

https://dspace.org/

Polymeric organoiron compounds with carcinostatic properties (branched hydrazone linkers)

Nkazi, Bavon Diakanua

2011-05-24
http://hdl.handle.net/10539/9920

Downloaded from a DSpace repository. https://dspace.org/

Polymeric Organoiron Compounds with Carcinostatic Properties (Branched Hydrazone Linkers)

Bavon Diakanua Nkazi

A dissertation submitted for the fulfillment of the requirements for the degree of Master of Science in the Department of Chemistry / Faculty of Science at the University of the Witwatersrand, Johannesburg.

Supervisor: Professor Eberhard W. Neuse

Johannesburg, 2011.

DECLARATION

I declare that this work is my own, unaided work. It is being submitted for the degree of Master of Science in the Faculty of Science, University of the Witwatersrand, Johannesburg, South Africa. It has not been submitted before for any degree or examination in any other University.

_____

Bavon Diakanua Nkazi

_____ day of _____, 2011.

"An education isn't how much you have committed to memory, or even how much you know. It's being able to differentiate between what you do know and what you don't."

Anatole France (1844-1924)

ABSTRACT

The insufficient efficaciousness of most currently used anticancer drugs has prompted worldwide efforts to reduce toxic and resistance effects, improve overall bioavailability, and widen the therapeutic window. A particularly promising technology to this end rests on the concept of polymer-drug conjugation, in which the bioactive agent is bound to a meticulously designed macromolecular water-soluble carrier through a biofissionable link.

Drug release in the cancerous cell, strongly pH dependant, proceeds hydrolytically in the acidic intracellular compartment, and this represents an advanced drug delivery method in cancer chemotherapy.

The synthesis of water-soluble macromolecular anticancer drugs composed of a polymeric carrier to which the antineoplastic agents are tied via biodegradable hydrazone links were investigated in this project.

Carriers were synthesized essentially by polyaddition and ring-opening methods, and polycondensation process was utilized, refined and routinely used. Polyaspartamides derived from polysuccinimide by aminolytic ring-opening was the parent carrier's structure, allowing for:

a) A non-immunogenic and non-toxic chain construction, which was amenable to biodegradation and ensured catabolic elimination of the duly fragment polymer upon drug release;
b) A highly flexible backbone and the presence of intrachain-type or side group-attached solubilizing groups, which ensured conjugate solution in aqueous media required for rapid dissipation in the central circulation system, even if the conjugated drug itself does not possess water solubility; and;
c) The presence of functional groups as binding sites, represented by the hydrazone entity, which ensured drug attachment and release, was introduced by treatment of polysuccinimide with hydrazine hydrate under specially developed experimental conditions, followed by treatment with selected, functionally active amines providing the aforementioned structural features.

Drug systems were modified so as to contain carbonyl functionality, the crucial reaction site in this hydrazone linking process, and bioactive aldehydes, such as ferrocenylpropenal. A cinnamaldehyde was the primary drug model. In order to illustrate the multidrug-binding capacity of the polyaspartamide type carriers, and at the same time ensuring target-specific drug delivery, folic acid, a potential cell entry facilitator, was co-conjugated to selected polymeric conjugate containing ferrocenylpropenal. Cell carrier and conjugate polymers were purified, fractionated by aqueous phase dialysis in membrane tubing with 12 000 – 14 000 molecular – mass cut - off, and isolated by freeze-drying in ultimate yields of 45 – 80 % as water-soluble materials; and they were structurally characterized by spectroscopic techniques. Inherent viscosities were in the range of 8 – 36 mL g^{-1}. The resulting cinnamaldehyde, curcumin and iron contents of the conjugates were in the range of 4 - 7 %, 10 – 14 % and 1.5 – 2.8 % respectively. In vitro experiments done under buffered solution (performed in polymer laboratory of school of chemistry of the university of the Witwatersrand) showed the released of drugs in cancer cell's pH (pH<7). The results of these tests suggest that in acidic environment PSI-hydrazine carriers drugs systems can release active drug such as ferrocenylpropenal, and on the other hand the polymers drugs systems showed higher stabilities under neutral conditions. Therefore drugs released under pH control can play an important role in future cancer therapy.

DEDICATION

This dissertation is dedicated to my wife Bijou Kanabwingi Nkazi, my daughters Elisa Mafwene, Plamedi, Glodi and Blessing Nkazi for their love, understanding, patience and encouragement and support. I could not have coped without them.

