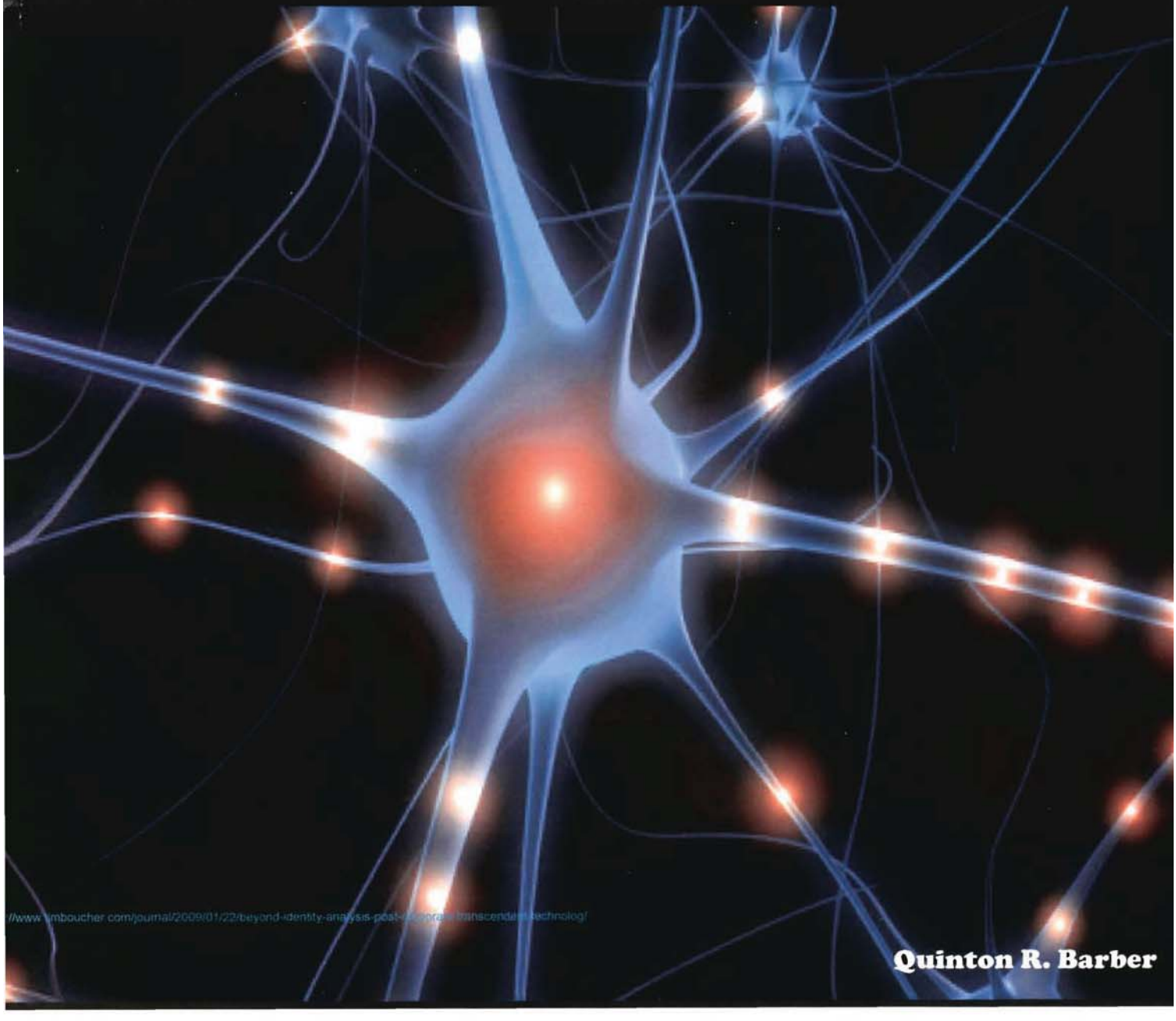
Polycyclic Propargylamines as Multi-Functional Neuro-Protective Agents



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Polycyclic Propargylamines as Multi-Functional Neuro-Protective Agents

