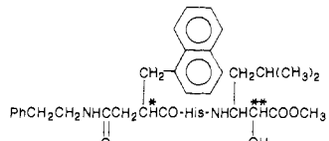
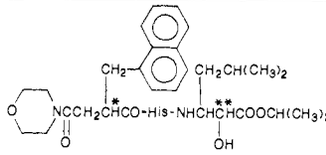
Table II. Renin Inhibitory Potencies of the Stereoisomers[a]



no.	*	**	IC_{50}, M, against human renin	IC_{50}, M, against human plasma renin
5 (KRI-1177)	+	*R,S* (7:3)	7.8×10^{-8}	9.0×10^{-8}
12[b]	−	*R,S* (7:3)	$>10^{-4}$	
13[c]	+	*R*	3.1×10^{-8}	7.7×10^{-8}
14[d]	+	*S*	1.3×10^{-6}	



no.	*	**	IC_{50}, M, against human renin	IC_{50}, M, against human plasma renin
9 (KRI-1230)	−	*R,S* (7:3)	2.5×10^{-8}	7.8×10^{-9}
15[e]	+	*R,S* (7:3)	$>10^{-4}$	

[a] The IC_{50} values of the inhibitors against isolated human renin and human plasma renin were measured by the method described in Table I. Anal.: [b] ($C_{37}H_{45}N_5O_6$) C, H, N. [c] ($C_{37}H_{45}N_5O_6$) C, H, N. [d] ($C_{37}H_{45}N_5O_6$) C, H, N. [e] ($C_{35}H_{47}N_5O_7 \cdot {}^1/_5CHCl_3$) C, H, N.

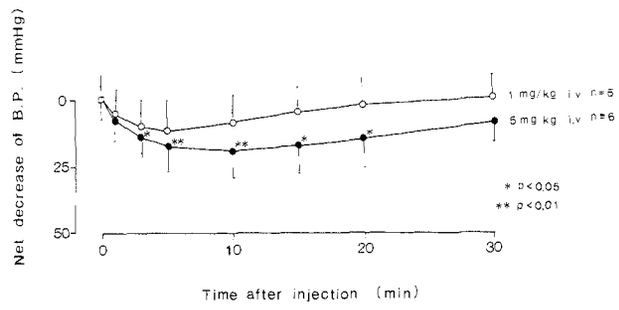


Figure 4. Effect of intravenous injection of **9** on blood pressure. Frosemide was applied by the method described in Figure 3 to sodium-depleted male marmosets. A catheter was inserted under anesthesia into the femoral artery. The catheter was connected to the pressure transducer for measurement of blood pressure. After come out from under the anesthesia, compound **9** was injected into the femoral vein as 1 mL/kg aqueous solution.

idue was moderately stable in the same condition. Compound **9** was stable also in the human plasma.

Oral administration of 30 mg/kg of **9** to common marmosets resulted in a lowering of mean blood pressure accompanying a reduction of the plasma renin activity (Figure 3). Figure 4 shows changes in blood pressure after intravenous injection of **9** in doses of 1 or 5 mg/kg. The lowering effect of a 5 mg/kg injection was comparable to that of oral administration of a 30 mg/kg dose. In the case of intravenous injection, the hypotensive response was dose dependent, and the maximum response occurred within 10 min after injection. On the other hand, long-lasting hypotensive effect was found when **9** was orally administered. The maximum response occurred 1 h after the administration and both blood pressure and plasma renin activity recovered gradually. However, recovery of the blood pressure was very slow, and even after 7 h the blood pressure was significantly depressed (Figure 3).

In conclusion, the present study shows that norstatine is a useful component of the renin inhibitors compared with statine and KRI-1230 is one of the most compact and highly potent renin inhibitors.

[†] Kissei Pharmaceutical Co., Ltd.
[‡] Kitasato University.
[§] Kyoto Pharmaceutical University.

