ANTICONVULSANT STUDIES OF THREE SYNTHESIZED DICHLORO-SUBSTITUTED PHENYL PROPANAMIDES AND THEIR ACTION ON VOLTAGE-GATED SODIUM CHANNELS ($Na_v1.6$)

BY

SANI MALAMI

DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS

FACULTY OF PHARMACEUTICAL SCIENCES

AHMADU BELLO UNIVERSITY, ZARIA

MARCH, 2015

ANTICONVULSANT STUDIES OF THREE SYNTHESIZED DICHLORO-SUBSTITUTED PHENYL PROPANAMIDES AND THEIR ACTION ON VOLTAGE-GATED SODIUM CHANNELS ($Na_v1.6$)

By

Sani MALAMI B.Pharm (ABU) 2001, MSc. (ABU) 2009

PhD/Pharm Sci/01357/09-10

A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA, IN PARTIAL FULFILMENT FOR THE AWARD OF DOCTOR OF PHILOSOPHY IN PHARMACOLOGY

DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS

AHMADU BELLO UNIVERSITY, ZARIA

MARCH, 2015

Declaration

I declare that the work in the dissertation entitled ‘‘Anticonvulsant Studies of Three Synthesized Dichloro-substituted Phenyl Propanamides and their Action on Voltage-gated Sodium Channels ($Na_v1.6$)’’has been performed by me in the Department of Pharmacology and Therapeutics.

The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at any institution.

Sani Malami

Name of student	Signature	Date

Certification

This dissertation entitled "SYNTHESIS AND ANTICONVULSANT STUDIES OF DICHLORO-SUBSTITUTED PHENYL PROPANAMIDES AND THEIR ACTION ON VOLTAGE-GATED SODIUM CHANNELS ($Na_v1.6$)'' by Sani MALAMI meets the regulations governing the award of the degree of Doctor of Philosophy of Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

_____ _____

Prof. J.A. Anuka Date
(Chairman, Supervisory Committee)

_____ _____

Prof. I.M. Hussaini Date
(Member, Supervisory Committee)

_____ _____

Dr. A.Y. Idris Date
(Member, Supervisory Committee)

_____ _____

Dr. A.U. Zezi Date
(Head of Department)

_____ _____

Prof. A.Z Hassan Date
(Dean, School of Postgraduate Studies)

Acknowledgement

Words are not enough to quantify my gratitude to the almighty Allah, whose help and mercy acted in me and made this work a success; without which my effort could have yielded nothing but a failure.

My sincere appreciation goes to my able and well known dedicated supervisors; Professor J.A. Anuka, Professor I.M. Hussaini and Dr. A.Y. Idris, for their enormous contributions toward the success of this work. Without being told, I would continue to cherish and remember our good relationship throughout the work. I am indeed very grateful.

I find it imperative to specially thank Prof. I.M. Hussaini for securing bench space for me at the University of Virginia, USA, where I worked as a Visiting Scholar for six months, and in addition to this, he linked me to other laboratories where I was exposed to essential biomedical research technique. Sir, I am grateful!

I thank and appreciate Dr Manoj K. Patel, Department of Anesthesiology, University of Virginia, Charlottesville, USA, for his good gesture and academic mentoring with free access to all consumables and laboratory equipments. Similarly I appreciate other members of his research Laboratory especially Mirko Rivera (PhD), Divina (PhD), Corinne, and Luqman, for their friendly reception and good guidance during my stay in the Lab. Also, I thank K. Zaman (PhD), Kamal (PhD), Benjamin Purow (PhD) and his technician, Aizhen, for what they taught me in their various research areas while I was in University of Virginia.

I must sincerely appreciate and acknowledge TETFund for awarding full sponsorship to me which I utilized for my bench work at the University of Virginia, Charlottesville, Virginia, USA, for 6 months.

I also wish to express my thanks to everybody who has contributed directly or indirectly toward the actualization of this achievement. In fact, a paragraph may appear too little to accommodate the full description of your kind support and contributions. Let me say a big 'Thank' to you all; especially to Dr. Abdullahi Hamza Yaro, Dr Jamilu Ya'u, Dr Nuhu M. Danjuma, Dr A.U. Zezi- Head of Department, Asma'u Hamza, Aliyu Ahmad and my colleaques in the Department.

It is also imperative on me to appreciate and thank the entire members of my family and friends, including my Grand mother (Hajiya Iya); for their prayers, support and understanding. May Almighty Allah reward them abundantly.

All can not be ended without extending my special and specific regards to my beloved and caring wife, for her magnanimous and enthusiastic support and well wishes. I extend same to my children; Mahmud, Hafsat, Ibrahim Khalil and Ahmad. Please, endeavor to keep it up.

Abstract

The field of antiepileptic drug development has become dynamic, affording many promising 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 is aimed at conducting anticonvulsant studies on three synthesized dichloro-substituted phenyl propanamides. Isomers of 2,3- (DCP23)- 2,5- (DCP25) and 3,4- (DCP34) Dichloro-substituted Phenyl Propanamides were synthesized from acrylamide and dichloro-substituted anilines. The products were formed by an addition reaction according to Michael's reaction. The physicochemical properties of the products were determined, and their structures were elucidated using standard analytical procedures; infrared (IR) and nuclear magnetic resonance (NMR) spectroscopy. The test compounds were evaluated for anticonvulsant effect in both acute and chronic animal models, via intraperitoneal (i.p.). In maximal electroshock test (MEST), the percentage protection offered by DCP23, DCP25 and DCP34, at 50 mg/kg, was 71.4%, 57.2% and 42.9% respectively. The middle dose (25 mg/kg) of the compounds offered protection of 42.9% (DCP23), 28.5% (DCP25) and 14.3% (DCP34), while the lowest dose (12.5 mg/kg) offered minimal / no protection. The highest dose (50 mg/kg) of DCP23, DCP25 and DCP34 used in pentylenetetrazole-induced seizure test, offered 66.7%, 66.7 and 0% protections against clonic seizures. Also, DCP23, at doses of 25 and 12.5 mg/kg, produced 16.7% protection while similar doses of DCP25 and DCP34 did not offer any protection. There was statistically significant difference in the mean onset of seizure exhibited by DCP23 at doses of 50 mg/kg ($p<0.001$) and 25 mg/kg ($p<0.001$). In 4-aminopyridine-induced seizure test, there was no protection offered by all the tested compounds, but DCP34 at doses of 50 mg/kg and 25 mg/kg

exhibited statistically ($p<0.05$) significant difference in the onset of seizures. All test 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% (50 mg/kg) respectively and 50.0% and 66.7% (25 mg/kg) respectively. There was no protection at 12.5 mg/kg. The medial effective dose (ED_{50}) for DCP23, DCP25 and DCP34 using MEST was found to be 25.12, 39.81 and 44.67 mg/kg respectively, while that of picrotoxin was 35.48 mg/kg (DCP23) and 28.18 mg/kg (DCP25). The median toxic doses (TD_{50}) were 100.0, 100.0 and 104.7 mg/kg for DCP23, DCP25 and DCP34 respectively. The protective index (MEST) was 3.98, 2.51 and 2.33 respectively while that of picrotoxin-induced seizure test was 2.82 (DCP23) and 3.55 (DCP25). In the single oral administration (100 mg/kg) evaluation, DCP23, DCP25 and DCP34 offered 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. Co-administration of DCP23 (50 mg/kg), DCP25 (50 mg/kg) and DCP34 (50 mg/kg) each with 5 mg/kg fluphenamic acid, resulted in potentiation as the percentage protection against THLE (MEST) were 100% for DCP23 and DCP25, and 50% for DCP34. When DCP23 and DCP25 at the doses of 25 mg/kg, were coadministered with nickel chloride (5 mg/kg) the percentage protection against PTZ-induced seizure were 66.67% and 33.33% respectively. Similarly, their co-administration produced significant ($p<0.05$) difference in the mean onset of seizure when compared with the control group. Cyproheptadine at the dose of 4 mg/kg did not affect the anticonvulsant effect of DCP23 (50 mg/kg) and DCP25 (50 mg/kg) against PTZ-induced seizure. DCP23 (50 mg/kg) and DCP25 (50 mg/kg), significantly ($p<0.001$) decreased the onset of sleep as well as increased the duration of sleep ($p<0.05$). All the compounds at the dose of 50 mg/kg significantly ($p<0.05$) reduced the

severity of seizure episodes induced by kindling. A 28-day sub-chronic study was conducted for DCP25 at doses of 50, 25 and 12.5 mg/kg. The results showed that DCP25 at 50 mg/kg only, caused significant ($p<0.05$) increase in urea, creatinine and aspartate aminotransferase levels. There was no significant ($p<0.05$) change in haematological indices, lipid profile parameters as well as other renal and liver function test parameters caused by DCP25. The possible mechanism of action was studied on voltage-gated sodium channels($Na_v1.6$) at different states of the channel: DCP23 at holding potential of -60 mV, produced concentration-dependent tonic blockade of sodium current of 9.73%, 18.04%, 46.80%, 68.46%, 95.64 and 98.10% at 10µM, 30µM, 60µM, 100µM, 300µM and 600µM respectively. At holding potential of -60 mV, DCP25 at 100µM and 600µM blocked the current by 21.63% and 83.03%; while DCP34 (100µM and 600µM) blocked the current minimally by 3.8% and 16.9%, respectively. DCP23 was further tested at a holding potential of -100 mV at the graded concentration (10µM, 30µM, 60µM, 100µM, 300µM and 600µM) and similarly blocked the sodium currents by 0%, 10%, 28.93%, 50.12%, 88.51% and 90.10% respectively. The IC_{50} values of DCP23 were 64.76 and 100.37 µM at resting and inactivated states respectively. The activation/inactivation pattern in the presence of DCP23 (100 µM) indicated that there was significant reduction in the elicited current even at depolarized potential where the sodium conductance was found to be highest. The results obtained from this work showed that the compounds possess anticonvulsant effects mediated partly via voltage-gated sodium channel blockade.

Table of Contents

Title	Page
Title page	ii
Declaration	iii
Certification	iv
Acknowledgement	v
Abstract	vii
Table of Contents	x
List of Tables	xv
List of Figures	xvii
List of Appendices	xviii
Abbreviations, Definitions, Glossary and Symbols	xix

1.0 INTRODUCTION…….. 1

1.1 Statement of Research Problems…….....2

1.2 Justification……4

1.3 Theoretical Frame Work……5

1.4 Objectives of the Study…….7

1.5 Research Hypothesis……..7

2.0 LITERATURE REVIEW……..8

2.1 Epilepsy……...8

2.1.1 Neural network oscillations……..8

2.1.2 Epileptogenesis and Ictogenesis……...9

2.2 Ion Channels……...11

2.3 Action Potential…….12

2.4 Current-Voltage Relationship……..14

2.5 Voltage-Gated Sodium Channels……14

2.5.1 Nomenclature of voltage-gated sodium channels……..15

2.5.2 Anatomical location of voltage-gated sodium channels……15

2.5.3 Structural function of voltage-gated sodium channels……...16

2.5.4 Sodium channel molecular pharmacology……..16

2.5.5 Biophysical modulation of voltage-gated sodium channels……....19

2.5.5.1 State dependent block……..20

2.5.5.2 Use dependent blockade……...21

2.5.5.3 Inactivation of Na^+ channels……21

2.5.5.4 Fast inactivation……....21

2.5.5.5 Recovery from inactivation…….....22

2.5.6 Voltage sensing and voltage-dependent activation……..22

2.5.7 Ion selectivity and conductance……....23

2.5.8 Phosphorylation of sodium channels……24

2.5.9 Channelopathies of sodium channels……25

2.6 Voltage-Gated Potassium Channels (VGKCs)……26

2.7 Voltage-Gated Calcium Channels (VGCCs)……...28

2.8 Chloride Channels……30

2.9 Neurotransmitter Mediated Mechanisms……..31

2.9.1 Gamma amino butyric acid (GABA)…….....31

2.9.1.1 GABA receptor subtypes……....33

2.9.2 Glycine……...34

2.9.3 Glutamate receptors……...35

2.9.3.1 Ionotropic glutamate receptors……...35

2.9.3.2 Metabotropic glutamate receptors…….35

2.10 Neuromodulators (neuropeptide Y and corticotropin-releasing hormone)…...36

2.10.1 Neuropeptide Y (NPY)……..36

2.10.2 Neuropeptide corticotrophin-releasing hormone (CRH)……...37

2.11 Other Modulators……..37

2.11.1 GABA transporters……37

2.11.2 Gene expression patterns……...37

2.11.3 Gap junction……...37

2.12 Structure Activity Relationship of Sodium Channel Blockers…….38

2.13 Michael Reaction……...43

2.14 Pharmacophoric Units for Clinically Available Antiepileptic Agents Acting via Sodium Channels……..43

3.0 MATERIALS AND METHODOLOGY…….....45

3.1 Materials, Equipment, Chemicals and Animals……45

3.1.1 Materials and equipment……..45

3.1.2 Chemicals……45

3.2 Synthesis and Chemical Analysis……46

3.2.1 Synthesis……..46

3.2.1.1 Procedure……..47

3.2.2 Thin layer chromatography.....……49

3.2.3 Identification and characterization of the compounds……49

3.3 Study Animals and Cell Lines……50

3.3.1 Animals……....50

3.3.2 Cell lines……50

3.4 Preparation and Administration of Drugs……....51

3.5 Toxicity Study……. 51

3.5.1 Acute toxicity study……..51

3.5.2 Sub-chronic toxicity study in rats…….52

3.5.2.1 Blood analysis……....52

3.5.3 Determination of median neurotoxicity (TD_{50})……....53

3.6 Anticonvulsant Studies……53

3.6.1 Maximal electroshock-induced seizure in mice……....53

3.6.2 Pentylenetetrazole-induced seizure test……54

3.6.3 4- aminopyridine-induced seizure test……..54

3.6.4 Strychnine –induced seizure test……...55

3.6.5 Picrotoxin-induced seizure test…….55

3.6.6 Pentylenetetrazole-induced kindling……....55

3.6.7 Determination of median effective dose (ED_{50})……..56

3.6.8 Tolerance study Using maximal electroshock test…….....57

3.7 Pharmacological Interaction……57

3.7.1 Effect of fluphenamic acid on anticonvulsant activity of DCP23, DCP25 and DCP34 in mice……..57

3.7.2 Effect of nickel chloride on anticonvulsant activity of DCP23, DCP25 and DCP34 in mice……58

3.7.3 Effect of cyproheptadine on anticonvulsant activity of DCP23, DCP25 and DCP34 in mice……58

3.7.4 Diazepam-induced sleeping time in mice……...59

3.8 Actions of DCP23, DCP25 and DCP34 on Voltage-gated (Nav 1.6) Sodium Channels Stably Expressed in Human Embryonic Kidney (HEK Cells 293) 59

4.0 RESULTS……..61

4.1 Purification, Identification and Characterization of the Test Compounds…… 61

4.2 Toxicity Study…….69

4.2.1 Acute toxicity study…….69

4.2.2 Subchronic Toxicity Study in Rats……...70

4.3 Anticonvulsant Studies…….75

4.3.1 Maximal electroshock-induced seizure in mice…….75

4.3.2 Pentylenetetrazole-induced seizure in mice……...77

4.3.3 4-Aminopyridine-induced seizure in mice…….79

4.3.4 Strychnine-induced seizure in mice……81

4.3.5 Picrotoxin-induced seizure in mice……....83

4.3.6 Pentylenetetrazole-induced kindling in mice…….85

4.3.7 Determination of median effective dose (ED_{50})……87

4.3.8 Tolerance study using maxmal electroshock test……...88

4.4 Pharmacological Interaction……..89

4.4.1 Effect of fluphenamic acid on anticonvulsant activity of DCP23, DCP25 and DCP34 in mice…….89

4.4.2 Effect of Nickel Chloride on DCP23 and DCP25 against Pentylenetetrazole-induced Seizure in Mice……91

4.4.3 Effect of Cyproheptadine on DCP23, DCP25 and DCP34 against Pentylenetetrazole-induced Seizure in Mice…….93

4.4.4 Effects of DCP23 and DCP25 on Diazepam-induced in Mice……95

4.5 Actions of DCP23, DCP25 and DCP34 ON Voltage-Gated Sodium Channels (Na_V 1.6)……98

4.5.1 States Dependent Actions……98

4.5.2 Use Dependent Action……105

5.0 DISCUSSION……...107

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION……127

6.1 Summary…….127

6.2 Conclusion……..129

6.3 Recommendation……130

REFERENCE……131

APPENDIX……147

LIST OF TABLES

Table 4.1: Physicochemical Properties of DCP23, DCP25 and DCP34……61

Table 4.2: Thin Layer Chromatographic Analysis of DCP23, DCP25 and DCP34 using Ethylacetate……62

Table 4.3: Pharmacophore Units of the Test Compounds and their Distances…..63

Table 4.4: Infrared Spectra Data for 2,3-, 2,5- and 3,4- Dichloro – 3(aminophenyl) propanamides……..64

Table 4.5: H^1 and ^{13}C NMR Interpretation of the DCP23 Isomer……..66

Table 4.6: H^1 and ^{13}C NMR Interpretation of the DCP25 Isomer……..67

Table 4.7: H^1 and ^{13}C NMR Interpretation of the DCP34 Isomer……..68

Table 4.8: LD_{50} Values of 2,3-, 2,5- and 3,4- Dichloro – 3(aminophenyl) Propanamides in Mice and Rats via Intraperitoneal and Oral Routes…….69

Table 4.9: Effect of 2,5- Dichloro – 3(aminophenyl) Propanamides on Liver Function Parameters after 28-Day Oral Admnistration in Rats…….71

Table 4.10:Effect of 2,5- Dichloro – 3(aminophenyl) Propanamides on Renal Parameters after 28-Day Oral Admnistration in Rats…….72

Table 4.11: Effect of 2,5- Dichloro – 3(aminophenyl) Propanamides on Lipid Parameters after 28-Day Oral Admnistration in Rats…….73

Table 4.12: Effect of 2,5- Dichloro – 3(aminophenyl) Propanamides on Haematological Parameters after 28-Day Oral Admnistration in Rats…….74

Table 4.13: Effect of DCP23, DCP25 and DCP34 Dichloro and Phenytoin on Maximal electroshock-induced Seizures in Mice……..76

Table 4.14: Effect of DCP23, DCP25 and DCP34 and Valproate on Pentylenetetrazole-induced Seizures in Mice……78

Table 4.15: Effect of DCP23, DCP25 and DCP34 and Phenobarbital on 4-aminopyridine-induced Seizures in Mice……80

Table 4.16: Effect of DCP23, DCP25 and DCP34 and Phenobarbital on Strychnine-induced Seizures in Mice…….82

Table 4.17: Effect of DCP23, DCP25 and DCP34 and Diazepam on Picrotoxin-induced Seizures in Mice……...84

Table 4.18: Protective Index Values of DCP23, DCP25 and DCP34 in Mice……87

Table 4.19: Effect of Single and Multiple Oral Administrations of DCP23, DCP25 and DCP34 on Maximal Electroshock-induced Seizure in Mice……88

Table 4.20: Effect of Fluphenamic Acid on DCP23, DCP25 and DCP34 against Maximal Electroshock-induced Seizure in Mice…….90

Table 4.21: Effect of Nickel Chloride on DCP23 and DCP25 against Pentylenetetrazole-induced Seizure in Mice……..92

Table 4.22: Effect of Cyproheptadine on DCP23, DCP25 and DCP34 against Pentylenetetrazole-induced Seizure in Mice……94

LIST OF FIGURES

Figure 1: Pharmacophore Model Proposed by Unverferth……6

Figure 2: 3-[(2,3-dichlorophenyl)amino]propanamide……..48

Figure 3: 3-[(2,5-dichlorophenyl)amino]propanamide……..48

Figure 4: 3-[(3,4-dichlorophenyl)amino]propanamide……..49

Figure 5: Effect of DCP23, DCP25 and DCP34 against Pentylenetetrazole-induced Kindling in Mice……86

Figure 6: Effect of DCP23 on Diazepam-induced in Mice……96

Figure 7: Effect of DCP25 on Diazepam-induced in Mice……97

Figure 8: Tonic Current Blockade by DCP23 on Na_v 1.6 at Resting Membrane Potential (-60 mV)……..99

Figure 9: Tonic Current Blockade by DCP25 on Na_v1.6 at Resting Membrane Potential (-60 mV)……...100

Figure 10: Tonic Current Blockade by DCP34 on Nav1.6 at Resting Membrane Potential (-60 mV)……101

Figure 11: Current Blockade by DCP23 on Nav1.6 at Inactivated Membrane Potential (-100 mV)……102

Figure 12: Concentration – Response Curves for DCP23 at Resting and Inactivated States of Nav1.6……103

Figure 13: Current Blockade by DCP25 at Resting and Inactivated States of Nav 104

Figure 14: Current-Voltage Curves obtained in Control Solution and in the Presence of DCP23 (100 µM) using Nav 1.6……106

LIST OF APPENDICES

APPENDIX A: Determination of TD_{50} for DCP23 Using Walking Beam Test for Motor Coordination Deficit……147

APPENDIX B: Determination of ED_{50} for DCP23 Using Maximal electroshock-induced Seizure Test…….148

APPENDIX C: Determination of TD_{50} for DCP25 Using Beam Walking Test for Motor Coordination Deficit……149

APPENDIX D: Determination of ED_{50} for DCP25 Using Maximal electroshock-induced Seizure Test……150

APPENDIX E: Determination of TD_{50} for DCP34 Using Beam Walking Test for Motor Coordination Deficit……151

APPENDIX F: Determination of ED_{50} for DCP34 Using Maximal electroshock-induced Seizure Test……..152

APPENDIX G: Determination of ED_{50} for DCP23 Using Picrotoxin-induced Test...153

APPENDIX H: Determination of ED_{50} for DCP25 Using Picrotoxin-induced Test…154

APPENDIX I: SAMPLE OF POLYGRAPHS RECORDINGS FOR Nav 1.6…….155
CURRENTS

APPENDIX J: ANALYTICAL SPECTRA……..163

ABBREVIATIONS, DEFINITIONS, GLOSSARY AND SYMBOLS

<	Less than
µg	microgram
^0C	Degree Centigrade
5HT	5-hydroxytryptophan
AAT	Alanine amino transferase
ABU	Ahmadu Bello University
ABUTH	Ahmadu Bello University Teaching Hospital
AEDs	Antiepileptic drugs
AMPA	Alpha amino- 3- hydroxyl- 5- methyl isoxazole- 4- pripionic acid
ANOVA	Analysis of variance
AST	Aspartate aminotrransferase
ATP	Adenosine triphosphate
BBB	Blood-brain barrier
BDH	British Drug House
BDZ	Benzodiazepines
BDZ	Benzodiazepine
Ca^{2+}	Calcium ions
$CaCl_2$	Calcium chloride
Cl-	Chloride ions
CNS	Central nervous system
Co	Company
CO_2	Carbondioxide
CRH	Corticotropin- releasing hormone
CYP	Cyproheptadine

DCP23	2,3 [(dichlorophenyl)amino] propanamide
DCP25	2,5 [(dichlorophenyl)amino] propanamide
DCP34	3,4 [(dichlorophenyl)amino] propanamide
DMSO	Dimethyl sulphoxide
DZ	Diazepam
ED_{50}	Mean Effective Dose
EEG	Electroencephalograph
Etc	Ecetra
FBS	Fetal Bovine Serum
g	grams
GABA	Gamma amino butyric acid
GAD	Glutamic acid decarbixylase
h	hour
H_1	Histamine receptor subtype 1
HDL	High Density Lipid
HEK	Human Embryonic Kidney
HEPES	Hydroxyethyl piperazine ethane sulfonic acid
HLTE	Hind limb tonic extension
HVA	High voltage activated
Hz	Herzt
i.p.	Intraperitoneal
IC_{50}	Mean Inhibitory Concentration
K^+	Potassium
KA	Kainite
KCNQ2	Gene encoding for potassium channels

Kg	Killogram
LD_{50}	Mean Lethal Dose
LDL	Low Density Lipid
Ltd	Limited
LVA	Low Voltage Activated
mA	Milli ampere
MES	Maximal electroshock
MEST	Maximal electroshock test
mg	Milligram
$MgCl_2$	Magnesium chloride
MGluR	Metabotropic receptors
ml	Milli litre
ms	Millisecond
MTLE	Mesial temporal lobe epilepsy
mV	Millivolt
n	Number of animal
Na^+	Sodium ions
NaCl	Sodium chloride
Na_V	Voltage-gated sodium channel
Ni^{+1}	Nickel
NMDA	N- methyl- D- aspartate
NPY	Neuropeptide Y
OECD	Organisation of Economic Countries and Development
p.o.	Per oral
pA	Pico ampere

PBS	Phosphate Buffered Saline
PCV	Packed Cell Volume
PHB	Phenobarbitone
PHT	Phenytoin
PI	Protective index
PTZ	Pentylenetetrazole
RBC	Red Blood Cell
R_f	Retardation factor
s.c.	Subcutaneous
SEM	Standard Error of Mean
SSRI	Selective serotonin reuptake inhibitors
TD_{50}	Mean Toxic Dose
TETFund	Tertiary Education Trust Fund
TLC	Thin Layer Chromatography
TLE	Temporal Lobe Epilepsy
T-type	Subtype of calcium current
USA	United States of America
VA	Sodium valproate
VGCCs	Voltage-gated calcium channels
VGKCs	Voltage-gated potassium channels
VGSCs	Voltage-gated sodium channels
WBC	White Blood Cell
WHO	World Health Organisation

CHAPTER ONE

1.0 INTRODUCTION

Epilepsy is defined as a condition characterized by recurrent (two or more) epileptic seizures, unprovoked by any immediate identified cause (Banerjee *et al*., 2009). It refers to a disorder of brain function characterized by the periodic and unpredictable occurrence of seizures. Epilepsy is currently defined as the occurrence of at least one seizure with an enduring alteration in the brain structure or function that increases the likelihood of future seizures (Gerlach and Krajewski, 2010). Seizures are transient alteration of behavior due to the disordered, synchronous and rhythmic firing of populations of brain neurons (McNamara, 2001). Multiple seizures occurring in 24 h period or an episode of status eilepticus are considered a single event. Individuals who have had only febrile seizures or only neonatal seizures (seizures in the first 30 days of life), and people with acute symptomatic seizures (seizures associated with acute systemic illness, intoxication, substance abuse or withdrawal, or acute neurological insults), and individuals with a single unprovoked seizure, are excluded from this category (Banerjee *et al*., 2009). Seizures are broadly classified as either generalized or partial depending on whether they involve widespread bilateral cortical regions at the onset or originate from a discrete focal area. This designation is based on both outward symptoms and electroencephalograph (EEG) patterns (Gerlach and Krajewski, 2010). Generalized seizures may be convulsive (tonic myoclonic, tonic clonic, depending on the characteristics of the muscle contraction) or non-convulsive, as in the case of petit mal where the paroxysmal discharge can be accompanied only by suspension of consciousness without motor phenomena (Pevarello *et al*., 1998). This group of disorders is diverse, and they all

appear to have in common, the feature of aberrant synchronized discharge of neurons leading to alteration in electroencephalograph (EEG) activity and behavior (Nicholas *et al*., 2002). The causes of seizures are many and include the full range of neurological diseases, from infection to neoplasm and head injury. In some sub-groups of epilepsy, hereditory is known to be a major contributing factor. These may explain why monotherapy in epilepsy is difficult (Roger and Brians, 2004).

1.1 Statement of Research Problems

Drugs in the Central nervous system (CNS) can be broadly classified according to whether they have a general stimulatory or depressant action, with further sub-division regarding specific actions such as anticonvulsant and psychopharmacological activities (Evans, 1996). Several types of insults such as status epilepticus, hypoxia and trauma are known to alter the normal function of the central nervous system (CNS). Modalities that protect the brain against such insults have been very difficult and challenging. It is important to know that, epilepsy, as one of such CNS disorders, alter the normal function of brain; and its treatment is all about neuroprotection, either to reduce the duration of seizures or to suppress the occurrence of seizures (Arzimanoglous *et al*., 2002).

The scientific understanding of seizure pathogenesis and propagation is far from complete and the mechanism of action of most available antiepileptic drugs (AEDs) is either unknown or involves multiple interactions (Gerlach and Krajewski, 2010). Epilepsy is one of the most common and widespread neurological disorders. Recent estimates suggest that it accounts for 1% of the global burden of disease and affects over 65 million people; more than 500 million people are indirectly affected by the disease.

Thus, epilepsy imposes a large economic burden on global health care systems and is a major public health problem in low and middle-income countries (Mbuba and Newton, 2009). About 2 million people in the United States have epilepsy and 3% of persons in the general population will have epilepsy at some point in their lives (Bernard *et al*., 2003). It has been established that the highest incidence of epilepsy is found in children and elderly (Pevarello *et al*., 1998).

Typical therapeutic strategy is to optimize the use of a single antiepileptic drug, given that about 60% of patients have become seizure free. As second line approach, concurrent treatment with more than one AED is employed. Unfortunately, only 5% of patients who fail to respond adequately to monotherapy experience long term freedom from seizures using polytherapy (Gerlach and Krajewski, 2010). Nearly 95% of prescriptions written by physicians worldwide for the treatment of epilepsy are from the old AEDS developed prior to 1975. Several new drugs have been approved e.g felbamate, lamotrigine, gabapentine, topiramate, vigabatrin and tiagabine. These drugs have been shown to be effective in reducing seizures in a number of patients, their efficacy does not appear to be superior to that of the drugs developed earlier (Unverferth *et al*., 1998).

Epilepsy is the most common non-infectious neurologic disease in developing African countries, including Nigeria and it remains a major medical and social problem. In many African countries, people with epilepsy are out-cast as Africans believe that the disease results from visitation of the evil, effect of witch-craft, the revenge of an aggrieved ancestral spirit or consumption of something harmful *in utero*. Suicide or attempted

suicide is not uncommon among Nigerians who suffer from epilepsy. The patient with epilepsy is likely to drop out of school, looses his job, finds it impossible to marry, loses his wife or husband, and be tormented to the extent of becoming a vagrant vagabond (Ogunrin, 2006). Untreated epilepsy can lead to impaired intellectual function or death and is typically accompanied with psychosocial prejudices and other psycho pathological consequences such as loss of self-esteem and poor quality of life (Idris *et al*., 2008c).

1.2 Justification

The need to develop phenytoin-like compounds or compounds with pharmacophore responsible for Na^+ channels blockade affinity relies on the need for alternative new drugs because of well established side effects of phenytoin; which include neurologic signs (ataxia, nystagmus, sedation or irritability, orofacial dyskinesia); heamatologic (leucopenia); immunologic (reduction of IgA, lupus syndrome); endocrinologic (hirsutism); and cell growth (gingival hypertrophy) dysfunctions (Vameq *et al.*, 2000).

Most of the current antiepileptic drugs are synthetic and they are still the promising agents employed in controlling seizures among epileptic patients. Therefore, the trend should be continued to source for more promising agents. Synthetic agents are target specific, as molecular structure can be designed based on possible prediction of its pharmacophore. Also, the production of synthetic compounds can be modified to give higher yield as required. Similarly, the side effects of synthetic compounds can be avoided or reduced by optimizing their structural moieties responsible for a particular untoward effect.

1.3 Theoretical Frame Work

The field of antiepileptic drug development has become dynamic, affording many promising research opportunities (Siddiqui *et al*., 2009). Continued efforts are being made in the development of antiepileptic drugs employing a range of strategies, including modification of the structure of existing drugs, targeting novel molecular substrates and non-mechanism-based drug screening of compounds in traditional and newer animal models (Gitto *et al*., 2009). The present AEDs predominantly target voltage gated channels (e.g. alpha-subunits of voltage-gated Na^+ channels, T-type voltage-gated Ca^{2+}) or influence GABA-mediated inhibition. Recently identified, new, and potentially interesting molecular targets include KCNQ-type K^+ channels, SV2A synaptic vesicle protein, ionotropic and metabotropic glutamate receptors, and gap junctions (Pasquale and Salvatore, 2009).

1.3.1 Pharmacophore model for blockers of sodium channels

Several attempts were made to postulate a general pharmacophore for the different anticonvulsant classes. The various postulated pharmacophore models show no uniform picture. Nevertheless, the presence of at least one aryl unit, one or two electron donor atoms, and/or an NH group in a special spatial arrangement seems to be recommended. Jones and Woodbury defined a model with two electron donors in some proximity to a bulky hydrophobic moiety. Coddling postulated a pharmacophore consisting of a linear arrangement of a rotated phenyl ring, an electron donor atom, and a hydrogen donor site which partially agrees with the model of Jones and Woodbury (Unverferth *et al*., 1998).

On the basis of molecular dynamics distance estimations, the suggested pharmacophore model for compounds acting as blockers of the voltage-dependent sodium channel is given as: electron donor D in relatively limited distance ranges of 3.2-5.1 A^0 to an aryl ring or other hydrophobic units R and of 3.9-5.5 A^0 to a hydrogen bond acceptor/donor (HAD) unit. The distance between R and HAD spans a wider range of 4.2-8.5 A^0. The hydrophobic unit R is not oriented in the same plane like the other essential elements. The rings are rotated in relation to the R-D-HAD plane by 10-400 (Unverferth *et al*., 1998).

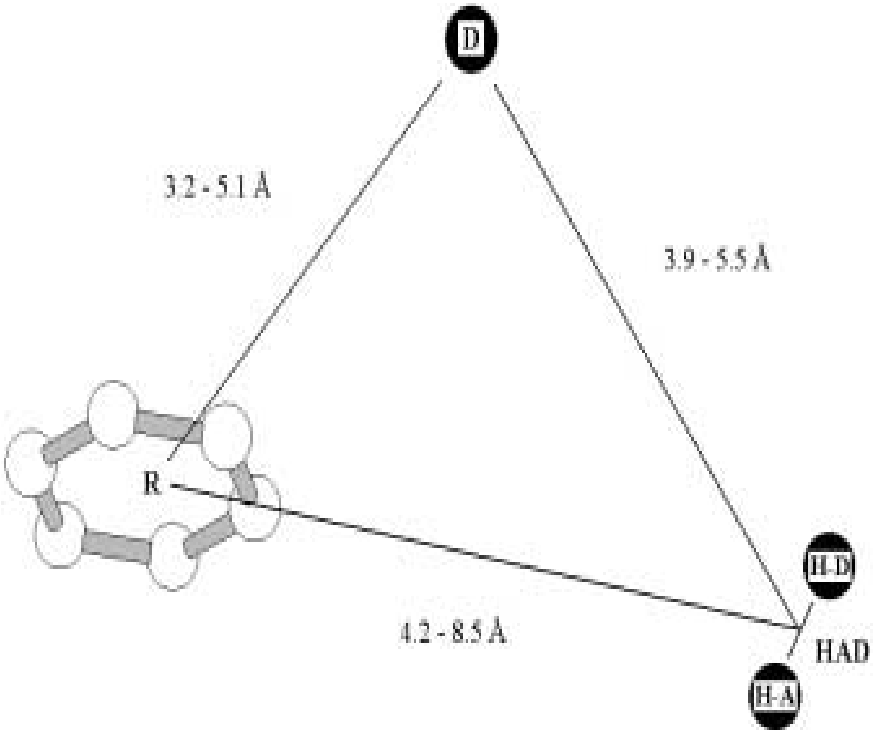


Figure 1: Pharmacophore model proposed by Unverferth *et al*., 1998

1.4 Aims and Objectives of the Study

This research is aimed at conducting anticonvulsant studies on three synthesized dichloro substituted phenyl propanamides.

The Specific objectives are as follows:

i. To synthesize, identify and characterize the compounds using standard analytical procedures (IR and NMR Spectroscopy)
ii. To determine median lethal dose (LD_{50}), median effective dose (ED_{50}), median toxic dose (TD_{50}) and protective index (PI) of the compounds to ascertain their safety profiles
iii. To establish anticonvulsant activity of the synthesized compounds in mice and rats using both acute and chronic models of convulsion
iv. To conduct subchronic toxicity studies on one representative isomer; aimed at evaluating its effect on renal, hepatic and hematologic indices as well as its effect on lipid profile
v. To determine the action of the synthesized compounds on voltage-gated sodium channels ($Na_v1.6$)

1.5 Hypothesis

Dichloro substituted phenyl propanamide isomers exert their anticonvulsant activity via voltage-gated sodium channels blockade.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Epilepsy

2.1.1 Neural network oscillations

The cortex is intimately connected with the thalamus, and the cortico-thalamo-cortical excitatory loop mediates network oscillations underlying epilepsies in man and in animal models (Paz, *et al*., 2011). Also, cortico-thalamo-cortical circuits mediate sensation, perception and consciousness, and generate neural network oscillations associated with physiological sleep-spindle activity and focal or generalized epileptic activities such as absence seizures. Communication between cerebral cortex and thalamic relay nuclei is mediated by reciprocally connected corticothalamic (CT) and thalamocortical (TC) glutamatergic excitatory pathways. Cortical input is thought to influence primary sensory thalamus by a feed-forward synaptic inhibition of TC relay neurons by GABAergic reticular thalamic nucleus (nRT) neurons. CT neurons excite nRT neurons, which subsequently inhibit TC relay neurons. Cortico-thalamo-cortical oscillations are initiated if this inhibition is followed by post-inhibitory rebound bursts of action potentials in TC neurons that in turn re-excite nRT neurons. As this sequence iterates, a network oscillation is sustained. These events in the thalamus, which is an important element in the generation of neural oscillations in cortico-thalamo-cortical circuits, rely on a stronger synaptic excitation in the CT-nRT than in the CT-TC pathway, allowing the inhibition of TC cells to overcome direct CT-TC excitation (Paz *et al.,* 2012).

2.1.2 Epileptogenesis and Ictogenesis

Epileptogenesis refers to the multiphase process in which a normal brain undergoes alterations to support the generation of spontaneous seizures. It may be initiated by brain damage produced by events such as head trauma, stroke, infection, or status epilepticus. Following such an initial insult, a latency phase without seizures follows and may last for weeks to years. During these initial stages, progressive brain alterations result in lowered seizure thresholds which eventually cause spontaneous seizures. Once seizures occur, the epileptic disease state probably continues to progress, with each seizure having the potential to induce additional neuronal alterations that may further lower seizure thresholds (Klitgaard and Pitkanen, 2003).

Drugs with antiepileptogenic properties, would act by blocking the initial epileptogenic process or by altering the epileptic disease state after the seizure onset. This would be by the ability of such drugs to reduce alterations in molecular, cellular, and network properties that occur during the epileptogenic process (Klitgaard and Pitkanen, 2003).

