74.18; H, 5.41; N, 7.52. Found: C, 73.99; H, 5.51; N, 7.36.

4.1.9. 1-(3,4,5-Trimethoxyphenyl)-2-(naphth-2-yl)-1,2-ethanedione (11)

4.1.9.1. Method 1. SeO_2 (161 mg, 1.45 mmol) was added to a mixture of ketone **1** (223 mg, 0.66 mmol) in toluene (15 mL). The reaction mixture was refluxed for 5 days. Then, it was allowed to cool at room temperature and sequentially washed with saturated aqueous $NaHCO_3$ and saturated aqueous NaCl, dried over anhydrous Na_2SO_4 and the solvent evaporated, yielding 231 mg (98%) of diketone **11**. ^{1}H NMR δ 3.89 (6H, s, 3-OCH_3 and 5-OCH_3), 3.96 (3H, s, 4-OCH_3), 7.28 (2H, s, Ar–H), 7.53–7.70 (3H, m, Ar–H), 7.89–8.03 (2H, m, Ar–H), 8.10 (1H, d, $J = 8.4$ Hz, Ar–H), 8.41 (1H, br s, Ar–H). ^{13}C NMR δ 56.3 (×2) (CH_3), 61.0 (CH_3), 107.3 (×2) (CH), 123.8 (CH), 127.2 (CH), 128.0 (CH), 128.5 (C), 128.7 (C), 129.2 (CH), 129.6 (CH), 130.0 (CH), 130.4 (C), 132.4 (C), 133.6 (CH), 136.4 (C), 153.4 (×2) (C), 193.4 (C), 194.4 (C). HRMS *m/z* found 350.1170; calcd for $C_{21}H_{18}O_5$ *m/z* 350.1154.

4.1.9.2. Method 2. $CuBr_2$ (34 mg, 0.15 mmol) was added to a solution of ketone **6** (30 mg, 0.09 mmol) in ethyl acetate (5 mL). The reaction mixture was refluxed for 48 h. Then, it was filtered to remove CuBr and the solvent was evaporated. The residue was redissolved in $CHCl_3$ and it was sequentially washed with saturated aqueous $Na_2S_2O_3$ and saturated aqueous NaCl, dried over anhydrous Na_2SO_4 and evaporated to dryness, yielding 22 mg (77%) of diketone **11**.

4.1.10. 2-(3,4,5-Trimethoxyphenyl)-3-(naphth-2-yl)pyrazine (12). Ethylenediamine (200 μL, 0.30 mmol) was added to a solution of diketone **11** (245 mg, 0.70 mmol) in glacial AcOH (8 mL). The reaction mixture was refluxed for 20 h. Next, AcOH was removed. Saturated $NaHCO_3$ was added and it was extracted with ethyl acetate. The organic layer was washed with saturated NaCl, dried over anhydrous Na_2SO_4 and the solvent removed under vacuum to yield 24 mg (46%, oil) of **12**. ^{1}H NMR δ 3.54 (6H, s, 3-OCH_3 and 5-OCH_3), 3.83 (3H, s, 4-OCH_3), 6.72 (2H, s, Ar–H), 7.26–7.51 (3H, m, Ar–H), 7.75 (1H, d, $J = 8.8$ Hz, Ar–H); 7.79–7.84 (2H, m, Ar–H), 8.12 (1H, br s, Ar–H), 8.62 (1H, d, $J = 2.6$ Hz, pyrazine–H), 8.63 (1H, d, $J = 2.6$ Hz, pyrazine–H). ^{13}C NMR δ 56.1 (×2) (CH_3), 61.1 (CH_3), 107.4 (×2) (CH), 126.6 (CH), 127.0 (CH), 127.8 (CH), 127.9 (CH), 128.7 (CH), 129.0 (CH), 129.4 (CH), 129.6 (CH), 132.4 (C), 132.7 (C), 133.3 (C), 133.4 (C), 133.7 (C), 136.5 (C), 136.6 (C), 142.2 (CH), 153.2 (×2) (C). HRMS *m/z* found 372.1504; calcd for $C_{23}H_{20}N_2O_3$ *m/z* 372.1474.

