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MACROMOLECULAR DERIVATIVES OF METHOTREXATE AND FERROCENE AS POTENTIAL PRODRUGS IN CANCER CHEMOTHERAPY

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A dissertation submitted to the Faculty of Science, University of the Witwatersrand, Johannesburg, in fulfillment of the requirements for the degree of Master of Science.

Supervisor: Professor E.W. Neuse

Johannesburg, 2010

DECLARATION

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

_____

Ilunga Mufula

_____day of _____, 2010

ABSTRACT

Cancerous diseases present a formidable health problem worldwide. While the chemotherapy of cancer, in conjunction with other treatment modalities, has reached a significant level of maturity, efficacious use of such agents is still restricted by numerous pharmacological deficiencies, such as poor solubility, short serum circulation lifetimes, and low bioavailability resulting from lack of affinity to cancer tissue and inadequate mechanisms of cell entry. More critically still, most drugs suffer from toxic side effects and a risk of drug resistance. In an attempt to enhance the therapeutic effectiveness of carcinostatic drugs, the concept of anchoring bioactive agents to polymeric carriers has proved to be a promising approach to overcome these deficiencies and was the main aim of this project.

Water-soluble, biodegradable macromolecular carriers used were *polyaspartamides*, prepared by an aminolytic ring-opening process of polysuccinimide; *polyamides* obtained by ester-amine base-catalyzed polyaddition; and *polyamidoamines* prepared by Michael-type addition polymerization. The drug-anchoring potential of carrier polymers was demonstrated by the coupling of methotrexate (MTX), ferrocene and platinum drug models.

MTX was linked to carrier *via* polymer attached amine by N-acylation of linear amine-functionalized polyaspartamide carriers with the acid group from methotrexate. Acylation was brought about by mediation of HBTU coupling agent. The resulting MTX content of the conjugates was in the range of 10-19% by mass.

In the present dissertation, series of water-soluble ferrocene conjugates were synthesized as for MTX by N-acylation of linear amine-functionalized polyaspartamide carriers with 4-ferrocenylbutanoic acid. Acylation was brought about again by mediation of HBTU coupling agent. The resulting iron content of the conjugates was in the range of 6-13% by mass.

Polymer-attached dihydroxylato-type ligands were used to anchor the platinum drug to the polymeric carriers. The platinum content of the conjugates was in the range of 6-8% by mass.

A member of selected conjugates was submitted to the Department of Immunology, University of Pretoria, and to the School of Pharmacy, University of California, Los Angeles, CA, for biomedical activity assessment.

In order to demonstrate the multidrug-binding capacity of the polyaspartamide-type carriers, ferrocene was co-conjugated to selected polymeric conjugates containing MTX or folic acid. The latter was used to ensure target-specific drug delivery.

DEDICATION

This dissertation is dedicated to my father for his prayers and constant support throughout my studies.

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LIST OF ABBREVIATIONS

AEE	2-(2-Aminoethoxy)ethanol
AEM	4-(2-aminoethyl)morpholine
APM	4-(3-aminopropyl)morpholine
aq	aqueous
d	day(s)
DACH	1,2-diaminocyclohexane
DCC	N,N′-dicyclohexylcarbodiimide
DEEA	2-(diethylamino)ethylamine
DEP	3-(diethylamino)-1-propylamine
DET	diethylenetriamine
Detart	diethyl L-tartrate
DMEA	2-(dimethylamino)ethylamine
DMF	N,N-dimethylformamide
DMP	3-(dimethylamino)-1-propylamine
DMSO	dimethyl sulphoxide
DNA	deoxyribonucleic acid
EA	ethanolamine
EDDA	2,2-(ethylenedioxy)-diethylamine
FA	folic acid
Fc	ferrocenyl
η_{inh}	inherent viscosity
HBTU	2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium fluorophosphates
MBA	methylenebisacrylamide
MTX	methotrexate
Net_3	triethylamine
NMR	nuclear magnetic resonance
PDA	1,3-propylenediamine
ppm	parts per million
PSI	poly(D,L-succinimide)

RNA ribonucleic acid

RT room temperature

TRIA 4,7,10-trioxa-1,13-tridecanediamine

SOLUMIX: mixture of (% w/w): Toluene (42.96%), m-xylene (14.39%), p-xylene (6.72%), o-xylene (6.39%), ethylbenzene (6.08%), heptane and isomers (10.01%), n-hexane (2.26%), hexane, mixture of isomers (1.60%), pentane (0.16%), isopentane and 2-methylbutane (0.12%).