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. 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, because aromatic ring-containing retinoid analogues, namely arotinoids, are known to interact with RAR and RXR. 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 synthesis 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. 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)\(_3\)·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\(_3\))\(_4\), Cul, 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

<table>
<thead>
<tr>
<th>entry</th>
<th>Pd source (mol%)</th>
<th>additive (mol%)</th>
<th>4a : 3 (eq.)</th>
<th>time (h)</th>
<th>5a (%) yield(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pd(_2)(dba)(_3)·CHCl(_3) (4)</td>
<td>AsPh(_3) (16), CsF (200)</td>
<td>1.1 : 1</td>
<td>24</td>
<td>5a (27)</td>
</tr>
<tr>
<td>2</td>
<td>Pd(PPh(_3))(_4) (10)</td>
<td>Cul (20), CsF (200)</td>
<td>1 : 1.3</td>
<td>3</td>
<td>5a (81)</td>
</tr>
<tr>
<td>3</td>
<td>Pd(PPh(_3))(_4) (10)</td>
<td>Cul (20), CsF (200)</td>
<td>1 : 1.3</td>
<td>15</td>
<td>5a (86)</td>
</tr>
</tbody>
</table>

* 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 arotoninoids 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

<table>
<thead>
<tr>
<th>Entry</th>
<th>5 (% yield)$^a$</th>
<th>6 (% yield)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5b (78)</td>
<td>6b (90)</td>
</tr>
<tr>
<td>2</td>
<td>5c (92)</td>
<td>6c (79)</td>
</tr>
<tr>
<td>3</td>
<td>5d (76)</td>
<td>6d (93)</td>
</tr>
<tr>
<td>4</td>
<td>5e (69)</td>
<td>6e (88)</td>
</tr>
<tr>
<td>5</td>
<td>5f (78)</td>
<td>6f (90)</td>
</tr>
<tr>
<td>6</td>
<td>5g (80)</td>
<td>6g (49)</td>
</tr>
<tr>
<td>7</td>
<td>5h (76)</td>
<td>6h (89)</td>
</tr>
<tr>
<td>8</td>
<td>5i (62)</td>
<td>6i (0)$^b$</td>
</tr>
<tr>
<td>9</td>
<td>5j (80)</td>
<td>6j (30)$^c$</td>
</tr>
<tr>
<td>10</td>
<td>5k (88)</td>
<td>6k (96)</td>
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<tr>
<td>11</td>
<td>5l (65)</td>
<td>6l (57)</td>
</tr>
<tr>
<td>12</td>
<td>5m (75)</td>
<td>6m (76)</td>
</tr>
</tbody>
</table>

$^a$ 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 RAR\(\beta\)-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 RXR\(\alpha\)-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). While 2-fluoranyl 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. $^1$H 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 $^1$H) and residual solvent signal (CDCl$_3$: δ = 77.0 ppm for $^{13}$C; DMSO-$d_6$: δ = 2.50 ppm for $^1$H, 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$_2$Cl$_2$ and water. After vigorous stirring, the mixture was filtered through Celite with CH$_2$Cl$_2$/EtOAc (1/1). The organic layer was separated, dried over Na$_2$SO$_4$, 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 (2E,4E,6Z)-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$_2$O = 30/1. Reaction time: 15 h.
Pale yellow oil; IR 1698, 1602 cm$^{-1}$; $^1$H 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 (2E,4E,6Z)-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(PPh₃)₄ (114 mg, 98.4 μmol) and CuI (37.5 mg, 0.197 mmol). Eluent: hexane/Et₂O = 19/1. Reaction time: 13 h.

Pale yellow oil; IR 1698, 1604 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) 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); ¹³C NMR (75 MHz, CDCl₃) 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 C₂₁H₂₂O₂ (M⁺) 306.1620. Found 306.1631.

Ethyl (2E,4E,6Z)-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(PPh₃)₄ (102 mg, 88.0 μmol) and CuI (33.5 mg, 0.176 mmol). Eluent: hexane/Et₂O = 19/1. Reaction time: 13 h.

