2-Alkyl 1-(4′-Benzhydrazide)aminomethylbenzimidazoles as MAO Inhibitors

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Abstract □ 2-Alkyl 1-(4′-benzhydrazide)aminomethylbenzimidazoles were synthesized and evaluated for their MAO inhibitory property, using kynuramine, tyramine, and 5-hydroxytryptamine as substrates. Anticonvulsant activity exhibited by these benzimidazole hydrazides against pentylenetetrazol-induced seizures was found to be unrelated to the MAO inhibitory ability of these compounds.

Keyphrases □ 1-(4′-Benzhydrazide)aminomethylbenzimidazoles, 2-alkyl substituted—synthesis, evaluated as MAO inhibitors, anticonvulsant activity □ Anticonvulsant agents, potential—synthesis and evaluation of 2-alkyl substituted 1-(4′-benzhydrazide)aminomethylbenzimidazoles □ MAO inhibitors, potential—synthesis and evaluation of 2-alkyl substituted 1-(4′-benzhydrazide)aminomethylbenzimidazoles

Hydrazine derivatives have been shown to be potent inhibitors of the enzyme MAO (1), and inhibitors of this enzyme have exhibited pronounced anticonvulsant properties (2). Benzimidazole derivatives also have been shown to possess CNS depressant activity (3, 4). These observations led to the synthesis of substituted benzimidazole hydrazides, which were prepared according to the steps outlined in Scheme I. In the present study, the ability of these compounds to inhibit MAO activity of rat brain homogenate was determined in an attempt to correlate the anticonvulsant activity of these compounds with their enzyme inhibitory property.

o-Phenylenediamine, with aliphatic acids, gave 2-alkyl benzimidazoles (I*a*) which, on Mannich reaction with ethyl *p*-aminobenzoate and formaldehyde, resulted in 2-alkyl 1-(4′-ethylbenzoate)aminomethylbenzimidazoles (I–V). Corresponding hydrazides (VI–X) were prepared by the reaction of esters (I–V) with hydrazine hydrate.

EXPERIMENTAL

Analyses for carbon, hydrogen, and nitrogen were performed. Melting points were taken in open capillary tubes and were corrected.

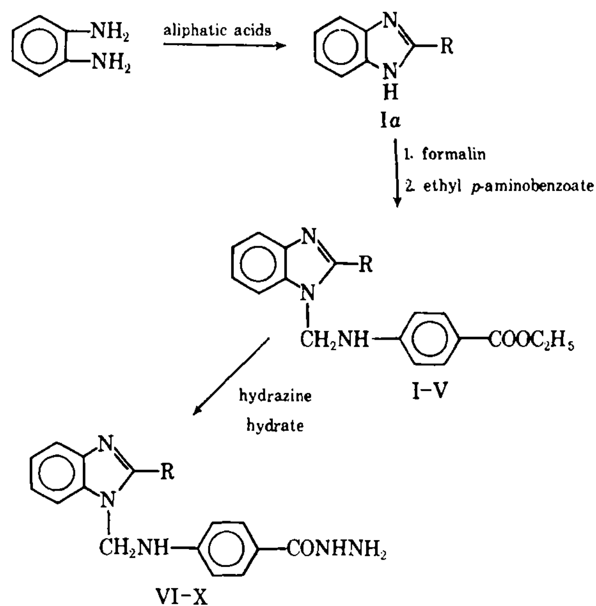
2-Alkyl Benzimidazoles (I*a*)—These compounds were prepared by the reaction of *o*-phenylenediamine with aliphatic acids (5).

2-Alkyl 1-(4′-Ethylbenzoate)aminomethylbenzimidazoles (I–V)—To a suspension of 2-alkyl benzimidazole (I*a*) (0.01 mole) in ethanol were added 0.012 mole of 37% formalin and 0.011 mole of ethyl *p*-aminobenzoate. During the addition of ethyl *p*-aminobenzoate, the reaction mixture was stirred on an ice bath for 2 hr. The product which separated out on cooling was left overnight, collected by filtration, and recrystallized from ethanol. The benzimidazoles recorded in Table I were characterized by their sharp melting points and elemental analyses.

2-Alkyl 1-(4′-Benzhydrazide)aminomethylbenzimidazoles (VI–X)—A mixture of appropriate 2-alkyl 1-(4′-ethylbenzoate)aminomethylbenzimidazole (I–V) (0.01 mole) and hydrazine hydrate (0.02 mole, 99–100%) was refluxed on a steam bath in absolute ethanol for 8–10 hr. The excess solvent was distilled off under reduced pressure, and the hydrazides which separated out were filtered and recrystallized from ethanol. These benzimidazole hydrazides (Table II) were characterized by their sharp melting points and elemental analyses.

