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COMMUNICATION

The furan route to tropolones: probing the antiproliferative effects of β-thujaplicin analogs†

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A direct route to analogs of the naturally occurring tropolone β-thujaplicin has been developed in just four steps from furan. Using this method, a series of derivatives were synthesized and evaluated. Several of these compounds demonstrated very high levels of potency against bacterial and fungal pathogens with good selectivity over mammalian cells.

Introduction

Natural products containing a tropolone nucleus have been known since the early 1950s when a novel non-benzenoid aromatic structure was independently proposed by Dewar and Nozoe for the plant derived metabolite β-thujaplicin (hinokitiol).[1] This nucleus also appears in more complex terpenes such as colchicine, which is used clinically for the treatment of gout (Fig. 1). Recently, there has been renewed interest in the monoterpenoid tropolones because of the wide range of biological activities associated with the parent natural products.[2] β-Thujaplicin and its positional isomers have been shown to possess significant antibacterial,[3] antifungal,[4] and anticancer[5] activities and may serve as exciting leads for new therapeutics. Despite widespread interest in the synthesis of these natural products,[6] there have been no attempts to probe key structure–activity relationships through the synthesis and evaluation of β-thujaplicin analogs.

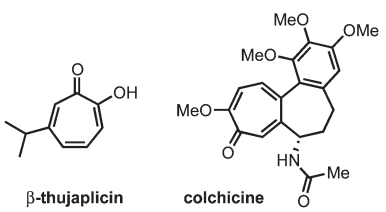

Fig. 1 Tropolone natural products.

The mechanism(s) by which these small natural products are able to elicit such a wide range of effects in both prokaryotic and eukaryotic systems is unclear but may relate to the ability of the tropolone unit to chelate metal ions. Accordingly, the antiproliferative activity is typically abrogated by etherification or acylation of the tropolone hydroxyl functionality.[2] Recent investigations have uncovered several specific pathways affected by thujaplicin including p27/Skp2 in a melanoma line,[7] androgen receptor signaling in prostrate cells[8] and Cyr1/Ras1 in *Candida ablicans*.[9] In addition, potential metalloenzyme targets such as carboxypeptidase A[10] have been identified, again highlighting the chelating potential of the tropolone in targeting a bound metal ion such as zinc. From the perspective of a drug development effort, thujaplicin is much more reminiscent of a lead structure, characterized by low molecular weight, minimal functionalization and multiple target effects.[11]

Results and discussion

Chemistry

We have been interested in developing an efficient and flexible route to substituted tropolones that would allow us to probe key structure–activity relationships between these lead-like compounds and the variety of biological functions associated with them. In doing so, we aim to achieve a route by which these leads can be developed into efficient, potent and selective agents. Herein, we describe a new route to substituted tropolones and its application to the synthesis of β-thujaplicin analogs.

Over the past several years, we have investigated the formal [4 + 3] cycloadduct produced from the reaction of furan and tetrabromocyclopropene (TBCP) (Scheme 1).[12] Hydrolysis of the primary adduct gives in high yield the dibromoenone **1**, an intermediate that can be used for a variety of applications.

This highly functionalized intermediate is both complimentary to and functionally distinct from the oxyallyl cation adduct of furan that had previously been used by Cha in an elegant synthesis of β-thujaplicin.[6a] Because of the renewed interest in substituted tropolones, we were intrigued by the possibility of using

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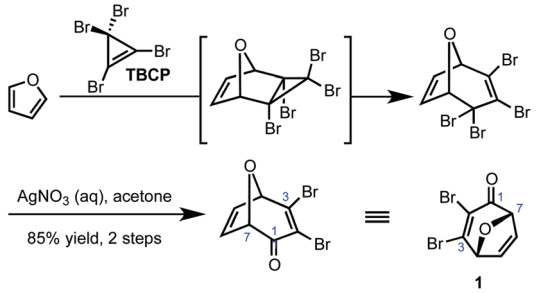
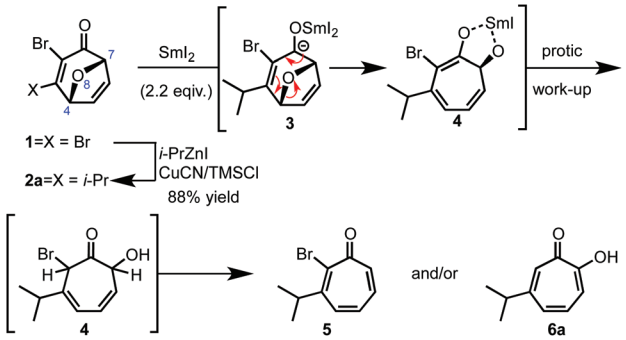


Scheme 1 Synthesis of an oxabicyclic building block.



Scheme 2 Direct synthesis of tropolones.

1 as a versatile precursor to β-thujaplicin analogs. Key to utilizing bridged adducts derived from **1** would be the development of a highly regioselective, reductive ether cleavage that would preserve the vicinal oxygen functionality at C1 and C7. This route would be strategically distinct from the elimination-based cleavages used with oxyallyl cation adducts which necessitates the prior incorporation of additional oxygen functionality. We initially set out to examine the inherent regioselectivity in the reductive cleavage of the ether bridge from a C1-ketyl and evaluate the potential of such a reaction *en route* to the tropolone nucleus.

