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Semi-Syntheses and Interrogation of Indole-Substituted *Aspidosperma* Terpenoid Alkaloids**

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** This manuscript is dedicated to the memory of our beloved colleague Dr. Rodrigo B. Andrade.

‡ Deceased May 24th, 2021.

Abstract

We demonstrated here a series of *Aspidosperma* terpenoid alkaloids can be quickly prepared using semisynthesis from naturally sourced tabersonine, featuring multiple oxygen-based substituents on the indole ring such as hydroxy and methoxy groups. This panel of complex compounds enabled the exploration of indole modifications to optimize the indole alkaloids' anticancer activity, generating lead compounds (e.g., with C15-hydroxy, C16-methoxy, and/or C17-methoxy derivatizations) that potently inhibit cancer cell line growth in the single-digit micromolar range. These results can help guide the development of *Aspidosperma* terpenoid alkaloid therapeutics. Furthermore, this synthetic approach features late-stage facile derivatization on complex natural product molecules, providing a versatile path to indole derivatization of this family of alkaloids with diverse chemical functionalities for future medicinal chemistry and chemical biology discoveries.

Introduction

Aspidosperma monoterpene indole alkaloids (MIAs) are an important class of plant natural products with architectural complexity and fascinating biological activities.[1-3] Their promising medicinal effects are best represented by the dimeric *Vinca* alkaloids vinblastine and vincristine, which are classic frontline drugs for cancer treatment.[4, 5] Mechanistically, *Aspidosperma* MIAs perturb microtubule dynamics by binding with tubulin, leading to broad-spectrum antitumor activity.[6-8] Nevertheless, their structural complexity makes the de novo synthesis of most MIAs challenging.[9-13] In addition, the limited quantities available from natural sources present significant bottlenecks to the biomedical applications of *Aspidosperma* MIAs.[9, 12-15] Thus, the discovery and facile synthesis of potent *Aspidosperma* MIAs, e.g., the melodinine and jerantinine series (Figure 1), are of high priority for anticancer drug development.[9, 16, 17] To date, most structure-activity studies of these natural products or synthetic analogues have focused on substitutions on rings D/E,[9, 16-18] while fewer efforts have exploited derivatizations on indole ring

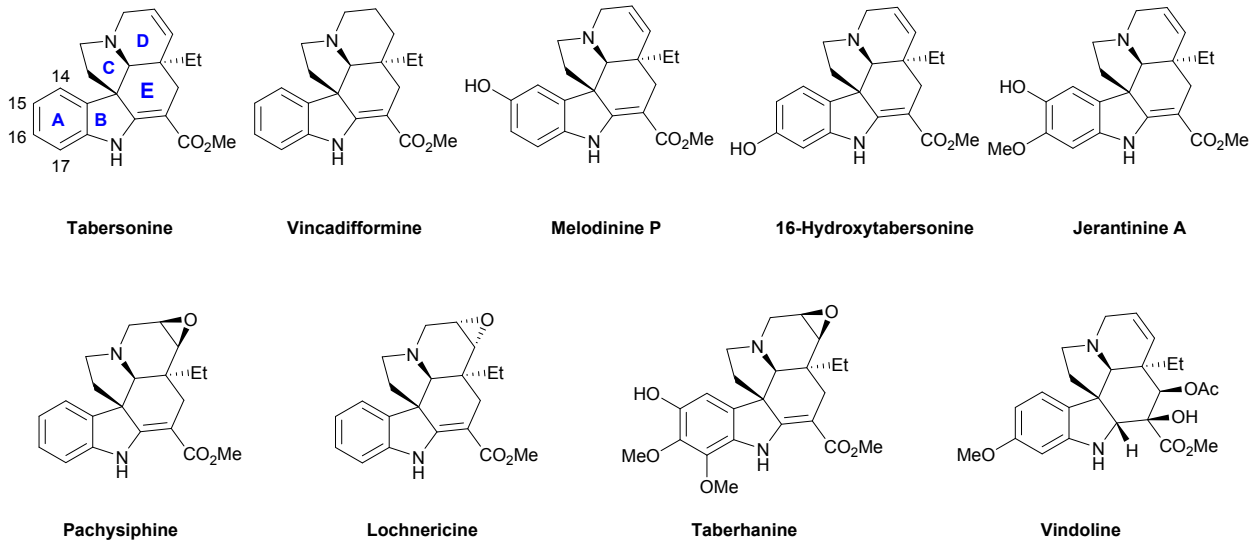


Fig. 1 Representative monoterpene indole alkaloids of the *Aspidosperma* class.

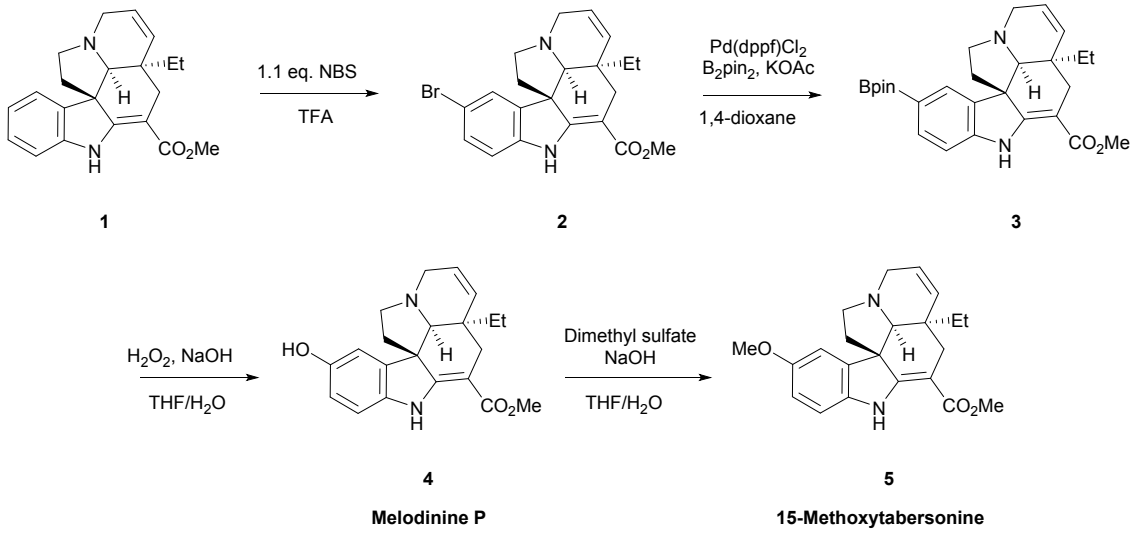
A. The diverse class of *Aspidosperma* MIAs (Figure 1) actually present varied hydroxy and methoxy substitution patterns on indole ring A, and the sparse preliminary studies revealed the possibility of significant tuning of anticancer activities based on these substitutions.[16, 17, 19] Herein, we report a unified semisynthesis approach that starts from readily accessible tabersonine and allows for ready preparation of *Aspidosperma* MIAs with indoles carrying multiple oxygen-based substitutions. Such an approach empowered us to systematically interrogate the structure-activity relationships of hydroxy/methoxy functional groups at key indole positions C15, C16, and C17, leading to the discovery of the most potent indole substitution pattern for development of future anticancer therapeutics.

Tabersonine has been reported as the single biosynthetic precursor for most *Aspidosperma* indole alkaloids including vinblastine, vincristine, melodinines, jerantinines, etc.[1, 19, 20] As the main alkaloid in *Voacanga* seeds and the *C. roseus* plant,[1, 17] tabersonine is naturally abundant and has been utilized as the leading starting material to prepare jerantinine A derivatives[9] and synthetic analogues[17] through chemical synthesis. Nevertheless, there is a lack of a systematic synthetic strategy to explore all the key positions (C15-C17) in the indole ring which have been observed in isolated natural alkaloids.[16, 18, 21] Specifically, no substitutions on the C17 position have ever been explored before, let alone the resulting effects on the alkaloids' anticancer activity.

