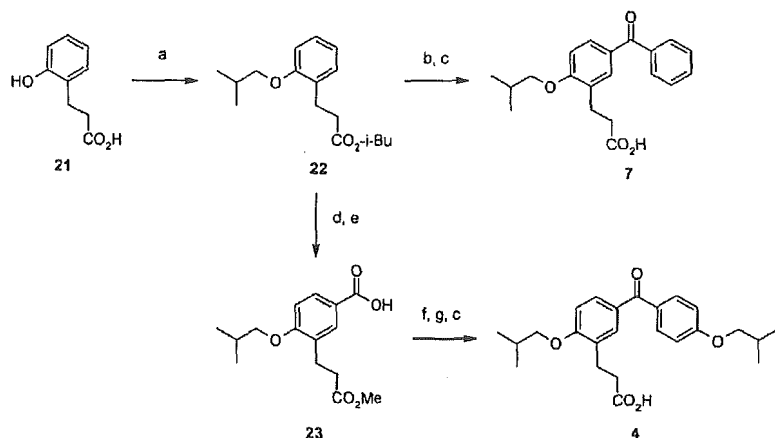
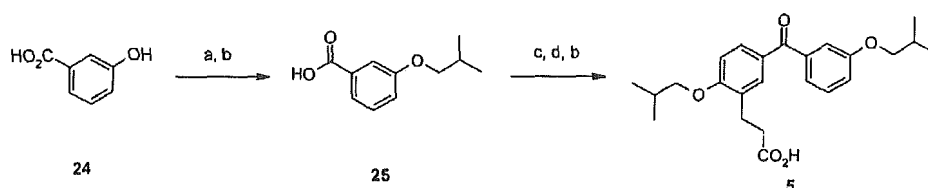
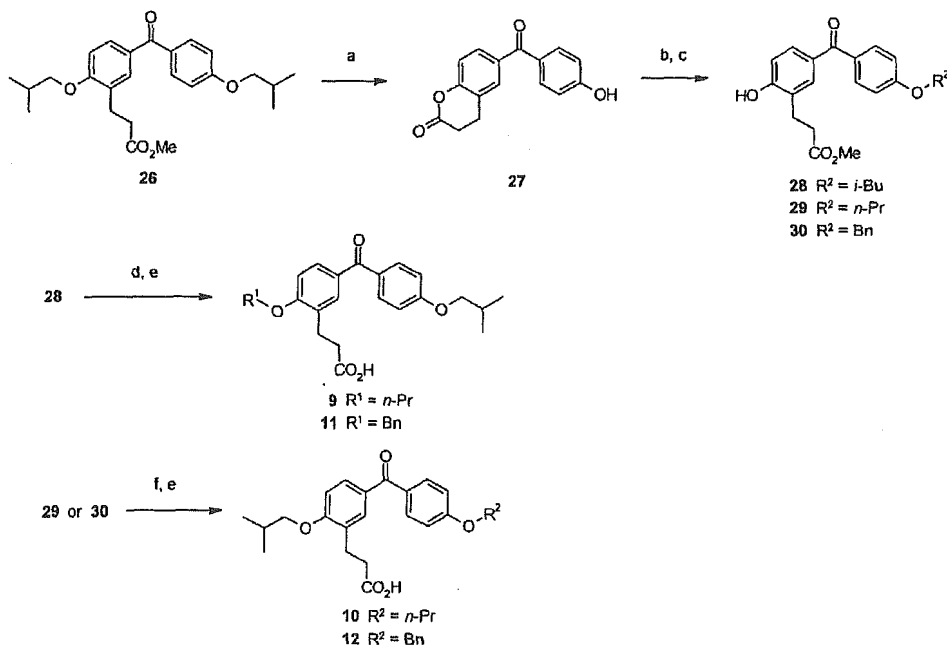


Scheme 3^a

^a Reagents: (a) isobutyl bromide, K_2CO_3 , DMF; (b) benzoyl chloride, $AlCl_3$, CH_2Cl_2 ; (c) $NaOH-H_2O$, acetone (for 4) or EtOH (for 7); (d) Cl_2CHOMe , $AlCl_3$, CH_2Cl_2 , then MeOH, Δ ; (e) $NaClO_2$, H_2O_2 , NaH_2PO_4 , aq. MeCN; (f) oxalyl chloride, DMF, CH_2Cl_2 ; (g) isobutoxybenzene, $AlCl_3$, CH_2Cl_2 .

Scheme 4^a

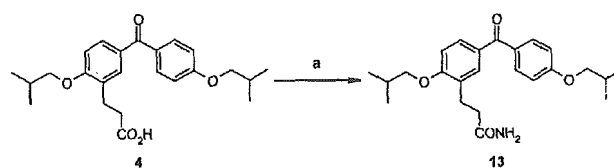
^a Reagents: (a) isobutyl bromide, K_2CO_3 , DMF; (b) $NaOH-H_2O$, acetone; (c) oxalyl chloride, DMF, CH_2Cl_2 ; (d) compound 22, $AlCl_3$, CH_2Cl_2 .

Scheme 5^a

^a Reagents: (a) $AlCl_3$, 1,2-dichloroethane; (b) R^2-OH , DIAD, Ph_3P , THF; (c) $MeONa-MeOH$, THF; (d) R^1-Br , K_2CO_3 , DMF; (e) $NaOH-H_2O$, acetone; (f) isobutyl bromide, K_2CO_3 , DMF.

catalyzed methanolysis to yield the corresponding alkyl ethers 28–30. Compound 28 was alkylated with R^1-Br (propyl bromide or benzyl bromide), followed by hydrolysis with alkali to yield 9 and 11 as depicted in Scheme 5. The regioisomeric analogues 10 and 12 were prepared similarly via 29 ($R^2 =$ propyl) and 30 ($R^2 =$ benzyl), respectively.

Synthesis of carboxamide of 4 (compound 13) was carried out as outlined in Scheme 6. Compound 4 was treated with oxalyl chloride and catalytic DMF leading to the acid chloride,

Scheme 6^a

^a Reagents: (a) oxalyl chloride, DMF, THF, then NH_4OH .

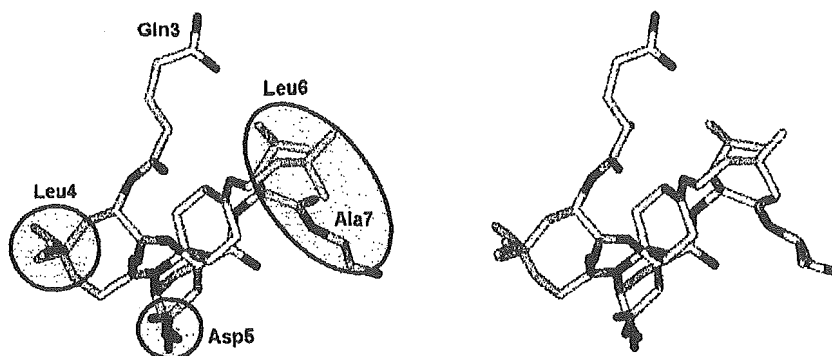


Figure 5. Stereoview of the superposition of Leu-Asp-Leu-Ala of peptide 1 (yellow) with compound 2 (white). The fitted points are shown as magenta-colored circles and an ellipse. Hydrogen atoms are not shown for clarity.