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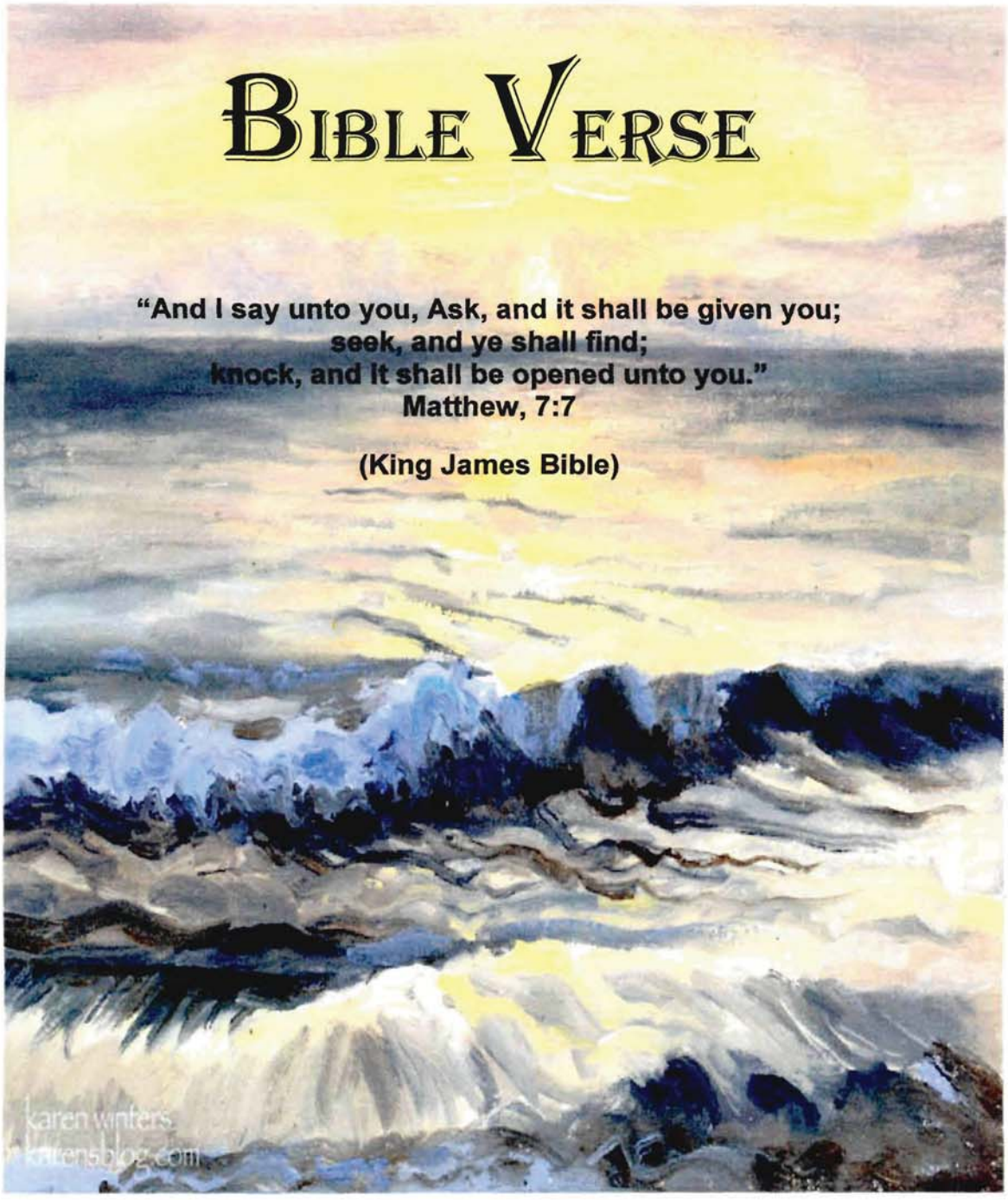
BIBLE VERSE
"And I say unto you, Ask, and it shall be given you;
seek, and ye shall find;
knock, and it shall be opened unto you."
Matthew, 7:7
(King James Bible)
karen winters

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ABSTRACT

The pathology of neurodegenerative disorders, such as Parkinson's disease (PD) and Alzheimer's disease (AD), is caused by the abnormal loss of neuronal cells in certain areas of the brain. It consequently causes an imbalance of certain neurotransmitter levels in the brain, giving rise to the characteristic signs and symptoms of these diseases. Ultimately it compromises the normal functionality and well being of the individual suffering from the disease, thus making it an absolute necessity to create compounds which would halt this neuronal breakdown process, but will also aid in treating the signs and symptoms.

