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*Iso*CombretaQuinazolines: Potent Cytotoxic Agents with Antitubulin Activity

Mohamed Ali Soussi,[a] Olivier Provot,*[a] Guillaume Bernadat,[a] Jérome Bignon,[b] Déborah Desravines,[b] Joëlle Dubois,[b] Jean-Daniel Brion,[a] Samir Messaoudi,*[a] Mouad Alami*[a]

ABSTRACT. *A series of novel isocombretaquinazolines (isoCoQ)* ***4*** *was quickly prepared using the coupling of N-tosylhydrazones with 4-chloroquinazolines under palladium catalysis. These compounds, which could be regarded as isoCA-4 analogues devoid of the 3,4,5-trimethoxyphenyl ring, displayed a nanomolar level of cytotoxicity against various human cancer cells and inhibited tubulin polymerization effectively. IsoCoQ* ***4b****,* ***4c*** *and* ***4d*** *having the greatest ressemblance to isoCA-4, isoFCA-4 and isoNH$_2$CA-4, respectively, led to the arrest of HCT116 cancer cell lines in the G$_2$/M phase of the cell cycle at a very low concentration. Preliminary in vitro antivascular results showed that* ***4d*** *has disrupted a network of capillary-like structures formed by human umbilical vein endothelial cells on Matrigel. All of these results clearly demonstrated that the replacement of the 3,4,5-trimethoxyphenyl ring of isoCA-4 by a quinazoline nucleus is possible and leads to new highly promising derivatives of potential for further development as antitubulin agents.*

Introduction

Combretastatin A-4 (CA-4, Figure 1), a *cis*-stilbene isolated from the South African tree *Combretum caffrum*,[1] strongly inhibits tubulin assembly by binding at the colchicine site.[2] CA-4 is also a highly cytotoxic agent at a nanomolar concentration against a variety of cancer cells, including multidrug-resistant cell lines.[3,4] Several studies have demonstrated that CA-4, by binding to β-tubulin, has deleterous effects on tumor vasculature causing a rapid vascular shutdown which led to central tumor necrosis.[5,6] However, this stilbene of very simple chemical structure, suffers from several drawbacks such as a low water solubility and a chemical instability of its *Z*-double bond,[7] which isomerizes during storage, administration[8] and metabolism.[9] The first drawback has been resolved by the synthesis of a phosphate water-soluble prodrug (CA-4P, fosbretabulin) and a serinamido derivative (AVE-8062, ombrabulin) which also cause vascular shutdown and reduction in tumor blood flow *in vivo*. These pro-drugs are currently used in clinical trials for advanced anaplastic thyroid carcinoma, even if some vascular side effects[10,11] were reported. Concerning the instability of the *Z*-double bond, a significant effort has been undertaking to develop CA-4 analogues in which the stilbene double bond was inserted in several cycles, particularly five-membered heterocyclic rings.[12,13,14,15] Our strategy in the CA-4 field has been focused on the replacement of the unstable *Z*-double bond by several linkers[16,17] of different sizes, from which the one-atom linker was found to be the optimal length of the bridge between the two aromatic A and B rings. These studies have revealed novel promising classes of non-isomerizable combretastatin A-4 analogues,[17] including 1,1-diarylethylenes **1**,[18,19] 1,1-diarylethanes **2**[20] and aza*iso*erianin derivatives **3**[21] (Figure 1). Thus, we have demonstrated that it is possible to replace the *Z*-1,2-diarylethylene scaffold of CA-4 by a 1,1-diarylethylene one giving *iso*CA-4, a structural isomer of CA-4, with biological activities comparable to that of the natural product.[18, 19, 22] We have next showed that it is possible to reduce the double bond of *iso*CA-4 to furnish (±)-*iso*erianin **2a**,[20] which also displayed excellent anti-cancer activities comparable to that of natural erianin.[23] Very recently, we have prepared aza*iso*erianin derivatives **3** and we were pleased to observe that such compounds were as potent as their *C*-congeners.[21] The structural features of all the related compounds **1-3** are (*i*) the conformational relationship of the A and B rings which must be inclined toward each other with a dihedral angle ranging from 50-80°[17] and (*ii*) the presence of the trimethoxyphenyl A-ring. This latter fragment is an essential component to induce cytotoxicity of the compounds **1**-**3** in addition to its crucial role in tubulin binding.[20,24] It is interesting to note that in the *iso*CA4 analogues **1**-**3**, substitution of the 3,4,5-trimethoxyphenyl A-ring with heterocyclic structures has received very little attention.[25,26,27]

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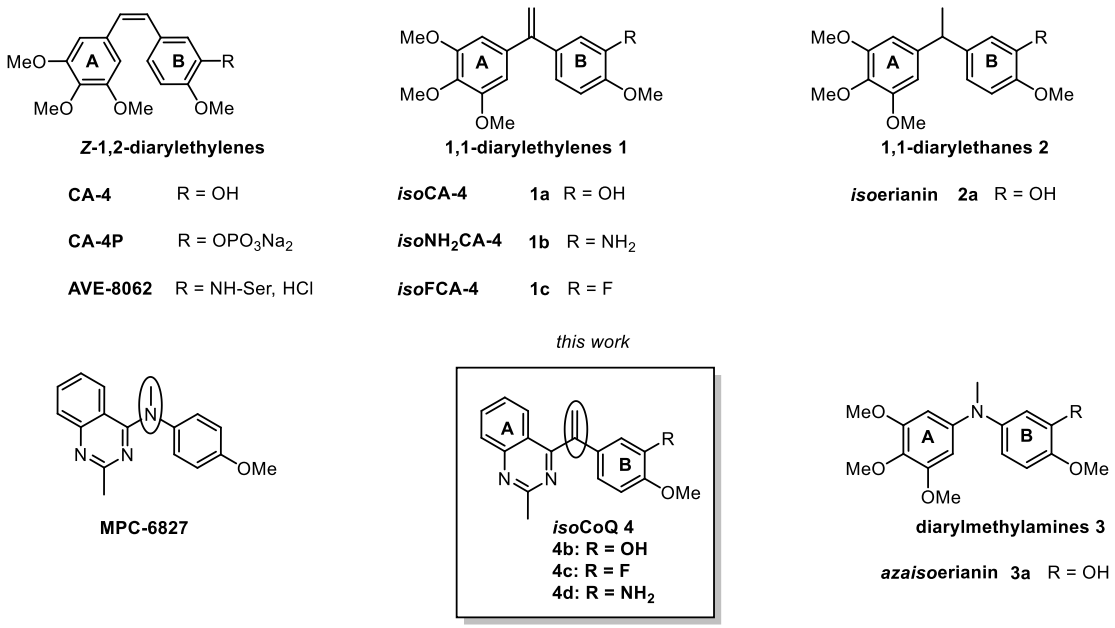


Figure 1. Representative inhibitors of tubulin polymerization and rational drug design from MPC-6827 and aza*iso*erianin **3** to novel 1-aryl-1-quinazoline ethylene analogues **4**.

In 2007, Cai[25,26] reported the discovery of MPC-6827, containing a quinazoline nucleus, as a potent cytotoxic (GI_{50} = 6 nM on HCT cells and others) and apoptosis small molecule (EC_{50} = 2 nM) that prevent the microtubule formation with potency equal to that of vinblastin. Due to structural resemblances between MPC-6827 and aza*iso*erianin derivatives **3**, we have prepared a series of novel derivatives **4**, as *iso*CA-4 analogues, in which a quinazoline ring replaced the "traditional" 3,4,5-trimethoxyphenyl unit.[28] Herein, we report the synthesis of *iso*combretaquinazolines **4** (*iso*CoQ) along with their biological properties that will allow us to conclude whether the 3,4,5-trimethoxyphenyl A-ring is as crucial as originally postulated, and about the possibility to replace it in these derivatives while maintaining an antitumor activity of first order. Our goal is the further definition of structure-activity relationships among this class of substituted quinazolines as effective agents for the treatment of solid tumors. Our initial results with 14 *iso*combretaquinazoline derivatives **4** are presented in this paper.

Results and Discussion

Chemistry.

The retrosynthetic analysis of the target *iso*CoQ **4** is outlined in Figure 2. According to *path a*, we first envisioned that quinazolines of type **4** could be prepared in a convergent manner from methylketones **6** through the palladium coupling reactions of their *N*-tosylhydrazones with various aryl halides according to a recent methodology developed by Barluenga.[29] Quinazolinemethylketones **6** should be available by the cyanation of 4-chloroquinazolines **5** followed by the addition of methylmagnesium halide on the nitrile function of **8**. An alternative approach (*path b*), consists in the palladium-catalyzed couplings of variously substituted-*N*-tosylhydrazones of type **7**, available from their corresponding ketones, with quinazolines **5a**,**b** bearing on the 4-position a chlorine atom.
At the outset of this work, and according to *path a* (Scheme 1), we first transformed under palladium catalysis, the 4-chloroquinazoline **5a** into 2-methylquinazoline-4-carbonitrile **8a** using $Zn(CN)_2$ as the nucleophile in a 82% yield.[30]

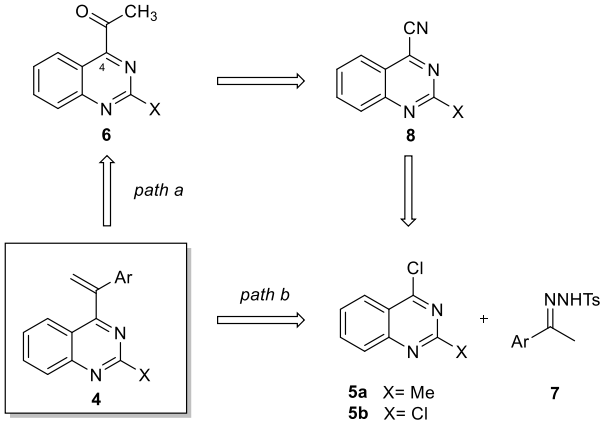
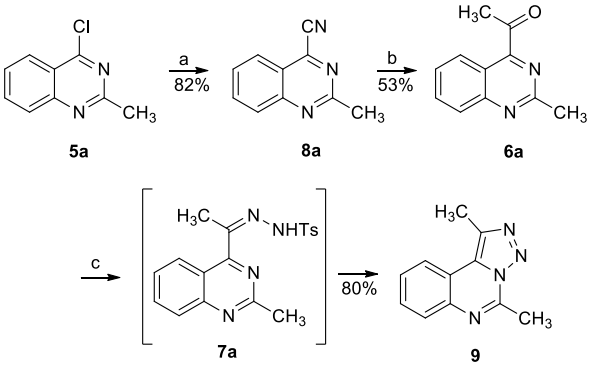


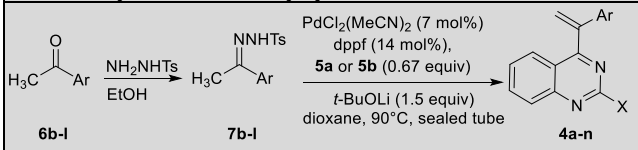
Figure 2. Retrosynthetic analysis of *iso*CoQ **4**.



