‘Covalent Drugs’ – 
Drugs acting by forming a covalent bond with their biological targets

Lecture 6. 1st Year Graduate students.
2016
Picture of the day

Hot outside?

shut up
General considerations

• pharmaceutical companies are reluctant to apply covalent mechanisms of inhibition

• off-target reactivity

• idiosyncratic drug-related toxicity (e.g., by causing immune response)

• >40 FDA-approved drugs bind covalently to their targets

• 1/3 of all enzyme drug targets have at least one example of approved covalent drug

• a majority of successful covalent drugs were discovered in biological assays by chance and covalent mechanism of action was established later on

• recently, a concept of TARGETED COVALENT INHIBITORS (TCI) was developed
Targeted covalent inhibitors (TCI)

BTK – Bruton’s tyrosine kinase, a target to treat B-cell malignancies
Discovered in 1993.
The only approved drug (13/11/2013) –
Ibrutinib (Johnson & Johnson, marketed by AbbVie):

First designed at Celera Genomics in 2007
Ibrutinib selectivity
Ibrutinib synthesis
Acalabrutinib – next generation
BTK inhibitor

- Phase III for chronic lymphocytic leukemia: > 95% response!
- Developed by Acerta Pharma
TCIs vs. reversible (classical) inhibitors

\[ K_i = \frac{k_{off}}{k_{on}} \]

Kinetic and thermodynamic parameters describing covalent and noncovalent inhibition.
Advantages of covalent inhibition

Particularly difficult targets:

- protein-protein interactions (PPI) \(\rightarrow\) subject of a standalone lecture next semester!
- kinase ATP binding site (due to high intracellular levels of ATP)

\[\downarrow\]

COVALENT MODIFICATION OF A BIOLOGICAL TARGET OFFERS OPPORTUNITY OF ESTABLISHING NON-EQUILIBRIUM BINDING KINETICS!
Covalent vs. reversible inhibitors

→ Extremely useful graphs

For conventional drugs, the pharmacodynamic effect is driven by the pharmacokinetics of the drug (left). For irreversible covalent drugs, the pharmacodynamic effect is driven by the turnover rate (de novo synthesis) of the protein target (right).

Reactivity of TCIs

- ‘warheads’ of TCIs are weakly electrophilic to non-selectively modify other biomolecules (off-targets)
- this makes TCIs very different from reactive intermediates

Sulfotransferases
(Liver, kidney, intestine)
Design and optimization of TCIs

1. Bioinformatic analysis to identify nucleophilic amino acid (e.g., kinase cysteinome bioinformatics)
   • Near functionally relevant binding site
   • More or less unique to that protein

2. Reversible inhibitor identified with a known binding mode (X-ray!)

3. Computer-aided design to properly position the reactive functionality

4. Iterative synthesis and testing

5. Counter-screen on closely related kinases

Afatinib (Boehringer-Ingelheim)
Inhibitor of EGFR, approved for NSCLC
Reversible covalent inhibitors (RCI)

\[ E + I \overset{k_{\text{on}}}{\underset{k_{\text{off}}}{\rightleftharpoons}} [EI] \overset{k_{\text{inact}}}{\rightleftharpoons} E-I \]

\[ K_i = k_{\text{on}} / k_{\text{off}} \]

- RCEI dissociate from protein faster than the rate of its degradation
- Similar potency/selectivity benefits as for true covalent inhibitors
- Minimized production of long-lived modified proteins (↓ immune response)
- Perfect example – serine hydrolases and proteases (see WARHEADS in previous lectures)
Serine protease mechanism
Why are these RCIs of serine proteases?

