Mechanisms For the Bioactivation of Cancer Promoieties

Outline

I. Introduction
   i. Why promoieties: Identifying key reasons promoieties are pursued in cancer drug development

II. Prodrugs – An Introduction
   i. Definition of prodrugs
   ii. General mechanism of action
   iii. Early key developments

III. Enzymatic prodrug release
   i. DT-diaphorase
      a. Indolequinone reduction
      b. Hydroquinone reduction
   ii. (FMN)-dependent-NADH-Azoreductase
      a. Reduction of azo bonds
   iii. Nitroreductase
      a. Nitroaryl reduction and cyclization
      b. Nitroaryl reduction and elimination

IV. Tumor microenvironment triggered prodrug release
   i. pH sensitive prodrug release
   ii. Glutathione sensitive prodrug release
   iii. Reactive oxygen species (ROS) sensitive linkers

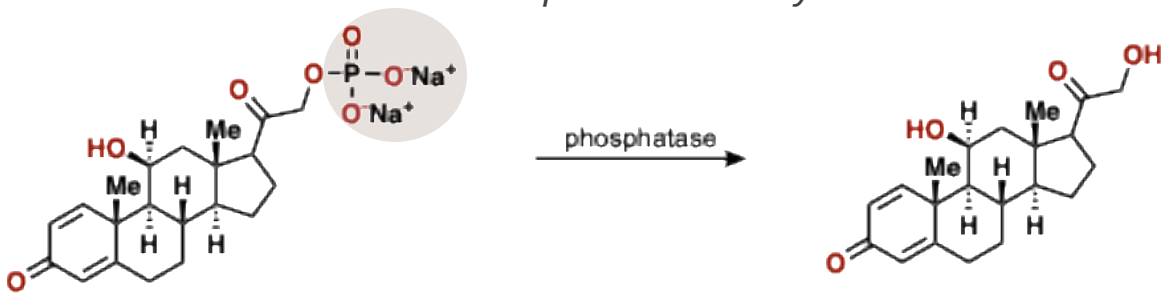
V. Antibody Drug Conjugates (ADCs) – An Introduction
   i. Structure of ADCs
   ii. Mechanism of Action
   iii. Linkers and payloads

VI. ADC drug release examples
   i. Cathepsin cleavable
   ii. Acid cleavable
   iii. Iron cleavable

Not covered in this topic: *I have omitted much discussion about the biology behind the drug mechanism of action, internalization, metabolism, etc.. I have not included the stories or "why" behind the design of each prodrug (i.e. overcoming solubility, toxicity, permeability challenges, etc.).These are important topics (but outside of the immediate purview of this topic). I also do not have an exhaustive discussion about ADCs – there has been a topic that covers the fundamentals and linker chemistry nicely (see website). The goal is to familiarize the audience with some common* ***mechanistic*** *designs. If there is continued interest in the topic or the areas not covered, I encourage reading the reviews/papers cited in this topic and the topic uploaded on our website. ☺*

Why Promoieties? Improving Drug Properties

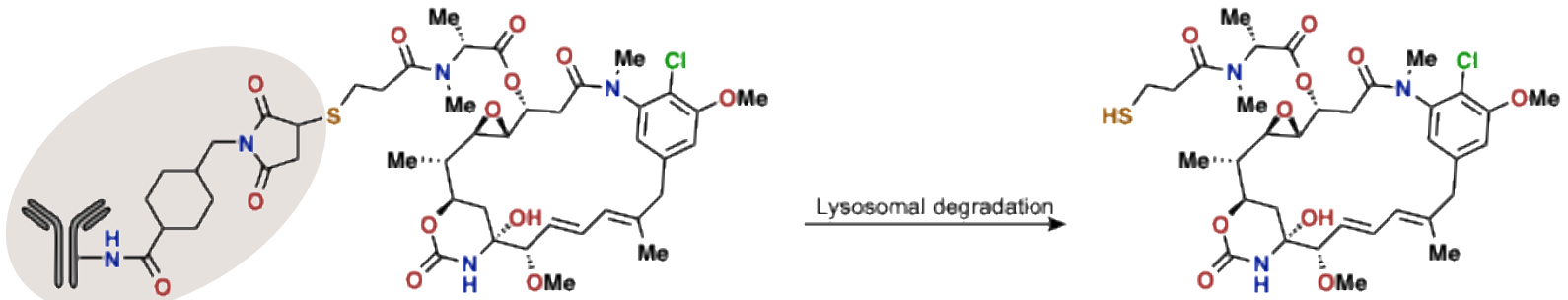
Increase Aqueous Solubility



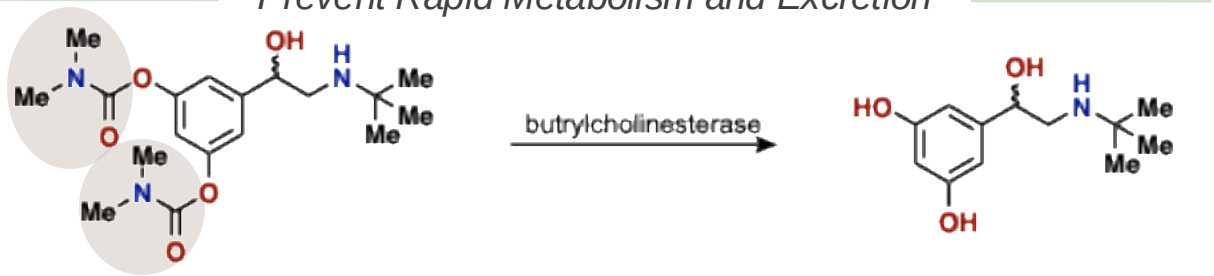
Increase Permeability and Absorption

- Increase oral bioavailability
- Improve drug delivery
- Support dose escalation
- Tailor rate of drug release
- Extend drug lifecycle

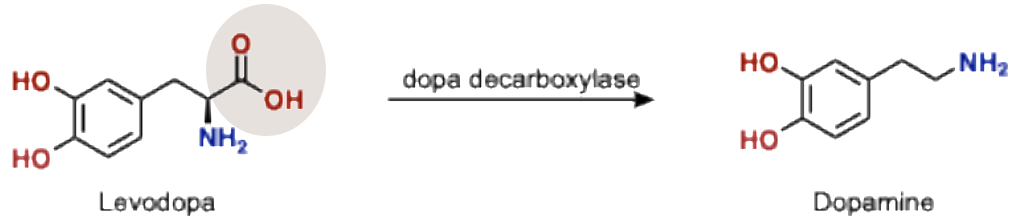
Overcoming Toxicity



Prevent Rapid Metabolism and Excretion



Change Distribution Profile

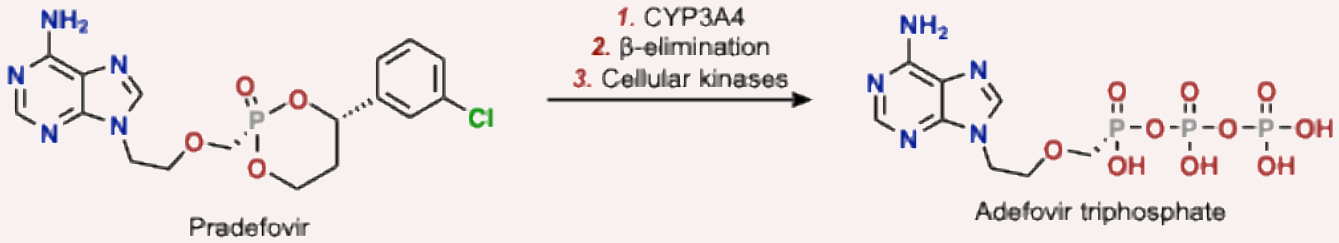


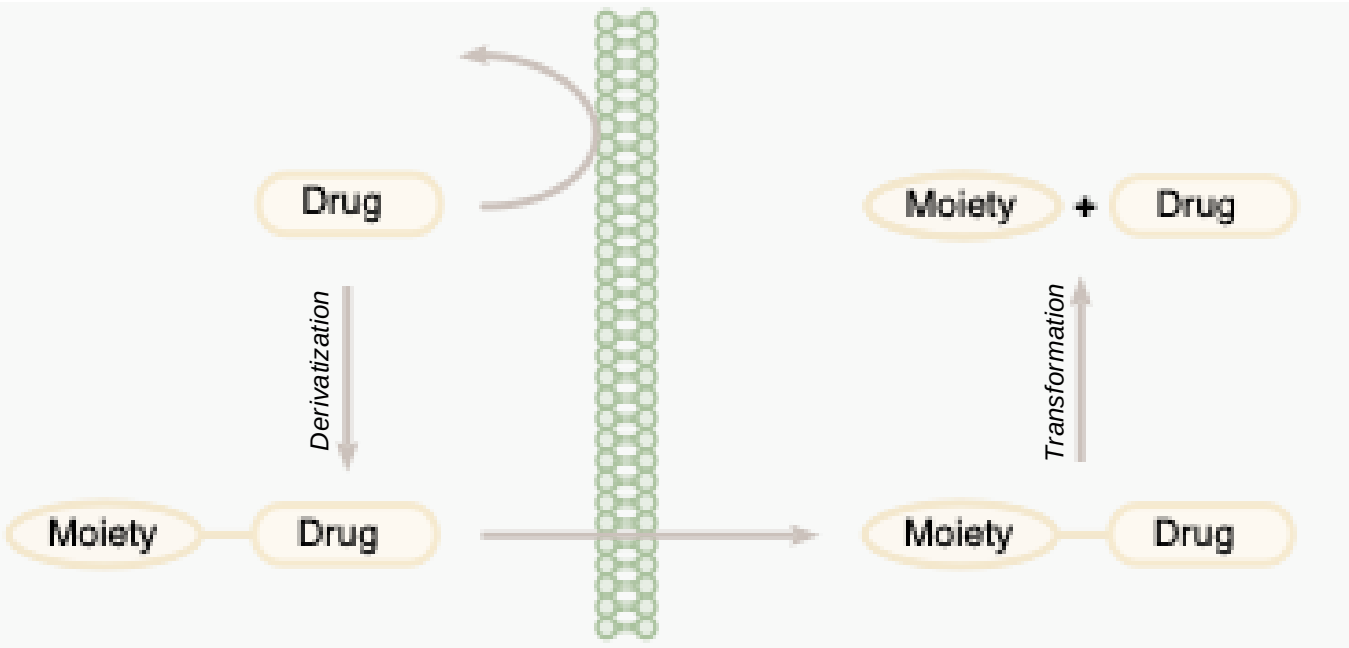
Adv. Drug. Deliv. Rev. **2007**, ***59***, 667., *Drugs* **2001**, ***61***, 263., *Expert. Opin. Biol. Ther..*, **2011**, ***6***, 807., *Clin. Pharmacokinet.* **1996**, ***4***, 246, *Adv. Drug. Deliv. Rev.* **2007**, ***7***, 677.

Prodrugs – An Introduction

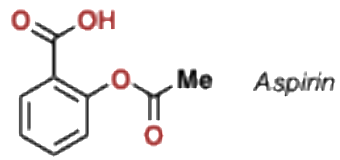
What Are Prodrugs?

