LETTER TO THE EDITOR

NOVEL INDOLYL PYRAZOLINES AS MONOAMINE OXIDASE INHIBITORS

Sir,

The significance of 5-hydroxytryptamine (5-HT) and other tryptamines in the functioning of central nervous system (CNS) has in recent years aroused interest in search of indole derivatives as potential psychotropic agents. Indole derivatives have been shown to exhibit anticonvulsant (2,9,1,8) and monoamine oxidase (MAO) inhibitory (15,7), properties. Furthermore diverse variety of CNS activities including tranquilizing, muscle relaxant, psychoanaleptic (12), hypnotic (3), anticonvulsant (2,4) and anti MAO (11) have been associated with pyrazole derivatives. Earlier studies have also reported anticonvulsant activity of MAO inhibitors (13,14). These observations led to the synthesis of some new 1-aryl-3-(4-methoxyphenyl)-5-[2-(4-methoxyphenyl) indol-3-yl-)]-Δ^2-pyrazolines and evaluation of their anticonvulsant and MAO inhibitory properties. Steps in the synthesis are summarized below under (A), (B), (C) and (D).

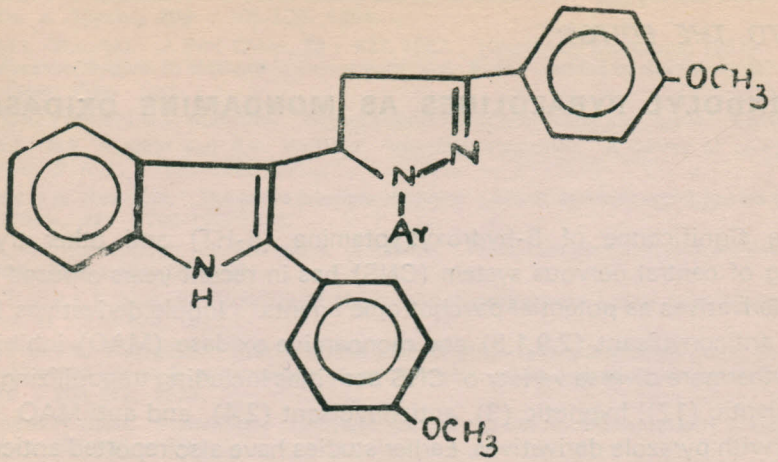
(A) *2-(4-methoxyphenyl) indole* - was synthesized by following the method reported in the literature (5).

(B) *2-(4-methoxyphenyl) indole-3-aldehyde* - was prepared following the method of Buchmann and Rossner (6).

(C) *1-[2-(4-methoxyphenyl) indol-3-yl]-2-(4-methoxypenzoyl) ethylene* - The method of Parmar *et al.* (11) was followed for the synthesis of this compound. A mixture of 4-methoxy acetophenone (0.01 mole) and 2-(4-methoxyphenyl) indol-3-aldehyde (0.01 mole) was stirred in ethanolic NaOH solution. The brown solid separating on cooling was filtered, washed several times with cold water and recrystallized from ethanol (M.P. 188°C; yield 85%).

(D) *1-Aryl-3-(4-methoxyphenyl)-5-[2(4-methoxyphenyl)indol-3-yl-]-Δ^2 pyrazolines:* Following the method of Parmar *et al.* (11) 1-[2-(4-methoxy phenyl) indol-3-yl]-2 (4-methoxybenzoyl) ethylene (0.01 mole) and appropriate aromatic hydrazine hydrochloride (0.02 mole) were refluxed in acetic acid (20 *ml*) for 4-5 hr. The reaction mixture was cooled and poured in ice water. The solid mass which separated out was filtered, washed with water and recrystallized from ethanol. All indolyl pyrazolines were characterized by their sharp melting points, elemental analysis and infrared spectra (Table I).

TABLE I MAO inhibitory and anticonvulsant properties of 1-aryl-3-(4-methoxyphenyl)-5-[2-(4-methoxyphenyl)-indol-3-yl-]Δ^2-pyrazolines.



