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Synthesis and Antidepressant Activity of 5-(4-Dimethylaminobenzyl)imidazolidine-2,4-dione

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Abstract □ 5-(4-Dimethylaminobenzyl)imidazolidine-2,4-dione was prepared by catalytic hydrogenation of the corresponding benzylidene compound. Antidepressant testing in mice indicated that its ED_{50} values for antagonism of tetrabenazine-induced ptosis and potentiation of levodopa-induced behavioral changes were 42 and 17 mg/kg po, respectively. *In vitro* neurochemical studies demonstrated that this compound did not inhibit the uptake of selected biogenic amines into crude synaptosomes of mouse whole brain, and it did not have significant monoamine oxidase inhibitory activity *in vivo* and *in vitro*. Thus, this compound possesses potential antidepressant activity with a mechanism different from that of the tricyclic antidepressants and monoamine oxidase inhibitors.

Keyphrases □ 5-(4-Dimethylaminobenzyl)imidazolidine-2,4-dione—synthesis and testing for antidepressant activity □ Antidepressant activity—5-(4-dimethylaminobenzyl)imidazolidine-2,4-dione, *in vitro* and *in vivo* testing

The tricyclic antidepressant drugs are the most useful drugs for the treatment of depression. However, these drugs are not effective in all patients and are not without compromising side effects (1, 2). Monoamine oxidase inhibitors also are effective antidepressants; however, because of their propensity to cause hypertensive crisis, they now have limited use in the treatment of depression (3).

As part of a program directed at the discovery and development of novel agents affecting the central nervous system (CNS), 5-(4-dimethylaminobenzyl)imidazolidine-2,4-dione (I) was synthesized as a chemical intermediate and unexpectedly demonstrated antidepressant activity in selected experimental models (4). This paper presents the synthesis and results of pharmacological testing of this compound.

EXPERIMENTAL[1]

Synthesis of 5-(4-Dimethylaminobenzyl)imidazolidine-2,4-dione (I)—A mixture of 69.3 g (0.30 mole) of 5-(4-dimethylaminobenzylidene)imidazolidine-2,4-dione (IV) (5), 600 ml of 1 *N* KOH, and 15 g of wet No. 28 Raney nickel catalyst was shaken with hydrogen at 3–4 atm for 28 hr until the theoretical quantity was consumed. The catalyst was washed with two 100-ml portions of 1 *N* KOH, and the filtrate and combined washings were acidified with acetic acid to give the crude product (60 g). Recrystallization from methanol gave 35 g (50%) of I, mp 180–186°; IR: 3.10, 3.21 (NH), 5.66, 5.90 (C=O), and 6.20 (C=C) μm; NMR (dimethyl sulfoxide-d_6): δ 2.75–2.85 [m, 8, $(CH_3)_2N$ and aryl CH_2], 4.20 (t, 1, CH_2CHNH), 6.60, 7.01 (d, 4, J = 9 Hz, aromatic CH), 7.78 (broad exchangeable s, 1, imidazolidine 1-NH), and 11.0 (broad exchangeable m, 1, imidazolidine 3-NH).

Anal.—Calc. for $C_{12}H_{15}N_3O_2$: C, 61.78; H, 6.48; N, 18.02. Found: C, 61.79; H, 6.45; N, 18.02.

Tetrabenazine Antagonism—The method used was similar to that described by Barnett *et al.* (6). Groups of five unfasted male mice (TAC:SW/N fBr), 20–27 g, were pretreated with the test compound at 50 mg/kg po as a 0.5% suspension in 0.5% methylcellulose 4000 cps[2] or methylcellulose alone. Thirty minutes later, each animal received tetrabenazine methanesulfonate[3] (36 mg/kg ip in saline) or saline at a volume of 10 ml/kg. After an additional 30 min, the degree of palpebral narrowing was estimated as a measure of ptosis and compared to the methylcellulose and tetrabenazine control groups. The degree of ptosis was evaluated using a rating scale of 0–4, with a score of 4 representing a normal palpebral opening and scores of 3, 2, 1, and 0 representing slight, moderate, marked, and complete active closure of the palpebral opening, respectively. The percent prevention of ptosis was calculated as described by Barnett *et al.* (6).

Compound I at a dose of 50 mg/kg antagonized the effect of tetrabenazine by 50% or greater and, therefore, was evaluated further for dose-response effect as an ED_{50} value. The method described by Litchfield and Wilcoxon (7) was used for determining the ED_{50} value.

Levodopa Potentiation—The levodopa potentiation model is a reflection of increased dopaminergic activity, and the method used was that described by Everett (8). Groups of unfasted male mice [TAC:(SW)fBr] were administered pargyline[4] at a dose of 40 mg/kg po (0.4% in 0.5% methylcellulose) 4 hr prior to the administration of graded doses of test drugs or methylcellulose only. One hour after administration of the test drugs, levodopa (100 mg/kg ip, 1% in saline) was administered to all mice.

