Synthesis and Antitumor Activity of Novel *O*-Carbamoylmethyloxime Derivatives of Radicicol

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Radicicol (**1**), a macrocyclic antifungal antibiotic, is the lead compound of a novel class of heat shock protein 90 (Hsp90) inhibitors that result in the inhibition or degradation of Hsp90-associated proteins, such as v-src and Raf-1 kinases. New *O*-carbamoylmethyloxime derivatives of **1** were synthesized and evaluated for their in vitro antiproliferative activities against v-src- and K-ras-transformed cells and for their inhibitory activity against v-src tyrosine kinase. *O*-(Piperidinocarbonyl)methyloxime **9b**, one of the most potent of these derivatives, exhibited more potent antiproliferative activity than **1** and its hydroxime KF25706 (**2**) and had an IC_{50} of 25 nM for the inhibition of v-src kinase activity. Compound **9b** was also found to decrease the Raf-1 protein level of KNRK5.2 cells. Furthermore, compound **9b** exhibited significant antitumor activity when tested against MX-1 and A431 xenografts in nude mice.

Introduction

An antifungal antibiotic radicicol (**1**), first isolated

1 (**Radicicol**): X = O
2 (**KF25706**): X = N-OH
3 (**KF29158**): X = N-OCH_2CONMe_2

4 (**GA**): R^1 = H, R^2 = H, R^3 = OMe
5 (**HA**): R^1 = Me, R^2 = OMe, R^3 = H
6 (**17-AAG**): R^1 = H, R^2 = H, R^3 = NH

7 (**PU3**)

from the fungus *Monosporium bonorden*,[1] was shown to inhibit v-src tyrosine kinase[2] and to suppress the transformation by various oncogenes such as src, ras, raf, mos, and fos.[3–5] It was also reported that **1** could inhibit K-ras-activated aberrant signaling pathway through the selective depletion of Raf-1 kinase and subsequent inhibition of mitogen-activated protein kinase (MAPK) pathway in K-ras-transformed rat cells (KNRK5.2 cells), leading to the inhibition of cell growth.[6] In addition, compound **1** was found to show potent in vitro antiproliferative activity against a wide variety of human tumor cell lines.[7]

Although compound **1** exhibited potent antiproliferative activity, the compound was inactive when tested against in vivo antitumor models.[7] It was also reported that the inhibitory effect of **1** against v-src kinase was abolished by reducing agents such as dithiothreitol.[2] Therefore, novel oxime derivatives such as KF25706 (**2**) and KF29158 (**3**), which are more stable than **1**, were designed and synthesized.[7,8] Compounds **2** and **3** were found to show more potent antiproliferative activities than **1** and significant in vivo antitumor activities in several human tumor xenograft models.

Soon after the present study was started, for the purpose of increasing the antitumor activity of oxime derivative **2**, it was revealed that one of the major molecular targets of **1** is the heat shock protein 90 (Hsp90) family, including Hsp90 and glucose-regulated protein 94 (Grp94),[9–11] which plays an essential role in the stability and function of important cellular proteins such as v-src, Raf-1, EGFR, $p185^{erbB2}$, Cdk4, steroid hormone receptors, and mutated p53.[12–22] Binding of **1** to the highly conserved N-terminal site of Hsp90 alters Hsp90 function, leads to the inhibition or degradation of these Hsp90-associated proteins, and results in cell death.[9–11] In addition, **2** was shown to deplete several Hsp90-associated proteins through the inhibition of the Hsp90 function, similar to **1**.[7] Hsp90 inhibitors that can simultaneously stimulate depletion of multiple oncogenic proteins may be of clinical benefit.

It has been also reported that ansamycin antibiotics, such as geldanamycin (GA, **4**) and herbimycin A (HA, **5**), are Hsp90 inhibitors, which destabilize many Hsp90-associated proteins.[14,16–19,22,23] Both antibiotics **1** and **4** were shown to directly bind to the N-terminal nucleotide-binding domain of Hsp90 by X-ray structures of

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Scheme 1[a]

[a] (a) $(H_2NOCH_2CO_2H)_2$·HCl, AcOH, Py, 40 °C; (b) amine, EDCI, HOBt, DMF; (c) amine, EDCI, DMAP, DMF; (d) amine, DCC, *N*-hydroxysuccinimide, DMF.

radicicol-Hsp90 and GA-Hsp90 complexes.[24,25] More recently, PU3 (**7**), a small-molecule inhibitor of Hsp90, was designed by computer modeling using X-ray structures of ligand–Hsp90 complexes.[26,27] 17-Allylamino-17-demethoxygeldanamycin (17-AAG, **6**), a less toxic derivative of **4**, is currently in phase I clinical trial.[28,29]

We concentrated our efforts on optimizing the oxime group of **2** to further increase in vivo antitumor activity. As the first approach, we optimized a carbamoyl group of **3**, using an easily accessible *O*-carboxymethyloxime **8**.[8] In addition, *O*-carbamoylmethyl derivatives with polar functional groups were synthesized for the purpose of improving the poor water solubility of **2** and **3**. We report here the synthesis, in vitro antiproliferative activity, the effects on Hsp90-associated proteins such as v-src and Raf-1, and in vivo antitumor activity of a new series of *O*-carbamoylmethyloxime derivatives of **1**.

Chemistry

As depicted in Scheme 1, *O*-carboxymethyloxime **8** was prepared from **1** by the modification of the method reported previously.[8] Compound **8** was then condensed with various amines in the presence of condensing agents to give carbamoyl derivatives **9a–v** of Table 1. Because **8** was a mixture of *E* and *Z* isomers on the oxime group ($E/Z = 1/4$), all carbamoyl derivatives synthesized were obtained as isomeric mixtures, which were not separated by TLC, in *E*/*Z* ratios varying from 1/2 to 1/10 as shown in Table 1. When compound **9b** was prepared by two different methods using 1,3-dicyclohexylcarbodiimide (DCC) and *N*-hydroxysuccinimide and using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) and 1-hydroxybenzotriazole hydrate (HOBt), a variation of the *E*/*Z* ratios was observed ($E/Z = 1/6$ and 1/2, respectively). It is suggested that the variation of the *E*/*Z* ratios is due to loss on purification using column chromatography or preparative TLC and not to reactivity between both isomers. The oxime configurations and the *E*/*Z* ratios of these compounds were identified by comparison with ^{1}H NMR data of both isomers of **2** and its methoxime,[8] which were assigned on the basis of NOESY and ^{13}C NMR data.[30]

The poor solubility of compounds was not improved by the introduction of polar functional groups into the oxime group (data not shown).

Results and Discussion

Compounds were first evaluated for their antiproliferative activities against normal rat fibroblast cells (3Y1-B cells), its v-src-transformed cells (SR-3Y1 cells), normal rat kidney epithelial cells (NRK cells), and its K-ras-transformed cells (KNRK5.2 cells) and for their inhibitory activity against the v-src-induced tyrosine autophosphorylation in SR-3Y1 cells because **1** was shown to suppress the growth of these transformed cell lines and to inhibit the tyrosine kinase.[2,6,7]

As shown in Table 1, compounds **9a–c** exhibited more potent antiproliferative activities against SR-3Y1 and KNRK5.2 cells than **1**. In particular, **9b**,**c** were found to exhibit a greater than 2-fold increase in the antiproliferative activity compared to compounds **2** and **3** (IC_{50} = 0.015 μM against SR-3Y1 cells) with a potent in vivo activity.[7,8]

However, although compound **1** exhibited strong antiproliferative activity against the transformed cells compared with their normal counterparts, these oximes inhibited the growth of 3Y1-B and NRK cells as well as their transformed cells. Compounds **9a–c** inhibited tyrosine phosphorylation with IC_{50} values of 0.070, 0.025, and 0.067 μM, respectively, compared to an IC_{50} value of 0.056 μM for **2**. Although compound **9c** showed more potent antiproliferative activity than **2** and **9b**, its inhibitory effect on tyrosine phosphorylation was less potent compared to the inhibitory effect of these compounds.