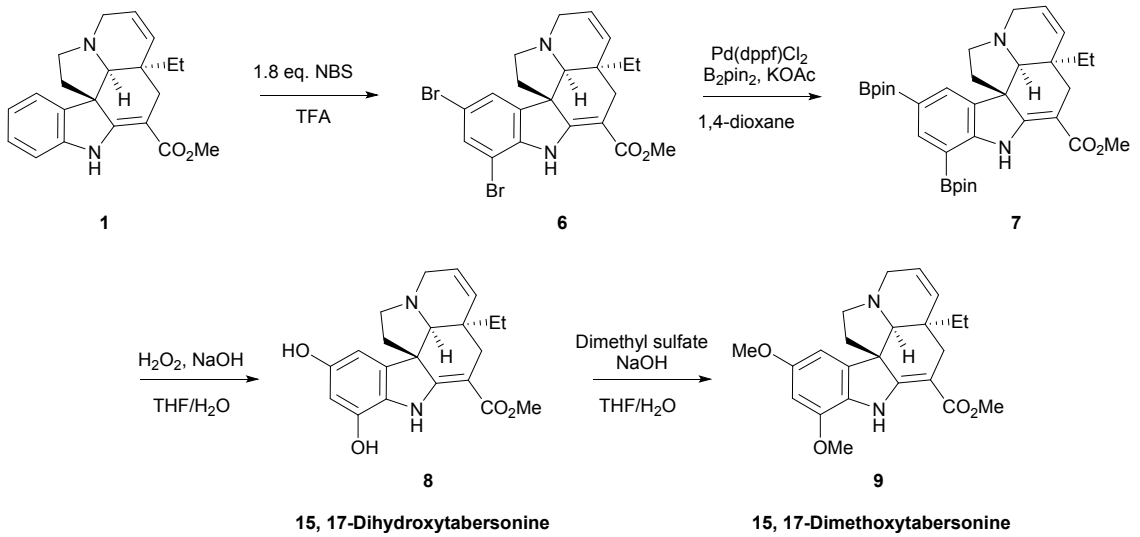
Results and discussion

Inspired by the reported direct halogenation on the indole ring,[9, 17] we treated tabersonine with 1.1 equivalents of NBS in TFA to achieve selective bromination on C15 (Scheme 1). The borate ester **3** was then synthesized in ~83% yield under Miyaura borylation conditions using

Pd(dppf)Cl_2.[9] Subsequent oxidation was performed with H_2O_2/NaOH at 0 °C to afford melodinine P (**4**) in ~73%



Scheme 1 Synthesis of Melodinine P and 15-Methoxytabersonine.



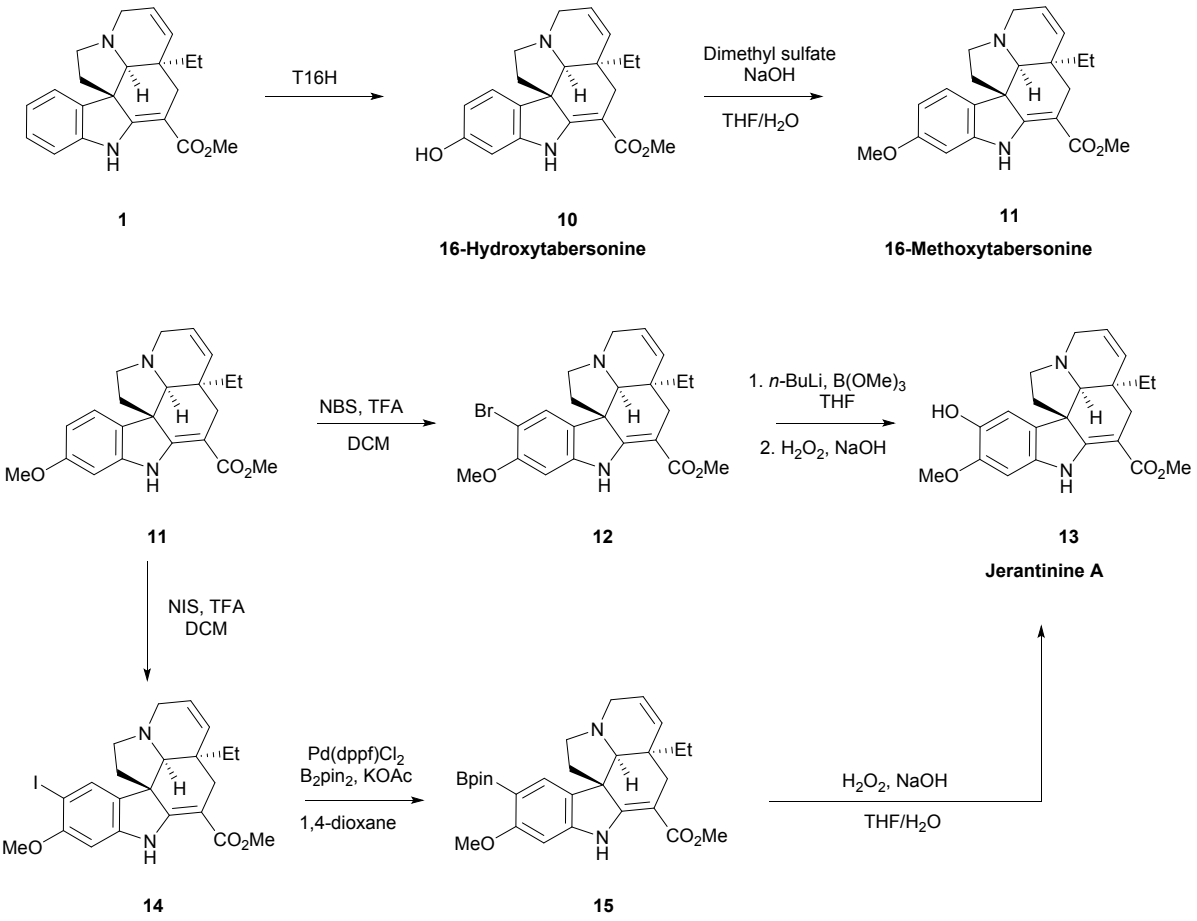
Scheme 2 Synthesis of 15,17-Dihydroxytabersonine and 15,17-Dimethoxytabersonine.

yield. Treatment of **4** with dimethyl sulfate and NaOH in THF/water resulted in the methylated product 15-Methoxytabersonine (**5**) with ~70% yield. As shown in Scheme 2, we also increased

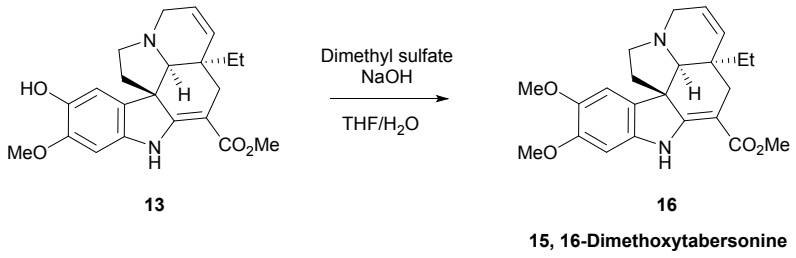
the initial amount of NBS to 1.8 equivalents to generate 15,17-Dibromotabersonine (**6**), which can be separated from 15-Bromotabersonine (**2**) and isolated in ~30%–40% yield. Attempts to optimize the dibromination reaction through increasing the amount of NBS, solvent screening (DCM, DMF, acetonitrile), or the adjustment of reaction temperature/steps did not afford any significantly better results. Subsequent Miyaura borylation resulted in the diborylated intermediate **7** at a yield of ~73%. Follow-up oxidation rendered the 15,17-Dihydroxytabersonine (**8**) in ~81% yield. We also pursued methylation to obtain the methylated analogue 15,17-Dimethoxytabersonine (**9**).

