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Research Article

SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SOME NEW SUBSTITUTED PYRIDAZINONE DERIVATIVES IN SEARCH OF POTENT ANTICONVULSANT AGENTS

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ABSTRACT

A series of novel 2-substituted phenyl-6-(substituted phenylamino) pyridazin-3(*2H*)-one were synthesized by the reaction of various substituted anilines with 6-chloro-2-substituted phenylpyridazin-3(*2H*)-one. The compounds were characterized by FT-IR, UV-Vis, mass and NMR. The compounds were tested for their anticonvulsant activity utilizing MES and scPTZ animal models and compared with the standard drug phenytoin. The majority of the compounds exhibited significant activity against both animal models; however, compounds 6-(p-toluidino)-2-(4-chlorophenyl) pyridazin-3(2H)-one **(II_d)** and 2-(4-chlorophenyl)-6-(4-fluorophenylamino) pyridazin-3(2H)-one **(II_f)** displayed promising activity and could be considered as leads for further investigations. Some of the selected compounds were evaluated for their neurotoxic effects, and some of these showed no sign of neurotoxicity.

Keywords: Pyridazinone, Aniline, Anticonvulsant and Neurotoxicity.

INTRODUCTION

Epilepsy is a common chronic neurological disorder characterized by recurrent unprovoked seizures [1]. These seizures are transient signs or symptoms of abnormal, excessive, synchronous neuronal activity in the brain [2]. Being one of the world's oldest recognized disorders, it is surrounded by fear, discrimination, social and frightening manifestation [3]. A global campaign against epilepsy conducted by World Health Organization (WHO) in partnership with International Bureau for Epilepsy (IBE) and International League Against Epilepsy (ILAE) suggests that around 1% of world population at any time (about 50 million people worldwide) is afflicted with this neurological disorder. Every year about 2.4 million new cases are added to these figures [4, 5]. Currently available antiepileptic drugs (AEDs) provide adequate seizure control in many patients, still about 28–30% of patients are estimated to be poorly treated[6,7]. Many investigations indicated that the presence of at least one aryl group, one or two electron donor atoms and/or an NH group in a spatial arrangement is necessary for anticonvulsant activity[8-10]. In recent years, pyridazinone nucleus showed promising potential in playing vital role in discovering novel drugs having potential anticonvulsant activity [11-18]. In the present work it is therefore thought to design and synthesize the combination of pyridazinone as a basic nucleus incorporated with substituted arylamine within a single molecule. Such combination is hoped to develop compounds having potential anticonvulsant activity.

MATERIAL AND METHODS

The chemicals used for experimental work were commercially procured from various chemical units Merck India Ltd., CDH ,S.D. Fine Chemicals and Qualigens. These

solvents and reagents were purified before use.

General procedure for Synthesis of 2-substituted phenyl-6-(substituted phenylamino) pyridazin-3(2H)-one derivatives (I_a-I_g, II_a- II_g)

To the solution of maleic anhydride (4.92g) in hydrochloric Acid (6ml) was added substituted phenyl hydrazine (4.5g) and the content is refluxed for 3 hours. Water is added in between slowly. Then, after the completion of reaction, reaction mixture was adjusted to pH 7 by saturated Na_2CO_3. After cooling, the solution was filtered to give buff cultured solid. The crude products were recrystallized from methanol. Then, above product (4.3g) and Phosphorus oxychloride ($POCl_3$) was heated on a stream bath for 6 hours. After heating, the mixture was carefully poured drop by drop on crushed ice. After making alkaline pH with NaOH solution, crude product was collected by filtration, washed with cold water to remove excess $POCl_3$ and recrystallized with ethanol.

The mixture of substituted aniline (0.005mol), Compound I_2 / II_2 (0.005mol) and Iso-propyl alcohol (15ml), in a conical bottle was introduced into microwave oven and irradiated for 15min (output power at 30%). The progress of the reaction is monitored by TLC. Then, the reaction mixture was cooled to room temperature and slowly added drop by drop on crushed ice. Crude product was filtered, washed with cold water to remove excess aniline and recrystallized by methanol. By adapting the above procedure the compounds I_a-I_g, II_a- II_g was prepared. (Scheme-1)

