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Design, Synthesis and Anticancer Properties of *Iso*CombretaQuinolines as Potent Tubulin Assembly Inhibitors

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Abstract

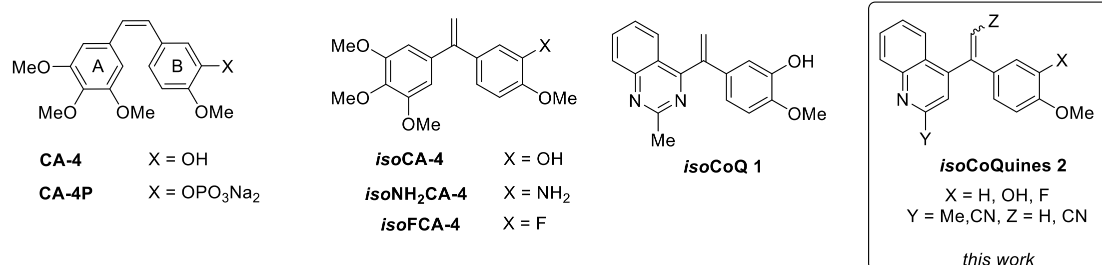
The synthesis and evaluation of a new series of *Iso*CombretaQuinolines (*Iso*CoQuines) **2** with a 2-substituted-quinoline in place of the 3,4,5-trimethoxyphenyl ring present in *iso*CA-4 and CA-4 are described. Most of these compounds displayed a potent cytotoxic activity (IC_{50} < 10 nM) against a panel of five human cancer cell lines and inhibited tubulin assembly at a micromolar level. The most potent analogue **2b**, having a 3-hydroxy-4-methoxyphenyl as B-ring, led to cell cycle arrest in G2/M phase. Docking studies indicate that **2b** showed a binding mode comparable to those previously observed with quinazoline analogous (IsoCoQ) and with *iso*CA-4 at the colchicine binding site of tubulin.

Keywords: Cancer, quinoline, *iso*CA-4, cytotoxicity, tubulin, binding.

1. Introduction

Combretastatin A-4 (CA-4) has been isolated from the African willow tree, *Combretum caffrum* by Pettit and co-workers [1] in 1989. This (*Z*)-stilbene was found to be a potent anticancer agent which affect microtubule dynamics. CA-4 inhibit the assembly of tubulin by binding to the colchicine site.[2] Besides its anti-tubulin activity, CA-4 displayed a potent cytotoxicity at a nanomolar level against a wide range of human cancer cells including multidrugs resistant (MDR) cells.[3] Moreover, it has been demonstrated that CA-4 displayed a selective toxicity towards the vascular network of tumors, inducing an irreversible shutdown of blood flow to neoplastic cells.[4] Currently, a water-soluble prodrug, CA-4P (fosbretabulin, Zybrestat^{TM,})[5] has received in 2016 orphan drug status (USA and Europe) to treat neuroendocrine tumors (NETs) and glioblastoma multiform (GBM).[6] However, CA-4 and its prodrugs are unstable since the (*Z*)-stilbene is prone to isomerization to provide the 100-fold less active *E*-isomer during storage, administration and metabolization.[7, 8] This problem and the structural simplicity of CA-4 encouraged the scientific community these last decades to synthesize a large number of stable CA-4 analogues, mainly by including the double bond into cycles and heterocycles.[9, 10] Moreover, numerous modifications on the B-ring of CA-4 and its analogues have been achieved to improve activity or solubility of novel classes of ligands. Our group, in an ongoing project aimed to develop stable CA-4 analogues[11], has recently definitively solved the stability problem of CA-4 by the discovery and the synthesis of *iso*CA-4, the 1,1-diarylethylene non-natural isomer of CA-4 which holds similar biological properties as CA-4.[12, 13]. This compound which was easily synthesized in four steps was shown to be chemically [14] and metabolically stable[15]. We have also prepared a large variety of new bioactive analogues by chemical modifications on the B-ring of *iso*CA-4, and identified other stable and promising antiproliferative agents such as *iso*NH_2CA-4, *iso*FCA-4,[16] or naphthyl,[17] chromen[18] and benzoxepin derivatives.[19] We are now interested in identifying novel potent drugs as *iso*CA-4 analogues by replacing the "traditional" 3,4,5-trimethoxyphenyl unit (A-ring) by nitrogen containing heterocycles. It should be noted that, in the CA-4 series, the replacement of the 3,4,5-trimethoxyphenyl A-ring [20, 21, 22] which is "crucial to obtain relevant cytotoxic and antitubulin responses"[9], by heterocyclic derivatives has received very little attention. Very recently, we have demonstrated for the first time, that changing the 3,4,5-trimethoxyphenyl A-ring by a quinazoline nucleus resulted in a novel series of antimitotic drugs,[23] with *iso*CoQ **1** as a lead compound, displaying a nanomolar level of cytotoxicity against various tumor cells. The possible binding mode on tubulin of the more potent quinazolines was studied and indicate that the N-1 atom of such quinazolines interacted with Cys β241 of tubulin whereas the position of the N-3 atom of these drugs was inadequate to interact with tubulin. On the basis of these observations, we were interested to synthesize other nitrogen containing heterocycles as A ring and particularly *iso*combretaquinolines (*iso*CoQuines[24]) of type **2** (Figure 1) to evaluate their biological activities. Preliminary *in vitro* cytotoxicity and inhibition of tubulin assembly of novel nitrogen-containing heterocycles (quinolines **2a-c** and **2g-n**, *iso*quinoline **2d** and quinoxaline **2e**) will be presented and discussed. The possible binding mode of the more bioactive substrate *iso*CoQuine **2b** and its superimposition with *iso*CA-4 on tubulin is also reported.

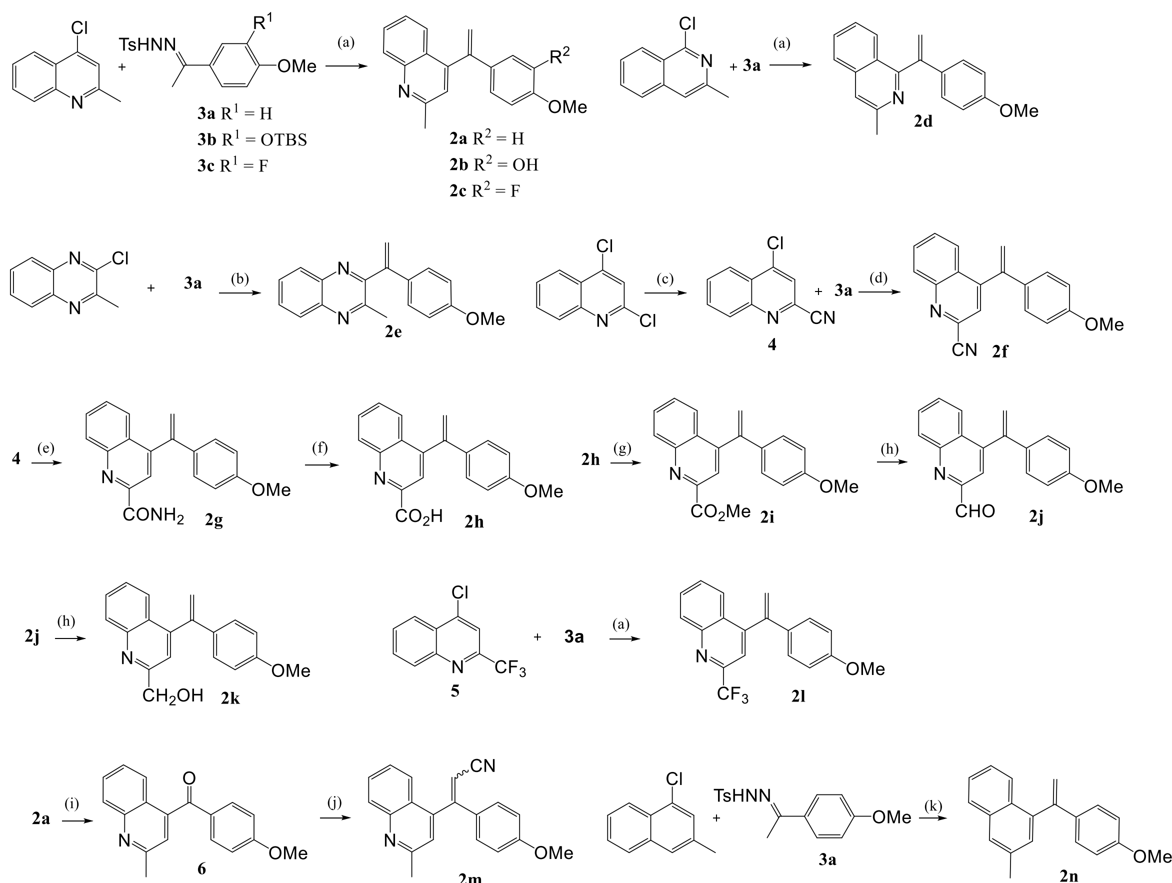
Figure 1. Structures of CA-4 and water soluble prodrugs, *iso*CA-4 and analogues, *iso*CoQ **1** and target *iso*CoQuines **2**.



