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Synthesis and biological activity of pyrrole analogues of combretastatin A-4

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ABSTRACT

A series of pyrrole analogues of combretastatin (CA-4) were synthesized and tested for their anti-proliferative activity. The highly diastereoselective acyl-Claisen rearrangement was used to provide 2,3-*syn* disubstituted morpholine amides which were used as precursors for the various analogues. This synthesis allows for the preparation of 1,2- and 2,3-diaryl-1*H*-pyrroles which are both geometrically similar to CA-4. These pyrrolic analogues were tested for their anti-proliferative activity against two human cell lines, K562 and MDA-MB-231 with 2,3-diaryl-1*H*-pyrrole **35** exhibiting the most potent activity with IC_{50} value of 0.07 μM against MDA-MB-231 cell line.

Combretastatin A-4 (**2**), a natural product isolated from the bark of the South African bush willow *Combretum caffrum*, is one of the most potent antimitotic agents and disrupts microtubulin dynamics.[1–13] Combretastatin A-4 binds to the colchicine (**1**) domain of tubulin and inhibits microtubule polymerisation, resulting in destabilisation of the microtubule cytoskeleton and inhibition of mitosis. The structural simplicity and potent cytotoxicity of CA-4 has led to the development of analogues of CA-4 as new anticancer agents (Fig. 1).[2,3,11–13]

Structure–activity-relationship (SAR) studies with CA-4 have indicated that the *cis*-configuration of the olefinic bridge between the two aromatic rings A and B is essential. The 3,4,5-trimethoxy group on ring A affects cytotoxic activity and 3-hydroxy-4-methoxy groups on ring B affects binding to tubulin.[1,4,5,8,13] In terms of the spatial relationship, the *cis*-orientation of the two rings of CA-4 is required for binding in the colchicine-binding site of tubulin. Nevertheless, it is notable that the *cis*-olefin in CA-4 can convert into the thermodynamically more stable, and inactive, *trans*-olefin due to its metabolic instability. This *cis–trans* isomerisation results in a loss of antimitotic activity and cytotoxicity.[1,2,5,7,9,14] The low water-solubility of CA-4 results in its low efficacy in vivo and therefore the water-soluble sodium phosphate prodrug of CA-4, fosbretabulin (CA-4P), was designed and is currently in phase II/III clinical trials.[5–11] Efforts to remove the possibility of *cis–trans* isomerisation have been attempted by replacing the *cis*-olefin with a five-membered heterocycle which result in a *cis*-like constrained configuration. CA-4 analogues having five-membered heterocyclic rings such as oxazoles, imidazoles and triazoles have been reported to have increased potency and bioactivity.[2,3,13,14]

Pyrrole-linked heterocyclic analogues have also been previously synthesised and their antimitotic activity evaluated with 3,4-diaryl-1*H*-pyrrole-2-carboxylates,[15] 4,5-diaryl-1*H*-pyrrole-2-carboxylates,[7] 3,5-diaryl-1*H*-pyrrole-2-carboxylates[16] and 1,2-diaryl-1*H*-pyrroles[10] being reported.

With the objective of synthesis of pyrrolic analogues of CA-4 in mind, herein, we report the synthesis of novel 1,2-diaryl and 2,3-diaryl-1*H*-pyrrole analogues of CA-4.

To prepare the desired analogues the Paal–Knorr pyrrole synthesis was chosen with an acyl-Claisen approach being used to form the diketone precursors. The acyl-Claisen rearrangement of acid chlorides with (*E*)-allylic morpholines is highly diastereoselective giving 2,3-*syn*-disubstitued amides.[17,18] The 2,3-*syn* arrangement of substituents in 1,4-dicarbonyl compounds is the preferred arrangement for heterocycle formation in Paal–Knorr synthesis.[20] For 1,2-diarylpyrroles, the two aryl groups found in CA-4, 3,4,5-trimethoxy on A and 4-methoxy-3-hydroxy substitution on B, would be introduced. We envisaged that previously prepared morpholine amide **7** would allow for the change in substitution pattern, ring A or B, by addition of different aryl organometallic reagents. The second aryl group would be added using the appropriate aniline in the Paal–Knorr condensation (Scheme 1).

