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Synthesis and Structure–Activity Relationships of 5,6,7,8-Tetrahydropyrido[3,4-*b*]pyrazine-based Hydroxamic Acids as HB-EGF Shedding Inhibitors

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Abstract—HB-EGF Shedding inhibitors have been expected to become effective medicines for skin diseases caused by the proliferation of keratinocytes. In order to discover novel HB-EGF shedding inhibitors and clarify their structure–activity relationships, 5,6,7,8-tetrahydronaphthylidine-based hydroxamic acid and 5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-based hydroxamic acids have been synthesized. Among the synthesized compounds, the ethoxyethoxy derivative **3o** and the methoxypropoxy derivative **3p** exhibited much more potent HB-EGF shedding inhibitory activity than CGS 27023A. The structural modification of 5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-based hydroxamic acids enabled us to establish the following structure–activity relationships; the existences of the hydroxamic acid, the sulfonamide, and the phenyl moieties are crucial for a potent HB-EGF shedding inhibitory activity, and the stereochemistry of the alpha carbon of hydroxamic acid is also important. In addition, from the comparison of their HB-EGF shedding inhibitory activities with their MMPs inhibitory activities, we found that the S1′ pocket of the responsible enzyme for HB-EGF shedding is deep unlike that of MMP-1.

Introduction

Keratinocytes are main components in the epidermis, and they play a central role in the barrier function of skin. In various skin diseases, such as psoriasis, atopic disease, and epidermal cancers, the aberrant proliferation of keratinocytes is observed. The proliferation of keratinocytes is driven by a number of growth factors,[1] including epidermal growth factor (EGF), transforming growth factor-α (TGF-α), amphiregulin (AR), heparin-binding epidermal growth factor-like growth factor (HB-EGF), fibroblast growth factor-1 (FGF-1), and hepatocyte growth factor (HGF). Recently, it has been reported that, among these growth factors, HB-EGF[2] might be the most important for the proliferation of keratinocytes.[3] The precursor of HB-EGF (proHB-EGF) is synthesized as a membrane-binding form, and it is shed to give the soluble active form, HB-EGF.[2] It has been reported that a disintegrin and metalloproteinases (ADAMs) are associated with the shedding of HB-EGF.[4] ADAMs consist of more than 30 enzymes, but it is still unclear which enzyme is responsible for HB-EGF shedding. It was already found that *N*-{DL-{2-(hydroxyaminocarbonyl)methyl}-4-methylpentanoyl}-L-3-(2′-naphtyl)-alanyl-L-alanine 2-aminoethylamide (TAPI), a peptide-type matrix metalloproteinase (MMP) inhibitor, inhibited the proteolytic cleavage of proHB-EGF.[5] Moreover, Tokumaru et al. have reported that other peptide-type MMP inhibitors suppressed the proliferation of keratinocytes in both in vitro and in vivo models.[3] Therefore, we have expected that MMP inhibitors would become effective medicines for skin diseases caused by the proliferation of keratinocytes.

In general, peptide-type MMP inhibitors have drawbacks concerning bioavailability, for example poor oral absorption. Such poor pharmacokinetic profiles of

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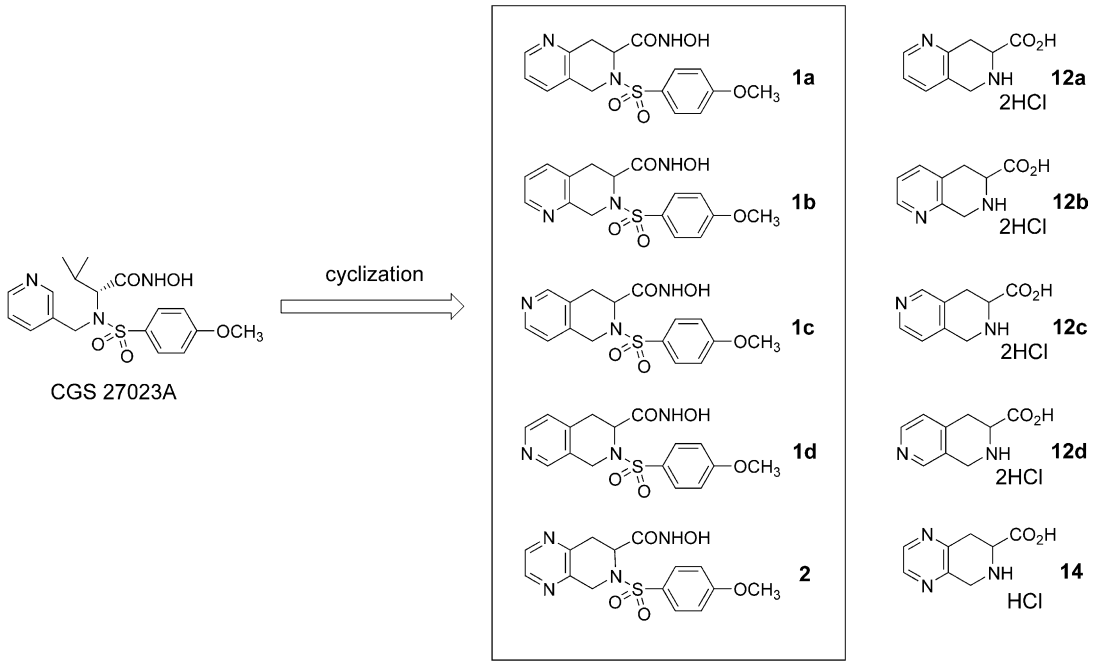


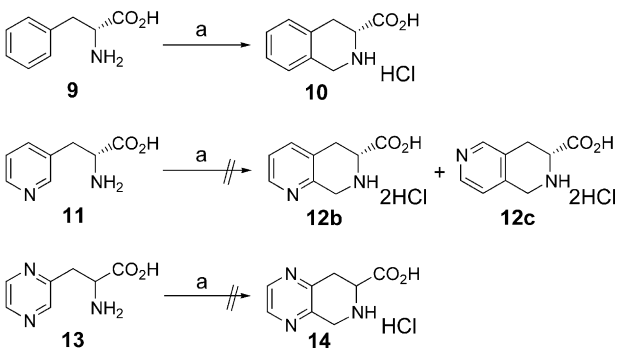
Figure 1. Design of bicyclic compounds from CGS 27023A.

peptide-type MMP inhibitors made us select another type of MMP inhibitors as our lead compound. CGS 27023A, a sulfonamide-type MMP inhibitor, is known to have a better pharmacokinetic profile,[6] so we chose CGS 27023A as our lead compound. The study on the conformation of CGS 27023A by NMR has showed that the pyridymethyl moiety and the isopropyl moiety of CGS 27023A are located close to each other.[7] Based on this information, we designed bicyclic compounds **1a–d** and **2** shown in Figure 1. Then, we synthesized them and evaluated their HB-EGF shedding inhibitory activity in order to discover novel HB-EGF shedding inhibitors with excellent potency. In addition, we tried to clarify their structure–activity relationships (SARs), because no research groups have not reported on the SARs of HB-EGF shedding inhibitors. Moreover, many of the synthesized compounds were tested for MMPs inhibitory activities in order to elucidate whether they are selective HB-EGF shedding inhibitors or not. In this paper, at first, we describe the synthesis of compounds **1a–d** and **2**. Then, the structural requirements for a potent HB-EGF shedding inhibitory activity are presented. From the comparison of the HB-EGF shedding activities with the MMPs inhibitory activities, we describe the S1′ pocket of the responsible enzyme for HB-EGF shedding.

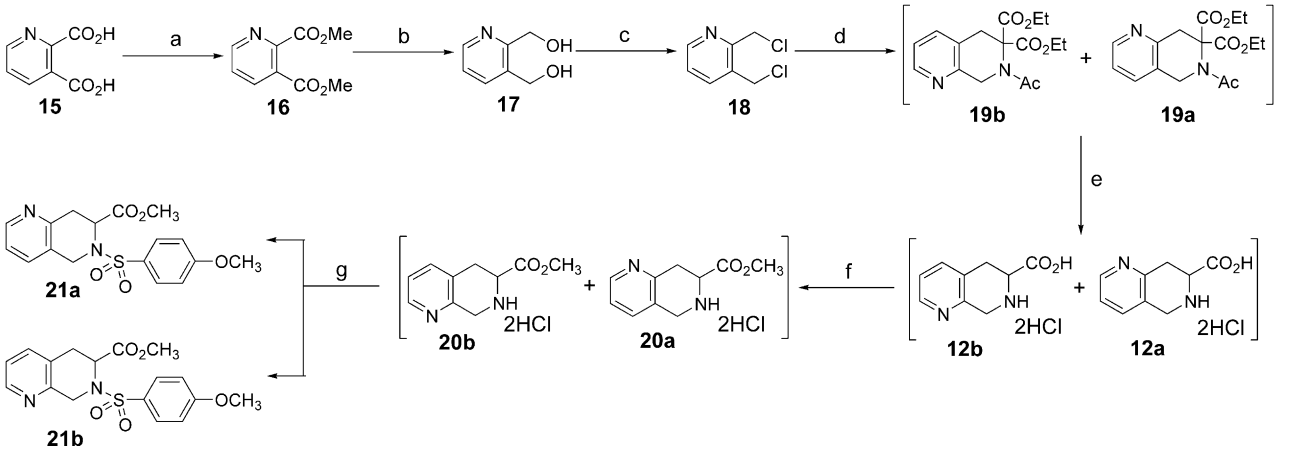
Chemistry

We selected compounds **12a–d** and **14** (Fig. 1) as starting materials for the synthesis of bicyclic compounds **1a–d** and **2**. However, these bicyclic amino acids **12a–d** and **14** had not been synthesized yet. Therefore, first of all, we tried to synthesize them. Compounds **12a–d** and **14** are structurally related to 1,2,3,4-tetrahydroisoqiunoline-3-carboxylic acid **10** (Scheme 1). It is known that compound **10** can be easily synthesized from phenylalanine **9** and formaldehyde in the presence of hydrochloric acid (Pictet–Spengler reaction[8]). We applied the typical reaction condition of Pictet–Spengler reaction to the synthesis of **12b**, **12c**, and **14**. However, the treatment of pyridylalanine or pyrazylalanine with formaldehyde in the presence of hydrochloric acid did not provide the target compounds **12b**, **12c**, and **14** at all.

As a result of exploring alternative synthetic routes for **12a–d** and **14**, we found the synthetic route shown in Scheme 2. At first, 2,3-pyridinedicarboxylic acid (**15**) was converted into 2,3-bis(chloromethyl)pyridine (**18**) by esterification, reduction, and chlorination. The alkylation of diethyl acetamidomalonate with compound **18** in the presence of sodium hydride gave bicyclic diester as a mixture of regioisomers **19a** and **19b**. Then, this mixture was heated with 6 N hydrochloric acid to afford a mixture of bicyclic amino acids **12a** and **12b**. Esterification of this mixture, and subsequent sulfonylation



Scheme 1. Reagents: (a) formaldehyde, hydrochloric acid.

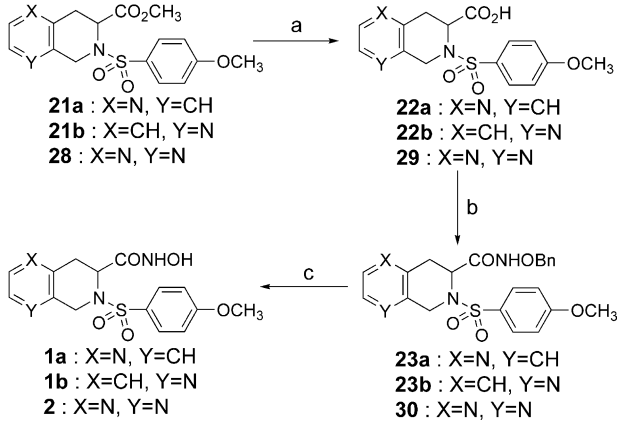


Scheme 2. Reagents: (a) conc H_2SO_4, MeOH; (b) $NaBH_4$; (c) (i) $SOCl_2$; (ii) $NaHCO_3$ aq; (d) diethyl acetamidomalonate, NaH; (e) 6 N HCl; (f) $SOCl_2$, MeOH; (g) 4-methoxybenzenesulfonyl chloride, 4-dimethylaminopyridine.

with 4-methoxybenzenesulfonyl chloride gave two sulfonylamino esters, which were separated by column chromatography to yield compounds **21a** and **21b**. The structures of **21a** and **21b** were confirmed by the measurement of their HMBC spectra.

Hydrolysis of **21a** under an alkaline condition, followed by condensation with *O*-benzylhydroxylamine in the presence of 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDCI) and 1-hydroxybenzotriazole hydrate (HOBt), and hydrogenolysis to give the target compound **1a** (Scheme 3). Compound **1b**, a regioisomer of **1a**, was synthesized from sulfonylamino ester **21b** in the same manner as **1a**. 3,4-Pyridinedicarboxylic acid was converted into compounds **1c** and **1d** in the same manner as 2,3-pyridinedicarboxylic acid (the route is not shown here).

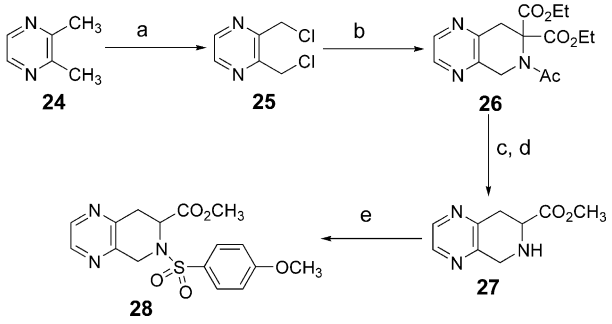
Next, this new synthetic method was applied to the synthesis of **2** with tetrahydropyrido[3,4-*b*]pyrazine skeleton (Scheme 4). To begin with, 2,3-dimethylpyrazine (**24**) was chlorinated into 2,3-bis(chloromethyl)pyrazine (**25**) by treatment with *N*-chlorosuccimide (NCS) in carbon tetrachloride. Then, the cyclization between diethyl acetamidomalonate and compound **25** in the presence of cesium carbonate gave compound **26** with



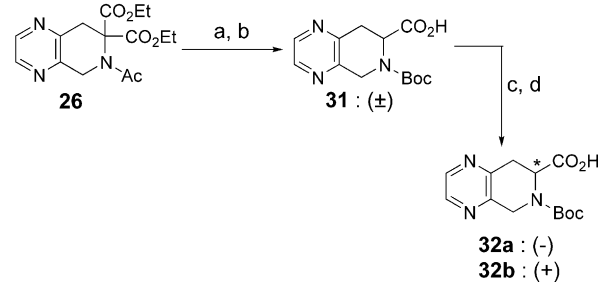
Scheme 3. (Method A). Reagents: (a) NaOH aq; (b) $H_2NOBn{\cdot}HCl$, EDCI, HOBt, Et_3N; (c) Pd/C, H_2.

5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine skeleton. Hydrolysis and decarboxylation of **26** was performed by heating **26** in hydrochloric acid, followed by esterification with thionyl chloride to give amino ester **27**. Sulfonylation of **27** with 4-methoxybenzenesulfonyl chloride afforded sulfonylamino ester **28**, which was converted into the target compound **2** in the same manner as **1a** (Scheme 3).

