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Synthesis of Indane Derivatives as Mechanism-Based Inhibitors of Dopamine β-Hydroxylase

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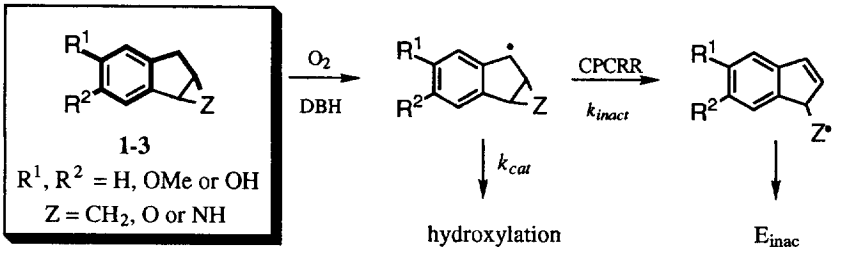
Abstract: A series of indane derivatives was synthesized and evaluated as mechanism-based inhibitors of dopamine β-hydroxylase (DBH).

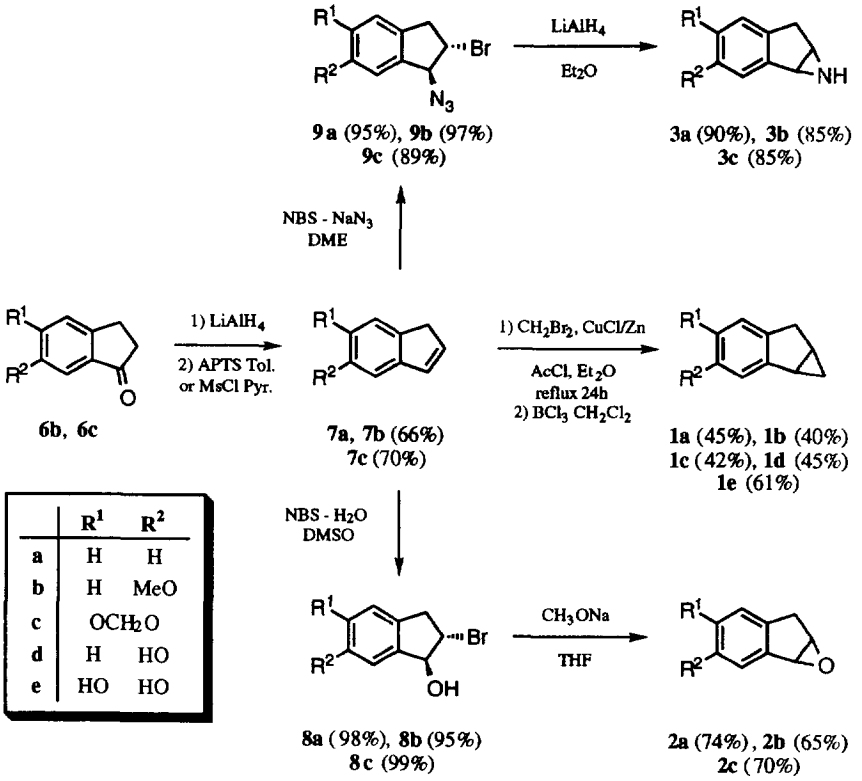
Dopamine β-hydroxylase (DBH; E. C. 1.14.17.1), a copper-containing monooxygenase present in a variety of mammalian tissues, catalyzes the benzylic hydroxylation of dopamine.[1] As it plays a key role in the biosynthetic production of noradrenaline, DBH presents an appealing target for the design of inhibitors as potential therapeutic agents for the modulation of adrenergic activity *in vivo*.[2] On the other hand, beyond the therapeutic interest, is the development of mechanism-based inhibitors useful as probes for the mechanism of C—H bond activation, a subject of current interest.[3,4,5]

Klinmann *et al.*[6] have recently proposed a mechanism where the determining step is the homolytic abstraction of the benzylic hydrogen of the substrate by a highly reactive copper oxygen species. Taking advantage of this benzylic radical formation, Villafranca *et al.*[3] have shown that benzyl cyclopropane compounds **4** and **5** (Table 1) were mechanism-based inhibitors of DBH and explained the inactivation by the well known cyclopropyl-carbinyl radical rearrangement (CPCRR).[7] However, when studying the CP_{450} mechanism with radical clock compounds, Ingold *et al.* [7b] have shown that the ring opening of α-cyclopropylbenzyl radical is slower than the ring closure of benzylhomoallyl radical.

In order to investigate the effect of both good substrate recognition and fast CPCRR, we have studied 1,2-cyclopropyl, epoxy and aziridinyl indane derivatives **1-3** of which the benzyl radicals are known to undergo rearrangements.[8,9,10] In this paper we describe the synthesis of these compounds and discuss their evaluation as mechanism-based inhibitors of DBH.



