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Halohydrin and Oxime Derivatives of Radicicol: Synthesis and Antitumor Activities

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Abstract—Novel halohydrin and oxime derivatives of radicicol (**1**) were prepared and evaluated for their v-src tyrosine kinase inhibitory, antiproliferative, and antitumor activities. Some of the resulting derivatives showed significantly improved antitumor activities than those of **1** in vitro as tested in a cell proliferation assay and in vivo using sc-inoculated human breast carcinoma and epidermoid tumor models. Design and synthesis of radicicol-based novel affinity probes are also described.
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Introduction

Radicicol,[1] also known as monorden,[2] a macrocyclic lactone antibiotic originally isolated from the fungus *Monosporium bonorden*,[3] has been received much attention because of its interesting biological activities, such as inhibition of $p60^{v\text{-}src}$ protein tyrosine kinase,[4] induction of differentiation of HL-60 cells to macrophage-like cells,[5] and in vivo angiogenesis inhibition.[6] More recently, we and others have reported that radicicol has the ability to suppress the morphological transformation of tumor phenotype by diverse oncogenes such as src,[7] ras, and mos.[8] We also revealed that radicicol leads to selective depletion of Raf-1 protein and subsequent inhibition of MAPK pathway in K-ras-transformed cells.[9] Because of its important biological activities, intensive chemical research has been initiated to establish the fundamental structure–activity relationship, to provide more biologically active analogues, and to elucidate the precise mechanism of actions (Fig. 1).

Kwon et al. reported that inhibitory effects of radicicol on the tyrosine kinase was abolished by reducing agents, such as dithiothreitol (DTT).[4] Therefore, weak antitumor activity of radicicol might be due to lack of stability because of the presence of epoxy and α,β,γ,δ-unsaturated carbonyl groups which are highly reactive to nucleophiles. In fact, model reaction of radicicol with DTT afforded 1,6-adduct of DTT to dienone moiety (Scheme 1). Increased chemical stability may be a key to improving not only its biological activity, but also its pharmacological behavior such as pharmacokinetics. Thus, we focused on the modification of these two functional groups to overcome the instability.

Results and Discussion

Synthesis of halohydrin derivatives of radicicol

We prepared the halohydrin derivatives, which were expected to revert to the epoxide under cell culture or administrative conditions, and examined their stability as well as their antiproliferative activities. Treatment of radicicol with two equivalents of phosphorus oxychloride or oxalyl chloride in DMF gave the chlorohydrin formate **3a** in moderate yield. Simple chlorohydrin **3b** and bromohydrin **3c** were obtained by the treatment of radicicol with concd HCl or concd HBr in dioxane, respectively. Chlorohydrin **3b** was found to be somewhat less stable than corresponding formyl ester **3a** and to slowly convert to 3-chloro-2,3-dihydro-6*H*-pyran derivative **4** at room temperature even in a solid state. Unexpectedly, treatment of radicicol with thionyl

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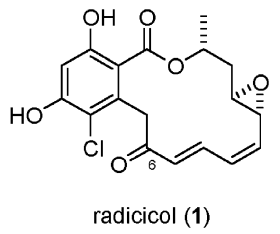


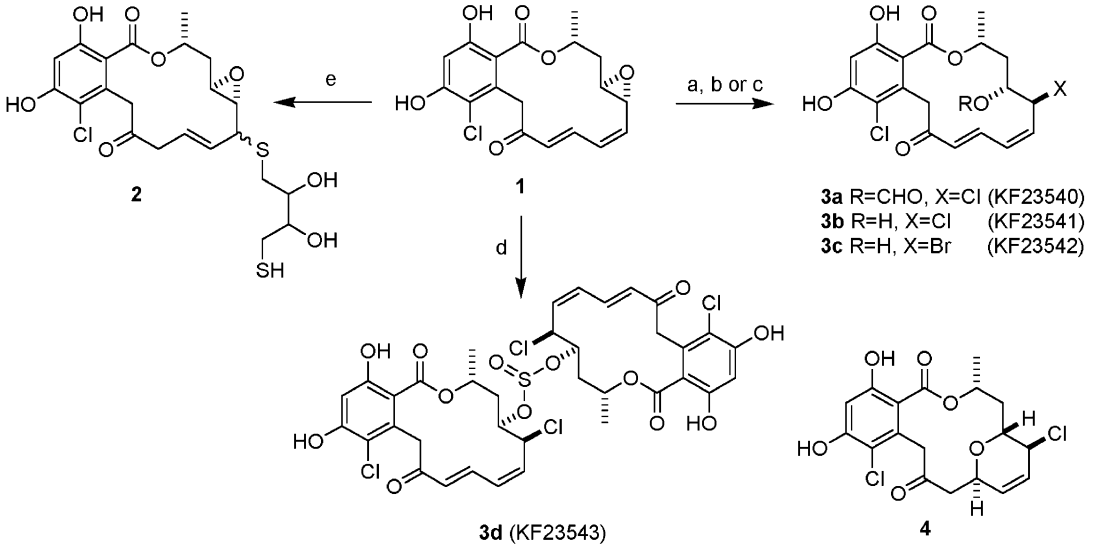
Figure 1. Structure of radicicol (**1**).

chloride in DMF gave the sulfite ester **3d** which was more stable than corresponding chlorohydrin **3b**.

To examine the conversion of halohydrins to parent epoxide **1**, compounds were incubated in 10 mM phosphate buffer (pH 7.25) and analyzed by HPLC. The results showed that bromohydrin **3c** was smoothly converted to the epoxide **1** within 1 h (Fig. 2b). Formyl ester **3a** was hydrolyzed to chlorohydrin **3b** and then converted to the epoxide **1** (Fig. 2a), while sulfite dimer **3d** was much more stable than the other halohydrins (data not shown). Similar biological activity of bromohydrin **3c** with parent compound in a cell proliferation assay is consistent with the rapid conversion to epoxide. On the other hand, formyl ester **3a**, chlorohydrin **3b**, and sulfite dimer **3d** were less active than the parent epoxide **1**. These data suggest that halohydrins themselves have little or no biological activities, and their observed activities are due to the conversion to the parent compound.

Synthesis of oxime derivatives of radicicol

As mentioned above, radicicol lacks stability in the presence of nucleophiles such as DTT. Kanda and Fukuyama reported that Michael addition of nucleophile to the conjugated carbonyl group could be prevented by the protection as a less electrophilic oxime.[10] Oxime derivatives of **1** were obtained by the treatment of **1** with hydroxylamine or *O*-alkyl hydroxylamines (Scheme 2). Transformation of α,β,γ,δ-conjugated carbonyl to oxime by the action of free hydroxylamine (hydroxylamine hydrochloride in the presence of pyridine) led to **5a** in low yields because of the formation of **6a**. The major product found in this reaction was 1,4-addition product at the β-position, and its structure including stereochemistry was confirmed by COSY and NOESY



Scheme 1. Reagents and conditions: (a) $POCl_3$, DMF, 0 °C to rt, 24 h, 51%; (b) concd HCl, dioxane, 0 °C to rt, 6 h, 45%; (c) concd HBr, dioxane, 0 °C to rt, 6 h, 56%; (d) $SOCl_2$, DMF, 0 °C to rt, 12 h, 62%; (e) DTT, 50% DMSO, 37 °C, 2 h.

