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Synthesis of antimicrotubule dibenzoxepines

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ABSTRACT

New dibenzoxepines **5a–i** bearing various substituents on B- and C-rings were synthesized in a straightforward manner using a Suzuki–Miyaura coupling, a Grignard addition, and a cyclodehydration as key steps. The antimicrotubule activity of all analogues was evaluated and compared to reference compounds. Compounds **5d–f** displayed the highest activity for this type of allocolchicinoids to date.

Vascular-disrupting agents (VDAs) are promising anticancer molecules which act by damaging existing tumor vasculature.[1] Among VDAs, compounds that bind to tubulin at the colchicine or vinblastin site and cause microtubule depolymerisation showed promising in vitro and in vivo activities.[2] In particular, two series of prodrugs of molecules that bind to the colchicine (**1**) site have undergone clinical trials (Fig. 1): prodrugs of the natural products combretastatin A-1 and A-4 (**2a–c**) and the allocolchicinoid ZD6126 (**3b**), a prodrug of *N*-acetylcolchinol (NAC, **3a**).[3]

Whereas the development of combretastatin analogues as VDAs has been the subject of extensive research in the past few years,[4] allocolchicinoid-type molecules have attracted much less attention. In this context, we recently reported the synthesis of analogues of NAC (**5**) having an oxepine medium B-ring, as well as their in vitro antimitotic and antivascular properties.[5] The most active analogues (R^1 = H or Et, R^2 = OMe, R^3 = H) showed activity profiles similar to that of NAC in various in vitro assays, but at higher doses.[5c] While the interaction of colchicine with tubulin has been characterized crystallographically,[6] the binding of allocolchicinoids such as NAC to tubulin still shows elements of uncertainty. In particular, it is known that the presence of the trimethoxybenzene A-ring is essential to this binding,[7] but the role of the acetamide and phenol substituents on B- and C-rings, respectively, is not clear. In addition, the comparison with the combretastatin series is not straightforward because of the limited three-dimensional overlap of both types of molecules. In order to shed light on this interaction and further optimize the antimitotic activity of our dibenzoxepine analogues, we decided to synthesize new compounds with more

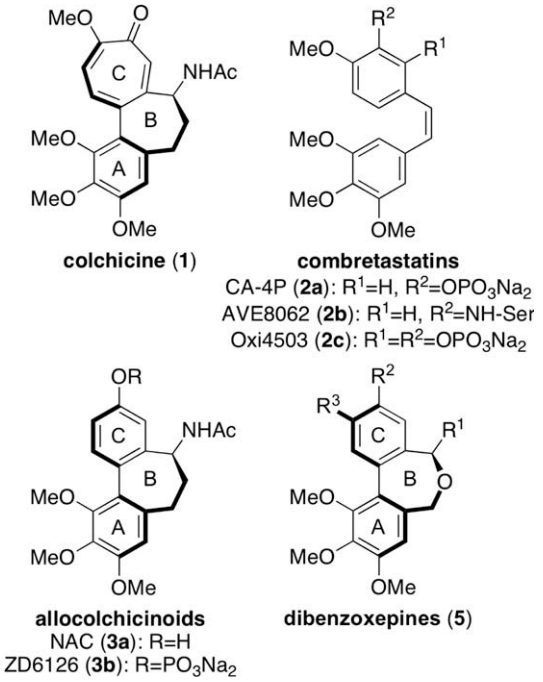

Figure 1.

