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Discovery of tetrahydrotetramethylnaphthalene analogs as adult T-cell leukemia cell-selective proliferation inhibitors in a small chemical library constructed based on multi-template hypothesis

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ABSTRACT

Adult T cell leukemia (ATL), caused by infection of human T-lymphotropic virus type 1 (HTLV-1), has a poor prognosis and curative therapy is unavailable, so it is important to find or design superior lead compounds for the drug treatment of ATL. We used our micro-reversed fragment-based drug design hypothesis and multi-template hypothesis to extract the tetrahydrotetramethylnaphthalene (TMN) skeleton from tamibarotene, a useful medicament for the treatment of acute promyelocytic leukemia (APL). Structural development of TMN yielded highly ATL cell-selective growth inhibitors, including 2-acetyl-3-hydroxy-5,6,7,8-tetrahydro-5,5,8,8-tetramethylnaphthalene (**6**). Structure–activity relationship analysis suggests the existence of a specific target molecule for ATL cell-selective inhibition of proliferation through G2 arrest.

1. Introduction

Adult T cell leukemia (ATL) is an intractable disease caused by infection of human T-lymphotropic virus type 1 (HTLV-1) and involves the expansion of leukemic cells and the development of immunodeficiency.[1,2] The geographic distribution of HTLV-1 has been well defined; it is highly prevalent in Japan, Africa, the Caribbean islands, and South America.[3] In Japan, the number of HTLV-1 carriers is estimated to be 1.2 million, and more than 700 cases of ATL are diagnosed every year.[4] Since conventional anticancer chemotherapy active against other lymphoid malignancies proved to be ineffective for treating aggressive types of ATL, it seems mandatory to find or design superior lead compounds for the development of drugs to treat this fatal disease.[5–7] HTLV-1 infection is the cause of ATL, and monoclonal expansion of HTLV-1-infected T cells is the reason for the malignancy of the disease. However, ATL cells generally do not express HTLV-1 gene. Therefore, it would be expected that an altered specific gene expression pattern caused by HTLV-1 infection resulted in the transformation of normal cells, suggesting that a molecular target(s) for inhibition of ATL cell-selective proliferation might exist.

Only a few medicaments which elicit their pharmacological effects at the gene expression level by targeting a specific molecule(s) are known. Tamibarotene (Am80), a synthetic retinoid derived from all *trans*-retinoic acid (ATRA), is one example (Fig. 1).[8–10] Tamibarotene has been clinically used to treat acute promyelocytic leukemia (APL), and its mechanism of action is considered to be the regulation of specific gene expression mediated by binding to aberrant nuclear retinoic acid receptor [PML-RAR fused protein resulting from chromosomal translocation t(15;17)] and/or normal nuclear retinoic acid receptor (RAR). Although tamibarotene inhibits ATL cell proliferation,[11,12] it also inhibits proliferation of other HTLV-1-negative malignant cells.[8,13,14] In our assay system, tamibarotene elicited almost non-selective (actually slightly selective for HL-60 cells) anti-proliferative activity against all of the following cell lines examined: S1T (a cell line established from an ATL patient,[7] IC_{50} = 15.9 μM), HL60 cells (IC_{50} = 6 μM), Jurkat cells (IC_{50} = 17.3 μM), U937 cells (IC_{50} = 20.2 μM) and MOLT-4 cells (IC_{50} = 19.5 μM). The amide bond isomer of tamibarotene, Am580, is also a superior ligand for both PML-RAR and RAR, like ATRA.[8,13] The three-dimensional structures of complexes formed by various ligands and the ligand binding domain (LBD) of RAR, as well as the structural requirement of ligands for high-affinity binding to RAR(LBD), have been

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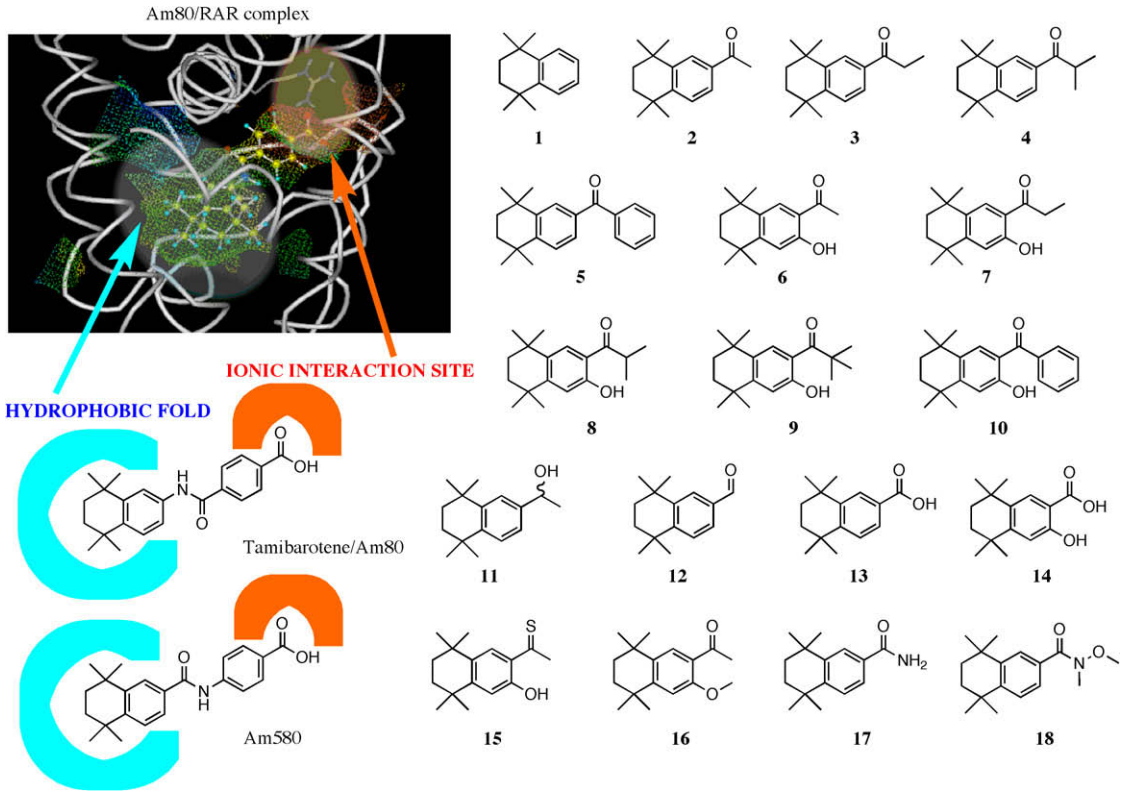


