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Synthesis and cytotoxic evaluation of *cis*-locked and constrained analogues of combretastatin and combretastatin A4

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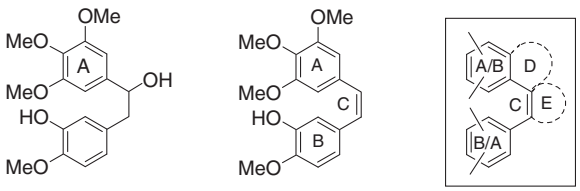
ABSTRACT

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.

1. Introduction

The combretastatins represent a group of structurally simple antimitotic agents known as potent inhibitors of tubulin assembly,[1] a research field that received a major impetus when Pettit and co-workers isolated (−)-combretastatin **1** (Fig. 1) from the bark of the South African bush willow tree *Combretum caffrum*.[2] Owing to its high affinity to the colchicine site[3] the most active of these, combretastatin A4 (CA4) (**2**) acts as a potent inhibitor of cancer cell growth inducing irreversible vascular shutdown within solid tumors while sparing normal vasculature.[4] Its more available prodrug disodium phosphate analogue is now in Phase III clinical trials.[5] Consequently significant efforts have been expended 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.[6,7] Structure–activity relationship studies (SAR) then led to the discovery of an array of biologically active analogues that were assessed as potential anticancer agents, some of which have recently been reviewed.[6,8] 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, that is, aromatic rings A and B and double bond C (Fig. 1).[6,8a] 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[16,9] 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 (Fig. 1D)[6,8] or in place of the olefinic bridge (Fig. 1E).[10]

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.[11] However, these SAR studies have been confined to 3-benzylidene



Combretastatin (**1**) Combretastatin A4 (CA4, **2**)

Figure 1. Combretastatins.

