Advances in covalent drug discovery

2022/10/12
M2 Zhai

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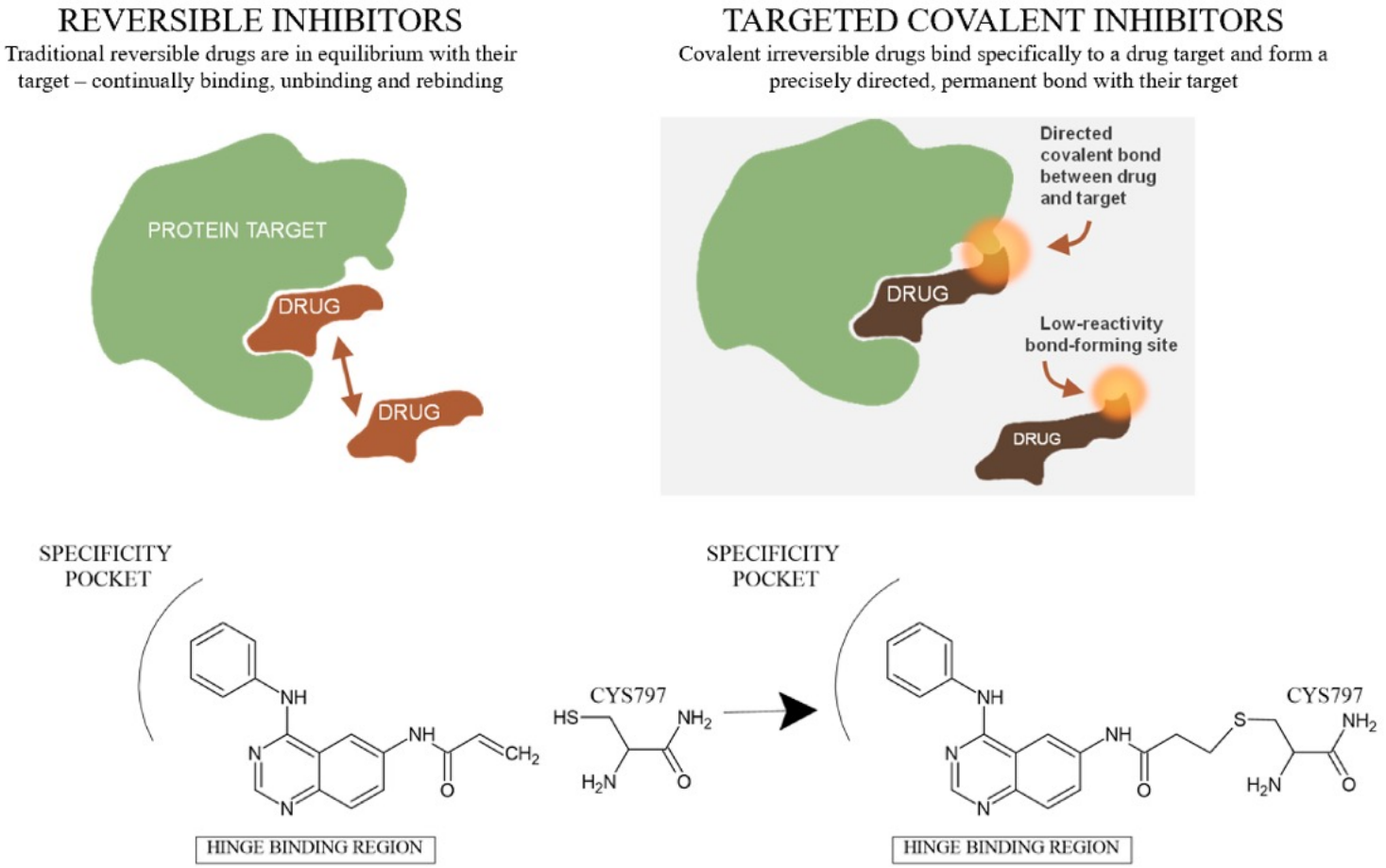
Introduction

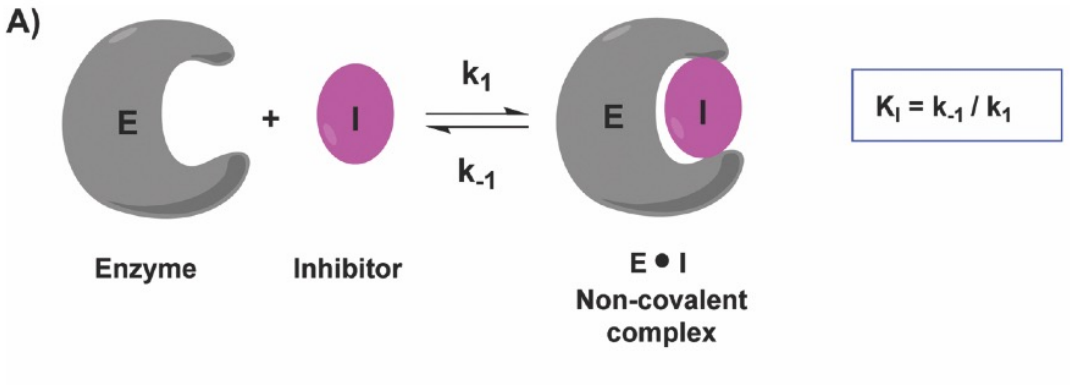
Compounds that by design are intended to form a covalent bond with a specific molecular target.



Juswinder Singh, *Journal of Medicinal Chemistry.* **2022**, 65, 5886

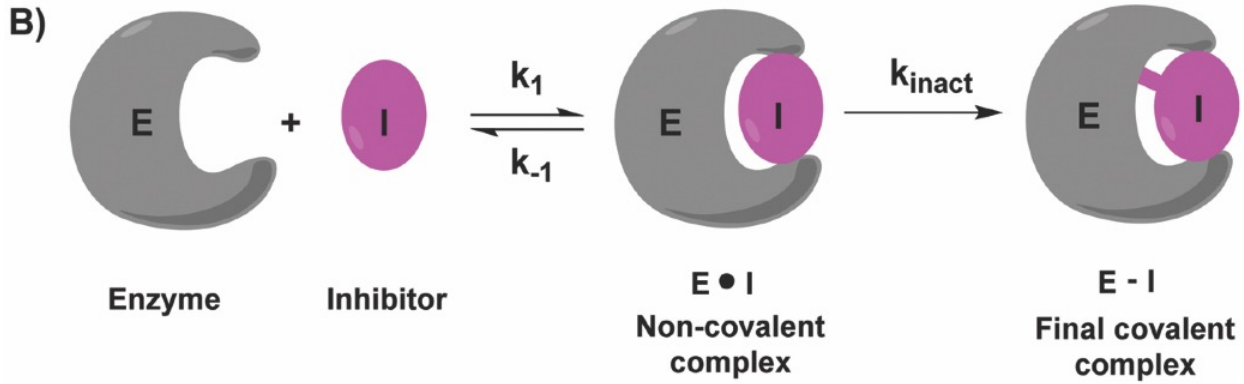
Non-Covalent and Covalent Inhibitors

Non-covalent inhibitor



Non-covalent inhibitors bind to their targets in equilibrium and in a reversible manner.

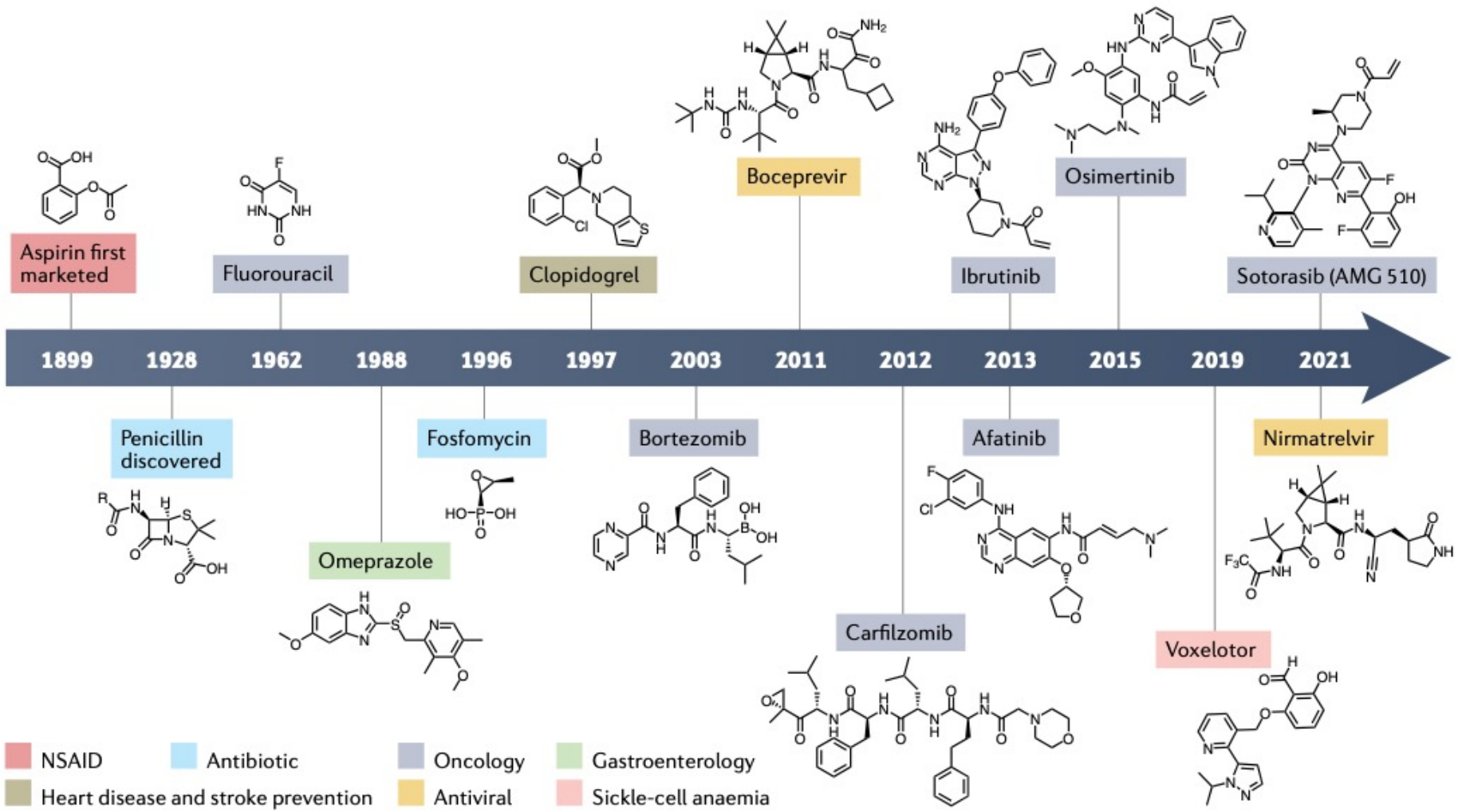
Covalent inhibitor



Covalent inhibitors bind to their targets in a two-step manner – the formation of initial non-covalent complex being reversible and formation of final covalent complex being irreversible.

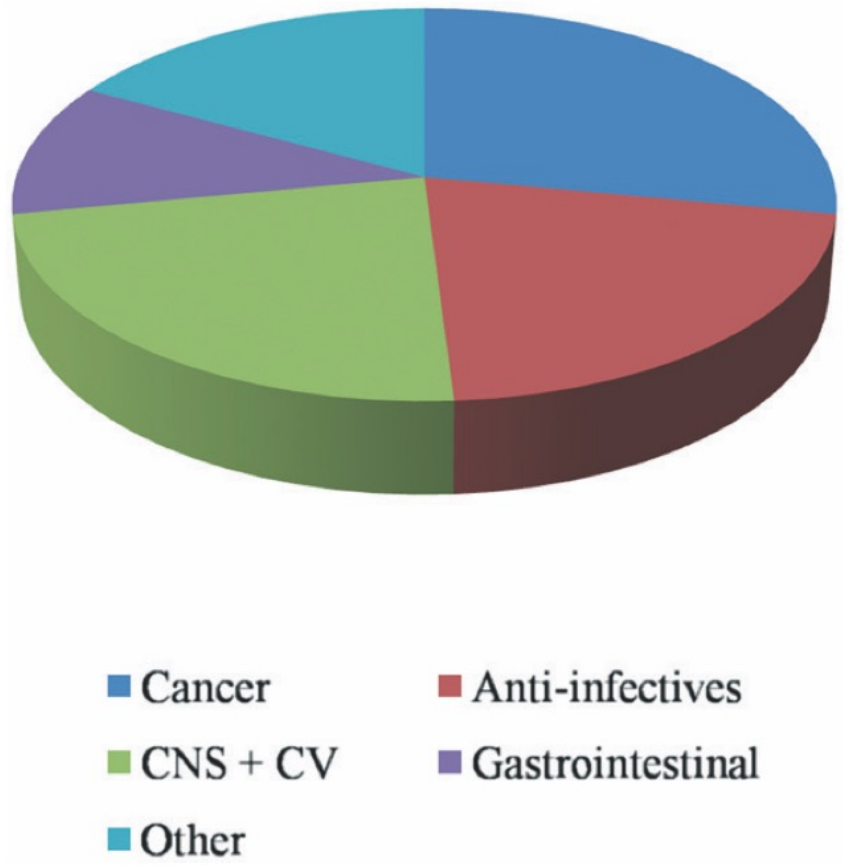
Ruddraraju, K. V.; Zhang, Z. *Mol. BioSyst*. **2017**, 13, 1257.

