



Faculty of Pharmacy

Molecular design and synthesis of certain heterocycles based compounds as potential anti-cancer targeted agents

Thesis

Presented by

Mai Ali Mohamed Ali Mansour

Bachelor of Pharmaceutical Sciences (2014)

Faculty of Pharmacy, Ain shams University

Submitted in Partial Fulfillment of the Requirements for the

Master Degree

In Pharmaceutical Sciences (**Pharmaceutical Chemistry**)

Under Supervision of

Prof. Dr. Khaled A. M. Abouzid

Professor of Pharmaceutical Chemistry

Faculty of Pharmacy, Ain Shams University

Dean of Faculty of Pharmacy, University of Sadat City

Prof. Dr. Hatem Mostafa Gaber

Professor of Organic Chemistry,

Head of Pharmaceutical Chemistry Department,

National Organization of Drug Control and Research

Assoc. Prof. Dr. Deena S. Lasheen

Associate Professor of Pharmaceutical chemistry,

Faculty of Pharmacy, Ain Shams University

Faculty of Pharmacy- Ain Shams University

2020

Acknowledgments

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

I would like to express my deepest thanks to **Prof. Dr. Khaled Abouzid**, Professor of pharmaceutical chemistry, for suggesting the topic of research and for providing continuous scientific supervision and follow up and for his valuable scientific supervision, constructive advice and continuous guidance throughout the work.

My deepest appreciation is expressed to **Prof. Dr. Hatem Gaber**, Head of the Pharmaceutical Chemistry department, National Organization of drug control and research, and his dear student **Dr. Zeinab Abdelaal**, for their divine support and for kindly supplying the laboratory facilities whenever needed.

I am also greatly indebted to **Dr. Deena Lasheen**, Associate Professor of Pharmaceutical Chemistry Department, Faculty of Pharmacy, Ain Shams University. For her valuable time and big effort are greatly appreciated. Her constructive criticism, guided me immensely throughout the work and during the revision of the paper and thesis.

I would like to express my appreciation to **Dr. Mamdouh Orabi**, Lecturer of Pharmacology department, Badr University in Cairo for performing the *in vivo* anticancer assessment for the synthesized active compounds and for his precise statistical analysis.

I would also like to thank my dear **colleagues** and to all the **workers** at the Faculty of Pharmacy, Badr University in Cairo as well as my **colleagues** in the Miscellaneous department, national organization of drug control and research. Besides, I would like to thank my **colleagues** at Faculty of Pharmacy, Pharmaceutical Chemistry Department, Ain Shams University for their support and providing help whenever needed.

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.....

Mai Ali Mansour

Part of this work was published in *RSC Advance*, 2020, **10**, 32103–32112.



RSC Advances

PAPER



Cite this: *RSC Adv.*, 2020, **10**, 32103

Elaborating piperazinyl-furo[2,3-*d*]pyrimidine based scaffolds as phosphoinositol-3-kinase enzyme alpha (PI3K α) inhibitors to combat pancreatic cancer†

Mai A. Mansour, *^a Deena S. Lasheen, ^b Hatem M. Gaber^c
and Khaled A. M. Abouzid *^{bd}

Phosphoinositol-3-kinase enzyme (PI3K) plays a crucial role in driving oncogenic growth in various mammalian cells, particularly pancreatic cells. In the current study a series of novel furo[2,3-*d*]pyrimidine based-compounds were designed and synthesized as potential PI3K- α inhibitors. In accordance to the structure–activity relationship (SAR) studies of known PI3K- α inhibitors, different linkers including amide, urea and ether were attached to a piperazinyl furo[2,3-*d*]pyrimidine core. The synthesized compounds that revealed moderate PI3K- α inhibitory activity were tested for their anti-proliferative activities against pancreatic carcinoma on the PANC-1 cell line. Compounds **7b** and **8a** showed the highest anti-proliferative activity with IC₅₀ values of 4.5 μ M and 6 μ M, respectively and relatively, the best *in vitro* PI3K inhibition ability within the newly synthesized compounds. Additionally, all the newly synthesized final compounds were tested on 60 human cancer cell lines. A docking study was carried out on the PI3K- α active site showing a comparable binding mode to that of FDA approved PI3K- α inhibitors. These newly discovered lipid kinase inhibitors could be considered as potential candidates for the development of new targeted anticancer agents.

