Synthesis and Biological Evaluation of 9*Z*-Retinoic Acid Analogs Having 2-Substituted Benzo[*b*]furan[1)]

Takashi Okitsu,[a] Daisuke Nakazawa,[a] Kimie Nakagawa,[b] Toshio Okano,[b] and Akimori Wada*,[a]

[a] *Department of Organic Chemistry for Life Science, Kobe Pharmaceutical University; and* [b] *Department of Hygienic Sciences, Kobe Pharmaceutical University; 4–19–1 Motoyamakita-machi, Higashinada, Kobe 658–8558, Japan.*
Received October 29, 2009; accepted December 21, 2009; published online December 25, 2009

Palladium-catalyzed cross-coupling reactions of a 2-substituted 3-iodobenzo[*b*]furans and stannanyl ester afforded the stereoselective production of 9*Z*-retinoic acid ester analogs in good yields. These esters were then converted to the corresponding acids *via* basic hydrolysis in excellent yields, and their biological activities were evaluated. The analog changed the connected position of polyene side chain from 2-position to 3-position of benzo[*b*]furan decreased the biological activities dramatically, and the introduction of various substituents at 2-position afforded almost no effect on the activities.

Key words retinoid X receptor; 9*Z*-retinoic acid analog; transcriptional assay; coupling reaction; 2-substituted 3-iodobenzofuran

All-*E*-retinoic acid (ATRA: all-*trans*-retinoic acid) **1** and 9*Z*-retinoic acid (9CRA: 9-*cis*-retinoic acid) **2** (Chart 1) are metabolites of vitamin A and these compounds are the well known ligand molecules of the retinoic acid receptors (RARα, β, γ) and retinoid X receptors (RXRα, β, γ), respectively.[2)] These receptors are members of the nuclear receptor superfamily and exhibit significant biological functions including cell differentiation, cell proliferation, embryonic development *etc.* through gene transcription.[3,4)] Much effort has been directed toward for the preparation of receptor-selective retinoids in order not only to define the functions of each receptor but also to develop therapeutic agents.[5—12)] We have also studied the stereoselective synthesis[13—16)] and structure–activity relationship of retinoid analogs.[1,17—19)] Recently, we showed that the 9*Z*-retinoic acid analog **3**, in which the cyclohexene ring and adjacent double bond in retinoic acid (**2**) were replaced by 2-benzo[*b*]furan, had no biological effects such as antiproliferative, differentiation-inducing, and apoptosis-inducing activities in HL-60 cells. Rather, compound **3** exhibited stronger transcriptional activity towards RXR as compared to native 9CRA.[18)] It was speculated that the low biological activities of **3** were attributed to no interaction between the amino acid residues of RAR and the lipophilic part (benzo[*b*]furan ring) of ligand molecule **3**. We are very interested in an influence towards the biological activities not only by changing the connected position of polyene side chain but also by introduction of substituent, which might cause the interaction with ligand-binding domain of nuclear receptor, on the benzo[*b*]furan ring. Here we wish to describe 9CRA analogs having the 2-substituted benzo[*b*]-furan **4** which were synthesized using a palladium-catalyzed cross-coupling reaction. We also evaluated the biological activities of the new compounds.

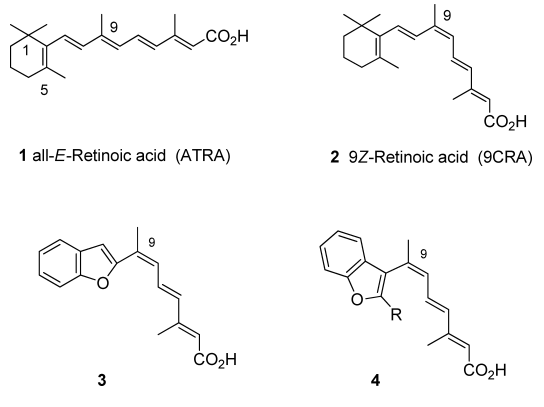

Chart 1. Structures of Retinoic Acids and Analogs

Chemistry Very recently, we have reported the efficient synthesis of 3-iodobenzo[*b*]furans by iodocyclization of 2-alkynyl-1-(1-ethoxyethoxy)benzenes.[20)] In addition, we have developed the cesium fluoride-promoted Stille coupling reaction of vinyl triflates with alkenylstannane having an electron withdrawing group.[21)] We adopted these methodologies for the preparation of the 9CRA analogs having a 2-substituted benzo[*b*]furan **4**. Treatment of 2-substituted 3-iodobenzofurans **6**, derived from 2-alkynyl-1-(1-ethoxyethoxy)benzenes **5**, with alkenylstannane **7** in the presence of cesium fluoride, copper iodide, and with tetrakis(triphenylphosphine)palladium(0) ($Pd(PPh_3)_4$) as the catalyst in *N*,*N*-dimethylformamide (DMF) afforded the coupled products **8** in good yield without an isomerization of double bonds.[21,22)] The structure of **8** was confirmed on the basis of its spectral data.

For the preparation of ester **8a**, without a substituent at the 2-position of benzo[*b*]furan, we pursued another pathway because no 3-iodobenzofuran **6a** was obtained by iodocyclization of **5a**. Thus, commercially available benzofuran-3(2*H*)-one **9** was transformed into the enol triflate **10**[23)] and was used for the coupling reaction. The coupling reaction of **10** with the stannanyl ester **7** proceeded smoothly under the above conditions to give the ester **8a** in 56% yield. Basic hydrolysis of the esters **8** was achieved using potassium hydroxide (10%) to afford the corresponding acids **4** in good to excellent yields (Chart 2). In both the coupling reaction and basic hydrolysis, an isomerization of double bonds was not observed at all and the structure of each compound was confirmed by its spectral data. The results of the coupling reactions and basic hydrolysis are listed in Table 1.

