Covalent Drugs: Trends, Mechanisms, & Warheads

Covalent drugs - a two step process


The term “covalent” has replaced the use of the term “irreversible” in the literature (chart looks eerily familiar…)

The evolution of covalent drugs - a timeline

1890s
Synthesis of Aspirin (Bayer)

1920s-1970s
Discovery of cephalosporin C

1980s
Fosfomycin inhibits MurA

1990s
Penicillin discovered

2000s
Omeprazole, a masked covalent proton pump inhibitor

2010s
Afatanib

2020s
Treatment for COVID

Pros of covalent inhibitors
- High efficacy may translate to lower doses
- Nonequilibrium binding might aid in overcoming competing exogenous [substrate]
- Covalent mechanism may slow the development of drug resistance
- Prolonged duration of action may lead to less frequent dosing
- Can address targets thought to be “undruggable”

Potential cons of covalent drugs
- Potential risk of toxicity
- Over-reactive warheads can cause off-target effects
- Small advantage for targets with a rapid resynthesis rate

What to expect in this GM:
- Highlighted examples of covalent inhibition used in drugs with a focus on diversity of mechanism
- The synthesis of said molecules
- Learn how covalent mech. enable new therapies
- Brief biological discussion and relevance

What NOT to expect in this GM:
- A rigorous discussion on biology or kinetics
- Chemical biology or reactive metabolites

Drug Discov., 2015, 20, 1061
More context and orientation: Over 40 covalent drugs are currently approved by the FDA, treating a variety of illnesses

FDA approved warheads and target nucleophiles are diverse in nature. Future Med. Chem. 2020, 13, 193.

Considerations in the development of covalent drugs

Covalent modification is a key and common element in regulating biological systems

<table>
<thead>
<tr>
<th>Targets of recent interest that have been investigated clinically:</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Oncology:</strong></td>
</tr>
<tr>
<td>· EGFR (receptor tyrosine kinase)</td>
</tr>
<tr>
<td>· BTK (cytoplasmic kinase)</td>
</tr>
<tr>
<td>· MEK1 (mitogen-activated protein kinase)</td>
</tr>
<tr>
<td>· HSP70 (heat shock protein)</td>
</tr>
<tr>
<td>· PI3K (phosphatidylinositol 3-kinase)</td>
</tr>
<tr>
<td>· Proteasome</td>
</tr>
<tr>
<td>· KRAS</td>
</tr>
<tr>
<td><strong>Non-oncology</strong> (as many as 80% of approved covalent drugs treat areas outside of oncology):</td>
</tr>
<tr>
<td>· Keap1-Nrf2 pathway (cellular stress response; MS, anti-inflammatories, etc.)</td>
</tr>
<tr>
<td>· GST (glutathione S-transferase; diuretic, combats resistance to cancer drugs)</td>
</tr>
<tr>
<td>· β-lactamase (novel antibiotics)</td>
</tr>
<tr>
<td>· HCV protease</td>
</tr>
<tr>
<td>· SARS-CoV-2 main protease</td>
</tr>
<tr>
<td>· and many more…</td>
</tr>
</tbody>
</table>

Drug Discov., 2015, 20, 1061

Pie chart of approved covalent drugs by indication

Nat. Rev. Drug Discov. 2011, 10, 307

![Pie chart of approved covalent drugs by indication](image)
**Covalent Drugs: Trends, Mechanisms, & Warheads**

**Mechanism bank 1: Acylation**

**Aspirin (Bayer)**

The world’s most popular drug

Aspirin

\[
\text{CO}_2 \text{H} \quad \text{OAc}
\]

\&

\[
\text{COX-1} \quad \text{COX-2}
\]

\( \text{OAc} \)

acylation of cyclooxygenase (COX) serine residues

\( \text{COX-1 inactivated; anti-thrombotic} \)

\( \text{COX-2 prostaglandin production inhibited; “turns-on” production of protective lipid mediators} \)

