1-(Thienylalkyl)imidazole-2(3*H*)-thiones as Potent Competitive Inhibitors of Dopamine β-Hydroxylase

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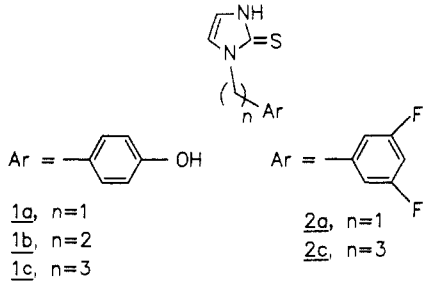
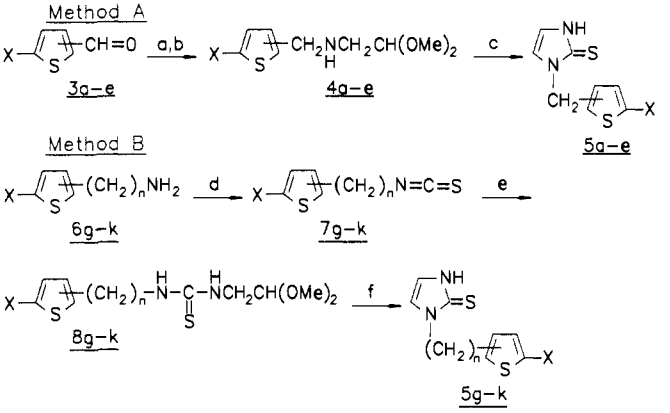
1-(2-Thienylalkyl)imidazole-2(3*H*)-thiones (**5a-k**) are competitive inhibitors of dopamine β-hydroxylase (DBH) and demonstrate the utility of thiophene in the design of potent competitive inhibitors of this enzyme. The structure-activity relationships for these compounds are discussed and compared with those of 1-phenylalkylimidazole-2(3*H*)-thiones (**1**). With the aid of molecular modeling, an idealized active-site conformer is proposed and an explanation for the difference in activity between the phenyl (**1**) and thienyl (**5**) DBH inhibitors is presented. The difference in activity is consistent with our proposal that thiophene may not always be a bioisostere for phenyl. The inhibitor of most interest, 1-[2-(2-thienyl)ethyl]imidazole-2(3*H*)-thione (**5g**), was selected for study in the spontaneously hypertensive rat. The changes in dopamine and norepinephrine levels that resulted from oral administration of **5g** correlated with the reduction of blood pressure.

Dopamine β-hydroxylase (DBH; EC 1.14.17.1) is a copper-dependent monooxygenase that catalyzes the conversion of dopamine (DA) to norepinephrine (NE) in the catecholamine biosynthetic pathway.[1] Inhibition of this enzyme provides an attractive target for the regulation of catecholamine levels.[2] A selective DBH inhibitor would be expected to lower NE levels while elevating DA levels, a combined effect that should modulate sympathetic drive and lead to new agents for the treatment of cardiovascular diseases.[3] In 1973 fusaric acid (5-butylpicolinic acid), an uncompetitive inhibitor of DBH (vs tyramine),[4] was reported to be an effective antihypertensive agent in man[5] with a combined mechanism of action of DBH inhibition and direct vasodilation.[6] Since the report on fusaric acid, a number of approaches have been taken to design inhibitors of DBH.[1a,7-9]

One approach that has received considerable attention recently is the design of competitive inhibitors of DBH. 1-Alkylimidazole-2(3*H*)-thiones are weak competitive inhibitors of DBH;[10] however, activity is dramatically increased by the introduction of a phenyl group onto the alkyl side chain.[11-13] For instance, DBH inhibitors 1000-fold more potent than the corresponding 1-alkyl-substituted imidazole-2(3*H*)-thiones were obtained when 1-[(4-hydroxyphenyl)methyl]-(**1a**) and 1-[3-(4-hydroxyphenyl)propyl]imidazole-2(3*H*)-thiones (**1c**) were prepared. Interestingly, the one carbon (**1a**) and three carbon bridge (**1c**) inhibitors were 10-fold more active than the two carbon bridge compound (**1b**). The authors postulated that the two carbon bridge compound (**1b**) was unable to attain a best-fit conformation for binding to DBH.[12] Similar results were reported for the metabolically stable 3,5-difluorophenyl analogues (**2a**,**2c**).[14]



Scheme I[a]



[a] (a) $H_2NCH_2CH(OMe)_2$, EtOH, TsOH; (b) $NaBH_4$; (c) KSCN, H_2O, HCl, EtOH, reflux; (d) Thiocarbonyldiimidazole, DMF; (e) $H_2NCH_2CH(OMe)_2$, DMF; (f) 10% HCl, H_2O, EtOH, reflux.

We have demonstrated that thiophene[15] and more recently the dithiolane ring[16] can be used in place of phenyl

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(1) (a) Rosenberg, R. C.; Lovenberg, W. *Essays Neurochem. Neuropharm.* **1980**, *4*, 163. (b) Ljones, T.; Skotland, T. In *Copper Proteins and Copper Enzymes*; Lontie, R., Ed.; CRC Press: Boca Raton, FL, 1984; Vol. 2, p 131. (c) Villafranca, J. J. In *Copper Proteins*; Spiro, T. G., Ed.; Wiley: New York, 1981; p 264. (d) Kaufman, S.; Friedman, S. *Pharmacol. Rev.* **1965**, *17*, 71.
(2) (a) Axelrod, J. *Pharmacol. Rev.* **1972**, *24*, 233. (b) Sole, M. J.; Kamble, A. B.; Hussian, M. N. *Circ. Res.* **1977**, *41*, 814.
(3) (a) Ohlstein, E. H.; Kruse, L. I.; Ezekiel, M.; Sherman, S. S.; Erickson, R.; DeWolf, W. E., Jr.; Berkowitz, B. A. *J. Pharmacol. Exp. Ther.* **1987**, *241*, 554. (b) Chidsey, C. A.; Sonnenblick, E. H.; Morrow, A. G.; Braunwald, E. *Circulation* **1966**, *33*, 43.
(4) (a) Nagatsu, T.; Hidaka, H.; Kuzuya, H.; Takeya, K. *Biochem. Pharmacol.* **1970**, *19*, 35.
(5) (a) Matta, R. J.; Wooten, G. F. *Clin. Pharmacol. Ther.* **1973**, *14*, 541. (b) Terasawa, F.; Hidaka, H. *Jpn. Circ. J.* **1976**, *40*, 785. (c) Terasawa, F.; Ying, L. H.; Kameyama, M. *Jpn. Circ. J.* **1976**, *40*, 1017. (d) Terasawa, F.; Ying, L. H.; Suzuki, T. *Jpn. Circ. J.* **1976**, *40*, 1025.
(6) Hidaka, H.; Asano, M. *J. Pharmacol. Exp. Ther.* **1976**, *199*, 620.
(7) Kruse, L. I.; Kaiser, C.; DeWolf, W. E., Jr.; Chambers, P. A.; Goodhart, P. J.; Ezekiel, M.; Ohlstein, E. H. *J. Med. Chem.* **1988**, *31*, 704 and references cited therein.
(8) Fitzpatrick, P. F.; Villafranca, J. J. *Arch. Biochem. Biophys.* **1987**, *257*, 231.
(9) May, S. W.; Wimalasena, K.; Herman, H. H.; Fowler, L. C.; Ciccarello, M. C.; Pollock, S. H. *J. Med. Chem.* **1988**, *31*, 1066.