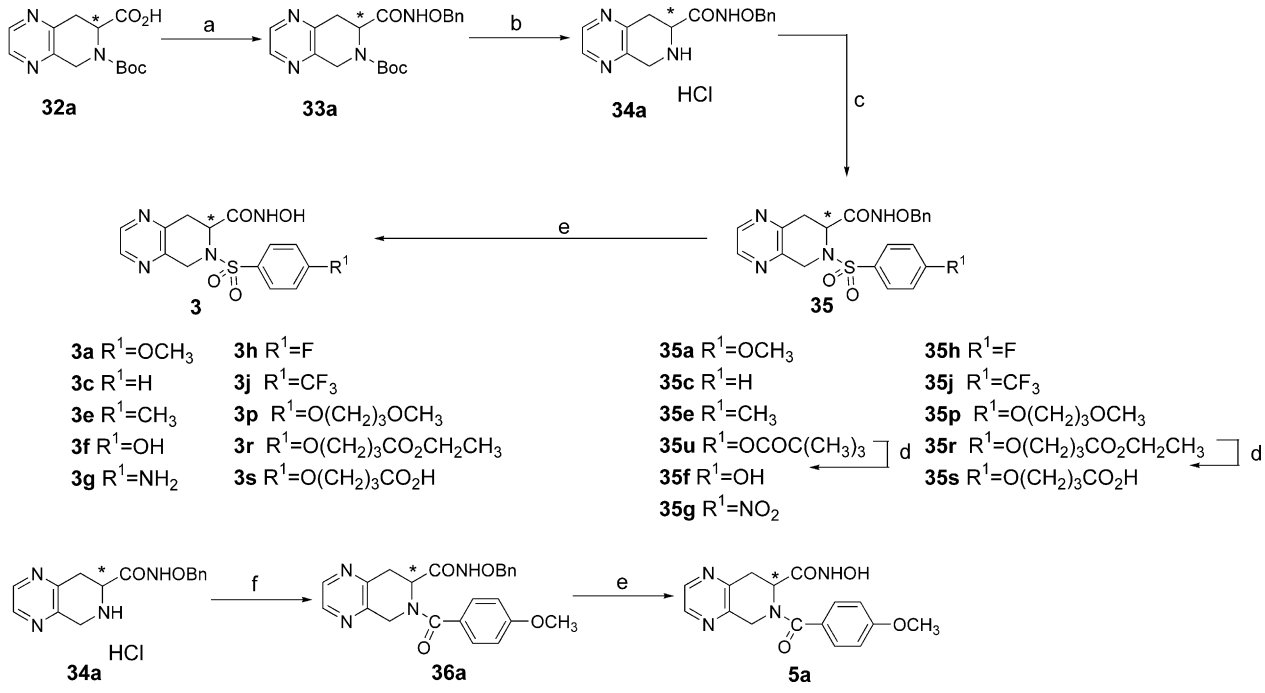
Schemes 5 shows the synthetic routes for optically active derivative of **2** (**3a**) and their related compounds (compounds **3c**, **3e–h**, **3j**, **3p**, **3r–s**, and **5a**) listed in Tables 2 and 3. First of all, diester **26** was heated with 6 N HCl, followed by acylation with di-*tert*-butyl



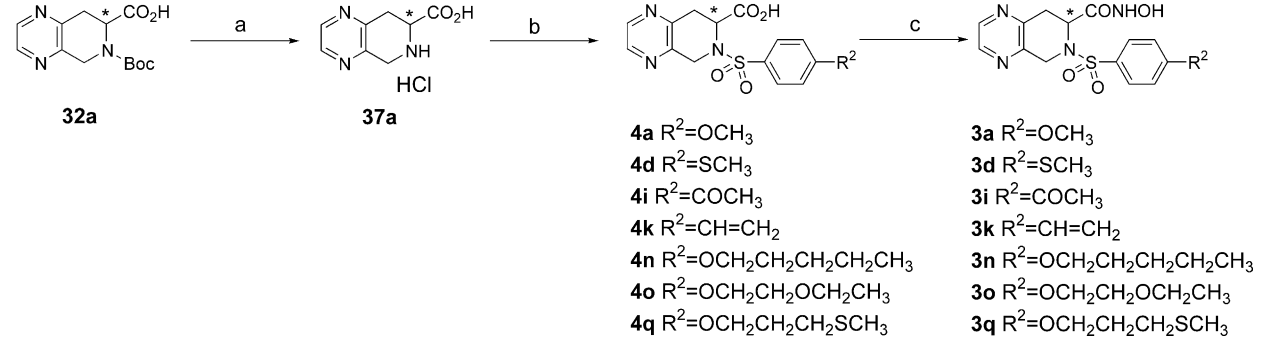
Scheme 4. Reagents: (a) *N*-chlorosuccimide, benzoyl peroxide; (b) diethyl acetamidomalonate, Cs_2CO_3; (c) 6 N HCl; (d) $SOCl_2$, MeOH; (e) 4-methoxybenzenesulfonyl chloride, 4-dimethylaminopyridine.



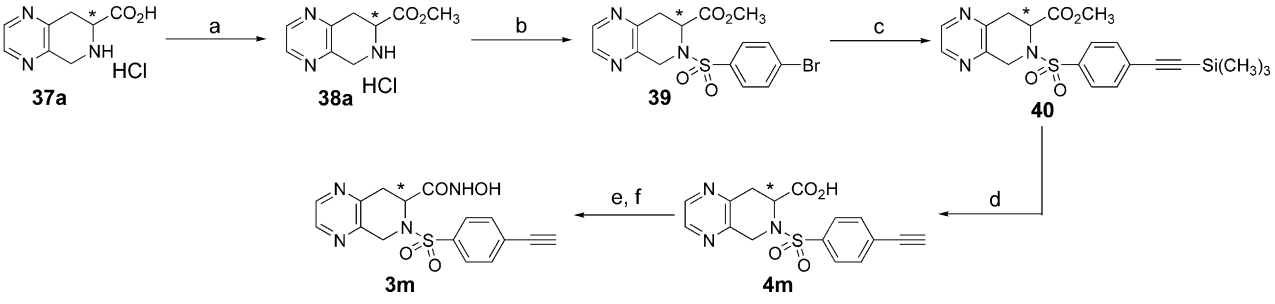
Scheme 5. Reagents: (a) 6 N HCl; (b) di-*tert*-dicarbonate, NaOH; (c) (*S*)-(−)-1-phenylethylamine or (*R*)-(+)-1-phenylethylamine; (d) citric acid.



Scheme 6. (Method B). Reagents: (a) $H_2NOBn{\cdot}HCl$, EDCI, HOBt, Et_3N; (b) 4 N HCl/ethyl acetate solution; (c) benzenesulfonyl chloride or 4-substituted benzenesulfonyl chloride Et_3N; (d) NaOH aq; (e) Pd/C, H_2; (f) 4-methoxybenzoyl chloride, Et_3N.



Scheme 7. (Method C). Reagents: (a) 4 N HCl/ethyl acetate solution; (b) 4-substituted benzenesulfonyl chloride; (c) (i) ClCOCOCl, (ii) H_2NOH aq.

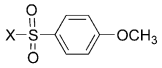


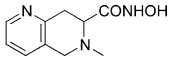
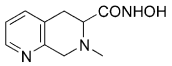
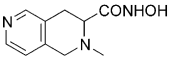
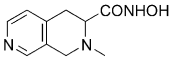
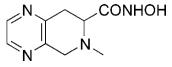
Scheme 8. (Method D). Reagents: (a) $SOCl_2$, MeOH; (b) bromobenzenesulfonyl chloride, Et_3N; (c) Et_3N, Ph_3P, CuI, trimethylsilylacetylene; (d) KOH aq; (e) ClCOCOCl; (f) H_2NOH aq.

dicarbonate to afford (±)-*tert*-butoxycarbonylamino acid **31**, as shown in Scheme 5. Optical resolution of **31** by use of (*S*)-(−)-1-phenylethylamine gave (−)-*tert*-butoxycarbonylamino acid **32a**, and on the other hand, that by use of (*R*)-(+)-1-phenylethylamine gave (+)-*tert*-butoxycarbonylamino acid **32b**.

The target compounds **3a**, **3c**, **3e–h**, **3j**, **3p**, and **3r–s** were prepared from (−)-*tert*-butoxycarbonylamino acid **32a** according to Method B shown in Scheme 6. At first, **32a** was condensed with *O*-benzylhydroxylamine in the presence of EDCI and HOBt to afford *N*-benzyloxy amide **33a**. Removal of the *tert*-butoxycarbonyl (Boc) moiety

Table 1. HB-EGF Shedding inhibitory activity of tetrahydronaphthylidine-based hydroxamic acids **1a–d**, tetrahydropyrido[3,4-*b*]pyrazine-based hydroxamic acid **2**, and CGS 27023A



Compd	X	Synthetic method	Sign of optical rotation	HB-EGF Shedding inhibitory activity[a]
				IC_{50} (μM)[b]
1a		A	±	0.094
1b		A	±	2.5
1c		A	±	0.37
1d		A	±	0.83
2		A	±	0.80
CGS 27023A				1.2

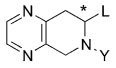
[a]The details of the assay are described in the Experimental.
[b]Concentration required for 50% inhibition of HB-EGF shedding. All values represent the mean of two determinations.

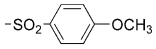
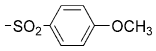
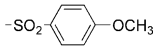
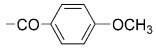
of **33a** by treatment with hydrochloric acid gave the unprotected compound **34a**, which was sulfonylated with various sulfonyl chlorides in the presence of a tertially amine to yield sulfonamides **35**. Finally, hydrogenolysis of **35** in the presence of palladium–carbon (Pd/C) gave the target hydroxamic acids. The hydroxy derivative **3f** and the carboxyl derivative **3s** were synthesized by treating the pivaloyloxy derivative **35u** and the ethyl ester derivative **35r**, respectively, with alkali, followed by hydrogenolysis of the obtained compounds **35f** and **35s**. The hexanesulfonamide derivative **6** was prepared in the same manner as benzenesulfonamide type compounds (the scheme is not shown here). The enantiomer of **3a** (**3b**) was synthesized from (+)-Boc-amino acid **32b** in the same manner as **3a** (the scheme is not shown here). The carboxamide derivative **5a** was prepared from **34a** and 4-methoxybenzoyl chloride via *N*-benzyloxyamide **36a**.

Scheme 7 shows the synthetic route (Method C) for compounds **3a**, **3d**, **3i**, **3k**, **3n**, **3o**, **3q**, and **4a**. The Boc moiety of (−)-Boc-carboxylic acid **32a** was removed by hydrochloric acid treatment to give amino acid **37a**. The sulfonylation of **37a** by various kinds of sulfonyl chloride gave sulfonylamino acids **4**. Finally, the carboxylic acid moiety of **4** was converted into hydroxamic acid moiety via carboxylic acid chloride.

The synthetic route of the ethynyl derivative **3m** is shown in Scheme 8. First, amino acid **37a** was treated

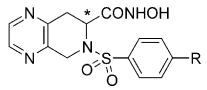
Table 2. HB-EGF Shedding inhibitory activity and MMPs inhibitory activity of tetrahydropyrido[3,4-*b*]pyrazine-based hydroxamic acid and related compounds



Compd	Y	L	Synthetic method	Sign of optical rotation	HB-EGF Shedding inhibitory activity[a]	MMPs Inhibitory activity, K_i (nM)[c]		
					IC_{50} (μM)[b]	MMP-1	MMP-3	MMP-9
3a		-CONHOH	B, C	+	0.35	61	15	45
3b		-CONHOH	B	−	>10[d]	>850	>650	>790
4a		$-CO_2H$	C	+	>10[e]	>850	>650	>790
5a		-CONHOH	B	+	>10[f]	>850	>650	>790
6	$-SO_2-(CH_2)_5CH_3$	-CONHOH	B	+	4.4	560	>650	740
CGS 27023A					1.2	28	9.8	36

[a]The details of the assay are described in the Experimental.
[b]Concentration required for 50% inhibition of HB-EGF shedding. All values represent the mean of at least two determinations.
[c]K_i Values were calculated from percent inhibition and K_m value of each MMPs to the substrate. All values represent the mean of two determinations. The details of the assays are described in the Experimental.
[d]15% Inhibition at 10 μM.
[e]7.9% Inhibition at 10 μM.
[f]<5% Inhibition at 10 μM.

Table 3. HB-EGF Shedding inhibitory activity and MMPs inhibitory activities of tetrahydropyrido[3,4-*b*]pyrazine-based hydroxamic acid and related compounds



Compd	R	Synthetic method	Sign of optical rotation	HB-EGF Shedding inhibitory activity[a]	MMPs Inhibitory activity, K_i (nM)[c]		
				IC_{50} (μM)[b]	MMP-1	MMP-3	MMP-9
3a	$-OCH_3$	B, C	+	0.35	61	15	45
3c	–H	B	+	0.51	190	270	180
3d	$-SCH_3$	C	+	1.5	170	31	250
3e	$-CH_3$	B	+	2.7	150	300	130
3f	–OH	B	+	0.30	42	120	52
3g	$-NH_2$	B	+	1.8	460	220	390
3h	–F	B	+	0.81	15	>650	370
3i	$-COCH_3$	C	+	2.2	22	4.8	15
3j	$-CF_3$	B	+	40	20	>650	210
3k	$-CH=CH_2$	C	+	1.1	35	46	44
3m	$-C\equiv CH$	D	+	0.13	14	14	5.1
3n	$-OCH_2CH_2CH_2CH_2CH_3$	C	+	0.12	290	0.20	0.70
3o	$-OCH_2CH_2OCH_2CH_3$	C	+	0.036	>850	77	43
3p	$-OCH_2CH_2CH_2OCH_3$	B	+	0.053	380	9.2	5.4
3q	$-OCH_2CH_2CH_2SCH_3$	C	+	0.10	110	0.90	0.60
3r	$-OCH_2CH_2CH_2CO_2CH_2CH_3$	B	+	1.1	260	4.8	29
3s	$-OCH_2CH_2CH_2CO_2H$	B	+	95	>850	500	200

[a]The details of the assay are described in the Experimental.
[b]Concentration required for 50% inhibition of HB-EGF shedding. All values represent the mean of at least two determinations.
[c]K_i Values were calculated from percent inhibition and K_m value of each MMPs to the substrate. All values represent the mean of two determinations. The details of the assays are described in the Experimental.

with thionyl chloride in methanol to give methyl ester **38a**. The sulfonylation of **38a** with 4-bromobenzenesulfonyl chloride to give sulfonylamino ester **39**. The cross coupling between **39** and trimethylsilylacetylene in the presence of palladium catalysis gave the trimethylsilylethynyl derivative **40**. The removal of trimethylsilyl and methyl group of **40** was simultaneously accomplished by treatment with alkali to give ethynyl carboxylic acid **4m**. Finally, **4m** was treated with oxalyl chloride, followed by addition of hydroxylamine to give hydroxamic acid **3m**.

HB-EGF Shedding inhibitory activity and MMPs inhibitory activities

All the hydroxamic acids synthesized here were evaluated in HB-EGF shedding inhibition assay by use of HT1080 cell. In addition, many of them were tested in MMPs inhibition assays. The results are shown in Tables 1–3.

Among four tetrahydronaphthylidine-base hydroxamic acids **1a–d**, compound **1a** exhibited the most potent HB-EGF shedding inhibitory activity (Table 1). Interestingly, compound **1b**, one of regioisomers of **1a**, showed 25 times weaker inhibitory activity than **1a**. Tetrahydropyrido[3,4-*b*]pyrazine-based hydroxamic acid **2** exhibited a moderate HB-EGF shedding inhibitory activity, which was not so potent as that of **1a**. However, we selected compound **2** as our lead compound, because the synthesis of **1a** was accompanied by the formation of regioisomer. In addition, compound **1a** was not stable in water compared to **2**.

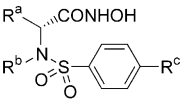
Then, we investigated the HB-EGF shedding inhibitory activities of optically active derivatives of **2** (**3a** and **3b**). Among them, the inhibitory activity of (+)-enantiomer **3a** was more potent than that of CGS 27023A, while the inhibitory activity of (−)-enantiomer **3b** was considerably weaker. This result means that the stereochemistry of the alpha carbon of the hydroxamic acid group is crucial to inhibit HB-EGF shedding. The corresponding carboxylic acid derivative of **3a** (**4a**) did not exhibit a strong inhibitory activity compared to **3a**, indicating that the hydroxamic acid group of **3a** plays an important role in the inhibition of HB-EGF shedding. The conversion of the sulfonamide group of **3a** into carboxamide group (**5a**) caused a large decrease in the inhibitory activity. The conversion of the 4-methoxyphenyl moiety of **3a** into *n*-hexyl moiety (**6**) diminished the inhibitory activity.

