Advances in Synthetic Approaches for the Preparation of 
Combretastatin-Based Anti-Cancer Agents

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Abstract: The natural product combretastatin A-4 (CA4) is a potent anti-cancer agent known for its antimitotic and antiangiogenic properties. The basic structure of CA4 has inspired the design and synthesis of a variety of medicinally active analogues that take advantage of the relatively simple stilbenoid architecture of the molecule. Here, we examine recent advances in the synthesis of various CA4-based analogues. A significant focus is placed on the modifications to the bridging alkene moiety of the stilbene scaffold for conformationally restricting the structure in a bioactive form. An effort is also made to discuss promising ring modifications and replacements, including the incorporation of indazole and oxindole rings, as well as the design and synthesis of amino-substituted analogues.

1 Introduction

The discovery and development of small-molecule tubulin assembly inhibitors began with studies of colchicine (1, Figure 1) in the early 1930s.1 Research in this field received a major impetus in 1982 when Pettit et al. discovered and isolated (−)-combretastatin from Combretum caffrum, a South African bush willow tree.2 The compound was found to be a potent inhibitor of cancer cell proliferation.3 Since then, a significant number of studies have been directed towards analysis of combretastatin and related medicinally active compounds.4 Combretastatin A-4 (CA4) or 2-methoxy-5-[2-(3,4,5-trimethoxyphenyl)vinyl]phenol along with combretastatin A-1 (CA1) and their phosphate analogues CA4P and CA1P form a family of some of the most potent combretastatins.5 After extensive preclinical evaluation, the water-soluble prodrug CA4P has emerged as a promising clinical candidate for treating cancer.6,7 Tubulin is a heterodimeric protein which is present in all eukaryotic cells.8 Assembly of tubulin and disassembly of the polymeric form is a dynamic process which leads to the formation of microtubules.9 The polymeric microtubules play an important role in the construction of the mitotic spindle during cell division.10 The microtubules also perform intracellular functions such as transport and maintaining cellular morphology.

Figure 1 Structures of colchicine, (−)-combretastatin, combretastatin A4 (CA4), combretastatin A-4 phosphate, combretastatin A-1 and combretastatin A-1 phosphate

Antimitotic drugs that inhibit growth of cancer cells by anti-tubulin action are classified into two categories: microtubule-stabilizing agents and microtubule-destabilizing agents. The microtubule-stabilizing agents work by interacting directly with microtubules, enhancing the tubulin polymerization and hence stabilizing them against depolymerization. This action disturbs the dynamic pro-
cess and is the foundation of the well-documented anti-cancer properties of drugs such as paclitaxel and docetaxel. In contrast to members of the taxoid family, the Vinca alkaloids and colchicinoids work by inhibiting the tubulin polymerization. Different binding sites for Vinca alkaloids (such as vinblastine and vincristine) and colchicinoids (such as colchicine and CA4) suggest that the two classes of compounds adopt different mechanisms of action. Furthermore, the failure of cancer chemotherapy is often associated with an acquired resistance to various anti-cancer drugs. The cause behind this multi-drug resistance characteristic has been identified as expression of the drug efflux transporter P-glycoprotein in increased quantities. However, CA4 is not recognized by this glycoprotein and thus renders the protein impotent in developing resistance to the drug.

Various structural analogues of combretastatin have been synthesized and analyzed for their anti-cancer activity. More prominent amongst them are the stilbenes, diaryl ethylenes, phenanthrene-based derivatives, macrocyclic lactones as well as CA4-based macrocyclic derivatives. General structures of the above-mentioned classes of anti-cancer compounds are presented in Figure 2. Amongst these classes, the stilbenes are more versatile and hence have been studied with much profound interest. Additionally, the heterocyclic-ring-containing stilbenes provide another sub-class with interesting steric effects worthy of analysis. This report focuses on various stilbenoids and diverse synthetic approaches employed to achieve these targeted anti-cancer compounds.

**Biographical Sketches**

**Rohit Singh** obtained his M.Sc. (Hons) degree from Panjab University, India in 2001. The next year, he joined the University of New Orleans and obtained a second M.Sc degree in 2003 for his work on organocatalysis via N-heterocyclic carbenes. He continued his research on the development of N-heterocyclic carbenes as organic catalysts and efficient ligands in palladium-catalyzed transformations and obtained his Ph.D. from the University of New Orleans under the supervision of Professor Steven P. Nolan in 2007. He then joined the Center for Drug Design (University of Minnesota) and worked on the synthesis of combretastatin-based pyrazoline anti-cancer therapeutic agents. In 2008, he joined the group of Professor Robert Vince (Director, Center for Drug Design, University of Minnesota) where he is presently working on the design and synthesis of Portmanteau inhibitors (reverse transcriptase/integrase) for the treatment of HIV-AIDS.

**Harneet Kaur** obtained her M.Sc. (Hons) degree from Panjab University, India in 2001. She joined the University of New Orleans in 2002 and obtained a second M.Sc. degree in 2003 for her work on the development of well-defined N-heterocyclic carbene–copper complexes under the supervision of Professor Steven P. Nolan. In 2003, she joined Professor Mark L. Trudell’s group and worked towards her Ph.D. on the design and synthesis of tropane derivatives as potential therapeutics for cocaine abuse. After the hurricanes in New Orleans, she continued her research at the National Institute of Drug Abuse under the guidance of Dr. Amy H. Newman (NIDA – IRP Associate Director Translational Research, NIH, Baltimore). She returned to the University of New Orleans to finish her degree in 2007. She then joined Professor David M. Ferguson’s group (Center for Drug Design and Department of Medicinal Chemistry, University of Minnesota) for post-doctoral research on the synthesis of substituted heterocyclic analogues as drug targets for cancer studies and catalytic inhibitors of human topoisomerase-II.
2 New Approaches for the Synthesis of CA4

Building upon their protocols for the synthesis of different combretastatins, Pettit and co-workers utilized a Wittig reaction in their first synthesis of CA4 in 1995 (Scheme 1). To obtain the desired product, isovanillin was silylated by tert-butyldimethylsilyl ether followed by reduction of the aldehyde with sodium borohydride to obtain benzyl alcohol. The alcohol was then brominated by trimethylsilyl chloride and lithium bromide. The phosphonium bromide for the key Wittig step was obtained by reaction of the bromide with triphenylphosphine. Phosphonium bromide was coupled with 3,4,5-trimethoxybenzaldehyde to obtain a mixture of the product with Z/E stereoselectivity of 1:1.5 in 93% yield. The cis-isomer was separated from the trans-isomer by chromatography. Tetrabutylammonium fluoride (TBAF) was employed for desilylation to obtain the desired compound CA4.

