A Novel Class of Enkephalinase Inhibitors Containing a C-Terminal Sulfo Group[1]

Tetsutaro Mimura,* Yasuhisa Nakamura, Junko Nishino, Tadahiro Sawayama, Toshio Komiya, Takashi Deguchi, Atsuko Kita, Hideo Nakamura, and Jun-ichi Matsumoto

Research Laboratories, Dainippon Pharmaceutical Co., Ltd., Enoki 33-94, Suita, Osaka 564, Japan. *Received March 12, 1991*

A new series of sulfonic acids were synthesized and tested for their enkephalinase inhibitory activity. Among them, the most potent was *N*-(2-benzyl-3-mercaptopropionyl)metanilic acid **10i** with an IC_{50} value of 0.27 nM. Several other analogues (**10a,b,j,n,o,gg,hh**) showed the inhibitory activity comparable to or greater than thiorphan (IC_{50} = 2.6 nM), a C-terminal carboxyl-containing inhibitor of enkephalinase. Thus compounds containing a C-terminal sulfo group, instead of the C-terminal carboxyl group, were found to show a remarkably high level of inhibition of enkephalinase. The analgesic activity of **10b**, (*S*)-**10b**, and (*R*)-**10b** was also evaluated by the phenylbenzoquinone writhing test.

The endogenous opioid pentapeptides, methionine-enkephalin (Tyr-Gly-Gly-Phe-Met) and leucine-enkephalin (Tyr-Gly-Gly-Phe-Leu), are rapidly hydrolyzed into inactive fragments by various peptidases after their synaptic release.[2] Among the peptidases, enkephalinase (EC 3.4.24.11) cleaving the Gly-Phe bond of enkephalins plays a major role in the degradation of enkephalins in the brain and also has a narrow substrate specificity.[2,3] This enzyme seems a neutral metalloendopeptidase containing a zinc atom in the catalytic site.[4] A variety of enkephalinase inhibitors with a Zn-chelating function consisting of a thiol,[5,6h] carboxyl,[5b,6] phosphoryl,[5f,6b,d,7] or hydroxamate[5f,7a,8] group have been studied.

Regarding the substrate recognition by enkephalinase, it has been suggested that an active site arginine 102 which recognizes the C-terminal carboxylate of enkephalins, as well as S_1' and S_2' subsites (according to the nomenclature by Schechter and Berger[9]), has a significant role.[10] Many potent enkephalinase inhibitors reported thus far have indeed a C-terminal carboxyl group, which may interact with the arginine 102 of the enzyme. This interaction may involve an electrostatic nature and, therefore, introduction of a more acidic sulfo group instead of the carboxyl group may produce a greater inhibitor–enzyme interaction, thereby resulting in enhancing the inhibitory activity. No inhibitors containing a sulfo group on the C-terminal residue, however, are known in the existing enkephalinase inhibitors.

We hence designed and synthesized a new series of enkephalinase inhibitors **10** containing the C-terminal sulfo group with the aim of obtaining potent inhibitors and of examining their selectivities for enkephalinase versus an-

(1) (a) Presented in part at the 108th Annual Meeting of the Pharmaceutical Society of Japan, Hiroshima, Japan, April 4–6, 1988; Abstract p 688. (b) Mimura, T.; Nakamura, Y.; Nishino, J.; Komiya, T.; Sawayama, T.; Nakamura, H. Japan Patent Kokai 87270555, 1987; *Chem. Abstr.* 1988, *109*, 109861b. (c) Mimura, T.; Nakamura, Y.; Nishino, J.; Sawayama, T.; Nakamura, H. Japan Patent Kokai 89254654, 1989; *Chem. Abstr.* **1990**, *112*, 197646a.

(2) Schwartz, J. C.; Malfroy, B.; De La Baume, S. Biological Inactivation of Enkephalins and the Role of Enkephalin-Dipeptidyl-Carboxypeptidase ("Enkephalinase") as Neuropeptidase. *Life Sci.* 1981, *29*, 1715–1740.

(3) (a) Malfroy, B.; Swerts, J. P.; Guyon, A.; Roques, B. P.; Schwartz, J. C. High-Affinity Enkephalin-Degrading Peptidase in Brain Is Increased after Morphine. *Nature (London)* 1978, *276*, 523–526. (b) Fournie-Zaluski, M. C.; Perdrisot, R.; Gacel, G.; Swerts, J. P.; Roques, B. P.; Schwartz, J. C. Inhibitory Potency of Various Peptides on Enkephalinase Activity from Mouse Striatum. *Biochem. Biophys. Res. Commun.* 1979, *91*, 130–135.

(4) Swerts, J. P.; Perdrisot, R.; Malfroy, B.; Schwartz, J. C. Is 'Enkephalinase' Identical with 'Angiotensin-Converting Enzyme'? *Eur. J. Pharmacol.* 1979, *53*, 209–210.

(5) (a) Roques, B. P.; Fournie-Zaluski, M. C.; Soroca, E.; Lecomte, J. M.; Malfroy, B.; Llorens, C.; Schwartz, J. C. The Enkephalinase Inhibitor Thiorphan Shows Antinociceptive Activity in Mice. *Nature (London)* 1980, *288*, 286–288. (b) Roques, B. P.; Fournie-Zaluski, M. C.; Florentin, D.; Waksman, G.; Sassi, A.; Chaillet, P.; Collado, H.; Costentin, J. New Enkephalinase Inhibitors as Probes to Differentiate "Enkephalinase" and Angiotensin-Converting-Enzyme Active Sites. *Life Sci.* 1982, *31*, 1749–1752. (c) Bindra, J. S. U.S. Patent 4 329 495, 1982; *Chem. Abstr.* 1982, *97*, 162612f. (d) Gordon, E. M.; Cushman, D. W.; Tung, R.; Cheung, H. S.; Wang, F. L.; Delaney, N. G. Rat Brain Enkephalinase: Characterization of the Active Site Using Mercaptopropanoyl Amino Acid Inhibitors, and Comparison with Angiotensin-Converting Enzyme. *Life Sci.* 1983, *33*, 113–116. (e) Roques, B. P.; Lucas-Soroca, E.; Chaillet, P.; Costentin, J.; Fournie-Zaluski, M. C. Complete Differentiation between Enkephalinase and Angiotensin-Converting Enzyme Inhibition by Retro-Thiorphan. *Proc. Natl. Acad. Sci. U.S.A.* 1983, *80*, 3178–3182. (f) Altstein, M.; Bachar, E.; Vogel, Z.; Blumberg, S. Protection of Enkephalins from Enzymatic Degradation Utilizing Selective Metal-Chelating Inhibitors. *Eur. J. Pharmacol.* 1983, *91*, 353–361. (g) Fournie-Zaluski, M. C.; Lucas, E.; Waksman, G.; Roques, B. P. Differences in the Structural Requirements for Selective Interaction with Neutral Metalloendopeptidase (Enkephalinase) or Angiotensin-Converting Enzyme. Molecular Investigation by Use of New Thiol Inhibitors. *Eur. J. Biochem.* 1984, *139*, 267–274. (h) Scott, W. L.; Mendelsohn, L. G.; Cohen, M. L.; Evans, D. A.; Frederickson, R. C. A. Enantiomers of (*R,S*)-Thiorphan: Dissociation of Analgesia from Enkephalinase A Inhibition. *Life Sci.* 1985, *36*, 1307–1313. (i) Mendelsohn, L. G.; Johnson, B. G.; Scott, W. L.; Frederickson, R. C. A. Thiorphan and Analogs: Lack of Correlation between Potency to Inhibit "Enkephalinase A" in Vitro and Analgesic Potency in Vivo. *J. Pharmacol. Exp. Ther.* 1985, *234*, 386–390. (j) Fournie-Zaluski, M. C.; Lucas-Soroca, E.; Devin, J.; Roques, B. P. ^1H NMR Configurational Correlation for Retro-Inverso Dipeptides: Application to the Determination of the Absolute Configuration of "Enkephalinase" Inhibitors. Relationships between Stereochemistry and Enzyme Recognition. *J. Med. Chem.* 1986, *29*, 751–757. (k) Benchetrit, T.; Fournie-Zaluski, M. C.; Roques, B. P. Relationship between the Inhibitory Potencies of Thiorphan and Retrothiorphan Enantiomers on Thermolysin and Neutral Endopeptidase 24.11 and their Interactions with the Thermolysin Active Site by Computer Modelling. *Biochem. Biophys. Res. Commun.* 1987, *147*, 1034–1040. (l) Giros, B.; Gros, C.; Schwartz, J. C.; Danvy, D.; Plaquevent, J. C.; Duhamel, L.; Duhamel, P.; Vlaiculescu, A.; Costentin, J.; Lecomte, J. M. Enantiomers of Thiorphan and Acetorphan: Correlation between Enkephalinase Inhibition, Protection of Endogenous Enkephalins and Behavioral Effects. *J. Pharmacol. Exp. Ther.* 1987, *243*, 666–673.

