Synthesis of Substituted Thiobenzoxazoles/Benzothiazoles: Inhibition of Cellular Respiratory and Monoamine Oxidase Activities and Anticonvulsant Property

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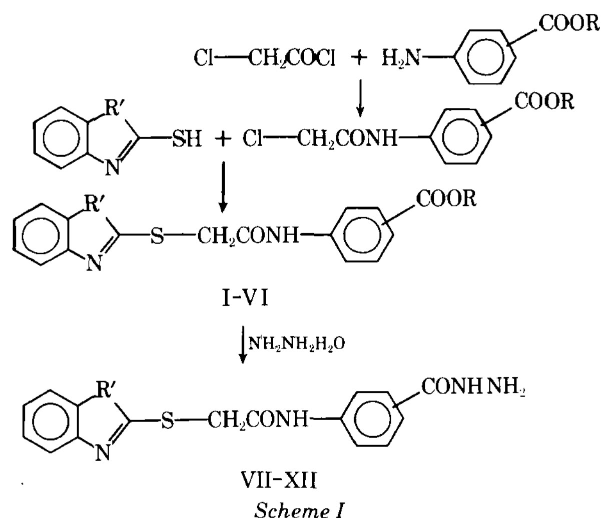
Abstract □ Several 2-(substituted alkoxy/hydrazinocarbonyl acetanilidothio)benzoxazoles/benzothiazoles were synthesized and characterized by their sharp melting points, elemental analyses, and IR spectra. All thiobenzoxazoles/benzothiazoles possessed low anticonvulsant activity, which was reflected by the 10–40% protection afforded by these compounds against pentylenetetrazol-induced convulsions. All thiobenzoxazoles/benzothiazoles inhibited selectively the nicotinamide adenine dinucleotide (NAD)-dependent oxidation of pyruvate, DL-isocitrate, and α-ketoglutarate by rat brain homogenates. NAD-independent oxidation of succinate remained unaltered. All 2-(substituted hydrazinocarbonyl acetanilidothio)benzoxazoles/benzothiazoles inhibited monoamine oxidase activity of rat brain homogenates. Greater monoamine oxidase inhibition was observed with thiobenzothiazoles than with the corresponding thiobenzoxazoles. The low anticonvulsant activity possessed by these thiobenzoxazoles/benzothiazoles was found to be unrelated with their ability to inhibit cellular respiratory and monoamine oxidase activities of rat brain homogenates.

Keyphrases □ Thiobenzoxazoles, substituted—synthesis and characterization, anticonvulsant activity, effect on NAD-dependent oxidation, monoamine oxidase activity □ Thiobenzothiazoles, substituted—synthesis and characterization, anticonvulsant activity, effect on NAD-dependent oxidation, monoamine oxidase activity □ Anticonvulsant activity—screened in substituted thiobenzoxazoles and thiobenzothiazoles □ Oxidation, NAD dependent—screened in substituted thiobenzoxazoles and thiobenzothiazoles □ Monoamine oxidase activity—screened in substituted thiobenzoxazoles and thiobenzothiazoles

Depressant effects of benzimidazole (1) and anticonvulsant properties of benzimidazoles and benzothiazoles (2) led to the development of zoxazolamine (3) and chlorozoxazone (4) as centrally acting muscle relaxants. Benzimidazole (5, 6) and benzoxazole (5, 7) derivatives have been reported to raise the threshold of electroshock convulsions. Earlier studies also indicated the anticonvulsant activity of benzothiazole, an isoester of benzoxazole and benzimidazole, which, in the form of a methyl derivative, raised the electroshock threshold in cats (5). Furthermore, diverse psychopharmacological properties exhibited by benzoxazoles (8) and benzothiazoles (2, 9) prompted synthesis of 2-(substituted alkoxy/hydrazinocarbonyl acetanilidothio)benzoxazoles/benzothiazoles.



Scheme I

In the present study, thiobenzoxazoles/benzothiazoles were evaluated for their anticonvulsant activity and ability to inhibit cellular respiratory and monoamine oxidase activities of rat brain homogenates.