The abnormal death of neurons in the central nervous system of individuals suffering from neurodegenerative diseases, takes place by an intrinsic cell suicide program known as apoptosis. This process is triggered by several stimuli, and consists of numerous pathways and cascades, each one having an influence on the other, ultimately leading to the death of the cells. In PD and AD it has been shown that there are elevated levels of monoamine oxidase B (MAO-B), which not only acts indirectly as a trigger to the apoptotic process, but also gives rise to some of the signs and symptoms of the diseases.

In the current study the approach was to develop multifunctional drugs, which would halt the neuronal breakdown process, but will also eliminate some of the signs and symptoms of diseases such as AD and PD. Keeping this in mind we focused on the structures of rasagiline, pentacyclo-undecane and amantadine. Rasagiline is a well known MAO-B inhibitor with promising neuroprotective activity, which can be attributed to its propargylamine moiety. The pentacyclo-undecane and amantadine polycyclic structures are highly non-polar compounds, with high potential to contribute in the transport of drugs across the blood-brain barrier and across cell membranes into cells. It was thus a rational decision to incorporate the structures of propargylamine and the polycyclic cages into the structures of the synthesised compounds.

In synthesising the test compounds, several experimental procedures and methods, both conventional and modern, were utilised. In most of the synthetic routes either propargylamine, propargylbromide or ethynyl magnesium bromide were utilised together with pentacyclo-undecane or amantadine as reagents. The reaction of these substances afforded the target compounds. Most of the reactions took place without the necessity of a catalysing agent, even though an external source of energy was necessary to provide heat for the reactions to take place. Each compound was synthesised to evaluate the activity and benefit

of the presence of a certain group of atoms in the molecule. Some of these groups include a terminal acetylene group, an acetylene group between two non-polar groups, a secondary propargylamine connected to a polycyclic cage and a tertiary propargylamine in an aza polycyclic structure.

These synthesised compounds were evaluated for anti-apoptotic as well as MAO-B inhibiting activity. The anti-apoptotic activity were evaluated *in vitro* using the Desphiper™ kit, which marks changes in the mitochondrial membrane potential, that takes place during apoptosis. The quantitative and qualitative detection of these processes were done by means of flow cytometry, which made it possible to determine what percentage of the cells in the samples were still viable after treatment with the synthesised compounds. For this purpose SK-N-BE(2) neuroblastoma cells were used, and apoptosis was induced using a serum-deprivation model.

Besides their anti-apoptotic activity, the synthesised compounds were also evaluated *in vitro* as competitive inhibitors of MAO-B using a spectrophotometric assay that utilised MMTP, an analogue of the neurotoxin MPTP as substrate, with baboon liver mitochondria serving as enzyme source. The potency of MAO-B inhibition was expressed as percentage inhibition of the enzyme.

In evaluating the activity of the synthesised compounds, there was one compound, 8-phenylethynyl-8-hydroxy-pentacyclo[5.4.1.$0^{2,6}$.$0^{3,10}$.$0^{5,9}$]undecane, that had significant MAO-B inhibiting activity as well as significant anti-apoptotic activity. The other compounds of the series all had moderate to weak anti-apoptotic activity, and showed limited MAO-B inhibition. Having moderate to weak anti-apoptotic activity, these compounds can be used as lead-compounds in the development and design of more potent inhibitors. It was confirmed that when linked to a polycyclic cage structure, propargylamine still displayed potent anti-apoptotic activity. It was also determined that a terminal acetylene group, as well as an acetylene group between two non-polar groups had comparable anti-apoptotic activity. The polycyclic propargylamines thus have potential as neuroprotective agents and further investigation is necessary to determine what their maximum benefit can be in the treatment of neurodegenerative diseases.