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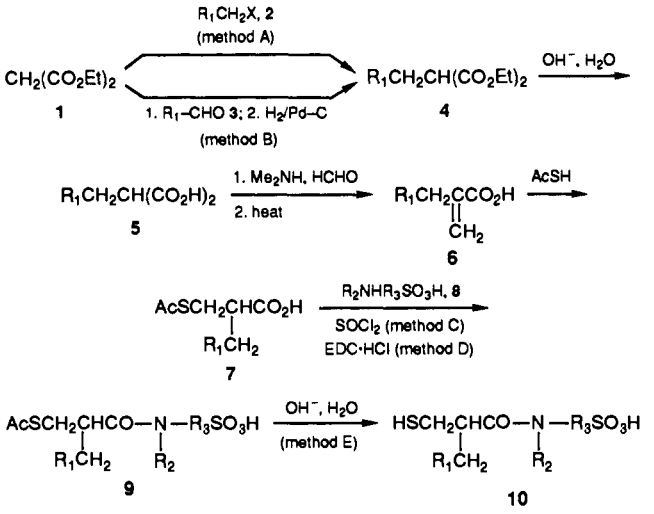
Scheme I



giotensin-converting enzyme (ACE). Their structure–activity relationships, involving the stereochemistry of the P_1' component,[9] were studied as well.

Chemistry

Scheme I illustrates the synthetic route to the sulfo-containing inhibitors **10** designed in the present study.

According to the literature, the substituted malonates **4** were prepared by the reaction of diethyl malonate **1** with halides **2** (method A)[11] or by the condensation of **1** with aldehydes **3** and subsequent catalytic hydrogenation (method B).[5c] Diacids **5**, obtained by the alkaline hydrolysis of **4**, were allowed to react with dimethylamine and formalin, followed by the pyrolysis, according to the procedure of Mannich and Ritsert,[12] to afford the acrylic acid derivatives **6**. Reaction of **6** with thioacetic acid by the method of Ondetti et al.[13] gave the propionic acid derivatives **7** (Table I). Acylation of amino sulfonic acids

(6) (a) Fournie-Zaluski, M. C.; Soroca-Lucas, E.; Waksman, G.; Llorens, C.; Schwartz, J. C.; Roques, B. P. Differential Recognition of "Enkephalinase" and Angiotensin-Converting Enzyme by New Carboxyalkyl Inhibitors. *Life Sci.* **1982**, *31*, 2947–2954. (b) Mumford, R. A.; Zimmerman, M.; ten Broeke, J.; Taub, D.; Joshua, H.; Rothrock, J. W.; Hirshfield, J. M.; Springer, J. P.; Patchett, A. A. Inhibition of Porcine Kidney "Enkephalinase" by Substituted-*N*-Carboxymethyl Dipeptides. *Biochem. Biophys. Res. Commun.* **1982**, *109*, 1303–1309. (c) Fournie-Zaluski, M. C.; Chaillet, P.; Soroca-Lucas, E.; Marcais-Collado, H.; Costentin, J.; Roques, B. P. New Carboxyalkyl Inhibitors of Brain Enkephalinase: Synthesis, Biological Activity, and Analgesic Properties. *J. Med. Chem.* **1983**, *26*, 60–65. (d) Almenoff, J.; Orlowski, M. Membrane-Bound Kidney Neutral Metalloendopeptidase: Interaction with Synthetic Substrates, Natural Peptides, and Inhibitors. *Biochemistry* **1983**, *22*, 590–599. (e) Murthy, L. R.; Glick, S. D.; Almenoff, J.; Wilk, S.; Orlowski, M. Inhibitors of an Enkephalin Degrading Membrane-Bound Metalloendopeptidase: Analgesic Properties and Effects on Striatal Enkephalin Levels. *Eur. J. Pharmacol.* **1984**, *102*, 305–313. (f) Chipkin, R. E.; Berger, J. G.; Billard, W.; Iorio, L. C.; Chapman, R.; Barnett, A. Pharmacology of SCH 34826, an Orally Active Enkephalinase Inhibitor Analgesic. *J. Pharmacol. Exp. Ther.* **1988**, *245*, 829–838. (g) Haslanger, M. F.; Sybertz, E. J.; Neustadt, B. R.; Smith, E. M.; Nechuta, T. L.; Berger, J. Carboxyalkyl Dipeptides with Atrial Natriuretic Factor Potentiating and Antihypertensive Activity. *J. Med. Chem.* **1989**, *32*, 737–739. (h) Ksander, G. M.; Diefenbacher, C. G.; Yuan, A. M.; Clark, F.; Sakane, Y.; Ghai, R. D. Enkephalinase Inhibitors. 1. 2,4-Dibenzylglutaric Acid Derivatives. *J. Med. Chem.* **1989**, *32*, 2519–2526. (i) Danilewicz, J. C.; Barclay, P. L.; Barnish, I. T.; Brown, D.; Campbell, S. F.; James, K.; Samuels, G. M. R. UK-69,578, a Novel Inhibitor of EC 3.4.24.11 which Increases Endogenous ANF Levels and Is Natriuretic and Diuretic. *Biochem. Biophys. Res. Commun.* **1989**, *164*, 58–65.

(7) (a) Hudgin, R. L.; Charleson, S. E.; Zimmerman, M.; Mumford, R.; Wood, P. L. Enkephalinase: Selective Peptide Inhibitors. *Life Sci.* **1981**, *29*, 2593–2601. (b) Altstein, M.; Blumberg, S.; Vogel, Z. Phosphoryl-Leu-Phe: A Potent Inhibitor of the Degradation of Enkephalin by Enkephalinase. *Eur. J. Pharmacol.* **1982**, *76*, 299–300. (c) Elliott, R. L.; Marks, N.; Berg, M. J.; Portoghese, P. S. Synthesis and Biological Evaluation of Phosphonamidate Peptide Inhibitors of Enkephalinase and Angiotensin-Converting Enzyme. *J. Med. Chem.* **1985**, *28*, 1208–1216.

(8) (a) Blumberg, S.; Vogel, Z.; Altstein, M. Inhibition of Enkephalin-Degrading Enzymes from Rat Brain and of Thermolysin by Amino Acid Hydroxamates. *Life Sci.* **1981**, *28*, 301–306. (b) Bouboutou, R.; Waksman, G.; Devin, J.; Fournie-Zaluski, M. C.; Roques, B. P. Bidentate Peptides: Highly Potent New Inhibitors of Enkephalin Degrading Enzymes. *Life Sci.* **1984**, *35*, 1023–1030. (c) Fournie-Zaluski, M. C.; Chaillet, P.; Bouboutou, R.; Coulaud, A.; Cherot, P.; Waksman, G.; Costentin, J.; Roques, B. P. Analgesic Effects of Kelatorphan, a New Highly Potent Inhibitor of Multiple Enkephalin Degrading Enzymes. *Eur. J. Pharmacol.* **1984**, *102*, 525–528. (d) Fournie-Zaluski, M. C.; Coulaud, A.; Bouboutou, R.; Chaillet, P.; Devin, J.; Waksman, G.; Costentin, J.; Roques, B. P. New Bidentates as Full Inhibitors of Enkephalin-Degrading Enzymes: Synthesis and Analgesic Properties. *J. Med. Chem.* **1985**, *28*, 1158–1169. (e) Hernandez, J. F.; Soleilhac, J. M.; Roques, B. P.; Fournie-Zaluski, M. C. Retro-Inverso Concept Applied to the Complete Inhibitors of Enkephalin-Degrading Enzymes. *J. Med. Chem.* **1988**, *31*, 1825–1831. (f) Inaoka, Y.; Naruto, S. Propioxatins A and B, New Enkephalinase B Inhibitors. IV. Characterization of the Active Site of the Enzyme Using Synthetic Propioxatin Analogues. *J. Biochem. (Tokyo)* **1988**, *104*, 706–711. (g) Fournie-Zaluski, M. C.; Hernandez, J. F.; Soleilhac, J. M.; Renwart, N.; Peyroux, J.; Xie, J.; Roques, B. P. Enkephalin-Degrading Enzyme Inhibitors. Crucial Role of the C-Terminal Residue on the Inhibitory Potencies of Retro-Hydroxamate Dipeptides. *Int. J. Pept. Protein Res.* **1989**, *33*, 146–153. (h) Xie, J.; Soleilhac, J. M.; Schmidt, C.; Peyroux, J.; Roques, B. P.; Fournie-Zaluski, M. C. New Kelatorphan-Related Inhibitors of Enkephalin Metabolism: Improved Antinociceptive Properties. *J. Med. Chem.* **1989**, *32*, 1497–1503. (i) Xie, J.; Soleilhac, J. M.; Renwart, N.; Peyroux, J.; Roques, B. P.; Fournie-Zaluski, M. C. Inhibitors of the Enkephalin Degrading Enzymes. Modulation of Activity of Hydroxamate Containing Compounds by Modifications of the C-Terminal Residue. *Int. J. Peptide Protein Res.* **1989**, *34*, 246–255.

