Pergamon

Bioorganic & Medicinal Chemistry 8 (2000) 2417–2425

BIOORGANIC & MEDICINAL CHEMISTRY

Synthesis and Biological Evaluation of Aryl Azide Derivatives of Combretastatin A-4 as Molecular Probes for Tubulin

Kevin G. Pinney,[a,*] Maria P. Mejia,[a] Victor M. Villalobos,[a] Brent E. Rosenquist,[a] George R. Pettit,[b] Pascal Verdier-Pinard[c] and Ernest Hamel[c]

[a]*Department of Chemistry and Biochemistry, Baylor University, Waco, TX 76798-7348, USA*
[b]*Cancer Research Institute and Department of Chemistry, Arizona State University, Tempe, AZ 85287-2404, USA*
[c]*Screening Technologies Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, Frederick Cancer Research and Development Center, Frederick, MD 21702, USA*

Received 14 January 2000; accepted 22 May 2000

Abstract—Two new aryl azides, (*Z*)-1-(3′-azido-4′-methoxyphenyl)-2-(3″,4″,5″-trimethoxyphenyl)ethene **9** and (*Z*)-1-(4′-azido-3′-methoxyphenyl)-2-(3″,4″,5″-trimethoxyphenyl)ethene **5**, modeled after the potent antitumor, antimitotic agent combretastatin A-4 (CA-4), have been prepared by chemical synthesis as potentially useful photoaffinity labeling reagents for the colchicine site on β-tubulin. Aryl azide **9**, in which the 3′-hydroxyl group of CA-4 is replaced by an azido moiety, demonstrates excellent in vitro cytotoxicity against human cancer cell lines (NCI 60 cell line panel, average $GI_{50} = 4.07 \times 10^{-8}$ M) and potent inhibition of tubulin polymerization ($IC_{50} = 1.4 \pm 0.1$ μM). The 4′-azido analogue **5** has lower activity (NCI 60 cell line panel, average $GI_{50} = 2.28 \times 10^{-6}$ M, and $IC_{50} = 5.2 \pm 0.2$ μM for inhibition of tubulin polymerization), suggesting the importance of the 4′-methoxy moiety for interaction with the colchicine binding site on tubulin. These CA-4 aryl azide analogues also inhibit binding of colchicine to tubulin, as does the parent CA-4, and therefore these compounds are excellent candidates for photoaffinity labeling studies.

Introduction

Tubulin is a heterodimeric protein consisting of α and β subunits, each approximately 50 kDa in size.[1,2] The α and β subunits have a high degree of homology (40–50%) to each other[3] and the primary sequence of these subunits has been determined from protists, plants, fungi and eukaryotes. The secondary and tertiary structures of the α and β subunits of tubulin have been elucidated by electron crystallography from protofilaments purified in the presence of zinc ions by the pioneering work of Nogales and Downing.[4] Upon binding of GTP, tubulin polymerizes into microtubules which are helical arrays of alternating α and β subunits with a cross-sectional diameter of approximately 24 nm. The polymerization and subsequent depolymerization of microtubules is responsible for ciliar and flagellar movement, vesicle movement in secretion, transport of organelles down the axons in nerve cells, chromosome separation during cell division (mitosis), and the generation and maintenance of cell shape.[1–3,5]

During cellular division, the interphase microtubule array largely disassembles, and the α,β-tubulin repolymerizes to form the microtubule framework of the mitotic spindle, which is essential for chromosome separation and formation of two daughter cells (Fig. 1(A)). When ligands that interact with α,β-tubulin or with microtubules are present, a reduction in cellular division is observed (Fig. 1(B)). Due to the key role played by tubulin during cellular division, ligands that interrupt the dynamic instability inherent to this system have been developed as antimitotic, anti-cancer drugs. Different types of naturally occurring ligands that inhibit tubulin polymerization have been reported in the literature for many years, and there has been a continuing discovery of new agents with pronounced structural diversity.[6]

Three distinct small-molecule binding sites are known, to date, for the tubulin system. These are the colchicine site and the vinca alkaloid domain, both located on monomeric unpolymerized α,β-tubulin, and the taxoid site on the polymerized microtubule. Ligands which interact at the taxoid site (such as paclitaxel and the epothilones) stabilize the microtubule, while ligands interacting at the vinca domain (such as vinblastine) or

*Corresponding author. Tel.: +1-254-710-4117; fax: +1-254-710-2403; e-mail: kevin_pinney@baylor.edu

