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Synthesis and Antimicrotubule Activity of Combretatropone Derivatives

Mark E. Janik[a] and Susan L. Bane[b,*]

[a]*Department of Chemistry, SUNY-Binghamton, Binghamton, NY 13902, USA*
[b]*Department of Chemistry, SUNY-Fredonia, Fredonia, NY 14063, USA*

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Abstract—Combretatropone is a hybrid of combretastatin and colchicine in which the *o*-methoxyphenol of dihydrocombretastatin A-4 is replaced by an α-methoxytropone. Derivatives of combretatropone have been synthesized and evaluated for antimicrotubule activity. All combretatropones were less active than the corresponding colchicine derivatives, supporting the idea that loss of ligand conformational entropy upon tubulin binding results in decreased potency for colchicinoid ligands. The structure–activity relationship of the combretatropone series was different than that of the colchicine series. These data indicate that conformationally mobile and conformationally rigid colchicinoids do not interact with the receptor site in the same manner.

Introduction

Microtubules are the cellular target for a number of useful chemotherapeutic agents.[1] These drugs are generally divided into three classes defined by the three known drug binding domains of tubulin, the major protein component of microtubules. Drugs that interact with the Taxol and vinblastine regions of the protein are used clinically in the treatment of cancer. Molecules that bind to the colchicine domain of tubulin, however, are not yet in standard use as antineoplastic agents.[2] The promise of the antiangeogenic compound combrestatin A-4, a colchicine site ligand, has sparked renewed interest in colchicine site drugs as potential anticancer drugs.[3]

The most studied ligand for the colchicine binding site on tubulin is colchicine itself (Fig. 1). Several hundred derivatives and analogues of colchicine (**1**) have been tested for antimicrotuble activity.[4] Attempts to gain a mechanistic understanding have taken different forms, from conventional SAR studies to intricate QSAR and thermodynamic analyses.[5–8] The primary pharmacophore appears to consist of the A and C rings of the parent molecule, but it is also clear that the B ring portion of the molecule contributes to the efficacy of the drug. The substituent on the B ring plays at least two important roles. In the absence of the C-7 substituent, the colchicine ring system exists in two conformational isomers with different axial chiralities. The amide in natural *S*-colchicine holds the aryl-tropone rings in the *aR* configuration, while the amide in unnatural *R*-colchicine places the aryl-tropone rings in the inactive *aS* conformation.[8–10] The C-7 substituent also affects the kinetics of colchicinoids binding to tubulin, presumably through contact with the protein in the transition state. The presence of an amine, substituted amine or amide at the C-7 position raises the activation energy of the association reaction by up to 5 kcal/mol.[11,12] The B ring itself, however, seems to play no enthalpic role in either the ground or transition state. Removal of the entire B ring decreases the affinity of the molecule for tubulin (MTC, 2), but the loss of activity is attributed to the conformational properties of the ligand and not directly to binding site interactions. Thermodynamic analyses of colchicine and MTC binding to tubulin have shown that the two ligands have identical binding enthalpies, indicating that the contacts made between colchicine and tubulin involve just the A and C rings.[13–15]

If the sole role of the B ring is to limit the conformational mobility of the A–C ring system, structure–activity relationships in the colchicinoid and MTC series should have a parallel correlation. For example, substituents that decrease the activity of the colchicine derivative

*Corresponding author. Tel.: +1-607-777-2927; fax: +1-607-777-4478; e-mail: sbane@binghamton.edu

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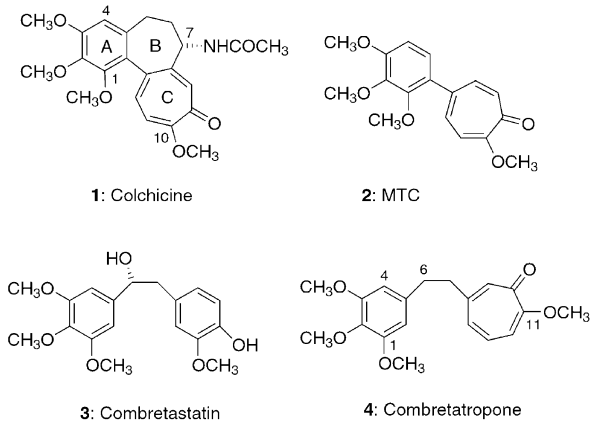


Figure 1. Structures of colchicine and analogues.

should cause an equivalent decrease in the activity of the MTC derivative. Structure–activity studies of tropone-substituted analogues in both the colchicine and MTC series have been performed. It was found that the nature of the C-10 substituent of colchicine has little effect on its ability to inhibit in vitro microtubule assembly.[16] In contrast, a similar series of MTC derivatives displayed a wide variation in activity as a function of the tropone substituent.[17]

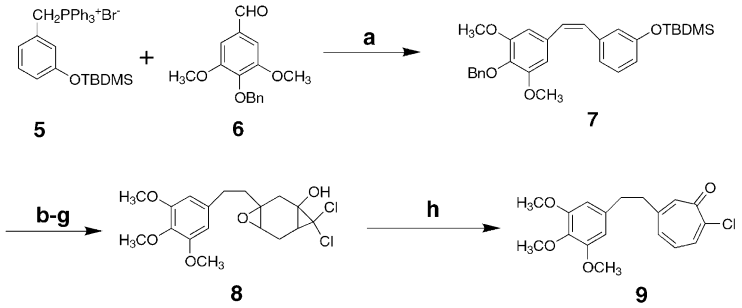
In order to explore the role of ligand conformational entropy in the binding of colchicinoids to tubulin, a structure–activity study of tropone-containing ligands with additional degrees of conformational freedom was undertaken. It was previously shown that a hybrid of the conformationally mobile combretastatin (**3**) and the tropone-containing colchicine, termed 'combretatropone' (**4**), retained tubulin-binding ability.[18] This skeleton provides a suitable scaffold on which to evaluate the relative role of conformational entropy in the affinity of colchicinoids for tubulin. Therefore, in this study, a series of substituted tropone derivatives of combretatropone were prepared and their ability to inhibit microtubule assembly in vitro was evaluated. The structure–activity relationship in the combretatropone series followed the SAR for bicyclic (MTC) compounds rather than the SAR in the corresponding tricyclic (colchicinoid) molecules. These data support the notion that conformationally mobile ligands interact with the colchicine binding site differently than the conformationally rigid colchicinoids.

Chemistry

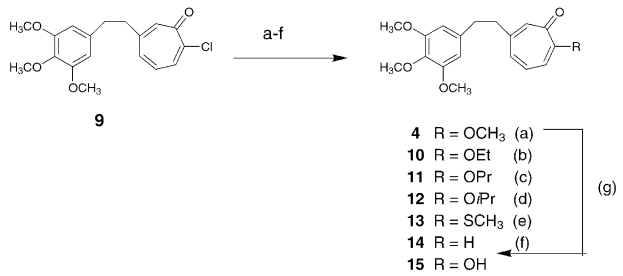
The synthesis of 10-chloro-10-demethoxycombretatropone (**9**) is shown in Scheme 1. Compounds **5**–**8** were previously reported by Andres et al.[18] and have been synthesized here using some procedural modifications. 3-Hydroxybenzaldehyde was protected, reduced and converted to the phosphonium salt (**5**) in 63% yield. Subsequent Wittig condensation of **5** with 3,5-dimethoxy-4-benzyloxybenzaldehyde (**6**) provided a mixture of *cis*- and *trans*-stilbene (**7**) in 58% yield. Ring expansion of the 3-silyloxyphenol of **7** into an α-chlorotropone was accomplished as modified from Andres et al.[18] as follows. Stilbene **7** was subjected to catalytic hydrogenation followed by Birch reduction. Methylation of the phenol was accomplished in high yield with diazomethane rather than methyl iodide. Subsequent dichlorocyclopropanation, desilylation and epoxidation provided **8** in 30% overall yield from **7**. Acid catalyzed ring expansion of **8** was then achieved with pTSA in benzene to produce **9** in 57% yield.