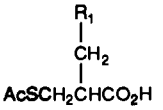
(9) Schechter, I.; Berger, A. On the Size of the Active Site in Proteases. I. Papain. *Biochem. Biophys. Res. Commun.* **1967**, *27*, 157–162.

(10) (a) Malfroy, B.; Schwartz, J. C. Properties of "Enkephalinase" from Rat Kidney: Comparison of Dipeptidyl-Carboxypeptidase and Endopeptidase Activities. *Biochem. Biophys. Res. Commun.* **1982**, *106*, 276–285. (b) Malfroy, B.; Schwartz, J. C. Enkephalinase from Rat Kidney. Purification, Characterization, and Study of Substrate Specificity. *J. Biol. Chem.* **1984**, *259*, 14365–14370. (c) Hersh, L. B.; Morihara, K. Comparison of the Subsite Specificity of the Mammalian Neutral Endopeptidase 24.11 (Enkephalinase) to the Bacterial Neutral Endopeptidase Thermolysin. *J. Biol. Chem.* **1986**, *261*, 6433–6437. (d) De la Baume, S.; Schwartz, J. C. Labelling and Exploration of the Active Site of Enkephalinase (EC 3.4.24.11) in Kidney Membranes with [^{3}H]Thiorphan as Ligand. *Eur. J. Pharmacol.* **1988**, *149*, 121–129. (e) Bateman, R. C., Jr.; Jackson, D.; Slaughter, C. A.; Unnithan, S.; Chai, Y. G.; Moomaw, C.; Hersh, L. B. Identification of the Active-Site Arginine in Rat Neutral Endopeptidase 24.11 (Enkephalinase) as Arginine 102 and Analysis of a Glutamine 102 Mutant. *J. Biol. Chem.* **1989**, *264*, 6151–6157.

(11) Cope, A. C.; Holmes, H. L.; House, H. O. The Alkylation of Esters and Nitriles. In *Organic Reactions*; Adams, R., Ed.; John Wiley and Sons, Inc.: New York, 1957; Vol. 9, pp 107–331.

(12) Mannich, C.; Ritsert, K. Condensation of Ethyl Diethylammonium Malonate and Formaldehyde. *Chem. Ber.* **1924**, *57B*, 1116–1118.

Table I. Physical Properties of 2-Substituted 3-(Acetylthio)propionic Acids (7)



compd[a]	R_1[b]	salt	mp, °C	formula[c]	anal.[d]	method[e]	% yield[f]
7a[g]	Ph	-	oil	$C_{12}H_{14}O_3S$	C,H,S	A	74
(*S*)-**7a**[h]	Ph	-	oil	$C_{12}H_{14}O_3S$	C,H,S	A	15[i]
(*R*)-**7a**[h]	Ph	-	oil	$C_{12}H_{14}O_3S$	C,H,S	A	18[j]
7b	*p*-MePh	-	74–75[k]	$C_{13}H_{16}O_3S \cdot 0.25H_2O$	C,H,S	A	35
7c	*p*-(MeO)Ph	-	oil	$C_{13}H_{16}O_4S$	C,H,S	B	13
7d	*p*-FPh	-	oil	$C_{12}H_{13}FO_3S$	C,H,F,S	A	20
7e	*p*-(CF_3)Ph	-	78–79[k]	$C_{13}H_{13}F_3O_3S$	C,H,F,S	A	14
7f	*p*-(NO_2)Ph	-	64–66[k,l]	$C_{12}H_{13}NO_5S \cdot 0.4H_2O$	C,H,N,S	A	11
7g	*p*-(Me_2N)Ph	-	121–122[k]	$C_{14}H_{19}NO_3S \cdot 0.25H_2O$	C,H,N,S	B	15
7h	*p*-PhPh	Na	lyoph[m]	$C_{18}H_{17}O_3SNa \cdot 0.6H_2O$	C,H,S,Na	A	16
7i	$Ph(CH_2)_2$	-	oil	$C_{14}H_{18}O_3S$	C,H,S	A	54
7j	1-naphthyl	-	oil	$C_{16}H_{16}O_3S \cdot 0.25H_2O$	C,H,S	A	36
7k	3-pyridyl	-	89–90[k]	$C_{11}H_{13}NO_3S \cdot 0.5H_2O$	C, H, N, S	B	15
7l	BI[n]	Na	lyoph[m]	$C_{13}H_{12}NO_4SNa \cdot 0.75H_2O$	C,H,N,S,Na	A	19
7m	Me_2CH	-	oil	$C_9H_{16}O_3S$	C,H,S	A	36

[a] All compounds were racemic unless otherwise noted. [b] Ph represents phenyl or phenylene. [c] All compounds exhibited IR and ^1H NMR spectra consistent with the assigned structures. [d] Analytical results were within ±0.4% of theoretical values unless otherwise noted. [e] See Scheme I and Experimental Section. [f] Yields are not optimized and represent an overall yield from 1 unless otherwise noted. [g] See ref 13. [h] See ref 5l. [i] Based on the *S* enantiomer containing in the racemic **7a**. [j] Based on the *R* enantiomer containing in the racemic **7a**. [k] Amorphous powder. [l] Reference 19 describes this as an oil. [m] Lyophilized powder. [n] 1,2-Benzisoxazol-3-yl.

8 with acid chlorides of **7** afforded the acetylthio sulfonic acids **9** except **9dd** (method C) (Table IV). Compound **9dd** was prepared by the condensation of **7k** with sulfanilic acid in the presence of 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl) (method D) (Table IV). The target compounds, mercapto sulfonic acids **10**, were obtained by the deacetylation of **9** (method E) (Table II).

Optical resolution of **7a** using (*R*)- and (*S*)-*N*-isopropylphenylalaninol (IPAOL)[14] as resolving agents gave (*S*)-**7a** and (*R*)-**7a**,[15] respectively, which were then converted into (*S*)-**10b** and (*R*)-**10b**.

Biological Results and Discussion

Table II shows the enkephalinase inhibitory potency of the synthesized mercapto sulfonic acids **10**. In order to know an effect of variation of the P_2' moiety (R_3) on enkephalinase inhibition, the moiety (R_3) was changed while the P_1' substituent (R_1) was fixed to a phenyl group corresponding to the P_1' (Phe) of enkephalins. The aminomethanesulfonic acid derivative **10a** exhibited a high activity comparable to the corresponding carboxylic acid analogue, thiorphan (*N*-(2-benzyl-3-mercaptopropionyl)-glycine).[5a] This finding suggests that a C-terminal sulfo group might be a bioisostere of the C-terminal carboxyl group in enkephalinase inhibitors. Varying the chain length from 1 (**10a**) to 2 (**10b**) caused no change of inhibitory activity, but a chain length of 3 (**10e**) induced a slight decrease in activity compared to **10a**. The same tendency has already been observed with hydroxamate-containing inhibitors by Xie et al.[8h] On the other hand, Gordon et al.[5d] and Fournie-Zaluski et al.[5g] synthesized a number of inhibitors possessing a natural amino acid as the P_2' residue; among them, the compound containing P_2' alanine or leucine was similar to the glycine counterpart in the inhibitory potency. In contrast to this, the sulfonic acid analogues **10f** and **10g** having the P_2' side chain corresponding to alanine and leucine, respectively, were remarkably less potent than **10a** with no P_2' side chain. Such notable reduction of activity is surprising, even if taking into account that **10f** and **10g** are diastereomeric mixtures.

