Bioorganic & Medicinal Chemistry Letters 21 (2011) 342–345

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Asymmetric synthesis of phenanthroindolizidine alkaloids with hydroxyl group at the C14 position and evaluation of their antitumor activities

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ARTICLE INFO

Article history:
Received 27 August 2010
Revised 28 October 2010
Accepted 1 November 2010
Available online 5 November 2010

Keywords:
Asymmetric synthesis
Phenanthroindolizidine alkaloid
Antitumor

ABSTRACT

The asymmetric total synthesis of the strongly cytotoxic phenanthroindolizidine alkaloid **3** was achieved. Using the same route, various derivatives were also synthesized. Cytotoxicity of those synthetic compounds was evaluated and compounds **19**, **23**, and **27** demonstrated potent cytotoxicities similar to that of **3**. The in vivo antitumor efficacy of selected compounds was also evaluated and **23** demonstrated moderate antitumor efficacy.

Phenanthroindolizidine alkaloids, isolated mainly from plants of the Asclepiadaceae family, are known to possess various biological activities including anti-arthritis,[1] antifungal,[2] anti-inflammatory,[3] and antitumor[4] activities. In particular, their significant antitumor activities make them attractive candidates for novel antitumor agents. Tylophorine (**1**) and tylocrebrine (**2**) are representative of this group of alkaloids (Fig. 1).[5] Compound **3** was recently isolated from a butterfly (*Ideopsis similis*) and has exhibited remarkable cytotoxicity against various human cancer cell lines.[6] The structural features of **3** include the presence of a hydroxyl group at the C14 position. This compound has attracted attention due to its unique origin, strong cytotoxicity, and structural features. Another unique aspect of this compound is that its related phenanthroindolizidine alkaloids are suggested to have a different antitumor mechanism from those of other antitumor agents that are currently being used clinically.[4,7] In this study, we report the asymmetric total synthesis of **3**[8] and its derivatives together with their in vitro cytotoxicities and in vivo antitumor activities.

Total synthesis of phenanthroindolizidine alkaloids has been reported by various groups;[9] however, those with stereoselectively constructed 14-hydroxyl groups are rare.[10] Buckley and Rapoport reported an asymmetric total synthesis of (*S*)-tylophorine (*ent*-**1**), and during this process they synthesized 14-α-hydroxyl (*S*)-tylophorine stereoselectively as an intermediate product.[11] In this study, we made few modifications to their synthetic route and successfully attained the asymmetric total synthesis of **3**. We also synthesized various derivatives of **3** using the same route.

Synthesis of **3** commenced with the condensation of commercially available 4-benzyloxybenzaldehyde (**4**) and 3,4-dimethoxybenzyl cyanide (**5**) to the stilbene (**6**) (Scheme 1). Buckley and Rapoport demonstrated that 2,3,6,7-substituted phenanthrene (so-called tylophorine-type) could be obtained by vanadium(V) oxidation of the corresponding stilbene; however, 2,3,6-substituted phenanthrene could not be synthesized in the same manner. Although Compound **3** is 3,6,7-substituted phenanthrene rather than 2,3,6-substituted phenanthrene, there is a possibility that vanadium(V) oxidation will not work because of the lack of tylophorine-type substituents. Therefore, we adopted the photo-induced electron cyclization reaction to construct the phenanthrene ring because of its feasibility.[12] Subsequently, we successfully obtained the desired phenanthrene (**7**) by this route.

Conversion of the nitrile (**7**) to the aldehyde (**8**) followed by the reductive amination with the glutamic acid unit produced the partially racemized **12** contrary to the report (Scheme 2, path A).[11] Thus, we changed the method to introduce the glutamic acid unit from the reductive amination of the aldehyde to the amination of the bromide (**11**) (path B). Fortunately, racemization was not observed via path B.

