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CONCISE PREPARATION AND BIOLOGICAL EVALUATIONS OF 9-*cis*-RETINOIC ACID ANALOGUES HAVING AN AROMATIC RING

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This paper is dedicated to Professor Yasuyuki Kita on the occasion of his 77th birthday.

Abstract – A series of 9-*cis*-retinoic acid analogues having an aromatic ring were prepared in only two steps, and were evaluated for transcriptional activities with retinoic acid response element (RARE) and retinoid X response element (RXRE). Among them, compound **6c**, bearing a 2-naphthyl substituent, exhibited the highest transcriptional activity with RXR selectivity.

INTRODUCTION

Retinoid X receptors (RXRs; isotypes α, β, and γ) belong to a nuclear receptor superfamily. RXRs exist as homodimers with themselves or heterodimers with other nuclear receptors, including retinoic acid receptors (RARs), liver X receptors (LXRs), peroxisome proliferator-activated receptors (PPARs), farnesoid X receptors (FXRs), thyroid receptors (TRs), and vitamin D receptors (VDRs).[1] Such dimers modulate gene transcription through binding of corresponding ligands. Although RXR agonists cannot solely activate RAR/RXR (non-permissive effect),[2] the use of the combination of RXR agonists and RAR agonists activates RAR/RXR heterodimers (retinoid synergist effect).[3] On the other hand, other RXR heterodimers can be activated by RXR agonists (permissive effect).[4] Thus, RXR-selective agonists might not modulate RAR/RXR, but can influence other RXR heterodimers, and are candidates for the treatment of metabolic syndrome through PPARs (lipid metabolism), LXRs (cholesterol metabolism), and FXRs (lipid metabolism).[5]

9-*cis*-Retinoic acid **1**, a stereoisomer of all-*trans*-retinoic acid **2** (ATRA), is a native agonist of RXRs, and also acts as a RAR agonist (Figure. 1). This result implies that **1** cannot be used as a drug for metabolic syndrome because it induces a side effect, so-called retinoic acid syndrome.[6] Therefore, the development of RXR-selective agonists based on the structure-activity relationship of **1** have been conducted. We have previously reported the preparation and biological evaluation of 9-*cis*-retinoic acid analogues, which replace the 2,2,6-trimethylcyclohexene ring or its adjacent C7-8 double bond with an aromatic ring,[7] because aromatic ring-containing retinoid analogues, namely arotinoids, are known to interact with RAR and RXR.[8] However, these synthetic routes included the tedious separation of undesired stereoisomers and were based on linear syntheses, making these earlier synthetic pathways unsuitable for the syntheses of a wider variety of their analogues. Aiming to improve these problems, we accomplished a highly efficient and rapid total synthesis of **1** and its analogues by CsF-promoted Stille coupling reaction as a key step.[9] This methodology enabled a convergent synthesis of 9-*cis*-retinoic acid analogues in only two steps from stannanyl ester **3** and vinyl triflates without the *cis-trans* isomerization of the double bonds. In the present study, we applied this synthetic strategy to the systematic preparation of arotinoids that contain either an aromatic or a heteroaromatic ring. Furthermore, these analogues were tested for transcriptional activities with RARE, RXRE, and RXRα-GAL4.

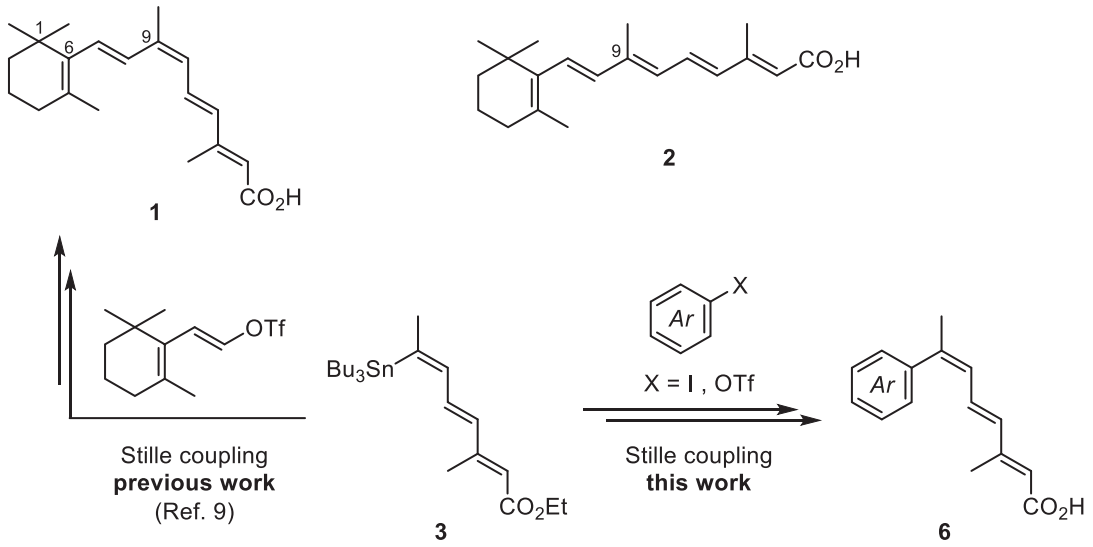
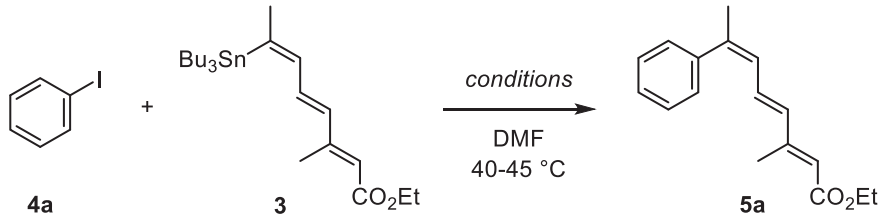


Figure 1. Structures of 9-*cis*-retinoic acid (**1**), all-*trans*-retinoic acid (**2**), and synthetic strategy for creating **1** and its analogues **6**

RESULTS AND DISCUSSION

For an optimization of the Stille coupling reaction conditions with stannanyl ester **3** and aromatic component, we selected iodobenzene **4a** as an aromatic partner (Table 1). A model study revealed that while our previous coupling conditions [$Pd_2(dba)_3 \cdot CHCl_3$, $AsPh_3$, CsF, DMF, 40-45 °C][9] yielded **5a** in only 27% after 24 h along with recovered **3** (40%) (entry 1), Baldwin's procedure [$Pd(PPh_3)_4$, CuI, CsF, DMF, 40-45 °C, 3 h][10] afforded **5a** in 81% yield (entry 2). Notably, the prolongation of the reaction time to 15 h in the Baldwin conditions did not affect the geometry of the *cis*-double bond and afforded **5a** in 86% yield (entry 3). Thus, we decided to employ the optimized conditions of entry 3 for all further studies.