Compounds **10**–**14** were prepared directly from compound **9** (Scheme 2). Combretatropone (**4**) was produced in 80% yield by reaction of magnesium methoxide with **9** at room temperature. The remaining alkoxy combretatropones (**10**–**12**) were prepared in the following manner. Compound **9** was reacted separately with either ethoxymagnesium bromide or propoxymagnesium bromide under reflux to afford the alkoxycombretatropones **10** and **11** in yields of 73 and 76%, respectively. The isopropoxy derivative **12** was prepared in 35% yield by the reaction of compound **9** with sodium isopropoxide. The remaining derivatives shown in Scheme 2 were prepared as follows. 10 - Demethoxy - 10 - thiomethylcombretatropone (**13**) was produced in 78% yield by the reaction of **9** with sodium thiomethoxide. Conversion of compound **9** into the tropone derivative **14** was accomplished using sodium borohydride in DMSO in a yield of 67%. Synthesis of combretatropolone (**15**) was carried out by acid catalyzed hydrolysis of **4** in 86% yield.

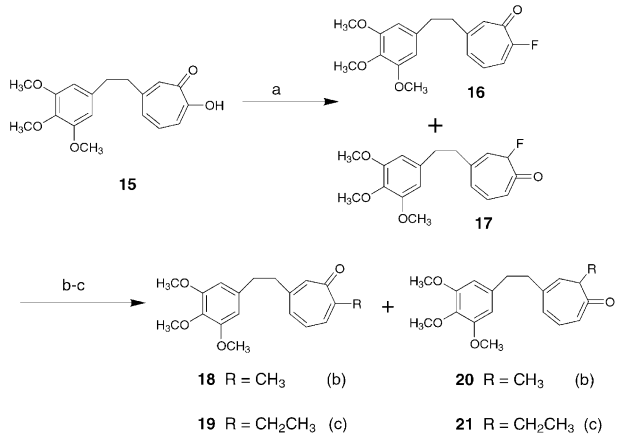
The synthesis of the fluorocombretatropones **16**–**17** and alkylcombretatropone derivatives **18**–**21** is shown in Scheme 3. Reaction of combretatropolone (**15**) with diethylaminosulfur trifluoride (DAST) at 0 °C resulted in the formation of two isomeric products (**16** and **17**) in a combined yield of 83%. The fluorocombretatropones



Scheme 1. (a) *n*BuLi, THF, 58%; (b) H_2, Pd/C, EtOH, 96%; (c) Li/NH_3, *t*-BuOH, THF, reflux, 90%; (d) CH_2N_2, CH_2Cl_2, 97%; (e) $NaO_2C_2Cl_3$, DME, reflux; (f) 20% HF, CH_3CN, 53%; (g) *m*CPBA, CH_2Cl_2, 67%; (h) *p*TSA, C_6H_6, reflux, 57%.



Scheme 2. (a) Mg, I_2, CH_3OH, 66%; (b) EtOH, EtMgBr, THF, 73%; (c) PrOH, PrMgBr, THF, 76%; (d) Na, *i*PrOH, 38%; (e) $NaSCH_3$, H_2O, CH_3OH, 78%; (f) $NaBH_4$, DMSO, 67%; (g) 2 N HCl, CH_3OH, reflux, 86%.



Scheme 3. (a) DAST, CH_2Cl_2, 83%; (b) CH_3MgBr, THF, 0 °C; (c) CH_3CH_2MgBr, THF, 0 °C.

16 and **17** were then reacted with the appropriate Grignard reagent to produce alkylcombretatropones **18**–**21**. Reaction of compound **16** with methyl magnesium bromide in THF at 0 °C produced 10-demethoxy-10-methylcombretatropone (**18**) in a yield of 51%. Similarly, reaction of **16** with ethyl magnesium bromide in THF at 0 °C produced 10-demethoxy-10-ethylcombretatropone (**19**) in a yield of 59%. The remaining two isomeric alkylcombretatropones (**20** and **21**) were prepared in an analogous manner from fluorocombretatropone **17** in yields of 48 and 56%, respectively.

The synthesis of the amine derivatives (**22**–**24**) was accomplished in the following manner. Combretatropone (**4**) was reacted with ammonia in a pressure bottle for 12 h at 85 °C to produce 10-amino-10-demethoxycombretatropone (**22**) in a yield of 82%. 10-Demethoxy-10-methylaminocombretatropone (**23**) was prepared in a yield of 84% through the reaction of **4** with methylamine for 24 h at 35 °C in a pressure bottle. Reaction of **4** with dimethylamine at 80 °C for 3 days in a pressure bottle afforded 10-demethoxy-10-dimethylaminocombretatropone (**24**) in a yield of 65%.

Biological Results and Discussion

The antimicrotubule activities of the combretatropone analogues were assessed by their ability to inhibit in vitro assembly of microtubule protein (Table 1). The I_{50} value is the concentration of ligand required to decrease the extent of microtubule polymerization to 50% of the control.

Table 1. Inhibition of in vitro microtubule assembly by C-11 substituted combretatropone derivatives

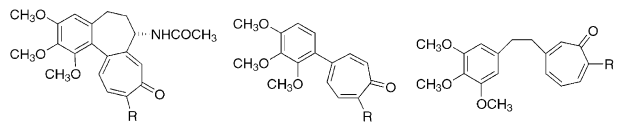
Compound	Substituent[a]	I_{50} (μM)[b]
4	OCH_3	54.3 ± 3.23[c]
9	Cl	300 ± 4.04
10	OCH_2CH_3	148 ± 1.06
11	$OCH_2CH_2CH_3$	235 ± 3.76
12	$OCH(CH_3)$	254 ± 3.61
13	SCH_3	64.1 ± 2.02
14	H	375 ± 5.12
15	OH	175 ± 4.82
16	F	182 ± 3.81
17	F[d]	187 ± 3.70
18	CH_3	92.2 ± 2.28
20	CH_3[d]	95.4 ± 2.56
19	CH_2CH_3	158 ± 2.70
21	CH_2CH_3[d]	165 ± 4.09
22	NH_2	180 ± 1.41
23	$NHCH_3$	38.2 ± 1.69
24	$N(CH_3)_2$	174 ± 2.08

[a]Substituent on the tropone ring.
[b]I_{50} is the concentration of ligand required to effect a 50% reduction in microtubule polymerization.
[c]Errors are expressed as standard deviation of the mean. Each compound was tested a minimum of four times.
[d]Isomer of compound in which the relative location of the C-10 and C-11 groups are interchanged; see Scheme 3.

It has been shown that both the absolute and relative magnitude of I_{50} values for antimicrotubule drugs are dependent on the conditions employed in the assay.[19] The assay conditions and procedures used in different laboratories must be taken into account when data from different sources are compared. The assay conditions used to evaluate the MTC analogues are significantly different from the standard assay employed in our laboratory. In order to make a precise comparison between the bicyclic and combretatropone SAR, some of the previously studied MTC derivatives were synthesized in our laboratory and subjected to our standard assay conditions. These values are included in Table 2.