Inactive or significantly less active form of an active metabolite released in vivo via chemical modification, enzymatic activation, or microenvironment conditions

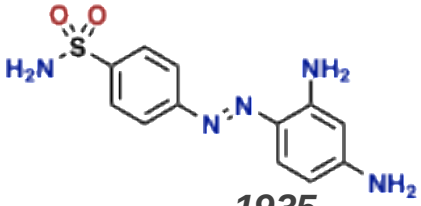




Key Developments



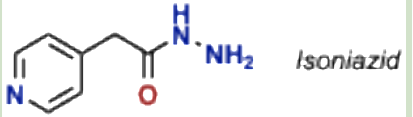
1899



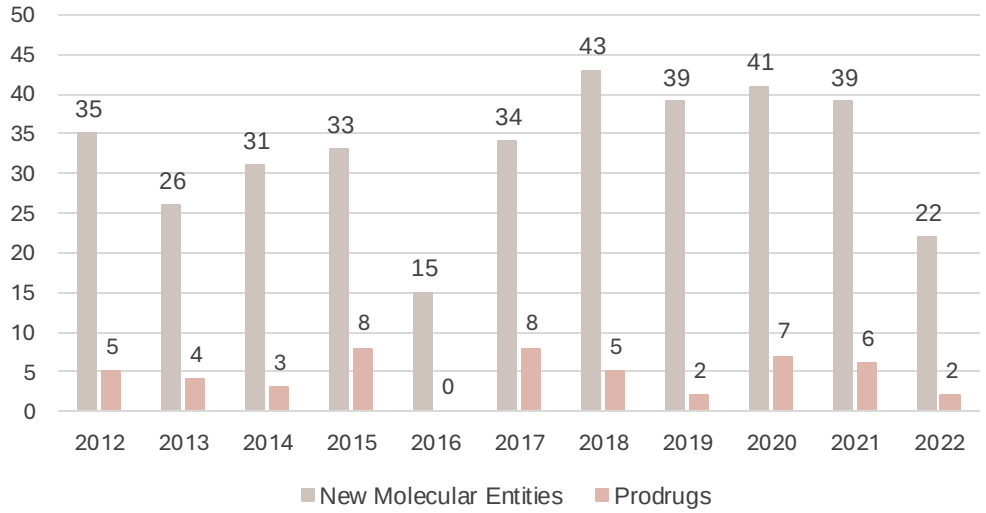
1935

"When a substance is administered…[it] is only a 'pro-drug' which has to be broken down to give the true drug."
- Adrien Albert

1958



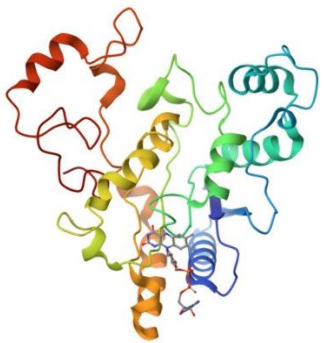
1992



Adv. Drug. Deliv. Rev. **2007**, ***59***, 667., *Drugs* **2001**, ***61***, 263., *Expert. Opin. Biol. Ther..*, **2011**, ***6***, 807., *Clin. Pharmacokinet.* **1996**, ***4***, 246, *Adv. Drug. Deliv. Rev.* **2007**, ***7***, 677.

DT-Diaphorase: Bioactivation of Indolequinones

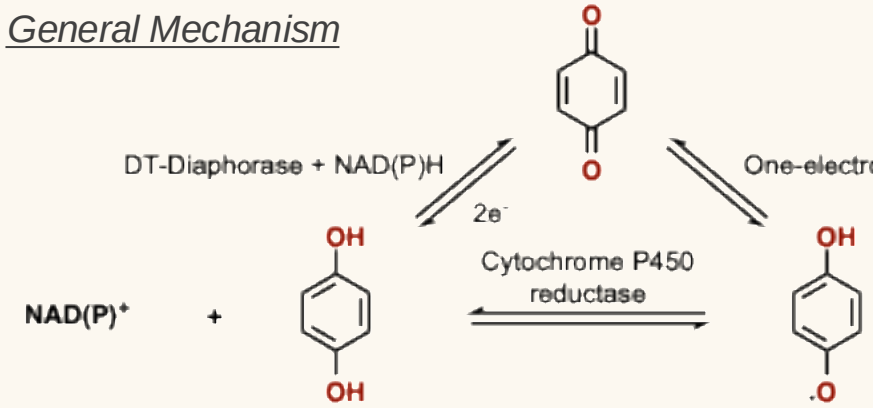
Background

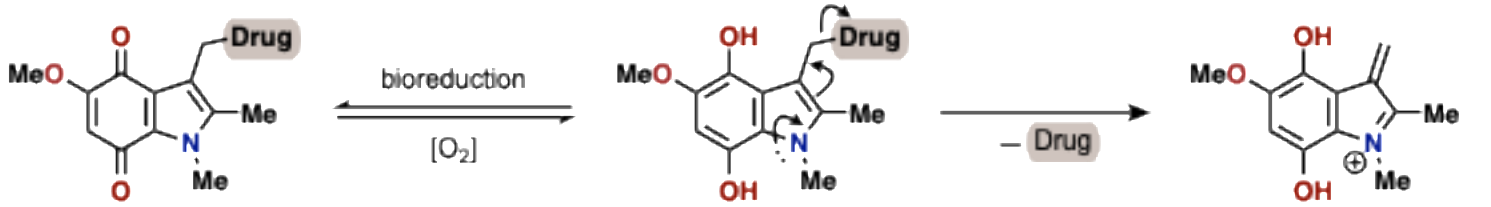


DT-Diaphorase

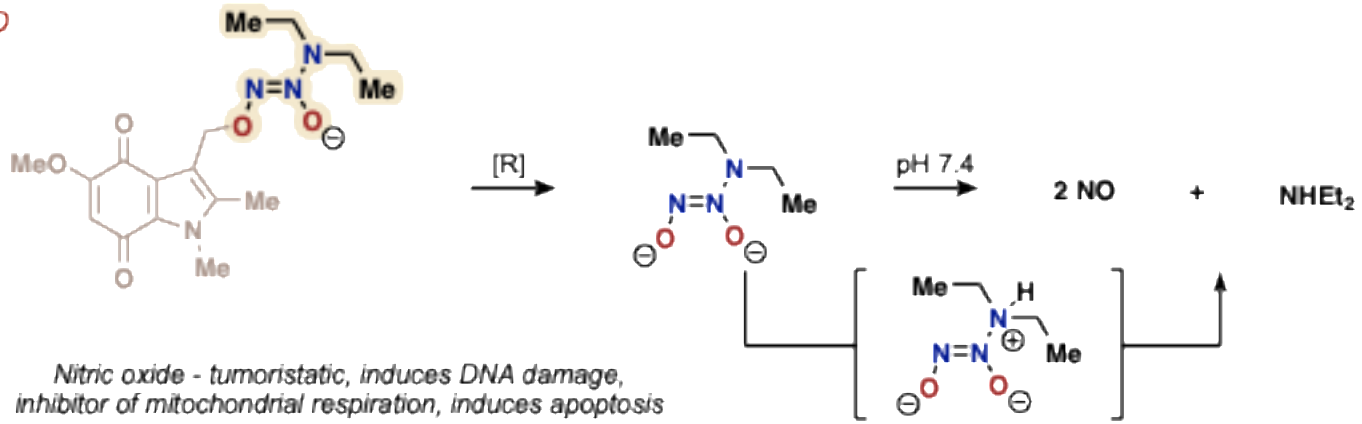
- *Catalyzes oxidation of NADH and NADHP*
- *Two electron reductase enzyme*
- *Upregulated in neoplastic tissues (NSCLC)*
- *Bioactivates chemotherapeutic quinones*

General Mechanism

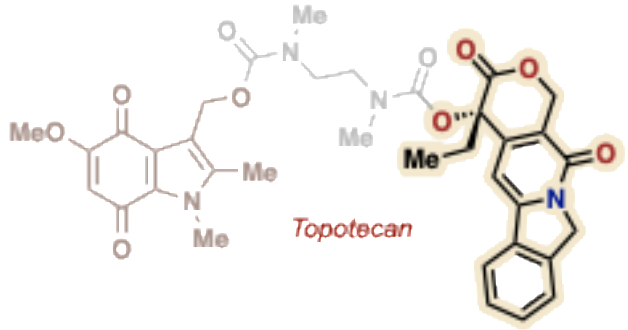




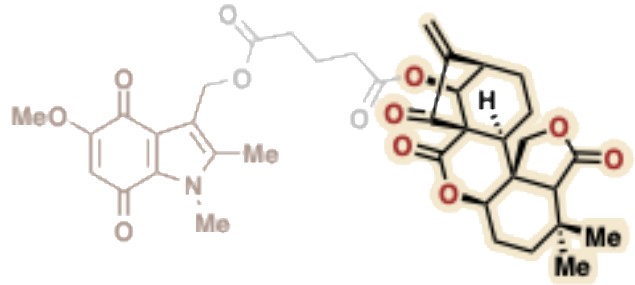
INDQ/NO



Nitric oxide - tumoristatic, induces DNA damage, inhibitor of mitochondrial respiration, induces apoptosis



Camptothecin derivative - inhibitor of DNA topoisomerase, leads to DNA strand breaks during replication

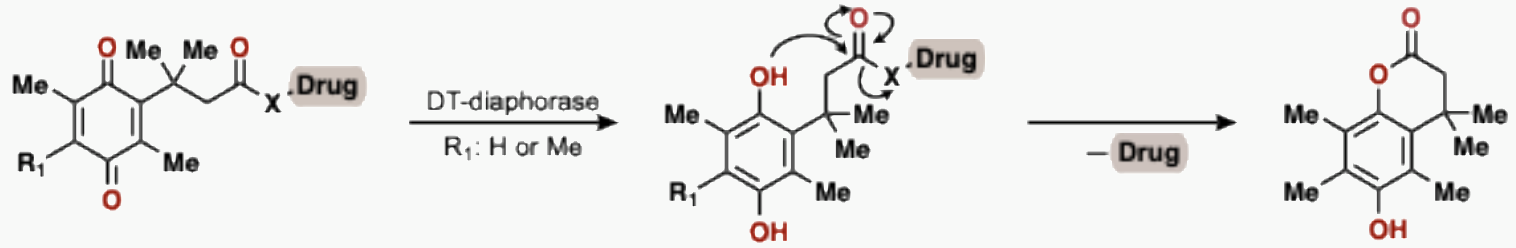


Oridonin derivative - inhibits cell cycle progression, disrupts mitochondrial function, induces apoptosis

J. Mater. Chem. B. **2022**, ***10***, 5504., *Org. Biomol. Chem.*. **2005**, ***3***, 1905., *Org. Lett.* **2013**, ***15***, 11, 2636., *Eur. J. Med. Chem.*, **2017**, ***132***, 310.