UITTREKSEL

Die patologie van neurodegeneratiewe versteurings, soos Parkinson se siekte (PS) en Alzheimer se siekte (AS), word deur abnormale verlies aan neurone in sekere dele van die brein veroorsaak. Dit veroorsaak 'n wanbalans in die vlakke van sekere neurotransmitters in die brein wat oorsprong gee aan die karakteristieke tekens en simptome van hierdie siektes. Uiteindelik tas dit die normale funksionering en welsyn van die individu wat aan die siekte ly aan, en daarom is dit absoluut noodsaaklik om verbindings te ontwikkel wat hierdie afbraakproses van neurone sal stop, asook sal help om die tekens en simptome te behandel.

Die abnormale afsterf van neurone in die sentrale senuwee stelsel van indiwidue wat aan neurodegeneratiewe siektes ly, geskied volgens 'n intrinsieke program van selfmoord van selle, bekend as apoptose. Hierdie proses word deur verskeie stimuli afgesit en bestaan uit talle weë en kaskades wat mekaar beïnvloed en uiteindelik tot die dood van die sel lei. Dit is aangetoon dat daar met PS en AS hoër vlakke monoamienoksidase B (MAO-B) is, wat nie net indirek as sneller vir die proses van apoptose optree nie, maar ook oorsprong gee aan sommige van die tekens en simptome van hierdie siektes.

In die betrokke studie was die benadering om multifunksionele geneesmiddels te ontwikkel wat die neuronale afbraakproses sal stop asook sekere van die tekens en simptome van siektes soos PS en AS sal elimineer. Met dit ingedagte is daar op die strukture van rasagilien, pentasiklo-undekaan en amantadien gefokus. Rasagilien is 'n bekende MAO-B-remmer met belowende neurobeskermende aktiwiteit wat aan die propargielamiendeel daarvan toegeskryf kan word. Die polisikliese pentasiklo-undekaan- en amantadienstrukture is hoogs nie-polêre verbindings, met groot potensiaal om by te dra tot die transport van geneesmiddels oor die bloedbreinskans en oor selmembrane, in selle in. Dit was dus 'n rasionele besluit om die strukture van beide propargielamien en die polisikliese hokkies in die strukture van die gesintetiseerde verbindings te inkorporeer.

Verskeie eksperimentele prosedures en metodes, sowel konvensioneel as modern, is vir die sintese van die teikenverbindings gebruik. In die meeste sinteseroetes is óf propargielamien, óf propargielbromied óf etynielmagnesiumbromied saam met pentasiklo-undekaan of amantadien as reagense geselekteer. Die reaksie van hierdie stowwe het die geselekteerde verbindings gelewer. Die meeste reaksies het sonder 'n katalisator verloop, hoewel 'n eksterne bron van energie nodig was om hitte te verskaf sodat die reaksies kon plaasvind. Die verbindings is gesintetiseer om die aktiwiteit en voordeel van sekere groepe in die

molekuul te beoordeel. Sommige van hierdie groepe behels 'n terminale asetileengroep, 'n asetileengroep tussen twee nie-polêre groepe, 'n sekondêre propargielamien verbind aan 'n polisikliese hokkie en 'n tersiêre propargielamien in 'n asapolisikliese struktuur.

Hierdie gesintetiseerde verbindings is getoets vir hulle aktiwiteit teen apoptose asook remming van MAO-B. Die anti-apoptotiese aktiwiteit is *in vitro* bepaal deur die Desphiper^{TM}-stel te gebruik, wat veranderinge in die mitochondriële membraanpotensiaal wat tydens apoptose voorkom merk. Die kwantitatiewe en kwalitatiewe opsporing van hierdie prosesse is deur middel van vloeisitometrie gedoen, wat kon bepaal watter persentasie selle in die monsters steeds lewendig was na behandeling met die gesintetiseerde verbindings. Vir hierdie doel was SK-N-BE(2)-neuroblastoomselle gebruik, waarin apoptose geïnduseer is deur gebruik te maak van die serumdeprivasiemodel.

Benewens hulle anti-apoptotiese aktiwiteit is die gesintetiseerde verbindings ook *in vitro getoets* as kompeterende remmers van MAO-B, deur gebruik te maak van 'n spektrofotometriese bepaling met MMTP, 'n analoog van die neurotoksien MPTP, as substraat, en lewerweefsel van bobbejane as bron van ensiem. Die mate van MAO-B-remming is uitgedruk as persentasie inhibisie van die ensiem.

