

# **Molecular Modelling and Synthesis of Certain Heterocyclic Compounds with Expected Biological Activity**

Thesis

Presented by

**Eman Zaglol ElRazaz**

*Assistant Lecturer of Pharmaceutical Chemistry  
Faculty of Pharmacy, Ain Shams University*

*Submitted in partial fulfillment of the  
**PHD Degree***

*In Pharmaceutical Sciences  
(Pharmaceutical Chemistry)*

Under the supervision of

**Prof. Dr. / Dalal A. Abou El Ella**

*Professor of Pharmaceutical Chemistry  
Faculty of pharmacy-Ain Shams University*

**Prof. Dr. / Khaled A. M. Abouzid**

*Professor of Pharmaceutical Chemistry &  
Vice Dean for the Educational & Student Affairs  
Faculty of pharmacy-Ain Shams University*

**Dr. / Nasser Saad**

*Assistant Professor of Pharmaceutical Chemistry  
Faculty of pharmacy-Ain Shams University*

**Dr. / Rabah A. Taha**

*Lecturer of Pharmaceutical Chemistry  
Faculty of pharmacy-Ain Shams University*

Faculty of Pharmacy

Ain Shams University

2015

## Acknowledgements

*I owe my deepest appreciation and truthful gratitude to **Professor Dr. Dalal A. Abou El Ella**, Professor of Pharmaceutical Chemistry, for her scientific supervision. I am really sincerely and profoundly indebted to her for her priceless guidance, endless support and immense knowledge during all stages of this work. I am heartily grateful to her indispensable opinion, real interest, invaluable advices, trust, caring, eminent guidance, and untiring help throughout the whole work. I truly thank her for his great efforts which allowed this thesis to appear in its final form.*

*I owe my deepest appreciation and truthful gratitude to **Professor Dr. Khaled Abouzeid Mohamed Abouzeid**, Professor of Pharmaceutical Chemistry and Vice Dean for Educational and Student Affairs, for his scientific supervision. I am really sincerely and profoundly indebted to him for his priceless guidance, endless support and immense knowledge during all stages of this work. I am heartily grateful to his indispensable opinion, real interest, invaluable advices, trust, caring, eminent guidance, and untiring help throughout the whole work. I truly thank him for his great efforts which allowed this thesis to appear in its final form.*

*I would like also to express my sincere thanks to **Dr. Nasser Saad**, Assistant Professor in Pharmaceutical Chemistry, for his scientific supervision, fruitful opinion, untiring help, valuable assistance and constant encouragement. My cordial gratitude extend to him for his invaluable guidance and assistance all throughout the time spent in this thesis work.*

*I am extremely grateful and sincerely appreciated to **Dr. Rabah Ahmed Taha**, Lecturer in Pharmaceutical Chemistry for her kindness, continuous encouragement, indispensable assistance, valuable guidance and constant support throughout the whole practical work and during writing this thesis. I really thank her for her great efforts and tremendous support.*

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

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

*Finally, I am profoundly indebted to my mother and my family for their unconditional love and aid, endless patience, understanding, encouragement and full support all throughout the whole long way.*

**Table of Contents**

<b>Acknowledgements .....</b>	<b>I</b>
<b>List of Figures. ....</b>	<b>V</b>
<b>List of Tables.....</b>	<b>VII</b>
<b>List of Abbreviations .....</b>	<b>VIII</b>
<b>Abstract .....</b>	<b>XI</b>
<b>1. Introduction .....</b>	<b>1</b>
1.1. Cancer .....	1
1.1.1 Overview .....	1
1.1.2 Development.....	1
1.1.3 Hallmarks of cancer .....	2
1.1.4 Aetiology and carcinogenic factors .....	3
1.1.5 Epidemiology.....	3
1.1.6 Treatment.....	4
1.2 Protein kinases as cancer targeted therapy.....	10
1.2.1 Overview on Protein kinases.....	10
1.2.2 Overview on Tyrosine kinases (TK) .....	11
1.2.3 Tyrosine kinase structure.....	12
1.2.4 Tyrosine kinase Inhibitors .....	14
1.2.5 Inhibitors of TKs with proangiogenic activity: VEGFR and related kinases .....	21
<b>2. Rationale and Design .....</b>	<b>37</b>
2.1 Structure Activity Relationship Study (SAR) .....	38
2.2 Design of novel thieno[2,3- <i>d</i> ]pyrimidine based VEGFR-2 inhibitors: .....	41
2.3 Preliminary evaluation of the designed compounds using Molecular modeling techniques:.....	45
2.4 Synthetic schemes for synthesis of the designed compounds:.....	49
<b>3. Results and Discussion.....</b>	<b>54</b>