Biological Activity Ligand molecules of the nuclear receptors act as transcriptional factors that bind to the corresponding responsive elements in the promotion regions of target genes. ATRA is the endogeneous low molecular weight ligand that modulates the transcriptional activity by

* To whom correspondence should be addressed. e-mail: a-wada@kobepharma-u.ac.jp

the RARs.[24—26] On the contrary, 9CRA, the natural ligand of RXRs, exhibits almost the same binding affinity to both RARs and RXRs and controls their transcriptional activities.[27—29] Therefore, the analogs prepared here (having 9*Z*-stererochemistry) were tested for potency of transcriptional assays of both RAR and RXR.

It is well documented that the RARβ subtype is essential for the anti-proliferative effect exerted by retinoid, the levels of RARβ increased in tumors, and epigenetic silence of the RARβ gene has been demonstrated in breast cancer.[30,31] Thus, initially we assessed the transcriptional activity towards a human RARβ gene promoter with three copies of retinoic acid responsive elements (RAREs) in transfected MG-63 cells. None of the 9*Z*-analogs **4a**—**f** at 10^{-6} M exhibited a comparable or even higher RARβ/RARE mediated gene expression than did ATRA, the natural ligand of RAR (Fig. 1).

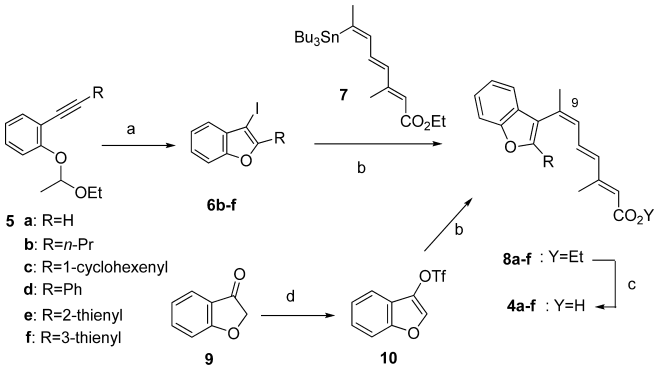

Chart 2. Synthetic Route for Retinoic Acid Analogs Having a 2-Substituted Benzo[*b*]furan

Table 1. Yields of Reactions for the Synthesis of Retinoic Acid Analogs

Run	Substituent R	Product	Yield (%)	Product	Yield (%)
1	H	**8a**	56	**4a**	93
2	*n*-Pr	**8b**	72	**4b**	85
3	1-Cyclohexenyl	**8c**	60	**4c**	89
4	Ph	**8d**	80	**4d**	92
5	2-Thienyl	**8e**	63	**4e**	92
6	3-Thienyl	**8f**	72	**4f**	75

In the transcriptional activities of analogs toward a rat cellular retinoic acid-binding protein II (CRABPII) gene including RXREs in transfected MG-63 cells, none of the analogs exhibited higher transcriptional activity than 9CRA, the natural ligand of RXR (Fig. 2). It is noteworthy that **4a**, which differs from **3** in the position of attachment of the polyene side chain, was markedly less active compared to **3**. In addition, introduction of a substituent at the 2-position of the benzo[*b*]furan ring decreased the transcriptional activity, and this remarkable effect was retained despite differences in the nature of the substituent (compounds **4b**—**f**).

Similarly, all the new analogs showed low transcriptional activity for human RXRα-GAL4 expressed in transfected MG-63 cells (Fig. 3). Thus, all the 9*Z*-analogs **4a**—**f** showed weak activity to RXRα as compared to the native ligand (9CRA), and among the analogs, non-substituted benzo[*b*]-furan analog **4a** exhibited the highest activity. This fact strongly corroborated the result obtained in the transcriptional assay of RXRE.

In summary, we have developed a novel method for the

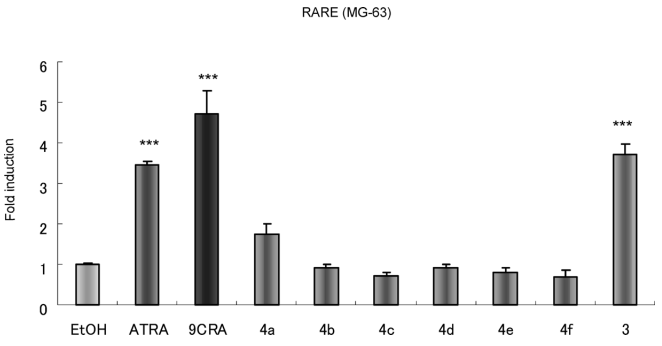

Fig. 1. Transcriptional Activity of ATRA, 9CRA, and Their Analogs toward a Human RARα-RARE Expression Gene in Transfected MG-63 Cells

The cells were cotransfected with a luciferase reporter plasmid (pGVB2 vector) containing a human RARα gene promoter including a RARE and a pRL-CMV vector as an internal control. Luciferase activities induced by ATRA, 9CRA and the analogs in MG-63 cells were quantified and are presented as fold increase in induction as compared to the luciferase activity observed in the ethanol treated control cells, which is defined as 1.0. Each result represents the mean of three experiments (values in column) at 10^{-6} M, and the vertical bars indicate the standard error of the mean, *** $p<0.001$.

