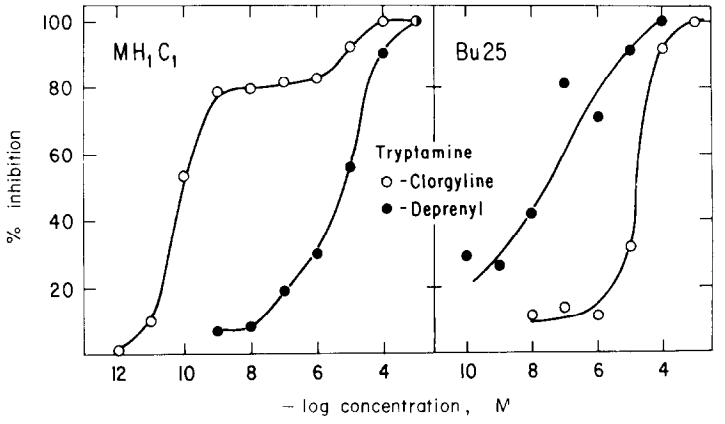

Fig. 4. Inhibition of MAO activity in crude mitochondrial preparations of rat hepatoma MH_1C_1 and human HeLa Bu25 cells using [^{3}H]tryptamine. Control activities were 1.6 and 0.008 nmoles/min/mg of protein in MH_1C_1 and Bu25 respectively. Data are shown from one of two similar experiments.

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Biochemical Pharmacology, Vol. 28, pp. 528–530.

0006-2952/79/0215-0528 $02.00/0

Inhibition *in vitro* of rabbit adrenal norepinephrine *N*-methyltransferase by 2,3,4,5-tetrahydro-1H-2-benzazepines

(*Received* 2 *February* 1978; *accepted* 13 *June* 1978)

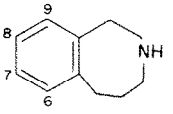
Previously we reported that phenylethylamine and benzylamine analogs with rigid conformation were inhibitors of norepinephrine *N*-methyltransferase (NMT) (EC 2.1.1.28), the epinephrine-forming enzyme. For example, 2-aminotetralins [1] and 1-aminoindans [2] were more potent inhibitors of norepinephrine *N*-methyltransferase than were their non-cyclized analogs, phenylethylamines [3, 4] and benzylamines [5]. The compounds described here, 2,3,4,5-tetrahydro-1H-2-benzazepines, can be viewed as benzylamine analogs with the amino group connected to the ortho position of the ring via a propyl group. The chlorinated compounds in this series are generally more active inhibitors of norepinephrine *N*-methyltransferase than are the corresponding benzylamines.

Rabbit adrenal glands were purchased from Pel-Freez Biologicals, Inc., Rogers, AR. Norepinephrine *N*-methyltransferase was prepared by ammonium sulfate fractionation of the supernatant fluid after high speed centrifugation of adrenal homogenates and assayed as described previously [6]. Enzyme activity was assayed radiometrically with L-norepinephrine bitartrate (Winthrop) as the methyl-accepting substrate (40 μM unless otherwise indicated) and *S*-adenosyl-L-methionine[methyl-^{14}C] (New England Nuclear) as the methyl donor (20 μM unless otherwise indicated). The formation of radioactive epinephrine was measured after precipitation of the unreacted methyl donor with Reinecke salt (ammonium tetrathiocyanoammonochromate). Inhibitors were tested at four to six concentrations and

pI_{50} values (negative logarithm of the molar concentration required for 50 per cent inhibition) were determined by interpolation on a graph of per cent inhibition (linear scale) versus inhibitor concentration (log scale). All of the inhibitors were synthesized in the Lilly Research Laboratories, and their identities and purity were verified by physicochemical methods.

Table 1 shows the per cent inhibition of norepinephrine *N*-methyltransferase by 2,3,4,5-tetrahydro-1H-2-benzazepine, the four monochloro-substituted position isomers, and three dichloro compounds. The pI_{50} values calculated from these data can be compared directly to those reported earlier [5], since all determinations were made with the same substrate concentrations. The p_{50} value for the unsubstituted compound was 1.53 units higher than that for benzylamine [5], i.e. restricting the conformation of benzylamine in this way increased its inhibitor potency more than 30-fold. Except for the 6-chloro compound, compounds in Table 1 were more potent inhibitors than the corresponding benzylamines [5]. The most improvement was with the 7,8-dichloro compound, whose pI_{50} was 1.71 units higher than that of 3,4-dichlorobenzylamine. This compound was chosen for further study, though two other inhibitors in Table 1 had pI_{50} values above 6 (caused 50 per cent inhibition of norepinephrine *N*-methyltransferase at concentrations less than 10^{-6} M).

