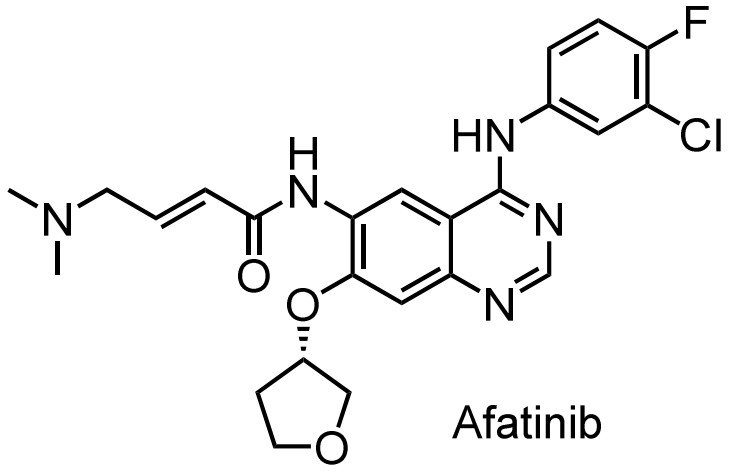
Covalent drugs

Litelature seminar
2014.10.4
Takushi Araya

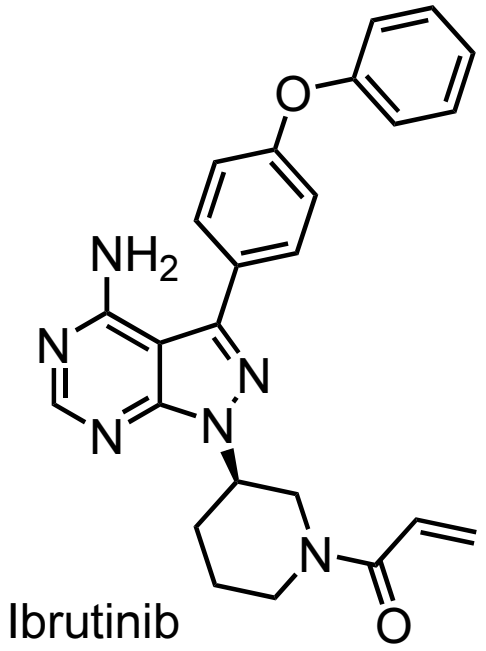
Recently approved targeted-covalent drugs



Afatinib

EGFR inhibitor
(Anti-NSCLC)
Firstly approved

Solca, F. *et al. J Pharmacol. Exp. Ther.* **2012**, *342*, 342.



Ibrutinib

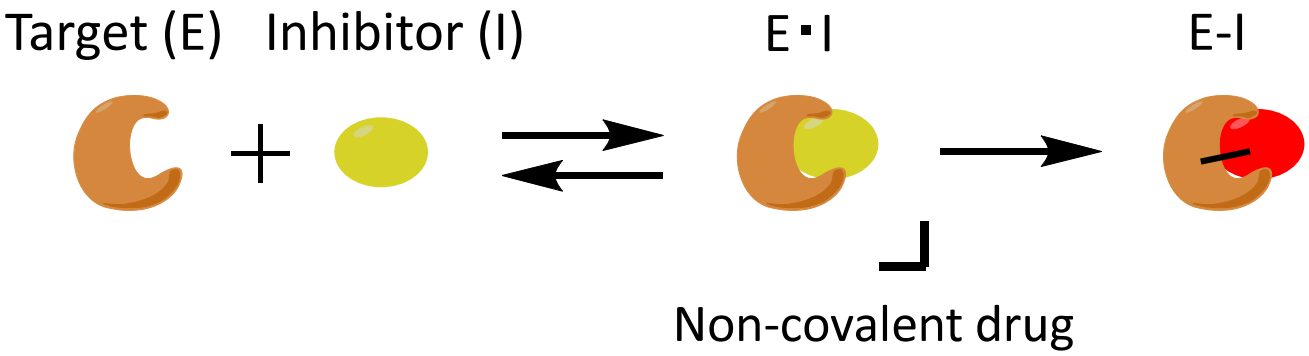
BTK inhibitor
(Anti-mantle cell lymphoma)
Secondly approved

Pan, Z. *et al. ChemMedChem* **2007**, *2*, 58

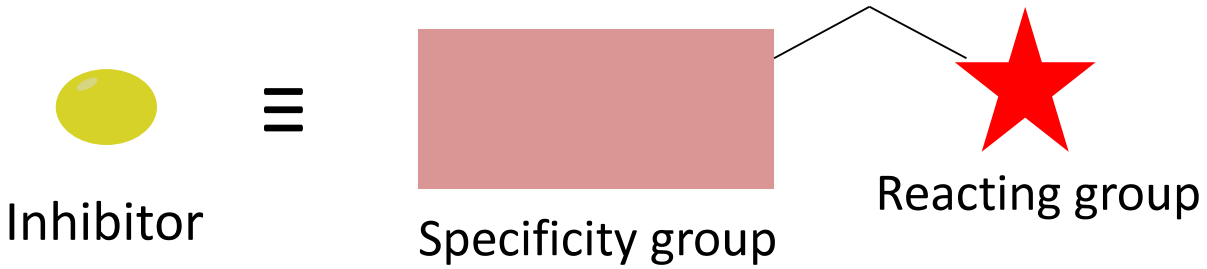
Contents

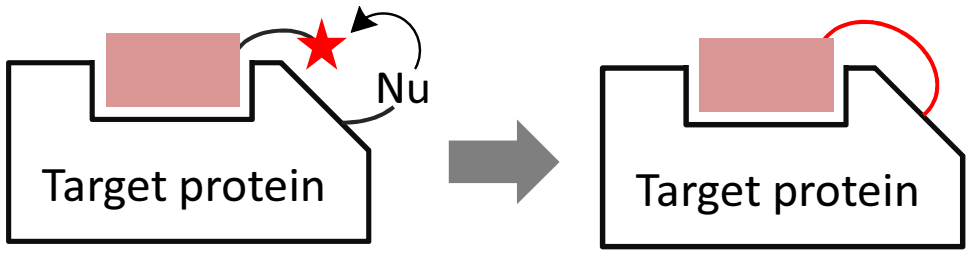
What is Covalent drug?

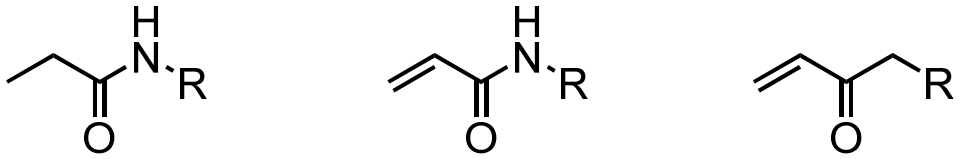
Compound to use as drug (medical use).
It has (or is going to have) **chemical reacting group**
and binds to target **covalently**.

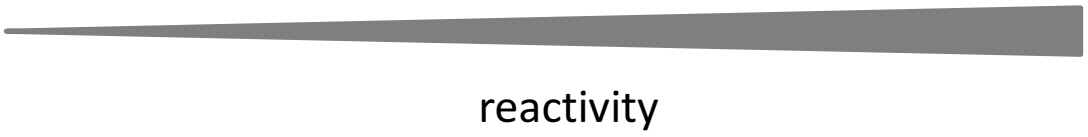


Concept

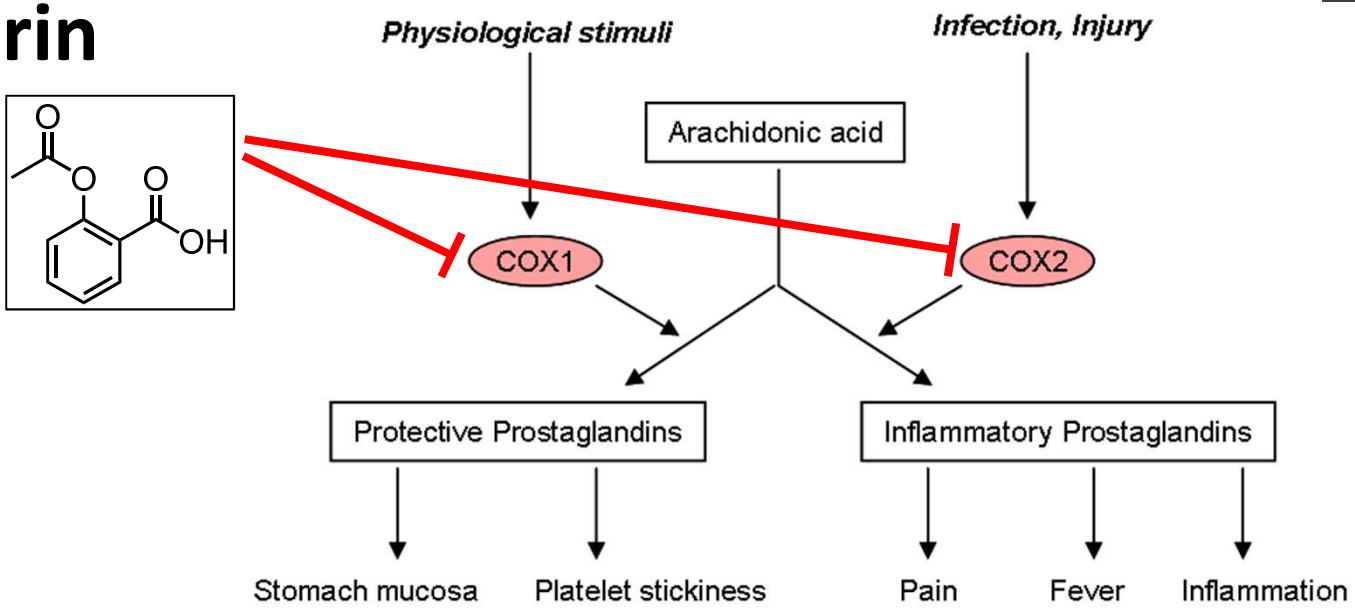

Inhibitor
≡
Specificity group
Reacting group

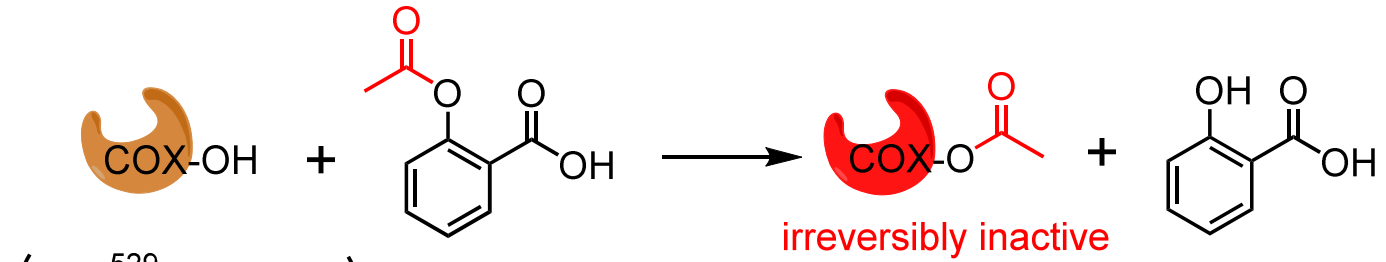

Nu
Target protein
Target protein


H
N
R
O
too low
moderate
too strong


reactivity

Aspirin





(Ser^{529} of COX-1 or Ser^{516} of COX-2)

COX-1

Funk, C. D. *et al. The FASEB Journal* **1991**, *5*, *2304.*

DeWitt, D. L. *et al. J. Biol. Chem.* **1990**, *265,* 5192.

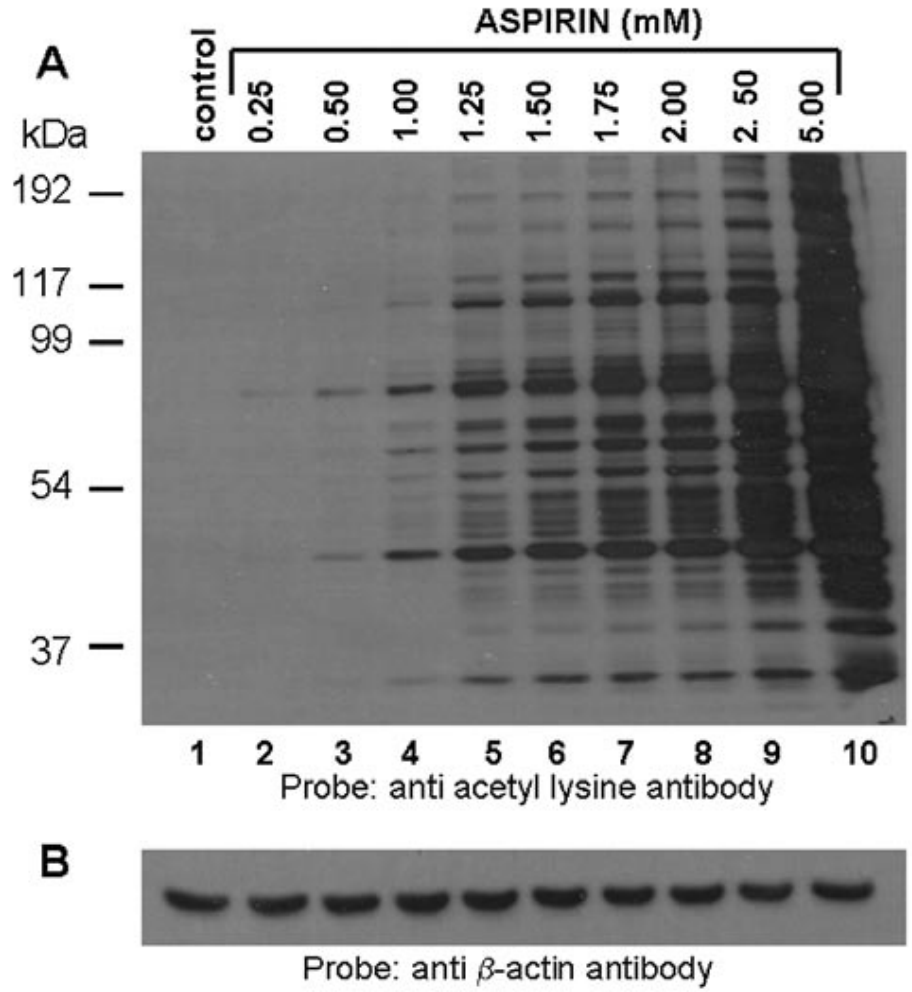
COX-2

Hla, T. *et al. Proc. Natl. Acad. Sci. USA* **1992**, *89*, 7384.

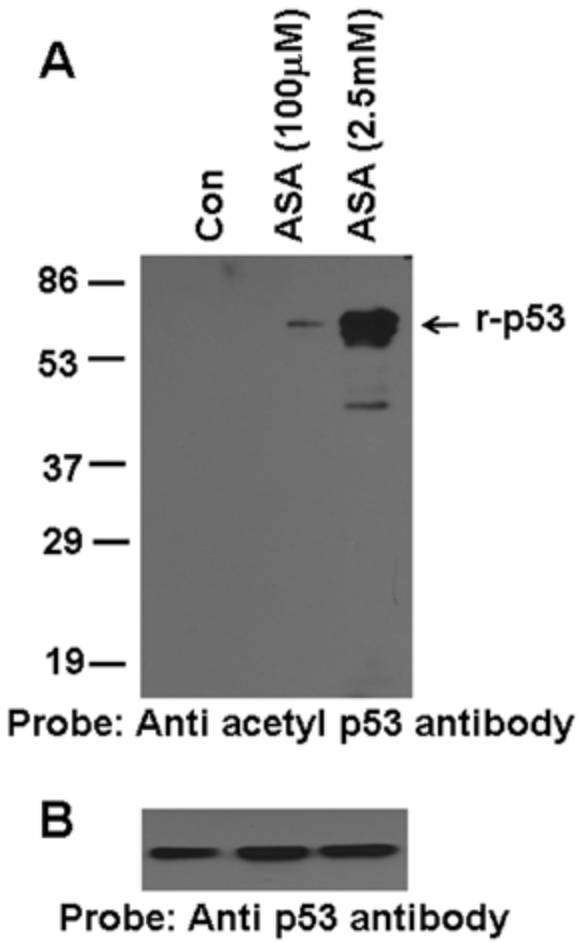
Figure: http://blog.metaome.com/use-case-2/different-strokes-common-painkillers-and-their-mechanisms/

Aspirin also acetylate other cellular proteins

Rat liver cell culture

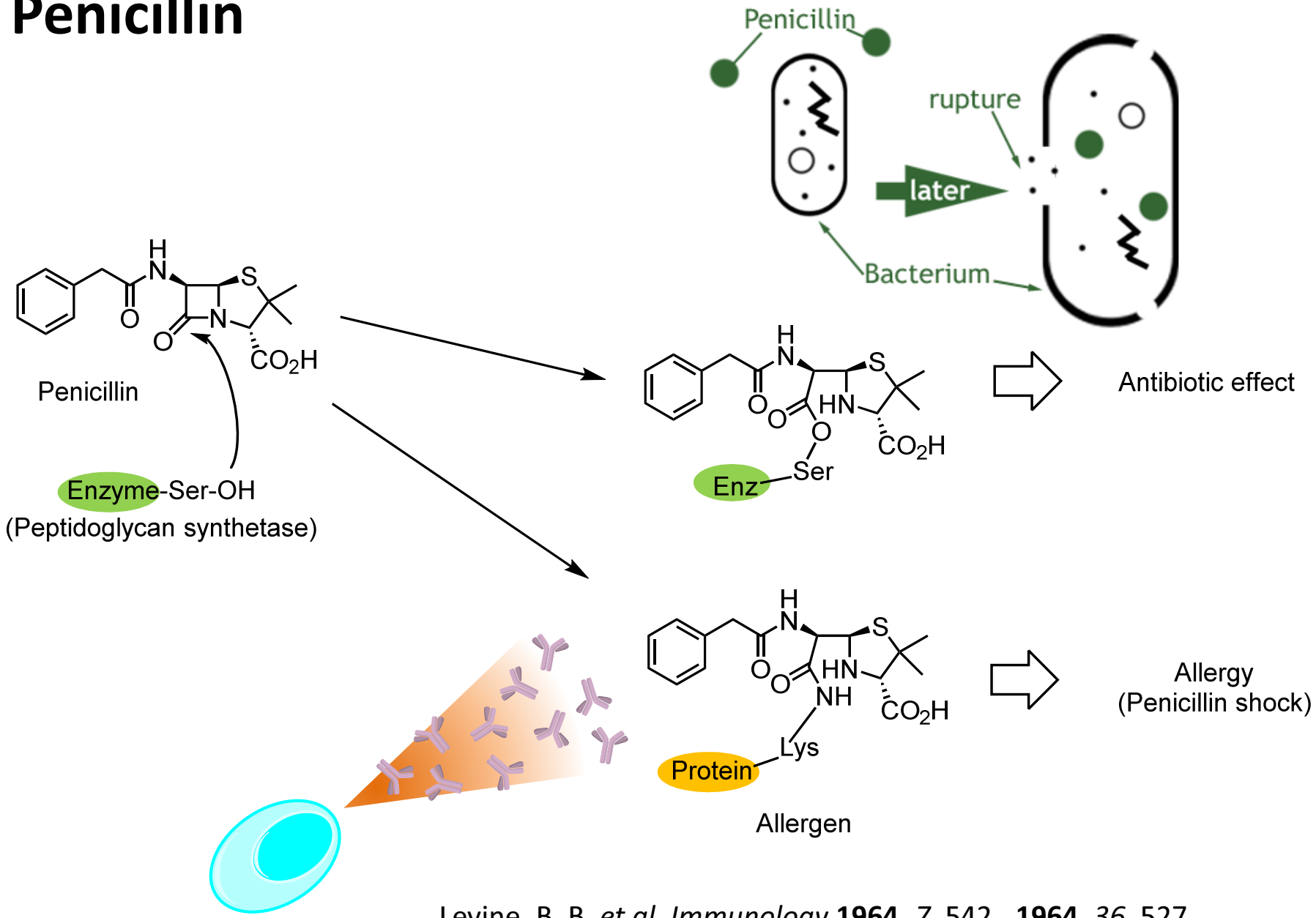


Recombinant p53



Review : Alfonso, L. F. *et al. Mol. Med. Reports*, **2009**, *2*, 533.
Original (inaccessible from UT): Alfonso, L. F. *et al. Int. J. Oncol.* **2009**, *34*, 597.