Hydrolysis of **12** produced the amido acid **13** with a good yield (Scheme 3). An intramolecular Friedel–Crafts acylation was completed successfully but partial cleavage of the 3-benzylether to the corresponding 3-hydroxyl compound was also observed.[13] The constructed carbonyl moiety was reduced to alcohol diastereoselectively using L-selectride. Reduction of the lactam moiety followed by hydrogenolysis of the benzylether afforded **3**. Synthetic **3** was confirmed as a single enantiomer by chiral HPLC analysis and

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doi:10.1016/j.bmcl.2010.11.008

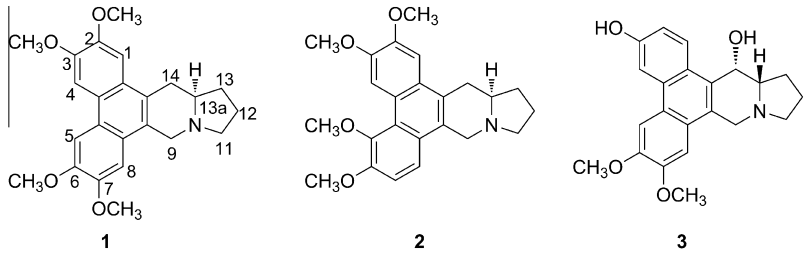
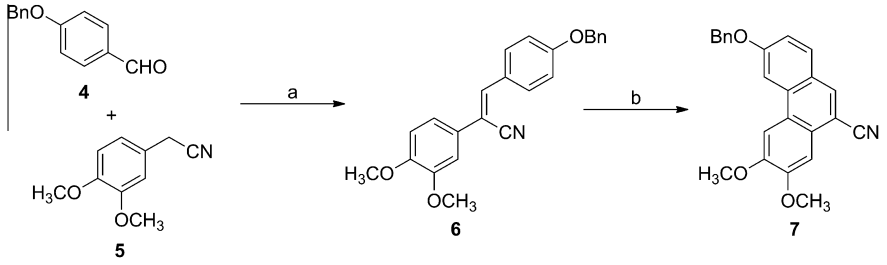


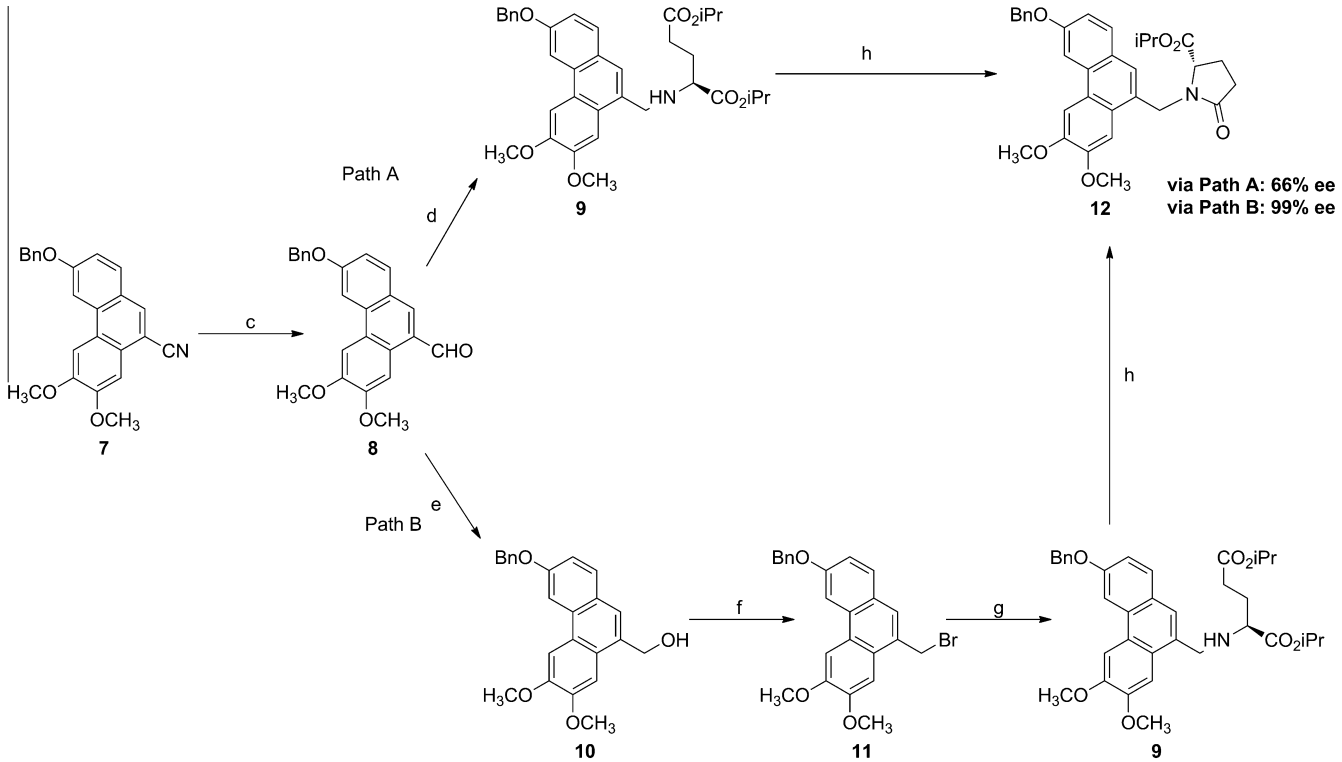
Figure 1. Chemical structures of phenanthroindolizidine alkaroids.