Table 1. Optimization for Stille coupling with iodobenzene **4a** and trienyl stannane **3**

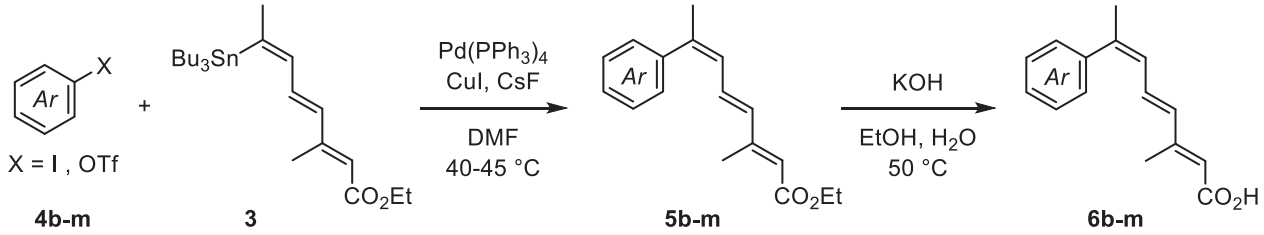


entry	Pd source (mol%)	additive (mol%)	**4a** : **3** (eq.)	time (h)	**5a** (% yield)[a]
1	$Pd_2(dba)_3 \cdot CHCl_3$ (4)	$AsPh_3$ (16), CsF (200)	1.1 : 1	24	**5a** (27)
2	$Pd(PPh_3)_4$ (10)	CuI (20), CsF (200)	1 : 1.3	3	**5a** (81)
3	$Pd(PPh_3)_4$ (10)	CuI (20), CsF (200)	1 : 1.3	15	**5a** (86)

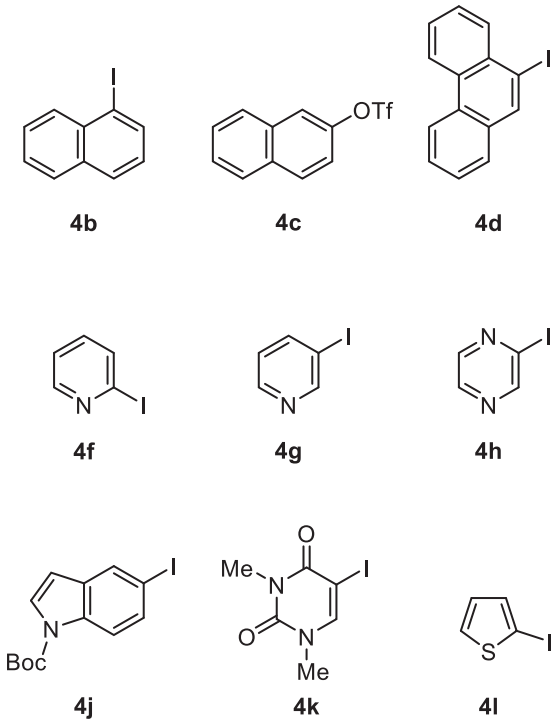
[a] Isolated yields.

We next examined the Stille coupling reactions of stannanyl ester **3** with various aromatic components **4b-m** (Table 2). Not only polycyclic aromatic iodides **4b**, **4d** and **4e**, but also triflate **4c** reacted smoothly under the optimized conditions to give the desired products **5b-e** in good to excellent yields (entries 1-4). Furthermore, in the case of using *N*- and *S*-heteroaromatic iodides **4f-m**, we obtained coupled products **5f-m** in good to high yields (entries 5-12). In our previous work, **5l** was prepared from 2-thienylboronic acid in 3 steps and 45% overall yield along with 13-*cis*-isomer of **5l**.[7a] Thus, we improved the synthetic route and reduced the number of steps. The contamination of tin residues derived from the Stille coupling reaction were removed completely by column chromatography using KF-silica as a stationary phase.[11] The coupled products **5b-m** were hydrolyzed under basic aqueous conditions to afford the desired arotinoids **6b-m** in good yields except for **6i**. On the occasion of the hydrolysis step of **5j**, the Boc-deprotected product **6j** was obtained due to the acidic quench (5% HCl aqueous solution). Compounds **6b-m** were relatively stable to light and could be stored for a long period in the freezer.

Table 2. Stille coupling with aromatic and heteroaromatic iodides **4b-m** and trienyl stannane **3**

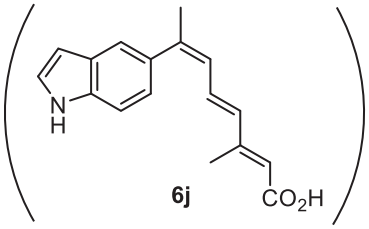


4b-m **3** **5b-m** **6b-m**



4b **4c** **4d** **4e** **4f** **4g** **4h** **4i** **4j** **4k** **4l** **4m**

entry	**5** (% yield)[a]	**6** (% yield)[a]
1	**5b** (78)	**6b** (90)
2	**5c** (92)	**6c** (79)
3	**5d** (76)	**6d** (93)
4	**5e** (69)	**6e** (88)
5	**5f** (78)	**6f** (90)
6	**5g** (80)	**6g** (49)
7	**5h** (76)	**6h** (89)
8	**5i** (62)	**6i** (0)[b]
9	**5j** (80)	**6j** (30)[c]
10	**5k** (88)	**6k** (96)
11	**5l** (65)	**6l** (57)
12	**5m** (75)	**6m** (76)



6j

[a] Isolated yields.
[b] Unknown products were obtained.
[c] Boc group-deprotected product **6j** was obtained.

With the 9-*cis*-retinoic acid analogues **6b-m** in hand, we evaluated them in three kinds of transcriptional assays (Figure. 2). In the transcriptional assays for a human RARβ gene retinoic acid responsive element (RARE), none of the analogues exhibited higher activity than that of ATRA and 9-*cis*-retinoic acid **1** (Figure. 2A). Using the transcriptional activities on a rat CRABPII-RXRE luciferase reporter gene, compounds **6c** (2-naphthyl), **6e** (2-fluorenyl), and **6j** (5-indolyl) showed higher activity than **1** (Figure. 2B). In order to clarify whether RXRE transcriptional activity was reflected by the binding affinity of RXR, the analogues were tested in the RXRα (mainly expressed in liver) binding assay using RARα-GAL4 hybrid luciferase assay (Figure. 2C). Among them, **6c** (2-naphthyl) exhibited the highest transcriptional activity.

