A Synopsis of the Applications of Prodrugs in Drug Discovery & Development

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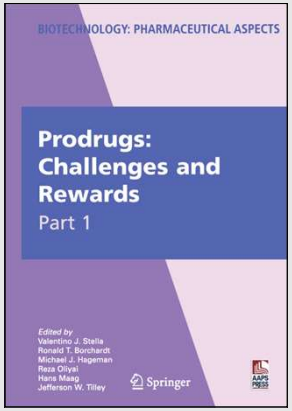
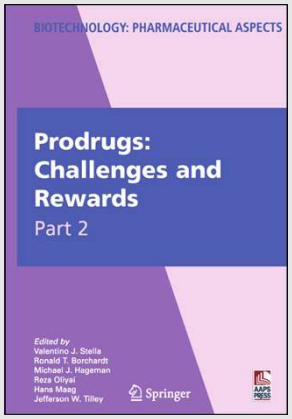
Outline

- Background
  - prodrug history & nomenclature
- Prodrug applications
  - basic prodrug design principles
  - prodrug approvals 2021-2022 & a selection of approved prodrugs
  - prodrug space & landscape
- Basic principles of prodrug design
  - prodrug strategies for common functionalities
- Prodrugs to address problems with membrane permeability
  - carboxylic acids, phosphonates, amides
  - antiviral nucleos(t)ide analogues: prodrugs of prodrugs
- Prodrugs to address problems with solubility
  - IV & PO administration
- Prodrugs to affect *in vivo* disposition
  - enhancing PK, tissue targeting
- Prodrugs & long-acting compounds
  - role of prodrugs in compound properties
- Antibody drug conjugates (ADCs)
  - a unique & growing class of prodrug
  - not an in-depth discussion
- Conclusion
  - acknowledgements

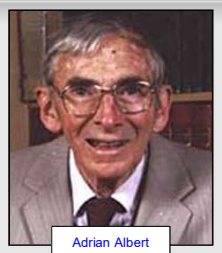




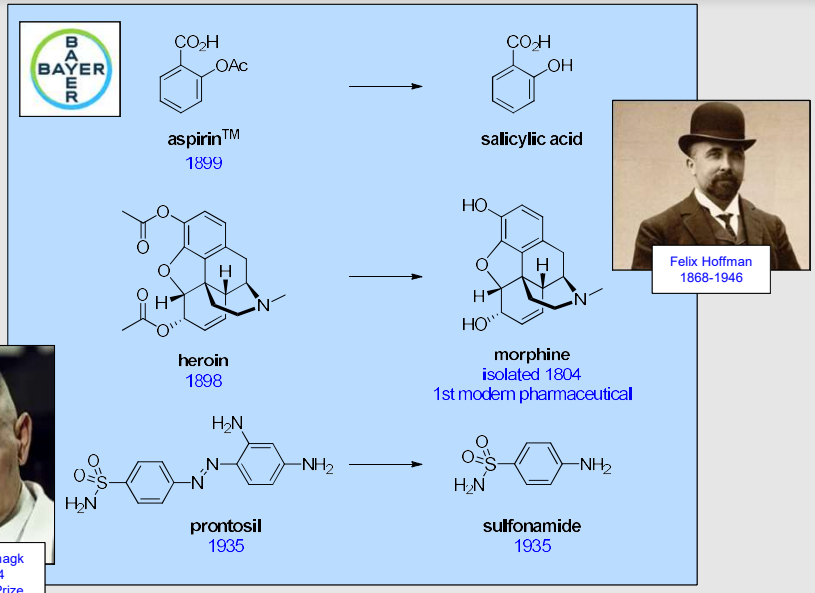
Background

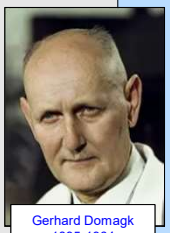
Prodrug Nomenclature & History

- The term "prodrug" was introduced by Adrian Albert in 1958
  - aspirin, heroin & prontosil are early examples
- Nomenclature expanded to include:
  - prodrug: metabolized or converted to an active drug
  - codrug: produced 2 active principles
  - soft drug: metabolized to an inactive substance
  - bioprecursor prodrug: intramolecular prodrug

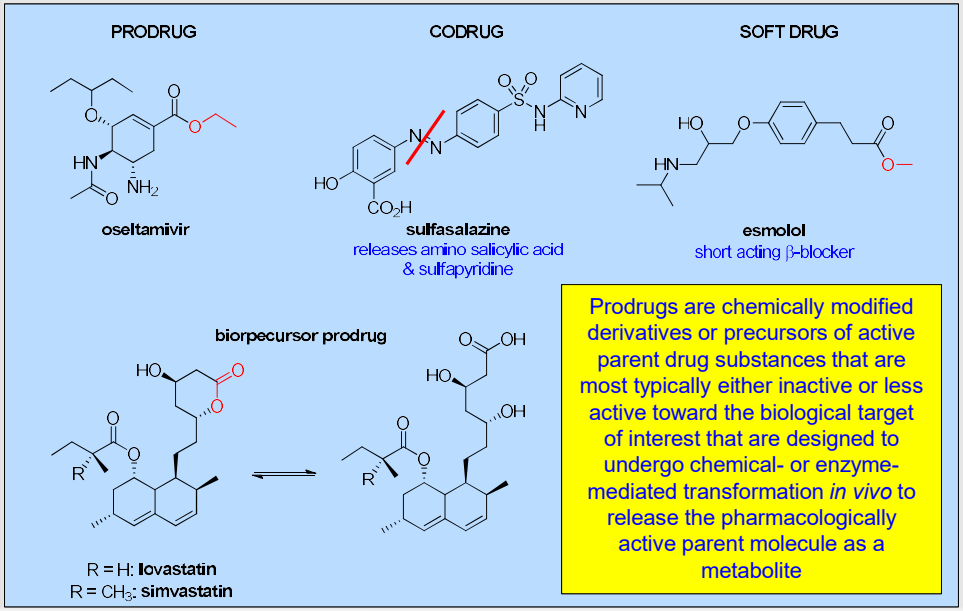


Adrian Albert
1907-1989





Gerhard Domagk
1895-1964
1939 Nobel Prize



Prodrugs are chemically modified derivatives or precursors of active parent drug substances that are most typically either inactive or less active toward the biological target of interest that are designed to undergo chemical- or enzyme-mediated transformation *in vivo* to release the pharmacologically active parent molecule as a metabolite

- Felix Hoffman (Bayer) acetylated salicylic acid & morphine in August 1897
  - afforded Aspirin™ (August 10th) & heroin (August 27th)
  - heroin originally prepared by C.R. Wright in 1874
  - both marketed by Bayer: both are prodrugs
- Prontosil discovered in 1932 at Bayer by Gerhard Domagk
  - 1st synthetic drug used to treat bacterial infection
  - opened up a new era in medicine

A. Albert, *Nature,* 1958, **182**, 421-422; J. Rautio, 2016 presentation, *personal communication*
Science History Institute: https://www.sciencehistory.org/education/scientific-biographies/felix-Hoffmann/; H. Beck *et al., Drug Discov. Today*, 2022, **27**, 1560-1574

Prodrug Applications & Recent Approvals

Improved ADMET Properties & Profile

- Formulation & administration
  - increased solubility for oral & IV administration
  - reduced solubility for sub-cutaneous & intramuscular delivery
  - to improve shelf life (solid or liquid)
- Absorption
  - improved membrane permeability
  - enhanced solubility to overcome dissolution issues
- Distribution
  - tissue targeting
- Metabolism & excretion
  - abrogate first-pass metabolism
- Toxicity mitigation
  - tissue targeting
  - *in vivo* distribution

- 348 Drugs approved by the FDA 2012-2022
  - 41 prodrugs: 12%
  - ~10% of all drugs approved worldwide are prodrugs
- Exploit a wide range of bioactivation mechanisms
  - alkaline phosphatase, CYP 450, nitroreductase
  - ligase, peptidase, esterase, kinase

No prodrugs approved in 2023 & 2024

- Recently approved prodrugs 2018-2022

2022
- omidenepag isopropyl (PGE_2 antagonist, ocular hypertension)
- terlipressin (vasopressin agonist, kidney function)

2021
- fexinidazole (NO_2-antimicrobial, sleeping sickness)
- brincidofovir (nucleoside analogue for smallpox)
- serdexmethylphenidate (CNS stimulant for ADHD)
- melphalan flufenamide (alkyating agent for cancer)

2020
- remdesivir (nucleoside analogue for COVID)
- nifurtimox (nitrofuran antimicrobial)
- triheptanoin (fatty acid source)
- fostemsavir (HIV-1 attachment inhibitor)
- artesunate (malaria)
- bempedoic acid (ACL inhibtor for atherosclerosis)

2019
- diroximel fumarate (NRF2 activator for MS)
- pretonamid (NO_2-mycolic acid synthesis inhibitor for TB)
- baloxavir marboxil (influenza endonuclease inhibitor)

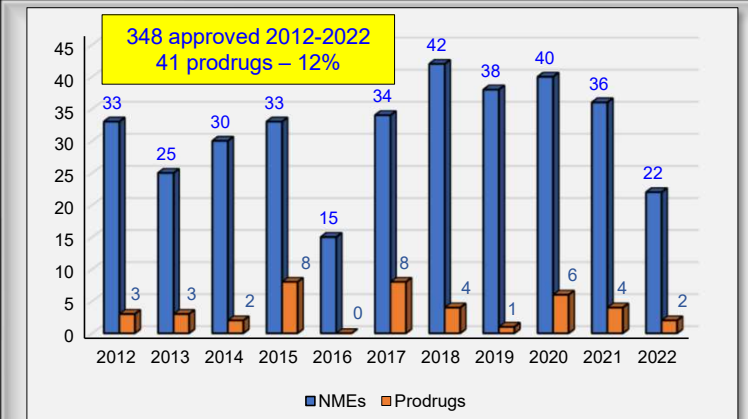
2018
- tafenoquine (aminoquinoline antimalarial)
- fosnetupitant (NK1 antagonist for emesis)
- fostamatinib (SYK kinase inhibitor for thrombocytopenia)

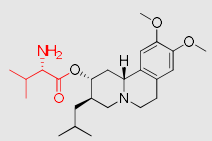
Applications of Prodrugs

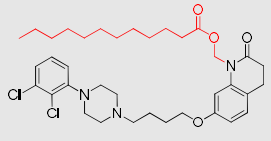
Specific Challenge	Underlying Issue	Prodrug Design Solution
Low oral bioavailability/exposure	Poor solubility and/or low permeability	Enhancing solubility or permeability
Non-linear exposure with ascending dose		
Inadequate exposure to support toxicological or clinical studies		
pH-dependent absorption	pH-dependent solubility	Enhancing solubility at higher pH
High pill burden	Poor aqueous solubility	Enhancing aqueous solubility
Formulation challenges		
Lack of feasibility of developing solid and/or IV dosage forms		
Low oral exposure due to high metabolic clearance	1st pass metabolism	Mitigating or bypassing metabolism
High intersubject PK variability		
Higher dosing frequency (BID or TID) of oral drugs	Short *in vivo* $t_{1/2}$	Reduce dosing frequency by extending half-life or sustained release
Reduced patient compliance with daily injections	PK-limiting QD dose	Weekly or monthly LAIs
Poor drug exposure at a specific site (tissue, organ, or microbe)	Non-selective delivery	Site-specific drug delivery
Dose-related toxicities from chemotherapeutic agents	Non-selective action on normal tissues	Targeted delivery to TME or cancer cells
Chemical instability	Poorly stable functional group	Mask the functional group

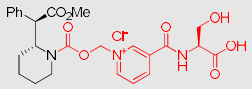
LAI: long-acting injectable; TME: tumor microenvironment

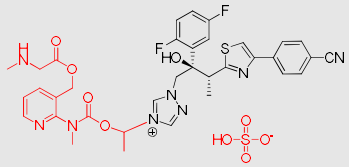
Prodrug Approvals 2012-2022

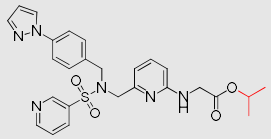


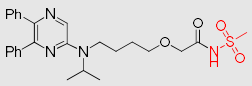

valbenazine
2017

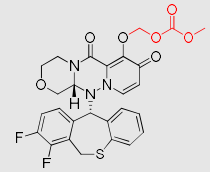

aripiprazole lauroxil
2015

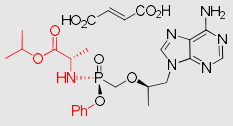

serdexmethylphenidate
2021
MW = 536; parent: 233
Prodrug moiety larger than the drug!

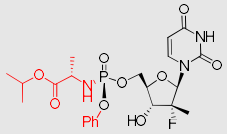

isavuconazonium sulfate
2015

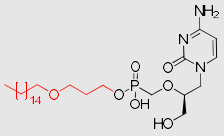

omidenepag isopropyl
2022

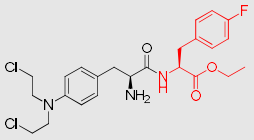

selexipag
2015

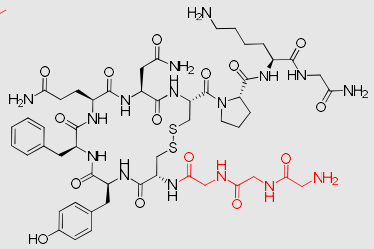

baloxavir marboxil
2018

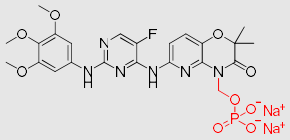

tenofovir alafenamide fumarate
2015

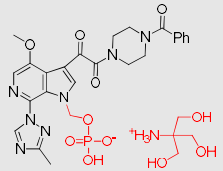

sofosbuvir
2013

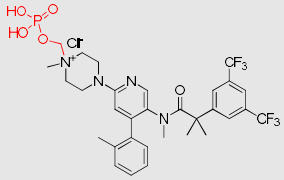

brincidofovir
2021

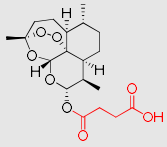

melphalan flufenamide
2021

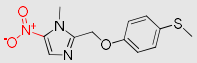

terlipressin [Gly-Gly-Gly tripeptide]
2022


fostamatinib
2018


fostemsavir
2020


fosnetupitant
2018


artesunate
2020


fexinidazole
2021

No prodrugs approved in 2023 & 2024

M.A.M. Subbaiah, J. Rautio & N.A. Meanwell, *Chem. Soc. Rev.*, 2024, **53**, 2099-2210

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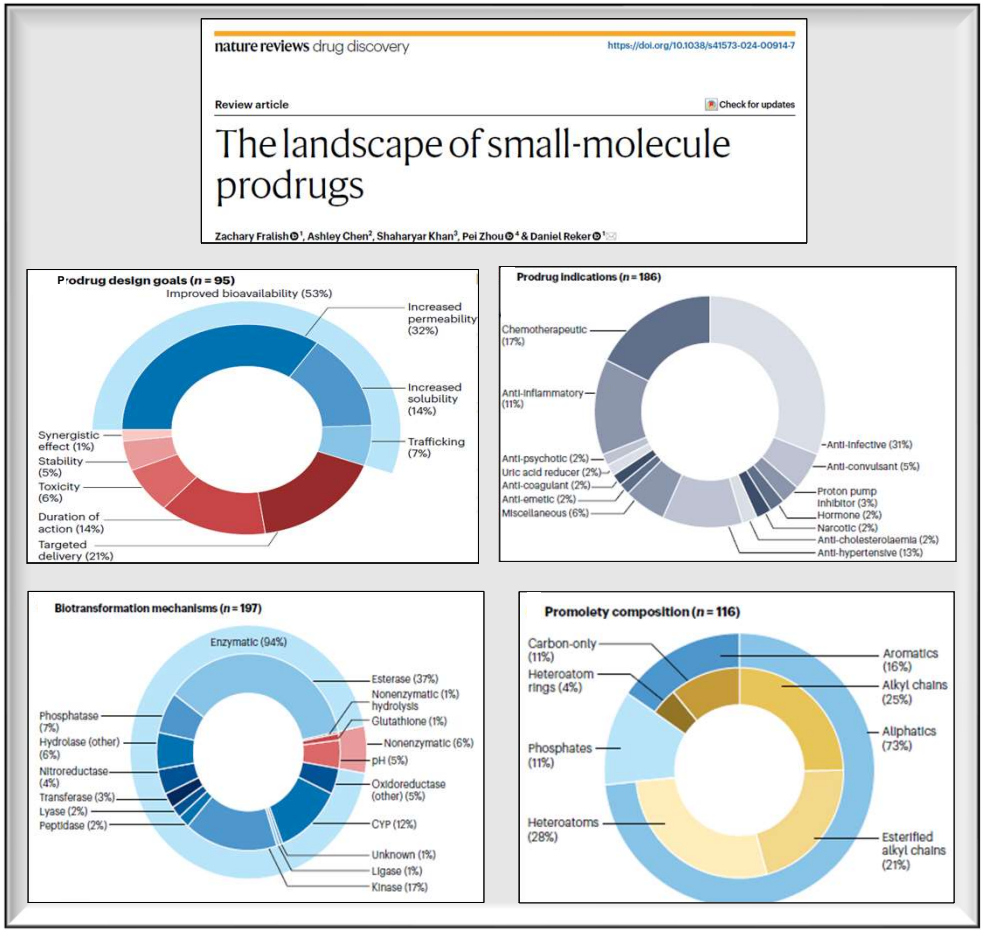
Prodrug Landscape 2024

