J. Med. Chem. 1984, 27, 1354–1357

(10). Boc-His-OH (0.32 g, 1.28 mmol), 1-hydroxybenzotriazole (0.34 g, 2.56 mmol), and 2 (0.6, 1.28 mmol) were dissolved in DMF (7 mL). The solution was cooled in an ice bath and then treated with *N*-methylmorpholine (0.13 g, 1.28 mmol) followed by a solution of dicyclohexylcarbodimide (0.26 g, 1.28 mmol) in $CH_2Cl_2$ (7 mL). The mixture was allowed to come to room temperature where it was stirred overnight. The mixture was filtered and the filtrate stripped of solvents in vacuo. The residue was partitioned between 1 M $NaHCO_3$ and EtOAc. The EtOAc layer was washed with 1 M $NaHCO_3$ followed by saturated NaCl solution. The EtOAc layer was then dried ($MgSO_4$). Removal of the solvent in vacuo yielded 0.74 g (86%) of the crude product. Recrystallization of this material from a mixture of EtOAc/MeOH provided 0.45 g of pure **10**: mp 191–192.5 °C; $[\alpha]^{25}_D$ −43.2° (*c* 1.23, $CH_3OH$); TLC ($CHCl_3$/MeOH, 9:1) $R_f$ 0.36; (1-propanol/$NH_4OH$, 4:1) $R_f$ 0.93; NMR ($CDCl_3$) δ 5.39 (br s, 2 H, CH=CH). Anal. ($C_{35}H_{52}N_6O_7$) C, H, N.

**L-Histidinyl-[5(*S*)-amino-7-methyl-3(*E*)-octenoyl]-L-valyl-L-phenylalanine Methyl Ester Hydrochloride (His-Leuψ[*E*-CH=CH]Gly-Val-Phe-OCH$_3$, 3).** The *tert*-butoxycarbonyl group was removed from **10** (0.21 g, 0.31 mmol) by using 10 mL of 4 N HCl in dioxane. Workup in the same manner as described above for **2** yielded 188 mg (94%) of hygroscopic product: $[\alpha]^{22}_D$ −2.1° (*c* 1.04, $CH_3OH$); TLC (1-propanol/$NH_4OH$, 4:1) $R_f$ 0.82; (butanol/HOAc/pyridine/$H_2O$, 5:1:3:4) $R_f$ 0.74; NMR ($Me_2SO$-$d_6$) δ 5.48 (dt, $J$ = 15.4 and 6.7 Hz, 1 H, CH=C*H*CH$_2$), 5.31 (dd, $J$ = 15.4 and 6.7 Hz, 1 H, NCHC*H*=CH). Anal. ($C_{30}H_{46}N_6O_5Cl_2\cdot{}^1/_2H_2O$) C, H, N.

**Renin Inhibition Studies.** The ability of compounds **2** and **3** to inhibit either hog kidney renin or human amniotic renin was measured by determining the inhibitory constant ($K_i$) of each compound. The $K_i$ and the type of inhibition of each compound were determined through the use of Dixon plots.[21] Data for these plots were obtained by measuring the reaction velocities of hog kidney renin or human amniotic renin at two concentrations of porcine angiotensinogen (0.1 and 0.05 μM) in the presence of varying concentrations of each inhibitor.

The enzymatic assay was carried out in a manner identical with that described previously.[13,14] Reaction velocities for hog kidney renin were expressed as the number of nanomoles of angiotensin I generated per unit of enzyme per minute, while the reaction velocities for human amniotic renin were expressed as the number of nanomoles of angiotensin I generated per milliliter per hour. The average values of three determinations for each inhibitor concentration at each substrate level were used to generate a Dixon plot ($1/v$ vs. inhibitor concentration) for each compound tested. All lines were calculated by linear regression analysis. The −[I] value at the intersection of the two substrate lines gave the $K_i$ value of each analogue. The competitive and noncompetitive nature of each inhibitor was assessed by whether the point of intersection of the two lines was above or on the $x$ axis, respectively.