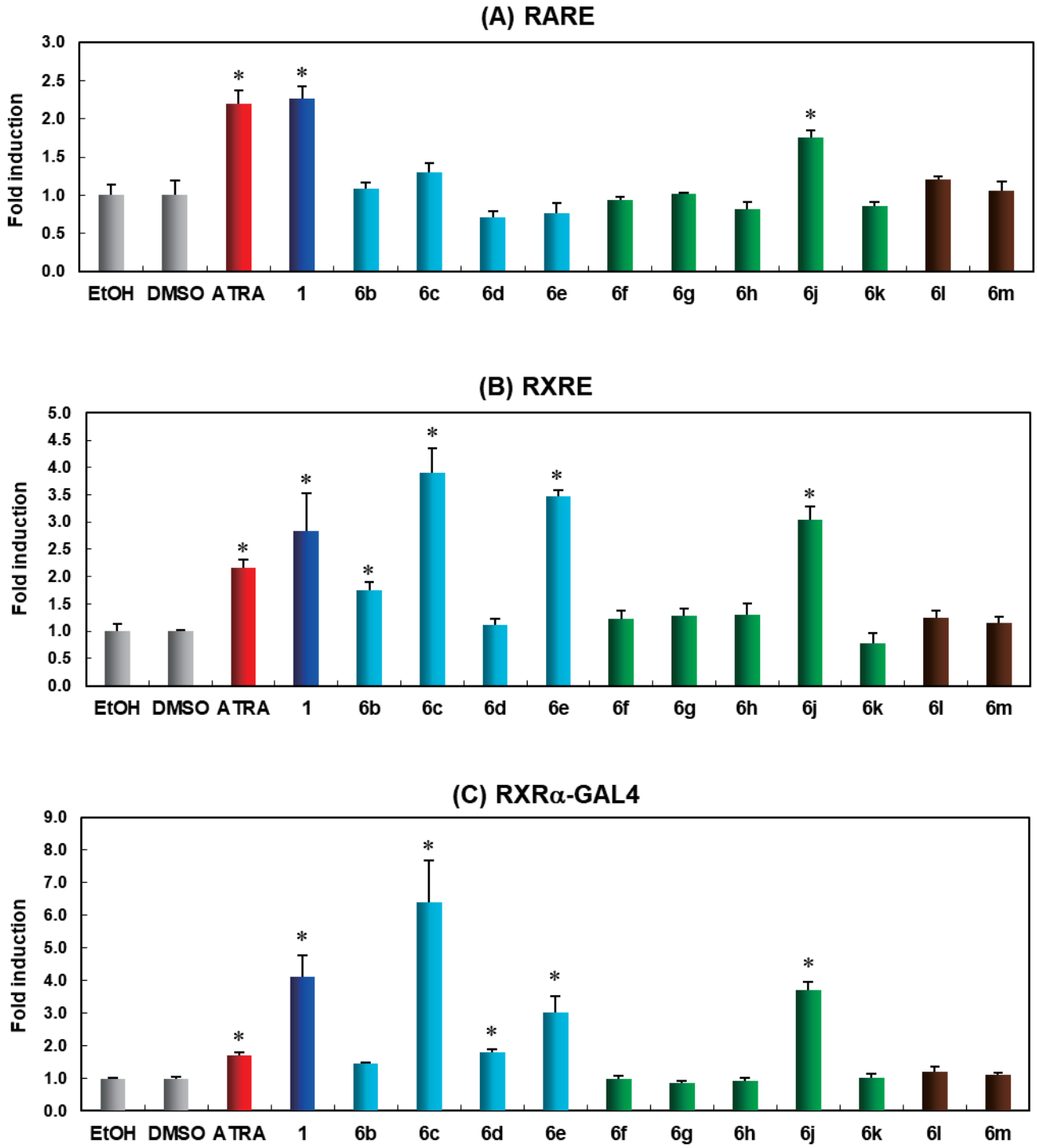


Figure 2. (A) Transcriptional potency of ATRA, **1**, and its analogues in a human RARβ-RARE luciferase reporter gene in transfected MG-63 cells. (B) Transcriptional potency of ATRA, **1**, and its analogues in a rat CRABPII-RXRE luciferase reporter gene in transfected MG-63 cells. (C) Transcriptional potency of ATRA, **1**, and its analogues on a human RXRα-GAL4 hybrid luciferase assay in transfected MG-63 cells. In all panels, light blue bars correspond to carbo-aromatic series **6b-e**, green bars correspond to *N*-heteroaromatic series **6f-k**, and brown bars correspond to thiophene series **6l-m**. Significant differences: ∗ $p < 0.05$ (vs EtOH), Dunnett's test.

To confirm in greater detail, dose-dependent transcriptional activity toward RXRα was tested for selected analogues **6c**, **6e** and **6j** (Figure. 3). Among them, 2-naphthyl analogue **6c** showed highest activity in RXRα-GAL4. It was noteworthy that **6c** expressed higher transcriptional activity than that of **1**, the native ligand of RXR. These results indicated that **6c** might be twisted at the naphthalene-polyene C-C bond to form an L-shaped conformation, a configuration which is crucial for adapting RXR ligand binding pocket (LBP).[2] While 2-fluorenyl analogue **6e** might be also twisted at the same C-C bond, it is bulkier than **1** and **6c**, and so may be less able to adopt a favorable conformation with RXR LBP. Compared with **6c**, 5-indolyl analogue **6j** showed poor results. That outcome implies that the indole part of **6j** is less fitted toward RXR LBP of the lipophilic domain than the naphthalene moiety of **6c**.

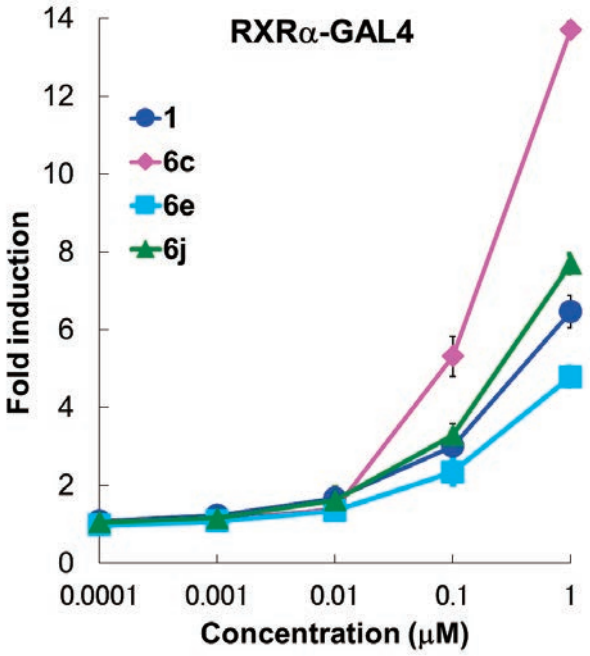


Figure 3. Dose-dependent transcriptional activities of RXRα-GAL4

In summary, we have prepared retinoic acid analogues **6b-m** by a CsF-promoted Stille coupling reaction. Our synthetic methodology easily provided a wide variety of aromatic and heteroaromatic ring-containing retinoic acid analogues. In the structure-activity relationship study, we found that 2-naphthyl analogue **6c** had a selective RXR transcriptional activity against RAR and a higher RXR-transcriptional activity than 9-*cis*-retinoic acid **1**. This research provides a powerful, convergent synthetic method for 9-*cis*-retinoic acid analogues and holds great implications for the research field of nuclear receptors. Additional investigations of other RXR-selective agonists, their isotype selectivity, and their efficacies in treating metabolic disease are ongoing.

EXPERIMENTAL

General

Melting points were determined on a micro melting point apparatus (Yanagimoto) and are uncorrected. UV-vis spectra were recorded on a JASCO Ubest-55 or JASCO V-650 instrument. IR spectra were measured on a Perkin Elmer FT-IR spectrometer, model Paragon 1000 or Horiba FT-IR spectrometer, model FT-720, using $CHCl_3$ unless otherwise noted. ^1H NMR and ^{13}C NMR spectra were determined on a Varian Gemini-300 or a Varian Mercury-300 or a Varian VXR-500 superconducting FT-NMR spectrometer. Chemical shifts (δ) are reported in ppm relative to tetramethylsilane as internal reference ($CDCl_3$: δ = 0 ppm for ^1H) and residual solvent signal ($CDCl_3$: δ = 77.0 ppm for ^{13}C; DMSO-d_6: δ = 2.50 ppm for ^1H, δ = 39.5 ppm for ^{13}C). *J*-Values are given in Hz. *J*-Values are given in Hz. Mass spectra were taken on a Hitachi M-4100 spectrometer. Column chromatography was performed using Kanto Silica Gel 60 N (spherical, neutral). All reagents were used as obtained commercially unless otherwise noted. Compounds **6l** and **6m** were previously synthesized by us.[7a]

General procedure for Stille coupling reaction (GP1)

According to Baldwin's procedure,[10] a mixture of **4** (1.0 equiv) and **3** (1.3 equiv) was dissolved in dry DMF (0.1 M), and then CsF (2.0 equiv), $Pd(PPh_3)_4$ (10 mol%) and CuI (20 mol%) were added. The flask was evacuated and refilled with argon five times. After the mixture was stirred at 45 °C for 12-15 h (except for **4e**, 3 h), it was cooled to room temperature, and diluted with CH_2Cl_2 and water. After vigorous stirring, the mixture was filtered through Celite with CH_2Cl_2/EtOAc (1/1). The organic layer was separated, dried over Na_2SO_4, filtered, and evaporated *in vacuo*. The residue was purified by column chromatography using neutralized SiO_2/powdered KF (9/1)[11] to give a coupled product **5**.

