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1,3-Dipolar cycloaddition route to novel isoxazole-type derivatives related to combretastatin A-4

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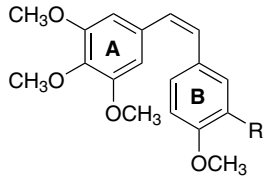
Abstract—A series of compounds related to combretastatin A-4, containing a five-membered heterocyclic ring interposed between the two phenyl groups have been prepared. Synthetic approach involves 1,3-dipolar cycloaddition of various 3,4,5-trimethoxyphenyl units with an in situ generated nitrile oxide from a suitable aldoxime using sodium hypochlorite. Depending on the nature of the dipolarophile, 3,5-diarylisoxazole derivatives were obtained along with the 3,4-regioisomeric isomers.

Combretastatin A-4 (CA4), a naturally occurring phenolic stilbene isolated from the South African willow tree, *Combretum caffrum*,[1] has been found to be a potent cytotoxic agent, which strongly inhibits the polymerization of tubulin by binding to the colchicine site.[2] Furthermore, CA4 displayed potent and selective vascular shutdown within solid tumours.[3] Two formulation prodrugs (CA4P and AVE8062) are currently under clinical evaluation as tumour vascular targeting agents (Fig. 1).[4]

Considerable efforts have been devoted to the synthesis of new CA4 analogues.[5] From these investigations, it was established that the *cis*-orientation of the two phenyl rings A and B is essential for the bioactivity of combretastatins. Furthermore, when heterocyclic rings were used as linkers in place of the CA4 double bond, both antitubulin activity and cytotoxicity could be improved.[6]



R = OH **CA4**
R = OPO_3Na_2 **CA4P**
R = NHSer, HCl **AVE8062**

Figure 1.

Our goal was to prepare a series of diaryl-heterocyclic systems, of the isoxazole type,[7] in order to obtain new analogues of CA4 with greater stability and higher affinity for endothelial cells within tumour vessels. To this end, 1,3-dipolar cycloaddition reactions[8] provided an interesting synthetic route to five-membered heterocyclic rings by addition of a dipole (here a nitrile oxide),[9] to an unsaturated system. First, we tested the behaviour of a benzonitrile oxide **1** bearing the substitution pattern of the B-ring, towards several 3,4,5-trimethoxyphenyl derivatives **2** (Scheme 1). Depending on the nature of the dipolarophile two regioisomeric cycloadducts could be expected, 3,5- (isomers **3**) and 3,4-diaryl-isoxazole derivatives (isomers **4**).[9,10]

The expected nitrile oxide **1** was generated in situ from the corresponding aldoxime using sodium hypochlorite,[11] and this in the presence of the dipolarophile, to form the cycloadduct directly. Isovanillin **5** was silylated[12] to give **6**, which was then converted into oxime **7** by treatment with hydroxylamine hydrochloride (Scheme 2). In an effort to reduce formation of furoxan via nitrile oxide dimerisation, oxime **7** dissolved in dichloromethane was added dropwise to a dichloromethane/aqueous sodium hypochlorite biphasic mixture containing the dipolarophile and catalytic triethylamine. The in situ generated nitrile oxide **1** underwent