0968-0896/00/$ - see front matter
PII: S0968-0896(00)00176-0

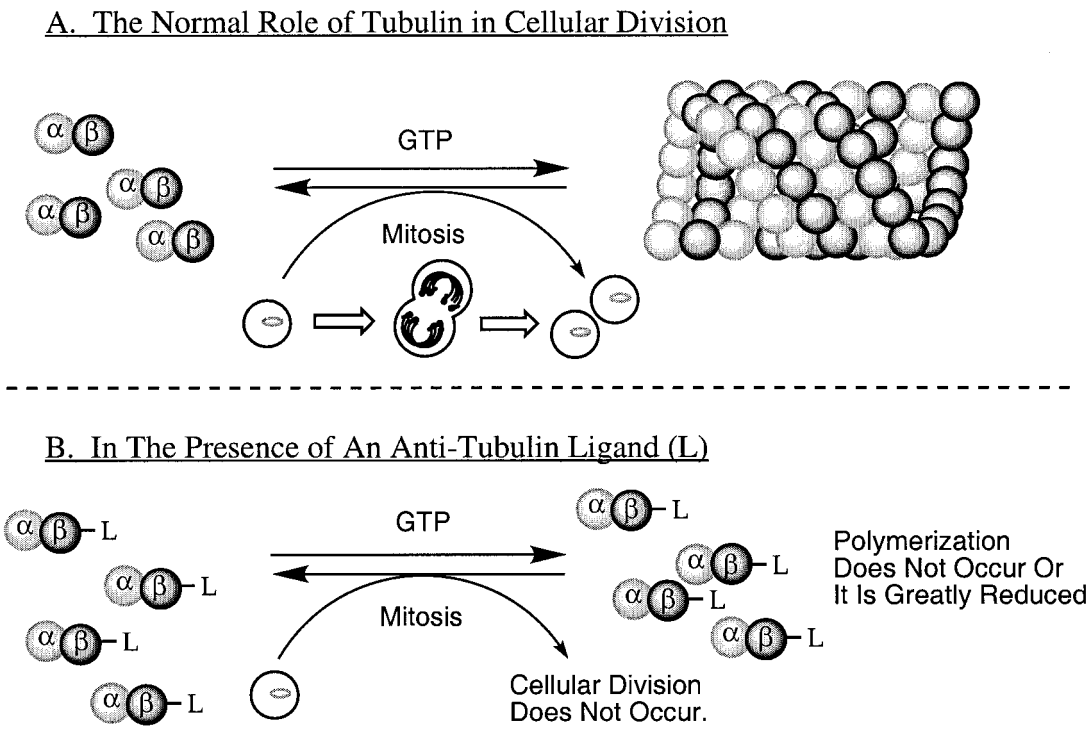


Figure 1. (A) Normal tubulin polymerization dynamics in cell division. (B) Disruption of dynamic polymerization in the presence of an anti-tubulin ligand (L).

the colchicine site (such as colchicine and combretastatin A-4 [CA-4]) disrupt the formation of microtubules. Treatment of cells with all these drugs can completely halt their replication. Representative naturally occurring antimitotic ligands include paclitaxel,[7] epothilone A,[8] vinblastine,[9] CA-4,[10–14] dolastatin 10,[15,16] and colchicine (Fig. 2).

In addition, there are a variety of synthetic compounds that also demonstrate efficient inhibition of tubulin polymerization (examples in Figure 3). Various photoaffinity and chemical affinity labeling reagents have been prepared in an effort to elucidate detailed structural information regarding these known tubulin binding sites. Among the most noteworthy of these ligands are those based on paclitaxel[17] and colchicine.[18–22] In addition, a diazoketone derivative of combretastatin has been prepared as well.[23] In each case, these affinity probes have provided useful information, but none have given a clear picture of the exact nature of their respective binding domains. For example, a 7-(benzoyldihydrocinnamoyl)-paclitaxel derivative has recently been shown to label amino acid residues 277–293 with specific interaction noted at Arg-282 in β-tubulin of the polymerized microtubule.[24] Earlier work with azido-paclitaxel analogues suggested binding interaction at amino acid residues 217–233 of β-tubulin (in the microtubule)[25] as well as the N-terminal 31 amino acid residues.[26] The results of these photoaffinity labeling studies are in agreement with the paclitaxel bound structure determined by electron crystallography.[4,26] In terms of the colchicine binding site, to date there is no crystal structure available. Uppuluri and co-workers[20] used direct photoaffinity labeling to crosslink [^{3}H]-colchicine with a peptide containing amino acid residues 1–36 or a peptide containing residues 214–241 of β-tubulin. In another approach, Bai and co-workers,[22] using a chemical (electrophilic) affinity probe in the A ring of a colchicine analogue, formed crosslinks between the analogue and Cys-354 (major crosslink) or Cys-239 (minor crosslink) of β-tubulin. An interpretation of these findings is that the A ring may bind to tubulin between the two cysteine residues, while the C ring may bind near the amino terminus of β-tubulin. It is our intention to further clarify and define the colchicine binding region of tubulin through the use of aryl azide derivatives of CA-4. Compound **9** should provide specific insight into the location of the binding region for the C ring.

CA-4[11] is the most potent anti-mitotic agent from the combretastatin family. The first member of the series, combretastatin, was isolated, and its structure elucidated by Pettit and co-workers in 1982 from the South African tree *Combretum caffrum*.[13] CA-4[11] is the simplest structure, to date, for a natural product that binds to tubulin. It is structurally analogous to colchicine and binds to the same site on β-tubulin.[23,27–31] Due in part to its structural simplicity, as well as its remarkable affinity for the colchicine binding site, we selected CA-4 as the molecular template for the development of new photoaffinity labeling reagents. CA-4 is of particular current interest since its phosphate prodrug is currently in phase I/II clinical trials.[32] The prodrug demonstrates a remarkable ability to selectively cause the disintegration of the vasculature of solid tumors.[14,28,33–38] Our understanding of this unusual biological activity may be increased if we obtain a more detailed characterization of the colchicine binding site of tubulin.

