Regular Article

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Synthesis and Evaluation of Fuligocandin B Derivatives with Activity for Overcoming TRAIL Resistance

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The tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) signaling pathway induces apoptosis in cancer cells but not in normal cells. Therefore, this pathway has attracted attention regarding possible clinical treatment of cancer. However, many cancer cells demonstrate TRAIL resistance. To overcome this problem, small molecules that sensitize cancer cells to TRAIL are desired. Heterocyclic derivatives of the natural product, fuligocandin B (2), with activity for overcoming TRAIL resistance were synthesized, and their activity was evaluated. Of the synthetic molecules, the quinoline derivative (10g) showed potent activity against TRAIL-resistant gastric adenocarcinoma cells. After a docking study of the target protein valosin-containing protein, 7′-amino fuligocandin B (10m) was designed and synthesized. Compound 10m also showed good activity for overcoming TRAIL resistance. 10m produced a 49.7% difference in viability with TRAIL at 30 μM compared to without TRAIL. This activity was better than that of fuligocandin B (2).

Key words natural product; cancer; inhibitor; cytotoxicity; tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)

Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), a member of the TNF superfamily, induces apoptosis in tumor cells through the activation of caspases. TRAIL binds death receptors (DR4 or DR5), and then the death-inducing signaling complex (DISC) is formed, which activates caspases (Fig. 1). On the other hand, normal cells are usually protected from TRAIL signaling by decoy receptors such as DcR1 (TRAIL-R3) and DcR2 (TRAIL-R4). DcR1 lacks a cytoplasmic region, and DcR2 has a truncated nonfunctional cytoplasmic death domain. Because TRAIL can affect cancer cells selectively, the TRAIL signaling pathway is a very attractive therapeutic target.[1–3] However, many cancer cells acquire resistance to TRAIL signaling. Therefore, small molecules that can sensitize cancer cells to TRAIL are needed for cancer treatment.[4,5]

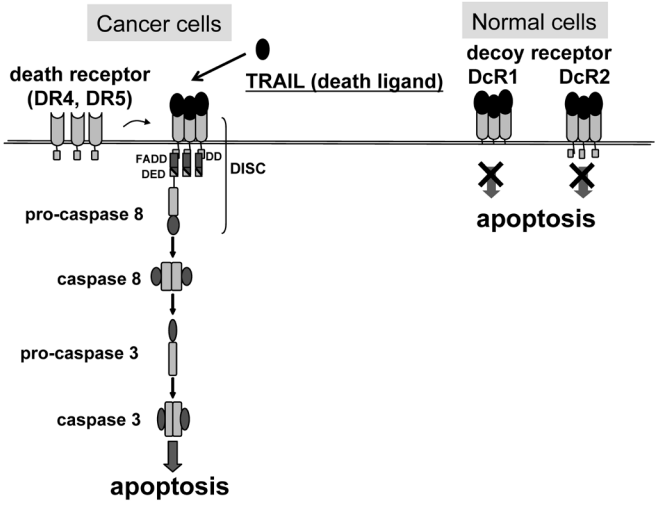

Fig. 1. Schematic Representation of the TRAIL Signaling Pathway

DR; death receptor, DcR; decoy receptor, FADD; Fas-associated protein with death domain, DD; death domain, DED; death effector domain, DISC; death-inducing signaling complex.

Fuligocandin A (**1**) and B (**2**) are novel natural cycloanthranilylproline derivatives that were isolated from the slime mold, *Fuligo candida*, in 2004 by our group[6] (Fig. 2). Fuligocandin B (**2**) has the ability to overcome TRAIL resistance in T-cell leukemia/lymphoma[7] and human gastric adenocarcinoma (AGS) cells,[8] however fuligocandin B (**2**) did not show the cytotoxicity against peripheral blood mononuclear cells.[7] Recently, we identified valosin-containing protein (VCP) as a target protein of 5′-I fuligocandin B (**3**) and elucidated the mechanism of the activity for overcoming TRAIL resistance.[9] 5′-I fuligocandin B (**3**) inhibits VCP function along with inducing accumulation of ubiquitinated proteins, which causes endoplasmic reticulum (ER) stress. The expression of DR5 is increased by accumulation of CCAAT/enhancer-binding protein (C/EBP) homologous protein (CHOP), a transcription factor for DR5, which is induced by ER stress. Therefore, TRAIL resistant cancer cells can catch the TRAIL by increased DR5, so that TRAIL induced cell death would be enhanced. Because substitution of the indole unit resulted in better activity, new derivatives were designed with various heterocyclic units instead of the indole unit to create more active compounds

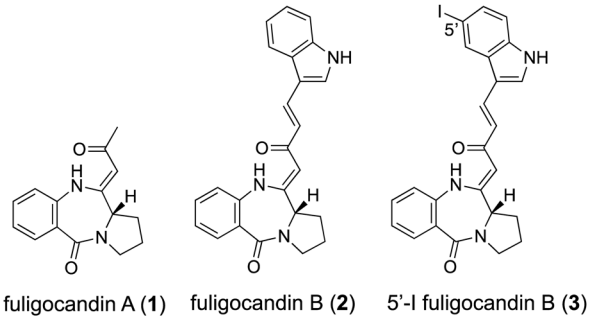

Fig. 2. The Structure of Fuligocandin A (**1**), Fuligocandin B (**2**) and 5′-I Fuligocandin B (**3**)

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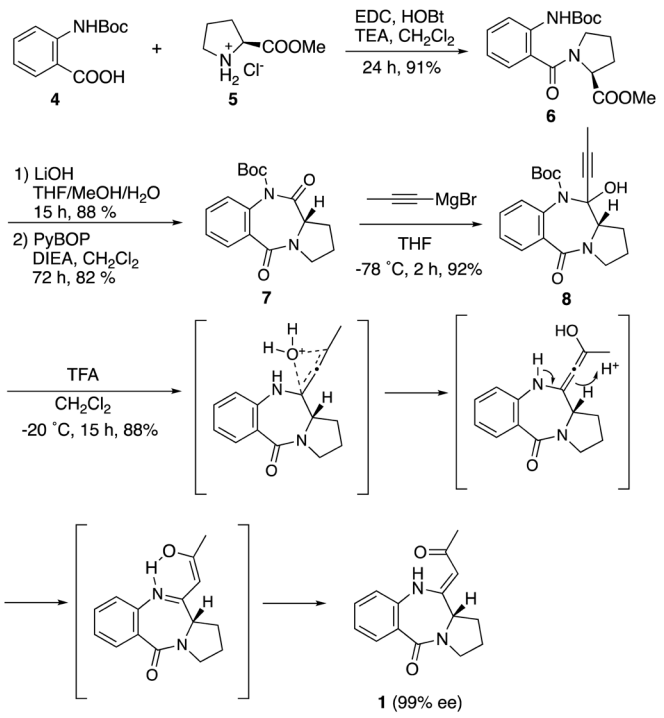


Chart 1. Synthesis of Fuligocandin A (**1**)

based on fuligocandin B (**2**). Here we report the synthesis of fuligocandin B derivatives and their activity for overcoming TRAIL resistance in AGS cells.