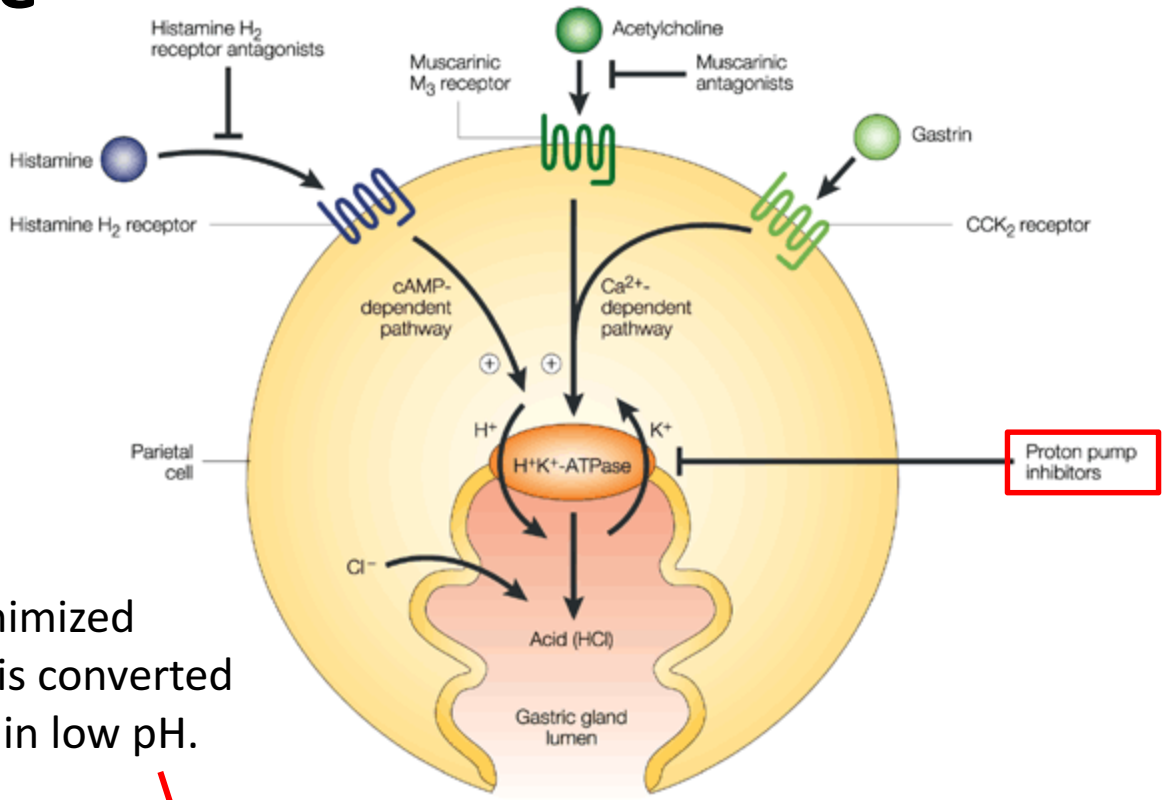
Penicillin



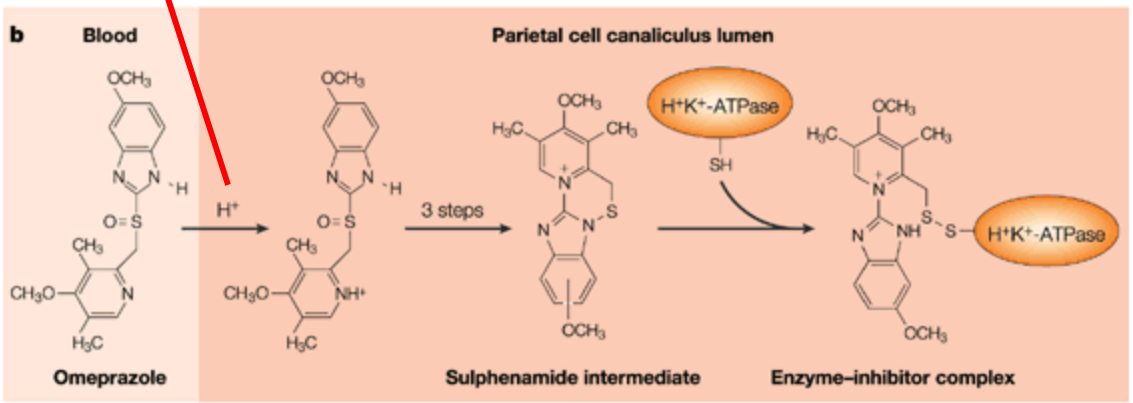
Levine, B. B. *et al. Immunology* **1964**, *7*, 542., **1964**, *36*, 527.

Figure: http://www.antibioticslist.com/images/design/penicillin_img.gif

Omeprazole

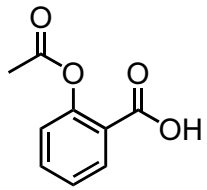


Side effect is minimized because Omeprazole is converted active intermediate in low pH.



Olbe, L. *et al*. *Nat. Rev. Drug Discov.* **2003**, *2*, 132.

History of covalent drugs

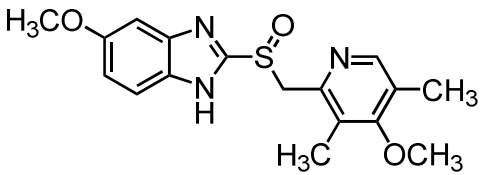


Aspirin

First synthetic and covalent drug

(Mechanism of action was discovered in 1970s.)

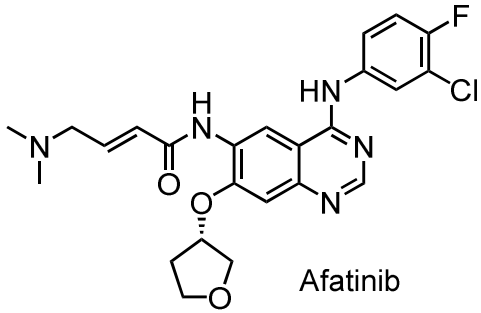
1899



Omeprazole

Blockbuster PPIs

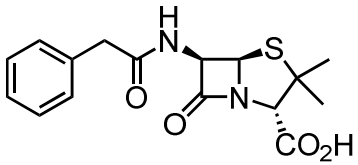
1980s



Afatinib

First-approved Targeted covalent drug

1990s 2013



Penicillin

First antibiotics

1940s

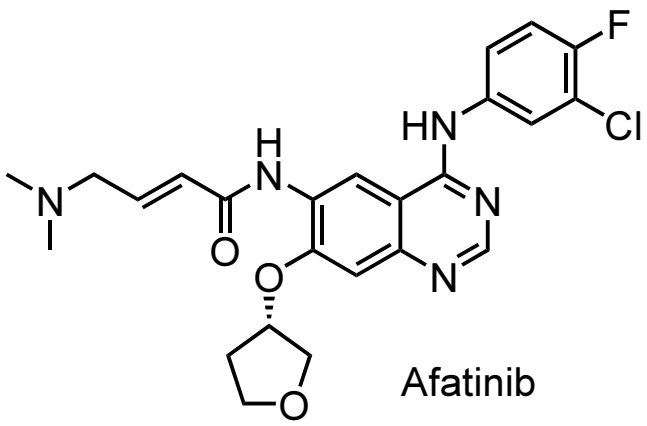
Development of Targeted covalent drugs for EGFR family

Until recently, covalent drugs were discovered by serendipity.
Their mechanisms of action were reported after a long time later.
Can we design "Targeted" covalent drug?

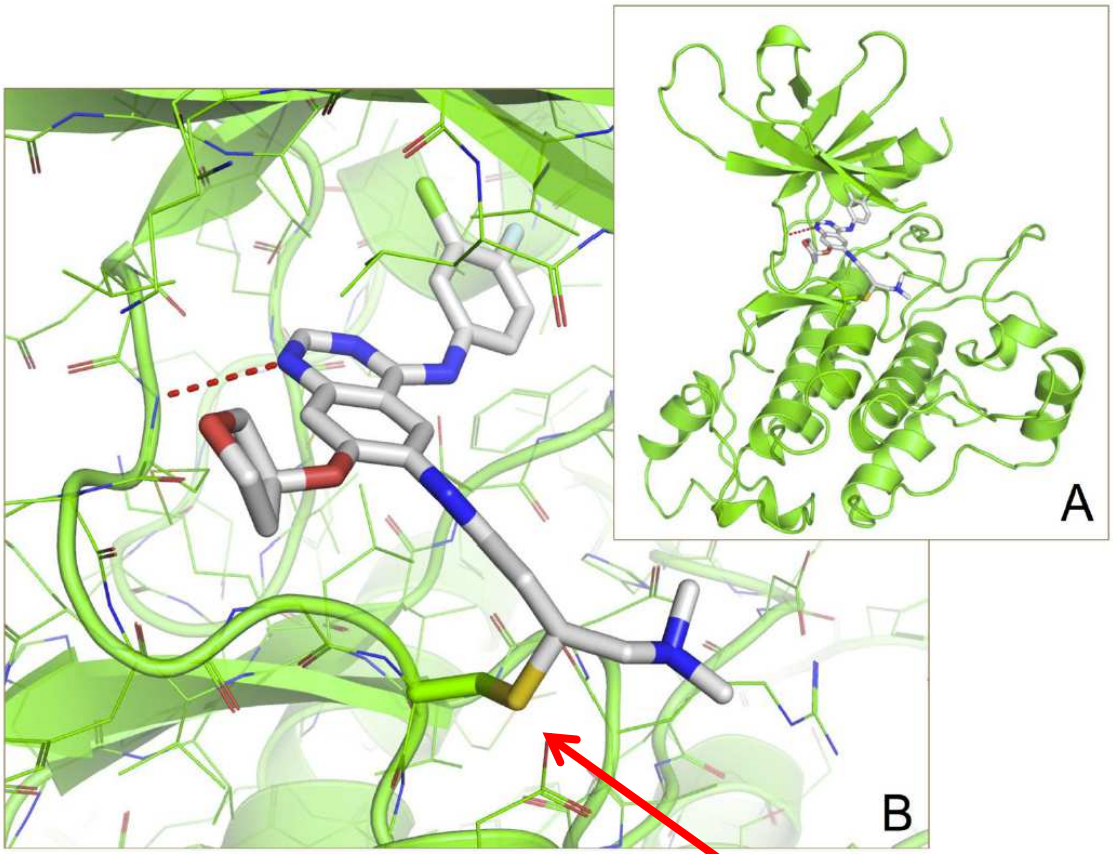
Singh, J. *et al*. *Nat. Rev. Drug Discov.* **2011**, *10*, 307.
Warner, T. D. *et al. Proc. Natl. Acad. Sci. USA* **2002**, *99*, 13371.

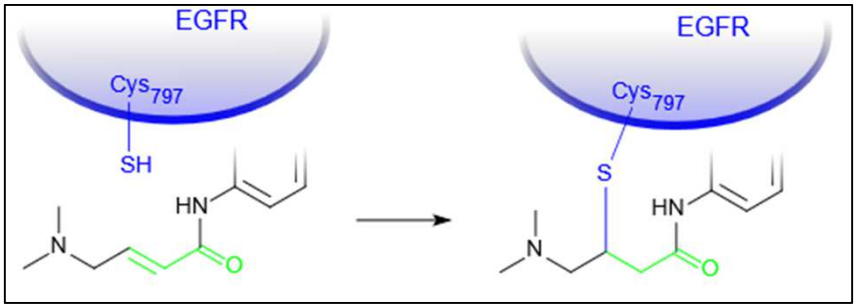
Contents

Afatinib



EGFR inhibitor
(Anti-NSCLC)
Firstly approved targeted covalent drug





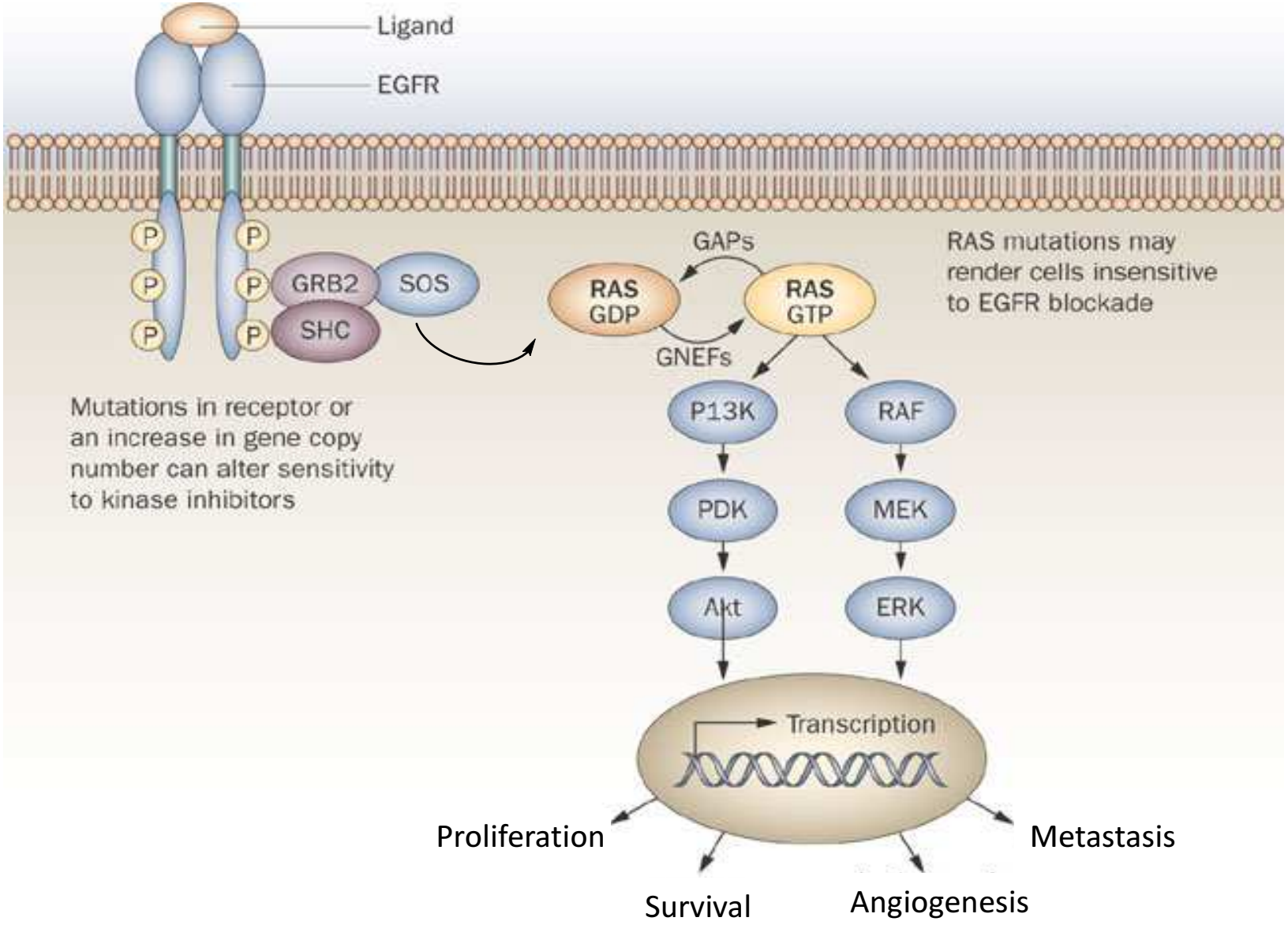
Afatinib covalently binds to Cys797

Lately, Cys 773 was corrected to Cys797.
Same Cys, but different numbering method.
In this material, they are described as “Cys797”.

Solca, F. *et al. J Pharmacol. Exp. Ther.* **2012**, *342*, 342.

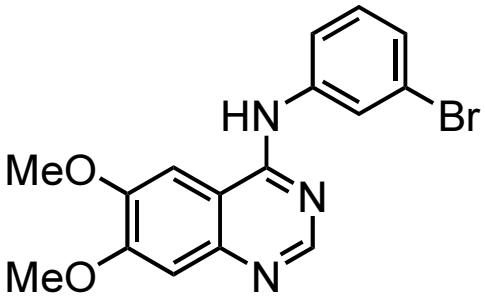
NSCLC : Non-Small-Cell Lung Cancer

EGFR mutation induces NSCLC

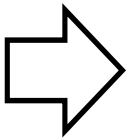


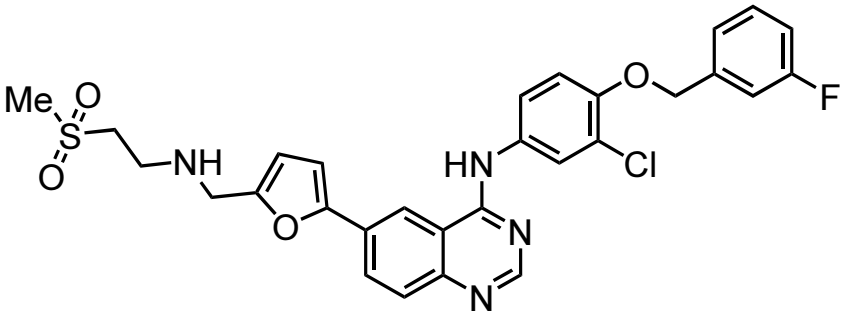
Kerr, D. J. *et al. Nat. Rev. Clin. Oncol.* **2009**, *6*, 499.

