University of North Carolina at Asheville
Journal of Undergraduate Research
Asheville, North Carolina
May 2014

Design and Synthesis of Heterocyclic Combretastatin Analogues

Benjamin J. Shields
Department of Chemistry
The University of North Carolina at Asheville
One University Heights
Asheville, North Carolina 28804 USA

Faculty Advisor: Dr. Herman Holt Jr.

Abstract

This article provides an overview of the small molecule cancer treatment drug Combretastatin A-4 (CA-4) followed by progress towards a divergent synthesis of three CA-4 analogues. CA-4 is the lead drug in a subclass of microtubule inhibitors known as vascular disrupting agents. Issues with drug longevity, water solubility and normal cell toxicity have lead to the search for analogues with CA-4 as a model. Analogues bearing different bridging groups and heterocyclic rings have proven to be more effective than Combretastatin A-4 against many cancer cell lines. With this in mind our group has set out to prepare 2-(1,2,3-trazolyl)indole, 2-aroylindole and 2-styryl indole analogues of CA-4. The Hemetsberger-Knittel method was used to prepare substituted indoles from α-azidocinnamates in good yields. Regioselective annulation was observed during thermolysis of unsymmetrical α-azidocinnamates yielding 2-methoxy-5,6-disubstituted indole esters. Subsequent oxidation state adjustments and coupling provided 2-aroylindoles. One carbon homologation gave substituted ethynyl indoles as functional dipolarophiles in catalyzed 1,3-dipolar cycloadditions to produce a 2-(1,2,3-triazolyl)indole.

1. Introduction

1.1. Medicinal Chemistry

Advances in research and development in pharmaceutical agents have progressed rapidly over the past century. In particular, a vast quantity of chemical and biological knowledge has been accumulated so that drugs may be designed and synthetically prepared. However in spite of the dramatic increase in synthetic prowess and physiological understanding, natural products still hold the mainstay in drug discovery and as of 2007 more than 70% of new drugs introduced since 1981 were isolated from natural sources.[1] Mother Nature is still the best drug designer but the value of synthetic techniques cannot be overstated in the optimization and production stages of drug development.

The correlation between molecular shape and biological activity can be evaluated through structure activity relationship (SAR) studies and can produce knowledge of the pharmocophore for a drug. In SAR studies hypotheses about binding mechanisms are made and tested by comparing biological activities of varying molecular shapes, substitution patterns, electronic and spatial arrangements. For this paper any mention of SAR study or activity results are grounded in quantitative comparison of IC_{50} (half maximal inhibitory concentration) values produced by other groups for tubulin inhibition and cytotoxicity towards various cancer cell lines.

1.2. Small Molecule Cancer Treatment Drugs

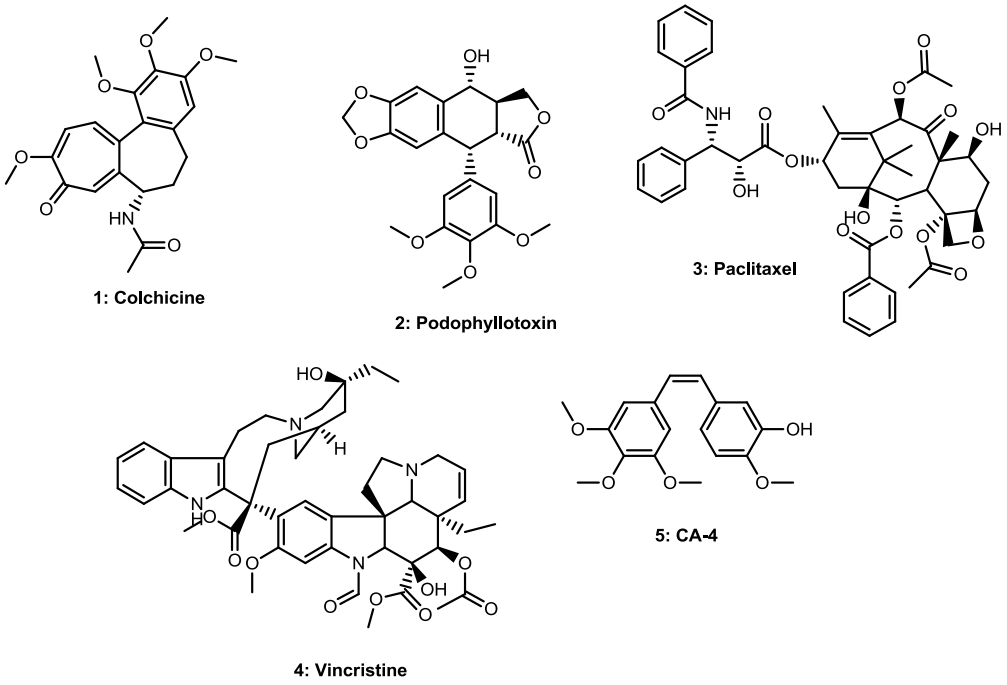


Figure 1. Structures of microtubule interfering agents. Colchicine domain binding agents: Colchicine (1), Podophyllotoxin (2) and Combretastatin A-4 (5). Taxol domain binding agent, Paclitaxel (3). Vinca alkaloid domain binding agent, Vincristine (4).

Medicinal chemistry has had far reaching effects on research in chemotherapeutics for the treatment of cancer. Cancer is a disease of the cell cycle, thus it was envisioned that treatments could been designed based on the events that lead up to cell replication. This assertion was based on the fact that neoplasm divide uncontrollably but still progress through the cell cycle similarly to normal cells. The progression of cellular events is highly ordered and requires that certain events must be completed before others are begun. For example beginning mitosis before completion of DNA replication would obviously be detrimental to the cell.[2,3]

The interruption of cell cycle events is typically discussed in terms of cell cycle check points, which for present purposes are events that are susceptible to external chemical manipulation. External as in not native to the chemical process underlying the biological event. For example during the first growth phase (G_1), a cell accumulates the components that it will use to synthesize DNA. Hence it was envisioned that if a cell's ability to accrue the necessary nutrients for DNA replication could be hampered cell cycle progression could be stopped at the end of G_1 and anti-metabolites have indeed been developed that accomplish this task.[4] Another strategy for halting the cell cycle is based on preventing the replication of DNA during S phase. Drugs like the platinum compound *cis* platin covalently bond to DNA and make cellular replication otherwise impossible and have been utilized in chemotherapy.[4] A third category of compounds known as the microtubule interfering agents affect events during the second growth phase (G_2) and mitosis (M). Drugs of this type like the colchicine (**1**), podophyllotoxin (**2**) and taxol (**3**) (figure 1) have been used in chemotherapy and it is this category that the synthetic targets of this work fall into.[5]

1.3. Interfering With Microtubule Dynamics

Microtubules are made of α- and β-tubulin dimers which polymerize together to form protofilaments which then associate into a cylindrical microtubule. Microtubules are multifunctional protein polymers that comprise the cytoskeleton which maintains cellular structure, form the mitotic spindle which aligns the chromosomes for separation during mitosis, and are involved in intracellular protein trafficking. Microtubules have been considered an ideal target for anticancer drugs for some time because of their essential role in mitosis in which they segregate the chromosomes so that each daughter cell has a full genome from the parent cell.[5,6] Microtubules are dynamic

polymers which alternate between periods of growth and shortening. The frenzied polymerization and disassociation of microtubules, known as dynamic instability, is energy expensive but fundamental in the diversity of their cellular functions.[5]

Drugs that target microtubule dynamics act by interfering with the delicate interplay between growth and shortening stages. Microtubule interfering agents are either microtubule stabilizers or microtubule destabilizers. Microtubule stabilizers like paclitaxel (3)(figure 1), which was isolated from the Pacific Yew tree and is the most successful drug of this type to date, promote growth and lead to over polymerization.[5] Microtubule destabilizing agents have a number of different binding mechanisms but the net result is the prevention of polymerization at low concentrations and induced disassociation at higher concentrations. The vinca alkaloids like vinblastine and vincristine (**4)** and colchicine domain binding agents like combretastatin A-4 CA-4) (**5**) and podophyllotoxin (**2**) are microtubule destabilizing agents (figure 1).[7] Inherent problems like lipophilicity and toxicity of drugs of this type has lead to problems with drug delivery and has spurred the search for more water soluble and less toxic counterparts.

1.4. Combretastatin A-4 (CA-4)

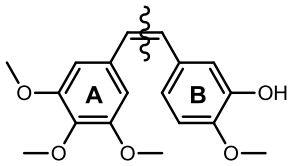


Figure 2. Structure of Combretastatin A-4.

The Combretastatins were first isolated and characterized by Dr. George Pettit *et al* from the South African Bush Willow (*Combretum caffrum*). Shrubs from the genus *Combretum* had been employed as primitive medicine in Africa and India for the treatment of leprosy, mental illness and cancer for some time.[8,9] The *cis* stilbene combretastatin analogue 4 (CA-4) (**5**) is by far is the most bioactive of the combretastatins (of the numerous compounds isolated and biologically evaluated by *Pettit et al*).[8,9] CA-4 is the lead compound of a class of colchicine domain binding microtubule interfering agents. The disodium phosphate prodrug (CA-4P) made it to stage 3 clinical trials, under the trade name zybrestat, for the treatment of solid tumors and phase 2 clinical trials for the treatment of macular degeneration.[10] CA-4's main interest is as a therapeutic vascular disrupting agent (VDA) though it does have some antiangiogenic affects as well. CA-4 causes the cells lining a tumors vascular network to swell which cuts of vital oxygen and nutrients. Moreover, CA-4 does so without many of the side effects associated with chemotherapy like hair loss and bone marrow damage. The main side effect of CA-4 is pain in the area of the tumor, as VDAs act through a necrotic pathway rather than the natural apoptotic pathway.

Since discovery three decades ago hundreds of CA-4 analogues have been synthesized and evaluated as potential chemotherapeutics. The driving forces of the explosion of research in this area is CA-4's potency as a therapeutic VDA, small size relative to other microtubule interfering agents and some intrinsic issues with the model drug. That is, CA-4 has poor metabolic stability, poor water solubility and observed normal cell toxicity.[11] One particular consequence of CA-4's small size is that it has undergone extensive SAR studies and has a well established pharmocophore. Specifically the 3,4,5-trimethoxy A-ring, *cis* confirmation bridge, and *para* methoxy on the B-ring are all requisite for binding at the active site.

1.5 Structure Activity Relationship

1.5.1. A-Ring

Various substitution patterns on the A-ring have been explored yielding specific steric and electronic properties that optimize effectiveness of CA-4 analogues. It is well established that the presence of trimethoxyphenyl moiety is vital for full activity based on the significant loss of tubulin inhibition that resulted from the exclusion of a meta or para methoxy or replacement with bulkier groups.[11] Substitution of larger ethoxy groups and the formation of a dioxolane ring resulted in significant loss in activity which suggest that the sterics and substituent mobility play pivotal roles in binding at the colchicine site.[11] Exceptions have been found in a series of fluorine containing

analogues that maintained activity. However, when bulkier halogens like bromine and chlorine were present a decrease in activity was found which further suggests that substituent size plays a large role.[10] It has also been demonstrated that when methoxys are substituted for methyls, cytotoxicity is lost but selective tubulin inhibition is maintained which presents a potential solution to the observed normal cell toxicity of CA-4.[11] However CA-4's damaging effects may be a result of some metabolite, in which case improving *in vivo* stability would play a more pivotal role in decreasing toxicity. CA-4's trimethoxy A-Ring has proven to be fundamental for colchicine domain binding by SAR studies and the reoccurrence of this motif in relevant natural products such as colchicine and podophyllotoxin.