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# Antihypertensive Activities of Phenyl Aminoethyl Sulfides, a Class of Synthetic Substrates for Dopamine β-Hydroxylase

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Four sulfur-containing analogues of phenylpropylamine were synthesized and evaluated as substrates for dopamine β-hydroxylase (DBH) and monoamine oxidase (MAO). All four phenyl aminoethyl sulfides were shown to be good substrates for DBH whereas only the two analogues not possessing a methyl group α to the terminal amino group were substrates for MAO. All four analogues were tested for acute antihypertensive activity in an animal model for hypertension, the spontaneously hypertensive rat (SHR). Two of the analogues, both of which should partition readily across the blood–brain barrier, did not appreciably reduce systemic blood pressure in the 6-h testing period. However, the two analogues that were designed to be relatively restricted to peripheral sites of action caused a dramatic drop in blood pressure in SHR of 25% within 1–1.5-h postinjection, with the analogue designed to be both restricted to the periphery and MAO inactive, causing a more prolonged antihypertensive activity.

Phenyl 2-aminoethyl sulfide (PAES, **4a**) was designed, synthesized, and characterized in our laboratories as a synthetic substrate for dopamine β-hydroxylase (DBH; EC 1.14.17.1). May and Phillips[1] have previously shown that DBH readily oxygenates PAES to the corresponding sulfoxide (PAESO), and the kinetics and mechanism of this reaction have been investigated.[2] We have also demonstrated that PAES possesses indirect sympathomimetic activity in vivo and inhibits the reflexive tachycardia induced by vasodilatory antihypertensives.[3] These findings suggested that PAES or structurally-similar derivatives might be effective in reversibly modifying peripheral adrenergic function, and thus might be useful in the control of hypertension.

In the present study, we have synthesized three additional PAES derivatives designed to provide information on structure–activity relationships of this class of sulfur-containing compounds (Scheme I). Our rationale was to prepare MEPAES, **4b**, which would be protected from catabolism by amine oxidase enzymes, HOPAES, **4c**, which would be relatively restricted to peripheral sites of action by virtue of its reduced ability to penetrate the blood–brain barrier,[4] and HOMEPAES, **4d**, possessing both of these

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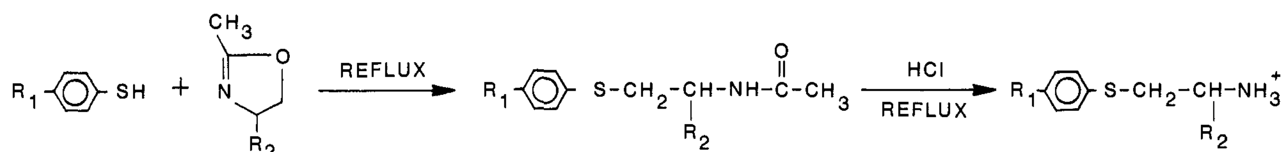
**Scheme I**



1.a. $R_1$=H
1.b. $R_2$=OH

2.a. $R_2$=H
2.b. $R_2$=$CH_3$

3.a. $R_1$=H, $R_2$=H
3.b. $R_1$=H, $R_2$=$CH_3$
3.c. $R_1$=OH, $R_2$=H
3.d. $R_1$=OH, $R_2$=$CH_3$

4.a. PAES, $R_1$=H, $R_2$=H
4.b. MEPAES, $R_1$=H, $R_2$=$CH_3$
4.c. HOPAES, $R_1$=OH, $R_2$=H
4.d. HOMEPAES, $R_1$=OH, $R_2$=$CH_3$

**Table I.** Enzymatic Kinetic Constants

| | DBH[a,b] | | | MAO[b] | | |
|---|---|---|---|---|---|---|
| compd | $K_m$, mM | $k_{cat}$, $s^{-1}$ | $k_{cat}/K_m$, $M^{-1}$ $s^{-1}$ | $K_m$, mM | $k_{cat}$, $s^{-1}$ | $k_{cat}/K_m$, $M^{-1}$ $s^{-1}$ |
| PAES, **4a** | 17.2 | 39 | $2.3 \times 10^3$ | 0.055 | 0.43 | $7.3 \times 10^3$ |
| (±)-MEPAES, **4b** | 19.1 | 20 | $1.0 \times 10^3$ | *c* | *c* | *c* |
| HOPAES, **4c** | 4.4 | 36 | $8.3 \times 10^3$ | 0.049 | 0.22 | $4.5 \times 10^3$ |
| (±)-HOMEPAES, **4d**[f] | 6.5 | 44 | $6.7 \times 10^3$ | *c* | *c* | *c* |
| tyramine[g] | 2.0 | 85 | $4.2 \times 10^4$ | *d* | *d* | *d* |
| phenylethylamine | 7.0[e] | 65[e] | $9.3 \times 10^3$[e] | 2.2 | 0.23 | $1.0 \times 10^2$ |
| phenylpropylamine | 20.4[e] | 12[e] | $5.9 \times 10^2$[e] | 0.29 | 0.17 | $5.8 \times 10^2$ |

