BIOCHEMICAL STUDIES ON THE MODE OF ACTION OF SOME ANTIMICROBIAL AGENTS

Thesis Submitted to the faculty of science
Ain Shams University

In partial fulfillment for the requirements of the degree of Master of Science in Biochemistry

By
Sally Ahmed Abd El Kader Khalil

Supervisors

Prof. Dr. Ibrahim R. Shimi

Professor of Biochemistry Faculty of Science Ain Shams University

Dr. Naglaa A. Mabrouk

Ass. Prof. of Biochemistry Faculty of Science Ain Shams University

Faculty of Science Ain Shams University

2005

ABSTRACT

The present study was carried out to search for some new antimicrobial and antifungal agents against broad-spectrum microorganisms and to show the probable mechanism through which the most potent derivative exerts its action on the test organism. From screening program, 2-amino-4,6dimethyl pyridine derivative of 5-nitro-2-thiophene carboxaldehyde given the abbreviated names DMNP exerting a pronounced antifungal activity against Candida albicans. Results of this study clearly indicated that, the target for action of DMNP is the cell membrane which causes its damage by the inhibition of ergosterol (the major sterol of fungi) biosynthesis. Moreover, DMNP cause also disturbance in fatty acid metabolism whish may accumulate in oil droplets. In addition, DMNP strongly curtailed the synthesis of Candida albicans soluble phosphorus, RNA, DNA besides a cytoplasmic pool target, protein synthesis.

This Thesis Has Not Been Submitted To This or Any Other University

Sally Ahmed A. Khalil

ACKNOWLEDGEMENT

First of All Cordial Thankfulness to Allah

I would like to express my deep appreciation and gratitude to *Prof. Dr. Ibrahim R. Shimi*, Professor of Biochemistry, Faculty of Science, Ain Shams University, for suggesting the subject, his consistent supervision, and above all for his moral support and fatherly attitude.

I would like also to express deepest thanks to *Prof. Dr. Nabil Abdel kader*, Professor of Biochemistry, Faculty of Science, Ain Shams University, for his valuable supervision, sincere guidance, constructive suggestions and wholehearted support throughout this work.

I am grateful to *Dr. Naglaa Abdel Zaher*, Ass. Prof. of Biochemistry, Faculty of Science, Ain Shams University, for her tutorial guidance, valuable assistance and efforts.

I am also deeply indebted to *Prof. Dr. Fahmy T. Ali*, Professor and head of Biochemistry Department, Faculty of Science, Ain Shams University, for his moral help, patience, meticulous observation, continuous encouragement and generous advice during this work.

Last, but not least, my sincere thanks to my Husband, Professors and Colleagues in the Biochemistry Department, Faculty of Science, Ain Shams University, for their unfailing support and encouragement.

Sally Ahmed A. Khalil

CONTENTS

	Page
• Acknowledgement.	_
• Abbreviations.	i
• List of Figures.	iii
• List of Tables.	vi
• List of Photos.	vii
• Abstract.	ix
• Introduction.	1
• Aim of the work.	4
Review of literature.	5
-Antibiotics and antimicrobials.	5
-General characteristics of antimicrobial agents.	7
-General principles for use of antibiotics.	9
-Factor influencing the effectiveness of antimicrobial	10
agents.	12
-Antimicrobial agents used in combination.	13
-General characteristics of antimicrobial agents.	13
# Morphological changes. # Inhibitors of cell wall synthesis.	13 16
# Cell membrane inhibitors.	10
# Protein synthesis inhibitors.	21
# Nucleic acid synthesis inhibitors.	22
-Antifungal agents.	28
-Mode of action of antifungal agents.	28
# Inhibitors of cell membrane synthesis.	30
# Nucleic acid synthesis inhibitors.	31
# Inhibitors of fungal cell wall synthesis.	33
-Clinical applications of antifungal therapy	34
# Amphotericin B.	37
n imphotentia b.	31

# Azoles.	42
# Terbinafin.	47
Materials and Methods.	49
- Chemicals.	49
- Organisms and culture conditions.	49
 Screening of various newly synthesized compounds against microbial organisms. 	50
- Preparation of new synthetic compounds (NTC) derivatives.	51
-Chromatographic behavior of DMNP using TLC technique.	54
-Studies on the mode of action on <i>Candida albicans</i> 2402-E cells which includes.	56
- Electron micrographs of <i>Candida albicans</i> 2402-E cells under effect of DMNP at MIC level.	56
- Amino acids analysis in the metabolism solution of treated and untreated <i>Candida albicans 2402-E</i> cells.	56
- Fractionation and extraction of intracellular components of <i>C</i> . albicans cells.	59
# Extraction of acid soluble phosphorous.	60
# Extraction of total proteins.	60
# Extraction of total lipids.	60
# Extraction of RNA.	60
# Extraction of DNA.	61
# Quantitative determination of acid soluble phosphorous.	62
#Quantitative determination of total proteins.	64
#Quantitative determination of total lipids.	65
#Quantitative determination of RNA.	67
#Quantitative determination of DNA.	68
- Effect of DMNP on thermal denaturation (T _m) of DNA.	70
- SDS-PAGE analysis of the intracellular proteins of <i>Candida albicans 2402-E</i> cells.	72

treated and untreated <i>Candida albicans 2402-E</i> cells. Results. Discussion. Summary. References.	7
 Discussion. Summary. 	
• Summary. 14	1
~ 	29
• References.	10
	13
• Arabic summary.	
Arabic abstract.	

