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# Design, synthesis and anticancer properties of 5-arylbenzoxepins as conformationally restricted *iso*Combretastatin A-4 analogues

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## Abstract

A series of novel benzoxepins **6** was designed and prepared as rigid-*iso*CA-4 analogues according to a convergent strategy using the coupling of *N*-tosylhydrazones with aryl iodides under palladium catalysis. The most potent compound **6b**, having the greatest resemblance to CA-4 and *iso*CA-4 displayed antiproliferative activity at nanomolar concentrations against various cancer cell lines and inhibited tubulin assembly at a micromolar range. In addition, benzoxepin **6b** led to the arrest of HCT116, K562, H1299 and MDA-MB231 cancer cell lines in the G<sub>2</sub>/M phase of the cell cycle, and strongly induced apoptosis at low concentrations. Docking studies demonstrated that benzoxepin **6b** adopt an orientation similar to that of *iso*CA-4 at the colchicine binding site on  $\beta$ -tubulin.

**Keywords:** Benzoxepin, Combretastatin, *iso*CA-4, Tubulin, Cytotoxicity, Apoptosis, Cancer

## 1. Introduction

Combretastatin A-4 (CA-4, Figure 1), a natural stilbene isolated by Pettit[1] from the South African tree *Combretum caffrum*, is a potent cytotoxic agent against a variety of cancer cells, including multidrug-resistant cell lines.[2,3] CA-4, which binds on  $\beta$ -tubulin at the colchicine binding site, is also an exceptionally strong inhibitor of tubulin polymerization with an  $IC_{50}$  value of 1.0  $\mu$ M.[4] Disruption of tubulin assembly results in rapid tumor endothelial cell damage followed by neovascular shutdown, and subsequent hemorrhagic necrosis.[5] A great interest in CA-4 is reflected in the fact that the water soluble phosphate prodrug CA-4P (fosbretabulin, **1b**)[6,7] is currently used for advanced anaplastic thyroid carcinoma, even if some vascular side effects were reported.[8,9] Despite its remarkable anticancer activity, CA-4 isomerizes into its inactive (*E*)-isomer[10] during storage, administration[11] and metabolism[12] leading to a significant loss of potency. The poor bioavailability and solubility in biological media associated with the *Z* to *E*-isomerization drawback,[11] together with the structural simplicity of CA-4, makes it very interesting from a medicinal chemistry point of view, thus stimulating the search for new and more potent compounds with improved pharmacological properties[13,14] Considerable effort has been gone into modifying the isomerizable *cis* carbon-carbon double bond. Several reviews outlined this vast array of chemistry focusing on the stabilization of the two aryl rings of CA-4 using one to three atom bridgeheads.[15-20]

### <Figure 1>

**Figure 1.** Representative inhibitors of tubulin polymerization and rational drug design from CA-4 and *iso*CA-4 to dihydrobenzoxepin analogues **6**.

In our efforts to discover novel CA-4 analogues having non-isomerizable linkers between the A- and B-rings,[21-26], we recently synthesized a series of 1,1-diarylethylene derivatives[27-29] with general structure **2**. The most active molecules in this series, *iso*CA-4 (**2a**), *iso*NH<sub>2</sub>CA-4 (**2b**), *iso*FCA-4 (**2c**) and **2d** displayed a nanomolar level of cytotoxicity against various cancer cell lines, inhibited tubulin polymerization (ITP) at a micromolar level, and arrested cancer cells in the G<sub>2</sub>/M phase of the cell cycle (Figure 1).[30-33] These 1,1-diarylethylene compounds **2** were found to be as active as their (*Z*)-1,2-ethylene isomers (CA-4 **1a**, NH<sub>2</sub>CA-4 **1c**, FCA-4 **1d**), clearly demonstrating that it is possible to replace the (*Z*)-double bond of CA-4 derivatives with a 1,1-ethylene unit with no loss of efficacy.

*iso*CA-4 was utilized as a starting point to design cyclic and heterocyclic derivatives **3-5** with restricted rotation to identify novel compounds with improved anticancer activities. Inserting the double bond of *iso*CA-4 into 6-membered rings led to (dihydro)naphthalene **3**[34-36] and chromene **4**[37,38] derivatives endowed with interesting anticancer activities (Figure 1). In 2008, Pinney reported the synthesis of benzosuberenes of type **5**[39,40] as highly cytotoxic agents, demonstrating that the double bond in *iso*CA-4 could be successfully included in a 7-membered ring to maintain potent activity against various cancer cell lines. In continuation of our earlier work, a novel series of restricted *iso*CA-4 derivatives **6** containing a 2,3-dihydrobenzoxepin ring was designed. Herein we report the synthesis, the biological evaluation and the possible binding mode of 5-aryldihydrobenzo[*b*]oxepins **6**.

## 2. Results and discussion

### 2.1. Chemistry.

The retrosynthetic analysis of the target 5-arylbenzoxepins **6** is outlined in Figure 2. We envisioned that the 5-aryl moiety in **6** could be installed through palladium catalyzed coupling reactions starting from (i) vinylstannanes **7** (Stille coupling, path *a*), (ii) vinyl iodides **8** (Suzuki coupling, path *a*), or (iii) *N*-tosylhydrazones **11** (path *b*) using a recent methodology developed by Barluenga and us[41,42] (Figure 2). To build up the 7-membered heterocyclic ring of compounds **7** and **8**, a ring-closing metathesis (RCM) reaction was planned as the key step from the corresponding diene derivatives **9**, **10** and **13**, respectively. An alternative route to **11** could be an intramolecular Mitsunobu reaction starting from phenol **12**.

<Figure 2>

**Figure 2.** Retrosynthetic analysis of 5-aryldihydrobenzo[*b*]oxepins **6**.

At the outset of this work, and according to path *a* (Scheme 1), we first studied the synthesis of diene **9a** ( $R^1 = R^2 = H$ ) through regioselective addition of  $Bu_3SnH$  on the alkyne triple bond[43] of terminal arylalkyne **14**. [44] As expected, we were pleased to observe a total regioselectivity in the palladium-catalyzed hydrostannylation of **14** leading exclusively to  $\alpha$ -vinylstannane **9a** in a 80% yield. Iododestannylation of **9a** with molecular iodine in  $CH_2Cl_2$  at room temperature afforded **10a** ( $R^1 = R^2 = H$ ) in a modest and non-optimized yield of 45%. Unfortunately, despite attempting a plethora of RCM conditions using the Grubbs I, Grubbs II, and Hoveyda-Grubbs catalysts (from 2% to 20%) in  $CH_2Cl_2$ , toluene at different temperatures (from 20 °C to reflux) and various concentrations, no macrocyclization of dienes **9a** and **10a** was ever observed.

<Scheme 1>

**Scheme 1.** Synthesis of vinylstannane **9a** and vinyl iodide **10a**. Reagent and conditions: a)  $PdCl_2(PPh_3)_2$  (2 mol%),  $Bu_3SnH$  (1.2 equiv), THF, 20 °C; b)  $I_2$ ,  $CH_2Cl_2$ , 20 °C.

The synthesis of benzoxepin derivatives **6** was next envisioned according to path *b* involving an intramolecular Mitsunobu reaction (Scheme 2). Initially, the compounds targeted **6b** and **6c** contain on the benzoxepin nucleus 9-hydroxy and 8-methoxy substituents which are present in the *isoCA-4* B-ring, and that were proved to be essential for bioactivity (Scheme 2).

<Scheme 2>

**Scheme 2.** Synthesis of dihydrobenzoxepins **6b,c**. Reagent and conditions: a)  $MOMCl$  (2 equiv),  $iPr_2NH$  (2 equiv),  $CH_2Cl_2$ ; b) But-3-yn-1-ol (1.1 equiv),  $PdCl_2(PPh_3)_2$  (8 mol%),  $CuI$  (15 mol%),  $Et_3N$ ; c)  $PTSA$  (1 equiv),  $H_2O$ , 100 °C, sealed tube; d)  $DEAD$  (1.5 equiv),  $PPh_3$  (1.5 equiv), THF; e)  $TsNHNH_2$  (2.5 equiv),  $PTSA$  (20 mol%),  $EtOH$  reflux; f)  $Pd_2dba_3$  (10 mol%),  $XPhos$  (20 mol%),  $tBuOLi$  (2 equiv),  $ArI$  (1 equiv), 90 °C, sealed tube; g)  $AlCl_3$  (5 equiv),  $CH_2Cl_2$ .

Arylalkynol **17**[45] was prepared from iodophenol **15** through a sequence of MOM-ether protection followed by a Sonogashira-Linstrumelle coupling reaction[46,47] with homopropargylic alcohol. The triple bond of **17** was regioselectively hydrated in the presence of  $PTSA$ [48-52] in water, and a subsequent intramolecular Mitsunobu reaction furnished the required benzoxepan-5-one **19** in a 63% yield. This latter was mixed with  $TsNHNH_2$  in the presence of  $PTSA$  to afford the key intermediate *N*-tosylhydrazone **11a**, which in turn was coupled with various aryl halides under  $Pd_2dba_3/XPhos$  as the catalytic system.[41,53] Accordingly, trimethoxyphenylbenzoxepin **6a** was formed in an excellent 98% yield.[54,55] Finally, the phenolic part of isopropylether **6a** was revealed by treatment with  $AlCl_3$  to give **6b** but in a low 15% yield. Target molecule **6c**, with a 3,5-dimethoxyphenyl nucleus as *A*-ring, was also prepared from *N*-tosylhydrazone **11a**, and the resulting crude coupling product treated with  $AlCl_3$  in  $CH_2Cl_2$  furnished the desired benzoxepin derivative **6c** in a modest 25% yield.

For benzoxepin derivatives **6d-g** having different substituents on C9, we envisioned their synthesis *via* a convergent approach starting from *N*-tosylhydrazone **11b** having a bromine atom at the C9 position, useful for subsequent functionalizations under palladium catalysis (Scheme 3)[32,36,56]. To this end, we planned the construction of the 7-membered ring of **11b** using RCM of diene **13a**. Briefly, benzaldehyde **21** was prepared from *isovanillin* through a sequence of regioselective *N*-methylpyrrolidin-2-one hydrotribromide (MPHT) bromination[57,58] and allyl ether formation. Further reaction with vinylmagnesium chloride afforded diene **13a**, which underwent ring-closing olefin metathesis using the Grubbs II catalyst in  $CH_2Cl_2$ . [59] The resulting dihydrobenzoxepin-5-ol **22** was subjected to a double bond reduction, PCC alcohol oxidation and then *N*-tosylhydrazone formation to give **11b**. Further palladium-catalyzed coupling reaction with 3,4,5-trimethoxyiodobenzene furnished the key 9-bromo-8-methoxybenzoxepin **6d** in a 68% yield. Having achieved efficient access to **6d**, we then turned studying various means of substitution on the C9-bromo atom. The bromo substituent of **6d** was first exploited in a coupling reaction using sodium azide as the amino source in the presence of a catalytic amount of  $CuI$  to give **6e** (75%). [60,61] Debromination of **6d** was achieved using catalytic amounts of  $Pd(OAc)_2$ ,  $PPh_3$  and  $K_2CO_3$  in hot *n*BuOH and produced **6f** in 69% yield. [62] For the synthesis of **6g** having a

butyn-1-ol chain, **6d** was coupled with but-3-yn-1-ol using PdCl<sub>2</sub>(PPh<sub>3</sub>)<sub>2</sub> and CuI catalysts under microwave irradiation at 120 °C in DMF (74%). Noteworthy that compound **6b** was also prepared in an acceptable yield of 55% by treating **6d** with KOH in the presence of Pd<sub>2</sub>dba<sub>3</sub> and *t*BuXPhos in a dioxane-H<sub>2</sub>O mixture according to a Buchwald protocol.[63] Finally, because the double bond present in *iso*CA-4 can be reduced to furnish *iso*erianin derivatives with no loss of anticancer properties,[23] we next focused our attention on the catalytic reduction of the carbon-carbon double bond of **6b** and **6g**. Thus, 5-aryltetrahydrobenzoxepins **25a** and **25b** were obtained in non-optimized yields using H<sub>2</sub> in the presence of Pd/C in MeOH (Scheme 3).

