Structure and biological activity of novel FN analogs as flowering inducers

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Abstract

(12*Z*,15*Z*)-9-Hydroxy-10-oxooctadeca-12,15-dienoic acid (**1**) and norepinephrine (**2**) undergo cycloaddition to afford FN1 (**3**) and FN2 (**4**), both of which induce flowering in *Lemna paucicostata*. Although derivatives of **1** were also suggested to yield FN-like compounds after reacting with **2**, their structures have not been elucidated. In this report, we present the structure and stereochemistry of seven novel FN analogs. These analogs were formed in the same regio- and stereocontrolled manner as FNs. The activity of these analogs on flower induction was examined and all (Compound no.), except for **8**, were found to be effective as flowering inducers in *L. paucicostata*.

Keywords: *Lemna paucicostata*; Flowering; FN; Cycloaddition; Structure-activity relationships.

1. Introduction

The control of transition from vegetative growth to flowering is important in agriculture, horticulture, and plant breeding because this transition is the first step of sexual reproduction in plants. FN1 (**3**) and FN2 (**4**) are artificial flowering inducers of *Lemna paucicostata*, which are formed by cycloaddion of (12*Z*,15*Z*)-9-hydroxy-10-oxooctadeca-12,15-dienoic acid (**1**) with norepinephrine (**2**) (Scheme 1). FNs strongly induce flowering in *L. paucicostata* at quite low doses; but, their effect seems to be restricted to few plant species only. It is well known that natural flowering signals in plants are proteins encoded by *FLOWERING LOCUS T* (*FT*) and its orthologs. Although orthologs of *FT* have not been found in *Lemna* plants, most genetical components known in *Arabidopsis thaliana*, a model plant, have been suggested to also play a role in *Lemna* flowering. Study on identifying the FN's mode of action will allow developing chemicals that induce flowering in many species of plants.

The structure-activity relationship (SAR) study of FN using the reaction products of **1** and its analogs with **2**/epinephrine (**5**) revealed that tricyclic structure is essential for biological activity. Other structural moieties derived from **1** are modifiable without total loss of activity.We tentatively identified the structures of reaction products by LC−PDA/MS and HRMS but determination of their complete structure and absolute configurations are essential for future chemical and biological studies. We report here the structural and stereochemical determinants of novel FN analogs **6**−**12**. Furthermore, we also describe the SAR study of FN for flowering induction in *L. paucicostata*.

2. Results and discussion

2.1. Synthesis and purification of FN analogs

Analogs of FN (**6**−**12**, Fig. 1) were prepared according to the previous method. Fatty acids **13**−**15** (Fig. 2) and **2** were reacted at 25ºC under O_2 atmosphere to give reaction mixtures containing desired analogs **6**−**8**, **11**, and **12**. These were purified by reverse-phase HPLC. C-9 Epimers of compound **6** could not be separated due to difficulty in HPLC resolution. *N*-Methylated analogs of FN, **11** and **12**, were obtained by cycloaddtion of **1** with **5** and subsequent purification by reverse-phase HPLC. These preparation and isolation techniques afforded pure compounds **6**−**8**, **11**, and **12** in 2−18 % yields. It should be noted that these yields do not reflect the real conversion yields because of an unavoidable loss due to their decomposition during the purification steps. Compounds **9** and **10** were easily prepared from FN1 and FN2, respectively, by methylation with (trimethylsilyl)diazomethane.

2.2. Structure elucidation of FN analogs

Compound **6** exhibited a pseudo molecular ion at *m/z* 518.2728 $[M+Na]^+$ (calcd for $C_{26}H_{41}NNaO_8$, 518.2730). The ^1H and ^{13}C NMR data of **6** (Table 1), except for signals due to saturation of 15-olefin, gave almost same results as those of FN1/2, which indicated that a similar structural relationship existed between **6** and FN1/2. The gross structure of **6** was deduced from detailed analysis of the ^1H and ^{13}C NMR data aided by 2D NMR experiments (^1H−^1H COSY, HSQC, and HMBC). ^1H−^1H connectivities of C-11 to C-12 and C-13 to C-18 and HMBC correlations of H-2′ to C-3′ and C-13 suggested that a tricyclic moiety was formed in same regiomanner as FN1/2. NOESY correlations of H-7′ at (*S*)-oxymethine to H-13 and H-14 and H-2′ to H-11 revealed that stereochemistry around the tricyclic system of **6** was identical to that of FN1/2 (Fig. 3).