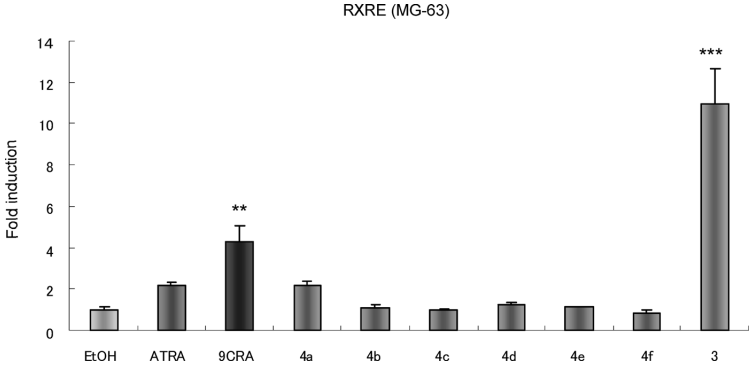

Fig. 2. Transcriptional Activity of ATRA, 9CRA, and Their Analogs toward a Rat CRBPII-RXRE Expression Gene in Transfected MG-63 Cells

The cells were cotransfected with a luciferase reporter plasmid (pGVB2 vector) containing a rat CRABPII gene promoter including a RXRE and a pRL-CMV vector as an internal control. Luciferase activities induced by ATRA, 9CRA and the analogs in MG-63 cells were quantified and are shown as the fold increase in induction as compared with luciferase activity observed in the ethanol treated control cells (defined as 1.0). Results represent the mean of three experiments (values in column) at 10^{-6} M, and the vertical bars indicate the standard error of the mean, ** $p<0.01$; *** $p<0.001$.

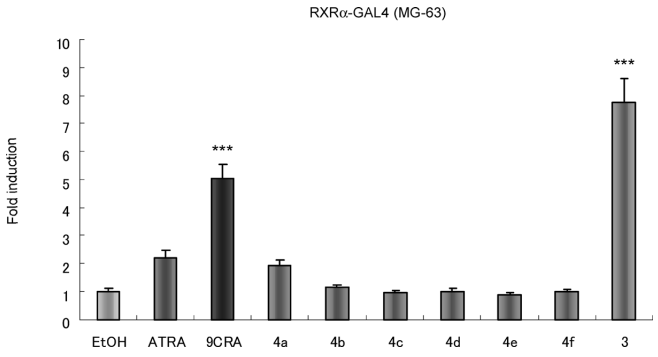

Fig. 3. Transcriptional Potency of ATRA, 9CRA, and Their Analogs in a Human RXRα-GAL4 Expression Gene in Transfected MG-63 Cells

The cells were cotransfected with an expression plasmid (pM vector) inserted with a human RXRα cDNA connected with GAL4-DNA binding domain, a luciferase reporter plasmid (pGVP2 vector) containing the GAL4-binding site, and a pRL-CMV vector as an internal control. Results represent the mean of three experiments (values in column) at 10^{-6} M and the vertical bars show the standard error of mean. The data are expressed as fold increase in induction, and are expressed relative to the ethanol-treated control, which is defined as 1.0. *** $p<0.001$.

stereoselective synthesis of 9*Z*-retinoic acid analogs **4a**—**f** having 2-subustituted benzo[*b*]furan moieties, and found that changing the site of the connecting position of the side chain decreased the transcriptional activity toward RXR. Furthermore, differences in the substituent at the 2-position of benzo[*b*]furan had little effect on the biological activities.

Experimental

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. UV spectra were recorded on a JASCO Ubest-55 instrument in EtOH. IR spectra were recorded on a Perkin-Elmer FT-IR Paragon 1000 spectrometer in $CHCl_3$. ^{1}H-NMR spectra were obtained on a Varian Gemini-300 or a Varian VXR-500 superconducting FT-NMR spectrometer with tetramethylsilane as an internal standard in $CDCl_3$. ^{13}C-NMR spectra were obtained on a Varian Gemini-300 (75 MHz) or a Varian VXR-500 (125 MHz) superconducting FT-NMR spectrometer in $CDCl_3$. Mass spectra were determined on a Hitachi M-4100 instrument. Column chromatography was performed using Kanto Silica Gel 60 N (spherical, neutral). All reactions were carried out under an argon atmosphere. Materials obtained from commercial suppliers were used without further purification. Standard workup means that the organic layers were finally washed with brine, dried over anhydrous sodium sulfate (Na_2SO_4), filtered, and concentrated *in vacuo* below 30 °C using a rotary evaporator.

General Procedure for the Stille Coupling (8a—f) A mixture of the iodide (1.0 eq) **6** or triflate **10**[22] and the stannanyl ester **7** (1.3 eq) was dissolved in dry DMF (0.1 M), then CsF (2.0 eq), $Pd(PPh_3)_4$ (10 mol%), and CuI (20 mol%) was added. The flask was evacuated and refilled with argon five times. The mixture was stirred at 40—45 °C for 12—15 h, 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/AcOEt (1 : 1). The organic layer was separated, followed by standard workup. The residue was purified by column chromatography using neutralized SiO_2/powdered KF (9 : 1) to give coupling product **8**.

Ethyl (2*E*,4*E*,6*Z*)-3-Methyl-7-(benzo[*b*]furan-3-yl)-2,4,6-octatrienoate (8a) This was prepared from **10** (108 mg, 0.407 mmol), **7** (248 mg, 0.529 mmol), $Pd(PPh_3)_4$ (47.0 mg, 40.7 μmol), CuI (15.6 mg, 82.1 μmol) and CsF (124 mg, 0.821 mmol) in 56% yield (67.5 mg) as a pale red oil. Eluent: hexane/AcOEt=30/1.