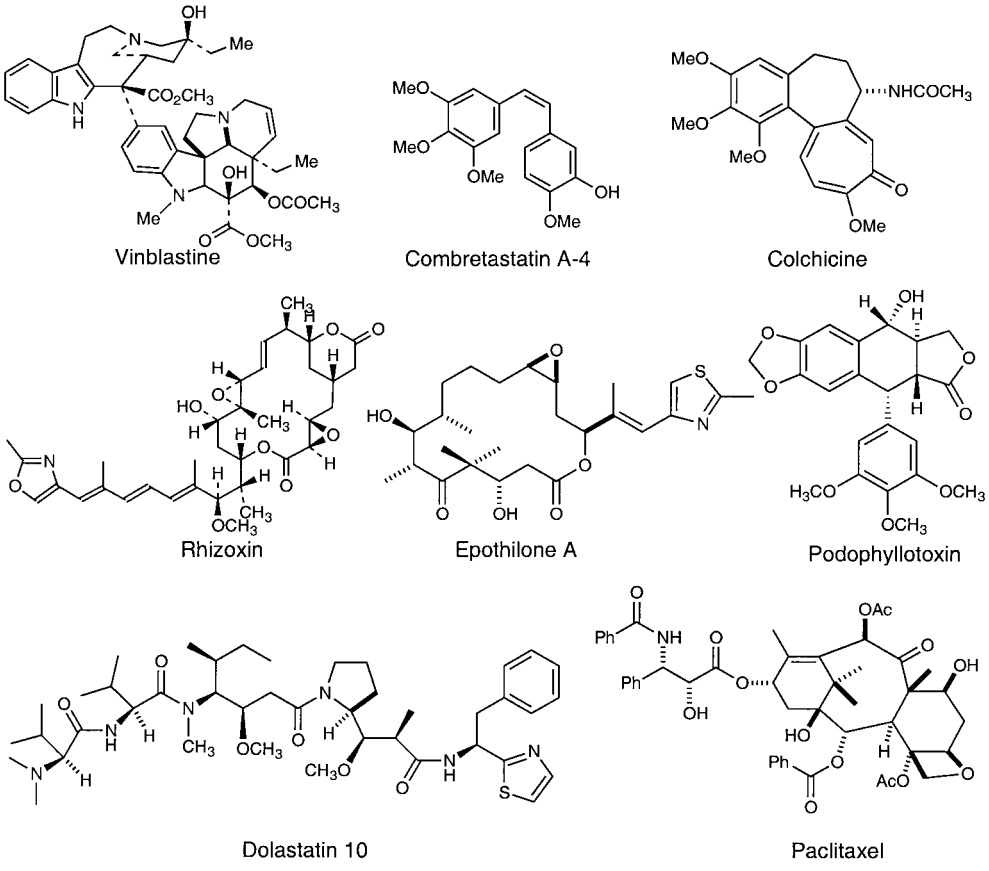


Figure 2. Representative antimitotic compounds which interact with tubulin.

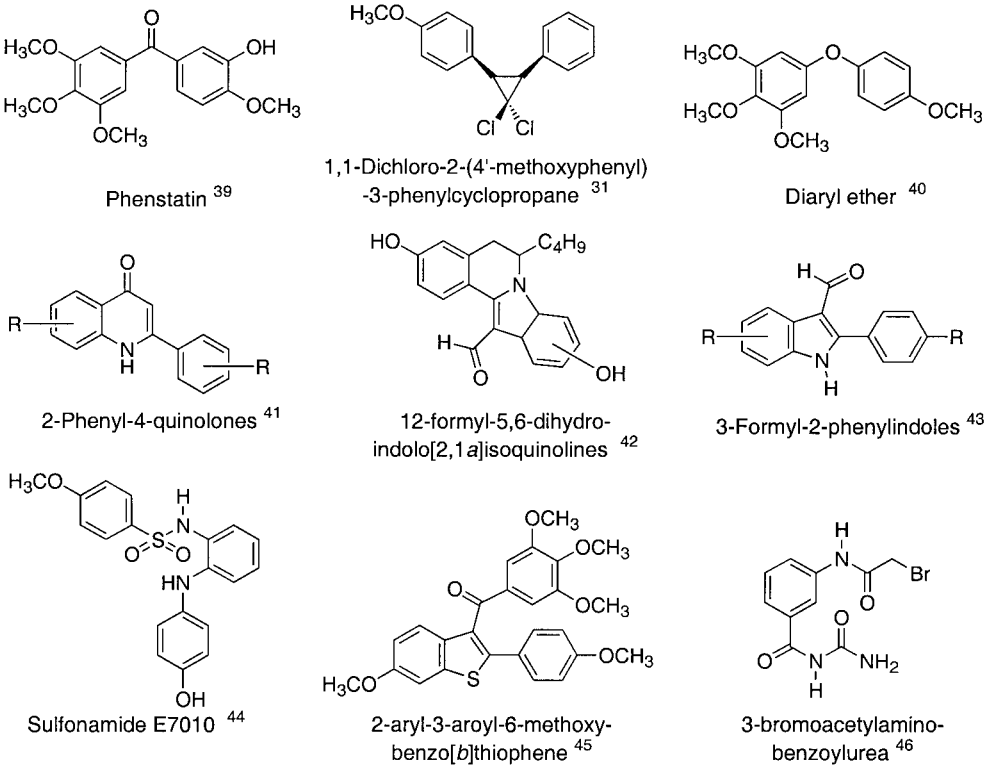


Figure 3. Synthetic anti-tubulin polymerization inhibitors.

Results and Discussion

In order to expand the current knowledge base concerning the structural and electronic nature of the colchicine binding site on tubulin, two new photoaffinity labeling reagents have been prepared based on CA-4. The synthesis of these compounds involved the preparation of the corresponding 3′- and 4′-nitro and amino[47] analogues of CA-4 (Fig. 4).

The nitro-combretastatin analogues **3A**, **3B**, **7A** and **7B** were obtained employing a Wittig reaction sequence (Schemes 1 and 2). Treatment of phosphonium salt **1** or **6** with the appropriate aldehyde in the presence of NaH resulted in a mixture of *E* and *Z* isomers that are separable by column chromatography. Photochemical isomerization of the *trans*- to *cis*-stilbene has been studied as a means of obtaining higher quantities of the desired *Z*-nitrostilbene isomer. Accordingly, the *E*-nitrostilbenes **3A** and **7A** were interconverted to the *Z*-nitro analogues **3B** and **7B**, respectively, in moderate yields using benzil as the sensitizer.[48,49] Following the Wittig reaction and careful separation of the isomers, the *Z*-nitro derivatives were reduced with sodium hydrosulfite to the corresponding *Z*-aryl amines **4** and **8**[47] in moderate yield. The somewhat reduced yield was