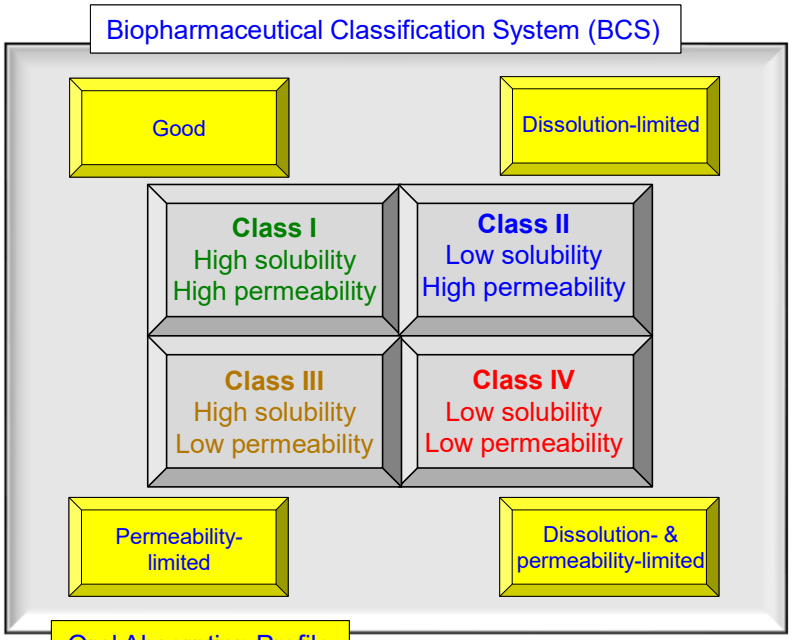
- 178 Prodrugs approved by the FDA
  - ~9% of the approved pharmacopeia
  - across all therapeutic indications
  - wide range of biotransformation mechanisms

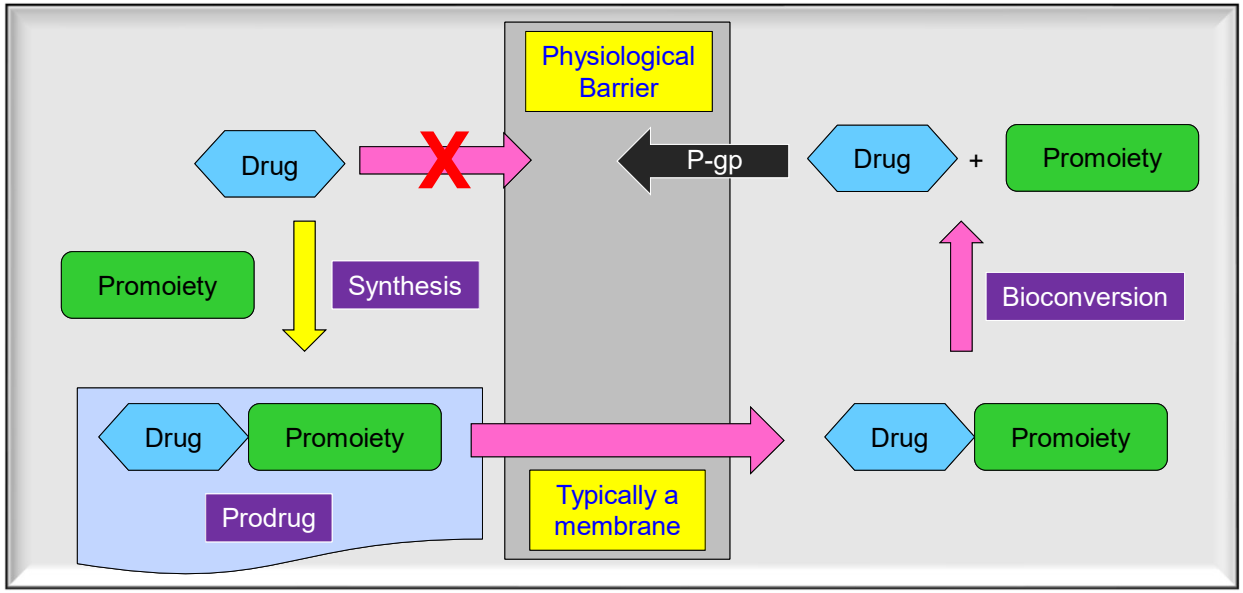
Prodrug Complexion

- Detailed data on design characteristics available for 85 prodrugs
  - 95 design objectives
  - some designs had multiple objectives
- 59% (50 prodrugs) designed to **enhance oral bioavailability**
  - 35% (30 prodrugs) designed to **increase membrane permeability**
  - 15% (13 prodrugs) designed to **enhance aqueous solubility**
  - 8% (7 prodrugs) to take advantage of **endogenous uptake mechanisms**
- 24% (20 prodrugs) designed for **targeted delivery**
  - 15% (13 prodrugs) designed to prolong duration of action
  - 7% (6 prodrugs) designed to minimize toxicity
  - 6% (5 prodrugs) designed to enhance chemical or metabolic stability
- **Enhancing membrane permeability & solubility most common prodrug design**
  - heavy focus on solving oral bioavailability deficiencies
- Since 2015, 5/7 approved prodrugs designed to address issues beyond %F
  - suggests a shift toward more sophisticated prodrug design
- 294 Investigational prodrugs analyzed to explore emerging trends
  - 42% (124/294) designed to enhance %F
  - 26.5% (78/294) focused on tissue targeting
    - an increase of 5% compared to approved prodrugs
- Data suggests that emerging prodrugs are:
  - being designed to solve more sophisticated problems



BCS Classification & Barriers to Absorption





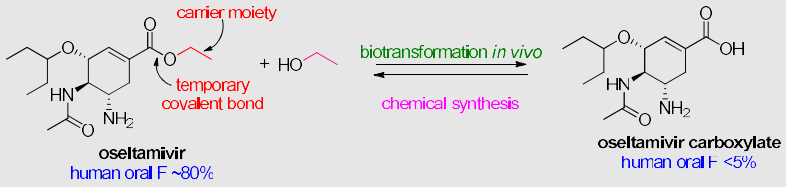
- Prodrugs can address Class II, III & IV BCS problems for orally administered drugs
  - promoiety can productively modulate physicochemical properties
- Only **membrane permeable** drugs are absorbed
  - prodrugs can mask problematic polarity
  - prodrug modification can access transporter-mediate uptake
- Only **soluble drug** is absorbed
  - solubility/dissolution can limit oral bioavailability
  - excipients can assist but prodrugs are applicable when this fails

- Prodrugs can address low membrane permeability & poor drug solubility

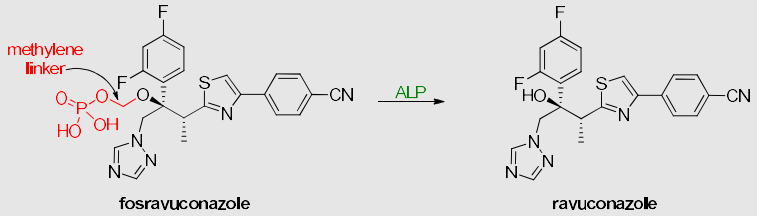
G.L. Amidon *et al.*, *Pharm. Res.*, 1995, **12**, 413-420; M.G. Papich & M.N. Martinez, *AAPS J.*, 2015, **17**, 948-964; M.A.M. Subbaiah *et al.*, *Chem. Soc. Rev.*, 2024, **53**, 2099-2210

Basic Prodrug Designs

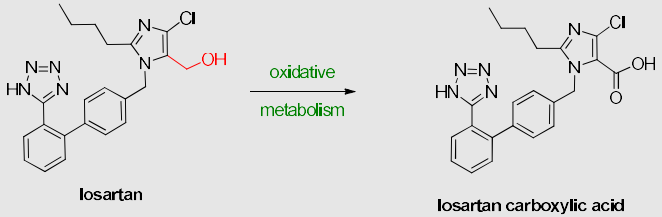
1. Prodrug that involves chemical derivatization with simple element



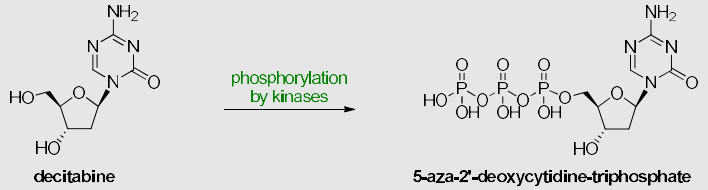
2. Prodrug design that relies upon chemical derivatization *via* a linker



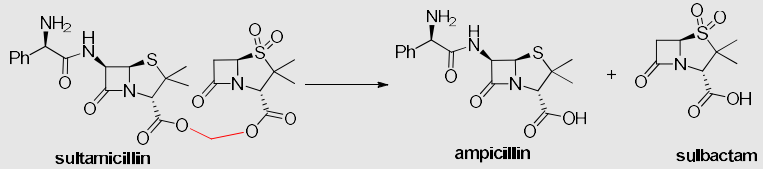
3. Prodrug design that relies upon metabolism *in vivo* to provide an active species



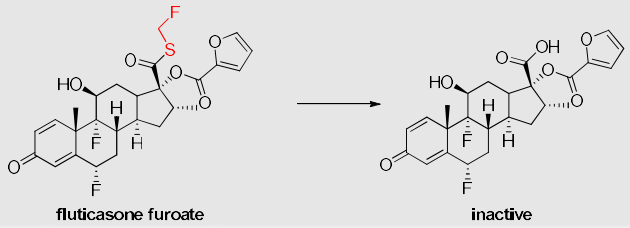
4. A prodrug that relies upon metabolic modifiction by addition of new elements *in vivo*



5. An example of codrug design that releases 2 active principles



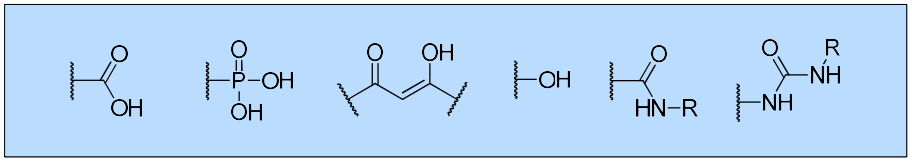
6. An example of soft drug design in which metabolism produces an inactive compound



M.A.M. Subbaiah, J. Rautio & N.A. Meanwell, *Chem. Soc. Rev.*, 2024, **53**, 2099-2210

Prodrugs to Address Poor Membrane Permeability

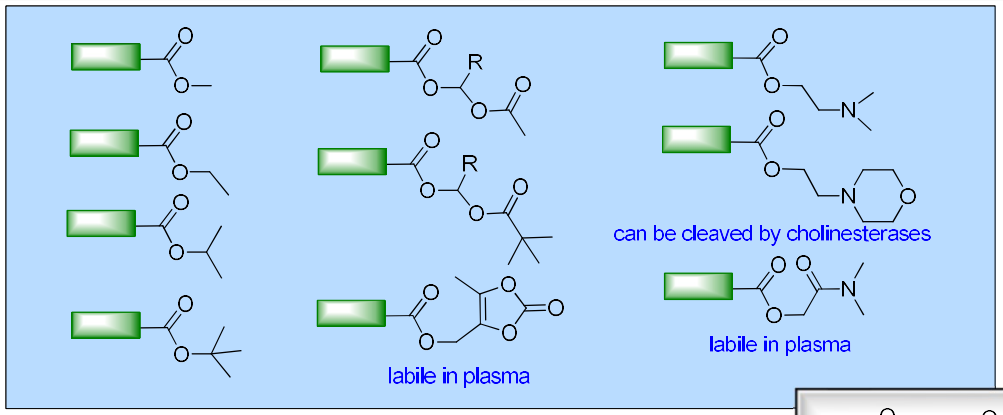
- Typically moieties with charge or multiple H-bond donors
  - eliminate charge, reduce solvation



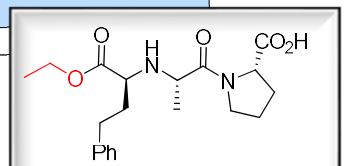
M.A.M. Subbaiah, J. Rautio & N.A. Meanwell, *Chem. Soc. Rev.*, 2024, **53**, 2099-2210

Prodrugs of Carboxylic Acids - Esters

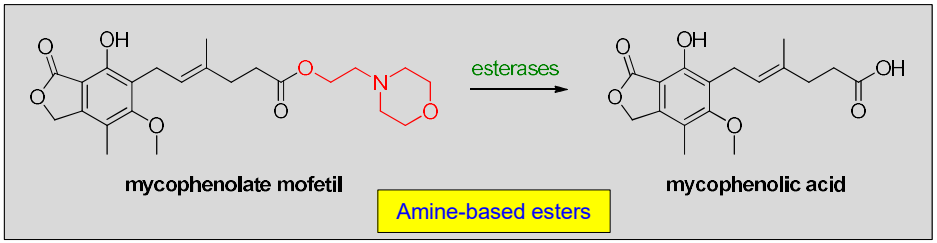
Membrane Permeability

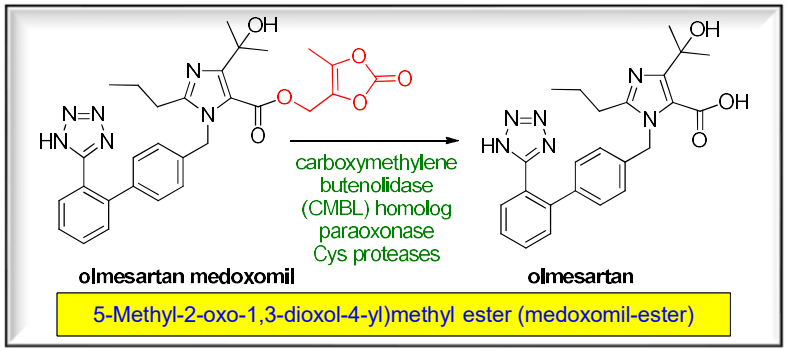


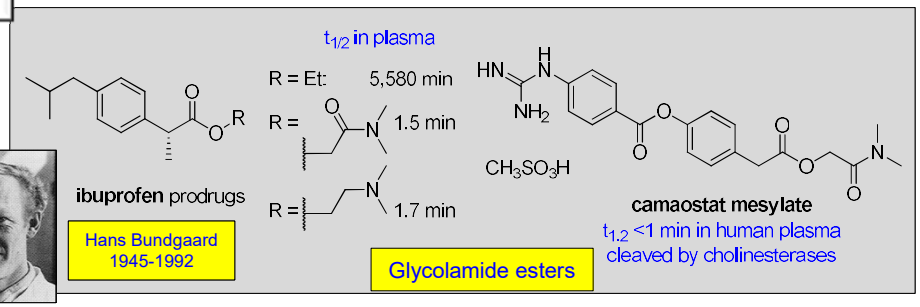
- Carboxylic acids are polar & ionized
  - can interfere with membrane permeability
- Simple esters can be configured as prodrugs
  - Et is the most prevalent in marketed drugs
- Common in ACE inhibitors (11 examples): maybe transported
  - stable in blood; may be cleaved in liver &/or kidney
- Amine-containing esters can invert physicochemical properties
  - ionizable center that facilitates dissolution & solubilization
- For sterically hindered acids, more elaborate prodrugs have been developed
  - project the cleavage site away from the drug
- Cleaved by esterases: Ser & Cys
  - cleavage rate depends on enzyme
  - CES1 in liver cleaves esters with small alcohol, large acyl moiety
  - CES2 in intestine cleaves esters with large alcohol, small acyl moiety



enalapril: F = 3%
enalaprilat: 53-74% absorbed
F of parent = 36-44%



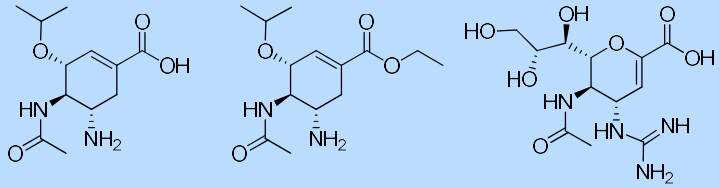




Hans Bundgaard
1945-1992

K. Beaumont *et al., Curr. Drug Metab.,* 2003, **4**, 461-485; B.M. Liederer & R.T. Borchardt, *J. Pharm. Sci.,* 2006, **95**, 1177-1195; W.A. Lee *et al., Pharm, Res.,* 1990, **7**, 161-166; T. Ishizuka *et al., J. Biol. Chem*., 2010, **285**, 11892–11902; N. Mork & H. Bundgaard, *Pharm. Res.* 1992, **9**, 492-496; J. Midgely *et al*., *Xenobiotica,* 1994, **24**, 79-92