A potential precursor to β-thujaplicin was prepared by conjugate addition/elimination of an isopropyl zinc cuprate to the dibromoenone **1**. Exposure of the enone to samarium diiodide resulted in a rapid reaction and complete consumption of starting material. Interestingly, neutral or mildly acidic work-up of the reaction directly yielded a mixture of two aromatic products, the bromotropone **5** and β-thujaplicin **6a** (Scheme 2).

It is assumed that both products initially arise from a highly regioselective opening of the C4–O8 bond to generate enolate **4**. The exclusive opening of this bond is likely driven by formation of this extended enolate in contrast to the cross-conjugated enolate that would arise from opening of the C7–O8 bond. Kinetic protonation of the enolate should initially lead to the diene **4** that is poised for easy aromatization either through dehydration to give **5** or dehydrohalogenation to give the tropolone **6a**. It was presumed that dehydration to produce the tropone would be more facile in acidic media while under more basic conditions the desired dehydrobromination to the tropolone would be preferred. Gratifyingly, switching to a strongly basic work-up with aqueous hydroxide led to direct formation of the tropolone and complete suppression of the bromotropone product. Utilizing this optimized method, β-thujaplicin was prepared in just four steps from furan in 56% overall yield.

With an effective synthesis of β-thujaplicin in hand, we turned our attention to exploiting this synthesis to access a small array of thujaplicin analogs. In addition to probing the scope of the tropolone synthesis, we would also be in position to develop some preliminary structure–activity relationships for these small but highly active natural products (Table 1).

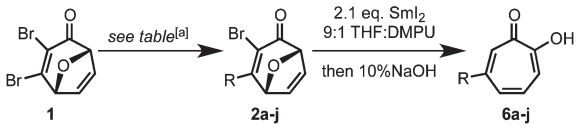
The introduction of the β-substituent after cycloaddition renders the generation of analogs particularly straightforward. In prior work with dibromoenone **1**, we had shown that the β-bromide could be regioselectively substituted either though nucleophilic addition/elimination or palladium catalyzed cross-coupling reactions. Accordingly, **1** was converted into nine different enones **2b–j** containing various alkyl and aryl groups at C3. Exposure of these compounds to samarium diiodide followed by a basic work-up as previously described gave the tropolones **6b–j** in very good yields. This route to β-thujaplicin analogs only requires two parallel operations from a common intermediate.

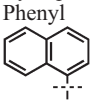
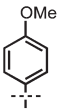
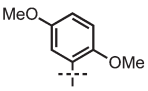
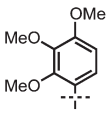
Biological activities

With a small array of β-thujaplicin analogs in-hand we were in a position to evaluate the role of β-substitution on the activity of the compounds (Table 2). A panel of cell-based assays was selected to evaluate the efficacy of the compounds in two important bacterial pathogens (methicillin-resistant *Staphylococcus aureus* (MRSA)[13] and *Enterococcus faecalis*[14]), a fungal pathogen (*Candida glabrata*[15]) that is resistant to most common antifungal agents and a human breast cancer line (MCF-7). The results of the assays are shown in Table 2 and demonstrate that key structure–activity relationships exist across this class of natural product analogs. Firstly, the assays confirm that the β-substituted tropolones function as very effective antimicrobial agents and show very high levels of activity against both bacterial and fungal pathogens. Several of the derivatives show MIC values <0.1 μg mL^{-1} against MRSA and *C. glabrata*, notoriously difficult organisms to target. For example, compounds **6a–6f** are ten-fold more potent against MRSA than vancomycin and compounds **6a–6d** are 3–5 times more potent than vancomycin against sensitive lines of *E. faecalis*. While several of the compounds show moderate activity against the cancer cell line, there is still very strong pathogenic selectivity with at least a 1000-fold difference relative to the antibacterial (MRSA) potency. Secondly, the lack of inhibition of a non-proliferating human dermal fibroblast cell line emphasizes that specific targets are involved rather than simple sequestration of metal ions. In general, structure–activity relationships show that increasing steric bulk at the β-position correlates strongly with activity and is sensitive to substitution (compare **6h** and **6i**). Finally, it is interesting that the SAR follows closely regardless of the organism, possibly indicating conserved or structurally similar targets in both prokaryotic and eukaryotic cells.

Through the use of the readily available dibromoenone **1**, we have been able to develop a highly flexible route to β-thujaplicin

Table 1 Synthesis of tropolones



Enone **2**	R	Method[a]	Yield[b] [%]	Tropolone **6**	Yield[b] [%]
2a	Isopropyl	A	88	**6a**	75
2b	Cyclopropyl	B	70	**6b**	61
2c	*sec*-Butyl	C	77	**6c**	71
2d	*tert*-Butyl	C	82	**6d**	77
2e	Cyclopentyl	A	92	**6e**	68
2f	Phenyl	D	80	**6f**	72
2g		D	78	**6g**	54
2h		D	73	**6h**	62
2i		D	69	**6i**	65
2j		D	72	**6j**	67

[a] A: RZnI, CuCN, TMSCl, $BF_3{\cdot}Et_2O$. B: RMgBr, CuI (1.1 equiv.). C: RLi, CuCN (1.1 equiv.), TMSCl. D: $RB(OH)_2$, $Pd(PPh_3)_2Cl_2$, Cs_2CO_3. [b] Yield of isolated products. THF = tetrahydrofuran. DMPU = *N*,*N*′-dimethylpropylene urea. TMS = trimethylsilyl.

analogs based on a one-pot reductive ether cleavage/aromatization strategy. The new route was adapted for the formation of several new tropolone derivatives that show compelling structure–activity relationships. High levels of potency and selectivity suggest that these compounds could serve as excellent leads in developing novel antimicrobial agents.

