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REGULAR ARTICLE

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Structure–activity relationship study of the anti-obesity natural product yoshinone A

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Abstract

Yoshinone A was derived from marine algae and shown to inhibit adipogenic differentiation. The natural compound is composed of a γ-pyrone ring and a side chain and that contains two asymmetric carbons. Although their absolute configuration has been determined, there is no information available on the stereoisomers and their bioactivities. To address this question, we synthesized all four stereoisomers and measured their activities. We also prepared three more derivatives of yoshinone A and found that the stereo-configuration inside the side chain, the γ-pyrone ring, and bulkiness of the side chain all played important roles in its activity. Our findings should help to elucidate the mechanism of action of yoshinone A.

KEYWORDS

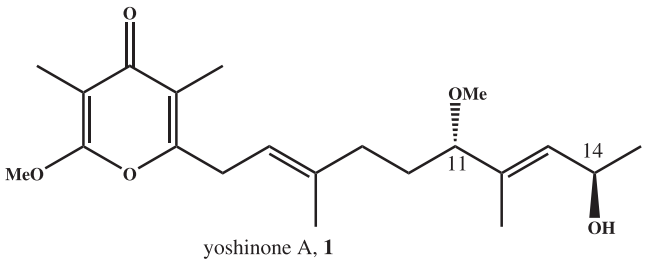
adipocytes, natural products, obesity, structure–activity relationship, yoshinone A

1 | INTRODUCTION

The chirality of bioactive molecules frequently influences their pharmacological activity. One of the most famous examples is thalidomide, which was first brought to the market to treat insomnia. However, it was soon revealed that the drug affected fetal development, in a condition called thalidomide embryosis. While the *R* enantiomer is believed to induce somnolence, the *S* enantiomer is thought to be teratogenic.[1,2] A recent study identified the cellular binding protein of the *S* enantiomer of thalidomide.[3,4] Another example is Bay-K-8644, which acts on a calcium channels. While the *S* enantiomer functions as an agonist, the *R* enantiomer acts as an antagonist.[5–7] Thus, it is important to investigate the differences in the activities of asymmetric compounds for drug development.

Yoshinone A (**1**, Figure 1), a natural product derived from marine blue-green algae, *Leptolyngbya* sp., has been identified as an inhibitor of adipogenic differentiation in the mouse fibroblastic cell line 3T3-L1.[8] It has also been shown to reduce the increase in body weight induced by the administration of a high-fat diet.[9] These results indicated that the natural product should be a promising candidate to treat obesity.

This Paper was dedicated to the late Professor Koji Nakanishi for his great contribution to natural products.



yoshinone A, **1**

FIGURE 1 Chemical structure of yoshinone A

In contrast to its pharmacological activity in vivo, a little was known about the structure of the compound that is responsible for this activity. Yoshinone A consists of a γ-pyrone ring and a side chain. Although our previous study showed that a conjugation state between the γ-pyrone ring and an olefin located in the side chain was important for blocking adipogenesis in 3T3-L1 cells,[8] the contributions of other parts of the compound to its inhibitory effects were not clear. Especially, there was no information on the relationship between the stereo-configuration of yoshinone A and its bioactivity, although the absolute configuration within the side chain region of the compound was determined through its total synthesis.[10] In this study, we tried to study the structure–activity relationship of yoshinone A using all four stereoisomers. We also tested if the γ-pyrone ring, hydroxy group, or methoxy group in the side chain was necessary for the activity. Our efforts contribute to the development of a yoshinone A-based anti-obesity medicine or research reagent for studying fat metabolism.

1.1 | General

All reagents were ultra-pure grade and purchased from WAKO Pure Chemical or Nacalai Tesque. NMR spectra were recorded by a JEOL JNM-ECS 400 FT NMR system. MS spectra were recorded with a Bruker APCI TOF-MS. Cell culture media and insulin were purchased from Gibco. Mouse fibroblastic 3T3-L1 cells were maintained in DMEM supplemented with 10% calf serum, and HeLa cells were maintained in DMEM supplemented with 10% fetal calf serum at 37°C in a 5% CO_2 atmosphere and divided every 2 or 3 days before they reached confluence.