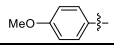
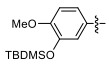
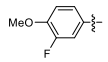
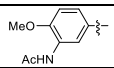
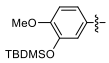
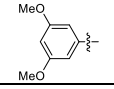
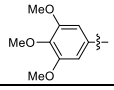
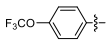
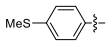
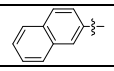
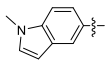
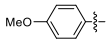
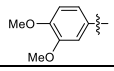
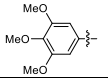
Scheme 1. Attempt to the synthesis of **7a**. Reagents and conditions: a) $Zn(CN)_2$, $Pd(PPh_3)_4$ (10 mol%), DMF, 120 °C; b) CH_3MgI (2 equiv), Et_2O, -15 °C, then HCl; c) $TsNHNH_2$ (2 equiv), EtOH, 80 °C.

Next, reaction of **8a** with CH_3MgI (2 equiv) at -15 °C in Et_2O furnished the methylketone **6a** (53%). However, despite all our efforts, we were unable to prepare the desired tosylhydrazone

Table 1. Synthesis of *N*-tosylhydrazones **7** and *iso*CoQ **4a-n**.

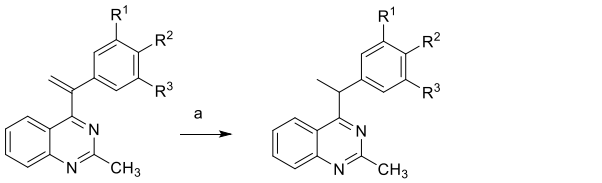


6b-l **7b-l** **4a-n**

Entry	Ar		Yield[c] (%)	X		Yield[c] (%)
1		**7b**	62	Me	**4a**	50
2		**7c**	73	"	**4b**	34[a]
3		**7d**	73	"	**4c**	46
4		**7e**	86	"	**4d**	27[b]
5		**7f**	79	"	**4e**	48
6		**7g**	86	"	**4f**	36
7		**7h**	81	"	**4g**	40
8		**7i**	93	"	**4h**	78
9		**7j**	82	"	**4i**	43
10		**7k**	82	"	**4j**	36
11		**7l**	76	"	**4k**	46
12		**7b**	62	Cl	**4l**	46
13		**7f**	79	"	**4m**	19
14		**7h**	81	"	**4n**	34

[a] The crude mixture was treated for 6 h with K_2CO_3 (2 equiv) in MeOH at rt. [b] The crude mixture was treated with KOH (20 equiv) in MeOH at 100 °C for 24 h in a sealed tube. [c] Yields of isolated products.

7a, useful for further coupling reactions under Pd-catalysis. Whatever the experimental conditions tested using methanol or ethanol as the solvents, by varying the amount of hydrazine, or by using with or without microwave irradiation at 80 °C, hydrazine **7a**, formed *in situ*, rearranged to triazole **9**, as it was previously reported by Reimlinger in the isoquinoline series.[31] Facing this difficulty, the synthesis of *iso*CoQ **4a-n** was next envisioned according to *path b* (Figure 2) involving the Pd-catalyzed coupling of 4-chloroquinazolines **5a**,**b** with several *N*-tosylhydrazones **7b-l** (Table 1). These latter compounds were prepared from their corresponding ketones in EtOH with satisfactory yields ranging from 62 to 93%. All *N*-tosylhydrazones **7** were successfully coupled with **5a** and **5b** by using $PdCl_2(MeCN)_2$ as the catalyst, 1,1'-bis(diphenylphosphino)ferrocene (dppf) as the ligand, *t*-BuOLi as the base in hot dioxane in a sealed tube.[32] All coupling yields were modest (19-78%) but afforded sufficient quantities of *iso*CoQ derivatives for preliminary biological tests. For the preparation of quinazolines having a OH or NH_2 substituent (**4b** and **4d**), their precursors hydrazones were used as silylated ether **7c** or acetamide **7e**, which were deprotected after the coupling step under basic conditions (entries 2 and 4). When the Pd-coupling sequence was achieved with **5b** bearing two chlorine atoms on the C2 and C4 position of the quinazoline nucleus, the results observed in entries 12-14 clearly indicate that the C4-Cl bond is more reactive than the C2-Cl bond, which remain unchanged under our experimental conditions after a careful examination of the crude mixture.



4a, 4e-g

10a, R^1 = H, R^2 = OMe, R^3 = H (99%)
10b, R^1 = OMe, R^2 = OMe, R^3 = H (97%)
10c, R^1 = OMe, R^2 = H, R^3 = OMe (89%)
10d, R^1 = OMe, R^2 = OMe, R^3 = OMe (97%)

Scheme 2. Reduction of **4a** and **4e**-**g** into **10a**-**d.** Reagents and conditions: a) H_2, Pd/C, EtOAc, 20 °C, 12 h.

Finally, because the double bond present in *iso*CA-4 can be reduced to furnish *iso*erianin derivatives with no significant loss of anticancer properties, we next reduced the ethylene double bond of *iso*CoQ derivatives **4a** and **4e-g** into **10a-d** with quantitative yields (Scheme 2).

Biology

(A) *In Vitro* Cell Growth Assay

Table 2. Cytotoxicities (GI_{50}) of compounds **4** and **10** against human colon carcinoma cell line (HCT116)[c]. Data are the mean of 3 experiments.

Cpnd	**4a**	**4b**	**4c**	**4d**	**4e**
GI_{50}[a] (nM)	35 ± 2	6.1 ± 0.5	14.8 ± 3.4	10.1 ± 3.2	219 ± 12
Cpnd	**4f**	**4g**	**4h**	**4i**	**4j**
GI_{50}[a] (nM)	Na[b]	Na[b]	3740 ± 230	50 ± 15	138 ± 37
Cpnd	**4k**	**4l**	**4m**	**4n**	**10a**
GI_{50}[a] (nM)	17.8 ± 2.6	36.7 ± 0.75	96 ± 4	Na[b]	146 ± 42
Cpnd	**10b**	**10c**	**10d**	CA-4	*iso*CA-4[d]
GI_{50}[a] (nM)	1270 ± 700	Na[b]	Na[b]	2.1 ± 1	2.4 ± 0.5

[a] A sample's concentration, which produces a 50% reduction in cell growth. [b] Non active. [c] HCT116, colon carcinoma; [d] The GI_{50} values for *iso*CA-4 and CA-4 were determined in this study.

All new quinazolines **4a**-**n** and **10a**-**d** were evaluated in a preliminary assay for their cytotoxic effects against human colon carcinoma (HCT116) cell line using CA-4,[33] and *iso*CA-4 as reference compounds (Table 2). Seven compounds **4a**, **4b**, **4c**, **4d**, **4i**, **4k** and **4l** were found to display strong growth

inhibitory activity against HCT116 cells with GI_{50} values lower to 50 nM. As we have previously observed with other *iso*CA-4 analogues,[Erreur ! Signet non défini.,21,34,35] compounds **4b, 4c** and **4d**, having the greatest resemblance to *iso*CA-4, *iso*FCA-4 and *iso*NH_2CA-4, respectively, possessed the highest potency, inhibiting the growth of HCT116 with GI_{50} values ranging from 10-18 nM. These GI_{50} values are comparable to those obtained with *iso*CA-4 clearly indicating that it is possible to replace the 3,4,5-trimethoxyphenyl A-ring of *iso*CA-4 by a quinazoline ring with no significant loss of biological activity. Comparing the 4-methoxy derivative **4a** with the 4-thiomethyl analogue **4i** indicates that it is also possible to replace the 4-methoxy substitutent of the B-ring with a minimal loss of activity (35 nM *vs* 50 nM, respectively). As it was reported in the CA-4 series,[27,36] our findings show that a *N*-methyl indole ring could be introduced as B-ring (**4k**; GI_{50} = 18 nM) in place of the 3-hydroxy-4-methoxyphenyl ring. Replacement of the 2-methyl group of quinazoline **4a** with a chlorine atom was examined and it is interesting to note that compound **4l** (GI_{50} = 37 nM), with a chlorine atom at the C-2 position, maintains a high cytotoxicity level as compared to **4a** (GI_{50} = 35 nM). Finally, reducing the ethylene bond of quinazolines **4a** and **4e-g** to give compounds **10a-d** resulted in a nearly complete loss of activity indicating that the double bond between the quinazoline and the B-ring is critical for activity.

