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Alkynes in covalent enzyme inhibitors: down the kinetic rabbit hole

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Alkynes in Covalent Enzyme Inhibitors: Down the Kinetic Rabbit Hole

Elma Mons

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Alkynes in Covalent Enzyme Inhibitors: Down the Kinetic Rabbit Hole

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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



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Abbreviations, Acronyms, and Symbols

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

NMM	N-methylmorpholine
NMP	N-methyl-2-pyrrolidone (<i>solvent</i>)
NMR	Nuclear Magnetic Resonance
NSCLC	Non-Small Cell Lung Carcinoma
OC	Osteoclast
ODN	Odanacatib
PBMC	Peripheral Blood Mononuclear Cell
PBS	Phosphate-Buffered Saline (<i>buffer</i>)
PD	Pharmacodynamics
PDB	Protein Data Bank
pEGFR	Phosphorylated EGFR
Phth-	Phthalimide
PK	Pharmacokinetics
ppm	Parts Per Million (<i>NMR unit</i>)
prepHPLC	Preparative HPLC
Prg	Propargyl (<i>warhead</i>)
PROTAC	Proteolysis-Targeting Chimera
Prp	Propyl (<i>warhead</i>)
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 κ B Ligand
REV	Reversible
Rf	Retention Factor (<i>TLC</i>)
RFU	Relative Fluorescence Unit
Rho-	5-carboxy-Rhodamine110 (<i>fluorophore</i>)
RP-HPLC	Reversed-Phase High-Performance Liquid Chromatography
Rt	Retention Time (<i>HPLC, LC-MS</i>)
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 (<i>fluorophore</i>)
SUMO	Small Ubiquitin-like Modifier
tBu-	<i>tert</i> -butyl
TCEP	Tris(2-carboxyethyl)phosphine
TCI	Targeted Covalent Inhibitor
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran (<i>solvent</i>)
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 (<i>buffer</i>)
Tween20	Polyoxyethylene (20) Sorbitan Monolaurate
Ub	Ubiquitin
Ubl	Ubiquitin-Like Modifier
UPLC	Ultra Performance Liquid Chromatography
UV	Ultraviolet
VME	Vinyl Methyl Ester (<i>warhead</i>)
VS	Vinyl Sulfone (<i>warhead</i>)
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 (<i>2-step IRREV</i>)
K _i ⁺	Steady-State Inhibition Constant
K _M	Michaelis Constant (<i>enzyme</i>)
k _{cat}	Substrate Turnover Number (<i>enzyme</i>)
k _{inact}	Maximum Rate of Inactivation (<i>2-step IRREV</i>)
k _{obs}	Observed Reaction Rate
λ _{ex} /λ _{em}	Emission/Excitation wavelength
t _{1/2}	Half-life

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