To synthesize new fuligocandin B derivatives, we performed several chemical steps to obtain optically pure fuligocandin A (**1**)[8,9] (Chart 1). Following our established protocol, a coupling reaction of *N*-*tert*-butoxycarbonyl (Boc)-anthranilic acid **4** and L-proline methyl ester (**5**) was performed. After hydrolysis of the methyl ester **6** with LiOH, intramolecular cyclization produced *N*-Boc-cycloanthranilylproline **7**. The reaction with propynylmagnesium bromide gave precursor **8** for the next reaction. The Meyer–Schuster rearrangement of **8** in trifluoroacetic acid (TFA)-containing solvent generated fuligocandin A (**1**) with 88% yield, 99% enantiomeric excess (ee), as a *Z*-isomer. The rearrangement proceeded through the corresponding hydroxyallene followed by protonation. Next, aldol condensation with various aldehydes was performed (Chart 2). A moderate yield (24%) was obtained for fuligocandin B (**2**) synthesis, and heterocyclic derivatives (**10a**–**k**) were synthesized in low yield (1–13%). We investigated many conditions such as other bases, solvents, and reaction temperatures and times but the yield remained low level. Despite this, we obtained a small library of heterocyclic fuligocandin B derivatives.

Next, the activity of these synthetic compounds for overcoming TRAIL resistance was evaluated (Fig. 3). When TRAIL-resistant AGS cells were treated with TRAIL only, the cells were not killed (control). When the cells were treated with TRAIL plus these synthesized compounds at 30 μM, the viability of AGS cells decreased in all cases. Compared to compound alone, the differences in viability with TRAIL plus the compounds (**10a**–**k**, **2**) were 47.1, 31.2, 23.3, 31.5, 31.8, 29.9, 51.6, 26.3, 28.9, 43.1, 42.3, and 33.9%, respectively. The quinoline derivative (**10g**) showed the strongest activity, with a 51.6% difference.

Because the quinoline ring resembles the indole ring, we next speculated about the structure based on simulation of docking of fuligocandin B (**2**) to VCP (Fig. 4). VCP has three domains: N, D1, and D2 (Fig. 4A), and exists as a hexamer. The D1 and D2 domains have ATP binding sites with a pocket for ATP. Although ATP binding sites of D2 domains were buried in the back on the model of VCP as a hexamer, ATP binding sites of D1 domains seems to be easy accessible from the upper side. According to the docking study, fuligocandin B appears to bind to the ATP binding site on the D1 domain (Fig. 4B). The docking analysis indicated a vacant space around the indole unit. If an additional hydrogen bond was available between Thr249 and additional atom on the indole unit, the derivative will have stronger binding to VCP and show better activity for overcoming TRAIL resistance. Interaction energy was shown in the Fig. 4D. When the NH_2 group was added at 7′ position, the energy became −56.2 kcal/mol, which indicated the complex of VCP with 7′-amino fuligocandin B (**10m**) is more stable than other complexes. To obtain more active compounds, we designed the molecule 7′-amino fuligocandin B (**10m**) with a nitrogen at the 7′ position.

As shown in Chart 3, 7-nitroindole-3-carbaldehyde (**9l**) was synthesized from 7-nitroindole (**11**) in 96% yield. 7-Aminoindole-3-carbaldehyde (**9m**) was obtained in reducing conditions from **9l**. Aldol condensation with fuligocandin A (**1**) gave the desired 7′-nitro fuligocandin B (**10l**) in 7% yield and 7′-amino fuligocandin B (**10m**) in 1% yield. The activity of **10l** and **10m** for overcoming TRAIL resistance was evaluated with AGS cells (Fig. 5). As we expected, both compounds showed better activity than that of fuligocandin B (**2**) (33.9%) at 30 μM. The activities of **10l** and **10m** at 30 μM were 34.9 and 49.7%, respectively.

Conclusion

In this article, we describe the synthesis of fuligocandin B derivatives with heterocycles. Eleven compounds were evaluated for their activity for overcoming TRAIL resistance in TRAIL-resistant AGS cells. Of the compounds, quinoline derivative (**10g**) showed good activity at 30 μM, with a 51.6% difference in viability between the presence and absence of TRAIL. From the docking study analysis, we speculated that 7′-amino fuligocandin B (**10m**), with an additional hydrogen bond, would be a good compound. Compound **10m** showed better activity for overcoming TRAIL resistance (49.7% difference) than fuligocandin B (33.9% difference) at 30 μM. These data suggest that other heterocyclic fuligocandin B derivatives may have significant potential for use as reagents with activity for overcoming TRAIL resistance.

Experimental

General Information NMR spectra were recorded on JEOL ECZ400 and ECZ600 spectrometers in a deuterated solvent whose chemical shift was taken as an internal standard. MS were obtained using AccuTOF LC-plus JMS-T100LP (JEOL). IR spectra were measured on attenuated total reflectance (ATR) on a JASCO FT-IR 230 spectrophotometer. Column chromatography was performed using silica gel PSQ100B (Fuji Silysia Chemical Ltd., Kasugai, Japan) and silica gel 60N (Kanto Chemical Co., Inc., Tokyo, Japan).

(*S*)-11-Hydroxy-5-oxo-11-prop-1-ynyl-2,3,11,11a-tetrahydro-1*H*,5*H*-benzo[*e*]pyrrolo[1,2-*a*][1,4]diazepine-10-car-

