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Original article

Discovery of azaisoerianin derivatives as potential antitumors agents

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ABSTRACT

A series of *N*-methyl-diarylamines **2** was designed and synthesized as a novel class of CA-4 and *iso*CA-4 analogues. Compounds **2b** and **2m** showed excellent antiproliferative activity with mean GI_{50} values at a nanomolar level in a diverse set of human cancer cells. These compounds also inhibited tubulin assembly at a micromolar range, arrested the cellular cycle in the G2/M phase and induced apoptosis at very low concentrations. Preliminary *in vitro* results revealed that **2b** and **2m** displayed substantial efficacy as potent antivascular agents. Docking studies indicates that these lead compounds showed a binding mode similar to those observed with *iso*CA-4 at the colchicine binding site of tubulin.

1. Introduction

Combretastatin A-4 (CA-4) a natural stilbene isolated from the African willow tree, *Combretum caffrum* in 1989 by Pettit [1] has been found to be a potent anticancer agent which strongly inhibits tubulin assembly by binding to the colchicine site. [2] CA-4 also shows potent cytotoxicity against a wide range of human cancer cells including multi drugs resistant (MDR) cancer cells [3]. Moreover, CA-4 has been demonstrated to selectively target the vascular network of tumors, inducing irreversible shutdown of blood flow to neoplastic cells [4]. Currently, two water-soluble prodrug derivatives are in clinical trials: disodium phosphate CA-4P (fosbretabulin, Zybrestat™) [5] and AVE8062 (ombrabulin) [6]. To date, CA-4P [7,8] either as a single agent or in combination therapy is undergoing several advanced clinical trials worldwide for the treatment of age-related macular degeneration or anaplastic thyroid cancer.

Despite their remarkable anticancer properties, the main problem associated with CA-4 and its prodrugs is the ready isomerization of the *Z*-double bond into the inactive *E*-form during storage, administration and metabolism [9,10]. In an ongoing project aimed at developing stable CA-4 analogues [11–19], we recently solved the stability problem of CA-4 by the discovery of the non-isomerizable *iso*combretastatin A-4 (*iso*CA-4) which holds biological activities comparable to that of CA-4 [20,21]. This structural isomer of the natural product, having a 1,1-diarylethylene scaffold, is chemically and metabolically stable [22] and easy to synthesize [23–25] at a multigrams scale without the need to control the olefin geometry. By chemical modifications on the B-ring of *iso*CA-4, we have also identified other stable and promising antiproliferative agents [21,26] such as *iso*NH_2CA-4 and *iso*FCA-4. We next have demonstrated that the reduction of the 1,1-ethylene bond of *iso*CA-4 led to (±)-*iso*erianin, in which racemic and both enantiomers displayed excellent anti-cancer activities comparable to that of the natural isomer erianin [27]. It seems that the carbon (Csp^3) linker in *iso*erianin is not involved in the interaction in the colchicine binding site. It serves only to position correctly the two aromatic rings of the molecule [27]. Herein, we planned to prepare a new class of *iso*erianin derivatives, named aza*iso*erianins (Fig. 1), in which the two aromatic

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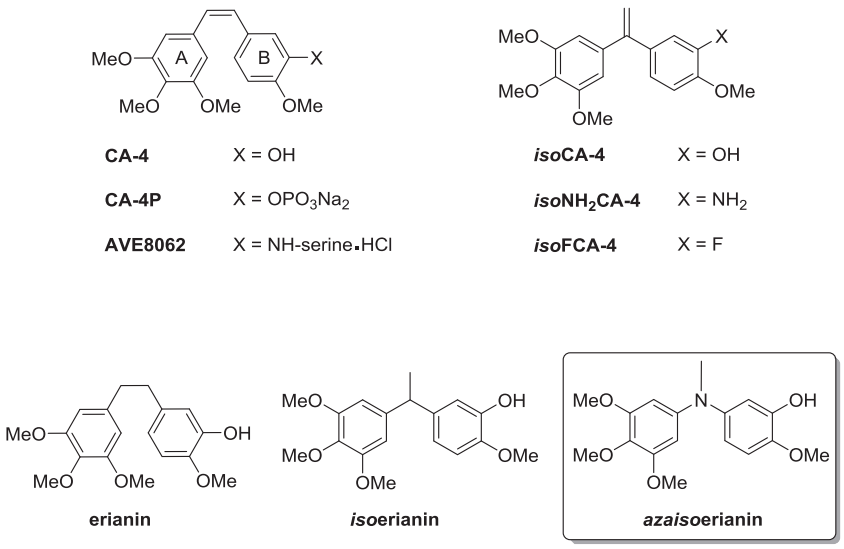


Fig. 1. Structures of CA-4 and synthetic prodrugs, *iso*CA-4 and derivatives, erianin, *iso*erianin and targeted aza*iso*erianin.

rings are connected through a nitrogen atom in place of the carbon linker in *iso*erianin compounds. Indeed, the diphenylamine skeleton is a common privileged structure found in biologically active compounds, and compounds derived from this scaffold manifest diverse activities, including anticancer activity [28,29]. This design has several advantages as follow: (*i*) the ease in synthesis where various side-arms can be introduced on nitrogen atom in a convergent access, (*ii*) the absence of a chiral center, (*iii*) the rapid access to weakly basic and chargeable derivatives in a solubilization strategy, (*iv*) the discovery of a third generation of candidates having promising antiproliferative and antitubulin activities and a better understanding of structure–activity relationships (SAR). In this article are described the synthesis and the biological evaluation of novel aza*iso*erianin derivatives **2** and **3**. Preliminary *in vitro* efficacy of these compounds in terms of cytotoxicity, inhibition of tubulin assembly, cell cycle, apoptosis and *in vitro* antivascular activity is described. The possible binding mode of the most bioactive substrates **2b** and **2m** on tubulin is also reported.

2. Results and discussion

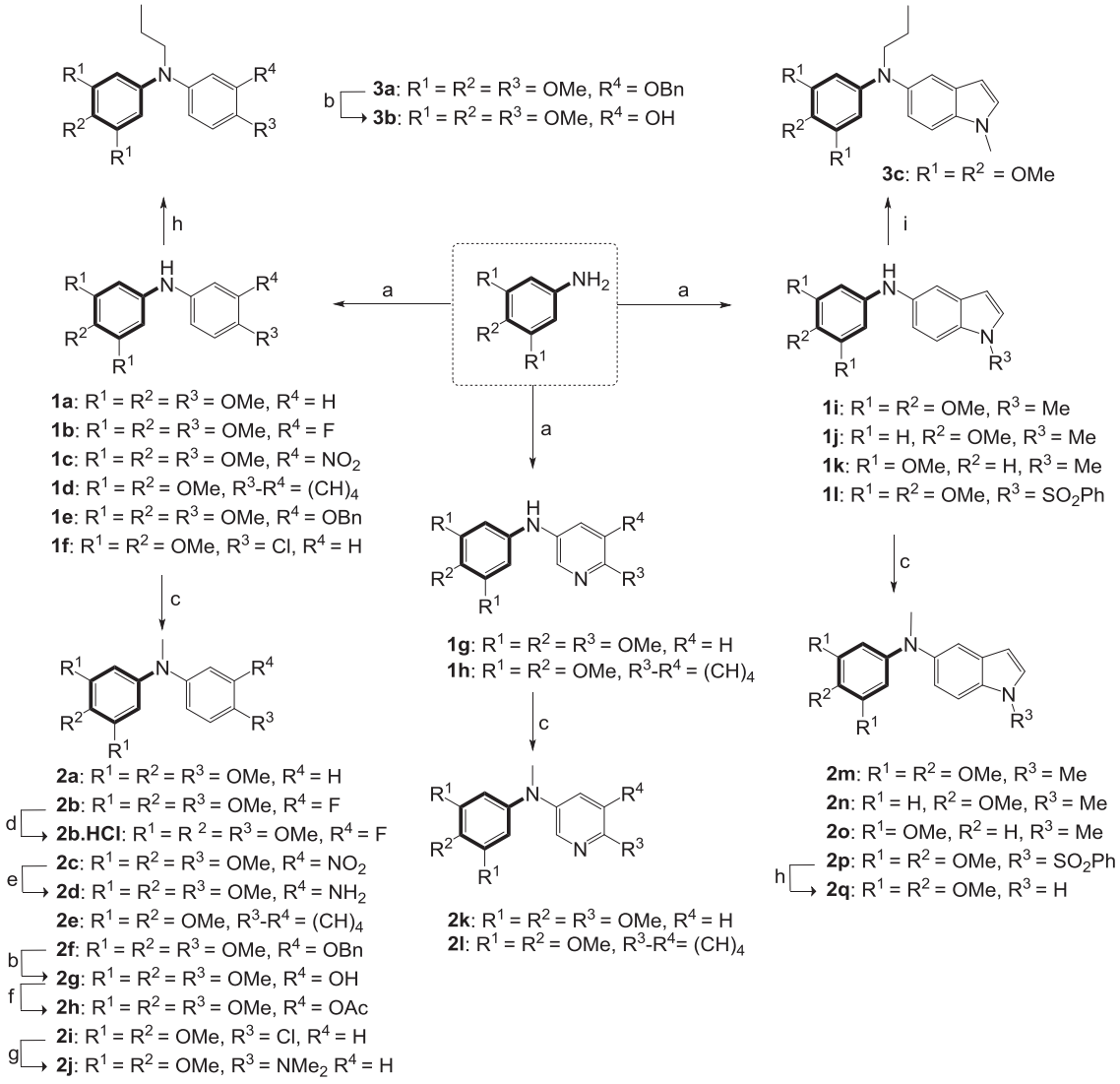
2.1. Chemistry

The synthesis of aza*iso*erianin derivatives **2a**–**q** and **3a**–**c**, is summarized in Scheme 1. Diarylanilines **1a**–**l** were prepared from commercially available methoxylated anilines which were coupled in a sealed tube with a variety of (hetero)aryl halides under our previously reported conditions ($Pd(OAc)_2$ as the catalyst, Xantphos as the ligand, Cs_2CO_3 as the base in hot dioxane) [30–33]. Treatment of the resulting diarylamines **1** with sodium hydride followed by reaction with MeI gave the best results for the *N*-alkylation process furnishing the desired trisubstituted amines **2**. By analogy with highly cytotoxic *N*-propylbenzylanilines, [34] *N*-propyl derivatives **3** were prepared using a large excess of *n*PrBr as the alkylating agent and NaH as the base. Reduction of the nitro group of **2c** using HCl and iron powder yielded aza*iso*erianin derivative **2d** with 3-amino-4-methoxy groups on the B-ring identical to the B-ring of AVE8062 drug. Debenzylation of the phenol function of **2f** was carried out by hydrogenation to afford aza*iso*erianin **2g** having the greatest resemblance with *iso*erianin and *iso*CA-4. Compound **2j** was synthesized from 4-chloro derivative **2i** using dimethylamine in the presence of $Pd_2(dba)_3.CHCl_3$ (5 mol%), and Johnphos (10 mol%) as the ligand and *t*BuONa as the base in hot toluene [35]. Finally, the removal of the *N*-protected tosyl group of **2p** was accomplished by the use of TBAF in DMF at 100 °C to afford **2q** (49%).

