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Inhibition of Pyruvic Acid Oxidation by 2,5-Substituted 1,3,4-Oxadiazoles

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Abstract □ Several 2-substituted amino-5-(dichlorophenoxymethyl)-1,3,4-oxadiazoles were synthesized by cyclizing various corresponding 1-(dichloro substituted phenoxyacetyl)-4-substituted thiosemicarbazines. These compounds exhibited the ability to inhibit oxidation of pyruvic acid by rat brain homogenate and showed gross behavioral depression in mice on intraperitoneal administration.

Keyphrases □ Pyruvic acid oxidation—2,5-substituted 1,3,4-oxadiazoles, synthesized and tested as inhibitors, rats, mice □ 1,3,4-Oxadiazoles, 2,5-substituted—synthesis, studied as inhibitors of pyruvic acid oxidation, rats, mice □ Thiosemicarbazines, substituted—synthesis, studied as inhibitors of pyruvic acid oxidation, rats, mice

Several 1-acyl-4-substituted thiosemicarbazines and their cyclized 1,3,4-oxadiazoles have been reported to possess diverse biological properties. The ability of 2,5-disubstituted 1,3,4-oxadiazoles to exhibit analgesic (1, 2), anti-inflammatory (2–4), antipyretic (2), muscle relaxant (5–7), tranquilizing (5, 6), and CNS depressant (8) activities led the present authors to synthesize several 2-substituted amino-5-aryl-1,3,4-oxadiazoles. Furthermore, selective inhibition of nicotinamide adenine dinucleotide-dependent oxidation by CNS depressants such as substituted 4-quinazolones (9–11) prompted the evaluation of the intermediate thiosemicarbazines and cyclized oxadiazoles for their ability to inhibit oxidation of pyruvic acid by rat brain homogenate. Attempts also were made to determine the structure–activity relation of these compounds to their enzyme inhibitory activity. The various oxadiazoles were synthesized according to Scheme I.

EXPERIMENTAL

Ethyl 2,6-Dichlorophenoxyacetate (I)—An equimolar quantity of 2,6-dichlorophenol (8.15 g.), ethyl chloroacetate (6.15 g.), and potassium carbonate (10.35 g.) in dry acetone (50 ml.) was refluxed under anhydrous conditions for 10 hr. The reaction mixture was filtered and the filtrate was poured into 50 ml. of chilled water. The ester was extracted with ether and dried over magnesium sulfate. On removing the excess ether, the remaining liquid was fractionally distilled. The fraction boiling at 180–185° was collected, yielding 9.90 g. (80%).

Table I—1-(Dichlorophenoxyacetyl)-4-substituted Thiosemicarbazines and Their Effect on the Oxidation of Pyruvic Acid

