Interaction of dopamine β -mono-oxygenase with substituted imidazoles and pyrazoles

Catalysis and inhibition

Sarath R. SIRIMANNE, Heath H. HERMAN and Sheldon W. MAY*

School of Chemistry, Georgia Institute of Technology, Atlanta, GA 30332, U.S.A.

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The interaction of dopamine β -mono-oxygenase (DBM) with substrate analogues possessing either imidazole or pyrazole functionalities at the alkyl chain terminus was investigated. 1-(4-Hydroxybenzyl)imidazole (4-HOBI) is an active substrate for DBM, and it exhibits the expected ascorbate- and fumarate-dependencies and normal kinetic behaviour at concentrations up to 10 mM. 4-Hydroxybenzaldehyde was identified as the product formed from 4-HOBI on the basis of h.p.l.c. and g.c.-m.s. analysis, and its formation exhibits the expected 1:1 stoichiometry with O₂ consumption. The 4-HOBI/DBM reaction is kinetically comparable with other DBM activities, and 4-HOBI is the first substrate analogue yet reported that exhibits substantial activity though lacking a terminal amino group. Introduction of a methyl substituent at the 2-position of the imidazole ring abolishes substrate activity, probably through a steric effect. 1-(4-Hydroxybenzyl)pyrazole, where imidazole is replaced by the isomeric pyrazole moiety, is a potent DBM inhibitor, and not a substrate. These results represent the first report of an active heterocyclic substrate or inhibitor for this enzyme, and establish the basis for the design of new classes of DBM substrates and inhibitors.

INTRODUCTION

Dopamine β -mono-oxygenase (EC 1.14.17.1, DBM) is a copper-containing mono-oxygenase that is localized in neurotransmitter vesicles of the adrenergic nervous system and in the chromaffin granules of adrenal medullary tissue, where it catalyses the benzylic hydroxylation of dopamine (3,4-dihydroxyphenethylamine) to produce the important neurotransmitter noradrenaline (norepinephrine) (Levin & Kaufman, 1961; Friedman & Kaufman, 1965; van der Shoot & Crevling, 1965). Although DBM is traditionally viewed as a 'specific hydroxylase', previous work in our laboratory has demonstrated several new kinetically ready mono-oxygenase activities for DBM: stereoselective sulfoxidation of phenylaminoalkyl sulphides (May & Phillips, 1980; May *et al.*, 1981b), selenoxidation of phenylaminoalkyl selenides (Padgett *et al.*, 1985b), oxygenative ketonization of benzylic *S*-alcohols (May *et al.*, 1981a, 1982), epoxidation of appropriately designed olefinic substrates (May *et al.*, 1983; Padgett *et al.*, 1985a), and oxidative *N*-dealkylation of a variety of molecules with benzylic amine functionalities (Padgett *et al.*, 1984, 1985a,b). In addition, DBM-catalysed oxidative ketonization of α -halophenethylamines has been reported by Klinman and co-workers (Klinman & Krueger, 1982; Mangold & Klinman, 1984), and alkyne oxidation has been reported by both us (Padgett *et al.*, 1985a) and Villafranca and co-workers (Colombo *et al.*, 1984a). More recently, Bargar *et al.* (1986) have shown that the phenyl ring of the substrate analogues can be replaced by heteroaromatics such as thienyl and furanyl groups.

In contrast, early work by van der Shoot & Crevling (1965) suggested that a terminal primary amino functionality is essential for substrate activity, since neither phenylethane, nor 4-hydroxyphenylethane, nor 2-(4-hydroxyphenyl)ethanol exhibited substrate activity. Similarly, we have found that removal of the amino group from the active olefinic substrate 2-(4-hydroxyphenyl)prop-2-enylamine almost completely abolishes substrate activity (Padgett *et al.*, 1985a). Although various types of inhibitors for DBM that lack a terminal amino group have been described (van der Shoot & Crevling, 1965; Rajashkar *et al.*, 1983; Rosenberg, 1983; Padgett *et al.*, 1985a; Dewolf & Kruse, 1985), these compounds exhibit little, if any, substrate activity. To date, appreciable substrate activities have been reported only for compounds having: (1) an aromatic nucleus (generally a phenyl ring, but including thienyl and furanyl nuclei); (2) a side chain of about two or three atoms in length; (3) a charged primary amine at the terminus of the side chain.