We recently demonstrated regioselective derivatization on the C16 position using site-selective enzymatic oxidation in yeast.[22, 23] As shown in Scheme 3, we achieved a highly efficient biotransformation (~70% yield) from tabersonine (**1**) to 16-Hydroxytabersonine (**10**) with T16H, a cytochrome P450 monooxygenase. Subsequent methylation using the above-mentioned dimethyl sulfate reagent afforded 16-Methoxytabersonine (**11**) with ~84% yield. To semi-synthesize jerantinine A derivatives, Smedley et al. have previously utilized ortho-formylation and sequential Dakin oxidation on *N*-Boc protected melodinine P, which took ~10 steps from tabersonine.[9] Since we can install the critical C16 methoxy group readily using the above-mentioned chemoenzymatic strategy, we first converted **11** to the C15 brominated intermediate **12** (Scheme 3). Treatment of **12** with *n*-BuLi at -78 °C generated the arene anion, which was then quenched with $B(OCH_3)_3$. The resulting mixture was directly subjected to the oxidation condition mentioned above to afford jerantinine A (**13**) in ~47% yield over the two steps. To facilitate future scaleup, we also converted **11** to the iodo substrate **14** with NIS and TFA in DCM.[24] In this case, the borate ester **15** was successfully achieved in ~67% yield with the classical Miyaura borylation conditions and was subsequently oxidized to give jerantinine A

(**13**) in ~71% yield. With a total of 5 steps, our synthetic route is free from protecting groups and provided by far the most efficient semisynthesis of jerantinine A and its derivatives.[9, 25] As demonstrated by Scheme 4, jerantinine A can be further methylated to furnish the dimethoxy substituted analogue **16**.



Scheme 3 Synthesis of 16-Hydroxytabersonine and 16-Methoxytabersonine and Jerantinine A.



Scheme 4 Synthesis of 15,16-Dimethoxytabersonine.

With a panel of indole-substituted *Aspidosperma* MIAs in hand, we set out to explore their potential as anticancer agents. Using the parent compound tabersonine (**1**), we first screened this

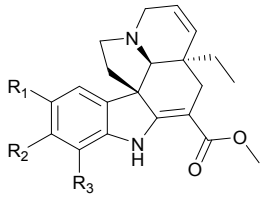
indole alkaloid's inhibitory activity on the growth of sixty human cancer cell lines (Supporting Information Figure S1, Figure S2). Tabersonine displayed broad-spectrum antitumor activities, with growth inhibition (GI_{50}) of most cancer cells at a similar level (single-digit μM), regardless of cancer type (Figure S2, Table 1). We therefore selected the prostate cancer cell line PC3 to move forward, which represented one of the most common, yet metastatic and fatal cancer types.[26, 27] As shown in Figure 2 and Table 2, jerantinine A is the most potent tabersonine derivative, with an EC_{50} of 2.8 ± 0.7 μM. Other analogues displayed potencies between those of tabersonine and jerantinine A.

Table 1 Growth Inhibition (GI_{50}) Values of Tabersonine in Cancer Cell Lines.

Cancer Cell Line	**GI_{50} (μM)**	**Cancer Cell Line**	**GI_{50} (μM)**	**Cancer Cell Line**	**GI_{50} (μM)**
Leukemia		Non-Small Cell Lung Cancer		Colon Cancer	
CCRF-CEM	2.7	A549	12.4	COLO205	5.6
HL-60	3.2	EKVX	6.0	HCC-2998	1.1
K-562	3.1	HOP-62	9.8	HCT-116	3.2
MOLT-4	3.2	HOP-92	2.3	HCT-15	2.7
RPMI-8226	3.2	NCI-H23	4.4	HT29	3.4
SR	2.6	NCI-H322M	10.9	KM12	3.6
		NCI-H460	4.1	SW-620	3.2
		NCI-H522	3.2		
CNS Cancer		Melanoma		Ovarian Cancer	
SF-268	4.6	LOX IMVI	1.9	IGROV1	5.8
SF-295	13.0	MALME-3M	3.4	OVCAR-3	2.4
SF-539	3.1	M14	3.0	OVCAR-4	4.4
SNB-19	10.7	MDA-MB-435	2.1	OVCAR-5	6.6
SNB-75	2.2	SK-MEL-2	4.4	OVCAR-8	3.8
U251	3.6	SK-MEL-28	4.5	NCI/ADR-RES	6.4
		SK-MEL-5	4.9		
		UACC-257	5.1		
		UACC-62	2.9		
Renal Cancer		Prostate Cancer		Breast Cancer	
786-0	3.7	PC-3	5.4	MCF7	3.3

A498	7.1	DU-145	3.8	MDA-MB-231	3.2
ACHN	2.1			HS 578T	3.0
CAKI-1	4.7			BT-549	8.7
RXF393	2.5			T-47D	2.8
SN12C	3.6			MDA-MB-468	3.4
TK-10	4.9				
UO-31	4.1				

Table 2 Chemical Structures of Indole Derivatives of Tabersonine and Inhibitory Activities Against the Growth of Prostate Cancer PC3 Cells.



Compound	**R_1**	**R_2**	**R_3**	**EC_{50} (μM)**
1 (Tabersonine)	H	H	H	76.0 ± 10.1
4 (Melodinine P)	OH	H	H	20.9 ± 2.7
5 (15-Methoxytabersonine)	OCH_3	H	H	59.5 ± 6.6
8 (15,17-Dihydroxytabersonine)	OH	H	OH	14.4 ± 18.1
9 (15,17-Dimethoxytabersonine)	OCH_3	H	OCH_3	26.8 ± 11.7
10 (16-Hydroxytabersonine)	H	OH	H	51.1 ± 5.1
11 (16-Methoxytabersonine)	H	OCH_3	H	35.7 ± 14.0
13 (Jerantinine A)	OH	OCH_3	H	2.8 ± 0.7
16 (15,16-Dimethoxytabersonine)	OCH_3	OCH_3	H	21.6 ± 6.1

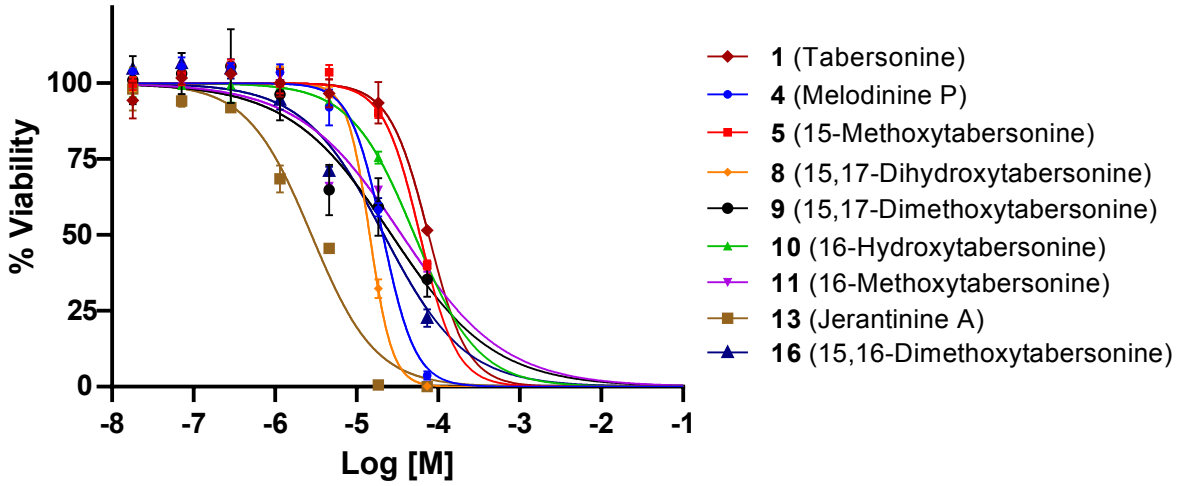


Fig. 2 Growth inhibition plots of the tabersonine derivatives on the representative cancer cell line PC3.