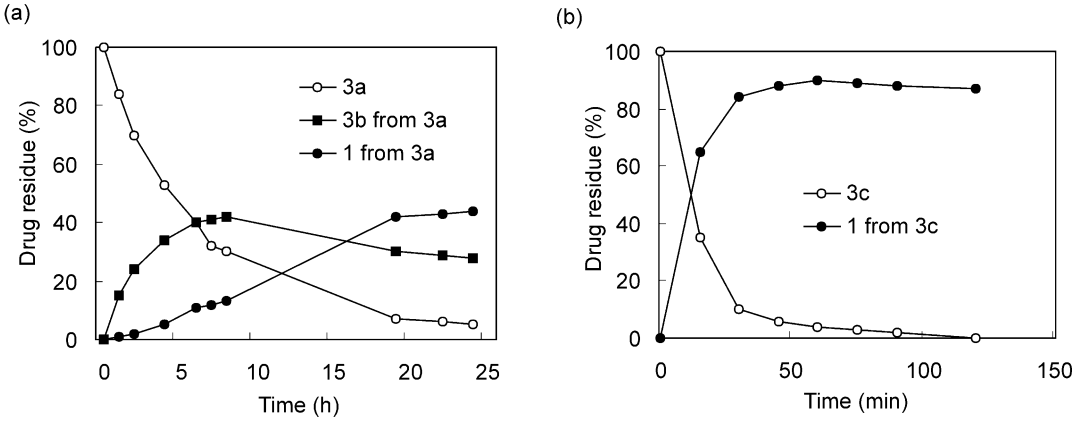
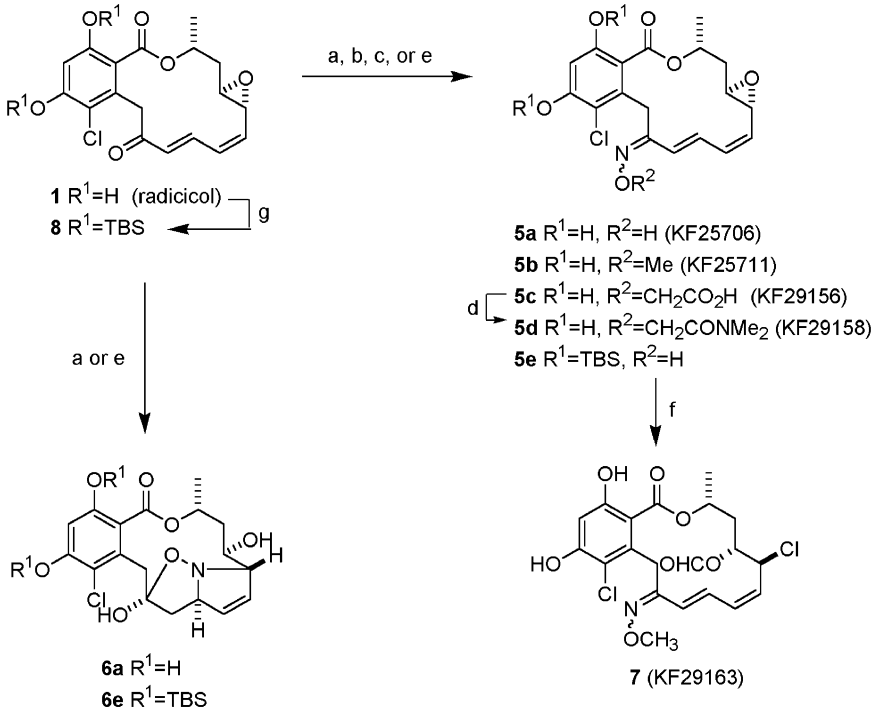


Figure 2. Time course of conversion of halohydrins to radicicol (**1**).



Scheme 2. Reagents and conditions: (a) $NH_2OH{\cdot}HCl$, pyridine, 50 °C, 8 h, 23% for **5a** and 31% for **6a**, respectively; (b) $NH_2OMe{\cdot}HCl$, pyridine, 80 °C, 1.5 h, 16%; (c) $NH_2OCH_2CO_2H{\cdot}1/2HCl$, pyridine, 60 °C, 1.5 h, 39%; (d) *N*-hydroxysuccinimide, DCC, DMAP, THF, rt, 2 h; then, $Me_2NH{\cdot}HCl$, Et_3N, CH_2Cl_2, rt, 12 h, 36%; (e) $NH_2OH{\cdot}HCl$, pyridine, CH_2Cl_2, 65 °C, 10 h, 24% for **5e** and 9.7% for **6e**, respectively; (f) $(COCl)_2$, DMF, 0 °C to rt, 15 h, 22%; (g) TBSCl, imidazole, DMF, rt, 24 h, 99%.

data of corresponding di-TBS ether **6e**. In attempts to modify the reactivity of hydroxylamine and minimize the formation of adduct, reaction was carried out with no added base other than excess hydroxylamine hydrochloride in methanol or tetrahydrofuran. However, no oxime formation was detected in latter cases.

Hydroxime **5a**, methoxime **5b** and carboxymethoxime **5c** were synthesized with usual manner, and **5c** was further converted to dimethylcarbamoylmethyloxime **5d**. All oxime derivatives were obtained as the mixtures of *E*- and *Z*-isomers at the C=N double bonds and each isomers were found to be difficult to separate. Halohydrin–oxime hybrid **7** was obtained by treatment of **5b** with oxalyl chloride in DMF.

We compared the stability of oxime derivatives with that of parent compound in mouse serum and DTT solution (Fig. 3). In mouse serum, although half-life of radicicol was approximately 2 h, remarkable improvement of half-life was observed in oxime derivative **5b** ($t_{1/2} > 24$ h). Although radicicol completely disappeared within 15 min in the presence of 100 mM DTT, approximately 60% of methoxime existed even after 120 min incubation. These data suggest the potential advantage of oxime derivatives in stability.

Biological evaluation of radicicol derivatives

Inhibitory activities on the v-src tyrosine kinase, antiproliferative activities against v-src transformed 3Y1

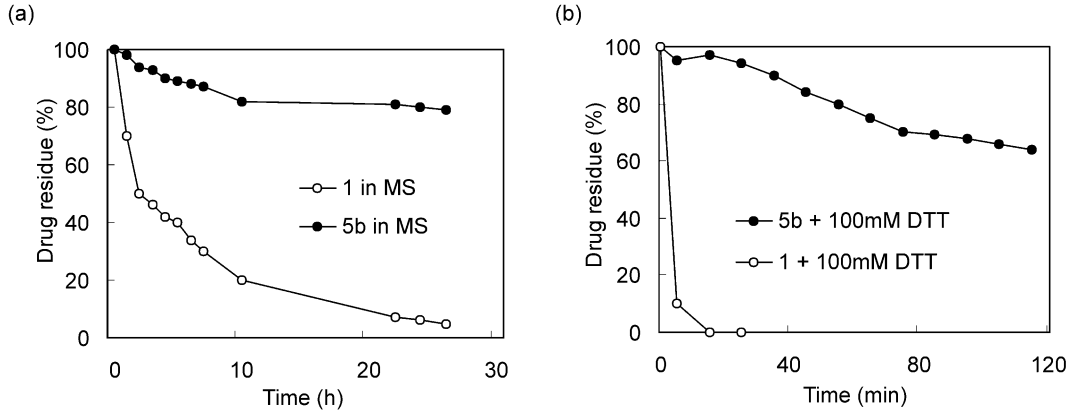


Figure 3. Comparison of the stability of radicicol (**1**) and 6-oxime (**5b**) in 10% DMSO-mouse serum (a) and in 100 mM DTT/10% DMSO–10 mM phosphate buffer solution (b).

cells (SR-3Y1), and antitumor activities against sc-inoculated human breast carcinoma cells (MX-1 and MCF-7) and human epidermoid cells (A431) are shown in Table 1. In halohydrin derivatives, antiproliferative activities against SR-3Y1 cells seemed to be consistent with the inhibitory activities against v-src tyrosine kinase. Although bromohydrin **3c** had comparable potency with parent compound in a cell proliferation assay due to the rapid conversion to parent epoxide **1**, more stable formyl ester **3a** and sulfite ester **3d** had less activities. Less antiproliferative activity found in derivative **3b** seemed to be due to another degradative pathway (e.g., to **4**).

Three oxime derivatives (**5a**, **5b**, and **5d**) showed more potent activities than parent compound in antiproliferative assay as well as in tyrosine kinase assay. Analogues **3a**, **5a**, **5b**, **5d** and **7** were evaluated in vivo using sc-inoculated human breast carcinoma (MX-1 and MCF-7) and epidermoid (A431) tumor models. The in vivo activity of halohydrin **3a** was equivalent to that observed in **1** and consider inactive (T/C values greater than 0.50). All three oximes showed in vivo activity in the MX-1 xenograft tumor model. In particular, hydroxime **5a** showed most potent antitumor activities in all models. No activity was observed in halohydrin–oxime hybrid **7**.

