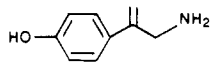
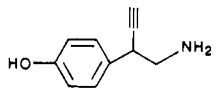
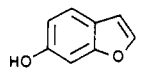
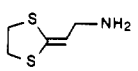
Table II. Comparison of Kinetic Parameters of Previously Published Mechanism-Based Inhibitors (6–8) of DβH

no.	structure	K_i, mM	k_{inact}, min^{-1}	k_{inact}/K_i (M^{-1} min^{-1})
6		0.52[a]	0.81[a]	1560
7		0.057[b]	0.184[b]	3230
4		0.040	0.23	5750
8		0.005[c]	0.15[c]	30000

[a] Apparent values at pH 5.0, 0.24 mM O_2, 37 °C.[10] [b] Apparent values at pH 5.0, 0.24 mM O_2, 37 °C.[14] [c] Apparent values at pH 5.0, 0.24 mM O_2, 37 °C.[15]

of a similar intermediate along the reaction pathway. With 6-hydroxybenzofuran (4), a 27-fold decrease in the partition ratio was found as compared to that of 1, whereas 5-hydroxybenzofuran (3), which binds with a K_i larger than that of *p*-hydroxystyrene (1), exhibits a 90-fold lower partition ratio.

A substantial change in the partition ratio may be explained by increased resonance stabilization of the radical cation intermediate. The net effect of this added stabilization would be to lower the transition state for formation of the intermediate and also to increase the probability of trapping the resonance-stabilized intermediate by an enzyme nucleophile (Scheme I). The oxygen in the furan ring of benzofurans 3–5 can serve to stabilize the radical cation as an oxycation radical (shown in Scheme I). The radical cation of 6-hydroxybenzofuran (4) can also be further stabilized by loss of a proton from the 6-hydroxyl group to form a "quinone-like" radical intermediate. Michael addition of an active-site nucleophile to the radical cation intermediate would result in the formation of a stable adduct at the active site of DβH. The difference in the partition ratio of benzofurans 3 and 4 could be either due to the difference in the positioning of these molecules in the active site or due to a difference in the ability of the enzyme to stabilize the radical cation intermediates during catalysis.

Table II provides a comparison of kinetic constants of 6-hydroxybenzofuran (4) versus several other published mechanism-based inhibitors. The k_{inact} for 4 was determined at 25 °C versus 37 °C for 6–8[10,14,15] and may be a factor of 2 larger at 37 °C. Even so the bicyclic analogue 4 is clearly a potent mechanism-based inhibitor of DβH on the basis of the criterion of a large k_{inact}/K_i value.[15]

In summary each of the bicyclic olefins (3–5) is able to stabilize a radical cation intermediate more effectively than *p*-hydroxystyrene (1) through resonance forms involving oxycations or other delocalized radical intermediates. This stabilization would potentially increase the life time of the reactive intermediate, leading to more effective enzyme inactivation. This added stabilization of the radical cation intermediate could be the explanation for the large decrease in the partition ratio with these analogues compared to those of the simple olefins 1 and 2.

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Registry No. 1, 2628-17-3; 2, 20649-39-2; 3, 13196-10-6; 4, 13196-11-7; 5, 4790-81-2; DβH, 9013-38-1.

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Carboxyalkyl Dipeptides with Atrial Natriuretic Factor Potentiating and Antihypertensive Activity

Sir:

The atrial natriuretic factors (ANF) are peptide hormones with powerful vasodilatory, diuretic, and natriuretic effects.[1] Much work has been done toward establishing their physiological and pathophysiological roles in blood pressure regulation and fluid-volume and electrolyte homeostasis.[2] Recent reports[3] demonstrating the ability of neutral endopeptidase (NEP) (EC 3.4.24.11) to inactivate ANF in vitro prompt us to report our work on NEP inhibitors that potentiate the hypotensive activity of exogenous ANF and express antihypertensive activity.

