

Polymeric Organoiron Compounds with Carcinostatic Properties (Branched Hydrazone Linkers)

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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.

III

“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-hydrazone 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.

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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
2.2 Polymer-drug conjugation	16
2.2.1 Conjugation 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
2.2.2.1.1 Hydrosolubility for intravenous delivery	20
2.2.2.1.1.2 Biodegradability	20
2.2.2.1.1.3 Biocompatibility	21
2.2.2.1.1.4 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: ^1H NMR of ferrocenyl drugs	38
Table 3.3: Summary of experimental data for copolyaspartamide carriers	48
Table 3.4: ^1H NMR of copolyaspartamide 2a (80) to 2f (90)	49
Table 3.5: Summary of experimental data for terpolyaspartamide carriers 3.....	54
Table 3.6: ^1H NMR of terpolyaspartamides 3a (50) to 3f (60)	55
Table 3.7: Summary of experimental data for terpolyaspartamide carriers 4.....	60
Table 3.8: ^1H NMR of terpolyaspartamides 4a (50) to 4f (60)	61
Table 3.9: Preparative and analytical data of drug-conjugates	71
Table 3.10: ^1H NMR of drug-conjugates	73
Table 3.11: Analytical data of drug-conjugates	77
Table 3.12: ^1H 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: polyaspartamide

PSI: polysuccinimide

RFC: Reduced folate carrier

RNA: Ribonucleic acid

RT: Room temperature

SOD: Superoxide dismutase

UV-Vis: Ultraviolet-visible

WHO: World health organization