Observations of the levodopa-induced response were recorded 25 min after administration on a scale similar to that described by Everett (8). The degree of potentiation was scored as: 0 = no unusual irritability; 1 = increased alertness and responsiveness to touch; 2 = aggressive response to touch (hyperreactive); and 3 = markedly aggressive response to touch, spontaneous fighting with salivation and piloerection, or near prostrate. The percent of levodopa potentiation was calculated as described by Everett (8). The ED_{50} values were obtained using the method described by Litchfield and Wilcoxon (7).

Tryptamine Potentiation—Tedeschi *et al.* (9) hypothesized that tryptamine increases brain serotonin levels but that convulsions do not occur because the destruction of serotonin by monoamine oxidase keeps pace with its formation. The interruption of the metabolism of serotonin (*i.e.*, by monoamine oxidase inhibitors) and tryptamine will cause convulsions. The method used was similar to that described by Tedeschi *et al.* (9). Groups of 10 unfasted male mice [TAC:(SW)fBr] were administered the test compounds suspended in 0.5% methylcellulose at a dose 10 times their levodopa potentiation ED_{50} values. Four hours later, tryptamine[5] was administered at 50 mg/kg iv. The compounds were considered to potentiate tryptamine if >50% of the animals in the treated group convulsed.

[1] Melting points were determined on a Mel-Temp apparatus and are corrected. The IR spectrum was determined as a mineral oil mull using a Perkin-Elmer 137B spectrophotometer. The NMR spectrum was obtained on a Varian A-60 instrument and was compared with tetramethylsilane as the internal standard.

[2] Methocel, Dow Chemical Co.
[3] Hoffmann-La Roche.
[4] Abbott Laboratories.
[5] Aldrich Chemical Co.

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Table I—Effect of I and Selected Compounds in Various Tests for Potential Antidepressant Activity

Compound	Anti-tetrabenazine[a] ED_{50}, mg/kg po	Levodopa[b] Potentiation ED_{50}, mg/kg po	Potentiation of Tryptamine[c]	Biogenic Amine[d] Uptake Inhibition *In Vitro* IC_{50}, μM			Inhibition[e] of Liver Monoamine Oxidase *In Vitro* IC_{50}, μM
				Norepinephrine	5-Hydroxy-tryptamine	Dopamine	
I	42 (14–126)	17 (8–38)	No	>100	>100	>100	44 ± 4.0
Desipramine	0.25 (0.03–0.58)	1.1 (0.5–2.3)	No	6.6 ± 1.3	0.6 ± 0.06	8.6 ± 1.3	75 ± 13.0
Imipramine	1.3 (0.8–2.1)	5.0 (2.3–11)	No	15 ± 3	0.11 ± 0.02	12 ± 1.0	23 ± 5.0
Pargyline	25 (3–81)	5.1 (2.2–12)	Yes[f]	Inactive	—	—	0.14 ± 0.01
Benzotropine	—	—	—	0.35 ± 0.11	11 ± 2.0	0.17 ± 0.02	—

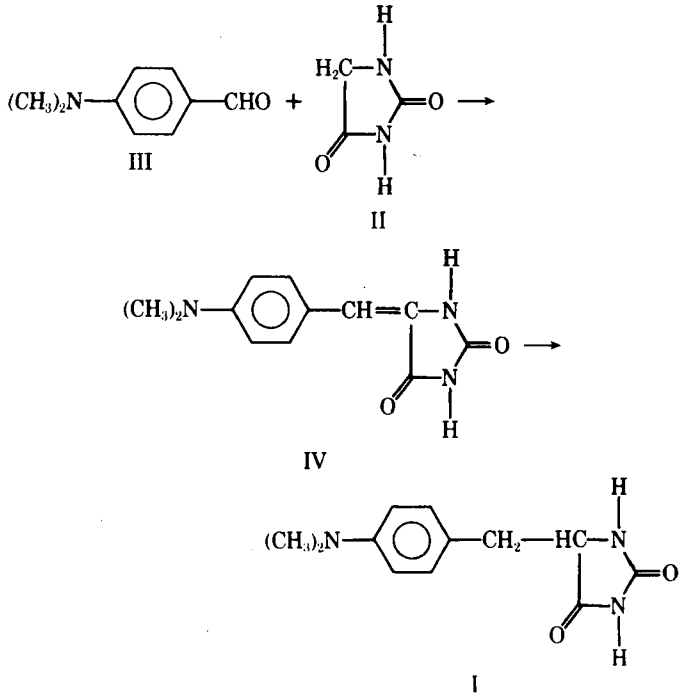
[a] Dose of the compound that inhibited tetrabenazine-induced ptosis in 50% of the mice. The 95% confidence limits are indicated in the parentheses. [b] Dose of the compound that potentiated the response of levodopa in 50% of the mice. The 95% confidence limits are indicated in the parentheses. [c] Dose of the compound that potentiated the response of tryptamine in mice. Tested at a dose 10 times or greater than the levodopa ED_{50}. [d] Concentration of the compound that caused 50% inhibition of the uptake in mice crude synaptosomes of norepinephrine, 5-hydroxytryptamine, and dopamine (mean ± *SE*). [e] Concentration of the compound that inhibited monoamine oxidase by 50% (mean ± *SE*). [f] Dose of pargyline was 100 mg/kg, approximately four times the antitetrabenazine ED_{50} value.