There have been a number of reports of sympathomimetic activity, cardiotonic effects and antihypertensive activity for substituted phenylalkyl-imidazoles and -imidazolines (Tuttle *et al.*, 1975; Lucas *et al.*, 1983; Machin *et al.*, 1984; Hamada *et al.*, 1985). We therefore turned our attention to the interaction of phenylalkyl heterocycles with DBM, since there has been increasing interest in this enzyme as a target for pro-drugs or inhibitors designed as possible modulators of adrenergic outflow (May *et al.*, 1981a; Herman *et al.*, 1983; Mangold & Klinman, 1984; Bargar *et al.*, 1986; Kruse *et al.*, 1986). In the present paper we report on the

Abbreviations used: DBM, dopamine β -mono-oxygenase; g.c., gas chromatography; BI, 1-benzylimidazole; 4-HOBI, 1-(4-hydroxybenzyl)-imidazole; 3,4-DiHOBI, 1-(3,4-dihydroxybenzyl)imidazole; 4-HOMeBI, 1-(4-hydroxybenzyl)-2-methylimidazole; PEI, 2-phenethylimidazole; 4-HOBP, 1-(4-hydroxybenzyl)pyrazole; 4-HOBA, 4-hydroxybenzaldehyde.

* To whom correspondence should be addressed.

interaction of DBM with substrate analogues possessing either imidazole or pyrazole functionalities at the alkyl chain terminus. We demonstrate a novel capability for DBM with 1-(4-hydroxybenzyl)imidazole, namely ready enzymic oxygenation leading to oxygenative dealkylation. On the other hand, we report that 1-(4-hydroxybenzyl)pyrazole is a potent reversible inhibitor of DBM. These findings represent the first report on the interaction of this important enzyme with heterocyclic compounds of this type, and establish the basis for new types of DBM substrates and inhibitors with moieties other than an amino functionality at the alkyl chain terminus.

EXPERIMENTAL

Synthesis of 4-HOBI

This compound was prepared by the method of Machin *et al.* (1984) by heating 4-hydroxybenzyl alcohol and imidazole at 160 °C on an oil bath. The solid was then recrystallized from diethyl ether/ethanol to give a pale-yellow crystalline solid. It had: m.p. 206–207 °C [lit. m.p. 206–208 °C (Machin *et al.*, 1984)]; ^1H n.m.r. δ (p.p.m.) 8.9 (1 H, s), 7.57 (2 H, s) and 7.2 (4 H, dd); m.s. (electron-impact) m/z 174 (M^+).

Synthesis of 4-HOBP

This substance was synthesized by the method of Machin *et al.* (1984). An equimolar mixture of 4-hydroxybenzyl alcohol and pyrazole was heated at 160 °C on an oil bath. The brown oil obtained was dissolved in conc. HCl and washed with chloroform. Recrystallization of the solid obtained on evaporation of water from diethyl ether/ethanol yielded a white crystalline solid. It had: m.p. 118–120 °C [lit. m.p. 113–115 °C (Machin *et al.*, 1984)]; ^1H n.m.r. δ (p.p.m.) 8.33 (1 H, s), 8.13 (1 H, s), 7.16 (4 H, dd) and 5.57 (2 H, s); m.s. (electron-impact) m/z 174 (M^+).

Synthesis of 4-HOMeBI

The synthesis of this compound was similar to that of the other imidazole derivatives and involved heating 4-hydroxybenzyl alcohol and 2-methylimidazole in 1:1 molar proportion at 160 °C. The resultant solid was dissolved in 5 M-HCl and washed well with chloroform. The aqueous phase was evaporated to dryness to yield a pale-yellow solid. Recrystallization of this from diethyl ether/ethanol gave a pale-yellow crystalline solid. It had: m.p. 146–147 °C; ^1H n.m.r. δ (p.p.m.) 8.9 (1 H, s), 7.5 (2 H, s), 7.26 (4 H, dd), 5.37 (2 H, s) and 2.8 (3 H, s); m.s. (electron-impact) m/z 188 (M^+).

Synthesis of 3,4-DiHOBI

An equimolar mixture of piperonyl alcohol and imidazole was heated at 160 °C in an oil bath, and the resultant solid was recrystallized from diethyl ether/ethanol. Removal of the methylenedioxy group was accomplished by reaction with BCl_3 in methylene chloride at 0 °C. The compound had: m.p. 171–172 °C; ^1H n.m.r. δ (p.p.m.) 8.84 (1 H, s), 7.57 (2 H, s), 7.02 (3 H, s) and 5.33 (2 H, s); m.s. (electron-impact) m/z 190 (M^+).