2. Results and discussion

2.1. Chemistry.

The synthesis of the targets *iso*CoQuines **2a-c** bearing a 3,4-disubstituted B-ring and having a great resemblance with *iso*CA-4, and *iso*FCA-4 were prepared from 4-chloroquinaldine which was coupled in a sealed tube with a variety of *N*-tosylhydrazones **3a-c** in the presence of $PdCl_2(CH_3CN)_2$ as the catalyst,[25] 1,1'-bis(diphenylphosphino)ferrocene (dppf) as the ligand, LiO*t*Bu (2.2 equiv) as the base at 100 °C. Similarly, isoquinoline **2d** and quinoxaline derivative **2e** were prepared from *N*-tosylhydrazone **3a** which reacted with 1-chloro-3-methylisoquinoline and 2-chloro-3-methylquinoxaline respectively, to furnish **2d** and **2e** with satisfactory yields. One can note that the replacement of dppf by di-*t*Bu(methyl)phosphonium tetrafluoroborate ($[(tBu)_2MePH]BF_4$) as the ligand for the coupling of 2-chloro-3-methylquinoxaline with **3a** slightly improved the yield of **2e** (65% *vs* 57%). Using this ligand in place of dppf for the coupling of 1-chloro-3-methylisoquinoline with **3a** was totally unsuccessful. To introduce some functionalities on the C-2 of quinaldines **2** in place of the methyl substituent, we have synthesized 4-chloro-2-cyanoquinoline **4** from 2,4-dichloroquinoline and $Zn(CN)_2$ in the presence of $Pd(PPh_3)_4$ in DMF. Using these experimental conditions, quinoline **4** was obtained with a good yield of 70% and with a total selectivity since only the chlorine atom on C-2 was substituted by a CN group.



Scheme 1. Synthesis of target compounds **2a-n** [a]Reagents and conditions: (a) $PdCl_2(CH_3CN)_2$ (10 mol%), dppf (20 mol%), dry LiOtBu (2.2 equiv), N-tosylhydrazone (1.5 equiv), dioxane, 100 °C, sealed tube. (b) $PdCl_2(CH_3CN)_2$ (10 mol%), $[(tBu)_2MePH]BF_4$ (20 mol%), LiOtBu (2.2 equiv), dioxane, 100 °C, sealed tube (c) $Zn(CN)_2$ (0.5 equiv), $Pd(PPh_3)_4$ (10 mol%), DMF, 90 °C (d) $PdCl_2(CH_3CN)_2$ (10 mol%), dppf (20 mol%), dry LiOtBu (1.8 equiv), *N*-tosylhydrazone (1.5 equiv), dioxane, 100 °C, sealed tube, 3 h. (e) $PdCl_2(CH_3CN)_2$ (10 mol%), dppf (20 mol%), LiOtBu (3.6 equiv), dioxane, 100 °C, sealed tube. (f) NaOH, 100 °C, sealed tube (g) $SOCl_2$, MeOH, -5 °C to 55 °C. (h) DIBAL (1 equiv), CH_2Cl_2, -78 °C. (i) O_3, PPh_3 (2 equiv), MeOH, -78°C. (j) $PO(OEt)_2CH_2CN$ (2 equiv), LiHMDS (2 equiv), THF, 0 °C to reflux. (k) $Pd_2dba_3.CHCl_3$ (2.5 mol%), XPhos (10 mol%), LiOtBu (2.2 equiv), *N*-tosylhydrazone (1.5 equiv), dioxane, 100 °C, microwave irradiation.

The 2-cyano-4-chloroquinoline **4** was then successfully coupled in the presence of dry LiO*t*Bu (1.8 equiv) with hydrazone **3a** to provide *iso*CoQuine **2f**. *Iso*CoQuine **2g** bearing a $CONH_2$ function on C-2 was successfully prepared (70 %) by using similar coupling conditions between *N*-tosylhydrazone **3a** and the cyano derivative **4** using an excess of LiO*t*Bu (3.6 equiv) in 1,4-dioxane. In this case and beside the expected Pd-coupling reaction, we were pleased to also observe a total hydration of the nitrile group providing the amide compound **2g**.[26] This later was transformed into its corresponding carboxylic acid **2h** using NaOH at 100 °C (63%) and into ester **2i** using $SOCl_2$ in MeOH.[27] *Iso*CoQuine **2j** was prepared from the DIBAL-reduction of the ester **2i** and a subsequent DIBAL-reduction of **2j** furnished *iso*CoQuine **2k** having a CH_2OH function on C-2 of quinoline nucleus. Quinoline **2l**, having a CF_3 group on C-2 was easily prepared from 4-chloro-2-(trifluoromethyl)quinoline **5** and *N*-tosylhydrazone **3a**. We next focused our attention on *iso*CoQuine **2m** possessing a tri-substituted ethylene bond and having structural analogies with a bioactive analogues of *iso*CA-4 previously prepared in our group.[12] This quinoline **2m** was synthetized in two steps from the ozonolysis of *iso*CoQuine **2a** and provided the expected 1,1-diarylketone **6**. The latter compound was then subjected to a Wittig-Horner olefination to provide give **2m** (49 %).[28] In view of structure-activity relationships and to evaluate the importance of the nitrogen atom of such heterocycles and particularly *iso*CoQuines, the naphthyl derivative **2n** was also prepared from the Pd-coupling reaction between **3a** and 1-chloro-3-methylnaphtalene in a sealed tube at 100 °C (79%).

2.2. Biological results.

In vitro cytotoxicity of the synthesized compounds **2** was investigated against U87 human glioblastoma cell line. A fluorimetry-based assay was used for determination of the drug concentration required to inhibit cell growth by 50% after incubation in the culture medium for 72 h. *Iso*CA-4 and *iso*CoQ **1** were included as reference compounds for comparisons. To investigate whether these derivatives were exerting their activity by interactions with microtubules, all drugs **2** were also evaluated for their anti-tubulin activities. The cytotoxicity results of novel derivatives **2** and 1,1-arylheteroarylketone **6** are presented in Table 1.