1st generation EGFR inhibitors (non-covalent)

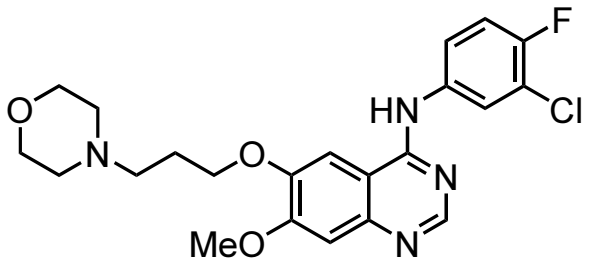


PD153035
EGFR inhibitor
IC_{50}=29 pM, K_i=5.2 pM

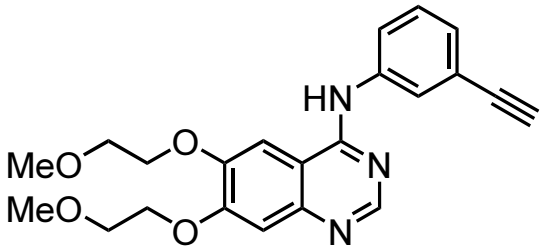




Lapatinib (Tykerb®)



Gefitinib (Iressa®)

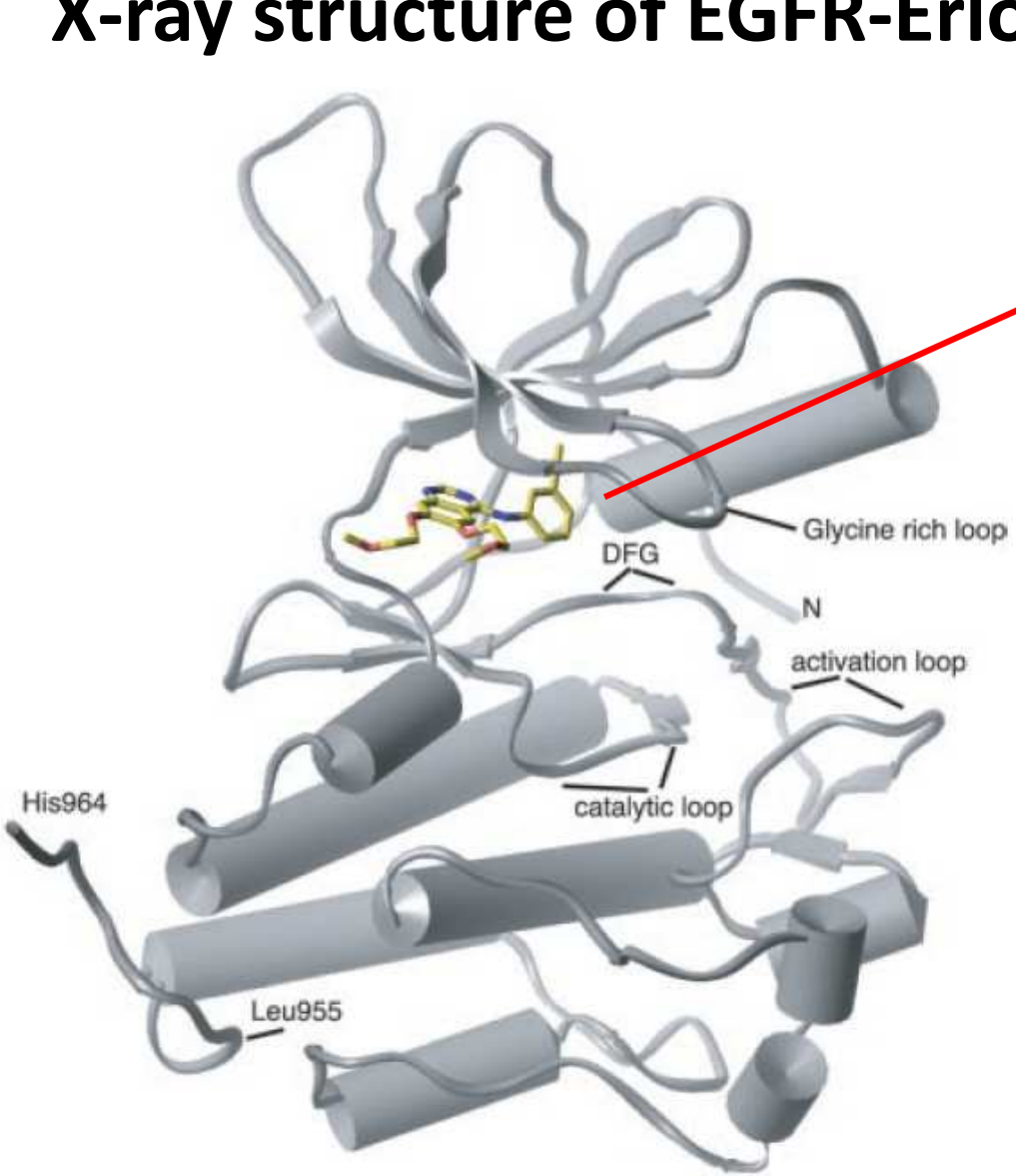


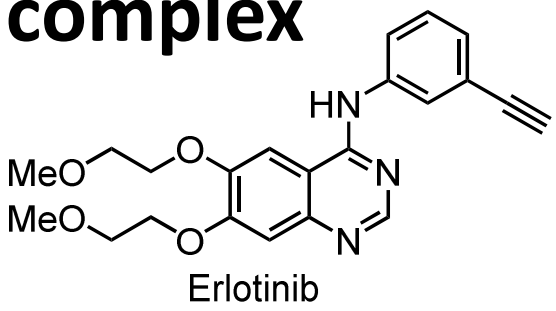
Erlotinib (Tarceva®)

etc...

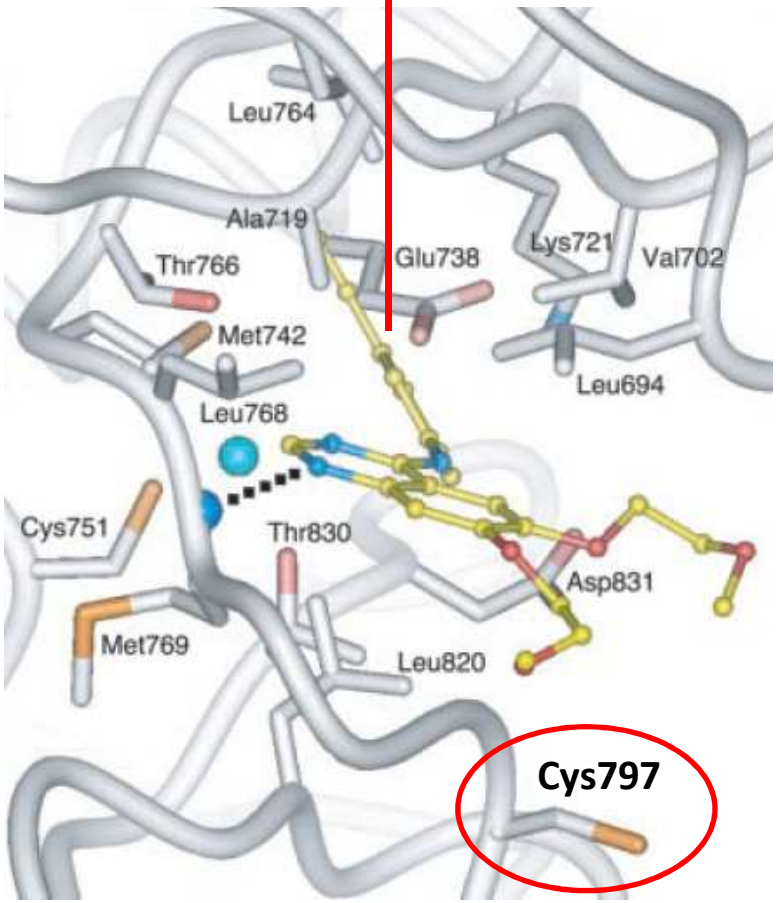
Fry, D. W. *et al. Science* **1994**, *265*, 1093.

X-ray structure of EGFR-Erlotinib complex





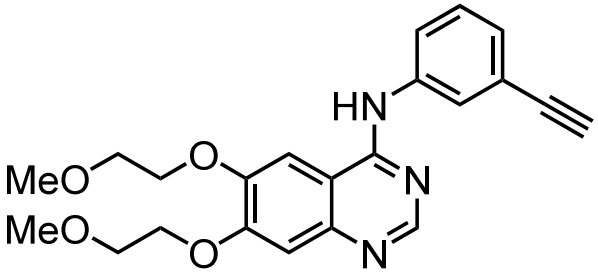
Erlotinib



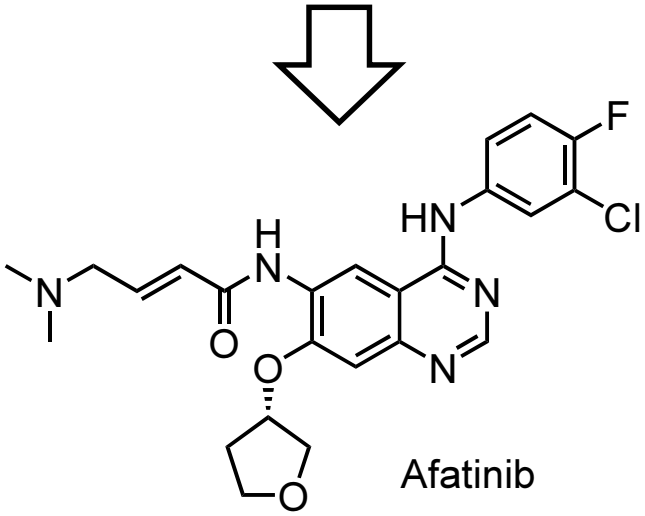
Eigenbrot, C. *et al. J. Biol. Chem.* **2002**, *277*, 46265.

PDB code:1M17

Afatinib : 2nd generation EGFR inhibitor (covalent)

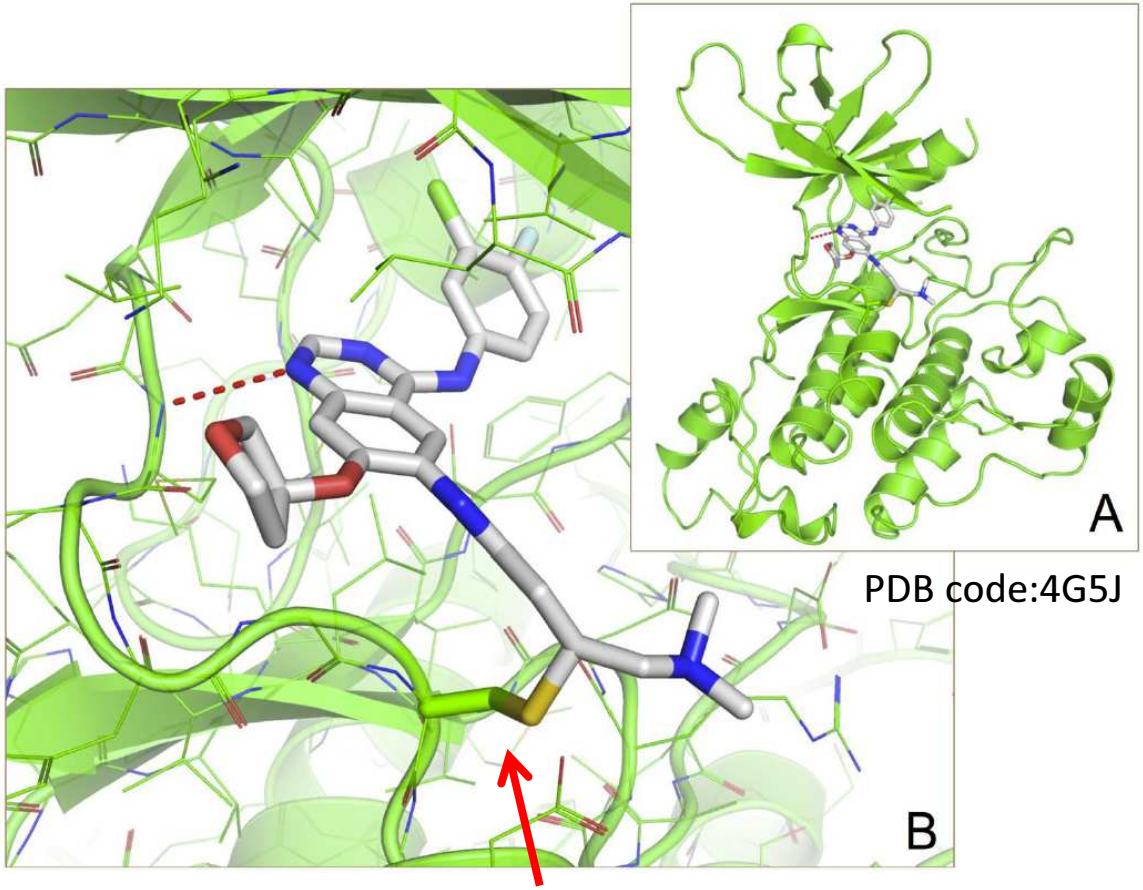


Erlotinib



Afatinib

X-ray structure of Afatinib-EGFR complex

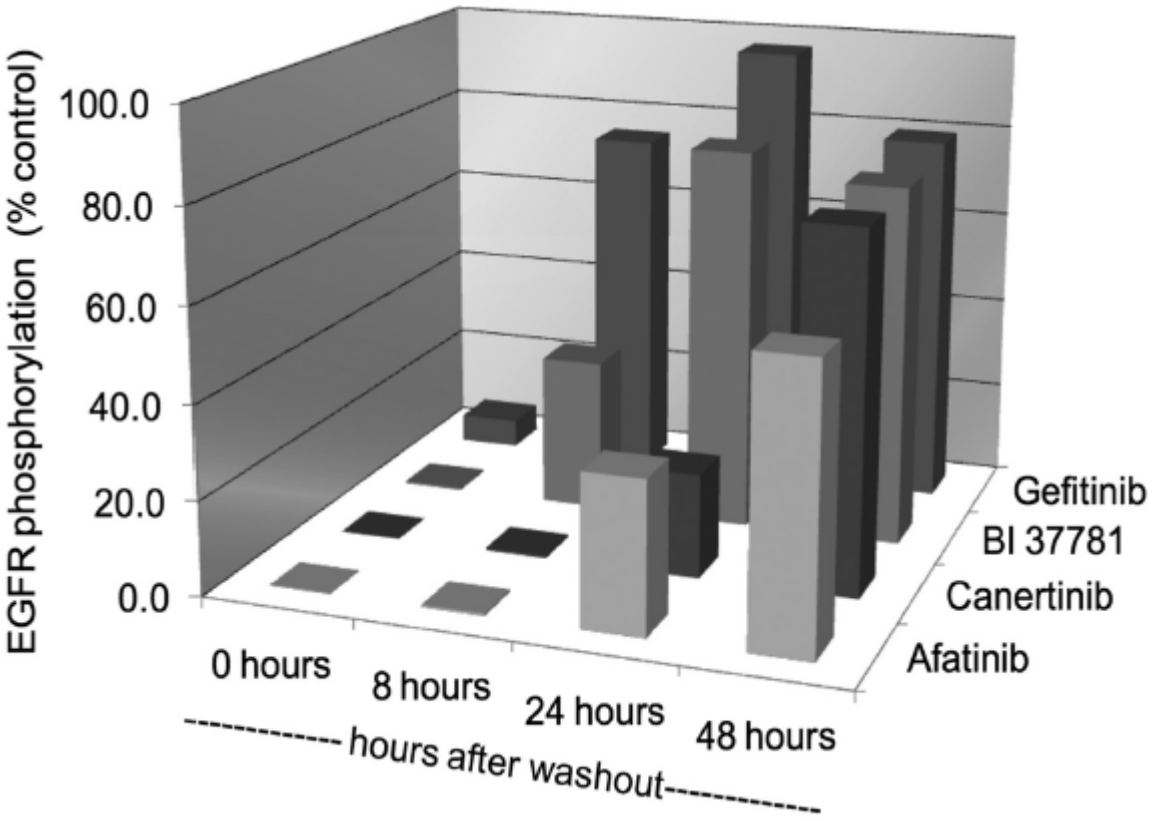


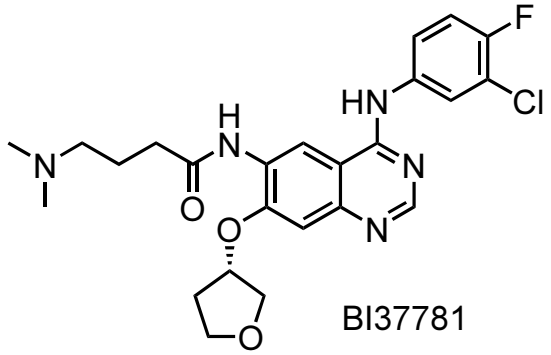
PDB code:4G5J

Afatinib covalently binds to Cys797
Also confirmed by MS/MS

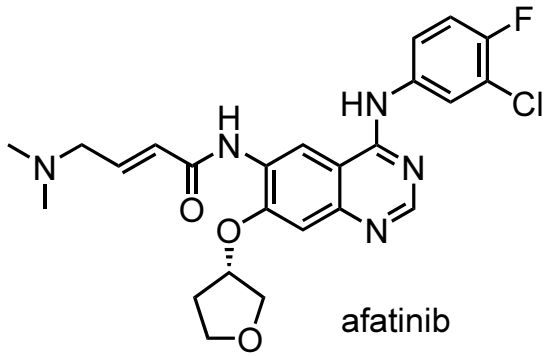
Solca, F. *et al. J Pharmacol. Exp. Ther.* **2012**, *342*, 342.

Afatinib inhibition continues after washout

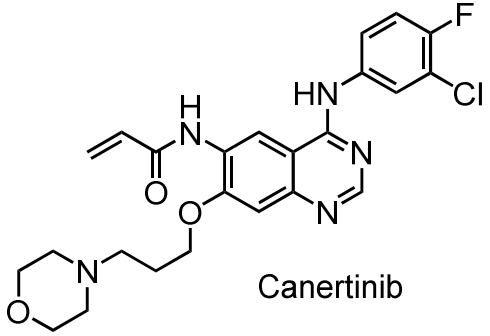




BI37781



afatinib

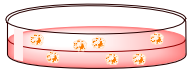


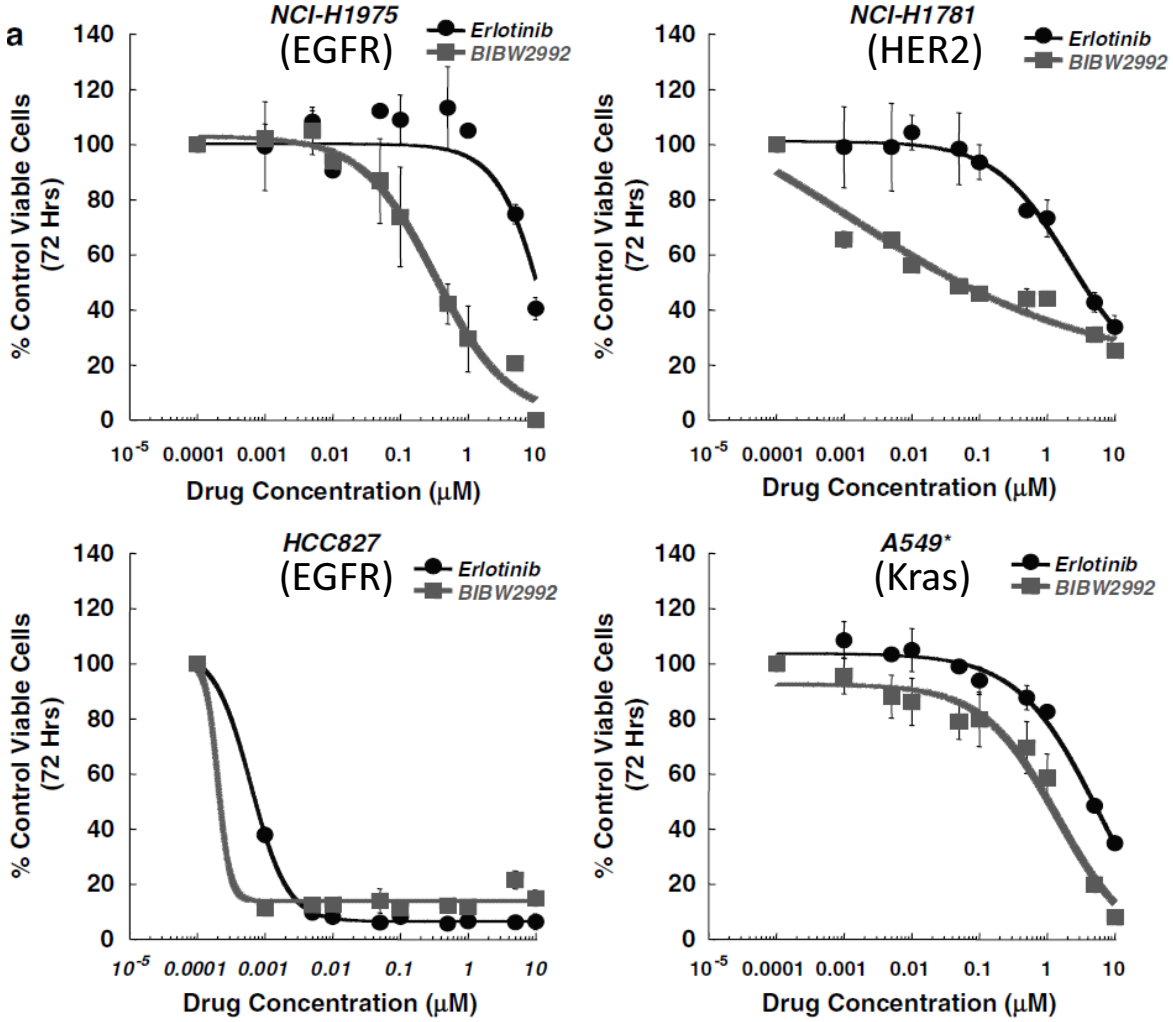
Canertinib

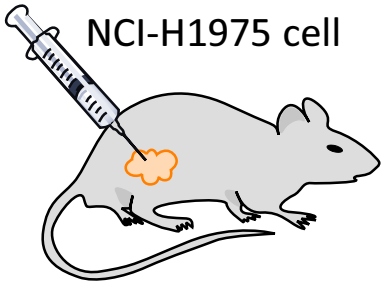
Solca, F. *et al. J Pharmacol. Exp. Ther.* **2012**, *342*, 342.