There are a few trends in the polymerization data that are notable. First, the combretatropones as a class are significantly less active than the analogous bicyclic colchicine derivatives, which are themselves less active than C-10 substituted colchicine analogues. The most active combretatropone is **23**, which has an I_{50} value of 38 μM. In contrast, the I_{50} values obtained under similar conditions for the colchicine derivatives corresponding to combretatropones **4**, **9**, **10**, **13**, **14**, **16**, **18**, and **19** fall between 2 and 8 μM. The least active colchicinoids, which correspond to the propoxy and isopropoxy combretatropones, have I_{50} values of 11 and 22 μM, respectively.[16] The I_{50} values for the analogous MTC derivatives tend to be in between those found for colchicine and the combretatropones (Table 2 and ref 17). These data support the conclusion of Menendez et al.[13] that the

Table 2. Inhibition of in vitro microtubule assembly by selected colchicinoids



I II III

Substituent	Colchicine (I)	MTC (II)	Combretatropone (III)
	I_{50}, μM[a]	I_{50}, μM[b]	I_{50}, μM
OCH_3	5.1	6.9±2.0[c]	54
$NHCH_3$	2.0	3.5±1.0	38
SCH_3	2.5	11±1.7	64
CH_2CH_3	4.0	63±3.0	160
Cl	4.0	~500[d]	300

[a]From ref 16.
[b]Determined in this laboratory.
[c]Errors are expressed as standard deviation of the mean. Each compound was tested a minimum of four times.
[d]Value from ref 17.

conformational entropy of the ligand is an important factor in the potency of colchicinoid drugs.

Differences in conformational mobility alone, however, cannot explain the trends in the combretatropone data. Table 2 compares the antimicrotubule activity of the analogous derivatives in the colchicine, MTC and combretatropone series. The nature of the tropone substituent has little effect on the potency of colchicinoids. In contrast, the biological activity of the MTC and combretatropone derivatives varied considerably with the tropone substituent. The most dramatic difference is seen in the chlorotropone series. 10-Chloro-10-demethoxycolchicine is highly potent—in fact, it is slightly more active than colchicine itself. The corresponding bicyclic and combretatropone compounds are considerably less active than the parent molecules. Furthermore, performing an isosteric substitution of ethyl for methoxy produced little effect on activity in the colchicine series but decreased activity in the bicyclic and combretatropone analogues. It appears that steric and electrostatic properties of the substituent play a role in its antimicrotubule activity in the absence of the tethering B ring. This observation is an important consideration in 3-D-QSAR analyses of colchicine site ligands. 3-D-QSAR analyses using CoMFA require the generation of alignment rules.[20,21] Our results indicate that an alignment rule generated for conformationally restricted ligands may not be suitable for the conformationally mobile ligands.

A somewhat surprising outcome from the combretatropone series is found in compounds **17**, **20**, and **21** (Table 1). The structural isomers in each pair of compounds are analogous to the colchicine and isocolchicine pair for the parent molecule. In the tricyclic series, isocolchicine isomers are inactive as inhibitors of microtubule assembly at sub-millimolar concentrations.[22,23] In the combretatropone series, though, the activities of the two isomers are essentially identical. It appears that the conformational mobility imparted by the methylene bridge in sufficient to allow the 'isocolchicine-like' isomer to interact with tubulin in a 'colchicine-like' manner. So, with respect to the tropone ring isomers, the entropic loss may be counterbalanced by the enthalpic gain.

Conclusions

A series of substituted combretatropones have been synthesized and evaluated for antimicrotubule activity. The SAR results support the idea that loss of ligand conformational entropy upon tubulin binding results in decreased potency for colchicinoid ligands. These results also support the notion that conformationally mobile colchicinoids may interact with the receptor site differently than conformationally restricted colchicinoids.[14] This possibility needs to be considered in 3-D-QSAR studies of colchicine site ligands.

Experimental

All NMR spectra were recorded on a Bruker AM 360 spectrometer in deuterochloroform. Chemical shifts are reported in ppm (δ) and *J* coupling constants are reported in Hz. Infrared spectra were measured in spectroscopy grade chloroform on a Perkin-Elmer 1600 series FTIR. UV spectra were obtained on a Hewlett Packard 8453 diode ray spectrometer. Extinction coefficients were determined in PME buffer (PME buffer = 100 mM Pipes, 2 mM EGTA, 1 mM $MgSO_4$, pH 6.90 at 23 °C) and are reported as M^{-1} cm^{-1}. Melting points were determined using an Electrothermal IA6304 open capillary melting point apparatus and are uncorrected. EI high-resolution mass spectra were obtained by Dr. Gordon Nicol at the University of Delaware.

All reactions were conducted under a protective atmosphere of either dry nitrogen or argon gas unless otherwise stated. All solvents were dried and distilled prior to use. All reagents were obtained pure from Aldrich Chemical Corporation or were purified according to known literature purification procedures before use. Column chromatography was performed using Baker silica gel 60–200 or 200–400 mesh. A chromatatron (Harrison Research) was used for radial chromatrography.

Purity criteria were established using two diverse systems of HPLC. The first method performed was reverse phase chromatography using a Waters Bondapak C18 column (3.9×300 mm). The solvent system employed with this technique was methanol/acetonitrile/water, 12.5:37.5:50. The second method used was liquid/solid adsorption chromatography. The adsorbent used was Keystone Silica 60 (particle size 10 nanometers, pore size 60 Å). The dimensions of the column were 40×5 mm, and the solvent system employed was 70:30 hexanes/isopropanol. The detection method for both systems was an ISCO V4 variable wavelength detector, connected to a Spectra-Physics SP4270 integrator. No impurities were detected in any combretatropone tested in the biological assays.

3-(*tert*-Butyldimethylsiloxy)benzyl-triphenylphosphonium bromide (5). The procedure of Andres et al.[18] was used. Briefly, 3-hydroxybenzaldehyde was treated with imidazole and *t*-butyldimethylchlorosilane in THF to produce 3-(*tert*-butyldimethylsiloxy)benzaldehyde in 87% yield. The aldehyde was reduced to 3-(*tert*-butyldimethylsiloxy)benzyl alcohol by catalytic hydrogenation (97%). 3-(*tert*-Butyldimethylsiloxy)benzyl bromide was prepared in two steps by reaction of the benzyl alcohol with trifluoroacetic anhydride (96%) followed by LiBr in THF (87%). Treatment of the benzyl bromide with triphenylphosphine in xylenes yielded compound 5 (88%). The structures of all products were confirmed by ^{1}H NMR.

4-Benzoyl-3,5-dimethoxybenzaldehyde (6). Syringe aldehyde (3.12 g, 17.1 mmol) was dissolved in 30 mL of THF and to the stirred solution was added potassuim hydroxide (1.07 g, 18.8 mmol). The resulting solution was refluxed for 24 h and five crystals of 18-crown-6 and benzyl bromide (2.24 mL, 18.8 mmol) were then added to the solution and refluxed continued for 24 h. The reaction was cooled to room temperature, worked up and purified using flash chromatography to yield 3.30 g of a pure yellow liquid. The product was confirmed by ^{1}H NMR.