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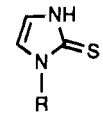
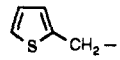
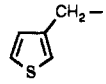
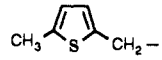
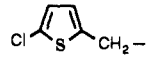
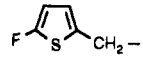
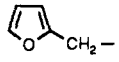
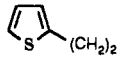
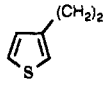
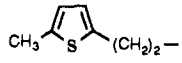
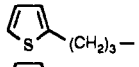
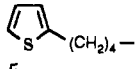
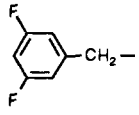
Table I. Kinetic Constants and Physical Properties of DBH Inhibitors



compd	R	K_i,[a] nM	IC_{50},[b] nM (n)	mp, °C	method prep.	recryst solvent
5a		740	4800 ± 500 (4)	128–130[c]	A	EtOH
5b			5300 ± 200 (3)	127–129	A	$PhCH_3$
5c			6000	149–150	A	$PhCH_3$
5d			1000	165–167	A	EtOH
5e			4600 ± 210 (4)	149–151	A	i-PrOH/H_2O
5f			6300 ± 430 (4)	105.5–108	A	EtOAc
5g		25.5	114.4 ± 19.8 (11)	136–137	B	EtOH/H_2O
5h		25.0	130 ± 56 (6)	148–149	B	$PhCH_3$
5i		160	491 ± 140 (4)	143–145	B	toluene
5j		480	1900 ± 200 (4)	100–1.5	B	$PhCH_3$
5k		1400	3600 ± 800 (3)	89–90	B	$PhCH_3$/cyclohexane
2a		60[d]	210 ± 40 (4)			

[a] The assay was performed at atmospheric pressure in 0.2 M acetate buffer, pH 5.5, in the presence of ascorbate, fumarate, copper, and catalase. K_i determinations were obtained from the inhibition of the conversion of tyramine to octopamine, which was followed by HPLC as described in ref 15. Each value was determined in duplicate and varied by <10%. Specific activity of DBH was 44 μM/min per mg of protein; K_m for tyramine was 1.2 mM in 3% DMSO. [b] IC_{50} concentrations inhibiting commercial DBH (Sigma) by 50% in duplicate were determined by oxygen consumption with a YSI Model 53 oxygen electrode, as described in the Experimental Section, where n = the number of duplicate determinations. [c] Literature[31] mp 129–130 °C. [d] Literature value from ref 12: K_i = 41 nM vs tyramine substrate at pH 4.5.

to produce dramatic increases in potency for time-dependent inhibitors of DBH. We now report that a series of 1-(2-thienylalkyl)imidazole-2(3*H*)-thiones[17] (**5a–k**) are potent competitive inhibitors of DBH in vitro and that in contrast to the phenylalkyl series (1) the two carbon bridge analogues are the most potent inhibitors. In addition, we report that analogue **5g** (MDL 43,925) produced significant increases in the tissue DA/NE ratio in the spontaneously hypertensive rat (SHR). These compounds demonstrate the utility of thiophene in the design of potent competitive inhibitors of DBH and highlight differences in binding of thiophene and substituted (phenylalkyl)imidazole-2(3*H*)-thiones to the active site. With the aid of molecular modeling, we offer an hypothesis concerning the region of conformational space where the active-site conformer exists for the thiophene inhibitors (**5**). The modeling results explain the difference in enzyme inhibition data between the thiophene (**5**) and phenyl (1) substituted analogues. This is noteworthy since these two ring systems are often considered to be bioisosteres.[18]

Chemistry

The preparation of the compounds in Table I was ac-

(10) (a) Stolk, J. M.; Hanlon, D. P. *Life Sci.* **1973**, *12*, 417. (b) Fuller, R. W.; Ho, P. P. K.; Matsumoto, C.; Clemens, J. A. In *Advances in Enzyme Regulation*; Weber, G., Ed.; Pergamon Press, Oxford, 1977; Vol. 15, p 267.

(11) 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.

(12) 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.

(13) 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.

(14) A number of biological studies have been reported on the 3,5-difluorophenyl methyl analogue **2a** (SKF 102698). See: Berkowitz, B. A.; Arleth, A. J.; Sung, C. P.; Kruse, L. I.; DeWolf, W. E., Jr.; Ohlstein, E. H. *J. Pharmacol. Exp. Ther.* **1988**, *245*, 850 and references cited therein.

(15) 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.

(16) Bargar, T. M.; Broersma, R. J.; Creemer, L. C.; McCarthy, J. R.; Hornsperger, J. M.; Attwood, P. V.; Jung, M. J. *J. Am. Chem. Soc.* **1988**, *110*, 2975.

(17) Matthews, D. P.; McCarthy, J. R.; Whitten, J. P.; Broersma, R. J., Jr. European Patent Application 87106440.8, filed 4/5/87.

(18) Pres, J. B. In *Thiophene and Its Derivatives*; Gronowitz, S., Ed.; Wiley: New York, 1985; Vol. 44, Part 1, p 353–456.

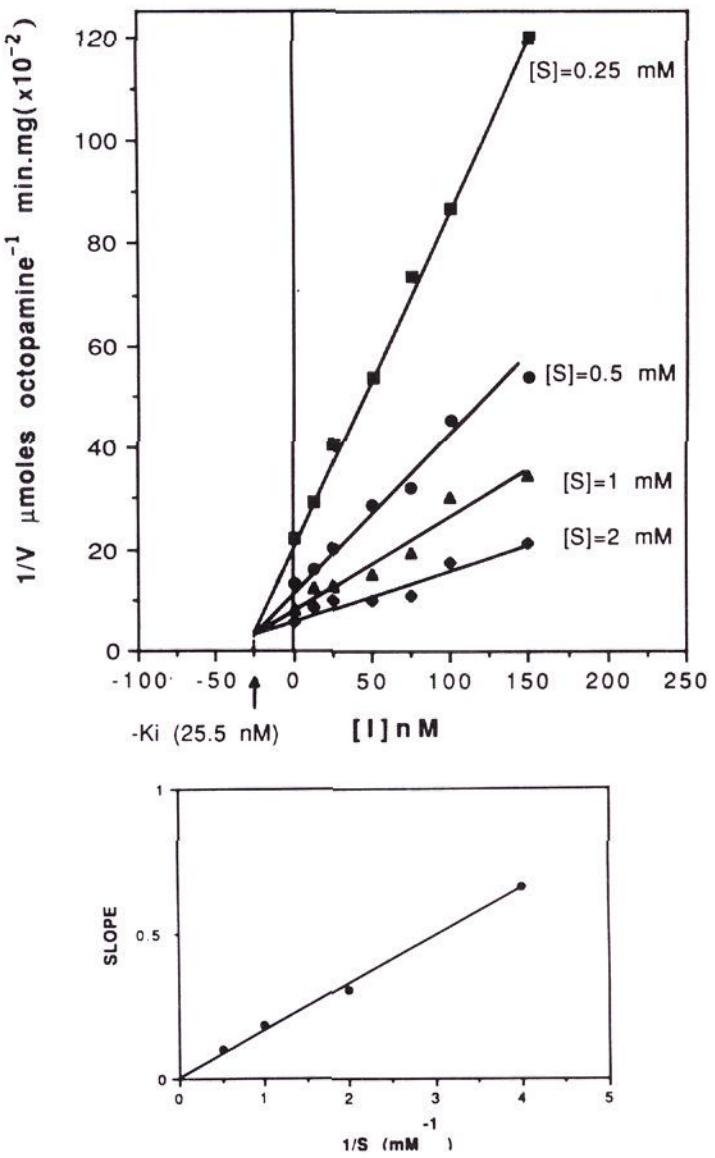


Figure 1. (Top) Dixon plot for DBH inhibition by **5g** at 37 °C, pH 5.5, 0.2 M acetate buffer in the presence of different fixed concentrations of substrate ([S] = tyramine). (Bottom) Replot of the slopes of Dixon plot versus $1/S$.