Experimentally induced status epilepticus can also be used as a model for epileptogenesis. Status epilepticus induces cell loss in specific neuronal populations in multiple brain regions, including the hippocampus, amygdala, and entorhinal cortex. The damage induced by kainic acid-induced status epilepticus is produced by the evoked seizure activity and not by direct activation of glutamate receptors by kainic acid. There are two phases of cell death following status epilepticus. Acute necrotic cell loss occurs during the prolonged seizure event, while other cells undergo delayed cell death hours or

days following seizure termination. Surviving brain cells undergo morphological alterations including axonal sprouting and altered density of dendritic spines. In addition, status epilepticus causes widespread changes in gene expression, the extracellular matrix, and neurogenesis. Also, it causes alteration in non-neuronal brain cells, such as changes in number and morphology of astrocytes and microglia. Functionally, status produces long-lasting deficits in cognition, behavior, and memory. Critical to the use of this model of epileptogenesis is that spontaneous seizures develop after a latency following status epilepticus (Klitgaard and Pitkanen, 2003).

Similarly, kindling, in which repeated seizures cause a reduction in seizure thresholds over time, may share features with the epileptogenic process in humans. It is possible that the long delay between trauma and seizure expression in posttraumatic epilepsy may reflect a slow kindling process. The main drawback with kindling as a model of epileptogenesis is that kindled seizures must be induced as the emergence of spontaneous seizue is rare. It is possible that the neuronal alterations produced by kindling, including cell loss and aberrant axonal sprouting, are relatively mild and may not be sufficient to mediate epileptogenesis. Again, it could be that the neuronal damage in the kindling model is as a result of seizures rather than it cause. Levetiracetam is known to delay the development of kindling, thus, has the potential to interfere with circuitry modifications underlying the progressive development of lowered seizure threshold (Klitgaard and Pitkanen, 2003).

Current drug treatment options for epilepsy predominantly combat ictogenesis, or the initiation of paroxysmal activity. Traditional AEDs have their effects by reducing the expression of epileptic seizures; nevertheless, their function invariably elicits some impairment of the normal neuronal excitability underlying cognitive function. The fact that ictogenesis and cognition are both mediated by neuronal excitability, it may not be possible to discover optimal non-impairing AEDs using traditional screens. This may be improved by performing drug screens in animal models of chronic epilepsy. Thus, by applying genetically modified or kindled animals it may be possible to discover new AEDs that inhibit the neuronal hypersynchronization leading to an ictal event, without interfering with normal neuronal excitability (Klitgaard and Pitkanen, 2003).

2.2 Ion Channels

It is known that every heartbeat, every nerve impulse, every movement and thought is critically dependent on the tightly controlled and precisely timed flow of ions across cell membranes (Nestler, *et al.*, 2009). Ion channels are important in cellular functions and are altered in many pathological conditions either directly or indirectly, as in the channelopathies (Camerino, *et al.*, 2007). Their role was most obvious in the membrane of electrically excitable cells, such as the neuron, the cardiac myocyte, and the skeletal muscle fiber. Consequently, a number of drugs able to modulate cell excitability by acting on voltage-gated or neurotransmitter-gated ion channels in these tissues have gained therapeutic relevance. Ion channel function is modulated by many natural agents that contribute to the dangerous effects of poisons or the beneficial effects of medicinal herbs. Once isolated, these lead compounds have served as the basis for the synthesis of

more specific ligands with fewer side effects. For instance, cocaine was therapeutically known to have analgesic properties, but the occurrence of CNS and cardiovascular toxicity led medicinal chemists to synthesize new derivatives, thus giving rise to the pharmaceutical class of local anesthetics, which are selective blockers of sodium channels (Camerino, *et al.,* 2007). Also, lidocaine and procaine are known to block voltage-gated sodium channels and prevent the conduction of nerve impulses that signal the occurrence of tissue damage and therefore pain. Other drugs like phenytoin and carbamazepine, potent antiepileptic agents, act by altering Na^+ channel kinetics (Nestler, *et al.,* 2009).

2.3 Action Potential

An action potential is a rapidly propagating depolarization of the axonal membrane that can lead to the release of neurotransmitter from axon terminals (Nestler, *et al.,* 2009). Neurons, cardiac, smooth muscle, skeletal muscle, and many endocrine cells have an excitable character, and thus, capable to generate and propagate electrical action potentials (Dekker, *et al.,* 2008).

It is this signal that is responsible for the transfer of information from one part of neuron to another. The threshold is important to ensure that small, random depolarizations of the membrane do not generate action potentials. Only stimuli of sufficient importance result in information transfer via action potential in the axon. Another essential feature of action potentials is that they are all-or-none events. The all-or-none law demonstrates that any stimulus large enough to produce an action potential produces the same size action

potential, regardless of stimulus strength. In other words, once the stimulus is above threshold, the amplitude of the response no longer reflects the amplitude of the stimulus. However, the latency, the time delay from the onset of the stimulus to the peak of action potential, is a function of stimulus strength (Levitan and Kaczmarek, 2002). Dendrites are the recipients of incoming synaptic activity and are said to be electrically active. They contain voltage-dependent Na^+ Ca^+ and K^+ channels and are capable of generating action potential and thus amplify incoming synaptic signals so that they can be propagated to the soma. Due to the presence of Na^+ channels along the length of axon, the action potential propagates down the axon and invades the presynaptic nerve terminals, where it triggers the influx of Ca^+ by activating voltage-dependent Ca^+ channels and subsequently leads to the Ca^+-dependent release of neurotransmitter(Nestler, *et al.*, 2009).

Voltage-gated (delayed rectifier) K^+ channels contribute to the rapid repolarisation phase of the action potential. Although membrane depolarization opens these channels, they open and close more slowly than do Na^+ channels in response to depolarization. Therefore, inward Na^+ current dominates the early (depolarization) phase of action potential, and outward K^+ current dominates the later (repolarisation) phase. Thus, action potential is characterized by an initial depolarization as a result of fast inward Na^+ current, followed by a prolonged repolarization caused by slower and more sustained outward K^+ current (Dekker, *et al.*, 2008).

2.4 Current-Voltage Relationship

The magnitude of current flowing between two points is determined by the potential difference between those two points and the resistance to current flow. Voltage is described as the potential energy or as the propensity for charge to flow from one area to another, while resistance is the obstacle to this flow. Deceased resistance allows greater ion flow and therefore increased current. Thus, current increases as voltage increases because a higher voltage results in a greater potential energy difference between the inside and outside of the cell, which in turn favors an increased rate of charge movement across the membrane (Dekker, *et al.*, 2008).

2.5 Voltage-Gated Sodium Channels (Na_V)

The primary function of voltage-gated sodium channels is to allow the propagation of action potentials. In mammals, there are nine different genes encoding voltage-gated sodium channels, many of which are more than 90% identical by sequence. On one hand, the sequence similarity of the channels presumably corresponds to similarity of their functional properties. On the other hand, the multiplicity of genes encoding sodium channels is responsible for many different mechanisms for generating an action potential (Elaine and Michael, 2007).

Voltage-gated sodium channels (Na_V) play key roles in determining neuronal excitability. They are involved in the generation of the neuronal action potential, as they mediate the initial inward current during depolarization. Similarly, they are responsible for this same process in cardiac tissue and other excitable cells. They represent the molecular site of action of various neurotoxins, local anesthetics, anticonvulsants, and antiarrhythmics

(Benjamin, *et al.,* 2006). Compounds such as these are very effective in treating clinical hyperexcitability states driven through the aberrant activity of sodium channels, in conditions such as epilepsy and neuropathic pain, or to produce complete nerve block in the case of the local anesthetics (Steve and Marcel, 2009).

2.5.1 Nomenclature of voltage-gated sodium channels

According to Ertel *et al.,* (2000) and Catterall, *et al*., (2005), a systematic nomenclature has also been adopted for voltage-gated sodium channels. In this numenclature the name of an individual channel consists of the chemical symbol of the principal permeating ion (Na) with the principal physiological regulator (voltage) indicated as a subscript (Na_V). The number following the subscript indicates the gene subfamily (currently only Na_V1), and the number following the full point identifies the specific channel isoform (e.g., $Na_V1.1$). This last number has been assigned according to the approximate order in which each gene was identified. Splice variants of each family member are identified by lowercase letters following the numbers (e.g., $Na_V1.1a$).

2.5.2 Anatomical location of voltage-gated sodium channels

Sodium channels are predominantly found in specific anatomical regions, suggesting that they might be tuned for specific functions. For example, the channels $Na_v1.1$, $Na_v1.2$, $Na_v1.3$, $Na_v1.6$, and $Na_v1.7$ are predominantly localized in the central and peripheral nervous systems; $Na_v1.8$ and $Na_v1.9$ primarily in the dorsal root ganglion; $Na_v1.4$ primarily at skeletal muscular junctions; $Na_v1.5$ primarily in cardiac tissue (Elaine and Michael, 2007).

2.5.3 Structural function of voltage-gated sodium channels (Nav)

Voltage-gated sodium channels ($Na_{v)}$ are heteromultimers and composed of α pore-forming subunit that can associate with auxiliary β subunits. Nine α subunits, named $Na_v1.1$ through $Na_v1.9$, have been cloned and expressed (Benjamin, *et al.,* 2006). The auxiliary β subunits, is known to modulate the gating kinetics of the channel (Catterall, 2000) there by modulating biophysical properties of the channels and regulate trafficking and anchoring of the channels at the cell membrane (Dib-Hajj, 2009). The major structural component of Na_v is a large (230–270 kDa) subunit, which is alone sufficient to form a functional Na^+ conducting channel (initiation and propagation of action potentials in excitable cells). This subunit contains four homologous domains (I–IV), each containing six membrane-spanning segments (S1–S6). In response to membrane depolarization, an outward movement of the positively charged S4 segments induces the conformational changes in the pore leading to the conducting activated state. The channels then enter inactivated states within a few milliseconds of channel opening. The nine subunit subtypes ($Na_v1.1$ to $Na_v1.9$) differ in their primary structure, ionic permeation, tissue distribution, functional properties, and pharmacology (Browne *et al.*, 2009).

2.5.4 Sodium channel molecular pharmacology

Voltage-gated sodium channels are the molecular targets for several important commonly used classes of drugs: local anesthetic and antiarrhythmic drugs, anticonvulsant drugs (antiepileptics), and antidepressants, even though the therapeutic goals for these drugs are quite different. All the three types of drugs block sodium currents with noticeable voltage

dependence, showing low affinity at resting states and strong block for open-inactivated states (Lipkind and Fozzard, 2010).

According to Ceste`le and Catterall, (2000) and Catterall, *et al.*, (2005), all of the pharmacological agents that act on sodium channels have receptor sites on the subunits. At least six distinct receptor sites for neurotoxins and one receptor site for local anesthetics and related drugs have been identified as follows:

Neurotoxin receptor site 1: This binds the non peptide pore blockers (tetrodotoxin and saxitoxin) and the peptide pore blocker (conotoxin). The receptor sites for these toxins are formed by amino acid residues in the pore loops and immediately on the extracellular side of the pore loops at the outer end of the pore.

Neurotoxin receptor site 2: Binds a family of lipid-soluble toxins, including batrachotoxin, veratridine, aconitine, and grayanotoxin, which enhance activation of sodium channels. The receptor site for batrachotoxin is at the transmembrane segments IS6 and IVS6.

Neurotoxin receptor site 3: This site binds the scorpion toxins and sea anemone toxins, which slow the coupling of sodium channel activation to inactivation. These peptide toxins bind to a complex receptor site that includes the S3-S4 loop at the outer end of the S4 segment in domain IV.

Neurotoxin receptor site 4: Binds the scorpion toxins, which enhance activation of the channels. The receptor site includes the S3-S4 loop at the extracellular end of the voltage-sensing S4 segments in domain II.

Neurotoxin receptor site 5: This site binds the complex polyether toxins brevetoxin and ciguatoxin, which are made by dinoflagellates and cause toxic red tides in warm ocean waters. Transmembrane segments IS6 and IVS5 are implicated in brevetoxin binding from photoaffinity labeling studies.

Neurotoxin receptor site 6: Binds conotoxins, which slow the rate of inactivation like the scorpion toxins. The location of neurotoxin receptor site 6 is unknown.

Local anesthetics and related antiepileptic and antiarrhythmic drugs: Bind to overlapping receptor sites located in the inner cavity of the pore of the sodium channel. Amino acid residues in the S6 (I, III and IV) segments from at least three of the four domains contribute to this complex drug receptor site, with the IVS6 segment playing the dominant role.

Anticonvulsants are characterized by their ability to block seizures in patients with epilepsy, mediated by their interaction and inhibition of brain Na channels. They have little effect on normal brain activity, and they block Na channels poorly at slower firing rates from normal resting potentials. Seizures, however, are characterized by depolarized resting potentials and high frequency trains of action potentials. The drugs are thought to

suppress seizures by inhibiting this high frequency repetitive firing, allowing the drugs to have selective action on hyperactive rather than normal neurons (McNamara, 2006). The functional factors that could underlie this voltage and use dependence are a higher affinity for depolarized cells and an off-rate slower than the repetition frequency of the action potentials (Lipkind and Fozzard, 2010).

2.5.5 Biophysical modulation of voltage-gated sodium channels (Na_v)

The strength of block and therefore the potency of a blocking drug can be influenced by membrane voltage. The increase in the strength of block induced by a change in membrane voltage has been termed voltage-dependent block. Strength of block can also be influenced by the frequency and duration of repetitive depolarizations during a train of action potentials or voltage clamp pulses. The increase in block induced by repetitive firing has been termed as use-dependent block. Use-dependent block can conveniently be thought of as having a depolarization phase during which block accumulates as drug molecules enter and block more Na channels, and a resting phase during which block dissipates as blocker leaves channels (Yeh and Teneick, 1987).

Small molecule sodium channel modulators frequently possess state- and use-dependency. A compound showing state-dependent pharmacology exhibits a higher binding affinity for one channel state over another. For the local anesthetics, affinities are highest for the open and inactivated states of the channel and lowest for the resting state; this seems to be true for most of the drug-like molecules described as sodium channel blockers. Again, rapidly firing nerve fibres were more sensitive to block by the local

anesthetics procaine than those firing at a slower rate, and this eventually became known as use-dependent (or phasic) block (Steve and Marcel, 2009).

Lamotrigine has a greater affinity for inactivated channels compared with those at rest. The compound binds slowly to fast-inactivated channels and the kinetics of lamotrigine binding to inactivated channels is faster than the development of the slow inactivated state (Kuo and Lu, 1997). These features mean that lamotrigine needs a sustained depolarization in a neurone to produce significant block. Long depolarizations are unusual in normal physiological systems but are characteristic of epileptic discharge (De Curtis and Avanzini, 2001). Therefore, it seems unlikely that a strongly use-dependent but non-selective channel modulator will be able to deliver the next generation of therapies for conditions such as epilepsy or pain (Steve and Marcel, 2009).

2.5.5.1 State dependent block

Clinically used sodium channel blockers are state dependent; that is, they bind with higher affinity to the inactivated state of the sodium channel than the resting state (Jones, *et al.,* 2007). This state dependence not only is considered important for seizure suppression but also is essential for a favorable side effect profile since it allows AEDs to inhibit action potential (AP) bursts that occur during seizures without affecting normal neuronal activity (Rogawski and Loscher, 2004).

2.5.5.2 Use dependent blockade

In addition to voltage-dependent block, AEDs often exhibit use-dependent block. This characteristic is considered important since it allows the enhanced block of high-frequency action potential discharges that occur during epileptic seizures (Rogawski and Loscher, 2004). Thus, it occurs when neurones fire frequently and the probability of a channel being in an inactivated state (i.e. one for which the drug has a higher affinity and that can be pharmacologically modified) is highest. Block can therefore accumulate as Local anesthetics have a slower dissociation rate from inactivated channels, compared with those in the resting state (Chernoff, 1990).

2.5.5.3 Inactivation of Na^+ channels

Sodium channels open in response to membrane depolarization, after which the channels enter a closed state in which they are inactivated (i.e. prevented from subsequent opening). This inactivation of Na^+ conductance, combined with the slowly decaying voltage-gated K^+ conductance produces dynamic changes in membrane excitability (Dekker, *et al.*, 2008).

2.5.5.4 Fast inactivation

Sodium channels are closed and inactive at rest but undergo structural changes in response to membrane depolarization, leading to cycling of the channel through activated (open), inactive, and repriming states. Transient channel opening allows a flow of sodium ions down their concentration gradient, thus generating an inward transmembrane current that depolarizes neurons, bringing them closer to the threshold for action potential

generation. Most channels rapidly inactivate, within milliseconds of opening, and then undergo conformational changes to recover from inactivation (Dib-Hajj, 2009).

According to Catterall, (2012), sodium channels in metazoans open in response to depolarization and then inactivate within 1–2ms. This fast inactivation process is required for repetitive firing of action potentials in neural circuits and for control of excitability in nerve and muscle cells. Studies with site-directed anti-peptide antibodies showed that the short intracellular loop connecting homologous domains III and IVof the sodium channel α subunit is responsible for fast inactivation. This fast inactivation gate serves as an intracellular blocking particle that folds into the channel structure and blocks the pore during inactivation. The inactivation gate bends at a key pair of glycine residues to fold into the intracellular mouth of the pore, bind and block sodium conductance as a hinged lid.

2.5.5.5 Recovery from inactivation

It occurs only when the membrane is repolarized, whereupon the Na^+ channels return to the closed, resting state from which they can open in response to a stimulus (Dekker, *et al.,* 2008).

2.5.6 Voltage sensing and voltage-dependent activation

Voltage dependence of sodium channel activation requires movement of three electrically charged particles across the cell membrane through the full extent of the transmembrane electric field (Catterall, 2012). The S4 transmembrane segments of sodium channels, which contain four to eight repeated motifs of a positively charged amino acid residue

(usually arginine) followed by two hydrophobic residues, was proposed to carry the gating charges of sodium channels in the sliding helix or helical screw model of voltage sensing. The S4 segment is proposed to be in a transmembrane position in both resting and activated states; the gating charges are stabilized in their transmembrane position by forming ion pairs with neighbouring negatively charged residues; and their outward movement is catalysed by exchange of these ion pair partners (Yarov-Yarovoy *et al.* 2006). The transmembrane position of the S4 segment in sodium channels has been shown to bind to the outer end of the S3–S4 loop of the voltage sensors in both resting and activated states, thereby establishing that the S4 segment remains in a transmembrane position in both of these states (Wang *et al.* 2011; Zhang *et al.* 2011). Covalent labelling and voltage clamp fluorescence studies show that the S4 segments of sodium channels move outward and rotate upon membrane depolarization and transport the gating charges from an inner water-accessible vestibule to an outer water-accessible vestibule (Chanda and Bezanilla, 2002).

2.5.7 Ion selectivity and conductance

Although the overall pore architecture of sodium and potassium channels is similar, the structures of their ion selectivity filters and their mechanisms of ion selectivity and conductance are completely different. Potassium channels select K^+ by direct interaction with a series of four ion coordination sites formed by the backbone carbonyls of the amino acid residues that comprise the ion selectivity filter. No water molecules intervene between K^+ and its interacting backbone carbonyls in the ion selectivity filter of potassium channels (Zhou *et al.* 2001). In contrast, the Na_vAb ion selectivity filter has a

high-field-strength site at its extracellular end, formed by the side chains of four glutamate residues (Payandeh *et al.* 2011), which are highly conserved and are key determinants of ion selectivity in vertebrate sodium and calcium channels (Heinemann *et al.* 1992). Considering its dimension of approximately 4.6°A square, Na^+ with two planar waters of hydration could fit in this high-field-strength site. This outer site is followed by two ion coordination sites formed by backbone carbonyls. These two carbonyl sites are perfectly designed to bind Na^+ with four planar waters of hydration but would be much too large to bind Na^+ directly. In fact, the Na_vAb selectivity filter is large enough to fit the entire potassium channel ion selectivity filter inside it (Payandeh *et al.* 2011). Thus, the chemistry of Na^+ selectivity and conductance is opposite to that of K^+: negatively charged residues interact with Na^+ to remove most (but not all) of its waters of hydration, and Na^+ is conducted as a hydrated ion interacting with the pore through its inner shell of bound waters. This structure of the ion selectivity filter of NavAb is remarkably similar to the four-barrier, three-site model of ion selectivity, which predicted an outer high-field-strength site that would partially dehydrate the permeating ion and two inner sites that would conduct and rehydrate the permeant Na^+ ion (Hille, 1975). This theory and structure gives clear insight into the chemistry and biophysics of sodium permeation.

2.5.8 Phosphorylation of Sodium Channels

Sodium channels in brain are rapidly phosphorylated by cAMP-dependent protein kinase, which reduces their ion conductance activity. Phosphorylation of domains I and II reduces peak sodium currents in brain neurons and in cells expressing cloned sodium channels without altering the voltage dependence of activation and inactivation, action

potential generation and peak sodium currents are reduced by dopamine acting on D1-like receptors and subsequent activation of the cAMP signaling pathway. Thus, there is clear evidence for involvement in dopamine regulation of the firing properties and input-output relationships of striatonigral neurons (Catterall, 2000). Also, sodium currents in nucleus accumbens neurons are persistently reduced after treatment with cocaine, a drug which increases dopaminergic neurotransmission by blocking dopamine reuptake (Zhang *et al*., 1998).

Sodium channels are also phosphorylated by protein kinase C, and this activation by diacylglycerols or by acetylcholine acting on muscarinic receptors slows sodium inactivation and reduces peak sodium currents. Also, as modulated by tyrosine phosphorylation, activation of tyrosine kinases causes a negative shift in the voltage-dependence of sodium channel inactivation (Catterall, 2000).

2.5.9 Channelopathies of sodium channels

In Dravet syndrome, and possibly also in generalized epilepsy with febrile seizures plus, loss-of-function mutations in $Na_v1.1$ channels selectively impair the excitability of GABAergic inhibitory neurons and thereby create hyperexcitability in neural circuits and cause epilepsy (Catterall *et al.* 2010).

In sensory neurons, mutations in $Na_v1.7$ channels cause erythromelalgia by shifting the voltage dependence of activation to more negative membrane potentials and cause paroxysmal extreme pain disorder by impairing sodium channel inactivation (Dib-Hajj *et*

al., 2009). In the rare recessive pain disorder congenital indifference to pain, loss-of-function mutations in both alleles of the gene encoding $Na_v1.7$ channels cause complete loss of pain sensation (Cox *et al.,* 2006).

Mutations of the gating charges in the voltage sensor of $Na_v1.4$ channels cause an ionic leak through the gating pore, resulting in excess Na^+ influx through the leaky voltage sensor, accumulation of intracellular Na^+, depolarization and conduction block that lead to episodic paralysis (Sokolov *et al.,* 2008).In skeletal muscle, mutations in $Na_v1.4$ channels increase channel activity by impairing fast and/or slow inactivation in paramyotonia congenita and hyperkalaemic periodic paralysis (Venance *et al.,* 2006).

Also, mutations in $Na_v1.5$ channels cause long QT syndrome by impairing sodium channel inactivation (Keating and Sanguinetti, 2001). In Brugada syndrome, loss-of-function mutations of $Na_v1.5$ channels create inhomogeneity of conduction across the ventricular wall and generate arrhythmias (Terrenoire *et al.,* 2007).

2.6 Voltage-Gated Potassium Channels (VGKCs)

Voltage-gated potassium channels indirectly modify presynaptic Ca^{2+} entry, neurotransmitter release and action potential. Hence, more subtle mechanism of seizure control than voltage-gated calcium channel. Presynaptic voltage-gated potassium regulates the release of both excitatory and inhibitory neurotransmitters throughout the brain. Generally, if VGKCs open; the membrane potential will move toward the potassium equilibrium potential, whereas if close, other ionic conductances active in

neurons will drive membrane potential changes, often, as in case of VGSCs and VGCCs, in a depolarizing direction thus, VGKCs openers can be considered as good antiepileptic because they speed the repolarization of the presynaptic terminals, which will shorten the duration of action potential toward potassium equilibrium potential (E_K). Examples of VGKCs opener are Flupirtine and Retigabine (Nicholas *et al*., 2002).

Voltage-gated (delayed rectifier) K^+ channels contribute to the rapid repolarisation phase of the action potential. Although membrane depolarization opens these channels, they open and close more slowly than do Na^+ channels in response to depolarization. Therefore, inward Na^+ current dominates the early (depolarization) phase of action potential, and outward K^+ current dominates the later (repolarisation) phase. Thus, action potential is characterized by an initial depolarization as a result of fast inward Na^+ current, followed by a prolonged repolarization caused by slower and more sustained outward K^+ current (Dekker, *et al.*, 2008).

Potassium channels play a major role in the control of all aspect of neuronal excitability that need to be exploited for the development of new AEDs, this may serve as an alternate or adjunct therapy for the treatment of drug-resistant or refractory epilepsy. For instance, inward rectifiers, is a class of potassium channels primarily functions to control neuronal excitability. These channels pass current over a hyperpolarized voltage range; this will lead to the maintenance of resting membrane potential, responsiveness to synaptic inputs and neurotransmitter release. Gating of some inward rectifiers is tightly regulated by intracellular ATP levels, providing a link between cellular metabolism and

neuronal excitability. Others are activated by G-protein coupled receptors; both are referred to as ATP-sensitive K^+ channels and G-protein activated K^+ channels respectively (Wickenden, 2002).

Another class of VGKCs is $KCNQ_2$ and $KCNQ_3$. They are predominantly expressed in the CNS, and are found both pre- and post-synaptically in brain regions that are known to be important for the control of neuronal network oscillations and synchronization (Cooper, *et al*., 2001).

2.7 Voltage-Gated Calcium Channels (VGCCs)

Calcium ion is an important signaling molecule that is present in low concentration in extracellular fluid and in minute concentration in most cell interiors. The opening of Ca^{2+} channels is the critical link between cell depolarization and Ca^{2+} entry which can result in its high concentration level. The subsequent binding of Ca^{2+} to intracellular molecules can lead to; muscle contraction, the triggering of neurotransmitter release from nerve terminals, the activation of second messenger system that cause many changes, including alteration in gene expression and in extreme cases, neuronal self-destruction. Some Ca^{2+} channels also impart electrical properties to the cells in which they are expressed, thus, may show action potentials in which the depolarizing current is carried predominantly by Ca^{2+} (Nestler, *et al*., 2009).

According to Nicholas, *et al*., (2002), VGCCs are key regulators of Ca^{2+} entry into neurons, and are known to control a variety of cellular processes that regulate neuronal

excitability. They are divided into two groups; high-voltage activated and low-voltage activated.

High voltage activated, also known as L-type (large current or long open time) controls the release of neurotranmitters such as the excitatory neurotransmitter glutamate. Whereas, low voltage activated, also known as T-type (tiny current or transient) controls membrane potential that lead to low threshold stimulation in thalamic neurons, which may underlie the synchronizing discharges characteristic of epilepsy. These channels have been shown to be blocked by known antiepileptic drugs such as ethosuximide, gabapentin and levetiracetam (Nicholas, *et al*., 2002).

Sodium valproate is also known to inhibit T-type Ca^{2+} channels in thalamic neurons (Lowestein, 2001). Similarly, zonisamide inhibits T-type Ca^{2+} currents and also inhibits the sustained, repetitive firing of spinal cord neurons presumably by pronlonging inactivation of voltage-gated Na^{+} channels in manners similar to actions of phenytoin and carbamazepine (McNamara, 2006). Overexpression of T-type channels appears to be linked to pathophysiological conditions such as absence epilepsy, and metallic ions such as Cd^{2+}, Co^{2+}, Ni^{+}, Pb^{2+} and Zn^{2+} have been found to inhibit Ca^{2+} permeation via voltage-dependent Ca^{2+} channels with different potencies. Among these cations Ni^{+1} was found to be selective blocker for low voltage-activated T-type Ca^{2+} channels (Kang *et al*., 2006).

2.8 Chloride Channels

Anion channels are proteinaceous pores in biological membranes that allow the passive diffusion of negatively charged ions along their electrochemical gradient. Chloride ion channel gating may depend on the transmembrane voltage (in voltage-gated channels), on cell swelling, on the binding of signaling molecules (as in ligand-gated anion channels of postsynaptic membranes), on various ions (e.g., anions, H^+ (pH), or Ca^{2+}), on the phosphorylation of intracellular residues by various protein kinases, or on the binding or hydrolysis of ATP. Plasma membrane Chloride ion currents are important for the regulation of excitability in nerve and muscle (Jentsch *et al*., 2002).

The intracellular Chloride ion concentration of neurons determines the response to the neurotransmitters glycine and GABA. Because glycine, $GABA_A$, and $GABA_C$ receptors are ligand-gated chloride ion channels, their activation can lead to a passive influx or efflux of chloride, depending on the electrochemical potential for Cl^-. Their activation can therefore lead to an excitatory, or to the more commonly observed inhibitory, response. Chloride ion channels activated by intracellular calcium are found in many cell types, including epithelial cells, neurons, cardiac and smooth muscle cells, as well as blood cells. In neurons and muscle cells, Ca^{2+} activated Cl^- channels may modulate excitability, e.g., by generating after potentials, and Ca^{2+} activated Cl^- channels are thought to regulate the tonus of smooth muscle (Jentsch *et al*., 2002).

2.9 Neurotransmitter Mediated Mechanisms

Synapses are known to mediate interneuronal communication via synaptic neurotransmitters and as such reduction of inhibitory synaptic activity might be expected to trigger occurrence of seizures. Amino acids including glycine as well as gamma amino butyric acid (GABA) are the inhibitory neurotransmitters. On the other hand, glutamate is the principal excitatory neurotransmitter acting through N-methyl-D-aspartate (NMDA), alpha amino-3-hydroxy-5-methyl isoxazole-4-propionic acid (AMPA) and kainite (KA) receptor subtypes (McNamara, 2001).

2.9.1 Gamma amino butyric acid (GABA)

GABA is the predominant inhibitory neurotransmitter in the brain, and the expression and function of GABA receptors also are developmentally regulated (Russell and Frances, 2001).

GABA receptors are pentameric ligand-gated anion channels. The subunits are derived from α, β, γ, δ, and ε gene families, some of which have multiple members such as α 1–6, β1–3, and γ1–3. The majority of the receptors appear to be composed of 2α, 2β, and γ or δ subunits. The sensitivity of the receptor to many modulators depends on its subunit composition. Subunit composition-dependent properties of GABA receptors relevant to status epilepticus and epilepsy are their sensitivity to benzodiazepines such as diazepam, midazolam and lorazepam, and also to the divalent cation Zn^{2+} and neurosteroids. Diazepam sensitivity requires the presence of a γ2 subunit and the relative affinity of diazepam to the receptor also depends on the subtype of α subunit. In contrast, sensitivity to Zn^{2+} is reduced by the presence of a γ subunit, but relative sensitivity to Zn^{2+} is also

regulated by the subtype of α subunit. The presence of a δ subunit confers higher GABA and neurosteroid affinity to the receptor (Joshi and Kapur, 2012).

Prolonged seizures (status epilepticus) result in alterations in the expression and membrane localization of several GABAR subunits (α1, α4, γ2, δ) in hippocampal dentate granule neurons. These alterations, which are associated with changes in phasic and tonic GABAR -mediated inhibition, and in GABAR modulation by benzodiazepines, neurosteroids, and zinc, begin soon after status epilepticus and continue as animals become epileptic (Zhang *et al*., 2007).

In addition to pharmacological modulation by different drugs, subunit composition also influences the kinetic properties of the receptors. For example, δ subunit-containing receptors desensitize slowly and more incompletely than γ2 subunit-containing receptors. Subunit composition also determines receptor targeting. Presence of a γ2 subunit is necessary for synaptic localization of GABA receptors, whereas GABA receptors composed of the δ subunit remain exclusively in the peri- and extra-synaptic membrane. Synaptic GABA receptors mediate fast inhibition in response to the high concentration of GABA released in the synaptic cleft, whereas extra-synaptic receptors respond to GABA spilled over from synapses and contribute to persistent background inhibition commonly referred to as tonic inhibition (Joshi and Kapur, 2012).

2.9.1.1 GABA receptor subtypes

Three types of GABA receptors, $GABA_A$, $GABA_B$ and $GABA_C$, are found in the mature central nervous system. $GABA_A$ and $GABA_C$ are ionotropic receptors, whereas, $GABA_B$ is a metabotropic receptor. Most fast synaptic inhibition in the mature brain is mediated by $GABA_A$ receptors whereas slow inhibition is mediated by $GABA_B$ receptors (Kayal-Brooks *et al*., 2009).

$GABA_A$ receptors mediate post synaptic responses to GABA in central neurons, and are expressed at embryonic stages (Laurie, *et al*., 1992). However, in the first postnatal week, activation of $GABA_A$ receptors causes membrane depolarization rather than hyperpolarisation typical of mature GABA-ergic synapses (Swann, *et al*., 1999). Thus, although functional GABA receptors are present very early in development, the delayed onset of GABA-ergic inhibition may contribute to the enhanced excitability of immature brain (Rivera, *et al*., 1999). $GABA_A$ receptors are selectively activated by muscimol and isoguvacin. Picrotoxin, a mixture of picrotin and picrotoxinin, is a noncompetitive inhibitor that reduces channel activity. It binds to a site different from the GABA-binding site (Jentsch *et al*., 2002).

The G-protein-coupled $GABA_B$ receptors are activated both pre- and postsynaptically, with opposite effects on transmission. Postsynaptic $GABA_B$ receptors mediate relatively slowly activating and long-lasting membrane hyperpolarisation through the activation of a K^+ conductance, whereas the activation of presynaptic $GABA_B$ receptors decreases neurotransmitter release through the inhibition of Ca^{2+} channels (Giarsa, *et al*., 1995).

$GABA_C$ receptors were first described in interneurons of the spinal cord and later also identified in the retina. In contrast to $GABA_A$ receptors, which are sensitive to bicuculline, and $GABA_B$ receptors, which are sensitive to baclofen, $GABA_C$ receptors are insensitive to either drug. Compared with $GABA_A$ receptors, $GABA_C$ receptors have a higher sensitivity to GABA. Their currents are smaller, and they do not desensitize. They share only 30– 38% amino acid sequence identity with the $GABA_A$ receptors subunits (Jentsch *et al*., 2002)

.

2.9.2 Glycine

Fast inhibitory neurotransmission in the mammalian central nervous system (CNS) is mediated primarily by the neurotransmitters GABA and glycine. Glycine is predominantly used in the spinal cord and the brain stem, whereas GABA is more commonly used in the brain. Their binding to their receptors opens intrinsic anion channels. In the adult CNS, this mostly leads to a Cl^- influx, which hyperpolarizes the neuron and thereby inhibits neuronal activity. Early in development, GABA and glycine induce a strong depolarizing response that can cause Ca^{2+} influx via voltage-gated Ca^{2+}channels and thus triggers neurotransmitter release (Reichling, *et al*., 1994).

Glycine receptors can be activated by glycine, alanine and taurine. They are selectively antagonized by strychnine. At least two different binding sites for agonists and strychnine were identified; one in the aminoterminal extracellular domain and a second in the second extracellular cysteine loop. The only known agonist that is not an amino acid is cesium. Apparent glycine affinity is strongly potentiated by Zn^{2+} but it is reduced at

higher concentrations. High concentrations of ethanol are found to potentiate glycine-activated currents in chick spinal cord neurons. Glycine receptors are also potentiated by the anesthetic isoflurane. The alkaloid strychnine is a highly selective and extremely potent competitive antagonist of glycine receptors. The only known glycine receptor channel blocker is cyanotriphenylborate (Jentsch *et al*., 2002).

2.9.3 Glutamate receptors

2.9.3.1 Ionotropic glutamate receptors

These include the NMDA, AMPA, and KA subtypes (Dingledine, *et al*., 1999). AMPA and KA receptors mediate fast excitatory signaling as they exhibit rapid activation and desensitization. NMDA receptors play a more modulatory role, as their activation requires concurrent glutamate binding and membrane depolarization and results in slower and longer-lasting excitation. NMDA-receptor channels are highly permeable to Ca^{2+}; in addition to Na^{+} and K^{+} (Aamodi and Constantine-paton, 1999).

2.9.3..2 Metabotropic glutamate receptors

There are at least eight cloned metabotropic glutamate receptors: mGluR1, mGluR2, mGluR3, mGluR4, mGluR5, mGluR6, mGluR7 and mGluR8 (Conn and Pin, 1997). These have been classified into three groups based on sequence homology, coupling to second-messenger systems and pharmacological sensitivities. Group 1 receptors are coupled to phosphoinositide (PI) hydrolysis that leads to Ca^{2+} mobilization from intracellular stores, whereas Groups 2 and 3 receptors are negatively coupled to adenylyl cyclase (AC) activity. Although the consequences of mGluR activation vary depending

on receptor type, neuronal type, or brain region, some general principles regarding the effects of mGluR activation in relation to seizures have emerged (Wong, *et al*., 1999). For instance, postsynaptic group 1 mGluR activation causes an increase in the intrinsic excitability of principal neurons (particularly in hippocampal CA1 and CA3 subfields), mainly by down modulation of voltage-gated potassium channels (Gerber and Gahwiler, 1994), and therefore, activation of PI-coupled mGluRs is likely to promote seizure activity. Conversely, presynaptic Groups 2 and 3 receptor activation tends to depress excitatory synaptic transmission by inhibiting glutamate release (Glaum and Miller, 1994), and therefore, activation of AC-coupled mGluRs is likely to inhibit seizure activity.

2.10 Neuromodulators (neuropeptide Y and corticotropin-releasing hormone)

2.10.1 Neuropeptide Y (NPY)

This is a neuromodulatory substance that appears to be of central importance in the regulation of neuronal excitability, particularly tuning interneuron discharge propensity (Baraban and Tallent, 2004). NPY is able to decrease synaptic transmission by reducing presynaptic calcium influx and can suppress epileptiform activity via Y2 receptor activation (Avoli-massimo, 2005). According to Tu, *et al*., (2005), tonically released, endogenous NPY may decrease excitability in recurrent mossy fibre projections in a limbic epilepsy model.

2.10.2 Neuropeptide corticotrophin-releasing hormone (CRH)

The excitatory neuropeptide CRH, is the most potent epileptogenic peptide, and may play a critical role in the triggering of seizures (Wasterlin and Mazarati, 1997).

2.11 Other Modulators

2.11.1 GABA transporters

GABA transporters may be altered in epilepsy as seen in human epileptogenic tissue from mesial temporal lobe epilepsy (MTLE), the research reveals the presence of reduced level of GABA transporters. The K^+ stimulated release of GABA is increased and glutamate induced Ca^{2+} independent release of GABA is decreased in the epileptogenic hippocampus (Avoli, *et al*., 2005).