The inhibitory activities of compounds **3a**, **3b**, **4a**, **5a**, and **6** against MMP-1, -3, and 9 enabled us to establish the same SARs as those observed in the HB-EGF shedding inhibition. Thus, the existences of the hydroxamic acid, the sulfonamide, and the 4-methoxyphenyl moieties were important for potent MMPs inhibitory activities, and the stereochemistry of the alpha carbon of the hydroxamic acid group was also crucial. The attempt to get a singular crystal of **3a** for X-ray structural analysis resulted in a failure, so we have not determined the absolute configuration of compound **3a** yet. However, Novartis' research group has reported that CGS 27023A (*R* isomer) was much more active as an MMP-3 inhibitor than the other enantiomer (*S* isomer).

Therefore, it was considered that the stereochemistry of the asymmetric carbon of **3a** is *R* configuration.

Next, our attention was focused on the optimization of the substituent on the benzene ring. We synthesized derivatives of **3a** bearing various kinds of substituents on the benzene ring, and evaluated their HB-EGF shedding inhibitory activity. The results are shown in Table 3. Both of the deletion of the methoxy group of **3a** (**3c**) and the deletion of the methyl group from the methoxy group (**3f**) had no influence on the HB-EGF shedding inhibitory activity. On the other hand, the replacement of the oxygen atom of the methoxy group with sulfur atom (**3d**) and the deletion of the oxygen atom from the methoxy group (**3e**) weakened the potency. Amino group is an electron-donating group as well as methoxy group (the σ_p value of methoxy group: −0.27, the σ_p value of amino group: −0.66), but the inhibitory activity of the amino derivative **3g** was not as strong as that of **3a**. Among three compounds having an electron-withdrawing group, the fluoro derivative **3h** exhibited a moderate HB-EGF shedding inhibitory activity, while the acetyl derivative **3i** and the trifluoromethyl derivative **3j** did not show a strong inhibitory activity. Interestingly, the replacement of the methoxy group with ethynyl group (**3m**) improved the inhibitory activity, whereas the replacement of that with ethenyl group (**3k**) weakened the inhibitory activity. It is obscure what property of ethynyl group contributed to the excellent inhibitory activity of **3m**. But, as one possibility, the improved inhibitory activity can be attributed to the straight orientation of the ethynyl group. The elongation of the methoxy group led to the discovery of the most potent HB-EGF shedding inhibitors, including the ethoxyethoxy derivative **3o** (IC_{50} = 36 nM, SD = 21 nM, n = 3) and the methoxypropoxy derivative **3p** (IC_{50} = 53 nM, SD = 17 nM, n = 4), which were much more stronger than CGS 27023A (IC_{50} = 1200 nM, SD = 37 nM, n = 3). The ethyl ester derivative **3r** was a moderately potent inhibitor, but hydrolysis of **3r** resulted in a large reduction in the inhibitory activity (**3s**).

Then, compounds **3c–s** were subjected to the assays for MMPs inhibitory activities. The results are shown in Table 3. Novartis' research group explored the binding mode of CGS 27023A to MMP-3 by use of NMR spectroscopy, and concluded that the 4-methoxyphenyl group of CGS 27023A occupies the S1′ pocket of MMP-3.[7] On the other hand, Agouron's research group performed an X-ray crystallographic analysis of the complex of CGS 25966 (a benzyl analogue of CGS 27023A. See Fig. 2) and MMP-1, and found that CGS 25966 binds to MMP-1 in the same binding mode that CGS 27023A does to MMP-3.[9] Thus, when CGS 25966 binds to MMP-1, its 4-methoxyphenyl group lies in the S1′ pocket of MMP-1. Many research group reported that the S1′ pocket of MMP-1 is comparatively shallow, while the S1′ pockets of MMP-3 and MMP-9 are deep and hydrophobic. If our 5,6,7,8-tetrahydropyrido[3,4-*b*] pyrazine-based hydroxamic acids bind to MMP-1 in the same binding mode as CGS 25966, the compounds bearing a long substituent on the benzene ring, for example **3n**, should not show a strong MMP-1 inhibitory activity because of the steric hindrance between the long substituent and the S1′ pocket. As a result of MMP-1 inhibition assay, all of the lengthy derivatives **3n–r** exhibited K_i values above 100 nM against MMP-1. This result means that our compounds bind to MMP-1 in the same binding mode as CGS 25966.



Compound	R^a	R^b	R^c
CGS 27023A	—$CH(CH_3)_2$	-CH_2-(4-pyridyl)	—OCH_3
CGS 25966	—$CH(CH_3)_2$	-CH_2-(4-pyridyl)	—OCH_3
40	—H	—$CH_2CH(CH_3)_2$	—OCH_3
41	—H	—$CH_2CH(CH_3)_2$	—$OCH_2CH_2CH_2CH_3$
42	—H	—$CH_2CH(CH_3)_2$	—$OCH_2CH_2OCH_2CH_3$

Figure 2. Structure of CGS 27023A and its related compounds.

Novartis' researchers have reported that the elongation of the methoxy group of compound **40** (a compound related to CGS 27023A, see Fig. 2) to butoxy group (**41**) strengthened the MMP-3 inhibitory activity, but that the replacement of the methoxy group of **40** with ethoxyethoxy group (**42**) weakened the inhibitory activity.[6] Among the lengthy derivatives **3n–r**, the pentyloxy derivative **3n** exhibited a potent MMP-3 inhibitory activity whose K_i values were below 1 nM, while the ethoxyethoxy derivative **3o** was less active than the methoxy derivative **3a**. These results were consistent with Novartis' ones. Interestingly, the methoxypropoxy derivative **3p** exhibited a strong MMP-3 inhibitory activity comparable to that of **3a**, and moreover, the methylthiopropoxy derivative **3q** and the ethoxycarbonylpropoxy derivative **3r** showed more potent MMP-3 inhibitory activity than **3a**. The MMP-9 inhibitory activities of **3n–r** allowed us to establish the same SAR as that from the MMP-3 inhibitory activities. Thus, the ethoxyethoxy derivative **3o** was a poor MMP-9 inhibitor compared to **3n** and **3p–r**.

Both of compounds **3o** and **3p** have an alkoxyalkoxy group, but the MMP-3 and MMP-9 inhibitory activities of **3o** were less potent than those of **3p**. Novartis' researchers have reported that the low inhibitory activity of **42** against MMP-3 must be due to its low hydrophobicity compared to that of the butoxy derivative **41**.[6] However, the calculated logP (ClogP) value of **3o** was a little greater than that of **3p** (**3o**: −0.288 versus **3p**: −0.434), meaning that the hydrophobicity of **3o** is higher than that of **3p**. Therefore, the poor MMP-3 and MMP-9 inhibitory activities of **3o** are not due to the low hydrophobicity of **3o**. The only structural difference between **3o** and **3p** is the position of oxygen atom in the alkoxyalkoxy group, so it can be considered that the difference in oxygen position is associated with the difference in the MMP-3 and MMP-9 inhibitory activities between **3o** and **3p**.

As mentioned above, all of the lengthy derivatives **3n–r** showed a strong HB-EGF shedding activity. On the other hand, all of **3n–r** showed a week MMP-1 inhibitory activity. The responsible enzyme for HB-EGF shedding has not been identified yet, but these results mean that the S1′ pocket of the HB-EGF shedding responsible enzyme is deep unlike that of MMP-1.

Through HB-EGF shedding inhibition and MMPs inhibition assays, we discovered three kinds of HB-EGF shedding inhibitors which are different in the selectivity. First one is selective-type HB-EGF shedding inhibitor, such as **3o**, whose MMPs inhibitory activities were comparatively weak compared to its potent HB-EGF shedding inhibitory activity. Second one is broad-type HB-EGF shedding inhibitor, such as **3m**, which also showed potent inhibitory activities against all of MMP-1, MMP-3, and MMP-9. Final one is HB-EGF shedding inhibitor with potent MMP-3 and MMP-9 inhibitory activity, such as **3n**. Recently, Tokumaru et al. have reported that HB-EGF plays a crucial role in the proliferation of keratinocytes,[3] which means that HB-EGF shedding inhibitors would become effective medicines for skin diseases caused by the proliferation of keratinocytes, such as psoriasis. On the other hand, the effect of each MMP on the proliferation of keratinocytes has not been clarified sufficiently yet. Therefore, all of the above three types of HB-EGF shedding inhibitors will be characterized in both in vitro keratinocytes proliferation inhibition test and in vivo psoriasis model.

In conclusion, we succeeded in the discovery of potent HB-EGF shedding inhibitors with 5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine skeleton, such as compounds **3o** and **3p**. In addition, we clarified their SARs, which would be very useful for design of more effective HB-EGF shedding inhibitors.

Experimental

Melting points were determined on a Yamato MR-21 capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra of all the compounds synthesized here were obtained on a Brucker DPX-250 spectrometer at 250 MHz (tetramethylsilane as an internal standard). MALDI-TOF MS spectra were obtained on a PerSeptive Biosystems Voyager-DE RP spectrometer. Column chromatography was performed using silica gel (YMC-GEL SIL-60A) under medium pressure. No attempt was made to maximize the yields.

Dimethyl 2,3-pyridinedicarboxylate (16)

Concentrated sulfuric acid (10 mL) was added dropwise in 5 min to a mixture of 2,3-pyridinedicarboxylic acid (25.0 g, 150 mmol) and methanol (100 mL) under ice-cooling, and then the resulting mixture was refluxed for 20 h. After cooling, the reaction mixture was concentrated in vacuo. A saturated solution of sodium hydrogen carbonate was added to the obtained residue carefully until pH showed 8, and then the whole was extracted with ethyl acetate. The organic layer was washed with water, dried over magnesium sulfate, and concentrated in vacuo to give the title compound as a brown solid (21.0 g, Y = 72%). ^{1}H NMR ($CDCl_3$) δ 3.94 (s, 3H), 4.00 (s, 3H), 7.50 (dd, J = 4.8, 7.9 Hz, 1H), 8.18 (dd, J = 1.6, 7.9 Hz, 1H), 8.77 (dd, J = 1.6, 4.8 Hz, 1H).

2,3-Bis(hydroxymethyl)pyridine (17). Sodium borohydride (15.0 g, 397 mmol) was added in portions to a solution of dimethyl 2,3-pyridinedicarboxylate (**16**) (15.5 g, 79.4 mmol) in ethanol (200 mL) under ice-cooling, and then the resulting mixture was refluxed for 17 h. After ethanol (200 mL) had been added to the hot reaction mixture, insoluble matter was removed by filtration while the diluted mixture was still hot. The filtrate was concentrated in vacuo. The obtained residue was purified by column chromatography (eluent: chloroform/methanol/triethylamine = 15:5:1) to give the title compound as an orange oil (9.40 g, Y = 85%). ^{1}H NMR ($CDCl_3$) δ 4.52 (s, 2H), 4.60 (s, 2H), 5.63 (br s, 2H), 7.08 (dd, J = 4.9, 7.4 Hz, 1H), 7.62 (d, J = 7.4 Hz, 1H), 8.10–8.40 (m, 1H).

2,3-Bis(chloromethyl)pyridine (18). Thionyl chloride (50 mL) was added to a solution of 2,3-bis(hydroxymethyl)pyridine (**17**) (9.40 g, 67.6 mmol) in dichloromethane (20 mL) under ice-cooling, and then the resulting mixture was stirred at 75 °C for 1 h. After having been cooled to room temperature, the reaction mixture was concentrated in vacuo to give hydrochloride salt of the title compound as a brown powder. The powder was dissolved in a saturated aqueous solution of sodium hydrogen carbonate, and then the solution was extracted with ethyl acetate. The organic layer was washed with a saturated aqueous solution of sodium chloride, dried over magnesium sulfate, and concentrated in vacuo. Finally, the obtained residue was purified by column chromatography (eluent: ethyl acetate) to give the title compound as a dark brown oil (3.80 g, Y = 26%). ^{1}H NMR ($CDCl_3$) δ 4.73 (s, 2H), 4.82 (s, 2H), 7.25 (dd, J = 4.8, 7.8 Hz, 1H), 7.72 (dd, J = 1.6, 7.8 Hz, 1H), 8.52 (dd, J = 1.6, 4.8 Hz, 1H).

Ethyl 6-acetyl-7-ethoxycarbonyl-5,6,7,8-tetrahydro[1,6]-naphthylidine-7-carboxylate (19a) and ethyl 7-acetyl-6-ethoxycarbonyl - 5,6,7,8 - tetrahydro[1,7]naphthylidine - 6-carboxylate (19b). Diethyl acetamidomalonate (4.69 g, 21.6 mmol) and sodium hydride (60%, 860 mg, 21.5 mmol) were successively added at room temperature to a solution of 2,3-bis(chloromethyl)pyridine (**18**) (3.80 g, 21.6 mmol) in dimethylformamide (DMF) (20 mL), and then the resulting mixture was stirred at room temperature for 30 min. Sodium hydride (60%, 860 mg, 21.5 mmol) was further added to the reaction mixture, and then the resulting mixture was stirred at room temperature for 14 h. The reaction mixture was diluted with water (500 mL), and then the resulting mixture was extracted with ethyl acetate. The organic layer was washed with a saturated aqueous solution of sodium chloride, dried over magnesium sulfate, and concentrated in vacuo. The obtained oil was purified with column chromatography (eluent: ethyl acetate/methanol = 20:1) to give a mixture of the title compounds

(**19a**/**19b** = 5:1) as a brown oil (2.50 g, Y = 36%). ^{1}H NMR ($CDCl_3$) δ 1.15–1.30 (m, 6H), 2.30 (s, 3H), 3.47 (s), 3.65 (s), 4.05–4.30 (m, 4H), 4.73 (s), 4.81 (s), 7.15–7.25 (m, 1H), 7.40–7.55 (m, 1H), 8.40–8.55 (m, 1H).

(±)-5,6,7,8-Tetrahydro[1,6]naphthylidine-7-carboxylic acid dihydrochloride (12a) and (±)-5,6,7,8-tetrahydro[1,7]naphthylidine-6-carboxylic acid dihydrochloride (12b). Hydrochloride (6 N, 15 mL) was added to a mixture of ethyl 6-acetyl-7-ethoxycarbonyl-5,6,7,8-tetrahydro[1,6]naphthylidine-7-carboxylate (**19a**) and ethyl 7-acetyl-6-ethoxycarbonyl-5,6,7,8-tetrahydro[1,7]naphthylidine-6-carboxylate (**19b**) (1.80 g, 5.62 mmol), and then the resulting mixture was refluxed for 3 h. After having been cooled to room temperature, the reaction mixture was concentrated in vacuo. Dioxane (150 mL) was added to the obtained residue, and the resulting mixture was concentrated in vacuo to give a mixture of the title compounds (**12a**/**12b** = 5:1) as a brown powder (1.40 g, Y = quant). ^{1}H NMR (DMSO-d_6–D_2O) δ 3.10–3.65 (m, 2H), 4.30–4.60 (m, 3H), 7.42 (dd, J = 4.9, 7.7 Hz), 7.65 (dd, J = 5.1, 7.8 Hz), 7.82 (d, J = 7.7 Hz), 8.08 (d, J = 7.8 Hz), 8.45 (d, J = 4.9 Hz), 8.60 (d, J = 5.1 Hz).