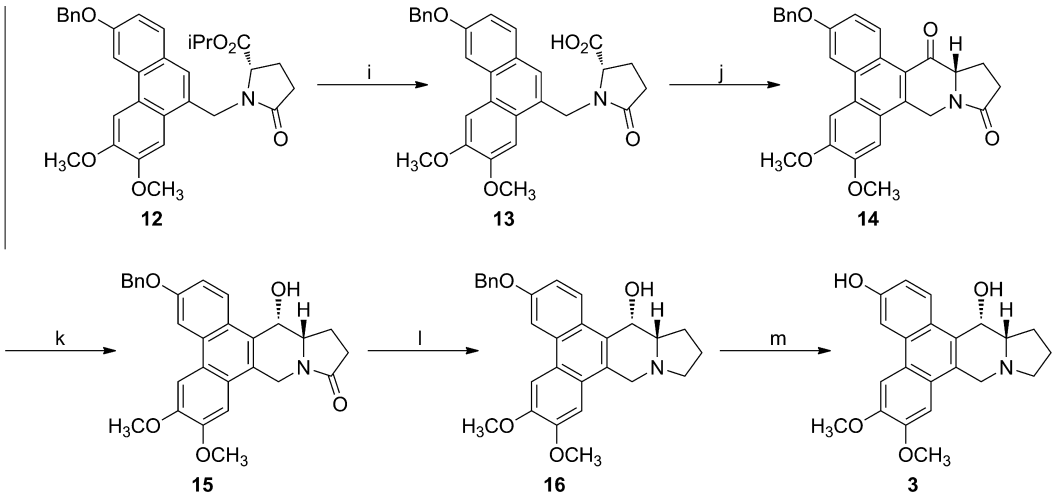
Scheme 1. Reagents and conditions: (a) NaOEt, EtOH (93%); (b) I_2, propylene oxide, hν, CH_3CN (79%).

was spectroscopically identical to the compound in a previous report.[6] Subsequently, 13% overall yield of Compound **3** was obtained starting from **4** with 12-steps. By changing the starting material appropriately, we also obtained derivatives of **3** that have different substituents on the phenanthrene ring.[14]

The compounds synthesized were evaluated for their cytotoxicities against three human cancer cell lines, KB (nasopharyngeal), A549 (lung), and HT-29 (colorectal) (Table 1).[15] Compound **3** exhibited potent cytotoxicity against all cell lines as previously reported.[6] Compounds **17** and **18** are derivatives of **3** that differed from **3** in the position of the hydroxyl group from R^2 to R^1 or R^3. Although **3** exhibited potent cytotoxicity against all cell lines tested, these two compounds exhibited cytotoxicities that were cell line-dependent. This result stimulated us to probe the efficacy of the substituent at R^2. Therefore, we changed the hydroxyl group of **3** to either a methoxy group (**19**), hydrogen atom (**20**), fluorine atom (**22**), ethyl group (**21**), or acetoxy group (**23**) and evaluated their cytotoxicities. Compounds **19** and **23** exhibited comparable activities to **3**, while **20** and **22** were found to be less active. In contrast, Compound **21** showed a remarkable decrease in activity.

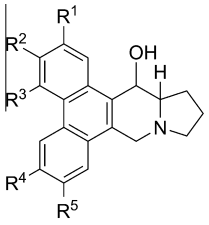


Scheme 2. Reagents and conditions: (c) DIBAL, CH_2Cl_2 (86%); (d) (L)-diisopropyl glutamate, $NaCNBH_3$, CH_2Cl_2; (e) $NaBH_4$, MeOH, 1,4-dioxane; (f) PBr_3, Et_3N, $CHCl_3$; (g) (L)-diisopropyl glutamate, K_2CO_3, benzene, DMF; (h) AcOH, MeOH (via Path A: 88%, two-steps, via Path B: 84%, four-steps).