Therefore, compound **6** has the structure as shown in Figure

Molecular formula of compound **7** was determined to be $C_{26}H_{39}NO_7$ by HRMS (*m/z* 500.26260 for $[M+Na]^+$), which indicated that **7** had one oxygen less than FN1/2. ^1H and ^{13}C NMR spectra (Table 1) revealed that **7** possessed an FN-like structure, where the characteristic signals of tricyclic moiety were observed. Different sets of signals for C-8, 9, 10 and 11were observed due to C-9 deoxygenation. $^1H-^1H$ COSY connectivities of C-11 to C-12 and C-13 to C-18 and HMBC correlations of H-2′ to C-3′ and C-13 and H-13 to C-1′ revealed that cycloaddition of **14** with **2** took place in the same regiomanner as that of **1** with **2**. The stereochemistry of tricyclic moiety in **7** was deduced from NOESY correlations of H-13, H-14, and H-17 to H-7′, H-8′ to H-13, H-12 to H-13, and H-2′ to H-11 to be identical to that of FN1/2 (Fig. 3). On the basis of above evidences, the structure of **7** was identified as 9-deoxy analog of FN1/2.

Molecular formula of compound **8** was determined to be $C_{26}H_{41}NO_7$ by HRMS. It revealed that **8** had two protons more than compound **7**. This was further confirmed by loss of C-15 olefine signals in ^1H and ^{13}C NMR of **8** (Table 2). Other signals were almost identical to those of **7**. NOESY correlations of H-13 and H-14 to H-7′, H-12 to H-13, and H-2′ to H-11 revealed that stereochemistry of the tricyclic ring system at C-1′, C-3′, C-12, and C-13 is same that of FN1/2 (Fig. 3).

Compounds **9** and **10** were prepared from FN1 and FN2, respectively, by methyl esterification and their molecular formulas were determined to be $C_{27}H_{41}NO_8$ by HRMS (*m/z* 530.27310 $[M+Na]^+$ for **9** and *m/z* 530.27316 $[M+Na]^+$ for **10**). Their ^1H and ^{13}C NMR spectra (Table 2) resembled those of FN1/2, except for the presence of one *O*-methyl group. Complete assignment of ^1H and ^{13}C NMR chemical shifts, as shown in table 2, was done by $^1H-^1H$ COSY, HSQC, and HMBC studies. The key NOESY correlations showed stereochemistry of **9** and **10** was same as that of FN1 and FN2,

respectively (data not shown). In a previous study, the ability of **9** and **10** for flowering was tentatively evaluated by the reaction products derived from methyl ester of **1** and **2**. Therefore, we investigated whether they are truly identical to **9**/**10** by comparison to their LC−PDA/MS data. The LC characteristics, UV spectra, and MS of analogs **9** and **10** same as those of the reaction products of methyl ester **1** and **2** (data not shown), indicating that **9** and **10** were identical to the compounds prepared in the previous study.

HRMS showed that compounds **11** and **12** have a molecular formula $C_{27}H_{41}NO_8$. ^1H and ^{13}C NMR data of **11** and **12** (Table 2), except for *N*-methyl group, gave almost same results as that of FN1/2. $^1H-^1H$ COSY, HSQC, HMBC, and NOESY experiments of **11** and **12** gave cross peaks as those of FN1/2 (Table 2 and Fig. 3). These results indicated that **11** and **12** have structures as depicted in Figure 1.

2.3. Biological activity of 6−12

Aanalogs **6**−**12** were evaluated for their ability to induce flowering in *L. paucicostata*. With exception of **8**, these compounds proved to be active (Fig. 4). Compound **6**, in which 15-olefinic bond is saturated, is significantly less active compared to FN1/2. As we have suggested in our previous study, 15-olefinic bond in **6** is not essential for its activity, but its presence is favorable for high activity. The effect of 9-hydroxy group on flowering was investigated with analog **7**. Elimination of **9** hydroxyl resulted in considerable decrease in activity. This suggested that 9-hydroxy group is also not essential for activity but is required to show high activity. Although, during our previous study, stereochemistry at C-9 of FN1/2 seemed to be important for biological activity but we could not observe any implication of stereochemistry in these isomers (**3** and **4**). This is consistent with the fact that other pair of C-9 epimers (**9** and **10**; **11** and **12**) showed almost identical effect on the induction of flowering (see below). Probably, the