2.2. Biological results

New synthesized aza*iso*erianin compounds were evaluated for their cytotoxic effects against HCT116 human carcinoma cell line and for tubulin polymerization inhibitory activity. *Iso*erianin [27], *iso*CA-4 [21] and CA-4 [36] were included as reference compounds. The cytotoxicity and inhibition of tubulin polymerization (ITP) results are presented in Table 1. Without any exception, all secondary diarylamines **1** were not cytotoxic (data not shown).

In the series of compounds **2** having a benzene substituted B-ring, our findings showed that compound **2b**, having a fluorine atom at the C3′-position, displayed the best cytotoxicity with a GI_{50} values of 20 nM comparable to that of the reference *iso*erianin. Additionally, the water-soluble hydrochloride salt **2b**-HCl displayed a high level of cytotoxicity against HCT116 cells (GI_{50} = 28 nM) comparable to that of the parent molecule **2b** (GI_{50} = 22 nM). Replacement of the F atom with H or a NH_2 on the C3′-position generated compounds **2a** and **2d** (GI_{50} = 64 nM and 66 nM, respectively) which were three fold less active than **2b**. Contrary to our expectations, a dramatic loss of antiproliferative activity was observed with aza*iso*erianin **2g**, having a OH group on C3′-position.This compound and its corresponding acetate **2h** displayed only modest cytotoxicity against HCT116 cells (GI_{50} = 650–700 nM). We next showed that the introduction of a pyridine or a quinoline nucleus as B-ring resulted in products **2k** (GI_{50} = 650 nM) and **2l** (GI_{50} = 2100 nM), respectively, with a significant loss of potency. We next investigated the role on cytotoxicity of derivatives having an indole nucleus which was found useful as B-ring in the CA-4 series [37]. It is significant to note that in the aza*iso*erianin series, *N*-methyl-indol-5-yl compound **2m** was consistently the more potent compound. This lead compound (**2m**) was about four times more cytotoxic than the reference *iso*erianin with a low GI_{50} value of 7 nM. The absence of the trimethoxyphenyl-ring in the 5-indoyl series reduced the cytotoxicity as it was observed with



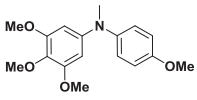
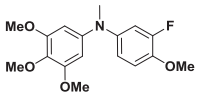
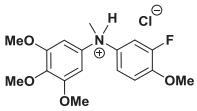
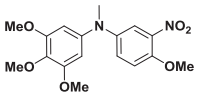
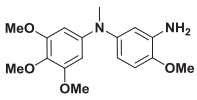
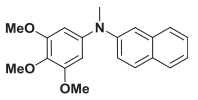
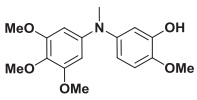
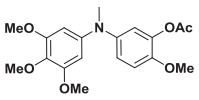
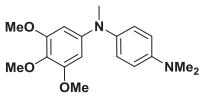
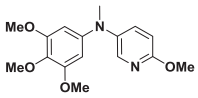
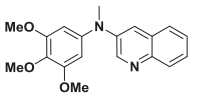
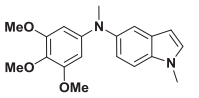
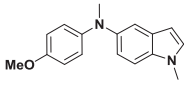
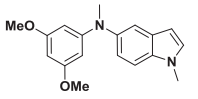
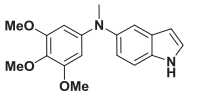
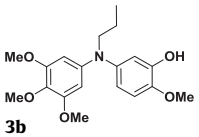
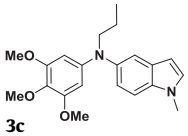
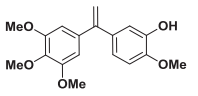
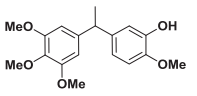
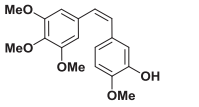
[a]Reagents and conditions: (a) $Pd(OAc)_2$, Xantphos, ArX, Cs_2CO_3, dioxane, 100-130 °C, sealed tube. (b) H_2, Pd/C, AcOEt. (c) NaH, MeI, DMF, RT. (d) HCl, MeOH, Et_2O, RT. (e) Fe, HCl, EtOH/H_2O. (f) Pyridine, DMAP, $(Ac)_2O$, CH_2Cl_2. (g) $Pd_2(dba)_3CHCl_3$, *t*-Bu_2P(*o*-biphenyl), $NHMe_2$, *t*BuONa, toluene, 100 °C. (h) TBAF, DMF, 100 °C. (i) NaH, *n*PrBr, DMF, RT.

Scheme 1. Synthesis of compounds **1**–**3**.

compounds **2n** (GI_{50} = 1100 nM) and **2o** (GI_{50} = 70 nM). Contrary to other observations, [37] the *N*-methyl substitution of the indole moiety in **2m** plays an important role for the antiproliferative activity. Indeed, its des-methyl analogue **2q** showed a 25-fold reduction in cytotoxicity. Finally, a marked loss in cytotoxicity was observed with compounds **3b** (GI_{50} = 1400 nM) and **3c** (GI_{50} = 200 nM) having a *N*-propyl group in comparison with their *N*-methyl analogues (e.g. **3c** *vs* **2m**; **3b** *vs* **2g**). To further characterize the cytotoxicity profiles of these compounds, we next investigated the effect of the most active substances **2a**, **2b**, **2d** and **2m** on the proliferation of three tumor cell lines (hormone-independent breast cancer (MDA-MB231)), human glioblastoma (U87-MG), myelogenous leukemia (K562) and on the normal primary human umbilical vein endothelial (HUVEC). As shown in Table 2, all examined compounds displayed very potent activity against all cell lines tested, in the nanomolar range. Again, compound **2b** and indole **2m** displayed the highest level of cytotoxicity with GI_{50} values ranging from 2 to 39 nM.

To investigate whether compounds **2** and **3** exert their activities by interacting with microtubules, their effect on the *in vitro* polymerization of purified tubulin was next examined (see Table 1). It is of interest to note that highly cytotoxic derivatives **2b** and *N*-methylindole **2m** were also very potent as tubulin inhibitors and were comparable to *iso*erianin with IC_{50} μM values (2.7 μM and 1.3 μM for **2b** and **2m**, respectively). Interestingly, derivative **2g**, having the greatest resemblance to *iso*CA-4 and *iso*erianin which was poorly cytotoxicity against HCT116 cells was one of the more potent agent as tubulin inhibitor with an IC_{50} value of 1.5 μM. Similarly, *N*-propyl derivatives **3b** and **3c** which displayed a modest level of cytotoxicity were very potent in the tubulin assay with IC_{50} values ranging from 2.8 to 3.6 μM. These results showed an important discrepancy between the potency of antitubulin activity and cytotoxicity of *N*-propyl derivatives **3**, indicating that with hindered substituents on the nitrogen atom of derivatives **3**, it might be possible to design news series of antivascular agents with low cytotoxicity.

Table 1
Cytotoxicity against HCT 116 cells[a] and ITP of selected compounds **2** and **3**.

				
Compound	**2a**	**2b**	**2b.HCl**	**2c**
Cytotoxicity GI_{50}[b] [nM]	64 ± 4	20 ± 1.5	28 ± 2	190 ± 15
ITP IC_{50}[c] [μM]	5.5 ± 0.5	2.7 ± 0.2	3 ± 0.2	28 ± 2
				
Compound	**2d**	**2e**	**2g**	**2h**
Cytotoxicity GI_{50}[b] [nM]	66 ± 5	170 ± 12	650 ± 55	700 ± 55
ITP IC_{50}[c] [μM]	3.0 ± 0.2	14 ± 1.8	1.5 ± 0.2	8.0 ± 0.5
				
Compound	**2j**	**2k**	**2l**	**2m**
Cytotoxicity GI_{50}[b] [nM]	250 ± 25	650 ± 62	2100 ± 180	7 ± 0.6
ITP IC_{50}[c] [μM]	18 ± 1.2	NA[d]	NA[d]	1.3 ± 0.1
				
Compound	**2n**	**2o**	**2q**	**3b**
Cytotoxicity GI_{50}[b] [nM]	1100 ± 120	70 ± 5	180 ± 14	1400 ± 145
ITP IC_{50}[c] [μM]	NA[d]	3.4 ± 0.5	NA[d]	2.8 ± 0.3
				
Compound	**3c**	***iso*CA-4**	***iso*erianin**	**CA-4**
Cytotoxicity IC_{50}[b] [nM]	200 ± 16	2 ± 0.2[e]	28 ± 2[e]	2 ± 0.2[e]
ITP IC_{50}[c] [μM]	3.6 ± 0.5	2.0 ± 0.3	3.0 ± 0.2[e]	1.0 ± 0.2[e]

[a] HCT116 Human colon carcinoma.
[b] GI_{50} is the concentration of compound needed to reduce cell growth by 50% following 72 h cell treatment with the tested drug (average of three experiments).
[c] IC_{50} is the concentration of compound required to inhibit 50% of the rate of microtubule assembly (average of three experiments).
[d] NA not active.
[e] The GI_{50} values for *iso*CA-4, *iso*erianin and CA-4 were determined in this study.