[a] The specific activity of the enzyme was determined for each kinetic experiment using 10 mM tyramine as the substrate under standard assay conditions. When enzyme activities for different experiments were not the same, the resulting $k_{cat}$ values were multiplied by 14.7/(specific activity for the experiment), where 14.7 units/mg represents an arbitrary reference specific activity. Therefore, in the absence of an active site titrant, $k_{cat}$ values will vary somewhat for different enzyme preparations. [b] Kinetic assay reaction conditions as detailed in the Experimental Section. [c] Compounds displayed no trace of substrate activity even at high enzyme concentrations. [d] Values not determined experimentally. [e] Kinetic values reported previously.[2] [f] HOMEPAES was found to be a weak inhibitor of benzylamine oxidation by MAO. [g] Hydroxylation by DBH of dopamine has been reported to proceed at 93% of tyramine activity.[19]

characteristics. In this report we demonstrate that all of these compounds are excellent DBH substrates, and we have determined their kinetic constants as substrates for both DBH and an important amine-catabolizing enzyme, monoamine oxidase (MAO). Finally, we have extended our initial pharmacological study of this class of compounds by examining their effects in a commonly used animal model for essential hypertension, the spontaneously hypertensive rat (SHR).

**Chemistry.** The synthetic route to the *N*-acetyl PAES derivatives, shown in Scheme I, was via the Wehrmeister reaction[5,6] of the appropriately substituted thiophenols (**1**) and 2-methyl-2-oxazolines (**2**). Hydrolysis of **3** with refluxing HCl gave the corresponding PAES derivatives **4**.

**Enzymatic Studies.** The substrate kinetic parameters of **4a–d** with DBH and MAO are shown in Table I. It is apparent that all of the PAES derivatives are good substrates for DBH, with $K_m$ and $k_{cat}$ values comparable to standard DBH substrates. Although tyramine, a standard DBH assay substrate, is a better substrate than any of the PAES derivatives, **4a–d** are better substrates than the corresponding carbon-containing analogue, phenylpropylamine. It is also apparent that ring hydroxylation decreases $K_m$ values for the sulfide substrates, just as is the case for the phenylalkylamines, tyramine, and β-phenylethylamine.[2]

As has been reported previously,[7] the presence of an α-methyl group in a monoamine compound abolishes MAO deamination. Thus, the fact that MEPAES and HOMEPAES showed no trace of substrate activity with the preparation of MAO utilized, even with high concentrations of enzyme, was in accord with our expectations. On the other hand, the nonmethylated derivatives, PAES and HOPAES, showed good MAO substrate activity, with the $k_{cat}$ for the ring hydroxylated HOPAES about half that of the nonhydroxylated PAES. The $K_m$ values for each of the MAO substrates were in the 50 μM range. It should be noted that a commercial preparation of MAO was used in these experiments, which is a mixture of the A and B isozymes. Although this undoubtably will effect the values of the kinetic parameters, the basic conclusion for our purposes, namely, that α-methylation abolishes MAO activity, remains valid.