presence of a hydroxy group allows a very specific interaction to take place with target protein. The character of this interaction is also illustrated by absence of activity in compound **8**. Analog **8**, which lacks both 15-olefine and 9-hydroxy group, was almost inactive. In addition to loss of 9-hydroxy group, saturation of 15-olefine no longer permits the molecular form of **8** to be correctly positioned in the binding protein. This was inconsistent with the previous suggestion that **8** retained flowering activity, indicating the presence of unknown active compounds in reaction mixture. Introduction of methyl group at terminal carboxy group (**9** and **10**) dramatically decreased activity at low concentrations compared to FN1/2. Carboxy group in FNs might work as a hydrogen bond donor to the target protein. The *N*-methylated derivatives (**11** and **12**) were considerably stronger than parent compounds **3** and **4**. Although primary effect of this portion would be an enhancement of hydrophobicity of molecule to bind the target protein. Other types of *N*-alkylated analogs should be synthesized to address the reason of this enhancement of biological activity.

3. Conclusions

In this report, we have elucidated the structure and stereochemistry of FN analogs **6**−**12** that were synthesized from fatty acids (**1** and **13**−**15**) and catecholamines (**2** and **5**). We observed that these compounds, except for **8**, displayed significant activity with respect to flowering in *L. paucicostata*. The results of SAR study of FN are summarized in Figure 5. Studies to design novel analogs are necessary to extend our knowledge of structural factors governing the biological activity in FNs.

4. Experimental

4.1. General

^{1}H and ^{13}C NMR spectra were recorded on a JNM λ500A spectrometer (JEOL, Tokyo, Japan). High-resolution mass spectra were obtained with a JMS-T100LC AccuTOF mass spectrometer (JEOL). HPLC separation was performed with a JASCO (Tokyo, Japan) LC system. Solvents for HPLC were purchased from Kanto Chemical (Tokyo, Japan). A two-solvent system was used to generate the mobile phase for HPLC: solvent A, 0.05% aq. TFA; solvent B, MeCN.

4.2. Preparation of FN analogs 6−12

*4.2.1. Analog **6***

To a solution of fatty acid **13** (40 mg, 128 μmol) in DMSO (1.2 mL), **2** (20 mM in water; 12.8 mL, 256 μmol), Tris-HCl buffer (1 M, pH 8.0, 6.4 mL), and water (38 mL) were added. Reaction was carried out at 25ºC for 15 h under O_2 atmosphere. After acidification of reaction mixture with 1% aq HCOOH, products were extracted with EtOAc (3×50 mL). EtOAc layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by HPLC [column, CAPCELL PAK UG120 20×250 mm (Shiseido, Tokyo, Japan); solvent, 35% B/(A+B); flow rate, 10 mL/min] to give **6** as a brown oil (4.0 mg, 8.1 μmol, 6%). HRMS (ESI^+) *m/z* 518.2728 $[M+Na]^+$ (calcd for $C_{26}H_{41}NNaO_8$). ^{1}H and ^{13}C NMR: Table 1. HMBC correlation peaks: H-2/C-1, C-3, H-3/C-1, C-2, H-17/C-16, C-18, H-18/C-17, C-16, H-2′/C-13, C-3′, C-4′.

*4.2.2. Analog **7***

Reaction procedure as in Section 4.2.1 was followed. HPLC: column, CAPCELL PAK UG120 20×250 mm; solvent, 40% B/(A+B); flow rate, 10 mL/min. Brown oil (2%). HRMS (ESI^+) *m/z* 500.2626 $[M+Na]^+$ (calcd for $C_{26}H_{39}NNaO_7$). ^1H and ^{13}C NMR: Table 1. HMBC correlation peaks: H-2/C-1, C-3, H-3/C-1, C-2, H-8/C-9, C-10, H-9/C-8, C-10, H-11/C-10, C-12, H-14/C-15, C-16, H-17/C-18, H-18/C-16, C-17, H-2′/C-13, C-3′, C-4′, C-6′, H-7′/C-6′, H-8′/C-1′, C-6′, C-7′.