Methyl (±)-5,6,7,8-tetrahydro[1,6]naphthylidine-7-carboxylate dihydrochloride (20a) and methyl (±)-5,6,7,8-tetrahydro[1,7]naphthylidine-6-carboxylate dihydrochloride (20b). Methanol (10 mL) and thionyl chloride (2.00 g, 16.8 mmol) were successively added at room temperature to a mixture of (±)-5,6,7,8-tetrahydro[1,6]naphthylidine-7-carboxylic acid dihydrochloride (**12a**) and (±)-5,6,7,8-tetrahydro[1,7]naphthylidine-6-carboxylic acid dihydrochloride (**12b**) (1.40 g, 5.58 mmol), and then the resulting mixture was refluxed for 20 h. After having been cooled to room temperature, the reaction mixture was concentrated in vacuo. Dioxane (50 mL) was added to the obtained residue, and then the resulting mixture was concentrated in vacuo to give a mixture of the title compounds (**20a**/**20b** = 4:1) as a brown solid (1.50 g, Y = quant). ^{1}H NMR (DMSO-d_6) δ 3.20–3.70 (m, 2H), 3.82 (s), 3.84 (s), 4.35–4.80 (m, 3H), 7.45–7.55 (m), 7.65–7.85 (m), 7.91 (d, J = 7.6 Hz), 8.20 (d, J = 7.7 Hz), 8.56 (d, J = 4.3 Hz), 8.70 (d, J = 4.9 Hz), 10.60 (br s, 3H).

Methyl (±)-6-(4-methoxybenzenesulfonyl)-5,6,7,8-tetrahydro[1,6]naphthylidine- 7-carboxylate (21a) and methyl (±)-7-(4-methoxybenzenesulfonyl)-5,6,7,8-tetrahydro[1,7]naphthylidine-6-carboxylate (21b). 4-Dimethylaminopyridine (2.06 g, 16.9 mmol) and 4-methoxybenzenesulfonly chloride (1.68 g, 8.37 mmol) were successively added at room temperature to a suspension of methyl (±)-5,6,7,8-tetrahydro[1,6]naphthylidine-7-carboxylate dihydrochloride (**20a**) and methyl (±)-5,6,7,8-tetrahydro[1,7]naphthylidine-6-carboxylate dihydrochloride (**20b**) (1.50 g, 5.66 mmol) in DMF (15 mL), and then the resulting mixture was stirred at room temperature for 5 days. After the reaction mixture had been poured into water (200 mL), pH of the resulting mixture was adjusted to 8 by addition of a saturated aqueous solution of sodium hydrogen carbonate. The neutralized mixture was extracted with ethyl acetate. The organic layer was washed with a saturated aqueous solution of sodium chloride, dried over magnesium sulfate, and concentrated in vacuo. The obtained oil was purified by column chromatography (eluent: ethyl acetate) to give the title compounds, methyl (±)-6-(4-methoxybenzenesulfonyl)-5,6,7,8-tetrahydro[1,6]naphthylidine-7-carboxylate (**21a**) as a colorless oil (1.00 g), and methyl (±)-7-(4-methoxybenzenesulfonyl)-5,6,7,8-tetrahydro[1,7]naphthylidine-6-carboxylate (**21b**) as a colorless oil (200 mg).

Physicochemical data of 21a. ^{1}H NMR ($CDCl_3$) δ 3.30–3.50 (m, 2H), 3.51 (s, 3H), 3.87 (s, 3H), 4.57 (d, J = 15.8 Hz, 1H), 4.79 (d, J = 15.8 Hz, 1H), 5.10–5.20 (m, 1H), 6.98 (d, J = 8.8 Hz, 2H), 7.14 (dd, J = 4.7, 7.8 Hz, 1H), 7.35–7.45 (m, 1H), 7.80 (d, J = 8.8 Hz, 2H), 8.35–8.50 (m, 1H). Anal. calcd for $C_{17}H_{18}N_2O_5S$: C, 56.34; H, 5.01; N, 7.73; found: C, 56.11; H, 4.75; N, 7.90.

Physicochemical data of 21a. ^{1}H NMR ($CDCl_3$) δ 3.10–3.35 (m, 2H), 3.42 (s, 3H), 3.84 (s, 3H), 4.48 (d, J = 16.5 Hz, 1H), 4.83 (d, J = 16.5 Hz, 1H), 5.10 (dd, J = 3.0, 5.6 Hz, 1H), 6.96 (d, J = 8.9 Hz, 2H), 7.09 (dd, J = 4.7, 7.7 Hz, 1H), 7.41 (d, J = 7.7 Hz, 1H), 7.79 (d, J = 8.9 Hz, 2H), 8.39 (d, J = 4.7 Hz, 1H). Anal. calcd for $C_{17}H_{18}N_2O_5S$: C, 56.34; H, 5.01; N, 7.73; found: C, 56.31; H, 5.09; N, 7.80.

Method A: (±)-6-(4-methoxybenzenesulfonyl)-5,6,7,8-tetrahydro[1,6]naphthylidine- 7-carboxylic acid hydrochloride (22a). An aqueous solution of sodium hydroxide (0.5 N, 11.0 mL, 5.52 mmol) was added to a solution of methyl (±)-6-(4-methoxybenzenesulfonyl)-5,6,7,8-tetrahydro[1,6]naphthylidine-7-carboxylate (**21a**) (1.00 g, 2.76 mmol) in dioxane (11 mL), and then the resulting mixture was stirred at room temperature for 25 h. pH of the reaction mixture was adjusted to 2 by addition of concentrated hydrochloric acid. The resulting mixture was concentrated in vacuo. Dioxane (30 mL) was added to the obtained residue, and then the resulting mixture was concentrated in vacuo to dryness. The obtained residue was stirred with a mixture of chloroform and methanol (10:1), and then insoluble matter was removed by filtration. The filtrate was concentrated in vacuo to give the title compound as an yellow solid (1.10 g, Y = quant). ^{1}H NMR (DMSO-d_6) δ 3.05–3.50 (m, 2H), 3.81 (s, 3H), 4.46 (d, J = 16.4 Hz, 1H), 4.71 (d, J = 16.4 Hz, 1H), 4.98 (dd, J = 3.0, 6.3 Hz, 1H), 7.06 (d, J = 8.8 Hz, 2H), 7.30 (dd, J = 4.8, 7.8 Hz, 1H), 7.65–7.75 (m, 1H), 7.77 (d, J = 8.8 Hz, 2H), 8.35–8.45 (m, 1H). Anal. calcd for $C_{16}H_{16}N_2O_5S{\cdot}0.5H_2O$: C, 53.77; H, 4.79; N, 7.84; found: C, 54.02; H, 5.08; N, 8.15.

(±)-*N*-Benzyloxy-6-(4-methoxybenzenesulfonyl)-5,6,7,8-tetrahydro[1,6]naphthylidine-7-carboxamide (23a). EDCI (997 mg, 5.20 mmol) and HOBt (796 mg, 5.20 mmol) were added to a solution of (±)-6-(4-methoxybenzenesulfonyl)-5,6,7,8-tetrahydro[1,6]naphthylidine-7-carboxylic acid hydrochloride (**22a**) (1.00 g, 2.60 mmol) in DMF (10 mL), and then the resulting mixture was stirred at room temperature for 15 min. A mixture of *O*-benzylhydroxylamine hydrochloride (830 mg, 5.20 mmol), triethylamine (789 mg, 7.80 mmol), and

DMF (10 mL) was added to the reaction mixture, and then the resulting mixture was stirred at room temperature for 64 h. The reaction mixture was poured into water (500 mL), and then the resulting mixture was extracted with ethyl acetate. The organic layer was washed with water, dried over magnesium sulfate, and concentrated in vacuo. The obtained residue was purified by column chromatography (eluent: chloroform/methanol = 10:1) to give an yellow powder. The powder was stirred with ethyl acetate (20 mL) for 10 min, and insoluble solid was collected by filtration to give the title compound as an yellow powder (590 mg, Y = 50%). ^{1}H NMR (DMSO-d_6) δ 2.85–3.10 (m, 2H), 3.79 (s, 3H), 4.45–4.75 (m, 5H), 7.04 (d, J = 8.7 Hz, 2H), 7.10–7.25 (m, 1H), 7.25–7.45 (m, 5H), 7.56 (d, J = 7.9 Hz, 1H), 7.74 (d, J = 8.7 Hz, 2H), 8.25–8.35 (m, 1H), 11.43 (s, 1H). Anal. calcd for $C_{23}H_{23}N_3O_5S$: C, 60.91; H, 5.11; N, 9.27; found: C, 60.78; H, 5.02; N, 9.39.

(±)-*N*-Hydroxy-6-(4-methoxybenzenesulfonyl)-5,6,7,8-tetrahydro[1,6]naphthylidine-7-carboxamide (1a). (±)-*N*-Benzyloxy-6-(4-methoxybenzenesulfonyl)-5,6,7,8-tetrahydro[1,6]naphthylidine-7-carboxamide (**23a**) (560 mg, 1.23 mmol) was dissolved in a mixture of methanol (45 mL) and dioxane (90 mL) with heating. A mixture of Pd/C (10%, 1.31 g) and dioxane (30 mL) was added to the solution, and then the resulting mixture was stirred at room temperature under hydrogen atmosphere for 17 h. The reaction mixture was diluted with methanol (500 mL), and then Pd/C was removed by filtration. The obtained filtrate was concentrated in vacuo, and then the obtained residue was purified by HPLC (eluent: water/acetonitrile = 2:1) to give the title compound as a colorless powder (210 mg, Y = 47%). ^{1}H NMR (DMSO-d_6) δ 2.90–3.05 (m, 2H), 3.81 (s, 3H), 4.50–4.75 (m, 3H), 7.02 (d, J = 8.9 Hz, 2H), 7.15 (dd, J = 4.8, 7.8 Hz, 1H), 7.50–7.60 (m, 1H), 7.72 (d, J = 8.9 Hz, 2H), 8.20–8.35 (m, 1H), 8.84 (s, 1H), 10.79 (s, 1H). Anal. calcd for $C_{16}H_{17}N_3O_5S{\cdot}0.5H_2O$: C, 51.60; H, 4.87; N, 11.28; found: C, 51.87; H, 4.91; N, 11.24. MALDI-TOF MS (M_r = 363.39): 364 $[M+H]^+$, 386 $[M+Na]^+$, 402 $[M+K]^+$.

Compound **1b** was synthesized from compound **21b** via compounds **22b** and **23b** in the same manner as compound **1a.** The physicochemical data of compounds **22b**, **23b**, and **1b** are shown below.

(±)-7-(4-Methoxybenzenesulfonyl)-5,6,7,8-tetrahydro[1,7]naphthylidine-6-carboxylic acid hydrochloride (22b). A colorless powder. ^{1}H NMR (DMSO-d_6) δ 3.00–3.30 (m, 2H), 3.82 (s, 3H), 4.54 (d, J = 17.1 Hz, 1H), 4.78 (d, J = 17.1 Hz, 1H), 4.94 (dd, J = 2.5, 6.3 Hz, 1H), 7.07 (d, J = 8.9 Hz, 2H), 7.44 (dd, J = 5.1, 7.6 Hz, 1H), 7.77 (d, J = 8.9 Hz, 2H), 7.89 (d, J = 7.6 Hz, 1H), 8.45–8.55 (m, 1H). Anal. calcd for $C_{16}H_{16}N_2O_5S{\cdot}0.5H_2O$: C, 53.77; H, 4.79; N, 7.84; found: C, 53.99; H, 5.04; N, 7.54.

(±)-*N*-Benzyloxy-7-(4-methoxybenzenesulfonyl)-5,6,7,8-tetrahydro[1,7]naphthylidine-6-carboxamide (23b). A colorless powder. ^{1}H NMR ($CDCl_3$) δ 2.64 (dd, J = 6.5, 15.9 Hz, 1H), 3.10–3.35 (m, 1H), 3.79 (s, 3H), 4.42 (d, J = 16.7 Hz, 1H), 4.55–4.85 (m, 4H), 6.85 (d, J = 9.0 Hz, 2H), 7.04 (dd, J = 4.8, 7.7 Hz, 1H), 7.20–7.40 (m, 6H), 7.67 (d, J = 9.0 Hz, 2H), 8.30–8.40 (m, 1H), 9.59 (s, 1H). Anal. calcd for $C_{23}H_{23}N_3O_5S$: C, 60.91; H, 5.11; N, 9.27; found: C, 60.90; H, 4.89; N, 9.20.

(±)-*N*-Hydroxy-7-(4-methoxybenzenesulfonyl)-5,6,7,8-tetrahydro[1,7]naphthylidine-6-carboxamide (1b). A colorless powder. ^{1}H NMR (DMSO-d_6) δ 2.90–3.00 (m, 2H), 3.81 (s, 3H), 4.45–4.70 (m, 3H), 7.04 (d, J = 8.9 Hz, 2H), 7.15 (dd, J = 5.0, 7.6 Hz, 1H), 7.45–7.55 (m, 1H), 7.72 (d, J = 8.9 Hz, 2H), 8.25–8.40 (m, 1H), 8.82 (s, 1H), 10.78 (s, 1H). Anal. calcd for $C_{16}H_{17}N_3O_5S{\cdot}0.5H_2O$: C, 51.60; H, 4.87; N, 11.28; found: C, 51.63; H, 4.84; N, 11.44. MALDI-TOF MS (M_r = 363.39): 364 $[M+H]^+$, 386 $[M+Na]^+$, 402 $[M+K]^+$.

Compounds **1c** and **1d** were synthesized from 3,4-pyridinedicarboxylic acid in the same manner as **1a** and **1b**. The physicochemical data of compounds **1c** and **1d** are shown below.

(±)-*N*-Hydroxy-6-(4-methoxybenzenesulfonyl)-5,6,7,8-tetrahydro[2,6]naphthylidine-7-carboxamide (1c). A colorless powder. ^{1}H NMR (DMSO-d_6) δ 2.75–3.10 (m, 2H), 3.80 (s, 3H), 4.40–4.75 (m, 3H), 7.02 (d, J = 8.9 Hz, 2H), 7.09 (d, J = 5.0 Hz, 1H), 7.73 (d, J = 8.9 Hz, 2H), 8.25 (d, J = 5.0 Hz, 1H), 8.34 (s, 1H), 8.85 (s, 1H), 10.76 (s, 1H). Anal. calcd for $C_{16}H_{17}N_3O_5S{\cdot}0.5H_2O$: C, 51.60; H, 4.87; N, 11.28; found: C, 51.82; H, 4.99; N, 11.28.

(±)-*N*-Hydroxy-7-(4-methoxybenzenesulfonyl)-5,6,7,8-tetrahydro[2,7]naphthylidine-6-carboxamide (1d). A colorless powder. ^{1}H NMR (DMSO-d_6) δ 2.80–3.30 (m, 2H), 3.82 (s, 3H), 4.50–4.90 (m, 3H), 7.05 (d, J = 8.9 Hz, 2H), 7.42 (d, J = 5.0 Hz, 1H), 7.74 (d, J = 8.9 Hz, 2H), 8.20–8.60 (m, 2H), 8.83 (br s, 1H), 10.83 (s, 1H). Anal. calcd for $C_{16}H_{17}N_3O_5S{\cdot}0.5H_2O$: C, 51.60; H, 4.87; N, 11.28; found: C, 51.62; H, 4.86; N, 11.53.

2,3-Bis(chloromethyl)pyrazine (25). *N*-Chlorosuccimide (205 g, 1.54 mol) and benzoyl peroxide (3.00 g) were added to a solution of 2,3-dimethylpyrazine (75.0 g, 0.720 mol) in carbon tetrachloride (750 mL), and then the resulting mixture was refluxed for 21 h. After removal of precipitating solid by filtration, the filtrate was concentrated in vacuo. The obtained oil was purified by column chromatography (eluent: *n*-hexane/acetone = 10:1) to give the title compound as a pale brown oil (55.7 g, Y = 44%). ^{1}H NMR ($CDCl_3$) δ 4.86 (s, 4H), 8.54 (s, 2H).