2.11.2 Gene expression patterns

Widespread changes of gene expression patterns do occur in human epileptic hippocampus, some of which overlap with changes found in pilocarpine-induced chronic MTLE condition. These include protein involved in, cell-matrix interactions, cell growth and differentiation, transcriptional regulation and cellular signaling (Becker, *et al.*, 2003).

2.11.3 Gap junction

Gap junctions play an important role in synchronizing neuronal networks under physiological and pathological conditions such as epileptic seizures (Nakese and Naus, 2004). They allow flow of electrical signals and small molecules including dyes, between cells, thus promoting neuronal synchrony. Procedures capable of enhancing or blocking

the function of gap junction increase or decrease epileptiform synchronization, respectively (Carlen, *et al*., 2000). It is important to note that, the constituent of gap junctions are proteins called *connexins* (Hormuzdi, *et al*., 2004).

2.12 Structure Activity Relationship of Sodium Channel Blockers

According to Tasso *et al.,* (2001), there are two different approaches in the field of rational drug design that allow searching for new structures with improved biological activity, targeting not only an increase in the potency, but also the simultaneous decrease of adverse side effects. New structures can be modeled on the basis of knowledge of the characteristics of the receptor site, or by means of comparison of different ligands, selected from those that interact with the receptor. For the applicability of the latter approach, all the molecules should be assumed to bind to the same domain of a given type of receptor protein. Within this framework, the complexity of the study is partially determined by the flexibility of the molecules, which defines many energetically accessible conformations that can coexist in equilibrium. When designing from the ligand, the goal is to identify the geometric and electronic features that, being shared by all the molecules, are most likely involved in receptor recognition and activation. Under these conditions, a comparative analysis, which should include active and non-active compounds, leads to the identification of the minimal requirements associated with the pharmacophoric pattern for a manifested activity. For the majority of antiepileptic drugs (AED) that are presently in clinical use, the mechanism of action responsible of the whole biological response remains unknown, mainly due to the fact that one receptor site can not be uniquely associated with a given action.

Anticonvulsant drugs typically have a tricyclic structure, with a polar amide in the middle. These molecules are wider and more rigid than local anesthetics. Although structurally different, anticonvulsant drugs interact with some of the same amino acid residues in the Na channel's inner pore and have similar overall open/inactivated state affinity (Tarnawa *et al*., 2007).

The anticonvulsant drugs with similar affinity are neutral and have different therapeutic effects. Neutral anticonvulsant drugs also seem to have rapid off-rates because they show little frequency dependent block at moderate concentrations and allow normal neuronal firing rates in the presence of the drugs (Lipkind and Fozzard, 2010).

In the closed state the four S6 helices at their C-ends form the so-called S6-crossing, producing an inner cavity of restricted size below the selectivity filter. Voluminous phenytoin practically fills this space in the closed channel, excluding water and making direct contacts with the walls of this cavity. Such low-affinity interactions would likely be only non bonded van der Waals interactions. In this case the interface between nonpolar surfaces of the drug and bulk water is lacking, and therefore the additional stabilizing effect of hydrophobic interactions is absent. This comparative analysis by binding of phenytoin in the open and closed states could explain why even neutral molecules of anticonvulsants show low affinities of binding in the rested state relative to the high affinity with the open/inactivated state of the Na channel (Lipkind and Fozzard, 2010).

Three typical anticonvulsant drugs, phenytoin, carbamazepine, and lamotrigine, share a common binding site in neuronal Na channels. Despite the differences in their chemical structure, it is likely that their mechanisms of block and pharmacological action seem to be similar (Lipkind and Fozzard, 2010).

Sodium channel activity of several mono- and bicyclic phenytoin analogues was investigated and thus established that, a free imide group, and a specific aromatic orientation are optimal for high binding affinity to the sodium channel. Five well known and structurally different compounds with anticonvulsant activity, carbamazepine, phenytoin, lamotrigine, zonisamide and rufinamide; have at least one aryl ring (R), one electron donor atom (D), and a second donor atom in close proximity to the NH group forming a hydrogen bond acceptor/donor unit (HAD); in most cases this is an amide bond. Most compounds (with exception of phenytoin) are able to realize two alternative conformational orientations of the HAD unit, the one with the H acceptor functions, the other with the H donor function cannot point to the aryl ring because of missing flexibility. If this HAD unit should be essential for sodium channel-blocking activity, then the receptor site for this group seems to be rather flexible (Unverferth *et al*., 1998).

Phenytoin (diphenylhydantoin) contains a hydantoin ring and two phenyl substitutions at C5 of the hydantoin, but the second aromatic ring is not obligatory. 5-alkyl-5-phenyl-hydantoins also have a comparable affinity for Na channels (Brown *et al.,* 1999), with an optimal length of the aliphatic chain corresponding to pentyl, hexyl, and heptyl. Most likely, this aliphatic chain and the corresponding aromatic ring participate in less specific

hydrophobic interactions, whereas the second aromatic ring has specific aromatic-aromatic interactions. Methyl (or alkyl) substitutions of the amide nitrogens of the hydantoin ring reduced binding significantly (Brown *et al.*, 1999), which underlines the importance of these hydrogens for binding. It has been proposed that one aromatic ring and one amide group of the hydantoin form the pharmacophore core of phenytoin (Unverferth *et al.*, 1998; Brown *et al.*, 1999).

Substitutions of the second aromatic ring of phenytoin by aliphatic hydrophobic chains produced derivatives with high affinity of binding with the Na^+ channel. Improvement of binding of the derivatives of phenytoin with increase in size of the 5-alkyl chains (from *n*-propyl to *n*-nonyl;) certainly reinforces the hydrophobic nature of their interactions with the inner pore in the open/inactivated state, where the wide opening fills the pore with bulky water (Brown *et al.*, 1999).

4-amino moiety played an important role in the molecular recognition process at the level of the receptor modulating the voltage-dependent sodium channel status, this was proved using batrachotoxin affinity assay. This moiety was therefore preserved in most pharmacomodulations (Vamecq *et al.*, 2000). Also, amides of substituted benzoic acids including some aminohalobenzamides have been reported to possess anticonvulsant activity. Series of 4-aminobenzanilides derived from ring-alkylated anilines have also known to exhibit anticonvulsant activity (Clark, 1985).

Anticonvulsant activity of 4-amino-N-cyclopropyl-3,5 dichloro benzamide and 4-amino-N-cyclopropyl-3,5-dibromobenzamide have been reported against strychnine, pentylenetetrazole and electroshock. Dihalogenation of the 2,6 positions produces effective antagonists to electroshock, while dihalagenation in the 3,5- positions increases the antipentamethylenetetrazole activity (Horrom, and Lynes, 1962). 4-aminobenzamides represent a fertile series for the discovery of anticonvulsant agents (Robertson *et al*., 1987).

Clark and coworkers demonstrated significant anticonvulsant potential for the amino-substituted benzamides derived from alkyl-, arylalkyl-, and arylamines. Optimal antiepileptic activity was found in those amides having primary amine in the 4-position of the benzamide moiety and an aromatic N-substitution of the 4-aminobenzamide pharmacophore. From this work emerged ameltolide which exhibits a phenytoin-like profile. Also, N-phenylpthlamide derivatives were shown to possess a similar degree of anticonvulsant potency also associated with a phenytoin-like profile, leading to the design of the phthalamide counterpart of ameltolide known as 4-amino-N-(2,6-dimethylphenyl) phthalamide (Vamecq *et al*., 2000).

Several works had been conducted on series of carboxamides (3-anilinopropanamides) and were reported to possess promising anticonvulsant activity; 3-anisidinopropanamides (Idris *et al*., 2008a), Chloro-3-anilinopropanamides (Idris *et al*., 2008b), 3-toluidinopropanamides (Idris *et al*., 2008c) and (N-benzyl-3-[(methoxyphenyl) amino] propanamides (Idris *et al*., 2009).

2.13 Michael Reaction

This is a nucleophilic addition reaction involving variety of reagents, in form of HZ, combined to activated alkene of type CH_2=CH-X to form Z-CH_2-CH_2-X. The prerequisite for this mode of addition to the alkenes is that X be an electron-attracting group such as aldehyde, ketone, carboxylic acid, ester, amide, nitrile, nitro or sulphonyl group. The expectation is that the carboxamide group in acrylamide would stimulate and direct the addition of nucleophilic reagents to the alkene. Apart from active methylene, HZ could include others moieties like acetophenone, certain hydrocarbon such as cyclopentadiene, and indenes that contain sufficiently reactive hydrogen atoms. Thus, the reaction of HZ with acrylamide would yields the β-propanamide derivative; Z- CH_2-CH_2 $CONH_2$ (Idris, 2008, unpublished Dissertation).

2.14 Pharmacophoric Units for Clinically Available Antiepileptic Agents Acting via Sodium Channels

According to Tasso *et al.*, 2001; the structural and electronic requirements imposed by the pharmacophore involve:

- A 3-atom portion characterized by a large polarization of the interatomic bonds (negative charge on atoms 1 and 3, positive charge on atom 2), bonded to a *sp*3 hybridized atom, that can be nitrogen (carbamazepine), carbon (sodium valproate, zonisamide, lamotrigine, phenytoin), or oxygen (topiramate, felbamate).

- A hydrophobic portion coordinated through atom 4 to the positive end of the polar group, which comprises at least three atoms, which can belong to aromatic (phenytoin, carbamazepine, zonisamide, lamotrigine) or aliphatic moieties (sodium valproate, felbamate, topiramate).

CHAPTER THREE

3.0 MATERIALS AND METHODOLOGY

3.1 Materials, Equipment, Chemicals and Animals

3.1.1 Materials and equipment

Electroconvulsive machine (Ugo Basile, model no. 7801), Evaporation disc, Filter paper, Beam walking apparatus- Flat ruler (80 × 3 cm) and Wooden beam rod (8 mm × 60 cm), Funnel, Metler balance (P162 Gallen Kamp, UK), Mortar and Pestle, Plastic animal cages, Spatula, Stop watch, Syringes (10, 5, 2 and 1 mls), Micropippette, Water bath (Gallenkamp), Weighing balance (Ohio, New York, USA), Glass wares (measuring cylinders, conical flasks, beakers, pipette, thermometer), Oven (BS model OV33 Gallenkamp), Microcapillary tubes (Drumen Scientific Company, USA), Thin Layer Chromatographic Plates (5 × 20 CM), Whatman filter paper, Melting Point Apparatus (Gallenkamp), Axopatch 200 amplifier (Molecular Devices, Sunnyvale, CA), pClamp 9 software and a Digidata 1322A (Molecular Devices), Microscope, Borosilicate glass pipettes, effendoff pipettes and tubes, Brown-Flaming puller (model P97; Sutter Instruments Company, Novato, CA), Incubator, Hood with air lamina flow and UV light

3.1.2 Chemicals

4-aminopyridine (Sigma Chemical Company, Louis Mo, USA), Diazepam (Roche Product Ltd. Welnyn Garden City), Pentylenetetrazole (Sigma Chemical Company, Louis Mo, USA), Strychnine (Sigma Chemical Company, Louis Mo, USA), Phenobarbitone (Sigma Chemical Company, Louis Mo, USA), Phenytoin sodium (Sigma Chemical Company, Louis Mo, USA), Fluphenamic acid (Sigma Chemical Company, Louis Mo,

USA), Nickel chloride (Sigma Chemical Company, Louis Mo, USA), Cyproheptadine (PT Kalbe Pharma, Bekasi, Indonesia), Sodium chloride (Fisher Scientific Co. USA), Sodium valproate (Epillim^{R}), Analytical grade of 2,3-, 2,5- and 3,4- dichloroanilines (BDH Chemicals Co.), Iodine crystals (BDH Chemicals Co.), Solvents – ethyl acetate, benzene, chloroform (BDH Chemicals Co.), Dulbecco's modified Eagle's medium/F-12 media (Invitrogen, Carlsbad, CA), 10% fetal bovine serum and Geneticin (Sigma Aldrich), Humam Embryonic Kidney Cells (HEK Cell 293) stably expressing voltage-gated sodium channels (Na_v 1.6), Dulbecco's modified Eagle's medium/F-12 media (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum and Geneticin (G418, 500 mg/ml (2.5 ml/500 ml media); Sigma Aldrich).

3.2 Synthesis and Chemical Analysis

3.2.1 Synthesis

All the protocols involved in the synthesis were carried out at the Research Laboratory in the Department of Pharmaceutical and Medicinal Chemistry, Ahmadu Bello University, Zaria.

The reactants were 2,3- dichloroaniline, 2,5- dichloroaniline, and 3,4- dichloroaniline. Each of these compounds was reacted with pure acrylamide (a 3-carbon atom compound containing an amide moeity). This is a Michael – type Addition reaction, as described by Idris *et al*., (2008).

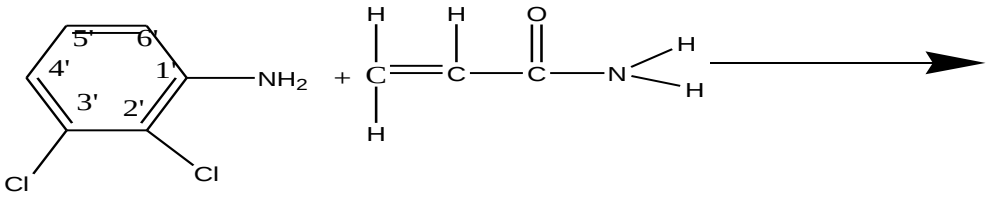
Progress of all the reactions was monitored by Thin Layer Chromatography (TLC) using ethylacetate as development solvent. The TLC was performed on Merck aluminium plate precoated with 0.2 mm silica gel. Iodine vapour (from iodine crystals) was used to locate the spot on the chromatograms to aid the clear appearance of the spot.

3.1.1.1 Procedure

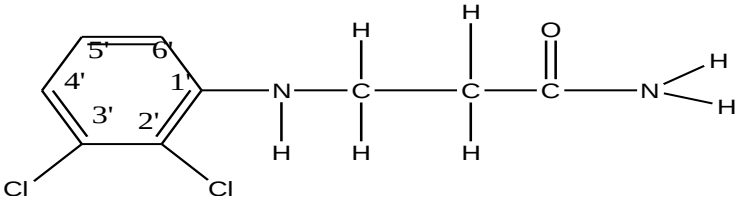
2,5- and 3,4- Substituted Isomers: A portion of each isomer (32.4 g, 0.2 mol) was mixed with pure acrylamide (14.2 g, 0.2 mol) in separate beakers of 250 ml capacity, and heated on a water bath (maintained at about 85^0C) for about 30 minutes until homogenous mixture was obtained. The mixture was left to stand at room temperature for 2 weeks. Benzene was used for recrystalization of each of the products. The residue was washed several times with benzene until a single spot on TLC was obtained for each.

2,3- Substituted Isomer: An equivalent volume based on its density was measured using pipette and mixed with 0.2 mol (14.2 g) of acrylamide in a 250 ml beaker. The flask containing the two reactants were heated on a water bath (maintained at about 85^0C) for about 25 minutes until homogenous solution was obtained. The mixture was left to stand at room temperature; on cooling, traces of crystals were beginning to form. This was left to stand for 3 weeks when the formed crystals had completely dissolved to form homogenous semi – solid. TLC profile of the product gave a single spot.

Scheme 1 (Figure 2**):**

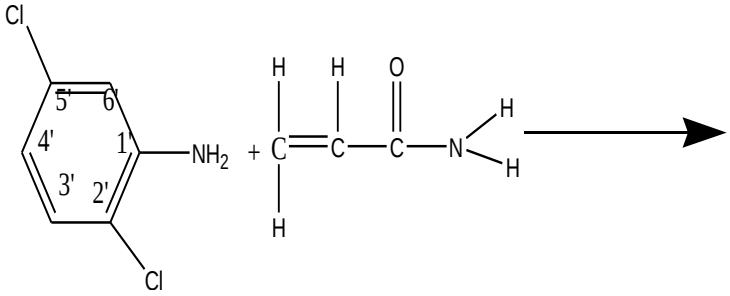


2,3-dichloroaniline acrylamide

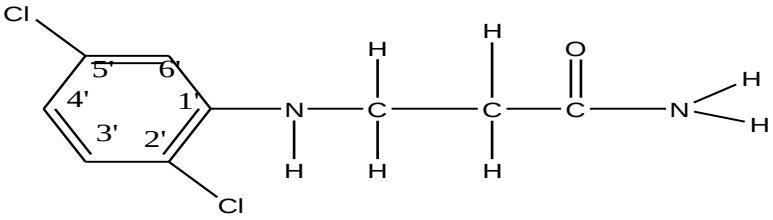


3-[(2,3-dichlorophenyl)amino]propanamide

Scheme 2 (Figure 3**):**

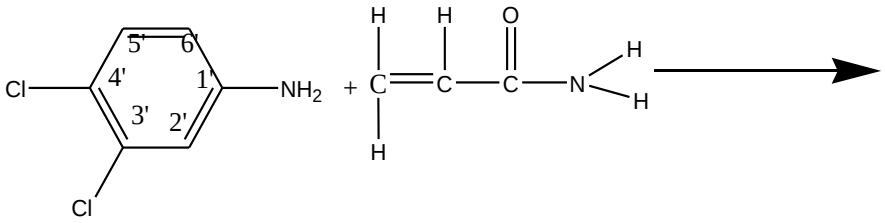


2,5-dichloroaniline acrylamide

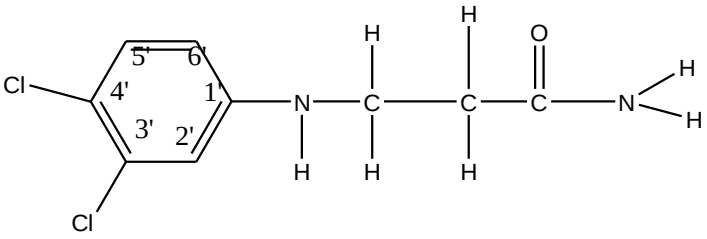


3-[(2,5-dichlorophenyl)amino]propanamide

Scheme 3 (Figure 4)**:**



3,4-dichloroaniline acrylamide



3-[(3,4-dichlorophenyl)amino]propanamide

3.2.2 Thin layer chromatography

Thin layer chromatographic (TLC) technique was used to monitor the purity of the product using ethylacetate as the development solvent. The chromatogram of each compound was developed and visualized with iodine vapour. The appearance of single spot on the chromatogram indicates the purity of the product.

3.2.3 Identification and characterization of the compounds

Percentage yield, form, appearance and melting point of each compound was determined and recorded. The melting point was determined using an electrothermal melting point apparatus (Model 2038 – England). Infrared (IR) spectroscopy was conducted at National Research Institute of Chemical Technology (NARICT), Basawa, Zaria, to find the type of functional groups in the compounds. This was achieved by recording the frequency of absorption (cm^{-1}) of each compound with the use of Parkin Elmer Paragon 1000 as KBr

disc. Nuclear Magnetic Resonance (NMR) was conducted to confirm the structure of the desired compounds. This was achieved through the analysis of proton and carbon-13 spectra. Data for both proton and carbon-13 NMR was reported as chemical shift in parts per million (ppm). It was conducted at the University of Kwazulu, South Africa.

3.3 Study Animals and Cell Lines

3.3.1 Animals

Rats and mice were employed in these studies. Adult rats (120- 180 g) and albino mice (18-24 g) of both sexes bred in the Animal House, Department of Pharmacology and Therapeutics, Ahmadu Bello Universty, Zaria, were used. They were maintained under normal environmental temperature with illumination of approximately 12 hours day and night cycle. They were fed with Growers mesh and water *ad libitum*, and maintained under good hygiene.

3.3.2 Cell lines

Humam Embryonic Kidney Cells (HEK Cell 293) stably expressing voltage-gated sodium channels (Na_v 1.6) were used. They were grown in Dulbecco's modified Eagle's medium/F-12 media (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum and Geneticin (G418, 500 mg/ml (2.5 ml/500 ml media); Sigma Aldrich). Also grown in a humidified atmosphere of 5% CO2 and 95% air at 37°C, in the Department of Anaesthesiology, University of Virginia, Charlottesville, Virginia, United States of America.

3.4 Preparation and Administration of Drugs

Freshly prepared solution of drugs and test compounds were used where possible for each experiment. Calculation of volumes to be administered was done based on the stock concentration of each prepared solution, doses required and the body weights of the experimental animals. The frequency and route of drug administration was followed as described for each specific experimental model. The test compounds were dissolved in 30% propylene glycol and 70% distilled water while standard drugs used for positive control were dissolved in distilled water.

3.5 Toxicity Study

3.5.1 Acute toxicity study

The method of Lorke (1983) was adopted using thirteen albino mice and rats; the determination involved two routes of administration (oral and intraperitoneal). The method was in two phases as follows: the first phase utilized three groups of three animals each, which were administered 10, 100 and 1000 mg/kg of the test compounds to ascertain the range of toxicity. The animals were observed for 24 hours for possible death and toxicity. Doses of the second phase were chosen based on the outcome of the first phase and administered to another four set of animals (one dose per animal) as described in the method. They were observed again for another 24 hours. From the outcome of this phase, LD_{50} value was determined by calculating the geometric mean of lowest dose that killed the animal and highest dose for which the animal survived.

3.5.2 Sub-chronic toxicity study in rats

Sub-chronic toxicity of 2,5- dichloro- (3- phenyl) propanamide (DCP25) was conducted in rats for 28 days via oral route as described by WHO (1992) and OECD (1995) guidelines. Briefly, Twenty four healthy rats of both sexes were grouped into four of 6 rats each and rats of different sexes were not caged together. Group 1 received 1 ml/kg of the vehicle (30% polyethylene glycol and 70% water) while groups 2, 3 and 4 received 12.5, 25 and 50 mg/kg of the compound respectively. The administration was daily for 28 days via oral route and the weights of the animals were taken at weekly interval. All animals were observed daily for signs of toxicity and mortality. At the 29^{th} day, animals were anaesthetized using chloroform; blood samples were collected via jugular vein into non-heparinized and EDTA containing plastic tubes for biochemical and haematological analyses respectively.

3.5.2.1 Blood analysis

Biochemical and Haematological analyses were performed at the Departments of Chemical Pathology and Haematology, Ahmadu Bello University Teaching Hospital (ABUTH), Shika, Zaria. Serum biochemical tests such as Urea and electrolyte levels (Marsh, 1965), liver function tests and lipid profile were determined (Sigma Diagnostic, 1985 and 1987). Similarly, packed cell volume, red blood cell count, white blood cell count and its differentials were determined using micro-haematocrit method, and blood smear stained with Giemsa (Schlam *et al*., 1975).

3.5.3 Determination of median toxic dose (TD_{50})

Graded doses of the test compounds were administered to groups of mice containing six mice per group such that two points were established between limits of 100% toxicity and 0% toxicity. The study was conducted according to method described by Stanley *et al*., (2005). The mice were trained to travel from a start platform along a ruler (80 cm long, 3 cm wide) elevated 30 cm above the bench by metal supports to a metal box. Trials were performed for each mouse, and were designed such that the mice to be tested would be aware that there was a box that could be reached. All the groups were treated with the test compounds (DCP23, DCP25 and DCP34). Thirty minutes post treatment, each mouse was placed at one end of wooden beam (8 mm in diameter, 60 cm long and elevated 30 cm above the bench by metal supports), and was allowed to walk to the box. The number of falls as an indicator of neurotoxicity for each group was counted and recorded. The percentage falls was found with its corresponding dose and converted into probit and log dose respectively. TD_{50} Value was determined using graphical method described by Miller and Tainter (1944).

3.6 Anticonvulsant Studies

3.6.1 Maximal electroshock-induced seizure in mice

The method of Swinyard and Kupferberg (1985) was adopted. Thirty five mice were divided into five groups of seven mice each. Group 1 served as control while groups 2, 3, and 4 received 50, 25 and 12.5 mg/kg (i.p.) of DCP23, DCP25 and DCP34, respectively, where as group 5 received phenytoin at the dose of 20 mg/kg (i.p.). One hour post treatment, maximal electroshock was delivered to each mouse to induce seizure using an

Ugobasile electro-convulsive machine (Model No. 7801) connected with corneal electrodes. The shock parameters were 50 (mA), 50 (Hz), 0.3 (s) and 0.4 (ms); the value for each parameter was predetermined through pilot study. Episode of tonic extension of the hind limb was regarded as full convulsion while lack of tonic extension of the hind limbs was considered as protection.

3.6.2 Pentylenetetrazole-induced seizure test

The method of Swinyard (1969) was adopted. Thirty adult albino mice weighing 18-24g were divided into five groups of six mice each. Group 1 served as control while mice in groups 2, 3, and 4 received 50, 25 and 12.5 mg/kg (i.p.) of DCP23, DCP25 and DCP34 respectively, where as mice in group 5 received sodium valproate at the dose of 200 mg/kg (i.p.). Thirty minutes later 90 m/kg (s.c.) of freshly prepared solution of pentylenetetrazole was administered to each mouse. The mice were observed for presence or absence of clonic seizures characterized by loss of righting reflex for at least 5 seconds.

3.6.3 4- aminopyridine-induced seizure test

The study was conducted according to method described by Yamaguchi and Rogawski (1992). Thirty adult albino mice were divided into five groups of six mice each. Group 1 served as control while mice in groups 2, 3, and 4 received 50, 25 and 12.5 mg/kg (i.p.) of DCP23, DCP25 and DCP34 respectively, where as group 5 received phenobarbitone at 30 mg/kg. Thirty minutes later, all the mice were administered 15 mg/kg s.c. 4-

aminopyridine and observed for presence or absence of tonic extension as well as onset of seizures.

3.6.4 Strychnine –induced seizure test

The method of Krall *et al*., (1978) was adopted. Thirty adult albino mice were divided into five groups of six mice each. Group 1 served as control while mice in groups 2, 3, and 4 received 50, 25 and 12.5 mg/kg (i.p.) of DCP23, DCP25 and DCP34 respectively; group 5 received phenobarbitone (30 mg/kg, i.p.). 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.

3.6.5 Picrotoxin-induced seizure test

The study was done according to method described by Swinyard *et al*., (1989). Thirty adult albino mice were divided into five groups of six mice each. Group 1 served as control while mice in groups 2, 3, and 4 received 50, 25 and 12.5 mg/kg (i.p.) of DCP23, DCP25 and DCP34 respectively; group 5 received diazepam at 10 mg/kg. All mice were administered 4 mg/kg (s.c) picrotoxin and observed for presence or absence of clonic convulsion as well as onset of seizures and protection against mortality.

3.6.6 Pentylenetetrazole-induced kindling test61

The method was as described by Gupta *et al*., (2001); Dhir *et al*., (2007) and Rajabzadeh *et al*., (2012). Pentylenetetrazole (PTZ) was administered at sub-convulsive dose (40 mg/kg, i.p.) on alternate days for a total period of 15 days. Twenty eight rats were divided

into four groups of seven rats each. Group 1 served as control while other groups (2, 3 and 4) received DCP23, DCP25 and DCP34 respectively; each at the dose of 50 mg/kg (i.p.). Thirty minutes post treatment; all the groups were administered the sub-convulsive dose of PTZ and were observed for a period of 20 minutes. Seizure activity was scored using a scoring system from 0-5 as follows:

Stage 0: no change in response

Stage 1: ear and facial twitching

Stage 2: convulsive twitching axially through the body

Stage 3: myoclonic jerks and rearing

Stage 4: turn over onto side position, wild running and wild jumping

Stage 5: generalized clonic-tonic seizures

3.6.7 Determination of median effective dose (ED_{50})

This was determined using two convulsant models; maximal electroshock- and subcutaneous picrotoxin- induced seizures, according to Swinyard and Kupferberg (1985); Swinyard *et al*., (1989). Groups of six mice each were selected, each group receiving graded doses (i.p.) of the test compound such that two points were established between the limits of 100% and 0% protection. The percentage protections obtained from each group were converted to probit values while the doses were converted to log dose values. The median effective dose (ED_{50}) was estimated using graphical method (Probit vs Log dose) as described by Miller and Tainter (1944).

3.6.8 Tolerance study using maximal electroshock test

This study was conducted as described by Stable and Kupferberg, (1997) with some modification. It involved administering the test compounds (DCP23 and DCP25) orally for five days to rats and then comparing the anticonvulsant activity between this group and a group that received single administration of the compound. These studies employed four groups of eight rats each. Two groups were treated for four days with vehicle alone; the next two groups received different doses of test compounds for four days. On the fifth day, each compound treated groups and one vehicle treated group received 50 mg/kg of DCP23, DCP25 and DCP34 orally. The compound's anticonvulsant activity for each group was determined 1 hour post treatment on the 5^{th} day. Activity in the chronically treated groups was compared to the acute treatment and the control group. The test model employed was maximal electroshock test as previously described in Swinyard and Kupferberg (1985).

3.7 Pharmacological Interaction Studies

3.7.1 Effect of Fluphenamic acid on anticonvulsant activity of DCP23, DCP25 and DCP34 in mice

The effect of fluphenamic acid, an inhibitor of sodium current (Hau-Jie *et al.*, 2010), on the outcome of anticonvulsant activity of DCP23, DCP25 and DCP34 was studied in MES-induced seizure test. Mice of either sex were divided into twelve groups of six mice per group. Group 1 served as control, while Groups 2, 3 and 4 received fluphenamic acid at graded doses of 20, 10 and 5 mg/kg via intraperitoneal route. One hour post fluphenamic acid administration these groups including the control, were electroshocked

via corneal electrodes clipped to both ears. The dose where least quantal protection was obtained was used for the interaction study. Thus, groups 6, 8 and 10 received fluphenamic acid at the dose of 5 mg/kg. Five minutes post fluphenamic acid administration, all the animals in groups 5 to 10 received 50 mg/kg of the test compounds at the dose of 50 mg/kg (i.p) and allowed for one hour. Thereafter, seizure was induced using electroshock machine as previously described in Swinyard and Kupferberg (1985).

3.7.2 Effect of nickel chloride on anticonvulsant activity of DCP23, DCP25 and DCP34 in mice

The effect of Nickel Chloride, specific T-type calcium channel blocker (Kang *et al*., 2006), was studied on the anticonvulsant activity of DCP23, DCP25 and DCP34 in PTZ-induced Seizure. Mice of either sex were divided into eight groups of six mice each. Group 1 served as control, while Groups 2, 4, 6 and 8 received nickel chloride at the dose of 5 mg/kg. Five minutes after nickel chloride administration, groups 3 and 4 received DCP23; groups 5 and 6 received DCP25 while groups 7 and 8 received DCP34, all at the dose of 25 mg/kg (i.p). 1 hour post treatment, all the mice from groups 1 to 8 were administered PTZ (90 mg/kg, sc); and were observed for presence or absence of seizures as previously described in Swinyard (1969).

3.7.3 Effect of cyproheptadine on anticonvulsant activity of DCP23, DCP25 and DCP34 in mice

The test compounds (DCP23, DCP25 and DCP34) were interacted with cyproheptadine (non selective serotonin and histamine receptor antagonist) to evaluate the possible

involvement of serotonergic/histaminergic pathways on their anticonvulsant effects. This was conducted using PTZ-induced seizures in mice. Mice of both sexes were divided into eight groups of six mice each. Group 1 served as control, while Groups 2, 4, 6 and 8 received cyproheptadine at the dose of 4 mg/kg (Yau, 2013). Fifteen minutes after cyproheptadine administration, groups 3 and 4 received DCP23 at 50 mg/kg; groups 5 and 6 received DCP25 at 50 mg/kg while groups 7 and 8 received DCP34 at 50 mg/kg, via intraperitoneal route. Thirty minutes post treatment all the mice from groups 1 to 8 were administered PTZ (90 mg/kg, s.c.); and were observed for presence or absence of threshold seizure as previously described by Swinyard (1969).

3.7.4 Diazepam-induced sleeping time in mice

Mice of either sex were divided into four groups of six mice each. Animals in group 1 received normal saline (10 ml/kg) and served as control, while those in Groups 2, 3 and 4 received DCP23 and DCP25 and DCP34 at 50, 25 and 12.5 mg/kg. Thirty minutes after treatment, all animals were administered diazepam (20 mg/kg, i.p.). Each mouse was observed for the onset and duration of sleep, with the criterion of sleep being loss of righting reflex (Wambebe, 1985; Amos *et al*., 2001). The time from the loss of righting reflex to recovery was recorded as sleeping time (Soulimani *et al*., 2001).

3.8 Actions of DCP23, DCP25 and DCP34 on Voltage-gated ($Na_v1.6$) Sodium Channels Stably Expressed in Human Embryonic Kidney (HEK Cells 293)

Human embryonic kidney cells (HEK Cells 293) stably expressing human Nav1.6 were grown in Dulbecco's modified Eagle's medium/F-12 media (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum and Geneticin (G418, 500 mg/ml (2.5 ml/500

ml media); Sigma Aldrich). Cells were grown in a humidified atmosphere of 5% CO2 and 95% air at 37°C. Sodium currents were recorded using the whole-cell configuration of the patch-clamp recording technique with an Axopatch 200 amplifier (Molecular Devices, Sunnyvale, CA). All voltage protocols were applied using pClamp 9 software (Molecular Devices) and a Digidata 1322A (Molecular Devices). Currents were amplified, low pass filtered (2 kHz), and sampled at 33 kHz. Borosilicate glass pipettes were pulled using a Brown-Flaming puller (model P97; Sutter Instruments Company, Novato, CA) and heat-polished to produce electrode resistances of 0.5 to 1.5 mΩ when filled with the following electrode solution: 130 mM CsCl, 1 mM MgCl2, 5 mM MgATP, 10 mM BAPTA, and 5 mM HEPES (pH adjusted to 7.4 with CsOH). Cells were plated on glass coverslips and superfused with solution containing the following composition: 130 mM NaCl, 4 mM KCl, 1 mM CaCl2, 5 mM MgCl2, 5 mM HEPES, and 5 mM glucose (pH adjusted to 7.4 with NaOH). All sodium channel current experiments were performed at room temperature (20–22°C). After establishing whole-cell configuration, a minimum series resistance compensation of 60% was applied, and cells were held at -60 mV or -100 mV for 5 minutes to account for any equilibrium gating shifts. After control recordings, compound solutions were applied for 5 min to allow for bath equilibration. Tonic block was assessed by comparing peak sodium current in drug-free conditions with peak current when drug was present. For the current – voltage experiment, the effect of the drug on the sodium currents was recorded during depolarization phase when the cells were not voltage clamped at either resting or inactivated states. Similarly the current reduction was assessed by comparing peak sodium current in drug-free conditions with peak current when drug was present.

CHAPTER FOUR

4.0 RESULTS

4.1 Purification, Identification and Characterization of the Test Compounds

DCP25 and DCP34 were in the crystal form while DCP23 was semi solid. The percentage yield of DCP23 was highest followed by DCP25 and then DCP34. Melting point ranges of DCP25 and DCP34 were 60-62^{0}C and 57-59^{0}C respectively (Table 4.1).

Table 4.1: Physicochemical Properties of DCP23, DCP25 and DCP34

Compound	Physical appearance	M. pt. (^{0}C)	% Yield
DCP23	Brown oily	Room temp	54.6
DCP25	Brown crystals	60 – 62	37.4
DCP34	Deep brown crystals	57 – 59	34.2

DCP23, DCP25 and DCP34 = 2,3-, 2,5- and 3,4- Dichloro – 3(aminophenyl) propanamides

Thin layer chromatographic profile using ethylacetate as solvent system confirmed the appearance of a single spot for each compound. The R_f values for DCP23, DCP25, and DCP34 were 0.64, 0.46 and 0.58, respectively (Table 4.2).

Table 4.2: Thin Layer Chromatographic Analysis of DCP23, DCP25 and DCP34 using Ethylacetate as Developing solvent

Compound	Solute movement (cm)	Solvent movement (cm)	R_f Value
DCP23	3.8	5.9	0.64
DCP25	2.4	5.2	0.46
DCP34	3.1	5.3	0.58

DCP23, DCP25 and DCP34 = 2,3-, 2,5- and 3,4- Dichloro – 3(aminophenyl) propanamides

The pharmacophore distance between hydrogen bond acceptor/donor groups (HAD), donor group (D) and aryl group (R) were 3.96, 4.36 and 6.49 A^0 respectively (Table 4.3).

Table 4.3: Pharmacophore Units of the Test Compounds and their Distances

Pharmacophores	Distance for the test set (Theoretical Distance) (A^0)
HAD – D	3.96 (3.9-5.5)
D – R	4.36 (3.2-5.1)
R – HAD	6.49 (4.2-8.5)

HAD = Hydrogen bond acceptor/donor group, D = Donor group, R = Aryl group

Infrared spectra revealed the presence of the expected functional groups within the theoretical frequency ranges (Table 4.4).

Table 4.4: Infrared Spectra Data for 2,3-, 2,5- and 3,4- Dichloro – 3(aminophenyl) propanamides

Compound	N-H-str	═C-Has	C═C	C═O Amide Band I	C-Nstr	C-Cl
DCP23	3362.04	3155.65	1430.26	1612.54	1276.92	721.4
DCP25	3354.32	3188.44	1428.34	1685.84	1291.39	672.21
DCP34	3390.97	3169.15	1439.91	1673.3	1291.39	682.82

DCP23, DCP25 and DCP34 = 2,3-, 2,5- and 3,4- Dichloro – 3(aminophenyl) propanamides

The chemical shift values for proton and carbon nuclear magnetic resonance (^{1}H and ^{13}C NMR) were assigned and presented in parts per million (ppm) and generally the values obtained from the spectra conformed to the theoretical values range (Tables 4.5, 4.6 and 4.7).

