



شبكة المعلومات الجامعية
التوثيق الإلكتروني والميكرو فيلم

بسم الله الرحمن الرحيم



MONA MAGHRABY



شبكة المعلومات الجامعية
التوثيق الإلكتروني والميكروفيلم



شبكة المعلومات الجامعية التوثيق الإلكتروني والميكروفيلم



MONA MAGHRABY



شبكة المعلومات الجامعية
التوثيق الإلكتروني والميكروفيلم

جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها
علي هذه الأقراص المدمجة قد أعدت دون أية تغيرات



يجب أن

تحفظ هذه الأقراص المدمجة بعيدا عن الغبار



MONA MAGHRABY



Pharmacological and Molecular Modeling Studies on the Effect of Biochanin-A in Colon Cancer Cells

Thesis presented by

Mohamed Mahmoud Abdel Rahman

Bachelor of pharmaceutical sciences (2009)
Faculty of Pharmacy, German University in Cairo

Submitted for Partial Fulfillment of the
Master degree
In Pharmaceutical Sciences
(Pharmacology and Toxicology)

Under the Supervision of

Prof. Dalal Abou El Ella, PhD

Professor of Pharmaceutical Chemistry Department,
Faculty of Pharmacy, Ain Shams University

Prof. Suher Kamal Zada, PhD

Professor of Immunology
Biology Department
American University in Cairo (AUC)

Associate. Prof. Mai Fathy Tolba, PhD

Associate Professor, Pharmacology and Toxicology Department,
Faculty of Pharmacy, Ain-Shams University

2020

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

وَعَلَّمَكَ مَا لَمْ تَكُن تَعْلَمُ ج
وَكَانَ فَضْلُ اللَّهِ عَلَيْكَ
عَظِيمًا

صدق الله العظيم

سورة النساء الآية (١١٣)

Acknowledgments

First, I thank "**Allah**" for granting me the power to accomplish this work.

I would like to express my deepest thanks to **Prof. Dalal Abou El Ella**, Professor of Pharmaceutical chemistry, Faculty of Pharmacy, Ain-Shams University, for her valuable scientific supervision, constructive advice and continuous guidance throughout the work.

My deepest gratitude and appreciation are expressed to **Prof. Suher Zada**, Professor of Immunology, Biology department, American University in Egypt, for her divine support and for kindly supplying the laboratory facilities whenever needed. Her constructive criticism, guided me immensely throughout the work and during the revision of the thesis.

I am also greatly indebted to **Dr. Mai Fathy Tolba**, associate professor of Pharmacology and Toxicology, Faculty of Pharmacy, Ain Shams University, for suggesting the topic of research and for providing continuous scientific supervision and follow up. Her valuable time and big effort are greatly appreciated. I also thank her for providing guidance especially throughout the genetics part in this study.

I would also like to thank my dear **colleagues** and to all the **workers** at the Faculty of Pharmacy, Ain Shams University for their help and support during this work.

Finally, my deepest everlasting thanks and appreciation are for my beloved **parents** for their continuous support and encouragement throughout my life.

والحمد لله رب العالمين.....

Mohamed Mahmoud abdel Rahman

Table of Contents

Table of contents.....	I
List of abbreviations.....	II
List of figures.....	VI
List of tables.....	VIII
Abstract.....	1
Introduction	3
Colorectal Cancer	4
Epidemiology.....	4
Etiology and risk factors.....	4
Colorectal cinogenesis.....	11
Colorectal cancer treatment.....	19
Apoptosis.....	22
5-fluorouracil	26
Chemistry.....	26
Pharmacokinetics.....	27
Pharmacodynamics.....	30
Toxicity.....	31
Drug interactions.....	32
Biochanin A	33
Chemistry.....	33
Pharmacokinetics.....	34
Pharmacodynamics.....	39
Toxicity.....	39
Drug interactions.....	40
Aim of the work	42
Materials and Methods	44
(A) - Design of the work:	44
(B) - Materials:	47
(C) - Methods:	51
Results	72
Discussion	149
Summary and Conclusion	157
References	159
Arabic summary	183

List of Abbreviations

5-FU	5-fluorouracil
ACE-I	Angiotensin converting enzyme inhibitors
AIF	Apoptosis inducing factor
APC	Adenomatous polyposis coli
ATM	Ataxia telangiectasia mutated
Bcl-2	B-cell lymphoma 2
Bio-A	Biochanin-A
BP	Bisphosphonate
BUB1	Benzimidazole-1
CAT	Catalase
CCL4	Carbon tetrachloride
ChK1	Checkpoint 1
CI	Confidence interval
CIN	Chromosomal instability
CRC	Colorectal cancer
DHFU	Dihydrofluorouracil
Disc	Death inducing signaling complex
DNA	Deoxyribonucleic acid