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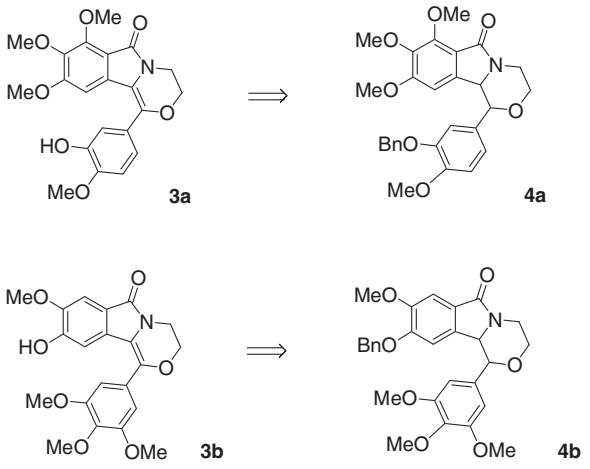
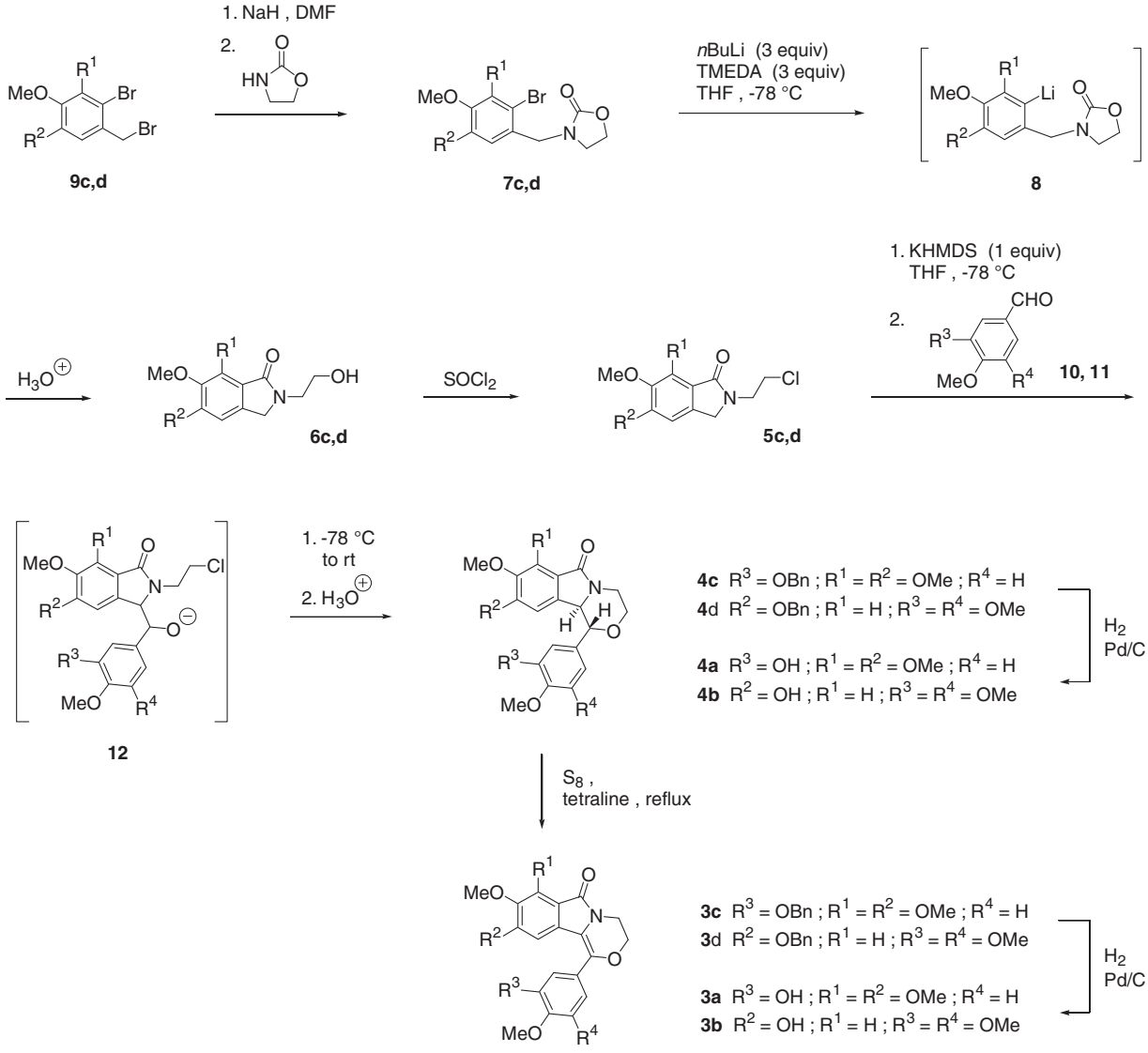


Scheme 1. Constrained analogues of combretastatin and combretastatin A4.

indolin-2-one derivatives (Fig. 1, AD = oxindole) and to the best of our knowledge the corresponding 3-benzylidene-isoindolinones (Fig. 1, AD = isoxindole) have been ignored by the scientific community. This is probably attributable to the difficulties associated with the assembly of *Z*-configured stilbene compounds which are prone to double bond isomerization during storage, leading to the *E*-isomers which display a dramatically reduced biological activity.[12]

In this Letter we report our exploration of the synthesis and biological evaluation of a number of combretastatin-like benzylideneisoindolinones and our attention was focused on a structural modification that maintained the *cis*-diaryl relationship of **2** (CA4). In this view we targeted the synthesis of configurationally *cis*-locked CA4 analogues **3a** and **3b** (Scheme 1) which are characterized by the presence of a bridging olefinic moiety embedded in a morpholine unit. It has been well established that the presence of heteroatoms in a cyclic moiety around the bridge helps in retaining the bioactive configuration of the molecule and leads to a broader variety of SAR studies.[8a] Furthermore the 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**.[13]



Scheme 2. Synthesis of combretastatin analogues **3a**,**b** and **4a**,**b**.