Table 1 Cytotoxicity against U87 cells[a] and ITP of compounds **2a-i**, **2k-n** and ketone **6**

Compound	**2a**	**2b**	**2c**	**2d**	**2e**	**2f**	**2g**	**2h**
Cytotoxicity IC_{50}[b] [nM]	21 ± 0.1	1.5 ± 0.05	4 ± 0.07	300 ± 1	690 ± 10	1 ± 0.1	240 ± 10	75 ± 1
ITP IC_{50}[c] [μM]	1.1± 0.2	3.9 ± 0.9	2.2 ± 0.4	[e]	[f]	1.1 ± 0.2	23.8± 3.3	[g]

Compound	**2i**	**2k**	**2l**	**2m**	**2n**	**6**	***iso*CA-4**	***iso*CoQ 1**
Cytotoxicity IC_{50}[b] [nM]	2.3 ± 0.1	390 ± 10	21.7 ± 0	0.25 ± 0.01	140 ± 1	25 ± 0.8	7.8 ± 0.1	6.1 ± 0.5
ITP IC_{50}[c] [μM]	3.9 ± 0.6	14.3 ± 1.4	-	1.9 ± 0.3	17.1 ± 2.3	7.2 ± 1.2	2.0 ± 0.1	0.6 ± 0.1

[a] U87 Human glioblastoma. [b] IC_{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] Not active. [e] 53 % of inhibition at a concentration of 100 μM. [f] 24 % of inhibition at a concentration of 100 μM. [g] 29 % of inhibition at a concentration of 100 μM.

*Iso*CoQuine **2a**, having a 4-methoxy substituent on B-ring exhibited a potent antiproliferative activity against U87 cells with an IC_{50} value of 21 nM. For comparison, its 2-methylquinazolinic analogue [23] displayed a slightly lowest cytotoxicity (IC_{50} = 40 nM), strengthening our ideas that nitrogen-containing heterocycles, as quinolines, are good surrogates for the 3,4,5-trimethoxyphenyl ring of *iso*CA-4. As it was previously observed in CA-4 and *iso*CA-4 series, introducing small substituents on the *meta*-position of the B-ring of **2a** as hydroxyle function or a fluorine atom led to more potent derivatives having IC_{50} values inferiors to 5 nM. It should be noted that phenol derivative **2b** (IC_{50} = 1.5 nM) is slightly more cytotoxic against U87 cells than *iso*CA-4 (IC_{50} = 7.8 nM) and than its quinazolinic analogue, *iso*CoQ **1** (IC_{50} = 6.1 nM). The IC_{50} values obtained for isoquinoline **2d** (IC_{50} = 300 nM) and for quinoxaline **2e** (IC_{50} = 690 nM) showed that these nitrogen containing heterocycles were less suitable for an improved cytotoxicity than their counterpart quinoline as **2a** which was similarly substituted (IC_{50} = 21 nM). Next, we have evaluated the importance of

various substituents on the C-2 of quinolines **2** by replacements of the methyl substituent. Interestingly, quinolines **2f** and **2i** having a CN or a CO_2Me group on the C-2 position respectively, also displayed a good level of cytotoxicity with IC_{50} values against U87 cells equal to 1 nM and 2.3 nM, respectively. However, introduction of other polar substituents as a $CONH_2$ present in (**2g**; IC_{50} = 240 nM), COOH (**2h**; IC_{50} = 75 nM) or CH_2OH (**2k**; IC_{50} = 390 nM) at the C-2 position resulted in an important decrease in the mean of IC_{50} values. Quinoline **2l** having on C-2 a CF_3 lipophilic substituent displayed an interesting IC_{50} value of 21.7 nM. As expected, and as observed in the *iso*CA-4 series, the introduction of a CN-function on the double bond of **2a** gave **2m** (as a mixture of inseparable isomers) which displayed an excellent level of cytotoxicity (**2m**, IC_{50} = 0.25 nM). This compound may constitutes a valuable payload for antibody drug conjugates (ADCs). Finally, the high IC_{50} value of naphthalene **2n** (140 nM) clearly demonstrated the importance of the nitrogen atom (N-1) in quinolines of type **2** for the design of novel chemotherapeutic drugs. To investigate whether these derivatives were exerting their activities by interactions with microtubules, compounds **2** were next evaluated for their antitubulin activities (Table 1). The results indicated clearly that tubulin is the intracellular target of nitrogen-containing heterocycles **2** because all of them were potent inhibitors of tubulin assembly with micromolar IC_{50} values. It is important to note that quinoline **2a** exhibited a IC_{50} value against tubulin (1.1 µM) substantially weaker than those of its analogous isoquinoline **2d**, quinoxaline **2e** and naphthalene **2n** reinforcing our ideas that a quinoline ring is important for improved anti-cancer activities in connection with a significant involvement of β-tubulin. As expected, we were pleased to observe that quinoline **2m** having a tri-substituted ethylene bond was as active as *iso*CA-4 against tubulin with an IC_{50} value of 1.9 µM. As observed with these preliminary cytotoxicity results, the replacement of the Me-substituent on C-2 (**2a**) by a CN (**2f**) or a CO_2Me (**2i**) group is authorized and led to an equipotent anti-tubulin drugs whereas other replacements on C-2 were less efficient (**2g**,**h**, **2k**,**l**). These preliminary cytotoxicity and inhibition of tubulin assembly results showed that quinolines **2a-c**, **2f**, **2i** and **2m** are promising drugs in this novel series of heterocyclic derivatives.[29] In this context, we next investigated their effects on the proliferation of four other tumor cell lines: chronic myeloid leukaemia in blast crisis (K562), doxorubicin-resistant K562 (K562R), human lung adenocarcinoma epithelial cells (A549) and human colon carcinoma (HCT116).

Table 2. Cytotoxicity against human cancer cell lines, U87, K562, K562R, A549 and HCT116.

	Cytotoxicity (IC_{50} nM)				
Compounds	U87	K562[b]	K562R[c]	A549[d]	HCT116[e]
2a	21 ± 0.1	17± 0.5	0.7± 0.03	18.5 ± 0.2	3 ± 0.2
2b	**1.5 ± 0.05**	**1.8 ± 4**	**2.4 ± 2**	**0.4 ± 0.04**	**0.9 ± 0.1**
2c	4 ± 0.07	5.0 ± 0.7	2.4 ± 0.3	9.5 ± 0.2	9.6 ± 0.5
2f	1 ± 0.1	9 ± 2	0.9 ± 0.1	50 ± 0.8	1.6 ± 0.1
2i	2.3 ± 0.1	88 ± 2	18 ± 0.5	nd[g]	160 ± 8
2m	0.25 ± 0.01	17 ± 0.3	6.6± 0.2	79 ± 2	13.2 ± 0.8
6	25 ± 0.8	139 ± 4	52 ± 1	nd[g]	130 ± 10
***Iso*CA-4**[f]	7.8 ± 0.06	0.8 ± 0.1	1.1 ± 0.2	8.2 ± 0.7	2.0 ± 0.1

[a] IC_{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] K562, chronic myeloid leukemia cells in blast crisis. [c] K562R, doxorubicin-resistant K562 cells, [d] A549, human lung epithelial cells. [e] HCT116 human colon carcinoma. [f] The IC_{50} values of *iso*CA-4 was determined in this study. [g] not determined.

The results depicted in Table 2 revealed that all selected *iso*CoQuines **2** were also strongly cytotoxic with nanomolar IC_{50} values. Quinoline **2b** (0.4 nM < IC_{50} < 2.4 nM) and in a lesser extent quinoline **2c** (2.4 nM < IC_{50} < 9.6 nM) displayed a constant high level of cytotoxicity against all tested cancer cell lines. On the contrary, quinoline **2f** having a CN-group on the C-2 position which was a very cytotoxic at a nanomolar level against U87 and K562R displayed a

lower level of cytotoxicity against the human lung epithelial cells (IC_{50} = 50 nM). One note that quinoline **2m** which was found to be the more cytotoxic agent against U87 cells with a picomolar IC_{50} value (250 pM) was dramatically 300-fold less cytotoxic against A549 cell lines (IC_{50} = 79 nM). *Iso*CoQuine **2b** which displayed against the five cancer cell lines U87, K562, K562R, A549 and HCT116 a high and homogeneous cytotoxicity level (0.4 nM $< IC_{50} <$ 2.4 nM) was next tested in dose-response experiments on A549 cell cycle distribution. A549 cells were treating for 24 h with increasing nanomolar concentrations (0.5, 1, 5 and 10 nM) and DMSO was used as control. As seen in Fig. 2, *iso*CoQuine **2b**, at a concentration of 10 nM, has arrested the entire population of A549 cells in the G2/M phase of the cellular cycle as it was previously observed for antimitotic derivatives in the *iso*CA-4 series.[11]

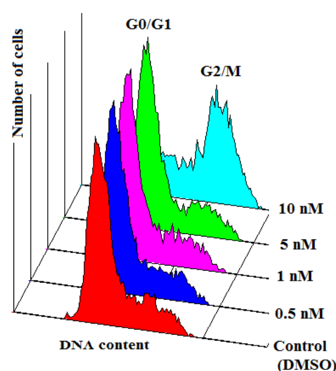


Fig. 2. Effects of **2b** on cell cycle distribution in A549 cells determined by flow cytometry analysis. DNA content was assessed *via* propidium iodide staining.

