Discovery and Structure−Activity Relationships of Novel Piperidine Inhibitors of Farnesyltransferase

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A novel piperidine series of farnesyltransferase (FTase) inhibitors is described. Systematic medicinal chemistry studies starting with the lead compound, discovered from a 5-nitropiperidin-2-one combinatorial library, resulted in a potent series of novel FTase inhibitors. We found that all of four substituents of the piperidine core played an important role for FTase inhibition. A 10-fold increase in potency was observed by changing the piperidine-2-one core to the corresponding piperidine core. This class of compounds was found to inhibit farnesyltransferase in a Ras competitive manner. Optical resolution of several potent inhibitors revealed that the (+)-enantiomers showed potent farnesyltransferase inhibition. (+)-**8** inhibited FTase with an IC_{50} of 1.9 nM.

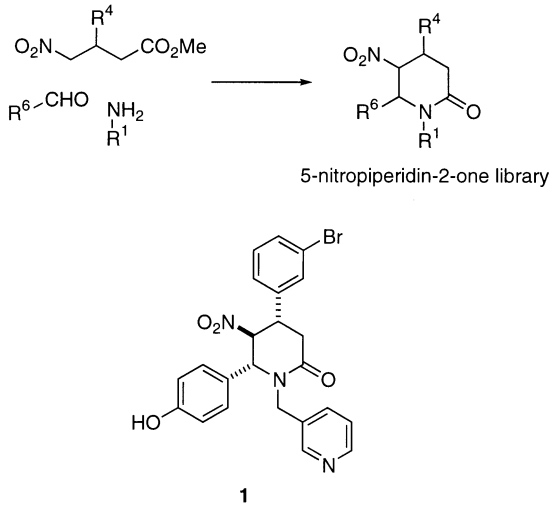
Introduction

Farnesyltransferase (FTase) inhibitors have emerged as promising anticancer drugs.[1] These compounds inhibit protein farnesyltransferase, an enzyme that catalyzes farnesylation of proteins ending with the CAAX motif (C is cystein, A is an aliphatic amino acid, and X is the C-terminal amino acid). FTase inhibitors inhibit anchorage-independent growth of a variety of transformed cells. A survey of cancer cell lines has shown that >70% of cells are sensitive to FTase inhibitors.[2] In addition, FTase inhibitors have been shown to inhibit the growth of tumors in a number of animal model studies.[3] These promising drugs, such as R115777,[3b] SCH66336,[3c] and BMS-214662,[3d] are currently being assessed in clinical trials and have been shown to have clinical efficacy for the treatment of human cancers.[4]

In our screening process of novel FTase inhibitors, we discovered piperidine derivative **1** with moderate FTase inhibition (IC_{50} = 420 nM) from a 5-nitropiperidin-2-one combinatorial library of ca. 3000 compounds (Chart 1). Although compound **1** was the only derivative to show activity, novel structure and chemical accessibility of this compound prompted us to conduct a systematic medicinal chemistry approach to improve the potency of FTase inhibition.

Herein, we report the synthesis and structure−activity relationships of a novel piperidine series of FTase inhibitors.

Chart 1



Chemistry

5-Nitropiperidin-2-ones were synthesized on the basis of known three-component coupling reactions.[5] The general synthetic method was illustrated for the synthesis of **1** (Scheme 1). Methyl 4-nitro-3-(3-bromophenyl)butyrate (**3**) was synthesized from 3-bromobenzaldehyde (**2**) by the Wittig reaction followed by the Michael addition of nitromethane[6] to the cinnamate intermediate in quantitative yield. A mixture of 4-nitrobutyrate **3**, benzaldehyde **4**, and amine **5** was refluxed in ethanol to give 5-nitropiperidin-2-one **1** in 61% yield as a single isomer. Various benzaldehydes, substituted alkylamines, and methyl 3-aryl-4-nitrobutyrates were used to prepare different substituents at N-1, C-4, and C-6 positions. A single diastereomer was obtained in each case, and the other diastereomer could not be detected in the reaction products. The relative stereochemistry was determined as 4,5-trans-5,6-trans stereochemistry by NMR analyses and was confirmed by the X-ray crystallographic analysis of compound (+)-**6**. The 4,5-trans-5,6-trans configuration seemed to be thermodynamically favorable because all substituents on the piperidine core could exist as equatorial.

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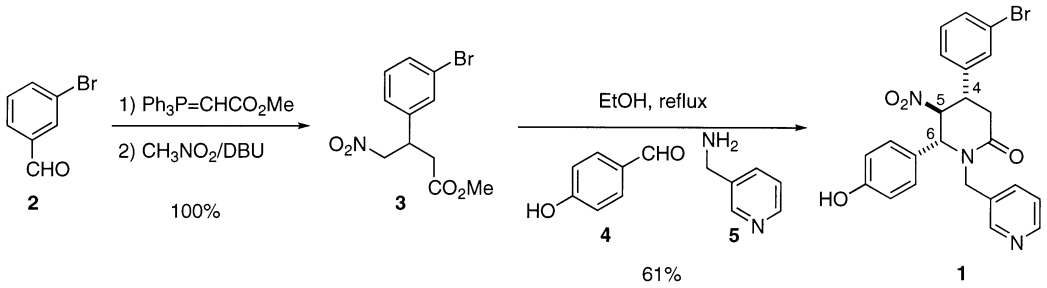
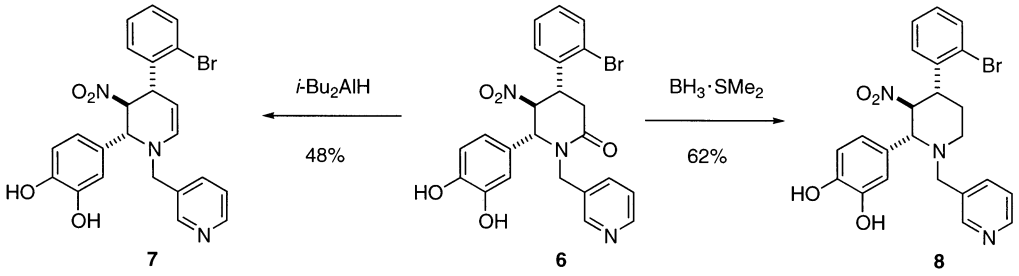
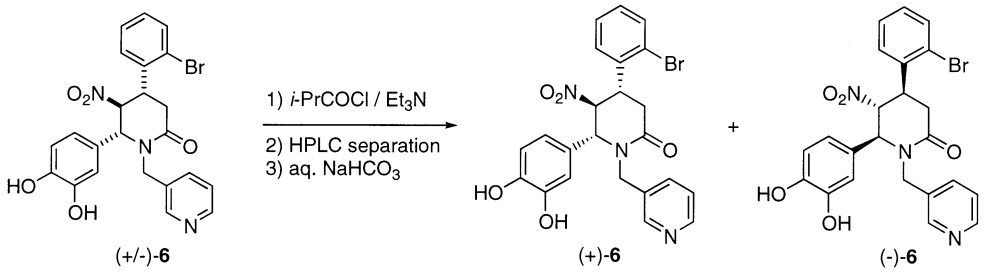
Scheme 1. Synthesis of 5-Nitropiperidin-2-one **1**