*4.2.3. Analog **8***

Reaction procedure as in Section 4.2.1 was followed. HPLC: column, CAPCELL PAK UG120 20×250 mm; solvent, 35% B/(A+B); flow rate, 10 mL/min. Brown oil (2%). HRMS (ESI^+) *m/z* 502.2783 $[M+Na]^+$ (calcd for $C_{26}H_{41}NNaO_7$). ^1H and ^{13}C NMR: Table 1. HMBC correlation peaks: H-2/C-1, C-3, H-3/C-1, C-2, H-8/C-10, H-9/C-8, C-10, H-11/C-10, C-12, C-13, H-13/C-1′, H-18/C-17, C-16, H-2′/C-13, C-1′, C-3′, C-4′, C-6′, H-7′/C-6′, C-8′, H-8′/C-7′.

*4.2.3. Analogs **9** and **10***

To a solution of **3**/**4** (3 mg, 6.0 μmol) in MeOH (1 mL), a solution of (trimethylsilyl)diazomethane (2 M in hexane; 500 mL) was added dropwise and stirred for 5 min. After removing the solvent and reagent under vacuum, the resulting oil was **9**/**10**. HRMS (ESI^+) *m/z* 530.2731 $[M+Na]^+$ (calcd for $C_{27}H_{41}NNaO_8$) for **9**, *m/z* 530.2732 $[M+Na]^+$ (calcd for $C_{27}H_{41}NNaO_8$) for **10**. ^1H and ^{13}C NMR: Table 1. HMBC correlation peaks (**9**): H-2/C-1, C-3, H-3/C-1, C-2, H-8/C-9, H-11/C-10, C-12, H-14/C-12, C-13, C-15, C-16, H-17/C-15, C-16, C-18, H-18/C-16, C-17, H-2′/C-13, C-1′, C-3′, C-4′, C-6′, H-7′/C-6′, H-8′/C-6′,C-7′, O*C*H_3/C-1.

*4.2.3. Analogs **11** and **12***

Reaction procedure as in Section 4.2.1 was followed. HPLC: column, CAPCELL PAK UG120 20×250 mm; solvent, 23% B/(A+B); flow rate, 10 mL/min. Brown oil (18% for **11**, 8.0% for **12**). HRMS (ESI^+) *m/z* 530.2732 $[M+Na]^+$ (calcd for $C_{27}H_{41}NNaO_8$) for **11**, *m/z* 530.2732 $[M+Na]^+$ (calcd for $C_{27}H_{41}NNaO_8$) for **12**. ^1H and ^{13}C NMR: Table 1. HMBC correlation peaks (**11**): H-2/C-1, C-3, H-3/C-1, C-2, H-9/C-8, C-7, H-11/C-10, C-12, C-13, H-13/C-11, C-12, C-14, C-1′, C-2′, H-14/C-13, C-15, C-16, H-15/C-14, C-17, H-16/C-14, C-17, H-17/C-15, C-16, C-18, H-18/C-16, C-17, H-2′/C-13, C-1′, C-3′, C-4′, C-6′, H-7′/C-6′, C-8′, H-8′/C-1′, C-6′, C-7′, NCH_3/C-6′, C-8′.

4.3. Flower induction assay

The flower induction assays were performed according to the previous study. A three-frond colony of *L. pucicostata* 151 (P151, a gift from Professor O. Tanaka) was placed on E medium containing test sample and 6-benzylaminopurine, and incubated for 10 days at 25ºC under continuous light. The percentage of fronds with flowers was determined. All experiments were performed with replicates and reproducibility was checked on different days.

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References and notes

Figure and scheme legends

Scheme 1.

Figure 1.

Structures of FN analogs **6**−**12**.

Figure 2.

Structures of fatty acids **13**−**15**.

Figure 3.

Selected NOESY correlations for **6**−**8**, **11**, and **12**.

Figure 4.

Flower-inducing activity of FN analogs. The error bars indicate the standard deviations of three replicates.

Figure 5.

Summary of SAR study of FNs.

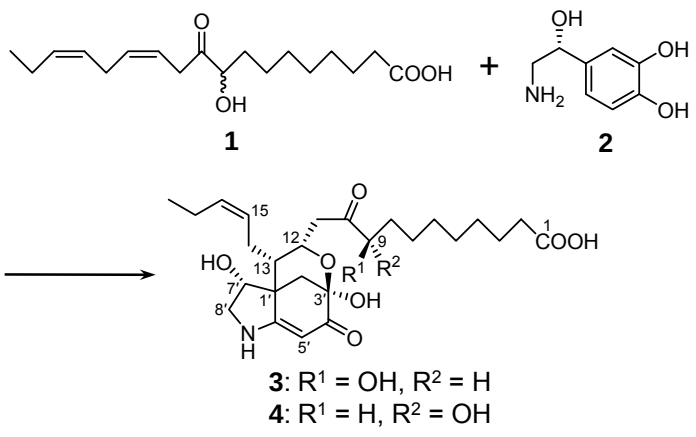
Table 1. NMR data of compounds **6**–**8**