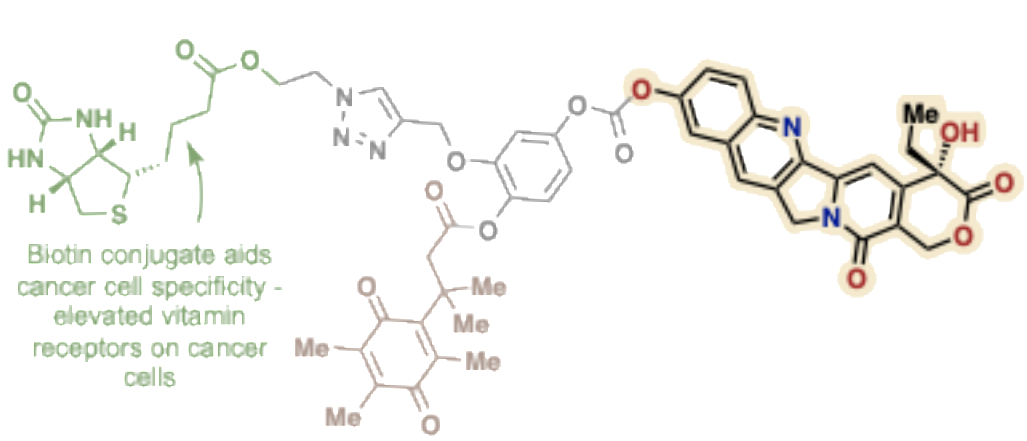
DT-Diaphorase: Bioactivation of Hydroquinones

Hydroquinone-Assisted Lactonization



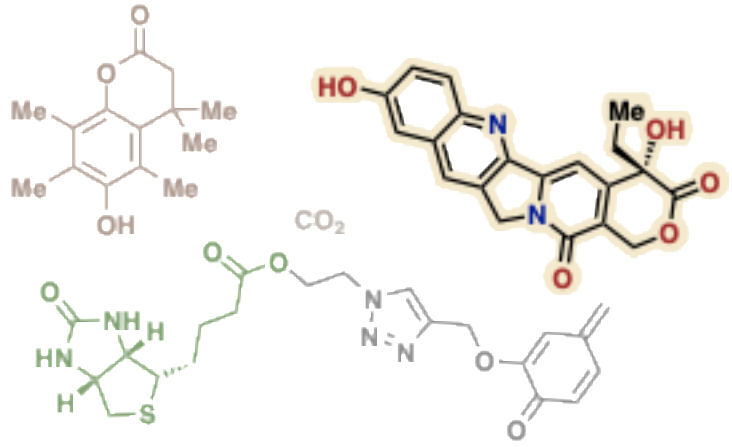
Lactonization induced via trialkyl (trimethyl) lock

Trimethyl lock: Lactonization is induced due to unfavorable steric interactions from three proximal methyl groups

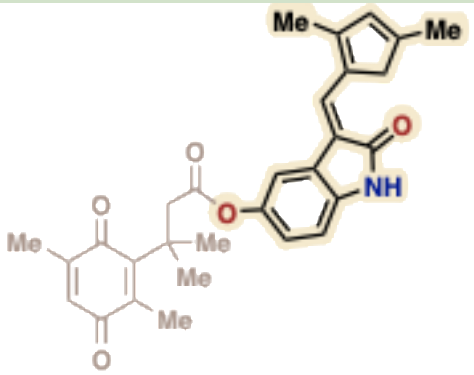


Biotin conjugate aids cancer cell specificity - elevated vitamin receptors on cancer cells

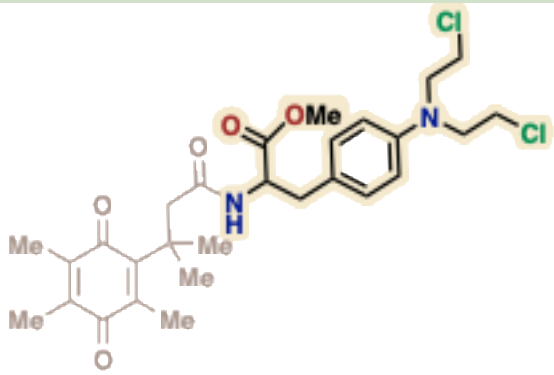
DT-diaphorase



Camptothecin derivative - topoisomerase I inhibitor, induces apoptosis, real time monitoring via fluorescence



6-hydroxy pyrrolylmethylidene oxindole - inhibition of VEGF, inhibition of angiogenisis



Melphalan derivative - binds to N7 of guanine and induced DNA strand break, inhibits DNA and RNA synthesis

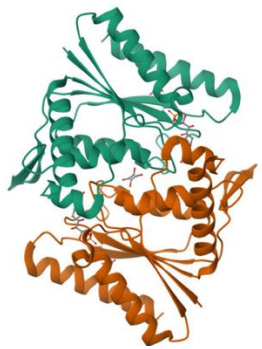
Key Idea: Site selectivity by targeting cancer receptors

Key Idea: Fluorescence readout via conjugation

J. Mater. Chem. B. **2022**, ***10***, 5504., *Curr. Med. Chem.* **2001**, ***9***, 1093., *Bioconjugate Chem..* **2016**, ***27***, 1419., *Biorg. Med. Chem. Lett.* **2007**, ***17***, 1575.

(FMN)-dependent NADH-AzoR: Bioactivation of Azo Groups

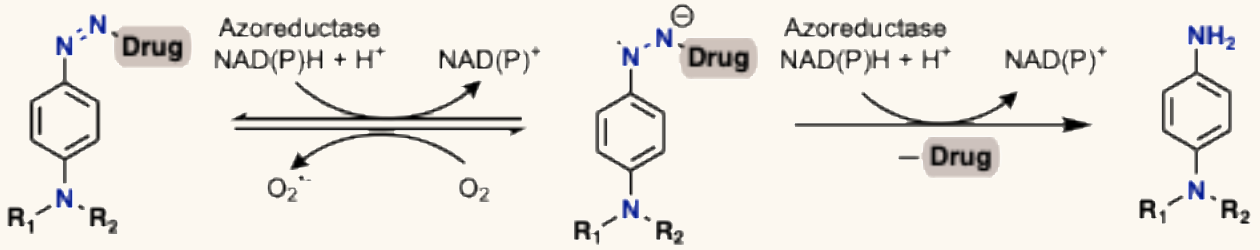
Background

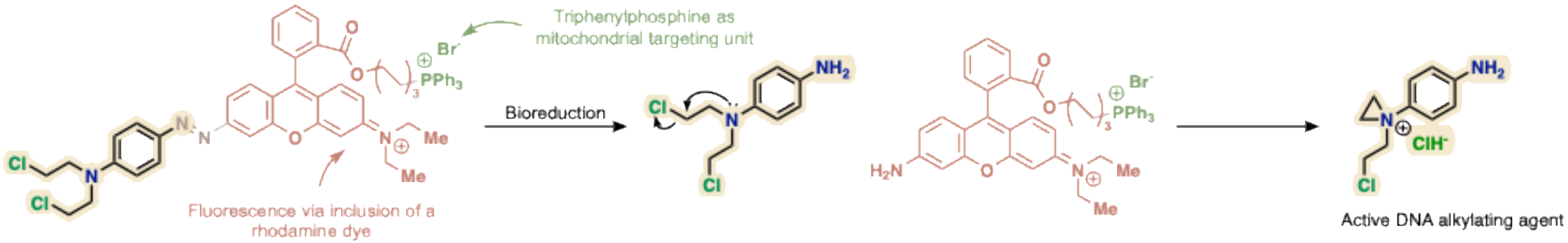


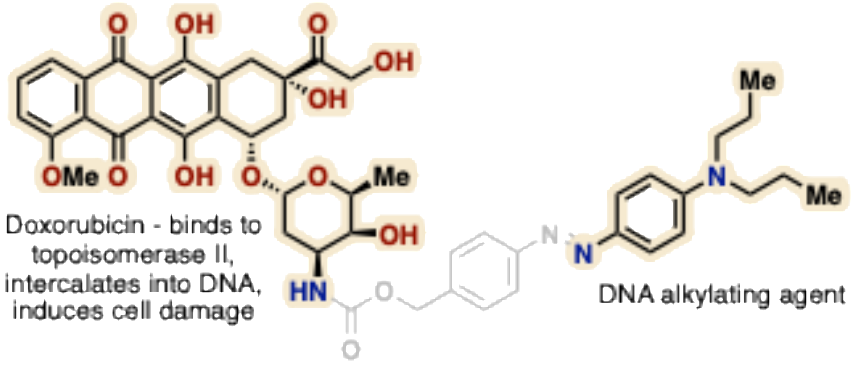
AzoR

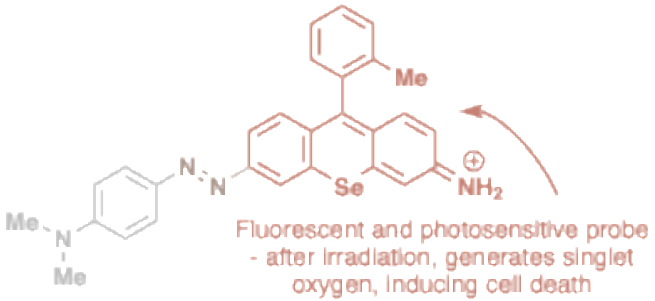
- Flavin mononucleotide reductase
- Found in hypoxic environments
- Highly expressed in breast/ovarian cancer
- Reduces azo bonds (N=N) to amine

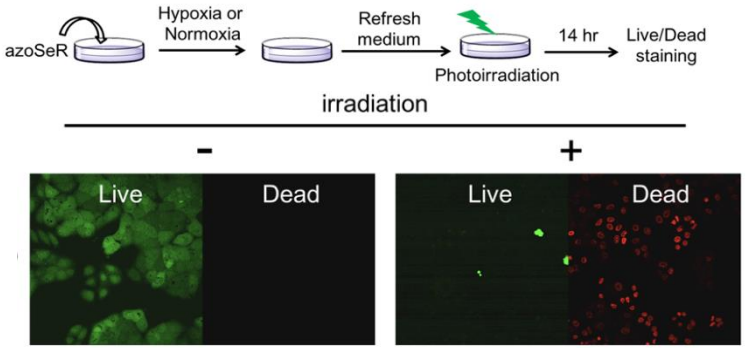
General Mechanism For Azo Reduction







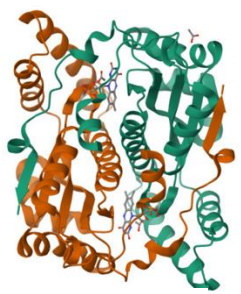




J. Mater. Chem. B. **2022**, ***10***, 5504., *Biomaterials.*. **2017**, ***115***, 104., *Chem. Commun.*, **2018**, ***54***, 7983., *J. Am. Chem. Soc.*, **2017**, ***139***, 13713.