EXPERIMENTAL[1]

Chemistry—The various 2-(substituted alkoxy/hydrazinocarbonyl acetanilidothio)benzoxazoles/benzothiazoles were synthesized by following the methods outlined in Scheme I (R′ = O or S). 2-Mercaptobenzoxazole or 2-mercaptobenzothiazole, on condensation with *N*-chloroacetylamino esters, resulted in the formation of 2-(substituted alkoxycarbonyl acetanilidothio)benzoxazoles/benzothiazoles. These compounds were treated with hydrazine hydrate to form the corresponding 2-(substituted hydrazinocarbonyl acetanilidothio)benzoxazoles/benzothiazoles.

Alkylamino Benzoates—These alkylamino benzoates were prepared by treating aminobenzoic acids with the appropriate alcohol according to the method reported earlier (10).

***N*-Chloroacetylaminoalkyl Esters**—To a solution of an appropriate aminoalkyl benzoate (0.04 mole) in 20 ml of dry benzene was gradually added chloroacetyl chloride (0.022 mole), and the mixture was refluxed on a steam bath for 2–4 hr. Excess benzene was distilled, and the resulting mixture was poured over ice-cold water. The desired *N*-chloroacetylaminoalkyl esters, which separated out, were reported earlier (11).

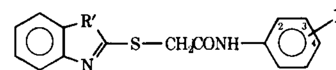
2-(Substituted Alkoxycarbonyl Acetanilidothio)benzoxazoles/benzothiazoles—A mixture of the appropriate 2-mercaptobenzoxazole or 2-mercaptobenzothiazole (0.01 mole) in 20 ml of dry acetone, anhydrous potassium carbonate (0.011 mole), and the suitable chloroacetylaminoalkyl ester (0.01 mole) was refluxed on a steam bath for 16–18 hr. The mixture was filtered to remove potassium chloride. Excess acetone from the filtrate was distilled; the crude products, which separated on cooling, were collected by filtration, washed with water, and recrystallized from ethanol.

The various 2-(substituted alkoxycarbonyl acetanilidothio)benzoxazoles/benzothiazoles were characterized by their sharp melting points and elemental analyses (Table I). The presence of characteristic bands for —C(=O)O (1735 cm^{-1}), CH_2 (2800 cm^{-1}), and CONH (1690 cm^{-1}) in the IR spectra of these compounds provided further confirmation of their molecular structure.

2-(Substituted Hydrazinocarbonyl Acetanilidothio)benzoxazoles/benzothiazoles—To a solution of the appropriate 2-(sub-

[1] All compounds were analyzed for their carbon, hydrogen, and nitrogen content. Melting points were taken in open capillary tubes with a partial immersion thermometer and are corrected. IR spectra were obtained with Perkin-Elmer Infracord spectrophotometer model 137 equipped with sodium chloride optics in potassium bromide films in the 700–3500-cm^{-1} range.

Table I—Physical Constants of 2-(Substituted Alkoxycarbonyl Acetanilidothio)-benzoxazoles/benzothiazoles



Compound	R′	R	Melting Point[a]	Yield, %	Molecular Formula	Analysis, % Calc.	Found
I	O	2-$COOCH_3$	116°	58	$C_{17}H_{14}N_2O_4S$	C 59.64 H 4.09 N 8.19	59.86 3.90 7.93
II	O	3-$COOC_2H_5$	90°	63	$C_{18}H_{16}N_2O_4S$	C 60.67 H 4.49 N 7.86	60.98 4.29 7.70
III	O	4-$COOC_2H_5$	140°	72	$C_{18}H_{16}N_2O_4S$	C 60.67 H 4.49 N 7.86	60.89 4.30 7.74
IV	S	2-$COOCH_3$	109°	60	$C_{17}H_{14}N_2O_3S_2$	C 56.98 H 3.91 N 7.82	56.66 4.10 7.64
V	S	3-$COOC_2H_5$	147°	66	$C_{18}H_{16}N_2O_3S_2$	C 58.06 H 4.30 N 7.52	58.28 4.16 7.40
VI	S	4-$COOC_2H_5$	135°	70	$C_{18}H_{16}N_2O_3S_2$	C 58.06 H 4.30 N 7.52	57.84 4.42 7.38

[a] Melting points were taken in open capillary tubes with a partial immersion thermometer and are corrected.

stituted alkoxycarbonyl acetanilidothio)benzoxazole/benzothiazole (0.01 mole) in 20 ml of absolute ethanol was added hydrazine hydrate (99–100%; 0.02 mole), and the mixture was refluxed on a steam bath for 10–12 hr. Excess ethanol was distilled; the crude products, which separated, were collected by filtration, dried, and recrystallized from ethanol.