List of Abbreviations

DPD	Dihydropyrimidine dehydrogenase
dTMP	Deoxythymidine monophosphate
dUMP	Deoxyuridine monophosphate
ER-α	Estrogen receptor alpha
FasL	Fas ligand
FdUMP	Fluorodeoxyuridine monophosphate
GalN	Galactosamine
GIT	Gastro-intestinal tract
GnRH	Gonadotropin releasing hormone
GPx	Glutathione peroxidase
GSH:	Reduced Glutathione
GY	Gray unit
H2AX	Histone family member X
HER-2	Human epidermal growth factor receptor -2
HNPCC	Hereditary non-polyposis colorectal cancer
HPS	Hyperplastic polyposis syndrome
HR	Hazard ratio
IBD	Inflammatory bowel disease
IgG-1	Insulin-like growth factor-1

List of Abbreviations

IL-1β	Interleukin1 beta
INR	International normalized ratio
KIF-11	Kinesin family member 11
KRas	Kirsten Rat Sarcoma
LEF	Lymphoid enhancer family
LOH	Loss of heterozygosity
LPS	Lipo-polysaccharide
MAD	Mitotic-arrest deficient
MDA	Malondialdehyde
MMP-2:	Metalloproteinase-2
MMR	Mismatch repair
MPT	Mitochondrial permeability transition
MSI	Microsatellite instability
NF-κB	Nuclear factor kabba B
NSAIDS	Non-steroidal anti-inflammatory drugs
PFS	Progression-free survival
PLGF	Placenta growth factor
PLK	Polo-like kinase
PTT	Partial thromoplastin time
RR	Relative Risk

List of Abbreviations

SOD	Superoxide dismutase
SRB	Sulforhodamine B
SSAs	Sessile serrated adenomas
TCF	T-cell factor
TIMP-1	Tissue inhibitor of Metalloproteinases-1
TNF-α	Tumor necrosis factor alpha
TP53	Tumor protein 53
TS	Thymidylate synthase
TSA	Traditional serrated adenoma
TXNIP	Thioredoxin-integrating protein
UC	Ulcerative colitis
VEGFR:	vascular endothelial growth factor receptor
Wnt	Wingless-related integration site

List of Figures

Figure 1: Genetic path of colonic neoplasia with loss of MMR function.....	11
Figure 2: Intrinsic and extrinsic apoptotic pathways.....	18
Figure 3: Chemical structure of 5-fluorouracil.....	22
Figure 4: Biochanin-A chemical structure.....	28
Figure 5: Standard calibration curve of Cyclin D1.....	56
Figure 6: Standard calibration curve of MMP-2.....	60
Figure 7: Standard calibration curve of Survivin.....	63
Figure 8: Concentration-response plots of 5-FU (A) and Bio-A (B) in Caco2 Colon cancer cell line after 72h treatment.....	71
Figure 9: Concentration-response plots of treatment with 5-FU/Bio-A combinations in 1:2 and 1:4 ratios in Caco2 Colon cancer cell line after 72h treatment.....	72
Figure 10: Concentration-response plots of 5-FU (A) and Bio-A (B) in HCT-116 Colon cancer cell line after 72h treatment.....	73
Figure 11: Concentration-response plots of treatment with 5-FU/Bio-A combinations in 1:2 and 1:4 ratios in HCT-116 Colon cancer cell line after 72h treatment.....	74
Figure 12: Effect of 5-FU/Bio-A on the ratio between phosphorylated Akt and total Akt using ELISA kit on Caco2 (A) and HCT-116 (B) cell lines.....	79
Figure 13: Effect of 5-FU/Bio-A on the ratio between phosphorylated β -Catenin and total β -Catenin using ELISA kit on Caco2 (A) and HCT-116(B) cell lines.....	82
Figure 14: Effect of the different treatments of 5-FU or Bio-A monotherapy on tumor volume.	86
Figure 15: Histopathological examination of tumor specimens from Control group.....	89
Figure 16: Histopathological examination of tumor specimens from 5-FU group..	90
Figure 17: Histopathological examination of tumor specimens from Bio-A group.....	91