Synthesis of radicicol-based affinity probes

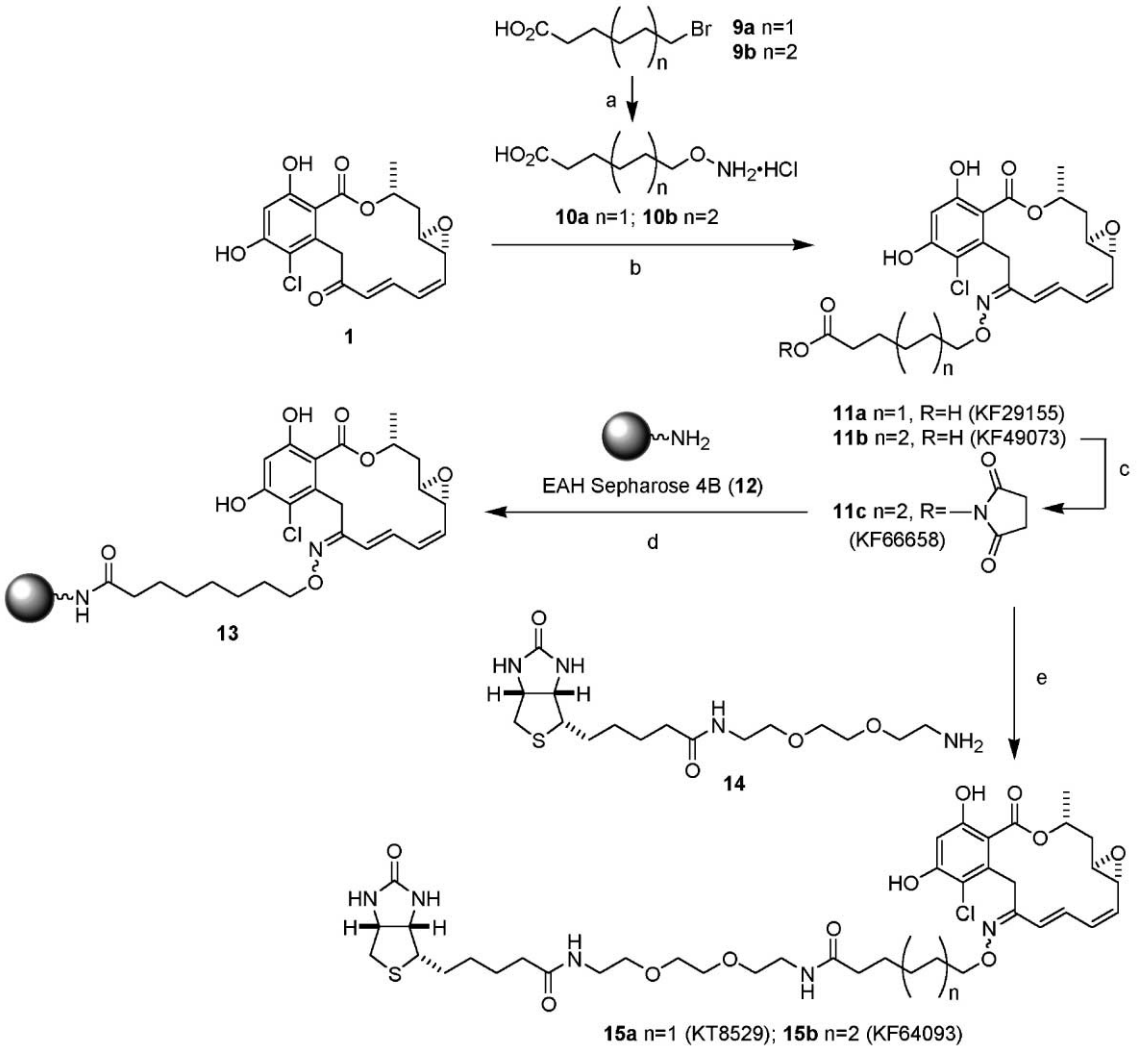
Identification of the molecular target is necessary for understanding the precise mechanism of actions for radicicol's various biological activities. We therefore attempted to design and synthesis of the radicicol-based affinity probes. The biotinylation or immobilization of a ligand is widely used in biochemistry and molecular biology for identification or purification of the specific binding protein.[11]

Table 1. Tyrosine kinase inhibition, antiproliferative activities and in vivo antitumor activities for compounds **1**–**7**

Compd	IC_{50} (μM)		T/C (dose, mg/kg)[c]		
	v-src[a]	SR-3Y1[b]	MX-1	MCF-7	A431
1	0.18	0.070	0.71 (100)	0.51 (150)	0.59 (150)
3a	2.5	0.17	0.61 (75)	0.78 (75)	0.89 (75)
3b	1.8	6.8	na[d]	na	na
3c	0.82	0.080	na	na	na
3d	3.0	0.64	na	na	na
5a	<0.050	0.084	0.24 (150)	0.39 (150)	0.38 (150)
5b	<0.050	0.033	0.41 (150)	0.44 (150)	0.58 (150)
5c	>5.0	na	na	na	na
5d	0.24	0.015	0.36 (150)	0.54 (150)	0.57 (150)
7	>5.0	0.35	0.93 (150)	0.89 (150)	0.94 (150)

[a]Inhibition of v-src induced tyrosine phosphorylation in SR-3Y1 cells was determined by Western blotting method using anti-phosphotyrosine antibody as described in ref 30.
[b]Antiproliferative activity against SR-3Y1 cells was determined by microculture tetrazolium (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Sigma Chemical Co.) assay. The cells were precultured for 24 h in 96-well microwell plates, and treated with the compounds for 72 h.
[c]MX-1 (8 mm^3), MCF-7 (27 mm^3), and A431 (8 mm^3) cells were inoculated sc on days −12, −20, and −18, respectively. Drugs were administered by five consecutive sc injections from day 0. Tumor sizes on day 0 were 145.0±41.3 (MX-1), 124.4±37.8 (MCF-7), and 206.8±76.7 (A431) mm^3, respectively.
[d]na, not available.

Some oxime derivatives of radicicol showed improved antiproliferative activities in cell-based assay as well as antitumor activities in animal models. Guided by these results, we have designed the *O*-alkylated oxime derivatives **11a** and **11b** for ligands those have carboxyl functionalities to covalently couple to a solid support or conjugate to a biotin reagent. Aliphatic linkers of suitable length to overcome any effects of steric hindrance and amide-forming transformations were used in the preparation of affinity probes. Bi-functional cross-linking agents, 6-(aminooxy)hexanoic acid (**10a**) and 8-(aminooxy)octanoic acid (**10b**), were prepared from 6-bromohexanoic acid (**9a**) and 8-bromooctanoic acid (**9b**) in four steps by protection of carboxyl group as *tert*-butyl ester, introduction of *N*-hydroxyphthalimidyl group, removing phthalimidyl group by hydrazine, and final deprotection of *tert*-butyl ester with trifluoroacetic acid. Two radicicol-based ligands **11a** and **11b**, which have 6- or 8-carbon spacer arms at the C-6 position via the oxime linkage, were obtained by the condensation of radicicol with thus obtained cross-linkers. These ligands have proved to possess comparable potency with radicicol **1** in antiproliferative assay (IC_{50} values against SR-3Y1 cells were 0.39 μM for **11a** and 0.066 μM for **11b**, respectively). Immobilization of radicicol on the solid support was achieved by the following manner; carboxyl functional group of **11b** was activated by the transformation to *N*-hydroxysuccinimidyl ester **11c** and coupled to EAH Sepharose beads **12** to yield **13**.[12] On the other hands, **11a** and **11b** were conjugated with the appropriate amine derivative of biotin containing a hydrophilic triethylene glycol linker terminated with amino group to afford **15a**[13] and **15b** (Scheme 3).[12] Using these affinity probes, detailed analyses of the radicicol-binding proteins have been carried out and the results have been already described in separate reports.[12–14] These reports revealed that one of the major targets of radicicol is the heat shock protein 90 (Hsp90). The Hsp90 family proteins are key mediators of protein folding in the cell under normal growth conditions as well as under stress conditions, and associate with a number of signaling proteins.[15] These proteins include steroid hormone receptors,[16] aryl hydrocarbon receptors,[17] mutated p53 tumor suppressor gene product,[18] tyrosine kinases such as EGFR, $p185^{erbB2}$, and v-src family kinases,[19–23] and serine/threonine kinases such as Raf-1[24] and Cdk4.[25] Association of these signaling proteins with Hsp90 is essential for their stability, and dissociation from Hsp90 induces the rapid degradation by the proteasome system. Benzoquinone ansamycin family of antibiotics such as geldanamycin and herbimycin A are reported to bind to the N-terminal domain of Hsp90 with high affinity and this binding leads to the degradation of signal transduction proteins.[26] 17-Allylamino-17-demethoxygeldanamycin (17AAG),[27] one of the geldanamycin derivatives, has shown potent antitumor activities in cell culture and animal models, and is currently in Phase I clinical trial. Therefore, radicicol, structurally distinct from ansamycin antibiotics, is the new chemotype of a second class of Hsp90 inhibitors.



