STUDIES ON NEUROPHARMACOLOGICAL AND BIOCHEMICAL PROPERTIES OF 5-SUBSTITUTED TETRAZOLES

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Summary : Neuropharmacological screening of 5-substituted tetrazoles revealed CNS depressant and anticonvulsant activities. The compounds also inhibited rat brain monoamine oxidase.

Key words : 5-substituted tetrazoles central nervous system (CNS) potentiation of sodium pentobarbitone sleeping time anticonvulsant activity monoamine oxidase activity (MAO)

INTRODUCTION

Tetrazoles have been used as a versatile nucleus for building CNS active compounds (3). Different sulphonamides were also found to possess anticonvulsant activity (2,4,7). These observations prompted us to synthesize 5-substituted sulphonamido tetrazoles. In our previous communication, we have reported the synthesis of various 5-[β-(N–alkyl–N–*p*–toluene sulphonamido) ethyl] tetrazoles, 5–(β–(N'–2–heterocyclic sulphonamido) ethyl] tetrazoles and 5–{β–(N'–(2–heterocyclic)–N^4–(arylidene amino benzene) sulphonamido] ethyl} tetrazoles (8,9). In the present study, attempts have been made to investigate their central nervous system and monoamine oxidase activity.

MATERIALS AND METHODS

Toxicity studies : Toxicity study was performed with the 5-substituted tetrazoles. The test compounds were administered intraperitoneally in albino mice of either sex weighing 20–25 *g* in 5% aqueous suspension of gum acacia at different doses and approximate LD_{50} were determined (11).

C.N.S. Activities :

(1) *Effect on gross behaviour :* Gross behaviour activity was performed according to the method of Irwin (5). The test compounds were administered ip in albino mice

weighing 20–25 *g* in 5% aqueous suspension of gum acacia at a dose of 1/5th of LD_{50}. The same amount of aqueous suspension of gum acacia given ip to the control group showed no marked effect on gross behaviour activity.

Spontaneous motor activity was done in albino mice weighing 20–25 *g* according to the method of Dews (1). The compounds were injected at 1/5th of the LD_{50}.

(2) *Potentiation of sodium-pentobarbitone sleeping time :* The ability of these 5-substituted tetrazoles to potentiate pentobarbitone induced hypnosis was investigated following the method of Winter(12). The albino mice weighing 20–25 *g* were taken in groups of six animals. Sodium pentobarbitone, administered ip in a dose of 40 *mg/kg* to the control group produced sleep. The test compounds were administered ip at a dose of 100 *mg/kg* one hr prior to the administration of sodium pentobarbitone. The animals were observed regularly for sleep as evidenced by the loss of righting reflex until the animal had awakened. The mean average sleeping time in each group and degree of potentiation was calculated.

(3) *Anticonvulsant activity :* The anticonvulsant activity against pentylene tetrazol-induced seizures was determined in albino mice weighing 25–30 *g* (6). The test compounds were aministered ip to a group of 10 amimals in 5% aqueous suspension of gum acacia at a dose of 100 *mg/kg*. One hr after the administration of these compounds, the mice were injected with pentylene tetrazol (90 *mg/kg*, sc). This dose of pentylene tetrazol had been shown not only to produce convulsions in almost all untreated mice but also to exhibit 100% mortality during 24 hr period. An episode of clonic spasm which persisted for at least 5 sec, was considered as threshold convulsions. Animals not exhibiting threshold convulsions during 60 min were considered protected. The number of animals protected in each group was recorded and percentage protection was determined. The mortality was recorded after 24 hr.

Monoamine oxidase activity : The monoamine oxidase activity of rat brain homogenate was determined by the method of Tabor *et al.* (10), using benzylamine hydrochloride as substrate. Adult rats weighing 100-150 *g* were killed by decapitation. Brains were quickly removed and homogenized in ice-cold 0.25 M sucrose solution to give a 10% 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 compounds were dissolved in propylene glycol (100%) and used at a final concentration of 1×10^{-4}M. The compounds were incubated for 10 min with brain homogenate (before the addition of benzylamine). The reaction mixture was further incubated for 30 min (after the addition of substrate). The enzyme reaction was stopped by

the addition of 1 *ml* of 10 % perchloric acid. Absorbance of the aliquot was measured and change in optical density was taken as an index of enzyme inhibition.

RESULTS

The approximate LD_{50} values of the 5-substituted tetrazoles were found in the range of 650– > 1000 *mg/kg* suggesting their relative low-toxicity. *In vivo* studies have indicated that all the tetrazoles produced depression in gross-behaviour as shown by slow or irregular respiration, decrease reactivity to sound and touch, depression of body and limbs, decrease of body temperature, loss of righting reflexes and the presence of ataxia. These compounds also produced decreased spontaneous motor activity of the animals. The results are shown in Table I.

All the 5-substituted tetrazoles were found to potentiate sodium pentobarbitone hypnosis in mice. Maximum potentiation was observed for the compound ***15*** and minimum to the compound ***5***. Their ability to potentiate pentobarbitone induced hypnosis ranged from 1.08 to 2.02 times that observed for normal control mice (Table I).

