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Synthetic approach to enyne and enediyne analogues of anticancer agents

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Abstract—The synthesis and the anticancer activity of a new kind of enynes **2** and **3** and enediynes **4**, analogues of Combretastatin A-4 **1** are reported and discussed.

Combretastatin A-4 (CA-4) **1**, a natural stilbene, has been extracted from the South African willow *Combretum caffrum* in 1987[1] and its synthesis has been published in 1995.[2] This substance, likeness to colchicine, has been found to be a cytotoxic agent which strongly inhibits the tubulin polymerization by binding to the colchicine site[2,3] and a pro-drug of CA-4, the water-soluble phosphate derivative CA-4P is now in phase II clinical trials. The structural simplicity of CA-4 combined with excellent antitumoural and antineoangiogenic activities encouraged the scientific community to synthesize numerous analogues. Among various studies on the structure activity relationship of CA-4, most of them concern the modification in aromatic rings and in linker alkene.[4] To our knowledge, very few studies concerning the elongation of the linker between the two aryl groups have been already published.

Hamel and co-workers[5] prepared a series of CA-4 analogues which differed only in the number of methylene units (ranging from none to four) separating the aryl moieties. With the exception of the biphenyl compound, all the synthetic combretastatin analogues had activity as inhibitors of tubulin polymerization and for example, the four bridge analogue was four times less effective than CA-4. Since it has been also well established that the cis-orientation of the two aryl rings in CA-4 is important for biological activity,[6] we were interested to investigate the structural changes of the linker having four or six carbon units in which the spacer constrains the two aryl rings in quasi 'cis' orientation that appears to be necessary.

In order to preserve the (*Z*) stereochemistry of the stilbene double bond and to maintain the π-conjugated system, we have prepared a series of CA-4 analogues incorporating between the double bond and the aromatic rings one or two triple bonds. These structural changes of the linker length would contribute to better understanding the structure–activity relationships associated with the presence of triple bonds in derivatives **2**–**4**. In this letter, we report the stereocontrolled synthesis of previously unknown trimethoxy substituted stilbene enyne **2** and **3** as well as enediyne **4** (Scheme 1) CA-4 analogues and comment on their biological activity to act as potential antimitotic agents and their ability to inhibit cancer cellular growth.

A straightforward approach to enynes and enediynes **2**–**4** could be the palladium-mediated coupling reactions from readily available *Z*-chloroenynes, an interesting class of compounds,[7] which are not photosensitive and more stable than the corresponding iodide and bromide derivatives. Previously,[8] we showed that the carbon–chlorine in these compounds is not inert to further coupling reactions and under appropriate transition metal

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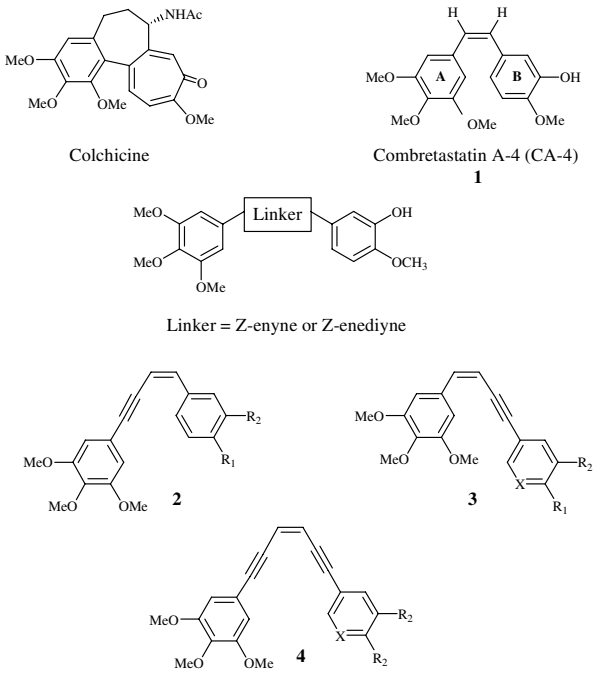
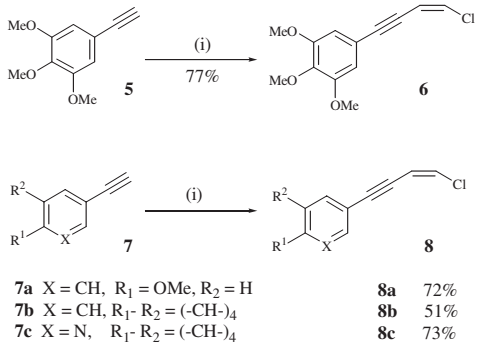


