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Synthesis of Anti-Microtubule Biaryls and Preliminary Evaluation as Vascular-Disrupting Agents

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A series of new dibenzoxepines were synthesized in a straightforward and efficient manner through diastereoselective biaryl Suzuki–Miyaura coupling and Brønsted-acid-mediated cyclodehydration as key steps. The vascular-disrupting potential of these molecules was evaluated with various in vitro assays: inhibition of microtubule assembly, antiproliferative activity against cancer cell lines and normal endothelial cells, modification of endothelial cell morphology, and disruption of endothelial cell cords. Two of these compounds showed promising activities in these assays, with profiles similar to that of the reference drug NAC and markedly different from that of colchicine. Altogether, these results show that dibenzoxepines represent promising new leads for the development of more selective vascular-disrupting agents.

Introduction

The targeting of tumor vasculature, first introduced by Denekamp in the early 1980s,[1] is a promising approach in anticancer therapy.[2] It is based on the concept that the destruction of blood vessels should cause tumor regression and prevent the invasion of normal tissues. Two types of vascular-targeting agents (VTAs) were designed for this approach: 1) anti-angiogenic substances that prevent the formation of new blood vessels from existing vessels, and 2) vascular-disrupting agents (VDAs) that destroy existing tumor vasculature. Among VDAs, small molecules that bind to tubulin at the colchicine or vinblastin site and cause microtubule depolymerization showed promising vascular-disrupting properties in vitro and in vivo. More particularly, two series of compounds that bind to the colchicine site in tubulin have undergone clinical trials (Figure 1): the allocolchicinoid ZD6126 (**1**), a prodrug of *N*-acetylcolchinol (NAC, **2**) as well as prodrugs of the natural products combretastatin A-1 and A-4 (**3**–**5**).

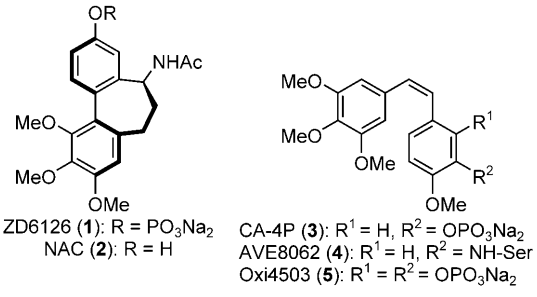

Figure 1. Selected anti-microtubule VDAs.

Whereas the development of combretastatin analogues as VDAs has been the subject of extensive research in the past few years,[3] allocolchicinoid-type molecules have attracted much less attention. This is probably due to synthetic issues, as allocolchicinoid-type molecules are less available than combretastatin analogues. NAC (**2**) itself is derived from colchicine by semisynthesis,[4] a procedure that intrinsically delivers a limited number of analogues.[5,6] Short and efficient total syntheses of allocolchicinoids that allow the introduction of a variety of functional groups on different parts of the molecule are therefore highly desirable in this context.[5b,7] We recently reported the asymmetric synthesis of hybrid analogues of NAC (**2**) and steganacin, a structurally related biaryl natural product that binds to tubulin at the colchicine site.[8] Compounds **6** (Figure 2), which bear a heterocyclic ring of varying size and different substituents, were synthesized and tested as anti-microtubule agents. Among them, dibenzoxepine **6a** showed the most potent anti-microtubule effect, with IC_{50}(NAC) < IC_{50}(**6a**) < IC_{50}(colchicine), but is nevertheless less cytotoxic toward cancer cells than both reference compounds by more than one order of magnitude. The ability of NAC to kill tumor endothelial cells seems to be directly related to its particular tubulin-binding properties.[9] However, the cardiotoxicity of this molecule evidenced in phase I clinical trials precluded its further development. As a result, the synthesis of NAC analogues with similar tubulin-binding properties but lower cell toxicities

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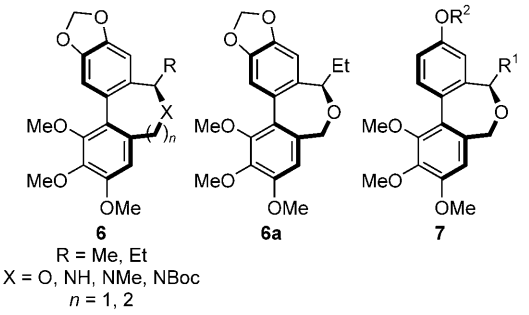


6 **6a** **7**

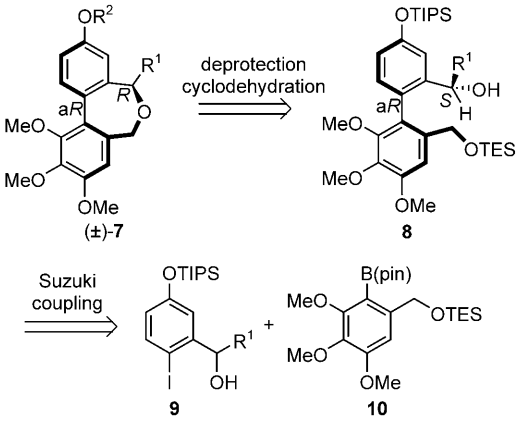
Figure 2. Previous (compounds **6**) and target (compounds **7**) heterocyclic analogues of NAC.

is of considerable interest. We therefore embarked on the synthesis of "second-generation" heterocyclic biaryls (compounds **7**, Figure 2) with closer structural similarity to NAC, that is, with a functionalizable phenol group in place of the dioxolane in compounds **6** and various benzylic (R^1) substituents. Herein we report a modified synthetic strategy to obtain these compounds and their preliminary biological evaluation as potential VDAs.

Results and Discussion

Synthesis of new dibenzoxepines

We previously disclosed the synthesis of racemic biaryls **6** as well as an enantioselective variant to access the biologically active *R*,a*R* enantiomers.[8] To perform more straightforward structure–activity relationship (SAR) studies, the target compounds **7** of this study were synthesized in their racemic form. We anticipate that our enantioselective variant should be easily transposed to the most active analogues if necessary for further development. Our retrosynthetic analysis is depicted in Scheme 1. We planned to form the target racemic dibenzoxepines **7** by a Brønsted-acid-mediated cyclodehydration of biaryl intermediate **8** as previously described.[8] Compound **8** would, in turn, arise from an atropo-diastereoselective Suzuki–

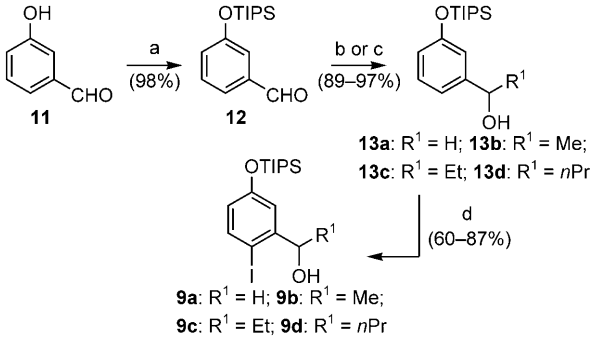


(±)-**7** **8** **9** **10**

Scheme 1. Retrosynthetic analysis of new dibenzoxepines. TIPS = triisopropylsilyl, TES = triethylsilyl, pin = pinacol (2,3-dimethyl-2,3-butanediol).

Miyaura coupling[10] of iodide **9** and pinacolboronate **10**. The rationale behind the choice of the triisopropylsilyl (TIPS) protecting group for the phenol moiety was threefold: 1) the steric shielding of both *ortho* positions to ensure a regioselective *para* iodination for the synthesis of **9**; 2) its stability toward alkaline bases, in particular barium hydroxide, which is used in our standard Suzuki coupling conditions; and 3) its possible concomitant cleavage during the acid-mediated cyclodehydration step. In other words, target **7** could possibly be produced directly as the free phenol (R^2 = H) from disilyl ether **8** by removal of both silyl groups and cyclodehydration in a single operation.