1-(3-{(1*Z* and 1*E*)-2-[3,5-Dimethoxy-4-(phenylmethoxy)-phenyl]vinyl}phenoxy)-1,1,2,2-tetramethyl-1-silapropane (7). Phosphonium bromide **5** (6.2 g, 11.1 mmol) was dissolved in 75 mL of dry THF and cooled to 0 °C. To this solution was added *n*-BuLi (7.6 mL, 12.2 mmol) and the resulting solution was stirred at 0 °C for 1.75 h. Aldehyde **6** (2.52 g, 9.23 mmol) was then added and the reaction was allowed to stir for 2 h at room temperature. The mixture was diluted with ether (40 mL), and washed first with $NaHSO_3$ (20 mL), followed by $NaHCO_3$ (20 mL), and finally with water (35 mL). The organic layer was dried with sodium sulfate, filtered and evaporated to yield a dark-yellow oil. The product was purified using flash chromatography (80:20 hexane/acetone) to yield 2.57 g (58%) of a clear oil. The structure of the compound was confirmed by ^{1}H NMR.

8,8-Dichloro-4-oxa-3-[2-(3,4,5-trimethoxyphenyl)ethyl]-tricyclo[5.1.0.0<3,5>]octan-1-ol (8). A modification of the procedure of Andres et al.[18] was employed. The letters b–g refer to the individual reactions shown in Scheme 1.

2,6-Dimethoxy-4-{2-[3-(1,1,2,2-tetramethyl-1-silapropoxy) phenyl]ethyl}phenol (reaction b). The mixture of $E+Z$ stilbenes **7** (3.24 g, 6.8 mmol) was dissolved in 15 mL of ethanol in a pressure bottle. To this solution was added 10 mol% Pd/C and the stilbene was allowed to react at 45 psi/H_2 over a 24-h time period. The solution was then vacuum filtered and stripped of solvent, yielding 2.52 g (96%) of a pure light-brown oil. The compound was confirmed by ^{1}H NMR.

2,6-Dimethoxy-4-{2-[5-(1,1,2,2-tetramethyl-1-silapropoxy) cyclohexa-1,4-dienyl]ethyl}phenol (reaction c). A 1000 mL three-neck round-bottom flask was cooled to −78 °C and ammonia (230 mL), *t*-butanol (20 mL) and 100 mL of dry THF were added to the flask. Stirring was initiated and lithium wire (1.40 g) was then added to the reaction. The hydrogenated stilbene (3.12 g, 8.04 mmol) was added and the reaction allowed to reflux for 1 h. The reaction was quenched and worked up to yield 2.81 g (90%) of a brown oil. The product was confirmed by ^{1}H NMR.

1,1,2,2-Tetramethyl-1-sila-1-{5-[2-(3,4,5-trimethoxyphenyl)ethyl]cyclohexa-1,4-dienyloxy}propane (reaction d). The cyclohexadienyl compound (3.05 g, 7.9 mmol) was dissolved in 20 mL of methylene and cooled to 0 °C with an ice bath. To this solution was added an excess of an etherial diazomethane solution (220 mL). The solution was allowed to stir for 6 h at 0 °C. The ice bath was then removed and the solution allowed to stir at room temperature for an additional 24 h. The solution was then evaporated and purified to yield 3.07 g (97%) a pure light-yellow oil. The compound was confirmed by ^{1}H NMR.

7,7-Dichloro-3-[2-(3,4,5-trimethoxyphenyl)ethyl]bicyclo [4.1.0]hept-3-en-1-ol (reactions e–f). Sodium trichloroacetate (1.10 g, 6.38 mmol) was added to a 25 mL two-neck round-bottom flask. To this flask was added the methylated dihydroaromatic (1.22 g, 3.1 mmol in 3 mL of dimethoxyethane). The resulting solution was then refluxed for 5 h. The reaction was cooled to room temperature and worked up. The crude product was then dissolved in 30 mL of acetonitrile and to this solution was added 6 mL of a 20% HF solution. The solution was allowed to stir at room temperature for 12 h, worked up and purified using flash chromatography to yield 0.590 g (53%) of a yellow oil. The product was confirmed by ^{1}H NMR.

Compound 8 (reaction g). The cyclopropyl alcohol (0.62 g, 1.6 mmol) was dissolved in 50 mL of dry methylene chloride and to this solution was added recrystalized MCPBA (0.432 g, 1.5 mmol). The reaction was allowed to stir at room temperature for 3.5 h, worked up and purified to yield 0.430 g (67%) of a yellow oil. The product was confirmed by ^{1}H NMR.

11-Chloro-11-demethoxycombretatropone (9). To a stirred solution of epoxide (**8**, 0.42 g, 1.1 mmol) in 40 mL of distilled benzene was added recrystalized pTSA (15 mg). The resulting solution was allowed to reflux for 5 h. The reaction was cooled to room temperature and diluted with ether (30 mL). The mixture was washed with water (25 mL) and then brine (20 mL). Dried the organic layer with sodium sulfate, filtered and stripped of solvent to yield a brown oil. The product was purified first by flash chromatography followed by radial chromatography (90:10 CH_2Cl_2/acetone) to yield 0.205 g (57%) of a white solid: $\varepsilon_{323} = 7.01 \times 10^3$; mp 114–116 °C. ^{1}H NMR δ 7.68 (d, 1H, H-12, $J = 9.3$ Hz), 7.16 (s, 1H, H-9), 6.89 (d, 1H, H-14, $J = 9.3$ Hz) 6.79 (t, 1H, H-13, $J = 9.3$ Hz), 6.34 (s, 2H, H-1 and H-5), 3.81 (s, 9H), 2.83 (s, 4H, H-7 and H-8); ^{13}C NMR δ 179.3, 153.2, 150.0, 148.7, 137.8, 137.0, 136.6, 135.2, 134.7, 129.9, 105.3, 60.7, 56.0, 42.3, 37.6. IR 3052–2812, 1720, 1620, 1595 cm^{-1}. LR-MS (*m*/*z*): 336 (10), 335 (5), 334 (M^+, 20), 182 (15), 181 (100), 148 (10), 135 (10), 69 (40). HRMS calcd for $C_{18}H_{19}O_4Cl$: 334.0970. Found: 334.0956.

2 - Methoxy - 6 - [2 - (3,4,5 - trimethoxyphenyl) ethyl] cyclohepta-2,4,6-trien-1-one (combretatropone, 4). Oven dried magnesium metal (0.057 g, 24 mmol) was added to a two-neck round-bottom flask. Dry methanol (6 mL) and five crystals of iodine were then added to the flask. The reaction was then refluxed for 1 h, at which point the magnesium had dissolved. Chlorocombretatropone **9** (80 mg, 0.24 mmol) was dissolved in 3 mL of dry methanol and syringed into the two-neck flask. The reaction was allowed to stir at room temperature for 7.5 h. The reaction was diluted with chloroform (20 mL) and neutralized with 1 N HCl (10 mL). The layers were seperated and the aqueous layer extracted five times with chloroform (5×15 mL). Combined organic layers, dried with sodium sulfate, and stripped of solvent to yield a dark-yellow oil. The product was purified first by flash chromatography followed by radial chromatography (80:20 CH_2Cl_2/acetone) to yield 52 mg (66%) of a light yellow oil: $\varepsilon_{333} = 7.20\times10^3$. ^{1}H NMR δ 7.16 (s, 1H, H-9), 6.93 (t, 1H, H-13, $J = 10.2$ Hz), 6.70 (d, 1H, H-14, $J = 10.2$ Hz), 6.62(d, 1H, H-12, $J = 10.2$ Hz), 6.33 (s, 2H, H-1 and H-5), 3.92 (s, 3H, 11-OCH_3), 3.83 (s, 9H), 2.81 (m, 4H, H-7 and H-8); ^{13}C NMR δ 179.9, 160.7, 148.9, 146.7, 132.2, 131.7, 127.1, 126.4, 124.1, 107.2, 101.1, 56.5, 51.8, 51.7, 38.3, 33.2. IR 3040–2820, 1720, 1627, 1600 cm^{-1}. LR-MS (*m*/*z*): 331 (15), 330 (M^+, 35), 315 (20), 312 (10), 300 (35), 182 (10), 181 (100), 148 (10). HRMS calcd for $C_{19}H_{22}O_5$: 330.1462. Found: 330.1458.