In the series of aminobenzenesulfonic acids **10h–j**, there is a 6-fold difference in activity of the meta isomer **10i** to the para isomer **10j** whereas the ortho derivative **10h** suffers a sharp drop in activity, the fact of which indicates the meta isomer is superior to the para derivative and far superior to the ortho derivative. Unlike our result, Ksander et al.,[6h] who studied on the carboxyl type inhibitors containing a C-terminal aminobenzoic acid, indicated that the meta isomer was more active than the ortho and para isomers, which had practically the same potency.

The phenylmethylene (**10k**) and 1,4-naphthylene (**10l**) derivatives were less potent than the foregoing para isomer **10j**. Introduction of a methyl (**10c**) or cyclopropyl (**10d**) group onto the amido nitrogen atom produced a dramatic loss of activity, which is consistent with the findings by other authors.[5g,8h,16]

SARs of the P_1' substituent (R_1) were then examined. We first introduced various substituents into the P_1' phenyl ring. Introduction of relatively small substituents, such as methyl (**10m–o**) and methoxy (**10p,q**) groups, and a fluorine atom (**10r,s**) did not largely affect the inhibitory activity, thus retaining a sufficiently potent inhibition with an IC_{50} value in nanomolar level. Nitro (**10u,v**), dimethylamino (**10w,x**), and phenyl (**10y**) groups caused a

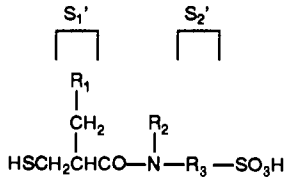
(13) Ondetti, M. A.; Conden, M. E.; Reid, J.; Sabo, E. F.; Cheung, H. S.; Cushman, D. W. Design of Potent and Specific Inhibitors of Carboxypeptidases A and B. *Biochemistry* **1979**, *18*, 1427–1430.

(14) Sawayama, T.; Tsukamoto, M.; Sasagawa, T.; Naruto, S.; Matsumoto, J.; Uno, H. An Improved Optical Resolution of 3-Acetylthio-2-methylpropionic Acid by Use of a New Chiral Amine, *N*-Isopropyl(phenylalaninol). *Chem. Pharm. Bull.* **1989**, *37*, 1382–1383.

(15) The enantiomers of **7a**, (*S*)-**7a** and (*R*)-**7a**, have already been prepared using (-)- and (+)-ephedrine, respectively, as the resolving agents by Giros et al. (see ref 5l).

(16) Llorens, C.; Gacel, G.; Swerts, J. P.; Perdrisot, R.; Fournie-Zaluski, M. C.; Schwartz, J. C.; Roques, B. P. Rational Design of Enkephalinase Inhibitors: Substrate Specificity of Enkephalinase Studied from Inhibitory Potency of Various Dipeptides. *Biochem. Biophys. Res. Commun.* **1980**, *96*, 1710–1716.

Table II. Physical Properties and Inhibitory Activities of Mercapto Sulfonic Acid Derivatives (10)



compd[a]	R_1[b]	R_2	R_3[b]	salt	formula[c]	anal.[d]	method[e]	% yield[f]	EK IC_{50},[g] nM	ACE IC_{50},[h] μM
10a	Ph	H	CH_2	–	$C_{11}H_{15}NO_4S_2 \cdot 1.5H_2O$	C,H,N,S	E	38	2.2	12
10b	Ph	H	$(CH_2)_2$	Na	$C_{12}H_{16}NO_4S_2Na \cdot H_2O$	C,H,N,S,Na	E	86	1.5	720
(*S*)-**10b**[i]	Ph	H	$(CH_2)_2$	Na	$C_{12}H_{16}NO_4S_2Na \cdot 0.5H_2O$	C,H,N,S,Na	E	67	0.82	430
(*R*)-**10b**[j]	Ph	H	$(CH_2)_2$	Na	$C_{12}H_{16}NO_4S_2Na \cdot 0.5H_2O$	C,H,N,S,Na	E	93	4.4	740
10c	Ph	Me	$(CH_2)_2$	Na	$C_{13}H_{18}NO_4S_2Na \cdot 0.25H_2O$	C,H,N,S,Na	E	57	490	–[k]
10d	Ph	Cy[l]	$(CH_2)_2$	Na	$C_{15}H_{20}NO_4S_2Na \cdot 0.25H_2O$	C,H,N,S,Na	E	81	4200	–
10e	Ph	H	$(CH_2)_3$	Na	$C_{13}H_{18}NO_4S_2Na \cdot H_2O$	C,H,N,S,Na	E	62	5.4	–
10f[m]	Ph	H	CH(Me)	Na	$C_{12}H_{16}NO_4S_2Na \cdot 0.75H_2O$	C,H,N,S,Na	E	75	31	–
10g[m]	Ph	H	$CH(CH_2CHMe_2)$	Na	$C_{15}H_{22}NO_4S_2Na \cdot 0.75H_2O$	C,H,N,S,Na	E	78	250	–
10h	Ph	H	*o*-Ph	Na	$C_{18}H_{16}NO_4S_2Na \cdot 0.5H_2O$	C,H,N,S,Na	E	65	140	–
10i	Ph	H	*m*-Ph	Na	$C_{16}H_{16}NO_4S_2Na \cdot 1.5H_2O$	C,H,N,S,Na	E	80	0.27	290
10j	Ph	H	*p*-Ph	Na	$C_{16}H_{16}NO_4S_2Na \cdot H_2O$	C,H,N,S,Na	E	70	1.7	210
10k	Ph	H	*p*-$PhCH_2$	Na	$C_{17}H_{16}NO_4S_2Na \cdot 0.5H_2O$	C,H,N,S,Na	E	69	11	–
10l	Ph	H	1,4-naphthylene	Na	$C_{20}H_{16}NO_4S_2Na \cdot 1.75H_2O$	C,H,N,S,Na	E	63	7.4	37
10m	*p*-MePh	H	$(CH_2)_2$	Na	$C_{13}H_{18}NO_4S_2Na \cdot H_2O$	C,H,N,S,Na	E	61	3.1	–
10n	*p*-MePh	H	*m*-Ph	Na	$C_{17}H_{18}NO_4S_2Na \cdot 1.75H_2O$	C,H,N,S,Na	E	78	2.5	–
10o	*p*-MePh	H	*p*-Ph	Na	$C_{17}H_{18}NO_4S_2Na \cdot 0.75H_2O$	C,H,N,S,Na	E	78	1.1	–
10p	*p*-(MeO)Ph	H	$(CH_2)_2$	Na	$C_{13}H_{18}NO_5S_2Na \cdot 0.5H_2O$	C,H,N,S,Na	E	49	4.4	–
10q	*p*-(MeO)Ph	H	*p*-Ph	Na	$C_{17}H_{18}NO_5S_2Na \cdot 0.5H_2O$	C,H,N,S,Na	E	56	3.2	–
10r	*p*-FPh	H	$(CH_2)_2$	Na	$C_{12}H_{15}FNO_4S_2Na \cdot H_2O$	C,H,F,N,S,Na	E	58	3.5	–
10s	*p*-FPh	H	*p*-Ph	Na	$C_{16}H_{15}FNO_4S_2Na \cdot 0.5H_2O$	C,H,F,N,S,Na	E	43	5.0	–
10t	*p*-(CF_3)Ph	H	*p*-Ph	Na	$C_{17}H_{15}F_3NO_4S_2Na \cdot 0.25H_2O$	C,H,F,N,S,Na	E	29	150	–
10u	*p*-(NO_2)Ph	H	$(CH_2)_2$	Na	$C_{12}H_{15}N_2O_8S_2Na \cdot H_2O$	C,H,N,S,Na	E	44	17	–
10v	*p*-(NO_2)Ph	H	*p*-Ph	Na	$C_{16}H_{15}N_2O_8S_2Na \cdot 1.3H_2O$	C,H,N,S,Na	E	44	14	–
10w	*p*-(Me_2N)Ph	H	$(CH_2)_2$	Na	$C_{14}H_{21}N_2O_4S_2Na \cdot 0.25H_2O$	C,H,N,S,Na	E	90	37	–
10x	*p*-(Me_2N)Ph	H	*p*-Ph	Na	$C_{13}H_{21}N_2O_4S_2Na \cdot 1.25H_2O$	C,H,N,S,Na	E	32	14	–
10y	*p*-Ph-Ph	H	*p*-Ph	Na	$C_{22}H_{20}NO_4S_2Na \cdot H_2O$	C,H,N,S,Na	E	53	52	–
10z	$Ph(CH_2)_2$	H	$(CH_2)_2$	–	$C_{14}H_{21}NO_4S_2 \cdot 0.75H_2O$	C,H,N,S	E	52	11	–
10aa	$Ph(CH_2)_2$	H	*p*-Ph	–	$C_{18}H_{21}NO_4S_2 \cdot 0.5H_2O$	C,H,N,S	E	38	110	–
10bb	1-naphthyl	H	$(CH_2)_2$	Na	$C_{18}H_{16}NO_4S_2Na \cdot 1.75H_2O$	C,H,N,S,Na[n]	E	57	8.4	–
10cc	1-naphthyl	H	*p*-Ph	Na	$C_{20}H_{16}NO_4S_2Na \cdot 2.25H_2O$	C,H,N,S,Na	E	74	34	–
10dd	3-pyridyl	H	*p*-Ph	–	$C_{15}H_{16}N_2O_4S_2 \cdot 0.5H_2O$	C,H,N,S	E	30	33	–
10ee	BI[o]	H	*p*-Ph	Na	$C_{17}H_{15}N_2O_5S_2Na \cdot H_2O$	C,H,N,S,Na	E	41	12	–
10ff	Me_2CH	H	$(CH_2)_2$	Na	$C_9H_{16}NO_4S_2Na \cdot H_2O$	C,H,N,S,Na	E	40	5.2	–
10gg	Me_2CH	H	*m*-Ph	Na	$C_{13}H_{16}NO_4S_2Na \cdot 1.5H_2O$	C,H,N,S,Na	E	63	2.4	–
10hh	Me_2CH	H	*p*-Ph	Na	$C_{13}H_{16}NO_4S_2Na \cdot 1.25H_2O$	C,H,N,S,Na	E	62	0.9	–
thiorphan[p]	Ph	Gly		–	$C_{12}H_{15}NO_3S$	C,H,N,S			2.6	0.42