Compared to tabersonine, derivatives **4** and **5** possessed much improved potencies, with EC_{50} of 20.9 ± 2.7 μM and 59.5 ± 6.6 μM, respectively. This trend suggests that C15 substitutions with hydroxy or methoxy groups enhanced *Aspidosperma* MIAs' activity, and hydroxy substitution is preferred. The preference for a hydroxy group on C15 was further evidenced by the almost 10-fold difference in EC_{50} between jerantinine A (**13**) and the dimethoxy derivative **16**. Direct comparison of EC_{50} between compounds **8** and **4** show a slight (~1.5-fold) enhancement of potency with hydroxy substitution at the C17 position. The trend is further corroborated by the > 2-fold enhanced potency of compound **9** versus **5**, which indicates a methoxy group at C17 would further improve the alkaloids' potency. Significant EC_{50} changes were also observed for compounds **10**/**11** when compared with the parent tabersonine, implying that oxygen-containing substitution at C16, particularly the methoxy group, increases the alkaloids' antitumor activity. To confirm the observed cancer growth inhibition by tabersonine derivatives is indeed through the suppression of tubulin polymerization, we also performed an *in*

vitro tubulin polymerization assay. As shown in Figure 3, all the representative derivatives significantly inhibited the polymerization in terms of the kinetics and the extent. Taken together, the above-mentioned substitutions at the C15-C17 positions universally enhanced the anticancer activity of tabersonine. The optimal substitution pattern could be C15-OH, C16-OMe, and C17-OMe. Consistent to this, such a tri-substituted tabersonine derivative, taberhanine, has been recently isolated as a natural product (Figure 1).[28] Although there is limited biological characterization for this monomer, the dimeric version, conophylline, has demonstrated much better anticancer activity than other *Aspidosperma* bisindole alkaloids.[18, 21, 29]

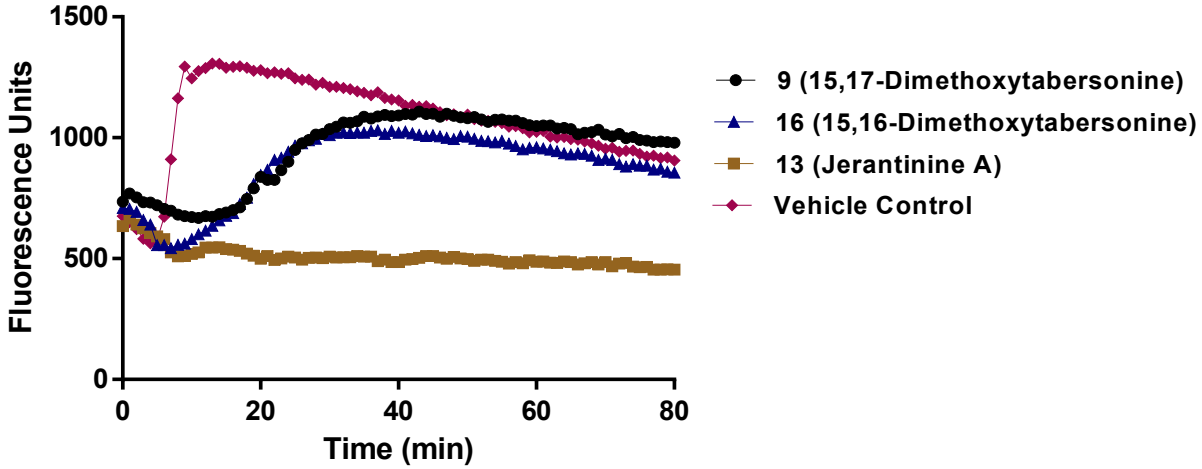


Fig. 3 Tubulin polymerization assay plots of the representative tabersonine derivatives.

Conclusions

In summary, we have invented an efficient semisynthesis of *Aspidosperma* monoterpene indole alkaloids from naturally sourced and abundant tabersonine using sequential halogenation, borylation, and oxidation. This strategy has resulted in a panel of MIAs with multiple oxygen-based substituents on the indole ring, and follow-up structure-activity relationship studies have

revealed the beneficial effects of hydroxy/methoxy derivatizations on the alkaloids' antitumor activities. These results can help guide the development of future cancer therapeutics. Furthermore, such a strategy in association with the panel of tool compounds could facilitate the medicinal chemistry advancement of *Aspidosperma* alkaloids in other disease areas including Alzheimer's disease.[17] Future work will focus on regioselective C17 functionalization with inspiration from precedent reports,[30, 31] and the exploration of halogenated derivatives to further expand the scope of this strategy.

Experimental section

General

All reactions using moisture or air sensitive reagents were performed in flame-dried glassware under nitrogen or argon. Chemical reagents and solvents were purchased from commercial resources and used directly without further purification. Analytical TLC was carried out with Silica Gel 60 F254 plates (Merck and Analtech). Detection was performed using UV light, $KMnO_4$ stain, iodine chamber, or PMA stain with subsequent heating. Compound purification was performed by normal phase flash column chromatography on silica gel grade 60 (230-400 mesh, Fisher Scientific) with indicated solvents and was further carried out on Waters semi-preparative HPLC. ^{1}H and ^{13}C NMR spectra were recorded on 400 MHz or 500 MHz Bruker Advance in $CDCl_3$ at 298K. The raw data were processed with MestReNova, with the chemical shifts indicated in parts per million (ppm) downfield from tetramethylsilane (TMS, $\delta = 0.00$) and

referenced to $CDCl_3$. Splitting patterns are abbreviated as the following: s (singlet), d (doublet), bs (broad singlet), bd (broad doublet), t (triplet), q (quartet), and m (multiplet). ESI-MS analysis was performed on an Agilent 6520 Accurate-Mass Quadrupole-Time-of-Flight (Q-TOF) coupled with an electrospray ionization source. LC-MS characterizations were carried out on a Waters system that operated on a 2767 sample manager, 2545 binary gradient module, and 2489 UV-vis detector and was equipped with an Atlantis T3 OBD column and an ACQUITY QDa mass detector. The purities of all compounds analyzed in the biological assays were evaluated by LC-MS analysis and were confirmed to be ≥ 95% unless otherwise specified.

15-Bromotabersonine (2) To the solution of **1** (500 mg, 1.5 mmol) dissolved in 3 mL of TFA was added NBS (318 mg, 1.8 mmol) at room temperature in one portion. The reaction mixture was stirred overnight at room temperature and then poured into 20 mL aq. $NaHCO_3$. The organic mixture was extracted with CH_2Cl_2 (2 × 10 mL), washed with brine (2 × 10 mL), and dried over anhydrous Na_2SO_4. The organic solution was concentrated under reduced pressure, and the residue was purified via flash chromatography eluting with 15-20% ethyl acetate/hexane to afford **2** (376 mg, 60% yield) as a white, amorphous solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.99 (s, 1H), 7.33 (d, J = 2.0 Hz, 1H), 7.24 (m, 1H), 6.69 (d, J = 8.0 Hz, 1H), 5.79 (ddd, J = 10.0, 5.0, 1.5 Hz, 1H), 5.70 (d, J = 10.0 Hz, 1H), 3.75 (s, 3H), 3.45 (dd, J = 16.0, 4.0 Hz, 1H), 3.19 (d, J = 16.0 Hz, 1H), 3.05 (t, J = 7.5 Hz, 1H), 2.69 (m, 1H), 2.63 (s, 1H), 2.55 (dd, J = 15.0, 2.0 Hz, 1H), 2.40 (d, J = 15.0 Hz, 1H), 2.05 (m, 1H), 1.82 (dd, J = 11.0, 4.0 Hz, 1H), 0.97 (m, 1H), 0.86 (m, 1H), 0.64 (t, J = 7.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) 169.0, 165.8, 142.4,

140.3, 132.8, 130.5, 124.9, 124.9, 112.9, 110.7, 93.0, 70.1, 55.3, 51.2, 51.1, 50.5, 44.6, 41.2, 28.6, 27.1, 7.6; HRMS (ESI) *m/z* calculated for $C_{21}H_{24}BrN_2O_2$ $[M+H]^+$ 415.1021, found 415.1023.