Scheme 1 Synthesis of CA4 via Wittig reaction by Pettit and co-workers

Harrowven and co-workers carried out the condensation of phosphonium salt with benzaldehyde derivative employing potassium tert-butoxide. The reaction provided (Z)-stilbenes with remarkable selectivity ranging from 9:1 to 45:1. Removal of the ortho-bromo directing groups by halogen–metal exchange with n-butyllithium followed by quenching with water afforded the stilbene derivatives.

Scheme 2 Synthesis of CA4 and analogues via Wittig reaction by Harrowven and co-workers

The Taylor group devised a concise route for the synthesis of CA4 utilizing the Ramberg–Backlund reaction for obtaining the key stilbene unit as shown in Scheme 3. Thiol was first prepared by reaction of 3,4,5-trimethoxybenzyl alcohol and Lawesson’s reagent and subsequently coupled with bromide using potassium hydroxide in ethanol. The resulting sulfide was oxidized to the sulfone using m-chloroperoxybenzoic acid.

Scheme 3 Synthesis of CA4 and analogues by application of Ramberg–Backlund conditions

Harrowven and co-workers carried out the condensation of phosphonium salt with benzaldehyde derivative employing potassium tert-butoxide. The reaction provided (Z)-stilbenes with remarkable selectivity ranging from 9:1 to 45:1. Removal of the ortho-bromo directing groups by halogen–metal exchange with n-butyllithium followed by quenching with water afforded the stilbene derivatives.

Scheme 2 Synthesis of CA4 and analogues via Wittig reaction by Harrowven and co-workers
The sulfone was then subjected to tandem halogenation–Ramberg–Backlund conditions as reported by Chan and co-workers (C₂Br₂, KOH–Al₂O₃, t-BuOH, 0 °C to r.t., 12 h).³¹ The one-pot process furnished the O-silylated product as a mixture of Z- and E-isomers (1:9) in 81% yield. CA₄ was obtained by deprotection via tetrabutyllammonium fluoride on silica gel. The reaction was also performed under Franck conditions (C₂F₄Br₂, KOH, t-BuOH, Δ, 12 h),³² and the original halogenation–Ramberg–Backlund conditions as reported by Meyers and co-workers (CCl₄, KOH, t-BuOH, H₂O).³³ While Franck conditions furnished the product with improved stereoselectivity (Z/E = 15:85) in 72% yield, Meyers conditions afforded the product with best stereoselectivity (Z/E = 53:47) in 69% yield.

Alami and co-workers devised a new hydrosilylation–protodesilylation process for obtaining combretastatin-based inhibitors of tubulin assembly.²² These authors recently demonstrated the efficacy of heterogeneous platinum oxide as a catalyst for the hydrosilylation of para- and ortho-substituted diarylalkynes in cis-fashion.²³ In contrast to the better-known stereoselective approach for the semi-reduction of diarylalkynes detailed by Lindlar,²⁴ which suffers from such shortcomings as formation of alkane due to over-reduction and Z-to-E isomerization, the reported method presents addition of H–Si bond in stereoselective cis-manner, and the regioselectivity of the reaction is independent of the choice of platinum catalyst but is instead governed by ortho-directing effects (ODE).²⁵

The generic synthetic scheme (Scheme 4) involved the silylation of alkynes 28 in the presence of a platinum catalyst, followed by desilylation by tetrabutyllammonium fluoride in tetrahydrofuran. Optimization studies included an analysis of the activity of various platinum sources such as Pt₂O, PtCl₂, Pt/C, PtCl₄ and Speier’s catalyst (H₂PtCl₆). The best conditions were found to be Pt₂O with loading of 7 mol%, 1.5 equivalents of HSiOEtMe₂ followed by treatment with 1.5 equivalents of tetrabutyllammonium fluoride at 0 °C. The synthetic route was modified to make it a one-pot process by exploiting the volatility of HSiOEtMe₂. A variety of CA₄ analogues with electron-donating as well as electron-withdrawing substituents were synthesized by this method. CA₄ was synthesized with a Z/E ratio of 9:1. Overall, this new method provided a mild, chemoselective protocol for the synthesis of (Z)-stilbenes.

Another efficient route to CA₄ and analogues uses the Perkin condensation between aldehydes and aryl acid derivatives mediated by acetic anhydride and triethylamine (Scheme 5).²⁶ Commercially available 4-methoxyphenylacetic acid (31) was first brominated to obtain the Perkin condensation precursor 3-bromo-4-methoxyphenylacetic acid (32). The aryl acid derivative 32 was then condensed with 3,4,5-trimethoxybenzaldehyde in a reaction mediated by acetic anhydride and triethylamine by heating at 130 °C for five hours to furnish 1,2-diarylacrylic acid 33 with excellent stereoselectivity (Z/E = 19:1). Hydroxylation was then performed to obtain the acrylic acid derivative 34, which underwent decarboxylation with assistance of quinoline and copper powder at 220 °C. CA₄ was obtained in 71% yield via this method.

