



Molecular Design, Synthesis and Biological Evaluation of Kinase Inhibitors Based on Pyrrolopyrimidine Scaffold

Thesis

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List of Abbreviations:

ABL: Abelson tyrosine kinase

ACK1: Activated Cdc42-associated kinase1

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

Akt: Protein kinase B (PKB), also known as Akt

ALL: Acute lymphoblastic leukemia

AlogP: Atomic logP (the logarithm of 1-octanol/water partition coefficient)

AMBER: Assisted Model Building with Energy Refinement (force field)

ATP: Adenine-5'-triphosphate

BBB: Blood brain barrier

BCG: bacillus calmette-guerin

BCR: breakpoint cluster region protein

BRCA1/A2: Breast cancer gene A1/A2

BRK: Breast tumor kinase

BSA: Bovine serum albumin

BTK: Bruton's tyrosine kinase

CDK: Cyclin dependant kinase

CDOCKER: CHARMM-based docker

C-Fms: Colony-Stimulating factor-1 receptor

CHARMm: Chemistry at HARvard Macromolecular Mechanics

c-MET: cellular mesenchymal to epithelial transition factor

¹³C NMR: Carbon-13 Nuclear Magnetic Resonance

CSF-1R: Colony Stimulating Factor 1 Receptor

CYP 450: Cytochrome P450

D₂O: Deuterium oxide

DCC: *N,N'*-Dicyclohexylcarbodiimide

DCM: dichloromethane

DFG: Aspartate- Phenylalanine- Glycine

DMAC: Dimethyl acetamide

DMCA: Dimethyl cyclohexylamine
DMAP: 4-(Dimethylamino)pyridine
DMF: Dimethylformamide
DMSO: Dimethyl sulfoxide
DNA: Deoxyribonucleic acid
DTP: Developmental Therapeutics Program
EphRs: Ephrin receptors
EGFR: Epidermal growth factor receptor
EI-MS: Electron Ionization Mass Spectrometry
FAK: Focal adhesion kinase
FDA: Food and Drug Administration
FGFR: Fibroblast growth factor receptor
FLT: FMS-like receptor tyrosine kinase
FPP: Field Point Pattern
HB: hydrogen bond
HBV: Hepatitis B virus
HCV: Hepatitis C virus
HIA: Human intestinal absorption
HIV: Human immunodeficiency virus
¹H NMR: Proton Nuclear Magnetic Resonance
HPV: Human papilloma virus
HRD: His- Arg- Asp
Hrs: hours
HUVEC: Human umbilical vein endothelial cells
Hz: Hertz
IC₅₀: Half-maximal inhibitory concentration
IGFR: Insulin-like growth factor receptor
ITK: interleukin-2-inducible T-cell kinase
JAK: Janus kinase
KDR: Kinase insert domain receptor
Ki: the inhibitor constant

Lck: Lymphocyte-specific protein tyrosine kinase
LC/MS: Liquid chromatography–mass spectrometry
m/z: mass-to-charge ratio
M⁺: Molecular ion
m.p.: Melting point
MD: Molecular Dynamics
MEK: mitogen-activated protein kinase
Min: Minutes
MMP's: Matrix mettaloproteinasae's
Mps1: Monopolar spindle 1 kinase
mTOR: mechanistic target of rapamycin
Mwt: Molecular Weight
MHz: Mega hertz
μM: Micromole
mmol: Millimole
μl: Microliter
MS: Mass spectroscopy
NCI: National Cancer Institute
NCR: National cancer registry
NIH: National Institutes of Health
nM: Nanomole
NMP: N-Methyl-2-pyrrolidone
NMR: Nuclear magnetic resonance
NRTK: Non-receptor tyrosine kinase
Pd-C: Palladium on carbon
PDB: Protien data bank
PDGFR: Platelet derived growth factor receptor
PDK1: Phosphoinositide-dependent kinase-1
PKC: protein kinase C
PKs: protein kinases
PPB: Plasma protein binding