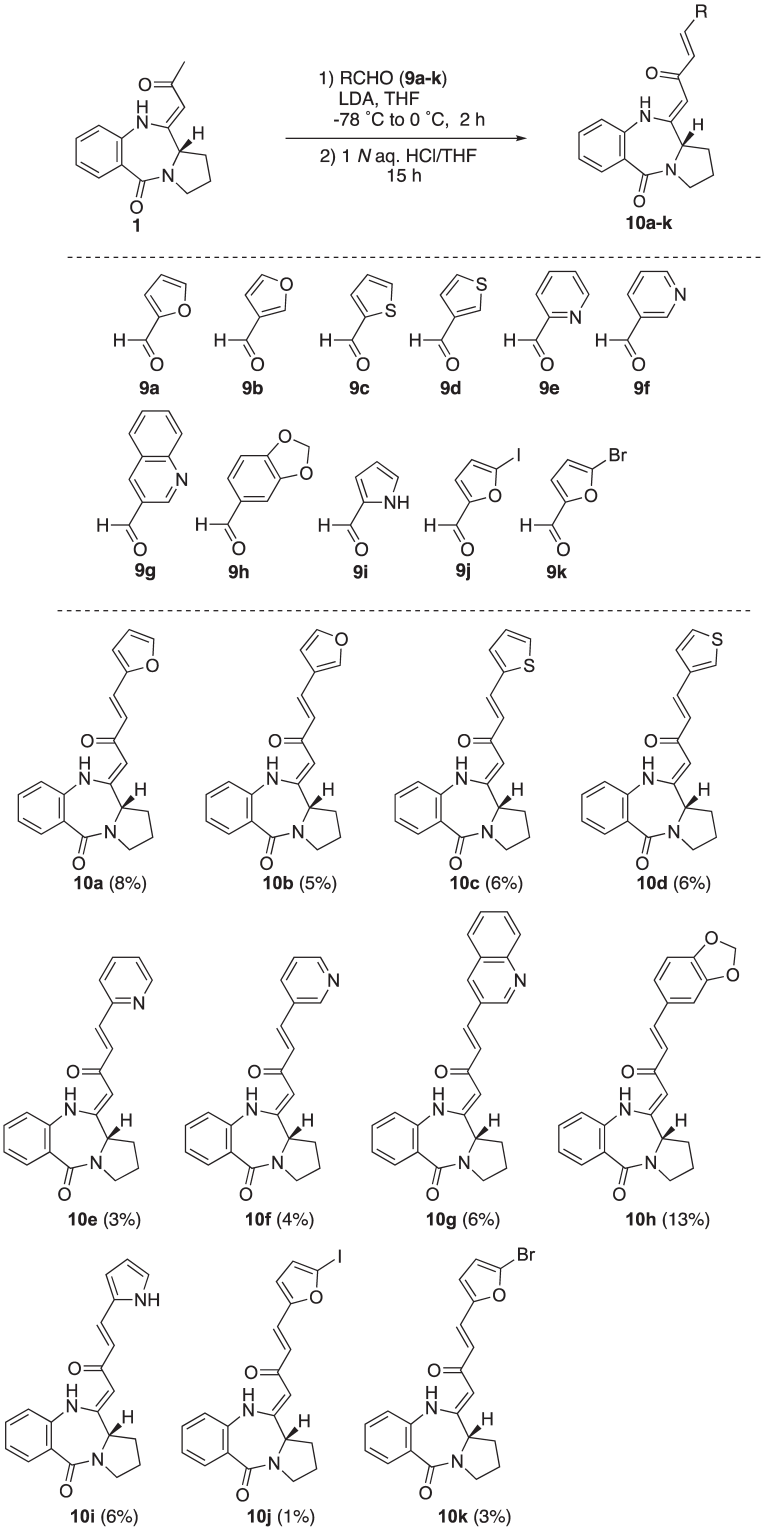


Chart 2. Synthesis of Heterocyclic Fuligocandin B Derivatives

boxylic Acid *tert*-Butyl Ester (8) To a solution of **7** (1.16 g, 3.67 mmol) in tetrahydrofuran (THF) (184 mL), propynyl magnesium bromide in THF (0.5 M, 11.0 mL, 5.50 mmol) was added at −78°C under an argon atmosphere. The mixture was stirred at −78°C for 60 min, and then saturated aq. NH_4Cl solution was added slowly. The mixture was extracted with EtOAc, and the organic extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was chromatographed on silica gel (hexane/EtOAc=2/1) to afford **8** (1.24 g, 3.49 mmol, 95%) as a white amorphous solid.

Spectral Data of **8**

$[\alpha]_D^{22}$ −51 (*c* 0.6, MeOH); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.42 (brs, 1H), 8.19 (d, *J*=8.4 Hz, 1H), 7.39–7.35 (m, 2H), 7.00 (dd, *J*=8.0, 6.8 Hz, 1H), 4.80 (dd, *J*=8.0, 6.4 Hz, 1H), 3.62–3.53 (m, 2H), 2.34–2.31 (m, 1H), 2.05 (s, 3H), 2.00–1.86 (m, 3H), 1.49 (s, 9H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 185.5,

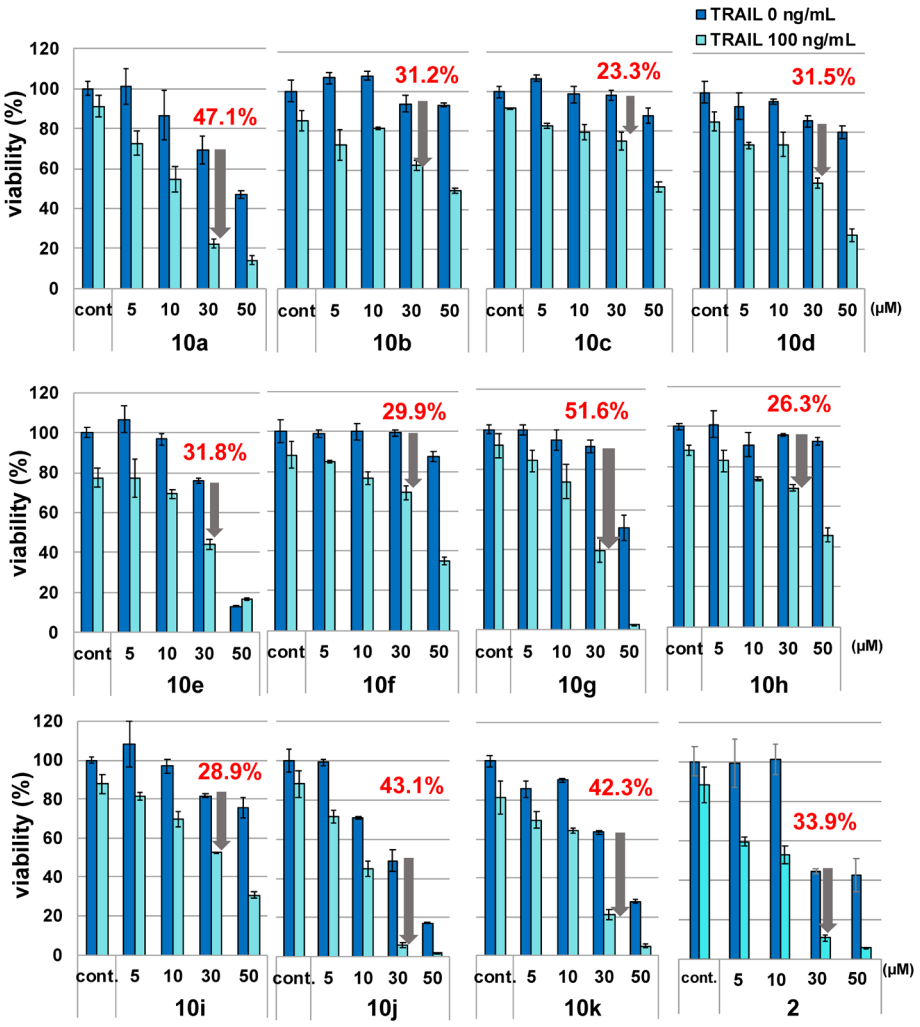
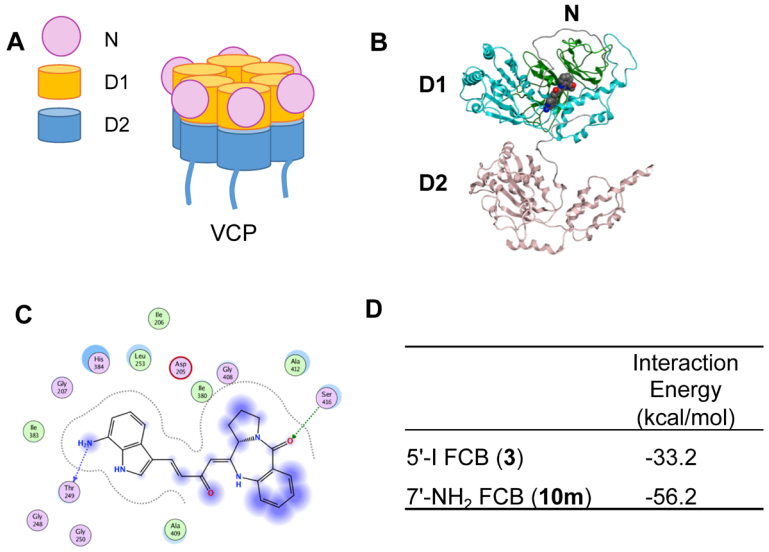


Fig. 3. Activity of Synthetic Compounds for Overcoming TRAIL Resistance

(Color figure can be accessed in the online version.)



	Interaction Energy (kcal/mol)
5'-I FCB (**3**)	-33.2
7'-NH_2 FCB (**10m**)	-56.2

Fig. 4. Docking Study of Fuligocandin B Derivative (**10m**) on VCP

A) Schematic representation of the VCP structure. B) Binding position of **10m** on VCP. C) An enlarged view of binding of **10m** to VCP in the ATP binding pocket of the D1 domain. D) Interaction energy of compounds with VCP. (Color figure can be accessed in the online version.)