Scheme 3. Reagents and conditions: (a) (i) $CCl_3C(=NH)O^tBu$, $BF_3{\cdot}OEt_2$, ^{c}hex.–CH_2Cl_2, rt, 14 h, 44% for $n=1$ and 47% for $n=2$, respectively; (ii) *N*-hydroxyphthalimide, $^i Pr_2NEt$, DMF, 100 °C, 3.5 h, 79% for $n=1$ and 81% for $n=2$, respectively; (iii) $NH_2NH_2{\cdot}H_2O$, $CHCl_3$, rt, 1 h, 95% for $n=1$ and 59% for $n=2$, respectively; (iv) TFA, CH_2Cl_2, rt, 1 h; then, 4 M HCl/EtOAc, 83% for $n=1$ and 86% for $n=2$, respectively; (b) **10a** or **10b**, pyridine, rt, 43 or 15 h, 60% for **11a** and 25% for **11b**, respectively; (c) *N*-hydroxysuccinimide, DCC, DMAP, CH_2Cl_2, rt, 1 h; (d) **11c**, EAH Sepharose 4B, THF, rt, 5 days;[12] (e) **11a** or **11b**, **14**, HOBt, Et_3N, EDC·HCl, DMF, rt, 63 or 19 h, 30% for **15a** and 22% for **15b**, respectively.

Recent report revealed that both geldanamycin and radicicol bind to the N-terminal nucleotide-binding domain of Hsp90, with radicicol displaying significantly higher affinity ($K_d = 19$ nM), and both antibiotics inhibit the ATPase activity of Hsp90 which is essential for its function.[28] Crystal structure determinations of Hsp90 N-terminal domain complexes with geldanamycin[29] and radicicol[28] have been reported and demonstrated their nucleotide mimetic interactions. Drugs that target Hsp90 in cancer cells and stimulate depletion of oncogenic proteins could be of clinical benefit.

Conclusion

In summary, a series of halohydrin and oxime derivatives of radicicol were prepared and evaluated for their antitumor activities in vitro and in vivo. Although halohydrin derivative **3a** was inactive, oxime derivatives showed in vivo antitumor activities, with hydroxime **5a** being the most active. KF25706 (**5a**) was further evaluated for its antitumor activity against various tumor models by iv injections.[30] As guided by the preliminary structure–activity relationships, we have designed and prepared radicicol-based novel affinity probes. Because of the involvements of Hsp90, one of the major target molecules of radicicol, in the stability of various signal transduction proteins, Hsp90 is considered to be an attractive target for antitumor drug development. In addition, with radicicol-based affinity probes in hands, these versatile tools enable us to elucidate the role and functions of Hsp90 in cellular events. More recently, small molecular weight compounds designed to bind to the nucleotide binding site of Hsp90 were reported.[31]

Further developments of radicicol 6-oximes are now in progress, in which we are trying to introduce soluble functionalities, and will be reported elsewhere.

Experimental

General procedure

^{1}H and ^{13}C NMR spectra were recorded on Bruker AM-500, JNM α400, or JNM LA300 spectrometer. Chemical shift values are given in ppm relative to TMS as an internal standard. FAB-MS spectra were obtained with JMS-HX110A spectrometer. TLC analysis was performed on Merck precoated silica gel 60 F-254 plates. Spots were visualized with 254 nm UV light and/or phosphomolybdic acid spray. Column chromatography was performed with silica gel (Merck or Wako). Unless otherwise noted, all materials were used as received from a commercial supplier without further purification. Radicicol (**1**) was obtained by fermentation in our laboratories.

HPLC analyses

Halohydrin derivatives **3a** and **3c** (1 mM) were dissolved in 10% DMSO/10 mM phosphate buffer solution (pH 7.25) and incubated at 37 °C. Conversion of halohydrins to radicicol was determined from the relative areas of the peaks corresponding to halohydrins and radicicol. HPLC analyses were performed using Shimadzu LC10A HPLC instrument (column: Unisil 5C18 250A; flow rate: 1 mL/min; elution buffer: 40 or 50% MeCN/50 mM phosphate buffer; detector: SPD10A).

Radicicol and methoxime **5b** (1 mM) were dissolved in 10% DMSO/10 mM phosphate buffer solution (pH 7.25) and incubated at 37 °C in the presence of DTT (100 mM). HPLC analyses were performed in a similar manner as that described above. Radicicol and methoxime **5b** (1 mM) were dissolved in 10% DMSO/mouse serum (pH 7.4, Funakoshi Co., Ltd.) and incubated at 37 °C. HPLC analyses were performed in a similar manner as that described above.

Radicicol-DTT adduct (2). Radicicol (**1**) was incubated with excess amount of DTT in 50% aqueous DMSO at 37 °C for 2 h. Major product was purified by preparative HPLC and Sephadex LH-20 column chromatography. Structure of **2** was confirmed by 2D-NMR data including DQF-COSY, NOESY, HSQC, and HMBC spectra. **2**: ^{1}H NMR (CD_3OD, major isomer) δ 6.36 (1H, s), 5.66 (1H, m), 5.30 (1H, m), 5.17 (1H, m), 4.15 (1H, d, J = 18.0 Hz), 3.95 (1H, d, J = 18.0 Hz), 3.69 (1H, m), 3.52 (1H, m), 3.20 (1H, dd, J = 12.9, 9.2 Hz), 2.95 (1H, m), 2.88 (1H, m), 2.86 (1H, m), 2.71 (1H, m), 2.64–2.70 (2H, m), 2.53 (2H, d, J = 7.1 Hz), 2.20 (1H, ddd, J = 14.6, 6.2, 3.5 Hz), 1.54 (1H, m), 1.30 (3H, d, J = 6.6 Hz); ^{13}C NMR (CD_3OD, major isomer) δ 206.8 (s), 170.2 (s), 160.3 (s), 159.5 (s), 135.5 (s), 133.9 (d), 127.6 (d), 116.5 (s), 111.0 (s), 104.2 (d), 75.3 (d), 72.8 (d), 72.5 (d), 62.7 (d), 55.5 (d), 51.4 (d), 46.2 (t, 2C), 38.0 (t), 34.8 (t), 28.2 (t), 19.8 (q); HRFAB-MS m/z 517.0766 $[M-H]^-$, calcd for $C_{22}H_{26}O_8S_2\,^{35}Cl$ m/z 517.0758.

Chlorohydrin formate (3a). Phosphorus oxychloride (1.0 mL, 10.7 mmol) was slowly added to ice-cooled DMF (5 mL) and the mixture was stirred at room temperature for 30 min. The resulting red-colored solution was slowly added to a solution of **1** (2 g, 5.5 mmol) in DMF at 0 °C. The resulting reaction mixture was stirred at room temperature for 24 h, diluted with EtOAc, washed with water, and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (2% MeOH/$CHCl_3$) to afford 1.2 g of **3a** (51%). **3a**: ^{1}H NMR (CD_3OD) δ 8.00 (1H, s), 7.14 (1H, ddd, J = 16.1, 11.2, 1.0 Hz), 6.44 (1H, s), 6.16 (1H, t, J = 10.8 Hz), 5.95 (1H, d, J = 16.1 Hz), 5.68 (1H, t, J = 10.0 Hz), 5.32 (1H, m), 5.25 (1H, m), 5.20 (1H, dd, J = 10.0, 5.6 Hz), 4.10 (1H, d, J = 16.1 Hz), 3.65 (1H, d, J = 16.1 Hz), 1.97 (1H, m), 1.42 (3H, d, J = 6.3 Hz); HRFAB-MS m/z 427.0369 $[M-H]^-$, calcd for $C_{19}H_{17}O_7\,^{35}Cl_2$ m/z 427.0351.

