UNIVERSITI PUTRA MALAYSIA

ANTIFUNGAL PEPTIDE MODELING, FOLDING AND MIMETIC DESIGN

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FBSB 2009 30

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By

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Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

October 2009

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of
the requirement for the degree of Master of Science

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MIMETIC DESIGN

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SHOEIB MORADI

October 2009

Chairman: **Nor Aripin Shamaan, PhD**

Faculty: **Biotechnology and Biomolecular Sciences**

The antifungal peptides represent diverse structures for drug design. Unfortunately, they
provide inferior drug candidates because of their low oral bioavailability, potential
immunogenicity, poor *in vivo* metabolic stability and high molecular weight. Recent
efforts have focused on the creation of non-natural peptide mimetics. Their artificial
backbone makes most peptidomimetics resistant to degradative enzymes, thus,
increasing the stability of peptidomimetic drugs in the body. In the present study, four
antifungal peptidomimetics structures named C_1 to C_4 were designed based on the
antifungal decapeptide crystallized structure of Pep-1 using bioinformatics tools.
Structures C_1 and C_2 belong to the N-terminal part of Pep-1 and C_3 and C_4 belong to the
C-terminal amino acid sequence part of Pep-1. Minimum inhibitory concentrations
(MIC) of these structures were estimated against *Aspergillus niger* N402, *Candida
albicans* ATCC 10231, and *Saccharomyces cerevisiae* PTCC 5052. Structures C_2 and
C_1 showed more potent antifungal activities against these fungal strains compared to C_3

and C_4, respectively. This demonstrated that the N-terminal part is more potent for antifungal activity and indicated that the N-terminal part of antifungal peptides is more active and important for antifungal activity than the C-terminal. Structure C_2 was demonstrated to be more active against these microorganisms and could be used as a potential target for future antifungal peptidomimetics studies. Important factors/descriptors of 63 antifungal peptides have been studied using Artificial Neural Network (ANN). The most important factors determined were amino acid number 1 (S1), Log P, and their α-helix contents. This is the first study on the structure of C_1 to C_4 peptidomimetics on *Aspergillus niger* N402, *Candida albicans* ATCC 10231, and *Saccharomyces cerevisiae* PTCC 5052.

Abstrak ini dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

PERMODELAN, PENGLIPATAN DAN REKABENTUK PEPTIDA MIMETIK ANTIKULAT

Oleh

SHOEIB MORADI

Oktober 2009

Pengerusi: **Nor Aripin Shamaan, PhD**

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Peptida antikulat merupakan struktur yang pelbagai bagi rekabentuk dadah. Malangnya, mereka membekalkan calon dadah yang berkualiti rendah disebabkan biopenggunaan oral yang rendah, potensi imunogenisiti, kestabilan metabolik *in vivo* yang rendah dan berat molekul yang tinggi. Usaha terkini tertumpu kepada penciptaan peptida mimetik yang tidak asli. Tulang belakang peptida mimetik yang tidak asli ini menyebabkan kebanyakan peptida mimetik rintang terhadap enzim degradatif dan meningkatkan kestabilan bahan dadah peptida mimetik tersebut didalam tubuh. Dalam kajian ini, empat struktur peptida mimetik antikulat ($C_1 - C_4$) di reka bentuk berasaskan struktur terhablur dekapeptida antikulat Pep-1 dengan menggunakan kaedah bioinformatik. Struktur C_1 dan C_2 terkandung dalam bahagian terminal-N struktur antikulat Pep-1 sementara struktur C_3 dan C_4 terkandung dalam bahagian jujukan asid amino terminal-C antikulat Pep-1. Kepekatan perencatan minimum (Minimum inhibitory concentration, MIC) struktur tersebut dianggar dengan menggunakan kulat *Aspergillus niger* N402, *Candida albicans* ATCC 10231 dan *Saccharomyces cerevisiae* PTCC 5052. Struktur C_2