Received 23rd July 2020
Accepted 21st August 2020

DOI: 10.1039/d0ra06428a

rsc.li/rsc-advances

Table of Contents

Contents

Table of Contents.....	I
List of Abbreviations	III
List of Figures.....	VI
List of Tables	VIII
List of Schemes.....	X
Abstract:.....	1
1. Introduction.....	6
1.1. Cancer	6
1.2. Pancreatic Cancer	8
1.3. PI3Kinase inhibition as cancer targeted therapy	16
2. Rationale and Design.....	43
2.1. Deliberation of previously explored lead compounds reported as pancreatic anti-proliferative agents	44
2.2. Deliberation of previously explored SAR (structure activity relationship) studies for the lead compounds reported as PI3K class I inhibitors:	46
2.3. Design of novel furo[2,3- <i>d</i>]pyrimidine based PI3K class I inhibitors:	50
2.4. Primary evaluation of some selected compounds using molecular docking:	53
2.5. Synthetic schemes for synthesis of the designed compounds:	60
3. Results and Discussion	64
3.1. Chemistry.....	64

Table of Contents

3.2 Biological Evaluation.....	77
3.3. Molecular modeling study	105
4. Conclusion.....	122
5. Experimental.....	124
5.1. Chemistry.....	124
5.2. Biological Evaluation	152
5.3. Molecular docking study	166
6. References.....	168
APPENDIX.....	202

List of Abbreviations

5-FU	5-Fluorouracil
ADM	Acinar-to-Ductal Metaplasia
ADMET	Absorption, distribution, metabolism, excretion and toxicity
AKT	Ak strain transforming
ATP	Adenosine triphosphate
BBB	Blood brain barrier
CHARMM	Chemistry at <i>HAR</i> vard <i>Macromolecular Mechanics</i>
CLL	Chronic lymphocytic leukemia
DMF	Dimethyl formamide
DMSO	Dimethyl sulphoxide
DOX	Doxorubicin
EAT	Ehrlich ascites tumor
EGFR	Epidermal growth factor receptor
ErbB3	Erb-B2 Receptor Tyrosine Kinase 3
FDA	Food and drug administration
FL	Follicular lymphoma
FT-IR	Fourier transform infrared spectroscopy
FLT-3	fms like tyrosine kinase 3

List of Abbreviations

GPCR	G protein-coupled receptor
GTPase	Guanosine triphosphatase
G_{βγ}	G protein betagamma
HBA	Hydrogen bond acceptor
HBD	Hydrogen bond donor
HDI	Human Development Index
hrs	hours
IGF	Insulin-like growth factor
IGF1R	Insulin-like growth factor 1 receptor
IRS	insulin receptor substrate
JFCR39	Japanese Foundation for Cancer Research 39
KRAS	Kirsten rat sarcoma
MAPK	Mitogen-activated protein kinase
MD	molecular dynamics
mTOR	mammalian target of rapamycin
NSCL	Non-Small Cell Lung Cancer
PDAC	Pancreatic Ductal Adenocarcinoma
PDB	Protein data bank
PDGFR	Platelet-derived growth factor receptor
PK-1	3'phosphoinositide- dependent kinase 1

List of Abbreviations

PI3K	Phosphatidylinositol 3-kinase
PIP2	Phosphatidylinositol-4,5-bisphosphate.
PIP3	phosphatidylinositol-3,4,5-trisphosphate
PSA	Polar surface area
PTEN	Phosphatase and TENsin homolog
RAS	Rat Sarcoma
rt	Room temperature
RTK	Receptor tyrosine kinases
RT-PCR	Reverse transcription polymerase chain reaction
SH2	Src Homology 2
SLL	Small Lymphocytic lymphoma
TEA	Triethyl amine
TGI	Tumor Growth inhibition
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TORC1	Target of rapamycin complex 1
TORC2	Target of rapamycin complex 2
Vps	vacuolar protein sorting

