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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 G₂/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 β -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 potently cytotoxic against a variety of cancer cells, including multidrug-resistant cell lines.[2,3] CA-4, which binds on β -tubulin at the colchicine binding site, is also an exceptionally strong inhibitor of tubulin polymerization with an IC_{50} value of 1.0 μ 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*NH₂CA-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 G₂/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**, NH₂CA-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 α -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 vinyliodide **10a**. Reagent and conditions: a) $\text{PdCl}_2(\text{PPh}_3)_2$ (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 *iso*CA-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), $i\text{Pr}_2\text{NH}$ (2 equiv), CH_2Cl_2 ; b) But-3-yn-1-ol (1.1 equiv), $\text{PdCl}_2(\text{PPh}_3)_2$ (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%), $t\text{BuOLi}$ (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 $\text{Pd}_2\text{dba}_3/\text{XPhos}$ 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 $\text{Pd}(\text{OAc})_2$, 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 PdCl₂(PPh₃)₂ 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 Pd₂dba₃ and *t*BuXPhos in a dioxane-H₂O 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 H₂ 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)₂ (1 equiv), NaBr (1 equiv), 50 °C, EtOH; b) MPHT (1 equiv), CH₃CN; c) 3-bromoprop-1-ene (1.2 equiv), K₂CO₃ (1.2 equiv), CH₃CN; d) vinylmagnesium chloride (1.2 equiv), THF; e) Grubbs II catalyst, 5 mol%, CH₂Cl₂, 20 °C; f) H₂, Pd/C, MeOH, AcOH cat.; g) PCC, CH₂Cl₂; h) TsNHNH₂ (1 equiv), PTSA (10 mol%), MgSO₄ (1 equiv), EtOH, reflux; i) Pd₂dba₃ (10 mol%), XPhos (40 mol%), *t*BuOLi (2 equiv), 3,4,5-trimethoxyiodobenzene (1.2 equiv), 90 °C, sealed tube; j) Pd₂dba₃ (5 mol%), *t*BuXPhos (15 mol%), KOH (3 equiv), dioxane-H₂O, 90 °C, sealed tube; k) CuI (10 mol%), TMEDA (6 mol%), NaN₃ (2 equiv), potassium ascorbate (6 mol%), DMSO-H₂O, 90 °C, sealed tube; l) Pd(OAc)₂ (1 mol%), PPh₃ (2.5 mol%), *n*BuOH, 100 °C, sealed tube; m) *n*But-3-yn-1-ol (1.2 equiv), PdCl₂(PPh₃)₂ (5 mol%), PPh₃ (15 mol%), CuI (15 mol%), Et₂NH (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^[a]

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 GI₅₀ values were found for both bromine derivative (**6d**, GI₅₀ = 170 nM) and compound **6g** having a butyn-ol chain on the C9 position (**6g**, GI₅₀ = 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 GI₅₀ value of 1.5 nM slightly inferior to those of *iso*CA-4 (GI₅₀ = 3 nM) and CA-4 (GI₅₀ = 2 nM). Contrary to Ohsumi's finding,[65] it appears that the introduction of a NH₂ substituent on C9 result in weaker cytotoxicity (**6e**, GI₅₀ = 22 nM). Compound **6f**, with no substitution on the C9 position showed a significant loss of activity with a GI₅₀ 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** (GI₅₀ = 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 (GI₅₀ = 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 NH₂ 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 GI₅₀ 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** (GI₅₀ = 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 NH₂ substituent on the C9 position resulted in a slight loss of cytotoxicity as observed with compound **6e** (GI₅₀ = 20 nM). Compound **6f**, with no substitution on the C9 position showed a slight loss of activity with a GI₅₀ value of 85 nM. Contrary to our expectation,[Erreur ! Signet non défini.] the alkynylation at the C9 position in benzoxepin **6g** with a butynol moiety, resulted in a moderate activity against HCT116 cells with a GI₅₀ value of 150 nM. Evaluation of tetrahydro-benzoxepin **25a** which was found to elicit a nanomolar level of cytotoxicity (GI₅₀ =

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 μ M, which were about two times greater than that of *iso*CA-4 ($IC_{50} = 1.5$ μ M). Consistent with earlier observations[66], the replacement of the 4-methoxy group in **6b** ($CI_{50} = 3.8$ μ M) with an hydrogen atom in **6c** ($CI_{50} = 3.2$ μ 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 G₂/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 G₂/M growth phase was observed. Cell-cycle arrest in the G₂/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) overlayed with *iso*CA-4 (blue) in the tubulin binding site.