Scheme 2. Reduction of 5-Nitropiperidin-2-one **6**



Scheme 3. Optical Resolution of 5-Nitropiperidin-2-one **6**



Some chemical modifications of compound **1** were also carried out (see Table 1). MCPBA oxidation of **1** gave pyridine *N*-oxide **10** in 59% yield. Reduction of the nitro group of **1** with zinc powder and hydrochloric acid afforded a mixture of 5-hydroxylaminopiperidin-2-one **11** (11% yield) and 5-aminopiperidin-2-one **12** (52% yield).

5,6-Dehydropiperidine **7** was prepared by the reduction of **6** with diisobutylaluminum hydride in 48% yield, while the reduction of **7** with the borane−methyl sulfide complex gave piperidine **8** in 62% yield (Scheme 2). Other piperidines were prepared by the reduction of the corresponding piperidine-2-ones.

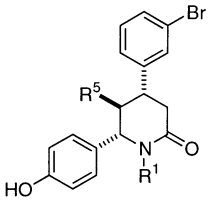
The optical resolution of several compounds (**6**, **8**, **17**, and **21**) was conducted (see Table 4). Although direct separation of (±)-**6** by chiral HPLC column failed, the diisobutyrate of **6** was found to be a good substrate for HPLC separation with a chiral column. Hydrolysis of each diisobutyrate under basic conditions afforded (+)-**6** and (−)-**6** (Scheme 3). The absolute stereochemistry of (+)-**6** was determined as 4*S*, 5*S*, and 6*R* by X-ray crystallographic analysis of the HCl salt of (+)-**6**. Other compounds were able to be resolved by a similar method or by direct HPLC separation using a chiral column. Conversion of (+)-**6** to the corresponding piperidine **8** by reduction with the borane−methyl sulfide complex revealed the absolute stereochemistry of the (+)-enantiomer of **8**.

Results and Discussion

FTase enzyme inhibition was determined by inhibition of farnesylation of viral K-Ras by bovine brain FTase as previously described.[7] Structures and FTase inhibition of N-1 and C-5 modified derivatives of **1** are shown in Table 1. The 3-pyridylmethyl group at the N-1 position (R^1) was found to be important for FTase inhibition. Changing the position of the nitrogen atom in the pyridine ring of **1** resulted in the complete loss of FTase inhibition (compound **9**). Compound **10**, the pyridine *N*-oxide of **1**, also lost FTase inhibition. The nitro group at the C-5 position (R^5) was also found to be important for FTase inhibition. Reduction of the nitro group of **1** resulted in inactive compounds **11** and **12**. We therefore decided to leave the N-1 and C-5 positions as the 3-pyridylmethyl and nitro groups, respectively, for further investigation.

Substitutions of the phenyl group at C-4 (R^4) were examined (Table 2). Compound **15**, which has a simple phenyl group at the C-4 position, showed less potent FTase inhibition than 3-bromophenyl compound **1**. Changing the 3-bromophenyl group of **1** to 3-chlorophenyl or 3-methylphenyl retained FTase inhibitory activity (**13** or **14**). Although the 4-bromophenyl compound **16** lost FTase inhibition, the 2-bromophenyl derivative **17** showed 3 times more potent FTase inhibition (IC_{50} = 140 nM) than **1**. The position and type of substitution of the C-4 phenyl group seemed to be important for the FTase inhibition.

Table 1. Modification of R^1 and R^5



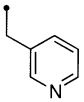
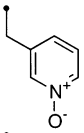
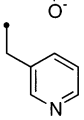
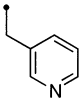
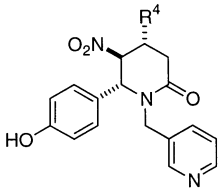
compound	R^1	R^5	FTase IC_{50} (nM)
1		NO_2	350
9		NO_2	>3700
10		NO_2	>3700
11		NHOH	>3700
12		NH_2	>3700

Table 2. Modification of R^4



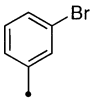
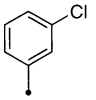
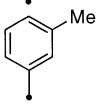
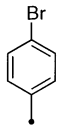
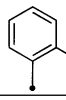
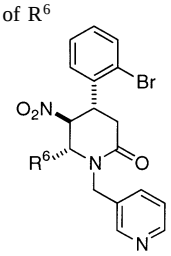
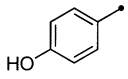
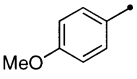
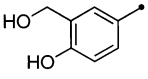
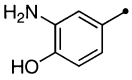
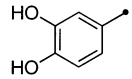
compound	R^4	FTase IC_{50} (nM)
1		420
13		320
14		410
15		1100
16		>3700
17		140

Table 3. Modification of R^6



compound	R^6	FTase IC_{50} (nM)
17		140
18		>1200
19		>1200
20		97
6		46

For the modification of C-6 phenyl group, compound **17** was selected as the starting point (Table 3). 4-Methoxyphenyl compound **18** lost FTase inhibition, and the compounds with other C-6 substituents, such as 4-fluorophenyl, 4-aminophenyl, and 3-cyanophenyl, also lost activity (data not shown). The para hydroxy group on the C-6 benzene ring seems to be important for the FTase inhibition. It is interesting that introduction of an amino or hydroxy group ortho to the hydroxy group of C-6 benzene ring resulted in an increase of FTase inhibition (compounds **20** and **6**), while introduction of a hydroxymethyl group caused a decrease of FTase inhibition (compound **19**). In particular, 3,4-dihydroxyphenyl derivative **6** showed 3 times more potent FTase inhibition (IC_{50} = 46 nM) than the corresponding 4-hydroxyphenyl compound **17**. Thus, we were able to obtain a 10-fold more potent compound **6** than the original hit compound **1** by simple modification of the two benzene rings at C-4 and C-6 positions.