Sl. No.	Ar	M.P. °C	Molecular formula	MAO inhibition % 1x10^{-2}	MAO inhibition % 5x10^{-4}	Anticonvulsant activity protection %	Pentylenetetrazol mortality during 24 hr %	ALD_{50} values (mg/kg)
1	2-$CH_3C_6H_4$	195	$C_{32}H_{29}N_3O$	73	65	60	40	>1000
2	3-$CH_3C_6H_4$	160	$C_{32}H_{29}N_3O_2$	60	60	20	80	1000
3	4-$CH_3C_6H_4$	180	$C_{32}H_{29}N_3O_2$	26	58	10	60	1000
4	2,4,6-$(CH_3)_3C_6H_4$	210	$C_{34}H_{33}N_3O_2$	53	45	10	60	1000
5	2-$OCH_3C_6H_4$	220	$C_{32}H_{29}N_3O_3$	79	45	40	80	1000
6	3-$OCH_3C_6H_4$	>280	$C_{32}H_{29}N_3O_3$	60	65	20	60	1000
7	4-$OCH_3C_6H_4$	>290	$C_{32}H_{29}N_3O_3$	40	38	10	60	1000
8	2-Cl.C_6H_4	235	$C_{31}H_{26}N_3ClO_2$	46	60	40	40	1000
9	3-Cl.C_6H_4	170	$C_{31}H_{26}N_3ClO_2$	36	32	30	60	>1000
10	2, 6-$(Cl)_2C_6H_3$	220	$C_{31}H_{25}N_3Cl_2O_2$	46	40	60	40	1000
11	2,4,6-$(Cl)_3C_6H_2$	>280	$C_{31}H_{24}N_3Cl_3O_2$	46	42	60	80	1000
12	2-(Cl),5$(NO)_2C_6H_3$	180	$C_{31}H_{25}N_4ClO_4$	66	66	40	40	1000
13	2-NO_2.C_6H_4	172	$C_{31}H_{26}N_4O_4$	26	15	20	80	1000
14	3-NO_2.C_6H_4	82-90	$C_{31}H_{26}N_4O_4$	30	25	40	50	1000
15	4-NO_2.C_6H_4	120-35	$C_{31}H_{26}N_4O_4$	46	36	50	60	1000
16	2,4-$(NO_2)_2C_6H_3$	223	$C_{31}H_{26}N_5O_6$	20	15	40	60	1000

Melting points were determined in open capillary tubes and are uncorrected. Yields were in the range of 59-79%. All compounds were analysed for their C,H and N analyses and the values obtained were within ±0.4% of the theoretical values.

Monoamine oxidase activity - MAO activity of rat brain homogenate was determined by the method of Tabor *et al.* (17) using benzylamine hydrochlpride (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) brain homogenate. The reaction mixture in a final volume 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 $1x10^{-3}$ 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.

Anticonvulsant activity : Anticonvulsant activity was determined (10) against pentylenetetrazol-induced seizures in mice of either sex weighing 25-30 *g*. The mice were divided into groups of 10 keeping the group weights as equal as possible. Each indolylpyrazoline was suspended in 5% aqueous gum acacia to give a concentration of 0.25%. The test compounds were injected intraperitoneally in a group of 10 animals at a dose of 100 *mg/kg*. Four hr after the administration of indolyl pyrazolines, the mice were injected with pentylenetetrazol (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 hr. The mice were then observed for 60 min for seizures and animals devoid of convulsions during 60 min were considered protected. The number of animals protected in each group was recorded and the anticonvulsant activity of these indolyl-pyrazolines was represented as percent protection. The animals were then observed for 24 hr and their mortality was observed.

Toxicity studies : The approximate LD_{50} values were determined by intraperitoneal administration of test compounds in albino mice following the method reported by Smith (16).

The MAO inhibitory activities of substituted indolyl pyrazoline using rat brain homogenate during oxidative deamination of benzylamine are shown in Table I. All compounds inhibited MAO activity *in vitro* at a final concentration of $1x10^{-3}$M, compound 5 producing maximum inhibition of 79%.

All indolyl pyrazolines at a dose of 100 *mg/kg* i.p. exhibited anticonvulsant activity which was reflected by their protection(10-60%) against pentylenetetrazol-induced seizures (Table I). Compounds 1,8,10 and 11 showed 60% anticonvulsant activity. Administration of these compounds failed to protect pentylenetetrazol treated animals against death since 40-80% mortality was observed during 24 hr period.

The shifting of CH_3/OCH_3 group from position 2 to 3 to 4 in the benzene ring attached to position 1 of the pyrazoline moiety decreased both MAO inhibitory and anticonvulsant properties accordingly (Compound 1,2,3,5,6,7). Such type of variations in both the biological activities were also observed with the movements of NO_2 group from position 2 to 3 to 4. The compound having two NO_2 groups at 2 and 4 positions (Compound 16) showed higher anticonvulsant activity than the compound containing one NO_2 at position 2 (Compound 13). The trend in the change of anticonvulsant activity was found to be similar to that of MAO inhibitory effectivness in the case of compounds possessing Cl group(s) (Compound 8-11). However, the introduction of NO_2 group in the chlorinated benzene ring (Compound 12) enhanced the MAO inhibition markedly. The replacement of three Cl groups (Compound 11) by three CH_3 groups (Compound 4) showed an increase in MAO inhibition and decrease in anticonvulsant activity. Furthermore, it was significant to note that all of these substituted indolyl pyrazolines possessed high approximate LD_{50} indicating their low toxicity.