To gain further insight the mechanism of action of aza*iso*erianin derivatives **2**, the most cytotoxic compounds **2b** and **2m** were assayed for their effects on cell cycle distribution. K562 and HCT116 cells were treated with **2b** and **2m** at different concentrations for 24 h. The results presented in Fig. 2, demonstrate that there was an accumulation of K562 and HCT116 cells in the G_2/M phase of the cellular cycle after treatment with **2b** and **2m** at very low concentrations. One note that there is a better perturbation of cycle cell progression in K562 cells after treatment with these drugs, and indolic derivative **2m** seems to be slightly more effective than **2b** in K562 and HCT116 cells.

Table 2
Cytotoxicity against human cancer cell lines, HCT116, MDA-MB231, U87-MG, K562 and HUVEC.

Compounds	Cytotoxicity (GI_{50} nM)[a]				
	HCT116[b]	MDA-MB231[c]	U87-MG[d]	K562[e]	HUVEC[f]
2a	64 ± 4	55 ± 4	30 ± 2	39 ± 4	35 ± 3
2b	20 ± 1.5	24 ± 2	7 ± 0.6	39 ± 5	22 ± 2
2d	66 ± 5	80 ± 7	20 ± 1.7	62 ± 5	25 ± 3
2m	7 ± 0.6	16 ± 1.8	2.2 ± 0.3	8 ± 0.7	7 ± 0.5
*Iso*erianin[g]	28 ± 2	35 ± 3	30 ± 3	25 ± 2	45 ± 5

[a] **GI_{50}** is the concentration of compound needed to reduce cell growth by 50% following 72 h cell treatment with the tested drug (average of three experiments).
[b] HCT116, colon carcinoma.
[c] MDA-MB-231 hormone-independent breast cancer.
[d] U87-MG Glioblastoma.
[e] K562, myelogenous leukaemia.
[f] HUVEC, Human umbilical vein endothelial cells.
[g] The GI_{50} values of *iso*erianin was determined in this study.

The ability of **2b** and **2m** to induce apoptosis in HCT116 cells was further characterized using caspase-3 and caspase-7 assays. The enzymatic activity of these caspases was measured by monitoring the cleavage of the fluorogenic substrate Z-DEVD-R110 in cancer cells. The results presented in Table 3 show a significant dose-dependent increase in proteolytic activity of caspases in the cells treated for 24 h with diarylmethylamines **2b** and **2m**. A spectacular 4-fold increase in apoptosis was evidenced by **2m** in HCT116 cells at a low concentration of 7.5 nM. These results indicate that, in addition to their antiproliferative and antitubulin effects, the treatment of cancer cells with **2b** and **2m** activate caspases system leading to programmed cell death.

To identify compounds that disrupt the neovascularization formation, an assay using endothelial colony-forming cells (ECFCs) was performed with a panel of diarylamines **2** and **3**. In the

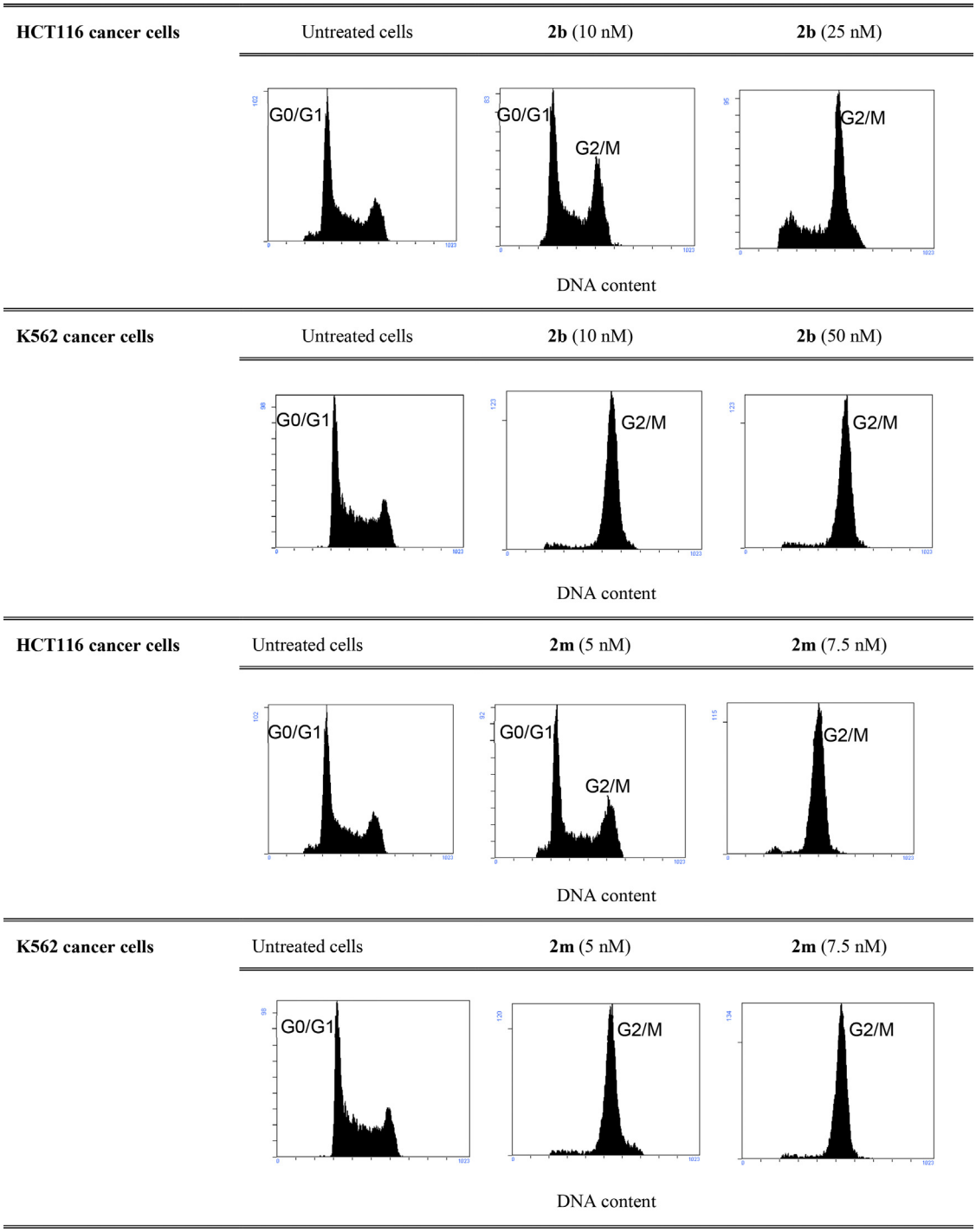


Fig. 2. Effects of **2b** and **2m** on cell cycle distribution in HCT116 and K562 cells determined by flow cytometry analysis. DNA content was assessed *via* propidium iodide staining.

presence of the vascular endothelial growth factor (VEGF), ECFCs form tube-like structures that express the endothelial marker (CD31) when co-cultured on an ADSC feeder layer [38]. The inhibition of endothelial tube formation (IC_{50}) was determined after 96 h of treatment using ECFCs, VEGF and compounds **2a-b**, **2d** and **2m** as angiogenesis inhibitors.

Compounds **2k**, **2l** having a pyridine and quinoline B-rings respectively, as well as **2n** were found to be inactive as angiogenesis inhibitors (Table 4). All other selected compounds inhibit the endothelial tube formation in a concentration-dependent manner (data not shown) with remarkable low IC_{50} values as for examples with **2b** or **2m** ($CI_{50} = 25$ and 8 nM, respectively). One can note that *N*-propyl derivatives **3b** and **3c** which displayed a low level of cytotoxicity (Table 1) inhibit angiogenesis with nanomolar IC_{50} values of 73 and 50 nM, respectively.

These results were next confirmed by a second *in vitro* model of angiogenesis [39] in which human umbilical vein endothelial cells (HUVECs), when seeded on Matrigel™, aggregate to form a reticular vascular network of capillary-like vessels. The results presented in Table 2 (see above) revealed that after 72 h of

Table 3
Apoptotic effects of **2b** and **2m** in HCT116 cells.

Rhodamine 110 relative fluorescence intensity[a] (% of control)			
2b 25 nM (300)	**2b** 50 nM (452)	**2m** 7.5 nM (393)	**2m** 15 nM (471)

[a] The results are expressed in the percentage of apoptotic cells detected following 24 h of treatment with **2b** and **2m** at different concentrations.

incubation, **2b** and **2m** exhibited good growth inhibition activity against HUVEC cells with GI_{50} values of 22 and 7 nM, respectively. To evaluate whether **2b** and **2m** could affect models of newly formed blood vessels, the HUVEC tube disruption assay was performed. Addition of **2b** at a concentration of 10 nM, and more significantly at 100 nM, rapidly disrupted the integrity of the network (Fig. 3). This effect was visible after only 3 h of treatment at doses of 10 and 100 nM which were not cytotoxic for such incubation time (data not shown). This antivascular effect is even more pronounced with indole derivative **2m** which destroyed neovascularization at a very low dose of 5 nM. All together these results suggested that aza*iso*erianin derivatives **2**, and particularly **2m** and **2b**, might be considered and evaluated as vascular disrupting agents for further *in vivo* studies.

2.3. Docking study

The biological IPT and antivascular results demonstrated that tubulin is the target of these diarylamines **2** and **3**. Molecular docking calculations were performed with **2b** and **2m** in order to investigate their possible binding mode in the colchicine binding site of tubulin. For this purpose, the X-ray structure of tubulin DAMA-colchicine complex (accession code 1SA0) [40] was used. Fig. 4 illustrates the docking-derived superimposition of **2b** and **2m**, with *iso*CA-4 (green). As expected, these diarylamines showed a binding pose matching the one calculated under the same conditions for *iso*CA-4, used as a reference compound with its A-trimethoxyphenyl ring placed in the proximity of Cys241. Additionally, the 4′-OMe substituent of **2b** accepts a stabilizing H-bond donated by a polar hydrogen atom belonging to the side-chain amide moiety of Asn101.

3. Conclusions

We designed and synthesized a series of original *N*-methyl- and *N*-propyldiarylamines **2** and **3**, respectively as aza-analogues of *iso*erianin and *iso*CA-4. High inhibition of cell growth was observed with compounds **2b** and **2m** in a range of human cancer cells. These potent drugs also inhibited tubulin assembly with micromolar IC_{50} values. Cell cycle analysis of K562 and HCT116 cells treated with nanomolar concentrations of **2b** and **2m** indicated that cells were arrested at the G2/M phase. Moreover, **2b** and **2m** induced apoptosis in HCT116 cells and destroyed *in vitro* models of neovasculature at very low doses. Docking studies revealed that these compounds adopted an orientation similar to that of *iso*CA-4 in the colchicine binding-site of tubulin.

