Monoamine Oxidase and Succinate Dehydrogenase Inhibitory Properties of Substituted 1,3,4-Oxadiazole-2-Thiones

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Fifteen new 3-arylaminomethyl-5-(2-hydroxy-3,5-dibromophenyl)-1,3,4-oxadiazole-2-thiones were synthesized and characterized by their sharp melting points, elemental analyses and ir spectra. These substituted oxadiazolylthiones were evaluated for their enzyme inhibitory activity. All compounds inhibited *in vitro* monoamine oxidase and succinate dehydrogenase activity of rat brain homogenates. The degree of monoamine oxidase inhibition ranged from 59-93% at a final concentration of 1×10^{-4} *M* whereas the inhibition of succinate dehydrogenase was 49-100% at a final concentration of 5×10^{-5} *M*.

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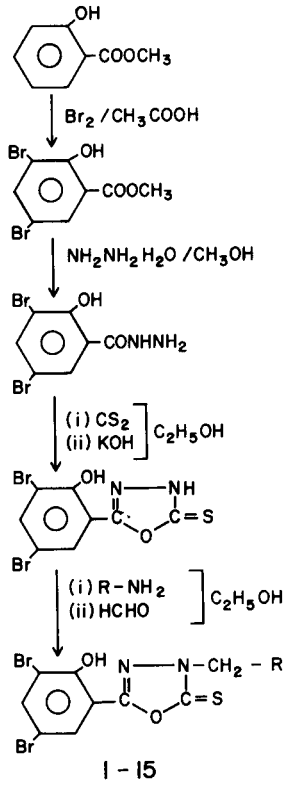
Variety of compounds including 1,3,4-oxadiazoles have been shown to possess central nervous system depressant activity (1). These 1,3,4-oxadiazoles have also been shown to exhibit analgesic (2,3), muscle relaxant (4), and tranquilizing properities (5). In earlier studies some mannich bases of 5-substituted phenyl-1,3,4-oxadiazole-2-thione have been reported to possess central nervous system depressant activity (6). These observations prompted synthesis of some mannich bases, 3-arylaminomethyl-5-(2-hydroxy-3,5-dibromophenyl)-1,3,4-oxadiazole-2-thiones, by condensation of 5-(2-hydroxy-3,5-dibromophenyl)-1,3,4-oxadiazole-2-thione with an appropriate amine as possible central nervous system depressants. The ability of central nervous system depressants to possess enzyme inhibitory effectiveness led to the evaluation of these compounds for their effects on monoamine oxidase and succinate dehydrogenase activity of rat brain homogenates.

Scheme I



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Materials and Methods.

Methyl 3,5-Dibromosalicylate.

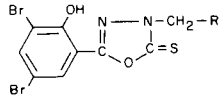
The preparation was carried out according to the method reported earlier (7). Ice-cold solution of bromine (5.3 ml) in glacial acetic acid (20 ml) was added slowly in small portions to an ice-cold solution of methyl salicylate (9.1 ml) in glacial acetic acid (30 ml) with thorough shaking. Bromination was carried out at room temperature for 1 hour. The reaction mixture was cooled in ice and poured into an excess of ice-cold water. A bulky white precipitate of methyl 3,5-dibromosalicylate was obtained along with a small quantity of methyl 5-bromosalicylate. The precipitate was filtered and dried (yield 90%).

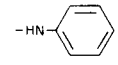
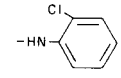
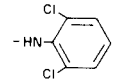
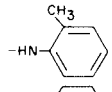
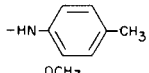
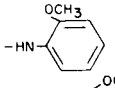
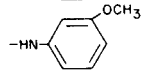
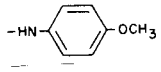
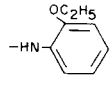
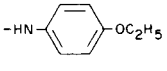
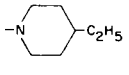
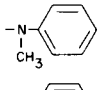
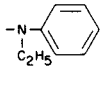
This crude product (0.08 mole) in glacial acetic acid (30 ml) was again subjected to bromination with 1.7 ml of bromine in glacial acetic acid (20 ml). This gave a precipitate of only methyl 3,5-dibromosalicylate which was filtered, dried, and recrystallized from aqueous methanol into colorless needles, mp 146° yield 80%.