**Biological Data.** In age-matched male SHR rats, ip injection of either 0.9% saline (2 $cm^3$/kg) or PAES (50 mg/kg, HCl salt) resulted in minor decreases in systolic blood pressure over a 6-h testing period. Similarly, the effect of ip injection of 5 mg/kg (HCl salt) of MEPAES, compared to saline controls, showed a modest decrease in blood pressure, although the duration of the effect was somewhat prolonged in comparison to PAES. At doses greater than 50 mg/kg of PAES, or 5 mg/kg of MEPAES, the potent indirect sympathomimetic activity of PAES or MEPAES precluded indirect blood-pressure measurement, presumably as a result of the constriction, by indirectly released norepinephrine (NE), of the tail-vein sphincter muscle.[8]

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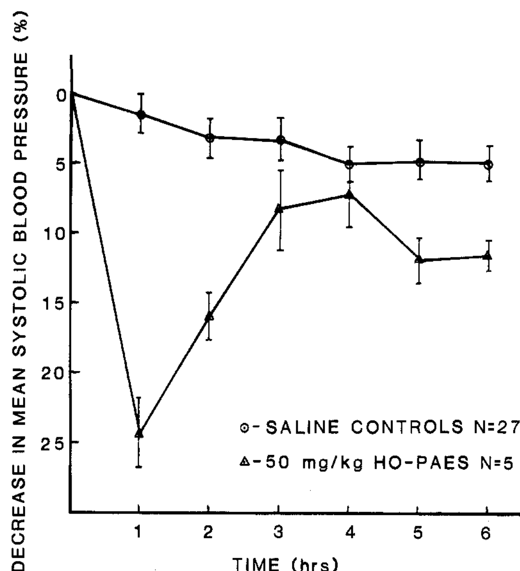


**Figure 1.** Effect of HOPAES (4c) on systolic blood pressure in SHR. At $t = 0$, SHR were injected intraperitoneally with 0.9% saline (2 $cm^3$/kg) or 50 mg/kg HOPAES (4c) after an initial blood-pressure average was obtained. Blood pressures were measured at 1-h intervals by using an indirect tail-cuff plethysmographic method (see Methods section). Each measurement was the average of 10–15 values collected over a 10-min span. The animals were age-matched male rats 11–15 weeks old, between 225–275 g in weight, and the mean blood pressure prior to infusion was 185 ± 15 mmHg. $N$, number of animals; bars, standard error of the mean (SEM).

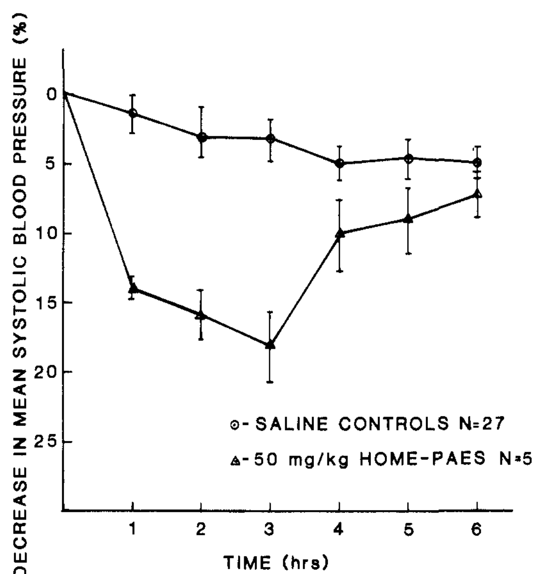


**Figure 2.** Effect of HOMEPAES (4d) on systolic blood pressure in SHR. Procedure was the same as in Figure 1, except that 50 mg/kg of HOMEPAES (4d) was injected.

The effects on systolic blood pressure of ip injection of either saline or HOPAES (50 mg/kg, HCl salt) are illustrated in Figure 1. The effect of HOPAES (4c) on systolic blood pressure is quite striking, and it is clear that the time course of this effect is limited to the first 3-h postinjection. In the group of animals that received HOPAES injections, the blood-pressure reduction reaches a maximum of 25% at 1-h postinjection, with a gradual return of the blood pressure to hypertensive levels after 3 h. The effects of ip injection of saline or HOMEPAES (50 mg/kg, HCl salt) on the systolic blood pressure of male SHR are shown in Figure 2. With this derivative, the time course of the blood-pressure reduction is broader, reaching a maximum at 3 h and lasting 4.5–5 h. Since these two compounds differ structurally only in that HOMEPAES possesses an $\alpha$-methyl group which makes it MAO inactive, the prolonged duration of the hypotensive activity of HOMEPAES may be consistent with the notion that MAO plays a role in the termination of the activity of those sulfur-containing derivatives not possessing an $\alpha$-methyl group. However, direct measurements of blood pressure effects are yet to be carried out.