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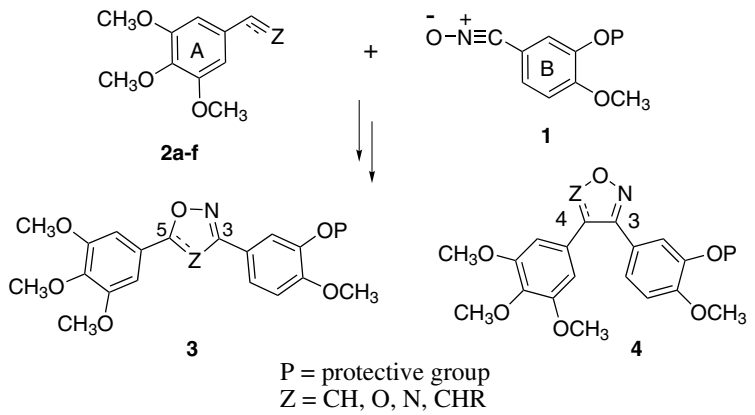
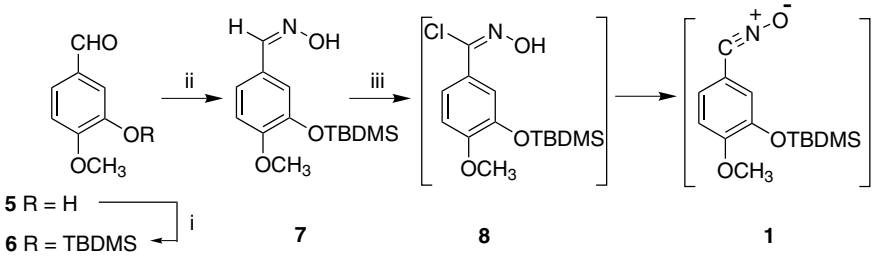


Scheme 1.



Scheme 2. Reagents and conditions: (i) Ref. 11: TBDMSCl, DIEA, THF, rt, 24 h, 95%; (ii) $NH_2OH \cdot HCl$, pyridine, EtOH, reflux, 1 h, 75%; (iii) aq NaOCl, Et_3N, CH_2Cl_2, O °C, rt, 24 h.

1,3-dipolar cycloaddition. Table 1 provides a summary of the type of dipolarophiles studied[13] (compounds **2a**–**f**), and of the yields and structures of cycloadducts obtained.

Cycloaddition to alkyne **2a** (entry 1) proceeded regioselectively to give the 3,5-diarylisoxazole **3a** in a moderate (38%) yield. As expected with alkenes, which are usually more reactive dipolarophiles than alkynes,[9] the cycloadduct **3b** was obtained from the ethylene derivative **2b** (entry 2) in a better yield (62%). Heteroatom-containing dipolarophiles often showed to be less reactive than the corresponding C=C unsaturated analogues.[9] Nevertheless 3,5-diaryldioxazole **3c** and 3,5-diaryloxadiazole **3d** could be obtained from aldehyde **2c** and nitrile **2d** (entries 3 and 4), but under more drastic conditions and in poor yields (10% and 6% yields, respectively). With the above mentioned dipolarophiles **2a**–**d**, formation of the isomeric 3,4-disubstituted derivatives **4** was never observed.

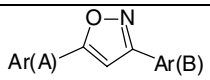
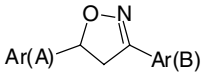
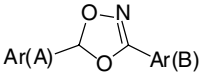
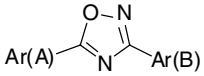
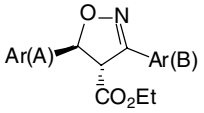
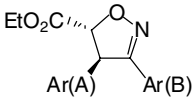
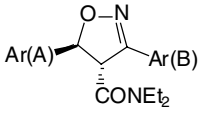
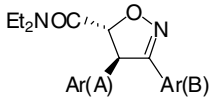
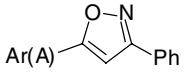
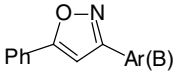
On the other hand, with 1,2-disubstituted alkenes, cycloaddition had reduced regioselectivity, leading to a mixture of the two possible regioisomeric δ^2-isoxazolines, in ratios resulting from a subtle interplay between steric and electronic factors.[9,16] Thus, *trans*-ethyl 3,4,5-trimethoxycinnamate **2e** (entry 5) afforded the 4-carboethoxydihydroisoxazole **3e** along with some amounts of the 5-carboethoxyisomer **4e** (ratio 85/15).[17] According to Weidner-Wells et al.[18] who have shown that using bulky tertiary cinnamides as the dipolarophiles results in a reversal of regioselectivity, cycloaddition was investigated with the *trans*-*N*,*N*-diethylcinnamide **2f**. In that case (entry 6) two cycloadducts were formed, **3f** and **4f**, in almost equal amounts (ratio: 45/55), the regioselectivity being effectively reversed.[19]