1.2 | Adipogenic differentiation

The induction of adipose cells from 3T3-L1 cells was performed as described elsewhere.[11] Briefly, 3T3-L1 cells were seeded onto 96-well plates at a density of 5×10^4 cells in each well. After 2 days, the cells were induced to undergo adipogenic differentiation by insulin in the presence of various concentrations of yoshinone A or its derivatives. The culture media were replaced with fresh media without insulin or yoshinone A derivatives every 2 days. After 7 days of induction, intracellular triglyceride levels were determined as an indicator of the degree of differentiation by a LabAssay Triglyceride Kit (WAKO) according to the manufacturer's instructions, and the IC_{50} values were then determined.

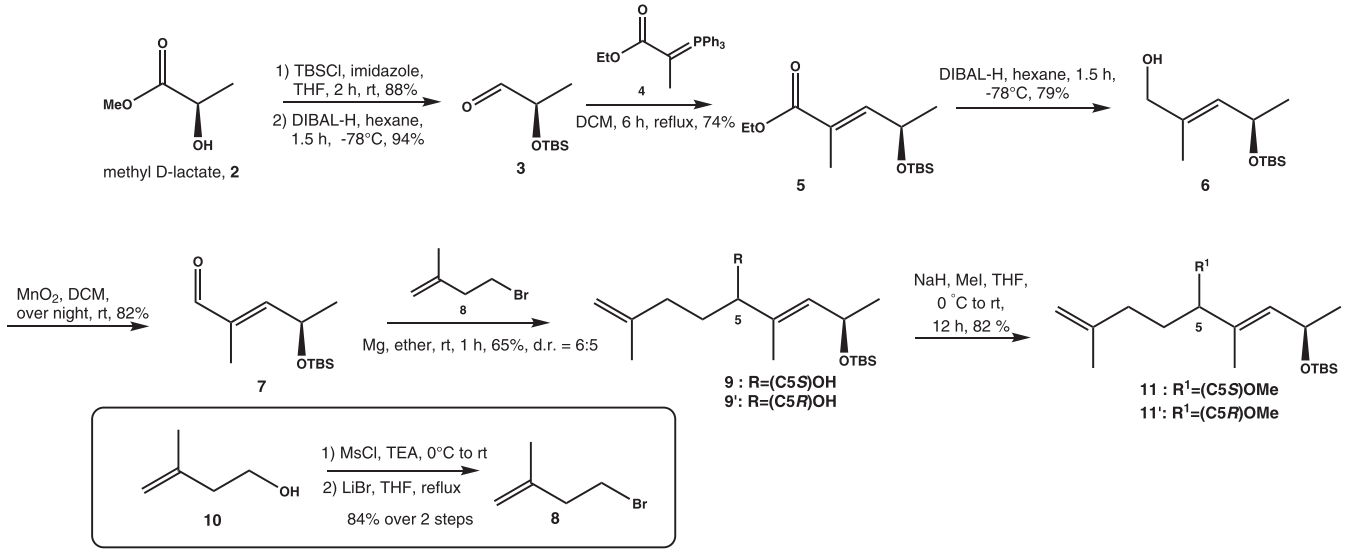
1.3 | Measurement of lactate levels

HeLa cells were placed in 96-well plates at a density of 1×10^4 cells in each well. On the next day, the culture media were replaced to with fresh media containing various concentrations of yoshinone A or its derivatives and cultured for an additional 2 days. Lactate levels in the culture media were measured with a Lactate Assay Kit-WST (Dojindo) according to the manufacturer's instructions. The EC_{50} values were then determined.