(B) Cytotoxicity and Inhibition of Tubulin Polymerization for Selected Compounds

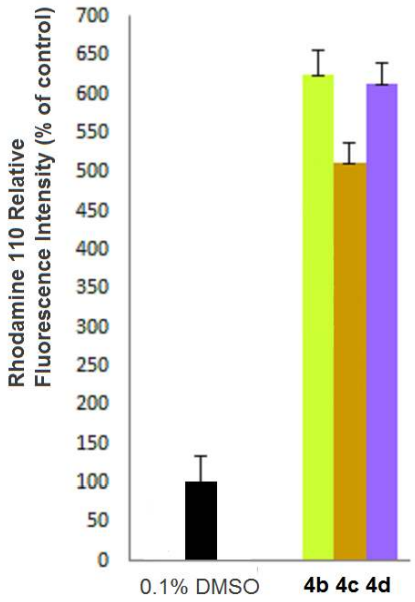
Table 3. Cytotoxic activity and inhibition of tubulin polymerization of selected *iso*CoQ compounds **4**. Data are the mean of 3 experiments.

	Cytotoxicity GI_{50}[a] [nM]			ITP[c]
Cpnd	HCT116	K562[b]	U87[b]	IC_{50} [μM]
4a	35 ± 2	25.1 ± 3.8	41 ±1.7	2.1 ± 0.3
4b	6.1 ± 0.5	6.2 ± 0.14	5.3 ± 0.2	0.6 ± 0.1
4c	14.8 ± 3.4	31.5 ± 1.45	10.7 ± 0.35	1.0 ± 0.15
4d	10.1 ± 3.2	9.5 ± 0.9	20.1 ± 7.2	2.3 ± 0.5
4i	50 ± 15	40.5 ± 1.75	93 ± 3.7	2.9 ± 0.4
4k	17.8 ± 2.6	20.7 ± 1.5	15.4 ± 4.1	1.6 ± 0.3
4l	36.7± 0.75	22.2 ± 3.0	41 ± 1.7	1.9 ± 0.15
*Iso*CA-4[d]	2.4 ± 0.5	4.98 ± 1.35	6.8 ± 0.1	1.0 ± 0.1

[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; K562, myelogenous leukaemia; U87, glioblastoma. [c] ITP, Inhibition of Tubulin Polymerization; IC_{50} is the concentration of compound required to inhibit 50% of the rate of microtubule assembly (average of three experiments). [d] The GI_{50} and IC_{50} values (cytotoxicity and ITP, respectively) for *iso*CA-4 was determined in this study.

We next have investigated the effect of the most bioactive derivatives (**4a-d**, **4i**, **4k**,**l**) having a low GI_{50} value on HCT116 cells, on the proliferation of two other tumor cell lines, myelogenous leukemia (K562) and human primary glioblastoma (U87). The results depicted in Table 3 revealed that all selected *iso*CoQ compounds **4** which retain a high level

Figure 4. Apoptotic effects of **4b** (green), **4c** (brown) and **4d** (purple) in U87 cells. The results are expressed in the percentage of apoptotic cells detected following 24 h of treatment with **4b**-**d** at 25 nM. Data are the mean of 3 experiments; errors bars represent the SD.

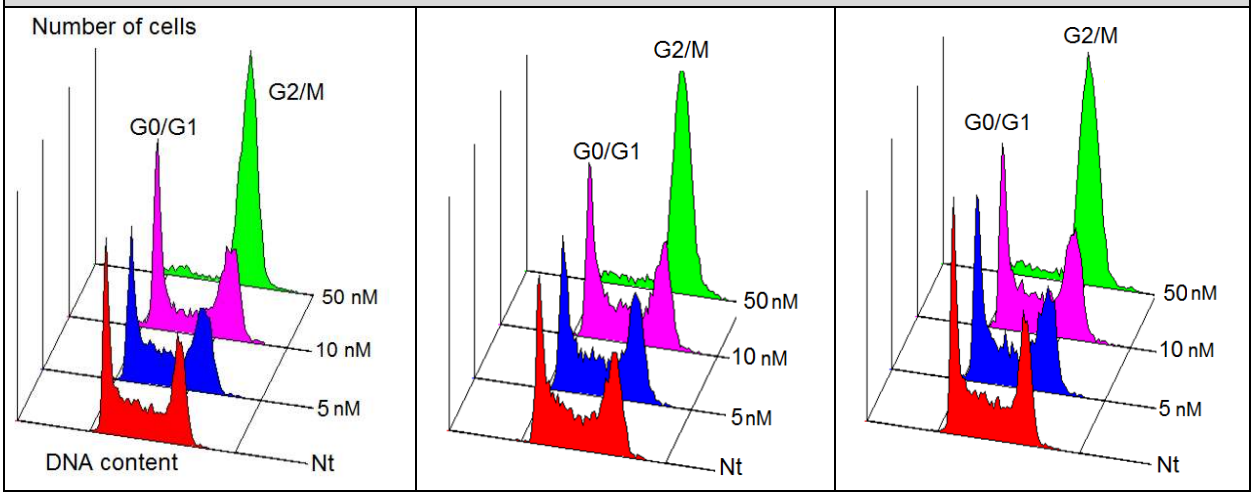


of cytotoxicity against HCT116 also displayed a nanomolar level of cytotoxicity regardless of the origin of the tumor cells. For example, the phenolic derivative **4b**, having the greatest resemblance with *iso*CA-4 and CA-4 remained the most cytotoxic agent against K562 and U87 with GI_{50} values inferior to 10 nM (5-6 nM). As it was previously described,[Erreur ! Signet non défini., 37] various modifications are possible on the B-ring of *iso*combretaquinazolines, as replacing the 3'OH group by a hydrogen, a fluorine atom or a NH_2 function, yielding potent compounds. To confirm that the antiproliferative activities of these derivatives, like those of CA-4 and *iso*CA-4, were related to an interaction with the microtubule system, all selected *iso*CoQ **4** having low IC_{50} values against various cancer cells were evaluated for their inhibitory effect on tubulin assembly. As expected, all tested compounds **4** strongly inhibited tubulin assembly with comparable IC_{50} values of 0.6-2.9 μM. Again, the most cytotoxic agent **4b** is also the most efficient derivative to inhibit tubulin polymerization with an IC_{50} value of 0.6 μM, two times lower than that of *iso*CA-4 (IC_{50} = 1.0 μM).

(C) Cell Cycle Analysis and Apoptosis

Because molecules exhibiting activity on tubulin should cause the alteration of cell cycle parameters leading to a preferential G_2/M arrest,[38] we next investigated the effects of the most potent *iso*combretaquinazolines **4b**-**d** on the cell cycle. Cancer cells were cultured without (not treated; Nt) or with **4b**-**d** at increasing concentrations and the cell cycle distribution was analyzed by flow cytometry after 24 h of treatment using the standard propidium iodide procedure. Cell cycle analysis on HCT116 cells (Figure 3) showed that similarly **4b**, **4c** and **4d** caused a massive cell accumulation in the G_2/M phase of the

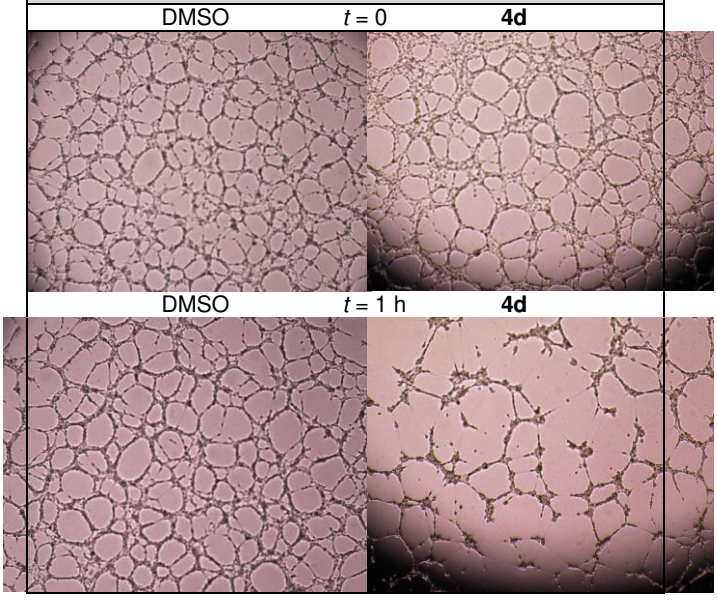
Figure 3. Effects of **4b** (left)**, 4c** (middle) and **4d** (right) at increasing concentrations on cell cycle distribution in HCT116 cell lines (Human Colon Carcinoma). Nt: no treatment.



cell cycle at the concentration of 50 nM. We examined whether the very bioactive quinazoline derivatives **4b**-**d**, in addition to arresting mitosis in the G_2/M phase, also triggered cell death in U87 cells using caspases 3 and 7 standard assays.[39] U87 cells were incubated for 24 h with **4b**, **4c** and **4d** at low concentrations and the activity of caspases was measured by monitoring the cleavage of the fluorogenic substrate Z-DEVD-R110 in U87 cells. As observed in Figure 4, **4b**, **4c** and **4d** can strongly induce caspases 3 and 7 at the concentration of 25 nM.

(D) Effects of *iso*CoQ 4d on HUVEC Organization

Figure 5. *In vitro* effects of **4d** on HUVEC cells (Human Umbilical Vein Endothelial Cells) at a concentration of 50 nM on new formed vessels after 1 h of treatment.



