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ANTICONVULSANT PROPERTIES OF ISOMERS OF DICHLOROPHENYL AMINO PROPANAMIDES IN MICE

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ABSTRACT

Antiepileptic drug development is a dynamic process, affording many research opportunities. Continued efforts are being made in the development of antiepileptic drugs employing a range of strategies, including modification of the structures of existing drugs, targeting novel molecular substrates and non-mechanism-based drug screening. This research was aimed at evaluating the anti-seizure effects of three isomers of diclorophenyl propanamides (DCP), viz; 2,3- (DCP23)-, 2,5- (DCP25) and 3,4- (DCP34) dichloro-substituted phenyl propanamides, using chemically- and electrically- induced seizure models. In 4-aminopyridine-induced seizure test, there was no protection offered by the three compounds, but DCP34 at doses of 50 mg/kg and 25 mg/kg exhibited significant ($p < 0.05$) increase in the onset of seizures. The compounds did not offer protection in strychnine-induced seizure test. In picrotoxin-induced seizure test, DCP23 and DCP25 offered protection against clonic convulsion of 66.7% and 83.3% at 50 mg/kg; 50.0% and 66.7% at 25 mg/kg, respectively. Single oral administration (100 mg/kg) of the compounds (DCP23, DCP25 and DCP34) produced 37.5%, 50% and 0.0% protections respectively against tonic hind limb extension (THLE) while a 5-day administration offered higher protection of 50%, 75% and 25% respectively. When DCP23 or DCP25 (25 mg/kg), was co-administered with nickel chloride (5 mg/kg), the percentage protection against PTZ-induced seizure was better (66.67% and 33.33% respectively) than when administered separately. These compounds possess anticonvulsant activity and could be optimized to develop potential lead compound with antiepileptic effect.

KEYWORDS: **Seizure, Dichloro phenyl propanamides, Nickel chloride.**

INTRODUCTION

Neurological disorders, including epilepsy, have been found as the leading causes of disability-adjusted life years, a measure of the global burden of disease [1]. Epilepsy is one of the more common and frequent neurological disorders, characterized by excessive temporary neuronal discharges resulting in uncontrolled convulsions [2]. It is a major public health problem especially in low income countries like sub-Saharan Africa where 75% of the affected people cannot afford the treatment [3]. Seizures play an important role in neuronal cell death by causing mitochondrial dysfunction with increased levels of reactive oxygen species and apoptotic neuronal cell death, resulting in oxidative stress, leading to the cycle of subsequent seizures [4]. Conventional antiepileptic drugs (AEDs) like phenobarbital, primidone,

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phenytoin, carbamazepine, ethosuximide and the benzodiazepines are widely used and are known to have dose-related toxicity and idiosyncratic side effects [5]. Currently, common methods employ in search for new anticonvulsants include modifications of clinically effective antiepileptic drugs and synthesis of entirely new structures [6]. Continued efforts are being made in the development of antiepileptic drugs employing a range of strategies such as non-mechanism-based drug screening of compounds using conventional animal models [7]. Antiepileptic agents predominantly target voltage-gated channels (e.g. voltage-gated Na^+ channels, T-type voltage-gated Ca^{2+} and K^+ channels); influence GABA-mediated inhibition and ionotropic and metabotropic glutamate receptors [8].

We have previously reported [9] the procedure for the synthesis and spectral analysis of the compounds; their anticonvulsant effects using primary screening methods (MEST and PTZ test) as well as their effects on voltage-gated sodium channels ($Na_V1.6$) stably expressing in HEK cells.

This research is aimed at evaluating the *in-vivo* effects of synthesized propanamides on some anticonvulsant targets.

MATERIALS AND METHODS

Materials

Strychinine, pentylenetetrazole and picrotoxin (Sigma chemical Co., St. Louis, USA) and 4-amino pyridine (Merch-schuchardt, Germany), phenobarbitone (Lab Renaudin France), Diazepam (Roche®), electroconvulsive machine (Ugo Basile, model No. 7801).

Animals

Swiss Albino mice (17 to 22 g) of either sex were obtained from Animal House, Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. The animals were kept in a well-ventilated condition at ambient temperature and fed with a standard animal feeds with access to water *ad-libitum* under laboratory conditions in accordance with National Academy of Science, Guide for the Care and Use of Laboratory Animals (1996).