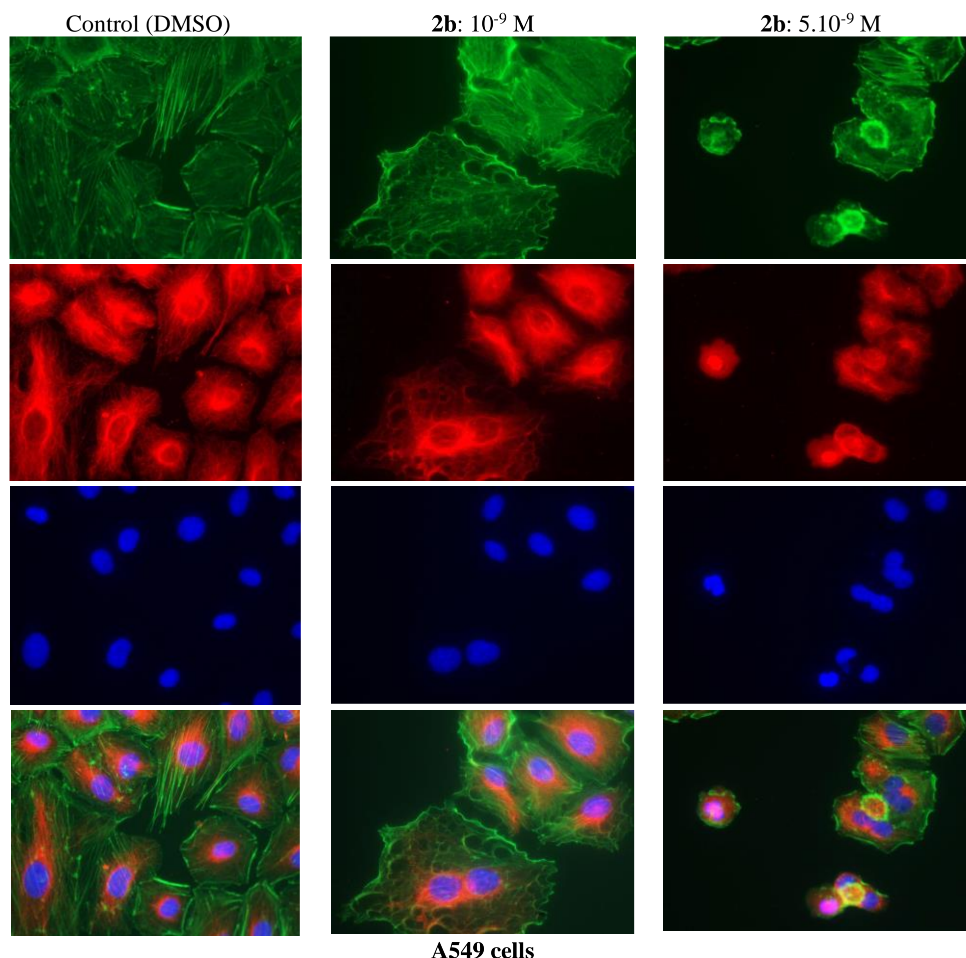


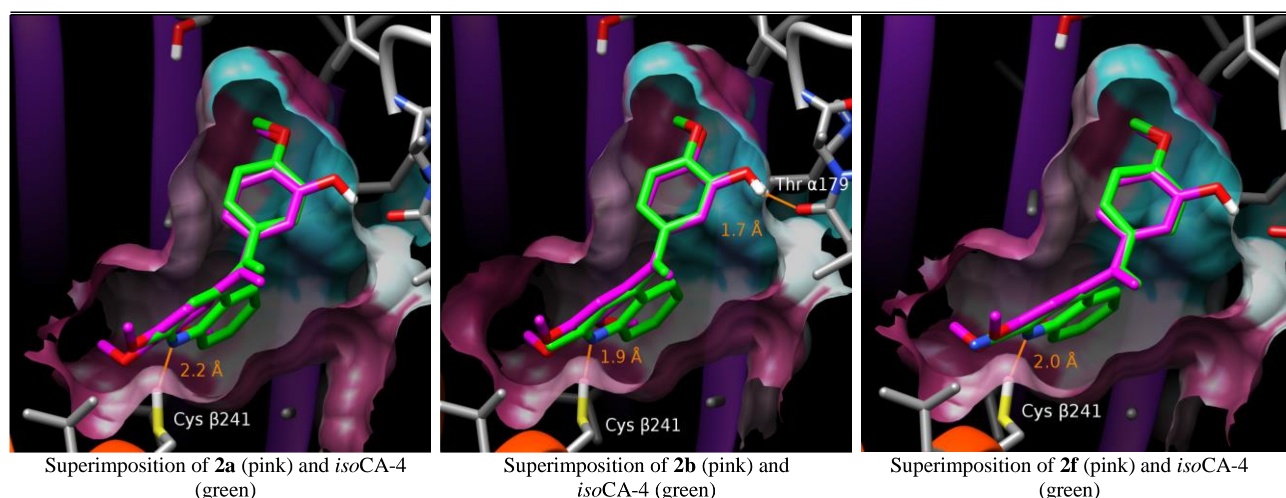
Fig. 3. Effect of **2b** on microtubule network organization. A549 cells were treated with **2b** for 24 h. After incubation, the cells were fixed and stained with monoclonal β-tubulin antibody (red), cell nuclei were stained with DAPI (blue) and Alexa Fluor® 488 Phalloidin (green) was used to visualize actin skeleton.

To support that *iso*CoQuines **2** and particularly *iso*CoQuine **2b** inhibit polymerization of β-tubulin, we evaluated its ability to alter the microtubule network of A549 cells using cell-base immunofluorescence staining assays (Fig. 3).We observed that, after 24 h of treatment, **2b** caused fragmentation of the microtubule network and disrupted mitotic spindle formation at a low concentration of 1 nM. A549 cells showed a dose-dependent disruption and loss of microtubules and, at a concentration of 5 nM, we observed a severe destruction of the microtubule system (red), a disorganization of actin network (green) and multinucleated cells (blue).

2.3. Docking study.

The ability for the colchicine binding site to accommodate *iso*CoQuines **2a,b** and compounds **2d-f** was investigated by molecular docking against five conformations of tubulin available [30] in the PDB database. Results for three of these compounds which displayed a high level of cytotoxicity (**2a,b** and **2f**) are shown in Figure 4.

Where A-ring of *iso*CA-4 possessed a 4-methoxy group establishing a hydrogen bond with Cys241 residue belonging to the β-subunit of tubulin, *iso*CoQuines of type **2** exhibit their nitrogen atoms capable of playing the same hydrogen bond acceptor role. In the case of *iso*CoQuine **2b**, an extra hydrogen bond is formed by the OH function with residue Thrα179. In low bio-active compounds isoquinoline **2d** and quinoxaline **2e**, though, the position of the aromatic nitrogen(s) is inadequate for interacting with Cysβ241 (data not shown). The replacement of the Me-substituent on C-2 of the quinoline ring in **2a** by a CN function (*iso*CoQuine **2f**) led to equivalent drugs (similar cytotoxicities and IPT IC_{50} values) which adopt a similar positioning in the colchicine binding site and establish a hydrogen bond between N-1 and Cys241.



