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Synthesis of 8β-hydroxy-9(11),13-abietadien-12-one from (+)-dehydroabietylamine and its AhR ligand activity

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ABSTRACT

8β-Hydroxy-9(11),13-abietadien-12-one (**1**), an abietane diterpenoid and an aryl hydrocarbon receptor (AhR) ligand, was synthesized in six steps from commercially available (+)-dehydroabietylamine (**2**). We used the hypervalent iodine catalyst phenyliodine dicarboxylate, a safer alternative to toxic organoselenide reagents, for the oxidative dearomatization of ferruginol (**7**) to compound **1**. Compounds **1** and **2**, as well as the synthetic intermediates (compounds **3**–**7**), were evaluated for AhR ligand activity. Only compounds **1** and **7** were active, which suggests that AhR affinity is influenced by the steric environment around the C-18 position of these compounds.

1. Introduction

Terpenoids, including diterpenoids, are common secondary plant metabolites. They are reported to have bioactive properties, including anticancer [1], antioxidant [1], antimicrobial [2], and anti-inflammatory activities [3]. Previously, we identified 7 diterpenoids with the abietane skeleton as AhR ligands from *Salvia officinalis* [4]. Although dioxins and tryptophan metabolites are well known AhR ligands [5–8], there are few reports on the AhR ligand activity of terpenoids including diterpenoids [9,10].

The AhR belongs to a family of basic helix-loop-helix PER-ARNT-SIM (bHLH-PAS) proteins containing a ligand-dependent transcription factor. Activation of AhR by a ligand, such as 2,3,7,8-tetrachlorodibenzo-dioxin (TCDD), induces CYP1A1 gene expression, which is important in xenobiotic metabolism [11]. Also, AhR activation relates to the adaptive immune system, especially naïve T cell differentiation into subpopulations of $CD4^+$ T cells, such as Th17 and regulatory T (Treg) cells [12]. Th17 cells secrete interleukin-17 (IL-17), which enhances host protective immunity against pathogenic microorganisms, while Treg cells contribute to immunotolerance by secreting IL-10. However, each AhR ligand promotes naïve T cell differentiation into distinct T cell subsets. For example, 6-formylindolo[3.2-*b*]carbazole (FICZ) induces differentiation of naïve T cells into Th17 cells via AhR activation, while 2,3,7,8-tetrachlorodibenzodioxin (TCDD) and naringenin, a flavonoid, promote their differentiation into Treg cells [13,14].

8β-Hydroxy-9(11),13-abietadien-12-one (**1**, Fig. 1), an AhR ligand we previously reported [4], was isolated from the seeds of *Cephalotaxus harringtonia* var. drupacea [15] and the aerial section of *Salvia pachyphylla* [16]. The physiological functions of this compound include antibacterial activity against gram-positive bacteria [15] and growth inhibition of A2780 ovarian cancer cells and HBL-100 breast cancer cells [16].

In our previous report, only 3.9 mg of compound **1** was isolated from 500 g of the dried aerial section of *S. officinalis* [4], which was insufficient to investigate the AhR-mediated physiological effects of compound **1**. Only one publication was found on the synthesis of compound **1**, in which benzeneseleninic anhydride [$(PhSeO)_2O$] was used for the oxidation of ferruginol (**7**) [17]. However, $(PhSeO)_2O$ is considered acutely toxic and carcinogenic by the Globally Harmonized System of Classification and Labeling of Chemicals (GSH). Herein we report a safer protocol using the hypervalent iodine compound, phenyliodine dicarboxylate (PIDA), for synthesizing compound **1**. In addition, the AhR ligand activities of compound **1** and its synthetic intermediates were evaluated.

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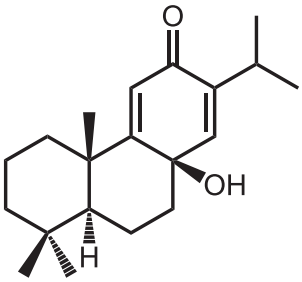


Fig. 1. Chemical structure of 8β-hydroxy-9(11),13-abietadien-12-one (**1**).