Along with the modifications of each subustituent of the piperidine-2-one core, we tried to change the piperidine-2-one core itself for further improvement of FTase inhibition potency (Chart 2). The reduction products of **6**, compounds **7** and **8**, showed significant increase in the potency of FTase inhibition. 5,6-Dehydropiperidine **7** showed more than a 3-fold increase in potency of FTase inhibition (IC_{50} = 13 nM), and piperidine **8** exhibited more than a 10-fold increase in potency (IC_{50} = 3.7 nM). Compound **21**, a piperidine version of compound **17**, also showed potent FTase inhibition (IC_{50} = 8.2 nM).

Biological Activities of Optically Active Piperidine Derivatives. The results of optically resolved piperidine derivatives are summarized in Table 4. In all cases examined, only the (+)-enantiomers showed potent FTase inhibition. For example, the (+)-enantiomer of **8** showed a 2-fold increase in potency (IC_{50} =

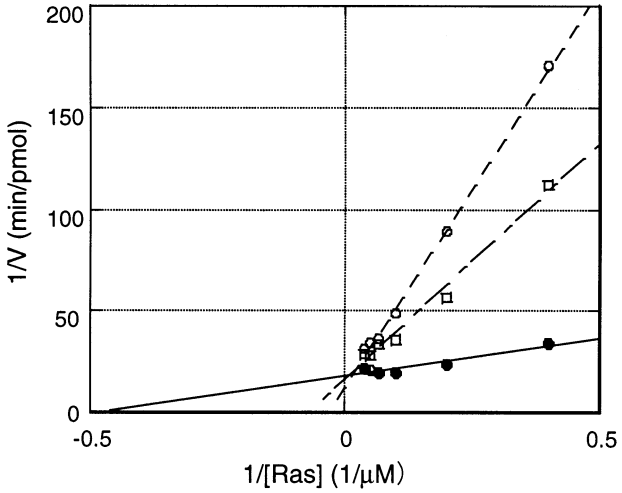
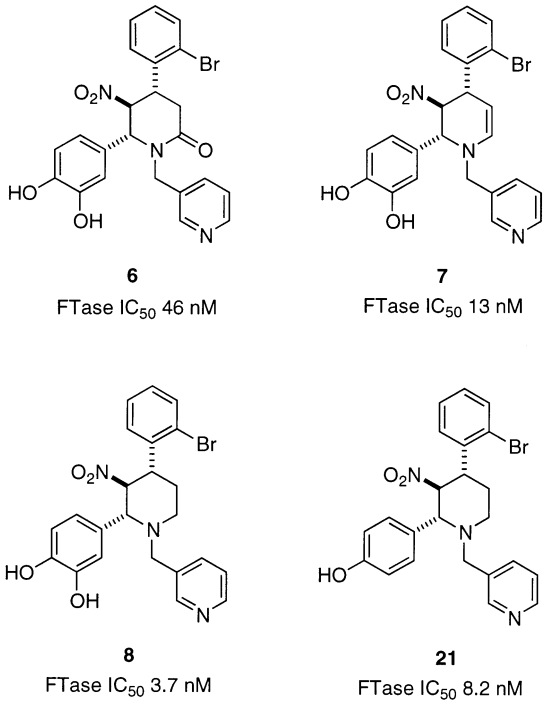


Figure 1. Lineweaver−Burk plot of FTase inhibition with (±)-**6**. Concentrations of (±)-**6** used were 0 (●), 50 (□), and 100 (○) nM.

Chart 2



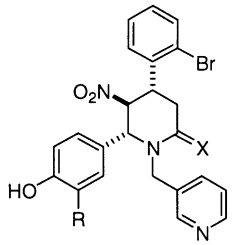
1.9nM), while the (−)-enantiomer of **8** showed weak FTase inhibition (IC_{50} > 310 nM). In cellular assays, (+)-**8** inhibited H-Ras processing in T24 bladder carcinoma cells with a minimum concentration of 16 nM.[8]

Kinetic analysis of FTase inhibition by (±)-**6** was performed.[7] Figure 1 shows the results of an experiment in which the concentration of the viral K-Ras protein was varied in the bovine brain FTase assay while the farnesyl pyrophosphate concentration was kept constant. The kinetic profile suggested that **6** inhibited FTase in a competitive manner with respect to the Ras protein substrate. The apparent K_i value was found to be 12.4 nM.

Conclusion

We have discovered a novel series of piperidine derivatives as potent FTase inhibitors. The kinetic analysis of FTase inhibition by 5-nitropiperidin-2-one **6** suggested that this class of compounds inhibited FTase in a competitive manner with respect to the Ras protein substrate. The potency of FTase inhibition was increased more than 100 times from the initial hit compound **1** (IC_{50} = 420 nM) to piperidine **8** (IC_{50} = 3.7nM). The optical resolution of this class of compounds revealed that only the (+)-enantiomers showed potent FTase inhibition. (+)-**8** inhibited FTase with an IC_{50} of 1.9 nM. In cellular assays, (+)-**8** inhibited H-Ras processing at a minimum concentration of 16 nM. Although the compounds described here are potent FTase inhibitors, preliminary pharmacokinetic studies suggested that the presence of phenolic hydroxy groups could be one of the reasons for the rapid clearance of this series of compounds. Detailed biochemical studies as well as further SAR studies for in vivo efficacy of this class of FTase inhibitors will be published elsewhere.

Table 4. FTase Inhibition of Optically Active Compounds



compd	X	R	FTase IC_{50} (nM)
(±)-**17**	O	H	140
(+)-**17**	O	H	75
(−)-**17**	O	H	950
(±)-**6**	O	OH	46
(+)-**6**	O	OH	30
(−)-**6**	O	OH	1200
(±)-**21**	H, H	H	8.2
(+)-**21**	H, H	H	5.8
(−)-**21**	H, H	H	>310
(±)-**8**	H, H	OH	3.7
(+)-**8**	H, H	OH	1.9
(−)-**8**	H, H	OH	>310

Experimental Section

General Methods. NMR spectra were obtained on a JEOL Lambda 300 (300 MHz) or a JEOL JNM 400 (400 MHz) NMR spectrometer. Mass spectra were recorded on a JEOL JMS HX/HX110 mass spectrometer. Elemental analyses were performed on a Perkin-Elmer series II analyzer (model 2400) or a Yamato MT-5 analyzer. Enantiomeric purity was determined by HPLC on a Chiralcel OD column.

