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**DISCOVERY OF POTENTIAL ANTICANCER
AND NEUROPROTECTIVE AGENTS
FROM NORTH QUEENSLAND PLANTS**

**Thesis submitted by
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in February 2005**

**for the degree of Doctor of Philosophy
in the Department of Chemistry
and the Department of Physiology and Pharmacology
James Cook University of North Queensland**

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STATEMENT OF THE CONTRIBUTION OF OTHERS

I, the undersigned and author of this work, acknowledge the contribution of others to this work. Financial support during this study was provided in the form of a stipend provided by the Queensland Cancer Fund (QCF George Roberts Scholarship for North Queensland), and research funding provided from two Doctoral Merit Research Grants by James Cook University. Substantial supervision was provided by Assoc. Prof. Bruce Bowden (School of Pharmacy and Molecular Sciences, JCU; primary supervisor) and Dr Anna-Marie Babey (School of Biomedical Sciences, JCU). Additional supervision was provided by Dr Vimal Kapoor (School of Medical Sciences, University of New South Wales), Dr Alan Nimmo (School of Biomedical Sciences, JCU) and Assoc. Prof. Betsy Jackes (School of Tropical Biology, JCU). Assoc. Prof. George Meehan (School of Pharmacy and Molecular Sciences, JCU) provided additional advice on some technical and theoretical aspects. Editorial assistance in the preparation of this thesis was provided by Assoc. Prof. Bruce Bowden, Dr Anna-Marie Babey and Ms Rachael Rutkowski (School of Pharmacy and Molecular Sciences, JCU). The laboratory of Dr Vimal Kapoor in the School of Medical Sciences, UNSW, was used during this study, in addition to facilities within the School of Pharmacy and Molecular Sciences and the School of Biomedical Sciences. Dr David Newman, through the National Cancer Institute (NCI), Washington DC, provided free testing of samples for cytotoxicity in a 60 cell line panel and interpretation of results, and some samples were tested personally by Ms Sarah Wisemiller. The NCI also provided five cancer cell lines to us (Dr Anna-Marie Babey, Assoc. Prof Bruce Bowden, myself) free of charge and at their expense. Mr Rick Willis at the Australian Institute of Marine Sciences, Townsville, performed mass spectrometry (ESI-MS) on samples free of charge. Assoc. Prof. George Meehan performed optical rotation ($[\alpha]_D$) experiments on samples using facilities within the School of Chemistry, University of Sydney.

Stephen Wright

February 2005

DECLARATION ON ETHICS

I, the undersigned and author of this work, declare that the research presented and reported in this thesis was conducted within the guidelines for research ethics outlined in the *James Cook University Policy on Experimentation Ethics. Standard Practices and Guidelines* (2001), and the *James Cook University Statement and Guidelines on Research Practice* (2001). Research involving the use of animals followed *The Australian Code of Practice for the Care and Use of Animals for Scientific Purposes* and the *Queensland Animal Care and Protection Act 2001*. The proposed research methodology for animal use received clearance from the James Cook University Experimentation Ethics Review Committee (approval number A618_00).

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ABSTRACT

Plants represent a largely untapped resource for drug discovery. There are approximately 25,000 plant species in Australia alone, and 9,000 of these are found in Queensland. This study aimed to discover drug leads from North Queensland plants by screening extracts for pharmacological activity. Two pharmacological targets were selected. The first screen aimed to find novel cytotoxic compounds with potential as anticancer agents. The second screen aimed to find compounds with therapeutic potential in schizophrenia or neurological disorders that involve neuroinflammation and neurodegeneration, such as Huntington's Disease and AIDS-related dementia. Various imbalances in the relative levels of kynurenine pathway metabolites, particularly kynurenic acid, quinolinic acid and 3-hydroxykynurenine, have been implicated in these conditions. In order to discover potential drug leads that might be applied to rectify these imbalances, the extracts were screened for inhibition of three key enzymes of this pathway, namely kynurenine-3-hydroxylase, kynureninase and kynurenine aminotransferase.

Samples (365) from 125 species of plants from North Queensland were collected and extracted. After removal of tannins to prevent interference with the assays, the plant extracts were screened for cytotoxicity and for enzyme inhibition. Cytotoxicity was assessed *in vitro* using the P388D1 mouse lymphoma cell line. The enzyme inhibition assays involved the use of crude enzyme preparations from rat liver or kidney, and products were quantified by HPLC with electrochemical or fluorescence detection. Extracts with the greatest kynurenine-3-hydroxylase inhibition and selected cytotoxic extracts were subjected to bioassay-guided fractionation in order to isolate and identify the active compounds. No extracts possessed sufficient kynureninase or kynurenine aminotransferase inhibition to warrant investigation.