ACKNOWLEDGEMENTS

I would like to thank the Almighty God, our creator, without whom I never would have been able to come thus far.

I wish to thank also the following people and organizations that so willingly helped and contributed to this project:

- Professor E.W. Neuse, my supervisor, for his patience, guidance, encouragement, commitment, helpfulness, and support during this throughout this project.
- MRC for financial support of the project.
- The National Research Foundation (NRF), Bradlow Postgraduate Bursary for their financial assistance.
- Mr. Richard Mampa of the School of Chemistry for running all NMR spectra that resulted in this work.
- My colleagues in the Polymer Research Laboratory for sharing their brilliant ideas with me and for their friendship, notably, Hembe Elie Mukaya, Jacques Kayembe, Jacob Masiala, Blessing Aderebrige, and Alain Didier Ilunga Mufula.
- My wife, my daughters and my parents who were helpful in many ways.
- My brothers, sisters and their families Rene Diakanua, Gee Diakanua, Fifi Diakanua, Mimi Diakanua, Bienvenu Kadima, Justine Kimbala, Aime Diakanua, Billy Ilunga, Abe Lunzitisa, Sophie Moza, Kelly Sungamena, Irene Bibomba, Jeannette Diakanua, Fatuma Kanabwingi, Nathan, and Joelle Twite for their love, support, courage, guidance and for their prayers.
- My families Pastor Abraham, Sarah Ilunga, Pastor Ruffin, Bibiche Binda, Raphael Kitiaka, Nana Masangu, Henry, Solange Lay Lay, Francis, Giselle, Apotre Andre, Angel Tandu, Samba, Ida, Julien, Mireille Lusilao, Elise Bakatula, Athur Nseka, and friends for their support and for their prayers.

- Prophet Kish and maman Fina, Bishop Timothy, and all members of Full fire Ministries for their love and their prayers.
- My brothers Neddy Lumanu Mulenda and Paul Lumanu Diobo for their love, encouragement and support.
- Kirsti Claassen for her friendship and assistance.
- Maman Kitty Banda for her kindness and assistance.
- Fortune and Aurora for their friendship and support.
- All who pray for me. Your prayers were not valueless.

Table of Contents

Declaration II
Abstract IV
Dedication VI
Acknowledgements VII
Table of contents VII
List of figures XII
List of schemes XII
List of tables XIII
List of symbols and abbreviations XIV

CHAPTER 1- INTRODUCTION 1

1.1 Cancer overview 2
1.2Aims of the project 4

CHAPTER 2- BACKGROUND AND LITERATURE REVIEW 5

2.1 Cancer chemotherapy 5
2.1.1 Introduction 5
.2.1.2Cancer chemotherapy 5
2.1.3Categories of chemotherapy drugs 6
2.1.3.1 Alkylating agents 6
2.1.3.2 Antimetabolites 7
2.1.3.3 Anthracyclines 7
2.1.3.4 Antitumor antibiotics 7
2.1.3.5 Monoclonal antibodies 8
2.1.3.6 Platinum drugs 8
2.1.3.7 Plant alkaloids 9
2.1.3.8 Ferrocenyl drug system 11
2.1.3.8.1 Ferrocene ferricenium system behavior in the biological environment 13

2.1.3.8.2 Ferrocene ferricenium system as candidate anticancer drug model 14
2.2Polymer-drug conjugation 16
2.2.1Conjugation systems 16
2.2.1.1 Lysosomotropic conjugates 18
2.2.1.2 Hydrazone conjugates 19
2.2.2. Polymers as drug carriers 19
2.2.2.1 Requirements for polymeric drug carriers 20
2.2.1.1.1 Hydrosolubility for intravenous delivery 20
2.2.1.1.2 Biodegradability 20
2.2.1.1.3 Biocompatibility 21
2.2.1.1.4 Chemical composition 21
2.2.2.2 Natural polymers as drug carriers 22
2.2.2.2.1 Protein-origin polymers 22
2.2.2.2.2 Polysaccharidic polymers 23
2.2.4 Synthetic polymers as drug carriers 25
2.2.4.1 Pharmacokinetic of macromolecular prodrugs 27
2.2.4.2 Pharmacokinetics of macromolecules at body and organ levels 29
2.2.4.3 Pharmacokinetics of macromolecules at the cellular level 29
2.2.4.4 Pharmacokinetic benefits of macromolecular prodrugs 31
2.2.5 Drug systems based on carrier-conjugated 31
2.3 Branched hydrazone linkers 32
2.3.1 Strategy for the synthesis of polymeric carriers containing the hydrazone bridge 33
2.3.2 Hydrazone linker's stability 34
2.3.3 Cancer cell's pH and ferrocenyl hydrazone drugs 34
2.3.3.1 pH and cancer cells 34
2.3.3.2 pH-responsive hydrazone-ferrocenyl conjugates 35