4.1.11. 2-*tert*-Butyldimethylsilyloxy-2-(naphth-2-yl)acetonitrile (13). To a solution of naphthalene-2-carbaldehyde (2.00 g, 12.8 mmol) in acetonitrile (20 mL), KCN (4.12 g, 64.1 mmol), *tert*-butyldimethylsilyl chloride (3.13 g, 9.90 mmol) and ZnI_2 (3.13 g, 9.90 mmol) were sequentially added with vigorous stirring. The reaction mixture was stirred at room temperature for 24 h and then filtered. The precipitate was repeatedly washed with diethyl ether. The solvent was then removed and the residue redissolved in diethyl ether. The organic layer was washed with water, dried over Na_2SO_4 and the solvent evaporated to yield 2.60 g (68%) of **13**. Mp: 40 °C (diethyl ether). ^{1}H NMR δ 0.19 (3H, s, Si–CH_3), 0.27 (3H, s, Si–CH_3), 0.98 (9H, s, $(CH_3)_3$–C), 5.69 (1H, s, CH–CN), 7.34–7.59 (3H, m, Ar–H), 7.86–7.92 (2H, m, Ar–H), 7.89 (1H, d, $J = 8,8$ Hz, Ar–H), 7.94 (1H, br s, Ar–H). ^{13}C NMR δ −5.3 (CH_3), −5.2 (CH_3), 18.1 (C), 25.4 (×3) (CH_3), 56.1 (×2) (CH_3), 60.8 (CH_3), 63.7 (CH), 102.9 (×2) (CH), 119.2 (C), 131.8 (C), 138.4 (C), 153.5 (×2) (C). HRMS *m/z* found 297.1561; calcd for $C_{18}H_{23}NOSi$ *m/z* 297.1549.

4.1.12. 2-*tert*-Butyldimethylsilyloxy-2-(3,4,5-trimethoxyphenyl)-1-(naphth-2-yl)ethanone (14). A solution of 2-*tert*-butyldimethylsilyloxy-2-(naphth-2-yl)acetonitrile (**13**) (1.00 g, 3.37 mmol) in THF (20 mL) was added to a solution of LDA in THF, prepared in turn from diisopropylamine (377 mg, 3.72 mmol) and *n*-BuLi (1.6 M in hexane, 2.3 mL, 3.7 mmol) in THF (17 mL), with vigorous stirring at −78 °C. Next, a solution of 3,4,5-trimethoxybenzaldehyde (660 mg, 3.37 mmol) in THF (10 mL) was added at the same temperature. After 14 h the reaction mixture was poured into ethyl acetate. The organic solution was washed with water, dried over anhydrous Na_2 SO_4 and the solvent was evaporated to produce a residue that was purified by column chromatography (hexane–ethyl acetate 4:0.5) to obtain 1.3 g (83%) of **14**. Mp: 83 °C (hexane–ethyl acetate). ^{1}H NMR δ 0.12 (6H, s, $(CH_3)_3CSi(CH_3)_2$), 0.91 (9H, s, $(CH_3)_3CSi(CH_3)_2$), 3.79 (3H, s, 4-OCH_3), 3.84 (6H, s, 3-OCH_3 and 5-OCH_3), 5.73 (1H, s, C*H*-OTBDMS), 6.82 (2H, s, Ar–H), 7.41–7.60 (3H, m, Ar–H), 7.77–7.88 (2H, m, Ar–H), 8.03 (1H, d, $J = 8.8$ Hz, Ar–H), 8.70 (1H, br s, Ar–H). ^{13}C NMR δ −5.14 (CH_3), −4.89 (CH_3), 18.4 (C), 25.9 (×3) (CH_3), 56.3 (×2) (CH_3), 60.9 (CH_3), 80.8 (CH), 102.6 (×2) (CH), 125.6 (CH), 126.7 (CH), 127.8 (CH), 128.0 (CH), 128.7

(CH), 129.8 (CH), 130.3 (C), 131.8 (C), 132.4 (CH), 134.8 (C), 135.6 (C), 137.5 (C), 153.6 (× 2) (C), 198.9 (C). HRMS *m*/*z* found 466.2216; calcd for $C_{27}H_{34}O_5Si$ *m*/*z* 466.2176.