Three cytotoxic quinonemethide triterpenes were isolated from *Maytenus cunninghamii* (Celastraceae). Netzahualcoyoic acid (**25**, IC₅₀ value = 0.12 μM, 0.11–0.13 μM 95% CI range) and Δ¹⁵-celastrol (**32**, IC₅₀ value not determined) are new structures, while celastrol

(**22**, IC₅₀ value = 0.37 μM, 0.30–0.45 μM 95% CI range) is a known cytotoxic agent.

Δ¹⁵-celastrol represents a possible biosynthetic intermediate between celastrol and netzahualcoyoic acid. Further work is needed to assess the cytotoxic profile of netzahualcoyoic acid and its acid-rearranged products in the National Cancer Institute's 60 cell line panel.

The known cytostatic compounds podophyllotoxin (**36**), deoxypodophyllotoxin (**37**) and picropodophyllotoxin (**38**) were isolated from *Callitris intratropica* (Cupressaceae). This is the first reported isolation of podophyllotoxins from this species.

Bioassay-guided fractionation of kynurenine-3-hydroxylase inhibitory extracts led to the isolation of two new triterpenes, 11α,28-dihydroxylupenone (**43**) and 2α,3β-dihydroxyfriedelan-29-oic acid (**53**), from *Maytenus disperma*, as well as two new 24-oxomaytenonic acids (**58** and **60**) and four known triterpenes from *M. cunninghamii*. A biosynthetic pathway was proposed for compounds isolated from *M. cunninghamii* and related compounds. Five other species afforded a total of five additional known triterpenoids.

Triterpenes were identified as a new class of potent and selective competitive inhibitors of kynurenine-3-hydroxylase. The most active was uncaric acid (**78**, K_{ic} = 0.023 ± 0.002 μM), isolated from *Dolichandrone heterophyllum* (Bignoniaceae), and it is one of the most potent kynurenine-3-hydroxylase inhibitors that has been reported. The next most active inhibitors of this study were the 24-oxofriedelan-29-oic acids (**60**, K_{ic} = 0.061 ± 0.005 μM; **62**, K_{ic} = 0.077 ± 0.011 μM; **58**, K_{ic} = 0.12 ± 0.02 μM) and celastrol (**22**, K_{ic} = 0.14 ± 0.03 μM). Important structure-activity relationships relating to triterpene skeleton, functional groups, and ring conformations were observed. It is proposed that the triterpene inhibitors would make ideal drugs for the treatment of many neuroinflammatory and neurodegenerative disorders, and that uncaric acid, 2α-hydroxy-24-oxomaytenonic acid (**60**) and celastrol should be investigated *in vivo*.

TABLE OF CONTENTS

LIST OF TABLES	xvi
LIST OF FIGURES	xvii
LIST OF SCHEMES	xvii
LIST OF ABBREVIATIONS	xviii
CHAPTER 1. INTRODUCTION	1
1.1 MEDICINAL PLANTS AND DRUG DISCOVERY.....	1
1.1.1 History of Medicinal Plant Use	1
1.1.2 Drug Discovery from Plants	3
1.1.3 Strategies for Drug Discovery from Plants	4
1.2 ANTICANCER DRUG DISCOVERY.....	6
1.2.1 Screening Plant Extracts for Anticancer Activity	6
1.2.2 Selection of the Cytotoxicity Screening Assay.....	8
1.3 NEURODEGENERATIVE DISORDERS AND THE KYNURENINE PATHWAY.....	9
1.3.1 Excitatory Amino Acids and Excitotoxicity	9
1.3.2 Binding-Sites on the NMDA Receptor	9
1.3.3 The Kynurenine Pathway of Tryptophan Metabolism	13
1.3.4 Implications of the Kynurenine Pathway in Neurodegenerative Disorders.....	15
1.3.5 Therapeutic Targets in the Kynurenine Pathway.....	17
1.3.6 Inhibitors of Kynurenine-3-hydroxylase.....	18
1.3.7 Screening for Inhibitors of Kynurenine-3-hydroxylase.....	21
1.4 DRUG DISCOVERY FROM AUSTRALIAN PLANTS	22
1.4.1 Selection of Plants for Pharmacological Screening.....	24
1.4.2 Considerations for Testing Plant Extracts for Pharmacological Activity	25
1.4.3 Overview and Aims of this Study	25