15-Bpin tabersonine (3) To the solution of **2** (462 mg, 1.1 mmol) dissolved in 5 mL of dry 1,4-dioxane was added B_2pin_2 (381 mg, 1.5 mmol), KOAc (294 mg, 3.0 mmol), $Pd(dppf)Cl_2$ (41 mg, 0.05 mmol) at room temperature under argon. The reaction was heated to 95–100 °C and then stirred at this temperature overnight under argon. The reaction mixture was filtered through Celite pad, rinsed with ethyl acetate (2 × 10 mL), washed with aq. $NaHCO_3$ (2 × 10 mL) and brine (2 × 10 mL), and finally dried over anhydrous Na_2SO_4. The organic solution was concentrated under reduced pressure, and the residue was purified via flash chromatography eluting with 15-30% ethyl acetate/hexane to afford **3** (462 mg, 89% yield) as a white, amorphous solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.08 (s, 1H), 7.66 (d, *J* = 8.0 Hz, 1H), 7.61 (s, 1H), 6.81 (d, *J* = 8.0 Hz, 1H), 5.78 (ddd, *J* = 10.0, 4.5, 1.5 Hz, 1H), 5.71 (d, *J* = 10.0 Hz, 1H), 3.77 (s, 3H), 3.46 (ddd, *J* = 16.0, 5.0, 1.5 Hz, 1H), 3.27 (d, *J* = 16.0 Hz, 1H), 3.02 (dd, *J* = 8.5, 6.0 Hz, 1H), 2.79 (m, 1H), 2.75 (s, 1H), 2.54 (dd, *J* = 15.0, 2.0 Hz, 1H), 2.44 (d, *J* = 15.0 Hz, 1H), 2.04 (m, 1H), 1.78 (dd, *J* = 11.5, 4.5 Hz, 1H), 1.34 (s, 12H), 0.97 (m, 1H), 0.83 (m, 1H), 0.63 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.1, 166.6, 146.1, 137.5, 135.7, 133.1, 127.4, 125.1, 108.9, 93.0, 83.7, 69.9, 55.0, 51.2, 51.0, 50.7, 44.8, 41.6, 28.3, 26.9, 25.0, 25.0, 7.6; HRMS (ESI) *m/z* calculated for $C_{27}H_{36}BN_2O_4$ $[M+H]^+$ 463.2768, found 463.2754.

15-OH tabersonine (4) To the solution of **3** (100 mg, 0.21 mmol) dissolved in 2 mL of THF was added 2.2 mL of 10% aq. NaOH and 0.25 mL of 30% aq. H_2O_2 at 0 °C. The mixture was stirred at 0 °C for 0.5 h. The reaction mixture was then diluted with 5 mL of aq. H_2O, neutralized with 1N HCl, extracted by ethyl acetate (2 × 10 mL), washed with brine (2 × 10 mL), and eventually dried over anhydrous Na_2SO_4. The organic solution was concentrated under

reduced pressure, and the residue was purified via flash column chromatography eluting with 35-50% ethyl acetate/hexane to afford **4** (56 mg, 73% yield) as a yellow, amorphous solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.84 (s, 1H), 6.85 (s, 1H), 6.64 (s, 2H), 5.80 (m, 1H), 5.71 (d, *J* = 10.0 Hz, 1H), 3.76 (s, 3H), 3.44 (ddd, *J* = 16.0, 5.0, 1.5 Hz, 1H), 3.17 (d, *J* = 16.0 Hz, 1H), 3.03 (t, *J* = 7.0 Hz, 1H), 2.68 (m, 1H), 2.63 (s, 1H), 2.55 (dd, *J* = 15.0, 2.0 Hz, 1H), 2.41 (d, *J* = 15.0 Hz, 1H), 2.07 (m, 1H), 1.81 (m, 1H), 0.99 (m, 1H), 0.86 (m, 1H), 0.64 (t, *J* = 7.5 Hz, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.3, 167.5, 150.3, 139.6, 136.7, 133.2, 124.7, 114.0, 110.3, 109.8, 91.3, 70.2, 55.7, 51.2, 51.1, 50.5, 44.4, 41.2, 28.8, 27.2, 7.6; HRMS (ESI) *m/z* calculated for $C_{21}H_{25}N_2O_3$ $[M+H]^+$ 353.1865, found 353.1862.

15-OMe tabersonine (5) To the solution of **4** (100 mg, 0.28 mmol) dissolved in 5 mL of 1:1 THF/H_2O (v/v) was added 227 μL of 10% aq. NaOH and 32 μL of dimethyl sulfate (0.34 mmol) at room temperature. The reaction was stirred for 1 h, quenched with 10 mL of saturated aq. NH_4Cl, extracted with ethyl acetate (2 × 5 mL), washed with brine (10 mL), and finally dried over anhydrous Na_2SO_4. The organic solution was concentrated under reduced pressure, and the residue was purified via flash column chromatography eluting with 20-30% ethyl acetate/hexane to afford **5** (73 mg, 70% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.89 (s, 1H), 6.86 (d, *J* = 2.5 Hz, 1H), 6.72 (d, *J* = 8.5 Hz, 1H), 6.67 (dd, *J* = 8.5, 2.5 Hz, 1H), 5.78 (ddd, *J* = 10.0, 4.5, 1.5 Hz, 1H), 5.71 (d, *J* = 10.0 Hz, 1H), 3.78 (s, 3H), 3.76 (s, 3H), 3.45 (dd, *J* = 16.0, 4.5 Hz, 1H), 3.18 (d, *J* = 16.0 Hz, 1H), 3.04 (t, *J* = 7.5 Hz, 1H), 2.69 (m, 1H), 2.64 (s, 1H), 2.54 (dd, *J* = 15.0, 2.0 Hz, 1H), 2.42 (d, *J* = 15.0 Hz, 1H), 2.108 (m, 1H), 1.80 (dd, *J* = 11.5, 4.5 Hz, 1H), 0.99 (m, 1H), 0.85 (m, 1H), 0.64 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.2, 167.5, 154.7, 139.8, 137.2, 133.2, 125.0, 111.5, 109.6, 109.5, 91.6, 70.3, 56.0, 55.6, 51.1, 50.7, 44.6, 41.5, 28.6, 27.1, 7.6; HRMS (ESI) *m/z* calculated for $C_{22}H_{27}N_2O_3$ $[M+H]^+$ 367.2022, found 367.2035.

15,17-Dibromotabersonine (6) To the solution of **1** (1.5 g, 4.5 mmol) dissolved in 8 mL of TFA was added NBS (1.5 g, 8.5 mmol) in one portion at room temperature. The resulting mixture was stirred at room temperature overnight and then poured into 50 mL of 10% aq. NaOH solution. The quenched reaction mixture was extracted with CH_2Cl_2 (2 × 10 mL), washed with brine (2 × 10 mL), and dried over anhydrous Na_2SO_4. The organic solution was concentrated under vacuum, and the residue was purified via flash column chromatography eluting with 5-20% ethyl acetate/hexane to afford **6** (751 mg, 34% yield) as a white, amorphous solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.01 (s, 1H), 7.43 (d, *J* = 2.0 Hz, 1H), 5.79 (ddd, *J* = 10.0, 5.0, 1.5 Hz, 1H), 5.70 (dt, *J* = 10.0, 2.0 Hz, 1H), 3.79 (s, 3H), 3.45 (ddd, *J* = 16.0, 5.0, 1.5 Hz, 1H), 3.19 (dt, *J* = 16.0, 2.0 Hz, 1H), 3.06 (t, *J* = 7.0 Hz, 1H), 2.67 (m, 1H), 2.62 (s, 1H), 2.56 (dd, *J* = 15.5, 2.0 Hz, 1H), 2.40 (d, *J* = 15.5 Hz, 1H), 2.07 (m, 1H), 1.85 (ddd, *J* = 12.0, 5.0, 1.5 Hz, 1H), 0.98 (m, 1H), 0.88 (m, 1H), 0.66 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.7, 163.9, 141.9, 141.0, 132.7, 132.6, 125.0, 123.8, 112.8, 103.0, 94.8, 70.3, 56.5, 51.4, 51.1, 50.5, 44.7, 41.1, 28.8, 27.2, 7.7; HRMS (ESI) *m/z* calculated for $C_{21}H_{23}Br_2N_2O_2$ $[M+H]^+$ 493.0126, found 493.0105.