4.1.13. 2-Hydroxy-2-(3,4,5-trimethoxyphenyl)-1-(naphth-2-yl)ethanone (15). Aqueous HCl (10%, 15 mL) was added to a solution of 2-*tert*-butyldimethylsilyloxy-2-(3,4,5-trimethoxyphenyl)-1-(naphth-2-yl)ethanone (**14**) (140 mg, 0.61 mmol) in THF (17 mL). The reaction mixture was stirred at room temperature for 48 h. Next, it was diluted with water and extracted with ethyl acetate (× 3). The combined organic layers were washed with saturated aqueous NaCl, dried over Na_2SO_4 and the solvent evaporated, yielding 82 mg (72%) of **15**. ^{1}H NMR δ 3.78 (3H, s, 4-OCH_3), 3.81 (6H, s, 3-OCH_3 and 5-OCH_3), 6.02 (1H, s, C*H*OH), 6.61 (2H, s, Ar–H), 7.50–7.64 (3H, m, Ar–H), 7.85 (1H, d, J = 8.8 Hz, Ar–H), 7.91–8.00 (2H, m, Ar–H), 8.48 (1H, br s, Ar–H). ^{13}C NMR δ 56.1 (× 2) (CH_3), 60.7 (CH_3), 76.3 (CH), 104.8 (× 2) (CH), 124.1 (CH), 127.0 (CH), 127.8 (CH), 128.6 (CH), 129.0 (CH), 129.6 (CH), 130.7 (C), 131.2 (CH), 132.1 (C), 134.5 (C), 135.8 (C), 137.6 (C), 153.7 (× 2) (C), 198.6 (C). HRMS *m*/*z* found 352.1299; calcd for $C_{21}H_{20}O_5$ *m*/*z* 352.1311.

4.1.14. 2-Methyl-5-(3,4,5-trimethoxyphenyl)-4-(naphth-2-yl)oxazole (16). Hydroxyketone **15** (192 mg, 0.55 mmol), acetic anhydride (165 μL, 1.72 mmol) and dimethylaminopyridine (4.0 mg, 0.03 mmol) were dissolved in CH_2Cl_2 (10 mL). The reaction mixture was kept at room temperature for 24 h. Then, it was diluted with ethyl acetate and washed with saturated NaCl, dried over anhydrous Na_2SO_4 and the solvent removed under vacuum to yield 210 mg (96%) of the corresponding acetylated derivative.

Ammonium acetate (574 mg, 5.33 mmol) and glacial acetic acid (6 mL) were added to this acetylated derivative (210 mg, 0.53 mmol). The reaction mixture was refluxed for 24 h. Next, 2 N NaOH was added and the solution extracted with ethyl acetate (× 3). The organic layer was washed with saturated NaCl, dried over Na_2SO_4 and the solvent evaporated. The remaining residue was purified by column chromatography, with hexane–ethyl acetate 3:1 as eluent, obtaining 80 mg (39%) of the oxazole derivative **16** as an oil. ^{1}H NMR δ 2.60 (3H, s, CH_3–oxazole), 3.72 (6H, s, 3-OCH_3 and 5-OCH_3), 3.94 (3H, s, 4-OCH_3), 6.86 (2H, s, Ar–H), 7.46–7,50 (3H, m, Ar–H), 7.74 (1H, d, J = 8.8 Hz, Ar–H), 7.80–7.85 (2H, m, Ar–H), 8.24 (1H, br s, Ar–H). ^{13}C NMR δ 13.9 (CH_3), 56.1 (× 2) (CH_3), 60.9 (CH_3), 103.8 (× 2) (CH), 124.4 (C), 125.7 (CH), 126.2 (CH), 126.3 (CH), 127.0 (CH), 127.6 (CH), 127.8 (CH), 128.1 (CH), 129.8 (C), 133.0 (C), 133.4 (C), 134.8 (C), 138.4 (C), 145.6 (C), 153.3 (× 2) (C), 160.0 (C). HRMS *m*/*z* found 375.1452; calcd for $C_{23}H_{21}NO_4$ *m*/*z* 375.1471.