2 | RESULTS AND DISCUSSION

2.1 | Synthesis of compound 14 (a synthetic yoshinone A) and its diastereomer 16

After protection of the secondary alcohol of methyl D-lactate **2** with TBSCl, reduction with DIBAL led to the formation of TBS-protected aldehyde **3** in 82% yield in two steps (Scheme 1). Aldehyde **3** was converted into the ethyl ester **5** in 74% yield by the Wittig reaction with phosphorane **4**. DIBAL reduction of ethyl ester **5** was expected to give the corresponding aldehyde as in the case of compounds **2** to **3**; however, the reaction gave corresponding allyl alcohol **6**. This was explained by considering that the α,β-unsaturated hemiaminal intermediate was rapidly decomposed to give an undesired alcohol even at low temperature. For conversion to an aldehyde, alcohol **6** was subjected to oxidation with MnO_2 under reflux to give the α,β-unsaturated aldehyde **7** in 64% yield in two steps. To link the two fragments, aldehyde **7** was exposed to a Grignard reaction with easily available bromide **8** to form exo-olefin **9** as a mixture of diastereomers with respect to the hydroxy group (**9′**). We separated each diastereomer by silica gel column chromatography and determined its stereochemistry by means of the modified Mosher's method. The proton NMR spectra of the two synthesized MTPA esters (Figures S1 and S2) were



SCHEME 1 Synthetic pathway to compounds **11** and **11′**

carefully compared to investigate their differences. As a result, the left and right signs were divided around the MTPA plane (Figure 2). Therefore, we concluded that the stereochemistry of the stereotype applied was *S*. Conversion of secondary hydroxyl to a methoxy group occurred smoothly by methylation using MeI and NaH. On the other hand, three-step synthesis from dimethyl 3-oxopentandioate gave allyl γ-pyrone **12**, as reported previously.[12]

To determine the full carbon frameworks, we turned to the cross-metathesis reaction with Hoveyda-Grubbs second generation catalyst (HG-II). A terminal olefin can easily homodimerize during the cross-metathesis reaction.[13] Diluted allyl γ-pyrone was slowly added to 4 equivalents of exo-olefin **11** to prevent homodimerization to give the desired coupling product **13**. Following the deprotection of **13** in AcOH/THF/H_2O = 3:1:1, we obtained the desired compound **14** (Scheme 2). Additionally, we synthesized **16** which is a diastereomer at the C11 methine of **14** from the epimer **11′** by the same reaction sequence.

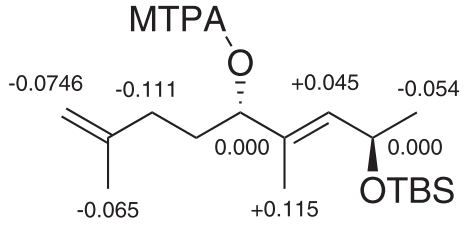


FIGURE 2 $\Delta\delta^{RS}$ values from the ^{1}H-NMR spectra of different MTPA esters