Inhibition of Biogenic Transmitter Uptake into Crude Synaptosomes of Mouse Brain *In Vitro*—A modification of the technique described by Kuhar (10) was used. The procedure included isolation of crude synaptosomes of mouse whole brain by differential centrifugation, incubation of synaptosomes with the test compound and a tracer amount of tritiated neurotransmitter[6] (norepinephrine, 5-hydroxytryptamine, and dopamine), separation of the free neurotransmitter by vacuum filtration, and radioactivity measurement of the bound neurotransmitter remaining on the cellulose membrane[7] filter by means of liquid scintillation spectrophotometry. The micromolar concentrations of test compound producing 50% inhibition of neurotransmitter uptake (IC_{50}) were estimated from log dose–response curves based on at least three concentrations with at least three determinations at each concentration.

***In Vitro* Monoamine Oxidase Inhibition in Mouse Liver—**The method used was essentially that described by Wurtman and Axelrod (11) with [side chain-2-^{14}C]tryptamine as the substrate. The micromolar concentrations of test compound producing 50% inhibition of monoamine oxidase (IC_{50}) were estimated from log dose–response curves on three concentrations with three determinations at each concentration.

RESULTS AND DISCUSSION

Condensation of imidazolidine-2,4-dione (II) with the aldehyde (III) gave rise to a benzylidene compound (IV) (5) which, upon catalytic hydrogenation in the presence of Raney nickel, afforded 5-(4-dimethylaminobenzyl)imidazolidine-2,4-dione (I) (Scheme I).



Scheme I

In preliminary testing at a dose of 50 mg/kg po, I inhibited tetrabenazine-induced ptosis by >90%. Therefore, a dose–response effect (7) was established and an antitetrabenazine ED_{50} value of 42 mg/kg was estimated (Table I).

This antidepressant activity of I was evaluated further using the levodopa potentiation test, which provides a measure of antidepressant activity more clearly related to CNS events (8) than to responses involving peripheral biogenic amines, such as the eyelid (ptosis) response to tetrabenazine. The usefulness of the levodopa potentiation model rests on its being a behavioral response and a reflection of the CNS effects of increased dopaminergic activity (8). However, this test is particularly sensitive to monoamine oxidase inhibitors because of the requirement for monoamine oxidase inhibitor pretreatment. In this test, I potentiated the levodopa response, and the estimated oral ED_{50} value was 17 mg/kg. Two tricyclic antidepressants, desipramine and imipramine, as well as a monoamine oxidase inhibitor, pargyline, also were effective with oral ED_{50} values of 1.1, 5.0, and 5.1 mg/kg, respectively.

Since the levodopa test is highly susceptible to monoamine oxidase inhibition, and since I was found to be an active potentiator of levodopa, I was evaluated for potentiation of tryptamine-induced convulsions. When administered intravenously, tryptamine causes an increase in brain 5-hydroxytryptamine levels (9). However, convulsions do not occur because the rate of destruction of 5-hydroxytryptamine by monoamine oxidase is directly related to the formation of 5-hydroxytryptamine. But in the presence of a monoamine oxidase inhibitor, with the metabolism of 5-hydroxytryptamine interrupted, tryptamine causes convulsions. This action is presumptive evidence of monoamine oxidase inhibitory activity (9). In this test, I at a dose of 174 mg/kg po (greater than 10 times the levodopa potentiating ED_{50}), as well as the tricyclic antidepressants, did not potentiate the tryptamine response, indicating that the potentiation of levodopa action was not a function of monoamine oxidase inhibition. On the other hand, pargyline at a dose of 100 mg/kg po did potentiate the tryptamine response, indicating the involvement of monoamine oxidase inhibition.