Effect of DCP23, DCP25 and DCP34 on 4-aminopyridine-induced Seizure

The study was conducted according to method described by Yamaguchi and Rogawski [10]. Sixty-six albino mice were divided into eleven groups of six mice each. Group 1 served as control and received the vehicle (30% propylene glycol and 70% distilled water). Groups 2-4 received DCP23 (50, 25 and 12.5 mg/kg, *i.p.*, respectively); Groups 5-7 received DCP25 (50, 25 and 12.5 mg/kg, *i.p.,* respectively); Groups 8-10 received DCP34 (50, 25 and 12.5 mg/kg, *i.p.,* respectively), while group 11 served as positive control and was administered phenobarbitone (30 mg/kg). Thirty minutes post-treatment, all the groups were administered 4-aminopyridine (15 mg/kg, *s.c*) and observed for presence or absence of tonic extension as well as onset of seizures.

Effect of DCP23, DCP25 and DCP34 on Strychnine –induced Seizure

The method of Krall *et al*. [11] was adopted. Sixty-six albino mice were divided into eleven groups of six mice each. Group 1 received the vehicle (30% propylene glycol and 70% distilled water) and served as negative control. Groups 2-4 received DCP23 (50, 25 and 12.5 mg/kg, *i.p.*, respectively); Groups 5-7 received DCP25 (50, 25 and 12.5 mg/kg, *i.p.,* respectively); Groups 8-10 received DCP34 (50, 25 and 12.5 mg/kg, *i.p.,* respectively), while group 11 served as positive control and was administered phenobarbitone (30 mg/kg). Thirty minutes later 1.0 mg/kg (*s.c.*) of strychnine was administered to all the groups. The mice were observed for presence or absence of convulsion and latency of death.

Effect of DCP23, DCP25 and DCP34 on Picrotoxin-induced Seizure

The study was done according to method described by Swinyard *et al*. [12]. Sixty-six albino mice were divided into eleven groups of six mice each. Group 1 received the vehicle (30% propylene glycol and 70% distilled water) and served as negative control. Groups 2-4 received DCP23 (50, 25 and 12.5 mg/kg, *i.p.*, respectively); Groups 5-7 received DCP25 (50, 25 and 12.5 mg/kg, *i.p.,* respectively); Groups 8-10 received DCP34 (50, 25 and 12.5 mg/kg, *i.p.,* respectively), while group 11 served as positive control and was administered diazepam (10 mg/kg).. Thirty minutes post-treatment, all the groups were administered picrotoxin (4 mg/kg, *s.c*) and observed for presence or absence of clonic convulsion as well as onset of seizures and protection against mortality.

Effect of Single and 5-day Oral Administrations of DCP23, DCP25 and DCP34 in Rats

Method of maximal electroshock test was conducted as described by Stable and Kupferberg,

[13]. This study employed seven groups of eight rats each. Group 1 was treated with (30% propylene glycol and 70% distilled water) and served as control group while groups 2, 3 and 4 were treated with DCP23, DCP25 and DCP34 (100 mg/kg, *p.o.*, each), for 5 days. Groups 5, 6 and 7 were treated for four days with vehicle alone and administered the test compounds (100 mg/kg) orally on the fifth day. The anticonvulsant activity of the compounds was determined 1 hour post treatment on the 5th day. Activity in the groups with multiple administrations of the test compounds was compared to that of single administration.

Effect of Nickel chloride on anticonvulsant activity of DCP23 and DCP25 in mice

Mice of either sex were divided into six groups of six mice each. Group 1 served as control and treated with 30% propylene glycol and 70% distilled water, while groups 2, 4 and 6 received nickel chloride (5 mg/kg, *i.p.*). Five minutes after nickel chloride administration, groups 3 and 4 received DCP23 (25 mg/kg, *i.p*); groups 5 and 6 received DCP25 (25 mg/kg, *i.p*). One hour post- treatment, all the groups were administered PTZ (90 mg/kg, *s.c.*) and observed for presence or absence of seizures [14].

Statistical analysis

Statistical analysis was carried out using SPSS (Version 20) and data obtained were expressed as Mean ± SEM. All analyses were done using analysis of variance (ANOVA), values with $p < 0.05$ were considered significant.

RESULTS

In 4-aminopyridine-induced seizure test, DCP34 (50 and 25 mg/kg) exhibited significant ($p < 0.05$) increase in the onset of seizures when compared with the control. There was no protection by DCP23 and DCP25 at all the tested doses. Phenobarbitone (30 mg/kg) offered 66.7% protection and produced significant ($p < 0.05$) increase in the mean latency to onset of seizure (Table 1).

The compounds did not protect against characteristic episode of tonic seizure or against the mortality in strychnine-induced seizure test. There was no significant difference ($p > 0.05$) in mean onset of seizure in all the treated groups as compared with the negative control group (Table 2).