The synthesis of iodoarene coupling partners **9a–d** was performed in a straightforward manner from commercially available 3-hydroxybenzaldehyde **11** (Scheme 2). The introduction

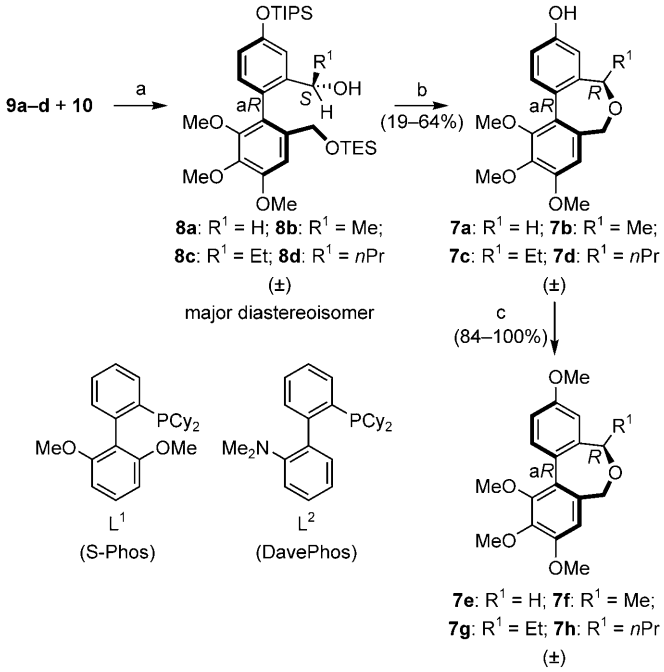


11 **12**

Scheme 2. Preparation of iodoarene building blocks **9a–d**. Reagents and conditions: a) TIPSCl (1.1 equiv), Et_3N (3 equiv), CH_2Cl_2, 0→20 °C, 14 h; b) R^1 = H: $NaBH_4$ (1.6 equiv), MeOH, 20 °C, 30 min (97%); c) R^1 = Me: MeMgCl (1.5 equiv), THF, 0 °C, 30 min; R^1 = Et, *n*Pr: EtMgBr or *n*PrMgBr (1.5 equiv), THF, −78 °C, 2 h (**13b**, 97%; **13c**, 89%; **13d**, 95%); d) I_2 (0.9 equiv), CF_3CO_2Ag (1.1 equiv), $CHCl_3$, 0 °C, 15 min (**9a**, 87%; **9b**, 67%; **9c**, 60%; **9d**, 64%).

of the TIPS group was followed either by reduction to the primary alcohol **13a** with sodium borohydride, or by reaction with a Grignard reagent to produce secondary alcohols **13b–d**. The reaction of ethyl and propyl Grignard reagents was performed at −78 °C with slow addition by syringe pump to avoid the competitive aldehyde reduction that produces **13a**. Finally, the iodination of **13a–c** occurred regioselectively in the presence of 0.9 equiv of iodine in order to limit the formation of regioisomers that were difficult to separate from the desired product. With this synthetic sequence, iodoarenes **9a–d** were obtained in 52–83% overall yield from **11**.

The palladium(0)-catalyzed coupling of iodoarenes **9a–d** with boronate **10** was performed under conditions that were previously optimized for biaryls **6** (Scheme 3).[8] The coupling of chiral racemic iodides **9b–d** gave rise, as observed before, to a mixture of two racemic diastereomers, with the major diastereomer having the *S*,a*R* relative configuration as evidenced by NOESY experiments. The coupling yield and diastereoselectivity varied with the palladium ligand used. For each iodoarene **9a–d**, phosphine ligands L^1 and L^2 of Buchwald and coworkers[11,12] that gave the highest reactivity in the previous



Scheme 3. Synthesis of dibenzoxepines **7a–h**. Reagents and conditions: a) **9a–d** (1.0 equiv), **10** (1.5 equiv), $Pd(OAc)_2$ (5 mol%), L^1 or L^2 (10 mol%), $Ba(OH)_2$ (1.1 equiv), dioxane/H_2O (9:1; $c = 1$ M), 100 °C, 1 h (see Table 1); b) 40% HF(aq)/CH_3CN (1:5), 20 °C, 48 h (yields for steps a–b: **7a**, 61%; **7b**, 64%; **7c**, 61%; **7d**, 19%); c) $NaN(SiMe_3)_2$ (5 equiv), MeI (5 equiv), THF, 20 °C, 15 h (**7e**, 87%; **7f**, 90%; **7g**, 84%; **7h**, 100%).

coupling system among other types of ligands[8] were tested. For iodides **9a–c**, ligand L^1 gave higher yields and diastereoselectivities (Table 1) than L^2 (data not shown), with the major diastereomers **8a–c** being isolated pure in 63–73% yield. The coupling of *n*-propyl-containing iodide **9d** proved troublesome, and owing to purification problems, gave a disappointing un-optimized yield of 27% for biaryl **8d** with ligand L^2. In addition, the diastereomeric ratio could not be measured reliably for this coupling. Overall, the present biaryl coupling seems to follow the same trend as before,[8] with lower yields and diastereoselectivities observed with increasing size of the benzylic alkyl group (R^1).

Table 1. Suzuki coupling of iodoarenes **9a–d** and boronate **10**.[a]

Iodoarene	Ligand	Product	Yield [%][b]	d.r.[c]
9a (R = H)	L^1	**8a** (R^1 = H)	73	–
9b (R = Me)	L^1	**8b** (R^1 = Me)	72	92:8
9c (R = Et)	L^1	**8c** (R^1 = Et)	63	80:20
9d (R = *n*Pr)	L^2	**8d** (R^1 = *n*Pr)	27[d]	n.d.

[a] Reagents and conditions: see Scheme 3. [b] Yield of the isolated major diastereomer **8a–d**. [c] Diastereomeric ratio measured by ^1H NMR analysis of the crude mixture (n.d. = not determined). [d] Un-optimized yield (see text).

Next, the cleavage of both silyl ethers and the cyclodehydration of intermediates **8a–d** were studied. Treatment of **8a** with trifluoroacetic acid (TFA), which was used for the cyclodehydration step in our previous work,[8] produced the corresponding TIPS-protected dibenzoxepine (Scheme 1, **7**, R^1 = H, R^2 = TIPS). This was deprotected with nBu_4NF to give target **7a** (Scheme 3) in 56% yield from **8a**. Alternatively, treating **8a** first with nBu_4NF removed both silyl groups, and upon treatment with TFA, **7a** was produced in 41% yield. Finally, treatment of **8a** with HF(aq) in CH_3CN resulted in the deprotection of both alcohols, and cyclodehydration occurred concomitantly to furnish dibenzoxepine **7a** in 83% yield (Scheme 3, step b). The same procedure applied to biaryls **8b–d** furnished alkyl-substituted analogues **7b–d** in high yield. With this synthetic sequence, targets **7a–d** were obtained in 60, 46, 33, and 16% yield, respectively, from 3-hydroxybenzaldehyde **11**. This decreasing overall yield with increasing size of the R^1 substituent reflects the negative effect of the alkyl group steric hindrance on the two most sensitive steps: the iodination and the Suzuki coupling. Finally, for SAR studies, the phenol group in **7a–d** was methylated to give analogues **7e–h** in high yield (Scheme 3, step c). Compounds **7a–g** occur as conformational mixtures of a*R* and a*S* atropisomers, like previous analogues, with the seven-membered ether bridging ring allowing free rotation around the biaryl bond.[8] Therefore, **7a** and **7e** are achiral, and **7b–d** and **7f–h** are racemic mixtures due to central chirality. In subsequent biological assays, only the racemic mixtures were evaluated, as racemic **6a** and its active *R* enantiomer showed similar potency in previous work.

Biological evaluation

As a pre-requisite for the assessment of the vascular-disrupting potential of dibenzoxepines **7a–h**, their anti-microtubule activity was evaluated using colchicine and NAC (**2**)[13] as reference molecules (Table 2). To compensate for variations over the different assays, results are reported as the IC_{50} value of the studied compound versus that of colchicine, which was tested in the same experiment. NAC is a potent anti-microtubule agent,

Table 2. Inhibition of microtubule assembly and growth of HCT116 cancer cells by dibenzoxepines **7a–h**.