The various 2-(substituted hydrazinocarbonyl acetanilidothio)-benzoxazoles/benzothiazoles were characterized by their sharp melting points and elemental analyses (Table II). The molecular structure of these compounds was further characterized by the presence of the characteristic bands for CONH (1660 cm^{-1}), CH_2 (2850 cm^{-1}), and NH_2 (3350 cm^{-1}) groups in their IR spectra.

Determination of Respiratory Activity of Rat Brain Homogenates[2]—Male albino rats, kept on an *ad libitum* diet, were used in all experiments. Rats, 100–150 g, were sacrificed by decapitation. The brains were removed immediately and homogenized[3] in ice-cold 0.25 *M* sucrose in a ratio of 1:9 (w/v). All incubations were carried out at 37° in the conventional Warburg manometric apparatus, using air as the gas phase (12). The oxygen uptake was measured at 10-min intervals.

Fresh brain homogenate (1 ml) equivalent to 100 mg of wet brain weight was added to the chilled Warburg vessels containing 6.7 m*M* magnesium sulfate, 20 m*M* sodium hydrogen phosphate buffer solution (pH 7.4), 1 m*M* adenosine monophosphate (sodium salt), 33 m*M* potassium chloride, and 500 μg of cytochrome c in a final volume of 3 ml unless otherwise stated. The central well contained 0.2 ml of 20% potassium hydroxide solution. Pyruvate, DL-isocitrate, α-ketoglutarate, and succinate were used at a final concentration of 10 m*M*. It was assumed that the endogenous NAD, present in brain homogenates, was sufficient for the cellular respiratory activity of rat brain homogenates.

All 2-(substituted alkoxy/hydrazinocarbonyl acetanilidothio)-benzoxazoles/benzothiazoles were dissolved in propylene glycol (100%) and were used at a final concentration of 2 m*M*. An equal volume of propylene glycol was added to the control vessels.

Determination of Anticonvulsant Activity—Anticonvulsant activity was determined in mice of either sex, 25–30 g, against pentylenetetrazol-induced convulsions. The mice were divided in groups of 10, keeping the group weights as near the same as possible. All 2-(substituted alkoxy/hydrazinocarbonyl acetanilidothio)benzoxazoles/benzothiazoles were suspended in 5% aqueous gum acacia to give a concentration of 1% (w/v). The test compounds were administered intraperitoneally in a dose of 100 mg/kg to a group of 10 mice.

Four hours after the administration of the test compounds, the mice were injected with pentylenetetrazol (90 mg/kg sc). This dose was shown to produce convulsions in almost all untreated mice, and the mice exhibited 100% mortality during 24 hr (12). No mortality was observed during 24 hr in mice treated with 100 mg/kg of thiobenzoxazoles/benzothiazoles alone.

The mice were observed for 60 min for the occurrence of seizures. An episode of clonic spasm persisting for at least 5 sec was considered a threshold convulsion. Transient intermittent jerks and tremulousness were not counted. Animals devoid of threshold convulsions during the 60-min period were considered protected. The number of animals protected in each group was recorded, and the anticonvulsant activity of 2-(substituted alkoxy/hydrazinocarbonyl acetanilidothio)benzoxazoles/benzothiazoles was represented as percent protection. The mice were then observed for 24 hr, and their mortality was recorded.

Determination of Monoamine Oxidase Activity—Monoamine oxidase activity of rat brain homogenates was determined spectrophotofluorometrically, using kynuramine as the substrate (10). Male albino rats, 100–150 g, were allowed food and water *ad libitum* and were sacrificed by decapitation. The brains were removed immediately and homogenized[3] in the ratio of 1:9 (w/v) in ice-cold 0.25 *M* sucrose. The monoamine oxidase activity of rat brain homogenates was determined by incubation at 37° in air for 30 min. The reaction mixture in a total volume of 3 ml consisted of 0.5 ml of phosphate buffer (0.02 *M*, pH 7.5), 0.01 m*M* kynuramine, and 0.5 ml of brain homogenates equivalent to 10 mg of wet weight of the tissue.