11-Ethoxy-11-demethoxycombretatropone (10). To a round-bottom flask was added 3 mL of dry THF and excess dry ethanol (0.5 mL). To this stirred solution (3.74 mL, 3.75 mmol) of a 1 M ethylmagnesium bromide solultion was slowly syringed in and the resulting solution was allowed to stir at room temperature for 20 min. Chlorocombretatropone **9** (50 mg, 0.15 mmol) was dissolved in 2 mL of dry THF and syringed into the reaction flask. The reaction was allowed to stir at room temperature for 12 h. The reaction was then diluted with chloroform (15 mL) and water (15 mL). The aqueous layer was neutralized with 0.5 N HCl (1 mL). The layers were separated and the aqueous layer was extracted four times with chloroform. The organic extracts were combined, dried with sodium sulfate, filtered and stripped of solvent. The product was first purified by flash chromatography followed with radial chromatography (80:20 CH_2Cl_2/acetone) to yield 37 mg (73%) of a colorless oil: $\varepsilon_{334} = 6.98\times10^3$. ^{1}H NMR δ 7.18 (s, 1H, H-9), 6.92 (t, 1H, H-13, $J = 10.5$ Hz,), 6.69 (d, 1H, H-14, $J = 10.5$ Hz), 6.63 (d, 1H, H-12, $J = 10.5$ Hz), 6.34 (s, 2H, H-1 and H-5), 4.12 (q, 2H, 11-OCH_2CH_3, $J = 7.1$ Hz), 3.80 (s, 9H), 2.82 (m, 4H, H-7 and H-8), 1.51 (t, 3H, 11-OCH_2CH_3, $J = 7.0$ Hz); ^{13}C NMR δ 175.4, 160.1, 148.8, 146.3, 133.5, 132.2, 131.8, 127.2, 126.1, 107.9, 101.1, 60.4, 56.4, 51.7, 38.3, 33.3, 9.9. IR 3040–2810, 1720, 1627, 1600 cm^{-1}. LR-MS (*m*/*z*). 345 (5), 344 (M^+, 10), 316 (10), 207 (15), 182 (15), 181 (100), 148 (10). HRMS calcd for $C_{20}H_{24}O_5$: 344.1626. Found: 344.1637.

11-Demethoxy-11-propoxycombretatropone (11). To a round-bottom flask was added 3 mL of dry THF and excess dry 1-propanol (0.4 mL). To the stirred solution, 1.9 mL (3.75 mmol) of a 2 M propylmagnesium bromide solution was slowly syringed in and the resulting solution was allowed to stir at room temperature for 20 min. Chlorocombretatropone **9** (50 mg, 0.15 mmol) was dissolved in 2 mL of THF and then syringed into the reaction flask. The reaction was then allowed to stir at room temperature for 24 h. The solution was diluted with chloroform (20 mL) and water (15 mL) and the aqueous layer was neutralized with 0.5 N HCl (1 mL). The layers were separated and the aqueous layer was extracted four additional times with chloroform. The product was purified by flash chromatography followed by radial chromatography (80:20 CH_2Cl_2/acetone) to yield 40 mg (76%) of a white solid: $\varepsilon_{323} = 6.49\times10^3$; mp 103–105 °C. ^{1}H NMR δ 7.20 (s, 1H, H-9), 6.93 (t, 1H, H-13, $J = 10.4$ Hz), 6.69 (d, 1H, H-14, $J = 10.3$ Hz), 6.65 (d, 1H, H-12, $J = 10.4$ Hz), 6.36 (s, 2H, H-1 and H-5), 4.02 (t, 2H, 11-$OCH_2CH_2CH_3$, $J = 6.8$ Hz), 3.81 (s, 9H), 2.83 (m, 4H, H-7 and H-8), 1.98–1.87 (m, 2H, 11-$OCH_2CH_2CH_3$), 1.05 (t, 3H, 11-$OCH_2CH_2CH_3$, $J = 7.1$ Hz); ^{13}C NMR δ 179.7, 164.7, 153.3, 150.8, 136.6, 136.2, 135.3, 131.7, 130.5, 112.5, 105.5, 70.8, 60.9, 56.1, 42.7, 37.7, 22.0, 10.4. IR 3041–2830, 1714, 1627, 1595 cm^{-1}. LR-MS (*m*/*z*): 359 (5), 358 (M^+, 20), 182 (20), 181 (100), 148 (15). HRMS calcd for $C_{21}H_{26}O_5$: 358.1772. Found: 358.1794.

11-Isopropoxy-11-demethoxycombretatropone (12). Into a two-neck round-bottom was added distilled isopropanol (3 mL) and sodium metal (0.030 g, 1.35 mmol). The resulting solution was then refluxed for 45 min at which point the sodium had dissolved. Chlorocombretatropone **9** (0.030 g, 0.090 mmol) was dissolved in 2 mL of isopropanol and syringed into the reaction flask and the reaction was allowed to stir at room temperature for 20 min. The reaction was diluted with methylene chloride (25 mL) and water (15 mL) and the aqueous layer was neutralized with 0.5 N HCl (0.5 mL). The layers were separated and the aqueous layer was extracted three additional times with methylene chloride. The organic layers were combined, dried with sodium sulfate, filtered and stripped of solvent. The product was purified first with flash chromatography followed by radial chromatography (80:20 CH_2Cl_2/acetone) to yield 12 mg (38%) of a white solid: $\varepsilon_{324} = 4.36\times10^3$; mp 99–102 °C. ^{1}H NMR δ 7.18 (s, 1H, H-9), 6.92 (t, 1H, H-13, $J = 10.5$ Hz), 6.69 (d, 1H, H-14, $J = 10.5$ Hz), 6.67 (d, 1H, H-12, $J = 10.4$ Hz), 6.36 (s, 2H, H-1 and H-5), 4.67 (m, 1H, 11-$OCH(CH_3)_2$), 3.81 (s, 9H), 2.83 (m, 4H, H-7 and H-8), 1.42 (d, 6H, 11-$OCH(CH_3)_2$, $J = 6.1$ Hz), ^{13}C NMR δ 180.1, 163.6, 153.1, 150.5, 136.5, 136.0, 132.3, 130.7, 129.1, 114.2, 105.4, 71.4, 60.7, 56.5, 42.5, 37.5, 21.5. IR 3040–2820, 1720, 1627, 1600 cm^{-1}. LR-MS (*m*/*z*): 359 (5), 358 (M^+, 20), 316 (10), 301 (15), 285 (20), 197 (15), 182 (20), 181 (100), 148 (5). HRMS calcd For $C_{21}H_{26}O_5$: 358.1772. Found: 358.1758.