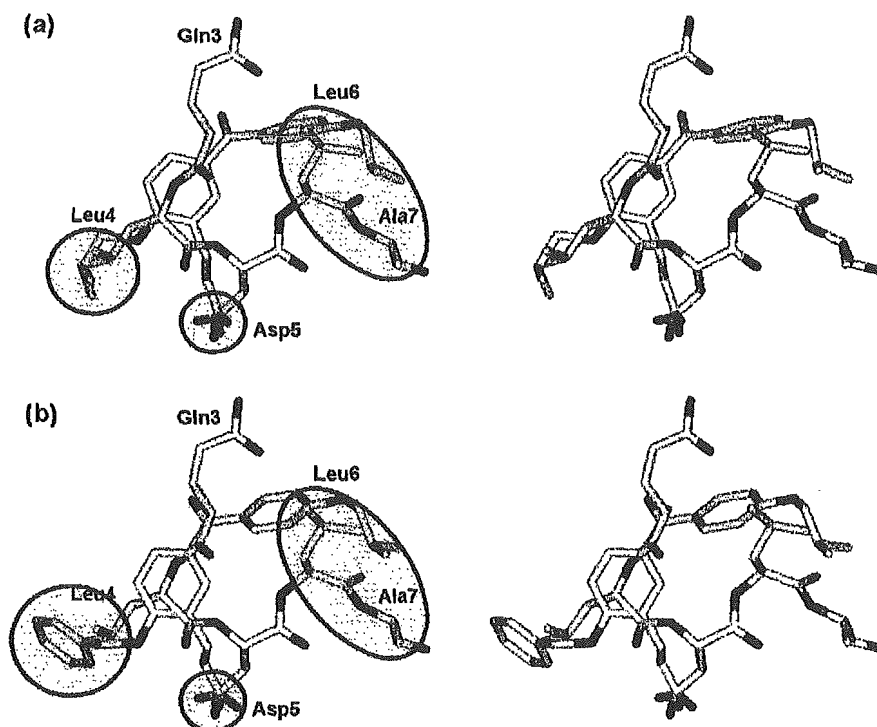


Figure 6. Stereoviews of the superposition of Leu-Asp-Leu-Ala of peptide 1 (yellow) with (a) compound 4 (white) and (b) compound 11 (white). The fitted points are shown as magenta-colored circles and an ellipse. Hydrogen atoms are not shown for clarity.

which was converted to propionamide **13** with ammonium hydroxide. Bis(4-isobutoxyphenyl)methanone (**8**) was prepared from commercially purchased bis(4-hydroxyphenyl)methanone by *O*-isobutylation with isobutyl bromide and K_2CO_3 in DMF.

Results and Discussion

Evaluation of Designed Compounds. The designed compounds were synthesized and evaluated using an ELISA-based AP-1 DNA-binding assay. Among these, compounds **2** and **3** bearing 1-thia-4-azaspiro[4.5]decane as a scaffold, and compounds **4** and **5**, bearing benzophenone as a scaffold, inhibited the binding of AP-1 bZIP and oligonucleotides containing the AP-1 binding site (Table 1; $IC_{50} = 460\text{--}650\ \mu M$). The three pharmacophoric elements of these compounds corresponded to Leu4, Asp5, and the centroid of Leu6 and Ala7 of peptide **1**. As examples, the best-fit superpositions of compounds **2** and **4** onto peptide **1** based on the pharmacophore model are shown in Figures 5 and 6a, respectively. The three pairs of pharmacophoric points that were used for overlapping are shown as circles and an ellipse in these figures. The three overlapped

points within compound **2** were the carbon atom of the carboxyl group plus the carbon atoms at the branched positions of the 4-methylpentanoyl and 4-methylpentylidene groups. The RMSD value resulting from the superposition of the three pharmacophoric points was $0.89\ \text{\AA}$, and the energy of this best-fitted conformer relative to the lowest energy was $2.01\ \text{kcal mol}^{-1}$. The three overlapped points within compound **4** were the carbon atom of the carboxyl group plus the carbon atoms at the branched positions of the isobutyl groups. The RMSD value resulting from the superposition of the three pharmacophoric points was $0.92\ \text{\AA}$, and the energy of this best-fitted conformer relative to the lowest energy was $2.46\ \text{kcal mol}^{-1}$. The structural isomers **2** and **3**, in which the lengths of methylene linkers between the 1-thia-4-azaspiro[4.5]decane scaffold and each isobutyl pharmacophore element were different, showed similar activity. The RMSD value obtained by the best-fit superposition of compound **3** and peptide **1** was $0.64\ \text{\AA}$, and the energy of the best-fitted conformer relative to the lowest energy was $1.73\ \text{kcal mol}^{-1}$; thus the 3D pharmacophore requirement was met by compound **3**, as well as compound **2**. Because compounds

4 and 5, in which only the substitution position of an isobutoxy moiety corresponding to the centroid of Leu6 and Ala7 differed, showed similar activity, there might be some allowance in the hydrophobic region of the pharmacophore. This was consistent with our assumption that Leu6 and Ala7 comprised one large hydrophobic element. These small-molecule compounds showed lower inhibitory activity than peptide 1 ($IC_{50} = 64 \mu M$). The results would be acceptable, given that these compounds have only three pharmacophore elements compared to the cyclic decapeptide.

Subsequently, we evaluated the effects of these compounds on the transactivation activity of AP-1 using a cell-based AP-1 reporter gene assay (Table 1). Compounds 2–5 showed dose-dependent inhibitory activities in this assay, similar to AP-1 DNA-binding inhibition. Thus, taking into account both the inhibitory activities and synthetic feasibility, we carried out further structural development of the benzophenone derivatives.

Conversion of Substituents on Benzophenone Derivatives. To verify the pharmacophoric elements, we designed and synthesized several derivatives based on compound 4, and evaluated their inhibitory activities on AP-1 DNA-binding (Table 2).

The conversion of each substituent of compound 4 to hydrogen (compounds 6–8) resulted in a considerable loss of inhibitory activity in the binding assay; thus, the importance of the three pharmacophore elements was confirmed.

In addition, the conversion of each isobutoxy group to an *n*-propoxy group (compounds 9 and 10) resulted in a moderate loss of inhibitory activity at 1 mM. This finding may suggest a requirement for bulkiness of the branched chain alkyl groups.

Furthermore, compounds 11 and 12, in which each isobutoxy group was substituted with a benzyloxy group, showed similar inhibitory activities to compound 4, which implied that both aliphatic and aromatic substituents were acceptable hydrophobic elements at this position. The best-fit superposition of compound 11 and peptide 1 is shown in Figure 6b. The three overlapped points within compound 11 are the carbon atom of the carboxyl group, the carbon atom at the branched position of the isobutyl group, and the carbon atom at the root of the benzene ring in the benzyl group. The RMSD value obtained by the superposition of the three pharmacophoric points was 0.93 Å, and the energy of this best-fitted conformer relative to the lowest energy was 4.58 kcal mol⁻¹. The conformation and orientation of compound 11 were similar to those of compound 4 (Figure 6).

Finally, we converted the R₃ substituent to investigate the importance of the carboxyl group. The conversion into carboxamide (compound 13) resulted in a considerable loss of inhibitory activity. This result clearly demonstrates the significance of the ionic interactions. This appears to support our initial hypothesis that the basic lysine and/or arginine in the basic region of bZIP domains might take part in the interactions with peptide 1.

The above-mentioned results demonstrate that the optimal arrangement of at least three pharmacophoric elements is fundamental. In addition, we suggest that it might be possible to enhance the inhibitory activity by the further introduction of a fourth pharmacophoric element corresponding to Gln3 of peptide 1 that showed more potent inhibitory activity.

Conclusions

We converted cyclic peptide 1 to small-molecule AP-1 inhibitors using a lead-hopping approach based on a 3D pharmacophore model that was derived from the peptide. New AP-1 inhibitors, 1-thia-4-azaspiro[4.5]decane and benzophenone

derivatives, were discovered using this combination of computational methods and medicinal chemistry intuition. Our study demonstrated the reliability of the 3D pharmacophore model and its utility in the discovery of AP-1 inhibitors. Additionally, the possibility of further improvement of the benzophenone derivatives was suggested by the results of the conversion of the substituents. The benzophenone derivatives were considered to be an attractive lead series, because they exhibited AP-1 inhibitory activities not only in the binding assay but also in the cell-based assay. The continuous optimization of this series might produce drugs that can be used against AP-1 for the treatment of various diseases.