### <Scheme 3>

**Scheme 3.** Synthesis of dihydrobenzoxepins **6b**, **6d-g** and tetrahydrobenzoxepins **25a,b**. Reagent and conditions: a) Hg(OAc)<sub>2</sub> (1 equiv), NaBr (1 equiv), 50 °C, EtOH; b) MPHT (1 equiv), CH<sub>3</sub>CN; c) 3-bromoprop-1-ene (1.2 equiv), K<sub>2</sub>CO<sub>3</sub> (1.2 equiv), CH<sub>3</sub>CN; d) vinylmagnesium chloride (1.2 equiv), THF; e) Grubbs II catalyst, 5 mol%, CH<sub>2</sub>Cl<sub>2</sub>, 20 °C; f) H<sub>2</sub>, Pd/C, MeOH, AcOH cat.; g) PCC, CH<sub>2</sub>Cl<sub>2</sub>; h) TsNHNH<sub>2</sub> (1 equiv), PTSA (10 mol%), MgSO<sub>4</sub> (1 equiv), EtOH, reflux; i) Pd<sub>2</sub>dba<sub>3</sub> (10 mol%), XPhos (40 mol%), *t*BuOLi (2 equiv), 3,4,5-trimethoxyiodobenzene (1.2 equiv), 90 °C, sealed tube; j) Pd<sub>2</sub>dba<sub>3</sub> (5 mol%), *t*BuXPhos (15 mol%), KOH (3 equiv), dioxane-H<sub>2</sub>O, 90 °C, sealed tube; k) CuI (10 mol%), TMEDA (6 mol%), NaN<sub>3</sub> (2 equiv), potassium ascorbate (6 mol%) DMSO-H<sub>2</sub>O, 90 °C, sealed tube; l) Pd(OAc)<sub>2</sub> (1 mol%), PPh<sub>3</sub> (2.5 mol%), *n*BuOH, 100 °C, sealed tube; m) *n*But-3-yn-1-ol (1.2 equiv), PdCl<sub>2</sub>(PPh<sub>3</sub>)<sub>2</sub> (5 mol%), PPh<sub>3</sub> (15 mol%), CuI (15 mol%), Et<sub>2</sub>NH (2 equiv), DMF, 120 °C, MWI.

## 2.2. Biological results.

### 2.2.1 In vitro cell growth assay

All the synthesized compounds were tested in a preliminary cytotoxic assay on a human colon carcinoma (HCT116) cell line using CA-4,[64] and *iso*CA-4 as reference compounds.

### <Table 1>

**Table 1.** Cytotoxic activity of dihydrobenzoxepins **6b-g** and tetrahydrobenzoxepins **25a,b** against HCT116<sup>[a]</sup>

In this assay, the benzoxepin derivatives **6** inhibited cellular growth to a varying degree. Except tetrahydrobenzoxepin **25b**, having a bulky butanol chain on C9 which was found inactive against HCT116 cells, all selected compounds displayed a nanomolar level of cytotoxicity. Lowest GI<sub>50</sub> values were found for both bromine derivative (**6d**, GI<sub>50</sub> = 170 nM) and compound **6g** having a butyn-ol chain on the C9 position (**6g**, GI<sub>50</sub> = 250 nM) (Table 1). These functional groups on C9 were bulky, so next we examined smaller substituents on the C9 position. The C9-OH benzoxepin derivative **6b**, having the greatest resemblance to *iso*CA-4 is undoubtedly the most cytotoxic compound in this series with a GI<sub>50</sub> value of 1.5 nM slightly inferior to those of *iso*CA-4 (GI<sub>50</sub> = 3 nM) and CA-4 (GI<sub>50</sub> = 2 nM). Contrary to Ohsumi's finding,[65] it appears that the introduction of a NH<sub>2</sub> substituent on C9 result in weaker cytotoxicity (**6e**, GI<sub>50</sub> = 22 nM). Compound **6f**, with no substitution on the C9 position showed a significant loss of activity with a GI<sub>50</sub> value of 85 nM. Concerning the A-ring, it seems that a 3,4,5-trimethoxyphenyl ring is required for strong activity, as compound **6c** (GI<sub>50</sub> = 25 nM) with only two MeO-substituents on the A-ring exhibited a lowest cytotoxicity in comparison with **6b**. Evaluation of tetrahydro-benzoxepin **25a** which was found to elicit a nanomolar level of cytotoxicity (GI<sub>50</sub> = 20 nM) demonstrated that the presence of the double bond in dihydrobenzoxepin compounds **6** was important but not essential for antiproliferative property as we previously showed by reducing the double bond of *iso*CA-4.[23]

The results reported in Table 1 showed that benzoxepins **6b** and **6e** having a OH and a NH<sub>2</sub> substituent on C9 position, respectively, displayed strong cytotoxicities. Benzoxepin **6b**, having the greatest resemblance to *iso*CA-4 is undoubtedly the most cytotoxic compound with a GI<sub>50</sub> value of 1.5 nM comparable to those of *iso*CA-4 and CA-4. Compound **6c** with only two MeO-substituents on the A-ring exhibited a decreased cytotoxicity in comparison with **6b** (GI<sub>50</sub> = 25 nM). Contrary to previous observations in the CA-4[1] and *iso*CA-4 series,[Erreur ! Signet non défini.] the replacement of the OH group by a NH<sub>2</sub> substituent on the C9 position resulted in a slight loss of cytotoxicity as observed with compound **6e** (GI<sub>50</sub> = 20 nM). Compound **6f**, with no substitution on the C9 position showed a slight loss of activity with a GI<sub>50</sub> value of 85 nM. Contrary to our expectation,[Erreur ! Signet non défini.] the alkylation at the C9 position in benzoxepin **6g** with a butynol moiety, resulted in a moderate activity against HCT116 cells with a GI<sub>50</sub> value of 150 nM. Evaluation of tetrahydro-benzoxepin **25a** which was found to elicit a nanomolar level of cytotoxicity (GI<sub>50</sub> =

20 nM) demonstrated that the presence of the double bond in dihydrobenzoxepin compounds **6** was important but not essential for antiproliferative property as we previously showed by reducing the double bond of *iso*CA-4. [Erreur ! Signet non défini.]

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### 2.2.2 Cytotoxicity and Inhibition of Tubulin Polymerization for Selected Compounds

To further characterize the cytotoxicity profiles of these compounds, we investigated the effect of the more cytotoxic benzoxepins **6b**, **6c**, **6e**, **6f**, and **25a** ( $GI_{50} < 85$  nM) on the proliferation of three human tumor cell lines: myelogenous leukemia (K562), non-small cell lung carcinoma (H1299) and hormone-independent breast cancer (MDA-MB231).

<Table 2>

**Table 2.** Cytotoxic activity and inhibition of tubulin polymerization of selected compounds

The results depicted in Table 2 revealed that all selected compounds which retain a high level of cytotoxicity against HCT116 were also strongly cytotoxic with no significant disparity against K562, H1299 and MDA-MB231 cancer cell lines. Again, benzoxepin **6b** was the most active compound inhibiting the growth of K562, H1299 and MDA-MB cancer cell lines with  $GI_{50}$  values inferior ranging from 1.5 to 8 nM comparable to that of *iso*CA-4 ( $GI_{50} = 3-5$  nM), thus clearly indicating an antiproliferative activity regardless of the origin of the tumor cells. 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, benzoxepins **6b,c**, **6e,f** and **25a** were evaluated for their inhibitory effects on tubulin assembly (Table 2). As expected, all tested compounds strongly inhibited tubulin assembly with comparable  $IC_{50}$  values of 3.2-3.9  $\mu$ M, which were about two times greater than that of *iso*CA-4 ( $IC_{50} = 1.5$   $\mu$ M). Consistent with earlier observations[66], the replacement of the 4-methoxy group in **6b** ( $CI_{50} = 3.8$   $\mu$ M) with an hydrogen atom in **6c** ( $CI_{50} = 3.2$   $\mu$ M) had no significant impact on tubulin assembly but reduced cytotoxicity by approximately 10 orders of magnitude.

### 2.2.3 Cell Cycle Analysis and Apoptosis

Because molecules exhibiting activity on tubulin should cause the alteration of cell cycle parameters leading to a preferential  $G_2/M$  blockade,[67] the effect of the most potent benzoxepin **6b** on the cell cycle of K562, MDA-MB231, H1299 and HCT116 cells was then investigated. Cancer cells were cultured without or with **6b** (Figure 3) and cell cycle distribution was analyzed by flow cytometry after 24 h of treatment using the standard propidium iodide procedure.

<Figure 3>

**Figure 2.** Effects of benzoxepin **6b** at a concentration of 5.0 nM on cell cycle distribution in various cancer cell lines.

After treatment with 5 nM of **6b**, a net increase in the number of cells arrested at the  $G_2/M$  growth phase was observed. Cell-cycle arrest in the  $G_2/M$  phase is frequently followed by DNA fragmentation and other morphological features of apoptosis. The potency of benzoxepin **6b** to induce apoptosis was further characterized by a specific apoptosis assay. Cleavage of pro-caspases to active caspases is one of the hall marks of apoptosis. HCT116 and H1299 cells were incubated with 1, 5, and 10 nM of **6b** for 24 h and caspases-3 and -7 activities were evaluated using the standard caspases assays.

<Figure 4>

**Figure 3.** Apoptotic effects of **6b** in HCT116 and H1299 cells. The results are expressed in the percentage of apoptotic cells detected following 24 h of treatment with **6b** at different concentrations.

The results depicted in Figure 4 show a significant dose-dependent increase in proteolytic activity of caspases in HCT116 and H1299 cells treated with **6b** at low concentrations of 5 and 10 nM. These data suggest that benzoxepin **6b**, in addition to its antiproliferative properties, also induced apoptosis in the tested cancer cell lines.

### 2.3. Docking study.

To rationalize the potential binding mode of benzoxepins **6** in tubulin, a docking study was carry out with **6b** as the most potent derivative in this series. For this purpose, the X-ray structure of tubulin-DAMA-colchicine complex (Code PDB: 1sa0)[68] was used.

<Figure 4>

**Figure 5.** Docked pose of **6b** (gray) overlaid with *isoCA-4* (blue) in the tubulin binding site.

The docking-derived superimposition of **6b** and *isoCA-4* was illustrated in Figure 5, and benzoxepin **6b** showed a binding pose similar to the one observed with *isoCA-4*. As it was previously observed with CA-4 and *isoCA-4*, the trimethoxyphenyl ring of **6b** was placed in proximity of Cys241 and the benzoxepin ring in proximity of Val181 to establish a hydrogen bond with its C9-OH substituent.

