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Synthesis and Cytotoxic Evaluation of Constrained Analogues of Combretastatin and Combretastatin A4.

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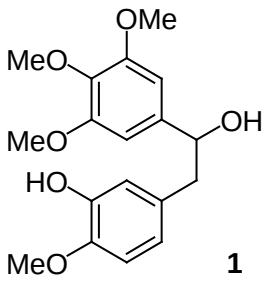
Abstract: *A series of cis-locked stilbenic compounds related to combretastatin and combretastatin A4 has been designed and synthesized. The cytotoxic effects of these rigid analogues were evaluated as well as their abilities to inhibit tubulin polymerization.*

Keywords: *combretastatins, isoindolinones, oxygen heterocycle, oxidation, anticancer agents.*

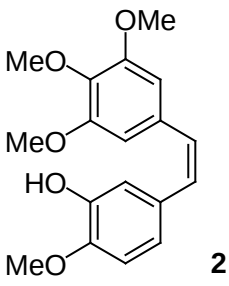
Introduction

The combretastatins represent a group of structurally simple antimitotic agents known as potent inhibitors of tubulin assembly [1]. Owing to their high affinity to the colchicine site [2] the most active of these, combretastatin (**1**) and combretastatin A4 (CA4) (**2**) (Figure 1), act as potent inhibitors of cancer cell growth inducing irreversible vascular shutdown within solid tumors while sparing normal vasculature [3].



1

Combretastatin



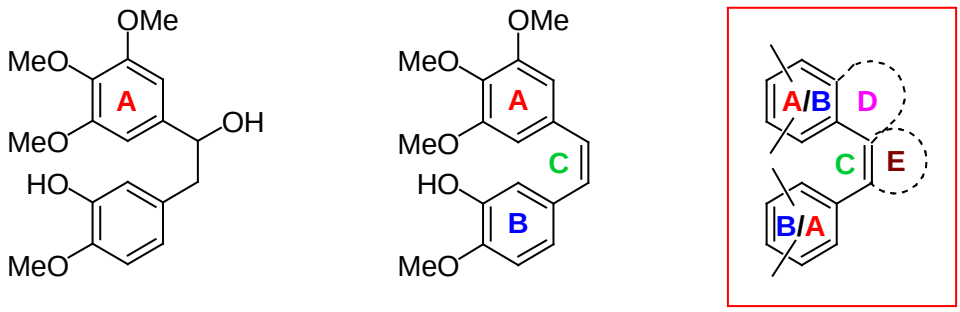
2

Combretastatin A4

Figure 1.

The more available prodrug disodium phosphate analogue of **2** is now in Phase III clinical trials [4]. Consequently significant efforts have been stimulated to develop combretastatin-type analogues and the *Z*-stilbenoid molecular scaffold has provided a simple structural template for the design of related compounds that retain the biological action of the parent molecules but provide improved pharmacokinetic properties [5-7].

The literature demonstrates the impressive possibilities for structural variability in analogues of this class of compounds and structure **2** has been modified in each of the three elements, i.e. aromatic rings A and B and double bond C (Figure 2) [5,7a]. Owing to the fact that the 3,4,5-trimethoxy substitution on the A ring, the cis orientation between the two aryl rings and the free hydroxyl functionality at ring B are essential requirements for efficient binding to tubulin and high levels of cytotoxicity [7,8], structural variations have been notably devoted to the synthesis and biological evaluation of derivatives with carbo and heterocyclic moieties fused with one of the aromatic units (Figure 2, D) [5-7] or in place of the olefinic bridge (Figure 2, E) [9].



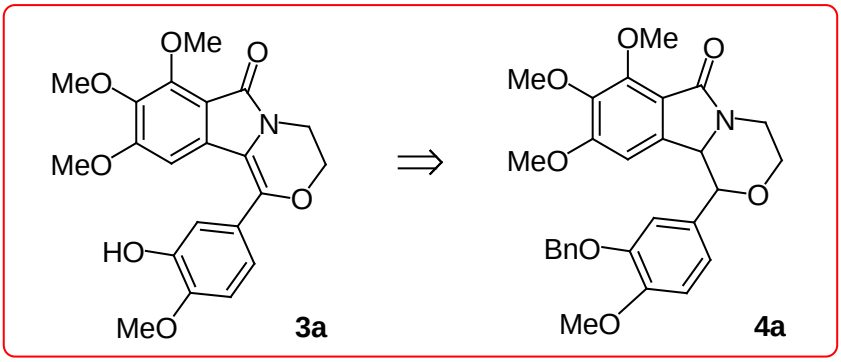
Combretastatin (**1**)

Combretastatin A4 (CA4, **2**)

Figure 2.