4-(3-Bromophenyl)-6-(4-hydroxyphenyl)-5-nitro-1-(3-pyridylmethyl)piperidin-2-one (1). Methyl 3-(3-bromophenyl)-4-nitrobutylate (302 mg, 1.0 mmol), 4-hydroxybenzaldehyde (120 mg, 1.0 mmol), and 3-(aminomethyl)pyridine (0.20 mL, 2.0 mmol) in EtOH were heated under reflux for 20 h. After removal of EtOH under reduced pressure, the residue was chromatographed on silica gel, eluting with $CHCl_3$/MeOH, 95:5, to afford **1** (293 mg, 61% yield). ^{1}H NMR (DMSO-d_6) δ 9.65 (br s, 1H), 8.42 (br d, J = 3.1 Hz, 1H), 8.16 (br s, 1H), 7.73 (m, 1H), 7.50−7.20 (m, 5H), 7.09 (m, 2H), 6.69 (m, 2H), 5.73 (dd, J = 11.1, 10.0 Hz, 1H), 4.79 (d, J = 10.0 Hz, 1H), 4.70 (d, J = 15.6 Hz, 1H), 3.94 (d, J = 15.6 Hz, 1H), 3.94 (m, 1H), 3.14 (dd, J = 17.0, 13.2 Hz, 1H), 2.75 (dd, J = 17.0, 3.7 Hz, 1H). FAB-MS (m/z): 484, 482 $(M + H)^+$.

4-(3-Bromophenyl)-6-(4-hydroxyphenyl)-5-nitro-1-(4-pyridylmethyl)piperidin-2-one (9). Methyl 3-(3-bromophenyl)-4-nitrobutylate (302 mg, 1.0 mmol), 4-hydroxybenzaldehyde (122 mg, 1.9 mmol), and 4-(aminomethyl)pyridine (0.20 mL, 2.0 mmol) afforded **9** (120 mg, 25% yield). ^{1}H NMR (DMSO-d_6) δ 9.67 (b s, 1H), 8.44 (m, 2H), 7.75 (br s, 1H), 7.50−6.60

(m, 9H), 5.76 (dd, J = 11.5, 9.9 Hz, 1H), 4.82 (d, J = 9.9 Hz, 1H), 4.67 (d, J = 16.2 Hz, 1H), 4.00 (m, 1H), 3.90 (d, J = 16.2 Hz, 1H), 3.17 (dd, J = 16.9, 13.0 Hz, 1H), 2.77 (dd, J = 16.9, 4.9 Hz, 1H). FAB-MS (m/z): 484, 482 $(M + H)^+$.

(±)-4-(3-Bromophenyl)-6-(4-hydroxyphenyl)-5-nitro-1-(3-(*N*-oxo)-pyridylmethyl)piperidin-2-one (10). *m*-Chloroperbenzoic acid (24 mg, 0.10 mmol) was added to a solution of **1** (12 mg, 0.025 mmol) in dichloromethane (1.0 mL). After being stirred for 1 h, the reaction mixture was concentrated and the residue was purified by preparative TLC, developed with $CHCl_3$/MeOH, 95:5, to give **10** (7.4 mg, 59% yield). ^{1}H NMR (DMSO-d_6) δ 9.66 (b s, 1H), 8.03 (br d, J = 6.2 Hz, 1H), 7.86 (br s, 1H), 7.72 (br s, 1H), 7.53−6.56 (m, 9H), 5.77 (dd, J = 10.5, 9.7 Hz, 1H), 4.90 (d, J = 9.7 Hz, 1H), 4.37 (d, J = 15.8 Hz, 1H), 4.12 (d, J = 15.8 Hz, 1H), 3.96 (m, 1H), 3.15 (dd, J = 16.5, 13.0 Hz, 1H), 2.75 (dd, J = 16.5, 4.5 Hz, 1H). FAB-MS (m/z): 500, 498 $(M + H)^+$.

(±)-4-(3-Bromophenyl)-6-(4-hydroxyphenyl)-5-hydroxyamino-1-(3-pyridylmethyl)piperidin-2-one (11) and (±)-5-Amino-4-(3-bromophenyl)-6-(4-hydroxyphenyl)-1-(3-pyridylmethyl)piperidine-2-one (12). Compound **1** (19 mg, 0.039 mmol) was dissolved in EtOH (1.5 mL) and hydrochloric acid (3 mol/L, 0.1 mL). Zinc powder (10 mg) was added to the solution, and the mixture was stirred for 40 min at room temperature. After removal of zinc powder by filtration, the filtrate was concentrated and purified by preparative TLC plate and developed with $CHCl_3$/MeOH, 95:5, to afford **11** (2.0 mg, 11% yield) and **12** (9.3 mg, 52% yield).

11 (hydroxylamine): ^{1}H NMR ($CDCl_3$ + CD_3OD) δ 8.44 (m, 1H), 8.08 (br s, 1H), 7.65 (m, 1H), 7.48−6.70 (m, 9H), 5.05 (d, J = 15 Hz, 1H), 4.59 (d, J = 8.8 Hz, 1H), 3.88 (d, J = 15 Hz, 1H), 3.40 (m, 1H), 3.10 (dd, J = 11.2. 8.8 Hz, 1H), 2.95−2.70 (m, 2H). FAB-MS (m/z): 470, 468 $(M + H)^+$.

12 (amine): ^{1}HNMR ($CDCl_3$) δ 8.49 (m, 1H), 8.01 (br s, 1H), 7.76 (m, 1H), 7.48−6.80 (m, 9H), 5.31 (d, J = 15 Hz, 1H), 3.89 (d, J = 8.8 Hz, 1H), 3.75 (d, J = 15 Hz, 1H), 3.33 (m, 1H), 3.02−2.72 (m, 3H). FAB-MS (m/z): 454, 452 $(M + H)^+$.