Camacho-Davila reported the first example of the utilization of a Kumada–Corriu cross-coupling reaction for the synthesis of CA₄ (Scheme 6).²⁷ The Grignard component of the Kumada–Corriu coupling, 38, was synthesized by bromination of the easily available guaiacol (35) in a three-step process. The free hydroxy was then protected with a tert-butylimidethylsilyl group followed by reaction with magnesium to obtain the key intermediate 38. The halide reagent was prepared by subjecting 3,4,5-trimethoxybenzaldehyde to Corey–Fuchs conditions (CB₃, P₃H) followed by palladium-catalyzed reduction to obtain the Z-isomer 41 in a stereoselective process. The key coupling step was performed with ferric acetylacetonate as catalyst to achieve the O-silylated product with retention of stereochemistry. The final product 3 was obtained.
by desilylation, mediated by potassium fluoride dihydrate, in 40% overall yield. The efficiency of the protocol, in achieving stereoselectivity in the synthesis of CA4 with a metal-catalyzed coupling reaction as key step, is noteworthy.

![Scheme 6](image)

**Scheme 6** Synthesis of CA4 by Kumada–Corriu cross-coupling reaction

### 3 Synthetic Approaches for the Preparation of Analogues of CA4

Since the initial discovery of combretastatin and the subsequent determination of its absolute configuration by Pettit et al. in 1987,28 a plethora of studies exploring the structure–activity relationship (SAR) of combretastatin-based compounds have been reported. These studies have focused on multiple substitutions on the phenyl rings (both ring A and ring B) as well as variations to the structure of the bridging moiety between them (Figure 3).

The structural features of CA4 required for the robust inhibition of tubulin assembly include a bridging moiety connecting two phenyl rings with a cis disposition. Although the conformation of tubulin-bound CA4 has not been analyzed completely, the fact that the tubulin binding site is very flexible has been confirmed.29 To maintain the structural integrity of the compounds that display inhibition of tubulin assembly, prevention of isomerization of the cis-compounds is mandatory. To achieve this goal, various methodologies have been adopted to lock the compound in the desired cis conformation.

### 3.1 Modification of the Bridging Moiety

Amongst the structural modifications envisaged on the CA4 structure, modification of the bridging moiety is a very appealing option since it opens avenues for incorporating a large variety of functional groups and substituents to achieve optimum biological results.30

To impart structural rigidity to the compounds, the incorporation of different functional groups, particularly cyclic moieties, at the bridge connecting the two phenyl rings has found many applications. A recent study exploiting this postulate has resulted in the synthesis of a series of cyclopentanone analogues of CA4.31 Jaggi and co-workers prepared 2,3-diaryl-4/5-hydroxycyclopent-2-en-1-one analogues of CA4 by replacing the cis double bond with 4/5-hydroxycyclopentanone. The synthetic route (Scheme 7) consists of the generation of furylithium from the reaction of furan with n-butyllithium in tetrahydrofuran and its subsequent condensation with a substituted benzaldehyde. The resultant 2-furylmethanol was then converted into 2-aryl-4-hydroxycyclopentenone by employing zinc chloride as a weak Lewis acid.32 Next, the hydroxy group on 44 was protected by tert-butyldimethylsilyl chloride. The protected cyclopentanone intermediate was then coupled with various aryl halides via palladium-catalyzed Heck reactions in the presence of potassium carbonate as base and tetrabutylammonium bromide (TBAB) as an additive to obtain the 4-hydroxy series of compounds 45.

The 5-hydroxy series of compounds was prepared by treating the cyclopentenone intermediate 46 with substituted aryl halide in the presence of magnesium or n-butyl-
Further reaction of the tertiary alcohol with pyridinium dichromate in dichloromethane afforded the desired product via allylic rearrangement of the chromate ester. Following the synthetic protocol, a series of 56 new compounds was synthesized and analyzed for biological activity. Assessment of their cytotoxicity, as well as apoptotic and tubulin assembly inhibition properties, was carried out. The in vitro cytotoxicity data indicated superior potential for the 5-hydroxycyclopent-2-en-1-one compounds 50 and 51, shown in Figure 4. These compounds demonstrated a very high cytotoxicity with IC_{50} < 2.7 nM in a panel of human cancer lines consisting of oral, larynx, ovary, colon, lung and pancreas cancer cells. Further analysis of these compounds for tubulin polymerization inhibition activity revealed a significant difference between 50 and 51. Compound 51 was found to be more strongly anti-tubulin with a low IC_{50} value of 1.75 μM.

In a separate study, Flynn and co-workers synthesized inhibitors of tubulin assembly with structural features similar to those of CA4. The work focused on incorporating structural rigidity analogous to CA4 by introducing indanone and indenone motifs in the desired molecules. Initially, aldehydes were brominated to obtain dibromostyrenes 55 and 56 (Scheme 8) which were then converted into lithium phenylacetylides by treatment with n-butyllithium. The lithium phenylacetylides were utilized for preparation of diarylpropynones 57 and 58. Next, the authors utilized their previously developed palladium-catalyzed hydrostannylation methodology for the preparation of chalcones. Synthesis of diarylpropynones was followed by the key step of palladium-catalyzed hydrostannylation coupling to obtain the desired chalcones 59. Although stereochemical retention is typically observed in the hydrostannylation reaction, the chalcones were isolated as a mixture of both Z- and E-isomers. The presence of a catalytic amount of triphenylphosphine has been acknowledged to be the reason for this stereochemical outcome. Next, the indanones 60 were prepared by performing a Nazarov cyclization of the 2-aryl- and 2-aroylchalcones using cupric triflate or methanesulfonic acid. The indanones were then oxidized to the corresponding indenones 61 by 2,3-dichloro-5,6-dicyanoquinone (DDQ). All indanone compounds were isolated as the trans isomers. Various chalcones, indanones and indenones prepared were tested for their tubulin polymerization inhibition activity and were compared to the activity of CA4. The chalcones were determined to possess better tubulin inhibitory properties than indanones and indenones and were also evaluated for activity in inhibition of MCF-7 breast cancer cells. However, none of the compounds displayed inhibition of these cancer cells, whereas CA4 itself performed well against them, with activity in the nanomolar range.
Pinney and co-workers have attempted to incorporate the structural features of combretastatin compounds by preparing benzosuberene analogues of combretastatin. The synthetic route involved the preparation of \( \text{68} \) and \( \text{70} \) (Scheme 9) as key intermediates. A hydroxy group was first introduced on 6-methoxy-1,2,3,4-tetrahydronaphthalene (\( \text{62} \)), followed by protection of the 5-hydroxy regiosomer \( \text{63} \) as the isopropoxide, leading to the formation of \( \text{65} \). The protected tetralin derivative was then regioselectively oxidized at the benzylic position to obtain tetralone \( \text{66} \). The ketone was transformed into a methylene unit by performing a Wittig reaction. Formation of \( \text{67} \) preceded the key ring-expansion step, which was achieved by utilization of cyanogen azide to obtain the important intermediate \( \text{68} \). Ketone \( \text{68} \) was converted into regioisomer \( \text{70} \) by subjection to Clemmensen reduction conditions, followed by oxidation via chromium trioxide. The key intermediates \( \text{68} \) and \( \text{70} \) were then coupled with lithiated trimethoxybenzene in parallel reactions. The coupling products were dehydrated through an acetic acid catalyzed process, then the hydroxy group was deprotected by the use of a Lewis acid to obtain the desired compounds \( \text{74} \) and \( \text{75} \).