Table I

Physical Constants of

3-Arylaminomethyl-5-(2-hydroxy-3,5-dibromophenyl)-1,3,4-oxadiazole-2-thiones



Compound No.	R	Melting Point °C	Molecular Formula	Yield %	Analysis					
					Calculated			*Found*		
					C	H	N	C	H	N
1		134	$C_{15}H_{11}Br_2N_3O_2S$	60	39.38	2.40	9.19	39.08	2.39	9.01
2		190	$C_{15}H_{10}Br_2ClN_3O_2S$	64	36.62	2.03	8.54	36.51	2.06	8.43
3		125	$C_{15}H_9Br_2Cl_2N_3O_2S$	60	34.22	1.71	7.98	34.10	1.66	7.88
4		140	$C_{16}H_{13}Br_2N_3O_2S$	50	40.76	2.76	8.91	40.81	2.81	8.89
5		110	$C_{16}H_{13}Br_2N_3O_2S$	55	40.76	2.76	8.91	40.66	2.72	8.83
6		118	$C_{16}H_{13}Br_2N_3O_3S$	60	39.42	2.66	8.62	39.33	2.58	8.52
7		205	$C_{16}H_{13}Br_2N_3O_3S$	50	39.42	2.66	8.62	39.01	2.61	8.60
8		110	$C_{16}H_{13}Br_2N_3O_3S$	64	39.42	2.66	8.62	39.40	2.63	8.58
9		80	$C_{17}H_{15}Br_2N_3O_3S$	60	40.71	2.99	8.38	40.63	3.01	7.93
10		150	$C_{17}H_{15}Br_2N_3O_3S$	65	40.71	2.99	8.38	40.67	2.89	8.27
11		115	$C_{14}H_{15}Br_2N_3O_2S$	76	37.41	3.34	9.35	37.32	3.26	9.30
12		148	$C_{16}H_{19}Br_2N_3O_2S$	65	40.25	3.98	8.80	40.18	3.84	8.79
13		235	$C_{13}H_{13}Br_2N_3O_3S$	50	34.58	2.88	9.31	34.61	2.92	8.99
14		132	$C_{16}H_{13}Br_2N_3O_2S$	70	40.76	2.76	8.91	40.81	2.66	8.82
15		117	$C_{17}H_{15}Br_2N_3O_2S$	65	42.06	3.09	8.65	41.98	3.08	8.46

3,5-Dibromosalicylhydrazide.

Methyl dibromosalicylate (0.015 mole) was dissolved in methanol (40 ml) and hydrazine hydrate (5 ml, 60%) was added. The mixture was refluxed on a water bath for 20 minutes. The solution was diluted with water and cooled. To the cold solution was added a few ml of dilute acetic acid when a voluminous white solid mass was obtained. The crude product thus obtained was filtered, washed with methanol, and crystallized from hot water, mp 217° dec, yield 80%.

5-(2-Hydroxy-3,5-dibromophenyl)-1,3,5-oxadiazole-2-thione.

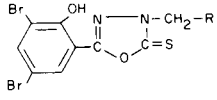
Following the method of Young and Wood (8) a mixture of 3,5-dibromosalicylhydrazide (0.2 mole), potassium hydroxide (0.2 mole), carbon disulfide (40 ml), and ethanol (200 ml) was heated under reflux until the evolution of hydrogen sulfide had nearly stopped. The excess of the solvent was removed by distillation and the residue was dissolved in water and acidified with dilute hydrochloric acid. The resulting oxadiazolylthione was collected by filteration, washed with water, and dried. The product was recrystallized from ethanol, mp 240°, yield 70%.

3-Arylaminomethyl-5-(2-hydroxy-3,5-dibromophenyl)-1,3,4-oxadiazole-2-thiones.

The method of Ram, *et al.*, (6) was followed for the synthesis of these compounds. To a mixture of ethanolic solution of 5-(2-hydroxy-3,5-dibromophenyl)-1,3,4-oxadiazole-2-thione (0.01 mole) and formaldehyde (0.015 mole, 40%), was slowly added with stirring an ethanolic solution of suitable amine (0.01 mole). The reaction was stirred for an hour and left overnight in a refrigerator. The solid mas obtained on cooling was filtered, washed with cold ethanol, and dried. All these reactions were routinely monitored by thin layer chromatography. The various 3-arylaminomethy-5-(2-hydroxy-3,5-dibromophenyl)-1,3,4-oxadiazole-2-thiones reported in Table I were recrystallized from ethanol and characterized by their sharp melting points, elemental analyses and infrared spectrum.