Nitroreductase: Bioactivation of Nitroaryl Groups

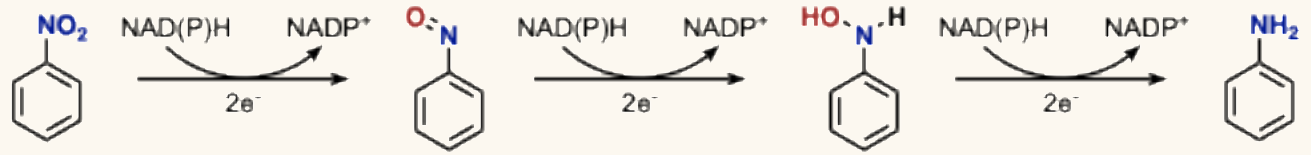
Background



NTR

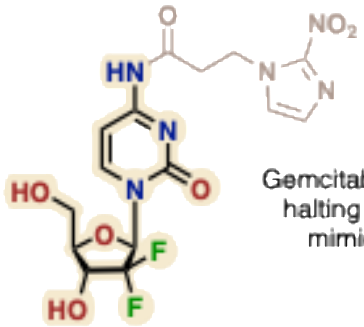
- Flavin mononucleotide reductase
- Found in hypoxic environments
- Highly expressed in breast/cervical cancer
- Reduces nitroaromatic compounds

General Mechanism For Nitro Bioreduction

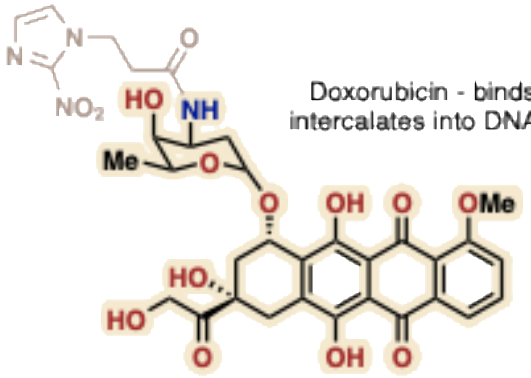


**Can also facilitate one electron reduction

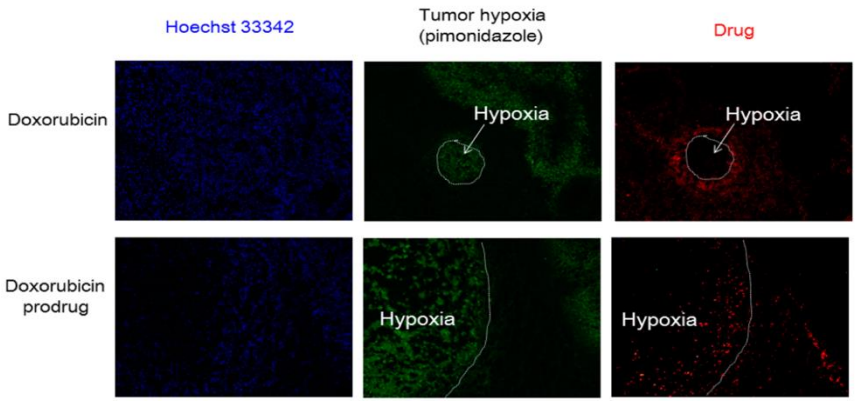
Can also induce cyclization and drug release before thiol attack on imidazole ring



Gemcitabine - masked chain termination, halting DNA and protein synthesis via mimicking DNA or RNA synthesis



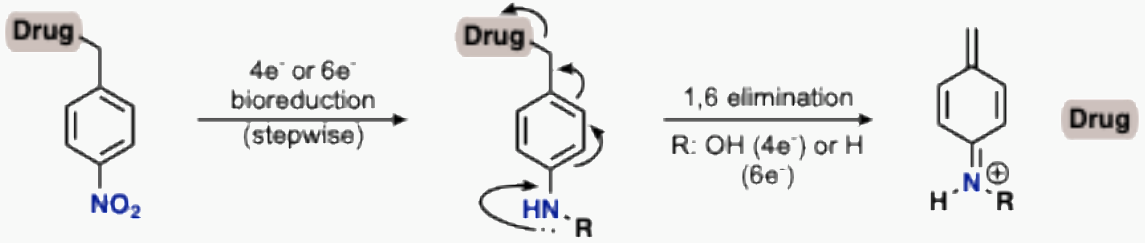
Doxorubicin - binds to topoisomerase II, intercalates into DNA, induces cell damage

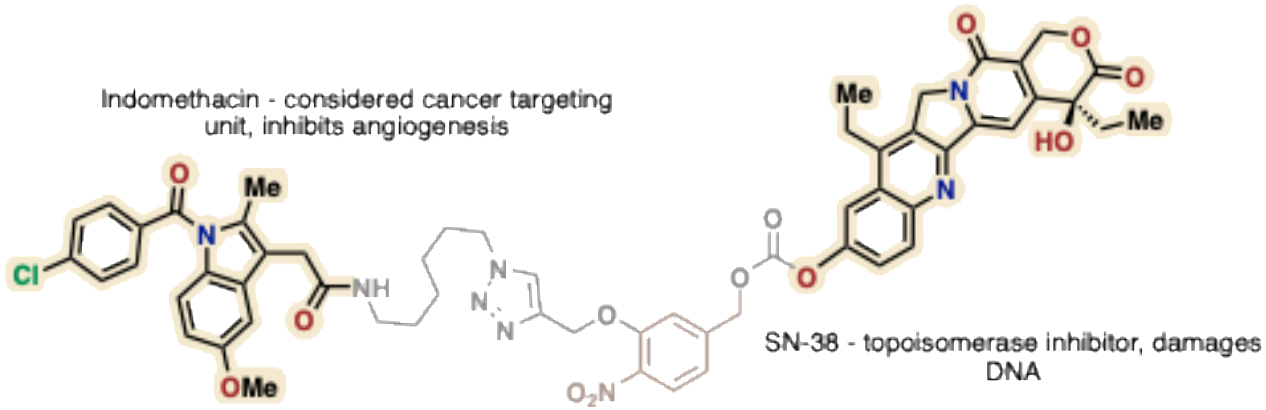


J. Mater. Chem. B. **2022**, ***10***, 5504., *Mol Pharmaceutics.*. **2016**, ***13***, 2283.

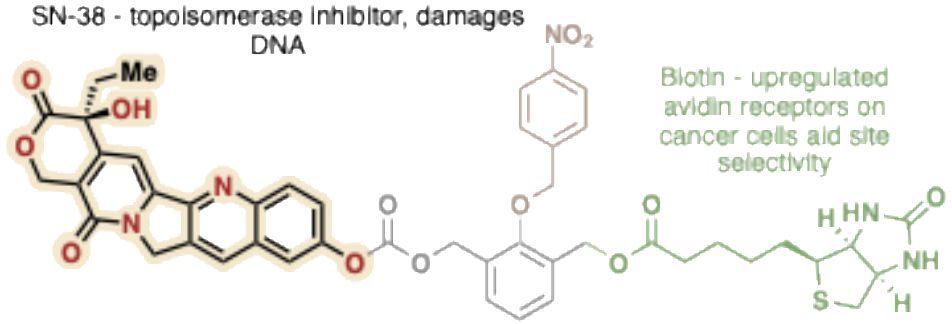
Nitroreductase: Bioactivation of Nitroaryl Groups

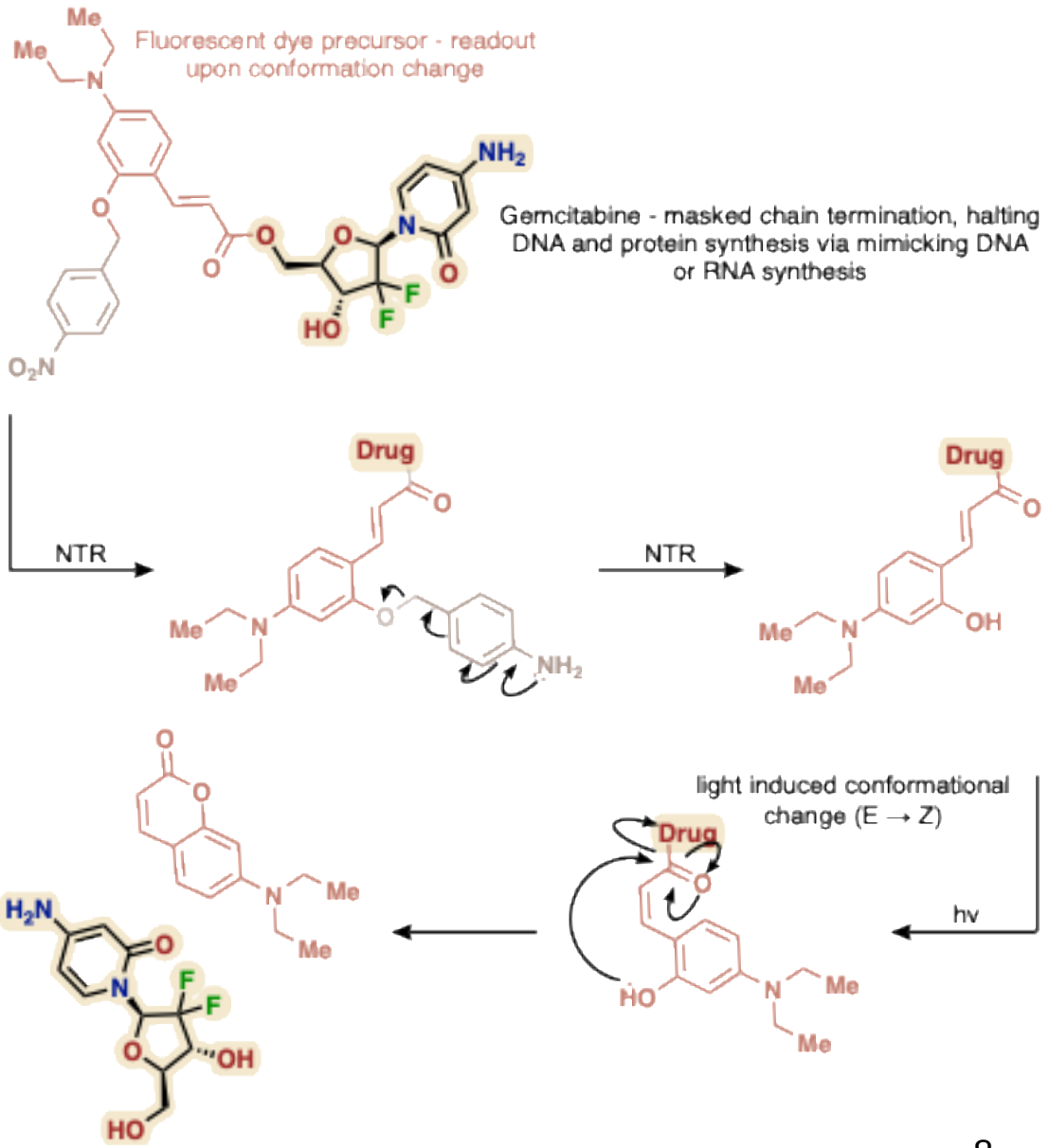
Nitro Reduction and Elimination





Bioactivation methods with similar mechanisms: β-galactosidase and β-glucuronidase based prodrugs





J. Mater. Chem. B. **2022**, ***10***, 5504., *Biomaterials.*. **2016**, ***104***, 119., *Biomaterials.*. **2018**, ***185***, 63., *Chem. Commun.*, **2016**, ***52***, 9434.

