Alkynes in covalent enzyme inhibitors: down the kinetic rabbit hole

Mons, E.

Alkynes in Covalent Enzyme Inhibitors: Down the Kinetic Rabbit Hole

Elma Mons

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Promotoren
prof. dr. H. Ovaa
prof. dr. J.J.C. Neefjes

Co-promotoren
dr. M.P.C. Mulder
dr. R.Q. Kim

Promotiecommissie
prof. dr. A.C.O. Vertegaal
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Wandering another path
often leads us where we need to go

Once Upon a Time [TV Series] – Season 7, Episode 15

IC50
Ub
Kinact

Table of Contents

Abbreviations, Acronyms, and Symbols

Ab	Antibody
ABP	Activity-Based Probe
ABPP	Activity-Based Protein Profiling
Ac-	Acetyl
ADME	Absorption, Distribution, Metabolism, and Excretion
AMC	7-amino-4-methylcoumarin
APT	Attached Proton Test (NMR)
aq	Aqueous
ATP	Adenosine Triphosphate
AU/A.U.	Arbitrary Units
BME	β-mercaptoethanol
Boc-	*tert*-butyloxycarbonyl
BODIPY	Fluorinated Boron-Dipyrromethene
BSA	Bovine Serum Albumin
calc.	Calculated
CatK	Cathepsin K
CD	Catalytic Domain
ChEF	Chelation-Enhanced Fluorescence
conc.	Concentration
cryo-EM	Cryogenic Electron Microscopy
CSox	Cysteine Modified with a Sox fluorophore
CuAAC	Copper-Catalyzed Alkyne–Azide Cycloaddition
Cy5	Cyanine-5 *(fluorophore)*
CYP	Cytochrome P450
CysDUB	Cysteine Deubiquitinating Enzyme
Da	Dalton *(mass unit)*
DCE	1,2-dichloroethane *(solvent)*
DCM	Dichloromethane *(solvent)*
DIAD	Diisopropyl azodicarboxylate
DIC	*N,N'*-diisopropylcarbodiimide
DIPEA	*N,N*-diisopropylethylamine
diUb	Diubiquitin
DMAc	*N,N*-dimethylacetamide *(solvent)*
DME	1,2-dimethoxyethane *(solvent)*
DMF	Dimethylformamide *(solvent)*
DMSO	Dimethyl sulfoxide *(solvent)*
DODt	3,6-dioxa-1,8-octanedithiol
DRC	Dose-Response Curve
DTT	1,4-dithiothreitol
DUB	Deubiquitinating Enzyme
E1	Ubiquitin Activating Enzyme
E2	Ubiquitin Conjugating Enzyme
E3	Ubiquitin Ligase
ECD	Extracellular Domain
EDC	*N*-ethyl-*N'*-(3-dimethylamino-propyl) carbodiimide
EDG	Electron-Donating Group
EDTA	Ethylenediaminetetraacetic acid
EGF	Epidermal Growth Factor
EGFR	Epidermal Growth Factor Receptor
eq	Equivalents
ESI	Electrospray Ionization
Et-	Ethyl
EWG	Electron-Withdrawing Group
FA	Formic acid
FCC	Flash Column Chromatography
FCS	Fetal Calf Serum
FDA	Food and Drug Administration USA
FI	Fluorescence Intensity
FL	Full-Length
Fmoc-	9-fluorenylmethyloxycarbonyl
FP	Fluorescence Polarization
FRET	Fluorescence Resonance Energy Transfer
GSH	Glutathione
GST-	Glutathione *S*-Transferase *(protein tag)*
HA-	Human Influenza Hemagglutinin *(protein tag)*
HATU	Hexafluorophosphate azabenzotriazole tetramethyl uronium
HCV	Hepatitis C Virus
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid *(buffer)*
HFIP	1,1,1,3,3,3-hexafluoroisopropylalcohol
His-	Polyhistidine *(protein tag)*
HRP	Horseradish Peroxidase
HRMS	High-Resolution Mass Spectrometry
HTS	High-Throughput Screening
IAc	Iodoacetamide
IEDDA	Inverse Electron-Demand Diels-Alder Reaction
iPr-	*iso*-propyl
IRREV	Irreversible
kPCA	Kinetic Probe Competition Assay
LC	Liquid Chromatography
M	mol/L *(concentration unit)*
M-CSF	Macrophage Colony Stimulating Factor
Me-	Methyl
MES	2-(*N*-morpholino)ethanesulfonic acid *(buffer)*
MMTS	*S*-methyl methanethiosulfonate
MOPS	3-(*N*-morpholino)propanesulfonic acid *(buffer)*
M^{pro}	Viral Main Protease
MS	Mass Spectrometry
Ms-	Mesyl/methanesulfonyl
NBS	Non-Binding Surface
NBS	*N*-bromosuccinimide
NEM	*N*-ethylmaleimide