1.5.2. *Cis* Olefin Bridge

The single most important factor for CA-4 like tubulin inhibition is the *cis* olefin bridge linking the A and B-rings. This is evident in the disparity in activity between *cis* and *trans* CA-4 where the *trans* isomer is more thermodynamically favorable but nearly void of activity. *Cis-trans* isomerization is intrinsic to stilbenes and anything from heat to light at ultraviolet and near visible wavelengths can induce a confirmation change. The implications of this are twofold in that drug longevity both *in vivo* and in storage is poor. For this reason maintenance of the *cis* olefin bridge is a fundamental problem with CA-4 and has been a major research topic. Much emphasis has been placed on stabilizing or "locking" the *cis* confirmation through structural modifications to the olefin like epoxidation or direct azirdination, modifications that have been explored by our group and others in the past. An attempted Jacobsen epoxidation of CA-4 by Pettit et al. yielded no epoxide but did produce phenstatin (**8**) as a side product which has a carbonyl bridge linking the A and B rings.[12] Phenstatin was the first nonstilbene CA-4 analogue that showed bioactivity comparable to that of CA-4. Another successful example of bridge modification are the chalcones. In fact a chalcone (**7**) with the same A and B ring substitution pattern as CA-4 proved to have greater activity than CA-4 by Ducki et al.[14] The significance of this finding is that few colchicine domain drugs display higher activity than CA-4 and that chalcones can be synthesized in a one step condensation between substituted benzaldehydes and acetophenones.

1.5.3. B-Ring

According to SAR studies the only substituent on CA-4's B ring that is fundamental for full bioactivity is the para methoxy. However methylation of the B ring methoxy position mirrors the affect of methylation of the methoxys on the A ring and results in decreased cytotoxicity but retained tubulin inhibition.[11] CA-4's B ring has the most susceptibility to change without adversely affect bioactivity. For this reason this is where numerous attempts to reconcile the inherent water solubility problems of CA-4 have been made. The presence of the meta hydroxyl group has allowed CA-4 salt prodrugs like CA-4P to be made with relative ease. However replacement with fluorine has given analogues with significant cytotoxic activity and it has also been proposed that the presence of the highly elecronegative fluorine should preclude electrophilic substitutions, thus leading to a more metabolically stable compound.[11] Unfortunately fluorine substitution precludes the prodrug approach again posing solubility issues.

1.5.4. Combretastatin Analogues

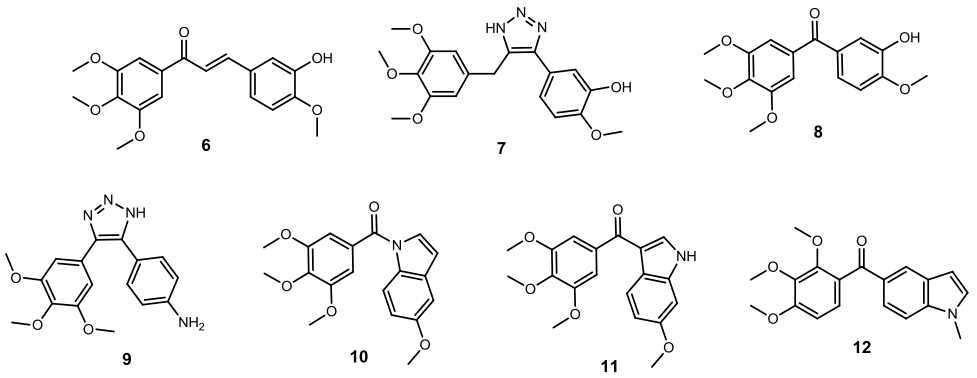


Figure 3. CA-4 analogues. Chalcone (6), triazoles (7) (9), phenstatin (8), indole phenstatins (10)(11)(12).

Many of the first modifications to CA-4s structure were centered around variation of the A and B-rings because most attempts to change the bridging group led to analogues void of activity.[11] However the advent of phenstatin[12] (**8**)(figure 3) and its noteworthy retention of activity catalyzed more research in bridge modifications. This together with the work of Oshumi and coworkers[13] who observed that when the structures of colchicine and CA-4 were superimposed, there was a good match, and postulated that the bridge region was amenable to expansion. Five-membered heterocyclic rings such as imidazoles, 2-cyclopenten-1-one, pyrazole, triazoles and numerous others were prepared and indeed most retained both antitublin and cytotoxic behavior and some even showed activity higher than that of CA-4 (figure 3),[11] The triazole (**7**, **9**) and indole (**10,11,12**)(figure 3) ring systems have proven to maintain activity but may also provide opportunities for hydrogen bonding and thus increase water solubility.[15] Moreover nitrogenous systems are ubiquitous throughout the human body (purines, pyrimidines and tryptophan for example) so it follows that their presence may provide increased metabolic stability.

2. Design of Indole and Triazol based CA-4 Analogues

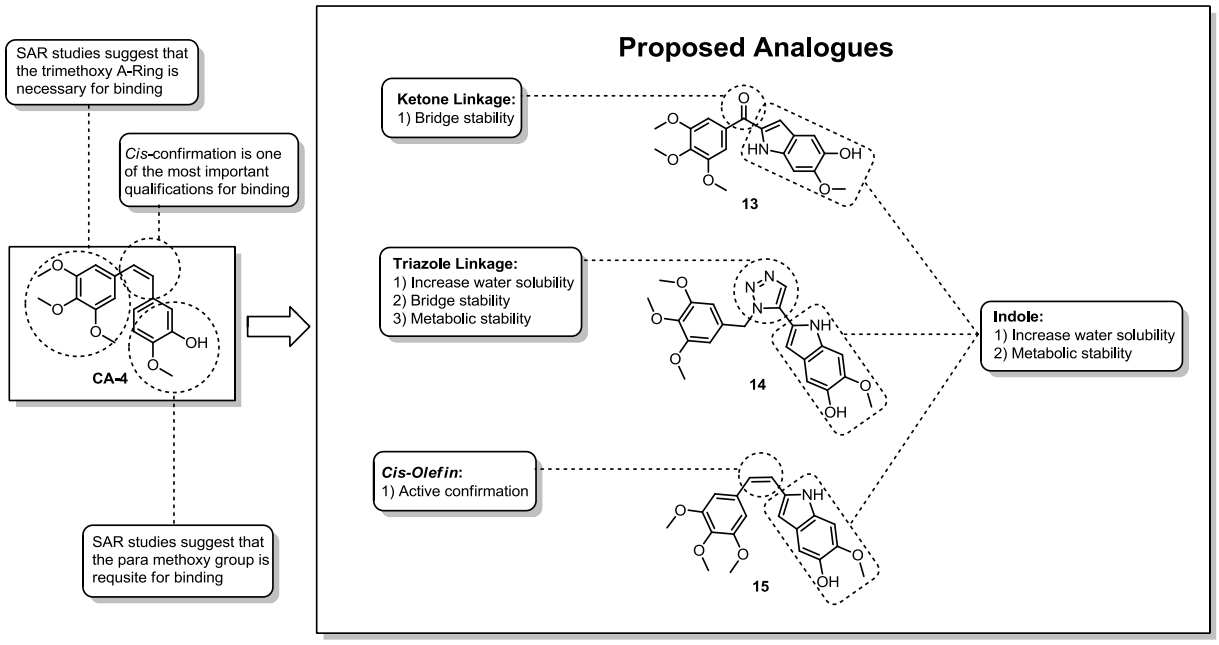


Figure 4. Design of novel indole and triazolyl indole based CA-4 analogues.

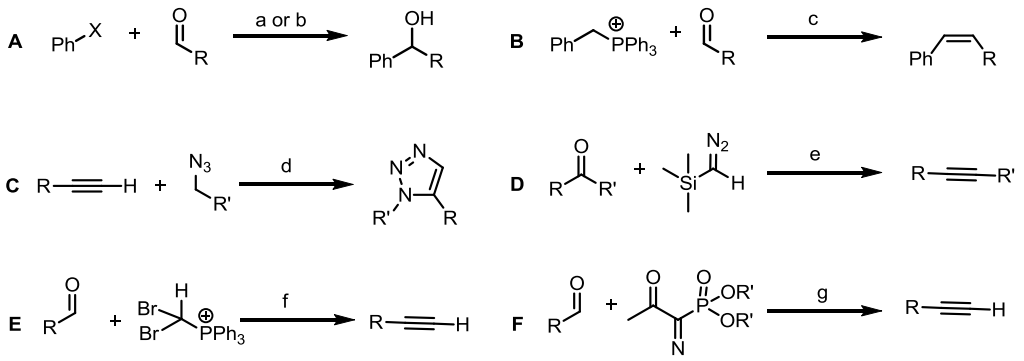
Recent work has shown that CA-4 analogues modification with both indole and triazole ring systems maintain activity and some indole phenstatin analogues even produce greater bioactivity (Figure 3).[16] In addition CA-4 substituted chalcones (**6**)(figure 3) have proven to be potent microtubule inhibitors. With these observations in mind the analogues displayed in figure 4 were proposed containing 5-hydroxy-6-methoxyindolyl B-rings and ketone or 1,2,3-triazole bridging groups. Phenstatin analogue **13** (figure 4) is a 2-aroylindole and has a structural backbone that resembles a confirmationally locked CA-4 substituted chalcone. The ketone linking group should add bridge stability and lock the 2-aroylindole analogue into an active confirmation. In addition the added hetero atoms may increase the water solubility. The 1,2,3-triazole moiety of analogue **14** (figure 4) may provide bridge stability and lock the molecule into an active confirmation as well as improve water solubility. In addition the triazole moiety may improve metabolic stability. It is noteworthy that there may be some entropic penalty for the presence of a benzyl group rather than a phenyl. 2-Styryl indole analogue **15** has a *cis*-olefin bridge, analogous to CA-4 and an indole B-ring which may affect water solubility and metabolic stability. All proposed analogues were designed around the indole moiety and thus synthetic planning naturally revolved around designing a divergent synthesis, based on a common indole precursor.

3. Synthesis

3.1. Retrosynthetic Analysis

Synthesis was centered around the reactivity of an aldehyde functionality. Thus synthetic planning was carried out based on the preparation of an indole aldehyde as the precursor to both target molecules. From the indole aldehyde there was a clear root to 2-aroylindole ketone analogue **13**. It was noted that a protecting group strategy would have to be developed for the labile indole hetero protons. For **13**, it was noticed that the ketone functionality was the basic retron of a organometallic coupling reaction (Scheme 1A) (e.g. Grignard or lithium halogen exchange)[17,18], which would produce a secondary alcohol that would have to be oxidized.