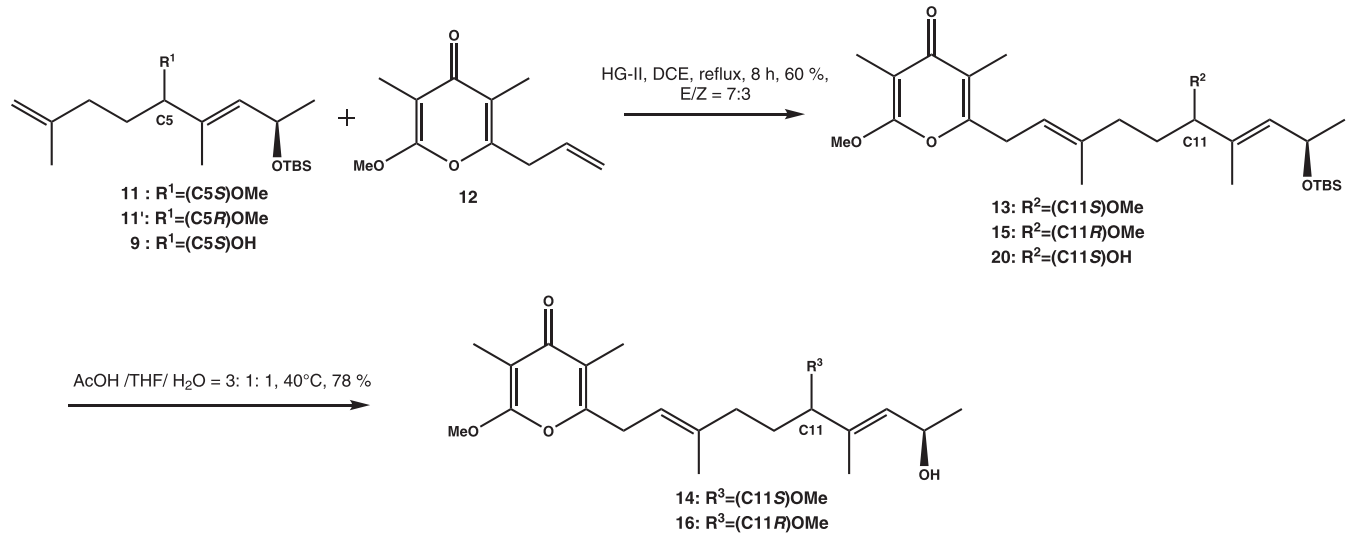
2.2 | Synthesis of compound 17, an enantiomer of compound 14, and its diastereomer 19

Yoshinone A contains two asymmetric carbon atoms, indicating that there are four stereoisomers including yoshinone A itself. We prepared two of these four compounds. To complete structure–activity relationship study of all stereoisomers of yoshinone A, next, we tried to synthesize the remaining two stereoisomers, **17** and **19**. We commenced the synthesis of **17** from methyl L-lactate **18** via the same pathway to **14**. Compound **19**, an enantiomer of **16**, was synthesized by oxidation of allyl alcohol by means of MnO_2 followed by Luche reduction (Scheme 3). Reduction gave a racemate of **14** and **19**. We separated the diastereomers by column chromatography and determined their identities by comparing their NMR spectra.

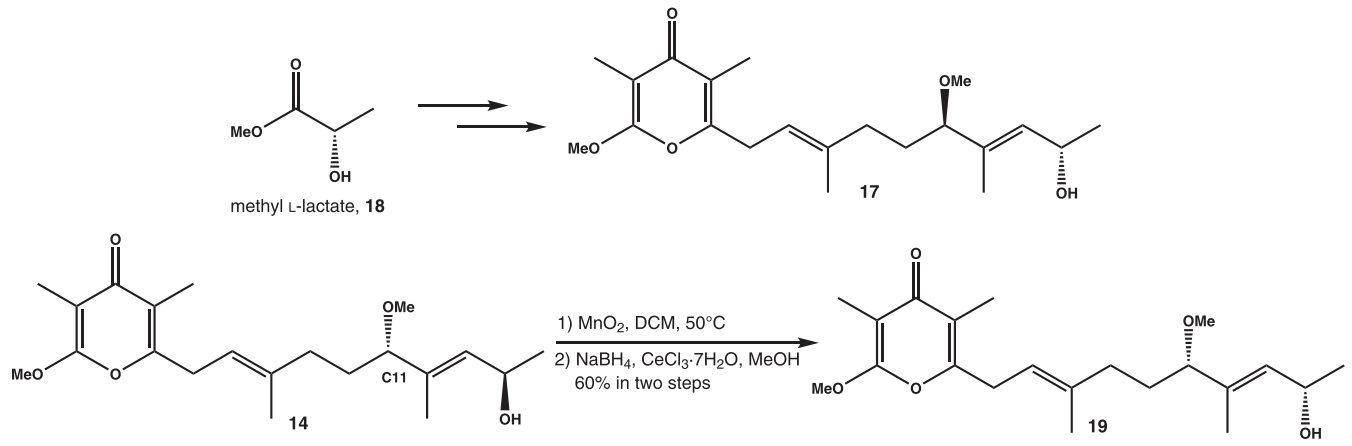
2.3 | Synthesis of compounds 20, 21, and 23

Next, we focused on the structure–bioactivity relationship around C11 and C14. A cross-metathesis reaction between **9** and **12** led to the synthesis of C14-protected allyl alcohol **20** (Scheme 2).