The *iso*combretaquinazoline **4d** bearing a NH_2 group on C3' position was evaluated for its ability to disrupt a network of capillary-like structures formed by human umbilical vein endothelial cells (HUVECs) on Matrigel. On this matrix, HUVECs spontaneously align forming a network of interconnecting cords, mimicking the breakable tumor vasculature. After a short reaction time of 1 h, **4d** disrupts the vessels-like structure and the entire integrity of the network at a concentration of 50 nM (Figure 5). One note that at this concentration of 50 nM and after one hour of treatment, **4d** was not cytotoxic against HUVECs (data not shown), indicating that the strong observed disrupting effects are only due to the possible antivascular properties of **4d**.

(E) Docking Study

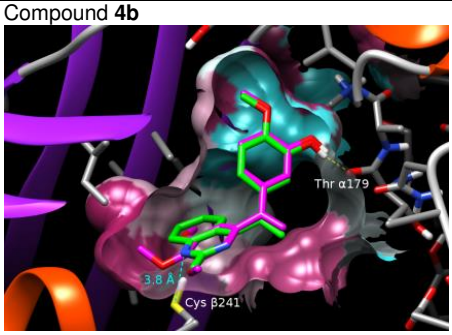
Docking experiments with **4b-d** and indolo-derivative **4k** showed that these bioactive *iso*combretaquinazoline compounds could adopt a similar conformation within the colchicine-binding site in tubulin (Figure 6). In this hypothetical common binding mode, the methylquinazoline ring would occupy one of the major lipophilic pockets belonging to the β subunit (which is also supposed to accomodate A-ring from *iso*CA-4). Within this pocket, N-1 nitrogen atom in the heteroaromatic system would be in a close enough proximity of the SH group belonging to Cys β241 to permit the establishment of a strong interaction with this residue. Study of the variations in the B-ring suggested that binding of derivatives **4b** and **4d** might be reinforced by the formation of a hydrogen bond between their O−H or N−H moiety (respectively) and the backbone oxygen belonging to Thr α179.

Conclusion

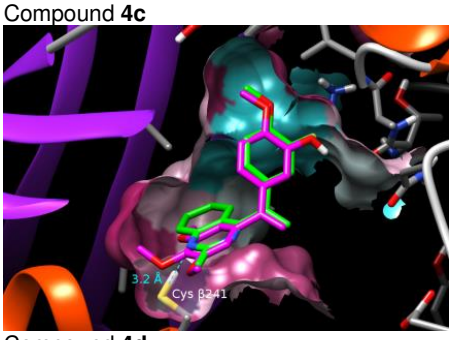
We have designed, synthesized and evaluated a series of *iso*combretaquinazolines devoid of the 3,4,5-trimethoxyphenyl ring. Many of these original compounds displayed a nanomolar level of cytotoxicity against various cancer cell lines and inhibited tubulin polymerization at a micro or submicromolar level. Our best derivatives arrested the cellular cycle in the G2/M phase at a low concentration of 50 nM in HCT116 cells. Moreover, preliminary *in vitro* results reveal that **4d** disrupt vessels-like structure on Matrigel and is a potent vascular disrupting agent.

Figure 6. Putative binding modes and superimposition of compounds **4b-d** and **4k** (green) and *iso*CA-4 (magenta) in the colchicine binding site of tubulin. Solvent-accessible surface is colored according to its local polarity (maroon: hydrophobic, cyan: polar). S(PLP) values[50] for *iso*CoQ **4b-d** and **4k**: -65.32, -63.30, -65.20, -65.80, respectively.

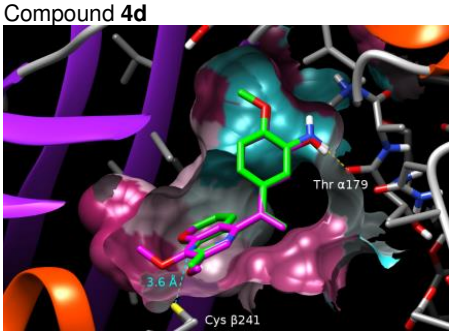
Compound **4b**



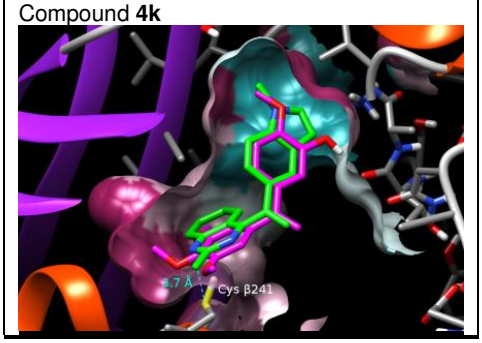
Compound **4c**



Compound **4d**



Compound **4k**



For the first time, all of these results reveal that the replacement of the 3,4,5-trimethoxyphenyl ring by a quinazoline nucleus is possible in the *iso*CA-4 series. Modification of heterocyclic systems is in progress in our lab and will be published later.

Experimental Section

Chemistry

General considerations

NMR spectra were performed on a Bruker AVANCE 300 (^{1}H, 300 MHz; ^{13}C, 75 MHz) or Bruker AVANCE 400 (^{1}H, 400 MHz; ^{13}C, 100 MHz). Unless otherwise stated, $CDCl_3$ was used as solvent. Chemical shifts δ are in ppm, and the following abbreviations are used: singlet (s), doublet (d), triplet (t), multiplet (m), broad singlet (brs). Mass spectra were obtained using a Bruker Esquire electrospray ionization apparatus. High resolution mass spectra were recorded on a MicrotofQ Bruker Daltonics. Thin-layer chromatography was performed on silica gel 60 plates with a fluorescent indicator and visualized under a UVP Mineralight UVGL-58 lamp (254 nm) and with a 7% solution of phosphomolybdic acid in ethanol. Flash chromatography was performed using silica gel 60 (40-63 µm, 230-400 mesh ASTM) at medium pressure (200 mbar). All solvents were distilled and stored over 4 Å molecular sieves before use. All reagents were obtained from commercial suppliers unless otherwise stated. Organic extracts were, in general, dried over magnesium sulphate ($MgSO_4$) or sodium sulphate (Na_2SO_4).

Procedure for the synthesis of 1-(2-Methylquinazolin-4-yl)ethanone 6a

A solution of methylmagnesium iodide solution in Et_2O [3M] (3.42 mL, 10.28 mmol) was added to a stirred solution of 4-cyano, 2-methylquinazoline **8** (870 mg, 5.14 mmol) in Et_2O (20 mL) at -15 °C for 1 h. Then, the reaction mixture was allowed to warm to room temperature. The reaction mixture was acidified with HCl 2N (8 mL) and the product was extracted with diethyl ether (2 x 15 mL). The organic layers were combined and washed with aqueous $NaHCO_3$ and brine. After concentration, the reaction mixture was purified by silica gel chromatography to afford **6a** as a pale yellow colored liquid (53% yield). Rf 0.6 (Cyclohexane/EtOAc, 7/3). IR (neat, cm^{-1}): 2927, 1696, 1613, 1557, 1484, 1358, 1144. ^{1}H NMR (300 MHz, $CDCl_3$): 8.67 (d, 1H, *J* = 8.5 Hz), 7.99 (d, 1H, *J* = 8.5 Hz), 7.88 (ddd, 1H, *J* = 8.5 Hz, *J* = 6.9 Hz, *J* = 1.4 Hz), 7.63 (ddd, 1H, *J* = 8.3 Hz, *J* = 6.9 Hz, *J* = 1.2 Hz), 2.94 (s, 3H), 2.83 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 202.3, 163.6, 159.6, 152.7, 134.2, 128.5, 128.3, 126.5, 118.8, 28.2, 26.4. *m/z* MS (APCI$^+$): 187 (M + H)$^+$. HRMS (ESI$^+$): m/z calculated for $C_{11}H_{11}N_2O$ [M+H]$^+$ 187.0866; found 187.0877.

Procedure for the synthesis of 1,5-Dimethyl-[1,2,3]triazolo[1,5-c]quinazoline 9

To a solution of **6a** (0.537 mmol) in EtOH (4 mL) was added *p*-toluenesulfonylhydrazide (200 mg, 1.07 mmol). The resulting solution was stirred at 80 °C for 30 min. After concentration, the reaction mixture was purified by silica gel chromatography to afford the compound **9** as a white solid (80% yield). M.p.: 158.2 °C. Rf 0.4 (Cyclohexane/EtOAc, 7/3). IR (neat, cm^{-1}): 1631, 1475, 1430, 1390, 1361, 1024. ^{1}H NMR (300 MHz, $CDCl_3$): 8.09 (dd, 1H, *J* = 7.8 Hz, *J* = 1.4 Hz), 7.93 (dd, 1H, *J* = 8.0 Hz, *J* = 1.1 Hz), 7.70-7.58 (m, 2H), 3.08 (s, 3H), 2.86 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 146.1, 140.0, 136.9, 130.1, 129.0, 128.4, 128.3, 122.8, 119.2, 19.9, 12.8. *m/z* MS (APCI+): 199 (M + H)$^+$.

General procedure for the synthesis of *N*-tosylhydrazones 7b-i

To a solution of ketones **6b-i** (1.75 mmol) in EtOH (20 mL) was added *p*-toluenesulfonylhydrazide (1.75 mmol) and the mixture

was stirred at reflux for 6 h. After cooling, the resulting *N*-tosylhydrazone was filtered and washed with Et_2O.