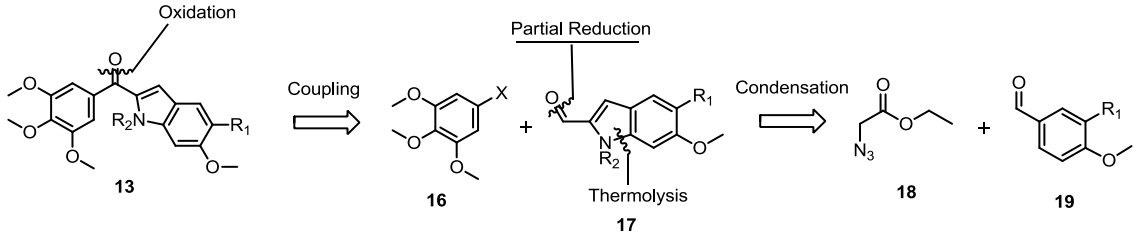
Scheme 1. Ring coupling and homologation reactions: A. organometalic coupling, B. 1,3-dipolar addition, C. Ru catalyzed cycloaddition, D. Colvin rearrangement, E. Corey-Fuchs homologation, F. Seyferth-Gilbert homologation.



Reagents and conditions: a) $Mg_{(s)}$,THF,-78°C , b) *t*-BuLi,THF,-78°C c) n-BuLi, THF, -78°C, d) Ru(cat.), e) THF,-78 °C, f) i. THF, *t*-BuOK, r.t. ii. *t*-BuOK, g) K_2CO_3 (2 eq), MeOH, r.t.

Applying a coupling transform to **13** (Scheme 1) 3,4,5-trimethoxyhalobenzene (**16**) was obtained, which was commercially available and thus dropped out of the retrosynthetic scheme. For indole aldehyde (**17**), it was recognized that a 2-alkyl substituted indole is the basic retron of the Hemetsberger-Knittel indole synthesis.[19,20] It was recognized that the Hemetsberger-Knittel method produces indole 2-carboxylic esters, thus the ester moiety would have to be reduced to the desired aldehyde. Applying the Hemetsberger-Knittel transform produces ethyl azidoacetate (**18**) and substituted benzaldheyde (**19**), both of which are commercially available. Details about the Hemetsberger-Knittel method may be found in the following section.

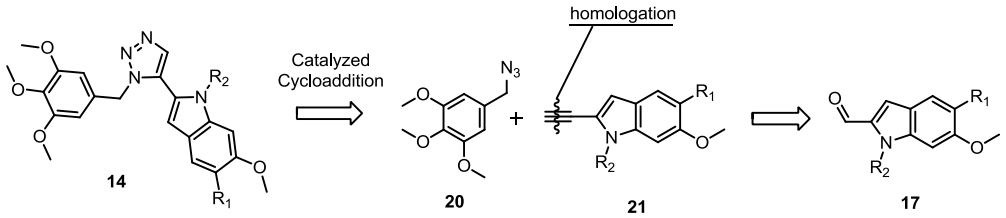
Scheme 2. Retrosynthetic analysis of indole phenstatin analogue **13**.



In regards to the specific coupling method, our group has attempted to couple substituted aromatic halides via lithium halogen exchange and Grignard methods. It was found that aromatic halides that bear electron donating groups undergoing magnesium exchange react in an unpredictable manner. However the lithium halogen exchange reaction approach has been used on similar substrates with success in the past. Thus the method of choice was a lithium halogen exchange reaction resulting in a secondary alcohol that would have to be oxidized to a ketone.

For analogue **14** (Scheme 3), it was recognized that that the 1-benzyl-1,2,3-triazole moiety was the basic retron of a catalyzed [2+4] cycloaddition[25] (Scheme 1C) between ethynyl indole **21** and readily available 3,4,5-trimethoxybenzylazide **20**. Finally, the alkyne functionality was to be prepared by homologation of **17**.

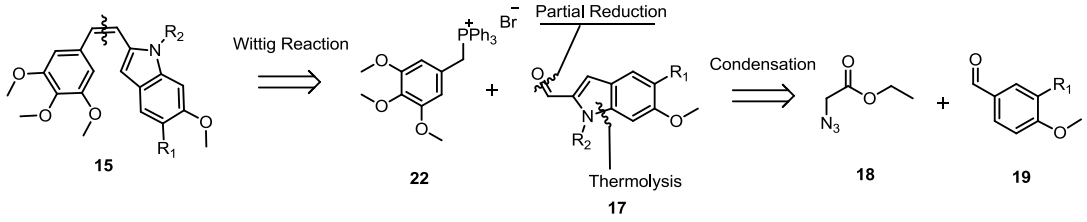
Scheme 3. Retrosynthetic analysis of 1,2,3-triazole analogue 1**4**.



In the past our group has prepared ethynyl indoles from indole alkynes via Corey-Fuchs homologation (Scheme 1E)[26,27] but with undesirably low yields and unavoidable chromatographic purification. Thus it was necessary to survey different homologation methods of which Seyferth-Gilbert homologation (Scheme 1F)[28,29,30] and the Colvin rearrangement (Scheme 1D)[31,32] were noticed as possible alternatives to the Corey-Fuchs method. The Seyferth-Gilbert method uses dimethyl (diazomethyl)phosphonates (the Seyferth-Gilbert reagent) for efficient, one-carbon homologation of aldehydes and ketones to alkynes with a simple saponification/extraction workup. The mechanism is presumed to be Wittig-like.[29] Despite the obvious value of this method in the past it has often been left out of synthetic procedures because of the multistep procedure required to prepare the Seyferth-Gilbert reagent. In light of this Ohira and Bestmann developed a modified methodology that is of particular interest with cheaper and more easily accessible precursors commercially available.[30] However, in lieu of the available reagents the Corey-Fuchs method was still an acceptable option, while the Seyferth-Gilbert method was being explored.

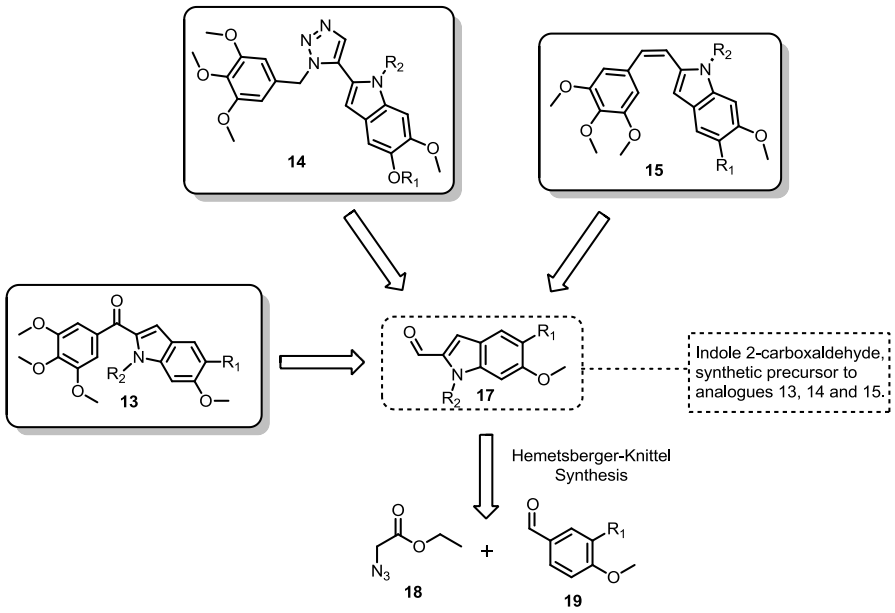
Carbene chemistry revolving around diazomethane has long been used for one carbon homologation. The Colvin rearrangement uses diazomethane for this transformation and offers by far the most efficiency with yields often reported near 100%.[31,32] However there are a number of safety issues associated with using and preparing diazomethane and several deaths have been reported. In particular diazomethane is a contact explosive that may detonate from as little as a scratch in the glassware used for its preparation. In recent years TMSdiazomethane has emerged as a less reactive alternative and has demonstrated similar efficiency in homologation. Though this modified reagent has little risk of detonation it has been found to be extremely toxic and several deaths have been reported due to its use in poorly ventilated areas. Thus the Colvin rearrangement was eliminated as a possibility due to the risks associated with these reagents.

Scheme 4. Retrosynthetic analysis of 2-styryl indole **15**.



The *cis*-olefin moiety on analogue **15** was identified as the retron of a Wittig reaction (Scheme 1B) with indole aldehyde **17** and aryl Wittig salt **22**. As in the previous retrosynthetic analyses, indole aldehyde **17** is retron of the Hemetsberger-Knittel indole synthesis. It was noted that the Wittig reaction would likely produce both *cis* and *trans* isomers of **15**, given the semi-stable ylide resulting from reactions with aromatic phosphonium salts. Thus chromatographic separation was precluded by this method. The combined retrosynthetic analysis can be seen in scheme 5. Notice that **17** is the synthetic precursor to all proposed analogues and thus was naturally the first major synthetic target.

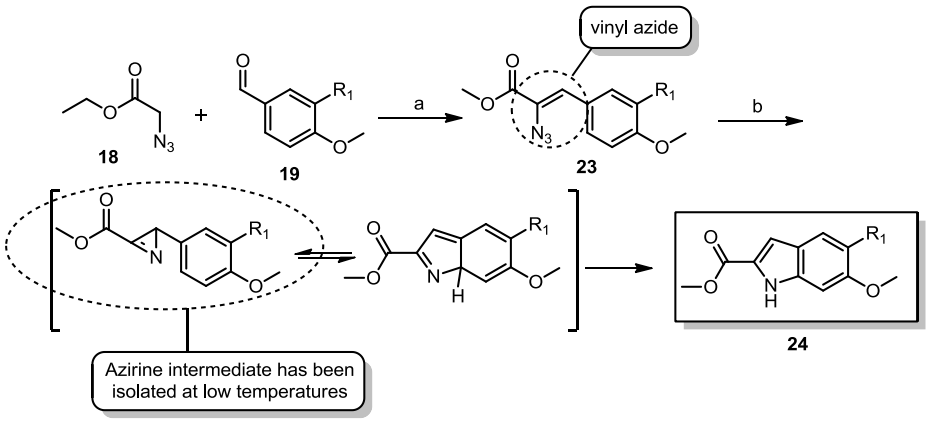
Scheme 5. Combined retrosynthetic schemes. Notice that indole aldehyde **16** is the synthetic precursor to compounds **13**, **14** and **15**.



3.2. B-Ring Synthesis

The optimized synthetic methodology for the preparation of methoxy and trimethoxy indoles were advanced by past group member Tom Graham. It was expected that the same techniques could be applied to preparing indole intermediate **17**, though the reaction scheme had never been carried out in the presence of a Hydroxyl group. Tom Graham precluded the viability of copper mediated aryl amination and Batcho-Leimgruber indole synthesis.[33] Copper mediated aryl amination, used by Fukuyama in a synthesis of the Duocarmycins, was ruled out because it required the synthesis of complex reagents.[34] Batcho-Leimgruber indole synthesis was barred for low overall yields and the use of fuming nitric acid in the nitration step. Even with a viable methodology in place it was necessary to briefly justify the practicality of the Hemetsberger-Knittel method by exploring other methods for preparing 2-subustiuted indoles. Specifically a synthesis starting from benzocyclobutenone[35] and the Madelung indole synthesis[36] were recognized as potential alternatives to our standing methodologies. However following a thorough literature search, it was determined that the Hemetsberger-Knittel method was still the best route. The benzocyclobutanone and Madelung indole synthesis were precluded by the lack of commercially available benzocyclobutenones and *o*-methylanilines.

