Small Molecule Inhibitors For Type III Receptor Tyrosine Kinases

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Degree of Doctor of Philosophy

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Declaration

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Abstract

Colony stimulating Factor-1 Receptor (CSF-1R, FMS) and FMS-like Tyrosine Kinase-3 (FLT3) are members of the type III receptor tyrosine kinase (RTK) family. They have been implicated in a wide range of physiological and pathological processes including cancer and inflammatory diseases. Therefore blockade of their kinase activity using small molecule inhibitors (SMIs) may be a helpful treatment strategy for diseases associated with aberrant expression of FMS and FLT3. In this study, a cellular system for evaluation of SMIs was established by separate expression of human FMS and FLT3 in murine factor dependent FDC-P1 early myeloid cells. cDNAs encoding wild-type (WT) human FMS and FLT3 as well as leukaemia-associated constitutively active mutant forms of FLT3 (internal tandem duplication (ITD), D835V and D835Y) in the expression vector MSCV-IRES-GFP were introduced into FDC-P1 cells by retroviral transduction. Transduced cells were selected by Fluorescence-activated cell sorting (FACS) for green fluorescent protein GFP and growth in CSF-1 (also known as M-CSF), FLT3 ligand (FLT3L) or, in the case of FLT3 mutants, in the absence of growth factor. The coding regions for the CSF-1 and FLT3L were cloned from RNA extracted from K562, human erythroleukaemia cells and recombinant growth factors were produced in the yeast, Pichia pastoris.

Several known SMIs of one or more Type III RTKs were evaluated for inhibition of FMS and FLT3 driven cell proliferation. Imatinib, dasatinib and sunitinib are potent inhibitors of c-KIT, while PKC412 and CEP701 are FLT3 inhibitors. The potency and selectivity of these SMIs were evaluated by inhibition of cell growth in presence of either mouse granulocyte macrophage colony-stimulating factor GM-CSF (control) or specific human growth factors (CSF-1 and FLT3L) and confirmed by inhibition of FMS and FLT3 phosphorylation upon stimulation by their cognate ligands. Each of these SMIs inhibited FMS kinase activity while FLT3 kinase (both WT and mutants) was inhibited by CEP701, PKC412 and to some degree by sunitinib, but not imatinib or dasatinib. The binding modes of the SMIs were predicted by molecular docking into homology models based on crystal structures of related kinases. Because kinase domains adopt different conformations in the inactive, active and inhibited states, multiple models of each kinase were evaluated. The binding mode data were correlated with selectivity and potency of the SMIs.

Each of the small molecule inhibitors studied in this project represent a unique mode of activity against kinases, but in general they can be classified into three main categories. Firstly, molecules interacting mainly with the catalytic area (such as imatinib) taking advantage of the relatively unique substrate recognition site to be relatively selective, but affected adversely by the conformational switch during activation of the kinase domain. Secondly, molecules which interact exclusively with the ATP binding area (such as PKC412 and CEP701) can be effective on both active and inactive forms of kinases by taking advantage of binding to the area with least conformational changes during activation. However, it comes at the cost of less selectivity as this area is widely conserved among different types of kinases. Dasatinib, on the other hand, seems to have benefited from a kind of balanced interaction with both of these areas enabling it to be potent as well as relatively selective for the kinases with a threonine as gate-keeper residue. These examples show that extension of the purine-like core structure is required for high potency; otherwise the inhibitor (a molecule such as sunitinib) will not be able to compete with high concentration of ATP for binding to the active conformation of kinase. Extensions toward the ribose and phosphate groups (in molecules such as PKC412 and CEP701) result in increased potency, but decreased selectivity. To achieve higher potency and relative selectivity at the same time, the core structure should be extended toward the catalytic area (i.e. dasatinib). However, it should be limited to the vicinity of gate-keeper residue; otherwise the molecule will be vulnerable to the conformational changes during activation as explained for imatinib.

The implications for design of SMIs of tyrosine kinases are discussed. Since the catalytic region is less stringently conserved and more influenced by conformational changes on activation, there is a high possibility of point mutations giving rise to resistance against SMIs targeting this region. If highly selective inhibitors are required, targeting of the catalytic area will be the choice, but if the aim is preventing or overcoming drug resistance in cancers due to mutations in the catalytic area (e.g. T670I in KIT) or strongly favouring the active conformation of the kinase domain (e.g. D816V in KIT or D835V/Y in FLT3), then the hinge region should be considered as the target area. It also will be possible to balance the selectivity and the potency by designing molecules that bridge the catalytic area and the hinge region. These findings will help in the design of new SMIs against the kinases according to each specific problem.