Compound	Microtubule assembly[a]	Cytotoxicity: IC_{50} [μM][c]
colchicine	1[b]	0.04 ± 0.005
NAC (**2**)	0.3 ± 0.1	0.1 ± 0.01
6a	0.7 ± 0.1	1.4 ± 0.07
7a (R^1 = H)	12 ± 4	3.0 ± 0.15
7b (R^1 = Me)	2.4 ± 0.3	2.5 ± 0.12
7c (R^1 = Et)	0.9 ± 0.1	2.0 ± 0.10
7d (R^1 = *n*Pr)	3.1 ± 0.7	3.5 ± 0.17
7e (R^1 = H)	1.0 ± 0.2	0.55 ± 0.05
7f (R^1 = Me)	1.0 ± 0.2	2.0 ± 0.12
7g (R^1 = Et)	0.5 ± 0.1	0.7 ± 0.09
7h (R^1 = *n*Pr)	0.7 ± 0.1	0.4 ± 0.08

[a] Expressed as the ratio: IC_{50}(compd)/IC_{50}(colchicine); IC_{50}(compd) is the concentration of compound required to inhibit 50% of the rate of microtubule assembly (average of three experiments). [b] IC_{50}(colchicine) = 8.2 μM. [c] IC_{50} is the concentration of compound corresponding to 50% growth inhibition after 72 h incubation (average of three experiments).

which is more than threefold as active as colchicine. Dibenzoxepines **7a**–**d**, with a free phenol group, were less active than their corresponding methyl ethers **7e**–**h**. All other structural elements being comparable, the presence of an alkyl group and an ether medium ring for **7a**–**d** relative to an acetamide and a carbocyclic medium ring for NAC seems detrimental for anti-microtubule activity. The alkyl group size showed a marked influence, with the lowest IC_{50} value observed for the ethyl-substituted analogue **7c**. Among methyl ether derivatives **7e**–**h**, the ethyl analogue **7g** again showed the strongest inhibition, with twofold greater activity over colchicine and a 1.6-fold lower activity relative to NAC. Compound **7g** was also slightly more active than its previously reported dioxolane analogue **6a** (Figure 2).[8b]

In vitro antiproliferative activity of all dibenzoxepines was first determined against the human colon carcinoma cell line (HCT116) using colchicine and NAC as reference compounds. The results presented in Table 2 indicate a rough correlation between the anti-microtubule activity and the antiproliferative activity of compounds **7e**, **7g**, and **7h** exhibiting the highest cytotoxicities. Relative to NAC, these compounds showed lower cytotoxicity, in line with their anti-microtubule properties. However, the most cytotoxic dibenzoxepines were less cytotoxic than colchicine by two orders of magnitude, in contrast to their respective anti-microtubule activities. The cytotoxicity of colchicine was also significantly higher than that of NAC.

Compounds **7e** and **7g**, which displayed different anti-microtubule/antiproliferative patterns (**7g** was more active than **7e** toward tubulin but less cytotoxic), were next evaluated against five other cancer cell lines of diverse origin. The cytotoxicity of both compounds against these cell lines were on the same order of magnitude as with HCT116 cells (Table 3), clearly indicating an antiproliferative activity regardless of the origin of the tumor cells. At the same time, the effects of compounds **7e** and **7g** on the proliferation of normal human umbilical vein endothelial cells (HUVEC) were determined (Table 3). The results revealed that after incubation for 72 h, compounds **7e** and **7g** exhibited marked growth inhibition against HUVEC proliferation, with IC_{50} values of 0.35 and 0.40 μM, respectively. However, after short incubation times (1, 3, or 6 h) neither NAC nor **7e** or **7g** exerted a cytotoxic effect on endothelial cells, the viability of which was evaluated at the end of each short incubation with the products as well as following the 72 h incubation (data not shown). Simultaneously, treatment of endothelial cells with colchicine for 3 h impaired cell proliferation (IC_{50} = 0.3 μM). The observed differences in the cytotoxicity of NAC and dibenzoxepines on one hand, and colchicine on the other, could be at least partially due to differences in their binding to tubulin. Indeed, members of the *N*-acetylcolchinol family have been shown to bind tubulin with rapidly reversible kinetics[9] when the interaction of colchicine with tubulin is slow and only poorly reversible.[14] Furthermore, variability in the uptake of the investigated compounds cannot be ruled out.

Table 3. Cytotoxicity of dibenzoxepines **7e** and **7g**.[a]

Cell line	IC_{50} [μM] Colchicine	NAC (**2**)	**7e**	**7g**
HCT116	0.04 ± 0.005	0.1 ± 0.01	0.55 ± 0.05	0.7 ± 0.09
B16F10	0.03 ± 0.004	0.07 ± 0.008	0.28 ± 0.05	0.42 ± 0.08
A549	0.045 ± 0.005	0.25 ± 0.02	1.0 ± 0.1	3.6 ± 0.2
U87	0.03 ± 0.004	0.2 ± 0.02	4.0 ± 0.2	4.0 ± 0.2
MDA-MB435	0.04 ± 0.005	0.14 ± 0.01	0.35 ± 0.03	1.0 ± 0.1
MDA-MB231	0.07 ± 0.008	0.7 ± 0.08	2.8 ± 0.15	2.8 ± 0.15
HUVEC	0.03 ± 0.004	0.09 ± 0.01	0.35 ± 0.03	0.4 ± 0.05

[a] IC_{50} is the concentration of compound corresponding to 50% growth inhibition after 72 h incubation (average of three experiments).

To further determine whether these compounds exhibit anti-vascular activity as previously described for NAC,[15] the effects of compounds **7e** and **7g** on endothelial cell morphology were investigated. To this purpose, compounds **7e** and **7g** were added to the top of a confluent layer of HUVECs over 40 min at nontoxic concentrations. As shown in Figure 3, **7e** and **7g** clearly induced rapid changes in the morphology of the treated cells as soon as 1 h after drug washout, with a decrease in the area of cultured cells by 53 and 42%, respectively.

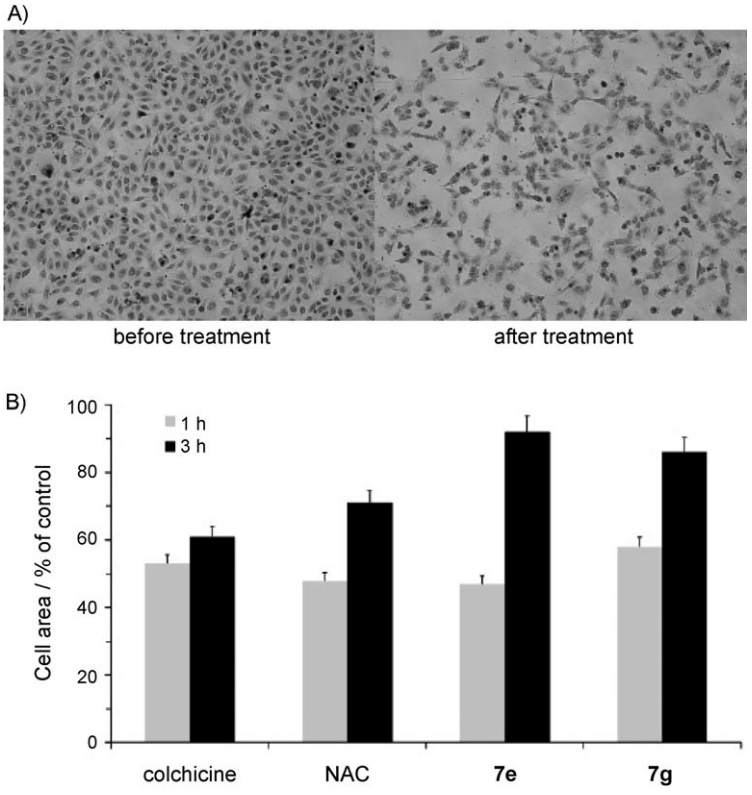


Figure 3. Reversible effect of dibenzoxepines **7e** and **7g** on endothelial cell morphology. HUVECs were exposed to the compounds (1 μM) for 40 min, and images were taken at 1 and 3 h after drug washout. A) Representative images (100×) of cells before (left) and after (right) 40 min treatment with **7e**. B) Cell spreading, evaluated as the cell area, is expressed as the percentage of control (average of three experiments).