Figure 1. Molecular anatomy of tamibarotene.

well characterized by X-ray analysis, docking studies and structure–activity relationship analysis (Fig. 1).[15–17]

The required interactions for the formation of stable ligand/RAR(LBD) complexes have been established to be ionic and hydrophobic interactions.[14–16] The former type of interaction is a point-to-point interaction which is specific for the amino acid sequence (or perhaps more precisely, for the chemical nature of the amino acid residues in the sequence) of RAR(LBD). On the other hand, the latter interaction can be regarded as more environmental in nature, that is, the fold structure of the hydrophobic pocket into which the tetrahydrotetramethylnaphthyl (TMN) moiety of tamibarotene (Am80) and/or Am580 fits (indicated by the blue band in Fig. 1) is an important factor. There is an enormous number of proteins (perhaps 50,000–70,000 in humans) from the standpoint of amino acid sequences, but it is thought that the three-dimensional spatial structures (i.e., fold structures) of proteins are far more highly conserved in evolution than are the amino acid sequences.[17,18] The number of fold structure types that comprise all the domains occurring in human proteins is thought to be as few as approximately 1000.[18–20] Therefore, one might expect that many proteins would contain a fold structure similar to that of the hydrophobic pocket of RAR(LBD) mentioned above, into which the TMN skeleton fits; this is the multi-template hypothesis. In addition, it might be expected that similar folds exhibit similar roles from the standpoint of biological responses to the corresponding ligands. On this basis,[21] we expected that the TMN skeleton might act as a multi-template for ATL cell-selective proliferation inhibitors, and so we prepared the TMN analogs listed in Figure 1 (compounds **1**–**18**).

In this paper, we describe our structural development study of TMN analogs as ATL cell-selective proliferation inhibitors. We also present preliminary data concerning their mechanism of action at the cellular level.

