species but is too unstable to be isolated.

Since many of the postulated structures are very lipophilic, the question of using a charged side chain for maximum water solubility was examined in detail. Our reevaluation of the influence of pH-dependent partitioning on drug accumulation by the target cells takes into account the fact that the transmembrane pH gradient in normal tissue is likely to be reversed in hypoxic regions of tumors. Thus weakly acidic HSA may be therapeutically superior to neutral or basic analogues.

In conclusion, nitrophenyl mustards can be seen as having potential as HSA if a number of design criteria are considered simultaneously. A study of weakly basic and weakly acidic derivatives of the dinitro compound **14**, the corresponding mononitro derivatives **12** and **13**, and various other isomeric compounds is now under way.

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Registry No. **1**, 100858-17-1; **2**, 74109-34-5; **3**, 13551-87-6; **4**, 4533-39-5; **5**, 443-48-1; **6**, 55743-71-0; **7**, 2067-58-5; **8**, 553-27-5; **9**, 21919-05-1; **10**, 24813-13-6; **11**, 100858-18-2; **15**, 61837-23-8; **16**, 61837-26-1; **17**, 70132-50-2; oxygen, 7782-44-7.

Communications to the Editor

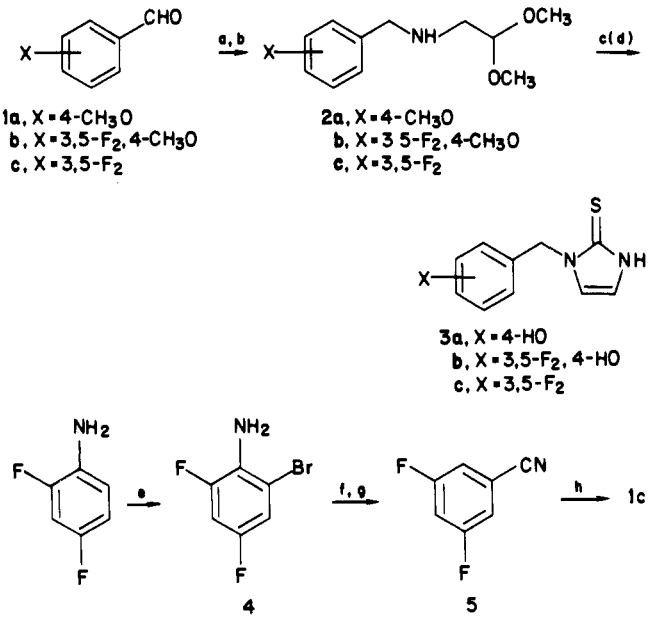
Substituted 1-Benzylimidazole-2-thiols as Potent and Orally Active Inhibitors of Dopamine β-Hydroxylase[1]

Sir:

Dopamine β-hydroxylase (DBH; EC 1.14.17.1), a copper-containing mixed-function oxidase, catalyzes the penultimate step in the catecholamine biosynthetic pathway, the benzylic oxidation of dopamine (DA) to norepinephrine (NE).[2] This enzymatic transformation affords a particularly attractive point for biochemical modulation of catecholamine levels in vivo. A selective DBH inhibitor would be expected to lower NE levels to blunt noradrenergic drive while simultaneously elevating levels of DA to provide the beneficial effects of peripheral dopamine receptor activation (i.e., renal vasodilation). An additional advantage of modulating this biochemical transformation derives from the known, limited potential for DBH upregulation.[3] This suggests a low probability of tachyphylaxis upon chronic administration of inhibitor, an important concern in the development of enzyme inhibitors as new therapeutic modalities.