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dan C_1 menunjukkan aktiviti antikulat yang kuat terhadap ketiga-tiga species kulat yang dikaji berbanding C_3 dan C_4. Ini membuktikan bahawa bahagian terminal-N mengandungi aktiviti antikulat yang lebih kuat berbanding terminal-C dan bahagian terminal-N peptida antikulat adalah lebih aktif dan penting dari terminal-C. Struktur C_2 mempamerkan aktiviti antikulat yang tinggi terhadap kulat yang dikaji dan berkemungkinan menjadi sasaran untuk kajian masa hadapan bagi peptida mimetik antikulat. Kajian ke atas 63 peptida antikulat telah dilakukan dengan menggunakan jaringan artifisial neural (Artificial Neural Network, ANN). Faktor paling penting yang ditentukan ialah asid amino pertama dalam jujukan (S1), Log P dan isi kandungan α-heliks. Kajian ini merupakan kajian pertama keatas struktur C_1 hingga C_4 peptida mimetik *Aspergillus niger* N402, *Candida albicans* ATCC 10231, dan *Saccharomyces cerevisiae* PTCC 5052.

ACKNOWLEDGEMENT

Hymn the praises be to thy Lord, who ever ready to show mercy, and refuge us from evil.

I specially thank Prof. Nor Aripin Shamaan who trusted and supported me during these 2 academic years.

I would like to thank Dr. Soroush Sardari for serving not only as my supervisor, but also in my life that changed my insight about my living manner and taught me how to see the world. He provides continuous guidance and support throughout during these two years graduate work.

I also thank my other committee member Dr. Mohd. Puad Abdullah**.**

I am grateful to my all lecturers during these two years of study in Malaysia especially Dr. Foo Hooi Ling for her help and recommendations.

Moreover, I should really thank my parents for their financial support in addition to being my sponsor during my stay in Malaysia and for their patience. I am grateful to them and the rest of family members my sisters and my little brother, and encouragement during difficult periods in my study and my life.

Finally I would like to thank all my friends in Pasteur Institute of Iran especially Mr. Saeed Soltani for his help during my study, and also thank Mohammad, Sina, Mahdi, Morteza, Amir.

APPROVAL

This thesis submitted to the Senate of University Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee are as follows:

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DECLARATION

I hereby declare that this thesis is based on my original work, except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or any other institution.

Shoeib Moradi
Date: 14th October 2009

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LIST OF ABBREVIATIONS

/	Per
°C	Degree Celsius
µg	Microgram
µH/µH max	Relative-amphipathicity
µM	Micro Molar
3D	Three-Dimentional
ACE	Angiotensin-converting enzyme
AFPs	Antifungal peptides
AMPs	Antimicrobial peptides
ANN	Artificial Neural Networks
BLAST	Basic local alignment search tool
C_α	Alpha-carbon
CAMD	Computer-Aided Molecular Design
C_β	Beta-carbon
Cys	Cysteine
CFU	Colony forming unit
Cont.	Continued
D	Dalton
DMSO	Dimethyl sulfoxide
DPM	Double prediction Method
Drs	Drosomycin
ECM	Extracellular matrix
h	Hour
HBD	Human beta-defensin
HIV	Human immunodeficiency virus

IC$_{50}$	Inhibitory concentration
IgA	Immunoglobulin A
ITR	Itraconazole
LAP	Lingual antimicrobial peptide
LPS	Lipopolysaccharide
LTA	Lipoteichoic acid
MIC	Minimum inhibitory concentration
ml	Milliliter
NMR	Nuclear magnetic resonance
NP	Neutrophils
pI	Isoelectric point
PDB	Protein Data Bank
PMN	Polymorphonuclear
QSAR	Quantitative structure activity relationship
RMSD	Root mean square deviation
SAR	Structure-activity relationships
SE	Syringomycin-E
SMB	Sabouraud maltose broth
TF	Tissue factor
TL	Thaumatin-like
TNF	Tumor necrosis factor

CHAPTER I

INTRODUCTION

1.1. Background and Objectives

In the past 10 years, the frequency of fungal disease has increased noticeably (Hong et al., 2001). Multiple-drug resistances have appeared leading to the discovery of novel drugs to combat the through diverse mechanisms of actions. Antimicrobial peptides (AMPs) are an evolutionarily conserved component of the innate immune response and are found among all classes of life. These peptides are potent, broad spectrum antibiotics which demonstrate potential as novel therapeutic agents. These AMPs are finding from diverse sources ranging from primary eukaryotes to mammalian having typically fewer than 60 amino acids long, containing cationic amino acid residues and an amphiphilic structure bound to the membrane (α-helical and/or β-sheet). Many of these peptides are unstructured in free solution, and fold into their final configuration upon partitioning into biological membranes.