**Orlistat (Roche, GSK), an anti-obesity medication**

- Reduces absorption of dietary fat by \( \sim 30\% \) through reversible inhibition of triacylglycerol lipase

\[
\text{Me} \quad \text{Me} \\
\text{O} \quad \text{O} \\
\text{OH} \quad \text{CHO} \\
\text{Ser 152} \\
\text{Ser 152}
\]

TAG-lipase inhibited

**Isoniazid forms an enzyme-bound cofactor adduct**

- First-line anti-tuberculosis agent

\[
\text{NAD}^+ \quad \text{NAD}^+ \\
\text{NAD}^+ \quad \text{NAD}^+
\]

**Second-generation process route to orlistat (Roche)**

*OPRD, 2007, 11, 524*

- \( \text{H}_2, \text{MeOH} \)
- \( [\text{RuCl}_6((R)-\text{MeOBIPHEP})] \)
- then \( \text{KHCO}_3, \text{DMAP}, \text{acyl halide} \)

\[
\text{C}_{11}\text{H}_{23} \quad \text{C}_{11}\text{H}_{23} \quad \text{C}_{11}\text{H}_{23} \quad \text{C}_{11}\text{H}_{23} \quad \text{C}_{11}\text{H}_{23} \quad \text{C}_{11}\text{H}_{23}
\]

\( \text{5 steps} \)

\( \text{90\%} \)

\( \text{85-94\%} \)

**Other acylating agents:**

- \( \beta\text{-lactams} \)
- warfarin
- disulfiram

**Aspirin Timeline and Impact**

- 3000 BC: Willow bark used by ancient civilizations
- 1853: Charles Frederic determines structure of salicylic acid and makes acetylsalicylic acid
- 1897: Felix Hoffman (Bayer) finds that adding acetyl group to salicylic acid improves properties
- 1918: Aspirin used in 1918 pandemic
- 2002: AHA recommends low-dose aspirin for prevention of heart attack and stroke

**Orlistat**

\[
\text{C}_{11}\text{H}_{23} \quad \text{C}_{11}\text{H}_{23} \quad \text{C}_{11}\text{H}_{23} \quad \text{C}_{11}\text{H}_{23} \quad \text{C}_{11}\text{H}_{23} \quad \text{C}_{11}\text{H}_{23}
\]

\( \text{80\%} \)

**Isonicotinic acyl-NADH**

*Science, 1998, 279, 98*

The drug was tested in a Navajo community in Arizona ca. 1950’s

**Discov. Med., 2009, 4, 470**
Mechanism bank 2: Disulfide or thioselenide formation

Clopidogrel (BMS/Sanofi), a blockbuster antiplatelet medication with a unique prodrug mechanism
- Inhibits P2Y12, a protein involved in platelet aggregation
- Reduces risk of heart disease and stroke for high-risk people
- 36th most prescribed drug in 2019; 19 million prescriptions

Propylthiouracil as a hyperthyroidism treatment
- Irreversibly inhibits thyroxine 5'-deiodinase
- Decreases the amount of thyroid hormone produced by the thyroid gland
- Came into use in the 1940s
- WHO List of Essential Medicines

A general improved route to Clopidogrel

OPRD, 2007, 11, 487

Not discussed in detail: omeprazole (see Natural Products as Covalent Inhibitors, He for the mechanism) J. Am. Chem. Soc. 2004, 126, 7800, and references therein
- Proton pump inhibitor; treats gastresophageal reflux disease
**Mechanism bank 3: Alkylation**

**Vigabatrin - a simple but effective medicine**
- Generic medication used to treat epilepsy and seizures
- Irreversibly binds to GABA-aminotransferase, inhibiting the breakdown of GABA

\[ \text{Vigabatrin} \rightarrow \text{plasmalogen} \rightarrow \text{covalently modified lysine residue} \]

\[ \text{J. Biol. Chem. 1991, 266, 20056} \]

**Nitrogen mustards as cytotoxic alkylating agents**
- Melphalan used as a prodrug for melphalan, a common chemotherapy agent that treats many cancers
- DNA alkylating mechanism through aziridinium species
  
  \[ \text{Expert Opin. Investig. Drugs. 2020, 29, 1069} \]