Structural assignment for regioisomers **3** and **4** was accomplished on the basis[10,20] of the relative chemical shifts and coupling constants of the C_4–H and C_5–H methine doublets, $\Delta\delta_{5,4}$ and $J_{4,5}$ being larger for the 3,5- than the 3,4-disubstituted isoxazolines.[21] The values of $J_{4,5}$ (<9.9 Hz) for cycloadducts obtained from the *trans*-olefins **2f** and **2d**, are consistent with the hitherto reported values for the *trans*-protons in δ^2-isoxazolines, ascertaining the stereoselectivity of the cycloaddition.

Deprotection of the phenol function of the cycloadducts **3a**–**f** and **4e**,**f** (yields ranging from 80% to 95%) carried out using TBAF gave eight new analogues of combretastatin A-4.[22]

In order to clarify the effect of the substitution pattern of the aromatic rings on both the nitrile oxide and the dipolarophile, cycloadditions were performed with phenyl oxime instead of **7** (entry 7) and phenylacetylene in place of the dipolarophile **2a** (entry 8).[23] In both cases, yields were enhanced (respectively, 58% and 53% vs 38%; entries 7 or 8 vs entry 1). These results show that the trimethoxy motif on A-ring and the functionalities

Table 1. Results of the 1,3-dipolar cycloaddition of in situ generated nitrile oxides with various dipolarophiles[a]

Entry	Reactants		Cycloadducts[b]			
	Dipolarophile	Oxime	Total yield, %[c]	Isomer **3**	Isomer **4**	Ratio **3/4**
1	**2a** Z = CH	**7**	38	 **3a**	—	100/0
2	**2b** Z = CH_2	**7**	62	 **3b**	—	100/0
3	**2c** Z = O	**7**	10[d]	 **3c**	—	100/0
4	**2d** Z = N	**7**	6[d]	 **3d**	—	100/0
5	**2e** Z = $CHCO_2Et$	**7**	63	 **3e**	 **4e**	85/15[e]
6	**2f** Z = $CHCONEt_2$	**7**	58	 **3f**	 **4f**	45/55[e]
7	**2a** Z = CH	PhCH=NOH[f]	58		—	100/0
8	Ph–C≡CH	**7**	53		—	100/0

[a] General procedure:[11] dipolarophile (1 equiv), Et_3N (0.1 equiv), aq NaOCl (1.6 equiv)/CH_2Cl_2 then oxime (1 equiv) at 0 °C, rt, 24 h.
[b] Ar(A): 3,4,5-trimethoxyphenyl; Ar(B): 3-OTBDMS,4-methoxyphenyl.
[c] Yields of adducts isolated after flash chromatography.
[d] Reaction run with additional reflux during 24 h.
[e] Determined by integration of the C_4–H and C_5–H in the NMR of the reaction mixture.
[f] Oxime prepared from benzaldehyde according to (ii), Scheme 2.

on B-ring are not very favourable for the cycloadditions studied, due to steric and/or electronic effects.

In summary, despite modest yields, 1,3-dipolar cycloaddition represents a convenient synthetic route for the preparation of five-membered heterocyclic analogues of combretastatin A-4. Extension of this work to the synthesis of 'inverse' cycloadducts by reaction of the 3,4,5-trimethoxybenzyl nitrile oxide with various dipolarophiles affording the aromatic B-ring, is currently in progress and will be reported in due course.