Table 1. Inhibition of norepinephrine *N*-methyltransferase by 2,3,4,5-tetrahydro-1H-2-benzazepines



Ring substituent	% Inhibition 0.1 μM	0.3 μM	1 μM	3 μM	10 μM	32 μM	pI_{50}
None			0	7	26	60	4.65
6-Chloro			1	11	29	62	4.68
7-Chloro		4	25	54	80	91	5.57
8-Chloro	13	37	72	90	96		6.31
9-Chloro	3	10	34	68	88	95	5.76
6,7-Dichloro	3	11	37	71			5.81
7,8-Dichloro	21	67	92	98	100		6.68
8,9-Dichloro		38	84	92	98	99	6.37

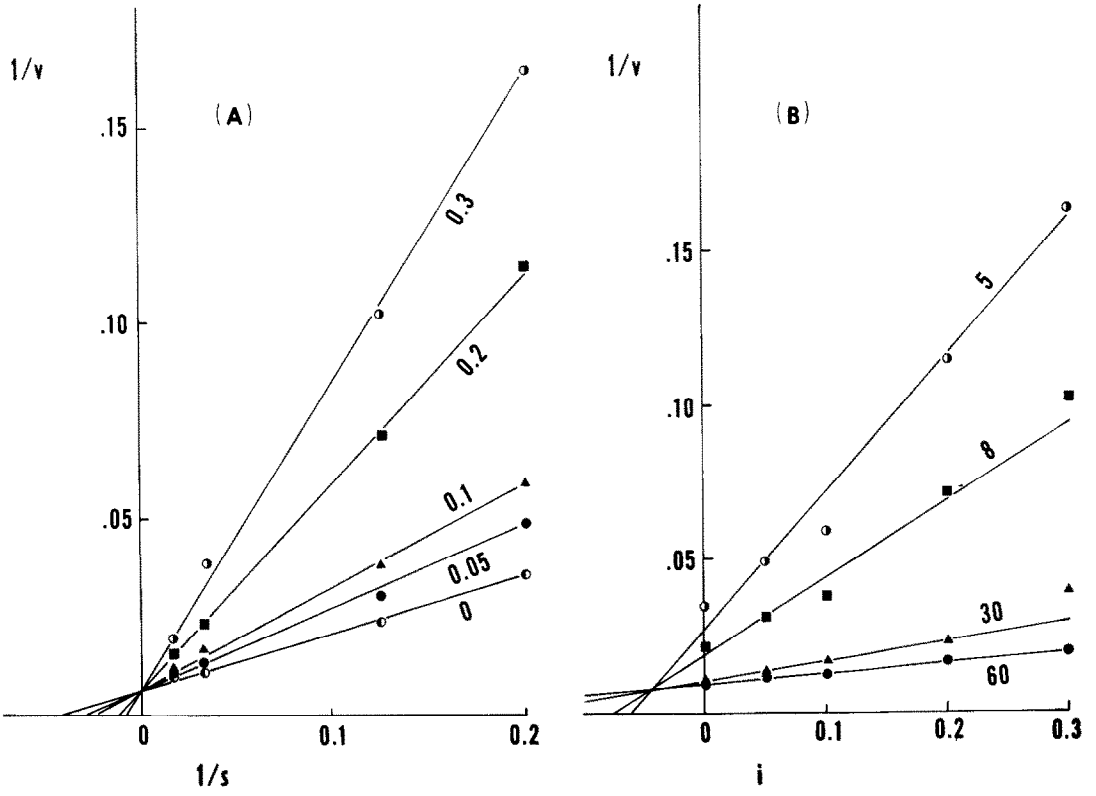


Fig. 1. Competitive kinetics in the inhibition of NMT by 7,8-dichloro-2,3,4,5-tetrahydro-1 H-2-benzazepine with L-norepinephrine as the variable substrate. (A) Lineweaver–Burk plot. L-Norepinephrine concentrations (*s*) were 5, 8, 12, 20 and 60 μM. Velocity (*v*) units were pmol product formed 30 min of incubation. The inhibitor concentration was 0, 0.05, 0.1, 0.2 or 3 μM as indicated. (B) Dixon plot for determination of the K_i value of 7,8-dichloro-2,3,4,5-tetrahydro-1H-2-benzazepine. The reciprocal of velocity (*v*), in pmol product formed 30 min of incubation, is plotted against the μM concentration of inhibitor (*i*). The L-norepinephrine concentration was 5, 8, 30 or 60 μM as indicated.