Synthesis of PEI

Phenethyl bromide was allowed to react with imidazole (sodium salt). The product was partitioned between

chloroform and HCl. The acidic phase was evaporated to dryness and crystallized from diethyl ether/ethanol. The compound had: m.p. 102–105 °C; ^1H n.m.r. δ (p.p.m.) 8.83 (1 H, s), 8.5 (1 H, s), 7.4 (6 H, m), 4.57 (2 H, t, $J = 3.1$ Hz) and 3.23 (2 H, t, $J = 3.1$ Hz); m.s. (electron-impact) m/z 172 (M^+).

Enzyme isolation and assay

DBM was isolated from bovine chromaffin granules obtained from fresh adrenal medulla. The granules were isolated by the method of Aunis *et al.* (1977), and the purification procedure of Ljones *et al.* (1976) was used with the omission of Triton X-100. The specific activity of DBM was 41 units ($\mu\text{mol}/\text{min}$)/mg. The concentration of DBM was determined spectrometrically by measuring the absorbance at 280 nm ($A_{280}^{1\%} = 12.4$). A polarographic oxygen-monitor assay was used in the determination of kinetic constants ($k_{\text{cat. (app.)}}$ and K_i) of both substrates and inhibitors. Kinetic constants ($k_{\text{cat. (app.)}}$ and K_m) of substrates were calculated by computer fit of the data to the hyperbolic form of the Michaelis–Menten equation (Cleland, 1967). O_2 consumption was measured with a Clark electrode and Yellow Springs Instrument Co. oxygen monitor. The standard oxygen-monitor assay mixture contained 0.12 M-sodium acetate buffer, pH 5.0, 10 mM-sodium fumarate, 0.1 mg of catalase/ml, 0.5 μM - CuSO_4 , 10 mM-ascorbate and 3–5 mg of DBM. In this assay mixture at atmospheric pressure O_2 saturation was found to be 260 μM . Initial rates were corrected for a small background due to ascorbic acid auto-oxidation. In cases where inhibitors were dissolved in methanol or dimethylformamide, the final concentration of organic solvent in the assay mixture was 5% (v/v), and controls were run in the same solvent mixture. 4-HOBP inhibition of the time-dependent inactivation activity of 2-phenylprop-2-enylamine was carried out as previously described (Padgett *et al.*, 1985a).

Determination of O_2 concentrations in buffer solutions saturated with different O_2/N_2 mixtures

The 0.12 M buffer was adjusted to pH 7.5 with 0.1 M-NaOH and equilibrated with the appropriate O_2/N_2 mixture for about 15 min. Then 2.5 ml of this solution was incubated at 37 °C with protococatechuic acid in the water bath of the Yellow Springs Instrument Co. oxygen monitor. Protocatechuate 3,4-dioxygenase (generously given by Ms. Joni Kaighobadi) was injected after the background had been recorded. With various amounts of protococatechuate present the O_2 consumption was recorded and the amount of protococatechuate required for total O_2 depletion in the assay solution was evaluated by extrapolation.

Detection and isolation of the enzymic product 4-HOBA from the DBM/4-HOBI reaction

Standard assay conditions were employed with 50 mM-4-HOBI and 0.03 mg of DBM in a total volume of 2.6 ml. Controls (enzyme omitted; boiled enzyme; ascorbate omitted; 4-HOBI omitted) were run in parallel. The reaction mixtures were incubated at 37 °C for 4 h open to air with shaking, filtered through a Centricon 30, and analysed by h.p.l.c. with an Excalibur C_{18} reverse-phase column (10 cm, 0.5 mm bore) with a 10 μl filled injection loop equilibrated with a mobile phase containing 60% 5 mM-sodium octanesulphonate/0.1 M-sodium acetate buffer, pH 5.75, and 40% methanol at

Fig. 1. H.p.l.c. analysis of DBM reaction with 4-HOBI

The procedure is given in the Experimental section. (a) Recorder trace after injection of a sample from a solution of 4-HOBI incubated at 37 °C with ascorbate. (b) Trace after injection of a sample from a solution of 4-HOBI incubated with DBM. (c) Trace after injection of a sample from a solution of 4-HOBI incubated with ascorbate and denatured DBM (boiled). (d) Trace after injection of a sample from a reaction mixture containing 4-HOBI, ascorbate and DBM. The first peak, eluted in the void volume, consists of fumarate, ascorbate, dehydroascorbate and imidazole, the second peak is 4-HOBA, and the third is unchanged 4-HOBI.