**Kinji Iizuka,*† Tetsuhide Kamijo,† Tetsuhiro Kubota†
Kenji Akahane,† Hideaki Umeyama,‡ Yoshiaki Kiso§**

Division of Medicinal Chemistry
Central Research Laboratories
Kissei Pharmaceutical Co., Ltd.
Nagano 399 Japan
School of Pharmaceutical Sciences
Kitasato University
Shirokane, Minato-Ku, Tokyo 108, Japan
Kyoto Pharmaceutical University
Yamashina-Ku, Kyoto 607, Japan

Received August 3, 1987

β-Substituted Phenethylamines as High-Affinity Mechanism-Based Inhibitors of Dopamine β-Hydroxylase

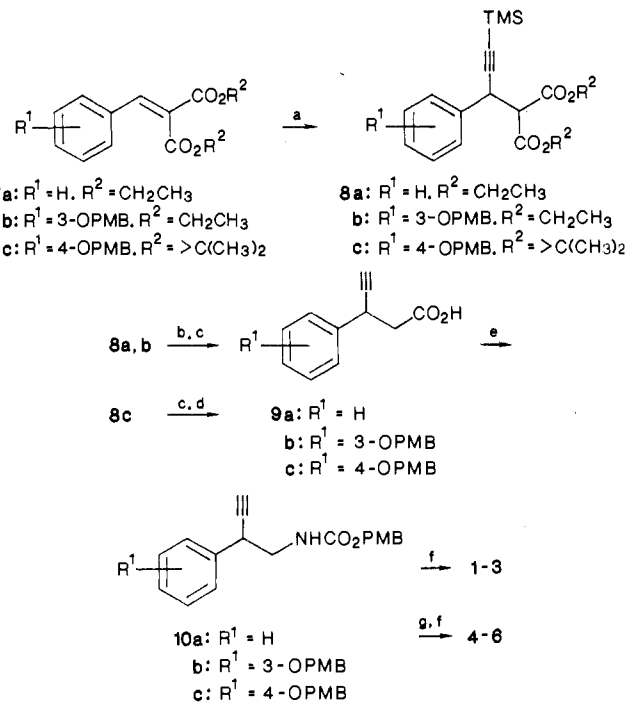
Sir:

Dopamine β-hydroxylase (DBH; E.C. 1.14.17.1) presents an appealing target for the design of inhibitors as potential new cardiovascular agents. We have recently reported potent, reversible inhibitors of DBH that are effective antihypertensive agents[1-4] and, in an alternative approach, have described several structurally simple mechanism-based inhibitors of DBH.[5,6] Whereas a multitude of other mechanism-based inhibitors of DBH have been reported previously,[7-14] the high, millimolar K_m for dopamine substrate makes critically important the design of k_{cat} inhibitors with enhanced binding to DBH. To date, only one class of mechanism-based inhibitors, some heterocyclic allylamines, appear to fulfill this criterion.[14] In this paper we describe a simple ethynyl-substituted tyramine that is an effective mechanism-based inhibitor of DBH; it binds enzyme in the micromolar range, nearly 100-fold more

