

ANTICONVULSANT AND MONOAMINE OXIDASE INHIBITORY ACTIVITY OF SUBSTITUTED OXADIAZOLTHIONES

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Summary : Several 1,3,4-oxadiazol-thiones were synthesised and characterized by their melting points elemental analysis and I.R. spectra. All the oxadiazol-thiones possessed anticonvulsant activity which was reflected by protection upto 80% against pentylenetetrazole induced seizures and 40% protection against maximal electroshock induced seizures. Substantutions at position-3 of oxadiazol-thiones have shown marked effect on MAO inhibitory activity. No definite correlation between monoamine oxidase inhibitory and anticonvulsant activity could be established. It was observed that by the substitution of one, two and three methyl groups in the phenyl ring of 2-arylamino methyl side chain anticonvulsant activity against both maximal electroshock induced convulsions and pentylenetetrazol induced convulsions decreases i.e. the order of activity was found to be unsubstituted > monomethyl > dimethyl > trimethyl.

INTRODUCTION

Various 1, 3, 4-oxadiazoles were shown to exhibit CNS depressant (2, 3) muscle relaxant (12) analgesic (1) tranquillizing (6) and aniconvulsant (4, 5) properties. Further some of the mannich bases of 1, 3, 4-oxadiazol-2-thiones have also been reported as pyruvate oxidase inhibitors (8). These observations prompted the synthesis of 1, 3, 4-oxadiazol-thiones having substitution at 5 and 3 positions of oxadiazole ring. Furthermore, the enzyme monoamine oxidase (MAO) has been considered to play an important

role in the activity of central nervous system (7). This could possible account for anti-convulsant properties of MAO inhibitors which have been shown to be closely associated with the elevation of 5-hydroxy-tryptamine and noradrenaline in brain (14). In the present study some newer 3-(arylaminoethyl)-5-(pyridin-4'-yl) 1,3,4-oxadiazol-2-thiones were synthesized and evaluated for their inhibitory effects on rat brain monoamine oxidase *in vitro* and anticonvulsant activity *in vivo* in order to correlate structure activity relationship.

MATERIALS AND METHODS

5-(pyridin-4'-yl)-1,3,4-oxadiazol-2-thiones :

Isonicotinyl hydrazide (0.2 mole), KOH (0.2 mole) and 40 ml of carbon disulfide in 200 ml of ethanol was refluxed until the evolution of H₂S subsided, solvent was removed by distillation and product obtained was dissolved in water and acidified with dil. HCl. Solid thus obtained was filtered washed with water and recrystallised from alcohol. M.P. 380; N% Calculated 23.46%, Found 23.52% (13).

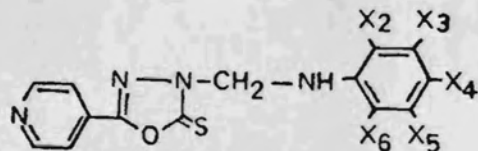
3-(Arylaminoethyl)-5-(pyridin-4'-yl)-1,3,4-oxadiazol-2-thiones :

To a mixture of oxadiazol-thione (0.01 mole) and formaldehyde (0.015 mol) ethanolic solution of suitable amine (0.01 mols) was added slowly with stirring. The reaction mixture was stirred for an hour and left in refrigerator over night. Solid thus obtained was recrystallized from ethanol. Sharp melting points, satisfactory elemental analysis (Table I), provided support for the structure of the different oxadiazol-thiones.

Determination of monoamine oxidase inhibitory activity :

MAO activity of rat brain homogenate was determined by the method of Tabor *et al.* (11) using benzylamine hydrochloride (0.1 M) as substrate. Adult rats weighing approximately 100-150 g were used in all experiments. They were killed by decapitation. Brains were quickly removed and homogenized in ice cold 0.25 M sucrose solution to give a 10% (w/v) solution of brain homogenate. The reaction mixture in a final volume

TABLE I : Physical constants, MAO inhibitory and anticonvulsant properties of 3- aryl amino methyl)-5-(pyridin-4-yl) 1,3,4-oxadiazol-2-thiones.



Compd. No.	X ₂	X ₃	X ₄	X ₅	X ₆	Melting point*	Molecular formula	Monoamine** oxidase inhibition %	Anticonvulsant activity*** % protection against			ALD ₅₀ ****
									Pentylenetetrazol	Maximal electroshock	Mortality after 24 hrs	
1	H	H	H	H	H	160	C ₁₄ H ₁₂ N ₄ OS	82.8 ± 0.88	60	30	40	>1000
2	OCH ₃	H	H	H	H	150	C ₁₅ H ₁₄ N ₄ O ₂ S	50.2 ± 0.19	60	40	40	>1000
3	H	H	OCH ₃	H	H	182	C ₁₅ H ₁₄ N ₄ O ₂ S	21.7 ± 0.92	80	40	20	1000
4	H	H	CH ₃	H	H	198	C ₁₅ H ₁₄ N ₄ OS	43.4 ± 0.68	30	30	60	1000
5	CH ₃	CH ₃	H	H	H	94	C ₁₆ H ₁₆ N ₄ OS	65.2 ± 0.34	40	40	40	500
6	H	CH ₃	H	CH ₃	H	222	C ₁₆ H ₁₆ N ₄ OS	30.5 ± 0.26	40	20	50	>1000
7	CH ₃	H	CH ₃	H	CH ₃	110	C ₁₇ H ₁₈ N ₄ OS	91.3 ± 0.52	20	20	50	1000
8	H	H	Cl	H	H	130	C ₁₄ H ₁₁ ClN ₄ OS	43.5 ± 0.44	20	10	60	500
9	H	Cl	H	Cl	H	188	C ₁₄ H ₁₀ Cl ₂ N ₄ OS	86.9 ± 0.50	20	Nil	80	500
10	H	H	NO ₂	H	H	200	C ₁₄ H ₁₁ N ₅ O ₃ S	43.4 ± 0.26	40	Nil	60	500

* Melting points were taken in open capillary tubes and are uncorrected and nitrogen analysis was found within the range.

