Substituted Benzoxazole/Benzthiazole-2-thiones: Interrelationship between Anticonvulsant Activity and Inhibition of Nicotinamide Adenine Dinucleotide-Dependent Oxidations and Monoamine Oxidase

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Abstract □ 3-Alkyl benzoate/benzhydrazide aminomethyl benzoxazole/benzthiazole-2-thiones were synthesized and evaluated for their ability to affect the respiratory activity of rat brain homogenate. All compounds were found to inhibit selectively the nicotinamide adenine dinucleotide (NAD)-dependent oxidation of DL-isocitrate, α-ketoglutarate, and pyruvate while NAD-independent oxidation of succinate remained unaltered. Inhibition of monoamine oxidase by 3-benzhydrazide aminomethyl benzoxazole/benzthiazole was greater with benzthiazoles as compared to the corresponding benzoxazoles. Anticonvulsant activity exhibited by these benzoxazole/benzthiazole derivatives against pentylenetetrazol-induced seizures was found to be unrelated to their enzyme inhibitory properties.

Keyphrases □ 3-Alkyl benzoate/benzhydrazide aminomethyl benzoxazole/benzthiazole-2-thiones—synthesis, anticonvulsant activity and relationship to inhibition of NAD-dependent oxidations and monoamine oxidase □ Benzoxazole/benzthiazole-2-thiones, 3-alkyl benzoate/benzhydrazide aminomethyl—synthesis, anticonvulsant activity and relationship to inhibition of NAD-dependent oxidations and monoamine oxidase □ Inhibition of NAD-dependent oxidations and monoamine oxidase—synthesis and evaluation of 3-alkyl benzoate/benzhydrazide aminomethyl benzoxazole/benzthiazole-2-thiones

Diverse psychopharmacological effects exhibited by benzoxazoles (1) and benzthiazole (2, 3) led to the synthesis of 3-alkyl benzoate/benzhydrazide aminomethyl benzoxazole/benzthiazole-2-thiones. In the present study, their ability to inhibit nicotinamide adenine dinucleotide (NAD)-dependent oxidation of DL-isocitrate, α-ketoglutarate, and pyruvate and the ability of 3-benzhydrazide aminomethyl benzoxazole/benzthiazole-2-thiones to inhibit monoamine oxidase activity were investigated. Attempts also were made to determine the anticonvulsant activity of these compounds to correlate their enzyme inhibitory properties with their ability to afford protection against pentylenetetrazol-induced seizures.

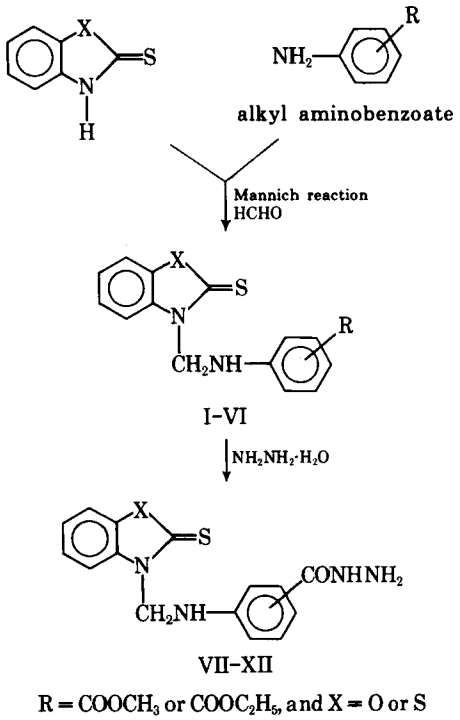
EXPERIMENTAL[1]

Alkyl Aminobenzoates—These were prepared by treating aminobenzoic acids with appropriate alcohols according to the method reported earlier (4).

3-Alkyl Benzoate Aminomethyl Benzoxazole/Benzthiazole-2-thiones (I–VI) (Scheme I)—A mixture of an appropriate alkyl aminobenzoate (0.011 mole) and 2-mercaptobenzoxazole/2-mercaptobenzthiazole (0.01 mole) in ethanol with 37% formalin (0.012 mole) was stirred on an ice bath for 3 hr. The reaction mixture was refrigerated overnight, and the solid mass which separated out was filtered, washed with water, dried, and recrystallized from ethanol. The various 3-alkyl benzoate aminomethyl benzoxazole/benzthiazole-2-thiones were characterized by their sharp melting points and elemental analyses (Table I). The presence of characteristic bands for $COOC_2H_5$ (1730 cm^{-1}), NH (3320 cm^{-1}), and CH_2 (2930 cm^{-1}) groups in their IR spectra provided further support for their structure.