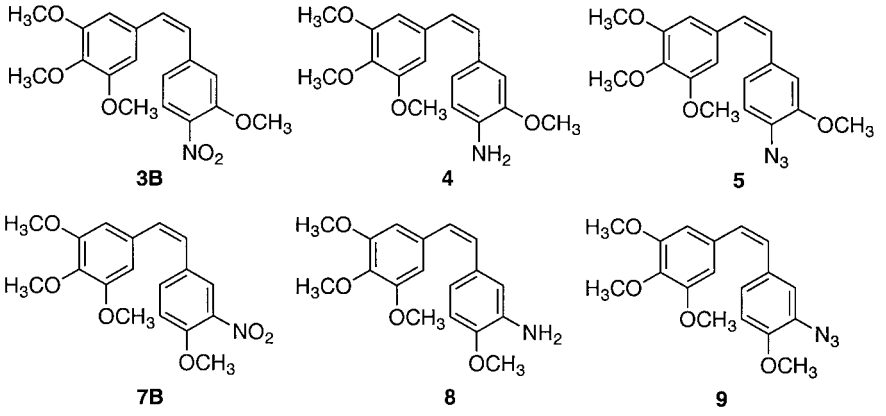
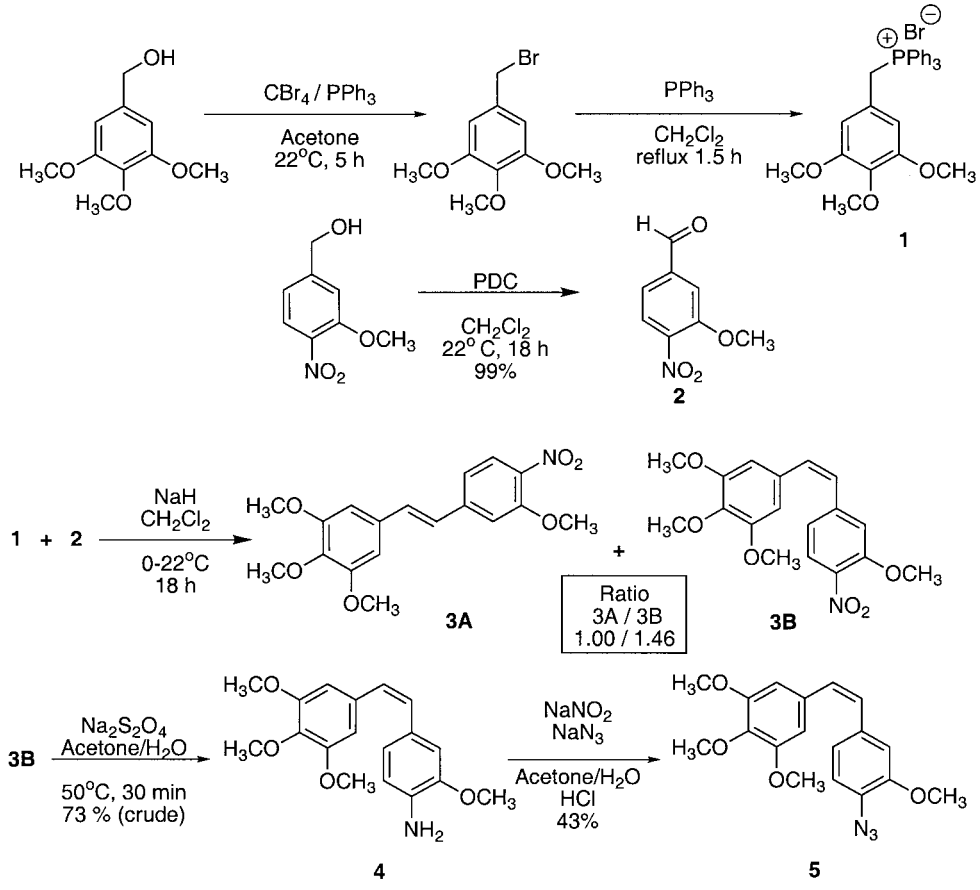
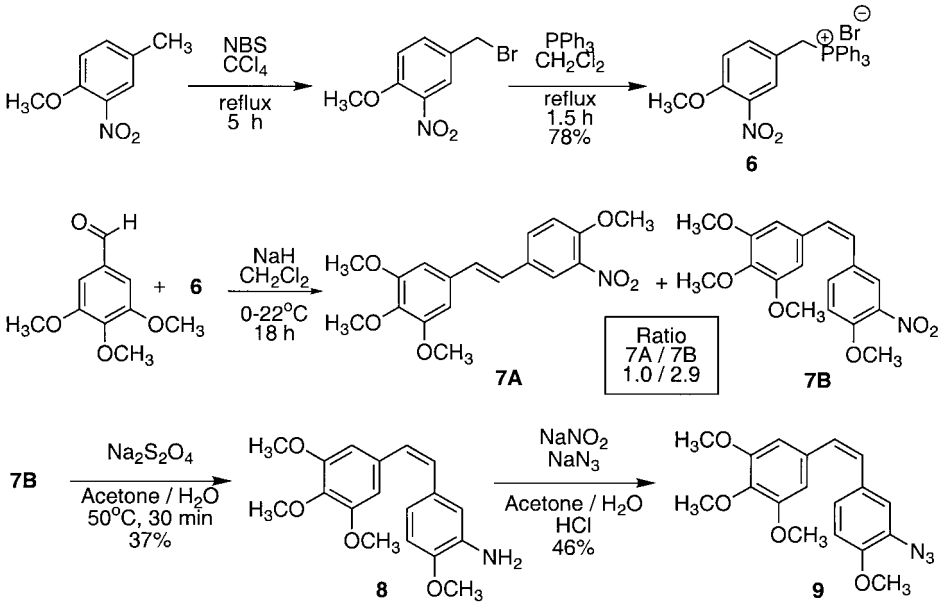


Figure 4. Nitrogen-containing analogues of combretastatin A-4.



Scheme 1. Synthesis of the 4′-azido combretastatin analogue.



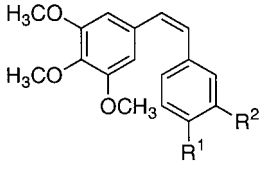
Scheme 2. Synthesis of the 3′-azido combretastatin analogue.

due, in part, to isomerization during the reduction, which was most likely induced by the heat necessary to drive the reaction to completion. We have previously reported an X-ray crystallographic study of the *E*-4′-nitro CA-4 analogue.[50] Aryl amine **8** was previously synthesized by reduction of **7B** using acetic acid and zinc powder.[47] Diazotization with sodium nitrite followed by treatment with sodium azide completed the synthesis to afford the azides **5** and **9**.