2. Results and discussion

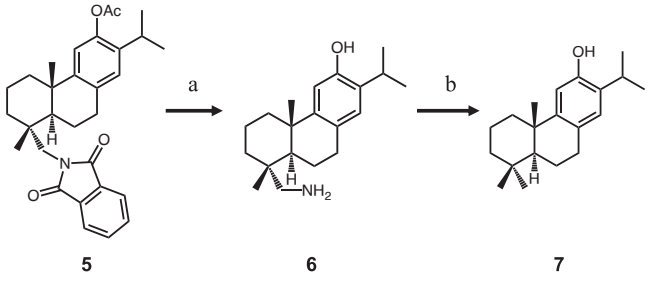
2.1. Synthesis of 8β-hydroxy-9(11),13-abietadien-12-one

The conversion of (+)-dehydroabietylamine (**2**) to 12-acetoxy-*N*-phthaloyldehydroabiethylamine (**5**) was based on the work of the Wiemann group (Scheme 1) [18]. The amino group of compound **2** was protected with a phthaloyl group to obtain compound **3** (66% yield). Next, compound **4** was obtained from compound **3** by Friedel-Crafts acylation (99% yield). To oxidize the keto group of compound **4** by Baeyer-Villiger oxidation, *m*-chloroperoxybenzoic acid was used, and compound **5** was yielded (74% yield). The next step was the synthesis of ferruginol (**7**) from compound **5** (Scheme 2), which was based on the work of González [19]. Initial hydrazine treatment cleaved the acetate group of compound **5** and deprotected the phthalimide to obtain compound **6** (65% yield). Reductive deamination with hydroxylamine-*o*-sulfonic acid (HOS) produced compound **7**. Thus, compound **7** was obtained in a yield of 40%.

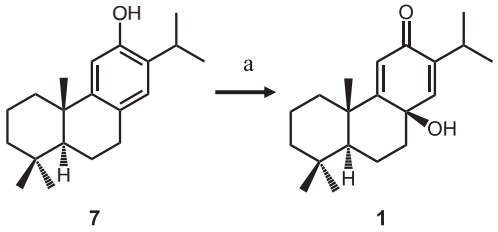
The key step in reaching compound **1** was the de-aromatization of compound **7**. Had et al. used $(PhSeO)_2O$ for this step [17]. The use of $(PhSeO)_2O$ requires strict controls and handling protocols due to its toxicity. To avoid using this reagent, PIDA, which is a hypervalent iodine (III) compound, was selected for the oxidative de-aromatization of compound **7** to compound **1** (Scheme 3). Based on previous reports [20,21], compound **1** was synthesized from compound **7** in a yield of 19%. The oxidations using hypervalent iodine compounds require the iodine to associate to the hydroxyl-group oxygen. The compound reported by Xu et al. [21] possessed a methyl group *ortho*- to the hydroxyl group. However, in the case presented here, an isopropyl group is bound to the *ortho*-position of compound **7**. This may raise activation energies for the PIDA iodine to bind to the hydroxyl-group oxygen, thus lowering reaction yield.

2.2. AhR ligand activity of compounds 1–7

The AhR ligand activity of compounds **1**–**7** was evaluated using HepG2 cells stably expressing AhR-dependent firefly luciferase reporter gene (HepG2-XRE cells) [4]. The *Renilla* luciferase reporter gene, which was expected to be constitutively expressed under the control of the cytomegalovirus (CMV) promoter in HepG2-XRE cells was lower after treatment with compound **2** than after treatment with vehicle, while the other compounds showed no inhibitory effect on *Renilla* luciferase expression (data not shown). Microscopically, HepG2-XRE cells treated with compound **2** shrank and exhibited a round morphology. Compound **2** is reported to induce metastatic melanoma cell death by inhibiting intracellular cholesterol transport [22]. As HepG2 cells are cancerous, like melanoma cells, it seemed likely that compound **2** impaired viability of HepG2-XRE cells as well. Compounds **1** and **7** both had AhR agonist activity, which was suppressed by the AhR antagonist CH-223191 (Fig. 2). This reveals that both compound **1** and compound **7** are AhR agonists.