The introduction of a polar functional group, such as hydroxyl, piperidino, and carbamoyl groups, into the piperidine ring (**9d–f**) and the conversion of the piperidine ring into a polar morpholine or *N*-methylpiperazine ring (**9g**,**h**) substantially reduced the antiproliferative activity against SR-3Y1 and KNRK5.2 cells. However, the inhibitory activity of **9h** against v-src kinase was more potent than that of **2**.

In the case of N-monosubstituted carbamoylmethyloximes, similar SARs were also observed. Compounds **9i–k** with a hydrophobic cycloalkyl group exhibited a more potent antiproliferative activity against SR-3Y1 cells than **2**, and compounds **9l–p** containing polar functional groups at the *N*-alkyl group exhibited a less potent in vitro activity. Compounds **9i** and **9k** strongly inhibited v-src tyrosine phosphorylation with IC_{50} values of 0.014 and 0.017 μM, respectively; however, the inhibitory effect of **9j** on v-src kinase was less potent.

On the other hand, **9q–v** with an aromatic ring or a heteroaromatic ring were about 2- to 11-fold less active against SR-3Y1 and KNRK5.2 cells than **2**. However, **9q**,**s**,**t** exhibited strong inhibitory activity against v-src kinase.

All of oxime derivatives, except **9d–f**, showed stronger antiproliferative activity against SR-3Y1 cells than against KNRK5.2 cells.

Among the derivatives synthesized, **9b**, one of the most potent, was further evaluated for its ability to

Table 1. Antiproliferative Activity and Inhibitory Activity against v-src Tyrosine Kinase of *O*-Carbamoylmethyloximes of **1**

compd	NRR'	E/Z[a]	3Y1-B	SR-3Y1	NRK	KNRK5.2	v-src
			IC_{50} (μM)[b]				IC_{50} (μM)[c]
1	–	–	0.70	0.059	0.29	0.11	0.18
2	–	1/2	0.090	0.026	0.080	0.039	0.056
9a	N	1/3	0.055	0.020	0.034	0.039	0.070
9b	N	1/6	0.0083	0.0061	0.018	0.014	0.025
9c	N	1/10	<0.0041	<0.0041	0.0073	0.0064	0.067
9d	N–OH	1/3	>3.0	>3.0	>3.0	>3.0	NT[d]
9e	N–N	1/8	>3.0	>3.0	3.0	>3.0	NT
9f	N–CONH$_2$	1/3	>3.0	>3.0	>3.0	>3.0	NT
9g	N O	1/4	0.31	0.13	0.34	0.34	0.14
9h	N NMe	1/4	1.0	0.47	1.2	1.5	0.013
9i	NH	1/3	0.041	0.0098	0.041	0.045	0.014
9j	NH	1/3	0.067	0.0057	0.19	0.073	0.21
9k	NH	1/4	0.18	0.020	0.21	0.14	0.017
9l	NH OH	1/5	2.8	1.5	>3.0	2.2	>1.0
9m	N(OH)$_2$	1/5	>3.0	1.1	>3.0	2.9	>1.0
9n	NH OEt	1/10	0.39	0.093	0.26	0.23	>1.0
9o	NH NEt$_2$	1/3	>3.0	2.3	>3.0	>3.0	>1.0
9p	NH CO$_2$Me	1/4	0.79	0.24	0.70	0.57	0.36
9q	NH	1/3	0.62	0.12	0.32	0.29	0.043
9r	NH–OMe	1/3	0.10	0.058	0.26	0.081	0.39
9s	NH–NEt$_2$	1/3	0.49	0.10	0.52	0.24	<0.010
9t	NH N	1/3	0.20	0.051	0.24	0.12	0.054
9u	NH N	1/3	0.67	0.29	0.51	0.48	>1.0
9v	NH N Me	1/3	0.60	0.084	1.2	0.38	>1.0

[a] *E*/*Z* ratio of oxime group was determined by ^{1}H NMR. [b] Drug concentration to inhibit the growth of 3Y1-B, SR-3Y1, NRK, and KNRK5.2 cells to 50% of control cultures, using a 72 h drug exposure. [c] IC_{50} value for the inhibition of v-src tyrosine autophosphorylation in SR-3Y1 cells after a 15 h drug exposure. [d] NT, not tested.

deplete Raf-1 levels and consequently inhibitory activity against K-ras-induced phosphorylation of MAPK (Erk2) in KNRK5.2 cells, to validate whether this compound would inhibit a MAPK pathway, like **1** and **2**.[6,7]

As shown in Table 2, **9b**, which showed stronger antiproliferative activity against KNRK5.2 cells than **2**, markedly decreased Raf-1 protein compared to **2** and significantly inhibited Erk2 phosphorylation when the

Table 2. Inhibition of K-Ras Signal Transduction Pathway by Selected Oxime **9b** in KNRK5.2 Cells

compd	Raf-1[a] % depletion at 0.2 μM	Erk2[b] % inhibition at 0.2 μM
2	29	81
9b[c]	74	95

[a] % depletion value of Raf-1 protein, after a 40 h drug exposure. [b] % inhibition value of formation of phosphorylated Erk2, after a 40 h drug exposure. [c] **9b** with *E*/*Z* ratio of 1/6 was used.

cells were treated at 0.2 μM. Compound **9b** did not affect the expression level of the Erk2 protein, which is not a client of Hsp90. These results suggest that **9b** inhibits the growth of KNRK5.2 cells through the depletion of Raf-1 and the resultant inhibition of Erk2 phosphorylation. The effects of **9b** on the Hsp90-associated proteins, such as v-src and Raf-1, suggest that **9b** exerts its biological effects by binding to Hsp90, similar to **1** and **2**.[6,7]

In this study, there was no correlation between the antiproliferative activity against SR-3Y1 cells and inhibitory activity against v-src kinase, although Hsp90 inhibitors are thought to result in their antiproliferative effects through the inhibition or degradation of the Hsp90-associated proteins. Compounds **9h**, **9q**, and **9s** strongly inhibited v-src kinase but were less active against SR-3Y1 cells. Contrary to this, compounds **9n** and **9v**, which had IC_{50} values of above 1 μM for v-src kinase inhibition, were found to moderately inhibit the growth of SR-3Y1 cells. In addition, several compounds (e.g., **9a**–**c** and **9g**) exhibited high sensitivity to both the transformed cells and their normal counterparts, although Hsp90 inhibitors are expected to selectively inhibit the growth of the transformed cells, which depend strongly on v-src or Raf-1 kinases for their survival.