Scheme 3. Reagents and conditions: (i) KOHaq, MeOH, 1,4-dioxane (99%); (j) $(COCl)_2$, DMF, CH_2Cl_2, then $SnCl_4$ (49%); (k) L-selectride, THF (82%); (l) BH_3–THF, THF (93%); (m) H_2, Pd-C, MeOH (62%).

Table 1
Cytotoxicity of synthetic compounds



Compound	R^1	R^2	R^3	R^4	R^5	Configuration of (**13a**,**14**)	IC_{50} (nM)		
							KB	A549	HT-29
3	H	OH	H	CH_3O	CH_3O	(*S*,*S*)	5.2	0.5	1.6
17	OH	H	H	CH_3O	CH_3O	(*S*,*S*)	43.8	4.7	6.8
18	H	H	OH	CH_3O	CH_3O	(*S*,*S*)	1.4	3.3	82.1
ent-3	H	OH	H	CH_3O	CH_3O	(*R*,*R*)	3.0	10.9	12.5
19	H	CH_3O	H	CH_3O	CH_3O	(*S*,*S*)	4.2	0.3	2.1
ent-**19**	H	CH_3O	H	CH_3O	CH_3O	(*R*,*R*)	47.4	15.5	20.8
20	H	H	H	CH_3O	CH_3O	(*S*,*S*)	17.5	7.4	10.6
ent-**20**	H	H	H	CH_3O	CH_3O	(*R*,*R*)	2031.9	603.8	583.8
21	H	CH_3CH_2	H	CH_3O	CH_3O	(*S*,*S*)	180.1	559.0	134.6
ent-**21**	H	CH_3CH_2	H	CH_3O	CH_3O	(*R*,*R*)	1589.5	267.6	320.5
22	H	F	H	CH_3O	CH_3O	(*S*,*S*)	49.0	11.2	19.6
ent-**22**	H	F	H	CH_3O	CH_3O	(*R*,*R*)	653.2	92.8	105.6
23	H	AcO	H	CH_3O	CH_3O	(*S*,*S*)	1.7	1.5	0.2
24	H	OH	H	H	H	(*S*,*S*)	1964.8	353.7	458.4
25	H	OH	H	$\vdash OCH_2O-$	$\vdash OCH_2O-$	(*S*,*S*)	25.5	54.4	134.5
26	H	OH	H	$\vdash OC(CH_3)_2O-$	$\vdash OC(CH_3)_2O-$	(*S*,*S*)	741.8	927.8	487.5
27	H	OH	H	CH_3CH_2O	CH_3CH_2O	(*S*,*S*)	10.9	0.7	1.2

Subsequently, we examined the enantiomers of **3**, **19**, **20**, **21**, and **22** to observe how the configuration at C-13a and C-14 influences cytotoxicity. As a result, enantiomers were found to be less active in almost all cases. We then probed the effect of substituents at R^4 and R^5. Changing the methoxy groups at R^4 and R^5 of **3** to hydrogen atoms (**24**) led to a remarkable decrease in activity. This demonstrated the importance of alkoxy groups at these positions. Thus, we examined three compounds that have alkoxy group(s) at R^4 and R^5. Compounds **25** and **26** that have a cyclic alkoxy group between R^4 and R^5 exhibited lower activity than **3**, while **27**, having ethoxy groups at R^4 and R^5, showed similar potent cytotoxicity to that of **3**.

Subsequently, we planned to explore the in vivo antitumor efficacy of these compounds.[16] Thus, we selected **3**, *ent*-**3**, **19**, **23**, and **27** as testing probes because of their potent in vitro activities.