Ester Prodrugs for Oral Delivery

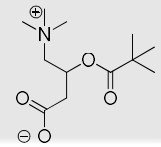


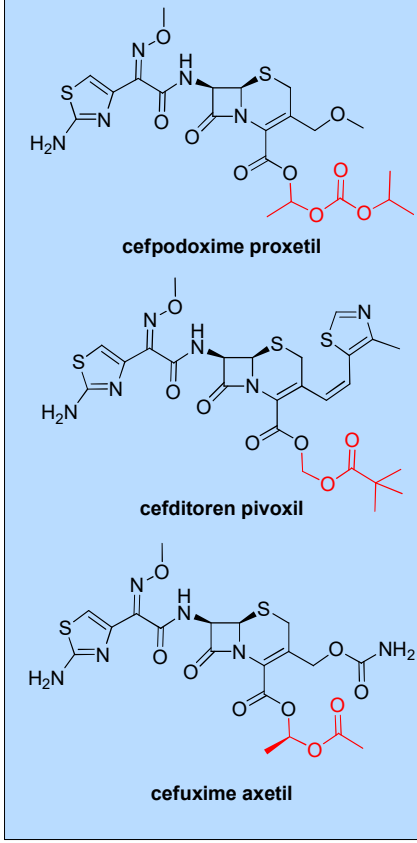
- Oseltamivir carboxylate is an influenza neuraminidase inhibitor
  - zwitterion with low membrane permeability
  - poor (4.3%) F in humans
- Ethyl ester efficiently delivers active carboxylate *in vivo*
  - metabolized by carboxylesterases (CES1)
  - confers 80% F for carboxylic acid
- Acid is detected in plasma within 30 minutes of dosing
  - t_{max} = 3-4 h
- Oseltamivir ester cleavage is species-dependent
  - %F lower in rodents
  - higher carboxylesterase activity in the GI tract
- More convenient dosing than zanamavir
  - topical delivery by inhaler
  - can be challenging for the elderly

Species	Compound	%F of acid
Mouse	Prodrug	30
Rat	Prodrug	35
Dog	Prodrug	73
Human	Acid	4.3
	Prodrug	80

- CO_2H prodrugs of β-lactam antibiotics
  - can be released in the face of steric encumbrance
- Cefpodoxime proxetil
  - bioconversion releases iPrOH, CO_2 & CH_3CHO
  - F = 50%
- Cefditoren pivoxil
  - releases pivalic acid & formaldehyde
  - rapidly cleaved in humans after PO administration
  - converted in intestine & liver
  - F with low fat meal = ~16%
  - F with high fat meal = ~25%
- Cefuxime axetil
  - CH_3CO_2H & CH_3CHO released as by products
  - converted in intestine & liver: not detected in plasma
  - $t_{1/2}$ in fresh human blood = 3.5 min
  - F fasted = 36%; F fed = 52%

Pivalic acid is converted to the carnitine ester – can interfere with the carnitine cycle & fatty acid oxidation in the heart & cause cardiotoxicity

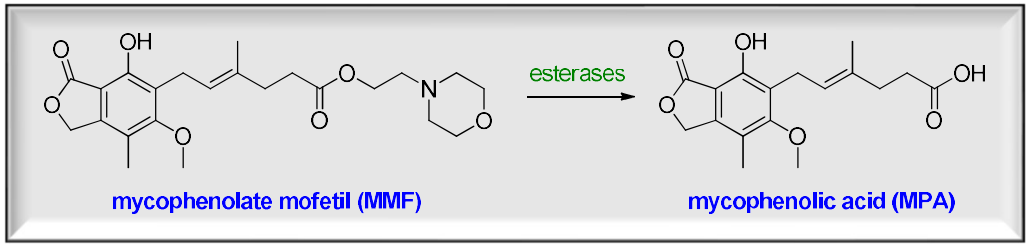




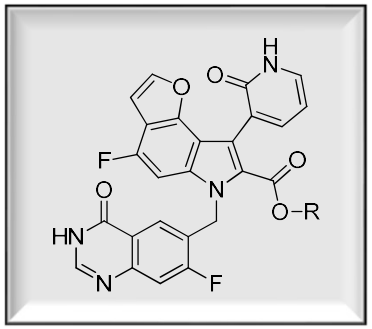
C.U. Kim *et al.*, *J. Am. Chem. Soc.*, 1997, **119**, 681-690; W. Li *et al.*, *Antimicrob. Agents Chemother.*, 1998, **42**, 647-653; D. Shi *et al.*, *J. Pharmacol. Exp. Ther.*, 2006, **319**, 1477-1484
J.E. Frampton *et al.*, *Drugs,* 1992, **44**, 889-917; W.M. Todd, *Int. J. Antimicrob. Agents*, 1994, **4**, 37-62; K. Wellington, *Drugs,* 2004, **64**, 2597-2618; C.M. Perry, *Drugs,* 1996, **52,** 125-158

Prodrugs of Carboxylic Acids - Amine-Based Esters

Membrane Permeability



- Mycophenolic acid (MPA) is an orally bioavailable immunosuppressive agent
  - inosine monophosphate dehydrogenase (IMPDH) inhibitor
- Oral bioavailability of MPA is <40%
  - see variability in monkeys: 10-fold range for C_{min}
  - presystemic metabolism?
  - readily forms the acyl glucuronide
- Low solubility, poor partition coefficient
  - properties almost identical to naproxen which has good oral F
  - not a full explanation of low %F
- Mofetil ester (MMF) exhibits improved oral F in monkeys: 94%
  - exposure of MPA is less variable, just 3x
- MMF is more soluble under acidic conditions in the gut
  - more facile partitioning into lipophilic phases
- MMF is stable in plasma
  - presystemic cleavage, likely the liver
  - $t_{1/2}$ of MMF in mouse liver homogenate <5 s
- Only MPA & AG seen in plasma after oral dosing of MMF to monkeys
  - AG subject to enterohepatic recirculation



R	IC_{50} (nM)	EC_{50} (nM)	AUC_{0-6h} of Acid (μM·h)
H	5	8	4.3 (10*)
	9	22	0.22
	150	5	22* **5x**
	110	11	2.3
	75	14	0.79

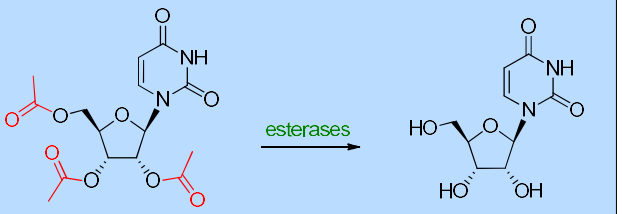
AUC data for 0−6 h from oral dosing of 10 mg/kg to rats; *0-24 h AUC data

- HCV NS5B polymerase inhibitor: potent in cell culture
- Modest PK profile after PO dosing to rats
  - AUC_{0-6h} = 4.3 μM·h at 10 mpk
  - oral F = 0.7%, 4% & 5% in rat, dog, cyno
- Prodrugs explored to address deficiencies
  - enhance both membrane permeability & solubility
- Lipophilic esters failed to deliver parent
  - ethyl ester 20-fold lower AUC than parent
  - attributed to poor solubility, low bioactivation
- Me_2N-CH_2-CH_2- ester afforded unique profile
  - active in cell culture: facile conversion to acid
- 5-Fold increase in AUC in rats
  - HCl salt solubility: 0.9 mM at pH = 3
  - oral F = 2%, 29%, 63% in rat, dog, cyno
- Homologous esters performed poorly

W.A. Lee *et al., Pharm, Res.,* 1990, **7**, 161-166; K. Beaumont *et al., Curr. Drug Metab.,* 2003, **4**, 461-485; K.X. Chen *et al., ACS Med. Chem. Lett.*, 2014, **5**, 244-248

Prodrugs of Alcohols & Enols – Esters & Carbonates

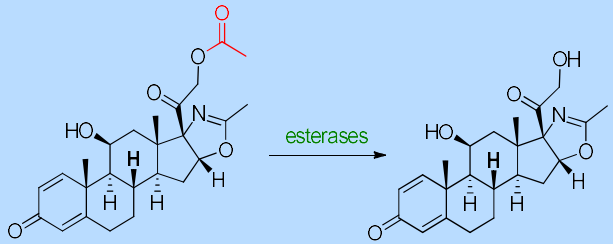
Membrane Permeability



uridine triacetate
marketed drug

uridine

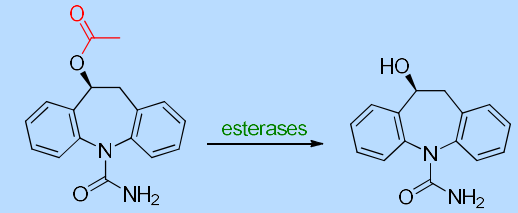
- Uridine is poorly bioavailable: F = 10%
  - triacetate delivers 5-10x in rodents
  - 4-6x in humans



deflazacort
marketed drug

desacetyldeflazacort

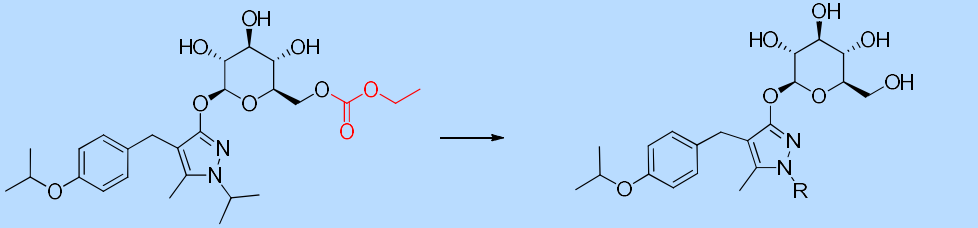
- Deflazacort: F = 92%, 37% & 43% in rat, dog, cyno
  - deacetylated in rat, dog, human plasma
- Highly membrane permeable
  - permeability of alcohol not disclosed



eslicarbazepine acetate
marketed drug

eslicarbazepine

- Eslicarbazepine
  - acetate believed to be designed as prodrug
  - prodrug rapidly cleaved in humans: levels BLQ
  - AUC dose proportional 400-1200 mg range

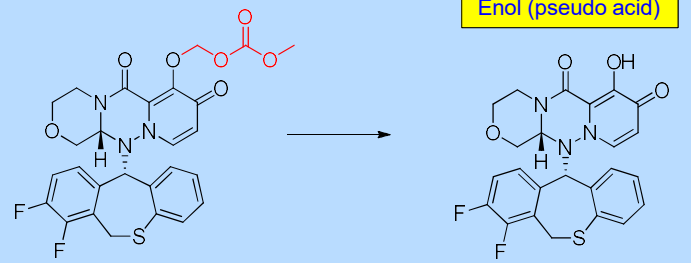


remogliflozin etabonate
marketed drug

R = iPr: **remogliflozin** → R = H: **active metabolite**
conversion
in vivo

- Remogliflozin: carbonate more permeable than alcohol in Caco-2 cells
  - cleaved during transit across Caco-2 cells
  - both prodrug & parent seen in rat plasma after PO dosing
  - plasma levels of parent lower after PO dosing of parent

Enol (pseudo acid)



baloxavir marboxil
marketed drug

baloxavir
influenza endonuclease inhibitor

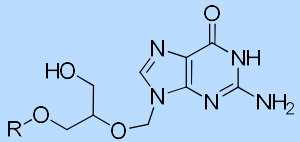
- Carbonate prodrug increases permeability of baloxavir
  - AUC of parent from prodrug increases dose-proportionally in cyno
  - similar profile in humans at doses 6-80 mg

G. Ison *et al., Clin. Cancer Res.*, 2016, **22**, 4545-4549; M. Banach *et al., Exp. Opin. Drug Metab. Toxicol.*, 2015, **11**, 639-648, K. McKeage *et al., Drugs Ther. Perspect.,* 2018, **34**, 16-22
K. Shimizu *et al., Bioorg. Med. Chem.*, 2021, **34**, 116033; T. Noshi *et al., Antivir. Res.,* 2018, **160**, 109-117

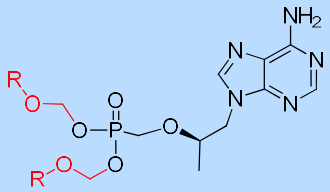
Prodrugs & Active Transport: PEP-T1

Membrane Permeability

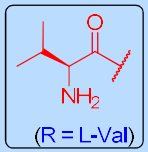
R = H: **aciclovir**
R = L-Val: **valaciclovir**



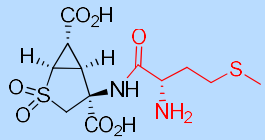
R = H: **ganciclovir**
R = L-Val: **valganciclovir**



bis(L-Val) ester
prodrug of tenofovir

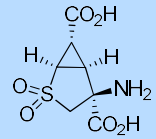


(R = L-Val)



LY2140023
clinical candidate
human F of parent = 68%

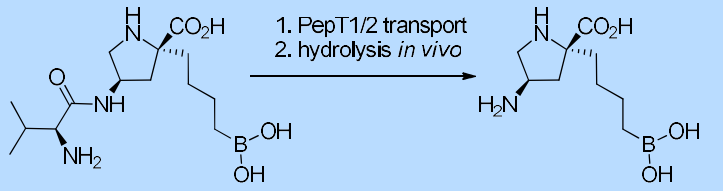
peptidases



LY404039
human F = 6%

- PepT1 transporters are expressed in the small intestine
  - high capacity; broad substrate specificity
- Transports dipeptides, tripeptides, β-lactam antibiotics, ACE inhibitors
  - plays a role in the oral absorption of these drugs
- Adding small amino acids to poorly permeable drugs can enhance absorption
  - valaciclovir & valganciclovir are classic examples
- Valaciclovir offers 3 to 5-fold increased exposure of aciclovir in humans
  - oral F of ganciclovir from valganciclovir is 60%; F= 10% for parent
  - cleavage in enterocytes, liver, plasma, other tissues

- Tenofovir prodrug exhibits 3x improved %F in rats compared to disoproxil
  - efficacy in duck HBV comparable to disoproxil
- LY2140023 displayed oral F of 6% in humans
  - HeLa cells expressing PepT1 accumulated prodrug
  - F = 68% in humans
- Influenza NA inhibitor GS 4416 could not be developed due to poor oral %F
  - simple ethyl ester ineffective: charged nature of guanidine
  - oral F of L-Val = 48% compared to 5% for parent

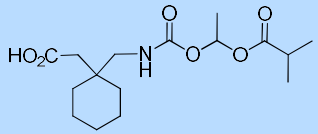


1. PepT1/2 transport
2. hydrolysis *in vivo*

Prodrug
Arg_1 IC_{50} = 328 nM
rat F of parent = 59%

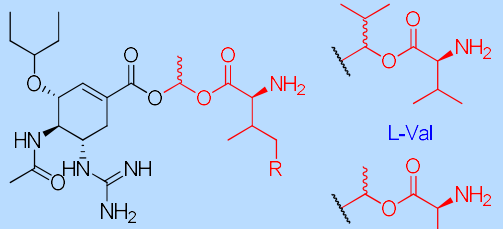
Arginase inhibitor

AZD0011
Arg_1 IC_{50} = 7 nM
rat F <5%



gabapentin enacarbil
transported by MCT1 & SMVT
cleaved by esterases

MCT1: monocarboxylic acid transporter-1 (SLC16A)
SMVT: Na-dependent vitamin transporter (SLC5A6)