A similar decrease in cell area was obtained after treatment with NAC (52%) or colchicine (47%). In fact, endothelial cell retraction is usually associated with alterations in the organization of both the tubulin and actin cytoskeletons, as shown previously for *N*-acetylcolchinol.[15] In contrast to the persistent modifications induced by colchicine, the effects of **7e** and **7g** on endothelial cell shape were reversible. Indeed, the treated cells were shown to regain their original shape 3 h after drug washout (Figure 3) and to cover 92 and 86% of the tissue culture dishes, respectively, as compared with 61% for colchicine. These findings indicate that compounds **7e** and **7g** affect the morphology of endothelial cells at concentrations that do not alter their proliferation and that this effect is reversible. This biological property of **7e** and **7g** may be beneficial in vivo, considering that brief treatment with these tubulin-binding agents is adequate to produce rapid damage of tumor vasculature, while inducing no side toxicity through prolonged disruption of microtubules in other tissues. This property demonstrated for **7e** and **7g** confers an advantage over other microtubule-destabilizing agents, such as colchicine, whose narrow therapeutic window has been ascribed to the pseudo-irreversible binding to tubulin.[9,16]

To further confirm that these compounds may affect newly formed vessels, their activities were evaluated with a tube-formation assay.[15] HUVECs seeded on a thick layer of Matrigel (a solubilized basement membrane preparation) rapidly align and form a network of cords corresponding to the newly formed capillaries. As shown in Figure 4, the addition of **7e** and **7g** to the formed cords at non-cytotoxic concentrations rapidly disrupted the integrity of the network in the same manner as NAC.

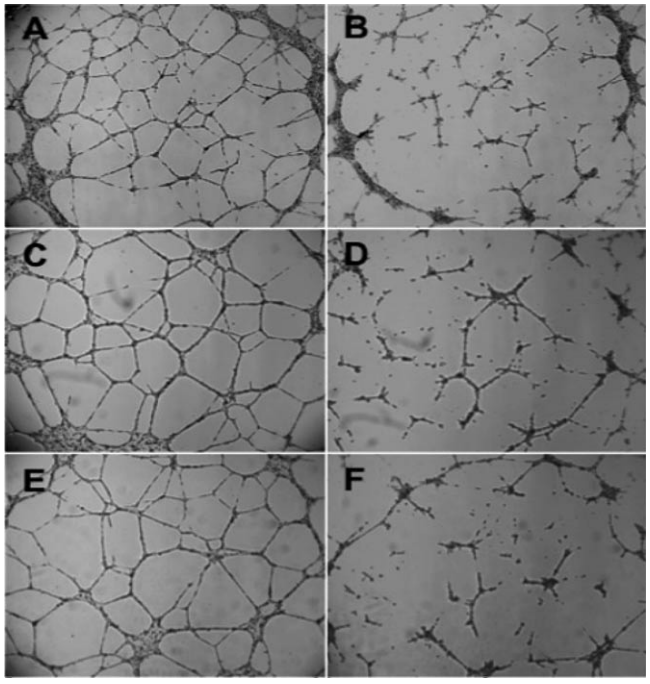


Figure 4. Effect of dibenzoxepines **7e** and **7g** on cords formed by HUVECs on Matrigel. Images (40×) were taken before (A, C, E) or 3 h after (B, D, F) addition of the compound. A, B) NAC (1 μM), C, D) **7e** (5 μM), E, F) **7g** (5 μM).

Conclusions

We have synthesized a series of new dibenzoxepines in a straightforward and efficient manner by using Suzuki–Miyaura biaryl coupling and Brønsted-acid-mediated cyclodehydration as the key steps. The vascular-disrupting potential of these molecules was evaluated with various in vitro assays. Compounds **7e** and **7g** showed an activity profile similar to that of the reference compound NAC at higher doses, and a markedly different profile from that of colchicine. Altogether, these results show that dibenzoxepines are promising new leads for the development of selective and nontoxic vascular-disrupting agents. Further research is underway to optimize the activity and improve the bioavailability of these molecules.

Experimental Section

Chemistry

General methods: Reagents were commercially available and used without further purification unless otherwise stated. All solvents were distilled from the appropriate drying agents immediately before use. Yields refer to chromatographically and spectroscopically homogeneous materials. Merck silica gel 60 (particle size 40–63 mM) was used for flash column chromatography, and 1- and 2-mm SDS silica-gel-coated glass plates (60 F_{254}) were used for preparative TLC using UV light as visualizing agent. Products that had been reported previously were isolated in >95% purity, as determined by ^{1}H NMR spectroscopy. NMR spectra were recorded on Bruker Avance 300 or Avance 500 instruments at 295 K, with tetramethylsilane or residual protiated solvent used as an internal reference for ^{1}H and ^{13}C spectra. The following abbreviations were used to designate the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. Assignments were made on the basis of DQF-COSY, NOESY or ROESY, HMQC, and HMBC experiments. IR spectra were recorded on a PerkinElmer Spectrum BX spectrometer. Mass spectra and high-resolution mass spectra (HRMS) were recorded under electrospray ionization (ESI) conditions at the Laboratoire de Spectrométrie de Masse, ICSN, Gif-sur-Yvette (France). Melting points (mp) are uncorrected and were recorded on a Büchi B-540 capillary melting point apparatus.

*3-Triisopropylsilyloxybenzaldehyde (**12**)*: Triethylamine (17.1 mL, 122.8 mmol) and triisopropylsilyl chloride (9.7 mL, 45.0 mmol) were added dropwise to a solution of 3-hydroxybenzaldehyde (5.0 g, 40.9 mmol) in CH_2Cl_2 (300 mL) at 0 °C. The mixture was stirred for 30 min at 0 °C, then 14 h at 20 °C, and then treated with a saturated solution of aqueous $NaHCO_3$. After extraction of the aqueous layer with CH_2Cl_2, the combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and evaporated under vacuum. The residue was purified by flash chromatography (silica gel, heptanes/EtOAc 95:5) to give **12** as an oil (11.15 g, 98%); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 9.95$ (s, 1H), 7.49–7.38 (m, 3H), 7.17 (ddd, $J =$ 7.8, 2.4, 1.2 Hz, 1H), 1.36–1.24 (m, 3H), 1.12 ppm (d, $J = 7.0$ Hz, 18H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 192.2$, 156.8, 137.9, 130.0, 126.3, 123.3, 119.9, 17.9, 12.6 ppm; IR (neat): $\tilde{\nu} = 2944$, 2866, 1702 cm^{-1}.

*3-Triisopropylsilyloxybenzyl alcohol (**13a**)*: $NaBH_4$ (785 mg, 20.7 mmol) was added portion-wise to a solution of aldehyde **12** (3.61 g, 13.0 mmol) in MeOH (50 mL) at 20 °C, and the mixture was stirred for 30 min. A solution of 1 M HCl(aq) was then added until the mixture reached pH 2. The aqueous layer was extracted with

CH_2Cl_2, and the combined organic layers were washed successively with H_2O, a saturated solution of $NaHCO_3$(aq), and brine. After drying over $MgSO_4$, filtration, and evaporation under vacuum, alcohol **13a** was obtained as a colorless oil (3.52 g, 97%); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.22 (t, J = 7.5 Hz, 1H), 6.95–6.92 (m, 2H), 6.79 (dd, J = 7.5, 2.1 Hz, 1H), 4.60 (d, J = 3.3 Hz, 2H), 2.00 (br s, 1H), 1.31–1.19 (m, 3H), 1.12 ppm (d, J = 6.9 Hz, 18H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 156.3, 142.5, 139.5, 119.4, 119.0, 118.4, 62.5, 17.9, 12.6 ppm; IR (neat): $\tilde{\nu}$ = 3312, 2943, 2865 cm^{-1}.

*α-Methyl-3-triisopropylsilyloxybenzyl alcohol (**13b**)*: A solution of methylmagnesium chloride (3 M in THF, 1.8 mL, 5.4 mmol) was added dropwise to a solution of aldehyde **12** (1.0 g, 3.6 mmol) in THF (15 mL) at 0 °C. The mixture was stirred for 30 min at 0 °C and was then allowed to warm up to room temperature. A solution of acetic acid (10% aq) was added, and the aqueous layer was extracted with Et_2O. The combined organic layers were washed with H_2O and a saturated solution of $NaHCO_3$(aq) until the aqueous phase reached pH 7, dried over $MgSO_4$, filtered, and evaporated under vacuum. The residue was purified by flash chromatography (silica gel, heptanes/EtOAc 9:1) to give alcohol **13b** as a colorless oil (1.03 g, 97%); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.18 (t, J = 8.1 Hz, 1H), 6.93–6.90 (m, 2H), 6.78 (ddd, J = 8.1, 2.7, 0.9 Hz, 1H), 4.83 (qd, J = 6.3, 3.3 Hz, 1H), 1.84 (d, J = 3.3 Hz, 1H), 1.46 (d, J = 6.3 Hz, 3H), 1.31–1.18 (m, 3H), 1.10 ppm (d, J = 6.9 Hz, 18H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 156.4, 147.7, 129.5, 119.0, 118.1, 117.0, 70.4, 25.3, 18.1, 12.9 ppm; IR (neat): $\tilde{\nu}$ = 3340, 2944, 2866 cm^{-1}.

