was added dropwise a solution of CAN (5.0 g, 9.1 mmol) in H_2O (5 mL) and the resulting solution was stirred at this temperature for 2 h. The mixture was diluted with H_2O (20 mL) and the aqueous layer was extracted with ether (3 × 100 mL). The extracts were combined, washed (brine, 3 × 100 mL), dried ($MgSO_4$), filtered, and concentrated. The brown residue was chromatographed using a flash column (silica gel, 250 g). Elution by 5% ether in *n*-hexane gave **49** (850 mg, 92%) as yellow needles; mp 37–38 °C; MS *m/e* 214 (M^+), 199, 172; IR (Nujol) 1654, 1520, 1400 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.20–8.00 (2 H, m, C_5-H, C_8-H), 7.80–7.50 (2 H, m, C_6-H, C_7-H), 6.65 (1 H, s, C_3-H), 3.00–2.80 (2 H, m, $ArCH_2CH_2CH_2CH_3$), 2.00–1.30 (4 H, m, $ArCH_2CH_2CH_2CH_3$), 1.20–0.95 (3 H, m, $ArCH_2CH_2CH_2CH_3$). Anal. ($C_{14}H_{14}O_2$) C, H. Quinones **50** and **51** were prepared in a similar fashion from **42** and **48**, respectively.

Registry No. **1a**, 27436-93-7; **1b**, 27436-99-3; **1c**, 27437-03-2; **1d**, 123332-48-9; **1e**, 123332-49-0; **1f**, 123332-50-3; **1g**, 123332-51-4; **1h**, 123357-80-2; **1i**, 84153-34-4; **1j**, 34741-93-0; **1k**, 62589-23-5; **2**, 99107-53-6; **3**, 107536-17-4; **4**, 107536-14-1; **5**, 107536-22-1; **6**, 107536-20-9; **7**, 123332-22-9; **8**, 123332-23-0; **9**, 123332-24-1; **10**, 107536-19-6; **11**, 99107-52-5; **12**, 123332-25-2; **13**, 123332-26-3; **14**, 107536-21-0; **15**, 99120-56-6; **16**, 99107-70-7; **17**, 123332-27-4; **18**, 121444-82-4; **19**, 121444-83-5; **20**, 123332-28-5; **21**, 123332-29-6; **22**, 123332-30-9; **23**, 123332-31-0; **24**, 123332-32-1; **25**, 123332-33-2; **26**, 123332-34-3; **27**, 123332-35-4; **28**, 123332-36-5; **29**, 123332-37-6; **30**, 123332-38-7; **31**, 123332-39-8; **32**, 123332-40-1; **33**, 123332-41-2; **34**, 123332-42-3; **35**, 99107-56-9; **36**, 99497-21-9; **37**, 99497-22-0; **38**, 99107-54-7; **39**, 99107-55-8; **40**, 99497-23-1; **41**, 99107-57-0; **42**, 99107-50-3; **43**, 123332-43-4; **44**, 120255-00-7; **45**, 123332-44-5; **46**, 123332-45-6; **47**, 99107-58-1; **48**, 99107-51-4; **49**, 34491-88-8; **50**, 123332-46-7; **51**, 123332-47-8; *o*-MeC_6H_4Br, 95-46-5; *m*-BuC_6H_4Br, 54887-20-6; $Cr(CO)_6$, 13007-92-6; BuC≡CBu, 1942-46-7; $CH_3(CH_2)_4C{\equiv}CCO_2Et$, 10519-20-7; BuC≡CH, 693-02-7; EtC≡CEt, 928-49-4; PhC≡CPh, 501-65-5; $HC{\equiv}C(CH_2)_7CH_3$, 764-93-2; 5-lipoxygenase, 80619-02-9; 2,5-dimethylbromobenzene, 553-94-6; 2,3,5-trimethylbromobenzene, 31053-99-3; 2,3,4,5-tetramethylbromobenzene, 40101-36-8; *N*-methylpyrrole, 96-54-8.

Some Benzyl-Substituted Imidazoles, Triazoles, Tetrazoles, Pyridinethiones, and Structural Relatives as Multisubstrate Inhibitors of Dopamine β-Hydroxylase. 4.[1] Structure–Activity Relationships at the Copper Binding Site

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Structure–activity relationships (SAR) were determined for novel multisubstrate inhibitors of dopamine β-hydroxylase (DBH; EC 1.14.17.1) by examining the effects upon in vitro inhibitory potencies resulting from structural changes at the copper-binding region of inhibitor. Attempts were made to determine replacement groups for the thione sulfur atom of the prototypical inhibitor 1-(4-hydroxybenzyl)imidazole-2-thione described previously. The synthesis and evaluation of oxygen and nitrogen analogues of the soft thione group demonstrated the sulfur atom to be necessary for optimal activity. An additional series of imidazole-2-thione relatives was prepared in an effort to probe the relationship between the pK_a of the ligand group and inhibitor potency. In vitro inhibitory potency was shown not to correlate with ligand pK_a over a range of approximately 10 pK_a units, and a rationale for this is advanced. Additional ligand modifications were prepared in order to explore bulk tolerance at the enzyme oxygen binding site and to determine the effects of substituting a six-membered ligand group for the five-membered imidazole-2-thione ligand.

Recently we reported some multisubstrate inhibitors of dopamine β-hydroxylase (DBH; EC 1.14.17.1), the mixed-function oxidase that catalyzes the hydroxylation of dopamine to norepinephrine.[1-5] Kinetic characterization of these inhibitors has led to structural insights to the active site of DBH[3] while the pharmacological activity of certain DBH inhibitors lends support to the notion that inhibiting DBH might offer a novel approach to the treatment of cardiovascular disorders such as hypertension.[1,2,5] Previous structure–activity relationship (SAR) studies of these DBH inhibitors demonstrated a striking dependence of potency upon substitution patterns at the portion of inhibitor that mimics the phenethylamine substrate,[5] as well as the dependence upon the length and substitution patterns of the bridging chain.[4] The prototype of this class of inhibitor, 1-(4-hydroxybenzyl)imidazole-2-thione, was designed on the basis of a capacity to bind a hypothesized binuclear active site as illustrated in Figure 1. Evidence in support of a direct binding of inhibitor to one copper atom has already been presented,[3,6] but further evidence to support the hypothesized binuclear copper site shown in Figure 1 is lacking. Indeed, EXAFS (extended X-ray absorption fine structure) studies have failed to detect a Cu···Cu interaction in either the resting Cu^{2+} or reduced, catalytically competent Cu^{1+} oxidation state of DBH.[6] For this reason and because of the stringent SAR shown at the bridging chain and phenethylamine mimic portions of inhibitor, it became of interest to explore inhibitory potency as a function of modifications of the copper-binding portion of inhibitor. In this paper we re-

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§ Deceased.

(1) For part 3 see: Ross, S. T.; Kruse, L. I.; Ohlstein, E. H.; Erickson, R. W.; Ezekiel, M.; Flaim, K. E.; Sawyer, J. L.; Berkowitz, B. A. *J. Med. Chem.* **1987**, *30*, 1987.
(2) 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.
(3) Kruse, L. I.; DeWolf, W. E., Jr.; Chambers, P. A.; Goodhart, P. J. *Biochemistry* **1986**, *25*, 7271.
(4) 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.
(5) 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.
(6) Scott, R. A.; Sullivan, J.; DeWolf, W. E., Jr.; Dolle, R. E.; Kruse, L. I. *Biochemistry* **1988**, *27*, 5411.

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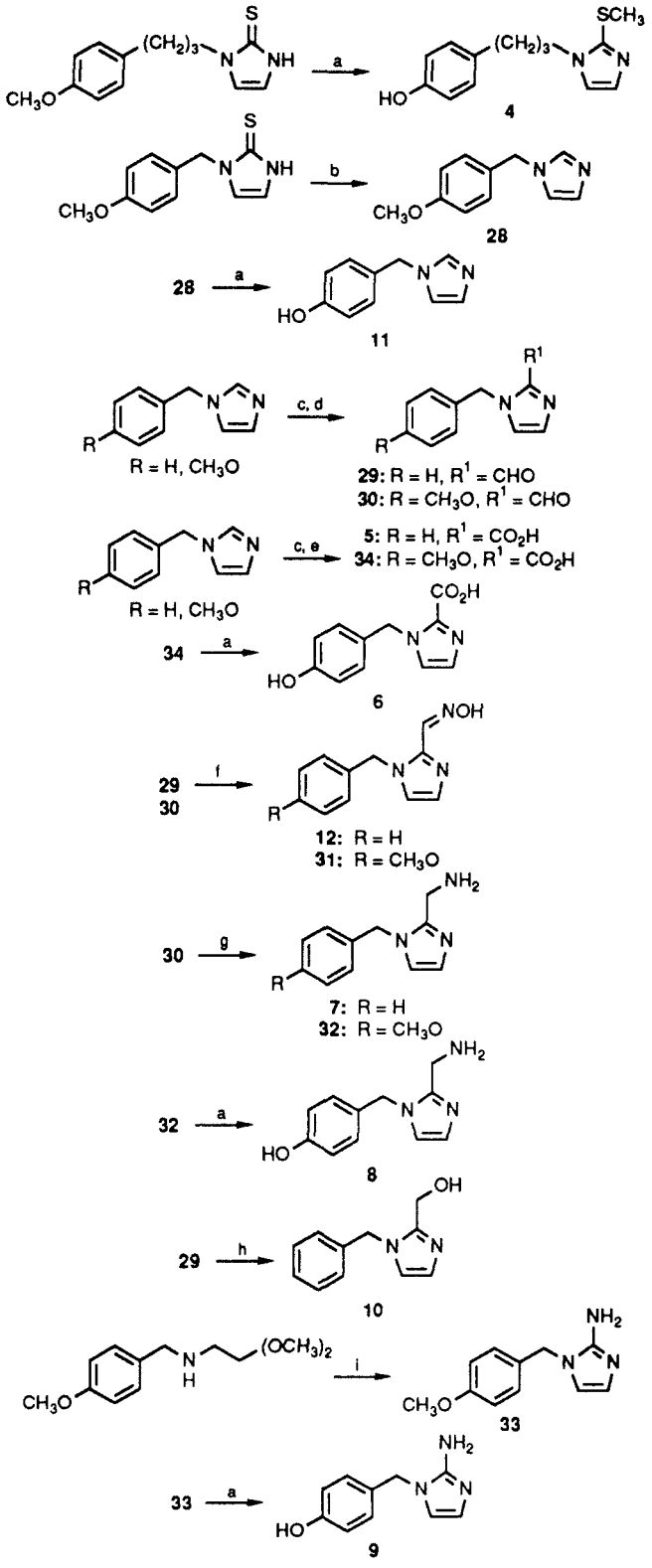
Scheme I. Synthesis of 2-Substituted 1-Aralkylimidazoles[a]