Synthesis. Compounds **1-3** were prepared in few steps from indenes **7a-c** by the pathways outlined in Scheme 1.[11] Commercially not available indenes **7b-c** were obtained by reduction of 1-indanones **6b-c** with lithium aluminum hydride in diethyl ether followed by dehydration of the corresponding alcohols with *para*-toluenesufonic acid in toluene for **7b**, or with methanesulfonyl chloride in pyridine for **7c**. Indanone **6c** were obtained in 3 steps from cinnamic acid according to Reeve and Myers.[12] Simmons-Smith cyclopropanation modified by Friedrich affords cyclopropylindanes **1a-c** in moderate yields.[13] Epoxy indanes **2a-c** were obtained by reaction of bromohydrins **8a-c** with MeONa in THF.[14] Bromohydrins **8a-c** were quantitatively obtained by bromination using NBS in DMSO/water.[15,16] The same reaction in the presence of NaN_3 in DME, gives rise to 1-azido-2-bromoindanes **9a-c** which after reduction with $LiAlH_4$ in diethyl ether afford in good yields aziridines **3a-c**.[17] Demethylation of compounds **7b** and **7c** to give respectively **1d** and **1e**, was performed according to the method of McOmie *et al.*.[18]



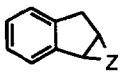
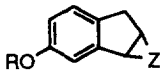
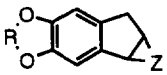
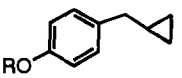
	R^1	R^2
a	H	H
b	H	MeO
c	OCH_2O	
d	H	HO
e	HO	HO

Scheme 1. Synthesis of Indane Derivatives **1a-e**, **2a-c** and **3a-c**.

Biochemistry. Kinetic experiments were conducted under the conditions defined in Table 1 with homogeneous DBH obtained from bovine adrenal medulla by modified Ljones' purification.[19] Upon incubation of purified DBH in air with varying concentrations of compounds **1-3** (at least five concentrations), the rate of loss of activity was determined by taking aliquots and measuring the residual activity with tyramine as substrate with a Clark oxygen electrode according to dioxygen consumption. The observed rates of inactivation fit with the hyperbolic form of the rate equation $1/k_{obs} = 1/k_{inact} + K_I/k_{inact}[I]$.

Discussion. Except for aziridines **3a** and **3c** which gave unclear results, all other compounds fulfill the minimal criteria for mechanism-based inhibitors.[20] The inactivation: (*i*) was first order, (*ii*) showed saturation kinetics, (*iii*) was dioxygen and ascorbate dependent, (*v*) was prevented in the presence of tyramine, and (*vi*) was not reversed by extensive dialysis. By plotting $1/k_{obs}$ as a function of 1/[I] the kinetic parameters k_{inact} and the apparent binding constant K_I have been estimated. These data as well as the k_{inact}/K_I ratio are given in Table 1.

Table 1. Kinetic Inhibition Parameters for Dopamine β-Hydroxylase by Indane Derivatives.

Inhibitors		R	Z	K_I (mM)	k_{inact} ($min.^{-1}$)	k_{inact}/K_I ($M^{-1}\ min.^{-1}$)
	**1a**	—	CH_2	3.03	0.20	70
	2a	—	O	1.06	0.08	70
	3a	—	NH	—	—	—
	**1b**	Me	CH_2	1.12	0.11	100
	1d	H	CH_2	0.15	0.23	1530
	2b	Me	O	0.65	0.07	110
	3b	Me	NH	0.40	0.03	70
	**1c**	CH_2	CH_2	0.16	0.06	370
	1e	H, H	CH_2	0.109	0.129	1180
	2c	CH_2	O	1.01	0.06	60
	3c	CH_2	NH	—	—	—
	**4**	Me	—	0.24	0.018	75
	5	H	—	12	1.8	150

Inactivation reactions were performed at 37°C in 100 mM MES (pH = 5.5) containing 250 μM O_2, 3 μM DBH, 10 mM fumarate, 10 mM ascorbate, 100 μg/mL catalase, 12 μM $CuSO_4$, 0.5-30 mM inhibitors in 15% DMF.

Some preliminary conclusions can be reached from the data in Table 1. First of all, as judged by the pharmacologically relevant ratio k_{inact}/K_I, cyclopropane compounds **1b** and **1d** demonstrate better inhibition efficiency than simple benzylic cyclopropane compounds **4** and **5** (compare **1b** *vs.* **4** and **1d** *vs.* **5**). For these compounds, the kinetic parameter k_{inact} decreases with the substitution of the aromatic ring by *para* hydroxy or methoxy groups (**1a** *vs* **1b** and **1d**) what is consistent with a stabilization of a benzylic radical by these oxygen groups. More interesting is the lack of substituent effects on the aromatic ring upon the kinetic parameter k_{inact} with epoxides **2a-c**. This suggest the occurrence of an inactivation mechanism which does not involve the formation of a benzylic radical. The possible inhibition mechanism by epoxide compounds could proceed via abstraction of one electron from epoxide by the copper oxygen species to form a radical cation epoxide which can be attacked by an enzyme nucleophile. Further work is currently in progress to define the mechanism of inhibition by cyclopropanes and epoxides using optically pure and deuterium or tritium labelled compounds.

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