---

3.1	Chemistry.....	54
3.1.1	Scheme 1 .....	54
3.1.2	Scheme 2 .....	62
3.1.3	Scheme 3 .....	66
3.2	Biological Evaluation.....	71
3.2.1	In vitro VEGFR-2 tyrosine kinase inhibitory activity.....	71
3.2.2	In vitro antiproliferative activity against NCI 60-cell line .....	77
3.3	Molecular modeling study.....	82
3.3.1	Docking study.....	82
3.3.2	QSAR study .....	102
<b>4.</b>	<b>Conclusion.....</b>	<b>105</b>
<b>5.</b>	<b>Experimental.....</b>	<b>106</b>
5.1	Chemistry.....	106
5.1.1	Materials and instrumentation.....	106
5.1.2	Synthesis .....	107
5.2	Biological evaluation:.....	142
5.2.1	In vitro VEGFR-2 tyrosine kinase activity .....	142
5.2.2	In vitro Anti-proliferative activity against 60 cell line panel.....	143
5.3	Molecular Modelling study .....	145
5.3.1	Field alignment study: .....	145
5.3.2	Molecular docking: .....	145
5.3.3	QSAR study:.....	146
<b>6.</b>	<b>References: .....</b>	<b>148</b>

## List of Figures.

<b>Figure 1.</b> Process of cancer development.....	2
<b>Figure 2.</b> The Hallmarks of Cancer .....	3
<b>Figure 3.</b> Therapeutic Targeting of the Cancer Hallmarks .....	10
<b>Figure 4</b> FDA-approved small-molecule kinase inhibitors. ....	11
<b>Figure 5.</b> A representative protein kinase (IRK) (PDB ID 1IR3). ....	12
<b>Figure 6.</b> (a) Ribbon diagram of ATP binding site with a DFG-in activation-loop conformation (b) Ribbon diagram of a representative for type II inhibitor binding mode showing the DFG-out activation-loop conformation .....	14
<b>Figure 7.</b> Left panel shows a DFG-in conformation of ABL kinase bound to dasatinib (28) and the right panel shows a DFG-out conformation of the ABL kinase domain bound to imatinib (36). ....	18
<b>Figure 8.</b> Different binding modes for different types of kinase inhibitors. ....	19
<b>Figure 9.</b> Kinase structure and different types of reversible small-molecule kinase inhibitor. ....	20
<b>Figure 10.</b> Schematic illustration of the expression patterns, ligand specificity and cellular/physiological effects of each of the vascular endothelial growth factor receptors (VEGFRs) .....	23
<b>Figure 11.</b> The binding mode of VEGF and VEGFR-2. ....	25
<b>Figure 12.</b> Tumour angiogenesis and inhibitors of VEGFR-2 signalling.....	26
<b>Figure 13.</b> SAR of various potent VEGFR-2 inhibitors.....	39
<b>Figure 14. (a)</b> Reported binding mode of lead compound pyrrolo[3,2-d]pyrimidine derivative (60) to VEGFR-2 <b>(b)</b> Binding mode of lenvatinib (48) to VEGFR-2 .....	42
<b>Figure 15.</b> Design of type II VEGFR-2 inhibitors based on pyrrolo[3,2-d]pyrimidine derivative (60) lead compound. ....	43
<b>Figure 16.</b> Design of VEGFR-2 inhibitors based on Lenvatinib (48) lead compound.....	44
<b>Figure 17.</b> Design of type II VEGFR-2 inhibitors based on (52) lead compound. ....	45
<b>Figure 18.</b> Cyclization of 2-methyl-4-nitroaniline into indazole through hydroxydiazine intermediate .....	57