The catalytic activity of DBH, hydroxylation of an unactivated C–H bond, is contingent upon the intermediacy of a powerful oxidizing species, presumably an activated copper–oxygen complex. The chemical reactivity of this species requires a proximity between the active site copper atom(s) and the enzyme-bound phenethylamine substrate to facilitate catalysis rather than single turnover autoxidation of enzyme. This rationale suggests compounds that are capable of binding to both the phenethylamine site and the active site copper atom(s) should be inhibitors of considerable potency. We have found that certain 1-benzylimidazole-2-thiols inhibit DBH by binding

Scheme I[a]



[a] Reagents and conditions: (a) $NH_2CH_2CH(OCH_3)_2$, ethanol; (b) $NaBH_4$, ethanol; (c) KSCN, HCl, H_2O, ethanol, reflux; (d) BBr_3, CH_2Cl_2; (e) Br_2, acetic acid; (f) $NaNO_2$, HBr, then $NaH_2PO_2 \cdot xH_2O$; (g) CuCN, *N,N*-dimethylacetamide, reflux; (h) Raney Ni alloy, formic acid, reflux.

in such a fashion.[1,4] Here we report the synthesis and pharmacological evaluation of several of these, e.g., **3a–c**, which are extraordinarily potent inhibitors of DBH. Indeed, **3b** binds DBH approximately 10^6-fold more tightly than the phenethylamine (dopamine, tyramine) substrates do. The preparation of compounds **3a–c** is illustrated in Scheme I. Condensation of the appropriate benzaldehydes **1a–c** with aminoacetaldehyde dimethyl acetal followed by $NaBH_4$ reduction afforded (70–80%) the (*N*-benzylamino)acetaldehyde dimethyl acetals **2a–c** as oils. Cyclization of the crude oils **2a–c** with acidic thiocyanate and subsequent BBr_3 demethylation of the anisoles afforded imidazole-2-thiols **3a** (65%, mp 188–190 °C), **3b** (48%, mp 213–215 °C) and **3c** (76%, mp 142–144 °C). The benzaldehyde **1c**, a known compound that is difficult to pre-

(1) Presented in part at the 190th National Meeting of the American Chemical Society: Chicago, IL, Sept. 1985; American Chemical Society: Washington, DC, 1985; MEDI 65, MEDI 66.

(2) For recent monographs, see: (a) Skotland, T.; Ljones, T. *Inorg. Perspect. Biol. Med.* **1979**, *2*, 151. (b) Rosenberg, R. C.; Lovenberg, W. In *Essays in Neurochemistry and Neuropharmacology*; Youdin, M. B. H., Lovenberg, W., Sharman, D. F., Lagnado, J. R., Eds.; Wiley: New York, 1980; Vol. 4, p 163. (c) Villafranca, J. J. In *Copper Proteins*; Spiro, T. G., Ed.; Wiley: New York, 1981; p 264. (d) Ljones, T.; Skotland, T. In *Copper Proteins and Copper Enzymes*; Lontie, R., Ed.; CRC Press: Boca Raton, 1984; Vol. 2, p 131.

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Table I. Inhibition of DBH in Vitro and in Vivo by **3a–c**

compd	K_i,[a] nM	DA/NE ratio[b]		mean arterial blood pressure[c]	
		treated	control	treated	control
3a	55 ± 2	0.100 ± 0.007***[e]	0.052 ± 0.002	137 ± 4.4* (N = 3)	155 ± 6.5 (N = 5)
3b	5.7 ± 0.6	0.082 ± 0.013*	0.031 ± 0.002	137 ± 9.0* (N = 3)	155 ± 6.5 (N = 5)
3c	41 ± 1	0.208 ± 0.011*** (N = 15)	0.041 ± 0.001 (N = 14)	105 ± 17.6* (N = 3)	143 ± 7.2 (N = 5)
		0.30 ± 0.03***[d] (N = 12)	0.04 ± 0.03[d] (N = 10)	110 ± 10***[d] (N = 12)	157 ± 6[d] (N = 10)
fusaric acid	149 ± 5				