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List of abbreviations

Abbreviation	Description	Synonyms
AB	Acidic Box	
ADP	Adenosine Di-Phosphate	
A-loop	Activation loop	
AML	Acute myelogenous leukemia	
Arg	Arginine	R
Asn	Asparagine	N
Asp	Aspartic acid	D
ATP	Adenosine Tri-Phosphate	
Axl	a Tyro3 PTK	
CadhD	Cadherin-Like Domain	
CML	Chronic myelogenous leukemia	
CRD	Cysteine-Rich Domain	
CSF-1	Colony Stimulating Factor-1	M-CSF, Macrophage – Colony Stimulating Factor
Cys	Cysteine	C
DAG	Diacylglycerol	
DC	dendritic cell	
DDR	Discoidin Domain Receptor	
DiscD	Discoidin-like Domain	
EGFD	Epidermal Growth Factor-like Domain	
EGFR	Epidermal Growth Factor Receptor	HER, Erb
EphR	Ephrin Receptor	
FGFR	Fibroblast Growth Factor Receptor	
FLT3	FMS-like (related) Tyrosine Kinase 3	FLK2, CD135, Stem cell tyrosine kinase 1 (STK1)
FLT3L	FLT3 ligand	SL Cytokine
FMS	Human Homolog of McDonough Feline Sarcoma Viral (v-FMS) Oncogene	CSF-1 Receptor, CD115
FNIII	Fibronectin Type III-Like Domain	
GIST	gastrointestinal stromal tumours	
Glu	Glutamic acid	E
Gly	Glycine	G
H-bond	Hydrogen-bond	
HGFR	Hepatocyte Growth Factor Receptor	
HTS	High-Throughput Screening	
IgD	Immunoglobulin-like Domain	
Ile	Isoleucine	I

InsR	Insulin Receptor	
ITD	Internal Tandem Duplication	
JM	Juxtamembrane	
KIT	Stem Cell Factor Receptor	Mast/Stem Cell Growth Factor Receptor, CD117
KLG/CCK	Colon Carcinoma Kinase	
KrinD	Kringle-like Domain	
LMR	Lemur	
LRD	Leucine-Rich Domain.	
LTK	Leukocyte Tyrosine Kinase	
Lys	Lysine	K
Met	Methionine	M
MMP	metalloproteinases	
MuSK	Muscle-Specific Kinase	
NF-1	neurofibromin	
NGFR	Nerve Growth Factor Receptor	
P. pastoris	*Pichia pastoris*	
PDB	Protein Data Bank	
PDGFR	Platelet-Derived Growth Factor Receptor	
Phe	Phenylalanine	F
PI3K	phosphoinositide-3-kinase	
PLCγ	phospholipase Cγ	
PLD	phospholipase D	
P-loop	Nucleotide binding loop	
PtdIns(4,5)P 2	phosphatidylinositol-4,5-bisphosphate	
RMSD	root mean square deviation	
ROR	Receptor Orphan	
RTK	Receptor Tyrosine Kinase	
SCF	Stem Cell Factor Receptor	
SHIP	SH2-containing inositol 5-phosphatase	
SHP2	Src-homology phosphatase type-2	
SMI	Small Molecule Inhibitor	
SOCS-1	Suppressor of cytokine signalling 1	
STAT	Signal Transducer and Activator of Transcription	
TAM	tumor-associated macrophages	
Thr	Threonine	T
TIE	Tyrosine Kinase With Immunoglobulin-like and EGF-like domains	Angiopoietin Receptors

TM	Transmembrane Domain	
Tyr	Tyrosine	Y
Val	Valine	V
VEGFR	Vascular Endothelial Growth Factor Receptor	FLT1
VHTS	Virtual High-Throughput Screening	
VS	Virtual Screening	

List of publications

1. The results of Chapter 5 were already published in this paper:
   Mashkani, B., Griffith, R. & Ashman, L.K. (2010) Colony stimulating factor-1 receptor as a target for small molecule inhibitors. Bioorg Med Chem, 18, 1789-1797.

2. The results of Chapter 6 is under preparation as a paper entitled: *Structure-activity relationship of small molecule inhibitors for FMS-Like Tyrosine Kinase 3 (FLT3)*

Conferences Abstracts

Baratali Mashkani, Renate Griffith, Leonie Ashman Small Molecule Inhibitors of Colony Stimulating Factor-1 Receptor 7th AFMC International Medicinal Chemistry Congress - August 23-27, 2009 in Cairns, QLD, Australia