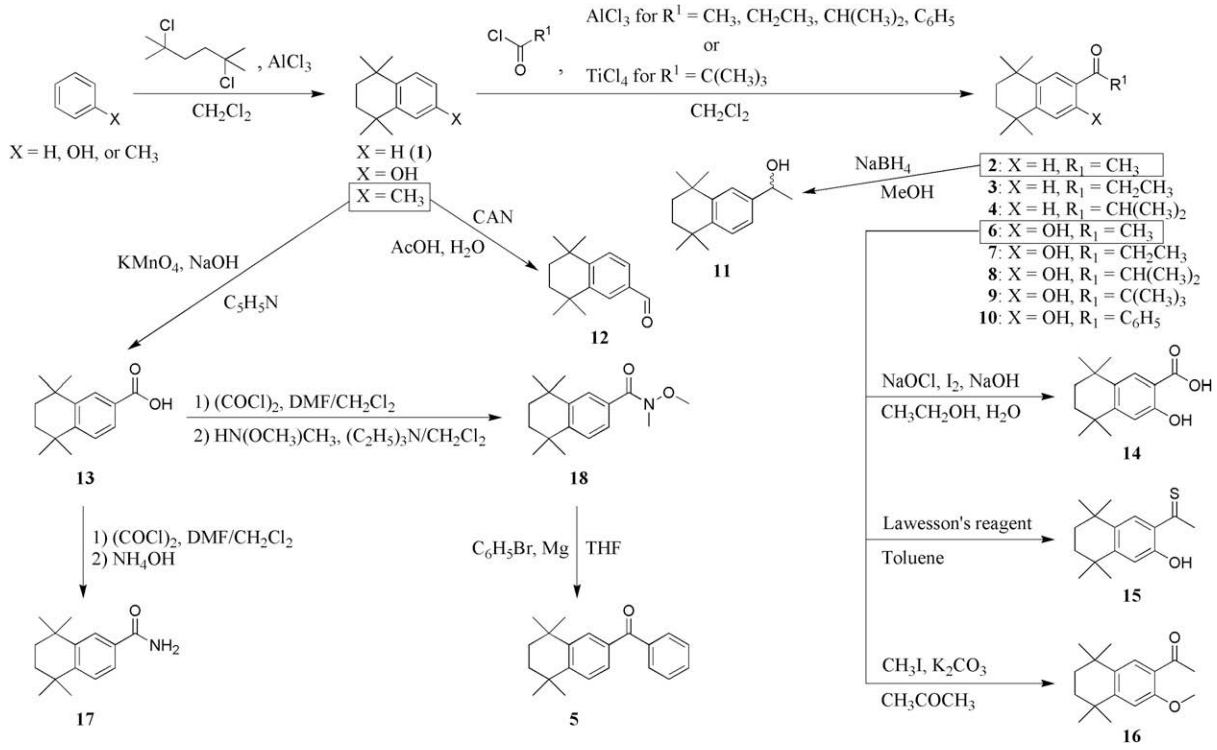
2. Results and discussion

2.1. Screening for ATL cell-selective proliferation inhibitors: structure–activity relationships

Compounds **1**–**18** were prepared by usual organic synthetic methods as shown in Scheme 1 (see Section 4). Briefly, compounds containing the 5,6,7,8-tetrahydro-5,5,8,8-tetramethylnaphthalene skeleton, including (**1**), were prepared by Friedel–Crafts alkylation as previously described.[8] Introduction of acyl groups was performed by Friedel–Crafts acylation catalyzed by $AlCl_3$ (compounds **2**–**4** and **6**–**8**), except for the pivaloyl group (**9**) and benzoyl group (**5**). Introduction of the pivaloyl group was smoothly achieved by the use of $TiCl_4$ as a catalyst instead of $AlCl_3$ to give compound **9**. Formyl and carboxylic acid derivatives (**12** and **13**, respectively) were obtained by selective oxidation of 2-methyl-5,6,7,8-tetrahydro-5,5,8,8-tetramethylnaphthalene using CAN and $KMnO_4$, respectively.

For screening of ATL cell-selective inhibitors, two cell lines were adopted, that is, S1T established from acute-type ATL[7,22] and MOLT-4 established from HTLV-1-negative T-cell leukemia. Chemical(s) which inhibits S1T cell proliferation, but not MOLT-4 cell proliferation might be suitable lead compound(s) for the development of drugs to treat ATL. The effects of compounds **1**–**18** on the growth and viability of S1T and MOLT-4 cells were examined. S1T and MOLT-4 cells were incubated in the absence or presence of various concentrations of test compounds for 4 days, viable cell number was determined by the MTT method, and IC_{50} values were calculated. The results are shown in Table 1.

The most simple hydrophobic skeleton, 5,6,7,8-tetrahydro-5,5,8,8-tetramethylnaphthalene (TMN: **1**) did not show apparent growth-inhibitory activity toward S1T cells or MOLT-4 cells. However, introduction of an acetyl group into the 2-position of TMN,



Scheme 1. Synthesis of TMN derivatives.

Table 1
Cell type-selective proliferation inhibitory activity of tetrahydrotetramethylnaphthalene derivatives.

Compds.	IC_{50} values (μM)		SI values[a]
	S1T cells	MOLT-4 cells	
1	>100	>100	—
2	6.9	66.0	9.6
3	4.7	21.5	4.6
4	3.8	34.6	9.l
5	7.0	11.8	1.7
6	6.7	82.9	12.4
7	4.7	32.3	6.9
8	6.0	31.9	5.3
9	41.5	43.2	1.0
10	15.2	28.3	1.9
11	393	34.4	0.87
12	33.0	24.8	0.75
13	>100	61.1	—
14	29.7	44.4	1.5
15	6.9	36.4	5.3
16	7.0	11.9	1.7
17	37.0	39.4	1.1
18	12.6	9.9	0.8

[a] IC_{50} for MOLT-4 cells/IC_{50} for SIT cells.

that is, compound **2**, resulted in appearance of the activity. Interestingly, compound **2** showed S1T cell-selective proliferation-inhibitory activity with a selectivity index (SI: IC_{50} value for MOLT-4 cells/IC_{50} value for S1T cells) value of 9.6, that is, S1T cells are one magnitude of order more sensitive to **2** than MOLT-4 cells. Further introduction of one and two methyl groups at the aliphatic β-carbon of the carbonyl moiety, that is, ethylcarbonyl (**3**) and isopropylcarbonyl (**4**) derivatives, respectively, caused enhancement of the growth-inhibitory activity towards both S1T and MOLT-4 cells with retention of S1T selectivity (SI values of 4.6 and 9.1, respectively). Deletion of the methyl group on the acetyl moiety of **2**, that is, the formyl derivative **12**, caused decreased activity toward S1T cells with retention of moderate activity toward MOLT-4 cells, resulting in loss of the cell type selectivity (SI value of 0.75: slightly MOLT-4 selective). Reduction of a carbonyl group on the acetyl moiety of **2**, that is, compound **11**, also caused decreased activity toward S1T cells with retention of moderate activity toward MOLT-4 cells, resulting in loss of the cell type selectivity. Among alkylcarbonyl derivatives (**2**–**4**), the activity toward S1T cells decreased in the order of **4** (isopropyl) > **3** (ethyl) > **2** (methyl), while the order for MOLT-4 cells was **3** (ethyl) > **4** (isopropyl) > **2** (methyl). The phenylcarbonyl derivative (**5**) showed about the same activity toward S1T cells as **2**, but the SI value was far lower than that of **2** (SI = 1.7). Overall, the acetyl group seemed to be the best substituent for further structural development from the standpoints of both S1T cell growth-inhibitory activity and cell type selectivity.