[a] K_i values (mean ± SEM) were determined vs. tyramine substrate at pH 4.5 in the absence of fumarate with use of homogeneous bovine DBH (sp act. 30–42 U/mg at pH 5.0). Experimental conditions: ionic strength, μ = 0.2; 50 mM sodium acetate buffer; 200 μg/mL bovine catalase; 10 μM Cu^{2+}; 10 mM ascorbic acid; 37 °C. Under these conditions the K_m apparent for tyramine substrate is 3.0 mM. Inhibition constants (K_{is} values for **3a–c**, K_{ii} value for fusaric acid) were determined by using the computer programs of Cleland (*Methods in Enzymology*; Purich, D. L., Ed.; Academic: New York, 1979; Vol. 63, pp 103–138). [b] SHF (N = 5 unless otherwise indicated) were dosed twice orally with vehicle (5% PEG 400 in 1% methocel) or vehicle and drug at 50 mg/kg. The two doses were ca. 18 h apart and animals were sacrificed 2 h after the second dose. The mesenteric artery was removed and homogenized and catecholamine levels were determined by enzymatic methylation with catechol *O*-methyltransferase and [*methyl*-^{3}H]-*S*-adenosylmethionine followed by chromatographic separation of methylated amine and scintillation counting as previously reported (Head, R. J.; Berkowitz, B. A. *Blood Vessels* **1979**, *16*, 320). Catecholamine levels are reported as mean ± SEM. [c] SHR were anesthetized with Brevital Na (10 mg/kg, iv). The femoral artery was cannulated with P.E. 50 intramedic tubing and was connected to a pressure transducer for recording. After a 1-h recovery from the short-acting anesthetic, mean arterial blood pressure (MAP) was measured at 15-min intervals for 1 h. The test compounds were administered (50 mg/kg, ip, unless otherwise noted), and MAP was measured at 15–30-min intervals for 4 h. The blood pressures reported here (mean ± SEM) are 150-min postdrug. [d] In this experiment SHR were dosed twice at 50 mg/kg, po, 18 and 2 h prior to measuring blood pressure and sacrifice for determining tissue DA/NE levels. The same rats were used for blood pressure (mean ± SEM) and DA/NE measurements. [e] (*) $p < 0.05$, (**) $p < 0.01$, (***) $p < 0.001$.

Table II. Dose Response of DA/NE Ratio to **3c** in Mesenteric Artery[a]

dose, mg/kg, po	DA, μg/g of tissue	NE, μg/g of tissue	DA/NE ratio
control (untreated, N = 10)	0.301 ± 0.019	7.148 ± 0.658	0.043 ± 0.003
control (fusaric acid)[b]	0.702 ± 0.041***[c]	6.55 ± 0.32	0.109 ± 0.009***
25	0.765 ± 0.030***	4.937 ± 0.214*	0.155 ± 0.004***
50 (N = 10)	0.809 ± 0.048***	4.004 ± 0.290**	0.204 ± 0.013***
100	1.059 ± 0.062***	3.051 ± 0.356***	0.378 ± 0.075**

[a] SHF (N = 5) were treated with the indicated dose of drug according to the protocol defined in Table I, footnote. Catecholamine levels are reported as mean ± SEM. [b] 50 mg/kg, po dose. [c] $p < 0.05$, (**) $p < 0.01$, (***) $p < 0.001$.

pare,[5] was synthesized on a large scale by bromination of 2,4-difluoroaniline to yield the hydrobromide salt of **4** (88%, mp 222–225 °C), which was diazotized and deaminated to 3,5-difluorobromobenzene (62%, bp 130–140 °C (760 torr)).[6] This trihalobenzene was converted to 3,5-difluorobenzonitrile (**5**; 72%, mp 83–85 °C) by heating (4 h) with CuCN in *N*,*N*-dimethylacetamide. Aldehyde **1c** was readily obtained (78%) from **5** by heating with Raney nickel alloy in aqueous formic acid.[7]