NMM	*N*-methylmorpholine
NMP	*N*-methyl-2-pyrrolidone *(solvent)*
NMR	Nuclear Magnetic Resonance
NSCLC	Non-Small Cell Lung Carcinoma
OC	Osteoclast
ODN	Odanacatib
PBMC	Peripheral Blood Mononuclear Cell
PBS	Phosphate-Buffered Saline *(buffer)*
PD	Pharmacodynamics
PDB	Protein Data Bank
pEGFR	Phosphorylated EGFR
Phth-	Phthalimide
PK	Pharmacokinetics
ppm	Parts Per Million *(NMR unit)*
prepHPLC	Preparative HPLC
Prg	Propargyl *(warhead)*
PROTAC	Proteolysis-Targeting Chimera
Prp	Propyl *(warhead)*
PTM	Post-Translational Modification
pY	Phosphorylated Tyrosine
PyBOP	Benzotriazol-1-yloxytripyrrolidino-phosphonium hexafluorophosphate
qABP	Quenched Fluorescent ABP
quant	Quantitative yield
RANKL	Receptor Activator of Nuclear Factor κB Ligand
REV	Reversible
Rf	Retention Factor *(TLC)*
RFU	Relative Fluorescence Unit
Rho-	5-carboxy-Rhodamine110 *(fluorophore)*
RP-HPLC	Reversed-Phase High-Performance Liquid Chromatography
Rt	Retention Time *(HPLC, LC-MS)*
rt	Room Temperature
RTK	Receptor Tyrosine Kinase
SAR	Structure-Activity Relationship
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SD	Standard Deviation
SDS-PAGE	Sodium Dodecyl Sulfate Poly-Acrylamide Gel Electrophoresis
SILAC	Stable-Isotope Labeling by Amino Acids in Cell Culture
Sox	Sulfonamido-oxine *(fluorophore)*
SUMO	Small Ubiquitin-like Modifier
tBu-	*tert*-butyl
TCEP	Tris(2-carboxyethyl)phosphine
TCI	Targeted Covalent Inhibitor
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran *(solvent)*
TIC	Total Ion Count
TKD	Tyrosine Kinase Domain
TKI	Targeted Kinase Inhibitor
TLC	Thin Layer Chromatography
TMEDA	Tetramethylethylenediamine
TAMRA/TMR	Tetramethylrhodamine
TMS-	Trimethylsilyl
TRAcP	Tartrate-Resistant Acid Phosphatase
TR-FRET	Time-Resolved Fluorescence Resonance Energy Transfer
Tris	Tris(hydroxymethyl)-aminomethane *(buffer)*
Tween20	Polyoxyethylene (20) Sorbitan Monolaurate
Ub	Ubiquitin
Ubl	Ubiquitin-Like Modifier
UPLC	Ultra Performance Liquid Chromatography
UV	Ultraviolet
VME	Vinyl Methyl Ester *(warhead)*
VS	Vinyl Sulfone *(warhead)*
WB	Western Blotting
WHO	World Health Organization
WT/^{WT}	Wild-Type

Amino Acids

Ala	A	Alanine
Arg	R	Arginine
Cys	C	Cysteine
Gly	G	Glycine
Lys	K	Lysine
Met	M	Methionine
Nle	–	Norleucine
Phe	F	Phenylalanine
Ser	S	Serine
Tyr	Y	Tyrosine

one- and three-letter abbreviations of amino acids according to IUPAC recommendations.

Symbols

IC_{50}	[Inhibitor] resulting in 50% inhibition
K_D	Equilibrium Dissociation Constant
K_i	Noncovalent Inhibition Constant
K_I	Covalent Inactivation Constant *(2-step IRREV)*
K_i^*	Steady-State Inhibition Constant
K_M	Michaelis Constant *(enzyme)*
k_{cat}	Substrate Turnover Number *(enzyme)*
k_{inact}	Maximum Rate of Inactivation *(2-step IRREV)*
k_{obs}	Observed Reaction Rate
$\lambda_{ex}/\lambda_{em}$	Emission/Excitation wavelength
t½	Half-life

Full list of symbols can be found in ***Chapter 3***