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Naphthylphenstatins as tubulin ligands: Synthesis and biological evaluation

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ABSTRACT

A new family of naphthalenic analogues of phenstatins with modifications on the ketone-bridge has been synthesised. The synthesised compounds have been assayed for tubulin polymerisation inhibitory activity as well as for cytotoxic activity against cancer cell lines. The naphthalene has been confirmed as a good surrogate for the isovanillin moiety (3-hydroxy-4-methoxyphenyl) of phenstatin, when combined with the 3,4,5-trimethoxyphenyl ring, but not when combines with the 2,3,4-trimethoxyphenyl ring. Binding models for the synthesised compounds have been generated and analysed in terms of a pharmacophore proposed for colchicine site ligands. The ketone is the optimal bridge substitution but *E*-acetyloximes or acetylhydrazones are also tolerated. Significant differences with indole substituted phenstatins are observed and discussed.

1. Introduction

Microtubules are essential cellular components involved in intracellular transport, motility, shape maintenance, and division.[1] The microtubules are mainly composed of α,β-tubulin dimers and drugs that interact with tubulin interfere with their dynamic equilibrium, cause mitotic arrest and, often, trigger cell death.[2] Ligands binding at the colchicine site of tubulin have received much attention and some of them are undergoing clinical trials as antitumour and/or vascular disrupting drugs, such as the combretastatin A-4 phosphate prodrug.[3] Many of these ligands have two non-coplanar polyoxygenated aromatic rings (most typically one trimethoxyphenyl and a 3-hydroxy-4-methoxyphenyl or related ring) linked by a bridge of zero to more than four atoms (Fig. 1).[4] Amongst them, much effort has been devoted to combretastatins (*Z* stilbenes and derivatives) and more recently to phenstatins (bisarylketones) and their structure–activity relationships.[3,4]

We have previously explored the possibility of replacing the aryl rings of combretastatins by naphthyl systems (Fig. 2).[5] Replacement of the 3,4,5-trimethoxyphenyl ring of combretastatin A-4 by a 2-naphthyl system, and almost any other one, is highly detrimental for the activity, but the 3-hydroxy-4-methoxyphenyl ring can be replaced with no substantial loss of activity.[5a,5b] The introduction of a 1-naphthyl system is highly detrimental, indicating a strong geometrical preference of tubulin. We have also replaced several carbon atoms of the 2-naphthyl system of naphthylcombretastatins by nitrogen atoms, thus preparing quinoline and quinoxaline analogues of combretastatins, and found that the position of the heteroatom is very influential on the activity but does not improve much the water solubility.[6]

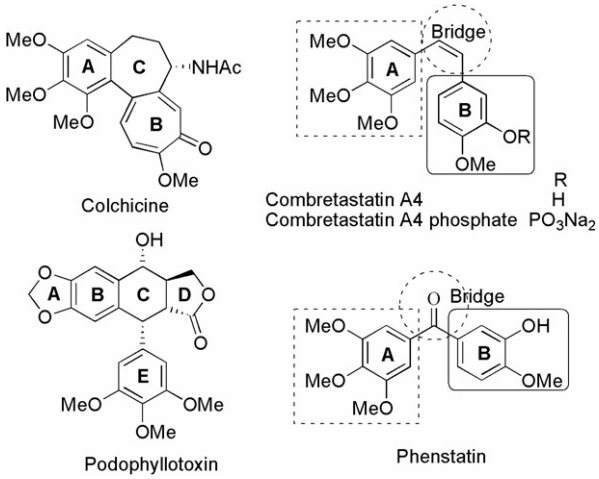

Figure 1. Structure of colchicine, podophyllotoxin, combretastatin A-4 and its phosphate prodrug and phenstatin.

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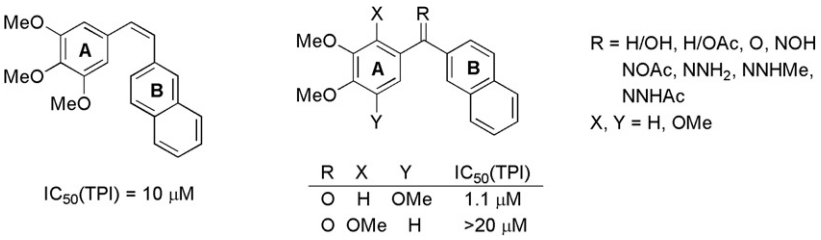


Figure 2. Structure and TPI activity of previously described naphthylcombretastatin and naphthylphenstatins (X = O). Chemical structures of the new derivatives of naphthylphenstatins modified on the bridge

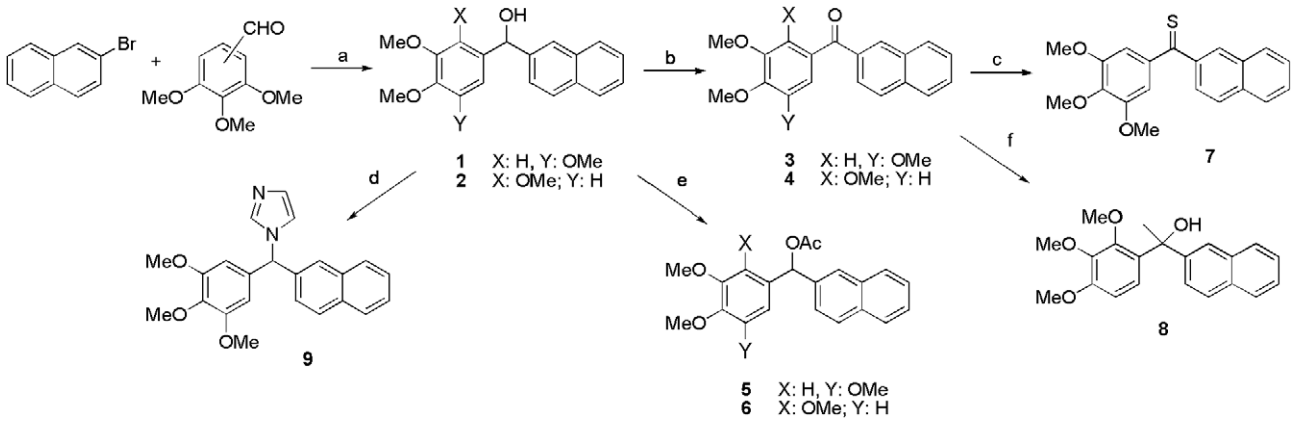
We have also shown in a preliminary communication that a 2-naphthophenone (naphthylphenstatin) bearing a 2-naphthyl system (in substitution for the 3-hydroxy-4-methoxyphenyl or related ring) combined with a 3,4,5-trimethoxyphenyl ring (3,4,5-TM; such as the one present in combretastatins and podophyllotoxin) is a potent inhibitor of tubulin polymerisation and cytotoxic compound.[7] On the other hand, the combination of a 2-naphthyl system with a 2,3,4-trimethoxyphenyl ring (2,3,4-TM, such as that found in colchicine) is highly detrimental for the activity of phenstatin analogues (Fig. 2). In this paper, we disclose a full account of the synthesis and biological activity of naphthylphenstatins and of new derivatives modified on the ketone-bridge with nitrogen containing substituents, in an attempt to further expand our knowledge on the structure–activity relationships of the naphthylphenstatins (Fig. 2).

2. Results and discussion

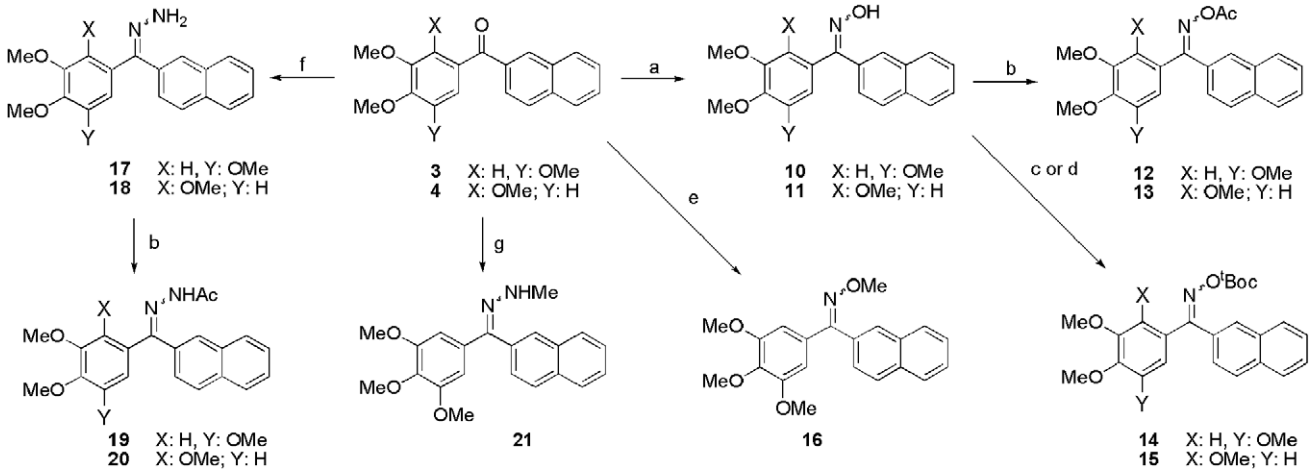
2.1. Chemistry

The synthesis of the naphthylphenstatins is depicted in Schemes 1–3. The compounds were grouped into two families depending on the position at which the trimethoxyphenyl ring is attached to the carbonyl group. Thus, family I comprises derivatives bearing a 3,4,5-trimethoxyphenyl ring, whereas family II includes compounds with a 2,3,4-trimethoxyphenyl moiety. We have recently described the preparation of the diarylmethanols, diarylmethylacetates and diarylketones.[7] Briefly, diarylmethanols **1** and **2** were prepared by reaction between naphthalen-2-yllithium and 2,3,4- or 3,4,5-trimethoxybenzaldehyde. The alternative approach, that is, the reaction between the corresponding 2,3,4- or 3,4,5-trimethoxyphenyllithium and 2-naphthaldehyde, was discarded due to the formation of dialkylated by-products which are difficult to separate from the desired compound. The best results were obtained by using *n*-BuLi for transmetallation, followed by addition of the arylaldehyde. The by-products formed from the direct addition of *n*-BuLi to the aldehyde were chromatographically removed. Ketones **3** and **4** were obtained in high yields (higher than 70%) by oxidation of the corresponding hydroxyderivatives with $KMnO_4$ in wet CH_2Cl_2 in the presence of a tetrabutylammonium phase transfer catalyst, provided that the usual method for oxidation used in phenstatin synthesis (PDC) proved unsuccessful. Diarylmethanols were acetylated with acetic anhydride in pyridine to yield the corresponding acetates **5** and **6**. Diarylmethanol **1** was also transformed into the imidazole derivative **9** by treatment with imidazole in the presence of triphenylphosphine and iodine. Ketone **3** was in turn the starting material to prepare the hydroxy derivative **8**, which bears a tetrasubstituted sp^3 carbon-bridge, by treatment with methylmagnesium bromide in THF.