Afatinib (BIBW2992) effect *in vivo*

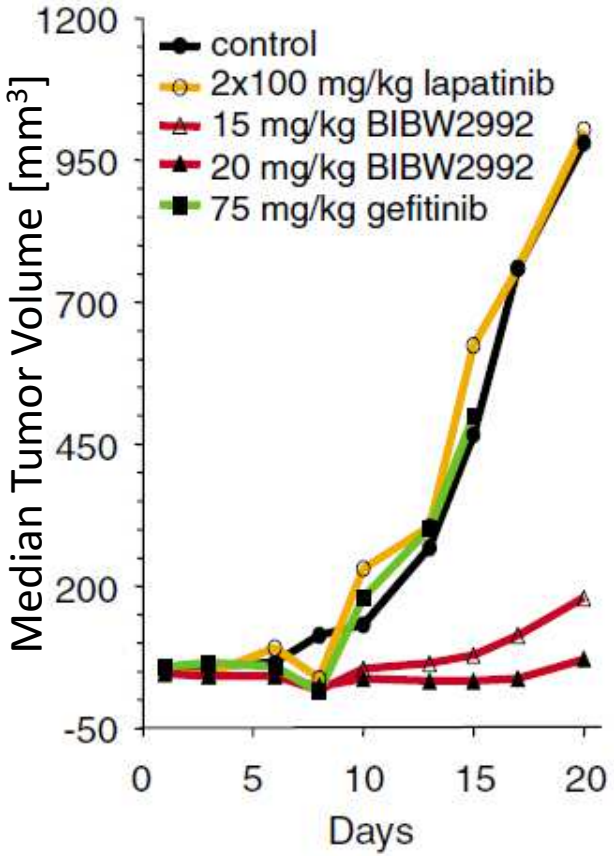
Human NSCLC cell lines (mutant proteins)





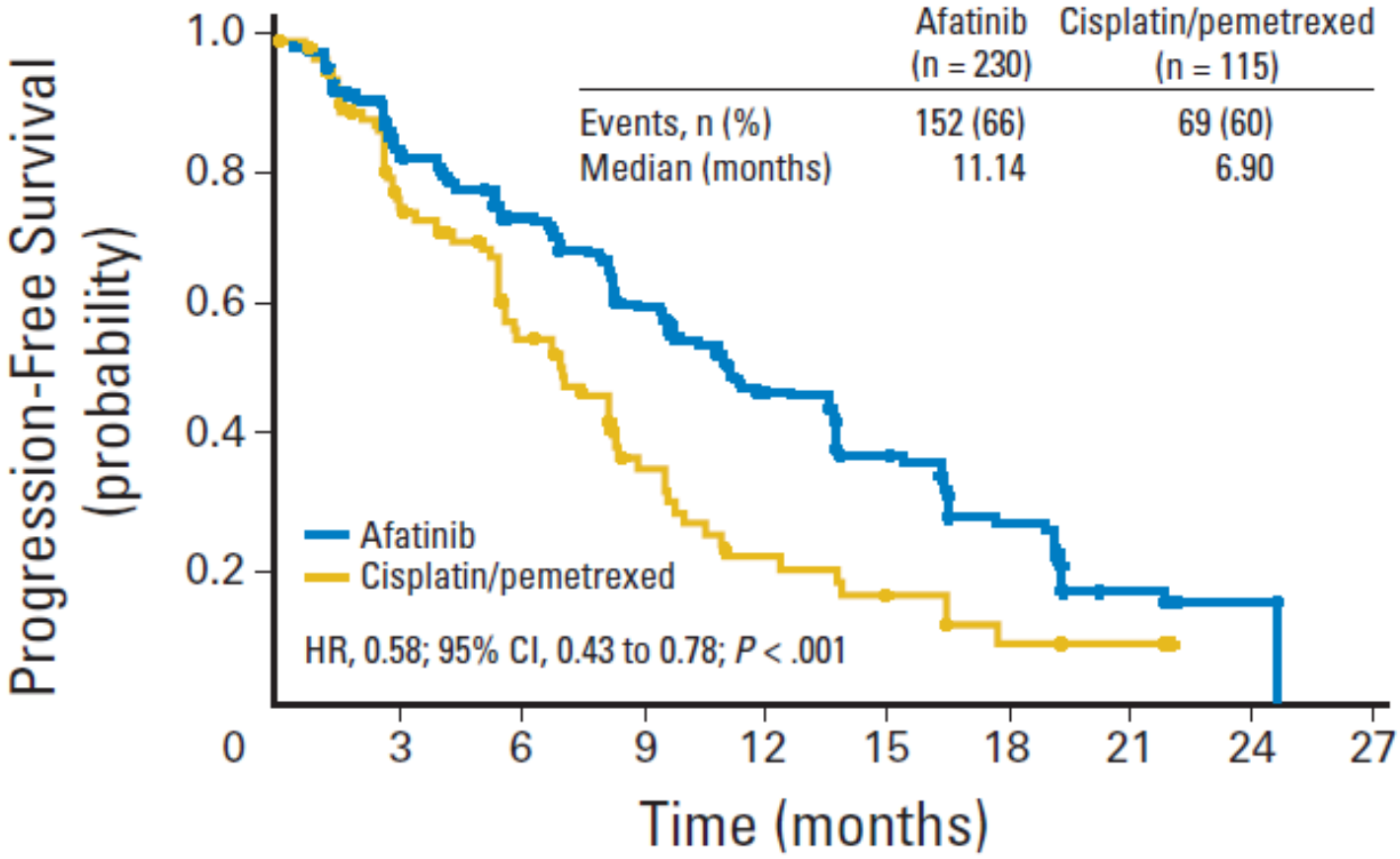


Mice xenograft model



Solca, F. *et al. Oncogene* **2008**, *27*, 4702.

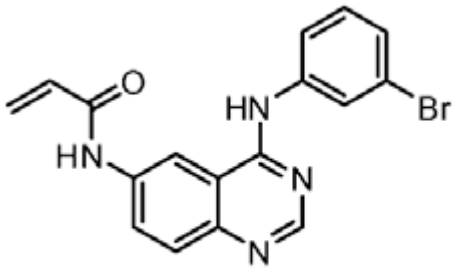
Phase III Study : Afatinib or chemotherapy



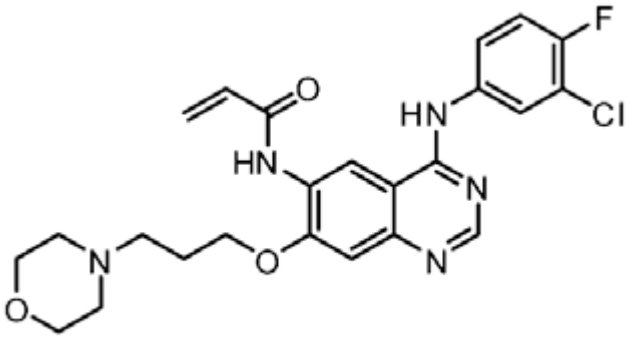
Direct comparisons between Afatinib and Gefitinib (or Erlotinib) in clinical trial are ongoing (Lux-Lung 7 (or 8)).

Yang, J. C.-H. *et al. J. Clin. Oncol.* **2013**, *31*, 3327.

Potential risk : alkylate Bmx kinase

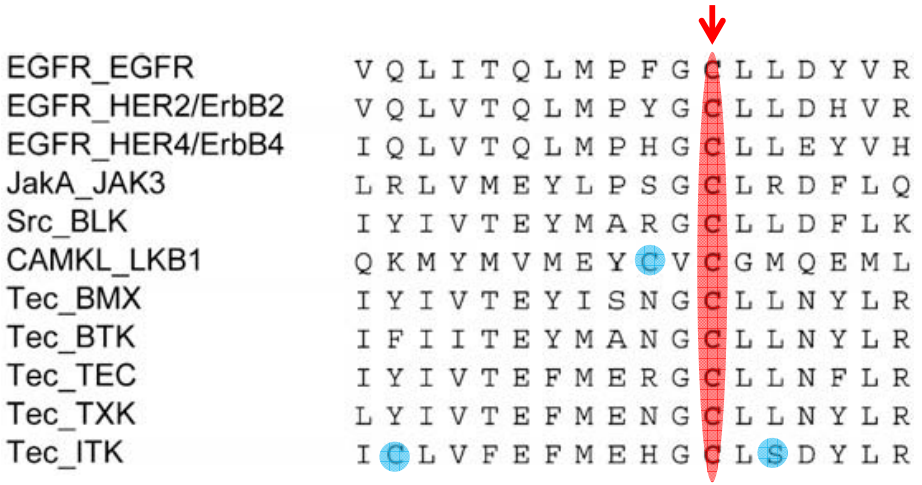


PD168393



CI-1033

Cys797 (EGFR) is highly conserved in TK family



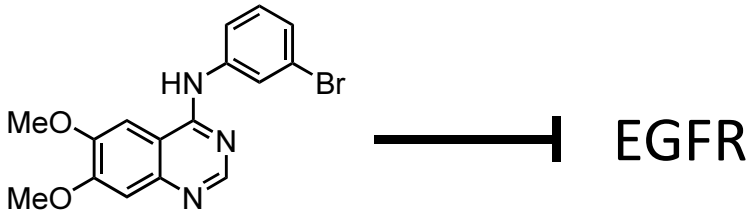
IC_{50} (μM)	Enzymatic assay		Tel-kinase Ba/F3	
	PD168393	CI-1033	PD168393	CI-1033
EGFR	<0.0015	0.0023	ND	ND
HER2	0.024	0.048	ND	ND
HER4	0.012	0.014	ND	ND
Jak3	3.14	3.88	>10	2
Blk	5.47	0.05	>10	0.029
Lkb1	>10	>10	–	–
Bmx	1.1	0.586	0.303	0.062
Btk	5.83	0.185	ND	ND
Tec, Txk	ND	ND	ND	ND
Itk	>30	5.65	ND	ND

No data about Afatinib, but there may be potential risk.
Non-conserved residue is desired for target.

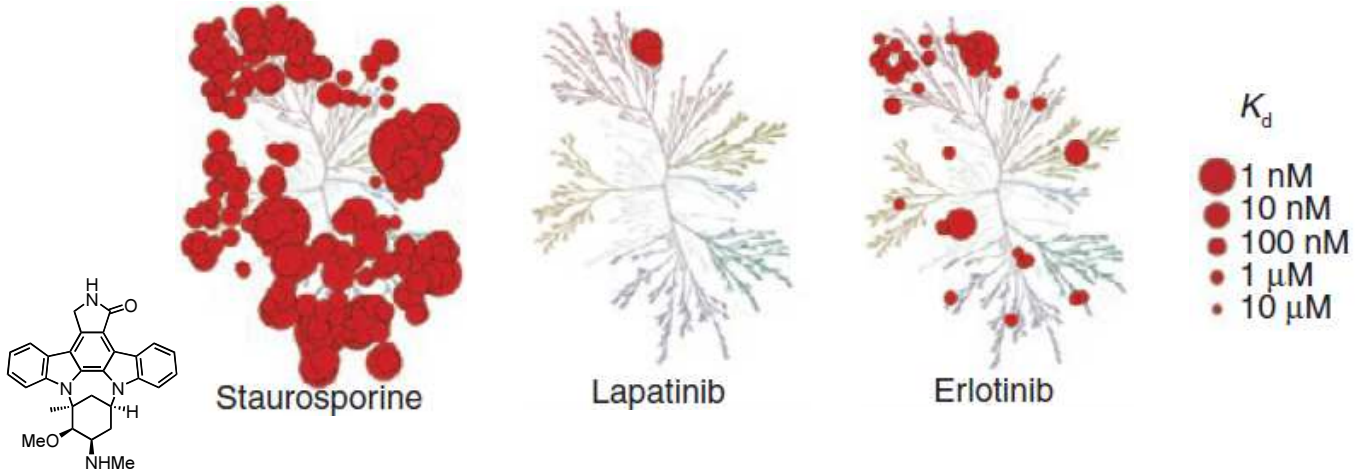
Gray, N. S. *et al. Bioorg. Med. Chem. Lett.* **2008**, *18*, 5916.

Points to obtain targeted covalent inhibitor (1)

1) Inhibitor known, Target known
→target-based HTS ⇔ phenotypic screening



2) High selectivity
minimized-interaction with off-target

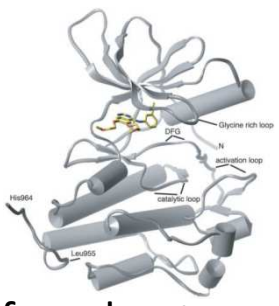


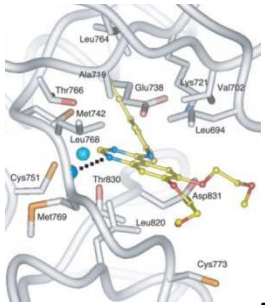
Zarrinkar, P. P. *et al. Nat. Biotech.* **2008**, *26*, 127.

Points to obtain targeted covalent inhibitor (2)

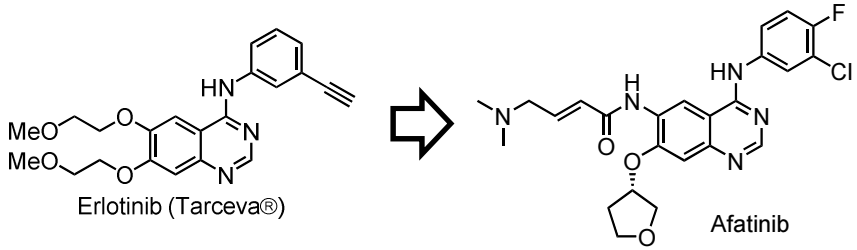
3) Introduce reactive warhead to inhibitor

Target-inhibitor structure, interaction known or homology modeling available

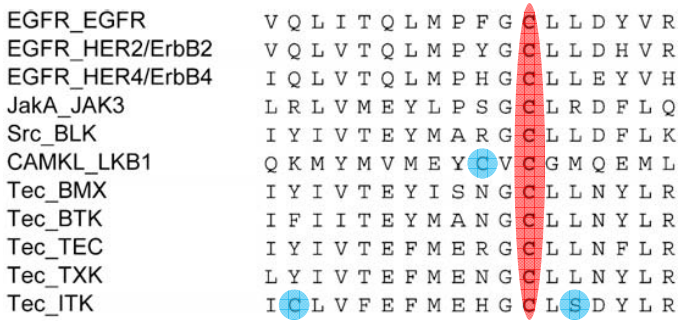




Warhead of moderate reactivity, at appropriate position



Target residue is preferred to non-conserved using protein database (PDB, UniProt, genomatix etc…)



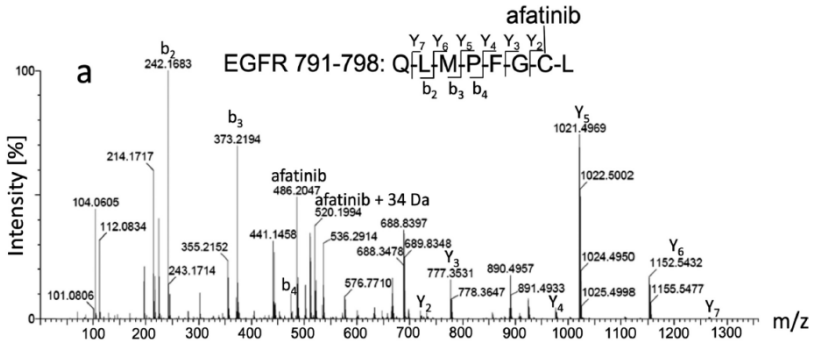
Computational chemistry

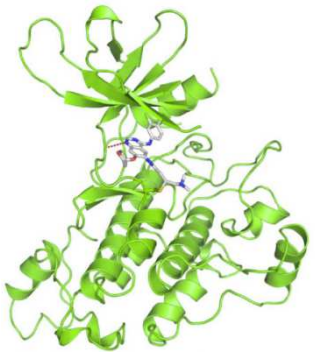
Bioinformatics

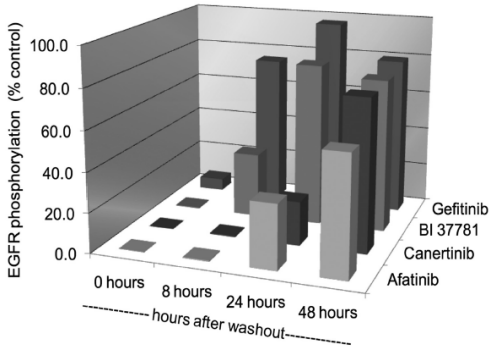
This step might be bottle neck.