N-(1-(3-((*tert*-Butyldimethylsilyl)oxy)-4-methoxyphenyl)ethylidene)-4-methylbenzenesulfonohydrazide **7c**

73% yield, white solid, Mp.: 171.3 °C. TLC: Rf 0.4 (Cyclohexane/EtOAc, 7/3). IR (neat, cm^{-1}): 3231, 1598, 1507, 1423, 1305, 1270, 1168. ^1H NMR (300 MHz, CD_3COCD_3): 9.17 (brs, 1H), 7.87 (d, 2H, *J* = 8.3 Hz), 7.38 (d, 2H, *J* = 8.0 Hz), 7.32 (d, 1H, *J* = 2.2 Hz), 7.25 (dd, 1H, *J* = 8.5 Hz, *J* = 2.2 Hz), 6.92 (d, 1H, *J* = 8.5 Hz), 3.83 (s, 3H), 2.40 (s, 3H), 2.05 (s, 3H), 1.02 (s, 9H), 0.17 (s, 6H). ^{13}C NMR (75 MHz, CD_3COCD_3): 153.6, 153.3, 145.6, 144.4, 137.7, 131.5, 130.1 (2C), 128.9 (2C), 121.3, 119.0, 112.2, 55.8, 26.3 (3C), 21.5, 19.0, 13.7, -4.41 (2C). *m/z* MS (ESI^+): 449 $(M + H)^+$. HRMS (ESI^+): *m/z* calculated for $C_{22}H_{32}N_2NaO_4SSi$ $[M+Na]^+$ 471.1744; found 471.1757

N-(1-(3-Fluoro-4-methoxyphenyl)ethylidene)-4-methylbenzene sulfonohydrazide **7d**

73% yield, white solid, M.p.: 200.1 °C. TLC: Rf 0.2 (Cyclohexane/EtOAc, 7/3). IR (neat, cm^{-1}): 3029, 1510, 1432, 1309, 1272, 1164. ^1H NMR (300 MHz, CD_3COCD_3): 7.88-7.76 (m, 3H), 7.69 (d, 1H, *J* = 12.2 Hz), 7.36 (d, 2H, *J* = 7.9 Hz), 7.23 (t, 1H, *J* = 8.5 Hz), 3.97 (s, 3H), 2.53 (s, 3H), 2.40 (s, 3H), NH not seen. ^{13}C NMR (75 MHz, CD_3COCD_3): 154.3, 151.0, 144.2 (2C), 137.7, 131.5, 130.0 (2C), 128.8 (2C), 126.8, 116.0 (d, 1C, *J* = 18.8 Hz), 113.7, 56.7, 26.4, 21.4. ^{19}F NMR (188, $CDCl_3$): -134.6 *m/z* MS (ESI^+): 359 $(M + Na)^+$. HRMS (ESI^+): *m/z* calculated for $C_{16}H_{17}FN_2NaO_3S$ $[M+Na]^+$ 359.0836; found 359.0854.

N-(2-Methoxy-5-(1-(2-tosylhydrazono)ethyl)phenyl)acetamide **7e**

86% yield, white solid, M.p.: 150.6 °C. TLC: Rf 0.7 (Cyclohexane/EtOAc, 3/7). IR (neat, cm^{-1}): 3179, 1692, 1583, 1536, 1486, 1423, 1325. ^1H NMR (300 MHz, DMSO): 10.27 (brs, 1H), 9.12 (brs, 1H), 8.34 (s, 1H), 7.82 (d, 2H, *J* = 8.3 Hz), 7.38 (d, 2H, *J* = 8.0 Hz), 7.31 (dd, 1H, *J* = 8.6 Hz, *J* = 2.2 Hz), 6.99 (d, 1H, *J* = 8.7 Hz), 3.83 (s, 3H), 2.36 (s, 3H), 2.09 (s, 6H). ^{13}C NMR (75 MHz, DMSO): 168.5, 152.9, 150.6, 143.1, 136.2, 129.6, 129.3 (2C), 127.7 (2C), 127.2, 122.3, 119.7, 110.5, 55.8, 23.9, 21.0, 14.0. *m/z* MS (ESI^+): 376 $(M + H)^+$, 398 $(M + Na)^+$. HRMS (ESI^+): *m/z* calculated for $C_{18}H_{21}N_3NaO_4S$ $[M+Na]^+$ 398.1145; found 398.1150.

N-(1-(3,4-Dimethoxyphenyl)ethylidene)-4-methylbenzene sulfonohydrazide **7f**

79% yield, white solid, M.p.: 177.4 °C. TLC: Rf 0.5 (Cyclohexane/EtOAc, 7/3). IR (neat, cm^{-1}): 3210, 2013, 1333, 1309, 1165, 1151. ^1H NMR (300 MHz, DMSO): 10.3 (brs, 1H), 7.83 (d, 2H, *J* = 8.3 Hz), 7.41 (d, 2H, *J* = 8.0 Hz), 7.17-7.14 (m, 2H), 6.92 (d, 1H, *J* = 8.3 Hz), 3.75 (s, 3H), 3.74 (s, 3H), 2.37 (s, 3H), 2.13 (s, 3H). ^{13}C NMR (75 MHz, DMSO): 153.2, 150.1, 148.4, 143.3, 136.1, 130.0, 129.3 (2C), 127.8 (2C), 119.3, 111.0, 108.7, 55.5, 55.3, 21.0, 14.0. m/z MS (ESI+): 371 $(M + Na)^+$. HRMS (ESI^+): *m/z* calculated for $C_{17}H_{20}N_2NaO_4S$ $[M+Na]^+$ 371.1036; found 371.1050.

4-Methyl-*N*-(1-(4-(trifluoromethoxy)phenyl)ethylidene)benzene sulfonohydrazide **7i**

93% yield, white solid, M.p.: 131.1 °C. TLC: Rf 0.3 (Cyclohexane/EtOAc, 7/3). IR (neat, cm^{-1}): 3219, 1597, 1509, 1339, 1315, 1258, 1161. ^1H NMR (300 MHz, $CDCl_3$): 8.38 (brs, 1H), 7.93 (d, 2H, *J* = 8.3 Hz), 7.66 (d, 2H, *J* = 8.9 Hz), 7.32 (d, 2H, *J* = 8.0 Hz), 7.16 (d, 2H, *J* = 8.1 Hz), 2.41 (s, 3H), 2.17 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 151,3, 150.2, 144.4, 136.0, 135.4, 129.8 (2C), 128.2 (2C), 127.9 (2C), 120.6 (2C), 120.5 (q, *J* = 257.6 Hz), 21.7, 13.6. ^{19}F NMR (188 MHz, $CDCl_3$): -58.16. HRMS (ESI^+): *m/z* calculated for $C_{16}H_{15}F_3N_2NaO_3S$ $[M+Na]^+$ 395.0648; found 395.0657.

4-Methyl-*N*-(1-(4-(methylthio)phenyl)ethylidene)benzene sulfonohydrazide **7j**

82% yield, white solid (unstable product, M.p.: 160.9 °C. TLC: Rf 0.4 (Cyclohexane/EtOAc, 7/3). IR (neat, cm-*1*): 1590, 1399, 1187, 1165, 1079, 1044. 1H NMR (300 MHz, CD_3COCD_3): 9.29 (brs, 1H), 7.87 (d, 2H, *J* = 8.3 Hz), 7.66 (d, 2H, *J* = 8.6 Hz), 7.38 (d, 2H, *J* = 8.2 Hz), 7.23 (d, 2H, *J* = 8.6 Hz), 2.49 (s, 3H), 2.38 (s, 3H), 2.22 (s, 3H). ^{13}C NMR (75 MHz, CD_3COCD_3): 153.2, 144.5, 141.5, 137.6, 135.1, 130.2 (2C), 128.8 (2C), 127.4 (2C), 126.3 (2C), 21.4, 15.0, 14.6. *m/z* MS (ESI+): 335 (M + H)+, 357 $(M + Na)^+$. HRMS (ESI^+): *m/z* calculated for $C_{16}H_{19}N_2O_2S_2$ $[M+Na]^+$ 335.0882; found 335.0888.

4-Methyl-*N*-(1-(naphthalen-2-yl)ethylidene)benzene sulfonohydrazide **7k**

82% yield, white solid, M.p.: 167.1 °C. TLC: Rf 0.4 (Cyclohexane/EtOAc, 7/3). IR (neat, cm^{-1}): 3027, 1596, 1406, 1332, 1163. ^1H NMR (300 MHz, CD_3COCD_3): 9.44 (brs, 1H), 8.16 (s, 1H), 8.01 (dd, 1H, *J* = 8.8 Hz, *J* = 1.8 Hz), 7.94-7.84 (m, 5H), 7.54-7.48 (m, 2H), 7.40 (d, 2H, *J* = 8.0 Hz), 2.37 (s, 6H). ^{13}C NMR (75 MHz, CD_3COCD_3): 153.4, 144.6, 137.7, 136.2, 134.8, 134.1, 130.3 (2C), 129.5, 129.0 (2C), 128.7, 128.5, 127.7, 127.3, 127.2, 124.4, 21.5, 13.8. *m/z* MS (ESI+): 339 $(M + H)^+$. HRMS (ESI^+): *m/z* calculated for $C_{19}H_{18}N_2NaO_2S$ $[M+Na]^+$ 361.0981; found 361.0979.