By following the presented synthetic scheme, the authors prepared various benzosuberene analogues by incorporating distinct functionalities on the aromatic rings of \( \text{74} \) and \( \text{75} \), and they performed biological testing to evaluate the products’ activity in tubulin polymerization and non-

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**Scheme 8** Synthesis of CA4-based indanone and indenone derivatives

**Scheme 9** Synthesis of benzosuberene anti-tubulin compounds
small cell lung cancer, prostate cancer and ovarian cancer cell lines. The SAR studies confirmed 75 as the most active compound. The cis disposition of the two aromatic rings analogous to CA4 potentiates the activity of this compound. The compound was found to have nanomolar activity against the three cancer cell lines and is thus a very promising candidate for further studies and development as an effective anti-cancer agent.

Apart from the above-discussed elaborate synthetic schemes, a few easy-to-prepare combretastatin analogues have also been explored. One such attempt led to the synthesis of carbazole sulfonamide analogues.37 The preparation of carbazole sulfonamides was preceded by the synthesis of the carbazole sulfonyl chloride intermediates 77 (Scheme 10) from commercially available carbazoles 76.38 The carbazole sulfonyl chloride precursors were then treated with different anilines in the presence of stoichiometric equivalents of triethylamine to furnish the desired carbazole sulfonamides 78 in moderate to excellent yields.

Scheme 10 Synthesis of carbazole sulfonamide anti-tubulin compounds

The general structure of the synthesized compounds and a few representative examples are presented in Figure 5. The synthesized compounds were evaluated for activity in human leukemia cells. The SAR studies revealed that the presence of an alkyl group on the nitrogen at the 9-position is required for strong activity in these compounds. On replacing carbazole with dibenzofuran (i.e., 82), a loss of potency was observed. Therefore, the presence of a carbazole moiety is central to the success of this series of compounds. The studies also revealed that, unlike in CA4 itself, the presence of the three methoxy substituents on ring A is not a pharmacophoric requirement for activity in this series of analogues, since the compounds with replaced methoxy groups on ring A were found to be equally potent.

Figure 5 General structure and representative examples of anti-tubulin carbazole sulfonamides

In a different study, Alami and co-workers replaced the cis double bond of CA4 with a 1,2-diketo functionality by way of a user-friendly synthetic protocol that involved a metal-mediated cross-coupling reaction (Scheme 11).39 The diarylalkynes prepared via Sonogashira cross-coupling reactions were oxidized to achieve the desired benzil compounds. The authors modified their previously reported oxidation protocol using iron tribromide and dimethylsulfoxide40 to include nitrile, phenols and anilines by replacing the iron catalyst with palladium(II) iodide. The modified catalytic system performed exceedingly well in furnishing the desired benzils with various functionalities including acetates, acetamides, fluorine and heterocycles on ring B in a user-friendly protocol. The compounds were found to possess excellent antiproliferative activity at nanomolar concentrations.
3.2 Modification of the Bridging Moiety via Heterocyclic Functionalities

In addition to the above-mentioned studies, a major volume of work related to the modification of the CA4 bridging moiety has focused on the use of heterocyclic rings for attaining the stereochemical congruity and structural features of CA4 which are required for optimum biological activity. The presence of heteroatoms in a cyclic moiety around the bridge not only helps in retaining the bioactive configuration of the molecule, it also makes the targeted compounds amenable to further manipulations, thus leading to a broader variety of SAR studies. Some of the different heterocyclic groups utilized for various SAR studies have included azetidinones,41 isoxazoles,42 imidazoles,43 triazoles,4g pyrazoles,44 epoxides,44 thiophenes,45 benzo[β]thiophenes46 and furanones.47

In a recent report, Welsh and co-workers studied the incorporation of a triazole ring at the bridge of CA4-based anti-tubulin compounds.48 Alteration of the CA4 B-ring has also been studied. The synthetic route consisted of preparation of amides 88 (Scheme 12) starting from the reaction of different amines with 3,4,5-trimethoxybenzoyl chloride (87). The amides were then converted into thioamides 89 by Curphey’s method, utilizing P4S10 and hexamethyldisiloxane.49 The method furnished thioamides with various B rings in quantitative yields. To obtain indolyl-ring-incorporated compounds, the use of Lawesson’s reagent facilitated obtaining the desirable thioamide product. Next, the thioamides were treated with hydrazine at 0 °C to obtain amidrazones 90 which were cyclized by the use of triethylorthoformate under acidic conditions.