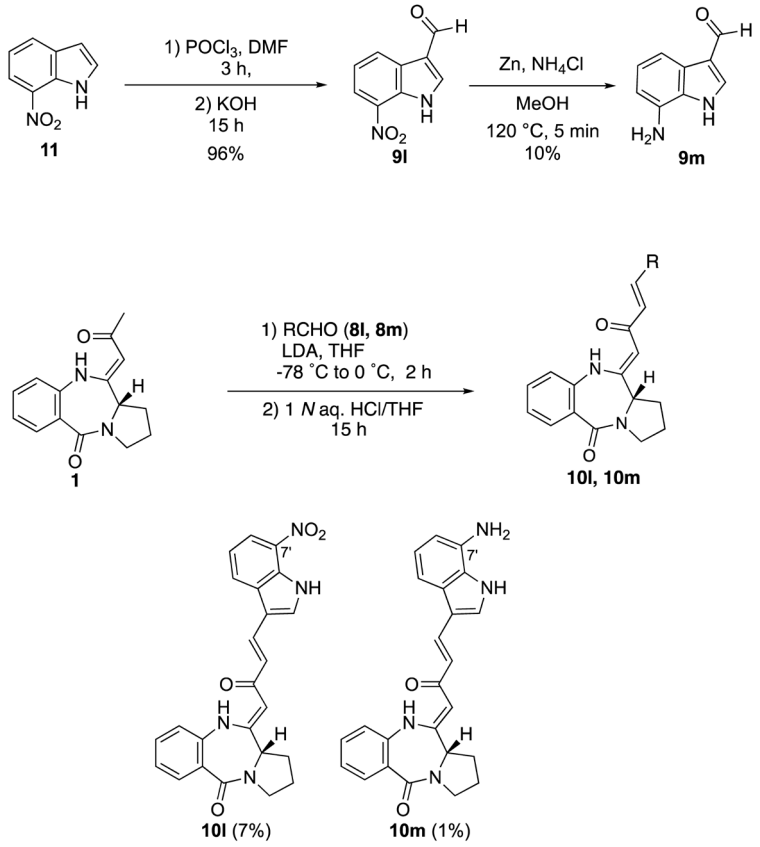


Chart 3. Synthesis of Heterocyclic Fuligocandin B Derivatives **10l** and **10m**

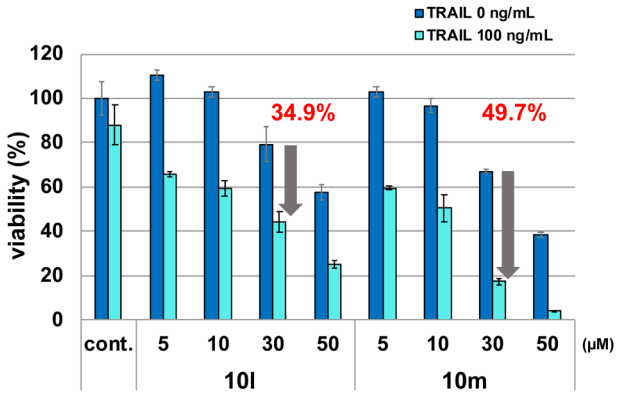


Fig. 5. Activity of **10l** and **10m** for Overcoming TRAIL Resistance
(Color figure can be accessed in the online version.)

168.8, 153.0, 137.6, 131.1, 127.3, 123.1, 121.5, 120.2, 92.9, 80.4, 78.1, 66.5, 46.8, 28.5, 28.3, 25.1, 4.3; IR (ATR) 3336, 2926, 2220, 1727, 1521, 1409, 1340, 1157 and 756 cm^{-1}; electrospray ionization-high resolution (ESI-HR)-MS Calcd 356.1736 [M^+]; Found 356.1730.

Fuligocandin A (1) To a solution of **8** (185 mg, 0.52 mmol) in CH_2Cl_2 (13 mL), TFA (13 mL) was added at −20°C. The reaction mixture was stirred for 8 h at −20°C. Toluene was then added, and the compound was concentrated *in vacuo*. Saturated aq. $NaHCO_3$ solution was then added. The mixture was stirred overnight at 0°C, and then allowed to warm to room temperature. The mixture was extracted with CH_2Cl_2, and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was chromatographed on silica gel (hexane/EtOAc=1/1) to afford **1** (123 mg, 0.48 mmol, 93%) as a yellow amorphous solid.

Spectral Data of **1**

$[\alpha]_D^{18}$ +225 (*c* 1.4, MeOH); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 12.63 (br s, 1H), 7.96 (dd, *J*=8.4, 1.6 Hz, 1H), 7.44 (td, *J*=8.4, 1.6 Hz, 1H), 7.21 (td, *J*=8.4, 1.4 Hz, 1H), 7.02 (dd, *J*=8.4, 1.4 Hz, 1H), 5.30 (s, 1H), 4.29 (dd, *J*=7.6, 2.0 Hz,1H), 3.84–3.80 (m, 1H), 3.68–3.61 (m, 1H), 2.45–2.38 (m, 1H), 2.20 (s, 3H), 2.16–2.04 (m, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 198.2, 165.7, 159.2, 136.9, 132.6, 131.2, 127.1, 124.6, 122.2, 91.1, 55.4, 47.0, 29.8, 27.0, 23.4; IR (ATR) 2987, 2877, 1629, 1591, 1557, 1406, 1262, 1250, 754, 733, 696 and 683 cm^{-1}; HR-EI-MS Calcd 256.1212 [M^+]; Found 256.1211.

General Procedure for Preparation of 10a–m To a solution of prepared lithium diisopropylamide (LDA) (1.95 mmol) in THF (3.9 mL), **1** (50.0 mg, 0.195 mmol) in THF was added *via* a cannula at −78°C under an argon atmosphere. The solution was stirred for 20 min at this temperature. The resulting solution was then transferred to a solution of corresponding aldehyde **9a–m** (0.585 mmol) in THF *via* a cannula at −78°C. The solution was stirred for 20 min at this temperature. The reaction mixture was allowed to warm to 0°C. The reaction mixture was stirred for 2 h at 0°C, and water was then added. The mixture was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. To the residue, solvent (THF/1 *N* aq. HCl=1/1, 3.2 mL) was added, and the solution was stirred at room temperature. After 15 h, saturated aq. $NaHCO_3$ was added to the mixture, which was extracted with EtOAc. The organic layer

was washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was separated with chromatography (hexane/EtOAc=3/1 to 0/1) to afford **10a**–**m**.

(*S,Z*)-11-((*E*)-4-(Furan-2-yl)-2-oxobut-3-en-1-ylidene)-1,2,3,10,11,11a-hexahydro-5*H*-benzo[*e*]pyrrolo[1,2-*a*][1,4]diazepin-5-one (**10a**)

8% yield after ODS purification as a yellow pigment.