The ability to associate with membranes is a definitive feature of antimicrobial peptides (Dhople, et al. 2006) although membrane permeabilisation is not necessary. These peptides have a variety of antimicrobial activities ranging from membrane permeabilization to action on a range of cytoplasmic targets. It appears as though many peptides initially isolated as and termed "antimicrobial peptides" have been shown to have more significant alternative functions *in vivo* (e.g. hepcidin) (Brogden, 2005). These peptides are excellent candidates for development as novel therapeutic agents and complements to conventional antibiotic therapy because in

contrast to conventional antibiotics they do not appear to induce antibiotic resistance while they generally have a broad range of activity, are bacteriocidal as opposed to bacteriostatic and require a short contact time to induce killing.

A number of naturally occurring peptides and their derivatives have been developed as novel anti-infective therapies for conditions as diverse as oral mucositis, lung infections associated with cancer (Amsterdam, 1996), and topical skin infections. Pexiganan has been shown to be useful to treat infection related diabetic foot ulcer. The application of peptides as drugs is difficult due to their poor oral and tissue absorption, quick proteolytic cleavage and weak half-life or stability. Since the majority of proteins and small peptides are simply proteolyzed, quickly excreted and poorly bioavailable, a lot attempt has been exhausted to discover ways to replace portions of peptides with non-peptide structures, termed peptidomimetics which are to mimic peptide in the expectation of achieving more bioavailable units. Their artificial backbone is resistant to proteases, as well. Peptidomimetics are one set of probes utilized in the shift pathway of tiny molecule drug design.

In recent times considerable advancement has been made in use of Computer-Aided Molecular Design (CAMD) of novel molecules. These techniques normally rely on two steps: the first being forward modelling, through which Quantitative Structure Activity Relationship (QSAR) procedure is accomplished by application of non-linear modelling procedures such as Artificial Neural Networks (ANN). The second step is model inversion/optimization which utilizes optimization of algorithms in exploitation of the first stage result in discovery of molecules with enhanced activity. Several factors have an effect on the activity of antifungal peptides like sequence, size, charge, degree of structure formation, cationicity, hydrophobicity,

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amphipathicity, hydrophobic moment and pH. ANN possibly will aid us to calculate approximately the significance of these structural parameters in bioactivity of AFPs and finally the most possible model of AFPs' mechanism of action.

In the current study, important factors/descriptors in antifungal peptides have been studied and 4 antifungal peptidomimetics structures were designed based on antifungal peptide structures, using different design methods by means of bioinformatics as a computational studies, rather than synthesize first and test antimicrobial peptidomimetics strategy.

Objectives:

Two objectives have been followed in this study which are:

1. *In silico* study of potential features/descriptors in antifungal peptides molecules.