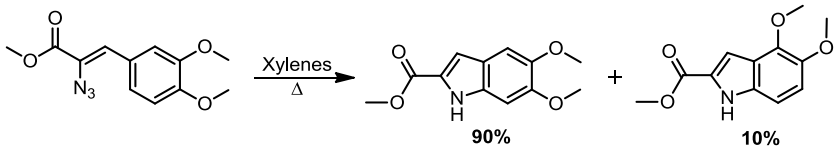
Scheme 6. Hemetsberger-Knittel indole synthesis.



Reagents and Conditions: a. NaOMe, MeOH, b. Xylenes,Δ.

Thermal decomposition of vinyl azides, specifically alkyl 3-aryl-2-azido-propenoates, to form 2-indole esters was first described by H. Hemetsberger and D. Knittel in the late 60s.[19,20] The Hemetsberger-Knittel method (Scheme 6) involves the condensation of a substituted benzaldehyde (**19**) and ethyl azidoacetate (**18**) to form a vinyl azide (**23**) which when dispersed in xylenes and heated to 140 °C produces indole esters (**24**), often in quantitative yields.[19,20,37]

Scheme 7. Regioselectivity of ring closure for 3,4-dimethoxy substrates.

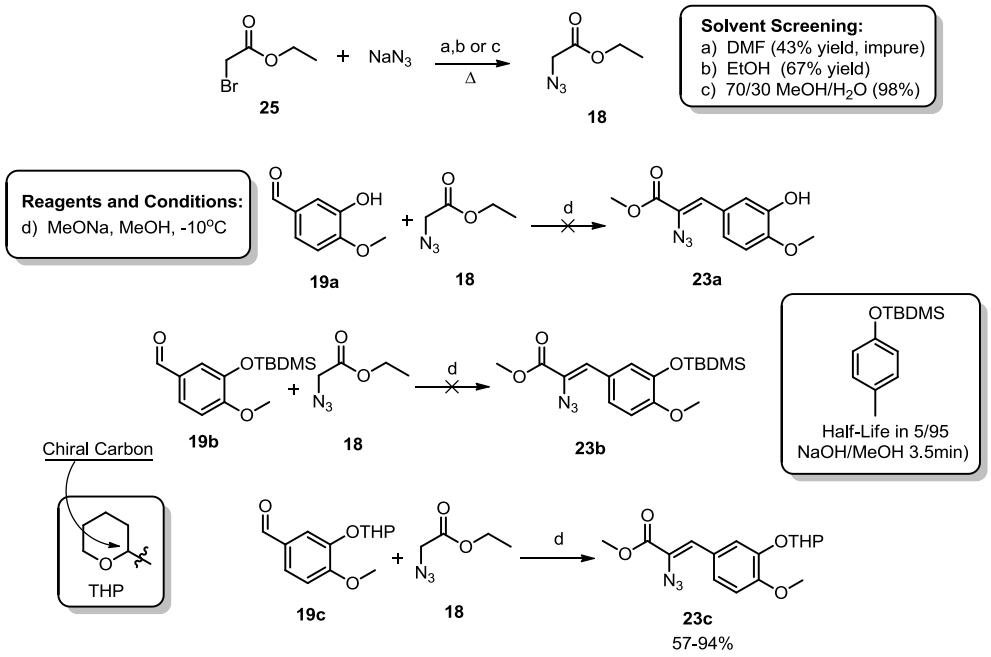


One drawback of the method was the reported lack of regioselectivity when alkyl 3-aryl-2-azidopropenoates having asymmetrically substituted rings were used (Scheme 7), the vary case of this synthesis.[37,38] For example when alkyl 3-(3,4-dimethoxyphenyl)-2-azidopropenoate was thermolized the reaction resulted in a 9:1 mixture of 5,6-dimethoxyindole-2-carboxylate and 6,7-dimethoxyindole-2-carboxylate.[38] This issue may stem from the accepted reaction mechanism (Scheme 6) which proceeds through a azirine intermediate and would be most stable in a staggered arrangement. However, ethyl azido acetate and 3-hydroxy-4-methoxybenzaldehyde are commercially available which makes this method ideal. In addition, the indole aldehyde (**17**) could be easily prepared from the resulting indole ester (**24**) via oxidation state adjustment.

4. Results and Discussion

4.1 Synthesis of Indole B-Ring

Scheme 8. Preparation of vinyl azide **23** and development of protecting group strategy.

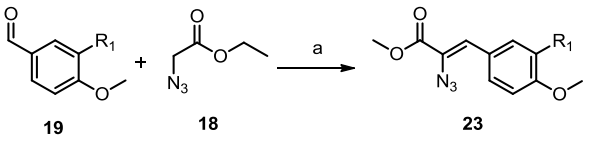


The synthesis of the indole B-ring began with substitution of ethyl bromoacetate **25** with sodium azide under S_N2 conditions (anhydrous DMF) to produce **18** in poor yields with an unknown inorganic impurity (Scheme 8). Through solvent optimization it was found that the yield and purity of the product could be drastically improved by the use of S_N1 type conditions (70/30 MeOH/H_2O, open air) which was likely the result of greater NaN_3 solvation.

The next step involved condensation of **18** and 3-alkyoxy-4-methoxybenzaldehyde **19** to produce vinyl azide **23** (Scheme 8). However, it was found that the reaction would not proceed in the presence of the unprotected hydroxyl group of **19a** and it was necessary to devise a protecting strategy. Initially a tert-butyldimethylsilyl ether (TBDMS) was chosen as the protecting group for the alcohol because of its ease of introduction and removal and its reported stability to a wide variety of reaction conditions.[39] Condensation reactions attempted with the TBDMS protected benzaldehyde, **19b** proved unsuccessful with evidence of deprotection. Closer examination of the literature showed that silyl ethers have undergone rapid base catalyzed hydrolysis in similar conditions (*p*-MeC_6H_4OSi-*t*-$BuMe_2$ Half-Life in 5/95 NaOH/MeOH 3.5min) (Scheme 8).[40] The literature showed that tetrahydropyranyl (THP) ethers had been successfully used as a alcohol protecting groups in condensation conditions similar to those found in this synthesis.[41] Thus, the chosen methodology for the protection of 3-hydroxy-4-methoxybenzaldehyde was with DCM as a solvent because of the non-polar nature of the substrate and dihydopyran (DHP). Pyridinium *p*-toluenesulfonate was used as a catalyst though almost any acidic reagent can be used to introduce the THP group.[40] It is notable that THP as a protecting group has a complicating effect on NMR spectra, in particular the region between 3-4 ppm in the ^1H NMR spectra, likely due to the introduction of a stereogenic center that accompanies its attachment.

Condensation of **18** and **19c** produced **23c** in good yields (Scheme 9). Azide decomposition was found to occur when condensation is carried out at temperatures above 10°C so the reaction is cooled in -10°C as a precaution. Azidoalcohols are formed at temperatures around -30°C so it is important to keep the reaction temperature cool enough that the azide doesn't decompose but not so cold that the desired azidocinnamate isn't produced.[19] Vinyl azides are reportedly light and heat sensitive so the crude product from this reaction is quickly carried on to the next step without purification.

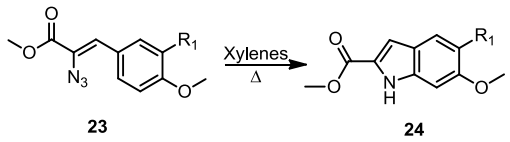
Scheme 9. Preparation of vinyl azide **23**. Note that condensation reactions with hydroxy and OTBDMS substituted benzaldehydes resulted in no product formation.



	Substituents	Percent Yield
23a	R_1=OH	NR
23b	R_1=OTBDMS	NR
23c	R_1=OTHP	57-94
23d	R_1=OMe	48-86
23e	R_1=H	11-43

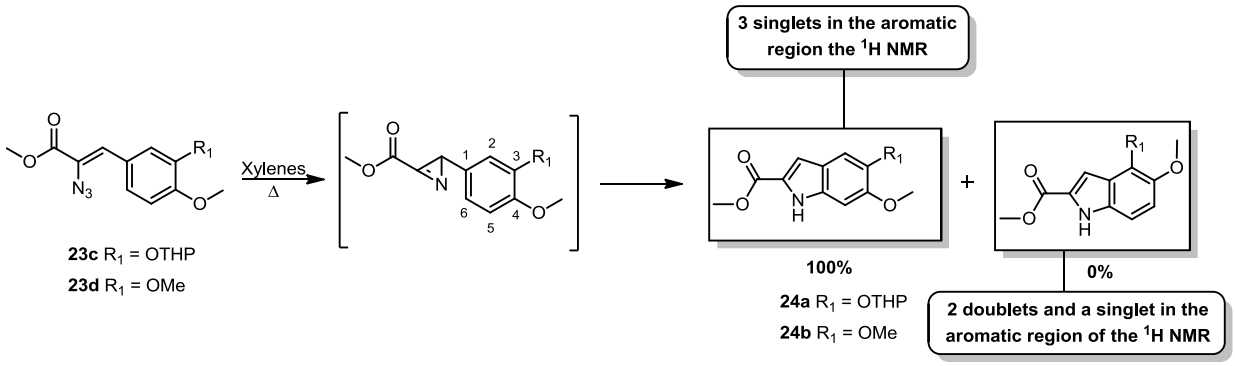
The final step of indole preparation is thermolysis. The substituted vinyl azide, when heated, undergoes an intramolecular rearrangement resulting in the loss of N_2 gas and the formation of an indole. The currently accepted mechanism proceeds through an azirine intermediate with a freely rotating σ-bond which initially was expected to yield two different indole isomers.[37] However the reactions carried out thus far on disubstituted substrates (Scheme 11), including THP protected (**23c**) and 3,4-dimethoxy (**23d**) vinyl azides, have yielded regioselective annulation and 2,5,6- tri substituted indoles contrary to what has been reported[38] in the literature (Scheme 7). The presence of the desired single regioisomer was determined from the splitting patterns in the ^{1}H NMR spectrum for the compounds.

Scheme 10. Thermolysis of vinyl azides.