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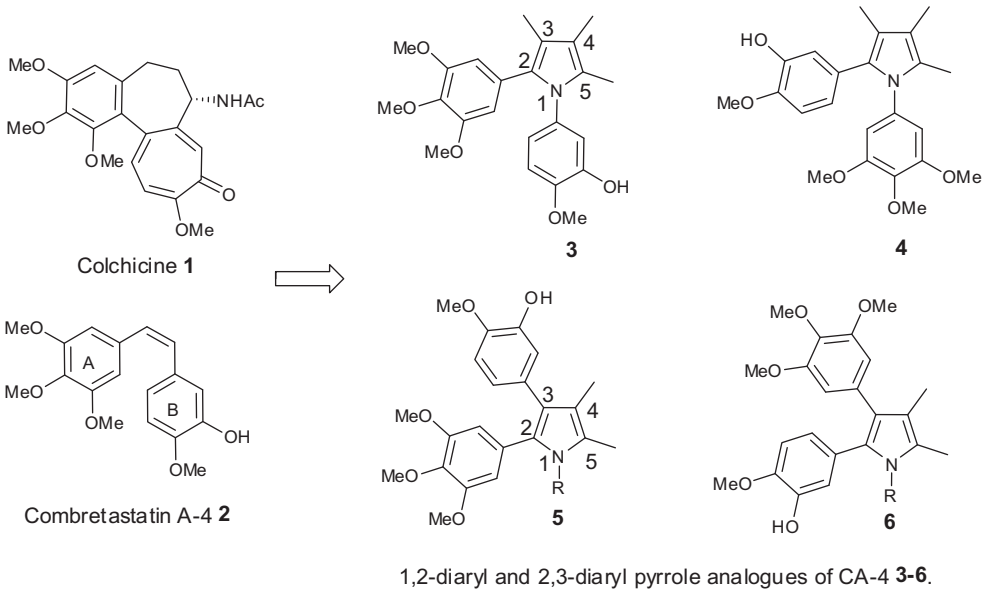
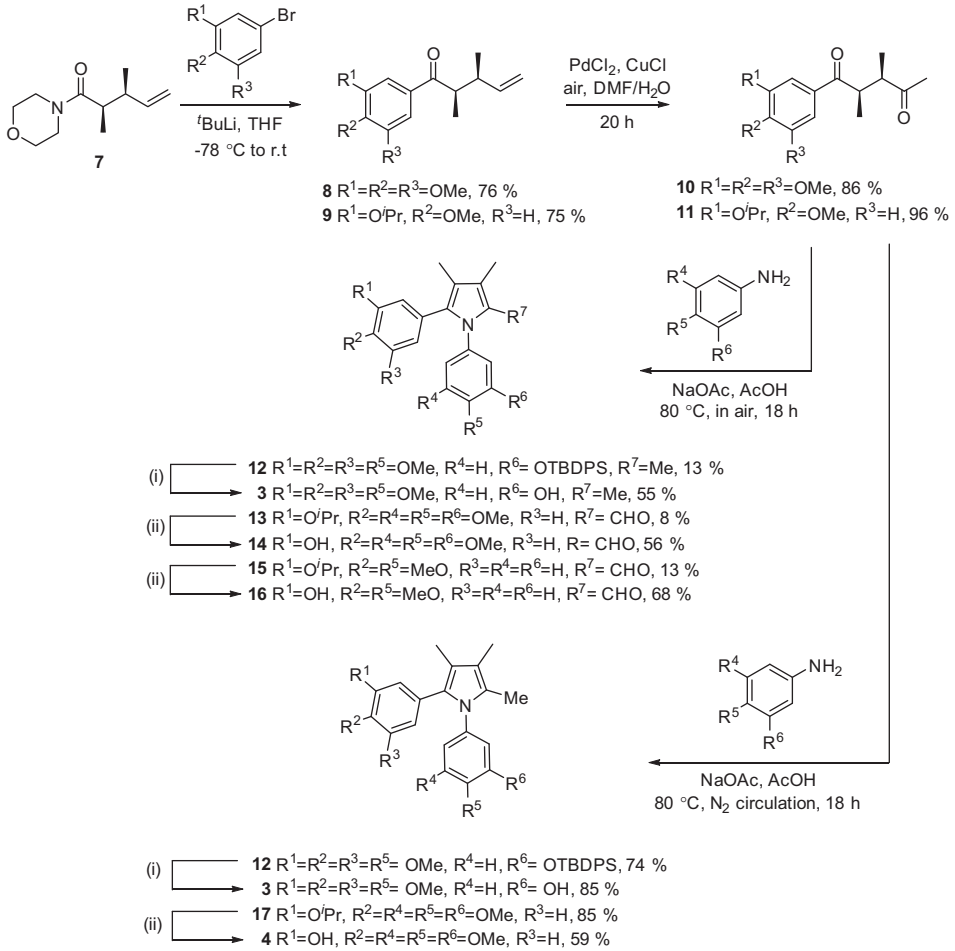