INSTRUMENTS

Melting points were determined in open glass capillary and are uncorrected. All the Fourier Transform Infra-Red (FTIR) spectra were recorded on Shimadzu 01799 IR-Affinity-1 Spectrophotometer using KBr pellets; v max values are given in cm^{-1}. The Proton Magnetic Resonance Spectra (1H-NMR) were recorded on Bruker DPX-300 (300 MHz) and Bruker Advance 400(400 MHz) instrument in DMSO-d6/CDCl3 using Tetramethylsilane [(CH3)4Si] as internal standard. Chemical shift were given in δ ppm (parts per million) scale. The mass spectra (MS) were recorded on Mass Spectrometer Jeol SX-102 (FAB), equipped with direct intel probe system. The m/z values of the more intense peaks are mentioned. The progress of the reaction was checked on thin layer chromatography (TLC) plates (silica gel G) using Toluene: Ethyl Acetate: Formic Acid (5:4:1), Benzene: Methanol (8:2) as solvent systems which were visualized by exposing to iodine vapors. Microwave irradiation was carried out with Catalyst Microsystems Microwave oven (650W, 2450MHz) at 30% output power.

Anticonvulsant Activity

The anticonvulsant activity was done MES method [19, 20] and sc. PTZ [21, 22] methods.

1. MES Test

Each compound was administered as an i.p. (intraperitoneal) injection at dose level of 30, 100 and 300 mg per kg and the anticonvulsant activity was assessed after 0.5 h and 4 h interval of administration.

Maximal electroshock seizures were elicited in mice by delivering a 60 Hz, 50 mA electric stimuli for 0.2 sec via ear clip electrode. The maximal electroshock seizures typically consist of a short period of tonic extension of the hind limbs and a final clonic episode. Blocked of the limbs tonic extensor component due to the drug treatment is taken as the end point [19].Phenytoin and test compound was given intraperitoneally to groups (standard, control and test compound) respectively. The animals are subjected to electro convulsions after 0.5 and 4 hours of administration of drugs.

Equipment: Electroconvulsometer

2. Pentylenetetrazol (Metrazol) test in mice and rats

This assay has been used primarily to evaluate antiepileptic drugs. However, it has been shown that most of anxiolytic agents are also able to prevent or antagonize Metrazole-induced convulsions. Inhibition of seizures and tonic-clonic convulsions are recorded by this method [21].

Pentylenetetrazole (85mg/Kg) produces seizures in >95% of mice, is administered as a 0.5% solutions sc. in the posterior midline. The animal was observed for 30 min., failure to observe even a threshold seizure (a single episode of clonic spasms of at least 5 second duration) was defined as protection.

The values are mentioned in Table 2 and Table 3 calculated from at least three different experiments in duplicate.

Neurotoxicity screening of new-substituted-pyridazinone derivatives (NT)

Toxicity induced by a compound was detected in mice using the standardized rotarod described by Dunham and Miya (1957). Untreated control mice, when placed on a 10 r.p.m. rotation rod (knurled plastic rod)[20] of 3.2cm in diameter, can maintain their equilibrium for prolonged period of time (more than 1 minute).

The mice were trained to stay on an accelerating rotarod that rotates at 10 rpm. The rod diameter was 3.2cm. Trained animals were injected intraperitoneally with the test compounds and standard drugs at doses of 100mg/Kg and 300mg/Kg. Neurotoxicity was indicated by the inability of the animal to maintain equilibrium on the rod for at least 1 min in each of the three trials. (Table 4)

RESULT AND DISCUSSION

Synthetic route depicted in Scheme 1 outline the chemistry of the present work. The key intermediate 6-chloro-2-substitutedphenylpyridazin-3(2H)-one (I_2/II_2) was obtained by reacting substituted phenyl hydrazine with maleic anhydride in acidic conditions for 3hr to yield 6-hydroxy-2-substitutedphenylpyridazin-3(2H)-one (I_1/II_1) which was then chlorinated with the help of $POCl_3$ (6hrs) to give the desired product (I_2/II_2). Then the title compounds 2-substituted phenyl-6-(substituted phenylamino) pyridazin-3(2H)-one **(Ia-Ig, IIa- IIg)** were obtained by the reaction of 6-chloro-2-substitutedphenylpyridazin-3(2H)-one (I_2/II_2) with a variety of substituted aniline. The formation of the title compound was indicated by the presence of peak due to NH, NH_2 of aniline in the IR and ^{1}H NMR spectrum of all the compounds **(Ia-Ig, IIa- IIg)**. The IR and ^{1}H NMR spectrum of these compounds showed the presence of peaks due to pyridazinones, carbonyl (C=O) and aryl groups. The mass spectra of the title compounds showed molecular ion peak corresponding to their molecular formulae. **(Table 1)**