4. Experimental

4.1. General considerations

The compounds were all identified by usual physical methods, i.e. ^{1}H NMR, ^{13}C NMR, IR, MS. ^{1}H and ^{13}C NMR spectra were measured in $CDCl_3$ with a Bruker Avance 300. ^{1}H chemical shifts are reported in ppm from an internal standard TMS or of residual chloroform (7.27 ppm). The following abbreviations are used: m (multiplet), s (singlet), d (doublet), br s (broad singlet), t (triplet), dd (doublet of doublet), td (triplet of doublet). ^{13}C chemical shifts are reported in ppm from the central peak of $CDCl_3$ (77.14). IR spectra were measured on a Bruker Vector 22 spectrophotometer (neat, cm^{-1}). Mass spectra were obtained with a LCT Micromass spectrometer. Analytical TLC was performed on Merck precoated silica gel 60F plates. Merck silica gel 60 (230–400 mesh) was used for column chromatography.

4.1.1. 3,4,5-Trimethoxy-N-(4-methoxyphenyl)-N-methylaniline *2a*

In a sealed tube and under an argon atmosphere were added successively, $Pd(OAc)_2$ (11 mg; 5 mol%), Xantphos (29 mg; 5 mol%), 1-bromo-4-methoxybenzene (187 mg; 1 mmol), 3,4,5-trimethoxyaniline (275 mg; 1.5 mmol), Cs_2CO_3 (652 mg; 2 mmol) in dioxane (2 mL). After heating at 100 °C for 12 h, the resulting suspension was cooled to room temperature and filtered through a pad of Celite eluting with ethyl acetate, and the inorganic salts were removed. The filtrate was concentrated and a rapid purification by silica gel column chromatography of the residue to eliminate the excess of 3,4,5-trimethoxyaniline gave the desired product **1a** which was immediately engaged in the alkylation step. **1a**: (75 mg, 26%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.04 (d, 2H, J = 9.0 Hz), 6.85 (d, 2H, J = 9.0 Hz), 6.15 (s, 2H), 5.52 (s, 1H), 3.79 (s, 6H), 3.76 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.1, 153.9 (2), 141.6, 136.1, 131.5, 121.8 (2), 114.7 (2), 93.7 (2), 61.1, 55.9 (2), 55.6. IR (neat) ν_{max}/cm^{-1}: 3278, 2993, 1600, 1501, 1224, 1124, 1007. MS (APCI) m/z: 290 $[M + H]^+$. To a diarylamine **1a** (50 mg; 0.17 mmol) solution in DMF (2 mL) was added at 0 °C, NaH (8 mg; 0.34 mmol), and iodomethane (48 mg; 28 μL, 0.34 mmol). The mixture was stirred at r.t. for a night and diluted in EtOAc (2 mL) and washed with aqueous $NaHCO_3$. The organic layer was dried, evaporated, and the residue was subjected to flash chromatography to give **2a** as a yellow oil (35 mg, 67%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.05 (d, 2H, J = 8.9 Hz), 6.88 (d, 2H, J = 8.9 Hz), 6.05 (s, 2H), 3.81 (s, 3H), 3.79 (s, 3H), 3.76 (s, 6H), 3.25 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.9, 153.6 (2), 146.5, 142.5, 131.6, 125.1 (2), 114.8 (2), 95.1 (2), 61.2, 56.1 (2), 55.6, 41.0. IR (neat) ν_{max}/cm^{-1}: 1575, 1503, 1465, 1419, 1242, 1127. MS (APCI) m/z: 304 $[M + H]^+$. HRMS calcd for $C_{17}H_{22}NO_4$ $[M + H]^+$ 304.1549, obsd. 304.1548.

4.1.2. N-(3-Fluoro-4-methoxyphenyl)-3,4,5-trimethoxy-N-methylaniline *2b*

By following the general procedure described above for **1a**. Heating at 100 °C 3,4,5-trimethoxyaniline (275 mg; 1.5 mmol) and 4-bromo-2-fluoro-1-methoxybenzene (205 mg; 1 mmol) for 5 h gave **1b** (181 mg; 59%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.92–6.84 (m, 2H), 6.76 (d, 1H, J = 8.7 Hz), 6.22 (s, 2H), 5.45 (s, 1H), 3.87 (s, 3H),

Table 4
Inhibition of endothelial tube formation using ECFCs.

	Inhibition of endothelial colony tube formation												
Compounds (IC_{50}) nM	**2a** 56	**2b** 25	**2c** 300	**2k** 2000	**2l** NA[a]	**2m** 8	**2n** NA[a]	**2o** 67	**2q** 250	**3b** 73	**3c** 50	***iso*erianin** 70	***iso*CA-4** 7

[a] Not active.

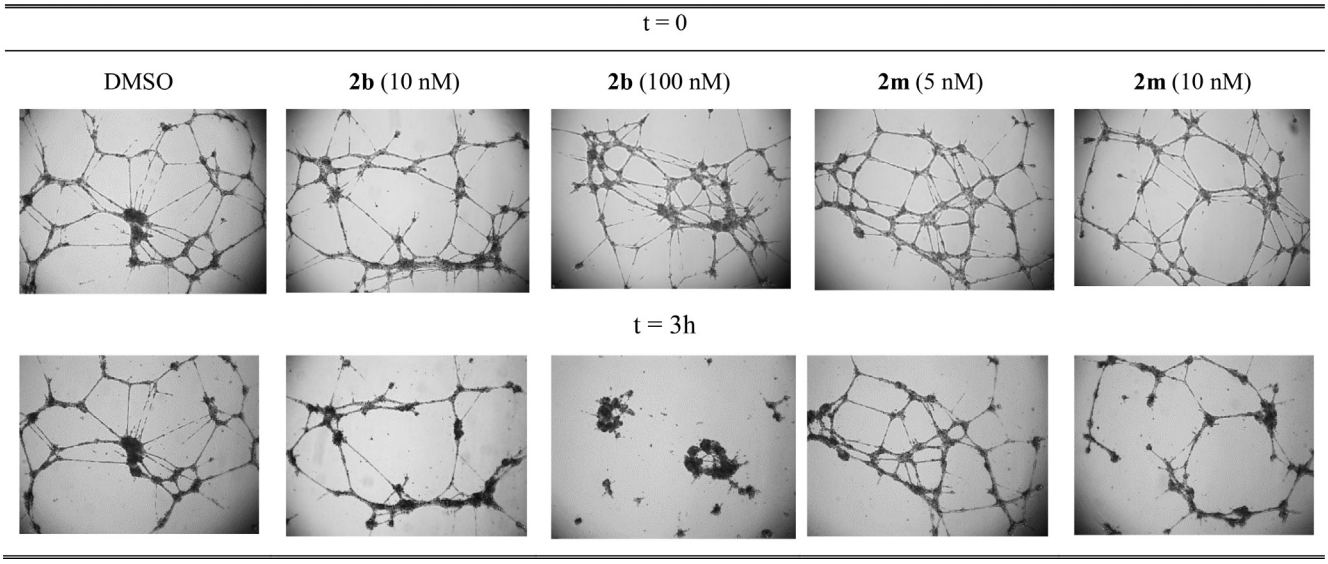


Fig. 3. Inhibition of HUVEC tube formation on a Matrigel by **2b** (at 10 and 100 nM) and **2m** (at 5 and 10 nM). Images were taken 3 h after addition of compound **2b** and **2m**.

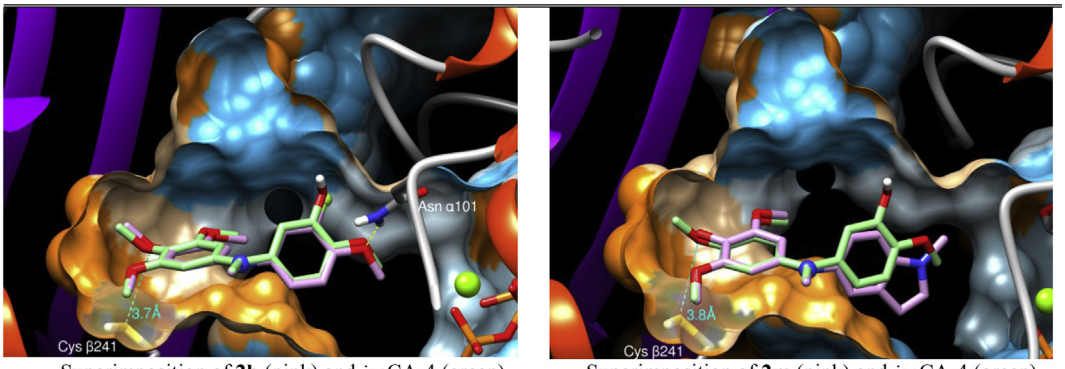
3.80 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 154.0 (2), 153.0 (d, $J = 244.5$ Hz), 142.4 (d, $J = 11.2$ Hz), 140.1, 137.6 (d, $J = 9.0$ Hz), 132.7, 115.1 (d, $J = 2.2$ Hz), 114.5 (d, $J = 2.2$ Hz), 107.8 (d, $J = 20.2$ Hz), 95.4 (2), 61.1, 57.1, 56.1 (2). ^{19}F NMR (188 MHz, $CDCl_3$) δ −131.0. IR (neat) ν_{max}/cm^{-1}: 1604, 1502, 1464, 1262, 1129, 1030. MS (APCI) *m/z*: 308 $[M + H]^+$. By following the general procedure described above for **2a**. Diarylamine **1b** (102 mg; 0.33 mmol), NaH (12 mg; 0.5 mmol) and CH_3I (71 mg; 0.5 mmol) gave **2b** as a yellow oil (80 mg; 75%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.87 (dd, 1H, $J = 9.3$ Hz, $J = 9.0$ Hz), 6.75 (dd, 1H, $J = 13.3$ Hz, $J = 2.7$ Hz), 6.71–6.66 (m, 1H), 6.18 (s, 2H), 3.84 (s, 3H), 3.80 (s, 3H), 3.76 (s, 6H), 3.22 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.6 (2), 152.7 (d, $J = 243.7$ Hz), 145.4, 143.4 (d, $J = 8.4$ Hz), 142.3 (d, $J = 11.1$ Hz), 133.2, 115.9 (d, $J = 2.7$ Hz), 114.6 (d, $J = 2.3$ Hz), 109.1 (d, $J = 20.5$ Hz), 98.2 (2), 61.0, 56.9, 56.1 (2), 40.8. ^{19}F NMR (188 MHz, $CDCl_3$) δ −133.4. IR (neat) ν_{max}/cm^{-1}: 2999, 1604, 1581, 1503, 1242, 1125. MS (APCI) *m/z*: 322 $[M + H]^+$. HRMS calcd for $C_{17}H_{21}FNO_4$ $[M + H]^+$ 322.1455, obsd. 322.1450.