No.	**6**[a]		**7**		**8**	
	^{13}C	^{1}H, multi., Hz	^{13}C	^{1}H, multi., Hz	^{13}C	^{1}H, multi., Hz
1	174. 8		174.7		175.1	
2	34.2	2.28, 2H, t,7.2	34.1	2.28, 2H, t, 7.3	34.2	2.27, 2H, t, 7.3
3	25.6, 25.7	1.59, 2H, m	25.6	1.59, 2H, t, 7.6	25.5	1.59, 2H, m
4						
5	30.0, 30.2, 30.5,	1.30−1.40	29.9 (2), 30.0,	1.20−1.30	29.9, 30.1, 30.2,	1.25−1.40
6	30.5		30.2		30.5	
7						
8	34.1	1.50, 1H, m	24.0	1.49, 2H, m	24.0	1.50, 2H, m
		1.73, 1H, m				
9	77.3, 77.7	4.03, 1H, m	43.1	2.37, 2H, m	43.1	2.43, 2H, m
10	211.7		208.4		208.7	
11	41.4, 41.6	2.63, 1H, dd, 5.5, 16.5	46.1	2.49, 1H, dd, 5.8, 15.8	46.1	2.53, 1H, dd, 5.2, 15.9
		2.97, 1H, dd, 7.6, 16.5		2.64, 1H, m		2.69, 1H, dd, 7.9, 15.9
12	70.7, 70.9	4.61, 1H, m	70.7	4.60, 1H, m	70.7	4.57, 1H, m
13	39.8, 39.5	1.27, 1H, m	39.9	1.45, 1H, m	39.8	1.48, 1H, m
14	26.8, 26.9	1.40, 1H, m	24.5	2.16, 1H, m	26.8	1.39, 1H, m
		1.81, 1H, m		2.64, 1H, m		1.79, 1H, m
15	32.0	1.30−1.40	130.5	5.26, 1H, m	32.1	
16	32.8		132.4	5.33, 1H, m	32.8	1.25−1.40
17	23.1, 23.2	1.25−1.45	21.5	2.16, 2H, m	23.2	
18	14.3	0.90, 3H, m	14.2	1.01, 3H, t, 7.6	14.3	0.90, 3H, t, 7.3
1′	57.5		58.7		57.4	
2′	32.4	1.96, 1H, d, 12.8	32.5	1.99, 1H, d, 12.5	32.4	1.95, 1H, d, 13.1
		2.05, 1H, m		2.08, 1H, dd, 1.6, 7.7		2.05, 1H, m
3′	93.9		93.7		93.8	
4′	187.4		186.3		187.7	
5′	93.9	5.47, 1H, br	93.7	5.45, 1H, br	n.d.	5.38
6′	174.7		173.0		174.6	
7′	73.0	4.36, 1H, d, 3.0	71.8	4.40, 1H, d, 3.7	73.0	4.35, 1H, m
8′	54.9	3.52, 1H, d, 12.2	54.7	3.50, 1H, d, 12.2	54.8	3.51, 1H, d, 11.0
		3.93, 1H, d, 9.2		3.91, 1H, dd, 3.7, 12.2		3.91, 1H, m

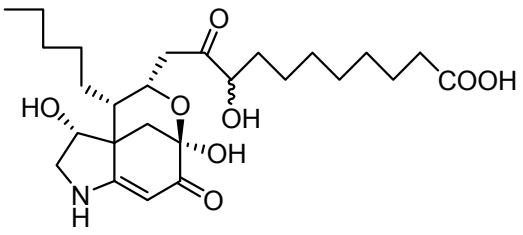
[a] Diastereomeric mixture.