IR ν_{max} cm^{-1}: 3684, 3619, 3487, 3019, 2980, 1698, 1601; ^{1}H-NMR (300 MHz) δ: 7.54—7.51 (2H, m), 7.36—7.31 (1H, m), 7.31—7.23 (2H, m), 6.80 (1H, dd, *J*=15.0, 10.8 Hz), 6.37 (1H, d, *J*=10.8 Hz), 6.28 (1H, d, *J*=15.0 Hz), 5.76 (1H, s), 4.15 (2H, q, *J*=7.2 Hz), 2.26 (3H, s), 2.11 (3H, d, *J*=1.2 Hz), 1.27 (3H, t, *J*=7.2 Hz); ^{13}C-NMR (75 MHz) δ: 167.1, 155.3, 152.6, 142.7, 134.7, 132.5, 132.2, 128.9, 126.9, 124.6, 122.8, 121.1, 118.8, 111.7, 77.2, 59.7, 25.3, 14.3, 13.7; HR-EI-MS Calcd for $C_{19}H_{20}O_3$ (M^+); 296.1412. Found 296.1408.

Ethyl (2*E*,4*E*,6*Z*)-3-Methyl-7-(2-propylbenzo[*b*]furan-3-yl)-2,4,6-octatrienoate (8b) This was prepared from **6b** (286 mg, 1.00 mmol), **7** (610 mg, 1.30 mmol), $Pd(PPh_3)_4$ (116 mg, 0.100 mmol), CuI (38.1 mg, 0.200 mmol) and CsF (304 mg, 2.00 mmol) in 72% yield (244 mg) as pale yellow oil. Eluent: hexane/AcOEt=40/1.

IR ν_{max} cm^{-1}: 3022, 2966, 2934, 2875, 1699, 1604; ^{1}H-NMR (300 MHz) δ: 7.46—7.43 (1H, m), 7.39—7.36 (1H, m), 7.28—7.17 (2H, m), 6.49 (1H, dd, *J*=15.0, 10.5 Hz), 6.39 (1H, dq, *J*=10.5, 1.2 Hz), 6.27 (1H, d, *J*=15.0 Hz), 5.75 (1H, s), 4.15 (2H, q, *J*=7.2 Hz), 2.62 (2H, td, *J*=7.5, 3.3 Hz), 2.20 (3H, s), 2.09 (3H, d, *J*=0.9 Hz), 1.75 (2H, sext, *J*=7.5 Hz), 1.27 (3H, t, *J*=6.9 Hz), 0.93 (3H, t, *J*=7.5 Hz); ^{13}C-NMR (75 MHz) δ: 167.1, 154.9, 154.2, 152.6, 134.5, 134.1, 132.3, 129.6, 128.7, 123.5, 122.4, 119.8, 118.8, 115.7, 110.9, 59.6, 29.1, 24.8, 21.2, 14.3, 13.8, 13.7; HR-EI-MS Calcd for $C_{22}H_{26}O_3$ (M^+); 338.1882. Found 338.1898.

Ethyl (2*E*,4*E*,6*Z*)-3-Methyl-7-(2-(cyclohexen-1-yl)benzo[*b*]furan-3-yl)-2,4,6-octatrienoate (8c) This was prepared from **6c** (210 mg, 0.649 mmol), **7** (396 mg, 0.844 mmol), $Pd(PPh_3)_4$ (75.1 mg, 64.9 μmol), CuI (24.8 mg, 130 μmol) and CsF (197 mg, 1.30 mmol) in 60% yield (145 mg) as a pale yellow oil. Eluent: hexane/AcOEt=40/1.

IR ν_{max} cm^{-1}: 3026, 2984, 2939, 2861, 1701, 1604; ^{1}H-NMR (300 MHz) δ: 7.44 (1H, d, *J*=7.8 Hz), 7.32 (1H, d, *J*=7.8 Hz), 7.25 (1H, td, *J*=7.8, 1.2 Hz), 7.18 (1H, dd, *J*=7.8, 1.2 Hz), 6.53—6.35 (3H, m), 6.37 (1H, d, *J*=15.0 Hz), 5.73 (1H, s), 4.14 (2H, q, *J*=7.2 Hz), 2.39 (2H, br s), 2.22 (2H, br s), 2.18 (3H, s), 2.05 (3H, d, *J*=1.2 Hz), 1.78—1.60 (4H, m), 1.26 (3H, t, *J*=7.2 Hz); ^{13}C-NMR (75 MHz) δ: 167.1, 153.4, 152.8, 134.8, 134.5, 132.4, 130.2, 129.7, 129.5, 128.5, 124.1, 122.5, 119.8, 118.7, 114.3, 110.8, 77.2, 59.6, 25.8, 25.5, 24.4, 22.5, 21.9, 14.3, 13.7; HR-EI-MS Calcd for $C_{25}H_{28}O_3$ (M^+); 376.2038. Found 376.2055.

Ethyl (2*E*,4*E*,6*Z*)-3-Methyl-7-(2-phenylbenzo[*b*]furan-3-yl)-2,4,6-octatrienoate (8d) This was prepared from **6d** (326 mg, 1.02 mmol), **7** (622 mg, 1.33 mmol), $Pd(PPh_3)_4$ (116 mg, 102 μmol), CuI (38.1 mg, 204 μmol) and CsF (310 mg, 2.04 mmol) in 80% yield (301 mg) as a pale yellow oil. Eluent: hexane/AcOEt=40/1.

