Molecular Design and Synthesis of Small Organic Molecules as Anticancer Targeting Agents

Thesis

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3D-QSAR pharmacophore modelling, virtual screening and docking studies for lead discovery of a novel scaffold for VEGFR 2 inhibitors: Design, synthesis and biological evaluation

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ABSTRACT

A series of novel 6,7-dihydro-5H-cyclopenta[*d*]pyrimidine derivatives was successfully designed, synthesized and evaluated as a new chemical scaffold with vascular endothelial growth factor receptor (VEGFR 2) inhibitory activity. Compounds **6c** and **6b** showed enzyme inhibition of 97% and 87% at 10 μM, respectively, and exhibited potent dose-related VEGFR 2 inhibition with IC_{50} values of 0.85 μM and 2.26 μM, respectively. The design of the 6,7-dihydro-5H-cyclopenta[*d*]pyrimidine scaffold was implemented via consecutive molecular modelling protocols prior to the synthesis and biological evaluation of the derivatives. First, sorafenib was docked in the binding site of VEGFR 2 to study its binding orientation and affinity, followed by the generation of a valid 3D QSAR pharmacophore model for use in the virtual screening of different 3D databases. Structures with promising pharmacophore-based virtual screening results were refined using molecular docking studies in the binding site of VEGFR 2. A novel scaffold was designed by incorporating the results of the pharmacophore model generation and molecular docking studies. The new scaffold showed hydrophobic interactions with the kinase front pocket that may be attributed to increasing residence time in VEGFR 2, which is a key success factor for ligand optimization in drug discovery. Different derivatives of the novel scaffold were validated using docking studies and pharmacophore mapping, where they exhibited promising results as VEGFR 2 inhibitors to be synthesized and biologically evaluated. 6,7-dihydro-5H-cyclopenta[*d*]pyrimidine is a new scaffold that can be further optimized for the synthesis of promising VEGFR 2 inhibitors.

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ABL1: Abelson murine leukemia viral oncogene homolog 1
Ala: Alanine
Asp: Aspartate
ATP: adenosine 5'-triphosphate
Asp: Aspartate
BCR-ABL: fusion between Abelson tyrosine kinase gene and break point cluster gene
BTK: Bruton's tyrosine kinase
CADD: Computer-aided drug design
c-FMS: Colony-stimulating factor-1 receptor
CHARMm: Chemistry at Harvard Macromolecular Mechanics
c-Kit: v-kit (Hardy-Zuckerman 4 feline) sarcoma viral oncogene
c-SRC: Cellular sarcoma (Schmidt-Ruppin A-2) viral oncogene
Cys: Cysteine
DCM: Dichloromethane
DFG: Aspartate- Phenylalanine- glycine
DIPEA: N, N-Diisopropylethylamine
DMF: Dimethyl Formamide
DMSO: Dimethyl sulphoxide
DNA: Deoxyribonucleic acid
EGFR: Epidermal growth factor receptor
ESI-MS: Electrospray ionization mass spectroscopy
FAK: Focal adhesion kinase
FDA: Food and Drug Administration
FGFR: fibroblast growth factor receptor
FLT-3: fms like tyrosine kinase 3
FT-IR: Fourier transform -Infrared
Glu: Glutamate
Gly: Glycine
GTP: Guanosine 5'-Triphosphate
HBA: Hydrogen bond acceptor
HBD: Hydrogen bond donor
His: Histidine
Hrs: Hours
HUVEC: Human umbilical vein endothelial cells
HYP: Hydrophobic
Hz: Hertz
IC_{50}: Half maximal inhibitory concentration
Ile: Isoleucine
Leu: Leucine
Lys: Lysine
MAPK: Mitogen Activated Protein Kinase
MEK: mitogen-activated protein kinase
m.p: Melting point
MHz: Mega hertz