The effect of introduction of a hydroxyl group into the alkylcarbonyl derivatives, that is, compounds **6**–**10**, was also investigated. Comparison of corresponding compounds with and without a hydroxyl group, that is, **2** versus **6**, **3** versus **7**, and **4** versus **8**, indicates that introduction of a hydroxyl group had little apparent effect on the activity. But, there seems to be some indication that the introduction of a hydroxyl group produced a very slight decrease of the activity toward MOLT-4 cells, because compound **6** possesses the highest SI value (12.4) among the compounds investigated. Introduction of a hydroxyl group into the phenylcarbonyl derivative (**5**), that is, compound **10**, potentiated the activity toward both S1T and MOLT-4 cells. The tertiary-butylcarbonyl analog (**9**) possessed moderate cell growth-inhibitory activity towards both S1T and MOLT-4 cells, but it showed almost no cell type selectivity.

The tetramethylcyclohexane moiety of the above-mentioned compounds seems to be essential for the activity, because the demethyl analog of **6**, that is, compound **19**, and its oxa-analogs (**20** and **21**), and dimethyl analogs of acetophenone (compounds **22**–**24**)

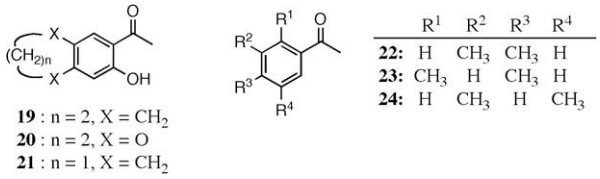


Chart 1.

were inactive (IC_{50} values of these compounds toward both S1T and MOLT-4 cells were larger than 100 μM) (Chart 1). Some other analogs **12**–**18** were also prepared and their activity was investigated. Although the thiocarbonyl analog of **6**, that is, compound **15**, showed almost the same potency toward S1T cells as **6**, its activity toward MOLT-4 cells was increased (IC_{50} value changed to 36.4 μM from 82.9 μM), resulting in S1T cell-selectivity with an SI value of 5.3. Other compounds were found to be almost non-selective with SI values of 0.75–1.7. Compound **16** is an analog of **6** in which the phenolic hydroxyl group is methylated, and the two compounds showed almost the same activity toward S1T cells, but the activity toward MOLT-4 cells of **16** was much more potent than that of **6**, resulting in loss of cell type selectivity. Compound **17** is an amide analog of **2**, whose activity toward S1T cells was much lower than that of **2**, while its activity toward MOLT-4 cells was more potent than that of **2**.

2.2. Correlation analysis

The correlation of the cell proliferation-inhibitory activities of the prepared compounds toward S1T cells and MOLT-4 cells was analyzed by plotting the IC_{50} values for S1T cells (vertical scale) and those for MOLT-4 cells (horizontal scale) (Fig. 2).

As shown in Figure 2, the active compounds whose IC_{50} values for S1T/MOLT-4 cells are less than 100 μM (**2**–**12** and **14**–**18**) could apparently be divided into two groups, that is, one group is the compounds with no cell type-selective activity (compounds **5**, **9**–**12**, **14**, and **16**–**18**: these compounds were located around the line defined by IC_{50}/S1T cells = IC_{50}/MOLT-4 cells in Fig. 2), and the other group is the compounds with S1T cell-selective toxicity [compounds **2**–**4**, **6**–**8**, and **15**: all of these possess IC_{50} values of 3.8–6.9 μM toward S1T cells with various IC_{50} values (21.5–82.9 μM) toward MOLT-4 cells, and they are located inside the dotted square in Fig. 2]. The apparent common structural feature of the compounds of the latter group is illustrated as a Markush structure in Figure 2. Among the compounds we prepared, all of the compounds which fitted the Markush structure (Fig. 2) were grouped into the latter group without exception. In other words, compounds with no hydrogen at the α-carbon of the carbonyl moiety are non-selective. The results suggests that (i) there exist at least two molecular bases for the cell proliferation-inhibiting activity elicited by our TMN derivatives, of which one is common to both S1T and MOLT-4 cells and the other is specific to S1T cells, (ii) there may be a specific target molecule in S1T cells which recognizes the structure shown as the Markush structure in Figure 2. The requirement of at least one hydrogen atom at the α-carbon of the carbonyl moiety might suggest that the active form is an enol-like form of the compounds.

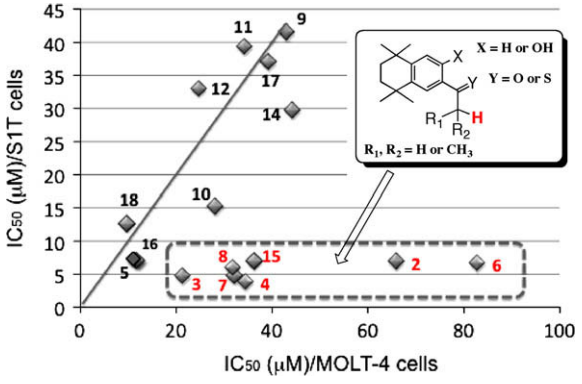


Figure 2. Correlation of S1T and MOLT-4 cells inhibitory activities of TMN derivatives towards S1T and MOLT-4 cells.