(±)-4-(3-Chlorophenyl)-6-(4-hydroxyphenyl)-5-nitro-1-(3-pyridylmethyl)piperidin-2-one (13). According to the procedure for the synthesis of **1**, methyl 3-(3-chlorophenyl)-4-nitrobutylate (260 mg, 1.0 mmol), 4-hydroxybenzaldehyde (120 mg, 1.0 mmol), and 3-(aminomethyl)pyridine (0.20 mL, 2.0 mmol) afforded **13** (107 mg, 24% yield). ^{1}H NMR (DMSO-d_6) δ 9.65 (s, 1H), 8.42 (d, J = 3.5 Hz, 1H), 8.16 (s, 1H), 7.59 (s, 1H), 7.43 (d, J = 7.9 Hz, 1H), 7.30−7.36 (m, 3H), 7.28 (dd, J = 17.6, 4.7 Hz, 1H), 7.09 (d, J = 7.7 Hz, 2H), 6.68 (d, J = 7.7 Hz, 2H), 5.73 (dd, J = 11.6, 10.1 Hz, 1H), 4.79 (d, J = 10.1 Hz, 1H), 4.69 (d, J = 15.7 Hz, 1H), 3.91−3.9 (m, 2H), 3.15 (dd, J = 16.9, 12.9 Hz, 1H), 2.75 (dd, J = 16.9, 5.0 Hz, 1H). FAB-MS (m/z): 438 $(M + H)^+$.

(±)-6-(4-Hydroxyphenyl)-4-(3-methylphenyl)-5-nitro-1-(3-pyridylmethyl)piperidin-2-one (14). According to the procedure for the synthesis of **1**, methyl 3-(3-methylphenyl)-4-nitrobutylate (240 mg, 1.0 mmol), 4-hydroxybenzaldehyde (100 mg, 0.8 mmol), and 3-(aminomethyl)pyridine (0.20 mL, 2.0 mmol) afforded **14** (47 mg, 11% yield). ^{1}H NMR (DMSO-d_6) δ 9.63 (s, 1H), 8.42 (dd, J = 5.0, 1.8 Hz, 1H), 8.15 (d, J = 1.8 Hz, 1H), 7.44 (d, J = 7.8 Hz, 1H), 7.28 (dd, J = 7.8. 4.9 Hz, 1H), 7.20−7.23 (m, 3H), 7.08−7.11 (m, 3H), 6.67 (d, J = 8.4 Hz, 2H), 5.65 (dd, J = 11.8, 9.8 Hz, 1H), 4.79 (d, J = 9.8 Hz, 1H), 4.70 (d, J = 15.4 Hz, 1H), 3.93 (d, J = 15.4 Hz, 1H), 3.83 (m, 1H), 3.15 (dd, J = 17.3, 12.6 Hz, 1H), 2.70 (dd, J = 17.3, 4.9 Hz, 1H), 2.28 (s, 3H). FAB-MS (m/z) 418 $(M + H)^+$.

(±)-6-(4-Hydroxyphenyl)-5-nitro-4-phenyl-1-(3-pyridylmethyl)piperidin-2-one (15). A mixture of **1** (20 mg, 0.041 mmol) and 10% Pd/C (4.3 mg) in MeOH (5.0 mL) was stirred under hydrogen atmosphere for 19 h at room temperature. After Pd/C was filtered out, the filtrate was concentrated and the residue was purified by preparative TLC plate, developed with $CHCl_3$/MeOH, 95:5, to afford **15** (4.0 mg, 24%). ^{1}H NMR ($CDCl_3$ + CD_3OD) δ 8.46 (br dd, J = 4.7, 1.4 Hz, 1H), 8.09 (br d, J = 1.4 Hz, 1H), 7.58 (m, 1H), 7.40−7.28 (m, 6H), 6.95 (m, 2H), 6.80 (m, 2H), 5.08 (dd, J = 11.7, 9.5 Hz, 1H), 5.07 (d, J = 15.0 Hz, 1H), 4.78 (d, J = 9.5 Hz, 1H), 3.94 (d, J = 15.0 Hz, 1H), 3.70 (m, 1H), 3.03−2.90 (m, 2H). FAB-MS (m/z): 404 $(M + H)^+$.

(±)-4-(4-Bromophenyl)-6-(4-hydroxyphenyl)-5-nitro-1-(3-pyridylmethyl)piperidin-2-one (16). According to the procedure for the synthesis of **1**, methyl 3-(4-bromophenyl)-4-nitrobutylate (300 mg, 1.0 mmol), 4-hydroxybenzaldehyde (120 mg, 1.0 mmol), and 3-(aminomethyl)pyridine (0.20 mL, 2.0 mmol) afforded **16** (130 mg, 27% yield). ^{1}H NMR (DMSO-d_6) δ 9.65 (b s, 1H), 8.42 (br d, J = 4.5 Hz, 1H), 8.15 (br s, 1H), 7.60−6.60 (m, 10H), 5.69 (dd, J = 11.5, 10.0 Hz, 1H), 4.79 (d, J = 10.0 Hz, 1H), 4.70 (d, J = 15.5 Hz, 1H), 4.93 (d, J = 15.5 Hz, 1H), 3.92 (m, 1H), 3.14 (dd, J = 17.0, 13.2 Hz, 1H), 2.73 (dd, J = 17.0, 5.0 Hz, 1H). FAB-MS (m/z): 484, 482 $(M + H)^+$.

4-(2-Bromophenyl)-6-(4-hydroxyphenyl)-5-nitro-1-(3-pyridylmethyl)piperidin-2-one (17). According to the procedure for the synthesis of **1**, methyl 3-(2-bromophenyl)-4-nitrobutylate (61 g, 0.20 mol), 4-hydroxybenzaldehyde (25 g, 0.21 mol), and 3-(aminomethyl)pyridine (42 mL, 0.41 mmol) afforded **17** (69 g, 73% yield). Analytically pure **17** was obtained by recrystallization from hot EtOH. ^{1}H NMR (DMSO-d_6) δ 9.62 (s, 1H), 8.38 (d, J = 4.8 Hz, 1H), 8.11 (s, 1H), 7.78 (d, J = 7.9 Hz, 1H), 7.61 (d, J = 7.9 Hz, 1H), 7.39−7.46 (m, 2H), 7.22−7.27 (m, 2H), 7.14 (d, J = 8.0 Hz, 2H), 6.64 (d, J = 8.0 Hz, 2H), 5.89 (dd, J = 11.0, 9.5 Hz, 1H), 4.94 (d, J = 9.5 Hz, 1H), 4.47 (d, J = 15.8 Hz, 1H), 4.38 (m, 1H), 4.18 (d, J = 15.8 Hz, 1H), 3.05 (dd, J = 16.8, 11.0 Hz, 1H), 2.74 (dd, J = 16.8, 4.7 Hz, 1H). FAB-MS (m/z): 484, 482 $(M + H)^+$. Anal. ($C_{23}H_{20}BrN_3O_4$) C, H, N. (C, 57.27; H, 4.19; N, 8.71%. Found: C, 57.12; H, 4.21; N, 8.67%).