4.1.15. 5-Methoxy-3-(3,4,5-trimethoxyphenyl)-2-(naphth-2-yl)-1*H*-indole (17). *p*-Methoxyphenyl hydrazine (497 mg, 2.85 mmol) and glacial AcOH (9 mL) were added to a solution of ketone **1** (479 mg, 1.43 mmol) in EtOH (30 mL). The mixture was refluxed for 1 h 30 min and then allowed to cool to room temperature. Saturated aqueous Na_2CO_3 was added and the resulting mixture extracted with diethyl ether (× 3). The organic layer was washed with saturated NaCl, dried over Na_2SO_4 and the solvent evaporated to dryness to obtain a residue that was subjected to column chromatography (hexane–ethyl acetate 2:1), yielding 155 mg (25%) of indole derivative **17**, which was further purified by crystallization from hexane–ethyl acetate. Mp: 142 °C (hexane–ethyl acetate). ^{1}H NMR δ 3.69 (6H, s, 3-OCH_3 and 5-OCH_3), 3.86 (3H, s, 4-OCH_3), 3.93 (3H, s, indole–OCH_3), 6.68 (2H, s, Ar–H), 6.94 (1H, dd, J_1 = 8.8 Hz, J_2 = 2.6 Hz, indole–H), 7.19 (1H, d, J = 2.6 Hz, indole–H), 7.37 (1H, d, J = 8.8 Hz, indole–H), 7.45–7.51 (3H, m, Ar–H), 7.47 (1H, d, J = 8.8 Hz, Ar–H), 7.72–7.82 (2H, m, Ar–H), 7.97 (1H, br s, Ar–H), 8.32 (1H, br s, NH). ^{13}C NMR δ 56.3 (× 3) (CH_3), 60.1 (CH_3), 101.8 (CH), 107.8 (× 2) (CH), 112.0 (CH), 113.2 (CH), 115.5 (C), 126.5 (× 3) (CH), 126.5 (C), 127.8 (× 2) (CH), 128.1 (CH), 129.3 (C), 129.4 (C), 129.8 (CH), 130.4 (C), 130.6 (C), 132.8 (C), 137.6 (× 2) (C), 153.6 (× 2) (C), 154.0 (C). MS *m*/*z* 439. Anal. Calcd for $C_{28}H_{25}NO_4$: C, 76.52; H, 5.73; N, 3.19. Found: C, 76.44; H, 5.67; N, 3.26.

4.1.16. 5-Methoxy-2-(naphth-2-yl)-3-(3,4,5-trimethoxyphenyl)-1-(oxiran-2-ylmethyl)-1*H*-indole (18). Aqueous NaOH (50%, 107 μL), tetrabutylammonium hydrogensulfate (31 μL, 0.09 mmol) and epichlorohydrine (35 μL, 0.38 mmol) were added to a suspension of indole **17** (81 mg, 0.19 mmol) in dry benzene (5 mL). The reaction mixture was stirred at room temperature for 24 h. Then, the solvent was removed, the residue dissolved in $CHCl_3$ and the organic solution washed with saturated NaCl, dried over Na_2SO_4 and the solvent evaporated. The obtained residue was purified by column chromatography (hexane–ethyl acetate 3:2), yielding 30 mg (32%) of **18** as an oil. ^{1}H NMR δ 2.50 (1H, dd, J_1 = 4.4 Hz, J_2 = 2.6 Hz, oxirane), 2.80 (1H, t, J = 4.4 Hz, oxirane), 3.27–3.32 (1H, m, oxirane), 3.65 (6H, s, 3-OCH_3 and 5-OCH_3), 3.83 (3H, s, 4-OCH_3), 3.88 (3H, s, indole–OCH_3), 4.06–4.23 (1H, m, N–C*H* H), 4.43 (1H, dd, J_1 = 12.4 Hz, J_2 = 2.6 Hz, N–CH*H*), 6.64 (2H, s, Ar–H), 6.98 (1H, dd, J_1 = 8.8 Hz, J_2 = 2.6 Hz, indole–H), 7.30 (1H, d, J = 2.6 Hz, indole–H), 7.33 (1 H. dd, J_1 = 8.8 Hz, J_2 = 1.4 Hz, indole–H), 7.39–7.45 (3H, m, Ar–H), 7.72 (1H, d, J = 8.8 Hz, Ar–H), 7.76–7.81 (2H, m, Ar–H), 7.89 (1H, br s, Ar–H). ^{13}C NMR δ 45.7 (CH_2), 46.4 (CH_2), 50.9 (CH), 51.0 (CH_3), 55.7 (× 2) (CH_3), 60.9 (CH_3), 101.5 (CH), 106.7 (× 2) (CH), 111.2 (CH), 112.8 (CH), 126.6 (CH), 127.4 (CH), 127.7 (CH), 128.1 (CH), 128.8 (C), 128.9 (C), 129.3 (CH), 130.5 (CH), 130.6 (CH), 132.8 (C), 133.1 (C), 133.6 (C), 136.0 (C), 142.4 (C), 142.5 (C), 142.7 (C), 152.9 (× 2) (C), 155.0 (C). HRMS *m*/*z* found 495.1987; calcd for $C_{31}H_{29}NO_5$ *m*/*z* 495.2046.