[a] Reagents and conditions: (a) BBr_3, CH_2Cl_2; (b) Raney Ni, EtOH; (c) *n*-BuLi, Et_2O; (d) DMF; (e) CO_2; (f) NH_2OH·HCl; (g) H_2/Raney Ni; (h) $NaBH_4$, MeOH; (i) NH_2CN, AcOH.

port the synthesis and in vitro DBH-inhibitory potency of some new multisubstrate inhibitors that resulted from a systematic exploration of ligand replacements.

Chemistry. Synthesis of compounds **4–12** (Table I) was accomplished from the corresponding 1-benzylimidazole-2-thiones by the sequences outlined in Scheme I. It was found that prolonged reaction of 1-[3-(4-methoxyphenyl)propyl]imidazole-2-thione with BBr_3 produced the *S*-methyl inhibitor **4** via the CH_3Br produced in situ. Desulfurization of the imidazole-2-thiones with Raney nickel afforded the parent imidazoles which, upon lithiation and reaction with the appropriate electrophile, yielded the inhibitors **5–12**.

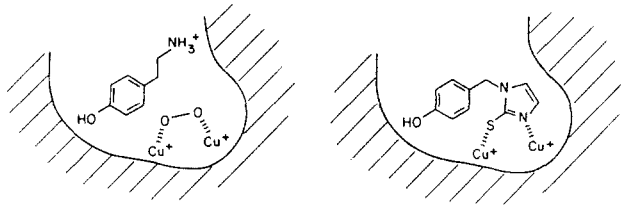
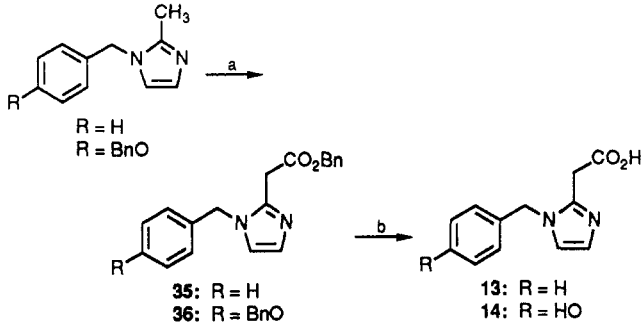


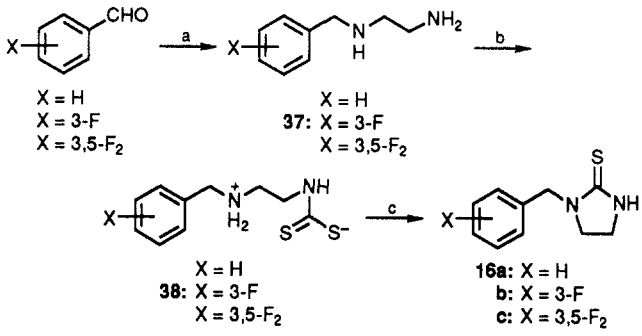
Figure 1.

Scheme II. Synthesis of 1-Benzylimidazole-2-acetic Acids[a]



[a] Reagents and conditions: (a) BnOCOCl, NEt_3, MeCN; (b) H_2, 10% Pd/C.

Scheme III. Synthesis of 1-Benzylimidazoline-2-thiones (**16a–c**)[a]



[a] Reagents and conditions: (a) $NH_2CH_2CH_2NH_2$, CH_3OH, $NaBH_4$; (b) CS_2, EtOAc; (c) Δ, *N*-methylpyrolidinone.

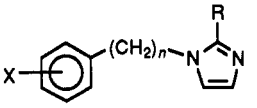
The imidazole-2-acetic acids **13** and **14** were prepared[7] by carboalkoxylation of the corresponding 1-benzyl-2-methylimidazole[8] followed by catalytic debenzylation (Scheme II).

The 1-benzylimidazoline-2-thiones **16a–c** were prepared by the procedure outlined in Scheme III. Condensation of a substituted benzaldehyde with ethylenediamine followed by sodium borohydride reduction yielded the intermediate *N*-benzylethylenediamines which upon reaction with CS_2 and pyrolysis provided **16a–c**.

The 1-benzyl-1,2,3,4-tetrazole-5-thiols (**20a,c**) and the 4-benzyl-1,2,4-triazole-3-thiones (**17a–d**) were prepared from the appropriate benzyl isothiocyanates as depicted in Scheme IV. The reaction of the intermediate isothiocyanate with sodium azide yielded the tetrazoles **20a,c** whereas reaction with *N*-formylhydrazine followed by cy-

(7) Macco, A. A.; Godefroi, E. F.; Drouen, J. J. M. *J. Org. Chem.* **1975**, *40*, 252.
(8) Guida, W. C.; Mathre, D. J. *J. Org. Chem.* **1980**, *45*, 3172.

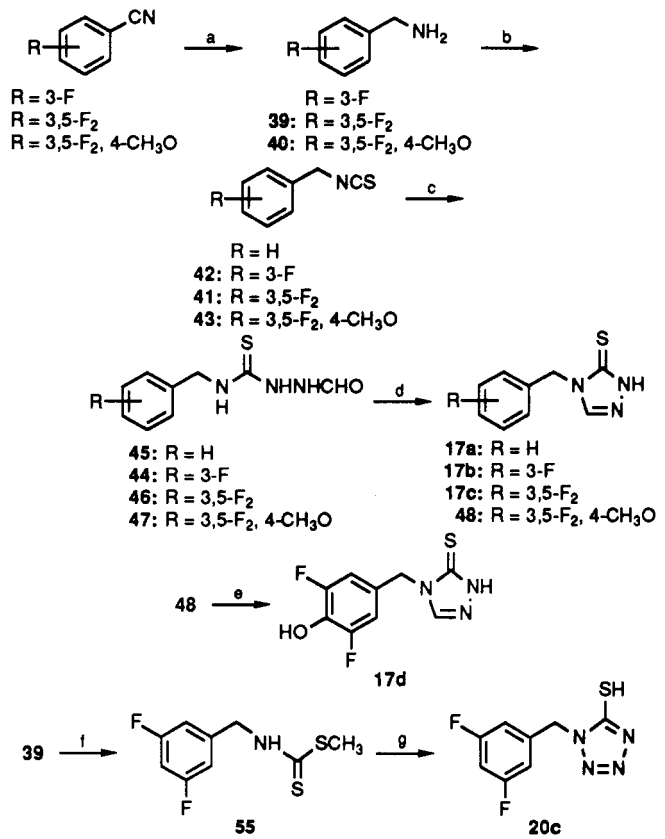
Table I. Structure, Physical Properties, and DBH-Inhibitory Activity of Some 2-Substituted 1-Aralkylimidazoles



compd	X	*n*	R	mp, °C	recryst solvent	yield,[a] %	formula[b]	IC_{50},[c] μM
1	H	1	SH					32 (20–46)[d]
2	4-OH	1	SH					2.6 (1.3–4.6)[d]
3	4-OH	3	SH					2.2 (1.8–2.8)[d]
4	4-OH	3	SCH_3	140–142	EtOH	8	$C_{13}H_{16}N_2OS$·HCl	79 (36–117)
5	H	1	CO_2H	128	MeOH–Et_2O	60	$C_{11}H_{10}N_2O_2$·HCl	57 (46–71)
6	4-OH	1	CO_2H	135 dec	MeOH–Et_2O	20	$C_{11}H_{10}N_2O_3$·HCl	112 (54–220)
7	H	1	CH_2NH_2	185–187	EtOH	29	$C_{11}H_{13}N_3$·2HCl	240 (120–390)
8	4-OH	1	CH_2NH_2	252–254	MeOH	10	$C_{11}H_{13}N_3O$·2HCl	165 (83–260)
9	4-OH	1	NH_2	203–210	MeOH–Et_2O	16	$C_{10}H_{11}N_3O$·HCl	4%[e]
10	H	1	CH_2OH	158	EtOH–Et_2O	70	$C_{11}H_{12}N_2O$	0%[e]
11	4-OH	1	H	212–213	EtOH	35	$C_{10}H_{10}N_2O$	17%[e]
12	H	1	CH=NOH	170–172	EtOH	58	$C_{11}H_{11}N_3O$	10%[e]
13	H	1	CH_2CO_2H	115–116	EtOH	6	$C_{12}H_{12}N_2O_2$	0%[e]
14	4-OH	1	CH_2CO_2H	172	H_2O	4	$C_{12}H_{12}N_2O_3$	0%[e]

[a] The overall yield is given. [b] All new compounds had C, H, N microanalyses within ±0.4% of the calculated values. [c] Values are given as IC_{50} in μM with upper and lower 95% confidence limits (mean ±SEM) shown in parentheses. [d] Data from ref 4. [e] Activity expressed as percent inhibition at a compound concentration of 10^{-4} M.