IR ν_{max} cm^{-1}: 3027, 2930, 2855, 1701, 1605; ^{1}H-NMR (500 MHz) δ: 7.83 (2H, d, *J*=8.0 Hz), 7.54 (1H, d, *J*=8.0 Hz), 7.42—7.38 (3H, m), 7.34—7.30 (2H, m), 7.24—7.21 (1H, m), 6.50—6.44 (2H, m), 6.26—6.22 (1H, m), 5.70 (1H, s), 4.12 (2H, q, *J*=7.5 Hz), 2.19 (3H, s), 1.92 (3H, d, *J*=1.0 Hz), 1.25 (3H, t, *J*=7.5 Hz); ^{13}C-NMR (125 MHz) δ: 167.0, 154.0, 152.6, 150.4, 135.1, 134.0, 132.1, 130.7, 130.6, 129.6, 128.7, 128.5, 126.5, 124.7, 122.9, 120.2, 119.0, 116.1, 111.2, 59.6, 24.0, 14.3, 13.6; HR-EI-MS Calcd for $C_{25}H_{24}O_3$ (M^+); 372.1725. Found 372.1721.

Ethyl (2*E*,4*E*,6*Z*)-3-Methyl-7-(2-(2-thienyl)benzo[*b*]furan-3-yl)-2,4,6-octatrienoate (8e) This was prepared from **6e** (303 mg, 0.930 mmol), **7** (567 mg, 1.21 mmol), $Pd(PPh_3)_4$ (108 mg, 93.0 μmol), CuI (35.4 mg, 186 μmol) and CsF (283 mg, 1.86 mmol) in 63% yield (222 mg) as a pale yellow oil. Eluent: hexane/AcOEt=40/1.

IR ν_{max} cm^{-1}: 3026, 2938, 1700, 1605; ^{1}H-NMR (300 MHz) δ: 7.52 (1H, d, *J*=7.5 Hz), 7.45 (1H, dd, *J*=6.9, 1.2 Hz), 7.40—7.29 (3H, m), 7.24 (1H, dd, *J*=7.5, 1.5 Hz), 7.07 (1H, dd, *J*=6.9, 5.1 Hz), 6.54—6.40 (2H, m), 6.28 (1H, d, *J*=14.1 Hz), 5.72 (1H, s), 4.12 (2H, q, *J*=6.9 Hz), 2.23 (3H, s), 1.95 (3H, s), 1.25 (3H, t, *J*=6.9 Hz); ^{13}C-NMR (75 MHz) δ: 167.0, 153.8, 152.5, 135.5, 133.2, 131.9, 131.5, 129.4, 127.6, 126.6, 125.8, 124.8, 123.1, 120.0, 119.1, 115.0, 111.1, 77.3, 59.6, 23.7, 14.3, 13.6; HR-EI-MS Calcd for $C_{23}H_{22}O_3S$ (M^+); 378.1290. Found 378.1316.

Ethyl (2*E*,4*E*,6*Z*)-3-Methyl-7-(2-(3-thienyl)benzo[*b*]furan-3-yl)-2,4,6-octatrienoate (8f) This was prepared from **6f** (391 mg, 1.20 mmol), **7** (732 mg, 1.56 mmol), $Pd(PPh_3)_4$ (139 mg, 0.120 mmol), CuI (45.7 mg, 0.240 mmol) and CsF (365 mg, 2.40 mmol) in 72% yield (327 mg) as a pale yellow oil. Eluent: hexane/AcOEt=40/1.

IR ν_{max} cm^{-1}: 3117, 3026, 2984, 2939, 1701, 1605; ^{1}H-NMR (300 MHz) δ: 7.71 (1H, dd, *J*=4.2, 1.2 Hz), 7.53—7.48 (2H, m), 7.39—7.29 (3H, m), 7.25—7.20 (1H, m), 6.52—6.40 (2H, m), 6.25 (1H, d, *J*=14.1 Hz), 5.71 (1H, s), 4.12 (2H, q, *J*=7.2 Hz), 2.22 (3H, s), 1.92 (3H, d, *J*=0.9 Hz), 1.25 (3H, t, *J*=7.2 Hz); ^{13}C-NMR (75 MHz) δ: 167.0, 153.8, 152.5, 147.4, 135.2, 133.8, 132.0, 131.6, 130.8, 129.4, 126.1, 125.8, 124.6, 122.9, 120.0, 119.0, 114.9, 111.1, 77.3, 59.6, 24.1, 14.3, 13.6; HR-EI-MS Calcd for $C_{23}H_{22}O_3S$ (M^+); 378.1290. Found 378.1306.

General Procedure for the Preparation of 9*Z*-Retinoic Acid Analogs (4a—f) A mixture of the ester (**8**, 1.0 eq) and 10% KOH (0.133 M) in ethanol (0.08 M) was heated at 50 °C for 2—3 h. After cooling, the reaction mixture was made acidic with 5% HCl, and the organic compounds were extracted with ethyl acetate followed by standard workup. The residue was purified by flash column chromatography on silica gel to give the acid (**4**).