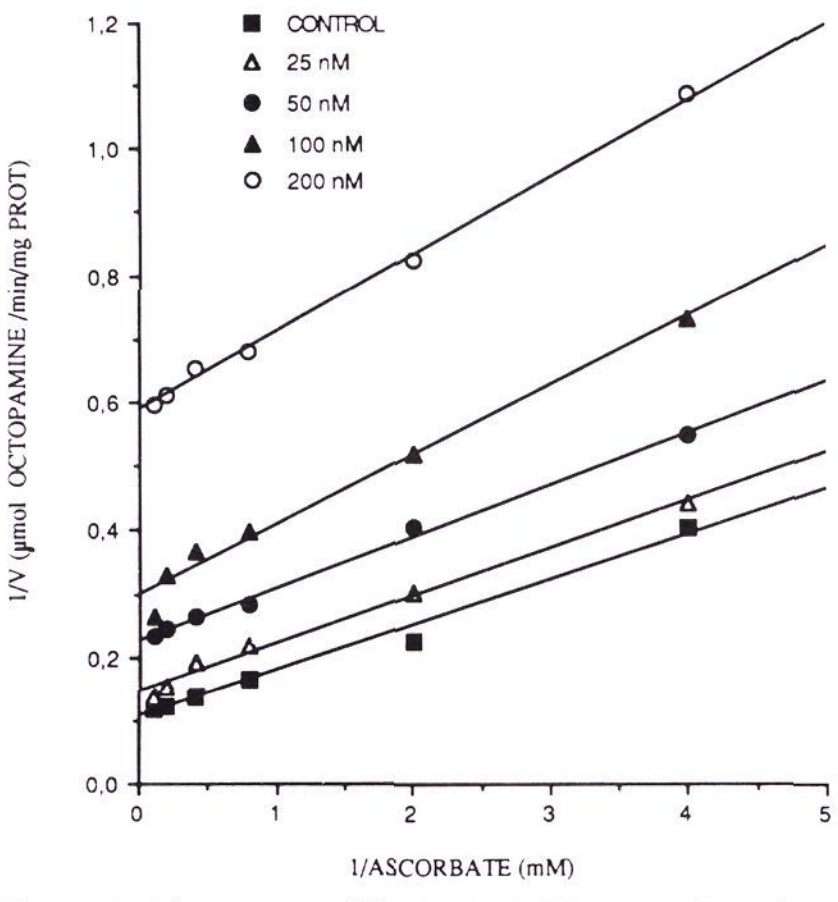


Figure 2. Lineweaver and Burk plot ($1/V$ versus 1/ascorbate) in the presence of different fixed concentrations of **5g**.

complished by two convenient synthetic routes (methods A and B) illustrated in Scheme I. In method A, 2- and 3-thiophenecarboxaldehydes (**3a–e**) were condensed with aminoacetaldehyde dimethyl acetal, and the Schiff bases were reduced to the corresponding aminoacetaldehyde dimethyl acetals **4a–e** with sodium borohydride. The

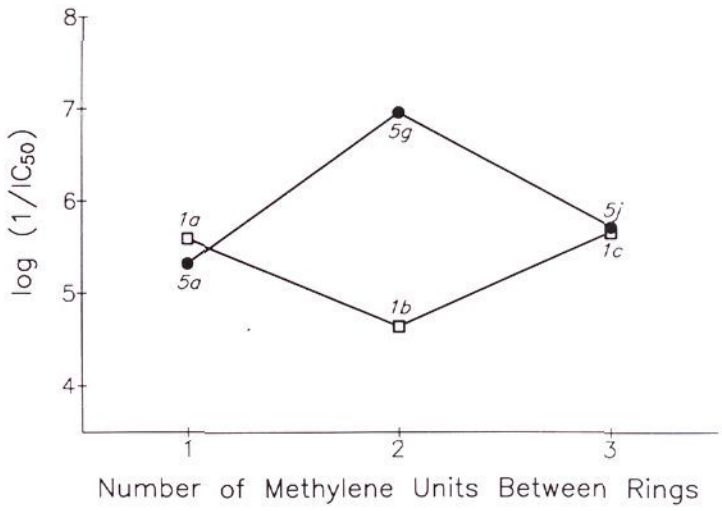


Figure 3. A comparison of the log ($1/IC_{50}$) values for the inhibition of DBH by 4-hydroxyphenylalkyl (**1a–c**) versus 2-thienylalkyl (**5a,g,j**) substituted imidazole-2(3*H*)-thiones. IC_{50} values for **1a–c** were obtained from ref 11.

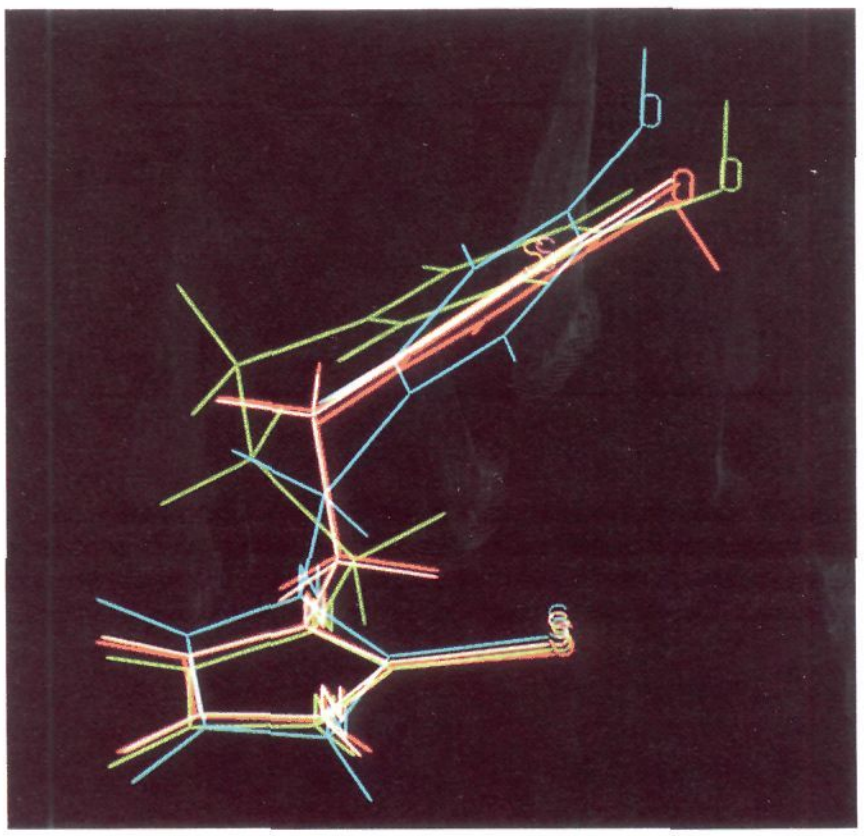


Figure 4. Overlay of compounds **1a** (blue), **1b** (red), **1c** (green), **5g**, and **5h** showing that similar molecular shapes are possible. Compounds **5g** (magenta) and **5h** (yellow) appear as white.