Scheme IV. Synthesis of 4-Benzyl-1,2,4-triazole-3-thiones (**17a–d**) and 1-(3,5-Difluorobenzyl)-1,2,3,4-tetrazole-5-thiol (**20c**)[a]

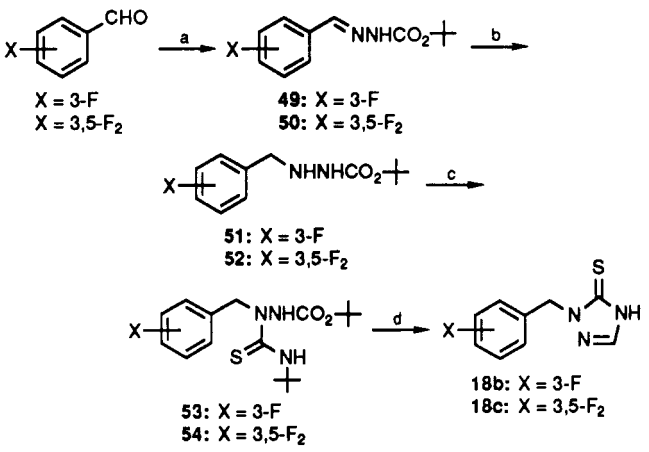


[a] Reagents and conditions: (a) H_2, Raney Ni, NH_3; (b) CS_2, DCC, Et_2O; (c) NH_2NHCHO, EtOH; (d) NaOEt, EtOH; (e) BBr_3, CH_2Cl_2; (f) KOH, CS_2, CH_3I; (g) NaN_3, H_2O.

clization under basic conditions provided the corresponding triazoles **17a–d**.

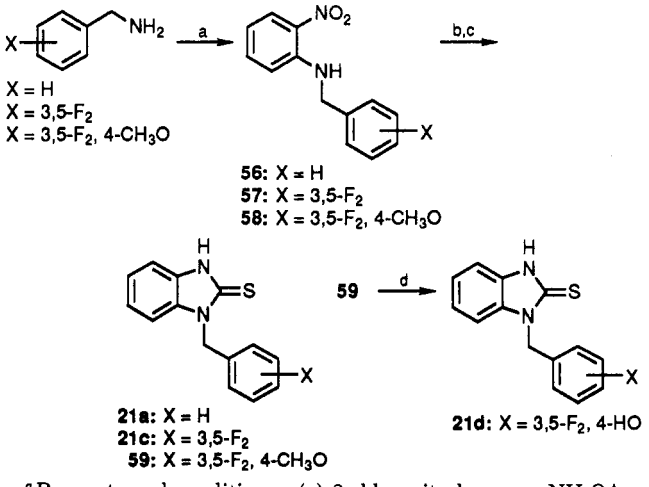
The 1-benzyl-1,2,4-triazole-5-thiones **18b,c** were prepared by the condensation of *tert*-butyl carbazate with the appropriate benzaldehyde followed by hydrogenation as shown in Scheme V. The resulting *tert*-butyl *N*-benzylcarbazates were reacted with *tert*-butyl isothiocyanate to give the protected thiosemicarbazates which upon heating with formic acid gave low yields of the triazoles **18b,c** (Scheme V).

Scheme V. Synthesis of 1-Benzyl-1,2,4-triazole-5-thiones (**18b,c**)[a]



[a] Reagents and conditions: (a) *t*-BuOCONHNH$_2$, *n*-hexane; (b) H_2, 10% Pd/C, MeOH; (c) *t*-BuNCS, EtOAc; (d) HCO_2H, Δ.

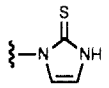
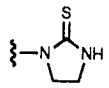
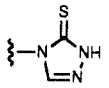
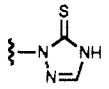
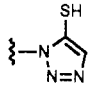
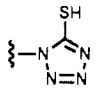
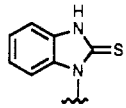
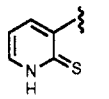
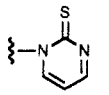
Scheme VI. Synthesis of 1-Benzylbenzimidazole-2-thiones (**21a,c,d**)[a]



[a] Reagents and conditions: (a) 2-chloronitrobenzene, NH_4OAc, Δ; (b) H_2, PtO_2, EtOH; (c) CS_2, KOH, H_2O; (d) BBr_3, CH_2Cl_2.

Synthesis of compound **19a** was accomplished via the known procedure.[9] The 1-benzylbenzimidazole-2-thiones

Table II. Structure, Physical Properties, and DBH-Inhibitory Activity of Some Aralkyl-Substituted Heterocycles

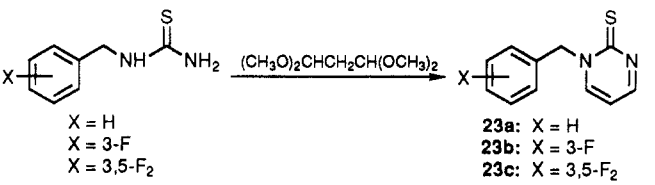
compd	ligand	side chain[a]	mp, °C	recryst solvent	yield,[b] %	formula[c]	IC_{50},[d] μM
1		A					32 (20–46)[e]
15b		B					5.6 (4.9–6.5)[e]
15c		C					1.2 (1.0–1.9)[e]
15d		D					0.074 (0.06–0.087)[e]
16a		A	177–182	MeOH–EtOAc	18	$C_{10}H_{12}N_2S$	5%[f]
16b		B	187–188	MeOH–$CHCl_3$	38	$C_{10}H_{11}FN_2S$	3.7 (2.4–5.2)
16c		C	186–187	MeOH–$CHCl_3$	32	$C_{10}H_{10}F_2N_2S$	1.4 (1.3–1.6)
17a		A	122–123	EtOH	21	$C_9H_9N_3S$	24 (19–29)
17b		B	125–126	EtOH–H_2O	4.6	$C_9H_8FN_3S$	1.9 (1.7–3.2)
17c		C	129–130	EtOH–H_2O	8.9	$C_9H_7F_2N_3S$	1.0 (0.93–1.1)
17d		D	184–185	EtOH–hexane	7.2	$C_9H_7F_2N_3OS$	0.035 (0.023–0.061)
18a		A			*g*		75 (70–80)
18b		B	155–156	Et_2O–hexane	6	$C_9H_8FN_3S$	15 (12.8–17.4)
18c		C	188–189	Et_2O–hexane	8	$C_9H_7F_2N_3S$	6.2 (5.0–7.8)
19a		A	85–87	Et_2O	21	$C_9H_9N_3S$	0%[f]
20a		A			*h*		16 (14–19)
20c		C	155–156	hexane–EtOAc	18	$C_8H_6F_2N_4S$	1.54 (1.2–2.0)
21a		A	185	EtOH	61	$C_{14}H_{12}N_2S$	61 (48–84)
21c		C	179–180	EtOH	42	$C_{14}H_{10}F_2N_2S$	6.2 (5.6–6.8)
21d		D	200	EtOAc–hexane	15	$C_{14}H_{10}F_2N_2OS$	0.66 (0.52–0.83)
22a		A	129–130	Et_2O–CH_2Cl_2	4.2	$C_{12}H_{11}NS$	26%[f]
22b		B	159–160	CH_2Cl_2–Et_2O–hexane	1.3	$C_{12}H_{10}FNS$	34%[f]
22c		C	195–197	CH_2Cl_2	3.9	$C_{12}H_9F_2NS$	13.4 (11.1–16.4)
23a		A	139.5–140.5	EtOH	32	$C_{11}H_{10}N_2S$	5%[f]
23b		B	182–183.5	MeOH	13	$C_{11}H_9FN_2S$·HCl	15%[f]
23c		C	183–184	CH_2Cl_2–Et_2O	10	$C_{11}H_8F_2N_2S$·HCl	2.8 (2.3–3.4)

[a] A = benzyl, B = 3-fluorobenzyl, C = 3,5-difluorobenzyl, D = 3,5-difluoro-4-hydroxybenzyl. [b] The overall yield is given. [c] All new compounds had C, H, N microanalyses within ±0.4% of the calculated values. [d] Values given are IC_{50} in μM with upper and lower 95% confidence limits (mean ± SEM) shown in parentheses. [e] Data from ref 5. [f] Activity expressed as percent inhibition at a compound concentration of 10^{-5} M. [g] Prepared according to ref 16. [h] Prepared according to ref 17.

were prepared by the route outlined in Scheme VI. Alkylation of the appropriate benzylamine with 2-chloronitrobenzene, followed by hydrogenation and cyclization with CS_2, yielded the target compounds **21a,c,d**.