4-Methyl-*N*-(1-(1-methyl-*1H*-indol-5-yl)ethylidene)benzene sulfonohydrazide **7l**

76% yield, white solid, M.p.: 189.5 °C. TLC: Rf 0.2 (Cyclohexane/EtOAc, 7/3). IR (neat, cm^{-1}): 1391, 1331, 1308, 1248, 1164. ^1H NMR (300 MHz, DMSO): 10.24 (brs, 1H), 7.87-7.80 (m, 3H), 7. 53 (dd, 1H, *J* = 8.7 Hz, *J* = 1.6 Hz), 7.42-7.38 (m, 3H), 7.32 (d, 1H, *J* = 3.0 Hz), 6.45 (d, 1H, *J* = 2.9 Hz), 3.78 (s, 3H), 2.37 (s, 3H), 2.22 (s, 3H). ^{13}C NMR (75 MHz, DMSO): 154.7, 143.1, 136.8, 136.4, 130.4, 129.3 (2C), 128.6, 127.6 (2C), 127.6, 119.2, 118.9, 109.5, 101.2, 32.5, 21.0, 14. 5. *m/z* MS (ESI^+): 342 $(M + H)^+$, 364 $(M + Na)^+$. HRMS (ESI^+): *m/z* calculated for $C_{18}H_{19}N_3NaO_2S$ $[M+Na]^+$ 364.1090; found 364.1105.

General procedure for the synthesis of products 4a-n

To a solution of *N*-tosylhydrazone **7b-i** (1.5 mmol), *t*-BuOLi (2.2 mmol), $PdCl_2(CH_3CN)_2$ (0.1 mmol), and dppf (0.2 mmol) in dioxane (1 mL) was added **5a**,**b** (1 mmol). The reaction vessel was sealed and then heated at 90 °C for 2 h. The resulting suspension was cooled to room temperature and filtered through a pad of Celite, eluting with EtOAc, to remove the inorganic salts. After concentration, the reaction mixture was purified by silica gel chromatography.

*4-[1-(4-Methoxy-phenyl)-vinyl]-2-methyl-quinazoline **4a:***

50% yield; yellow oil, Rf 0.3 (Cyclohexane/EtOAc, 7/3). IR (neat, cm^{-1}): 1608, 1554, 1511, 1250, 1180. ^1H NMR (300 MHz, $CDCl_3$): 7.97 (d, 1H, *J* = 8.5 Hz), 7.87 (d, 1H, *J* = 8.5 Hz), 7.81 (td, 1H, *J* = 8.5 Hz, *J* = 0.9 Hz), 7.41 (td, 1H, *J* = 8.3 Hz, *J* = 0.9 Hz), 7.23 (d, 2H, *J* = 8.9 Hz), 6.82 (d, 2H, *J* = 8.9 Hz), 6.04 (s, 1H), 5.46 (s, 1H), 3.78 (s, 3H), 2.93 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 169.9, 164.1, 159.8, 151.1, 145.0, 133.9, 131.4, 128.1, 128.0 (2), 127.1, 126.7, 121.7, 116.9, 114.1 (2C), 55.4, 26.8. *m/z* MS (ESI+): 277 $(M + H)^+$. HRMS (ESI^+): *m/z* calculated for $C_{18}H_{17}N_2O$ $[M+H]^+$ 277.1335; found 277.1335.

2-Methoxy-5-(1-(2-methylquinazolin-4-yl)vinyl)phenol ***4b***

Compound **4b** was prepared by the coupling of *N*-tosylhydrazone **7a** with **5a** (see the general procedure) followed by the deprotection of the –OTBS group: The resulting crude mixture after the coupling of **7a** with **5a** was dissolved in MeOH (1mL) and K_2CO_3 (2 mmol) was added to the mixture, and stirring was continued for 6 h. The resulting suspension was filtered and purified by silica gel chromatography. 34% yield, yellow oil, Rf 0.1 (Cyclohexane/EtOAc, 7/3). IR (neat, cm^{-1}): 1615, 1554, 1512, 1439, 1279, 1135. ^1H NMR (300 MHz, $CDCl_3$): 7.98 (d, 1H, *J* = 8.4 Hz), 7.87 (d, 1H, *J* = 8.3 Hz), 7.81 (td, 1H, *J* = 7.1 Hz, *J* = 1.2 Hz), 7.42 (t, 1H, *J* = 7.4 Hz), 6.92 (s, 1H), 6.75 (s, 2H), 6.12 (brs, 1H), 6.04 (s, 1H), 5.46 (s, 1H), 3.87 (s, 3H), 2.90 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 170.1, 164.0, 150.7, 147.1, 145.9, 145.0, 134.1, 132.3, 127.8, 127.1, 126.9, 121.8, 118.9, 117.4, 113.0, 110.8, 56.1, 26.5. *m/z* MS (ESI^+): 293 $(M + H)^+$. HRMS (ESI^+): *m/z* calculated for $C_{18}H_{17}N_2O_2$ $[M+H]^+$ 293.1285; found 293.1293.

4-[1-(3-Fluoro-4-methoxyphenyl)-vinyl]-2-methyl-quinazoline ***4c***

46% yield, yellow oil, Rf 0.2 (Cyclohexane/EtOAc, 7/3). IR (neat, cm^{-1}): 1615, 1554, 1517, 1275, 1134, 1027. ^1H NMR (300 MHz, $CDCl_3$): 7.99 (d, 1H, *J* = 8.3 Hz), 7.86-7.80 (m, 2H), 7.46-7.41 (td, 1H, *J* = 7.2 Hz, *J* = 1.0 Hz), 7.13 (dd, 1H, *J* = 12.5 Hz, *J* = 2.2 Hz), 6.96-6.92 (m, 1H), 6.85 (t, 1H, *J* = 8.5 Hz), 6.06 (s, 1H), 5.51 (s, 1H), 3.86 (s, 3H), 2.93 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 169.3, 164.1, 154.1, 150.9 (d, 1C, *J* = 23.7 Hz), 147.9 (d, 1C, *J* = 11.1 Hz), 144.1, 134.1, 131.9 (d, 1C, *J* = 6.3 Hz), 128.2, 126.9, 126.8, 122.9 (d, 1CH, *J* = 3.3 Hz), 121.6, 118.1, 114.3 (d, 1C, *J* = 19.4 Hz), 113.3 (d, 1C, *J* = 1.9 Hz), 56.4, 26.7. ^{19}F NMR (188 MHz, CDCl3): -132.7. *m/z* MS ($APCI^+$): 295 $(M + H)^+$. HRMS (ESI^+): *m/z* calculated for $C_{18}H_{16}FN_2O$ $[M+H]^+$ 295.1241; found 295.1252.

2-Methoxy-5-(1-(2-methylquinazolin-4-yl)vinyl)aniline ***4d***

Compound **4d** was prepared by the coupling of *N*-tosylhydrazone **7e** with **5a** (see the general procedure) followed by the deprotection of the –NHAc group: The resulting crude mixture after the coupling of **7e** with **5a** was dissolved in MeOH (1mL) and KOH (20 mmol) was added. The mixture was heated and stirred in sealed tube at 100 °C for 12 h. The resulting suspension was filtered and purified by silica gel chromatography to furnished compound **4d** as a yellow oil, 27% yield, Rf 0.1 (Cyclohexane/EtOAc, 7/3). IR (neat, cm^{-1}): 1614, 1567, 1553, 1441, 1330, 1219. ^1H NMR (300 MHz, CD_3COCD_3): 7.94-7.84 (m, 3H), 7.52-7.46 (m, 1H), 6.76 -6.72 (m, 2H), 6.54 (dd, 1H, *J* = 8.3 Hz, *J* = 2.3 Hz), 5.96 (s, 1H), 5.33 (s, 1H), 4.41 (brs, 2H), 3.81 (s, 3H), 2.79 (s, 3H). ^{13}C NMR (75 MHz, CD_3COCD_3): 170.7, 164.6, 152.1, 148.2, 147.1, 138.5, 134.4, 133.1, 128.9, 127.8, 127.3, 122.9, 116.6, 116.1, 113.0, 111.0, 55.8, 26.6. *m/z* MS (APCI+): 292 $(M + H)^+$. HRMS (ESI^+): *m/z* calculated for $C_{18}H_{18}N_3O$ $[M+H]^+$ 292.1444; found 292.1457.

4-[1-(3,4-Dimethoxyphenyl)-vinyl]-2-methyl-quinazoline ***4e***

48% yield, yellow oil, Rf 0.1 (Cyclohexane/EtOAc, 7/3). IR (neat, cm^{-1}): 1554, 1514, 1464, 1326, 1221, 1143. ^1H NMR (300 MHz, $CDCl_3$): 7.96 (d, 1H, *J* = 8.4 Hz), 7.87-7.78 (m, 2H), 7.41 (t, 1H, *J* = 7.6 Hz), 6.97 (d, 1H, *J* = 1.6 Hz), 6.76-6.68 (m, 2H), 6.03 (s, 1H), 5.48 (s, 1H), 3.84 (s, 3H), 3.41 (s, 3H), 2.93 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 169.7, 164.1, 151.1, 149.5, 149.1, 145.3, 133.9, 131.8, 128.1, 127.0, 126.7, 121.7, 120.1, 117.4, 111.1, 109.5, 56.0 (2C), 26.8. *m/z* MS ($APCI^+$): 307 $(M + H)^+$. HRMS (ESI^+): *m/z* calculated for $C_{19}H_{19}N_2O_2$ $[M+H]^+$ 307.1441; found 307.1452.

4-[1-(3,5-Dimethoxy-phenyl)-vinyl]-2-methyl-quinazoline ***4f***

36% yield, white solid, M.p.: 89.1-91.2 °C. Rf 0.3 (Cyclohexane/EtOAc, 7/3). IR (neat, cm^{-1}): 1592, 1460, 1424, 1294, 1206, 1161. ^1H NMR (300 MHz, $CDCl_3$): 7.96 (d, 1H, *J* = 8.4 Hz), 7.87-7.78 (m, 2H), 7.42 (td, 1H, *J* = 8.1 Hz, *J* = 1.0 Hz), 6.45 (d, 2H, *J* = 2.1 Hz), 6.41 (d, 1H, *J* = 2.0 Hz), 6.12 (s, 1H), 5.58 (s, 1H), 3.72 (s, 6H), 2.93 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 169.3, 164.1, 161.0 (2C), 151.1, 145.7, 141.0, 134.0, 128.1, 127.0, 126.8, 121.7, 119.5, 105.4 (2C), 100.2, 55.5 (2C), 26.8. *m/z* MS ($APCI^+$): 307 $(M + H)^+$. HRMS (ESI^+): *m/z* calculated for $C_{19}H_{19}N_2O_2$ $[M+H]^+$ 307.1441; found 307.1446.