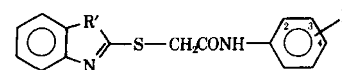
The various 2-(substituted hydrazinocarbonyl acetanilidothio)-benzoxazoles/benzothiazoles were dissolved in propylene glycol (100%) and added to the brain homogenates to produce a final concentration of 0.1 m*M*. An equivalent amount of propylene glycol was added to the control tubes, and the mixture was incubated for 10 min before the addition of kynuramine. The reaction mixture, after addition of kynuramine, was further incubated for 30 min. The reaction was stopped by the addition of 1 ml of 10% trichloroacetic acid (w/v), and the precipitated proteins were removed by centrifugation.

Suitable 1-ml aliquots of the supernatant solution were taken in 2 ml of 1 *N* NaOH solution and were assayed for 4-hydroxyquinoline, formed during oxidative deamination of kynuramine by rat brain homogenates. The 4-hydroxyquinoline formed was measured fluorometrically in a spectrophotofluorometer[4], using activating light of 315 nm and measuring fluorescence at the maximum of 380 nm. 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 calcu-

[2] Commercial chemicals were used. Sodium pyruvate, trisodium DL-isocitrate, sodium α-ketoglutarate, adenosine monophosphate (sodium salt), and cytochrome c were obtained from Sigma Chemical Co., St. Louis, Mo. Other common chemicals were obtained from British Drug House, Bombay, India.
[3] Potter–Elvehjem.

[4] Aminco Bowman.

Table II—Physical Constants of 2-(Substituted Hydrazinocarbonyl Acetanilidothio)-benzoxazoles/benzothiazoles



Compound	R'	R	Melting Point[a]	Yield, %	Molecular Formula	Analysis, % Calc.	Found
VII	O	2-CONHNH$_2$	160°	45	$C_{16}H_{14}N_4O_3S$	C 56.14 H 4.09 N 16.37	56.02 4.20 16.18
VIII	O	3-CONHNH$_2$	270°	50	$C_{16}H_{14}N_4O_3S$	C 56.14 H 4.09 N 16.37	56.32 3.90 16.24
IX	O	4-CONHNH$_2$	188°	56	$C_{16}H_{14}N_4O_3S$	C 56.14 H 4.09 N 16.37	55.90 4.20 16.18
X	S	2-CONHNH$_2$	185°	48	$C_{16}H_{14}N_4O_2S_2$	C 53.63 H 3.91 N 15.64	53.90 3.76 15.52
XI	S	3-CONHNH$_2$	230°	52	$C_{16}H_{14}N_4O_2S_2$	C 53.63 H 3.91 N 15.64	53.34 3.98 15.40
XII	S	4-CONHNH$_2$	195°	58	$C_{16}H_{14}N_4O_2S_2$	C 53.63 H 3.91 N 15.64	53.80 3.82 15.46

[a] Melting points were taken in open capillary tubes with a partial immersion thermometer and are corrected.

Table III—Effects of 2-(Substituted Alkoxycarbonyl Acetanilidothio)benzoxazoles/benzothiazoles on the Oxidation of Pyruvate, DL-Isocitrate, α-Ketoglutarate, and Succinate by Rat Brain Homogenates and Their Anticonvulsant Activity

Compound	Inhibition[a], % Pyruvate	DL-Isocitrate	α-Keto-glutarate	Anti-convulsant Activity[b], % Protection	Pentylene-tetrazol Mortality[b], %
I	30.0 ± 0.4	26.2 ± 0.3	23.7 ± 0.5	20	40
II	52.6 ± 0.8	51.0 ± 0.8	51.0 ± 0.7	10	50
III	71.5 ± 1.1	58.8 ± 1.0	55.3 ± 0.8	20	40
IV	23.4 ± 0.3	21.3 ± 0.3	20.3 ± 0.3	40	30
V	35.9 ± 0.4	42.5 ± 0.5	24.6 ± 0.4	20	40
VI	59.3 ± 0.9	50.8 ± 0.8	34.2 ± 0.5	30	30

