



Computer Based Design and Synthesis of Certain Quinazoline Derivatives as Tyrosine Kinase Inhibitors with Potential Anticancer Activity

Thesis

Presented by

Wegdan Mohamed Metwally sayed

BSc. In Pharmaceutical Sciences (May 2006)

Teaching Assistant of Pharmaceutical Chemistry

Modern Science and Arts University

Submitted for the partial fulfillment of the

Master Degree

In Pharmaceutical Sciences

(Pharmaceutical Chemistry)

Under the supervision of

Prof. Dr. / Dalal A. Abou El Ella

Professor of Pharmaceutical Chemistry

Head of Pharmaceutical Chemistry Department

Ain Shams University

Assoc.Prof. Dr. / Gehan Hegazy

*Associate professor of Pharmaceutical
Chemistry*

Cairo University

Dr. / Rabah A. Taha

*Lecturer of Pharmaceutical Chemistry
Ain Shams University*

Faculty of Pharmacy

Ain Shams University

2014

To
My Mother

Acknowledgements

*I owe my deepest appreciation and truthful gratitude to **Prof. Dr. Dalal Ahmed Abou El Ella**, Professor of Pharmaceutical Chemistry and Head of Pharmaceutical Chemistry Department Ain Shams University, for her scientific supervision, innovative ideas, fruitful opinion, invaluable advices, precious suggestions, continuous encouragement and untiring help. I am really sincerely and profoundly indebted to her for her priceless guidance and endless support throughout the whole work and during writing this thesis. I truly thank her for her great efforts which allowed this thesis to appear in its final form.*

*I would like also to express my sincere thanks to **Ass. Prof. Dr. Gehan Hegazy**, Associate professor of Pharmaceutical Chemistry Cairo University, for her kindness, continuous encouragement, indispensable assistance, valuable guidance and constant support throughout the whole work, especially in the practical work.*

*I am extremely grateful and sincerely appreciated to **Dr. Rabah Ahmed Taha** Lecturer of Pharmaceutical Chemistry Ain Shams University, for her kindness, continuous interest, encouragement, indispensable comments, and real support throughout the molecular modeling parts in this work.*

*I am thankful to **Prof. Dr. Khaled A. M. Abouzid** Professor of Pharmaceutical Chemistry & vice Dean for Educational & Student Affairs, Ain Shams University for his kind support.*

*I am thankful to **Dr. Sameh Eid** department of Pharmaceutical Sciences, University of Basel for his kind support and assistance in molecular modeling.*

I acknowledge with thankfulness all members of the Pharmaceutical Chemistry department; Ain Shams University for their friendly cooperation, support, unconditional love and aid. I am indebted to all of them who have helped me.

*My husband and my son, **Sameh** and **Kareem**, they form the backbone and the origin of my happiness. I'd like to thank **Sameh** for supporting me in carrying out this thesis. His love and support without any complaint or regret has enabled me to complete this work. **Kareem**, the most important, most difficult and most rewarding experiment I have ever undertaken. He has made me smile when I might just have been taking myself a little too seriously.*

Finally, I am profoundly indebted to my father, my father-in-law, my mother-in-law and my brothers for their endless patience, understanding, encouragement and support all throughout the whole long way

Contents:

Acknowledgements:.....	ii
Contents:.....	iv
List of figures:	vii
List of tables:	ix
List of Abbreviations:	x
Abstract:	1
1. Introduction:	5
1.1 Cancer:	5
1.1.1 Definition:	5
1.1.2 Causes of cancer:	5
1.1.3 Tumorigenesis:	5
1.1.4 Cancer hallmarks:	6
1.2 Cancer therapy:	8
1.2.1 Surgery:	8
1.2.2 Radiotherapy:	8
1.2.3 Cancer chemotherapy:	9
1.2.3.1 Antimetabolites:	9
1.2.3.2 Alkylating agents:	10
1.2.3.3 DNA intercalators and topoisomerase inhibitors:	10
1.2.3.4 Antitumor antibiotics:	11
1.2.3.5 Anticancer that targets hormonal actions:	11
1.2.3.6 Corticosteroids:	12
1.2.3.7 Targeted therapy:	12
1.2.3.7.1. Proteasome inhibitors:	15
1.2.3.7.2 Tolmerase inhibitors:	16

1.2.3.7.3. Antitense oligonucleotides:	18
1.2.3.7.4. Targetting protein kinases:	19
1.2.3.7.4.1. EGFR tyrosine kinase inhibitors:	25
1.2.3.7.4.2. Inhibitors of TKs with pro-angiogenic activity VEGFR and related kinases:	29
2. Rational and design:	38
2.1 Identification of the key interactions with the binding site of VEGFR2:	40
2.1.1. Binding mode of the cosrystallized benziimidazole urea in VEGFR2 binding site:	41
2.1.2. Binding mode of the redocked benziimidazole urea in VEGFR2 binding site:	43
2.1.3. Docking of the reference compound (patent) with VEGFR2 binding site:	44
2.1.4. Structural basis of VEGFR2 inhibitors:	45
2.2. Exploration SAR and modifications:	46
3. Results and discussion:	50
3.1. Chemistry:	50
3.2-Biological evaluation:	57
3.2.1. In-Vitro anticancer activity:	57
3.2.2. Enzyme inhibition assay:	59
3.3. Molecular Modeling:	60
3.3.1. Docking study:	60
3.3.1.1 Docking study using Glide program:	60
3.3.1.2 Docking study using Accelry's discovery:	60
3.3.2 Design process:	60
4. Conclusion:	68
5. Experimental:	71
5.1. Chemistry:	71
5.2. Biological evaluation of compounds:	87
5.2.1. In-Vitro anticancer evaluation:	87