1.00 ml/min flow rate. Column eluent was monitored at 225 nm. The reaction mixtures were spiked with authentic 4-HOBA, 4-HOBI or imidazole for the identification of the products. For the purposes of g.c. and g.c.-m.s. analyses, enzymic reaction mixtures were extracted with diethyl ether, concentrated and chromatographed on silica gel before analysis by capillary g.c. or g.c.-m.s.

O_2 /product stoichiometry of the DBM/4-HOBI reaction

The assay mixture used in this experiment contained 0.127 M-sodium acetate buffer, pH 5.0, 10 mM-fumarate, 0.1 mg of catalase/ml, 5.7 μ M- $CuSO_4$, 10 mM-4-HOBI and 30 mM-ascorbate. The enzymic reaction was initiated

by addition of 0.05 mg of DBM, and O_2 consumption was monitored until there was no further noticeable consumption. Parallel controls were run under identical conditions without enzyme and worked up identically. The reaction mixtures were filtered through a Centricon 30 and analysed by reverse-phase h.p.l.c. under the same conditions used in the detection of products. Peak areas were measured with a Hewlett-Packard HP 3390A integrator. Enzymically produced 4-HOBA was quantified by using standard curves obtained under identical analytical conditions. Standard curves based on plots of peak area versus nmol of 4-HOBA were linear over the range used (5–40 nmol).

RESULTS AND DISCUSSION

Substrate activity of 4-HOBI

Incubation of DBM with 4-HOBI under the standard assay conditions resulted in an enzyme-dependent

Fig. 2. G.c. analysis of products of the DBM/4-HOBI reaction

The procedure is given in the Experimental section. (a) Recorder trace after injection of a sample from the ether extract of the enzymic reaction. (b) Trace after injection of authentic 4-HOBA. (c) Trace after injection of a sample from the ether extract of the control (DBM omitted).

consumption of O_2 , which was strictly dependent on the presence of substrate and the reducing agent ascorbate. The substrate activity was also stimulated by the DBM activator fumarate, and displayed normal Michaelis-Menten kinetics at substrate concentrations up to 10 mM.

Initial identification of the products of the enzymic reaction was accomplished by analysing reaction mixtures by h.p.l.c. and comparing the products with authentic compounds (Fig. 1). The h.p.l.c. chromatograms showed formation of a product peak with a retention time corresponding to that of authentic 4-HOBA. Confirmation of the identity of this product was obtained by g.c.-m.s. analysis. Large-scale enzymic reaction mixtures were extracted with diethyl ether, and unchanged substrate was removed by silica-gel chromatography on a micro column. G.c. analysis (Fig. 2) gave

rise to a single peak with a retention time corresponding to that of authentic 4-HOBA. The diethyl ether was evaporated off, and the residue was redissolved in methanol and then subjected to both h.p.l.c. and g.c.-m.s. analysis. H.p.l.c. again resulted in the single expected peak, and g.c.-m.s. of the enzymic product and authentic 4-HOBA gave identical results (Fig. 3), with an M^+ peak at m/z 122. Control reaction mixtures from which DBM was omitted did not show any detectable amount of 4-HOBA under standard reaction conditions.

It is clear from the above that enzymic processing of 4-HOBI produces 4-HOBA. Presumably, initial hydroxylation at the benzylic methylene group of 4-HOBI is occurring to give 4-hydroxy- α -(imidazol-1-yl)benzyl alcohol as the immediate enzymic product, which then undergoes rapid cleavage to 4-HOBA and imidazole. If

Fig. 3. G.c.-m.s. analysis of products of the DBM/4-HOBI reaction

(a) Authentic 4-HOBA. (b) Ether extract of the enzymic reaction mixture.

Table 1. Kinetic constants for DBM substrates

The reaction system contained 0.5 μ M-CuSO₄, 10 mM-fumarate, 10 mM-ascorbate, 0.1 mg of catalase/ml, 3–5 μ g of DBM and various amounts of substrate in 0.12 M-sodium acetate buffer, pH 5.0. The reaction was followed by measuring O₂ consumption at 37 °C with a Yellow Springs Instrument Co. model 53 oxygen monitor.