(1) Kruse, L. I.; Kaiser, C.; DeWolf, W. E., Jr.; Frazee, J. S.; Erickson, R. W.; Ezekiel, M.; Ohlstein, E. H.; Ruffolo, R. R., Jr.; Berkowitz, B. A. *J. Med. Chem.* **1986**, *29*, 887.
(2) Kruse, L. I.; Kaiser, C.; DeWolf, W. E., Jr.; Frazee, J. S.; Garvey, E.; Hilbert, E. L.; Faulkner, W. A.; Flaim, K. E.; Sawyer, J. L.; Berkowitz, B. A. *J. Med. Chem.* **1986**, *29*, 2465.
(3) Kruse, L. I.; Kaiser, C.; DeWolf, W. E., Jr.; Frazee, J. S.; Ross, S. T.; Wawro, J.; Wise, M.; Flaim, K. E.; Sawyer, J. L.; Erickson, R. W.; Ezekiel, M.; Ohlstein, E. H.; Berkowitz, B. A. *J. Med. Chem.* **1987**, *30*, 486.
(4) Kruse, L. I.; DeWolf, W. E., Jr.; Chambers, P. A.; Goodhart, P. J. *Biochemistry* **1986**, *25*, 7271.
(5) DeWolf, W. E., Jr.; Goodhart, P. J.; Kruse, L. I. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1986**, *45*, 1537.
(6) Goodhart, P. J.; DeWolf, W. E., Jr.; Kruse, L. I. *Biochemistry* **1987**, *26*, 2576.
(7) Klinman, J. P.; Krueger, M. *Biochemistry* **1982**, *21*, 67.
(8) May, S. W.; Mueller, P. W.; Padgette, S. R.; Herman, H. H.; Phillips, R. S. *Biochem. Biophys. Res. Commun.* **1983**, *110*, 161.
(9) Rajashekhar, B.; Fitzpatrick, P. F.; Colombo, G.; Villafranca, J. J. *J. Biol. Chem.* **1984**, *259*, 6925.
(10) Mangold, J. B.; Klinman, J. P. *J. Biol. Chem.* **1984**, *259*, 7772.
(11) Colombo, G.; Villafranca, J. J. *J. Biol. Chem.* **1984**, *259*, 15017.
(12) Padgette, S. R.; Wimalasena, K.; Herman, H. H.; Sirimanne, S. R.; May, S. M. *Biochemistry* **1985**, *24*, 5826.
(13) Fitzpatrick, P. F.; Villafranca, J. J. *J. Biol. Chem.* **1986**, *261*, 4510.
(14) Bargar, T. M.; Broersma, R. J.; Creemer, L. C.; McCarthy, J. R.; Hornsperger, J.-M.; Palfreyman, M. G.; Wagner, J.; Jung, M. J. *J. Med. Chem.* **1986**, *29*, 315.

0022-2623/88/1831-0704$01.50/0

Scheme I[a]

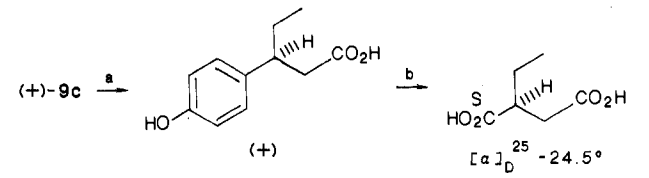


[a] Reagents and conditions: (a) TMSC≡CMgBr, then HCl; (b) NaOH, H_2O/EtOH, reflux, then HCl; (c) H_2O/C_5H_5N, 100 °C, then HCl; (d) KF, DMF, 50 °C, then HCl; (e) $(PhO)_2PON_3$, NEt_3, PMBOH, toluene, 100 °C; (f) HCl, Et_2O/EtOAc; (g) H_2, Pd/$BaCO_3$, CH_2Cl_2.

tightly than dopamine substrate.