Table 1
Compounds **6c**,**d**, **5c**,**d**, **4a**–**d**, **3a**–**d**, produced via Scheme 2

	R^1	R^2	R^3	R^4	Mp (yield)			
					6	**5**	**4**	**3**
a	OMe	OMe	OH	H	—	—	194–196 °C (87%)	170–172 °C (69%)
b	H	OH	OMe	OMe	—	—	227–229 °C (65%)	221–223 °C (55%)
c	OMe	OMe	OBn	H	146–148 °C (54%)	113–115 °C (96%)	165–167 °C (91%)	152–154 °C (54%)
d	H	OBn	OMe	OMe	175–177 °C (52%)	159–161 °C (97%)	210–212 °C (56%)	198–200 °C (52%)

2. Results/discussion

The key step for the assembly of the targeted compounds **3a**,**b** was the preliminary elaboration of the suitably substituted *N*-chloroethylisoindolinones **5c**,**d** (Scheme 2). We surmised that these compounds would be readily accessible from the corresponding alcohols **6c**,**d** which could be in turn conceivably assembled by an anionic cyclization process applied to the *N*-bromobenzyl substituted oxazolidinones **7c**,**d**.[14] Indeed we conjectured that the interception of the aryllithiated species **8** derived from **7c**,**d** by the oxazolidinone ring system, that is, a cyclic carbamate acting as the internal electrophile would provide the potential for a direct access to a properly substituted isoindolinone with the concomitant installation of the mandatory hydroxyethyl appendage, therefore providing the N-functionalized models **6c**,**d**.

Initially the cyclic carbamate precursors **7c**,**d** were readily assembled by N-alkylation of 2-oxazolidinone with the suitably substituted *ortho*-bromobenzylbromide derivatives **9c**,**d** obtained by the standard chemical manipulation of the corresponding benzyl alcohols. Optimal conditions for the key ring-opening/ring -closing reaction had to be determined and we found that adding the parent compound (**7c**,**d**, 1 equiv) in THF to *n*BuLi (3 equiv) and TMEDA (3 equiv) in degassed THF at −78 °C for 30 min led to complete consumption of the starting material and isolation solely of the targeted hydroxyethyl chain tethered isoindolinones **6c**,**d** in fairly good yields (Scheme 1, Table 1). Conversion of alcohols **6c**,**d** into the key intermediates **5c**,**d** by treatment with thionyl chloride proceeded uneventfully to deliver very satisfactory yields of the requisite *N*-chloroethylisoindolinones **5c**,**d** (Table 1). The assembling of the highly fused models **4c**,**d** could be fortunately performed as a single one-pot reaction. For this purpose, compounds **5c**,**d** were smoothly deprotonated with KHMDS in THF at −78 °C and subsequently allowed to react with the appropriate aldehydes **10**, **11**. To trigger off the intramolecular O-alkylation process the reaction mixture containing the transient oxanion **12** was warmed to rt followed by standard aqueous workup. Gratifyingly conducting this reaction according to this procedure brought about the intramolecular cyclization and afforded straightforwardly and solely the annulated compounds **4c**,**d**. The isolation of a single crystal of **4c** gave access to its X-ray analysis (Fig. 2). The ORTEP view clearly established the *trans* configuration of the hydrogen atoms at C-2 and C-19 with a dihedral angle of 176.08°.[15]

With these highly fused compounds in hand we were only one deprotection away from the targeted compounds **4a**,**b** which can be regarded as constrained analogues of combrestatin **1**. Thus the 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**. The synthesis of the strict structural analogues **3a**,**b** of combretastatin A4 (**2**) from the corresponding mono-protected and saturated compounds **4c**,**d**, respectively, revealed itself to be 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. One can reasonably assume that this elimination reaction is fraught with difficulties associated with the *trans* configuration of benzylic