Timeline of Covalent Inhibitor Drugs



Boike, L.; Henning, J.N. *Nature Reviews*. **2022**, 13, 1257.

Application of Covalent Inhibitor Drugs



Approved covalent drugs by therapeutic indication.

FDA-approved covalent drugs (2011–2019)

No	Name	Therapeutic area	Warhead	Year
1.	Telaprevir	Anti-HCV	α-Ketoamide	2011
2.	Boceprevir	Anti-HCV	α-Ketoamide	2011
3.	Abiraterone	Anticancer	—	2011
4.	Afatinib	Anticancer	α,β-Unsaturated carbonyl	2013
5.	Dimethyl fumarate	Multiple sclerosis	α,β-Unsaturated carbonyl	2013
6.	Ibrutinib	Anticancer	α,β-Unsaturated carbonyl	2014
7.	Osimertinib	Anticancer	α,β-Unsaturated carbonyl	2015
8.	Olmutinib	Anticancer	α,β-Unsaturated carbonyl	2015
9.	Narlaprevir	Anti-HCV	α-Ketoamide	2016
10.	Acalabrutinib	Anticancer	α,β-Unsaturated propargylamide	2017
11.	Neratinib	Anticancer	α,β-Unsaturated carbonyl	2017
12.	Dacomitinib	Anticancer	α,β-Unsaturated carbonyl	2018
13.	Selinexor	Anticancer	α,β-Unsaturated carbonyl	2019
14.	Zanubrutinib	Anticancer	α,β-Unsaturated carbonyl	2019

Sutanto, F.; Konstantinidou, M.; Dömling, A., *RSC Medicinal Chemistry*. **2020**, 11, 876.

The advantages and disadvantages

Advantages

- Improving efficiency.
- Lowering the dose.
- Increasing compliance due to less-frequent dosing.
- Reducing the possibility of drug resistance.
- Targeting shallow binding sites.

Disadvantages

- May cause unexpected toxicity or hypersensitivity.
- May cause drug-induced toxicity.
- May not be suitable for targets that are rapidly turned over/ degraded by enzymes.
- May cause problems in choosing the correct warhead targeting.

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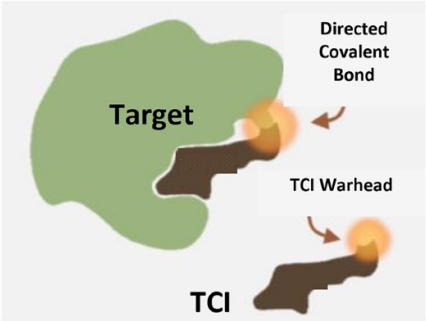
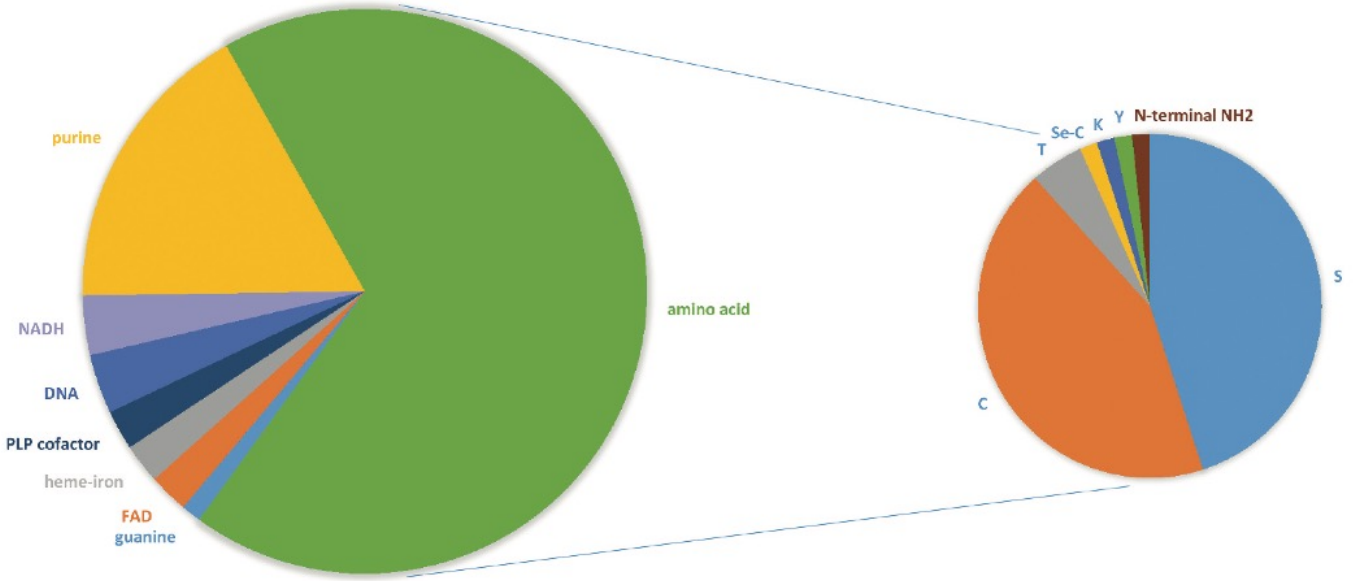
Different Warheads of TCIs



TCI Drug Approvals		
2013 EGFR	afatinib	Lung cancer
2013 BTK	ibrutinib	Lymphoma
2015 EGFR	osimertinib	Lung Cancer
2017 BTK	acalabrutinib	Lymphoma
2017 HER2	neratinib	Breast Cancer
2018 EGFR	dacomitinib	Lung Cancer
2019 BTK	zanubrutinib	Lymphoma
2021 KRAS	sotorasib	Lung Cancer



The distribution of the FDA approved drugs according to their targets and labeled residues.

The distribution of reaction mechanisms from the CovPDB database

Mechanism of action	Number of warheads
nucleophilic aliphatic substitution	18
nucleophilic acyl substitution	17
composite reaction	14
nucleophilic addition to double bond	13
ring opening	13
michael addition	11
nucleophilic aromatic substitution	7
phosphorylation	6
cyclohemiaminoacetalization	4
hemi(thio)acetalization	4
lactone addition	4
nucleophilic addition to triple bond	4
borylation	3
disulfide formation	3
hemiaminalization	2
imine condensation	2
sulfonylation	2
aziridine ring opening	1
beta-lactam addition	1
epoxide ring opening	1
imidazolidinone ring opening	1

Péczka,N.; Orgován, Z.; et al, *Expert Opinion on Drug Discovery*, **2022**, 17:4, 413

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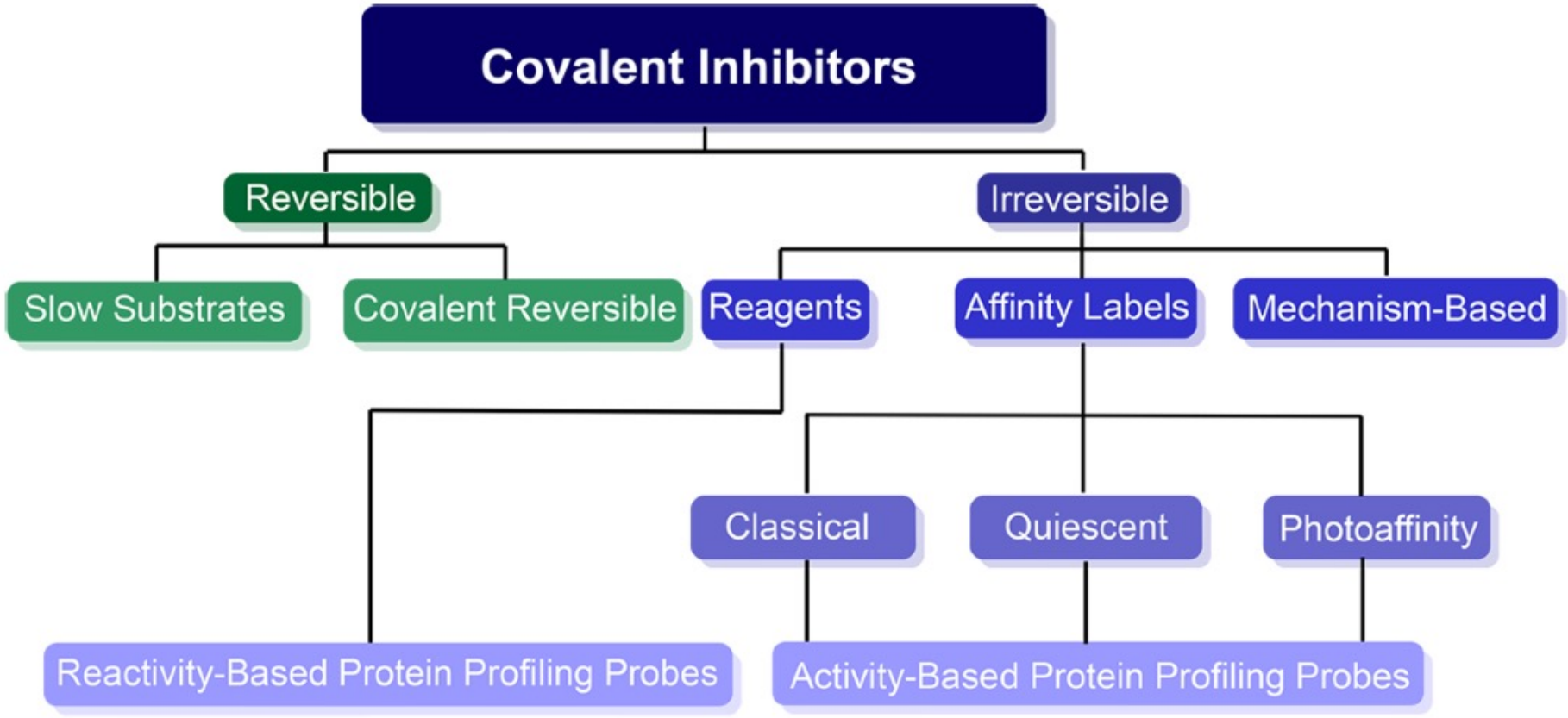
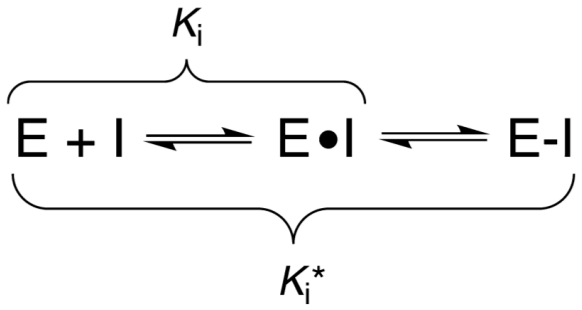
Classification of Covalent Inhibitors

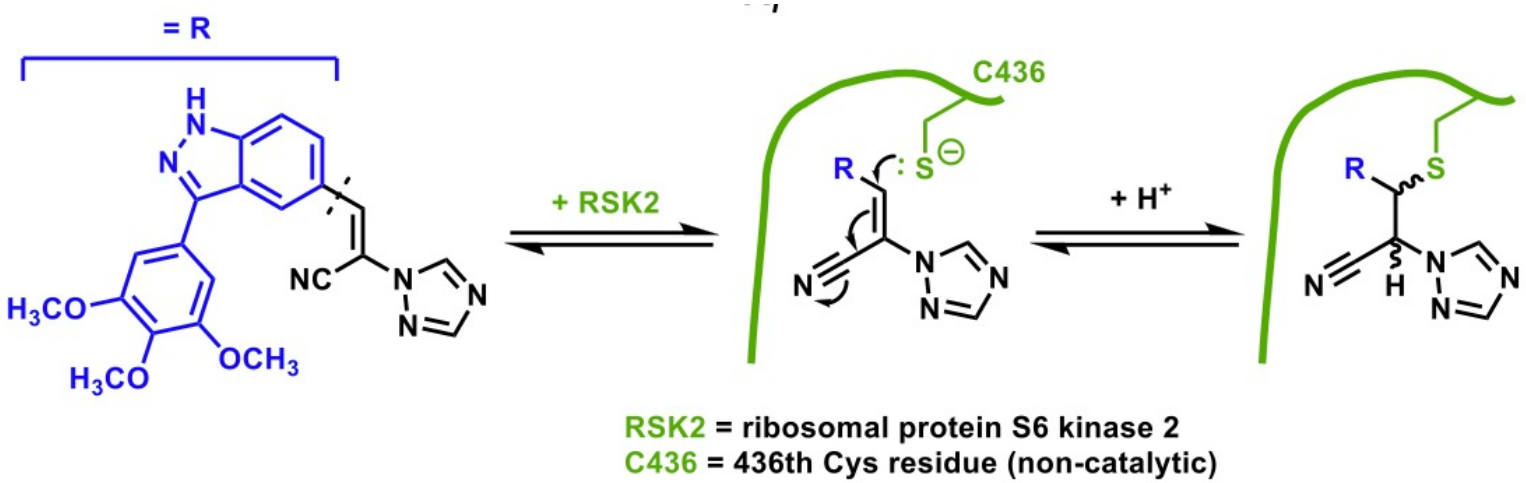


Tuley, A.; Fast, W. *Biochemistry*. **2018**, 57, 3326.