Table 4.5: H^1 and ^{13}C NMR Interpretation of the DCP23 Isomer

Position	δ_H	δ_C
1	-	168.08
2	6.15	113.70
3	6.28	117.37
1'	-	114.72
2'	-	133.00
3'	-	130.28
4'	6.75	127.58
5'	6.64	127.53
6'	6.94	119.39
-NH2	4.25	-
-NH	5.6	-

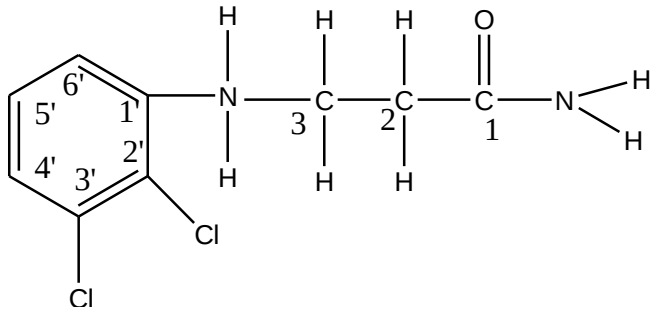


Table 4.6: H^1 and ^{13}C NMR Interpretation of the DCP25 Isomer

Position	δ_H	δ_C
1	-	167.82
2	6.09	115.35
3	6.16	118.73
1'	-	143.88
2'	-	133.08
3'	7.10	130.14
4'	7.09	130.19
5'	-	117.38
6'	7.28	127.65
-NH2	4.12	-
-NH	5.7	-

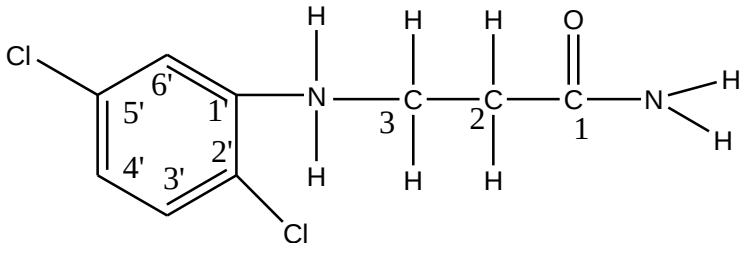
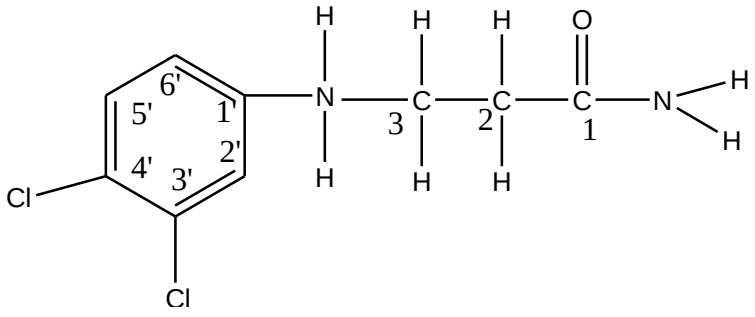


Table 4.7: H^1 and ^{13}C NMR Interpretation of the DCP34 Isomer

Position	δ_H	δ_C
1	-	167.76
2	6.13	114.61
3	6.27	116.38
1'	-	146.10
2'	6.74	130.16
3'	-	132.63
4'	-	130.69
5'	6.46	127.69
6'	7.16	120.97
-NH2	3.75	-
-NH	5.66	-



4.2 Toxicity Study

4.2.1 Acute Toxicity Study: Species differences and route of administration were the determinant of the median lethal dose for the test compounds. The values were 177.48 mg/kg (i.p) and 471 mg/kg (p.o.) in mice; 288.53 mg/kg (i.p.) and 471.17 mg/kg (p.o.) in rats. However, the value for oral DCP34 in rats was 774.59 mg/kg and was higher than the other values.

Table 4.8: LD_{50} Values of 2,3-, 2,5- and 3,4- Dichloro – 3(aminophenyl) Propanamides in Mice and Rats via Intraperitoneal and Oral Routes

Compound	Mice		Rats	
	i.p. (mg/kg)	p.o. (mg/kg)	i.p. (mg/kg)	p.o. (mg/kg)
DCP23	177.48	471.17	288.53	471.17
DCP25	177.48	471.17	288.53	471.17
DCP34	177.48	471.17	288.53	774.59

DCP23, DCP25 and DCP34 = 2,3-, 2,5- and 3,4- Dichloro – 3(aminophenyl) propanamides

4.2.2 Subchronic Toxicity Study in Rats

Effects of DCP25 on some biochemical and haematological indices were evaluated after 28 days daily oral administraton. The results of this study indicated that DCP25 did not cause significant toxic effects on most of the indices evaluated. There was a general increase in liver function tests (ALT, AST and alkaline phosphatase) indices but the increase was only significant ($p<0.05$) in the ALT at 50 mg/kg (Table 4.9). Similarly, at the highest dose, there was significant increase ($p<0.05$) in the level of creatinine and urea, indices for renal function test, but the level of electrolytes was found to be insignificant ($p<0.05$) (Table 4.10). There was no statistical significant ($p<0.05$) difference in lipid profile (Table 4.11) parameters (total cholesterol, total glyceride, LDL and HDL) as well as in haematological (Table 4.12) indices (RBCs, PCV and WBC indices). This was true for all doses (50, 25 and 12.5 mg/kg) tested as compared with the control group.

Table 4.9: Effect of 2,5- Dichloro – 3(aminophenyl) Propanamides on Liver Function Test after 28-Day Oral Admnistration in Rats

Parameters	**Treatment**			
		2,5- Dichloro – 3(aminophenyl) Propanamides		
	Control	12.5 mg/kg	25 mg/kg	50 mg/kg
Aspartate Aminotransferase	8.25 ± 0.75	7.50 ± 2.06	5.50 ± 0.86	13.25 ± 1.17*
Alanine Aminotransferase	6.75 ± 2.14	7.00 ± 1.91	6.25 ± 2.17	9.00 ± 1.58
Alkaline Phosphatase	58.25 ± 13.64	61.00 ± 8.50	60.25 ± 12.45	68.75 ± 10.62

Data presented as mean ± SEM, n = 4 rats per group, statistical significant difference ($p<0.05$), ANOVA test.

Table 4.10: Effect of 2,5- Dichloro – 3(aminophenyl) Propanamides on Renal Parameters after 28-Day Oral Admnistration in Rats

Parameters	**Treatment**			
(mMol/L)		2,5- Dichloro – 3(aminophenyl) Propanamides		
	Control	12.5 mg/kg	25 mg/kg	50 mg/kg
Urea	3.75 ± 0.56	3.25 ± 0.43	5.08 ± 0.60	6.73 ± 0.78*
Sodium	136.50 ± 0.50	140.00 ± 1.78	137.75 ± 3.25	137.25 ± 3.33
Potassium	3.80 ± 0.15	4.58 ± 0.18	5.15 ± 0.39	4.45 ± 0.49
Chloride	98.25 ± 0.85	104.75 ± 0.85	104.25 ± 2.39	101.25 ± 3.15
Bicarbonate	25.50 ± 0.87	22.75 ± 1.11	20.25 ± 2.50	24.25 ± 2.06
Creatinine	53.25 ± 7.94	51.25 ± 9.56	82.50 ± 18.71	88.75 ± 12.73*
Calcium	2.36 ± 0.06	2.43 ± 0.09	2.49 ± 0.13	2.41 ± 0.14
Phosphorus	1.10 ± 0.07	1.02 ± 0.03	1.33 ± 0.06	1.19 ± 0.12

Data presented as mean ± SEM, n = 4 rats per group, statistical significant difference at ($p<0.05$), ANOVA test.

Table 4.11: Effect of 2,5- Dichloro – 3(aminophenyl) Propanamides on Lipid Profile after 28-Day Oral Admnistration in Rats

Parameters (mMol/L)	**Treatment** 2,5- Dichloro – 3(aminophenyl) Propanamides			
	Control	12.5 mg/kg	25 mg/kg	50 mg/kg
Total Cholesterol	4.70 ± 0.53	3.93 ± 0.53	5.93 ± 1.38	4.55 ± 0.36
High Density Lipoprotein	1.55 ± 0.43	1.60 ± 0.29	1.93 ± 0.67	1.38 ± 0.25
Low Density Lipoprotein	2.28 ± 0.27	2.18 ± 0.61	3.20 ± 0.90	2.40 ± 0.43
Triglyceride	0.85 ± 0.13	1.17 ± 0.17	1.43 ± 0.35	1.05 ± 0.18

Data presented as mean ± SEM, n = 4 rats per group, no statistical significant difference using ANOVA test.

Table 4.12: Effect of 2,5- Dichloro – 3(aminophenyl) Propanamides on Haematological Parameters after 28-Day Oral Admnistration in Rats

Parameters (mMol/L)	Units	Treatment			
			2,5- Dichloro – 3(aminophenyl) Propanamides		
		Control	12.5 mg/kg	25 mg/kg	50 mg/kg
Haemaglobin	g/dL	12.18 ± 0.62	12.95 ± 0.33	12.93 ± 0.32	13.15 ± 0.53
PCV	%	36.50 ± 2.10	38.75 ± 0.75	38.50 ± 0.65	38.50 ± 1.55
RBC	$\times 10^{12}$/L	4.13 ± 0.24	4.30 ± 0.09	4.23 ± 0.09	4.35 ± 0.19
Platelets	$\times 10^{9}$/L	440.00 ± 38.13	463.50 ± 88.75	339.00 ± 78.54	350.25 ± 89.42
WBC	$\times 10^{9}$/L	15.30 ± 3.40	16.55 ± 2.72	15.15 ± 4.30	15.85 ± 4.34
Neutrophils	%	35.00 ± 12.07	33.50 ± 1.71	29.50 ± 3.30	35.75 ± 2.71
Lymphocytes	%	61.00 ± 12.66	62.75 ± 3.04	67.50 ± 4.11	64.50 ± 4.73
Monocytes	%	2.75 ± 1.10	1.75 ± 0.25	3.25 ± 0.94	2.75 ± 0.85

Data presented as mean ± SEM, n = 4 rats per group, no statistical significant difference using ANOVA test.

4.3 Anticonvulsant Studies

4.3.1 Effect of DCP23, DCP25 and DCP34 on Maximal Electroshock-induced Seizure Test in Mice

The compounds, DCP23, DCP25 and DCP34, demonstrated good anticonvulsant activity against maximal electroshock-induced seizure as determined in mice. They showed dose dependent protection against tonic hind limb extension (THLE); the percentage protections against seizure exhibited by DCP23, DCP25 and DCP34, at 50 mg/kg, were 71.4%, 57.2% and 42.9% respectively. Where as at 25 mg/kg their percentage protections were 42.9%, 28.5% and 14.3% respectively, while the lowest dose (12.5 mg/kg) offered minimal / no protection. Thus, DCP23 provided the greatest protection while DCP34 offered the lowest protection. Also, phenytoin, the standard drug, protected all the animals against THLE at the dose of 20 mg/kg. Although, there was no statistically significant difference in the recovery from seizures between the control and the compound treated groups, no mortality was observed in all the experimental groups (Table 4.13).

Table 4.13: Effect of DCP23, DCP25 and DCP34 and Phenytoin on Maximal Electroshock-induced Seizures in Mice

Treatment (mg/kg)	Recovery from Seizures (min)	Quantal Protection	Protection against Seizures (%)	Protection from mortality (%)
Control	6.75 ± 0.31	0/7	0.0	100
DCP23 (50)	8.13 ± 1.14	5/7	71.4	100
DCP25 (50)	6.88 ± 1.06	4/7	57.2	100
DCP34 (50)	8.63 ± 0.65	3/7	42.9	100
DCP23 (25)	8.75 ± 0.84	3/7	42.9	100
DCP25 (25)	7.00 ± 0.42	2/7	28.5	100
DCP34 (25)	7.88 ± 0.35	1/7	14.3	100
DCP23 (12.5)	7.25± 0.53	1/7	14.3	100
DCP25 (12.5)	7.13 ± 0.90	0/7	0.0	100
DCP34 (12.5)	7.25± 0.45	0/7	0.0	100
PHT (20)	-	7/7	100.0	100

Values are presented as Mean ± SEM, n = 7; DCP23, DCP25 and DCP34 = 2,3-, 2,5- and 3,4- Dichloro – 3(aminophenyl) propanamides respectively, PHT = Phenytoin; Control = 30% propylene glycol, 70% distil water; No Significant difference in the mean time to recovery from seizures between control (vehicle) group and treated groups (ANOVA).

4.3.2 Pentylenetetrazole-induced Seizure Test in Mice

The highest dose (50 mg/kg) of DCP23, DCP25 and DCP34 as used in pentylenetetrazole-induced seizure test, offered 66.7%, 66.7 and 0% protections against clonic seizures. Also, DCP23, at doses of 25 and 12.5 mg/kg, gave 16.7% protection; while DCP25 and DCP34 at both 25 and 12.5 mg/kg, did not offer protection. There was a statistically significant ($p<0.001$) difference exhibited by DCP23 at doses of 50 mg/kg and 25 mg/kg ($p<0.001$). Similarly, sodium valproate (200 mg/kg) gave 83.3% protection against episode of clonic seizure. However, there was no statistically significant difference in latency to onset of seizures for DCP25 and DCP34 at all the tested doses. There was however a reduction in mortality at the most active doses (Table 4.14).

Table 4.14: Effect of DCP23, DCP25 and DCP34 and Valproate on Pentylenetetrazole-induced Seizures in Mice

Treatment (mg/kg)	Mean Latency of Seizures (min)	Quantal Protection	(%) Protection against Seizures	Protection against Mortality (%)
Control	8.00 ± 1.29	0/6	0.0	50.0
DCP23 (50)	27.00 ± 1.00**	4/6	66.7	100.0
DCP25 (50)	12.00 ± 1.00	4/6	66.7	100.0
DCP34 (50)	6.83 ± 1.35	0/6	0.0	16.7
DCP23 (25)	19.4 ± 2.18*	1/6	16.7	100.0
DCP25 (25)	11.67 ± 1.98	0/6	0.0	50.0
DCP34 (25)	8.67 ± 2.83	0/6	0.0	16.7
DCP23 (12.5)	8.6 ± 1.29	1/6	16.7	0.0
DCP25 (12.5)	6.83 ± 1.30	0/6	0.0	33.3
DCP34 (12.5)	9.17± 1.51	0/6	0.0	50.0
VA (200)	13.00 ± 00	5/6	83.3	100.0

Values are presented as Mean ± SEM, n = 6; DCP23, DCP25 and DCP34 = 2,3-, 2,5- and 3,4- Dichloro – 3(aminophenyl) propanamides respectively, VA = Valproate; Control = 30% propylene glycol, 70% distil water; Significant difference in the mean latency to onset of seizures between control (vehicle) groupand treated groups at *$p<0.001$ and **$p<0.000$ (ANOVA) followed by Post hoc (Scheffe) for multiple comparism.

4.3.3 4-Aminopyridine-induced Seizure in Mice

4-aminopyridine is a chemoconvulsant agent known to block potassium channels. In 4-aminopyridine-induced seizure test, there was no protection offered by all the tested compounds against characteristic episode of tonic hind limb extension. But a statistically significant ($p<0.05$) difference was exhibited by DCP34 at the doses of 50 mg/kg and 25 mg/kg when compared with the control. Phenobarbitone offered 66.7% protection with a significant ($p<0.05$) difference in the mean latency to onset of seizure, as compared with the control. There was no protection against mortality in the control and compound treated groups; while 100% protection in mortality was recorded in phenobarbitone treated group (Table 4.15).

Table 4.15: Effect of DCP23, DCP25 and DCP34 and Phenobarbitone on 4-aminopyridine-induced Seizures in Mice

Treatment (mg/kg)	Mean latency of Seizures (min)	Quantal Protection	(%) Protection against Seizures	Protection against Mortality (%)
Vehicle	10.00 ± 1.03	0/6	0.0	0.0
DCP23 (50)	12.67 ± 1.53	0/6	0.0	0.0
DCP25 (50)	17.00 ± 2.51	0/6	0.0	0.0
DCP34 (50)	17.17± 1.82*	0/6	0.0	0.0
DCP23 (25)	11.33 ± 0.76	0/6	0.0	0.0
DCP25 (25)	13.17 ± 0.87	0/6	0.0	0.0
DCP34 (25)	16.67 ± 1.23*	0/6	0.0	0.0
DCP23 (12.5)	12.0± 1.29	0/6	0.0	0.0
DCP25 (12.5)	14.0± 1.26	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

Values are presented as Mean ± SEM, n = 6 per group; DCP23, DCP25 and DCP34 = 2,3-, 2,5- and 3,4- Dichloro – 3(aminophenyl) propanamides respectively, PHB = Phenobarbitone; Control = 30% propylene glycol, 70% distil water; Significant difference in the mean onset of seizures between control (vehicle) group and treated groups at *$p<0.05$ (ANOVA) followed by Post hoc (Scheffe) for multiple comparism.

4.3.4 Strychnine-induced Seizure Test in Mice

Strychnine is a competitive antagonist of the inhibitory amino acid glycine in the CNS and hence, induces convulsion which usually results to death. The compounds (DCP23, DCP25 and DCP34) neither offered protection against characteristic episode of tonic seizure nor against the mortality in strychnine-induced seizure test. Similarly, there was not statistically significant difference in mean onset of seizure in all the treated groups as compared with the control group. However, a significant ($p<0.05$) difference was recorded in the phenobarbitone (30 mg/kg) treated group when compared to the control with 66.7% protection in mortality (Table 4.16).

Table 4.16: Effect of DCP23, DCP25 and DCP34 and Phenobarbital 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
DCP25 (50)	8.33 ± 0.84	0/6	0.0	0.0
DCP34 (50)	8.67 ± 1.05	0/6	0.0	0.0
DCP23 (25)	9.33 ± 0.30	0/6	0.0	0.0
DCP25 (25)	8.00 ± 0.77	0/6	0.0	0.0
DCP34 (25)	6.33 ± 0.76	0/6	0.0	0.0
DCP23 (12.5)	6.83 ± 0.31	0/6	0.0	0.0
DCP25 (12.5)	7.17 ± 0.54	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

Values are presented as Mean ± SEM, n = 6; DCP23, DCP25 and DCP34 = 2,3-, 2,5- and 3,4- Dichloro – 3(aminophenyl) propanamides respectively, PHB = Phenobabitone; Control = 30% propylene glycol, 70% distil water; Significant difference in the mean onset of seizures between control (vehicle) group and treated groups at *$p<0.05$ (ANOVA) followed by Post hoc (Scheffe) for multiple comparism.

4.3.5 Picrotoxin-induced seizure in mice

Picrotoxin is a non-competitive GABA antagonist that blocks the chloride channel. In Picrotoxin-induced seizure test there was good protection offered by DCP23 and DCP25 against clonic convulsion and mortality. The percentage protections recorded at the dose of 50 mg/kg for DCP23 and DCP25 were 66.7% and 83.3%, respectively, while at 25 mg/kg the protections were 50.0% (DCP23) and 66.7% (DCP25). Where as, protection against mortality at 50 mg/kg was 100% for both DCP23 and DCP25; while at 25 mg/kg the protections were 50% and 66.7% for DCP23 and DCP25 respectively. However, the compounds did offer protection against convulsion and mortality at 12.5 mg/kg. Diazepam at the dose of 10 mg/kg gave 100% protection against convulsion. Also, DCP25 at doses of 50 mg/kg and 25 mg/kg showed statistically significant ($p<0.005$) difference as compared to the control. Similarly, DCP23 showed statistical significant ($p<0.05$) difference at 50 mg/kg only (Table 4.17).

Table 4.17: Effect of DCP23, DCP25 and DCP34 and Diazepam 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
DCP25 (50)	21.00 ± 00**	5/6	83.3	100.0
DCP23 (25)	17.00 ± 0.58	3/6	50.0	50.0
DCP25 (25)	24.50 ± 0.50**	4/6	66.7	66.7
DCP23 (12.5)	17.12 ± 0.58	0/6	0.0	0.0
DCP25 (12.5)	15.83 ± 1.51	0/6	0.0	0.0
DZ (10)	0.0	6/6	100.0	100.0

Values are presented as Mean ± SEM, n = 6 per group; DCP23, DCP25 and DCP34 = 2,3-, 2,5- and 3,4- Dichloro – 3(aminophenyl) propanamides respectively, DZ = Diazepam; Control = 30% propylene glycol, 70% distil water; Significant difference in the mean onset of seizures between control (vehicle) group and treated groups at *$p<0.05$ and *$p<0.005$ (ANOVA) followed by Post hoc (Scheffe) for multiple comparism.

4.3.6 Pentylenetetrazole-induced Kindling in Mice

DCP23, DCP25 and DCP34 (50 mg/kg) retarded the severity of seizure episodes induced by sub convulsive dose (40 mg/kg) of pentylenetetrazole which was administered at alternate day for 15 days. The retardation as recorded by seizure scoring mode (0 to 5), was statistically significant ($p<0.05$) throughout the treatment days for all the compounds. There was graded increase in the severity of seizure from Day 1 to Day 5 where it reached plateau, there after the severity dropped at Day 6 and was maintained through Day 7 up to the last treatment day (Figure 5).

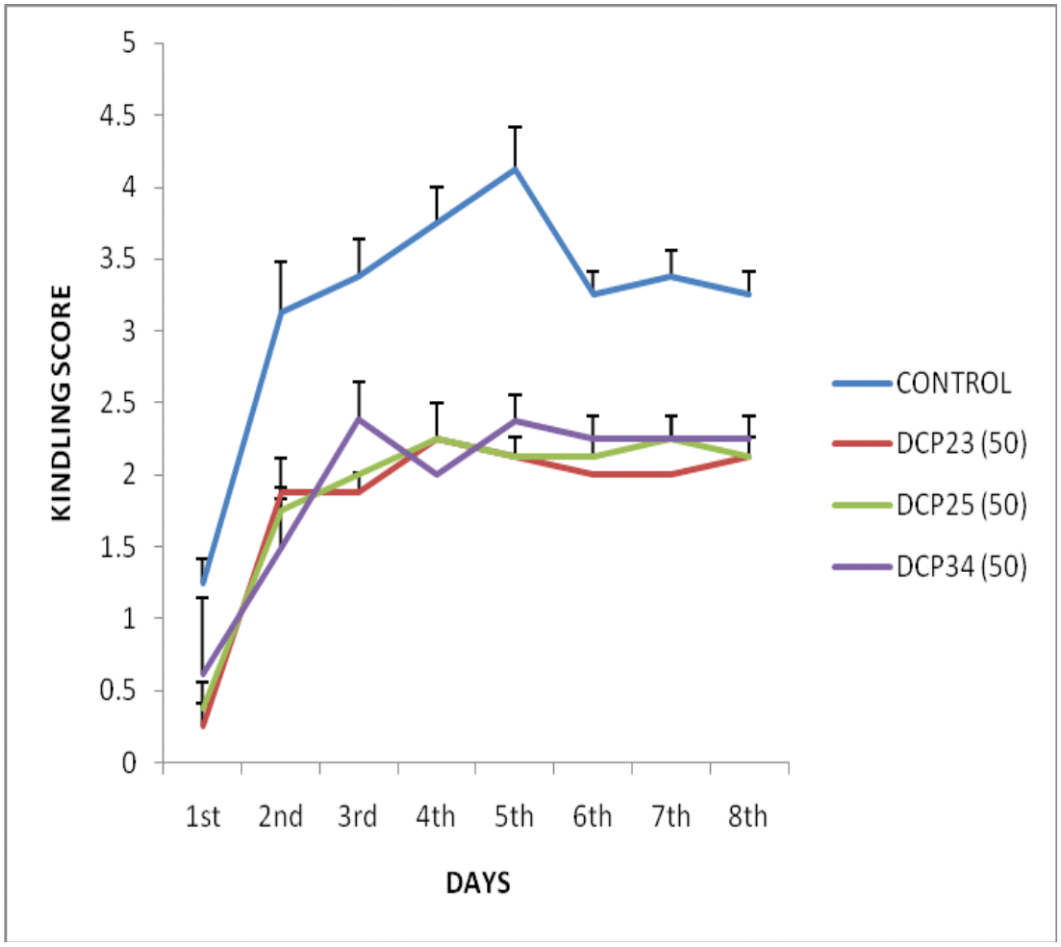


Figure 5: Effect of DCP23, DCP25 and DCP34 against Pentylenetetrazole-induced Kindling in Mice
n = 7 rats per group

4.3.7 Determination of median effective dose (ED_{50})

The medial effective doses (ED_{50}) for DCP23, DCP25 and DCP34 using MEST were 25.12 mg/kg, 39.81 mg/kg and 44.67 mg/kg, respectively, while that of picrotoxin were 35.48 mg/kg (DCP23) and 28.18 mg/kg (DCP25). Whereas, their medial toxic doses as determined by walking beam test for motor coordination deficit, were 100.0 mg/kg, 100.0 mg/kg and 104.7 mg/kg, respectively. Accordingly, their protective index values (MEST) were 3.98, 2.51 and 2.33, respectively; while that of picrotoxin test were 2.82 (DCP23) and 3.55 (DCP25) (Table 4.18).

Table 4.18: Protective Index Values of DCP23, DCP25 and DCP34 in Mice

Compound	$^{M}ED_{50}$ (mg/kg)	$^{P}ED_{50}$ (mg/kg)	TD_{50} (mg/kg)	PI (MEST)	PI (Picrotoxin)
DCP23	25.12	35.48	100.0	3.98	2.82
DCP25	39.81	28.18	100.0	2.51	3.55
DCP34	44.67	ND	104.7	2.33	ND
Phenytoin	12.2	ND	I52.0	9.46	ND

2,3 DCP, 2,5 DCP and 3,4 DCP = 2,3-, 2,5- and 3,4- Dichloro – 3(aminophenyl) propanamides respectively, $^{M}ED_{50}$ = Median effective dose for maximal electroshock model, $^{P}ED_{50}$ = Median effective dose for picrotoxin model, TD_{50} = Median toxic dose, PI (MEST) = Protective index for maximal electroshock test, PI (Picrotoxin) = Protective index for picrotoxin, n = 6 mice per group, ND = Not determined.

4.3.8 Tolerance study using maxmal electroshock test

The three compounds (DCP23, DCP25 and DCP34) administered via oral route, at the dose of 100 mg/kg, offered protection against Tonic Hind Limb Extension according to the frequency of their administration. For the single administration the percentage protection were 37.5%, 50% and 0.0% respectively. While that of daily administration for 5 days offered higher protections of 50%, 75% and 25%, respectively (Table 4.19).

Table 4.19: 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

4.4 Pharmacological Interaction

4.4.1 Effect of fluphenamic acid on anticonvulsant activity of DCP23, DCP25 and DCP34 in mice

Fluphenamic acid modulates neuronal sodium channels there by reducing sodium current, thus, prevent seizure generation. Fluphenamic acid treated groups (20 mg/kg, 10 mg/kg and 5 mg/kg) gave 100%, 100% and 0% against Tonic Hind Limb Extension (THLE). When the group that was treated with fluphenamic acid at the dose of 5 mg/kg (0% protection) was co-administered with DCP23 (50 mg/kg), DCP25 (50 mg/kg) and DCP34 (50 mg/kg), the protection against THLE was 100% for DCP23 and DCP25, while that of DCP34 was 50%. The groups treated with DCP23, DCP25 and DCP34 gave 66.7%, 50% and 16.7% respectively. Also, when phenytoin (10 mg/kg) was coadministered with fluphenamic acid (5 mg/kg) there was an enhanced activity than when the two at 5 mg/kg each (Table 4.20).

Table 4.20: Effect of Fluphenamic Acid on Anticonvulsant Activity of DCP23, DCP25 and DCP34 in Maximal Electroshock-induced Seizure in Mice

Treatment (mg/kg)	Quantal Protection	% Protection Against Seizure	% Mortality Protection
Control	0/6	0.0	100.0
FA (20)	6/6	100.0	100.0
FA (10)	6/6	100.0	100.0
FA (5)	0/6	0.0	100.0
DCP23 (50)	4/6	66.67	100.0
FA (5) + DCP23 (50)	6/6	100.0	100.0
DCP25 (50)	3/6	50.0	100.0
FA (5) + DCP25 (50)	6/6	100.0	100.0
DCP34 (50)	1/6	16.67	100.0
FA (5) + DCP34 (50)	3/6	50.0	100.0
FA (5) + PHT (5)	0/6	0.0	100.0
FA (5) + PHT (10)	6/6	100.0	100.0

DCP23, DCP25 and DCP34 = 2,3-, 2,5- and 3,4- Dichloro – 3(aminophenyl) propanamides respectively, FA = Fluphenamic acid, n = 6 per group.

4.4.2 Effect of Nickel Chloride on DCP23 and DCP25 against Pentylenetetrazole-induced Seizure in Mice

Nickel chloride is a selective blocker for low voltage-activated T-type Ca2+ channels. Nickel chloride at the dose of 5 mg/kg offered 16.7% and 83.3% protection against clonic convulsions and death (mortality) respectively. When DCP23 (25 mg/kg) and DCP25 (25 mg/kg) were interacted with same dose of nickel chloride the percentage protections against seizure and mortality for DCP23 were 66.67% and 83.33 respectively; while that of DCP25 were 33.33% and 66.67% respectively. Similarly the co-administration of the two compounds led to statistically significant difference ($p<0.05$) in the mean onset of seizure when compared to the control group; using ANOVA Test followed by Post hoc Test (Scheffe). Also, DCP23 and DCP25 offered accordance protection against convulsion and mortality of 33.3% and 50%, 33.3 and 66.7% each, respectively. Same dose of nickel chloride potentiated the effect of sodium valproate (100 mg/kg) from 33.3% to 83.3% (Table 4.21).

Table 4.21: 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
VA (100)	15.82 ± 2.37*	33.33	100.0
VA (100) + NKL (5)	14.00 ± 0.0	83.33	100.0

Values are presented as Mean ± SEM, n = 6 per group; DCP23, DCP25 and DCP34 = 2,3-, 2,5- and 3,4- Dichloro – 3(aminophenyl) propanamides respectively, DZ = Diazepam; Control = 30% propylene glycol, 70% distil water; Significant difference in the mean onset of seizures between control (saline) group at *$p<0.05$ using ANOVA.

4.4.3 Effect of Cyproheptadine on DCP23, DCP25 and DCP34 against Pentylenetetrazole-induced Seizure in Mice

Cyproheptadine induces seizure by blocking both serotonin (5HT1 and 5HT2) and histamine (H1). Cyproheptadine at the dose of 4 mg/kg did not enhance or reduce the protection ability of both DCP23 (50 mg/kg) and DCP25 (50 mg/kg), when compared to the groups treated with the compounds alone and that treated with cyproheptadine and the compounds; as each gave the same percentage protection (50%). However, DCP34 offered 16.67% protection which increased to 33.3% when coadministered with cyproheptadine. All the treated groups except cyproheptadine treated, showed statistically significant difference ($p<0.05$) in the mean onset of seizure when compared to the control group using ANOVA followed by Post hoc Test (Scheffe) for multiple comparism (Table 4.22).

Table 4.22: Effect of Cyproheptadine on DCP23, DCP25 and DCP34 against Pentylenetetrazole-induced Seizure in Mice

Treatment (mg/kg)	Mean Onset of Seizure (Minutes)	% Protection	Mortality Protection (%)
Control	5.67 ± 0.98	0.00	16.67
CYPRO (4)	6.17 ± 1.11	0.00	83.33
DCP23 (50)	9.67 ± 1.86*	50.00	50.00
CYPRO (6) + DCP23 (50)	10.00 ± 1.00*	50.00	33.33
DCP25 (50)	13.67 ± 2.40*	50.00	16.67
CYPRO (6) + DCP25 (50)	15.00 ± 1.00*	50.00	50.00
DCP34 (50)	13.80 ± 1.59*	16.67	66.67
CYPRO (6) + DCP34 (50)	18.25 ± 2.55*	33.33	66.67

Values are presented as Mean ± SEM, n = 6 per group; DCP23, DCP25 and DCP34 = 2,3-, 2,5- and 3,4- Dichloro – 3(aminophenyl) propanamides respectively, CYPRO = cyproheptadine, DZ = Diazepam; Vehicle = 30% propylene glycol, 70% distil water; Significant difference in the mean onset of seizures between control (saline) group at *$p<0.05$ using Post hoc (Scheffe) for multiple comparism after ANOVA.

4.4.4 Effects of DCP23 and DCP25 on Diazepam-induced Sleep in Mice

DCP23 (50 mg/kg) and DCP25 (50 mg/kg), produced statistical significant decrease in the onset of sleep (($p<0.001$) as well as significant increase in the duration of sleep ($p<0.05$); using ANOVA followed by Post hoc test. Thus, the compounds potentiated sleep induced by diazepam at the highest dose tested. There was no statistically significant difference in either of the two indices at the middle and lowest doses when compared with the control group (Figure 6 and 7).

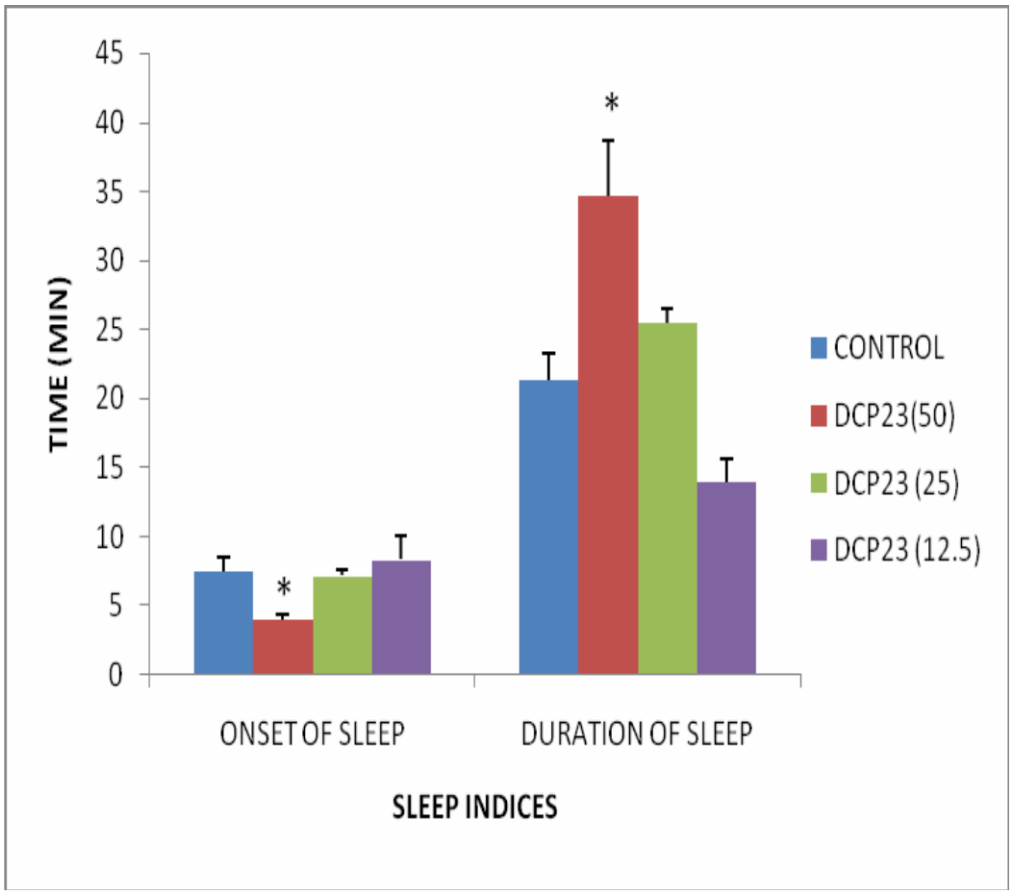


Figure 6: Effect of DCP23 on Diazepam-induced Sleep in Mice

DCP23 = 2,3- Dichloro – 3(aminophenyl) propanamide, n= 6 mice per group, significant difference in the mean onset and duration of sleep at *$p<0.05$ using ANOVA followed by Post hoc (Scheffe) for multiple comparism.

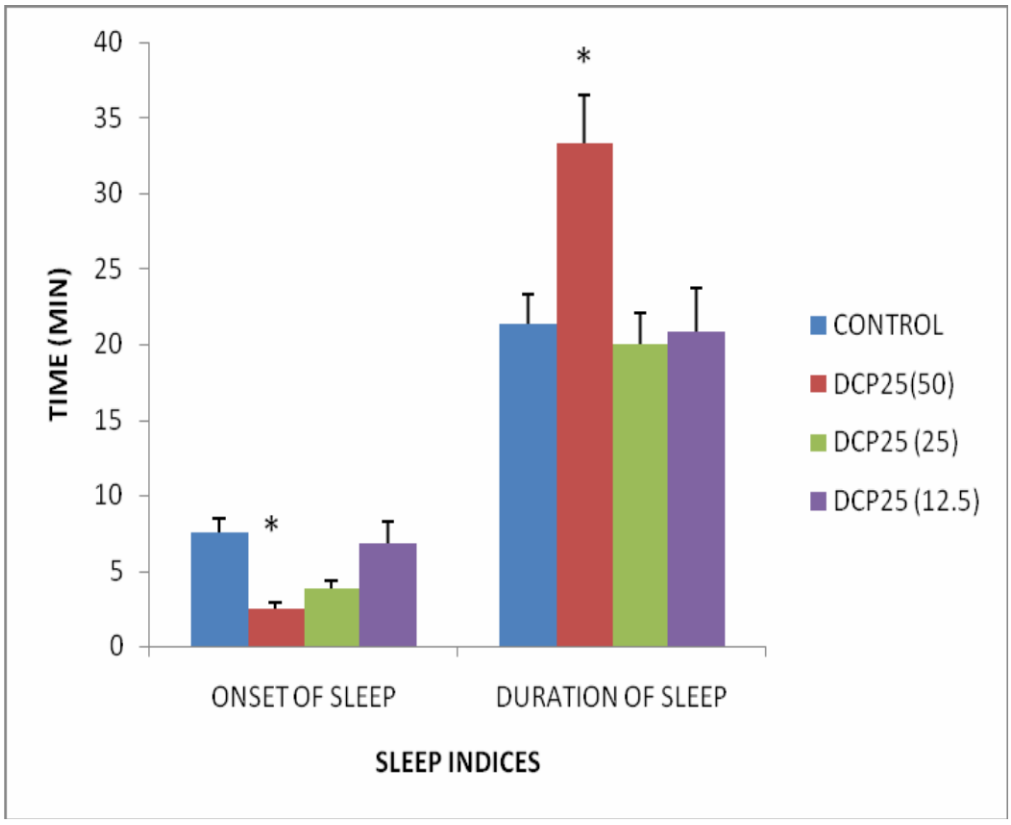


Figure 7: Effect of DCP25 on Diazepam-induced Sleep in Mice

DCP25 and DCP34 = 2,5- Dichloro – 3(aminophenyl) propanamide, n= 6 mice per group, significant difference in the mean onset and duration of sleep at *p<0.05 using ANOVA followed by Post hoc (Scheffe) for multiple comparism.

4.5 Actions of DCP23, DCP25 and DCP34 on Voltage-Gated Sodium Channels (Na_V 1.6)

4.5.1 States Dependent Actions

The compounds (DCP23, DCP25 and DCP34) as tested on voltage-gated sodium channels (Na_V 1.6), showed graded degree of channel blockade. They reduced the sodium currents which indicated an action at sodium channels. DCP23 was found to have the highest potency when tested at the resting state (-60 mV) of the sodium channels followed by DCP25 and DCP34 (least potent). DCP23 at holding potential of -60 mV, caused concentration-dependent tonic blockade of 9.73%, 18.04%, 46.80%, 68.46%, 95.64 and 98.10%; for 10µM, 30µM, 60µM, 100µM, 300µM and 600µM respectively (Figure 8). Whereas at the same holding potential, DCP25 at 100µM and 600µM blocked the current by 21.63% and 83.03% respectively (Figure 9); while DCP34 at similar concentrations blocked the current minimally by 3.8% and 16.9% respectively (Figure 10). DCP23 was selected and tested in another state of the sodium channels (holding potential of -100 mV) using same graded concentrations, it blocked the sodium currents by 0%, 10%, 28.93%, 50.12%, 88.51% and 90.10% (Figure 11). The IC_{50} values of DCP23 were determined at both resting/closed and inactivated/opened states of the sodium channels (Nav 1.6). Dose response curves (Figure 12) for each state of the channel were automatically plotted (fractional block vs concentration) as provided by Boltzsman equation. The values for resting and inactivated states were found to be 64.76 µM and 100.37 µM respectively.