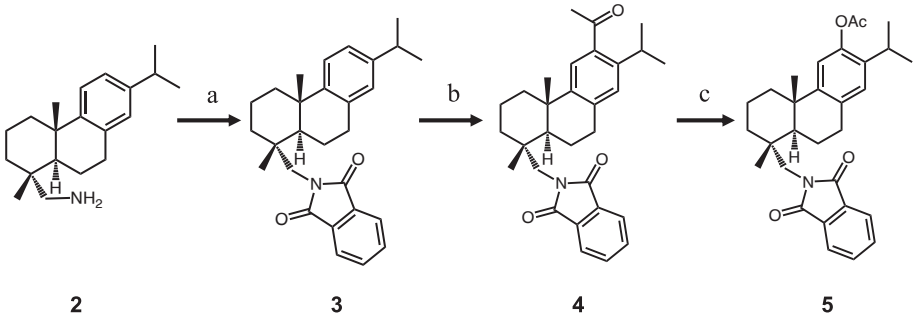
Scheme 2. Synthetic scheme for ferruginol (**7**) from 12-acetoxy-*N*-phthaloyldehydroabiethylamine (**5**): (a) $N_2H_4 \cdot H_2O$, EtOH, reflux, 3 h, 65%; (b) HOS, EtOH, 2.5 M NaOH rt, 2 h, 40%.



Scheme 3. Synthetic scheme for 8β-hydroxy-9(11),13-abietadien-12-one (**1**) from ferruginol (**7**): (a) PIDA, MeCN/H_2O (4:1), 0℃, 4 h, 19%.

2.3. Docking simulations

Compounds **1** and **7** showed AhR agonist activity, while compounds **3**–**6** did not. At the C-18 position of their structures, compounds **1** and **7** both have a methyl group, whereas compound **6** has an amino group, and compounds **3**–**5** have a large phthalimide group. Of note, the AhR agonist activities of compounds **1** and **7** were not significantly different regardless of the presence or absence of a hydroxyl group at the C-8



Scheme 1. Synthetic scheme for 12-acetoxy-*N*-phthaloyldehydroabiethylamine (**5**) from (+)-dehydroabietylamine (**2**): (a) phthalic anhydride, pyridine, reflux, rt, 3 h, 66%; (b) AcCl, $AlCl_3$, DCM, rt, 2 h, 99%; (c) *m*CPBA, TFA, DCM, rt, 16.5 h, 74%.

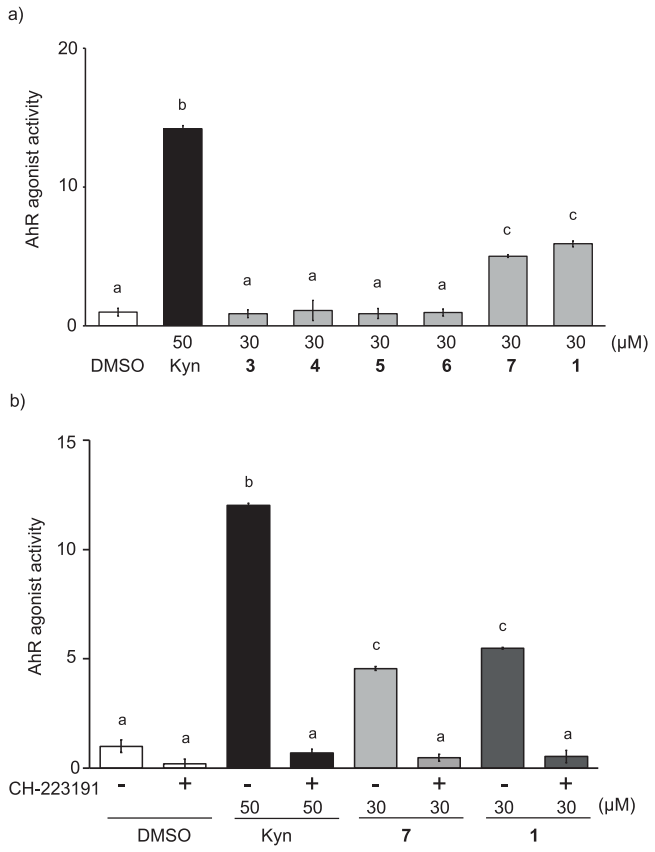


Fig. 2. AhR ligand activity of compounds **1** and **3**–**7**. (a) DMSO was the vehicle. Kynurenine (Kyn, 50 μM) was employed as a positive control. (b) CH-223191 was added at 1 μM as an AhR antagonist. Values are means ± SE, n = 3. Different letters (a, b, and c) indicate statistically significant difference (p less than 0.05).

position. Based on these differences, it is reasonable that steric hinderance at the C-18 position influences binding to AhR. AhR has both PAS-A and PAS-B domains. The PAS-A domain plays a role in heterodimerization with proteins that have PAS domains, such as the aryl hydrocarbon receptor nuclear translocator (ARNT) [23]. The PAS-B domain is a ligand-binding domain (LBD) with a ligand-binding pocket [24]. However, the crystal structure of AhR has not been reported so far.