[a] All compounds were lyophilized and racemic unless otherwise noted. [b–e] See footnotes in Table I. [f] Based on the immediate precursor (**9**). Yields are not optimized. [g] Enkephalinase inhibition. [h] ACE inhibition. [i] $[\alpha]_D$ = +36.8° (*c* 0.51, MeOH). [j] $[\alpha]_D$ = –32.1° (*c* 0.50, MeOH). [k] Not tested. [l] Cyclopropyl. [m] A mixture of diastereomers. [n] Na: calcd, 5.65; found, 6.09. [o] 1,2-Benzisoxazol-3-yl. [p] MP: 137–138 °C (MeCN) (lit.[5g] mp 138 °C (amorphous)).

discernible decrease in activity. Interestingly, an unexpectedly remarkable decrease in activity in the trifluoromethyl analogue **10t** was observed, probably being partly due to the large steric effect (E_s)[17] of the trifluoromethyl group. We next examined an effect of substitution of the P_1' phenyl group with different groups on enkephalinase inhibition. Replacement with a hydrophilic pyridyl group (**10dd**) decreased the activity. Similarly, substitution by bulkier naphthyl (**10bb,cc**) and benzisoxazolyl (**10ee**) groups and insertion (**10z,aa**) of an ethylene group between the phenyl ring and the inhibitor backbone resulted in a distinct decrease in activity. Substitution by an isopropyl group (**10ff–hh**), on the whole, maintained the activity comparable to that of the parent compounds **10b,i,j**. These results support that enkephalinase possesses a relatively large and hydrophobic S_1' subsite. This is in fair agreement with findings concerning the nature of the S_1' by many workers.[2,5d,f,g,6b,d,e,7a,10d,16]

ACE inhibitory activity of the sulfonic acids **10a,b,i,j,l** tested was five thousand to a million times lower than their enkephalinase inhibitory activity as shown in Table II. On the other hand, other authors[5d,g,h] previously demonstrated that thiorphan and its analogues, containing the C-terminal carboxyl group, showed only 1–200-fold difference between inhibitory potencies toward both enzymes. In the present experiment with thiorphan, an approximately 200-fold difference between activities against two enzymes was actually detected. These data suggest that the sulfonic acid derivative inhibits more selectively enkephalinase than the carboxylic acid derivative.[18]

It is of interest to see an influence of the stereochemistry

(17) Hansch, C.; Leo, A. *Substituent Constants for Correlation Analysis in Chemistry and Biology*; John Wiley and Sons, Inc.: New York, 1979; pp 9–12.

(18) *m*-((2-Benzyl-3-mercaptopropionyl)amino)benzoic acid, the carboxylic analogue of **10i**, inhibits enkephalinase and ACE with IC_{50}'s of 2.1 nM and 2.5 μM, respectively (unpublished results). A full, detailed description of these observations will be reported.

(19) Beaumont, A.; Hernandez, J. F.; Chaillet, P.; Crine, P.; Roques, B. P. Irreversible Photolabeling of Active Site of Neutral Endopeptidase-24.11 "Enkephalinase" by Azidothiorphan and [^{14}C]-Azidothiorphan. *Mol. Pharmacol.* **1987**, *32*, 594–599.

Table III. Antiwrithing Activities of 10b, (S)-10b, and (R)-10b in the Phenylbenzoquinone Writhing Test in Mice

compd	antiwrithing activity, ED_{50} (95% CL) ic (μg/mouse)		iv (mg/kg)	
10b	20.0 (12.6–31.8)	n^a = 18	18.0 (10.6–30.5)	n = 24
(S)-10b	11.8 (7.3–19.1)	n = 15	8.5 (6.8–10.5)	n = 36
(R)-10b	17.7 (10.1–31.0)	n = 18	43.3 (33.7–55.8)	n = 36
thiorphan	17.0 (10.9–26.6)	n = 24	72.0 (40.1–129.5)	n = 32

[a] Number of mice used.

of the P_1' component on the inhibitory activity against enkephalinase. Thus comparison of the optically active (S)-10b with (R)-10b shows that the S isomer is superior to the R isomer in inhibiting enkephalinase (Table II). A similar result of the stereochemistry–activity relationship for thiorphan has been reported by Scott et al.[5h]

Finally, the analgesic activity of the selected inhibitors (10b, (S)-10b, and (R)-10b) was evaluated using the phenylbenzoquinone writhing test in mice. As shown in Table III, intracisternal and intravenous injections of 10b as well as thiorphan[5e] produced an efficient inhibition of the writhe counts; compound 10b is approximately 4 times more potent than thiorphan when administered intravenously (iv). The analgesic activity of (S)-10b administered iv is approximately 5 times greater than that of (R)-10b. This result shows that the analgesic activity by the intravenous administration seemingly parallels the in vitro enkephalinase inhibitory potency. When administered intracisternally (ic), (S)-10b is 1.5 times more active than (R)-10b; this situation does not sufficiently reflect the difference between their in vitro inhibitory activities. Therefore, it is necessary to further study a correlation between the enkephalinase inhibitory activity and the analgesic effect.

Among the series of sulfonic acids prepared in the present study, the most potent inhibitor of enkephalinase was the m-aminobenzenesulfonic acid derivative 10i[18] with an IC_{50} value of 0.27 nM. Besides this, the potent compounds were 10a,b,j,n,o,gg,hh, which displayed the inhibitory activity comparable to or greater than thiorphan. From these findings, it follows that the C-terminal sulfo group is a good replacement of the C-terminal carboxyl group and causes a remarkably potent inhibition against enkephalinase.

Experimental Section

Melting points were determined on a Yanaco micro melting point apparatus and are uncorrected. Rotations at the Na D line were observed at ambient temperature by using a JASCO DIP-4 digital polarimeter. The proton nuclear magnetic resonance (^1H NMR) and infrared (IR) spectra were recorded on a Varian FT-80A spectrometer (with tetramethylsilane as an internal standard) and a JASCO A-102 spectrophotometer, respectively. Gas chromatography was carried out on an SE-54 capillary column (30 m × 0.25 mm i.d.) at 230 °C with a Hewlett-Packard 5840A gas chromatograph. The carrier gas was He ($\bar{u}$ = 24.2 cm/s). The highly porous polystyrene resin (Diaion HP20 or CHP20P) was purchased from Mitsubishi Chemical Ind., Japan. Solutions were dried over anhydrous $MgSO_4$, and solvents were evaporated under reduced pressure.