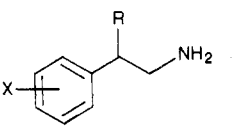
Chemistry.[15] Compounds **1–6** were prepared by the route outlined in Scheme I. The conjugate addition of (trimethylsilyl)ethynyl Grignard to diesters **7a–c** yielded **8a** (60%), **8b** (47%), and **8c** (100%). Saponification followed by decarboxylation provided the acetylenic acids **9a** (58%), **9b** (43%), and **9c** (44%) from **8a–c**. The reaction of carboxylic acids **9a–c** with diphenyl phosphorazidate and 4-methoxybenzyl alcohol in a modified Curtius procedure[16] afforded carbamates **10a** (52%), **10b** (50%), and **10c** (62%). The deprotection of **10a–c** with HCl in ether/ethyl acetate mixtures provided crystalline hydrochloride salts of **1–3**. Controlled hydrogenation[17] of **10a–c** afforded the corresponding olefins that were deprotected by HCl treatment to give the corresponding β-vinyltyramines **4–6**. The use of the 4-methoxybenzyl (PMB) group to protect the phenolic hydroxyl and carbamate groups was critical to the success of the synthetic scheme. A resolution of the acetylenic inhibitor **3** was accomplished by fractional crystallization (48% yield) of the (1*R*,2*S*)- and (1*S*,2*R*)-2-amino-1-(4-nitrophenyl)-1,3-propanediol salts of intermediate **9c** from 2-PrOH: (+)-**9c**, $[\alpha]^{25}_D$ +19.0° (*c* 1.5, DMF); (−)-**9c**, $[\alpha]^{25}_D$ −19.4° (*c* 1.5, DMF). Curtius rearrangement of (+)-**9c** and (−)-**9c** yielded the chiral carbamates: (+)-**10c**, $[\alpha]^{25}_D$ +25.4° (*c* 1.5, DMF); (−)-**10c**, $[\alpha]^{25}_D$ −26.5° (*c* 1.5, DMF). Deprotection yielded the enantiomers of **3**: (+)-**3**, $[\alpha]^{25}_D$ +14.1° (*c* 1.5, DMF); (−)-**3**, $[\alpha]^{25}_D$ −17.1° (*c* 1.5, DMF). The absolute configuration of **3** was determined by chemical degradation (Scheme II) of the intermediate (+)-**9c** to (*S*)-(−)-ethylsuccinic acid [mp 94–95 °C, $[\alpha]^{25}_D$ −24.5° (*c* 3.0, acetone) (lit.[18a] mp 94–96 °C, lit.[18b] $[\alpha]^{25}_D$ −24.0° (c 3.0, acetone))] of known absolute configuration.[18c] This chemical correlation establishes the absolute configuration of the mechanism-based inactivator (−)-**3** as *S* (Scheme II).

Scheme II[a]



[a] Reagents and conditions: (a) H_2, Pd/C, EtOH; (b) $RuCl_3$, $NaIO_4$, MeCN/H_2O/CCl_4.

Table I. DBH Inhibitory Properties of Some β-Substituted Tyramines



no.	X	R	K_{is},[a,b] μM	K_I,[b,c] μM	k_{inact},[d] min^{-1}
1	H	HC≡C	160 ± 10	*e*	*e*
2	3-OH	HC≡C	190 ± 5	*e*	*e*
(±)-**3**	4-OH	HC≡C	13.6 ± 0.8	15.7 ± 2.3	0.023 ± 0.001
(+)-**3**	4-OH	HC≡C	7.9 ± 0.3	*e*	*e*
(−)-**3**	4-OH	HC≡C	33.9 ± 1.4	57 ± 8	0.184 ± 0.015
4	H	H_2C=CH	670 ± 70	*e*	*e*
5	3-OH	H_2C=CH	1270 ± 80	*e*	*e*
6	4-OH	H_2C=CH	82 ± 5	*e*	*e*

[a] K_{is} values (mean ± SEM) were determined vs tyramine substrate in the absence of fumarate with the use of homogeneous bovine DBH (sp act. 30–42 units/mg at pH 5.0). Inhibition constants were determined by using the computer programs of Cleland (*Methods in Enzymology*; Purich, D. L., Ed.; Academic: New York, 1979; Vol. 63, pp 103–138). [b] Experimental conditions: pH 5.0; ionic strength, μ = 0.2; 50 mM sodium acetate buffer; 1 mg/mL bovine catalase; 10 μM Cu^{2+}; 10 mM ascorbic acid; 37 °C. [c] K_I and k_{inact} values (mean ±SEM) were determined from plots of $1/k_{inact}$ (observed) vs 1/[inhibitor] for a minimum of four inhibitor concentrations. Values of k_{inact} (observed) were determined from a plot of log (percent enzyme activity remaining) vs times. As determined here, the K_I value may not be a true dissociation constant since, under the conditions similar to those used here, a substantial commitment to catalysis has been shown for several tyramine substrates (Miller, S. M.; Klinman, J. P. *Biochemistry* **1985**, *24*, 2114). [d] These are k_{inact} (apparent) values since the concentration of oxygen cosubstrate was held constant at 0.24 mM. [e] Minimal time-dependent inactivation was observed under the experimental conditions.