To clarify these discrepancies, both isomers of each compound need to be separated and evaluated for their biological activity. We have recently clarified that the *E* configuration of the oxime group is more important for the binding affinity to Hsp90, the effects on Hsp90-associated proteins such as Raf-1 and erbB2, and antiproliferative activity against human breast tumor cell lines by separating both isomers of an *O*-[2-(2-pyrrolidonyl)ethyl]oxime derivative of **1**.[31,32] The active form of **9b** was also assumed to be *E* configuration. When **9b** was resynthesized by using the method with EDCI and HOBt to investigate the effects on in vivo xenograft models, a compound with an *E*/*Z* ratio of 1/2 was obtained, as described above. This compound exhibited stronger antiproliferative activity (IC_{50} = 0.0029 μM) against SR-3Y1 cells than that with an *E*/*Z* ratio of 1/6. The result suggests that the *E* isomer of **9b** is a more potent inhibitor than its *Z* isomer. Compound **9b** with an *E*/*Z* ratio of 1/2 also showed the selectivity for the transformed cells (IC_{50} = 0.0081 μM against 3Y1-B cells) compared to that with an *E*/*Z* ratio of 1/6. The result suggests that *Z* isomers, in which the inhibitory effects on the Hsp90 function are less potent, might cause growth inhibition not correlated to v-src inhibitory activity, and nonselective antiproliferative activity. No correlation between the antiproliferative activity and v-src inhibition might also result from the different stability of the compounds in the cells (in antiproliferative and v-src assays, the cells were treated with each compound for 72 and 15 h, respectively). To clarify these possibilities, further investigations are currently in progress.

Table 3. Antitumor Activity of Selected Oxime **9b** against Human Tumor Xenograft Models[a]

compd	tumor	dose (mg/kg)	frequency	*T*/*C* minimum[b] (on day)	mortality
2[c]	MX-1	100	(1/day) × 5	0.49 (14)	0/5
9b[d]	MX-1	50	(1/day) × 5	0.17 (11)[e]	0/5
	MX-1	25	(1/day) × 5	0.54 (11)[e]	0/5
	A431	50	(1/day) × 5	0.44 (7)[e]	0/5

[a] BALB/c-nu/nu mice (n = 5) transplanted with tumor cells were treated with oxime derivatives by daily iv injections for 5 consecutive days. [b] *T*/*C* value was calculated as described in Experimental Section. [c] Data from ref 7. [d] **9b** with *E*/*Z* ratio of 1/2 was used. [e] $P < 0.02$ by Mann–Whitney *U* test.

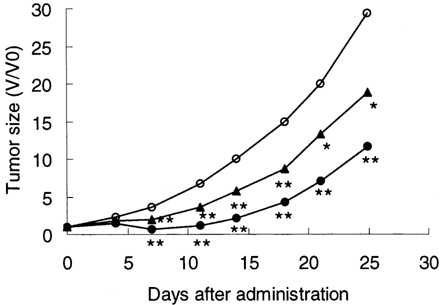

Figure 1. Tumor growth-inhibitory effect of **9b** against human breast tumor MX-1 xenograft model in vivo. MX-1 cells were inoculated sc into nude mice on day −14. Compound **9b** (*E*/*Z* = 1/2) was administered by daily iv injections on days 0–4: (○) control; (▲) **9b**, 25 mg/kg; (●) **9b**, 50 mg/kg; (**) $P <$ 0.02 and (*) $P < 0.05$ by Mann–Whitney *U* test.

Compound **9b** with an *E*/*Z* ratio of 1/2 was evaluated for the antitumor activities against subcutaneously inoculated human breast carcinoma MX-1 cells and human epidermoid carcinoma A431 cells in nude mouse models.[7] As shown in Table 3 and Figure 1, **9b** given at a dose of 50 mg/kg once daily over five consecutive iv injections exhibited strong antitumor activity against MX-1 cells compared to **2**, which was more potent than **3** in this animal model.[7,8] In addition, visual toxicity such as body weight loss was not observed at a dose of 50 mg/kg **9b**. However, compound **9b** exhibited lethal toxicity at a dose of 100 mg/kg.

Furthermore, **9b** at a dose of 50 mg/kg exhibited significant antitumor activity against A431 cells (Table 3), which were known to express high levels of EGFR and mutated p53, and its antitumor effect was more potent than that of **2**, as shown in our previous study.[7,8]

Conclusions

In this study, we have introduced various carbamoylmethyl groups into the oxime group of the lead **2**. The introduction of hydrophobic carbamoylmethyl groups enhanced the antiproliferative activity compared to **2**. In contrast, the introduction of carbamoylmethyl groups containing a hydrophilic functional group or an aromatic ring resulted in a decrease in antiproliferative activity. Compound **9b** was one of the most potent inhibitors with strong inhibitory activities against both the transformed cells and v-src kinase. Furthermore, **9b** was found to decrease the Raf-1 protein level in KNRK5.2 cells and to exhibit significant in vivo antitumor activities against MX-1 and A431 xenograft models. The effects of **9b** on

the Hsp90-associated proteins, such as v-src and Raf-1, suggested that it targeted Hsp90, similar to **1** and **2**.

Compound **9b**, which exhibited potent antitumor activities with no visual toxicity, is one of the most promising in the series of oxime derivatives of **1**. Further optimization of the oxime group of **2** has been carried out[31,32] and will be reported in detail in another paper.

Experimental Section

^{1}H NMR spectra were measured on JEOL JNM-GX270 and JNM-EX270 spectrometers. The spectra were referenced to Me_4Si or residual protonated solvent residues as an internal standard. Mass spectra were measured with a JEOL JMS-DX303 spectrometer. Elemental analyses were performed with a Perkin-Elmer 2400 C, H, N analyzer. For column chromatography, silica gel (SiO_2, Merck silica gel 60 or YMC GEL SIL-120-S50) was used. For further purification, preparative TLC was carried out on glass plates coated with Merck silica gel 60 F_{254}. Usual workup refers to washing of organic layers with brine, drying over anhydrous Na_2SO_4, and evaporating of the solvents under reduced pressure. ^{1}H NMR data of major *Z* isomers are only reported except for **8** and **9b**.

Radicicol 6-[*O*-(Carboxymethyl)oxime] (8). To a solution of compound **1** (5.00 g, 13.7 mmol) in Py/AcOH (5:1, 48 mL) was added carboxymethoxyamine hemihydrochloride (5.66 g, 51.8 mmol), and the mixture was then stirred at 40 °C. After 2 h, more carboxymethoxyamine hemihydrochloride (0.53 g, 4.9 mmol) was added. The mixture was stirred at 40 °C for an additional 4.5 h, then diluted with 0.5 M HCl and extracted with AcOEt, and then worked up as usual. The residue was purified by column chromatography, eluting with $CHCl_3$/MeOH (20:1 to 8:1) to give **8** as an *E/Z* mixture of 1/4 (2.82 g, 47%): ^{1}H NMR (CD_3OD, as a *Z* isomer) δ 1.53 (d, *J* = 6.6 Hz, 3H), 1.60 (ddd, *J* = 4.2, 9.0, 14.4 Hz, 1H), 2.42 (m, 1H), 3.02 (m, 1H), 3.34 (m, 1H), 3.82 (d, *J* = 16.4 Hz, 1H), 3.91 (d, *J* = 16.4 Hz, 1H), 4.64 (s, 2H), 5.31 (m, 1H), 5.61 (dd, *J* = 3.4, 10.5 Hz, 1H), 6.17 (dd, *J* = 10.5, 11.2 Hz, 1H), 6.42 (s, 1H), 6.82 (d, *J* = 16.1 Hz, 1H), 7.27 (dd, *J* = 11.2, 16.1 Hz); ^{1}H NMR (CD_3OD, as a *E* isomer) δ 1.53 (d, *J* = 6.6 Hz, 3H), 2.07 (m, 1H), 2.42 (m, 1H), 3.02 (m, 1H), 3.34 (m, 1H), 3.46 (d, *J* = 16.9 Hz, 1H), 3.72 (d, *J* = 16.9 Hz, 1H), 4.67 (s, 2H), 5.31 (m, 1H), 5.47 (dd, 1H), 6.00−6.17 (m, 2H), 6.42 (s, 1H), 7.19 (dd, 1H); FAB-MS *m/z* (M + H)$^+$ 438.