1-(thienylmethyl)imidazole-2(3*H*)-thiones **5a–e** were obtained by treatment of the crude intermediates **4a–e** with potassium thiocyanate in 10% aqueous hydrochloric acid and ethanol.[19] The products were readily isolated by pouring the reaction onto ice, filtering the resulting precipitate, and recrystallizing. In addition, 1-(2-furanylmethyl)imidazole-2(3*H*)-thione (**5f**) was prepared by this method.

The imidazole-2(3*H*)-thiones **5g–k** were obtained by starting with thiophenealkylamines (**6g–k**). These amines were synthesized by diborane reduction of the corresponding nitriles. Amines **6g–k** were transformed into isothiocyanates **7g–k** and the resulting crude intermediates were condensed with aminoacetaldehyde dimethyl acetal in DMF. The DMF was removed from the resulting thioureas **8g–k** and these intermediates were cyclized to **5g–k** in refluxing 10% aqueous hydrochloric acid and ethanol (method B).

Biochemistry

DBH from bovine adrenal medulla was isolated and purified by following a described procedure[20] or obtained

(19) Cannon, W. N.; Powell, C. E.; Jones, R. G. *J. Org. Chem.* **1957**, *22*, 1323.

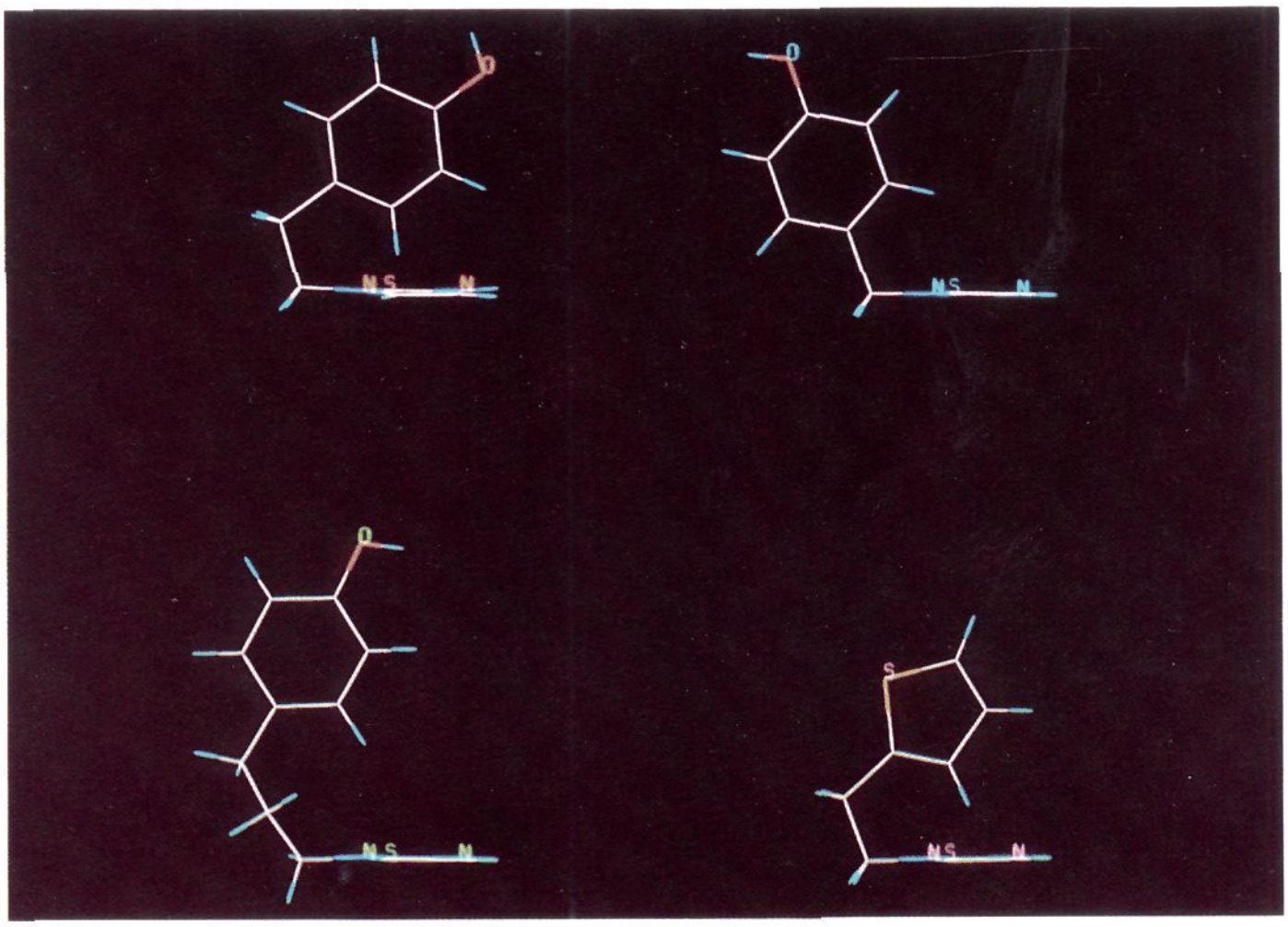


Figure 5. Orthogonal conformations of compounds **1a**, **1b**, **1c**, and **5g** possessing standard bond lengths and valence angles. (Compounds illustrated counterclockwise, **1a** in upper right.)

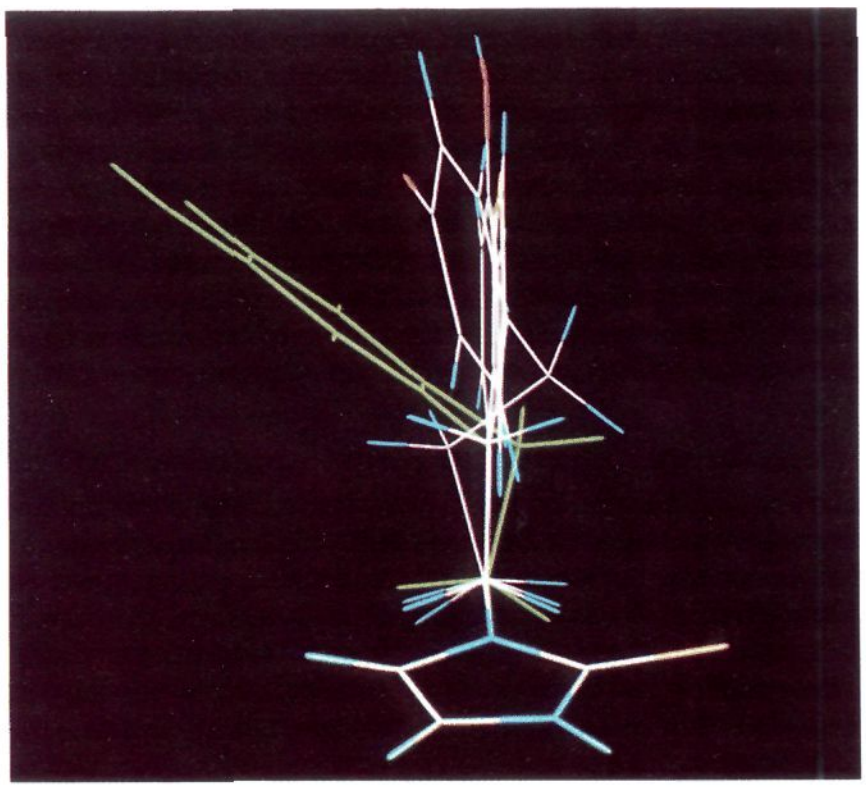


Figure 6. Local minima found for full geometry optimizations of "orthogonal" starting conformations of compounds **1a–c**, and **5g,h**. Compound **1b** is shown in green.

from a commercial source (Sigma). Dixon plots were used to identify the type of inhibition and to determine the K_i values reported in Table I. The values were obtained with purified DBH with tyramine as a substrate and by measuring the formation of octopamine by HPLC as previously reported.[16] All compounds for which K_i values were obtained were competitive inhibitors. IC_{50} values were determined with commercial enzyme versus tyramine as substrate with an oxygen electrode.[16]

Computational Chemistry

Computer-assisted molecular modeling was used in the development of the proposed model of the active conformer for DBH inhibitors. The following modeling techniques were employed: method 1, constrained conformational searching; method 2, molecular mechanics geometry optimization; method 3, semiempirical molecular orbital geometry optimization; and method 4, systematic conformational searching (see the Experimental Section for additional details of these methods). The commercially available computational chemistry (CC) software SYBYL 5.1[21] was used along with AMPAC 1.0[22] (as distributed with SYBYL).