Biological evaluation of these nitrogen-modified, CA-4 analogues demonstrates that these ligands interact with the colchicine binding site on β-tubulin, as does CA-4 itself. *Z*-Azido CA-4 analogue **9** demonstrates excellent in vitro cytotoxicity (Table 1) against human cancer cell lines (for example, $GI_{50} = 5.0 \times 10^{-3}$ μg/mL against a human pancreas cell line (BXPC-3)).[51] Furthermore, azide **9** displays excellent in vitro cytotoxicity against the entire NCI 60 cell line panel, average $GI_{50} = 4.07 \times 10^{-8}$ M,[52] and demonstrates potent inhibition of tubulin polymerization ($IC_{50} = 1.4 \pm 0.1$ μM, Table 2). The ability to inhibit cell growth is greater for the nitrogen-containing analogues at the 3′ position **7B**, **8** and **9** (nitro, amine and azide groups respectively in the *Z* orientation of CA-4) compared to the 4′ position analogues **3B** and **5** (nitro and azide groups respectively in the *Z* orientation of CA-4), which is not surprising due to their resemblance to the natural product combretastatin A-4.

Each of the CA-4 analogues modified with nitrogen at the 3′ position (nitro **7B**, amine **8**, and azide **9**) were strong inhibitors of tubulin polymerization (Table 2), with the IC_{50} value for amine **8** identical to that obtained for CA-4 (IC_{50} values, 1.2 μM). The azido ligand **9** was almost as potent ($IC_{50} = 1.4$ μM) and should prove very useful as a photoaffinity labeling reagent for the colchicine binding site on tubulin. In contrast, the 4′-nitrogen modified CA-4 ligands are not as active with tubulin.

Table 1.[51] In vitro human cancer cell line study of nitrogen-containing CA-4 analogues. GI_{50} values are reported as concentrations in μg/mL



Compound	R^1	R^2	GI_{50} (μg/mL)					
			BXPC-3 Pancreas	SK-N-SH Neuroblast	SW1736 Thyroid	NCI-H460 Lung-NSC	FADU Pharynx	DU-145 Prostate
3B	NO_2	OCH_3	1.9	2.1	3.3	2.3	1.1	2.6
5	N_3	OCH_3	3.0×10^{-1}	2.4×10^{-1}	4.5×10^{-1}	3.5×10^{-1}	4.2×10^{-1}	7.0×10^{-1}
7B	OCH_3	NO_2	3.7×10^{-2}	ND[a]	ND	4.3×10^{-2}	2.6	4.6×10^{-2}
8	OCH_3	NH_2	1.3×10^{-3}	$< 1.0 \times 10^{-3}$	$< 1.0 \times 10^{-3}$	6.8×10^{-4}	$< 1.0 \times 10^{-3}$	9.6×10^{-4}
9	OCH_3	N_3	5.0×10^{-3}	3.3×10^{-3}	1.2	5.6×10^{-3}	7.6×10^{-3}	9.1×10^{-3}

[a]ND, not determined.

Table 2.[53] Inhibition of tubulin polymerization and colchicine binding. IC_{50} is defined as the inhibitory constant and measures the concentration that inhibits 50% of tubulin polymerization

Compound	R^1	R^2	IC_{50} (μM) (tubulin assembly)	% Inhibition (colchicine binding) inhibitor: colchicine		
				0.2:1	1:1	10:1
3B	NO_2	OCH_3	>40	ND[a]	ND	33±5
5	N_3	OCH_3	5.2±0.2	ND	33±20	65±10
7B	OCH_3	NO_2	1.8±0.1	ND	73±6	ND
8	OCH_3	NH_2	1.2±0.06	79±1	97±4	ND
9	OCH_3	N_3	1.4±0.1	ND	85±2	ND
CA-4	OCH_3	OH	1.2±0.06	83±2	98±1	ND

[a]ND, not determined.

The nitro analogue **3B** demonstrates negligible inhibition of tubulin polymerization, while its azide counterpart **5** is a modest inhibitor ($IC_{50}=5.2$ μM). Although this value is significantly higher than that obtained with CA-4 itself ($IC_{50}=1.2$ μM), it is still low enough to suggest that azide **5** is also interacting at the colchicine binding site on tubulin, and may, therefore, be useful as a photoaffinity labeling reagent for mapping this important binding domain.

The CA-4 analogues were also evaluated for their ability to inhibit binding of [^{3}H]-colchicine to tubulin (Table 2). Relative activities corresponded completely with their relative activities as inhibitors of tubulin polymerization. Again, compound **8** had activity virtually identical to that of CA-4.

Conclusions

We have prepared two new aryl azide CA-4 analogues, both of which interact with tubulin. The *Z*-azido analogue **9**, which contains the azide function at the 3′ position, interacts more strongly with tubulin and also has significant cytotoxic activity against human tumor cell lines. The *Z*-azido analogue **5**, which contains the azide function at the 4′ position, interacts more weakly with tubulin and has limited cytotoxicity. These results are readily understandable due to the closer structural resemblance of azide **9** to the parent CA-4 molecule. Synthetic efforts are currently underway to prepare these analogues in high specific activity radiolabeled form for further evaluation as photoaffinity labeling reagents for the colchicine binding site on tubulin.

Experimental

General methods

Proton (^{1}H) and carbon (^{13}C) NMR were obtained at 360 MHz and 90 MHz respectively in the solvent indicated. Chemical shifts are expressed in ppm (δ). Elemental analyses were carried out by Atlantic Microlab Inc. (Norcross, Georgia) and are within ±0.4% of the theoretical values unless otherwise indicated. All reagents and solvents were obtained from commercial sources and used without further purification unless indicated. Tetrahydrofuran (THF) was distilled from potassium and methylene chloride (CH_2Cl_2) was distilled from calcium hydride (CaH_2) immediately prior to use.

3,4,5-Trimethoxybenzyl-triphenylphosphonium bromide (1). To a well-stirred solution of CBr_4 (10.20 g, 30.76 mmol) in acetone (80 mL) at 0 °C under nitrogen, 3,4,5-trimethoxybenzyl alcohol (4.45 g, 22.45 mmol) and triphenylphosphine (8.00 g, 30.50 mmol) were added. After 12 h the mixture was filtered through Celite, and the solvent was removed under reduced pressure to afford 3,4,5-trimethoxybenzyl bromide as a brown oil. 3,4,5-Trimethoxybenzyl bromide was dissolved in CH_2Cl_2 (50 mL) and triphenylphosphine (6.48 g, 24.70 mmol) was added. After 14 h at reflux, water was added, and the product was isolated by extraction with CH_2Cl_2. The organic phase was washed with brine and dried over $MgSO_4$. The product was purified by trituration with ether and immediately used in the Wittig reaction.

