Molecular Design and Synthesis of Certain Indazole Derivatives with Potential Anticancer Activity

Thesis

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Besides the work presented in this thesis, the candidate successfully passed general and special postgraduate courses in Pharmaceutical Chemistry for one year during academic year 2010/2011 with the following grades

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7) Selected Topics in Pharmaceutical Chemistry	**Very Good**
8) Drug Stereochemistry	**Excellent**

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List of Abbreviations

2D: Two dimensional
5-FU: 5-Fluorouuracil
6-MP: 6-Mercaptopurine
Å: Angstroms
AcOH: Acetic acid
AcONa: Sodium acetate
ADP: Adenosine di phosphate.
AIDS: acquired immune deficiency syndrome
ATP: Adenosine triphosphate
BCR-ABL: Breakpoint cluster region-Abelson
CagA: Cytotoxin-associated gene A
CDK: Cycline dependant kinase
CDOCKER: CHARMm-based DOCKER
CHARMm: Chemistry at HARvard macromolecular mechanics
DCM: Dichloro methane
DFG: Aspartate- Phenylalanine- Glycine
DHFR: Dihydrofolate reductase
DIPEA: *N*,*N*-Diisopropylethylamine
DMF: Dimethyl formamide
DMSO: Dimethylsulfoxide
DNA: Deoxyribo neucleic acid
EGFR: Epidermal Growth Factor Receptor
ErbB-2: Human Epidermal Growth Factor Receptor 2
Et_2O: Diethyl ether
EtOAc: Ethyl acetate
FDA: Food and Drug Administration
FGFR: Fibroblast growth factor receptor
FLT kinase: FMS-Like tyrosine kinase
FT-IR: Fourier transform-Infrared
GA: Genetic algorithm
GI_{50}: Growth Inhibition
GIST: gastrointestinal stromal tumors
GIT: Gastrointestinal tract
GLIDE: Grid-based ligand docking with energetics
GOLD: Genetic optimization of ligand docking
Gl.: Glacial
Glide: Grid-based ligand docking with energetic

GSH: Glutathione
GST: Glutathione S-transferase
HCC: Hepatocellular carcinoma
HER2: Human epidermal growth factor receptor 2
HIF: Hipoxia Inducible Factor
HIV: Human immunodeficiency virus
Hr: Hour
HUVEC: Human umbilical vein endothelial cell
IC50: 50% Maximal ihibitory concentration.
i-PrOH: isopropanol
KDR: Kinase insert domain
LC_{50}: Lethal Dose
MAPk: Mitogen-activated protein kinase
MC: Monte Carlo
MD: Molecular dynamics
MEK: Mitogen/extracellular signal-regulated kinase
MHz: Mega hertz
MS: Mass spectroscopy
NCI: National Cancer Institute.
nM: nanomole
NMR: Nuclear magnetic resonance
NRTK: Non- receptor tyrosine kinase
NSCLc: Non-small-cell lung carcinoma
Pd: Palladium
PDB: Protein data bank
PDGF: Platelet-derived growth factor
PDGFR: Platelet-derived growth factor receptor
Pet ether: Petroleum ether
PK: Protein Kinase
ppm: Part per million
PSA: Polar surface area
p-TsOH: Para toluene sulfonic acid
PIGF: Placental growth factor
RAF: Rapidly Accelerated Fibrosarcoma
RMSD: Root mean square deviation
rt: Room temperature
RNA: ribo nucleic acid
RTK: Receptor tyrosine kinase
SAR: Structure Activity Relationship
SERMs: Selective estrogen receptor modulators

Smac: Second mitochondrial activator of caspases
SMI: Small molecule inhibitors
SRC: Sarcoma
TEA:Triethyl amine
THF: Tetrahydrofuran
TK: Tyrosine kinase
TLC: Thin layer chromatography
USA: United States of America
VDA: Vascular disrupting agents
VEGF: Vascular endothelial growth factor
VEGFR: Vascular endothelial growth factor receptor
WHO: World Health Organization
µM: Micromole

Abstract:

Cancer is one of the major health problems as it is one of the most common causes of death worldwide. Development of targeted anticancer therapy has recently received more attention in order to inhibit some overexpressed molecular targets (enzymes and receptors) that are related to the abnormal nature of cancerous cell. Antiangiogenic therapy was introduced as promising anticancer treatment making use of the continuous need of tumor cell to nutrients and oxygen received through activating certain signalling pathways to provide high micro vessel density. Vascular endothelial growth factor (VEGFa) and its receptor VEGFR-2 are identified as key regulators of angiogenesis. Therefore, inhibition of VEGFR-2 tyrosine kinase (also known as kinase insert domain KDR) through design of small molecule inhibitors was the aim of this study.