Covalent Reversible Inhibitors

- Reversible
- Selective
- K_i* describes the overall dissociation constant of the two steps





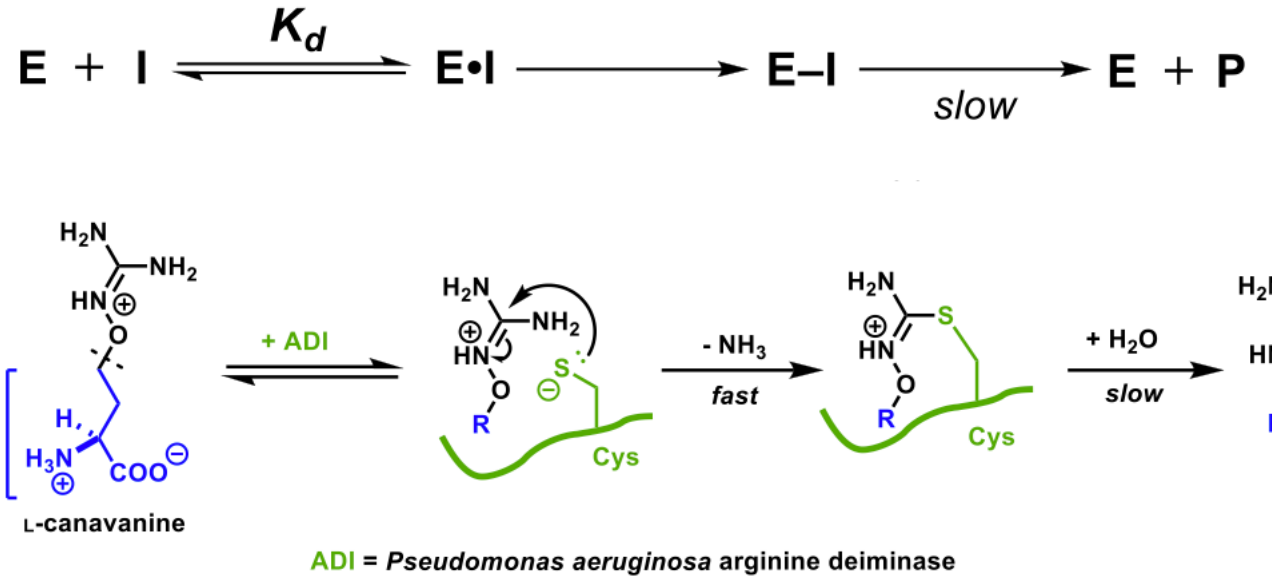
RSK2 = ribosomal protein S6 kinase 2
C436 = 436th Cys residue (non-catalytic)

The low pK_a of the α-proton makes the reaction reversible.

Tuley, A.; Fast, W. *Biochemistry*. **2018**, 57, 3326.

Slow Substrates

- Reversible
- Inhibitor recognized as substrate for the enzyme
- Covalent intermediate further decomposes into free enzyme and non-active product (P)

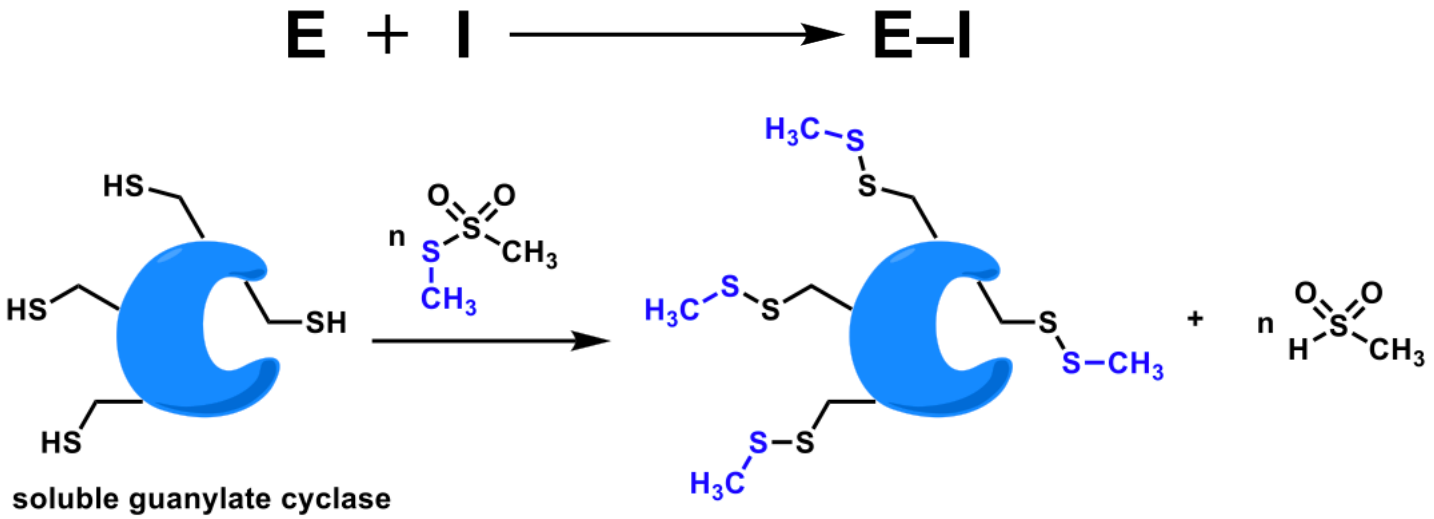


Slow hydrolysis of the pseudo thiourea through the normal catalytic mechanism leads to release of O-ureido and recovered active enzyme.

Tuley, A.; Fast, W. *Biochemistry*. **2018**, 57, 3326.

Residue-Specific Reagents

- Irreversible
- The least selective
- Used only in vitro as biochemical tools
- Influenced by chemo selectivity for particular nucleophiles instead of noncovalent affinity

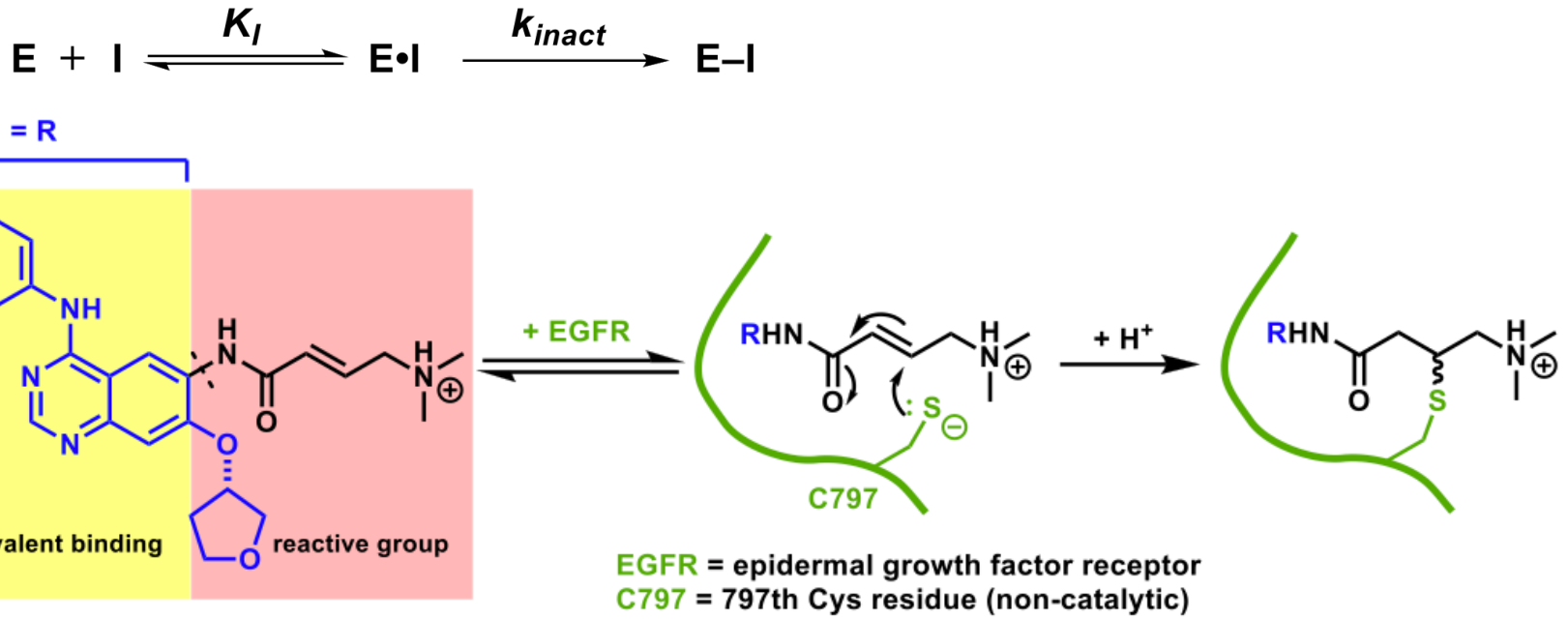


High concentration leads to nonspecific enzyme inhibition, illustrating the nonselective nature of residue-specific reagents.

Tuley, A.; Fast, W. *Biochemistry*. **2018**, 57, 3326.

Affinity Labels

- Irreversible
- Site selective inhibition
- Moiety with non-covalent binding affinity + reactive group (typically a poor electrophile)
- Dissociation from covalent complex E–I to non-covalent complex E•I can be ignored

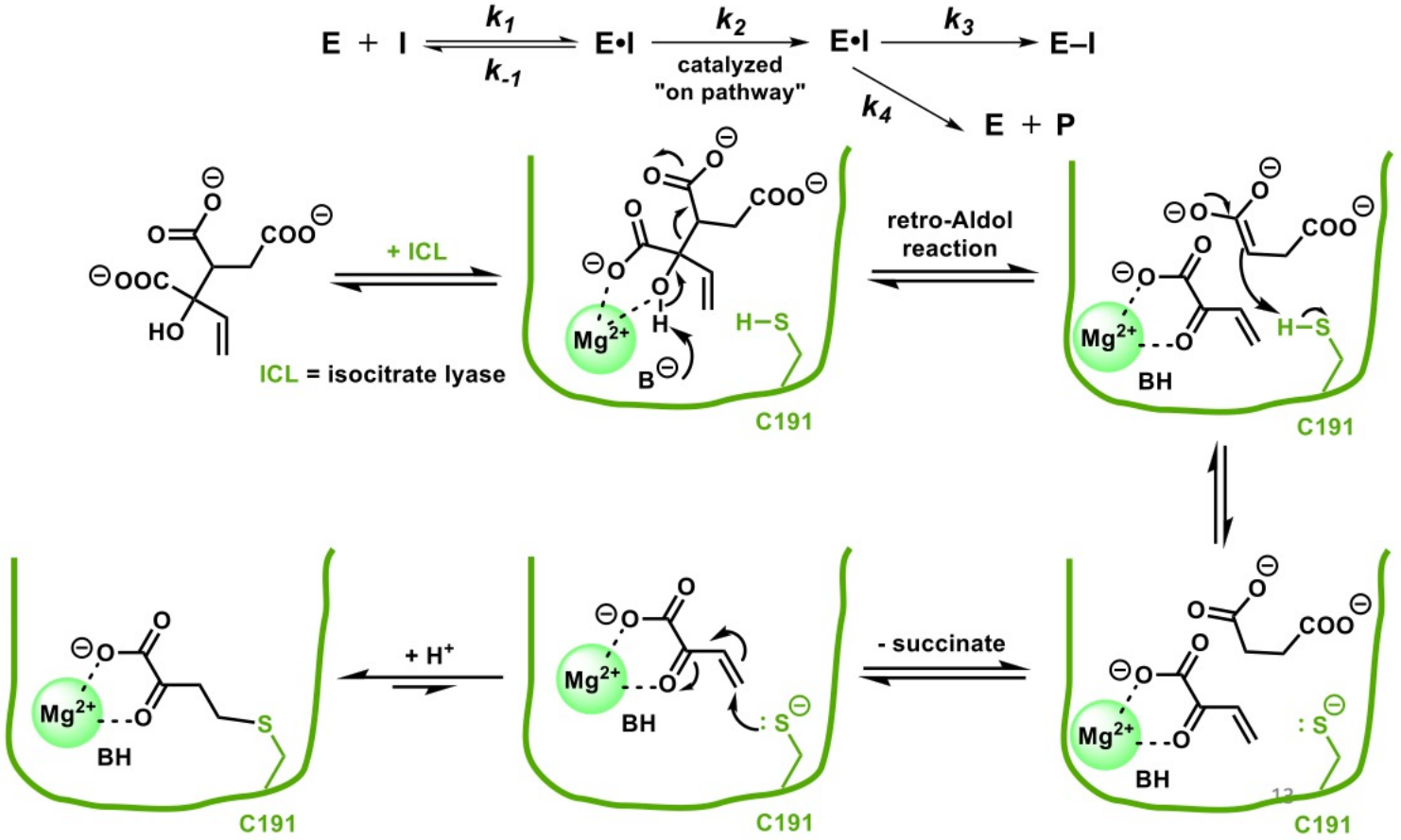


The effective molarity of the reactive group near the site of enzyme modification is raised by the non-covalent binding.