*α-Ethyl-3-triisopropylsilyloxybenzyl alcohol (**13c**)*: A solution of ethylmagnesium bromide (1 M in THF, 10.8 mL, 10.8 mmol) was added dropwise over 1.5 h via syringe pump to a solution of aldehyde **12** (2.01 g, 7.2 mmol) in THF (40 mL) at −78 °C. The mixture was stirred for 30 min at −78 °C and was then allowed to warm to room temperature. A solution of acetic acid (10% aq) was added, and the aqueous layer was extracted with Et_2O. The combined organic layers were washed with H_2O and a saturated solution of $NaHCO_3$(aq) until the aqueous phase reached pH 7, dried over $MgSO_4$, filtered, and evaporated under vacuum. The residue was purified by flash chromatography (silica gel, heptanes/EtOAc 9:1) to give alcohol **13c** as a colorless oil (1.03 g, 97%); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.17 (t, J = 7.8 Hz, 1H), 6.90–6.87 (m, 2H), 6.78 (ddd, J = 7.8, 2.7, 1.8 Hz), 4.52 (t, J = 6.9 Hz, 1H), 1.95 (br s, 1H), 1.84–1.66 (m, 2H), 1.34–1.19 (m, 3H), 1.10 (d, J = 6.3 Hz, 18H), 0.86 ppm (t, J = 7.3 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 156.3, 146.4, 129.4, 119.1, 118.7, 117.6, 76.0, 32.0, 18.1, 12.9, 10.2 ppm; IR (neat): $\tilde{\nu}$ = 3352, 2942, 2866 cm^{-1}; HRMS (ESI) calcd for $C_{19}H_{32}O_2NaSi$ [M+Na$^+$]: 331.2069; found: 331.2076.

*α-Propyl-3-triisopropylsilyloxybenzyl alcohol (**13d**)*: Compound **13d** was obtained in the same manner as above for **13c**, starting from **12** (1.5 g, 5.4 mmol) and *n*-propylmagnesium bromide (2 M in Et_2O, 4.0 mL, 8.1 mmol). It was isolated as a colorless oil (1.65 g, 95%) after flash chromatography (silica gel, heptanes/EtOAc 95:5, then 4:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.17 (t, J = 7.8 Hz, 1H), 6.90–6.88 (m, 2H), 6.79 (ddd, J = 7.8, 2.4, 0.9 Hz, 1H), 4.59 (t, J = 6.6 Hz, 1H), 2.09 (br s, 1H), 1.82–1.59 (m, 2H), 1.47–1.16 (m, 5H), 1.12 (d, J = 6.6 Hz, 18H), 0.92 ppm (t, J = 7.5 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 156.2, 146.7, 129.3, 118.9, 118.7, 117.5, 74.3, 41.2, 19.0, 18.0, 14.1, 12.8 ppm; IR (neat): $\tilde{\nu}$ = 3354, 2944, 2866 cm^{-1}; HRMS (ESI) calcd for $C_{19}H_{34}O_2NaSi$ [M+Na$^+$]: 345.2226; found: 345.2233.

*2-Iodo-5-triisopropylsilyloxybenzyl alcohol (**9a**)*: Silver trifluoroacetate (2.59 g, 11.7 mmol) and iodine (2.56 g, 10.1 mmol) were added in one portion to a solution of alcohol **13a** (2.99 g, 10.7 mmol) in chloroform at 0 °C. After stirring for 15 min at 0 °C, the mixture was filtered through Celite and washed with a saturated solution of Na_2SO_3(aq). The organic layer was dried over $MgSO_4$, filtered, and evaporated under vacuum. The residue was purified by flash chromatography (silica gel, heptanes/EtOAc 95:5) to give alcohol **9a** as a colorless oil (3.78 g, 87%); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.61 (d, J = 8.7 Hz, 1H), 7.03 (d, J = 2.7 Hz, 1H), 6.58 (dd, J = 8.7, 2.7 Hz, 1H), 4.60 (d, J = 5.4 Hz, 2H), 2.06 (t, J = 5.4 Hz, 1H), 1.33–1.17 (m, 3H), 1.10 ppm (d, J = 6.9 Hz, 18H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 156.9, 143.7, 139.6, 120.9, 120.4, 85.9, 69.1, 17.9, 12.6 ppm; IR (neat): $\tilde{\nu}$ = 3356, 2942, 2865 cm^{-1}.

*2-Iodo-α-methyl-5-triisopropylsilyloxybenzyl alcohol (**9b**)*: Compound **9b** was obtained in the same manner as above for **9a**, starting from **13b** (473 mg, 1.61 mmol), silver trifluoroacetate (390 mg, 1.77 mmol), and iodine (367 mg, 1.45 mmol). The residue was purified by flash chromatography (silica gel, heptanes/EtOAc 95:5) to give alcohol **9b** as a colorless oil (453 mg, 67%); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.58 (d, J = 8.4 Hz, 1H), 7.12 (d, J = 3.0 Hz, 1H), 6.55 (dd, J = 8.4, 3.0 Hz, 1H), 4.98 (qd, J = 6.3, 3.0 Hz, 1H), 1.91 (d, J = 3.0 Hz, 1H), 1.42 (d, J = 6.3 Hz, 3H), 1.33–1.17 (m, 3H), 1.09 ppm (d, J = 6.3 Hz, 18H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 157.2, 148.8, 139.9, 121.2, 118.4, 86.0, 73.7, 23.8, 18.1, 12.8 ppm; IR (neat): $\tilde{\nu}$ = 3378, 2943, 2866 cm^{-1}; HRMS (ESI) calcd for $C_{17}H_{28}IOSi$ [M+H−H_2O^+]: 403.0954; found: 403.1001.

*2-Iodo-α-ethyl-5-triisopropylsilyloxybenzyl alcohol (**9c**)*: Compound **9c** was obtained in the same manner as above for **9a**, starting from **13c** (1.73 g, 5.62 mmol), silver trifluoroacetate (1.36 g, 6.17 mmol), and iodine (1.28 g, 5.05 mmol). The residue was purified by flash chromatography (silica gel, heptanes/EtOAc 95:5, then 4:1) to give alcohol **9c** as a white solid (1.47 g, 60%); mp: 43 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.59 (d, J = 6.0 Hz, 1H), 7.06 (d, J = 1.8 Hz, 1H), 6.55 (dd, J = 6.0, 1.8 Hz), 4.75 (m, 1H), 1.94 (d, J = 2.7 Hz, 1H), 1.81–1.75 (m, 2H), 1.52–1.29 (m, 3H), 1.09 (d, J = 6.3 Hz, 18H), 1.01 ppm (t, J = 5.7 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 157.0, 143.7, 139.8, 121.3, 119.1, 86.7, 78.5, 30.8, 18.1, 12.8, 10.3 ppm; IR (neat): $\tilde{\nu}$ = 3282, 2941, 2864 cm^{-1}; HRMS (ESI) calcd for $C_{18}H_{31}IO_2NaSi$ [M+Na$^+$]: 457.1036; found: 457.1060.

