Demethylation Studies. VII. A Unique Effect of the *N*-Cyclopropyl Group in a New Series of Inhibitors of Oxidative Microsomal Demethylation

ROBERT E. MCMAHON, HILMAN W. CULP, and JACK MILLS

The Lilly Research Laboratories, Indianapolis, Indiana 46206

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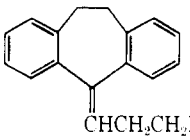
The hepatic microsomal oxygenases that mediate the *in vivo* oxidation of many foreign chemicals are sensitive to certain highly lipid-soluble amines. Among the most potent and widely studied of these inhibitors are 2,4-dichloro-6-phenylphenoxyethylamine, (**1**)[1,2a,b] and β-diethylaminoethyl α,α-diphenylvalerate.[1,3]

Recently, studies on the *in vivo* demethylation of the antidepressant drug, 5-(α-methylaminopropylidene)-dibenzo[*a,d*]cyclohepta[1,4]diene (**2a**)[4] were reported.[5]



2a, R = Me
b, R = cyclopropyl
c, R = H

In these studies it was found that while **2a** was demethylated much more slowly than the corresponding tertiary amine,[6] **2a** appeared to have a higher affinity for the enzyme and to have an inhibitory effect. In order to exploit this lead, a number of analogs of **2a** have been prepared and their activity as *in vitro* demethylase inhibitors has been studied. These studies, which are described below, led to the discovery of a new *in vitro* microsomal inhibitor, the *N*-cyclopropyl analog **2b**, which was more potent than any of the inhibitors so far reported.

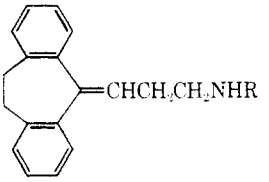
Experimental Section

Compounds.—The compounds were prepared by standard laboratory procedures and purified by recrystallization of the HCl salts. All of them gave a single spot when chromatographed (as the free base) on silica gel in a system of C_6H_6 (4 parts) and EtOAc (1 part).

In Vitro **Studies.**—The source of enzyme in all cases was the 15,000*g* supernatant from rat liver homogenate prepared as described previously.[2a]

Assays for inhibitor activity were performed as follows. Into each 20-ml beaker was placed 1 ml of 15,000*g* supernatant (equivalent to 200 mg of liver), 200 μmoles of TPN$^+$, 11 μmoles of glucose 6-phosphate, 45 μmoles of neutralized semicarbazide, 50 μmoles of $MgCl_2$, 50 μmoles of nicotinamide, substrate, and sufficient H_2O to yield a final volume of 3 ml. In addition, the inhibitor to be tested was added at two concentrations (1×10^{-3} *M* and 5×10^{-5} *M*). Th substrates used and their concentrations are shown in the tables. The reaction mixture was incubated in air with shaking at 37° for 30 min. CH_2O formation was determined by the procedure of Axelrod and Cochin.[7] The results are reported in Tables I and II as per cent inhibition of control rates.

TABLE I
INFLUENCE OF *N*-ALKYL SUBSTITUENTS ON INHIBITOR ACTIVITY



	% inhibition of demethylation of					
	Propoxyphene[a]		Diphenyl-2-dimethylaminoethane[a]		Botyramine[b]	
R	A[c]	B[d]	A[c]	B[d]	A[c]	B[d]
H	6	21	22	58	47	75
CH_3	4	7	10	23	23	
C_2H_5	1	9	1	9	11	41
CH_2CH_2OH	2	7	2	9	10	44
n-C_3H_8	0	10	1	16	18	51
i-C_3H_8	2	4	2	18	26	52
cyclo-C_3H_5	24	51	60	88	76	95
$CH_2CH{=}CH_2$	6	7	9	26	32	65
$CH_2C{\equiv}CH$	0	2	0	7	2	29
$C(CH_3)_2C{\equiv}CH$	0	4	12	40	38	40
CH_2Ph	0	6	0	6	50	61

[a] Concn = 1×10^{-3} *M*. [b] Concn = 6.7×10^{-3} *M*. [c] Inhibitor concn = 1×10^{-5} *M*. [d] Inhibitor concn = 5×10^{-5} *M*.