4.1.3. *N-(3-fluoro-4-methoxyphenyl)-3,4,5-trimethoxy-N-methylbenzenaminium chloride* ***2b.HCl***

To a solution of **2b** (97 mg; 1 mmol) in MeOH–Et_2O (1:1) (10 mL) was added HCl_g within 10 min and then the mixture was stirred for 1 h at RT. The precipitated salt **2b.HCl** (92 mg; 86%) was isolated as green amorphous solid after filtration. ^{1}H NMR (300 MHz, DMSO) δ 7.06 (t, $J = 9.4$ Hz, 1H), 6.83 (dd, $J = 13.8$, 2.5 Hz, 1H), 6.73 (d, $J = 8.9$ Hz, 1H), 6.60 (s, 1H), 6.25 (s, 2H), 3.78 (s, 3H), 3.69 (s, 6H), 3.61 (s, 3H), 3.19 (s, 3H). ^{13}C NMR (75 MHz, DMSO) δ 153.86 (2C), 152.19 (d, $J = 233.9$ Hz), 145.37, 143.40 (d, $J = 8.5$ Hz), 141.91 (d, $J = 11.1$ Hz), 133.19, 116.20, 115.32, 108.60 (d, $J = 20.2$ Hz), 99.00 (2C), 60.56, 56.88, 56.28 (2C), 41.02. IR (neat) ν_{max}/cm^{-1}: 2999, 1612, 1521, 1488, 1275, 1122, 1005. MS (APCI) *m/z*: 322 $[M]^+$. HRMS calcd for $C_{17}H_{21}FNO_4$ $[M]^+$ 322.1449, obsd. 322.1442.

4.1.4. *3,4,5-Trimethoxy-N-(4-methoxy-3-nitrophenyl)-N-methylaniline* ***2c***

By following the general procedure described above for **1a**. Heating at 100 °C 3,4,5-trimethoxyaniline (275 mg; 1.5 mmol) and 4-iodo-1-methoxy-2-nitrobenzene (279 mg; 1 mmol) in the presence of $Pd(OAc)_2$ (22 mg; 10 mol%), Xantphos (58 mg; 10 mol%) for 1 h 30 gave **1c** (307 mg; 92%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.55 (d, 1H, $J = 2.8$ Hz), 7.21 (dd, 1H, $J = 9.0$ Hz, $J = 2.8$ Hz), 7.01 (d, 1H, $J = 9.0$ Hz), 6.25 (s, 2H), 5.59 (s, 1H), 3.92 (s, 3H), 3.81 (s, 3H), 3.80 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 154.2 (2), 147.6, 140.2, 139.0, 137.3, 133.4, 124.2, 115.3, 114.9, 96.3 (2), 61.2, 57.2, 56.2 (2). IR (neat) ν_{max}/cm^{-1}: 1600, 1535, 1347, 1302, 1230, 1122, 1021. MS (APCI) *m/z*: 335 $[M + H]^+$.



Superimposition of **2b** (pink) and *iso*CA-4 (green) Superimposition of **2m** (pink) and *iso*CA-4 (green)

Fig. 4. Calculated binding mode for **2b** and **2m** in the colchicine binding site of tubulin and superimposition of the latter with that of *iso*CA-4. (Central nitrogen atom and (if applicable) fluorine atom are represented as spheres in order to increase their visibility.)

By following the general procedure described above for **2a**. Diarylamine **1c** (167 mg; 0.5 mmol), NaH (18 mg; 0.75 mmol) and CH_3I (106 mg; 0.75 mmol) gave **2c** as an orange oil (172 mg; 99%). ^1H NMR (300 MHz, $CDCl_3$) δ 7.41 (d, 1H, J = 2.9 Hz), 7.09 (dd, 1H, J = 9.1 Hz, J = 2.9 Hz), 6.97 (d, 1H, J = 9.1 Hz), 6.26 (s, 2H), 3.91 (s, 3H), 3.83 (s, 3H), 3.79 (s, 6H), 3.27 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 154.0 (2), 146.9, 144.6, 143.0, 140.1, 134.6, 124.6, 115.0, 114.8, 100.3 (2), 61.1, 57.2, 56.2 (2), 40.9. IR (neat) ν_{max}/cm^{-1}: 1589, 1525, 1502, 1443, 1364, 1254, 1234, 1122. MS (APCI) m/z: 349 $[M + H]^+$. HRMS calcd for $C_{17}H_{21}N_2O_6$ $[M + H]^+$ 349.1400, obsd. 349.1392.

4.1.5. 4-Methoxy-N-methyl-N-(3,4,5-trimethoxyphenyl)benzene-1,3-diamine **2d**

To a (EtOH/H_2O: 4/1) solution (1.5 mL) of **2c** (140 mg; 0.4 mmol) were added iron (225 mg; 4 mmol) and a drop of HCl 12N. After refluxing for 2 h, the mixture was filtered through a pad of Celite, concentrated to give a residue which was subjected to flash chromatography to give **2d** as a brown oil (96 mg, 76%). ^1H NMR (300 MHz, $CDCl_3$) δ 6.74 (d, 1H, J = 8.3 Hz), 6.49–6.44 (m, 2H), 6.09 (s, 2H), 3.84 (s, 3H), 3.79 (s, 3H), 3.76 (s, 6H), 3.21 (s, 3H), NH_2 not seen. ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.6 (2), 146.6, 143.8, 143.2, 136.9, 131.7, 113.3, 111.3, 110.8, 95.7 (2), 61.1, 56.2 (2), 55.9, 41.0. IR (neat) ν_{max}/cm^{-1}: 1612, 1581, 1502, 1148, 1248, 1126. MS (APCI) m/z: 319 $[M + H]^+$. HRMS calcd for $C_{17}H_{23}N_2O_4$ $[M + H]^+$ 319.1658, obsd. 319.1654.

4.1.6. N-Methyl-N-(3,4,5-trimethoxyphenyl)naphthalen-2-amine **2e**

By following the general procedure described above for **1a**. Heating at 100 °C 3,4,5-trimethoxyaniline (275 mg; 1.5 mmol) and 2-iodonaphtalene (254 mg; 1 mmol) in the presence of $Pd(OAc)_2$ (22 mg; 10 mol%), Xantphos (58 mg; 10 mol%) for 3 h gave **1d** (213 mg; 69%). ^1H NMR (300 MHz, $CDCl_3$) δ 7.77 (d, 2H, J = 8.5 Hz), 7.67 (d, 1H, J = 8.2 Hz), 7.46–7.42 (m, 2H), 7.36–7.22 (m, 2H), 6.45 (s, 2H), 5.86 (s, 1H), 3.89 (s, 3H), 3.84 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.9 (2), 141.4, 139.2, 134.7, 133.2, 129.2, 129.1, 127.7, 126.5, 126.5, 123.5, 119.9, 111.5, 96.4 (2), 61.1, 56.1 (2). IR (neat) ν_{max}/cm^{-1}: 1631, 1596, 1503, 1465, 1223, 1127, 1006. MS (APCI) m/z: 310 $[M + H]^+$. By following the general procedure described above for **2a**. Diarylamine **1d** (155 mg; 0.5 mmol), NaH (18 mg; 0.75 mmol) and CH_3I (106 mg; 0.75 mmol) gave **2e** as a yellow oil (140 mg; 87%). ^1H NMR (300 MHz, $CDCl_3$) δ 7.72 (t, 2H, J = 7.8 Hz), 7.66 (d, 1H, J = 9.0 Hz), 7.45–7.39 (td, 1H, J = 8.6 Hz, J = 1.3 Hz), 7.33–7.28 (m, 1H), 7.25 (d, 1H, J = 2.5 Hz), 7.17 (dd, 1H, J = 8.9 Hz, J = 2.4 Hz), 6.39 (s, 2H), 3.87 (s, 3H), 3.79 (s, 6H), 3.41 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.9 (2), 146.9, 145.4, 134.8, 134.4, 128.7, 128.5, 127.6, 126.7, 126.4, 123.5, 121.0, 112.5, 100.9 (2), 61.1, 56.2 (2), 41.0. IR (neat) ν_{max}/cm^{-1}: 1629, 1589, 1502, 1240, 1128, 1005. MS (APCI) m/z: 324 $[M + H]^+$. HRMS calcd for $C_{20}H_{22}NO_3$ $[M + H]^+$ 324.1600, obsd. 324.1602.

4.1.7. N-(3-(benzyloxy)-4-methoxyphenyl)-3,4,5-trimethoxy-N-methylaniline **2f**

Compound **2f** was prepared from diarylaniline **1e** (yield 61%) according to literature [41]. ^1H NMR (300 MHz, $CDCl_3$) δ 7.40–7.27 (m, 5H), 6.86 (d, 1H, J = 8.4 Hz), 6.67–6.62 (m, 2H), 6.06 (s, 2H), 5.08 (s, 2H), 3.88 (s, 3H), 3.81 (s, 3H), 3.72 (s, 6H), 3.20 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.6 (2), 148.7, 146.1, 145.8, 142.9, 137.1, 128.6 (2), 127.9, 127.4 (2), 115.1, 112.9, 110.1, 96.2 (2), 71.1, 61.1, 56.5, 56.1 (2), 41.0. IR (neat) ν_{max}/cm^{-1}: 1579, 1502, 1452, 1411, 1230, 1125. MS (ESI) m/z: 432.3 $[M + Na]^+$. HRMS calcd for $C_{24}H_{28}NO_5$ $[M + H]^+$ 410.1967, obsd. 410.1964.