Points to obtain targeted covalent inhibitor (3)

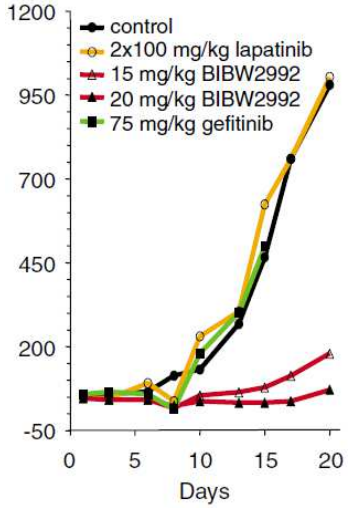
4) Check affinity and covalent bond formation
by MS/MS, X-ray crystal, wash-out experiment etc…

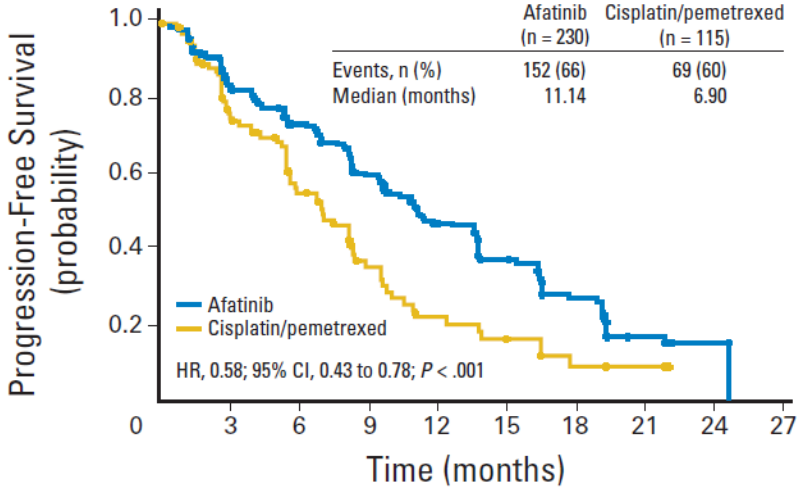






5) Therapeutic effects vs adverse effect
in vitro (enzyme), *in vivo* (cell culture), and clinical stage (patients)

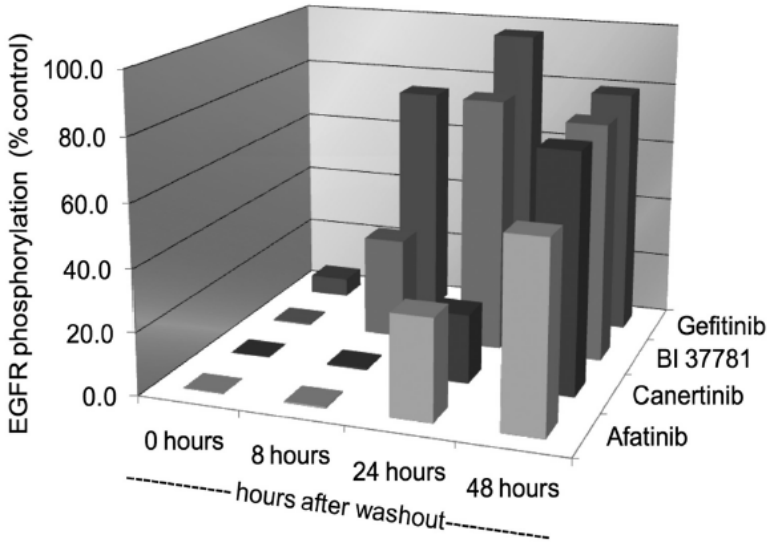


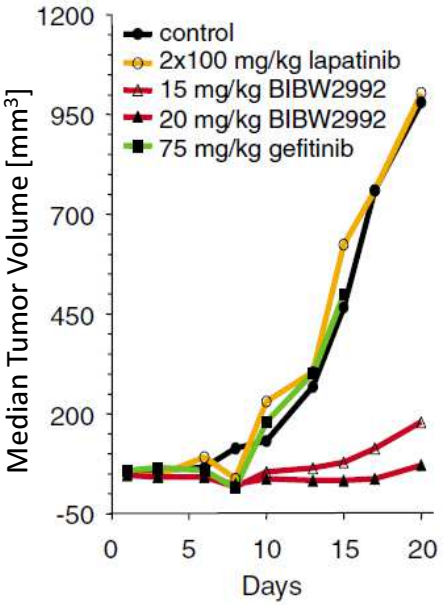


Characteristics (1)

・Strong and prolonged pharmacodynamic activity

1) More complete target inhibition





2) Lower dose

<u>Afatinib: 50 mg/day</u> <u>(Mw:486)</u>

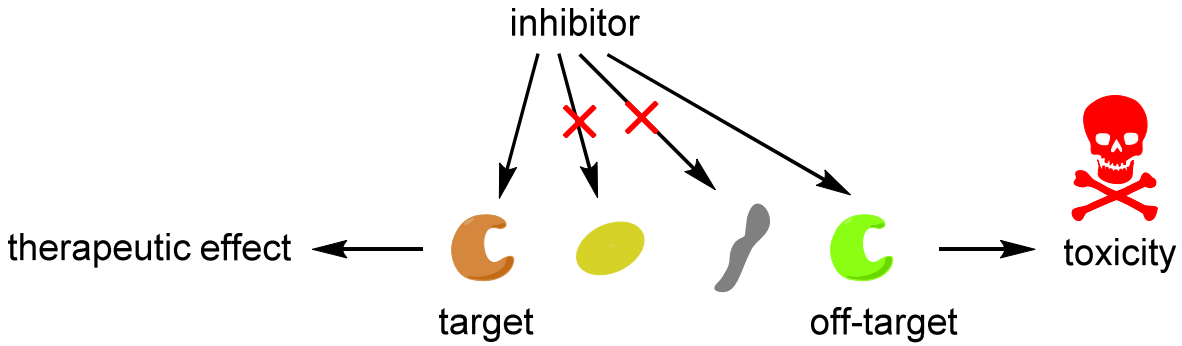
Lapatinib: 1250 mg/day (Mw:943)

Gefitinib: 250 mg/day (Mw:447)

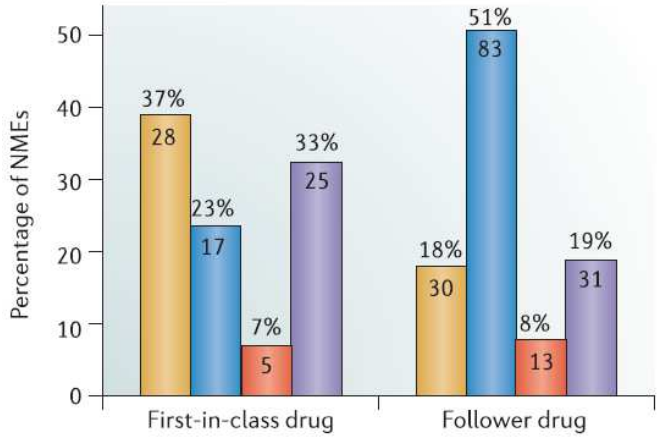
Erlotinib: 150 mg/day (Mw:486)

Characteristics (2)

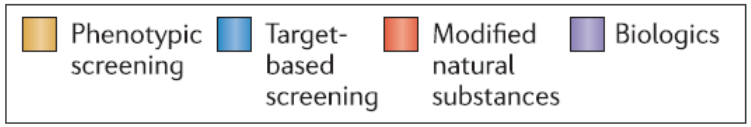
・Toxicity due to off-target・・・Current target is limited to severe (fatal) disease.
Does covalent type give more severe toxicity?
→Necessity of comprehensive study
Targeting non-conserved residue, using moderate reacting group.



・Can be Best-in-class, but can not be First-in-class
(common issue in target-based drugs)

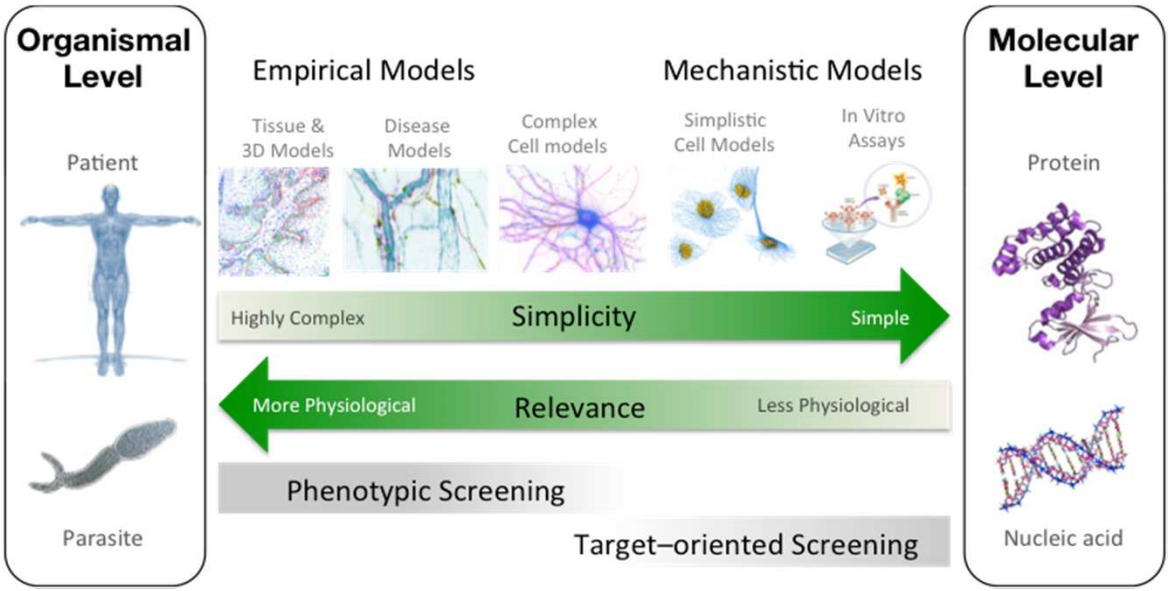


Distribution of new drugs (1999-2008)

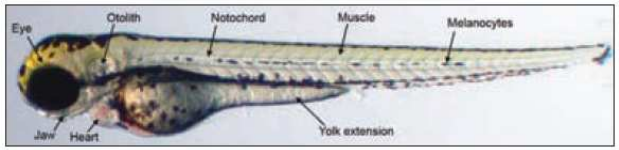


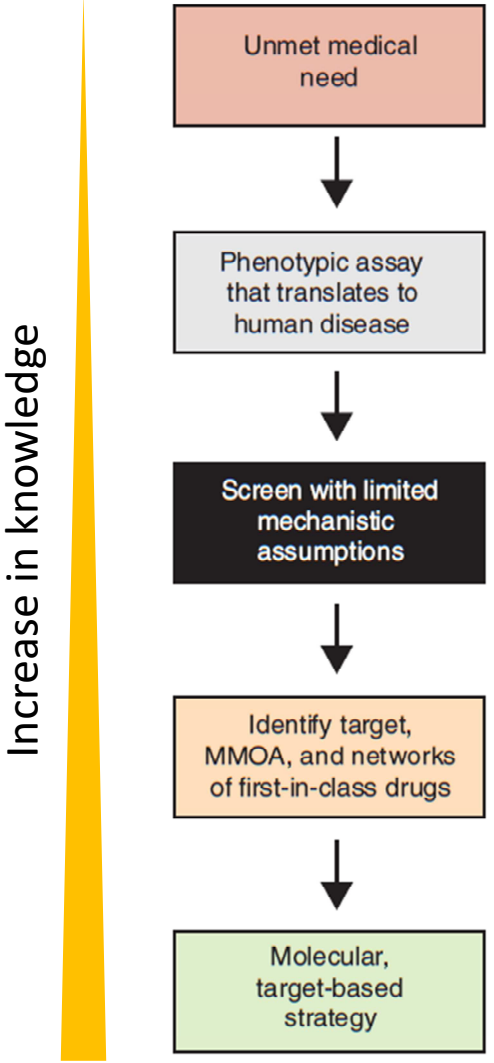
Swinney, D. C. *et al*.
Nat. Rev. Drug Discov. **2011**, *10*, 507.

Drug discovery: Phenotipic and Target-oriented



http://www.sulsa.ac.uk/research-facilities/uk-npsc/phenotypic-screening





e.g) Phenotipic screening using Zebrafish larvae in 96-well plate

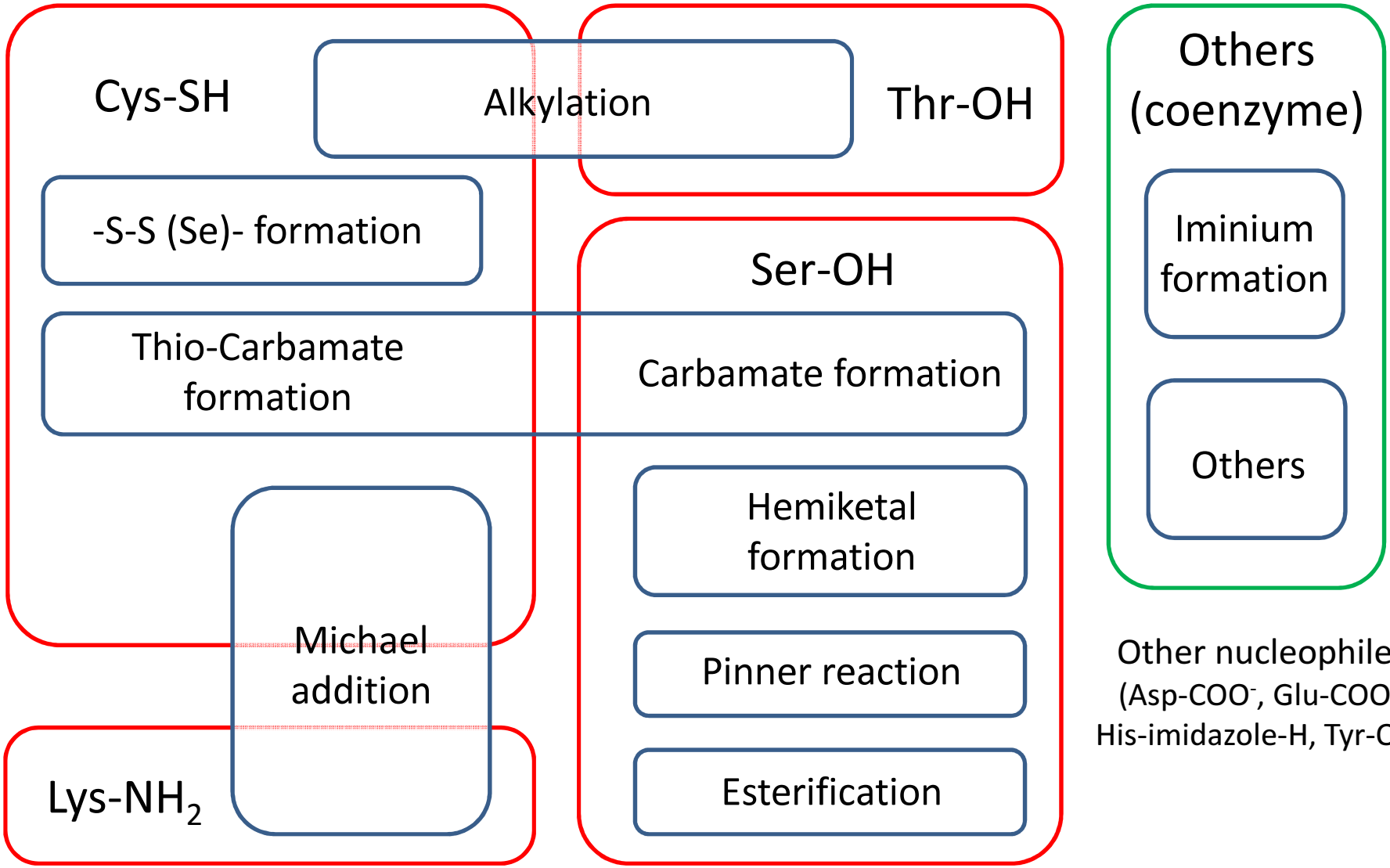
http://www.ddw-online.com/chemistry/p102797-zebrafish:-a-versatile-in-vivo-model-for-drug-safety-assessmentfall-06.html

Swinney, D. C. *Clin. Pharmacol. Ther.* **2013**, *93*, 299.

Contents

Target reactions and residues

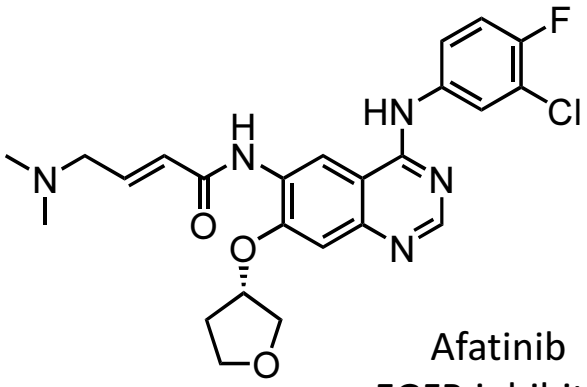
*DNA-targeted drugs are excepted



(In all cases, compound after phase II are picked up and derivatives are omitted.)

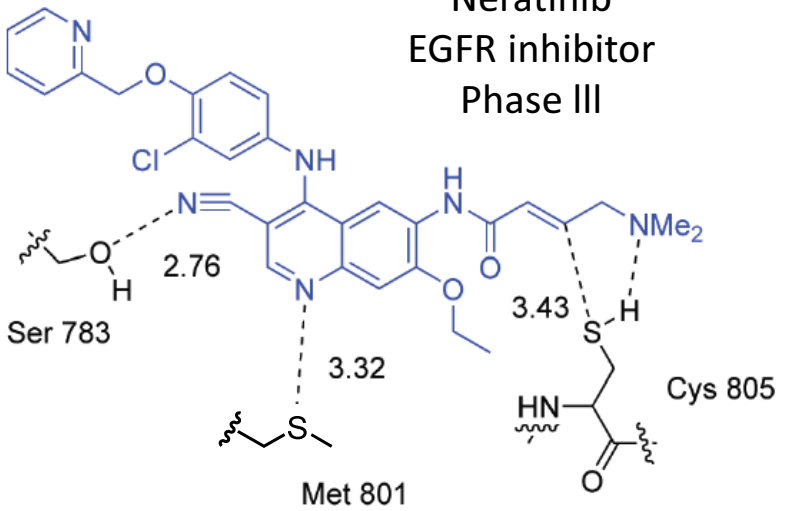
Potashman, M. H. *et al. J. Med. Chem.* **2009**, *52*, 1231.; Barf, T. *et al. J. Med. Chem.* **2012**, *55*, 6243.

1. Michael addition

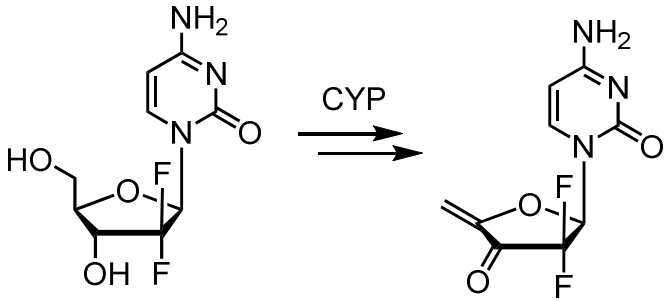


Afatinib
EGFR inhibitor
(Anti-NSCLC)

Neratinib
EGFR inhibitor
Phase lll



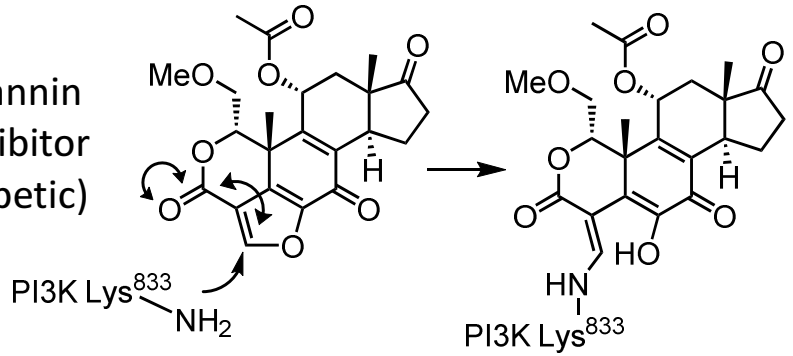
Tsou, H.-R. *et al. J. Med. Chem.* **2005**, *48*, 1107.



Gemcitaine (prodrug)
DNA synthetase inhibitor
(Anti-cancer)

Stubbe, J. *et al. J. Med. Chem.* **1991**, *34*, 1879.