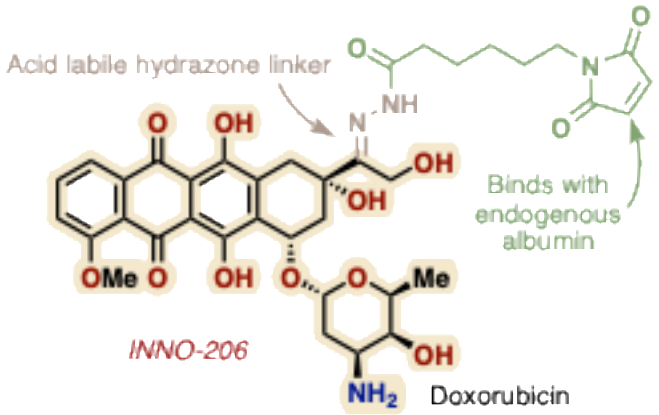
pH Sensitive Prodrugs

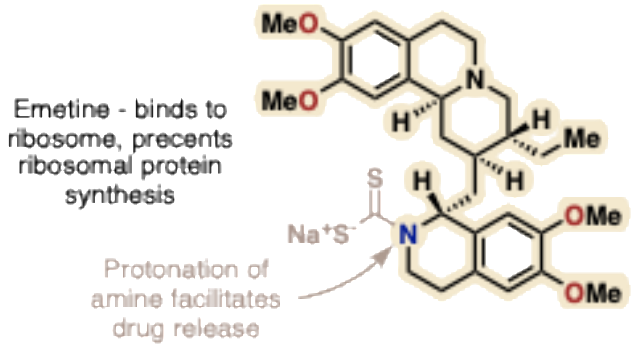
Why use pH differences?

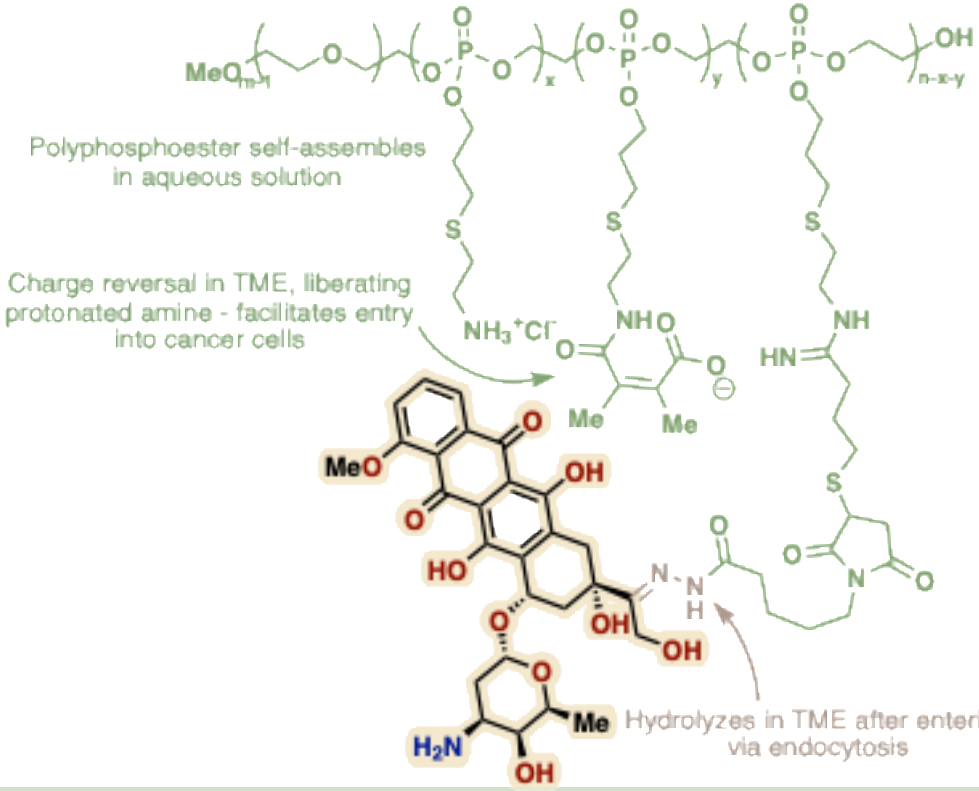
TME has lower pH than normal cells 6.5-6.8 vs. 7.4

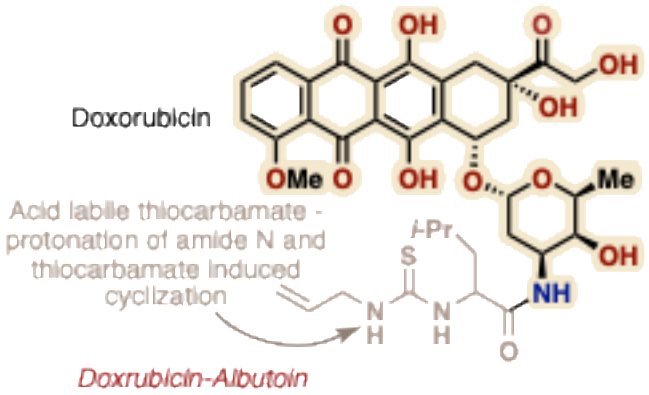
Lower pH related to hypoxic environment

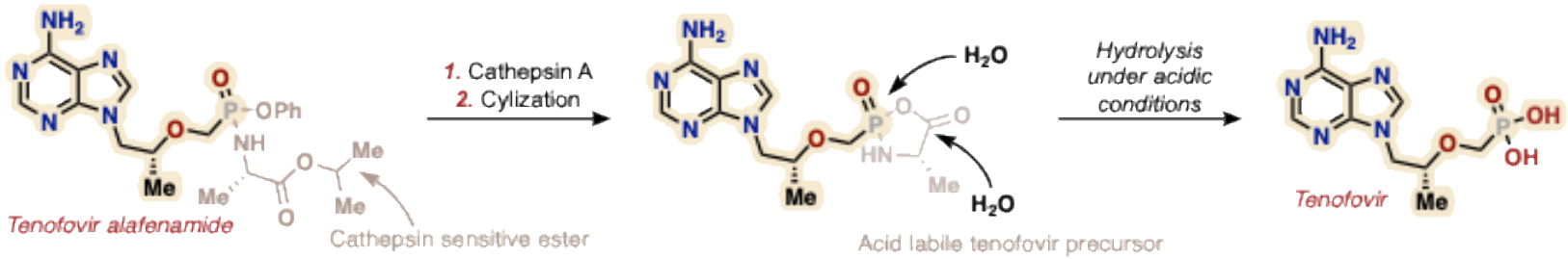
Prodrugs pursued that can be degrade in acidic mediums









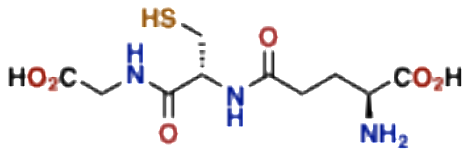


Other acid sensitive groups: imines, carboxylic acid esters, orthoesters, borate ester, acetals, phenyl vinyl ether, inorganic materials, nanoparticles

Eur. J. Med. Chem. **2017**, ***139***, 542., *Expert Opin. Investig. Drugs.* **2007**, ***16***, 855., *J. Am. Chem. Soc.*, **2011**, ***133***, 17560., *Bioconjugate Chem.*, **2019**, ***30***, 741., *AIDS Res. Hum. Retroviruses.*, **2018**, ***34***, 337.

Glutathione Based Bioactivation

Glutathione Background

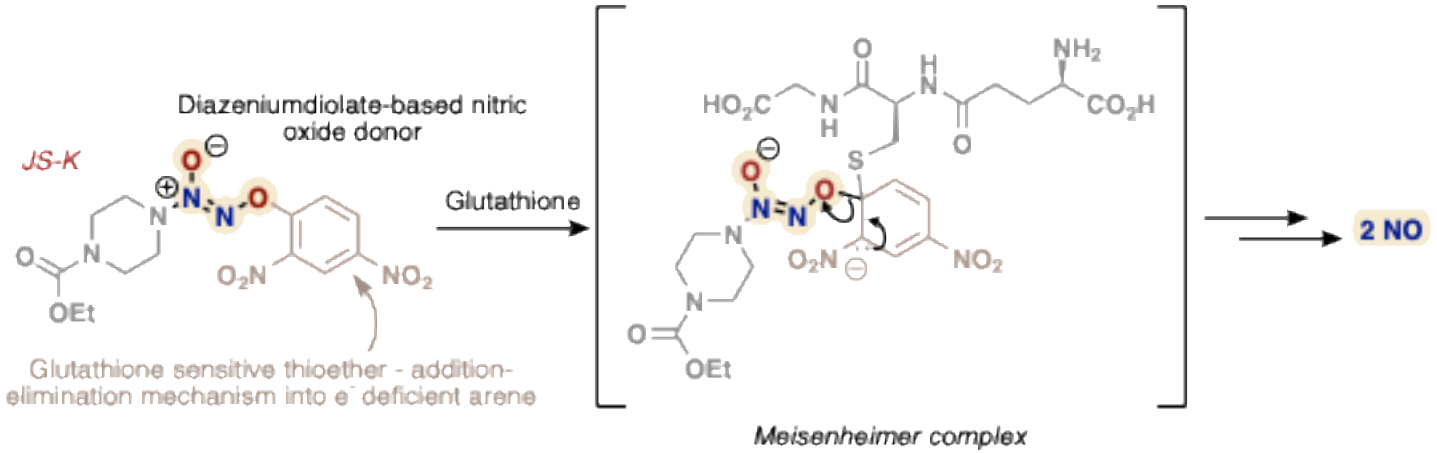


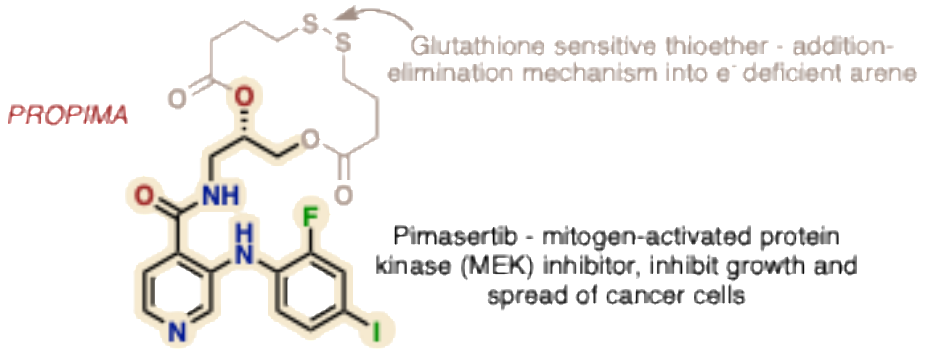
Glutathione

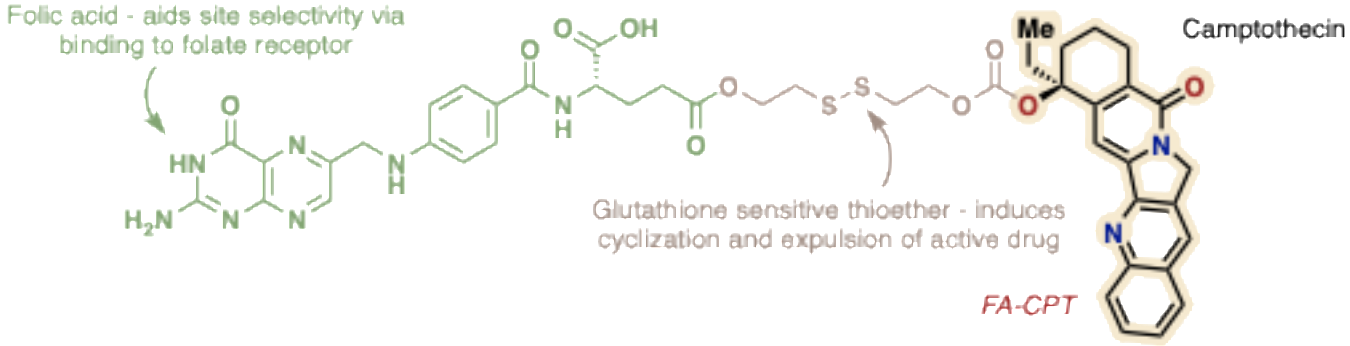
Thiol-containing endogenous tripeptide – helps repair damaged cells, regulate immune system