Table 2. NMR data of compounds **9**−**12**

No.	**9**		**10**		**11**		**12**	
	^{13}C	^{1}H, multi., Hz	^{13}C	^{1}H, multi., Hz	^{13}C	^{1}H, multi., Hz	^{13}C	^{1}H, multi., Hz
1	174.1		174.2		174.7		174.8	
2	34.3	2.29, 2H, t, 7.3	34.3	2.29, 2H, t, 7.3	34.1	2.28, 2H, t, 7.6	34.2	2.29, 2H, t, 7.4
3	25.6	1.59, 2H, m	25.6	1.59, 2H, m	25.6	1.60, 2H, m	25.6	1.60, 2H, m
4	30.4, 30.5,	1.25-1.42	30.4,	1.25-1.40	29.7 (2),	1.25-1.40	29.7 (2),	1.25-1.40
5	29.3-30.5		30.5 (2),		30.0 (2)		30.0 (2)	
6			30.6					
7								
8	34.0	1.45, 1H, m	34.1	1.50, 1H, m	33.9	1.49, 1H, m	34.1	1.49, 1H, m
		1.72, 1H, m		1.70, 1H, m		1.71, 1H, m		1.70, 1H, m
9	77.7	4.01, 1H, m	77.3	3.98, 1H, m	77.7	4.01, 1H, dd, 4.1, 7.6	77.3	4.00, 1H, br
10	211.4		210.2		211.3		211.4	
11	41.7	2.68, 1H, dd, 4.9, 15.9	41.6	2.59, 1H, dd, 4.9, 17.1	41.7	2.47, 1H, dd, 4.6, 16.8	41.5	2.52, 1H, dd, 4.0, 16.5
		2.92, 1H, dd, 8.5, 15.9		2.92, 1H, dd, 7.3, 17,1		2.92, 1H, dd, 8.2, 16.4		2.95, 1H, dd, 9.5, 16.5
12	70.9	4.62, 1H, m	70.7	4.65, 1H, m	70.9	4.56, 1H, m	70.8	4.59, 1H, br
13	40.3	1.48, 1H, m	39.9	1.48, 1H, m	40.3	1.49, 1H, m	40.0	1.49, 1H, m
14	24.6	2.21, 1H, m	24.5	2.20, 1H, m	24.6	2.20, 1H, m	24.5	2.19, 1H, m
		2.65, 1H, m		2.65, 1H, m		2.64, 1H, m		2.65, 1H, m
15	130.5	5.30, 1H, m	130.4	5.27, 1H, m	130.3	5.30, 1H, m	130.2	5.26, 1H, m
16	132.6	5.35, 1H, m	132.6	5.34, 1H, m	132.6	5.35, 1H, m	132.7	5.35, 1H, m
17	21.5	2.18, 2H, m	21.5	2.18, 2H, m	21.5	2.17, 2H, m	21.5	2.17, 2H, m
18	14.2	1.02, 3H, t, 7.3	14.2	1.02, 3H, t, 7.3	14.2	1.01, 3H, t, 7.3	14.2	1.01, 3H, t, 7.6
1′	57.3		56.6		58.7		58.9	
2′	32.3	2.01, 1H, d, 12.2	32.3	2.00-2.01, 2H, br	32.5	1.99, 1H, d, 12.8	32.6	2.05, 1H, d, 12.8
		2.08, 1H, m				2.07, 1H, m		2.13, 1H, m
3′	93.8		93.8		93.9		94.1	
4′	187.4		187.4		186.3		186.4	
5′	n.d.	n.d.	n.d.		93.9	5.47, 1H, br	94.1	5.47, 1H, br
6′	174.4		174.3		173.0		173.5	
7′	73.0	4.42, 1H, d, 3.7	73.0	4.42, 1H, m	71.8	4.38, 1H, d, 3.7	71.8	4.39, 1H, br
8′	54.7	3.51, 1H, d, 12.2	54.7	3.51, 1H, d, 12.2	62.9	3.48, 1H, d, 12.5	63.0	3.50, 1H, br
		3.93, 1H, dd, 3.7, 12.2		3.92, 1H, m		4.18, 1H, dd, 3.7, 12.5		4.19, 1H, br
Me	51.4	3.61, 3H, s	51.4	3.61, 3H, s	33.8	3.09, 3H, s	34.0	3.11, 3H, s
	(*O*-Me)		(*O*-Me)		(*N*-Me)		(*N*-Me)	



Scheme 1. Kai et al.