CHAPTER 2. POTENTIAL ANTICANCER AGENTS FROM NORTH QUEENSLAND PLANTS: RESULTS AND DISCUSSION	27
2.1 QUINONEMETHIDE TRITERPENES FROM <i>MAYTENUS CUNNINGHAMII</i>	27
2.1.1 Background	27
2.1.2 Characterisation of Compounds Isolated from <i>Maytenus cunninghamii</i>	33
2.1.2.1 Netzahualcoyoic acid (25)	33
2.1.2.2 Δ^{15} -Celastrol (32)	35
2.1.2.3 Celastrol (22)	38
2.1.3 Biosynthetic Aspects	39
2.1.4 Biological Activity and the Relationship with Structure	39
2.1.5 Further Work	41
2.2 PODOPHYLLOTOXINS FROM <i>CALLITRIS INTRATROPICA</i>	42
CHAPTER 3. POTENTIAL ANTICANCER AGENTS FROM NORTH QUEENSLAND PLANTS: EXPERIMENTAL	44
3.1 GENERAL	44
3.1.1 High-Performance Liquid Chromatography	44
3.1.2 Spectroscopy	44
3.2 PLANT EXTRACTS FOR CYTOTOXICITY SCREENING	45
3.2.1 Plant Material	45
3.2.2 Sample Extraction	45
3.2.3 Extract Treatment	46
3.3 CYTOTOXICITY ASSAYS	46
3.3.1 Cells and Cell Maintenance	46
3.3.2 Cytotoxicity Screening	47
3.3.2.1 Seeding plates	47
3.3.2.2 Extract preparation and application	47
3.3.2.3 Cell fixation	48
3.3.2.4 Cell staining and measurement	50
3.3.2.5 Determination of cytotoxicity	50
3.3.3 IC ₅₀ Determination of Isolated Compounds	52
3.3.3.1 Extract preparation and application	52
3.3.3.2 Analysis of cytotoxicity data	52

3.4	ISOLATION OF CYTOTOXIC COMPOUNDS	53
3.4.1	<i>Maytenus cunninghamii</i>	53
3.4.1.1	Plant material and extraction.....	53
3.4.1.2	Crude extract treatment	54
3.4.1.3	Bioassay-guided fractionation.....	54
3.4.2	<i>Callitris intratropica</i>	58
3.4.2.1	Plant material and extraction.....	58
3.4.2.2	Crude extract treatment	58
3.4.2.3	Bioassay-guided fractionation.....	59

CHAPTER 4. POTENTIAL NEUROPROTECTIVE AGENTS FROM NORTH QUEENSLAND PLANTS: RESULTS AND DISCUSSION.....60

4.1	ISOLATION AND CHARACTERISATION OF KYNURENINE-3-HYDROXYLASE INHIBITORS	60
4.1.1	Compounds from <i>Maytenus disperma</i>	60
4.1.1.1	11 α ,28-Dihydroxylupenone (43).....	61
4.1.1.2	2 α ,3 β -Dihydroxyfriedelan-29-oic acid (53).....	65
4.1.2	Compounds from <i>Maytenus cunninghamii</i>	71
4.1.2.1	2 β -Hydroxy-24-oxomaytenonic acid (58).....	71
4.1.2.2	2 α -Hydroxy-24-oxomaytenonic acid (60).....	75
4.1.2.3	Other compounds.....	79
4.1.2.4	Biosynthetic aspects	80
4.1.3	Compounds from <i>Dolichandrone heterophylla</i>	81
4.1.4	Compounds from <i>Lophostemon grandiflorus</i>	84
4.1.5	Compounds from <i>Lagerstroemia speciosa</i>	86
4.1.6	Compounds from <i>Hyptis suaveolens</i>	86
4.1.7	Compounds from <i>Melaleuca viridiflora</i>	87
4.1.8	Other compounds investigated	87
4.2	ACTIVITY OF ENZYME INHIBITORS AND STRUCTURE-ACTIVITY RELATIONSHIPS.....	89
4.2.1	Inhibition of Kynurenine-3-hydroxylase by Triterpenoids and Relation to Structure	89
4.2.1.1	Relationship of triterpenoid-type with activity.....	89
4.2.1.2	Relationship of variations in ring E with activity.....	93
4.2.1.3	Relationship of variations in ring A with activity	95
4.2.1.4	Comparison of inhibitor structures with 3-hydroxykynurenine.....	96
4.2.2	Inhibition of Kynurenine Aminotransferase by Triterpenoids and Relation to Structure	97
4.2.3	Inhibition Studies of Kynureninase.....	98