Ethyl (2*E*,4*E*,6*Z*)-3-methyl-7-phenylocta-2,4,6-trienoate (5a)

According to **GP1**, **5a** (22.7 mg, 86%) was obtained from **4a** (20.9 mg, 0.102 mmol), **3** (72.1 mg, 0.154 mmol), CsF (31.1 mg, 0.205 mmol), $Pd(PPh_3)_4$ (11.8 mg, 10.2 μmol) and CuI (3.9 mg, 20.5 μmol). Eluent: hexane/Et_2O = 30/1. Reaction time: 15 h.

Pale yellow oil; IR 1698, 1602 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.41-7.24 (m, 5H), 6.70 (dd, *J* = 15.3, 11.1 Hz, 1H), 6.25 (br d, *J* = 15.3 Hz, 2H), 5.74 (s, 1H), 4.15 (q, *J* = 7.2 Hz, 2H), 2.19 (s, 3H), 2.15 (d, *J* = 0.6 Hz, 3H), 1.27 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.1, 152.8, 143.0, 141.0, 134.7, 132.3, 128.23, 128.18, 127.5, 127.1, 118.5, 59.6, 25.7, 14.3, 13.8; HR-EIMS Calcd for $C_{17}H_{20}O_2$ (M^+) 256.1463. Found 256.1471.

Ethyl (2*E*,4*E*,6*Z*)-3-methyl-7-(naphthalen-1-yl)octa-2,4,6-trienoate (5b)

According to **GP1**, **5** (236 mg, 78%) was obtained from **4b** (250 mg, 0.984 mmol), **3** (600 mg, 1.28 mmol), CsF (299 mg, 1.97 mmol), $Pd(PPh_3)_4$ (114 mg, 98.4 μmol) and CuI (37.5 mg, 0.197 mmol). Eluent: hexane/Et_2O = 19/1. Reaction time: 13 h.

Pale yellow oil; IR 1698, 1604 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.90-7.78 (m, 3H), 7.51-7.45 (m, 3H), 7.27-7.25 (m, 1H), 6.49 (d, *J* = 9.6 Hz, 1H), 6.24 (d, *J* = 15.3 Hz, 1H), 6.16 (dd, *J* = 15.6, 9.6 Hz, 1H), 5.70 (s, 1H), 4.11 (q, *J* = 7.2 Hz, 2H), 2.26 (d, *J* = 0.9 Hz, 3H), 1.89 (d, *J* = 0.9 Hz, 3H), 1.24 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.0, 152.7, 142.3, 139.2, 134.4, 133.7, 132.2, 130.7, 128.9, 128.4, 127.6, 126.1, 125.8, 125.5, 125.44., 125.41, 118.6, 59.5, 26.6, 14.3, 13.6; HR-EIMS Calcd for $C_{21}H_{22}O_2$ (M^+) 306.1620. Found 306.1631.

Ethyl (2*E*,4*E*,6*Z*)-3-methyl-7-(naphthalen-2-yl)octa-2,4,6-trienoate (5c)

According to **GP1**, **5** (248 mg, 92%) was obtained from **4c** (243 mg, 0.880 mmol), **3** (537 mg, 1.14 mmol), CsF (267 mg, 1.76 mmol), $Pd(PPh_3)_4$ (102 mg, 88.0 μmol) and CuI (33.5 mg, 0.176 mmol). Eluent: hexane/Et_2O = 19/1. Reaction time: 13 h.

Yellow oil; IR 1698, 1602 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.87-7.82 (m, 3H), 7.71 (d, *J* = 1.2 Hz, 1H), 7.52-7.49 (m, 2H), 7.40 (dd, *J* = 8.4, 1.8 Hz, 1H), 6.76 (dd, *J* = 15.3, 11.1 Hz, 1H), 6.34 (d, *J* = 11.1 Hz, 1H), 6.30 (d, *J* = 15.3 Hz, 1H), 5.76 (s, 1H), 4.15 (q, *J* = 7.2 Hz, 2H), 2.28 (s, 3H), 2.12 (d, *J* = 0.9 Hz, 3H), 1.27 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.1, 152.8, 142.8, 138.5, 134.9, 133.2, 132.6, 132.3, 128.0, 127.8, 127.6, 127.5, 127.1, 126.4, 126.3, 126.1, 118.6, 59.6, 25.7, 14.3, 13.8; HR-EIMS Calcd for $C_{21}H_{22}O_2$ (M^+) 306.1620. Found 306.1601.

Ethyl (2*E*,4*E*,6*Z*)-3-methyl-7-(phenanthren-9-yl)octa-2,4,6-trienoate (5d)

According to **GP1**, **5d** (222 mg, 76%) was obtained from **4d** (250 mg, 0.822 mmol), **3** (501 mg, 1.07 mmol), CsF (250 mg, 1.64 mmol), $Pd(PPh_3)_4$ (94.8 mg, 82.0 μmol) and CuI (31.3 mg, 0.164 mmol). Eluent: hexane/Et_2O = 19/1. Reaction time: 13 h.

Colorless crystals; mp 125-127 °C (EtOAc/hexane); IR 1698, 1604 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.75 (d, *J* = 8.5 Hz, 1H), 8.70 (d, *J* = 8.0 Hz, 1H), 7.88-7.84 (m, 2H), 7.69-7.674 (m, 2H), 7.63-7.56 (m, 2H), 7.52 (s, 1H), 6.53 (dd, *J* = 10.0, 1.0 Hz, 1H), 6.33-6.23 (m, 2H), 5.70 (s, 1H), 4.10 (q, *J* = 7.0 Hz, 2H), 2.28 (d, *J* = 1.0 Hz, 3H), 1.88 (d, *J* = 1.0 Hz, 3H), 1.23 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.0, 152.7, 142.3, 137.8, 134.7, 132.1, 129.3, 128.5, 126.79, 126.77, 126.62, 126.61, 126.3, 126.0, 123.0, 122.5, 118.6, 59.6, 26.5, 14.3, 13.7; HR-EIMS Calcd for $C_{25}H_{24}O_2$ (M^+) 356.1798. Found 356.1776.

Ethyl (2*E*,4*E*,6*Z*)-7-(9*H*-fluoren-2-yl)-3-methylocta-2,4,6-trienoate (5e)

According to **GP1**, **5e** (206 mg, 69%) was obtained from **4e** (253 mg, 0.865 mmol), **3** (528 mg, 1.13 mmol), CsF (263 mg, 1.73 mmol), $Pd(PPh_3)_4$ (100 mg, 86.5 μmol) and CuI (33.0 mg, 0.173 mmol). Eluent: hexane/Et_2O = 19/1. Reaction time: 3 h.

Pale yellow crystals; mp 178-181 °C (EtOAc/hexane); IR 1699, 1601 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.79 (d, *J* = 8.0 Hz, 1H), 7.78 (d, *J* = 8.0 Hz, 1H), 7.55 (d, *J* = 7.5 Hz, 1H), 7.44 (d, *J* = 1.0 Hz, 1H), 7.39 (t, *J* = 7.5 Hz, 1H), 7.31 (td, *J* = 7.5, 1.0 Hz, 1H), 7.27 (dt, *J* = 7.5, 1.0 Hz, 1H), 6.76 (dd, *J* = 15.0, 11.0 Hz, 1H), 6.29 (d, *J* = 11.0 Hz, 1H), 6.28 (d, *J* = 15.0 Hz, 1H), 5.75 (s, 1H), 4.15 (q, *J* = 7.0 Hz, 2H), 3.92 (s, 2H), 2.24 (s, 3H), 2.15 (d, *J* = 1.0 Hz, 3H), 1.27 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.1, 152.9, 143.4, 143.3, 143.2, 141.3, 141.2, 139.6, 134.6, 132.5, 127.13, 127.09, 126.8 (2C), 125.1, 124.8, 119.9, 119.5, 118.4, 59.6, 36.9, 25.8, 14.3, 13.8; HR-EIMS Calcd for $C_{24}H_{24}O_2$ (M^+) 344.1776. Found 344.1789.