Table 2
Antitumor efficacy in vivo

Compound	Inhibition rate (%)	Total dose (mg/kg)	Mortality
3	30.6	25	0/5
	–	50	5/5
ent-**3**	7.2	25	0/5
	12.5	50	0/5
19	4.3	25	2/5
	–	50	5/5
23	54.9	25	0/5
	39.3	50	2/5
27	−3.1	25	0/5
	−10.6	50	0/5
CPT-**11**	46.2	50	0/5
	78.3	100	0/5

Contrary to the promising results in an in vitro screening, all compounds showed low to moderate tumor growth inhibition rates (Table 2). Only **23** showed moderate antitumor activity, but due to inherent toxicity, higher dosing examination could not be performed. The same toxicity was observed in the cases of **3** and **19**, while *ent*-**3** and **27** showed neither activity nor toxicity.

In summary, we completed asymmetric total synthesis of **3**. This synthetic route could be applicable for various substrates, thus we could acquire various derivatives of **3** in view of the substituents on the phenanthrene ring and/or configurations at the C13a and C14 stereocenters. Evaluation of in vitro cytotoxicities of these synthetic compounds showed that the substituents on the phenanthrene ring and configuration strongly affected the activity. In vivo antitumor efficacy was explored using compounds that showed potent cytotoxicity in vitro. In this examination, **23** showed moderate antitumor efficacy, but almost all other compounds showed inherent toxicity, thus limiting higher dosing examination. To overcome this problem, new research is currently underway.

Acknowledgments

The authors thank Miyuki Uchida and Fukiko Nishisaka for their excellent technical assistance.