3-Methoxy-4-nitrobenzaldehyde (2). A solution of 3-methoxy-4-nitrobenzyl alcohol (0.200 g, 1.09 mmol) and PDC (0.626 g, 1.66 mmol) in CH_2Cl_2 (30 mL) was stirred at 23 °C under a nitrogen atmosphere. After 16 h the reaction mixture was filtered through silica gel/Celite to afford 3-methoxy-4-nitrobenzaldehyde (0.196 g, 1.08 mmol, 99%) as a pale yellow solid (mp 97–98 °C). ^{1}H NMR ($CDCl_3$, 360 MHz) δ 10.06 (s, 1H, C$\underline{H}$O), 7.93 (d, $J=8.1$ Hz, 1H, Ar$\underline{H}$), 7.61 (d, $J=1.5$ Hz, 1H, Ar$\underline{H}$), 7.55 (dd, $J=8.1$, 1.5 Hz, 1H, Ar$\underline{H}$), 4.04 (s, 3H, -OC$\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 90 MHz) δ 190.2, 153.0, 143.4, 139.7, 125.9, 122.6, 112.6, 56.8. HRMS (EI) M^+ calcd for $C_8H_7NO_4$ 181.0375, found 181.0379. Anal. calcd for $C_8H_7NO_4$: C, 53.04, H, 3.90, N, 7.73. Found: C, 52.99, H, 3.92, N, 7.67.

***(E/Z)*-1-(3′-Methoxy-4′-nitrophenyl)-2-(3″,4″,5″-trimethoxyphenyl)ethene (3A/B).** A solution of 3,4,5-trimethoxybenzyltriphenylphosphonium bromide **1** (1.50 g, 2.86 mmol) and 3-methoxy-4-nitrobenzaldehyde **2** (0.518 g, 2.86 mmol) in CH_2Cl_2 (50 mL) was stirred under a nitrogen atmosphere. NaH (0.412 g, 17.16 mmol) was added. After 16 h water was added, and the product was isolated by extraction with CH_2Cl_2. The organic phase was washed with brine and dried over $MgSO_4$. The solvent was removed under reduced pressure to afford ethene **3** as a mixture of isomers (*Z:E* 1.46:1.00 as determined by careful integration of ^{1}H NMR signals).

Purification and characterization of (*E*) isomer 3A.[50] Purification by flash chromatography (silica gel, 70:30 hexanes:ethyl acetate) afforded (*E*)-ethene **3A** (0.274 g, 0.793 mmol, 28%) as a bright yellow solid. Recrystallization (hexanes/CH_2Cl_2) gave an analytically pure sample of (*E*)-ethene **3A** (mp 187–188 °C). ^{1}H NMR ($CDCl_3$, 360 MHz) δ 7.92 (d, J = 8.6 Hz, 1H, ArH), 7.16 (m, 2H, ArH), 7.15 (d, J = 16.4 Hz, 1H, vinyl CH), 6.99 (d, J = 16.4 Hz, 1H, vinyl CH), 6.77 (s, 2H, ArH), 4.03 (s, 3H, OCH_3), 3.93 (s, 6H, OCH_3), 3.89 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$, 90 MHz) δ 153.7, 153.5, 143.8, 138.9, 138.0, 132.9, 131.8, 126.6, 125.9, 117.9, 111.0, 104.1, 61.0, 56.5, 56.2. HRMS (EI) M^+ calcd for $C_{18}H_{19}NO_6$ 345.1212, found 345.1220. Anal. calcd for $C_{18}H_{19}NO_6$: C, 62.60, H, 5.55, N, 4.06. Found: C, 62.51, H, 5.53, N, 3.95.

Purification and characterization of (*Z*) isomer 3B. Purification by flash chromatography (silica gel, 70:30 hexanes:ethyl acetate) afforded (*Z*)-ethene **3B** (0.319 g, 0.923 mmol, 32%) as a bright yellow solid. Recrystallization (hexanes/CH_2Cl_2) afforded an analytically pure sample of ethene **3B** (mp 83–85 °C). ^{1}H NMR ($CDCl_3$, 360 MHz) δ 7.80 (d, J = 8.4 Hz, 1H, ArH), 6.98 (d, J = 1.3 Hz, 1H, ArH), 6.95 (dd, J = 8.6 Hz, 1.4 Hz, 1H, ArH), 6.72 (d, J = 12.1, 1H, vinyl CH), 6.54 (d, J = 12.1, 1H, vinyl CH), 6.46 (s, 2H, ArH), 3.84 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 3.71 (s, 6H, OCH_3); ^{13}C NMR ($CDCl_3$, 90 MHz) δ 153.2, 153.0, 143.9, 138.0, 137.9, 133.5, 131.6, 127.9, 125.9, 121.1, 113.8, 106.0, 60.9, 56.3, 56.1. HRMS (EI) M^+ calcd for $C_{18}H_{19}NO_6$ 345.1212, found 345.1229. Anal. calcd for $C_{18}H_{19}NO_6$: C, 62.60, H, 5.55, N, 4.06. Found: C, 62.68, H, 5.58, N, 4.05.

(*Z*)-1-(4′-Amino-3′-methoxyphenyl)-2-(3″,4″,5″-trimethoxy-phenyl)ethene (4). (*Z*)-Ethene **3B** (0.454 g, 1.31 mmol) was dissolved in acetone:water (10:5 mL) and heated at 50 °C. After 30 min, $Na_2S_2O_4$ (4.57 g, 26.28 mmol) was added slowly, and the mixture was heated at reflux (50 °C, 1 h), cooled to room temperature, and water was added. The product was rinsed with $NaHCO_3$, and then isolated by extraction with ethyl acetate. The organic layer was dried over Na_2SO_4. The solvent was removed under reduced pressure, and the crude product (0.302 g, 0.956 mmol, 73%) obtained was used in the azide formation.