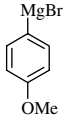
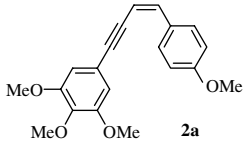
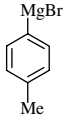
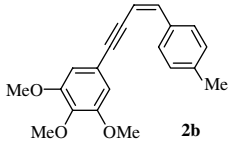
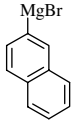
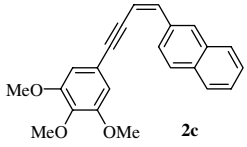
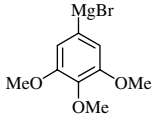
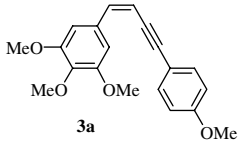
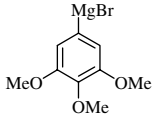
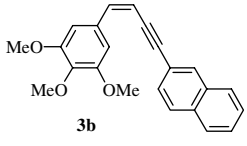
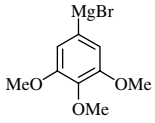
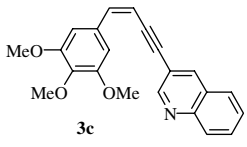
Scheme 1.

catalysis, isomerically pure chloroenynes react rapidly and cleanly with organometallic reagents to afford the corresponding coupling products. Thus, in the presence of Et_3N and a catalytic amount of $PdCl_2(PPh_3)_2$, isomerically pure conjugated enynes were obtained from Grignard reagents. Moreover, under weakly ligated palladium complexes $PdCl_2(PhCN)_2$ and CuI, they



Scheme 2. Reagents and conditions: (i) 5 mol % $PdCl_2(PPh_3)_2$, 10 mol % CuI, 2 equiv (*Z*)-1,2-dichloroethylene, 2 equiv *n*-$BuNH_2$, Et_2O, 20 °C.

Table 1. Coupling reaction of *Z*-chloroenyne **6** and **8** with Grignard reagents under palladium catalysis[a]

Entry	Chloroenyne	Grignard reagent	Enyne **2a–c**, **3a–c**[15]	Yield[b] (%)
1	**6**		 **2a**	56
2	**6**		 **2b**	93
3	**6**		 **2c**	64
4	**8a**		 **3a**	61
5	**8b**		 **3b**	51
6	**8c**		 **3c**	77

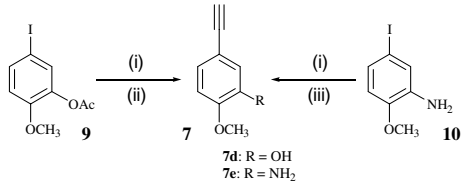
[a] All reactions were performed with 2 equiv of ArMgBr, 5 mol % of $PdCl_2(PPh_3)_2$ in THF at room temperature.
[b] Isolated yields.

undergo rapid coupling with terminal alkynes to afford enediynes derivatives in high yields.[9] Therefore, the scope of these methods has been explored towards the synthesis of target enynes and enediynes **2**–**4** in the context of a brief structure–activity relationship study of analogues of CA-4 **1**.

The key precursors pure *Z*-chloroenynes **6** and **8** were obtained in good yields (51–77%) as already reported by Sonogashira–Linstrumelle (S–L)[10] coupling reactions between (*Z*)-1,2-dichloroethylenes with terminal alkynes **5**[11] and **7** in the presence of bis(triphenylphosphine)-palladium chloride, copper iodide and *n*-butylamine in diethylether (Scheme 2).

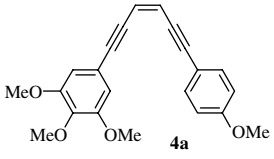
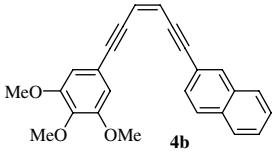
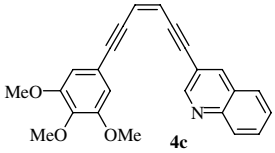
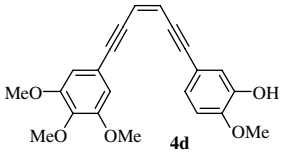
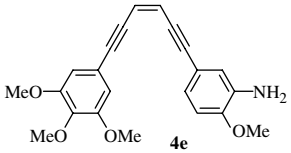
The target enyne derivatives were then obtained by further coupling of the remaining carbon–chlorine bond according to our previous synthetic strategy. Thus, reactions of *Z*-chloroenyne **6** with various aryl Grignard reagents[12] under a catalytic amount of $PdCl_2(PPh_3)_2$, in THF at room temperature furnished the expected biaryl-enyne adducts **2** in moderate to good yields and with retention of the configuration of the *Z*-double bond (Table 1). Under similar conditions, aryl-enynes **3** have been successfully prepared by the coupling of chloroenynes **8a**–**c** with 3,4,5-trimethoxyarylmagnesium bromide. Although the synthetic strategy to enyne **3** was less convergent than the route leading to enynes **2**, it was nevertheless efficient. It should be noted that *Z*-enyne derivatives **2** and **3** were stable and no isomerization of the *Z*-double bond has been noticed after a prolonged exposure at room temperature.