** Assay procedure was as described in the text. Each experiment was done in duplicate and results indicate the ± standard errors of mean value. Compounds were used at a final concentration of 5 × 10⁻⁴ M.

*** Compounds were administered at a dose of 100 mg/kg. ip.

**** Represents approximate lethal dose in mg/kg.

of 2 ml consisted of 0.4 ml phosphate buffer (pH 7.2, 0.5 M) 0.2 ml of 10% brain homogenate and 0.1 ml benzylamine hydrochloride. The various compounds were dissolved in propylene glycol (100%) and used at the final concentration of 5×10^{-4} M. Compounds under assay were generally incubated for 10 min at 37°C with brain homogenate before the addition of benzylamine. The reaction mixture was further incubated for 30 min at 37°C after the addition of the substrate. Enzyme reaction was stopped by the addition of 1 ml of 10% perchloric acid and the precipitated proteins were removed by centrifugation. Absorbance of the aliquot was measured in Hitachi Perkin-Elmer Spectrophotometer at 250 nm. Change in optical density was taken as an index of enzyme inhibition.

Pharmacological studies :

Determination of Anticonvulsant activity :

(i) Against pentylenetetrazol induced seizures :

Anticonvulsant activity was determined against pentylenetetrazole induced seizures in mice of either sex weighing 25-30 gms. The mice were divided in groups (10 animal in each group) keeping the group weight as equal as possible. Each substituted oxadiazol-thione was suspended in aqueous gum acacia. The test compounds were injected intraperitoneally in a group of 10 animals at a dose of 100 mg/kg. One group of animals was treated with phenobarbitone as standard (30 mg/kg, s.c.). Four hrs after the administration of oxadiazol-thione the mice were injected with pentylenetetrazole (90 mg/kg, s.c.). This dose of pentylenetetrazole has been shown not only to produce convulsions in almost all untreated mice but also to exhibit 100% mortality during 24 hrs. The mice were then observed for 60 mins for seizures and animals devoid of convulsions during 60 mins were considered protected. The number of animals protected in each group was recorded and the anticonvulsant activity of these quinazolone was represented as percent protection. The animals were then observed for 24 hrs and their mortality was observed.

(ii) Maximal electroshock seizures :

The protective effect of the compounds against the tonic phase of maximal electroshock seizures (MES) was tested according to the procedure of Swinyard *et al.* (10). Group of 10 mice of either sex, weighing between 20-25 g were given test compounds intraperitoneally (100 mg/kg) one hour prior to being subjected to supramaximal electro-

shock delivered through ear electrodes (mA, 0.3 sec.) The resultant normal seizures showed a tonic phase of limb flexion followed by full tonic extension and a few clonic jerks thereafter. The number of mice devoid of hand limb extension phase of seizures were considered protected and anticonvulsant activity was represented as percent protection.

Toxicity studies :

The approximate LD_{50} values were determined by intraperitoneal administration of test compounds in albino mice following the method reported by Smith (9).

RESULTS AND DISCUSSION

In the present study various substituted oxadiazolthiones were screened for their monoamine oxidase inhibitory activity during oxidative deamination of benzylamine at a final concentration of $5 \times 10^{-4}M$ and their ability to protect against convulsions produced by pentylenetetrazol and maximal electroshock. Oxadiazol-thiones with an (unsubstituted phenyl amino) methyl group at position-3 showed a marked inhibitory activity of 82.6% but substitution of trimethyl group increased the degree of inhibition to 91.3%. Among methyl substituents, the MAO inhibitory activity increased from mono to di to trimethyl with the exception of 3,5-dimethyl substituent which showed inhibition less than monomethyl substituent. Methoxy substitution showed a further decrease in degree of inhibition. Dichloro substituents showed same inhibition as that of unsubstituted one, where as replacement of 4-methyl group by 4-chloro or 4-nitro group exhibited no change in inhibitory activity.

Substituted oxadiazol-thiones at a dose of 100 mg/kg, ip showed a varied degree of protection against pentylenetetrazol and maximal electroshock induced seizures. Maximum protection (80%) was exhibited by 3-(o-methoxy phenyl amino) methyl-5-(pyridin-4-yl)-1,3,4-oxadiazol-2-thione. Replacements of methoxy group by methyl group exhibited a marked decrease in degree of protection. Monochloro and dichloro substituents also showed lower degree of protection.

All the substituted oxadiazol-thiones also showed protection against maximal electroshock seizures ranging from 20-40% except dichloro and nitro substituent which showed no protection against MES.

The low toxicity of these oxadiazol-thiones was reflected by their high ALD₅₀ values.

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