TABLE II
INHIBITION OF MICROSOMAL DEMETHYLATION OF VARIOUS SUBSTRATES

	% inhibition by			
	Compound 2b at		Compound 1 at	
Substrate[a]	10^{-3} *M*	5×10^{-5} *M*	10^{-3} *M*	5×10^{-5} *M*
Amitriptyline	57	86	13	37
Imipramine	61	84	35	62
Dimethylaniline	25	50	0	21
Anisonitrile	47	69	44	72
Aminopyrine	53	88	33	63
Ethylmorphine	69	90	7	66
Diamylmethylamine	88	100	41	87
Meperidine	85	97	31	67
Meperidine[b]	77		35	
Meperidine[c]	20	84	68	92
3-(*p*-Chlorophenyl)-dimethylpropylamine	89	92	70	91
3-(*p*-Chlorophenyl)-dimethylpropylamine[c]	12	54	41	67

[a] All substrate concentrations were 1×10^{-3} *M*. [b] This study was carried out in rat liver slices. [c] This study was carried out with liver microsomes from guinea pig.

Results and Discussion

Studies on the structure–activity relations in the phenoxyethylamine series showed that optimal activity

(1) The amine, 2,4-dichloro-6-phenylphenoxyethylamine has been referred to in the literature as DPEA or Lilly 32391. Similarly, β-diethylaminoethyl α,α-diphenylvalerate is widely known by the code designation of SKF525.

(2) (a) R. E. McMahon, J. Mills, H. W. Culp, W. R. Gibson, W. M. Miller, and F. J. Marshall, *J. Med. Chem.*, **12**, 207 (1969); (b) R. E. McMahon and J. Mills, *ibid.*, **4**, 211 (1961).

(3) (a) J. Axelrod, J. Reichenthal, and B. B. Brodie, *J. Pharmacol. Exp. Ther.*, **112**, 49 (1954); (b) J. R. Cooper, J. Axelrod, and B. B. Brodie, *ibid.*, **112**, 55 (1954).

(4) Nortriptyline.

(5) R. E. McMahon, *Life Sci.*, **3**, 235 (1964); R. E. McMahon, F. J. Marshall, H. W. Culp, and W. M. Miller, *Biochem. Pharmacol.*, **12**, 1207 (1963).

(6) Amitriptyline.

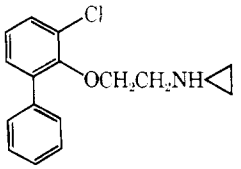
(7) J. Cochin and J. Axelrod, *J. Pharmacol. Exp. Ther.*, **125**, 105 (1959).

resided in the primary amines.[2] For that reason the present study was designed to assess the effect of N substitution on the activity of the primary amine **2c**. Three model substrates were chosen (a) propoxyphene, an excellent substrate that is difficult to inhibit, (b) diphenyldimethylaminoethane, also a very active substrate but more easily inhibited, and (c) butynamine, a less active substrate that is very readily inhibited. The results summarized in Table I show that, indeed, substitution on N does diminish activity in accord with the earlier studies.[2a,b] There was, however, one conspicuous exception, that of the *N*-cyclopropyl analog, **2b** which was substantially more active than **2c**.

The activity of **2b** was further investigated by a comparative study with 2,4-dichloro-6-phenylphenoxyethylamine (**1**), the most active compound in the earlier study. The relative activities of the two inhibitors in the presence of a number of substrates are shown in Table II. In the rat liver microsomal system, **2b** was clearly a more effective inhibitor than was **1**. It also appeared to be more effective in the liver slice incubation. In guinea pig microsomes, however, **2b** was a less active inhibitor.

Preliminary *in vivo* studies with **2b** were also conducted. In order to insure a maximum effect **2b** was given by ip injection 5 min prior to administration of substrate. These studies showed **2b** to be less effective than **1** both in prolonging hexobarbital sleeping time in mice and in inhibiting the *in vivo* demethylation of imipramine (see McMahon, *et al.*,[2a] for a description of *in vivo* methodology). Because of these results, further studies of **2b** in the intact animal did not appear warranted. However, its activity in the rat liver microsomal system suggests that it may be a very valuable tool in the study of the mechanism by which these interesting oxygenases oxidize drugs.

The effect of cyclopropyl substitution in the phenoxyethylamine series was also investigated. Thus *N*-cyclopropyl-2-chloro-6-phenylphenoxyethylamine (**3**) was synthesized, and its activity was compared with that of the corresponding primary amine. Compound **3** was found to be significantly more active than the corresponding primary amine in rat liver microsomes. However, it appeared to have very little *in vivo* activity.