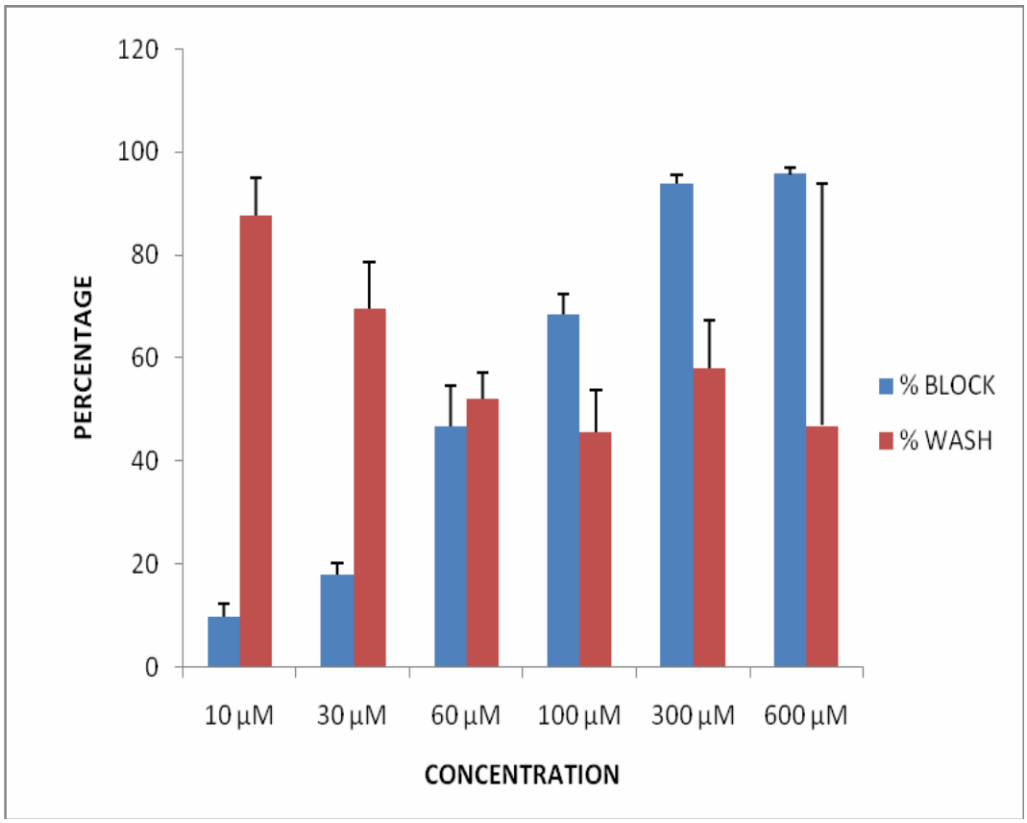


Figure 8: Tonic Current Blockade by DCP23 on Nav 1.6 at Resting Membrane Potential (-60 mV)
n= 5 cells per each concentration

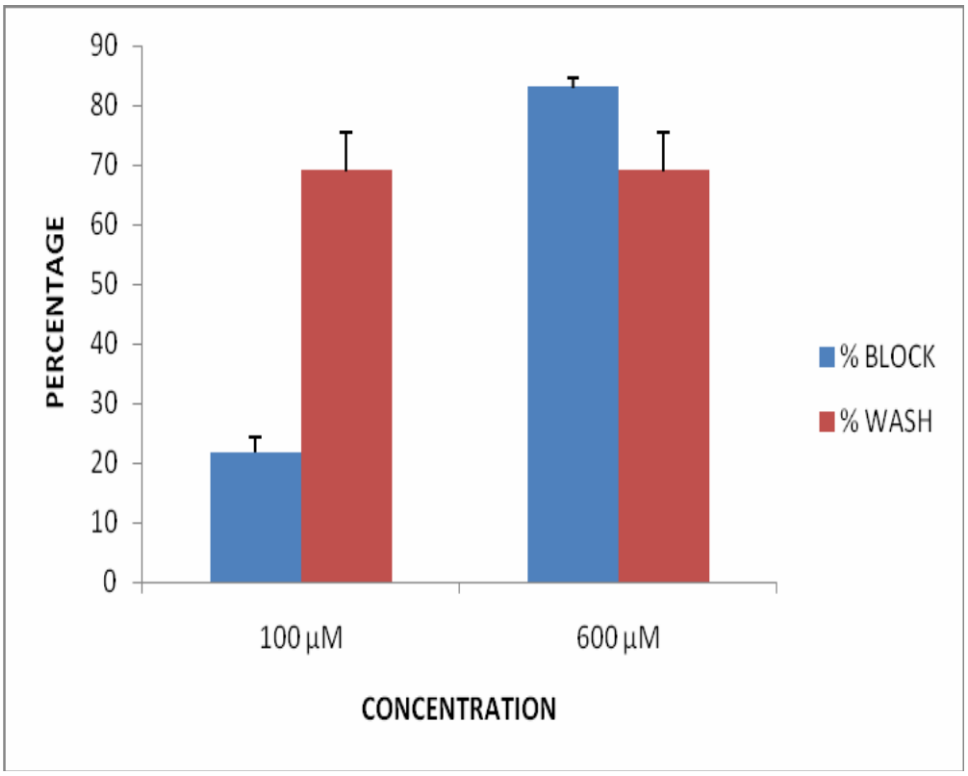


Figure 9: Tonic Current Blockade by DCP25 on Nav1.6 at Resting Membrane Potential (-60 Mv)
n= 4 cells per each concentration

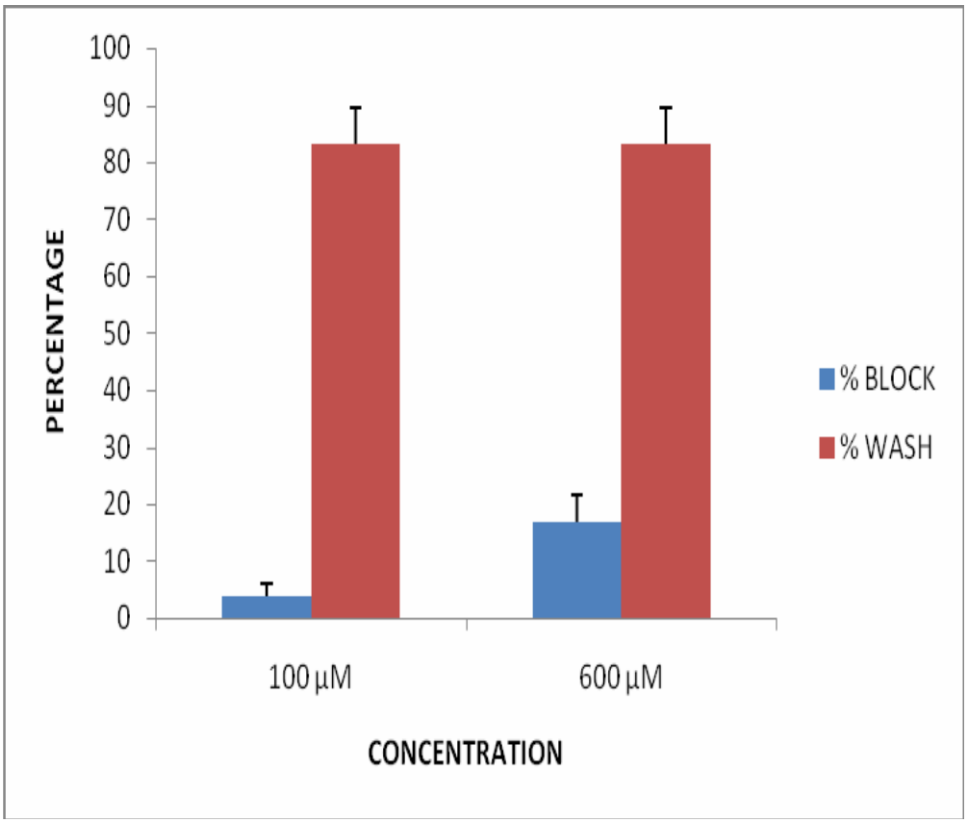


Figure 10: Tonic Current Blockade by DCP34 on $Na_v1.6$ at Resting Membrane Potential (-60 mV)
n = 4 cells per each concentration

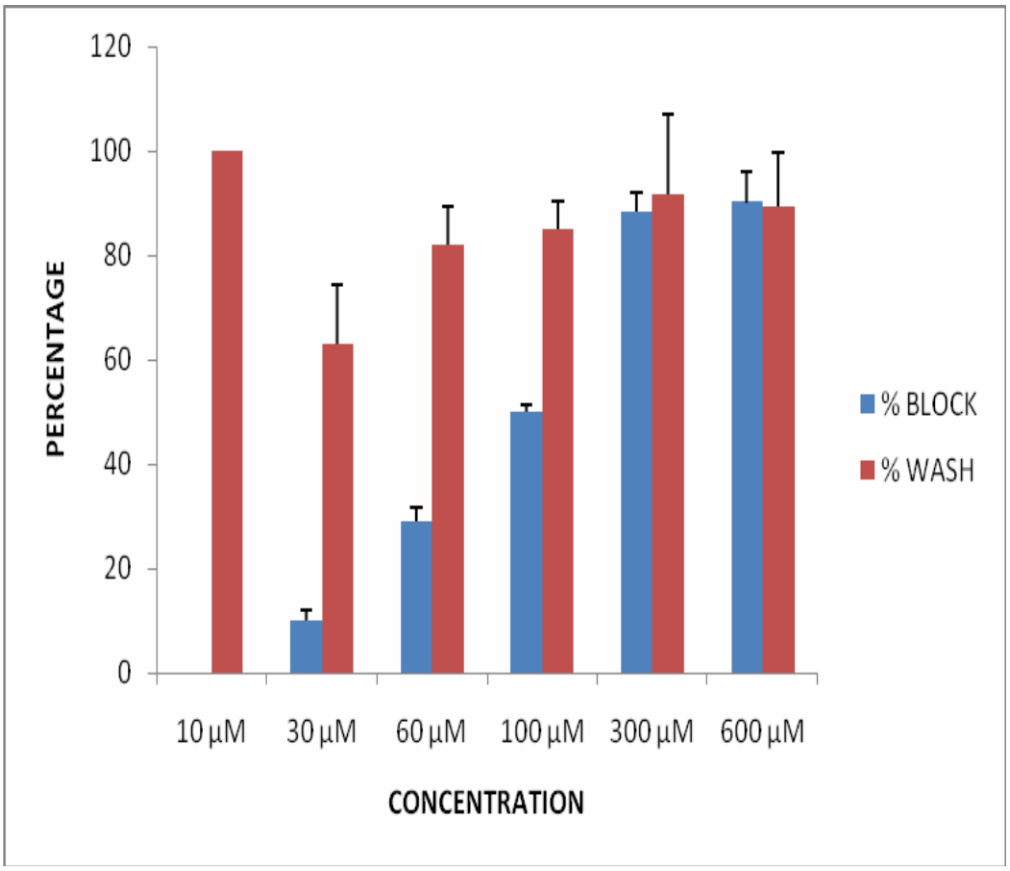


Figure 11: Current Blockade by DCP23 on $Na_v1.6$ at Inactivated Membrane Potential (-100 mV)
n = 5 cells per each concentration

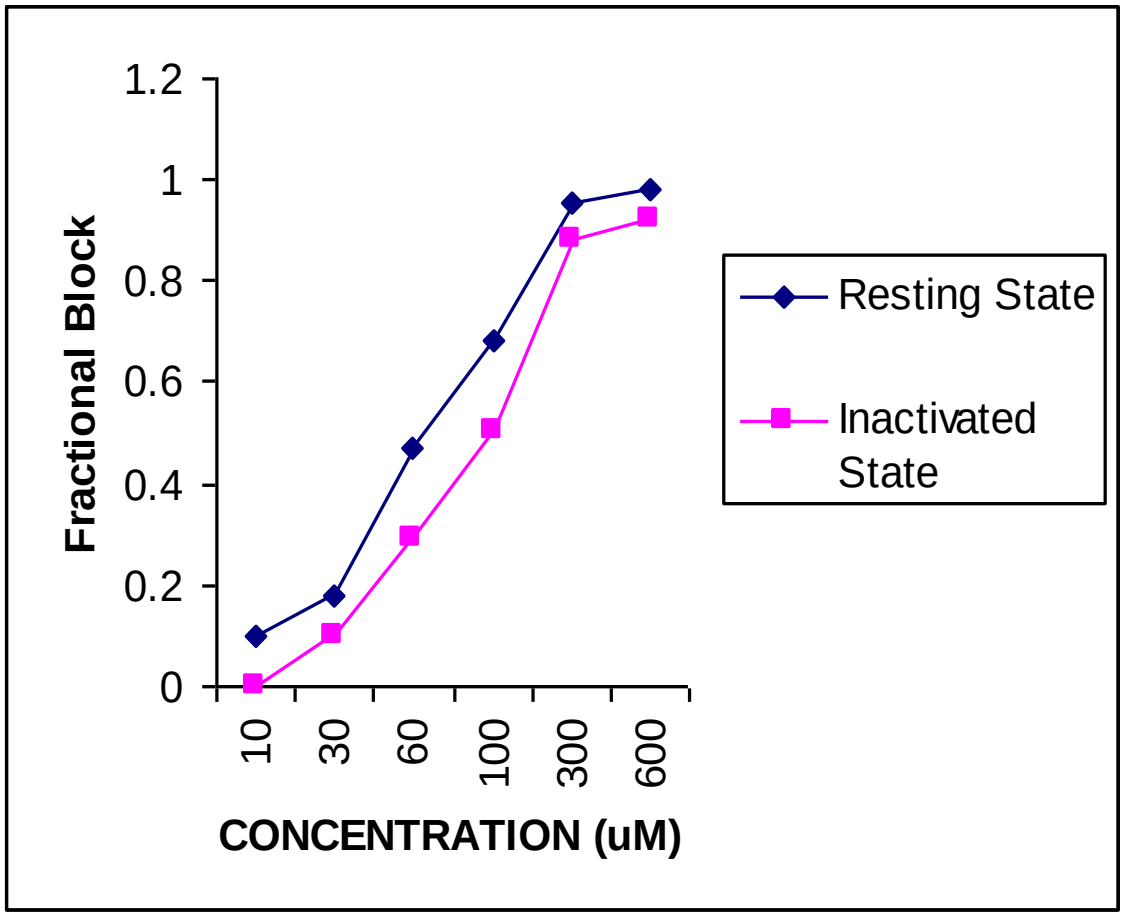


Figure 12: Concentration – Response Curves for DCP23 at Resting and Inactivated States of $Na_v1.6$

Resting State (-60mV): IC_{50} (µM) = 64.76 µM

Inactivated State (-100 mV): IC_{50} (µM) = 100.37 µM

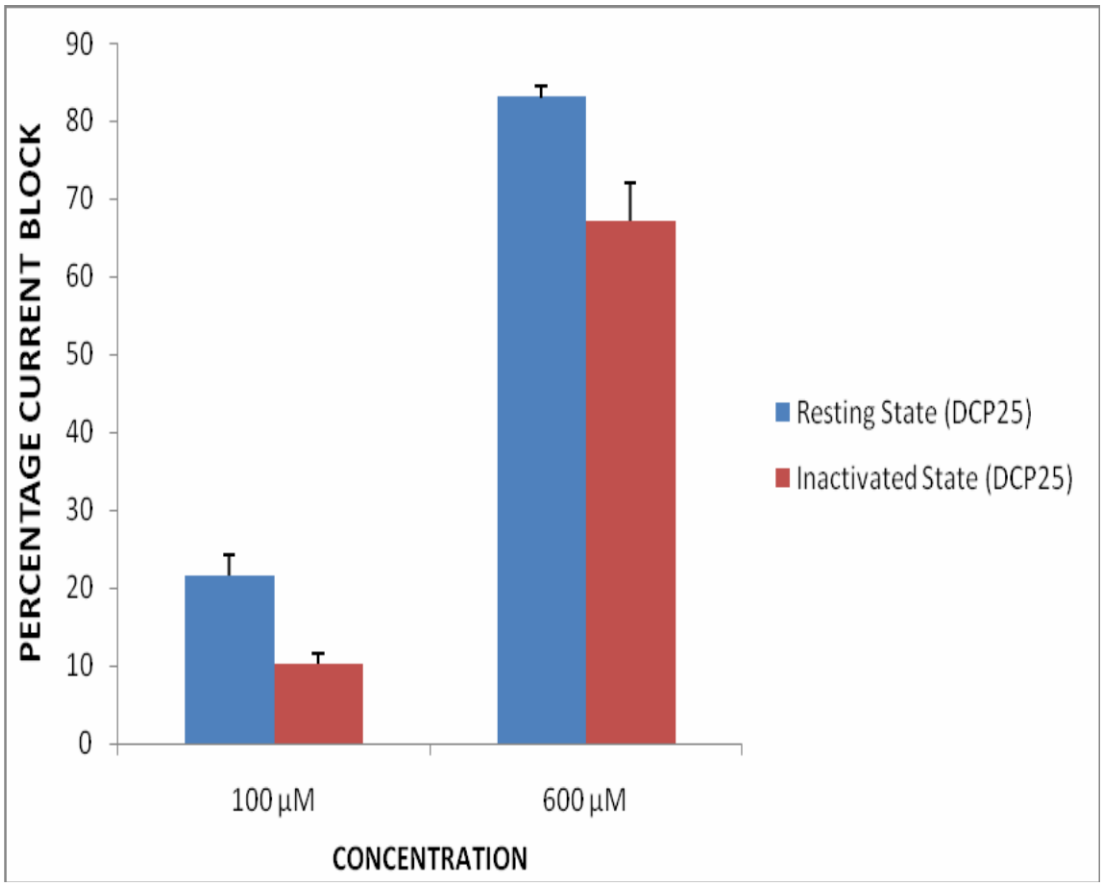


Figure 13: Current Blockade by DCP25 at Resting and Inactivated States of Nav

n = 4 cells per concentration

4.5.2 Use Dependent Action

The relation between current and voltage was established in both control solution and in the presence of 100 µM 2,3- dichloro- 3 (aminophenyl) propanamides (DCP23). The curve of the control solution indicated how sodium channels were activated by passing depolarized voltages in a step wise manner to when the channels were steadily inactivated. There was corresponding increase in the elicited current as the voltage changed from hyperpolarized potential to depolarized potential; up to when plateau was reached where there was highest elicited sodium current at about +10 mV. At this point, the conductance of sodium ions through the channels became saturated and further depolarization resulted to a reduction in sodium current, and thus, passed the channels to inactivated states where virtually the conductance of sodium ions had completely stopped at about +60 mV but zero current. The activation/inactivation pattern in the presence of DCP23 (100 µM) indicated that, there was significant reduction in the elicited current even at depolarized potential where the sodium conductance was found to be highest. Similarly, as the channels went into inactivation state indicated by the corresponding reduction in elicited current recorded for the control solution that was also reflected in the presence of 100 µM of DCP23. The action of DCP23 at 100 µM concentration even at depolarized voltage where sodium conductance was at its peak, suggested its use-dependent action (Figure 14).

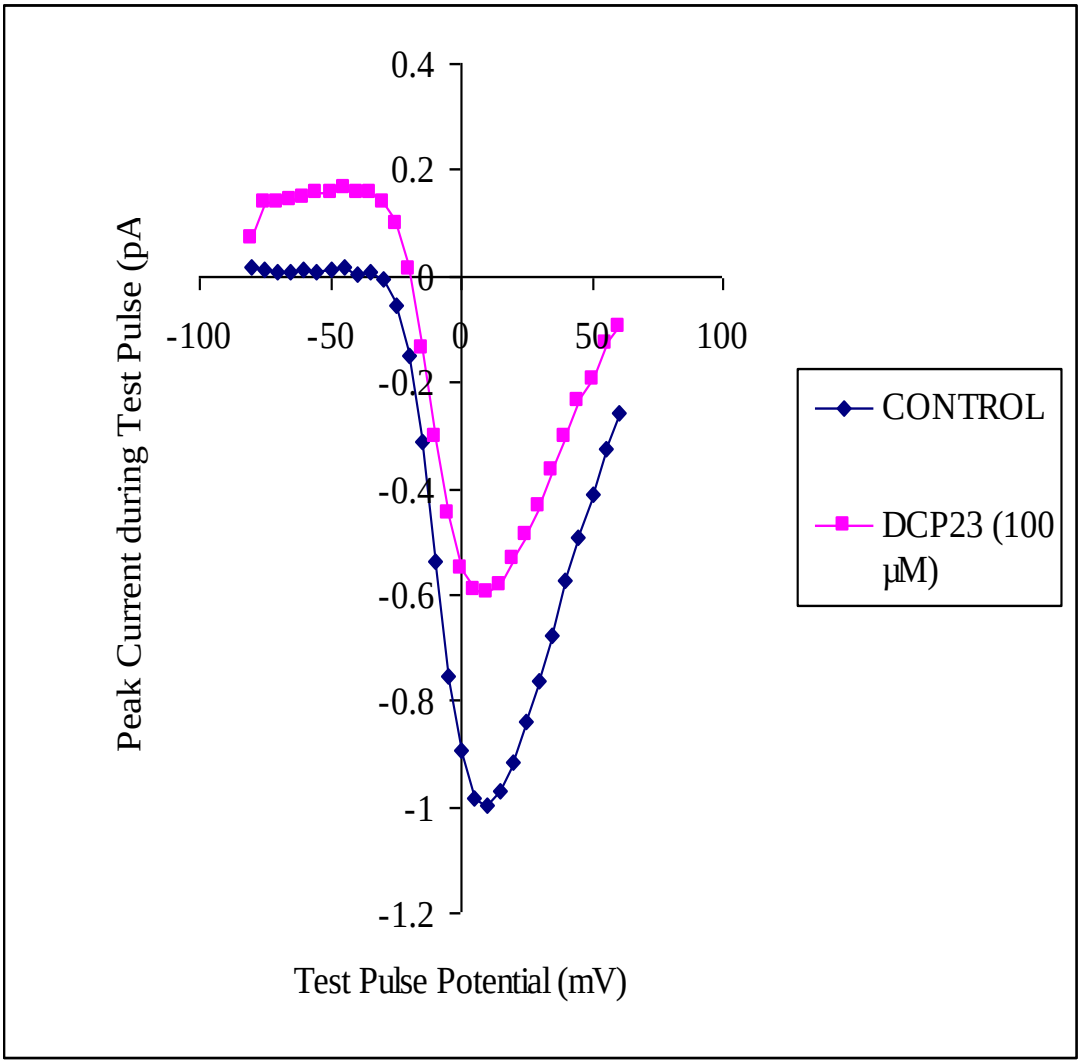


Figure 14: Current-Voltage Curves obtained in Control Solution and in the Presence of DCP23 (100 µM) using Nav 1.6 (n = 5)

CHAPTER FIVE

5.0 DISCUSSION

The synthesis of biologically active dichloro substituted propanamides gave final products that have different physical appearance and the percentage yields were appreciable. The melting point for each product was determined and the close range obtained for each indicated good purity of the final products. Similarly, appearance of single spot for each compound as obtained from thin layer chromatographic profile, further confirmed the purity of the final product.

Infrared spectroscopy (IR) revealed the presence of expected functional groups in the compounds. This was achieved by comparing the peak values of absorption frequency obtained from each spectrum to the theoretical values. More importantantly, the IR spectra confirmed the presence of C – N bond which was the point of the nucleophilic attachment; an indication of final product formation. This bond was not present in the starting reagents, either acrylamide or dichloroanilines. The absorption peak values obtained for the C – N bond were 1276.92, 1291.39 and 1291.39 cm^{-1} for DCP23, DCP25 and DCP34, respectively. The theoretical range was at 1200 - 1300 cm^{-1}, thus the values for the products were within the theoretical range (Kemp, 1991).

Nuclear magnetic resonanace (NMR) spectra for the three compounds were recorded and the spectral analysis revealed the structures of the intended products. Proton NMR spectra was analysed to elucidate the number of hydrogen atoms, position of hydrogen, environment of hydrogen (splitting pattern) and attachment of hydrogen – carbon (H – C

attachment). Whereas, Carbon- 13 NMR spectra was used to find the number of carbon atoms present in the compounds as well as the environment of carbon atoms. All the three isomers (DCP23, DCP25 and DCP34) were to have 9 carbon atoms and 7 protons at different chemical environments. The values as expressed in parts per million (ppm) for each isomer conformed to the expected range of values, thus, elucidate the structure of the final product. However, there was an abnormal shift obtained in the values of the carbon present in CH_2 from the range of 0 - 50 ppm to the regions of about 115 ppm, close to the aromatic carbon regions. Synthesis of dichlorophenylpropanamides through Michael reaction between acrylamide and the anilines came with some challenges. The reaction appeared not thermodynamically favourable due to the observed abnormal frequency of $-CH_2-$ from the Carbon- 13 NMR spectra. Theoretically, $-CH_2-$ vibration frequency was absent at its supposed chemical environment as there were no peaks observed at that chemical region. Therefore, Carbon- 13 NMR spectra indicated some level of degradation in the final products after being formed. This would affect the level potency of the synthesized compounds and hence, probable decrease in biological activity. However, the splitting pattern as indicated in COSY, a proton NMR spectrum, revealed the pattern for protons presence in CH_2-CH_2 where the two protons in the CH_2 gave three peaks (triplet) because they are neighbouring protons (n + 1) and suggests the presence of CH_2-CH_2 in the product.

The molecular distance estimation obtained from the 3- dimensional structure of each isomer conformed to the suggested pharmacophore models according to Unverferth *et al.,*

(1998) and Shindikar *et al.*, (2006). The distances for all the pharmacophore units were found to be the same for all the three isomers.

The determination of LD_{50} is of significant importance in the sense that, it is a valuable tool employed to compare toxicities of compounds relative to their therapeutic doses (Berkowitz, 2004). In studying unknown compounds (e.g. a plant extract, synthetic compounds), appropriate doses should be determined by preliminary studies of acute toxicity. Such studies are also essential to prevent over dose of drug which may interfere with results of experiment, and provide a clue for the toxicity profiles of the test compounds (Ozbek *et al*., 2004). Thus, LD_{50} values of DCP23, DCP25 and DCP34 were determined to ascertain their relative toxicities. The result showed that route of administration was a key factor in determining toxicity, as oral route was safer than the parenteral route (i.p.), and species differences as well play a critical role in assessing toxicity of compounds. According to Matsumura (1975) and Corbett *et al* (1984), the test compounds could be said to be moderately toxic in mice and rats via both intraperitoneal and oral routes of drug administration. However the value of DCP 34 oral LD_{50} could be said to be slightly toxic in rats.

Subchronic toxicity study was conducted on one isomer (DCP25) to evaluate its effects on some biochemical and haematological parameters. Generally, the compound was relatively safe as there was no statistically significant increase or decrease in most of the indices after 28-day daily oral administration.

Alanine aminotransferase (ALT), aspartate aminotranferase (AST) and alkaline phosphatase (AP) are liver enzymes of which their increase in level indicates possible liver damage (Stables and Kupferberg, 1997). ALT and AST are essential predictive measure of intracellular hepatic enzymes that have leaked into systemic circulation and serve as a marker of hepatocyte injury, whereas, AP acts as a marker for biliary function and cholestasis (Harris, 2005). Also, clinically used antiepileptic drugs like carbamazepine, sodium valproate and felbamate are known to cause liver damage in rare cases (Rang *et al*., 2007). Therefore, it could be said that DCP25 at 50 mg/kg could have toxic effect on liver as indicated by significant ($p<0.05$) increase in ALT level.

Renal function test was to evaluate the level of electrolytes, urea and creatinine present in the serum. Kidney has several functions including the excretion of water, soluble wastes such as urea and creatinine, and foreign materials such as drugs or the metabolites. It is responsible for the composition and volume of circulating fluids with respect to water and electrolyte balance and acid-base status (Yusuf *et al*., 2010). In renal failure, blood urea nitrogen and serum creatinine usually rise and this implies pre-renal azotemia, but may be caused by increased protein catabolism or an excessive protein load (Feinfeld, 2002). Carbamazepine, a standard antiepileptic drug is known to cause water retention (Rang *et al*., 2007), signifying its unwanted effect on kidney. From the results of this test, DCP25 at 50 mg/kg caused significant increase in the level of creatinine and urea, an indication of toxic effect on the kidney.

Results obtained from the lipid profile tests indicated no significant change in the levels of total cholesterol (TC), total glyceride (TG), Low density lipid (LDL) as well as high density lipid (HDL). They are essential markers for metabolic disease like diabetes and for coronary artery disease. Increase in the level of TC, TG and LDL with corresponding decrease in HDL, are essentially noticed in uncontrolled diabetes; and contribute to coronary artery disease in some diabetic patients (Arvind, *et al*., 2002, Yerima, 2013). DCP25 did not seem to have significant effect on all the tested lipid parameters at the administered doses.

The haematological parameters evaluated are essential for haematopoietic and leucopoietic systems; and are very sensitive to toxic compounds and serve as an important index of the physiological and pathological status for both animals and humans (Adeneye *et al*., 2006). Some clinically useful antiepileptic drugs are known to have unwanted effect on either haematopoietic or leucopoietic systems; phenytoin causes megaloblastic anaemia, carbamazepine is known to cause leucopenia while felbamate results to aplastic anaemia (Rang *et al*., 2007). This is a possible indication of DCP25's free unwanted effects on these systems and may further ascertain its non mimicking unwanted properties to some standard antiepileptic agents sighted.

Median toxic dose (TD_{50}) of the test compounds was determined using beam walking test. This was to measure extent of motor deficits caused by effect to the motor cortex, as an index of neurotoxicity. Walking-beam assay is more sensitive in the sense that, a significant deficit can be observed at about 25-30% $GABA_A$ receptor occupancy whereas

rotarod assay, about 70% receptor occupancy with a full benzodiazepine agonist is required to provide a significant impairment on the rotarod (Stanley *et al*., 2005). The results showed that phenytoin was less toxic than the test compounds and thus, lesser tendency to cause damage to the motor cortex. This also may further explain the possible interaction of the test compounds to the $GABA_A$ receptor complex.

Similarly, median effective dose (ED_{50}) of the test compounds was established using MES- and Picrotoxin- induced seizure tests. The values found showed that the test compounds especially DCP23 could be compared to the ED_{50} of phenytoin. Also, some ED_{50} values found by Idris *et al*., (2008a, 2008b, 2008c) on different isomers of 3-anilinopropanamides could favourably be compared to that of the test compounds. The values of TD_{50} and ED_{50} were used to determine therapeutic index (TI); in this case described as protective index (PI), and it is an index for safety margin (Brummelen, 2001). The safety margin for the test compounds as determined from the ratio between TD_{50} and ED_{50} was more to DCP23 (PI: 4), followed by DCP25 (PI: 3) and then DCP34 (PI: 2). According to the work of Shindikar *et al*., (2006), the protective indexes of phenytoin and carbamazepine were found to be approximately 7 and 6, respectively. In this work, the protective indexes were approximately 4, 3 and 2; for DCP23, DCP25 and DCP34 respectively. The values could have been compared favourably to that of Shindikar *et al*., 2006 if the model for TD_{50} was same to that of this research work. The fact that walking-beam test (Stanley *et al*., 2005); as used in this research work was more sensitive (about 30% receptor occupancy) than the rotarod test (about 70% receptor occupancy) as used by Shindikar *et al*., 2006, means that, motor impairment as an

indication of neurotoxicity must have been manifested at lower doses of the test compounds. Thus, resulted to lower TD_{50} values and hence, led to lower protective index values.

Maximal electroshock causes several changes at the cellular level, which can disrupt the signal transduction in the neurons. One of the most important mechanism by which it causes cellular damage is facilitation of Ca^{2+} entry into the cells in large amount and thus, prolonging the duration of convulsions (Inan and Buyukafsar, 2008). Apart from the Ca^{2+} ions, MES may also facilitate the entry of other positive ions like Na^{+} and thus, its blockade can prevent the MES-induced tonic extension (Bum *et al.*, 2010). On the contrary, potentiation of opioid and GABA receptors are also reported to protect against MES-induced seizures (Sandrini, 1992). Currently available anticonvulsant drugs like sodium valproate and phenytoin act by modulation of these ion channels (Rang *et al.*, 2003).

The MES is a model for generalized tonic-clonic seizures. The behavioral and electrographic seizures generated in this model are consistent with the human disorder (grand mal). This model identifies those compounds which prevent the spread of seizures. Protection against hind limb tonic extension (HLTE) in the MEST predicts anticonvulsant activity of antiepileptic drugs that prevent the spread of seizure discharge from an epileptic focus during seizure activity (e.g phenytoin, carbamazepine, oxcarbazepine, and lamotrigine) (Browning, 1992). Thus, indicate the ability of the antiepileptic agent to serve in the treatment of generalized tonic-clonic and partial

seizures (Raza *et al*., 2001). It has been suggested that, the induction of seizures by electroshock machine is through inhibitory current breakdown and voltage-dependent sodium channels in these electrically induced stimuli (McNamara, 1996). Antiepileptic drugs that act via this pathway are able to limit the repetitive firing of action potentials by slowing the rate of recovery of voltage-activated sodium ions channels from inactivation and suppress hind limb tonic extension in maximal electroshock seizures (Rho and Sankar, 1999). Also, increased levels noradrenaline, dopamine and 5-hydroxytrytamine protect against elecro-convulsion in chicks and rats (Osuide and Wambebe, 1979). All the test compounds (DCP 23, DCP 25 and DCP 34) demonstrated good efficacy against MES-induced seizure in a dose dependent manner. Evidently from the graded doses used, 2,3- dichloro substituted compound appeared to be the most potent. The activity was due to their ability to inhibit tonic hindlimb extension (THLE) of the animals and thus prevented the spread of seizure by possibly limiting the repetitive firing of action potentials. Though the model is not specific to one mechanism as described, generally could be closely linked to sodium channels mediated mechanism. This could be confirmed by the efficacy of the standard drug (phenytoin) used, which is known to act via sodium channel. Therefore, the test compounds may serve as lead compounds for the development of newer antiepileptic agents in the management of generalized tonic-clonic and partial seizures.

Pentylenetetrazole (PTZ) is believed to be an antagonist at GABA pathway in the CNS resulting in an imbalance between the ionic concentrations of the membrane (Nagakannan *et al*., 2011). Like other rodent models of absence seizures, PTZ-induced

seizures are potentiated by g-aminobutyric acid (GABA) antagonist. With some minor exceptions, the pharmacological profile of the scPTZ seizure model is consistent with the human condition (petit mal) (Malawska, 2005). Studies have shown that PTZ induces seizures by blocking the major inhibitory pathways mediated by the predominant inhibitory neurotransmitter GABA, at all levels of the CNS (DeSarro *et al*., 1999). It has also been shown that seizures induced by PTZ, can be blocked by drugs such as ethosuximide that reduces T-type Ca^{2+} currents (Rho and Saukar, 1999), and standard drugs such as diazepam and phenobarbitone are thought to produce their effects by enhancing GABA-mediated inhibition in the brain (Rogawski and Porter, 1990). It has been reported that dopamine reduces the threshold of PTZ convulsions in mice and pimozide (specific dopamine receptor antagonist) protected experimental animals against PTZ-induced seizures (Amabeoku, 1989). In addition, activation of N-methyl-D-aspartate (NMDA) receptor system appears to be involved in the initiation and propagation of pentylenetetrazole-induced seizures (Velisek *et al*., 1999). Drugs such as felbamate that block glutamatergic excitation mediated by NMDA receptor, have shown anticonvulsant activity against PTZ-induced seizures (White, 1997). Anticonvulsant activity in scPTZ test identifies compounds that can raise the seizure threshold in the brain (White *et al*., 1998). Antiepileptic drugs effective in the therapy of generalized seizures of (absence or myoclonic) petitmal type such as phenobarbital, and benzodiazepines, are capable of raising seizure threshold induced by pentylenetetrazole (Loscher *et al*., 1991). It is therefore possible that the anticonvulsant effects shown by DCP 23 ans DCP 25 against the PTZ-induced seizures might be due to activation of GABA neurotransmissions, blockade of glutamatergic neurotransmission mediated by NMDA receptor, or blockade

of the dopaminergic receptor system in the CNS. However, DCP34 may lack these properties as it did not show activity against this test model. Therefore, the two compounds with good anticonvulsant activity in this test model, which was comparable to sodium valproate (standard drug), could be optimized and serve as potential lead compounds for the development of antiepileptic agents against clonic seizure and thus may be beneficial in absence seizure.

4-aminopyridine induces clonic-tonic convulsions by blocking potassium channels (Yamaguchi and Rogawski, 1992). Potassium channels play a vital role in the control of neuronal excitability and seizure susceptibility, and will prove important for the suppression of seizure initiation and spread (Wickenden, 2002). The potassium channel activators which more readily penetrate the central nervous system (CNS) may have therapeutic potential in the treatment of epilepsy. The test compounds may not have activity against this test model, as they did not offer protection against seizures generated by 4-aminopyridine contrary to the standard drug (Phenobarbitone) and thus, could not act to block potassium channels. However, the little activity demonstrated by the 3,4-dichlorosubstituted compound might be an evidence to show some properties against this pathway.

Strychnine is a competitive antagonist of the inhibitory amino acid glycine (Larson, 1969). The absence of anticonvulsant activity shown by all the test compounds and phenobarbitone in the seizures generated by strychnine, suggests that the compounds and the standard drug at the dose tested, might not have interaction with glycine receptor.

The test compounds (DCP 23 and DCP 25) showed good activity against picrotoxin-induced seizures in a dose dependent manner. Picrotoxin is a non-competitive GABA antagonist that blocks the chloride channel (Takeuchi and Takeuchi, 1969). Antiepilepic agents such as sodium valproate, phenobarbitone, benzodiazepine and ethosuximide suppress seizures induced by picrotoxin at different doses (Porter *et al*., 1984). Similarly, among the new antiepileptic drugs, gabapentin and tiagabine suppress seizures induced by picrotoxin (Taylor, 1995). These agents are known to have different mechanisms of action at the molecular level in relation to GABA (Rho and Sankar, 1999). The efficacy of the test compounds against this model further suggests their possible ability to interact with chloride channel, and the interaction may be related to that of benzodiazepine and other allied agents as evidently shown when diazepam was employed as the standard drug in the study. Therefore, the test compounds could be predicted to act via GABA-mediated mechanisms.