In this regard a group of five-membered benzolactamic compounds has been designed and reported with potent growth inhibitory activities on hormone-independent prostate and breast cancer lines with IC_{50} values in low to subnanomolar range [10]. However these SAR studies have been confined to 3-benzylidene indolin-2-one derivatives (Figure 2, AD = oxindole) whereas the corresponding 3-benzylidene isoindolinones (Figure 2, AD = isoxindole) have been ignored by the scientific community.

In this letter we have targeted the synthesis of configurationally *cis*-locked CA4 analogues **3a** and **3b** (Retrosynthetic Scheme 1) which are characterized by the presence of a bridging olefinic moiety embedded in a morpholine unit. Elaboration of these targeted compounds offered a double advantage since we surmised that such compounds could be accessed from the corresponding saturated compounds **4a, 4b** respectively, which possess the main structural feature of the inhibitor of cancer cell proliferation combretastatin **1** [11].



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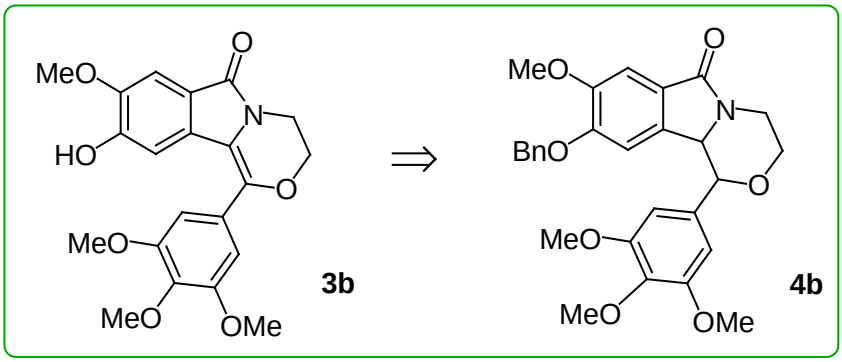


Retrosynthetic Scheme 1.