4.1.17. 5-Methoxy-3-(3,4,5-trimethoxyphenyl)-2-(naphth-2-yl)-1-methyl-1*H*-indole (19). To a solution of indole **17** (30 mg, 0.06 mmol) in dry DMF (2 mL), NaH (80% in oil, 2 mg, 0.1 mmol) and MeI (11 μL, 0.2 mmol) were added. The reaction mixture was stirred at room temperature for 14 h. Then, DMF was removed and the res-

idue dissolved in $CHCl_3$. The organic layer was washed with saturated NaCl, dried over Na_2SO_4 and the solvent evaporated to yield a residue that was purified by column chromatography (hexane–ethyl acetate 2:1), obtaining 23 mg (75%) of **19** as an oil. ^{1}H NMR δ 3.54 (6H, s, 3-OCH_3 and 5-OCH_3), 3.70 (3H, s, N–CH_3), 3.82 (3H, s, 4-OCH_3), 3.88 (3H, s, indole–OCH_3), 6.53 (2H, s, Ar–H), 6.99 (1H, dd, J_1 = 8.8 Hz, J_2 = 2.2 Hz, indole–H), 7.32 (1H, d, J = 2.2 Hz, indole–H), 7.41 (1H, d, J = 8.8 Hz, indole–H), 7.49–7.54 (3H, m, Ar–H), 7.81 (1 H. d, J = 8.8 Hz, Ar–H), 7.83–7.87 (2H, m, Ar–H), 7.87 (1H, br s, Ar–H). ^{13}C NMR δ 31.2 (CH_3), 55.7 (× 2) (CH_3), 56.0 (CH_3), 60.9 (CH_3), 101.4 (CH), 106.7 (× 2) (CH), 110.5 (CH), 112.5 (CH), 114.2 (C), 115.0 (C), 126.4 (CH), 126.6 (CH), 127.0 (C), 127.7 (CH), 127.9 (CH), 128.1 (CH), 128.6 (CH), 129.5 (C), 130.3 (CH), 132.7 (C), 133.1 (C), 135.8 (C), 138.3 (C), 152.9 (× 2) (C), 154.8 (C). HRMS *m/z* found 453.1897; calcd for $C_{29}H_{27}NO_4$ *m/z* 453.1940.

4.1.18. 5-Methoxy-2-(3,4,5-trimethoxyphenyl)-3-(naphth-2-yl)-1*H*-indole (20). From ketone **6** (250 mg, 0.74 mmol), compound **20** (172 mg, 53%) was obtained by the previously described procedure, isolated as a solid after chromatography (hexane–ethyl acetate 3:2) and purified by crystallization from hexane–ethyl acetate. Mp: 104 °C (hexane–ethyl acetate). ^{1}H NMR δ 3.51 (6H, s, 3-OCH_3 and 5-OCH_3), 3.79 (3H, s, 4-OCH_3), 3.84 (3H, s, indole–OCH_3), 6.63 (2H, s, Ar–H), 6.91 (1H, dd, J_1 = 8.8 Hz, J_2 = 2.2 Hz, indole–H), 7.14 (1H, d, J = 2.2 Hz, indole–H), 7.32 (1H, d, J = 8.8 Hz, indole–H), 7.52 (1H, d, J = 8.4 Hz, Ar–H), 7.54–7.64 (3H, m, Ar–H), 7.75–7.95 (2H, m, Ar–H), 8.00 (1H, br s, Ar–H), 8.68 (1H, br s, NH). ^{13}C NMR δ 55.8 (× 3) (CH_3), 60.8 (CH_3), 101.0 (CH), 105.1 (× 2) (CH), 111.8 (CH), 112.9 (CH), 114.6 (C), 125.5 (CH), 125.9 (CH), 127.6 (× 2) (CH), 127.7 (CH), 127.8 (C), 128.4 (CH), 129.0 (CH), 129.3 (C), 131.0 (C), 132.0 (C), 132.1 (C), 133.7 (C), 135.0 (C), 137.4 (C), 153.1 (× 2) (C), 154.7 (C). HRMS *m/z* found 439.1726; calcd for $C_{28}H_{25}NO_4$ *m/z* 439.1784. Anal. Calcd for $C_{28}H_{25}NO_4$: C, 76.52; H, 5.73; N, 3.19. Found: C, 76.46; H, 5.68; N, 2.97.