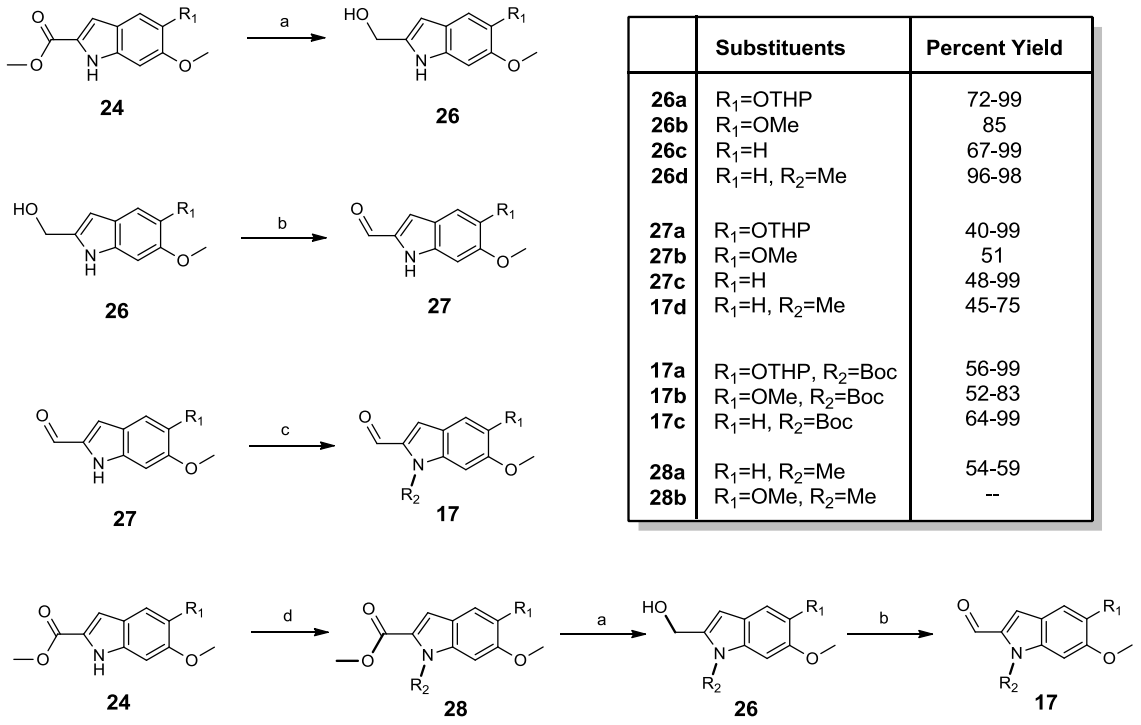
	Substituents	Percent Yield
24a	R_1=OTHP	64-80
24b	R_1=OMe	48-99
24c	R_1=H	67-76

Scheme 11. Thermolysis of unsymmetrical vinylazides.



Next it was necessary to transform the ester moiety of **24** to an aldehyde. Past group members tried to accomplished this conversion with a chemoselective reduction using a bulky reducing agent like diisobutylaluminium hydride (DIBAL) with mixed results. The alternative and chosen method was total reduction of the ester to a 1° alcohol followed by partial oxidation to the aldehyde with 2-Iodoxybenzoic acid (IBX) (Scheme 10). One downside to the IBX oxidation is that it demands the use of DMSO as a solvent, for its poor organic solubility, which can be difficult to remove from the product requiring techniques like lyophilization for removal and contributes to an aggregation of impurities in the final synthetic products. One alternative may be the use Dess-Martin periodinane (DMP), a similar hypervalent iodine reagent, as it has greater solubility in organic solvents.[42]

Scheme 12. Preparation and protection of indole aldehyde.

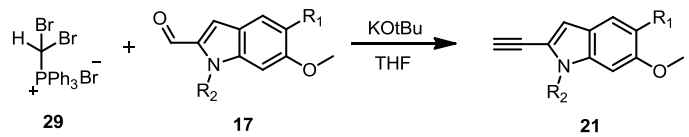


	Substituents	Percent Yield
26a	R_1=OTHP	72-99
26b	R_1=OMe	85
26c	R_1=H	67-99
26d	R_1=H, R_2=Me	96-98
27a	R_1=OTHP	40-99
27b	R_1=OMe	51
27c	R_1=H	48-99
17d	R_1=H, R_2=Me	45-75
17a	R_1=OTHP, R_2=Boc	56-99
17b	R_1=OMe, R_2=Boc	52-83
17c	R_1=H, R_2=Boc	64-99
28a	R_1=H, R_2=Me	54-59
28b	R_1=OMe, R_2=Me	--

Reagents and Conditions: a. $LiAlH_4$, THF, 0°C, b. IBX, DMSO, c. Boc_2O, DMAP, DCM, d. KOH, CH_3I, DMSO

With **27** in hand it was recognized that a protecting group strategy would have to be developed because of the basic conditions necessitated by subsequent homologation and coupling reactions. *t*-Butyl Carbamate (Boc) is a commonly used amine protecting group and is stable to basic conditions. The synthetic methodology chosen for the introduction of the Boc group is with DMAP as a catalyst. It has been show that 1° and 2° amines undergo acylation several orders of magnitude more quickly in the presence of DMAP.[43] The DMAP-Boc complex N-(1-(tert-butoxycarbonyl)pyridin-4(1H)-ylidene)-N-methylmethanaminium is the active catalyst where DMAP functions as a good leaving group.[39] This reaction has produced mixed results where sometimes it has formed pure *N*-Boc indoles and others unidentified impurities are present that complicate ^{1}H NMR spectra. This may indicate that the reaction is more time sensitive than expected and may be due to a side reaction forming isocyanates; a problem reported for sterically hindered amines.[39] Isocyanate formation can be avoided using *N*-methylimidazole rather than DMAP as a catalyst.[39]

Scheme 13. Homologation.

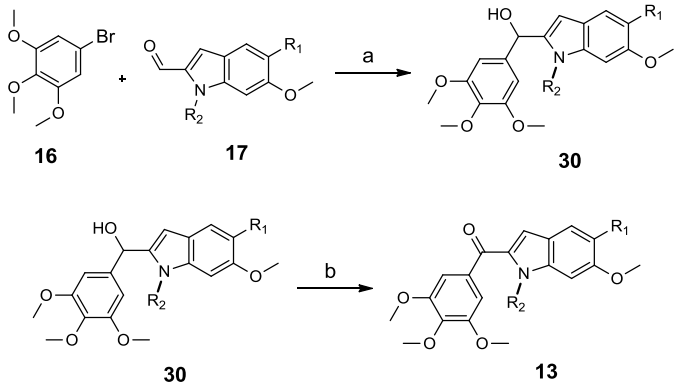


	Substituents	Percent Yield
21a	R_1=OTHP, R_2=Boc	–
21b	R_1=OMe, R_2=Boc	23
21c	R_1=H, R_2=Boc	23
21d	R_1=H, R_2=Me	56

The Corey-Fuchs homologation methodology produced ethynyl indoles in low yields (Scheme 13). Future effort will be focused on applying the Seyferth-Gilbert or Ohira-Bestmann reagents.

4.2. Linkage of A and B-Rings

Scheme 14. Preparation of phenstatin analogue **13** via lithium halogen exchange. Note that the indole nitrogen of 30a and 30b were deprotected under BuLi conditions. This lead to low yields and impure products.

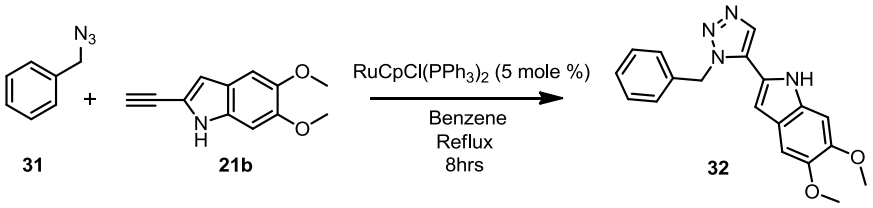


	Substituents	Percent Yield
30a	R_1=OTHP, R_2=Boc	<5
30b	R_1=H, R_2=Boc	<5
30c	R_1=H, R_2=Me	97
13a	R_1=OTHP, R_2=Boc	--
13b	R_1=H, R_2=Boc	--
13c	R_1=H, R_2=Me	89

Reagents and Conditions: a. *t*-BuLi, THF, -78°C, b. IBX, DMSO

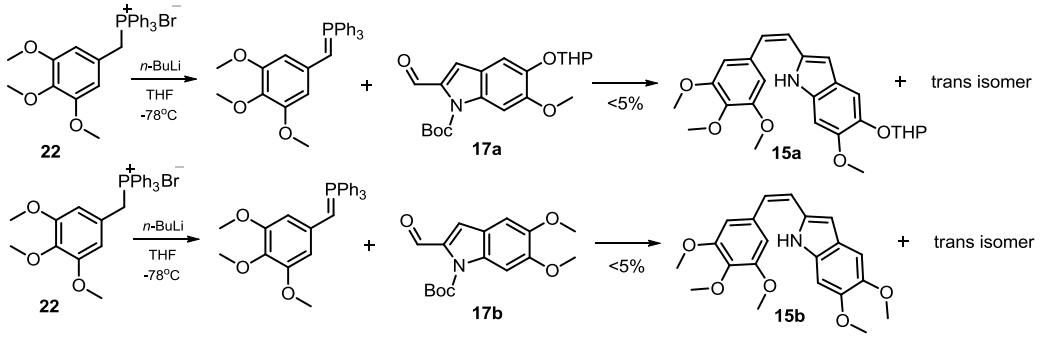
With protected indole aldehyde **17** in hand the next step was to couple **16** via lithium halogen exchange to produce the desired 2° alcohol, **30** (Scheme 14). When **17a** was subjected to lithium halogen exchange conditions, only qualitative formation of the desired product **30a** was observed. Moreover, it was apparent that the indole nitrogen had been deprotected during the course of the reaction. Following this result **17b** and **17c** were prepared to assess the efficacy of Boc protecting groups under BuLi conditions. The results suggest that the Boc group is not stable (Scheme 14). Thus future effort will be placed on designing an alternate protecting group strategy. Subsequent IBX oxidation of the 2° alcohol produced **13c** in good yields.

Scheme 15. Test substrate Ru mediated dipolar addition.



To date, the ruthenium mediated dipolar addition has only been attempted on ethynyl indole test substrate **21b**. This is due to issues associated with preparing both $RuCpCl(PPh_3)_2$ and benzyl azide **31**. The reaction (Scheme 15) produced an impure product but gave promising results. The poor yield may have been due to low catalytic turn over or catalyst poisoning. Having developed methods for preparing $RuCpCl(PPh_3)_2$ and **31**, future efforts will be focused on the optimizing reaction conditions and extending the method to desired analogue **14**.

Scheme 16. Wittig reaction. Note that the indole nitrogen was deprotected during the course of the reaction.



The final step towards the synthesis of 2-styrylindole analogue **15** was a Wittig reaction. When indole aldehydes **17**(**a** and **b**) were subjected to Wittig conditions (Scheme 16), only qualitative product formation was observed. Moreover, as with the previously discussed lithium halogen exchange reaction the indoles were deprotected during the course of the reaction. Due to substrate optimization (Scheme 14) it is now known that the Boc group is unstable in alkyl lithium conditions. Future efforts will be focused on attempting Wittig reactions on methylated indole aldehydes and on developing an alternative protecting group strategy for **17a**.

5. Conclusion

Our group has set out to prepare 2-(1,2,3-trazolyl)indole, 2-aroylindole and 2-styryl indole analogues of CA-4 (compounds **13**, **14** and **15)**. The Hemetsberger-Knittel method was used to prepare substituted indoles from α-azidocinnamates in good yields. Regioselective annulation was observed during thermolysis of unsymmetrical α-azidocinnamates yielding 2-methoxy-5,6-disubstituted indole esters. This finding was contrary to previous reports in the literature. Subsequent oxidation state adjustments and coupling provided 2-aroylindoles and a Wittig reaction produced 2-styryl indoles. One carbon homologation via the Corey-Fuchs method provided substituted ethynyl indoles in moderate yields. A catalyzed 1,3-dipolar cycloaddition reaction of a 5,6-dimethoxyethynyl indole and benzyl azide produced promising results. Future efforts will be focused on optimizing reaction conditions, developing alternate protecting group strategies and evaluating the compounds prepared for antitubulin activity.