The extremely potent inhibitory action of **3a–c** on bovine DBH in vitro is indicated by the data presented in Table I. Furthermore, under other assay conditions (pH 6.6, no fumarate) where oxygen and tyramine substrates appear to bind randomly,[8] competitive inhibition of **3a** is observed with these substrates (data not shown). All three compounds are orally active in vivo as evidenced by significant changes in the DA/NE ratio after oral administration to spontaneously hypertensive rats (SHR) (Table I). The effects of an increase in DA/NE ratio for **3a–c** are associated with a reduction in mean arterial blood pressure (Table I). Interestingly, the magnitude of shift observed in the DA/NE ratios for these compounds does not correlate with in vitro enzyme inhibition (K_{is}) since **3c** is substantially more potent in vivo than either of the phenolic inhibitors **3a** and **3b**, even though **3c** is less potent than **3b** against purified DBH in vitro. At present we hypothesize that this may result from rapid metabolic conjugation of the phenolic group of **3a** and **3b** in vivo. Compound **3c**, which lacks a hydroxyl, will be resistant toward this mode of metabolism. As indicated by the data

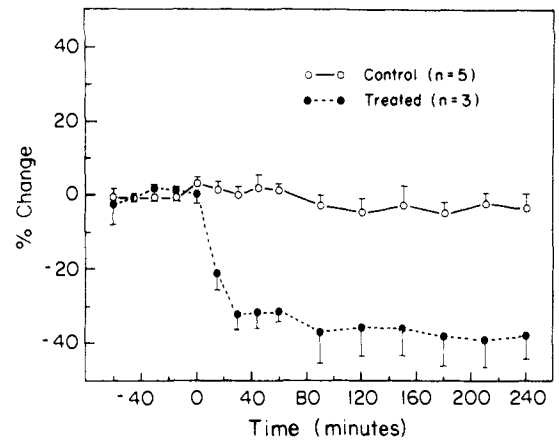

Figure 1. Effect of **3c**, 50 mg/kg, ip, on mean arterial blood pressure of conscious SHR (mean ± SEM).

tabulated in Table II, a dose-dependent increase in DA/NE ratio is observed following oral administration of **3c**. This derives from a statistically significant decrease in NE as well as a marked increase in DA. The in vivo data reported in Table II shows **3c** to be more potent than the standard, fusaric acid, a natural product that has been evaluated as a DBH inhibitor in human clinical trials.[9] Compound **3c** also shows a rapid onset of pharmacological activity, as evidenced by a rapid decrease in blood pressure, when administered ip to SHR (Figure 1). Lastly, extended (8–10 week) evaluation of **3c** in SHR confirm the predicted lack of tachyphylaxis for a DBH inhibitor.[10]

Clearly the DBH inhibitory potencies of the imidazole-2-thiols reported in this communication demonstrate

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that the nature of the substituent in position one of the imidazole-2-thiol moiety plays an important role in determining inhibitory activity. Thus **3a–c** are 10^2–10^3-fold more potent inhibitors of DBH than previously described simple 1-alkylimidazole-2-thiols.[11] Kinetic characterization of the DBH inhibitory activity of **3a** and **3b**, compounds whose extraordinary potency may derive from a "multisubstrate" mode of interaction with DBH, will be described elsewhere.[4]

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Registry No. **1a**, 123-11-5; **1b**, 654-11-5; **1c**, 32085-88-4; **2a**, 54879-77-5; **2b**, 101471-18-5; **2c**, 101471-19-6; **3a**, 95333-64-5; **3b**, 95333-60-1; **3c**, 95333-81-6; **4**·HBr, 101471-20-9; **5**, 64248-63-1; DBH, 9013-38-1; $H_2NCH_2CH(OCH_3)_2$, 22483-09-6; 2,4-difluoroaniline, 367-25-9; 3,5-difluorobromobenzene, 461-96-1.