<b>Figure 19.</b> Synthetic approaches for thieno[2,3-d]pyrimidines.....	61
<b>Figure 20.</b> Example of mean graph produced from NCI 60 cell line screening program.....	78
<b>Figure 21.</b> The alignment between the X-ray bioactive conformer of the lead compound ( <b>60</b> ) and the redocked pose of the same compound at VEGFR-2 binding site .....	83
<b>Figure 22.</b> Predicted versus experimental -logIC <sub>50</sub> values of the training set according to QSAR Equation .....	103

**List of Tables.**

<b>Table 1.</b> Type I kinase inhibitors approved by the FDA .....	16
<b>Table 2</b> Examples of thieno[2,3- <i>d</i> ]pyrimidine based protein kinase inhibitors. ....	35
<b>Table 3.</b> Field alignment of chosen compounds with reference molecules.....	47
<b>Table 4.</b> Percent inhibition of VEGFR-2 enzymatic activity achieved by the targeted compounds at 10 $\mu$ M and the $IC_{50}$ values for selected compounds. ....	72
<b>Table 5.</b> Cell growth percentage of NCI 60 cancer cell lines exhibited by investigated final compounds (XIIIg, XVb, XVIId, XVIe, XVIIa, XIXa, XIXb): .....	79
<b>Table 6.</b> The binding interactions of the docked compounds together with their binding energies .....	84
<b>Table 7.</b> Experimental activity of the synthesized compounds against the predicted activity according to the model equation. ....	104