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

Fig. 4. Calculated binding mode for derivatives **2a**, **2b** and **2f** in the colchicine binding site of tubulin and superimposition of the latter with that of *iso*CA-4. The color of solvent-accessible surface indicates its polarity (cyan means polar and purple means nonpolar). Orange lines represent hydrogen bonds.

3. Conclusion

In this work, we have designed and synthesized a series of *iso*CA-4 derivatives having a quinoline A-ring and found that these compounds show good growth inhibition activities against a range of human cancer cells. We demonstrated that replacing a quinazoline-A-ring by a quinoline resulted in more potent derivatives. The more interesting *iso*CoQuine **2b**, having the greatest resemblance to *iso*CA-4 and quinazoline **1** displayed a nano- and subnanomolar level of cytotoxicity against five cancer cell lines and inhibited tubulin polymerization with micromolar IC_{50} values. These results demonstrated that incorporation of a quinoline as A-ring in **2b** in place of a quinazoline in **1** improved anticancer activity, characterized by a high level of cytotoxicity. After 24 h of treatment with *iso*CoQuin **2b**, A549 cells were arrested in the G2/M phase at a concentration of 10 nM as it was observed with other *iso*CA-4 derivatives.[31, 32] Docking studies show that *iso*CoQuin **2b** adopts a similar orientation to that of *iso*CA-4 when bound to β-tubulin. We

also observe that the nitrogen atom N-1 of the quinoline A-ring establish an important hydrogen bond with Cys241 residue belonging to the β-subunit of tubulin as it was previously showed with the 4-MeO substituent of the A-ring in the CA-4 and *iso*CA-4 series.

4. Experimental

4.1 General considerations

The compounds were all identified by usual physical methods, i.e. ^{1}H NMR, ^{13}C NMR, ^{19}F NMR, IR, HRMS. ^{1}H, ^{13}C and ^{19}F NMR spectra were measured in $CDCl_3$ with a Bruker AMX 200 (^{1}H, ^{13}C and ^{19}F) or with a Bruker Avance 300 (^{1}H and ^{13}C). ^{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), brs (broad singlet), t (triplet), ^{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 pre-coated silica gel 60F plates. Merck silica gel 60 (230-400 mesh) was used for column chromatography.

4.2.1. Synthesis of 4-chloroquinoline-2-carbonitrile **4** (93%)

To a solution of 2,4-dichloroquinoline (444 mg, 2.24 mmol) in DMF (5 mL) was added zinc cyanide (117 mg, 1.14 mmol) and $Pd(PPh_3)_4$ (259 mg, 0.22 mmol). The reaction mixture was stirred at 120 °C for 1 h. A saturated NH_4Cl solution (20 mL) was added and the mixture was extracted with ethyl acetate (3x5 mL). The organic layer was washed with a NaCl solution (5mL) and dried over sodium sulfate, filtered and evaporated. The product was further purified by chromatography on silica gel eluting with cyclohexane/ethyl acetate (95:5) to give **4** (392 mg, 2.085 mmol, 93 %) as a white solid.

White solid. F = 108.9-109.3 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.12 (d, *J* = 8.4 Hz, 1H), 8.06 (d, *J* = 8.4 Hz, 1H), 7.81 (t, *J* = 7.6 Hz, 1H), 7.70 (t, *J* = 7.6 Hz, 1H), 7.61 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 148.9, 144.3, 133.4, 132.2, 130.7 (2C), 127.3, 124.4, 123.4, 116.8. IR neat $\nu_{max/}$ cm^{-1}: 3093, 2344, 2240, 1570, 1491, 1403, 1292, 1024. HRMS calcd for $C_{10}H_6N_2Cl$ $[M+H]^+$ 189.0220, obsd. 189.0217.

4.2.2. Synthesis of isoCoQuines **2**

General procedure for the synthesis of compounds **2a***,* **2b***,* **2c***,* **2d***,* **2f***,* **2l**

In a sealed tube and under an argon atmosphere were added successively, ArCl (0.5 mmol), hydrazone **3a** or **3c** (for **2c**) (0.75 mmol) $PdCl_2(CH_3CN)_2$ (10 mol%), dppf (20 mol%) in dry dioxane (4 mL) and the mixture was stirred for 5 min. at rt. Then dry LiO*t*Bu (2.2 mmol) (1.8 for **2f**) was added and the mixture was stirred at 100° C for 3 h. The resulting suspension was cooled to room temperature, filtered through a pad of Celite eluting with ethyl acetate and the inorganic salts were removed. The filtrate was concentrated and the crude was purified by silica gel column chromatography.

4.2.2.1. 4-(1-(4-Methoxyphenyl)vinyl)-2-methylquinoline **2a** (65 %)

Beige solid, F = 94.5-95.2 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.08 (d, *J* = 9.1 Hz, 1H), 7.75 (d, *J* = 9.1 Hz, 1H), 7.66 (t, *J* = 8.4 Hz, 1H), 7.36 (t, *J* = 8.4 Hz, 1H), 7.24 (d, *J* = 8.9 Hz, 2H), 6.84 (d, *J* = 8.9 Hz, 2H), 5.94 (s, 1H), 5.32 (s, 1H), 3.81 (s, 3H), 2.80 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.7, 158.9, 148.9, 148.3, 145.6, 132.5, 129.4, 129.0 (2), 127.9, 126.1, 125.7, 125.5, 122.5, 115.1, 114.0 (2), 55.4, 25.4. IR (neat) ν_{max}/ cm^{-1}: 2961, 2929, 1651, 1591, 1510, 1440, 1351. HRMS calcd for $C_{19}H_{18}NO$ $[M+H]^+$ 276.1388, obsd. 276.1388.

4.2.2.2. 2-Methoxy-5-(1-(2-methylquinolin-4-yl)vinyl)phenol **2b** (45% for the coupling and the cleavage of the O-Si bond)

Beige solid, F = 107.5-108.3 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.00 (s, 1H), 7.89 (d, *J* = 8.2 Hz, 1H), 7.71 (d, *J* = 8.2 Hz, 1H), 7.52 (t, *J* = 8.1 Hz, 1H), 7.31 (t, *J* = 8.1 Hz, 1H), 7.10 (s, 1H), 7.03 (d, *J* = 8.4 Hz, 1H), 6.88 (d, *J* = 8.4 Hz, 1H), 6.72 (s, 1H), 5.95 (s, 1H), 5.24 (s, 1H), 3.93 (s, 3H), 2.52 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.4, 149.7, 147.7, 147.0, 146.5, 145.3, 132.6, 129.6, 127.7, 126.2, 125.8, 125.5, 122.5, 117.2, 114.8, 114.0, 111.4, 56.1, 24.3. IR (neat) ν_{max}/ cm^{-1}: 3162, 2840, 2363, 1594, 1560, 1515, 1461, 1350. HRMS calcd for $C_{19}H_{18}NO_2$ $[M+H]^+$ 292.1338, obsd. 292.1330.

4.2.2.3. 4-(1-(3-Fluoro-4-methoxyphenyl)vinyl)-2-methylquinoline **2c** (37 %)

Pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.03 (d, *J* = 8.4 Hz, 1H), 7.64 (d, *J* = 8.4 Hz, 1H), 7.56 (t, *J* = 7.3 Hz, 1H), 7.26 (t, *J* = 7.3 Hz, 1H), 7.16 (s, 1H), 7.06 (d, *J* = 12.5 Hz, 1H), 6.84 (d, *J* = 8.5 Hz, 1H), 6.72 (t, *J* = 8.5 Hz, 1H), 5.84 (s, 1H), 5.28 (s, 1H), 3.75 (s, 3H), 2.71 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.8, 153.0 (d, *J* = 244.5 Hz), 148.2, 148.0, 147.6 (d, *J* = 10.5 Hz), 144.8, 132.9 (d, *J* = 6.0 Hz), 129.3, 129.0, 125.8, 125.7, 125.2, 122.7 (d, *J* = 3.0 Hz), 122.4, 116.1, 114.0 (d, *J* = 19.5 Hz), 113.1, 56.1, 25.3. ^{19}F NMR (188 MHz, $CDCl_3$) δ -135.2. IR (neat) ν_{max}/ cm^{-1}: 2841, 1616, 1594, 1516, 1463, 1433. HRMS calcd for $C_{19}H_{17}FNO$ $[M+H]^+$ 294.1294, obsd. 294.1289.