(*Z*)-1-(4′-Azide-3′-methoxyphenyl)-2-(3″,4″,5″-trimethoxyphenyl)ethene (5). (*Z*)-Amino ethene **4** (0.166 g, 0.526 mmol) was dissolved in acetone (7.5 mL) in the dark. The mixture was cooled to 0 °C, and HCl (0.22 M, 7.5 mL) was added. After 10 min of stirring under a nitrogen atmosphere, $NaNO_2$ (0.160 g, 2.31 mmol) was added, followed 30 min later by adding NaN_3 (0.427 g, 6.58 mmol) while maintaining the reaction mixture at 0 °C. Fifteen min later, ether (7.5 mL) was added as an overlay. Water was added 45 min later, and the product was isolated by extraction with ether. The organic layer was extracted with brine and dried over $MgSO_4$. The solvent was removed under reduced pressure and purified by flash chromatography (silica gel, 70:30 hexanes:ethyl acetate). (*Z*)-Ethene **5** was obtained as a colorless solid (0.077 g, 0.226 mmol, 43%) with mp 61–63 °C. ^{1}H NMR ($CDCl_3$, 360 MHz) δ 6.89 (s, 2H, ArH), 6.81 (s, 1H, ArH), 6.54 (d, J = 12.1 Hz, 1H, vinyl CH), 6.50 (s, 2H, ArH), 6.49 (d, J = 12.1 Hz, 1H, vinyl CH), 3.83 (s, 3H, OCH_3), 3.71 (s, 6H, OCH_3), 3.67 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$, 90 MHz) δ 153.0, 151.3, 137.3, 134.7, 132.5, 130.2, 129.1, 127.1, 122.3, 119.8, 112.5, 105.9, 60.8, 55.9, 55.7. HRMS (EI) M^+ calcd for $C_{18}H_{19}N_3O_4$ 341.1376, found 341.1360. Anal. calcd for $C_{18}H_{19}N_3O_4$: C, 63.33, H, 5.62, N, 12.31. Found: C, 63.23, H, 5.62, N, 12.21.

3-Nitro-4-methoxybenzyl-triphenylphosphonium bromide (6). To a well stirred solution of 4-methyl-2-nitroanisole (4.16 g, 24.87 mmol) in CCl_4 (50 mL) were added AIBN (40.8 mg, 0.25 mmol), and NBS (4.43 g, 24.87 mmol). The reaction mixture was heated at reflux for 18 h, water was added, and the product was isolated by extraction with CH_2Cl_2. The organic phase was washed with brine and dried over $MgSO_4$. 3-Nitro-4-methoxybenzyl bromide (6.02 g, 24.47 mmol) was obtained as a mixture of 4-methyl-2-nitroanisole, and the monobromo and dibromo derivatives (8.5:14.8:1.0 respectively, ratios determined by ^{1}H NMR). This mixture was dissolved in CH_2Cl_2 (60 mL), and triphenylphosphine (6.42 g, 24.48 mmol) was added. The reaction mixture was heated at reflux for 5 h. The solvent was removed under reduced pressure, and the triphenylphosphonium salt **6** (9.68 g, 19.05 mmol, 78%) was obtained as colorless crystals after trituration in ether.

(*E*/*Z*)-1-(4″-Methoxy-3″-nitrophenyl)-2-(3′,4′,5′-trimethoxyphenyl)ethene (7A/B). 3,4,5-Trimethoxybenzaldehyde (1.17 g, 5.98 mmol) and 3-methoxy-4-nitrobenzyl triphenylphosphonium bromide **6** (3.01 g, 5.92 mmol) were dissolved in CH_2Cl_2 (40 mL) and stirred under a nitrogen atmosphere. NaH (0.710 g, 29.6 mmol) was added. After 14 h water was added carefully, and the product was isolated by extraction with CH_2Cl_2. The organic phase was washed with brine and dried over $MgSO_4$ to afford a mixture of ethenes **7B:7A** (*Z*:*E* 2.9:1.0).

Purification and characterization of (*E*)-ethene 7A. Purification by flash chromatography (silica gel, 70:30 hexanes:ethyl acetate) afforded ethene **7A** (0.541 g, 1.57 mmol, 27%) as a bright yellow solid. Recrystallization (hexanes/CH_2Cl_2) afforded an analytically pure sample of ethene **7A** (mp 147–148 °C). ^{1}H NMR ($CDCl_3$, 360 MHz) δ 8.01 (d, J = 2.2 Hz, 1H, ArH), 7.65 (dd, J = 8.7, 2.3 Hz, 1H, ArH), 7.15 (d, J = 8.7 Hz, 1H, ArH), 7.00 (d, J = 16.2 Hz, 1H, vinyl CH), 6.93 (d, J = 16.2 Hz, 1H, vinyl CH), 6.73 (s, 2H, ArH), 3.99 (s, 3H, OCH_3), 3.92 (s, 6H, OCH_3), 3.88 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$, 90 MHz) δ 153.5, 150.2, 140.0, 138.5, 132.4, 131.8, 130.4, 129.6, 125.2, 123.1, 113.8, 103.8, 61.0, 56.7, 56.2. HRMS (EI) M^+ calcd for $C_{18}H_{19}NO_6$ 345.1212, found 345.1206. Anal. calcd for $C_{18}H_{19}NO_6$: C, 62.60, H, 5.55, N, 4.06. Found: C, 62.60, H, 5.60, N, 3.97.

Purification and characterization of (*Z*)-ethene 7B. Purification by flash chromatography (silica gel, 70:30 hexanes:ethyl acetate) afforded (*Z*)-ethene **7B** (1.44 g, 4.16 mmol, 73%) as a light yellow solid. Recrystallization (hexanes/CH_2Cl_2) afforded an analytically pure sample of ethene **7B** (mp 119–121 °C). ^{1}H NMR ($CDCl_3$, 360

MHz) δ 7.80 (d, $J=2.2$ Hz, 1H, ArH), 7.43 (dd, $J=8.6$, 2.2 Hz, 1H, ArH), 6.94 (d, $J=8.6$ Hz, 1H, ArH), 6.58 (d, $J=12.2$ Hz, 1H, vinyl CH), 6.45 (d, $J=13.3$ Hz, 1H, vinyl CH), 6.47 (s, 2H, ArH), 3.94 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.72 (s, 6H, OCH_3); ^{13}C NMR ($CDCl_3$, 90 MHz) δ 153.2, 151.7, 139.5, 137.7, 134.6, 131.8, 131.3, 129.7, 126.8, 125.9, 113.1, 105.9, 60.9, 56.5, 56.0. HRMS (EI) M^+ calcd for $C_{18}H_{19}NO_6$ 345.1212, found 345.1191. Anal. calcd for $C_{18}H_{19}NO_6$: C, 62.60, H, 5.55, N, 4.06. Found: C, 62.53, H, 5.61, N, 3.97.