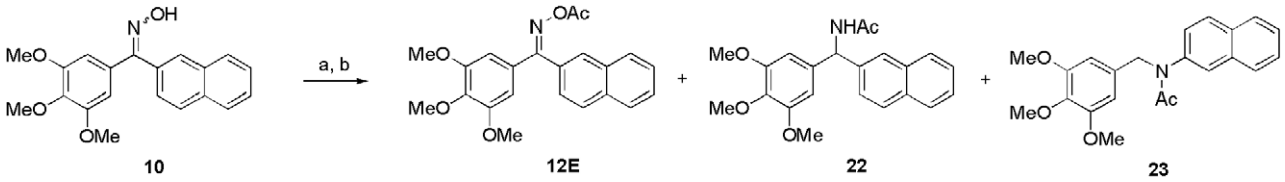
Diarylketones **3** and **4** are key intermediates towards the synthesis of analogues bearing a sp^2 carbon-bridge. Thus, thioketone **7** was prepared from ketone **3** by treatment with Lawesson's reagent under microwave irradiation. Nitrogenated derivatives were synthesised from the corresponding ketones following standard methodologies, through reaction with hydroxylamine, methoxyamine, hydrazine and methylhydrazine to yield oximes **10** and **11**, methoxime **16**, hydrazones **17** and **18** and methylhydrazone **21**, respectively. These derivatives were mainly obtained as mixtures of *Z* and *E* isomers (see experimental section), which could not be chromatographically resolved. For compounds of family I, in which the methoxy groups are attached at positions 3,4,5 of the phenyl ring, the *Z*/*E* ratio of isomers does not show a clear preference towards any of them. However, in the case of family II, with a 2,3,4-trimethoxyphenyl ring, one of them is always preferred, as it is observed for oxime **11**, with a 2:1 ratio, and for hydrazone **18**, with only one isomer detected. Oximes and hydrazones were further derivatised by formation of the acetylated analogues **12** and **13** (acetoximes) and **19** and **20** (acetylhydrazones). Oximes were



Scheme 1. Synthesis of naphthylphenstatins **1**–**9**. Reagents and conditions: (a) *n*-BuLi, THF, −78 °C; (b) $KMnO_4$, $Bu_4N^+HSO_4^-$, CH_2Cl_2; (c) Lawesson's reagent, microwave irradiation; (d) imidazole, PPh_3, I_2, CH_2Cl_2; (e) Ac_2O, pyridine; (f) MeMgBr, THF.



Scheme 2. Synthesis of naphthylphenstatins **10**–**21**. Reagents and conditions: (a) $NH_2OH{\cdot}HCl$, pyridine, MeOH; (b) Ac_2O, pyridine; (c) $(^tBoc)_2O$, Na_2CO_3, dioxane/H_2O (1:1); (d) $(^tBoc)_2O$, NaH, THF; (e) $MeONH_2{\cdot}HCl$, NaAcO, THF/MeOH (1:1); (f) NH_2NH_2, AcOH, MeOH; (g) $MeNHNH_2$, AcOH, MeOH.



Scheme 3. Products derived from oxime **10**. Reagents: (a) $LiAlH_4$, THF; (b) Ac_2O, pyridine.

also transformed into the corresponding *O*-*tert*-butoxycarbonyl derivatives **14** and **15**. In that way, the size and the donor–acceptor pattern of the bridge are also modified, so their influence on biological activity could be observed. The two geometrical isomers have been separated in some cases, for example, acetoxime **13** and acetylhydrazone **19**, and they are stable in solution for days (data not shown). The stereochemistry has been assigned based upon the ^1H NMR chemical shifts of H3 of the 2-naphthyl system and H2,6 of the 3,4,5-trimethoxyphenyl ring (H6 of 2,3,4-TM). For a related indole substituted series, we have previously shown that H6 of the 5-indolyl moiety is deshielded in the *Z* isomer related to the *E* one, whereas H2,6 of 3,4,5-TM show the opposite trend.[8] Hence, in compounds of family II the preferred isomer is the *Z* isomer.

Attempts to obtain further nitrogenated derivatives by reacting ketone **3** with several amines (ethylamine, benzylamine, 1,1-dimethylhydrazine) in different conditions were unsuccessful. Thioketone **7**, more reactive, could not be used as starting material due to its instability. Then, we decided to enlarge the group of nitrogenated analogues by reducing the already obtained oximes. Thus, the *Z* + *E* mixture of oximes **10** was treated with $LiAlH_4$, and the reaction crude was acetylated before further purification for an easier separation of the products (Scheme 3). Three compounds were isolated from this reaction mixture. The major one, acetamide **23**, produced by Beckmann rearrangement of the starting *Z* oxime **10*Z*** followed by reduction and acetylation. The minor compounds are the expected acetamide **22** and acetoxime **12*E***, produced by acetylation of unreacted *E* isomer **10*E***.

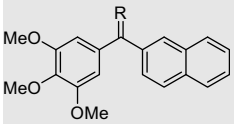
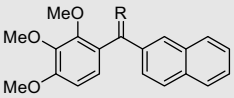
2.2. Biological evaluation

The synthesised compounds were evaluated in vitro as inhibitors of tubulin polymerisation as previously described.[5b] When two geometric isomers were possible (*Z* and *E*), they were usually tested as a mixture (indicated by the corresponding number in Table 1). In the case of one or both isomers being isolated, they were assayed as individual compounds (indicated by *E* or *Z* following the number). The compounds were first assayed at a single concentration (20–40 μM), and their degree of inhibition of tubulin polymerisation indicated as a percentage of the control (which represents 100% polymerisation). Phenstatin was used as reference. The results are shown in Table 1 and are in agreement with the structure–activity relationships for naphthylcombretastatins, which indicate that the 2-naphthyl ring is a bioisostere of the 3-hydroxy-4-methoxyphenyl ring.[5] Expressed in terms of a proposed pharmacophore of the colchicine site ligands,[4] the binding models generated for naphthylphenstatins (Figs. 3 and 4) show that the 2-naphthylphenstatins occupy the R1 pharmacophoric planar group with the naphthalene ring, the H2 hydrophobic centre and the A2 hydrogen bond acceptor with the 3,4,5-trimethoxyphenyl ring and, depending upon the bridge substitution (see below), the A3 hydrogen bond acceptor with, for instance, the carbonyl oxygen.

The most potent compound was ketone **3**, for which an IC_{50} value of 1.1 μM was determined. This value makes it even more potent than phenstatin (IC_{50} = 2.5 μM) and CA-4 (IC_{50} = 3–4 μM). The ten fold potency increase observed for naphthylphenstatin (**3**) when compared with naphthylcombretastatin (Fig. 2) is consistent with previous comparisons between equally substituted combretastatins and phenstatins.[9] An explanation for the greater TPI potency of phenstatins relative to combretastatins has been suggested.[4] The presence of an additional hydrogen bond acceptor (point A3) in phenstatins (the carbonyl oxygen) which could hydrogen bond to the backbone N–H group of residues β248–β249–β250 would explain their greater potency. The binding models that we have constructed for **3** are in good agreement with such an explanation (Fig. 3).

Except for acetates **5** and **6**, compounds of family I (bearing a 3,4,5-trimethoxyphenyl ring) are more potent inhibitors of tubulin polymerisation than those of family II (bearing a 2,3,4-trimethoxy-

Table 1
Inhibition of tubulin polymerisation (TPI) and cytotoxicity results for the synthesised compounds

		% TPI concentration assayed (μM)	HeLa	A-549	HT-29
Family I	Family II				
1 (R = H, OH)		0 (40)			
	2 (R = H, OH)	0 (20)	>10	>10	>10
3 (R = O)		100 (40)	0.043	0.25	
	4 (R = O)	4 (20)	1.7	>10	>10
5 (R = H, OAc)		5 (20)	3.8	>10	1.2
	6 (R = H, OAc)	26 (20)	2.7		>10
	8 (R = Me, OH)	3 (20)	1–10		>10
9 (R = H, Im)		0 (20)			>10
10 (R = N–OH)		0 (30)	1.5	>10	1.8
	11 (R = N–OH)	0 (30)	0.4	>10	1–10
12 (R = N–OAc)		10 (20)			
12*E* (R = N–OAc)		38 (20)	0.001–0.1		1–10
	13*Z* (R = N–OAc)	0 (20)	1.2	>10	>10
14 (R = N–O^{t}Boc)		15 (20)			>10
	15 (R = N–O^{t}Boc)	0 (20)	0.3		>10
	15*Z* (R = N–O^{t}Boc)				>10
16 (R = N–OMe)		4 (20)			1–10
17 (R = N–NH_2)		0 (30)			10
	18*Z* (R = N–NH_2)	0 (20)			
19 (R = N–NHAc)		35 (30)	>10	>10	>10
19*Z* (R = N–NHAc)		0 (20)			
	20*Z* (R = N–NHAc)	0 (20)			
21 (R = N–NHMe)		31(30)			
CA-4		100 (20)	2.7	0.003	0.032

TPI is expressed as the percentage of inhibition observed at the concentration assayed, related to the control; the values are the mean of at least three experiments. Cytotoxicity is expressed as IC_{50} (μM); the values shown represent the mean of at least six experiments.