2-Methyl-4-[1-(3,4,5-trimethoxyphenyl)-vinyl]-quinazoline ***4g***

40% yield, yellow solid, M.p.: 127-128 °C. Rf 0.2 (Cyclohexane/EtOAc, 7/3). IR (neat, cm^{-1}): 1553, 1506, 1464, 1410, 1331, 1242, 1128. ^1H NMR (300 MHz, $CDCl_3$): 7.97 (d, 1H, *J* = 8.5 Hz), 7.90-7.80 (m, 2H), 7.45 (td, 1H, *J* = 8.2 Hz, *J* = 1.1 Hz), 6.54 (s, 2H), 6.07 (s, 1H), 5.56 (s, 1H), 3.84 (s, 3H), 3.72 (s, 6H), 2.94 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 169.3, 164.1, 153.4 (2C), 151.2, 145.7, 138.6, 134.7, 134.0, 128.1, 127.0, 126.9, 121.7, 119.0, 104.5 (2C), 61.0, 56.3 (2C), 26.8. *m/z* MS (ESI^+): 359 $(M + Na)^+$. HRMS (ESI^+): *m/z* calculated for $C_{20}H_{21}N_2O_3$ $[M+H]^+$ 337.1547; found 337.1547.

2-Methyl-4-(1-(4-(trifluoromethoxy)phenyl)vinyl)quinazoline ***4h***

78%, yellow oil, Rf 0.3 (Cyclohexane/EtOAc, 7/3). IR (neat, cm^{-1}): 1554, 1509, 1490, 1254, 1207, 1164. ^1H NMR (300 MHz, $CDCl_3$): 8.00 (d, 1H, *J* = 8.2 Hz), 7.85-7.82 (m, 2H), 7.49-7.43 (td, 1H, *J* = 7.4 Hz, *J* = 1.1 Hz), 7.36 (d, 2H, *J* = 8.9 Hz), 7.15 (d, 2H, *J* = 8.1 Hz), 6.16 (s, 1H), 5.62 (s, 1H), 2.93 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 169.2, 164.1, 151.1, 149.3, 144.3, 137.4, 134.3, 128.3 (2C), 128.2, 127.1, 126.7, 121.6, 121.1 (2C), 120.4 (q, *J* = 256.5 Hz), 119.9, 26.8. ^{19}F NMR (188 MHz, $CDCl_3$): -58.23. *m/z* MS ($APCI^+$): 331 $(M + H)^+$. HRMS (ESI^+): *m/z* calculated for $C_{18}H_{14}F_3N_2O$ $[M+H]^+$ 331.1053; found 331.1064.

2-Methyl-4-(1-(4-(methylthio)phenyl)vinyl)quinazoline ***4i***

43% yield, yellow oil, Rf 0.4 (Cyclohexane/EtOAc, 7/3). IR (neat, cm^{-1}): 1751, 1554, 1492, 1326, 1215. ^1H NMR (300 MHz, $CDCl_3$): 7.96 (d, 1H, *J* = 8.4 Hz), 7.83-7.76 (m, 2H), 7.42-7.36 (m, 1H), 7.20 (d, 2H, *J* = 8.6 Hz), 7.14 (d, 2H, *J* = 8.7 Hz), 6.09 (s, 1H), 5.52 (s, 1H), 2.91 (s, 3H), 2.42 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 169.4, 164.1, 151.1, 145.0, 139.2, 135.4, 133.9, 128.1, 127.0 (2C), 126.9, 126.8, 126.3 (2C), 121.6, 118.1, 26.7, 15.5. *m/z* MS ($APCI^+$): 293 $(M + H)^+$. HRMS (ESI^+): *m/z* calculated for $C_{18}H_{17}N_2S$ $[M+H]^+$ 293.1107; found 293.1119.

2-Methyl-4-(1-(naphthalen-2-yl)vinyl)quinazoline ***4j***

36% yield, white solid, Mp.: 113.6 °C. TLC: Rf 0.2 (Cyclohexane/EtOAc, 7/3). IR (neat, cm^{-1}): 1614, 1568, 1490, 1325, 1168, 906. ^1H NMR (300 MHz, $CDCl_3$): 8.01 (d, 1H, *J* = 8.4 Hz), 7.86 (d, 1H, *J* = 8.4 Hz), 7.83-7.77 (m, 3H), 7.68-7.65 (m, 1H), 7.60-7.57 (m, 2H), 7.46-7.34 (m, 3H), 6.28 (s, 1H), 5.68 (s, 1H), 2.98 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 169.6, 164.1, 151.1, 145.6, 136.1, 134.0, 133.3, 133.1, 128.5, 128.4, 128.0, 127.6, 127.0, 126.8, 126.5, 126.4, 126.4, 124.1, 121.7, 119.3, 26.8. *m/z* MS (ESI^+): 297 $(M + H)^+$. HRMS (ESI^+): *m/z* calculated for $C_{21}H_{17}N_2$ $[M+H]^+$ 297.1386; found 297.1393.

2-Methyl-4-(1-(1-methyl-1H-indol-5-yl)vinyl)quinazoline ***4k***

46% yield, red oil, Rf 0.2 (Cyclohexane/EtOAc, 7/3). IR (neat, cm^{-1}): 1612, 1565, 1490, 1332, 1246. ^1H NMR (300 MHz, $CDCl_3$): 8.00 (d, 1H, *J* = 8.4 Hz), 7.91 (dd, 1H, *J* = 8.4 Hz, J = 0.7 Hz), 7.83-7.77 (ddd, 1H, *J* = 8.4 Hz, *J* = 6.9 Hz, *J* = 1.4 Hz), 7.50-7.49 (m, 1H), 7.40-7.34 (ddd, 1H, *J* = 8.4 Hz, *J* = 6.7 Hz, *J* = 1.3 Hz), 7.30-7.27 (m, 2H), 7.03 (d, 1H, *J* = 3.1 Hz), 6.41 (d, 1H, *J* = 3.1 Hz), 6.12 (d, 1H, *J* = 0.7 Hz), 5.53 (d, 1H, *J* = 0.7 Hz), 3.77 (s, 3H), 2.99 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 170.6, 164.1, 151.0, 146.8, 136.7, 133.7, 130.7, 129.7, 128.6, 127.9, 127.4, 126.6, 121.9, 120.6, 119.7, 116.7, 109.5, 101.7, 33.0, 26.8. *m/z* MS ($APCI^+$): 300 $(M + H)^+$. HRMS (ESI^+): *m/z* calculated for $C_{20}H_{18}N_3$ $[M+H]^+$ 300.1495; found 300.1500.

2-Chloro-4-[1-(4-methoxyphenyl)-vinyl]-quinazoline ***4l***

46% yield, yellow brown oil, Rf 0.4 (Cyclohexane/EtOAc, 7/3). IR (neat, cm^{-1}): 1673, 1597, 1511, 1246, 1175. ^1H NMR (300 MHz, $CDCl_3$): 8.0 (dd, 1H, *J* = 8.5 Hz, *J* = 1.1 Hz), 7.90-7.85 (m, 2H), 7.49 (td, 1H, *J* = 8.5 Hz, *J* = 1.1 Hz), 7.23 (d, 2H, *J* = 8.8 Hz), 6.84 (d, 2H, *J* = 8.8 Hz), 6.06 (s, 1H), 5.55 (s, 1H), 3.79 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 173.1, 160.1, 157.3, 152.7, 144.2, 135.1, 131.1, 128.1 (2C), 128.1, 127.9, 127.5, 122.4, 118.6, 114.3 (2C), 55.4. *m/z* MS ($APCI^+$): 297 $(M + H)^+$.

2-Chloro-4-[1-(3,4-dimethoxyphenyl)-vinyl]-quinazoline ***4m***

19% yield, yellow oil, Rf 0.3 (Cyclohexane/EtOAc, 7/3). IR (neat, cm-1): 1665, 1595, 1563, 1514, 1465, 1264, 1143. 1H NMR (300 MHz, CDCl3): 8.00 (d, 1H, J = 8.9 Hz), 7.91-7.85 (m, 2H), 7.49 (td, 1H, J = 8.2 Hz, J = 1.2 Hz), 6.95 (d, 1H, J = 2.0 Hz), 6.77 (d, 1H, *J* = 8.4 Hz), 6.71 (dd, 1H, J = 8.3 Hz, J = 2.0 Hz), 6.05 (s, 1H), 5.59 (s, 1H), 3.86 (s, 3H), 3.83 (s, 3H). 13C NMR (75 MHz, $CDCl_3$): 172.8, 157.2, 152.7, 149.7, 149.2, 144.5, 135.2, 131.5, 128.0, 128.0, 127.4, 122.4, 120.2, 119.2, 111.2, 109.6, 56.1, 56.0 . *m/z* MS ($APCI^+$): 327 $(M + H)^+$. HRMS (ESI^+): *m/z* calculated for $C_{18}H_{16}ClN_2O_2$ $[M+H]^+$ 327.0895; found 327.0904.