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13. Debenzyl derivative could also be utilized for synthesis of **3**.
14. *Compound* **3**: yellow powder, ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.75–1.88 (3H, m), 2.12–2.20 (1H, m), 2.30–2.42 (2H, m), 3.40–3.49 (2H, m), 3.92 (3H, m), 3.99 (3H, m), 4.53 (1H, d, J = 16.1 Hz), 4.58–4.61 (1H, m), 4.90–4.92 (1H, m), 7.09 (1H, d, J = 2.2, 9.0 Hz), 7.19 (1H, s), 7.91 (1H, s), 7.91 (1H, s), 8.12 (1H, d, J = 9.0 Hz), 9.63 (1H, br s); HRMS (ESI) calcd for $C_{22}H_{24}NO_4$ [M+H]$^+$, 366.1705. Found: 366.1711; $[\alpha]_D^{25}$ +102.30 (c = 0.12, $CHCl_3$: CH_3OH = 1:1).
Compound **17**: yellow powder, ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.78–1.91 (3H, m), 2.10–2.26 (1H, m), 2.28–2.45 (2H, m), 3.20–3.35 (1H, m), 3.47 (1H, d, J = 15.6 Hz), 3.90 (3H, s), 3.98 (3H, s), 4.52 (1H, d, J = 10.0 Hz), 4.57 (1H, d, J = 15.6 Hz), 4.80 (1H, dd, J = 2.0, 10.0 Hz), 7.09 (1H, dd, J = 2.6, 8.9 Hz), 7.19 (1H, s), 7.62 (1H, d, J = 2.6 Hz), 8.0 (1H, s), 8.55 (1H, d, J = 8.9 Hz), 9.62 (1H, br s); HRMS (ESI) calcd for $C_{22}H_{24}NO_4$ [M+H]$^+$, 366.1705. Found: 366.1700; $[\alpha]_D^{28}$ +137.83 (c = 0.11, $CHCl_3$: CH_3OH = 1:1).
Compound **18**: yellow powder, ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.75–1.91 (3H, m), 2.10–2.26 (1H, m), 2.28–2.45 (2H, m), 3.50 (1H, d, J = 15.9 Hz), 3.91 (3H, s), 3.93 (3H, s), 4.10–4.15 (1H, m), 4.52–4.58 (1H, m), 4.59 (1H, d, J = 15.9 Hz), 4.89 (1H, dd, J = 2.0, 10.0 Hz), 7.06 (1H, s), 7.36 (1H, t, J = 7.8 Hz), 7.64–7.75 (1H, m), 7.84 (1H, d, J = 7.8 Hz), 9.48 (1H, s), 10.45 (1H, br s); HRMS (ESI) calcd for $C_{22}H_{24}NO_4$ [M+H]$^+$, 366.1705. Found: 366.1713; $[\alpha]_D^{28}$ +88.17 (c = 0.11, $CHCl_3$: CH_3OH = 1:1).
Compound **19**: yellow powder, ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.86–2.06 (3H, m), 2.30–2.56 (4H, m), 3.30–3.42 (2H, m), 3.94 (3H, s), 4.04 (3H, s), 4.11 (3H, s), 5.00–5.08 (1H, m), 6.67 (1H, s), 7.28 (1H, dd, J = 8.8, 2.4 Hz), 7.76 (1H, s), 7.83 (1H, d, J = 2.4 Hz), 8.38 (1H, d, J = 8.8 Hz); HRMS (ESI) calcd for $C_{23}H_{26}NO_4$ [M+H]$^+$, 380.1862. Found:380.1686; $[\alpha]_D^{28}$ +106.09 (c = 0.11,$CHCl_3$).
Compound **20**: white powder, ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.76–1.90 (3H, m), 2.14–2.24 (1H, m), 2.32–2.48 (2H, m), 3.28–3.36 (1H, m), 3.51 (1H, d, J = 15.4 Hz), 3.94 (3H, s), 4.01 (3H, s), 4.61 (1H, d, J = 15.4 Hz), 4.66–4.67 (1H, m), 4.96–5.01 (1H, m), 7.26 (1H, s), 7.55–7.60 (2H, m), 8.16 (1H, s), 8.26–8.32 (1H, m), 8.72–8.76 (1H, m); HRMS (ESI) calcd for $C_{22}H_{24}NO_3$ [M+H]$^+$, 350.1756. Found: 350.1754; $[\alpha]_D^{28}$ +115.11 (c = 0.10, $CHCl_3$).
Compound **21**: white powder, ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.32 (3H, t, J = 7.8 Hz), 1.80–1.88 (3H, br s), 2.10–2.46 (3H, m), 2.86 (2H, q, J = 7.8 Hz), 3.29–3.38 (1H, m), 3.49 (1H, d, J = 15.6 Hz), 3.93 (3H, s), 4.02 (3H, s), 4.58 (1H, d, J = 15.6 Hz), 4.62 (1H, d, J = 10.2 Hz), 4.91–4.99 (1H, m), 7.23 (1H, s), 7.40–7.48 (1H, m), 8.15 (1H, s), 8.32 (1H, d, J = 8.8 Hz), 8.52 (1H, s); HRMS (ESI) calcd for $C_{24}H_{28}NO_3$ [M+H]$^+$, 378.2069. Found: 378.2079; $[\alpha]_D^{29}$ +91.29 (c = 0.02, $CHCl_3$).
Compound **22**: yellow powder, ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.84 (3H, br s), 2.10–2.52 (3H, m), 3.30–3.33 (1H, m), 3.50 (1H, d, J = 15.6 Hz), 3.94 (3H, s), 4.01 (3H, s), 4.61 (1H, d, J = 15.6 Hz), 4.75 (1H, d, J = 9.76 Hz), 4.93–4.99 (1H, m), 7.27 (1H, s), 7.41–7.48 (1H, m), 8.11 (1H, s), 8.30–8.37 (1H, m), 8.55–8.61 (1H, m); HRMS (ESI) calcd for $C_{22}H_{23}FNO_3$ [M+H]$^+$, 368.1662. Found: 368.1621; $[\alpha]_D^{27}$ +159.42 (c = 0.34, $CHCl_3$).