[a] Assay procedures and the contents of the reaction mixture are as indicated in the text. Each experiment was done in duplicate, and the values represent mean values of percent inhibition with ± *SE* of the mean calculated from three separate experiments. Inhibition was determined by the decrease in the oxygen uptake/100 mg wet brain weight/hr. All substrates and the test compounds were used at a final concentration of 10 and 2 m*M*, respectively. The oxygen uptake in the control experiments, in the absence of added test compounds, was 96.4 ± 5.8, 116.2 ± 6.3, 102.5 ± 6.7, and 171.8 ± 10.6 μl/100 mg wet tissue weight/hr using pyruvate, DL-isocitrate, α-ketoglutarate, and succinate, respectively, as substrates. The percent inhibition by succinate was nil. [b] Screening procedures are as described in the text. The test compounds were administered (100 mg/kg ip) 4 hr prior to the administration of pentylenetetrazol (90 mg/kg sc). Mortality in pentylenetetrazol-treated animals was observed for 24 hr.

lated from the decrease observed in absorbance, and this value provided an index of the monoamine oxidase inhibitory property of these 2-(substituted hydrazinocarbonyl acetanilidothio)benzoxazoles/benzothiazoles.

RESULTS AND DISCUSSION

The effects of 2-(substituted alkoxycarbonyl acetanilidothio)-benzoxazoles/benzothiazoles on the cellular respiratory activity of rat brain homogenates are recorded in Table III. All compounds selectively inhibited the *in vitro* NAD-dependent oxidations of pyruvate, DL-isocitrate, and α-ketoglutarate by rat brain homogenates. However, NAD-independent or flavine-dependent oxidation of succinate remained unaltered.

As is evident from Table III, substituted thiobenzoxazoles (I–III) possessed greater inhibitory effectiveness than the corresponding thiobenzothiazoles (IV–VI) during oxidation of all substrates. These 2-(substituted alkoxycarbonyl acetanilidothio)benzoxazoles/benzothiazoles possessed low anticonvulsant activity, and the protection afforded by these compounds against pentylenetetrazol-induced convulsions ranged from 10 to 40%. Data on anticonvulsant activity and 24-hr pentylenetetrazol mortality provided evidence for some association between increased protection from convulsions and decreased pentylenetetrazol-induced mortality by these compounds in experimental animals.

The selective inhibitory effects of 2-(substituted hydrazinocarbonyl acetanilidothio)benzoxazoles/benzothiazoles during NAD-dependent oxidations of pyruvate, DL-isocitrate, and α-ketoglutarate by rat brain homogenates are recorded in Table IV. Contrary to such a selective inhibition of NAD-dependent oxidations by the precursors, 2-(substituted alkoxycarbonyl acetanilidothio)benzoxazoles/benzothiazoles, the 2-(substituted hydrazinocarbonyl acetanilidothio)benzothiazoles possessed greater inhibitory effectiveness than the corresponding 2-(substituted hydrazinocarbonyl acetanilidothio)benzoxazoles only during oxidation of pyruvate and DL-isocitrate. The inhibition of the oxidation of α-ketoglutarate by X and XI was an exception to such an increased effectiveness of 2-(substituted hydrazinocarbonyl acetanilidothio)benzothiazoles, since these compounds possessed lower inhibitory activity as compared to their corresponding 2-(substituted hydrazinocarbonyl acetanilidothio)benzoxazoles (VII and VIII). The oxidation of succinate, as was observed with their precursors (I–VI), was not inhibited by these compounds (VII–XII).

The various 2-(substituted hydrazinocarbonyl acetanilidothio)-benzoxazoles/benzothiazoles also provided low protection against pentylenetetrazol-induced convulsions. The low anticonvulsant activity possessed by these compounds ranged from 10 to 40%. These results indicated that, in general, the compounds possessing higher anticonvulsant activity also provided greater protection against pentylenetetrazol mortality in experimental mice.

The monoamine oxidase inhibitory activity of 2-(substituted hydrazinocarbonyl acetanilidothio)benzoxazoles/benzothiazoles is recorded in Table V. The inhibition of rat brain monoamine oxidase was found to be higher with 2-(substituted hydrazinocarbonyl ac-

Table IV—Effects of 2-(Substituted Hydrazinocarbonyl Acetanilidothio)benzoxazoles/benzothiazoles on the Oxidation of Pyruvate, DL Isocitrate, α-Ketoglutarate, and Succinate by Rat Brain Homogenates and Their Anticonvulsant Activity