4.2.2.4. 1-(1-(4-Methoxyphenyl)vinyl)-3-methylisoquinoline **2d** (41 %)

White solid, F = 65.7-66.3 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.92 (d, *J* = 8.2 Hz, 1H), 7.75 (d, *J* = 8.2 Hz, 1H), 7.60 (t, *J* = 8.2 Hz, 1H), 7.50 (s, 1H), 7.37 (t, *J* = 8.2 Hz, 1H), 7.26 (d, *J* = 8.7 Hz, 2H), 6.81 (d, *J* = 8.7 Hz, 2H), 6.04 (s, 1H), 5.41 (s, 1H), 3.79 (s, 3H), 2.75 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.0, 159.5, 151.0, 146.7, 137.3, 132.6, 130.1, 127.9 (2), 127.6, 126.3, 126.2, 125.5, 118.3, 115.5, 113.8 (2), 55.3, 24.6. IR (neat) ν_{max}/ cm^{-1}: 2955, 2835, 1621, 1606, 1510, 1464, 1252. HRMS calcd for $C_{19}H_{18}NO$ $[M+H]^+$ 276.1388, obsd. 276.1385.

4.2.2.5. 4-(1-(4-Methoxyphenyl)vinyl)quinoline-2-carbonitrile **2f** (62%)

White solid, F = 112.4-113.2 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.19 (d, *J* = 8.5 Hz, 1H), 7.89-7.72 (m, 2H), 7.63 (s, 1H), 7.54 (t, *J* = 8.5 Hz, 1H), 7.16 (d, *J* = 8.9 Hz, 1H), 6.83 (d, *J* = 8.9 Hz, 2H), 5.99 (s, 1H), 5.34 (s, 1H), 3.79 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 159.9, 150.8, 148.5, 144.1, 133.4, 131.6, 131.0, 130.2, 129.3, 127.8 (2), 126.3, 123.7, 117.5, 116.6 (2), 114.1 (2), 55.3. IR (neat) ν_{max}/ cm^{-1}: 3062, 2838, 2236, 1605, 1576, 1441, 1218. HRMS calcd for $C_{19}H_{15}N_2O$ $[M+H]^+$ 287.1184, obsd. 287.1180.

4.2.3. In a sealed tube and under an argon atmosphere were added successively, 2-chloro-3-methylquinoxaline (0.5 mmol), hydrazone **3a** (0.75 mmol) $PdCl_2(CH_3CN)_2$ (10 mol%), $[(tBu)_2MePH]BF_4$ (20 mol%) in dry dioxane (4 mL) and the mixture was stirred for 5 min. at rt. Then dry LiO*t*Bu (1.8 mmol) was added and the mixture was stirred at 100° C for 3 h. The resulting suspension was cooled to room temperature, filtered through a pad of Celite eluting with ethyl acetate and the inorganic salts were removed. The filtrate was concentrated and the crude was purified by silica gel column chromatography.

2-(1-(4-Methoxyphenyl)vinyl)-3-methylquinoxaline **2e** (57 %)

Beige solid, F = 102.2-102.9 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.14 (d, *J* = 9.6 Hz, 1H), 8.05 (d, *J* = 9.6 Hz, 1H), 7.84 – 7.62 (m, 2H), 7.20 (d, *J* = 8.9 Hz, 2H), 6.85 (d, *J* = 8.9 Hz, 2H), 5.93 (s, 1H), 5.46 (s, 1H), 3.80 (s, 3H), 2.49 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.9, 155.7, 153.5, 147.1, 141.8, 136.6, 131.1, 129.9, 129.5, 129.3, 128.5, 127.8 (2),

116.2, 114.2 (2), 55.4, 23.5. IR (neat) ν_{max}/ cm^{-1}: 2929, 2836, 1606, 1511, 1440, 1248. HRMS calcd for $C_{19}H_{17}N_2O$ $[M+H]^+$ 277.1341, obsd. 277.1336.

4.2.4. 4-(1-(4-Methoxyphenyl)vinyl)quinoline-2-carboxamide **2g** (70%)

In a sealed tube and under an argon atmosphere were added successively, **4** (1 mmol), hydrazone **3a** (1.5 mmol) $PdCl_2(CH_3CN)_2$ (10 mol%), dppf (20 mol%) in dioxane (4 mL) and the mixture was stirred for 5 min. at rt. Then LiO*t*Bu (3.6 mmol) was added and the mixture was stirred at 100° C for 3 h. The resulting suspension was cooled to room temperature, filtered through a pad of Celite eluting with ethyl acetate and the inorganic salts were removed. The filtrate was concentrated and the crude was purified by silica gel column chromatography.

White solid, F = 184.1-184.9 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.25 (s, 1H), 8.13 (d, *J* = 7.9 Hz, 1H), 7.83 (d, *J* = 7.9 Hz, 1H), 7.72 (t, *J* = 7.6 Hz, 1H), 7.47 (t, *J* = 7.6 Hz, 1H), 7.18 (d, *J* = 8.8 Hz, 2H), 6.80 (d, *J* = 8.8 Hz, 2H), 5.95 (s, 1H), 5.76 (s, 2H), 5.34 (s, 1H), 3.78 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.2, 159.8, 150.5, 149.2, 147.2, 145.3, 132.5, 130.3, 130.0, 128.4, 128.1, 128.0 (2), 126.4, 119.5, 116.1, 114.1 (2), 55.4. IR (neat) ν_{max}/ cm^{-1} : 2838, 2236, 1675, 1605, 1441, 1218, 1153. HRMS calcd for $C_{19}H_{17}N_2O_2$ $[M + H]^+$ 305.1290, obsd. 305.1283

4.2.5. 4-(1-(4-Methoxyphenyl)vinyl)quinoline-2-carboxylic acid **2h** (63%)

In a sealed tube was added **2g** (100 mg, 0.33 mmol) in a 3M solution of NaOH (8 mL) and the mixture was stirred at 100 °C for 4.5 h. EtOAc was added to the cooled mixture which was neutralized with HCl 1N until pH = 2. After extraction, organic layers were dried (Na_2SO_4) and concentrated *in vacuo*. The crude was purified by preparative HPLC to give **2h** (63 mg, 63%).

Pale yellow solid, F = 114.2-115.1 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.22-8.20 (m, 2H), 7.89 (d, *J* = 8.5 Hz, 1H), 7.78 (t, *J* = 7.4 Hz, 1H), 7.55 (t, *J* = 7.4 Hz, 1H), 7.17 (d, *J* = 8.7 Hz, 2H), 6.82 (d, *J* = 8.7 Hz, 2H), 5.98 (s, 1H), 5.35 (s, 1H), 3.79 (s, 3H) OH not seen. ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.5, 160.0, 152.3, 146.3, 145.8, 145.0, 132.1, 131.0, 129.8, 129.1, 129.0, 128.0 (2), 126.6, 119.9, 116.6, 114.2 (2), 55.5. IR (neat) ν_{max}/ cm^{-1} :3291, 2959, 1696, 1606, 1511, 1441, 1250. HRMS calcd for $C_{19}H_{16}NO_3$ $[M + H]^+$ 306.1130, obsd. 306.1134.

4.2.6. Methyl 4-(1-(4-methoxyphenyl)vinyl)quinoline-2-carboxylate **2i** (100 %).

At -5 °C were successively added in MeOH (1 mL), $SOCl_2$ (72 mL, 1 mmol), and after 30 min amide **2g** (171 mg, 0.62 mmol). The solution was then stirred at 55 °C for 6 h. After cooling to rt, the solution was concentrated and water and EtOAc were added to the crude. After extraction and drying on Na_2SO_4, the residue was purified by silica gel column chromatography to give **2i** (203 mg, 100%).