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 (Alvan *et al*., 2001). It can result from increased drug metabolism or from adaptations in the neural elements that respond to the drugs initially, thus, causes diminished effect of the drug after repeated administration at the same dose (Nestler *et al*., 2009). Daily oral administration of the test compounds for 5 days is a recommended study to evaluate possible development of tolerance for potential anticonvulsant agents (Stables and Kupferberg, 1997). The results of this study showed that, all the test compounds were able to offer higher protection against MES-induced

seizure after 5-day of their daily administration. The protection was higher than those that received single dose of the test compounds. This would further show that there was no diminished effect of the compounds, and hence, indicated non development of tolerance.

Kindling is a well-established model of abnormal plasticity leading to prolonged seizures and to epilepsy (Rivera *et al*., 2012). It is a model of epilepsy and epileptogenesis where repeated administration of a subconvulsive dose of PTZ produced a progressive increase in convulsant activity, culminating in generalized seizures in animals (Dhir, *et al*., 2007). The generalized tonic-clonic seizure in this model resemble that of epilepsy in humans (Becker, *et al*., 1992) and hippocampus is the brain area that participate in seizure generation following kindling (Takemiya, *et al*., 2003). The test compounds were able to retard the severity of seizure by not allowing the seizure progression to classical convulsion stage (stage 3-5). Since PTZ induces seizures by blocking the major inhibitory pathways mediated by the predominant inhibitory neurotransmitter GABA, at all levels of the CNS (DeSarro *et al*., 1999) further suggests the potential of the test compounds in enhancing GABA transmission or inhibit the seizure generation following kindling in hippocampus area.

Fluphenamic acid has an interesting modulatory effect on neuronal sodium channels, reducing sodium current availability and slowing down inactivation and recovery from inactivation, leading to diminished repetitive and burst firing (Hau-Jie *et al*., 2010). Antiepileptic drugs that act via MEST model are able to limit the repetitive firing of action potentials by slowing the rate of recovery of voltage-activated sodium ions

channels from inactivation and suppress hind limb tonic extension in maximal electroshock seizures (Rho and Sankar, 1999). The ability of fluphenamic acid to offer dose dependent protection against MES model confirmed its modulatory effect on neuronal sodium channels. This is clearly demonstrated by its effect to potentiate the protective outcome of the test compounds (DCP 23 and DCP 25) when interacted at the lowest dose where it offered no protection. The potentiation was similarly observed with phenytoin suggesting similar possible mechanism of action between the test compounds, phenytoin and fluphenamic acid.

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. Among these cations Ni^{+1} was found to be selective blocker for low voltage-activated T-type Ca^{2+} channels (Kang *et al*., 2006). *In-vitro* studies showed that it selectively blocked T-type current of number of cell types including sensory neurons (Todorovic and Lingle, 1998). Pharmacological studies have shown that low voltage activated T-type of calcium ion channels are involved in the genesis of absence seizures (Van Luijtelaar *et al*., 2000). Drugs that act by inhibiting neuronal T-type calcium ion currents like sodium valproate have potential activity against absence seizures (Loscher *et al*., 1991). Also, study conducted by Rehni and Singh, (2009), 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 DCP 23 and DCP 25 there was reduction in severity of seizure as evidently demonstrated by the significant

increase in the latency of seizure. Accordingly, the decrease in the severity was more with the DCP 23 as it appreciably increased the protective outcome. Therefore, the test compounds could be said to act via calcium channels by possibly blocking the neuronal T-Type calcium ion channels. Thus, the compounds might mimic the action of sodium valproate and hence could be exploited in the management of absence seizure.

Study of serotonergic and histaminergic pathways show that the decrease neurotransmission of serotonin and histamine in the brain reduces seizures threshold. Since cyproheptadine interferes with these pathways via antagonizing subtypes of $5HT_{1,}$ $5HT_2$ and H_1 receptors (Singh and Goel, 2010), therefore it is considered as proconvulsant. From the interaction study, cyproheptadine did not seem to augment the convulsive effect of PTZ; the outcome of seizure protection and the mean onset of seizure were not affected. Therefore, the interaction study between cyproheptadine and the test compounds suggested that the mechanism of action of the compounds may not be via serotonergic and histaminergic pathway as shown from the outcome of seizure protection.

Hypnotic effects are classically considered to involve more pronounced depression of the central nervous system than sedation, and this can typically be achieved by increasing the dose of sedative-hypnotic drugs and therefore bring about shortening of sleep latency, increase of total sleep time and sleep efficiency (Tobler *et al*., 2001). The test compounds (DCP 23 and DCP 25) at the highest dose potentiated the hypnotic effect of diazepam by decreasing the onset of sleep and enhancing the duration of sleep. This effect may be attributed to an action on the central mechanisms involved in the regulation of sleep

(N'Gouemo *et al*., 1994; Amos *et al*., 2001) or an inhibition of diazepam metabolism (Kaul and Kulkarni, 1978). Sleep is also reduced when there is a decrease in serotonin concentration or destruction of the dorsal raphe nucleus in the brain stem, which contains most of the brain's serotonergic cell bodies. On the other hand, increased firing of norepinephrine-containing neurons in the locus ceruleus will result in a reduction of rapid eye movement sleep and increased wakefulness (Curtis and Jermain, 2002). It has also been reported that activation of $GABA_A$ receptor in the CNS is known to favour sleep (Gottesmann, 2002). Thus, the compounds possessed pharmacophore that may be responsible for central depressive action. Therefore, could be linked partly to serotonergic and noradrenergic pathways; but more to GABAergic pathway as shown by potentiating the hypnotic effect of diazepam.

Voltage-gated sodium channels (VGSCs) play a critical role in regulating cellular excitability and controlling many of the physiological processes associated with neuronal activity (Catterall, 2000). The design of the test compounds (DCP23, DCP25 and DCP34) was on the basis of suggested pharmacophore model for compounds acting as blockers of the voltage-dependent sodium channel as described by Unverferth *et al*., (1998). Therefore, their supposed molecular target would be via votage-gated sodium channels (VGSCs). Thus, VGSCs was selected to possibly prove the conformity of the test compounds to the pharmacophore model. In animal models of epilepsy, the Nav1.6 sodium channel isoform, which is heavily expressed in the central nervous system, has been shown to be upregulated (Hargus *et al.,* 2011). Similarly, Nav1.6 channels have a particularly low threshold for spike initiation and are abundantly expressed along the axon initial segment, the site of action potential initiation. They have been shown to

contribute to the spike trigger and also to regulate the repetitive discharge properties of hippocampal CA1 pyramidal neurons. It was also found that kindling is associated with higher expression of Nav1.6 sodium channels in hippocampal CA3 neurons (Rivara *et al*., 2012). This is a clearly evidence to show that, Nav1.6 isoform plays an important role in controlling neuronal firing and as such, its inhibition would accordingly decrease neuronal firing. Also, the fact that the Nav1.6 sodium channel isoform has been heavily implicated in epilepsy, therefore, its selective pharmacological inhibition could increase the efficacy of anti-epileptic drugs (Rivera *et al*., 2012). Thus, the choice of Nav1.6 isoform to test for the efficacy of DCP23, DCP25 and DCP34 at different concentrations; was on the basis of these facts.

This study was conducted to compare the potency of phenytoin, lamotrigine and carbamazepine on sodium channel of neuroblastoma cell, at resting membrane potential. The outcome of the results showed that phenytoin is the most potent followed by lamotrigine and then carbamazepine. This was depicted by their IC_{50} values of 58, 91 and 140 µM, respectively, and this has also proven that phenytoin and lamotrigine offered tonic inhibition of sodium channels over a similar dose range (Lang, *et al*., 1993). Similarly, all the test compounds were found to produce tonic inhibition of the sodium channels at different potency level when their efficacies were tested at a resting membrane potential of -60mV. Their ability to produce tonic inhibition of sodium currents when voltage clamped at -60mV, though at different potency level, was an indication to prove their efficacy against sodium channels and may act as blockers of the channel. The IC_{50} value of the most active compound (DCP23) among the test

compounds was found to be 64.8 µM. This suggests that the potency of DCP23 could be favorably compared to that of phenytoin and lamotrigine and it appeared to be more potent than lamotrigine and carbamazepine. In another study, the action of Propofol; which is also known to modulate neuronal sodium channels as its major determinant for AED activity in addition to GABA and Ca^{2+} channels modulation (Martella *et al*., 2005), was evaluated on hippocampal cultured neuron. The IC_{50} was found to be 17.7 µM while that of other hydroxyamide analogs of propofol; HS245 and HS357, were 100 and 24 µM respectively (Jones *et al*., 2007). Also, study was conducted to evaluate a novel lactam (YW192) containing anticonvulsant pharmacophore in comparism to lamotrigine using NaV 1.2, the IC_{50} values for lamotrigine and YW192 at resting membrane potential, were found as 172 and 57 µM respectively (Jones, *et al*., 2009). These results could additionally show good potency of DCP23.

Generally, antiepileptic drugs (phenytoin-like drugs) blocked sodium channels at specific receptor site which is formed by amino acid residues in the S6 segments in domains I, III and IV. These drugs bind to a receptor site in the pore of sodium channels and impede ion permeation (Catterall, 2012). The ability of DCP23, DCP25 and DCP34 to reduce sodium channel currents was an indication to show that they bound to a specific receptor site in the pore of sodium in these segments and domains, hence, blocked sodium ion permeation.

A common feature of many AEDs is their ability to bind with greater affinity to open and inactivated channels over channels at rest (Kuo *et al*., 1997). Antiepileptic drugs such as

diphenyl hydantoins, carbamazepine and lamotrigine, are thought to be effective because of a higher affinity for the inactivated state of the channel over the resting state of the channel (Kuo and Bean, 1994). This characteristic is best described by the modulated receptor hypothesis that states that drug affinity for the channel receptor is dependent on the channel state, transitioning from a low- to high affinity site during inactivation of the channel i.e sodium channels accumulate in high-affinity drug biding conformations (Hondeghem and Katzung, 1977), thus, results in stabilization of the inactivated state and minimization of the persistent current (Kuo *et al.* 1997). The action of DCP23 and DCP25 were tested at open/inactivated state of the channels; voltage clamped at -100Mv. The outcome of the results showed their states-dependent action on NaV 1.6 as indicated by their lower sodium current inhibition in inactivated state but higher current inhibition at resting state. The evidence for this phenomenon would be the outcome of IC_{50} of DCP23 which was found to be100 µM higher that (64 µM) found at the resting potential. This could be seen from Dose – Response Curves of DCP23 at the two states of the channel, where there was right shift of the curve obtained at inactivated state when compared to that of the resting state. This was an indication of higher binding affinity of the compound to inactivated channels where sodium channels accumulate in high-affinity drug binding conformations. The increased fraction of inactivated channels limits the number of sodium channels ready to open during repetitive firing, which is believed to occur when the seizure activity spreads (Rogawski and Loscher, 2004). Similarly, DCP25 showed twice higher affinity to inactivated state than to the resting state as evidently shown at the concentration of 100 µM, thus, conformed to the similar action of DCP23. However, to compare this to the peculiarity of other phenytoin-like drugs e.g,

lamotrigine; it was found to demonstrate much higher affinity at open/inactivated state (Jones *et al*., 2009). Carbamazepine also limits repetitive firing of nerve action potentials and it shows neuronal sodium channel blocking characteristics similar to those of phenytoin i.e low-affinity resting block and high affinity for the inactivated state (Lipkind and Fozzard, 2010). This showed that the test compounds share similar state-dependent characteristics to that of phenytoin, lamotrigine as well as carbamazepine but with lower degree of potency. Several studies have highlighted the importance of Na channel inactivation in regulating neuronal activity, synaptic integration and neuronal spiking. In cortical pyramidal neurons, inactivation of Na channels accounted for the reduction in spike rate during high frequency stimulation (Fleidervish *et al.*, 1996). Disruptions in Na channel inactivation mechanisms alter membrane excitability leading to epileptiform activity and the generation of action potential bursts in generalized epilepsy with febrile seizures plus and severe myoclonic epilepsy of infancy (Spampanato *et al.*, 2001). Therefore, the test compounds could be exploited in regulating neuronal activity, synaptic integration and neuronal spiking due to peculiar transitioning characteristics of the channel at the inactivated state.

The use-dependent inhibition of sodium current by phenytoin and carbamazepine is an important mechanism for inhibiting the sustained high-frequency repetitive firing of action potentials that occurs during a seizure thus limiting the spread of the seizure (Rogawski and Porter, 1990). However, carbamazepine possess weak use-dependent block when compared to that of phenytoin (Lipkind and Fozzard, 2010). DCP23 being the most potent, was tested at the high frequency driven state of the sodium channel and

similarly was found to demonstrate appreciable frequency-dependent block commonly known as use-dependent block. It was shown to reduce the inward conduction of sodium ions as indicated by significant reduction of the normalized current recorded during steady state activation. The voltage dependence of activation of the sodium channel was due to outward movement of gating charges in response to changes in the membrane electric field (Catterall, 2000). Therefore, DCP23 shifted the steady state activation curve to more hyperpolarized voltage, and that could be a good indicator for suppression of epileptiform activity during high frequency neuronal firing.

Generally, this voltage- and frequency-dependent block is considered critical for the selective suppression of epileptiform activity prevalent during epileptic seizures, while sparing normal channel function (Rogawski and Loscher, 2004). Hence, the various actions demonstrated by the test compounds on Nav1.6 were corroborated with their observed anti-MES activity in animals. Thus, these compounds could be optimized and exploited as lead compound in developing newer antiepileptic agents.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 Summary

2,3 (DCP23)-, 2,5 (DCP25)- and 3,4 (DCP34)- Dichloro– 3(aminophenyl) propanamides were designed and synthesized based on proposed anticonvulsant pharmacophore units according to Unverferth *et al*., 1998 and Shindikar, 2006. They were found to conform to the distance range estimations according to these pharmacophore models.

The compounds were tested on different acute models for anticonvulsant screening and thus, their anticonvulsant effects were established most especially in maximal electroshock- and pentylenetetrazole- induced seizure models, which are the recommended primary screening models.

Median lethal dose (LD_{50}), median toxic dose (TD_{50}) and median effective dose (ED_{50}) of all the synthesized compounds were determined. These indices were accordingly applied for the subsequent determination of compounds' therapeutic index (TI), as a measure for their margin of safety.

Pharmacological interaction between the compounds and some specific receptor or channel blockers was conducted. Data obtained from these interactions suggested that the compounds could have their possible mechanism of action via sodium and calcium channels blockade; as they were able to potentiate the blocking effect of Fluphenamic acid, an inhibitor of neuronal firing via sodium ion channel blockade; and nickel chloride,

a known specific blocker of T-type calcium ion channels. Similarly, the results obtained from diazepam-induced sleep test when interacted with DCP23 and DCP34; could be another predictive measure of their ability to interact with benzodiazepine receptor site.

Also, from the interaction study conducted between the test compounds and cyproheptadine, histamine (H_1) and serotonin ($5HT_1$ and $5HT_2$) blocker; it was an evidence to predict non interaction of the compounds with either of the two pathways. Thus, their anticonvulsant effect was not mediated via histaminergic or serotonergic pathways.

The test compounds offered highly appreciable retardation in the pentylenetetrazole-induced kindling, a chronic model of convulsion that is closely mimicking human pathologic condition.

The *in vitro* study conducted on voltage-gated sodium channels ($Na_v1.6$), stably expressed in Human Embryonic Kidney (HEK Cells 293), the synthesized compounds showed concentration-dependent current blockade of voltage-gated sodium channels (VGSCs).

DCP23 and DCP25 were found to be more potent than DCP34, and both showed state dependent action by exhibiting higher affinity to inactivated state than the resting state, an important gating characteristic of sodium channel blockers used in the management of epilepsy.

DCP23 was found to be most potent and hence, its action was evaluated at depolarized state of the channels (activated state). The outcome of this action from an established current-voltage relationship was that, DCP23 exhibited 'use dependent action', also known as frequency dependent action.

The results obtained from biochemical and haematological as well as lipid profile assays after 28 days oral administration of DCP25, showed no significant deleterious effects in all the indices evaluated, an indication of relative safety of the compound.

6.2 Conclusion

The test compounds possess pharmacophores which are responsible for their anticonvulsant properties as demonstrated in both acute and chronic animal models of convulsion. The primary molecular target for their action was via voltage-gated sodium channels, and hence, they could be exploited and optimized to serve as lead compounds for the development of newer antiepileptic agents known to block sodium channels. Therefore, the design conformed to the proposed pharmacophore models according to Unverferth *et al.,* (1998) and Shindikar *et al.,* (2006).

6.3 Recommendation:

Structural modification should be done to improve the potency as well as to reduce the toxicity of these compounds probably by computer modeling to study other molecular descriptors that are not stated by Unverferth *et al.*, (1998) and Shindikar *et al.*, (2006).

Also, the synthetic pathway as adopted in this research should be modified by either introducing a coupling reagent or by substituting an acrylamide with another reagent containing amide moiety. This may eventually improve the thermal stability of the compounds there by improving their potencies.

There is the need to further investigate the action of these compounds on cultured hippocampal neurons using electrophysiological measurement of sodium channel currents.

Also, indepth chronic toxicity studies should be carried out to ascertain their safety or otherwise, on structural and functional integrity of vital organs of the body.

Similarly, the potential of these compounds as antinociceptive should be evaluated in both *in vivo* and *in vitro* models.

REFERENCE

Aamodi, S.M. and Constantine-Paton, M. (1999). The role of neural activity in synaptic development and its implications for adult brain function. *Advance Neurology*, 79: 133-144.

Adeneye, A.A., Ajagbonna, O.P., Adeleke, T.I. and Bello, S.O. (2006). Preliminary toxicity and phytochemical studies of the stem bark aqueous extract of Musanga cecropioides in rats. *Journal of Ethnopharmacology*, 105, 374-379.

Alvan, G., Paintaud, G. and Wakelkamp, M. (2001). Clinical pharmacodynamics. In: *Drug Benefits and Risks*, Boxtel, C.J., Santoso, B. and Edwards, I.R. (Eds). John Willey and Sons LTD, New York, USA, p. 97; p. 152.

Amabeoku, G.J., Leng, M.J. and Syce, J.A. (1989). Antimicrobial and anticonvulsant activities of Viscum capense. *Journal of Ethnopharmacology*, 61: 237-241.

Amos, S., Adzu, B., Binda, L., Wambebe, C. and Gamaniel, K. (2001). Neuropharmacological effect of the aqueous extract of *Sphoeranthus senegalensis* in mice. *Journal of Ethnopharmacolg*, 78: 33-37.

Amos, S., Akah, P.A., Enwerem, N., Chindo, B.A., Hussaini, I.M., Wambebe, C., Gamaniel, K. (2004). Behavioral effect of pavetta crassipes extract on rodents. *Pharmacology, Biochemistry and Behavior*, 77: 751-759.

Arvind, K., Pradeepa, R., Deapa, R. and Mohan, V. (2002). Diabetes and coronary artery diseases. *Indian Journal of Medical Research*, 116: 163-176.

Arzimanoglou, A., Hirsch, E., Nehlig, A., Castelnau, P., Gressens, P., Vasconcelos, A. (2002). Epilepsy and neuroprotection. *Epileptic Disorder*, 3: 173-182.

Avoli, M., Louvel, J., Pumain, R., Kohling, R. (2005). Cellular and molecular mechanisms of epilepsy in the human brain. *Progress in Neurobiology*, 77: 166-200.

Banerjee, P.N., Filippi, D. and Hauser, W.A. (2009). The descriptive epidemiology of epilepsy- A review. *Epilepsy Research*, 85: 31-45.

Baraban, S.C. and Tallent, M.K. (2004). Interneuron diversity series: interneuronal neuropeptides-endogenous regulators of neuronal excitability. *TINS*, 27: 135-142.

Becker, A., Grecksch, G., Ruthrich, H.L., Pohle, W., Marx, B. and Mathies, H. (1992). Kindling and its consequences on learning in rats. *Behavior Neural Biology*, 57: 37-43.

Becker, A.J., Chen, J., Zien, A., Sochivico, D., Normann, S., Schramm, J., Elger, C.E., Wiestler, O.D. and Blumcice, I. (2003). Correlated stage and subfield associated hippocampal gene expression patterns in experimental and human temporal lobe epilepsy. *Europian Journal of Neuroscience*, 18: 2792-2802.

Benjamin, E.R., Fruthi, F. Olanrewaju, S., Ilyin, V.I., Crumley, G., Kutlini, E., Valenzano, K.J. and Woodward, R.M. (2006). State-Dependent Compound Inhibition of NaV 1.2 Sodium Channels Using the FLIPR Vm Dye: On-Target and Off-Target Effects of Diverse Pharmacological Agents. *Journal of Biomolecular Screening*, 11(1): 29-39.

Berkowitz, B.A. (2004). *In*: B.G. Katzung. *Basic and clinical evaluation of new drugs*. Lange Medical Books, Mc Graw-Hill Medical Publishing Division New York Chicago, pp. 67.

Bernard, S., Chang, M.D., Daniel, H. and Lowentein, M.D. (2003). Mechanism of disease epilepsy. *New England journal of medicine*, 349: 1257-1266.

Brown, M.L., Brown, G.B. and Brouillette, W.J. (1999). Comparative molecular field analysis of hydantoin binding to the neuronal voltage-dependent sodium channel. *Journal of Medicial Chemistry*, 40**:**602–607.

Browne, L.E., Blaney, F.E., Yusaf, S.P., Clare, J.J. and Wray, D. (2009). Structural Determinants of Drugs Acting on the NaV 1.8 Channels. *Journal of Biological Chemistry*, 284(16): 10523-10536.

Browning, R. (1992). The electroshock model, neuronal network and antiepileptic drugs. *In*: *Drugs for Control of Epilepsy: Actions on Neuronal Networks in Seizure Disorders*, Faingold, C.L., Fromm, G.H. (Eds). CRC press: Boca Raton, FL, pp. 195-211.

Brummelen, P.V. (2001). Drug development. In: Drug Benefits and Risks, Boxtel, C.J., Santoso, B. and Edwards, I.R. (Eds). John Willey and Sons LTD, New York, USA, p. 97.

Bum, E.N., Nkantchoua, G.N., Njikam, N., Taiwe, G.S., Ngoupaye, G.T. and Pelanken, M.M. (2010). Anticonvulsant and sedative activity of leaves Senna spectabilis in mice. *International Journal of Pharmacology*, 6: 123-128.

Camerino, D.C., Tricarico, D. and Desaphy, J.F. (2007). Ion Channel Pharmacology. *Neurotherapeutics*, 4: 184-198.

Carlen, P.L., Skinnner, F., Zhang, L., Naus, C., Kushnir, M. and Perez velaquez, J.L. (2000). The role of gap junctions in seizures. *Brain Research Revised*. 32: 235-241.

Catterall, W.A. (2000) From ionic currents to molecular mechanisms: the structure and function of voltage-gated sodium channels. *Neuron* 26**:**13–25.

Catterall, W.A., Goldin, A.I. and Waxman, S.G. (2005). Nomenclature and structure-function relationships of voltage-gated sodium channel. *International Union of Pharmacology XLVII*, 27: 7520–7531.

Catterall, W.A**.** (2012). Voltage-gated sodium channels at 60: structure, function and pathophysiology. *Journal of Physiology*, 590(11): 2577–2589.

Catterall, WA, Kalume, F. and Oakley, J.C. (2010). NaV1.1 channels and epilepsy. *Journal of Physiology*, 588, 1849–1859.

Cest'ele, S. and Catterall, W.A. (2000). Molecular Mechanisms of Neurotoxin Action on Voltage-Gated Sodium Channels. *Biochimie*, 82: 883-892.

Chanda B and Bezanilla F (2002). Tracking voltage-dependent conformational changes in skeletal muscle sodium channel during activation. *Journal of General Physiology*, 120: 629–645.

Chernoff, D.M. and Strichartz, G.R. (1990). Kinetics of local anesthetic inhibition of neuronal sodium currents pH and hydrophobicity dependence. *Biophysical Journal*, 58: 69-81.

Clark, C.R., Sansom, R.T., Lin, C.M. and Norris, G.N. (1985). Anticonvulsant activity of some 4-aminobenzanilides. *Journal of Medicinal Chemistry*, 28: 1259-1262.

Conn, P.J. and Pin, J. (1997). Pharmacology and functions of metabotropic glutamate receptors. *Annual Revised Phamacology and Toxicology*, 37: 205-237.

Cooper, E.C., Harrington, E., Jan, Y.N. and Jan, L.Y. (2001). M Channel KCNQ2 subunits are localizedto key sites for control of neuronal network oscillations and synchronization in mouse brain. *Journal of Neuroscience*, 21: 9529-9540.

Corbett, J.R., Wright, K. and Bailie, A.C. (1984). *The Biochemical Mode of Action of Pesticides*. 2nd Edition. Academic Press, London.

Cosford, N.D.P.; Meinke, P.T.; Stauderman, K.A.; Hess, S.D. (2002). Recent advances in the modulation of voltage-gated ion channels for the treatment of epilepsy. *Current Drug Target on CNS and Neurological Disorder, 1*: 81-104.

Cox JJ, Reimann F, Nicholas AK, Thornton G, Roberts E, Springell K, Karbani G, Jafri H, Mannan J, Raashid Y, Al-Gazali L, Hamamy H, Valente EM, Gorman S,Williams R, McHale DP,Wood JN, Gribble FM &Woods CG (2006). An SCN9A channelopathy causes congenital inability to experience pain. *Nature,* 444: 894–898.

Curtis, J.L. and Jermaine, D.M. (2002). Sleep Disorders. In: *Pharmacotherapy*. A Physiological Approach, 5th edition, Dipiro, J.T., Talbert, R.L., Yee, G.C., Matzke, G.R., Wells, B.G. and Posey, L.M. (eds). McGraw-Hill, New York, pp. 1323-1333.

DeCurtis, M. and Avanzini, G. (2001). Interictal Spikes in Focal Epileptogesis. *Progress Neurobiology,* 63(5): 541-567.

Dekker, J., Ty, M. and Strichartz, G.R. (2008). Principles of Cellular Excitability and Electrochemical Transmission. In: *Principles of Pharmmacology, the Pathophysiologic Basis of Drug Therapy*, 2nd edition, Golan, D.E., Tashjian, A.H., Armstrong, E.J. and Armstrong, A.W. (eds). Lippincott Williams and Wilkins, Baltimore, pp. 79-89.

DeSarro, A., Cecchetti, V., Fravolin., Naccari, F., Tabarrini, O. and DeSarro, G. (1999). Effects of novel 6-defluroquinolones and classic quinolones on Pentylenetetrazole-induced seizures in mice. *Antimicrobial agents and Chemotherapy*, 43: 1729-1736.

Dhir, A., Naida, P.S. and Kulkarni, S.K. (2007). Neuroprotective effect of nimesulide, a preferencial COX-2 inhibitor against pentylenetetrazole PTZ-induced chemical parameters in mice. *Seizure,* 16: 691-697.

Dib-Hajj, S.D., Black, J.A. and Waxman, S.G. (2009). Voltage-Gated Sodium Channels: Therapeutic Targets for Pain. *Pain Medicine,* 10(7): 1260-1269.

Dingledine, R., Borges, K. and Bowie, D. (1999). The glutamate receptor ion channels. *Pharmacology Review,* 51: 7-61.

Elaine, A. and Michael, P.B. (2007). Excitability Constraints on Voltage-Gated Sodium Channels. *PLOS Computational Biology,* 3(9): 1751-1760.

Ertel, E.A., Campbell, K.P., Harpold, M.M., Hofmann, F., Mori, Y., Perez-Reyes, E., Schwartz, A. Snutch, T.P., Tanabe, T. and Birnbauma, L. (2000). Nomenclature of Voltage-Gated Calcium Channels. *Neuron,* 25: 533-535.

Evans, W.C. (1996). *Trease and Evans Pharmacognosy* 14th ed. London: Saunders, pp. 780.

Feinfeld, D.A., Barqouthi, H., Niaz, Q. and Carvounis, C.P. (2002). Massive and disproportionate elevation of blood urea nitrogen in acute azotemia. *International Urology and Nephrology,* 34 (1): 143-145.

File, S. and Pellow, S. (1985). The effect of triazolobenzodiazepines in two animal tests of anxiety and on the hole-board. *British Journal of Pharmacology*, 86: 729-735.

File, S. and Wardill, A.G. (1975). Validity of head-dipping as a measure of exploratory modified hole-board. *Psychopharmacology*. 53-59.

Fleidervish, I.A., Friedman, A. and Gutnick, M.J. (1996). Slow inactivation of Na+ current and slow cumulative spike adaptation in mouse and guinea-pig neocortical neurones in slices. *Journal of Physiology,* 493(1): 83–97.

Fujimori, H. (1965). Potentiation of barbital hypnosis as an evaluation method for central nervous system depressant. *Psychopharmacology*, 7: 374-377.

Gerber, U. and Gahwiler, B.H. (1994). Modulalation of ionic currents by metabotropic glutamate receptors. *In*: Conn, P.J., Patel, J. (Eds). *The Metabotropic Glutamate Receptors*. Totowa, N.J. Human Press, pp.125-146.

Gerlach, A.C. and Krajewski, J.L. (2010). Antiepileptic drug discovery and development: What have we learned and where are we going? *Pharmaceuticals*, 3: 2884-2899.

Giarsa, J.L., McLean, H. and Conger, P. (1995). Postnatal maturation of GABA-A and B-mediated inhibition in CA3 hippocampal region in the rat. *Journal of Neurobiology*, 26: 339-349.

Gitto, R., Pagano, B., Citraro, R., Scicchitano, F., DeSarro, G. and Chimirri, A. (2009). Solution phase parallel synthesis and evaluation of anticonvulsant activity of N-substituted-3,4-dihydroisoquinoline-2(1H)-carboxamides. *European Journal of Medicinal Chemistry*, 44: 1349-1354.

Glaum, S.R., Miller, R.J. (1994). Acute regulation of synaptic transmission by metabotropic glutamate receptors. *In*: Conn, P.J., Patel, J. (Eds). *The Metabotropic Glutamate Receptors*. Totowa, N.J. Humana Press, pp. 147-172.

Goldin AL (2003) Mechanisms of sodium channel inactivation. *Current Opinion in Neurobiology,* 13**:**284–290.

Gottesmann, C. (2002). GABA mechanisms and sleep. *Neuroscience,* 111: 231-239.

Gupta, Y.K., Shirma, M. and Chaudhary, G. (2001). Antiepileptic activity of *Panax Ginseng* against pentylenetetrazole-induced kindling in rats. *Indian Journal of Pharmacology*, 45(4): 502-506.

Hamil, O.P., Marty, A., Neher, E., Sakmann, B. Sigworth, F. (1981). Improved patch clamp technique for high resolution current recordings from cells and cell free membranes. *Pflugers Arch*., 391: 85-100.

Hargus, N.J., Merrick, E.C., Nigam, A., Kalmar, C.L., Baheti, A.R., Bertram, E.H. and Patel, M.K. (2011). Temporal Lobe Epilepsy Induces Intrinsic Alterations in Na Channel Gating in Layer II Medial Entorhinal Cortex Neurons, *Neurobiology Disorder*, 41(2): 361–376.

Harris, E.H. (2005). Elevated liver function tests in type 2 diabetes. *Clinical Diabetes*, 23(3): 115-119.

Hau-Jie, Y., Baranauskas, G. and Martina, M. (2010) Flufenamic acid decreases neuronal excitability through modulation of voltage-gated sodium channel gating. *Journal of Physiology*, 588(20): 3869-3882.

Heinemann, S.H., Terlau, H., St¨uhmer, W., Imoto, K. and Numa, S. (1992). Calcium channel characteristics conferred on the sodium channel by single mutations. *Nature,* 356, 441–443.

Hille, B. (1975). Local anesthetics: hydrophilic and hydrophobic pathways for the drug-receptor reaction. *Journal of General Physiology,* 69**:**497–515.

Hondeghem, L.M. and Katzung, B.G. (1977). Time- and voltage-dependent interactions of antiarrhythmic drugs with cardiac sodium channels. *Biochim Biophys Acta* 472: 373–398.

Hormuzdi, S.G., Filippov, M.A., Mitropoulou, G.I., Monyer, H. and bruzzone, R. (2004). Electrical synapses: a dynamic signalling system that shapes the activity of neuronal networks. *Biochemisrty Biophysics Acta*, 1662: 113-137.

Horrom, B.W. and Lynes, T.E. (1962). The anticonvulsant properties of some substituted benzamides. *Federation Proc*., 6: 528-532.

Idris, A.Y. (2008). Synthesis and Anticonvulsant Studies of 3-Anilinopropanamides and their N-Benzyl Derivatives. Unpublished Dissertation, Department of Pharmaceutical and Medicinal Chemistry, Ahmadu Bello University, Zaria.

Idris, A.Y., Haruna, A.K., Sule, M.I., Yaro, A.H., Sallau, M.S. and Musa, A.M. (2008a). Synthesis and anticonvulsant studies of 3-anisidinopropanamides. *International Journal of Pure and Applied Sciences,*, 2(3): 132-140.

Idris, A.Y., Haruna, A.K., Sule, M.I., Yaro, A.H., Sallau, M.S. and Musa, A.M. (2008b). Synthesis and anticonvulsant studies of Chloro-3-anilinopropanamides. *Journal of Biological and Environmental Sciences for the Tropics*, 5(3): 168-176.

Idris, A.Y., Haruna, A.K., Sule, M.I., Yaro, A.H., Sallau, M.S. and Musa, A.M. (2008c). Synthesis and anticonvulsant studies of 3-toluidinopropanamides. *Nigerian Journal of Pharmaceutical Sciences*, 7(1): 93-107.

Idris, A.Y., Sule, M.I. and Sallau, M.S. (2009). Synthesis and anticonvulsant studies of N-benzyl-3-anisidinopropanamides (N-benzyl-3-[(methoxyphenyl)amino]propanamides). *Nigerian Journal of Pharmaceutical Sciences*, 8(1): 118-127.

Inan, S.Y. and Buyukafsar, K. (2008). Antiepileptic effects of two Rho-kinase inhibitors, Y-27632 and fusudil, in mice. *British Journal of Pharmacology*, 155: 44-51.

Jentsch, T.J., Stein, V. Weinreich, F. and Zdebik, A.A. (2002). Molecular structure and physiological function of chloride channels. *Physiology Review*, 82: 503–568.

Jones, P.J., Merrick, E.C., Batts, T.W. Hargus, N.J., Wang, Y., Stables, J.P. Bertram, E.H. Brown, M.L. and Patel, M.K. (2009). Modulation of sodium channel inactivation gating by a novel Lactam: Implications for seizure suppression in chronic Limbic Epilepsy. *Journal of Pharmacology and Experimental Pharmacology*, 328(1): 201-212.

Jones, P.J., Wang, Y., Smith, M.D., Hargus, N.J., Eidam, H.S. White, S., Kapur, J., Brown, M.L and Patel, M.K. (2007). Hydroxyamide analogs of propofol exhibit state-dependent block of sodium channels in hippocampal neurons: Implications for anticonvulsant activity. *Journal of Pharmacology and Experimental Pharmacology*, 320 (2): 828-836.

Joshi, S. and Kapur, J. (2012). GABAA Receptor Plasticity During Status Epilepticus. Eds. Noebels, J.L., Avoli, M., Rogawski, M.A., Olsen, R.W. and Delgado-Escueta, A.V., In: *Jasper's Basic Mechanism of Epilepsies*, pp. 1-12.

Kang, H., Park, J., Jeong, S., Kim, J., Moon, H., Perez-Reyes, E. and Lee, J. (2006). A molecular determinant of nickel inhibition in Ca_v 3.2 T-type calcium channels. *Journal of Biological Chemistry*, 281(8): 4823-4830.

Kaul, P.N. and Kulkarni, S.K. (1978). New drug metabolism inhibitor of marine origin. *Journal of Pharmaceutical Sciences*, 67: 1293-1296.

Kayal-Brooks, A.R., Raol,Y.H. and Russek, S.J. (2009). Alteration of Epileptogenesis Genes. *Neurotherapeutics*, 6(2): 312-318.

Keating MT & Sanguinetti MC (2001). Molecular and cellular mechanisms of cardiac arrhythmias. *Cell*, 104, 569–580.

Kemp, W. (1991). Applications of Infrared Spectroscopy- Identification of Functional Groups. *In: Organic Spectroscopy*, 3rd Ed., McMillan Press LTD, Houndmills and London, pp. 56-87.

Klitgaard, H. and Pitkanen, A. (2003). Antiepileptogenesis, neuroprotection, and disease modification in the treatment of epilepsy: focus on levetiracetam. *Epileptic Disorder*, 5(suppl.1): 9-16.

Krall, R.L., Penry, J.K., White, B.G., Kupferberg, H.J. and Swinyard, E.A. (1978). Antiepileptic drug development: II. Anticonvulsant drug screening. *Epilepsia*, 19: 409-428.

Kuo, C.C. and Bean, B.P. (1994). Slow binding of phenytoin to inactivated sodium channels in rat hippocampal neurons. *Molecular Pharmacology*, 46: 716–725.

Kuo, C.C. and Lu, I. (1997). Characterization of Lamotrigine Inhibition of Na+ Channels in Rat Hippocampal Neurons. *British Journal of Pharmacology*, 121: 1231-1238.

Kuo, C.C., Chen, R.S., Lu L. and Chen R.C. (1997). Carbamazepine inhibition of neuronal Na currents: quantitative distinction from phenytoin and possible therapeutic implications. *Molecular Pharmacology*, 51:1077–1083.

Kupferberg, H.J. (1989). Antiepileptic drug development program: A cooperative effort of government and industry. *Epilepsia*, 30(suppl. 1), S51-S56.

Lang, D.G., Wang, C.M. and Cooper, B.A. (1993) Lamotrigine, Phenytoin and Carbamazepine interactions on the sodium current present in N4TG1 mouse neuroblastoma cells. *of Pharmacology and Experimental Pharmacology*, 266(2): 829-835.

Lang, D.G., Wang, C.M. and Cooper, B.A. (1993). Lamotrigine, Phenytoin and Carbamazepine Interactions on the Sodium Current Present in N4TG1 Mouse Neuroblastoma Cells. *Journal of Pharmacology and Experimental Therpeutics*, 266(2): 829-835.

Larson, M.D. (1969). An analysis of the action of strychnine on the recurrent IPSP and amino acid induced inhibitions in the cat spinal cord. *Brain Research*, 15: 185-200.