A novel series of indazole-based compounds was designed, synthesized and biologically evaluated for their antiangiogenic and anticancer activity. The design process was based on comprehensive SAR study of various potent VEGFR-2 kinase inhibitors and supported by a field alignment study using Cresset BMD FieldAlign application.

The thesis describes the process of design, synthesis and biological evaluation of this new series of compounds covering the following topics:

1. Introduction

A brief account on cancer was given, describing the development of the disease, its main hallmarks and different ways of treatment. Also, an overview on tumor angiogenesis as a target for anticancer therapy was given, highlighting the role of VEGFR-2 as a therapeutic target. Additionally, an account on the medicinal chemistry of indazole as a scaffold was included.

2. Rationale and Design

A comprehensive SAR study was performed in order to determine the essential features required for the design of potent VEGFR-2 inhibitors. The process of design was described based on bioisosteric replacement and scaffold hopping approaches. The results of field alignment study was included; supporting the design rationale.

3. Chemistry

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

1. *N*-(2-Chloropyrimidin-4-yl)-1*H*-indazol-5-amine**(IV)**
2. *N*-(1*H*-Indazol-5-yl)-6,7-dimethoxyquinazolin-4-amine **(VIIIb)**
3. 1-(6,7-Dimethoxyquinazolin-4-yl)-1*H*-indazol-5-amine **(XII)**

Also, it comprises the following new intermediates:

1. 6,7-Dimethoxy-4-(5-nitro-1*H*-indazol-1-yl)quinazoline **(XI)**
2. 1-(2-Chloropyrimidin-4-yl)-5-nitro-1*H*-indazole **(XIV)**
3. *N*-(4-Methoxyphenyl)-4-(5-nitro-1*H*-indazol-1-yl)pyrimidin-2-amine **(XVa)**
4. *N*-(4-Chlorophenyl)-4-(5-nitro-1*H*-indazol-1-yl)pyrimidin-2-amine **(XVb)**
5. 4-(5-Nitro-1H-indazol-1-yl)-*N*-(3,4,5-trimethoxyphenyl)pyrimidin-2-amine **(XVc)**

Moreover, these new target compounds were synthesized:

1. N^4-(1*H*-Indazol-5-yl)-N^2-(p-tolyl)pyrimidine-2,4-diamine **(Va)**
2. N^2-(4-Fluorophenyl)-N^4-(1*H*-indazol-5-yl)pyrimidine-2,4-diamine **(Vb)**
3. N^2-(4-Chlorophenyl)-N^4-(1H-indazol-5-yl)pyrimidine-2,4-diamine **(Vc)**
4. N^2-(3,4-Dichlorophenyl)-N^4-(1*H*-indazol-5-yl)pyrimidine-2,4-diamine **(Vd)**
5. N^4-(1*H*-Indazol-5-yl)-N^2-(3-methoxyphenyl)pyrimidine-2,4-diamine **(Ve)**
6. N^4-(1*H*-Indazol-5-yl)-N^2-(4-methoxyphenyl)pyrimidine-2,4-diamine **(Vf)**
7. *N*-(4-((4-((1*H*-Indazol-5-yl)amino)pyrimidin-2-yl)amino)phenyl)acetamide **(Vg)**
8. *N*-(5-((4-((1*H*-Indazol-5-yl)amino)pyrimidin-2-yl)amino)-2-ethylphenyl)acetamide **(Vh)**
9. 4-((4-((1*H*-Indazol-5-yl)amino)pyrimidin-2-yl)amino)benzenesulfonamide **(Vi)**
10. N^4-(1*H*-Indazol-5-yl)-N^2-(2-methyl-5-nitrophenyl)pyrimidine-2,4-diamine **(Vj)**
11. 5-((2-Chloropyrimidin-4-yl)amino)-*N*-phenyl-1*H*-indazole-1-carboxamide **(VI)**
12. *N*-(3-Chloro-4-methylphenyl)-5-(quinazolin-4-ylamino)-1*H*-indazole-1-carboxamide **(IXa)**
13. *N*-(3,4-Dichlorophenyl)-5-(quinazolin-4-ylamino)-1*H*-indazole-1-carboxamide **(IXb)**
14. *N*-(3-Bromophenyl)-5-(quinazolin-4-ylamino)-1*H*-indazole-1-carboxamide **(IXc)**
15. *N*-(5-Chloro-2,4-dimethoxyphenyl)-5-(quinazolin-4-ylamino)-1*H*-indazole-1-carboxamide **(IXd)**