Pale yellow solid, F = 94.2-94.9 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.33 (d, *J* = 8.0 Hz, 1H), 8.13 (s, 1H), 7.83 (d, *J* = 8.0 Hz, 1H), 7.73 (t, *J* = 7.5 Hz, 1H), 7.49 (t, *J* = 7.5 Hz, 1H), 7.18 (d, *J* = 8.9 Hz, 2H), 6.81 (d, *J* = 8.9 Hz, 2H), 5.96 (s, 1H), 5.34 (s, 1H), 4.09 (s, 3H), 3.78 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.2, 159.9, 150.4, 148.1, 147.9, 145.2, 132.3, 131.1, 130.2, 128.6, 128.4, 128.0 (2), 126.2, 121.7, 116.0, 114.2 (2), 55.4, 53.3. IR (neat) ν_{max}/ cm^{-1} : 3001, 2952, 1720, 1605, 1511, 1414, 1249. HRMS calcd for $C_{20}H_{18}NO_3$ $[M + H]^+$ 320.1287, obsd. 320.1278.

4.2.7. 4-(1-(4-Methoxyphenyl)vinyl)quinoline-2-carbaldehyde **2j** (69%, sensitive compound which must be used rapidly after purification)

At -78 °C under argon, was added a 1N solution of DIBAL (0.06 mL) to a CH_2Cl_2 solution (1 mL) of **2i** (20 mg, 0.06 mmol). The mixture was stirred at this temperature for 0.5 h and quenched with MeOH (5 mL). After concentration, the crude was purified by silica gel column chromatography to give **2j** (17 mg, 69%).

White solid. F = 105.3-106.0 °C ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.27 (s, 1H), 8.28 (d, J = 8.5 Hz, 1H), 7.96 (s, 1H), 7.85 (d, J = 8.5 Hz, 1H), 7.76 (t, J = 8.2 Hz, 1H), 7.53 (t, J = 8.2 Hz, 1H), 7.18 (d, J = 8.8 Hz, 2H), 6.81 (d, J = 8.8 Hz, 2H), 5.96 (s, 1H), 5.34 (s, 1H), 3.79 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 194.1, 159.9, 152.5, 150.5, 148.5, 145.3, 132.3, 130.9, 130.4, 129.2, 128.0 (2), 126.6, 126.3, 118.0, 116.2, 114.2 (2), 55.5.

4.2.8. (4-(1-(4-Methoxyphenyl)vinyl)quinolin-2-yl)methanol **2k** (66%)

2k was prepared according to the same reducing protocole used for the synthesis of **2j**

Brown oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.11 (d, J = 7.6 Hz, 1H), 7.76 (d, J = 7.6 Hz, 1H), 7.68 (t, J = 7.6 Hz, 1H), 7.39 (t, J = 7.6 Hz, 1H), 7.32 – 7.15 (m, 3H), 6.81 (d, J = 8.9 Hz, 2H), 5.93 (s, 1H), 5.30 (s, 1H), 4.95 (s, 2H), 3.78 (s, 3H) 3.46 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.8, 158.8, 149.9, 147.1, 145.5, 132.3, 129.8, 128.9, 128.0 (2), 126.7, 126.4 (2), 118.9, 115.5, 114.1 (2), 64.2, 55.4. IR (neat) ν_{max}/ cm^{-1}: 3056, 2789, 2289, 1615, 1579, 1535, 1412, 1270. HRMS calcd for $C_{19}H_{18}NO_2$ $[M+H]^+$ 292.1338, obsd. 292.1343.

4.2.9. N-(4-methoxyphenyl)-N-methyl-2-(trifluoromethyl)quinolin-4-amine **2l** (92%)

In a sealed tube and under an argon atmosphere were added successively, 4-chloro-2-(trifluoromethyl)quinoline **5** (232 mg, 1 mmol), hydrazone **3a** (478 mg, 1.5 mmol) $PdCl_2(CH_3CN)_2$ (26mg, 10 mol%), dppf (111 mg, 20 mol%) in dry dioxane (10 mL) and the mixture was stirred for 5 min. at rt. Then dry LiO*t*Bu (176 mg, 2.2 mmol) was added and the mixture was stirred at 100° C for 3 h. The resulting suspension was cooled to room temperature, filtered through a pad of Celite eluting with ethyl acetate and the inorganic salts were removed. The filtrate was concentrated and the crude was purified by silica gel column chromatography (Cyclohexane/AcOEt, 95/5) to give **2l** (303 mg, 0.92 mmol, 92 %).

Brown oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.07 (d, J = 8.5 Hz, 1H), 7.67 (d, J = 8.5 Hz, 1H), 7.58 (t, J = 7.7 Hz, 1H), 7.49 (s, 1H), 7.33 (t, J = 7.7 Hz, 1H), 7.00 (d, J = 8.5 Hz, 2H), 6.64 (d, J = 8.5 Hz, 2H), 5.80 (s, 1H), 5.16 (s, 1H), 3.61 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.8, 150.2, 146.7 (q, J = 38 Hz) 146.5, 143.8 (2), 130.8, 129.5, 129.3, 127.4, 126.8 (2), 125.2, 120.6 (q, J = 274 Hz), 116.2, 115.1, 113.0 (2), 54.3. ^{19}F NMR (188 MHz, $CDCl_3$) δ -68.2. IR (neat) ν_{max}/ cm^{-1} : 2961, 2904, 2358, 1606, 1511, 1442, 1419, 1382, 1341, 1250, 1179, 1033. HRMS calcd for $C_{19}H_{15}NOF_3$ $[M + H]^+$ 330.1106 found 330.1110.

4.2.10. (4-Methoxyphenyl)(2-methylquinolin-4-yl)-methanone **6** (49%)

Into a three neck round bottom flask provided with a drying tube and a gas dispersion tube, a solution of **2a** (874 mg, 3.15 mmol) in 50 mL of a mixture of CH_2Cl_2/MeOH (9/1) was prepared. The solution was then cooled to -78 °C and saturated with O_2. Then, a stream of O_3/O_2 (approximately 1% of O_3) was applied to the solution. After 15 min, the mixture become green-blue. The ozonizer was set to 0 V, and the solution was purged with O_2 for 15 min. After disappearance of **2a** (judged by TLC), PPh_3 (1.65 g, 6.30 mmol) was added by small portions at -78 °C. After 4 h of stirring, the reaction mixture was returned to rt and PPh_3 (826 mg, 3.15 mmol) was added again. The resulting orange solution was stirred overnight and concentrated under reduced pressure. The crude residue was purified by chromatography on silica gel (Cyclohexane/AcOEt, 8/2 to 4/6) to give 431 mg of **5** (1.54 mmol, 49 %).

Brown solid, F: 121-124 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.01 (d, J = 8.4 Hz, 1H), 7.73 (d, J = 8.9 Hz, 2H), 7.66 (d, J = 9.2 Hz, 1H), 7.60 (dd, J = 8.3 Hz, J = 1.2 Hz, 1H), 7.35 (t, J = 7.6 Hz, 1H), 7.18 (s, 1H), 6.84 (d, J = 8.9 Hz, 2H), 3.78 (s, 3H), 2.70 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 194.8, 164.6, 158.4, 148.3, 145.5, 132.80 (2), 130.0, 129.8, 129.2, 126.6, 125.4, 123.5, 120.1, 114.1 (2), 55.7, 25.5. IR (neat) ν_{max}/ cm^{-1} : 1731, 1657, 1597, 1511, 1462, 1422, 1379, 1342, 1250, 1170, 1029. HRMS calcd for $C_{20}H_{16}NO_2$ $[M + H]^+$ 278.1181 found 278.1179.