3-Benzhydrazide Aminomethyl Benzoxazole/Benzthiazole-2-thiones (VII–XII)—3-Alkyl benzoate aminomethyl benzoxazole/benzthiazole-2-thione (0.01 mole) dissolved in absolute ethanol was heated under reflux in a water bath with 0.02 mole of 99–100% hydrazine hydrate for 10–12 hr. Excess solvent was removed by distillation. The hydrazides which separated out on cooling were collected by filtration, dried, and recrystallized from ethanol. The various 3-benzhydrazide aminomethyl benzoxazole/benzthiazole-2-thiones thus obtained were characterized by their sharp melting points and elemental analyses (Table II). These hydrazides were further characterized by the presence of the characteristic bands for CONH (1660 cm^{-1}), NH_2 (3350 cm^{-1}), NH (3200 cm^{-1}), and CH_2 (2840 cm^{-1}) in their IR spectra.

Assay of Respiratory Activity of Rat Brain Homogenate—Male albino rats, allowed food and water *ad libitum*, were used in all experiments. Rats weighing 100–150 g were sacrificed by decapitation. The brains were removed immediately and homogenized[2] in the ratio of 1:9 (w/v) in cold 0.25 *M* sucrose. All incubations were carried out in the conventional Warburg manometric apparatus at 37° with air as the gas phase. The oxygen uptake



Scheme I

[1] All compounds were analyzed for their carbon, hydrogen, and nitrogen content. Melting points were taken in open capillary tubes and are corrected. Commercial chemicals were used in the present study. DL-Isocitrate, α-ketoglutarate, pyruvate, succinate, kynuramine, AMP (sodium salt), and cytochrome c were obtained from Sigma Chemical Co., St. Louis, Mo.

[2] Potter-Elvehjem homogenizer.

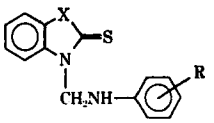


Table I—3-Alkyl Benzoate Aminomethyl Benzoxazole/Benzthiazole-2-thiones

Compound	R	X	Yield, %	Melting Point	Molecular Formula	Analysis, % Calc.	Found
I	2-$COOCH_3$	O	66	150°	$C_{16}H_{14}N_2O_3S$	C 61.14 H 4.46 N 8.92	60.96 4.62 8.70
II	3-$COOC_2H_5$	O	72	180°	$C_{17}H_{16}N_2O_3S$	C 62.19 H 4.88 N 8.54	62.40 4.76 8.42
III	4-$COOC_2H_5$	O	83	169°	$C_{17}H_{16}N_2O_3S$	C 62.19 H 4.88 N 8.54	62.34 4.70 8.38
IV	2-$COOCH_3$	S	68	172°	$C_{16}H_{14}N_2O_2S_2$	C 58.18 H 4.24 N 8.48	58.46 4.08 8.60
V	3-$COOC_2H_5$	S	76	135°	$C_{17}H_{16}N_2O_2S_2$	C 59.30 H 4.65 N 8.14	59.52 4.50 7.98
VI	4-$COOC_2H_5$	S	80	155°	$C_{17}H_{16}N_2O_2S_2$	C 59.30 H 4.65 N 8.14	59.12 4.74 8.00

[a] Melting points were taken in open capillary tubes and are corrected.

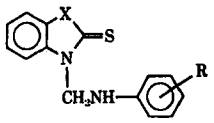


Table II—3-Benzhydrazide Aminomethyl Benzoxazole/Benzthiazole-2-thiones

Compound	R	X	Yield, %	Melting Point[a]	Molecular Formula	Analysis, % Calc.	Found
VII	2-$CONHNH_2$	O	44	248°	$C_{15}H_{14}N_4O_2S$	C 57.32 H 4.46 N 17.83	57.60 4.28 17.62
VIII	3-$CONHNH_2$	O	52	172°	$C_{15}H_{14}N_4O_2S$	C 57.32 H 4.46 N 17.83	57.14 4.60 17.73
IX	4-$CONHNH_2$	O	57	140°	$C_{15}H_{14}N_4O_2S$	C 57.32 H 4.46 N 17.83	57.10 4.62 17.78
X	2-$CONHNH_2$	S	50	200°	$C_{15}H_{14}N_4OS_2$	C 54.54 H 4.24 N 16.96	54.78 4.10 16.65
XI	3-$CONHNH_2$	S	54	197°	$C_{15}H_{14}N_4OS_2$	C 54.54 H 4.24 N 16.96	54.66 4.02 16.70
XII	4-$CONHNH_2$	S	60	203°	$C_{15}H_{14}N_4OS_2$	C 54.54 H 4.24 N 16.96	54.32 4.40 16.74