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REFERENCES

1. Agarwal, V.K., A.K. Chaturvedi, T.K. Gupta, S.S. Parmar and Boer Benjamih De. Anticonvulsant activity of substituted indole alkyl amines. *J. Med. Chem.*, **17** : 377-380, 1974.
2. Agarwal, V.K., T.K. Gupta and S.S. Parmar. Substituted indole benzyl hydrazines as anticonvulsant. *J. Med. Chem.*, **15** : 1000-1001, 1972.
3. Avakumov, M.V. and Y.M. Batulin. Mechanism of underlying potentiation of the hypnotic effect of hexobarbital by 3,5-dimethyl-4-bromopyrazole. *Farmacol. Toksikol.*, **31** : 402, 1968 through *Chem. Abstr.*, **69** : 75439H, 1968.
4. Batulin, Y.M. Mechanism of anticonvulsant activity of some pyrazole derivatives. *Farmakol. Toksikol.*, 1968, **31** : 533, Through *Chem. Abstr.*, **70** : 2236a, 1969.
5. Blades, C.E. and A.L. Wilds. The preperation of indoles from diazoketones. *J. Org. Chem.*, **21** : 1013-1021, 1966.
6. Buchmann, G. and D. Rossner. Chemistry of 2-phenyl acylated 2-phenyl indole and their reactivity, *J. Prakt. Chem.*, **25** : 117-134, 1964.
7. Gujrati, V., N. Srivastava, K. Shanker, K. Kishor and K.P. Bhargava. Monoamine oxidase (MAO) inhibitory activity of indole 3-acetamido 4-aryl carboxylic acid alkylesters. *Curr. Sci.*, **47** : 300-301, 1978.
8. Keasling, H.H., Willette and J. Szmuszkuiez. The anticonvulsant activity of 3-acyl indoles compared with phenobarbital. *J. Med. Chem.*, **7** : 94-96, 1964.
9. Nagar, S., V.K. Agarwal and S.S. Parmar. Substituted indoles hydrazines as possible anticonvulsants. **41** : 215-216, 1972.

10. Pandey, B.R., S.S. Parmar, S. Kumar, S.J. Brumleve. Anticonvulsant activity and selective inhibition of NAD dependent oxidation by 1,2, 4-trisubstituted-5-imidazolone. *Pharmacology*, **16** : 344-346, 1978.

11. Parmar, S.S., B.R. Panday, C. Dwivedi and R.D. Harbinson. Anticonvulsant activity and monoamine oxidase inhibitory properties of 1,3,5-trisubstituted pyrazolones. *J. Pharm. Sci.*, **65** : 1152-1155, 1974.

12. Polevoi, L.G. Relation between the chemical structure and Pharmacological activity of aminopyrazoles. Tr. Nauch. Knof. Aspir. ordin. I. Yi (Peryi) *Mosk. Med. Inst. Moscow*, **159** : 1964 Through *Chem., Abstr.*, **65** : 19147d, 1966.

13. Polevoi, L.G., K.N. Kudrin, I.I. Grandburg and A.N. Kost. Relation of aminopyrazole structure to their neurotropic activity. *Timiryazev. Selskokhos. Akd.*, **199** : 1968. Through *Chem. Abstr.*, **70** : 1557g, 1969.

14. Prockop, D.J., P.A. Shore and B.B. Brodie. Anticonvulsant properties of monoamine oxidase inhibitors. *Ann. N.Y. Acad. Sci.*, **80** : 643-651, 1959.

15. Shanker, K., V.K. Agarwal, R.J. Selveraj and S.S. Parmar. Synthesis of indole hydrazines as monoamine oxidase inhibitors. *J. Med. Chem.*, **12** : 324-326, 1969.

16. Smith, C.C. A Short term chronic test. *J. Pharmacol. Exptl. Therap.*, **100** : 408, 1950.

17. Tabor, C.W., H. Tabor and S.M. Rosenfall. Methods in Enzymology. Vol. II. p. 390, 1955.