Peptide hormones are usually eliminated in various ways: uptake by target tissues and intracellular degradation, inactivation by blood proteases or by the liver or by the kidneys. Although the metabolism of ANF is not completely understood, studies to date indicate that ANF may be metabolized within the kidney, liver, lung, and vasculature.[4,5] The kidney is one of the target organs where the activity of ANF is expressed. Major among the brush border peptidases of the kidney proximal tubule is NEP, a zinc-containing protease that cleaves peptide bonds on the amino side of the residues with hydrophobic side chains.[6] However, NEP has a heterogeneous distribution and has been found in a variety of tissues.[7] Thus, we speculated that NEP, in the kidney and elsewhere, might be important in the degradation of ANF in vivo. We hypothesized that inhibition of this enzyme could modulate the in vivo activity of both exogenously administered as well as endogenously generated ANF. After the initiation of this project, several publications disclosed that ANF is a substrate for NEP and that the enzyme rapidly inactivates this peptide in vitro.[3] These results are consistent with our hypothesis.

In order to test this hypothesis, we required compounds that are potent and selective inhibitors of NEP, since inhibition of related metallopeptidases, such as angiotensin

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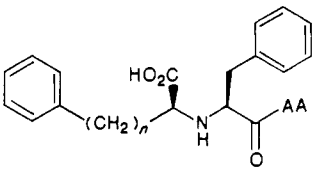
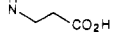
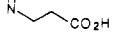
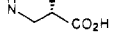
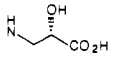
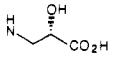
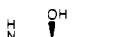
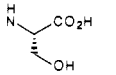
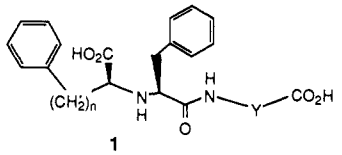
Table I. Biological Activity of Carboxyalkyl Dipeptides



no.	AA	n	NEP IC_{50}, nM	ACE IC_{50}, nM	ANF potentiation: ΔBP, mmHg
6a		1	20	>1000	20
6b		2	16	>1000	17
6c		1	11	>1000	17
6d		2	11	>1000	25
6e		2	15	>1000	35
6f		2	21	640	28
6g		2	393	320	ns[a]

[a] Not significant.

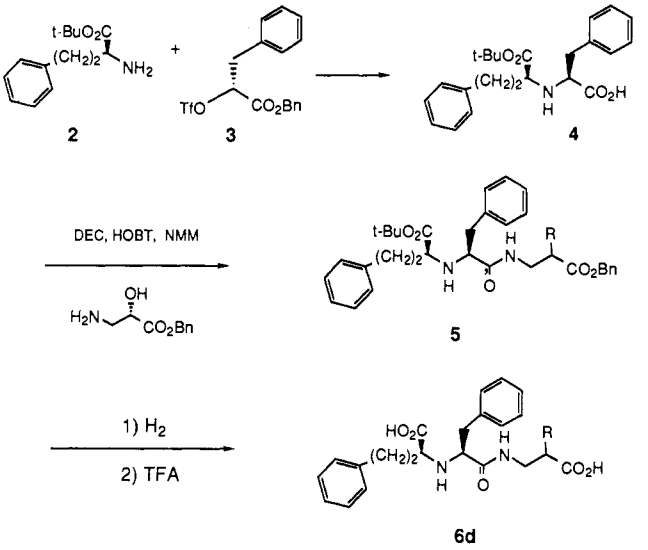
converting enzyme (ACE), would complicate the interpretation of in vivo results. This selectivity requirement precluded the use of thiorphan, the prototype NEP inhibitor. NEP is a zinc metallopeptidase homologous to thermolysin,[8] and previous work provided some precedent for design of inhibitors.[9] The design of NEP selective carboxyalkyl inhibitors of the general structure **1** follows from the NEP subsite preferences.[10,11] The compounds shown in Table I were made to explore the subsite generalizations and test our hypothesis.