Spectral Data of **10a**

$[\alpha]_D^{20}$ +262 (*c* 0.1, MeOH); ^{1}H-NMR (400 MHz, acetone-d_6) δ: 13.34 (brs, 1H), 7.89 (dd, *J*=8.4, 1.6 Hz, 1H), 7.68 (d, *J*=1.4 Hz, 1H), 7.53 (ddd, *J*=8.6, 8.4, 1.6 Hz, 1H), 7.40 (d, *J*=15 Hz, 1H), 7.24 (td, *J*=8.4, 1.2 Hz, 1H), 7.15 (d, *J*=8.6 Hz, 1H), 6.79 (d, *J*=2.8 Hz, 1H), 6.77 (d, *J*=15 Hz, 1H), 6.58 (dd, *J*=2.8, 1.4 Hz, 1H), 5.76 (s, 1H), 4.45 (d, *J*=7.6 Hz, 1H), 3.72–3.66 (m, 1H), 3.60–3.53 (m, 1H), 2.61–2.55 (m, 1H), 2.32–2.22 (m, 1H), 2.13–2.06 (m, 2H); ^{13}C-NMR (100 MHz, acetone-d_6) δ: 188.9, 165.6, 161.7, 152.9, 145.6, 138.1, 133.2, 131.9, 128.5, 126.8, 126.6, 124.9, 122.9, 115.2, 113.3, 93.3, 56.1, 47.5, 27.6, 24.1; IR (ATR); 3127, 2917, 1600, 1416, 1352, 1310, 1249, 1138 and 629 cm^{-1}; ESI-HR-MS Calcd for $C_{20}H_{19}N_2O_3$ 335.1396 $[M+H]^+$; Found 335.1348.

(*S,Z*)-11-((*E*)-4-(Furan-3-yl)-2-oxobut-3-en-1-ylidene)-1,2,3,10,11,11a-hexahydro-5*H*-benzo[*e*]pyrrolo[1,2-*a*][1,4]diazepin-5-one (**10b**)

The compound was synthesized according to the general procedure in 5% yield after octadecyl silica (ODS) purification as a yellow pigment.

Spectral Data of **10b**

$[\alpha]_D^{20}$ +292 (*c* 1.0, MeOH); ^{1}H-NMR (400 MHz, acetone-d_6) δ: 13.31 (s, 1H), 7.94 (s, 1H), 7.89 (dd, *J*=8.2, 1.8 Hz, 1H), 7.62 (d, *J*=1.4 Hz, 1H), 7.54 (d, *J*=16 Hz, 1H), 7.53 (td, *J*=8.2, 1.8 Hz, 1H), 7.24 (td, *J*=8.2, 1.2 Hz, 1H), 7.14 (d, *J*=8.2 Hz, 1H), 6.82 (d, *J*=1.4 Hz, 1H), 6.75 (d, *J*=16 Hz, 1H) 5.69 (s, 1H), 4.44 (d, *J*=8.4 Hz, 1H), 3.70–3.65 (m 1H), 3.63–3.53 (m, 1H), 2.54–2.30 (m, 1H), 2.27–2.22 (m, 1H), 2.12–2.06 (m, 2H); ^{13}C-NMR (150 MHz, acetone-d_6) δ: 189.5, 161.6, 145.9, 145.5, 138.2, 133.2, 131.9, 130.3, 128.9, 128.5, 124.9, 124.4, 122.8, 108.4, 92.8, 56.1, 47.5, 27.6, 14.1; IR (ATR);3127, 1741, 1644, 1516, 1465, 1367, 1217, 705 and 649 cm^{-1}; ESI-HR-MS Calcd for $C_{20}H_{18}N_2NaO_3$ 357.1215 $[M+Na]^+$; Found 357.1233.

(*S,Z*)-11-((*E*)-2-Oxo-4-(thiophen-2-yl)but-3-en-1-ylidene)-1,2,3,10,11,11a-hexahydro-5*H*-benzo[*e*]pyrrolo[1,2-*a*][1,4]diazepin-5-one (**10c**)

The compound was synthesized according to the general procedure in 6% yield as a yellow pigment.

Spectral Data of **10c**

$[\alpha]_D^{20}$ +491 (*c* 1.0, MeOH); ^{1}H-NMR (400 MHz, acetone-d_6) δ: 13.33 (brs, 1H), 7.90 (dd, *J*=7.8, 1.4 Hz, 1H), 7.75 (d, *J*=16 Hz, 1H), 7.56 (dd, *J*=5.6, 1.2 Hz, 1H), 7.54 (td, *J*=7.8, 1.4 Hz, 1H), 7.41 (d, *J*=3.4 Hz, 1H), 7.25 (td, *J*=7.8, 0.8 Hz, 1H), 7.15 (d, *J*=7.8 Hz, 1H), 7.12 (dd, *J*=5.6, 3.4 Hz, 1H), 6.76 (d, *J*=16 Hz, 1H), 5.77 (s, 1H), 4.45 (d, *J*=7.6 Hz, 1H), 3.72–3.66 (m, 1H), 3.60–3.53 (m, 1H), 2.59–2.55 (m, 1H), 2.30- 2.22 (m, 1H), 2.14–2.06 (m, 2H); ^{13}C-NMR (150 MHz, acetone-d_6) δ: 188.8, 165.6, 161.8, 141.7, 138.1, 133.2, 132.8, 131.9, 131.8, 129.1, 128.9, 128.6, 127.9, 124.9, 122.9, 93.2, 56.1, 47.5, 27.6, 24.2; IR (ATR); 3334, 2931, 1741, 1644, 1540, 1516, 1458, 1367, 1217 and 619 cm^{-1}; ESI-HR-MS Calcd for $C_{20}H_{18}N_2NaO_2S$ 373.0987 $[M+Na]^+$; Found 373.1001.

(*S,Z*)-11-((*E*)-2-Oxo-4-(thiophen-3-yl)but-3-en-1-ylidene)-1,2,3,10,11,11a-hexahydro-5*H*-benzo[*e*]pyrrolo[1,2-*a*][1,4]diazepin-5-one (**10d**)

The compound was synthesized according to the general procedure in 6% yield after ODS purification as a yellow pigment.

Spectral Data of **10d**

$[\alpha]_D^{20}$ +627 (*c* 0.1, MeOH); ^{1}H-NMR (400 MHz, acetone-d_6) δ: 13.34 (s, 1H), 7.89 (dd, *J*=8.0, 1.8 Hz, 1H), 7.78 (dd, *J*=3.0, 1.4 Hz, 1H), 7.63 (d, *J*=16 Hz, 1H), 7.54 (dd, *J*=5.2, 3.0 Hz, 1H), 7.53 (td, *J*=8.0, 1.8 Hz, 1H), 7.48 (dd, *J*=5.2, 1.4 Hz, 1H), 7.24 (td, *J*=8.0, 1.2 H, 1H), 7.15 (d, *J*=8.0 Hz, 1H), 6.87 (d, *J*=16 Hz, 1H), 5.74 (s, 1H), 4.45 (d, *J*=8.0, 1H), 3.71–3.66 (m, 1H), 3.61–3.53 (m,1H), 2.56–2.51 (m, 1H), 2.33–2.23 (m, 1H), 2.13–2.06 (m, 2H); ^{13}C-NMR (150 MHz, acetone-d_6) δ: 189.7, 165.6, 161.6, 139.6, 138.1, 133.9, 133.2, 131.9, 128.8, 128.7, 128.5, 127.9, 126.2, 124.9, 122.8, 93.0, 56.1, 47.5, 27.6, 24.1; IR (ATR); 2974, 1741, 1644, 1548, 1516, 1461, 1367, 1224, 705 and 653 cm^{-1}; ESI-HR-MS Calcd for $C_{20}H_{18}N_2NaO_2S$ 373.0987 $[M+Na]^+$; Found 373.0999.

