

***C. elegans*-based screen identifies lysosome-damaging alkaloids that induce
STAT3-dependent lysosomal cell death**

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Experiment Section

Extraction and Identification of Natural Compounds—All alkaloids used in this study were isolated from *E. chinensis* by the investigators involved in this research and compound structures were determined by means of 1D and 2D NMR as well as MS.

The extraction and isolation procedures are as follows: the powder of *E. chinensis* (7.5 g) were extracted three times with EtOH–H₂O (95:5, v/v). The combined extracts were concentrated under reduced pressure, followed by partitioning between EtOAc and 3% tartaric acid. The aqueous phase was adjusted to pH 9–10 with saturated Na₂CO₃ and then extracted with CHCl₃ to give crude alkaloids (40.0 g). The crude alkaloid extract was then subjected to a silica gel CC (300–400 mesh) using a petroleum ether–Me₂CO gradient (1:0–0:1) to obtain four major fractions A–D. Fraction B (3.8 g) was further purified by silica gel CC (300–400 mesh) with petroleum ether–Me₂CO–Et₂NH (10:1:0.2–4:1:0.2, v/v), then followed by Sephadex LH-20 (MeOH) to afford ervachinine A (20.0 mg), ervachinine C (25.0 mg), and decarbomethoxyvoacamine (60.0 mg). Fraction D (4.4 g) was further purified by reversed phase chromatography on a C18 column (MeOH–H₂O, 20:80–100:0) to give three subfractions (D1–D3). Subfraction D2 (1.2 g) was subjected to silica gel CC eluted with petroleum ether–EtOAc (10:1–3:1, v/v), then followed by Sephadex LH-20 CC (MeOH) to afford ervachinine B (17.0 mg), and ervachinine D (17.5 mg). Subfraction D3 (1.0 g) was further purified by silica gel eluting with petroleum ether–Me₂CO (8:1–4:1, v/v), and then Sephadex LH-20 (CHCl₃–MeOH, 1:1) to yield tabernaecorymbosine A (30.0 mg).

HEC-16 (Tabernaecorymbosine A): ¹H-NMR (500 MHz, CD₃OD) δ (ppm): 5.30 (1H, dd, *J* = 13.0, 3.5 Hz, H-3), 3.81 (1H, t, *J* = 9.0 Hz, H-5), 3.43 (2H, dd, *J* = 15.8, 3.5 Hz, H-6), 7.62 (1H, d, *J* = 7.5 Hz, H-9), 3.80 (1H, d, *J* = 11.5 Hz, H-17a), 3.63 (1H, d, *J* = 11.5 Hz, H-17b), 1.64 (3H, d, *J* = 7.5 Hz, Me-18), 5.33 (1H, q, *J* = 7.0 Hz, H-19), 2.88 (1H, d, *J* = 14.0 Hz, H-21a), 3.52 (1H, d, *J* = 14.0 Hz, H-21b), 2.35 (3H, s, Me-COOMe), 2.55 (3H, s, Me-NMe), 7.15 (1H, d, *J* = 8.0 Hz, H-9'), 6.81 (1H, d, *J* = 8.0 Hz, H-10'), 0.81 (3H, t, *J* = 7.5 Hz, H-18'), 1.29 (2H, q, *J* = 7.5, 1.42 Hz, H-19'), 3.36 (1H, s, H-21'), 3.66 (3H, s, Me-COOMe'), 3.95 (3H, s, Me-11'); ¹³C-NMR (125 MHz, CD₃OD) δ (ppm): 138.8 (C-2), 36.7 (C-3), 61.8 (C-5), 19.0 (C-6), 110.4 (C-7), 130.9 (C-8), 118.9 (C-9), 119.8 (C-10), 122.7 (C-11), 111.1 (C-12), 138.3 (C-13), 35.4 (C-14), 36.3 (C-15), 54.1 (C-16), 70.2 (C-17), 12.3 (C-18), 120.6 (C-19), 138.4 (C-20), 52.9 (C-21), 50.6 (Me-COOMe), 175.0 (CO-COOMe), 42.6 (C-NMe), 136.5 (C-2'), 52.9 (C-3'), 54.2 (C-5'), 22.7 (C-6'), 110.0 (C-7'), 125.7 (C-8'), 117.7 (C-9'), 106.4 (C-10'), 153.3 (C-11'), 116.4 (C-12'), 136.7 (C-13'), 28.6 (C-14'), 33.0 (C-15'), 55.6 (C-16'), 35.7 (C-17'), 11.9 (C-18'), 27.6 (C-19'), 40.0 (C-20'), 57.5 (C-21'), 52.9 (Me-COOMe'), 175.9 (CO-COOMe'), 57.4 (C-OMe'). ESI-MS: *m/z* 735 [M + H]⁺.