11-Demethoxy-11-thiomethylcombretatropone (13). In a round-bottom flask, sodium methane thiolate (15 mg, 2.0 mmol) was dissolved in water (1 mL) and stirring was initiated. Chlorocombretatropone **9** (26 mg, 0.078 mmol) was dissolved in methanol (3 mL) and then syringed into the reaction flask. The reaction was allowed to stir at room temperature for 4 h. The reaction was then diluted

with methylene chloride (25 mL) and water (15 mL) and the aqueous layer was acidified with 0.5 N HCl (0.5 mL). The layers were separated and the aqueous layer was extracted three additional times with methylene chloride. The organic extracts were combined, dried with sodium sulfate, filtered and evaporated. Purification of the product was first by flash chromatography followed by radial chromatography (60:40 hexanes/acetone) yielding 21 mg (78%) of a light-yellow oil: $\varepsilon_{346} = 8.87 \times 10^3$. ^{1}H NMR δ 7.04 (s, 1H, H-9), 6.97 (d, 1H, H-12, $J = 9.8$ Hz), 6.90 (t, 1H, H-13, $J = 9.8$ Hz), 6.74 (d, 1H, H-14, $J = 9.8$ Hz), 6.35 (s, 2H, H-1 and H-5), 3.81 (s, 9H), 2.84 (s, 4H, H-7 and H-8), 2.37 (s, 3H, 11-SCH_3); ^{13}C NMR δ 178.0, 155.6, 148.9, 146.3, 132.3, 131.6, 129.8, 128.0, 126.8, 121.7, 101.1, 56.5, 51.7, 38.2, 33.3, 10.9. IR 3030–2840, 1670, 1620, 1590 cm^{-1}. LR-MS (*m*/*z*): 348 (5), 347 (10), 346 (M^+, 30), 341 (20), 339 (10), 315 (15), 299 (20), 230 (10), 182 (15) 181 (100), 148 (10), 137 (15). HRMS calcd for $C_{19}H_{22}O_4S$: 346.1237. Found: 346.1236.

11-Demethoxycombretatropone (14). In a round-bottom flask, chlorocombretatropone **9** (17 mg, 0.051 mmol) was dissolved in distilled DMSO (2 mL) and stirring initiated. To this solution was added sodium borohydride (5.1 mg, 0.134 mmol). The reaction was allowed to stir at room temperature for 6 h. The reaction was then diluted with methylene chloride (15 mL) and water (10 mL). The layers were separated and the aqueous layer extracted three additional times with methylene chloride. Combined the organic extracts, dried with sodium sulfate, filtered and evaporated. The product was purified first by flash chromatography followed by radial chromatography (90:10 ethyl acetate/hexanes) yielding 10 mg (67%) of a colorless oil: $\varepsilon_{312} = 6.19 \times 10^3$. ^{1}H NMR δ 7.05 (s, 1H, H-9), 7.03 (d, 1H, H-11, $J = 11.5$ Hz), 7.01 (t, 1H, H-12, $J = 11.5$ and 11.3 Hz), 6.86 (t, 1H, H-13, $J = 11.5$ and 11.3 Hz), 6.82 (d, 1H, H-14, $J = 11.3$ Hz), 6.35 (s, 2H, H-1 and H-5), 3.82 (s, 9H), 2.83 (m, 4H, H-7 and H-8); ^{13}C NMR δ 187.1, 153.1, 150.2, 142.0, 141.0, 137.9, 136.6, 135.6, 135.4, 133.1, 105.4, 60.7, 56.0, 42.4, 37.1. IR 3020–2835, 1650, 1610, 1595 cm^{-1}. LR-MS (*m*/*z*): 301 (5), 300 (M^+, 40), 285 (25), 269 (20), 194 (10), 182 (15), 181 (100), 148 (10), 91 (10). HRMS calcd for $C_{18}H_{20}O_4$: 300.1360. Found: 300.1361.

Combretatropolone (15). Combretatropone (**4**, 60 mg, 0.18 mmol) was dissolved in a 1:1 ratio of methanol/2 N HCl (5 mL of each solvent). Stirring was initiated and the solution was refluxed for 16 h. The reaction was cooled to room temperature and diluted with methylene chloride (25 mL) and water (15 mL). The aqueous layer was neutralized with 5% sodium bicarbonate (5 mL) and the layers were separated. The aqueous layer was extracted four additional times with methylene chloride and the organic extracts were combined, dried with sodium sulfate, filtered and evaporated. Purification of the product was first by flash chromatography followed by radial chromatography (80:20 CH_2Cl_2/acetone) to yield 49 mg (86%) of a yellow solid: $\varepsilon_{330} = 1.02 \times 10^4$; mp 107–111 °C. ^{1}H NMR δ 7.49 (s, 1H, H-9), 7.41 (d, 1H, H-14 $J = 9.3$ Hz), 7.37 (t, 1H, H-13, $J = 9.3$ Hz), 6.94 (d, 1H, H-12, $J = 9.4$ Hz), 6.33 (s, 2H, H-1 and H-5), 3.80 (s, 9H), 2.89 (m, 4H, H-7 and H-8), 1.66 (broad s, 1H, 11-OH); ^{13}C NMR δ 177.0, 155.6, 153.1, 139.3, 136.5, 136.0, 128.6, 126.9, 124.0, 122.0, 105.3, 60.7, 56.0, 43.4, 38.3. IR 3564–3346, 3052–2825, 1714, 1610 cm^{-1}. HRMS calcd for $C_{19}H_{23}O_5$: 316.1310. Found: 316.1320.

11-Fluoro-11-demethoxycombretatropone and 10-fluoro-10-demethoxyisocombretatropone (16 and 17). Combretatropolone (**15**, 30 mg, 0.095 mmol) was dissolved in dry methylene chloride (3 mL) and cooled to 0 °C. Diethylaminosulfur trifluoride (38 μL, 0.285 mmol) was then slowly syringed into the reaction and the solution allowed to stir at room temperature for 7 h. The reaction was then diluted with methylene chloride (40 mL) and washed two times with water (2×10 mL). The organic layer was dried with sodium sulfate, filtered and evaporated. The product was purified first by flash chromatography followed by radial chromatography (50:50 ethyl acetate/petroleum ether) to yield 13 mg of a white solid (**16**) and 12 mg of a second white solid (**17**) (83% overall yield): Compound **16**: $\varepsilon_{328} = 7.24 \times 10^3$; mp 104–107 °C. ^{1}H NMR δ 7.24 and 7.19 [dd, 1H, H-12, $J = 22$ Hz (F-H), $J = 10.1$ Hz (H-H], 7.21 (t, 1H, H-13, $J = 10.1$ Hz), 7.12 (s, 1H, H-9), 6.83 (d, 1H, H-14, $J = 10.1$ Hz), 6.37 (s, 2H, H-1 and H-5), 3.83 (s, 6H, 2-OCH_3 and 4-OCH_3), 3.81 (3H, 3-OCH_3), 2.86 (s, 4H, H-7 and H-8); ^{13}C NMR δ 177.6, 163.7, 153.2, 138.4, 138.2, 136.8, 135.2, 131.0, 122.3, 122.0, 105.4, 60.7, 56.0, 42.2, 37.1. IR 3040–2835, 1720, 1630, 1610 cm^{-1}. LR-MS (*m*/*z*): 319 (5), 318 (M^+, 45), 290 (25), 182 (10), 181 (100), 148 (5), 109 (10). HRMS calcd for $C_{19}H_{22}O_4F$: 318.1266. Found: 318.1271. Compound **17**: $\varepsilon_{320} = 4.34 \times 10^3$; mp 109–112 °C. ^{1}H NMR δ 7.25 [d, 1H, H-9, $J = 26$ Hz (F–H)], 7.16 (t, 1H, H-13, $J = 9.9$ Hz), 7.11 and 7.08 [dd, 1H, H-12, $J = 9.9$ Hz (H–H) and 7.9 Hz (F–H)], 6.89 (d, 1H, H-14, $J = 9.9$ Hz), 6.35 (s, 2H, H-1 and H-5), 3.82 (s, 9H), 2.87 (s, 4H, H-7 and H-8); ^{13}C NMR δ 177.2, 164.2, 153.2, 139.5, 139.3, 135.4, 129.8, 129.6, 118.7, 118.3, 105.4, 60.7, 56.0, 42.8, 37.2. IR 3040–2810, 1725, 1640, 1610 cm^{-1}. LR-MS (*m*/*z*): 319 (15), 318 (M^+, 55), 305 (30), 301 (20), 287 (40), 207 (10), 182 (15), 181 (100), 148 (10), 109 (15). HRMS calcd for $C_{19}H_{22}O_4F$: 318.1266. Found: 318.1250.