Compound	Inhibition[a], %			Anticonvulsant Activity[a], % Protection	Pentylenetetrazol Mortality[a], %
	Pyruvate	DL-Isocitrate	α-Ketoglutarate		
VII	52.5 ± 0.7	46.3 ± 0.7	69.5 ± 1.1	30	60
VIII	43.9 ± 0.4	33.2 ± 0.5	65.3 ± 1.0	30	50
IX	56.9 ± 0.6	31.4 ± 0.5	53.1 ± 0.8	30	60
X	72.1 ± 1.1	51.3 ± 0.9	31.7 ± 0.4	40	10
XI	65.8 ± 0.9	36.6 ± 0.4	51.7 ± 0.5	10	80
XII	74.2 ± 1.6	65.8 ± 1.0	56.4 ± 0.7	20	70

[a] Assay procedures for the cellular respiratory activity of rat brain homogenates and screening procedures for the determination of anticonvulsant activity are as described in Table III. The percent inhibition by succinate was nil.

etanilidothio)benzothiazoles (X–XII) than with the corresponding 2-(substituted hydrazinocarbonyl acetanilidothio)benzoxazoles (VII–IX). The monoamine oxidase inhibitory effectiveness of these compounds was similar to their ability to inhibit the cellular respiratory activity during NAD-dependent oxidations of pyruvate and DL-isocitrate by rat brain homogenates.

These results indicated that the conversion of 2-(substituted alkoxycarbonyl acetanilidothio)benzoxazoles/benzothiazoles (I–VI) into 2-(substituted hydrazinocarbonyl acetanilidothio)benzoxazoles/benzothiazoles (VII–XII) in no way altered the low anticonvulsant activity against pentylenetetrazol-induced convulsions. However, opposite effects were observed by such a conversion with respect to their ability to inhibit the cellular respiratory activity of rat brain homogenates. In the present study, 2-(substituted hydrazinocarbonyl acetanilidothio)benzothiazoles (X–XII) exhibited greater inhibition of the NAD-dependent oxidations of pyruvate and DL-isocitrate as compared to 2-(substituted hydrazinocarbonyl acetanilidothio)benzoxazoles (VII–IX).

On the other hand, such an inhibition of NAD-dependent oxidations was greater with 2-(substituted alkoxycarbonyl acetanilidothio)benzoxazoles (I–III) than with the corresponding 2-(substituted alkoxycarbonyl acetanilidothio)benzothiazoles (IV–VI). These results indicated an altered inhibition of cellular respiratory activity by the conversion of the COOR (I–VI) to $CONHNH_2$ (VII–XII) group during oxidation of pyruvate and DL-isocitrate by brain homogenates. The increased basicity of 2-(substituted hydrazinocarbonyl acetanilidothio)benzoxazoles/benzothiazoles (VII–XII) compared to the corresponding precursors, 2-(substituted alkoxycarbonyl acetanilidothio)benzoxazoles/benzothiazoles (I–VI), may presumably account for the generally greater selective inhibitory effectiveness of 2-(substituted hydrazinocarbonyl acetanilidothio)benzoxazoles/benzothiazoles.

Table V—Monoamine Oxidase Inhibitory Activity of 2-(Substituted Hydrazinocarbonyl Acetanilidothio)benzoxazoles/benzothiazoles

Compound	Monoamine Oxidase Inhibition[a], %
VII	33.4 ± 0.5
VIII	46.0 ± 0.7
IX	52.3 ± 0.5
X	35.3 ± 0.3
XI	57.8 ± 0.6
XII	64.1 ± 0.9

[a] Assay procedure and the contents of the reaction mixture are as indicated in the text. Each experiment was done in triplicate, and the values are the mean values with ± *SE* of the mean calculated from three separate experiments. Kynuramine and the test compounds were used at a final concentration of 0.1 m*M*. The enzyme activity observed in the absence of the test compounds ranged from 21.3 to 24.9% change in transmission/10 mg wet brain weight/30 min.

These results failed to provide any correlation between the *in vitro* selective inhibition of NAD-dependent oxidations and monoamine oxidase inhibition and the low anticonvulsant activity of these thiobenzoxazoles/benzothiazoles. Further studies dealing with detailed pharmacological and toxicological properties may account for the enzyme inhibitory effectiveness of 2-(substituted alkoxy/hydrazinocarbonyl acetanilidothio)benzoxazoles/benzothiazoles.

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