In vitro and *in vivo* tests of phenylephrine and phenylpropanolamine products, obtained by the facilitated molecular entrapment method, confirmed the pharmaceutical utility of this technique.

These results indicate that the drug–polymer entrapment products prepared by the facilitation method possess considerable potential for exploitation as sustained-action or controlled-release pharmaceuticals.

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Selected Pharmacological Studies of a Series of Substituted Imidazo(4,5-*d*)pyridazines

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Abstract □ A general pharmacological screening program was applied to a series of 10 substituted imidazo(4,5-*d*)pyridazine compounds. All of the compounds produced depression of spontaneous activity in rats, and six of the compounds produced significant lengthening of hexobarbital "sleeping times." In addition, two of the compounds produced a partial reversal of reserpine hypothermia, measured by a rectal temperature monitoring apparatus, and seven of the compounds produced significant inhibition of monoamine oxidase using a Warburg apparatus. One compound produced monoamine oxidase inhibition when a spectrophotometric assay was employed.

Keyphrases □ Imidazo(4,5-*d*)pyridazines—pharmacological screening □ CNS activity—imidazo(4,5-*d*)pyridazines □ Monoamine oxidase inhibition—imidazo(4,5-*d*)pyridazines □ Reserpine-induced hypothermia—imidazo(4,5-*d*)pyridazine blocking effect

In recent years, considerable interest has arisen in the development of compounds having potential antineoplastic effects. Robins (1), for example, reported the synthesis of a series of over 1300 purine derivatives and included antineoplastic screening data on active compounds. The compounds studied in this report, the imidazo(4,5-*d*)pyridazines, were synthesized by Gerhardt *et al.* (2) as possible purine antimetabolites and are representative of 75 compounds in that series (Table I).

Little has been reported concerning the pharmacology of imidazopyridazines or similar compounds, other than antineoplastic screening data. Dimmling and Hein (3) found that certain types of imidazole derivatives decreased contractility of the frog heart and produced damage to leukocytes and macrophages. Certain pyrimidazole derivatives have shown local anesthetic effects (4), and other pyridine and pyridine–pyrrolidine imidazole compounds have been shown to have CNS depressant effects (5). Rinaldi *et al.* (6) showed some protection of mice to the effects of X-ray exposure with imidazole and benzimidazole derivatives. Certain derivatives of phenylimidazopyridine carboxylic acid have been shown to have diuretic effects (7). It was felt that a survey of the general pharmacological properties of this series of compounds might be useful to further consideration of them as possible antineoplastic agents. In this paper, studies of certain effects of these compounds on the CNS are reported.

Table I—Chemical Names of the Compounds

Compound	Chemical Name
1	1*H*-Imidazo[4,5-*d*]pyridazine-2,4,7-triol
2	1-Benzyl-2-mercapto-1*H*-imidazo[4,5-*d*]pyridazine-4,7-diol
3	1-Benzyl-2-(isopentylthio)-1*H*-imidazo[4,5-*d*]pyridazine-4,7-diol
4	1-Benzyl-2-(cyclopentylthio)-1*H*-imidazo[4,5-*d*]pyridazine-4,7-diol
5	1-Benzyl-2-[(*p*-fluorobenzyl)thio]-1*H*-imidazo[4,5-*d*]-pyridazine-4,7-diol
6	1-Benzyl-2-[(*m*-fluorobenzyl)thio]-1*H*-imidazo[4,5-*d*]-pyridazine-4,7-diol
7	1-Benzyl-2-[(2,4-dichlorobenzyl)thio]-1*H*-imidazo[4,5-*d*]-pyridazine-4,7-diol
8	1-Benzyl-2-[(2,6-dichlorobenzyl)thio]-1*H*-imidazo-[4,5-*d*]pyridazine-4,7-diol
9	1-Benzyl-2-[(*p*-bromobenzyl)thio]-1*H*-imidazo[4,5-*d*]-pyridazine-4,7-diol
10	1-Benzyl-2-[(*o*-iodobenzyl)thio]-1*H*-imidazo[4,5-*d*]-pyridazine-4,7-diol

EXPERIMENTAL

Evaluation of Gross CNS Effects—Adult Houston-Cheek albino rats, weighing between 200 and 350 g., were used for measurements of spontaneous activity and for determinations of hexobarbital "sleeping time." Spontaneous activity measurements were done using a "light box" similar to that proposed by Dews (8). Each animal was injected with a dose intraperitoneally of drug suspended in alkalinized distilled water (pH 9), placed in a stimulus-free chamber for 15 min. to allow for absorption of the drug, then placed in the activity box, and monitored for spontaneous activity for 1 hr.