6. Acknowledgments

The author would like to express his appreciation to Holt group members past and present for their efforts in laying the groundwork for this project. Additionally, he would like to recognize the University of North Carolina Asheville Chemistry department faculty and staff for their patient guidance. Funding for this project has come from University of North Carolina Asheville URP, Furman Universities NSF REU program and the Philip G. Carson Distinguished Chair of Natural Science.

7. Supplemental Information

7.1. Instrumentation

NMR spectra were obtained with $CDCl_3$ or deuterated DMSO as the solvents on a Varian Gemini 2000 with an Oxford Instruments 200MHz superconducting magnet, Varian Unity INOVA with an Oxford Instruments 400MHz superconducting magnet and Varian INOVA with an Oxford Instruments 500MHz superconducting magnet. A

Thermo-Fischer Scientific Nicolet (Madison, WI, USA) iS10 FT-IR spectrometer , equipped with a Germanium crystal for attenuated total reflectance was employed for infrared measurements. Spectra treatment and data manipulation were carried out on Omnic (Thermo Nicolet Corp., Madison, WI, USA) software.

7.2. Materials

All reagents were purchased from commercial sources and used as received. Prior to use in water sensitive reactions solvents (methanol, DMF, DCM, DMSO, THF, xylenes) were dried over activated 3Å molecular sieves and allowed to sit for 24 hrs before use. THF was also distilled over sodium metal and collected on activated 3Å molecular sieves.

7.3. Experimental Methods

*7.3.1. ethyl azidoacetate (**18**)*

(Note: organic azides are thermally unstable and potentially explosive; reaction should be carried out behind a blast shield.) A 500mL three neck RBF was fitted with a reflux condenser, thermometer adapter and magnetic stir bar. The flask was charged with 35mL of methanol and ethyl bromoacetate (46.76g, 280mmol) and set to stir. A slurry of Sodium azide (20.00g, 307.65mmol) and 20mL of water was added via funnel and the residual was rinsed with an additional 5mL of water. The suspension was then set to stir and refluxed at 70°C over night (~8hr). The reaction mixture was allowed to cool to room temperature, washed with 200mL of water and extracted with 3x150mL of diethyl ether. The organic extracts were pooled, dried over sodium sulfate and condensed under reduced pressure to yield the desired product; a pale oil (35.46g, 274mmol, 98% yield). ^{1}H NMR ($CDCl_3$): δ 4.27 (2H, q), 3.85 (2H, s), 1.31 (3H, d) (ppm). ^{13}C NMR ($CDCl_3$): δ 168.45, 62.01, 50.47, 14.25 (ppm)

*7.3.2. 4-methoxy-3-((tetrahydro-2H-pyran-2-yl)oxy)benzaldehyde (**19c**)*

A 250mL two neck RBF was flame dried, cooled under a stream of nitrogen and fitted with a magnetic stir bar and rubber septum. The flask was charged with 140mL of anhydrous DCM and 3-hydroxy-4-methoxybenzaldehyde (4.75g, 31.22mmol) followed by a catalytic amount of pyridinium *p*-toluenesulfonate (PPTS) (0.20g, 0.80mmol) *(Note: PPTS prepare by adding p-toluenesulfonic acid (1eq) to stirred pyridine (5eq), removing excess pyridine in vacuo and recrystallizing in acetone).* The resulting mixture was set to stir until complete dissolution occurred. 3,4-dihydro-2H-pyran (DHP)(7.84mL,7.23g, 89.95mmol) was dispersed in 10mL of DCM in a separate flame dried, nitrogen flushed two neck RBF and added to the mixture drop wise via syringe. The reaction mixture stirred at room temperature for 2 hours and TLC indicated the presence of starting material so an additional portion of DHP(1.00mL, 0.92g, 10.96mmol) was added drop wise via syringe. After an additional hour of stirring TLC indicated the reaction had gone to completion and the mixture was concentrated under reduced pressure. The concentrate was then washed with 200mL of water and extracted with 2x100mL of diethyl ether. The ethereal extracts where washed with 6x50mL of saturated K_2CO_3 *(Note: A color change from opaque to yellow is observed when the unprotected 3-hydroxy-4-methoxybenzaldehyde is deprotonated, Sat. K_2CO_3 washes were continued until aqueous layer was clear).* The combined organic extracts were dried over sodium sulfate and concentrated under reduced pressure to afford the title compound, a clear oil (6.53g, 27.65mmol, 89% yield). ^{1}H NMR ($CDCl_3$): δ 9.84 (1H, s), 7.64 (1H, s), 7.54 (1H, d), 7.00 (1H, d), 5.47 (1H, t), 3.94 (3H, s), 3.65 (2H, m), 2-1.5 (6H, multiple peaks) (ppm). ^{13}C NMR ($CDCl_3$): δ 191.23, 155.75, 146.78, 130.22, 126.93, 116.76, 111.48, 97.62, 62.62, 56.41, 30,44, 25.34, 19.08 (ppm).

7.3.3. Vinyl azides, general procedure

*7.3.3.1. Methyl-2-azido-3-(4-methoxy-3-((tetrahydro-2H-pyran-2-yl)oxy)phenyl) acrylate (**23c**)*

(Note: Organic azides are thermally unstable and potentially explosive; reaction should be carried out behind a blast shield.) A 100mL three neck RBF, reflux condenser and addition funnel were flame dried and cooled under a stream of nitrogen. The flask was charged with sodium metal (2.07g, 90.00mmol), fitted with a thermometer

adapter and 20mL of anhydrous methanol was added through the addition funnel. The mixture was set to stir until complete dissolution occurred during which a vigorous exotherm was observed *(Note: H_2 gas evolved).* The resulting sodium methoxide solution was cooled to -10°C in a methanol dry ice bath. In a separate flame dried RBF 4-methoxy-3-((tetrahydro-2H-pyran-2-yl)oxy)benzaldehyde (6.50g, 27.53mmol) and ethyl azidoacetate (8.39g, 65mmol) were dissolved in 15mL of dry methanol and stirred until homogenous. The resulting mixture was transferred to the addition funnel via cannulation and the addition funnel was set to a slow drip. The internal reaction mixture was carefully monitored and maintained at -10°C for 4 hours. TLC indicated the presence of starting material so the reaction was left to stir over night (~8hrs) and gradually come to room temperature. TLC indicated completion and the final reaction mixture, a suspension of yellow liquid and tacky yellow-brown precipitate which was poured over saturated NH_4Cl, filtered via vacuum filtration through a Buchner funnel and rinsed with additional saturated NH_4Cl. The filtrate was poured over saturated NH_4Cl and extracted with 4x50mL of ethyl acetate *(Note: the filtrate extracts are impure and should be kept separate from the residue initially collected).* The supernatant was dissolved in ethyl acetate, washed with 200mL of water and the organic extracts collected. Pure product was dried over sodium sulfate and concentrated under reduced pressure to afford the title compound, a gummy brown solid (5.26g, 15.78mmol, 57% yield) which was used without farther purification.
^{1}H NMR ($CDCl_3$): δ 7.73 (1H, s), 7.52 (1H, d), 6.94 (1H, d), 6.90 (1H, s), 5.46 (1H, t), 3.93 (3H, s), 3.92 (3H, s), 3.65 (2H, m), 2.14-1.94 (6H, multiple peaks) (ppm) (note: long range coupling seen in aromatic protons). ^{13}C NMR ($CDCl_3$): δ 164.52, 151.67, 145.92, 126.38, 126.29, 126.16, 123.39, 120.06, 111.77, 97.93, 62.63, 56.22, 53.06, 30.53, 25.43, 19.20 (ppm).

*7.3.3.2. Methyl-2-azido-3-(3,4-dimethoxyphenyl) acrylate (**23d**)*

^{1}H NMR ($CDCl_3$): δ 7.53 (1H, s), 7.37 (1H, d), 6.87 (1H, s), 6.84 (1H, d), 3.93 (3H, s), 3.92 (3H, s), 3.91 (3H, s) (ppm)

7.3.4. Thermolysis, general procedure

*7.3.4.1. Methyl 6-methoxy-5-((tetrahydro-2H-pyran-2-yl)oxy)-1H-indole-2-carboxylate (**24a**)*

A 3 neck 1000mL RBF was flame dried and cooled under a stream of nitrogen. The flask was fitted with a jacketed condenser, addition funnel, thermometer adapter and magnetic stir bar. The flask was then charged with 300mL of dry xylenes via cannulation through the addition funnel. The xylenes were then heated to 140°C with a heating mantel and began to reflux and gently boil. In a separate flame dried, nitrogen flushed 500mL RBF Methyl-2-azido-3-(4-methoxy-3-((tetrahydro-2H-pyran-2-yl)oxy)phenyl) acrylate (13.93g, 41.79mmol) was dissolved in 200mL of dry xylenes producing a light yellow solution which was transferred to the addition funnel via cannulation. The addition funnel was set to a slow drip so that addition was complete over the next 1.5 hrs. After 15 min of addition the reaction mixture was a dark yellow-brown. After addition was complete the reaction mixture was left to reflux for an additional 3 hrs after which TLC confirmed complete consumption of starting material. Heat was removed, the reaction mixture allowed to cool to 70°C and then transferred to a 1 neck 1000mL RBF. 5/6 of the xylenes were removed via high vacuum rotary evaporation and 500mL of hexanes was added as an anti-solvent, immediately precipitating the solid product. The suspension was then placed in the freezer over night and the solid collected in a Buchner funnel and rinsed with 3x30mL of hexanes to afford the title compound as a dark yellow crystalline solid (10.22g, 33.47mmol, 80% yield). ^{1}H NMR ($CDCl_3$): δ 8.70 (1H, s), 7.36 (1H, s), 7.10 (1H, s), 6.84 (1H, s), 5.35 (1H, t), 3.92 (3H, s), 3.91 (3H, s), 3.65 (2H, m), 2.1-1.8 (6H, multiple peaks) (ppm). ^{13}C NMR ($CDCl_3$): δ 162.57, 151.55, 143.15, 133.47, 126.07, 120.89, 110.73, 109.35, 98.64, 94.25, 62.51, 56.36, 52.08, 30.69, 25.56, 19.21 (ppm).

*7.3.4.2. Methyl 5,6-dimethoxy-1H-indole-2-carboxylate (**24b**)*

^{1}H NMR ($CDCl_3$): δ 8.70 (1H, s), 7.11 (1H, s), 7.05 (1H, s), 6.85 (1H, s), 3.94 (3H, s), 3.93 (3H, s), 3.91 (3H, s) (ppm).