**Generic nitrogen mustard drug mechanism:**
- Further alkylation
- Prone to hydrolysis and fragmentation

**Cycloserine as a covalently reactive anti-tuberculosis treatment**
- Discovered in 1954 from a *streptomyces*
- WHO List of Essential Medicines
- Inhibits cell wall synthesis via covalent modification of PLP, a cofactor used by alanine racemase

\[ \text{Cycloserine in the news:} \]
In 2015, the price of CS increased from $500 for 30 pills to $10,800 after the Chao Center for Industrial Pharmacy changed ownership to Rodelis. The price was then lowered to $1,050 for 30 pills, twice what was originally charged
**Mechanism bank 4a: Conjugate additions - A case study on KRAS\(^{G12C}\) inhibitors. A decades-long therapeutic challenge**

- Member of the RAS family, a key regulator in signalling pathways responsible for cell proliferation, differentiation, and survival (GTPase)
- KRAS is the most frequently mutated oncogene in human cancer
- \(\text{KRAS}^{G12C}\) mutations occur in about 13% of NSCLC patients and ~1-3% of colorectal and other solid tumors.
- Glycine-to-cysteine mutation at codon 12 favors the activated state of KRAS, which amplifies pathways that lead to cancer development

https://www.amgenoncology.com/targets/kras.html

**Sotorasib (Amgen) - the first KRAS inhibitor approved by the FDA**

- Approved in May 2021 for NSCLC
- Acts by binding with cysteine 12 of mutated KRAS

**Discovery route:** *J. Med. Chem.* **2020**, 63, 52

**Mirati in on the gold-rush: MRTX849**

- Covalent KRAS\(^{G12C}\) inhibitor in clinical trials (Cys12)
- Breakthrough Therapy Designation by FDA in June 2021

**Discovery route:** *J. Med. Chem.* **2020**, 63, 6679
**Mechanism bank 4b: More conjugate additions**

**Gemcitabine - a hidden vinyl ketone for cancer treatment**
- First intended as an antiviral, but preclinical results showed that it killed leukemia cells *in vitro*.
- Blocks the synthesis of DNA via covalent modification of a cysteine residue in ribonucleotide diphosphate reductase

*First synthesis of gemcitabine (Lilly)*
*JOC 1988, 53, 2406*

1. Dowex-50 MeOH, H₂O
2. TBSOTf, lutidine, 92%

**Silegiline undergoes SET to form a covalent adduct with FAD**
- Treats Parkinson’s and major depressive disorder by acting as a monoamine oxidase inhibitor
- Forms a Michael-type adduct with the FAD cofactor, subsequently inhibiting MAO-B mediated degradation of dopamine

**Finasteride as a covalent inhibitor of 5-α-reductase**
- Used to treat hair loss and benign prostatic hyperplasia in men
- Works as an antiandrogen by decreasing production of dihydrotestosterone
- Available as a generic medication; >8 million prescriptions in 2019

**Finasteride**
*J. Am. Chem. Soc. 1996, 118, 2359*

**[Similar to reduction of testosterone]**
- 5-α-reductase
- Covalent adduct

**Flavin** + **NHTMS**
**Flavin-MAO**
**Mechanism bank 5: Aldehydes - enabling first-in-class medications**

**Voxelotor - a reversible aldehyde for sickle cell anemia** (Global Blood Therapeutics)

- Approved by the FDA in 2019 for sickle cell disease as a first-in-class medication
- Acts as an allosteric effector of sickle cell hemoglobin, consequently increasing the affinity of hemoglobin for oxygen
- Increased affinity for oxygen inhibits polymerization of hemoglobin under hypoxic conditions

**Discovery:** *ACS Med. Chem. Lett.* 2017, 8, 321 (Global Blood Therapeutics)

- Sickle cell disease is caused by a mutation in the β chain of hemoglobin, where a Glu residue has been swapped for a Val residue
- Affects ~100,000 Americans

![Figure from Dennis Hu's "Drug Hunter"](https://www.cdc.gov/nchbddd/sicklecell/data.html)