The docking-derived superimposition of **6b** and *iso*CA-4 was illustrated in Figure 5, and benzoxepin **6b** showed a binding pose similar to the one observed with *iso*CA-4. As it was previously observed with CA-4 and *iso*CA-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-*iso*CA-4 analogues and evaluated their biological activities. Compound **6b**, the hybrid most closely resembling *iso*CA-4 in structure, have potent antimitotic 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 *iso*CA-4. Flow cytometry analysis indicated that benzoxepin **6b** at a low concentration of 5 nM, arrested the cell cycle in the G₂/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 *iso*CA-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 (¹H, 300 MHz; ¹³C, 75 MHz) or Bruker AVANCE 400 (¹H, 400 MHz; ¹³C, 100 MHz). Unless otherwise stated, CDCl₃ was used as solvent. Chemical shifts δ 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 μ 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 (MgSO₄) or sodium sulphate (Na₂SO₄).

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 PdCl₂(PPh₃)₂ (246 mg, 0.35 mmol, 10 mol%) and Bu₃SnH (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 Et₃N) afforded compound **9a** as a colorless oil (1.25 g; 2.8 mmol; 80%). R_f (cyclohexane/EtOAc : 9/1) = 0.77. ¹H NMR: (CDCl₃, 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). ¹³C NMR (100 MHz, CDCl₃): 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⁻¹. MS (APCI) [M+H]⁺ = 465.0.

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

To a CH₂Cl₂ (10 mL) solution containing **9a** (597 mg, 1.29 mmol) was added in one portion I₂ (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 Et₂O, washed with a Na₂S₂O₃ solution (10 mL). The combined organic layers were dried over MgSO₄, 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. ¹H NMR: (CDCl₃, 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). ¹³C NMR (100 MHz, CDCl₃): 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): 2927, 2927, 1661, 1594, 1487, 1447, 1283, 1243, 1162, 1113 cm⁻¹. MS (APCI) [M+H]⁺ = 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 Et₂NH (1.41 mL; 9.72 mmol) and MOMCl (0.75 mL; 9.72 mmol) in CH₂Cl₂ (15 mL) for 1 h at room temperature. The medium was quenched with H₂O (45 mL) and extracted with CH₂Cl₂ (3 x 15 mL). The combined organic layers were dried over MgSO₄, 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_f (cyclohexane/EtOAc: 8/2) = 0.68. ¹H NMR: (CDCl₃, 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). ¹³C NMR (100 MHz, CDCl₃): 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]⁺ = 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), PdCl₂(PPh₃)₂ (112 mg; 0.16 mmol), CuI (44 mg; 0.30 mmol) was stirred in Et₃N (10 mL) for 2 h at room temperature. EtOAc (20 mL) was added to the crude mixture which was washed with a saturated NH₄Cl solution. After extraction with CH₂Cl₂, the combined extracts were dried (MgSO₄) and concentrated. Purification by flash chromatography on silica gel afforded alkyne **17** as a brown oil (538 mg; 1.80 mmol; 90%). R_f (cyclohexane/EtOAc: 7/3) = 0.22. ¹H NMR: (CDCl₃, 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. ¹³C NMR (100 MHz, CDCl₃): 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⁻¹. MS (ESI) [M+Na]⁺ = 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 H₂O (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 CH₂Cl₂. The organic layers were dried over MgSO₄, 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_f (cyclohexane/EtOAc: 5/5) = 0.30. ¹H NMR: (CDCl₃, 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. ¹³C NMR (100 MHz, CDCl₃): 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⁻¹. MS (APCI) [M+H]⁺ = 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 PPh₃ (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_f (cyclohexane/EtOAc: 5/5) = 0.60. ¹H NMR: (CDCl₃, 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). ¹³C NMR (100 MHz, CDCl₃): 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⁻¹. MS (ESI) [M+Na]⁺ = 273.1.