2-Chloro-4-[1-(3,4,5-trimethoxyphenyl)-vinyl]-quinazoline ***4n***

34% yield, white solid, M.p.: 135.1-136 °C. TLC: Rf 0.4 (Cyclohexane/EtOAc, 7/3). IR (neat, cm^{-1}): 1580, 1528, 1401, 1316, 1277, 1243, 1121. ^1H NMR (300 MHz, $CDCl_3$): 8.01 (d, 1H, *J* = 8.9 Hz), 7.92-7.87 (m, 2H), 7.55-7.49 (m, 1H), 6.52 (s, 2H), 6.09 (s, 1H), 5.66 (s, 1H), 3.85 (s, 3H), 3.66 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): 172.4, 157.2, 153.5 (2C), 152.7, 144.9, 138.8, 135.3, 134.3, 128.1 (2C), 127.3, 122.3, 120.8, 104.5 (2C), 61.1, 56.4 (2C). *m/z* MS (ESI^+): 357 $(M + H)^+$. HRMS (ESI^+): *m/z* calculated for $C_{19}H_{18}ClN_2O_3$ $[M+H]^+$ 357.1000; found 357.1010.

General procedure for the synthesis of quinazolines 10a-d

A solution of vinylquinazolines **4a**, **4e**, **4f** and **4g** (1 mmol) in EtOAc (1mL) was hydrogenated, at atmospheric pressure, in the presence of Pd/C (20%). After filtration over a pad of Celite, the solution was concentrated under reduced pressure, and the residue was purified by flash chromatography.

4-[1-(4-Methoxyphenyl)-ethyl]-2-methyl-quinazoline ***10a***

99% yield, brown oil, Rf 0.4 (Cyclohexane/EtOAc, 7/3). IR (neat, cm^{-1}): 2925, 1612, 1564, 1511, 1463, 1247, 1178. ^1H NMR (300 MHz, $CDCl_3$): 8.07 (d, 1H, *J* = 8.4 Hz), 7.91 (d, 1H, *J* = 8.4 Hz), 7.75-7.69 (m, 1H), 7.45-7.39 (m, 1H),7.24 (d, 2H, *J* = 8.7 Hz), 6.79 (d, 2H, *J* = 8.7 Hz), 4.95 (q, 1H, *J* = 6.9 Hz), 3.71 (s, 3H), 2.92 (s, 3H), 1.78 (d, 3H, *J* = 6.9 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): 172.4, 163.8, 158.2, 150.7, 136.5, 133.0, 128.6 (2C), 128.5, 126.4, 124.6, 121.4, 114.1 (2C), 55.2, 42.2, 26.8, 21.3. *m/z* MS ($APCI^+$): 279 $(M + H)^+$. HRMS (ESI^+): *m/z* calculated for $C_{18}H_{19}N_2O$ $[M+H]^+$ 279.1492; found 279.1505.

4-[1-(3,4-dimethoxyphenyl)-ethyl]-2-methyl-quinazoline ***10b***

97% yield, yellow oil, Rf 0.2 (Cyclohexane/EtOAc, 7/3). IR (neat, cm^{-1}): 1563, 1514, 1491, 1418, 1328, 1262, 1140. ^1H NMR (300 MHz, $CDCl_3$): 8.10 (d, 1H, *J* = 8.2 Hz), 7.90 (d, 1H, *J* = 8.3 Hz), 7.75 (ddd, 1H, *J* = 8.2 Hz, *J* = 6.9 Hz, *J* = 1.2 Hz), 7.45 (ddd, 1H, *J* = 8.2 Hz, *J* = 6.8 Hz, *J* = 1.1 Hz), 6.92 (d, 1H, *J* = 2.0 Hz), 6.86 (dd, 1H, *J* = 8.2 Hz, *J* = 2.0 Hz), 6.75 (d, 1H, *J* = 8.2 Hz), 4.94 (q, 1H, *J* = 6.9 Hz), 3.82 (s, 3H), 3.81 (s, 3H), 2.91 (s, 3H), 1.78 (d, 3H, J = 6.9 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): 172.3 , 163.8, 150.8, 149.1, 147.8, 137.0, 133.2, 128.6, 126.5, 124.7, 121.4, 119.8, 111.3, 111.0, 56.0, 55.9, 42.6, 26.9, 21.4. *m/z* MS ($APCI^+$): 309 $(M + H)^+$. HRMS (ESI^+): *m/z* calculated for $C_{19}H_{21}N_2O_2$ $[M+H]^+$ 309.1598; found 309.1604.

4-[1-(3,5-Dimethoxyphenyl)-ethyl]-2-methyl-quinazoline ***10c***

89% yield, brown oil, Rf 0.3 (Cyclohexane/EtOAc, 7/3). IR (neat, cm^{-1}): 1609, 1593, 1564, 1428, 1205, 1157. ^1H NMR (300 MHz, $CDCl_3$): 8.08 (d, 1H, *J* = 8.2 Hz), 7.90 (d, 1H, *J* = 8.4 Hz), 7.77-7.72 (ddd, 1H, *J* = 8.2 Hz, J = 6.9 Hz, *J* = 1.3 Hz), 7.44 (ddd, 1H, *J* = 8.3 Hz, *J* = 7.0 Hz, *J* = 1.0 Hz), 6.49 (d, 2H, *J* = 2.2 Hz), 6.27 (t, 1H, *J* = 2.2 Hz), 4.90 (q, 1H, *J* = 6.9 Hz), 3.73 (s, 6H), 2.91 (s, 3H), 1.78 (d, 3H, *J* = 6.9 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): 171.8, 163.8, 161.0 (2C), 150.7, 146.9, 133.2, 128.5, 126.6, 124.7, 121.6, 106.2 (2C), 98.0, 55.4 (2C), 43.4, 26.9, 21.1. *m/z* MS ($APCI^+$): 309 $(M + H)^+$. HRMS (ESI^+): *m/z* calculated for $C_{19}H_{21}N_2O_2$ $[M+H]^+$ 309.1598; found 309.1610.

2-Methyl-4-[1-(3,4,5-trimethoxyphenyl)-ethyl]-quinazoline ***10d***

97% yield, yellow oil, Rf 0.2 (Cyclohexane/EtOAc, 7/3). IR (neat, cm^{-1}): 1587, 1564, 1492, 1461, 1420, 1328, 1238, 1122. ^1H NMR (300 MHz, $CDCl_3$): 8.1 (d, 1H, *J* = 8.2 Hz), 7.91 (d, 1H, *J* = 8.3 Hz), 7.76 (ddd, 1H, *J* = 8.2 Hz, J = 7.1 Hz, *J* = 1.3 Hz), 7.47 (ddd, 1H, *J* = 8.2 Hz, *J* = 7.0 Hz, *J* = 1.2 Hz), 6.58 (s, 2H), 4.91 (q, 1H, *J* = 6.9 Hz), 3.79 (s, 6H), 3.77 (s, 3H), 2.91 (s, 3H), 1.78 (d, 3H, *J* = 6.9 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): 172.0, 163.8, 153.3 (2C), 150.8, 140.0, 136.8, 133.2, 128.6, 126.6, 124.6, 121.5, 105.0 (2C), 60.9, 56.2 (2C), 43.2, 26.8, 21.5. *m/z* MS ($APCI^+$): 339 $(M + H)^+$. HRMS (ESI^+): *m/z* calculated for $C_{20}H_{23}N_2O_3$ $[M+H]^+$ 339.1703; found 339.1705.

Biology

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. Human K562 leukemia, U87 glioblastoma, MCF7 breast cancer 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. 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 GI_{50} corresponds to the concentration of the tested compound that caused a decrease of 50% in fluorescence of drug treated cells compared with untreated cells. Nonlinear regression graph was plotted between % cell inhibition and Log10 concentration, and IC_{50} was determined using GraphPad Prism software. Experiments were performed in triplicate. The GI_{50} values for all compounds were compared to the GI_{50} of CA-4 and *iso*CA-4 and were measured the same day under the same conditions.

Tubulin Binding Assay

Sheep brain tubulin was purified according to the method of Shelanski [40] 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 [41] 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.

Cell Cycle Analysis

Exponentially growing HCT116 cancer cells were incubated with **4b-d** at a concentration of 5, 10 and 50 nM or DMSO for 24 h. Cell-cycle profiles were determined by flow cytometry on a FC500 flow cytometer (Beckman-Coulter, France) as described previously.[42]

Apoptosis

Apoptosis was measured by the Apo-one homogeneous caspase-3/7 assay (Promega Co, WI) according to the manufacturer's recommendations. Briefly, U87 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 **4b**-**d** at a concentration of 25 nM or 0.1% DMSO (as negative control). The U87 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.

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. Quinazoline **4d** (50 nM) or DMSO (vehicle) were added to the formed cords and left for 1 h. Images were taken 1 h after the addition of compounds.

Molecular modeling

X-ray structures of four different tubulin co-crystals were retrieved from the PDB [43] (accession codes 1SA0, 1SA1, 3HKC, 3HKD and 3HKE) and prepared using Protein Preparation Wizard workflow from Schrödinger suite[44], including optimization of the hydrogen bond network and a short minimization with position restraints on heavy atoms using OPLS_2005 force field[45]. Coordinates for compounds 4b-d and 4k were generated using Standardizer from JChem suite v6.3.1[46] and geometries were refined at the HF/STO-3G level[47] using NWChem v6.1[48] Ligands were then freely docked in the colchicine binding site located between chains C and D using the ensemble docking procedure available in GOLD v5.2.2[49] over the 5 aligned tubulin structures. CHEMPLP with default parameters was used as an objective function.[50] Structures of complexes were exported, subjected to hydrogen bond network optimization and a short minimization with position restraints on heavy atoms using Protein Preparation Wizard, and loaded in Chimera v1.9[51] for examination (including hydrogen bond detection, close contact analysis and representation of solvent-accessible surface) and depiction.

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