4.1.19. 5-Methoxy-3-(naphth-2-yl)-2-(3,4,5-trimethoxyphenyl)-1-(oxiran-2-ylmethyl)-1*H*-indole (21). Compound **21** (16 mg, 46%) was prepared from indole **20** (30 mg, 0.07 mmol), according to the previously described procedure, and obtained as a solid which was purified by crystallization from hexane–ethyl acetate. Mp: 142 °C (hexane–ethyl acetate). ^{1}H NMR δ 2.37 (1H, dd, J_1 = 4.4 Hz, J_2 = 2.6 Hz, oxirane), 2.72 (1H, t, J = 4.4 Hz, oxirane), 3.10–3.15 (1H, m, oxirane), 3.54 (6H, s, 3-OCH_3 and 5-OCH_3), 3.82 (3H, s, 4-OCH_3), 3.88 (3H, s, indole–OCH_3), 4.01–4.24 (1H, m, N–C*H* H), 4.35 (1H, dd, J_1 = 15.4 Hz, J_2 = 4.4 Hz, N–CH*H*), 6.53 (2H, s, Ar–H), 7.00 (1H, dd, J_1 = 8.8 Hz, J_2 = 2.6 Hz, indole–H), 7.33 (1H, d, J = 2.6 Hz, indole–H), 7.41–7.55 (3H, m, Ar–H), 7.42 (1H, d, J = 8.8 Hz, indole–H), 7.71–7.84 (2H, m, Ar–H), 7.86 (1H, d, J = 8.8 Hz, Ar–H), 7.90 (1H, br s, Ar–H). ^{13}C NMR δ 45.5 (CH_2), 45.7 (CH_2), 50.9 (CH), 56.0 (× 3) (CH_3), 60.9 (CH_3), 101.5 (CH), 108.6 (× 2) (CH), 110.9 (CH), 112.6 (CH), 115.4 (C), 125.3 (CH), 125.7 (CH), 126.6 (C), 127.6 (× 3) (CH), 127.6 (C), 127.7 (CH), 128.4 (CH), 132.5 (C), 132.7 (C), 133.6 (× 2) (C), 137.9 (C), 138.6 (C), 153.0 (× 2) (C), 155.1 (C). HRMS *m/z* found 495.2063; calcd for $C_{31}H_{29}NO_5$ *m/z* 495.2046. Anal. Calcd for $C_{31}H_{29}NO_5$: C, 68.95; H, 5.79; N, 3.22. Found: C, 68.90; H, 5.82; N, 3.04.

4.1.20. 3-[5-Methoxy-3-(naphth-2-yl)-2-(3,4,5-trimethoxyphenyl)-1*H*-indol-1-yl]-1-dimethylamino-propan-2-ol (22). Compound **21** (12 mg, 0.03 mmol) was added to a 30% solution of dimethylamine in ethanol. The reaction mixture was stirred at room temperature for 24 h and the solvent was removed, yielding 14 mg (90%) of **22** as a solid. ^{1}H NMR δ 1.5–1.9 (2H, m, Me_2N–CH_2), 2.20 (6H, s, $N(CH_3)_2$, 3.5–3.6 (1H, m, C*H*OH), 3.66 (6H, s, 3-OCH_3 and 5-OCH_3), 3.83 (3H, s, 4-OCH_3), 3.88 (3H, s, indole–OCH_3), 4.0–4.4 (2H, m, indole–CH_2), 6.64 (2H, s, Ar–H), 6.97 (1H, dd, J_1 = 8.4 Hz, J_2 = 2.2 Hz, indole–H), 7.28–7.47 (3H, m, Ar–H), 7.32 (1H, d, J = 8.4 Hz, indole–H), 7.45 (1H, d, J = 2.2 Hz, indole–H), 7.72 (1H, d, J = 8.4 Hz, Ar–H), 7.74–7.82 (2H, m, Ar–H), 7.87 (1H, br s, Ar–H). ^{13}C NMR δ 45.2 (× 2) (CH_3), 48.3 (CH_2), 56.1 (× 2) (CH_3), 60.9 (× 2) (CH_3) 62.9 (CH_2), 66.6 (CH), 101.5 (CH), 108.8 (× 2) (CH), 111.3 (CH), 112.5 (CH), 115.4 (C), 125.2 (CH), 125.8 (CH), 127.1 (C), 127.5 (× 3) (CH), 127.7 (CH), 128.5 (CH), 131.5 (C), 131.7 (C), 132.4 (C), 132.8 (C), 133.6 (C), 138.3 (C), 138.5 (C), 153.0 (× 2) (C), 154.9 (C). MS *m/z* 541. Anal. Calcd for $C_{33}H_{36}N_2O_5$: C, 73.31; H, 6.71; N, 5.18. Found: C, 73.24; H, 6.69; N, 5.00.