4.1.8. AzaisoCA-4 **2g**

Compound **2g** was prepared from diarylaniline **2f** using H_2 and Pd/C (10 mol%) in EtOAc (yield 98%) according to literature [1]. ^1H NMR (300 MHz, $CDCl_3$) δ 6.79 (d, 1H, J = 8.7 Hz), 6.67 (d, 1H, J = 2.7 Hz), 6.53 (dd, 1H, J = 8.7 Hz, J = 2.7 Hz), 6.14 (s, 2H), 5.64 (s, 1H), 3.87 (s, 3H), 3.80 (s, 3H), 3.77 (s, 6H), 3.22 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.6 (2), 146.3, 146.1, 143.7, 142.3, 132.4, 113.4, 111.5, 109.2, 97.0 (2), 61.1, 56.4, 56.2 (2), 41.0. IR (neat) ν_{max}/cm^{-1}: 2959, 1581, 1501, 1447, 1249. MS (APCI) m/z: 342 $[M + Na]^+$. HRMS calcd for $C_{17}H_{22}NO_5$ $[M + H]^+$ 320.1498, obsd. 320.1494.

4.1.9. 2-Methoxy-5-(methyl(3,4,5-trimethoxyphenyl)amino)phenyl acetate **2h**

At 0 °C, to a solution of **2g** (101 mg; 0.316 mmol) in CH_2Cl_2 (1 mL) were added pyridine (54 μL), and DMAP (0.016 mmol) and Ac_2O (42 μL). After one hour of stirring, H_2O (3 mL) was added to the mixture. After extraction with EtOAc (3 × 3 mL), the organic layer was dried, evaporated, and the residue was subjected to flash chromatography to give **2h** as a yellow oil (76 mg, 67%). ^1H NMR (300 MHz, $CDCl_3$) δ 6.92–6.84 (m, 2H), 6.75 (d, J = 2.4 Hz, 1H), 6.17 (s, 2H), 3.80 (s, 6H), 3.77 (s, 6H), 3.24 (s, 3H), 2.28 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.0, 153.7 (2), 146.4, 145.7, 142.9, 140.2, 132.8, 119.3, 116.8, 113.3, 97.4 (2), 61.1, 56.4, 56.2 (2), 40.9, 20.7. IR (neat) ν_{max}/cm^{-1}: 1721, 1574, 1452, 1375, 1204. MS (ESI) m/z: 362 $[M + H]^+$. HRMS calcd for $C_{19}H_{23}NO_6Na$ $[M + Na]^+$ 384.1423, obsd. 384.1426.

4.1.10. N-(4-Chlorophenyl)-3,4,5-trimethoxy-N-methylaniline **2i**

By following the general procedure described above for **1a**. Heating at 100 °C 3,4,5-trimethoxyaniline (275 mg; 1.5 mmol) and 1-chloro-4-iodobenzene (59 mg; 0.25 mmol) in the presence of $Pd(OAc)_2$ (22 mg; 10 mol%), Xantphos (58 mg; 10 mol%) for 12 h gave **1f** as an orange solid (40 mg; 55%). m.p. = 109 °C. ^1H NMR (300 MHz, $CDCl_3$) δ 7.20 (d, 2H, J = 8.9 Hz), 6.95 (d, 2H, J = 8.9 Hz), 6.30 (s, 2H), 3.82 (s, 3H), 3.80 (s, 6H), NH not seen. ^{13}C NMR (75 MHz, $CDCl_3$) δ 154.0 (2), 142.5, 138.9, 133.3, 129.4 (2), 125.3, 118.6 (2), 96.8 (2), 61.1, 56.1 (2). IR (neat) ν_{max}/cm^{-1}: 1611, 1590, 1508, 1228, 1119. MS (APCI) m/z: 294, 296 $[M + H]^+$. By following the general procedure described above for **2a**. Diarylamine **1f** (29 mg; 0.1 mmol), NaH (5 mg; 0.2 mmol) and CH_3I (28 mg; 0.2 mmol) gave **2i** as a yellow-orange solid (27 mg; 88%). m.p. = 91 °C. ^1H NMR (300 MHz, $CDCl_3$) δ 7.17 (d, 2H, J = 8.5 Hz), 6.82 (d, 2H, J = 8.5 Hz), 6.31 (s, 2H), 3.84 (s, 3H), 3.79 (s, 6H), 3.26 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.9 (2), 147.9, 144.8, 134.6, 129.0 (2), 124.6, 118.9 (2), 101.0 (2), 61.0, 56.1 (2), 40.6. IR (neat) ν_{max}/cm^{-1}: 2999, 1609, 1579, 1510, 1451, 1237.

4.1.11. N,N,N′-Trimethyl-N′-(3,4,5-trimethoxyphenyl)benzene-1,4-diamine **2j**

In a sealed tube and under an argon atmosphere were added successively, $Pd_2(dba)_3.CHCl_3$ (52 mg; 5 mol%), Johnphos (30 mg; 10 mol%), dimethylamine (large excess), **2i** (307 mg; 1 mmol), NaO*t*Bu (134 mg; 1.4 mmol) in toluene (10 mL). After heating at 100 °C for 24 h, the resulting suspension was cooled to room temperature and filtered through a pad of Celite eluting with ethyl acetate, and the inorganic salts were removed. The filtrate was concentrated and a rapid purification by silica gel column chromatography of the residue to eliminate the excess of 3,4,5-trimethoxyaniline gave the desired product **2j** as a white solid (98 mg, 31%). m.p. = 75 °C. ^1H NMR (300 MHz, $CDCl_3$) δ 7.05 (d, 2H, J = 7.3 Hz), 6.75 (d, 2H, J = 7.3 Hz), 6.01 (s, 2H), 3.78 (s, 3H), 3.76 (s, 6H), 3.24 (s, 3H), 2.95 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.6 (2), 147.9, 147.8, 146.9, 139.1, 126.0 (2), 113.9 (2), 93.8 (2), 61.2, 56.1 (2), 41.1 (2), 41.0. IR (neat) ν_{max}/cm^{-1}: 1611, 1583, 1504, 1128. MS (ESI) m/z: 339 $[M + Na]^+$. HRMS calcd for $C_{18}H_{25}N_2O_3$ $[M + H]^+$ 317.1865, obsd. 317.1860.

4.1.12. 6-Methoxy-N-methyl-N-(3,4,5-trimethoxyphenyl)pyridin-3-amine **2k**

By following the general procedure described above for **1a**. Heating at 100 °C 3,4,5-trimethoxyaniline (275 mg; 1.5 mmol) and 5-bromo-2-methoxypyridine (187 mg; 1 mmol) in the presence of $Pd(OAc)_2$ (11 mg; 5 mol%), Xantphos (29 mg; 5 mol%) for 5 h gave **1g** as a yellow oil (160 mg; 54%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.98 (d, 1H, J = 2.3 Hz), 7.42 (dd, 1H, J = 8.8 Hz, J = 2.8 Hz), 6.72 (d, 1H, J = 8.8 Hz), 6.10 (s, 2H), 5.36 (s, 1H), 3.92 (s, 3H), 3.79 (s, 3H), 3.78 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.1, 154.1 (2), 141.2, 139.0, 133.5, 133.1, 132.2, 111.2, 93.9 (2), 61.2, 56.2 (2), 53.8. IR (neat) ν_{max}/cm^{-1}: 1599, 1486, 1367, 1230, 1125, 1028. MS (APCI) m/z: 291 $[M + H]^+$. By following the general procedure described above for **2a**. Diarylamine **1g** (290 mg; 1 mmol), NaH (48 mg; 2 mmol) and CH_3I (284 mg; 2 mmol) gave **2k** as a yellow oil (267 mg; 88%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.93 (d, 1H, J = 2.8 Hz), 7.34 (dd, 1H, J = 8.8 Hz, J = 2.8 Hz), 6.68 (d, 1H, J = 8.8 Hz), 6.03 (s, 2H), 3.90 (s, 3H), 3.76 (s, 3H), 3.74 (s, 6H), 3.22 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.2, 153.7 (2), 145.8, 141.2, 139.7, 134.9, 132.2, 111.2, 95.6 (2), 61.0, 56.1 (2), 53.6, 41.0. IR (neat) ν_{max}/cm^{-1}: 1587, 1491, 1275, 1235, 1127, 1028. MS (APCI) m/z: 305 $[M + H]^+$. HRMS calcd for $C_{16}H_{21}N_2O_4$ $[M + H]^+$ 305.1501, obsd. 305.1501.

4.1.13. N-Methyl-N-(3,4,5-trimethoxyphenyl)quinolin-3-amine **2l**

By following the general procedure described above for **1a**. Heating at 100 °C 3,4,5-trimethoxyaniline (275 mg; 1.5 mmol) and 3-bromoquinoline (207 mg; 1 mmol) in the presence of $Pd(OAc)_2$ (44 mg; 20 mol%), Xantphos (29 mg; 5 mol%) and Cs_2CO_3 (534 mg; 1.64 mmol) for 3 h gave **1h** as a yellow oil (167 mg; 54%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.69 (d, 1H, J = 2.7 Hz), 8.00–7.97 (m, 1H), 7.64 (d, 1H, J = 2.7 Hz), 7.61–7.58 (m, 1H), 7.50–7.40 (m, 2H), 6.41 (s, 3H), 3.84 (s, 3H), 3.77 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 154.0 (2), 144.9, 143.4, 138.2, 137.8, 133.6, 129.0, 129.0, 127.2, 126.4 (2), 116.7, 97.1 (2), 61.1, 56.1 (2). IR (neat) ν_{max}/cm^{-1}: 3338, 1602, 1506, 1237, 1124. MS (APCI) m/z: 311 $[M + H]^+$. By following the general procedure described above for **2a**. Diarylamine **1h** (155 mg; 0.5 mmol), NaH (18 mg; 0.75 mmol) and CH_3I (107 mg; 0.75 mmol) gave **2l** as a yellow oil (110 mg; 68%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.62 (d, 1H, J = 2.8 Hz), 8.00–7.94 (m, 1H), 7.68–7.63 (m, 1H), 7.51–7.42 (m, 2H), 7.39 (d, 1H, J = 2.8 Hz), 6.38 (s, 2H), 3.85 (s, 3H), 3.78 (s, 6H), 3.38 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 154.1 (2), 145.4, 144.2, 142.7, 142.6, 135.2, 129.0, 127.1, 126.5, 126.3, 117.3, 101.5 (2), 61.1, 56.2 (2), 40.82 (one C missing). IR (neat) ν_{max}/cm^{-1}: 1588, 1504, 1245, 1229, 1128. MS (APCI) m/z: 325 $[M + H]^+$.