Ethyl (2*E*,4*E*,6*Z*)-3-methyl-7-(pyridin-2-yl)octa-2,4,6-trienoate (5f)

According to **GP1**, **5f** (216 mg, 78%) was obtained from **4f** (220 mg, 1.07 mmol), **3** (654 mg, 1.40 mmol), CsF (326 mg, 2.15 mmol), $Pd(PPh_3)_4$ (124 mg, 0.107 mmol) and CuI (40.9 mg, 0.215 mmol). Eluent: hexane/EtOAc = 4/1. Reaction time: 13 h.

Yellow oil; IR 1699, 1604 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.67 (dq, *J* = 4.8, 1.2 Hz, 1H), 7.71 (td, *J* = 7.8, 1.8 Hz, 1H), 7.30 (dt, *J* = 6.9, 0.9 Hz, 1H), 7.21 (ddd, *J* = 7.8, 4.8, 0.9 Hz, 1H), 7.03 (dd, *J* = 15.3, 11.1 Hz, 1H), 6.40 (br d, *J* = 11.1 Hz, 1H), 6.31 (d, *J* = 15.3 Hz, 1H), 5.78 (s, 1H), 4.16 (q, *J* = 7.2 Hz, 2H), 2.26 (s, 3H), 2.21 (d, *J* = 1.2 Hz, 3H), 1.28 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.0, 158.7, 152.6, 149.3, 140.4, 136.5, 136.1, 131.7, 129.6, 123.4, 122.1, 119.1, 59.6, 24.0, 14.3, 13.7; HR-EIMS Calcd for $C_{16}H_{19}NO_2$ (M^+) 257.1438. Found 257.1416.

Ethyl (2*E*,4*E*,6*Z*)-3-methyl-7-(pyridin-3-yl)octa-2,4,6-trienoate (5g)

According to **GP1**, **5g** (220 mg, 80%) was obtained from **4g** (220 mg, 1.07 mmol), **3** (654 mg, 1.40 mmol), CsF (326 mg, 2.15 mmol), $Pd(PPh_3)_4$ (124 mg, 0.107 mmol) and CuI (40.9 mg, 0.215 mmol). Eluent: hexane/EtOAc = 4/1. Reaction time: 13 h.

Yellow oil; IR 1701, 1605 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.56-8.51 (m, 2H), 7.56 (td, *J* = 8.1, 2.1 Hz, 1H), 7.32 (ddd, *J* = 6.6, 4.8, 0.9 Hz, 1H), 6.58 (dd, *J* = 15.0, 11.1 Hz, 1H), 6.35-6.26 (m, 2H), 5.76 (s, 1H), 4.15 (q, *J* = 7.2 Hz, 2H), 2.19 (s, 3H), 2.15 (d, *J* = 1.2 Hz, 3H), 1.27 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.9, 157.7, 152.1, 149.0, 148.5, 138.5, 136.1, 135.5, 130.7, 128.7, 123.2, 119.3, 59.6, 25.3, 14.2, 13.7; HR-EIMS Calcd for $C_{16}H_{19}NO_2$ (M^+) 257.1438. Found 257.1432.

Ethyl (2*E*,4*E*,6*Z*)-3-methyl-7-(pyrazin-2-yl)octa-2,4,6-trienoate (5h)

According to **GP1**, **5h** (223 mg, 76%) was obtained from **4h** (233 mg, 1.13 mmol), **3** (690 mg, 1.47 mmol), CsF (344 mg, 2.26 mmol), $Pd(PPh_3)_4$ (131 mg, 0.113 mmol) and CuI (43.0 mg, 0.226 mmol). Eluent: hexane/EtOAc = 3/1. Reaction time: 13 h.

Yellow oil; IR 1702, 1605 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.65-8.61 (m, 2H), 8.48 (d, *J* = 2.4 Hz, 1H), 7.07 (d, *J* = 15.3, 11.1 Hz, 1H), 6.51 (d, *J* = 11.1 Hz, 1H), 6.39 (d, *J* = 15.3 Hz, 1H), 5.81 (s, 1H), 4.17 (q, *J* = 7.2 Hz, 2H), 2.29 (s, 3H), 2.23 (d, *J* = 1.5 Hz, 3H), 1.29 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.9, 154.4, 152.0, 144.3, 144.0, 142.6, 138.2, 136.3, 131.7, 130.5, 120.0, 59.7, 23.5, 14.3, 13.8; HR-EIMS Calcd for $C_{15}H_{18}N_2O_2$ (M^+) 258.1368. Found 258.1384.

***tert*-Butyl 4-((2*Z*,4*E*,6*E*)-8-ethoxy-6-methyl-8-oxoocta-2,4,6-trien-2-yl)-1*H*-pyrazole-1-carboxylate(5i)**

According to **GP1**, **5i** (178 mg, 62%) was obtained from **4i** (245 mg, 0.833 mmol), **3** (508 mg, 1.08 mmol), CsF (253 mg, 1.67 mmol), $Pd(PPh_3)_4$ (96.3 mg, 83.3 μmol) and CuI (31.7 mg, 0.166 mmol). Eluent: hexane/EtOAc = 5/1. Reaction time: 13 h.

Pale yellow oil; IR 1748, 1702, 1602 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.06 (s, 1H), 7.77 (s, 1H), 6.90 (dd, *J* = 15.0, 11.4 Hz, 1H), 6.29 (d, *J* = 15.3 Hz, 1H), 6.20 (d, *J* = 11.4 Hz, 1H), 5.77 (s, 1H), 4.15 (q, *J* = 7.2 Hz, 2H), 2.25 (d, *J* = 1.2 Hz, 3H), 2.12 (s, 3H), 1.65 (s, 9H), 1.27 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.9, 152.1, 147.4, 143.3, 135.8, 130.8, 130.6, 128.4, 127.9, 124.2, 119.3, 85.7, 59.6, 27.8, 24.7, 14.3, 13.7; HR-EIMS Calcd for $C_{19}H_{26}N_2O_4$ (M^+) 346.1881. Found 346.1893.

***tert*-Butyl 5-((2*Z*,4*E*,6*E*)-8-ethoxy-6-methyl-8-oxoocta-2,4,6-trien-2-yl)-1*H*-indole-1-carboxylate (5j)**

According to **GP1**, **5j** (280 mg, 80%) was obtained from **4j** (302 mg, 0.880 mmol), **3** (537 mg, 1.14 mmol), CsF (267 mg, 1.76 mmol), $Pd(PPh_3)_4$ (102 mg, 88.3 μmol) and CuI (33.5 mg, 0.176 mmol). Eluent: hexane/Et_2O = 19/1. Reaction time: 13 h.

Yellow oil; IR 1729, 1702, 1601 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.13 (d, *J* = 8.4 Hz, 1H), 7.62 (d, *J* = 3.6 Hz, 1H), 7.44 (d, *J* = 1.8 Hz, 1H), 7.21 (dd, *J* = 8.4, 1.5 Hz, 1H), 6.73 (dd, *J* = 15.3, 10.8 Hz, 1H), 6.57 (d, *J* = 3.0 Hz, 1H), 6.29-6.24 (m, 2H), 5.74 (s, 1H), 4.15 (q, *J* = 7.2 Hz, 2H), 2.23 (s, 3H), 2.13 (d, *J* = 0.9 Hz, 3H), 1.68 (s, 9H), 1.27 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.2, 153.0, 149.6, 143.5, 135.6, 134.4 (2C), 132.7, 130.5, 126.9, 126.5, 124.7, 120.5, 118.2, 114.8, 107.3, 83.8, 59.5, 28.2, 26.2, 14.3, 13.8; HR-EIMS Calcd for $C_{24}H_{29}NO_4$ (M^+) 395.2097. Found 395.2089.