Tuley, A.; Fast, W. *Biochemistry*. **2018**, 57, 3326.

Mechanism-Based Enzyme Inactivators

- Irreversible
- Selectively Bind to active site of enzymes
- Processed by catalytic mechanism to give reactive species



Tuley, A.; Fast, W. *Biochemistry*. **2018**, 57, 3326.

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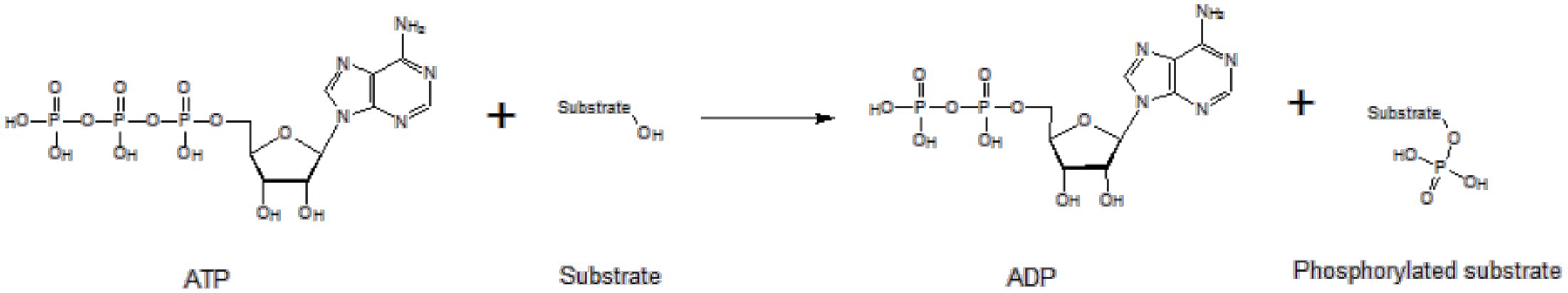
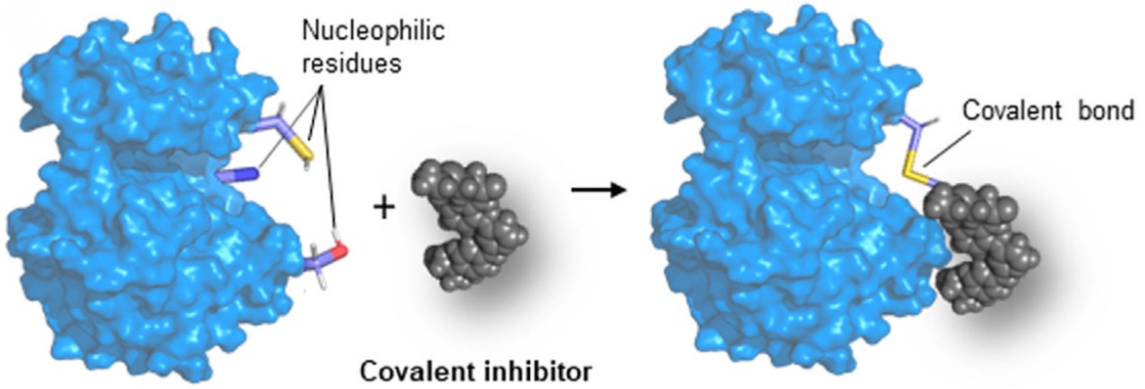
Kinase-targeting Covalent Inhibitors

Kinases play crucial roles in regulating various cellular activities by catalyzing the phosphorylation of biomacromolecules.



Covalent inhibitors use electrophilic warhead groups to react with nucleophilic kinase residues such as cysteine to form covalent bonds to inhibits ATP binding.

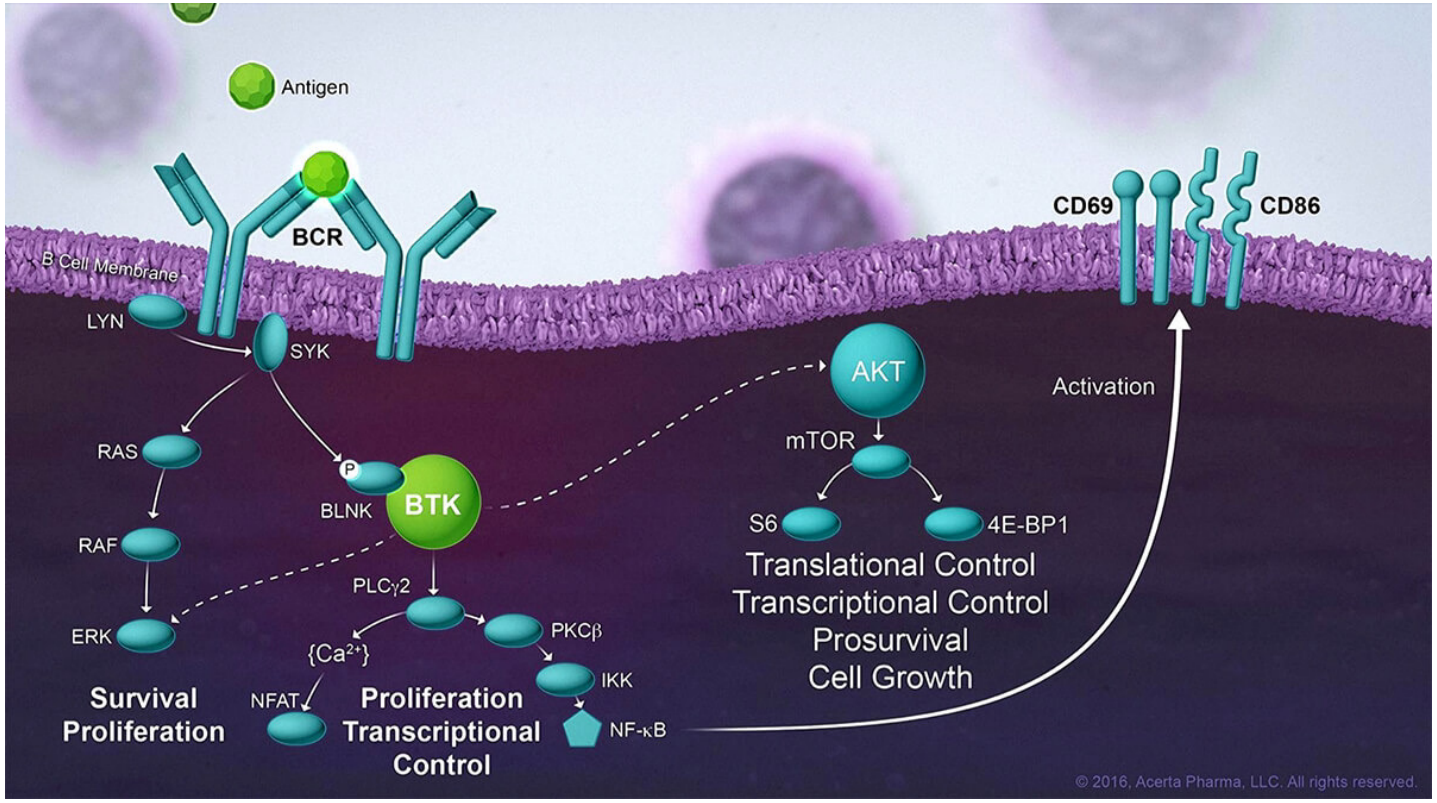


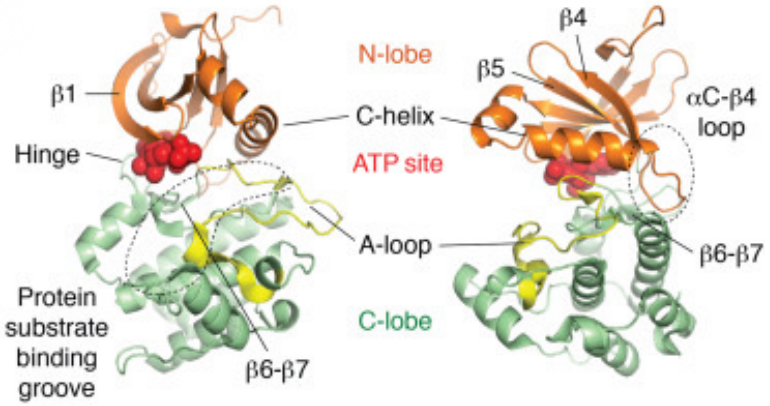
Goebel, G.; Wu, P. *Trends Pharmacol. Sci*. **2022**, 43, 10.

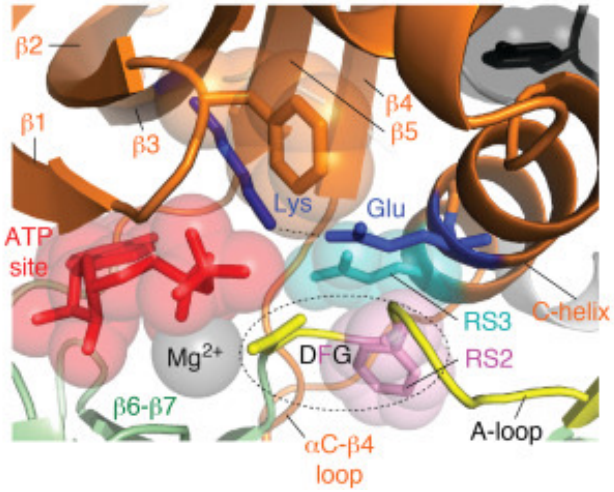
Kinase-targeting Covalent Inhibitors

Bruton's tyrosine kinase (BTK)

BTK plays a crucial role in B cell development as it is required for transmitting signals from the pre-B cell receptor that forms after successful immunoglobulin heavy chain rearrangement.



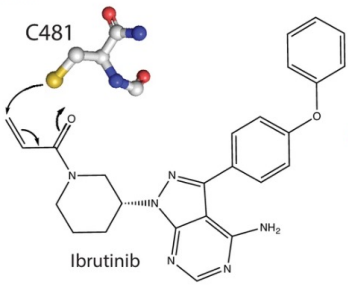


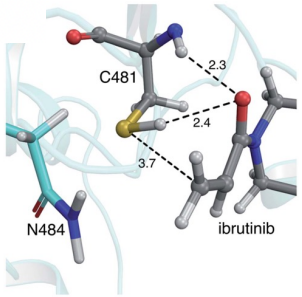


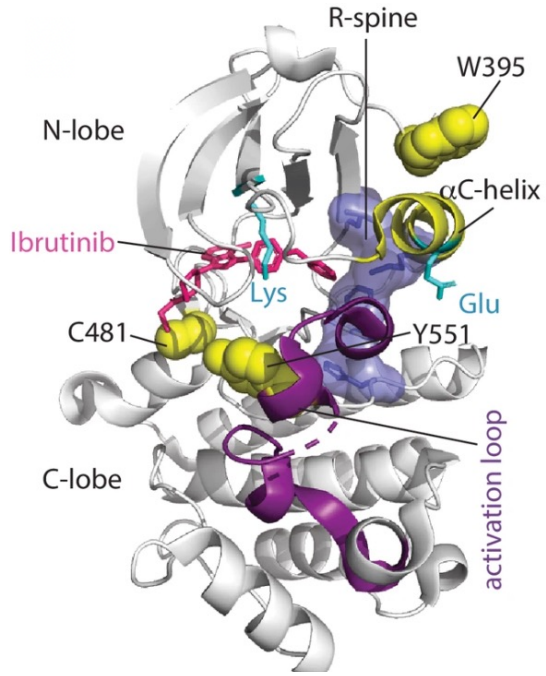
Arter, C.; Trask, L. etc. *J. Biol. Chem.* **2022**, 298, 102247.