Chlorohydrin (3b). To an ice-cooled solution of **1** (2 g, 5.5 mmol) in 1,4-dioxane (70 mL) concd HCl (36%, 1.3 mL) was slowly added and the mixture was stirred at room temperature for 6 h. The resulting reaction mixture was diluted with cold water, carefully neutralized with saturated $NaHCO_3$, and extracted with EtOAc. The organic layer was separated, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (2% MeOH/$CHCl_3$) to give 1.0 g of **3b** (45%). **3b**: ^{1}H NMR (CD_3OD) δ 7.25 (1H, ddd, J = 16.4, 11.3, 1.0 Hz), 6.50 (1H, s), 6.21 (1H, dt, J = 1.0, 11.7 Hz), 5.99 (1H, d, J = 16.4 Hz), 5.79 (1H, dt, J = 1.0, 11.7 Hz), 5.42 (1H, m), 5.17 (1H, ddd, J = 11.7, 5.9, 1.0 Hz), 4.25 (1H, d, J = 16.4 Hz), 4.03 (1H, br dd, J = 8.1, 5.9 Hz), 3.70 (1H, d, J = 16.4 Hz), 2.07 (1H, ddd, J = 15.1, 6.8, 1.2 Hz), 1.93 (1H, ddd, J = 15.1, 8.1, 3.7 Hz), 1.46 (3H, d, J = 6.3 Hz); HRFAB-MS m/z 399.0414 $[M-H]^-$, calcd for $C_{18}H_{17}O_6\,^{35}Cl_2$ m/z 399.0402.

Bromohydrin (3c). In a similar manner as that described above, radicicol (**1**, 2.5 g, 6.9 mmol) was treated with concd HBr (47%, 1.0 mL) to give 1.7 g of **3c** (56%). **3c**: ^{1}H NMR (CD_3OD) δ 7.28 (1H, dd, J = 16.0, 10.8 Hz), 6.51 (1H, s), 6.13 (1H, t, J = 10.8 Hz), 6.00 (1H, d, J = 16.0 Hz), 5.96 (1H, t, J = 10.8 Hz), 5.40 (1H, m), 5.33 (1H, dd, J = 10.8, 5.2 Hz), 4.24 (1H, d, J = 16.1 Hz), 4.18 (1H, m), 3.71 (1H, d, J = 16.1 Hz), 2.08 (1H, m), 1.92 (1H, m), 1.45 (3H, d, J = 6.4 Hz); HRFAB-MS m/z 442.9908 $[M-H]^-$, calcd for $C_{18}H_{17}O_6\,^{79}Br^{35}Cl$ m/z 442.9897.

Dimeric chlorohydrin sulfite (3d). To an ice-cooled solution of **1** (1.4 g, 3.8 mmol) in DMF (75 mL) was slowly added thionyl chloride (0.5 mL, 6.9 mmol). The resulting reaction mixture was stirred at room temperature for 12 h, diluted with EtOAc, washed with water, and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the resulting residue was purified by silica gel column chromatography (4% MeOH/$CHCl_3$) to afford 1.0 g of **3d** (62%) and 0.2 g of **3b** (13%), respectively. **3d**: ^{1}H NMR (CD_3OD) δ 7.15 (2H, dd, J = 16.1, 10.8 Hz), 6.52 (2H, s), 6.27 (2H, t, J = 10.8 Hz), 6.05 (2H, d, J = 16.1 Hz), 5.73 (2H, t, J = 10.8 Hz), 5.39 (2H, m), 5.35 (2H, m), 4.88 (2H, m), 4.28 (2H, d, J = 16.4 Hz), 3.74 (2H, d, J = 16.4 Hz), 2.27 (2H, m), 2.10 (2H, m), 1.50 (6H, d, J = 6.3 Hz); HRFAB-MS m/z 845.0408 $[M-H]^-$, calcd for $C_{36}H_{33}O_{13}S^{35}Cl_4$ m/z 845.0396.

Degradation product of 3b (4). Chlorohydrin (**3b**, 28.8 mg, 0.072 mmol) was allowed to stand at room temperature with protection from light and moisture. After several months, TLC analysis ($CHCl_3$–MeOH, 19:1) revealed that **3b** (R_f = 0.16) was converted to **1** (R_f = 0.37) and **4** (R_f = 0.48). This mixture was separated by silica gel preparative TLC ($CHCl_3$–MeOH, 50:1) to afford **4** (10.4 mg, 36%), **1** (4.3 mg, 16%), and **3b** (1.8 mg, 6.3%), respectively. Structure of **4** was confirmed by 2D-NMR data including DQF-COSY, NOESY, HSQC, and HMBC spectra. **4**: ^{1}H NMR (CD_3OD) δ 6.45 (1H, s), 5.88 (1H, ddd, J = 10.3, 2.4, 1.2 Hz), 5.84 (1H, ddd, J = 10.3, 1.7, 1.3 Hz), 5.29 (1H, m), 4.84 (1H, m), 4.57 (1H, d, J = 18.3 Hz), 4.25 (1H, ddd, J = 8.3, 1.3, 1.2 Hz), 4.18 (1H, d, J = 18.3 Hz), 3.65 (1H, dd, J = 8.3, 7.8 Hz), 2.97 (1H, dd, J = 15.6, 11.2 Hz), 2.49 (1H, dd, J = 15.6, 3.7 Hz), 2.33 (1H, ddd, J = 15.1, 4.8, 2.0 Hz), 1.79 (1H, ddd, J = 15.1, 9.3, 7.8 Hz), 1.40 (3H, d, J = 6.4 Hz); ^{13}C NMR (CD_3OD) δ 206.8 (s), 171.1 (s), 158.8 (s, 2C), 135.7 (s), 132.1 (d), 129.1 (d), 126.5 (s), 116.0 (s), 103.9 (d), 74.0 (d), 73.9 (d), 71.1 (d), 56.3 (d), 47.8 (t), 44.1 (t), 37.7 (t), 21.6 (q); HRFAB-MS m/z 401.0542 $[M+H]^+$, calcd for $C_{18}H_{19}O_6{}^{35}Cl_2$ m/z 401.0559.

Radicicol 6-oxime (5a). Radicicol (**1**, 42.0 mg, 0.115 mmol) and hydroxylamine hydrochloride (20.0 mg, 0.288 mmol) were dissolved in pyridine (2 mL) and the resulting solution was stirred at 50 °C for 8 h. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography ($CHCl_3$–MeOH, 25:1) to give **5a** (10.0 mg, 23%) and **6a** (14.0 mg, 31%), respectively. **5a**: ^{1}H NMR (CD_3OD, major isomer) δ 7.22 (1H, dd, J = 16.2, 11.3 Hz), 6.83 (1H, d, J = 16.2 Hz), 6.43 (1H, s), 6.16 (1H, t, J = 11.3 Hz), 5.58 (1H, dd, J = 11.3, 3.6 Hz), 5.30 (1H, m), 3.91 (1H, d, J = 16.1 Hz), 3.81 (1H, d, J = 16.1 Hz), 3.35 (1H, m), 3.02 (1H, m), 2.42 (1H, m), 1.60 (1H, m), 1.53 (3H, d, J = 6.6 Hz); HRFAB-MS m/z 380.0901 $[M+H]^+$, calcd for $C_{18}H_{19}NO_6{}^{35}Cl$ m/z 380.0900; **6a**: ^{1}H NMR (CD_3OD) δ 6.43 (1H, s), 5.99 (1H, m), 5.90 (1H, d, J = 6.0 Hz), 5.70 (1H, m), 4.49 (1H, m), 4.18 (1H, d, J = 9.5 Hz), 3.67 (1H, d, J = 13.4 Hz), 3.49 (1H, d, J = 13.4 Hz), 3.43 (1H, dd, J = 9.5, 2.6 Hz), 2.53 (1H, m), 2.37 (1H, d, J = 15.2 Hz), 2.03 (1H, t, J = 12.9 Hz), 1.88 (1H, br d, J = 15.2 Hz), 1.37 (3H, d, J = 6.2 Hz); HRFAB-MS m/z 398.1022 $[M+H]^+$, calcd for $C_{18}H_{21}NO_7{}^{35}Cl$ m/z 398.1006.