- Telaprevir: $189,000 per treatment course
- Boceprevir: ~$200,000 per treatment course
- Both agents were voluntarily withdrawn ‘due to overwhelming superiority of newer direct-acting antiviral agents, such as ledipasvir/sofosbuvir.'
Lyrical digression: Gilead Sciences

Ledipasvir/sofosbuvir (trade name Harvoni)

- HCV Nucleotide replication inhibitor of
- phosphoprotein viral RNA
- NS5A inhibitor polymerase

Harvoni: $90,000 per treatment course
>95% cure rates for genotype 1 HCV

a.k.a. Sovaldi
Lyrical digression: Approved NS5A inhibitors

- Daclatasvir (Bristol-Myers-Squibb)
- Ombitasvir (AbbVie)
- Elbasvir (Merck)

- >10 other advanced compounds in development
- All approved for HCV
Back to RCEI: FAAH inhibitors

Endocannabinoid system

2-AG = 2-arachidoyl glycerol
AEA = arachidoyl ethanolamine (anadamide)
MGL = monoglycerol lipase
FAAH = fatty acid amide hydrolase (serine protease)

Pain
Mood
Appetite
Very simple ketone FAAH inhibitors (RCEI type)

FAAH $IC_{50} = 4.7 \text{nM}$

FAAH $IC_{50} = 22 \mu \text{M}$

What about substituent effects?

$R = \text{COOMe, CF}_3; IC_{50} = 400-800 \text{ pM!}$

- > 300-fold selectivity over other Ser hydrolases
- increases anandamide levels in vivo
- potent analgesic activity in pain models
Covalent modification of Serine by carbamoylation

\[
\text{F}_3\text{C}-\text{O}-\text{N} = \text{N}-\text{N} = \text{N}\text{-O} + \text{Me}_2
\]

- \( h\text{FAAH kinact/Ki} = 40,300 \)
- \( h\text{FAAH IC}_50 = 7.2 \text{ nM} \)

- \( h\text{MGL IC}_50 = 54 \text{ nM} \)
- \( h\text{FAAH IC}_50 = 37 \text{ nM} \)

LY 2183240
Other representative examples of reactive groups employed in TCI design

GSK3 (glycogen synthase kinase 3)
\( IC_{50} = 5 \text{ nM} \)

Cathepsin B (cystein protease, inflammation target):
\( IC_{50} = 13 \text{ nM} \)

Carfilzomib (approved)
\( K_{inact}/K_i = 34,000 \)
\( h20S \text{ proteasome 1h } IC_{50} = 6 \text{ nM} \)
Lyrical digression: proteosome as a drug target (to chase latest lecture)

- Protein complex in cells (often viewed as organelle)
- Responsible for degradation of proteins
- In particular, in various cancers, proteasome degrades proteins responsible for apoptosis (programmed cell death)
- Therefore, proteasome (catalytic 20S subunit) was considered a drug target for cancer

Bortezomib (Velcade®)
First approved (2003) proteasome inhibitor
By Millennium Pharmaceuticals
(for multiple myeloma)
Recalling: omeprazole – covalent modifier of PROTON PUMP

These are inhibitors of gastric hydrogen potassium ATPase, also known as H+/K+ ATPase, an enzyme whose purpose is to acidify the stomach.
Importance of the pKa for activation of omeprazole
What happens next...

Cys813

Cys813

Cys813

Clopidogrel (Plavix) – best-selling anticoagulant drug

- Prevents platelet (thrombocyte) aggregation and is used to treat blood clotting
- Prescribed to patients at risk of heart attack and stroke
- Acts by blocking P2Y₁₂ receptor on platelet surface
- 2010 global sales >$9 bln!
- Only works in people with ‘good’ CYP 2C19 metabolism!
- Is a prodrug
How does clopidogrel work?

- Only one stereoisomer is reactive
- The reactive thiol quickly forms a disulfide bond with P2Y12 Cys
- Prasugrel (Effient, Daiichi Sankyo) – easy version for poor CYP2C19 metabolizers:

Metabolized by intestine esterases
Finally, the aspirin story

However, aspirin itself is COX inhibitory, *in vitro* where metabolic activation is not possible…

Unlike salicylic acid, aspirin acts as a covalent modifier of COX-1/2 Ser\(^{530}\):
Conclusions

- Covalent drugs are important
- Many of the known covalent drugs were discovered serendipitously
- Chances of discovering new covalent inhibitors via screening nowadays are nil
- Targeted covalent inhibitor (TCIs) are designed via bioinformatics and *in silico* modeling
- TCIs can act irreversibly by forming covalent bond with unique nucleophilic residues
- TCIs can act as reversible covalent inhibitors (RCIs) which appears a better alternative to covalent inhibitors
QUESTIONS?