Experimental

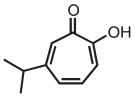
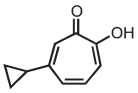
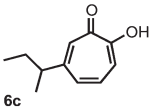
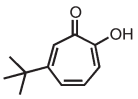
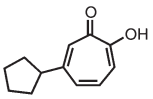
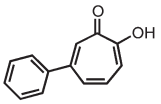
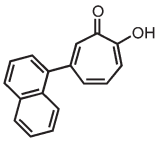
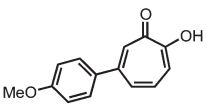
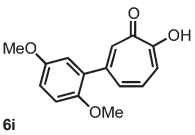
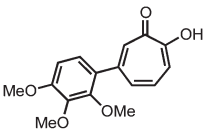
Synthesis

General. All reactions were carried out under an inert argon atmosphere with dry solvents under anhydrous conditions unless otherwise noted. Commercial grade reagents and solvents were used without further purification except as indicated below. Hexanes, tetrahydrofuran (THF), diethyl ether (Et_2O), and dichloromethane (CH_2Cl_2) were used directly from a Baker cycletainer system. Reagents were purchased at the highest commercial quality and used without further purification, unless otherwise noted. Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogenous materials, unless otherwise stated. Dibromoenone **1** and α-bromoenones **2f**, **2g**, and **2h** were prepared according to literature procedures. Reactions were monitored by thin layer chromatography (TLC) carried out on Whatman Partisil K6F silica gel 60 Å precoated plates using UV light as the visualizing agent and an acidic mixture of anisaldehyde or basic aqueous potassium permanganate ($KMnO_4$) and heat as developing agents. Flash chromatography was performed using Baker silica gel (60 Å particle size). NMR spectra were recorded on Bruker-500 and calibrated using residual undeuterated solvent as an internal reference ($CHCl_3$ at δ 7.26 ppm ^{1}H NMR, d 77.0 ppm ^{13}C NMR). The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, hept = heptet, b = broad. High-resolution mass spectra (HRMS) were provided by the Notre Dame Mass Spectrometry Laboratory by electrospray ionization of flight reflectron experiments. IR Spectra were recorded on Shimadzu FTIR 8400 spectrometer. Melting points (m.p.) are uncorrected and were recorded on a Mel-Temp 3.0 digital melting point apparatus.

General procedure for zinc cuprate additions

To a suspension of activated zinc dust (4.2 g, 64.3 mmol) in 50 mL THF was added chlorotrimethylsilane (0.61 mL, 4.6 mmol) at room temperature. After stirring for 10 min, the iodoalkane (30.8 mmol) was added dropwise at room temperature and stirred for 1 h. A separate round bottomflask, charged with freshly purified CuCN (2.89 g, 30.8 mmol) and lithium chloride (2.79 g, 64.3 mmol), was flame dried and allowed to cool to room temperature before the addition of THF (45 mL). The resulting light green solution was stirred for 30 min at room temperature, cooled to 0 °C, and the organozinc reagent was added dropwise *via* cannula. After 30 min, the

Table 2 Biological evaluation of thujaplicin analogs

Tropolone **6**	Fibroblasts	MCF-7[a]	*E. faecalis*	MRSA	*C. glabrata*
 **6a**	NA[b]	42.3	1.3	0.04	0.7
 **6b**	ND[c]	58.0	1.3	0.03	2.6
 **6c**	NA[b]	46.9	1.4	0.09	0.7
 **6d**	NA[b]	33.5	0.7	0.09	0.4
 **6e**	NA[b]	47.7	24	0.09	0.8
 **6f**	NA[b]	53.8	25	0.1	0.8
 **6g**	ND[c]	NA[b]	248	31	65
 **6h**	NA[b]	39.1	28	14	3.6
 **6i**	NA[b]	NA[b]	258	64	NA[b]
 **6j**	ND[c]	98.0	36	18	4.6
Vancomycin	ND[c]	ND[c]	4	1	ND[c]

[a] Inhibition of human strains reported with IC_{50} values in μM, activity against pathogenic species is shown as MIC values (μg mL^{-1}). [b] NA = Not active at 100 μM. [c] Not determined.

solution was cooled to −78 °C and $BF_3{\cdot}Et_2O$ (8.07 mL, 10.3 mmol) was added followed by the dropwise addition of dibromoenone 1 (3.00 g, 10.3 mmol) in 5 mL THF. The reaction was stirred at this temperature for 45 min before the solution was poured over sat. aq. NH_4Cl (150 mL) and 25% aq. NH_3 (30 mL). The resulting mixture was stirred for 1 h at room temperature before it was extracted with EtOAc (3 × 50 mL). The combined organics were washed with sat. aq. NH_4Cl (25 mL), H_2O (25 mL), and brine (25 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Flash chromatography of the crude

residue (SiO_2, EtOAc in hexanes) provided the desired α-bromoenones.