Radicicol di-TBS ether (8). To a solution of **1** (100 mg, 0.275 mmol) and imidazole (60.0 mg, 0.882 mmol) in DMF (1.5 mL) was added *tert*-butyldimethylsilyl chloride (TBSCl, 100 mg, 0.663 mmol), and the mixture was stirred at room temperature for 24 h. The reaction mixture was diluted with EtOAc, washed with water, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane–EtOAc, 5:1) to give radicicol di-TBS ether (**8**, 162 mg, 99%). **8**: ^{1}H NMR ($CDCl_3$) δ 7.56 (1H, dd, J = 16.1, 10.8 Hz), 6.36 (1H, s), 6.10 (1H, ddd, J = 10.8, 10.8, 1.7 Hz), 6.01 (1H, d, J = 16.1 Hz), 5.76 (1H, dd, J = 10.8, 3.5 Hz), 5.29 (1H, m), 3.86 (1H, d, J = 16.3 Hz), 3.67 (1H,d, J = 16.3 Hz), 3.37 (1H, m), 3.00 (1H, m), 2.42 (1H, ddd, J = 14.5, 2.9, 2.9 Hz), 1.51 (3H, d, J = 6.6 Hz), 1.46 (1H, m), 0.97 (9H, s), 0.92 (9H, s), 0.21 (3H, s), 0.19 (3H, s), 0.18 (3H, s), 0.17 (s, 3H); HRFAB-MS m/z 593.2531 $[M+H]^+$, calcd for $C_{30}H_{46}O_6Si_2{}^{35}Cl$ m/z 593.2521.

Radicicol 6-oxime di-TBS ether (5e). To a solution of **8** (129.2 mg, 0.218 mmol) in dichloromethane (1.0 mL) were added pyridine (1.0 mL) and hydroxylamine hydrochloride (50.0 mg, 0.720 mmol), and the mixture was stirred at 65 °C for 10 h. The reaction mixture was diluted with EtOAc, washed with dil HCl, satd $NaHCO_3$, and brine, dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by silica gel column chromatography (*n*-hexane–EtOAc, 5:1) to give **5e** (31.6 mg, 24%) and **6e** (13.3 mg, 9.7%). Structure of **6e** was confirmed by 2D-NMR data including DQF-COSY, NOESY, HSQC, and HMBC spectra. **5e**: ^{1}H NMR ($CDCl_3$) (major isomer) δ 6.37 (1H, s), 5.91 (1H, m), 5.82 (1H, ddd, J = 6.2, 1.8, 1.8 Hz), 5.43 (1H, m), 4.62 (1H, dd, J = 9.1, 5.0 Hz), 4.44 (1H, m), 4.09 (1H, d, J = 18.3 Hz), 4.00 (1H, d, J = 18.3 Hz), 3.60 (1H, m), 2.80 (1H, dd, J = 13.4, 5.5 Hz), 2.31 (1H, dd, J = 13.4, 7.2 Hz), 2.13 (1H, m), 1.81 (1H, m), 1.41 (3H, d, J = 6.6 Hz), 1.020 (9H, s), 1.015 (9H, s), 0.246 (3H, s), 0.240 (3H, s), 0.237 (3H, s), 0.227 (3H, s); (minor isomer) δ 7.13 (1H, dd, J = 16.0, 11.2 Hz), 6.36 (1H, s), 6.08 (1H, t, J = 11.2 Hz), 6.02 (1H, d, J = 16.1 Hz), 5.53 (1H, dd, J = 11.2, 3.1 Hz), 5.26 (1H, m), 4.85 (1H, d, J = 16.3 Hz), 3.39 (1H, m), 3.01 (1H, d, J = 16.3 Hz), 2.99 (1H, m), 1.54 (3H, d, J = 6.5 Hz), 1.49 (2H, m), 0.94 (9H, s), 1.00 (9H, s), 0.230 (3H, s), 0.212 (3H, s), 0.209 (3H, s), 0.197 (3H, s); HRFAB-MS m/z 608.2643 $[M+H]^+$, calcd for $C_{30}H_{47}NO_6Si_2{}^{35}Cl$ m/z 608.2630; **6e**: ^{1}H NMR ($CDCl_3$) δ 6.41 (1H, s), 5.94 (1H, m), 5.84 (1H, d, J = 6.5 Hz), 5.68 (1H, m), 4.54 (1H, m), 4.12 (1H, m), 3.74 (1H, d, J = 13.6 Hz), 3.61 (1H, m), 3.56 (1H, d, J = 13.6 Hz), 2.62 (1H, s), 2.58 (1H, dd, J = 12.2, 6.2 Hz), 2.50 (1H, ddd, J = 15.9, 2.6, 2.6 Hz), 2.03 (1H, t, J = 12.2 Hz), 1.89 (1H, ddd, J = 15.9, 4.6, 4.6 Hz), 1.60 (1H, s), 1.44 (3H, d, J = 6.5 Hz), 1.02 (9H, s), 0.96 (9H, s), 0.240 (3H, s), 0.230 (3H, s), 0.227 (3H, s), 0.224 (3H, s); ^{13}C NMR ($CDCl_3$) δ 167.7 (s), 152.7 (s), 152.2 (s), 137.7 (d), 133.0 (s), 129.2 (d), 123.1 (s), 120.4 (s), 110.9 (d), 108.6 (s), 74.4 (d), 72.9 (d), 69.8 (d), 65.4 (d), 47.0 (t), 40.6 (t), 39.0 (t), 25.9 (q, 3C), 25.6 (q, 3C), 24.3 (q), 18.7 (s), 18.4 (s), −3.5 (q), −3.9 (q), −4.3 (q, 2C); HRFAB-MS m/z 626.2758 $[M+H]^+$, calcd for $C_{30}H_{49}NO_7Si_2{}^{35}Cl$ m/z 626.2736.

Radicicol 6-(*O*-methyloxime) (5b). To a stirred solution of **1** (200 mg, 0.549 mmol) in pyridine (1.0 mL) was added *O*-methylhydroxylamine hydrochloride (100 mg, 1.20 mmol), and the resulting mixture was stirred at 80 °C for 1.5 h. The reaction mixture was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (1% MeOH/$CHCl_3$) to give radicicol 6-(*O*-methyloxime) (**5b**, 34.0 mg, 16%). **5b**: ^{1}H NMR (CD_3OD, major isomer) δ 7.23 (1H, dd, J = 16.2, 11.3 Hz), 6.70 (1H, d, J = 16.2 Hz), 6.42 (1H, s), 6.14 (1H, t, J = 11.3 Hz), 5.58 (1H, dd, J = 11.3, 3.6 Hz), 5.30 (1H, m), 3.904 (1H, d, J = 16.1 Hz), 3.901 (3H, s), 3.80 (1H, d, J = 16.1 Hz), 3.33 (1H, m), 3.01 (1H, m), 2.42 (1H, ddd, J = 14.5, 3.5, 3.5 Hz), 1.59 (1H, ddd,

$J=14.5$, 9.0, 4.1 Hz), 1.52 (3H, d, $J=6.5$ Hz); HRFAB-MS m/z 394.1064 $[M+H]^+$, calcd for $C_{19}H_{21}NO_6{}^{35}Cl$ m/z 394.1058.

Radicicol 6-(*O*-carboxymethyloxime) (5c). Radicicol (**1**, 1.5 g, 4.1 mmol) and carboxymethoxylamine hemihydrochloride ($NH_2OCH_2CO_2H{\cdot}1/2HCl$, 1.0 g, 9.2 mmol) were dissolved in pyridine, and the mixture was stirred at 60 °C for 1.5 h. The reaction mixture was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (2% MeOH/$CHCl_3$) to give **5c** (692 mg, 39%). **5c**: ^1H NMR (CD_3OD, major isomer) δ 7.27 (1H, dd, $J=16.1$, 11.2 Hz), 6.82 (1H, d, $J=16.1$ Hz), 6.42 (1H, s), 6.17 (1H, dd, $J=11.2$, 10.5 Hz), 5.61 (1H, dd, $J=10.5$, 3.4 Hz), 5.31 (1H, m), 4.64 (2H, s), 3.91 (1H, d, $J=16.4$ Hz), 3.82 (1H, d, $J=16.4$ Hz), 3.34 (1H, m), 3.02 (1H, m), 2.42 (1H, m), 1.60 (1H, ddd, $J=14.4$, 9.0, 4.2 Hz), 1.53 (3H, d, $J=6.6$ Hz); HRFAB-MS m/z 438.0978 $[M+H]^+$, calcd for $C_{20}H_{21}NO_8{}^{35}Cl$ m/z 438.0955.

