New Metal Chelators for Chelation Therapy in Neurodegenerative Diseases

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under the supervision of
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CERTIFICATE OF ORIGINAL AUTHORSHIP

I, *Mahmoud El Safadi* declare that this thesis, is submitted in fulfilment of the requirements for the award of *Doctor of Philosophy*, in the *School of Mathematical and Physical Sciences* at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise referenced or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

This document has not been submitted for qualifications at any other academic institution.

This research is supported by an Australian Government Research Training Program.

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List of Abbreviations

Å: Angstrom

δ: Chemical Shift (NMR)

λ: Wavelength

ν_{max}: Maximum absorbance

$[M]^+$: Molecular ion

$[M+H]^+$: Protonated molecular ion

8-HQ: 8-Hydroxyquinoline

Aβ: Amyloid-beta

AD: Alzheimer's disease

ADMET: Absorption, Distribution, Metabolism, Excretion, and Toxicity

AICD: APP intracellular domain

ALA: Alanine

AMPA: 2- amino- 3- (3-hydroxy 5-methyl- isoxazol-4-yl propanoic acid

APP: Amyloid precursor protein

ASP: Aspartic acid

ATP: Adenosine triphosphate

BBB: Blood-brain barrier

BCA: Bicinchoninic acid assay

BSA: Bovine Serum Albumin

$CDCl_3$: Deuterated chloroform

CNS: Central nervous system

COX17: Cytochrome oxidase coper chaperone

CQ: Clioquinol

CTR1: Copper transport protein

Cyclam: 1,4,8,11-Tetraazacyclotetradecane or 14 ane- N4

Cyclen: 1, 4, 7, 10-tetrazacyclododecane or 12 ane- N4

d: Doublet (NMR)

dd: Doublet of doublets (NMR)

DA: Dopamine

DAT: Dopamine transporter

DFP: Deferiprone

DPPH: 2,2-diphenyl-1-picrylhydrazyl

DMSO: Dimethyl sulfoxide

DNA: Deoxyribonucleic acid

FTIR: Fourier transform infrared spectroscopy

GABA: Gamma-amino butyric acid

GC: Gas chromatography

GLU: Glutamic acid

HIS: Histidine

HRMS: High resolution mass spectroscopy

Hz: Hertz

IC50: The half maximal inhibitory concentration

ITC: Isothermal titration colorimetry

J: Coupling constant (NMR)

M: Multiplet (NMR)

m/z: Mass to charge ratio

MAO: Monoamine oxidase inhibitory

mg: Milligrams

mL: Millilitre

mmol: Millimole

MLCT: Metal ligand charge transfer

MS: Mass spectroscopy

MTF: Membrane- bound transferrin

MTT: Microculture Tetrazolium Assay

NMDA: N-Methyl-D-aspartic acid

NMR: Nuclear magnetic resonance

NTBI: Non transferrin-bound iron

ORTEP: Oak Ridge Thermal Ellipsoid Plot Program

PBS: Phosphate-buffered saline

PD: Parkinson's Disease

PEAs: Phenylethylamines

PSA: Polar surface area

q: quartet (NMR)

Q-TOF: Quadrupole Time-of-Flight

R_f: Retention factor

RNA: Ribonucleic acid

ROS: Reactive oxygen species

SERT: Serotonin transporter

SFN: Small fibre neuropathy

s: Singlet (NMR)

SOD: Superoxide dismutase

SN: Substantia nigra

TETA: Triethylenetetramine

TF-Fe^{3+}: Iron-bound transferrin

TLC: Thin layer chromatography

Tyr: Tyrosinase

UV: Ultraviolet

Publications from This Thesis

M. E Safadi, M. Bhadbhade, R. Shimmon, A. T. Baker, A. M. McDonagh.

Cyclen-based chelators for the inhibition of Aβ aggregation: Synthesis, antioxidant and aggregation evaluation.

Inorganica Chimica Acta, 467**, 2017** 343-350. https://doi.org/10.1016/j.ica.2017.07.060.

ABSTRACT

In this thesis, a series of four new metal chelating compounds (**MS1-4**) based on the cyclen macrocycle that bear pendant arms to modify the molecules' properties are evaluated as candidate drugs for Alzheimer's disease. These compounds have been designed to bind to the misregulated metals associated with Alzheimer's disease. The corresponding Cu^{2+}, Zn^{2+} and Ni^{2+} complexes were synthesized and characterized to examine the ability of the chelators to bind to the metal centres. $A\beta_{40}$ de-aggregation by the cyclen compounds was assessed using turbidometry, and the re-solubilization of $A\beta_{40}$ was also examined. The results show that the cyclen compounds have the ability to effectively chelate Cu^{2+} and Zn^{2+} metal ions and de-aggregate $A\beta_{40}$ that has been aggregated due to the presence of these ions. The antioxidant properties of the cyclen compounds were tested using the DPPH scavenging assay, and the results show that some of the compounds can decrease oxidative stress especially MS1 with IC_{50} equals to 71 µM. Molecular modelling studies revealed the behaviour of the cyclen compounds within the E2 domain of APP and its interaction with Cu and Zn. Pharmacokinetics parameters were also promising for the compounds. Overall, these compounds exhibit promising properties as candidates for the treatment of Alzheimer's disease.

A study of drug candidates for Parkinson's disease investigated a series of new iron chelating compounds based on deferiprone and 8-hydroxyquinoline that have amphetamine-like structures. This work addresses a significant problem in Parkinson's disease research, the issue of targeted delivery of chelating compounds into dopaminergic cells where iron may accumulate. The corresponding Fe^{2+} complexes were synthesised and characterised to examine the ability of the chelators to bind to metal centres. Thermodynamic parameters between the chelators and iron were calculated by using Isothermal titration calorimetry.

The results show that 8-hydroxyquinoline-based compounds are more potent chelators than deferiprone-based compounds. N27 dopaminergic cell culture study was conducted to investigate whether the chelators can be transported within cells to prevent dopaminergic cell death caused by iron toxicity. The 8-hydroxyquinoline-based compounds showed the most promising results to rescue dopaminergic cells from iron-induced stress. The antioxidant properties of the compounds were tested using the DPPH scavenging assay, and the results show that 8-hydroxyquinoline-based compounds were superior in decreasing oxidative stress compared to the deferiprone-based compounds. Overall, 8-hydroxyquinoline-based compounds showed favourable properties as candidates for future Parkinson's disease research.