R = H, L-Val
R = Me, L-Ile

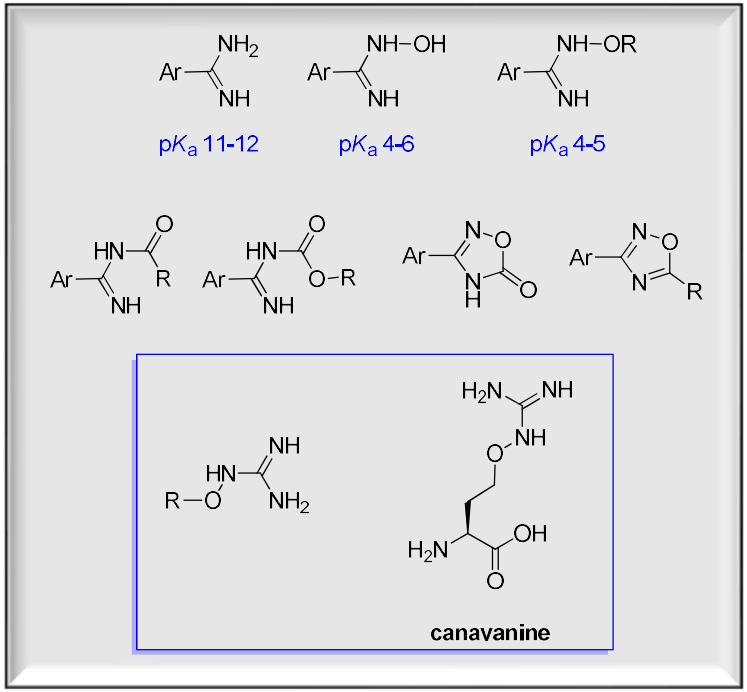
L-Val

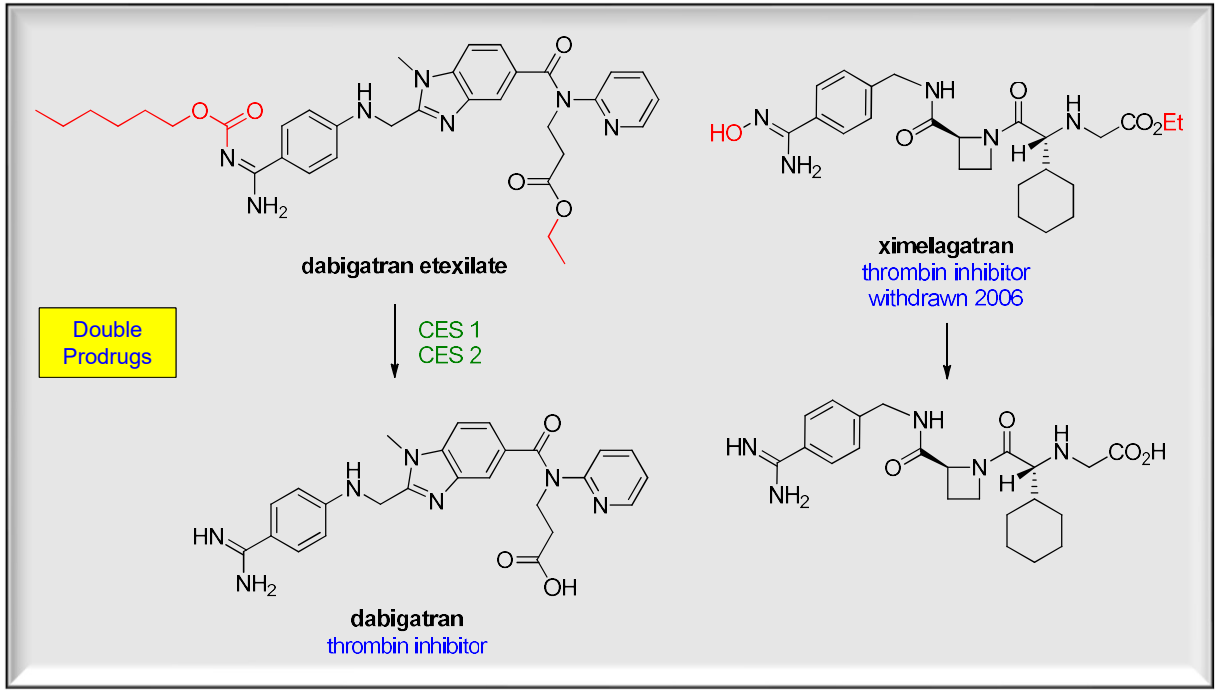
L-Leu

GS 4416
influenza neuraminidase inhibitor

M. Killer *et al., Sci. Adv.*, 2021, **7**, eabk3259; D. Pescovitz *et al., Antimicrob. Agents Chemother.*, 2000, **44**, 2811-2815; R.D. Moulton *et al., Drug Metab. Dispos.*, 2015, **43**, 756-761; M. Sugawara *et al., J. Pharm. Sci.,* 2000, **89**, 781-789; A. Wang *et al., ACS MCL,* 2019, **10**, 991-995; D. Gupta *et al., Mol. Pharmaceutics*, 2013, **10**, 512-522; S.N. Mlynarski *et al., J. Med. Chem.*, 2024, **67**, 20827-20841

Amidine/Guanidine Prodrugs





- Guanidines & amidines are highly basic
  - protonated at physiological pH
  - poor membrane permeability
- Acylated & hydroxylated derivatives less basic
  - have been exploited as prodrugs

- Acyl amidines/guanidiines cleaved by esterases
  - OH derivatives can be reduced in liver & extra-hepatic microsomes
  - CYP 450 enzymes, mammalian molybdoprotein mARC1 (Mo enzyme)
- Some N-O bonds can be reduced by in the gut by bacteria in the microflora
  - isoxazoles, 1,2,4-oxadiazoles

L. Peterlin-Mašič *et al., Curr. Pharmaceuitcal Des*., 2006, **12**, 73-91; S. Gruenewald *et al., J. Med. Chem*., 2008, **51**, 8173-8177
P. Indorf et al., *J. Med. Chem*., 2019, **63**, 6538-6546; D.-H. Kim, *Drug Metab. Dispos*., 2015, **43**, 1581-1589

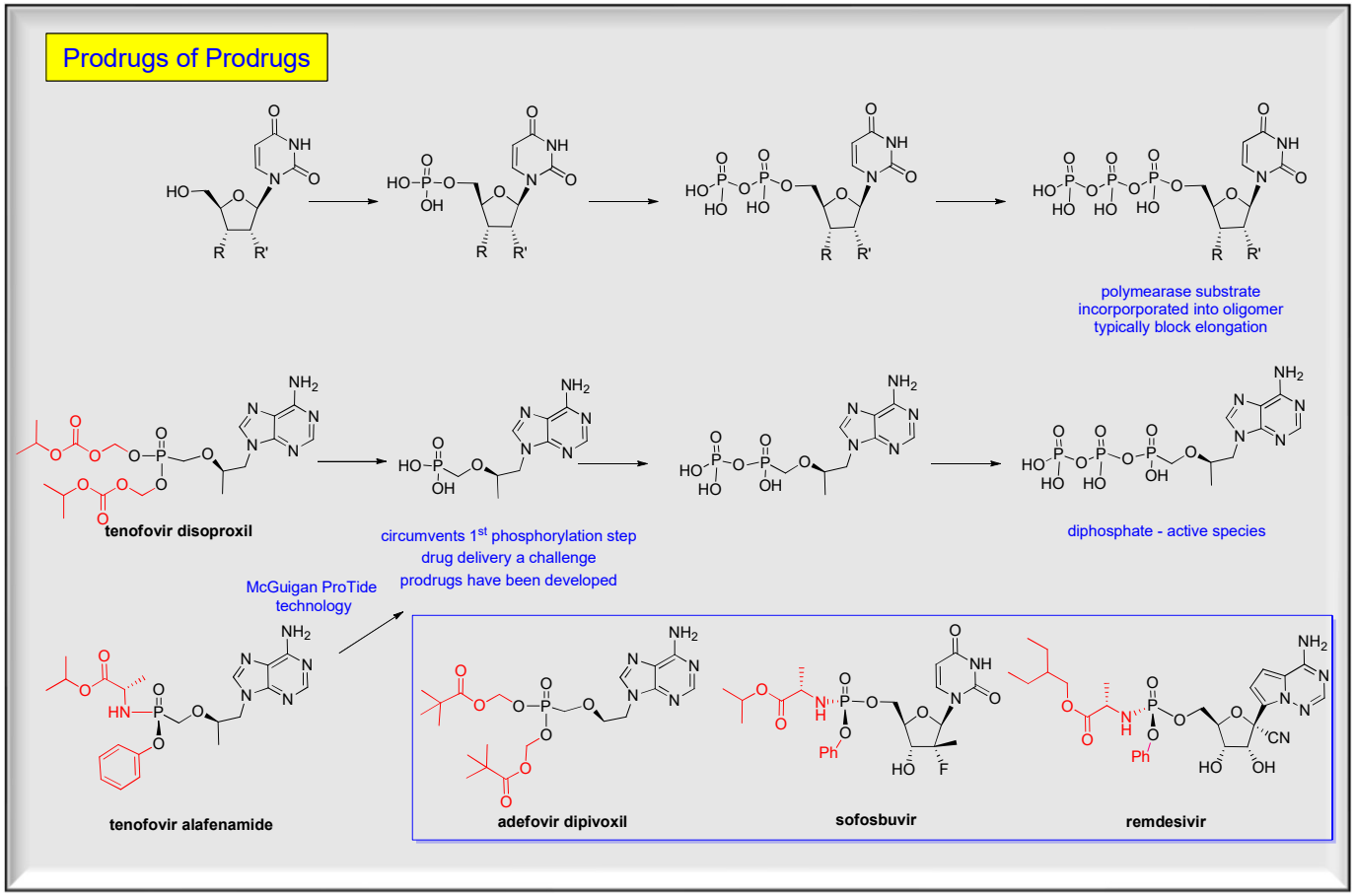
Antiviral Nucleos(t)ide Analogues – Special Cases

Prodrugs of Prodrugs

Design of Prodrugs for Virus Targeting

Membrane Permeability

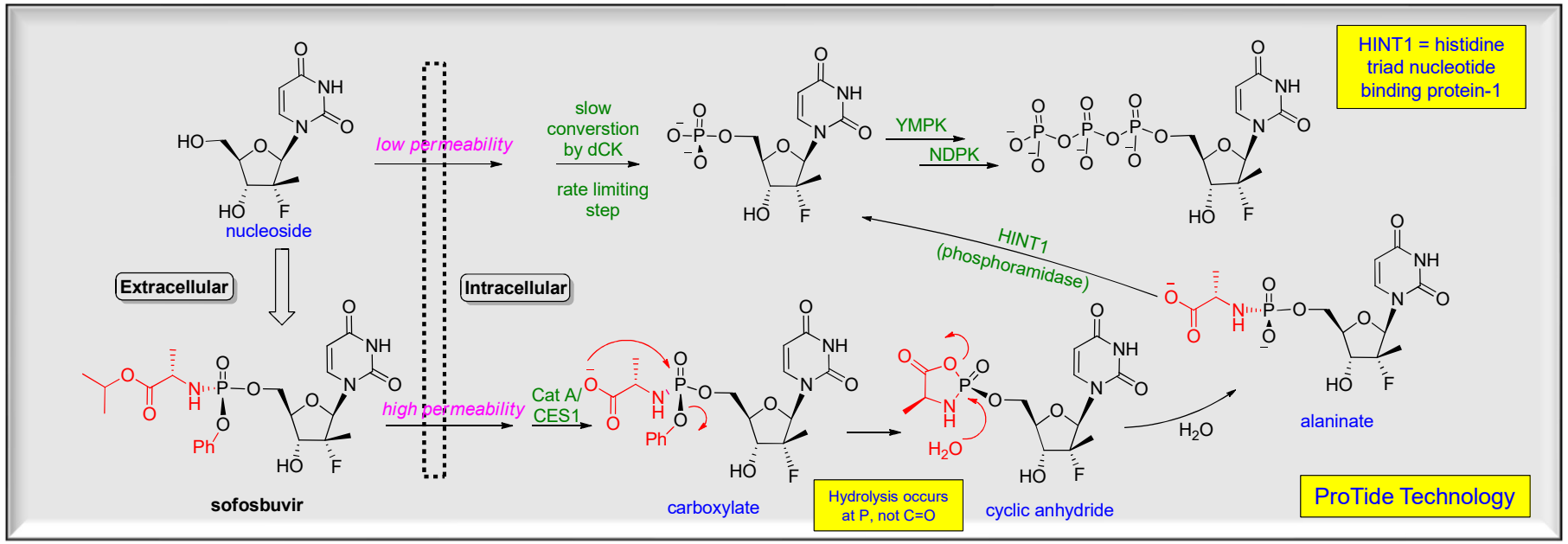
- Nucleoside analogues are polymerase inhibitors
  - block oligonucleotide synthesis
  - substrates that cause termination
- Depend upon phosphorylation
  - 3 consecutive phopsphorylation steps
  - 1st step commonly the slow step
- Nucleoside phosphonates can circumvent
  - polarity limits oral bioavailability, cell penetration
- Prodrugs developed
  - cleaved in plasma, tissue, target cells
- Pivalic acid is cardiotoxic
  - converted to CoA ester
  - interferes with fatty acid oxidation
- McGuigan ProTide phosphoramidate technology
  - delivers mono phosph(on)ate prodrug
  - complex unmasking process
  - occurs intracellularly
- For tenofovir alafenamide, dose is 10-25 mg
  - tenofovir disoproxil dose is 300 mg
- ProTide critical to the discovery of sofosbuvir
  - HCV NS5B inhibitor
  - nucleoside not phosphorylated in hepatocytes
  - prodrug unmasked in liver
    - & presumably other tissues



P.J. Thornton *et al., J. Med. Chem.*, 2016, **59**, 10400-10410; Y. Mehellou *et al., J. Med. Chem.*, 2017, **60**, 2211-2226; M. Serpi *et al., Exp. Opin. Drug Discov.*, 2021, **16**, 1149-1161
E.S. Rudge *et al., RSC Med. Chem.*, 2022, 13, 375-391; M. Krečmerová *et al., Front. Chem.*, 2022, **10**, 889737

Nucleoside Phosphoramidate (McGuigan) Prodrugs

Membrane Permeability



- Nucleoside not converted to mono-phosphate in liver cells
  - ProTide technology resolved the metabolic block
- Complex unmasking process
  - ester cleaved by cathepsin A and/or carboxylesterase-1
- Unmasked carboxylic acid ejects phenol
  - generates a chemically reactive anhydride: spontaneous hydrolysis

- The phosphoramidase HINT1 cleaves the alanine
  - generates the monophosphate in cells
- Monophosphate efficiently converted to triphosphate
  - host cell kinases
  - recognized by HCV NS5B polymerase
  - incorporated, terminates chain extension

Y. Mehellou *et al., ChemMedChem,* 2009, **4**, 1779 -1791; *J. Med. Chem.*, 2018, **61**, 2211-2226; M.J. Sofia *et al., J. Med. Chem.*, 2010, **53**, 7202-7218
T.C. Appleby *et al., Science*, 2015, **347**, 771-775; I. Gentle *et al., Exp. Opin. Drug Discov.*, 2015, **10**, 1363-1377; K.M. Glockzin *et al., Biochemistry*, 2024, **63**, 1774-1782

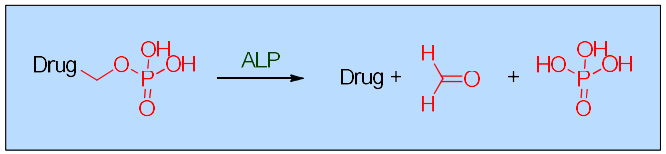
Prodrugs Designed to Address Low Drug Solubility