Table 2
Effect of compound **6** (20 μM) on S1T/MOLT-4 cell cycle (2-1) and apoptosis induction (2-2)

	Cell population (%)		
	Phase	SIT cells	MOLT-4 cells
2-1			
Non-treated	G0/G1	41.39	40.27
	G2/M	18.08	12.42
	S	40.53	47.31
Treated for 24 h	G0/G1	37.0	42.14
	G2/M	23.0	11.10
	S	39.95	46.76

	Apoptosis (%)			
	S1T cells		MOLT-4 cells	
	None	+ compd **6**	None	+ compd **6**
2-2				
Treated for 24 h	7.9	10.**1**	6.9	7.0
Treated for 48 h	4.9	10.1	4.0	4.0

2.3. Cell level analysis

Among the compounds prepared, compound **6** showed superior activity with the highest SI value of 12.4 (Table 1). Cell cycle and apoptosis analyses were performed by fluorescence flow cytometry (Table 2). As shown in Table 2-1, treatment of S1T cells with 20 μM compound **6** for 24 h resulted in increase of the population of G2 M stage cells and decrease of the population in both G0/G1 and S-phase, indicating that **6** induced G2-arrest of S1T cells. By contrast, treatment of MOLT-4 cells with **6** had no apparent effect on the cell cycle. Similarly, as shown in Table 2-2, treatment of S1T cells with compound **6** for 24 and 48 h induced increased apoptotic cell death [the ratio of apoptosis (%) in S1T cells untreated and treated with **6** for 24 or 48 h was 1.3 or 2.1, respectively], while 24 and 48 h treatment of MOLT-4 cells had no effect on the ratio of apoptosis. These results suggest that **6** selectively induces G2-arrest and apoptosis of S1T cells. The selectivity of **6** for ATL cells was confirmed by the use of several HTLV-1-positive and negative cell lines (unpublished observations). The target molecule(s) of **6** and related compounds and the molecular mechanism of G2-arrest/apoptosis elicited by **6** remain to be elucidated.

3. Conclusion

In conclusion, we extracted the TMN skeleton from our synthetic retinoids based on the multi-template hypothesis and micro-reversed fragment-based drug design hypothesis (molecular anatomy to extract small protopharmacophore skeletons which are applicable as multi-templates).[18] Preparation and screening of TMN derivatives resulted in discovery of ATL cell-selective proliferation inhibitors, including 2-acetyl-3-hydroxy-5,6,7,8-tetrahydro-5,5,8,8-tetramethylnaphthalene (**6**) which selectively induced G2-arrest/apoptosis in S1T cells. Structure–activity relationship analysis of the TMN derivatives suggested the existence of a specific target molecule(s) for eliciting S1T cell-selective growth-inhibitory activity. Studies to identify the putative target molecule(s) and the molecular mechanism are under way.

4. Experimental

4.1. Cells

4.1.1. Cell culture

The HTLV-1 carrying T-cell line S1T was established from the peripheral blood mononuclear cells of an ATL patient, as described previously.[7,22] The HTLV-1-negative T-cell line MOLT-4 was also used.[7] These cell lines were maintained in RPMI1640 medium supplemented with 10% v/v heat-inactivated fetal bovine serum, 100 U/ml penicillin G, and 100 μg/ml streptomycin.

4.1.2. Cell cycle analysis

Cells were seeded at a density of 1×10^6 cells per ml in 24-well plate and incubated in the absence or the presence of 2-acetyl-3-hydroxy-5,6,7,8-tetrahydro-5,5,8,8-tetramethylnaphthalene (**6**) at a concentration of 20 μM. After 24 h, cells were washed with phosphate buffered saline (PBS), fixed with 1% paraformaldehyde, permeabilized with 70% ethanol and incubated with propidium iodide/RNA staining buffer (BD Biosciences, San Diego, CA) for 15 min. The stained cells were analyzed by FACS Calibur (Becton Dickinson, San Jose, CA). The distribution of cell cycle phase was analyzed by MODFIT software.

4.1.3. Assays for apoptosis

Cells were seeded at a density of 1×10^6 cells per ml in 24-well plate and incubated in the absence or the presence of 2-acetyl-3-hydroxy-5,6,7,8-tetrahydro-5,5,8,8-tetramethylnaphthalene (**6**) at a concentration of 20 μM. After 24 or 48 h, cells were washed with PBS and incubated with fluorescein isothiocyanate-conjugated Annexin V (Invitrogen, Eugene, OR) in annexin-binding buffer (10 mM HEPES, 140 mM NaCl, and 2.5 mM $CaCl_2$, pH 7.4) for 15 min. After washing, the stained cells were analyzed by FACS Calibur (Becton Dickinson).

4.2. Chemistry

4.2.1. General

^{1}H NMR spectra were recorded on a JEOL JNM-GX500 (500 MHz) spectrometer. Chemical shifts are expressed in parts per million relative to tetramethylsilane. Mass spectra were recorded on a JEOL JMS-DX303 spectrometer.

4.2.2. 5,6,7,8-Tetrahydro-2-hydroxy-5,5,8,8-tetramethylnaphthalene[23]

To a solution of phenol (1.02 g, 10.8 mmol) in CH_2Cl_2 (5.0 mL) was added aluminum chloride (144 mg, 10.8 mmol) and 2,5-dichloro-2,5-dimethylhexane (2.18 g, 11.9 mmol) at room temperature, and the mixture was stirred at the same temperature for 19 h. The reaction mixture was poured into ice-water and extracted with ethyl acetate. The extract successively washed with water, saturated aqueous $NaHCO_3$ and brine and then dried over $MgSO_4$. The solvent was evaporated, and the residue was purified by recrystallization from hexane to give the title compound (1.83 g, 83%) as a white solid. FAB-MS *m/z*: 204 $(M+H)^+$; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.17 (d, 1H, *J* = 8.5 Hz), 6.75 (d, 1H, *J* = 3.0 Hz), 6.62 (dd, 1H, *J* = 8.5, 3.0 Hz), 4.49 (s, 1H), 1.66 (s, 4H), 1.25 (s, 6H), 1.24 (s, 6H).