$ArOCH_2CONHNHC(=S)NHR$

Compound Number	R	Melting Point[a]	Yield, %	Formula	Analysis, % Calc.	Found	Percent Inhibition[b]
			Ar = 2,6-Dichlorophenyl				
III	n-C_4H_9	151°	72	$C_{13}H_{17}Cl_2N_3O_2S$	N 12.00	11.93	13.3 ± 1.0
IV	$CH_2CH{=}CH_2$	176–177°	69	$C_{12}H_{13}Cl_2N_3O_2S$	N 12.59	12.31	62.7 ± 0.4
V	C_6H_{11}	172–174°	70	$C_{15}H_{19}Cl_2N_3O_2S$	N 11.17	11.50	75.0 ± 0.8
VI	C_6H_5	180°	64	$C_{15}H_{13}Cl_2N_3O_2S$	N 11.35	11.57	31.3 ± 0.7
VII	p-BrC_6H_4	197°	70	$C_{15}H_{12}BrCl_2N_3O_2S$	C 40.09	39.91	73.5 ± 1.2
					H 2.67	2.55	
					N 9.33	8.92	
VIII	p-IC_6H_4	175°	85	$C_{15}H_{12}Cl_2IN_3O_2S$	N 8.46	8.08	78.1 ± 1.0
IX	o-$CH_3C_6H_4$	171°	70	$C_{16}H_{15}Cl_2N_3O_2S$	C 50.76	50.56	40.3 ± 1.2
					H 3.85	3.65	
					N 10.93	10.63	
X	p-$CH_3C_6H_4$	196°	65	$C_{16}H_{15}Cl_2N_3O_2S$	N 10.93	10.91	42.1 ± 1.0
XI	m-$OCH_3C_6H_4$	167°	68	$C_{16}H_{15}Cl_2N_3O_3S$	N 10.50	10.84	35.3 ± 1.0
XII	p-$OCH_3C_6H_4$	180–181°	70	$C_{16}H_{15}Cl_2N_3O_3S$	N 10.50	10.86	63.5 ± 1.0
			Ar = 3,4-Dichlorophenyl				
XIII	n-C_4H_9	129°	65	$C_{13}H_{17}Cl_2N_3O_2S$	C 50.32	50.17	15.4 ± 0.8
					H 5.48	5.32	
					N 12.00	12.32	
XIV	$CH_2CH{=}CH_2$	125°	68	$C_{12}H_{13}Cl_2N_3O_2S$	N 12.59	12.64	76.6 ± 0.8
XV	C_6H_{11}	149°	70	$C_{15}H_{19}Cl_2N_3O_2S$	N 11.17	10.77	84.4 ± 0.9
XVI	C_6H_5	165°	72	$C_{15}H_{13}Cl_2N_3O_2S$	N 11.35	11.21	60.9 ± 1.2
XVII	p-BrC_6H_4	180°	78	$C_{15}H_{12}BrCl_2N_3O_2S$	N 9.33	8.96	85.2 ± 1.1
XVIII	p-IC_6H_4	182°	70	$C_{15}H_{12}Cl_2IN_3O_2S$	C 36.29	35.90	84.2 ± 1.0
					H 2.41	2.23	
					N 8.46	8.83	
XIX	o-$CH_3C_6H_4$	123°	70	$C_{16}H_{15}Cl_2N_3O_2S$	N 10.93	11.34	21.3 ± 0.5
XX	p-$CH_3C_6H_4$	160°	66	$C_{16}H_{15}Cl_2N_3O_2S$	N 10.93	11.35	39.7 ± 0.5
XXI	m-$OCH_3C_6H_4$	154°	75	$C_{16}H_{15}Cl_2N_3O_3S$	N 10.50	10.80	67.2 ± 0.8
XXII	p-$OCH_3C_6H_4$	167°	75	$C_{16}H_{15}Cl_2N_3O_3S$	N 10.50	10.82	30.1 ± 1.0

[a] All melting points were taken in open capillary tubes and are corrected. [b] Each experiment was done in duplicate, and the values are the mean of three separate experiments. The oxygen uptake was measured at 5-min. intervals during a 1-hr. incubation period. The percentage inhibition was calculated from the decrease in the oxygen uptake/125 mg. wet brain weight. The final concentrations of pyruvic acid and the thiosemicarbazines were 10 and 1 mM, respectively. The percent inhibition of 37.3 ± 0.8% observed with an anticonvulsant, 2-methyl-3-O-tolyl-4-quinazolone, in a final concentration of 1 mM under similar experimental conditions was determined for the sake of comparison.

Anal.—Calc. for $C_{10}H_{10}Cl_2O_3$: C, 48.19; H, 4.01. Found: C, 48.00; H, 3.80.

2,6-Dichlorophenoxyacetylhydrazine (II*a*)—To a solution of 7.05 g. of methyl 2,6-dichlorophenoxyacetate (0.03 mole) in absolute ethanol (20 ml.) was added 2.25 g. of 99–100% hydrazine hydrate (0.045 mole), and the mixture was refluxed under anhydrous conditions on a water bath for 2 hr. On cooling, a white solid mass which separated out was collected by filtration and recrystallized from ethanol into a white crystalline solid, m.p. 139°, yield 9.4 g. (80%).

Anal.—Calc. for $C_8H_8Cl_2N_2O_2$: N, 11.91. Found: N, 11.67.