Een van die gesintetiseerde verbindings, 8-fenieletyniel-8-hidroksie-pentasiklo [5.4.1.$0^{2,6}$.$0^{3,10}$.$0^{5,9}$]undekaan het beduidende aktiwiteit getoon as MAO-B-remmer asook anti-apoptotiese middel. Die ander verbindings in die reeks het almal matig tot swak anti-apoptotiese aktiwiteit getoon, met beperkte MAO-B-remming. Hierdie verbindings kan egter verder gebruik word as leidraad verbindings in die ontwikkeling en ontwerp van meer potente inhibeerders. Dit is bevestig dat as dit aan 'n polisikliese hokkiestruktuur gekoppel is, propargielamien steeds sterk anti-apoptotiese aktiwiteit uitoefen. Dit is ook vasgestel dat 'n terminale asetileengroep, asook 'n asetileengroep tussen twee nie-polêre groepe, vergelykbare anti-apoptotiese aktiwiteit het. Die polisikliese propargielamiene het dus potensiaal as neurobeskermende middels en verdere ondersoek is nodig om te bepaal wat hulle maksimum voordeel vir die behandeling van neurodegeneratiewe siektes kan wees.

LIST OF ABBREVIATIONS

6-OHDA	-	6-hydroxydopamine
AD	-	Alzheimer's disease
apo	-	apo-lipoprotein
APP	-	amyloid precursor protein
ATP	-	adenosine triphosphate
Aβ	-	β–Amyloid
BACE	-	beta-site APP-cleaving enzyme
Bak	-	bcl-2 homologous antagonist/killer
Bax	-	bcl-2–associated X protein
Bcl-2	-	basal cell lymphoma-2
Bcl-xL	-	basal cell lymphoma-extra large
BDNF	-	brain-derived neurotrophic factor
CAD	-	caspase activated DNAse
CAG	-	cytosine adenine guanine
CNS	-	central nervous system
DA	-	dopamine
DFF-40	-	DNA Fragmentation Factor-40
DFF-45	-	DNA Fragmentation Factor-45
DNA	-	deoxyribonucleic acid
ER	-	endoplasmic reticulum
ERK	-	extra cellular signal-regulated kinases
FAS	-	apoptosis stimulating fragment
FBS	-	foetal bovine serum
FZ	-	fungizone
GAPDH	-	glyceraldehyde-3-phosphate dehydrogenase
GDNF	-	glial cell line-derived neurotrophic factor
GFR	-	growth factor receptor
GMP	-	cyclic guanosine 5'-monophosphate
GRB	-	growth factor receptor-bound
H_2O_2	-	Hydrogen peroxide
ICAD	-	inhibitor of caspase activated DNAse
JNK	-	c-Jun amino-terminal kinases
L-DOPA	-	levodopa
MAO	-	monoamine oxidase

MAO-A	-	monoamine oxidase A
MAO-B	-	monoamine oxidase B
MAPK	-	mitogen-activated protein kinase
MFI	-	mean fluorescence intensity
MP	-	mononuclear phagocytes
MPP^+	-	1-methyl-4-phenylpyridinium
NADPH	-	nicotinamide adenosine dinucleotide phosphate
NF	-	neurotrophic factor/neurotrophin
NFT	-	neurofibrillary tangles
NF-κB	-	nuclear factor kappa-light-chain-enhancer of activated B cells
NMDA	-	N-methyl-D-aspartic acid
NO	-	nitric oxide
NOS	-	nitric oxide synthase
OXPHOS	-	oxidative phosphorylation
p75	-	low affinity neurotrophin receptor
PARP	-	poly(ADP-ribose) polymerase
PC12	-	pheochromocytoma cell line 12 culture cells
PCP	-	phencyclidine
PD	-	Parkinson's disease
PEA	-	phenylethylamine
Pen/Strep	-	penicillin/streptomycin
PKC	-	protein kinase C
PTP	-	permeability transition pores
ROS	-	reactive oxygen species
RT	-	room temperature
sAPPα	-	secreted amyloid precursor protein alpha
SEM	-	standard error of mean
SOD	-	super oxide dismutase
SOS	-	Son of Sevenless
TLC	-	thin layer chromatography
Trk	-	tyrosine kinase receptor
UV	-	ultraviolet
$\Delta\Psi m$	-	electrochemical gradient across the mitochondrial membrane