Laurie, D.J., Wisden, W., Seeburg, P.H. (1992). The distribution of thirteen $GABA_A$ receptor sub-unit mRNAs in the rat brain. Iii. Embryonic and postnatal development. *Journal of Neuroscience*, 12: 4151-4172.

Levitan, I.B. and Kaczmarek, L.K. (2002). *The Neuron: Cell and Molecular Biology*, 3rd Ed. Oxford University Press, New York, pp. 45-139.

Lipkind, G.M. and Fozzard, H.A. (2010). Molecular Model of Anticonvulsant Drug Binding to the Voltage-Gated Sodium Channel Inner Pore. *Molecular Pharmacology*, 78(4): 631-638.

Lorke, D.A. (1983). A new approach to practical acute toxicity testing. *Archives of Toxicology*, 54 (4): 275-287.

Loscher, W. and Meldrum, B. (1984). Evaluation of anticonvulsant drugs in genetic animal models of epilepsy. *Fed. Proc.*, 43(2): 276-284.

Loscher, W., Honack, D., Fassbender, C.P. and Nolting, B. (1991). The role of technical, biological and pharmacological factors in the laboratory evaluation of L-type calcium current by propofol in cortical neurons: implications for its antiepileptic activity. *Epilepsia*, 46:624–635.

Lowestein, D.H. (2001). Seizures and Epilepsy In: (Braunwald, E., Fauci, A.S., Kasper, D.L., Hauser, S.L., Longo, D.L., and Jameson, J.L. (Eds) Harrison's Principles of Internal Medicine 15th edition McGraw-Hill medical publishing division part 14, section 2, 360.

Malawska, B. (2005). Anticonvulsant Agents. *Topics in Medicinal Chemistry,* 5: 69-85.

Marsh, W.H. (1965). Automated and manual direct methods for the determination of blood urea. *Clinical Chemistry*, 2: 624-625.

Martella, G., De Persis, C., Bonsi, P., Natoli, S., Cuomo, D., Bernardi, G., Calabresi, P., and Pisani, A. (2005). Inhibition of persistent sodium current fraction and voltage-gated

Matsumura, F. (1975). *Toxicology of Insecticides*. Plenum Press, New York, p. 263.

Mbuba, C.K. and Newton, C.R. (2009). Packages of care for epilepsy in low and middle-income countries. *PLos Medicine*, 6(10): 1-7.

McNamara, J.O. (1996). Drugs effective in the management of epilepsies. In: Goodman and Gilman's. *The pharmacological Basis of Therapeutics*. Hardman, J.G., Limbird, L.E., Molinoff, P.B., Ruddon, R.W. and Gilman, A.G. (Eds). McGraw-Hill Co. Inc. pp 461-486.

McNamara, J.O. (2001). Drugs effective in the therapy of the epilepsies. In: Gilman's, G. (Ed), *The Pharmacological Basis of Therapeutics*, pp. 521 – 523.

McNamara, J.O. (2006) Pharmacotherapy of the epilepsies, in *Goodman & Gilman's Pharmacological Basis of Therapeutics*, 11th ed (Brtunton LL, Lazo JS, Parker KL eds) pp 501–526, McGraw-Hill, New York.

Miller, L.C. and Tainter, M.L. (1994). Estimation of ED_{50} and its error by log. Probit graph paper. *Proceedings of the Royal Society for Experimental Biology and Medicine*, 57: 261-264.

Nagakannan, P., Shivasharan, B.D. Veerapur, V.P. and Thppeswamy, B.S. (2011). Sedative and antiepileptic effects of *Anthocephalus cadamba* Roxb. in mice and rats. *Indian Journal of Pharmacology*, 43(6): 699-702.

Nakese, T., Naus, C.C. (2004). Gap junctions and neurological disorders of the central nervous system. *Biochemistry Biophysics Acta*, 1662: 149-158.

Nestler, E.J., Hyman, S.E. and Malenka, R.C. (2009). The electrical properties of neurons. In: *Molecular Neuropharmaacology, a Foundation for Clinical Neuroscience*, pp. 24-48; p. 367.

N'Gouemo, P., Nguemby-bina, C., Baldy-moulinier, M. (1994). Some neuropharmacological effects of an ethanolic extract of *Maprounea Africana* in rodents. *Journal of Ethnopharmacology,* 43: 161-166.

Nicholas, D.P., Peter, T.M., Kenneth, A.S. and Stephen, D.H. (2002). Recent advances in the modulation of voltage-gated ion channels for the treatment of epilepsy. *Current Drug Targets-CNS and Neurological Disorders*, 1: 81-104.

OECD (1995). Repeated Dose 28-day Oral Toxicity Study in Rodents. *OECD guideline for testing of chemical*, 407: 1-8.

Ogunrin, A.O. (2006). Epilepsy in Nigeria – A review of etiology, epidemiology and management. *Benin Journal of Postgraduate Medicine*, 8(1): 27-51.

Osuide, G.E. and Wambebe, C. (1979). The influence of intraperitoneally administered 6-hydrodopamine on electroshock seizure in chicks and rats. *Clinical and Experimental Pharmacology and physiology*, 6: 367-372.

Ozbek, H., Ozturk, M., Ozturk, A., Ceylan, E. and Yener, Z. (2004). Determination of Lethal Doses of Volatile and Fixed Oils of Several Plants. *Eastern Journal of Medicine*, 9 (1): 04-06.

Pasquale, S. and Salvatore, S. (2009). New and investigational antiepileptic drugs. *Expert Opinion Investigational Drugs*, 18(12): 1875-1884.

Payandeh J, Scheuer, T, Zheng, N. and Catterall, W.A. (2011). The crystal structure of a voltage-gated sodium channel. *Nature,* 475, 353–358.

Paz, J.T., Bryant, A.S., Peng, K., Fenno, L., Yizhar, O., Frankel, W.N., Deisseroth**,** K. and Huguenard J.R. (2011). A new mode of corticothalamic transmission revealed in the *Gria4*$^{-/-}$ model of absence epilepsy. *Nature Neuroscience,* 14(9): 1167-1175.

Paz, J.T., Davidson, T.J., Frechette, E.S., Delord, B., Parada, I., Peng, K., Deisseroth K. and Huguenard, J.R. (2012). Closed-loop optogenetic control of thalamus as a tool for interrupting seizures after cortical injury. *Nature Neuroscience,*1-10.

Pedersen M.E., Vestergaard, H.T., Hansen, S.L. Bah, S., Diallo, D. and Jager, A.K. (2009). Pharmacological screening of Malian medicinal plants used against epilepsy and convulsions. *Journal of Ethnopharmacology,* 121: 472-475.

Perez, R.M., Perez, J.A., Garcia L.M., Sossa, H.M. (1998). Neuropharmacological activity of solanum nigrum fruit. *Journal of ethnopharmacology*, 62: 43-48.

Pevarello, P., Bonsignori, A., Dostert, P., Heidempergher, F., Pinciroli, V., Colombo, M., McArthur, R.A., Salvati, P., Post, C., Fariello, R.G. and Varasi, M. (1998). Synthesis and

anticonvulsant activity of a new class of 2-[(arylalkyl)amino] alkanamide derivatives. *Journal of Medicinal Chemistry*, 41: 579-590.

Porter, R.J., Cereghino, J.J. and Gladding, G.D. (1984). Antiepileptic drug development program. *Cleve Clinical*, 51: 293-305).
Rajabzadeh, A., Bideskan, A.E., Fazel, A., Sankian, M., Rafatpanah, H. and Haghir, H. (2012). The effect of PTZ-induced epileptic seizures on hippocampal expression of PSA-NCAM in offspring born to kindled rats. *Journal of Biomedical Science*, 19(1): 56-64.

Rang, H.P., Dale, M.M., Ritter, J.M. and Moore, P.K. (2003). *Pharmacology*, 5th ed. Philadelphia: Churchil Livingstone, Elsevier Science Ltd.

Rang, H.P., Dale, M.M., Ritter, J.M. and Moore, P.K. (2007). *Pharmacology*, 6th ed. Philadelphia: Churchil Livingstone, Elsevier Science Ltd. pp.575-587.

Raza, M., Shaheen, F., Choudhary, M.I., Suria, A., Attaur-Rahman, Sombati, S. and DeLorenzo, R.J. (2001). Anticonvulsant activities of the FS-1 subfraction isolated from roots of Delphinium denudatum. *Phytotherapy Research*, 15: 426-430.

Rehni, A.K. and Singh, N. (2009). Reversal of PTZ-induced seizure activity in mice by nickel chloride. *Indian Journal of Pharmacology*, 41(1): 15-18.

Reichling, D.B., Kyrozis, A., Wang, J. and Macdermott, A.B. (1994). Mechanisms of GABA and glycine depolarization-induced calcium transients in rat dorsal horn neurons. *Journal of Physiology*, 476: 411–421.

Rho, J.M. and Sankar, R. (1999). The pharmacological basis of antiepileptic drug action. *Epilepsia*, 40: 1471-1483.

Rivera, M., Patel, M.K. and Zuliani, V. (2012). Inhibition of NaV1.6 sodium channel currents by a novel series of 1,4-disubstituted-triazole derivatives obtained via copper-catalyzed click chemistry. *Bioorganic and Medicinal Chemistry*, 22: 6401-6404.

Rivera, C., Voipio, J. and Payne, J.A. (1999). The K/Cl co-transporter KCC2 renders GABA hyperpolarizing during neuronal maturation. *Nature*, 397: 251-255.

Robertson, D.W., Leander, J.T., Lawson, R., Beedle, E.E., Clark, C.R., Potts, B.D. and Parli, C.J. (1987). Discovery and anticonvulsant activity of the potent metabolic inhibitor 4-amino-N-(2,6-dimethylphenyl)-3,5-dimethylbenzamide. *Journal of Medicinal Chemistry*, 30(10):1742-1746.

Rogawski MA and Loscher W (2004) The neurobiology of antiepileptic drugs. *Nature Review Neuroscience*, 5**:**553–564.

Rogawski, M.A. and Porter**,** R.J. (1990). Antiepileptic drugs: Pharmacological mechanisms and clinical efficacy with consideration of promising developmental stage compounds. *Pharmacology Review*, 42: 223-286.

Roger, J.P. and Brians, S.M. (2004). Antiseizure Drugs. *In*: Katzung, B.G, (Ed). *Basic and Clinical Evaluation of New Drugs*, Lange Medical Books/ McGraw-Hill Medical Publishing Division.

Russel, M.S. and Frances, E.J. (2001). Maturstional aspects of epilepsy mechanisms and consequences for the immature brain. *Epilepsia*, 42(5): 577-585.

Sandrini, M. Marrama, D., Vergoni, A.V. and Bertolini, A. (1992). Repeated administration of triiodothyronine enhances the susceptibility of rats to isoniazid- and picrotoxin-induceed seizures. *Life Science*, 51: 765-770.

Schlam, O.W., Jain, N.C. and Caroll, E.J. (1975). Veterinary haematotology, 3rd ed. Lea and Tebiger Publishers, Philadelphia, 207-209.

Shindikar, A.V., Khan, F. and Viswanathan, C.L. (2006). Design, synthesis and invivo anticonvulsant screening in mice of novel phenylacetamides. *European Journal of Medicinal Chemistry*, 41: 786-792.

Siddiqui, N., Rana, A., Khan, S.A., Haque, S.E., Arshad, M.F., Ahmed, S. and Ahsan, W. (2009). Synthesis and preliminary screening of benzothiazol-2-yl thiadiazole derivatives for anticonvulsant activity. *Acta Pharma*, 59: 441-451.

Sigma Diagnostic (1985). Transaminase (ALT/GPT) and (AST/GOT). Procedure No. 505.

Sigma Diagnostic (1987). ALP Optimised Alkaline Phosphatase. Procedure No. DG 1245.

Singh, D. and Goel, R.K. (2010). Proconvulsant potential of cyproheptadine in experimental animal models. *Fundamental and Clinical Pharmacology*, 24(4): 451-455.

Sokolov, S., Scheuer, T. and Catterall, W.A. (2008). Depolarization-activated gating pore current conducted by mutant sodium channels in potassium-sensitive normokalemic periodic paralysis. *Proc Natl Acad Sci USA* 105, 19980–19985.

Soulimani, R., Younus, C., Jamouni-Idrissi, S.,Bousa, D., Khalonki, F. and Haila, A. (2001). Behavioral and pharmacological study of Papaver rhoeas L. in mice. *Journal of Ethnopharmacology*, 74: 265-274.

Spampanato, J., Escayg, A., Meisler, M.H. and Goldin, A.L. (2001). Functional effects of two voltage-gated sodium channel mutations that cause generalized epilepsy with febrile seizures plus type 2. *Journal of Neuroscencei* 21**:**7481–7490.

Stables, J.P. and Kupferberg, H.J. (1997). The NIH anticonvulsant drug development (ADD) program: preclinical anticonvulsant screening project. In: Avanzini, G., Regesta, G., Tanganelli, I. and Avoli, M. (Eds). *Molecular and Cellular Target for antiepileptic drugs*. John Libbey and Company Ltd. pp. 191-198.

Stanley, J.L., Lincoln, R.J., Brown, I.A., McDonald, L.M., Dawson, G.R. and Reynolds, D.S. (2005). The mouse beam walking assay offers improved sensitivity over the mouse rotarod in determining motor coordination deficits induced by benzodiazepine. *Journal of Psychopharmacology*, 19(3): 221-227).

Steve, E. and Marcel, J.D.G (2009). Subtype-selective Targetting of Voltage-gated Sodium Channels. *British Journal of Pharmacology*, 158: 1413-1425.

Swann, J.W., Pierson, M.G. and Smith, K.L. (1999). Developmental neuroplasticity: roles in early life seizures and chronic epilepsy. *Advance Neurology*, 79: 203-216.

Swinyard, E.A. (1969). Laboratory evaluation of antiepileptic drugs. Reviews of Laboratory methods. *Epilepsia*, 10: 107-119.

Swinyard, E.A. (1972). Electrically induced convulsions. *In*: Purpura, D.P., Penry, J.K., Tower, D.B., Woodbury, D.M. and Walter, R.D. (Eds). *Experimental Models of Epilepsy*. A manual for the laboratory worker, Raven press: New York, pp. 433-458.

Swinyard, E.A. and Kupberg, H.J. (1985). Antiepileptic drugs: detection, quantification and evaluation. *Fed proc.* 44: 39-43.

Swinyard, E.A., Woodhead, J.H., White, H.S. and Franklin, M.R. (1989). General principles: experimental selection, quantification and evaluation of anticonvulsants. In: *Antiepileptic Drugs*, Third Edition, Levy, H., Mattson, R.H., Melrum, B., Penry, J.K. and Dreifuss, F.E. (eds.), New York, Raven Press, pp. 85-102.

Takeda, H., Tsugi, M.,Matsumiya, T. (1998). Changes in head dipping behaviour in the hole-board test reflect the anxiogenic and/or anxiolytic state in mice. *Europe Journal of Pharmacology*, 350: 21-29.

Takemiya, T., Suzuki, K. Sugiura, H. Yasuda, S., Yamagata, K. and Kawakami, Y. (2003). Inducible brain COX-2 facilitates the recurrence of hippocampal seizures in mouse rapid kindling. *Prostaglandin other Lipid Mediator*, 71: 205-216.

Takeuchi, A. and Takeuchi, N. (1969). A study of the action of picrotoxin on the inhibitory neuromuscular junction of the crayfish. *Journal of Physiology*, 205: 377-391.

Tarnawa I, Bo¨lcskei H, and Kocsis P (2007) Blockers of voltage-gated sodium channels for the treatment of central nervous system diseases. *Recent Pat CNS Drug Discovery* 2**:**57–78.

Tasso, S.M., Bruno-Blanch, L.E. and Estiú, G.L. (2001). Pharmacophore model for antiepileptic drugs acting on sodium channels. *Journal of Molecular Model,* 7:231–239.

Taylor, C.P. (1995). Gabapentin: mechanism of action. In: *Antiepileptic Drugs*, 4th ed. Levy, R.H., Mattson, R.H. and Meldrum, B.S. (eds). Raven Press, New York, pp. 829-841.

Terrenoire C, Simhaee D and Kass RS (2007). Role of sodium channels in propagation in heart muscle: how subtle genetic alterations result in major arrhythmic disorders. *J Cardiovascular Electrophysology,* 18, 900–905.

Tobler, I., Koff, C., Deboer, T. and Rudolph, U. (2001). Diazepam-induced changes in sleep: Role of the α1 $GABA_A$ receptor subtype. *PNAS*, 98(11): 6464-6469.

Todorovic, S.M. and Lingle, C.J. (1998). Pharmacological properties of T-type Ca^{+2} current in adult rat sensory neurons: Effect of anticonvulsant and anesthetic agents. *Journal of Neurophysiology*, 79: 240-252.

Tu, B., Timofeeva, O., Jiao, Y., Nadler, J.V. (2005). Spontaneous release of neuropeptide Y tonically inhibits recurrent mossy fiber synaptic transmission in epileptic brain. *Journal of Neuroscience*, 25: 1718-1729.

Unverferth, K., Engel, J., Hofgen, N., Rostock, A., Gunther, R., Lankau, H.J., Menzer, M., Rolfs, A., Liebscher, J., Muller, B. and Hofmann, H.J. (1998). Synthesis, anticonvulsant activity, and structure-activity relationships of sodium channel blocking 3-aminopyrroles. *Journal of Medicinal Chemistry*, 41: 63-73.

Vamecq, J., Bac, P., Herrenknecht, C., Maurois, P., Delcourt, P. and Stables J.P. (2000). Synthesis and anticonvulsant and neurotoxic properties of substituted N-phenyl derivatives of the phthalimide pharmacophore. *Journal ofMedicinal Chemistry*, 43: 1311-1319.

Vamecq, J., Lambert, D., Poupaert, J.H., Masereel, B. and Stables, J.P. (1998). Anticonvulsant activity and interactions with neuronal voltage-dependent sodium channel of analogues of ameltolide. *Journal of Medicinal Chemistry*, 41: 3307-3313.

Van Luijtelaar, G., Wiaderna, D., Elants, C. and Scheenen, W. (2000). Opposite effects of T- and L-type Ca^{+2} channels blockers in generalized absence epilepsy. *European Journal of Pharmacology*, 406: 381-389.

Velisek, L., Kusa, R., Kulovana, M. and Mares, P. (1999). Excitatory amino acid antagonists and Pentylenetetrazole-induced seizures during ontogenesis 1. The effects of 2-amino-7-phosphonoheptanoate. *Life Sciences*, 46:1349-1357.

Venance SL, Cannon SC, Fialho D, Fontaine B, Hanna MG, Ptacek LJ, Tristani-Firouzi M, Tawil R & Griggs RC (2006). The primary periodic paralyses: diagnosis, pathogenesis and treatment. *Brain*, 129, 8–17.

Wambebe, C. (1985). Influence of some agents that affect 5-HT metabolism and receptors and nitrazepam-induced sleep in mice. *British Journal of Pharmacology*, 84:185-191.

Wang, J., Yarov-Yarovoy, V., Kahn, R., Gordon, D., Gurevitz, M., Scheuer, T. and Catterall, W.A. (2011). Mapping the receptor site for α-scorpion toxins on a Na+ channel voltage sensor. *Proc Natl Acad Sci U S A* 108, 15426–15431.

Wasterlain, C.G. and Mazarati, A.M. (1997). Neuromodulators and second messengers. *In*: Engel, J.Jr., Pedley, T.A. (Eds). *Epilepsy: a comprehensive textbook*. Philadelphia: Lippincott-Raven, pp. 277-289.

White, H.S. (1997). New mechanisms for antiepileptic drugs II. In: *Epilepsies*. Porter, R. and Chadwick, D. (Eds). Butterworth Heinimann, Boston, pp. 1-30.

White, H.S., Wolf, H.H., Woodhead, J.H., Kupferberg, H.J. (1998). The National Institute of Health anticonvulsant drug development programm: Screening for efficacy. *In*: French, J., Leppik, I.E. and Dichter, M.A. (Eds). *Antiepileptic Drug Development. Advances in Neurology*. Lippincott-Raven publishing; Philadelphia; pp. 29-39.

WHO (1992). Research guidelines for evaluating the safety and efficacy of herbal medicine. WHO regional office for Western Pacific Manila, Philippine, p.38.

Wickenden, A.D. (2002). Potassium channels as antiepileptic drug targets. *Neuropharmacology*, 43: 1055-1060.

Wong, R.K., Bianchi, R. and Taylor, G.W. (1999). Role of metabotropic receptors in epilepsy. *Advance Neurology*, 79: 685-698.

Yamaguchi, S.I. and Rogawski, M.A. (1992). Effects of anticonvulsant drugs on 4-aminopyridine-induced seizures in mice. *Epilepsy Research*, 11: 9-16.

Yarov-Yarovoy V., Baker, D. and Catterall, W.A. (2006). Voltage sensor conformations in the open and closed states in ROSETTA structural models of K+ channels. *Proc Natl Acad Sci U S A* 103, 7292–7297.

Yau, J. (2013). Anticonvulsant and Antitumour Properties of Carissa Edulis (VAHL) Root Bark Extracts in Laboratory Animals. Unpublished PhD Dissertation in Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria.

Yeh, J.Z. and Teneick, R.E. (1987). Molecular and Structural Basis of Resting and Use-Dependent Block of Sodium Current Defined Using Disopyramide Analogues. *Biophysics,* 51: 123-135.

Yerima, M. (2013): Antihyperglycemic Activity of the Methanol Stem-Bark Extract of *Tamarindus Indica* L. (Caesalpiniaceae) on Experimentally Induced Hyperglycemic Wistar Rats. Unpublished Dissertation, Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria.

Yusuf, R., Aliyu, I.S., Anaja, P.O. and Muktar, H.M. (2010). Serum electrolytes, calcium, phosphate and uric acid in HIV/AIDS patients on HAART in a tertiary hospital in Northern Nigeria. *Nigerian Journal of Pharmaceutical Sciences*, 9(1): 101-108.

Zhang, X.F., Hu, X.T. and White, F.J. (1998). Whole-cell plasticity in cocaine withdrawal: reduced sodium currents in nucleus accumbens neurons. *Journal of Neuroscience*, 18: 488–498.

Zhang, J.Z., Yarov-Yarovoy, V., Scheuer, T., Karbat, I., Cohen L, Gordon D, Gurevitz M and CatterallWA (2011). Structure-function map of the receptor site for β-scorpion toxins in domain II of voltage-gated sodium channels. *Journal of Biology and Chemistry,* 286, 33641–33651.

Zhang, N., Wei, W., Mody, I. and Houser, C.R.(2007). Altered localization of GABA(A) receptor subunits on dentate granule cell dendrites influences tonic and phasic inhibition in a mouse model of epilepsy. *Journal of Neurosciece*,27(28): 7520-7531.

Zhou, Y., Morais-Cabral, J.H., Kaufman, A. and MacKinnon, R. (2001). Chemistry of ion coordination and hydration revealed by a potassium channel-Fab complex at 2.0 A resolution. *Nature,* 414: 43–48.

APPENDIX A: Determination of TD_{50} for DCP23 Using Walking Beam Test for Motor Coordination Deficit

Log Dose – Probit Values for TD_{50} of DCP23

Log Dose	Probit
1.7	3.25
1.88	3.98
2	5
2.18	6.64

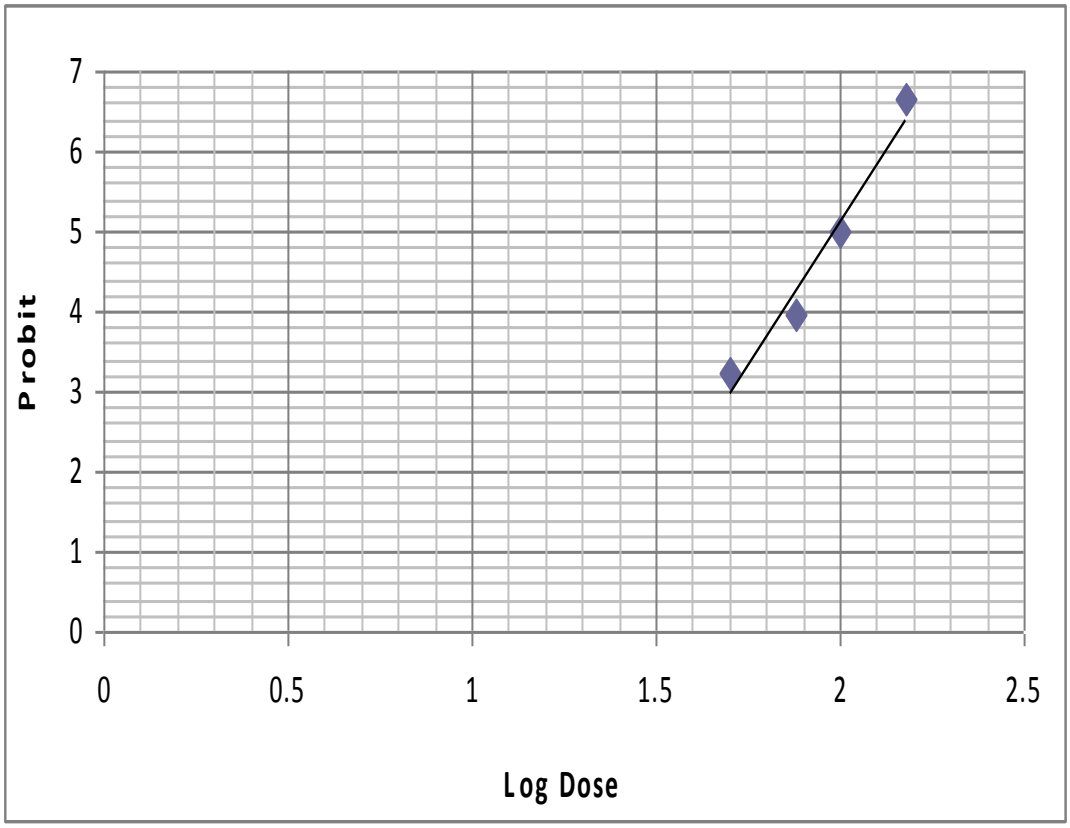


FIGURE 1: Graphical Determination of TD_{50} for DCP23 using Beam Walking Test for Motor Coordination Deficit

APPENDIX B: Determination of ED_{50} for DCP23 Using Maximal electroshock-induced Seizure Test

Log Dose – Probit Values for ED_{50} of DCP23

Log Dose	Probit
0.7	3.19
1.1	3.9
1.4	4.8
1.7	5.53
1.88	6.7

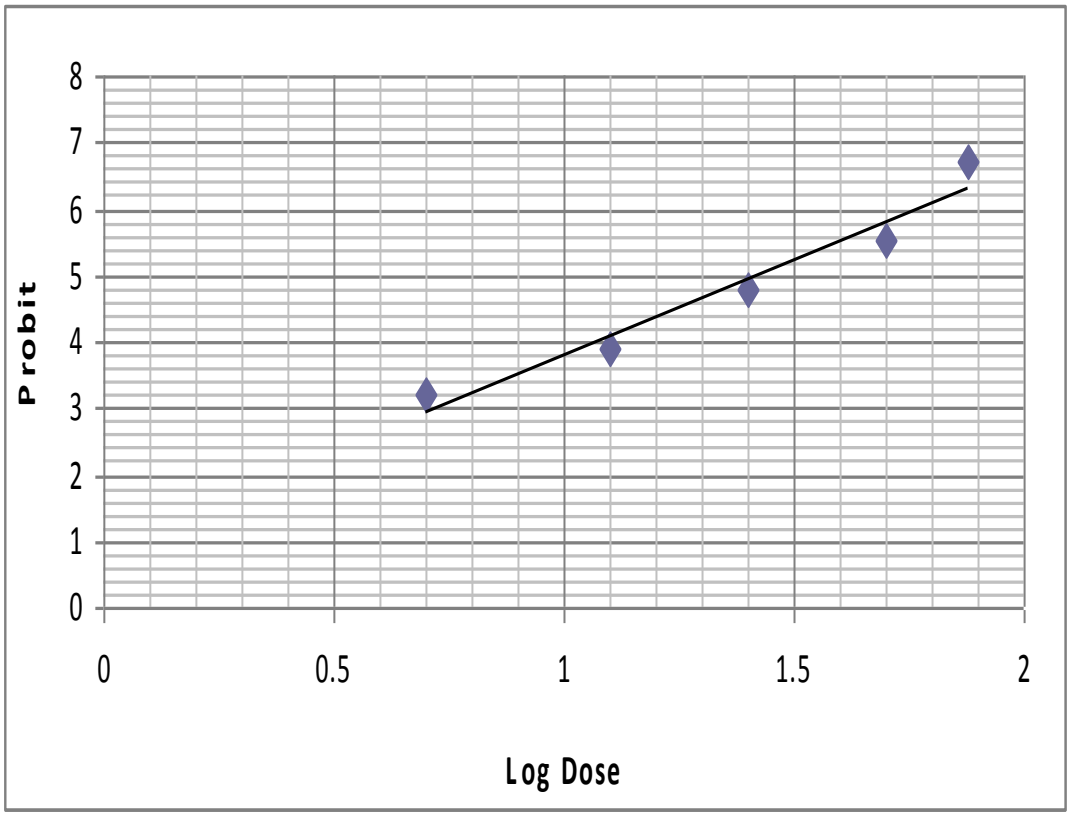


FIGURE 2: Graphical Determination of ED_{50} for DCP23 Using Maximal electroshock-induced Seizure Test

APPENDIX C: Determination of TD_{50} for DCP25 Using Beam Walking Test for Motor Coordination Deficit

Log Dose – Probit Values for TD_{50} of DCP25

Log Dose	Probit
1.7	3.25
1.88	4.53
2	5
2.18	6.64

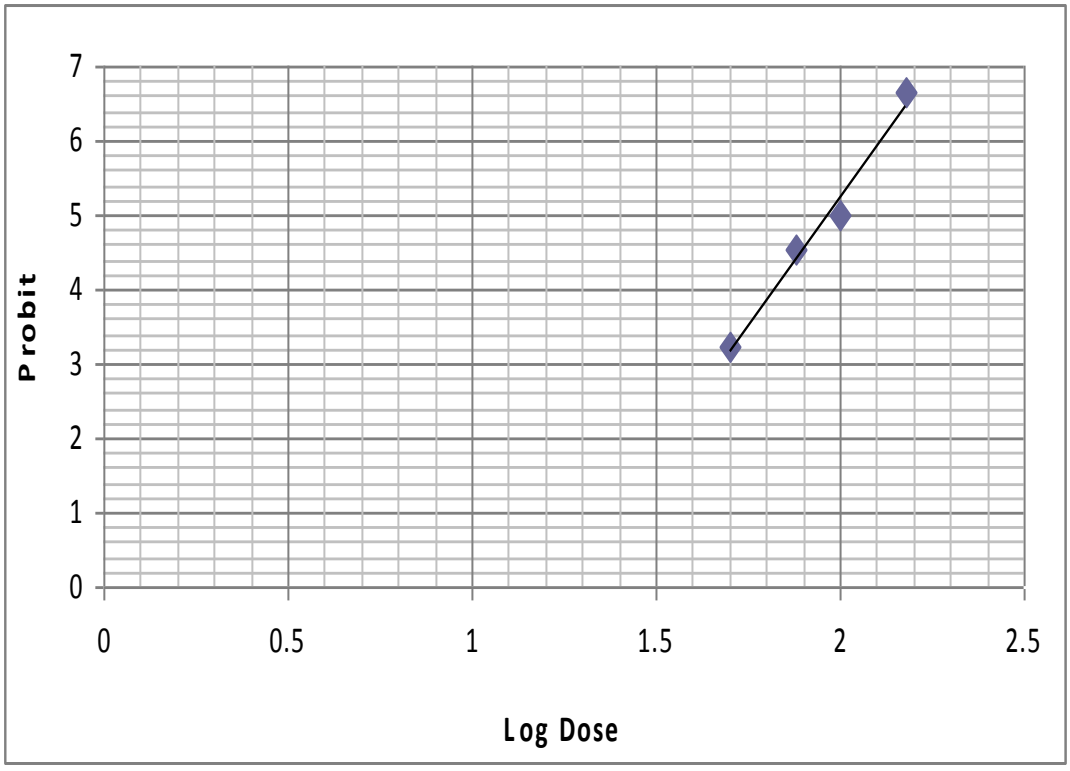


FIGURE 3: Graphical Determination of TD_{50} for DCP25 using Beam Walking Test for Motor Coordination Deficit

APPENDIX D: Determination of ED_{50} for DCP25 Using Maximal electroshock-induced Seizure Test

Log Dose – Probit Values for ED_{50} of DCP25

Log Dose	Probit
1.1	3.19
1.4	4.41
1.7	5.2
1.88	5.53
2	6.7

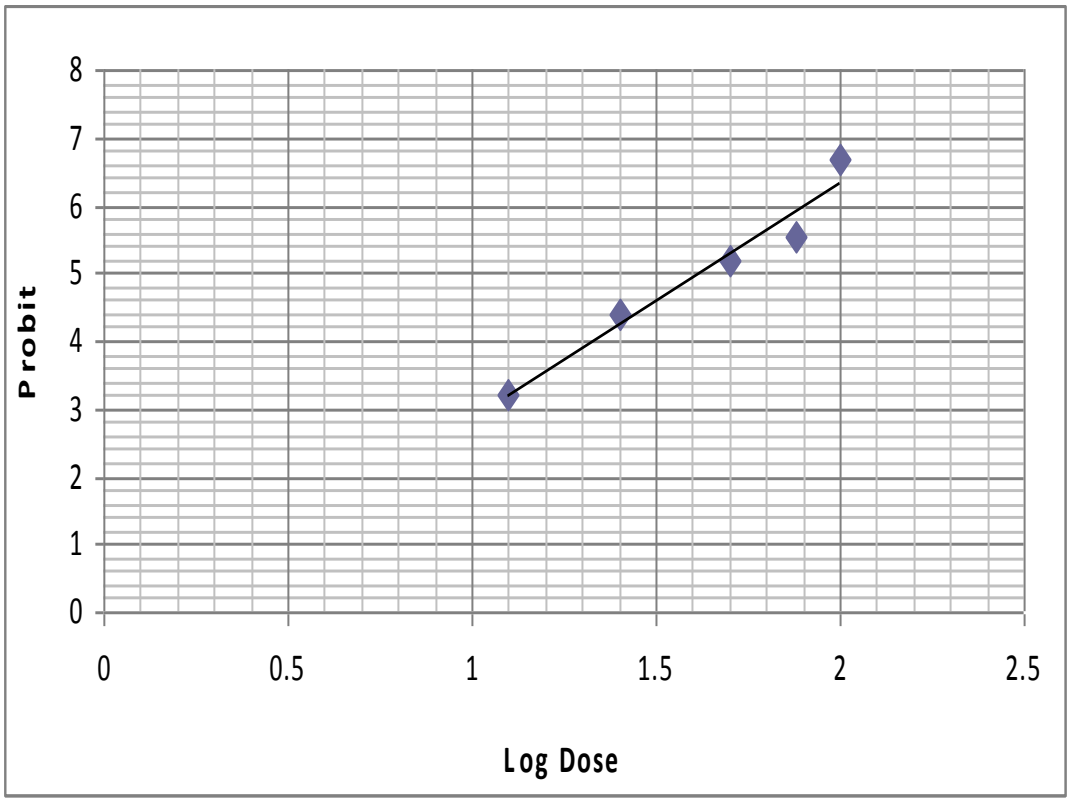


FIGURE 4: Graphical Determination of ED_{50} for DCP25 Using Maximal electroshock-induced Seizure Test

APPENDIX E: Determination of TD_{50} for DCP34 Using Beam Walking Test for Motor Coordination Deficit

Log Dose – Probit Values for TD_{50} of DCP34

Log Dose	Probit
1.7	3.25
1.88	3.98
2	4.53
2.18	5.92
2.24	6.64

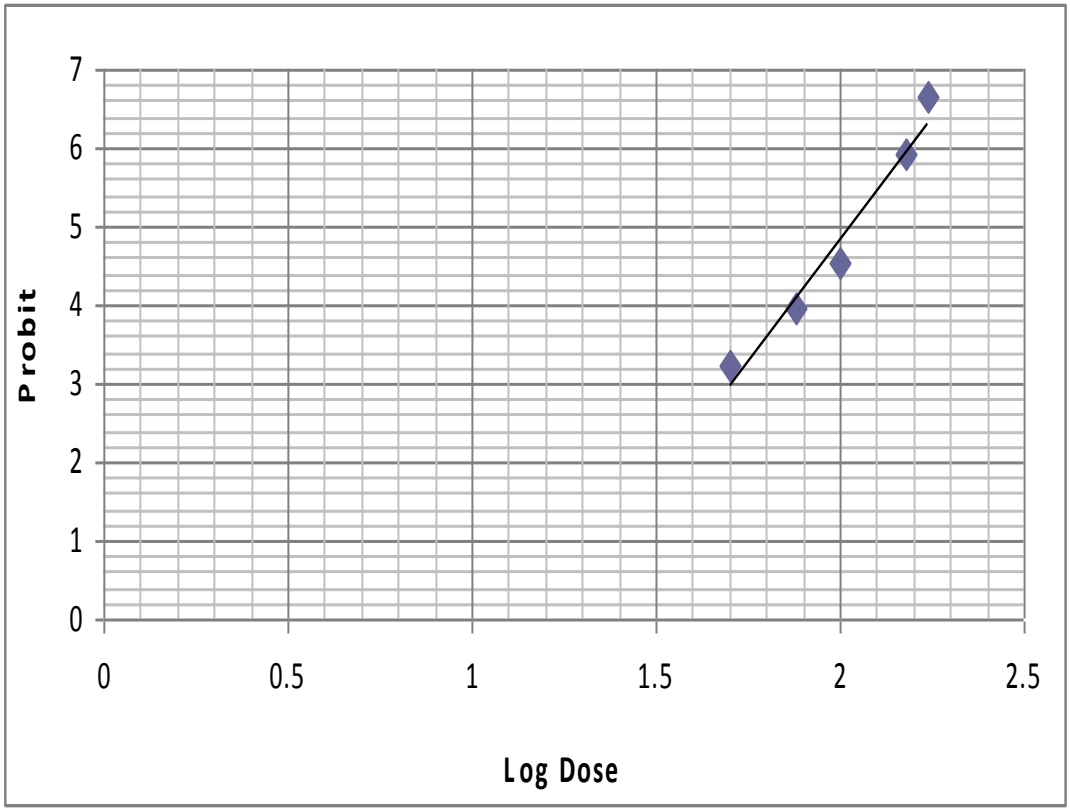


FIGURE 5: Graphical Determination of TD_{50} for DCP25 using Beam Walking Test for Motor Coordination Deficit