### 3. Conclusion

We have designed and synthesized a series of benzoxepin compounds **6** as rigid-*isoCA-4* analogues and evaluated their biological activities. Compound **6b**, the hybrid most closely resembling *isoCA-4* in structure, have potent antimetabolic and cytotoxic properties. Benzoxepin **6b**, which was prepared from isovanillin according to a convergent synthetic strategy in 9 steps (7.8% overall yield), displayed a nanomolar level of cytotoxicity against various cancer cell lines ( $GI_{50} = 1.5-8$  nM), and showed similar antitubulin activities than that of *isoCA-4*. Flow cytometry analysis indicated that benzoxepin **6b** at a low concentration of 5 nM, arrested the cell cycle in the G<sub>2</sub>/M phase in H1299, K562, HCT116 and MDA-MB231 cells. Furthermore, **6b** was characterized as a strong apoptotic agent by inducing the activation of procaspases in HCT116 and H1299 cells. All these results are well supported by molecular modelling studies, where it was observed that benzoxepin **6b** and *isoCA-4* can be perfectly superimposed in the tubulin binding-site. On the basis of its excellent properties, compound **6b** is worthy of further *in vitro* and *in vivo* evaluations.

### 4. Experimental

#### 4.1 General considerations

The synthesis of **6g** was achieved using a Biotage Initiator microwave synthesizer. NMR spectra were performed on a Bruker AVANCE 300 (<sup>1</sup>H, 300 MHz; <sup>13</sup>C, 75 MHz) or Bruker AVANCE 400 (<sup>1</sup>H, 400 MHz; <sup>13</sup>C, 100 MHz). Unless otherwise stated, CDCl<sub>3</sub> was used as solvent. Chemical shifts  $\delta$  are in ppm, and the following abbreviations are used: singlet (s), doublet (d), triplet (t), multiplet (m), doublet of triplet (dt), quintuplet (qt), broad singlet (br s). Elemental analyses (C, H, N) were performed with a Perkin-Elmer 240 analyzer and were within 0.4% of the theoretical values otherwise stated. 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  $\mu$ m, 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<sub>4</sub>) or sodium sulphate (Na<sub>2</sub>SO<sub>4</sub>).

#### 4.2 Synthesis of 1-(2-(but-3-en-1-yloxy)phenyl)vinyltributylstannane **9a**

To a solution of 1-(but-3-en-1-yloxy)-2-ethynylbenzene[69] (600 mg, 3.5 mmol) in THF (10 mL) were successively added PdCl<sub>2</sub>(PPh<sub>3</sub>)<sub>2</sub> (246 mg, 0.35 mmol, 10 mol%) and Bu<sub>3</sub>SnH (1.15 mL, 4.2 mmol, 1.2 equiv). The mixture was stirred at room temperature for 1 h and concentrated. Purification by flash chromatography on silica gel (neutralized with 1% of Et<sub>3</sub>N) afforded compound **9a** as a colorless oil (1.25 g; 2.8 mmol; 80%).  $R_f$  (cyclohexane/EtOAc : 9/1) = 0.77. <sup>1</sup>H NMR: (CDCl<sub>3</sub>, 300 MHz): 7.15 (dt, 1H,  $J = 1.8$  Hz,  $J = 7.6$  Hz), 7.03 (dd, 1H,  $J = 7.5$  Hz,  $J = 1.8$  Hz), 6.89 (m, 1H), 6.79 (d, 1H,  $J = 7.6$  Hz), 5.89-5.86 (m, 2H), 5.39 (m, 1H), 5.13 (m, 2H), 4.01 (t, 2H,  $J = 7.3$  Hz), 2.65-2.55 (m, 2H), 1.55-1.30 (m, 12H), 1.20-0.65 (m, 15H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): 155.6, 131.2, 128.3, 126.1, 120.2, 116.6, 113.8, 111.5, 110.8, 67.1, 33.3, 28.7 (3), 26.8 (3), 13.3 (3), 9.8 (3), one C missing. IR (neat): 2955, 2926, 1577, 1464, 1237, 1117, 1030 cm<sup>-1</sup>. MS (APCI) [M+H]<sup>+</sup> = 465.0.

#### 4.3 Synthesis of 1-(but-3-en-1-yloxy)-2-(1-iodovinyl)benzene **10a**

To a CH<sub>2</sub>Cl<sub>2</sub> (10 mL) solution containing **9a** (597 mg, 1.29 mmol) was added in one portion I<sub>2</sub> (326 mg, 1.29 mmol) at 0°C. The mixture was then stirred at rt for 1 h. Then a saturated KF solution (10 mL) was added to the mixture which was extracted with Et<sub>2</sub>O, washed with a Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> solution (10 mL). The combined organic layers were dried over MgSO<sub>4</sub>, filtered, and the solvent was then removed. Purification by flash chromatography afforded compound **10a** as a brown oil (213 mg; 0.71 mmol; 45%).  $R_f$  (cyclohexane/EtOAc : 9/1) = 0.54. <sup>1</sup>H NMR: (CDCl<sub>3</sub>, 300 MHz): 7.28-7.24 (m, 2H), 6.92-6.82 (m, 2H), 6.28 (s, 1H), 6.14 (s, 1H),

5.94 (m, 1H), 5.19-5.11 (m, 2H), 4.07 (m, 2H), 2.62 (m, 2H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): 134.6, 130.2, 130.1, 129.9, 120.2, 117.7, 117.3, 117.1, 112.1, 100.5, 67.8, 33.7. IR (neat): 29272927, 1661, 1594, 1487, 1447, 1283, 1243, 1162, 1113 cm<sup>-1</sup>. MS (APCI) [M+H]<sup>+</sup> = 301.0.

#### 4.4 Synthesis of 1-iodo-3-isopropoxy-4-methoxy-2-(methoxymethoxy)benzene **16**.

Phenol **15**[**Erreur ! Signet non défini.**] (1.5 g, 4.86 mmol) was mixed with Et<sub>2</sub>NH (1.41 mL; 9.72 mmol) and MOMCl (0.75 mL; 9.72 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (15 mL) for 1 h at room temperature. The medium was quenched with H<sub>2</sub>O (45 mL) and extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 x 15 mL). The combined organic layers were dried over MgSO<sub>4</sub>, filtered, and the solvent was then removed. Purification by flash chromatography afforded compound **16** as a colorless oil (1.67 g; 4.77 mmol; 98%). R<sub>f</sub> (cyclohexane/EtOAc: 8/2) = 0.68. <sup>1</sup>H NMR: (CDCl<sub>3</sub>, 300 MHz): 7.36 (d, 1H, *J* = 8.8 Hz) 6.42 (d, 1H, *J* = 8.8 Hz), 5.14 (s, 2H), 4.33 (m, 1H), 3.75 (s, 3H), 3.60 (s, 3H), 1.21 (d, 6H, *J* = 6.2 Hz). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): 155.0, 140.1, 132.8, 124.0, 109.7, 99.3, 81.5, 75.9, 58.6, 56.1, 22.4 (2C). MS (ESI) [M+Na]<sup>+</sup> = 375.0.

#### 4.5 Synthesis of 4-(3-isopropoxy-4-methoxy-2-(methoxymethoxy)phenyl)but-3-yn-1-ol **17**.

A solution of **16** (700 mg; 2 mmol), but-3-yn-1-ol (0.2 mL, 2.42 mmol), PdCl<sub>2</sub>(PPh<sub>3</sub>)<sub>2</sub> (112 mg; 0.16 mmol), CuI (44 mg; 0.30 mmol) was stirred in Et<sub>3</sub>N (10 mL) for 2 h at room temperature. EtOAc (20 mL) was added to the crude mixture which was washed with a saturated NH<sub>4</sub>Cl solution. After extraction with CH<sub>2</sub>Cl<sub>2</sub>, the combined extracts were dried (MgSO<sub>4</sub>) and concentrated. Purification by flash chromatography on silica gel afforded alkyne **17** as a brown oil (538 mg; 1.80 mmol; 90%). R<sub>f</sub> (cyclohexane/EtOAc: 7/3) = 0.22. <sup>1</sup>H NMR: (CDCl<sub>3</sub>, 300 MHz): 7.09 (d, 1H, *J* = 8.6 Hz), 6.61 (d, 1H, *J* = 8.6 Hz), 5.23 (s, 2H), 4.39 (m, 1H), 3.83 (s, 3H), 3.80 (t, 2H, *J* = 6.2 Hz), 3.61 (s, 3H), 2.69 (t, 2H, *J* = 6.2 Hz), 1.27 (d, 6H, *J* = 6.2 Hz), OH not seen. <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): 154.6, 127.7, 114.9, 110.8, 107.4, 99.0, 97.3, 88.9, 78.9, 75.6, 61.1, 57.6, 55.9, 24.1, 22.4 (2C). IR (neat): 3894, 3807, 3634, 3489, 2976, 2106, 2021, 1594, 1491, 1437, 1381, 1290, 1207, 1157, 1099, 1060, 968, 921, 803, 774, 693 cm<sup>-1</sup>. MS (ESI) [M+Na]<sup>+</sup> = 317.0.

#### 4.6 Synthesis of 4-hydroxy-1-(2-hydroxy-3-isopropoxy-4-methoxyphenyl)butan-1-one **18**.

To a solution of **17** (400 mg; 1.36 mmol) in EtOH (272 μL) was added PTSA (260 mg; 1.36 mmol) and H<sub>2</sub>O (2.45 mL). The solution was then stirred at 100° C in a sealed tube for 0.5 h. After cooling, the crude mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub>. The organic layers were dried over MgSO<sub>4</sub>, filtered, and the solvent was then removed. Purification by flash chromatography afforded compound **18** as a brown oil (228 mg; 0.84 mmol; 62%). R<sub>f</sub> (cyclohexane/EtOAc: 5/5) = 0.30. <sup>1</sup>H NMR: (CDCl<sub>3</sub>, 300 MHz): 12.53 (brs, 1H), 7.53 (d, 1H, *J* = 9.1 Hz), 6.46 (d, 1H, *J* = 9.1 Hz), 4.43 (m, 1H), 3.88 (s, 3H), 3.71 (t, 2H, *J* = 6.1 Hz), 3.05 (t, 2H, *J* = 7.1 Hz), 1.97 (m, 2H), 1.29 (d, 6H, *J* = 6.2 Hz), OH not seen. <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): 205.2, 158.9, 157.6, 134.5, 126.0, 114.7, 102.8, 75.1, 61.9, 55.9, 34.5, 27.1, 22.4 (2C). IR (neat): 2976, 1631, 1505, 1429, 1261, 1131, 1092, 944, 685, 619 cm<sup>-1</sup>. MS (APCI) [M+H]<sup>+</sup> = 269.0.

#### 4.7 Synthesis of 9-isopropoxy-8-methoxy-3,4-dihydrobenzo[*b*]oxepin-5(2H)-one **19**.

Compound **18** (300 mg; 1.11 mmol) was stirred for 1 h with DEAD (0.27 mL; 1.68 mmol) and PPh<sub>3</sub> (441 mg; 1.68 mmol) in THF (30 mL). After concentration under reduced pressure, the crude mixture was purified by flash chromatography to afford benzoxepin-5-one **19** as a pale yellow oil (174 mg; 0.69 mmol; 63%). R<sub>f</sub> (cyclohexane/EtOAc: 5/5) = 0.60. <sup>1</sup>H NMR: (CDCl<sub>3</sub>, 300 MHz): 7.51 (d, 1H, *J* = 8.9 Hz), 6.66 (d, 1H, *J* = 8.9 Hz), 4.34 (m, 1H), 4.22 (t, 2H, *J* = 6.7 Hz), 3.85 (s, 3H), 2.83 (t, 2H, *J* = 6.9 Hz), 2.15 (m, 2H), 1.28 (d, 6H, *J* = 6.2 Hz). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): 199.4, 157.7, 156.6, 137.9, 124.4, 123.9, 106.4, 75.7, 72.8, 55.9, 40.2, 25.9, 22.4 (2C). IR (neat): 2975, 2023, 1674, 1587, 1493, 1433, 1372, 1270, 1218, 1196, 1169, 1093, 1008, 964 cm<sup>-1</sup>. MS (ESI) [M+Na]<sup>+</sup> = 273.1.