Radicicol 6-{*O*-[(Pyrrolidin-1-ylcarbonyl)methyl]oxime} (9a). To a solution of **8** (200 mg, 0.457 mmol) in DMF (2.5 mL) were added HOBt (77 mg, 0.50 mmol), EDCI (96 mg, 0.50 mmol), and pyrrolidine (0.042 mL, 0.50 mmol), and the mixture was then stirred at room temperature for 19 h. The resulting solution was diluted with 0.01 M phosphoric buffer (pH 7), extracted with AcOEt, and then worked up as usual. The residue was purified by column chromatography, eluting with $CHCl_3$/MeOH (80:1 to 50:1) to give **9a** as an *E/Z* mixture of 1/3 (109 mg, 49%): ^{1}H NMR (DMSO-d_6, as a *Z* isomer) δ 1.21−1.52 (m, 1H), 1.43 (d, *J* = 6.3 Hz, 3H), 1.70−1.91 (m, 4H), 2.43 (m, 1H), 3.05 (m, 1H), 3.27−3.54 (m, 6H), 3.80 (d, *J* = 15.8 Hz, 1H), 4.68 (s, 2H), 5.15 (m, 1H), 5.63 (dd, *J* = 3.5, 10.4 Hz, 1H), 6.23 (dd, *J* = 10.4, 11.2 Hz, 1H), 6.51 (s, 1H), 6.74 (d, *J* = 15.8 Hz, 1H), 7.14 (dd, *J* = 11.2, 15.8 Hz, 1H), 10.00 (br s, 1H), 10.34 (br s, 1H); FAB-MS *m/z* (M + H)$^+$ 491. Anal. ($C_{24}H_{27}ClN_2O_7$·$1.0H_2O$) C, H, N.

Radicicol 6-{*O*-[(Piperidinocarbonyl)methyl]oxime} (9b). This compound (0.53 g, 14%, *E/Z* = 1/2) was prepared from **8** (3.26 g, 7.45 mmol) and piperidine (1.48 mL, 15.0 mmol) in the same manner as described for **9a**: ^{1}H NMR (DMSO-d_6, as a *Z* isomer) δ 1.20−1.72 (m, 10H), 2.43 (m, 1H), 3.02 (m, 1H), 3.25−3.57 (m, 5H), 3.46 (d, *J* = 16.2 Hz, 1H), 3.80 (d, *J* = 16.2 Hz, 1H), 4.75 (s, 2H), 5.14 (m, 1H), 5.62 (dd, *J* = 3.5, 10.8 Hz, 1H), 6.22 (t, *J* = 10.9 Hz, 1H), 6.51 (s, 1H), 6.72 (d, *J* = 16.2 Hz, 1H), 7.14 (dd, *J* = 10.9, 16.2 Hz, 1H), 9.98 (s, 1H), 10.33 (s, 1H); ^{1}H NMR (DMSO-d_6, as an *E* isomer) δ 1.20−1.72 (m, 10H), 2.43 (m, 1H), 3.02 (m, 1H), 3.25−3.57 (m, 6H), 4.53 (d, *J* = 16.2 Hz, 1H), 4.82 (m, 2H), 5.14 (m, 1H), 5.49 (dd, *J* = 3.6, 10.9 Hz, 1H), 6.17 (m, 2H), 6.51 (s, 1H), 7.02 (dd, *J* = 10.9, 16.2 Hz, 1H), 10.01 (s, 1H), 10.33 (s, 1H); FAB-MS *m/z* (M + H)$^+$ 505. Anal. ($C_{25}H_{29}ClN_2O_7$·$0.2H_2O$) C, H, N.

Radicicol 6-{*O*-[(Hexamethyleneiminocarbonyl)methyl]-oxime} (9c). This compound (41 mg, 17%, *E/Z* = 1/10) was prepared from **8** (200 mg, 0.457 mmol) and hexamethyleneimine (0.062 mL, 0.55 mmol) in the same manner as described for **9a**: ^{1}H NMR ($CDCl_3$, as a *Z* isomer) δ 1.18−1.36 (m, 4H), 1.46−1.65 (m, 2H), 1.53 (d, *J* = 6.9 Hz, 3H), 1.74 (m, 2H), 1.95 (ddd, *J* = 4.0, 8.9, 15.2 Hz, 1H), 2.31 (dt, *J* = 3.6, 15.2 Hz, 1H), 2.94 (ddd, *J* = 2.3, 2.6, 8.9 Hz, 1H), 3.16 (m, 1H), 3.37−3.62 (m, 4H), 3.98 (m, 1H), 4.69 (m, 1H), 4.79 (d, *J* = 13.9 Hz, 1H), 4.85 (d, *J* = 13.9 Hz, 1H), 5.46 (m, 1H), 5.60 (dd, *J* = 3.0, 10.6 Hz, 1H), 6.09 (dd, *J* = 10.6, 11.6 Hz, 1H), 6.60 (s, 1H), 6.75 (d, *J* = 15.8 Hz, 1H), 7.14 (dd, *J* = 11.6, 15.8 Hz, 1H), 7.86 (br s, 1H), 10.78 (br, 1H); FAB-MS *m/z* (M + H)$^+$ 519. Anal. ($C_{26}H_{31}ClN_2O_7$·$1.1H_2O$) C, H, N.

Radicicol 6-{*O*-[(4-Hydroxypiperidinocarbonyl)methyl]-oxime} (9d). This compound (83 mg, 35%, *E/Z* = 1/3) was prepared from **8** (200 mg, 0.457 mmol) and 4-hydroxypiperidine (56 mg, 0.55 mmol) in the same manner as described for **9a**: ^{1}H NMR ($CDCl_3$, as a *Z* isomer) δ 1.20−1.39 (m, 4H), 1.54 (d, *J* = 6.6 Hz, 3H), 1.90 (m, 1H), 2.30 (m, 1H), 2.96 (m, 1H), 3.12−3.38 (m, 3H), 3.78 (m, 1H), 3.87−4.15 (m, 3H), 4.63 (br, 1H), 4.80 (s, 2H), 5.48 (m, 1H), 5.63 (br d, *J* = 11.2 Hz, 1H), 6.11 (dd, *J* = 10.2, 11.2 Hz, 1H), 6.57 (s, 1H), 6.70 (d, *J* = 16.2 Hz, 1H), 7.16 (m, 1H); FAB-MS *m/z* (M + H)$^+$ 521. Anal. ($C_{25}H_{29}ClN_2O_8$·$0.4H_2O$·$0.7CHCl_3$) C, H, N.

