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

Thesis

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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

^{13}C NMR: Carbon-13 Nuclear Magnetic Resonance

CSF-1R: Colony Stimulating Factor 1 Receptor

CYP 450: Cytochrome P450

D_2O: 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

^{1}H NMR: Proton Nuclear Magnetic Resonance

HPV: Human papilloma virus

HRD: His- Arg- Asp

Hrs: hours

HUVEC: Human umbilical vein endothelial cells

Hz: Hertz

IC_{50}: 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: Quantitatve structure activity relationship

Raf: v-raf murine sarcoma viral oncogene

RMSD: Root mean square deviation

RNA: Riboneucleic 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 IC_{50} values in nanomolar range. The pyrrolo[2,3-*d*]pyrimidine based-derivatives **(XVIId** and **XXc)** showed the highest potent nanomolar VEGFR-2 inhibition (IC_{50} 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.