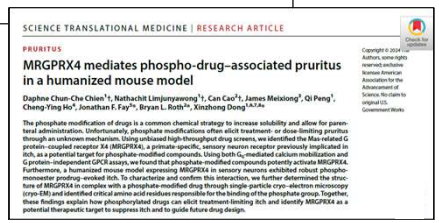
IV & PO Drug Delivery Applications

Prodrugs to Address Poor Solubility Challenges

Drug Solubility

- Only solubilized drug absorbed in the gut
  - oral bioavailability can be limited by solubility & dissolution issues
- IV administration demands high solubility
  - opportunity will depend on the dose
- Approach: add a solubilizing element to a drug molecule
  - requires a functional chemical handle to install
  - wide array of functionality has been demonstrated
- Many solubilizing motifs & approaches have been developed
  - can be cleaved pre-systemically in the gut
  - can be cleaved during or after absorption
- General strategy is to add an ionizable center
  - phosphate, acid, amine
- Append directly or *via* a linker
  - will depend on the available functionality/handle in molecule
  - formaldehyde linker prominent
- Phosphates a popular approach
  - can be associated with pruritus
  - recently attributed to MRGPRX4 inhibition
    - a GPCR activated by bile acids




SCIENCE TRANSLATIONAL MEDICINE | RESEARCH ARTICLE

PRURITUS

MRGPRX4 mediates phospho-drug–associated pruritus in a humanized mouse model

Daphne Chun-Che Chien[1†], Nattachit Limjunyawong[1†], Can Cao[2†], James Meixiong[3], Qi Peng[1], Cheng-Ying Ho[4], Jonathan F. Fay[5*], Bryan L. Roth[5*], Xinzhong Dong[1,6,7,8*]

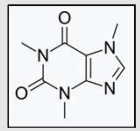
The phosphate modification of drugs is a common chemical strategy to increase solubility and allow for parenteral administration. Unfortunately, phosphate modifications often elicit treatment- or dose-limiting pruritus through an unknown mechanism. Using unbiased high-throughput drug screens, we identified the Mas-related G protein–coupled receptor X4 (MRGPRX4), a primate-specific, sensory neuron receptor previously implicated in itch, as a potential target for phosphate-modified compounds. Using both G_q-mediated calcium mobilization and G protein–independent GPCR assays, we found that phosphate-modified compounds potently activate MRGPRX4. Furthermore, a humanized mouse model expressing MRGPRX4 in sensory neurons exhibited robust phosphomonoester prodrug–evoked itch. To characterize and confirm this interaction, we further determined the structure of MRGPRX4 in complex with a phosphate-modified drug through single-particle cryo–electron microscopy (cryo-EM) and identified critical amino acid residues responsible for the binding of the phosphate group. Together, these findings explain how phosphorylated drugs can elicit treatment-limiting itch and identify MRGPRX4 as a potential therapeutic target to suppress itch and to guide future drug design.

Formaldehyde Release

	Dose	$CH_2C=O$ released	
formaldehyde from fostemsavir	600 mg	15.33 mg	0.22 mpk
	800 mg	20.44 mg	0.29 mpk
	1200 mg	30.66 mg	0.44 mpk

Drug	Dose	$CH_2C=O$ released/dose	
fosphenytoin	~1400 mg	103 mg	1.5 mpk
tenofovir dipivoxil	300 mg	28.1 mg	0.4 mpk
caffeine in 8 oz coffee	95-200 mg	14.7-30.9 mg*	0.21-0.44 mpk*

All mpk based on 70 kg patient; * based on 80% metabolized

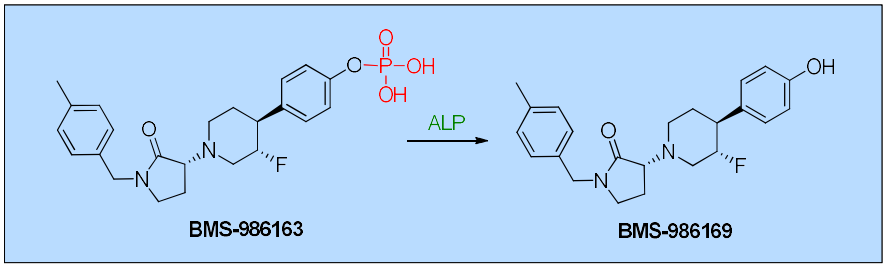


"Calculations suggest that the body daily turnover of formaldehyde is a surprising 31-59 g"

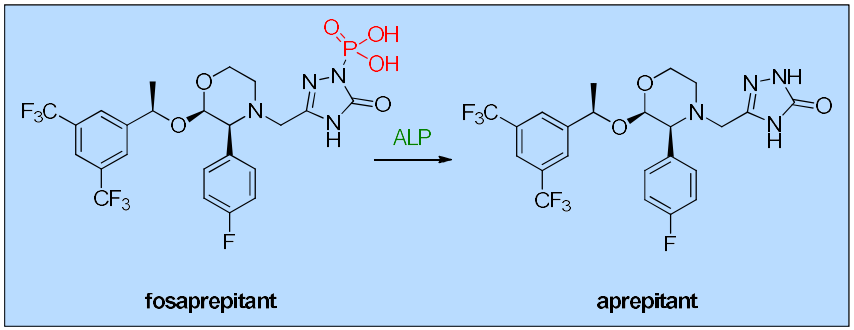
M.A.M. Subbaiah, J. Rautio & N.A. Meanwell, *Chem. Soc. Rev.*, 2024, **53**, 2099-2210; D.C.-C. Chen *et al., Sci, Transl. Med.*, 2024, **16**, adk8198
S.S. Dhareshwar & V.J. Stella, *J. Pharm. Sci.,* 2008, **97**, 4184-4193

Phosphate Prodrugs for IV Delivery

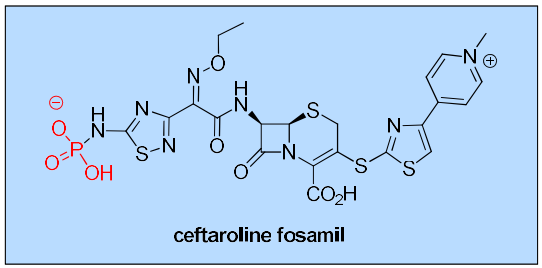
Drug Solubility: IV Dosing



- BMS-986169 is a GluN2B NAM
  - explored as an IV therapy for treatment-resistant depression
- Poor aqueous solubility would not support dosing in toxicology studies
  - solubility = 2 µg/mL
- Phenol offered convenient handle for derivatization
  - solubility of crystalline zwitterion = 19.9 mg/mL (100,000x ↑)
  - rapidly converted in rat, dog, cynomolgus monkey, human blood
- Administered IV to cynomolgus monkeys at 1.2 mpk
  - prodrug converted within 10 minutes
  - dose-dependent efficacy in mice



- Aprepitant is a neurokinin-1 antagonist
  - prevention of chemotherapy-induced nausea & vomiting
- Fosaprepitant designed for IV drug delivery
  - solubility increased from 0.5 µg/mL to 90 mg/mL: **180,000x** ↑
- Fosaprepitant retains activity at NK-1
  - IC_{50} = 1.2 nM: 10x ↓ aprepitant
  - rapidly converted in rat blood with $t_{1/2}$ of ~30 minutes
- Stable in human & dog blood; rapidly converted in HLM
  - 97% conversion in 15 minutes
  - complete conversion in 30 minutes in humans after IV dosing



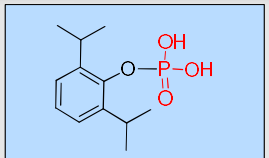
- *N*-Phosphono prodrug of ceftaroline enhanced solubility
  - from 2.3 mg/mL to >100 mg/mL
- Allowed for intravenous administration
  - ceftraoline fosamil approved, October 2010

L.R. Marcin *et al., ACS Med. Chem. Lett.*, 2018, **9**, 472-477; L.A. Sorbera *et al., Drugs Future*, 2002, **27**, 211-222
S.J.P. Van Belle & V. Cocquyt, *Exp. Opin. Pharmacother.*, 2008, **9**, 3261-3270; T. Ishikawa *et al., Bioorg. Med. Chem.*, 2003, **11**, 2427-2437

Phosphonooxymethyl Prodrugs for IV Delivery

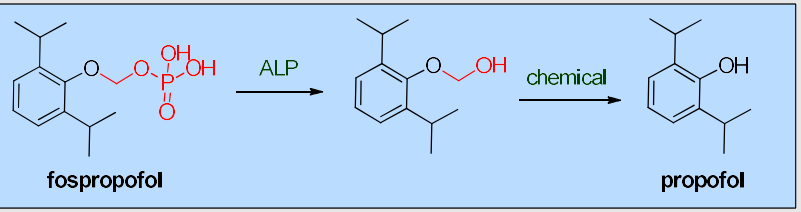
Drug Solubility: IV Dosing

- Propofol
  - anesthetic
- Aq. solubility is 0.13 mg/mL at pH 7.4
- Formulated as an oil/H_2O emulsion
  - pain on injection
- Prone to bacterial contamination



propofol phosphate

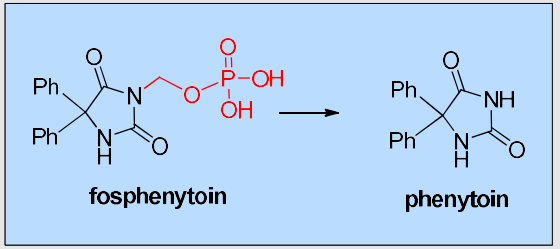
Propofol phosphate cleavage is much slower than for fospropofol



fospropofol propofol

- Fospropofol
  - approved 2008
- Aq. solubility is ~500 mg/mL at pH 7.4
  - readily formulated as an aqueous solution
  - no pain on injection
- $t_{1/2}$ *in vivo* = 1-5 minutes
- Slower onset of anesthesia than propofol
  - limited clinical uptake

- Phenytoin
  - anticonvulsant
- Aq. solubility is 20-25 µg/mL at pH 7.4
- Na salt can be used for IV dosing
  - solubility = 50 mg/mL
  - 46 mg/mL in 40% propylene glycol & 10% EtOH, pH = 12



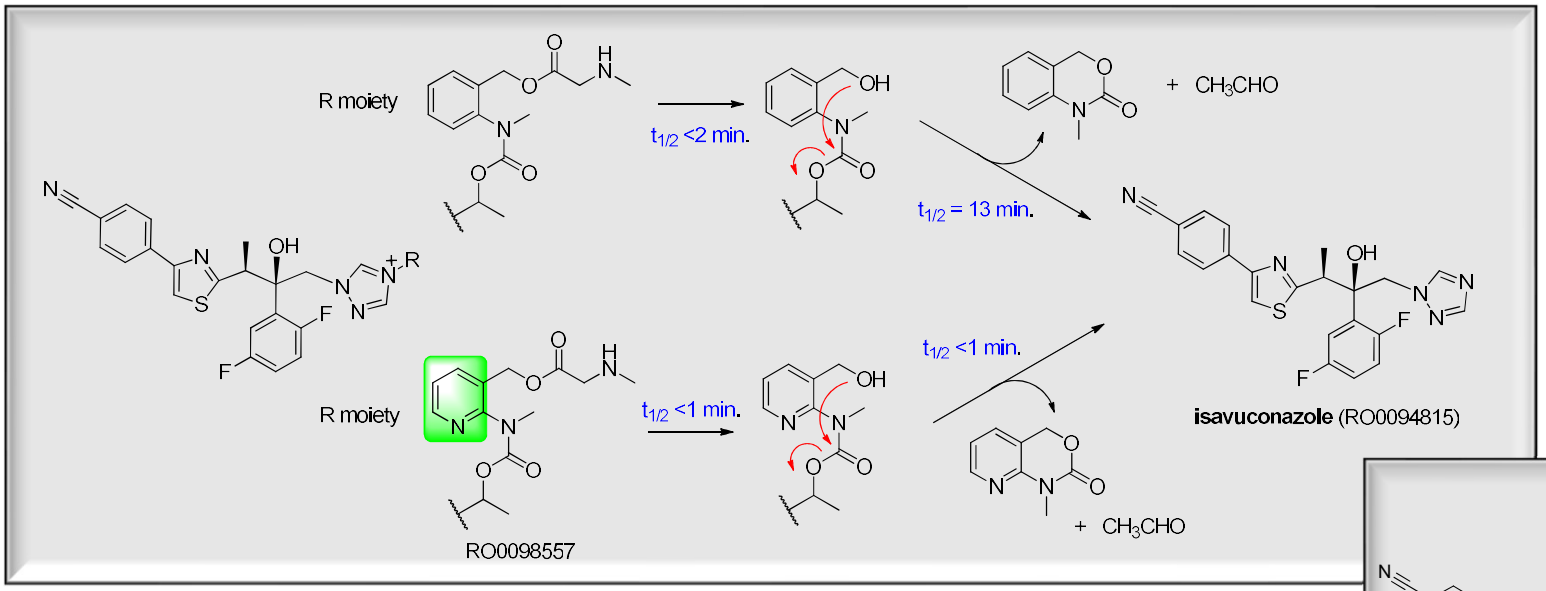
fosphenytoin phenytoin

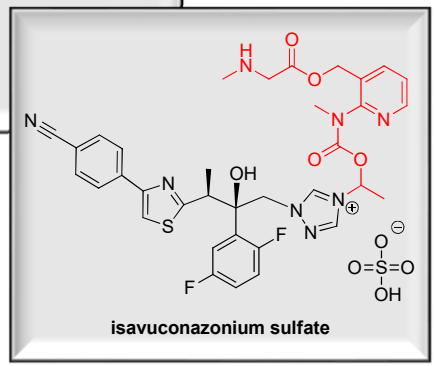
- Fosphenytoin
  - approved 1996
- aq. solubility is 142 mg/mL at pH 7.4
  - no irritation on injection
- $t_{1/2}$ *in vivo* = 8-7 minutes in humans
  - quantitative release after IV & IM administration
- Can be administered orally with similar PK to parent

V.J. Stella, *Adv. Drug Deliv. Rev.*, 1996, **19**, 311-330; M.D. Krasowski, *Curr. Opin. Investig. Drugs,* 2005, **6**, 90-98
M.G. Banaszczyk *et al., Anesth. Analg.,* 2002, **95**, 1285-1292; P. Revill *et al., Drugs Future,* 2005, **31**, 859-863

A Triazole-Based Prodrug for IV Delivery

Drug Solubility: IV Dosing





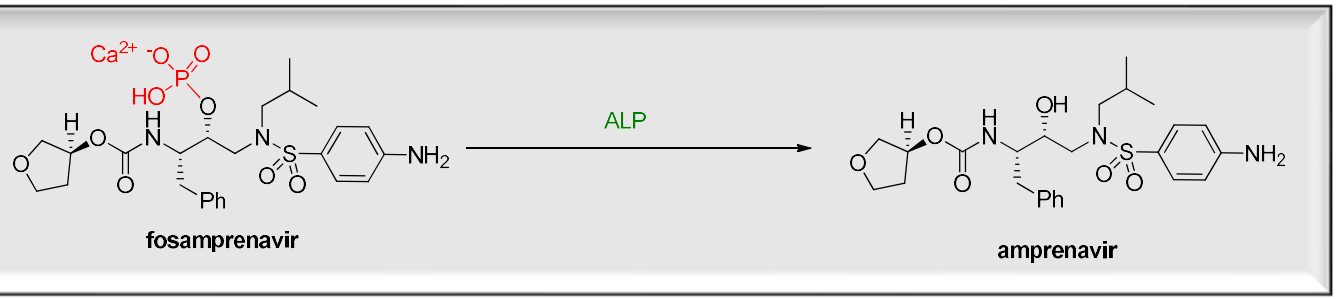
- Isavuconazole
  - azole antifungal agent approved by FDA & EMA in 2015
- Prodrug to increase aqueous solubility for IV administration
  - complex design that alkylates triazole
- Pyridine offered superior release performance to phenyl
  - 20x rate of cyclization
  - conformational &/or electronic effects
- Isavuconazonium sulfate approved by FDA in December 2023

	Lead	Isostere
	Phenyl	**Pyridyl**
Solubility	1 mg/mL	>100 mg/mL
Prodrug $t_{1/2}$ in rat plasma	<2 min.	<1 min.
Alcohol $t_{1/2}$ in rat plasma	13 min.	<1 min. (undetected)