3,4-Dichlorophenoxyacetylhydrazine (II*b*)—A mixture of 3,4-dichlorophenol (8.15 g., 0.05 mole), ethyl bromoacetate (7.65 g., 0.05 mole), and potassium carbonate (10.35 g., 0.075 mole) in dry acetone (40 ml.) was refluxed under anhydrous conditions for 10 hr. The reaction mixture was filtered hot, and the excess of solvent was removed by distillation under reduced pressure. To the residue was added 99–100% hydrazine hydrate (3.0 g.) and absolute ethanol (25 ml.). The mixture was refluxed under anhydrous conditions for 3 hr. Ethanol was removed by distillation. On cooling, a white solid mass which separated out was filtered, washed with a little cold ethanol, dried, and recrystallized from ethanol, m.p. 141°, yield 9.9 g. (85%).

Anal.—Calc. for $C_8H_8Cl_2N_2O_2$: N, 11.91. Found: N, 11.80.

Substituted Phenoxyacetylthiosemicarbazines (III–XXII)—An equimolar quantity (0.005 mole) of substituted phenoxyacetylhydrazine and different alkyl or aryl isothiocyanate was refluxed in ethanol (10 ml.) for 2–3 hr. The solvent was removed by distillation. On cooling, the solid products which separated out were filtered and dried. Recrystallization from ethanol gave pure compounds (Table I).

2-Substituted Amino-5-(substituted phenoxymethyl)-1,3,4-oxadiazoles (XXIII–XLII)—To an ethanolic suspension of an appropriate thiosemicarbazine (0.01 mole) was added 5 ml. of 4 N sodium hydroxide with continuous shaking. Iodine in 5% potassium iodide solution was gradually added to the clear solution with stirring until the color of iodine persisted at room temperature. The contents were then refluxed on a water bath, and more iodine solution was added until a permanent tinge of excess iodine remained. The reaction mixture was then poured into ice-cold water (500 ml.), and the precipitated solid mass which separated out was filtered and washed with water and then with warm carbon disulfide. The crude product was finally recrystallized from ethanol. The compounds thus prepared are recorded in Table II.

BIOCHEMICAL STUDIES

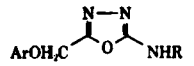
Materials—Commercial chemicals[1] were used in the present study.

Assay of Pyruvic Acid Oxidation by Rat Brain—Male albino rats, kept on an *ad libitum* diet, were used in all experiments. Rat brains isolated from decapitated animals were immediately homogenized in ice-cold 0.25 M sucrose in a Potter–Elvehjem homogenizer. All incubations were carried out at 37°, and the oxygen uptake was measured by the conventional Warburg manometric technique with air as the gas phase. Fresh rat brain homogenate equivalent to 125 mg. wet weight was added to chilled Warburg vessels containing 6.7 mM magnesium sulfate, 20 mM sodium hydrogen phosphate buffer solution (pH 7.4), 1 mM adenosine monophosphate (sodium salt), 33 mM potassium chloride, 10 mM pyruvic acid, and 500 mcg. of cytochrome c in a final volume of 3 ml. The central well contained 0.2 ml. of 20% potassium hydroxide solution. All of the compounds under assay were dissolved in propylene glycol (100%), and an equal volume of the solvent was added in the control vessels.

Effects of Oxadiazoles on Gross Behavior—The studies on the gross behavior in mice were done according to the scheme outlined by Irwin (12). The drugs were taken in 5% gum acacia suspension

[1] Sodium pyruvate, adenosine monophosphate, and cytochrome c were obtained from Sigma Chemical Co., St. Louis, Mo. Other common chemicals were obtained from British Drug House, Bombay, India.

Table II—2-Substituted Amino-5-dichlorophenoxymethyl-1,3,4-oxadiazoles and Their Effect on the Oxidation of Pyruvic Acid