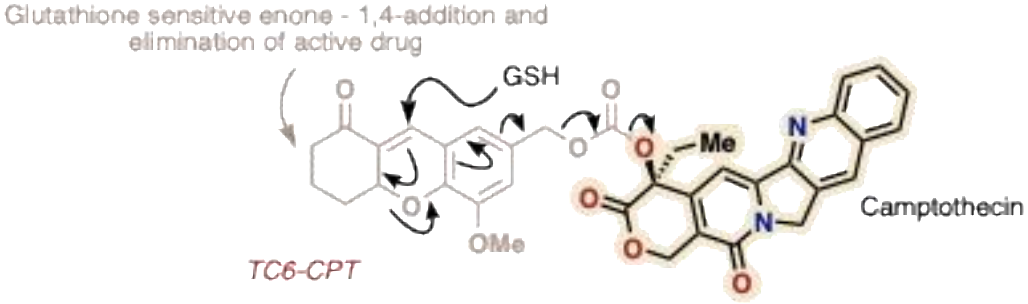
Present in all tissue, however elevated in cancer tissue

Possesses nucleophilic thiol often leveraged to activate prodrugs









Eur. J. Med. Chem. **2017**, ***139***, 542., *Biomater. Sci.*, **2017**, ***5***, 444., *RSC Med. Chem.*, **2024**, *Advanced article*., - *Bioorg. Chem.*, **2022**, ***129***, 106221.

Reactive Oxygen Species Sensitive Linkers

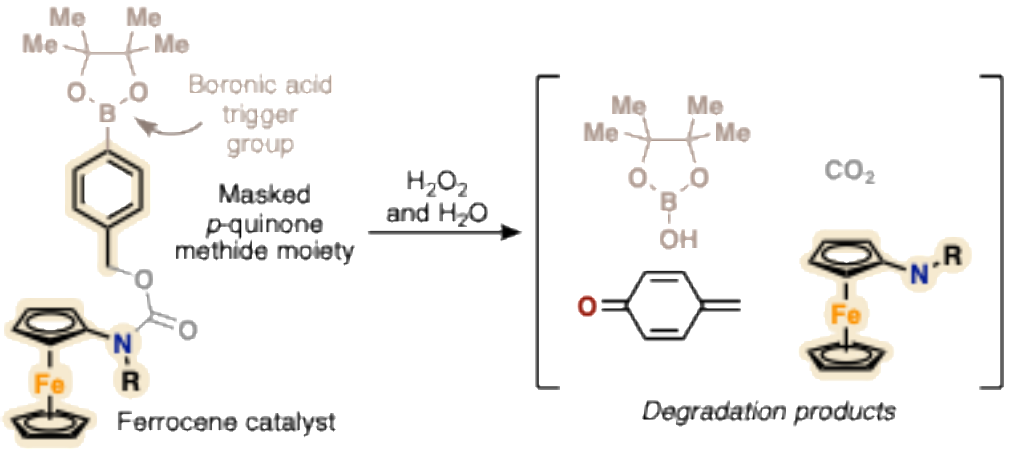
ROS and Cancer

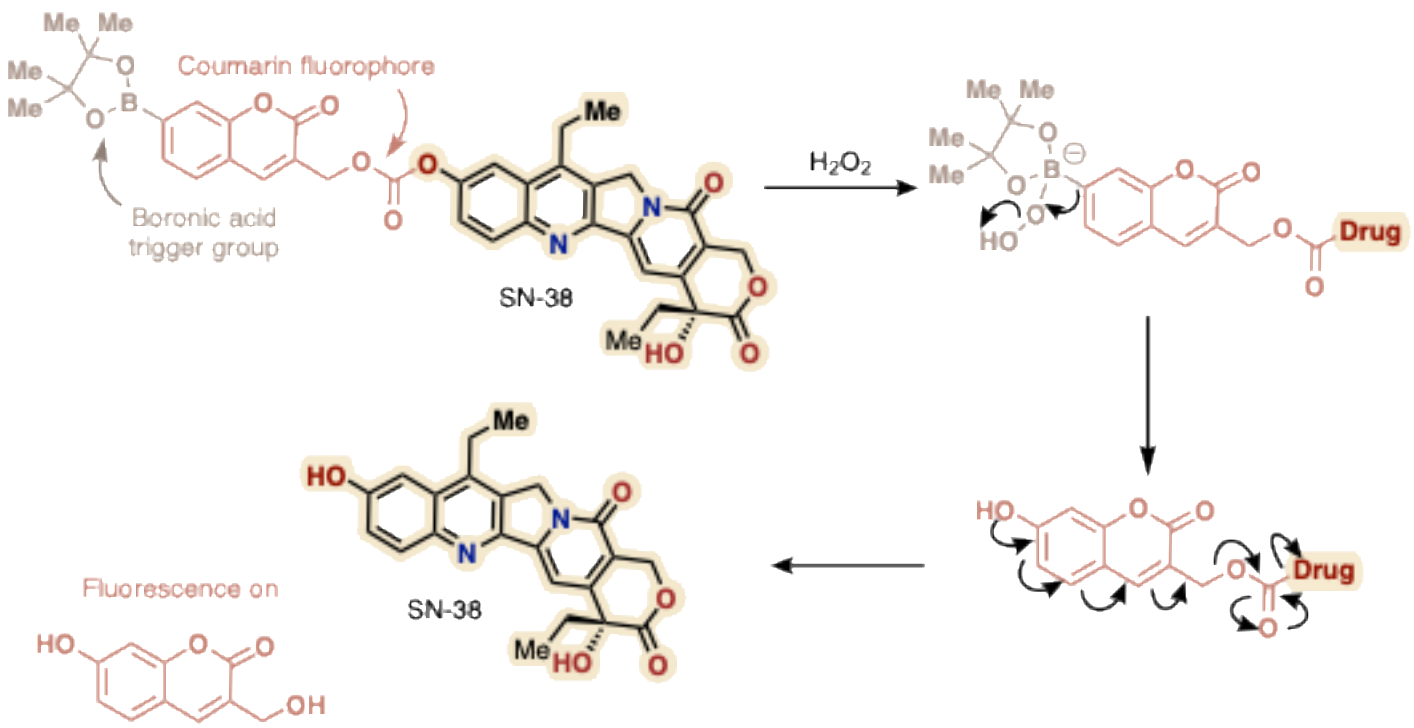
ROS: hydrogen peroxide, superoxide, hydroxyl radical

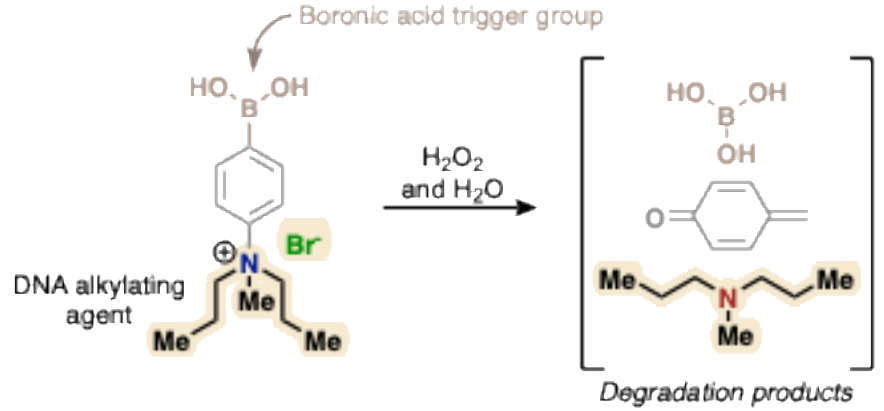
ROS are byproducts of cellular metabolism

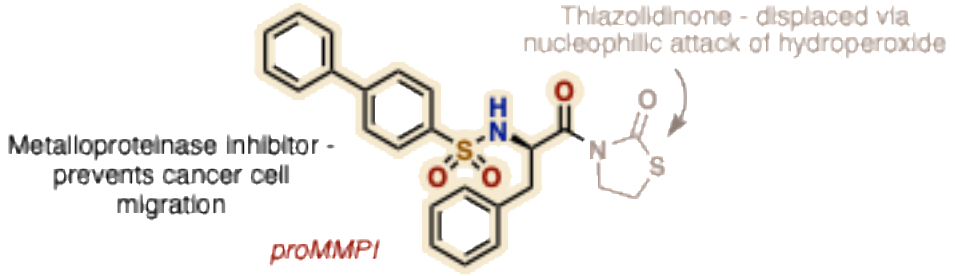
Greater concentration of ROS in cancer cells than normal tissue