Experimental Section

Molecular Modeling. All molecular modeling was performed using the SYBYL software package version 6.5²² running on a Silicon Graphics Power Indigo2 workstation. All of the compounds designed for use in this study were built using the SKETCH module. The carboxyl group of the designed compounds was deprotonated to imitate physiological conditions. The designed compounds were minimized using the Powell method in the MAXIMIN2 module of SYBYL to reach a final convergence of 0.01 kcal mol⁻¹ Å⁻¹; the MMFF94 force field and the MMFF94 charges²³ were employed for the energy minimizations. Electrostatic interactions were taken into consideration using a distance-based dielectric constant of 78.3 to simulate an aqueous environment.

Conformational analysis of each of the designed compounds was carried out using the Random Search module implemented in SYBYL. An absolute energy cutoff of 300 kcal mol⁻¹ was used to remove high energy conformations. The energy values during the optimization were computed with the MMFF94 force field and the MMFF94 charges. The number of maximum cycles was set to 1000. Within each cycle, the bonds that were defined as rotatable were set to random torsion angles and the compounds were then minimized. The number of maximum hits was set to 6. Unique conformers were distinguished by setting a RMS threshold of 0.2 Å, with chirality checking if necessary. The systematical superposition of collected low-energy conformers was carried out using the FIT routine in SYBYL.

The overlapping volume and the volume of the corresponding residues in peptide 1 as a template were calculated using the MVOLUME and VOLUME routines in SYBYL, respectively.

Chemistry. Melting points were determined using a B-545 melting point apparatus (Büchi Lab., Flawil, Switzerland) and were uncorrected. Proton NMR (¹H NMR) spectra were recorded on a JEOL JNM-AL 400 spectrometer (JEOL Ltd., Tokyo, Japan) using tetramethylsilane as the internal standard. Chemical shifts are reported in ppm (δ units), and the signal patterns are designated as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and br (broad). Column chromatography was carried out using PSQ100B silica gel of 75–200 μm (Fuji Silysia Chemical, Aichi, Japan). Low-resolution mass spectra (MS) were obtained on a Hitachi M-8000 mass spectrometer system equipped with an electrospray ionization interface (Hitachi Ltd., Tokyo, Japan). Elemental analysis (C, H, and N) was performed at Toyama Industrial Technology Center (Toyama, Japan), and all results were within $\pm 0.4\%$ of the theoretical values. Unless otherwise noted, all solvents, chemicals, and reagents were obtained commercially and used without purification. Peptide 1 has been previously described.⁹

4-(4-Methylpentylidene)cyclohexanone (15). To a stirred and cooled ($-45^\circ C$) suspension of 4-methylpentyltriphenylphosphonium bromide (13.1 g, 30.7 mmol) in THF (40 mL) was added dropwise *n*-butyllithium (1.6 M in hexane, 19.2 mL, 30.7 mmol). The mixture was then warmed to 0 $^\circ C$ over 20 min. A solution of 1,4-cyclohexanedione monoethylene ketal (14) (4.00 g, 25.6 mmol) in THF (20 mL) was added dropwise to the resulting phosphorane solution at 0–5 $^\circ C$. Subsequently, the reaction mixture was allowed to warm to room temperature and was stirred for 1 h. The mixture was quenched with water (100 mL) and extracted with EtOAc (1

× 100 mL). The aqueous layer was extracted with EtOAc (1 × 50 mL). The combined organic extracts were washed with water (1 × 100 mL) and brine (1 × 100 mL), dried over MgSO₄, and concentrated under reduced pressure. The residue was purified by chromatography (silica gel 150 g, 5:1 hexane/EtOAc) to give 8-(4-methylpentylidene)-1,4-dioxaspiro[4.5]decane (5.40 g, 94%) as a colorless oil: ¹H NMR (400 MHz, CDCl₃) δ 0.87 (6H, d, *J* = 6.6 Hz), 1.21 (2H, q, *J* = 7.5 Hz), 1.46–1.62 (1H, m), 1.62–1.72 (4H, m), 1.99 (2H, q, *J* = 7.5 Hz), 2.21 (2H, t, *J* = 6.3 Hz), 2.27 (2H, t, *J* = 6.5 Hz), 3.97 (4H, s), 5.13 (1H, t, *J* = 7.5 Hz).

A mixture of the ketal (1.00 g, 4.46 mmol) obtained above and 6 M HCl (5 mL) in 1,4-dioxane (10 mL) was stirred at room temperature for 9 h before dilution with CHCl₃ (50 mL) and water (30 mL). The aqueous layer separated was extracted with CHCl₃ (1 × 30 mL), and the combined organic extracts were washed with water (1 × 30 mL) and brine (1 × 30 mL), dried over MgSO₄, and concentrated under reduced pressure. The residue was purified by chromatography (silica gel 100 g, 10:1 hexane/EtOAc) to give 15 (0.77 g, 96%) as a colorless oil: ¹H NMR (400 MHz, CDCl₃) δ 0.90 (6H, d, *J* = 6.6 Hz), 1.16–1.32 (2H, m), 1.48–1.66 (1H, m), 2.04 (2H, q, *J* = 7.6 Hz), 2.36–2.56 (8H, m), 5.30–5.38 (1H, m).

4-(3-Methylbutylidene)cyclohexanone (17). Compound 14 was treated by the procedure described for 15 except using 3-methylbutyltriphenylphosphonium iodide to afford 17 (65%) as a colorless oil: ¹H NMR (400 MHz, CDCl₃) δ 0.91 (6H, d, *J* = 6.6 Hz), 1.56–1.72 (1H, m), 1.90–1.97 (2H, m), 2.36–2.54 (8H, m), 5.37 (1H, t, *J* = 7.3 Hz); MS *m/z* 167 (M + H)⁺.

(R)-8-(4-Methylpentylidene)-1-thia-4-azaspiro[4.5]decane-3-carboxylic Acid (16). A mixture of 15 (1.20 g, 6.66 mmol) and L-cysteine (1.21 g, 9.99 mmol) in a mixture of 7:3 EtOH/water (36 mL) was stirred at room temperature for 3 h and at 40 °C for 5 h. The reaction mixture was evaporated under reduced pressure before dilution with water (100 mL). The product precipitated was collected by filtration and washed with water (3 × 30 mL) and hexane (3 × 30 mL) to afford 16 (1.18 g, 63%) as a light brown solid: ¹H NMR (400 MHz, CDCl₃) δ 0.88 (6H, d, *J* = 6.6 Hz), 1.20 (2H, q, *J* = 7.3 Hz), 1.46–1.60 (1H, m), 1.84–2.64 (10H, m), 3.22–3.34 (1H, m), 3.36–3.50 (1H, m), 4.33 (1H, t, *J* = 7.2 Hz), 5.10–5.22 (1H, m), 5.30–5.70 (2H, br); MS *m/z* 284 (M + H)⁺, 282 (M - H)⁻.

(R)-8-(3-Methylbutylidene)-1-thia-4-azaspiro[4.5]decane-3-carboxylic Acid (18). Compound 17 (5.00 g, 30.1 mmol) was treated by the procedure described for 16 to afford 18 (4.40 g, 54%) as a white solid: ¹H NMR (400 MHz, CDCl₃) δ 0.87 (6H, d, *J* = 6.3 Hz), 1.50–1.64 (1H, m), 1.82–2.14 (6H, m), 2.16–2.64 (4H, m), 3.22–3.32 (1H, m), 3.42 (1H, dd, *J* = 11.3, 7.4 Hz), 3.78 (2H, br s), 4.32 (1H, t, *J* = 7.4 Hz), 5.19 (1H, t, *J* = 7.3 Hz); MS *m/z* 268 (M - H)⁻.