Table II—Effect of the Compounds on Spontaneous Activity in Rats (counts/hr.)[a]

Compound	Control	5 mg./kg.	10 mg./kg.	20 mg./kg.	40 mg./kg.	80 mg./kg.
1	186 ± 14[b]	133 ± 12	261 ± 33	133 ± 33	162 ± 38	131 ± 38
2	186 ± 14	123 ± 22	125 ± 24	135 ± 16	95 ± 15	117 ± 11
3	186 ± 14	118 ± 13	94 ± 12	100 ± 15	72 ± 13	57 ± 11
4	186 ± 14	130 ± 23	123 ± 19	87 ± 18	80 ± 17	83 ± 10
5	186 ± 14	127 ± 24	73 ± 18	69 ± 19	76 ± 17	96 ± 25
6	186 ± 14	95 ± 16	134 ± 23	116 ± 13	64 ± 11	38 ± 9
7	186 ± 14	69 ± 5	85 ± 15	56 ± 8	78 ± 11	99 ± 23
8	186 ± 14	92 ± 14	84 ± 22	62 ± 8	57 ± 18	57 ± 16
9	186 ± 14	71 ± 16	95 ± 11	55 ± 7	50 ± 18	22 ± 9
10	186 ± 14	76 ± 16	64 ± 10	34 ± 6	40 ± 14	23 ± 4

[a] Each value represents the mean of five trials. [b] Standard error of the mean.

Results were compared to controls having identical treatment except for the use of the test drug.

As a corollary study, rats were tested for the effect of the compounds on the hexobarbital sleeping time. The animals were injected with 20 mg./kg., i.p., of the compound to be tested 2 hr. prior to the administration of 50 mg./kg., i.p., hexobarbital. The length of sleep between the loss and regaining of the righting reflex (sleeping time) was then determined for each animal, and the results were compared to controls (9, 10).

Evaluation of Monoamine Oxidase Inhibition—Initial screening for possible monoamine oxidase (MAO) inhibition was done using the "reserpine reversal" technique of Pletscher (11). Adult Lemberger albino mice, weighing between 25 and 40 g., were restrained in plastic holders; their rectal temperatures were monitored using an electronic thermometer equipped with temperature-sensing probes.[1]

The ability of the compounds to reverse the hypothermic effect of reserpine was measured, using four mice for each trial: two control animals received 2.5 mg./kg., i.p., reserpine and two experimental animals were pretreated with 100 mg./kg., i.p., of the compound to be tested 1 hr. prior to treatment with 2.5 mg./kg. reserpine. A small number of animals received 100 mg./kg., i.p., iproniazid to serve as a standard of MAO inhibition. The rectal temperatures were monitored for 4 hr., and the effects of the drugs on the reserpine-induced hypothermia were noted.

As a further evaluation of possible MAO inhibition, the effects of the compounds on rat brain mitochondria were investigated. Brain mitochondria were prepared, according to the method of Brody and Bain (12), and MAO activity was determined in the presence or absence of inhibitor by conventional manometric techniques (13) or spectrophotometrically (14). In the manometric determinations, Warburg flasks contained in the main compartment a final concentration of 0.01 *M* phosphate buffer (pH 7.8), 0.017 *M* compound, and the mitochondrial suspension representing 50 mg. wet weight of tissue in a total volume of 1.8 ml. The side arm of the flask contained 0.3 ml. of 0.1 *M* tyramine. After the flasks were allowed to equilibrate at 37° for 15 min., the manometer valves were closed and the side arm contents of each flask were tipped in. Oxygen uptake was recorded at 10-min. intervals for a total period of 90 min.