ROS induce oxidative stress and apoptosis in cells









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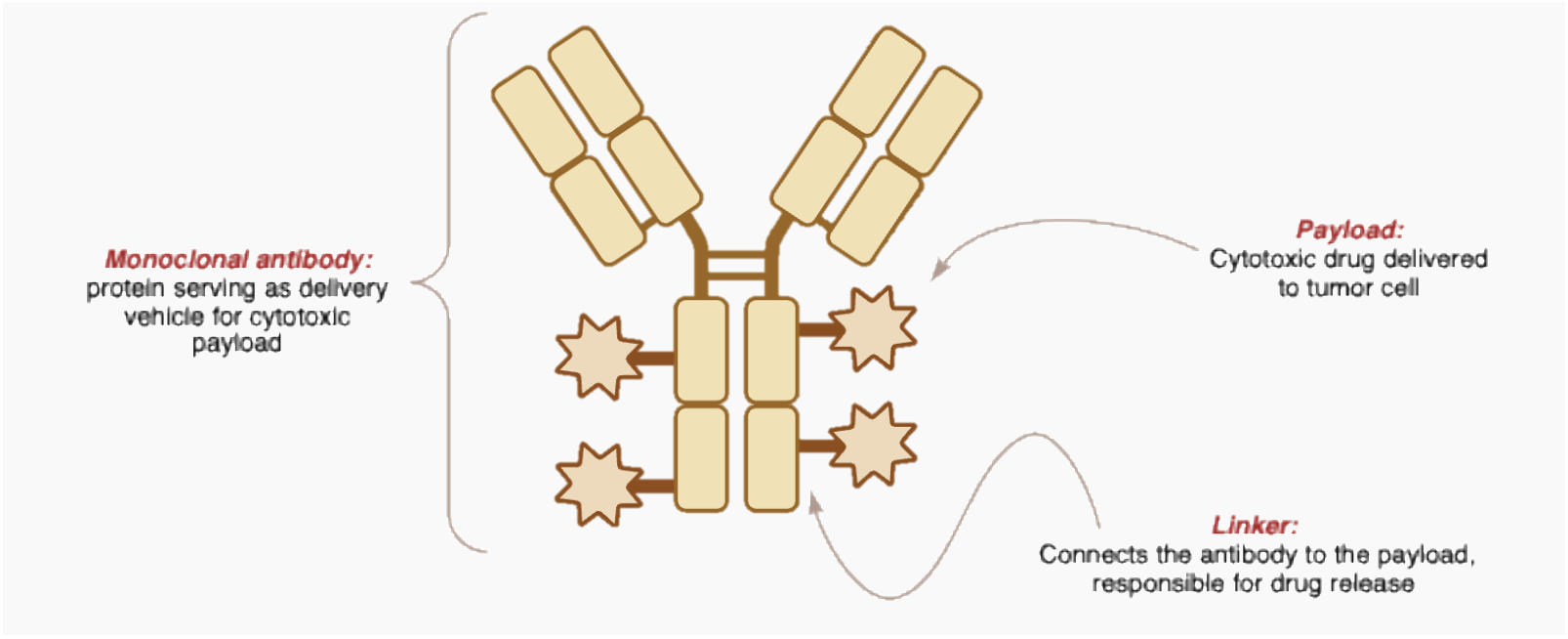
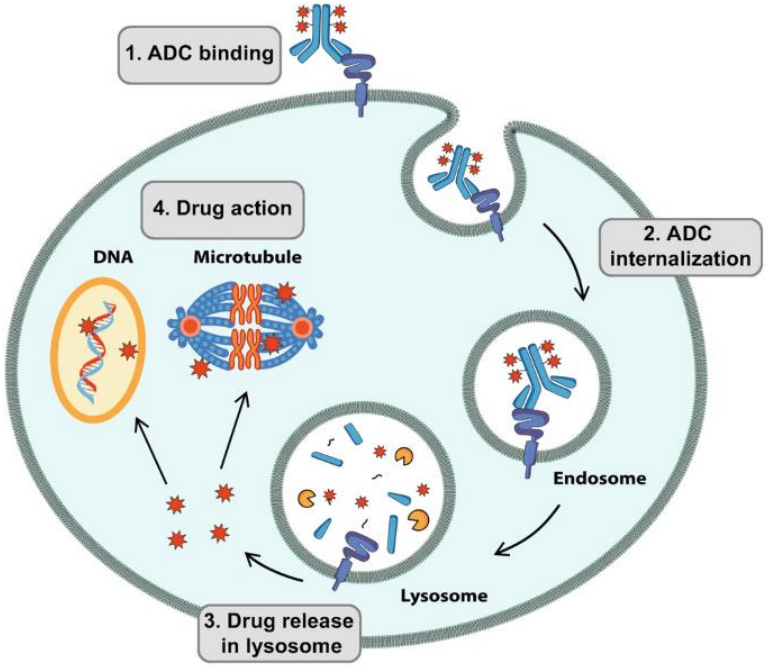
Antibody-Directed Enzyme Prodrug Therapy

Antibody Drug Conjugate Structure, Key Components, and Mechanism of Action





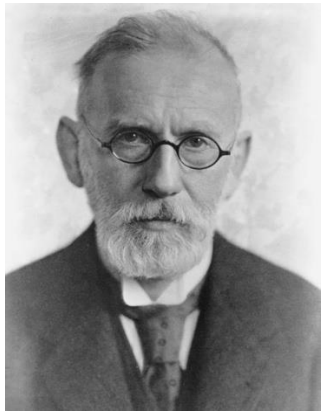
Paul Ehrlich's "Magic Bullet"

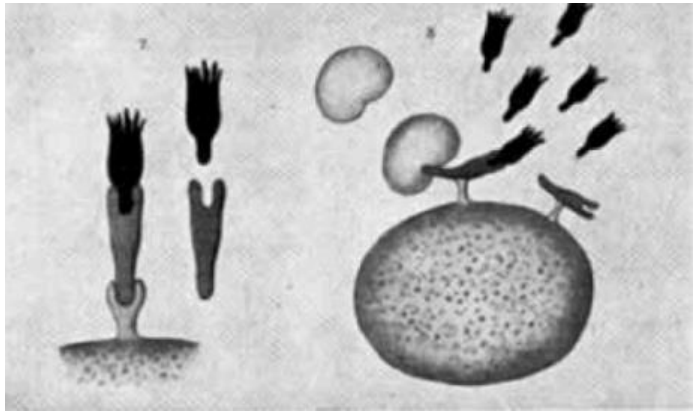
Decrease off target toxicity

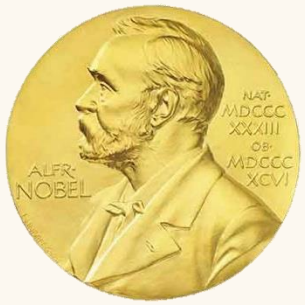
Increases half life of therapeutic agent

Can use highly cytotoxic chemotherapeutics

Overall increase of therapeutic window







1908 Nobel Prize for Medicine

Mol. Immunol. **2022**, ***150***, 90., -- *Biomaterials.*. **2017**, ***115***, 104., *Chem. Commun.*, **2018**, ***54***, 7983., *J. Am. Chem. Soc.*, **2017**, ***139***, 13713.

Antibody Drug Conjugates: Linkers and Payloads

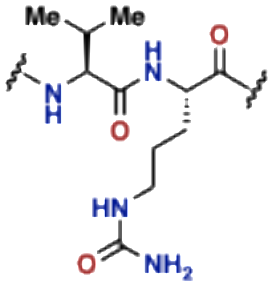
Linkers

Cleavable and noncleavable linkers are used

Ideal linker: stable in circulatory system, tumor specific release

80% clinically approved ADCs are cleavable

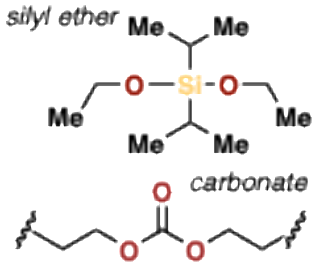
Cathepsin cleavable



Cleaved by cathepsin B, K, and L - cathepsin B upregulated in cancer

cBu-Cit linker selectively cleaved by cathepsin B

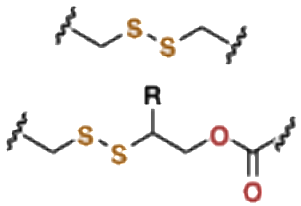
Acid cleavable



TME more acidic than normal tissue

Challenge with nonspecific release

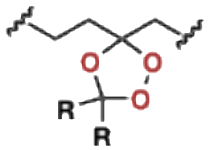
Glutathione cleavable



Disulfide most common

Difficult balance between high circulatoryand efficient intracellular release

Iron (II) cleavable



Recently disclosed trioxolane moiety, used to trigger a Spangler Fenton reaction

Not yet incorporated into an approved ADC

Payloads

Common targets: DNA, RNA, Mitosis disrupters

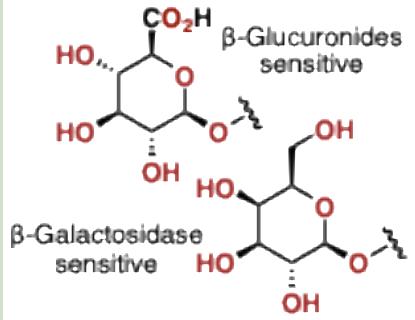
Common antimitotics: maytansinoids, auristatins

Common DNA/RNA targets: calicheamicin, amatoxins

80% clinically approved ADCs are cleavable

Important payload characteristics:
• High cytotoxicity • Intracellular target • Small molecule •
• Aqueous solubility • Allow linker conjugation •
• Stable in plasma •

Enzyme cleavable



Many enzymes are upregulated in cancer cells and/or work more efficiently in the TME - these enzymes can be targeted

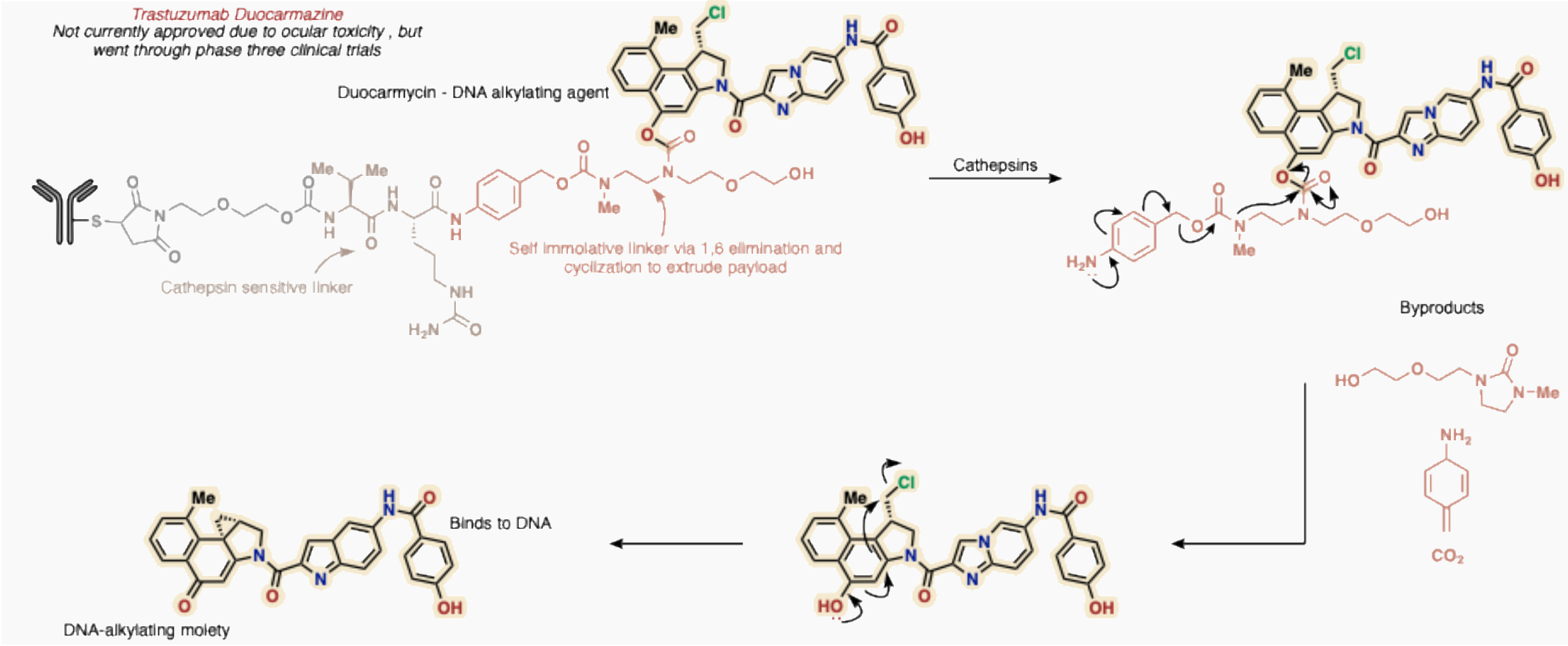
These categories are not exhaustive, but covers some of the most common or unique

Some not included: maleimide linkers, photoresponsive, and bioorthogonal linkers

Mol. Immunol. **2022**, ***150***, 90., -- *Biomaterials.*. **2017**, ***115***, 104., *Chem. Commun.*, **2018**, ***54***, 7983., *J. Am. Chem. Soc.*, **2017**, ***139***, 13713.