4.2.2. Enzyme inhibition assay:	88
5.3. Molecular modeling:	88
5.3.1. Protein preparation for docking using glide program:	89
5.3.2 Protein preparation for docking using Accelry's discovery studio 2.5 program:	89
5.3.3. Ligand preparation for docking using glide program:	90
5.3.4. Ligand preparation for docking using Accelry's discovery syudio 2.5 program:	90
6- References:	91

List of figures

Figure 1: The main hallmarks of cancer:	7
Figure 2: Therapeutic targeting of the cancer hallmarks:	13
Figure 3: Mechanisms of targeted therapies. The molecular targets in this figure are not over-expressed in a single cell type, but rather on various malignant and normal tissues:	14
Figure 4a: Biological function of telomerase:	16
Figure 4b: Addition of telomeres to the 3' end of DNA by telomerase:	17
Figure 5: DNA G-quadruplex in telomeric DNA:	18
Figure 6: Binding mode of ATP molecule to the hinge region through hydrogen bonding (dark area):.....	21
Figure 7: The difference between type 1 binding with PP1inhibitor (a) and type 2 with STI-571 inhibitor (b) binding modes.....	24
Figure 8: Signal transduction pathways controlled by the activation of EGFR:	26
Figure 9: Binding of ATP at the TK domain of EGFR:	28
Figure 10: Schematic illustration of the cellular/physiological effects of each of the vascular endothelial growth factor receptors (VEGFRs):	30
Figure 11: Up; type 1 VEGFR tyrosine kinase inhibitors and their binding modes. down; type 2 VEGFR tyrosine kinase inhibitors and their binding modes. Hydrogen bonds between the inhibitor and the protein are shown with black dotted lines:	32
Figure 12: Binding of AAL-993 (ZK-260255) to VEGFR-2:	35
Figure 13: Binding modes of kinase inhibitors. (a) Schematic representation of the ATP binding site. (b) Schematic representation of the allosteric binding site. (c) Ribbon diagram of ATP binding site with a DFG-in activation-loop conformation (active conformation). (d) Ribbon diagram of a representative of type 2 binding mode showing the DFG-out activation-loop conformation (inactive conformation):	41
Figure 14: Binding mode of the co-crystallized benzimidazole-urea 27 inhibitor in complex	

with VEGFR2 (PDB code 2OH4) show the hydrogen bonds between the urea, Glu883 and ASP1044 of the DFG motif:.....	42
Figure 15: Re-docking of benziimidazole-urea inhibitor 27 in complex with VEGFR2 (PDB 2OH4); white carbons: crystal structure pose, green carbons: top-ranked docking pose. Protein atoms and cartoons are colored orange. Hydrogen bonds are represented by red dotted lines:.....	43
Figure 16: Alignment between the co-crystallized benzimidazole urea inhibitor 27 (colored in purple) and its redocked pose (colored in green):.....	44
Figure 17: Docking of the reference compound 31 in complex with VEGFR2 (PDB code 2OH4 shows the formation of hydrogen bond with Glu883 represented by the dotted line:.....	45
Figure 18: Similarities between reference compound 31 and target compounds IVa-f, Va-d, VIa-d and VIIa-b:	47
Figure 19: Top-ranked docking poses of compound VIa to VEGFR2 maintain the key interactions exhibited by the co-crystallized inhibitor in PDB 2OH4. Protein atoms and cartoons are colored orange. Hydrogen bonds are represented by red dotted line:.....	62

List of tables:

Table 1: The activity of the tested compounds on hepatic and breast cell lines:58

Table 2: Percentage inhibition of enzymatic activity for the targeted compounds of VEGFR2 kinase:
.....59

Table 3: Molecular docking simulation study results of selected designed compounds in VEGFR2
active site:63

List of abbreviations

ABL1: Abelson kinase

ANLL: Acute Non-Lymphocytic Leukemia

ATP: Adenosine tri phosphate

ASO: Antisense oligonucleotides

BCL-2: B-cell lymphocyte 2

BR-I: Binding region-I

BR-II: Binding region-II

DFG: Aspartate- Phenylalanine- Glycine

EGFR: epidermal growth factor receptor

FT-IR: Fourier transform-Infrared

GIST: Gastrointestinal stromal tumors

GOLD: Genetic optimization of ligand docking

HEPG2: Hepatocellular carcinoma cell line.

IC₅₀: 50% Inhibitory concentration

IgG1: Immunoglobulin G1

MCF7: Human breast adenocarcinoma cell line.

KDR: Kinase insert domain

mAbs: Monoclonal antibodies

MHz: Mega hertz

MS: Mass spectroscopy

NSCLC: Non-small lung cell cancer

NF-KB: Nuclear factor-KB