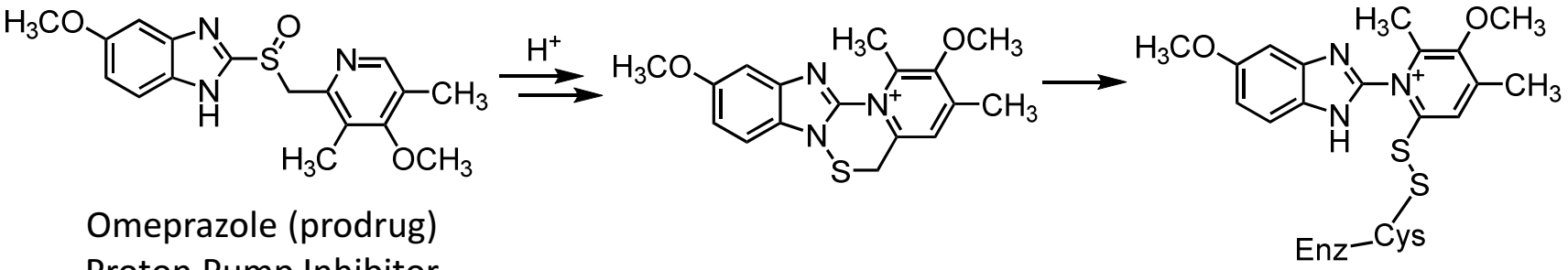
Wortmannin
PI3K inhibitor
(antidiabetic)



Furan-ring opening

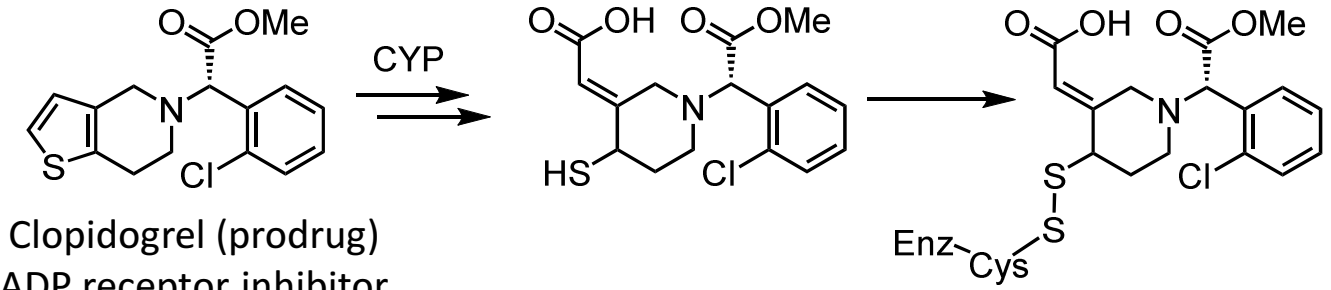
Wymann, M. P. *et al. Mol. Cell. Biol.* 1996, 16, 1722.

2. -S-S(Se)- formation



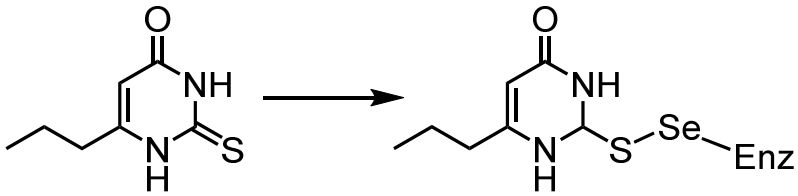
Omeprazole (prodrug)
Proton Pump Inhibitor

Olbe, L. *et al*. *Nat. Rev. Drug Discov.* **2003**, *2*, 132.



Clopidogrel (prodrug)
ADP receptor inhibitor
(Anti-platelet aggregation)

Herbert, J. M. *et al. Thromb. Haemostasis* **2000**, *84*, 891.

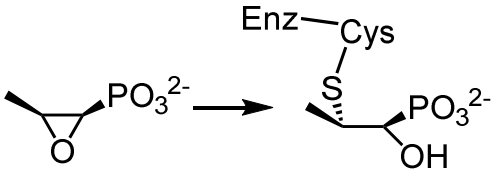


Propylthiouracil
thyroxine 5'-deionase inhibitor
(Anti-hyperthyroidism)

(Target amino acid is Seleno-cystein)

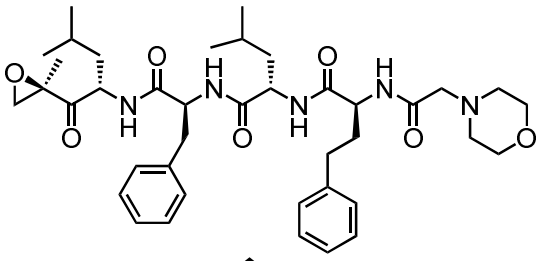
Sies, H. *et al. Org. Biomol. Chem.* **2003**, *1*, 2848.

3. Alkylation



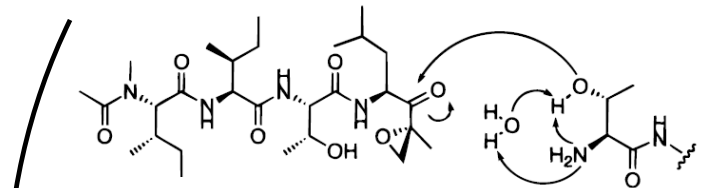
Fosfomycin
MurA inhibitor
(antibiotic)

Macheroux, P. *et al.*
Eur. J. Biochem. **2004**, *271*, 2682.

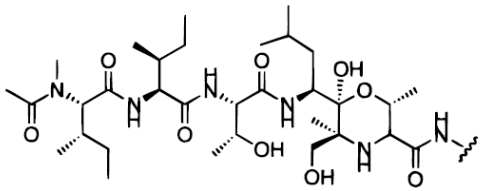


Carfizomib
Proteasome inhibitor
(anti-cancer)

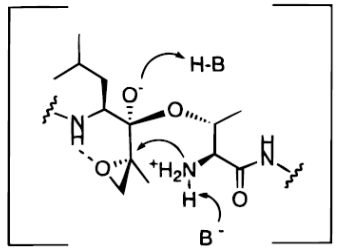
Orlowski, R. Z. *et al.*
Blood. **2007**, *110*, 3281.



Epoxomicin

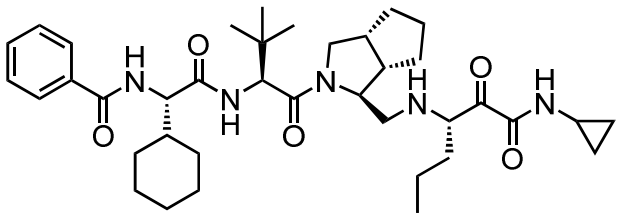


(by X-ray structure)

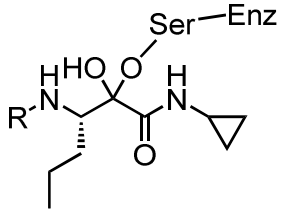


Crews, C. M. *et al. J. Am. Chem. Soc.* **2000,** *122,* 1237.

4. Hemiketal formation (reversible)

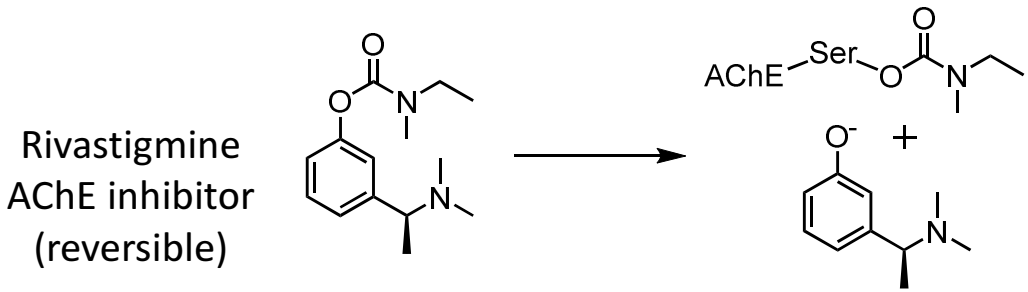


Telaprevir
HCV NS3·4A inhibitor



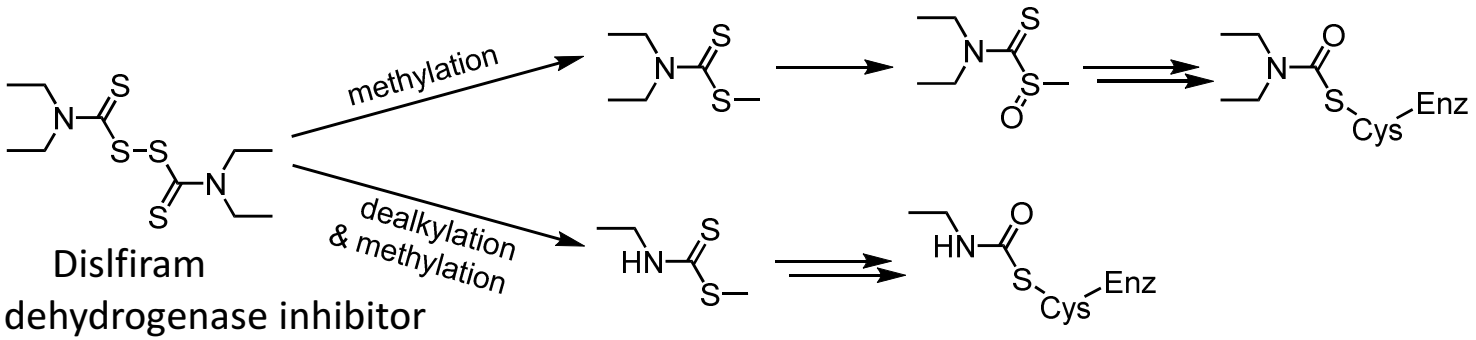
Lin, C. *et al. J. Biol. Chem.* **2004**, *279*, 17508.

5. (Thio)Carbamate formation



Rivastigmine
AChE inhibitor
(reversible)

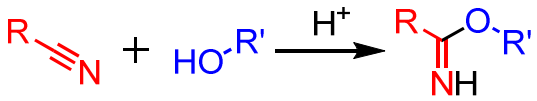
Silman, I. *et al. Biochemistry,* **2002,** *41,* 3555.

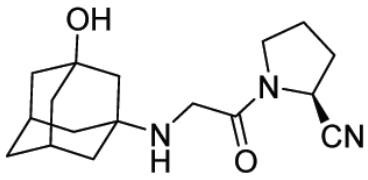


Dislfiram
Aldehyde dehydrogenase inhibitor

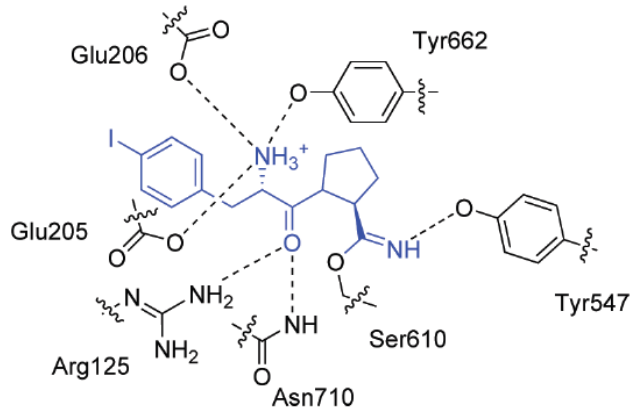
Naylor, S. *et al. Biochem. Pharmacol.* **2001**, *61*, 537.

6. Pinner reaction (reversible)



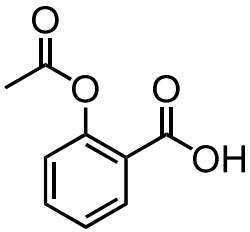


Vildagliptin
DPP-4 inhibitor

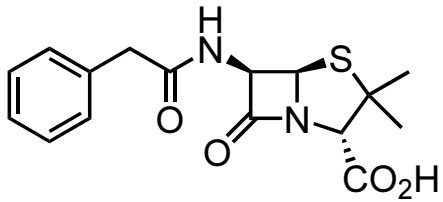


Peters, J.-U. *Curr. Top. Med. Chem.* **2007**, *7*, 579.

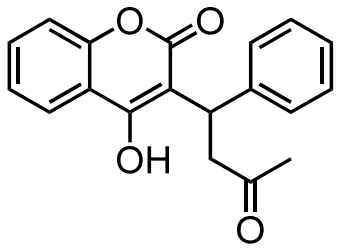
7. Esterification (reversible)



Aspirin
COX-2 inhibitor

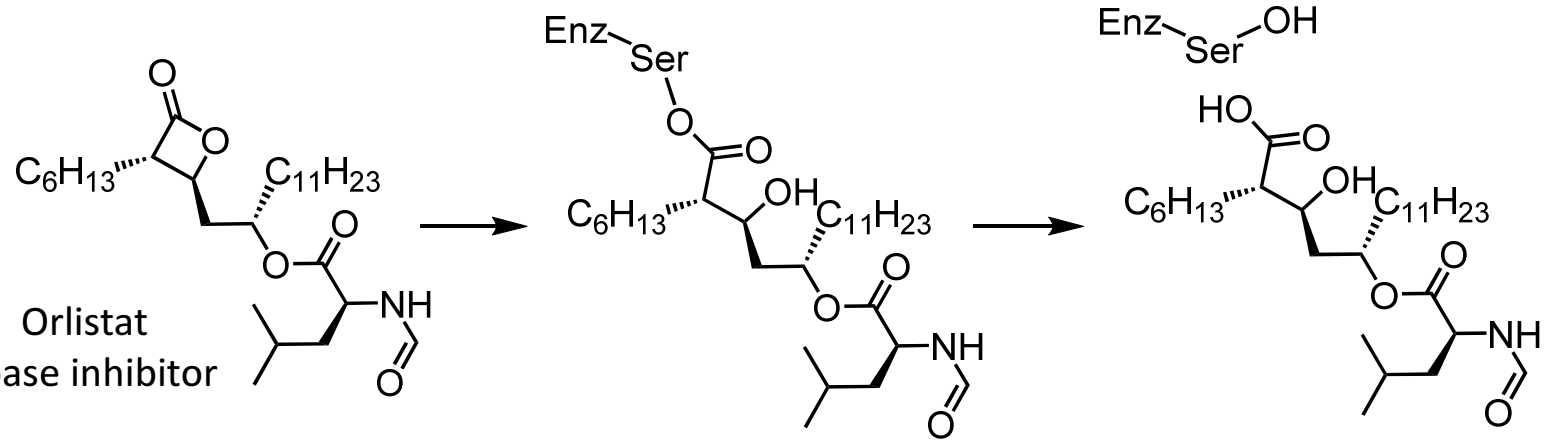


Penicillin
Peptidoglycan synthetase inhibitor



Warfarin
V.K. reductase inhibitor
(Anticoagulant)

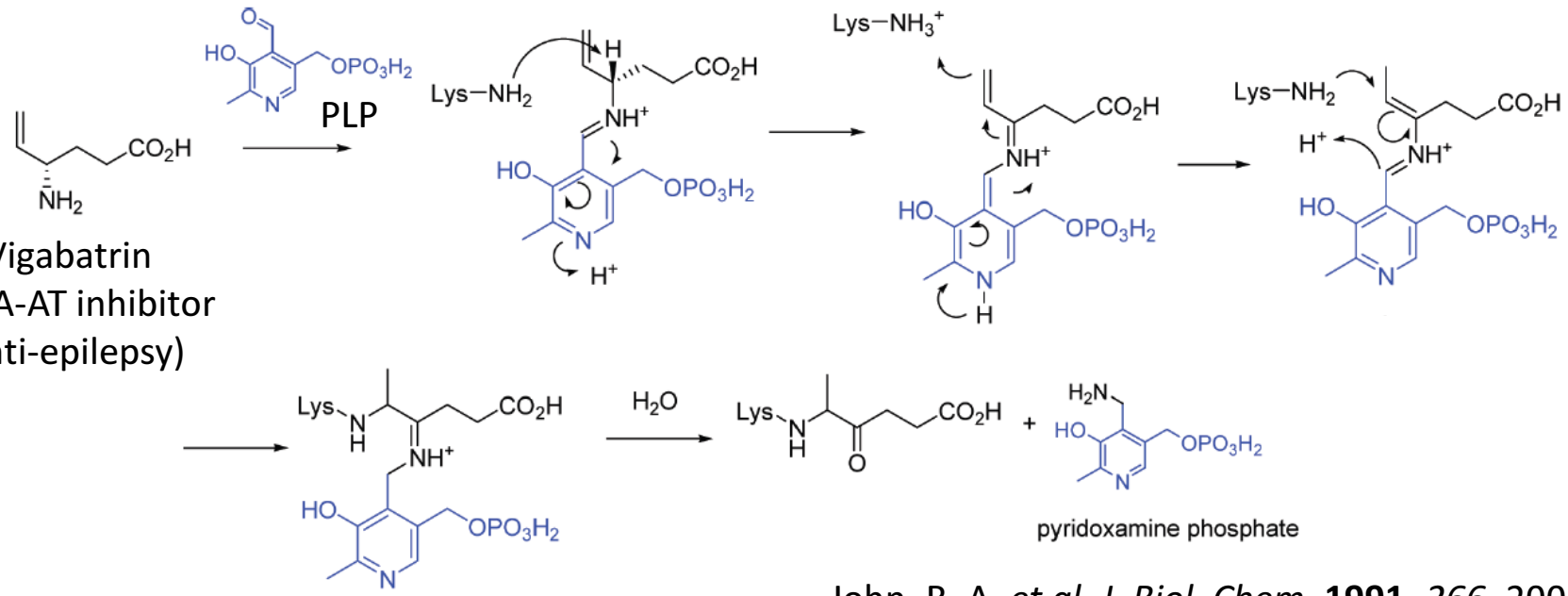
Fasco, M. J. *et al. J. Biol. Chem.* **1982**, *257*, 4894.



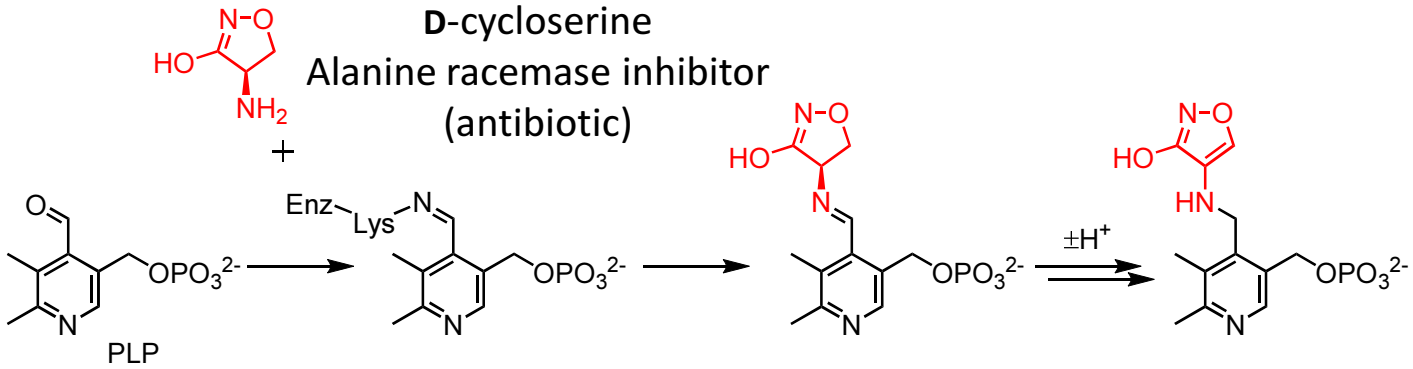
Orlistat
Lipase inhibitor

Hadvary, P. et al. *J. Biol. Chem.* **1991**, *266*, 2021.