Those compounds which showed significant MAO inhibition using the manometric technique were then tested using a direct spectrophotometric assay for MAO activity. The 3 ml. reaction mixture contained in a final concentration 0.03% commercial emulsifier,[2] 0.09 *M* phosphate buffer (pH 7.4), 0.00016 *M* compound, and 0.017 *M* *p*-dimethylaminobenzylamine hydrochloride, with mitochondrial suspension representing 50 mg. wet weight of tissue. The conversion of *p*-dimethylaminobenzylamine hydrochloride to its aldehyde derivative by MAO was observed by determining the increase in density of the solution at 355 mμ, according to the method of Deitrich and Erwin (14). Readings were taken at 1-min. intervals for 5 min. after the reaction began.

RESULTS AND DISCUSSION

All imidazopyridazine derivatives, except Compound 1, produced significant depression of spontaneous activity at all dosage levels employed (5–80 mg./kg.). Compound 1 produced significant depression of spontaneous activity at all dosages except 10 mg./kg. (Table II). Compounds 1, 2, 4, 6, 8, and 10 produced significant prolongation of hexobarbital sleeping time (Table III).

Table III—Effects of the Compounds on the Hexobarbital "Sleeping Times" of Rats (Hexobarbital 50 mg./kg.)

Compound	Number of Animals	Dose, mg./kg.	Sleeping Time, min.	*p*
Control	28	—	44 ± 3	—
1	12	20	68 ± 6[a]	0.001
2	12	20	64 ± 4[a]	0.001
3	9	20	55 ± 5	0.10
4	10	20	65 ± 4[a]	0.001
5	12	20	34 ± 4	0.10
6	12	20	64 ± 5[a]	0.01
7	12	20	43 ± 4	0.30
8	11	20	73 ± 5[a]	0.001
9	12	20	46 ± 6	0.30
10	12	20	57 ± 4[a]	0.05

[a] Significant lengthening of sleeping time.

None of the 10 compounds produced complete reversal of reserpine-induced hypothermia in mice. Compounds 5 and 8 produced a partial reversal of the induced hypothermia, although the results were not significant at the $p = 0.05$ level (Table IV).

Table IV—Effects of the Compounds in Blocking Reserpine-Induced Hypothermia in Mice

Compound	Control[a]	Experimental
1	−9.0°[b]	−7.8°
	−8.4°	−10.0°
2	−7.6°	−7.6°
	−9.4°	−6.1°
3	−8.6°	−8.4°
	−8.7°	−12.4°
4	−10.9°	−9.4°
	−8.5°	−6.5°
5	−9.6°	−6.8°
	−10.0°	−4.5°
6	−8.1°	−11.4°
	−8.7°	−5.6°
7	−10.1°	−7.8°
	−8.9°	−10.8°
8	−6.4°	−3.4°
	−9.5°	−6.5°
9	−5.6°	−7.8°
	−6.5°	−6.2°
10	−9.4°	−7.6°
	−7.8°	−8.8°
Iproniazid	−7.2°	+1.4°
	−7.9°	−2.2°

[a] "Controls" were treated with 2.5 mg./kg., i.p., reserpine and received 0.3 ml. normal saline solution to replace the compound. [b] Values represent change in rectal temperature for 4-hr. period.

[1] TRI-R model TML, TRI-R Instruments.
[2] Lubrol 90, I.C.I./Organics/Inc., Providence, RI 02901

Table V—Effect of the Compounds on the Oxidation of Tyramine by Rat Brain Mitochondria[a]

Compound[b]	Time, min. 10 *p*	30 *p*	50 *p*	70 *p*	90 *p*
1	5[a] ± 1[b] 0.01	15[c] ± 1 0.10	25[c] ± 1 0.30	31[c] ± 1 0.20	41[c] ± 2 0.30
2	4 ± 1 0.01	13 ± 1 0.01	20 ± 1 0.01	27 ± 2 0.01	39[c] ± 2 0.20
3	2 ± 1 0.001	10 ± 1 0.01	19 ± 1 0.01	25 ± 3 0.01	37[c] ± 2 0.10
4	0 ± 1 0.001	2 ± 1 0.001	3 ± 1 0.001	7 ± 1 0.001	9 ± 2 0.001
5	2 ± 1 0.001	6 ± 1 0.001	10 ± 1 0.001	14 ± 2 0.001	19 ± 2 0.001
6	1 ± 1 0.001	5 ± 1 0.001	10 ± 1 0.001	13 ± 2 0.001	19 ± 2 0.001
7	3 ± 1 0.01	11 ± 1 0.01	18 ± 1 0.01	24 ± 2 0.01	34 ± 2 0.01
8	4 ± 1 0.01	7 ± 1 0.001	13 ± 2 0.01	19 ± 2 0.01	27 ± 2 0.01
9	5 ± 1 0.01	14 ± 1 0.01	22 ± 1 0.01	29 ± 1 0.01	36 ± 1 0.01
10	3 ± 1 0.01	9 ± 1 0.01	17 ± 1 0.01	22 ± 1 0.01	28 ± 1 0.01
Control	8 ± 1 —	18 ± 1 —	26 ± 1 —	34 ± 1 —	42 ± 1 —