[a] Melting points were taken in open capillary tubes and are corrected.

was measured at 10-min intervals. Fresh rat brain homogenate, equivalent to 125 mg wet weight, was added to the chilled Warburg vessels containing 6.7 m*M* $MgSO_4$, 20 m*M* Na_2HPO_4, 1 m*M* adenosine monophosphate (AMP) (sodium salt), 33 m*M* KCl, buffer solution (pH 7.4), and 500 μg of cytochrome c in a final volume of 3 ml. The central well contained 0.2 ml of 20% KOH solution. All substrates were used at a final concentration of 10 m*M* while the final concentration of the various benzoxazole/benzthiazole derivatives was 2 m*M*. The compounds were dissolved in propylene glycol (100%), and an equivalent amount of the solvent was added to the control vessels.

Determination of Monoamine Oxidase Activity—A spectrophotofluorometric method was used for the determination of monoamine oxidase activity of the rat brain homogenate, using kynuramine as the substrate (5). The 4-hydroxyquinoline formed during oxidative deamination of kynuramine was measured fluorometrically in a spectrophotofluorometer[3], using activating light of 315 nm and measuring fluorescence at the maximum of 380 nm. The reaction mixture in a final concentration consisted of 0.5 ml of phosphate buffer (0.2 *M*, pH 7.5), 1×10^{-4} *M* kynuramine, and 0.5 ml of brain homogenate (equivalent to 10 mg of wet weight of the tissue). The monoamine oxidase activity was determined by incubation at 37° in air for 30 min. The various 3-benzhydrazide aminomethyl benzoxazole/benzthiazole-2-thiones were added to the brain homogenate to produce a final concentration of 1×10^{-4} *M* and then were incubated for 10 min before the addition of kynuramine. The mixture was then incubated for an additional 30 min. The reaction was stopped by the addition of 1 ml of 10% trichloroacetic acid (w/v), and the precipitated proteins

[3] Aminco Bowman.

Table III—Biochemical and Pharmacological Properties of 3-Alkyl Benzoate Aminomethyl Benzoxazole/Benzthiazole-2-thiones

Compound	Inhibition[a], %				Anticonvulsant Activity[b], % Protection	Mortality after 24 hr, %
	DL-Isocitrate	α-Ketoglutarate	Pyruvate	Succinate		
I	66.1 ± 0.8	53.5 ± 0.9	49.2 ± 0.4	Nil	40	20
II	55.2 ± 0.6	31.4 ± 0.5	60.9 ± 0.8	Nil	20	30
III	59.1 ± 0.7	24.9 ± 0.4	68.0 ± 1.0	Nil	30	30
IV	63.8 ± 0.9	42.8 ± 0.5	54.8 ± 0.7	Nil	40	10
V	70.1 ± 1.2	47.9 ± 0.6	61.5 ± 0.9	Nil	10	50
VI	61.7 ± 0.9	38.2 ± 0.4	77.4 ± 1.3	Nil	20	40

[a] Each experiment was done in duplicate. Assay conditions and vessel contents were as reported in the text. All values represent mean values of percent inhibition with ±standard error calculated from three separate experiments. Inhibition was determined by the decrease in the oxygen uptake with 125 mg wet tissue weight/hr. The final concentrations of the compounds and the substrates were 2 and 10 m*M*, respectively. The oxygen uptake in the control experiments, in the absence of added 3-alkyl benzoate aminomethyl benzoxazole/benzthiazole-2-thiones, was 142.0 ± 7.6, 125.0 ± 8.8, 113.0 ± 6.5, and 210.0 ± 11.4 μl/125 mg wet tissue weight/hr using DL-isocitrate, α-ketoglutarate, pyruvate, and succinate, respectively, as substrates. [b] Compounds were administered at the dose of 100 mg/kg ip 4 hr before administration of pentylenetetrazol (90 mg/kg sc).