4.1.21. 2-(3,4,5-Trimethoxyphenyl)-3-(naphth-2-yl)quinoxaline (23). Glacial AcOH (500 μL) and *o*-phenylendiamine (100 μL, 0.15 mmol) were added to a solution of diketone **11** (25 mg, 0.07 mmol) in dry EtOH (2 mL). The reaction mixture was refluxed for 24 h. Next, EtOH was removed under vacuum. Water was added and the mixture extracted with ethyl acetate (× 3). The combined organic layers were sequentially washed with 2 N HCl, saturated $NaHCO_3$ and saturated NaCl, dried over anhydrous Na_2SO_4 and the solvent evaporated to obtain 29 mg (98%) of the quinoxaline derivative **23** as a thick oil. ^{1}H NMR δ 3.55 (6H, s, 3-OCH_3 and 5-OCH_3), 3.84 (3H, s, 4-OCH_3), 6.79 (2H, s, Ar–H), 7.45–7.56 (3H, m, Ar–H), 7.77–7.87 (3H, m, Ar–H), 7.78 (1H, d, J = 8.4 Hz, Ar–H), 8.17–8.23 (2H, m, Ar–H), 8.22 (1H, br s, Ar–H). ^{13}C NMR δ 56.1 (× 2) (CH_3), 61.1 (CH_3), 107.7 (× 2) (CH), 126.6 (CH), 127.1 (CH), 127.8 (× 2) (CH), 127.9 (CH), 128.7 (CH), 129.4 (× 2) (CH), 129.6 (CH), 130.2 (CH), 131.1 (CH), 132.2 (C), 133.4 (× 2) (C), 136.9 (C), 137.0 (C), 139.2 (C), 141.4 (C), 141.5 (C), 153.2 (× 2) (C), 153.6 (C). HRMS *m/z* found 422.1668; calcd for $C_{27}H_{22}N_2O_3$ *m/z* 422.1630.

4.1.22. 2-(3,4,5-Trimethoxyphenyl)-3-(naphth-2-yl)pyrido[2,3-*b*]pyrazine (24) and 3-(3,4,5-trimethoxyphenyl)-2-(naphth-2-yl)pyrido[2,3-*b*]pyrazine (25). Diketone **11** (245 mg, 0.70 mmol) was dissolved in glacial AcOH (15 mL) and 2,3-diaminopyridine (164 mg, 1.50 mmol) was added to the solution. The reaction mixture was refluxed for 4 days. Next, AcOH was evaporated. HCl