Preparation of the 3-benzylpyridine-2-thiones **22a–c** was accomplished as shown in Scheme VII. The requisite 3-benzylpyridines were prepared from 3-cyanopyridine, and these were converted to the corresponding 2-pyridone by oxidation and rearrangement of the pyridine *N*-oxide. The desired regioisomer was isolated and reacted with Lawesson's reagent to yield the corresponding pyridine-2-thiones.

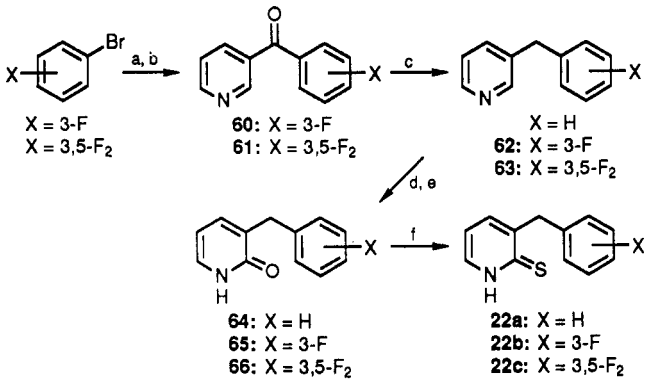
The final class of inhibitors, the 1-benzyl-1,2-dihydropyrimidine-2-thiones **23a–c**, was prepared by cyclizing the appropriate *N*-benzylthiourea with propane-1,3-dial bis(dimethyl acetal).



Enzymology. The compounds in Tables I and II were screened for DBH-inhibitory activity with use of commercially available bovine enzyme. Enzymatic assays and data analyses were performed by the methods previously

(9) Wiley, R. H.; Hussung, K. F.; Moffatt, J. *J. Org. Chem.* **1956**, *21*, 190.

Scheme VII. Synthesis of 3-Benzylpyridine-2-thiones **22a–c**[a]



[a] Reagents and conditions: (a) *t*-BuLi; (b) 3-cyanopyridine; (c) Zn/Hg, HCl, Δ; (d) *m*-CPBA, $CHCl_3$; (e) Ac_2O, Δ, base; (f) [4-$CH_3OC_6H_4P(S)S]_2$, PhMe, Δ.

described[4,5] to yield the indicated IC_{50} values and confidence limits.

Results and Discussion

One objective of the present study was to explore the dependence of DBH-inhibitory activity upon the thione group of the multisubstrate inhibitor **2**. The design of **2** was based upon the hypothesis that the ligand portion of inhibitor binds the "soft" Cu^{1+} oxidation state of enzyme in a fashion that mimics the binding of oxygen substrate during normal catalysis.[4] Detailed kinetic[3] and X-ray fluorescence studies[6] have subsequently supported this hypothesis. In light of these results, the compounds in Table I were prepared to evaluate the potential of nitrogen or oxygen ligands to substitute for the sulfur-containing thione. In the most interesting cases, the relative activities of 1-benzyl- and 1-(4-hydroxybenzyl)-substituted inhibitors were compared since in the case of the imidazole-2-thione inhibitors, an increase in potency results from the 4-hydroxyl group (cf. **1** vs **2**). This has been demonstrated to result from a greater resemblance of inhibitor to phenethylamine substrate. The results in Table I indicate the soft sulfur atom is required for optimal activity. Methylation of the sulfur as in **4** significantly decreases activity relative to **2** or **3**, inhibitors of identical potency.[4] Replacement of the sulfur by an oxygen ligand as in **5**, **6**, **10**, **12**, **13** and **14** greatly diminishes activity. The alcohol **10**, the aldoxime **12**, and the imidazole-2-acetic acids **13** and **14** are much less active, whereas the carboxylic acids **5** and **6** retain significant activity. Interestingly, the 4-hydroxyl group slightly *decreases* the potency of **6** relative to **5** to suggest inhibition of enzyme by these inhibitors occurs in a fashion different from the parent inhibitor **2**, perhaps via chelation of solution copper ions, as is known to be the case with fusaric acid.[10] The aminomethyl compounds **7** and **8** are significantly less potent than **1** and **2**, but in this case the presence of the 4-hydroxyl group does marginally improve inhibitory potency. The amine **9** is of greatly reduced potency, as is the simple imidazole **11**. The various heteroatoms that were explored as potential replacements for the sulfur ligand in inhibitor **2** appear to be inferior to the parent inhibitor. Perhaps this is not surprising in view of the direct binding of the ligand portion of **2** to the very "soft" Cu^{1+} from of DBH.[3,6]

Having established the necessity of the soft thione functional group, it was of interest to explore structural variations of this ligand. The collection of inhibitors found in Table II represents an attempt to vary the following structural parameters: (1) ligand pK_a, (2) ring size, (3) bulk, and (4) an acidic proton at N-3. The previously determined, optimal[5] fluorinated benzyl substituents were used to calibrate activity trends for the ligand substitutions reported in Table II.

The inhibitor thiol/thione pK_a was varied by increasing the number of nitrogens present in the heterocyclic ring. It was anticipated that the family of azoles (**2**, **17a–d**, **18a–c**, **19a**, **20a,c**) resulting from this study would be nearly isosteric. With the exception of **19a**, which was of greatly diminished activity, the other inhibitors were essentially identical in potency, a striking observation in light of the range of pK_a (ca. 10 units) for these ligands. The apparent independence of inhibitor potency upon ligand pK_a is of interest with respect to the binding mode hypothesized in Figure 1. If, as illustrated, the binding *does* involve the electron pair on N-3, then the deprotonated, anionic form of inhibitor must be that which binds enzyme. There appear to be two explanations for the lack of correlation between inhibitor pK_a and potency. Firstly, the hypothesized interaction between the lone-pair electrons on N-3 and the second copper atom or another enzymatic group might not occur, making deprotonation at N-3 unnecessary. Alternatively, an initial ligation of the thione sulfur to a copper atom could labilize the N-3 hydrogen toward removal, thereby rendering irrelevant the pK_a of these inhibitors in solution.[11] The available data do not allow a distinction between these possibilities at present. Compound **19a**, which lacks the lone-pair electrons at position 4 (corresponding to position 3 in **1** and **2**) and which should therefore be a critical test compound, undergoes a facile air-oxidation of the thiol to a disulfide under the DBH assay conditions. The "dihydro" analogues **16a–c**, which also have a labile N-3 hydrogen, were found to be comparable in potency to the parent imidazole-2-thiones.

The six-membered ligand groups in **22a–c** and **23a–c** were prepared in an attempt to explore the effects of ligand ring size upon DBH inhibitory potency. However, the ligands present in **22a–c** and **23a–c** do not afford an ideal comparison since several structural changes are present relative to **2** in addition to the desired ring expansion. Thus, **22a–c** lack the N-1 heteroatom while **23a–c** lack the labile N-3 hydrogen present in **2**. Other, more analogous six-membered ring counterparts to **2** were not immediately obvious. Whereas **22a–c** have reduced inhibitory potency relative to **2**, **23a–c** are of comparable potency although the effects that cause this are unclear due to the multiple structural changes present in these inhibitors. It is worth noting, however, that both classes of inhibitors have a lone pair of electrons at the position corresponding to N-3 in **2**: compounds **22a–c** by deprotonation and compounds **23a–c** in the neutral molecule.

The extremely large inhibitors **21a–c** appear to define a location of considerable bulk tolerance at the DBH active site. Whereas earlier studies demonstrated a lack of bulk

(10) Brenner, M.; Krueger, M.; Klinman, J. P. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1983**, *42*, 2059.

(11) An alternative explanation would have enzyme binding that fraction of inhibitor which is present in solution in the deprotonated, anionic form. The fraction of inhibitor in this form at pH 5.5 will vary from >90% for **20a,c** (pK_a ca. 3.3) to $1/10^7$ for **2** (pK_a ca. 12.5). Since the K_i for **2** has been determined at pH 5.5 to be 6×10^{-8} M, if the true inhibitory species were the small fraction of **2** present as the anion under these conditions, then this must bind enzyme with an implausibly low K_i of ca. 10^{-15} M. An inhibitor of this potency would exhibit essentially irreversible inhibition or at minimum, tight binding behavior. Neither has been observed (see ref 3).

tolerance at the phenethylamine binding site and the intersite bridging methylene chain, the enzyme appears to tolerate considerable steric bulk at the "backside" of the oxygen mimic. This may become particularly relevant to the design of DBH inhibitors bearing additional or more complex pharmacophores.

In summary, this study has afforded several insights to the structural requirements for the inhibitor group that binds the DBH copper atom. Firstly, the presence of a "soft" ligand atom, such as sulfur, confers maximal inhibitory potency. Other atoms or functional groups have proved decidedly inferior. Secondly, the ligand pK_a is of little importance as convincingly shown by the data for nitrogen-containing isosteres of **2**. Lastly, the enzymatic site near the copper atom shows considerable bulk tolerance, such that even very large "oxygen mimics" bind tightly.

Experimental Section

Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. When appropriate, all solvents used in reaction mixtures were dried and/or purified by standard procedures.[12] IR spectra were recorded on a Perkin-Elmer 727 spectrophotometer as neat oils or Nujol mulls calibrated with the 1601 cm^{-1} absorption of polystyrene film. NMR spectra were obtained as $CDCl_3$ solutions on a Hitachi Perkin-Elmer R-24 spectrometer and/or a Varian EM 390 spectrometer using Me_4Si as an internal reference; IR and NMR spectra were obtained for all new compounds and were judged to be consistent with the assigned structure.