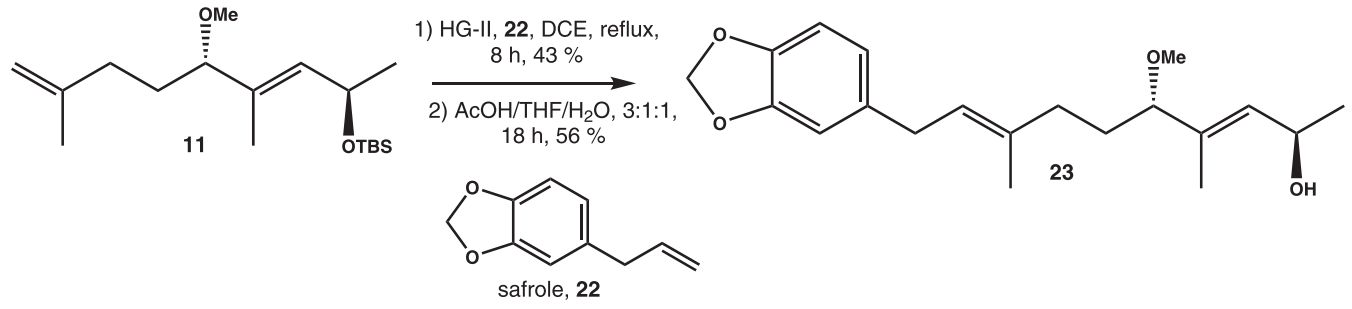
Furthermore, we examined the effect of the pyrone ring at the terminal moiety of yoshinone A. Commercially available safrole **21** was exposed to cross-metathesis followed by deprotection of silyl ether to give the desired derivative **22** in 24% yield over two steps (Scheme 4).



SCHEME 2 Synthetic pathway to the stereoisomers



SCHEME 3 Synthetic pathway to stereoisomers **17** and **19**



SCHEME 4 Synthesis of yoshinone A derivative **22**

2.4 | Activities of natural and synthetic yoshinone A (compounds 1 and 14)

Finally, we evaluated the activities of natural and synthetic yoshinone A and its derivatives. The adipogenic differentiation of 3T3-L1 cells has been used for purpose.[8] Although this system was sensitive enough to evaluate yoshinone A and its derivatives, it took more than 1 week, indicating that it was not suitable for processing many samples. Thus, we tried to

develop a novel evaluation system to estimate the activities of the derivatives. In a previous study, we showed that the lactate level in the culture medium increased in parallel with the dose of yoshinone A.[9] Based on this observation, we speculated that the lactate level could be helpful for evaluating yoshinone A and its derivatives. To verify this idea, we tried to compare the lactate-producing effect to the adipogenic differentiation-inhibitory effect of yoshinone A. We measured the lactate levels in the HeLa cell culture medium after 2 days of incubation or triglyceride levels in 3T3-L1 cell adipocytes after 7 days of induction with insulin in the presence of various concentrations of natural yoshinone A. As a result, the EC_{50} value of the lactate-producing activity was comparable with the IC_{50} value of the adipogenesis-inhibitory activity of yoshinone A (130 and 308 nM, respectively; Figure 3A, B). We also compared the EC_{50} and IC_{50} values of synthetic yoshinone A, compound **14**. Again, these values were comparable (150 and 297 nM, respectively; Figure 3C,D). These results suggested that lactate-producing activity should be a simple tool for evaluating yoshinone A derivatives.

2.5 | Evaluation of yoshinone A derivatives

Next, we examined the lactate-producing activities of yoshinone A stereoisomers, compounds **16**, **17**, and **19**. Compounds **16** and **17** did not exhibit strong lactate-producing activity and showed higher EC_{50} values (64.5 mM for compound **16**, 1.15 mM for compound **17**; Figure 4A,B). On the other hand, compound **19** hardly showed lactate-producing activity. As shown in Figure 4C, the effect was limited, and only a trace level of lactate production was observed.