(*S,Z*)-11-((*E*)-2-Oxo-4-(pyridin-2-yl)but-3-en-1-ylidene)-1,2,3,10,11,11a-hexahydro-5*H*-benzo[*e*]pyrrolo[1,2-*a*][1,4]diazepin-5-one (**10e**)

The compound was synthesized according to the general procedure in 3% yield after ODS purification as a yellow pigment.

Spectral Data of **10e**

$[\alpha]_D^{20}$ +467 (*c* 0.1, MeOH); ^{1}H-NMR (400 MHz, acetone-d_6) δ: 13.41 (brs, 1H), 8.64 (ddd, *J*=6.2, 1.2, 0.8 Hz, 1H), 7.91 (dd, *J*=7.6, 1.8 Hz, 1H), 7.82 (td, *J*=7.6, 1.6 Hz, 1H), 7.62 (dd, *J*=7.5, 1.0 Hz, 1H), 7.59 (d, *J*=16 Hz, 1H), 7.55 (td, *J*=7.6, 1.2 Hz, 1H), 7.39 (d, *J*=16 Hz, 1H), 7.33 (ddd, *J*=7.5, 6.2, 1.0 Hz, 1H), 7.27 (td, *J*=7.5, 1.0 Hz, 1H), 7.18 (d, *J*=7.6 Hz, 1H), 5.86 (s, 1H), 4.47 (d, *J*=7.6 Hz, 1H), 3.73–3.68 (m, 1H), 3.61–3.54 (m, 1H), 2.65–2.60 (m, 1H), 2.32- 2.24 (m, 1H), 2.20–2.06 (m, 2H); ^{13}C-NMR (150 MHz, acetone-d_6) δ: 189.2, 165.6, 162.3, 154.8, 150.9, 139.1, 138.0, 137.7, 133.2, 132.5, 131.9, 128.7, 125.1, 125.0, 124.7, 123.0, 93.4, 56.1,47.5, 27.6, 24.2; IR (ATR); 3281, 1743, 1692, 1644, 1548, 1516, 1461, 1423 and 637 cm^{-1}; ESI-HR-MS Calcd for $C_{21}H_{19}N_3NaO_2$ 368.1375 $[M+Na]^+$; Found 368.1392.

(*S,Z*)-11-((*E*)-2-Oxo-4-(pyridin-3-yl)but-3-en-1-ylidene)-1,2,3,10,11,11a-hexahydro-5*H*-benzo[*e*]pyrrolo[1,2-*a*][1,4]diazepin-5-one (**10f**)

The compound was synthesized according to the general procedure in 4% yield after ODS purification as a yellow pigment.

Spectral Data of **10f**

$[\alpha]_D^{20}$ +238 (*c* 1.0, MeOH); ^{1}H-NMR (400 MHz, acetone-d_6) δ: 13.36 (brs, 1H), 8.83 (d, *J*=2.4 Hz, 1H), 8.55 (dd, *J*=4.6, 1.6 Hz, 1H), 8.06 (ddd, *J*=7.8, 2.4, 1.6 Hz, 1H), 7.90 (dd, *J*=8.0, 1.6 Hz, 1H), 7.62 (d, *J*=16 Hz, 1H), 7.55 (ddd, *J*=8.0, 7.0, 1.6 Hz, 1H), 7.41 (dd, *J*=7.8, 4.6 Hz, 1H), 7.26 (td, *J*=8.0, 0.8 Hz, 1H), 7.18 (d, *J*=7.0 Hz, 1H), 7.15 (d, *J*=16 Hz, 1H), 5.80 (s, 1H), 4.46 (d, *J*=6.4 Hz, 1H), 3.73–3.67 (m, 1H), 3.62–3.54 (m, 1H), 2.58- 2.52 (m, 1H), 2.34- 2.24 (m, 1H), 2.12–2.07 (m, 2H); ^{13}C-NMR (150 MHz, acetone-d_6) δ: 188.8, 165.6, 162.3, 151.2, 150.6, 138.0, 136.4, 134.8, 133.2, 132.1, 131.9, 130.9, 128.6, 125.1, 124.6, 122.9, 93.1, 56.1, 47.5, 27.6, 24.2; IR (ATR); 3010, 1739, 1533, 1437, 1366, 1219, 1093 and 903 cm^{-1}; ESI-HR-MS Calcd for $C_{42}H_{38}N_6NaO_4$ 713.2852 $[2M+Na]^+$;

Found 713.2810.

(*S*,*Z*)-11-((*E*)-2-oxo-4-(quinolin-3-yl)but-3-en-1-ylidene)-1,2,3,10,11,11a-hexahydro-5*H*-benzo[*e*]pyrrolo[1,2-*a*][1,4]diazepin-5-one (**10g**)

The compound was synthesized according to the general procedure in 6% yield after ODS purification as a yellow pigment.

Spectral Data of **10g**

$[\alpha]_D^{20}$ +269 (*c* 0.1, MeOH); ^{1}H-NMR (400 MHz, acetone-d_6) δ: 13.40 (s, 1H), 9.23 (d, *J*=2.2 Hz, 1H), 8.53 (d, *J*=2.2 Hz, 1H), 8.05 (d, *J*=8.4 Hz, 1H), 7.99 (d, *J*=7.8 Hz, 1H), 7.91 (dd, *J*=8.0, 1.6 Hz, 1H), 7.81 (d, *J*=16 Hz, 1H), 7.78 (ddd, *J*=8.0, 7.2, 1.6 Hz, 1H), 7.63 (ddd, *J*=8.4, 7.2, 1.2 Hz, 1H), 7.56 (ddd, *J*=7.8, 7.2, 1.2 Hz, 1H), 7.34 (d, *J*=16 Hz, 1H), 7.27 (td, *J*=8.0, 1.2 Hz, 1H), 7.20 (d, *J*=7.2 Hz, 1H), 5.86 (s, 1H), 4.48 (d, *J*=7.6 Hz, 1H), 3.74–3.68 (m, 1H), 3.62–3.55 (m, 1H), 2.60–2.55 (m, 1H), 2.34–2.26 (m, 1H), 2.15–2.06 (m, 2H); ^{13}C-NMR (150 MHz, acetone-d_6) δ: 188.8, 165.6, 162.3, 150.5, 149.3, 136.7, 135.8, 133.2, 131.9, 130.9, 130.8, 130.1, 130.0, 129.6, 129.3, 128.8, 128.7, 128.1, 125.1, 123.0, 93.3, 56.1, 47.5, 27.6, 24.2; IR (ATR); 3202, 2977, 1741, 1646, 1516, 1367, 1217, 705 and 637 cm^{-1}; ESI-HR-MS Calcd for $C_{25}H_{22}N_3O_2$ 396.1712 $[M+H]^+$; Found 396.1748.

(*S*,*Z*)-11-((*E*)-4-(Benzo[*d*][1,3]dioxol-5-yl)-2-oxobut-3-en-1-ylidene)-1,2,3,10,11,11a-hexahydro-5*H*-benzo[*e*]pyrrolo-[1,2-*a*][1,4]diazepin-5-one (**10h**)

The compound was synthesized according to the general procedure in 13% yield as a yellow pigment.