11-Demethoxy-11-methylcombretatropone (18). Fluorocombretatropone **16** (30 mg, 0.094 mmol) was dissolved in dry THF (5 mL) and cooled to 0 °C. To the cooled solution was slowly added 3 M methylmagnesium bromide (130 μL, 0.188 mmol). The reaction was allowed to stir at 0 °C for 45 min. The reaction was diluted with methylene chloride (30 mL) and water (10 mL) and the aqueous layer was acidified with 0.5 N HCl (1 mL). The layers were separated and the aqueous layer was extracted three additional times with methylene chloride. The organic extracts were combined, dried with sodium sulfate and evaporated. Purification of the product was first by flash chromatography followed by radial chromatography (90:10 CH_2Cl_2/acetone) yielding 15 mg (51%) of a white solid: $\varepsilon_{326} = 8.65 \times 10^3$; mp 98–101 °C. ^{1}H NMR δ 7.23 (s, 1H, H-9), 7.03 (d, 1H, H-14, $J = 9.3$ Hz), 6.99 (t, 1H, H-13, $J = 9.3$ Hz), 6.71 (d, 1H, H-12, $J = 9.3$ Hz), 6.34 (s, 2H, H-1 and H-5), 3.82 (s, 9H), 2.83 (s, 4H, H-7 and H-8), 2.29 (s, 3H, 11-CH_3); ^{13}C NMR δ 186.5, 153.1, 151.2, 147.7, 139.1, 138.2, 137.7,

135.8, 135.3, 130.9, 105.5, 60.7, 56.0, 42.6, 37.5, 23.1. IR 3041–2830, 1720, 1610 cm^{-1}. LR-MS (*m*/*z*): 315 (5), 314 (M^+, 10), 182 (15), 181 (100), 148 (15). HRMS calcd for $C_{20}H_{25}O_4$: 314.1516. Found: 314.1519.

11-Ethyl-11-demethoxycombretatropone (19). Fluorocombretatropone **16** (28 mg, 0.088 mmol) was dissolved in dry THF (4 mL) and cooled to 0 °C. To the cooled solution was slowly added 1 M ethylmagnesium bromide (0.415 mL, 0.194 mmol) and the reaction was allowed to stir at 0 °C for 10 min. The ice bath was then removed and the reaction allowed to stir at room temperature for an additional 10 min. The reaction was diluted with methylene chloride (25 mL) and water (10 mL) and the aqueous layer was acidified with 0.5 N HCl (0.5 mL). The layers were separated and the aqueous layer was extracted three additional times with methylene chloride. The organic extracts were combined, dried with sodium sulfate and evaporated. The product was purified first by flash chromatography followed by radial chromatography (98:2 CH_2Cl_2/acetone) to yield 17 mg (59%) of a light-yellow solid: $\varepsilon_{332} = 6.68 \times 10^3$; mp 104–107 °C. ^{1}H NMR δ 7.07 (d, 1H, H-14, $J = 9.2$ Hz), 7.05 (s, 1H, H-9), 6.67 (d, 1H, H-12, $J = 9.2$ Hz), 6.41 (t, 1H, H-13, $J = 9.2$ Hz), 6.32 (s, 9H), 3.82 (s, 6H, 2-OCH_3 and 4-OCH_3), 3.80 (s, 3H, 3-OCH_3), 2.82 (s, 4H, H-7 and H-8), 2.69 (m, 2H, 11-C$H_2$$CH_3$, $J = 7.4$ Hz), 1.17 (t, 3H, 11-$CH_2$$CH_3$, $J = 7.4$ Hz); ^{13}C NMR δ 181.3, 149.3, 148.8, 148.4, 140.9, 135.4, 132.2, 131.8, 128.9, 125.9, 101.2, 56.4, 51.7, 37.8, 34.5, 25.3, 9.5. IR 3030–2825, 1725, 1610, 1595 cm^{-1}. LR-MS (*m*/*z*): 329 (5), 328 (M^+, 10), 182 (15), 181 (100), 148 (15). HRMS calcd for $C_{21}H_{27}O_4$: 328.1672. Found: 328.1682.

10-Demethoxy-10-methylisocombretatropone (20). This compound was synthesized as described **18** except that the isocombretatropone isomer **17** (27 mg, 0.085 mmol) was used as the starting compound. The product was purified first by flash chromatography followed by radial chromatography (90:10 CH_2Cl_2/acetone) to yield 13 mg (48%) of a white solid: $\varepsilon_{321} = 3.72 \times 10^3$; mp 107–109 °C. ^{1}H NMR δ 7.22 (d 1H, H-12, $J = 8.8$ Hz), 7.04 (s, 1H, H-9), 6.99 (d, 1H, H-14, $J = 8.7$ Hz), 6.81 (t, 1H, H-13, $J = 8.8$ Hz), 6.35 (s, 2H, H-1 and H-5), 3.82 (s, 9H), 2.81 (m, 4H, H-7 and H-8), 2.27 (s, 3H, 10-CH_3); ^{13}C NMR δ 186.4, 153.1, 149.4, 146.6, 138.9, 136.5, 135.9, 134.2, 132.2, 130.7, 105.3, 60.7, 56.0, 42.2, 37.3, 22.5. IR 3040–2830, 1720, 1610 cm^{-1}. LR-MS (*m*/*z*): 315 (5), 314 (M^+, 10), 207 (10), 182 (15), 181 (100), 148 (10). HRMS calcd for $C_{20}H_{25}O_4$: 314.1516. Found: 314.1529.