1-[3-(4-Hydroxyphenyl)propyl]-2-(methylthio)imidazole Hydrochloride (4). A solution of 1-[3-(4-methoxyphenyl)-propyl]imidazole-2-thione[4] (1.2 g, 0.0046 mol) in CH_2Cl_2 (40 mL) was stirred at 22 °C as a solution of BBr_3 (3.5 g, 0.014 mol) in CH_2Cl_2 (10 mL) was added. After 4 h, excess BBr_3 was destroyed by the careful addition of MeOH, and the reaction mixture was stirred for an additional 16 h. The solution was concentrated and the residue was dissolved in H_2O and washed with EtOAc. The aqueous phase was basified with $NaHCO_3$ and extracted with EtOAc. The extracts were washed with H_2O and dried, and the solvent was evaporated. The residue was dissolved in 5 mL of EtOH, and the solution was treated with ethereal HCl. The crystalline product was filtered and recrystallized from EtOH to give 0.61 g (45%) of **4** (Table I).

1-(4-Methoxybenzyl)imidazole (28). A mixture of 1-(4-methoxybenzyl)imidazole-2-thione[4] (15.0 g, 0.068 mol) and Raney Ni (80 g) in EtOH (400 mL) was heated at reflux for 4 h. The mixture was cooled and filtered, and the filtrate was concentrated. The residue was dissolved in 1 N HCl (100 mL) and washed with Et_2O. The aqueous phase was basified and extracted with Et_2O. The extracts were combined, dried (Na_2SO_4), and concentrated to yield **28** as an oil: 8.7 g (63%).

1-Benzylimidazole-2-carboxaldehyde (29). A solution of 1-benzylimidazole (7.9 g, 0.05 mol) in Et_2O (150 mL) was stirred at −45 °C while a solution of *n*-BuLi in hexanes (44.5 mL, 1.6 M, 0.071 mol) was added. After stirring for 30 min, the yellow solution was cooled to −75 °C and DMF (4.38 g, 0.06 mol) was added. The cooling bath was replaced with an ice–H_2O bath, and after 10 min the reaction mixture was poured into saturated NH_4Cl. The mixture was extracted with Et_2O, the extracts were washed with H_2O and dried (Na_2SO_4), and the solvent was concentrated to give **29** as an oil: 7.2 g (77%).

1-(4-Methoxybenzyl)imidazole-2-carboxaldehyde (30). The reaction of **28** as in the preparation of **29** yielded **30** (68%) as an oil.

1-Benzylimidazole-2-carboxaldehyde Oxime (12). A solution of **29** (1.80 g, 0.0097 mol) in H_2O (50 mL) was stirred vigorously during the addition of NH_2OH·HCl (2.0 g, 0.029 mol) and NaOAc (5.0 g). After 10 min the solution became cloudy, and an oil separated and then solidified. The solid was filtered, washed with H_2O, and recrystallized from EtOH to give 1.72 g (75%) of **12** (Table I).

(12) Perrin, D. D.; Armarego, W. F. L. *Purification of Laboratory Chemicals*; Pergamon: Oxford, 1966.

1-(4-Methoxybenzyl)imidazole-2-carboxaldehyde Oxime (31). The reaction of **30** as in the preparation of **12** yielded (68%) **31**: mp 201–204 °C.

2-(Aminomethyl)-1-benzylimidazole Dihydrochloride (7). Oxime **12** (1.25 g, 0.0053 mol) was dissolved in warm EtOH (125 mL), Raney Ni (2 mL of an aqueous slurry) was added, and the mixture was hydrogenated at 50 psi H_2 for 4.5 h. The mixture was filtered, the filtrate was treated with an ethereal HCl solution, and the solvents were concentrated until crystallization commenced. The crystalline precipitate was filtered and recrystallized from EtOH to give 0.76 g (49%) of **7** (Table I).

2-(Aminomethyl)-1-(4-methoxybenzyl)imidazole Dihydrochloride (32). The reduction of **31** as in the preparation of **7** yielded (22%) **32**: mp 147–157 °C.

1-Benzyl-2-(hydroxymethyl)imidazole (10). A solution of **29** (1.86 g, 0.01 mol) in MeOH (20 mL) was stirred at 0 °C while $NaBH_4$ (1.0 g) was added. After stirring at 0 °C for 2.5 h the solution was concentrated. The residue was taken up in Et_2O, washed with H_2O, dried (Na_2SO_4), and concentrated. The residue was dissolved in EtOH and treated with ethereal HCl. Crystallization was induced by the addition of Et_2O and cooling to give 1.7 g (91%) of 1-benzyl-2-(hydroxymethyl)imidazole hydrochloride (Table I).

2-Amino-1-(4-methoxybenzyl)imidazole Hydrochloride (33). A mixture of *N*-(4-methoxybenzyl)aminoacetaldehyde dimethyl acetal[4] (4.5 g, 0.02 mol) and cyanamide (3.36 g, 0.08 mol) in glacial AcOH (1.13 mL, 0.02 mol) and H_2O (4 mL) was heated at 40 °C for 16 h and then at 60 °C for 2 h. The solution was concentrated and the residue was triturated with a mixture of Me_2CO and Et_2O and filtered. The solid was dissolved in concentrated HCl (5 mL), heated to 50 °C for 20 min, and then concentrated. The solid was recrystallized from a mixture of MeOH and Et_2O to give 1.6 g (33%) of **33**: mp 216–218 °C.

1-Benzylimidazole-2-carboxylic Acid Hydrochloride (5). A solution of 1-benzylimidazole (3.16 g, 0.02 mol) in Et_2O (100 mL) was cooled to −50 °C and treated with a solution of *n*-BuLi in *n*-hexane (12.5 mL, 1.6 M, 0.02 mol). After stirring at −50 °C for 30 min, a stream of dry CO_2 was passed into the reaction. After 10 min, the color of the reaction mixture had changed from yellow to clear and a white precipitate formed. The mixture was warmed to ambient temperature and filtered and the precipitate was washed with Et_2O. This white powder was dissolved in H_2O (10 mL), the pH was adjusted to 3, and the mixture was cooled to induce crystallization. The resulting 1-benzylimidazole-2-carboxylic acid was filtered and dried. The hydrochloride salt was formed by treating a methanolic solution of the imidazole with ethereal HCl and inducing crystallization by the addition of Et_2O (Table I).

1-(4-Methoxybenzyl)imidazole-2-carboxylic Acid Hydrochloride (34). The reaction of **28** as in the preparation of **5** yielded (78%) **34**: mp 115 °C dec.

General Method A. Cleavage of *O*-Methyl Ethers. A solution of the appropriate *O*-methyl ether (0.05 mol) in CH_2Cl_2 (150 mL) was cooled to −20 °C and a solution of BBr_3 (0.10 mol) in CH_2Cl_2 (50 mL) was added. The cooling bath was replaced with an ice–water bath, and the reaction was followed by TLC until completion (generally 2–4 h). When completed, the reaction was cooled to −20 °C, and MeOH was *cautiously* added to decompose excess BBr_3. The solvents were evaporated under reduced pressure, and the residue was recrystallized from the appropriate solvent.

1-(4-Hydroxybenzyl)imidazole-2-carboxylic Acid Hydrochloride (6). The demethylation of **34** by general procedure A yielded (41%) **6** (Table I).

2-(Aminomethyl)-1-(4-hydroxybenzyl)imidazole Dihydrochloride (8). The demethylation of **32** by general procedure A yielded (27%) **8** (Table I).

2-Amino-1-(4-hydroxybenzyl)imidazole Hydrochloride (9). The demethylation of **33** by general procedure A yielded (47%) **9** (Table I).

1-(4-Hydroxybenzyl)imidazole (11). The demethylation of **28** by general procedure A yielded (56%) **11**.

Benzyl 1-Benzylimidazole-2-acetate (35).[7] A solution of 1-benzyl-2-methylimidazole[8] (2.8 g, 16.3 mmol) and triethylamine (23.0 mL, 0.163 mol) in MeCN (15 mL) was stirred at 0 °C during the dropwise addition of benzyl chloroformate (23 mL, 0.163 mol)

and then heated at reflux for 18 h. The resulting mixture was partitioned between Et_2O (50 mL) and H_2O (150 mL) and the aqueous phase was washed with Et_2O. The combined Et_2O washes were dried (Na_2SO_4) and concentrated, and the residue was purified by silica gel chromatography using CH_2Cl_2 and then 1% MeOH–CH_2Cl_2 as eluent to yield 1.2 g (24%) of **35** as an oil, which solidified.

Benzyl 1-[4-(benzyloxy)benzyl]imidazole-2-acetate (36)[7] was prepared in 39% yield as an oil, which solidified from 1-[4-(benzyloxy)benzyl]-2-methylimidazole[8] by the method used to prepare **35**.

1-Benzylimidazole-2-acetic Acid (13). A solution of **35** (1.1 g, 3.6 mmol) in EtOH (50 mL) was hydrogenated (50 psi) over 10% palladium on carbon (0.2 g) for 2 h and then filtered and concentrated. The residue was recrystallized from EtOH to yield 0.18 g (23%) of **13** (Table I).