Optical Resolution of (±)-17. (±)-**17** (10 mg, 0.02 mmol) was separated by preparative HPLC [Chiralcel OD (0.46 cm × 25 cm), *i*-PrOH/*n*-hexane/Et_2NH, 40:60:0.1, 6.0 mL/min, UV 254 nm] to afford (+)-**17** (2.1 mg, 21% yield) and (−)-**17** (1.3 mg, 13% yield). (+)-**17**: $[\alpha]^{26}_D$ +90.7°(*c* 0.10, MeOH); t_R = 17 min (*i*-PrOH/*n*-hexane/Et_2NH, 40:60:0.1, 1.0 mL/min); 98%ee. (−)-**17**: $[\alpha]^{26}_D$ −85.5°(*c* 0.04, MeOH); t_R - 22 min (*i*-PrOH/*n*-hexane/Et_2NH = 40:60:0.1, 1.0 mL/min); 86% ee.

(±)-4-(2-Bromophenyl)-6-(4-methoxyphenyl)-5-nitro-1-(3-pyridylmethyl)piperidin-2-one (18). According to the procedure for the synthesis of **1**, methyl 3-(2-bromophenyl)-4-nitrobutyrate (300 mg, 1.0 mmol), 4-methoxybenzaldehyde (0.10 mL, 0.9 mmol) and 3-(aminomethyl)pyridine (0.20 mL, 2.0 mmol) afforded **18** (220 mg, 45% yield). ^{1}H NMR (DMSO-d_6) δ 8.38 (dd, J = 4.8, 1.5 Hz, 1H), 8.11 (d, J = 1.5 Hz, 1H), 7.78 (d, J = 7.0 Hz, 1H), 7.61 (dd, J = 8.1, 1.1 Hz, 1H), 7.44 (t, J = 7.0 Hz, 1H), 7.37 (d, J = 8.1 Hz, 1H), 7.20−7.29 (m, 4H), 6.83 (d, J = 8.8 Hz, 2H), 5.92 (dd, J = 11.5, 10.1 Hz, 1H), 5.02 (d, J = 10.1 Hz, 1H), 4.35−4.48 (m, 2H), 4.21 (d, J = 15.4 Hz, 1H), 3.70 (s, 3H), 3.04 (dd, J = 16.8, 12.8 Hz, 1H), 2.75 (dd, J = 16.8, 5.2 Hz, 1H). FAB-MS (m/z): 498, 496 $(M + H)^+$.

(±)-4-(2-Bromophenyl)-6-(3-hydroxymethyl-4-hydroxyphenyl)-5-nitro-1-(3-pyridylmethyl)piperidin-2-one (19). According to the procedure for the synthesis of **1**, methyl 3-(2-bromophenyl)-4-nitrobutylate (436 mg, 1.4 mmol), 4-hydroxy-3-(hydroxymethyl)benzaldehyde (198 mg, 1.3 mmol), and 3-(aminomethyl)pyridine (0.29 mL, 2.8 mmol) afforded **19** (24 mg, 3.2% yield). ^{1}H NMR (DMSO-d_6) δ 9.61 (s, 1H), 8.40 (dd, J = 5.0, 1.8 Hz, 1H), 8.13 (d, J = 1.8 Hz, 1H), 7.82−7.79 (m, 1H), 7.62−7.59 (m, 1H), 7.45−7.39 (m, 2H), 7.30−7.19 (m, 3H), 6.99−6.96 (m, 1H), 6.63 (d, J = 8.3 Hz, 1H), 5.90−5.83 (m, 1H), 5.01 (br s, 1H), 4.90 (d, J = 9.7 Hz, 1H), 4.58 (d, J = 15 Hz, 1H), 4.43−4.32 (m, 3H), 4.05 (d, J = 15 Hz, 1H), 3.26−3.20 (m, 1H), 2.74 (dd, J = 17, 5.3 Hz, 1H). FAB-MS (m/z): 514, 512 $(M + H)^+$.

(±)-6-(3-Amino-4-hydroxyphenyl)-4-(2-bromophenyl)-5-nitro-1-(3-pyridylmethyl)piperidin-2-one (20). A mixture of 6-(4-hydroxy-3-nitrophenyl)-4-(2-bromophenyl)-5-nitro-1-(3-pyridylmethyl)piperidin-2-one (10 mg, 2.0 mmol) and Pd/C (5.0 mg) in EtOH (5.0 mL) was stirred under hydrogen atmosphere for 4 h at room temperature. The mixture was filtered, and the filtrate was concentrated. The residue was chromatographed on silica gel, eluting with $CHCl_3$/MeOH, 97:3, to afford **20** (4.1 mg, 44% yield). ^{1}H NMR ($CDCl_3$) δ 8.51 (br

s, 1H), 8.04 (br s, 1H), 7.83 (d, J = 7.9 Hz, 1H), 7.56 (d, J = 7.3 Hz, 1H), 7.41−7.26 (m, 3H), 7.11 (m, 1H), 6.96−6.95 (m, 2H), 6.22 (d, J = 7.3 Hz, 1H), 5.31−5.20 (m, 2H), 4.63 (d, J = 9.4 Hz, 1H), 4.32 (m, 1H), 3.98 (d, J = 15 Hz, 1H), 3.07 (dd, J = 18 Hz, 5.0 Hz, 1H), 2.70 (m, 1H). FAB-MS (m/z): 499, 497 $(M + H)^+$.