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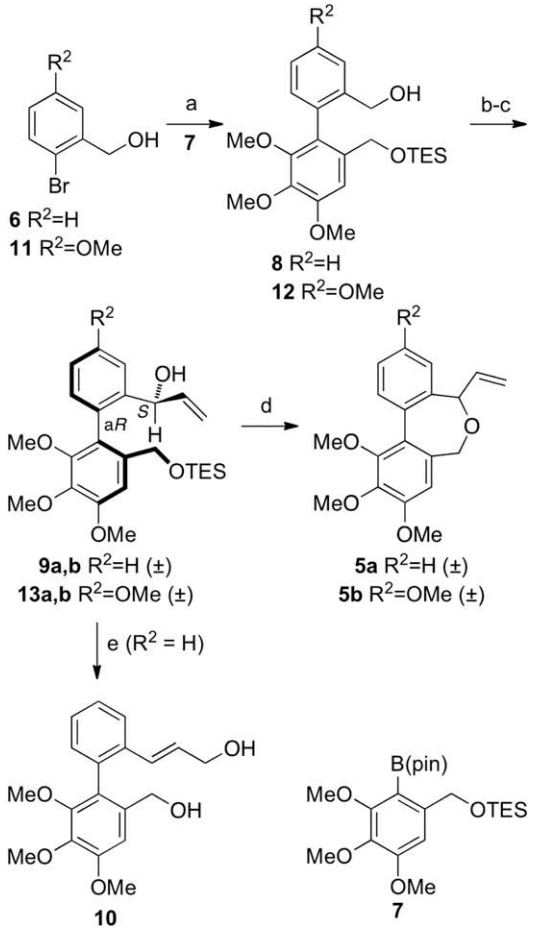
structurally varied B- and C-ring substituents. In a previous Letter, we reported a novel and straightforward synthetic strategy to access these dibenzoxepines.[8] Herein, we describe the extension of this strategy to the synthesis of analogues **5a**–**i** (R^2 = H, OMe, R^3 = H), and their preliminary evaluation as antimitotic agents.

The synthesis of racemic dibenzoxepine **5a** was first performed, both to adapt our previously described synthetic route and to investigate the effect of the R^2 substituent on the antimitotic activity (Scheme 1). Suzuki–Miyaura coupling of benzyl alcohol **6** with arylboronate **7**[9] under conditions previously optimized in our laboratory,[5] which employed $Pd(OAc)_2$/S-Phos[10] as the catalyst and barium hydroxide as the base, gave rise to biaryl **8** in moderate yield. The oxidation of the primary alcohol to the corresponding aldehyde was better performed using excess manganese oxide. The addition of vinylmagnesium bromide to this aldehyde gave rise to diastereomeric allylic alcohols **9a**,**b**, containing a stereogenic axis and a stereogenic center, in 75% yield and 97:3 ratio in favor of the (*S*,a*R*) diastereoisomer. The relative configuration of the major diastereoisomer was assigned by analogy with previous work.[8] The diastereoisomeric mixture **9a**,**b** was subjected to our Brønsted acid-mediated cyclodehydration protocol.[5] Interestingly, in the presence of aq HF in acetonitrile **9a**,**b** underwent cyclodehydration to give the desired dibenzoxepine **5a** in 57% yield, whereas in the presence of TFA in dichloromethane conjugated allylic alcohol **10**, arising from allylic rearrangement,[11] was formed selectively.