(2*E*,4*E*,6*Z*)-3-Methyl-7-(benzo[*b*]furan-3-yl)-2,4,6-octatrienoic Acid (4a) This was prepared from **8a** (63.0 mg, 0.213 mmol) in 93% yield (53.2 mg) as yellow crystals. Eluent: hexane/AcOEt=4/1.

mp 170—171 °C (hexane/AcOEt), IR ν_{max} cm^{-1}: 3690, 2939, 1678, 1586,

UV–vis λ_{max} 308 (ε 31200) nm; ^{1}H-NMR (300 MHz) δ: 7.61 (1H, s), 7.55—7.51 (2H, m), 7.34 (1H, ddd, J=7.8, 7.5, 1.8 Hz), 7.26 (1H, td, J=7.5, 1.2 Hz), 6.85 (1H, dd, J=15.3, 11.1 Hz), 6.39 (1H, d, J=11.1 Hz), 6.31 (1H, d, J=15.3 Hz), 5.79 (1H, s), 2.27 (3H, s), 2.12 (3H, d, J=1.2 Hz); ^{13}C-NMR (75 MHz) δ: 172.1, 155.2 (2C), 142.7, 134.5, 133.3, 133.1, 128.8, 126.8, 124.7, 122.8, 121.1, 121.0, 118.0, 111.7, 25.3, 13.9; HR-EI-MS Calcd for $C_{17}H_{16}O_3$ (M^+); 268.1099. Found 268.1104.

(2*E*,4*E*,6*Z*)-3-Methyl-7-(2-propylbenzo[*b*]furan-3-yl)-2,4,6-octatrienoic Acid (4b) This was prepared from **8b** (244 mg, 0.721 mmol) in 95% yield (213 mg) as yellow crystals. Eluent: hexane/AcOEt=7/3.

mp 129—131 °C (hexane/AcOEt), IR ν_{max} cm^{-1}: 3674, 3523, 3022, 2967, 2876, 1679, 1598, UV–vis λ_{max} 299 (ε 60900) nm; ^{1}H-NMR (300 MHz) δ: 7.46—7.43 (1H, m), 7.38—7.36 (1H, m), 7.28—7.17 (3H, m), 6.53 (1H, dd, J=15.0, 10.8 Hz), 6.40 (1H, dd, J=10.8, 1.5 Hz), 6.29 (1H, d, J=15.0 Hz), 5.77 (1H, s), 2.61 (1H, t, J=7.5 Hz), 2.60 (1H, t, J=7.5 Hz), 2.20 (3H, s), 2.09 (3H, d, J=1.5 Hz), 1.75 (2H, sext, J=7.5 Hz), 0.93 (3H, t, J=7.5 Hz); ^{13}C-NMR (75 MHz) δ: 172.6, 155.2, 154.9, 154.2, 134.9, 134.3, 133.2, 129.5, 128.6, 123.6, 122.5, 119.8, 118.0, 115.6, 110.9, 29.1, 24.8, 21.2, 13.9, 13.8; HR-EI-MS Calcd for $C_{20}H_{22}O_3$ (M^+); 310.1569. Found 310.1568.

(2*E*,4*E*,6*Z*)-3-Methyl-7-(2-(cyclohexen-1-yl)benzo[*b*]furan-3-yl)-2,4,6-octatrienoic Acid (4c) This was prepared from **8c** (144 mg, 0.382 mmol) in 89% yield (110 mg) as yellow crystals. Eluent: hexane/AcOEt=7/3.

mp 161—163 °C (hexane/AcOEt), IR ν_{max} cm^{-1}: 3677, 3523, 3022, 2967, 2876, 1679, 1598, UV–vis λ_{max} 294 (ε 55200) nm; ^{1}H-NMR (300 MHz) δ: 7.44 (1H, d, J=7.2 Hz), 7.33—7.18 (3H, m), 6.58—6.37 (3H, m), 6.27 (1H, d, J=7.2 Hz), 5.75 (1H, s), 2.44—2.34 (2H, m), 2.28—2.21 (2H, m), 2.20 (3H, s), 2.06 (3H, s), 1.76—1.60 (4H, m); ^{13}C-NMR (75 MHz) δ: 172.1, 155.4, 153.4, 152.8, 135.7, 134.3, 133.3, 130.1, 129.8, 129.5, 128.5, 124.1, 122.5, 119.7, 117.8, 114.2, 110.8, 25.8, 25.5, 24.5, 22.5, 21.9, 13.9; HR-EI-MS Calcd for $C_{23}H_{24}O_3$ (M^+); 348.1725. Found 348.1734.

(2*E*,4*E*,6*Z*)-3-Methyl-7-(2-phenylbenzo[*b*]furan-3-yl)-2,4,6-octatrienoic Acid (4d) This was prepared from **8d** (298 mg, 0.800 mmol) in 92% yield (253 mg) as yellow crystals. Eluent: hexane/AcOEt=7/3.

mp 190—191 °C (hexane/AcOEt), IR ν_{max} cm^{-1}: 3692, 3524, 3028, 1678, 1601, UV–vis λ_{max} 301 (ε 60100) nm; ^{1}H-NMR (300 MHz) δ: 7.84—7.81 (2H, m), 7.54 (1H, d, J=7.8 Hz), 7.44—7.30 (6H, m), 6.52—6.49 (2H, m), 6.31—6.24 (1H, m), 5.72 (1H, s), 2.20 (3H, s), 1.91 (3H, s); ^{13}C-NMR (75 MHz) δ: 172.2, 155.1, 154.0, 150.4, 134.9, 134.8, 132.9, 130.6, 130.5, 129.5, 128.7, 128.5, 126.4, 124.8, 122.9, 120.2, 118.2, 116.0, 111.2, 24.1, 13.7; HR-EI-MS Calcd for $C_{23}H_{20}O_3$ (M^+); 344.1412. Found 344.1426.