In picrotoxin-induced seizure test, DCP23 and DCP25 (50 mg/kg) offered 66.7% and 83.3% protection respectively, while at 25 mg/kg the protections were 50.0 % and 66.7% respectively. Diazepam (10 mg/kg) gave 100% protection. DCP23 (50 mg/kg) and DCP25 (25 and 50 mg/kg) significantly ($p < 0.05$) increased the mean onset of seizures as compared to the control (Table 3).

Single administration of the compounds offered 37.5%, 50% and 0.0% protection for DCP23, DCP25 and DCP34 respectively. For a 5-day daily administration the protection by DCP23, DCP25 and DCP34 was 50%, 75% and 25%, respectively (Table 4).

Nickel chloride (5 mg/kg) offered 16.7% and 83.3% protection against clonic convulsions and mortality respectively. When DCP23 (25 mg/kg) and DCP25 (25 mg/kg) were co-administered with nickel chloride, percentage protections against seizure and mortality for DCP23 were 66.67% and 83.33; while that of DCP25 were 33.33% and 66.67%, respectively. There was significant ($p < 0.05$) increase in the mean onset of seizure when the compounds were co-administered with nickel, as compared to the control (Table 5).

DISCUSSION

4-Aminopyridine blocks voltage-gated K^+ (Kv) channels, which leads to prolonged depolarization [15]; facilitates Ca^{2+} influx into presynaptic terminals and release of transmitter [16]; and subsequently, enhances inter-neuronal and neuromuscular synaptic transmission [17]. It induces clonic-tonic convulsions by blocking potassium channels [10]. Potassium channels play a vital role in the control of neuronal excitability and seizure susceptibility, and plays important role in the suppression of seizure initiation and spread [18]. The test compounds (DCP23 and DCP25) may not have activity against this test model, as they did not offer protection against seizures generated by 4-aminopyridine. However, significant increase in latency of seizures demonstrated by DCP34 might be an evidence to show some anticonvulsant properties in this pathway.

Strychnine acts by antagonizing glycine receptors, thereby increasing the rate of neuronal excitability [19,20]. The absence of anticonvulsant activity by these compounds against seizures induced by strychnine, suggests non interaction with glycine receptor.

Gamma amino butyric acid (GABA) is the principal neurotransmitter which attenuates seizures by binding to GABA receptors involves in activating the inhibitory cascades at different regions of the CNS and thus, plays an important role in reducing the

Table 1: Effect of DCP23, DCP25 and DCP34 on 4-aminopyridine-induced seizures in mice

Treatment (mg/kg)	Mean latency of seizures (min)	Quantal protection	(%) Protection against seizures	Protection against mortality (%)
Control	10.00 ± 1.03	0/6	0.0	0.0
DCP23 (50)	12.67 ± 1.53	0/6	0.0	0.0
DCP23 (25)	11.33 ± 0.76	0/6	0.0	0.0
DCP23 (12.5)	12.0± 1.29	0/6	0.0	0.0
DCP25 (50)	17.00 ± 2.51	0/6	0.0	0.0
DCP25 (25)	13.17 ± 0.87	0/6	0.0	0.0
DCP25 (12.5)	14.0± 1.26	0/6	0.0	0.0
DCP34 (50)	17.17± 1.82*	0/6	0.0	0.0
DCP34 (25)	16.67 ± 1.23*	0/6	0.0	0.0
DCP34 (12.5)	12.50± 1.52	0/6	0.0	0.0
PHB (30)	19.5 ± 0.50*	4/6	66.7	100.0

DCP23, DCP25 and DCP34 = 2,3-, 2,5- and 3,4- Dichloro – 3(aminophenyl) propanamides respectively, PHB = Phenobarbitone; Control = 30% propylene glycol, 70% distilled water; at *p < 0.05, n=6.

Table 2: Effect of DCP23, DCP25 and DCP34 on strychnine-induced seizures in mice

Treatment (mg/kg)	Mean latency of seizures (min)	Quantal protection	(%) Protection against seizures	Protection against mortality (%)
Control	6.50 ± 0.43	0/6	0.0	0.0
DCP23 (50)	7.00 ± 0.26	0/6	0.0	0.0
DCP23 (25)	9.33 ± 0.30	0/6	0.0	0.0
DCP23 (12.5)	6.83 ± 0.31	0/6	0.0	0.0
DCP25 (50)	8.33 ± 0.84	0/6	0.0	0.0
DCP25 (25)	8.00 ± 0.77	0/6	0.0	0.0
DCP25 (12.5)	7.17 ± 0.54	0/6	0.0	0.0
DCP34 (50)	8.67 ± 1.05	0/6	0.0	0.0
DCP34 (25)	6.33 ± 0.76	0/6	0.0	0.0
DCP34 (12.5)	6.33 ± 0.61	0/6	0.0	0.0
PHB (30)	14.83 ± 1.49*	0/6	0.0	66.7

PHB = Phenobarbitone *p<0.05, n=6.