Anticonvulsant activity

The tested compounds showed anticonvulsant activity ranging from 33 % to 100 % against MES and sc.PTZ method **(Table 3).** The standard drug phenytoin showed 100% anticonvulsant activity after 30 min and 4 hours. The compounds **II_c (R=4-Cl,R'=4-Cl), II_f (R=4-Cl, R'=4-F),** showed potent anticonvulsant activity in MES test at dose 30mg/kg after 30 min comparable with phenytoin and carbamazepine. Compound **II_f (R=4-Cl, R'=4-F)** showed potent anticonvulsant activity in MES test at dose 30mg/kg after 4 hours comparable with phenytoin and carbamazepine. The compounds **I_b,I_c ,I_d ,I_f ,I_g,II_b ,II_d ,II_g** also showed potent anticonvulsant activity after 30 min and compound **II_c** showed potent anticonvulsant activity after 4 hours in MES test at dose 100mg/kg comparable with phenytoin and carbamazepine.

Then the compounds were evaluated for Pentylene tetrazole induced seizure (sc.PTZ) test by administering PTZ as a 0.5% solutions subcutaneously (*sac*) in the posterior midline and the test compounds *i.p* at dose level 30mg/kg in mice. Out of all compounds, **II_c** and **II_g** were found active in PTZ test due to electron withdrawing property.

Activity can be concluded in this decreasing order: 4-Cl > 4-F > 3,4-$DiCl_2$ > 2-Cl > 4-CH_3 > 2-F > H.

Neurotoxicity

The minimal motor impairment was assessed for active compounds and it was observed that all the compounds tested except I_b, Ib_{is} were less neurotoxic than the standard drug phenytoin. The most active compound of the series II_d and II_f did not show any sign of neurotoxicity except in case of compound II_f which was found to be neurotoxic at 300 mg/kg after 0.5 h. **(Table 4)**

CONCLUSION

In summary, synthesis of new series of 2-substituted phenyl-6-(substituted phenylamino) pyridazin-3(2H)-ones have been described. Most of the compounds have displayed considerable anticonvulsant activity in both the seizure models. They were also found to be less neurotoxic as indicative of the better tolerability of the compounds. They have strong future commitments. Compound Si_c and II_f exhibited maximum protection and offers potential for further optimization and development to new anticonvulsant agents.