4.8 Synthesis of N'-(9-isopropoxy-8-methoxy-3,4-dihydrobenzo[b]oxepin-5(2H)-ylidene)-4-methylbenzenesulfonohydrazide **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-methylbenzenesulfonohydrazide (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_f (cyclohexane/EtOAc: 5/5) = 0.60. F = 192-194 °C. ¹H NMR: (CDCl₃, 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}C NMR (100 MHz, 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 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 CH_3CN 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 NaHCO_3 solution. After extraction with EtOAc (3 x 100 mL), the combined extracts were dried (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 CH_3CN solution of **20** (4.6 g; 20 mmol) was added K_2CO_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 H_2O . After extraction with EtOAc, the combined extracts were dried (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. ^1H NMR: (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}C NMR (100 MHz, 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 cm^{-1} . MS (APCI) $[\text{M}+\text{H}]^+ = 271.0$ (^{79}Br), 273.5 (^{81}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 H_2O . After extraction with CH_2Cl_2 , the combined extracts were dried (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. ^1H NMR: (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}C NMR (100 MHz, 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 cm^{-1} . MS (APCI) $[\text{M}+\text{H}]^+ = 299.1$ (^{79}Br), 301.1 (^{81}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-imidazolidinylidene]dichloro(phenylmethylene)(tricyclohexylphosphine)ruthenium, 231 mg; 0.27 mmol) in CH_2Cl_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. ^1H NMR: (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}C NMR (100 MHz, 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 cm^{-1} . MS (APCI) $[\text{M}+\text{H}]^+ = 271.3$ (^{79}Br), 273.0 (^{81}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 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. ^1H NMR: (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}C NMR (100 MHz, 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 cm^{-1} . MS (APCI) $[\text{M}+\text{H}]^+ = 273.0$ (^{79}Br), 275.1 (^{81}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 CH₂Cl₂ (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_f (cyclohexane/EtOAc: 8/2) = 0.60. ¹H NMR: (CDCl₃, 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). ¹³C NMR (100 MHz, CDCl₃): 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⁻¹. MS (APCI) [M+H]⁺ = 271.4 (⁷⁹Br), 273.5 (⁸¹Br).

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), MgSO₄ (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 H₂O (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_f (cyclohexane/EtOAc: 7/3) = 0.30. F = 201-203 °C. ¹H NMR: (CDCl₃, 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). ¹³C NMR (100 MHz, CDCl₃): 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⁻¹. MS (APCI) [M+H]⁺ = 439.0 (⁷⁹Br), 441.0 (⁸¹Br).

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), Pd₂dba₃ (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_f (cyclohexane/EtOAc: 7/3) = 0.60. ¹H NMR: (CDCl₃, 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). ¹³C NMR (100 MHz, CDCl₃): 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⁻¹. MS (APCI) [M+H]⁺ = 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 CH₂Cl₂ (5 mL) was stirred with AlCl₃ (92 mg; 0.28 mmol) for 1 h at room temperature. Then another portion of AlCl₃ (92 mg) was added to the crude mixture. After 1 h of stirring, the medium was hydrolyzed by H₂O (10 mL). After extraction with CH₂Cl₂ (3 x 10 mL), the combined extracts were dried (MgSO₄) 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), Pd₂dba₃ (46 mg; 0.005 mmol) and *t*BuXPhos (8 mg; 0.015 mmol) in a mixture of dioxane/H₂O: 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 NH₄Cl (10 mL) and extracted with EtOAc (3 x 10 mL). The combined organic layers were then dried over MgSO₄, filtered and evaporated to dryness. Purification by flash chromatography afforded compound **6b** as a pale yellow oil (47 mg, 0.13 mmol, 55%). R_f (cyclohexane/EtOAc: 7/3) = 0.26. ¹H NMR: (CDCl₃, 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). ¹³C NMR (100 MHz, CDCl₃): 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⁻¹. MS (APCI) [M+H]⁺ = 359.2. HRMS calcd for C₂₀H₂₃O₆ [M+H]⁺ 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), Pd₂dba₃ (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 CH₂Cl₂ and stirred for 2 h with AlCl₃ (22 mg; 0.24 mmol).

