Part of the work described in this thesis has been included in the following publications:

1- Published paper "Diels Alder/ Thiol-Olefin Co-Oxidation Approach to the Antimalarial 2,3-dioxabicyclo [3.3.1] nonane pharmacophore", Paul M. O'Neill; Edite Verissimo; Stephen A. Ward ; Jill Davies; Mario D. Bachi ; Edward E. Korshin; Nuna Araujo; Matthew Pugh; M. Lurdes S. Cristiano; Paul A. Stocks; *Bioorganic and Medicinal Chemistry Letters*; 2006; 16; 2991-2995.

2- Published paper "Design and Syntheses of Endoperoxide Antimalarial Pro-Drug Models: Prototypes for Selective Intraparasitic Generation of Cysteine Protease Inhibitors and Other Parasitocidal Species" Paul M. O'Neill, Paul A. Stocks, Matthew D. Pugh, Jamie F. Bickley, Edward E. Korshin, Stephen A. Ward, Patrick G. Bray, Erica Pasini, Jill Davies, Nuna C. Araújo, Edite Veríssimo, and Mario D. Bachi, *Angewandte Chemie International Edition;* 2004; 43; 4193-4197.

3- Poster communication "Peptidomimetic Inhibitors of Plasmodium falciparum: Development of new antimalarials", presented in the international conference "Sci ESOR X" , in Rome (July 2005) - awarded with the first prize for the best poster communication.

4- Poster communication "New strategies Towards Fighting Malaria: Design and Synthesis of Pharmacologically Active Compounds", presented in the national meeting "Químico-Sul", in Faro (May 2005)

5- Poster communication "Endoperoxide Cysteine Protease Inhibitor Pro-Drugs: a Novel "Trojan Horse Approach" to Antimalarial Chemotherapy", presented in

the conference Reaction Mechanisms VIII, in Dublin (July 2004)- awarded with the fifth prize in the poster competition.

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Name: Edite Manuela da Graça Veríssimo

Faculty: Faculdade de Ciências e Tecnologia

Supervisors*:* Doutora Maria de Lurdes Cristiano (Universidade do Algarve) e Dr. Paul O'Neill (University of Liverpool)

Date*:* November of 2006

Thesis Title: "New Approaches to Antimalarial Chemotherapy: Design and Synthesis of Bicyclic Endoperoxides and of Peptide and Peptidomimetic Carbonyl Containing Cysteine Protease Inhibitors"

ABSTRACT

The emphasis of the research described within this thesis was on finding new strategies towards fighting malaria. The introductory chapter makes a presentation of the disease, providing a general description of the current status of malaria worldwide, the symptoms caused by the disease and the biology of the malaria parasite and also reviewing on antimalarial drugs, new parasite biochemical targets and new viable drug combinations.

The concept of achieving combination therapy by a single entity was the common theme to the projects developed and was pursued in a two-fold manner: in chapter 2, synthetic approaches to the development of bicyclic endoperoxide pro-drugs capable of releasing a carbonyl unit and a C-centered radical *in tandem* are described, and, in chapter 3, the synthesis of novel carbonyl containing cysteine protease inhibitors that can potentially be incorporated within a pro-drug endoperoxide unit is addressed. Biomimetic Fe(II) chemistry is included in the investigation within chapter 2 and, with respect to the protease inhibitors in chapter 3, both peptide and peptidomimetic systems were designed and synthesised, and the antimalarial *in vitro* activity of target peptidomimetics was assessed.

Key-words: malaria, antimalarial chemotherapy, combination chemotherapy, ECPI drugs, peptidic and peptidomimetic cysteine protease inhibitors.

Título da Tese: "New Approaches to Antimalarial Chemotherapy: Design and Synthesis of Bicyclic Endoperoxides and of Peptide and Peptidomimetic Carbonyl Containing Cysteine Protease Inhibitors"

RESUMO

A procura de novas estratégias de combate à Malária constitui o objectivo da investigação descrita nesta tese. O capítulo introdutório apresenta alguns aspectos relevantes da doença, tais como a sua contextualização no panorama global, os sintomas e o ciclo biológico do parasita. É feita uma abordagem aos fármacos antimaláricos disponíveis, referindo novos alvos bioquímicos e novas combinações eficazes de fármacos.

Os projectos desenvolvidos no âmbito da investigação descrita nesta tese enquadram-se no conceito do desenvolvimento de fármacos bifuncionais que permitam uma terapia combinada contra a malária, mediante a utilização de uma entidade química única. Este objectivo foi abordado através de duas estratégias distintas

No capítulo 2 é apresentado e discutido o design e a síntese de endoperoxidos bicíclicos, pró-fármacos com apetência para libertar um composto carbonílico em simultâneo com radicais centrados no carbono, uma vez activados pelo Fe(II) existente no vacúolo alimentar do parasita. No capítulo 3, são apresentadas e discutidas as estratégias adoptadas na preparação de inibidores de proteases Cisteínicas, baseados em compostos carbonílicos, aos quais haverá a possibilidade de posteriormente acoplar uma unidade endoperoxídica. No capítulo 2 são ainda discutidos os resultados dos estudos da biomimética do Fe (II). Os sistemas peptídicos e peptidomiméticos preparados no capítulo 3 foram ainda submetidos a testes de actividade antimalárica "*in vitro*".

Key-words: malaria, quimioterapia antimalárica, terapia combinada, fármacos ECPI, inibidores peptídicos e peptidomiméticos de proteases cisteínicas.

CONTENTS

Definitions and Abbreviations

Ac- acyl

ACN- acetonitrile

Aq- aqueous

Ar- aryl

Bn- benzyl

Bu- butyl

CC- combination chemotherapy

Cbz- carbobenzyloxy

CPI- cysteíne protease inhibitors

CQ- chloroquine

3D7- *P. falciparum* strain sensitive to chloroquine

DCM- dichloromethane

Dess-Martin-Periodinane: 1,1,1-Triacetoxy-1,1-dihydro-1,2-benziodoxol-3 (1H)-one

Diazald: N-methyl-N-nitroso-p-toluenesulfonamide

DMAP- dimethylaminopyridine

DMF- *N,N*-dimethylformamide

DMSO- dimethyl sulphoxide

ECPI- endoperoxide cysteine protease inhibitors

EDC- 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide

EtOAc- ethyl acetate

Et_2O- diethyl ether

EtOH- ethanol

eq- molar equivalents

FV- food vacuole

FP-2- falcipain 2

h- hour (s)

HOBt- hydroxybenzotriazole

HPLC- high performance liquid chromatography

IC_{50}- a dose which causes 50% of the maximum possible inhibition of a response to a given drug.

M- molar

Me- methyl

MeOH- methanol

m.p.- melting point

MTBE- *t*-butyl methyl ether

NaOH- sodium hydroxide

NMM- *N*-methylmorpholine

NPCF- 4- nitrophenylchloroformate

OTf- triflate

Ph- phenyl

py- pyridine

RT- room temperature

SAR- structure-activity relationship

TEMPO- 2,2,6,6-tetramethylpiperidin-1-oxyl

THF- tetrahydrofuran

TLC- thin layer chromatography

TM6- *P. falciparum* strain resistant to chloroquine

TMS- trimethylsilane

δ- chemical shift