16. *N*-(3-Chloro-4-methylphenyl)-5-((6,7-dimethoxyquinazolin-4-yl)amino)-1*H*-indazole-1-carboxamide **(Xa)**

17. *N*-(3,4-Dichlorophenyl)-5-((6,7-dimethoxyquinazolin-4-yl)amino)-1*H*-indazole-1-carboxamide **(Xb)**

18. *N*-(3-Bromophenyl)-5-((6,7-dimethoxyquinazolin-4-yl)amino)-1*H*-indazole-1-carboxamide **(Xc)**

19. *N*-(5-chloro-2,4-dimethoxyphenyl)-5-((6,7-dimethoxyquinazolin-4-yl)amino)-1*H*-indazole-1-carboxamide **(Xd)**

20. 5-((6,7-Dimethoxyquinazolin-4-yl)amino)-N-phenyl-1*H*-indazole-1-carboxamide **(Xe)**

21. 5-((6,7-Dimethoxyquinazolin-4-yl)amino)-N-phenyl-1*H*-indazole-1-carbothioamide **(Xf)**

22. 1-(1-(6,7-Dimethoxyquinazolin-4-yl)-1*H*-indazol-5-yl)-3-phenylurea **(XIIIa)**

23. 1-(3-Chloro-4-methylphenyl)-3-(1-(6,7-dimethoxyquinazolin-4-yl)-1*H*-indazol-5-yl)urea **(XIIIb)**

24. 1-(3,4-Dichlorophenyl)-3-(1-(6,7-dimethoxyquinazolin-4-yl)-1*H*-indazol-5-yl)urea **(XIIIc)**

25. 1-(3-Bromophenyl)-3-(1-(6,7-dimethoxyquinazolin-4-yl)-1*H*-indazol-5-yl)urea **(XIIId)**

26. 1-(5-Chloro-2,4-dimethoxyphenyl)-3-(1-(6,7-dimethoxyquinazolin-4-yl)-1*H*-indazol-5-yl)urea **(XIIIe)**

27. 1-(5-Chloro-2,4-dimethoxyphenyl)-3-(1-(2-((4-methoxyphenyl)amino)pyrimidin-4-yl)-1*H*-indazol-5-yl)urea **(XVIIa)**

28. 1-(5-Chloro-2,4-dimethoxyphenyl)-3-(1-(2-((4-chlorophenyl)amino)pyrimidin-4-yl)-1*H*-indazol-5-yl)urea **(XVIIb)**

29. 1-(5-Chloro-2,4-dimethoxyphenyl)-3-(1-(2-((3,4,5-trimethoxyphenyl)amino) pyrimidin-4-yl)-1*H*-indazol-5-yl)urea **(XVIIc)**

4. Biological evaluation

The biological activity of the compounds was evaluated at both molecular and cellular levels. The target compounds were biologically evaluated for their activity against VEGFR-2 kinase. Most of the target compounds exhibited excellent inhibitory activity against the enzyme. Compounds **(XIIIb)**, **(XIIIc)** and **(XIIIe)** displayed significant potency against VEGFR-2 kinase; where they showed IC_{50} of 1.4, 1.3 and 8.1 nM respectively. Compound **(Vi)** (IC_{50}=24.5 nM) was further evaluated for its cellular antiangiogenic activity against HUVEC cell line showing IC_{50} of 1.37µM.