1-(4-Hydroxybenzyl)imidazole-2-acetic acid (14) was prepared in 10% yield by hydrogenation of **36** by the procedure used to prepare **13** (Table I).

***N*-(3-Fluorobenzyl)ethylenediamine (37).** A solution of ethylenediamine (45.3 g, 0.754 mol) in MeOH (500 mL) was stirred at 0 °C during the dropwise addition of 3-fluorobenzaldehyde (23.4 g, 0.189 mol). After the addition was completed, the solution was stirred and maintained at 0 °C while $NaBH_4$ (7.13 g, 0.189 mol) was added *cautiously* in small portions. The reaction mixture was allowed to warm to ambient temperature and was stirred overnight. The resulting cloudy solution was filtered, the filtrate was concentrated and the residual oil was partitioned between EtOAc and H_2O. The EtOAc layer was dried (Na_2SO_4) and concentrated to give a viscous oil, which was distilled to yield **37** (21.6 g, 68%): bp 89–91 °C (0.7 mmHg).

***N*-(3-Fluorobenzyl)ethylenediaminecarbodithioic Acid (38).** A solution of **37** (5.0 g, 0.03 mol) in EtOAc (50 mL) was cooled to 0 °C and stirred during the dropwise addition of a solution of CS_2 (2.26 g, 0.03 mol) in EtOAc (10 mL). The reaction mixture was diluted with MeOH and filtered and the filtrate was concentrated to yield 6.28 g (87%) of **38** as an amorphous white solid. Trituration with hot MeOH yielded a white solid: mp 178–182 °C.

1-(3-Fluorobenzyl)imidazoline-2-thione (16b). A 5.73 g (0.024 mol) quantity of **38** was heated as a neat melt to 165 °C in an oil bath for several minutes. *N*-Methyl-2-pyrrolidinone (5 mL) was added followed by heating to 185 °C for 15 min. The resulting semisolid mass was cooled, triturated with EtOAc, and filtered to yield 2.3 g of solid. The filtrate was concentrated and the residue was triturated with H_2O and filtered to yield an additional 2.1 g of product. The combined crops were recrystallized from MeOH–$CHCl_3$ to yield 3.15 g (64%) of **16b** (Table II).

1-Benzylimidazoline-2-thione (16a) was prepared (18% yield) from benzaldehyde by the procedure described for the preparation of **16b**.

1-(3,5-Difluorobenzyl)imidazoline-2-thione (16c) was prepared (32% yield) from 3,5-difluorobenzaldehyde[5] by the procedure described for the preparation of **16b**.

3,5-Difluorobenzylamine (39). A solution of 3,5-difluorobenzonitrile[5] (13.9 g, 0.1 mol) in ammonia-saturated MeOH (140 mL) was hydrogenated (50 psi) over Raney Ni for 1.5 h. The resulting mixture was filtered and concentrated, and the residue was dissolved in EtOAc. The solution was extracted twice with 1 N HCl, and the aqueous extracts were washed with EtOAc. The aqueous solution was made basic with 2.5 N NaOH and extracted three times with EtOAc. The extracts were washed with H_2O and brine and then dried (Na_2SO_4) and concentrated to yield **39** as a colorless oil: 11.7 g (82%).

3,5-Difluoro-4-methoxybenzylamine (40). The hydrogenation of 3,5-difluoro-4-methoxybenzonitrile[3] as in the preparation of **39** yielded **40** (98%) as a colorless oil.

3,5-Difluorobenzyl Isothiocyanate (41). A solution of **39** (11.6 g, 0.081 mol) in Et_2O (20 mL) was added dropwise to a cooled (−5 to −10 °C) solution of dicyclohexylcarbodiimide (16.7 g, 0.081 mol) and CS_2 (32.4 mL, 0.539 mol) in Et_2O (100 mL), and the resulting mixture was stirred overnight at ambient temperature. The mixture was filtered, the filtrate was concentrated, and the residual oil was dissolved in 19:1 *n*-hexane–Et_2O and filtered. The filtrate was concentrated and the residue was purified by flash chromatography[13] using *n*-hexane as eluant to yield 5.40 g (36%) of **41** as a colorless oil.

3-Fluorobenzyl isothiocyanate (42) was prepared as a colorless oil in 51% yield from 3-fluorbenzylamine by the procedure used to prepare **41**.

3,5-Difluoro-4-methoxybenzyl isothiocyanate (43) was prepared as a colorless oil in 79% yield from 3,5-difluoro-4-methoxybenzylamine by the procedure used to prepare **41**.

4-(3-Fluorobenzyl)-1-formyl-3-thiosemicarbazide (44). A solution of **42** (4.27 g, 25.5 mmol) and formylhydrazine (1.69 g, 28.7 mmol) was heated at reflux in EtOH (30 mL) for 1 h. The resulting mixture was concentrated and the residue was triturated with 1:1 hexane–EtOAc and filtered. The crude product was recrystallized from EtOH–hexane to yield 1.29 g (22%) of **44**: mp 124–125 °C.

4-Benzyl-1-formyl-3-thiosemicarbazide (45) was prepared in 38% yield from benzyl isothiocyanate by the procedure used to prepare **44**. The crude product was recrystallized from EtOH: mp 162–165 °C.

4-(3,5-Difluorobenzyl)-1-formyl-3-thiosemicarbazide (46) was prepared in 35% yield from **41** by the procedure used to prepare **44**. A sample was purified by flash chromatography using 9:1 CH_2Cl_2–EtOAc as eluant followed by recrystallization from CH_2Cl_2–hexane: mp 129–130 °C.

4-(3,5-Difluoro-4-methoxybenzyl)-1-formyl-3-thiosemicarbazide (47) was prepared in 62% yield from **43** by the procedure used to prepare **44**. A sample was recrystallized from EtOH–hexane: mp 124–126 °C.

4-(3-Fluorobenzyl)-1,2,4-triazole-3-thione (17b). A solution of **44** (1.29 g, 5.68 mmol) in ethanolic sodium ethoxide [prepared from sodium (0.26 g, 11.0 g-atom) and EtOH (25 mL)] was heated at reflux for 24 h. The resulting mixture was filtered and concentrated and the residue was dissolved in H_2O. The aqueous solution was acidified to pH 3 with 10% HCl and the precipitate was filtered and recrystallized from EtOH–H_2O to yield 0.48 g (41%) of **17b** (Table II).

4-Benzyl-1,2,4-triazole-3-thione (17a). The reaction of **45** as in the preparation of **17b** yielded (56%) **17a** (Table II).

4-(3,5-Difluorobenzyl)-1,2,4-triazole-3-thione (17c). The reaction of **46** as in the preparation of **17b** yielded **17c** (71%) (Table II).

4-(3,5-Difluoro-4-methoxybenzyl)-1,2,4-triazole-3-thione (48). The reaction of **47** using the same procedure as described for preparation of **17b** yielded (65%) **48**: mp 124–126 °C.

4-(3,5-Difluoro-4-hydroxybenzyl)-1,2,4-triazole-3-thione (17d) was prepared in 23% yield from **48** by demethylation according to general procedure A.

3-Fluorobenzaldehyde (*tert*-Butoxycarbonyl)hydrazone (49). A solution of 3-fluorobenzaldehyde (6.21 g, 0.05 mol) and *tert*-butyl carbazate (6.61 g, 0.05 mol) in *n*-hexane (50 mL) was heated at reflux for 30 min. The mixture was cooled to 0 °C and filtered. The product was recrystallized from ethanol to yield (8.79 g, 74%) **49**: mp 165–168 °C.

3,5-Difluorobenzaldehyde (*tert*-butoxycarbonyl)hydrazone (50) was prepared in 58% yield from 3,5-difluorobenzaldehyde by the procedure used to prepare **49**: mp 190–191 °C.

1-(3-Fluorobenzyl)-2-(*tert*-butoxycarbonyl)hydrazine (51). A suspension of **49** (1.02 g, 4.28 mmol) in MeOH (40 mL) was hydrogenated (40 psi) over 10% palladium on carbon (0.22 g) for 1 h. The mixture was filtered and concentrated to give 1.0 g (97%) of **51** as an oil.

1-(3,5-Difluorobenzyl)-2-(*tert*-butoxycarbonyl)hydrazine (52). The hydrogenation (4.5 h) of **50** as in the preparation of **51** yielded (95%) **52** as a colorless oil.

1-(*tert*-Butoxycarbonyl)-2-(3-fluorobenzyl)-4-*tert*-butylthiosemicarbazide (53). A solution of **51** (2.29 g, 9.53 mmol) and *tert*-butyl isothiocyanate (2.41 mL, 19.1 mmol) in EtOAc (15 mL) was heated at reflux for 7 h. The mixture was concentrated and the residue was triturated to yield 1.28 g (38%) of **53** as an amorphous solid.

1-(*tert*-Butoxycarbonyl)-2-(3,5-difluorobenzyl)-4-*tert*-butylthiosemicarbazide (54). The reaction of **52** as in the

(13) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

preparation of **53** yielded (65%) **54** as an amorphous solid.

1-(3-Fluorobenzyl)-1,2,4-triazole-5-thione (18b). A solution of **53** (1.28 g, 3.6 mmol) in 98% HCO_2H (15 mL) was heated at reflux for 4 h and then concentrated. The residue was purified by flash chromatography using 4:1 CH_2Cl_2–EtOAc followed by trituration with Et_2O–hexane to yield 0.165 g (22%) of **18b** (Table II).