4.3	THERAPEUTIC POTENTIAL OF THE KYNURENINE-3-HYDROXYLASE INHIBITORS.....	98
4.3.1	Potential of Some Traditional Herbal Medicines Containing Kynurenine-3-hydroxylase Inhibitors	98
4.3.2	Potential of Kynurenine-3-hydroxylase Inhibitors as Drugs.....	100
4.3.3	Further Work.....	104
CHAPTER 5. POTENTIAL NEUROPROTECTIVE AGENTS FROM NORTH QUEENSLAND PLANTS: EXPERIMENTAL		106
5.1	GENERAL	106
5.2	PLANT EXTRACTS FOR SCREENING FOR ENZYME INHIBITORS	106
5.3	KYNURENINE-3-HYDROXYLASE INHIBITION ASSAYS	107
5.3.1	Screening Plant Extracts for Inhibition of Kynurenine-3-hydroxylase.....	107
5.3.1.1	Crude enzyme preparation.....	107
5.3.1.2	Incubation procedure	108
5.3.1.3	HPLC quantification of 3HK in incubated samples	108
5.3.2	Bioassay-guided Fractionation of Kynurenine-3-hydroxylase Inhibitors.....	110
5.3.2.1	Crude enzyme preparation.....	110
5.3.2.2	Total protein determination of crude enzyme preparation.....	110
5.3.2.3	Incubation procedure	110
5.3.2.4	HPLC quantification of 3HK in incubated samples.....	111
5.3.3	Determination of Inhibition Constants of Isolated Compounds.....	112
5.3.3.1	Optimisation of incubation conditions.....	112
5.3.3.2	Testing of isolated compounds to determine inhibition constants.....	113
5.3.3.3	Analysis of kynurenine-3-hydroxylase inhibition data	115
5.4	KYNURENINE AMINOTRANSFERASE AND KYNURENINASE INHIBITION ASSAYS	116
5.4.1	Screening Plant Extracts for Inhibition of Kynurenine Aminotransferase and Kynureninase.....	116
5.4.1.1	Crude enzyme preparation.....	116
5.4.1.2	Total protein determination of crude enzyme preparation.....	117
5.4.1.3	Incubation procedure	117
5.4.1.4	HPLC quantification of KYNA and AA in incubated samples	118
5.4.2	Testing of Kynurenine-3-hydroxylase Inhibitors for Inhibition of Kynurenine Aminotransferase and Kynureninase.....	119
5.4.2.1	Crude enzyme preparation.....	119
5.4.2.2	Total protein determination of crude enzyme preparation.....	119
5.4.2.3	Incubation procedure	120
5.4.2.4	HPLC quantification of KYNA and AA in incubated samples	121
5.4.2.5	Analysis of kynurenine aminotransferase and kynureninase inhibition data ...	122

5.5	ISOLATION OF KYNURENINE-3-HYDROXYLASE INHIBITORS.....	123
5.5.1	<i>Maytenus disperma</i>	123
5.5.1.1	Plant material and extraction.....	123
5.5.1.2	Crude extract treatment	123
5.5.1.3	Bioassay-guided fractionation.....	124
5.5.2	<i>Maytenus cunninghamii</i>	125
5.5.2.1	Plant material, extraction and isolation.....	125
5.5.3	<i>Dolichandrone heterophylla</i>	127
5.5.3.1	Plant material and extraction.....	127
5.5.3.2	Crude extract treatment	128
5.5.3.3	Bioassay-guided fractionation.....	128
5.5.4	<i>Lophostemon grandiflorus</i>	129
5.5.4.1	Plant material and extraction.....	129
5.5.4.2	Crude extract treatment	129
5.5.4.3	Bioassay-guided fractionation.....	130
5.5.5	<i>Lagerstroemia speciosa</i>	131
5.5.5.1	Plant material and extraction.....	131
5.5.5.2	Crude extract treatment	131
5.5.5.3	Bioassay-guided fractionation.....	132
5.5.6	<i>Hyptis suaveolens</i>	133
5.5.6.1	Plant material and extraction.....	133
5.5.6.2	Crude extract treatment	133
5.5.6.3	Bioassay-guided fractionation.....	133
5.5.7	<i>Melaleuca viridiflora</i>	134
5.5.7.1	Plant material and extraction.....	134
5.5.7.2	Crude extract treatment	135
5.5.7.3	Bioassay-guided fractionation.....	135
CHAPTER 6.	CONCLUSIONS.....	137
6.1	DISCOVERY OF POTENTIAL ANTICANCER AGENTS FROM NORTH QUEENSLAND PLANTS.....	137
6.2	DISCOVERY OF POTENTIAL NEUROPROTECTIVE AGENTS FROM NORTH QUEENSLAND PLANTS.....	138
REFERENCES		142