8. Iminium formation

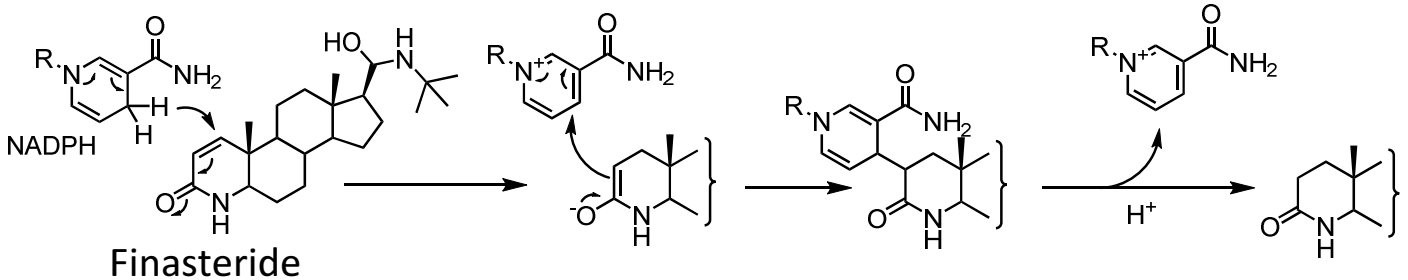


John, R. A. *et al. J. Biol. Chem.* **1991**, *266*, 20056.



Ringe, D. *et al. Biochemistry* **2003,** *42,* 5775.

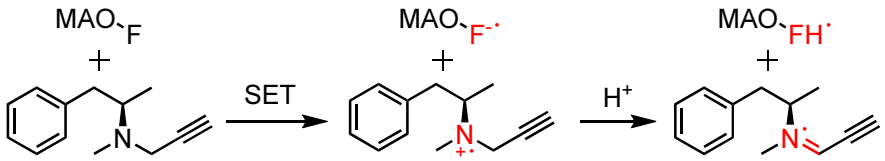
9. Others



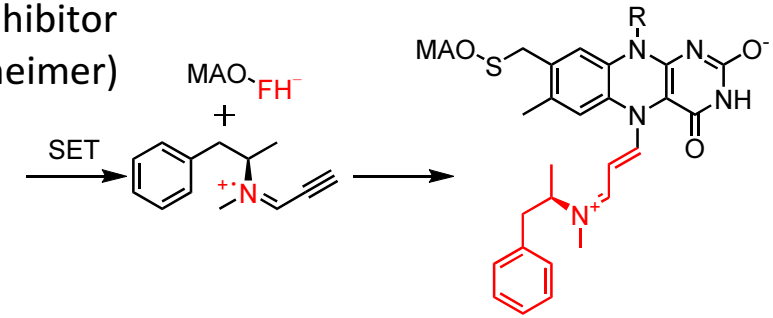
Finasteride
5-α-reductase inhibitor
(Anti-AGA)

(reversible)

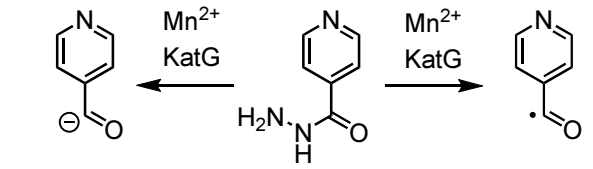
Bull, H. G. *et al. J. Am. Chem. Soc.* **1996,** *118,* 2359.



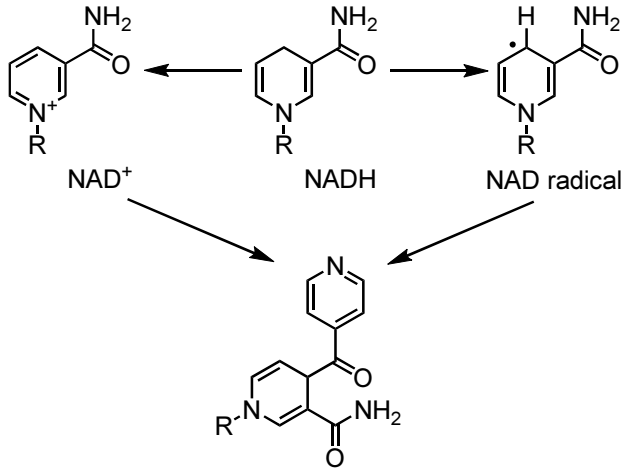
Selegiline
MAO-B inhibitor
(Anti-Alzheimer)



Mariano, P. S. *et al. J. Am. Chem. Soc.* **1998,** *120,* 5864-5872.

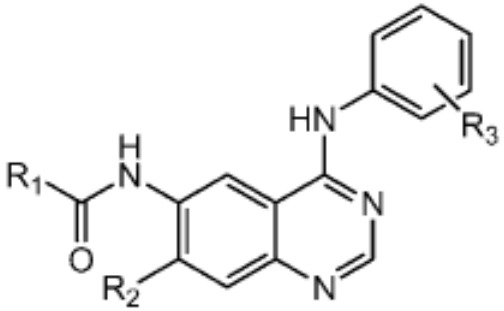


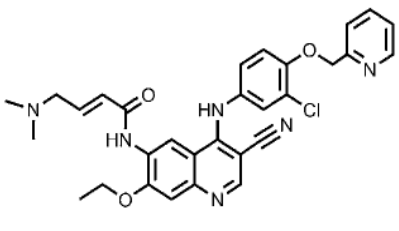
Isoniazid (prodrug)
InhA inhibitor
Anti-tuberculous



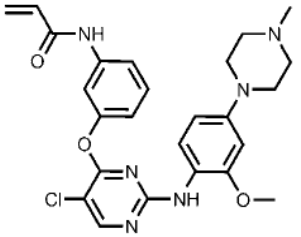
Sacchettini, J. C. *et al. Science*, **1998**, *279*, 98.

Kinetic analysis of MA-covalent inhibitors

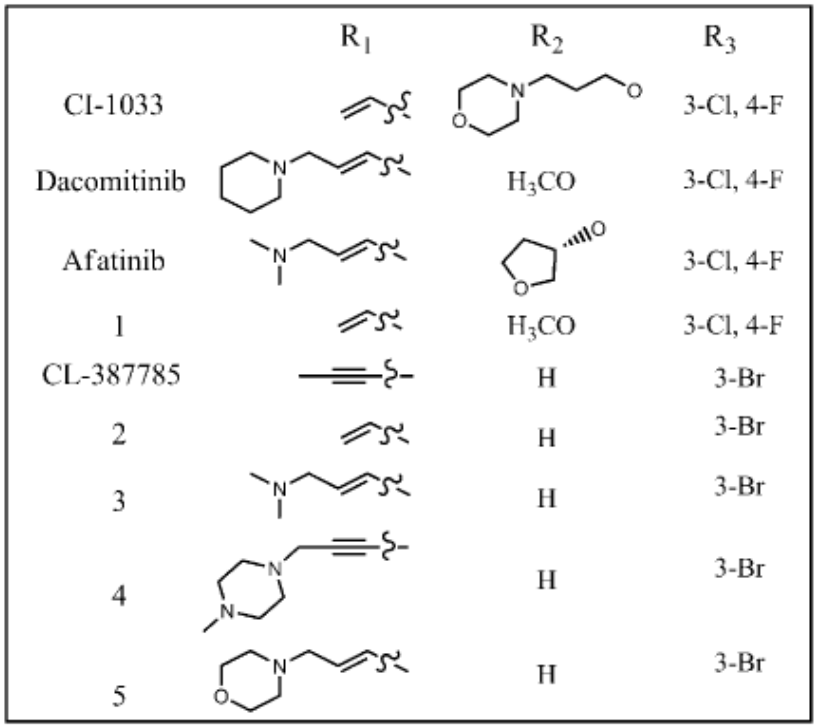




Neratinib



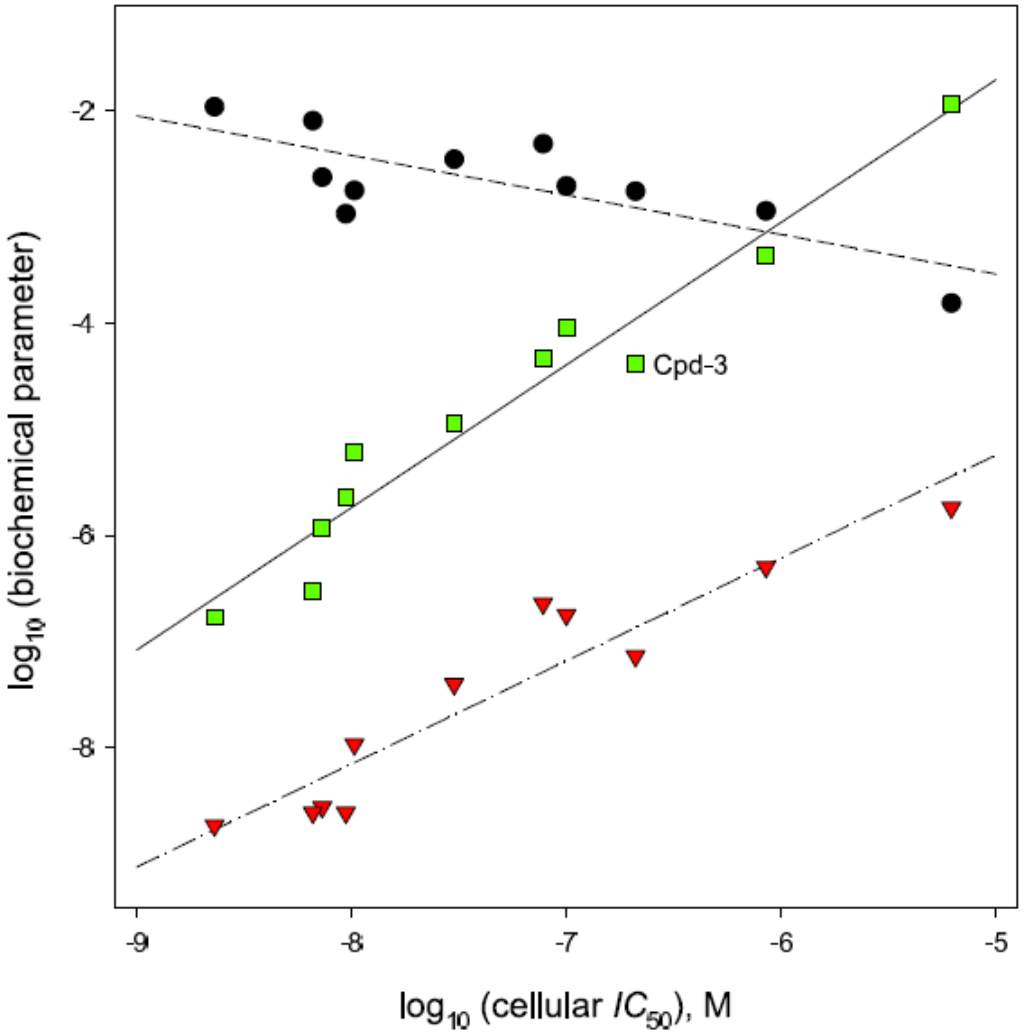
WZ4002



	EGFR-L858R/T790M				WT EGFR			
Inhibitor	K_i (nM)	k_{inact} (ms^{-1})	k_{inact}/K_i ($\mu M^{-1}s^{-1}$)	H1975 IC_{50} (nM)	K_i (nM)	k_{inact} (ms^{-1})	k_{inact}/K_i ($\mu M^{-1}s^{-1}$)	A549 IC_{50} (nM)
CI-1033	0.11 ± 0.03	11.0 ± 0.2	100 ± 20	2.3 ± 0.5	0.093 ± 0.002	2.9 ± 1.9	23 ± 1	4.9 ± 0.4
Dacomitinib	0.63 ± 0.05	1.8 ± 0.1	2.8 ± 0.3	10.3 ± 1.1	0.16 ± 0.01	1.5 ± 0.1	9.9 ± 0.8	2.5 ± 0.1
Afatinib	0.16 ± 0.03	2.4 ± 0.3	15 ± 4	7.3 ± 1.1	0.15 ± 0.01	0.9 ± 0.1	6.3 ± 0.8	11.5 ± 2.4
Neratinib	0.14 ± 0.03	1.1 ± 0.2	7 ± 2	9.4 ± 4.0	7.1 ± 0.4	1.8 ± 0.1	0.25 ± 0.01	5.2 ± 0.9
1	0.14 ± 0.07	8 ± 4	60 ± 40	6.6 ± 0.2	0.18 ± 0.01	2.3 ± 0.2	13 ± 1	5.8 ± 2.5
WZ-4002	13 ± 3	5.0 ± 0.1	0.40 ± 0.10	75 ± 25	28 ± 1	2.0 ± 0.1	0.089 ± 0.005	1,400 ± 400
CL-387785	10 ± 2	2.0 ± 0.3	0.21 ± 0.10	100 ± 7				
2	2.3 ± 0.3	3.5 ± 0.6	1.5 ± 0.3	30 ± 2				
3	4.0 ± 1.0	1.8 ± 0.1	0.40 ± 0.10	210 ± 3				
4	108 ± 20	1.5 ± 0.2	0.0014 ± 0.0003	6,200 ± 3,200				
5	30 ± 3	1.1 ± 0. 1	0.04 ± 0.01	850 ± 90				

Murraya, B. W. *et al.*
Proc. Natl. Acad. Sci. USA **2014**, *111*, 173.

Kinetic analysis of MA-covalent inhibitors

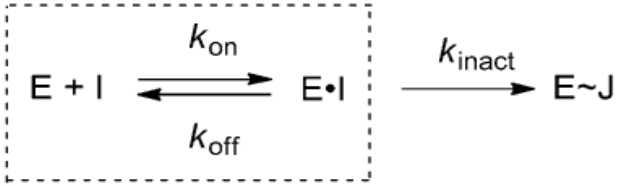


K_i^* / k_{inact}, M.s

k_{inact}, s^{-1}

K_i^*, M

$K_i = k_{off} / k_{on}$

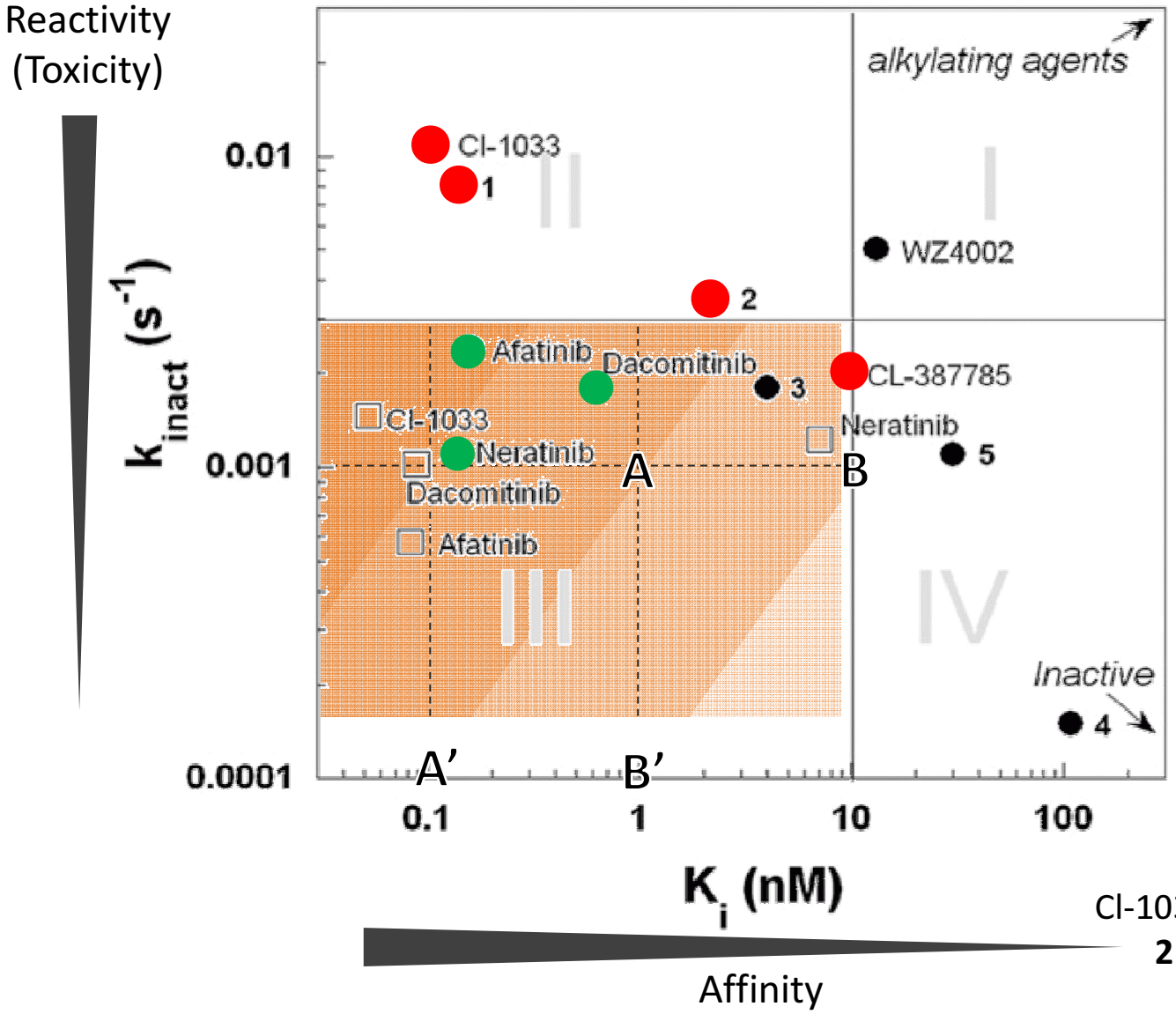


Murraya, B. W. *et al.* *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 173.