#### 4.8 Synthesis of *N'*-(9-isopropoxy-8-methoxy-3,4-dihydrobenzo[*b*]oxepin-5(2H)-ylidene)-4-methylbenzenesulfonylhydrazide **11a**.

To a solution of benzoxepin-5-one **19** (173 mg; 0.69 mmol) and PTSA (28 mg; 0.14 mmol) in ethanol (5 mL) was added 4-methylbenzenesulfonylhydrazide (321 mg; 1.72 mmol). The resulting mixture was stirred under reflux for 1 h. The medium was diluted with EtOAc and filtered over a pad of Celite. The solvent was next removed and the residue was purified by crystallization in ethanol to afford **11a** as a white solid (147 mg; 0.35 mmol; 51%). R<sub>f</sub> (cyclohexane/EtOAc: 5/5) = 0.60. F = 192-194 °C. <sup>1</sup>H NMR: (CDCl<sub>3</sub>, 300 MHz): 7.76 (d, 2H, *J* = 8.1 Hz), 7.60 (brs, 1H), 7.31 (d, 2H, *J* = 8.1 Hz), 6.87 (d, 1H, *J* = 8.3 Hz), 6.53 (d, 1H, *J* = 8.3 Hz), 4.20 (m, 1H), 4.10 (m, 1H), 3.80 (m, 1H), 3.76 (s, 3H), 2.60 (t, 2H, *J* = 6.8 Hz), 2.37 (s, 3H), 1.80 (m, 2H), 1.19 (d, 6H, *J* = 6.3



Hz).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ): 157.1, 155.3, 151.6, 144.1, 138.8, 135.5, 129.7 (2C), 128.2 (2C), 123.9, 122.8, 107.1, 75.8, 71.0, 56.1, 26.3, 24.9, 22.5 (2C), 21.7. IR (neat): 3803, 3676, 2972, 1595, 1494, 1435, 1373, 1321, 1288, 1219, 1164, 1097, 911, 812, 728, 665  $\text{cm}^{-1}$ . MS (APCI)  $[\text{M}+\text{H}]^+ = 419.0$ .

#### 4.9 Synthesis of 3-bromo-2-hydroxy-4-methoxybenzaldehyde **20**[70]

To a solution of *isovanillin* (2.5 g; 21.55 mmol) in EtOH (25 mL) were added a drop of AcOH and  $\text{Hg}(\text{OAc})_2$  (6.85 g; 21.55 mmol). After 20 min of stirring at 50°C, an aqueous solution of NaBr (2.21 g; 21.55 mmol) was added and the precipitate was filtered. This latter was dissolved in  $\text{CH}_3\text{CN}$  and MPHT (8.45 g; 21.55 mmol) was added in one portion. After 20 min of stirring at room temperature, the medium was concentrated and hydrolyzed with a saturated aqueous  $\text{NaHCO}_3$  solution. After extraction with EtOAc (3 x 100 mL), the combined extracts were dried ( $\text{MgSO}_4$ ) and concentrated. Purification by flash chromatography on silica gel afforded **20** as a beige solid (2.34 g; 12.05 mmol; 56%).

#### 4.10 Synthesis of 2-(allyloxy)-3-bromo-4-methoxybenzaldehyde **21**.

To a  $\text{CH}_3\text{CN}$  solution of **20** (4.6 g; 20 mmol) was added  $\text{K}_2\text{CO}_3$  (3.32 g; 24 mmol) and allylbromide (2.08 mL; 24 mmol). The mixture was stirred for 1 h under reflux and after cooling to room temperature, the medium was filtered, concentrated and hydrolyzed with  $\text{H}_2\text{O}$ . After extraction with EtOAc, the combined extracts were dried ( $\text{MgSO}_4$ ) and concentrated. Purification by flash chromatography on silica gel afforded **21** as a colorless oil (5.2 g; 19.2 mmol, 96%).  $R_f$  (cyclohexane/EtOAc: 8/2) = 0.48.  $^1\text{H}$  NMR: ( $\text{CDCl}_3$ , 300 MHz): 10.15 (s, 1H), 7.77 (d, 1H,  $J = 8.8$  Hz), 6.74 (d, 1H,  $J = 8.8$  Hz), 6.06 (m, 1H), 5.36-5.26 (m, 2H), 4.55 (m, 2H), 3.91 (s, 3H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ): 188.3, 162.2, 160.5, 132.2, 128.8, 124.6, 119.6, 107.9, 107.5, 76.6, 56.8. IR (neat): 3082, 2938, 2847, 2362, 1675, 1584, 1485, 1452, 1437, 1414, 1391, 1359, 1291, 1246, 1199, 1178, 1127, 1073  $\text{cm}^{-1}$ . MS (APCI)  $[\text{M}+\text{H}]^+ = 271.0$  ( $^{79}\text{Br}$ ), 273.5 ( $^{81}\text{Br}$ ).

#### 4.11 Synthesis of 1-(2-(allyloxy)-3-bromo-4-methoxyphenyl)prop-2-en-1-ol **13a**.

To a solution of **21** (1.0 g; 3.7 mmol) in THF (10 mL) was added at 0°C 4.5 mL of a 1N solution of vinylmagnesium bromide (4.5 mmol) in THF. After stirring for 1 h at room temperature, the medium was hydrolyzed with  $\text{H}_2\text{O}$ . After extraction with  $\text{CH}_2\text{Cl}_2$ , the combined extracts were dried ( $\text{MgSO}_4$ ) and concentrated. Purification by flash chromatography on silica gel afforded **13a** as a yellow oil (1.05 g; 3.51 mmol; 95%).  $R_f$  (cyclohexane/EtOAc: 7/3) = 0.50.  $^1\text{H}$  NMR: ( $\text{CDCl}_3$ , 300 MHz): 7.22 (d, 1H,  $J = 8.7$  Hz), 6.64 (d, 1H,  $J = 8.7$  Hz), 6.16-5.90 (m, 2H), 5.43-5.13 (m, 5H), 4.47 (m, 2H), 3.81 (s, 3H), 2.40 (d, 1H,  $J = 5.4$  Hz).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ): 156.8, 154.8, 139.7, 133.2, 129.9, 127.0, 118.2, 114.9, 107.8, 107.3, 74.7, 69.8, 56.5. IR (neat): 2947, 1675, 1584, 1485, 1319, 1291, 1246, 1199, 1178, 1127, 998  $\text{cm}^{-1}$ . MS (APCI)  $[\text{M}+\text{H}]^+ = 299.1$  ( $^{79}\text{Br}$ ), 301.1 ( $^{81}\text{Br}$ ).

#### 4.12 Synthesis of 9-bromo-8-methoxy-2,5-dihydrobenzo[*b*]oxepin-5-ol **22**

Compound **13a** (828 mg; 2.79 mmol) was stirred under argon with Grubbs II catalyst ([1,3-bis-(2,4,6-trimethylphenyl)-2-imidazolidynylidene]dichloro(phenylmethylene)(tricyclohexylphosphine)ruthenium, 231 mg; 0.27 mmol) in  $\text{CH}_2\text{Cl}_2$  (60 mL) for 1 h at room temperature. The crude mixture was concentrated and the residue was purified by flash chromatography on silica gel to afford **22** as a colorless oil (558 mg; 2.04 mmol; 73%).  $R_f$  (cyclohexane/EtOAc: 7/3) = 0.30.  $^1\text{H}$  NMR: ( $\text{CDCl}_3$ , 300 MHz): 7.11 (d, 1H,  $J = 8.6$  Hz), 6.55 (d, 1H,  $J = 8.6$  Hz), 5.87 (m, 1H), 5.34-5.30 (m, 2H), 4.50 (m, 2H), 3.80 (s, 3H), 2.80 (d, 1H,  $J = 7.0$  Hz, OH).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ): 156.4, 153.6, 133.0, 131.8, 127.1, 124.2, 107.2, 106.5, 69.8, 68.5, 56.5. IR (neat): 2362, 1675, 1584, 1291, 1246, 1199, 1178, 1127, 1073, 956, 873  $\text{cm}^{-1}$ . MS (APCI)  $[\text{M}+\text{H}]^+ = 271.3$  ( $^{79}\text{Br}$ ), 273.0 ( $^{81}\text{Br}$ ).

#### 4.13 Synthesis of 9-bromo-8-methoxy-2,3,4,5-tetrahydrobenzo[*b*]oxepin-5-ol **23**

A mixture of **22** (480 mg, 1.76 mmol), Pd/C (48 mg), MeOH (20 mL) and 1 drop of AcOH was stirred under an  $\text{H}_2$  atmosphere for 1 h at room temperature. The mixture was then filtered over a pad of Celite and concentrated. Purification by flash chromatography on silica gel afforded **23** as a colorless oil (318 mg; 1.16 mmol; 66%).  $R_f$  (cyclohexane/EtOAc: 9/1) = 0.33.  $^1\text{H}$  NMR: ( $\text{CDCl}_3$ , 300 MHz): 7.24 (d, 1H,  $J = 7.2$  Hz), 6.61 (d, 1H,  $J = 7.2$  Hz), 4.86 (m, 1H), 4.17-3.99 (m, 2H), 3.87 (s, 3H), 2.50-2.15 (m, 2H), 2.10-1.93 (m, 2H), OH not seen.  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ): 156.3, 156.1, 131.3, 126.3, 106.7, 76.4, 73.3, 72.5, 56.6, 34.2, 27.0. IR (neat): 2978, 1595, 1490, 1435, 1355, 1321, 1288, 1231, 1206, 1164, 812, 730, 665, 641  $\text{cm}^{-1}$ . MS (APCI)  $[\text{M}+\text{H}]^+ = 273.0$  ( $^{79}\text{Br}$ ), 275.1 ( $^{81}\text{Br}$ ).

#### 4.14 Synthesis of 9-bromo-8-methoxy-3,4-dihydrobenzo[b]oxepin-5(2H)-one **24**

To a solution of **23** (240 mg; 0.87 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (15 mL) was added in 3 portions PCC (192 mg) at the beginning of the reaction, a second drop of PCC (192 mg) was next added to the mixture after 1 h of stirring and a last drop of PCC (96 mg) was finally added to complete the oxidation reaction after 3 h of stirring. The mixture was filtered over a pad of Celite and concentrated. Purification by flash chromatography on silica gel afforded **24** as brown oil (228 mg; 0.84 mmol; 96%). R<sub>f</sub> (cyclohexane/EtOAc: 8/2) = 0.60. <sup>1</sup>H NMR: (CDCl<sub>3</sub>, 300 MHz): 7.83 (d, 1H, *J* = 8.8 Hz), 6.77 (d, 1H, *J* = 8.8 Hz), 4.38 (t, 2H, *J* = 6.8 Hz), 4.02 (s, 3H), 2.94 (t, 2H, *J* = 6.8 Hz), 2.27 (m, 2H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): 198.8, 160.6, 159.5, 129.6, 124.0, 106.4, 104.7, 73.0, 56.7, 40.1, 25.6. IR (neat): 2813, 1795, 1490, 1462, 1399, 1321, 1287, 1219, 1164, 812, 728, 652 cm<sup>-1</sup>. MS (APCI) [M+H]<sup>+</sup> = 271.4 (<sup>79</sup>Br), 273.5 (<sup>81</sup>Br).