A series of B-ring substituted compounds were synthesized, including compound 96 (Figure 6) in which the B-ring was substituted with an indole group. The X-ray crystal structure of compound 96 was also elucidated.50 Biological evaluation of the compounds revealed potent inhibition of tubulin polymerization and cytotoxicity against an array of cancer cell lines including multi-drug-resistant (MDR) cancer cell lines. Inhibitor–protein interactions were also studied by molecular modeling and dynamics simulations. The molecular dynamics simulations on the tubulin–96 complex suggested that 96 interacts comprehensively with β-tubulin, but, in α-tubulin, it is involved in hydrophobic interactions with only Ala and Val residues. The conclusions supported the general consensus that colchicine binds preferentially to β-tubulin.33,51

![Scheme 12](image)

**Scheme 12** Synthesis of triazole-based inhibitors of tubulin polymerization

In another recently reported study, the triazole moiety was utilized for the bridge modification in anti-tubulin compounds. Hansen and co-workers prepared a series of triazolium-CA4 analogues. Huisgen cycloaddition served as the key reaction in the preparation of these compounds (Scheme 13).52

Initialy, 3,4,5-benzaldehyde was converted into the terminal alkyne 98 through the use of Colvin rearrangement conditions (LDA, TMSCHN2, −78 °C).53 The magnesium acetylide of 98 was generated by treating it with ethyl magnesium chloride. The acetylide thus generated then reacted with azide 99 in the Huisgen cycloaddition44 as the key reaction in the synthetic scheme. The process led to the formation of the desired triazole derivatives of CA4 in
moderate to excellent yields. Following this method, a series of regioisomeric cis-triazole compounds was prepared and evaluated for cytotoxicity. After initial screening against leukemia cancer cell line (K562), selected compounds were analyzed for their tubulin-assembly inhibition activity. Although both 107 and 111 displayed tubulin assembly inhibition at micromolar concentrations, they were found to be less active than CA4. Compound 111 was nevertheless tested in six human cancer lines. It displayed nanomolar activity in the assays and was found to be comparable in activity to CA4. Molecular modeling studies revealed that the observed activity of 111 was due to its binding affinity to the colchicine binding site of α,β-tubulin.

In another instance of nitrogen-based heterocyclic compounds being utilized for bridge modification in anti-tubulin compounds, Moses Lee and co-workers studied various pyrazoline-based CA4 derivatives (Figure 7).55 A previous study in Lee’s laboratories pertaining to pyrazole-based CA4 derivatives revealed an unanticipated attenuation of bioactivity of the compounds.44 X-ray crystallographic analysis suggested that the aromaticity-induced planar structure of the pyrazole ring was the mitigating factor in pyrazole-based compounds, as compared to the twisted geometry of CA4.56 As a non-aromatic substitute for the pyrazole ring, the authors explored the utilization of pyrazoline moiety in the targeted compounds. A number of pyrazoline derivatives were synthesized by the reaction of hydrazine hydrate with the corresponding chalcones (Scheme 14). Positive activity of N-acylated pyrazolines in the inhibition of kinesin spindle protein (KSP) prompted the authors to synthesize N-acylated derivatives of CA4 and test them as potential therapeutic agents for the treatment of cancer. The synthesized compounds were subjected to in vitro cytotoxicity screening. The bioactivity data indicated that pyrazoline compounds are, indeed, suitable candidates for anti-cancer studies. The results also revealed that the presence of an acetyl group on the pyrazoline nitrogen is detrimental to the biological activity of the compounds.

Scheme 13 Synthesis of triazole-based CA4 analogues with a Huisgen cycloaddition as the key reaction

Scheme 14 Synthesis of pyrazoline-based anti-tubulin derivatives

Figure 7 Pyrazoline derivatives of CA4
Pui-Kai Li and co-workers reported the use of an inhibitor of tyrosine kinase, SU5416,53 which is a receptor for the vascular endothelial growth factor, as a model for the design of analogues of CA4.54 In preceding work, the authors reported the 2-indolinocontaining compound 122 (Figure 8) as a growth inhibitor for prostate and breast cancer cell lines with nanomolar IC₅₀ values.55 The authors recognized the structural congruency of SU5416, compound 122 and CA4 and decided to study the biological activity of compound 122 and analogues.

![Figure 8 Structures of SU5416 and SU5416-based CA4 analogues](image)

For the synthesis of the 3-benzylideneindolin-2-ones 123, the precursor 6-alkoxyindolin-2-ones 127 were first prepared by literature procedures as depicted in Scheme 15.56

Synthesis of 6-substituted 3-benzylideneindolin-2-ones was achieved via coupling of the prepared indolin-2-ones 127 and substituted benzaldehydes in the presence of piperidine. The coupling reaction furnished predominantly the 6'-isomer of the final product. The configuration of the compounds was assigned based upon the chemical shifts of protons at the C-2' and C-6' positions in the phenyl ring and NOE experiments. The chemical shifts for the protons are approximately 7.45–7.84 ppm for the E-isomers and 7.85–8.53 ppm for the Z-isomers.

The synthesized compounds were tested for biological activity with a special focus on the activity of compound 122. A screening of compound 122 in 53 different cancer lines revealed that the compound was very effective with GI₅₀ below 10 nm in 46 out of the 53 cancer lines. The compound was found to be active in colon, renal, prostate and CNS cancer lines with concentrations of less than 10 nm. To determine the effect of 122 on the progression of cancer cells, a DNA profile comparison of pancreatic cancer cells at different concentrations was performed. The analysis with podophyllotoxin, a known anti-mitotic, anti-microtubule agent as a control, revealed that 122 caused G₂/M-phase cycle arrest in pancreatic cancer cells (PC-3).

The series of synthesized compounds was included in the SAR for biological activity against prostate and breast cancer cell lines. The SAR showed that the presence of three methoxy groups on the benzylidene ring plays an important role in imparting the activity to the compound. It was found that the number of methoxy groups required for optimum activity is indeed three. Moreover, the three methoxy groups are most active at 3-, 4- and 5-positions, as any alteration of number of methoxy groups or their positions on the benzylidene rings diminished the compound’s activity. Furthermore, substitution of the three methoxy groups with three methyl or three ethyl groups also led to a loss of cytotoxicity. Conceivably, this effect is due to a change in electronics of the aromatic ring as well as an incorporation of a different hydrogen-bonding capacity. A similar decrease in cytotoxicity was observed upon substitution of the 6'-methoxy on the 2-indolino ring of compound 122. A complete loss of cytotoxicity was observed upon replacement of the 6'-methoxy with hydrogen. Replacement of the methoxy group with other longer and branched derivatives also resulted in a decline in cytotoxicity. Only a 6'-ethoxy substituent maintained activity comparable to that of the methoxy group. In agreement with 122 being the most cytotoxic compound of the series, it was also established as the most potent in its ability to inhibit tubulin polymerization with an IC₅₀ of 4.5µM.