Spectral Data of **10h**

$[\alpha]_D^{20}$ +366 (*c* 1.0, MeOH); ^{1}H-NMR (400 MHz, acetone-d_6) δ: 13.34 (brs, 1H), 7.89 (dd, *J*=7.6, 1.6 Hz, 1H), 7.54 (d, *J*=16 Hz, 1H), 7.53 (ddd, *J*=8.4, 7.6, 1.6 Hz, 1H), 7.24 (td, *J*=7.6, 1.6 Hz, 1H), 7.23 (dd, *J*=8.0, 1.6 Hz, 1H), 7.15 (dd, *J*=8.4, 1.6 Hz, 1H), 7.14 (d, *J*=1.6 Hz, 1H), 6.91 (d, *J*=16 Hz, 1H), 6.89 (d, *J*=8.0 Hz, 1H), 6.06 (s, 2H), 5.74 (s, 1H), 4.45 (d, *J*=6.4 Hz, 1H), 3.72–3.66 (m, 1H), 3.61–3.54 (m 1H), 2.56–2.51 (m, 1H), 2.33–2.23 (m, 1H), 2.12–2.06 (m, 2H);^{13}C-NMR (150 MHz, acetone-d_6) δ: 189.4, 165.7, 161.5, 150.2, 149.4, 140.0, 138.2, 133.2, 131.9, 130.9, 128.5, 127.2, 125.2, 124.8, 122.8, 109.2, 107.0, 102.5, 93.2, 56.1, 47.5, 27.6, 24.1; IR (ATR); 2976, 1741, 1644, 1549, 1516, 1465, 1367, 665 and 626 cm^{-1}; ESI-HRM Calcd for $C_{23}H_{20}N_2NaO_4$ 411.1321 $[M+Na]^+$; Found 411.1272.

(*S*,*Z*)-11-((*E*)-2-Oxo-4-(1*H*-pyrrol-2-yl)but-3-en-1-ylidene)-1,2,3,10,11,11a-hexahydro-5*H*-benzo[*e*]pyrrolo[1,2-*a*][1,4]diazepin-5-one (**10i**)

The compound was synthesized according to the general procedure in 6% yield after ODS purification as a yellow pigment.

Spectral Data of **10i**

$[\alpha]_D^{20}$ +574 (*c* 0.5, MeOH); ^{1}H-NMR (400 MHz, acetone-d_6) δ: 13.31 (brs, 1H), 10.67 (brs, 1H), 7.88 (dd, *J*=8.0, 1.2 Hz, 1H), 7.52 (td, *J*=8.0, 1.2 Hz, 1H), 7.51 (d, *J*=16 Hz, 1H), 7.21 (td, *J*=8.0, 1.2 Hz, 1H), 7.12 (d, *J*=8.0 Hz, 1H), 7.00 (dd, *J*=2.8, 1.6 Hz, 1H), 6.62 (d, *J*=16 Hz, 1H), 6.56 (dd, *J*=3.6, 1.6 Hz, 1H), 6.21–6.19 (m, 1H), 5.56 (s, 1H), 4.43 (d, *J*=8.0 Hz, 1H), 3.70–3.65 (m, 1H), 3.61–3.54 (m, 1H), 2.52–2.47 (m, 1H), 2.31–2.21 (m, 1H), 2.11–2.06 (m, 2H); ^{13}C-NMR (100 MHz, acetone-d_6) δ: 189.7, 165.8, 160.6, 138.4, 133.2, 131.9, 130.7, 130.3, 128.3, 124.5, 123.3, 122.7, 122.5, 114.5, 111.1, 92.9, 56.0, 47.5, 27.6, 24.1; IR (ATR); 3015, 1739, 1547, 1508, 1441, 1368, 1221 and 828 cm^{-1}; ESI-HR-MS Calcd for $C_{40}H_{38}N_6NaO_4$ 689.2852 $[2M+Na]^+$; Found 689.2851.

(*S*,*Z*)-11-((*E*)-4-(5-Iodofuran-2-yl)-2-oxobut-3-en-1-ylidene)-1,2,3,10,11,11a-hexahydro-5*H*-benzo[*e*]pyrrolo[1,2-*a*][1,4]diazepin-5-one (**10j**)

The compound was synthesized according to the general procedure in 1% yield after ODS purification as a yellow pigment.

Spectral Data of **10j**

$[\alpha]_D^{20}$ +361 (*c* 0.1, MeOH); ^{1}H-NMR (400 MHz, acetone-d_6) δ: 13.34 (brs, 1H), 7.90 (dd, *J*=8.0, 1.6 Hz, 1H), 7.54 (td, *J*=8.0, 1.6 Hz, 1H), 7.32 (d, *J*=16 Hz, 1H), 7.25 (td, *J*=8.0, 1.2 Hz, 1H), 7.15 (d, *J*=8.0 Hz, 1H), 6.81 (d, *J*=3.4 Hz, 1H), 6.76 (d, *J*=16 Hz, 1H), 6.73 (d, *J*=3.4 Hz, 1H), 5.81 (s, 1H), 4.45 (d, *J*=6.8 Hz, 1H), 3.70–3.67 (m, 1H), 3.60–3.53 (m, 1H), 2.64–2.59 (m, 1H), 2.30–2.19 (m, 1H), 2.14–2.06 (m, 2H); ^{13}C-NMR (150 MHz, acetone-d_6) δ: 188.5, 165.6, 162.0, 158.2, 138.0, 133.2, 131.9, 128.6, 127.2, 125.4, 125.0, 124.2, 122.9, 117.6, 93.5, 93.4, 56.1, 47.5, 27.6, 24.2; IR (ATR); 3019, 1740, 1547, 1512, 1459, 1366, 1222 and 864 cm^{-1}; ESI-HR-MS Calcd for $C_{40}H_{34}I_2N_4NaO_6$ 943.0465 $[2M+Na]^+$; Found 943.0503.

(*S*,*Z*)-11-((*E*)-4-(5-Bromofuran-2-yl)-2-oxobut-3-en-1-ylidene)-1,2,3,10,11,11a-hexahydro-5*H*-benzo[*e*]pyrrolo-[1,2-*a*][1,4]diazepin-5-one (**10k**)

The compound was synthesized according to the general procedure in 3% yield after ODS purification as a yellow pigment.

Spectral Data of **10k**

$[\alpha]_D^{20}$ +196 (*c* 0.1, MeOH); ^{1}H-NMR (600 MHz, acetone-d_6) δ: 13.33 (brs, 1H), 7.90 (dd, *J*=8.4, 1.6 Hz, 1H), 7.54 (td, *J*=8.4, 1.6 Hz, 1H), 7.31 (d, *J*=16 Hz, 1H), 7.25 (td, *J*=8.4, 1.8 Hz, 1H), 7.16 (d, *J*=8.4 Hz, 1H), 6.81 (d, *J*=3.6 Hz, 1H), 6.78 (d, *J*=16 Hz, 1H), 6.63 (d, *J*=3.6 Hz, 1H), 5.81 (s, 1H), 4.45 (d, *J*=8.4 Hz, 1H), 3.71–3.68 (m, 1H), 3.59–3.54 (m, 1H), 2.62–2.59 (m, 1H), 2.31–2.24 (m, 1H), 2.15–2.06 (m, 2H); ^{13}C-NMR (150 MHz, acetone-d_6) δ: 188.5, 165.6, 162.0, 155.1, 138.0, 133.2, 131.9, 128.6, 127.1, 125.5, 125.1, 125.0, 122.9, 117.4, 115.4, 93.5, 56.1, 47.5, 27.6, 24.1; IR (ATR); 3012, 1740, 1515, 1462, 1423, 1366, 1218 and 853 cm^{-1}; ESI-HR-MS Calcd for $C_{40}H_{34}{}^{79}Br_2N_4NaO_6$ 847.0743 $[2M+Na]^+$; Found 847.0792.