Ethyl (2*E*,4*E*,6*Z*)-7-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-3-methylocta-2,4,6-trienoate (5k)

According to **GP1**, **5k** (241 mg, 88%) was obtained from **4k** (228 mg, 0.857 mmol), **3** (523 mg, 1.11 mmol), CsF (260 mg, 1.71 mmol), $Pd(PPh_3)_4$ (99.4 mg, 86.0 μmol) and CuI (32.6 mg, 0.171 mmol). Eluent: hexane/EtOAc = 2/1. Reaction time: 13 h.

Colorless crystals; mp 159-161 °C (EtOAc/hexane); IR 1702, 1653, 1606 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.01 (s, 1H), 6.52 (dd, *J* = 15.3, 10.8 Hz, 1H), 6.27-6.22 (m, 2H), 5.76 (s, 1H), 4.16 (q, *J* = 7.2 Hz, 2H), 3.43 (s, 3H), 3.39 (s, 3H), 2.22 (s, 3H), 2.10 (s, 3H), 1.28 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.0, 161.7, 152.0, 151.4, 141.0, 135.5, 135.1, 130.4, 130.3, 119.2, 113.3, 59.7, 36.9, 28.0, 24.0, 14.2, 13.9; HR-EIMS Calcd for $C_{17}H_{22}N_2O_4$ (M^+) 318.1580. Found 318.1571.

Ethyl (2*E*,4*E*,6*Z*)-3-methyl-7-(thiophen-2-yl)octa-2,4,6-trienoate (5l)

According to **GP1**, **5l** (45.7 mg, 65%) was obtained from **4l** (56.0 mg, 0.267 mmol), **3** (163 mg, 0.347 mmol), CsF (80.9 mg, 0.533 mmol), $Pd(PPh_3)_4$ (30.8 mg, 26.7 μmol) and CuI (10.3 mg, 54.1 μmol). Eluent: hexane/Et_2O = 19/1. Reaction time: 13 h.

Yellow oil; IR 1697, 1598 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.35 (dd, *J* = 4.8, 1.2 Hz, 1H), 7.26 (dd, *J* = 15.0, 11.1 Hz, 1H), 7.11-7.05 (m, 2H), 6.34 (d, *J* = 15.3 Hz, 1H), 6.24 (d, *J* = 11.4 Hz, 1H), 5.79 (s, 1H), 4.17 (q, *J* = 7.2 Hz, 2H), 2.31 (d, *J* = 0.9 Hz, 3H), 2.25 (s, 3H), 1.29 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.1, 152.7, 142.7, 136.0, 133.7, 131.8, 127.4, 127.0, 126.7, 125.8, 119.0, 59.7, 26.2, 14.3, 13.9; HR-EIMS Calcd for $C_{15}H_{18}O_2S$ (M^+) 262.1028. Found 262.1032.

Ethyl (2*E*,4*E*,6*Z*)-3-methyl-7-(thiophen-3-yl)octa-2,4,6-trienoate (5m)

According to **GP1**, **5m** (54.1 mg, 75%) was obtained from **4m** (58.0 mg, 0.276 mmol), **3** (168 mg, 0.359 mmol), CsF (83.8 mg, 0.552 mmol), $Pd(PPh_3)_4$ (32.4 mg, 28.0 μmol) and CuI (10.5 mg, 55.1 μmol). Eluent: hexane/Et_2O = 19/1. Reaction time: 13 h.

Pale yellow oil; IR 1697, 1599 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.34 (dd, *J* = 4.8, 3.0 Hz, 1H), 7.21 (dd, *J* = 3.0, 1.5 Hz, 1H), 7.12 (dd, *J* = 5.1, 1.2 Hz, 1H), 6.95 (dd, *J* = 15.3, 11.1 Hz, 1H), 6.30-6.20 (m, 2H), 5.76 (s, 1H), 4.16 (q, *J* = 7.2 Hz, 2H), 2.24 (d, *J* = 1.2 Hz, 3H), 2.17 (s, 3H), 1.28 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.1, 152.7, 141.4, 136.9, 134.9, 132.2, 127.8, 127.3, 125.4, 123.1, 118.6, 59.6, 25.5, 14.3, 13.8; HR-EIMS Calcd for $C_{15}H_{18}O_2S$ (M^+) 262.1028. Found 262.1032.

General procedure for the hydrolysis (GP2)

A mixture of **5** (1.0 equiv) and 10% KOH (0.133 M) aqueous solution in EtOH (0.08 M) was heated at 50 °C overnight. After cooling, the reaction mixture was made acidic or neutral by addition of 5% HCl aqueous solution at 0 °C. The mixture was extracted with EtOAc, and the organic layer was washed with brine, dried over Na_2SO_4, filtered, and evaporated *in vacuo*. The residue was purified by flash column chromatography on silica gel to give the carboxylic acid **6**.

(2*E*,4*E*,6*Z*)-3-Methyl-7-(naphthalen-1-yl)octa-2,4,6-trienoic acid (6b)

According to **GP2**, **6b** (133 mg, 90%) was obtained from **5b** (163 mg, 0.532 mmol) and 10% KOH (4.0 mL). Eluent: hexane/EtOAc = 3/1. Reaction time: 3 h.

Pale yellow crystals; mp 178-182 °C (EtOAc/hexane); UV-vis λ_{max} 304 ($\varepsilon = 37800$), 220 nm; IR 3022, 1679, 1599 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.90-7.76 (m, 3H), 7.51-7.45 (m, 3H), 7.25 (d, *J* = 5.7 Hz, 1H), 6.49 (d, *J* = 8.4 Hz, 1H), 6.28-6.16 (m, 2H), 5.70 (s, 1H), 2.26 (s, 3H), 1.89 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.0, 155.3, 143.2, 139.1, 134.2, 133.7, 133.1, 130.7, 128.9, 128.4, 127.7, 126.2, 125.9, 125.51, 125.46, 125.43, 117.6, 26.7, 13.8; HR-EIMS Calcd for $C_{19}H_{18}O_2$ (M^+) 278.1307. Found 278.1314.

(2*E*,4*E*,6*Z*)-3-Methyl-7-(naphthalen-2-yl)octa-2,4,6-trienoic acid (6c)

According to **GP2**, **6c** (142 mg, 79%) was obtained from **5c** (198 mg, 0.646 mmol) and 10% KOH (4.8 mL). Eluent: hexane/Et_2O = 2/1. Reaction time: 3 h.

Yellow crystals; mp 178-182 °C (EtOAc/hexane); UV-vis λ_{max} 320 ($\varepsilon = 32900$), 218 nm; IR 3018, 1679, 1599 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.88-7.83 (m, 3H), 7.71 (d, *J* = 1.2 Hz, 1H), 7.52-7.49 (m, 2H), 7.40 (dd, *J* = 8.4, 1.8 Hz, 1H), 6.80 (dd, *J* = 15.0, 11.1 Hz, 1H), 6.36 (dd, *J* = 11.1, 0.9 Hz, 1H), 6.33 (d, *J* = 14.7 Hz, 1H), 5.78 (s, 1H), 2.29 (s, 3H), 2.12 (d, *J* = 1.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.3, 155.4, 143.7, 138.4, 134.7, 133.3, 133.2, 132.7, 128.0, 127.9, 127.7, 127.5, 127.1, 126.39, 126.36, 126.2, 117.7, 25.8, 14.0; *Anal*. Calcd for $C_{19}H_{18}O_2$: C, 81.99; H, 6.52. Found: C, 81.94; H, 6.79; HR-EIMS Calcd for $C_{19}H_{18}O_2$ (M^+) 278.1307. Found 278.1320.