[a] Values represent mean of the microliters of oxygen uptake in 12 individual flasks. [b] Standard error of the mean. [c] Nonsignificant.

In studies using the Warburg respirometer, Compounds 4–10 produced significant inhibition of monoamine oxidase at the end of 90 min. (Table V). Upon further testing of these compounds with the spectrophotometric technique, it was found that Compound 9 produced noncompetitive MAO inhibition (Fig. 1).

While Compounds 4–10 produced significant MAO inhibition in the Warburg studies, only Compound 9 produced significant inhibition of the enzyme in the spectrophotometric assays. Several factors may be involved in the explanation of these findings. The concentrations of the drugs used were quite different in the two studies. The concentration of the compounds used in the Warburg studies was approximately 100 times that of the compounds used in the spectrophotometric assays. The substrates used were different in the two studies, and thus the influence of the inhibiting drug on the reaction system might have been different in each case. Finally, if the inhibition seen was irreversible, the 15-min. preequilibration in the Warburg flasks in the absence of substrate would be quite different from the conditions in the spectrophotometric studies, in which the inhibitor and substrate were added essentially together with no preequilibration time allowed.

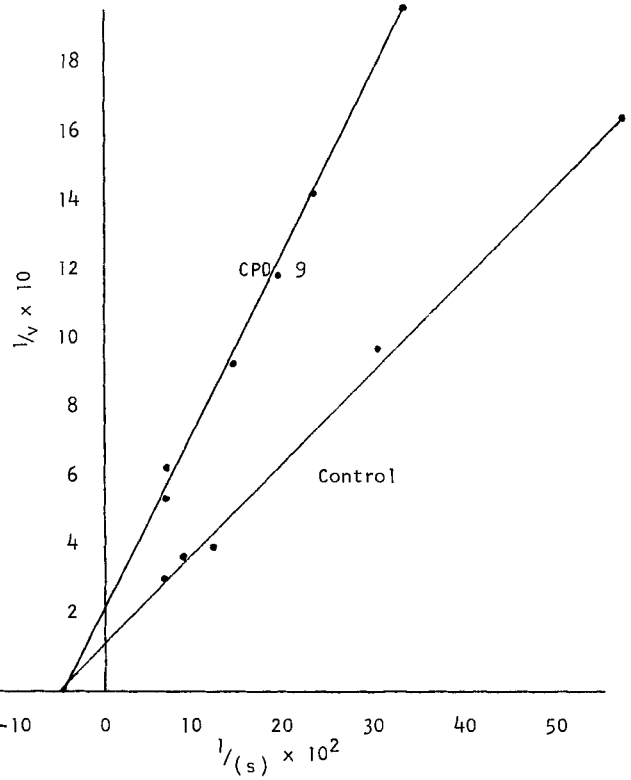

Figure 1—*MAO inhibition of Compound 9.*

SUMMARY

This study involved the evaluation of pharmacological activity of a group of 10 imidazo(4,5-*d*)pyridazine compounds which were originally synthesized as antineoplastic agents.

All of the compounds were found to exert CNS depressant effects. Compounds 1, 2, 4, 6, 8, and 10 prolonged the hexobarbital sleeping time in rats. Compounds 5 and 8 produced a partial reversal of reserpine-induced hypothermia in mice.

Compounds 4–10 inhibited MAO in Warburg studies, and Compound 9 inhibited MAO in a spectrophotometric assay technique.

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