2. Design of antifungal peptidomimetics structure using cheminformatics/bioinformatics tools.

CHAPTER II

LITERATURE REVIEW

2.1. Antimicrobial Peptides

2.1.1. Introduction

Multi-drug resistance against generally used antibiotics has turn into an essential community health trouble all over the world (Lohner, 2001; Novak et al., 1999). Although the occurrence of resistance is not new, being first proposed by Sir Alexander Fleming with regard to penicillin more than 60 years in the past, it has become of greater than ever concern as more and more antibiotics are caused to be ineffective. Furthermore, resistance at this moment also includes effective antibacterial agents which are used as a last resort, e.g. methicillin and vancomycin. Around 30% of hospital strains of *Enterococci* are vancomycin resistant and almost half of the infections caused by *Staphylococcus aureus* are methicillin resistant (Straus and Hancock, 2006). In the last two decades, the occurrence of human fungal infections has increased significantly; in parallel with the broad extend of untreatable infectious diseases connected with antibiotic resistant bacteria (Jang et al., 2006). Of the thousands of recognized fungal species, just about 175 are pathogenic and generate mycotic infections in humans and animals (Duggineni et al., 2007). As of 2003, over 57% of *Staphylococcus aureus* infections in US intensive care units were resistant to multiple antibiotics (National Nosocomial Infections Surveillance (NNIS) System Report). Fungal infections are significant reasons of morbidity and mortality in hospitalized patients: candidiasis is the fourth most general blood culture isolates in US hospitals (Pfaller et al., 1998), pulmonary aspergillosis is the leading cause of

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death in bone marrow transplant recipients (Panutti et al., 1992), and *Pneumocystis carinii* pneumonia is the leading cause of death in AIDS patients in North America and Europe fungal diseases have been converted into an emergent hazard, principally in immunocompromised patients, for which few or no effective drugs are currently obtainable (Lupetti et al., 2002). The incidence of fungal infections that have been seen in the increasing populations of immunocompromised hosts, counting individuals infected with HIV, aging, organ transplantation, and patients with cancer, has increased dramatically in the last few decades (Peara & Patterson, 2002). The AIDS epidemic, enhanced life-sustaining therapy, and aggressive anticancer therapy have supplied to the increase in the quantity of severely immunocompromised patients (Helmerhorst et al., 1999). This has led to a raise in mucosal and systemic fungal infections, and the simultaneous increased usage of antifungal agents for prophylaxis is most likely the main reason of the improvement of antifungal drug resistance (White et al., 2001).

The yeast fungus, *Cryptococcus neoformans*, has been recognized as the fourth most regular from of life-threatening infection in AIDS patients (White et al., 2001). Potentially fatal infections with *Candida albicans* and other species of *Candida* are in addition identified (Kovacs et al., 1985). Additionally, antifungal drugs frequently make use of various unfavourable effects and are irregularly dose-limiting. In such conditions, a novel scheme is for the control of fungal infections. Aspergillosis and candidiasis, which are typically caused by *Aspergillus fumigatus* and *Candida albicans*, respectively, are the most repeated of fungal infections (Clark & Hajjeh, 2002). In agriculture, enormous economic losses are caused by the infestation of crop plants with fungal pathogens (Szappanos et al., 2006). The infection with plant pathogens such as *Fusarium* spp. not only causes severe yield losses, crop damage

and decreases in quality of grain, but far more considerably, may also pollute the grain with mycotoxins, which are dangerous to animal and human wellbeing (Edwards, 2004). Human fungal pathogens are exceedingly different group of fungal species, and *Candida albicans*, *Aspergillus fumigatus*, and *Crytococcus neoformans* are the three most significant pathogens causing severe systemic infections among the immunocompromised population (Groll & Walsh, 2001).

At this time, efficient drug therapy to treat fungal infections is very limited and dominated by the azole class of antifungals, which selectively inhibit lanosterol demethylase activity, a late step in ergosterol biosynthesis (Geogopapadakou & Walsh, 1996; Green et al., 1999; Sheehan et al., 1999). Although the azole antifungals have admirable safety profiles and are orally bioavailable, they are not fungicidal and therefore have need of long therapeutic treatment. Subsequently, their extensive use has led to an increasing incidence of drug resistance (Sussman et al., 2004).

Scientists have consequently concentrated their efforts on the design and advancement of new classes of antimicrobial agents that acquire novel modes of action that could conquer recognized mechanisms of antibiotic resistance. An attractive approach consists of probing for naturally occurring antibiotic molecules and both the plant and animal kingdom are rich sources. For that reason, in the search for an alternative form of treatment for fungal infections, the last decade has seen a spreading out in innovative approaches, like therapeutic antibodies and peptide molecules (Hancock, 1999; Kumar et al., 2005). Accordingly, there is a greater concern in the pharmacological application of antimicrobial peptides to treat infection, and the development of a new class of anti-infective agents.