15,17-DiBpin tabersonine (7) To the solution of compound **6** (244 mg, 0.49 mmol) dissolved in 5 mL of dry 1,4-dioxane was added B_2pin_2 (316 mg, 1.24 mmol), KOAc (244 mg, 2.49 mmol), and Pd(dppf)Cl_2 (34 mg, 0.04 mmol) at room temperature under argon. The reaction was heated to 95–100 °C and then stirred at this temperature overnight under argon. The reaction mixture was filtered through Celite pad, rinsed with ethyl acetate (2 × 10 mL), washed with H_2O (2 × 10 mL), and dried over anhydrous Na_2SO_4. The organic solution was concentrated under reduced pressure, and the residue was purified via flash column chromatography eluting with 10-20% ethyl acetate/hexane to afford compound **7** (214 mg, 73%

yield) as a white, amorphous solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.64 (s, 1H), 8.06 (s, 1H), 7.65 (s, 1H), 5.78 (dd, *J* = 10.0, 5.0 Hz, 1H), 5.72 (d, *J* = 10.0 Hz, 1H), 3.82 (s, 3H), 3.45 (dd, *J* = 16.0, 5.0 Hz, 1H), 3.27 (d, *J* = 16.0 Hz, 1H), 3.02 (m, 1H), 2.80 (m, 1H), 2.73 (s, 1H), 2.60 (dd, *J* = 15.0, 2.0 Hz, 1H), 2.43 (d, *J* = 15.0 Hz, 1H), 2.03 (m, 1H), 1.77 (dd, *J* = 11.5, 4.5 Hz, 1H), 1.40 (d, *J* = 2.5 Hz, 12H), 1.33 (s, 13H), 1.01 (m, 1H), 0.85 (m, 1H), 0.64 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.3, 164.7, 152.7, 142.0, 136.9, 135.7, 133.3, 130.0, 125.0, 93.1, 84.0, 83.6, 69.7, 54.9, 51.1, 51.0, 50.6, 45.1, 41.4, 28.9, 27.0, 25.3, 25.1, 25.0, 7.6; HRMS (ESI) *m/z* calculated for $C_{33}H_{47}B_2N_2O_6$ $[M+H]^+$ 589.3620, found 589.3621.

15,17-Dihydroxytabersonine (8) To the solution of compound **7** (155 mg, 0.26 mmol) dissolved in 4 mL of THF was added 2.6 mL of aq. NaOH solution (1M) and 0.3 mL of 30% aq. H_2O_2 at 0 °C. The mixture was stirred at 0 °C for 1 h and then neutralized with 1N HCl. The organic components were extracted with ethyl acetate (2 × 10 mL), washed with brine (2 × 10 mL), and dried over anhydrous Na_2SO_4. The organic solution was concentrated under reduced pressure, and the residue was purified via flash column chromatography eluting with 40-70% ethyl acetate/hexane to afford **8** (79 mg, 81%) as a pale yellow, amorphous solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.95 (s, 1H), 6.46 (s, 1H), 6.45 (s, 1H), 5.76 (ddd, *J* = 10.0, 4.5, 1.5 Hz, 1H), 5.70 (d, *J* = 10.0 Hz, 1H), 3.73 (s, 3H), 3.43 (dd, *J* = 16.0, 4.5 Hz, 1H), 3.17 (d, *J* = 16.0 Hz, 1H), 3.01 (t, *J* = 7.5 Hz, 1H), 2.66 (m, 2H), 2.53 (d, *J* = 15.0 Hz, 1H), 2.39 (d, *J* = 15.0 Hz, 1H), 2.04 (m, 1H), 1.78 (dd, *J* = 12.0, 4.5 Hz, 1H), 0.97 (m, 1H), 0.86 (m, 1H), 0.61 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.8, 167.9, 151.8, 140.6, 133.2, 124.7, 124.4, 103.4, 102.3, 91.1, 75.8, 70.2, 56.5, 51.5, 51.2, 50.5, 44.3, 41.2, 28.8, 27.2, 7.6; HRMS (ESI) *m/z* calculated for $C_{21}H_{25}N_2O_4$ $[M+H]^+$ 369.1814, found 369.1815.

15,17-Dimethoxytabersonine (9) To the solution of **8** (137 mg, 0.37 mmol) dissolved in 6 mL of THF/H_2O solution (1:1 v/v) was added 0.75 mL of 10% aq. NaOH and 0.11 mL of dimethyl sulfate (1.12 mmol) at room temperature. The reaction was stirred at room temperature for 1 h and quenched with 10 mL of saturated aq. NH_4Cl. The organic fractions were extracted with ethyl acetate (2 × 10 mL), washed with brine (2 × 10 mL), and dried over anhydrous Na_2SO_4. The organic solution was concentrated under reduced pressure, and the residue was purified via flash column chromatography eluting with 15-30% ethyl acetate/hexane to afford compound **9** (103 mg, 69%) as a yellow, amorphous solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.77 (s, 1H), 6.47 (d, *J* = 2.0 Hz, 1H), 6.37 (d, *J* = 2.0 Hz, 1H), 5.77 (dd, *J* = 10.0, 4.0 Hz, 1H), 5.72 (d, *J* = 10.0 Hz, 1H), 3.84 (s, 3H), 3.79 (s, 3H), 3.76 (s, 3H), 3.45 (dd, *J* = 16.0, 5.0 Hz, 1H), 3.17 (dd, *J* = 16.0, 2.5 Hz, 1H), 3.03 (t, *J* = 7.5 Hz, 1H), 2.67 (m, 1H), 2.62 (s, 1H), 2.55 (dd, *J* = 15.0, 2.0 Hz, 1H), 2.41 (d, *J* = 15.0 Hz, 1H), 2.08 (td, *J* = 11.5, 6.5 Hz, 1H), 1.80 (dd, *J* = 11.5, 4.5 Hz, 1H), 0.99 (m, 1H), 0.85 (m, 1H), 0.63 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.9, 167.1, 155.6, 144.6, 139.5, 133.3, 125.9, 124.9, 99.8, 97.8, 91.8, 70.2, 56.3, 56.1, 55.7, 51.2, 51.0, 50.7, 44.5, 41.4, 28.6, 27.0, 7.6; HRMS (ESI) *m/z* calculated for $C_{23}H_{29}N_2O_4$ $[M+H]^+$ 397.2127, found 397.2130.

16-OH tabersonine (10) 16-OH tabersonine (**10**) was prepared from tabersonine (**1**) with T16H in yeast following the reported procedure.[23]

16-Methoxytabersonine (11) To the solution of **10** (1.0 g, 2.8 mmol) in 10 mL of THF/H_2O (1:1 v/v) was added 5.6 mL of 10% aq. NaOH and 0.3 mL of dimethyl sulfate (3.1 mmol) at room temperature. The reaction mixture was stirred at room temperature for 1 h, followed by neutralization with 1N HCl. The organic fraction was extracted with ethyl acetate (2 × 10 mL), washed with brine (2 × 10 mL), and dried over anhydrous Na_2SO_4. The organic phase was

concentrated under vacuum, and the residue was purified via flash column chromatography eluting with 20-40% ethyl acetate/hexane to afford **11** (878 mg, 84% yield) as a white, amorphous solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.96 (s, 1H), 7.10 (d, *J* = 8.0 Hz, 1H), 6.40 (m, 2H), 5.78 (ddd, *J* = 10.0, 4.5, 1.5 Hz, 1H), 5.70 (dt, *J* = 10.0, 2.0 Hz, 1H), 3.78 (s, 3H), 3.77 (s, 3H), 3.45 (ddd, *J* = 16.0, 5.0, 1.5 Hz, 1H), 3.17 (d, *J* = 16.0 Hz, 1H), 3.03 (t, *J* = 7.0 Hz, 1H), 2.67 (m, 1H), 2.62 (s, 1H), 2.53 (dd, *J* = 15.0, 2.0 Hz, 1H), 2.42 (d, *J* = 15.0 Hz, 1H), 2.05 (m, 1H), 1.76 (dd, *J* = 11.5, 4.5 Hz, 1H), 1.00 (m, 1H), 0.86 (m, 1H), 0.64 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.2, 167.4, 160.2, 144.6, 133.3, 130.7, 125.4, 121.9, 105.1, 96.8, 92.6, 70.3, 55.6, 54.6, 51.2, 51.1, 50.8, 44.7, 41.6, 28.6, 27.0, 7.6; HRMS (ESI) m/z calculated for $C_{22}H_{27}N_2O_3$ $[M+H]^+$ 367.2022, found 367.2020.