#### 4.15 Synthesis of N'-(9-bromo-8-methoxy-3,4-dihydrobenzo[b]oxepin-5(2H)-ylidene)-4-methylbenzenesulfonohydrazide **11b**

A solution of **24** (665 mg; 2.5 mmol), PTSA (53 mg; 0.25 mmol), MgSO<sub>4</sub> (296 mg; 2.5 mmol) 4-methylbenzenesulfonohydrazide (460 mg; 2.5 mmol) was stirred under reflux for 2 h. The medium was filtered over a pad of Celite, concentrated, diluted with EtOAc and washed with H<sub>2</sub>O (3 x 10 mL). After extraction, the crude was precipitated in EtOH to afford **11b** as a white solid (0.98 g; 2.22 mmol, 88%). R<sub>f</sub> (cyclohexane/EtOAc: 7/3) = 0.30. F = 201-203 °C. <sup>1</sup>H NMR: (CDCl<sub>3</sub>, 300 MHz): 8.14 (s, 1H, NH), 7.71 (d, 2H, *J* = 8.0 Hz), 7.24 (d, 1H, *J* = 8.8 Hz), 7.13 (d, 2H, *J* = 8.0 Hz), 6.46 (d, 1H, *J* = 8.8 Hz), 3.95 (t, 2H, *J* = 6.6 Hz), 3.71 (s, 3H), 2.43 (t, 2H, *J* = 6.6 Hz), 2.23 (s, 3H), 1.75 (m, 2H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): 158.2, 155.9, 154.2, 144.2, 135.3, 129.6, 128.0, 127.9, 124.3 (2C), 107.0, 105.4, 70.8, 56.5, 26.1, 24.2, 21.6, one C missing. IR (neat): 2967, 1595, 1494, 1435, 1373, 1321, 1288, 1219, 1164, 812, 728, 665 cm<sup>-1</sup>. MS (APCI) [M+H]<sup>+</sup> = 439.0 (<sup>79</sup>Br), 441.0 (<sup>81</sup>Br).

#### 4.16 Synthesis of 9-isopropoxy-8-methoxy-5-(3,4,5-trimethoxyphenyl)-2,3-dihydrobenzo[b]oxepin **6a**

To a dioxane (2 mL) solution of *N*-tosylhydrazone **11a** (60 mg, 0.14 mmol), *t*BuOLi (23 mg; 0.28 mmol), Pd<sub>2</sub>dba<sub>3</sub> (13 mg; 0.014 mmol), and XPhos (13 mg; 0.028 mmol) was added 3,4,5-trimethoxyphenyliodide (42 mg, 0.14 mmol). The mixture was stirred at 90 °C in a sealed tube for 1 h. After cooling at room temperature, the crude mixture was filtered over a pad of Celite and concentrated. The residue was purified by silica gel chromatography to yield **6a** as a brown oil (55 mg; 0.137 mmol; 98%). R<sub>f</sub> (cyclohexane/EtOAc: 7/3) = 0.60. <sup>1</sup>H NMR: (CDCl<sub>3</sub>, 400 MHz): 6.65 (d, 1H, *J* = 8.8 Hz), 6.56 (d, 1H, *J* = 8.8 Hz), 6.50 (s, 2H), 6.14 (t, 1H, *J* = 5.9 Hz), 4.48-4.43 (m, 3H), 3.87 (s, 3H), 3.83 (s, 3H), 3.81 (s, 6H), 2.50 (m, 2H), 1.35 (d, 6H, *J* = 5.6 Hz). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): 153.3, 152.8 (2C), 152.2, 141.2, 139.7 (2C), 138.9, 137.3, 126.3, 125.3, 106.5, 106.0 (2C), 76.5, 75.8, 60.9, 56.1 (2C), 55.9, 30.4, 22.7 (2C). IR (neat): 2936, 1710, 1659, 1582, 1549, 1493, 1411, 1232 cm<sup>-1</sup>. MS (APCI) [M+H]<sup>+</sup> = 401.0.

#### 4.17 Synthesis of 8-Methoxy-5-(3,4,5-trimethoxyphenyl)-2,3-dihydrobenzo[b]oxepin-9-ol **6b**

According to Scheme 2: A solution of **6a** (110 mg; 0.28 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (5 mL) was stirred with AlCl<sub>3</sub> (92 mg; 0.28 mmol) for 1 h at room temperature. Then another portion of AlCl<sub>3</sub> (92 mg) was added to the crude mixture. After 1 h of stirring, the medium was hydrolyzed by H<sub>2</sub>O (10 mL). After extraction with CH<sub>2</sub>Cl<sub>2</sub> (3 x 10 mL), the combined extracts were dried (MgSO<sub>4</sub>) and concentrated. Purification by flash chromatography on silica gel afforded benzoxepin **6b** as a pale yellow oil (16 mg; 0.04 mmol; 15%).

According to Scheme 3: A solution of **6d** (100 mg; 0.24 mmol), KOH (5 mmol), Pd<sub>2</sub>dba<sub>3</sub> (46 mg; 0.005 mmol) and *t*BuXPhos (8 mg; 0.015 mmol) in a mixture of dioxane/H<sub>2</sub>O: 1/1 (2 mL) was stirred at 90 °C in a sealed tube. After 1 h, the medium was cooled to room temperature, quenched with NH<sub>4</sub>Cl (10 mL) and extracted with EtOAc (3 x 10 mL). The combined organic layers were then dried over MgSO<sub>4</sub>, filtered and evaporated to dryness. Purification by flash chromatography afforded compound **6b** as a pale yellow oil (47 mg, 0.13 mmol, 55%). R<sub>f</sub> (cyclohexane/EtOAc: 7/3) = 0.26. <sup>1</sup>H NMR: (CDCl<sub>3</sub>, 300 MHz): 6.51 (d, 1H, *J* = 8.8 Hz), 6.42 (m, 3H), 6.08 (t, 1H, *J* = 5.9 Hz), 5.73 (br s, 1H, OH), 4.47 (t, 2H, *J* = 5.9 Hz), 3.83 (s, 3H), 3.80 (s, 3H), 3.74 (s, 6H), 2.49 (q, 2H, *J* = 5.9 Hz). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): 152.8 (2C), 146.7, 144.7, 141.2, 138.6 (2C), 137.6, 126.2, 125.0, 121.3, 106.3, 106.0 (2C), 76.6, 60.9, 56.1 (3C), 30.3, IR (neat): 3853, 3618, 3441, 2937, 1580, 1504, 1444, 1411, 1333, 1233, 1124, 1089, 1007 cm<sup>-1</sup>. MS (APCI) [M+H]<sup>+</sup> = 359.2. HRMS calcd for C<sub>20</sub>H<sub>23</sub>O<sub>6</sub> [M+H]<sup>+</sup> 359.1495, obsd 359.1510.

#### 4.18 Synthesis of 5-(3,5-dimethoxyphenyl)-8-methoxy-2,3-dihydrobenzo[b]oxepin-9-ol **6c**

To a dioxane (1 mL) solution of *N*-tosylhydrazone **11a** (100 mg, 0.24 mmol), *t*BuOLi (38 mg; 0.48 mmol), Pd<sub>2</sub>dba<sub>3</sub> (20 mg; 0.02 mmol), and XPhos (20 mg; 0.04 mmol) was added 3,5-dimethoxyphenyliodide (52 mg, 0.24 mmol). The mixture was stirred at 90 °C in a sealed tube for 1 h. After cooling, the crude mixture was diluted in CH<sub>2</sub>Cl<sub>2</sub> and stirred for 2 h with AlCl<sub>3</sub> (22 mg; 0.24 mmol).

The medium was then hydrolyzed by H<sub>2</sub>O (10 mL). After extraction with CH<sub>2</sub>Cl<sub>2</sub>, the combined extracts were dried (MgSO<sub>4</sub>) and concentrated. Purification by flash chromatography on silica gel afforded benzoxepin **6c** as a pale yellow oil (20 mg; 0.06 mmol; 25%). R<sub>f</sub> (cyclohexane/EtOAc: 7/3) = 0.40. <sup>1</sup>H NMR: (CDCl<sub>3</sub>, 300 MHz): 6.41 (d, 1H, *J* = 8.9 Hz), 6.33 (d, 1H, *J* = 8.9 Hz), 6.28 (m, 3H), 6.01 (t, 1H, *J* = 5.9 Hz), 5.66 (s, 1H), 4.37 (t, 2H, *J* = 5.9 Hz), 3.74 (s, 3H), 3.61 (s, 6H), 2.42 (q, 2H, *J* = 5.9 Hz). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): 160.5 (2C), 146.7, 145.1, 144.9, 141.0, 137.6, 126.7, 124.8, 121.4, 107.0, 106.4 (2C), 99.5, 76.3, 56.2, 55.4 (2C), 30.6. IR (neat): 3876, 2938, 1590, 1450, 1204, 1153, 1090 cm<sup>-1</sup>. MS (APCI) [M+H]<sup>+</sup> = 329.6. Compound **6c**, however, does not give completely satisfactory HRMS analyses despite repeated attempts at purification, but all other spectral data were consistent with this structure.

#### 4.19 Synthesis of 9-bromo-8-methoxy-5-(3,4,5-trimethoxyphenyl)-2,3-dihydrobenzo[b]oxepin **6d**

To a dioxane (3 mL) solution of *N*-tosylhydrazone **11b** (800 mg; 1.84 mmol), *t*BuOLi (296 mg; 3.68 mmol), Pd<sub>2</sub>dba<sub>3</sub> (168 mg; 0.16 mmol), and XPhos (344 mg; 0.72 mmol) was added 3,4,5-trimethoxyphenyliodide (632 mg, 2.16 mmol). The mixture was stirred at 90 °C in a sealed tube for 1 h. After cooling at room temperature, the crude mixture was filtered over SiO<sub>2</sub> and concentrated. The residue was purified by silica gel chromatography to yield **6d** as brown oil (536 mg; 1.26 mmol; 68%). R<sub>f</sub> (cyclohexane/EtOAc: 8/2) = 0.33. <sup>1</sup>H NMR: (CDCl<sub>3</sub>, 300 MHz): 6.86 (d, 1H, *J* = 8.7 Hz), 6.56 (d, 1H, *J* = 8.7 Hz), 6.41 (s, 2H), 6.19 (t, 1H, *J* = 6.3 Hz), 4.58 (t, 2H, *J* = 6.1 Hz), 3.84 (s, 3H), 3.80 (s, 3H), 3.74 (s, 6H), 2.37 (m, 2H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): 156.3, 155.1, 152.9 (2C), 141.2, 138.0, 137.4, 130.0, 127.2, 126.5, 106.7, 106.6, 105.6 (2C), 78.0, 60.9, 56.4, 56.1 (2C), 29.3. IR (neat): 2874, 1985, 1579, 1485, 1445, 1441, 1386, 1333, 1233, 1122, 1004, 975 cm<sup>-1</sup>. MS (APCI) [M+H]<sup>+</sup> = 421.0 (<sup>79</sup>Br), 423.5 (<sup>81</sup>Br). HRMS calcd for C<sub>20</sub>H<sub>22</sub>BrO<sub>5</sub> [M+H]<sup>+</sup> 421.0651, obsd 421.0663.