![Scheme 15 Synthesis of SU5416-based CA4 analogues](image)

Pontikis and co-workers used a metal-mediated tandem Heck–carbocyclization–Suzuki coupling protocol to prepare oxindole-based combretastatin analogues.61 In a previous study, the authors reported the synthesis of diene derivatives 128 (Figure 9).62

To restrict the structural flexibility of the two aromatic rings on 128, the authors decided to incorporate aromatic substituents between the double bond via a tandem Heck–Suzuki coupling approach. Similar domino methodologies have been reported in stereoselective syntheses of heterocyclic derivatives such as indolines,60 isoindolines,64 indanes65 and indoles.60 The key reaction for obtaining the
desired compounds involved the coupling of commercially available 133 or 135 with suitable alkynamides 132 for cyclization to obtain (E)-3-arylmethylenoxindoles 134 and (E,E)-3-alkylideneoxindoles 136 (Scheme 16).

For the preparation of alkynamides, 2-iodoanilines 67 (130, Scheme 16) were coupled with propynoic acid or but-2ynoic acid using dicyclohexylcarbodiimide. 68 The resulting anilides 131 were then reacted with benzyl bromide or methyl iodide with sodium hydride as the base to get the desired alkynamides 132.

With the alkynamides in hand, the key step of palladium-catalyzed coupling was then performed with the respective boronic acid. The process was catalyzed by 5 mol% palladium(II) acetate and 10 mol% triphenylphosphine in the presence of a base. Use of cesium fluoride as base afforded an excellent stereocontrol over the reaction, furnishing exclusively the desired E-isomer. The product was obtained with yields as high as 80%.

Duan, Matteucci and co-workers postulated that the presence of a nitrogen at the 2-position along with the oxygen of the carbonyl group could promote chelation with metals (137) or hydrogen-bonding with water (138) (Figure 10). Such chelation would reduce the conformational flexibility of the molecule, making it susceptible to mimicking a tubulin inhibitor such as CA4. To test their theory, they synthesized a series of 3-arylindazoles with a focus on substitutions on the C-7 position of the indazole moieties. 69

![Figure 9](image-url) General structure for diene derivatives of combretastatins

![Figure 10](image-url) Metal-chelation and hydrogen-bonding in indazole derivatives and general structure of the synthesized compounds

The synthesis of the desired compounds was achieved by the coupling of 5-iodo-1,2,3-trimethoxybenzene (140) with trimethylsilylacetylene, and palladium as catalyst, followed by desilylation to give 141 (Scheme 17). 70 Next, 5-ethynyl-1,2,3-trimethoxybenzene (141) was coupled with 4-iodo-3-nitroanisole in a palladium-catalyzed, modified Sonogashira-type coupling to give 142 which was reduced by iron to yield 5-methoxy-2-(3,4,5-trimethoxyphenylethynyl)phenylamine 143. The indazole core in 144 was obtained by cyclization of 143. 71

The iodination of (6-methoxy-1H-indazol-3-yl)-(3,4,5-trimethoxyphenyl)methanone (144) was performed with good regioselectivity to produce the 7-iodoindazole 145 derivative with 85% yield (Scheme 18). A second Sonogashira coupling was performed with terminal alkynes to get the desired 7-substituted indazoles 146, which were further derivatized to obtain 147 and 148.

All synthesized compounds were analyzed for cytotoxicity towards a human non-small lung cancer cell line, H460. A comparison revealed that the presence of the acetylene moiety in the indazole compounds vastly improved the cytotoxicity. Compounds 149, 150 and 151 (Figure 11) were found to be the most active, with IC50 values of 1 nM, 8 nM and 3 nM respectively.
Compounds 149, 150 and 151 were further studied for activity in multi-drug-resistant cancer cell lines (MDR-1 and MRP-1). All three compounds displayed strong antiproliferative activities, with IC\textsubscript{50} values between 1.6 nM and 3.9 nM.

In addition to the nitrogen heterocycles, sulfur has also been incorporated into the structural modifications to achieve optimum anti-cancer activity. In a study with thia-

diazole–linker-based combretastatin analogues, Yang, Ding and co-workers reported the design and synthesis of two series of 4,5-disubstituted 1,2,3-thiadiazole analogues of CA4 (152 and 153, Figure 12). The synthetic route for achieving the synthesis of thiadiazole derivatives of the general structure 152, as shown in Scheme 19, involved the phosphonation of 3,4,5-trimethoxybenzaldehyde (154) by Pudovik reaction with dibutylamine. 155 was then protected by tetrahydropyran to obtain 156 before it was condensed with the desired benzaldehyde 157. The condensation was followed by acidic hydrolysis to obtain deoxybenzoins 158. In the final step, the substituted deoxybenzoins 158 were condensed with toluenesulfonohydrazide in ethanol and the intermediates were treated with thionyl chloride in the key cyclization reaction to obtain the desired compounds 152.

For the synthesis of thiadiazides of general structure 153, 3,4,5-trimethoxybenzaldehyde (154) was first transformed into its tosylhydrazone 159 and then treated with the substituted benzaldehydes 157 to yield the desirable deoxybenzoins 158 in the final step, the substituted deoxybenzoins 158 were condensed with toluenesulfonohydrazide in ethanol and the intermediates were treated with thionyl chloride in the key cyclization reaction to obtain the desired compounds 152.