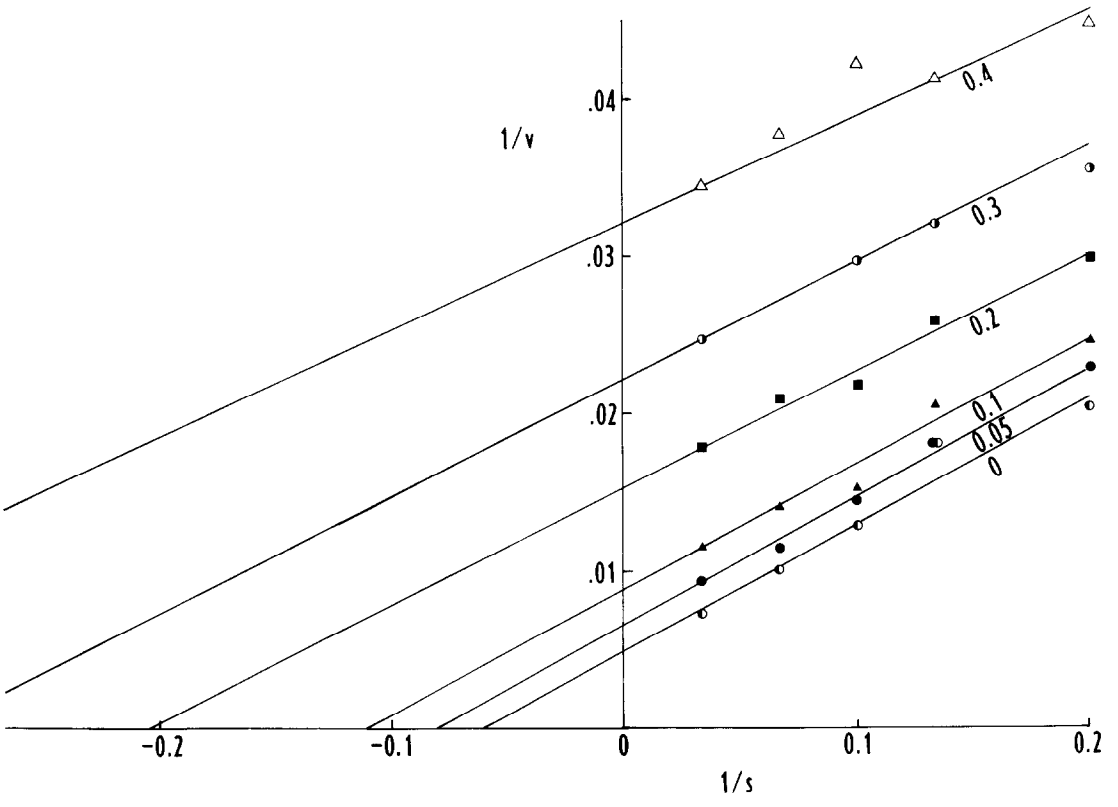


Fig. 2. Uncompetitive kinetics in the inhibition of NMT by 7,8-dichloro-2,3,4,5-tetrahydro-1H-2-benzazepine with *S*-adenosylmethionine as the variable substrate (Lineweaver–Burk plot). *S*-adenosylmethionine concentrations (*s*) were 5, 7.5, 10, 15 and 30 μM. Velocity (*v*) units were pmol product formed/30 min of incubation. The inhibitor concentration was 0.05, 0.1, 0.2, 0.3 or 0.4 μM as indicated.

The inhibition of norepinephrine *N*-methyltransferase by the 7,8-dichloro compound was competitive with L-norephrine as the variable substrate (Fig. 1A). Similar kinetic properties were observed earlier for benzylamines [5]. Figure 1B shows a Dixon plot, from which a K_i value of 0.043 μM was calculated. This inhibitor, 7,8-dichloro-2,3,4,5-tetrahydro-1H-2-benzazepine, is similar in structure and inhibitory potency to 7,8-dichloro-1,2,3,4-tetrahydroisoquinoline, an inhibitor of norepinephrine *N*-methyltransferase recently reported by Pendleton *et al.* [7]. 7,8-Dichloro-2,3,4,5-tetrahydro-1H-2-benzazepine was tested as a substrate for norepinephrine *N*-methyltransferase at 10^{-6}, 10^{-5}, 10^{-4} and 10^{-3} M concentrations, but no substrate activity was detected. With *S*-adenosylmethionine as the variable substrate, the inhibition showed uncompetitive kinetics (Fig. 2). This property also is similar to that of 7,8-dichloro-1,2,3,4-tetrahydroisoquinoline [7].

Acknowledgement—We thank Betty W. Roush for technical assistance.

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