Table II

Monoamine Oxidase and Succinate Dehydrogenase Inhibitory Properties of 3-Arylaminomethyl-5-(2-hydroxy-3,5-dibromophenyl)-1,3,4-oxadiazole-2-thiones in Rat Brain Homogenates



Compound	Percent Inhibition (a)	
	Monoamine Oxidase Activity (1×10^{-4} *M*)	Succinate Dehydrogenase Activity (5×10^{-5} *M*)
1	60.0	48.5
2	62.5	66.0
3	67.0	100.0
4	58.5	80.0
5	67.0	45.0
6	93.0	100.0
7	82.0	22.5
8	84.0	68.5
9	80.0	68.0
10	81.0	60.0
11	79.5	69.0
12	83.0	81.0
13	92.0	59.0
14	60.5	66.0
15	60.0	69.0

(a) The values reported as percent inhibitaion are mean values of two separate experiments done in duplicate. The test compounds were dissolved in propylene glycol and the assay procedures are as recorded in the text.

Biological Studies.

Adult albino rats weighing approximately 100-150 g were killed by cervical dislocation. The brain was dissected out, the adhering tissue and the blood was cleaned off and kept in ice bath until homogenization. The brains were homogenized in 0.25 *M* sucrose using a Potter-Elvehjem homogenizer under cold conditions. The enzyme inhibitory activities of these compounds were determined using brain homogenates as the source of enzymes.

Determination of Monoamine Oxidase Activity.

The spectrophotoflorometric method using kynuramine as the substrate was used for the determination of monoamine oxidase activity (9). The 4-hydroxyquinoline formed during oxidative deamination of kynuramine followed by the spontaneous cyclization of the deaminated product was measured as an index of the enzyme activity.

The reaction mixture in a final volume of 2.0 ml contained 1.0 ml of phosphate buffer (0.5 *M*, *p*H 7.4), a suitable aliquot of the enzyme preparation, 20.0 μg of kynuramine, the compound to be tested at a final concentration of 1×10^{-4} *M* and water. The reaction was initiated by the addition of the substrate after a preincubation period of 10 minutes. After an incubation of 30 minutes, at 37° C, in a water bath, the reaction was stopped by the addition of 1 ml of trichloroacetic acid solution (10%, w/v). The precipitated proteins were separated by centrifugation at 700 × gravity for 10 minutes and 1 ml of the clear supernatant was added to 2 ml of sodium hydroxide and the fluorescence was measured in an Aminco Bowman Spectrophotofluorometer using an activating light of 315 nm with a fluorescence wavelength of 380 nm.

Determination of Succinate Dehydrogenase Activity.

Succinate dehydrogenase activity was determined using sodium succinate as the substrate by following the spectrophotometric method of Slater and Bonner (10).

The reaction mixture in a final volume of 2 ml contained 0.4 ml of phosphate buffer (0.5 *M*, *p*H 7.2). 0.4 ml of potassium ferricyanide solution (0.01 *M*), 0.2 ml of sodium cyanide (0.1 *M*), a suitable aliquot of brain homogenate, 0.1 ml of sodium succinate (0.1 *M*), the test compounds to be tested, and water. The reaction was started by the addition of the substrate after a preincubation of 10 minutes at 37° C in a water bath. After incubation for 30 minutes, the reaction was stopped by the addition of 2 ml of trichloroacetic acid solution (10%, w/v). The contents of the reaction mixture were centrifuged at 700 × gravity for 10 minutes and the optical density of the clear supernatant

was observed at 400 mμ using Hitachi Perkin-Elmer UV-VIS spectrophotometer.