Compound Number	R	Melting Point[a]	Yield, %	Formula	Analysis, % Calc.	Found	Percent Inhibition[b]
				Ar = 2,6-Dichlorophenyl			
XXIII	n-C_4H_9	145°	65	$C_{12}H_{15}Cl_2N_3O_2$	C 49.36	48.95	55.5 ± 0.9
					H 4.74	4.50	
					N 13.29	12.93	
XXIV	$CH_2CH{=}CH_2$	191–193°	58	$C_{12}H_{11}Cl_2N_3O_2$	C 48.00	48.31	22.5 ± 0.9
					H 3.66	3.22	
					N 14.00	13.90	
XXV	C_6H_{11}	123–125°	54	$C_{15}H_{17}Cl_2N_3O_2$	C 52.63	52.39	3.5 ± 0.8
					H 4.97	5.01	
					N 12.28	11.96	
XXVI	C_6H_5	133–135°	60	$C_{15}H_{11}Cl_2N_3O_2$	C 53.57	53.19	9.1 ± 0.5
					H 3.27	3.52	
					N 12.50	12.16	
XXVII	p-BrC_6H_4	204°	53	$C_{15}H_{10}BrCl_2N_3O_2$	C 43.37	43.10	30.6 ± 1.0
					H 2.40	2.10	
					N 10.12	10.46	
XXVIII	p-IC_6H_4	195°	50	$C_{15}H_{10}Cl_2IN_3O_2$	C 38.96	38.63	42.9 ± 1.1
					H 2.16	2.01	
					N 9.09	8.81	
XXIX	o-$CH_3C_6H_4$	157°	61	$C_{16}H_{13}Cl_2N_3O_2$	C 54.85	54.51	22.6 ± 0.8
					H 3.71	3.80	
					N 12.00	11.68	
XXX	p-$CH_3C_6H_4$	180°	60	$C_{16}H_{13}Cl_2N_3O_2$	C 54.85	54.60	46.2 ± 0.9
					H 3.71	3.30	
					N 12.00	11.70	
XXXI	m-$OCH_3C_6H_4$	135°	56	$C_{16}H_{13}Cl_2N_3O_3$	C 52.45	52.10	—
					H 3.55	3.33	
					N 11.47	11.50	
XXXII	p-$OCH_3C_6H_4$	148–150°	53	$C_{16}H_{13}Cl_2N_3O_3$	C 52.45	52.70	20.6 ± 0.5
					H 3.55	3.79	
					N 11.47	11.22	
				Ar = 3,4-Dichlorophenyl			
XXXIII	n-C_4H_9	190–192°	55	$C_{12}H_{15}Cl_2N_3O_2$	C 49.36	49.60	18.8 ± 1.1
					H 4.74	4.90	
					N 13.29	12.90	
XXXIV	$CH_2CH{=}CH_2$	170°	80	$C_{12}H_{11}Cl_2N_3O_2$	C 48.00	48.36	47.1 ± 1.1
					H 3.66	3.42	
					N 14.00	13.78	
XXXV	C_6H_{11}	133°	60	$C_{15}H_{17}Cl_2N_3O_2$	C 52.63	52.80	73.9 ± 0.9
					H 4.97	5.20	
					N 12.28	12.44	
XXXVI	C_6H_5	155°	63	$C_{15}H_{11}Cl_2N_3O_2$	C 53.57	53.80	8.5 ± 0.9
					H 3.27	3.56	
					N 12.50	12.76	
XXXVII	p-BrC_6H_4	194°	60	$C_{15}H_{10}BrCl_2N_3O_2$	N 10.12	9.97	Nil
XXXVIII	p-IC_6H_4	196–198°	55	$C_{15}H_{10}Cl_2IN_3O_2$	C 38.96	38.84	52.6 ± 0.9
					H 2.16	2.39	
					N 9.09	9.36	
XXXIX	o-$CH_3C_6H_4$	165°	60	$C_{16}H_{13}Cl_2N_3O_2$	C 54.85	54.48	11.1 ± 0.8
					H 3.71	3.36	
					N 12.00	11.80	
XL	p-$CH_3C_6H_4$	190–192°	57	$C_{16}H_{13}Cl_2N_3O_2$	C 54.85	55.10	57.4 ± 0.0
					H 3.71	3.96	
					N 12.00	12.30	
XLI	m-$OCH_3C_6H_4$	171–173°	73	$C_{16}H_{13}Cl_2N_3O_3$	C 52.45	52.04	59.3 ± 0.8
					H 3.55	3.46	
					N 11.47	11.74	
XLII	p-$OCH_3C_6H_4$	154–155°	65	$C_{16}H_{13}Cl_2N_3O_3$	C 52.45	52.72	73.8 ± 1.2
					H 3.55	3.70	
					N 11.47	11.03	