Figure 1. Structure of colchicine **1**, combretastatin A-4 **2** and the target pyrrolic derivatives of the natural product.

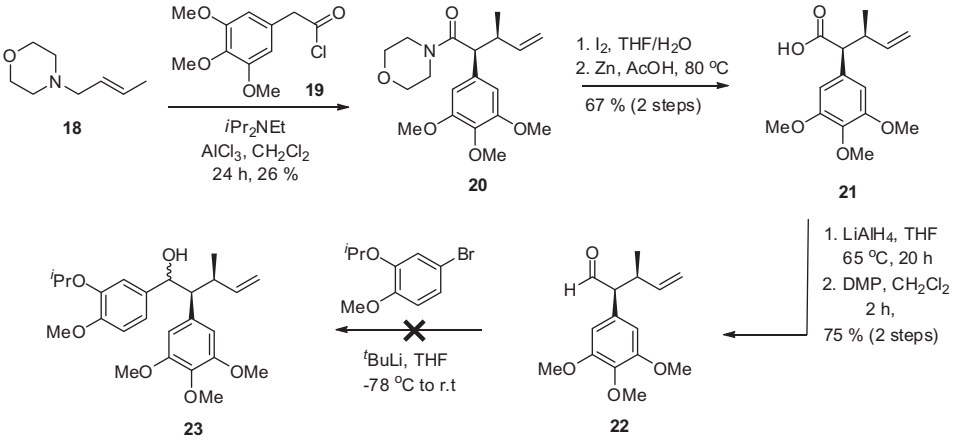


Scheme 1. Synthesis of 1,2-diaryl-pyrrolic analogues of combretastatin A-4. (i) TBAF, THF, rt, 18 h; (ii) $AlCl_3$, CH_2Cl_2, rt, 16 h.

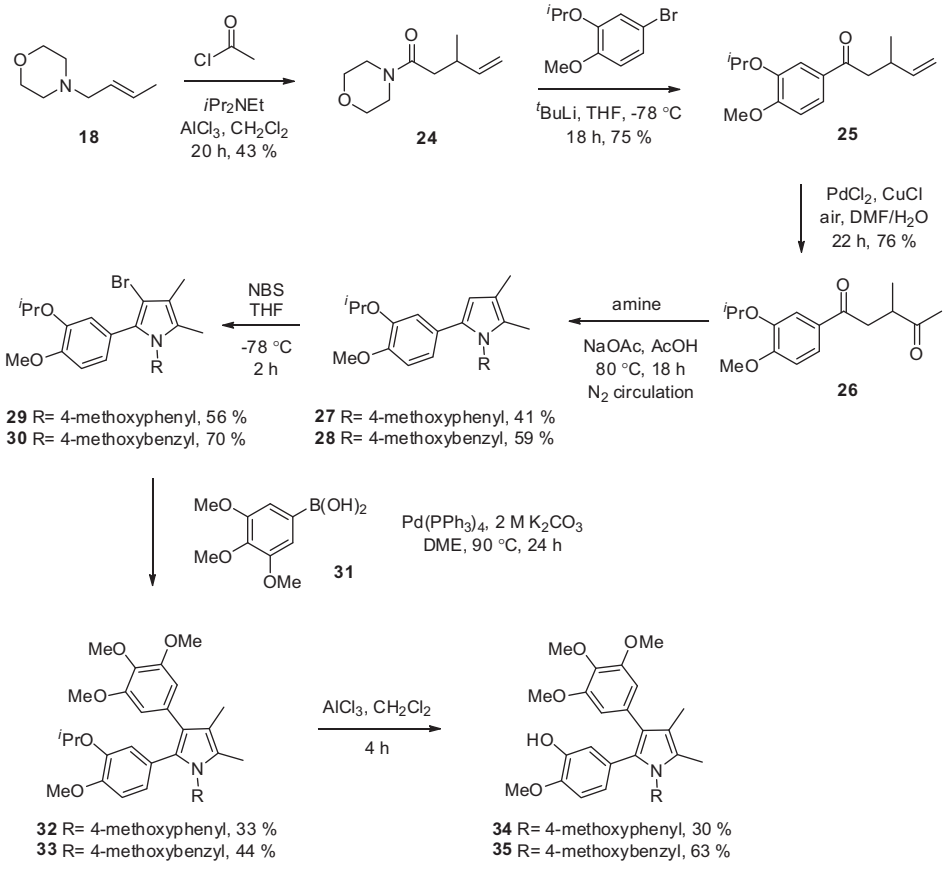
The 2,3-*syn*-dimethyl morpholine amide **7**[19] was substituted using lithiated arenes and gave ketones **8** and **9**, containing 3,4,5-trimethoxy or 3-isopropyl-4-methoxy phenyl groups respectively. Wacker–Tsuji oxidation of **8** and **9** using $PdCl_2$ and CuCl in DMF-water, open to the air gave diketones **10** and **11** in excellent yields. Condensation of diketone **10** with 3-TBDPSO-4-methoxyaniline in air, gave a complex mixture of products from which the expected pyrrole **12** was obtained in only 13% yield.