Radicicol 6-(dimethylcarbamoylmethyloxime) (5d). To a stirred solution of **5c** (5.20 g, 11.9 mmol), *N*-hydroxysuccinimide (2.50 g, 21.7 mmol) and 4-(dimethylamino)pyridine (DMAP, 310 mg, 2.54 mmol) in THF (100 mL) was slowly added a solution of 1,3-dicyclohexylcarbodiimide (DCC, 4.50 g, 21.8 mmol) in THF (30 mL). The mixture was stirred at room temperature for 2 h and precipitated 1,3-dicyclohexylurea (DCC urea) was removed by filtration. The filtrate was concentrated under reduced pressure to give crude succinimidyl ester, which was dissolved in dichloromethane (100 mL) and treated with triethylamine (4.5 mL, 32.3 mmol) and dimethylamine hydrochloride (2.0 g, 24.5 mmol). The mixture was stirred at room temperature for 12 h and concentrated under reduced pressure. The residue was dissolved in EtOAc, washed with dil HCl and brine, and dried over anhydrous Na_2SO_4. Purification by silica gel column chromatography (2% MeOH/$CHCl_3$) gave 2.0 g of **5d** (36%). **5d**: ^1H NMR (CD_3OD, major isomer) δ 7.27 (1H, dd, $J=16.1$, 11.3 Hz), 6.81 (1H, d, $J=16.1$ Hz), 6.42 (1H, s), 6.17 (1H, dd, $J=11.3$, 10.5 Hz), 5.61 (1H, dd, $J=10.5$, 3.5 Hz), 5.30 (1H, m), 3.91 (1H, d, $J=16.1$ Hz), 3.82 (1H, d, $J=16.1$ Hz), 3.34 (1H, m), 3.08 (3H, s), 3.02 (1H, ddd, $J=8.9$, 3.7, 2.2 Hz), 2.95 (3H, s), 2.42 (1H, ddd, $J=14.5$, 3.7, 3.6 Hz), 1.60 (1H, ddd, $J=14.5$, 8.9, 4.1 Hz), 1.52 (3H, d, $J=6.6$ Hz); HRFAB-MS m/z 465.1449 $[M+H]^+$, calcd for $C_{22}H_{26}N_2O_7{}^{35}Cl$ m/z 465.1429.

Halohydrin-oxime hybrid (7). To a stirred solution of **5b** (362 mg, 0.921 mmol) in DMF (7 mL) was slowly added oxalyl chloride (160 μL, 1.83 mmol) at 0 °C, and the mixture was stirred at room temperature for 15 h. The reaction mixture was diluted with EtOAc, washed with water, and dried over anhydrous Na_2SO_4. The organic layer was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (2% MeOH/$CHCl_3$) to give 94.0 mg of **7** (22%). **7**: ^1H NMR (CD_3OD, major isomer) δ 8.10 (1H, s), 6.87 (1H, d, $J=16.0$ Hz), 6.75 (1H, dd, $J=16.0$, 11.1 Hz), 6.49 (1H, s), 6.19 (1H, t, $J=11.1$ Hz), 5.58 (1H, t, $J=11.1$ Hz), 5.45 (1H, m), 5.32 (1H, m), 5.27 (1H, dd, $J=11.1$, 4.5 Hz), 3.91 (3H, s), 3.85 (1H, d, $J=15.9$ Hz), 3.75 (1H, d, $J=15.9$ Hz), 2.03 (1H, m), 1.95 (1H, dd, $J=14.4$, 4.8 Hz), 1.51 (3H, d, $J=6.4$ Hz); HRFAB-MS m/z 458.0775 $[M+H]^+$, calcd for $C_{20}H_{22}NO_7{}^{35}Cl_2$ m/z 458.0774.

6-(Aminooxy)hexanoic acid hydrochloride (10a). To a solution of 6-bromohexanoic acid (**9a**, 1.0 g, 5.1 mmol) in cyclohexane (10 mL)-dichloromethane (1 mL) mixture were added *tert*-butyl 2,2,2-trichloroacetimidate[32] (2.2 g, 10 mmol) and boron trifluoride diethyletherate (100 μL). The resulting solution was stirred at room temperature for 14 h and the reaction was quenched by addition of solid $NaHCO_3$ (100 mg). 2,2,2-Trichloroacetamide was removed by filtration and the resulting filtrate was concentrated and purified by silica gel column chromatography (*n*-hexane–EtOAc, 4:1) to give 562 mg of 6-bromohexanoic acid *tert*-butyl ester (44%). ^1H NMR ($CDCl_3$) δ 3.33 (2H, t, $J=6.8$ Hz), 2.15 (2H, t, $J=7.3$ Hz), 1.80 (2H, quint, $J=6.8$ Hz), 1.54 (2H, m), 1.38 (2H, m), 1.37 (9H, s); FAB-MS m/z 251, 253 $[M+H]^+$.

To a solution of 6-bromohexanoic acid *tert*-butyl ester (562 mg, 2.3 mmol) in DMF (3.0 mL) were added *N*-hydroxyphthalimide (550 mg, 3.4 mmol) and *i*-Pr_2NEt (750 μL, 4.3 mmol). The solution was stirred at 100 °C for 3.5 h, diluted with EtOAc, and washed with water, dil HCl, satd $NaHCO_3$ and brine. The organic layer was separated, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane–EtOAc, 4:1) to yield 603 mg of 6-(phthalimidooxy)hexanoic acid *tert*-butyl ester (79%). ^1H NMR ($CDCl_3$) δ 7.62–7.71 (4H, m), 4.07 (2H, t, $J=6.6$ Hz), 2.12 (2H, t, $J=7.5$ Hz), 1.67 (2H, quint, $J=7.5$ Hz), 1.53 (2H, m), 1.40 (2H, m), 1.32 (9H, s); FAB-MS m/z 334 $[M+H]^+$.

To a stirred solution of 6-(phthalimidooxy)hexanoic acid *tert*-butyl ester (272.9 mg, 0.820 mmol) in $CHCl_3$ (2 mL) was added hydrazine monohydrate (50 μL, 1.03 mmol). After stirring at room temperature for 1 h, the resulting reaction mixture was filtered to remove precipitated phthalhydrazide and the filtrate was concentrated under reduced pressure and purified by silica gel column chromatography (5% MeOH/$CHCl_3$) to give 157.9 mg of 6-(aminooxy)hexanoic acid *tert*-butyl ester (95%). ^1H NMR ($CDCl_3$) δ 5.24 (2H, br s), 3.52 (2H, t, $J=6.6$ Hz), 2.09 (2H, t, $J=7.5$ Hz), 1.43–1.53 (4H, m), 1.32 (9H, s), 1.23 (2H, m); FAB-MS m/z 204 $[M+H]^+$.

6-(Aminooxy)hexanoic acid *tert*-butyl ester (371.2 mg, 1.83 mmol) was dissolved in dichloromethane (2 mL) and the resulting solution was treated with TFA (1 mL). After stirring at room temperature for 1 h, the reaction mixture was concentrated under reduced pressure. The residue was treated with 4 M hydrochloride/EtOAc to give 278.6 mg of **10a** (83%). **10a**: ^1H NMR (D_2O) δ 4.04 (2H, t, $J=6.4$ Hz), 2.37 (2H, t, $J=7.3$ Hz), 1.55–1.70 (4H, m), 1.39 (2H, m); HRFAB-MS m/z 148.0984 $[M+H]^+$, calcd for $C_6H_{14}NO_3$ m/z 148.0974.

6-(Aminooxy)octanoic acid hydrochloride (10b). In a similar manner as that described above, 8-(aminooxy)-

octanoic acid hydrochloride (**10b**) was obtained in 19% overall yield from 8-bromooctanoic acid (**9b**). **10b**: ^{1}H NMR (D_2O) δ 4.03 (2H, t, J = 6.6 Hz), 2.34 (2H, t, J = 7.5 Hz), 1.69–1.52 (4H, m), 1.40–1.26 (6H, m); HRFAB-MS m/z 176.1290 $[M+H]^+$, calcd for $C_8H_{18}NO_3$ m/z 176.1287.