APPENDIX 1. Structures Discussed in the Results and Discussion Chapters	156
APPENDIX 2. List of Plant Species and Organs Collected.....	163

LIST OF TABLES

Table 2.1.	^{13}C and ^1H NMR shifts (δ), and <i>g</i> COSY, NOESY and <i>g</i> HMBC correlations for netzahualcoyoic acid (25) in CDCl_3	34
Table 2.2.	Selected ^1H NMR shifts (δ) for Δ^{15} -celastrol (32) and celastrol (22) in CDCl_3	37
Table 4.1.	^{13}C and ^1H NMR shifts (δ), and <i>g</i> COSY, NOESY and <i>g</i> HMBC correlations for $11\alpha,28$ -dihydroxylupenone (43) in CDCl_3	62
Table 4.2.	Observable ^1H NMR shifts (δ) and <i>g</i> COSY, NOESY and <i>g</i> HMBC correlations for $2\alpha,3\beta$ -dihydroxyfriedelan-29-oic acid (53) in CDCl_3	66
Table 4.3.	^{13}C NMR shifts (δ) for $2\alpha,3\beta$ -dihydroxyfriedelan-29-oic acid (53) in CDCl_3	66
Table 4.4.	^{13}C and ^1H NMR shifts (δ), and <i>g</i> COSY, NOESY and <i>g</i> HMBC correlations for 2β -hydroxy-24-oxomaytenonic acid (58) in CDCl_3	72
Table 4.5.	^{13}C and ^1H NMR shifts (δ), and <i>g</i> COSY, NOESY and <i>g</i> HMBC correlations for 2α -hydroxy-24-oxomaytenonic acid (60) in $\text{C}_5\text{D}_5\text{N}$	78
Table 4.6.	Enzyme inhibition of kynurenine-3-hydroxylase, kynurenine aminotransferase (KAT) and kynureninase by isolated compounds.	90
Table 4.7.	Summary of structure-activity relationships of the triterpene inhibitors of kynurenine-3-hydroxylase.	92
Table 4.8.	Cytotoxicity (IC_{50}) of compounds tested against the P388D1 cell line <i>in vitro</i>	101

LIST OF FIGURES

Figure 1.1.	Structures of NMDA receptor antagonists (1-9) and a kynureninase inhibitor (10).	10
Figure 1.2.	Stylised diagram of the ion channel-coupled NMDA receptor showing binding-sites.	11
Figure 1.3.	Kynurenine pathway of tryptophan metabolism.	14
Figure 1.4.	Structures of inhibitors of enzymes in the kynurenine pathway of tryptophan metabolism.	19
Figure 2.1.	Three-dimensional stick diagrams showing conformation of rings D and E and the position of the carboxylic acid groups in celastrol (22 , a) and netzahualcoyoic acid (25 , b), viewed from above the β face.	36
Figure 3.1.	96-well plate design, showing position of wells A1–H12, for cytotoxicity testing of 7 extracts or drugs at two concentrations (<i>D1a–D7a</i> and <i>D1b–D7b</i>).	49
Figure 3.2.	Flow-chart of general strategy for bioassay-guided fractionation of selected extracts following bulk collection, extraction and removal of polyphenols and quinones.	55
Figure 4.1.	Three-dimensional stick diagrams showing the conformations of rings D and E and the position of the carboxyl group in some triterpene acids.	70
Figure 4.2.	Graphical representation of kynurenine-3-hydroxylase inhibition by triterpenoids with K_{ic} values less than 2 μ M.	91
Figure 4.3.	Summary of features in ursanes, oleananes and friedelanes that increase inhibition of kynurenine-3-hydroxylase.	92