#### 4.20 Synthesis of 8-methoxy-5-(3,4,5-trimethoxyphenyl)-2,3-dihydrobenzo[b]oxepin-9-amine **6e**

A solution of **6d** (100 mg; 0.24 mmol), NaN<sub>3</sub> (31 mg; 0.48 mmol), CuI (3 mg; 0.02 mmol), DMEDA (0.13 mg; 0.012 mmol) and sodium ascorbate (8 mg; 0.04 mmol) in a mixture of DMSO/H<sub>2</sub>O: 5/1 (2 mL) was stirred at 60 °C in a sealed tube. After 12 h, the medium was cooled, quenched with NH<sub>4</sub>Cl and extracted with EtOAc (3 x 10 mL). The combined organic layers were dried over MgSO<sub>4</sub>, filtered and evaporated to dryness. Purification by flash chromatography afforded **6e** as a colorless oil (64 mg; 0.18 mmol, 75%). R<sub>f</sub> (cyclohexane/EtOAc: 7/3) = 0.26. <sup>1</sup>H NMR: (CDCl<sub>3</sub>, 300 MHz): 6.64-6.43 (m, 3H), 6.37 (d, 1H, *J* = 8.6 Hz), 6.17 (t, 1H, *J* = 6.0 Hz), 4.60-4.45 (t, 2H, *J* = 6.0 Hz), 3.88 (s, 6H), 3.81 (s, 6H), 2.48 (q, 2H, *J* = 6.0 Hz). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): 152.9 (2C), 147.6, 144.9, 141.9, 138.9, 137.3, 128.1, 125.7, 119.9, 106.0 (2C), 105.5, 76.6, 61.0, 56.2 (2C), 55.8, 30.1, 1C missing. IR (neat): 2444, 1985, 1579, 1485, 1415, 1333, 1233, 1122, 1004, 975 cm<sup>-1</sup>. MS (APCI) [M+H]<sup>+</sup> = 358.0. HRMS calcd for C<sub>20</sub>H<sub>24</sub>NO<sub>5</sub> [M+H]<sup>+</sup> 358.1654, obsd 358.1665.

#### 4.21 Synthesis of 8-methoxy-5-(3,4,5-trimethoxyphenyl)-2,3-dihydrobenzo[b]oxepin **6f**

A *n*BuOH (2 mL) solution of **6d** (105 mg; 0.24 mmol), Pd(OAc)<sub>2</sub> (1.5 mg; 0.0015 mmol), PPh<sub>3</sub> (7.5 mg; 0.006 mmol), was stirred for 1 h at 100 °C in a sealed tube. The medium was cooled, H<sub>2</sub>O (8 mL) was added to the crude mixture. After extraction with EtOAc (3 x 5 mL), the combined organic layers were dried over MgSO<sub>4</sub>, filtered and evaporated to dryness. Purification by flash chromatography afforded **6f** as a brown oil (57 mg; 0.165 mmol, 69%). R<sub>f</sub> (cyclohexane/EtOAc: 7/3) = 0.64. <sup>1</sup>H NMR: (CDCl<sub>3</sub>, 300 MHz): 6.82 (d, 1H, *J* = 6.9 Hz), 6.61 (m, 1H), 6.48 (m, 1H), 6.38 (s, 2H), 6.06 (t, 1H, *J* = 6.7 Hz), 4.38 (t, 2H, *J* = 6.7 Hz), 3.81 (s, 3H), 3.74 (s, 9H), 2.47 (q, 2H, *J* = 6.7 Hz). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): 159.8, 159.2, 152.9 (2C), 140.9, 139.1, 137.1, 132.2, 126.5, 109.3, 106.7, 105.9 (2C), 76.6, 60.9, 56.1, 55.4 (2C), 30.6, 1C missing. IR (neat): 2937, 1607, 1580, 1499, 1461, 1411, 1356, 1234, 1125, 1007, 826 cm<sup>-1</sup>. MS (APCI) [M+H]<sup>+</sup> = 343.0. HRMS calcd for C<sub>20</sub>H<sub>23</sub>O<sub>5</sub> [M+H]<sup>+</sup> 343.1545, obsd 343.1552.

#### 4.22 Synthesis of 4-(8-methoxy-5-(3,4,5-trimethoxyphenyl)-2,3-dihydrobenzo[b]oxepin-9-yl)but-3-yn-1-ol **6g**

A DMF (1 mL) solution of **6d** (80 mg; 0.19 mmol), but-3-yn-1-ol (0.17 mL; 0.23 mmol), PdCl<sub>2</sub>(PPh<sub>3</sub>)<sub>2</sub> (7 mg; 0.001 mmol), PPh<sub>3</sub> (7 mg; 0.03 mmol), CuI (2 mg; 0.03 mmol) and Et<sub>2</sub>NH (0.4 mL; 0.38 mmol) was stirred at 120 °C under microwave irradiation. After 25 min, the medium was cooled down to room temperature, quenched with NH<sub>4</sub>Cl and extracted with EtOAc (3 x 10 mL). The combined organic layers were then dried over MgSO<sub>4</sub>, filtered and evaporated to dryness. Purification by flash chromatography afforded compounds **6g** as a colorless oil (59 mg; 0.14 mmol; 74%). R<sub>f</sub> (cyclohexane/EtOAc: 4/6) = 0.42. <sup>1</sup>H NMR: (CDCl<sub>3</sub>, 300 MHz): 6.90 (d, 1H, *J* = 8.9 Hz), 6.57 (d, 1H, *J* = 8.9 Hz), 6.46 (s, 2H), 6.20 (t, 1H, *J* = 6.0 Hz), 4.56 (t, 2H, *J* = 6.0 Hz), 3.93-3.82 (m, 8H), 3.80 (s, 6H), 2.80 (t, 2H, *J* = 6.0 Hz), 2.49 (q, 2H, *J* = 6.0 Hz). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): 160.1, 159.4, 152.9 (2C), 140.8,

138.5, 137.3, 131.2, 126.7, 125.3, 105.7 (2C), 105.3, 94.6, 77.3, 76.0, 60.9 (2C), 56.1 (3C), 29.9, 24.6, 1C missing. IR (neat): 3531, 2939, 1582, 1504, 1486, 1463, 1412, 1356, 1333, 1283, 1236, 1126, 1102, 1055, 1007, 911, 829, 731, 696. MS (APCI)  $[M+H]^+$  = 411.0. HRMS calcd for  $C_{24}H_{27}O_6$   $[M+H]^+$  411.1808, obsd 411.1808.

#### 4.23 Synthesis of 8-methoxy-5-(3,4,5-trimethoxyphenyl)-2,3,4,5-tetrahydrobenzo[b]oxepin-9-ol **25a**

Compound **6b** (72 mg, 0.2 mmol) in MeOH (8 mL) was stirred under a  $H_2$  pressure of 20 bars for 1 h at room temperature. The mixture was then filtered over a pad of Celite and concentrated. Purification by flash chromatography on silica gel afforded **25a** as a pale brown oil (46 mg; 0.126 mmol; 63%).  $R_f$  (cyclohexane/EtOAc: 7/3) = 0.30.  $^1H$  NMR: ( $CDCl_3$ , 300 MHz): 6.48 (d, 1H,  $J$  = 6.7 Hz), 6.40 (s, 2H), 6.21 (d, 1H,  $J$  = 6.7 Hz), 5.83 (brs, 1H, OH), 4.22-4.10 (m, 2H), 4.02-3.95 (m, 1H), 3.86 (s, 3H), 3.85 (s, 3H), 3.81 (s, 6H), 2.25-2.10 (m, 2H), 2.05-1.95 (m, 2H).  $^{13}C$  NMR (100 MHz,  $CDCl_3$ ): 153.0 (2C), 146.4, 146.1, 139.4, 137.7, 136.2, 130.2, 119.3, 106.6, 105.4 (2), 73.8, 60.9, 56.1 (3C), 48.8, 33.1, 29.7. IR (neat): 3374, 2932, 2160, 1998, 1590, 1462, 1238, 1127, 1085, 1044  $cm^{-1}$ . MS (APCI)  $[M+H]^+$  = 361.0. Compound **25a**, however, does not give completely satisfactory HRMS analyses despite repeated attempts at purification, but all other spectral data were consistent with this structure.

#### 4.24 Synthesis of 4-(8-methoxy-5-(3,4,5-trimethoxyphenyl)-2,3,4,5-tetrahydrobenzo[b]oxepin-9-yl)butan-1-ol **25b**

A mixture of **6g** (90 mg, 0.21 mmol), Pd/C (9 mg), MeOH (30 mL) and 3 drops of AcOH was stirred under a  $H_2$  atmosphere for 3 h at room temperature. The mixture was then filtered over a pad of Celite and concentrated. Purification by flash chromatography on silica gel afforded **25b** as a colorless oil (30 mg; 0.007 mmol; 34%).  $R_f$  (cyclohexane/EtOAc: 9/1) = 0.33.  $^1H$  NMR: ( $CDCl_3$ , 300 MHz): 6.47 (d, 1H,  $J$  = 8.5 Hz), 6.39 (m, 3H), 4.11 (m, 2H), 3.80-3.75 (m, 1H), 3.78 (s, 3H), 3.74 (s, 6H), 3.70 (s, 3H), 3.62 (t, 2H,  $J$  = 5.8 Hz), 2.64 (t, 2H,  $J$  = 6.9 Hz), 1.99 (m, 4H), 1.54 (m, 5H).  $^{13}C$  NMR (100 MHz,  $CDCl_3$ ): 158.3, 157.0, 153.2 (2C), 140.2, 136.4, 130.7, 127.1, 123.6, 105.7 (2C), 105.5, 73.1, 63.1, 61.0, 56.2 (2C), 55.7, 48.6, 33.2, 32.7, 30.1, 26.1, 23.3. IR (neat): 2938, 1590, 1450, 1153, 1090  $cm^{-1}$ . MS (APCI)  $[M+H]^+$  = 417.0. HRMS calcd for  $C_{24}H_{33}O_6$   $[M+H]^+$  417.2277, obsd 417.2288.

#### 4.25 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 MDA-MB231 and H1299 cells were grown in Dulbecco minimal essential medium (DMEM) containing 4.5 g/L glucose supplemented with 10% FCS and 1% glutamine. Human K562 leukemia and HCT116 colorectal carcinoma cells were grown in RPMI 1640 containing 10% FCS and 1% glutamine. 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 ( $5 \times 10^3$  cells/well) containing 50  $\mu L$  growth medium. After 24 h of culture, the cells were supplemented with 50  $\mu L$  of the tested compound dissolved in DMSO (less than 0.1% in each preparation). After 72 h of incubation, 20  $\mu L$  of resazurin was added for 2 h before recording fluorescence ( $\lambda_{ex}$  = 560 nm,  $\lambda_{em}$  = 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. Experiments were performed in triplicate. The  $GI_{50}$  values for all compounds were compared to the  $GI_{50}$  of CA-4 and isoCA-4 and were measured the same day under the same conditions.

#### 4.26 Tubulin Binding Assay

Sheep brain tubulin was purified according to the method of Shelanski[71] 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[72] 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 isoCA-4 and measured the same day under the same conditions.

#### 4.27 Cell Cycle Analysis

Exponentially growing cancer cells (K562, H1299, HCT116, MDA-MB231) were incubated with benzoxepine **6b** at a concentration of 5 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.[73]

#### 4.28 Apoptosis Assay

Apoptosis was measured by the Apo-one homogeneous caspase-3/7 assay (Promega Co, WI) according to the manufacturer's recommendations. Briefly, HCT116 and H1299 cells were subcultured on a 96-well plate with  $5 \times 10^4$  cells/well in 100  $\mu$ L medium. After 24 h of incubation, the medium in the 96-well plate was discarded and replaced with medium containing different concentrations of benzoxepin **6b** (1, 5, and 10 nM) or 0.1% DMSO (as negative control). The treated cells were incubated for 24 h, each well then received 100  $\mu$ L of a mixture of caspase substrate and Apo-one caspase 3/7 buffer. After 1 h of incubation, the fluorescence of sample was measured using a Victor microtiter plate fluorimeter (Perkin-Elmer, USA) at 527 nm.