## **List of Abbreviations**

**ABL:** Abelson tyrosine kinase

**ADMET:** Absorption, Distribution, Metabolism, Excretion, and Toxicity study

**ALK:** Anaplastic lymphoma kinase

**ANLL:** Acute Non-Lymphocytic Leukemia

**Asp:** Aspartate

**ATP:** Adenine-5'-triphosphate

**BAECs :** Bovine aortic endothelial cells

**BBB:** Blood brain barrier

**BCR:** breakpoint cluster region protein

**BSA:** Bovine serum albumin

**BRMs:** Biological response modifiers

**C-Fms:** Colony-Stimulating factor-1 receptor

**C-kit:** v-kit (Hardy-Zuckerman 4 feline) sarcoma viral oncogene

**CHARMm:** Chemistry at HARvard Macromolecular Mechanics

**CTLA4 mab:** Cytotoxic T-lymphocytes 4A monoclonal antibody

**CYP 450:** Cytochrome P450

**Cys:** Cysteine

**D<sub>2</sub>O:** Deuterium oxide

**DFG:** Aspartate- Phenylalanine- Glycine

**DIPEA:** Diisopropyl ethylamine

**DMF:** Dimethyl formamide

**DMSO:** Dimethyl sulfoxide

**DNA:** Deoxyribonucleic acid

**EC:** Endothelial cell

**EI-MS:** Electron impact mass spectroscopy

**EGFR:** Epidermal growth factor receptor

**5-FU:** 5-Fluorouracil

**Fab:** Fragment antigen-binding

**FDA:** Food and Drug Administration  
**FGFR:** Fibroblast growth factor receptor  
**FLT:** FMS-like receptor tyrosine kinase  
**FT-IR:** Fourier transform-Infrared  
**Glu:** Glutamate  
**HER-2:** Human epidermal growth factor receptor-2  
**HIA:** Human intestinal absorption  
**His:** Histidine  
**Hrs:** hours  
**HUVEC:** Human umbilical vein endothelial cells  
**Hz:** Hertz  
**IC<sub>50</sub>:** Half-maximal inhibitory concentration  
**IGFR:** Insulin-like growth factor receptor  
**IRK:** Insulin receptor kinase  
**JAK:** Janus kinase  
**KDa:** Kilo Dalton  
**KDR:** Kinase insert domain receptor  
**Lys:** Lysine  
**6-MP:** 6-Mercaptopurine  
**m.p.:** Melting point  
**Min:** Minutes  
**MHz:** Mega hertz  
**μM:** Micromole  
**mmol:** Millimole  
**μl:** Microliter  
**MS:** Mass spectroscopy  
**NCI:** National Cancer Institute  
**NIH:** National Institutes of Health  
**nM:** Nanomole  
**NMR:** Nuclear magnetic resonance  
**NRTK:** Non-receptor tyrosine kinase

**NSCLC:** Non-Small Lung Cell cancer  
**PARP:** Poly ADP ribose polymerase  
**Pd-C:** Palladium on carbon  
**PDB:** Protein data bank  
**PDGFR:** Platelet derived growth factor receptor  
**PDT:** Photodynamic therapy  
**Phe:** Phenyl alanine  
**PM:** Picometre  
**PPB:** Plasma protein binding  
**Ppm:** Part per million  
**PSA:** Polar surface area  
**Raf:** v-raf murine sarcoma viral oncogene  
**Ras:** Rat sarcoma  
**RMSD:** Root mean square deviation  
**RNA:** Ribonucleic Acid  
**rt:** Room temperature  
**RTK:** Receptor tyrosine kinase  
**SAR:** Structure activity relationship  
**SMART:** string matching algorithms research tool  
**SRC:** Sarcoma (Schmidt-Ruppin A-2) Viral Oncogene  
**TEA:** Triethyl amine  
**THF:** Tetrahydrofuran  
**Tie-2:** Tyrosine kinase with immunoglobulin-like and EGF-like domains 2  
**TK:** Tyrosine kinase  
**TLC:** Thin layer Chromatography  
**TP53:** Tumor protein 53  
**U.V:** Ultra violet  
**VEGFR:** Vascular endothelial growth factor receptor

## **Abstract**

*Title of thesis:*

**"Molecular modeling and Synthesis of Certain Heterocyclic  
Compounds with Expected Biological Activity"**

*Name of candidate:*

**Eman Zaglol ElRazaz**

*Assistant Lecturer of Pharmaceutical Chemistry  
Ain Shams University*

*Under the supervision of*

**Prof. Dr. / Dalal A. Abou El Ella**

*Professor of Pharmaceutical Chemistry  
Faculty of pharmacy-Ain Shams University*

**Prof. Dr. / Khaled A. M. Abouzid**

*Professor of Pharmaceutical Chemistry &  
Vice Dean for the Educational & Student Affairs  
Faculty of pharmacy-Ain Shams University*

**Dr. / Nasser Saad**

*Assistant Professor of Pharmaceutical Chemistry  
Faculty of pharmacy-Ain Shams University*

**Dr. / Rabah A. Taha**

*Lecturer of Pharmaceutical Chemistry  
Faculty of pharmacy-Ain Shams University*

Cancer, also known as a malignant tumor, is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body. A major problem in treating cancer is the fact that it is not a single disease. There are more than 200 different cancers resulting from different cellular defects. The growth of new blood vessels (angiogenesis) is one of the well established hallmarks in the process of carcinogenesis. Vascular endothelial growth factor receptor-2 (VEGFR-2) plays a crucial role in cancer angiogenesis. By targeting VEGFR-2, angiogenesis is greatly inhibited leading to the death of the tumor cells.

In this study, thienopyrimidine derivatives have been designed and synthesized as targeted angiogenesis inhibitors. The design focused on exploration of the previous revealed SAR studies, bioisosteric modifications of the lead compounds both in market and in clinical studies, and identification of the key interactions with the binding site *in silico*.

Synthesis of the designed compounds was then accomplished & their structures were confirmed by various spectral and microanalytical data.