Based on these results, the activity of compound **14** very closely matched that of the natural product yoshinone A, suggesting that the configuration of yoshinone A should be the same as that of compound **14**. This conclusion is consistent with the stereochemistry determined by total synthesis.[10]

Next, we focused on the structure–activity relationship around C11 and C14. We changed the C11 methoxy group to a hydroxy group (compound **20**) or introduced a TBS group at a C14 hydroxyl group

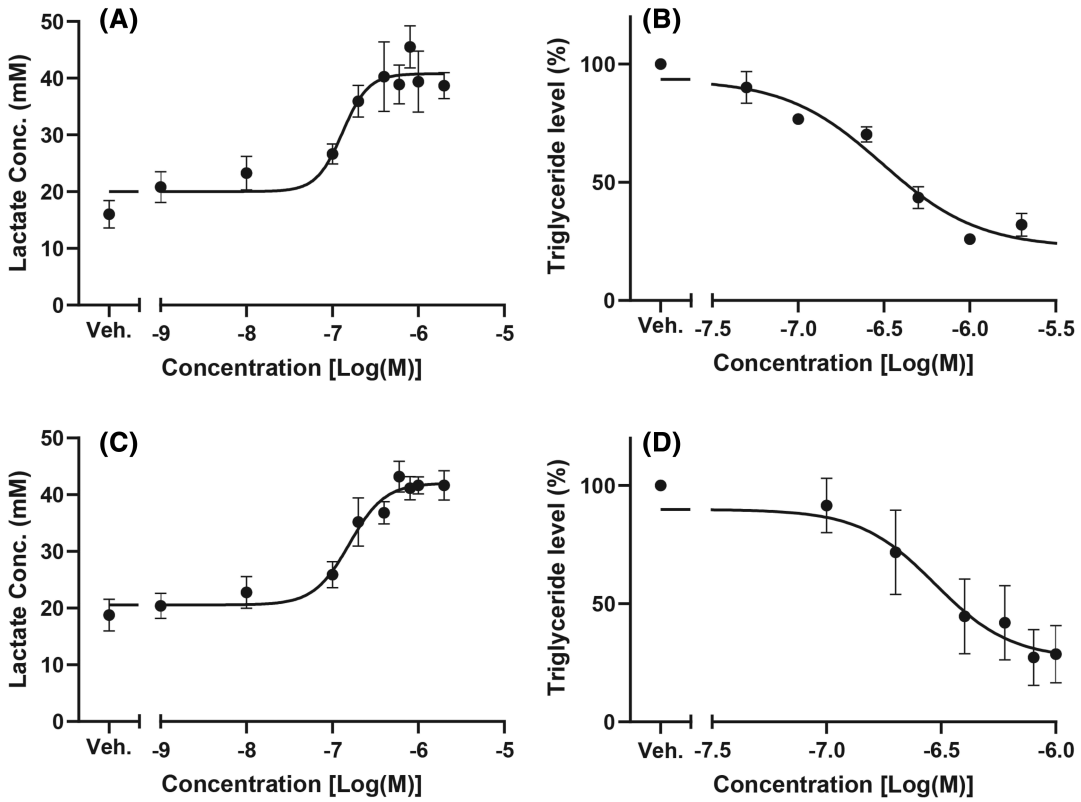


FIGURE 3 Activities of yoshinone A and compound **14**. Cells were treated with yoshinone A (A and B) or compound **14** (C and D), their lactate-producing activity (A and C) and adipogenesis-inhibiting activity (B and D) were measured. Each value is the mean ± SE of triplicate determinations