Yellow oil; IR 1698, 1602 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) 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); ¹³C NMR (75 MHz, CDCl₃) 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 C₂₁H₂₂O₂ (M⁺) 306.1620. Found 306.1601.

Ethyl (2E,4E,6Z)-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 (267 mg, 1.76 mmol), Pd(PPh₃)₄ (94.8 mg, 82.0 μmol) and CuI (31.3 mg, 0.164 mmol). Eluent: hexane/Et₂O = 19/1. Reaction time: 13 h.

Colorless crystals; mp 125-127 °C (EtOAc/hexane); IR 1698, 1604 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 8.75 (d, J = 8.5 Hz, 1H), 8.70 (d, J = 8.0 Hz, 1H), 7.88-7.84 (m, 2H), 7.69-7.67 (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); ¹³C NMR (125 MHz, CDCl₃) 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 C₂₅H₂₄O₂ (M⁺) 356.1798. Found 356.1776.
Ethyl (2E,4E,6Z)-7-(9H-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$_2$O = 19/1. Reaction time: 3 h.

Pale yellow crystals; mp 178-181 °C (EtOAc/hexane); IR 1699, 1601 cm$^{-1}$; $^1$H 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, 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 (2E,4E,6Z)-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$^{-1}$; $^1$H 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 167.1, 152.9, 143.4, 143.3, 143.2, 141.3, 141.2, 139.6, 134.6, 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$_{16}$H$_{19}$NO$_2$ (M$^+$) 257.1438. Found 257.1416.

Ethyl (2E,4E,6Z)-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$^{-1}$; $^1$H 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 (2E,4E,6Z)-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(PPh₃)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, CDCl₃) 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); ¹³C NMR (75 MHz, CDCl₃) 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 C₁₅H₁₈N₂O₂ (M⁺) 258.1368. Found 258.1384.

tert-Butyl 4-((2Z,4E,6E)-8-ethoxy-6-methyl-8-oxoocta-2,4,6-trien-2-yl)-1H-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(PPh₃)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, CDCl₃) 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); ¹³C NMR (75 MHz, CDCl₃) 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 C₁₉H₂₆N₂O₄ (M⁺) 346.1881. Found 346.1893.

tert-Butyl 5-((2Z,4E,6E)-8-ethoxy-6-methyl-8-oxoocta-2,4,6-trien-2-yl)-1H-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(PPh₃)₄ (102 mg, 88.3 µmol) and CuI (33.5 mg, 0.176 mmol). Eluent: hexane/Et₂O = 19/1. Reaction time: 13 h.

Yellow oil; IR 1729, 1702, 1601 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) 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); ¹³C NMR (75 MHz, CDCl₃) 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 C₂₄H₂₉NO₄ (M⁺) 395.2097. Found 395.2089.
Ethyl (2\textit{E},4\textit{E},6\textit{Z})-7-(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-3-methylocta-2,4,6-trienoate (5k)

According to \textbf{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\textsubscript{3})\textsubscript{4} (99.4 mg, 86.0 \textmu mol) 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\textsuperscript{-1}; \textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}) δ 7.01 (s, 1H), 6.52 (dd, \textit{J} = 15.3, 10.8 Hz, 1H), 6.27 - 6.22 (m, 2H), 5.76 (s, 1H), 4.16 (q, \textit{J} = 7.2 Hz, 2H), 3.43 (s, 3H), 3.39 (s, 3H), 2.22 (s, 3H), 2.10 (s, 3H), 1.28 (t, \textit{J} = 7.2 Hz, 3H); \textsuperscript{13}C NMR (75 MHz, CDCl\textsubscript{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\textsubscript{17}H\textsubscript{22}N\textsubscript{2}O\textsubscript{4} (M\textsuperscript{+}) 318.1580. Found 318.1571.