J. Ohwada *et al., Bioorg. Med. Chem. Lett.,* 2003, **13**, 191-196

Prodrugs for Improved Pharmaceutical Properties

Drug Solubility: Formulation



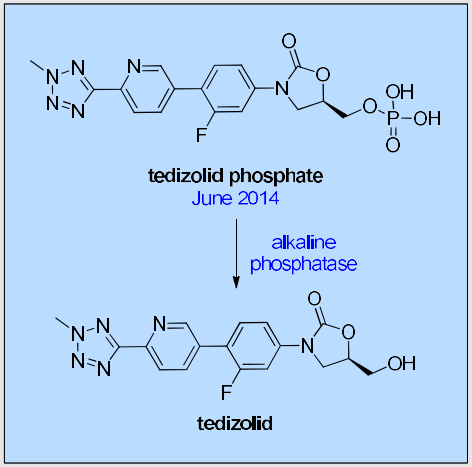
- Fosamprenavir
  - aq. solubility = 0.31 mg/mL
- Ca^{2+} salt is >100 mg/mL at pH 3-4
  - cleaved pre-systemically
  - ALP at brush border membrane
- Oral F comparable to amprenavir
  - identical preclinical profile
  - C_{max} 27% lower
- Full toxicology study required
  - altered complexion of PK profile
  - small amounts of fosamprenavir in plasma
- Improved pharmaceutics
  - 2 x 700 mg tablets BID

- Amprenavir
  - aq. solubility = 0.041 mg/mL
  - high oral F: ~80%
- High excipient content due to low solubility
  - 8 150 mg capsules BID

D. Fleisher *et al., Drug Deliv. Rev.*, 1996, **19**, 115-130; T.M. Chapman *et al., Drugs*, 2004, **64**, 2101-2124; C. Falcoz *et al., J. Clin. Pharmacol.*, 2002, **42**, 887-898

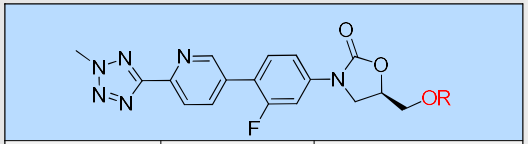
Prodrugs for Delivering Antibacterial Oxazolidinones

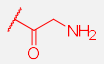
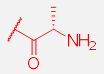
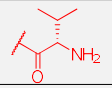
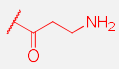
Drug Solubility: PO Dosing

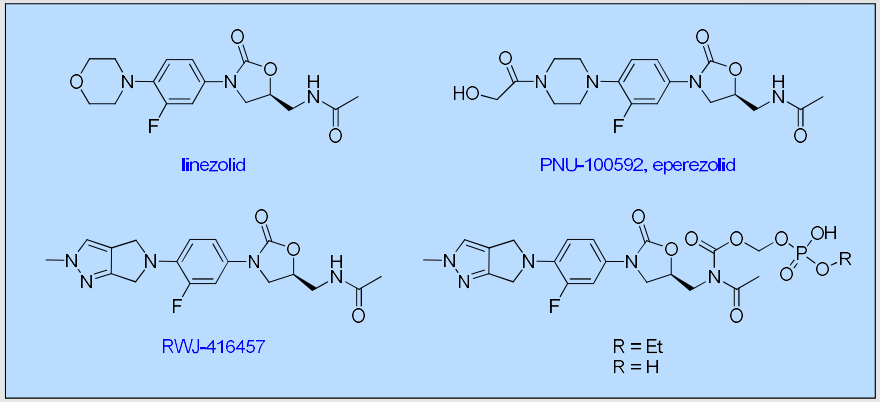


Alcohol handle

Illustrates strategies associated with linker-based & direct installation of phosphate moiety depending on functional group availability



Prodrug	Solubility (mg/mL)	% remaining after 8 h at pH 3/5/7
	12	95.1/93.3/11.6
	>50	98.0/88.1/7.5
	4.2	99.9/97.7/60.9
	20.4	99.9/94.9/50.5
	<1.6	81.9/65.2/0.2
	>50	99.9/99.9/99.9



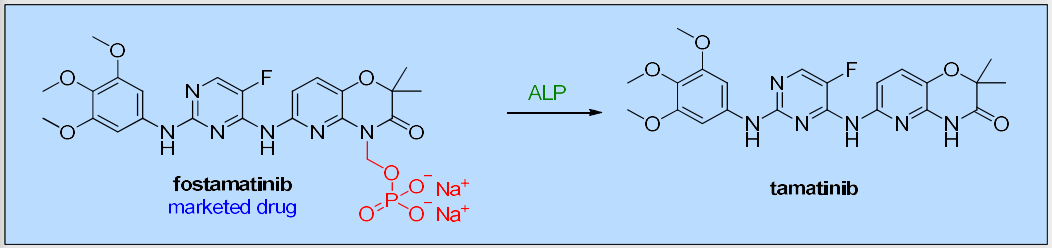
Amide handle

- Pyrrolo[3,4-*c*]pyrazole exploited as a morpholine mimic
  - oxazolidinone antibacterial agent based on linezolid & eperezolid
- Physicochemical properties presented a challenge
  - parent <10 µg/mL at pH = 7; 33 µg/mL in 0.1N HCl
    - weak base: pK_a of pyrrolidine = 1.83
  - crystalline material soluble at 5.8 ng/mL: mp = 234-238 °C
  - prodrugs designed to overcome solubility issues for IV & PO dosing
- RWJ-416457 advanced into Phase 1 clinical trials
  - development abandoned for business reasons

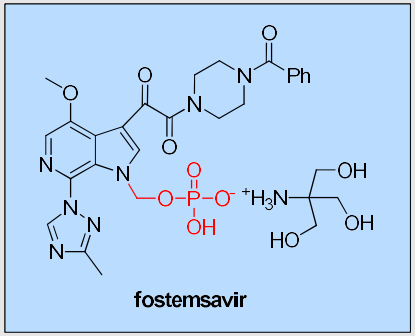
S.D. Paget & D.J. Hlasta, *U.S. Pat. Appl.*, US 20020161029 A1, Oct 31st, 2002; K. Bush *et al.*, *Antimicrob. Agents Chemother.*, 2007, **51**, 361-365
W.B. Im *et al.*, *Eur. J. Med. Chem.*, 2011, **46**, 1027-1039; S. Flanagan *et al.*, *Pharmacotherapy*, 2014, **34**, 891-900

Phosphonooxymethyl Prodrugs: Amide as a Handle

Drug Solubility: PO Dosing



- Tamatinib is an orally active spleen tyrosine kinase (SYK) inhibitor
  - therapeutic for immune thrombocytopenia (ITP)
- Poor solubility
  - FASSIF solubility = 5 µg/mL
  - limited potential to develop a solid dosage form
- TPGS/propylene glycol formulation gave dose-related AUC increase
  - 80-400 mg but no further increase from 400-600 mg
- Phosphonooxymethyl prodrug explored
  - rapid conversion to tamatinib in human intestinal microsomes
- In clinical studies, prodrug delivered parent comparable to solution dosing of parent
  - approved by FDA in 2018



Tris salt prodrug of fostemsavir	
pH	solubility
1.5	>11 mg/mL
4.1	>11 mg/mL
8.2	>11 mg/mL
Parent drug	
2-9	0.020 mg/mL

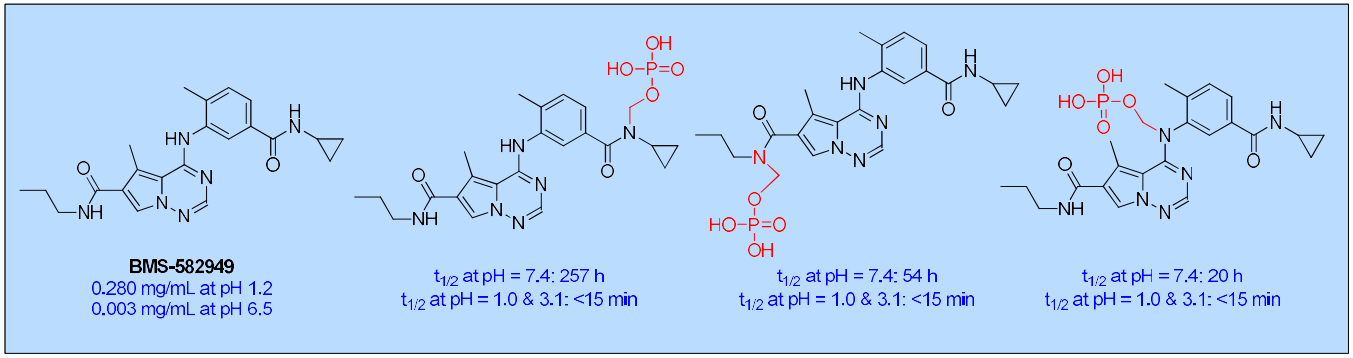
- Temsavir is a potent HIV-1 attachment inhibitor
  - low solubility, high membrane permeability
- High dose drug
  - low intrinsic solubility limited dose escalation
- Phosphonooxymethyl prodrug enhanced solubility
  - 250 mg/mL: 12,500x ↑ over parent
- Good dose escalation in preclinical species
  - translated to humans
- Delivery efficiency unmasked rapid metabolism
  - extended release formulation developed
  - gave optimal C_{min} value, moderated C_{max}
- Approved by FDA in July 2020
  - EMA in 2021

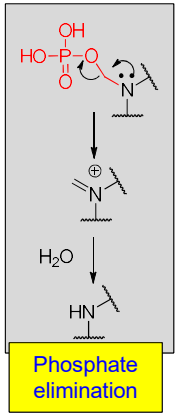
BCS Class 2

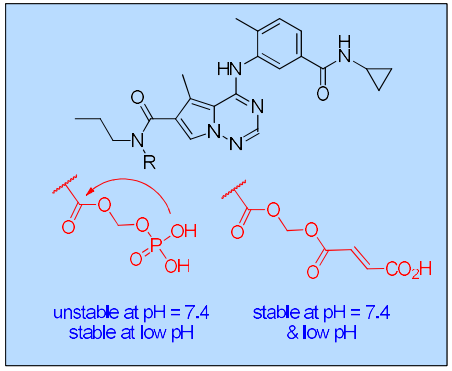
S.P. McAdoo *et al., Drugs Future*, 2011, **36**, 273-280; N.A. Meanwell *et al.*, *J. Med. Chem.*, 2018, **61**, 62-80; T. Wang, J.F. Kadow, *et al., J. Med. Chem.*, 2018, **61**, 6308-6327

Prodrugs of a p38 MAP Kinase Inhibitor

Drug Solubility: PO Dosing



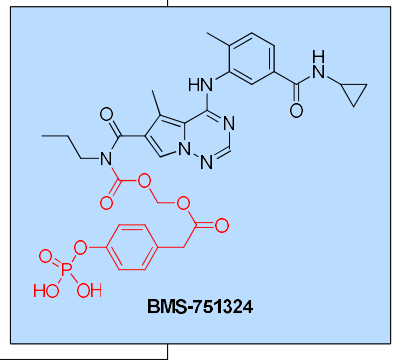


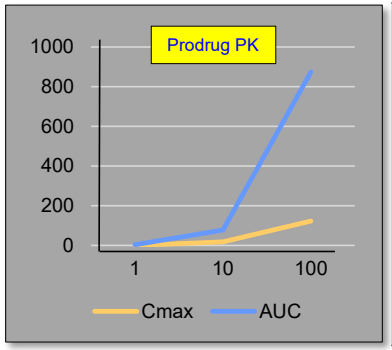


- BMS-582949 a p38 MAP kinase inhibitor
  - evaluated clinically for the treatment of rheumatoid arthritis
- Demonstrated pH-dependent absorption
  - 50% of RA patients take acid lowering agents
  - oral exposure fell by 70% when dosed with famotidine in humans
- Phosphonooxymethyl moieties installed at each NH
  - all unstable at low pH; elimination of phosphate
- OCH_2 linker homologue unstable at pH = 7.4
  - intramolecular attack of phosphate on C=O
  - fumarate stable with ↑ solubility
- Led to the design of BMS-751324
  - good solubility at low & neutral pH
  - cleaved sequentially by ALP, porcine liver esterase
- Exposure responded well to dose escalation
  - C_{max} & AUC greater than dose-proportional
  - significant improvement on parent dosing

Illustrates the importance of exploring linker & site of attachment to address stability issues

$t_{1/2}$ at 37 °C	solubility
21 h at pH = 1.1	0.1 mg/mL at pH = 1.2
37 h at pH = 7.4	3.03 mg/mL at pH = 6.7



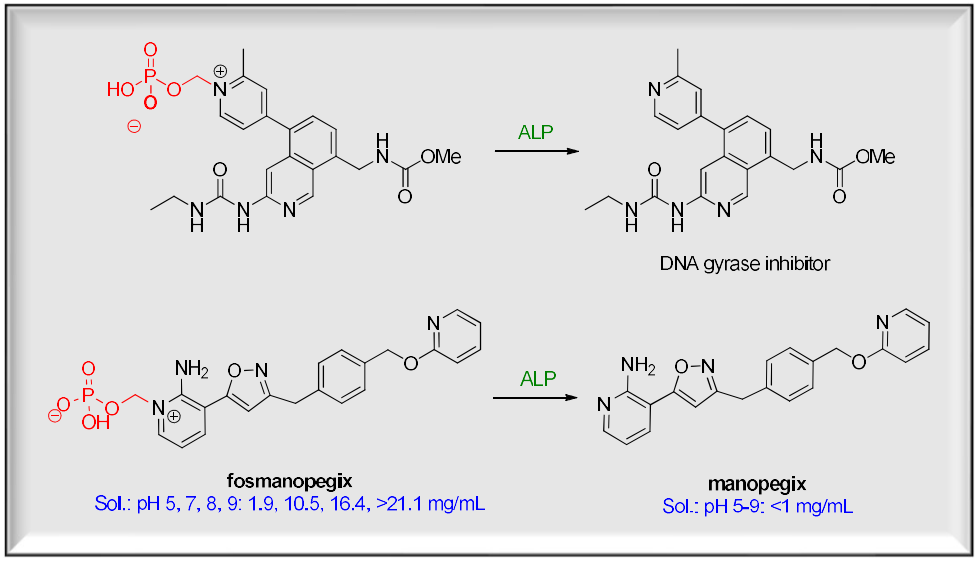


Methocel suspension	Dose (mpk)	C_{max} (μM)	AUC_{0-24} (μM·h)	%F
Parent	1	0.8	4.2	55
	10	4.3	23	31
	100	3.0	3.0	4.3
Rat PK				
Prodrug	1	0.4	3.1	41
	10	18	78	103
	100	122	875	115

C. Liu *et al.*, *J. Med. Chem.*, 2015, **58**, 7775-7784

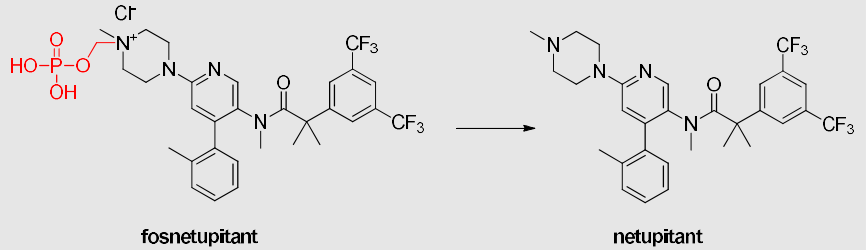
Phosphonooxymethyl Prodrugs Derived from Amines

Drug Solubility



- Antibacterial DNA gyrase inhibitor
  - poorly soluble in phosphate buffer: 20 µg/mL
  - prevented development of an IV formulation
- Pyridinium POM prodrug soluble at 12.7 mg/mL
  - $t_{1/2}$ of 0.3-1.6 h in human, rat, mouse whole blood
  - efficacious in a mouse model of thigh infection given IV
- Manopegix is an antifungal agent
  - 1st in class mechanism in P3 trials
- Low solubility prevented IV formulation
  - pyridinium POM prodrug fosmanopegix enhanced solubility

Inhibits glycosylphosphatidylinositol-anchored cell wall protein transfer 1 enzyme