*7.3.4.3. Methyl 6-methoxy-1H-indole-2-carboxylate (**24c**)*

^{1}H NMR ($CDCl_3$): δ 8.65 (1H, s), 7.49 (1H, d), 7.19 (1H, s), 6.77 (1H, s), 6.75 (1H, d), 3.86 (3H, s), 3.80 (3H, s) (ppm)

7.3.5. Indole ester reduction, general procedure

*7.3.5.1. (6-Methoxy-5-((tetrahydro-2H-pyran-2-yl)oxy)-1H-indol-2-yl)methanol (**26a**)*

A 2 neck 50mL RBF was fitted with a magnetic stir bar, flame dried and cooled under a stream of nitrogen. The flask was charged with lithium aluminum hydride (0.16g, 4.21mmol) and 8mL of dry THF, placed in an ice water bath and set to stir. In a separate flame dried 1 neck RBF Methyl 6-methoxy-5-((tetrahydro-2H-pyran-2-yl)oxy)-1H-indole-2-carboxylate (0.50g, 1.63mmol) was dissolved in 8mL of dry THF and added to the reaction flask drop wise via syringe. Upon addition an effervescent exotherm was observed. The reaction mixture was allowed to stir for 20min after which TLC confirmed complete consumption of starting material. The reaction mixture was then diluted with 20mL of diethyl ether, fitted with a vent needle and 3mL of saturated NH_4Cl was added drop wise via syringe *(Note: Sat. NH_4Cl was added until effervescence ceased)*. The reaction mixture was then dried over sodium sulfate, filtered through a course frit and rinsed with 2x20mL of diethyl ether. The organic extracts were collected and freed of solvent under reduced pressure to afford the title compound as a yellow oil (0.45g, 1.62mmol, 99% yield) which was used without any farther purification. ^{1}H NMR ($CDCl_3$): δ 8.21 (1H, s), 7.20 (1H, s), 6.78 (1H, s), 6.21 (1H, s), 5.25 (1H, t), 4.68 (2H, s), 3.80 (3H, s), 3.65 (2H, m), 2.1-1.8 (6H, multiple peaks) (ppm). ^{13}C NMR ($CDCl_3$): δ 148.71, 142.06, 132.51, 121.26, 110.44, 100.95, 99.03, 95.05, 68.24, 62.53, 58.97, 56.64, 30.77, 25.85, 19.31 (ppm).

*7.3.5.2. 5,6-Dimethoxy-1H-indol-2-yl)methanol (**26b**)*

^{1}H NMR ($CDCl_3$): δ 8.25 (1H, s), 7.03 (1H, s), 6.85 (1H, s), 6.30 (1H, s), 5.30 (1H, s), 4.77 (2H, s), 3.92 (3H, s), 3.73 (3H, s) (ppm)

*7.3.5.3. 6-Methoxy-1H-indol-2-yl)methanol (**26c**)*

^{1}H NMR ($CDCl_3$): δ 8.14 (1H, s), 7.39 (1H, d), 6.79 (1H, s) 6.71 (1H, d), 6.28 (1H, s), 4.72 (2H, s), 3.78 (3H, s), 3.62 (1H, s) (ppm)

7.3.6. Indole alcohol oxidation to aldehyde, general procedure

*7.3.6.1. 6-Methoxy-5-((tetrahydro-2H-pyran-2-yl)oxy)-1H-indole-2-carbaldehyde (**27a**)*

A 1 neck RBF was fitted with a magnetic stir bar and charged with (6-methoxy-5-((tetrahydro-2H-pyran-2-yl)oxy)-1H-indol-2-yl)methanol (0.45g, 1.62mmol) in one portion followed by 35mL of DMSO. IBX (1.00g, 3.57mmol) was added and the reaction mixture was allowed to stir over night *(~10hrs)*. The reaction mixture turned a dark brown after 30min. TLC confirmed that the reaction had gone to completion and the reaction mixture was diluted with 30mL of ethyl acetate and 30mL of saturated $NaHCO_3$ *(pH ~ 8)* and gravity filtered through a course pad to remove excess IBX. The filter was rinsed with 30mL of ethyl acetate and the mixture farther diluted with 30mL of sat. $NaHCO_3$. Aqueous and organic layers were separated and the aqueous was extracted with 2x30mL of ethyl acetate. The combined organic extracts were washed with brine, dried over sodium sulfate and concentrated under reduced pressure to afford the title compound as a dark brown residue (0.40g, 1.45mmol, 89% yield). ^{1}H NMR ($CDCl_3$): δ 9.61 (1H, s), 9.05 (1H, s), 7.33 (1H, s), 7.09 (1H, s), 6.77 (1H, s), 5.30 (1H, t), 3.85 (3H, s), 3.59 (2H, m), 2.1-1.8 (6H, multiple peaks) (ppm). ^{13}C NMR ($CDCl_3$): δ 181.20, 153.12, 143.56, 135.68, 135.57, 120.90, 116.22, 110.73, 98.56, 94.32, 62.52, 56.34, 30.66, 25.53, 19.16 (ppm).

*7.3.6.2. 5,6-Dimethoxy-1H-indole-2-carbaldehyde (**27b**)*

^{1}H NMR ($CDCl_3$): δ 9.69 (1H, s), 9.11 (1H, s), 7.17 (1H, s), 7.08 (1H, s), 6.85 (1H, s), 3.96 (3H, s), 3.92 (3H, s) (ppm).

*7.3.6.3. 6-Methoxy-1H-indole-2-carbaldehyde (**27c**)*

^{1}H NMR ($CDCl_3$): δ 9.69 (1H, s), 8.90 (1H, s), 7.58 (1H, d), 7.18 (1H, s), 6.80 (1H, s), 6.79 (1H, d), 3.85 (3H, s) (ppm).

7.3.7. Introduction of Boc protecting group, general procedure

*7.3.7.1. tert-Butyl 2-formyl-6-methoxy-5-((tetrahydro-2H-pyran-2-yl)oxy)-1H-indole-1-carboxylate (**17a**)*

A 2 neck RBF was fitted with a magnetic stir bar, flame dried and cooled under a stream of nitrogen. Boc_2O (0.440g, 2.02mmol) and DMAP (0.025g, 0.204mmol) were added in one portion followed by 25mL of anhydrous DCM. In a separate flame dried RBF 6-Methoxy-5-((tetrahydro-2H-pyran-2-yl)oxy)-1H-indole-2-carbaldehyde (0.390g, 1.417mmol) was dissolved in 5mL of anhydrous DCM and added to the reaction flask drop wise via syringe. Effervescence and evolution of gas observed upon addition of indole/DCM mixture which continued for 15min after addition. The reaction mixture stirred for 2.5 hrs after with TLC indicated complete consumption of starting materials. The reaction mixture was then diluted with 40mL of DCM, washed with 3x40mL of saturated $NaHCO_3$ and dried over sodium sulfate. Rotary evaporation afforded the title compound as a dark brown crude mixture (0.336g, .895mmol, 63% yield) which was purified via column chromatography *(Note: silica deprotected the indole nitrogen, silica columns were run with TEA).* ^{1}H NMR ($CDCl_3$): δ 10.33 (1H, s), 7.75 (1H, s), 7.37 (1H, s), 7.35 (1H, s), 5.30 (1H, t), 4.02-3.96 (5H, multiple peaks), 2.05-1.70 (15H, multiple peaks) (ppm). ^{13}C NMR ($CDCl_3$): δ 183.62, 152.76, 150.16, 144.66, 137.24, 134.73, 120.78, 117.64, 110.40, 99.26, 98.20, 62.49, 56.40, 30.59, 28.45, 28.14, 25.47, 19.09 (ppm).

*7.3.7.2. tert-Butyl 2-formyl-5,6-dimethoxy-1H-indole-1-carboxylate (**17b**)*

^{1}H NMR ($CDCl_3$): δ 10.38 (1H, s), 7.75 (1H, s), 7.40 (1H, s), 7.03 (1H, s), 4.01 (3H, s), 3.95 (3H, s), 1. 75 (9H, s) (ppm).

*7.3.7.3. tert-Butyl 2-formyl-6-methoxy-1H-indole-1-carboxylate (**17c**)*

^{1}H NMR ($CDCl_3$): δ 10.30 (1H, s), 7.70 (1H, s), 7.55 (1H, d), 7.39 (1H, s), 6.90 (1H, d), 3.88 (3H, s), 1.71 (9H, s) (ppm).

7.3.8. Ethynyl Indole preparation, general procedure

*7.3.8.1. 2-Ethynyl-6-methoxy-5-((tetrahydro-2H-pyran-2-yl)oxy)-1H-indole (**21a**)*

^{1}H NMR ($CDCl_3$): δ 8.06 (1H, s), 7.29 (1H, s), 6.80 (1H, s), 6.70 (1H, s), 5.33 (1H, t), 4.10 (2H, m), 3.87 (3H, s), 3.31 (1H, s), 2.10-1.60 (6H, multiple peaks) (ppm). ^{13}C NMR ($CDCl_3$): δ 140.17, 142.84, 132.16, 120.66, 116.33, 110.05, 109.99, 98.82, 94.64, 80.57, 76.94, 62.50, 56.49, 30.72, 25.56, 19.24 (ppm).

*7.3.8.2. 2-Ethynyl-5,6-dimethoxy-1H-indole (**21b**)*

^{1}H NMR ($CDCl_3$): δ 8.06 (1H, s), 6.96 (1H, s), 6.80 (1H, s), 6.64 (1H, s), 3.88 (3H, s), 3.83 (3H, s), 3.29 (1H, s) (ppm). ^{13}C NMR ($CDCl_3$): δ 148.81, 146.01, 130.72, 120.28, 116.03, 109.78, 101.95. 93.86, 56.39, 56.29, 31.83, 22.91 (ppm).

*7.3.8.2. 2-Ethynyl-6-methoxy-1H-indole (**21c**)*

^{1}H NMR ($CDCl_3$): δ 8.01 (1H, s), 7.43 (1H, d), 6.78 (1H, s), 6.75 (1H, s), 6.72 (1H,d), 3.81 (3H, s), 3.29 (1H, s) (ppm).

*7.3.8.4. 2-Ethynyl-6-methoxy-1-methyl-1H-indole (**21d**)*

^{1}H NMR ($CDCl_3$): δ 7.42 (1H, d), 6.77 (1H, d), 6.75 (1H, s), 6. 64 (1H, s), 3.86 (3H, s), 3.78 (3H, s), 3.45 (1H, s) (ppm).

7.3.9. 2-Aroyl indole preparation, general procedure

*7.3.9.1. (6-Methoxy-1-methyl-1H-indol-2-yl)(3,4,5-trimethoxyphenyl)methanone (**13c**)*

A 2 neck RBF was flame dried, allowed to cool under a stream of nitrogen and equipped with rubber septa and a magnetic stir bar. The flask was charged with 20mL of dry THF followed by 3,4,5-trimethoxybromobenzene (0.57g, 2.33mmol) and set to stir. The reaction mixture was then cooled to -78°C by way of a acetone/dry ice bath and t-BuLi (1.5M in pentanes) (5.65mL, 6.98mmol) was added drop wise via syringe. A solution of 6-Methoxy-1-methyl-1H-indole-2-carbaldehyde (0.40g, 2.11mmol) dissolved in 5mL of dry THF was then added to the cooled reaction mixture drip wise via syringe. After 1hr, TLC indicated the reaction had gone to completion, the reaction mixture was allowed to gradually come to r.t. and 20mL of NH_4Cl (aq) was added to quench any remaining t-BuLi. The reaction mixture was then extracted with ethyl ether (3x50mL) and washed with brine. The combined organic extracts where then dried over sodium sulfate and condensed under reduced pressure to afford (6-methoxy-1-methyl-1H-indol-2-yl)(3,4,5-trimethoxyphenyl)methanol (0.73g, 2.04mmol, 97% yield) which was carried on to the next step without further purification.