CHAPTER 3- RESULTS AND DISCUSSION 36

3.1 Synthesis of aldehydes and ketones drugs 36
3.1.1 Synthesis of Ferrocenylpropenal 36
3.1.2 Synthesis of acetylferrocene 37

3.2 Synthesis of macromolecular carriers 39
3.2.1 Introduction..... 39
3.2.1 Polyaspartamides..... 40
3.2.1.1 Poly-DL-succinimide..... 41
3.2.1.2 Poly-α,β-DL-aspartamides..... 41
3.2.1.2.1 Polyaspartamide carriers bearing one hydrosolubilizing group 44
3.2.1.2.2 Terpolyaspartamide carriers..... 51
3.2.2 Polymer-drug anchoring..... 63
3.2.3 Polymer multidrug conjugation 75
3.3 Evaluation of the stability of hydrazone linkage at various pH..... 79
3.3.1 In vitro stability test at pH 7.4 79
3.3.2 In vitro released test at pH 6.5..... 79
3.3.3 Discussion..... 82

CHAPTER 4- EXPERIMENTAL 83

4.1 General Procedures 83
4.2 Reagents and Solvents 84
4.3 Experimental Procedures..... 84
4.3.1 Preparation of ferrocenyl drugs 84
4.3.1.1 Ferrocenylpropenal 84
4.3.1.2 Acetylferrocene 85
4.3.2 Preparation of polymeric carriers..... 85
4.3.2.1 Poly-DL-succinimide..... 85
4.3.2.2 Synthesis of copolyaspartamides..... 86
4.3.2.3 Synthesis of terpolyaspartamides..... 91
4.3.3 Preparation of polymeric conjugates 99
4.3.3.1 Polyaspartamides-ferrocenyl anchoring 99
4.3.3.2 Polyaspartamides multidrug conjugation..... 101

CHAPTER 5-CONCLUSION AND FUTURE WORK..... 102

Reference..... 104

Appendix 115

^{1}H-NMR Spectra..... 116

LISTS OF FIGURES

Figure 1.1: Cancer cells 1
Figure 1.2: Cell cycle phases 3
Figure 2.1: Mechanism of action for anticancer drugs 10
Figure 2.2: Ferroquine and ferrocifen structures 12
Figure 2.3: Pathophysiology of tumor tissue 18
Figure 2.4: Ringsdorf model of synthetic polymer drugs 26
Figure 2.5: Examples of targets, barriers and strategies for intracellular drug delivery 28
Figure 2.6: Schematic representation of the intracellular fate of macromolecule drug conjugate after endocytosis 31
Figure 2.7: Formation of hydrazone bridge 33
Figure 2.8: pH control of hydrazone-ferrocenyl conjugates 35
Figure 3.1: Models of hydrazone-conjugates 64
Figure 3.2: Multidrugs conjugation 75
Figure 3.3: Percentage of hydrazone formation at ph values from 3.5 to 7 80
Figure 3.4: Fcp concentrations against times at neutral and acid pH 80
Figure 3.5: Fcp concentrations against times at different pH 81
Figure 3.6: Released of aldehydes and ketones drugs at pH 6.5 81