Determination of MAO Activity—*Spectrophotofluorometric Method*—The method described by Krajl (6) was used for the determination of MAO activity of rat brain homogenate, using kynuramine as a substrate. The 4-hydroxyquinoline formed during oxidative deamination of kynuramine was measured fluorometrically in a spectrophotofluorometer[1], using activating light of 315 nm. and measuring fluorescence at the maximum of 380 nm. The reaction mixture consisted of 0.5 ml. of phosphate buffer (pH 7.4, 0.5 *M*), 0.5 ml. of kynuramine (1×10^{-4} *M*), and 0.5 ml. of brain homogenate (corresponding to 10 mg. of wet tissue weight). MAO activity of the brain homogenate was determined by incubation for 30 min. at 37°. The various inhibitors used at the final concentration of 5×10^{-4} *M* were incubated with the brain homogenate for 10 min. before the addition of kynuramine. The mixture was further incubated for 30 min. The reaction was stopped by the addition of 1 ml. of 10% trichloroacetic acid, and the precipitated proteins were removed by centrifugation. Suitable aliquots of the supernate were taken in 1 *N* NaOH solution and were assayed for 4-hydroxyquinoline, which was taken as an index of the enzyme activity. The percent inhibition was calculated from the decrease observed in the absorbance.

Warburg Manometric Method—MAO activity of rat brain homogenate was determined by a conventional Warburg manometric technique as described earlier (7), using tyramine and 5-hydroxytryptamine as substrates. The decrease in the oxygen uptake during oxidative deamination of tyramine or 5-hydroxytryptamine in the presence of benzimidazoles was used as an index of the enzyme inhibition. The reaction mixture, in a total volume of 3 ml., was 66 m*M* phosphate buffer (pH 7.4), 10 m*M* tyramine or 5-hydroxytryptamine, and the homogenate equivalent to 140 mg. of fresh tissue. The inhibitors were incubated with the enzyme preparation for 20 min. before the addition of either substrate. The enzyme system was further incubated at 37°, using oxygen as the gas phase for an additional hour. The readings of the oxygen uptake were



Scheme I

[1] Aminco-Bowman.

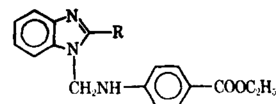


Table I—2-Alkyl 1-(4′-Ethylbenzoate)aminomethylbenzimidazoles

Compound Number	R	Melting Point	Yield, %	Molecular Formula	Analysis, % Calc.	Found
I	H	171°	80	$C_{17}H_{17}N_3O_2$	C 69.15 H 5.76 N 14.23	68.80 5.91 13.93
II	CH_3	176°	72	$C_{18}H_{19}N_3O_2$	C 69.90 H 6.15 N 13.59	70.05 5.98 13.46
III	C_2H_5	188°	65	$C_{19}H_{21}N_3O_2$	C 70.58 H 6.50 N 13.00	70.62 6.38 12.92
IV	n-C_3H_7	192°	60	$C_{20}H_{23}N_3O_2$	C 71.21 H 6.82 N 12.46	71.58 7.02 12.28
V	iso-C_3H_7	185°	55	$C_{20}H_{23}N_3O_2$	C 71.21 H 6.82 N 12.46	71.06 7.16 12.57

taken at 10-min. intervals. Benzimidazole hydrazides were used at a final concentration of 1×10^{-3} M.

Determination of Anticonvulsant Activity—Anticonvulsant activity was determined in mice of either sex weighing 20–27 g. (8). The mice were divided in groups of 10, keeping the weight of the groups equal as far as possible. One group of animals was used for each compound. The compounds were administered intraperitoneally in 5% gum acacia suspension in a dose of 100 mg./kg. Four hours after the administration of these compounds, the mice were injected with pentylenetetrazol (80 mg./kg.) subcutaneously under the loose skin of the back. This dose of pentylenetetrazol has been shown to produce convulsions in almost all untreated mice. The mice were then observed for 60 min. for the occurrence of seizures. An episode of clonic spasm persisting for 5 sec. was considered a threshold convulsion. Transient intermittent jerks and tremulousness were not taken into account. Animals devoid of even a threshold convulsion during 60 min. were considered protected. The number of animals protected in each group was recorded, and the percentage of protection was calculated (Table III).