Results and Discussion

The compounds in Table I were initially analyzed as inhibitors of bovine adrenal DBH obtained from Sigma to determine potency and relative activity. Comparison of IC_{50} values indicated that the two carbon bridge analogues are more potent inhibitors of DBH in this series. The order of inhibitory activity of the unsubstituted 1-(2-thienylalkyl)imidazole-2(3*H*)-thiones incorporating different carbon bridge lengths on DBH inhibition is **5g** ($n = 2$) > **5j** ($n = 3$) $\simeq$ **5a** ($n = 1$) > **5k** ($n = 4$). It should be noted that for the one and two carbon bridge analogues attachment of the alkyl chain at either the 2-position (**5a** and **5g**) or the 3-position (**5b** and **5h**) provided compounds of similar activity and that substitution of the functional groups at the 5-position of the thiophene ring (**5c,d,e,i**) only produced small changes on the inhibitory activity of DBH. For the one-carbon series, halogen substitution increased activity 2–5-fold, while a 5-methyl group (**5c**) decreased activity slightly. Similar results were observed in the two carbon bridge series where a 5-methyl group (**5i**) decreased activity 5-fold. Comparison of the IC_{50} values

(20) Aunis, D.; Miras-Portugal, M. T.; Mandel, P. *J. Neurochem.* **1975**, *24*, 425.

(21) SYBYL 5.2 (1988), Tripos Associates, 1699 S. Hanley Road, Suite 303, St. Louis, MO 63144.

(22) (a) Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902. (b) Stewart, J. J. P. *QCPE Bull.* **1985**, *5*, 62.

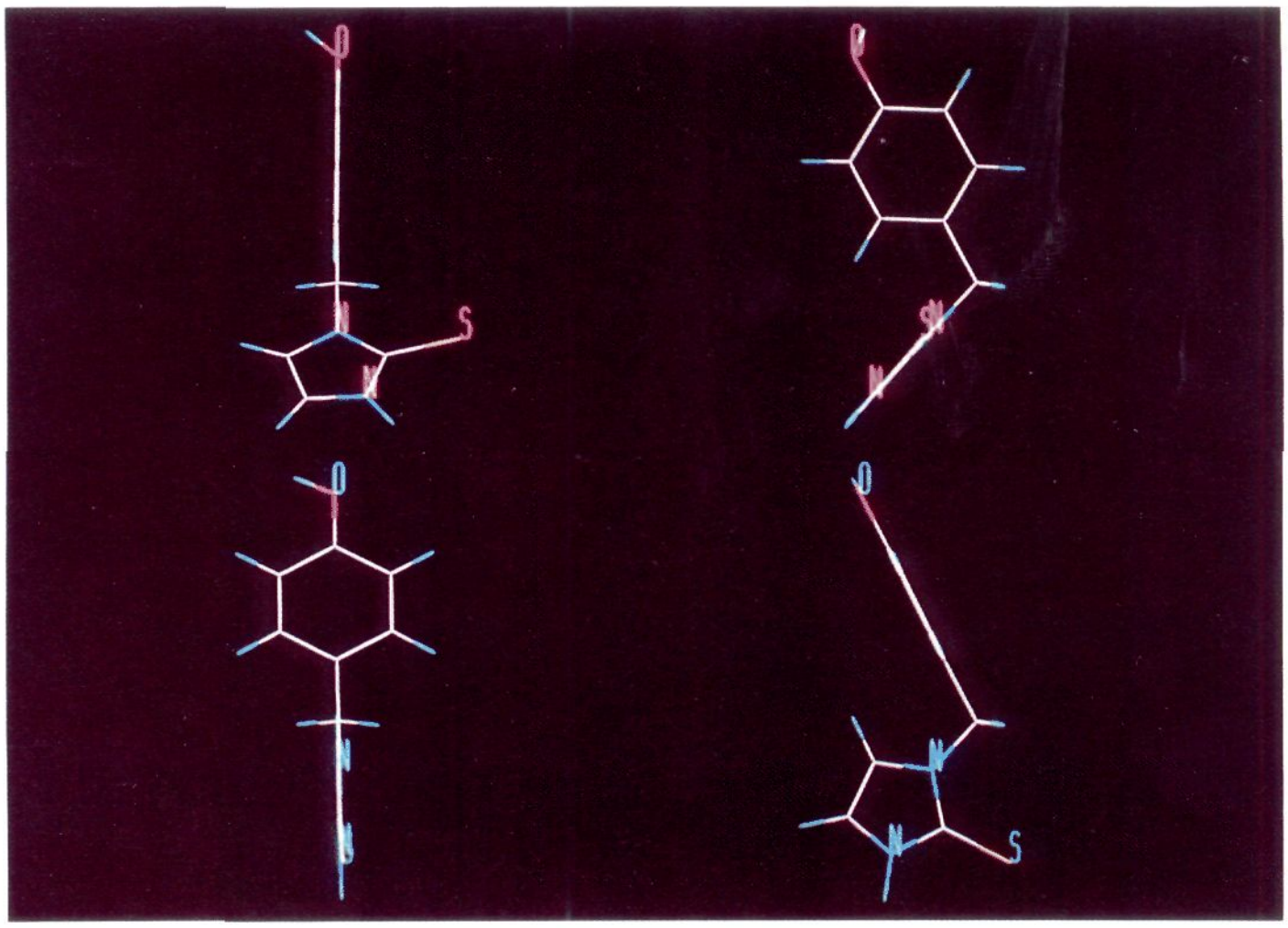


Figure 7. Possible orthogonal conformations for **1a**: case I (above) and case II (below). Orthogonal projections are shown.

for furanylmethyl (**5f**) and thienylmethyl (**5a**) compounds indicated that furan was a less effective substitution than thiophene. Therefore, thiophene was selected for a more in-depth study of structure–activity relationships.

A comparison of K_i values confirmed the IC_{50} results, i.e. that two carbon bridge analogues bind to DBH more tightly. Steady-state kinetic studies demonstrate that **5g** binds DBH competitively with the substrate tyramine. Figure 1 (top) shows a Dixon plot of the inhibition of DBH by compound **5g** and from this experiment a K_i of 25.5 nM was determined. Figure 1 (bottom) also shows a replot of slope versus the corresponding $1/S$ which is a straight line through the origin, indicating true competitive inhibition with respect to tyramine. Similar K_i values were obtained with either source of enzyme for **5g**, i.e. K_i = 16 nM with commercial DBH versus 25.5 nM with purified DBH. The inhibitor is uncompetitive with ascorbic acid (Figure 2), indicating it binds to reduced copper–enzyme, which is similar to that observed for the normal substrate.[12] The enhanced activity of two carbon bridge analogues may be due to the attainment of an optimal placement of the two ring systems in the active site, which facilitates binding to DBH and leads to increased potency (see below). The most potent thiophene analogues have a two-carbon bridge in contrast to the results reported for a series of phenyl analogues (**1a–c**). In the phenyl series the one carbon (**1a**) and three carbon (**1c**) analogues are 1 order of magnitude more potent than the two carbon bridge analogue (**1b**) (see Figure 3).