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13. Aldehyde **2c** and nitrile **2d** are commercially available. Alkyne **2a** and olefin **2b** were prepared according to literature methods.[14,15] Ethyl cinnamate **2e** was obtained from the corresponding carboxylic acid (EtOH, H_2SO_4 cat., reflux): mp 68–69 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.60 (d, 1H, $J = 15.9$ Hz), 6.75 (s, 2H), 6.35 (d, 1H, $J = 15.9$ Hz), 4.26 (q, 2H, $J = 7.1$ Hz), 3.88 (s, 9H), 1.34 (t, 3H, $J = 7.1$ Hz). Cinnamide **2f** was obtained by treatment with diethylamine (2 equiv, toluene, rt, 1 h) of 3,4,5-trimethoxycinnamic chloride prepared from the corresponding acid and oxalic chloride (1.1 equiv, toluene, reflux, 3 h): mp 132–134 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.62 (d, 1H, $J = 15.3$ Hz), 6.73 (s, 2H), 6.70 (d, 1H, $J = 15.3$ Hz), 3.89 (s, 6H), 3.87 (s, 3H), 3.49 (q, 4H, $J = 7.1$ Hz), 1.23 (br s, 6H).
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19. Typical procedure for the cycloadditions: Oxime **7** (562 mg, 2.0 mmol) in dichloromethane (2 mL) at 0 °C was dropwise added, in 1 h, to a mixture of amide **2f** (587 mg, 2.0 mmol), triethylamine (28 μL, 0.2 mmol) and 13% aqueous sodium hypochlorite (1.9 mL, 3.2 mmol) in dichloromethane (5 mL). After stirring for 24 h at room temperature, the reaction mixture was diluted with water and extracted with dichloromethane. The combined extracts were dried ($MgSO_4$) and concentrated under reduced pressure. The residue was subjected to flash chromatography on silica gel (cyclohexane/ethyl acetate: 6/4 to 4/6) to give 660 mg (58%) of a mixture of the two regioisomers **3f** and **4f**, which could be separated after another two tedious chromatographies (cyclohexane/ethyl acetate: 7/3). Cycloadduct **3f**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.17 (d, 1H, $J = 2.1$ Hz), 7.13 (dd, 1H, $J = 8.3$, 2.1 Hz), 6.81 (d, 1H, $J = 8.3$ Hz), 6.62 (s, 2H), 5.65 (d, 1H, $J = 9.9$ Hz), 4.59 (d, 1H, $J = 9.9$ Hz), 3.84 (s, 9H), 3.81 (s, 3H), 3.41 (q, 2H, $J = 7.1$ Hz), 3.19 (m, 2H), 1.25 (t, 3H, $J = 7.1$ Hz), 1.15 (t, 3H, $J = 7.1$ Hz), 0.99 (s, 9H), 0.14 (s, 6H). Cycloadduct **4f**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.24 (dd, 1H, $J = 8.5$, 2.1 Hz), 7.07 (d, 1H, $J = 2.1$ Hz), 6.75 (d, 1H, $J = 8.5$ Hz), 6.46 (s, 2H), 5.54 (d, 1H, $J = 5.7$ Hz), 5.05 (d, 1H, $J = 5.7$ Hz), 3.79 (s, 9H), 3.77 (s, 3H), 3.45 (m, 4H), 1.27 (t, 3H, $J = 7.0$ Hz), 1.25 (t, 3H, $J = 7.0$ Hz), 0.92 (s, 9H), 0.05 (s, 3H), 0.02 (s, 3H).
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21. So, for amide derivatives spacing, $\Delta\delta_{5,4}$ is larger for isomer **3f** (1.06) than for isomer **4f** (0.49).
22. All the deprotected cycloadducts give satisfactory elemental analyses, ^{1}H, ^{13}C NMR and mass data. Assessment of their ability to inhibit in vitro polymerization is now under investigation.
23. Cycloadditions were also attempted with the 1,3-dipole entity having the phenolic group protected as a 4-methoxybenzyl (PMB).[24] With both dipolarophiles investigated, **2a** and **2d**, coupling was slightly more efficient (respective yields: 46% vs 38%, 11% vs 6%), probably due to a lesser steric hindrance. But the deprotecting step, realized with trifluoroacetic acid, was less effective. At last protection of the phenol function with a silyl group seems more effective.
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