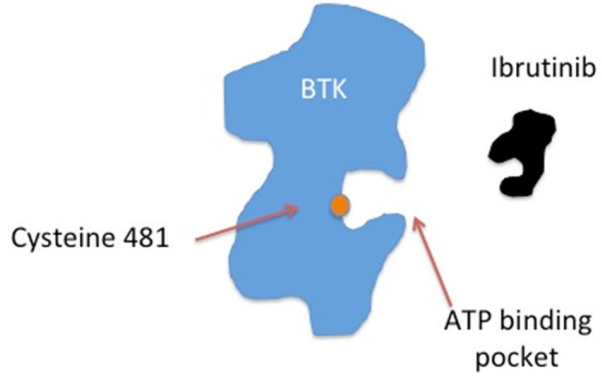
Kinase-targeting Covalent Inhibitors

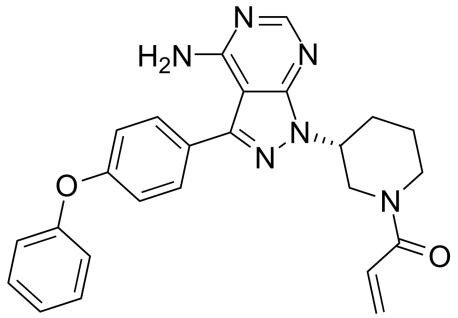
BTK-targeting covalent inhibitors block signaling through BTK inhibition by forming a covalent bond with Cys-481 in the ATP binding domain of BTK.



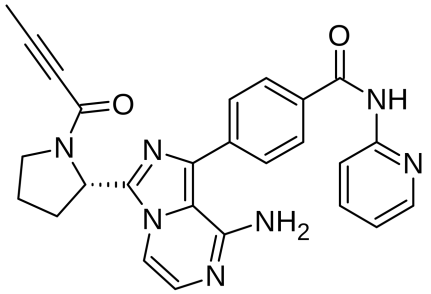




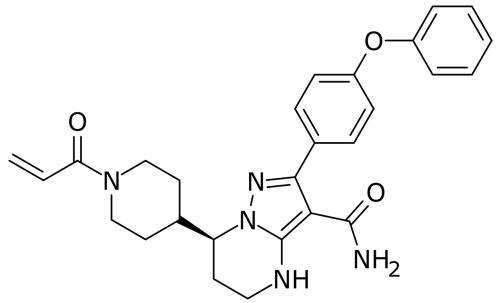




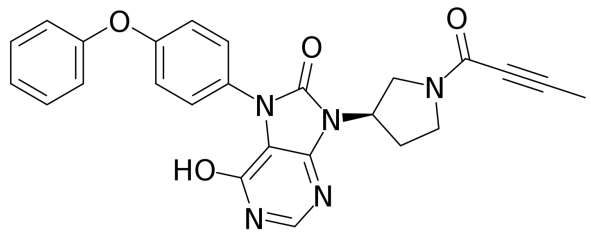
Ibrutinib



Acalabrutinib



Zanubrutinib



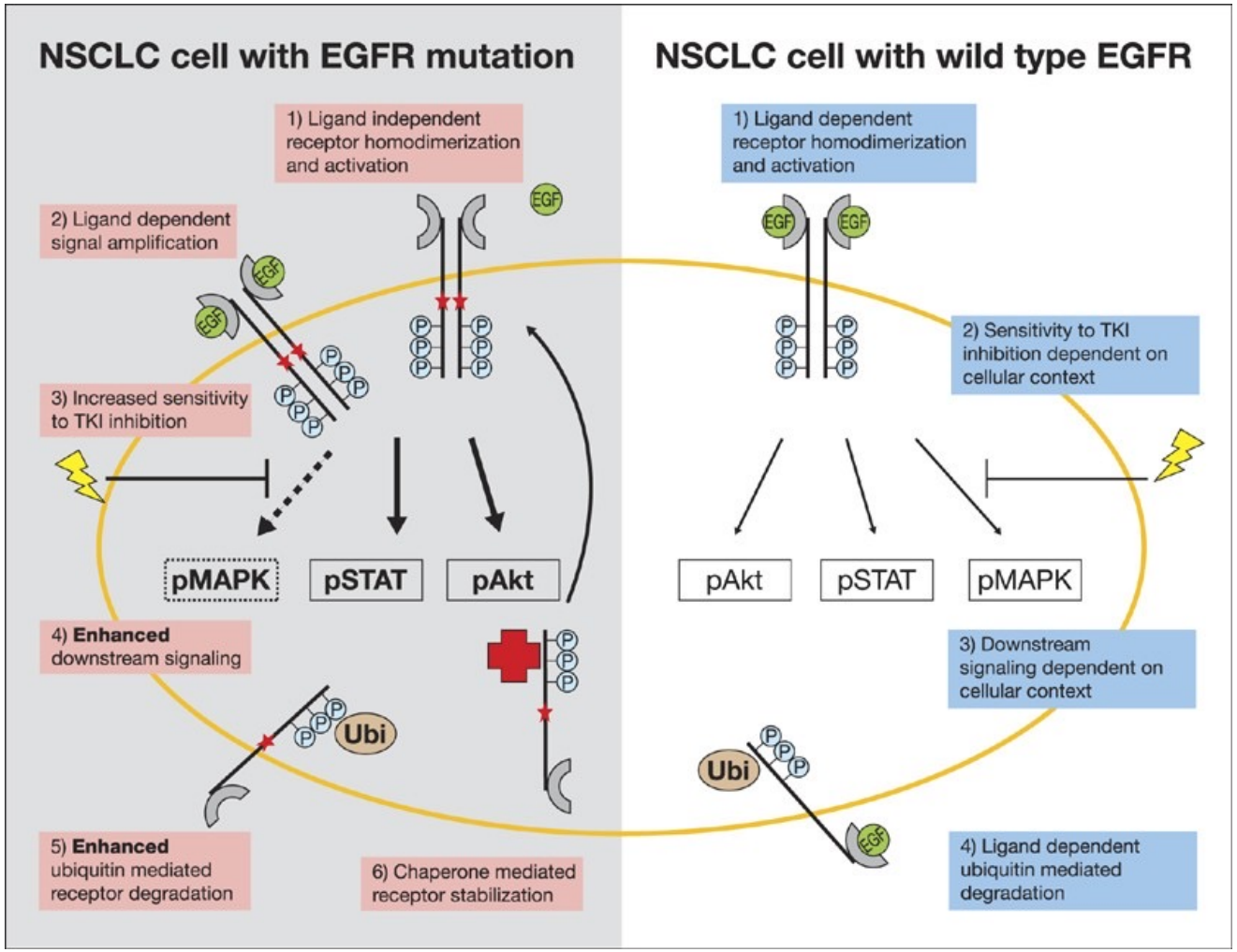
Tirabrutinib

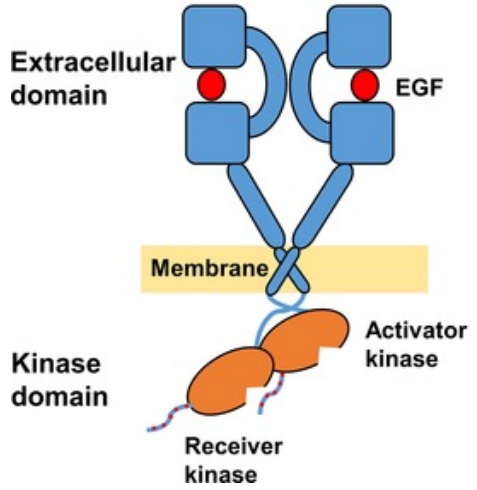
Approved drugs that treat cancers, including mantle cell lymphoma, chronic lymphocytic leukemia, and Waldenström's macroglobulinemia.

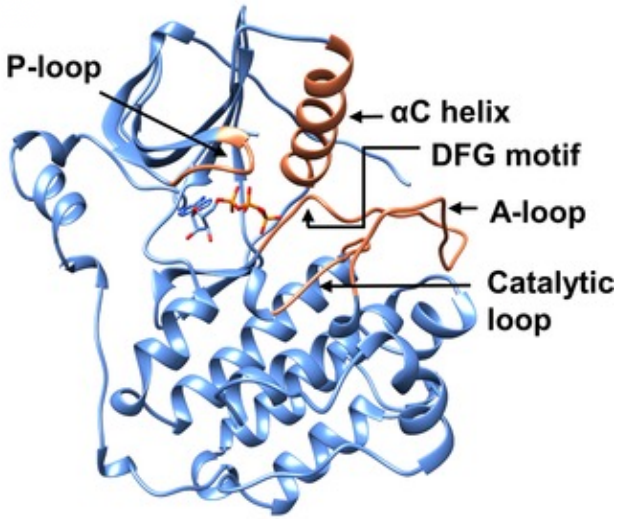
Joseph R et al. *eLife*, **2022**, 9:e60470.

Kinase-targeting Covalent Inhibitors

Epidermal growth factor receptor (EGFR) is a transmembrane protein that is activated by binding of its specific ligands, including epidermal growth factor and transforming growth factor α (TGFα).





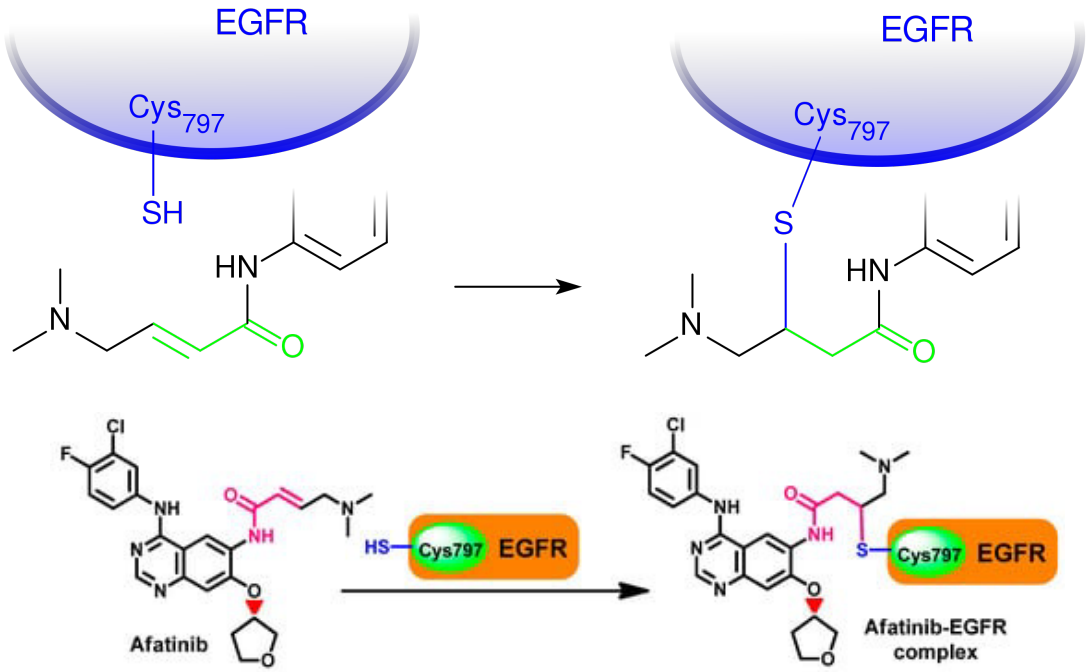


Tamirat, MZ., et al. *PLoS ONE*, **2019**, 14: e0222814.

Kinase-targeting Covalent Inhibitors

EGFR-targeting covalent inhibitors

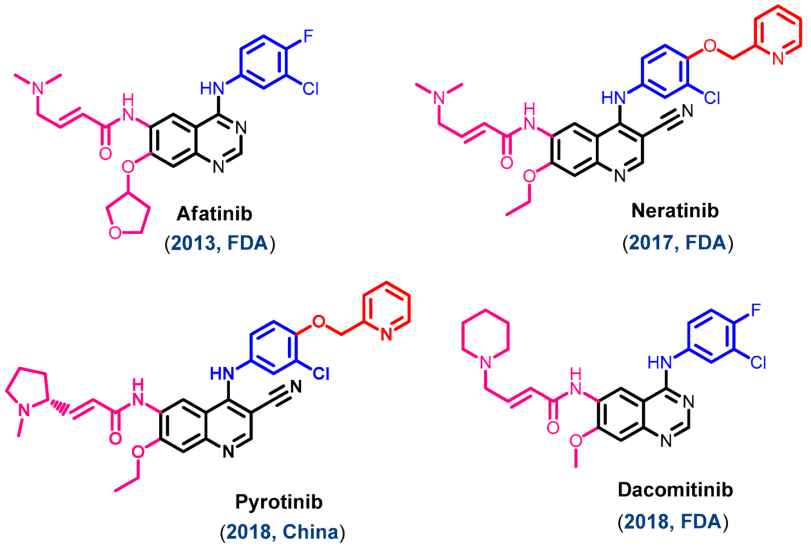
Targeting cys-797 in EGFR with covalent inhibitors is associated with the treatment of a wide variety of tumors.