(±)-4-(2-Bromophenyl)-6-(3,4-dihydroxyphenyl)-5-nitro-1-(3-pyridylmethyl)piperidin-2-one (6). According to the procedure for the synthesis of **1**, methyl 3-(2-bromophenyl)-4-nitrobutyrate (81 g, 0.27 mol), 3,4-dihydroxybenzaldehyde (37 g, 0.27 mol), and 3-(aminomethyl)pyridine (54 mL, 0.53 mol) in EtOH (200 mL) afforded **6** (78 g, 60% yield). Analytically pure **6** was obtained by recrystallization from hot EtOH. ^{1}H NMR (DMSO-d_6) δ 9.23 (s, 1H), 8.95 (s, 1H), 8.43 (dd, J = 4.6, 1.5 Hz, 1H), 8.18 (d, J = 2.0 Hz, 1H), 7.79 (d, J = 7.2 Hz, 1H), 7.61 (dd, J = 7.8, 1.1 Hz, 1H), 7.41−7.47 (m, 2H), 7.29 (dd, J = 7.8, 4.7 Hz, 1H), 7.22 (dt, J = 7.8, 1.5 Hz, 1H), 6.75 (d, J = 2.0 Hz, 1H), 6.64 (d, J = 8.1 Hz, 1H), 6.56 (dd, J = 8.1, 2.0 Hz, 1H), 5.84 (dd, J = 11.3, 9.8 Hz, 1H), 4.80 (d, J = 9.8 Hz, 1H), 4.65 (d, J = 15.6 Hz, 1H), 4.35 (m, 1H), 3.99 (d, J = 15.6 Hz, 1H), 3.02 (dd, J = 16.9, 12.5 Hz, 1H), 2.72 (dd, J = 16.9, 4.6 Hz, 1H). FAB-MS (m/z): 500, 498 $(M + H)^+$. Anal. ($C_{23}H_{20}BrN_3O_5$) C, H, N. (Calcd: C, 55.43; H, 4.05; N, 8.43%. Found: C, 55.29; H, 4.03 N, 8.35%).

Optical Resolution of (±)-6. According to the procedure for the separation of (±)-**8**, the diisobutyl ester of (±)-**6** was resolved by HPLC on a Chiralcel OD column followed by hydrolysis of diisobutyl ester. (+)-Diisobutyl ester of **6**: t_R = 12.5 min (*i*-PrOH/*n*-hexane/Et_2NH, 40:60:0.1, 1.0 mL/min), >98% ee. (−)-Diisobutyl ester of **6**: t_R = 20.1 min (*i*-PrOH/*n*-hexane/Et_2NH, 40:60:0.1, 1.0 mL/min), 98% ee. (+)-**6** (23 mg, 63%yield): $[\alpha]^{28}_D$ +83° (*c* 0.66, MeOH). (−)-**6** (18 mg, 48% yield): $[\alpha]^{28}_D$ −90° (*c* 0.30, MeOH).

(±)-4-(2-Bromophenyl)-6-(3,4-dihydroxyphenyl)-5-nitro-1-(3-pyridylmethyl)-2,3-dehydropiperidine (7). To a solution of **6** (100 mg, 0.20 mmol) in THF (5.0 mL) was added diisobutylaluminum hydride (1.0 mol/L in THF, 1.0 mL) at 0 °C. After being stirred for 12 h at room temperature, the reaction mixture was quenched with aqueous HCl, extracted with $CHCl_3$, dried with magnesium sulfate, and concentrated. The residue was chromatographed on silica gel, eluting with $CHCl_3$/MeOH, 99:1, to afford **7** (48 mg, 50% yield). ^{1}H NMR (DMSO-d_6) δ 9.12 (br s, 1H), 9.01 (br s, 1H), 8.50 (d, J = 4.2 Hz, 1H), 8.31 (s, 1H), 7.60−7.54 (m, 3H), 7.42−7.38 (m, 2H), 7.19 (t, J = 7.6 Hz, 1H), 6.77 (d, J = 2.4 Hz, 1H), 6.71 (d, J = 8.0 Hz, 1H), 6.61 (m, 1H), 6.49 (dd, J = 8.0, 2.4 Hz, 1H), 5.29 (dd, J = 9.5, 8.6 Hz, 1H), 4.63 (d, J = 9.3 Hz, 1H), 4.32 (d, J = 8.6 Hz, 1H), 4.21−4.13 (m, 2H), 3.73 (d, J = 15.2 Hz, 1H). FAB-MS (m/z): 484, 482 $(M + H)^+$.

(±)-4-(2-Bromophenyl)-2-(3,4-dihydroxyphenyl)-3-nitro-1-(3-pyridylmethyl)piperidine (8). To a solution of **6** (41 g, 82 mmol) in THF (1.6 L) was added the borane−methyl sulfide complex (30 mL, 320 mmol), and the mixture was refluxed for 10 h. After the mixture was cooled to 4 °C, the reaction was quenched by the addition of water (50 mL) and the mixture was concentrated. The residue was heated with hydrochloric acid (3.0 mol/L, 400 mL) and neutralized with NaOH solution (3.5 mol/L, 300 mL) after cooling to room temperature. The solid formed was recrystallized from EtOH to give analytically pure **8** (24.7 g, 62% yield). Mp 211−212 °C; IR (KBr) 2825, 1610, 1549, 1473, 1444, 1369, 1286, 1209, 1045, 762 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.05 (s, 1H), 8.97 (s, 1H), 8.44 (m, 2H), 8.42 (m, 1H), 7.75 (m, 1H), 7.66−7.56 (m, 2H), 7.40−7.32 (m, 2H), 7.17 (m, 1H), 6.94 (br s, 1H), 6.72 (m, 1H), 5.25 (dd, J = 9.4, 11.6 Hz, 1H), 3.86 (dt, J = 4.0, 11.6 Hz, 1H), 3.64 (d, J = 13.8 Hz, 1H), 3.63 (d, J = 9.4 Hz, 1H), 3.05 (d, J = 13.8 Hz, 1H), 2.88 (m, 1H), 2.36 (m, 1H), 1.86−1.71 (m, 2H); ^{13}C NMR (DMSO-d_6, 67.70 MHz) δ 149.66, 148.26, 145.66, 145.44, 138.48, 136.07, 134.02, 132.91, 129.32, 128.25, 128.06, 123.61, 123.41, 115.65, 93.45, 70.35, 54.49, 51.36, 46.16, 30.89. FAB-MS (m/z): 486, 484 $(M + H)^+$. Anal. ($C_{23}H_{22}BrN_3O_4$·1.0EtOH): C, H, N. (C, 56.60; H, 5.33; N, 7.92%. Found: C, 56.38; H, 5.58 N, 7.91%).