Ppm: Part per million

PSA: Polar surface area

PTKs: protein tyrosine kinases

QSAR: Quantitative structure activity relationship

Raf: v-raf murine sarcoma viral oncogene

RMSD: Root mean square deviation

RNA: Ribonucleic Acid

RPMI: Roswell Park Memorial Institute medium

rt: Room temperature

RTK: Receptor tyrosine kinase

SAR: Structure activity relationship

SFK: Src family of protein tyrosine kinase

SMKIs: Small molecule kinase inhibitors

SRC: Sarcoma (Schmidt-Ruppin A-2) Viral Oncogene

TEA: Triethyl amine

TGF: Transforming growth factor

THF: Tetrahydrofuran

Tie-2: Tyrosine kinase with immunoglobulin-like and EGF-like domains 2

TK: Tyrosine kinase

TLC: Thin layer Chromatography

TMS: Tetramethylsilane

TNF: Tumour necrosis factor

TP53: Tumor protein 53

Tris: tris(hydroxymethyl)aminomethane

TrkB : Tropomyosin receptor kinase B

U.V: Ultra violet

VEGFR: Vascular endothelial growth factor receptor

WHO:World Health Organization

Abstract

Cancer is a genetic disease characterized by two features: unregulated cell growth and tissue invasion (metastasis). Angiogenesis is a complex process in which there is growth of new blood vessels from the pre-existing ones and is an essential phenomenon for the growth and survival of solid neoplasms. Vascular endothelial-derived growth factor (VEGF) is one of the most important and potent angiogenic molecules which play an integral role in tumor angiogenesis. VEGFR-2 inhibition has been considered as an effective strategy for the prevention of angiogenesis. Antiangiogenic therapy based on VEGFR-2 inhibition is a powerful clinical treatment of cancers and several small molecule tyrosine kinase inhibitors of VEGF receptor have been approved for the treatment of several types of cancer. The research objective is to design and synthesize new selective inhibitors targeting VEGFR-2 with promising anti-cancer activity. Building on the classical kinase inhibitors design, profound literature survey, SAR studies and molecular modeling, a series of novel pyrrolopyrimidine-based compounds were designed. The designed compounds were synthesized, purified and structurally confirmed by different analytical and spectral techniques.

In vitro biological evaluation was accomplished through testing both anticancer activity and VEGF enzyme inhibition activity of the newly synthesized compounds. Many of the synthesized compounds showed good to potent VEGFR-2 inhibitory potency. Most of the tested urea compounds with diphenylurea moiety at the C4-position of the pyrrolo[2,3-*d*]pyrimidine core linked via an oxygen or NH linker demonstrated highly potent dose-related VEGFR-2 inhibition with IC₅₀ values in nanomolar range. The pyrrolo[2,3-*d*]pyrimidine based-derivatives (**XVIIId** and **XXc**) showed the highest potent nanomolar VEGFR-2 inhibition (IC₅₀ of 11.9 nM and 13.7 nM respectively). 13 of the final Compounds were selected by the National Cancer Institute “NCI” for single dose screening program at 10 µM in the full NCI 60 cell panel. The pyrrolo[2,3-*d*]pyrimidine-based derivative (**XXf**) showed the lowest cell growth promotion, indicating good anti-proliferative activity against different cell lines.

Finally, a thorough molecular docking, using C-DOCKER protocol in Discovery Studio 2.5 software was attempted to investigate the binding mode of the targeted compounds and interpret

their variable inhibitory activity. Moreover, a computer aided ADMET study on the active compounds was done using Accelrys discovery studio 2.5 software. The thesis involves 260 references showing the literature survey for this research.

1. Introduction

1.1. Cancer

1.2. Overview

Cancer is a genetic disease characterized by two main features: unregulated cell growth and tissue invasion/metastasis. The malignant phenotype requires mutations in several genes that regulate cell proliferation, motility, survival, DNA repair, invasion, and angiogenesis. Cancer-causing mutations activate signal transduction pathways leading to aberrant cell proliferation and perturbations of the tissue specific differentiation programs. The normal cell has protective mechanisms that repair any DNA damage that occurs during DNA synthesis and mitosis and also in response to environmental mutagens which are usually abnormal in cancer cells. Too much damage of a normal cell activates a suicide pathway to prevent the damage of the organ. Such pathway is usually altered in cancer cells, leading to the survival of the damaged cells that normally die. Cancer cells exist under conditions of low oxygen tension (hypoxia) and nutrient deprivation, this leads to the outgrowth of neoplastic variants that can survive under these conditions through the upregulation of a series of hypoxia-inducible genes. The novel phenotypic characteristics includes those that facilitate invasion and metastasis, such as the ability to break through basement membranes, migrate through the extracellular matrix and into the vascular compartment, and generate new blood vessels to support colonization in distant sites [1].

1.2.1. *Types of cancer genes*

There are three main classes of genes that are important in controlling cell growth and play a role in cancer cell development. People may inherit a mutated form of one of these genes, which may make them more likely to develop a particular type of cancer.

Oncogenes

Oncogenes cause cells to grow out of control. They promote cancer cell growth. Oncogenes are damaged versions of normal genes called proto-oncogenes which are normal genes involved in the control of cell growth and division that can mutate to an oncogene.