(2*E*,4*E*,6*Z*)-3-Methyl-7-(phenanthren-9-yl)octa-2,4,6-trienoic acid (6d)

According to **GP2**, **6d** (135 mg, 93%) was obtained from **5d** (157 mg, 0.440 mmol) and 10% KOH (3.3 mL). Eluent: hexane/EtOAc = 3/1. Reaction time: 6 h.

Colorless crystals; mp 200-203 °C (EtOAc/hexane); UV-vis λ_{max} 298 ($\varepsilon = 39200$), 251, 218 nm; IR 3018, 1679, 1601 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 8.91 (d, *J* = 8.0 Hz, 1H), 8.86 (d, *J* = 8.5 Hz, 1H),

8.01 (dd, *J* = 8.0, 1.0 Hz, 1H), 7.82 (dd, *J* = 8.0, 1.0 Hz, 1H), 7.73 (td, *J* = 7.5, 1.0 Hz, 1H), 7.72 (td, *J* = 7.5, 1.0 Hz, 1H), 7.69-7.65 (m, 2H), 7.67 (s, 1H), 6.64 (dd, *J* = 11.0, 1.0 Hz, 1H), 6.43 (d, *J* = 15.5 Hz, 1H), 6.14 (dd, *J* = 15.5, 11.0 Hz, 1H), 5.74 (s, 1H), 2.27 (s, 3H), 1.72 (d, *J* = 1.0 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 167.5, 150.8, 141.6, 137.2, 134.7, 131.1 (2C), 130.0, 129.33, 129.30, 128.8, 128.5, 127.1, 127.0, 126.92, 126.89, 125.8, 125.7, 123.4, 122.8, 119.8, 26.1, 13.0; *Anal*. Calcd for $C_{23}H_{20}O_2$, 84.12; H, 6.14. Found: C, 83.85; H, 6.07; HR-EIMS Calcd for $C_{23}H_{20}O_2$ (M^+) 328.1463. Found 328.1471.

(2*E*,4*E*,6*Z*)-7-(9*H*-Fluoren-2-yl)-3-methylocta-2,4,6-trienoic acid (6e)

According to **GP2**, **6e** (153 mg, 88%) was obtained from **5e** (189 mg, 0.549 mmol) and 10% KOH (4.2 mL). Eluent: CH_2Cl_2/MeOH = 30/1. Reaction time: 3 h.

Yellow crystals; mp 240-244 °C (CH_2Cl_2/MeOH); UV-vis λ_{max} 329 (ε = 35400) nm; IR (KBr) 2925, 1677, 1587 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.93 (d, *J* = 8.0 Hz, 1H), 7.92 (d, *J* = 8.0 Hz, 1H), 7.60 (d, *J* = 7.5 Hz, 1H), 7.53 (s, 1H), 7.40 (t, *J* = 7.5 Hz, 1H), 7.39-7.31 (m, 2H), 6.70 (dd, *J* = 15.0, 11.0 Hz, 1H), 6.43 (d, *J* = 15.0 Hz, 1H), 6.37 (d, *J* = 11.0 Hz, 1H), 5.77 (s, 1H), 3.96 (s, 2H), 2.23 (s, 3H), 2.06 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 167.7, 151.3, 143.3, 143.2, 142.6, 140.7, 140.6, 139.0, 134.7, 131.6, 127.0, 126.9, 126.8, 126.7, 125.1, 124.7, 120.1, 119.7, 119.5, 39.0, 25.4, 13.2; HR-EIMS Calcd for $C_{22}H_{20}O_2$ (M^+) 316.1463. Found 316.1476.

(2*E*,4*E*,6*Z*)-3-Methyl-7-(pyridin-2-yl)octa-2,4,6-trienoic acid (6f)

According to **GP2**, **6f** (172 mg, 90%) was obtained from **5f** (216 mg, 0.839 mmol) and 10% KOH (6.3 mL). Eluent: CH_2Cl_2/MeOH = 20/1. Reaction time: 3 h.

Pale brown crystals; mp 128-130 °C (EtOAc/hexane); UV-vis λ_{max} 314 (ε = 24200) nm; IR 3018, 1681, 1600 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.71 (br d, *J* = 5.1 Hz, 1H), 7.72 (td, *J* = 7.8, 1.8 Hz, 1H), 7.31-7.21 (m, 2H), 7.00 (dd, *J* = 15.3, 11.4 Hz, 1H), 6.40 (d, *J* = 11.4 Hz, 1H), 6.33 (d, *J* = 15.3 Hz, 1H), 5.79 (s, 1H), 2.26 (s, 3H), 2.20 (d, *J* = 1.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.5, 158.6, 154.6, 149.2, 140.8, 136.39, 136.36, 132.4, 129.7, 123.5, 122.3, 118.6, 24.1, 13.9; *Anal*. Calcd for $C_{14}H_{15}NO_2$: C, 73.34; H, 6.59; N, 6.11. Found: C, 73.27; H, 6.76; N, 6.04; HR-EIMS Calcd for $C_{14}H_{15}NO_2$ (M^+) 229.1103. Found 229.1113.

(2*E*,4*E*,6*Z*)-3-Methyl-7-(pyridin-3-yl)octa-2,4,6-trienoic acid (6g)

According to **GP2**, **6g** (85.0 mg, 49%) was obtained from **5g** (196 mg, 0.762 mmol) and 10% KOH (5.7 mL). Eluent: CH_2Cl_2/MeOH = 15/1. Reaction time: 3 h.

Pale orange crystals; mp 156-159 °C (EtOAc/hexane); UV-vis λ_{max} 310 (ε = 38200) nm; IR 3016, 1682, 1602 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.56 (br s, 2H), 7.61 (dt, *J* = 7.8, 1.8 Hz, 1H), 7.36 (dd, *J* = 7.8, 4.8 Hz, 1H), 6.61 (dd, *J* = 15.3, 10.8 Hz, 1H), 6.35 (d, *J* = 11.1 Hz, 1H), 6.33 (d, *J* = 15.3 Hz, 1H), 5.81 (s, 1H), 2.20 (s, 3H), 2.16 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.0, 153.8, 148.7, 148.1, 138.7, 136.9, 136.2, 135.9, 131.2, 128.9, 123.5, 119.1, 25.4, 13.8; *Anal*. Calcd for $C_{14}H_{15}NO_2$: C, 73.34; H, 6.59; N, 6.11. Found: C, 73.15; H, 6.63; N, 6.01; HR-EIMS Calcd for $C_{14}H_{15}NO_2$ (M^+) 229.1103. Found 229.1088.

(2*E*,4*E*,6*Z*)-3-Methyl-7-(pyrazin-2-yl)octa-2,4,6-trienoic acid (6h)

According to **GP2**, **6h** (151 mg, 89%) was obtained from **5h** (190 mg, 0.735 mmol) and 10% KOH (5.5 mL). Eluent: CH_2Cl_2/MeOH = 15/1. Reaction time: 2 h.