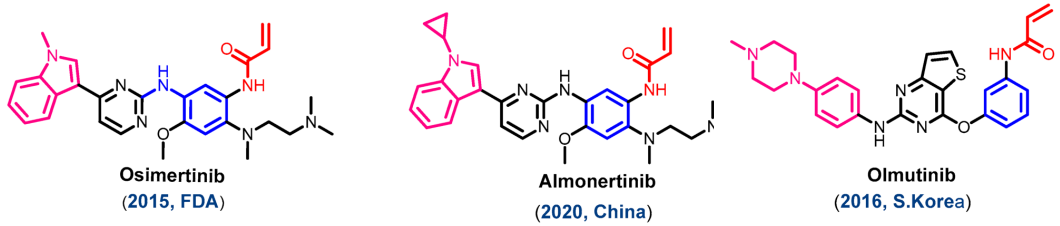
Afatinib covalently binds to cysteine 797 of EGFR via a Michael addition (IC50 = 0.5 nM)

Abourehab, M. *et al. Molecules,* **2021**, *26*, 6677

Approved EGFR covalent inhibitors

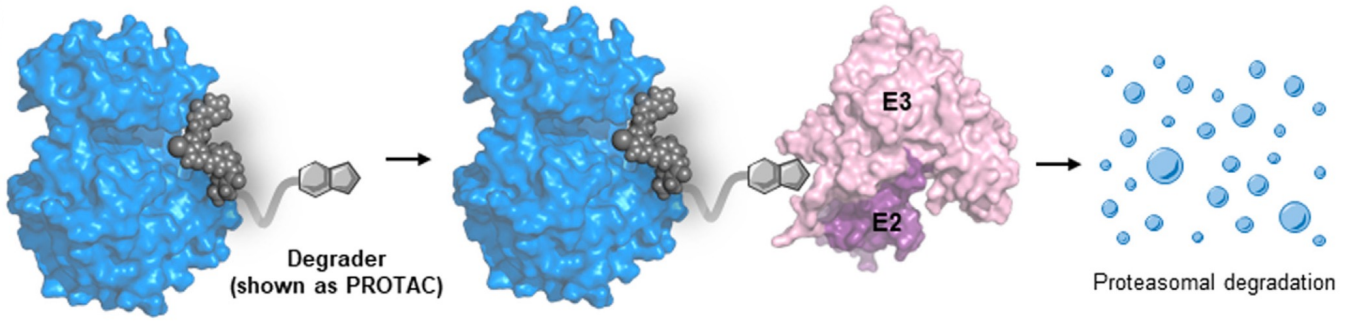


T790M EGFR covalent inhibitors

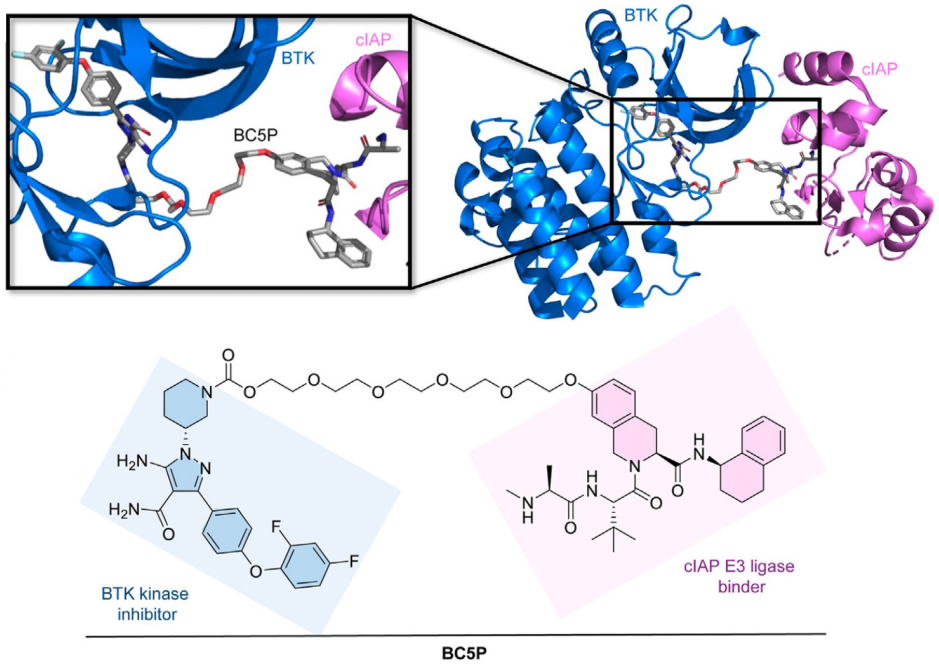


Kinase-targeting Covalent Inhibitors

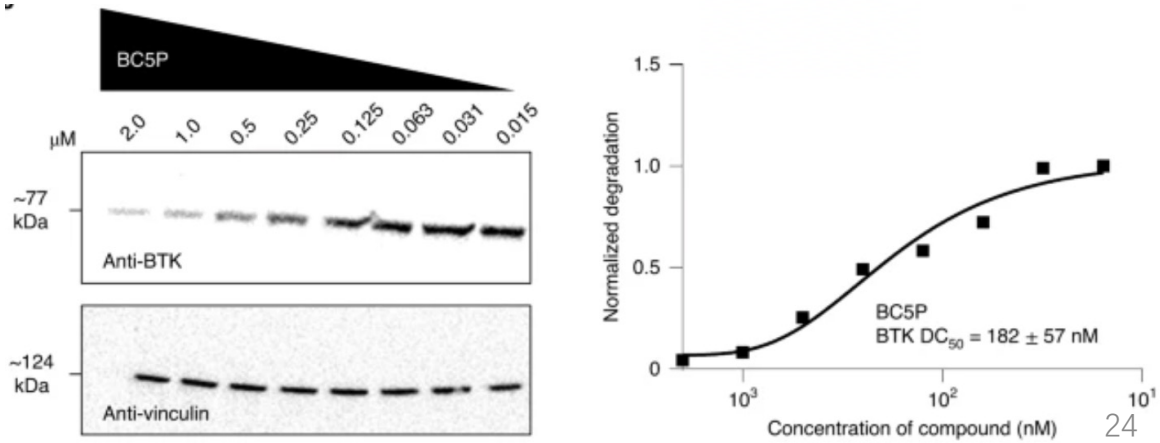
PROTACs are bifunctional molecules with a ligand at one end binding to a target of interest and an E3 ubiquitin ligase binder at the other end to recruit E3–E2 ligases for ubiquitin- mediated proteasomal degradation.



Covalent kinase inhibitors were further developed as kinase-targeting PROTACs.



- BC5P was developed by linking a aminopyrazole based BTK inhibitor to a known ligand of the E3 ligase cIAP via five polyethylene glycol (PEG) molecules.
- Treatment of THP-1 cells with BC5P led to a dose-dependent loss of BTK, with a half-maximum degradation concentration (DC50) of 182 ± 57 nM.



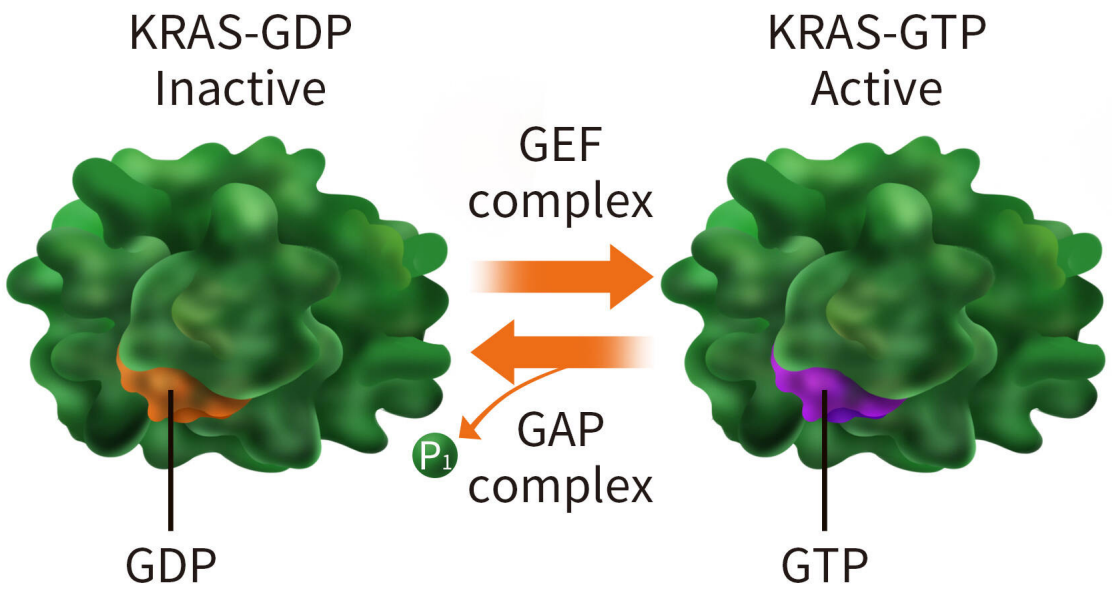
Schiemer, J. *et al. Nat Chem Biol,* **2021**, 17, 152–160.

Contents

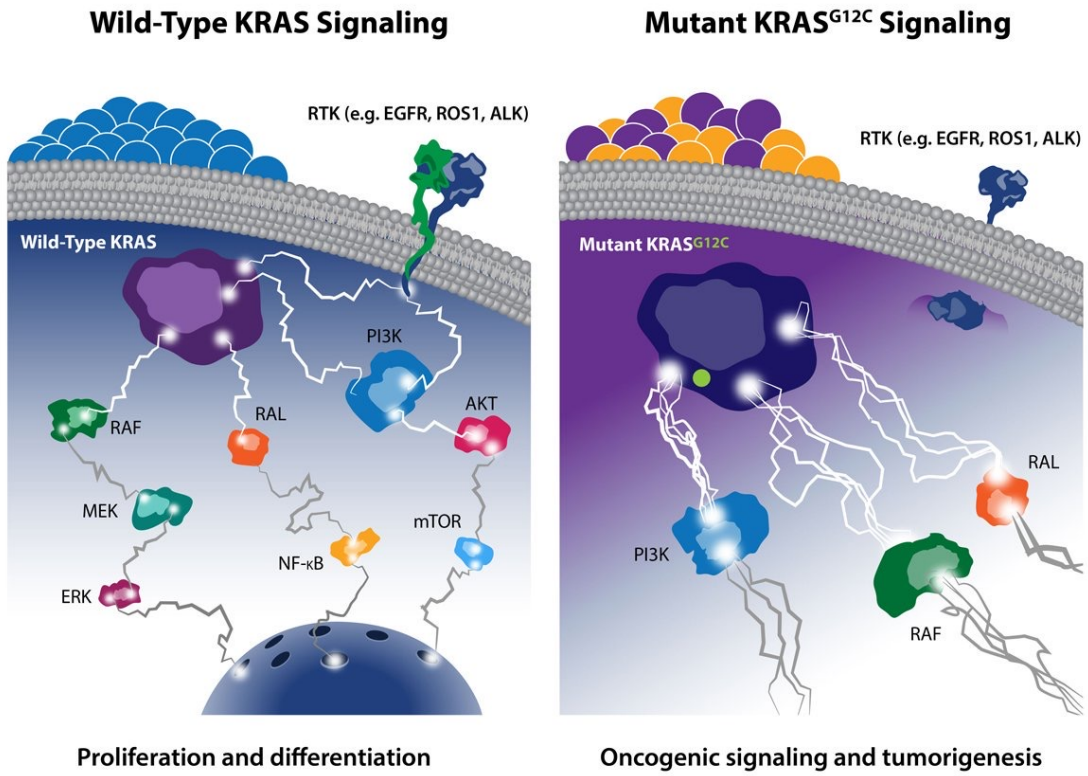
K-Ras-targeting covalent inhibitors

K-Ras and its biological roles

The K-Ras protein is a GTPase which converts the nucleotide GTP into GDP.