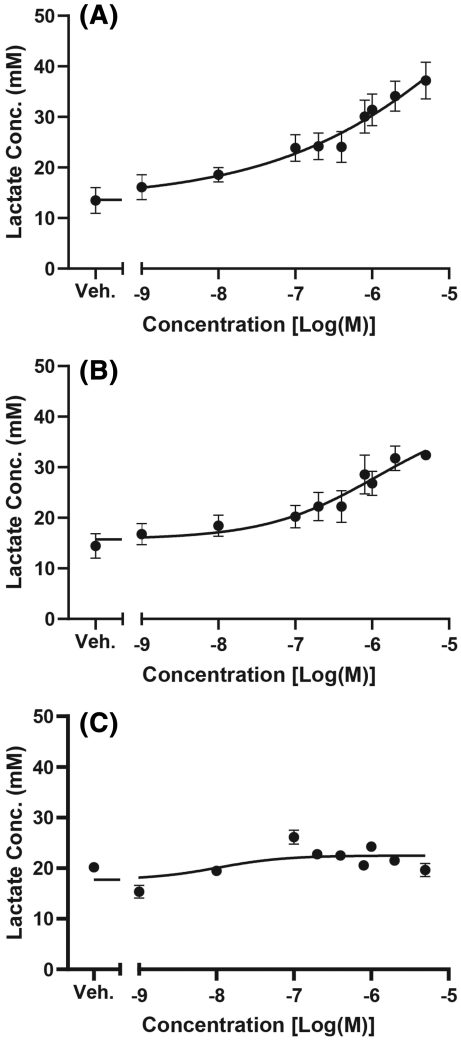


FIGURE 4 Activities of yoshinone A stereoisomers. Lactate-producing activities of compounds **16** (A), **17** (B), and **19** (C) were examined. Each value is the mean ± SE of triplicate determinations

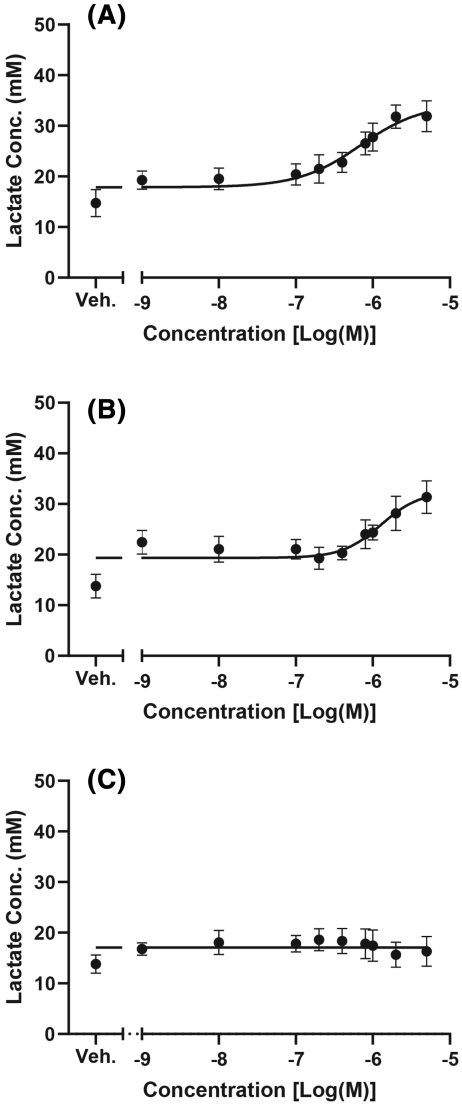


FIGURE 5 Activities of yoshinone A derivatives. Lactate-producing activities of compounds **20** (A), **13** (B), and **22** (C) were examined. Each value is the mean of triplicate determinations

(compounds **20** and **13**). While the introduction of silyl group to a medicine or its candidate is rare, there are some examples, including synthetic retinoid TAC-101 and DNA topoisomerase inhibitor karenitecin.[14] Both compounds showed weak activity only at a higher concentration (EC_{50} values were 685 nM and 1270 nM, respectively), indicating that the introduction of a bulky group at C14 abolished the activity (Figure 5A,B). Furthermore, we tried to determine the importance of the pyrone ring at the terminal moiety of yoshinone A and modified it (compound **22**). Interestingly, compound **22** did not enhance lactate production at all in HeLa cells (Figure 5C). This result indicated that the pyrone ring was indispensable for this activity.