HEC-19 (Ervachinine A): ¹H-NMR (500 MHz, CDCl₃) δ (ppm): 5.07 (1H, br d, *J* = 13.0, H-3), 3.99 (1H, t, *J* = 8.0 Hz, H-5), 3.48 (1H, dd, *J* = 13.8, 9.6 Hz, H-6a), 3.26 (1H, dd, *J* = 13.8, 8.0 Hz, H-6b), 7.46 (1H, br d, *J* = 7.5 Hz, H-9), 7.01 (1H, m, H-10), 7.00 (1H, m, H-11), 6.97 (1H, m, H-12), 2.42 (1H, m, H-14a), 1.95 (1H, m, H-14b),

3.43 (1H, m, H-15), 3.69 (1H, d, $J = 11.0$ Hz, H-17a), 3.46 (1H, d, $J = 11.0$ Hz, H-17b), 1.58 (3H, d, $J = 8.0$ Hz, H-18), 5.27 (2H, q, $J = 8.0$ Hz, H-19), 3.58 (1H, d, $J = 11.0$ Hz, H-21a), 3.04 (1H, d, $J = 11.0$ Hz, H-21b), 7.62 (1H, br s, NH), 2.32 (3H, s, OMe), 2.59 (3H, s, NMe), 2.81 (1H, br d, $J = 10.0$ Hz, H-3'a), 2.64 (1H, br d, $J = 10.0$ Hz, H-3'b), 3.30 (1H, m, H-5'a), 3.07 (1H, ddd, $J = 18.0, 12.0, 6.0$ Hz, H-5'b), 3.02 (1H, br d, $J = 9.6, 6.0$ Hz, H-6'a), 2.90 (1H, br d, $J = 9.6, 6.0$ Hz, H-6'b), 6.85 (1H, s, H-9'), 6.59 (1H, s, H-12'), 1.69 (1H, m, H-15'a), 1.02 (1H, m, H-15'b), 2.38 (1H, br d, $J = 16.5$ Hz, H-17'a), 1.66 (1H, br d, $J = 16.5$ Hz, H-17'b), 0.80 (3H, t, $J = 8.0$ Hz, H-18'), 1.56 (1H, m, H-19'a), 1.34 (1H, m, H-19'b), 1.19 (1H, m, H-20'), 3.44 (1H, br s, H-21'), 3.91 (3H, s, 10'-OMe), 3.54 (3H, s, OMe). ^{13}C -NMR (125 MHz, CDCl_3) δ (ppm): 138.0 (C-2), 37.2 (C-3), 60.0 (C-5), 17.4 (C-6), 109.9 (C-7), 129.8 (C-8), 117.5 (C-9), 118.9 (C-10), 120.4 (C-11), 110.2 (C-12), 136.0 (C-13), 36.4 (C-14), 35.7 (C-15), 52.0 (C-16), 70.3 (C-17), 12.2 (C-18), 121.7 (C-19), 136.1 (C-20), 51.8 (C-21), 50.4 (*Me*-COOMe), 173.9 (*CO*-COOMe), 42.0 (C-NMe), 137.2 (C-2'), 51.9 (C-3'), 53.0 (C-5'), 22.1 (C-6'), 110.2 (C-7'), 127.3 (C-8'), 99.1 (C-9'), 150.1 (C-10'), 129.9 (C-11'), 110.4 (C-12'), 130.2 (C-13'), 27.3 (C-14'), 31.9 (C-15'), 54.8 (C-16'), 36.4 (C-17'), 11.6 (C-18'), 26.7 (C-19'), 38.9 (C-20'), 57.0 (C-21'), 52.4 (*Me*-COOMe'), 175.2 (*CO*-COOMe'), 56.0 (C-OMe'). ESI-MS: m/z 735 [$\text{M} + \text{H}$] $^+$.