(1*S*,5*R*)-3-Bromo-4-isopropyl-8-oxa-bicyclo[3.2.1]octa-3,6-dien-2-one (2a). Dibromoenone **1** (3.0 g, 10.3 mmol) was subject to the zinc-cuprate conditions with 2-iodopropane to afford **2a** as a pale yellow solid (2.3 g, 88%): R_f = 0.44 (15% hexanes in EtOAc); mp = 74–76 °C; IR (NaCl): *v*: 2968, 2872, 1698, 1588, 1465, 1325, 1242, 1179, 1071, 1020, 926 cm^{-1}; 1H NMR (500 MHz, $CDCl_3$) δ 6.76 (ddd, *J* = 5.8, 2.0, 0.7 Hz, 1H), 6.49 (dd, *J* = 5.7, 2.2 Hz, 1H), 5.30 (d, *J* = 2.0 Hz, 1H), 5.06 (dd, *J* = 2.3, 0.6 Hz, 1H), 3.15 (hept, *J* = 6.9 Hz, 1H), 1.22 (d, *J* = 6.9 Hz, 3H), 1.07 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 186.12, 168.47, 140.48, 130.39, 113.51, 86.80, 79.79, 33.12, 19.76, 18.35; HRMS (ESI) calcd for $C_{10}H_{12}BrO_2$ $[M + H]^+$: 243.0021; found: 243.0016.

(1*S*,5*R*)-3-Bromo-4-cyclopentyl-8-oxa-bicyclo[3.2.1]octa-3,6-dien-2-one (2e). Dibromoenone **1** (500 mg, 1.79 mmol) was subject to the zinc–cuprate conditions with iodocyclopentane to afford **2e** as a white solid (474 mg, 92%): R_f = 0.47 (15% hexanes in EtOAc); mp = 70–73 °C; IR (KBr): *v*: 2973, 2874, 1697, 1577, 1066, 927 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.78–6.73 (m, 1H), 6.48 (dd, *J* = 5.7, 2.2 Hz, 1H), 5.20 (d, *J* = 1.8 Hz, 1H), 5.05 (d, *J* = 2.1 Hz, 1H), 3.20–3.10 (m, 1H), 2.09–1.99 (m, 1H), 1.83–1.64 (m, 5H), 1.55 (ddd, *J* = 18.7, 12.5, 8.7 Hz, 1H), 1.35 (ddd, *J* = 12.3, 10.5, 5.4 Hz, 1H). ^{13}C NMR (126 MHz, CDCl3) δ 185.82, 167.22, 140.26, 130.46, 114.39, 86.76, 80.49, 44.31, 30.88, 29.23, 25.95, 25.35; HRMS (ESI) calcd for $C_{12}H_{13}BrO_2$ $[M + H]^+$: 269.0177; found: 269.0172.

Representative procedure for higher-order cuprate additions. Freshly purified and flame-dried CuCN (88 mg, 0.982 mmol) was suspended in dry THF (4.0 mL) and treated with the appropriate organolithium or organomagnesium reagent (1.96 mmol) at −78 °C. The resulting solution was stirred at −78 °C for 1 h before the addition of chlorotrimethylsilane (0.45 mL, 3.6 mmol) and dropwise addition of dibromoenone **1** (250 mg, 0.893 mmol) in THF (2 mL). The reaction mixture was stirred for 45 min before being quenched with sat. aq. NH_4Cl (5 mL). The mixture was extracted with EtOAc (3 × 20 mL) and the combined organics were washed with brine (20 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Flash chromatography of the crude residue (SiO_2, EtOAc in hexanes) provided the desired α-bromoenones.

(1*S*,5*R*)-3-Bromo-4-cyclopropyl-8-oxa-bicyclo[3.2.1]octa-3,6-dien-2-one (2b). Dibromoenone **1** (250 mg, 0.893 mmol) was subject to the higher-order cuprate conditions with cyclopropylmagnesium bromide to afford **2b** as a white solid (151 mg, 70%): R_f = 0.24 (10% hexanes in EtOAc); mp = 115–117 °C IR (KBr): *v*: 1689, 1570, 1257, 1008, 930, 845 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.69 (dd, *J* = 5.7, 1.9 Hz, 1H), 6.53 (dd, *J* = 5.7, 2.2 Hz, 1H), 5.06 (d, *J* = 2.2 Hz, 1H), 4.62 (d, *J* = 1.9 Hz, 1H), 2.13 (tt, *J* = 8.4, 5.1 Hz, 1H), 1.24 (tdd, *J* = 8.7, 6.7, 5.0 Hz, 1H), 1.10 (tdd, *J* = 8.5, 6.8, 5.2 Hz, 1H), 1.00 (ddt, *J* = 9.8, 6.7, 5.0 Hz, 1H), 0.89–0.81 (m, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 185.14, 165.60, 139.53, 131.25, 114.19, 86.91, 78.82, 15.74, 8.28, 6.81; HRMS (ESI) calcd for $C_{10}H_{10}BrO_2$ $[M + H]^+$: 240.9864; found: 240.9859.