4.1.14. N,1-Dimethyl-N-(3,4,5-trimethoxyphenyl)-1H-indol-5-amine **2m**

To an Emrys Optimizer were added 3,4,5-trimethoxyaniline (260 mg; 1.42 mmol), 6-bromo-1-methyl-1*H*-indole (150 mg; 0.71 mmol), $Pd(OAc)_2$ (16 mg; 10 mol%), Xantphos (41 mg; 10 mol %), Cs_2CO_3 (463 mg; 1.42 mmol) in dioxane (5 mL). The reaction vessel was then placed in the Emrys Optimizer and exposed to microwave irradiation according to the following specifications: temperature: 130 °C, time: 3 h, fixed hold time: on, sample absorption: high, pre-stirring: 60 s. After cooling to room temperature, the mixture was filtered through a pad of Celite eluting with ethyl acetate, and the inorganic salts were removed. The filtrate was concentrated and a rapid purification by silica gel column chromatography of the residue to eliminate the excess of 3,4,5-trimethoxyaniline gave the desired product **1i** as a yellow oil which was immediately engaged in the alkylation step. **1i**: (108 mg, 49%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 (s, 1H), 7.23 (s, 1H), 7.05–7.02 (m, 2H), 6.38 (d, 1H, J = 3.0 Hz), 6.15 (s, 2H), 5.50 (brs, 1H), 3.77 (s, 3H), 3.76 (s, 3H), 3.74 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 154.0 (2), 143.0, 135.1, 133.8, 131.3, 129.6, 129.2, 117.9, 113.4, 110.0, 100.6, 93.3 (2), 61.2, 56.1 (2), 33.1. IR (neat) ν_{max}/cm^{-1}: 3370, 1605, 1505, 1247, 1125, 1009. MS (APCI) m/z: 313 $[M + H]^+$. By following the general procedure described above for **2a**. Diarylamine **1i** (31 mg; 0.1 mmol), NaH (12 mg; 0.5 mmol) and CH_3I (71 mg; 0. 5 mmol) gave **2m** as a yellow oil (21 mg; 65%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43 (d, 1H, J = 2.0 Hz), 7.30 (d, 1H, J = 8.6 Hz), 7.08–7.05 (m, 2H), 6.45 (d, 1H, J = 3.0 Hz), 6.03 (s, 2H), 3.81 (s, 3H), 3.80 (s, 3H), 3.74 (s, 6H), 3.33 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.5 (2), 147.4, 141.7, 134.4, 130.7, 129.6, 129.3, 120.7, 117.4, 110.1, 101.0, 93.6 (2), 61.1, 56.1 (2), 41.5, 33.1. IR (neat) ν_{max}/cm^{-1}: 1602, 1581, 1505, 1490, 1418, 1234, 1123. MS (APCI) m/z: 327 $[M + H]^+$. HRMS calcd for $C_{19}H_{23}N_2O_3$ $[M + H]^+$ 327.1709, obsd.327.1698.

4.1.15. N-(4-Methoxyphenyl)-N,1-dimethyl-1H-indol-5-amine **2n**

By following the general procedure described above for **1i**. Heating at 130 °C under microwave irradiation 4-methoxyaniline (123 mg; 1 mmol) and 6-bromo-1-methyl-1*H*-indole (105 mg; 0.5 mmol) for 3 h gave **1j** as a yellow oil (50 mg; 40%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37 (d, 1H, J = 1.7 Hz), 7.30 (d, 1H, J = 8.7 Hz), 7.08–7.02 (m, 4H), 6.94–6.89 (m, 2H), 6.47 (d, 1H, J = 3.0 Hz), 5.31 (br s, 1H), 3.86 (s, 3H), 3.79 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.7, 139.5, 136.9, 133.2, 129.4, 129.2, 118.6 (2), 116.2, 114.7 (2), 110.6, 109.8, 100.3, 55.7, 32.9. IR (neat) ν_{max}/cm^{-1}: 1618, 1571, 1509, 1422, 1240. MS (APCI) m/z: 253 $[M + H]^+$. By following the general procedure described above for **2a**. Diarylamine **1j** (63 mg; 0.25 mmol), NaH (12 mg; 0.5 mmol) and CH_3I (71 mg; 0.5 mmol) gave **2n** as a white solid (34 mg; 51%). m.p. = 113 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.25 (d, 1H, J = 1.9 Hz), 7.17–7.14 (m, 1H), 6.94 (d, 1H, J = 2.9 Hz), 6.90 (dd, 1H, J = 8.7 Hz, J = 2.0 Hz), 6.78–6.69 (m, 4H), 6.32 (d, 1H, J = 3.0 Hz), 3.68 (s, 6H), 3.20 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.4, 144.9, 143.0, 133.7, 129.5, 129.3, 119.2 (2), 119.1, 114.8, 114.6 (2), 110.0, 100.8, 55.8, 41.8, 33.0. IR (neat) ν_{max}/cm^{-1}: 1620, 1508, 1487, 1422, 1234, 1031. MS (APCI) m/z: 267 $[M + H]^+$. HRMS calcd for $C_{17}H_{19}N_2O$ $[M + H]^+$ 267.1497, obsd. 267.1494.

4.1.16. N-(3,5-Dimethoxyphenyl)-N,1-dimethyl-1H-indol-5-amine **2o**

By following the general procedure described above for **1i**. Heating at 130 °C under microwave irradiation 3,5-dimethoxyaniline (153 mg; 1 mmol) and 6-bromo-1-methyl-1*H*-indole (105 mg; 0.5 mmol) for 3 h gave **1k** as a yellow oil (35 mg; 25%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45 (d, 1H, J = 1.9 Hz), 7.30 (d, 1H, J = 9.4 Hz), 7.13–7.07 (m, 2H), 6.45 (d, 1H, J = 3.0 Hz), 6.14 (d, 2H, J = 2.1 Hz), 6.01 (t, 1H, J = 2.1 Hz), 5.68 (br s, 1H), 3.81 (s, 3H), 3.76 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.2 (2), 148.7, 134.2, 134.1, 129.6, 129.1, 118.6, 114.5, 109.9, 100.7, 93.6 (2), 91.3, 55.3 (2), 33.0. IR (neat) ν_{max}/cm^{-1}: 1592, 1518, 1448, 1154. MS (APCI) m/z: 305 $[M + Na]^+$. By following the general procedure described above for **2a**. Diarylamine **1k** (28 mg; 0.1 mmol), NaH (12 mg; 0.5 mmol) and CH_3I (71 mg; 0.5 mmol) gave **2o** as a yellow oil (27 mg; 93%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 (d, 1H, J = 2.0 Hz), 7.32 (d, 1H, J = 8.6 Hz), 7.09 (d, 2H, J = 6.9 Hz, J = 2.5 Hz), 6.46 (d, 1H, J = 3.1 Hz), 5.94 (s, 3H), 3.81 (s, 3H), 3.70 (s, 6H), 3.32 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.4 (2), 152.5, 141.2, 134.8, 129.6, 129.3, 121.5, 118.9, 110.2, 101.1, 93.6 (2), 89.6, 55.3 (2), 41.2, 33.1. IR (neat) ν_{max}/cm^{-1}: 3433, 1608, 1578, 1419, 1236, 1138. MS (APCI) m/z: 297 $[M + H]^+$. HRMS calcd for $C_{18}H_{21}N_2O_2$ $[M + H]^+$ 297.1603, obsd. 297.1601.

4.1.17. N-Methyl-1-(phenylsulfonyl)-N-(3,4,5-trimethoxyphenyl)-1H-indol-5-amine **2p**

By following the general procedure described above for **1i**. Heating at 130 °C under microwave irradiation 3,4,5-trimethoxyaniline (183 mg; 1 mmol) and 5-bromo-1-(phenylsulfonyl)-1*H*-indole (167 mg; 0.5 mmol) for 4 h gave **1l** as a yellow oil (162 mg; 74%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.89–7.84 (m, 3H),

7.53–7.47 (m, 2H), 7.43–7.37 (m, 2H), 7.17 (d, 1H, J = 2.0 Hz), 7.02 (dd, 1H, J = 2.1 Hz, J = 8.8 Hz), 6.53 (dd, 1H, J = 3.6 Hz, J = 0.5 Hz), 6.26 (s, 2H), 5.74 (s, 1H), 3.80 (s, 3H), 3.74 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.9 (2), 140.3, 139.8, 138.2, 133.8, 132.5, 131.9, 130.2, 129.2 (2), 127.0, 126.7 (2), 117.4, 114.3, 109.9, 109.3, 95.6 (2), 61.0, 56.0 (2). IR (neat) ν_{max}/cm^{-1}: 2927, 1600, 1504, 1450, 1336, 1227. MS (ESI) m/z: 461 [M + Na]$^+$. By following the general procedure described above for **2a**. Diarylamine **1l** (110 mg; 0.25 mmol), NaH (12 mg; 0.5 mmol) and CH_3I (71 mg; 0. 5 mmol) gave **2p** as a yellow oil (81 mg; 72%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.89–7.86 (m, 3H), 7.55–7.50 (m, 2H), 7.42 (dd, 2H, J = 10.4 Hz, J = 4.7 Hz), 7.13 (d, 1H, J = 2.2 Hz), 7.02 (dd, 1H, J = 8.9 Hz, J = 2.2 Hz), 6.57 (d, 1H, J = 3.6 Hz), 6.16 (s, 2H), 3.82 (s, 3H), 3.73 (s, 6H), 3.28 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.7 (2), 146.1, 145.7, 138.3, 133.8, 133.0, 131.9, 130.4, 129.3 (2), 127.0, 126.8 (2), 119.6, 114.2, 112.7, 109.4, 98.0 (2), 61.1, 56.1 (2), 41.2. IR (neat) ν_{max}/cm^{-1}: 1586, 1504, 1446, 1368, 1123. MS (ESI) m/z: 453 [M + H]$^+$, 475 [M + Na]$^+$. HRMS calcd for $C_{25}H_{27}N_2O_5S$ [M + H]$^+$ 453.1484, obsd.453.1487.