The following compounds were prepared according to the literatures cited: N-methyltaurine and N-cyclopropyltaurine,[20] α-aminoethanesulfonic acid and α-aminoisopentanesulfonic acid,[21–23] p-aminobenzenesulfonic acid,[24,25] and thiorphan.[5g]

Method A: 3-(Acetylthio)-2-benzylpropionic Acid (7a). According to the literature,[11] reaction of diethyl malonate 1 (160 g, 1 mol) with benzyl bromide 2a (171 g, 1 mol) gave crude diethyl benzylmalonate 4a. Alkaline hydrolysis of 4a afforded benzylmalonic acid 5a, which was converted into crude benzylacrylic acid 6a (126 g) by the procedure of Mannich and Ritsert.[12] A stirred mixture of 6a (126 g) and thioacetic acid (117 g) was heated at 80 °C for 1 h. After removal of excess thioacetic acid in vacuo, the crude product was subjected to column chromatography on Diaion HP20 with a 40% aqueous MeCN solution as an eluent. After evaporation of MeCN under reduced pressure, the residue was extracted with EtOAc. The organic layer was dried and evaporated to yield 7a (176 g, 74%) as a pale yellow oil.

Compounds 7b,d–f,h–j,l,m were prepared in a similar manner (Table I), but 7h,l were purified by column chromatography as Na salts.

Optical Resolution of 3-(Acetylthio)-2-benzylpropionic Acid (7a). A solution of 7a (89.5 g, 0.376 mol) and (S)-IPAOL[14] (50.8 g, 0.263 mol) in 2-propanol (600 mL) was allowed to stand for 3 days at room temperature. The precipitate (117 g) was collected by filtration and recrystallized several times from 2-propanol to give (R)-7a·(S)-IPAOL (14.5 g) as colorless needles: mp 138.5–139.5 °C; $[\alpha]_D$ = +24.4° (c 0.50, MeOH), 97.2% ee. A suspension of (R)-7a·(S)-IPAOL (13 g) in ice–water was acidified with 10% H_2SO_4 and extracted with EtOAc. The extract was washed with H_2O, dried, and evaporated to afford the free acid (R)-7a (7.3 g, 18%) as a pale yellow oil, $[\alpha]_D$ = +35.2° (c 0.67, EtOH).

The other enantiomeric salt (S)-7a·(R)-IPAOL (colorless needles, mp 140–141 °C, $[\alpha]_D$ = −25.4° (c 0.50, MeOH), 98.2% ee), was obtained in a similar manner using (R)-IPAOL[14] as a resolving agent. The corresponding free acid (S)-7a [a pale yellow oil, $[\alpha]_D$ = −37.2° (c 0.62, EtOH) (lit.[51] $[\alpha]_D$ = −36.4° (c 1.3, MeOH))] was liberated by the above-mentioned acidic treatment.

The optical purity was determined by capillary gas chromatographic analysis for 3-(acetylthio)-2-benzyl-N-(α-methylbenzyl)propionamide derived from 7a and (R)-(+)-α-methylbenzylamine.

Method B: 3-(Acetylthio)-2-(p-(dimethylamino)benzyl)propionic Acid (7g). A solution of diethyl malonate 1 (160 g, 1 mol), p-(dimethylamino)benzaldehyde 3g (149 g, 1 mol), and piperidine (5 mL) in toluene (650 mL) was refluxed for 10 h using a Dean–Stark apparatus. After evaporation of the toluene under reduced pressure, the residue was recrystallized from EtOH to yield the intermediate olefinic compound (234 g) as yellow needles. A solution of this olefin in THF (1 L) was hydrogenated over 5% palladium on charcoal (15 g) at room temperature. The catalyst was removed by filtration, and the filtrate was concentrated under reduced pressure to give the crude product 4g (220 g), which was converted into 7g (42.9 g, 15%) by method A.

Compounds 7c,k were prepared in a similar manner (Table I).

Method C: N-(3-(Acetylthio)-2-benzylpropionyl)taurine Sodium Salt (9b). Thionyl chloride (7.14 g, 0.06 mol) was added dropwise to a stirred solution of 7a (11.9 g, 0.05 mol) in $CHCl_3$ (80 mL) and DMF (0.1 mL) at room temperature. After the solution was refluxed for 30 min, the $CHCl_3$ and excess thionyl chloride were evaporated in vacuo to give the residue. To a stirred, ice-cooled solution of the residue in THF (80 mL) was added a solution of taurine (6.25 g, 0.05 mol) in a pH 10 aqueous NaOH solution (25 mL). The stirred mixture was kept at pH 7.5–8.5 with 1 N NaOH for 15 min under ice-cooling and for an additional 30 min at room temperature. After evaporation of the THF under reduced pressure, the resulting aqueous solution was subjected to column chromatography on Diaion HP20 with a 5% aqueous

(20) Marvel, C. S.; Bailey, C. F. Taurine. In *Organic Syntheses*; Blatt, A. H., Ed.; John Wiley and Sons, Inc.: New York, 1943; Collect. Vol. II, pp 563–565.

(21) Backer, H. J.; Mulder, H. Acetylation of Some 1,1-Aminosulfonic Acids and 1,1-Hydrazinesulfonic Acids. *Recl. Trav. Chim. Pays-Bas* **1934**, *53*, 1120–1127.

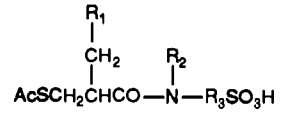
(22) McIlwain, H. Amino-Sulphonic Acid Analogues of Natural Amino-Carboxylic Acids. *J. Chem. Soc.* **1941**, 75–77.

(23) Frankel, M.; Moses, P. Syntheses of Amino Alkyl Sulphonic Acids and their Peptide Analogues. *Tetrahedron* **1960**, *9*, 289–294.

(24) Clutterbuck, P. W.; Cohen, J. B. The Aryl and Alkyl Sulphonamides. *J. Chem. Soc.* **1923**, *123*, 2507–2515.

(25) Schroeter, L. C.; Higuchi, T. Bisulfite Reduction of p-Nitrobenzyl Alcohol. *J. Pharm. Sci.* **1962**, *51*, 888–894.

Table IV. Physical Properties of Acetylthio Sulfonic Acid Derivatives (9)