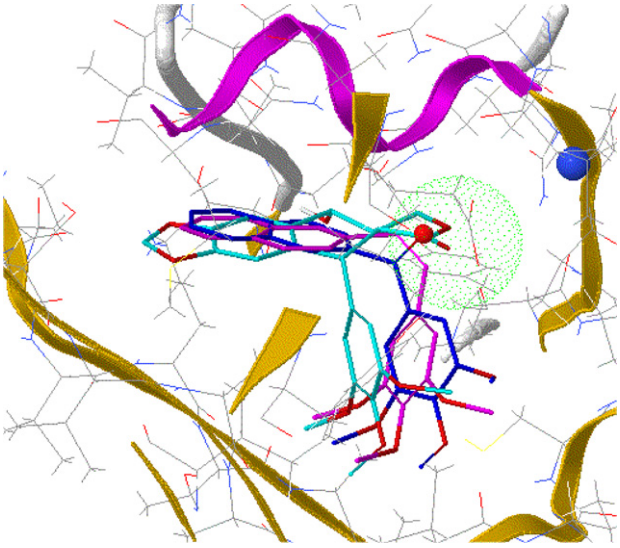


Figure 3. Superimposed binding models of naphthylcombretastatin (carbons in magenta), naphthylphenstatin (**3**, carbons in blue) and podophyllotoxin (carbons in cyan) in the colchicine site of tubulin. Tubulin is shown as ribbons and wireframe.[23] The carbonyl oxygen of naphthylphenstatin (**3**) and the backbone N of β250 are highlighted as cpk. The proposed acceptor site A3 of the pharmacophore is indicated by the light green dotted sphere.[4]

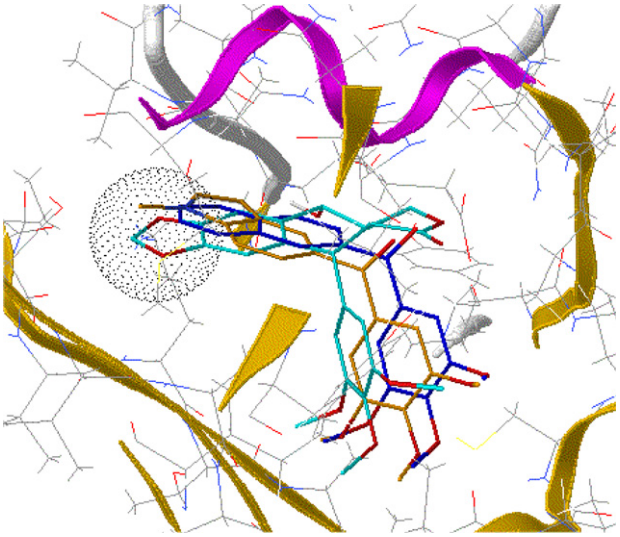


Figure 4. Superimposed binding models of naphthylphenstatin (**3**, carbons in blue), *N*-methyl-5-indolyl analogue[8] (carbons in orange) and podophyllotoxin (carbons in cyan) in the colchicine site of tubulin. Tubulin is shown as ribbons and wireframe.[23] The proposed hydrophobic site H1 of the pharmacophore is indicated by the dotted sphere.[4]

phenyl ring). The binding models of compounds of family II (not shown) satisfy either pharmacophoric point H2 (trimethoxyphenyl ring of podophyllotoxin) or R1 (plane of the methylenedioxyphenyl ring of podophyllotoxin), thus suggesting that the molecular scaffold precludes them from simultaneously fulfilling the overall geometric and steric requirements of binding represented by pharmacophoric points H2 and R1,[4] and accounting for the lower TPI potency.

With respect to bridge modifications, for compounds with a sp^2 bridge carbon, only acetoxime **12*E***, acetylhydrazone (**19**) and

methylhydrazone (**21**), all of them belonging to family I, showed moderate inhibitory activity. These results are in sharp contrast with the results of TPI recently described by us for a related series of compounds bearing a *N*-methyl-5-indolyl ring in place of the naphthalene one.[8] In the indolyl series, the oximes were even more potent than the parent ketones, and their acetylation lead to a potency decrease. A very different trend is seen here for the naphthylphenstatins, whose oximes show almost no TPI activity and their acetylation recovers some of it. A similar behaviour is observed for the hydrazones and their acetates and for the sp^3 bridged analogues (alcohols and acetates). For them, the indole series show higher potency for the hydrazones or alcohols compared to their acetates, while the reverse is true for the naphthalene series. These results suggest that, despite of the apparent high structural resemblance of the N-methylated-5-indole ring and the naphthalene one, they behave in a very different way in these series and that comparisons of families with such a replacement should be cautious. Their docking models show differences which might provide an explanation: the *N*-methyl group of the *N*-methyl-5-indolyl analogue occupies pharmacophoric point H1, which is not occupied in the naphthylphenstatins (Fig. 4), thus allowing for a different overall arrangement of the two fused ring systems. This in turn allows for more bulky substituents on the bridge for the naphthalene series. For the indole series, smaller substitutions are preferred, due to the tighter fit of the bicyclic system in the H1 and R1 sites.

Whenever possible, a comparison of the tubulin polymerisation inhibitory effect of mixtures of stereoisomers with isolated stereoisomers indicates that *E* isomers are more potent than the corresponding *Z* isomers (as seen in Table 1 for acetyloxime **12** and acetylhydrazone **19**). This stereochemistry is the opposite to that proposed for an oxime analogue of curacin[10] on the basis of a generated binding model.[4a] However, this discrepancy only reflects the very different scaffold characteristics of both families of compounds. The preferred stereochemistry allows in each case for the 3,4,5-trimethoxyphenyl ring to occupy the pharmacophoric H2 hydrophobic centre and the A2 hydrogen bond acceptor site. For the naphthylphenstatin analogues here described, the *E* isomers place the O-substituents toward the space occupied by ring C and D of podophyllotoxin.

The compounds were also evaluated as cytotoxic agents against three human tumour cell lines: HeLa (cervix carcinoma), A-549 (lung carcinoma) and HT-29 (colon adenocarcinoma). The results are shown in Table 1, expressed as IC_{50} (μM). All the compounds were tested in a preliminary assay, and only the ones that showed some cytotoxicity were taken further for determining their IC_{50} values. Combretastatin A-4 was used as reference. The most sensitive cell line was HeLa, whereas A-549 and HT-29 were more resistant to these agents, in contrast to the cytotoxicity shown by combretastatin A-4 but in agreement with our previous reports on naphthylcombretastatins[5] and indolephenstatins.[8] The compounds eliciting a more remarkable cytotoxicity belong to family I, bearing the ketone (**3**) and the acetoxime group (**12*E***) as the bridge between the aromatic systems, in good agreement with the above discussed TPI results. The IC_{50} values found for these analogues are comparable to those of the reference compound CA-4, and follow previously described trends for phenstatin analogues, which display lower cytotoxicity than equally substituted combretastatins even though they usually elicit higher TPI responses.[8]

3. Conclusions

The naphthalene system is a good subrogate of the B ring of combretastatins and phenstatins in the parent compounds (combretastatin A-4 and phenstatin), with a *cis*-olefin or ketone bridges. As previously demonstrated for combretastatins and now proven for phenstatins, further modifications at the bridge combined with the presence of naphthalene moiety, are detrimental for the inhibitory of tubulin polymerisation and inhibitory tumour cell growth activities. For nitrogen derivatives at the bridge having the possibility of *Z*,*E*-stereoisomerism, those with the *E*-configuration (N-substituent directed towards the naphthalene ring) showed to be more active than those with a *Z*-configuration. The substitution pattern represented by 2,3,4-trimethoxy in the ring A produced compounds with very low or no activity in both types of assays, as it is usually observed for these classes of antimitotics. These findings enlarge the knowledge of the structure–activity relationships of the phenstatins, which are different than those displayed by the related combretastatins, and show substantial departures in the structure–activity relationships of 2-naphthyl substituted from *N*-methyl-5-indolyl phenstatin derivatives. These findings have important bearings on the design of new families of colchicine site ligands, in particular of phenstatin analogues.

4. Experimental

4.1. Chemistry

IR spectra were recorded on a Nicolet Impact 410 instrument, as a thin film unless otherwise stated. ^1H 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 mm or 0.040–0.063 mm) silica gel. 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 acetonitrile/water. Commercial THF was distilled over Na, under argon, prior to use.

4.1.1. General method A: synthesis of diarylmethanols

To a stirred suspension of the corresponding arylhalide (15.4–45.2 mmol) in THF (35–60 mL), 1.6 M *n*-BuLi in hexanes (11.6–34.0 mL, 1.2 mmol per mmol of halide) was added at −78 °C, under argon. After 1.5 h, the arylaldehyde (1.2 mmol per mmol of halide) was added, and the mixture was allowed to reach room temperature. After 12–24 h, the reaction mixture was poured onto ice and extracted with dichloromethane. The combined organic layers were dried (Na_2SO_4) and the solvent evaporated.

