

"Molecular Modeling, Synthesis and Anticancer Activity of Fused Triazine Derivatives"

Thesis

Presented by

Khulood Hayal Oudah

BSc. In Pharmaceutical Sciences (2002)
College of Pharmacy, Mosul University
Submitted for the partial fulfillment of the

Master Degree

In Pharmaceutical Sciences (Pharmaceutical Chemistry)

Under the supervision of

Prof. Dr. / Khaled A. M. Abouzid

Professor of Pharmaceutical Chemistry &

Head of the Pharmaceutical Chemistry Department

Faculty of Pharmacy- Ain Shams University

Dr. / Rabah A. Taha Serya

Assistant Professor of Pharmaceutical Chemistry Faculty of Pharmacy- Ain Shams University

Dr. / Nermin Samir Abdou

Lecturer of Pharmaceutical Chemistry
Faculty of Pharmacy- Ain Shams University

Faculty of pharmacy
Ain Shams University
2017

Acknowledgements

Thanks to **Almighty Allah** for giving me strength and ability to understand, learn and complete this work.

First and foremost I would like to express my deepest sense of gratitude to **Professor Dr. Khaled Abouzid**, Professor of Pharmaceutical Chemistry and Head of the Pharmaceutical

Chemistry Department, who has the attitude and the substance of genius. I am sincerely

grateful to his devotion to his students' education and success. The door of Dr. Khaled's office

was always opened whenever I ran into a trouble spot or had a question about my research.

He was the one who taught me how to be multitasking. I owe him a huge debt of gratitude for

his guidance, endless support and immense knowledge.

I owe deep appreciation and truthful gratitude to **Dr. Rabah Ahmed Taha Serya**, Associate Professor of Pharmaceutical Chemistry, for her kindness, continuous encouragement, indispensible assistance, valuable guidance and constant support throughout the whole work.

It is my genuine pleasure to express my heartiest thanks to **Dr. Nermin Samir**, lecturer in pharmaceutical Chemistry, for her fruitful opinion, untiring help, valuable assistance and constant encouragement during writing this thesis.

I acknowledge with thankfulness all my friends in Pharmaceutical Chemistry Department, for their friendly cooperation, support and their unconditional love and aid.

Also I would like to express my gratitude to the National Cancer Institute, Maryland, U.S.A for performing the cytotoxicity assay of the synthesized compounds.

Finally, I am profoundly indebted to my parents, my beloved husband, and my little kids for their unconditional love and aid, endless patience, understanding, encouragement and full support all throughout the whole long way.



Ismail whose passion for teaching set a new standard for anyone involved in education, research and supervision or any other endeavor in which one seeks to support the development of another. His versatile knowledge in pharmaceutical chemistry and drug design and his unique teaching style has developed our knowledge and cleared many ambiguities. I am so grateful to him for assisting this challenging dissertation. Finally I would like to express my deepest gratitude to his vision and foresight which inspired me to conceive this thesis.

May Allah Bless His Soul

General and special postgraduate courses in Pharmaceutical Chemistry

Besides the work presented in this thesis, the candidate successfully passed general and special postgraduate courses in Pharmaceutical Chemistry for one year during academic year 2014/2015 with the general grade <u>Very good</u> as following:

1.	Instrumental Analysis	Very Good
2.	Physical Chemistry	Passed
3.	Computer Sciences	Excellent
4.	Statistics	Good
5.	Pharmaceutical Chemistry	Excellent
6.	Drug Stereochemistry	Excellent
7.	Drug Spectroscopy	Good
8.	Selected Topics in	Good
]	Pharmaceutical Chemistry	

Table of contents:

Acknowledgements	I
General and special postgraduate courses in Pharmaceutical Chemistry	III
Table of contents	IV
List of Figures	VII
List of Tables	IX
List of Abbreviations	X
Abstract	XII
1. Introduction	1
1.1. Cancer	1
1.1.1. Overview	1
1.1.2. Development	1
1.1.3. Hallmarks of cancer	3
1.1.4. Etiology and carcinogenic factors	4
1.1.5. Epidemiology	5
1.1.6. Treatment	5
1.2. Protein kinases as cancer targeted therapy	13
1.2.1. Overview on Protein kinases	13
1.2.2. Cycline-Dependent Kinases	13
1.2.3. Role of Cycline-Dependent Kinases in cell cycle	14
1.2.4. The structure of Cyclin2-dependent kinases and its activation	16
1.2.5. CDK2 inhibitors as important target in cancer therapy	18
1.2.6. Strategies in developing cyclin-dependent kinase2 Inhibitors	18

2. Rationale and Design2	29
2.1. Consideration of the previously explored SAR and determination of the key interactions between the binding site and ATP competitive inhibitor	30
2.2. Design of novel pyrazolo(1,5-a)(1,3,5)triazine based CDK2 inhibitors	33
2.3. Primary evaluation of some selected compounds by using Molecular docking 3	36
2.4. Synthetic schemes for synthesis of the designed compounds	39
2.4.1 Scheme (1): preparation of Ethyl 2-(ethylthio)-4-substituted phenylpyrazolo [1,5- α][1,3,5] triazine-8-carboxylate (VIIIa-c)40	
2.4.2 Scheme (2): preparation of compounds (IXa-o) & (Xa-c(4	1
3. Results and Discussion4	ł2
3.1. Chemistry ²	42
3.1.1. Scheme 14	12
3.1.2. Scheme 2	19
3.2. Biological Evaluation5	52
3.2.1. In vitro CDK2/CyclinA2 Kinase inhibitory Activity5	52
3.2.2. <i>In vitro</i> antiproliferative activity against NCI 60-cell line5	56
3.2.3 <i>In vitro</i> cytotoxic activity against MCF-7 cancer cell lines6	56
3.3. Molecular modeling study	57
3.3.1. Docking study	67
3.3.2. In silico ADMET study7	77
4. Conclusion8	80
5. Experimental	32
5.1. Chemistry	32
5.1.1. Materials and instrumentation	32