Ethyl (2\textit{E},4\textit{E},6\textit{Z})-3-methyl-7-(thiophen-2-yl)octa-2,4,6-trienoate (5l)

According to \textbf{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\textsubscript{3})\textsubscript{4} (30.8 mg, 26.7 \textmu mol) and CuI (10.3 mg, 54.1 \textmu mol). Eluent: hexane/Et\textsubscript{2}O = 19/1. Reaction time: 13 h. Yellow oil; IR 1697, 1598 cm\textsuperscript{-1}; \textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}) δ 7.35 (dd, \textit{J} = 4.8, 1.2 Hz, 1H), 7.26 (dd, \textit{J} = 15.0, 11.1 Hz, 1H), 7.11 - 7.05 (m, 2H), 6.34 (d, \textit{J} = 15.3 Hz, 1H), 6.24 (d, \textit{J} = 11.4 Hz, 1H), 5.79 (s, 1H), 4.17 (q, \textit{J} = 7.2 Hz, 2H), 2.31 (d, \textit{J} = 0.9 Hz, 3H), 2.25 (s, 3H), 1.28 (t, \textit{J} = 7.2 Hz, 3H); \textsuperscript{13}C NMR (75 MHz, CDCl\textsubscript{3}) δ 167.0, 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\textsubscript{15}H\textsubscript{18}O\textsubscript{2}S (M\textsuperscript{+}) 262.1028. Found 262.1032.

Ethyl (2\textit{E},4\textit{E},6\textit{Z})-3-methyl-7-(thiophen-3-yl)octa-2,4,6-trienoate (5m)

According to \textbf{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\textsubscript{3})\textsubscript{4} (32.4 mg, 28.0 \textmu mol) and CuI (10.5 mg, 55.1 \textmu mol). Eluent: hexane/Et\textsubscript{2}O = 19/1. Reaction time: 13 h. Pale yellow oil; IR 1697, 1599 cm\textsuperscript{-1}; \textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}) δ 7.34 (dd, \textit{J} = 4.8, 3.0 Hz, 1H), 7.21 (dd, \textit{J} = 3.0, 1.5 Hz, 1H), 7.12 (dd, \textit{J} = 5.1, 1.2 Hz, 1H), 6.95 (dd, \textit{J} = 15.3, 11.1 Hz, 1H), 6.30 - 6.20 (m, 2H), 5.76 (s, 1H), 4.16 (q, \textit{J} = 7.2 Hz, 2H), 2.24 (d, \textit{J} = 1.2 Hz, 3H), 2.17 (s, 3H), 1.28 (t, \textit{J} = 7.2 Hz, 3H); \textsuperscript{13}C NMR (75 MHz, CDCl\textsubscript{3}) δ 167.1, 152.7, 142.7, 136.0, 133.7, 131.8, 127.4, 127.0, 126.7, 125.8, 119.0, 59.6, 25.5, 14.3, 13.8; HR-EIMS Calcd for C\textsubscript{15}H\textsubscript{18}O\textsubscript{2}S (M\textsuperscript{+}) 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 Na₂SO₄, filtered, and evaporated in vacuo. The residue was purified by flash column chromatography on silica gel to give the carboxylic acid 6.

(2E,4E,6Z)-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 λmax 304 (ε = 37800), 220 nm; IR 3022, 1679, 1599 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) 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); ¹³C NMR (75 MHz, CDCl₃) 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 C₁₉H₁₈O₂ (M⁺) 278.1307. Found 278.1314.

(2E,4E,6Z)-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/Et₂O = 2/1. Reaction time: 3 h.

Yellow crystals; mp 178-182 °C (EtOAc/hexane); UV-vis λmax 320 (ε = 32900), 218 nm; IR 3018, 1679, 1599 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 7.88-7.83 (m, 3H), 7.71 (d, J = 1.2 Hz, 1H), 7.52-7.49 (m, 3H), 7.71 (d, J = 1.2 Hz, 1H), 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); ¹³C NMR (75 MHz, CDCl₃) 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 C₁₉H₁₈O₂: C, 81.99; H, 6.52. Found: C, 81.94; H, 6.79; HR-EIMS Calcd for C₁₉H₁₈O₂ (M⁺) 278.1307. Found 278.1320.