1-(3,5-Difluorobenzyl)-1,2,4-triazole-5-thione (18c). The reaction of **54** using the identical conditions as described for the preparation of **18b** yielded (22%) **18c** (Table II).

1-Benzyl-1,2,3-triazole-5-thione (19a). A solution of 1-benzyl-1,2,3-triazole[9] (3.2 g, 0.02 mol) in THF (40 mL) was cooled to −80 °C and stirred under nitrogen during the dropwise addition of a 2.6 M *n*-butyllithium solution in *n*-hexane (8.8 mL, 0.023 mol). After stirring for 15 min sublimed sulfur (0.64 g, 0.02 mol) was added in one portion. The reaction mixture was stirred for 45 min at −20 °C and then poured into Et_2O and filtered. The hygroscopic solid product was dissolved in H_2O, and the resulting solution was washed twice with EtOAc and acidified with 3 N HCl. The aqueous solution was extracted with EtOAc, and the EtOAc extracts were dried (Na_2SO_4) and concentrated to give a solid product which was recrystallized from Et_2O to yield 0.8 g (21%) of **19a** (Table II).

Methyl *N*-(3,5-Difluorobenzyl)dithiocarbamate (55). A solution of **39** (4.2 g, 29.7 mmol) and KOH (1.66 g, 29.7 mmol) in a mixture of H_2O (20 mL) and EtOH (18 mL) was stirred during the additon of CS_2 (8.9 mL, 0.148 mol) and then heated at reflux for 1 h. Iodomethane (1.8 mL, 29.7 mmol) was added and the resulting mixture was stirred at ambient temperature for 48 h and then concentrated. The residue was dissolved in EtOAc and the solution was washed with H_2O, dried (Na_2SO_4), and concentrated. The residue was purified by flash chromatography using 6:1 hexane–EtOAc as eluant to yield 4.7 g (68%) of **55** as a yellow oil.

1-(3,5-Difluorobenzyl)-1,2,3,4-tetrazole-5-thiol (20c). A solution of **55** (4.2 g, 0.018 mol) and NaN_3 (1.8 g, 0.027 mol) in H_2O (35 mL) was heated at reflux overnight. The solution was cooled, extracted with EtOAc, acidified to pH 1 with 10% HCl, and extracted with EtOAc. The EtOAc extracts were dried (Na_2SO_4) and concentrated, and the residue was recrystallized from hexane–EtOAc to give 1.3 g (32%) of **20c** (Table II).

***N*-Benzyl-2-nitroaniline (56).** A mixture of 1-chloro-2-nitrobenzene (10.0 g, 0.063 mol), benzylamine (40 g, 0.37 mol), and ammonium acetate (4.6 g, 0.06 mol) was heated at 100 °C for 24 h. The reaction mixture was cooled and dissolved in EtOAc. The solution was washed three times with 3 N HCl and once with brine, then dried (Na_2SO_4), and concentrated to yield 14.0 g (97%) of **56**: mp 65–67 °C.

***N*-(3,5-Difluorobenzyl)-2-nitroaniline (57)** was prepared in 99% yield from 3,5-difluorobenzylamine by following the procedure used for **56**: mp 105–108 °C.

***N*-(3,5-Difluoro-4-methoxybenzyl)-2-nitroaniline (58)** was prepared in 100% yield from 3,5-difluoro-4-methoxybenzylamine by following the procedure used for preparation of **56**: oil which solidified.

1-Benzylbenzimidazole-2-thione (21a). Platinum oxide (0.38 g) was hydrogenated (50 psi) in EtOH (50 mL) for 30 min. Compound **56** (1.0 g, 4.4 mmol) was added and the solution was hydrogenated (50 psi) for 3 h. The solution was filtered directly into a 250-mL flask containing KOH (0.38 g) and CS_2 (1.1 mL, 18.3 mmol) in H_2O (10 mL). The resulting mixture was heated at reflux for 2 h and then diluted with H_2O and acidified with 3 N HCl. The product was filtered and recrystallized from EtOH to yield 0.65 g (63%) of **21a** (Table II).

1-(3,5-Difluorobenzyl)benzimidazole-2-thione (21c) was prepared from **57** in 42% yield by the procedure used in the preparation of **21a** (Table II).

1-(3,5-Difluoro-4-methoxybenzyl)benzimidazole-2-thione (59) was prepared from **58** in 32% yield by the procedure used in the preparation of **21a**. The crude product was purified by flash chromatography using CH_2Cl_2 as eluant to give a yellow oil which solidified.

1-(3,5-Difluoro-4-hydroxybenzyl)benzimidazole-2-thione (21d) was prepared from **59** in 47% yield by general procedure A (Table II).

3-(3-Fluorobenzoyl)pyridine (60). A solution of 3-bromofluorobenzene (25.0, 0.143 mol) in ether (250 mL) was cooled to −78 °C and stirred during the dropwise addition of a 2.3 M solution of *t*-BuLi in pentane (62 mL, 0.143 mol). After the addition was completed, stirring was continued for 15 min and a solution of 3-cyanopyridine (14.9 g, 0.143 mol) in ether (125 mL) was added dropwise. The resulting mixture was allowed to warm to 15 °C and a solution of concentrated HCl (36 mL) in H_2O (90 mL) was added dropwise. After the resulting light yellow solution had stirred for 30 min, 10% NaOH was added to give a pH of 10, and the Et_2O layer was separated. The aqueous phase was extracted twice with Et_2O, the combined Et_2O extracts were concentrated, and the residue was distilled to yield 21.3 g (74%) of **60**: bp 112–128 °C (0.45 mmHg).

3-(3,5-Difluorobenzoyl)pyridine (61). The reaction of 1-bromo-3,5-difluorobenzene as in the preparation of **60** yielded (42%) of the ketone **61**: bp 110–137 °C (0.4 mmHg).

3-(3-Fluorobenzyl)pyridine (62). Zn(Hg) amalgam was prepared by stirring zinc (41 g) and $HgCl_2$ (4.1 g) in a mixture of H_2O (25 mL) and concentrated HCl (60 mL) and then ketone **60** (19.0 g, 0.095 mol) was added. The resulting mixture was stirred and heated at reflux for 30 h. Occasionally during the course of the reaction HCl gas was passed through the mixture, and after 20 h, additional zinc metal (41 g) was added. After 30 h, the reaction mixture was cooled, decanted, made strongly basic with 10% NaOH, and extracted three times with CH_2Cl_2. The extracts were dried (Na_2SO_4) and concentrated, and the residue was distilled to yield 5.2 g (29%) of **62**: bp 100–110 °C (0.6 mmHg).

3-(3,5-Difluorobenzyl)pyridine (63). The reaction of ketone **61** as in the preparation of **62** yielded (61%) **63**: bp 90–110 °C (0.6 mmHg).

3-Benzyl-2-pyridone (64). A solution of 3-benzylpyridine (50 g, 0.3 mol) in $CHCl_3$ (500 mL) was stirred during the portionwise addition of 3-chloroperbenzoic acid (60 g, 0.325 mol). The reaction mixture was stirred for 2 h and then diluted with H_2O (200 mL) and made basic to pH 8 with 10% NaOH. The $CHCl_3$ layer was separated, extracted twice with 5% $NaHCO_3$, and concentrated to give 57.5 g of crude *N*-oxide which was used without purification. The crude *N*-oxide (19.5 g, 0.1 mol) was added *cautiously* to Ac_2O (200 mL) which had been heated to 85 °C. After the addition was completed, the solution was heated at reflux for 45 min, then cooled, and concentrated. The residual oil was dissolved in Et_2O and the resulting solution was stirred with EtOH and 40% NaOH until the precipitation of solid was completed. The product was filtered to yield 2.26 g (12% overall) of **64** as a white solid: mp 158.5–160 °C.

3-(3-Fluorobenzyl)-2-pyridone (65). The reaction of **62** as in the preparation of **64** yielded (18%) **65**: mp 110 °C.

3-(3,5-Difluorobenzyl)-2-pyridone (66). The reaction of **63** as in the preparation of **64** yielded (44%) **66**: amorphous solid; TLC R_f 0.3 with 10% MeOH–$CHCl_3$.

3-Benzylpyridine-2-thione (22a). A mixture of **64** (777 mg, 4.2 mmol) and Lawesson's reagent[14] in toluene (10 mL) was heated at reflux for 1 h, then cooled, and decanted. The residue was washed with CH_2Cl_2, and the combined organic layers were concentrated. The resulting solid was purified by flash chromatography using 5% MeOH–CH_2Cl_2 as eluant followed by recrystallization from CH_2Cl_2–Et_2O–hexane to yield 320 mg (35%) of **22a** (Table II).

3-(3-Fluorobenzyl)pyridine-2-thione (22b). The reaction of **65** as in the preparation of **22a** yielded (35%) **22b** (Table II).

3-(3,5-Difluorobenzyl)pyridine-2-thione (22c). The reaction of **66** as in the preparation of **22a** yielded (35%) **22c** (Table II).