(1*S*,5*R*)-4-*sec*-Butyl-3-bromo-8-oxa-bicyclo[3.2.1]octa-3,6-dien-2-one (2c). Dibromoenone **1** (500 mg, 1.79 mmol) was subject to the higher-order cuprate conditions with *sec*-butyllithium to afford **2c** as a colorless solid (354 mg, 77%, 1 : 1 mixture of diastereomers): R_f = 0.49 (15% hexanes in EtOAc); mp = 68–71 °C; IR (KBr): *v*: 2964, 2931, 1697, 1587, 1577, 1460, 1070, 927 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.76 (d, *J* = 5.7 Hz, 1H), 6.56–6.45 (m, 1H), 5.25 (dd, *J* = 10.0, 1.9 Hz, 1H), 5.08 (s, 1H), 3.06–2.93 (m, 1H), 1.61 (dd, J = 7.4, 1.5 Hz, 1H), 1.42 (ddt, *J* = 21.5, 13.7, 7.3 Hz, 1H), 1.22 (d, *J* = 6.9 Hz, 1.5H), 1.04 (d, *J* = 6.9 Hz, 1.5H), 0.99 (t, *J* = 7.5 Hz, 1.5H), 0.87 (t, *J* = 7.4 Hz, 1.5H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 186.07, 185.93, 168.03, 167.74, 140.67, 140.50, 130.32, 130.30, 114.94, 114.77, 86.88, 86.84, 80.04, 79.76, 40.69, 40.00, 28.05, 26.52, 17.62, 16.58, 12.05, 11.78; HRMS (ESI) calcd for $C_{11}H_{14}BrO_2$ $[M + H]^+$: 257.0177; found: 257.0172.

(1*S*,5*R*)-4-*tert*-Butyl-3-bromo-8-oxa-bicyclo[3.2.1]octa-3,6-dien-2-one (2d). Dibromoenone **1** (500 mg, 1.79 mmol) was subject to the higher-order cuprate conditions with *tert*-butyllithium to afford **2d** as a pale yellow solid (377 mg, 82%): R_f = 0.51 (15% hexanes in EtOAc); mp = 68–71 °C; IR (KBr): *v*: 2966, 2879, 1701, 1552, 1211, 1083, 935 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.84–6.79 (m, 1H), 6.48 (dd, *J* = 5.7, 2.2 Hz, 1H), 5.49 (d, *J* = 1.9 Hz, 1H), 5.05 (d, *J* = 2.2 Hz, 1H), 1.38 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 185.93, 169.29, 140.47, 130.08, 114.47, 86.15, 82.54, 77.29, 77.04, 76.79, 36.69, 28.32; HRMS (ESI) calcd for $C_{11}H_{14}BrO_2$ $[M + H]^+$: 257.0177; found: 257.0172.

General procedure for Suzuki coupling. Dibromoenone **1** (500 mg, 1.79 mmol), aryl boronic acid (2.86 mmol), and cesium carbonate (700 mg, 2.15 mmol) were added to 10 : 1 THF–H_2O (10 mL) and the mixture was thoroughly degassed by bubbling argon through the solution (10 min). Bis(triphenylphosphine) palladium(II) dichloride (146 mg, 10 mol%) was added and the mixture was again degassed with argon. The homogenous solution was heated at 70 °C for 5 h before being cooled to room temperature. Water was added and the mixture was extracted with EtOAc (3 × 15 mL). The combined organic layers were washed with brine (25 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Flash chromatography of the crude residue (SiO_2, EtOAc in hexanes) provided the desired α-bromoenones.

(1*S*,5*R*)-3-Bromo-4-(2,5-dimethoxyphenyl)-8-oxa-bicyclo[3.2.1]-octa-3,6-dien-2-one (2i). Dibromoenone 1 (500 mg, 1.79 mmol) was subject to the coupling conditions with 2,5-dimethoxyphenylboronic acid to afford **2i** as a white solid (416 mg, 69%): R_f = 0.31 (15% hexanes in EtOAc); mp = 129–132 °C; IR (KBr): *v*: 2974, 1699, 1494, 1463, 1286, 1228, 1211, 1047 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.98–6.93 (m, 2H), 6.90 (d, *J* = 9.0 Hz, 1H), 6.86 (d, *J* = 2.9 Hz, 1H), 6.55 (dd, *J* = 5.7, 2.2 Hz, 1H), 5.41 (d, *J* = 1.8 Hz, 1H), 5.17 (dd, *J* = 5.4, 4.8 Hz, 1H), 3.84 (s, 3H), 3.78 (d, *J* = 3.4 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 186.18, 161.14, 153.38, 150.22, 141.27, 129.80, 124.65, 116.57, 115.86, 115.11, 112.46, 86.80, 84.34, 56.12, 55.90; HRMS (ESI) calcd for $C_{15}H_{13}BrNaO_4$ $[M + Na]^+$: 358.9895; found: 358.9889.