Table of contents

7. References	129
6. Supplementary Data	108
5.2.3. Docking process	106
5.3.2. Ligand preparation for docking	106
5.3.1. Protein preparation for docking	106
5.3. Molecular docking study	106
5.2.3. In vitro cytotoxic activity against MCF-7 cancer cell lines	105
5.2.2. In vitro Anti-proliferative activity against 60 cell line panel	104
5.2.1. In vitro CDK2/CyclinA activity	103
5.2. Biological evaluation	103
5.1.2. Synthesis	83

<u>List of figures:</u>

Figure 1: Three- phase process of carcinogenesis2
Figure 2: The Hallmarks of Cancer3
Figure 3: Milestones in the identification of the cell cycle14
Figure 4: Cycline-dependent kinase function in cell-cycle15
Figure 5: Structure biology of cyclin-dependent kinase 2- cyclin A17
Figure 6: Strategies for targeting Cyclin-dependent kinases18
Figure 7: Exploiting the CDK2–ANS interaction to identify small-molecule ligands27
Figure 8: Pharmacophoric model of kinase active site (ATP binding site)30
Figure 9: A selection of protein kinase inhibitors for which CDK2/inhibitor structures have been determined32
Figure 10: Reported binding mode of lead compound roscovitine (28) with CDK2/cyclin A33
Figure 11: Design of novel CDK2 inhibitors based on Roscovitine (28) lead compound
Figure 12: Mechanism of cyclization of 5-aminopyrazole43
Figure 13: Synthesis of pyrazolo[1,5-a][1,3,5]triazine through two-bond cyclization46
Figure 14: Synthesis of 4-amino-2-methyl-7-phenylpyrazolo[1,5-a][1,3,5]triazine through two-bond cyclization46
Figure 15: Synthesis of 7-oxo-4-thioxopyrazolo[1,5-a][1,3,5]triazine46

Figure 16: Pyrazolo[1,5-a][1,3,5]triazine scaffold generated by transformation of the 1,3,5-
thiadiazine ring47
Figure 17: Mechanism of synthesis of ethyl 2-(ethylthio)-4-substituted phenylpyrazolo [1,5- a]
[1,3,5]triazine-8 carboxylate (VIIIa-c)48
Figure 18: Structure of NCI selected compounds57
Figure 19: Example of mean graph produced from NCI 60 cell lines screening program. Mean
graph of compound (IXI) colour code are given for each cell line58
Figure 20: %Inhibition of compound IXI against (26 cell line from 56 NCI cell line) that exhibit $\%$
inhibition range from (40%-115%)63
Figure 21: % inhibition of compound Xc against (17 cell line from 56 NCI cell line) that exhibit
% inhibition range from (43%-92%)64
Figure 22: % Inhibition of compound IXg against (9 cell line from 56 NCI cell line) that exhibit
% inhibition range from (42%-81%)65

List of Tables:

Table 1. Docking energy and amino acid involved in the binding interactions of some of the
designed compounds with compared with Roscovitine (28). (PDB code 3ddq)36
Table 2.percentage inhibition of CDK2 enzymatic activity showed by compounds VIII (a-c)
Table 3. Percentage inhibition of CDK2 enzymatic activity showed by compounds IX (a-o)
Table 4. Percentage inhibition of CDK2 enzymatic activity showed by compounds X (a-c)
Table 5. The IC ₅₀ value for compounds (IXg, IXh, IXk, IXl, IXm, Xa and Xc)56
Table 6. Cell growth percentage of NCI 60 cancer cell lines exhibited by some of the investigated final compounds (VIIIa, VIIIc, IXb, IXg, IXh, and IXk)59
Table 7. Cell growth percentage of NCI 60 cancer cell lines exhibited by some of the investigated final compounds (IXI, IXm, IXn, IXo, Xa and Xc)
Table 8. Cytotoxic activity of the remaining compounds against MCF-7 cancer cell lines
Table 9. Molecular docking investigation study of synthesized compounds in CDK2 active site (PDB code 3ddq) compared to Roscovitine (28)70
Table 10. Computer aided ADMET screening of the synthesized compounds78

List of Abbreviations

2D: Two-dimensional

3D: Three-dimensional

Å: Angstroms

ADMET: absorption, distribution, metabolism, excretion and toxicity study

ATP: Adenosine triphosphate

CAK: CDK-Activated kinase

CDK: Cycline dependent kinase

CDKI: Cycline dependent kinase inhibitors

CDOCKER: CHARMm-based DOCKER

CHARMm: Chemistry at HARvard macromolecular mechanics

CIP: CDK interacting protein

DCM: Dichloromethane

DMF: Dimethyl formamide

DNA: Deoxyribonucleic acid

EGFR: Epidermal growth factor receptor

ELISA: Enzyme-linked ImmunoSorbent Assay

FDA: Food and Drug Administration

FT-IR: Fourier transform-Infrared

HAKRRLIF: Histidine, Alanine, Lysine, Arginine, Arginine, Leucine, Isoleucine,

Phenylalanine

H-bond: hydrogen bond

Hr.: Hour **Hz:** Hertz

IC₅₀: Half Maximal inhibitory concentration

INK4: Inhibitor of Kinase 4

kDa: unified atomic mass unit (symbol: u) or dalton (symbol: Da)

KIP: Kinase inhibitory protein

MD: Molecular dynamics

MHz: Mega hertz