4.1.18. NN-Methyl-N-(3,4,5-trimethoxyphenyl)-1H-indol-5-amine **2q**

A THF (1 mL) solution of **2p** (47 mg; 0.1 mmol) was added 0.5 mL of a TBAF solution in THF (0.5 mmol) and stirred at 100 °C for 6 h. After cooling, the solution was filtered through a pad of Celite, eluting with ethyl acetate. The filtrate was concentrated and a purification by silica gel column chromatography gave the desired product **2q** as a white solid (15 mg, 49%). m.p. 166 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.34 (s, 1H), 7.44 (s, 1H), 7.36 (d, 1H, J = 8.6 Hz), 7.22 (t, 1H, J = 2.7 Hz), 7.02 (dd, 1H, J = 8.6 Hz, J = 1.9 Hz), 6.52–6.51 (m, 1H), 6.04 (s, 2H), 3.80 (s, 3H), 3.74 (s, 6H), 3.33 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.6 (2), 147.4, 142.1, 133.4, 130.8, 128.8, 125.1, 121.0, 117.1, 112.0, 102.7, 93.8 (2), 61.2, 56.1 (2), 41.5. IR (neat) ν_{max}/cm^{-1}: 3433, 1608, 1578, 1505, 1450, 1419, 1236. MS (ESI) m/z: 335 [M + Na]$^+$. HRMS calcd for $C_{18}H_{21}N_2O_3$ [M + H]$^+$ 313.1552, obsd. 331.1548.

4.1.19. N-(3-(benzyloxy)-4-methoxyphenyl)-3,4,5-trimethoxy-N-propylaniline **3a**

To a diarylamine **1e** (197 mg; 0.5 mmol) solution in DMF (5 mL) was added at 0 °C, NaH (24 mg; 1 mmol), and 1-bromopropane (122 mg; 1 mmol). The mixture was stirred at r.t. for 2 h, diluted in EtOAc (5 mL) and washed with aqueous $NaHCO_3$. The organic layer was dried, evaporated, and the residue was subjected to flash chromatography to give **3a** as a brown oil (208 mg, 95%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40–7.27 (m, 5H), 6.86–6.83 (m, 1H), 6.63–6.59 (m, 2H), 6.00 (s, 2H), 5.09 (s, 2H), 3.89 (s, 3H), 3.80 (s, 3H), 3.71 (s, 6H), 3.50–3.44 (m, 2H), 1.61–1.59 (m, 2H), 0.89 (t, 3H, J = 7.4 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.7 (2), 148.7, 145.8, 145.4, 141.6, 137.1, 131.8, 128.6 (2), 127.9, 127.4 (2), 116.0, 112.8, 111.0, 96.3 (2), 71.1, 61.2, 56.5, 56.1 (2), 54.7, 21.0, 11.6. IR (neat) ν_{max}/cm^{-1}: 2925, 1610, 1502, 1464, 1225, 1127. MS (APCI) m/z: 438 [M + H]$^+$. HRMS calcd for $C_{26}H_{32}NO_5$ [M + H]$^+$ 438.2280, obsd. 438.2285.

4.1.20. 2-methoxy-5-(propyl(3,4,5-trimethoxyphenyl)amino) phenol **3b**

3b brown oil (140 mg; 99%) was prepared by following the general procedure described above for **2g**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.78 (d, 1H, J = 8.7 Hz), 6.65 (d, 1H, J = 2.6 Hz), 6.51 (dd, 1H, J = 8.7 Hz, J = 2.6 Hz), 6.11 (s, 2H), 5.60 (s, 1H), 3.88 (s, 3H), 3.81 (s, 3H), 3.77 (s, 6H), 3.57–3.52 (m, 2H), 1.70–1.60 (m, 2H), 0.94 (t, 3H, J = 7.4 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.7 (2), 146.3, 145.2, 142.6, 142.2, 132.3, 114.1, 111.6, 109.7, 97.5 (2), 61.2, 56.4, 56.2 (2), 54.7, 21.0, 11.6. IR (neat) ν_{max}/cm^{-1}: 2959, 1581, 1501, 1466, 1261, 1126, 1027. MS (ESI) m/z: 370 [M + Na]$^+$. HRMS calcd for $C_{19}H_{26}NO_5$ [M + H]$^+$ 348.1811, obsd. 348.1812.

4.1.21. 1-Methyl-N-propyl-N-(3,4,5-trimethoxyphenyl)-1H-indol-5-amine **3c**

3c yellow oil (120 mg; 34%) was prepared from **1i** by following the general procedure described above for **3a**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45 (d, 1H, J = 1.7 Hz), 7.32 (d, 1H, J = 8.6 Hz), 7.08–7.05 (m, 2H), 6.47 (d, 1H, J = 3.0 Hz), 5.99 (s, 2H), 3.81 (s, 6H), 3.73 (s, 6H), 3.66 (t, 2H, J = 6.5 Hz), 1.76–1.60 (m, 2H), 0.97 (t, 3H, J = 7.4 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.5 (2), 146.7, 139.9, 134.5, 130.2, 129.4, 129.3, 121.8, 118.7, 110.1, 100.9, 93.3 (2), 61.1, 56.0 (2), 55.0, 33.0, 21.0, 11.6. IR (neat) ν_{max}/cm^{-1}: 1606, 1580, 1507, 1487, 1237, 1125. MS (ESI) m/z: 377 [M + Na]$^+$. HRMS calcd for $C_{21}H_{27}N_2O_3$ [M + H]$^+$ 355.2022, obsd. 355.2018.

4.2. Biology

4.2.1. Cell culture and proliferation assay

Cancer cell lines were obtained from the American type Culture Collection (Rockville, MD) and were cultured according to the supplier's instructions. Briefly, A549 lung carcinoma, U87-MG human glioblastoma, MDA-MB231 cells were grown in Dulbecco minimal essential medium (DMEM) containing 4.5 g/L glucose supplemented with 10% FCS and 1% glutamine. Human K562 leukemia and HCT116 colorectal carcinoma cells were grown in RPMI 1640 containing 10% FCS and 1% glutamine. Human umbilical vein endothelial cells (HUVECs) were obtained from Clonetics (Lonza, Walkersville, MD, USA) and cultured according to the supplier's instructions. Briefly, HUVECs from three to six passages were subcultured to confluence onto 0.2% gelatin coated tissue culture flasks in endothelial cell growth medium (EGM2) containing growth factors and 2% FCS. All cell lines were maintained at 37 °C in a humidified atmosphere containing 5% CO_2. Cell viability was assessed using Promega CellTiter-Blue TM reagent according to the manufacturer's instructions. Cells were seeded in 96-well plates (5 × 103 cells/well) containing 50 μL growth medium. After 24 h of culture, the cells were supplemented with 50 μL of the tested compound dissolved in DMSO (less than 0.1% in each preparation). After 72 h of incubation, 20 μL of resazurin was added for 2 h before recording fluorescence (λex = 560 nm, λem = 590 nm) using a Victor microtiter plate fluorimeter (Perkin–Elmer, USA). The IC50 corresponds to the concentration of the tested compound that caused a decrease of 50% in fluorescence of drug treated cells compared with untreated cells. Experiments were performed in triplicate.

4.2.2. Tubulin binding assay

Sheep brain tubulin was purified according to the method of Shelanski [42] by two cycles of assembly-disassembly and then diluted in the assembly buffer containing 0.1 M MES, 0.5 mM $MgCl_2$, 1 mM EGTA, and 1 mM GTP, pH 6.6 to a final concentration around 2–3 mg/mL. Tubulin assembly was monitored by fluorescence according to reported procedure [43] using DAPI as fluorescent molecule. Assays were realized on 96-well plates prepared with Biomek NKMC and Biomek 3000 from Beckman Coulter and read at 37 °C on Wallac Victor fluorimeter from Perkin Elmer. The IC_{50} value of each compound was determined as the concentration required to decrease the maximum assembly rate of tubulin by 50% compared to the rate in the absence of compound. The IC_{50} values for all compounds were compared to the IC_{50} of *iso*CA-4 and *iso*erianin measured the same day under the same conditions.

4.2.3. Cell cycle analysis

Exponentially growing cancer cells (K562, HCT116) were incubated with tested compound or DMSO for 24 h. Cell-cycle profiles

were determined by flow cytometry on a FC500 flow cytometer (Beckman–Coulter, France) as described previously [44].

4.2.4. Apoptosis assay

Apoptosis was measured by the Apo-one homogeneous caspase-3/7 assay (Promega Co, WI) according to the manufacturer's recommendations. Briefly, cells were subcultured on a 96-well plate with 5×10^4 cells/well in 100 μL medium. After 24 h of incubation, the medium in the 96-well plate was discarded and replaced with medium containing different concentrations of **2b** and **2m** (7.5, 15, 25 and 50 nM). The treated cells were incubated for 24 h, each well then received 100 μL of a mixture of caspase substrate and Apo-one caspase 3/7 buffer. After 1 h of incubation, the fluorescence of sample was measured using a Victor microtiter plate fluorimeter (Perkin–Elmer, USA) at 527 nm.

4.2.5. Inhibition of endothelial tube formation using ECFCs

The protocol was achieved according to ref. [38].

4.2.6. Cord disruption assay

HUVECs (2×10^4 cells per well) were plated in 96-well plates on a thick layer of Matrigel (Becton Dickinson; 10 mg mL^{-1}, 60 μL per well) and allowed to align for 24 h. **2b**, **2m** or vehicle (DMSO) were added to the formed cords and left for 3 h. Images were taken 3 h after the addition of compounds.

4.2.7. Molecular modelling

Coordinates for compounds **2b**, **2m** and *iso*CA-4 were generated using CORINA v3.44 software [45]. Molecules were then freely docked in the colchicine binding site between chains C and D from PDB structure 1SA0 using GOLD v5.1 software [46]. CHEMPLP with default parameters was used as an objective function [47]. Structures of the complexes were exported for further examination and depiction with Chimera v1.8.1 software [48], including hydrogen bonds detection, close contact analysis and representation of the solvent-accessible surface colored according to polarity.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.03.032.

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