Radicicol 6-{*O*-[(4-Piperidinopiperidinocarbonyl)-methyl]oxime} (9e). This compound (31 mg, 11%, *E/Z* = 1/8) was prepared from **8** (200 mg, 0.457 mmol) and 4-piperidinopiperidine (0.092 mL, 0.55 mmol) in the same manner as described for **9a**: ^{1}H NMR ($CDCl_3$/CD_3OD, as a *Z* isomer) δ 1.25−2.02 (m, 11H), 1.44 (d, *J* = 6.6 Hz, 3H), 2.25 (m, 1H), 2.42−2.80 (m, 6H), 2.80−3.05 (m, 2H), 3.20 (br, 1H), 3.71−4.10 (m, 2H), 4.49 (d, *J* = 16.8 Hz, 1H), 4.55 (d, *J* = 16.8 Hz, 1H), 4.68 (s, 2H), 5.34 (m, 1H), 5.55 (m, 1H), 6.06 (dd, *J* = 9.9, 11.6 Hz, 1H), 6.34 (s, 1H), 6.62 (d, *J* = 15.8 Hz, 1H), 7.12 (m, 1H); FAB-MS *m/z* (M + H)$^+$ 588. Anal. ($C_{30}H_{38}ClN_3O_7$·$2.7H_2O$) C, H, N.

Radicicol 6-{*O*-[(4-Carbamoylpiperidinocarbonyl)methyl]oxime} (9f). This compound (67 mg, 27%, *E/Z* = 1/3) was prepared from **8** (200 mg, 0.457 mmol) and isonipecotamide (70 mg, 0.55 mmol) in the same manner as described for **9a**: ^{1}H NMR ($CDCl_3$/CD_3OD, as a *Z* isomer) δ 1.43−1.90 (m, 5H), 1.47 (d, *J* = 6.6 Hz, 3H), 2.20−2.44 (m, 2H), 2.66 (m, 1H), 2.82−3.12 (m, 2H), 3.17 (br s, 1H), 3.80−4.04 (m, 2H), 4.30−4.54 (m, 2H), 4.70 (s, 2H), 5.37 (m, 1H), 5.57 (dd, *J* = 3.0, 10.2 Hz, 1H), 6.06 (dd, *J* = 10.2, 11.4 Hz, 1H), 6.41 (s, 1H), 6.64 (d, *J* = 16.2 Hz, 1H), 7.13 (dd, *J* = 11.4, 16.2 Hz, 1H), 9.67 (br, 1H); FAB-MS *m/z* (M + H)$^+$ 548. Anal. ($C_{26}H_{30}ClN_3O_8$·$1.1H_2O$·$0.6CHCl_3$) C, H, N.

Radicicol 6-{*O*-[(Morpholinocarbonyl)methyl]oxime} (9g). To a solution of **8** (300 mg, 0.685 mmol) in DMF (2 mL) were added DCC (155 mg, 0.753 mmol), *N*-hydroxysuccinimide (87 mg, 0.75 mmol), and morpholine (0.090 mL, 0.75 mmol), and the mixture was then stirred at room temperature for 39 h. The resulting solution was diluted with saturated NH_4Cl, extracted with AcOEt, and then worked up as usual. The residue was purified by column chromatography, eluting with $CHCl_3$/MeOH (20:1) to give **9g** as an *E/Z* mixture of 1/4 (42 mg, 12%): ^{1}H NMR (CD_3OD, as a *Z* isomer) δ 1.52 (d, *J* = 6.9 Hz, 3H), 1.94 (m, 1H), 2.42 (ddd, *J* = 3.5, 4.0, 14.4 Hz, 1H), 3.05 (m, 1H), 3.34 (m, 1H), 3.57−3.71 (m, 8H), 3.82 (d, *J* = 16.0 Hz, 1H), 3.87 (d, *J* = 16.0 Hz, 1H), 4.82 (s, 2H), 5.30 (m, 1H), 5.62 (dd, *J* = 3.0, 10.4 Hz, 1H), 6.17 (dd, *J* = 10.4, 11.4 Hz, 1H), 6.42 (s, 1H), 6.78 (d, *J* = 16.0 Hz, 1H), 7.28 (dd, *J* = 11.4, 16.0 Hz, 1H); FAB-MS *m/z* (M + H)$^+$ 507. Anal. ($C_{26}H_{30}ClN_3O_8$·$0.5H_2O$·$0.3CHCl_3$) C, H, N.

Radicicol 6-{*O*-[(4-Methylpiperazin-1-ylcarbonyl)methyl]oxime} (9h). This compound (24 mg, 20%, *E/Z* = 1/4) was prepared from **8** (100 mg, 0.288 mmol) and 1-methylpiperazine (0.028 mL, 0.25 mmol) in the same manner as described for **9g**: ^{1}H NMR (CD_3OD, as a *Z* isomer) δ 1.52 (d, *J* = 6.4 Hz, 3H), 1.60 (m, 1H), 2.32 (s, 3H), 2.38−2.51 (m, 5H),

3.02 (m, 1H), 3.35 (m, 1H), 3.51–3.75 (m, 4H), 3.82 (d, J = 16.3 Hz, 1H), 3.94 (d, J = 16.3 Hz, 1H), 4.82 (s, 2H), 5.30 (m, 1H), 5.62 (dd, J = 4.0, 10.4 Hz, 1H), 6.17 (t, J = 10.9 Hz, 1H), 6.43 (s, 1H), 6.79 (d, J = 15.8 Hz, 1H), 7.28 (dd, J = 11.4, 15.8 Hz, 1H); FAB-MS m/z $(M + H)^+$ 520. Anal. ($C_{25}H_{30}ClN_3O_7\cdot 2.0H_2O$) C, H, N.

Radicicol 6-{*O*-[(Cyclopentylcarbamoyl)methyl]oxime} (9i). This compound (89 mg, 39%, E/Z = 1/3) was prepared from **8** (200 mg, 0.457 mmol) and cyclopentylamine (0.054 mL, 0.55 mmol) in the same manner as described for **9a**: ^1H NMR ($CDCl_3$, as a Z isomer) δ 1.38–1.75 (m, 6H), 1.55 (d, J = 6.6 Hz, 3H), 1.82–2.10 (m, 3H), 2.33 (dt, J = 3.3, 15.2 Hz, 1H), 2.96 (m, 1H), 3.19 (br s, 1H), 3.99 (d, J = 15.8 Hz, 1H), 4.26 (m, 1H), 4.58 (s, 2H), 4.67 (d, J = 15.8 Hz, 1H), 5.49 (m, 1H), 5.68 (dd, J = 3.1, 10.2 Hz, 1H), 6.14 (dd, J = 10.2, 11.4 Hz, 1H), 6.32 (d, J = 7.6 Hz, 1H), 6.61 (s, 1H), 6.67 (d, J = 16.2 Hz, 1H), 7.23 (dd, J = 11.4, 16.2 Hz, 1H), 8.50 (br, 1H), 10.75 (br, 1H); FAB-MS m/z $(M + H)^+$ 505. Anal. ($C_{25}H_{29}ClN_2O_7\cdot 1.9H_2O$) C, H. N: found 4.76, calcd 5.20.