Ethyl 6-acetyl-7-ethoxycarbonyl-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxylate (26). Cesium carbonate (514.0 g, 1.58 mol) and diethyl acetamidomalonate (171.4 g, 0.789 mol) were successively added in potions at 70 °C to a solution of 2,3-bis(chloromethyl)pyrazine (25) (140.0 g, 0.791 mol) in acetonitrile (2500 mL) with vigorous stirring. After completion of the addition, the mixture was stirred at reflux temperature for 4 h. Cesium carbonate (80.0 g, 0.246 mol) was further added to the reaction mixture, and then the resulting mixture was refluxed for 1 h with vigorous stirring. After the reaction mixture had been cooled to room temperature, insoluble matter was removed by filtration. The insoluble matter

was washed with ethyl acetate (2600 mL × 2). The obtained filtrate and wash were combined, and concentrated in vacuo. The obtained oil was dissolved in ethyl acetate (200 mL), and the resulting solution was allowed to stand at room temperature overnight. After removal of precipitating solid by filtration, the filtrate was concentrated in vacuo. The obtained oil was purified by column chromatography (eluent: first, *n*-hexane/ethyl acetate = 1:1, then 1:5) to give the title compound as a red oil (152.3 g, Y = 60%). ^{1}H NMR ($CDCl_3$) δ 1.21 (t, J = 7.1 Hz, 6H), 2.31 (s, 3H), 3.70 (s, 2H), 4.10–4.40 (m, 4H), 4.85 (s, 2H), 8.44 (d, J = 2.6 Hz, 1H), 8.46 (d, J = 2.6 Hz, 1H). Anal. calcd for $C_{16}H_{19}N_3O_5$: C, 56.07; H, 5.96; N, 13.08; found: C, 56.10; H, 6.12; N, 13.01.

Methyl (±)-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxylate hydrochloride (27). A mixture of 6 N hydrochloric acid (40 mL) and ethyl 6-acetyl-7-ethoxycarbonyl-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxylate (**26**) (3.60 g, 11.2 mmol) was refluxed for 1 h. After the reaction mixture had been concentrated in vacuo to dryness, methanol (30 mL) was added to the obtained residue. To the resulting mixture was added dropwise thionyl chloride (3.0 mL) under ice-cooling, and then the resulting mixture was refluxed for 5 h. The reaction mixture was concentrated in vacuo to give the title compound as a colorless solid (2.40 g, Y = 93%). ^{1}H NMR (DMSO-d_6) δ 3.30–3.50 (m, 2H), 3.85 (s, 3H), 4.30–4.50 (m, 2H), 4.60–4.70 (m, 1H), 8.50–8.70 (m, 2H). Anal. calcd for $C_9H_{12}ClN_3O_2 \cdot 1.0H_2O$: C, 43.64; H, 5.70; N, 16.97; found: C, 43.59; H, 5.55; N, 16.69.

Methyl (±)-6-(4-methoxybenzenesulfonyl)-5,6,7,8-tetrahydropyrido[3,4-*b*] pyrazine-7-carboxylate (28). 4-Dimethylaminopyridine (0.70 g, 5.73 mmol) and 4-methoxybenzenesulfonyl chloride (0.60 g, 2.90 mmol) were successively added to a solution of methyl (±)-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxylate (**27**) (0.64 g, 2.79 mmol) in DMF (10 mL), and then the resulting mixture was stirred at room temperature overnight. The reaction mixture was acidified with an aqueous solution citric acid to pH 3, and then extracted with ethyl acetate. The organic layer was washed with water and a saturated aqueous solution of sodium chloride, and dried over magnesium sulfate. After removal of magnesium sulfate by filtration, the filtrate was concentrated in vacuo, and the obtained residue was purified by TLC (developing solvent: *n*-hexane/ethyl acetate = 1:1) to give the title compound as an yellow oil (220 mg, Y = 22%). ^{1}H NMR (CDCl3) δ 3.30–3.50 (m, 2H), 3.47 (s, 3H), 3.87 (s, 3H), 4.54 (d, J = 16.9 Hz, 1H), 4.90 (d, J = 16.9 Hz, 1H), 5.19 (dd, J = 2.8, 5.9 Hz, 1H), 6.97 (d, J = 8.9 Hz, 2H), 7.79 (d, J = 8.9 Hz, 2H), 8.39 (s, 2H). Anal. calcd for C16H17N3O5S: C, 52.88; H, 4.72; N, 11.56; found: C, 52.64; H, 4.77; N, 11.39.

Compound **2** was synthesized from compound **28** via compounds **29** and **30** in the same manner as compound **1a.** The physicochemical data of compounds **29**, **30**, and **2** are shown below.

(±)-6-(4-Methoxybenzenesulfonyl)-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxylic acid (29). A colorless powder. ^{1}H NMR ($CDCl_3$) δ 3.41 (d, J = 4.3 Hz, 2H), 3.87 (s, 3H), 4.57 (d, J = 17.0 Hz, 1H), 4.88 (d, J = 17.0 Hz, 1H), 5.22 (t, J = 4.3 Hz, 1H), 6.96 (d, J = 8.9 Hz, 2H), 7.81 (d, J = 8.9 Hz, 2H), 8.38 (s, 2H). Anal. calcd for $C_{15}H_{15}N_3O_5S$: C, 51.57; H, 4.33; N, 12.03; found: C, 51.90; H, 4.33; N, 11.91.

(±)-*N*-Benzyloxy-6-(4-methoxybenzenesulfonyl)-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxamide (30). A colorless powder. ^{1}H NMR ($CDCl_3$) δ 2.86 (dd, J = 6.1, 17.2 Hz, 1H), 3.44 (d, J = 17.2 Hz, 1H), 3.83 (s, 3H), 4.40 (d, J = 18.0 Hz, 1H), 4.70–5.00 (m, 4H), 6.87 (d, J = 8.8 Hz, 2H), 7.37 (s, 5H), 7.66 (d, J = 8.8 Hz, 2H), 8.33 (s, 2H), 9.17 (s, 1H). Anal. calcd for $C_{22}H_{22}N_4O_5S$: C, 58.14; H, 4.88; N, 12.33; found: C, 58.01; H, 4.89; N, 12.10.

(±)-*N*-Hydroxy-6-(4-methoxybenzenesulfonyl)-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxamide (2). A colorless powder. ^{1}H NMR (DMSO-d_6) δ 2.95 (dd, J = 2.0, 17.4 Hz, 1H), 3.16 (d, J = 7.0, 17.4 Hz, 1H), 3.82 (s, 3H), 4.59 (d, J = 16.8 Hz, 1H), 4.67 (d, J = 16.8 Hz, 1H), 4.78 (dd, J = 2.0, 7.0 Hz, 1H), 7.05 (d, J = 8.9 Hz, 2H), 7.75 (d, J = 8.9 Hz, 2H), 8.30–8.50 (m, 2H), 8.88 (s, 1H), 10.91 (s, 1H). Anal. calcd for $C_{15}H_{16}N_4O_5S \cdot 0.5H_2O$: C, 48.25; H, 4.59; N, 15.00; found: C, 48.11; H, 4.57; N, 14.71.

(±)-6-*tert*-Butoxycarbonyl-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxylic acid (31). A mixture of ethyl 6-acetyl-7-ethoxycarbonyl-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxylate (**26**) (65.0 g, 202 mmol) and 6 N hydrochloric acid (260 mL) was refluxed for 3 h. After having been cooled to room temperature, the reaction mixture was concentrated in vacuo. An aqueous solution of sodium hydroxide (2 N) was added under ice-cooling to the obtained residue until pH of the residue showed above 10. A solution of di-*tert*-butyl dicarbonate (53.0 g, 243 mmol) in dioxane (130 mL) was added dropwise in 20 min to the alkaline mixture, and then the resulting mixture was stirred at room temperature for 3 days. The reaction mixture was diluted with water (260 mL), and then the diluted mixture was washed with diethyl ether twice. The resulting acidic aqueous layer was acidified with 10% aqueous solution of citric acid to pH 3, and then the resulting mixture was extracted with ethyl acetate three times. The organic layers were combined, washed with a saturated aqueous solution of sodium chloride, and dried over magnesium sulfate. Activated charcoal (3.0 g) was added to the solution, and then the resulting mixture was stirred for 5 min. After removal of activated charcoal by filtration, the filtrate was concentrated in vacuo. Diisopropyl ether (100 mL) was added to the obtained residue, and the resulting crystals were collected by filtration to give the title compound as a brown powder (24.6 g, Y = 44%). ^{1}H NMR ($CDCl_3$) δ 1.52 (s, 9H), 3.34 (dd, J = 6.7, 17.2 Hz, 1H), 3.53 (d, J = 17.2 Hz, 1H), 4.50–4.70 (m, 1H), 4.90–5.10 (m, 1H), 5.10–5.50 (m, 1H), 8.40–8.50 (m, 2H). Anal. calcd for $C_{13}H_{17}N_3O_4$: C, 55.91; H, 6.14; N, 15.05; found: C, 56.21; H, 6.11; N, 14.78.

(−)-6-*tert*-Butoxycarbonyl-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxylic acid (32a). (±)-6-*tert*-Butoxycarbonyl-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxylic acid (**31**) (35.0 g, 125 mmol) was dissolved in ethyl acetate (900 mL) with heating, and then *S*-(−)-1-phenylethylamine (16.0 mL, 125 mmol) was added to the solution. The mixture was allowed to stand at room temperature overnight. The precipitating solid was collected by filtration, and it was dissolved in ethyl acetate (1000 mL) with heating. The resulting solution was allowed to stand at room temperature overnight. The precipitating solid was collected by filtration, and then the obtained solid was stirred with a 10% aqueous solution of citric acid (300 mL). The whole was extracted with ethyl acetate. The organic layers were combined, washed with water and a saturated saline solution, and dried over magnesium sulfate. Concentration of obtained solution in vacuo gave the title compound as a colorless solid (9.00 g, Y = 26%). ^{1}H NMR (DMSO-d_6) δ 1.52 (s, 9H), 3.34 (dd, J = 6.7, 17.2 Hz, 1H), 3.53 (d, J = 17.2 Hz, 1H), 4.50–4.70 (m, 1H), 4.90–5.10 (m, 1H), 5.10–5.50 (m, 1H), 8.40–8.50 (m, 2H). Anal. calcd for $C_{13}H_{17}N_3O_4$: C, 55.91; H, 6.14; N, 15.05; found: C, 55.96; H, 6.12; N, 14.98. $[\alpha]_D = -38$ (*c* 1.0, methanol).

Method B: (+)-*N*-benzyloxy-6-*tert*-butoxycarbonyl-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxamide (33a). EDCI (1.16 g, 6.05 mmol) and HOBt (927 mg, 6.05 mmol) were successively added to a solution of (−)-6-*tert*-butoxycarbonyl-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxylic acid (**32a**) (1.30 g, 4.65 mmol) in DMF (10 mL) under ice-cooling, and then the resulting mixture was stirred for 1 h under ice-cooling. A mixture of *O*-benzylhydroxylamine hydrochloride (966 mg, 6.05 mmol), triethylamine (612 mg, 6.05 mmol), and DMF (15 mL) were added to the reaction mixture, and then the resulting mixture was stirred at room temperature for 48 h. After the reaction mixture had been poured into water (500 mL), the whole was extracted with ethyl acetate. The organic layers were combined, washed with a saturated aqueous solution of sodium hydrogen carbonate, a 10% aqueous solution of citric acid, and water, and dried over magnesium sulfate. After concentration of the solution in vacuo, the obtained residue was purified by column chromatography (eluent: first, *n*-hexane/ethyl acetate = 1:1, then only ethyl acetate) to give the title compound as a colorless oil (1.80 g, Y = quant). ^{1}H NMR ($CDCl_3$) δ 1.47 (s, 9H), 3.20 (dd, J = 6.3, 17.5 Hz, 1H), 3.42 (d, J = 17.5 Hz, 1H), 4.36 (d, J = 17.8 Hz, 1H), 4.70–5.10 (m, 4H), 7.34 (s, 5H), 8.30–8.50 (m, 2H), 8.92 (br s, 1H). Anal. calcd for $C_{20}H_{24}N_4O_4$: C, 62.49; H, 6.29; N, 14.57; found: C, 62.51; H, 6.21; N, 14.36. $[\alpha]_D = +27$ (*c* = 1.0, methanol).

(+)-*N*-Benzyloxy-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxamide hydrochloride (34a). A solution of hydrochloric acid in ethyl acetate (4 N, 15 mL) was added to (+)-*N*-Benzyloxy-6-*tert*-butoxycarbonyl-5,6,7,8-tetrahydropyrido[3,4-b]pyrazine-7-carboxamide (**33a**) (1.80 g, 4.68 mmol) under ice-cooling, and then the resulting mixture was stirred for 1.5 h under ice-cooling. The precipitating solid was collected by filtration to give the title compound as a pale brown powder (1.41 g, Y = 95%). ^{1}H NMR (DMSO-d_6) δ 3.19 (dd, J = 11.4, 17.7 Hz, 1H), 3.36 (dd, J = 5.2, 17.7 Hz, 1H), 4.20–4.40 (m, 2H), 4.50 (d, J = 16.6 Hz, 1H), 4.86 (d, J = 11.1 Hz, 1H), 4.91 (d, J = 11.1 Hz, 1H), 7.30–7.50 (m, 5H), 8.50–8.60 (m, 2H), 9.90–10.70 (br, 1H), 12.20 (br s, 1H). Anal. calcd for $C_{15}H_{17}ClN_4O_2 \cdot 1.0H_2O$: C, 53.18; H, 5.65; N, 16.54; found: C, 53.10; H, 5.57; N, 16.46. $[\alpha]_D = +88$ (*c* 1.0, methanol).

(+)-*N*-Benzyloxy-6-(4-methoxybenzenesulfonyl)-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxamide (35a). (+)-*N*-Benzyloxy-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxamide hydrochloride (**34a**) (651 mg, 2.03 mmol) was dissolved in a mixture of water (15 mL) and dioxane (15 mL). Triethylamine (515 mg, 5.09 mmol) and 4-methoxybenzenesulfonyl chloride (463 mg, 2.24 mmol) were successively added to the solution under ice-cooling, and then the resulting mixture was stirred at room temperature for 3 h. After concentration of the reaction mixture in vacuo, the obtained residue was acidified with 1 N hydrochloric acid to pH 3, and then the whole was extracted with ethyl acetate. The organic layers were combined, washed with 1 N hydrochloric acid, water, and a saturated aqueous solution of sodium chloride, and dried over magnesium sulfate. After concentration of the solution in vacuo, the obtained residue was purified by column chromatography (eluent: chloroform/methanol = 30:1) to give the title compound as a pale yellow powder (592 mg, Y = 64%). ^{1}H NMR ($CDCl_3$) δ 2.86 (dd, J = 6.1, 17.2 Hz, 1H), 3.44 (d, J = 17.2 Hz, 1H), 3.83 (s, 3H), 4.40 (d, J = 18.0 Hz, 1H), 4.70–5.00 (m, 4H), 6.87 (d, J = 8.8 Hz, 2H), 7.37 (s, 5H), 7.66 (d, J = 8.8 Hz, 2H), 8.33 (s, 2H), 9.17 (s, 1H). Anal. calcd for $C_{22}H_{22}N_4O_5S$: C, 58.14; H, 4.88; N, 12.33; found: C, 58.34; H, 4.85; N, 12.28. $[\alpha]_D = +41$ (*c* 1.0, methanol).