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

- 1) 1-(4-Nitrophenyl)-3-phenylurea (**Ia**)
- 2) 1-(3-Methoxyphenyl)-3-(4-nitrophenyl)urea (**Ib**)
- 3) 1-(4-Nitrophenyl)-3-(*m*-tolyl)urea (**Ic**)
- 4) 1-(4-Acetylphenyl)-3-(4-nitrophenyl)urea (**Ie**)
- 5) 1-(4-Chlorophenyl)-3-(4-nitrophenyl)urea (**Ig**)
- 6) *N*1-(3-Bromophenyl)-3-(4-nitrophenyl)urea (**Ih**)
- 7) 1-(4-Ethylphenyl)-3-(4-nitrophenyl)urea (**Ii**)
- 8) 1-(3,4-diChlorophenyl)-3-(4-nitrophenyl)urea (**Ij**)
- 9) 1-(3-trifluoromethyl-4-chlorophenyl)-3-(4-nitrophenyl)urea (**Ik**)
- 10) 1-(4-Aminophenyl)-3-phenylurea (**IIa**)
- 11) 1-(4-Aminophenyl)-3-(3-methoxyphenyl)urea (**IIb**)
- 12) 1-(4-Aminophenyl)-3-(*m*-tolyl)urea (**IIc**)
- 13) 1-(4-Aminophenyl)-3-(4-chlorophenyl)urea (**Ilg**)
- 14) 1-(4-Aminophenyl)-3-(3-bromophenyl)urea (**IIh**)
- 15) 1-(4-aminophenyl)-3-(3,4-diChlorophenyl) urea (**IIj**)

- 16) 1-(4-aminophenyl)-3-(3-trifluoromethyl-4-chlorophenyl) urea (**IIIk**)
- 17) 1-(4-Hydroxyphenyl)-3-phenylurea (**IIIa**)
- 18) 1-(3-Bromophenyl)-3-(4-hydroxyphenyl)urea (**IIIb**)
- 19) 1-(4-Hydroxyphenyl)-3-(3-methoxyphenyl)urea (**IIIc**)
- 20) 1-(4-Chlorophenyl)-3-(4-hydroxyphenyl)urea (**IIId**)
- 21) 11-(3,4-Dichlorophenyl)-3-(4-hydroxyphenyl)urea (**IIIf**)
- 22) 1-(4-Chloro-3-(trifluoromethyl)phenyl)-3-(4-hydroxyphenyl)urea (**IIIg**)
- 23) 5-Nitroindazole (**IV**)
- 24) 5-Aminoindazole (**V**)
- 25) 5-Amino benzimidazole (**VI**)
- 26) N-(4-Nitrophenyl)-2-phenylacetamide (**VII**)
- 27) N-(4-aminophenyl)-2-phenylacetamide (**VIII**)
- 28) N-(4-Hydroxyphenyl)-2-phenylacetamide (**IX**)
- 29) Diethyl (5-amino-3-methylthiophene)-2,4-dicarboxylate (**X**)
- 30) Ethyl (5-methyl-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine)-6-carboxylate (**XI**)
- 31) Ethyl (4-chloro-5-methylthieno[2,3-d]pyrimidine)-6-carboxylate (**XII**)
- 32) 3-((6-(Ethoxycarbonyl)-5-methylthieno[2,3-d]pyrimidin-4-yl)amino)benzoic acid (**XXII**)

Also, it comprised the following new intermediates:

- 1) 1-(3-Acetylphenyl)-3-(4-nitrophenyl)urea (**Id**)
- 2) 1-(3-Chloro-4-methylphenyl)-3-(4-nitrophenyl)urea (**If**)
- 3) 1-(3-Acetylphenyl)-3-(4-aminophenyl)urea (**IId**)
- 4) 1-(4-Acetylphenyl)-3-(4-aminophenyl)urea (**IIf**)
- 5) 1-(4-Aminophenyl)-3-(3-chloro-4-methylphenyl)urea (**IIIf**)
- 6) 1-(4-Aminophenyl)-3-(4-ethylphenyl)urea (**IIIi**)
- 7) 1-(3-Chloro-4-methylphenyl)-3-(4-hydroxyphenyl)urea (**IIIe**)
- 8) 5-Methyl-4-((4-(3-phenylureido)phenyl)amino)thieno[2,3-d]pyrimidine-6-carboxylic acid (**XIVa**)
- 9) 4-((4-(3-(3-methoxyphenyl)ureido)phenyl)amino)-5-methylthieno[2,3-d]pyrimidine-6-carboxylic acid (**XIVb**)

Also, the study involved the synthesis and the characterization of the following new-targeted compounds:

- 1) Ethyl 5-methyl-4-((4-(3-phenylureido)phenyl)amino)thieno[2,3-d]pyrimidine-6-carboxylate (**XIIIa**)
- 2) Ethyl 4-((4-(3-(3-methoxyphenyl)ureido)phenyl)amino)-5-methylthieno[2,3-d]pyrimidine-6-carboxylate (**XIIIb**)
- 3) Ethyl 5-methyl-4-((4-(3-(*m*-tolyl)ureido)phenyl)amino)thieno[2,3-d]pyrimidine-6-carboxylate (**XIIIc**)
- 4) Ethyl 4-((4-(3-(3-acetylphenyl)ureido)phenyl)amino)-5-methylthieno[2,3-d]pyrimidine-6-carboxylate (**XIIId**)
- 5) Ethyl 4-((4-(3-(4-acetylphenyl)ureido)phenyl)amino)-5-methylthieno[2,3-d]pyrimidine-6-carboxylate (**XIIIe**)
- 6) Ethyl 4-((4-(3-(3-chloro-4-methylphenyl)ureido)phenyl)amino)-5-methylthieno[2,3-d]pyrimidine-6-carboxylate (**XIIIf**)
- 7) Ethyl 4-((4-(3-(4-chlorophenyl)ureido)phenyl)amino)-5-methylthieno[2,3-d]pyrimidine-6-carboxylate (**XIIIg**)
- 8) Ethyl 4-((4-(3-(3-bromophenyl)ureido)phenyl)amino)-5-methylthieno[2,3-d]pyrimidine-6-carboxylate (**XIIIh**)
- 9) Ethyl 4-((4-(3-(4-ethylphenyl)ureido)phenyl)amino)-5-methylthieno[2,3-d]pyrimidine-6-carboxylate (**XIIIi**)
- 10) Ethyl 4-((4-(3-(3,4-dichlorophenyl)ureido)phenyl)amino)-5-methylthieno[2,3-d]pyrimidine-6-carboxylate (**XIIIj**)
- 11) Ethyl 4-((4-(3-(4-chloro-3-(trifluoromethyl)phenyl)ureido)phenyl)amino)-5-methylthieno[2,3-d]pyrimidine-6-carboxylate (**XIIIk**)
- 12) 5-Methyl-4-((4-(3-phenylureido)phenyl)amino)-*N*-propylthieno[2,3-d]pyrimidine-6-carboxamide (**XVa**)
- 13) 4-((4-(3-(3-Methoxyphenyl)ureido)phenyl)amino)-5-methyl-*N*-propylthieno[2,3-d]pyrimidine-6-carboxamide (**XVb**)
- 14) Ethyl 5-methyl-4-(4-(3-phenylureido)phenoxy)thieno[2,3-d]pyrimidine-6-carboxylate (**XVIa**)
- 15) Ethyl 4-(4-(3-(3-bromophenyl)ureido)phenoxy)-5-methylthieno[2,3-d]pyrimidine-6-carboxylate (**XVIb**)
- 16) Ethyl 4-(4-(3-(3-methoxyphenyl)ureido)phenoxy)-5-methylthieno[2,3-d]pyrimidine-6-carboxylate (**XVIc**)