Pale yellow crystals; mp 175-177 °C (MeOH/hexane); UV-vis λ_{max} 310 (ε = 30000) nm; IR 3018, 1682, 1601 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.66-8.63 (m, 1H), 8.49 (d, *J* = 1.8 Hz, 1H), 7.10 (dd, *J* = 15.3, 11.1 Hz, 1H), 6.52 (d, *J* = 11.4 Hz, 1H), 6.41 (d, *J* = 15.3 Hz, 1H), 5.84 (s, 1H), 2.30 (s, 3H), 2.24 (d, *J* = 0.9 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.6, 153.4, 151.0, 144.12, 144.09, 143.1, 137.8, 136.2, 131.2, 130.6, 120.6, 23.2, 13.2; *Anal*. Calcd for $C_{13}H_{14}N_2O_2$: C, 67.81; H, 6.13; N, 12.17. Found: C, 67.78; H, 6.06; N, 12.11; HR-EIMS Calcd for $C_{13}H_{14}N_2O_2$ (M^+) 230.1055. Found 230.1046.

(2*E*,4*E*,6*Z*)-7-(1*H*-Indol-5-yl)-3-methylocta-2,4,6-trienoic acid (6j)

According to **GP2**, **6j** (49.5 mg, 30%) was obtained from **5j** (247 mg, 0.625 mmol) and 10% KOH (4.7 mL). Eluent: CH_2Cl_2/MeOH = 10/1. Reaction time: 2 h.

Brown crystals; mp 164-168 °C (CH_2Cl_2/hexane); UV-vis λ_{max} 326 (ε = 33900), 219 nm; IR 3018, 1678, 1594 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.21 (br s, 1H), 7.54 (d, *J* = 0.9 Hz, 1H), 7.40 (d, *J* = 8.7 Hz, 1H), 7.25 (d, *J* = 2.7 Hz, 1H), 7.11 (dd, *J* = 8.4, 1.5 Hz, 1H), 6.86 (dd, *J* = 15.3, 10.8 Hz, 1H), 6.58-6.57 (m, 1H), 6.29 (d, *J* = 15.3 Hz, 1H), 5.76 (s, 1H), 2.26 (s, 3H), 2.14 (d, *J* = 0.9 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.5, 155.9, 145.4, 135.2, 134.3, 133.6, 132.7, 127.7, 126.3, 124.8, 122.8, 120.4, 117.0, 110.7, 102.9, 26.4, 14.1; HR-EIMS Calcd for $C_{17}H_{17}NO_2$ (M^+) 267.1259. Found 267.1241.

(2*E*,4*E*,6*Z*)-7-(1,3-Dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-3-methylocta-2,4,6-trienoic acid (6k)

According to **GP2**, **6k** (131 mg, 96%) was obtained from **5k** (150 mg, 0.471 mmol) and 10% KOH (3.5 mL). Eluent: CH_2Cl_2/MeOH = 20/1. Reaction time: 3 h.

Pale yellow crystals; mp 223-226 °C (acetone/hexane); UV-vis λ_{max} 300 (ε = 24800) nm; IR 3018, 1703, 1654, 1602 cm^{-1}; ^1H NMR (300 MHz, DMSO-*d*$_6$) δ 7.59 (s, 1H), 6.64 (dd, *J* = 15.3, 11.1 Hz, 1H), 6.34 (d, *J* = 15.3 Hz, 1H), 6.25 (d, *J* = 11.1 Hz, 1H), 5.75 (s, 1H), 3.33 (s, 3H), 3.19 (s, 3H), 2.15 (s, 3H), 2.02 (s, 3H); ^{13}C NMR (75 MHz, DMSO-*d*$_6$) δ 167.8, 161.4, 151.7, 151.2, 142.8, 135.7, 134.6, 131.5, 129.5, 119.3, 111.1, 36.3, 27.5, 23.8, 13.5; *Anal*. Calcd for $C_{15}H_{18}N_2O_4$: C, 62.06; H, 6.25; N, 9.65. Found: C, 62.00; H, 6.17; N, 9.42; HR-EIMS Calcd for $C_{15}H_{18}N_2O_4$ (M^+) 290.1267. Found 290.1244.

Transfection and luciferase activity assay (RARE)

Human osteosarcoma MG-63 cells, which are positive for RXR gene expression, were maintained in Dulbecco's modified Eagle's medium (Gibco BRL) supplemented with 1% penicillin, 1% streptomycin, and 10% dextran-coated charcoal-treated FCS (Gibco BRL). The day before transfection, cells were seeded on six-well culture plates at a density of 2×10^5 cells per well, so that they were confluent on the day of transfection. The retinoid-responsive luciferase reporter construct, human RARβ-RARE3-SV40-Luc, was generated by cloning three copies of the RARE from the RARβ promoter (59/33: GGGTAAAGTTCACCGAAAGTTCACTCG). The pRL-CMV vector was used an internal control. After transfection using the Tfx-50 reagent, the cells were incubated with retinoid (10^{-6} M) for 2 days. Luciferase activity of the cell lysates was measured with a luciferase assay system (Toyo Ink Co., Ltd.), according to the manufacturer's instructions. Transactivation determined from the luciferase activity was standardized with the luciferase activity of the same cells measured with the Sea Pansy luciferase assay system (Toyo Ink Co., Ltd.) as a control. Each set of experiments was repeated at least three times, and the results are presented in terms of fold induction as mean ± S.E.

Transfection and luciferase activity assay (RXRE)

Human osteosarcoma MG-63 cells, which are positive for RXR gene expression, were maintained in Dulbecco's modified Eagle's medium (Gibco BRL) supplemented with 1% penicillin, 1% streptomycin, and 10% dextran-coated charcoal-treated FCS (Gibco BRL). The day before transfection, cells were seeded on six-well culture plates at a density of 2×10^5 cells per well so that they were confluent on the day of transfection. The retinoid-responsive luciferase reporter construct, rat CRBPII-RXRE-SV40-Luc, was generated by cloning three copies of the RXRE from the rat CRBPII promoter (639/605: GCTGTCACAGGTCACAGGTCACAGGTCACAGTTCA) in the pGL3 vector. The pRL-CMV vector was used as an internal control. After transfection using the Tfx-50 reagent, the cells were incubated with retinoids (10^{-6} M) for 2 days. Luciferase activity of the cell lysates was measured with a luciferase assay system (Toyo Ink Co., Ltd.), according to the manufacturer's instructions. Transactivation determined

from the luciferase activity was standardized with the luciferase activity of the same cells measured with the Sea Pansy luciferase assay system (Toyo Ink Co., Ltd.) as a control. Each set of experiments was repeated at least three times, and the results are presented in terms of fold induction as mean ± S.E.

Transfection and luciferase activity assay (RXRα-GAL4)

Human osteosarcoma MG-63 cells, which are positive for RXR gene expression, were maintained in Dulbecco's modified Eagle's medium (Gibco BRL) supplemented with 1% penicillin, 1% streptomycin and 10% dextran-coated charcoal-treated FCS (Gibco BRL). Cells (2×10^5) were suspended in 2 mL of medium and transfected with 1.0 μg of a one-hybrid plasmid (pM vector, Promega Corp., Madison, WI, USA) containing a human RXRα cDNA linked with a yeast GAL4 DNA-binding domain cDNA (GAL-DBD), 0.5 μg of luciferase reporter plasmid (pGVP2 vector, Toyo Ink Co., Ltd.) containing GAL4 binding site (GAL-BS) and pRL-CMV vector as an internal control, using the Tfx-50 reagent (Promega Corp.). The cells were incubated with retinoids (10^{-6} M) for 2 days. The luciferase activities of the cell lysates were measured with a luciferase assay system (Toyo Ink Co., Ltd.), according to the manufacturer's instructions. Transactivation measured by luciferase activity was standardized with the luciferase activity of the same cells determined by the Sea Pansy luciferase assay system (Toyo Ink Co. Ltd.) as a control. Each set of experiments was repeated at least three times, and the results are presented in terms of fold induction as mean ± S.E.

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