HEC-20 (Ervachinine C): ^1H -NMR (400 MHz, CD_3OD) δ (ppm): 5.11 (1H, br d, $J = 12.8$, H-3), 3.74 (1H, t, $J = 10.4$ Hz, H-5), 3.51 (1H, dd, $J = 16.2, 7.2$ Hz, H-6a), 3.23 (1H, dd, $J = 16.2, 8.4$ Hz, H-6b), 7.51 (1H, dd, $J = 7.0, 2.5$ Hz, H-9), 6.98 (1H, m, H-10), 6.98 (1H, m, H-11), 7.10 (1H, dd, $J = 7.2, 2.8$ Hz, H-12), 2.48 (1H, m, H-14a), 2.00 (1H, m, H-14b), 3.62 (1H, dd, $J = 12.0, 5.6$ Hz, H-15), 3.78 (1H, d, $J = 10.8$ Hz, H-17a), 3.56 (1H, d, $J = 10.8$ Hz, H-17b), 1.65 (3H, d, $J = 6.0$ Hz, H-18), 5.29 (2H, q, $J = 6.0$ Hz, H-19), 3.48 (1H, d, $J = 10.0$ Hz, H-21a), 2.75 (1H, d, $J = 10.0$ Hz, H-21b), 2.28 (3H, s, OMe), 2.39 (3H, s, NMe), 3.10 (1H, dd, $J = 13.2, 7.2$ Hz, H-3'a), 2.81 (1H, dd, $J = 13.2, 6.4$ Hz, H-3'b), 2.66 (1H, dd, $J = 9.6, 7.2$ Hz, H-5'a), 2.56 (1H, m, H-5'b), 2.71 (1H, t, $J = 7.2$ Hz, H-6'a), 2.59 (1H, t, $J = 7.2$ Hz, H-6'b), 6.70 (1H, s, H-9'), 6.83 (1H, s, H-12'), 1.55 (1H, m, H-14'), 1.59 (1H, m, H-15'a), 0.96 (1H, m, H-15'b), 2.55 (1H, br d, $J = 14.0$ Hz, H-17'a), 1.71 (1H, br d, $J = 14.0$ Hz, H-17'b), 0.81 (3H, t, $J = 7.2$ Hz, H-18'), 1.42 (1H, m, H-19'a), 1.32 (1H, m, H-19'b), 1.24 (1H, m, H-20'), 3.40 (1H, br s, H-21'), 3.92 (3H, s, 11'-OMe), 3.62 (3H, s, OMe). ^{13}C -NMR (100 MHz, CD_3OD) δ (ppm): 139.7 (C-2), 39.1 (C-3), 62.1 (C-5), 18.7 (C-6), 111.3 (C-7), 131.3 (C-8), 118.4 (C-9), 119.2 (C-10), 122.0 (C-11), 111.0 (C-12), 138.0 (C-13), 38.6 (C-14), 36.4 (C-15), 54.0 (C-16), 70.3 (C-17), 12.4 (C-18), 120.3 (C-19), 138.7 (C-20), 52.9 (C-21), 50.3 (*Me*-COOMe), 174.6 (*CO*-COOMe), 42.5 (C-NMe), 136.7 (C-2'), 54.5 (C-3'), 53.7 (C-5'), 22.7 (C-6'), 110.4 (C-7'), 123.5 (C-8'), 118.3 (C-9'), 128.5 (C-10'), 154.5 (C-11'), 93.9 (C-12'), 136.6 (C-13'), 28.6 (C-14'), 33.0 (C-15'), 56.1 (C-16'), 36.9 (C-17'), 12.1 (C-18'), 27.8 (C-19'), 39.9 (C-20'), 58.0 (C-21'), 52.8 (*Me*-COOMe'), 176.4 (*CO*-COOMe'), 56.2 (C-OMe'). ESI-MS: m/z 735 [$\text{M} + \text{H}$] $^+$.