Next, unsymmetrical (*Z*)-enediynes **4** (opening form of biologically active triphenylene analogues)[13] have been investigated. For their preparation, we planned to introduce alkynyl moieties by means of the palladium-mediated S–L coupling reaction starting from key precursors pure *Z*-chloroenynes **6** and **8**. To this end, we showed previously that the use of $PdCl_2(PhCN)_2$ as catalyst associated with piperidine or pyrrolidine improved the reactivity of terminal alkynes with vinyl



Scheme 3. Reagents and conditions: (i) CuI 10 mol %, $PdCl_2(PPh_3)_2$ 5 mol %, trimethylsilylacetylene 2 equiv, piperidine, THF, 12 h; (ii) NaOH, MeOH, 20 °C; (iii) K_2CO_3, MeOH, 0 °C.

Table 2. Coupling reaction between (*Z*)-chloroenyne and 1-alkyne: synthesis of enediyne **4a**–**e**

Entry	Chloroenyne	Alkyne	Enediyne **4**[15]	Yield[a] (%)
1	**8a**	**5**	 **4a**	86
2	**6**	**7b**	 **4b**	71
3	**6**	**7c**	 **4c**	38
4	**6**	**7d**	 **4d**	73
5	**6**	**7e**	 **4e**	61

[a] Isolated yields.

chlorides since large rate enhancements were observed reducing the reaction time to 0.5–2 h at room temperature compared to 5–12 h when using palladium catalyst ligated with triphenylphosphine.[9] Consequently, we undertook to use these conditions in our synthesis of CA-4 analogues.

The desired *Z*-enediynes **4a**–**e** were prepared from pure trimethoxyphenylchloroenyne **6**. The synthesis of functionalized 1-alkynes **7d** and **7e** as second partners for these coupling reactions were obtained from functionalized aryl iodides **9** and **10** using the two steps sequence S–L-coupling with trimethylsilylacetylene[14] followed by desilylation under alkaline conditions as outlined in Scheme 3.

When *Z*-trimethoxychloroenyne **6** was treated under the reaction conditions previously reported (5 mol % $PdCl_2(PhCN)_2$, 10 mol % CuI, piperidine), we were pleased to observe that the expected cross-coupled enediynes **4** were obtained in reasonable to good yields (38–73% unoptimized). In order to check the versatility of this coupling methodology, we have prepared enediyne **4a** this time, from trimethoxyaryl alkyne **5** and the *Z*-chloroenyne **8a** in an excellent overall yield (86%, Table 2, entry 1).

Compounds **2**, **3** and **4** were tested for their ability to interact with tubulin (polymerization and depolymerization) and for cytotoxicity against KB, MCF7 and MCF7R cell lines.

However, most compounds did not inhibit the tubulin polymerization and depolymerization. Only enyne **2b** and enediyne **4e** were found to be active (e.g., 6.5×10^{-5} and 6.8×10^{-5} M, respectively), but 50-fold less active than CA-4. Moreover, if the cytotoxicity of compounds **2**–**4** against KB, MCF7 and MCF7R cell lines was comparable to other previously described analogues, it was clearly less cytotoxic than combretastatin.

In summary, the purpose of this study was to investigate and develop methods for the preparation of original enynes and enediynes as analogues of Combretastatin A-4. We have presented here, the synthesis and the evaluation of new enynes **2** and **3** as well as enediynes **4**. We have developed an efficient route to the preparation of eleven new compounds via palladium cross-coupling reactions. Enyne **2b** and enediyne **4e** inhibited tubulin polymerization with an IC_{50} 60–70 μM while the other compounds did not reveal any significant activity. At a concentration of 10^{-5} M, these two compounds showed a marginal activity towards KB, MCF7 and MCF7R cells. In this series of enynes and enediynes-types analogues, examination of the anticancer results revealed that, in these analogues, the incorporation of one or two triple bonds does not play a role for maximal anticancer activity.

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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.2005.10.001.

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