List of Figures

Figure 1: Cancer causing agents and the transformation phases from normal cell to cancerous cells -----	7
Figure 2: Bar Chart of Region-Specific Incidence Age-Standardized Rates by Sex for Pancreatic Cancer in 2018. -----	9
Figure 3: ADM, acinar-to-ductal metaplasia. Schematic indicating points where cellular stress is leading to the dedifferentiation of mature acinar cells.-----	10
Figure 4: Illustration of RAS and phosphatidylinositol 3-kinase (PI3K) Signaling along with a combinatorial approach with agents may overcome resistance. -----	17
Figure 5: PI3K/ PTEN/ AKT/ mTOR pathway-----	18
Figure 6: Classes and isoforms within the PI3K family.-----	21
Figure 7: The ATP Binding Site of PI3K γ . -----	23
Figure 8: Class IA PI3K molecular contexts revealing applications for isoform-selective PI3K inhibitors' -----	34
Figure 9: Piperazinyl based derivatives, designed as anti-proliferative agents. -----	45
Figure 10: Amuvatinib (11) and its analogs designed as pancreatic cancer targeting anti-proliferative agents.-----	46
Figure 11: Binding mode of Wortmannin (13) and LY294002 (14) with PI3K -----	47
Figure 12: SAR and binding mode of imidazopyridines with PI3K- α related to compound HS-173 (39) -----	48
Figure 13: SAR and binding mode of triazolopyrimidines with PI3K- α related to PKI402 (35) -----	49
Figure 14: SAR and binding mode of AS-605240 (48) with PI3K- γ ¹³⁹ -----	49
Figure 15: Reported docking poses of HS- 173 (39) and PKI- 402 (35) analog in PI3K- α homology model based on PI3K γ crystal structures. -----	51
Figure 16: Reported docking pose of AS-605240 (48) in PI3K- γ -----	52

List of Figures

Figure 17: Design of PI3K- α inhibitors based on HS-173 (39) as a lead compound and combining some features from PKI-402 (35) analogs. -----	53
Figure 18: Mechanism of the addition elimination nucleophilic substitution reaction.-	65
Figure 19: Mechanism of cyclization of compound 1a -----	66
Figure 20: Byproduct formed as a result of lower reaction temperature-----	66
Figure 21: Mechanism of N-alkylation using nucleophilic substitution reaction. -----	70
Figure 22: Mechanism of Claisen–Schmidt condensation reaction.-----	76
Figure 23: Example of mean graph produced from NCI 60 cell line screening program. Mean graph of compound (7a) color codes are given for each cell line -----	84
Figure 24: Dose response curve for compound 9a in 9 Cell panels -----	94
Figure 25: Dose response curve for compound 9i in 9 Cell panels-----	95
Figure 26: Cell cycle analysis of MCF7 cells after treatment with compounds 9a and 9i for 24 hrs using flowcytometry. -----	99
Figure 27: Effect of compounds 9i and 9a on tumor volume.-----	100
Figure 28: Microscopic pictures of H&E stained muscles from control negative group -----	102
Figure 29: Microscopic pictures of H&E stained muscles from untreated Ehrlich ascites tumor (EAT) group -----	103
Figure 30: Caspase-3 immune-expression in tumor sections of treated groups (caspase-3 antibody) -----	104
Figure 31: Effect of compounds 9i and 9a on oxidative stress status of EAT mice----	105
Figure 32: The alignment between the co-crystalized bioactive conformer of the Inhibitor (52) (red) and its docked pose within PI3K- α binding site -----	107
Figure 33: The alignment between the co-crystalized bioactive conformer of the AS- 605240 (48) (green) and its docked pose within PI3K- γ binding site. -----	108
Figure 34: Superimposition of inhibitor (52) (red) and the lead compound HS-173 (39) -----	108