APPENDIX F: Determination of ED_{50} for DCP34 Using Maximal electroshock-induced Seizure Test

Log Dose – Probit Values for ED_{50} of DCP34

Log Dose	Probit
1.1	3.19
1.4	3.9
1.7	4.8
1.88	5.53
2	6.7

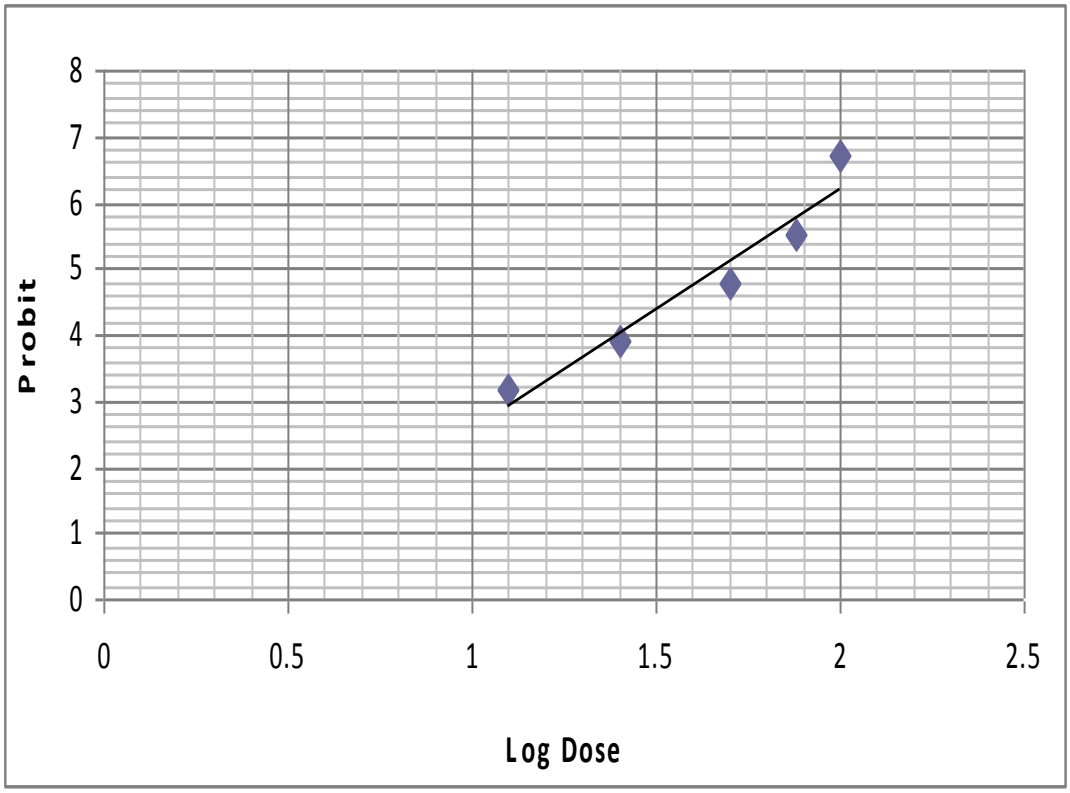


FIGURE 6: Graphical Determination of ED_{50} for DCP34 Using Maximal electroshock-induced Seizure Test

APPENDIX G: Determination of ED_{50} for DCP23 Using Picrotoxin-induced Test

Log Dose – Probit Values for ED_{50} of DCP23

Log Dose	Probit	PICROTOXIN
1.1	3.25	
1.4	5	
1.7	5.41	
1.88	5.92	
2	6.64	

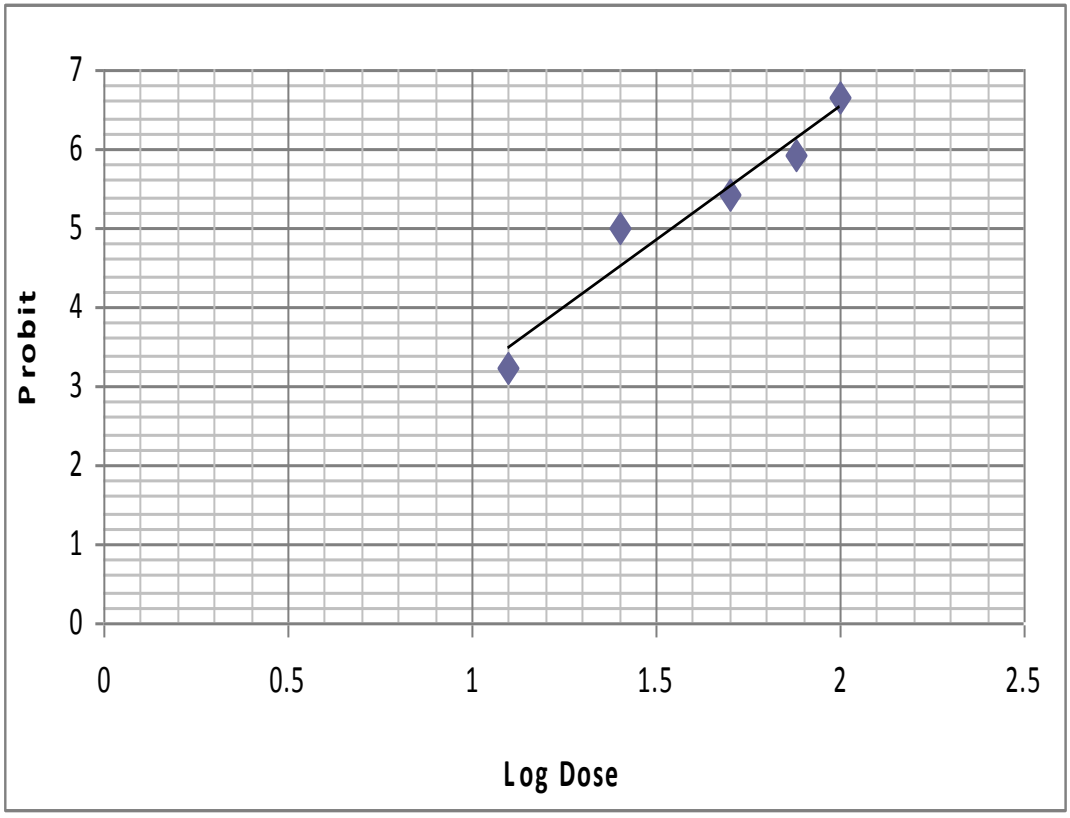


FIGURE 7: Graphical Determination of ED_{50} for DCP23 Using Picrotoxin-induced Seizure Test

APPENDIX H: Determination of ED_{50} for DCP25 Using Picrotoxin-induced Test

Log Dose – Probit Values for ED_{50} of DCP25

Log Dose	Probit	PICROTOXIN
1.1	3.25	
1.4	5.41	
1.7	5.92	
1.88	6.64	

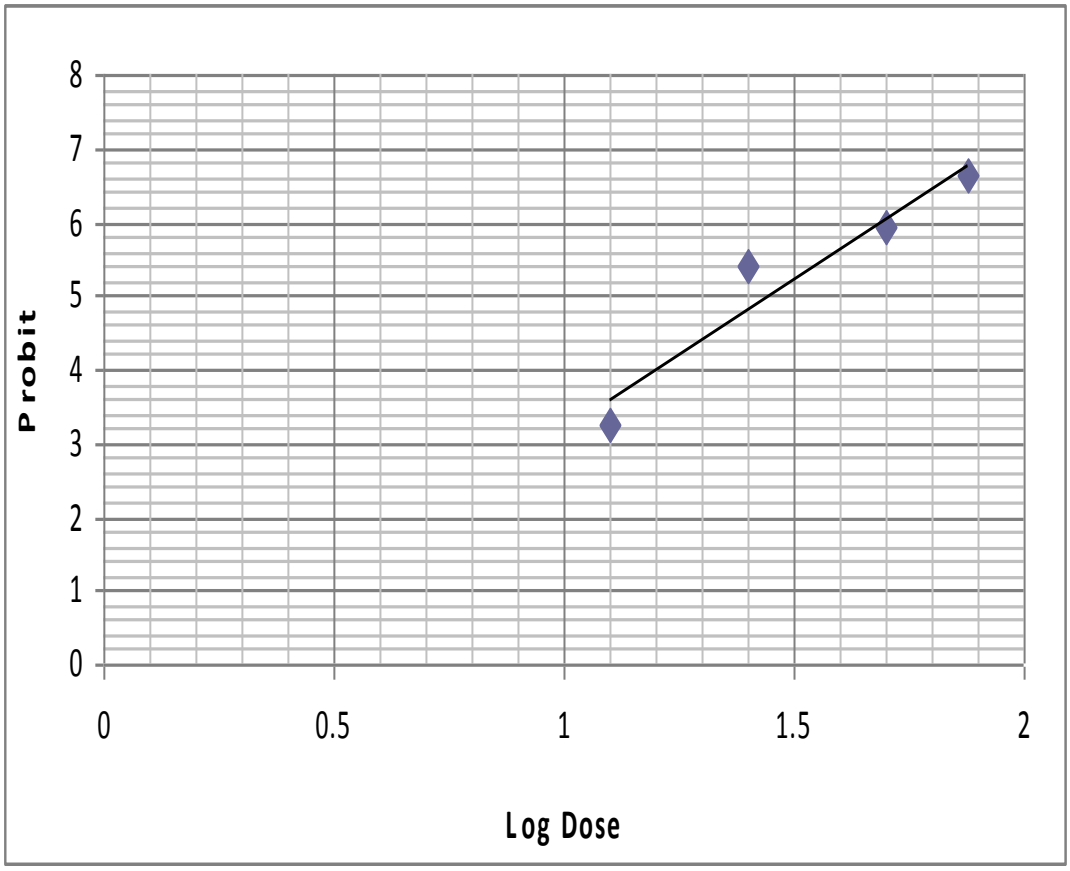
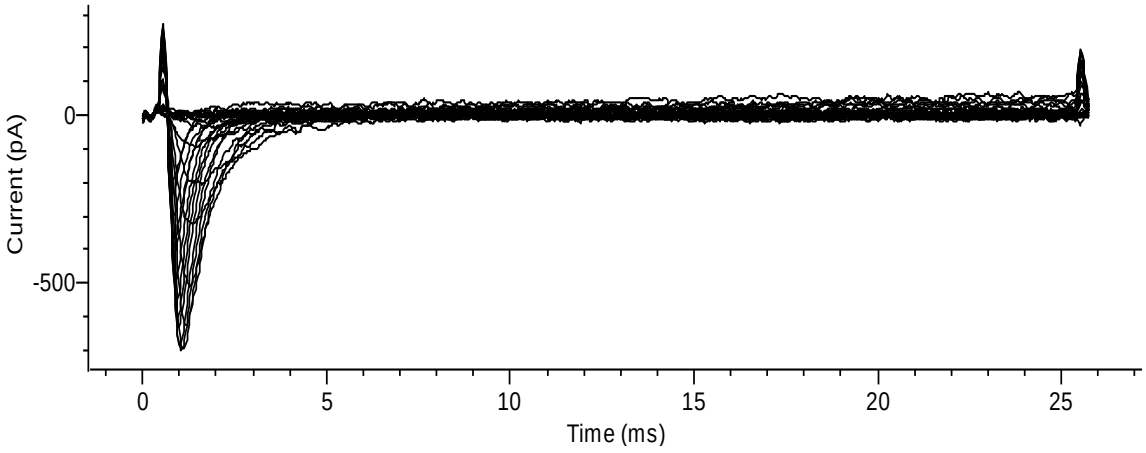


FIGURE 8: Log Dose - Probit Graphical Determination of ED_{50} for DCP25 Using Picrotoxin-induced Seizure Test

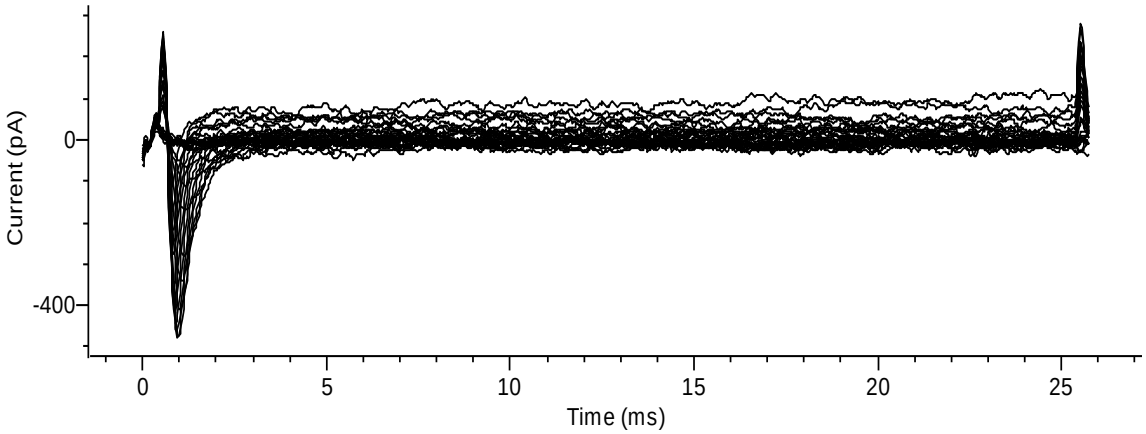
APPENDIX I: SAMPLE OF POLYGRAPHS RECORDINGS FOR Nav 1,6 CURRENTS

Polygraphs for the Effect of 100 µM DCP23 on Current-Voltage (I-V) of Na_v 1.6

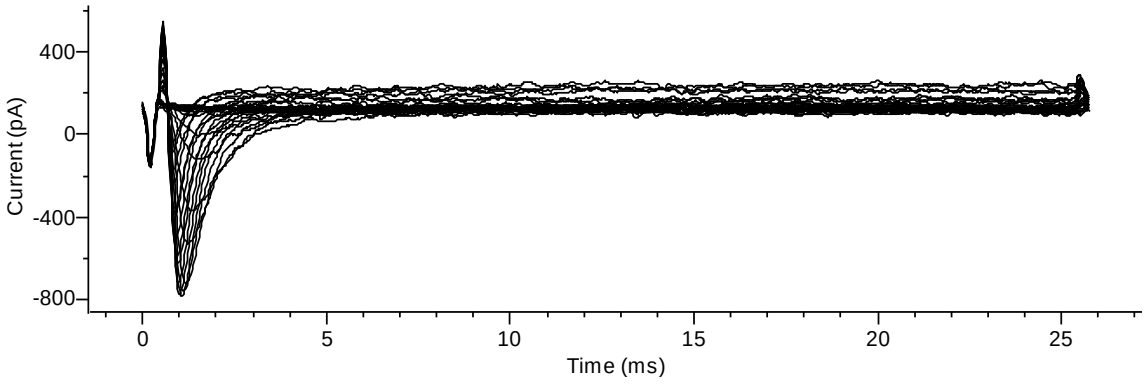
Control

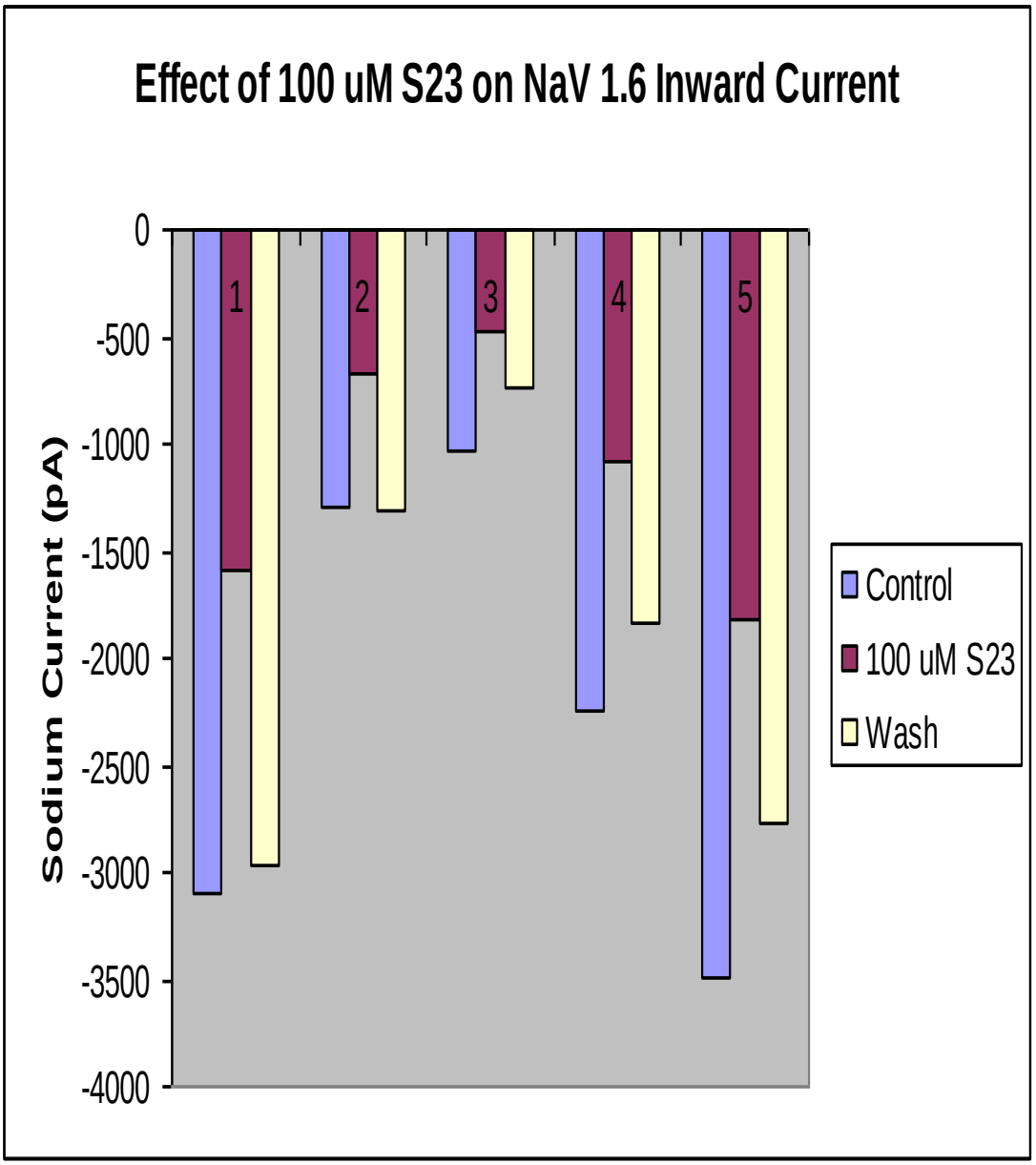


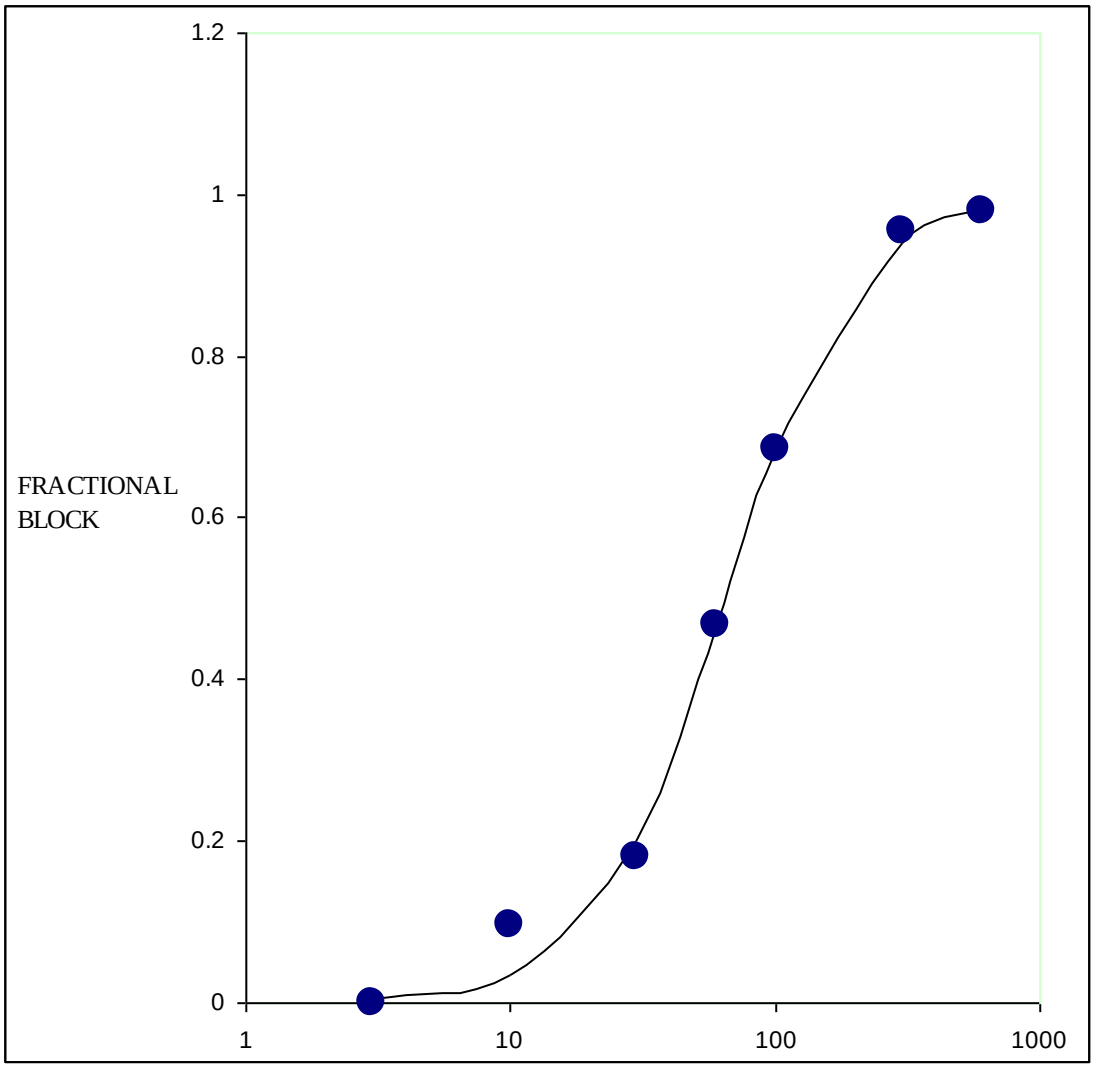
DCP23



After wash

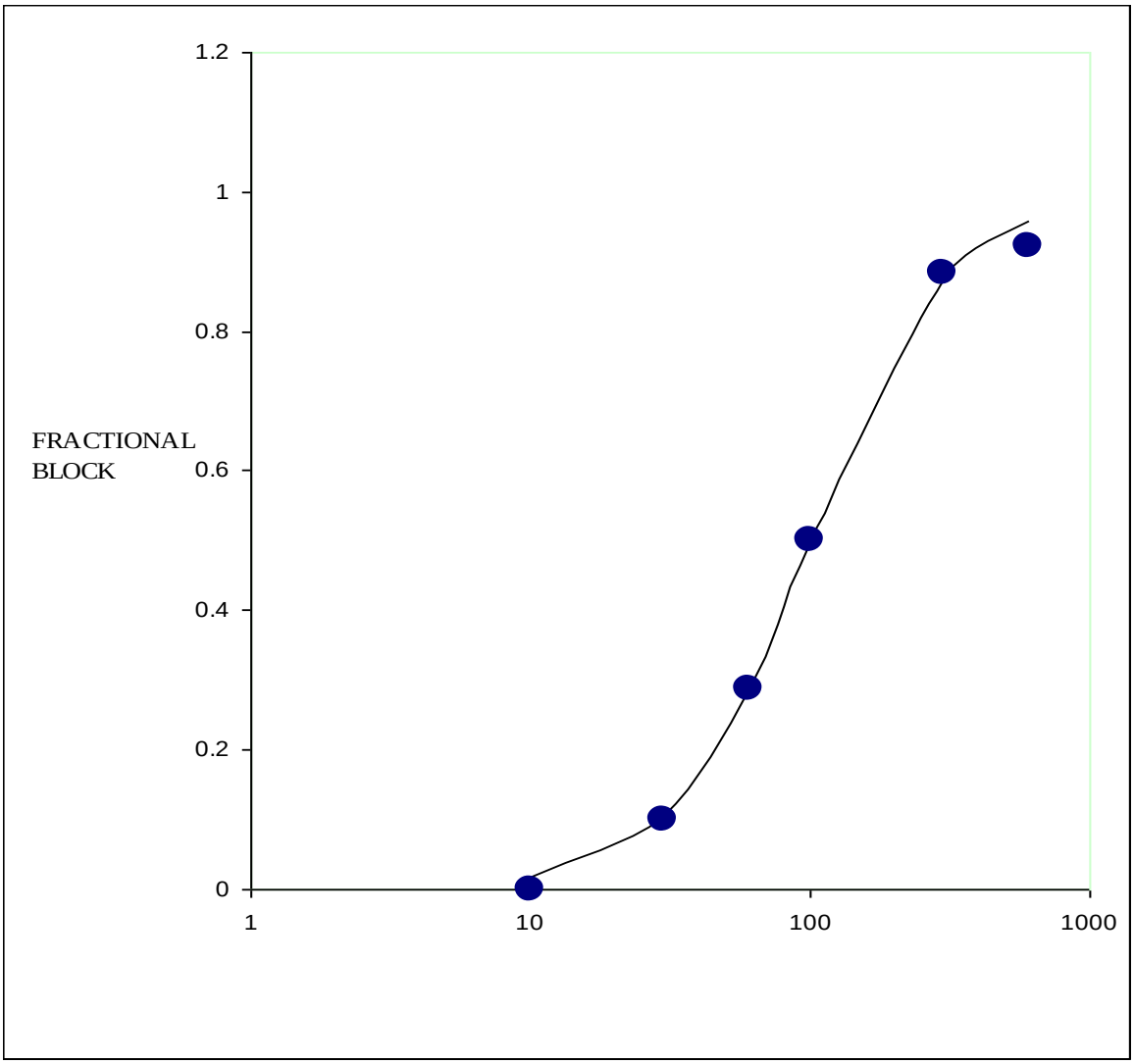



Effect of 100 uM S23 on NaV 1.6 Inward Current
Sodium Current (pA)
0
-500
-1000
-1500
-2000
-2500
-3000
-3500
-4000
1
2
3
4
5
Control
100 uM S23
Wash



X – Axis = Concentration; IC_{50} (µM) = 64.76; k = 1.795137

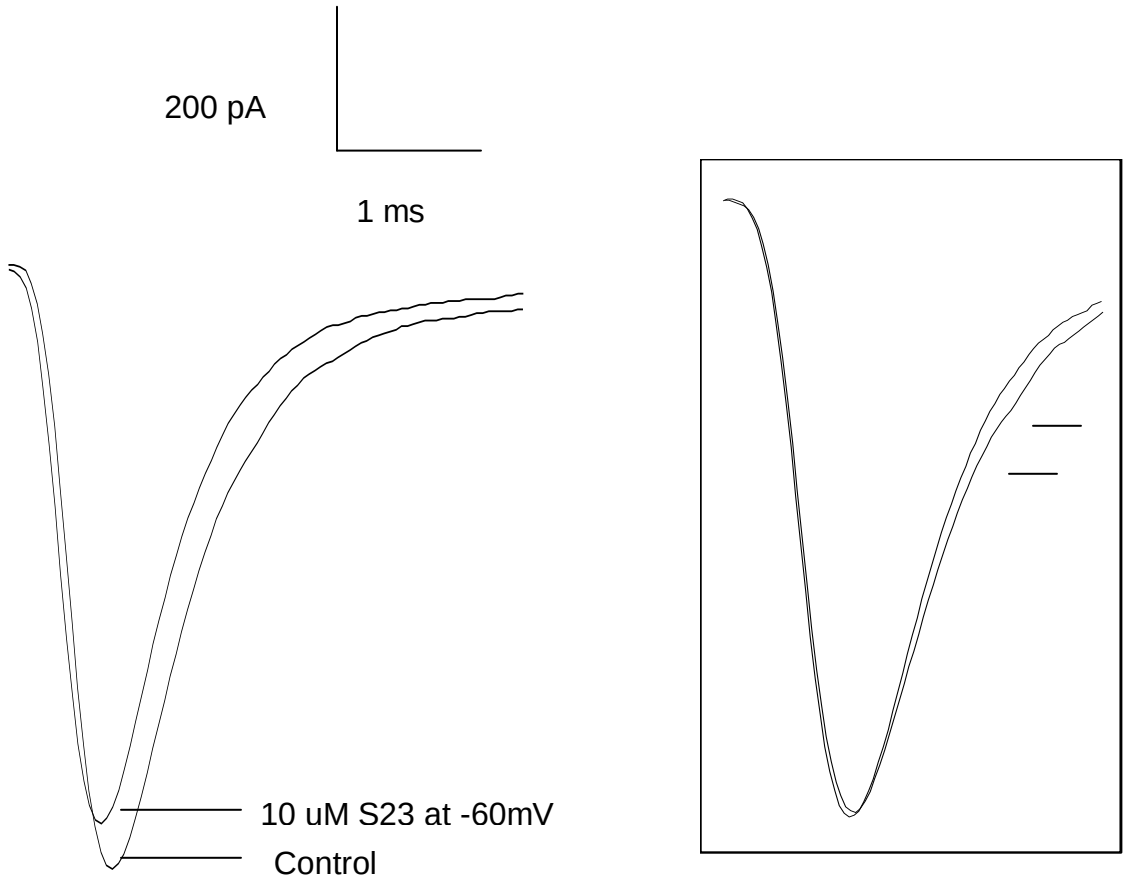
RESTING STATE (-60mV)

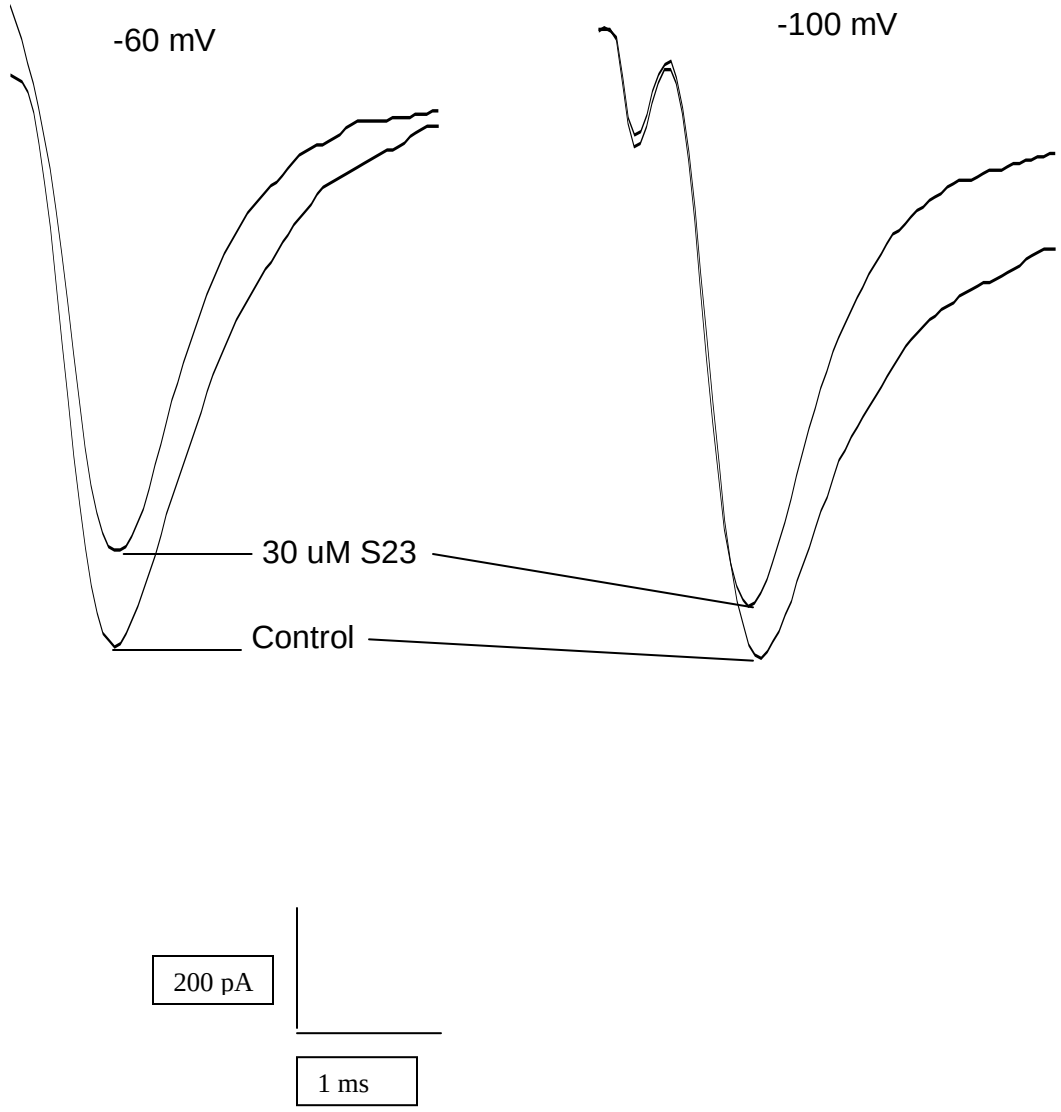


X – axis = Concentration (µM); IC_{50} (µM) = 100.37; k = 1.765335

INACTIVATED STATE (-100 mV)

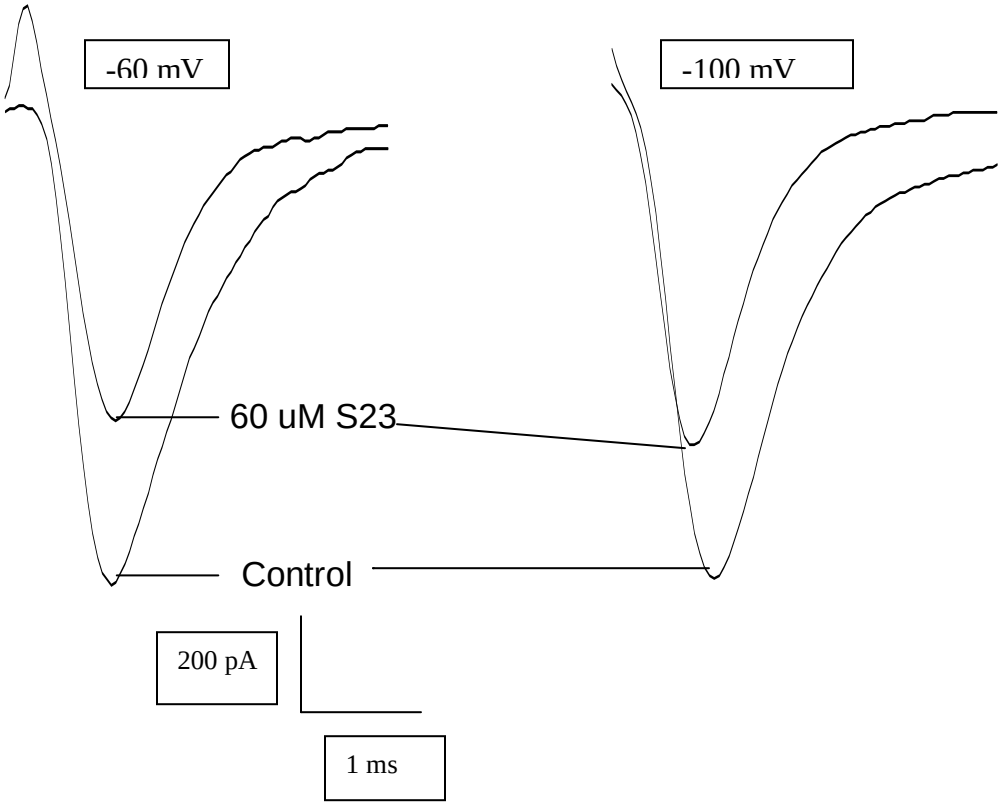
Voltage-dependent Action of 10 uM S23 at -60 mV and -100 mV



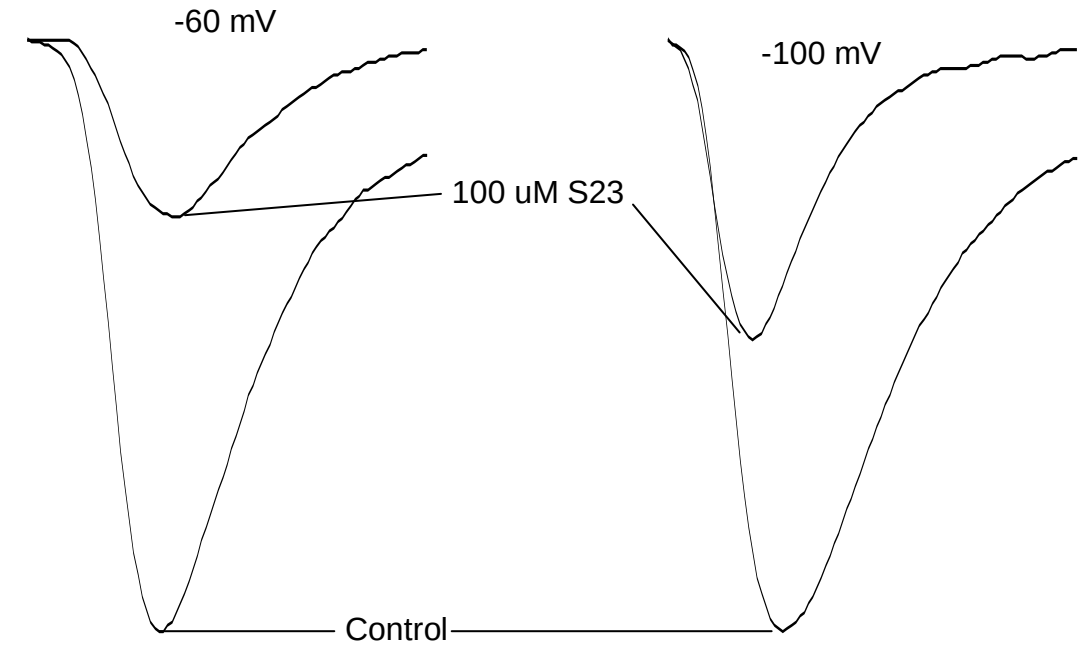


Effect of 30 uM S23 at -60 mV and -100 mV on NaV 1.6

150



Effect of 60 uM S23 at -60 mV and -100 mV on Na_V 1.6



Effect of 100 uM S23 at -60 mV and -100 mV on NaV 1.6

APPENDIX J: ANALYTICAL SPECTRA