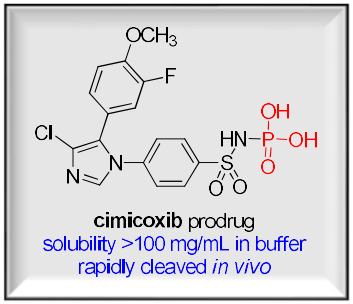
Analyte	Plasma AUC (ng.h/mL)	Brain AUC (ng.h/mL)	Plasma $t_{1/2}$ (h)
Prodrug	418	2.11	0.1
Parent	28700	19.1	26

- Netupitant is a substance P/neurokinin 1(NK1) antagonist
  - marketed in 2015
  - for use with $5HT_3$ antagonist for chemotherapy-induced nausea
- Parent exhibits low aqueous solubility
  - 0.25 mg/mL: too low for IV formulation
- Fosnetupitant offers 40-60-fold higher aqueous solubility
  - moderate chemical stability
- Rapid & complete conversion in rats after IV dosing
  - $t_{1/2}$ = 6 minutes
- Similarly rapid conversion in humans
  - approved by FDA in 2018
  - FDC with $5HT_3$ antagonist palonsteron for IV administration

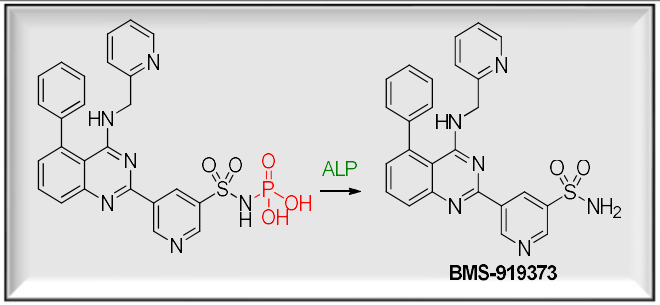
P. Panchaud *et al., J. Med. Chem.*, 2017, **60**, 3755-3775; J.A. Covel *et al., Med. Chem. Rev.*, 2019, **54**, 221-239; K.J. Shaw & A.S. Ibrahim, *J. Fungi*, 2020, **6**, 239
M. Shirley *et al., Drugs,* 2021, **81**, 1331-1342; T. Tyler *et al., Clin. Pharmacol. Drug Dev.,* 2022, **11**, 1405-1418, V.J. Stella *et al., U.S. Patent* US-2015-0011510, 2015

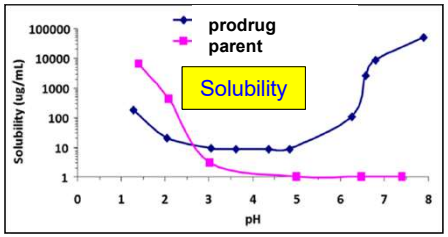
Sulfonamide Prodrugs to Enhance Solubility

Drug Solubility: PO Dosing

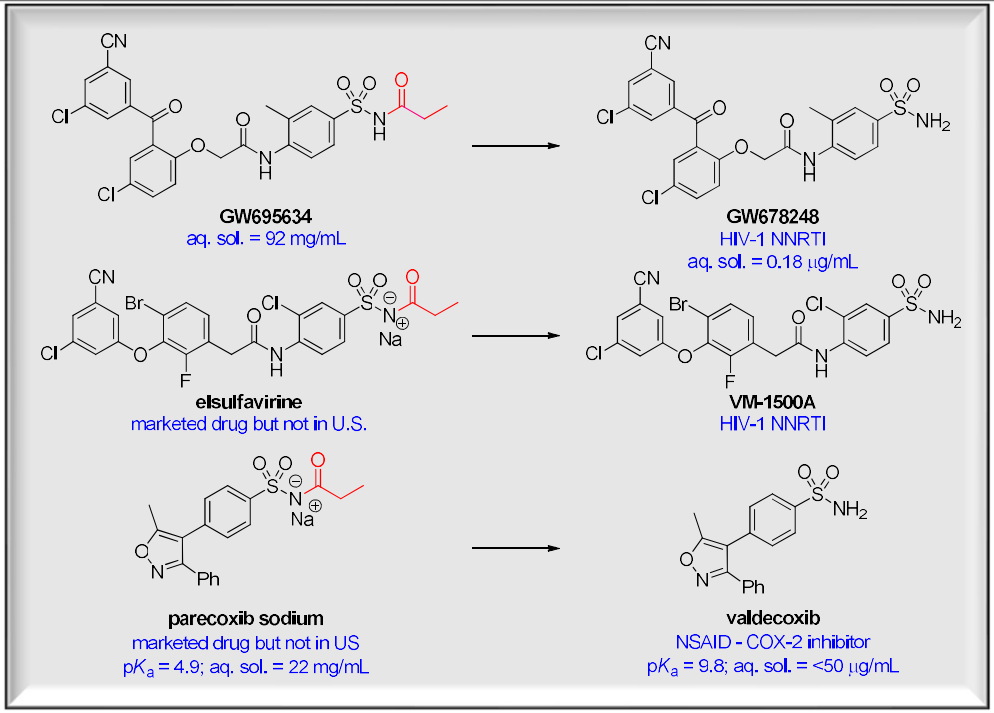


cimicoxib prodrug
solubility >100 mg/mL in buffer
rapidly cleaved *in vivo*





- Phosphoramidate concept originally explored with COX-2 inhibitor cimicoxib
  - developed for IV administration
- Kv1.5 (I_{kur}) inhibitor for the treatment of atrial fibrillation
  - exhibited pH-dependent absorption
- BMS-919373 exhibited pH-dependent absorption in cynomolgus monkey
  - attributed to pH-dependent solubility
- SO_2NH_2 exploited as prodrug handle
  - phosphoramidate: direct attachment
- Abrogated pH-dependent absorption
  - no circulating prodrug
  - pre-systemic cleavage by ALP in gut



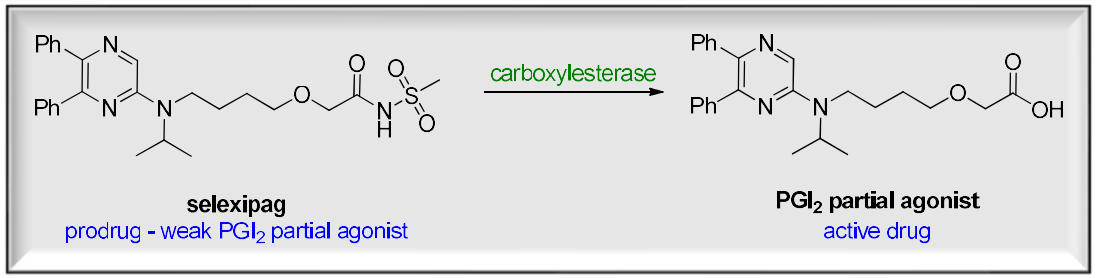
- GW695634 exhibited poor solubility of 0.18 µg/mL & low oral F
  - acylsulfonamide: 92 mg/mL; 46%, 20% & 29% absorption in rat, mice, cyno
- Elsulvarine developed in Russia June 2017: long acting - parent $t_{1/2}$ ~6 days
- Parecoxib examined as IV dosing form of COX-2 inhibitor
  - acetyl derivative poorly cleaved in dog, cyno
  - propionamide cleaved in rat, dog, cyno & in LMs

C. Almansa *et al., J. Med. Chem.*, 2004, **47**, 5579-5582; P. Gunaga *et al., J. Med. Chem.*, 2017, **60**, 3795-3803; H. Bundegaard *et al., Int. J. Pharm.*, 1987, **37**, 87-95;1988, **47**, 103-110
M. De Serres *et al., Xenobiotica*, 2010, **40**, 437-445; Z.T. Al-Salama, *Drugs*, 2017, **77**, 1811-1816; J.J. Talley *et al., J. Med. Chem.*, 2000, **43**, 1661-1663

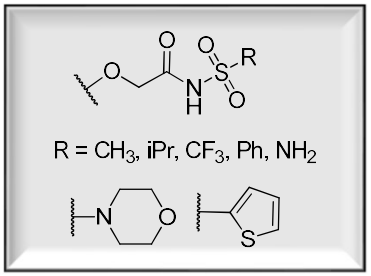
Prodrugs to Modulate Drug Disposition

Carboxylic Acid Prodrugs – Acyl Sulfonamide

Drug Disposition



- Selexipag is an orally bioavailable prostacyclin I_2 (PGI_2) receptor partial agonist
  - approved for the treatment of pulmonary arterial hypertension (PAH)
- Inhibits human blood platelet aggregation
  - IC_{50} = 200 nM
  - also an arterial smooth muscle vasodilator
- CH_3 acylsulfonamide is 16x less potent toward PGI_2 receptor
  - IC_{50} CO_2H = 11 nM; IC_{50} of acylsulfonamide = 177 nM
- Acyl sulfonamide converts slowly to CO_2H in LMs
  - blocked by phenylmethylsulfonyl fluoride, a serine hydrolase inhibitor
  - consistent with carboxylesterase as the hydrolase
- PK studies in cynomolgus monkeys confirmed release of CO_2H *in vivo*
  - acylsulfonamides afforded 10-30% lower C_{max}, 1.5x longer $t_{1/2}$ of CO_2H
  - t_{max} longer for CH_3 acylsulfonamide prodrug & parent CO_2H
- Buffered release of parent acid minimizes side effects
  - reduced GI effects, vasodilation
- Selexipag approved by the FDA in December 2015 for PAH (orphan drug status)
  - exhibits therapeutic effects in the treatment of frostbite
  - purchased by J&J in June 2016 (part of Actelion PAH franchise for $30 bn)



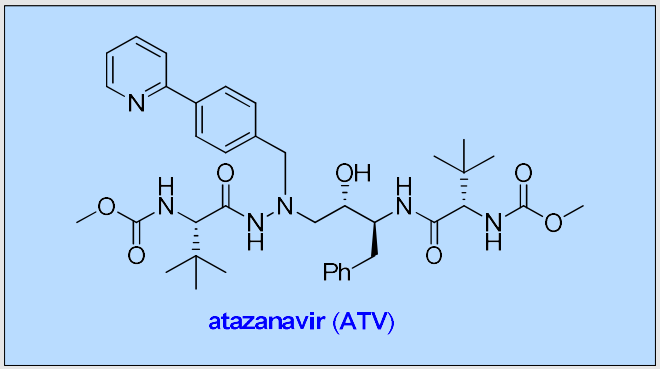
R	Analyte	t_{max} (h)	C_{max} (ng/mL)	AUC_{0-24h} (ng·h/mL)	$t_{1/2}$ (h)
acid		2.3	105	652	5.6
CH_3	prodrug	6.7	47	384	4.9
	acid	14.0	35	859	10.7
iPr	prodrug	10.0	17	128	2.3
	acid	10.0	13	170	14.5
CF_3	acid	4	31	308	8.5
NH_2	acid	6	20	374	*

cynomolgus monkey PK data; * acid conc. continued to increase at 24 h

T. Asaki *et al., J. Med. Chem.*, 2015, **58**, 7218-7137

Prodrugs of the HIV-1 PI Atazanavir

Drug Solubility & Disposition



atazanavir (ATV)

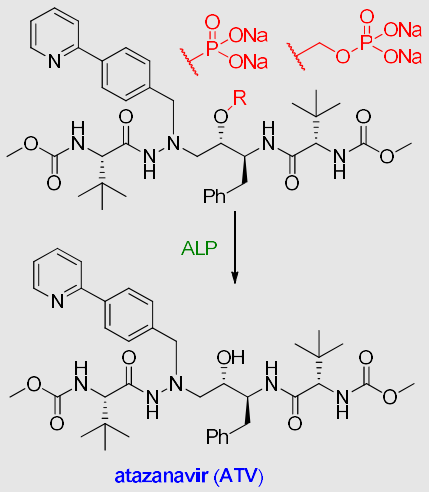
- ATV – a potent HIV-1 PI
  - sub-optimal PK profile
- pH-dependent absorption
  - weak base, poor solubility at neutral pH
  - 75% ↓ exposure with famotidiine
- CYP 450 substrate
  - combined with RTV or cobicistat for QD dosing

M.A.M. Subbaiah *et al., Eur. J. Med. Chem.*, 2017, **139**, 865–883; *J. Med. Chem.*, 2018, **61**, 4176-4188; *J. Med. Chem.*, 2019, **62**, 3553-3574; *J. Med. Chem.,* 2022, **65**, 11150-11176

Prodrugs of the HIV-1 PI Atazanavir

Drug Solubility & Disposition

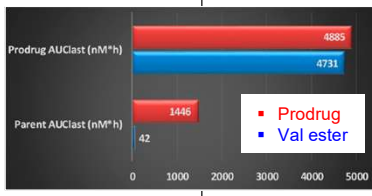
1st Generation Prodrugs



atazanavir (ATV)

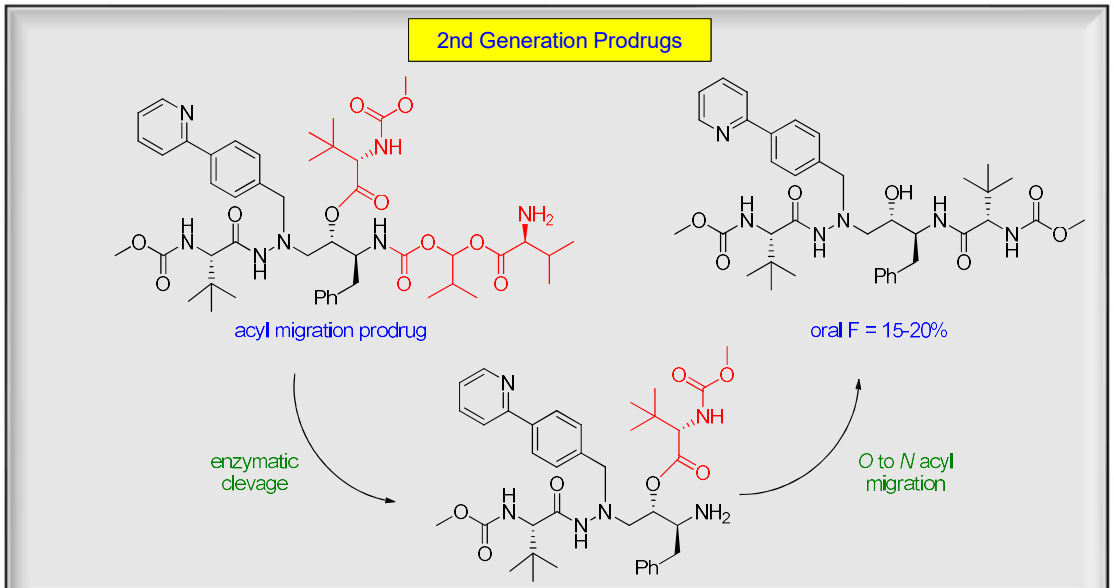
- 1st Generation approach
  - phosphate-ALP approach
- Enhanced aqueous solubility
  - failed to deliver ATV to rat plasma after PO dosing
- Phosphate sterically encumbered
  - CH_2 linker less stable at higher pH
  - PK similar to parent: did not address 1st pass effect

- 2nd Generation approach - acyl migration strategy
- Relies upon amine at terminal to enhance aqueous solubility - can form a salt
- Enzymatic cleavage of Val exposes terminal amine
  - 1,5-acyl migration of ester to generate ATV
  - dosing of intermediate gave poor results
- Mitigated pH-dependent absorption in rats
  - prodrug acted as a circulating depot with sustained release of ATV
  - prodrug & some intermediate detected in plasma
- Prodrug active in cell culture – efficient release



Val ester - Val attached directly to 2° OH

2nd Generation Prodrugs



M.A.M. Subbaiah *et al., Eur. J. Med. Chem.*, 2017, **139**, 865–883; *J. Med. Chem.*, 2018, **61**, 4176-4188; *J. Med. Chem.*, 2019, **62**, 3553-3574; *J. Med. Chem.,* 2022, **65**, 11150-11176