LIST OF FIGURES

Figure		Page
1	The major modes of action by which antibiotics exert their effect on bacterial cells.	15
2	Ergosterol the major sterol in fungal cell membranes.	29
3	Structure of amphotericin B.	40
4	Polyene cell membrane interactions.	41
5	Sterol 14 α-demethylation.	46
6	Ultraviolet spectrum of DMNP.	85
7	Infrared spectrum of NADP.	86
8	Mass spectroscopy of DMNP.	87
9	Suggested molecular structures of DMNP.	88
10	Amino acids profile in the metabolism solution of untreated <i>C. albicans</i> cells.	98
11	Amino acids profile in the metabolism solution of DMNP treated <i>C. albicans</i> cells.	99
12	Gas liquid chromatographic pattern of fatty acid derivatives of untreated <i>C. albicans</i> cells.	102
13	Gas liquid chromatographic pattern of fatty acid derivatives of DMNP treated <i>C. albicans</i> cells.	103
14	Gas liquid chromatographic pattern showing standard ergosterol.	104
15	Gas liquid chromatographic pattern showing sterol of untreated <i>C. albicans</i> cells.	105
16	Gas liquid chromatographic pattern showing sterol of DMNP treated <i>C. albicans</i> cells.	106

ir .		
17	Effect of the DMNP on the growth rate of <i>C. albicans</i> cells.	108
18	Effect of the DMNP on the acid soluble phosphorous content of <i>C. albicans</i> cells.	110
19	Effect of the DMNP on the total proteins content of <i>C. albicans</i> cells.	111
20	Effect of the DMNP on the total lipids content of <i>C. albicans</i> cells.	113
21	Effect of the DMNP on the RNA content of <i>C. albicans</i> cells.	114
22	Effect of the DMNP on the DNA content of <i>C. albicans</i> cells.	115
23	Effect of DMNP (5 μg/ml) on thermal denaturation and renaturation of <i>Salmon testis</i> DNA.	117
24	Effect of DMNP (10 μg/ml) on thermal denaturation and renaturation of <i>Salmon testis</i> DNA.	118
25	Effect of DMNP (15 μg/ml) on thermal denaturation and renaturation of <i>Salmon testis</i> DNA.	119
26	Effect of DMNP (20 μg/ml) on thermal denaturation and renaturation of <i>Salmon testis</i> DNA.	120
27	Effect of DMNP (5 μg/ml) on thermal denaturation and renaturation of <i>Salmon sperm</i> DNA.	121
28	Effect of DMNP (10 μg/ml) on thermal denaturation and renaturation of <i>Salmon sperm</i> DNA.	122
29	Effect of DMNP (15 μg/ml) on thermal denaturation and renaturation of <i>Salmon sperm</i> DNA.	123

30	Effect of DMNP (20 µg/ml) on thermal denaturation and renaturation of <i>Salmon sperm</i> DNA.	
31	Analysis of molecular weight of intracellular proteins in comparison with protein markers	127

LIST OF TABLES

Table		Page
1	The investigated derivatives of 5-nitro-2-thiophene carboxaldehyde.	82
2	Antibacterial and antifungal activities of the different compounds.	83
3	R _f values of parent compounds NTC, 2-amino4,6-dimethylpyridine and DMNP on silica gel plates using various developing solvents.	89
4	Amino acids content in metabolism solution of DMNP treated and untreated <i>C. albicans</i> cells	97
5	Change in proteins content extracted from untreated and DMNP treated <i>C. albicans</i> cells	128

LIST OF PHOTOS

Photo		Page
1	Scanning electron micrograph of untreated <i>C</i> .albicans 2402E cells (X 8000).	91
2	Scanning electron micrograph of DMNP-treated <i>C. albicans</i> 2402 <i>E</i> cells (X 8000).	91
3	Transmission electron micrograph of untreated <i>C. albicans</i> 2402E cells (X25.000, 20.000 respectively).	92
4	Transmission electron micrograph of untreated <i>C</i> .albicans 2402E cells (X25.000, 20.000 respectively).	92
5	Transmission electron micrograph of DMNP -treated <i>C .albicans 2402E</i> cells (X 20.000, 25.000 respectively).	93
6	Transmission electron micrograph of DMNP-treated <i>C</i> .albicans 2402E cells (X 20.000, 25.000 respectively).	93
7	Transmission electron micrograph of DMNP-treated <i>C</i> .albicans 2402E cells (X 20.000, 25.000 respectively).	94
8	Transmission electron micrograph of DMNP-treated <i>C</i> .albicans 2402E cells (X 20.000, 25.000 respectively).	94
9	Transmission electron micrograph of <i>C</i> .albicans 2402E cells showing budding (X 25.000).	95
10	Transmission electron micrograph of <i>C</i> .albicans 2402E cells showing budding (X 25.000).	95

11	Transmission electron micrograph of <i>C</i> .albicans 2402E cells showing budding (X 25.000).	95
12	SDS.PAGE analysis of intracellular proteins of untreated and DMNP treated <i>C. albicans</i> cells.	126

ABBREVIATIONS

 A^{o} Angstrom

ATP : Adenosine Tri Phosphate

A. niger : Aspergillus niger : boiling point b. p. B. subtilis : Bacillus subtilis

 \mathbf{C} Cytosine

C. albicans : Candida albicans

: Compound Cpd

DMSO : Dimethyl sulphoxide

DMNP : 2,4 dimethyl-5-N(2-methyl-5-nitrothiofyl)-

pyridine

: Deoxyribonucleic acid DNA

E. coli : Escherichia coli

G : Guanine

: Gas Chromatography GC

: Gas Liquid Chromatography GLC : Human Immunodeficiency Virus HIV **Highly Performance Liquid HPLC**

Chromatography

I.R. : Infrared Molar M

MIC Minimum inhibitory concentration

mM Millimolar

Mol. W. : Molecular weight

Messenger ribonucleic acid mRNA

: nanogram ng : nanometer nm No : Number

NTC : 5-nitro-2-thiophene carboxaldehyde

: Optical density O.D.