List of Figures

Figure 18: Histopathological examination of tumor specimens from Combination (5-FU/Bio-A) group.....	92
Figure 19: Effect of the different treatments on the percentage of tumor necrosis.....	94
Figure 20: Effect of the different treatments on Cyclin D1 protein level in tumor tissue using ELISA analysis.....	97
Figure 21: Effect of combination therapy 5-FU/ Bio-A on metalloproteinase-2 (MMP-2) in tumor tissue from Ehrlich-bearing mice using ELISA analysis.....	100
Figure 22: Effect of different treatments on vascular endothelial growth factor (VEGF) using immunohistochemical staining assay.....	103
Figure 23: Immunohistochemical staining of VEGF samples from control group.....	104
Figure 24: Immunohistochemical staining of VEGF samples from 5-FU single treatment group.....	105
Figure 25: Immunohistochemical staining of VEGF samples from Bio-A single treatment group.....	106
Figure 26: Immunohistochemical staining of VEGF samples from (5-FU/Bio-A) combination group.....	107
Figure 27: Effect of different treatments on Survivin levels in tumor specimens from Ehrlich-bearing mice using ELISA analysis.....	110
Figure 28: Effect of different treatments on the protein level of caspase-3 in tumor tissues from Ehrlich-bearing mice using immunohistochemical staining assay...	113
Figure 29: Immunohistochemical staining of caspase-3 in specimens from control group.....	114
Figure 30: Immunohistochemical staining of caspase-3 in specimens from 5-FU group.....	115
Figure 31: Immunohistochemical staining of caspase-3 in specimens from Bio-A group.....	116
Figure 32: Immunohistochemical staining of caspase-3 in specimens from (5-FU/Bio-A) combination group.....	117
Figure 33: Histological evaluation of intestinal specimens from Control group...	119
Figure 34: Histological evaluation of intestinal specimens from 5-FU group.....	120

List of Figures

Figure 35: Histological evaluation of intestinal specimens from Bio-A group.....	121
Figure36: Histological evaluation of intestinal specimens from Combination group.....	122
Figure 37: Effect of different treatments on the level of GSH in intestine samples from Tumor-bearing mice using colorimetric analysis.....	126
Figure 38: Effect of different treatments on the protein levels of MDA in intestine tissues of Ehrlich-bearing mice using colorimetric analysis.....	129
Figure 39: Binding site comparison using molecular surface representation of PyMOL.....	130
Figure 40: Ribociclib bound to the ATP binding domain of the CDK4 subunit of the CCND1-CDK4 complex.....	131
Figure 41: Generated poses from local docking of Ribociclib to the ATP binding domains of CDK6 and CCND1-CDK4 complex.....	132
Figure 42: Generated pose from local docking of Biochanin A to the ATP binding domain of the CDK4 subunit of CCND1-CDK4 complex	133
Figure 43: Generated poses from local docking of reference inhibitors to the ATP binding site of CDK4 of the CCND1-CDK4 complex	135
Figure 44: Generated pose from local redocking of Sorafenib to the ATP binding domain of VEGFR2.....	137
Figure 45: Generated poses from local docking of Biochanin A and reference inhibitors to the ATP binding pocket of the VEGFR2.....	139
Figure 46: Generated pose from local docking of Biochanin A to the ATP binding pocket of VEGFR2.....	141

List of Tables

Table 1: Colon cancer staging.....	15
Table 2: IC50 of 5FU and Bio-A either monotherapy or combination in Caco-2 and HCT-116 cell lines.....	75
Table 3: Synergy analysis and dose reduction index (DRI) of 5-FU/Bio-A combination ratio 1:2 in: A) Caco2 and B) HCT-116 colon cancer cell lines using Calcosyn software.....	76
Table 4: Synergy analysis and dose reduction index (DRI) of 5-FU/Bio-A combination ratio 1:4 in: A) Caco2 and B) HCT-116 colon cancer cell lines using Calcosyn software.....	77
Table 5: Effect of 5-FU/Bio-A on P.Akt/ total Akt ratio in CRC cells.....	80
Table 6: Effect of 5-FU/Bio-A on P. β-Catenin / totalβ-Catenin ratio in CRC cells.....	83
Table 7: Effect of single and combination treatments on tumor volume.....	87
Table 8: The effect of the different treatments on the percentage of tumor cells necrosis.....	93
Table 9: The effect of treatment (single and combination) on Cyclin D1 protein level.....	96
Table 10: The effect of treatment (single and combination) on MMP-2 protein level.....	99