(2*E*,4*E*,6*Z*)-3-Methyl-7-(2-(2-thienyl)benzo[*b*]furan-3-yl)-2,4,6-octatrienoic Acid (4e) This was prepared from **8e** (212 mg, 0.561 mmol) in 92% yield (181 mg) as yellow crystals. Eluent: hexane/AcOEt=7/3.

mp 189—190 °C (hexane/AcOEt), IR ν_{max} cm^{-1}: 3692, 3527, 3024, 1679, 1602, UV–vis λ_{max} 306 (ε 49800) nm; ^{1}H-NMR (300 MHz) δ: 7.52 (1H, d, J=8.1 Hz), 7.44 (1H, dd, J=4.2, 1.2 Hz), 7.39—7.34 (2H, m), 7.31 (1H, dd, J=8.1, 1.2 Hz), 7.21 (1H, d, J=8.1 Hz), 7.07 (1H, dd, J=5.1, 4.2 Hz), 6.54—6.44 (2H, m), 6.30 (1H, d, J=14.4 Hz), 5.73 (1H, s), 2.23 (3H, s), 1.95 (3H, s); ^{13}C-NMR (75 MHz) δ: 172.2, 155.2, 153.8, 146.3, 135.2, 134.0, 132.8, 132.1, 131.4, 129.3, 127.6, 126.6, 125.9, 124.8, 123.1, 120.0, 118.2, 114.9, 111.1, 23.7, 13.8; HR-EI-MS Calcd for $C_{21}H_{18}O_3S$ (M^+); 350.0977. Found 350.0990.

(2*E*,4*E*,6*Z*)-3-Methyl-7-(2-(3-thienyl)benzo[*b*]furan-3-yl)-2,4,6-octatrienoic Acid (4f) This was prepared from **8f** (303 mg, 0.800 mmol) in 75% yield (210 mg) as yellow crystals. Eluent: hexane/AcOEt=7/3.

mp 190—192 °C (hexane/AcOEt), IR ν_{max} cm^{-1}: 3692, 3524, 3026, 1678, 1600, UV–vis λ_{max} 299 (ε 45000) nm; ^{1}H-NMR (300 MHz) δ: 7.71 (1H, dd, J=3.0, 1.2 Hz), 7.52 (1H, d, J=8.1 Hz), 7.48 (1H, dd, J=8.1, 1.2 Hz), 7.40—7.20 (4H, m), 6.51—6.44 (2H, m), 6.32—6.25 (1H, m), 5.73 (1H, s), 2.23 (3H, s), 1.92 (3H, s); ^{13}C-NMR (75 MHz) δ: 171.8, 155.2, 153.8, 147.5, 135.0, 134.7, 132.9, 131.6, 130.7, 129.3, 126.1, 125.8, 124.6, 123.0, 120.0, 118.1, 114.9, 111.1, 24.1, 13.8; HR-EI-MS Calcd for $C_{21}H_{18}O_3S$ (M^+); 350.0977. Found 350.1000.

Transcriptional Activity Assay of RARE and RXRE Human osteosarcoma MG-63 cells, which are positive for RXR gene expression, were maintained in Dulbecco's modified Eagle 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 day of transfection. The retinoid-responsive luciferase reporter constructs human RARβ-RARE3-SV40-Luc and rat CRBPII-RXRE-SV40-Luc were generated by cloning three copies of the retinoic acid response element (RARE) from the RARβ promoter (59/33: GGGTAAAGTTCACCGAAAGTTCACTCG) or the RXRE from the rat CRBPII promoter (639/605: GCTGTCACAGGTCACAGGTCACAGGTCACAGTTCA) in the pGL3 vector.[8,9] The pRL-CMV vector was an internal control using the Tfx-50 reagent. After transfection, the cells were incubated with retinoids (10^{-6} M) for 2 d. 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 respect to the luciferase activity of the same cells measured with the Sea Pansy luciferase assay system as a control (Toyo Ink Co., Ltd.). Each set of experiments was repeated at least three times, and the results (means±S.E.M.) are presented in terms of folds in increase induction compared to the vehicle (ethanol)-treated control, which is expressed as 1.0.

Transcriptonal Activity Assay of RXRα-GAL4 Human osteosarcoma MG-63 cells, which are positive for RXR gene expression, were maintained in Dulbecco's modified Eagle 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 day of transfection. The cells were cotransfected with 1.0 μg of a one-hybrid plasmid (pM vector) containing a human RXR cDNA connected with a yeast GAL4-DNA binding domain cDNA, 0.5 mg of luciferase reporter plasmid (pGVP2 vector) containing GAL4-binding site and a pRL-CMV vector as an internal control. Each set of experiments was repeated at least three times using 10^{-6} M of the retinoid compound, and the results presented as means±S.E.M., represented the fold increase in induction.

Statistical Analysis Statistical significances were determined using Dunnett's test and are expressed as means±S.E.M. The data were compared to EtOH-treated control cells, and levels of significance were determined as *** $p<0.001$ or ** $p<0.01$.

Acknowledgment This work was supported in part by Grant-in-Aid for Scientific Research (C) (A.W.) and for Young Scientist (B) (T.O.) from the Ministry of Education, Culture, Sports, Science and Technology of Japan. We thank the Science Research Promotion Fund of the Japan Private School Promotion Foundation for research grants.