(1*S*,5*R*)-3-Bromo-4-(2,3,4-trimethoxyphenyl)-8-oxa-bicyclo[3.2.1]-octa-3,6-dien-2-one (2j). Dibromoenone **1** (500 mg, 1.79 mmol) was subject to the coupling conditions with 2,3,4-trimethoxyphenylboronic acid to afford **2j** as a pale yellow solid (473 mg, 72%): R_f = 0.28 (15% hexanes in EtOAc); mp = 105–108; IR (KBr): *v*: 2840, 1699, 1595, 1490, 1463, 1411, 1299, 1209, 1076, 1012 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.98 (d, J = 8.7 Hz, 1H), 6.95 (dd, J = 5.6, 1.6 Hz, 1H), 6.71 (d, J = 8.7 Hz, 1H), 6.53 (dd, J = 5.7, 2.2 Hz, 1H), 5.37 (d, J = 1.8 Hz, 1H), 5.14 (dd, J = 2.2, 0.5 Hz, 1H), 3.89–3.87 (m, 9H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 186.17, 161.17, 155.22, 150.99, 142.11, 141.10, 129.88, 124.39, 121.85, 115.45, 107.16, 86.64, 84.52, 61.49, 60.99, 55.97; HRMS (ESI) calcd for $C_{16}H_{15}BrNaO_5$ $[M + Na]^+$: 389.0001; found: 388.9995.

General procedure for SmI_2-mediated ring opening (purification A)

The α-bromoenone (250 mg, 1.03 mmol) was dissolved in THF (20.0 mL, 0.05 M) and freshly distilled DMPU (2.00 mL) was added. Argon was bubbled through the solution for 20 min, after which time SmI_2 (30.9 mL, 3.09 mmol, 3.0 equiv., 0.1 M in THF) was rapidly added at room temperature. After 30 s, the reaction was quenched with 10% NaOH (20 mL) and stirred for 6 h at room temperature. THF was removed by rotary evaporation and the resulting aqueous layer was cooled to 0 °C, acidified to pH = 2 with 2 N HCl and extracted with Et_2O (3 × 25 mL). The combined organics were washed with sat. aq. Rochelle's salt (3 × 20 mL) and H_2O (3 × 20 mL). [Note: It is necessary to wash excessively with Rochelle's salt to break up samarium chelates and wash with water to remove all traces of DMPU.] The alkyl tropolones were extracted from the organics by washing the ether layer with 10% NaOH (3 × 20 mL) then acidifying the alkaline extract to pH = 2 with 2 N HCl. The acidic aqueous layer was washed once with ether followed by extraction with CH_2Cl_2 (3 × 25 mL). The combined organics were washed with brine, dried over Na_2SO_4, filtered and concentrated *in vacuo* to give essentially pure tropolones.

General procedure for SmI_2-mediated ring opening (purification B)

The general procedure was followed as above without extracting the tropolones from the ether layer (attempts at the acid/base purification led to significantly lower yields due to partitioning of the tropolones between the aqueous and organic layers). After extraction with ether (3 × 25 mL), the combined organics were washed with sat. aq. Rochelle's salt (3 × 20 mL), H_2O (3 × 20 mL), brine (25 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Flash chromatography (SiO_2) of the crude material using 10% EtOAc in hexanes as the eluent provided the substituted tropolones. [Note: R_f values for the tropolones are not reported because the compounds streak on TLC plates and accurate values could not be obtained.]

β-Thujaplicin (6a). The α-bromoenone **2a** (200 mg, 0.823 mmol) was subject to the general procedure and purification A to afford β-thujaplicin as a pale yellow oil (101 mg, 75%): (a sample of **6a** was sublimed (80 °C, ~0.1 mmHg) to give pale yellow crystals). A mixed melting point of the sublimate and authentic β-thujaplicin melted at 48–51 °C: IR (KBr): *v*: 3209, 2962, 2929, 1610, 1541, 1473, 1270, 1220, 1105, 948 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.34–7.29 (m, 2H), 7.22 (d, J = 10.7 Hz, 1H), 6.95 (d, J = 10.1 Hz, 1H), 2.89 (hept, J = 6.9 Hz, 1H), 1.28 (s, 3H), 1.27 (s, 3H) ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.27, 170.90, 159.97, 137.22, 127.62, 123.23, 122.24, 38.91, 23.36.; HRMS (ESI) calcd for $C_{10}H_{13}O_2$ $[M + H]^+$: 165.0916; found: 165.0906.

(2*E*,4*Z*,6*Z*)-6-Cyclopropyl-2-hydroxycyclohepta-2,4,6-trienone (6b). The α-bromoenone **2b** (215 mg, 0.891 mmol) was subject to the general procedure and purification A to afford **6b** as a yellow oil (88 mg, 61%): IR (KBr): *v*: 3197, 2947, 2929, 1605, 1530, 1482, 1269, 967 cm^{-1}; ^1H NMR (500 MHz, CDCl3) δ 7.26 (d, J = 10.3 Hz, 1H), 7.20 (d, J = 10.7 Hz, 1H), 7.13–7.08 (m, 1H), 6.83 (d, J = 10.2 Hz, 1H), 1.96 (ddd, J = 13.4, 8.4, 5.1 Hz, 1H), 1.17–1.12 (m, 2H), 0.90–0.86 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.75, 170.71, 156.33, 136.69, 126.90, 121.83, 121.54, 20.10, 10.96.; HRMS (ESI) calcd for $C_{10}H_{11}O_2$ $[M + H]^+$: 163.0759; found: 163.0754.