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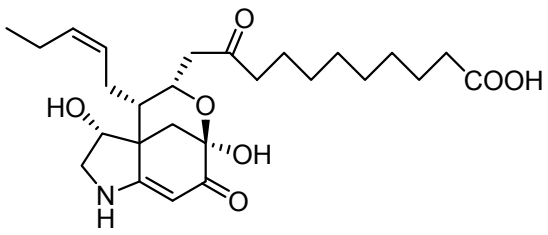

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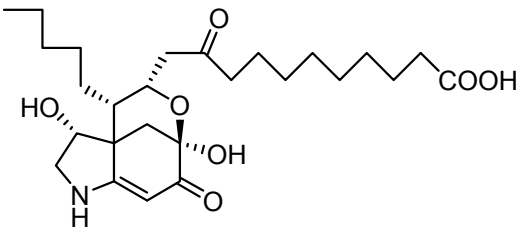

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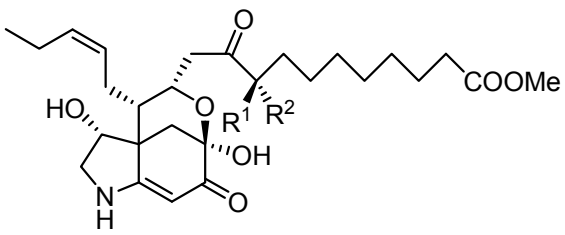
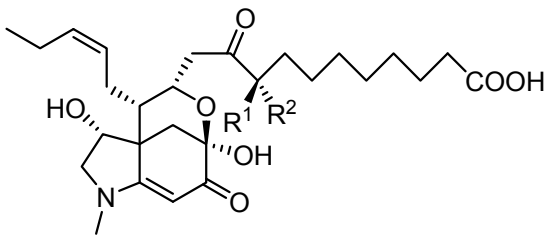
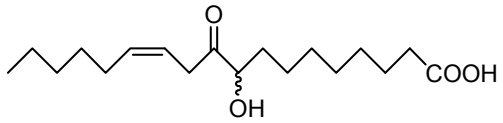


9: R^1 = OH, R^2 = H
10: R^1 = H, R^2 = OH

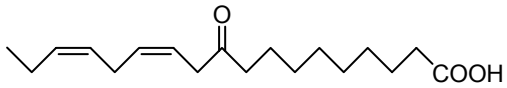


11: R^1 = OH, R^2 = H
12: R^1 = H, R^2 = OH

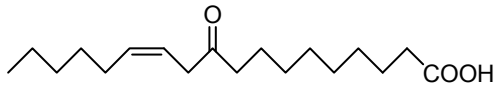
Figure 1. Kai et al.



13



14



15

Figure 2. Kai et al.