4.2.3. 5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthyl methyl ketone (2): General procedure for the synthesis of compounds 2–4, 6–10

To a solution of 5,6,7,8-tetrahydro-5,5,8,8-tetramethylnaphthalene (**1**)[8] (377 mg, 2.00 mmol) in CH_2Cl_2 (3.0 mL) was added aluminum chloride (296 mg, 2.22 mmol) and acetyl chloride (155 μL, 2.18 mmol) at room temperature, and the mixture was stirred at 50 °C for 2 h. The reaction mixture was poured into ice-water and extracted with ethyl acetate. The extract successively washed with water, saturated aqueous $NaHCO_3$ and brine and then dried over $MgSO_4$. The solvent was evaporated, and the residue was purified by silica gel column chromatography (hexane/CH_2Cl_2 = 10/1) to give **2** (359 mg, 78%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.92 (d, 1H, *J* = 1.9 Hz), 7.70 (dd, 1H, *J* = 8.0, 1.9 Hz), 7.38 (d, 1H, *J* = 8.0 Hz), 2.55 (s, 3H), 1.69 (s, 4H), 1.30 (s, 6H), 1.28 (s, 6H); HRMS (FAB) calcd for $C_{16}H_{23}O$ 231.1749; found: 231.1780 $(M+H)^+$.

4.2.4. 5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthyl ethyl ketone (3)

27% (colorless oil); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.94 (d, 1H, *J* = 2.0 Hz), 7.70 (dd, 1H, *J* = 8.0, 2.0 Hz), 7.38 (d, 1H, *J* = 8.0 Hz), 2.97 (q, 2H, *J* = 7.3 Hz), 1.70 (s, 4H), 1.31 (s, 6H), 1.29 (s, 6H), 1.22 (t, 3H, *J* = 7.3 Hz); HRMS (FAB) calcd for $C_{17}H_{25}O$ 245.1905; found: 245.1924 $(M+H)^+$.

4.2.5. 5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthyl isopropyl ketone (4)

38% (pale yellow oil); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.95 (d, 1H, *J* = 1.8 Hz), 7.70 (dd, 1H, *J* = 8.0, 1.8 Hz), 7.38 (d, 2H, *J* = 8.0 Hz), 3.54 (septet, 1H, *J* = 7.0 Hz), 1.70 (s, 4H), 1.31 (s, 6H), 1.29 (s, 6H), 1.21 (d, 6H, *J* = 7.0 Hz); HRMS (FAB) calcd for $C_{18}H_{27}O$ 259.2062; found: 259.2047 $(M+H)^+$.

4.2.6. 2-Acetyl-3-hydroxy-5,6,7,8-tetrahydro-5,5,8,8-tetramethylnaphthalene (5,6,7,8-Tetrahydro-3-hydroxy-5,5,8,8-tetramethyl-2-naphthyl methyl ketone) (6)[24]

Pale yellow solid; ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.85 (s, 1H), 7.63 (s, 1H), 6.88 (s, 1H), 2.59 (s, 3H), 1.66 (s, 4H), 1.27 (s, 6H), 1.25 (s, 6H); HRMS (FAB) calcd for $C_{16}H_{23}O_2$ 247.1698; found: 247.1705 $(M+H)^+$.

4.2.7. 5,6,7,8-Tetrahydro-3-hydroxy-5,5,8,8-tetramethyl-2-naphthyl ethyl ketone (7)

45% (white solid); ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.96 (s, 1H), 7.68 (s, 1H), 6.90 (s, 1H), 3.03 (q, 2H, *J* = 7.3 Hz), 1.68 (s, 4H), 1.28 (s, 6H), 1.27 (s, 6H), 1.25 (t, 3H, *J* = 7.3 Hz); Anal. Calcd for $C_{17}H_{24}O_2$: C, 78.42; H, 9.29; O, 12.29. Found: C, 78.19; H, 9.23; O, 12.58.

4.2.8. 5,6,7,8-Tetrahydro-3-hydroxy-5,5,8,8-tetramethyl-2-naphthyl isopropyl ketone (8)

47% (colorless oil); ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.11 (s, 1H), 7.71 (s, 1H), 6.91 (s, 1H), 3.60 (septet, 1H, *J* = 7.0 Hz), 1.68 (s, 4H), 1.29 (s, 6H), 1.27 (s, 6H), 1.24 (d, 6H, *J* = 7.0 Hz); HRMS (FAB) calcd for $C_{18}H_{27}O_2$ 275.2011; found: 275.2032 $(M+H)^+$.

4.2.9. 5,6,7,8-Tetrahydro-3-hydroxy-5,5,8,8-tetramethyl-2-naphthyl *tert*-butyl ketone (9)

9% (yellow oil); ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.30 (s, 1H), 7.97 (s, 1H), 6.92 (s, 1H), 2.05 (s, 1H), 1.68 (s, 4H), 1.45 (s, 9H), 1.29 (s, 6H), 1.27 (s, 6H); HRMS (FAB) calcd for $C_{19}H_{29}O_2$ 289.2168; found: 289.2135 $(M+H)^+$.

4.2.10. 5,6,7,8-Tetrahydro-3-hydroxy-5,5,8,8-tetramethyl-2-nap-hthyl phenyl ketone (10)

9% (pale yellow oil); ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.63 (s, 1H), 7.69 (s, 1H), 7.67 (s, 1H), 7.61–7.58 (m, 2H), 7.55–7.49 (m, 3H), 6.99 (s, 1H), 2.05 (s, 1H), 1.70–1.65 (m, 4H), 1.30 (s, 6H), 1.17 (s, 6H); HRMS (FAB) calcd for $C_{21}H_{25}O_2$ 309.1855; found: 309.1863 $(M+H)^+$.