Computational chemistry was applied in an attempt to understand the differences in the activity profiles between the thienyl and phenyl series of compounds. Conformational searches on **1a–c** and **5g** using SYBYL[21] (CC, method 1) show that it is possible for all compounds in the series to fit into a common shape (Figure 4). However, the enzyme inhibition data is not consistent with the structural overlays shown in Figure 4.

Literature reports[13] indicated that ortho-substituted analogues, such as 1-[(2,6-dichlorophenyl)methyl]-imidazole-2(3*H*)-thione (**1a**, where Ar = 2,6-dichlorophenyl), were very poor inhibitors. Combining this information with the conformational data discussed above led to the hypothesis that the phenyl ring approaches an orthogonal position above the imidazole ring when binding in the active site of DBH. The ortho substituents might sterically hinder or prevent the orthogonal positioning of the two rings, thus accounting for the observed lower activities. This hypothesis was applied to compounds **1a–c**, **5g**, and **5h**. Molecules **1a–c** and **5g** were initially placed in a conformation such that their rings were orthogonal to each other (Figure 5). The molecular geometry of each molecule was then optimized with the Tripos force field[23] in SYBYL (CC, method 2) to locate the nearest local minimum. The results of the optimizations are shown in Figure 6. Each compound, with the exception of **1b**, retained its original conformation (rings essentially orthogonal to one another). This suggested that the "orthogonal" conformation of compound **1b** was energetically inaccessible. These data also suggest that the phenyl and thiophene rings show important differences in their potential energy surfaces in this region of conformational space hypothesized to contain the "active" conformer. Examination of the models of **1b** and **5g** found in Figures 5 and 6, as well as space filling models, show that the location of the *o*-hydrogen on the phenyl ring of **1b** (adjacent to the imidazole-2(3*H*)-thione ring) is more directed into the face of the imidazole-2(3*H*)-thione ring than *o*-hydrogens on **5g**. This leads to greater steric repulsions for the orthogonal conformation of **1b**. These models clearly demonstrate why thiophene may not always be a bioisostere for phenyl.

Semiempirical molecular orbital calculations were used to define which of the two "orthogonal" conformations of the inhibitors was the "correct" one. Idealized orthogonal

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Table II. DA/NE Ratios and Antihypertensive Activity of **5g** in SHR

dose, mg/kg (po)	mesenteric artery DA/NE ratio[a] (n)	mean arterial blood pressure, mmHg		duration,[c] h
		control (n)	treatment[b] (n)	
vehicle	0.072 ± 0.029 (5)			
25	0.214 ± 0.017[d] (5)	148 ± 9 (4)	137 ± 7[e] (4)	<1
50	0.269 ± 0.047[d] (4)	172 ± 5 (5)	137 ± 9[e] (5)	8
100	0.333 ± 0.052[d] (5)	170 ± 11 (5)	126 ± 10[e] (5)	20

[a] DA/NE ratios (mean ± SD) were determined for fasted SHR, 6 h after dosing. DA/NE ratios were compared to control values using 2-tailed t test. [b] Compared (paired t test) to vehicle values (mean ± SEM) using a cross-over design. [c] $p \leq 0.05$ compared to vehicle-treated animals. [d] $p \leq 0.001$. [e] $p \leq 0.05$.

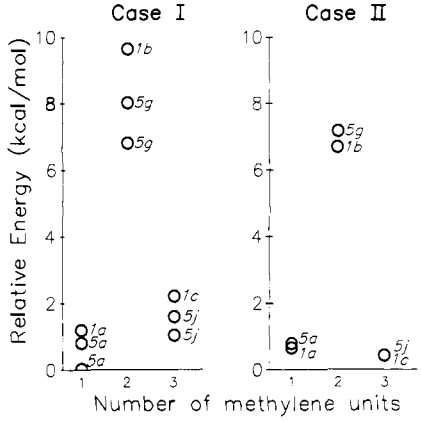

Figure 8. Active conformer differentiation: AMPAC AM1 energy above the global minimum (compounds **1a–c** and **5a,g,j**).

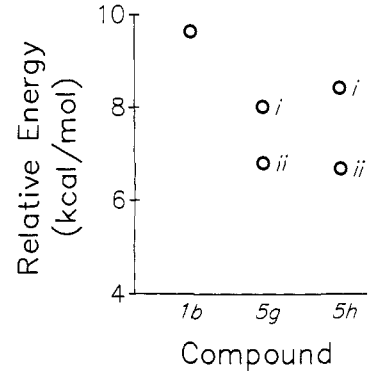

Figure 9. Energetic similarities of compounds **5g** and **5h** relative to **1b** in the idealized active conformation (case I, the two possible orthogonal rotamers of the thiophene ring system, i, ii), using the AMPAC AM1 energy above the global minimum.

orientations were chosen as an index of the energetic accessibility of this region of conformational space since a systematic exploration using semiempirical molecular orbital techniques would have been prohibitively time-consuming. As shown in Figure 7, in the first conformer for **1a** (case I), the phenyl ring is orthogonally positioned above the imidazole ring, while for the second conformer (case II), the imidazole is orthogonally positioned above the phenyl. Constrained AM1 geometry optimizations were run on all possible conformers containing orthogonal ring systems for molecules **1a–c** and **5a,g,j** (CC, method 3). The global energy minimum was located by performing a conformational search on all compounds (CC, method 4). The results of these calculations are shown in Figure 8. The imidazole-2(3*H*)-thione sulfur orthogonal to the phenyl ring was energetically inaccessible and therefore was not considered further. In case II there are no appreciable energy differences among the conformers. However, in case I there is a notable difference in conformational energy.

The conformers of one and three methylene unit compounds were within about 1 kcal/mol of one another, consistent with their IC_{50} values. However, an energy difference was noted between the conformers of the two methylene unit compounds (**1b** and **5g**). Compound **5g** had two possible conformers for case I due to the nonsymmetric thiophene ring. One of these conformers is shown in Figure 5. These conformers were 1.6 and 2.6 kcal/mol lower in energy than the corresponding conformer for the phenyl compound (**1b**). The energy differences in the proposed active conformation of DBH inhibitors qualitatively correlate with the IC_{50} values for the two methylene series and support the hypothesis that the phenyl or thiophene ring approaches an orthogonal position above the imidazole ring in the active site. However, our model does not explain the difference in potency among the one, two, and three methylene chain series. Only the energetic differences between thiophene- and phenyl-substituted compounds within a series are predictive of the relative potency of the compounds. The pharmacophore fit is also important in predicting activity. Energy alone is not the determining factor.

To compare the effect of position of substitution on the thiophene ring by the alkylene chain, the geometry of compound **5h** was also optimized as described above. Its relative conformational energies are compared to those of **1b** and **5g** in Figure 9. The figure shows that the two possible orthogonal conformations (i and ii) are energetically equivalent, which is consistent with the identical K_i values found for **5g** and **5h** (Table I).