Radicicol 6-{*O*-[(Cyclohexylcarbamoyl)methyl]oxime} (9j). This compound (118 mg, 50%, E/Z = 1/3) was prepared from **8** (200 mg, 0.457 mmol) and cyclohexylamine (0.063 mL, 0.55 mmol) in the same manner as described for **9a**: ^1H NMR ($CDCl_3$, as a Z isomer) δ 1.08–1.39 (m, 6H), 1.54 (d, J = 6.6 Hz, 3H), 1.58–1.75 (m, 2H), 1.80–2.01 (m, 3H), 2.33 (dt, J = 3.3, 14.8 Hz, 1H), 2.96 (m, 1H), 3.18 (br s, 1H), 3.80 (m, 1H), 3.97 (d, J = 14.5 Hz, 1H), 4.57 (s, 2H), 4.66 (d, J = 14.5 Hz, 1H), 5.48 (m, 1H), 5.68 (dd, J = 3.1, 10.6 Hz, 1H), 6.14 (dd, J = 10.6, 11.2 Hz, 1H), 6.27 (d, J = 8.6 Hz, 1H), 6.60 (s, 1H), 6.67 (d, J = 16.2 Hz, 1H), 7.23 (dd, J = 11.2, 16.2 Hz, 1H), 8.90 (br, 1H), 10.75 (br, 1H); FAB-MS m/z $(M + H)^+$ 519. Anal. ($C_{26}H_{31}ClN_2O_7\cdot 1.7H_2O$) C, H, N.

Radicicol 6-{*O*-[((Cyclohexylmethyl)carbamoyl)methyl]-oxime} (9k). This compound (40 mg, 16%, E/Z = 1/4) was prepared from **8** (200 mg, 0.457 mmol) and (aminomethyl)-cyclohexane (0.071 mL, 0.55 mmol) in the same manner as described for **9a**: ^1H NMR ($CDCl_3$, as a Z isomer) δ 1.10–1.34 (m, 4H), 1.49 (m, 1H), 1.56 (d, J = 6.6 Hz, 3H), 1.60–1.80 (m, 6H), 1.97 (m, 1H), 2.35 (dt, J = 3.3, 15.2 Hz, 1H), 2.99 (m, 1H), 3.05–3.27 (m, 3H), 4.04 (d, J = 14.2 Hz, 1H), 4.61 (s, 2H), 4.70 (d, J = 14.2 Hz, 1H), 5.50 (m, 1H), 5.69 (dd, J = 3.1, 10.6 Hz, 1H), 6.16 (dd, J = 10.6, 11.2 Hz, 1H), 6.42 (t, J = 6.1 Hz, 1H), 6.61 (s, 1H), 6.68 (d, J = 16.0 Hz, 1H), 7.24 (dd, J = 11.2, 16.0 Hz, 1H), 8.17 (br, 1H), 10.75 (br, 1H); FAB-MS m/z $(M + H)^+$ 533. Anal. ($C_{27}H_{33}ClN_2O_7\cdot 0.6H_2O$) C, N. H: found 6.94, calcd 6.34.

Radicicol 6-{*O*-[((2-Hydroxyethyl)carbamoyl)methyl]-oxime} (9l). To a solution of **8** (200 mg, 0.457 mmol) in DMF (1 mL) were added EDCI (88 mg, 0.46 mmol), ethanolamine (0.025 mL, 0.46 mmol), and 4-(dimethylamino)pyridine (DMAP) (56 mg, 0.46 mmol), and the mixture was then stirred at room temperature for 25 h. The resulting solution was evaporated under reduced pressure. The residue was purified by column chromatography, eluting with $CHCl_3$/MeOH (20:1 to 9:1) to give **9l** as an E/Z mixture of 1/5 (87 mg, 40%): ^1H NMR (CD_3OD, as a Z isomer) δ 1.52 (d, J = 6.4 Hz, 3H), 1.61 (m, 1H), 2.43 (m, 1H), 3.02 (m, 1H), 3.31 (m, 1H), 3.31–3.50 (m, 2H), 3.54–3.70 (m, 2H), 3.85 (d, J = 16.3 Hz, 1H), 3.96 (d, J = 16.3 Hz, 1H), 4.58 (s, 2H), 5.31 (m, 1H), 5.63 (dd, J = 3.0, 10.9 Hz, 1H), 6.19 (dd, J = 10.9, 11.4 Hz, 1H), 6.44 (s, 1H), 6.85 (d, J = 16.0 Hz, 1H), 7.30 (dd, J = 11.4, 16.0 Hz, 1H); FAB-MS m/z $(M + H)^+$ 481. Anal. ($C_{22}H_{25}ClN_2O_8\cdot 1.4H_2O$) C, H, N.

Radicicol 6-{*O*-[(Bis(2-hydroxyethyl)carbamoyl)methyl]-oxime} (9m). This compound (45 mg, 13%, E/Z = 1/5) was prepared from **8** (300 mg, 0.685 mmol) and diethanolamine (97 mg, 0.69 mmol) in the same manner as described for **9l**: ^1H NMR (CD_3OD, as a Z isomer) δ 1.53 (d, J = 6.4 Hz, 3H), 1.60 (m, 1H), 2.44 (dt, J = 3.5, 14.3 Hz, 1H), 3.00 (m, 1H), 3.34 (m, 1H), 3.42–3.64 (m, 4H), 3.64–3.90 (m, 4H), 3.82 (d, J = 16.3 Hz, 1H), 3.92 (d, J = 16.3 Hz, 1H), 4.91 (s, 2H), 5.29 (m, 1H), 5.60 (dd, J = 3.5, 10.4 Hz, 1H), 6.17 (dd, J = 10.4, 10.9 Hz, 1H), 6.42 (s, 1H), 6.83 (d, J = 15.8 Hz, 1H), 7.26 (dd, J = 10.9, 15.8 Hz, 1H); FAB-MS m/z $(M + H)^+$ 525. Anal. ($C_{24}H_{29}ClN_2O_9\cdot 1.6H_2O$) C, H, N.

Radicicol 6-{*O*-[((2-Ethoxyethyl)carbamoyl)methyl]-oxime} (9n). This compound (11 mg, 5%, E/Z = 1/10) was prepared from **8** (200 mg, 0.457 mmol) and 2-ethoxyethylamine (0.062 mL, 0.55 mmol) in the same manner as described for **9a**: ^1H NMR ($CDCl_3$, as a Z isomer) δ 1.14 (t, J = 7.1 Hz, 3H), 1.58 (d, J = 6.6 Hz, 3H), 2.00 (ddd, J = 4.0, 8.6, 15.2 Hz, 1H), 2.36 (ddd, J = 3.3, 3.6, 15.2 Hz, 1H), 2.99 (ddd, J = 2.3, 2.6, 8.6 Hz, 1H), 3.22 (br s, 1H), 3.40–3.61 (m, 6H), 4.10 (br d, 1H), 4.64 (s, 2H), 4.75 (br d, 1H), 5.51 (m, 1H), 5.70 (dd, J = 3.1, 10.4 Hz, 1H), 6.16 (dd, J = 10.4, 11.2 Hz, 1H), 6.60 (s, 1H), 6.67 (br, 1H), 6.69 (d, J = 16.2 Hz, 1H), 6.84 (br, 1H), 7.23 (dd, J = 11.2, 16.2 Hz, 1H), 10.76 (br, 1H); FAB-MS m/z $(M + H)^+$ 509.