LISTS OF SCHEMES

Scheme 2.1: Reversible oxidation/reduction 12
Scheme 2.2: Biological interactions involving the ferrocene-ferricenium system..... 14
Scheme 2.3: Ferricenium reduction by NADH..... 14
Scheme 2.4: Flow chart describing drug conjugate pharmacokinetics 27
Scheme 2.5: Interaction between hydrazine and aldehyde groups 34
Scheme 3.1: Synthesis of 3-ferrocenylpropenal 36
Scheme 3.2: Synthesis of acetylferrocene 37
Scheme 3.3: Basic carrier model..... 40
Scheme 3.4: Polycondensation of DL-aspartic acid 41
Scheme 3.5: Reaction scheme of formation of homopoly(α, β-DL-aspartamides)..... 42
Scheme 3.6: Aminolytic ring opening reaction leading to the formation of copolyaspartamide 42
Scheme 3.7: Synthesis of copolyaspartamides carriers 2a (80) – 2f (80)..... 46
Scheme 3.8: Synthesis of copolyaspartamides carriers 2a (80) – 2f (80)..... 47
Scheme 3.9: Synthesis of terpolyaspartamides carriers 3a (50) – 3e (60) 52
Scheme 3.10: Synthesis of terpolyaspartamides carriers 3a (60) – 3e (60) 53
Scheme 3.11: Synthesis of terpolyaspartamides carriers 4a (60) – 4e (60) 58
Scheme 3.12: Synthesis of terpolyaspartamides carriers 4a (50) – 4e (50) 59
Scheme 3.13: Synthesis of conjugates 2a (80)-Fcp – 2f (90)-Fcp..... 67
Scheme 3.14: Synthesis of conjugates 3a (60)-Fcp – 3e (50)-Fcp..... 68
Scheme 3.15: Synthesis of conjugates 4a (60)-Fcp – 4e (50)-Fcp..... 69
Scheme 3.16: Synthesis of conjugates with Fca, Fcc and Cyn 70
Scheme 3.17: Synthesis of co-conjugates 2a (80)- FA-Fcp, 2a (90)-FA-Fcp and 4c (60)- Cur- Fcp 76

LISTS OF TABLES

Table 3.1: Summary of experimental data for ferrocenyl drugs 38
Table 3.2: ^{1}H NMR of ferrocenyl drugs 38
Table 3.3: Summary of experimental data for copolyaspartamide carriers 48
Table 3.4: ^{1}H NMR of copolyaspartamide 2a (80) to 2f (90) 49
Table 3.5: Summary of experimental data for terpolyaspartamide carriers 3 54
Table 3.6: ^{1}H NMR of terpolyaspartamides 3a (50) to 3f (60) 55
Table 3.7: Summary of experimental data for terpolyaspartamide carriers 4 60
Table 3.8: ^{1}H NMR of terpolyaspartamides 4a (50) to 4f (60) 61
Table 3.9: Preparative and analytical data of drug-conjugates 71
Table 3.10: ^{1}H NMR of drug-conjugates 73
Table 3.11: Analytical data of drug-conjugates 77
Table 3.12: ^{1}H NMR for selected PAsA co-conjugates 78

LISTS OF ABBREVIATIONS

AEE: 2-(2-Aminoethoxy)ethanol

ATP: 4-amino-2,2,6,6-tetramethylpiperidine

Boc: *tert*-butoxycarbonyl

Calcd: Calculated

Cur: Curcumin

Cyn: Cinnamaldehyde

DCC: Dicyclohexylcarbodiimide

DEEA: 2-(diethylamino)ethylamine

DMEA: 2-(dimethylamino)ethylamine

DEP: 3-(diethylamino)propylamine

DET: Diethylenetriamine

DHFR: Dihydrofolate reductase

DMF: N,N-Dimethylformamide

DMP: 3-(N,N-Dimethylamino)propylamine

DMSO: Dimethyl sulfoxide

DNA: Deoxyribonucleic acid

EPR: Enhanced permeability and retention

EtAc: Ethyl acetate

FA: Folic acid

FAO: Food and agriculture organization

FDA: Food and Drug Administration

Fca: Acetylferrocene

Fcc: Ferrocenecarboxyaldehyde

Fcp: Ferrocenylpropenal

Fcp': Ferrocenylpropenal (with different feeding ratio than Fcp)

FR: Folate receptor

GPC: Gel permeation chromatography

GSH: Glutathione

Hex: Hexane

Hy: Hydrazine

Inh: Inherent

IR: Infrared

MTX: Methotrexate

NMP: N-Methylpyrrolidone

NMR: Nuclear magnetic resonance

PAA: Poly(amidoamine)

PAsA: polyasapartamide

PSI: polysuccinimide

RFC: Reduced folate carrier

RNA: Ribonucleic acid

RT: Room temperature

SOD: Superoxide dismutase

UV-Vis: Ultraviolet-visible

WHO: World health organization