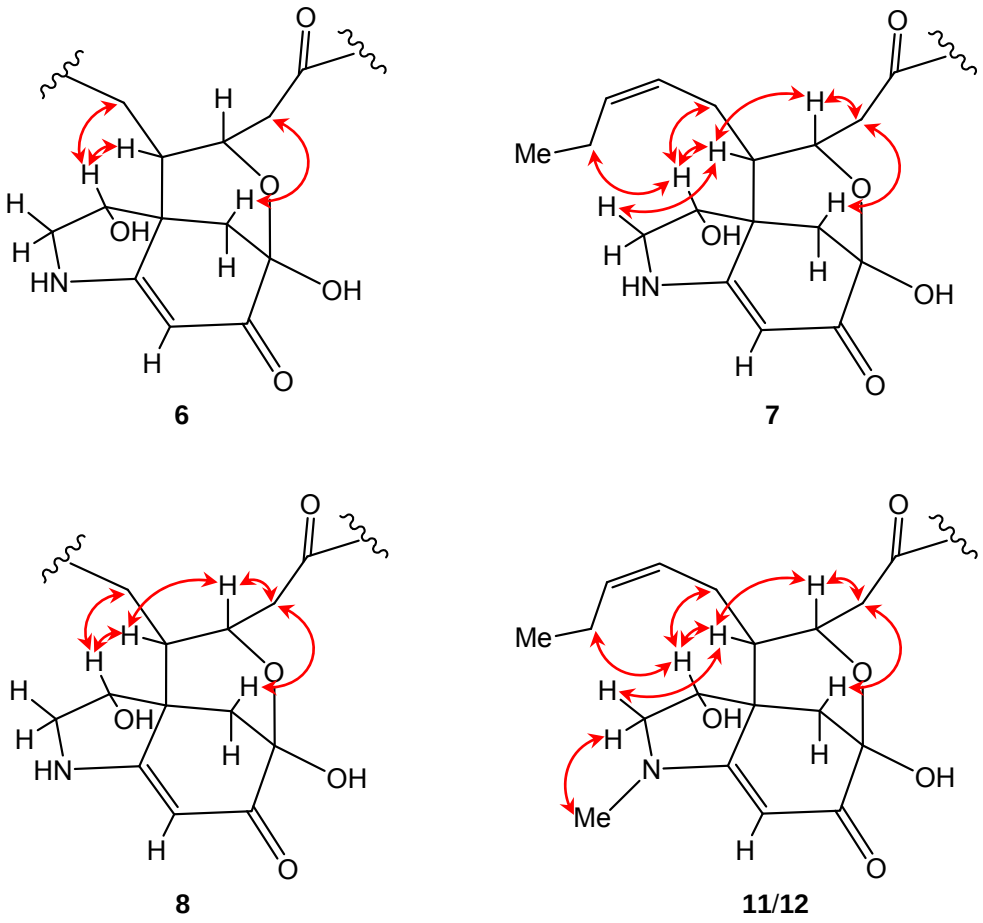


Figure 3. Kai et al.

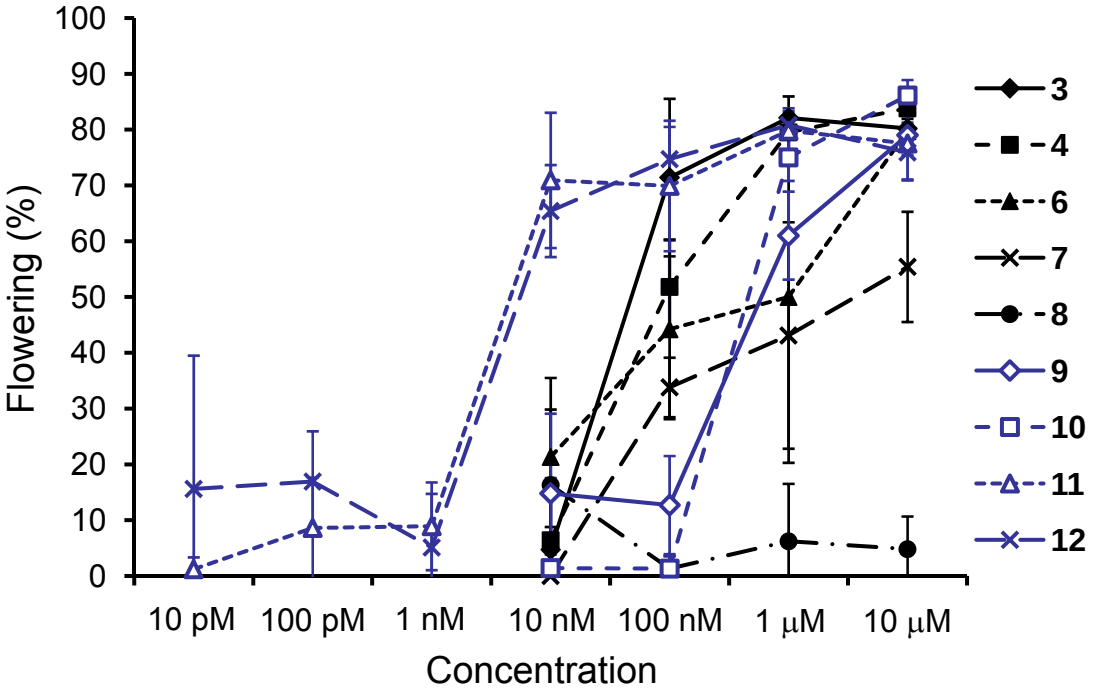


Figure 4. Kai et al.

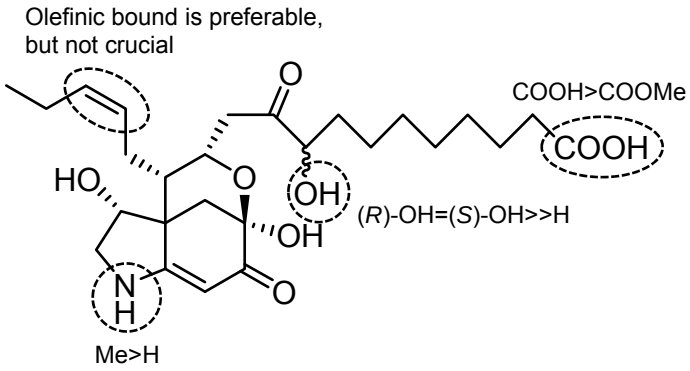


Figure 5. Kai et al.