(+)-*N*-Hydroxy-6-(4-methoxybenzenesulfonyl)-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxamide (3a). (+)-*N*-Benzyloxy-6-(4-methoxybenzenesulfonyl)-5,6,7,8-tetrahydropyrido[3,4-b]pyrazine-7-carboxamide (**35a**) (580 mg, 1.28 mmol) was dissolved in a mixture of methanol (8 mL) and dioxane (8 mL), and then the solution was stirred with Pd/C (10%, 290 mg) under hydrogen atmosphere at room temperature overnight. After removal of Pd/C by filtration, the obtained filtrate was concentrated in vacuo. The obtained residue was purified by column chromatography (eluent: chloroform/methanol = 10:1) to give the title compound as an yellow powder (342 mg, Y = 74%). ^{1}H NMR (DMSO-d_6) δ 2.95 (dd, J = 2.0, 17.4 Hz, 1H), 3.16 (dd, J = 7.0, 17.4 Hz, 1H), 3.82 (s, 3H), 4.59 (d, J = 16.8 Hz, 1H), 4.67 (d, J = 16.8 Hz, 1H), 4.78 (dd, J = 2.0, 7.0 Hz, 1H), 7.05 (d, J = 8.9 Hz, 2H), 7.75 (d, J = 8.9 Hz, 2H), 8.30–8.50 (m, 2H), 8.88 (s, 1H), 10.91 (s, 1H). Anal. calcd for $C_{15}H_{16}N_4O_5S \cdot 0.5H_2O$: C, 48.25; H, 4.59; N, 15.00; found: C, 48.21; H, 4.62; N, 14.70. MALDI-TOF MS (M_r = 364.38): 365 $[M+H]^+$, 387 $[M+Na]^+$, 403 $[M+K]^+$. $[\alpha]_D = +30$ (*c* 1.0, methanol).

Compounds **3c**, **3e**, **3h**, **3j**, **3p**, **3r**, and **6** were synthesized from compound **34a** and the corresponding sulfonyl chloride via the corresponding sulfonylamino benzyloxy-

amides **35** by use of reaction conditions similar to those used for the preparation of **3a**. The physicochemical data of **3c**, **3e**, **3h**, **3j**, **3p**, **3r**, and **6** were shown below.

(+)-*N*-Hydroxy-6-benzenesulfonyl-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxamide (3c). A colorless powder. ^{1}H NMR ($CDCl_3$) δ 2.80–3.00 (m, 1H), 3.41 (d, J = 16.4 Hz, 1H), 4.62 (d, J = 17.7 Hz, 1H), 4.82 (d, J = 17.7 Hz, 1H), 4.90–5.10 (m, 1H), 7.30–7.60 (m, 3H), 7.78 (d, J = 7.6 Hz, 2H), 8.20–8.40 (m, 2H), 9.91 (br, 1H). Anal. calcd for $C_{14}H_{14}N_4O_4S{\cdot}0.25H_2O$: C, 49.62; H, 4.31; N, 16.53; found: C, 49.52; H, 4.40; N, 16.32. MALDI-TOF MS (M_r = 334.35): 335 $[M+H]^+$, 357 $[M+Na]^+$, 373 $[M+K]^+$.

(+)-*N*-Hydroxy-6-(4-methylbenzenesulfonyl)-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxamide (3e). A colorless powder. ^{1}H NMR (DMSO-d_6) δ 2.34 (s, 3H), 2.93 (dd, J = 1.8, 17.3 Hz, 1H), 3.14 (dd, J = 6.8, 17.3 Hz, 1H), 4.58 (d, J = 16.8 Hz, 1H), 4.67 (d, J = 16.8 Hz, 1H), 4.78 (dd, J = 1.8, 6.8 Hz, 1H), 7.34 (d, J = 8.2 Hz, 2H), 7.69 (d, J = 8.2 Hz, 2H), 8.30–8.50 (m, 2H), 8.91 (br s, 1H), 10.94 (br s, 1H). Anal. calcd for $C_{15}H_{16}N_4O_4S{\cdot}0.25H_2O$: C, 51.06; H, 4.71; N, 15.88; found: C, 51.11; H, 4.96; N, 15.62. MALDI-TOF MS (M_r = 348.38): 349 $[M+H]^+$, 371 $[M+Na]^+$, 387 $[M+K]^+$.

(+)-*N*-Hydroxy-6-(4-fluorobenzenesulfonyl)-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxamide (3h). A colorless powder. ^{1}H NMR ($CDCl_3$) δ 2.80–3.10 (m, 1H), 3.37 (d, J = 17.8 Hz, 1H), 4.62 (d, J = 17.3 Hz, 1H), 4.77 (d, J = 17.3 Hz, 1H), 4.90–5.10 (m, 1H), 7.00–7.20 (m, 2H), 7.70–7.90 (m, 2H), 8.10–8.40 (m, 2H), 10.11 (br s, 1H). Anal. calcd for $C_{14}H_{13}FN_4O_4S{\cdot}0.25H_2O$: C, 47.12; H, 3.81; N, 15.70; found: C, 47.03; H, 3.95; N, 15.48. MALDI-TOF MS (M_r = 352.34): 353 $[M+H]^+$, 375 $[M+Na]^+$, 391 $[M+K]^+$

(+)-*N*-Hydroxy-6-(4-trifluoromethylbenzenesulfonyl)-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxamide (3j). A colorless powder. ^{1}H NMR (DMSO-d_6) δ 2.99 (dd, J = 1.8, 17.4 Hz, 1H), 3.15–3.35 (m, 1H), 4.62 (d, J = 16.5 Hz, 1H), 4.74 (d, J = 16.5 Hz, 1H), 4.83 (dd, J = 1.8, 6.9 Hz, 1H), 7.93 (d, J = 8.4 Hz, 2H), 8.05 (d, J = 8.4 Hz, 2H), 8.35–8.50 (m, 2H), 8.90 (s, 1H), 10.94 (s, 1H). Anal. calcd for $C_{15}H_{13}F_3N_4O_4S{\cdot}0.5H_2O$: C, 43.80; H, 3.43; N, 13.62; found: C, 43.91; H, 3.49; N, 13.62. MALDI-TOF MS (M_r = 402.35): 403 $[M+H]^+$, 425 $[M+Na]^+$, 441 $[M+K]^+$

(+)-*N*-Hydroxy-6-[4-(3-methoxypropoxy)benzenesulfonyl]-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxamide (3p). A pale brown colorless powder. ^{1}H NMR (DMSO-d_6) δ 1.94 (tt, J = 6.3, 6.4 Hz, 2H), 2.80–3.00 (m, 1H), 3.00–3.20 (m, 1H), 3.23 (s, 3H), 3.45 (t, J = 6.3 Hz, 2H), 4.07 (t, J = 6.4 Hz, 2H), 4.50–4.70 (m, 2H), 4.70–4.80 (m, 1H), 7.04 (d, J = 8.9 Hz, 2H), 7.73 (d, J = 8.9 Hz, 2H), 8.39 (d, J = 2.6 Hz, 1H), 8.41 (d, J = 2.6 Hz, 1H), 8.87 (s, 1H), 10.91 (s, 1H). Anal. calcd for $C_{18}H_{22}N_4O_6S{\cdot}0.6H_2O$: C, 49.90; H, 5.40; N, 12.93; found: C, 49.92; H, 5.37; N, 12.63. MALDI-TOF MS (M_r = 422.46): 423 $[M+H]^+$, 445 $[M+Na]^+$, 461 $[M+K]^+$.

(+)-*N*-Hydroxy-6-[4-(3-ethoxycarbonylpropoxy)benzenesulfonyl]-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxamide (3r). A colorless powder. ^{1}H NMR (DMSO-d_6) δ 1.17 (t, J = 7.1 Hz, 3H), 1.90–2.10 (m, 2H), 2.45 (t, J = 7.2 Hz, 2H), 2.90–3.00 (m, 1H), 3.16 (dd, J = 7.0, 17.3 Hz, 1H), 4.05 (t, J = 6.3 Hz, 2H), 4.06 (q, J = 7.1 Hz, 2H), 4.58 (d, J = 16.6 Hz, 1H), 4.67 (d, J = 16.6 Hz, 1H), 4.70–4.80 (m, 1H), 7.04 (d, J = 8.9 Hz, 2H), 7.74 (d, J = 8.9 Hz, 2H), 8.30–8.50 (m, 2H), 8.92 (br s, 1H), 10.95 (br s, 1H). Anal. calcd for $C_{20}H_{24}N_4O_7S{\cdot}0.25H_2O$: C, 51.22; H, 5.27; N, 11.95; found: C, 51.26; H, 5.52; N, 11.75. MALDI-TOF MS (M_r = 464.49): 465 $[M+H]^+$, 487 $[M+Na]^+$, 503 $[M+K]^+$.

(+)-*N*-Hydroxy-6-hexylsulfonyl-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxamide (6). A colorless powder. ^{1}H NMR (DMSO-d_6) δ 0.83 (t, J = 6.6 Hz, 3H), 1.15–1.45 (m, 6H), 1.55–1.70 (m, 2H), 3.00–3.50 (m, 4H), 4.55–4.80 (m, 3H), 8.46 (s, 2H), 8.98 (s, 1H), 10.91 (s, 1H). Anal. calcd for $C_{14}H_{22}N_4O_4S{\cdot}0.25H_2O$: C, 48.47; H, 6.54; N, 16.15; found: C, 48.52; H, 6.64; N, 16.06. MALDI-TOF MS (M_r = 342.42): 343 $[M+H]^+$, 365 $[M+Na]^+$, 381 $[M+K]^+$.

(+)-*N*-Benzyloxy-6-(4-nitrobenzenesulfonyl)-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxamide (35g). The title compound (a yellow powder) was synthesized from compound **34a** and 4-nitrobenzenesulfonyl chloride by use of reaction condition similar to that used for the preparation of **35a**. ^{1}H NMR ($CDCl_3$) δ 2.90–3.10 (m, 1H), 3.30–3.50 (m, 1H), 4.40–4.70 (m, 1H), 4.80–5.10 (m, 4H), 7.20–7.50 (m, 5H), 7.94 (d, J = 8.9 Hz, 2H), 8.29 (d, J = 8.9 Hz, 2H), 8.37 (s, 2H), 8.91 (br s, 1H). Anal. calcd for $C_{21}H_{19}N_5O_6S$: C, 53.73; H, 4.08; N, 14.92; found: C, 53.58; H, 4.06; N, 15.11.

(+)-*N*-Hydroxy-6-(4-aminobenzenesulfonyl)-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxamide (3g). The title compound (a colorless powder) was synthesized from compound **35g** by use of reaction condition similar to that used for the preparation of **3a**. ^{1}H NMR (DMSO-d_6) δ 2.80–3.20 (m, 2H), 4.40–4.70 (m, 2H), 4.70 (dd, J = 1.7, 6.7 Hz, 1H), 6.07 (br s, 2H), 6.40–6.70 (m, 2H), 7.30–7.50 (m, 2H), 8.30–8.50 (m, 2H), 8.91 (br s, 1H), 10.93 (br s, 1H). Anal. calcd for $C_{14}H_{15}N_5O_4S{\cdot}0.75H_2O$: C, 46.34; H, 4.58; N, 19.30; found: C, 46.38; H, 4.82; N, 19.12. MALDI-TOF MS (M_r = 349.37): 350 $[M+H]^+$, 372 $[M+Na]^+$, 388 $[M+K]^+$.

(+)-*N*-Benzyloxy-6-(4-pivaloyloxybenzenesulfonyl)-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxamide (35u). The title compound (a colorless powder) was synthesized from compound **34a** and 4-pivaloyloxybenzenesulfonyl chloride by use of reaction condition similar to that used for the preparation of **35a**. ^{1}H NMR ($CDCl_3$) δ 1.35 (s, 9H), 2.91 (dd, J = 6.6, 17.8 Hz, 1H), 3.44 (d, J = 17.8 Hz, 1H), 4.43 (d, J = 17.2 Hz, 1H), 4.70–5.00 (m, 4H), 7.15 (d, J = 8.5 Hz, 2H), 7.30–7.50 (m, 5H), 7.76 (d, J = 8.5 Hz, 2H), 8.35 (s, 2H), 9.15 (s, 1H). Anal. calcd for $C_{26}H_{28}N_4O_6S$: C, 59.53; H, 5.38; N, 10.68; found: C, 59.50; H, 5.39; N, 10.70.

(+)-*N*-Benzyloxy-6-(4-hydroxybenzenesulfonyl)-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxamide (35f). An aqueous solution of sodium hydroxide (1 N, 2.0 mL) was added to a solution of (+)-*N*-benzyloxy-6-(4-pivaloyloxybenzenesulfonyl)-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxamide (**35u**) (350 mg, 0.667 mmol) in dioxane (5 mL), and then the resulting mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with a 10% aqueous solution of citric acid (100 mL), and extracted with ethyl acetate. The organic layer was washed with water, and a saturated aqueous solution of sodium chloride, dried over magnesium sulfate, and concentrated in vacuo. The obtained residue was purified by column chromatography (eluent: *n*-hexane/ethyl acetate = 1:2) to give the title compound as a colorless solid (210 mg, Y = 71%). ^{1}H NMR ($CDCl_3$) δ 2.80–3.00 (m, 1H), 3.40–3.60 (m, 1H), 4.40 (d, J = 17.1 Hz, 1H), 4.70–5.00 (m, 4H), 6.81 (d, J = 8.5 Hz, 2H), 7.30–7.50 (m, 5H), 7.62 (d, J = 8.5 Hz, 2H), 8.37 (s, 2H), 9.11 (s, 1H). Anal. calcd for $C_{21}H_{20}N_4O_5S$: C, 57.26; H, 4.58; N, 12.72; found: C, 57.01; H, 4.55; N, 13.00.

(+)-*N*-Hydroxy-6-(4-hydroxybenzenesulfonyl)-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxamide (3f). The title compound (a colorless powder) was synthesized from compound **35f** by use of reaction condition similar to that used for the preparation of **3a**. ^{1}H NMR (DMSO-d_6) δ 2.94 (d, J = 16.9 Hz, 1H), 3.00–3.30 (m, 1H), 4.50–4.70 (m, 2H), 4.70–4.80 (m, 1H), 6.84 (d, J = 8.4 Hz, 2H), 7.64 (d, J = 8.4 Hz, 2H), 8.30–8.50 (m, 2H), 8.90 (br s, 1H), 10.78 (br s, 1H). Anal. calcd for $C_{14}H_{14}N_4O_5S{\cdot}0.4H_2O$: C, 47.03; H, 4.17; N, 15.67; found: C, 47.25; H, 4.41; N, 15.33. MALDI-TOF MS (M_r = 350.35): 351 $[M+H]^+$, 373 $[M+Na]^+$, 389 $[M+K]^+$.

(+)-*N*-Hydroxy-6-[4-(3-carboxyproproxy)benzenesulfonyl]-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxamide (3s). The title compound (a colorless powder) was synthesized from compound **34a** and 4-(3-ethoxycarbonylpropoxy)benzenesulfonyl chloride via compound **35r** and the corresponding *N*-benzyloxyamide **35s** by use of reaction conditions similar to those used for the preparation of **3f**. ^{1}H NMR (DMSO-d_6) δ 1.85–2.00 (m, 2H), 2.38 (t, J = 7.3 Hz, 2H), 2.95 (d, J = 15.6 Hz, 1H), 3.00–3.25 (m, 1H), 4.05 (t, J = 6.4 Hz, 2H), 4.50–4.80 (m, 3H), 7.00–7.10 (m, 2H), 7.70–7.80 (m, 2H), 8.35–8.45 (m, 2H), 8.90 (br s, 1H), 10.94 (br s, 1H). Anal. calcd for $C_{18}H_{20}N_4O_7S{\cdot}0.5H_2O$: C, 48.53; H, 4.75; N, 12.58; found: C, 48.31; H, 4.79; N, 12.60. MALDI-TOF MS (M_r = 436.44): 437 $[M+H]^+$, 459 $[M+Na]^+$, 475 $[M+K]^+$.

(+)-*N*-Benzyloxy-6-(4-methoxybenzoyl)-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxamide (36a). The title compound (a colorless powder) was synthesized from compound **34a** and 4-methoxybenzoyl chloride by use of reaction condition similar to that used for the preparation of **35a**. ^{1}H NMR ($CDCl_3$) δ 3.15–3.35 (m,1H), 3.56 (d, J = 17.9 Hz, 1H), 3.85 (s, 3H), 4.39 (d, J = 17.0 Hz, 1H), 4.80–5.00 (m, 3H), 5.43 (br s, 1H), 6.91 (d, J = 7.7 Hz, 2H), 7.20–7.40 (m, 7H), 8.35 (s, 1H), 8.45 (s, 1H), 9.71 (s, 1H). Anal. calcd for $C_{23}H_{22}N_4O_4$: C, 60.02; H, 5.30; N, 13.39; found: C, 59.73; H, 5.28; N, 13.60.