The mechanisms by which these sulfur-containing compounds are causing the observed hypotensive effects are as yet unclear. If these compounds are capable of gaining entrance to adrenergic neurons, then it is possible that, by competing with dopamine for oxygenation by DBH, these PAES derivatives decrease the net synthesis and storage of NE, and thus diminish the ability of the peripheral adrenergic nervous system to maintain vascular tone. If the sulfoxide products that result are released in place of NE, then these sulfur compounds may be acting as "false transmitters", particularly since we have already demonstrated that the DBH-catalyzed product of PAES oxygenation is itself inactive at adrenergic receptors.[3] The difference in the antihypertensive potency observed between the phenyl derivatives (PAES, **4a**, and MEPAES, **4b**) on the one hand and hydroxyphenyl derivatives (HOPAES, **4c**, and HOMPAES, **4d**) on the other hand could be partially related to the efficiency with which DBH oxygenates these substrates. 4-Hydroxylation does decrease the apparent $K_m$ of **4c** and **4d** for DBH, and the change in $k_{cat}/K_m$ (a measure of the catalytic efficiency of the enzymic process) is increased 4–7 times when one compares the analogous phenyl and hydroxyphenyl derivatives (Table I). However, it is risky to compare the in vitro kinetics of these compounds to their in vivo activities. Another possible explanation of the disparity in the observed antihypertensive effects could be related to the ability of a selected derivative from this series to penetrate into the CNS. Both PAES (**4a**) and MEPAES (**4b**) should partition readily across the blood–brain barrier, where the effects of a central inhibition of adrenergic neuronal inputs to hypothalamic vasomotor nuclei is known to cause an *increased* vasomotor outflow, which would tend to increase systemic blood pressure.[17,18] Thus, it is possible that the minor antihypertensive activity served in these indirect measurements with **4a** and **4b** is a reflection of competing, mutually-opposing effects on blood pressure arising from both central and peripheral sites. In the case of the hydroxyphenyl derivatives **4c** and **4d**, however, we expect very little penetration of the blood–brain barrier.

## Experimental Section

NMR spectra were obtained on a Varian T-60 NMR spectrometer. Samples run in $D_2O$ had 3-(trimethylsilyl)propionic acid, sodium salt, as internal standard, and all other NMR spectra had $Me_4Si$ as internal standard. Mass spectra were obtained on

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a Finnigan MAT 112S instrument with SS200 data system; isobutane was used as ionizing gas in CI spectra. Microanalyses were performed by Atlantic Microlabs, Atlanta, GA, and analytical results obtained for elements were within ±0.4% of theory unless otherwise indicated.

Thiophenol, 2-methyl-2-oxazoline, (±)-2-amino-1-propanol, and 4-mercaptophenol (redistilled) were commercially available.

***N*-[2-(Phenylthio)ethyl]acetamide (3a) and 2-(Phenylthio)ethylamine Hydrochloride (4a, PAES).** These compounds were synthesized as previously described.[1,6]

**(±)-*N*-[1-(Phenylthio)-2-propyl]acetamide (3b).** *N*-Acetyl-MEPAES, mp 87–89.5 °C (lit.[6] mp 88.5–89.5 °C), was prepared by the method of Wehrmeister[6] via in situ generation of 2,4-dimethyl-2-oxazoline in the presence of thiophenol.

**(±)-1-(Phenylthio)-2-propylamine Hydrochloride (4b, MEPAES).** MEPAES free amine was prepared by the method of Wehrmeister[6] and converted to the HCl salt by treatment with 1.1 equiv of concentrated HCl in EtOH, followed by crystallization (EtOH/ether), to give **4b**: mp 154–155.5 °C; $^1$H NMR ($D_2O$) δ 7.45 (m, 5 H), 3.72–3.12 (m, 3 H), 1.38 (d, 3 H); mass spectrum (EI), *m/e* 167 ($M^+$), (CI) *m/e* 168 (M + 1). Anal. ($C_9H_{13}NS{\cdot}HCl$) C, H, N.