4.1.2. General method B: synthesis of diarylketones

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

4.1.3. General method C: acetylation

A 10 mol excess of acetic anhydride was added to a 1 M solution of the compound to be acetylated (0.3–1.0 mmol) in pyridine. After 2–4 h, the reaction mixture was diluted with ethyl acetate and washed with 2 N HCl and saturated $NaHCO_3$. The organic layer was dried over Na_2SO_4 and the solvent evaporated.

4.1.4. General method D: synthesis of oximes

A mixture of hydroxylamine hydrochloride (10 mmol per mmol of ketone), pyridine (2–3 drops) and a 0.05–0.06 M solution of ketone in MeOH (15–25 mL) was refluxed for 12 h and then the solvent was evaporated. The crude product was dissolved in dichloromethane and washed with water. The organic layer was dried (Na_2SO_4) and evaporated.

4.1.5. General method E: synthesis of hydrazones

Hydrazine hydrate (10 mmol per mmol of ketone) and acetic acid (2–3 drops) were added to a 0.04–0.06 M solution of ketone in MeOH (15–25 mL). The reaction mixture was refluxed for 12–48 h. During this time, a large excess of hydrazine hydrate was added to the reaction mixture in four portions. The solvent was evaporated and the crude product was dissolved in dichloromethane and washed with water. The organic layer was dried (Na_2SO_4) and evaporated.

4.1.6. (2-Naphthyl)(3,4,5-trimethoxyphenyl)methanol (1)

Following general method A, 2-bromonaphthalene (3.2 g, 16.3 mmol) in dry THF (35 mL), 1.6 M *n*-BuLi in hexanes (11.6 mL, 18.6 mmol) and 3,4,5-trimethoxybenzaldehyde (3.6 g, 18.4 mmol) were reacted to obtain alcohol **1** (4.7 g, 14.5 mmol, 89%), which was purified by crystallisation from dichloromethane/hexane. ^{1}H NMR δ 3.82 (6H, s, 3′-OCH_3, 5′-OCH_3), 3.83 (3H, s, 4′-OCH_3), 5.94 (1H, br s, C*H*–*OH),* 6.65 (2H, s, H2′, H6′), 7.43–7.51 (3H, m, H3, H6, H7), 7.80–7.88 (3H, m, H4, H5, H8), 7.89 (1H, br s, H1). ^{13}C NMR δ 56.2 (2×) (CH_3), 60.9 (CH_3), 76.4 (CH), 104.1 (2×) (CH), 124.8 (CH), 125.1 (CH), 126.1 (CH), 126.2 (CH), 127.7 (CH), 128.1 (CH), 128.4 (CH), 133.0 (C), 133.3 (C), 137.3 (C), 139.5 (C), 141.1 (C), 153.4 (2×) (C). IR (cm^{-1}) 1130, 1600, 3425. HRMS *m*/*z* found 347.1235; calcd for $C_{20}H_{20}O_4$ (+Na) 347.1254. Mp 134–136 °C (CH_2Cl_2/hexane).

4.1.7. (2-Naphthyl)(2,3,4-trimethoxyphenyl)methanol (2)

Following general method A, 2-bromonaphthalene (9.4 g, 45.2 mmol) in dry THF (60 mL), 1,6 M *n*-BuLi in hexane (34.0 mL, 54.2 mmol) and 2,3,4-trimethoxybenzaldehyde (10.6 g, 54.2 mmol) were reacted to obtain alcohol **2** (13.5 g, 41.6 mmol, 92%). ^{1}H NMR δ 3.65 (3H, s, 2′-OCH_3), 3.83 (3H, s, 3′-OCH_3 or 4′-OCH_3), 3.85 (3H, s, 4′-OCH_3 or 3′-OCH_3), 6.11 (1H, br s, C*H*-OH), 6.62 (1H, d, *J* = 8,6, H5′), 6.99 (1H, d, *J* = 8,6, H6′), 7.43–7.48 (3H, m, H3, H6, H7), 7.72–7.88 (3H, m, H4, H5, H8), 7.91 (1H, br s, H1). ^{13}C NMR δ 56.0 (CH_3), 60.8 (2×) (CH_3), 72.4 (CH), 107.1 (CH), 122.6 (CH), 124.8 (CH), 125.0 (CH), 125.8 (CH), 126.1 (CH), 127.7 (CH), 128.0 (CH), 128.1 (CH), 129.9 (C), 132.8 (C), 133.3 (C), 141.5 (C), 142.2 (C), 151.5 (C), 153.5 (C). IR (cm^{-1}) 1600, 3425. HRMS *m*/*z* found 347.1248; calcd for $C_{20}H_{20}O_4$ (+Na) 347.1254.

4.1.8. (2-Naphthyl)(3,4,5-trimethoxyphenyl)methanone (3)

Following general method B, alcohol **1** (3.0 g, 9.3 mmol) in dichloromethane (40 mL) was oxidised with $KMnO_4$ (1.5 g, 9.3 mmol) in the presence of tetra-*n*-butylammonium hydrogensulfate (30 mg, 0.088 mmol), yielding ketone **3** (2.2 g, 6.8 mmol, 73%), which is purified by crystallisation from dichloromethane/hexane. ^{1}H NMR δ 3.88 (6H, s, 3′-OCH_3, 5′-OCH_3), 3.97 (3H, s, 4′-OCH_3), 7.13 (2H, s, H2′, H6′), 7.58–7.64 (3H, m, H3, H6, H7), 7.85–8.05 (3H, m, H4, H5, H8), 8.29 (1H, br s, H1). ^{13}C NMR δ 56.4 (2×) (CH_3), 61.0 (CH_3), 108.0 (2×) (CH), 125.9 (CH), 126.9 (CH), 127.9 (CH), 128.3 (2×) (CH), 129.4 (CH), 131.4 (CH), 132.3 (C), 132.9 (C), 135.1 (C), 135.2 (C), 142.0 (C), 153.0 (2×) (C), 195.7 (C). IR (cm^{-1}) 1600. HRMS *m*/*z* found 345.1080; calcd for $C_{20}H_{18}O_4$ (+Na) 345.1097. Mp 108–110 °C (CH_2Cl_2/hexane).

4.1.9. (2-Naphthyl)(2,3,4-trimethoxyphenyl)methanone (4)

Following general method B, alcohol **2** (1.7 g, 5.2 mmol) in dichloromethane (50 mL) was oxidised with $KMnO_4$ (829 mg, 5.25 mmol) in the presence of tetra-*n*-butylammonium hydrogensulfate (170 mg, 0.501 mmol), yielding ketone **4** (1.42 g, 4.42 mmol, 85%). ^{1}H NMR δ 3.75 (3H, s, 2′-OCH_3), 3.92 (3H, s, 3′-OCH_3 or 4′-OCH_3), 3.95 (3H, s, 4′-OCH_3 or 3′-OCH_3), 6.76 (1H, d, *J* = 8,6, H5′), 7.20 (1H, d, *J* = 8,6, H6′), 7.51–7.59 (3H, m, H3, H6, H7), 7.87–7.95 (3H, m, H4, H5, H8), 8.23 (1H, br s, H1). ^{13}C NMR δ 56.2 (CH_3), 61.0 (CH_3), 61.8 (CH_3), 106.8 (CH), 125.2 (2×) (CH), 125.9 (C), 126.7 (CH), 127.9 (CH), 128.1 (CH), 128.4 (CH), 129.6 (CH), 131.9 (CH), 132.4 (C), 135.6 (C), 135.8 (C), 142.2 (C), 152.8 (C), 156.3 (C), 195.5 (C). IR (cm^{-1}) 1590, 1652. HRMS *m*/*z* found 345.1097; calcd for $C_{20}H_{20}O_4$ (+Na) 345.1091.

4.1.10. [(2-Naphthyl)(3,4,5-trimethoxyphenyl)methyl]acetate (5)

Following general method C, compound **1** (100 mg, 0.31 mmol) in pyridine (300 μL) was acetylated by treatment with acetic anhydride (300 μL, 3.1 mmol), yielding acetate **5** (80 mg, 0.22 mmol, 70%). ^{1}H NMR δ 2.22 (3H, s, $COCH_3$), 3.82 (6H, s, 3′-OCH_3, 5′-OCH_3), 3.83 (3H, s, 4′-OCH_3), 6.63 (2H, s, H2′, H6′), 7.00 (1H, s, CH-OAc), 7.41–7.53(3H, m, H3, H6, H7), 7.81–7.88 (3H, m, H4, H5, H8), 7.84 (1H, br s, H1). ^{13}C NMR δ 21.5 (CH_3), 56.2 (2×) (CH_3), 60.9 (CH_3), 77.2 (CH), 104.6 (2×) (CH), 124.9 (CH), 125.9 (CH), 126.4 (2×) (CH), 127.8 (CH), 128.2 (CH), 128.5 (CH), 133.0 (C), 133.1 (C), 135.7 (C), 137.4 (C), 137.8 (C), 153.4 (2×) (C), 170.2 (C). IR (cm^{-1}) 1592, 1739. HRMS *m*/*z* found 389.1358; calcd for $C_{22}H_{22}O_5$ (+Na) 389.1359.