(+)-*N*-Hydroxy-6-(4-methoxybenzoyl)-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxamide (5a). The title compound (a colorless powder) was synthesized from compound **36a** by use of reaction condition similar to that used for the preparation of **3a**. ^{1}H NMR (DMSO-d_6) δ 3.10–3.50 (m, 2H), 3.81 (s, 3H), 4.50–5.60 (m, 3H), 7.00–7.10 (m, 2H), 7.40–7.60 (m, 2H), 8.45 (s, 2H), 8.92 (s, 1H), 10.90 (s, 1H). Anal. calcd for $C_{16}H_{16}N_4O_4{\cdot}0.5H_2O$: C, 56.97; H, 5.08; N, 16.61; found: C, 56.88; H, 5.26; N, 16.52. MALDI-TOF MS (M_r = 328.32): 329 $[M+H]^+$, 351 $[M+Na]^+$, 367 $[M+K]^+$

(+)-6-*tert*-Butoxycarbonyl-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxylic acid (32b). (±)-6-*tert*-Butoxycarbonyl-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxylic acid (**31**) was treated with *R*-(+)-1-phenylethylamine in the same manner that **32a** was prepared from **31** to give the title compound as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 1.52 (s, 9H), 3.34 (dd, J = 6.7, 17.2 Hz, 1H), 3.53 (d, J = 17.2 Hz, 1H), 4.50–4.70 (m, 1H), 4.90–5.10 (m, 1H), 5.10–5.50 (m, 1H), 8.40–8.50 (m, 2H). Anal. calcd for $C_{13}H_{17}N_3O_4$: C, 55.91; H, 6.14; N, 15.05; found: C, 55.78; H, 5.99; N, 15.23. $[\alpha]_D$ = +38 (*c* 1.0, methanol).

(−)-*N*-Benzyloxy-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxamide hydrochloride (34b). The title compound (a colorless solid) was synthesized from (+)-6-*tert*-butoxycarbonyl-5,6,7,8-tetrahydropyrido[3,4-b]pyrazine-7-carboxylic acid (**32b**) via (−)-*N*-benzyloxy-6-*tert*-butoxycarbonyl-5,6,7,8-tetrahydropyrido[3,4-b]pyrazine-7-carboxamide (**33b**) in the same manner as **34a**. ^{1}H NMR (DMSO-d_6) δ 3.19 (dd, J = 11.4, 17.7 Hz, 1H), 3.36 (dd, J = 5.2, 17.7 Hz, 1H), 4.20–4.40 (m, 2H), 4.50 (d, J = 16.6 Hz, 1H), 4.86 (d, J = 11.1 Hz, 1H), 4.91 (d, J = 11.1 Hz, 1H), 7.30–7.50 (m, 5H), 8.50–8.60 (m, 2H), 9.90–10.70 (br, 1H), 12.20 (bs, 1H). Anal. calcd for $C_{15}H_{17}ClN_4O_2{\cdot}1.0H_2O$: C, 53.18; H, 5.65; N, 16.54; found: C, 53.04; H, 5.44; N, 16.66. $[\alpha]_D$ = −88 (*c* 1.0, methanol).

(−)-*N*-Hydroxy-6-(4-methoxybenzenesulfonyl)-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxamide (3b). The title compound (a colorless powder) was synthesized from (−)-*N*-benzyloxy-5,6,7,8-tetrahydro pyrido[3,4-b]pyrazine-7-carboxamide hydrochloride (**34b**) via (−)-*N*-benzyloxy-6-(4-methoxybenzenesulfonyl)-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxamide (**35b**) in the same manner as **3a**. ^{1}H NMR (DMSO-d_6) δ 2.95 (dd, J = 2.0, 17.4 Hz, 1H), 3.16 (dd, J = 7.0, 17.4 Hz, 1H), 3.82 (s, 3H), 4.59 (d, J = 16.8 Hz, 1H), 4.67 (d, J = 16.8 Hz, 1H), 4.78 (dd, J = 2.0, 7.0 Hz, 1H), 7.05 (d, J = 8.9 Hz, 2H), 7.75 (d, J = 8.9 Hz, 2H), 8.30–8.50 (m, 2H), 8.88 (s, 1H), 10.91 (s, 1H). Anal. calcd for $C_{15}H_{16}N_4O_5S{\cdot}0.5H_2O$: C, 48.25; H, 4.59; N, 15.00; found: C, 48.11; H, 4.68; N, 14.82. MALDI-TOF MS (M_r = 364.38): 365 $[M+H]^+$, 387 $[M+Na]^+$, 403 $[M+K]^+$. $[\alpha]_D$ = −30 (*c* 1.0, methanol).

Method C: (+)-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxylic acid hydrochloride (37a). A solution of hydrochloric acid in ethyl acetate (4 N, 15 mL) was added to (−)-6-*tert*-butoxycarbonyl-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxylic acid (**32a**) (2.00 g, 7.16 mmol) under ice-cooling, and then the resulting mixture was stirred at room temperature for 4 h. The precipitating solid was collected by filtration to give the title compound as a pale brown powder (1.58 g, Y = quant). ^{1}H NMR (DMSO-d_6) δ 3.30–3.50 (m, 2H), 4.30–4.60 (m, 2H), 4.60–4.70 (m, 1H), 8.50–8.70 (m, 2H). Anal. calcd for $C_8H_{10}ClN_3O_2 \cdot 1.0H_2O$: C, 41.12; H, 5.18; N, 17.98; found: C, 41.41; H, 5.10; N, 17.85. $[\alpha]_D = +79$ (*c* 0.51, methanol).

(+)-6-[4-(3-Methylthiopropoxy)benzenesulfonyl]-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxylic acid (4q). (+)-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxylic acid hydrochloride (**37a**) (1.20 g, 5.56 mmol) was dissolved in a mixture of water (10 mL) and dioxane (10 mL). Triethylamine (1.40 g, 13.8 mmol) and 4-(3-methylthiopropoxy)benzenesulfonyl chloride (1.80 g, 6.41 mmol) were successively added to the solution under ice-cooling, and then the resulting mixture was stirred at room temperature for 4 h. After the reaction mixture had been acidified with diluted hydrochloric acid to pH 3, the whole was extracted with ethyl acetate. The organic layers were combined, washed with water and a saturated aqueous solution of sodium chloride, and dried over magnesium sulfate. After concentration of the solution in vacuo, the obtained residue was purified by column chromatography (eluent: chloroform/methanol = 10:1) to give the title compound as a colorless powder (790 mg, Y = 34%). ^{1}H NMR ($CDCl_3$) δ 2.13 (s, 3H), 2.00–2.20 (m, 2H), 2.69 (t, J = 7.0 Hz, 2H), 3.40–3.50 (m, 2H), 4.14 (t, J = 6.1 Hz, 2H), 4.61 (d, J = 17.4 Hz, 1H), 4.93 (d, J = 17.4 Hz, 1H), 5.20–5.30 (m, 1H), 6.98 (d, J = 8.8 Hz, 2H), 7.80 (d, J = 8.8 Hz, 2H), 8.30–8.50 (m, 2H). Anal. calcd for $C_{18}H_{21}N_3O_5S_2$: C, 51.05; H, 5.00; N, 9.92; found: C, 49.94; H, 5.02; N, 9.68. $[\alpha]_D = +8.6$ (*c* 1.0, methanol).

(+)-6-(4-Methoxybenzenesulfonyl)-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxylic acid (4a). The title compound (a colorless solid) was synthesized from compound **37a** and 4-methoxybenzenesulfonyl chloride by use of reaction condition similar to that used for the preparation of **4q**. ^{1}H NMR (DMSO-d_6) δ 3.10–3.35 (m, 2H), 3.81 (s, 3H), 4.51 (d, J = 17.1 Hz, 1H), 4.68 (d, J = 17.1 Hz, 1H), 4.95–5.05 (m, 1H), 7.07 (d, J = 9.0 Hz, 2H), 7.79 (d, J = 9.0 Hz, 2H), 8.40–8.50 (m, 2H). Anal. calcd for $C_{18}H_{21}N_3O_6S$: C, 53.06; H, 5.20; N, 10.31; found: C, 52.88; H, 5.19; N, 10.17. MALDI-TOF MS (M_r = 349.36): 350 $[M+H]^+$, 372 $[M+Na]^+$, 388 $[M+K]^+$.

(+)-*N*-Hydroxy-6-[4-(3-methylthiopropoxy)benzenesulfonyl]-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxamide (3q). Oxalyl chloride (260 mg, 2.05 mmol) and DMF (two drops) were added to a solution of (+)-6-[4-(3-methylthiopropoxy)benzenesulfonyl]-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxylic acid (**4q**) (0.75 g, 1.77 mmol) in dichloromethane (10 mL) under ice-cooling. The resulting mixture was stirred for 30 min under ice-cooling and further stirred at room temperature for 2 h. 1,2-Dimethoxyethane (5 mL) and an aqueous solution of hydroxylamine (50%, 1.5 mL) were successively added to the reaction mixture under ice-cooling, and the resulting mixture was stirred for 2.5 h under ice-cooling. After the reaction mixture had been acidified with diluted hydrochloric acid to pH 3, the whole was extracted with ethyl acetate three times. The organic layers were combined, washed with water and a saturated aqueous solution of sodium chloride, and dried over magnesium sulfate. After concentration of the solution in vacuo, the obtained residue was purified by column chromatography (eluent: chloroform/methanol = 10:1) to give the title compound as a colorless powder (350 mg, Y = 45%). ^{1}H NMR (DMSO-d_6) δ 1.90–2.10 (m, 2H), 2.05 (s, 3H), 2.60 (t, J = 7.2 Hz, 2H), 2.94 (d, J = 17.5 Hz, 1H), 3.15 (dd, J = 7.1, 17.5 Hz, 1H), 4.10 (t, J = 6.2 Hz, 2H), 4.57 (d, J = 16.8 Hz, 1H), 4.67 (d, J = 16.8 Hz, 1H), 4.70–4.80 (m, 1H), 7.04 (d, J = 8.8 Hz, 2H), 7.73 (d, J = 8.8 Hz, 2H), 8.40–8.50 (m, 2H), 8.91 (s, 1H), 10.94 (s, 1H). Anal. calcd for $C_{18}H_{22}N_4O_5S_2$: C, 49.30; H, 5.06; N, 12.78; found: C, 48.99; H, 5.23; N, 12.52. MALDI-TOF MS (M_r = 438.53): 439 $[M+H]^+$, 461 $[M+Na]^+$, 477 $[M+K]^+$. $[\alpha]_D = +20$ (*c* 0.50, methanol).

Compounds **3a**, **3d**, **3i**, **3k**, **3n**, and **3o** were synthesized from compound **37a** and the corresponding sulfonyl chloride via the corresponding sulfonylamino acids **4** by use of reaction conditions similar to those used for the preparation of **3q**. The physicochemical data of **3a**, **3d**, **3i**, **3k**, **3n**, and **3o** were shown below.

(+)-*N*-Hydroxy-6-(4-methoxybenzenesulfonyl)-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxamide (3a). A colorless powder. ^{1}H NMR (DMSO-d_6) δ 2.95 (dd, J = 2.0, 17.4 Hz, 1H), 3.16 (dd, J = 7.0, 17.4 Hz, 1H), 3.82 (s, 3H), 4.59 (d, J = 16.8 Hz, 1H), 4.67 (d, J = 16.8 Hz, 1H), 4.78 (dd, J = 2.0, 7.0 Hz, 1H), 7.05 (d, J = 8.9 Hz, 2H), 7.75 (d, J = 8.9 Hz, 2H), 8.30–8.50 (m, 2H), 8.88 (s, 1H), 10.91 (s, 1H). MALDI-TOF MS (M_r = 364.38): 365 $[M+H]^+$, 387 $[M+Na]^+$, 403 $[M+K]^+$. The $[\alpha]_D$ value (*c* 1.0, methanol) of **3a** prepared by Method C was equal to that of **3a** prepared by Method B.

(+)-*N*-Hydroxy-6-(4-methylthiobenzenesulfonyl)-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxamide (3d). A colorless powder. ^{1}H NMR (DMSO-d_6) δ 2.50 (s, 3H), 2.95 (dd, J = 1.7, 17.4 Hz, 1H), 3.19 (dd, J = 6.8, 17.4 Hz, 1H), 4.58 (d, J = 16.6 Hz, 1H), 4.67 (d, J = 16.6 Hz, 1H), 4.78 (dd, J = 1.7, 6.8 Hz, 1H), 7.36 (d, J = 8.7 Hz, 2H), 7.70 (d, J = 8.7 Hz, 2H), 8.39 (d, J = 2.7 Hz, 1H), 8.42 (d, J = 2.7 Hz, 1H), 8.91 (d, J = 1.6 Hz, 1H), 10.95 (d, J = 1.6 Hz, 1H). Anal. calcd for $C_{15}H_{16}N_4O_4S_2 \cdot 0.5H_2O$: C, 46.26; H, 4.40; N, 14.39; found: C, 46.46; H, 4.62; N, 14.18. MALDI-TOF MS (M_r = 380.44): 381 $[M+H]^+$, 403 $[M+Na]^+$, 419 $[M+K]^+$.

(+)-*N*-Hydroxy-6-(4-acetylbenzenesulfonyl)-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxamide (3i). A colorless powder. ^{1}H NMR (MeOH-d_4) δ 2.21 (s, 3H), 3.00–3.30 (m, 2H), 4.67 (d, J = 16.9 Hz, 1H), 4.80–5.10 (m,

2H), 7.79 (d, $J=9.1$ Hz, 2H), 7.84 (d, $J=9.1$ Hz, 2H), 8.33 (d, $J=2.5$ Hz, 1H), 8.38 (d, $J=2.5$ Hz, 1H). Anal. calcd for $C_{16}H_{16}N_4O_5S\cdot0.5H_2O$: C, 49.86; H, 4.45; N, 14.54; found: C, 49.50; H, 4.29; N, 14.30. MALDI-TOF MS ($M_r=376.39$): 377 $[M+H]^+$, 399 $[M+Na]^+$, 415 $[M+K]^+$.

(+)-*N*-Hydroxy-6-(4-vinylbenzenesulfonyl)-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxamide (3k). A colorless powder. ^{1}H NMR (DMSO-d_6) δ 2.97 (d, $J=1.7$, 17.6 Hz, 1H), 3.19 (dd, $J=6.7$, 17.6 Hz, 1H), 4.62 (d, $J=16.7$ Hz, 1H), 4.72 (d, $J=16.7$ Hz, 1H), 4.82 (dd, $J=1.7$, 6.7 Hz, 1H), 5.46 (d, $J=11.0$ Hz, 1H), 6.01 (d, $J=17.7$ Hz, 1H), 6.80 (dd, $J=11.0$, 17.7 Hz, 1H), 7.64 (d, $J=8.5$ Hz, 2H), 7.80 (d, $J=8.5$ Hz, 2H), 8.35–8.50 (m, 2H), 8.93 (s, 1H), 10.96 (br s, 1H). Anal. calcd for $C_{16}H_{16}N_4O_4S\cdot0.5H_2O$: C, 52.02; H, 4.64; N, 15.17; found: C, 52.27; H, 4.76; N, 15.13. MALDI-TOF MS ($M_r=360.39$): 361 $[M+H]^+$, 383 $[M+Na]^+$, 399 $[M+K]^+$.