*2-Iodo-α-propyl-5-triisopropylsilyloxybenzyl alcohol (**9d**)*: Compound **9d** was obtained in the same manner as above for **9a**, starting from **13d** (1.24 g, 3.85 mmol), silver trifluoroacetate (934 mg, 4.23 mmol), and iodine (878 mg, 3.46 mmol). The residue was purified by flash chromatography (silica gel, heptanes/EtOAc 95:5) to give alcohol **9d** as a colorless oil (1.11 g, 64%); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.58 (d, J = 8.7 Hz, 1H), 7.07 (d, J = 3.3 Hz, 1H), 6.54 (dd, J = 8.7, 3.3 Hz, 1H), 4.83 (dt, J = 3.6, 3.6 Hz, 1H), 1.89 (d, J = 3.6 Hz, 1H), 1.75–1.33 (m, 4H), 1.30–1.17 (m, 3H), 1.09 (d, J = 6.6 Hz, 18H), 0.97 ppm (t, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 157.0, 148.0, 139.8, 121.2, 119.0, 86.5, 77.1, 40.0, 19.2, 18.1, 14.1, 12.8 ppm; IR (neat): $\tilde{\nu}$ = 3271, 2943, 2865 cm^{-1}; HRMS (ESI) calcd for $C_{19}H_{33}IO_2NaSi$ [M+Na$^+$]: 471.1206; found: 471.1192.

*Biaryl **8a***: A sealed tube was charged with alcohol **9a** (504 mg, 1.24 mmol), boronate **10**[8] (816 mg, 1.86 mmol), $Pd(OAc)_2$ (13.8 mg, 0.062 mmol), S-Phos L[1] (51.0 mg, 0.12 mmol), $Ba(OH)_2{\cdot}8H_2O$ (433 mg, 1.36 mmol), and degassed dioxane/H_2O (9:1, [**9a**] = 1 M). The tube was sealed and placed in an oil bath pre-heated at 100 °C and stirred for 2.5 h. After cooling to room temperature, the mixture was filtered through Celite and $MgSO_4$. The filtrate was concentrated, and the residue was purified by flash chromatography (silica gel, heptanes/EtOAc 85:15, then 75:25, then 7:3) to give biaryl **8a** as an oil (538 mg, 73%); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.09 (d, J = 2.4 Hz, 1H), 6.98 (d, J = 8.1 Hz, 1H), 6.96 (s, 1H), 6.87 (dd, J = 8.1, 2.4 Hz, 1H), 4.35 (d, J = 12.9 Hz, 1H), 4.21 (d, J = 5.7 Hz,

2H), 4.19 (d, $J=12.9$ Hz, 1H), 3.92 (s, 3H), 3.90 (s, 3H), 3.51 (s, 3H), 3.05 (t, $J=5.7$ Hz, 1H), 1.36–1.20 (m, 3H), 1.13 (d, $J=6.9$ Hz, 18H), 0.87 (t, $J=7.8$ Hz, 9H), 0.55 ppm (q, $J=7.8$ Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=156.0$, 153.0, 150.8, 141.4, 141.3, 135.3, 131.3, 127.4, 125.5, 121.4, 119.4, 107.3, 64.1, 62.7, 61.2, 61.1, 56.0, 18.1, 12.8, 6.9, 4.4 ppm; IR (neat): $\tilde{\nu}=3441$, 2945, 2867, 1483 cm^{-1}; HRMS (ESI) calcd for $C_{32}H_{54}O_6NaSi_2$ [M+Na$^+$]: 613.3357; found: 613.3342.

Biaryl ***8b***: Compound **8b** was obtained in the same manner as above for **8a**, starting from **9b** (214 mg, 0.51 mmol), **10** (335 mg, 0.76 mmol), $Pd(OAc)_2$ (5.7 mg, 0.025 mmol), S-Phos L[1] (20.9 mg, 0.051 mmol), and $Ba(OH)_2{\cdot}8H_2O$ (177 mg, 0.56 mmol). The diastereomeric ratio of the coupling product (92:8) was determined by ^{1}H NMR spectroscopic analysis of the crude reaction mixture. The residue was purified by flash chromatography (silica gel, heptanes/EtOAc 98:2, then 94:6, then 9:1) to give biaryl **8b** as an oil (222 mg, 72%); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=7.18$ (d, $J=2.0$ Hz, 1H), 7.04 (s, 1H), 6.93 (d, $J=8.5$ Hz, 1H), 6.86 (dd, $J=8.5$, 2.0 Hz, 1H), 4.47 (q, $J=6.5$ Hz, 1H), 4.44 (d, $J=13.5$ Hz, 1H), 4.13 (d, $J=13.5$ Hz, 1H), 3.92 (s, 3H), 3.90 (s, 3H), 3.47 (s, 3H), 3.10 (br s, 1H), 1.36 (d, $J=6.5$ Hz, 3H), 1.36–1.26 (m, 3H), 1.13 (d, $J=7.0$ Hz, 18H), 0.91 (t, $J=8.0$ Hz, 9H), 0.56 ppm (q, $J=8.0$ Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=156.4$ (C4), 153.0, 150.5, 145.4, 141.0, 135.6, 131.3, 126.5, 124.9, 119.2, 117.5, 106.5, 66.7, 62.5, 61.3, 61.1, 56.0, 21.9, 18.0, 12.8, 6.9 ppm; IR (neat): $\tilde{\nu}=2945$, 2868 cm^{-1}; HRMS (ESI) calcd for $C_{33}H_{56}O_6NaSi_2$ [M+Na$^+$]: 627.3513; found: 627.3527.

Biaryl ***8c***: Compound **8c** was obtained in the same manner as above for **8a**, starting from **9c** (100 mg, 0.23 mmol), **10** (151 mg, 0.35 mmol), $Pd(OAc)_2$ (2.6 mg, 0.012 mmol), S-Phos L[1] (9.5 mg, 0.023 mmol), and $Ba(OH)_2{\cdot}8H_2O$ (80 mg, 0.25 mmol). The diastereomeric ratio of the coupling product (80:20) was determined by ^{1}H NMR spectroscopic analysis of the crude reaction mixture. The residue was purified by flash chromatography (silica gel, heptanes/EtOAc 96:4, then 9:1) to give biaryl **8c** as an oil (90 mg, 63%); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=7.12$ (d, $J=2.4$ Hz, 1H), 7.06 (s, 1H), 6.96 (d, $J=8.4$ Hz, 1H), 6.85 (dd, $J=8.4$, 2.4 Hz, 1H), 4.48 (d, $J=13.2$ Hz, 1H), 4.17–4.12 (m, 2H), 3.93 (s, 3H), 3.91 (s, 3H), 3.45 (s, 3H), 3.14 (t, $J=6.0$ Hz, 1H), 1.84–1.77 (m, 1H), 1.72–1.62 (m, 1H), 1.29–1.22 (m, 3H), 1.12 (d, $J=9.2$ Hz, 18H), 0.92 (t, $J=7.6$ Hz, 9H), 0.71 (t, $J=7.6$ Hz, 3H), 0.56 ppm (q, $J=7.6$ Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=156.3$, 152.9, 150.5, 144.2, 140.9, 135.5, 131.3, 127.4, 124.9, 119.4, 117.9, 106.3, 72.4, 62.5, 61.4, 61.1, 56.0, 28.3, 18.0, 12.8, 10.4, 6.9, 4.5 ppm; IR (neat): $\tilde{\nu}=3468$, 2937, 2868, 1462 cm^{-1}; HRMS (ESI) calcd for $C_{34}H_{58}O_6NaSi_2$ [M+Na$^+$]: 641.3670; found: 641.3664.

Biaryl ***8d***: Compound **8d** was obtained in the same manner as above for **8a**, starting from **9d** (238 mg, 0.53 mmol), **10** (349 mg, 0.80 mmol), $Pd(OAc)_2$ (6.0 mg, 0.026 mmol), Dave-Phos L[2] (21 mg, 0.053 mmol), and $Ba(OH)_2{\cdot}8H_2O$ (184 mg, 0.58 mmol). The residue was purified twice by flash chromatography (silica gel, heptanes/EtOAc 98:2, then 96:4) to give biaryl **8d** as an oil (92 mg, 27%); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=7.12$ (d, $J=2.4$ Hz, 1H), 7.04 (s, 1H), 6.95 (d, $J=8.1$ Hz, 1H), 6.84 (dd, $J=8.1$, 2.4 Hz, 1H), 4.47 (d, $J=13.5$ Hz, 1H), 4.24 (t, $J=7.2$ Hz, 1H), 4.12 (d, $J=13.5$ Hz, 1H), 3.92 (s, 3H), 3.90 (s, 3H), 3.44 (s, 3H), 3.07 (br s, 1H), 1.79–1.53 (m, 4H), 1.35–1.22 (m, 3H), 1.12 (d, $J=6.9$ Hz, 18H), 0.92 (t, $J=8.1$ Hz, 9H), 0.78 (t, $J=7.2$ Hz, 3H), 0.56 ppm (q, $J=8.1$ Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=156.3$, 152.9, 150.5, 144.5, 140.9, 135.5, 131.3, 127.2, 124.9, 119.9, 117.8, 106.3, 70.8, 62.5, 61.4, 61.1, 56.0, 37.8, 19.4, 18.0, 14.1, 12.8, 6.9, 4.5 ppm; IR (neat): $\tilde{\nu}=3459$, 2953, 2868, 1462 cm^{-1}; HRMS (ESI) calcd for $C_{35}H_{60}O_6NaSi_2$ [M+Na$^+$]: 655.3826; found: 655.3850.