List of Tables

Table 1: PI3Kinases classification depending on its different catalytic and regulatory subunits	19
Table 2: Docking energy and amino acids residues involved in the binding interactions of some of the designed compounds with PI3K- α (PDB code 4ZOP)	54
Table 3: Docking energy and amino acids residues involved in the binding interactions of some of the designed compounds with PI3K- γ (PDB code 2A5U)	57
Table 4: Percent inhibition of PI3K- α enzymatic activity achieved by the designed compounds assessed at 10 μ M.....	78
Table 5: Percent inhibition of PI3K- γ enzymatic activity achieved by the designed compounds assessed at 5 μ M.....	80
Table 6: Percent inhibition of FLT-3 enzymatic activity achieved by the designed compounds assessed at 10 μ M.....	82
Table 7: NCI 60 cancer cell lines cell growth percentage exhibited by some of the investigated final compounds (5b, 7b, 7c, 7d, 8a, 8b, 8c)	85
Table 8: Cell growth NCI 60 cancer cell lines cell growth percentage exhibited by some of the investigated final compounds (9a, 9c, 9e, 9f, 9g, 9h, 9i, 9k).....	87
Table 9: GI ₅₀ values of compounds 9a and 9i against 59 NCI cell line	91
Table 10: IC ₅₀ values of selected target compounds and Paclitaxel on PANC-1 cell line.	97
Table 11: IC ₅₀ values of selected target compounds and Doxorubicin on MCF-7-ADR cell line.....	98
Table 12: Tumor indices and TGI% of EAT control and treated mice	101
Table 13: Molecular docking investigational study of series 5, 7 and 8 in PI3K- α active site (PDB code: 4ZOP) compared to HS-173 (39)	109
Table 14: Molecular docking investigational study of series 9 in PI3K- γ active site (PDB code: 2A5U) compared to AS-605240 (48)	114

Table 15: Computer aided ADMET screening of the synthesized target compounds . 120

List of Schemes

Scheme 1.....	60
Scheme 2.....	61
Scheme 3.....	62
Scheme 4.....	63

Abstract:

Title of thesis:

“Molecular design and synthesis of certain heterocycles based compounds as potential anti-cancer targeted agents”

Name of candidate:

Mai Ali Mohamed Ali Mansour
Instructor of Pharmaceutical Chemistry
Faculty of Pharmacy- Badr University in Cairo

Thesis supervised by:

Prof. Dr. Khaled A. M. Abouzid (PhD)
Professor of Pharmaceutical Chemistry
Faculty of Pharmacy- Ain Shams University
Dean of Faculty of pharmacy- University of Sadat City

Prof. Dr. Hatem Mostafa Gaber (PhD)
Professor of Organic Chemistry,
Head of Pharmaceutical Chemistry Department
National Organization of drug Control and Research

Assoc. Prof. Dr. Deena S. Lasheen (PhD)
Associate Professor of Pharmaceutical Chemistry,
Faculty of Pharmacy- Ain Shams University

Cancer is considered rapidly propagating disease, in which the cells divide uncontrollably and abnormally, surpassing its usual boundaries and can as well extend to other organ and metastasize. In the meantime, pancreatic cancer is the seventh leading cause of cancer associated death, world widely, and predicted to be the second cause in the developed world by 2030.

PI3K family is involved in the normal physiological cell processes such as cell growth, proliferation, adhesion and survival through the production of lipid second messengers, binding to and activating downstream effector proteins. Among that family, the PI3K- α isoform was suggested to be the most frequently altered in human tumors; breast (27%), endometrial (23%), colorectal (14%), urinary tract (17%) and ovarian (8%) cancers. Meanwhile, overexpression of the upstream effectors of PI3K such as K-ras appeared within pancreatic ductal adenocarcinomas that consequently over activates the PI3K pathway, particularly the alpha isoform. Another member of the PI3K family is the PI3K- γ isoform was reported to be over activated by tumor-derived chemo-attractant signals, contributing in subsequent myeloid cell adhesion and invasion into tumors. Therefore, inhibiting such pathway represents an interesting class of rational targets for anticancer agents development.

The current study aimed to design novel Furo[2,3-*d*]pyrimidine based compounds targeting PI3K- α and PI3K- γ isoforms. The design focused on exploring the SAR studies, previously revealed by of the lead compounds possessing anti-cancer activity along with bioisosteric modifications to study the SAR of the newly designed series of compounds. The designed compounds were synthesized and their structures were confirmed by various spectral and micro-analytical methods.

This study involved the synthesis of the following unavailable reported intermediates:

- 1) 2-Chloro-N-phenylacetamide (**Ia**)
- 2) 2-Chloro-N-(p-tolyl)acetamide (**Ib**)
- 3) 2-Chloro-N-(4-methoxyphenyl)acetamide (**Ic**)
- 4) 2-Chloro-N-(4-nitrophenyl)acetamide (**Id**)