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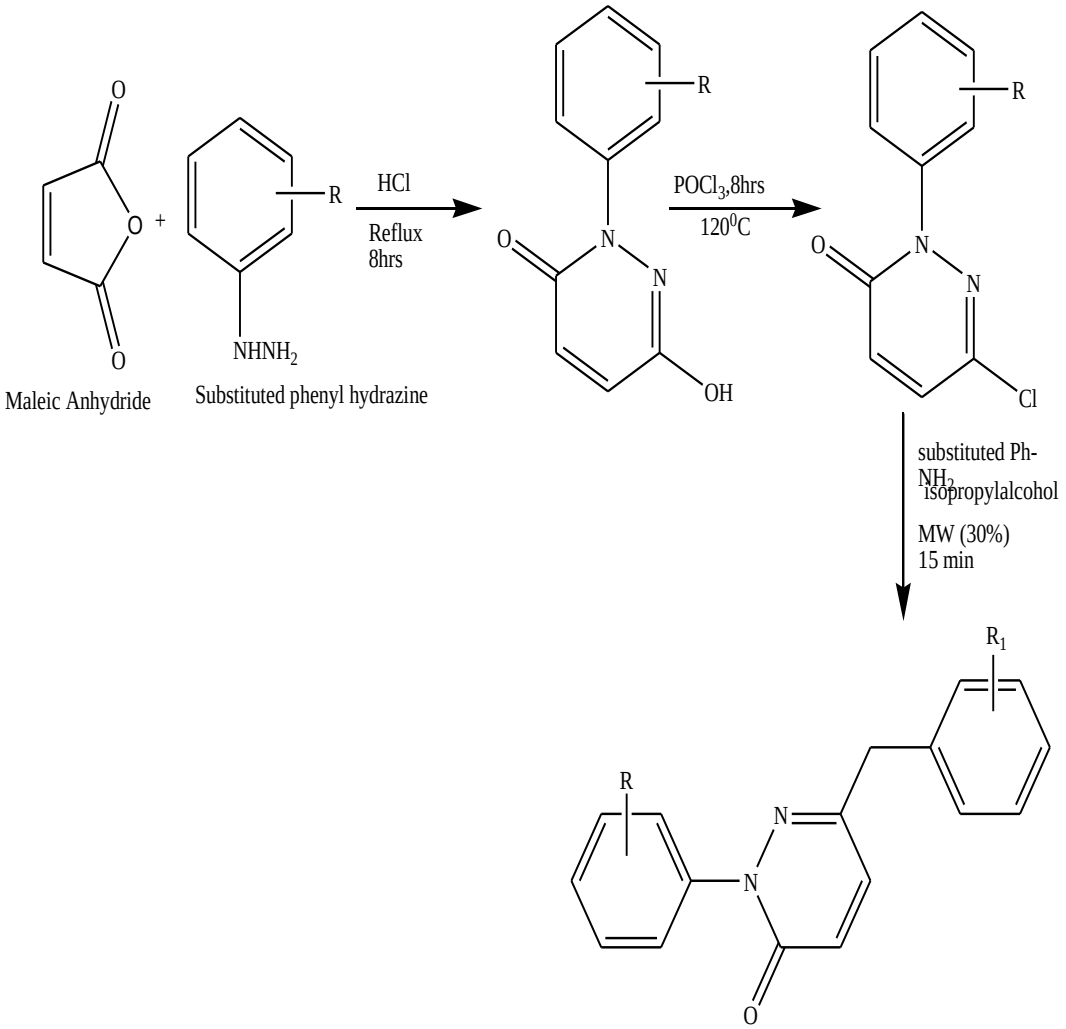
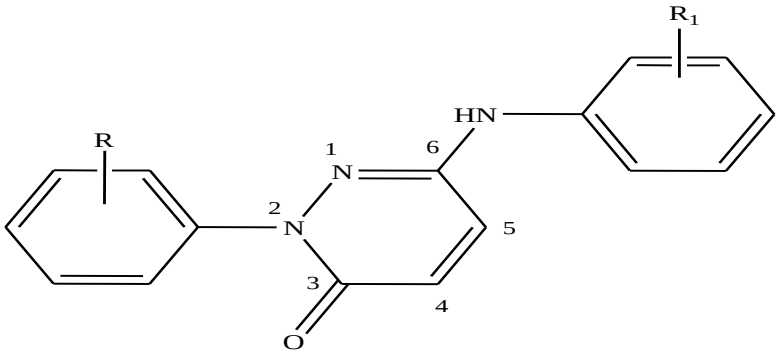


R= H, 4-Cl
R'= H, 3-Cl, 4-Cl, 4-CH_3, 2-F, 4-F, 3, 4-$DiCl_2$

Compound	R	R_1	Compound	R	R_1
Ia	H	H	**IIa**	4-Cl	H
Ib	H	3-Cl	**IIb**	4-Cl	3-Cl
Ic	H	4-Cl	**IIc**	4-Cl	4-Cl
Id	H	4-CH_3	**IId**	4-Cl	4-CH_3
Ie	H	2-F	**IIe**	4-Cl	2-F
If	H	4-F	**IIf**	4-Cl	4-F
Ig	H	3,4-$DiCl_2$	**IIg**	4-Cl	3,4-$DiCl_2$

Scheme. 1: Schematic representation of 2-substituted phenyl-6-(substituted phenylamino) pyridazin-3(2H)-one derivatives

Table 1: Characterization data of 2-substituted phenyl-6-(substituted phenylamino) pyridazin-3(2H)-one derivatives