HEC-21 (Ervachinine D): ^1H -NMR (400 MHz, CD_3OD) δ (ppm): 5.13 (1H, br d, $J = 13.0$, H-3), 3.78 (1H, t, $J = 11.2$ Hz, H-5), 3.56 (1H, m, H-6a), 3.26 (1H, dd, $J = 14.8, 8.0$ Hz, H-6b), 7.53 (1H, br d, $J = 7.0$ Hz, H-9), 7.00 (1H, m, H-10), 7.00 (1H, m,

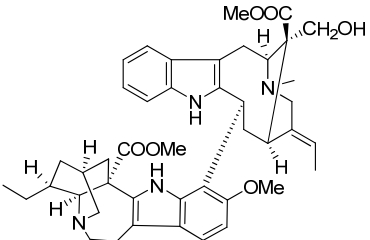
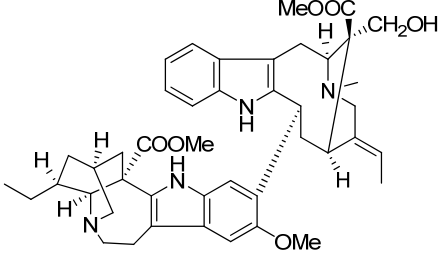
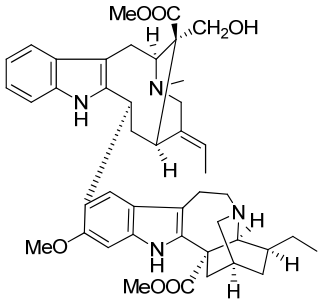
H-11), 7.10 (1H, dd, $J = 7.2, 2.4$ Hz, H-12), 2.51 (1H, m, H-14a), 2.01 (1H, m, H-14b), 3.63 (1H, dd, $J = 11.6, 6.8$ Hz, H-15), 3.81 (1H, d, $J = 12.0$ Hz, H-17a), 3.60 (1H, d, $J = 12.0$ Hz, H-17b), 1.69 (3H, d, $J = 6.4$ Hz, H-18), 5.36 (2H, q, $J = 6.4$ Hz, H-19), 3.57 (1H, d, $J = 13.2$ Hz, H-21a), 2.87 (1H, d, $J = 13.2$ Hz, H-21b), 2.31 (3H, s, OMe), 2.49 (3H, s, NMe), 2.90 (1H, br d, $J = 8.4$ Hz, H-3'a), 2.75 (1H, br d, $J = 8.4$ Hz, H-3'b), 3.04 (1H, br d, $J = 13.2$ Hz, H-5'a), 2.82 (1H, br d, $J = 13.2$ Hz, H-5'b), 3.00 (1H, m, H-6'a), 2.20 (1H, m, H-6'b), 6.67 (1H, s, H-9'), 6.83 (1H, s, H-12'), 1.69 (1H, m, H-14'), 1.79 (1H, m, H-15'a), 1.12 (1H, m, H-15'b), 2.89 (1H, br d, $J = 8.4$ Hz, H-16'), 2.01 (1H, br d, $J = 12.4$ Hz, H-17'a), 1.48 (1H, br d, $J = 12.4$ Hz, H-17'b), 0.88 (3H, t, $J = 6.8$ Hz, H-18'), 1.48 (1H, q, $J = 6.8$ Hz, H-19'a), 1.42 (1H, q, $J = 6.8$ Hz, H-19'b), 1.53 (1H, m, H-20'), 2.70 (1H, br s, H-21'), 3.95 (3H, s, 11'-OMe), $^{13}\text{C-NMR}$ (100 MHz, CD_3OD) δ (ppm): 139.9 (C-2), 39.1 (C-3), 62.1 (C-5), 18.7 (C-6), 111.2 (C-7), 131.4 (C-8), 118.2 (C-9), 119.2 (C-10), 122.0 (C-11), 111.0 (C-12), 138.0 (C-13), 38.7 (C-14), 36.4 (C-15), 54.1 (C-16), 70.3 (C-17), 12.3 (C-18), 120.2 (C-19), 138.9 (C-20), 53.1 (C-21), 50.4 (*Me*-COOMe), 174.5 (*CO*-COOMe), 42.6 (C-*NMe*), 141.5 (C-2'), 50.6 (C-3'), 55.4 (C-5'), 21.3 (C-6'), 108.6 (C-7'), 124.7 (C-8'), 118.1 (C-9'), 128.2 (C-10'), 154.0 (C-11'), 93.7 (C-12'), 135.6 (C-13'), 27.7 (C-14'), 33.0 (C-15'), 41.9 (C-16'), 35.3 (C-17'), 12.3 (C-18'), 28.5 (C-19'), 43.3 (C-20'), 59.4 (C-21'), 56.3 (C-OMe'). ESI-MS: m/z 677 [$\text{M} + \text{H}$] $^+$.