4.2.11. 5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-methylnaphthalene[8]

To a suspension of aluminum chloride (200 mg, 1.50 mmol) in toluene (10 mL) was added 2,5-dichloro-2,5-dimethylhexane

(4.70 g, 25.7 mmol) at room temperature, and the mixture was stirred at the same temperature for 24 h. The reaction mixture was poured into ice-water and extracted with ethyl acetate. The extract was successively washed with water, saturated aqueous $NaHCO_3$ and brine and then dried over $MgSO_4$. The solvent was evaporated to give the title compound (4.90 g, 94%) as a pale yellow oil. This crude product was used for the next step without further purification. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.20 (d, 1H, J = 8.0 Hz), 7.10 (d, 1H, J = 1.2 Hz), 6.95 (dd, 1H, J = 8.0, 1.2 Hz), 2.29 (s, 3H), 1.67 (s, 4H), 1.28 (s, 6H), 1.26 (s, 6H).

4.2.12. 5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthaldehyde (12)

To a solution of 5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-methylnaphthalene (202 mg, 1.00 mmol) in AcOH (8.2 mL) was added cerium ammonium nitrate (2.40 g, 4.37 mmol) at room temperature and the mixture was stirred at 100 °C for 1 h. The reaction mixture was poured into ice-water and extracted with ethyl acetate. The extract was successively washed with water, saturated aqueous $NaHCO_3$ and brine and then dried over $MgSO_4$. The solvent was evaporated, and the residue was purified by silica gel column chromatography (hexane/ethyl acetate = 15/1) to give **12** (106 mg, 49%) as a pale yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.95 (s, 1H), 7.83 (d, 1H, J = 2.0 Hz), 7.62 (dd, 1H, J = 8.0, 2.0 Hz), 7.46 (d, 1H, J = 8.0 Hz), 1.72 (s, 4H), 1.32 (s, 6H), 1.31 (s, 6H); HRMS (FAB) calcd for $C_{15}H_{21}O$ 217.1592; found: 217.1619 $(M+H)^+$.

4.2.13. 5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthoic acid (13)

To a solution of 5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-methylnaphthalene (3.52 g, 17.4 mmol) in pyridine (12 mL) was added $KMnO_4$ (6.70 g, 42.4 mmol) and sodium hydroxide (1.00 g, 25.0 mmol) at room temperature, and the mixture was stirred at 95 °C for 5 h. The reaction mixture was filtered through a Celite pad and the filtrate was added to diluted HCl to acidify it. The mixture was extracted with ethyl acetate and then dried over $MgSO_4$. The solvent was evaporated, and the residue was purified by silica gel column chromatography (hexane/ethyl acetate = 4/1) to give **13** (142 mg, 3.0%) as a colorless solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.05 (d, 1H, J = 1.8 Hz), 7.82 (dd, 1H, J = 8.0, 1.8 Hz), 7.40 (d, 1H, J = 8.0 Hz), 1.71 (s, 4H), 1.32 (s, 6H), 1.30 (s, 6H); HRMS (FAB) calcd for $C_{15}H_{21}O_2$ 233.1542; found: 233.1563 $(M+H)^+$.

4.2.14. 5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthylamide (17)

To a solution of **13** (465 mg, 2.00 mmol) in CH_2Cl_2 (5.0 mL) was added oxalyl chloride (250 μL, 2.95 mmol) and DMF (3 drops) at 0 °C, and the mixture was stirred at the same temperature. After 1 h, to this was added 25% aqueous ammonia and the mixture was stirred at room temperature for 15 h. The reaction was quenched by adding water and extracted with CH_2Cl_2. The extract was successively washed with water and brine, and then dried over $MgSO_4$. The solvent was evaporated, and the residue was purified by recrystallization from ethyl acetate to give **17** (453 mg, 98%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.81 (d, 1H, J = 2.0 Hz), 7.49 (dd, 1H, J = 8.0, 2.0 Hz), 7.37 (d, 1H, J = 8.0 Hz), 1.70 (s, 4H), 1.31 (s, 6H), 1.29 (s, 6H); HRMS (FAB) calcd for $C_{15}H_{22}NO$ 232.1701; found: 232.1718 $(M+H)^+$.

4.2.15. 5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthyl-*N*-methoxy-*N*-methylamide (18)

To a solution of **13** (232 mg, 1.00 mmol) in CH_2Cl_2 (10 mL) was added oxalyl chloride (294 mg, 2.40 mmol) and DMF (1 drop) at room temperature, and the mixture was stirred at the same temperature. After 5 h, the volatile material was removed under reduced pressure. The residue was dissolved in CH_2Cl_2 (10 mL), and to this was added *N*-methoxy-*N*-methylamine hydrochloride (116 mg, 1.20 mmol) and triethylamine (4.0 mL, 28.7 mmol). The mixture was stirred at room temperature for 17 h. The solvent was removed under reduced pressure and the residue was diluted with ethyl acetate. The organic solution successively washed with diluted HCl, water, saturated aqueous $NaHCO_3$ and brine, and then dried over $MgSO_4$. The solvent was evaporated, and the residue was purified by silica gel column chromatography (hexane/ethyl acetate = = 10/1) to give **18** (139 mg, 50%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34 (d, 1H, J = 1.8 Hz), 7.30 (d, 1H, J = 8.0 Hz), 7.16 (dd, 1H, J = 8.0, 1.8 Hz), 3.10 (br s, 1H), 3.00 (br s, 1H), 1.68 (s, 4H), 1.29 (s, 6H), 1.28 (s, 6H); HRMS (FAB) calcd for $C_{17}H_{26}NO_2$ 276.1964; found: 276.1991 $(M+H)^+$.