Dibenzoxepine ***7a***: A solution of HF (40% aq, 2 mL) was added to a solution of biaryl **8a** (129 mg, 0.22 mmol) in CH_3CN (10 mL) at 20 °C, and the mixture was stirred for 48 h. It was cautiously poured into a solution of saturated $NaHCO_3$(aq), and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and evaporated under vacuum. The residue was purified by preparative TLC (silica gel, heptanes/EtOAc 1:1) to give dibenzoxepine **7a** as a beige solid (55 mg, 83%); mp: 179 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=7.61$ (d, $J=8.4$ Hz, 1H), 6.96–6.92 (m, 2H), 6.76 (s, 1H), 5.80 (br s, 1H), 4.41 (m, 2H), 4.11 (m, 2H), 3.95 (s, 3H), 3.92 (s, 3H), 3.68 ppm (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=$ 15.6, 152.9, 150.7, 142.9, 136.5, 131.2, 131.0, 129.4, 126.5, 116.3, 115.6, 109.0, 67.9, 67.6, 61.3, 61.0, 56.2 ppm; IR (neat): $\tilde{\nu}=3190$, 1449 cm^{-1}; HRMS (ESI) calcd for $C_{17}H_{18}O_5Na$ [M+Na$^+$]: 325.1052; found: 325.1063.

Dibenzoxepine ***7b***: Compound **7b** was obtained in the same manner as above for **7a**, starting from **8b** (140 mg, 0.23 mmol). The residue was purified by flash chromatography (silica gel, heptanes/EtOAc 4:1, then 3:1) to give dibenzoxepine **7b** as a white solid (65 mg, 89%, ~95:5 mixture of interconverting atropisomers);[8] mp: 144 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=7.56$ (d, $J=8.4$ Hz, 1H), 7.02 (d, $J=2.4$ Hz, 1H), 6.94 (dd, $J=8.4$, 2.4 Hz, 1H), 6.76 (s, 1H), 4.44 (d, $J=11.1$ Hz, 1H), 4.36 (q, $J=6.3$ Hz, 1H), 4.06 (d, $J=11.1$ Hz, 1H), 3.96 (s, 3H), 3.92 (s, 3H), 3.70 (s, 3H), 1.56 ppm (d, $J=6.3$ Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=155.9$, 152.9, 150.5, 142.7, 138.8, 131.1, 130.9, 129.0, 126.3, 115.5, 112.2, 108.5, 69.1, 68.2, 61.2, 61.0, 56.2, 17.7 ppm; IR (neat): $\tilde{\nu}=3429$, 1487 cm^{-1}; HRMS (ESI) calcd for $C_{18}H_{20}O_5Na$ [M+Na$^+$]: 339.1208; found: 339.1234.

Dibenzoxepine ***7c***: Compound **7c** was obtained in the same manner as above for **7a**, starting from **8c** (44 mg, 0.071 mmol). The residue was purified by flash chromatography (silica gel, heptanes/EtOAc 3:2) to give dibenzoxepine **7b** as a brown solid (23 mg, 97%, ~9:1 mixture of interconverting atropisomers); mp: 151 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=7.55$ (d, $J=8.4$ Hz, 1H), 6.97 (d, $J=2.4$ Hz, 1H), 6.90 (dd, $J=8.4$, 2.4 Hz, 1H), 6.75 (s, 1H), 5.26 (s, 1H), 4.40 (d, $J=10.8$ Hz, 1H), 4.00–3.92 (m, 8H), 3.59 (s, 3H), 1.98 (dq, $J=7.2$, 7.2 Hz, 2H), 0.97 ppm (t, $J=7.2$ Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=155.6$, 152.9, 150.5, 142.7, 138.2, 131.1, 131.0, 128.9, 126.4, 115.0, 112.4, 108.6, 74.7, 68.1, 61.3, 61.0, 56.2, 24.7, 11.1 ppm; IR (neat): $\tilde{\nu}=3362$, 2935, 1484 cm^{-1}; HRMS (ESI) calcd for $C_{19}H_{22}O_5Na$ [M+Na$^+$]: 353.1365; found: 353.1322.

Dibenzoxepine ***7d***: Compound **7d** was obtained in the same manner as above for **7a**, starting from **8d** (63 mg, 0.10 mmol). The residue was purified by preparative TLC (silica gel, heptanes/EtOAc 3:2) to give dibenzoxepine **7b** as a colorless oil (24 mg, 71%, ~85:15 mixture of interconverting atropisomers); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=7.50$ (d, $J=8.4$ Hz, 1H), 6.93 (d, $J=2.7$ Hz, 1H), 6.87 (dd, $J=8.4$, 2.7 Hz, 1H), 6.71 (s, 1H), 4.37 (d, $J=10.8$ Hz, 1H), 4.04 (t, $J=7.2$ Hz, 1H), 3.96 (d, $J=10.8$ Hz, 1H), 3.92 (s, 3H), 3.88 (s, 3H), 3.64 (s, 3H), 1.93–1.79 (m, 2H), 1.47–1.24 (m, 2H), 0.84 ppm (t, $J=7.5$ Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=155.7$, 152.9, 150.5, 142.7, 138.3, 131.1, 129.7, 126.4, 115.1, 112.5, 108.6, 72.9, 68.1, 61.3, 61.1, 56.2, 33.9, 19.8, 14.3 ppm; IR (neat): $\tilde{\nu}=3356$, 2957, 2934, 1486 cm^{-1}; HRMS (ESI) calcd for $C_{20}H_{24}O_5Na$ [M+Na$^+$]: 367.1521; found: 367.1544.

Dibenzoxepine ***7e***: $NaN(SiMe_3)_2$ (2 M in THF, 112 μL, 0.22 mmol) and iodomethane (14 μL, 0.22 mmol) were added to a solution of dibenzoxepine **7a** (14 mg, 0.045 mmol) in THF (2 mL) at 20 °C, and the mixture was stirred for 15 h. A saturated solution of NH_4Cl(aq) was added, and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered, and

evaporated under vacuum. The residue was purified by flash chromatography (silica gel, heptanes/EtOAc 4:1) to give dibenzoxepine **7e** as a white solid (12 mg, 87%); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta =$ 7.65 (d, $J = 8.7$ Hz, 1H), 7.02–6.92 (m, 2H), 6.76 (s, 1H), 4.43 (m, 2H), 4.18 (m, 1H), 4.06 (m, 1H), 3.95 (s, 3H), 3.92 (s, 3H), 3.88 (s, 3H), 3.67 ppm (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 159.1$, 152.8, 150.7, 142.8, 136.6, 131.2, 130.9, 129.4, 126.5, 114.6, 114.0, 108.9, 67.9, 61.3, 61.0, 56.2, 55.5 ppm; IR (neat): $\tilde{\nu} = 2926$, 2847 cm^{-1}; HRMS (ESI) calcd for $C_{18}H_{20}O_5Na$ [M+Na$^+$]: 339.1208; found: 339.1191.