A Few ADC Examples

Cathepsin Cleavable

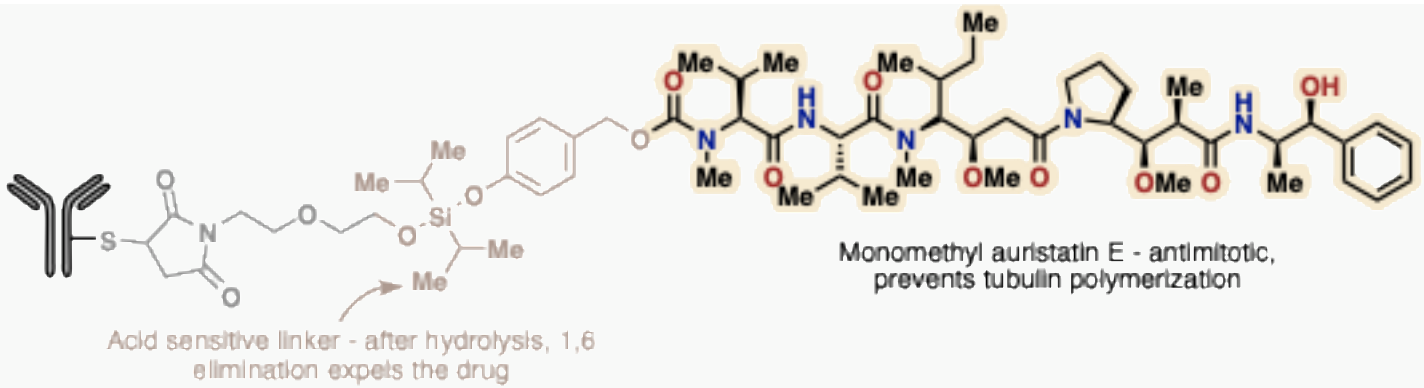


Approved ADCs with valine-citrulline linker: Adcetris (brentuximab vedotin), Polivy (polatuzumab vedotin), and Padcev (enfortumab vedotin)

Mol. Immunol. **2022**, ***150***, 90., -- *Biomaterials.*. **2017**, ***115***, 104., *Chem. Commun.*, **2018**, ***54***, 7983., *J. Am. Chem. Soc.*, **2017**, ***139***, 13713.

A Few ADC Examples

Acid Cleavable



Antibody type: Humanized

Antigen: HER2

HER2: tyrosine kinase, helps cell grow and divide

Currently no ABCs with acid labile silyl cleavable linker

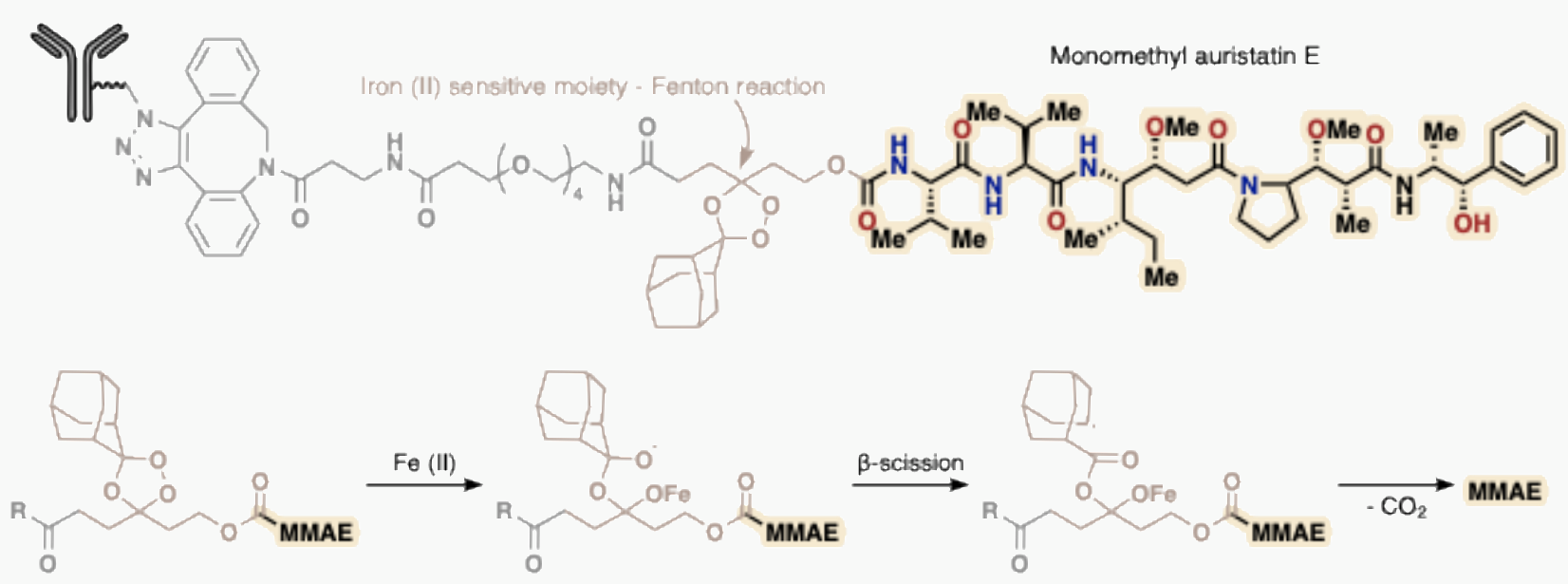
Iron (II) Cleavable

Antibody type: Humanized, trastuzumab

Antigen: HER2

Found trioxolane to be as stable as Val-Cit

Some instability observed depending on distance between trioxolane unit and AB



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Summary

Refresher: Why Promoieties

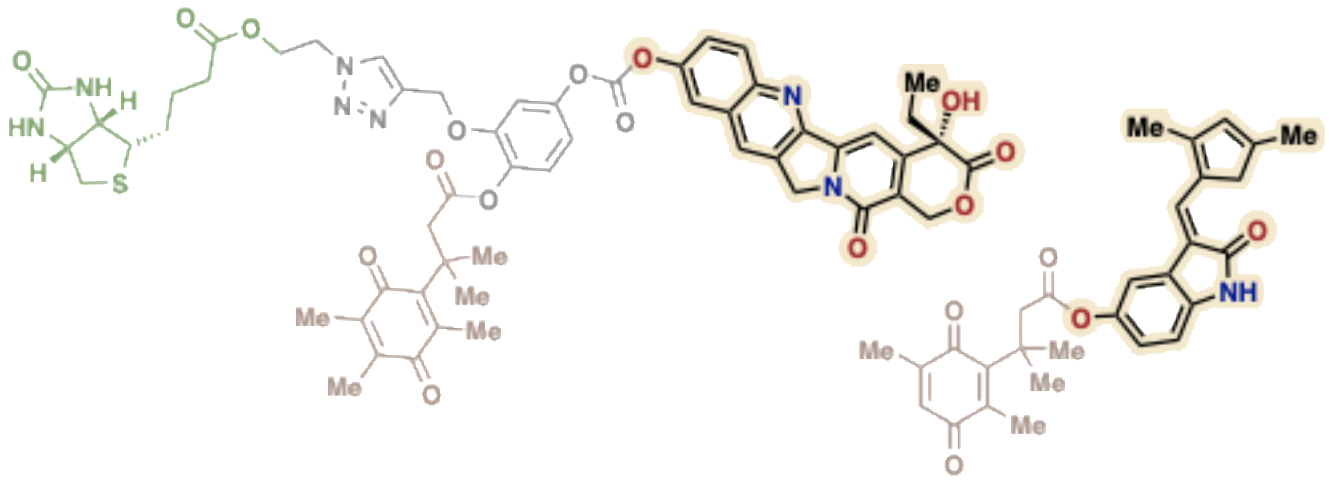
Increase the therapeutic window of cytotoxic agents by:

Increase site selectivity and decrease off-target effects

Overcome challenges with permeability and solubility

Increase circulatory stability and extend half lives

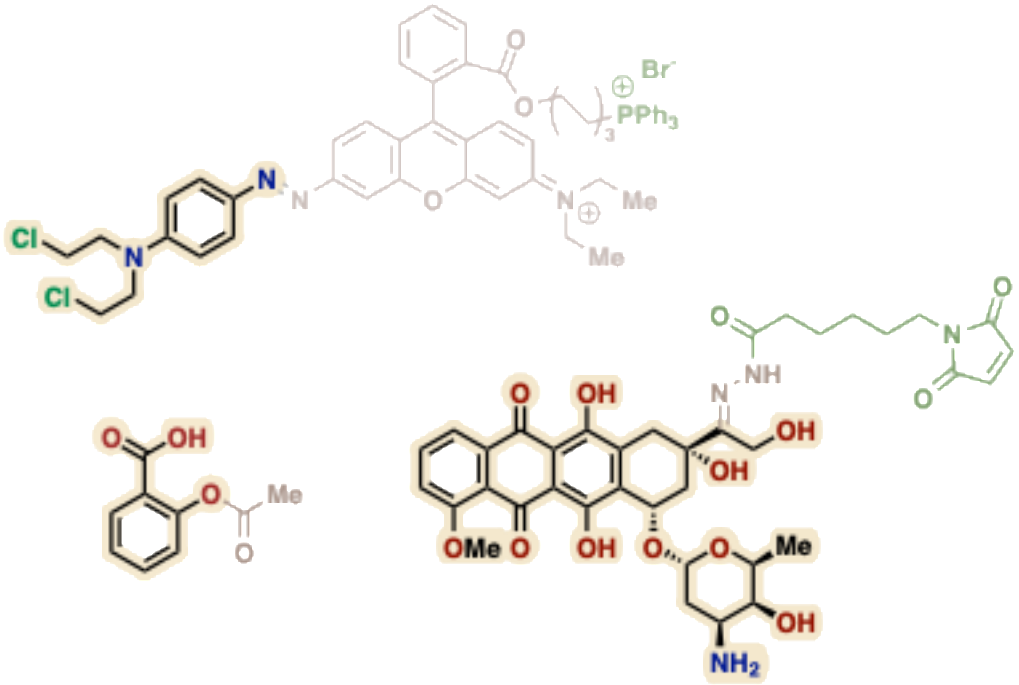
Many more reasons! Prodrugs aren't simply "an act of desperation"



1899

2024

125 Years of Moving from serendipity to rational design



What should we take away?

*Prodrug and ADC development is ripe with opportunity for ingenuity (**especially by synthetic organic chemists**)!*

Key chemical principles regarding reactivity and synthesis can be exploited to design cytotoxins!

Acta Pharm. Sin. **2021**, ***11***, 3889., *Cancers.*. **2019**, ***11***, 957.