References

1) Wada A., Matsuura N., Mizuguchi Y., Nakagawa K., Ito M., Okano T., *Bioorg. Med. Chem.*, **16**, 8471—8481 (2008).
2) Mangelsdorf D. J., Umesono K., Evans R. M., "The Retinoids," 2nd ed., ed. by Sporn M. B., Roberts A. B., Goodman D. S., Raven Press, New York, 1994, pp. 319—350.
3) Germain P., Chambon P., Eichele G., Evans R. M., Lazar M. A., Leid M., de Lera Á. R., Lotan R., Mangelsdorf D. J., Gronemeyer H., *Pharmacol. Rev.*, **58**, 712—725 (2006).
4) Germain P., Chambon P., Eichele G., Evans R. M., Lazar M. A., Leid M., de Lera Á. R., Lotan R., Mangelsdorf D. J., Gronemeyer H., *Pharmacol. Rev.*, **58**, 760—772 (2006).
5) Morishita K., Yakushiji N., Ohsawa F., Takamatsu K., Matsuura N., Makishima M., Kawahata M., Yamaguchi K., Tai A., Sasaki K., Kakuta H., *Bioorg. Med. Chem. Lett.*, **19**, 1001—1003 (2009).
6) Charton J., Deprez-Poulain R., Hennuyer N., Tailleux A., Staels B., Deprez B., *Bioorg. Med. Chem. Lett.*, **19**, 489—492 (2009).
7) García J., Khanwalkar H., Pereira R., Erb C., Voegel J. J., Collete P., Mauvais P., Bourguet W., Gronemeyer H., de Lera Á. R., *ChemBioChem*, **10**, 1252—1259 (2009).
8) Álvarez S., Pazos-Randulfe Y., Khanwalkar H., Germain P., Álvarez R., Gronemeyer H., de Lera Á. R., *Bioorg. Med. Chem.*, **16**, 9719—9728 (2008).
9) Sakaki J., Konishi K., Kishida M., Gunji H., Kanazawa T., Uchiyama H., Fukaya H., Mitani H., Kimura M., *Bioorg. Med. Chem. Lett.*, **17**, 4808—4811 (2007).
10) Sun W., Desai S., Piao H., Carroll P., Canney D. J., *Heterocycles*, **71**, 557—567 (2007).
11) Walker J. R., Alshafie G., Nieves N., Ahrens J., Clagett-Dame M., Abou-Issa H., Curley R. W. Jr., *Bioorg. Med. Chem.*, **14**, 3038—3048 (2006).
12) Ikegami S., Iimori T., Sudo M., Kitsukawa M., Foroumadi A., Yonemura T., Takahashi H., Kizaki K., Ishii H., *Bioorg. Med. Chem.*, **14**, 5099—5109 (2006).
13) Wada A., Wang F., Ito M., *Chem. Pharm. Bull.*, **56**, 112—114 (2007).
14) Wada A., Ieki Y., Nakamura S., Ito M., *Synthesis*, **2005**, 1581—1588 (2005).

15) Wada A., Babu G., Shimomoto S., Ito M., *Synlett*, **2001**, 1759—1762 (2001).
16) Wada A., Fujioka N., Tanaka Y., Ito M., *J. Org. Chem.*, **65**, 2438—2443 (2000).
17) Wada A., Mizuguchi Y., Miyake H., Niihara M., Ito M., Nakagawa K., Okano T., *Lett. Drug Des. Discov.*, **4**, 442—445 (2007).
18) Wada A., Mizuguchi Y., Shinmen M., Ito M., Nakagawa K., Okano T., *Lett. Drug Des. Discov.*, **3**, 118—122 (2006).
19) Wada A., Fukunaga K., Ito M., Mizuguchi Y., Nakagawa K., Okano T., *Bioorg. Med. Chem.*, **12**, 3931—3942 (2004).
20) Okitsu T., Nakazawa D., Taniguchi R., Wada A., *Org. Lett.*, **10**, 4967—4970 (2008).
21) Okitsu T., Iwatsuka K., Wada A., *Chem. Commun.*, **2008**, 6330—6332 (2008).
22) Mee S. P. H., Lee V., Baldwin J. E., *Chem. Eur. J.*, **11**, 3294—3308 (2005).
23) Morice C., Garrido F., Mann A., Suffert J., *Synlett*, **2002**, 501—503 (2002).
24) Petkiovich M., Brand N. J., Krust A., Chambon P., *Nature* (London), **330**, 444—450 (1987).
25) Giguere V., Ong E. S., Segui P., Evans R. M., *Nature* (London), **330**, 624—629 (1987).
26) Krust A., Kastner P., Petkiovich M., Zelent A., Chambon P., *Proc. Natl. Acad. Sci. U.S.A.*, **86**, 5310—5314 (1989).
27) Heyman R. A., Mangelsdorf D. J., Dyck J. A., Stein R. B., Eichele G., Evans R. M., Thaller C., *Cell*, **68**, 397—406 (1992).
28) Levin A. A., Sturzenbecker L. J., Kazmer S., Bosakowski T., Huselton C., Allenby G., Speck J., Kratzeisen C., Rosenberger M., Lovey A., Grippo J. F., *Nature* (London), **355**, 359—361 (1992).
29) Allenby G., Bocquel M. T., Sanders M., Kazmer S., Speck J., Rosenberger M., Lovey A., Kastner P., Grippo J. F., Chambon P., Levin A. A., *Proc. Natl. Acad. Sci. U.S.A.*, **90**, 30—34 (1993).
30) Alvarez S., Germain P., Alvarez R., Rodríguez-Barrios F., Gronemeyer H., de Lera A. R. *Int. J. Biochem. Cell Biol.*, **39**, 1406—1415 (2007).
31) Sirchia S. M., Ren M., Pili R., Sironi E., Somenzi G., Ghidoni R., Toma S., Nicolò G., Sacchi N., *Cancer Res.*, **62**, 2455—2461 (2002).