This hampered studies on the binding mode of AhR to its ligands, but increasing knowledge of structurally homologous proteins within the bHLH-PAS family enables understanding of the molecular structure of HIF2α, which has the highest similarity to AhR of reported PAS structures [25–28]. The PAS domain of HIF2α has 51% amino acid homology with that of AhR [28], with some compounds binding to both HIF2α and AhR [28]. HIF2α undergoes hererodimerization with ARNT, and the PAS-B domain of HIF2α contains a ligand binding pocket [28,29]. In a previous report, modelling of AhR LBD was performed using the HIF2α NMR as a structural template [30]. The authors validated their model by assessing the effect of artificial point mutations in the AhR LBD on the bindings between TCDD and AhR, and between TCDD-liganded AhR and its target DNA sequence [30]. These results were consistent with the proposed model, showing that the buried cavity in the modeled domain functioned as a ligand binding pocket. For the above reason, the PAS-B domain of HIF2α has been used as a binding study template between AhR and ligands, instead of that of AhR [31–34]. Therefore, docking simulations were performed between compounds **1**, **5**, **6**, and **7** and the binding pocket of HIF2α (PDB ID: 3H82) by molecular docking program, AutoDock Vina [34,35]. The scores of the compounds **1**, **5**, **6**, and **7** were −3.6, 14.5, −0.8, and −2.9 kcal/mol, respectively (Table 1). These

Table 1
Docking score of compounds **1**, **5**, **6**, and **7** with AhR.

Compound	Docking score (kcal/mol)
1	−3.6
5	14.5
6	−0.8
7	−2.9

results support the importance of the conformation around the C-18 position for ligand association with AhR.

3. Conclusion

In this paper, we reported a safer protocol for synthesizing compound **1** that used commercially available compound **2**, and showed the importance of the conformation around the C-18 position for association with AhR by evaluating the AhR ligand activity of the intermediates. Because few diterpenoids have been reported as AhR ligands, the relationship between their structures and AhR-stimulating activities has not been analyzed.

The activation of AhR is implicated in immune cell differentiation regulation. AhR activation is also reportedly involved in many physiological effects, including modulation of intestinal epithelial barrier functions [36–38]. A safer protocol for synthesizing compound **1** contributes to the investigation of the AhR-mediated physiological functions of this compound. The effects of compounds **1** and **7** on differentiation of immune cells and modulation of intestinal epithelial barrier function are now under investigation.

4. Experimental section

4.1. General procedures

Reagents and solvents were purchased from Nacalai Tesque or Fujifilm Wako Pure Chemical, Japan. ^{1}H and ^{13}C NMR spectra were recorded with an Avance III 400 or 500 (Bruker Japan, Japan). ESI-MS data were obtained on LCMS-8030 (Shimadzu, Japan). Silica gel (Wakogel® C-200, Fujifilm Wako Pure Chemical, Japan) was used for column chromatography. RP-HPLC was performed on a Chromaster® (Hitachi High-Tech Science, Japan) using a YMC-Triart C-18 column (10 mm × 250 mm, 5 μm, YMC, Japan). Analytical thin-layer chromatography (TLC) was carried out with TLC Silica gel 60 F_{254} plates (Merck Millipore, Deutschland). Spots were visualized by UV irradiation at 254 nm and fluorescence irradiation at 365 nm, and spraying with 5% H_2SO_4 in EtOH followed by heating.