1) K_i correlates to cellular IC_{50} (R^2=0.89)
2) k_{inact} correlates to cellular IC_{50} (R^2=0.60)
3) K_i / k_{inact} strongly correlates to cellular IC_{50} (R^2=0.95)

Low K_i
High k_{inact}
Low K_i / k_{inact}

Kinetic analysis of MA-covalent inhibitors



1) Low K_i

Moderate

2) ~~High~~ k_{intact}

3) Low K_i / k_{inact}

$K_i = k_{off} / k_{on}$

$$E + I \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} E{\bullet}I \xrightarrow{k_{inact}} E{\sim}J$$

☐ WT, ● Mutant

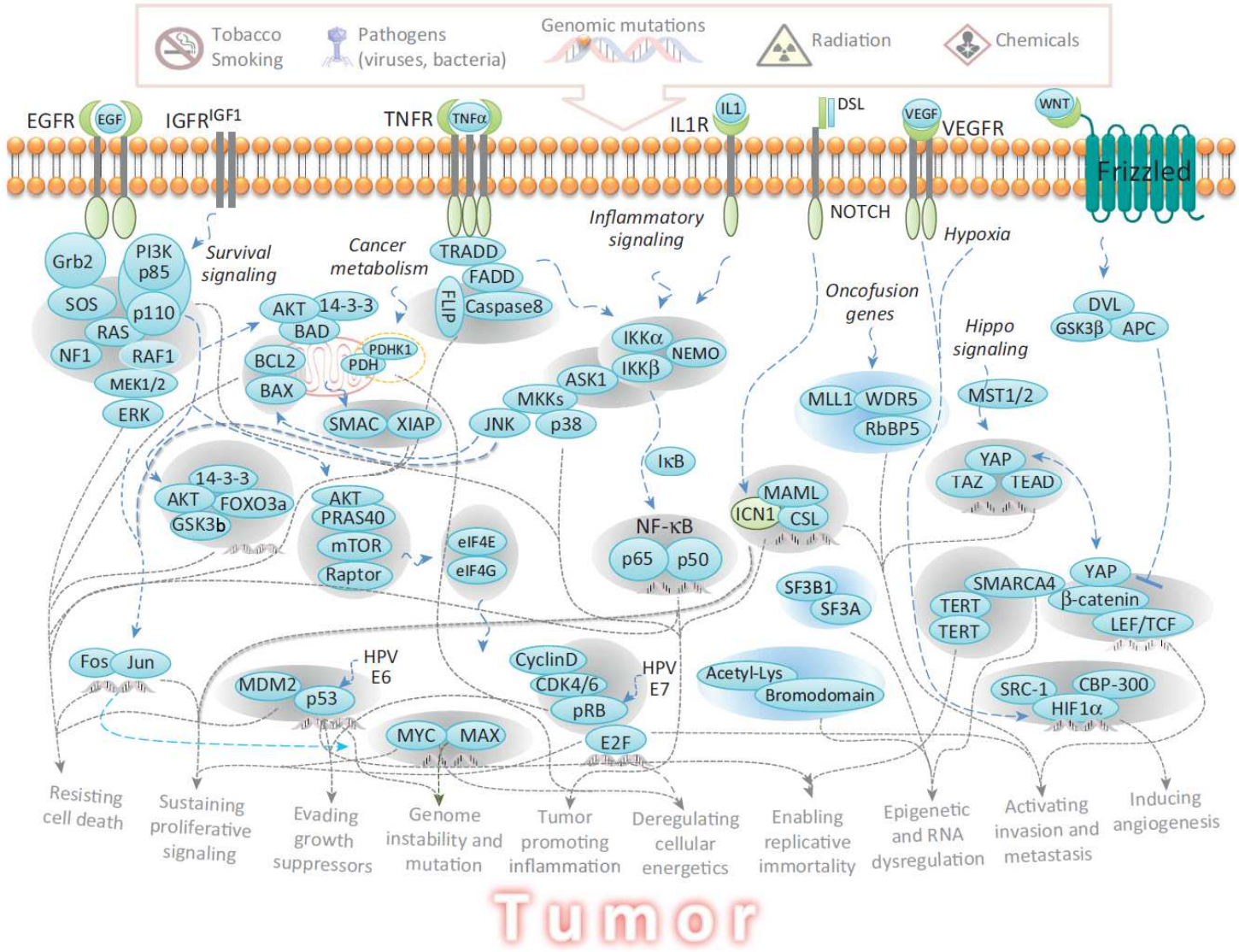
● Effective, ● Drop

Cl-1033 (Canertinib) drop (phase2)
2 (PD168393), CL-387785 drop
Dacomitinib (Phase2)
Neratinib (FDA application pending)

Murraya, B. W. *et al. Proc. Natl. Acad. Sci. USA* **2014**, *111*, 173.

Contents

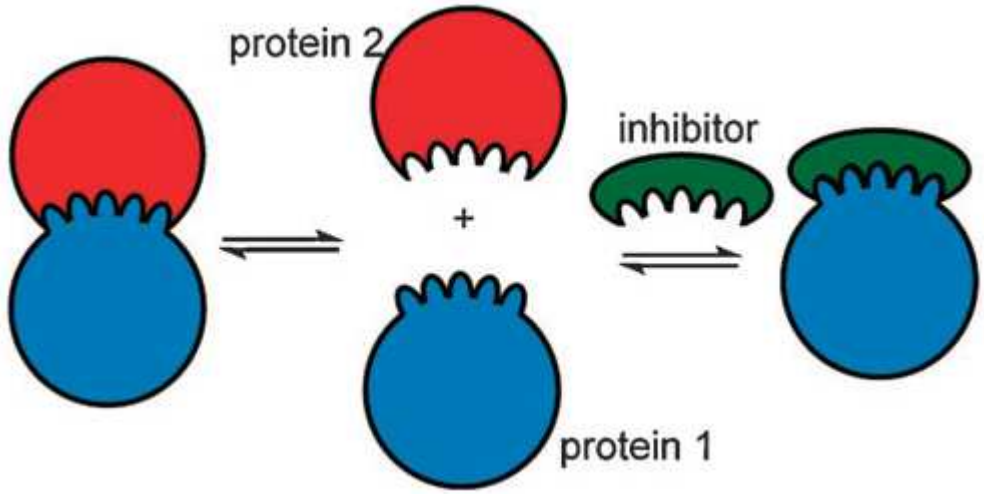
PPIs are important drug target cf. Literature Seminar, Shimizu, 2014



There are 375,000 PPIs (estimated), 32,000 PPIs (reported)

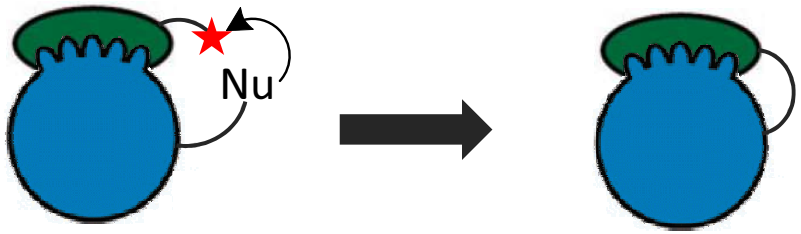
Mooney, E. M. *et al. Genome Biology* **2005**, *6*, R40.; Fu, H. *et al. Trends Pharmacol. Sci.* **2013**, *34*, 393.

PPIs + covalent inhibitor



Shallow, large binding surface (600-1300 Å ^{2})

Wilson, A. J. *Chem. Soc. Rev.* **2009**, *38*, 3289.



weak interaction strong interaction

Covalent modifying approach may increase potency.
May be a breakthrough of PPI inhibitor?

Way, J. C. *Curr. Opin. Chem. Biol.* **2000**, *4*, 40

Summary

Section 1

・Historically, many covalent drugs give us lots of benefit, in spite of serendipity.
Their mechanisms of action were determined at later stage.

Section 2

・Afatinib is first approved Targeted covalent inhibitor.
"Targeted covalent inhibitor can be designed by medicinal chemists"
・Targeted covalent inhibitor is irreversible, the strongest inhibitor class.
It may be a best-in-class method, although there are some limitations.
・Especially, potential off-target toxicity is remained, but there are few data.
Necessity to compare with conventional drugs. Currently under investigation.

Section 3

・Covalent drugs can be classified into some target and reaction types
・Drug candidate should pursue the low K_i, moderate k_{inact} and low K_i / k_{inact} value.

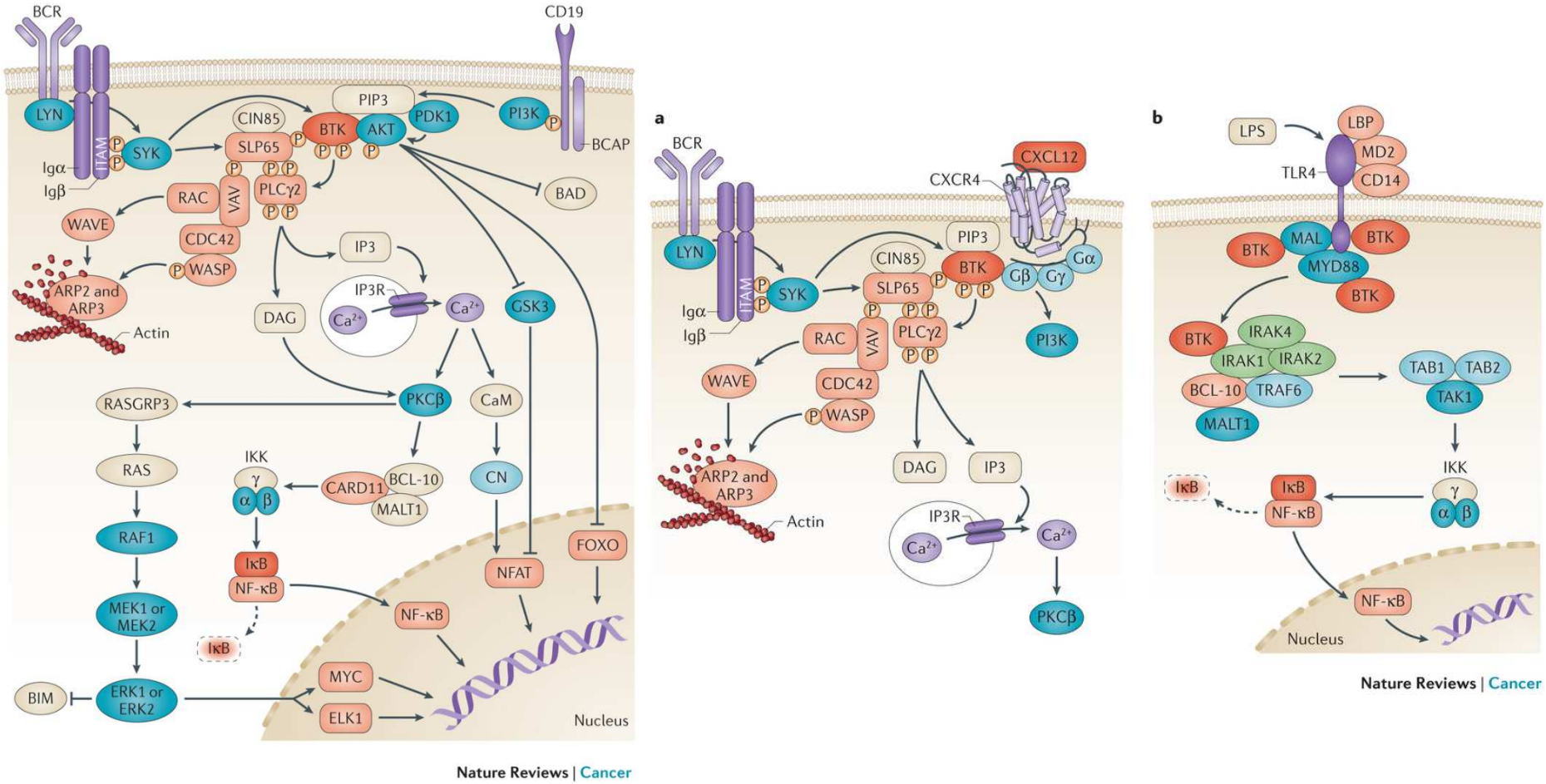
Section 4

・Covalent modifying method will bring a new perspective into PPI inhibition.

That’s all,
thank you for your kind attention!

Appendix

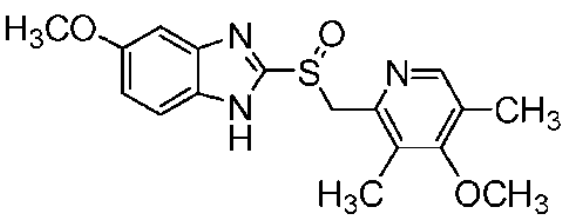
BTK relates to cancer



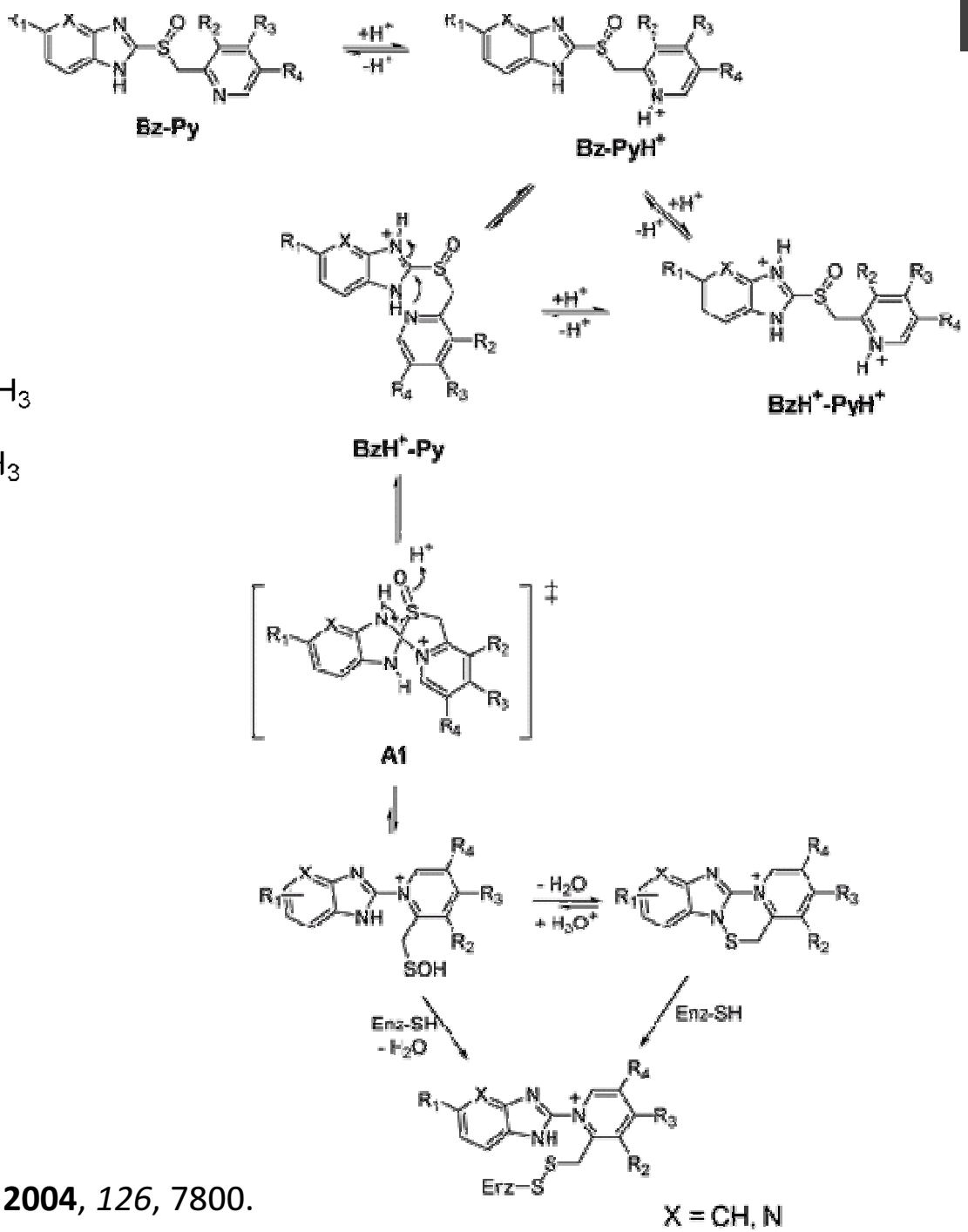
Hendriks, R. W. *et al. Nat. Rev. Cancer* **2014**, *14*, 219.

Appendix

PPI

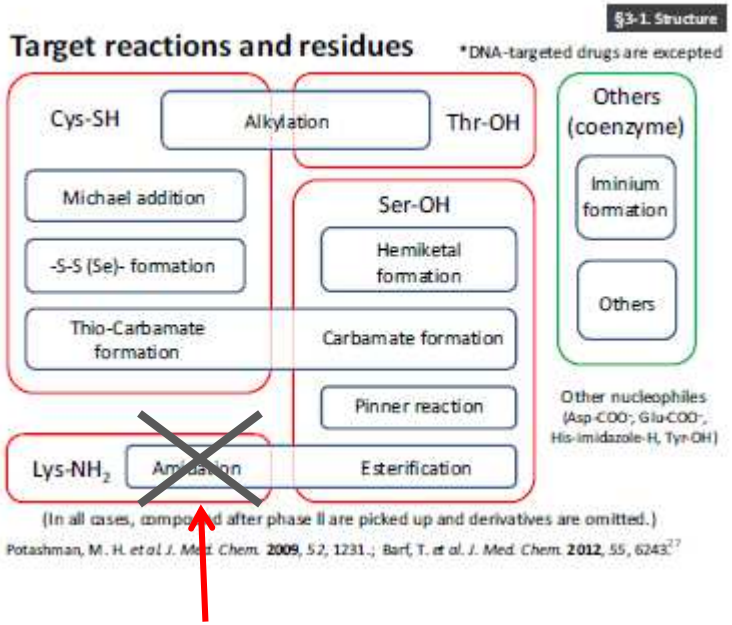


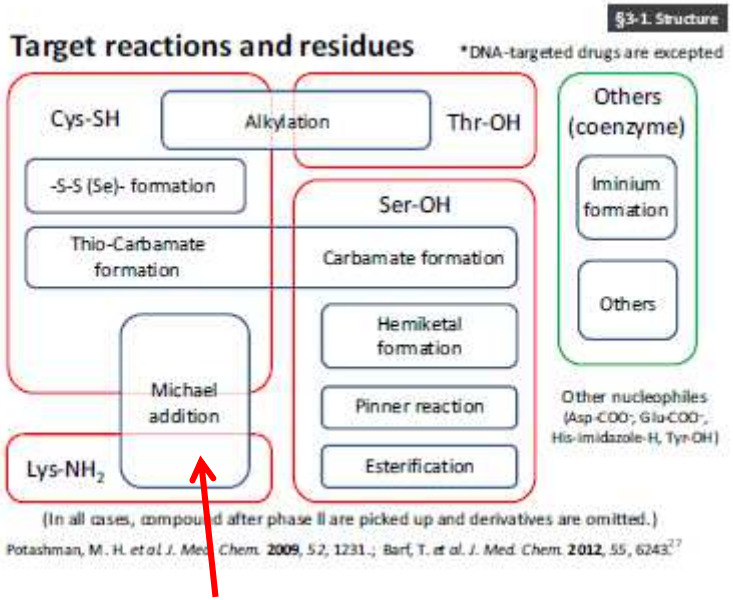
Omeprazole

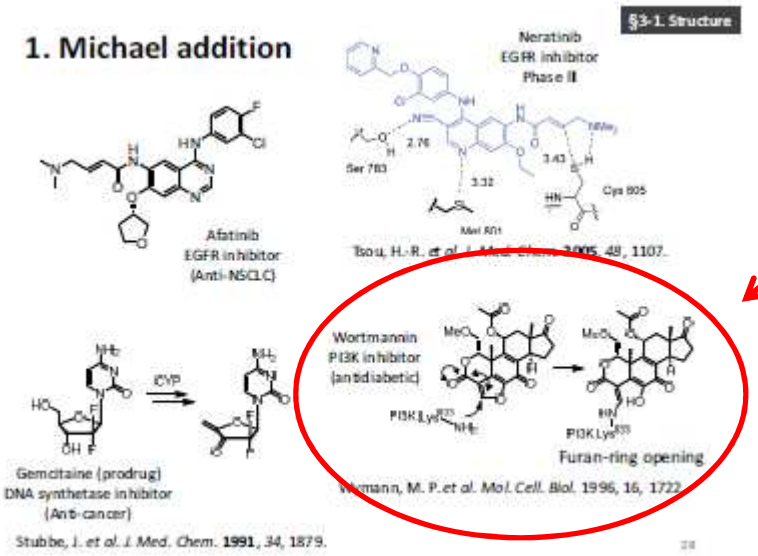


Shin, J. M. *et al. J. Am. Chem. Soc*. **2004**, *126*, 7800.

Revision







In previous version (and in my presentation)
Wortomannin was Amidation type,
but actually, Michael addition type.
There are no Amidation type.

Sorry to tell you mistakes.