compd[a]	R_1[b]	R_2	R_3[b]	salt	formula[c]	anal.[d]	method[e]	% yield[f]
9a	Ph	H	CH_2	Na	$C_{13}H_{16}NO_5S_2Na·0.5H_2O$	C,H,N,S,Na	C	47
9b	Ph	H	$(CH_2)_2$	Na	$C_{14}H_{18}NO_5S_2Na$	C,H,N,S,Na	C	50
(*S*)-**9b**[g]	Ph	H	$(CH_2)_2$	Na	$C_{14}H_{16}NO_5S_2Na·0.5H_2O$	C,H,N,S,Na	C	47
(*R*)-**9b**[h]	Ph	H	$(CH_2)_2$	Na	$C_{14}H_{18}NO_5S_2Na·H_2O$	C,H,N,S,Na	C	50
9c	Ph	Me	$(CH_2)_2$	Na	$C_{15}H_{20}NO_5S_2Na·0.5H_2O$	C,H,N,S,Na	C	60
9d	Ph	Cy[i]	$(CH_2)_2$	Na	$C_{17}H_{22}NO_5S_2Na·1.25H_2O$	C,H,N,S,Na	C	65
9e	Ph	H	$(CH_2)_3$	Na	$C_{15}H_{20}NO_5S_2Na·0.75H_2O$	C,H,N,S,Na[j]	C	17
9f[k]	Ph	H	CH(Me)	Na	$C_{14}H_{18}NO_5S_2Na·H_2O$	C,H,N,S,Na	C	28
9g[k]	Ph	H	$CH(CH_2CHMe_2)$	Na	$C_{17}H_{24}NO_5S_2Na·1.75H_2O$	C,H,N,S,Na	C	10
9h	Ph	H	*o*-Ph	Na	$C_{16}H_{18}NO_5S_2Na·1.5H_2O$	C,H,N,S,Na	C	14
9i	Ph	H	*m*-Ph	Na	$C_{16}H_{18}NO_5S_2Na·1.5H_2O$	C,H,N,S,Na	C	12
9j	Ph	H	*p*-Ph	Na	$C_{16}H_{16}NO_5S_2Na·1.5H_2O$	C,H,N,S,Na	C	89
9k	Ph	H	*p*-$PhCH_2$	Na	$C_{19}H_{20}NO_5S_2Na·1.25H_2O$	C,H,N,S,Na	C	76
9l	Ph	H	1,4-naphthylene	Na	$C_{22}H_{20}NO_5S_2Na·1.75H_2O$	C,H,N,S,Na	C	22
9m	*p*-MePh	H	$(CH_2)_2$	Na	$C_{15}H_{20}NO_5S_2Na·0.75H_2O$	C,H,N,S,Na	C	12
9n	*p*-MePh	H	*m*-Ph	Na	$C_{19}H_{20}NO_5S_2Na·1.2H_2O$	C,H,N,S,Na	C	29
9o	*p*-MePh	H	*p*-Ph	Na	$C_{19}H_{20}NO_5S_2Na·H_2O$	C,H,N,S,Na	C	9
9p	*p*-(MeO)Ph	H	$(CH_2)_2$	Na	$C_{15}H_{20}NO_6S_2Na·0.5H_2O$	C,H,N,S,Na	C	24
9q	*p*-(MeO)Ph	H	*p*-Ph	Na	$C_{19}H_{20}NO_6S_2Na·H_2O$	C,H,N,S,Na	C	63
9r	*p*-FPh	H	$(CH_2)_2$	Na	$C_{14}H_{17}FNO_5S_2Na·0.5H_2O$	C,H,F,N,S,Na	C	36
9s	*p*-FPh	H	*p*-Ph	Na	$C_{18}H_{17}FNO_5S_2Na·0.75H_2O$	C,H,F,N,S,Na	C	50
9t	*p*-(CF_3)Ph	H	*p*-Ph	Na	$C_{19}H_{17}F_3NO_5S_2Na·H_2O$	C,H,F,N,S,Na	C	14
9u	*p*-(NO_2)Ph	H	$(CH_2)_2$	Na	$C_{14}H_{17}N_2O_7S_2Na·0.5H_2O$	C,H,N,S,Na	C	46
9v	*p*-(NO_2)Ph	H	*p*-Ph	–	$C_{16}H_{16}N_2O_7S_2·2.5H_2O$	C,H,N,S	C	30
9w	*p*-(Me_2N)Ph	H	$(CH_2)_2$	Na	$C_{16}H_{23}N_2O_5S_2Na·2H_2O$	C,H,N,S,Na	C	15
9x	*p*-(Me_2N)Ph	H	*p*-Ph	Na	$C_{20}H_{23}N_2O_5S_2Na·1.5H_2O$	C,H,N,S,Na	C	26
9y	*p*-PhPh	H	*p*-Ph	Na	$C_{24}H_{22}NO_5S_2Na·H_2O$	C,H,N,S,Na	C	23
9z	$Ph(CH_2)_2$	H	$(CH_2)_2$	Na	$C_{16}H_{22}NO_5S_2Na·0.25H_2O$	C,H,N,S,Na	C	50
9aa	$Ph(CH_2)_2$	H	*p*-Ph	Na	$C_{20}H_{22}NO_5S_2Na·0.5H_2O$	C,H,N,S,Na	C	55
9bb	1-naphthyl	H	$(CH_2)_2$	Na	$C_{18}H_{20}NO_5S_2Na$	C,H,N,S,Na	C	34
9cc	1-naphthyl	H	*p*-Ph	Na	$C_{22}H_{20}NO_5S_2Na·2.75H_2O$	C,H,N,S,Na	C	21
9dd	3-pyridyl	H	*p*-Ph	Na	$C_{17}H_{17}N_2O_5S_2Na·0.25H_2O$	C,H,N,S,Na[l]	D	2
9ee	BI[m]	H	*p*-Ph	Na	$C_{19}H_{17}N_2O_6S_2Na·1.5H_2O$	C,H,N,S,Na	C	39
9ff	Me_2CH	H	$(CH_2)_2$	Na	$C_{11}H_{20}NO_5S_2Na·1.75H_2O$	C,H,N,S,Na	C	38
9gg	Me_2CH	H	*m*-Ph	Na	$C_{15}H_{20}NO_5S_2Na·1.25H_2O$	C,H,N,S,Na	C	35
9hh	Me_2CH	H	*p*-Ph	Na	$C_{15}H_{20}NO_5S_2Na·1.2H_2O$	C,H,N,S,Na	C	29

[a] See Table II. [b-e] See footnotes in Table I. [f] Based on the immediate precursor (7). Yields are not optimized. [g] $[\alpha]_D$ = −34.5° (*c* 0.63, MeOH). [h] $[\alpha]_D$ = +33.1° (*c* 0.51, MeOH). [i] Cyclopropyl. [j] C: calcd, 45.62; found, 46.10; S: calcd, 16.24; found, 15.79. [k] A mixture of diastereomers. [l] Na: calcd, 5.46; found, 5.01. [m] 1,2-Benzisoxazol-3-yl.

dioxane solution as an eluent. The eluate was lyophilized to give **9b** (9.18 g, 50%): ^{1}H NMR (Me_2SO-d_6) δ 7.75 (t, *J* = 6 Hz, 1 H), 7.2 (m, 5 H), 2.3–3.5 (9 H), 2.30 (s, 3 H).

Compounds **9a,c–cc,ee–hh**, (*S*)-**9b**, and (*R*)-**9b** were prepared in a similar manner (Table IV), but **9v** was purified by column chromatography as a free acid.

Method D: *N*-[3-(Acetylthio)-2-(3-pyridylmethyl)propionyl]sulfanilic Acid Sodium Salt (9dd). Sulfanilic acid (1.7 g, 0.01 mol) and EDC·HCl (2.1 g, 0.011 mol) were added to an ice-chilled solution of **7k** (2.5 g, 0.01 mol) in MeCN (30 mL) and $CHCl_3$ (20 mL). After the mixture was stirred for 1.5 h at room temperature, the organic solvents were distilled off under reduced pressure. Water (70 mL) was added to the residue, and the mixture was washed with $CHCl_3$. The aqueous layer was adjusted to pH 8, subjected to column chromatography on CHP20P, and eluted with a gradient of 0–5% dioxane in H_2O under medium pressure. The eluate was lyophilized to give **9dd** (0.1 g, 2%): ^{1}H NMR (Me_2SO-d_6) δ 10.00 (s, 1 H), 8.4 (m, 2 H), 7.2–7.7 (6 H), 2.5–3.2 (5 H), 2.30 (s, 3 H).

Method E: *N*-(2-Benzyl-3-mercaptopropionyl)taurine Sodium Salt (10b). A stirred solution of **9b** (2 g, 5.4 mmol) in H_2O (15 mL) was kept at pH 13 with 1 N NaOH for 30 min at room temperature under an N_2 atmosphere. The solution was adjusted to pH 8.5 with HCl, subjected to column chromatography on CHP20P, and eluted with a gradient of 5–10% dioxane in H_2O under medium pressure. The eluate was lyophilized to afford **10b** (1.6 g, 86%): ^{1}H NMR (Me_2SO-d_6) δ 7.78 (t, *J* = 6 Hz, 1 H), 7.2 (m, 5 H), 2.3–3.5 (9 H), 2.21 (t, *J* = 8 Hz, 1 H).

Compounds **10a,c–hh**, (*S*)-**10b**, and (*R*)-**10b** were prepared in a similar manner (Table II), but **10a,z,aa,dd** were purified by column chromatography as free acids.

Enkephalinase Inhibitory Activity in Vitro. (1) Separation of Enzyme. Enkephalin-degrading enzymes were separated according to the method of Gorenstein and Snyder.[26]

Male Std:Wistar rats were decapitated and the striata were removed. These were homogenized in 30 volumes of 50 mM Tris-HCl buffer (pH 7.7) and centrifuged for 15 min at 1000*g*. The supernatant was centrifuged for 15 min at 50000*g*. The pellet was washed three times with the same buffer and then suspended in 15 volumes of 50 mM Tris-HCl buffer (pH 7.7) containing 1% Triton X-100, followed by incubation for 45 min at 37 °C. After centrifugation for 1 h at 100000*g*, the supernatant was applied onto DEAE-cellulose chromatography column. The fraction containing enkephalin dipeptidylcarboxypeptidase was used as an enkephalinase sample.