#### 4.29 Molecular modeling

For ligand preparation, compound **6b** was built using Chemdraw and converted to 3D using the dbtranslate program from Unity module of Sybyl (Tripos International, S.L., Missouri, USA). The 3D structure was minimized and a conformational analysis was performed (systematic search) selecting all possible rotatable bonds. The most stable structure of each compound was selected and used for docking. For the receptor preparation, the x-ray structure of tubulin-colchicine complex (PDB: 1sa0) was used for this study downloaded from the Protein Data Bank. Hydrogen atoms were added and an energy minimisation using AMBER 8.0[74] was performed keeping the  $\alpha$ -carbons constrained by a constraint-force of 50 kcal/mol  $\text{\AA}^2$ , which permitted to side-chains free to move and kept the same secondary structures. Over the refined model we defined the active site of tubulin as 12  $\text{\AA}$  around the crystallized colchicine. We performed a docking of all compounds using the program GOLD 4.1.2.[75]

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## Legends to Figures, Schemes and Tables

**Figure 1.** Representative inhibitors of tubulin polymerization and rational drug design from CA-4 and *iso*CA-4 to dihydrobenzoxepin analogues **6**.

**Figure 2.** Retrosynthesis of 5-arylbenzoxepins **6**.

**Scheme 1.** Synthesis of vinylstannane **9a** and vinyliodide **10a**. Reagent and conditions: a) PdCl<sub>2</sub>(PPh<sub>3</sub>)<sub>2</sub> (2 mol%), Bu<sub>3</sub>SnH (1.2 equiv), THF, 20 °C; b) I<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>, 20 °C.

**Scheme 2.** Synthesis of dihydrobenzoxepins **6b,c**. Reagent and conditions: a) MOMCl (2 equiv), *i*Pr<sub>2</sub>NH (2 equiv), CH<sub>2</sub>Cl<sub>2</sub>; b) But-3-yn-1-ol (1.1 equiv), PdCl<sub>2</sub>(PPh<sub>3</sub>)<sub>2</sub> (8 mol%), CuI (15 mol%), Et<sub>3</sub>N; c) PTSA (1 equiv), H<sub>2</sub>O, 100 °C, sealed tube; d) DEAD (1.5 equiv), PPh<sub>3</sub> (1.5 equiv), THF; e) TsNHNH<sub>2</sub> (2.5 equiv), PTSA (20 mol%), EtOH reflux; f) Pd<sub>2</sub>dba<sub>3</sub> (10 mol%), XPhos (20 mol%), *t*BuOLi (2 equiv), ArI (1 equiv), 90 °C, sealed tube; g) AlCl<sub>3</sub> (5 equiv), CH<sub>2</sub>Cl<sub>2</sub>.

**Scheme 3.** Synthesis of dihydrobenzoxepins **6b, 6d-g** and tetrahydrobenzoxepins **25a,b**. Reagent and conditions: a) (i) Hg(OAc)<sub>2</sub> (1 equiv), NaBr (1 equiv), 50 °C, EtOH; (ii) MPHT (1 equiv), CH<sub>3</sub>CN; b) 3-bromoprop-1-ene (1.2 equiv), K<sub>2</sub>CO<sub>3</sub> (1.2 equiv), CH<sub>3</sub>CN; c) vinylmagnesium chloride (1.2 equiv), THF; d) Grubbs II catalyst, 5 mol%, CH<sub>2</sub>Cl<sub>2</sub>, 20 °C; e) H<sub>2</sub>, Pd/C, MeOH, AcOH cat.; f) PCC, CH<sub>2</sub>Cl<sub>2</sub>; g) TsNHNH<sub>2</sub> (1 equiv), PTSA (10 mol%), MgSO<sub>4</sub> (1 equiv), EtOH, reflux; h) Pd<sub>2</sub>dba<sub>3</sub> (10 mol%), XPhos (40 mol%), *t*BuOLi (2 equiv), 3,4,5-trimethoxyiodobenzene (1.2 equiv), 90 °C, sealed tube; i) Pd<sub>2</sub>dba<sub>3</sub> (5 mol%), *t*BuXPhos (15 mol%), KOH (3 equiv), dioxane-H<sub>2</sub>O, 90 °C, sealed tube; j) CuI (10 mol%), TMEDA (6 mol%), NaN<sub>3</sub> (2 equiv), potassium ascorbate (6 mol%) DMSO-H<sub>2</sub>O, 90 °C, sealed tube; k) Pd(OAc)<sub>2</sub> (1 mol%), PPh<sub>3</sub> (2.5 mol%), *n*BuOH, 100 °C, sealed tube; l) *n*But-3-yn-1-ol (1.2 equiv), PdCl<sub>2</sub>(PPh<sub>3</sub>)<sub>2</sub> (5 mol%), PPh<sub>3</sub> (15 mol%), CuI (15 mol%), Et<sub>2</sub>NH (2 equiv), DMF, 120 °C, MWI.

**Table 1.** Cytotoxic activity of dihydrobenzoxepins **6b-g** and tetrahydrobenzoxepins **25a,b** against HCT116<sup>a</sup>

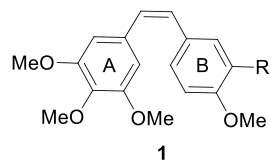
**Table 2.** Cytotoxic activity and inhibition of tubulin polymerization of selected compounds

**Figure 3.** Effects of benzoxepin **6b** at a concentration of 5.0 nM on cell cycle distribution in various cancer cell lines.

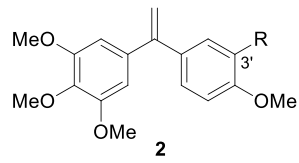
**Figure 4.** Apoptotic effects of **6b** in HCT116 and H1299 cells. The results are expressed in the percentage of apoptotic cells detected following 24 h of treatment with **6b** at different concentrations.

**Figure 5.** Docked pose of **6b** (gray) overlaid with *iso*CA-4 (blue) in the tubulin binding site.

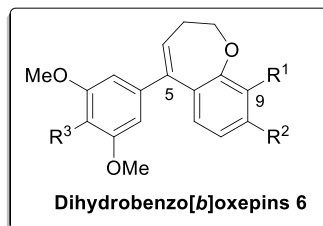
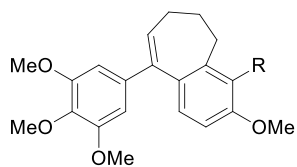
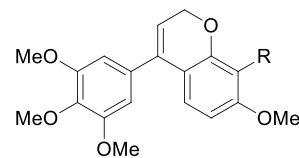
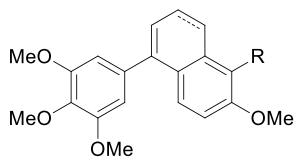




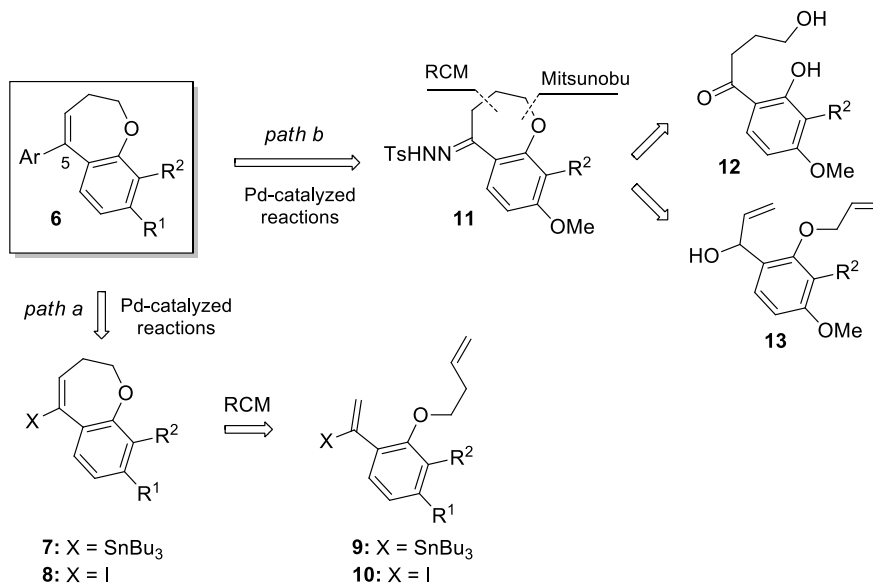
- CA-4**     **1a** R = OH  
**CA-4P**    **1b** R = OPO<sub>3</sub>Na<sub>2</sub>  
**NH<sub>2</sub>CA-4** **1c** R = NH<sub>2</sub>  
**FCA-4**     **1d** R = F



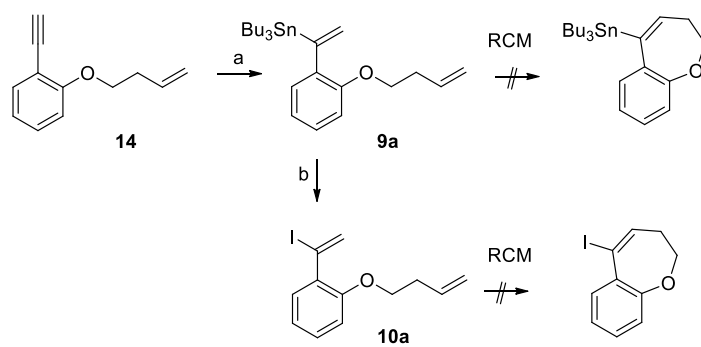
- isoCA-4**    **2a** R = OH  
**isoNH<sub>2</sub>CA-4** **2b** R = NH<sub>2</sub>  
**isoFCA-4**    **2c** R = F  
                  **2d** R = C≡C-CH<sub>2</sub>OH