(R)-4-(4-Methylpentanoyl)-8-(4-methylpentylidene)-1-thia-4-azaspiro[4.5]decane-3-carboxylic Acid (2). To a stirred solution of 4-methylpentanoic acid (225 mg, 1.94 mmol) in CH₂Cl₂ (5 mL) at room temperature was added dropwise oxalyl chloride (246 mg, 1.94 mmol) followed by the addition of DMF (50 μL), and the mixture was stirred at the same temperature for 2 h. The mixture was cooled in an ice bath before the sequential addition of 16 (500 mg, 1.76 mmol) and Et₃N (535 mg, 5.29 mmol), and then the mixture was stirred at room temperature for 2 h. The resulting mixture was treated with CHCl₃ (30 mL) and water (30 mL), followed by acidification to pH 2 with 6 M HCl, and the layers were separated. The aqueous layer was extracted with CHCl₃ (30 mL), and the combined organic extracts were washed with water (1 × 30 mL) and brine (1 × 30 mL), dried over MgSO₄, and concentrated under reduced pressure. The residue was purified by chromatography (silica gel 50 g, 10:1 CHCl₃/MeOH) to give 2 (380 mg, 56%) as a white solid, which was crystallized from CH₂Cl₂/hexane to give colorless crystals, mp 144–145 °C: ¹H NMR (400 MHz, CDCl₃) δ 0.87 (6H, d, *J* = 6.6 Hz), 0.88 (6H, d, *J* = 5.6 Hz), 1.20 (2H, q, *J* = 7.0 Hz), 1.34–1.78 (5H, m), 1.86–2.72 (9H, m), 2.84–3.38 (4H, m), 4.90–5.02 (1H, m), 5.06–5.16 (1H, m), 5.25–5.45 (1H, br); MS *m/z* 381 (M - H)⁻. Anal. (C₂₁H₃₅NO₃S) C, H, N.

(R)-8-(3-Methylbutylidene)-4-(5-methylhexanoyl)-1-thia-4-azaspiro[4.5]decane-3-carboxylic Acid (3). Compound 18 (500 mg, 1.86 mmol) was treated by the procedure described for 2 except using 5-methylhexanoic acid to afford 3 (305 mg, 43%) as a white solid, which was crystallized from CH₂Cl₂/hexane to give colorless crystals, mp 135–138 °C: ¹H NMR (400 MHz, CDCl₃) δ 0.87 (12H, d, *J* = 6.8 Hz), 1.10–1.24 (2H, m), 1.48–1.74 (5H, m), 1.76–2.50 (7H, m), 2.56–2.72 (1H, m), 2.84–3.34 (5H, m), 4.90–5.00 (1H, m), 5.10–5.20 (1H, m); MS *m/z* 382 (M + H)⁺, 380 (M - H)⁻. Anal. (C₂₁H₃₅NO₃S) C, H, N.

3-(2-Ethoxycarbonyl)ethyl)benzoic Acid (20). To a stirred suspension of NaH (60% dispersion in mineral oil, 1.37 g, 34.3 mmol) in DMF (75 mL) at 10–15 °C was added dropwise ethyl diethylphosphonoacetate (7.69 g, 34.3 mmol). The mixture was then stirred at room temperature for 30 min. To the stirred and ice-cooled mixture was added dropwise benzyl 3-formylbenzoate (19) (7.50 g, 31.2 mmol) before being stirred at room temperature for 30 min. The reaction mixture was poured into a mixture of EtOAc (100 mL) and 1 M HCl (50 mL), and the aqueous layer separated was extracted with EtOAc (1 × 50 mL). The organic extracts were washed with water (1 × 100 mL) and brine (1 × 100 mL), dried over MgSO₄, and concentrated under reduced pressure. The residue was purified by chromatography (silica gel 100 g, 3:1 hexane/EtOAc) to give benzyl 3-((*E*)-2-ethoxycarbonylvinyl)benzoate (9.12 g, 94%) as a colorless oil: ¹H NMR (400 MHz, CDCl₃) δ 1.34 (3H, t, *J* = 7.1 Hz), 4.27 (2H, q, *J* = 7.1 Hz), 5.38 (2H, s), 6.50 (1H, d, *J* = 16.0 Hz), 7.31–7.56 (6H, m), 7.70 (1H, d, *J* = 8.1 Hz), 7.70 (1H, d, *J* = 16.0 Hz), 8.08 (1H, d, *J* = 7.8 Hz), 8.22 (1H, s); MS *m/z* 311 (M + H)⁺.

A suspension of the α,β-unsaturated ester (8.50 g, 274 mmol) obtained above in EtOH (85 mL) was stirred at 40 °C under the atmospheric pressure of H₂ in the presence of 10% Pd-C (1.70 g). The mixture was filtered through Celite after 3 h, and the residue was washed with EtOH (3 × 20 mL). The filtrate was concentrated under reduced pressure to afford the semisolid residue, which was triturated with hexane (100 mL) to give 20 (5.87 g, 96%) as an off-white solid: ¹H NMR (400 MHz, CDCl₃) δ 1.24 (3H, t, *J* = 7.2 Hz), 2.67 (2H, t, *J* = 7.7 Hz), 3.03 (2H, t, *J* = 7.7 Hz), 4.14 (2H, q, *J* = 7.2 Hz), 7.41 (1H, t, *J* = 7.7 Hz), 7.47 (1H, d, *J* = 7.7 Hz), 7.92–8.03 (2H, m); MS *m/z* 221 (M - H)⁻.

Isobutyl 3-(2-Isobutoxyphenyl)propionate (22). A mixture of 3-(2-hydroxyphenyl)propionic acid (21) (10.0 g, 60.2 mmol), K₂CO₃ (41.6 g, 301 mmol), and isobutyl bromide (33.0 g, 241 mmol) in DMF (100 mL) was heated with vigorous stirring at 140 °C for 1 h. After cooling to room temperature, the reaction mixture was treated with EtOAc (150 mL) and water (300 mL) followed by acidification to pH 4 with 6 M HCl. The organic extract was washed with water (1 × 50 mL) and brine (1 × 50 mL), dried over MgSO₄, and concentrated under reduced pressure. The residue was purified by chromatography (silica gel 300 g, 5:1 hexane/EtOAc) to give 22 (15.4 g, 92%) as a colorless oil: ¹H NMR (400 MHz, CDCl₃) δ 0.91 (6H, d, *J* = 7.1 Hz), 1.05 (6H, d, *J* = 7.1 Hz), 1.84–1.96 (1H, m), 2.06–2.18 (1H, m), 2.63 (2H, t, *J* = 7.9 Hz), 2.97 (2H, t, *J* = 7.9 Hz), 3.74 (2H, d, *J* = 6.4 Hz), 3.85 (2H, d, *J* = 6.6 Hz), 6.78–6.88 (2H, m), 7.12–7.20 (2H, m); MS *m/z* 279 (M + H)⁺.

4-Isobutoxy-3-(2-methoxycarbonyl)ethyl)benzoic Acid (23). To a stirred solution of 22 (10.0 g, 35.9 mmol) in CH₂Cl₂ (50 mL) at -30 °C were successively added AlCl₃ (9.58 g, 71.8 mmol) and dichloromethyl methyl ether (4.96 g, 43.1 mmol), and the mixture was stirred at -10 °C for 1 h. After addition of MeOH (50 mL), the resulting mixture was stirred under reflux for 2 h before cooling to room temperature. The reaction mixture was poured into ice-water (200 mL), and the organic layer was sequentially washed with 1 M HCl (1 × 50 mL) and water (1 × 50 mL), and concentrated under reduced pressure to give aldehyde as a brown oil, which was used without further purification. The aldehyde thus obtained was dissolved in CH₃CN (20 mL). To the stirred solution were added a solution of NaH₂PO₄·2H₂O (15.1 g, 96.8 mmol) in water (20 mL) and 30% aqueous H₂O₂ (6.11 g, 53.9 mmol) in sequence. A solution of NaClO₂ (80% purity, 6.50 g, 57.5 mmol) in water (10 mL) was added dropwise over 10 min to the ice-cooled

mixture with stirring before being stirred at room temperature for 1 h. The solid material that had precipitated during the reaction was filtered and washed with water (2 × 20 mL) to give **23** (7.32 g, 73%) as a pale yellow solid: ¹H NMR (400 MHz, CDCl₃) δ 1.06 (6H, d, *J* = 6.6 Hz), 2.08–2.22 (1H, m), 2.64 (2H, t, *J* = 7.8 Hz), 3.00 (2H, t, *J* = 7.8 Hz), 3.69 (3H, s), 3.82 (2H, d, *J* = 6.6 Hz), 6.86 (1H, d, *J* = 8.7 Hz), 7.91 (1H, d, *J* = 2.2 Hz), 7.98 (1H, dd, *J* = 8.7, 2.2 Hz); MS *m/z* 279 (M – H)[–].