1-Benzyl-1,2-dihydropyrimidine-2-thione (23a). A solution of *N*-benzylthiourea[15] (8.0 g, 0.0482 mol) and malondialdehyde tetramethyl acetal (7.91 g, 0.048 mol) in H_2O (65 mL), concentrated HCl (16 mL), and EtOH (65 mL) was heated at reflux for 25 min. The solution was cooled, washed with Et_2O, and then made basic with 10% NaOH. The resulting solution was extracted

(14) Shabana, R.; Scheibye, S.; Clausen, K.; Olesen, S. O.; Lawesson, S. O. *Nouv. J. Chim.* **1980**, *4*, 47.
(15) Prepared by the method of: Neville, R. G.; McGee, J. J. *Can. J. Chem.* **1963**, *41*, 2123.
(16) Blackman, A. J.; Polya, J. B. *J. Chem. Soc. C* **1971**, 1016.
(17) Altland, H. W. *J. Org. Chem.* **1976**, *41*, 3395.

twice with $CHCl_3$, and the combined $CHCl_3$ extracts were dried (Na_2SO_4) and concentrated. The residue was recrystallized from EtOH to give 3.2 g (32%) of **23a** (Table II).

1-(3-Fluorobenzyl)-1,2-dihydropyrimidine-2-thione hydrochloride (23b) was prepared from *N*-(3-fluorobenzyl)thiourea[15] in 13% yield by the method used to prepare **23a**.

1-(3,5-Difluorobenzyl)-1,2-dihydropyrimidine-2-thione hydrochloride (23c) was prepared from *N*-(3,5-difluorobenzyl)thiourea[15] in 10% yield by the same method used for the preparation of **23a**.

pK_a Determinations were obtained by titration of the compounds in 2:1 MeOH-H_2O (to overcome solubility constraints).

Enzymology. In vitro IC_{50} determinations were made as previously reported.[4] The IC_{50} is defined as the concentration of compound that produces 50% inhibition of product formation when compared to uninhibited control.

Registry No. **1**, 23269-10-5; **2**, 95333-64-5; **3**, 95333-65-6; **4**, 95333-56-5; **4**·HCl, 95359-66-3; **5**, 16042-26-5; **5**·HCl, 123566-30-3; **6**, 95460-25-6; **6**·HCl, 123566-31-4; **7**, 26163-58-6; **7**·2HCl, 22600-75-5; **8**, 95460-13-2; **8**·2HCl, 123593-05-5; **9**, 123593-06-6; **9**·HCl, 123566-32-5; **10**, 5376-10-3; **10**·HCl, 5272-57-1; **11**, 41833-17-4; **12**, 10045-64-4; **13**, 123566-33-6; **14**, 123566-34-7; **15b**, 95333-80-5; **15c**, 95333-81-6; **15d**, 95333-60-1; **16a**, 123566-44-9; **16b**, 123566-45-0; **16c**, 123566-46-1; **17a**, 23289-13-6; **17b**, 107186-77-6; **17c**, 107186-78-7; **17d**, 107186-80-1; **18a**, 29983-31-1; **18b**, 112961-33-8; **18c**, 112961-34-9; **19a**, 68700-73-2; **20a**, 33898-72-5; **20c**, 105219-26-9; **21a**, 31493-51-3; **21c**, 105968-95-4; **21d**, 105968-97-6; **22a**, 123566-55-2; **22b**, 123566-56-3; **22c**, 123566-57-4; **23a**, 123566-58-5; **23b**, 123566-63-2; **23b**·HCl, 123566-60-9; **23c**, 123566-64-3; **23c**·HCl, 123566-62-1; **28**, 56643-95-9; **29**, 10045-65-5; **30**, 95460-12-1; **31**, 95460-14-3; **32**, 95460-15-4; **33**, 123566-35-8; **34**, 95460-23-4; **35**, 123566-36-9; **36**, 123566-37-0; **37**, 123566-39-2; **38**, 123566-42-7; **39**, 90390-27-5; **40**, 105969-16-2; **41**, 107186-81-2; **42**, 63351-94-0; **43**, 107186-82-3; **44**, 107186-83-4; **45**, 93114-14-8; **46**, 107186-84-5; **47**, 107186-85-6; **48**, 107186-79-8; **49**, 112961-36-1; **50**, 112961-39-4; **51**, 112961-37-2; **52**, 123566-47-2; **53**, 123566-48-3; **54**, 123566-49-4; **55**, 105219-25-8; **56**, 5729-06-6; **57**, 105969-13-9; **58**, 105969-11-7; **59**, 105968-93-2; **60**, 79568-07-3; **61**, 123566-50-7; **62**, 123566-51-8; **63**, 123566-52-9; **64**, 32967-14-9; **65**, 123566-53-0; **66**, 123566-54-1; *p*-$MeOC_6H_4CH_2NHCH_2CH(OMe)_2$, 54879-77-5; NH_2CN, 420-04-2; PhCHO, 100-52-7; *m*-FC_3H_4CHO, 456-48-4; 3,5-$F_2C_6H_3CHO$, 32085-88-4; $NH_2CH_2CH_2NH_2$, 107-15-3; $PhCH_2NHCH_2CH_2NH_2$, 4152-09-4; 3,5-$F_2C_6H_3CH_2NHCH_2CH_2NH_2$, 123566-40-5; $PhCH_2NHCH_2CH_2NHC(=S)SH$, 123566-41-6; 3,5-$F_2C_6H_3CH_2NHCH_2CH_2NHC(=S)SH$, 123566-43-8; 3,5-$F_2C_6H_3CN$, 64248-63-1; *m*-$FC_6H_4CH_2NH_2$, 100-82-3; $PhCH_2NCS$, 622-78-6; *t*-$BuOCONHNH_2$, 870-46-2; *o*-$NO_2C_6H_4Cl$, 88-73-3; $PhCH_2NH_2$, 100-46-9; *m*-BrC_6H_4F, 1073-06-9; 3,5-$F_2C_6H_3Br$, 461-96-1; *m*-$FC_6H_4CH_2NHC(=S)NH_2$, 123566-59-6; 3,5-$F_2C_6H_4CH_2NHC(=S)NH_2$, 123566-61-0; 1-[3-(4-methoxyphenyl)propyl]imidazole-2-thione, 95333-89-4; 1-(4-methoxybenzyl)imidazole-2-thione, 95460-09-6; 1-benzylimidazole, 4238-71-5; 1-benzyl-2-methylimidazole, 13750-62-4; 1-[4-(benzyloxy)benzyl]-2-methylimidazole, 123566-38-1; 3,5-difluoro-4-methoxybenzonitrile, 104197-15-1; 1-benzyl-1,2,3-triazole, 4368-68-7; 3-pyridinecarbonitrile, 100-54-9; 3-benzylpyridine, 620-95-1; *N*-benzylthiourea, 621-83-0; malonaldehyde tetramethyl acetal, 102-52-3; dopamine β-hydroxylase, 9013-38-1; 3-benzylpyridine *N*-oxide, 32361-74-3.

Synthesis and Pharmacological Evaluation of a Series of Dibenzo[*a*,*d*]cycloalkenimines as *N*-Methyl-D-aspartate Antagonists

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A series of 73 dibenzo[*a*,*d*]cycloalkenimines were synthesized and evaluated for their ability to displace (+)-10,11-dihydro-5-methyl-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine ([^{3}H]-(+)-**10**) from its specific binding site on rat cortical membranes. A number of the more active compounds (K_i ranging from 0.006 to 0.21 μM) were evaluated for *N*-methyl-D-aspartate (NMDA) antagonist activity in the rat cortical slice (K_b ranging from 0.08 to 0.9 μM) and anticonvulsant activity in the mouse against NMDA induced convulsions. The ED_{50} values ranged from 0.22 to 7.76 mg/kg and correlated reasonably well with the K_b determination. In the dibenzo[*a*,*d*]cyclohepten-5,10-imine series, the (+)-5*S*,10*R* enantiomer displayed consistently higher levels of biological activity. While substitution at the 3-position of (+)-**10** with electronegative atoms generally increased in vitro activity, a loss of potency relative to (+)-**10** (MK-801) was observed in vivo for all of the compounds tested.

Sometime ago a number of 1,4-dihydronaphthalen-1,4-imines were synthesized in our laboratory to explore the biologic properties of these molecules.[1] Interest in these rigid heterocycles had been stimulated by the observation that 9-methyl-5,6,7,8-tetrafluoro-1,4-dihydronaphthalen-1,4-imine (**1**) displayed modest antiseizure and apparent anxiolytic activity when orally administered to rodents. In the course of pursuing this study, the structurally related 9,10-dihydroanthracen-9,10-imines[2] were found to have similar biological properties. Placement of methyl groups on the bridgehead positions of this construction gave rise to compounds which were surprisingly more potent than the lead structure **1**, and also exhibited a high level of central sympathomimetic activity.[3]

Sedation is a frequently encountered side effect of available anticonvulsant drugs. The spectrum of phar-

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(1) Anderson, P. S.; Christy, M. E.; Engelhardt, E. L.; Lundell, G. F.; Ponticello, G. S. *J. Heterocycl. Chem.* **1977**, *14*, 213.
(2) Anderson, P. S.; Christy, M. E.; Colton, C. D.; Halczenko, W.; Ponticello, G. S.; Shepard, K. L. *J. Org. Chem.* **1979**, *44*, 1519.
(3) Anderson, P. S.; Clineschmidt, B. v.; Christy, M. E.; Evans, B. E.; Yarbrough, G. C. In *Natural Products and Drug Development*; P. Krogsgaard-Larsen, H. Kofod, and S. B. Christenen, Eds.; Munksgard: Copenhagen, 1984; pp 98-107.

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