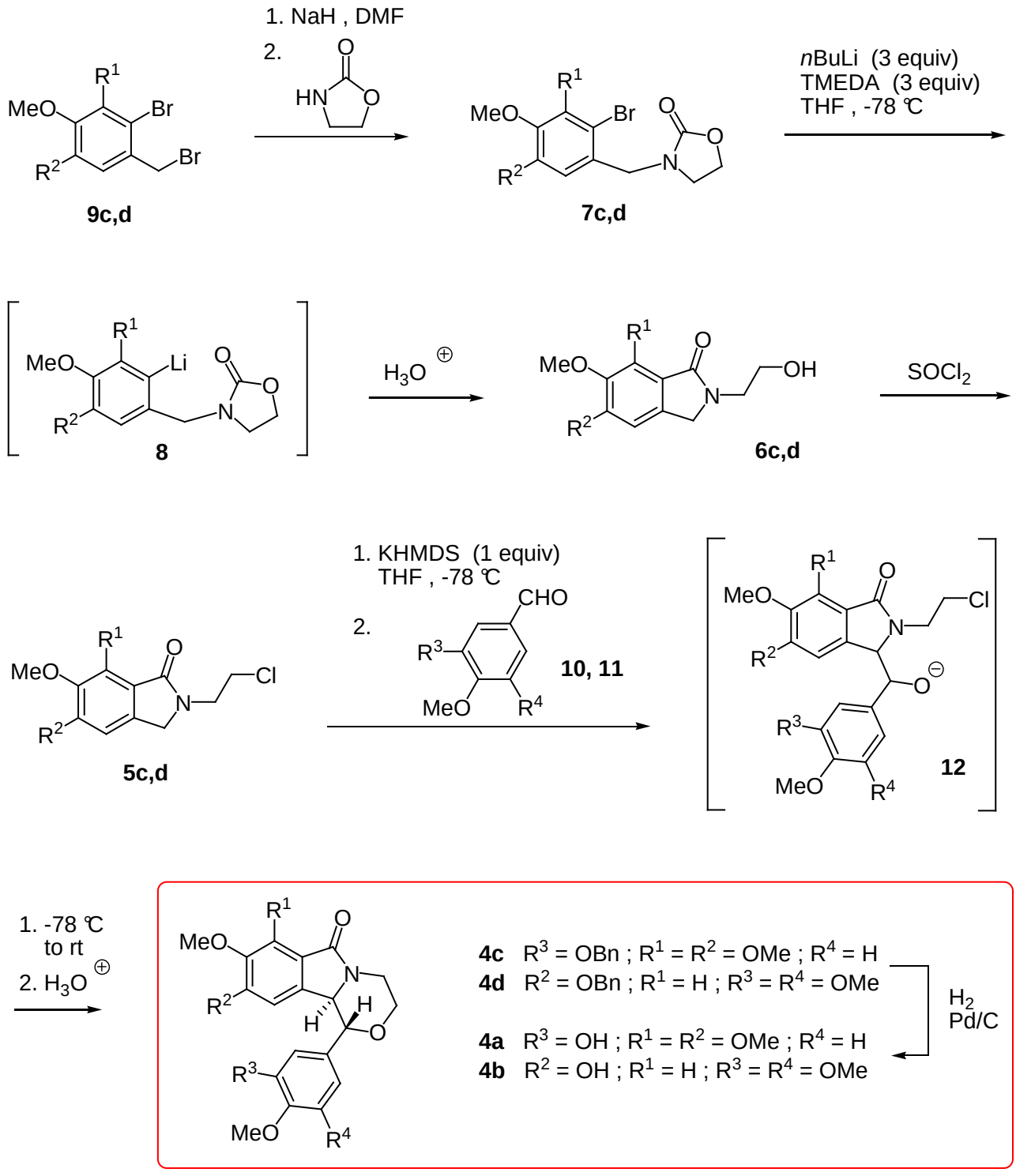
Results and Discussion

1. Synthesis of constrained analogues of combretastatin 1

The first facet of the synthesis which is depicted in Scheme 2 was the elaboration of the *N*-functionalized models **6c,d**. These compounds were readily assembled by an anionic cyclization process applied to the *N*-bromobenzylated oxazolidinones **7c,d** [12]. Interception of the aryllithiated species **8** by the cyclic carbamate acting as the internal electrophile provided a direct access to a properly substituted isoindolinone with the concomitant installation of the mandatory hydroxyethyl appendage (Table 1). The assemblage of the highly fused models **4c,d** could be performed as a single one-pot reaction from the corresponding *N*-chloroethylisoindolinones **5c,d** (Scheme 2, Table 1). Deprotonation of **5c,d** with KHMDS followed by addition of appropriate aldehydes **10, 11** and subsequent warming to trigger an intramolecular *O*-alkylation process, that is cyclization of transient oxanion **12**, afforded quite satisfactory yields of annulated compounds **4c,d** (Table 1). At last treatment of **4c,d** with Pd/C under H_2 atmosphere under mild conditions triggered off the cleavage of the benzyl protecting group and delivered excellent yields of the desired compounds **4a,b** which can be regarded as constrained analogues of combretastatin **1**.

Table 1. Compounds **6c,d, 5c,d, 4a-d,** Produced *via* Scheme 2.

	R^1	R^2	R^3	R^4	Mp (Yield %)		
					6	**5**	**4**
a	OMe	OMe	OH	H	-	-	194-196 ℃ (87)
b	H	OH	OMe	OMe	-	-	227-229 ℃ (65)
c	OMe	OMe	OBn	H	146-148 ℃ (54)	113-115 ℃ (96)	165-1 67 ℃ (91)
d	H	OBn	OMe	OMe	175-177 ℃ (52)	159-161 ℃ (97)	210-2 12 ℃ (56)