4.1.11. [(2-Naphthyl)(2,3,4-trimethoxyphenyl)methyl]acetate (6)

Following general method C, compound **2** (200 mg, 0.62 mmol) in pyridine (600 μL) was acetylated by treatment with acetic anhydride (600 μL, 6.2 mmol), obtaining acetate **6** (180 mg, 0.49 mmol, 79%). ^{1}H NMR δ 2.19 (3H, s, $COCH_3$), 3.80 (3H, s, 2′-OCH_3), 3.85 (3H, s, 3′-OCH_3 or 4′-OCH_3), 3.87 (3H, s, 4′-OCH_3 or 3′-OCH_3), 6.66 (1H, d, *J* = 8,8, H5′), 7.06 (1H, d, *J* = 8,8, H6′), 7.31 (1H, s, CH-OAc), 7.44–7.49 (3H, m, H3, H6, H7), 7.75–7.90 (4H, m, H1, H4, H5, H8). ^{13}C NMR δ 21.3 (CH_3), 56.0 (CH_3), 60.7 (2×) (CH_3), 72.1 (CH), 107.4 (CH), 122.1 (CH), 125.1 (CH), 125.8 (CH), 126.1 (2×) (CH), 127.7 (CH), 128.1 (2×) (CH), 133.0 (C), 133.1 (C), 137.9 (C), 142.4 (C), 145.9 (C), 153.8 (2×) (C), 169.8 (C). IR (cm^{-1}) 1601, 1741. HRMS *m*/*z* found 389.1359; calcd for $C_{22}H_{22}O_5$ (+Na) 389.1359.

4.1.12. (2-Naphthyl)(3,4,5-trimethoxyphenyl)methanothione (7)

Lawesson's reagent (32 mg, 0.08 mmol) was added to ketone **3** (50 mg, 0.16 mmol) in a test tube, that was placed into a vessel filled with alumina and irradiated at 750 W for 10 min (5 periods of 2 min). The reaction progress was checked by TLC until completion. The reaction mixture was then passed through silica gel, using ethyl acetate as eluent, yielding thioketone 7 (30 mg, 0.089 mmol, 55%). ^{1}H NMR δ 3.85 (6H, s, 3′-OCH_3, 5′-OCH_3), 3.98 (3H, s, 4′-OCH_3), 7.08 (2H, s, H2′, H6′), 7.54–7.62 (3H, m, H3, H6, H7), 7.87–7.95 (3H, m, H4, H5, H8), 8.16 (1H, br s, H1). MS *m*/*z* (rel. int.) 338 (M^+, 100).

4.1.13. 1-(2-Naphthyl)-1-(2,3,4-trimethoxyphenyl)ethanol (8)

A 3 M solution of methylmagnesium chloride in THF (3.1 mL, 9.33 mmol) was added to a solution of ketone **4** (1.0 g, 3.1 mmol) in THF (40 mL), at -40 °C. The crude product was percolated through silica gel using dichloromethane as eluent, obtaining compound **8** (550 mg, 1.63 mmol, 52%). ^{1}H NMR δ 1.90 (3H, s, CCH_3), 3.07 (3H, s, 2′-OCH_3), 3.81 (3H, s, 3′-OCH_3 or 4′-OCH_3), 3.90 (3H, s, 4′-OCH_3 or 3′-OCH_3), 4.75 (1H, br s, OH), 6.64 (1H, d, *J* = 8.8, H5′),7.27 (1H, d, *J* = 8.8, H6′), 7.40–7.47 (3H, m, H3, H6, H7), 7.73–7.84 (3H, m, H4, H5, H8), 7.89 (1H, s, H1). ^{13}C NMR δ 30.2 (CH_3), 56.0 (CH_3), 60.1 (CH_3), 60.6 (CH_3), 76.2 (C), 106.4 (CH), 121.0 (CH), 123.0 (CH), 124.1 (CH), 125.6 (CH), 126.0 (CH), 127.6 (2×) (CH), 128.1 (CH), 132.3 (C), 133.2 (C), 133.4 (C), 142.9 (C), 148.2 (C), 151.8 (C), 153.6 (C). IR (cm^{-1}) 1599, 3513. HRMS *m*/*z* found 361.1436; calcd for $C_{21}H_{22}O_4$ (+Na) 361.1410.

4.1.14. 1-[(2-Naphthyl)(3,4,5-trimethoxyphenyl)methyl]-1*H*-imidazole (9)

To a solution of compound **1** (100 mg, 0.30 mmol) in CH_2Cl_2 (2.0 mL), imidazole (84 mg, 1.2 mmol), triphenylphosphine (79 mg, 0.30 mmol) and iodine (312 mg, 1.23 mmol) were added. The reaction mixture was stirred at room temperature, under argon, for 8 h. It was diluted with CH_2Cl_2 and washed with 10% sodium thiosulfate and water. The organic layer was dried over Na_2SO_4 and the solvent evaporated. The crude product was chromatographed on silica using CH_2Cl_2/MeOH (9:1) as eluent, obtaining imidazole derivative **9** (30 mg, 0.080 mmol, 27%). ^{1}H NMR δ 3.73 (6H, s, 3′-OCH_3, 5′-OCH_3), 3.86 (3H, s, 4′-OCH_3), 6.35 (2H, s, H2′, H6′), 6.62 (1H, br s, $(Ar)_2$-CH-im), 6.92 (1H, br s, H3 im or H4 im), 7.14 (1H, br s, H4 im or H3 im), 7.25 (1H, dd, J_1 = 8.6, J_2 = 1.8, H3), 7.46 (1H, m, H6 or H7), 7.48 (1H, s, H1), 7.52 (1H, s, H2 im), 7.52 (1H, m, H7 or H6), 7.63 (1H, m, H5 or H8), 7.78 (1H, m, H8 or H5), 7.84 (1H, d, J = 8.6, H4). ^{13}C NMR δ 56.2 (2×) (CH_3), 60.8 (CH_3), 65.3 (CH), 105.4 (2×) (CH), 119.4 (CH), 125.5 (CH), 126.7 (CH), 127.7 (CH), 128.1 (CH), 128.4 (CH), 128.7 (CH), 129.0 (CH), 131.9 (CH), 132.1 (CH), 133.0 (C), 133.1 (C), 134.3 (C), 136.3 (C), 137.4 (C), 153.6 (2×) (C). IR (cm^{-1}) 1592, 1713. HRMS m/z found 397.1504; calcd for $C_{23}H_{22}N_2O_3$ (+Na) 397.1523.

4.1.15. (*Z* + *E*)-(2-Naphthyl)(3,4,5-trimethoxyphenyl) methanone oxime (10)

According to general method D, to a solution of ketone **3** (260 mg, 0.81 mmol) in methanol (15 mL) were added hydroxylamine (535 mg, 8.1 mmol) and pyridine (2 drops), yielding oxime **10** (250 mg, 0.74 mmol, 91%) as a 1:1 mixture of *Z* and *E* isomers. ^{1}H NMR δ 3.74 (6H, s, 3′-OCH_3, 5′-OCH_3, *Z* or *E* isomer), 3.83 (6H, s, 3′-OCH_3, 5′-OCH_3, *E* or *Z* isomer), 3.89 (3H, s, 4′-OCH_3, *Z* or *E* isomer), 3.96 (3H, s, 4′-OCH_3, *E* or *Z* isomer), 6.70 (2H, s, H2′, H6′, *Z* isomer), 6.76 (2H, s, H2′, H6′, *E* isomer), 7.46–7.58 (6H, m, H3, H6, H7), 7.78–7.95 (8H, m, H1, H4, H5, H8). ^{13}C NMR δ 56.2 (4×) (CH_3), 61.0 (2×) (CH_3), 105.5 (2×) (CH), 106.6 (2×) (CH), 124.4 (CH), 126.5 (2×) (CH), 126.8 (CH), 127.0 (2×) (CH), 127.9 (4×) (CH), 128.2 (CH), 128.6 (3×) (CH), 129.2 (C), 130.0 (C), 133.5 (C), 133.6 (C), 143.5 (C), 144.0 (C), 153.1 (4×) (C), 158.0 (2×) (C). IR (cm^{-1}) 1852, 3419. HRMS m/z found 360.1212; calcd for $C_{20}H_{19}NO_4$ (+Na) 360.1206.

4.1.16. (*Z* + *E*)-(2-Naphthyl)(2,3,4-trimethoxyphenyl) methanone oxime (11)

According to general method D, to a solution of ketone **4** (500 mg, 1.55 mmol) in methanol (25 mL) were added hydroxylamine (1.02 g, 15.5 mmol) and pyridine (3 drops), yielding oxime **11** (448 mg, 1.33 mmol, 86%) as a 2:1 mixture of *Z* and *E* isomers. ^{1}H NMR (DMSO) δ 3.17 (3H, s, 2′-OCH_3, *E* isomer), 3.57 (3H, s, 2′-OCH_3, *Z* isomer), 3.67 (3H, s, 3′-OCH_3 or 4′-OCH_3, *E* isomer), 3.77 (3H, s, 3′-OCH_3 or 4′-OCH_3, *Z* isomer), 3.82 (3H, s, 4′-OCH_3 or 3′-OCH_3, *E* isomer), 3.85 (3H, s, 4′-OCH_3 or 3′-OCH_3, *Z* isomer), 6.83 (1H, d, J = 8.4, H5′, *Z* isomer), 6.87 (1H, d, J = 8.4, H5′, *E* isomer), 6.91 (1H, d, J = 8.4, H6′, *Z* isomer), 7.13 (1H, d, J = 8.4, H6′, *E* isomer), 7.44–7.56 (6H, m, H3, H6, H7), 7.80–7.90 (8H, m, H1, H4, H5, H8). ^{13}C NMR δ 55.9 (2×) (CH_3), 60.4 (4×) (CH_3), 107.6 (CH), 107.9 (CH), 123.4 (2×) (CH), 124.0 (2×) (CH), 124.9 (CH), 126–128 (11×) (CH) and (2×) (C), 132.4 (C), 132.6 (2×) (C), 133.0 (C), 134.4 (2×) (C), 141.9 (2×) (C), 150.7 (C), 151.8 (C), 153.1 (C), 153.5 (C), 153.7 (C), 154.3 (C). HRMS m/z found 360.1239; calcd for $C_{20}H_{19}NO_4$ (+Na) 360.1206.