LIST OF SCHEMES

Scheme 2.1	30
Scheme 2.2	40
Scheme 4.1	77
Scheme 4.2	82

LIST OF ABBREVIATIONS

[I]	inhibitor concentration
[S]	substrate concentration
1D	one dimensional
2D	two dimensional
3HA	3-hydroxyanthranilic acid
3HAO	3-hydroxyanthranilic acid oxidase
3HK	3-hydroxykynurenine
AA	anthranilic acid
AD	Alzheimer's Disease
AMPA	α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
aq	aqueous
br d	broad doublet
br s	broad singlet
BRI	Herbarium of Queensland, Brisbane
BSA	bovine serum albumin
C ₅ D ₅ N	deuterated pyridine
CDCl ₃	deuterated chloroform
CH ₂ Cl ₂	dichloromethane
CH ₃ COOH	acetic acid
CHCl ₃	chloroform
CI	confidence interval
CNS	central nervous system
CSF	cerebrospinal fluid
CSIRO	Commonwealth Scientific and Industrial Research Organisation
d	doublet
dd	doublet of doublets
ddd	doublet of doublet of doublets
DEPT	Distortionless Enhancement by Polarisation Transfer
dm	doublet of multiplets
DMSO	dimethylsulfoxide
EAs	excitatory amino acids
EDTA	ethylenediaminetetraacetic acid
ESI-MS	Electrospray Ionisation Mass Spectrometry
EtOAc	ethyl acetate
EtOH	ethanol

FCS	foetal calf serum
<i>g</i> COSY	gradient Correlated Spectroscopy
<i>g</i> HMBC	gradient Heteronuclear Multiple-Bond Coherence
<i>g</i> HMQC	gradient Heteronuclear Multiple-Quantum Coherence
<i>g</i> HSQC	gradient Heteronuclear Single-Quantum Coherence
HD	Huntington's Disease
HPLC	High-Performance Liquid Chromatography
IC ₅₀	with respect to cytotoxicity: defined as the concentration of compound that inhibits cell-replication by 50% relative to the control
IC ₅₀	with respect to enzyme inhibition: defined as the concentration of inhibitor that reduces the formation of product by 50% relative to the control
i.d.	internal diameter
IDO	indoleamine-2,3-dioxygenase
IR	Infrared
<i>J</i>	coupling constant
JCT	Herbarium of James Cook University
JCU	James Cook University
KAT	kynurenine aminotransferase
K _{ic}	the specific (competitive) inhibition constant
K _{iu}	the catalytic (uncompetitive) inhibition constant
K _m	the concentration of substrate at which the rate of product formation is equal to half the maximum, or limiting, rate of product formation(V)
K _m ^{app}	the apparent K _m in the presence of an inhibitor
KYN	L-kynurenine
KYNA	kynurenic acid
m	multiplet
MeCN	acetonitrile
MeOH	methanol
min	minutes
mNBA	(<i>m</i> -nitrobenzoyl)alanine (13)
mp	melting point
NAD	β-nicotinamide adenosine dinucleotide
NADPH	β-nicotinamide adenine dinucleotide phosphoric acid
NAL	nicotinylalanine (11)
NCI	National Cancer Institute, Washington DC
NMDA	<i>N</i> -methyl-D-aspartate
NMR	Nuclear Magnetic Resonance
nOe	nuclear Overhauser effect

NOESY	Nuclear Overhauser Effect Spectroscopy
OD	optical density
oMBA	(<i>o</i> -methoxybenzoyl)alanine (12)
PCP	phencyclidine (1)
PD	Parkinson's Disease
PDA	photodiode-array
PLP	pyridoxal-5'-phosphate
q	quartet
QUIN	quinolinic acid
s	singlet
SAR	structure-activity relationship
sp.	species (singular)
spp.	species (plural)
SRB	sulforhodamine B
syn.	synonym
t	triplet
TCA	trichloroacetic acid
td	triplet of doublets
TDO	tryptophan-2,3-dioxygenase
Tris	tris(hydroxymethyl)aminomethane
UV	Ultraviolet
v br	very broad
V	the maximum, or limiting, rate of product formation
ν	the measured rate of product formation
V^{app}	the apparent maximum rate of product formation in the presence of an inhibitor
var.	variety
Vis	Visible light