KRAS Mutants are Prominent Oncogenic Drivers

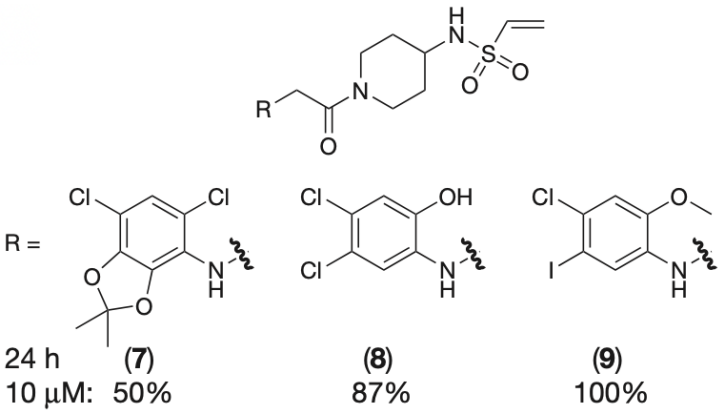


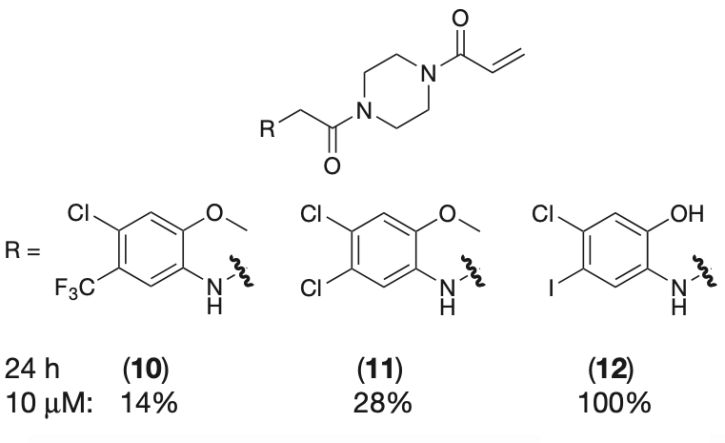
KRAS was considered undruggable because of its relatively smooth surface as well as the high affinity of GTP to the GTP/GDP-binding pocket

KRAS-targeting Covalent Inhibitors

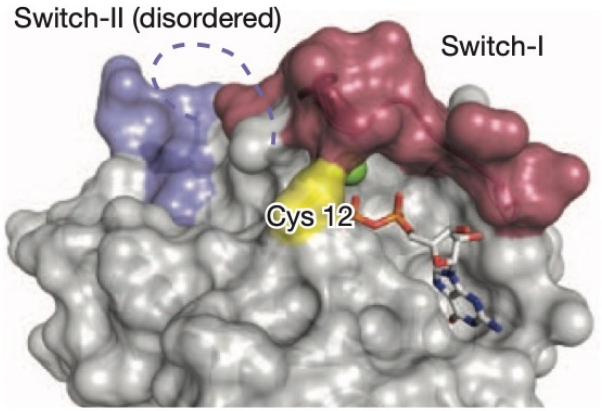
Covalent KRAS(G12C) inhibitors

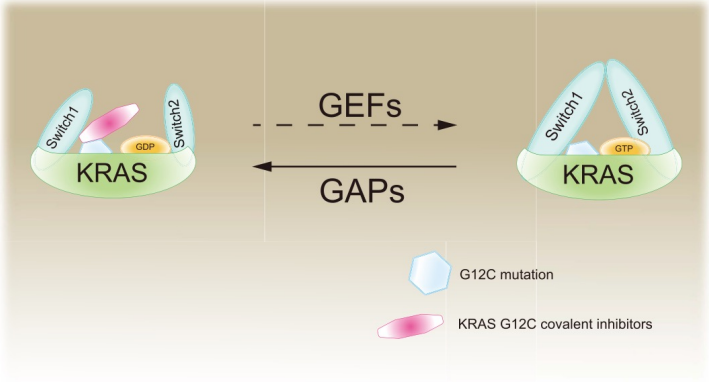
Compounds that covalently and irreversibly bound to the cysteine residue of the $KRAS^{G12C}$.



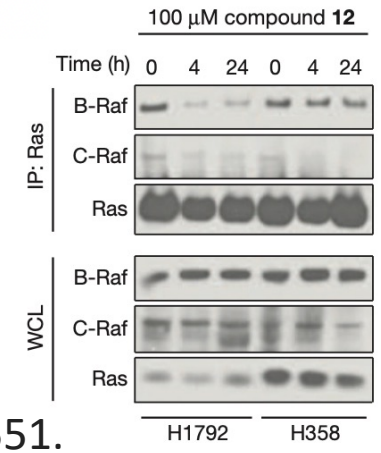


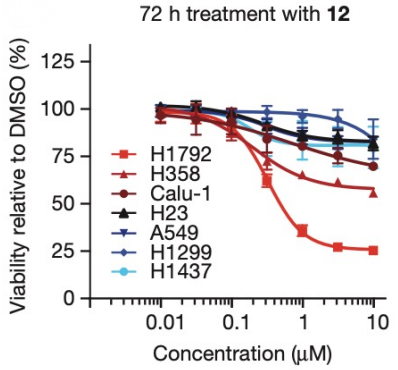
An allosteric pocket beneath the switch II region near the mutant cysteine was discovered

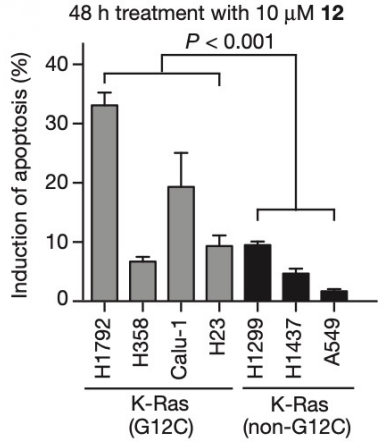


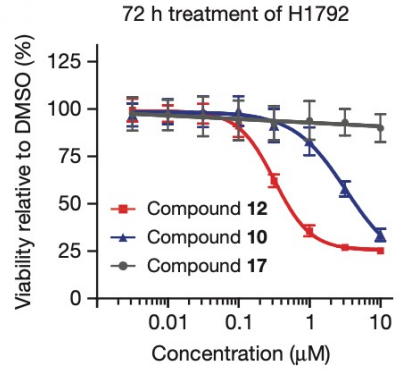


Compounds block K-Ras(G12C) interactions, decrease viability and increase apoptosis of G12C-containing lung cancer cell lines.







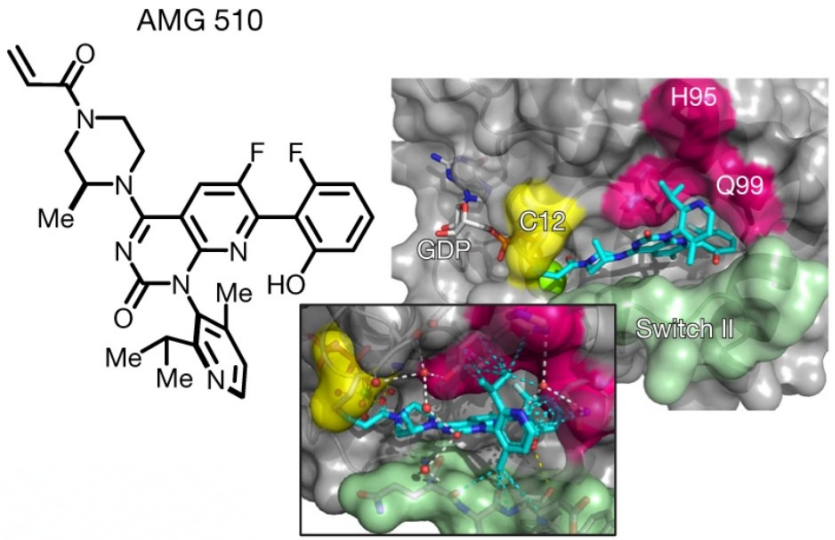


Ostrem, JM. et al. *Nature,* **2013**, 503, 548–551.

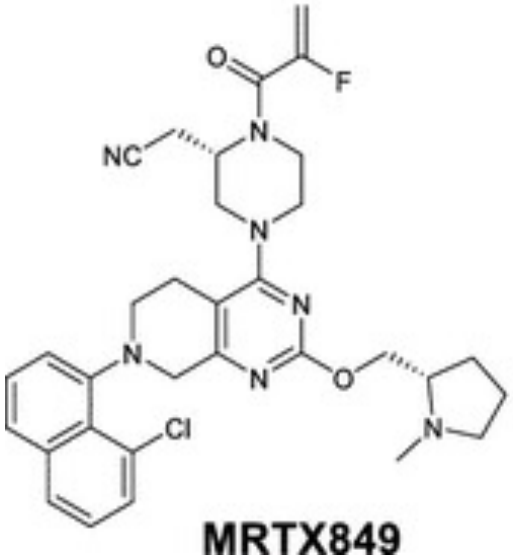
KRAS-targeting Covalent Inhibitors

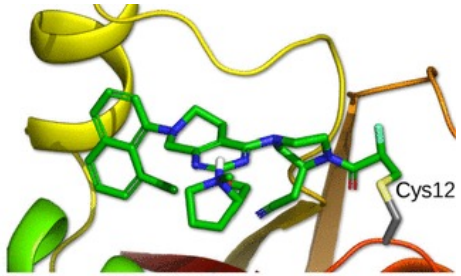
Covalent KRAS(G12C) inhibitors

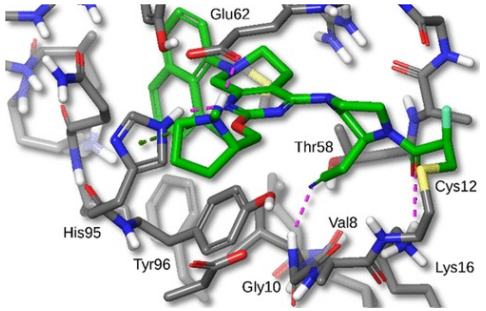
Sotorasib (AMG510) obtained FDA approval in 2021 to become the first therapy to directly target the KRAS oncoprotein in tumors



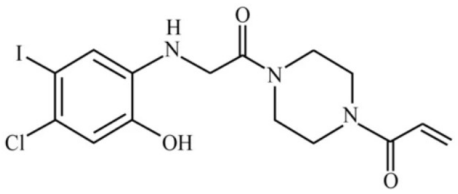
Adagrasib (MRTX849) and other direct KRAS^{G12C} inhibitors are currently being investigated in multiple clinical trials.

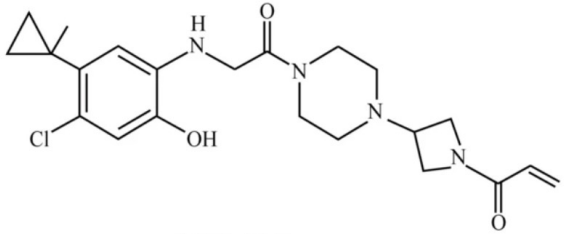


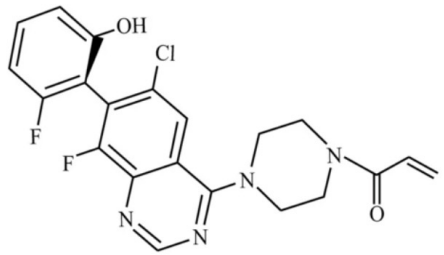




Preclinical compounds

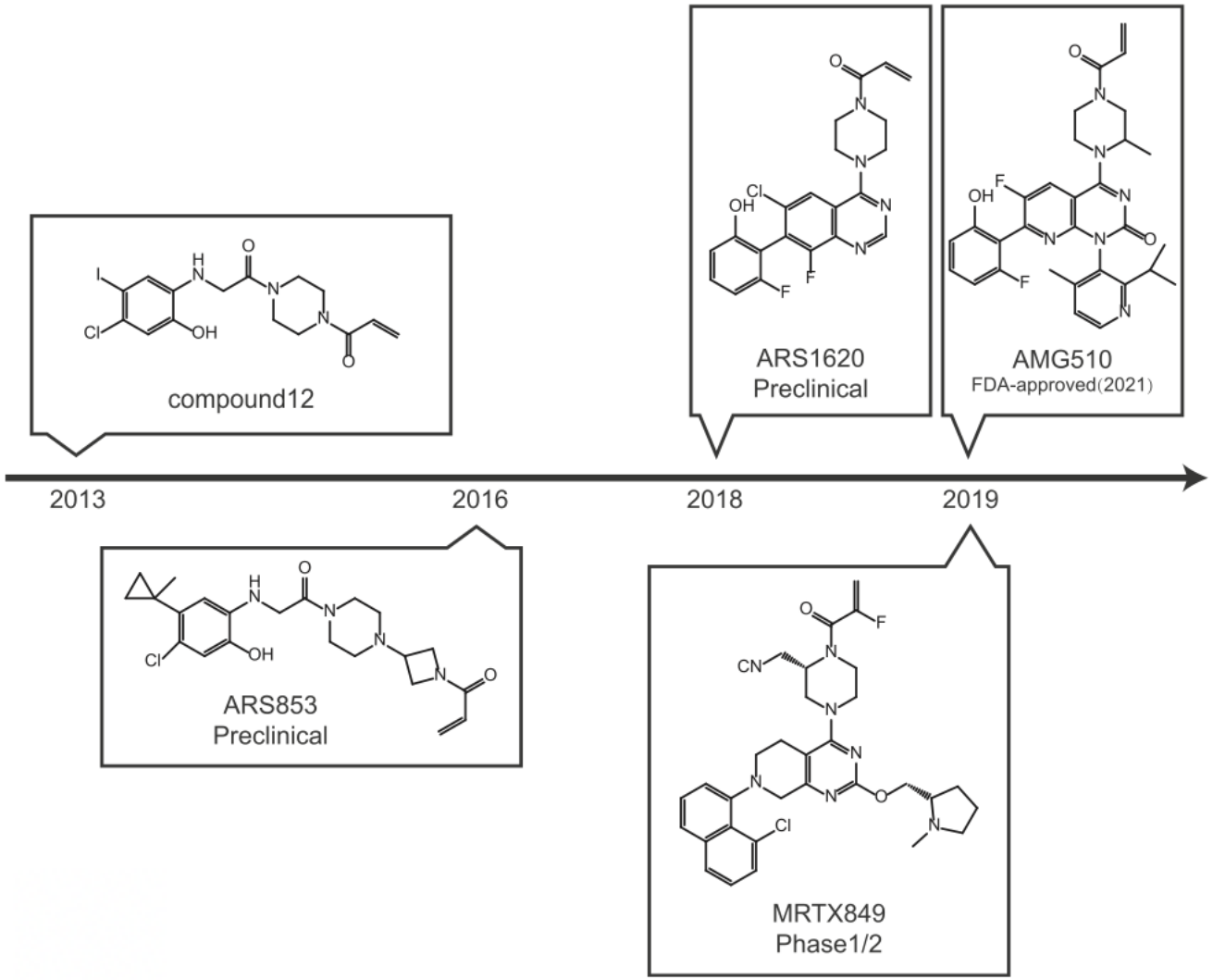






Canon, J. et al. *Nature*, **2019**, 575, 217–223.