**Roblitinib (Novartis) - A first in-class FGFR4 inhibitor clinical candidate**

- Inhibits fibroblast growth factor receptor 4 (FGFR4) via reversible hemithioacetal formation with Cys552
- In clinical phase I/II trials for the treatment of hepatocellular carcinoma (HCC)

**Discovery:** *J. Med. Chem.* 2020, 63, 12542

![Synthesis of A](image)

![Synthesis of B](image)
Mechanism bank 6: Boronation as an effective strategy to treat multiple ailments

There are currently 5 drugs approved by the FDA containing boronic acids (many more undergoing development)

**General reversible mechanism:**

For a fantastic review, see: Eur. J. Med. Chem. 2020, 195, 112270

---

**Bortezomib**

Forms covalent complex with threonine residue in 20s proteasome (2005; multiple myeloma)

**Ixazomib**

(similar mechanism to bortezomib) (2015; multiple myeloma)

**Tavaborole**

(similar mechanism to bortezomib) (2014; onychomycosis)

**Crisaborole**

(2016; eczema)

**Vaborbactam**

(2017; combination with Meropenem to treat bacterial infections)

---

**Vaborbactam mimics tetrahedral TS used in β-lactamase**

- Used in combination with Meropenem to prevent its hydrolysis by β-lactamase enzymes
- Broad-spectrum; restores activity of valuable carbapenems

**Discovery synthesis (Rempex)**

*J. Med. Chem.* 2015, 58, 3682

---

**Tavaborole - a unique covalent mechanism to inhibit aminoacyl-tRNA synthetase**

- Traps tRNA<sub>Leu</sub> in the active site of Leucyl-tRNA synthetase, a proofreading synthetase
- Treats onychomycosis, a fungal nail infection *Science* 2007, 316, 1759 (Anacor, now AN2)

---

**Oxyanion hole:** Common pocket found in active sites of enzymes that stabilize TS negative charges on alkoxides. Typically consists of backbone amides or positively charged amino acid residues.
**Mechanism bank 7a: Nitrile additions - A look at PF-07321332 (Nirmatrelvir)**

**Nirmatrelvir (Pfizer)**
- Orally bioavailable SARS-CoV-2 main protease inhibitor
- *In vitro* pan-human coronavirus antiviral activity
- Used in combination with ritonavir; sold as Paxlovid - FDA EUA for mild to moderate COVID.

**Discovery route:**

1. **Attachment and entry**
   - Host cell membrane
   - Host cell infection

2. **Translation of viral proteins**
   - Viral RNA
   - Ribosomes
   - Polyprotein chains

3. **Proteolysis**
   - Main protease
   - Viral proteins

**Initial disclosure:** *Science 2021*, 374, 1586

**Process chemistry (BMS):**

**Mechanism bank 7b: Nitrile additions via Pinner**

**Saxagliptin, a cyanopyrrolidine for the treatment of type II diabetes**
- Reacts reversibly with Ser630 in dipeptidyl peptidase IV (DPP-IV), forming an imidate adduct
- Serves as an effective glucose regulator

**ACS Catal. 2019**, 9, 2292
Covalent Drugs: Trends, Mechanisms, & Warheads

Brendyn Smith

1/22/2022

Some compounds of note not discussed:

- telaprevir (Vertex)
- dimethyl fumarate
- rivastigmine (Alzheimer's)
- several - "ibs"
- kinase inhibitors (see Kinase Inhibitors: An Introduction, Peters)
- epoxide-based compounds (e.g. fosfomycin)

An incredibly useful resource used throughout: J. Med. Chem. 2009, 52, 1231

What else is trending?

Development of new warheads for use in med-chem and chemical biology
J. Med. Chem. 2019, 62, 5673

 curse or cure? a perspective on the developability of aldehydes as active pharmaceutical ingredients

"Covalent inhibition has a rich history in drug discovery and continues to be a highly successful strategy for addressing diverse targets and disease areas"

Drug Discov. 2015, 20, 1061