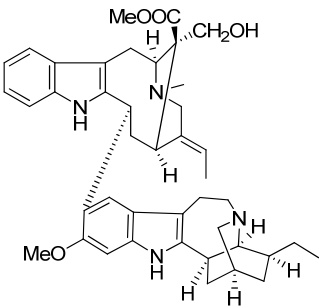
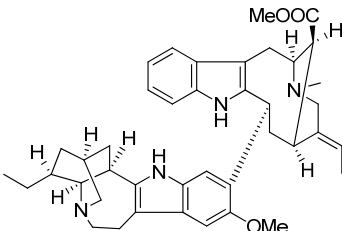
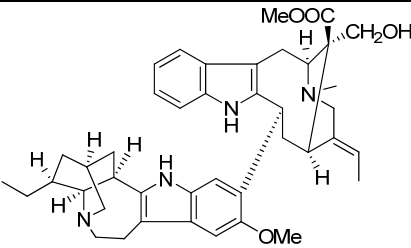
HEC-22 (Decarbomethoxyvoacamine): $^{13}\text{C-NMR}$ (150 MHz, CD_3OD) δ (ppm): 139.3 (C-2), 39.5 (C-3), 61.3 (C-5), 20.7 (C-6), 111.2 (C-7), 131.2 (C-8), 118.3 (C-9), 120.3 (C-10), 122.2 (C-11), 110.9 (C-12), 138.0 (C-13), 38.3 (C-14), 33.4 (C-15), 48.2 (C-16), 12.6 (C-18), 119.5 (C-19), 139.3 (C-20), 53.4 (C-21), 50.0 (*Me*-COOMe), 172.5 (*CO*-COOMe), 42.4 (C-*NMe*), 143.2 (C-2'), 49.7 (C-3'), 55.9 (C-5'), 21.6 (C-6'), 108.7 (C-7'), 129.4 (C-8'), 99.7 (C-9'), 152.2 (C-10'), 129.3 (C-11'), 111.2 (C-12'), 130.2 (C-13'), 27.6 (C-14'), 32.8 (C-15'), 41.7 (C-16'), 35.1 (C-17'), 12.1 (C-18'), 28.7 (C-19'), 42.4 (C-20'), 59.7 (C-21'), 56.6 (C-OMe'). ESI-MS: m/z 647 [$\text{M} + \text{H}$] $^+$.