Compound I then was evaluated for effects on the uptake of selected biogenic amines *in vitro* (10). At a concentration of 100 μM, I did not prevent the uptake of norepinephrine, 5-hydroxytryptamine, and dopamine into crude synaptosomes prepared from the whole mouse brain, whereas the reference drugs all were effective. Desipramine inhibited norepinephrine uptake with an IC_{50} value of 6.6 μM, imipramine inhibited 5-hydroxytryptamine uptake with an IC_{50} value of 0.11 μM, and benzotropine inhibited dopamine uptake with an IC_{50} value of 0.17 μM.

In vitro monoamine oxidase of mouse liver (11) was inhibited by I with an IC_{50} value of 44 μM. However, I was more than 100 times less active than pargyline (IC_{50} 0.14 μM) under the same conditions.

Table I summarizes the results of the pharmacological evaluation presented in this paper.

[6] New England Nuclear Corp.
[7] Millipore type HA, 0.45 μm.

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Evaluation of 2-Azabicyclo[2.2.2]octane Analogs of 4-Anilidopiperidine Analgesics

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Abstract □ Eight analogs of the fentanyl-type analgesics, in which the piperidine ring is restricted into a boat conformation, were evaluated for analgesic activity. All analogs were less active than fentanyl, but interesting conformational and structural relationships were observed. Results of the study are discussed.

Keyphrases □ Analgesic activity—evaluation of 2-azabicyclo[2.2.2]-octane analogs of 4-anilidopiperidines, mice □ 4-Anilidopiperidines—2-azabicyclo[2.2.2]octane analogs, evaluation for analgesic activity, mice □ Structure–activity relationships—evaluation of 2-azabicyclo[2.2.2]-octane analogs of 4-anilidopiperidines for analgesic activity, mice

Studies designed to clarify the structural and conformational factors influencing the interactions of centrally acting analgesic agents with analgesic receptors have received considerable attention (1), and the recent discovery of the natural opiates has intensified this interest (2). Structural modification of methadone and methadone derivatives led to the development of a new series of agents, the acyclic basic anilides (3), which are represented by diampromide (I). Subsequent development (4) of the 4-anilidopiperidines, represented by fentanyl (II), led to a potent new class of analgesics. Differences in the modes of receptor binding of the various classes have been suggested.

BACKGROUND

The acyclic basic anilides have been proposed to differ from the methadone-type analgesics (5), and the 4-anilidopiperidines apparently differ from the acyclic basic anilides and the 4-phenylpiperidines, although they more closely resemble the former group (6). Differences in the modes of receptor binding between the acyclic basic anilides and the 4-anilidopiperidines have been proposed on the basis of structure–activity relationships and may be due to differences in receptor conformations (6). Based on spectroscopic evidence (7), a conformation of I has been suggested in which, among other features, the protonated basic center lies in close proximity to the anilido nitrogen. The preferred conformation of II has been suggested (6) as that in which the anilido function assumes the equatorial position of the chair form of the piperidine ring. Such a conformation would place the protonated basic center at a greater distance from the anilido nitrogen in II than in I. Additionally, this conformation of II would differ from the preferred conformation of the 4-phenylpiperidines, which has been suggested to be a skew boat conformation (8). However, the possibility that boat-type conformations of II may contribute to receptor interactions has not been tested directly. Analogs of fentanyl in which the flexibility of the piperidine ring has been restricted through incorporation of the tropane nucleus, designed to simulate frozen chair conformations of fentanyl, were reported recently (9). The 3-β-(propanilido) isomers were more potent than the 3-α-(propanilido) isomers and were nearly as potent as fentanyl itself.

The 2-azabicyclo[2.2.2]octane ring system was employed previously to restrict acetylcholine (10) and procaine (11) in *trans-* and *gauche-*conformations. More recently, this ring system was employed to restrict the piperidine ring of prodine-type analgesics in boat conformations (12). This report concerns the pharmacological evaluation of boat conformers of fentanyl-type analgesics. Casy *et al.* (6) reported the effects of substituents on the ring nitrogen atom of 4-anilidopiperidines and noted that analgesic activity increases significantly in the order $CH_3 < CH_2C_6H_5 < CH_2CH_2C_6H_5$. The analogs prepared in this study would permit the evaluation of this effect as well as the effect of positional isomerism in the boat conformers III–X. The synthesis and stereochemical analyses of these analogs were reported previously (13).

EXPERIMENTAL

The analgesic potency of the fentanyl analogs was determined by a modification of the D'Amour–Smith tail-flick method (14) using male albino ICR mice[1], 25–30 g, and an analgesiometer. All mice had their tails blackened with a black magic marker at least 15 min prior to testing to ensure a short, uniform tail-flick response time. The challenge to thermal stimulus occurred 30 min postinjection with the vehicle alone or the respective drugs. A control group of 10 mice was used to establish the baseline response time.

The criterion for analgesia (antinociception) was defined as a tail-flick response time greater than or equal to the mean response time of the

[1] Harlan Industries, Cumberland, Ind.

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