The degree of antiproliferative activity of the complete series of compounds was determined against human myeloid leukemia cells, human colon adenocarcinoma cells and human microvascular endothelial cell lines. The most potent cytotoxic compounds from the synthesized series were analyzed for interactions with the microtubule system and compared with the control compound CA4. The inhibition potency in the polymerization of tubulin was also tested. Several compounds displayed activity that was equipotent to that of CA4 in the study. The effects of the compounds on tubulin were also confirmed via indirect immuno-staining.

Figure 11  7-Acetylene-substituted aroylindazole analogues of CA4

Figure 12  Thiadiazole derivatives of CA4
The authors utilized a flow cytometry assay to investigate the thiadiazole-targeted phase of the cell cycle which causes disruption leading to the antiproliferative activity of the compounds. The studies displayed an increase in cells in G2/M phase along with a concurrent reduction in S and G1 cells as a function of concentration of the thiadiazole compound being tested. The results were compared with those of the control compound CA4, indicating that the thiadiazole compounds lead to G2/M arrest in the cell cycle causing the aforementioned antiproliferative activity.74

Moses Lee and co-workers synthesized thioxopyrimidine analogues of CA4 with a concise synthetic approach (Scheme 21).75 The classic base-catalyzed Claisen–Schmidt condensation of substituted acetophenones 161 and benzaldehydes 162 was performed to obtain chalcones 163.76 The chalcones were then refluxed in ethanol in the presence of excess thiourea and potassium carbonate to obtain the desired series of 1,2,3,4-tetrahydro-2-thioxopyrimidine (164) analogues of CA4. Molecular modeling studies performed on the synthesized compounds revealed that, indeed, the prepared thioxopyrimidine compounds possessed the twisted geometry essential for bioactivity in CA4-based compounds.56 However, in comparison to CA4, the compounds were found to be less active in biological studies. Nevertheless, the protocol provided a user-friendly route of preparing CA4 analogues with increased solubility in aqueous media.

With regard to oxygen-containing heterocycles, Barbier and co-workers reported on the interaction of 4-arylcoumarin analogues of combretastatins with the microtubule network and analyzed their binding to tubulin.77 Compounds 165, 166 and 167 (Figure 13) were prepared by way of a method previously outlined by the authors.78 The synthesis involved treatment of 4-hydroxycoumarins 168 with triflic anhydride to obtain the 4-trifluoromethylsulfonyloxycoumarins 169.79 Next, coupling of various boronic acids was performed with the triflates 169 via Suzuki–Miyaura cross-coupling protocol to obtain the desired coumarin analogues of CA4 (Scheme 22).
Amongst the various cross-coupling reactions, the Suzuki–Miyaura reaction is one of the most valuable tools in synthetic chemistry.\(^8\)\(^0\) It has been extensively studied and reported to include both heteroaryl as well as sterically hindered unactivated substrates.\(^8\)\(^1\) Barbier and co-workers took advantage of the versatility of Suzuki–Miyaura reaction in a process catalyzed by palladium with copper as co-catalyst to prepare the coumarin analogues of CA4.\(^8\)\(^2\)

The cytotoxicity of the synthesized 4-arylcoumarin combretastatin analogues was analyzed in human breast cells. The study revealed that compounds 165 and 166 altered the cell cycle progression and induced apoptosis, while compound 167 did not show any viable activity in binding tubulin.

To further analyze the effects of CA4 and its coumarin analogues on the microtubule network of human breast cancer cell line (HBL100), immunofluorescence staining of the microtubule network was performed. Visualization and DNA content analysis of the microtubule network revealed compound 165 to be more active than compound 166. However, the concentration required to obtain an activity comparable to that of CA4 was a 100-fold excess.

Ampac 7.0 software was utilized to optimize compounds 165, 166, 167 and CA4 with the semiempirical AM1 method for molecular modeling studies. Superimposition of CA4, 165, 166 and 167 in the tubulin colchicine site indicated that the methoxy group in the 7-position of ring A plays an important role in imparting activity to the compounds (Figure 13). Furthermore, an increase in hydrogen-bonding capabilities by the substituents on ring C also play a vital role in imparting potency to these compounds with respect to the anti-tubulin activity they display.

Utilizing a similar triflate–boronic acid/ester Suzuki–Miyaura route, Beletskaya, Combes and co-workers synthesized polymethoxylated-4-heteroarylcoumarins.\(^8\)\(^3\) The protocol employed a Pd(dppf)Cl\(_2\)/K\(_2\)CO\(_3\)/TBAB catalytic system (Scheme 23) in place of the Pd(PPh\(_3\))\(_4\)/Na\(_2\)CO\(_3\)/CuI system used by the Barbier group.\(^7\)\(^7\) The authors were able to synthesize various compounds with heteroaryl boronic acids or esters, including benzofuran, indole, pyrimidine, pyridine and quinoline analogues, with excellent yields ranging from 71% to 98%. Evaluation of the products’ biological activity in breast cancer cell lines revealed the indole-substituted coumarin analogues to be the most potent amongst the tested compounds.

### 3.3 Macrocyclic Rings Affording Conformational Restriction to Combretastatin Analogues

Pettit and co-workers reported some pioneering work in the isolation and characterization of macrocyclic diaryl ether lactone inhibitors of tubulin polymerization.\(^8\)\(^4\) Medarde, Pelaez and co-workers studied the feasibility of retaining the structural conformation and restricting the compound to cis-form, essentially preventing isomerization without modifying the bridging double bond.\(^8\)\(^5\) Medarde reported the synthesis of macrocyclic structures in which the para-positions of the A- and B-rings of combretastatin are linked through a five- or six-atom-chain linker, thereby affording conformational restriction to the compound. Two families of compounds with different linkers were synthesized (Figure 14).