Radicicol 6-(*O*-hexanoic acid)oxime (11a). Radicicol (**1**, 100 mg, 0.275 mmol) and 6-(aminooxy) hexanoic acid hydrochloride (**10a**, 70.4 mg, 0.385 mmol) were dissolved in pyridine (0.5 mL) and the solution was stirred for 43 h at room temperature. The reaction mixture was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (2.5–10% MeOH/$CHCl_3$) to give 80.7 mg of **11a** (60%). **11a**: ^{1}H NMR (CD_3OD, major isomer) δ 7.22 (1H, dd, J = 16.1, 11.4 Hz), 6.72 (1H, d, J = 16.1 Hz), 6.42 (1H, s), 6.14 (1H, dd, J = 11.4, 10.6 Hz), 5.57 (1H, dd, J = 10.6, 3.5 Hz), 5.29 (1H, m), 4.05–4.19 (2H, m), 3.92 (1H, d, J = 16.0 Hz), 3.81 (1H, d, J = 16.0 Hz), 3.33 (1H, m), 3.00 (1H, m), 2.41 (1H, ddd, J = 3.5, 3.5, 14.5 Hz), 2.30 (1H, t, J = 7.5 Hz), 1.44–1.76 (7H, m), 1.51 (H, d, J = 6.4 Hz); HRFAB-MS m/z 494.1586 $[M+H]^+$, calcd for $C_{24}H_{29}NO_8$ ^{35}Cl m/z 494.1581.

Radicicol 6-(*O*-octanoic acid)oxime (11b). In a similar manner as that described above, radicicol 8-(*O*-octanoic acid)oxime (**11b**) was obtained in 25% yield by condensation of radicicol (**1**) with 8-(aminooxy)octanoic acid hydrochloride (**10b**). **11b**: ^{1}H NMR (CD_3OD, major isomer) δ 7.23 (1H, dd, J = 16.1, 11.2 Hz), 6.72 (1H, d, J = 16.1 Hz), 6.42 (1H, s), 6.15 (1H, dd, J = 11.2, 10.6 Hz), 5.58 (1H, dd, J = 10.6, 3.5 Hz), 5.29 (1H, m), 4.91–4.03 (2H, m), 3.91 (1H, d, J = 16.1 Hz), 3.79 (1H, d, J = 16.1 Hz), 3.33 (1H, m), 3.01 (1H, ddd, J = 9.0, 3.7, 2.2 Hz), 2.41 (1H, ddd, J = 14.5, 3.7, 3.7 Hz), 2.30–2.24 (2H, m), 1.68 (1H, m), 1.63–1.58 (4H, m), 1.52 (3H, d, J = 6.4 Hz), 1.46–1.34 (6H, m); HRFAB-MS m/z 520.1732 $[M-H]^-$, calcd for $C_{26}H_{31}NO_8$ ^{35}Cl m/z 520.1738.

***N*-Hydroxysuccinimidyl ester of 11b (11c).** To a stirred solution of **11b** (14.6 mg, 0.0280 mmol), *N*-hydroxysuccinimide (7.7 mg, 0.0669 mmol), and DMAP (8.5 mg, 0.0696 mmol) in dichloromethane (0.5 mL) was added DCC (10.0 mg, 0.0485 mmol). The reaction mixture was stirred at room temperature for 1 h and precipitated DCC urea was removed by filtration. The filtrate was concentrated under reduced pressure to give crude *N*-hydroxysuccinimidyl ester (**11c**, 18.5 mg). Analytical sample was prepared by silica gel preparative TLC ($CHCl_3$–MeOH, 10:1). **11c**: ^{1}H NMR (CD_3OD, major isomer) δ 7.23 (1H, dd, J = 11.6. 16.1 Hz), 6.72 (1H, d, J = 16.1 Hz), 6.42 (1H, s), 6.15 (1H, dd, J = 11.6, 10.6 Hz), 5.58 (1H, dd, J = 10.6, 3.3 Hz), 5.29 (1H, m), 4.59–4.06 (2H, m), 3.91 (1H, d, J = 16.1 Hz), 3.79 (1H, d, J = 16.1 Hz), 3.34 (1H, m), 3.02 (1H, m), 2.82 (4H, s), 2.66–2.60 (2H, m), 2.42 (1H, m), 1.86–1.57 (5H, m), 1.52 (3H, d, J = 6.6 Hz), 1.42–1.11 (6H, m); HRFAB-MS m/z 617.1932 $[M-H]^-$, calcd for $C_{30}H_{34}N_2O_{10}$ ^{35}Cl m/z 617.1902.

Radicicol–biotin conjugate (15a). To a solution of **11a** (31.7 mg, 0.0643 mmol) and (+)-biotinyl-3,6-dioxaoctanediamine (**14**, EZ-Link™ Biotin-PEO-Amine; PIERCE, 24.1 mg, 0.0644 mmol) in DMF (1 mL) were added 1-hydroxybenzotriazole hydrate (HOBt, 10.9 mg, 0.0807 mmol), Et_3N (10 μL, 0.0717 mmol), and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl, 15.5 mg, 0.0809 mmol). The resulting solution was stirred at room temperature for 63 h. The mixture was diluted with water and extracted with EtOAc. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (2.5–10% MeOH–$CHCl_3$) to yield biotin conjugate **15a** (16.3 mg, 30%). **15a**: ^{1}H NMR (CD_3OD, major isomer) δ 7.23 (1H, dd, J = 15.5, 11.4 Hz), 6.72 (1H, d, J = 15.5 Hz), 6.43 (1H, s), 6.16 (1H, dd, J = 11.4, 10.6 Hz), 5.59 (1H, dd, J = 10.6, 3.3 Hz), 5.29 (1H, m), 4.47 (1H, dd, J = 7.5, 4.6 Hz), 4.28 (1H, dd, J = 7.9, 4.6 Hz), 4.09–4.20 (2H, m), 3.91 (1H, d, J = 16.0 Hz), 3.78 (1H, d, J = 16.0 Hz), 3.45–3.63 (8H, m), 3.33–3.37 (5H, m), 3.18 (1H, m), 3.01 (1H, m), 2.90 (1H, dd, J = 12.7, 5.0 Hz), 2.69 (1H, d, J = 12.7 Hz), 2.43 (1H, m), 2.18–2.24 (4H, m), 1.42–1.73 (13H, m), 1.52 (3H, d, J = 6.6 Hz); HRFAB-MS m/z 850.3477 $[M+H]^+$, calcd for $C_{40}H_{57}N_5O_{11}S$ ^{35}Cl m/z 850.3464.

Radicicol–biotin conjugate (15b). In a similar manner as that described above, radicicol–biotin conjugate (**15b**) was obtained in 22% yield by condensation of radicicol 6-(*O*-octanoic acid)oxime (**11b**) with (+)-biotinyl-3,6-dioxaoctanediamine (**14**). **15b**: ^{1}H NMR (CD_3OD, major isomer) δ 7.23 (1H, dd, J = 16.1, 11.0 Hz), 6.72 (1H, d, J = 16.1 Hz), 6.43 (1H, s), 6.15 (1H, dd, J = 11.0, 10.6 Hz), 5.59 (1H, dd, J = 10.6, 3.7 Hz), 5.29 (1H, m), 4.47 (1H, ddd, J = 7.9, 5.0, 0.9 Hz), 4.28 (1H, dd, J = 7.9, 4.4 Hz), 4.16–4.07 (2H, m), 3.91 (1H, d, J = 16.0 Hz), 3.78 (1H, d, J = 16.0 Hz), 3.63–3.51 (8H, m), 3.38–3.33 (5H, m), 3.18 (1H, m), 3.02 (1H, m), 2.91 (1H, dd, J = 12.8, 5.0 Hz), 2.69 (1H, d, J = 12.8 Hz), 2.43 (1H, ddd, J = 14.1, 4.0, 4.0 Hz), 2.23–2.17 (4H, m), 1.76–1.31 (17H, m), 1.52 (3H, d, J = 6.6 Hz); HRFAB-MS m/z 876.3622 $[M-H]^-$, calcd for $C_{42}H_{59}O_{11}N_5S$ ^{35}Cl m/z 876.3620.

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