(2 N) was added to the residue and the aqueous layer was washed with ethyl acetate. The acidic aqueous solution was basified with saturated $NaHCO_3$ and extracted with ethyl acetate. The organic layer was then dried over anhydrous Na_2SO_4 and the solvent removed under reduced pressure, yielding 91 mg (31%) of a solid constituted by a 1:1 mixture of the regioisomers **24** and **25**. ^{1}H NMR δ 3.52 and 3.58 (6H, s, 3-OCH_3 and 5-OCH_3), 3.86 (3H, s, 4-OCH_3), 6.83 and 6.92 (2H, s, Ar–H), 7.51–7.61 (3H, m, pyridopyrazine–H), 7.51–7.61 (1H, m, Ar–H), 7.80–7.89 (2H, m, Ar–H), 7.82 (1H, d, $J = 8.4$ Hz, Ar–H), 8.21 and 8.35 (1H, br s, Ar–H), 8.53 and 8.57 (1H, t, $J = 1.8$ Hz, pyridopyrazine–H), 9.18 and 9.21 (1H, d, $J = 1.8$ Hz, pyridopyrazine–H). ^{13}C NMR δ 56.0 (× 2) (CH_3), 61.0 (CH_3), 107.6 (× 2) (CH), 108.0 (× 2) (CH), 125.4 (CH), 126.5 (CH), 126.7 (CH), 126.9 (CH), 127.3 (× 2) (CH), 127.7 (CH), 128.0 (CH), 128.6 (CH), 129.5 (CH), 130.4 (CH), 132.9 (C), 133.0 (C), 133.1 (C), 133.4 (C), 135.7 (C), 136.2 (C), 136.4 (C), 138.1 (CH), 139.4 (C), 139.5 (C), 149.8 (C), 153.0 (× 2) (C), 153.3 (× 2) (C), 155.8 (× 2) (C), 154.2 (CH), 154.6 (C), 156.2 (C). HRMS *m/z* found 423.1618; calcd for $C_{26}H_{21}N_3O_3$ *m/z* 423.1583. Anal. Calcd for $C_{26}H_{21}N_3O_3$: C, 73.74; H, 5.00; N, 9.92. Found: C, 73.59; H, 4.95; N, 9.73.

4.1.23. 3-(3,4,5-Trimethoxyphenyl)-2-(naphth-2-yl)quinoxaline-6-carboxylic acid (26) and 2-(3,4,5-trimethoxyphenyl)-3-(naphth-2-yl)quinoxaline-6-carboxylic acid (27). Diketone **11** (240 mg, 0.68 mmol) was dissolved in glacial AcOH (16 mL) and 3,4-diaminobenzoic acid (169 mg, 1.12 mmol) was added to the solution. The reaction mixture was refluxed for 4 days. Next, 10% NaOH was added and the aqueous layer was washed with ethyl acetate. The basic aqueous solution was then acidified with 2 N HCl and extracted with ethyl acetate. The organic layer was washed with saturated NaCl, dried over Na_2 SO_4 and the solvent removed under vacuum to yield 42 mg (18%) of a solid constituted by a 1:1 mixture of the regioisomers **26** and **27**. ^{1}H NMR δ 3.56 (6H, s, 3-OCH_3 and 5-OCH_3), 3.85 (3H, s, 4-OCH_3), 6.82 and 6.83 (2H, s, Ar–H), 7.47–7.58 (3H, m, Ar–H), 7.81 (1H, d, $J = 8.8$ Hz, Ar–H), 7.85–7.89 (2H, m, Ar–H), 8.21 (1H, br s, Ar–H), 8.27 (1H, d, $J = 8.8$ Hz, quinoxaline–H), 8.41 (1H, dd, $J_1 = 8.8$ Hz, $J_2 = 1.8$ Hz, quinoxaline–H), 9.00 (1H, s, quinoxaline–H). HRMS *m/z* found 466.1632; calcd for $C_{28}H_{22}N_2O_5$ *m/z* 466.1529.

4.2. Tubulin polymerization assays

Calf brain microtubule protein (MTP) was purified by two cycles of temperature-dependent assembly/disassembly, according to the method of Shelanski et al.[27] modified as described in the literature.[28] The MTP solution was stored at −80 °C. Protein concentration was determined by the method of Bradford,[29] using BSA as standard.

The in vitro self-assembly of tubulin was monitored turbidimetrically using a thermostated Thermospectronic Helios α spectrophotometer fitted with a Peltier temperature controller and a circulating water bath. The increase in turbidity was followed at 450 nm instead of 350 nm in order to avoid light absorption by the ligands. Each turbidimetry measure was carried out for a batch of six cuvettes simultaneously, always including a control (i.e., with no ligand) in the batch. Four different MTP preparations were used in these assays. The assayed ligands were dissolved in DMSO and the resultant solutions stored at −20 °C.

Cuvettes contained 1.5 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 corresponding ligand concentration. The maximum amount of DMSO in the assay cuvettes was 4%, which is reported not to interfere with the assembly process.[30] The samples were pre-incubated for 30 min at 20 °C in order to allow binding of the ligand, and subsequently placed on ice for 10 min. The cuvettes were then transferred to the spectrophotometer at 4 °C and the baseline registered. The assembly process was started by shifting the temperature to 37 °C.

IC_{50} was calculated as the concentration of drug causing 50% inhibition of polymerization after 20 min incubation, and was determined graphically.

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