The two families differed in the length of the linker chain as well as in the presence or absence of an extra oxygen atom as part of the linker. The substituent X on the B-ring was limited to H and OH. A third modification included replacement of the B-ring itself with the more structurally...
rigid indolyl group, which had shown potent activity in a previous study.86

The synthesis of the desired compounds followed the synthetic strategy outlined in Scheme 24. Starting from a double Mitsunobu reaction87 of the linker diol with the corresponding phenolic aldehydes, the di-aldehydes 178–183 were obtained. These were then subjected to the intramolecular McMurry pinacol conditions88 to furnish the desired macrocyclic CA4 analogues 184 and 185.89 However, the presence of a nitro group proved detrimental to the reaction outcome and resulted in the formation of a mixture of side-products. Amongst the synthesized compounds, 184 resulted in the formation of two diastereomers, while better stereoselectivity was observed in the case of 185 as only the trans isomer was obtained.

A conformational analysis of the compounds revealed strict restrictions on the macrocyclic compounds as compared to CA4.90 The aromatic rings were found to be less orthogonal than in colchicines and padophyllotoxin. The rotation of rings in these compounds is extremely hampered because of the linkers and the presence of substituents on rings. These restrictions are somewhat attenuated for the compounds of family II as compared to those of family I with the shorter 3-oxapentamethylene linker. Consequently, family I compounds did not show any significant biological activity. Although the compounds belonging to family II were found to be active in displaying anti-tubulin activity, they performed with less potency than CA4. The authors proposed that, contrary to the desired increase in potency of the compounds via the use of linkers, the presence of sterically hindered linkers in the para-position could be the reason for low potency of these compounds. Although previous studies have shown that CA4 analogues with bulky groups on the B-ring maintain moderate to high potency,91 the presence of linkers on the para-position of the aromatic rings of macrocyclic analogues of CA4 was found to be detrimental to the optimum activity of the compounds.

4 Amine Substituents on Aryl Rings of Combretastatin Analogues

The effect of the presence of various substituents on the aryl rings of the CA4 structure has been studied. In these cases the absence of a functionality to lock the compound in the cis form makes the structure susceptible to a possibly detrimental isomerization. However, the presence of the various substituents helps in docking the drug molecule at the colchicine binding site, thereby making these analogues potent for inhibition of tubulin assembly.

Following a report by Pinney and co-workers detailing the synthesis of various nitrogen-containing combretastatin derivatives including compound 188,92 Liou and co-workers synthesized a series of amino-combretastatins and evaluated their anti-tumor activity.93

The synthetic strategy for the preparation of these compounds involved a Wittig reaction between nitro-substituted benzaldehyde 187 or 190 (Scheme 25) and 3,4,5-trimethoxybenzyl triphenylphosphonium chloride (186) or 4-methoxybenzyl triphenylphosphonium bromide (191) as the key reaction. The Wittig reaction was followed by the reduction of the nitro group using zinc in acetic acid to furnish 188 and 189, and 192–194, respectively.
Scheme 25 Synthesis of aminocombretastatin analogues

For the synthesis of compounds 198 and 200 (Scheme 26), the ylide 196 was first prepared via reaction of triphenylphosphine with 1-bromomethyl-3,4,5-trimethoxy-2-nitrobenzene (195). Wittig reaction of 197 and 199 with 196 was followed by reduction with zinc and acetic acid to obtain the desired compounds 198 and 200.

The synthesized compounds were tested against five human cancer cell lines as well as multi-drug-resistant cell lines for anti-tumor activity. Compounds 189, 192, 198 and 200 displayed significant antiproliferative activity with IC50 values ranging from 11 to 55 nM. Upon being investigated for in vitro tubulin polymerization inhibitory activities and colchicine binding activities, compounds 189, 198 and 200 displayed activity comparable to that of CA4. The compounds were found to be superior to colchicine.

In a separate study, Pinney and co-workers elaborated upon their previous extensive work to present the synthesis and biological studies of similar amino-combretastatin compounds. Once again, the key step for the strategy adopted by the authors involved a Wittig reaction. The precursors for Wittig reaction were prepared by the steps outlined in Scheme 27.

Scheme 27 Synthesis of precursors for Wittig reaction leading to the preparation of amino-combretastatins

3,4,5-Trimethoxybenzyl alcohol (201) was brominated with phosphorous tribromide followed by treatment with triphenylphosphine to yield 3,4,5-trimethoxybenzyl triphenylphosphonium bromide 202. To prepare nitrobenzaldehyde precursors 205 and 206, benzyl bromide 203 was hydrolyzed by refluxing it in acetone–water mixture. The benzyl alcohol thus obtained was treated with pyridinium chlorochromate to obtain the benzaldehyde intermediate 204. Nitration of 204 gave a mixture of nitrobenzaldehydes 205 and 206 which were separated by column chromatography.

Precursor 202 was treated with sodium hydride to generate ylide 207 (Scheme 28) which was then treated with 205 or 206 under Wittig reaction conditions. This yielded both Z- and E-isomers of the nitro-substituted compounds 208. The stereoisomers were separated by flash chromatography and the nitro groups were reduced by zinc and acetic acid, to provide the desired amino-substituted compounds.
tuated combretastatin analogues 209. Biological testing of the compounds that revealed di-amino substitution on the 2′- and 3′-positions of the B-ring provided the most potent anti-tumor activity.

5 Conclusions

A survey of recent studies highlighting significant advances in the development of various structural motifs and methodologies for the synthesis of anti-cancer stilbenoids has been presented. Structural requirements outlining profound effects on the desired biological activity have been studied in these reports. The structural features targeted in these studies have included optimization of molecular volume produced by additional atoms between the aromatic rings corresponding to the original structural template of CA4, restrictions imposed upon the aryl rings, and variation in functional group compatibility.

Diverse synthetic transformations (such as Wittig reaction, Suzuki–Miyaura coupling, and Huisgen cycloaddition) have been used to achieve the synthesis of biologically active compounds with the incorporation of these structural features. The variation of synthetic strategies has allowed for the efficient preparation of CA4 analogues with the desired stereo-, regio- and chemoselectivity.

While these developments underscore the significance of efforts being directed towards finding better methodologies with a goal of achieving biologically active anti-cancer combretastatin-based compounds, further research aimed towards achieving greater cytotoxicity and anti-mitotic activity in CA4 analogues is foreseeable.

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