Compound	$k_{\text{cat.}}/K_m$ (s ⁻¹ ·mM ⁻¹)	$k_{\text{cat.}}$ (s ⁻¹)	K_m (mM)
1-(4-Hydroxybenzyl)imidazole (4-HOBI)	5.0	9.5	1.9
2-Phenylprop-2-enylamine*	2.1	14.0	6.7
2-(4-Hydroxyphenyl)prop-2-enylamine*	43.0	56.0	1.3
4-Hydroxy- α -methylstyrene*	0.2	0.7	3.7
Phenylaminoethyl sulphide†	2.3	39.0	17.2
Tyramine*	60.5	121.0	2.0
Dopamine‡	42.4	110.0	2.6
3-Phenylpropylamine†	1.7	20.4	12.2
N-Phenylethylenediamine*	2.3	21.0	9.1
1-Phenylpropyne§	0	0	—
4-Hydroxyphenylpropene	0.84	0.81	0.97
Phenylacetaldehyde¶	0.07	0.6	7.9

* Padgett *et al.* (1985a).

† Padgett *et al.* (1984b).

‡ Although we include kinetic values for dopamine for comparison purposes, possible kinetic complications have been reported in the literature (Levin & Kaufman, 1961).

§ Rajashkar *et al.* (1983).

|| Colombo *et al.* (1984b).

¶ These values obtained at elevated O₂ concentration (0.5 mM) (Bossard & Klinman, 1985).

so, a 1:1 stoichiometry of O₂ consumption to 4-HOBA formation should pertain. Quantification of 4-HOBA produced was accomplished by h.p.l.c. under the same conditions used in the analysis of products of the enzymic reaction, by using standard curves prepared with analytically pure 4-HOBA. An O₂/product ratio of 1.1:1 was determined; as expected, no 4-HOBA was detectable in parallel controls (DBM omitted; ascorbate omitted; boiled enzyme). K₄Fe(CN)₆, an alternative electron donor for DBM oxygenation, is generally employed in the determination of O₂/electron stoichiometry (May *et al.*, 1981b, 1982). This was not possible in the 4-HOBI/DBM reaction, as imidazole derivatives form 1:1 donor–electron-acceptor complexes with Fe(CN)₆⁴⁻ ions (Gerber *et al.*, 1973). In fact, the oxygenation of tyramine by DBM in the presence of K₄FeCN₆ was completely abolished instantly by the addition of 4-HOBI or any other imidazole derivative (results not shown).

The results to this point establish 4-HOBI as the prototype for a new type of DBM substrates: compounds that undergo benzylic oxygenation resulting in substrate dealkylation. As shown in Table 1, the 4-HOBI/DBM reaction is kinetically comparable with other well-characterized DBM-catalysed reactions, i.e. hydroxylation, sulphoxidation and olefin epoxidation. It is important to note that 4-HOBI is a far more active substrate than any previously reported compound that does not possess a terminal amino functionality.

In steady-state kinetic experiments, marked decreases in initial rates were observed at 4-HOBI concentrations greater than 10 mM, giving rise to deviations from linearity of the double-reciprocal plots (apparent K_i for substrate inhibition 58 mM). To check for possible irreversible time-dependent enzyme inactivation, incubation mixtures of DBM and 4-HOBI with all standard

assay components were periodically assayed by dilution into excess tyramine (Padgett *et al.*, 1985a). No loss of enzyme activity was detectable in these dilution assays. The aldehyde product, 4-HOBA, is not a substrate for DBM, but is a weak competitive inhibitor (Table 2). Although 4-hydroxyphenylacetaldehyde has been reported to be a weak substrate (Bossard & Klinman, 1985), prolonged incubation of 4-HOBA with DBM under the standard enzymic reaction conditions resulted in no h.p.l.c.-detectable product formation. The other product

Table 2. Kinetic constants for DBM inhibitors

See Table 1 and the Experimental section for assay conditions.

Compound	K_i (mM)
1-Benzylimidazole (BI)	11.9
1-(3,4-Dihydroxybenzyl)imidazole (3,4-DiHOBI)	4.6
1-(4-Hydroxybenzyl)-2-methylimidazole (4-HOMeBI)	2.9
2-Phenethylimidazole (PEI)	0
1-(4-Hydroxybenzyl)pyrazole (4-HOBP)	0.16
4-Hydroxybenzaldehyde (4-HOBA)	2.3
2-Phenylprop-2-enylamine*	4.7
2-(4-Hydroxyphenyl)prop-2-enylamine*	0.52
4-Hydroxy- α -methylstyrene*	2.5
N-Ethylaniline†	6.8
N-Phenylethylenediamine†	1.1
1-Phenylpropyne‡	2.5
3-Phenylpropene§	3.6

* Padgett *et al.* (1985a).