(*S*,*Z*)-11-((*E*)-4-(7-Nitro-1*H*-indol-3-yl)-2-oxobut-3-en-1-ylidene)-1,2,3,10,11,11a-hexahydro-5*H*-benzo[*e*]pyrrolo-[1,2-*a*][1,4]diazepin-5-one (**10l**)

The compound was synthesized according to the general procedure in 7% yield as a yellow pigment.

Spectral Data of **10l**

$[\alpha]_D^{20}$ +266 (*c* 0.025, MeOH); ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 13.18 (brs, 1H), 12.39 (brs, 1H), 8.59 (d, *J*=8.2 Hz, 1H), 8.21 (d, *J*=8.2 Hz, 1H), 8.09 (s, 1H), 7.83 (d, *J*=16 Hz, 1H), 7.82 (dd, *J*=8.2, 1.6 Hz, 1H), 7.55 (td, *J*=8.2, 1.6 Hz, 1H), 7.41 (t, *J*=8.2 Hz, 1H), 7.25 (td, *J*=8.2, 1.2 Hz, 1H), 7.17 (d, *J*=8.2 Hz, 1H), 7.13 (d, *J*=16 Hz, 1H), 5.86 (s, 1H), 4.46 (d, *J*=7.8 Hz, 1H), 3.67 −3.63 (m, 1H), 3.53–3.49 (m, 1H), 3.44–3.38 (m, 1H), 2.22–2.15 (m, 1H), 2.08–2.03 (m, 2H); ^{13}C-NMR (150 MHz, DMSO-d_6) δ: 188.7, 164.7, 159.8, 137.0, 133.3, 133.1, 132.6, 132.0, 130.8, 129.5, 129.2, 128.6, 126.9, 124.9, 124.0, 122.0, 120.3, 119.6, 114.0, 92.9, 55.1, 46.8, 26.5, 23.2; IR (ATR); 3006, 1739, 1533, 1515, 1451, 1368, 1220 and 846 cm^{-1}; ESI-HR-MS Calcd for $C_{24}H_{21}N_4O_4$ 429.1563 $[M+H]^+$; Found 429.1604.

(*S*,*Z*)-11-((*E*)-4-(7-Amino-1*H*-indol-3-yl)-2-oxobut-3-en-1-ylidene)-1,2,3,10,11,11a-hexahydro-5*H*-benzo[*e*]pyrrolo-[1,2-*a*][1,4]diazepin-5-one (**10m**)

The compound was synthesized according to the general procedure in 1% yield after ODS purification as a yellow pigment.

Spectral Data of **10m**

$[\alpha]_D^{20}$ +257 (*c* 0.1, MeOH); ^{1}H-NMR (400 MHz, acetone-d_6) δ: 13.40 (brs, 1H), 10.67 (brs, 1H), 7.88 (dd, *J*=8.6, 1.6 Hz, 1H), 7.87 (d, *J*=15 Hz, 1H), 7.71 (d, *J*=2.8 Hz, 1H), 7.51 (ddd, *J*=8.6, 8.4, 1.6 Hz, 1H), 7.32 (d, *J*=7.6 Hz, 1H), 7.19 (td, *J*=8.6, 1.2 Hz, 1H), 7.12 (d, *J*=8.4 Hz, 1H), 6.96 (t, *J*=7.6 Hz, 1H), 6.94 (d, *J*=15 Hz, 1H), 6.57 (dd, *J*=7.6, 0.8 Hz, 1H), 5.78 (s, 1H), 4.44 (d, *J*=6.4 Hz, 1H), 3.72–3.67 (m, 1H), 3.62–3.55 (m, 1H), 2.61–2.57 (m, 1H), 2.30–2.06 (m, 3H); ^{13}C-NMR (150 MHz, acetone-d_6) δ: 190.4, 165.8, 160.2, 138.7, 135.5, 134.9, 133.1, 131.9, 130.6, 128.2, 127.3, 124.3, 124.1, 123.3, 122.9, 122.6, 115.0, 110.6, 108.3, 93.5, 56.2, 47.5, 27.6, 24.2; IR (ATR); 3008, 2963, 1740, 1515, 1459, 1423, 1366, 1222, 1024 and 860 cm^{-1}; ESI-HR-MS Calcd for $C_{24}H_{22}N_4NaO_2$ 421.1640 $[M+Na]^+$; Found 421.1664.

7-Nitroindole-3-carbaldehyde (9l) $POCl_3$ (0.418 mL, 3.13 mmol) was added dropwise to *N*,*N*-dimethylformamide (DMF) (2.08 mL) at 0°C under an argon atmosphere. The mixture was stirred for 5 min, and then 7-nitroindole (200 mg, 1.25 mmol) was added as a DMF solution (10 mL per 1 g indole). The mixture was then allowed to warm to room temperature and stirred for 3 h. Then, 3.8 M aq. KOH (3.29 mL, 12.5 mmol) was added, and the mixture was stirred for 15 h. Saturated aq. $NaHCO_3$ and EtOAc were then added to the mixture until the mixture became clear and the organic layer separated. The mixture was extracted with EtOAc, and the organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was chromatographed on silica gel (hexane/EtOAc=1/5) to afford **9l** (228 mg, 1.20 mmol, 96%) as a yellow amorphous solid.

7-Aminoindole-3-carbaldehyde (9m) To a solution of **9l** (26 mg, 0.137 mmol) in MeOH (1.37 mL), zinc dust (53.7 mg, 0.822 mmol) and saturated aq. NH_4Cl (1.37 mL) were added. The solution was heated to 120°C for 5 min. Saturated aq. $NaHCO_3$ was then added to the reaction mixture, which was extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was chromatographed on silica gel (hexane/EtOAc=1/5) and ODS (60% MeOH) to afford **9m** (2.1 mg, 0.0131 mmol, 10%) as a white amorphous solid.

Cell Cultures and Viability Assay (Activity for Overcoming TRAIL Resistance) AGS cells were purchased from ATCC and cultured in Roswell Park Memorial Institute-1640 medium (Wako, Osaka, Japan) with 10% fetal bovine serum and 1% penicillin-streptomycin. Cultures were maintained in a humidified incubator at 37°C in 5% CO_2/95% air. Cell viability was assessed using a fluorometric microculture cytotoxicity assay in the presence and absence of TRAIL using TRAIL-resistant AGS cells. Cells were seeded in 96-well culture plates (6×10^3 cells per well) in 200 μL medium containing 10% fetal bovine serum. They were then incubated at 37°C in a 5% CO_2 incubator for 24 h. Test samples at different concentrations with or without TRAIL (100 ng/mL) were added to each well. After 24 h of incubation, the cells were washed with phosphate buffered saline (PBS), and 200 μL PBS containing fluorescein diacetate (10 μg/mL) was added to each well. The plates were incubated at 37°C for 1 h, and fluorescence at 538 nm with excitation at 485 nm was measured using Fluoroskan Ascent (Thermo Fisher Scientific, Waltham, MA, U.S.A.).

The Docking Simulation of Fuligocandin B Derivatives to VCP Construction of the three-dimensional structure of fuligocandin B derivatives and VCP were performed with standard geometric parameters of MOE (version 2016.08; Chemical Computing Group, Montreal, Canada) and based on the Brookhaven Protein Databank 5FTJ, respectively.

The molecular docking simulation of fuligocandin B derivatives to VCP were performed with the docking module of MOE.

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Conflict of Interest The authors declare no conflict of interest.

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