The molecular modeling results reinforced the important differences observed in the inhibition of DBH by phenyl and thiophene 1-substituted imidazole-2(3*H*)-thiones. Therefore, the potent DBH inhibitor, **5g** (MDL 43,925), was selected for in-depth evaluation. The effectiveness of **5g** in vivo was illustrated in separate studies by significant decreases in blood pressure and mesenteric artery catecholamine alterations after acute administration to SHR. Dose-related antihypertensive activity was demonstrated after oral administration of 25–100 mg/kg to conscious SHR (Table II). Mean arterial blood pressure was significantly lower compared to that of vehicle-treated animals (Figure 10). The antihypertensive activity was gradual in onset while duration of activity was dose related. Maximal activity was seen approximately 4.1, 6.5, and 16 h after the 25, 50, and 100 mg/kg doses of **5g**, respectively. Significant changes in mean arterial pressure from the corresponding vehicle control group lasted <1 h for 25 mg/kg, 8 h for 50 mg/kg, and 20 h for 100 mg/kg of **5g** (Table II). Reflex tachycardia did not accompany the antihypertensive effect of **5g**.

The antihypertensive action of **5g** was associated with inhibition of DBH as demonstrated by its ability to increase mesenteric artery levels of dopamine (DA) and decrease norepinephrine (NE) levels, thus increasing the DA/NE ratio. Doses (25–100 mg/kg) of **5g** caused significant dose-related increases in the DA/NE ratios of mesenteric artery 6 h after oral administration (Table II).

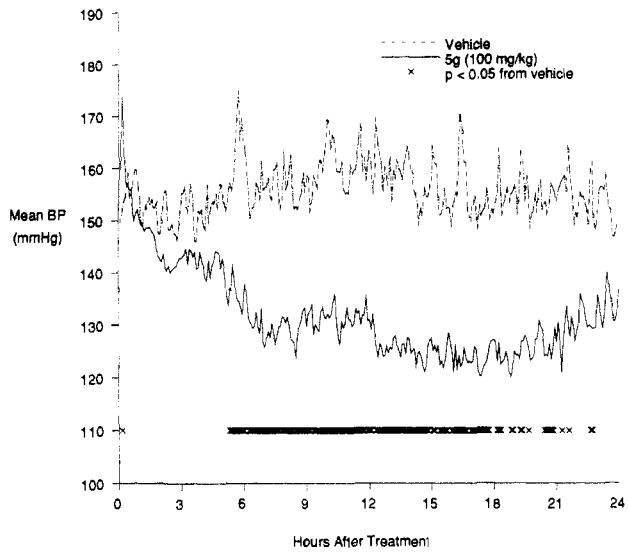

Figure 10. Effect of **5g** on mean blood pressure in conscious SHR (5 min averages, $n = 5$).

The DA/NE ratio of mesenteric artery at 100 mg/kg was increased approximately 4.5-fold from that of control values.

In summary, 1-(thienylalkyl)imidazole-2(3*H*)-thiones (**5a–k**) are competitive inhibitors of DBH. Comparison of DBH inhibitory activity of previously reported[11–13] 1-(phenylalkyl)imidazole-2(3*H*)-thiones with the thiophene compounds of the same methylene bridging chain length demonstrates that thiophene is not always a bioisostere for phenyl. In the thiophene series of DBH inhibitors, a two carbon bridging chain length was most active. Trends shown in the molecular modeling calculations were used to develop a qualitative model of the active-site conformation for DBH inhibitors in which the thiophene or phenyl ring approaches an orthogonal position above the imidazole ring. This idealized conformation explains the difference between (thienylethyl)- (**5g** and **5h**) and (phenylethyl)imidazole-2(3*H*)-thiones (**1b**) for optimal binding to the active site of DBH. Compound **5g** is a potent competitive inhibitor of DBH and has been selected for further evaluation. The results of these studies should lead to a significant increase in the understanding of the importance of DBH and catecholamines in disease states.

Experimental Section

All melting points are uncorrected. The IR spectra were recorded with a Perkin-Elmer Model 710B spectrophotometer, ^{1}H NMR spectra were determined with a Varian EM-360 and Varian VXR-300 (multinuclear probe) spectrometers. Mass spectra were obtained with a Finnigan MAT Model 4600 (electron-impact and chemical-ionization) mass spectrometer. Combustion analyses for C, H, and N were performed by Merrell Dow Analytical Laboratories, Cincinnati, Ohio. Starting materials were commercially available or were previously reported in the literature: **3e**,[24] **6i**,[25] and **6j**.[26]

1-(3-Thienylmethyl)imidazole-2(3*H*)-thione (5b) (Method A). A mixture of 3-thiophenecarboxaldehyde (22.4 g, 0.2 mol), aminoacetaldehyde dimethyl acetal (26.6 g, 0.2 mol), and *p*-toluenesulfonic acid monohydrate (100 mg) in absolute ethanol (200 mL) was heated at reflux for 2 h. The reaction was cooled, $NaBH_4$ (7.6 g, 0.2 mol) was added, and the mixture was refluxed for an additional 2 h, concentrated, diluted with water (150 mL), and extracted with EtOAc (2 × 300 mL). The combined organic layers were dried (Na_2SO_4) and concentrated to provide 42.6 g (93%) of crude amino acetal **4b** as a tan oil; ^{1}H NMR ($CDCl_3$) δ 2.13 (br s, 1), 2.75 (d, 2), 3.40 (s, 6), 3.82 (s, 2), 5.60 (t, 1), 6.63–7.32 (m, 3); MS (CI) m/z 230 (MH^+).

A mixture of **4b** (33 g, 0.144 mol), KSCN (15.5 g, 0.16 mol), 10% HCl (55 mL), and EtOH (150 mL) was heated at reflux for 6 h. The cooled reaction was poured onto 1 L of crushed ice and the resulting gray–white solid of **5b** (23.2 g, 82%) was collected by filtration, washed with water, and dried. Recrystallization from toluene provided analytically pure material: mp 127–129 °C; ^{1}H NMR (DMSO-d_6) δ 5.05 (s, 2), 6.65–7.52 (m, 5); MS (EI) m/z 196 (M^+). Anal. ($C_8H_8N_2S_2$) C, H, N.

1-[2-(2-Thienyl)ethyl]imidazole-2(3*H*)-thione (5g) (Method B). The free base of 2-thiopheneethanamine (**6g**,[27] 91 g, 0.715 mol) was dissolved in DMF (500 mL) and poured into an ice-cooled solution of 90% 1,1-thiocarbonyldiimidazole (142 g, 0.715 mol) in DMF (500 mL). The reaction was stirred at ambient temperature for 18 h and poured onto brine (4 L). The solution was extracted with EtOAc (3 × 600 mL) and the combined organic layers were washed with H_2O (1 L), dried (Na_2SO_4), and concentrated in vacuo to provide isothiocyanate **7g** as an oil; IR (thin film) 2100 cm^{-1}. Aminoacetaldehyde dimethyl acetal (75 g, 0.714 mol) was added to a solution of **7g** in DMF (300 mL) and the solution exothermed to 70 °C. The reaction was heated at 80 °C for 2.5 h and cooled, and the DMF was removed under vacuum. The resulting thiourea **8g** was heated at reflux with 10% aqueous HCl (150 mL) and EtOH (150 mL) for 2 h. The cooled solution was poured onto 3 L of ice with stirring. The solution was seeded with **5g** and the resulting crystals were collected by filtration and dried under vacuum (75.2 g, 50%): mp 136–137 °C (EtOH/H_2O or toluene): IR (KBr) 3420 (br), 3180, 3140, 3030, 2940, 1580, 1480 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.23 (t, 2, J = 7.4 Hz), 4.16 (t, 2, J = 7.4 Hz), 6.85–7.0 (12 lines, 3, ABX system), 7.34 (1, d, J = 1.2 Hz), 7.36 (1, d, J = 1.2 Hz), 12.11 (1, br s, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 28.24, 47.14, 114.1, 118.5, 124.4, 125.6, 127.0, 139.99, 160.62; MS (EI) m/z 210 (M^+). Anal. ($C_9H_{10}N_2S_2$) C, H, N.