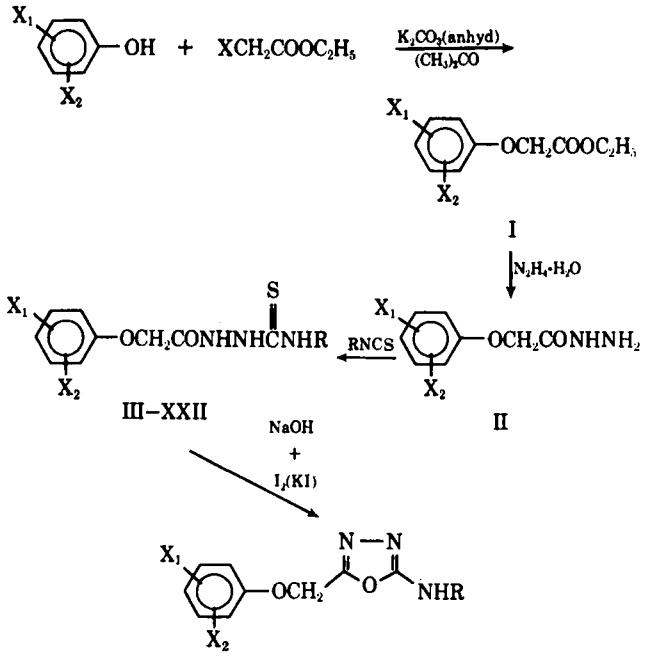
[a] All melting points were taken in open capillary tubes and are corrected. [b] Each experiment was done in duplicate, and the values are the mean of three separate experiments. The oxygen uptake was measured at 5-min. intervals during a 1-hr. incubation period. The percentage inhibition was calculated from the decrease in the oxygen uptake/125 mg. wet brain weight. The final concentrations of pyruvic acid and the oxadiazoles were 10 and 1 mM, respectively.

and administered intraperitoneally. The effect of these compounds on the simple reflexes and gross behavior was observed.

RESULTS AND DISCUSSION

All of the 1-(dichlorophenoxyacetyl)-4-substituted thiosemicarbazines were found to inhibit oxidation of pyruvic acid by rat brain homogenate (Table I). All of the compounds possessing a p-bromophenyl, p-iodophenyl, allyl, or cyclohexyl substituent at position R exhibited greater inhibition. Such an increase in their inhibitory effect was independent of the relative position of the two chlorine atoms present in the phenoxyacetyl moiety of these compounds.

Cyclization of thiosemicarbazines into corresponding oxadiazoles resulted in a significant lowering of their ability to inhibit oxidation of pyruvic acid (Table II). Some oxadiazoles were found to be inactive; a few, however, exhibited greater inhibitory effects than their corresponding thiosemicarbazines. These results failed to provide any direct correlation between the open-chain thiosemicarbazines and their cyclized oxadiazoles. At present, it is difficult



Scheme I

to provide any suitable explanation for the low inhibitory effects of these cyclized oxadiazoles. In general, the steric factors associated with the heterocyclic oxadiazole ring could presumably cause obstruction in the attachment of oxadiazoles on the active site(s) of the enzyme molecule and could, therefore, account for their decreased inhibitory effects. Such a decrease in enzyme inhibitory activities of the oxadiazoles as compared to the thiosemicarbazines could also be due to the loss of C=S, as well as the two acidic hydrogen atoms next to C=O and C=S, respectively, when the thiosemicarbazines are cyclized to their corresponding oxadiazoles. Otherwise, the sulfur and hydrogen atoms in these thiosemicarbazines would form hydrogen bonds with the enzymes to cause greater enzyme inhibition. *In vivo* studies have indicated that almost all compounds produced depression in the gross behavior of experimental mice. Loss of righting reflex was observed with 2-(cyclohexylamino)-5-(3′,4′-dichlorophenoxymethyl)-1,3,4-oxadiazole. In the present study, no correlation could be observed between the enzyme inhibitory activity of substituted thiosemicarbazines and oxadiazoles with behavioral effects.

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