The results on the anticonvulsant activity exhibited by these 5-substituted tetrazoles are shown in Table. I. All the compounds afforded protection ranging from 10–70% Maximum protection was observed with compound ***16*** while compound ***6*** exhibited the lowest anticonvulsant activity. These compounds however, were unable to provide protection against death. 20–90% mortality was observed during 24 hr in pentylene tetrazol treated animals.

The data on the inhibitory effect of 5-substituted tetrazoles on monoamine oxidase activity of rat brain homogenate are given in Table I. The maximum inhibitory activity was observed with compound ***11*** and minimum with compound ***6***.

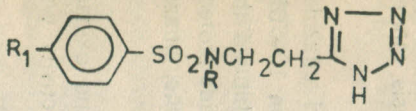
DISCUSSION

The approximate LD_{50} values ranging from 650– > 1000 *mg/kg* reflected low toxicity for the 5-substituted tetrazoles. All the tetrazoles produced behavioural depression. The CNS depressant property of these compounds was also reflected by their ability to possess anticonvulsant activity and to potentiate sodium pentobarbitone sleeping time.

All the compounds were found to quicken the effect of sodium pentobarbitone hypnosis in mice by 39.0 to 72.5 min.

The results of anticonvulsant activity showed that the elongation of alkyl chain at R (*1–5*) decreased the protecting ability of these compounds. On the other hand, attachment of different heterocyclic substituents at R and NH_2 at R_1 (*6–10*) was found to play

TABLE I : Pharmacological and biochemical properties of 5-substituted tetrazoles.



Compound No.	R	Approximate LD_{50} mg/kg ip	SMA decrease in activity %	Potentiation of pentobarbitone sleeping time min. Mean±SD	Increase* time control	Anticonvulsant activity: Protection %	Anticonvulsant activity: Pentylene tetrazol mortality during 24 hr %	Monoamine oxidase inhibition % Mean±SE
	$R_1 = CH_3$							
1	Methyl	825	53.0	58.3±5.2	1.62	50	50	58.4±0.3162
2	Ethyl	>1000	45.2	48.5±3.8	1.35	40	50	56.2±0.4183
3	n-Propyl	830	48.0	41.0±3.0	1.14	30	60	54.9±0.3512
4	iso-Propyl	830	50.0	49.4±5.9	1.37	30	40	55.1±0.2739
5	n-Butyl	900	45.0	39.0±2.8	1.08	20	70	50.2±0.5837
	$R_1 = NH_2$							
6	Pyridyl	700	32.6	55.5±6.7	1.52	10	90	23.2±0.3873
7	Thiazolyl	780	43.0	48.5±4.5	1.34	20	80	40.8±0.3542
8	Pyrimidyl	950	52.4	57.0±6.4	1.58	30	50	35.2±0.1582
9	4-Methyl pyrimidyl	1000	57.0	52.0±5.4	1.44	60	40	46.0±0.2730
10	4 6–Dimethyl pyrimidyl	>1000	50.8	39.5±2.8	1.09	60	30	50.0±0.2472
	$R_1 = C_6H_5CH=N-$							
11	Thiazolyl	800	49.6	49.3±5.8	1.37	20	60	70.2±0.5535
12	4–Methyl pyrimidyl	1000	47.4	44.5±3.4	1.23	40	50	60.2±0.3528
13	4 6–Dimethyl pyrimidyl	>1000	50.2	49.2±5.9	1.37	50	40	62.8±0.4739
	$R_1 = p$–Cl $C_6H_4CH=N-$							
14	Thiazolyl	>1000	43.0	62.8±6.5	1.74	50	40	33.5±0.2236
15	4–Methyl pyrimidyl	>1000	48.0	72.5±9.0	2.02	60	30	28.4±0.5162
16	4,6–Dimethyl pyrimidyl	>1000	55.6	64.5±7.0	1.79	70	20	23.8±0.0234
	$R_1 = p$–OH. C_6H_4 $CH=N-$							
17	Thiazolyl	650	36.2	49.0±5.3	1.36	20	60	—
18	4–Methyl pyrimidyl	1000	30.2	56.5±6.9	1.57	40	60	—
19	4,6–Dimethyl pyrimidyl	1000	32.8	60.5±2.8	1.67	50	50	—

*Control values for pentobarbitone (40 *mg/kg*) sleeping time was 36 min and test compounds was administered at a does of 100 *mg/kg* which was taken as 1 for comparison.

a definite role in their degree of protection. The compounds possessing *p*-chloroarylidene moiety at R_1 (*14–16*) produced more protection than the corresponding compounds containing unsubstituted arylidene moiety (*11–13*) or *p*-hydroxyarylidene moiety (*17–19*) at R_1. As is evident from Table I, all 5-substituted tetrazoles exhibiting a greater degree of protection elicited a lower pentylene tetrazol-induced mortality during 24 hr in the experimental animals.

All the 5-substituted tetrazoles produced inhibition of rat brain monoamine oxidase ranging from 23.2—70.2%. Increase in the number of carbon atom in the side chain at R (*1–5*) resulted in a decrease of inhibitory activity. Similar decrease in enzyme inhibitory property was observed with compounds having NH_2 group at R_1 and heterocyclic groups at R (*6–10*). The presence of unsubstituted arylidene moiety at R_1 and heterocyclic group at R (*11–13*) produced a greater degree of enzyme inhibition as compared to compounds having chloro group at R (*14–16*).

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