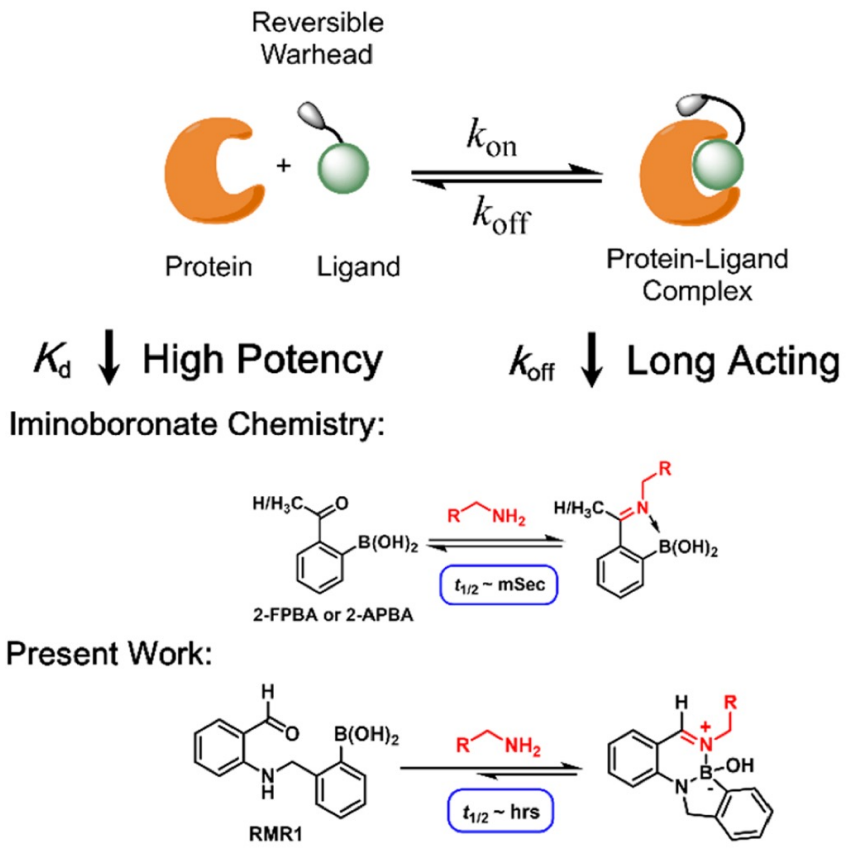
KRAS-targeting Covalent Inhibitors



A chronicle of discovery and development of $KRas^{G12C}$ covalent inhibitors.

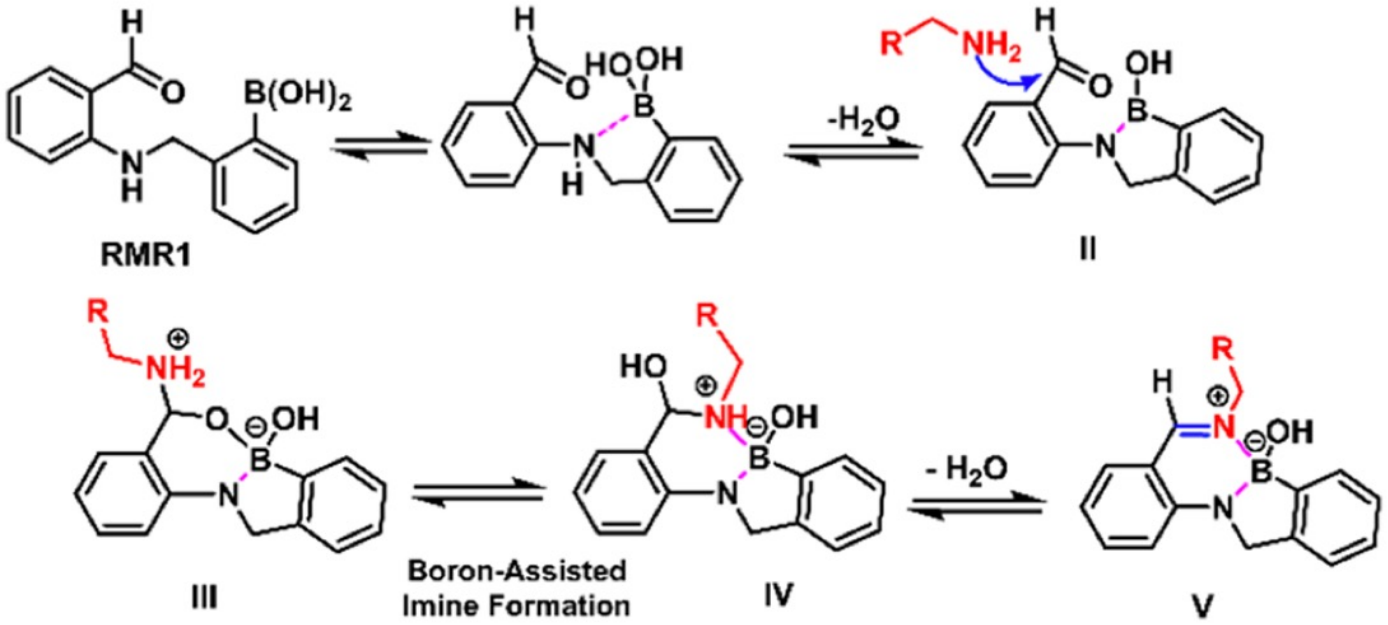
Contents

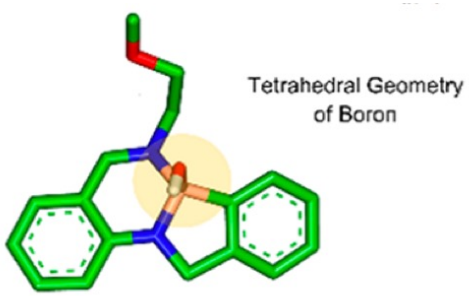
Lysine-Targeting Reversible Covalent Inhibitors



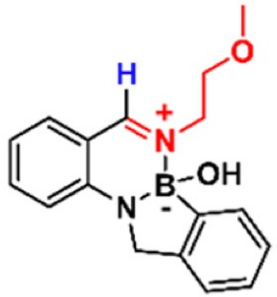
- Reversible covalent inhibitors
- Low K_{off} to maximize the benefit of the warhead, and also to achieve long-lasting inhibition.
- Diazaborines and related B−N heterocycles have also been explored as enzyme inhibitors and as reversible linkers for drug delivery to cancer cells

Lysine-Targeting Reversible Covalent Inhibitors





X-Ray Structure of RMR1-2MEA Conjugate

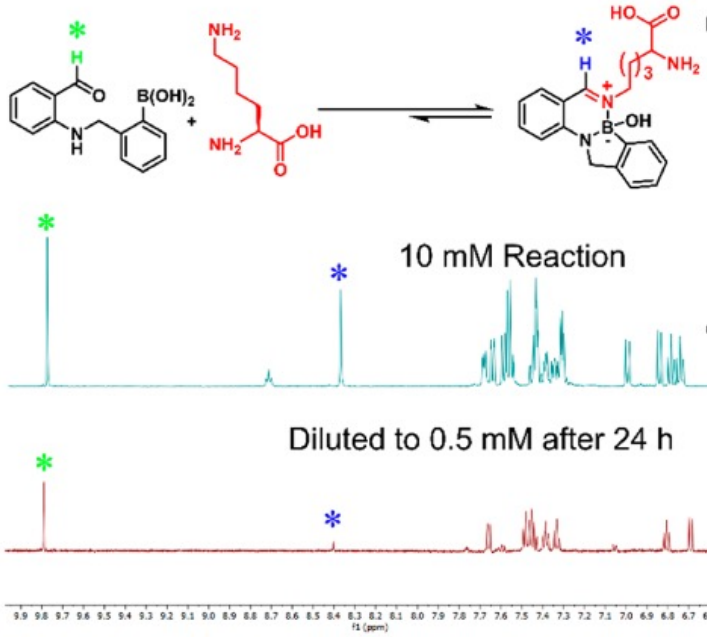


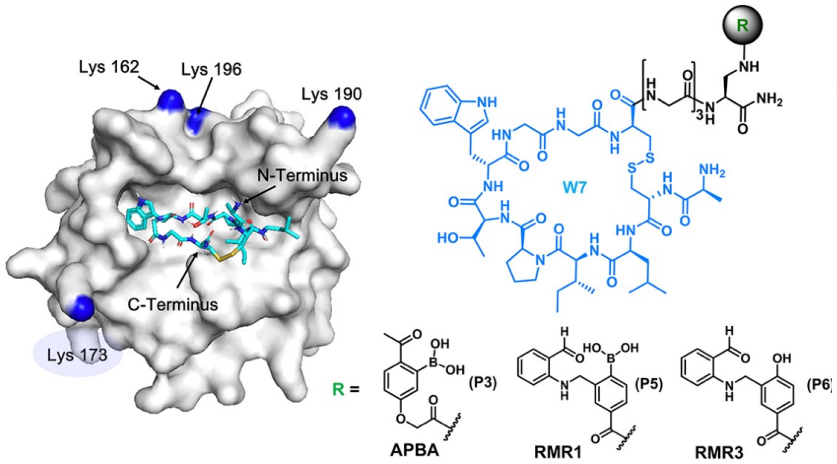
Chemical Structure of RMR1-2MEA Conjugate

- A new warhead RMR1

Reja, Rahi.m. et al, *J. Am. Chem. Soc.* **2022**, 144, 1152

Lysine-Targeting Reversible Covalent Inhibitors





Peptide	Peptide Sequence	IC_{50} (μM)
W7	ACLIPTWGGC	17.0 ± 0.3
W7-Linear	AC#LIPTWGGC#	>250
P1	ACLIPTWGGCGDap(APBA)	50.3 ± 1.0
P2	ACLIPTWGGCGGDap(APBA)	12.5 ± 0.9
P3	ACLIPTWGGCGGGDap(APBA)	4.6 ± 0.2
P4	ACLIPTWGGCGGGDap(alloc)	66.6 ± 0.8
P5¥	ACLIPTWGGCGGGDap(RMR1)	1.3 ± 0.2
P6	ACLIPTWGGCGGGDap(RMR3)	23.7± 0.8
P7	APBA-GGGACLIPTWGGC	4.5 ± 0.3
P8	RMR1-GGGACLIPTWGGC	3.8 ± 0.2

Replacing the APBA warhead with RMR1 (P5) gave even greater potency with an IC50of 1.3 μM.

The reversibility of the diazaborine conjugation was confirmed

- A novel lysine conjugation chemistry
- RMR1 can be grafted on to a peptide scaffold to create potent reversible covalent inhibitors.

Reja, Rahi.m. et al, *J. Am. Chem. Soc.* **2022**, 144, 1152

Contents

Conclusions

- Expanded covalent warhead toolbox allows for selective targeting of specific amino acid residues.
- The approvals of successful drugs showcase the evolution of covalent drug discovery from a serendipitous effort to a field with established roadmaps for success.
- Potential in new modalities such as PROTACs.