15-Bromo-16-methoxytabersonine (12) To the solution of **11** (300 mg, 0.82 mmol) in 4 mL of TFA was added NBS (145 mg, 0.82 mmol) in one portion at room temperature. The mixture was stirred at room temperature overnight, and the reaction was quenched by the addition of 20 mL of 10% aq. NaOH. The organic components were extracted with CH_2Cl_2 (2 × 10 mL), washed with brine (2 × 10 mL), and dried over anhydrous Na_2SO_4. After concentrating under reduced pressure, the residue was purified via flash column chromatography eluting with 20-35% ethyl acetate/hexane to afford **12** (244 mg, 67% yield) as a white, amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.00 (s, 1H), 7.34 (s, 1H), 6.46 (s, 1H), 5.78 (ddd, *J* = 10.0, 5.0, 1.6 Hz, 1H), 5.69 (d, *J* = 10.0 Hz, 1H), 3.87 (s, 3H), 3.77 (s, 3H), 3.44 (dd, *J* = 16.0, 1.5 Hz, 1H), 3.18 (d, *J* = 16.0 Hz, 1H), 3.03 (t, *J* = 7.0 Hz, 1H), 2.67 (m, 1H), 2.60 (s, 1H), 2.53 (dd, *J* = 15.0, 2.0 Hz, 1H), 2.39 (d, *J* = 15.0 Hz, 1H), 2.05 (m, 1H), 1.79 (m, 1H), 0.97 (m, 1H), 0.86 (m, 1H), 0.64 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.1, 166.5, 155.9, 143.9, 132.9, 131.5,

126.0, 125.1, 101.8, 95.1, 93.3, 70.3, 56.6, 54.9, 51.3, 51.1, 50.6, 44.6, 41.4, 28.6, 27.1, 7.6; HRMS (ESI) m/z calculated for $C_{22}H_{26}BrN_2O_3$ $[M+H]^+$ 445.1127, found 445.1129.

Jerantinine A (13) To the solution of **12** (160 mg, 0.36 mmol) dissolved in 5 mL of THF was added *n*-BuLi (0.33 mL, 0.79 mmol) at -78 °C. The reaction mixture was stirred at this temperature for 1 h. To the above mixture was then added $B(OMe)_3$ (0.16 mL, 1.44 mmol) at -78 °C. The mixture was stirred and allowed to slowly warm up to room temperature. After an additional 1 h of stirring at room temperature, the reaction was quenched with 10 mL of saturated aq. NH_4Cl. The organic components were extracted with ethyl acetate (2 × 10 mL), washed with brine (2 × 10 mL), and dried over Na_2SO_4. The organic fraction was concentrated, dried under vacuum, and redissolved in 4 mL of THF. For the subsequent oxidation step, 1.5 mL of 10% aq. NaOH solution and 0.4 mL of 30% aq. H_2O_2 at 0 °C were added to the solution. After stirring at 0 °C for 1 h, the reaction mixture was quenched with 10 mL of aq. $Na_2S_2O_3$ and neutralized with saturated aq. NH_4Cl. The organic components were extracted with ethyl acetate (2 × 5 mL), washed with brine (2 × 10 mL), and dried over anhydrous Na_2SO_4. The resulting organic fraction was finally concentrated under reduced pressure, and the residue was purified via flash column chromatography using 20-30% ethyl acetate/hexane to afford **13** (65 mg, 47% yield over two steps) as a pale yellow, amorphous solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.86 (s, 1H), 6.88 (s, 1H), 6.45 (s, 1H), 5.78 (ddd, *J* = 10.0, 5.0, 1.5 Hz, 1H), 5.70 (d, *J* = 10.0 Hz, 1H), 5.28 (s, 1H), 3.87 (s, 3H), 3.76 (s, 3H), 3.44 (dd, *J* = 16.0, 4.0 Hz, 1H), 3.15 (d, *J* = 16.0 Hz, 1H), 3.02 (t, *J* = 6.0 Hz, 1H), 2.67 (m, 1H), 2.59 (s, 1H), 2.53 (dd, *J* = 15.0, 2.0 Hz, 1H), 2.41 (d, *J* = 15.0 Hz, 1H), 2.05 (m, 1H), 1.76 (dd, *J* = 11.5, 4.5 Hz, 1H), 0.98 (0, 1H), 0.86 (m, 1H), 0.63 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.2, 168.0, 146.0, 140.0, 136.2, 133.2, 130.2,

125.1, 108.9, 94.6, 91.9, 70.3, 56.5, 55.4, 51.1, 51.0, 50.8, 44.5, 41.6, 28.5, 27.0, 7.6; HRMS (ESI) m/z calculated for $C_{22}H_{27}N_2O_4$ $[M+H]^+$ 383.1971, found 383.1968.

15-Iodo-16-OMe tabersonine (14) To the solution of **11** (796 mg, 2.20 mmol) in 10 mL of DCM was added TFA (1.8 mL, 21.8 mmol) and NIS (587 mg, 2.61 mmol) at room temperature. The reaction was stirred at room temperature for 3 h and quenched with saturated $NaHCO_3$ aqueous solution (50 mL). After extraction by DCM (10 mL × 3), the organic phase was washed with brine (20 mL) and dried over Na_2SO_4. After vacuum concentration, the residue was purified via flash column chromatography eluting with 20-30% ethyl acetate/hexane to afford **14** (651 mg, 60%) as a pale yellow, amorphous solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.01 (s, 1H), 7.52 (s, 1H), 6.41 (s, 1H), 5.77 (m, 1H), 5.68 (d, *J* = 10.0 Hz, 1H), 3.84 (s, 3H), 3.76 (s, 3H), 3.43 (m, 1H), 3.18 (d, *J* = 16.0 Hz, 1H), 3.02 (m, 1H), 2.66 (m, 1H), 2.58 (s, 1H), 2.52 (dd, *J* = 15.0, 2.0 Hz, 1H), 2.39 (d, *J* = 15.0 Hz, 1H), 2.03 (m, 1H), 1.78 (m, 1H), 0.97 (m, 1H), 0.84 (m, 1H), 0.64 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.0, 166.4, 158.1, 145.0, 132.9, 132.5, 131.6, 125.0, 94.3, 93.2, 73.7, 70.2, 56.7, 54.6, 51.2, 51.0, 50.6, 44.6, 41.3, 28.5, 27.1, 7.6; HRMS (ESI) m/z calculated for $C_{22}H_{26}IN_2O_3$ $[M+H]^+$ 493.0988, found 493.0999.