HEC-23 (Ervachinine B): $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ (ppm): 5.13 (1H, br d, $J = 13.0$, H-3), 3.91 (1H, t, $J = 9.0$ Hz, H-5), 3.52 (1H, dd, $J = 15.0, 9.0$ Hz, H-6a), 3.27 (1H, dd, $J = 15.0, 6.6$ Hz, H-6b), 7.55 (1H, br d, $J = 6.0$ Hz, H-9), 7.06 (1H, m, H-10), 7.05 (1H, m, H-11), 7.08 (1H, br d, $J = 6.6$, H-12), 2.49 (1H, m, H-14a), 2.02 (1H, ddd, $J = 9.6, 6.6, 2.4$, H-14b), 3.51 (1H, t, $J = 9.6$, H-15), 3.71 (2H, t, $J = 12.0$ Hz, H-17), 1.66 (3H, d, $J = 6.0$ Hz, H-18), 5.37 (2H, q, $J = 6.0$ Hz, H-19), 3.57 (1H, d, $J = 12.0$ Hz, H-21a), 2.94 (1H, d, $J = 12.0$ Hz, H-21b), 7.67 (1H, br s, NH), 2.40 (3H, s, OMe), 2.55 (3H, s, NMe), 3.01 (1H, br d, $J = 9.0$ Hz, H-3'a), 2.95 (1H, br d, $J = 9.0$ Hz, H-3'b), 3.35 (1H, m, H-5'a), 3.08 (1H, dt, $J = 13.2, 4.2$ Hz, H-5'b), 3.30 (1H, t, $J = 4.2$ Hz, H-6'a), 2.59 (1H, br d, $J = 13.2$ Hz, H-6'b), 6.92 (1H, s, H-9'), 6.62 (1H, s, H-12'), 1.79 (1H, m, H-14'), 1.75 (1H, br t, $J = 11.4$, H-15'a), 1.16 (1H, m, H-15'b), 2.77 (1H, m, H-16'), 1.94 (1H, br t, $J = 12.0$ Hz, H-17'a), 1.50 (1H, m, H-17'b), 0.86 (3H, t, $J = 6.6$ Hz, H-18'), 1.50 (1H, m, H-19'a), 1.42 (1H, m, H-19'b), 1.50 (1H, m, H-20'), 2.76 (1H, br s, H-21'), 3.98 (3H, s, 10'-OMe). $^{13}\text{C-NMR}$ (100 MHz, CDCl_3) δ (ppm): 138.2 (C-2), 37.3 (C-3), 60.1 (C-5), 17.2 (C-6), 110.5 (C-7), 129.9 (C-8), 117.5 (C-9), 118.9 (C-10), 121.7 (C-11), 110.0 (C-12), 136.1 (C-13), 36.6 (C-14), 36.0 (C-15), 52.0 (C-16), 70.7 (C-17), 12.2 (C-18), 119.9 (C-19), 136.7 (C-20), 52.0

(C-21), 50.3 (*Me*-COOMe), 174.0 (*CO*-COOMe), 42.0 (C-*NMe*), 136.8 (C-2'), 49.9 (C-3'), 54.2 (C-5'), 20.7 (C-6'), 108.9 (C-7'), 128.3 (C-8'), 98.6 (C-9'), 150.9 (C-10'), 128.8 (C-11'), 110.3 (C-12'), 129.2 (C-13'), 26.4 (C-14'), 32.0 (C-15'), 41.3 (C-16'), 34.1 (C-17'), 11.9 (C-18'), 27.8 (C-19'), 41.9 (C-20'), 57.7 (C-21'), 55.9 (C-OMe').
ESI-MS: m/z 677 [M + H]⁺.

Supplementary Table 1. **HEC compounds induced lysosome enlargement and cell death in both *C. elegans* and mammalian cells.**

| Compounds | Name (CAS number) | Structure | % of coelomocytes with enlarged lysosomes | No. of cell corpses per gonad arm | % of HeLa cells with enlarged lysosomes | % of cell death in total cells |
|-----------|---|--|---|---|---|--------------------------------------|
| HEC-16 | Tabernaecorymbosine A (1262306-81-9) |  | 1.8 | 1.0 | 3.1 | 1.6 |
| HEC-19 | Ervachinine A (1346017-27-3) |  | 58.6 | 1.9 | 32.9 | 30.6 |
| HEC-20 | Ervachinine C (1346017-29-5) |  | 72.5 | 2.3 | 55.3 | 51.8 |

| | | | | | | |
|--------|---|---|------|-----|------|------|
| HEC-21 | Ervachinine D (1346017-30-8) |  | 90.3 | 8.3 | 70.5 | 72.3 |
| HEC-22 | Decarbomethoxyvoacamine (30890-52-9) |  | 1.5 | 1.2 | 3.8 | 2.1 |
| HEC-23 | Ervachinine B (1346017-28-4) |  | 97.2 | 9.5 | 78.6 | 83.5 |
| DMSO | | | 0.0 | 1.2 | 2.2 | 0.5 |

(>25 worms or >100 cells per group were scored.)