(+)-*N*-Hydroxy-6-(4-pentyloxybenzenesulfonyl)-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxamide (3n). A colorless powder. ^{1}H NMR (DMSO-d_6) δ 0.88 (t, $J=7.1$ Hz, 3H), 1.20–1.50 (m, 4H), 1.60–1.80 (m, 2H), 2.80–3.00 (m, 1H), 3.05–3.30 (m, 1H), 4.01 (t, $J=6.4$ Hz, 2H), 4.58 (d, $J=16.5$ Hz, 1H), 4.66 (d, $J=16.5$ Hz, 1H), 4.70–4.80 (m, 1H), 7.03 (d, $J=8.9$ Hz, 2H), 7.72 (d, $J=8.9$ Hz, 2H), 8.30–8.50 (m, 2H), 8.90 (br s, 1H), 10.93 (br s, 1H). Anal. calcd for $C_{19}H_{24}N_4O_5S\cdot0.25H_2O$: C, 53.70; H, 5.81; N, 13.18; found: C, 53.60; H, 6.01; N, 13.00. MALDI-TOF MS ($M_r=420.49$): 421 $[M+H]^+$, 443 $[M+Na]^+$, 459 $[M+K]^+$

(+)-*N*-Hydroxy-6-[4-(2-ethoxyethoxy)benzenesulfonyl]-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxamide (3o). A colorless powder. ^{1}H NMR (DMSO-d_6) δ 1.09 (t, $J=7.0$ Hz, 3H), 2.93 (dd, $J=1.8$, 17.4 Hz, 1H), 3.14 (dd, $J=7.0$, 17.4 Hz, 1H), 3.46 (q, $J=7.0$ Hz, 2H), 3.60–3.80 (m, 2H), 4.00–4.20 (m, 2H), 4.57 (d, $J=16.8$ Hz, 1H), 4.66 (d, $J=16.8$ Hz, 1H), 4.75 (dd, $J=1.8$, 7.0 Hz, 1H), 7.04 (d, $J=9.0$ Hz, 2H), 7.72 (d, $J=9.0$ Hz, 2H), 8.30–8.50 (m, 2H), 8.86 (br s, 1H), 10.91 (br s, 1H). Anal. calcd for $C_{18}H_{22}N_4O_6S\cdot0.25H_2O$: C, 50.11; H, 5.37; N, 12.99; found: C, 50.31; H, 5.44; N, 12.79. MALDI-TOF MS ($M_r=422.46$): 423 $[M+H]^+$, 445 $[M+Na]^+$, 461 $[M+K]^+$.

Method D: methyl (+)-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxylate hydrochloride (38a). Thionyl chloride (3.26 g, 27.4 mmol) was added to a solution of (+)-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxylate (37a) (1.15 g, 5.33 mmol) in methanol (20 mL) under ice-cooling, and then the resulting mixture was refluxed for 3 h. After having been cooled to room temperature, the reaction mixture was concentrated in vacuo. Toluene (40 mL) and methanol (10 mL) were added to the obtained residue, and the resulting mixture was concentrated in vacuo to give the title compound as a brown powder (1.20 g, Y = 98%). ^{1}H NMR (DMSO-d_6) δ 3.30–3.50 (m, 2H), 3.85 (s, 3H), 4.30–4.50 (m, 2H), 4.60–4.70 (m, 1H), 8.50–8.70 (m, 2H). Anal. calcd for $C_9H_{12}ClN_3O_2\cdot1.0H_2O$: C, 43.64; H, 5.70; N, 16.97; found: C, 43.49; H, 5.64; N, 16.73.

Methyl (+)-6-(4-bromobenzenesulfonyl)-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine- 7-carboxylate (39). Triethylamine (1.62 g, 16.0 mmol) and 4-bromobenzenesulfonyl chloride (1.77 g, 6.93 mmol) were successively added to a solution of methyl (+)-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxylate hydrochloride (38a) (1.15 g, 5.01 mmol) in water (20 mL) and dioxane (20 mL), and then the resulting mixture was stirred at room temperature for 15 h. The reaction mixture was diluted with 10% aqueous solution of citric acid (100 mL), and then the whole was extracted with ethyl acetate three times. The organic layers were combined, washed with 10% aqueous solution of citric acid, a saturated aqueous solution of sodium hydrogen carbonate, and water successively, dried over magnesium sulfate, and concentrated in vacuo. The obtained oil was purified by column chromatography (eluent: *n*-hexane/ethyl acetate = 1:2) to give the title compound as a pale yellow oil (950 mg, Y = 46%). ^{1}H NMR ($CDCl_3$) δ 3.40–3.50 (m, 5H), 4.53 (d, $J=16.9$ Hz, 1H), 4.92 (d, $J=16.9$ Hz, 1H), 5.15–5.25 (m, 1H), 7.60–7.80 (m, 4H), 8.35–8.45 (m, 2H). Anal. calcd for $C_{15}H_{14}BrN_3O_4S$: C, 43.70; H, 3.42; N, 10.19; found: C, 43.49; H, 3.48; N, 9.94.

Methyl (+)-6-(4-trimethylsilylethynylbenzenesulfonyl)-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxylate (40). Triethylamine (1 mL), Pd/C (10%, 36 mg, 33.8 μmol), triphenylphosphine (28 mg, 107 μmol), copper (I) iodide (6.5 mg, 34.1 μmol), and trimethylsilylacetylene (80 mg, 814 μmol) were added to a solution of methyl (+)-6-(4-bromobenzenesulfonyl)-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxylate hydrochloride (39) (280 mg, 679 μmol) in acetonitrile (1 mL), and then the resulting mixture was refluxed for 2.5 h. After having been cooled to room temperature, the reaction mixture was diluted with methanol (20 mL). Insoluble matter in the resulting mixture was removed by filtration. The obtained filtrate was concentrated in vacuo, and the residue was purified by column chromatography (eluent: first, *n*-hexane/ethyl acetate = 3:1, then 2:1) to give the title compound as a colorless crystalline solid (190 mg, Y = 65%). ^{1}H NMR ($CDCl_3$) δ 0.21 (s, 9H), 3.30–3.45 (m, 5H), 4.48 (d, $J=16.8$ Hz, 1H), 4.89 (d, $J=16.8$ Hz, 1H), 5.10–5.20 (m, 1H), 7.54 (d, $J=8.5$ Hz, 2H), 7.75 (d, $J=8.5$ Hz, 2H), 8.30–8.40 (m, 2H). Anal. calcd for $C_{20}H_{23}N_3O_4SSi$: C, 55.92; H, 5.40; N, 9.78; found: C, 55.89; H, 5.32; N, 9.84.

(+)-6-(4-Ethynylbenzenesulfonyl)-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxylic acid (4m). An aqueous solution of potassium hydroxide (1 N, 2.30 mL, 2.30 mmol) was added to a solution of methyl (+)-6-(4-trimethylsilylethynylbenzenesulfonyl)-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxylate (40) (190 mg, 0.442 mmol) in dioxane (1.5 mL), and then the resulting mixture was stirred at room temperature for 2 h. After the reaction mixture had been diluted with water (50 mL), pH of the resulting mixture was adjusted to 3 by 10% aqueous solution of citric acid. The whole was extracted with ethyl acetate three times, and then the

organic layers were combined, washed with a saturated aqueous solution of sodium chloride, dried over magnesium sulfate, and concentrated in vacuo. The obtained residue was purified with column chromatography (eluent: first, chloroform/methanol = 30:1, then 10:1) to give the title compound as an yellow solid (80 mg, Y = 53%). ^{1}H NMR (DMSO-d_6) δ 3.19 (dd, J = 2.3, 17.4 Hz, 1H), 3.25–3.40 (m, 1H), 4.48 (d, J = 17.0 Hz, 1H), 4.51 (s, 1H), 4.74 (d, J = 17.0 Hz, 1H), 5.08 (dd, J = 2.3, 6.4 Hz, 1H), 7.65 (d, J = 8.3 Hz, 2H), 7.87 (d, J = 8.3 Hz, 2H), 8.40–8.50 (m, 2H). Anal. calcd for $C_{16}H_{13}N_3O_4S$: C, 55.97; H, 3.82; N, 12.24; found: C, 55.75; H, 3.79; N, 12.10.

(+)-*N*-Hydroxy-6-(4-ethynylbenzenesulfonyl)-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxamide (3m). Oxalyl chloride (148 mg, 1.17 mmol) and DMF (two drops) were added to a solution of (+)-6-(4-ethylnylbenzenesulfonyl)-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxylic acid (**4m**) (80 mg, 0.233 mmol) in dichloroethane (2 mL) under ice-cooling. The resulting mixture was stirred for 1 h under ice-cooling. 1,2-Dimethoxyethane (2 mL) and an aqueous solution of hydroxylamine (50%, 0.924 mL) were successively added to the reaction mixture under ice-cooling, and then the resulting mixture was stirred for 2 h under ice-cooling. After the reaction mixture had been diluted with 10% aqueous solution of citric acid (100 mL), the whole was extracted with ethyl acetate three times. The organic layers were combined, washed with a saturated aqueous solution of sodium chloride, and dried over magnesium sulfate. After concentration of the solution in vacuo, the obtained residue was purified by column chromatography (eluent: first, chloroform/methanol = 100:1, then 25:1) to give the title compound as a colorless powder (42 mg, Y = 50%). ^{1}H NMR (DMSO-d_6) δ 2.90–3.05 (m, 1H), 3.15–3.25 (m, 1H), 4.50–4.60 (m, 1H), 4.61 (d, J = 16.5 Hz, 1H), 4.72 (d, J = 16.5 Hz, 1H), 4.75–4.85 (m, 1H), 7.63 (d, J = 8.6 Hz, 2H), 7.82 (d, J = 8.6 Hz, 2H), 8.40–8.50 (m, 2H), 8.92 (br s, 1H), 10.93 (br s, 1H). Anal. calcd for $C_{16}H_{14}N_4O_4S{\cdot}0.8H_2O$: C, 51.55; H, 4.22; N, 15.03; found: C, 51.74; H, 4.28; N, 14.65. MALDI-TOF MS (M_r = 358.37): 359 $[M+H]^+$, 381 $[M+Na]^+$, 397 $[M+K]^+$.

Shedding inhibition assay by EGF receptor ligand–AP fusion protein

Expression vector of HB-EGF fused with human placental alkaline phosphatase (AP) that were constructed as described previously[3] was a generous gift from Dr. Higashiyama (School of Medicine, Osaka University, Osaka, Japan). The vector was transfected into HT1080 cells (American Type Culture Collection, Rockville, MD, USA) by lipofection using lipofectamine system (Gibco/BRL, Gaithersburg, MD, USA) according to the manufacturer's directions. Stable transfectants were selected by growth in G418. These transfectants were maintained in Minimum Essential Medium without phenol red (Gibco/BRL, Gaithersburg, MD, USA) supplemented with 10% heat-inactivated fetal calf serum (MEM). Stable transfectants (2×10^5 cells/well) expressing EGF receptor ligand–AP fusion protein were plated into 96-well culture plates and incubated for 24 h. After aspiration of conditioned media and wash with PBS (−), cells were treated with 0.2 mL of compound solutions in duplicate. After incubation for 30 min at 37 °C, 60 nM TPA containing compounds in MEM was added. After further incubation for 60 min at 37 °C, 100 μL of conditioned media were transferred from each well to individual well of new 96-well plates. In order to inactivate endogenous phosphatase, the plates were heated at 65 °C for 10 min. A hundred μL of AP buffer (1 M diethanolamine, 0.01% $MgCl_2$, 1 mg/mL *p*-nitrophenylphosphate, pH 9.8) were added to each well and incubated for 60–120 min at room temperature. AP activity was measured as the increase of absorption at 405 nm. IC_{50} values in the shedding inhibition were calculated by using GraphPad Prism Version 3.0 (GraphPad Software, Inc.) from concentration–inhibition curves.

MMP Inhibition assay

DNA fragments coding catalytic domain of human MMP-1 and human MMP-9 and a DNA fragment coding from pro-domain to catalytic domain of human MMP-3 were amplified by polymerase chain reaction (PCR) from cDNA of HT1080 cells stimulated with 0.01 μM of TPA. The 5′-end of each PCR primers was added a sequence for appropriate restriction enzyme site. Amplified DNA fragments were cloned into cloning vector, and then introduced into commercially available expression vector containing His-6 tag sequence at the end of N-terminus. Recombinant proteins were expressed in *Escherichia coli* cells and purified by Ni-NTA resin (Qiagen Inc.) and refolded. Recombinant MMP-3 was activated by incubating with 1 mM *p*-aminophenylmercuric acetate for 1 h at 37 °C.

Test compounds were dissolved in DMSO and diluted with reaction buffer (50 mM Tris, 150 mM NaCl, 10 mM $CaCl_2$, 0.05% Brij-35, pH 7.5). Twenty-five μL of compound solution was mixed with 25 μL of diluted enzyme solution in a well of 96-well half-area black microplate (Costar), and incubated for 10 min at 37 °C. The reaction was started by adding 50 μL of fluorescence-quenching peptide substrate solution to the well, and incubated for 2 h (MMP-1 and MMP-3) or 3 h (MMP-9) at 37 °C. Five μM of MOCAc-Pro-Leu-Gly-Leu-A2pr(Dnp)-Ala-Arg-NH_2[10] (Peptide Institute, Inc.) was used as a substrate for MMP-1 and MMP-9, and 5 μM of MOCAc-Arg-Pro-Lys-Pro-Val-Glu-Nva-Trp-Arg-Lys(Dnp)-NH_2[11] (Peptide Institute, Inc.) was used as a substrate for MMP-3. After incubation, fluorescence intensities (E_x/E_m = 320/405 nm) of the wells were measured by fluorescence microplate reader (Polarstar; BMG LabTechnologies, Germany). K_i values were calculated from percent inhibition and K_m value of each MMPs to the substrate by using GraphPad Prism.

References and Notes

1. Piepkorn, M.; Pittelkow, M. R.; Cook, P. W. *J. Invest. Dermatol.* **1998**, *115*, 715.
2. Higashiyama, S.; Abraham, J. A.; Miller, J.; Fiddes, J. C.; Klagsbrun, M. *Science* **1991**, *251*, 936.

3. Tokumaru, S.; Higashiyama, S.; Endo, T.; Nakagawa, T.; Miyagawa, J.; Yamamori, K.; Hanakawa, Y.; Ohmoto, H.; Yoshino, K.; Shirakata, Y.; Matsuzawa, Y.; Hashimoto, K.; Taniguchi, N. *J. Cell Boil.* **2000**, *151*, 209.
4. Izumi, Y.; Hirata, M.; Hasuwa, H.; Iwamoto, R.; Umata, T.; Miyado, K.; Tamai, Y.; Kurisaki, T.; Sehara-Fujisawa, A.; Ohno, S.; Mekada, E. *EMBO J.* **1998**, *17*, 7260.
5. Lanzrein, M.; Garred, Ø.; Olsnes, S.; Sandvig, K. *Biochem. J.* **1995**, *310*, 285.
6. MacPherson, L. J.; Bayburt, E. K.; Capparelli, M. P.; Carroll, B. J.; Goldstein, R.; Justice, M. R.; Zhu, L.; Hu, S.; Melton, R. A.; Fryer, L.; Goldberg, R. L.; Doughty, J. R.; Spirito, S.; Blancuzzi, V.; Wilson, D.; O'Byrne, E. M.; Ganu, V.; Parker, D. T. *J. Med. Chem.* **1997**, *40*, 2525.
7. Gonnella, N. C.; Li, Y. C.; Zhang, X.; Paris, C. G. *Bioorg. Med. Chem.* **1997**, *5*, 2193.
8. Whaley, W. M.; Govindachari, T. R. In: *Organic Reactions*; Adams, R., Ed.; Johnson Wiley & Sons: London, 1951; Vol. 6, p 151.
9. Babine, R. E.; Bender, S. L. *Chem. Rev.* **1997**, *97*, 1359.
10. Knight, C. G.; Willenbrock, F.; Murphy, G. *FEBS Lett.* **1992**, *296*, 263.
11. Nagase, H.; Fields, C. G.; Fields, G. B. *J. Biol. Chem.* **1994**, *269*, 20952.