Table 3: Effect of DCP23 and DCP25 on picrotoxin-induced seizures in mice

Treatment (mg/kg)	Mean latency of seizures (min)	Quantal protection	(%) Protection against seizures	Protection against mortality (%)
Control	13.33 ± 0.95	0/6	0.0	0.0
DCP23 (50)	20.50 ± 2.50*	4/6	66.7	100.0
DCP23 (25)	17.00 ± 0.58	3/6	50.0	50.0
DCP23 (12.5)	17.12 ± 0.58	0/6	0.0	0.0
DCP25 (50)	21.00 ± 00**	5/6	83.3	100.0
DCP25 (25)	24.50 ± 0.50**	4/6	66.7	66.7
DCP25 (12.5)	15.83 ± 1.51	0/6	0.0	0.0
DZ (10)	0.0	6/6	100.0	100.0

DZ = Diazepam; *p<0.05 and *p<0.005.

Table 4: Effect of single and multiple oral administrations of DCP23, DCP25 and DCP34 on maximal electroshock-induced seizure in mice

Compound (dose mg/kg)	% Protection against seizure (single oral administration)	% Protection against seizure (5-day oral administration)
DCP23 (100)	37.50	50.0
DCP25 (100)	50.0	75.0
DCP34 (100)	0.0	25.0

DCP23, DCP25 and DCP34 = 2,3-, 2,5- and 3,4- Dichloro – 3(aminophenyl) propanamides respectively; n = 8 mice per group.

Table 5: Effect of nickel chloride on DCP23 and DCP25 against pentylenetetrazole-induced seizure in mice

Treatment (mg/kg)	Mean onset of seizure (minutes)	% Protection	Mortality protection (%)
Control	5.50 ± 0.85	0.00	16.67
NKL (5)	12.60 ± 1.96*	16.67	83.33
DCP23 (25)	15.25 ± 2.13*	33.33	50.00
NKL (5) + DCP23 (25)	20.50 ± 7.50*	66.67	83.33
DCP25 (25)	10.00 ± 1.78*	33.33	66.67
NKL (5) + DCP25 (25)	17.50 ± 2.25*	33.33	66.67

NKL = Nickel chloride, *p < 0.05, n=6.

excitatory tone. Picrotoxin interacts with distinct domains of the GABA receptors and block GABA-mediated Cl^- current [21]. DCP23 and DCP25 showed significant activity against picrotoxin-induced seizures in a dose dependent manner. Their efficacy against this model showed possible blockade of chloride channel.

Tolerance development has been most frequently demonstrated for drugs that act upon the central nervous system, such as opiate analgesics, nicotine, benzodiazepines, ethanol, cocaine and amphetamine [22]. It can result from increased drug metabolism or from adaptations in the neural elements that respond to the drugs [23]. Daily oral administration of the test compounds for 5 days is a useful model to evaluate possible development of tolerance for potential anticonvulsant agents [13]. The results of this study showed that the compounds offered protection against MES-induced seizure after 5-day daily administration. The protection was better than that of received single dose administration, thus, indicates non development of tolerance.

Overexpression of T-type channels appears to be linked to pathophysiological conditions such as absence seizure, and metallic ions such as Cd^{2+}, Co^{2+}, Ni^{+1}, Pb^{2+} and Zn^{2+} have been found to inhibit Ca^{2+} permeation via voltage-dependent Ca^{2+} channels with different potencies [24]. Among these cations Ni^{+1} was found to be selective blocker for low voltage-activated T-type Ca^{2+} channels [24]. Pharmacological studies have shown that low voltage activated T-type of calcium ion channels are involved in the genesis of absence seizures [25]. Drugs that act by inhibiting neuronal T-type calcium ion currents like sodium valproate have potential activity against absence seizures [26]. Also, study conducted by Rehni and Singh [27], showed reversal of PTZ-induced seizure activity in mice by nickel chloride. In this study nickel chloride showed significant delay in latency of seizure, and when interacted with DCP23 and DCP25 there was reduction in severity of seizure as evidently demonstrated by the significant increase in the latency of seizure. Therefore, these compounds could be acting via calcium channels by possibly blocking the neuronal T-Type calcium ion channels.

CONCLUSION

The compounds possess anti-seizure activity in animal models of convulsion and the anticonvulsant effect could be via calcium channels blockade. Thus, these isomers could be optimized towards developing potential lead compound with antiepileptic effects.

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