A single neck RBF, equipped with a magnetic stir bar, was charged with (6-Methoxy-1-methyl-1H-indol-2-yl)(3,4,5-trimethoxyphenyl)methanol (0.65g, 1.82mmol) and 20mL of DMSO followed by IBX (1.52g, 5.46mmol). The reaction mixture was set to sir over night (8hrs). TLC indicated complete consumption of starting material and 200mL of water was added and the reaction mixture was gravity filtered with course filter paper. The resulting mixture was then extracted with ethyl ether (3x100mL) and the combined organic extracts washed with 200mL of $NaHCO_3$ (aq). The resulting ethereal extracts were dried over sodium sulfate and concentrated in vacuo to afford the title compound as a brown solid (0.58g, 89% yield). ^{1}H NMR ($CDCl_3$): δ 7.53 (1H, d), 7.15 (1H, s), 6.97 (1H, d), 6.57 (1H, s), 6.54 (1H, s), 4.05-3.80 (15H, multiple peaks) (ppm). ^{13}C NMR ($CDCl_3$): δ 159.22, 153.72, 152.96, 124.18, 123.80, 115.35, 112.78, 112.40, 111.12, 107.33, 105.31, 92.16, 61.22, 56.51, 56.26, 55.80, 44.54, 32.97, 32.24, 29.13 (ppm).

7.3.10. $RuCpCl(PPh_3)_2$

Procedure was adapted from Chloro (η^5-cyclomentadienyl)bis(triphenylphosphene)Ruthenium(II).[44]

^{1}H NMR ($CDCl_3$): δ 7.36 (12H, t), 7.23 (6H, d), 7.127 (12H, t) , 4.10 (5H, s) (ppm). ^{13}C NMR ($CDCl_3$): δ 128.53, 126.86, 126.75, 123.43, 123.14, 122.22, 76.13 (ppm)

8. References

1. Newman J. D.; Cragg G. M. Natural Products as Sources of New Drugs over the Last 25 Years. *Journal of Natural Products* **2007**, *70*, 461-477.
2. Ravelli R. B. G.; Gigant B.; Curmi P. A.; Jourdain I.; Lachkar S.; Sobel A.; Knossow M. Insight into tubulin regulation from a complex with colchicines and a stathmin-like domain. *Letters to Nature* **2004**, *428*, 198-202.
3. Andreeff M.; Goodrich D. W.; Pardee A. B. Cell Proliferation, Differentiation, and Apoptosis. In *Holland-Frei Cancer Medicine;* Bast RC Jr, Kufe DW, Pollock RE, et al., Ed.; Hamilton (ON): BC Decker; 2000; 5th edition.
4. Thomas G. Fundamentals of Medicinal Chemistry. *John Wiley & Sons Ltd* **2003**.
5. Stanton A. S.; Gernert K. M.; Nettles J. H.; Aneja R. Drugs that Target Dynamic Microtubules: A New Molecular Perspective. *Medical Research Reviews* **2011**, 444-481.
6. Downing K. H.; Nogales E. Tublin and microtubule structure. *Current Opinion in Cell Biology* **1998**, *10*, 16-22.
7. Cortese F.; Bhattacharyya B.; Wolff J. Podophyllotoxin as a Probe for Colchicine Binding Site of Tubulin. *The Journal of Biological Chemistry* **1977**, *252*, 1134-1140.
8. Pettit G. R.; Singh S. B.; Niven H. E.; Schmidt J. M. Isolation, Structure, and Synthesis of Combretastatins A-1 and B-1, Potent new Inhibitors of Microtubule Assembly, Derived from *Combretum Caffrum*. *Journal of Natural Products* **1989**, *50*, 119-131.
9. Pettit *et al. Can J. Chem.* **1982**, *610*, 1374
10. Hong-xue L.; Xiao-rong L.; Wen-ping Z.; Cang Z.; Hong-bin S. A review of the current status of combretastatin A-4 phosphate (CA4P). *Zhongguo Xinyao yu Linchuang Zazhi* **2010**, *29*, 816-821.
11. Tron G. C.; Pirali T.; Sorba G.; Pagliai F.; Busacca S.; Genazzani A. A.; Medical Chemistry of Combretastatin A4: Present and Future Directions. *J. Med. Chem.* **2006**, *49*, 3033-3044.
12. Pettit G. R.; Toki B.; Herald D. L.; Verdier-Pinard P.; Boyd M. R.; Hammel E.; Pettit R. K. Antineoplastic Agents. 369. Synthesis of Phinstatin Phosphate. *J. Med. Chem.* **1998**, *41*, 1688-1695.
13. Ohsumi, K.; Hatanaka, T.; Fujita, K.; Nakagawa, R.; Fukuda, Y.; et al. Syntheses and antitumor activity of cis-restricted combretastatins: 5-membered heterocyclic analogues. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3153-3158.
14. Ducki S.; Rennison D.; Woo M.; Kendall A.; Dit Chabert J. F.; McGown A. T.; Lawrence N. J. Combretastatin-like Chalcones as Inhibitors of Microtubule Polymerization Part 1: Synthesis and Biological Evaluation of Antivascular Activity. *Bioorganic & Medicinal Chemistry* **2009**.
15. Pati, H. N.; Wicks, M.; Holt H. L, Jr.; LeBlanc, R.; Weisbrunch, P.; et al. Synthesis and biological evaluation of *cis*-combretastatin analogs and their novel 1,2,3-triazole derivatives. *Heterocycl. Commun.* **2005**, *11*, 117-120.
16. Yu-Shan Wu,; et al. Synthesis and Evaluation of 3-Aroylindoles as Anticancer Agents: Metabolite Approach. *J. Med. Chem.* **2009**, *52*, 4941-4945.
17. Grignard, V. Some new organometallic combinations of magnesium and their application to the synthesis of alchols adn hydrocarbons. *C. R. Acad. Sci.* **1900**, 1322-1324.
18. Knochel P.; et al. Highly Functionalized Organomagnesium Reagents Prepared through Halogen-Metal Exchange. *Angewandte Chemie*. **2003**, *42*, 4302-4320.
19. Murakami Y.; Watanabe T.; Suzuki H.; Kotake N.; Takahashi T.; Toyonari K.; Ohno M.; Takase K.; Suzuki T.; Kondo. New Findings on the Hemetsberger-Knittel Reaction (Synthetic studies on Indoles and Related Compounds. XLIII). *Chem. Pharm. Bull.* **1997**, *45*, 1739-1744.
20. Hemtsberger, H.; Knittel, D.; Wiedmann, H. *Monatshefte Chemie* **1970**, *101*, 161-165.
21. Mesenzani O.; et al. Replacement of the double bond in antitubulin chalcones with triazoles and tetrazoles: Synthesis and biological evaluation. *Bioorganic & Medicinal Chemistry Letters*. **2011**, *21*, 764-768,
22. Farooq T.; Hang B. E.; Sydnes L. K.; Tornroos K. W. 1,3-Dipolar cycloaddition of benzyl azide to two highly functionalized alkynes. *Montash Chem.* **2012**, *143*, 505-512.
23. Sonogashira K. Coupling Reactions Between sp-2 and sp Carbon Centers. *Comp. Org. Synth.* **1991**, *3*, 521-549.
24. Stephens R. D.; Castro C. E. The substitution of aryl iodides with cuprous acetylides. A synthesis of tolanes and heterocyclics. *J. Org. Chem.* **1963**, *28*, 3313-3315.
25. Zhang L.; Chen X.; Xue P.; Sun H. H. Y.; Williams I. D.; Sharpless B. K.; Folkin V. V.; Jia G. Ruthenium-Catylized Cycloaddition of Alkynes and Organic Azides. *J. Am. Chem. Soc.* **2005**, *127*, 15998-15999.
26. Corey, E. J.; Fuchs, P. L.; Synthetic method for conversion of formyl groups into ethynyl groups(RCHO --> RCCH or RCCR1). *Tetrahedron Lett.* **1972**, 3769-3772.
27. Eymery F.; Iorga B.; Savignac P.; The usefulness of phosphorus compounds in alkyne synthesis. *Synthesis*, **2000**, 185-213.

28. Seyferth, D.; Gilbert, P,; Marmor, R. S. Novel diazo alkanes and the first carbene containing the dimethyl phosphite group. *J. Am. Chem. Soc.* **1967**, *89*, 4811-4812.
29. Seyferth, D., Marmor, R, S. Dimethyl diazomethylphosponate: its preparation and reactions. *Tetrahedron Lett.* **1970**, 2493-2496.
30. Taber D. F.; Bai S.; Guo P. A convenient reagent for aldehyde to alkyne homologation. *Tetrahedron Lett.* **2008**, *49*, 6904-6906.
31. Colvin, E. W.; Hammil B. J.; One-step conversion of carbonyl compounds into acetylenes. *J. Chem. Soc., Chem. Commun.* **1973**, 151-152.
32. Colvin, E. W.; Hamil B. J. A simple procedure for the elaboration of carbonyl compounds into homologous alkynes. *J. Chem. Soc., Perkin Trans. 1* **1977**, 869-874.
33. Bartoli B.; Palmieri G.; Bosco M.; Dalpozzo R. The reaction of vinyl grignard reagents with 2-substitued nitroarenes: a new approach to the synthesis of 7-substituted indoles. *Tetrahedron Lett.* **1989**, *30*, 2129-2132.
34. Kubo T.; Katoh C.; Yamada K.; Okano K.; Tokuyama H.; Fukuyama T. A mild inter- and intramolecular amination of aryl halides with a combination of CuI and CsOAc. *Tetrahedron Lett.* **2008**, *64*, 11230-11236.
35. Bansal R. K. Heterocyclic Chemistry, *New Age International*, **1999**, *3*, 280.
36. Madelung W. A new synthesis of substituted indoles. *Ber.* **1912**, *45*, 1128-1134.
37. Soderberg G. C. B. Synthesis of Heterocycles via Intramolecular Annulation of Nitrene Intermediates. *Current Organic Chemistry* **2000**, *4*, 727-764.
38. Reddy M. R.; Cook J. M. Synthesis of Roeharmine and (-)-1,2,3,4-Tetrahydroroeharmine *Tetrahedron Lett.* **1994**, *35*, 5413-6.
39. Wuts P. G. M.; Greene T. W. Greene's Protective Groups in Organic Synthesis, *Wiley*, **4th Edition**, (2007).
40. Davies J. S.; Higginbotham C. L.; Tremeer E. J.; Brown C.; Treadgold R. C. *J. Chem. Soc., Perkin Trans. I*, **1992**, 3043.
41. Nam N. H.; Kim Y.; You Y. J.; Hong D. H.; Kim H. M.; Ahn B. Z. *European J. Med. Chem.*, **2003**, *38*, 179-187.
42. Swift W. J.; Y. Luan Y Strategic Applications of Named Reactions in Organic Synthesis, *Elsevier*, (2005)
43. Ragnarsson U.; Grehn L. *Acc. Chem. Res.*, **1998**, *32*, 494-501.
44. Fackler J. P. Inorganic Synthesis, *Wiley*, **1982**, *21*, 79-80.