4.1.17. (*Z* + *E*)-(2-Naphthyl)(3,4,5-trimethoxyphenyl) methanone acetoxime (12)

Following general method C, oxime **10** (350 mg, 1.04 mmol) in pyridine (900 μL) was acetylated with acetic anhydride (900 μL, 9.45 mmol), yielding acetoxime **12** (340 mg, 0.90 mmol, 87%) as a mixture 1:1 of *Z* and *E* isomers. ^{1}H NMR δ 2.05 (3H, s, CH_3CO, *Z* or *E* isomer), 2.17 (3H, s, CH_3CO, *E* or *Z* isomer), 3.75 (6H, s, 3′-OCH_3, 5′-OCH_3, *Z* or *E* isomer), 3.81 (6H, s, 3′-OCH_3, 5′-OCH_3, *E* or *Z* isomer), 3.88 (3H, s, 4′-OCH_3, *Z* or *E* isomer), 3.96 (3H, s, 4′-OCH_3, *E* or *Z* isomer), 6.59 (2H, s, H2′, H6′, *Z* isomer), 6.84 (2H, s, H2′, H6′, *E* isomer), 7.42–7.58 (6H, m, H3, H6, H7), 7.75–7.95 (8H, m, H1, H4, H5, H8). ^{13}C NMR δ 19.7 (CH_3), 19.9 (CH_3), 56.3 (4×) (CH_3), 61.0 (2×) (CH_3), 106.4 (2×) (CH), 106.8 (2×) (CH), 125.0 (CH), 126.0 (CH), 126.6 (CH), 126.8 (CH), 127.5 (CH), 127.8 (CH), 127.9 (4×) (CH), 128.3 (CH), 128.6 (CH), 128.8 (CH), 130.0 (2×) (C), 130.2 (C), 130.4 (CH), 132.1 (C), 132.6 (C), 132.8 (C), 133.7 (C), 134.5 (C), 139.0 (C), 140.8 (C), 153.1 (4×) (C), 164.5 (C), 164.9 (C), 168.7 (C), 168.9 (C). IR (cm^{-1}) 1577, 1653, 1741. HRMS m/z found 402.1326; calcd for $C_{22}H_{21}NO_5$ (+Na) 402.1312.

4.1.18. (*Z*)-(2-Naphthyl)(2,3,4-trimethoxyphenyl)methanone acetoxime (13*Z*)

Following general method C, oxime **11** (200 mg, 0.59 mmol) in pyridine (600 μL) was acetylated with acetic anhydride (600 μL, 6.35 mmol), yielding acetoxime **13** (180 mg, 85%) as a 2:1 mixture of *Z* and *E* isomers. This mixture was subjected to chromatography on silica using CH_2Cl_2 as eluent, only allowing the isolation of isomer *Z* (40 mg, 0.11 mmol, 19%). ^{1}H NMR δ 2.13 (3H, s CH_3CO), 3.69 (3H, s, 2′-OCH_3), 3.91 (3H, s, 3′-OCH_3 or 4′-OCH_3), 3.94 (3H, s, 4′-OCH_3 or 3′-OCH_3), 6.76 (1H, d, J = 8.6, H5′), 6.86 (1H, d, J = 8.6, H6′), 7.45–7.51 (2H, m, H6, H7), 7.75–7.92 (4H, m, H1, H4, H5, H8), 7.89 (1H, dd, J_1 = 8.6, J_2 = 1.8, H3). ^{13}C NMR δ 20.0 (CH_3), 56.2 (CH_3), 61.1 (2×) (CH_3), 107.1 (CH), 123.8 (CH), 124.6 (CH), 126.5 (CH), 127.5 (CH), 127.9 (CH), 128.3 (CH), 128.9 (CH), 129.6 (CH), 132.3 (C), 132.9 (C), 134.6 (C), 142.2 (C), 147.7 (C), 151.4 (C), 155.0 (C), 162.4 (C), 169.0 (C). IR (cm^{-1}) 1652, 1761. HRMS m/z found 402.1312; calcd for $C_{22}H_{21}NO_5$ (+Na) 402.1311.

4.1.19. (*Z* + *E*)-(2-Naphthyl)(3,4,5-trimethoxyphenyl) methanone *tert*-butoxycarbonyloxime (14)

Di-*tert*-butyl dicarbonate (196 mg, 0.90 mmol) and Na_2CO_3 (300 mg, 2.83 mmol) were added to a solution of oxime **10** (300 mg, 0.90 mmol) in 1:1 dioxane/water (14 mL). The reaction mixture was stirred at room temperature for 48 h. After extraction with dichloromethane (3×), the combined organic layers were dried (Na_2SO_4) and the solvent evaporated to yield *tert*-butoxycarbonyloxime **14** (290 mg, 0.66 mmol, 74%) as a 1:1 mixture of *Z* and *E* isomers. ^{1}H NMR δ 1.53 (18H, s, $(CH_3)_3C$), 3.77 (6H, s, 3′-OCH_3, 5′-OCH_3, *Z* or *E* isomer), 3.82 (6H, s, 3′-OCH_3, 5′-OCH_3, *E* or *Z* isomer), 3.89 (3H, s, 4′-OCH_3, *Z* or *E* isomer), 3.97 (3H, s, 4′-OCH_3, *E* or *Z* isomer), 6.62 (2H, s, H2′, H6′, *Z* isomer), 6.83 (2H, s, H2′, H6′, *E* isomer), 7.44–7.60 (4H, m, H6, H7), 7.46 (1H, dd, J_1 = 8.4, J_2 = 1.8, H3, *Z* or *E* isomer), 7.80 (1H, d, J = 8.4, H4, *E* or *Z* isomer), 7.80–7.95 (4H, m, H5, H8), 7.88 (1H, dd, J_1 = 8.4, J_2 = 1.8, H3, *E* or *Z* isomer), 7.92 (1H, d, J = 8.4, H4, *Z* or *E* isomer), 7.92 (2H, br s, H1). ^{13}C NMR δ 27.4 (3×) (CH_3), 27.8 (3×) (CH_3), 56.3 (4×) (CH_3), 61.0 (2×) (CH_3), 83.7 (C). 85.2 (C), 106.7 (4×) (CH), 125.1 (CH), 126.3 (CH), 126.6 (2×) (CH), 127.3 (CH), 127.5 (CH), 127.7 (3×) (CH), 127.9 (CH), 128.1 (CH), 128.7 (CH), 129.0 (CH), 130.1 (2×) (CH) and (2×) (C), 132.3 (2×) (C), 132.8 (2×) (C), 133.7 (2×) (C), 134.5 (2×) (C), 146.6 (2×) (C), 153.1 (4×) (C), 163.5 (2×) (C). IR (cm^{-1}) 1582, 1773. HRMS m/z found 460.1736; calcd for $C_{25}H_{27}NO_6$ (+Na) 460.1731.

4.1.20. (*Z* + *E*)-(2-Naphthyl)(2,3,4-trimethoxyphenyl) methanone *tert*-butoxycarbonyloxime (15)

To a mixture of NaH (29 mg, 1.2 mmol) in THF (15 mL), oxime **11** (200 mg, 0.56 mmol) and di-*tert*-butyl dicarbonate (118 mg, 0.54 mmol) were added. The reaction mixture was stirred at room temperature, under argon, for 8 h. The mixture was then percolated through silica, using CH_2Cl_2 as eluent. The organic layer

was washed with water and dried over Na_2SO_4, yielding *tert*-butoxycarbonyloxime **15** (200 mg, 0.46 mmol, 82%) as a 2:1 mixture of *Z* and *E* isomers. This mixture was chromatographed on silica, with hexane/ethyl acetate (95:5) as eluent, allowing the separation of the *Z* isomer **15*Z*** (20 mg, 8%). **15*Z*** ^{1}H NMR δ 1.51 (9H, s, $(CH_3)_3C$), 3.73 (3H, s, 2′-OCH_3), 3.92 (3H, s, 3′-OCH3 or 4′-OCH_3), 3.94 (3H, s, 4′-OCH_3 or 3′-OCH_3), 6.77 (1H, d, J = 8.6, H5′), 6.89 (1H, d, J = 8.6, H6′), 7.44–7.51 (2H, m, H6, H7), 7.75–7.86 (3H, m, H1, H5, H8), 7.81 (1H, d, J = 8.4, H4), 7.95 (1H, dd, J_1 = 8.4, J_2 = 1.8, H3). **15*E*** (from *Z* + *E* mixture) ^{1}H NMR δ 1.51 (9H, s, $(CH_3)_3C$), 3.27 (3H, s, 2′-OCH_3), 3.77 (3H, s, 3′-OCH3 or 4′-OCH_3), 3.91 (3H, s, 4′-OCH_3 or 3′-OCH_3), 6.73 (1H, s, H5′), 7.34 (1H, d, J = 8.6, H6′), 7.47–8.00 (6H, m, H1, H4, H5, H6, H7, H8), 7.62 (1H, dd, J_1 = 8.7, J_2 = 1.4, H3). ^{13}C NMR (*Z* + *E* mixture) δ 27.9 (6×) (CH_3), 56.1 (2×) (CH_3), 61.0 (4×) (CH_3), 83.5 (2×) (C), 107.0 (2×) (CH). 119.3 (2×) (C), 123.9 (2×) (CH), 124.6 (2×) (CH), 126.4 (2×) (CH), 127.3 (2×) (CH), 127.7 (2×) (CH), 128.1 (2×) (CH), 128.8 (2×) (CH), 129.3. (2×) (CH), 132.5 (2×) (C), 132.9 (2×) (C), 134.5 (2×) (C), 142.2 (2×) (C), 151.5 (2×) (C) , 152.4 (2×) (C), 154.9 (2×) (C), 161.3 (2×) (C). IR (cm^{-1}) 1596, 1772. HRMS *m*/*z* found 460.1735; calcd for $C_{25}H_{27}NO_6$ (+Na) 460.1731.