Optical Resolution of (±)-8. To a solution of the HCl salt of **8** (600 mg, 1.1 mmol) in $CHCl_3$ (10 mL) and triethylamine (5.0 mL) was added isobutyrchloride (1.0 mL, 9.5 mmol) at room temperature. After being stirred for 7 h, the mixture was concentrated and the residue was chromatographed on silica gel, eluting with $CHCl_3$/MeOH, 95:5, to give the diisobutyl ester of **8** (375 mg, 56% yield). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.50−8.48 (m, 2H), 7.72−7.51 (m, 2H), 7.40−7.20 (m, 6H), 7.01 (m, 1H), 4.93 (dd, J = 9.6, 11.2 Hz, 1H), 4.04 (m, 1H), 3.85 (d, J = 9.6 Hz, 1H), 3.82 (d, J = 13.1 Hz, 1H), 3.06 (d, J = 13.1 Hz, 1H), 3.03 (m, 1H), 2.77 (m, 2H), 2.42 (m, 1H), 2.03 (m, 1H), 1.71 (m, 1H), 1.31 (d, J = 7.0 Hz, 6H), 1.28 (d, J = 7.0 Hz, 6H). FAB-MS (m/z): 626, 624 $(M + H)^+$.

The diisobutyl ester of **8** (300 mg) was resolved by HPLC according to the procedure of the separation of (±)-**21**. (+)-Diisobutyl ester of **8** (79 mg, 26%): $[\alpha]^{28}_D$ +76.7° (*c* 0.343, MeOH); t_R = 13.3 min (*i*-PrOH/*n*-hexane/Et_2NH, 33:66:0.1, 1.0 mL/min), >98% ee. (−)-Diisobutyl ester of **8** (52 mg, 17%): $[\alpha]^{26}_D$ −75.8° (*c* 0.360, MeOH); t_R = 15.8 min (*i*-PrOH/*n*-hexane/Et_2NH, 33:66:0.1, 1.0 mL/min), >98% ee.

Diisobutyl esters of (+)-**8** and (−)-**8** were treated with $NaHCO_3$ solution in MeOH to give (+)-**8** and (−)-**8**, respectively. (+)-**8** (20 mg, 76% yield): $[\alpha]^{28}_D$ +68.0° (*c* 0.225, MeOH). FAB-MS (m/z): 486, 484 $(M + H)^+$. (−)-**8** (9.5 mg, 68% yield): $[\alpha]^{26}_D$ −66.5° (*c* 0.224, MeOH). FAB-MS (m/z): 486, 484 $(M + H)^+$.

(±)-4-(2-Bromophenyl)-2-(4-hydroxyphenyl)-3-nitro-1-(3-pyridylmethyl)piperidine (21). According to the procedure for synthesis of **8**, **17** (40.0 g, 83 mmol) and borane−methyl sulfide complex (30 mL, 320 mmol) in THF (2.0 L) afforded **21** (35 g, 90% yield). Mp 229−230 °C; IR (KBr) 2823, 1614, 1560, 1554, 1473, 1373, 1280, 1250, 1022, 761 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.53 (s, 1H), 8.44 (m, 1H), 8.16 (br s, 1H), 7.73 (m, 1H), 7.65−7.56 (m, 2H), 7.40−7.31 (m, 4H), 7.18 (m, 1H), 6.78 (br s, 1H), 6.75 (br s, 1H), 5.34 (dd, J = 9.4, 11.7 Hz, 1H), 3.87 (dt, J = 4.0, 11.7 Hz, 1H), 3.73 (d, J = 9.4 Hz, 1H), 3.59 (d, J = 13.9 Hz, 1H), 3.08 (d, J = 13.9 Hz, 1H), 2.90 (m, 1H), 2.39 (m, 1H), 1.89−1.67 (m, 2H); ^{13}C NMR (DMSO-d_6, 67.70 MHz) δ 157.61, 149.55, 148.22, 138.47, 135.95, 133.98, 132.87, 129.63, 129.28, 128.27, 128.21, 127.35, 123.57, 123.38, 115.51, 93.21, 70.15, 54.44, 51.47, 46.14, 30.89. FAB-MS (m/z): 470, 468 $(M + H)^+$. Anal. ($C_{23}H_{22}BrN_3O_3$·$0.3H_2O$) C, H, N. (C, 58.31; H, 4.81; N, 8.87%. Found: C, 58.36; H, 5.02; N, 8.83%).

Optical Resolution of (±)-21. The HCl salt of (±)-**21** (200 mg, 0.37 mmol) was separated by preparative HPLC [Chiralcel OD (2.0 cm × 25 cm), *i*-PrOH/*n*-hexane/Et_2NH, 33:66:0.1, 6.0 mL/min, UV 254 nm] to afford (+)-**21** (36 mg, 18% yield) and (−)-**21** (48 mg, 24% yield). (+)-**21**: $[\alpha]^{26}_D$ +63.9° (*c* 0.227, MeOH). FAB-MS (m/z): 470, 468 $(M + H)^+$. t_R = 19 min (*i*-PrOH/*n*-hexane/Et_2NH, 33:66:0.1, 1.0 mL/min), >98%ee. (−)-**21**: $[\alpha]^{26}_D$ −68.5°(*c* 0.200, MeOH). FAB-MS (m/z): 470, 468 $(M + H)^+$. t_R = 23 min (*i*-PrOH/*n*-hexane/Et_2NH, 33:66:0.1, 1.0 mL/min), >98% ee.

Biological Assays. Farnesyltransferase Enzyme Assay. Bovine brain farnesyltransferase was partially purified according the method described in the literature.[7a] The resulting dialysate was used as a crude enzyme of farnesyltransferase. Farnesyltransferase assays were carried out using viral K-Ras protein and [^{3}H]farnesyl pyrophosphate as described.[7b] The concentration of the sample inhibiting the farnesylation by 50% (IC_{50}) was calculated by comparing the enzyme inhibitory activity of an untreated group with those of groups treated with the sample having known concentrations.

H-Ras Processing Assay. T24 cells were treated with various concentrations of (+)-**8** for 40 h. The cell lysates were analyzed by Western blotting with monoclonal antibody specific to H-Ras. (+)-**8** inhibited H-Ras processing at concentrations as low as 16 nM.

Supporting Information Available: X-ray crystallographic data of compound (+)-**6**, including an ORTEP drawing. This material is available free of charge via the Internet at http://pubs.acs.org.

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(8) (+)-**8** showed anchorage-independent growth inhibition of HCT116 cells with an IC_{50} of 39 nM. Detailed results of cellular assays as well as biochemical studies of this class of compounds will be described elsewhere. Hara, M.; et al. Manuscript in preparation.

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