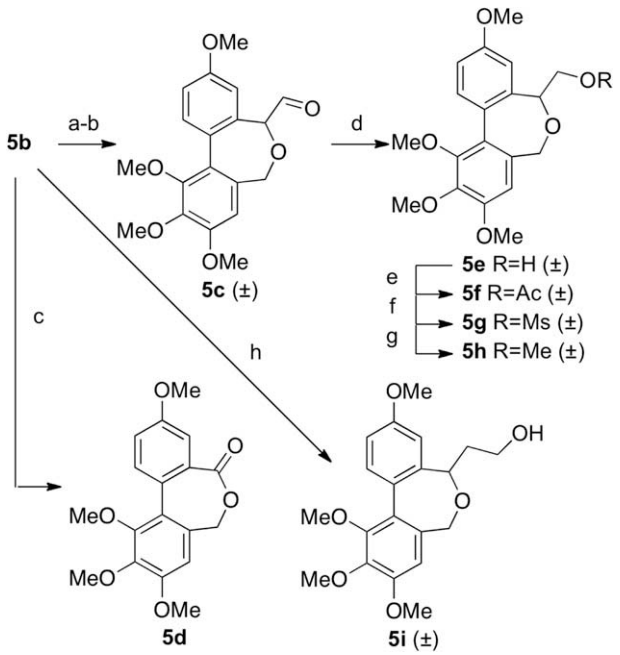


Scheme 1. Synthesis of dibenzoxepines **5a**–**b**. Reagents and conditions: (a) $Pd(OAc)_2$ (5 mol %), S-Phos (10 mol %), $Ba(OH)_2$ (1.1 equiv), dioxane/H_2O (9:1), 100 °C, 49% for **8**, 81% for **12**; (b) MnO_2 (12 equiv), CH_2Cl_2, 20 °C, 24 h; (c) $H_2C{=}CHMgBr$ (3 equiv), THF, −78 °C, 3 h, 75% for **9a**,**b** (dr 97:3), 81% for **13a**,**b** (dr 94:6); (d) 50% HF (aq)/CH_3CN (1:5), 20 °C, 48 h, 57% for **5a**, 95% for **5b**; (e) CF_3CO_2H/CH_2Cl_2 (1:2), −78 °C, 3.5 h, 67%.

This synthetic sequence was then applied to dibenzoxepine **5b** (Scheme 1). Of note, better yields were achieved for each step in this series compared to the previous series with R^2 = H, and thus compound **5b** was obtained in 62% overall yield from alcohol **11**.

Pivotal compound **5b** containing a functionalizable olefin was further elaborated to build a small library of analogues bearing various substituents on ring B (Scheme 2). A dihydroxylation was first performed under standard conditions to give the corresponding diol, which underwent oxidative cleavage in the presence of sodium periodate to give aldehyde **5c** (65% yield for two steps). The direct conversion of alkene **5b** to aldehyde **5c** using catalytic OsO_4 and excess $NaIO_4$ failed,[12] giving lactone **5d** as the only isolable product in 32% yield. Compound **5d** might originate from a complex sequence including olefin dihydroxylation, oxepine ring-opening, oxidative cleavage, lactol formation, and oxidation. Reduction of aldehyde **5c** with sodium borohydride delivered primary alcohol **5e** in high yield. Acetate **5f**, mesylate **5g**, and methyl ether **5h** were obtained by derivatization of **5e** under standard conditions. All attempts at reacting mesylate **5g** with various nitrogen nucleophiles failed. Finally, the oxidative hydroboration of **5b** furnished primary alcohol **5i** as the major regioisomer in 66% yield.

The antimicrotubule activity of new dibenzoxepines **5a**–**i** was next evaluated, using colchicine (**1**) and *N*-acetylcolchinol (NAC, **3a**) as references (Fig. 1, Table 1). The activity of the most active dibenzoxepine **5j** from previous studies was also printed for comparison (entry 3). 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.[5] NAC was found to be 1.7 more active than colchicine in these assays (entry 2). Dibenzoxepine **5a** deprived of substituent on ring C was found to be inactive (entry 4). In contrast, compounds **5b**–**i** bearing a methoxy substituent on ring C showed



Scheme 2. Synthesis of dibenzoxepines **5c**–**i**. Reagents and conditions: (a) OsO_4 (10 mol %), NMO (1.1 equiv), acetone/H_2O (1:1), 20 °C, 5.5 h; (b) $NaIO_4$ (1.0 equiv), MeOH/H_2O (3:1), 20 °C, 1 h, 65% for two steps; (c) OsO_4 (2 mol %), $NaIO_4$ (4 equiv), 2,6-lutidine (2 equiv), dioxane/H_2O (3:1), 20 °C, 24 h, 32%; (d) $NaBH_4$ (2 equiv), MeOH, 0 °C, 9 h, 96%; (e) Et_3N (1.2 equiv), AcCl (1.1 equiv), CH_3CN, 0→20 °C, 14 h, 79%; (f) Et_3N (2 equiv), CH_3SO_2Cl (1.5 equiv), CH_2Cl_2, 0→20 °C, 14 h, 81%; (g) NaHMDS (7 equiv), MeI (7 equiv), THF, 20 °C, 24 h, 88%; (h) $BH_3{\cdot}SMe_2$ (1.5 equiv), THF, 0→20 °C, 2 h, then 3 N aq NaOH (1.5 equiv), 35% aq H_2O_2 (1.5 equiv), 20 °C, 3 h, 66%.