Compound	R	R_1	M.F., M.Wt., M.pt(°C) ,R_F ,IR(cm^{-1}) ,1H-NMR(δ ppm), MS(m/z)
Ia	H	H	$C_{16}H_{13}N_3O$, 263.29;110;0.85; 1660(C=O), 1560(C=N), 1310 (N-N), 1593(C=C), 3450(NH); 3.7(s, 1H, NH), 7.048-7.08(d, 2H, CH=CH), 7.28-7.6(m, 10H, Ar-H); 261(M^++1)
Ib	H	3-Cl	$C_{16}H_{12}ClN_3O$, 297.74 ;120;0.91; 1660(C=O), 1560(C=N), 1310(N-N), 1593(C=C), 3450(NH), 745(C-Cl); 3.5 (s, 1H, NH), 6.9-7.0(d, 2H, CH=CH), 7.2-7.6(m, 9H, Ar-H)
Ic	H	4-Cl	$C_{16}H_{12}ClN_3O$, 297.74 ;117;0.92; 1660(C=O), 1560(C=N), 1310(N-N), 1593(C=C), 3450(NH), 765(C-Cl); 3.5(s, 1H, NH), 7.02-7.05(d, 2H, CH=CH), 7.2-7.6(m, 9H, Ar-H); 296 (M^+-1)
Id	H	4-CH_3	$C_{17}H_{15}N_3O$, 277.32 ;116;0.90; 1660(C=O), 1560(C=N), 1310(N-N), 1593(C=C), 3450(NH), 2932(CH_3); 2.57(s, 3H, CH_3), 3.58(s, 1H, NH), 7.02-7.05(d, 2H, CH=CH), 7.2-7.6(m, 9H, Ar-H); 277(M^+)
Ie	H	2-F	$C_{16}H_{12}FN_3O$, 281.28 ;112;0.87; 1680(C=O), 1560(C=N), 1310(N-N), 1593(C=C), 3450(NH), 1100(C-F), 699(Ar); 3.53(s, 1H, NH), 6.8-6.9(d, 2H, CH=CH), 7.03-7.61(m, 9H, Ar-H)
If	H	4-F	$C_{16}H_{12}FN_3O$, 281.28 ;109;0.86; 1660(C=O), 1560(C=N), 1310(N-N), 1593(C=C), 3450(NH), 1210(C-F); 3.25(s, 1H, NH), 6.9-7.06(d, 2H, CH=CH), 7.2-7.7(m, 9H, Ar-H); 280(M^+-1)
Ig	H	3,4-$DiCl_2$	$C_{16}H_{11}Cl_2N_3O$ 332.18 ;98;0.96; 1660(C=O), 1560(C=N), 1310(N-N), 1593(C=C), 3450(NH), 755(C-Cl); 3.5-3.7(s, 1H, NH), 6.4-7.05(d, 2H, CH=CH), 7.1-7.60(m, 8H, Ar-H); 331(M^+-1)
IIa	4-Cl	H	$C_{16}H_{12}ClN_3O$, 297.74 ;101;0.88; 1660(C=O), 1560(C=N), 1310(N-N), 1593(C=C), 3450(NH), 825(C-Cl);3.69(s, 1H, NH), 7.01-7.05(d, 2H, CH=CH), 7.1-7.6(m, 9H, Ar-H); 297 /298(M^+/M^++1)
IIb	4-Cl	3-Cl	$C_{16}H_{11}Cl_2N_3O$, 332.18 ;116;0.91; 1660(C=O), 1560(C=N), 1310(N-N), 1593(C=C), 3450(NH), 845(C-Cl); 3.6(s, 1H, NH), 6.8-7.0(d, 2H, CH=CH), 7.1-7.6(m, 8H, Ar-H)
IIc	4-Cl	4-Cl	$C_{16}H_{11}Cl_2N_3O$, 332.18 ;120;0.89; 1660(C=O), 1560(C=N), 1310(N-N), 1593(C=C), 3450(NH), 765(C-Cl); 3.6(s, 1H, NH), 6.8-7.01(d, 2H, CH=CH), 7.05-7.60(m, 8H, Ar-H); 333(M^++1)
IId	4-Cl	4-CH_3	$C_{17}H_{14}ClN_3O$, 311.77 ;103;0.82; 3520(OH), 1660(C=O), 1660(C=N), 1310(N-N), 1593(C=C) 2.25(s, 3H, CH_3), 3.4(s, 1H, NH), 6.6-6.9(d, 2H, CH=CH), 7.02-7.7(m, 8H, Ar-H) ; 311/312(M^+/M^++1)
IIe	4-Cl	2-F	$C_{16}H_{11}ClFN_3O$, 315.73 ;114;0.85; 1680(C=O), 1560(C=N), 1310(N-N), 1593(C=C), 3450(NH), 1100(C-F), 699(Ar), 842(C-Cl); 3.6(s, 1H, NH), 6.9-7.1(d, 2H, CH=CH), 7.2-7.60(m, 8H, Ar-H)
IIf	4-Cl	4-F	$C_{16}H_{11}ClFN_3O$, 315.73 ;112;0.84; 1660(C=O), 1560(C=N), 1310(N-N), 1593(C=C), 3450(NH), 1210(C-F); 3.6(s, 1H, NH), 6.4-6.9(d, 2H, CH=CH), 7.01-7.60(m, 8H, Ar-H); 315/315(M^+/M^++1)
IIg	4-Cl	3,4-$DiCl_2$	$C_{16}H_{10}Cl_3N_3O$, 366.63 ;104;0.86; 1660(C=O), 1560(C=N), 1310(N-N), 1593(C=C), 3450(NH), 755(C-Cl); 3.72(s, 1H, NH), 6.4-6.7(d, 2H, CH=CH), 7.01-7.60(m, 7H, Ar-H); 365(M^++1)