1

The synthesis of optically active carboxyalkyl dipeptide **6d**, outlined in Scheme I, is typical of the route used to prepare these compounds. The optically active triflate **3** is obtained by treating the corresponding lactate ester[12] with triflic anhydride and pyridine in dry methylene chloride at −20 °C, followed by warming to 10 °C, concentration, and rapid column filtration. The triflate **3** was used immediately without further purification. The homophenylalanine ester **2**, prepared from the commercially available amino acid and isobutylene, is added to a solution of triflate **3** at ambient temperature in methylene chloride

Scheme I. Carboxyalkyl Dipeptide Synthesis



containing Proton Sponge (Aldrich) to give the expected alkylation product as a single diastereomer.[13] Subsequent hydrogenolysis of the benzyl ester (H_2, 50 psi, 10% Pd/C in ethanol) gives intermediate **4**, in 94% yield after crystallization from wet methylene chloride. Coupling of acid **4** with the amino ester under standard conditions (water-soluble carbodiimide, hydroxybenzotriazole, *N*-methylmorpholine in dry DMF) gives diester **5** in 75% yield. Hydrogenolysis of the benzyl ester (H_2, 50 psi, 10% Pd/C in absolute ethanol) and then hydrolysis of the *tert*-butyl ester with trifluoroacetic acid in methylene chloride gives the diacid **6d**. The diacid *N*-[*N*-[1(*S*)-carboxyl-3-phenylpropyl]-(*S*)-phenylalanyl]-(*S*)-isoserine is purified by crystallization from 90% aqueous methanol (75% yield from **5d**, mp 235–8 °C dec, $[\alpha]_D$ +45.2° (c = 0.4, MeOH)). The diastereomeric purity of this diacid as well as intermediate **5d** is easily established by ^1H NMR. (Diastereomers from independent syntheses were available for comparison.) The 200-MHz spectra of both compounds showed no detectable diastereomeric impurities.

The carboxyalkyl dipeptides shown in Table I were tested against purified rabbit kidney NEP[9] and shown to be potent inhibitors of this enzyme. Except for analogues **6f** and **6g**, these compounds were not inhibitors of ACE at 1 μM. Thus, these compounds appeared to be appropriate probes to explore the effect of NEP inhibition on ANF activity and degradation in vivo. The weak mixed activity of the α-amino acid derivative **6g** contrasts with selectivity observed for the β-amino acid compounds. In a series of experiments, the effects of these NEP inhibitors on the mean arterial blood pressure (MAP) responses to 30 μg/kg ANF 103–125 (administered intravenously) were evaluated in conscious spontaneously hypertensive rats (SHR). In contrast to vehicle treated groups, the magnitude and duration of the hypotensive response to ANF 103–125 was increased in those animals treated subcutaneously with 30 mg/kg of the NEP inhibitors 30 min prior to ANF challenge. The ability of compound **6d** to potentiate the hypotensive activity of ANF is illustrated in Figure 1. Similar results were obtained when ANF 99–126 was used. The compounds listed in Table I were evaluated by this method. The ANF potentiation data given in Table I represent the maximum increases in the hypotensive response to ANF, over that of the control group. These

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(13) Effenberger, F.; Burkard, U.; Willfahrt, J. *Angew. Chem., Int. Ed. Engl.* **1983**, *22*, 65.

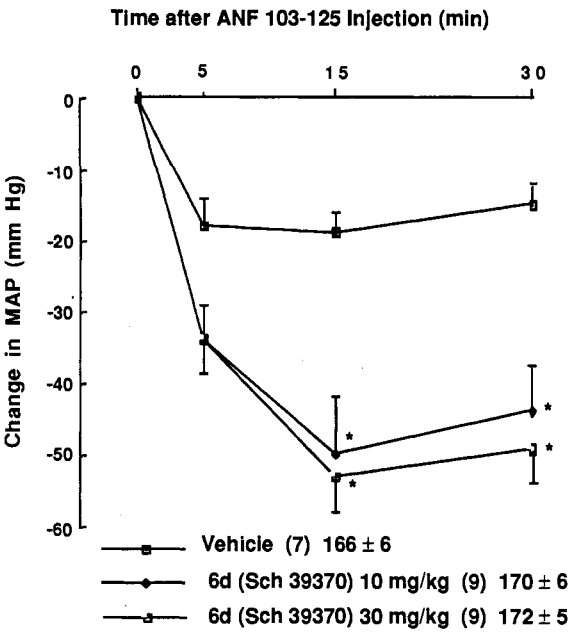

Figure 1. Potentiation of the antihypertensive effect of ANF 103–125, in SHR, by **6d** (Sch 39370); numbers in parentheses are number of animals in group, followed by baseline MAP ± SE.

results show that selective NEP inhibitors can enhance the magnitude and duration of the hypotensive activity of ANF.