(2*E*,4*Z*,6*Z*)-6-*sec*-Butyl-2-hydroxycyclohepta-2,4,6-trienone (6c). The α-bromoenone **2c** (202 mg, 0.786 mmol) was subject to the general procedure and purification A to afford **6c** as a yellow oil (99 mg, 71%): IR (KBr): *v*: 3196, 2962, 2930, 1610, 1548, 1471, 1265, 943 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.35–7.25 (m, 2H), 7.21 (d, J = 10.7 Hz, 1H), 6.90 (d, J = 9.9 Hz, 1H), 2.58 (dt, J = 14.0, 6.9 Hz, 1H), 1.60 (dd, J = 14.7, 7.3 Hz, 2H), 1.23 (d, J = 6.9 Hz, 3H), 0.82 (t, J = 7.4 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.04, 170.93, 158.92, 137.15, 128.21, 123.65, 122.38, 46.35, 30.45, 21.35, 12.04; HRMS (ESI) calcd for $C_{11}H_{15}O_2$ $[M + H]^+$: 179.1072; found: 179.1069.

(2*E*,4*Z*,6*E*)-6-*tert*-Butyl-2-hydroxycyclohepta-2,4,6-trienone (6d). The α-bromoenone **2d** (200 mg, 0.778 mmol) was subject to the general procedure and purification A to afford **6d** as a yellow oil (107 mg, 77%): IR (KBr): *v*: 3210, 2995, 1608, 1498, 1475, 1265 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.56 (d, J = 1.2 Hz, 1H), 7.32 (d, J = 10.6 Hz, 1H), 7.22 (d, J = 10.8 Hz, 1H), 7.16 (d, J = 10.1 Hz, 1H), 1.34 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.45, 170.34, 161.85, 136.97, 126.35, 123.28, 122.18, 38.22, 30.94; HRMS (ESI) calcd for $C_{11}H_{15}O_2$ $[M + H]^+$: 179.1072; found: 179.1062.

(2*E*,4*Z*,6*Z*)-6-Cyclopentyl-2-hydroxycyclohepta-2,4,6-trienone (6e). The α-bromoenone **2e** (210 mg, 0.780 mmol) was subject to the general procedure and purification B to afford **6e** as a thick yellow oil (100 mg, 68%); IR (KBr): *v*: 3192, 2984, 1608, 1542, 1471, 1263, 733 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.35 (d, J = 1.5 Hz, 1H), 7.31–7.26 (m, 1H), 7.24–7.18 (m, 1H), 7.01–6.95 (m, 1H), 3.04–2.94 (m, 1H), 2.14–2.06 (m, 2H), 1.88–1.81 (m, 2H), 1.75–1.69 (m, 2H), 1.65–1.59 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.07, 170.79, 158.27, 137.00, 128.24, 123.63, 122.38, 50.54, 34.75, 25.87; HRMS (ESI) calcd for $C_{12}H_{15}O_2$ $[M + H]^+$: 191.1072; found: 191.1067.

(2*E*,4*Z*,6*E*)-2-Hydroxy-6-phenylcyclohepta-2,4,6-trienone (6f). The α-bromoenone **2f** (200 mg, 0.722 mmol) was subject to the general procedure and purification B to afford **6f** as a white solid (103 mg, 72%): mp = 87–91 °C IR (KBr): *v*: 3186, 1606, 1498,

1465, 1436, 1245, 920 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.61 (s, 1H), 7.56–7.52 (m, 2H), 7.52–7.37 (m, 4H), 7.32 (d, J = 10.9 Hz, 1H), 7.23 (d, J = 10.0 Hz, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.23, 169.79, 151.61, 142.68, 137.36, 129.13, 128.92, 128.66, 127.82, 123.79, 123.43; HRMS (ESI) calcd for $C_{13}H_{11}O_2$ $[M + H]^+$: 199.0759; found: 199.0753.

(2*E*,4*Z*,6*E*)-2-Hydroxy-6-(naphthalen-1-yl)cyclohepta-2,4,6-trienone (6g). The α-bromoenone **2g** (200 mg, 0.611 mmol) was subject to the general procedure and purification B to afford **6g** as pale yellow solid (82 mg, 54%): mp = 129–134 °C IR (KBr): *v*: 3096, 1697, 1681, 1290, 1265, 802 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.06 (dd, J = 7.9, 1.3 Hz, 1H), 7.88 (d, J = 8.2 Hz, 1H), 7.84 (d, J = 8.3 Hz, 1H), 7.61 (td, J = 7.5, 1.4 Hz, 1H), 7.53–7.45 (m, 2H), 7.45–7.44 (m, 1H), 7.43 (d, J = 2.1 Hz, 1H), 7.38–7.32 (m, 2H), 7.28 (dd, J = 7.0, 1.1 Hz, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.33, 141.97, 139.19, 133.27, 132.22, 132.16, 131.97, 130.86, 130.04, 128.16, 127.65, 127.56, 125.97, 125.58, 125.48, 125.07; HRMS (ESI) calcd for $C_{17}H_{13}O2$ $[M + H]^+$: 249.0916; found: 249.0902.

(2*E*,4*Z*,6*E*)-2-Hydroxy-6-(4-methoxyphenyl)cyclohepta-2,4,6-trienone (6h). The α-bromoenone **2h** (200 mg, 0.651 mmol) was subject to the general procedure and purification B to afford **6h** as an pale yellow solid (92 mg, 62%): mp = 116–118 °C: IR (KBr): *v*: 3192, 1601, 1514, 1456, 1292, 1180 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.59 (d, J = 1.6 Hz, 1H), 7.52–7.47 (m, 2H), 7.41 (dd, J = 14.5, 6.7 Hz, 1H), 7.26 (dd, J = 9.2, 7.4 Hz, 1H), 7.24–7.19 (m, 1H), 7.01–6.93 (m, 2H), 3.85 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.93, 169.76, 160.44, 151.24, 137.25, 134.82, 129.16, 128.21, 123.11, 123.09, 114.35, 55.36; HRMS (ESI) calcd for $C_{14}H_{13}O_3$ $[M + H]^+$: 229.0865; found: 229.0860.