The medium was then hydrolyzed by H₂O (10 mL). After extraction with CH₂Cl₂, the combined extracts were dried (MgSO₄) and concentrated. Purification by flash chromatography on silica gel afforded benzoxepin **6c** as a pale yellow oil (20 mg; 0.06 mmol; 25%). R_f (cyclohexane/EtOAc: 7/3) = 0.40. ¹H NMR: (CDCl₃, 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). ¹³C NMR (100 MHz, CDCl₃): 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⁻¹. MS (APCI) [M+H]⁺ = 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), Pd₂dba₃ (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 SiO₂ and concentrated. The residue was purified by silica gel chromatography to yield **6d** as brown oil (536 mg; 1.26 mmol; 68%). R_f (cyclohexane/EtOAc: 8/2) = 0.33. ¹H NMR: (CDCl₃, 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). ¹³C NMR (100 MHz, CDCl₃): 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⁻¹. MS (APCI) [M+H]⁺ = 421.0 (⁷⁹Br), 423.5 (⁸¹Br). HRMS calcd for C₂₀H₂₂BrO₅ [M+H]⁺ 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), NaN₃ (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/H₂O: 5/1 (2 mL) was stirred at 60 °C in a sealed tube. After 12 h, the medium was cooled, quenched with NH₄Cl and extracted with EtOAc (3 x 10 mL). The combined organic layers were dried over MgSO₄, filtered and evaporated to dryness. Purification by flash chromatography afforded **6e** as a colorless oil (64 mg; 0.18 mmol, 75%). R_f (cyclohexane/EtOAc: 7/3) = 0.26. ¹H NMR: (CDCl₃, 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). ¹³C NMR (100 MHz, CDCl₃): 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⁻¹. MS (APCI) [M+H]⁺ = 358.0. HRMS calcd for C₂₀H₂₄NO₅ [M+H]⁺ 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)₂ (1.5 mg; 0.0015 mmol), PPh₃ (7.5 mg; 0.006 mmol), was stirred for 1 h at 100 °C in a sealed tube. The medium was cooled, H₂O (8 mL) was added to the crude mixture. After extraction with EtOAc (3 x 5 mL), the combined organic layers were dried over MgSO₄, filtered and evaporated to dryness. Purification by flash chromatography afforded **6f** as a brown oil (57 mg; 0.165 mmol, 69%). R_f (cyclohexane/EtOAc: 7/3) = 0.64. ¹H NMR: (CDCl₃, 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). ¹³C NMR (100 MHz, CDCl₃): 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⁻¹. MS (APCI) [M+H]⁺ = 343.0. HRMS calcd for C₂₀H₂₃O₅ [M+H]⁺ 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), PdCl₂(PPh₃)₂ (7 mg; 0.001 mmol), PPh₃ (7 mg; 0.03 mmol), CuI (2 mg; 0.03 mmol) and Et₂NH (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 NH₄Cl and extracted with EtOAc (3 x 10 mL). The combined organic layers were then dried over MgSO₄, filtered and evaporated to dryness. Purification by flash chromatography afforded compounds **6g** as a colorless oil (59 mg; 0.14 mmol; 74%). R_f (cyclohexane/EtOAc: 4/6) = 0.42. ¹H NMR: (CDCl₃, 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). ¹³C NMR (100 MHz, CDCl₃): 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 (5×10^3 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 ($\lambda_{ex} = 560$ 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 *iso*CA-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 *iso*CA-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 5×10^4 cells/well in 100 μ 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 μ 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 α -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) $\text{PdCl}_2(\text{PPh}_3)_2$ (2 mol%), Bu_3SnH (1.2 equiv), THF, 20 °C; b) I_2 , CH_2Cl_2 , 20 °C.

Scheme 2. Synthesis of dihydrobenzoxepins **6b,c**. Reagent and conditions: a) MOMCl (2 equiv), *i*Pr₂NH (2 equiv), CH_2Cl_2 ; b) But-3-yn-1-ol (1.1 equiv), $\text{PdCl}_2(\text{PPh}_3)_2$ (8 mol%), CuI (15 mol%), Et₃N; c) PTSA (1 equiv), H₂O, 100 °C, sealed tube; d) DEAD (1.5 equiv), PPh₃ (1.5 equiv), THF; e) TsNHNH₂ (2.5 equiv), PTSA (20 mol%), EtOH reflux; f) Pd_2dba_3 (10 mol%), XPhos (20 mol%), *t*BuOLi (2 equiv), ArI (1 equiv), 90 °C, sealed tube; g) AlCl_3 (5 equiv), CH_2Cl_2 .