Biochemistry. Kinetic experiments were conducted with homogeneous bovine DBH[19] (sp act. 30–42 units/mg at pH 5.0) under the conditions defined in Table I by using the previously described assay.[2] All of the compounds are competitive inhibitors vs tyramine substrate and show a considerable affinity for DBH, as judged from K_{is} values. A 4-hydroxyl group enhances binding (cf. **3** vs **1** and **6** vs **4**) whereas a 3-hydroxy group decreases binding (cf **2** vs **1** and **5** vs **4**) relative to the unsubstituted parent phenethylamines. A similar general trend is observed for simple tyramine substrates.[20] The presence of a β-vinyl (**4–6**) and to a greater extent a β-ethynyl (**1–3**) group substantially

(15) All new compounds were characterized by IR, NMR, and mass spectrometry and had C, H, and N microanalyses within ±0.4% of the theoretical values.

(16) (a) Shiori, T.; Ninomiya, K.; Yamada, S. *J. Am. Chem. Soc.* **1972**, *94*, 6203. (b) Ninomiya, K.; Shiori, T.; Yamada, S. *Tetrahedron* **1974**, *30*, 2151.

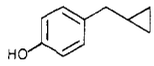
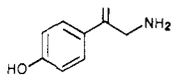
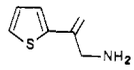
(17) Fieser, L. F.; Fieser, M. *Reagents for Organic Synthesis*; Wiley: New York, 1967; Vol. 1, pp 566–567.

(18) (a) Baldwin, J. E.; Barton, D. H. R.; Sutherland, J. K. *J. Chem. Soc.* **1965**, 1787. (b) Bettoni, G.; Cellucci, C.; Berardi, F. *J. Heterocycl. Chem.* **1980**, *17*, 603. (c) Listowsky, I.; Avigad, G.; Englard, S. *J. Org. Chem.* **1970**, *35*, 1080.

(19) DeWolf, W. E., Jr.; Kruse, L. I., unpublished results.

(20) Miller, S. M.; Klinman, J. P. *Biochemistry* **1985**, *24*, 2114.

Table II. Comparison of Kinetic Parameters for (−)-**3** with Other Reported Mechanism-Based DBH Inhibitors

compd	K_I, μM	k_{inact}, min^{-1}	k_{inact}/K_I, M^{-1} min^{-1}
	12000[a]	1.8[a]	150
	520[b]	0.81[b]	1560
	35[c]	0.124[c]	3540
(−)-**3**	57[d]	0.184[d]	3230

[a] Apparent values at pH 5.5, 1.21 mM O_2; data from Fitzpatrick, P. F.; Villafranca, J. J. *J. Am. Chem. Soc.* **1985**, *107*, 5022. [b] Apparent values at pH 5.0, 0.24 mM O_2; data from ref 12. [c] Apparent values at pH 5.0, 0.24 mM O_2; data from ref 14. [d] Apparent values.