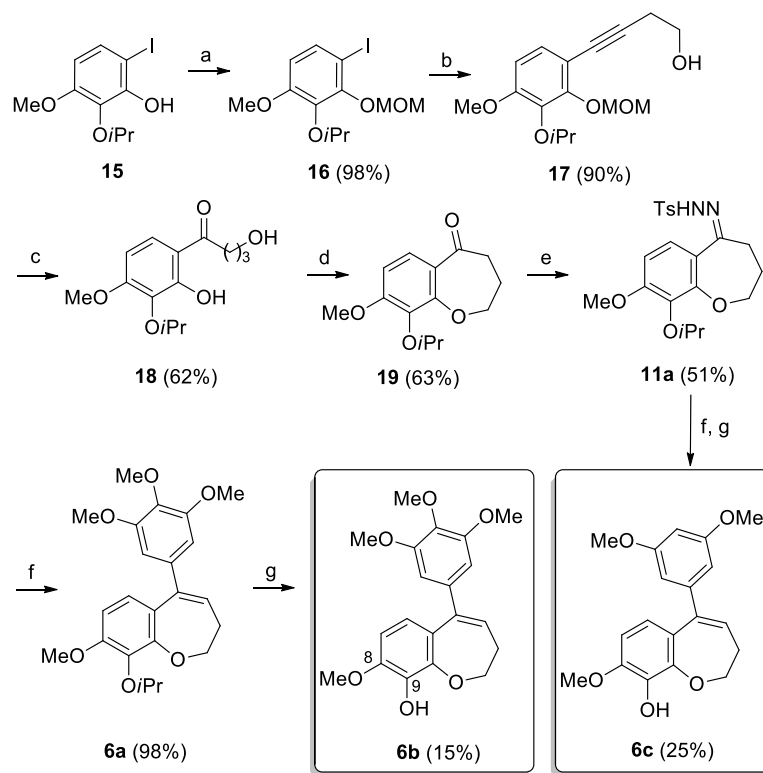
<Figure 1>



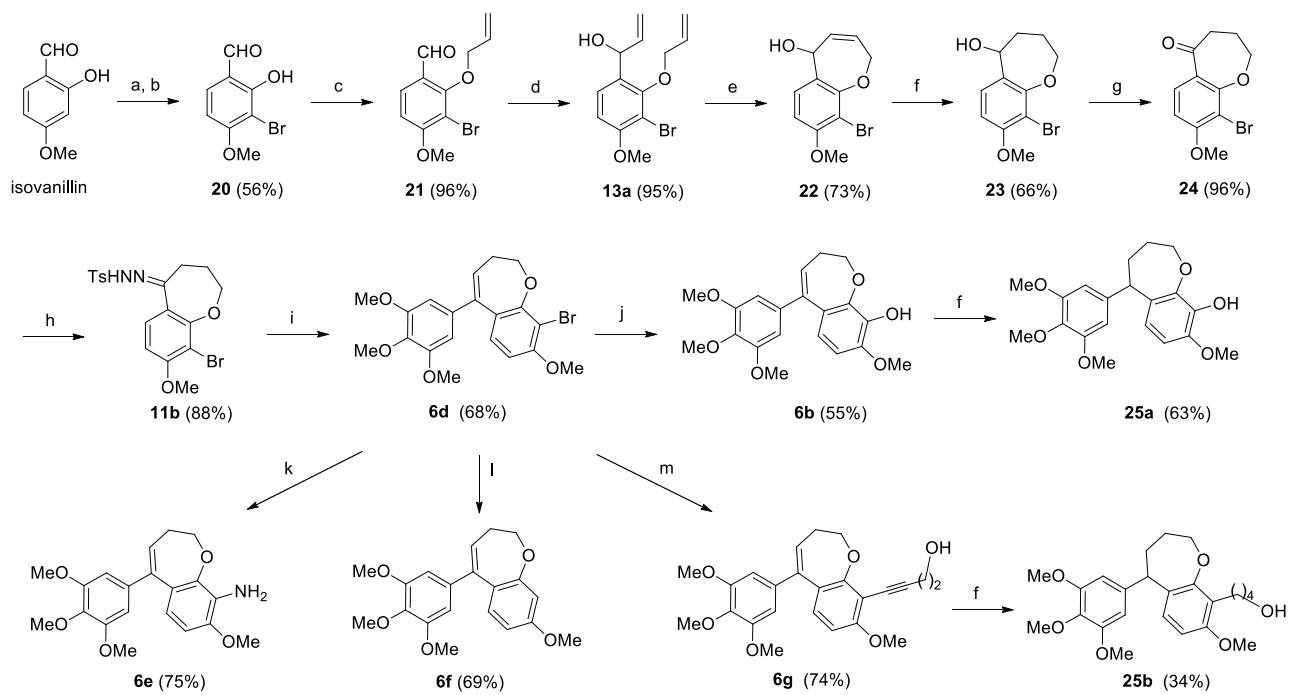
<Figure 2>



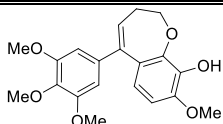
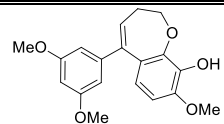
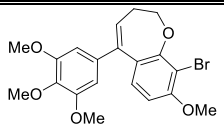
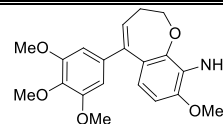
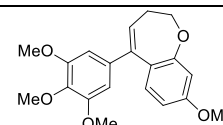
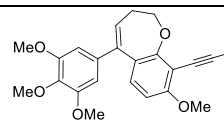
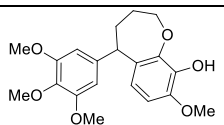
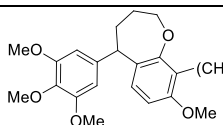
<Scheme 1>



<Scheme 2>



<Scheme 3>

Cytotoxicity against HCT-116 <sup>a</sup> cells				
<b>Cpnd</b>				
<b>GI<sub>50</sub><sup>b</sup> [nM]</b>	<b>6b:</b> 1.5 ± 0.1	<b>6c:</b> 25 ± 3	<b>6d:</b> 170 ± 18	<b>6e:</b> 22 ± 3
<b>Cpnd</b>				
<b>GI<sub>50</sub><sup>b</sup> [nM]</b>	<b>6f:</b> 85 ± 10	<b>6g:</b> 250 ± 22	<b>25a:</b> 20 ± 3	<b>25b:</b> Na <sup>c</sup>
<b>GI<sub>50</sub><sup>b</sup> [nM]</b>	<b>isoCA-4:</b> 3 ± 0.2 <sup>d</sup>	<b>CA-4:</b> 2 ± 0.2 <sup>d</sup>		

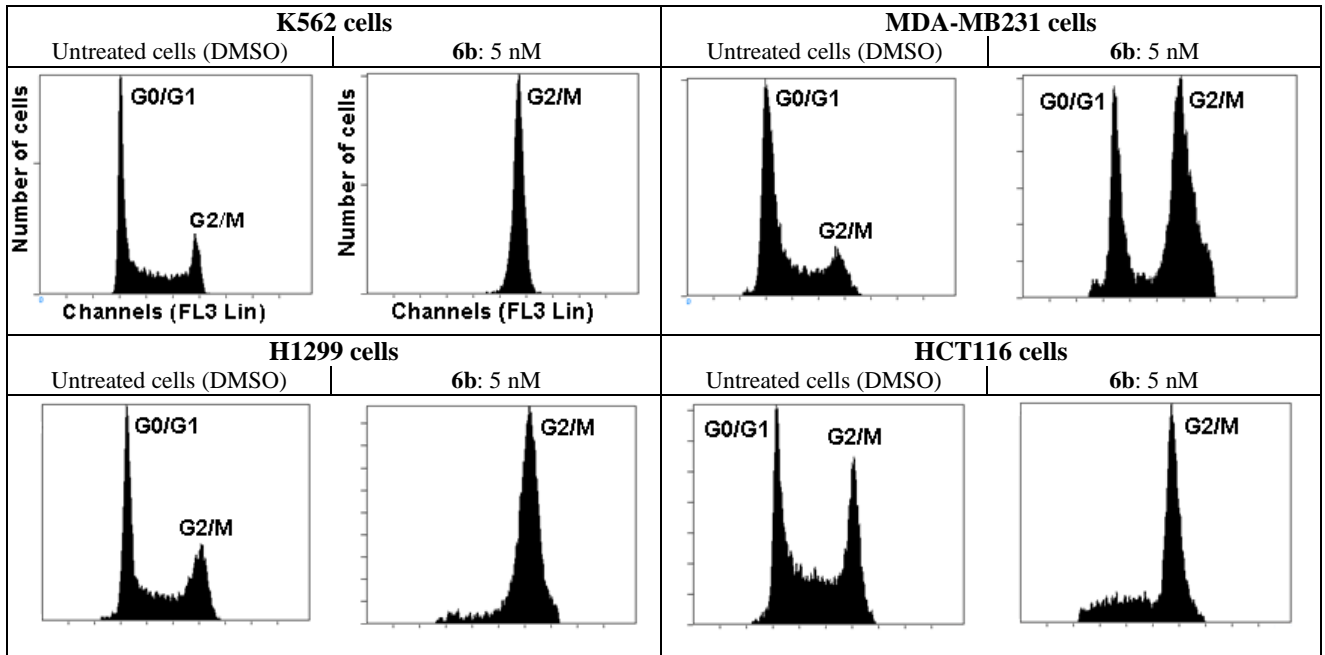
<sup>a</sup>HCT-116 Human colon carcinoma. <sup>b</sup>GI<sub>50</sub> is the concentration of compound needed to reduce cell growth by 50% following 72 h cell treatment with the tested drug (average of three experiments). <sup>c</sup>Non active. <sup>d</sup>The GI<sub>50</sub> values for CA-4, isoCA-4 were determined in this study.

<Table 1>

Cpnd	Cytotoxicity GI <sub>50</sub> <sup>a</sup> [nM]				Inhibition of tubulin polymerization IC <sub>50</sub> <sup>c</sup> [μM]
	HCT116 <sup>b</sup>	K562 <sup>b</sup>	H1299 <sup>b</sup>	MDA-MB231 <sup>b</sup>	
<b>6b</b>	1.5 ± 0.1	8 ± 1	4.5 ± 0.3	3 ± 0.2	3.8 ± 0.4
<b>6c</b>	25 ± 3	30 ± 4	32 ± 3	28 ± 4	3.2 ± 0.3
<b>6e</b>	22 ± 3	40 ± 4	30 ± 2	8 ± 1	3.6 ± 0.4
<b>6f</b>	85 ± 10	100 ± 9	180 ± 15	250 ± 23	3.9 ± 0.4
<b>25a</b>	20 ± 3	50 ± 5	7 ± 1	15 ± 2	3.3 ± 0.3
<i>isoCA-4</i> <sup>d</sup>	3 ± 0.2	5 ± 0.3	3 ± 0.2	4 ± 0.3	1.5 ± 0.2

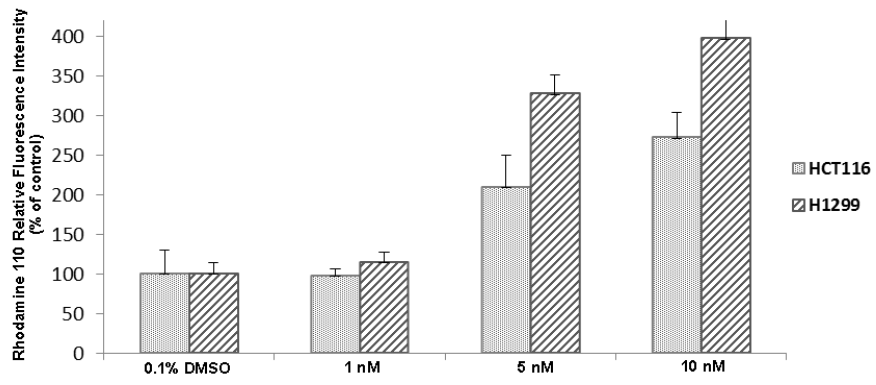
<sup>a</sup>GI<sub>50</sub> is the concentration of compound needed to reduce cell growth by 50% following 72 h cell treatment with the tested drug (average of three experiments). <sup>b</sup>HCT116, colon carcinoma; K562, myelogenous leukaemia; H1299, non-small cell lung carcinoma; MDA-MB231 hormone-independent breast cancer. <sup>c</sup>ITP, Inhibition of Tubulin Polymerization; IC<sub>50</sub> is the concentration of compound required to inhibit 50% of the rate of microtubule assembly (average of three experiments). <sup>d</sup>The GI<sub>50</sub> and IC<sub>50</sub> values (cytotoxicity and ITP, respectively) for *isoCA-4* were determined in this study.

<Table 2>

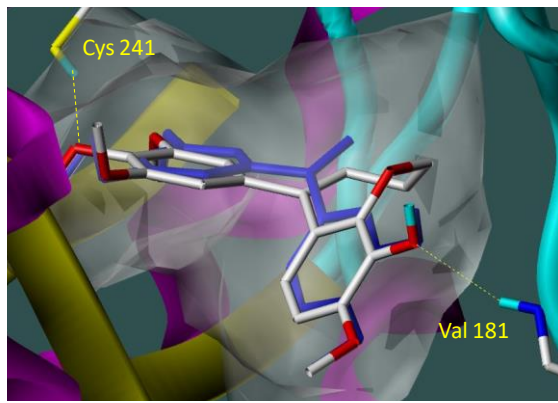


<Figure 3>





<Figure 4>



<Figure 5>