Compound **23**: white powder, ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.78–1.92 (3H, m), 2.15–2.25 (1H, m), 2.30–3.05 (2H, m), 2.36 (3H, s), 3.25–3.40 (1H, m), 3.48–3.63 (1H, m), 3.94 (3H, m), 4.01 (3H, m), 4.59–5.05 (3H, m), 7.26 (1H, s), 7.35 (1H, dd, J = 2.2, 9.0 Hz), 8.08 (1H, s), 8.32 (1H, d, J = 9.0 Hz), 8.48 (1H, d, J = 2.2 Hz); HRMS (ESI) calcd for $C_{24}H_{26}NO_5$ [M+H]$^+$, 408.1811. Found: 408.1850; $[\alpha]_D^{27}$ +102.72 (c = 0.016, $CHCl_3$).
Compound **24**: white powder, ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.76–1.90 (3H, m), 2.12–2.25 (1H, m), 2.30–2.48 (2H, m), 3.25–3.47 (1H, m), 3.49 (1H, d, J = 15.9 Hz), 4.60 (1H, d, J = 15.9 Hz), 4.64–4.69 (0.5H, m), 4.90–4.96 (1H, m), 7.16 (1H, dd, J = 2.4, 8.8 Hz), 7.57–7.66 (2H, m), 7.90–7.96 (1H, m), 8.01 (1H, d, J = 2.4 Hz), 8.18 (1H, d, J = 8.8 Hz), 8.55–8.62 (1H, m); HRMS (ESI) calcd for $C_{20}H_{20}NO_2$ [M+H]$^+$, 306.1494. Found: 306.1462; $[\alpha]_D^{28}$ +97.1 (c = 0.04, CH_3OH : $CHCl_3$ = 1 : 1).
Compound **25**: white powder, ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.75–1.87 (3H, m), 2.08–2.24 (1H, m), 2.30–2.41 (2H, m), 3.27–3.32 (1H, m), 3.39 (1H, d, J = 15.4 Hz), 4.47 (1H, d, J = 15.4 Hz), 4.58 (1H, d, J = 10.0 Hz), 4.89 (1H, dd, J = 1.9, 10.0 Hz), 6.17 (2H, d, J = 4.0 Hz), 7.10 (1H, dd, J = 2.4, 8.8 Hz), 7.33 (1H, s), 7.82 (1H, d, J = 2.4 Hz), 8.01 (1H, s), 8.12 (1H, d, J = 8.8 Hz), 9.73 (1H, br s); HRMS (ESI) calcd for $C_{21}H_{20}NO_4$ [M+H]$^+$, 350.1392. Found: 350.1391.
Compound **26**: yellow powder, ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.71 (3H, s), 1.74 (3H, s), 1.76–1.86 (3H, m), 2.09–2.21 (1H, m), 2.30–2.40 (2H, m), 3.25–3.31 (1H, m), 3.39 (1H, d, J = 15.1 Hz), 4.46 (1H, d, J = 15.1 Hz), 4.54 (1H, d, J = 9.8 Hz), 4.89 (1H, dd, J = 2.2, 9.8 Hz), 7.08 (1H, dd, J = 2.4, 9.0 Hz), 7.25 (1H, s), 7.80 (1H, d, J = 2.4 Hz), 7.91 (1H, s), 8.11 (1H, d, J = 9.0 Hz), 9.67 (1H, br s); HRMS (ESI) calcd for $C_{23}H_{24}NO_4$ [M+H]$^+$, 378.1705. Found: 378.1700.
Compound **27**: white powder, ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.41 (3H, t, J = 7.0 Hz), 1.43 (3H, t, J = 7.0 Hz), 1.72–1.87 (3H, m), 2.08–2.23 (1H, m), 2.28–2.42 (2H, m), 3.21–3.34 (1H, m), 3.38 (1H, d, J = 15.5 Hz), 4.16 (2H, dq, J = 7.0, 11.7 Hz), 4.27 (2H, q, J = 7.0 Hz), 4.43 (1H, d, J = 15.5 Hz), 4.60 (1H, d, J = 8.9 Hz), 4.88 (1H, d, J = 8.9 Hz), 7.08 (1H, dd, J = 2.2, 8.8 Hz), 7.13 (1H, s), 7.88 (1H, d, J = 2.2 Hz), 7.89 (1H, s), 8.11 (1H, d, J = 8.8 Hz), 9.60 (1H, s);.
15. Cell viability was assayed in a 96-well plate using a TetraColor ONE (Seikagaku Corp., Tokyo, Japan), according to the manufacturer's protocol. Briefly, exponentially growing KB, A549 or HT-29 cells were seeded into 96-well plates at a density of 10^3 cells/well, respectively. The next day, serial diluted compounds were added. After 96 h incubation, 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium monosodium salt: WST-8 reagent was added to each well and incubated at 37 °C for 1 h. Absorbance at 450 nm was measured with SPECTRA Max PLUS384 (Molecular Devices, Sunnyvale, CA). Cell viability IC_{50} was defined as the concentration of compound that inhibited cell viability by 50% compared to solvent-treated control cells.
16. Meth A cells (sarcoma, 2.5 × 10^5 cells/mouse) were inoculated sc into 7-wk old male BALB/c mice (5/group), and samples were injected iv on days 1, 5, and 9. Tumors were weighed on day 21 after tumor inoculation. The inhibition rate (%) was calculated by the formula[1 − (mean tumor weight of tested mice)/(mean tumor weight of control mice] × 100.