MS: Mass spectroscopy
NMR: Nuclear magnetic resonance
NRTK: Non-receptor tyrosine kinase
PDB: Protein data bank
Pd-C: Palladium on cardon
PDGFR: Platelet derived growth factor receptor
Phe: Phenyl alanine
PIGF: Placental growth factor
ppm: Part per million
3D QSAR: Three Dimensional Quantitative Structure-Activity Relationship
RA: Ring aromatic
Raf: v-raf murine sarcoma viral oncogene
Ras: Rat sarcoma
RET: Rearranged during transfection proto-oncogene
RMS: Root mean square
RMSD: Root mean square deviation
RNA: Ribonucleic acid
rt: Room temperature
RTK: Receptor tyrosine kinase
SAR: Structure activity relationship
TEA: Triethyl amine
THF: Tetrahydrofuran
Tie-2: Tyrosine kinase with immunoglobulin-like and EGF-like domains 2
TLC: Thin layer chromatography
Tp53: Tumor protein 53
UV: Ultra violet
Val: Valine
VEGF: Vascular endothelial growth factor
VEGFR: Vascular endothelial growth factor receptor

Abstract:

Title of thesis:

"Molecular Design and Synthesis of Small Organic Molecules as Anticancer Targeting Agents"

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Cancer is a collection of complex diseases. It begins when some of normal body cells start to divide and grow out of control, forming a mass or sheet of cells called tumor. It is a major cause of death throughout the world. According to the American Cancer Society, the number of deaths caused by cancer is second only to cardiovascular diseases. Although great strides have been made in the treatment of cancer over the past years, it continues to be a major health concern. Therefore, extensive efforts have been devoted to searching for new therapeutic approaches. 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 (VEGFR2) plays an essential role in cancer angiogenesis. Where, targeting VEGFR2 will inhibit angiogenesis causing tumor cell death.

In this study, a novel series of 6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidine derivatives was successfully designed and synthesized as a new chemical scaffold with vascular endothelial growth factor receptor (VEGFR2) inhibitory activity. The design of the novel scaffold was implemented via consecutive molecular modelling protocols; molecular docking, 3D QSAR pharmacophore model generation protocol and virtual screening, and was also focused on the exploration of the previously revealed SAR studies and bioisosteric modifications of lead compounds.

Designed compounds were then synthesized and their structures were confirmed through different spectral and microanalytical data.

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

1. 1-(4-Nitrophenyl)-3-phenylurea **(Ia)**
2. 1-(3-Choloro-4-methylphenyl)-3-(4-nitrophenyl)urea **(Ib)**
3. 1-(3-Trifluoromethyl-4-chlorophenyl)-3-(4-nitrophenyl)urea **(Ic)**

4. 1-(3-Methoxyphenyl)-3-(4-nitrophenyl)urea **(Id)**
5. 1-(4-Nitrophenyl)-3-(3-(trifluoromethyl)phenyl)urea **(Ie)**
6. 1-(4-Aminophenyl)-3-phenylurea **(IIa)**
7. 1-(4-Aminophenyl)-3-(3-choloro-4-methylphenyl)urea **(IIb)**
8. 1-(4-Aminophenyl)-3-(3-trifluoromethyl-4-chlorophenyl)urea **(IIc)**
9. 1-(4-Aminophenyl)-3-(3-methoxyphenyl)urea **(IId)**
10. 1-(4-Aminophenyl)-3-(3-(trifluoromethyl)phenyl)urea **(IIe)**
11. 2-Aminocyclopent-1-ene carbonitrile **(III)**
12. 4-Chloro-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidine **(V)**
13. 6,7-Dihydro-3*H*-cyclopenta[*d*]pyrimidin-4(5*H*)-one **(IV)**

Furthermore, it has comprised the synthesis and characterization of the following new targeted compounds:

1. 1-(4-(6,7-Dihydro-5*H*-cyclopenta[*d*]pyrimidin-4-ylamino)phenyl)-3-phenylurea **(VIa)**
2. 1-(3-Chloro-4-methylphenyl)-3-(4-(6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidin-4-ylamino)phenyl)urea **(VIb)**
3. 1-(4-Chloro-3-(trifluoromethyl)phenyl)-3-(4-(6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidin-4-ylamino)phenyl)urea **(VIc)**
4. 1-(4-(6,7-Dihydro-5*H*-cyclopenta[*d*]pyrimidin-4-ylamino)phenyl)-3-(3-methoxyphenyl)urea **(VId)**
5. 1-(4-(6,7-Dihydro-5*H*-cyclopenta[*d*]pyrimidin-4-ylamino)phenyl)-3-(3-(trifluoromethyl)phenyl)urea **(VIe)**

The biological evaluation was accomplished through testing of enzyme inhibition activity against VEGFR2 tyrosine kinase. The enzymatic assay was