3 | CONCLUSION

We isolated yoshinone A, which is an anti-obesity candidate derived from cyanobacteria in Okinawa, Japan. The compound showed clear inhibition of the adipogenic differentiation of 3T3-L1 cells.[8] Although its stereochemistry was determined by total synthesis,[10] there was no information available about the structure–activity relationship of the stereoisomers. In this report, we synthesized all four stereoisomers of yoshinone A and compared their activities. We demonstrated that the 11*S*, 14*R* isomer (same as the natural product) showed much stronger bioactivity than the others. Our findings support the absolute configuration of yoshinone A, which was previously determined through total synthesis, from the

point of view of bioactivities, suggesting that studies on the structure–activity relationship of chiral compounds can help to determine the absolute configuration of natural compounds. We also showed that both the pyrone ring and the bulkiness of the side chain were important for this activity of yoshinone A. These results will be helpful for elucidating the mechanism of action of yoshinone A.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding authors.

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REFERENCES

1. Franks ME, Macpherson GR, Figg WD. Thalidomide. *Lancet*. 2004;363(9423):1802-1811.
2. Vargesson N. The teratogenic effects of thalidomide on limbs. *J Hand Surg Eur Vol*. 2019;44(1):88-95.
3. Ito T, Ando H, Suzuki T, et al. Identification of a primary target of thalidomide teratogenicity. *Science*. 2010;327(5971):1345-1350.
4. Asatsuma-Okumura T, Ando H, De Simone M, et al. p63 is a cereblon substrate involved in thalidomide teratogenicity. *Nat Chem Biol*. 2019;15(11):1077-1084.
5. Bechem M, Schramm M. Calcium-agonists. *J Mol Cell Cardiol*. 1987;19(Suppl 2):63-75.
6. Triggle DJ, Langs DA, Janis RA. Ca^{2+} channel ligands: structure-function relationships of the 1,4-dihydropyridines. *Med Res Rev*. 1989;9(2):123-180.
7. Triggle DJ, Rampe D. 1,4-Dihydropyridine activators and antagonists: structural and functional distinctions. *Trends Pharmacol Sci*. 1989;10(12):507-511.
8. Inuzuka T, Yamamoto K, Iwasaki A, et al. An inhibitor of the adipogenic differentiation of 3T3-L1 cells, yoshinone A, and its analogs, isolated from the marine cyanobacterium Leptolyngbya sp. *Tetrahedron Lett*. 2014;55(49):6711-6714.
9. Koyama T, Kawazoe Y, Iwasaki A, Ohno O, Suenaga K, Uemura D. Anti-obesity activities of the yoshinone A and the related marine γ-pyrone compounds. *J Antibiot*. 2016;69(4):348-351.
10. Shinomiya S, Iwasaki A, Ohno O, Suenaga K. Total synthesis and stereochemical determination of yoshinone A. *Phytochemistry*. 2016;132:109-114.
11. Choi Y, Kawazoe Y, Murakami K, Misawa H, Uesugi M. Identification of bioactive molecules by adipogenesis profiling of organic compounds. *J Biol Chem*. 2003;278(9):7320-7324.
12. De Paolis M, Rosso H, Henrot M, Prandi C, d'Herouville F, Maddaluno J. A concise route to α′-methoxy-γ-pyrones and verticipyrone based upon the desymmetrization of α,α′-dimethoxy-γ-pyrone. *Chem a Eur J*. 2010;16(37):11229-11232.
13. Chatterjee AK, Choi T-L, Sanders DP, Grubbs RH. A general model for selectivity in olefin cross metathesis. *J am Chem Soc*. 2003;125(37):11360-11370.
14. Ramesh R, Reddy DS. Quest for novel chemical entities through incorporation of silicon in drug scaffolds. *J Med Chem*. 2018;61(9):3779-3798.

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