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

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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.

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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.

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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