Radicicol 6-{*O*-[((2-(Diethylamino)ethyl)carbamoyl)-methyl]oxime} (9o). This compound (46 mg, 37%, E/Z = 1/3) was prepared from **8** (100 mg, 0.288 mmol) and *N*,*N*-diethyl-ethylenediamine (0.035 mL, 0.25 mmol) in the same manner as described for **9g**: ^1H NMR (CD_3OD, as a Z isomer) δ 1.13 (t, J = 7.2 Hz, 6H), 1.51 (d, J = 6.4 Hz, 3H), 1.67 (m, 1H), 2.60 (m, 1H), 2.73–2.90 (m, 6H), 3.00 (m, 1H), 3.36 (m, 1H), 3.46 (m, 2H), 3.96 (br s, 2H), 4.58 (s, 2H), 5.30 (m, 1H), 5.63 (dd, J = 3.5, 10.9 Hz, 1H), 6.18 (t, J = 10.9 Hz, 1H), 6.39 (s, 1H), 6.84 (d, J = 16.0 Hz, 1H), 7.30 (dd, J = 11.4, 16.0 Hz, 1H); FAB-MS m/z $(M + H)^+$ 537. Anal. ($C_{26}H_{34}ClN_3O_7\cdot 0.4H_2O\cdot 0.2CHCl_3$) C, H. N: found 5.29, calcd 7.41.

Radicicol 6-{*O*-[((Methoxycarbonylmethyl)carbamoyl)-methyl]oxime} (9p). This compound (89 mg, 39%, E/Z = 1/4) was prepared from **8** (200 mg, 0.457 mmol) and glycine methyl ester hydrochloride (97 mg, 0.50 mmol) in the same manner as described for **9l**: ^1H NMR (CD_3OD, as a Z isomer) δ 1.53 (d, J = 6.4 Hz, 3H), 1.65 (m, 1H), 2.44 (dt, J = 3.5, 14.3 Hz, 1H), 3.03 (m, 1H), 3.36 (m, 1H), 3.73 (s, 3H), 3.83 (d, J = 16.3 Hz, 1H), 3.96 (d, J = 16.3 Hz, 1H), 4.02 (d, J = 2.5 Hz, 2H), 4.63 (s, 2H), 5.31 (m, 1H), 5.63 (dd, J = 4.0, 10.4 Hz, 1H), 6.21 (dd, J = 10.4, 10.9 Hz, 1H), 6.43 (s, 1H), 6.87 (d, J = 15.8 Hz, 1H), 7.30 (dd, J = 10.9, 15.8 Hz, 1H); FAB-MS m/z $(M + H)^+$ 509. Anal. ($C_{23}H_{25}ClN_2O_9\cdot 0.8H_2O$) C, H, N.

Radicicol 6-{*O*-[(Phenylcarbamoyl)methyl]oxime} (9q). This compound (16 mg, 7%, E/Z = 1/3) was prepared from **8** (200 mg, 0.457 mmol) and aniline (0.050 mL, 0.55 mmol) in the same manner as described for **9a**: ^1H NMR ($CDCl_3$, as a Z isomer) δ 1.57 (d, J = 6.9 Hz, 3H), 1.99 (ddd, J = 4.0, 8.6, 15.2 Hz, 1H), 2.36 (ddd, J = 3.3, 3.6, 15.2 Hz, 1H), 2.99 (ddd, J = 2.6, 3.3, 8.6 Hz, 1H), 3.23 (br s, 1H), 4.12 (br d, J = 15.2 Hz, 1H), 4.73 (s, 2H), 4.80 (br, 1H), 5.51 (m, 1H), 5.74 (dd, J = 3.6, 10.2 Hz, 1H), 6.21 (dd, J = 10.2, 11.6 Hz, 1H), 6.61 (s, 1H), 6.77 (d, J = 16.2 Hz, 1H), 7.07–7.20 (m, 2H), 7.25–7.37 (m, 3H), 7.45–7.58 (m, 2H), 7.97 (s, 1H), 10.76 (br, 1H); FAB-MS m/z $(M + H)^+$ 513. Anal. ($C_{26}H_{25}ClN_2O_7\cdot 0.4H_2O$) C, H. N: found 6.11, calcd 5.39.

Radicicol 6-{*O*-[((4-Methoxyphenyl)carbamoyl)methyl]-oxime} (9r). This compound (123 mg, 50%, E/Z = 1/3) was prepared from **8** (200 mg, 0.457 mmol) and *p*-anisidine (68 mg, 0.55 mmol) in the same manner as described for **9a**: ^1H NMR ($CDCl_3$, as a Z isomer) δ 1.53 (d, J = 6.9 Hz, 3H), 1.94 (ddd, J = 4.0, 8.9, 15.2 Hz, 1H), 2.33 (ddd, J = 3.3, 3.6, 15.2 Hz, 1H), 2.96 (ddd, J = 2.3, 3.6, 8.9 Hz, 1H), 3.20 (br s, 1H), 3.75 (s, 3H), 4.04 (d, J = 14.5 Hz, 1H), 4.67 (d, J = 14.5 Hz, 1H), 4.70 (s, 2H), 5.47 (m, 1H), 5.69 (dd, J = 3.3, 10.6 Hz, 1H), 6.16 (dd, J = 10.6, 11.5 Hz, 1H), 6.56 (s, 1H), 6.75 (d, J = 16.2 Hz, 1H), 6.83 (d, J = 9.1 Hz, 2H), 7.26 (dd, J = 11.5, 16.2 Hz, 1H), 7.42 (d, J = 9.1 Hz, 2H), 7.96 (s, 1H), 10.60 (br, 1H); FAB-MS m/z $(M + H)^+$ 543. Anal. ($C_{27}H_{27}ClN_2O_8\cdot 2.1H_2O$) C, H, N.

Radicicol 6-{*O*-[((4-(Diethylamino)phenyl)carbamoyl)-methyl]oxime} (9s). This compound (80 mg, 30%, E/Z = 1/3)-was prepared from **8** (200 mg, 0.457 mmol) and *N*,*N*-diethyl-1,4-phenylenediamine (0.091 mL, 0.55 mmol) in the same manner as described for **9a**: ^1H NMR ($CDCl_3$, as a Z isomer) δ 1.12 (t, J = 7.1 Hz, 6H), 1.55 (d, J = 6.6 Hz, 3H), 1.96 (ddd, J = 4.0, 8.9, 15.2 Hz, 1H), 2.34 (ddd, J = 3.3, 3.6, 15.2 Hz, 1H), 2.99 (ddd, J = 2.3, 2.6, 8.9 Hz, 1H), 3.21 (br s, 1H), 3.31 (q, J = 7.1 Hz, 4H), 4.04 (d, J = 16.8 Hz, 1H), 4.71 (s, 2H), 4.72 (d, J = 16.8 Hz, 1H), 5.48 (m, 1H), 5.71 (dd, J = 3.0, 10.2 Hz, 1H), 6.18 (dd, J = 10.2, 11.9 Hz, 1H), 6.58 (s, 1H), 6.64 (d,

J = 8.9 Hz, 2H), 6.75 (d, J = 16.2 Hz, 1H), 7.27 (m, 1H), 7.32 (d, J = 8.9 Hz, 2H), 7.85 (s, 1H); FAB-MS m/z $(M + H)^+$ 584. Anal. ($C_{30}H_{34}ClN_3O_7$·1.2H_2O) C, N. H: found 6.83, calcd 6.06.