15-Bpin-16-OMe tabersonine (15) To the solution of compound **14** (100 mg, 0.2 mmol) in 4 mL of DMF, was added KOAc (199 mg, 2.0 mmol), B_2pin_2 (254 mg, 1.0 mmol), and $Pd(dppf)Cl_2$ (34 mg, 0.04 mmol) at room temperature under argon. The mixture was further purged with argon, heated to 80 °C, and stirred at 80 °C overnight under argon. The reaction mixture was diluted with ethyl acetate (10 mL), filtered through Celite, washed with brine (10 mL), and dried over Na_2SO_4. The organic phase was concentrated under reduced pressure, and the residue was purified via flash column chromatography eluting with 20-40% ethyl acetate/hexane to afford **15** (67 mg, 67%) as a white, amorphous solid. ^{1}H NMR (500 MHz,

$CDCl_3$) δ 9.06 (s, 1H), 7.43 (s, 1H), 6.38 (s, 1H), 5.78 (ddd, *J* = 10.0, 5.0, 1.5 Hz, 1H), 5.72 (d, *J* = 10.0 Hz, 1H), 3.80 (s, 3H), 3.76 (s, 3H), 3.46 (ddd, *J* = 16.0, 5.0, 1.5 Hz, 1H), 3.26 (d, *J* = 16.0 Hz, 1H), 3.02 (t, *J* = 7.5 Hz, 1H), 2.76 (m, 1H), 2.69 (s, 1H), 2.53 (dd, *J* = 15.0, 2.0 Hz, 1H), 2.42 (d, *J* = 15.0 Hz, 1H), 2.00 (m, 1H), 1.76 (dd, *J* = 11.5, 4.5 Hz, 1H), 1.34 (s, 12H), 0.97 (m, 1H), 0.81 (m, 1H), 0.63 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.1, 167.2, 165.9, 147.4, 133.1, 129.6, 129.1, 125.0, 93.6, 93.0, 83.2, 70.0, 56.3, 54.5, 51.2, 51.0, 50.8, 44.8, 41.7, 28.1, 26.8, 25.0, 24.9, 7.6; HRMS (ESI) m/z calculated for $C_{28}H_{38}IN_2O_5$ $[M+H]^+$ 493.2874, found 493.2870.

Jerantinine A (13) Compound **15** (140 mg, 0.28 mmol) was dissolved in 4 mL of THF, and then 2.8 mL of aq. NaOH (1M) and 0.3 mL of 30% aq. H_2O_2 were added at 0 °C. The reaction mixture was stirred at 0 °C for 1 h and then neutralized with saturated aq. NH_4Cl. The organic components were extracted with ethyl acetate (2 × 10 mL), washed with brine (2 × 10 mL), and dried over anhydrous Na_2SO_4. The organic phase was concentrated under reduced pressure, and the residue was purified via flash column chromatography eluting with 40-70% ethyl acetate/hexane to afford **13** (76 mg, 71% yield) as a pale yellow, amorphous solid.

15,16-Dimethoxytabersonine (16) Compound **13** (100 mg, 0.26 mmol) was dissolved in 2 mL of THF/H_2O (1:1 v/v). Then 0.52 mL of aq. NaOH (1 M) and 30 µL of dimethyl sulfate (0.31 mmol) were added at room temperature. The reaction was stirred at room temperature for 1 h, followed by quenching with 10 mL of saturated aq. NH_4Cl. The organic components were extracted with ethyl acetate (2 × 10 mL), washed with brine (2 × 10 mL), and dried over anhydrous Na_2SO_4. The organic fraction was concentrated under reduced pressure, and the residue was purified via flash column chromatography eluting with 15-30% ethyl acetate/hexane to afford compound **16** (35 mg, 34% yield) as a yellow, amorphous solid. ^{1}H NMR (500 MHz,

$CDCl_3$) δ 8.87 (s, 1H), 6.84 (s, 1H), 6.48 (s, 1H), 5.78 (ddd, *J* = 10.0, 5.0, 1.5 Hz, 1H), 5.71 (dt, *J* = 10.0, 2.0 Hz, 1H), 3.86 (s, 6H), 3.76 (s, 3H), 3.45 (ddd, *J* = 16.0, 5.0, 1.5 Hz, 1H), 3.21 (d, *J* = 16.0 Hz, 1H), 3.04 (t, *J* = 7.0 Hz, 1H), 2.70 (m, 1H), 2.63 (s, 1H), 2.54 (d, *J* = 15.0, 1H), 2.40 (d, *J* = 15.0 Hz, 1H), 2.06 (m, 1H), 1.79 (m, 1H), 1.01 (m, 1H), 0.86 (m, 2H), 0.64 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.2, 167.9, 149.4, 143.8, 137.2, 133.2, 129.2, 124.8, 107.5, 95.4, 92.0, 70.3, 57.3, 56.4, 55.6, 51.2, 50.6, 44.5, 41.3, 28.7, 27.2, 7.6; MS (ESI) m/z calculated $C_{23}H_{29}N_2O_4$ for $[M+H]^+$ 397.2, found 397.3.

Cell line screening

The parent compound tabersonine (**1**) was prepared in DMSO/glycerol at a v/v ratio of 9:1 to make a stock solution with the concentration of 40 mM. It was then submitted to the Developmental Therapeutics Program sponsored by the National Cancer Institute and was evaluated using the NCI-60 Human Tumor Cell Lines Screen platform. Generally, the compound was serially diluted to 2x solutions with the complete medium (RPMI 1640 media containing 5% FBS, 2 mM L-glutamine, and 50 μg/mL gentamicin). It was then incubated with each of the human cancer cell lines (60 in total) at the final compound concentrations of 100 μM, 10 μM, 1 μM, 0.1 μM, and 0.01 μM. Each cell line was plated 24 h before the assay at the optimal density (5,000–40,000 cells/well) and three wells (in triplicate) were fixed in situ with TCA to measure the cell population at time zero (right before compound addition). After the addition of compounds, the sample plates were incubated for 48h at 37 °C, 5% CO_2. Cold TCA was then added to each sample mixture and was incubated for 60 min at 4 °C to quench the assay and fix the cells. The supernatant was removed, and the plate was washed five times with Milli-Q water. To the air-dried plate was finally added 0.4% w/v sulforhodamine B (SRB) solution with 1% acetic acid at 100 μL/well, followed by washing with 1% acetic acid. The protein-

bound stain was eventually solubilized by 10 mM trizma base, with the absorbance at the wavelength of 515 nm measured on a plate reader. The resulting data were reported as the cell growth relative to the non-drug control (normalized as 100% positive control) and relative to the number of cells at time zero (as 0% negative control). The mean graph of the percent growth of treated cells was shown in Figure S1, and the quantitative data were summarized in Figure S2.

Prostate cancer growth inhibition assay

PC-3 cells (ATCC) were seeded in a 96 well plate at a density of 10,000 cells per well in DMEM/F-12 media supplemented with 10% FBS, 100 IU/mL penicillin, and 100 μg/mL streptomycin. About 24 hours after seeding, each well was treated with one of nine experimental compounds at final concentrations that were serially diluted ranging from 73 μM to 0.018 μM with a final DMSO concentration of 1% (v/v). Cells were incubated after treatment for 5 days at 37 °C with 5% CO_2. After 5 days, the number of metabolically active/ viable cells was determined by measuring the concentration of ATP using the CellTiter-Glo® Luminescent Cell Viability Assay (Promega). Briefly, 30 μL CellTiter-Glo® reagent was added to each well. The plates were vigorously shaken for 2 minutes, and the luminescent signal was measured on a BioTek Synergy H1 microplate reader. Signals were normalized to the vehicle control (100%) and nonlinear regression was used to fit a curve to the data and calculate EC_{50} values (GraphPad Prism).

Tubulin polymerization inhibition assay

The effects of the representative compounds on tubulin polymerization were assessed using a fluorescence-based assay kit (Cytoskeleton Inc. Cat. # BK011P) following assay conditions for inhibitor detection. Briefly, compound stocks were dissolved in DMSO and added to a 96-well

plate. Purified porcine tubulin and the fluorescent polymerization reporter in reaction buffer were added to the wells to yield a final concentration of 50 μM compound and 5% (v/v) DMSO. The well plate was shaken for 5 seconds, and the fluorescent signal was monitored for 80 minutes at 37 °C on a BioTek Synergy H1 microplate reader.

Conflicts of interest

There are no conflicts to declare.

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