Tissue Catecholamines. Male, spontaneously hypertensive rats (SHR) were dosed orally with vehicle (50% PEG 400) or compound suspended in this vehicle. Six hours after dosing, the animals were sacrificed and the mesenteric arteries were removed. Each mesenteric artery was homogenized in 0.7 mL of chilled aqueous 0.2 M $HClO_4$ containing 10 mM DTT, 1 mM EGTA, and 0.05075 mg of 3,4-dihydroxybenzylamine hydrobromide (DHBA) as internal standard. The homogenates were centrifuged at 40000*g* for 20 min and 0.5 mL of supernatant or catecholamine standard solution was added to 0.5 mL of 2 M Tris·HCl (pH 8.6) containing 15 mg of activated alumina. After shaking, the supernatant was removed and the alumina was washed three times with water. The catecholamines were desorbed from the alumina with 250 μL of 0.1 M H_3PO_4. The samples were separated by HPLC on a 4.6 mm × 150 mm reverse-phase Ultremex (Phenomerex) column (3 μm, C18) with a mobile phase of 0.15 M monochloroacetic acid, 2 mM EDTA, 150 mg/L of 1-octanesulfonic acid, and 4% acetonitrile (pH 3.0). Detection was by electrochemical detection at a working electrode potential of 0.7 V. Peak areas were converted to nanograms per gram of tissue by using a sequence multi-point calibration data entry reporting system with DHBA as the internal standard.

Blood Pressure Measurements. Arterial blood pressure was continuously monitored in unrestrained, conscious, male Wistar-Okamoto SHR's purchased from Charles River, Wilmington, MA (23–26 weeks of age weighing 325–375 g). Arterial blood pressure measurements were obtained from the carotid artery via a catheter (PE 50) externalized at the nape of the neck. Rats were allowed approximately 48 h to recover from surgery before administration of vehicle (50% PEG 400) or compound suspended in vehicle. Animals were dosed again the next day at the same time (09:00) but the treatments were reversed. All animals were fasted from approximately 18:00 the day before treatment to 13:00 of the treatment day. Blood pressure (systolic and diastolic) and heart rate were monitored and recorded as 5-min interval averages

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from 1-min sampling intervals. The statistical significance of the difference between 5 min posttreatment averages of compound and vehicle was determined with a t test for paired observations. A p value <0.05 was considered to be a significant difference. Compound suspensions were prepared fresh daily in 50% PEG 400 and were administered in a volume of 0.4 mL/100 g of body weight.

In Vitro IC_{50} Determination and Kinetic Assays with Commercial DBH. The compounds in Table I were evaluated for DBH inhibitory activity with a commercially available bovine enzyme (Sigma). To measure enzyme activity, oxygen consumption at 25.0 °C was measured with a YSI Model 53 oxygen electrode. The assay mixture in a final volume of 3 mL contained 2 mM ascorbic acid, 10 mM sodium fumarate, ca 1000 units/assay of catalase (380 μg/mL) and 10 mM tyramine, in 0.1 M sodium acetate buffer at pH 5.0. Enzymatic analyses were performed to determine the concentrations that inhibited DBH activity by 50% (IC_{50}). IC_{50} data were determined in duplicate in at least three separate experiments to obtain a mean (±SD) value. Kinetic constants were determined with the computer programs of Cleland. When either ascorbate or tyramine was used as the substrate, enzyme activity was measured as in the IC_{50} assay. After the assay mixture had been equilibrated with air by stirring at 37 °C and the nonenzymatic endogeneous rate for ascorbate oxidation had been established, the assay was started by the addition of 10 μL of enzyme solution. All enzymatic rates were corrected for the nonenzymatic rate of ascorbate oxidation.

Kinetic Assays with Purified DBH. DBH was isolated from beef adrenal medula glands and purified following a described procedure[20] (sp act. 44 μmol min^{-1} mg^{-1} at pH 5.5 in the tyramine assay). DBH activity was determined by measuring the formation of octopamine from tyramine by reverse-phase HPLC with fluorometric detection (λ_{exc} = 275 nm, λ_{emi} = 315 nm). Kinetic experiments were performed at atmospheric pressure and 37 °C in a reaction mixture containing 0.2 M acetate buffer, pH 5.5, 10 mM ascorbic acid, 20 mM sodium fumarate, 2.5 mM $CuSO_4$, and 1300 units of catalase. K_i values were derived from the degree to which conversion of tyramine to octopamine was inhibited (followed by the HPLC assay). For each determination, six concentrations of the compound and four concentrations of substrate (0.25 nM to 2 mM) were assayed. Each value was determined in duplicate and varied by less than 6%. All compounds were dissolved in DMSO and added to the incubation mixture in such a way that the final concentration of DMSO did not exceed 3%.

Computational Chemistry, Method 1. The SYBYL search[28] on **1a** was carried out using a "hard-sphere" approximation. The van der Waals radii were relaxed to prevent exclusion of high-energy conformers. A step size of 10° was used in all conformational searches. The following distances were tabulated: (a) center of phenyl ring to center of imidazole ring, (b) center of phenyl ring to sulfur and (c) normal of phenyl ring to normal of imidazole ring. With the distance ranges (calculated above) as constraints, conformational searches were performed on **1b**, **1c**, and **5g**. By employing a "constrained search", only those conformers common to all structures are retained. The distance ranges were reapplied as constraints on compound **1a**. The subset of conformers generated was then visually examined to find the most similar conformers of the different compounds found in the search. A root mean square fit of the compounds was performed with the centers of the rings, the normals to the ring planes, and the sulfur atoms with compound **1a** as the reference.

Computational Chemistry, Method 2. Geometry optimizations were performed with the maximin2 minimizer in SYBYL.[23] Convergence was achieved when the energy changed by less than 10^{-6} kcal/mol. No electrostatic term was employed. Default settings were used for all other parameters.

Computational Chemistry, Method 3. Geometry optimizations were done with the AM1 Hamiltonian in AMPAC.[22] In these optimizations, the torsion angles representing the rotation about the bonds connecting the two rings were constrained to their initial values (all sets of torsion angles representing orthogonal conformers were calculated). All other geometrical parameters were optimized. Default settings were used for all other variable parameters. AM1 was chosen over MNDO since it provides a general improvement over the MNDO method and is capable of reproducing hydrogen bonds.[22,29]

Computational Chemistry, Method 4. AM1[22] charges were calculated for a starting geometry determined by using CC method 2. A systematic conformational search was performed with SYBYL.[21] Each rotatable bond was searched at 10° increments.[28] Each global minimum from the search results was fully optimized with AM1.

Acknowledgment. We thank Professor A. J. Hopfinger for early discussions and Jim Hughes, Carol Robideau, Eileen Heminger, and Tres Kutcher for technical assistance.

(28) SYBYL search details: van der Waals scaling factors 0.7, 0.7, 0.65 for general, 1–4, and H-bond, respectively; no charges; distance map created.

(29) One systematic error noted was that geometry optimization of the imidazole-2(3*H*)-thione consistently calculated the carbon–sulfur double bond to be about 1.59 Å in length compared to its normally reported value of about 1.69 Å.[30] This value was systematically underestimated for all compounds. Therefore, we felt that this would not adversely affect the trends noted in the energy calculations.

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