(*Z*)-1-(3′-Amino-4′-methoxyphenyl)-2-(3″,4″,5″-trimethoxyphenyl)ethene (8).[47] Ethene **7B** (1.24 g, 3.58 mmol) was dissolved in acetone:water (40:20 mL) and heated at 50 °C. After 30 min $Na_2S_2O_4$ (12.47 g, 71.61 mmol) was added slowly. After 30 min of reflux at 50 °C the mixture was cooled to room temperature, and water was added. The product was isolated by extraction with ethyl acetate. The organic layer was dried over Na_2SO_4. The solvent was removed under reduced pressure, and the product was purified by flash chromatography (70:30, hexanes:ethyl acetate) to afford a pure sample of arylamine (0.423 g, 1.34 mmol, 37%) **8** (mp 64–66 °C). ^1H NMR ($CDCl_3$, 360 MHz) δ 6.71 (s, 1H, ArH), 6.68 (s, 2H, ArH), 6.55 (s, 2H, ArH), 6.46 (d, $J=12.2$ Hz, 1H, vinyl CH), 6.37 (d, $J=12.0$ Hz, 1H, vinyl CH), 3.84 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 3.71 (s, 6H, OCH_3); ^{13}C NMR ($CDCl_3$, 90 MHz) δ 152.9, 146.7, 137.2, 135.8, 133.0, 130.2, 130.0, 128.4, 119.5, 115.3, 110.1, 106.2, 60.9, 56.0, 55.5. HRMS (EI) M^+ calcd for $C_{18}H_{21}NO_4$ 315.1471, found 315.1466.

(*Z*)-1-(3′-Azido-4′-methoxyphenyl)-2-(3″,4″,5″-trimethoxyphenyl)ethene (9). (*Z*)-Amino ethene **8** (0.327g, 1.04 mmol) was dissolved in acetone (15 mL) and cooled to 0 °C, and HCl (0.22 M, 15 mL) was added. After 10 min of stirring under a nitrogen atmosphere, $NaNO_2$ (0.316 g, 4.58 mmol) was added, and 30 min later, NaN_3 (0.852 g, 13.10 mmol) was added. The reaction mixture temperature was maintained at 0 °C, and 15 min later, ether (17 mL) was added as an overlay. Following 45 min of stirring, the mixture was extracted with brine, and the product was dried over $MgSO_4$. The solvent was removed under reduced pressure and the product was purified by flash column chromatography (70:30 hexanes:ethyl acetate). The product was obtained as a yellow oil (0.163 g, 0.478 mmol, 46%). ^1H NMR ($CDCl_3$, 360 MHz) δ 7.03 (dd, $J=8.4$ Hz, 2.1 Hz, 1H, ArH), 6.97 (d, $J=2.1$ Hz, 1H, ArH), 6.76 (d, $J=8.4$ Hz, 1H, ArH), 6.49 (s, 2H, ArH), 6.49 (d, $J=11.9$ Hz, 1H, vinyl CH), 6.43 (d, $J=12.2$ Hz, 1H, vinyl CH), 3.84 (s, 6 H, OCH_3), 3.71 (s, 6 H, OCH_3); ^{13}C NMR ($CDCl_3$, 90 MHz) δ 152.9, 150.7, 137.3, 132.4, 130.3, 129.6, 128.3, 127.8, 126.5, 120.4, 111.5, 105.8, 60.8, 55.8. HRMS (EI) M^+ calcd for $C_{18}H_{19}N_3O_4$ 341.1376, found 341.1376. Anal. calcd for $C_{18}H_{19}N_3O_4$: C, 63.33, H, 5.61, N, 12.31. Found: C, 63.38, H, 5.64, N, 12.28.

Photochemical isomerization

(*E*)-Nitroethene **3A** (0.274 g, 0.793 mmol) and benzil (0.833 g, 3.96 mmol) were dissolved in benzene (50 mL) and flushed with argon gas. After 16 h the solution was irradiated at 254 nm for 14 h. Removal of the solvent and flash column chromatography afforded the (*Z*)-nitrostilbene **3B** (0.091 g, 0.263 mmol, 33%). (*E*)-Nitroethene **7A** (0.803 g, 2.33 mmol) and benzil (2.44 g, 11.63 mmol) were dissolved in benzene (233 mL) and flushed with argon gas. After 12 h the solution was irradiated at 254 nm for 4 h. Removal of the solvent and flash column chromatography afforded the (*Z*)-nitrostilbene **7B** (0.251 g, 0.73 mmol, 31%) and recovered the (*E*)-nitrostilbene **7A**.

Tubulin studies

Inhibition of tubulin polymerization and of colchicine binding to tubulin were performed as described previously.[53] In the polymerization studies, reaction mixtures contained 10 μM (1.0 mg/mL tubulin) and varying inhibitor concentrations. The IC_{50} value was defined as the drug concentration required to inhibit extent of assembly by 50% after a 20 min incubation. In the colchicine binding studies, reaction mixtures contained 1.0 μM tubulin (0.1 mg/mL), 5.0 μM [^3H]-colchicine, and inhibitors at 1.0, 5.0, or 50 μM, as indicated in Table 2.

Acknowledgements

K.G.P. thanks The Robert A. Welch Foundation (Grant No. AA-1278), Baylor University, and Baylor University Research Committee for financial support of this project. G.R.P. thanks The Arizona Disease Control Research Commission, Outstanding Investigator Grant CA-44344-06-11 awarded by the DCTD, National Cancer Institute, DHHS, Drs. J.-C. Chapuis, F. Hogan, and J. M. Schmidt.

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