4.2. Synthesis

4.2.1. (-)-N-Phthaloyldehydroabietylamine (3)

(+)-Dehydroabietylamine (**2**; GC-purity: 90%, 1.8 g, 5.7 mmol, Tokyo Chemical Industry, Japan) was dissolved in pyridine (30 mL) and phthalic anhydride (4.15 g, 28 mmol, 4.9 equiv) was added at 0 °C. The mixture was allowed to warm up to RT and was then refluxed for 3 h. After cooling to RT, the mixture was poured into 5% HCl solution (100 mL) and filtered. The filtrate was dried with $MgSO_4$ and then concentrated under reduced pressure. The product was purified by column chromatography (silica gel, n-hexane/ethyl acetate = 9:1) to yield compound **3** (1.56 g, 66%).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.05 (s, 3H, 15-H), 1.21 (d, J = 6.9 Hz, 6H, 23-H, 24-H), 1.22 (s, 3H, 21-H), 1.32–1.89 (m, 7H, 1-H_a, 2-H_a, 2-H_b, 3-H_a, 3-H_b, 5-H, 6-H_a), 2.20–2.30 (m, 2H, 1-H_b and 6-H_b), 2.89 (sept, J = 6.9 Hz, 1H, 22-H), 2.97–3.03 (m, 2H, 7-H_a, 7-H_b), 3.51 (d, J = 13.8 Hz, 1H, 16-H_a), 3.69 (d, J = 13.8 Hz, 1H, 16-H_b), 6.91 (s, 1H, 14-H), 6.95 (d, J = 8.1 Hz, 1H, 12-H), 7.12 (d, J = 8.1 Hz, 1H, 11-H), 7.68–7.70 (m, 2H, 19-H, 20-H), 7.79–7.82 (m, 2H, 17-H, 18-H).

4.2.2. 12-Acethyl-N-phthaloyldehydroabiethylamine (4)

To a solution of **3** (1.56 g, 3.8 mmol) and AcCl (1.5 mL, 21 mmol, 5.5 equiv) in CH_2Cl_2 (1.5 mL), $AlCl_3$ (4.15 g, 30 mmol, 7.9 equiv) was added at 0 °C. The mixture was allowed to warm to RT and stirred for 2 h under N_2. Saturated $KHCO_3$ solution (100 mL) was added to quench the reaction. The mixture was extracted with CH_2Cl_2 (3×70 mL). The organic phase was washed with H_2O (50 mL) and brine (70 mL), and dried over $MgSO_4$. The solvent was concentrated under reduced pressure. The product was purified by column chromatography (silica gel, n-hexane/ethyl acetate = 8:2) to yield compound **4** (1.77 g, 99%).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.07 (s, 3H, 15-H), 1.18 (d, J = 6.8 Hz, 3H, 25-H), 1.21 (d, J = 6.8 Hz, 3H, 26-H), 1.23 (s, 3H, 21-H), 1.29–1.41 (m, 3H, 1-H_a, 3-H_a, 5-H), 1.51 (m, 1H, 3-H_b), 1.65–1.87 (m, 3H, 2-H_a, 2-H_b, 6-H_a), 2.22–2.32 (m, 2H, 1-H_b, 6-H_b), 2.50 (s, 3H, 23-H), 2.96–3.09 (m, 2H, 7-H_a, 7-H_b), 3.45 (sept, J = 6.8 Hz, 1H, 24-H), 3.50 (d, J = 13.8 Hz, 1H, 16-H_a), 3.69 (d, J = 13.8 Hz, 1H, 16-H_b), 7.08 (s, 1H, 14-H), 7.35 (s, 1H, 11-H), 7.68–7.72 (m, 2H, 19-H, 20-H), 7.80–7.83 (m, 2H, 17-H, 18-H).

4.2.3. 12-Acetoxy-N-phthaloyldehydroabiethylamine (5)