Table 1
Antimicrotubule activity and cytotoxicity of dibenzoxepines **5a–i**

Entry	Compd	R^1	R^2	Inhibition of microtubule assembly[a,c]	Cytotoxicity[b,c] IC_{50} (μM)			
				IC_{50} (compd)/IC_{50} (**1**)	HCT116	K562	MDA	HUVEC
1	**1** (colchicine)			1	0.02	0.009	0.04	0.02
2	**3a** (NAC)			0.6	0.04	0.05	0.10	0.07
3	**5j**[d]	Et	OMe	0.5	0.7		2.8	0.4
4	**5a**	CH=CH_2	H	In				
5	**5b**	CH=CH_2	OMe	1.9	0.35	0.3	0.8	0.4
6	**5d**	=O	OMe	1.0	0.07	0.2	0.4	0.25
7	**5e**	CH_2OH	OMe	0.7	0.2	0.3	0.45	0.25
8	**5f**	CH_2OAc	OMe	0.5	0.2	0.25	0.35	0.3
9	**5g**	CH_2OMs	OMe	2.7	0.7	0.3	0.8	0.45
10	**5h**	CH_2OMe	OMe	In				
11	**5i**	$(CH_2)_2OH$	OMe	1.7				

[a] IC_{50} (compd) is the concentration of compound required to inhibit 50% of the rate of microtubule assembly, average of three experiments; IC_{50} (**1**) = 7.3 μM.
[b] IC_{50} is the concentration of compound corresponding to 50% growth inhibition after 72 h incubation, average of three experiments; cell lines: HCT116 = human colon carcinoma, K562 = human myelogenous leukemia, MDA-MB231 = human breast cancer, HUVEC = human umbilical vein endothelial cells.
[c] In = inactive (or IC_{50} not measurable).
[d] See Ref. 5c.

a pronounced antimicrotubule activity (entries 5–11) except for compound **5h** (entry 10), in agreement with previous results on other methoxy-substituted analogues such as **5j** (entry 3). In particular, compounds **5e** (entry 7) and **5f** (entry 8) bearing a hydroxymethyl and an acetoxymethyl B-ring substituent, respectively, were found to be approximately as active as NAC **3a** and dibenzoxepine **5j** (entries 2 and 3), and twice more active than colchicine **1** (entry 1).

The in vitro antiproliferative activity of dibenzoxepines **5b–g**, which showed the best antimicrotubule activities, was next evaluated on a panel of cancer cell lines and on HUVEC endothelial cells, as a prerequisite for future evaluation of vascular-disrupting properties (entries 5–9).[5c] Colchicine **1** and NAC **3a** were again tested as references (entries 1 and 2). Compounds **5b–g** were found to be cytotoxic with average IC_{50} values in the 0.1–0.8 μM range. A more pronounced cytotoxicity was found for compounds **5d–f** (entries 6–8), which are also the most potent antimicrotubule analogues. These three analogues were also significantly less cytotoxic than colchicine and NAC on this panel of cell lines. Compounds **5d–f** are the most cytotoxic allocolchicinoid-type molecules synthesized to date in our group,[5] with a cytotoxicity being significantly higher than that of dibenzoxepine **5j** (entry 3) on the same cell lines. This suggests that a polar group on this position of ring B has a positive influence on the antimicrotubule and antiproliferative properties. Further in vitro assays are underway to assess the potential activity of these new analogues as vascular-disrupting agents.

In conclusion, we have synthesized a new series of dibenzoxepines **5a–i** with various substituents on ring B. A comparative evaluation of the antimicrotubule and antiproliferative properties of these and previously synthesized compounds showed that new analogues having either a lactone B-ring (compound **5d**) or bearing a polar substituent (CH_2OH, CH_2OAc) on the oxepine B-ring (compounds **5e–f**) displayed the highest activity for this type of allocolchicinoids to date. Further biological evaluation is underway to assess the potential activity of these new analogues as vascular-disrupting agents.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.04.039.

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