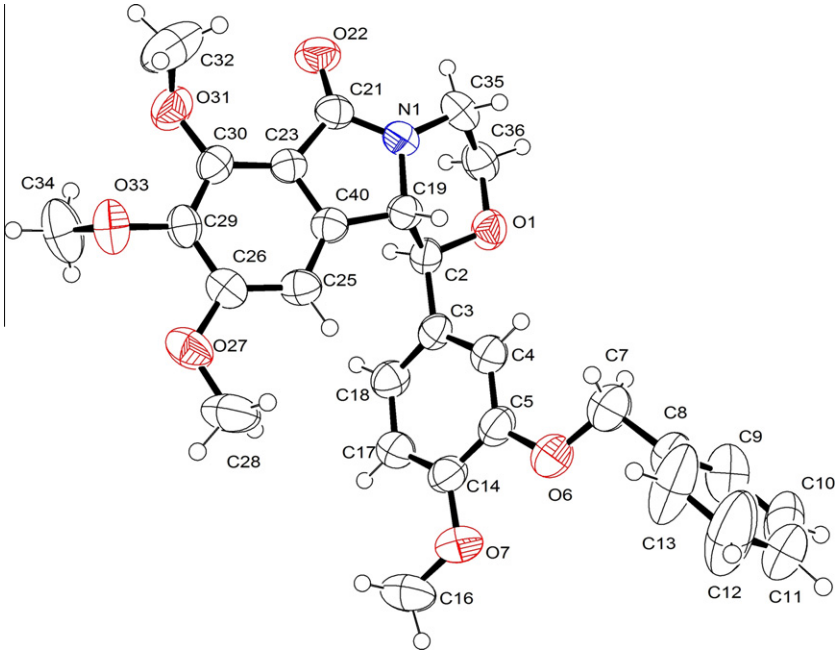


Figure 2. ORTEP view of the crystal structure of **4c**.

Table 2
Cytotoxicity on KB cells[a] and antitubulin activity for stilbenoid analogues **3a**,**b**, **4a**,**b**[b]

Compound	Cytotoxicity IC_{50}[c] [μM]	% 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 72 h incubation.
[d] Inhibition of tubulin polymerization of the tested compound compared to that of CA4 (i.e., 100%).

hydrogen atoms of these rather congested models. At last we found that this rather simple chemical transformation could be readily secured by treatment of the saturated compounds **4c**,**d** with sulfur in refluxing tetraline for a short period (5 min). This operation delivered quite satisfactory yields of the mono-protected oxidized compounds **3c**,**d** (Scheme 1, Table 1). Regeneration of the hydroxyl phenolic function from **3c**,**d** afforded excellent yields of the required *Z*-configured stilbenic derivatives **3a**,**b**.

Table 2 displays the cytotoxicity on human KB cells and the antitubulin activity values of the various analogues synthesized.[16,17] Despite their structural similarity with combretastatin A4, we were surprised to notice that three of them (**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 of this compound to inhibit tubulin polymerization.

3. Conclusion

We 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 environmentally diverse analogues of *cis* CA4 from appropriately substituted isoindolinones.

Acknowledgments

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Supplementary data

Supplementary data (copies of ^{1}H and ^{13}C NMR spectra for tested compounds) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.07.120.

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15. *X-ray data for* **4c**: $C_{28}H_{29}NO_7$, M = 491.52, $F(000)$ = 1040, colorless crystal, D_x = 1.263 g.cm^{-3}, $\mu(MoK_\alpha)$ = 0.091 cm^{-1} orthorhombic, *Pna2*, Z = 4, a = 11.0823 (5), b = 27.2073 (15), c = 8.5764 (5) Å, α = 90.00°, β = 90.00°, γ = 90.00°, V = 2586.0(2) Å^3. Further details of the X-ray structure data are available on request from the Cambridge Crystallographic Data Centre (deposition number CCDC 776601).
16. *Experimental protocol*: KB (human epidermoid carcinoma) cells were grown in Dulbecco's modified Eagle's medium supplemented with 25 mM glucose, 10% (v/v) fetal calf serum, 100 UI penicillin, 100 μg/ml streptomycin and 1.5 μg/ml fungizone and were kept under 5% CO_2 at 37 °C. 96-well plates were seeded with 500 KB cells per well in 200 μl medium. Twenty four hours later, chemicals dissolved in DMSO were added for 72 h at a final concentration (10^{-5} M) in a fixed volume of DMSO (1% final concentration). Controls received an equal volume of DMSO. The number of viable cells measured at 490 nm with the MTS reagent (Promega, Madison, WI) and IC_{50} was calculated as the concentration of compound eliciting a 50% inhibition of cell proliferation.
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