Scheme 3. Synthesis of dihydrobenzoxepins **6b**, **6d-g** and tetrahydrobenzoxepins **25a,b**. Reagent and conditions: a) (i) $\text{Hg}(\text{OAc})_2$ (1 equiv), NaBr (1 equiv), 50 °C, EtOH; (ii) MPHT (1 equiv), CH_3CN ; b) 3-bromoprop-1-ene (1.2 equiv), K_2CO_3 (1.2 equiv), CH_3CN ; c) vinylmagnesium chloride (1.2 equiv), THF; d) Grubbs II catalyst, 5 mol%, CH_2Cl_2 , 20 °C; e) H₂, Pd/C, MeOH, AcOH cat.; f) PCC, CH_2Cl_2 ; g) TsNHNH₂ (1 equiv), PTSA (10 mol%), MgSO_4 (1 equiv), EtOH, reflux; h) Pd_2dba_3 (10 mol%), XPhos (40 mol%), *t*BuOLi (2 equiv), 3,4,5-trimethoxyiodobenzene (1.2 equiv), 90 °C, sealed tube; i) Pd_2dba_3 (5 mol%), *t*BuXPhos (15 mol%), KOH (3 equiv), dioxane-H₂O, 90 °C, sealed tube; j) CuI (10 mol%), TMEDA (6 mol%), NaN_3 (2 equiv), potassium ascorbate (6 mol%) DMSO-H₂O, 90 °C, sealed tube; k) $\text{Pd}(\text{OAc})_2$ (1 mol%), PPh₃ (2.5 mol%), *n*BuOH, 100 °C, sealed tube; l) *n*But-3-yn-1-ol (1.2 equiv), $\text{PdCl}_2(\text{PPh}_3)_2$ (5 mol%), PPh₃ (15 mol%), CuI (15 mol%), Et₂NH (2 equiv), DMF, 120 °C, MWI.

Table 1. Cytotoxic activity of dihydrobenzoxepins **6b-g** and tetrahydrobenzoxepins **25a,b** against HCT116^a

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 = OPO₃Na₂
NH₂CA-4 **1c** R = NH₂
FCA-4 **1d** R = F

- isoCA-4** **2a** R = OH
isoNH₂CA-4 **2b** R = NH₂
isoFCA-4 **2c** R = F
2d R = C≡C-CH₂OH

<Figure 1>

<Figure 2>

<Scheme 1>

<Scheme 2>

<Scheme 3>

Cytotoxicity against HCT-116 ^a cells				
Cpnd				
GI₅₀^b [nM]	6b: 1.5 ± 0.1	6c: 25 ± 3	6d: 170 ± 18	6e: 22 ± 3
Cpnd				
GI₅₀^b [nM]	6f: 85 ± 10	6g: 250 ± 22	25a: 20 ± 3	25b: Na ^c
GI₅₀^b [nM]	isoCA-4: 3 ± 0.2 ^d	CA-4: 2 ± 0.2 ^d		

^aHCT-116 Human colon carcinoma. ^bGI₅₀ 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). ^cNon active. ^dThe GI₅₀ values for CA-4, isoCA-4 were determined in this study.

<Table 1>

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

^aGI₅₀ 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). ^bHCT116, colon carcinoma; K562, myelogenous leukaemia; H1299, non-small cell lung carcinoma; MDA-MB231 hormone-independent breast cancer. ^cITP, Inhibition of Tubulin Polymerization; IC₅₀ is the concentration of compound required to inhibit 50% of the rate of microtubule assembly (average of three experiments). ^dThe GI₅₀ and IC₅₀ values (cytotoxicity and ITP, respectively) for *isoCA-4* were determined in this study.

<Table 2>

<Figure 3>

<Figure 4>

<Figure 5>