(2*E*,4*Z*,6*E*)-2-Hydroxy-6-(2,5-dimethoxyphenyl)cyclohepta-2,4,6-trienone (6i). The α-bromoenone **2i** (130 mg, 0.386 mmol) was subject to the general procedure and purification B to afford **6i** as a thick yellow oil (65 mg, 65%): IR (KBr): *v*: 3206, 1659, 1503, 1229, 1160, 980 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.52 (s, 1H), 7.40 (dd, J = 12.8, 8.0 Hz, 1H), 7.35–7.30 (m, 1H), 7.15 (d, J = 9.9 Hz, 1H), 6.93 (t, J = 3.3 Hz, 2H), 6.84 (t, J = 1.6 Hz, 1H), 3.80 (s, 3H), 3.77 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.17, 169.53, 153.68, 150.12, 149.25, 136.72, 132.61, 130.65, 125.47, 124.09, 116.15, 114.85, 112.68, 56.23, 55.85; HRMS (ESI) calcd for $C_{15}H_{15}O_4$ $[M + H]^+$: 259.0970; found: 259.0965.

(2*E*,4*Z*,6*E*)-2-Hydroxy-6-(2,3,4-trimethoxyphenyl)cyclohepta-2,4,6-trienone (6j). The α-bromoenone **2i** (130 mg, 0.386 mmol) was subject to the general procedure and purification B to afford **6i** as a thick yellow oil (65 mg, 65%): IR (KBr): *v*: 3206, 1659, 1503, 1229, 1160, 980 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.52 (s, 1H), 7.40 (dd, J = 12.8, 8.0 Hz, 1H), 7.35–7.30 (m, 1H), 7.15 (d, J = 9.9 Hz, 1H), 6.93 (t, J = 3.3 Hz, 2H), 6.84 (t, J = 1.6 Hz, 1H), 3.80 (s, 3H), 3.77 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.17, 169.53, 153.68, 150.12, 149.25, 136.72, 132.61, 130.65, 125.47, 124.09, 116.15, 114.85, 112.68, 56.23, 55.85; HRMS (ESI) calcd for $C_{15}H_{15}O_4$ $[M + H]^+$: 259.0970; found: 259.0965.

Anti-proliferative effects of tropolones against MCF-7 and hDF. MCF-7 cells were maintained in a 1 : 1 mixture of Dulbecco's modified Eagle's medium : Ham's F-12 (Gibco) supplemented with non-essential amino acids, L-glutamine (2 mM), streptomycin (500 μg mL^{-1}), penicillin (100 units mL^{-1}) and 10% fetal bovine serum. Human adult fibroblasts (hDF) were cultured in DMEM (Life Technologies) supplemented with 10% FBS. Cells were grown to confluence in a humidified atmosphere (37 °C, 5% CO_2). MCF-7 cells were seeded at a density of 3000/well whereas hDF cells were seeded at 5000/well in clear 96-well plates and allowed to attach (37 °C, 5% CO_2). Compounds, at varying concentrations in DMSO, were added (1% DMSO final concentration) and cells returned to the incubator (37 °C, 5% CO_2) for 72 h. At 72 h, the number of viable cells was determined using an MTS/PMS cell proliferation kit (Promega) per the manufacturer's instructions. Cells incubated in 1% DMSO were used as 100% proliferation and values were adjusted accordingly. Data was subjected to graphical and statistical analysis using SigmaPlot or GraphPad software package and GI_{50} values obtained for each compound are the result of at least two separate experiments performed in triplicate.

Antifungal assay. *C. glabrata* was stored as a suspension in 50% glycerol at −78 °C. For susceptibility testing, a streak of stock culture was made on SDA agar and grown at 30 °C for 48 h. One pure colony of the test organism was recovered from the plate, suspended in appropriate media and grown in a 5 mL shake flask culture. A sample of the shake flask culture was diluted to 1×10^5 cells mL^{-1} in media and added to 96-well test plates (100 μL per well) containing test compounds dispensed in DMSO (1 μL). Amphotericin and ketoconazole were used as controls. After an incubation period determined from the strain specific doubling time, Alamar Blue (10 μL) was added, allowed to incubate; each well was scored for dye reduction (Davey *et al.*, 1998). The MIC value was taken as the lowest concentration of test compound that inhibits growth such that less than 1% reduction of the blue resazurin (λ_{max} 570 nm) component of the Alamar Blue to the pink resorufin (λ_{max} 600 nm) was observed.

Antibacterial assay. Minimum inhibitory concentrations (MIC) against *E. faecalis* and MRSA were determined using a broth microdilution approach based upon CLSI (formerly NCCLS) standards and the use of the colorimetric reporter Alamar Blue. The MIC value is the lowest concentration of test compound that inhibits growth such that less than 1% reduction of the blue resazurin (λ_{max} 570 nm) component of the Alamar Blue to the pink resorufin (λ_{max} 600 nm) is observed.

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