Dibenzoxepine ***7f***: Compound **7f** was obtained in the same manner as above for **7e**, starting from **7b** (43 mg, 0.14 mmol), $NaN(SiMe_3)_2$ (340 μL, 0.68 mmol), and iodomethane (42 μL, 0.68 mmol). The residue was purified by flash chromatography (silica gel, heptanes/EtOAc 4:1) to give dibenzoxepine **7f** as a colorless oil (41 mg, 90%, ~95:5 mixture of interconverting atropisomers); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.61$ (d, $J = 8.4$ Hz, 1H), 7.06 (d, $J = 2.4$ Hz, 1H), 6.98 (dd, $J = 8.4$, 2.4 Hz, 1H), 6.74 (s, 1H), 4.39 (d, $J = 11.3$ Hz, 1H), 4.33 (q, $J = 6.6$ Hz, 1H), 4.01 (d, $J = 11.3$ Hz, 1H), 3.94 (s, 3H), 3.92 (s, 3H), 3.88 (s, 3H), 3.68 (s, 3H), 1.59 ppm (d, $J = 6.3$ Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 159.4$, 153.0, 150.6, 142.8, 139.0, 131.2, 130.9, 129.5, 126.3, 113.0, 111.0, 108.5, 69.0, 68.3, 61.2, 61.0, 56.2, 55.5, 17.9 ppm; IR (neat): $\tilde{\nu} = 2936$, 1486 cm^{-1}; HRMS (ESI) calcd for $C_{19}H_{22}O_5Na$ [M+Na$^+$]: 353.1365; found: 353.1369.

Dibenzoxepine ***7g***: Compound **7g** was obtained in the same manner as above for **7e**, starting from **7c** (15 mg, 0.045 mmol), $NaN(SiMe_3)_2$ (114 μL, 0.23 mmol), and iodomethane (13 μL, 0.23 mmol). The residue was purified by flash chromatography (silica gel, heptanes/EtOAc 7:3) to give dibenzoxepine **7g** as a colorless oil (13 mg, 84%, ~9:1 mixture of interconverting atropisomers); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.59$ (d, $J = 8.7$ Hz, 1H), 7.01 (d, $J = 2.4$ Hz, 1H), 6.96 (dd, $J = 8.7$, 2.4 Hz, 1H), 6.75 (s, 1H), 4.36 (d, $J = 12.9$ Hz, 1H), 4.00–3.88 (m, 11H), 3.68 (s, 3H), 2.02 (dq, $J = 7.2$, 7.2 Hz, 2H), 0.98 ppm (t, $J = 7.2$ Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 159.2$, 152.9, 150.6, 142.7, 138.2, 131.3, 130.9, 130.0, 126.4, 112.9, 111.2, 108.5, 74.7, 68.2, 61.3, 61.0, 56.2, 55.5, 25.0, 11.2 ppm; HRMS (ESI) calcd for $C_{20}H_{24}O_5Na$ [M+Na$^+$]: 367.1521; found: 367.1539.

Dibenzoxepine ***7h***: Compound **7h** was obtained in the same manner as above for **7e**, starting from **7d** (16 mg, 0.047 mmol), $NaN(SiMe_3)_2$ (117 μL, 0.24 mmol), and iodomethane (15 μL, 0.24 mmol). The residue was purified by preparative TLC (silica gel, heptanes/EtOAc 3:2) to give dibenzoxepine **7g** as a white solid (17 mg, 100%, ~85:15 mixture of interconverting atropisomers); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.59$ (d, $J = 8.4$ Hz, 1H), 7.02 (d, $J =$ 2.7 Hz, 1H), 6.96 (dd, $J = 8.4$, 2.7 Hz, 1H), 6.74 (s, 1H), 4.37 (d, $J =$ 11.4 Hz, 1H), 4.07 (t, $J = 6.9$ Hz, 1H), 3.95 (d, $J = 11.4$ Hz, 1H), 3.95 (s, 3H), 3.92 (s, 3H), 3.88 (s, 3H), 3.67 (s, 3H), 2.03–1.90 (m, 2H), 1.57–1.25 (m, 2H), 0.92 ppm (t, $J = 7.2$ Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 159.2$, 152.9, 150.5, 142.7, 138.4, 131.2, 130.8, 129.9, 126.3, 112.9, 111.3, 108.5, 72.9, 68.1, 61.3, 60.9, 56.2, 55.5, 34.1, 19.8, 14.4 ppm; IR (neat): $\tilde{\nu} = 2955$, 2933, 1485, 1455 cm^{-1}; HRMS (ESI) calcd for $C_{21}H_{26}O_5Na$ [M+Na$^+$]: 383.1678; found: 383.1694.

Biological assays

Inhibition of microtubule assembly: The drug was dissolved in DMSO at various concentrations and pre-incubated with a solution of tubulin at 37 °C for 10 min. The solution was then cooled to 0 °C for 5 min to achieve complete tubulin depolymerization. The solution was placed in a temperature-controlled cell at 37 °C (microtubule assembly), and the increase in optical density was monitored in a UV spectrophotometer at 350 nm for 1 min. The maximum rate of assembly was recorded and compared with a sample without drug. The IC_{50} value is the concentration of compound required to inhibit 50% of the rate of microtubule assembly. It was calculated from the effect of several concentrations, and was compared with the IC_{50} value of colchicine obtained within the same day with the same tubulin preparation. The reported values are averages of three experiments.

Cell culture: Cancer cell lines were obtained from the American Type Culture Collection (Rockville, MD, USA) and were cultured according to the supplier's instructions. Briefly, A549 human lung carcinoma, U87 human glioblastoma, MDA-MB231 and MDA-MB435 human breast carcinomas, and mouse B16F10 melanoma cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing glucose (4.5 g L^{-1}) supplemented with fetal calf serum (FCS, 10%) and glutamine (1%). Human HCT116 colorectal carcinoma cells were grown in RPMI 1640 containing 10% FCS and 1% glutamine. Human umbilical vein endothelial cells (HUVECs) were obtained from Clonetics (Lonza, Walkersville, MD, USA) and cultured according to the supplier's instructions. Briefly, HUVECs from three to six passages were subcultured to confluence onto 0.2% gelatin-coated tissue culture flasks in endothelial cell growth medium (EGM2) containing growth factors and 2% FCS.

Proliferation assay: Cell viability was determined by using Promega CellTiter-Blue reagent according to the manufacturer's instructions. Briefly, cells were seeded in 96-well plates (5×10^3 cells per well) containing 50 μL growth medium. After 24 h culture, the cells were supplemented with 50 μL of the studied compound dissolved in DMSO (< 0.1% in each preparation). After 72 h incubation, 20 μL resazurin was added for 2 h before recording fluorescence ($\lambda_{ex} =$ 560 nm, $\lambda_{em} = 590$ nm) using a Victor microtiter plate fluorimeter (PerkinElmer, USA). Alternatively, HUVECs were incubated with the studied compounds for 3 h, washed with medium, and incubated for an additional 3 days. The IC_{50} value corresponds to the concentration of the studied compound that caused a 50% decrease in fluorescence of drug-treated cells relative to untreated cells. Experiments were performed in triplicate.

Analysis of cell morphology: 96-well plates were coated with 1% gelatin (Sigma, St-Quentin Fallavier, France) for 2 h at 37 °C. After washing in phosphate-buffered saline (PBS), nonspecific sites were blocked with 1% bovine serum albumin (BSA) in PBS (30 min at 37 °C). HUVECs (2.5×10^4 cells per well) in DMEM + 0.1% BSA were added, and allowed to adhere for 4 h at 37 °C. Adherent cells were then exposed to the studied compounds for 40 min (three wells for each condition). Following washing with DMEM + 0.1% BSA to remove detached cells, the cell layers were stained with crystal violet (0.5% in 20% EtOH), rinsed with H_2O, and air-dried. Cells were analyzed by inverted light microscope (TE 2000E, Nikon, Champigny-sur-Marne, France) and computer image analysis (NIS-Elements, Nikon, Champigny-sur-Marne, France). The degree of cell spreading was evaluated as the cultured cell area (area of the plate covered by the cells normalized to the number of adherent cells). To evaluate the number of adhered cells, the stain was eluted with a 1:1 solution of EtOH/0.1 M sodium citrate, and absorbance at 540 nm was read.

Cord disruption assay: HUVECs (2×10^4 cells per well) were plated in 96-well plates on a thick layer of Matrigel (Becton Dickinson; 10 mg mL^{-1}, 60 μL per well) and allowed to align for 24 h. Colchicine, NAC, **7e**, **7g**, or vehicle were added to the formed cords and

left for 3 h. Images were taken 3 h after the addition of compounds.

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Keywords: biaryl compounds · cross-coupling · endothelial cells · tubulin · vascular-disrupting agents

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