Condensation of diketone **11** with 3,4,5-trimethoxyaniline and 4-methoxyaniline was undertaken in air and gave 5-formyl pyrroles **13** and **15** in 8% and 13% yield, respectively. We have previously[17] found that the 5-methyl group in similar fully substituted pyrroles is susceptible to oxidation in the presence of oxygen under Paal–Knorr condition. It has been proposed that use of electron rich anilines results in the formation of a reactive enamine which reacts with oxygen giving the formyl group. Therefore, we conducted the condensation reactions under nitrogen to avoid formation of aldehyde and to hopefully increase yield. The reaction of diketones **10** and **11** with 3-TBDPSO-4-methoxyaniline and 3,4,5-trimethoxyaniline under nitrogen gave only fully substituted methyl pyrroles **12** and **17** in good yields of 74% and 85%, respectively, showing that the removal of oxygen minimises various side products and contributes to an increase in pyrrole formation. Finally, removal of the TBDPS group in **12** using TBAF, or removal of the isopropyl group in **17** using $AlCl_3$, provided the pyrrolic derivatives of CA-4, **3** and **4**, in 85% and 59%, respectively. Similarly removal of the isopropyl group in 5-formylpyrroles **13** and **15** gave additional analogues **14** and **16**, respectively.

Following the successful synthesis of 1,2-diaryl-pyrroles, we next sought to synthesise 2,3-diaryl-pyrroles to compare the activity of 1,2-diaryl and 2,3-diaryl-1*H*-pyrroles.

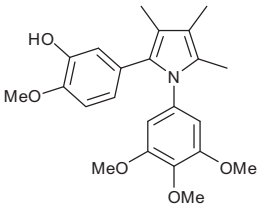
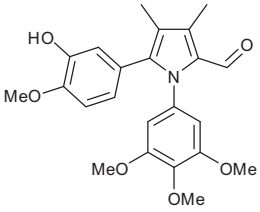
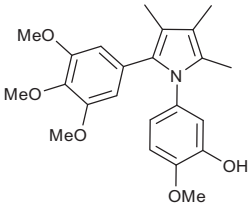
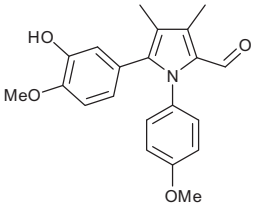
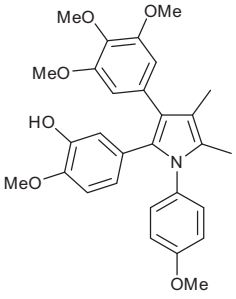
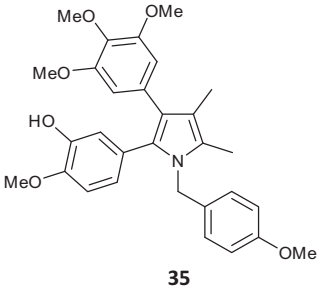


Scheme 2. Synthesis of aldehyde **22**.