4.2.11. (E/Z)-3-(4-Methoxyphenyl)-3-(2-methylquinolin-4-yl)-acrylonitrile **2m** (50%)

A 1.0 M solution of LiHMDS (1.20 mL, 1.20 mmol) was added dropwise to a cooled (0 °C) solution of diethyl (cyanomethyl)phosphonate (213 mg, 1.20 mmol) in dry THF (5 mL). The yellow reaction mixture was returned to rt and was stirred for 45 minutes. Then, a solution of **5** (166 mg, 0.60 mmol) in THF (5 mL) was slowly cannulated into the ylide solution. After 15 min. of stirring, the reaction flask was heated to reflux for 12 h, and was then cooled to rt. The reaction was quenched using H_2O (20 mL), and the solvent was removed under vacuum. After extraction with AcOEt (3 x 10 mL), the organic phase was washed with brine (15 mL), dried over $MgSO_4$ and concentrated. The crude residue was purified by chromatography on silica gel (Cyclohexane/AcOEt, 8/2 to 4/6) to afforded 90 mg of **2m** (0.30 mmol, 50 %) as a mixture of inseparable diastereoisomers (*E*/*Z* = 62/38).

White solid, F: 134-137 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.10 (t, *J* = 9.9 Hz, 1H), 7.76-7.64 (m, 1H), 7.62-7.49 (m, 1.7 H), 7.40 (t, *J* = 7.1 Hz, 1H), 7.32-7.20 (m, 2.3 H), 6.92 (d, *J* = 8.9 Hz, 0.76 H), 6.86 (d, *J* = 8.9 Hz, 1.24 H), 6.11 (s, 0.62 H), 5.56 (s, 0.38 H), 3.85 (s, 1.14 H), 3.82 (s, 1.86 H), 2.84 (s, 1.86 H), 2.80 (s, 1.14 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 162.1, 161.7, 159.0, 158.9, 158.8, 158.7, 148.5, 146.4, 143.7, 130.6, 130.0, 129.9, 129.5, 129.4, 129.0, 128.9, 126.6, 125.2, 125.0, 124.4, 124.3, 122.3, 122.1, 117.6, 117.1, 114.7, 114.4, 96.3, 95.4, 55.6, 55.5, 25.5, 25.4. IR (neat) ν_{max}/ cm^{-1} : 2925, 2851, 2214, 1603, 1512, 1449, 1359, 1299, 1256, 1179, 1029. HRMS calcd for $C_{20}H_{17}NO_2$ $[M + H]^+$ 301.1341 obsd. 301.1335.

4.2.12. 1-(1-(4-Methoxyphenyl)vinyl)-3-methylnaphthalene **2n** (79%)

A 20 mL microwave tube containing a stir bar was charged with 1-bromo-3-methylnaphthalene (221 mg, 1.0 mmol, 1.0 eq), N'-(1-(4-methoxyphenyl)ethylidene)-4-methylbenzenesulfonohydrazide (382 mg, 1.2 mmol, 1.2 eq), Pd_2dba_3.$CHCl_3$ (26 mg, 2.5 mol %), XPhos (48 mg, 10 mol %) and lithium tert-butoxide (176 mg, 2.2 mmol, 2.2 eq). The tube was capped and purged with argon three time, then dioxane (5-10 mL) was added *via* syringe. After sealing the tube, it was put into a pre-heated oil bath at 100 °C and stirred for 2 h. Then, the reaction mixture was allowed to cool to rt and EtOAc was added to the mixture, which was filtered through Celite. The solvents were evaporated under reduce pressure and the crude residue was purified by flash chromatography on silica gel (Cyclohexane/AcOEt 10/0 to 7/3) to give **2n** 216 mg (0.79 mmol, 79 %).

Yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 7.84 (dd, *J* = 8.1 Hz, *J* = 5.0 Hz, 2H), 7.70 (s, 1H), 7.53-7.42 (m, 1H), 7.39-7.32 (m, 3H), 6.87 (d, *J* = 8.8 Hz, 2H), 5.96 (s, 1H), 5.37 (s, 1H), 3.82 (s, 3H), 2.61 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 159.3, 147.7, 140.0, 135.1, 134.1, 133.8, 130.3, 129.4, 127.9 (2), 127.6, 126.9, 126.4, 125.8, 125.1, 114.2, 113.8 (2), 55.3, 21.7. IR (neat) ν_{max}/ cm^{-1} : 2959, 2834, 1604, 1509, 1462, 1441, 1342, 1290, 1246, 1177, 1117, 1097, 1033. HRMS (ESI) calcd for $C_{20}H_{19}O$ $(M+H)^+$ 275.1536 obsd. 275.1527.

4.3. Biolology

4.3.1. 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, U87, K562, K562R and HCT116 cells were grown in RPMI 1640 containing 10% FCS and 1% glutamine. A549 lung carcinoma, cells were grown in Dulbecco minimal essential medium (DMEM) containing 4.5 g/L glucose supplemented with 10% FCS and 1% glutamine.

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 IC_{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. Experiments were performed in triplicate.

4.3.2. Tubulin Binding Assay

Sheep brain tubulin was purified according to the method of Shelanski [33] by two cycles of assembly-disassembly and then dissolved in the assembly buffer containing 0.1 M MES, 0.5 mM $MgCl_2$, 1 mM EGTA, and 1 mM GTP, pH 6.6 (the concentration of tubulin was about 2-3 mg/mL). Tubulin assembly was monitored by fluorescence according to reported procedure [34] 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 which decreased 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 measured the same day under the same conditions.

4.3.3. Cell Cycle Analysis

Exponentially growing cancer A549 cells were incubated with **2b** at different concentrations (0.5, 1, 5 and 10 nM) or in DMSO alone for 24 h. Cell-cycle profiles were determined by flow cytometry on a FC500 flow cytometer (Beckman-Coulter, France) as described previously. [35]

4.3.4. Immunofluorescence Staining Assays

A549 cells were seeded on Lab-Tek™ *Chamber* Slides (Thermo Fisher Scientific) and incubated for 24 h in the presence or absence **2b**. Cells were then fixed for 30 min. with 2% paraformaldehyde at room temperature and washed with Dulbecco's phosphate-buffered saline (DPBS).

After permeabilization with 0.25% Triton X-100 for 5 min., A549 cells were washed in DPBS and incubated for 1 h in DPBS containing 1% Bovine Serum Albumine (BSA) and 10% normal goat serum (DAKO cytomation) for blocking. Cells were then incubated overnight at 4 °C with the mouse anti-β tubulin primary antibody (Thermo Fisher Scientific). Thereafter, the cells were washed with 0.2% Tween in DPBS, and incubated simultaneous for 2 h at room temperature with Alexa-Fluor® 647 goat anti-mouse IgG secondary antibody and Hoechst 33258 (Thermo Fisher Scientific). Cells were then washed with DPBS and incubated for 30 min at room temperature with Alexa-Fluor® 488 Phalloidin (Thermo Fisher Scientific) to perform F-actin labeling. Fluorescence images were collected by Nikon TE2000 inverted microscope with Nikon 1.4 NA DIC optics, 60X oil immersion objective (Nikon).

4.3.5. Molecular Modelling

X-ray structures of five different tubulin co-crystals were retrieved from the PDB [36] (accession codes 1SA0, 1SA1, 3HKC, 3HKD, and 3HKE) and prepared using the Protein Preparation Wizard workflow from Schrödinger suite, [37] including optimization of the hydrogen bond network and a short energy minimization with position restraints on heavy atoms using the OPLS_2005 force field. [38] Initial coordinates for compounds **2a,b** and **2d**–**f** were generated using MarvinSketch from JChem suite v15.12.7, [39]. 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 [40] over the five (aligned) tubulin structures. CHEMPLP with default parameters was used as an objective function.[41] Structures of the complexes were exported, subjected to hydrogen bond network optimization, and a short minimization with position restraints on heavy atoms using Protein Preparation Wizard, then loaded into Chimera v1.10.2 [42] for examination (including hydrogen bond detection, close contact analysis, and representation of solvent-accessible surface) and depiction.

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