Compound **4** (1.77 g, 3.87 mmol) and *m*-chloroperbenzoic acid (*m*CPBA, 1.75 g, 15.8 mmol, 4.1 equiv) were dissolved in CH_2Cl_2 (100 mL). At 0 °C, trifluoroacetic acid (0.8 mL, 10.4 mmol, 2.7equiv) was added dropwise. The mixture was allowed to warm to RT and stirred for 16.5 h under N_2. The suspension was washed with 10% Na_2SO_3 solution (120 mL), H_2O (120 mL), 10% $KHCO_3$ solution (120 mL) and brine (120 mL). The solvent was dried with $MgSO_4$ and evaporated under reduced pressure. The product was purified by column chromatography (silica gel, n-hexane/ethyl acetate = 8:2) to yield compound **5** (1.36 g, 74%).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.03 (s, 3H, 15-H), 1.15 (d, J = 6.8 Hz, 3H, 25-H), 1.18 (d, J = 6.8 Hz, 3H, 26-H), 1.22 (s, 3H, 21-H), 1.28–1.42 (m, 3H, 1-H_a, 3-H_a, 5-H), 1.48 (m, 1H, 3-H_b), 1.58–1.70 (m, 2H, 2-H_a, 2-H_b), 1.74–1.85 (m, 1H, 6-H_a), 2.11 (m, 1H, 1-H_b), 2.28 (s, 3H, 23-H), 2.24–2.30 (m, H, 6-H_b), 2.88 (sept, J = 6.9 Hz, 1H, 24-H), 2.94–3.00 (m, 2H, 7-H_a, 7-H_b), 3.46 (d, J = 13.8 Hz, 1H, 16-H_a), 3.70 (d, J = 13.8 Hz, 1H, 16-H_b), 6.77 (s, 1H, 11-H), 6.96 (s, 1H, 14-H), 7.69–7.71 (m, 2H, 19-H, 20-H), 7.77–7.80 (m, 2H, 17-H, 18-H).

4.2.4. 18-Aminoferruginol (6)

Compound **5** (1.36 g, 2.87 mmol) and hydrazine monohydrate (1.1 mL, 23 mmol, 8.0 equiv) were dissolved in hot ethanol (35 mL) and refluxed for 3 h. The mixture was filtered without cooling and the white solid was washed with fresh ethanol. The filtrate was concentrated under reduced pressure. The solid was treated with 2.5 M NaOH (50 mL) and stirred for 1 h. To neutralize the reaction, 2 M HCl (68 mL) was added to the solution, and then the solution was extracted with dichloromethane (3×45 mL). The extract was dried with $MgSO_4$ and concentrated to yield compound **6** (560 mg, 65%).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.88 (s, 3H, 15-H), 1.20 (s, 3H, 17-H), 1.21 (d, J = 6.8 Hz, 3H, 19-H), 1.23 (d, J = 6.8 Hz, 3H, 20-H), 1.25–1.42 (m, 3H, 1-H_a, 3-H_a, 5-H), 1.48 (m, 1H, 3-H_b), 1.60–1.79 (m, 3H, 2-H_a, 2-H_b, 6-H_a), 2.17 (m, 1H, 1-H_b), 2.43 (d, J = 13.2 Hz, 1H, 16-H_a), 2.62 (d, J = 13.2 Hz, 1H, 16-H_b), 2.72–2.88 (m, 3H, 6-H_b, 7-H_a, 7-H_b), 3.13 (sept, J = 6.9 Hz, 1H, 18-H), 6.62 (s, 1H, 11-H), 6.82 (s, 1H, 14-H).

4.2.5. Ferruginol (7)

Compound **6** (328 mg, 1.09 mmol) was dissolved in 2.5 M NaOH (3 mL) and ethanol (3 mL) and cooled to 0 °C. Hydroxylamine-*O*-sulfonic acid (HOS, 300 mg, 2.6 mmol, 2.4 equiv) was added. After stirring for 30 min, 2.5 M NaOH (1.5 mL), ethanol (1.5 mL) and HOS (15 mg) were added. After stirring at RT for 2 h, the solution was neutralized dropwise with 2.5 M HCl (~3 mL), and then extracted with ethyl acetate (3×10 mL). The organic phase was washed with brine (20 mL), dried with $MgSO_4$, and concentrated. The product was purified by column chromatography (silica gel, n-hexane/ethyl acetate = 84:16) to yield compound **7** (124 mg, 40%).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.92 (s, 3H, 15-H), 0.94 (s, 3H, 16-H), 1.17 (s, 3H, 17-H), 1.22 (d, J = 6.8 Hz, 3H, 19-H), 1.24 (d, J = 6.8 Hz, 3H, 20-H), 1.28–1.42 (m, 3H, 1-H_a, 3-H_a, 5-H), 1.47 (m, 1H, 3-H_b), 1.60–1.79 (m, 4H, 2-H_a, 2-H_b, 6-H_a, 6-H_b), 2.17 (m, 1H, 1-H_b), 2.72–2.88 (m, 2H, 7-H_a, 7-H_b), 3.11 (sept, J = 6.8 Hz, 1H, 18-H), 6.63 (s, 1H, 11-H), 6.83 (s, 1H, 14-H).