Antimicrobial peptides are capable candidates for novel therapeutic agents because of their low toxicity against mammalian cells, do not easily select resistant mutants (Duggineni et al., 2007), and are therapeutic agents against microbes and their unique biological mechanisms of perturbing the membrane of the pathogen (Hong et al., 2001). They are a new resource of potential new antibiotic drugs because they were proposed to interact directly with lipid bilayers, resulting in pore-forming or alteration of the cell membrane permeability and eventually cell death (Sitaram & Nagaraj, 1999; Shai, 1999). On the other hand, some of these agents have got to clinical trials, whereas others are undergoing detailed preclinical testing (Hancock 1999), the search for such antifungal peptides continues, especially in selecting an effective candidate for the development of a new type of antibiotic. Antimicrobial peptides (AMPs) make natural antibiotics the basic element of a novel generation of drugs for the treatment of bacterial and fungal infections (Delucca, 2000; Welling et al., 2000; Selitrennikoff, 2001). Figure 2.1 shows that these peptides are made by many cells in skin and have multiple functions. Peptides are expressed in layers providing antimicrobial barrier at surface when secreted by eccrine glands and can be made by keratinocytes when activated. Resident and recruited bone marrow-derived cells such as mast cells and neutrophils also express high levels of antimicrobial peptides. Functions attributed to these peptides extend well beyond activity as simple antibiotic. Select molecules will influence cytokine release and synthesis of components of the extracellular matrix (ECM), and are chemotactic and angiogenic (Izadpanah & Gallo, 2005).
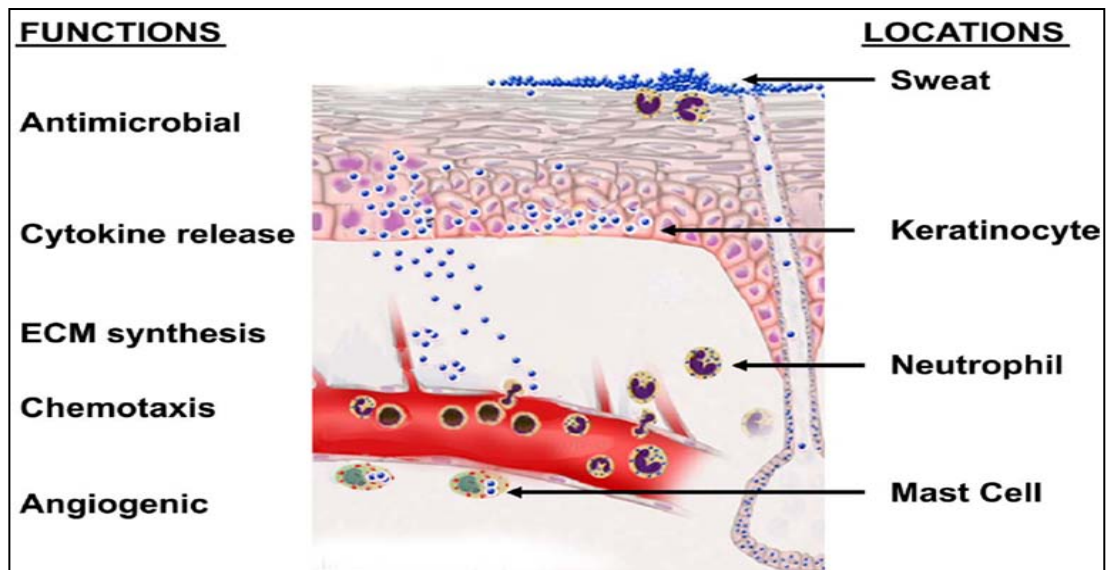
Figure 2.1. Antimicrobial peptides are made by many cells in the skin and have multiple functions. (Source: Izadpanah & Gallo, 2005)

AMPs, effectors molecules of the innate immune system, present a first line of defense against invading pathogens, promise to be a solution to this problem (Ganz, 1999; Lohner & Epand, 1997). Antimicrobial peptides are small molecules that serve in the vertebrate and invertebrate world for both offensive and defensive purposes as part of the immune defense system (Shai, 1999). Figure 2.2 describes some functions of AMPs.