***N*-[2-[(4-Hydroxyphenyl)thio]ethyl]acetamide (3c).** A mixture of 2-methyl-2-oxazoline (16.9 mL, 0.197 mol) and 4-mercaptophenol (24.85 g, 0.197 mol) was heated under reflux (neat) for 2 h at about 130 °C under Ar (spontaneous heating occurred upon mixing). Upon cooling of the reaction mixture to 0 °C, a white solid precipitated, which was collected and recrystallized from dilute EtOH to give white **3c** (36.2 g, 87%): mp 123–125 °C; $^1$H NMR ($(CD_3)_2$=O) δ 6.99 (d, d, 4 H), 3.50–2.60 (m, 5 H), 1.87 (s, 3 H); mass spectrum (EI), *m/e* 211 ($M^+$), (CI) *m/e* 212 (M + 1). Anal. ($C_{10}H_{13}NO_2S$) C, H, N.

**2-[(4-Hydroxyphenyl)thio]ethylamine Hydrochloride (4c, HOPAES).** *N*-Acetyl-HOPAES (**3c**) (10.7 g, 50.5 mmol) was refluxed in concentrated HCl (20 mL) under Ar for 12 h. After cooling to room temperature, the reaction mixture was diluted with $H_2O$ (20 mL), extracted with ether (2 × 15 mL), and the aqueous phase was evaporated to dryness and twice dissolved in $H_2O$ (20 mL) and evaporated. The resulting solid was recrystallized twice (EtOH/ether), giving white, crystalline **4c** (7.3 g, 70%): mp 128–129 °C; $^1$H NMR ($D_2O$) δ 7.17 (d, d, 4 H), 3.18 (s, 4 H); mass spectrum (EI), *m/e* 169 ($M^+$); (CI) *m/e* 170 (M + 1). Anal. ($C_8H_{11}NOS{\cdot}HCl$) C, H, N. No trace of free thiol was detected in the pure product with use of 2,2′-dithiodipyridine spectrophotometric thiol reagent.

**(±)-2,4-Dimethyl-2-oxazoline (2b).** The following procedure is based on the general method of Meyers et al.[9] To a mixture of ethyl iminoacetate hydrochloride (38.0 g, 0.308 mol) and dry $CH_2Cl_2$ (220 mL) at 0 °C was added (±)-2-amino-1-propanol (19.8 mL, 0.248 mol) with mechanical stirring. The resulting solution was stirred at 0 °C for 6 h and was then poured into ice water (300 mL). The layers were separated, the aqueous phase was extracted with $CH_2Cl_2$ (100 mL, 50 mL, and 50 mL), and the combined extracts were dried ($MgSO_4$) and filtered. The resulting solution was fractionally distilled by using a 40-cm column packed with glass helices, yielding **2b** (12.3 g, 50%), bp 109–112 °C, as a foul-smelling, clear, colorless liquid: $^1$H NMR (neat) δ 4.39–3.48 (m, 3 H), 1.89 (s, 3 H), 1.17 (d, 3 H).

**(±)-*N*-[1-[(4-Hydroxyphenyl)thio]-2-propyl]acetamide (3d).** A mixture of 2,4-dimethyl-2-oxazoline (3.5 g, 35 mmol) and 4-mercaptophenol (3.5 g, 28 mmol) was heated at reflux (about 140 °C) for 2 h under Ar. To the cooled reaction mixture was added $CHCl_3$ (25 mL) with heating on a steam bath. Upon slow cooling to room temperature a white solid was deposited. The crude **3d** (5.3 g, 84%) was crystallized by triturating a hot $CHCl_3$ solution of the amide with a small portion of $C_6H_6$ to give **3d**: mp 120–122 °C; $^1$H NMR ($(CD_3)_2C$=O) δ 7.00 (d, d, 5 H), 4.30–3.67 (m, 1 H), 3.27–2.53 (m, 2 H), 1.94 (s, 3 H) 1.20 (d, 3 H); mass spectrum (EI), *m/e* 225 ($M^+$), (EI) *m/e* 226 (M + 1). Anal. ($C_{11}H_{15}NO_2S$) H, N; C: calcd, 58.64; found, 57.73.