Additional evidence that an NEP inhibitor prolongs the half-life of ANF was obtained by studying the plasma half-life of immunoreactive ANF in conscious rats following ANF 99–126 infusion (0.1 and 1 μg/kg per min for 30 min). The disappearance of immunoreactive ANF from the plasma was significantly prolonged in animals pretreated with compound **6d**.[14] Having several pieces of evidence that NEP inhibitors could potentiate exogenous ANF, experiments were next performed to determine if NEP inhibition could elicit a hypotensive response consistent with potentiation of endogenous ANF. The effects of the isoserine analogue **6d** were evaluated in rats treated with deoxycorticosterone acetate and salt (DOC salt rats), a volume-dependent model of hypertension in which endogenous levels of ANF are known to be elevated.[15] Treatment of these hypertensive rats with isoserine compound **6d** (30 mg/kg subcutaneously) resulted in a significant reduction in arterial pressure (Figure 2). The onset of hypotensive response was within 1–2 h of injection and the effect was sustained for the 4-h duration of the study. In spite of the fall in blood pressure, the heart rate was not altered significantly by **6d**. A high dose of the ACE inhibitor captopril (30 mg/kg subcutaneously) did not alter the blood pressure significantly in these DOC salt rats.[14]

The results of these studies provide evidence that NEP inhibition prevents degradation of ANF, enhances its hypotensive activity, and lowers blood pressure in a model of volume-dependent hypertension, the DOC salt rat. Although the precise role of ANF in the antihypertensive action of compound **6d** (Sch 39370) remains to be established, these results suggest that inhibition of NEP represents a novel mechanism by which to reduce arterial blood pressure. The clinical significance of this action remains to be established.

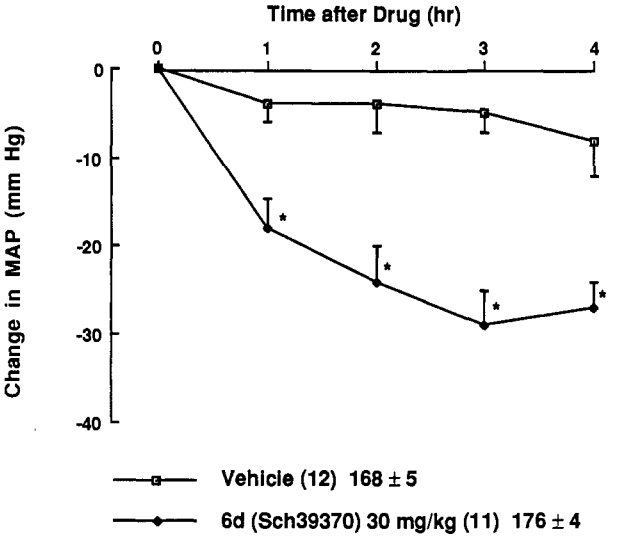

Figure 2. Hypotensive effect of **6d** (Sch 39370) as compared to vehicle in DOC salt rats; numbers in parentheses are number of animals in group, followed by baseline MAP ± SE.

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Hybrid Cholecystokinin (CCK) Antagonists: New Implications in the Design and Modification of CCK Antagonists†

Sir:

Recently two potent cholecystokinin (CCK) antagonists have been disclosed, CR 1409 (1), a proglumide derivative, which possesses enhanced potency over its progenitor,[1] and L-364,718 (2), a novel benzodiazepine[2a] with increased potency over CR 1409 and good selectivity[3] for peripheral CCK receptors[4] (CCK type A receptors). These disclosures have prompted considerable interest in understanding the structural interrelationships among the various classes of nonpeptide CCK antagonists as well as their relationship to CCK peptide counterparts. We report here our own efforts directed toward establishing a link between the proglumide and benzodiazepine series which has resulted

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