† Wimalasena & May (1986).

‡ Colombo & Villafranca (1984).

§ Rajashkar *et al.* (1983).

of the 4-HOBI/DBM reaction, imidazole, has been reported to be a stimulator of DBM (Brown & Harralson, 1976), and we have confirmed this effect (results not shown).

The nature of the substituents on the phenyl ring greatly affects the interaction of the imidazole compounds with DBM. BI exhibited less than 5% of the activity of 4-HOBI under standard assay conditions; as a consequence it behaves as a weak competitive inhibitor with respect to tyramine (Table 2). The same is true for the dihydroxylated derivative 3,4-DiHOBI. PEI, where the methylene chain is extended by one carbon atom, also exhibited no detectable substrate activity and only very weak inhibitory potency.

DBM inhibition by 4-HOMeBI and 4-HOBP

Although 4-HOBI is a good substrate for DBM, 4-HOMeBI, where a methyl substituent has been introduced at position 2 of the imidazole ring, exhibits no substrate activity under standard assay conditions. This lack of activity cannot be attributed to a change in pK_a of the imidazole moiety, since the pK_a values for the imidazole ionization in 4-HOBI and 4-HOMeBI were determined to be 6.4 and 6.8 respectively. 4-HOMeBI did exhibit weak competitive inhibitory potency, with a K_i of 2.9 mM. It seemed likely that the 2-methyl substituent interferes with catalytic turnover as the consequence of a deleterious steric interaction within the active site, possibly in the copper-ligation region. This notion is supported by the hypothesis that potent DBM inhibition by 1-(4-hydroxybenzyl)imidazole-2-thiol is due to ligation of the copper by the 2-thiol substituent (Dewolf & Kruse, 1985). We therefore turned to 4-HOBP, a structural isomer of 4-HOBI that contains a ring nitrogen atom at the position analogous to the 2-position of the imidazole ring, since pyrazoles are known to be good copper ligands (Sorrel & Jameson, 1983). 4-HOBP exhibited much more potent competitive inhibition than the imidazole compounds (Table 2), and showed no detectable substrate activity under standard assay conditions. The potency of 4-HOBP as a reversible inhibitor was further demonstrated by examining its ability to protect DBM against irreversible inactivation by the mechanism-based inhibitor 2-phenylprop-2-enylamine (May *et al.*, 1983; Padgett *et al.*, 1985a). Incubation of DBM with 2-phenylprop-2-enylamine under standard inactivation conditions (May *et al.*, 1983; Padgett *et al.*, 1985a) in the presence of 1.5 mM 4-HOBP resulted in a 76% decrease in the second-order rate constant for inactivation. It should be noted that 4-HOBP also showed non-competitive inhibition with respect to O_2 when assayed at O_2 concentrations in the range 105–400 μ M.

There has been increasing interest in the design of substrate analogues and inhibitors for DBM. Several groups have recognized the attractiveness of DBM as a target point for modulation of catecholamine concentrations (May *et al.*, 1981a; Herman *et al.*, 1983; Padgett *et al.*, 1984b; Bargar *et al.*, 1986; Kruse *et al.*, 1986). The pharmacological potential of substrate analogues capable of competing with dopamine for DBM oxygenation and thereby diluting noradrenaline concentrations pre-synaptically, and of inhibitors that would lower noradrenaline concentrations directly, has become quite clear. The results reported in the present paper establish a basis for the design of new types of substrates and

inhibitors for DBM. 4-HOBI represents the first prototype for active substrates that lack an amino functionality at the alkyl chain terminus, and 4-HOBP is likewise a prototypical potent reversible heterocyclic inhibitor. We note here that there are reports of either α - or β -agonistic activity as well as β -antagonistic activity for derivatives of imidazole and imidazoline-containing catechols and phenols (Miller *et al.*, 1984; Machin *et al.*, 1984; Hamada *et al.*, 1985), and these reports may have relevance to the activity *in vivo* of the compounds we describe here and their congeners.

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