4.2.6. 8β-Hydroxy-9(11),13-abietadien-12-one (1)

Compound **7** (48 mg, 168 μmol) was dissolved in a mixture of MeCN/H_2O (4:1, 6.6 mL), treated with PIDA (140 mg, 435 μmol, 2.6 equiv), and then stirred for 4 h at 0 °C under N_2. The reaction mixture was partially concentrated by excluding MeCN, diluted with saturated $KHCO_3$ (3 mL), and extracted with EtOAc (3×8 mL). The combined EtOAc layers were washed with saturated $KHCO_3$ (7 mL) and brine (5 mL), and then dried with $MgSO_4$. After filtration, the filtrate was concentrated and the product was purified by chromatography (silica gel, n-hexane/ethyl acetate = 90:10) and RP-HPLC (MeOH:0.1% CH_3COOH = 82:18, 4 mL/min, UV 254 nm, t_R = 16.5 min) to yield compound **1** (9.7 mg, 19 %).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.89 (s, 3H, 18-H), 0.94 (s, 3H, 19-H), 1.05 (d, 3H, J = 6.8 Hz, 17-H), 1.07 (d, 3H, J = 6.8 Hz, 16-H), 1.16 (m, 1H, 1-H_a), 1.36 (s, 3H, 20-H), 1.37–1.46 (m, 3H, 1-H_b, 3-H_a, 3-H_b), 1.58 (m, 2H, 2-H_a, 2-H_b), 1.68–1.75 (m, 3H, 5-H, 6-H_a, 7-H_a), 1.91 (dddd, 1H, J = 3.2, 13.2, 13.2 and 13.2, 6-H_b), 2.12 (ddd, 1H, J = 3.2, 3.2 and 13.6, 7-H_b), 2.92 (sep, 1H, J = 7.0, H-15), 6.02 (s, 1H, H-11), 6.38 (s, 1H, H-14); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 17.7 (C-6), 18.6 (C-2), 20.2 (C-20), 21.5 (C-19), 21.7 (C-17), 21.8 (C-16), 25.6 (C-15), 33.5 (C-18), 34.2 (C-4), 37.8 (C-1), 39.8 (C-10), 41.3 (C-7), 41.8 (C-3), 54.8 (C-5), 69.3 (C-8), 122.0 (C-11), 142.0 (C-13), 145.3 (C-14), 168.8 (C-9), 187.2 (C-12); ESI-MS: $[M + H]^+$ = 303.3.

4.3. AhR ligand assay

HepG2-XRE cells were seeded in 96-well plates (1.0×10^4/100 μL per well). After 18 h incubation, the medium was replaced by Dulbecco's Modified Eagle Medium containing 10% fetal bovine serum and one of compounds **1**–**7**. CH-223191 (Sigma-Aldrich, USA) was added as an AhR antagonist. Kynurenine (Sigma-Aldrich, USA) and DMSO were used as positive and vehicle control, respectively. After 4 h, the cells were lysed using a passive lysis buffer (Promega, USA), and lysates were centrifuged. The supernatants were then analyzed using the Dual-Luciferase® Assay System (Promega, USA) for firefly and *Renilla* luciferase activities. These luciferase activities were measured with a luminometer (Lumat^3 LB9508, Berthold Technologies, Deutschland). The firefly luciferase activity was normalized by the *Renilla* luciferase activity for the evaluation of AhR ligand activity. The AhR ligand activity of each compound was evaluated relative to the activity of the sample treated with DMSO.

4.4. Docking simulation

The crystal structure of HIF2α was obtained from Protein Data Bank (PDB ID:3H82). The PAS-B domain of HIF2α from 3h82 was used for docking simulation. The simulations with the compounds were performed using AutoDock Vina (The Scripps Research Institute).

4.5. Statistics

Statistical significance was tested by analysis of variance (ANOVA) and Tukey-Kramer Multiple Comparisons Test using R (ver. 1.1.383, RStudio). Differences were considered statistically significant if the p-value was less than 0.05.

Declaration of Competing Interest

The authors declare that they have no known competing financial

interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rechem.2023.100912.

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