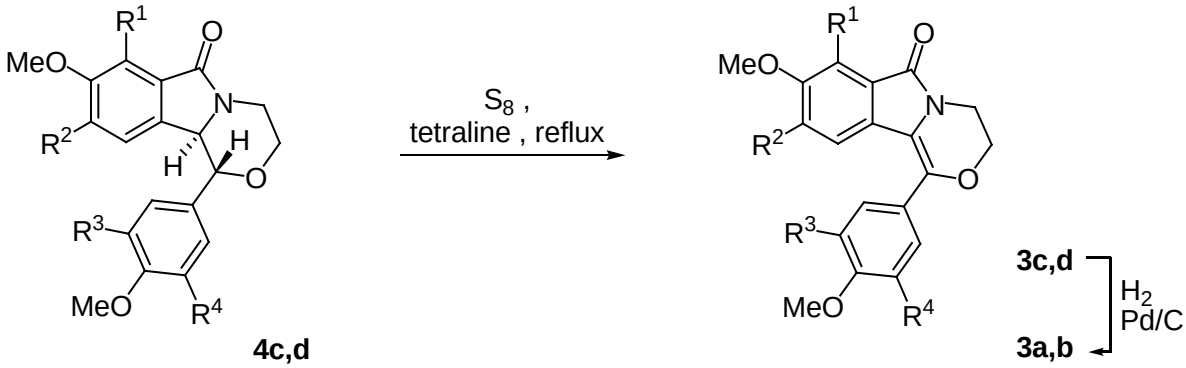


Scheme 2.

2. Synthesis of constrained analogues of combretastatin A4 (2)

The synthesis of the strict structural analogues **3a,b** of combretastatin A4 (**2**) from the corresponding monoprotected and saturated compounds **4c,d** respectively revealed much more problematic than we had anticipated. Several attempts to ensure the creation of the central double bond including treatment with oxone, DDQ, Pd/C in refluxing nitrotoluene, NBS then AIBN, a multistep sequence involving metallation/capture with

PhSeCl/oxidation/elimination, and oxidation with neat Se failed, probably due to the *trans* configuration of benzylic hydrogen atoms in **4c,d** [13]. At last we found that treatment of the saturated compounds **4c,d** with sulfur in refluxing tetraline for a short period delivered satisfactory yields of the monoprotected oxidized compounds **3c,d** (Scheme 3, Table 2). Regeneration of the hydroxylphenolic function from **3c,d** afforded excellent yields of the *Z*-configured stilbenic compounds **3a,b** strict analogues of combretastatin A4 (**2**) (Table 2).



4c,d

Scheme 3.

Table 2. Compounds **3a-d,** Produced *via* Scheme 3.

R^1	R^2	R^3	R^4	**4**	**3**	Mp	(Yield)
OMe	OMe	OH	H		**3a**	194-196 ℃	(87%)
H	OH	OMe	OMe		**3b**	227-229 ℃	(65%)
OMe	OMe	OBn	H	**4c**	**3c**	165-167 ℃	(91%)
H	OBn	OMe	OMe	**4d**	**3d**	210-212 ℃	(56%)

3. Biological evaluation

Table 3 displays the cytotoxicity on human KB cells and the antitubulin activity values of the various analogues synthesized. Despite their structural similarity with combretastatin A4, compounds (**3b**, **4a,b**) were devoid of any appreciable cytotoxic activity. Rather disappointingly, only compound **3a** featuring an isoxindole fused with a trimethoxyaryl ring and incorporating a *cis*-locked alkene seemed to gather the structural requirements for an improved cytotoxicity (IC_{50} = 0.16 μm). This is probably a consequence of the ability for this compound to inhibit tubulin polymerization.

Table 3. Cytotoxicity on KB cells[a] and antitubulin activity for stilbenoid analogs **3a,b**, **4a,b.**[b]

Compound	Cytotoxicity IC_{50} [μm][c]	% ITP[d]
Combretastatin A4	0.003	100
3a	0.16	23
3b	>100	-
4a	>100	30
4b	>100	-

[a]KB = cervical carcinoma cells. [b]Concentration 10^{-5} M in DMSO. [c]IC_{50} is the concentration of compound inducing 50% cell growth after 72h incubation. [d]Inhibition of tubulin polymerization of the tested compound compared to that of CA4 (*i e* 100%)

Conclusion

We have developed a new high-yielding synthetic route to highly fused combretastatin derivatives incorporating a five-membered lactamic unit and a morpholine moiety and we were also able to prepare the reversed analogues of *cis* CA4 from appropriately substituted isoindolinones.

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