10 - Ethyl - 10 - demethoxyisocombretatropone (21). This compound was synthesized as described **19** except that the isocombretatropone isomer **17** (26 mg, 0.082 mmol) was employed as the starting material. The product was purified first by flash chromatography followed by radial chromatography (98:2 CH_2Cl_2/acetone) to yield 15 mg (56%) of a light-yellow solid: $\varepsilon_{319} = 3.80 \times 10^3$; mp 111–114 °C. ^{1}H NMR δ 6.99 (d, 1H, H-12, $J = 9.0$ Hz), 6.73 (d, 1H, H-14, $J = 9.1$ Hz), 6.71 (s, 1H, H-9), 6.40 (t, 1H, H-13, $J = 9.0$ Hz), 6.36 (s, 2H, H-1 and H-5), 3.83 (s, 6H, 2-OCH_3 and 4-OCH_3), 3.81 (s, 3H, 3-OCH_3), 2.82 (m, 4H, H-7 and H-8), 2.70 (m, 2H, 11-C$H_2$$CH_3$ $J = 7.2$ Hz), 1.15 (t, 3H, 11-CH_2CH_3, $J = 7.2$ Hz); ^{13}C NMR δ 184.3, 149.0, 148.9, 146.1, 140.1, 132.3, 132.1, 131.7, 126.8, 125.4, 101.1, 56.5, 51.8, 35.0, 32.8, 24.5, 9.7. IR 3045–2825, 1714, 1610, 1595 cm^{-1}. LR-MS (*m*/*z*): 329 (5), 328 (M^+, 10), 221 (10), 182 (20), 181 (100), 148 (10). HRMS calcd for $C_{21}H_{27}O_4$: 328.1672. Found: 328.1684.

11 - Amino - 11 - demethoxycombretatropone (22). In a pressure tube, combretatropone (**4**, 22 mg, 0.067 mmol) was dissolved in distilled methanol (3 mL) and cooled to 0 °C. Ammonia (3 mL) was then condensed into the pressure tube and the reaction was stirred on an oil bath at 85 °C for 12 h. The solution was then evaporated to yield a brown oil. The product was purified first by flash chromatography followed by radial chromatography (70:30 ethyl acetate/acetone) yielding 17 mg (82%) of a yellow oil: $\varepsilon_{336} = 9.32 \times 10^3$. ^{1}H NMR δ 7.23 (s, 1H, H-9), 7.02 (t, 1H, H-13, $J = 10.1$ Hz), 6.78 (d, 1H, H-14, $J = 10.1$ Hz), 6.61 (d, 1H, H-12, $J = 10.1$ Hz), 6.36 (s, 2H, H-1 and H-5), 3.81 (s, 9H), 2.85 (s, 4H, H-7 and H-8); ^{13}C NMR δ 174.6, 156.5, 153.0, 151.9, 136.4, 136.2, 135.5, 131.3, 127.6, 112.6, 105.4, 60.7, 56.0, 43.0, 38.1. IR 3510, 3357, 3030–2825, 1676, 1610, 1595 cm^{-1}. LR-MS (*m*/*z*): 316 (10), 315 (M^+, 40), 300 (20), 298 (15), 284 (25), 182 (10), 181 (100), 148 (10). HRMS calcd for $C_{19}H_{24}O_4N$: 315.1469. Found: 315.1465.

11-Demethoxy-11-methylaminocombretatropone (23). In a pressure tube, combretatropone (**4**, 23 mg, 0.070 mmol) was dissolved in distilled methanol (5 mL). To this solution was added 2 M methylamine (2.8 mL, 0.140 mmol) and the reaction was then stirred at 35 °C on an oil bath for 24 h. The solution was then evaporated to yield a black solid. Purification of the product was carried out first by flash chromatography followed by radial chromatography (80:20 CH_2Cl_2/acetone) yielding 19 mg (84%) of a yellow solid: $\varepsilon_{338} = 1.04 \times 10^4$; mp 111–113 °C. ^{1}H NMR δ 7.23 (broad s, 1H, NH), 7.16 (s, 1H, H-9), 7.12 (t, 1H, H-13, $J = 10.0$ Hz), 6.57 (d, 1H, H-14, $J = 9.9$ Hz), 6.39 (d, 1H, H-12, $J = 10.0$ Hz), 6.36 (s, 2H, H-1 and H-5), 3.81 (s, 9H), 3.04 (d, 3H, NHCH_3, $J = 5.1$ Hz), 2.84 (m, 4H, H-7 and H-8); ^{13}C NMR δ 172.4, 152.6, 148.8, 147.6, 132.3, 132.1, 130.8, 125.0, 120.0, 103.0, 101.1, 56.4, 51.7, 38.9, 34.0, 25.1. IR 3335, 3020–2825, 1670, 1610 cm^{-1}. LR-MS (*m*/*z*): 330 (10), 329 (M^+, 60), 312 (10), 298 (15), 182 (15), 181 (100), 148 (10). HRMS calcd for $C_{20}H_{26}O_4N$: 329.1625. Found: 329.1637.

11 - Demethoxy - 11 - dimethylaminocombretatropone (24). Recrystallized pTSA (5 mg) was added to a pressure tube. To the tube was added combretatropone (**4**, 25 mg, 0.076 mmol) dissolved in dry THF (2 mL). To the solution was added 2 M dimethylamine (0.5 mL, 0.152 mmol) and the reaction was stirred at 80 °C for 72 h. The reaction was cooled to room temperature and diluted with methylene chloride. The organic layer was washed first with 1% sodium bicarbonate (15 mL) and then water (15 mL). Dried the organic layer with sodium sulfate, filtered and evaporated. The product was purified using flash chromatography followed by radial

chromatography (80:20 CH_2Cl_2/acetone) yielding 17 mg (65%) of a yellow oil: $\varepsilon_{354} = 9.08 \times 10^3$. ^1H NMR δ 7.04 (s, 1H, H-9), 6.96 (t, 1H, H-13, $J = 9.8$ Hz), 6.52 (d, 1H, H-14, $J = 9.8$ Hz), 6.41 (d, 1H, H-12, $J = 9.8$ Hz), 6.37 (s, 2H, H-1 and H-5), 3.82 (s, 6H, 2,4-OCH_3), 3.81 (s, 3H, 3-OCH_3), 3.10 (s, 6H, N(CH_3)$_2$) 2.85 (m, 4H, H-7 and H-8); ^{13}C NMR δ 180.4, 158.9, 153.0, 149.4, 136.5, 136.4, 132.6, 132.3, 125.4, 113.2, 105.4, 60.7, 55.9, 42.1, 41.7, 37.7. IR 3040–2812, 1710, 1610 cm^{-1}. LR-MS (*m*/*z*): 344 (10), 343 (M^+, 65), 207 (10), 182 (10), 181 (100), 162 (60), 148 (10), 134 (20). HRMS calcd for $C_{21}H_{28}O_4N$: 343.1781. Found: 343.1799.

Routine preparation of MTP and determination of protein concentration. Microtubule protein (MTP), consisting of tubulin and microtubule associated proteins, was purified from bovine brain by two cycles of assembly/disassembly.[24] The purified MTP was drop frozen and stored in liquid nitrogen until use. The frozen MTP was prepared daily for polymerization assays by desalting the thawed protein into PME buffer.

Microtubule assembly assays. MTP at a concentration of 2 mg/mL was incubated with the appropriate ligand at the desired concentration in PME buffer for 20 min at room temperature. GTP was then added to achieve the final concentrations of 0.1 mM. Assembly was initiated by warming the solution to 37 °C and the polymerization process was monitored by observing the change in turbidity of the solution (apparent absorption at 400 nm on a Beckman Model 25/UV–visible spectrophotometer containing a thermostated cell holder). The I_{50} (ligand concentration required to produce 50% inhibition of microtubule assemble relative to a control without ligand) was determined by interpolation from a plot of percent inhibition versus ligand concentration. The percent inhibition was determined using the plateau value after polymerization of the MTP was at steady state. The I_{50} value for each compound was measured a minimum of four times. The activities reported are the mean and standard deviation of these data.

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