4.2.16. 5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthyl phenyl ketone (5)

To a solution of **18** (55.0 mg, 200 mmol) in diethyl ether (2.0 mL) was added phenylmagnesium bromide (prepared from bromobenzene and magnesium in THF) at room temperature, and the mixture was stirred at the same temperature for 22 h. The reaction mixture was poured into saturated aqueous NH_4Cl and extracted with ethyl acetate. The extract was successively washed with water, saturated aqueous $NaHCO_3$ and brine and then dried over $MgSO_4$. The solvent was evaporated, and the residue was purified by silica gel column chromatography (hexane/ethyl acetate = 2/1) to give **5** (14.9 mg, 25%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.80 (d, 1H, J = 7.3 Hz), 7.79 (s, 1H), 7.57 (t, 1H, J = 7.3 Hz), 7.55 (dd, 1H, J = 8.0, 1.8 Hz), 7.47 (t, 1H, J = 8.0 Hz), 7.39 (d, 1H, J = 8.0 Hz), 1.72 (s, 4H), 1.31 (s, 6H), 1.29 (s, 6H); HRMS (FAB) calcd for $C_{21}H_{25}O$ 293.1905; found: 293.1882 $(M+H)^+$.

4.2.17. *rac*-1-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthyl)ethanol (11)

To a solution of **2** (50.0 mg, 217 μmol) in MeOH (1.0 mL) was added $NaBH_4$ (10.0 mg, 264 μmol) at room temperature, and the mixture was stirred at the same temperature for 30 min. The reaction mixture was poured into water and extracted with ethyl acetate. The extract was successively washed with water, saturated aqueous $NaHCO_3$ and brine and then dried over $MgSO_4$. The solvent was removed in vacuo to give **11** as a colorless oil (47.9 mg, 95%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.30 (s, 1H), 7.29 (d, 1H, J = 6.0 Hz), 7.14 (dd, 1H, J = 6.0, 2.0 Hz), 4.85 (q, 1H, J = 6.7 Hz), 1.68 (s, 4H), 1.50 (d, 3H, J = 6.7 Hz), 1.29 (s, 6H), 1.27 (s, 6H); HRMS (FAB) calcd for $C_{16}H_{25}O$ 233.1905; found 233.1930 $(M+H)^+$.

4.2.18. 5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-3-hydroxy-2-naphthoic acid (14)

To a solution of **6**[24] (98.5 mg, 400 μmol) in EtOH (2.0 mL) was added aqueous NaOCl solution (active chlorine concentration: 5%, 3 mL) at room temperature, and the mixture was stirred at 70 °C for 3 h. To this was added NaOH (300 mg, 7.50 mmol) and the mixture was stirred at the same temperature. After 1 h, to this was added I_2 (305 mg, 1.20 mmol) and the mixture was stirred at the same temperature for 3 h. After the reaction mixture had cooled to room temperature, the mixture was back-extracted with CH_2Cl_2. The aqueous layer was acidified by the addition of 2 N HCl, extracted with CH_2Cl_2, and then dried over $MgSO_4$. The solvent was evaporated, and the residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 9/1) to give **14** (8.0 mg, 8.1%) as a pale yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.73 (br s, 1H), 10.84 (br s, 1H), 7.69 (s, 1H), 6.85 (s, 1H), 1.58–1.64 (m, 4H), 1.22 (s, 6H), 1.20 (s, 6H); HRMS (FAB) calcd for $C_{15}H_{21}O_3$ 249.1491; found: 249.1522 $(M+H)^+$.

4.2.19. 1-(5,6,7,8-Tetrahydro-3-hydroxy-5,5,8,8-tetramethyl-2-naphthyl)ethanethione (15)

To a solution of **6**[24] (13.3 mg, 53.9 μmol) in toluene (1.0 mL) was added Lawesson's reagent (32 mg, 80.9 μmol) at room temperature, and the mixture was stirred at 120 °C for 24 h. The reaction mixture was poured into water and extracted with CH_2Cl_2. The solvent was evaporated, and the residue was purified by silica gel column chromatography (hexane/ethyl acetate = 12/1) to give **15** as a yellow solid (4.2 mg, 30%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.99 (s, 1H), 7.82 (s, 1H), 6.99 (s, 1H), 3.12 (s, 3H), 1.69 (s, 4H), 1.30 (s, 6H), 1.29 (s, 6H); HRMS (FAB) calcd for $C_{16}H_{23}OS$ 263.1470; found: 263.1446 $(M+H)^+$.

4.2.20. 5,6,7,8-Tetrahydro-3-methoxy-5,5,8,8-tetramethyl-2-naphthyl methyl ketone (16)

To a solution of **6**[24] (123 mg, 500 μmol) in acetone (2.0 mL) was added K_2CO_3 (138 mg, 1.00 mmol) and MeI (400 μL, 6.42 mmol) at room temperature, and the mixture was stirred at 70 °C for 10 h. The reaction mixture was filtered, washed with acetone, and evaporated. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 20/1) to give **16** (92.4 mg, 71%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.71 (s, 1H), 6.82 (s, 1H), 3.87 (s, 3H), 2.57 (s, 3H), 1.69–1.62 (m, 4H), 1.28 (s, 6H), 1.25 (s, 6H); HRMS calcd for $C_{17}H_{25}O_2$: 261.1855; found: 261.1893 $(M+H)^+$.

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