Table 2: Anticonvulsant screening of synthesized compound by MES and sc.PTZ method

Group (n=6)	Compound	MES screen[a]		sc. PTZ screen[a]	
		0.5hours	4 hours	0.5 hours	4 hours
1	Control	-	-	-	-
2	Ia	-	-	-	-
3	Ib	100	-	100	100
4	Ic	100	-	30	30
5	Id	100	-	100	-
6	Ie	-	-	-	-
7	If	100	-	100	-
8	Ig	100	-	30	100
9	IIa	-	-	-	-
10	IIb	100	-	100	-
11	IIc	30	100	30	-
12	IId	100	-	100	-
13	IIe	-	-	-	-
14	IIf	30	30	30	100
15	IIg	100	-	100	-
16	Phenytoin	30	30	-	-
17	Carbamazepine	-	-	100	300

a- Doses of 30mg/Kg and 100mg/Kg were administered i.p.

The data indicates the minimum dose whereby bioactivity was demonstrated in half or more of the mice. The animals were examined at 0.5 and 4 hours after injection were made. A dash (-) indicates an absence of activity at maximum dose.

Table 3: Anticonvulsant activity of synthesized Compounds % Protection

Compound	MES screen[a]		sc. PTZ screen[a]	
	% Protection	Recovery	% Protection	Recovery
Ia	-	-	-	-
Ib	50	Late	66	Late
Ic	83	Soon	83	Soon
Id	66	Late	83	Late
Ie	-	-	-	-
If	83	Very soon	66	Soon
Ig	33	Soon	33	Soon
IIa	-	-	-	-
IIb	50	Soon	33	Late
IIc	100	Very soon	100	Soon
IId	83	Late	50	Late
IIe	-	-	-	-
IIf	100	Very soon	100	Soon
IIg	67	Late	67	Late
Phenytoin	100	Very soon	100	Very soon

b- Doses of 30mg/Kg and 100mg/Kg were administered i.p.

c- The data indicates the minimum dose whereby bioactivity was demonstrated in half or more of the mice. The animals were examined at 0.5 and 4 hours after injection were made. A dash (-) indicates an absence of activity at maximum dose.

Table 4: Neurotoxicity screening of synthesized compound by rotarod method

Group (n=6)	Compound	Neurotoxicity screening at	
		0.5 hours	4 hours
1	control	-	-
2	Ia	-	-
3	Ib	100	100
4	Ic	-	300
5	Id	-	300
6	Ie	-	-
7	If	300	-
8	Ig	-	-
9	IIa	-	-
10	IIb	100	100
11	IIc	-	300
12	IId	300	-
13	IIe	-	-
14	IIf	300	-
15	IIg	-	-
16	Phenytoin	100	100
17	Carbamazepine	300	300

Doses of 100mg/Kg and 300mg/Kg were administered i.p. The data indicates the minimum dose whereby bioactivity was demonstrated in half or more of the mice. The animals were examined at 0.5 and 4 hours after injection were made. A dash (-) indicates
50% or more failed the neurotoxicity screening.

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