(2) Measurement of Inhibitory Activity. After the test compound and the enkephalinase sample (50 μL; approximately 0.1 mg protein/mL) were preincubated for 5 min at 37 °C, [^{3}H]leucine-enkephalin (substrate; Km: 60 μM) was added to the reaction mixture. The mixture was incubated for 1 h at 37 °C (final volume: 100 μL; final concentration of substrate: 20 nM). Ice-cold 0.2 N HCl was added to the mixture to stop the reaction. The substrate and metabolites were separated with Porapak Q

(26) Gorenstein, C.; Snyder, S. H. Two Distinct Enkephalinases: Solubilization, Partial Purification and Separation from Angiotensin Converting Enzyme. *Life Sci.* **1979**, *25*, 2065–2070.

column by the method of Vogel and Alstein.[27] Enzyme activity was estimated from the production rate of [^{3}H]Tyr-Gly-Gly and 14–24% hydrolysis of substrate was routinely detected. The IC_{50} value of the test compound was calculated from the inhibitory rates, estimated from the difference between the enzyme activities in the absence and the presence of the test compound. All IC_{50}'s were computed by log probit analysis of three or four concentrations of the test compounds and determined two or three times with an average standard error of the mean of ±30%. The results are shown in Table II.

ACE Inhibitory Activity in Vitro. ACE was prepared from the rabbit lung by the method of Cushman and Cheung,[28] and the inhibitory activity of the test compound was determined by the procedure of Takeyama et al.[29] The results are shown in Table II.

Analgesic Activity (Phenylbenzoquinone Writhing Test). Writhing was induced by an intraperitoneal injection of 10 mL/kg of 0.03% phenylbenzoquinone in a 5% aqueous EtOH solution in female ddY mice (18–22 g), and the number of writhes was counted for 15 min, beginning from 5 min after the phenylbenzoquinone injection.[30,31] The test compound was given ic or iv, 1 or 5 min, respectively, before the phenylbenzoquinone injection. The intracisternal injection was carried out according to the method of Ueda et al.[32] A 10-μL aqueous solution was slowly injected into the cisterna magna through the intact skin at the back of the neck of conscious animals. Mice showing a behavioral change immediately after the intracisternal injection were excluded. A reduction in writhe counts greater than 50% of the vehicle control value was considered to be effective. The ED_{50} value and 95% confidence limits were calculated from the effective rates according to the method of Litchfield and Wilcoxon.[33] The results are shown in Table III.

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Registry No. 1, 105-53-3; **2a**, 100-39-0; **3g**, 100-10-7; **4g**, 58353-47-2; **7a**, 80969-99-9; (*S*)-**7a**, 124815-67-4; (*S*)-**7a**·(*R*)-IPAOL, 138062-85-8; (*R*)-**7a**, 116111-79-6; (*R*)-**7a**·(*S*)-IPAOL, 138062-84-7; **7b**, 138062-86-9; **7c**, 135793-81-6; **7d**, 81110-10-3; **7e**, 138062-87-0; **7f**, 113346-68-2; **7g**, 138062-88-1; **7h**, 138062-89-2; **7i**, 138062-90-5; **7j**, 138062-91-6; **7k**, 138062-92-7; **7l**, 138062-93-8; **7m**, 78383-52-5; **9a**, 138062-94-9; **9b**, 138062-95-0; (*S*)-**9b**, 116112-13-1; (*R*)-**9b**, 116112-14-2; **9c**, 138062-96-1; **9d**, 138062-97-2; **9e**, 138062-98-3; (*R**,*S**)-**9f**, 138062-99-4; (*R**,*R**)-**9f**, 138063-00-0; (*R**,*S**)-**9g**, 138063-01-1; (*R**,*R**)-**9g**, 138063-02-2; **9h**, 138063-03-3; **9i**, 138063-04-4; **9j**, 138063-05-5; **9k**, 138063-06-6; **9l**, 138063-07-7; **9m**, 138063-08-8; **9n**, 138063-09-9; **9o**, 138063-10-2; **9p**, 138063-11-3; **9q**, 138063-12-4; **9r**, 138063-13-5; **9s**, 138063-14-6; **9t**, 138063-15-7; **9u**, 138063-16-8; **9v**, 138063-17-9; **9w**, 138063-18-0; **9x**, 138063-19-1; **9y**, 138063-20-4; **9z**, 138063-21-5; **9aa**, 138063-22-6; **9bb**, 138063-23-7; **9cc**, 138063-24-8; **9dd**, 138063-25-9; **9ee**, 138063-26-0; **9ff**, 138063-27-1; **9gg**, 138063-28-2; **9hh**, 138063-29-3; **10a**, 138063-30-6; **10b**, 138063-31-7; (*S*)-**10b**, 116111-86-5; (*R*)-**10b**, 116111-85-4; **10c**, 138063-32-8; **10d**, 138089-56-2; **10e**, 138063-33-9; (*R**,*R**)-**10f**, 138063-34-0; (*R**,*S**)-**10f**, 138063-35-1; (*R**,*R**)-**10g**, 138063-36-2; (*R**,*S**)-**10g**, 138063-37-3; **10h**, 138063-38-4; **10i**, 138063-39-5; **10j**, 138063-40-8; **10k**, 138063-41-9; **10l**, 138063-42-0; **10m**, 138063-43-1; **10n**, 138063-44-2; **10o**, 138063-45-3; **10p**, 138063-46-4; **10q**, 138063-47-5; **10r**, 138063-48-6; **10s**, 138063-49-7; **10t**, 138063-50-0; **10u**, 138063-51-1; **10v**, 138063-52-2; **10w**, 138063-53-3; **10x**, 138063-54-4; **10y**, 138063-55-5; **10z**, 138063-56-6; **10aa**, 138063-57-7; **10bb**, 138063-58-8; **10cc**, 138063-59-9; **10dd**, 138063-60-2; **10ee**, 138063-61-3; **10ff**, 138063-62-4; **10gg**, 138063-63-5; **10hh**, 138063-64-6; 3-(bromomethyl)-1,2-benzisoxazole, 37924-85-9; enkephalin dipeptidyl carboxypeptidase, 70025-49-9; 4-methylbenzyl bromide, 104-81-4; 4-methoxybenzaldehyde, 123-11-5; 4-fluorobenzyl bromide, 459-46-1; 4-(trifluoromethyl)benzyl bromide, 402-49-3; 4-nitrobenzyl bromide, 100-11-8; 4-phenylbenzyl bromide, 2567-29-5; (3-bromopropyl)benzene, 637-59-2; 1-(bromomethyl)naphthalene, 3163-27-7; 3-pyridinecarboxaldehyde, 500-22-1; 1-bromo-2-methylpropane, 78-77-3; thioacetic acid, 507-09-5; taurine, 107-35-7; sulfanilic acid, 121-57-3; angiotensin-converting enzyme, 9015-82-1.

(27) Vogel, Z.; Altstein, M. The Adsorption of Enkephalin to Porous Polystyrene Beads: A Simple Assay for Enkephalin Hydrolysis. *FEBS Lett.* **1977**, *80*, 332–336.
(28) Cushman, D. W.; Cheung, H. S. Spectrophotometric Assay and Properties of the Angiotensin-Converting Enzyme of Rabbit Lung. *Biochem. Pharmacol.* **1971**, *20*, 1637–1648.
(29) Takeyama, K.; Minato, H.; Fukuya, F.; Kawahara, S.; Hosoki, K.; Kadokawa, T. Antihypertensive Activity of Alacepril, an Orally Active Angiotensin Converting Enzyme Inhibitor, in Renal Hypertensive Rats and Dogs. *Arzneim.-Forsch./Drug Res.* **1985**, *35*, 1502–1507.
(30) Siegmund, E.; Cadmus, R.; Lu, G. A Method for Evaluating Both Non-Narcotic and Narcotic Analgesics. *Proc. Soc. Exp. Biol. Med.* **1957**, *95*, 729–731.
(31) Nakamura, H.; Ishii, K.; Yokoyama, Y.; Imazu, C.; Shimoda, A.; Kadokawa, T.; Shimizu, M. Central Actions of AD-1211, an Analgesic Lacking Common Opiate Features. *Eur. J. Pharmacol.* **1984**, *106*, 345–356.
(32) Ueda, H.; Amano, H.; Shiomi, H.; Takagi, H. Comparison of the Analgesic Effects of Various Opioid Peptides by a Newly Devised Intracisternal Injection Technique in Conscious Mice. *Eur. J. Pharmacol.* **1979**, *56*, 265–268.
(33) Litchfield, J. T.; Wilcoxon, F. A Simplified Method of Evaluating Dose-Effect Experiments. *J. Pharmacol. Exp. Ther.* **1949**, *96*, 99–113.