Table IV—Biochemical and Pharmacological Properties of 3-Benzhydrazide Aminomethyl Benzoxazole/Benzthiazole-2-thiones

Compound	Inhibition[a], %					Anticonvulsant Activity[a], % Protection	Mortality after 24 hr, %
	DL-Isocitrate	α-Ketoglutarate	Pyruvate	Succinate	Monoamine Oxidase[b]		
VII	14.7 ± 0.3	56.5 ± 0.8	18.8 ± 0.2	Nil	37.2 ± 0.4	20	60
VIII	22.4 ± 0.3	42.7 ± 0.7	21.6 ± 0.3	Nil	46.3 ± 0.5	Nil	90
IX	40.1 ± 0.4	49.1 ± 0.5	37.3 ± 0.7	Nil	58.4 ± 0.7	30	40
X	35.9 ± 0.6	55.8 ± 0.7	38.5 ± 0.8	Nil	54.1 ± 0.7	10	80
XI	52.1 ± 0.8	60.4 ± 0.8	32.2 ± 0.4	Nil	60.4 ± 1.0	20	70
XII	38.5 ± 0.5	64.1 ± 1.0	44.1 ± 0.6	Nil	70.2 ± 1.1	10	80

[a] Assay conditions were as described in Table III. [b] Each experiment was done in duplicate, and the values indicate mean values with ±standard error of three separate experiments. Final concentrations of kynuramine and the various 3-benzhydrazide aminomethyl benzoxazole/benzthiazole-2-thiones were 1×10^{-4} *M*.

were removed by centrifugation. Suitable 1-ml aliquots of the supernate were taken in 2 ml of 1 *N* NaOH solution and were assayed for 4-hydroxyquinoline. An increase in absorbance provided a direct measurement of 4-hydroxyquinoline formation, which was taken as an index of the monoamine oxidase activity. The percentage inhibition was calculated from the decrease observed in absorbance, and this provided an index of the inhibitory property of these 3-benzhydrazide aminomethyl benzoxazole/benzthiazole-2-thiones.

Determination of Anticonvulsant Activity—Anticonvulsant activity was determined against pentylenetetrazol-induced seizures in mice of either sex weighing between 25 and 30 g. The mice were divided into groups of 10, keeping the group weights as near the same as possible. All compounds (100 mg/kg) were suspended in 5% aqueous gum acacia to give a concentration of 0.25% (w/v). A 100-mg/kg ip dose of the test compound was administered to 10 animals. Four hours after the administration of these compounds, the mice were injected with pentylenetetrazol (90 mg/kg sc). This dose of pentylenetetrazol has been shown to produce convulsions in almost all untreated animals and to exhibit 100% mortality in mice during 24 hr. On the other hand, no mortality was observed during 24 hr in animals treated with 100 mg/kg of the test compounds alone. The mice were observed for 60 min for the occurrence of seizures. An episode of clonic spasm persisting for a minimum of 5 sec was considered a threshold convulsion. Transient intermittent jerks or tremulousness were not counted. Animals devoid of threshold convulsions during 60 min were considered protected. The number of animals protected in each group was recorded, and the anticonvulsant activity of these compounds was represented as percentage protection. The animals were then observed for 24 hr and the mortality was recorded.

RESULTS AND DISCUSSION

Selective *in vitro* inhibition of NAD-dependent oxidation of DL-isocitrate, α-ketoglutarate, and pyruvate by rat brain homogenate was observed in the present study with all 3-alkyl benzoate aminomethyl benzoxazole/benzthiazole-2-thiones (Table III). However, NAD-independent oxidation of succinate was found to be unaffected. Maximum inhibition of DL-isocitrate oxidation was observed with Compound V, while maximum inhibition of α-ketoglutarate oxidation was observed with Compound I. Compound V produced maximum inhibition of the oxidation of pyruvate. Anticonvulsant activity exhibited by 3-alkyl benzoate aminomethyl benzoxazole/benzthiazole-2-thiones is recorded in Table III. All compounds, when used at the dose of 100 mg/kg, possessed low anticonvulsant activity, as evidenced by their ability to afford only 10–40% protection against pentylenetetrazol-induced seizures. Maximum protection was observed with I and IV, while V was the least effective compound. 3-Alkyl benzoate aminomethyl benzoxazole/benzthiazole-2-thiones, possessing low anticonvulsant activity, showed higher mortality in experimental mice treated with pentylenetetrazol during 24 hr.