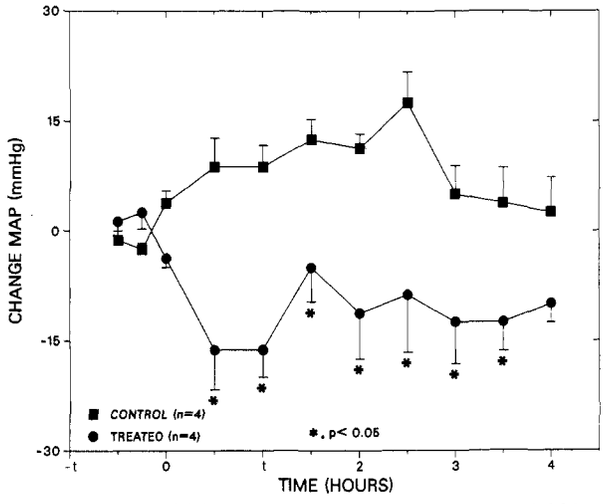


Figure 1. Effect of (−)-**3**, 100 mg/kg, ip, on mean arterial blood pressure of conscious spontaneously hypertensive rats (mean ± SEM).

increases binding to enzyme relative to *p*-tyramine (K_m = 1–2 mM). Of the inhibitors in Table I, **3** demonstrates both the highest affinity for the enzyme and an efficient time-dependent inactivation. Interestingly, both (+)-**3** and (−)-**3** inhibitors bind to DBH much more tightly than *p*-tyramine substrate, but time-dependent inactivation occurs only with the (−)-**3** isomer. The *S* absolute configuration of (−)-**3** retains the *pro-R* benzylic hydrogen of dopamine substrate which normally undergoes oxidation.[21] This implies support for time-dependent inactivation that arises from an abortive benzylic oxidation. The time-dependent inactivation of DBH by (±)-**3** is, as expected, considerably slower than that observed for (−)-**3** since, in the racemate, the competitive inhibitor (+)-**3** partially protects enzyme from time-dependent inactivation by the (−)-**3** isomer.[22] The time-dependent inactivation of DBH by (−)-**3** is irreversible, as evidenced by a failure to reactivate upon prolonged dialysis of enzyme, and is strictly dependent upon oxygen and ascorbate cosubstrates. A comparison of kinetic constants for (−)-**3** with the kinetic constants reported by others for representatives of various classes of DBH inactivators (Table II) shows (−)-**3** to be exceptionally effective. Inhibitor (−)-**3** combines a good rate of inactivation, k_{inact}(app), with a very high affinity for enzyme, K_I(app). Thus (Table II), of the numerous inactivators of DBH reported to date,[7-14] only one class of heterocyclic allylamines[14] is of comparable or greater effectiveness, as judged by the pharmacologically relevant ratio k_{inact}/K_I. Of equal importance is the observation that inactivation of DBH by (−)-**3** occurs with a partition ratio of <5:1.[19]

Pharmacology. The ip administration of (−)-**3**, 100 mg/kg, to spontaneously hypertensive rats in the previously defined protocol[1,3] produced a significant reduction (11%) in mean arterial blood pressure from the 150 mmHg level prior to drug dosing (Figure 1).

This paper establishes the ethynyltyramine (−)-**3** as an effective time-dependent inactivator of DBH that has a considerable affinity for enzyme. The tyramine structure of **3** may lead in vivo to an active uptake and concentration of inhibitor in the target organelle, the chromaffin vesicle.

† Address correspondence to this author at SK&F Research Limited, The Frythe, Welwyn, Hertfordshire, England, AL6 9AR.
‡ Department of Pharmacology.

(21) The apparent change in absolute configuration results from a change in substituent priorities at the benzylic carbon.
(22) Cleland, W. W.; Gross, M.; Folk, J. E. *J. Biol. Chem.* **1971**, *248*, 6541.

Lawrence I. Kruse,*† Carl Kaiser
Walter E. DeWolf, Jr., Pamela A. Chambers
Paula J. Goodhart, Mildred Ezekiel,‡ Eliot H. Ohlstein‡
Departments of Medicinal Chemistry and Pharmacology
Research and Development Division
Smith Kline & French Laboratories
709 Swedeland Road
Swedeland, Pennsylvania 19406
Received November 30, 1987