3-Isobutoxybenzoic Acid (25). A mixture of 3-hydroxybenzoic acid (**24**) (10.0 g, 72.4 mmol), K₂CO₃ (25.0 g, 181 mmol), and isobutyl bromide (29.8 g, 217 mmol) in DMF (100 mL) was heated with stirring at 120 °C for 2 h. The reaction mixture was then cooled to room temperature, treated with water (300 mL) followed by acidification to pH 4 with 6 M HCl, and extracted with EtOAc (100 mL). The organic layer was washed with water (1 × 100 mL) and brine (1 × 100 mL), dried over MgSO₄, and evaporated under reduced pressure to afford di-*O*-isobutylated product as a pale yellow oil. The di-*O*-isobutylated product thus obtained was dissolved in acetone (100 mL), and then 20 wt % aqueous NaOH (50.0 g) was added to the mixture at room temperature. The reaction mixture was allowed to cool to room temperature after being stirred at 50 °C for 2 h, and then water (300 mL) was added. The mixture was acidified to pH 1 with 12 M HCl and stirred at room temperature for 30 min, and the resulting precipitate was collected by filtration and washed with water (2 × 50 mL) to give **25** (9.85 g, 70%) as a white solid: ¹H NMR (400 MHz, CDCl₃) δ 1.05 (6H, d, *J* = 6.4 Hz), 2.04–2.18 (1H, m), 3.78 (2H, d, *J* = 6.6 Hz), 7.16 (1H, ddd, *J* = 7.9, 2.6, 0.5 Hz), 7.37 (1H, t, *J* = 7.9 Hz), 7.62 (1H, dd, *J* = 2.6, 1.6 Hz), 7.70 (1H, d, *J* = 7.9 Hz); MS *m/z* 193 (M – H)[–].

3-[2-Isobutoxy-5-(4-isobutoxybenzoyl)phenyl]propionic Acid (4). To a stirred solution of **23** (2.00 g, 7.13 mmol) and DMF (0.1 mL) in CH₂Cl₂ (20 mL) at room temperature was added oxalyl chloride (1.09 g, 8.59 mmol). After continuous stirring for 1 h, the mixture was cooled with ice–water before the sequential addition of AlCl₃ (2.09 g, 15.7 mmol) and isobutoxybenzene (1.29 g, 8.59 mmol). After being stirred at 5–10 °C for 30 min, the reaction mixture was poured into a mixture of ice (20 g) and 6 M HCl (30 mL). The layers were separated, and the organic layer was washed with water (1 × 20 mL) and brine (1 × 20 mL), dried over MgSO₄, and concentrated under reduced pressure. The residue was purified by chromatography (silica gel 50 g, 5:1 hexane/EtOAc) to give methyl 3-[2-isobutoxy-5-(4-isobutoxybenzoyl)phenyl]propionate (**26**) (2.36 g, 80%) as a white crystalline solid, mp 78–79 °C: ¹H NMR (400 MHz, CDCl₃) δ 1.05 (6H, d, *J* = 6.8 Hz), 1.07 (6H, d, *J* = 6.8 Hz), 2.06–2.22 (2H, m), 2.64 (2H, t, *J* = 7.8 Hz), 3.00 (2H, t, *J* = 7.8 Hz), 3.67 (3H, s), 3.80 (2H, d, *J* = 6.6 Hz), 3.82 (2H, d, *J* = 6.3 Hz), 6.87 (1H, d, *J* = 8.3 Hz), 6.95 (2H, d, *J* = 8.7 Hz), 7.64 (1H, d, *J* = 2.0 Hz), 7.67 (1H, dd, *J* = 8.3, 2.0 Hz), 7.76 (2H, d, *J* = 8.7 Hz); MS *m/z* 413 (M + H)⁺.

A mixture of **26** (10.4 g, 25.2 mmol) and 20 wt % aqueous NaOH (10.4 g) in acetone (30 mL) was stirred at 50 °C for 2 h before dilution with water (100 mL). The product that was precipitated by acidification to pH 1 with 12 M HCl was collected by filtration and washed with water (2 × 50 mL) to afford **4** (9.44 g, 94%) as a white solid, which was crystallized from CH₂Cl₂/hexane to give colorless crystals, mp 102–103 °C: ¹H NMR (400 MHz, CDCl₃) δ 1.04 (6H, d, *J* = 6.6 Hz), 1.07 (6H, d, *J* = 6.8 Hz), 2.06–2.22 (2H, m), 2.71 (2H, t, *J* = 7.7 Hz), 3.00 (2H, t, *J* = 7.7 Hz), 3.80 (2H, d, *J* = 6.4 Hz), 3.82 (2H, d, *J* = 6.6 Hz), 6.87 (1H, d, *J* = 8.5 Hz), 6.95 (2H, d, *J* = 8.8 Hz), 7.66 (1H, d, *J* = 2.2 Hz), 7.69 (1H, dd, *J* = 8.5, 2.2 Hz), 7.76 (2H, d, *J* = 8.8 Hz); MS *m/z* 397 (M – H)[–]. Anal. (C₂₄H₃₀O₅) C, H.

3-[2-Isobutoxy-5-(3-isobutoxybenzoyl)phenyl]propionic Acid (5). Compounds **22** and **25** were treated by the procedure described for **4** to afford **5** (85%) as a white solid, which was crystallized from CH₂Cl₂/hexane to give colorless crystals, mp 77–78 °C: ¹H NMR (400 MHz, CDCl₃) δ 1.03 (6H, d, *J* = 6.8 Hz), 1.07 (6H, d, *J* = 6.8 Hz), 2.04–2.22 (2H, m), 2.70 (2H, t, *J* = 7.7 Hz), 3.00 (2H, t, *J* = 7.7 Hz), 3.77 (2H, d, *J* = 6.4 Hz), 3.83 (2H, d, *J* = 6.3 Hz), 6.87 (1H, d, *J* = 9.3 Hz), 7.10 (1H, dd, *J* = 7.9, 2.6 Hz),

7.22–7.32 (2H, m), 7.35 (1H, t, *J* = 7.9 Hz), 7.68–7.78 (2H, m); MS *m/z* 397 (M – H)[–]. Anal. (C₂₄H₃₀O₅) C, H.

3-[3-(4-Isobutoxybenzoyl)phenyl]propionic Acid (6). Compound **20** and isobutoxybenzene were treated by the procedure described for **4** to afford **6** (77%) as a white solid, which was crystallized from CH₂Cl₂/hexane to give colorless crystals, mp 98–99 °C: ¹H NMR (400 MHz, CDCl₃) δ 1.05 (6H, d, *J* = 6.6 Hz), 2.06–2.18 (1H, m), 2.73 (2H, t, *J* = 7.7 Hz), 3.03 (2H, t, *J* = 7.7 Hz), 3.80 (2H, d, *J* = 6.6 Hz), 6.95 (2H, d, *J* = 8.8 Hz), 6.91–6.98 (3H, m), 7.36–7.46 (2H, m), 7.54–7.64 (2H, m), 7.80 (2H, d, *J* = 8.8 Hz); MS *m/z* 327 (M + H)⁺, 325 (M – H)[–]. Anal. (C₂₀H₂₂O₄) C, H.