The selective inhibitory effect of 3-benzhydrazide aminomethyl benzoxazole/benzthiazole-2-thiones on NAD-dependent oxidation of DL-isocitrate, α-ketoglutarate, and pyruvate by rat brain homogenate is recorded in Table IV. As was observed with their precursors, 3-alkyl benzoate aminomethyl benzoxazole/benzthiazole-2-thiones, these compounds were devoid of an inhibitory effect on the oxidation of succinate. Maximum inhibition of the oxidation of DL-isocitrate was observed with Compound XI, while maximum inhibition of the oxidation of α-ketoglutarate and pyruvate was observed with Compound XII. On the other hand, all compounds inhibited monoamine oxidase activity in the 37.2–

70.2% range when used at a final concentration of 1×10^{-4} *M* during oxidative deamination of kynuramine. Compound XII produced maximum inhibition. The degree of monoamine oxidase inhibition was influenced by the position of the hydrazide group on the phenyl nucleus attached at the aminomethyl portion of the benzoxazole or benzthiazole moiety. The presence of the hydrazide group at position 4 of the phenyl nucleus produced greater inhibition of monoamine oxidase in benzoxazole or benzthiazole derivatives. The ability of these hydrazides to inhibit monoamine oxidase was greater with 3-benzhydrazide aminomethyl benzthiazole-2-thiones as compared to their corresponding benzoxazole derivatives. All of these hydrazides possessed low anticonvulsant activity, lower than that of their precursor esters (I–VI). The low anticonvulsant activity of these hydrazides corresponded with high pentylenetetrazol mortality, which ranged from 40 to 90% in experimental mice treated with hydrazide derivatives during 24 hr. None of these compounds exhibited any appreciable sedative or central nervous system (CNS) depressant effect nor 24-hr mortality at 100 mg/kg. These observations failed to provide any correlation between *in vitro* enzyme inhibitory activity of these compounds and their low anticonvulsant activity.

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Mechanism for Riboflavin Enhancement of Bilirubin Photodecomposition *In Vitro*

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Abstract □ Riboflavin increases the rate of bilirubin photodecomposition as much as 25-fold under aerobic conditions. In the absence of oxygen, bilirubin photodecomposition is virtually arrested, with or without riboflavin. It is proposed that singlet oxygen generated by the riboflavin triplet species is primarily responsible for the observed rate enhancement. The occurrence of similar photochemical reactions *in vivo* might provide a means for improving the efficiency of bilirubin phototherapy through systemic administration of appropriate agents.

Keyphrases □ Riboflavin—enhancement of bilirubin photodecomposition *in vitro*, mechanism □ Bilirubin, photodecomposition—mechanism for riboflavin enhancement *in vitro* □ Photodecomposition, bilirubin—mechanism of enhanced rates under aerobic conditions with riboflavin *in vitro*

Bilirubin is a tetrapyrrolic dicarboxylic acid produced *in vivo*, mainly by the hepatic and splenic reticuloendothelial systems, from hemoglobin released from senescent erythrocytes (1). Nonhemolytic neonatal hyperbilirubinemia is ascribed to deficient glucuronidation of bilirubin arising from either capacity-limited hepatic uptake of bilirubin (2, 3) or deficiency of the hepatic enzyme bilirubin uridine diphosphate glucuronyltransferase (4), which catalyzes the conjugation of lipophilic bilirubin to its hydrophilic diglucuronide. Facile elimination of this diglucuronide occurs *via* biliary and urinary excretion.

Phototherapy has become a common procedure in neonatal hyperbilirubinemia (5–8). Despite the well-established efficacy of light therapy in reducing plasma bilirubin levels, reservations have been expressed with respect to exposing infants to light intensities of 300–500 foot-candles, often continuously for several days. Conceivably, an enhancement of bilirubin phototherapy may be accomplished by administration of suitable agents which would promote bilirubin photodecomposition *in vivo*. An enhancement of bilirubin phototherapy could mean either a reduction of exposure time or a reduction of light intensity, thereby reducing some potential hazards (9) of this treatment.

In view of its clinical importance, several investigators have studied bilirubin photochemistry in the presence and absence of a complexing polymeric molecule such as a serum albumin (10–12). Recent studies (13) showed that oxygen is essential for bilirubin photodecomposition. In the presence of oxygen, bilirubin undergoes irreversible photooxidation to a variety of products (14–18), including methylvinylmaleimide, biliverdin, and water- or methanol-propentdyopent adducts. Furthermore, under nitrogen and in the presence of a suitable nucleophilic substrate such as *N*-acetyl-1-cysteine, bilirubin undergoes an irreversible reaction with the substrate to give hydrophilic photoaddition products (19, 20). In the presence of oxygen, light-excited bilirubin sensitizes the formation of singlet oxygen which then attacks ground-state bilirubin with resultant bilirubin photooxidation (21). The same author (21) also showed that singlet oxygen-generating compounds