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Articles

Synthesis and Opioid Activity of Dermorphin Tetrapeptides Bearing D-Methionine *S*-Oxide at Position 2†

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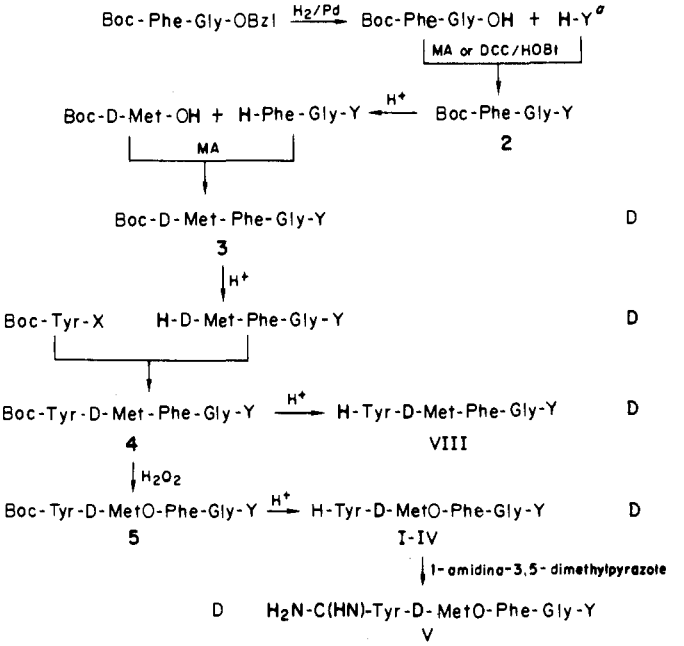
Eight new dermorphin tetrapeptides, X-Tyr-D-MetO-Phe-aa-Y (X = H, $H_2N{=}C(NH)$; aa = Gly, 2-aminoethanol, sarcosine; Y = NH_2, NH-alkyl), were prepared and tested for opioid activity. They show dose-related naloxone-reversible opioid effects in vitro and in vivo. H-Tyr-D-MetO-Phe-Gly-NH_2 (I) (guinea pig ileum IC_{50} = 13.6 nM; tail-flick ED_{50} = 1.97 pmol/mouse, icv, and 0.65 μmol/kg, sc), though less effective in the pheriphery, has central activities higher than those of dermorphin H-Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2. Following intracerebroventricular or subcutaneous administrations in mice, I is about respectively 1500 and 17 times as potent an analgesic as morphine.

Dermorphin (H-Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2) and its Hyp[6] analogue are opioid heptapeptides, originally isolated from frog skin[1] and characterized by the presence of a D-amino acid residue along their sequence.[2] Synthetic dermorphins have been prepared by different methods.[3] Opioid activities have been reported in the isolated longitudinal muscle strip of guinea pig ileum and mouse vas deferens,[3b,4] in cell-line preparations,[5] and in vivo in rat,[4a] mouse,[3b,4a] and human.[6] Our previous investigation concerned the synthesis and study of structure–activity relationships[4a,7] of 80 "small dermorphins"; we observed that tetrapeptides X-Tyr-D-Ala-Phe-aa-Y are potent analgesics after intracerebroventricular (icv) injection but relatively weak after subcutaneous (sc) administration.[7e,8] It has been reported that [D-Arg^2]- or [D-MetO^2]enkephalin[9,10] and the corresponding dermorphin peptides[11] retain high potency in vitro and/or in sc analgesia, the D-MetO^2 residue playing an important role.[12]

Scheme I. Synthesis of [D-Met^2]- and [D-MetO^2]dermorphin Tetrapeptide Analogues



[a] Y: see Table I (compounds I–V and VIII).

† Abbreviations according to IUPAC–IUB Commission on Biochemical Nomenclature, *Eur. J. Biochem.* **1984**, *138*, 9–37, are used throughout. Other abbreviations used are as follows: AcOH, acetic acid; Boc, *tert*-butyloxycarbonyl; DMF, dimethylformammide; Et_2O, diethyl ether; EtOAc, ethyl acetate; EtOH, ethyl alcohol; HPLC, high-performance liquid chromatography; IC, inhibitory concentration; MA, mixed anhydride; MeOH, methyl alcohol; mp, melting point; NMM, *N*-methylmorpholine; OMe, methyl ester; OSu, *N*-hydroxysuccinimidyl ester; PE, petrol ether; TEA, triethylamine; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TLC, thin-layer chromatography.

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Accordingly, we report the synthesis and opioid activity

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