All substituted 3-arylaminomethyl-5-(2-hydroxy-3,5-dibromophenyl)-1,3,4-oxadiazole-2-thiones inhibited monoamine oxidase activity of rat brain homogenates when used at a final concentration of 0.1 m*M*. In the present study compound **1**, without any substituent at the phenyl nucleus, showed 60% inhibition. Introduction of a substituent in the phenyl nucleus, in general, was found to increase the degree of monoamine oxidase inhibition with the exception of *o*-methyl substituted compound (**4**). Substitution of a methoxy group at the *ortho* (compound **6**), *meta* (compound **7**), and *para* (compound **8**) positions increased the precent degree of inhibition to 93, 82, and 84, respectively. Such an increase was also reflected by introduction of an ethoxy-substituent at *ortho* (compound **9**) and *para* (compound **10**) where 80% and 81% inhibition, respectively, of monoamine oxidase was observed. Introduction of halogen (compounds **2** and **3**) and methyl substituent (compound **5**) although increased the inhibitory effectiveness which, however, was not of a high order. On the other hand, use of *N*-methylamine (compound **14**) and *N*-ethylamine (compound **15**) during formation of these Mannich bases was found to have no effect on their ability to inhibit monoamine oxidase as compared to the degree of inhibition observed with compound **1**. However, it was interesting to note that replacement of the phenyl nucleus with a heterocyclic moiety like piperidine (compound **12**) and morpholine (compound **13**) significantly increased their ablity to inhibit monoamine oxidase which was reflected by 80%, 83%, and 92% inhibition, respectively.

All substituted oxadiazolylthiones inhibited succinate dehydrogenase activity of rat brain homogenates and the degree of inhibition ranged from 23-100%. The effect of the introduction of substituent in the phenyl nucleus, increased degree of inhibitory effectiveness in all compounds with the exception of *para*-methyl (compound **5**), *meta*-methoxy (compound **7**) substitution which showed 45% and 23% inhibition, respectively, as compared to 49% inhibition observed with the parent compound **1** with unsubstituted phenyl nucleus. Maximum inhibition observed with compound possessing chloro substituents at both *ortho*-positions (**3**) and *ortho*-methoxy substituent (**6**) in the phenyl nucleus. The replacement of the phenyl nucleus with a heterocyclic moiety also increased succinate dehydrogenase inhibitory activity. The degree of inhibition with piperidine (compound **11**), 4-ethylpiperidine (compound **12**), and morpholine (compound **13**) observed was 69%, 81%, and 59%, respectively. Contrary to their ability to inhibit monoamine oxidase where no change was observed it was found that compounds **14** and **15** produced greater degree of inhibition of succinate dehydrogenase of 66% and 69%, respectively, as compared to 49% inhibition observed with compound **1**.

These results have failed to provide clear-cut structure-activity relationship of substituted 3-arylaminomethyl-5-(2-hydroxy-3,5-dibromophenyl)-1,3,4-oxadiazole-2-thiones with respect to their ability to inhibit monoamine oxidase and succinate dehydrogenase activity of rat brain homogenates. In addition, no correlation was observed between monoamine oxidase and succinate dehydogenase inhibitory properties of these substituted oxadiazolylthiones.

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REFERENCES AND NOTES

(1) J. Maillard, M. Vincent, R. Moris and M. Bernhard, French Patent M. 379 (Jan. 1962); *Chem. Abstr.*, **57**, 15251g (1962).

(2) I. Angilini, L. Angilini and F. Sparaco, British Patent, 1,161,801 (August 1969); *Chem. Abstr.*, **71**, 112936g (1969).

(3) H. Najar, R. Giudicelli, C. Moral and J. Menin, *Bull. Soc. Chim. France*, 153 (1966).

(4) H. L. Yale and R. Losee, *J. Med. Chem.*, **9**, 478 (1966).

(5) J. J. Piala and H. L. Yale, U.S. Patent, 3,142,021 (July 1964); *Chem. Abstr.*, **61**, 8317b (1964).

(6) V. J. Ram and R. N. Pandey, *J. Indian Chem. Soc.*, **11**, 634 (1974).

(7) R. M. Acheson and M. J. T. Robinson, *J. Chem. Soc.*, 232 (1953).

(8) R. W. Young and K. H. Wood, *J. Am. Chem. Soc.*, **77**, 400 (1955).

(9) M. Krajl, *Biochem. Pharmacol.*, **14**, 1684 (1965).

(10) E. C. Slater and W. D. Bonner, *Biochem. J.*, **52**, 185 (1952).