**1-[(4-Hydroxyphenyl)thio]-2-propylamine Hydrochloride (4d, HOMEPAES).** *N*-Acetyl-HOMEPAES (**3d**) (4.0 g, 18 mmol) was refluxed in concentrated HCl (50 mL) for 12 h under Ar. After extraction with ether (2 × 25 mL) the aqueous phase was evaporated to give **4d** (3.7 g, 95%). An analytical sample of **4d** was obtained after two recrystallizations (EtOH/ether): mp 198–200 °C; $^1$H NMR ($D_2O$) δ 7.20 (d, d, 4 H), 4.64–2.97 (m, 3 H), 1.27 (d, 3 H); mass spectrum (EI), *m/e* 183 ($M^+$), (CI) *m/e* 184 (M + 1). Anal. ($C_9H_{13}NOS$ HCl) C, H, N.

**Determination of Kinetic Constants.** Dopamine β-hydroxylase was isolated and purified from bovine adrenals[10] and exhibited a specific activity of 9.9–20 units/mg (1 unit is defined as 1 μmol/min of oxygen consumed with 10 mM tyramine as substrate in the standard oxygen monitoring assay). Tyramine hydrochloride, ascorbic acid, sodium fumarate, catalase (C-10, specific activity 1600 units/mg solid), and bovine plasma monoamine oxidase (specific activity 46 units/g of protein) were obtained from Sigma Chemical Co.

Kinetic constants ($k_{cat}$ and $K_m$) of substrates with dopamine β-hydroxylase were determined by using the polarographic oxygen monitor assay as described previously[12] and were calculated by computer fit of the data to the hyperbolic form of the Michaelis–Menton equation.[12] The standard DBH kinetic assay mixture consisted of 0.1 M sodium acetate buffer, pH 5.0, in the presence of 10 mM sodium fumarate, 0.3 mg/mL of catalase, 5.6 μM $CuSO_4$, and 10 mM ascorbic acid, at atmospheric oxygen saturation in a total volume of 2.5 mL. In our assay system, atmospheric oxygen saturation was found to be 250 μM.[11] Enzymatic reactions were initiated with substrate, and the initial rate was measured as the rate of oxygen consumption minus the small background ascorbic acid autooxidation rate. The concentration of purified DBH was estimated from the absorbance at 280 nm ($A_{280}{}^{1\%}$ = 9.0).[10] Determinations of $k_{cat}$ values are based on a molecular weight for DBH of 290 000.[13]

Kinetics of the reactions of the sulfur compounds with monoamine oxidase were performed on a standard oxygen monitor apparatus, according to a literature procedure.[14] The oxygen monitor experiments were performed in 0.1 M potassium phosphate buffer, pH 7.2, at atmospheric oxygen saturation at 37 °C, in a total volume of 2.5 mL. As has been previously noted, bovine erythrocyte MAO is a mixture of both the A and B isozymes.[15] Kinetic constants ($k_{cat}$ and $K_m$) of substrates were calculated by computer fit of the data to the hyperbolic form of the Michaelis–Menton equation.[12] Determinations of $k_{cat}$ values are based on total protein concentration, assuming a molecular weight for MAO of 290 000.[16]

**SHR Bioassays.** Spontaneously hypertensive rats (SHR) were obtained from Charles River Breeding Laboratories (SHR/NCrlBR). All tested animals were males, 11–15 weeks of age, 245–300-g weight. Systolic blood pressure was measured by using an indirect tail-cuff plethysmographic method (Narco Biosystems, Inc., Houston, TX). Animals were housed in an appropriate caging facility, maintained on a constant 12-h light cycle beginning at 6 a.m., were allowed food and water ad libitum, and were conscious and unrestrained at all times except during 10–15-min blood-pressure measuring periods. Animals were acclimated to the blood-pressure measuring protocol by daily measurement over a 5–7-day period until reproducible base line blood pressure values were obtained. After an initial base line blood pressure was obtained, animals were injected intraperitoneally with the indicated doses (hydrochloride salts) of the test compounds in 0.9% saline (2 mL/kg) at $t = 0$. Each blood-pressure value was the average of 10–15 measurements taken over a 10-min-span. The standard error of the mean (SEM) was calculated on the basis of the number of animals tested at each dose level ($N$) and in all cases $N = 5$ or greater.

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