4.1.21. (*Z* + *E*)-(2-naphthyl)(3,4,5-trimethoxyphenyl) methanone methoxime (16)

Methoxyamine hydrochloride (84 mg, 6.4 mmol) and NaAcO·3H_2O (850 mg, 6.25 mmol) were added to a solution of ketone **3** (100 mg, 0.31 mmol) in 1:1 THF/MeOH (10 mL). The reaction mixture was stirred at room temperature for 10 days, checking by TLC until completion. The solvent was evaporated, the residue dissolved in CH_2Cl_2 and the resulting solution washed with water, dried (Na_2SO_4) and evaporated to yield methoxime **16** (75 mg, 0.21 mmol, 69%) as a 35:65 mixture of *Z* and *E* isomers. ^{1}H NMR δ 4.02 (3H, s, CH_3O–N, *E* isomer), 4.07 (3H, s, CH_3O–N, *Z* isomer), 3.79 (6H, s, 3′-OCH_3, 5′-OCH_3, *E* isomer), 3.84 (6H, s, 3′-OCH_3, 5′-OCH_3, *Z* isomer), 3.89 (3H, s, 4′-OCH_3, *E* isomer), 3.96 (3H, s, 4′-OCH_3, *Z* isomer), 6.62 (2H, s, H2′, H6′, *Z* isomer), 6.77 (2H, s, H2′, H6′, *E* isomer), 7.48–7.55 (6H, m, H3, H6, H7), 7.76–7.85 (8H, m, H1, H4, H5, H8). ^{13}C NMR δ 56.2 (4×) (CH_3), 61.0 (2×) (CH_3), 62.6 (2×) (CH_3), 105.5 (2×) (CH), 106.5 (2×) (CH), 124.6 (CH), 126.0–129.0 (13×) (CH), 130.7 (2×) (C), 132.1 (2×) (C), 132.9 (2×) (C), 133.0 (C), 133.8 (C), 139.4 (2×) (C), 153.1 (4×) (C), 156.6 (2×) (C). IR (cm^{-1}) 1585, 1653. HRMS *m*/*z* found 374.1364; calcd for $C_{21}H_{21}NO_4$ (+Na) 374.1363.

4.1.22. (*Z* + *E*)-(2-naphthyl)(3,4,5-trimethoxyphenyl) methanone hydrazone (17)

Following general method E, ketone **3** (200 mg, 0.62 mmol) in methanol (15 mL) reacted with excess hydrazine in the presence of acetic acid (2 drops) to give hydrazone **17** (200 mg, 0.59 mmol, 95%) as a 6:4 mixture of *Z* and *E* isomers. ^{1}H NMR δ 3.74 (6H, s, 3′-OCH_3, 5′-OCH_3, *E* isomer), 3.86 (6H, s, 3′-OCH_3, 5′-OCH_3, *Z* isomer), 3.86 (3H, s, 4′-OCH_3, *E* isomer), 3.96 (3H, s, 4′-OCH_3, *Z* isomer), 6.52 (2H, s, H2′, H6′, *Z* isomer), 6.76 (2H, s, H2′, H6′, *Z* isomer), 7.30–7.50 (6H, m, H3, H6, H7), 7.53 (1H, br s, H1, *E* isomer), 7.70–8.00 (7H, m, H1, *Z* isomer, H4, H5; H8). IR (cm^{-1}) 1588, 3403. HRMS *m*/*z* found 359.1361; calcd for $C_{20}H_{20}N_2O_3$ (+Na) 359.1366.

4.1.23. (*Z*)-(2-naphthyl)(2,3,4-trimethoxyphenyl)methanone hydrazone (18*Z*)

Following general method E, ketone **4** (500 mg, 1.55 mmol) in methanol (25 mL) reacted with excess hydrazine in the presence of acetic acid (3 drops) to give the *Z* hydrazone **18*Z*** (480 mg, 1.43 mmol, 92%). ^{1}H NMR δ 3.74 (3H, s, 2′-OCH_3), 3.94 (3H, s, 3′-OCH_3 or 4′-OCH_3,), 3.95 (3H, s, 4′-OCH_3 or 3′-OCH_3,), 5.60 (2H, br s, NH_2), 6.82 (1H, d, J = 8.4, H5′), 6.88 (1H, d, J = 8.4, H6′), 7.35-7.50 (2H, m, H6, H7), 7.58 (1H, d, J = 1.8, H1), 7.68-7.81 (2H, m, H5, H8), 7.79 (1H, d, J = 8.8, H4), 8.02 (1H, dd, J_1 = 8.8, J_2 = 1.8, H3). ^{13}C NMR δ 56.2 (CH_3), 61.1 (2×) (CH_3), 108.3 (CH), 118.9 (C), 123.7 (CH), 124.6 (CH), 126.1 (3×) (CH), 127.6 (CH), 127.9 (CH), 128.4 (CH), 133.3 (2×) (C), 136.5 (C), 142.9 (C), 146.7 (C), 151.8 (C), 154.6 (C). IR (cm^{-1}) 1597, 3405. HRMS *m*/*z* found 359.1362; calcd for $C_{20}H_{20}N_2O_3$ (+Na) 359.1366.

4.1.24. (*Z* + *E*)-(2-naphthyl)(3,4,5-trimethoxyphenyl) methanone acetylhydrazone (19)

Following general method C, hydrazone **17** (200 mg, 0.60 mmol) in pyridine (600 μL) was treated with acetic anhydride to obtain acetylhydrazone **19** (170 mg, 0.45 mmol, 75%) as a 6:4 mixture of *Z* and *E* isomers. This mixture was chromatographed on silica, allowing the isolation of *Z* isomer **19*Z*** (20 mg, 0.053 mmol, 9%). **19*Z*** ^{1}H NMR δ 2.50 (3H, s, CH_3CO), 3.85 (6H, s, 3′-OCH_3, 5′-OCH_3), 3.97 (3H, s, 4′-OCH_3), 6.47 (2H, s, H2′, H6′), 7.45–7.52 (2H, m, H6, H7), 7.63 (1H, br s, H1), 7.70–7.80 (2H, m, H5, H8), 7.84 (1H, d, J = 8.4, H4), 8.10 (1H, dd, J_1 = 8.4, J_2 = 1.8, H3). **19*E*** (from *Z* + *E* mixture): 2.46 (3H, s, CH_3CO), 3.76 (6H, s, 3′-OCH_3, 5′-OCH_3), 3.87 (3H, s, 4′-OCH_3), 6.83 (2H, s, H2′, H6′), 7.28 (1H, dd, J_1 = 8.4, J_2 = 1.8, H3), 7.60 (2H, m, H6, H7), 7.70–7.90 (2H, m, H5, H8), 7.78 (1H, br s, H1), 8.02 (1H, d, J = 8.4, H4). ^{13}C NMR δ 20.8 (2×) (CH_3), 56.3 (4×) (CH_3), 61.0 (2×) (CH_3), 105.2 (4×) (CH), 123.3 (CH), 125.2 (CH), 126.6 (CH), 126.8 (2×) (C), 127.2 (CH), 127.3 (CH), 127.7 (2×) (CH), 128.1 (CH), 128.3 (3×) (CH), 128.7 (2×) (CH), 130.0 (CH), 132.6 (C), 133.0 (C), 133.4 (C), 133.7 (C), 134.0 (C), 134.3 (C), 138.8 (C), 139.5 (C), 150.1 (C), 150.3 (C), 153.2 (2×) (C), 154.6 (2×) (C), 172.8 (C), 173.0 (C). IR (cm^{-1}) 1583, 1683, 3317. HRMS *m*/*z* found 401.1464; calcd for $C_{22}H_{22}N_2O_4$ (+Na) 401.1472.

4.1.25. (*Z*)-(2-naphthyl)(2,3,4-trimethoxyphenyl)methanone acetylhydrazone (20*Z*)

Following general method C, hydrazone **18*Z*** (200 mg, 0.60 mmol) in pyridine (600 μL) was treated with acetic anhydride to obtain acetylhydrazone **20*Z*** (180 mg, 0.48 mmol, 80%). ^{1}H NMR δ 2.50 (3H, s, CH_3CO), 3.69 (3H, s, 2′-OCH_3), 3.93 (3H, s, 3′-OCH_3 or 4′-OCH_3), 3.95 (3H, s, 4′-OCH_3 or 3′-OCH_3), 6.83 (2H, s, 5′H, 6′H), 7.42–7.48 (2H, m, H6, H7), 7.62 (1H, br s, H1), 7.65–7.90 (2H, m, H5, H8), 7.83 (1H, d, J = 8.8, H4), 8.09 (1H, dd, J_1 = 8.8, J_2 = 1.8, H3), 8.45 (1H, br s, NH). ^{13}C NMR δ 20.8 (CH_3), 56.3 (CH_3), 61.1 (2×) (CH_3), 108.4 (CH), 117.4 (C), 123.4 (CH), 124.1 (CH), 126.4 (CH), 126.9 (CH), 127.7 (CH), 128.0 (CH), 128.2 (CH), 128.6 (CH), 133.0 (C), 134.0 (C), 134.9 (C), 143.0 (C), 148.1 (C), 151.5 (C), 155.6 (C), 173.0 (C). IR (cm^{-1}) 1595, 1670. HRMS *m*/*z* found 401.1477; calcd for $C_{22}H_{22}N_2O_4$ (+Na) 401.1472.