Scheme 3. Synthesis of 2,3-diaryl pyrrolic analogues of combretastatin A-4.

Table 1
Antiproliferative activity (IC_{50} values) of pyrrole compounds against K562 and MDA-MB-231 cell lines

Compounds	K562 IC_{50}[a] (μM)	MDA-MB-231 IC_{50}[a] (μM)
 **4**	⩾10	⩾10
 **14**	⩾10	⩾10
 **3**	5.40 (±0.02)	1.66 (±0.02)
 **16**	⩾10	8.43 (±1.57)
 **34**	3.82 (±0.49)	1.74 (±0.14)
 **35**	0.21 (±0.02)	0.07 (±0.02)
Combretastatin A-4 (**1**)	0.003 (±0.0005)	0.03 (±0.0001)

[a] Experiments were performed in triplicate; stated values are the averages of three independent determinations. See Supplementary data for details.

Acyl-Claisen rearrangement of crotyl morpholine **18** and acid chloride **19** gave 2-aryl-3-methylamide **20** (Scheme 2), however, addition of lithiated arenes to amide **20** gave no ketone, presumably due to steric hindrance. It was therefore decided to convert amide **20** into a more reactive aldehyde, and this was achieved utilising iodolactonisation of amide **20** followed by reductive ring opening with zinc in acetic acid, giving acid **21**. Acid **21** was then reduced, to the primary alcohol followed by oxidation to give aldehyde **22**. Unfortunately lithiated arene addition to aldehyde **22** was also unsuccessful and it was again assumed that the aryl group at the α-position was again hindering the addition.

The synthesis was then revised to introduce aromatic ring B after pyrrole formation. The synthesis began from acyl-Claisen rearrangement of crotylmorpholine **18** and acetyl chloride, giving morpholine amide **24** in 43% yield (Scheme 3). Reaction of amide **24** with the lithiate of 3-isopropyl-4-methoxy bromobenzene gave ketone **25** in good yield. Alkene oxidation of **25** using the Wacker–Tsuji oxidation gave diketone **26** in 75% yield. Condensation of diketone **26** with 4-methoxyaniline and 4-methoxybenzylamine gave pyrroles **27** and **28** in 41% and 59% yields, respectively. To prevent oxidation at the 5-methyl group, pyrrole formation was again conducted under a nitrogen atmosphere. Bromination of pyrroles **27** and **28** using NBS in THF at −78 °C gave 3-bromo pyrroles **29** and **30**, with no poly-brominated products obtained. Suzuki coupling reaction between 3-bromopyrroles **29** and **30** and 3,4,5-trimethoxyphenylboronic acid **31** provided the desired arylated pyrroles **32** and **33**. Finally, removal of isopropyl protecting groups of **32** and **33** using $AlCl_3$ provided the desired pyrrolic derivatives of CA-4 **34** and **35**.

Following the synthesis of the pyrrole analogues, their antiproliferative activity was investigated against two human cell lines, K562, a human leukaemia cell line and MDA-MB-231, a breast cancer cell line and their IC_{50} values determined (Table 1). These cell lines were chosen as they have shown previous susceptibility to CA-4.[2,6,12,21,22]

The results show that 2,3-diaryl pyrroles **34** and **35** were more active than the prepared1,2-diaryl pyrroles. The most active compound **35** had IC_{50} values of 0.21 μM and 0.07 μM against K-562 and MDA-MB-231 cell lines respectively. All compounds were less active than combretastatin **2** itself with combretastatin being twice as active as compound **35** against the MDA-MB-231 cell line. Comparison of compounds **34** and **35** show that modification of the substituent on the pyrrole nitrogen can effect activity and that structure **34** with its 1,2,3-triaryl motif appears less bioactive.

In conclusion the synthesis of a number of pyrrole analogues of combretastatin A-4 has been achieved. An acyl-Claisen approach to the desired diketones followed by Paal–Knorr condensation with appropriate aniline gave the desired pyrrole analogues. 2,3-Diaryl pyrroles mimicking the structure of CA-4 were found to be more active than 1,2-diaryl analogues. The route used to prepare these analogues is easily adaptable to the preparation of other analogues with modification of the *N*-substituents easily achievable.

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Supplementary data

Supplementary data (experimental procedures, ^{1}H and ^{13}C NMR spectra and cell proliferation assay information) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.05.026.

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