3-(5-Benzoyl-2-isobutoxyphenyl)propionic Acid (7). Compound **22** and benzoyl chloride were treated by the procedure described for **4** to afford **7** (40%) as a white solid, which was crystallized from CH₂Cl₂/hexane to give colorless crystals, mp 95–97 °C: ¹H NMR (400 MHz, CDCl₃) δ 1.07 (6H, d, *J* = 6.6 Hz), 2.08–2.24 (1H, m), 2.71 (2H, t, *J* = 7.7 Hz), 3.00 (2H, t, *J* = 7.7 Hz), 3.83 (2H, d, *J* = 6.3 Hz), 6.88 (1H, d, *J* = 8.3 Hz), 7.44–7.52 (2H, m), 7.52–7.60 (1H, m), 7.68–7.80 (4H, m); MS *m/z* 327 (M + H)⁺, 325 (M – H)[–]. Anal. (C₂₀H₂₂O₄) C, H.

Methyl 3-[2-Hydroxy-5-(4-isobutoxybenzoyl)phenyl]propionate (28). A mixture of **26** (1.00 g, 2.42 mmol) and AlCl₃ (1.94 g, 14.5 mmol) in 1,2-dichloroethane (10 mL) was stirred under reflux for 8 h before cooling to room temperature. The mixture was diluted with 2-butanone (50 mL) and then poured into a mixture of ice (15 g) and 12 M HCl (15 mL). The organic layer separated was sequentially washed with 1 M HCl (2 × 10 mL), water (1 × 10 mL), and brine (1 × 10 mL), dried over MgSO₄, and concentrated under reduced pressure. The residual solid was triturated with hexane (10 mL) to give **27** (0.55 g) as a pale red solid: ¹H NMR (400 MHz, DMSO-*d*₆) δ 2.85 (2H, t, *J* = 7.3 Hz), 3.08 (2H, t, *J* = 7.3 Hz), 6.90 (2H, d, *J* = 8.7 Hz), 7.19 (1H, d, *J* = 8.3 Hz), 7.59 (1H, dd, *J* = 8.3, 2.2 Hz), 7.65–7.67 (1H, m), 7.66 (2H, d, *J* = 8.7 Hz), 10.43 (1H, s); MS *m/z* 269 (M + H)⁺, 267 (M – H)[–]. To a suspension of **27** (0.55 g), isobutyl alcohol (0.18 g, 2.43 mmol), and triphenylphosphine (0.65 g, 2.48 mmol) in THF (6 mL) at temperatures below 40 °C was added dropwise DIAD (0.50 g, 2.47 mmol). After being stirred for 30 min, NaOMe (28 wt % in MeOH, 1.98 g, 10.3 mmol) was added dropwise to the ice-cooled mixture with stirring, and then the resulting mixture was stirred at room temperature for 30 min. The reaction mixture was treated with toluene (10 mL) and 1 M HCl (20 mL). The organic layer was sequentially washed with water (1 × 10 mL) and brine (1 × 10 mL), dried over MgSO₄, and concentrated under reduced pressure. The residue was purified by chromatography (silica gel 100 g, 4:1 hexane/EtOAc) to give **28** (0.60 g, 69%) as a white solid: ¹H NMR (400 MHz, CDCl₃) δ 1.05 (6H, d, *J* = 6.8 Hz), 2.06–2.18 (1H, m), 2.76 (2H, t, *J* = 6.3 Hz), 2.95 (2H, t, *J* = 6.3 Hz), 3.72 (3H, s), 3.80 (2H, d, *J* = 6.6 Hz), 6.94 (1H, d, *J* = 8.3 Hz), 6.95 (2H, d, *J* = 8.7 Hz), 7.58 (1H, dd, *J* = 8.3, 2.2 Hz), 7.63 (1H, d, *J* = 2.2 Hz), 7.76 (2H, d, *J* = 8.7 Hz), 7.95 (1H, br s); MS *m/z* 357 (M + H)⁺, 355 (M – H)[–].

Methyl 3-[2-Hydroxy-5-(4-propoxybenzoyl)phenyl]propionate (29). Compound **26** (1.00 g, 2.42 mmol) was treated by the procedure described for **28** except using *n*-propyl alcohol to afford **29** (0.56 g, 67%) as a white solid: ¹H NMR (400 MHz, CDCl₃) δ 1.06 (3H, t, *J* = 7.3 Hz), 1.80–1.90 (2H, m), 2.76 (2H, t, *J* = 6.2 Hz), 2.95 (2H, t, *J* = 6.2 Hz), 3.72 (3H, s), 4.00 (2H, t, *J* = 6.6 Hz), 6.94 (1H, d, *J* = 8.4 Hz), 6.95 (2H, d, *J* = 8.8 Hz), 7.57 (1H, dd, *J* = 8.4, 2.1 Hz), 7.63 (1H, d, *J* = 2.1 Hz), 7.76 (2H, d, *J* = 8.8 Hz), 7.92 (1H, s); MS *m/z* 341 (M – H)[–].

Methyl 3-[5-(4-Benzoyloxybenzoyl)-2-hydroxyphenyl]propionate (30). Compound **26** (1.00 g, 2.42 mmol) was treated by the procedure described for **28** except using benzyl alcohol to afford **30** (0.66 g, 70%) as a white solid: ¹H NMR (400 MHz, CDCl₃) δ 2.76 (2H, t, *J* = 6.2 Hz), 2.95 (2H, t, *J* = 6.2 Hz), 3.72 (3H, s), 5.15 (2H, s), 6.94 (1H, d, *J* = 8.4 Hz), 7.03 (2H, d, *J* = 8.9 Hz), 7.32–7.48 (5H, m), 7.58 (1H, dd, *J* = 8.4, 2.2 Hz), 7.64 (1H, d, *J* = 2.2 Hz), 7.77 (2H, d, *J* = 8.9 Hz), 7.94 (1H, s); MS *m/z* 391 (M + H)⁺, 389 (M – H)[–].

3-[5-(4-Isobutoxybenzoyl)-2-propoxyphenyl]propionic Acid (9). To a stirred suspension of **28** (3.00 g, 8.42 mmol) and K_2CO_3 (1.16 g, 8.39 mmol) in DMF (15 mL) at room temperature was added *n*-propyl bromide (1.55 g, 12.6 mmol), and the mixture was stirred at 90–100 °C for 1 h. After cooling to room temperature, the reaction mixture was treated with toluene (15 mL) and water (60 mL) followed by acidification to pH 4 with 6 M HCl. The organic layer was washed with water (1 × 20 mL) and brine (1 × 20 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by chromatography (silica gel 75 g, 2:1 hexane/EtOAc) to give methyl 3-[5-(4-isobutoxybenzoyl)-2-propoxyphenyl]propionate (2.73 g, 81%) as a white solid: 1H NMR (400 MHz, $CDCl_3$) δ 1.02–1.12 (9H, m), 1.81–1.93 (2H, m), 2.00–2.20 (1H, m), 2.64 (2H, t, $J = 7.8$ Hz), 2.99 (2H, t, $J = 7.8$ Hz), 3.67 (3H, s), 3.80 (2H, d, $J = 6.3$ Hz), 4.02 (2H, t, $J = 6.3$ Hz), 6.87 (1H, d, $J = 8.4$ Hz), 6.95 (2H, d, $J = 8.7$ Hz), 7.64 (1H, d, $J = 2.2$ Hz), 7.67 (1H, dd, $J = 8.4, 2.2$ Hz), 7.77 (2H, d, $J = 8.7$ Hz); MS m/z 400 ($M + H$)⁺.