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The basis for the effect of cyclopropyl substitution in the two series of microsomal inhibitors remains obscure. The effect is not simply the effect of branching on the α-C since the *i*-Pr compound is no more active than *n*-Pr. The electron-attracting properties of cyclopropyl would be expected to lower the pK_a of the amine. However, this effect alone cannot explain the unique effect of cyclopropyl since other electron-withdrawing groups such as benzyl, allyl, and dimethylpropargyl[8] have a very minimal effect. A number of other workers[9] have investigated the effect of small ring alkyl substituents but as yet no substantive clues to their unique effects have emerged.

Acknowledgment.—We wish to thank Mr. W. M. Miller and Mrs. Catherine Cobb for expert technical assistance. We also thank Dr. W. Lacefield for advice on the chemical aspects of the study.

(8) R. E. McMahon and N. R. Easton, *J. Med. Pharm. Chem.*, **4**, 437 (1961).

(9) A. Burger, R. T. Standridge, and E. J. Ariens, *ibid.*, **6**, 221 (1963); J. Mills, R. Kattau, I. H. Slater, and R. W. Fuller, *ibid.*, **11**, 95 (1968).

Pyrazine Diuretics. VIII. *N*-Amidino-3-aminopyrazinecarboxamide 4-Oxides

JAMES H. JONES AND EDWARD J. CRAGOE, JR.

Merck Sharp and Dohme Research Laboratories, Division of Merck & Co., Inc. West Point, Pennsylvania 19486

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The interesting diuretic–saluretic properties of the *N*-amidino-3-amino-pyrazinecarboxamides[1] prompted the preparation of some corresponding *N*-amidino-3-aminopyrazinecarboxamide 4-oxides (IIIa, IIIb, V). These compounds were prepared by the reaction of the corresponding pyrazine ester with guanidine according to the method previously reported[2] (see Scheme I). The methyl 3-aminopyrazinecarboxylate 4-oxides (IIa, b) were obtained from the appropriate pyrazine esters by treatment with *m*-chloroperbenzoic acid. The position of the oxide function was established by the preparation of IV and VII from IIa and IIb by treatment with $POCl_3$. Thus, our experiences with py-

TABLE I

BIOLOGICAL RESULTS

Compd	Rat DOCA-inhibn[a] score[c]	Normal rat[b] score[d]
IIIa	+1	+1
IIIb	±	±
IIIc	±	+2
IV	0	+2
VIIIa[e]	0	+2
VIIIb[f]	+3	+3
VIIIc[g]	+3	+2

[a] Drs. M. S. Glitzer and S. L. Steelman and their associates supplied part of this data; the remainder was supplied by Dr. J. E. Baer and his associates. [b] Dr. J. E. Baer and his associates supplied these data. [c] The DOCA-inhibition score[1] is the dose producing reversal of the DOAC Na/K effect: +3 = 10–50 μg/rat; +2 = 51–100; +1 = 101–800; ± = >800; 0 = inactive at any dose. [d] Activity [E. J. Cragoe, Jr., O. W. Woltersdorf, Jr., J. E. Baer, and J. M. Sprague, *J. Med. Chem.*, **5**, 896 (1962)] is based upon increase in urinary electrolyte and volume over control values referred to standards: +3 = activity of 100 mg/kg hydrochlorothiazide; +2 = activity of 100 mg/kg of chlorothiazide, 0 = controls. Compounds with activities between chlorothiazide and controls are scored +1 or ±. [e] J. B. Bicking, C. M. Robb, S. F. Kwong, and E. J. Cragoe, Jr., *J. Med. Chem.*, **10**, 598 (1967). [f] See ref 1. [g] K. L. Shepard, J. W. Mason, O. W. Woltersdorf, Jr., J. H. Jones, and E. J. Cragoe, Jr., *J. Med. Chem.*, **12**, 280 (1969).

(1) J. B. Bicking, J. W. Mason, O. W. Woltersdorf, Jr., J. H. Jones, S. F. Kwong, C. M. Robb, and E. J. Cragoe, Jr., *J. Med. Chem.*, **8**, 638 (1965).

(2) E. J. Cragoe, Jr., O. W. Woltersdorf, Jr., J. B. Bicking, S. F. Kwong, and J. H. Jones, *ibid.*, **10**, 66 (1967).