(2E,4E,6Z)-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 λmax 298 (ε = 39200), 251, 218 nm; IR 3018, 1679, 1601 cm⁻¹; ¹H NMR (500 MHz, DMSO-d₆) 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-\( d_6 \)) \( \delta \) 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\(_2\), C, 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.

(2E,4E,6Z)-7-(9H-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\(_2\)Cl\(_2\)/MeOH = 30/1. Reaction time: 3 h. Yellow crystals; mp 240-244 °C (CH\(_2\)Cl\(_2\)/MeOH); UV-vis \( \lambda_{max} \) 329 (\( \varepsilon = 35400 \)) nm; IR (KBr) 2925, 1677, 1587 cm\(^{-1} \); \(^1\)H NMR (500 MHz, DMSO-\( d_6 \)) \( \delta \) 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-\( d_6 \)) \( \delta \) 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.

(2E,4E,6Z)-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\(_2\)Cl\(_2\)/MeOH = 20/1. Reaction time: 3 h. Pale brown crystals; mp 128-130 °C (EtOAc/hexane); UV-vis \( \lambda_{max} \) 314 (\( \varepsilon = 24200 \)) nm; IR 3018, 1681, 1600 cm\(^{-1} \); \(^1\)H NMR (300 MHz, CDCl\(_3\)) \( \delta \) 8.71 (br d, \( J = 5.1 \) Hz, 1H), 7.72 (td, \( J = 8.0 \) Hz, 1H), 7.67 (s, 1H), 7.40 (t, \( J = 7.5 \) Hz, 1H), 7.39-7.31 (m, 2H), 6.70 (dd, \( J = 11.0, 1.0 \) 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 (75 MHz, CDCl\(_3\)) \( \delta \) 171.5, 158.6, 154.6, 149.2, 140.8, 136.39, 136.36, 132.4, 129.7, 123.5, 123.2, 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.

(2E,4E,6Z)-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\(_2\)Cl\(_2\)/MeOH = 15/1. Reaction time: 3 h.
Pale orange crystals; mp 156-159 °C (EtOAc/hexane); UV-vis $\lambda_{\text{max}}$ 310 (ε = 38200) nm; IR 3016, 1682, 1602 cm$^{-1}$; $^1$H 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.

(2E,4E,6Z)-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$_2$Cl$_2$/MeOH = 15/1. Reaction time: 2 h.

Pale yellow crystals; mp 175-177 °C (MeOH/hexane); UV-vis $\lambda_{\text{max}}$ 310 (ε = 30000) nm; IR 3018, 1682, 1601 cm$^{-1}$; $^1$H 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$_2$O$_2$: 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$_2$O$_2$ (M$^+$) 230.1055. Found 230.1046.

(2E,4E,6Z)-7-(1H-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$_2$Cl$_2$/MeOH = 10/1. Reaction time: 2 h.

Brown crystals; mp 164-168 °C (CH$_2$Cl$_2$/hexane); UV-vis $\lambda_{\text{max}}$ 326 (ε = 33900), 219 nm; IR 3018, 1678, 1594 cm$^{-1}$; $^1$H 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.

(2E,4E,6Z)-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$_2$Cl$_2$/MeOH = 20/1. Reaction time: 3 h.
Pale yellow crystals; mp 223-226 °C (acetone/hexane); UV-vis $\lambda_{\text{max}}$ 300 ($\varepsilon = 24800$) nm; IR 3018, 1703, 1654, 1602 cm$^{-1}$; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 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-$d_6$) $\delta$ 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$_2$O$_4$: 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$_2$O$_4$ (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 $2 \times 10^5$ cells per well, so that they were confluent on the day of transfection. The retinoid-responsive luciferase reporter construct, human RAR$\beta$-RARE3-SV40-Luc, was generated by cloning three copies of the RARE from the RAR$\beta$ promoter (59/33: GGGTAAAGTTCCACCGAAAGTTCACTCG). The pRL-CMV vector was used as 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 $2 \times 10^5$ 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: GCTGTCACAGGTCACAGGTCACAGGTTCA) 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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