RESULTS AND DISCUSSION

All benzimidazole hydrazides (VI–X) were found to inhibit MAO activity during oxidative deamination of both kynuramine and 5-hydroxytryptamine. The degree of enzyme inhibition in general was not only of a low order when tyramine was used as the substrate, but Compounds VI and VII were devoid of inhibitory effects under similar experimental conditions. These results reflected the greater sensitivity of benzimidazole hydrazides to inhibit MAO during oxidative deamination of kynuramine as compared to tyramine or 5-hydroxytryptamine. Thus, significant differences observed with the inhibitory effects of benzimidazole hydrazides with the choice of the substrate in *in vitro* studies could possibly account for their different affinity and degrees of competition with the individual substrate for the secondary site(s) on the enzyme MAO where attachment of the inhibitor to these site(s) is responsible for greater inhibition as well as prolonged duration of action (9, 10). Similar results were reported (11) for 2-phenylcyclopropylamine during *in vitro* studies using different biogenic amines as substrates with rat liver mitochondrial MAO. Different degrees of enzyme inhibition were also observed when tyramine, serotonin, tryptamine, and dopamine were used as the substrates (12).

All of the benzimidazole hydrazides (VI–X) exhibited low anticonvulsant activity, which was found to be maximum with Compound VIII where 50% protection against pentylenetetrazol-induced seizures was observed (Table III). The toxicity of these compounds, as reflected by their approximate LD_{50} values, was found to

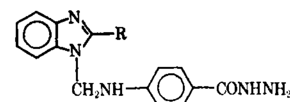


Table II—2-Alkyl 1-(4′-Benzhydrazide)aminomethylbenzimidazoles

Compound Number	R	Melting Point	Yield, %	Molecular Formula	Analysis, % Calc.	Found
VI	H	222°	56	$C_{15}H_{15}N_5O$	C 64.04 H 5.34 N 24.91	63.82 5.68 24.62
VII	CH_3	217°	50	$C_{16}H_{17}N_5O$	C 65.09 H 5.76 N 23.73	64.92 6.00 23.55
VIII	C_2H_5	100°	46	$C_{17}H_{19}N_5O$	C 66.01 H 6.15 N 22.65	66.21 5.96 22.43
IX	n-C_3H_7	92°	38	$C_{18}H_{21}N_5O$	C 66.87 H 6.50 N 21.67	66.82 6.36 21.58
X	iso-C_3H_7	197°	35	$C_{18}H_{21}N_5O$	C 66.87 H 6.50 N 21.67	67.06 6.42 21.36

Table III—MAO Inhibitory Property[a], Behavioral Effects[b], and Anticonvulsant Activity[b] of 2-Alkyl 1-(4′-Benzhydrazide)-aminomethylbenzimidazoles

Compound Number	MAO Inhibition, %: Kynuramine[c]	MAO Inhibition, %: Tyramine[d]	MAO Inhibition, %: Serotonin[d] (5-Hydroxytryptamine)	Behavioral Effects	Anticonvulsant Activity, %: Protection	Anticonvulsant Activity, %: Mortality after 24 hr.	Approximate LD_{50}, mg./kg.
VI	64.5 ± 0.6	Nil	32.8 ± 1.1	General excitation, increased motor activity, circling movements, ataxia, seizures, and death following extensor phase of hind limb in two mice	20	30	250
VII	63.0 ±0.5	Nil	11.5 ± 1.2		10	70	250
VIII	52.2 ± 0.4	22.3 ± 1.0	49.5 ± 0.9	Slight depression of spontaneous motor activity	50	50	1000
IX	51.4 ± 0.4	20.6 ± 0.8	45.5 ± 0.9		30	60	500
X	63.2 ± 0.6	17.0 ± 0.8	54.0 ± 0.8	Nil	20	40	>1500

[a] Vessel contents and assay procedures are as indicated in the text. Each experiment was done in triplicate. Figures indicate mean values ± standard error. [b] Compounds were administered at the dose of 100 mg./kg. i.p. [c] Final concentration of kynuramine and inhibitors was 1×10^{-4} *M* and 5×10^{-4} *M*, respectively. The enzyme activity observed in the absence of added inhibitors was equivalent to change in absorbance (ΔA) from 2.78 to 3.12/10 mg. fresh tissue/30 min. [d] Final concentration of tyramine or serotonin (5-hydroxytryptamine) was 1×10^{-2} *M*, while that of 2-alkyl 1-(4′-benzhydrazide)aminomethylbenzimidazoles was 1×10^{-3} *M*. The oxygen uptake in the absence of added inhibitors ranged from 67.56 to 72.12 µl. and from 52.05 to 57.18 µl./140 mg. of fresh tissue during oxidative deamination of tyramine and serotonin, respectively.

be of low order. As is evident from Table III, the behavioral effects of the benzimidazole hydrazides were found to correspond to some extent with their toxicity. These results provide further support to our earlier observation (13) that MAO is presumably not the only enzyme that could be responsible for the anticonvulsant property of such MAO inhibitors. In the present study the *in vitro* enzyme inhibitory activity of these hydrazides was in no way parallel with their ability to afford protection against pentylenetetrazol-induced seizures or with their *in vivo* gross behavioral effects.

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