A mixture of the ester (1.00 g, 2.51 mmol) obtained above and 20 wt % aqueous NaOH (1.50 g) in acetone (4 mL) was stirred at 50 °C for 1 h before dilution with water (12 mL). The product that was precipitated by acidification to pH 1 with 6 M HCl was collected by filtration and washed with water (2 × 5 mL) to afford **9** (0.91 g, 94%) as a white crystalline solid, mp 107–108 °C: 1H NMR (400 MHz, $CDCl_3$) δ 1.04 (6H, d, $J = 6.6$ Hz), 1.07 (3H, t, $J = 7.4$ Hz), 1.80–1.94 (2H, m), 2.04–2.20 (1H, m), 2.71, 2.99 (each 2H, t, $J = 7.6$ Hz), 3.79 (2H, d, $J = 6.6$ Hz), 4.01 (2H, t, $J = 6.5$ Hz), 6.88 (1H, d, $J = 8.3$ Hz), 6.95 (2H, d, $J = 8.7$ Hz), 7.66 (1H, d, $J = 2.1$ Hz), 7.69 (1H, dd, $J = 8.3, 2.1$ Hz), 7.76 (2H, d, $J = 8.7$ Hz); MS m/z 383 ($M - H$)⁻. Anal. ($C_{23}H_{28}O_5$) C, H.

3-[2-Benzyloxy-5-(4-isobutoxybenzoyl)phenyl]propionic Acid (11). Compound **28** was treated by the procedure described for **9** except using benzyl bromide to afford **11** (67%) as a white solid, which was crystallized from hexane/diisopropyl ether to give colorless crystals, mp 100 °C: 1H NMR (400 MHz, $CDCl_3$) δ 1.04 (6H, d, $J = 6.6$ Hz), 2.02–2.18 (1H, m), 2.72 (2H, t, $J = 7.6$ Hz), 3.04 (2H, t, $J = 7.6$ Hz), 3.79 (2H, d, $J = 6.6$ Hz), 5.17 (2H, s), 6.91–6.98 (3H, m), 7.30–7.48 (5H, m), 7.64–7.70 (2H, m), 7.76 (2H, d, $J = 8.8$ Hz); MS m/z 431 ($M - H$)⁻. Anal. ($C_{27}H_{28}O_5$) C, H.

3-[2-Isobutoxy-5-(4-propoxybenzoyl)phenyl]propionic Acid (10). Compound **29** was treated by the procedure described for **9** except using isobutyl bromide to afford **10** (85%) as a white crystalline solid, mp 128–129 °C: 1H NMR (400 MHz, $CDCl_3$) δ 1.00–1.12 (9H, m), 1.76–1.92 (2H, m), 2.08–2.24 (1H, m), 2.71 (2H, t, $J = 7.6$ Hz), 3.00 (2H, t, $J = 7.6$ Hz), 3.82 (2H, d, $J = 6.4$ Hz), 4.00 (2H, t, $J = 6.6$ Hz), 6.87 (1H, d, $J = 8.4$ Hz), 6.95 (2H, d, $J = 8.7$ Hz), 7.66 (1H, d, $J = 2.1$ Hz), 7.69 (1H, dd, $J = 8.4, 2.1$ Hz), 7.76 (2H, d, $J = 8.7$ Hz); MS m/z 383 ($M - H$)⁻. Anal. ($C_{23}H_{28}O_5$) C, H.

3-[5-(4-Benzyloxybenzoyl)-2-isobutoxyphenyl]propionic Acid (12). Compound **30** was treated by the procedure described for **9** except using isobutyl bromide to afford **12** (79%) as a white solid, which was crystallized from CH_2Cl_2 /hexane to give colorless crystals, mp 150–151 °C: 1H NMR (400 MHz, $CDCl_3$) δ 1.06 (6H, d, $J = 6.6$ Hz), 2.08–2.20 (1H, m), 2.71 (2H, t, $J = 7.7$ Hz), 3.00 (2H, t, $J = 7.7$ Hz), 3.82 (2H, d, $J = 6.3$ Hz), 5.15 (2H, s), 6.87 (1H, d, $J = 8.3$ Hz), 7.03 (2H, d, $J = 8.7$ Hz), 7.30–7.48 (5H, m), 7.60–7.72 (2H, m), 7.77 (2H, d, $J = 8.7$ Hz); MS m/z 432 ($M - H$)⁻. Anal. ($C_{27}H_{28}O_5$) C, H.

Bis(4-isobutoxyphenyl)methanone (8). To a stirred suspension of bis(4-hydroxyphenyl)methanone (2.00 g, 9.34 mmol) and K_2CO_3 (3.87 g, 28.0 mmol) in DMF (20 mL) at room temperature was added isobutyl bromide (3.84 g, 28.0 mmol), and then the mixture was heated with stirring at 100 °C for 1 h. Additional K_2CO_3 (1.29 g, 9.33 mmol) and isobutyl bromide (1.28 g, 9.34 mmol) were then added, and the heating was continued for a further 1.5 h. The reaction mixture was cooled to room temperature before filtration, and the residue was rinsed with EtOAc (3 × 30 mL). The filtrate was treated with water (50 mL) followed by acidification to pH 2 with 6 M HCl, and the aqueous layer separated was

extracted with EtOAc (1 × 30 mL). The combined organic extracts were washed with water (1 × 30 mL) and brine (1 × 30 mL), followed by the addition of $CHCl_3$ (30 mL). The solution was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was triturated with hexane (20 mL) to afford **8** (2.88 g, 94%) as a white solid, which was crystallized from CH_2Cl_2 /hexane to give colorless crystals, mp 118–119 °C: 1H NMR (400 MHz, $CDCl_3$) δ 1.05 (12H, d, $J = 6.8$ Hz), 2.04–2.20 (2H, m), 3.80 (4H, d, $J = 6.6$ Hz), 6.95 (4H, d, $J = 8.5$ Hz), 7.77 (4H, d, $J = 8.5$ Hz); MS m/z 327 ($M + H$)⁺. Anal. ($C_{21}H_{26}O_3$) C, H.

3-[2-Isobutoxy-5-(4-isobutoxybenzoyl)phenyl]propionamide (13). To a stirred solution of **4** (5.00 g, 12.5 mmol) in THF (50 mL) at room temperature was sequentially added oxalyl chloride (1.91 g, 15.0 mmol) and DMF (50 μ L), and the mixture was stirred for 3 h. The resulting mixture was slowly poured into ice-cooled 25 wt % NH_4OH (50 mL) with vigorous stirring. After being stirred at 5 °C for 30 min, the mixture was adjusted to pH 6 with 6 M HCl and extracted with EtOAc (1 × 100 mL). The aqueous layer separated was extracted with EtOAc (1 × 50 mL), and the organic extracts were washed with water (1 × 50 mL) and brine (1 × 50 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The residual solid was triturated with hexane (100 mL) to afford **13** (4.67 g, 94%) as a white solid, which was crystallized from CH_2Cl_2 /hexane to give colorless crystals, mp 123 °C: 1H NMR (400 MHz, $CDCl_3$) δ 1.05 (6H, d, $J = 6.8$ Hz), 1.08 (6H, d, $J = 6.6$ Hz), 2.06–2.22 (2H, m), 2.56 (2H, t, $J = 7.7$ Hz), 3.02 (2H, t, $J = 7.7$ Hz), 3.80 (2H, d, $J = 6.6$ Hz), 3.83 (2H, d, $J = 6.3$ Hz), 5.29 (1H, br s), 5.38 (1H, br s), 6.88 (1H, d, $J = 8.1$ Hz), 6.95 (2H, d, $J = 8.5$ Hz), 7.62–7.70 (2H, m), 7.76 (2H, d, $J = 8.5$ Hz); MS m/z 399 ($M + H$)⁺. Anal. ($C_{24}H_{31}NO_4$) C, H, N.