4.1.26. (*E* + *Z*)-(2-naphthyl)(3,4,5-trimethoxyphenyl) methanone methylhydrazone (21)

Methylhydrazine (286 mg, 6.2 mmol) and acetic acid (2 drops) were added to a solution of ketone **3** (200 mg, 0.62 mmol) in MeOH (20 mL). The reaction mixture was refluxed for 48 h, and in this time a large excess of methylhydrazine was added in four portions. The solvent was evaporated, the residue dissolved in CH_2Cl_2 and the resulting solution washed with water, dried (Na_2SO_4) and evaporated to yield methylhydrazone **21** (200 mg, 0.57 mmol, 92%) as a 40:60 mixture of *Z* and *E* isomers. ^{1}H NMR δ 3.03 (3H, s, CH_3NH, *E* isomer), 3.10 (3H, s, CH_3NH, *Z* isomer), 3.75 (6H, s, 3′-OCH_3, 5′-OCH_3, *E* isomer), 3.85 (9H, s, 4′-OCH_3, *E* isomer, 3′-OCH_3, 5′-OCH_3, *Z* isomer), 3.97 (3H, s, 4′-OCH_3, *Z* isomer), 5.47 (1H, br s, NH, *Z* or *E* isomer), 5.60 (1H, br s, NH, *E* or *Z* isomer), 6.52 (2H, s, 2′H, 6′H, *Z* isomer), 6.77 (2H, s, 2′H, 6′H, *E* isomer), 7.33–7.46 (4H, m, H5, H8), 7.35 (1H, dd, J_1 = 8.6, J_2 = 1.8, H3, *E* isomer), 7.53 (1H, br s, H1, *E* isomer), 7.75 (1H, br s, H1, *Z* isomer), 7.77–8.12 (4H, m, H6, H7), 7.78 (1H, d, J = 8.6, H4, *Z* isomer), 7.99 (1H, d, J = 8.6, H4, *E* isomer), 8.08 (1H, dd, J_1 = 8.6, J_2 = 1.8, H3, *Z* iso-

mer). ^{13}C NMR δ 38.1 (2×) (CH_3), 56.2 (4×) (CH_3), 61.0 (2×) (CH_3), 103.8 (2×) (CH), 105.8 (2×) (CH), 123.7 (CH), 126.0 (CH), 126.1 (CH), 126.2 (2×) (CH), 126.5 (CH), 126.7 (CH), 127.0 (CH), 127.7 (CH), 128.0 (CH), 128.3 (2×) (CH), 128.8 (CH), 129.0 (C), 129.4 (CH), 130.9 (C), 133.1 (C), 133.3 (2×) (C), 133.6 (C), 134.5 (C), 136.1 (C), 138.1 (C), 139.0 (C), 144.8 (2×) (C), 153.1 (2×) (C), 154.4 (2×) (C). HRMS *m*/*z* found 373.1517; calcd for $C_{21}H_{22}N_2O_3$ (+Na) 373.1523.

4.1.27. (*E*)-(2-naphthyl)(2,3,4-trimethoxyphenyl)methanone oxime (10*E*), *N*-[(2-naphthyl)(3,4,5-trimethoxyphenyl) methyl]acetamide (22) and *N*-(2-naphthyl)-*N*-(3,4, 5-trimethoxybenzyl)acetamide (23)

A solution of oxime **10** (200 mg, 0.59 mmol) in THF was treated with $LiAlH_4$ (236 mg, 5.90 mmol) at room temperature for 2 h. The reaction mixture was filtered through silica and the solvent evaporated. The crude product was acetylated according to general method C, by treatment with acetic anhydride (600 μL, 6.35 mmol) in pyridine (600 μL). The reaction product was then chromatographed on silica, using hexane/ethyl acetate as eluent, yielding 60 mg (0.16 mmol, 28%) of acetamide **23**, 20 mg (0.053 mmol, 9%) of acetoxime **12*E*** and 10 mg (0.027 mmol, 5%) of acetamide **22**.

Compound ***12E***. ^{1}H NMR δ 2.06 (3H, s, CH_3CO), 3.77 (6H, s, 3′-OCH_3, 5′-OCH_3), 3.89 (3H, s, 4′-OCH_3), 6.84 (2H, s, H2′, H6′), 7.42-7.65 (4H, m, H6, H7), 7.44 (1H, br d, *J* = 8.4, H3), 7.81-7.91 (3H, m, H4, H5, H8), 7.95 (1H, br s, H1). ^{13}C NMR δ 20.0 (CH_3), 56.3 (2×) (CH_3), 61.0 (CH_3), 106.8 (2×) (CH), 126.0 (CH), 126.8 (CH), 127.4 (CH), 127.9 (2×) (CH), 128.6 (CH), 128.8 (CH), 130.0 (C), 130.1 (C), 132.6 (C), 133.7 (C), 140.8 (C), 153.1 (2×) (C), 164.9 (C), 168.7 (C). IR (cm^{-1}) 1573, 1653, 1769. HRMS *m*/*z* found 402.1302; calcd for $C_{22}H_{21}NO_5$ (+Na) 402.1312.

Compound ***22***. ^{1}H NMR δ 2.10 (3H, s, CH_3CO), 3.76 (6H, s, 3′-OCH_3, 5′-OCH_3), 3.83 (3H, s, 4′-OCH_3), 6.23 (1H, br d, *J* = 8.0, NH), 6.34 (1H, d, *J* = 8.0, AcNH-C*H*), 6.48 (2H, s, H2′, H6′), 7.32 (1H, dd, J_1 = 8.4, J_2 = 1.8, H3), 7.45-7.55 (2H, m, H6, H7), 7.68 (1H, br s, H1), 7.75–7.90 (3H, m, H4, H5, H8). ^{13}C NMR δ 23.5 (CH_3), 56.2 (2×) (CH_3), 60.9 (CH_3), 57.3 (CH), 104.9 (2×) (CH), 125.6 (CH), 125.9 (CH), 126.2 (CH), 126.4 (CH), 127.7 (CH), 128.0 (CH), 128.6 (CH), 132.8 (C), 133.3 (C), 137.2 (C), 137.3 (C), 138.6 (C), 153.5 (2×) (C), 169.2 (C). IR (cm^{-1}) 1592, 1651, 3287. HRMS *m*/*z* found 388.1504; calcd for $C_{22}H_{23}NO_4$ (+Na) 388.1519.

Compound ***23***. ^{1}H NMR δ 1.91 (3H, s, CH_3CO), 3.71 (6H, s, 3′-OCH_3, 5′-OCH_3), 3.81 (3H, s, 4′-OCH_3), 4.87 (1H, s, CH_2–N), 6.42 (2H, s, H2′, H6′), 7.09–7.53 (2H, m, H6, H7), 7.11 (1H, br d, *J* = 8.0, H3), 7.73–7.85 (4H, m, H1,H4, H5, H8). ^{13}C NMR δ 22.9 (CH_3), 56.1 (2×) (CH_3), 60.9 (CH_3), 106.2 (2×) (CH), 126.2 (CH), 126.9 (3×) (CH), 127.8 (2×) (CH), 129.7 (CH), 132.5 (C), 133.1 (C), 133.6 (C), 137.4 (C), 140.2 (C), 153.1 (2×) (C), 170.6 (C). IR (cm^{-1}) 1660, 1714. HRMS *m*/*z* found 388.1515; calcd for $C_{22}H_{23}NO_4$ (+Na) 388.1519.

4.2. Biological evaluation

4.2.1. Cytotoxicity. XTT procedure

One hundred micro litres 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 (3,3̀-[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} was then calculated as the drug concentration causing a 50% inhibition of cell proliferation. At least six experiments were carried out for each compound assayed.

4.2.2. Isolation of microtubular protein

Calf brain microtubule protein (MTP) was purified by two cycles of temperature-dependent assembly/disassembly, according to the method of Shelanski,[11] modified as described in the literature.[12] Protein concentrations were determined by the method of Bradford,[13] using BSA as standard. Six different MTP preparations were used in the tubulin assembly assays.

4.2.3. Tubulin assembly

In vitro tubulin self-assembly was monitored turbidimetrically at 450 nm, using a thermostated Thermospectronic Helios λ spectrophotometer fitted with a Peltier temperature controller and a circulating water bath. The ligands were dissolved in DMSO and stored at −20 °C. The amount of DMSO in the assays was 4%. The increase in turbidity was followed simultaneously in a batch of six or seven cuvettes (containing 1.0 mg/mL MTP in 0.1 M MES buffer, 1 mM EGTA, 1 mM MgCl2, 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 then 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 polymerisation after 20 min of incubation and was determined graphically. At least three independent experiments with different MTP preparations were carried out for each compound tested.

4.2.3.1. Molecular modelling. The compounds were docked into the colchicine site of tubulin following a described protocol.[14] The X-ray structures of the tubulin complexes with podophyllotoxin and DAMA-colchicine were retrieved from the protein data bank[15], 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.[16] We initially restrained the backbone, and then it was set free. The relaxed structures were superimposed and a combined site was generated by shifting the tubulin–podophyllotoxin complex 30 Å along the *X* axes.[17] The combined tubulin sites and the podophyllotoxin and DAMA-colchicine ligands were used to generate a combined protomol with the Surflex docking program.[18] The individual sites were also used in separate docking experiments with AutoDock 3.[19] The synthesised compounds together with roughly 300 combretastatins and phenstatin analogues were manually constructed in silico[20] and docked into the combined sites (cross-docking), in an attempt to better reproduce the receptor flexibility by using different configurations of the protein.[21] Additionally, the test set of ACD compounds used in the Surflex validation were equally docked.[18] These compounds were considered a negative control group: that is, lacking biological activity. The combined results were analysed by receiver operating characteristics (ROCs);[22] 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.[23]

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