Radicicol 6-{*O*-[(Pyrid-3-ylcarbamoyl)methyl]oxime} (9t). This compound (46 mg, 20%, E/Z = 1/3) was prepared from **8** (200 mg, 0.457 mmol) and 3-aminopyridine (52 mg, 0.55 mmol) in the same manner as described for **9a**: ^1H NMR ($CDCl_3$, as a Z isomer) δ 1.56 (d, J = 6.9 Hz, 3H), 1.97 (ddd, J = 4.0, 8.9, 15.2 Hz, 1H), 2.34 (dt, J = 3.3, 15.2 Hz, 1H), 2.97 (m, 1H), 3.20 (br, 1H), 4.03 (d, J = 16.5 Hz, 1H), 4.70 (d, J = 16.5 Hz, 1H), 4.76 (m, 1H), 4.77 (d, J = 16.5 Hz, 1H), 5.48 (m, 1H), 5.72 (dd, J = 3.0, 10.2 Hz, 1H), 6.17 (dd, J = 10.2, 10.9 Hz, 1H), 6.52 (s, 1H), 6.76 (d, J = 16.2 Hz, 1H), 7.28−7.44 (m, 2H), 8.25−8.40 (m, 2H), 8.46 (m, 1H), 8.56 (d, J = 7.9 Hz, 1H); FAB-MS m/z $(M + H)^+$ 514. Anal. ($C_{25}H_{24}ClN_3O_7$·0.2H_2O·0.4$CHCl_3$) C, H. N: found 6.79, calcd 7.43.

Radicicol 6-{*O*-[((Pyrid-3-ylmethyl)carbamoyl)methyl]-oxime} (9u). This compound (106 mg, 44%, E/Z = 1/3) was prepared from **8** (200 mg, 0.457 mmol) and 3-(aminomethyl)-pyridine (0.051 mL, 0.55 mmol) in the same manner as described for **9a**: ^1H NMR ($CDCl_3$, as a Z isomer) δ 1.52 (d, J = 6.9 Hz, 3H), 1.94 (m, 1H), 2.31 (dt, J = 3.3, 15.2 Hz, 1H), 2.94 (m, 1H), 3.15 (br s, 1H), 3.90 (d, J = 15.2 Hz, 1H), 4.48−4.68 (m, 5H), 5.44 (m, 1H), 5.67 (dd, J = 3.0, 10.2 Hz, 1H), 6.10 (dd, J = 10.2, 11.2 Hz, 1H), 6.46 (s, 1H), 6.65 (d, J = 16.2 Hz, 1H), 6.93 (t, J = 6.3 Hz, 1H), 7.20 (dd, J = 11.2, 16.2 Hz, 1H), 7.36 (dd, J = 4.6, 7.9 Hz, 1H), 7.81 (ddd, J = 1.7, 2.0, 7.9 Hz, 1H), 8.47 (d, J = 4.6 Hz, 1H), 8.57 (s, 1H); FAB-MS m/z $(M + H)^+$ 528. Anal. ($C_{26}H_{26}ClN_3O_7$·0.8H_2O·0.5$CHCl_3$) C, H. N: found 6.29, calcd 6.98.

Radicicol 6-{*O*-[(2-(1-Methyl-1*H*-pyrrol-2-yl)ethylcarbamoyl)methyl]oxime} (9v). This compound (97 mg, 39%, E/Z = 1/3) was prepared from **8** (200 mg, 0.457 mmol) and 2-(2-aminoethyl)-1-methylpyrrole (0.069 mL, 0.55 mmol) in the same manner as described for **9a**: ^1H NMR ($CDCl_3$, as a Z isomer) δ 1.55 (d, J = 6.6 Hz, 3H), 1.96 (ddd, J = 4.0, 8.9, 16.2 Hz, 1H), 2.34 (dt, J = 3.3, 16.2 Hz, 1H), 2.81 (m, 2H), 3.00 (m, 1H), 3.23 (br s, 1H), 3.41−3.60 (m, 2H), 3.54 (s, 3H), 4.06 (br d, 1H), 4.53−4.70 (m, 3H), 5.47 (m, 1H), 5.68 (dd, J = 3.0, 10.2 Hz, 1H), 5.85−5.98 (m, 2H), 6.15 (dd, J = 10.2, 11.2 Hz, 1H), 6.46−6.70 (m, 3H), 6.60 (s, 1H), 7.22 (dd, J = 11.2, 15.8 Hz, 1H), 9.02 (br, 1H), 10.70 (br, 1H); FAB-MS m/z $(M + H)^+$ 544. Anal. ($C_{27}H_{30}ClN_3O_7$·0.4H_2O) C, H, N.

Antiproliferative Assays. 3Y1-B, SR-3Y1, NRK, and KNRK5.2 cell lines were obtained as described previously,[7] and the cell cultures were performed at 37°C in a humidified atmosphere of 5% CO_2. The cells were precultured in Dulbecco's modified Eagle's medium containing 10% fetal calf serum for 24 h in 96-well microwell plates (Nunc) and then were incubated with each drug for another 72 h. The cell viability was determined using an MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay.[7] IC_{50} is defined as the concentration of drug required for 50% inhibition of cell growth.

Src Kinase Assays. SR-3Y1 cells were incubated with each drug for 15 h and were lysed for 20 min by the addition of ice-cold lysis buffer [50 mM Tris HCl (pH 7.5), 150 mM NaCl, 1% Triton X-100, 0.1% sodium dodecyl sulfate, 1% sodium deoxycholate, 2 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 20 μM leupeptin, 0.15 unit/mL aprotinin, 1 mM Na_3VO_4] on ice. The cell lysate was clarified by centrifugation (10 min at 14 000 rpm). The cell lysate adjusted to a certain amount of proteins was electrophoresed by SDS−PAGE, transferred to a nitrocellulose membrane, and immunoblotted with primary mouse polyclonal phosphotyrosine antibody MX-pTYR (Kyowa Medex). For detection, the blots were incubated with secondary mouse IgG antibody conjugated with horseradish peroxidase (BIO-RAD), and tyrosine-phosphorylated protein was developed using an enhanced chemiluminesence detection system (Amersham) according to the instructions of the manufacturer. IC_{50} is defined as the concentration of drug required for 50% inhibition of tyrosine phosphorylation.

Raf-1 Depletion and Phosphorylated-Erk2 Inhibition Assays. KNRK5.2 cells were incubated with 0.2 μM selected drug for 40 h. After the cells were lysed, the resulting cell lysate, which was adjusted to a certain amount of proteins, was analyzed by Western blot using anti-Raf-1 rabbit polyclonal antibody (C-12, Santa Cruz), phosphospecific MAPK antibody (New England Biolabs), and anti-Erk2 monoclonal antibody (clone 1B3B9; Upstate Biotechnology) in duplicate, in the same manner as described previously.[7]

In Vivo Antitumor Assays. MX-1 and A431 cell lines were obtained as described previously.[7] These tumors were inoculated subcutaneously in the flanks of adult BALB/c nu/nu mice. The drug was dissolved in a solution of cremophor EL, *N,N*-dimethylacetamide, and physiological saline (7.5:5:87.5) and was administered daily by iv injections for 5 days from day 0 to day 4. MX-1 and A431 cells were transplanted on days −14 and −18, respectively.

For the evaluation of antitumor activity, the length and width of the tumors were measured and the tumor volume was calculated using the following formula, according to the method of the National Cancer Institute.[33]

$$\text{tumor volume (mm}^3) = \frac{\text{length (mm)} \times [\text{width (mm)}]^2}{2}$$

Drug efficacy was expressed as the ratio of the mean experimental V/V_0 value to that of the control group (T/C ratio), where V is the tumor volume at the day of evaluation and V_0 is the tumor volume at the day of the initial treatment with the drug. The criterion for effectiveness is a T/C ratio of 0.50 or less, and statistical significance was determined by the Mann−Whitney U test.

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Supporting Information Available: HPLC analytical data of compounds **9i**,**k**,**n**,**o**,**q**,**s**,**t**,**u**. This material is available free of charge via the Internet at http://pubs.acs.org.

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