ELISA-Based AP-1 Binding Assay: Preparation of c-Fos and c-Jun bZIP Peptides. The resin and all of the protected amino acids and coupling reagents were purchased from Watanabe Chemical Industries (Hiroshima, Japan). All of the reagents and solvents were reagent grade or better and were used without further purification. High-performance liquid chromatography (HPLC) was performed using a Hitachi L-7100 apparatus equipped with an L-7400 UV detector (peak detection at 230 nm) and a PROTEINER-*RP* column (YMC-Pack, YMC Co., Kyoto, Japan) of 250 × 20 mm for preparative HPLC or 150 × 4.6 mm for analytical HPLC. c-Jun bZIP peptide [c-Jun (263–324) Cys278Ser] and biotinylated c-Fos bZIP peptide [*N*-biotinyl-(Gly)₄-c-Fos (139–200) Cys154Ser]⁵ were synthesized manually using standard solid-phase peptide chemistry with *tert*-butoxycarbonyl (Boc)-protected amino acids on Boc-His(benzyloxymethyl)-PAM resin²⁴ at a 0.30 mmol scale. The Boc protecting group was removed by treatment with trifluoroacetic acid (10 mL) at room temperature for 3 min. Coupling with Boc-protected amino acids (4 equiv) was then performed in the presence of *N,N*-diisopropylethylamine (6 equiv) and 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (3.8 equiv) in *N*-methyl-2-pyrrolidone (8 mL) at room temperature for 30 min.²⁵ Subsequently, the peptide resin was treated with decanoic anhydride (0.3 mol/L in 1:1 DMF/ CH_2Cl_2 , 10 mL) at room temperature for 30 min. The protected c-Jun bZIP peptide resin (0.50 g) was treated with a reagent containing anhydrous HF (1.5 mL) and dimethyl sulfide (4.5 mL) with stirring at 0 °C for 1.5 h to remove the remaining O- and N-protecting groups.²⁶ After the evaporation of HF, ether was added to the resin, which was then filtered, washed in CH_2Cl_2 (5 × 10 mL), followed by dried in vacuo. The resin was treated with a reagent containing anhydrous HF (4.5 mL) and *p*-cresol (1.5 mL) with stirring at 0 °C for 1.5 h to induce cleavage of the peptide from the resin. The precipitate was collected by centrifugation and dissolved in water. The exhausted resin was filtered, and the filtrate was purified by reverse phase HPLC eluted with a linear gradient of acetonitrile in water containing 0.1% trifluoroacetic acid at a flow rate of 7.0 mL/min before lyophilization. Biotinylated c-Fos bZIP peptide was synthesized according to the procedure that was used to prepare the c-Jun bZIP peptide.

The peptides were separately dissolved in 20 mM Tris-HCl buffer (pH 7.5) containing 50 mM KCl, 1 mM ethylenediaminetetraacetic acid (EDTA), 10 mM $MgCl_2$, 1 mM dithiothreitol (DTT), 0.5 M

guanidine HCl, and 30% glycerol. Equimolar quantities of both of the solutions were mixed together and the resulting mixture was used as the AP-1 bZIP peptide.

Binding Assay of AP-1 bZIP Peptide and AP-1 Double-Stranded Oligonucleotides. The inhibitory activities of the synthetic compounds on the DNA-binding activity of AP-1 were evaluated using an ELISA-based AP-1 binding assay with synthetic double-stranded oligonucleotides that contained the AP-1 binding site (shown in bold) and the AP-1 bZIP peptide. The AP-1 bZIP peptide (10 pmol/well) was added to an avidin-coated 96-well ELISA plate, washed, and then blocked with bovine serum albumin. Digoxigenin-labeled double-stranded oligonucleotides (22-mer) containing an AP-1 binding sequence (5'-CTAGT**GATGAGT-CAGCCGGATC**-3' and 5'-GATCCGGCT**GACTCATCACTAG**-3') (Nissinbo Industries, Inc., Tokyo, Japan) were reacted in the presence or absence of each sample at room temperature for 1 h in a binding reaction solution [25 mM Tris-HCl (pH 7.9) containing 0.5 mM EDTA, 0.05% Nonidet P-40 and 10% glycerol]. Subsequently, the unbound labeled oligonucleotide was washed out with HEPES-KOH (pH 7.9) buffer containing 0.5 mM EDTA, 50 mM KCl, 10% glycerol, 0.1% BSA, and 0.05% Tween-20. The peroxidase-conjugated anti-digoxigenin antibody (Roche Diagnostics, Indianapolis, IN) was then added and reacted with the labeled oligonucleotide bound to AP-1. After washing out the excess antibody with HEPES-KOH buffer containing 0.05% Tween-20, the residue was reacted for a predetermined period of time in 100 mM citrate buffer (pH 5.0) containing 0.03% H₂O₂ using *o*-phenylenediamine as a substrate. After adding sulfuric acid solution to each well, the absorbance at 492 nm was measured with a Microplate spectrophotometer (Bio-Rad Laboratories, Hercules, CA). Taking the absorbance in the absence of sample as 100%, the inhibition rate of each sample was calculated from the absorbance in the presence of the sample. The IC₅₀ values were calculated based on a logistic concentration-response curve using the SAS system version 8.2 (SAS Institute, Cary, NC).

Cell-Based Assay: Plasmids. Reporter plasmids containing the firefly luciferase reporter gene driven by a basic promoter element (TATA box) plus a defined inducible cis-enhancer element were utilized (PathDetect in Vivo Signal Transduction Pathway *cis*-Reporting Systems, Stratagene, La Jolla, CA). The firefly luciferase gene in the reporter plasmid (pAP-1-Luc) was controlled by synthetic enhancer sequences comprising seven repeats of the binding sites for AP-1; TGACTAA. A control plasmid expressing *Renilla reniformis* luciferase driven by the herpes simplex virus thymidine kinase promoter, phRL-TK Vector (Promega, Madison, WI), was used to correct the efficiency of transfection.

Cell Culture. The murine fibroblast cell line NIH3T3 was obtained from the American Type Culture Collection (ATCC No. CRL-1658; Rockville, MD) and grown in Dulbecco's modified Eagle's medium (DMEM; Nissui Pharmaceutical, Tokyo, Japan) supplemented with 4 mM l-glutamine (Wako Pure Chemical, Osaka, Japan), 1.5 g/L glucose (Wako Pure Chemical), 10% heat-inactivated fetal calf serum (FCS; JRH Biosciences, Lenexa, KS), and 60 µg/mL kanamycin sulfate (Sigma-Aldrich, St. Louis, MO) at 37 °C and 5% CO₂.

Transient Transfection and Reporter Gene Assay. Approximately 1 × 10⁴ cells in 100 µL of 10% FCS-DMEM were plated into a 96-well tissue culture plate (Iwaki Glass, Chiba, Japan) 24 h before transfection (day 0). On day 1, the cells in each well were transiently cotransfected with 40 ng of pAP-1-Luc plasmid and 4 ng of phRL-TK Vector using the FuGENE 6 transfection reagent (Roche Diagnostics, Indianapolis, IN). After transfection for 6 h at 37 °C, the cells were cultured in 0.5% FCS-DMEM. On day 2, the medium was exchanged with 0.5% FCS-DMEM containing the compounds. After 1 h, phorbol 12-myristate 13-acetate (TPA) was added to each well (100 ng/mL). Three hours after TPA stimulation, whole cell lysates were examined for luciferase activity (Dual-Luciferase Reporter Assay System, Promega) with a luminometer (Luminous CT-9000D; DIA-IATRON,

Tokyo, Japan) according to the manufacturer's protocol. The firefly luciferase activity was normalized for transfection efficiency based on the Renilla luciferase activity. Taking the activity in the absence of sample as 100%, the inhibition rate of each sample was calculated from the absorbance in the presence of the sample. The IC₅₀ values were calculated based on a logistic concentration-response curve using the SAS system version 8.2.

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Supporting Information Available: Characterization data for esters of compounds 5-7 and 10-12 plus elemental analysis data for compounds 2-13. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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