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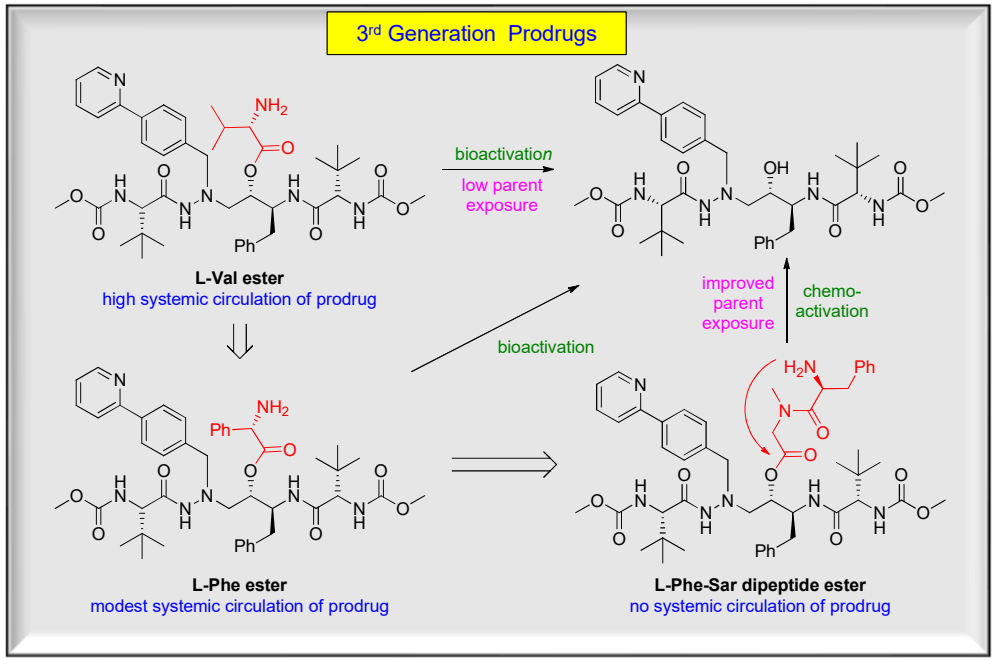
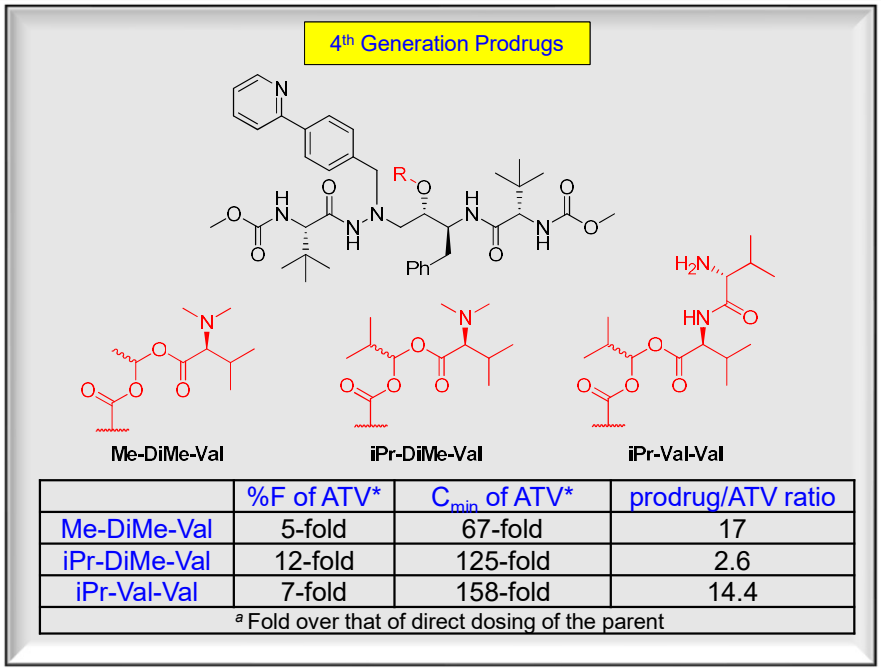
Prodrugs of the HIV-1 PI Atazanavir

Drug Solubility & Disposition





	%F of ATV*	C_{min} of ATV*	prodrug/ATV ratio
Me-DiMe-Val	5-fold	67-fold	17
iPr-DiMe-Val	12-fold	125-fold	2.6
iPr-Val-Val	7-fold	158-fold	14.4

[a] Fold over that of direct dosing of the parent

- 3rd Generation approach
  - amino acid esters of 2° pharmacophoric alcohol
  - poor enzymatic release
- Bis amino acid esters
  - can degrade by chemical bioactivation to release ATV
  - 4x ↑ in exposure of ATV in rats

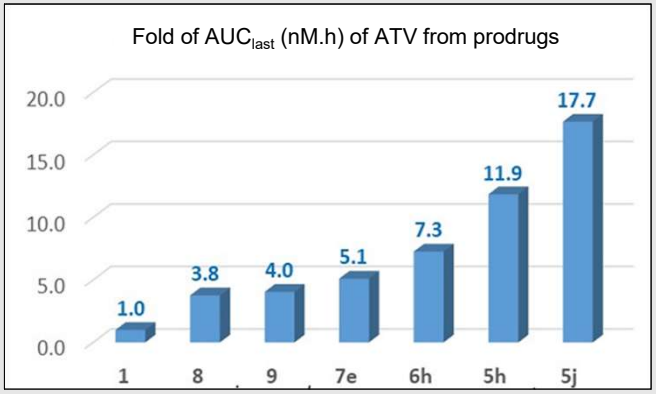
- 4th Generation approach
  - bis-amino acid prodrugs with acetal linker
  - amino acid identity tailors release kinetics
- Enhances oral F, C_{min} of ATV
  - prodrugs act as a circulating depot in plasma
- Measured release of ATV
  - prolongs $t_{1/2}$ by avoiding liver metabolism

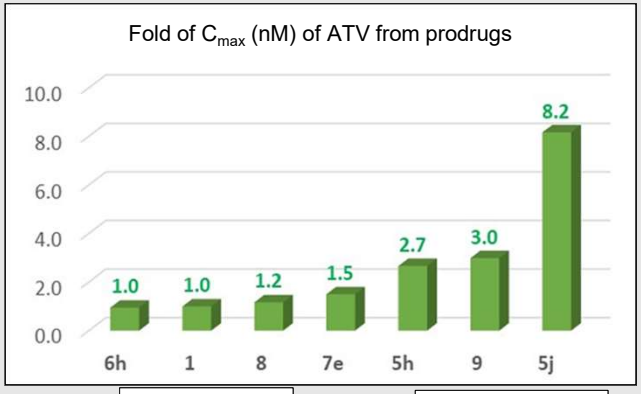
M.A.M. Subbaiah *et al., Eur. J. Med. Chem.*, 2017, **139**, 865–883; *J. Med. Chem.*, 2018, **61**, 4176-4188; *J. Med. Chem.*, 2019, **62**, 3553-3574; *J. Med. Chem.,* 2022, **65**, 11150-11176

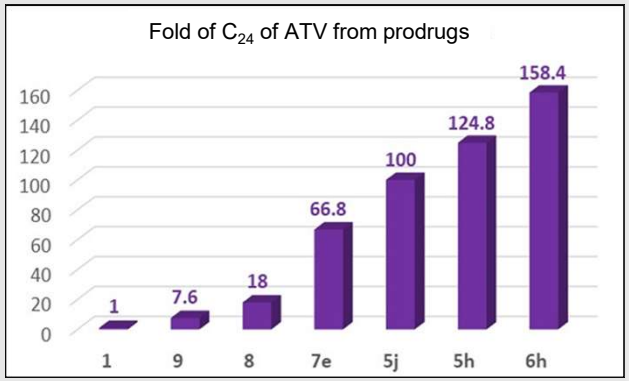
NuArq MedChem Consulting LLC

Evolution of Prodrugs of the HIV-1 PI Atazanavir

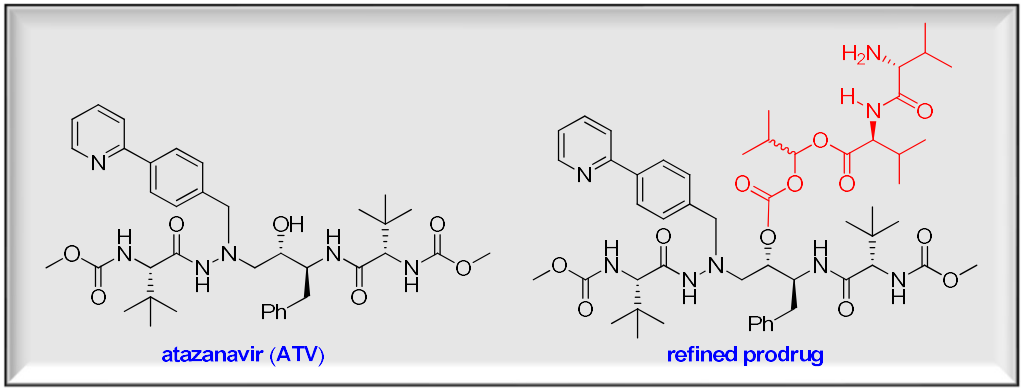
Drug Solubility & Disposition







- Iterative cycles of design & evaluation
  - *in vivo* profile improved with each design iteration
- Optimized compound solved pharmaceutics issues
  - absorbed & circulated in plasma of rats
  - acted as a depot to release ATV
  - enzymatic process
- Not evaluated in higher species
  - potential not fully understood



M.A.M. Subbaiah *et al., Eur. J. Med. Chem.*, 2017, **139**, 865–883; *J. Med. Chem.*, 2018, **61**, 4176-4188; *J. Med. Chem.*, 2019, **62**, 3553-3574; *J. Med. Chem.,* 2022, **65**, 11150-11176

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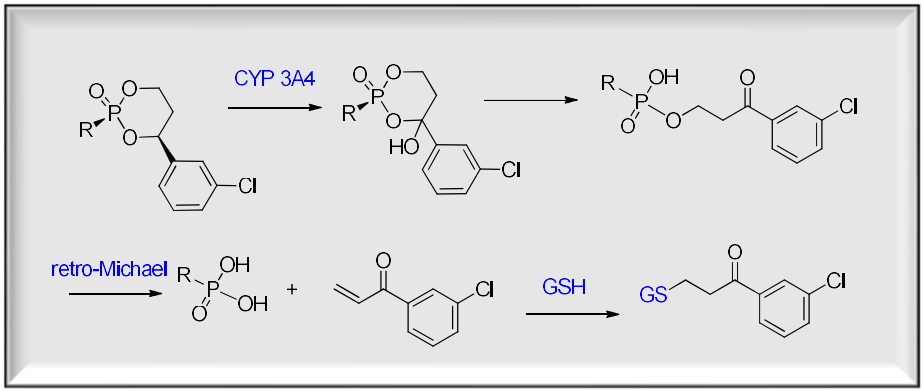
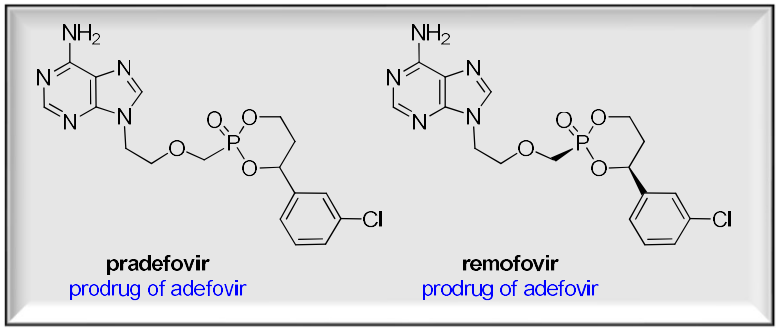
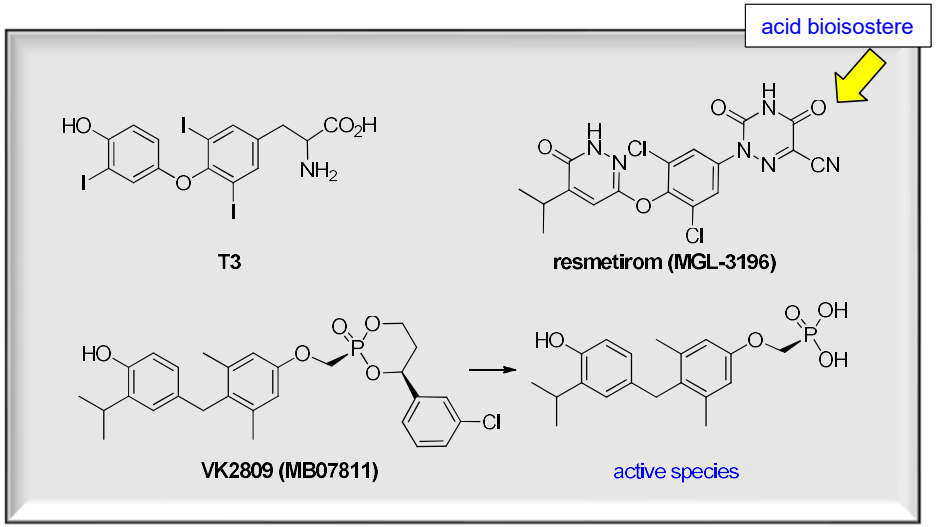
HepDirect Prodrugs - Liver Targeting of Phosphonates

Drug Disposition





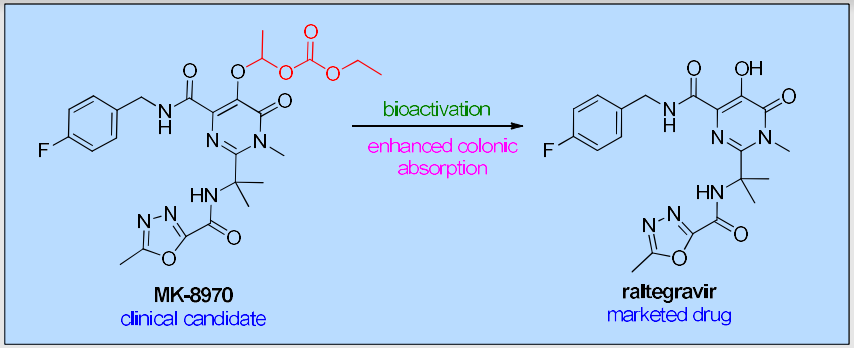
- Phosphate/phosphonate prodrug designed to be unmasked by CYP 450
  - pradefovir developed as prodrug of adefovir for HBV
  - liver targeting: 30, 45, 60 & 75 mg dose vs 10 mg for adefovir
  - filed in China August 2023 for HBV
- T3 thyromimetic drug resmetirom discovered by Roche
  - 3,5-dioxo-1,2,4-triazine-6-carbonitrile is CO_2H isostere
  - developed by Madrigal for NASH
  - approved by FDA on March 14, 2024
- VK2809 (MB07811) developed as liver-targeting T3 mimetic
  - phosphonate derivative unmasked by CYP 450
    - likely trapped in hepatocytes
  - orally bioavailable
  - recent P2 clinical data revealed positive effects in NASH



M.D. Erion *et al., J. Am. Chem. Soc.*, 2004, **126**, 5154-5163; *Curr. Opin. Investig. Drugs*, 2006, **7**,109-117; M.J. Kelly *et al., J. Med. Chem.*, 2014, **57**, 3912-3923

Prodrugs Designed to Target Colonic Delivery

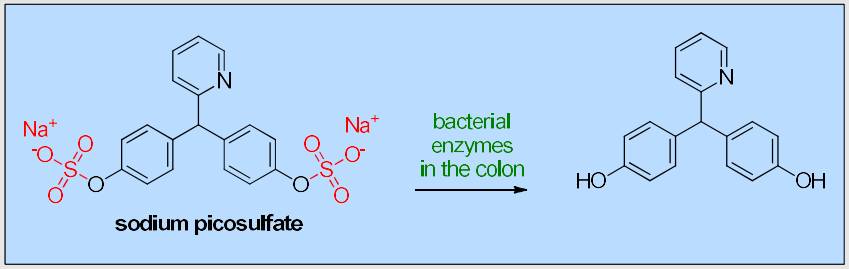
Membrane Permeability



	HPLC Log *D* at pH 7.4	LLC-PK1 P_{app} (*10^{-6} cm/s)	FaSSIF soly. (mg/mL)	Oral F	Relative colonic F*
RAL	1.4	11.6	-	-	9
MK-8970	2.4	5.9	0.33	48%	40

* relative colonic bioavailability = (AUC_{colon}/AUC_{oral}) x 100

- Raltegravir exhibits poor oral absorption & a short $t_{1/2}$
  - 400 mg BID dosing clinical regimen
- Explored prodrugs of enol moiety
  - stable in FASSIF
  - stable human & dog plasma; hydrolyzed in rat plasma
  - active in cell culture in presence of human serum: 2x ↓
- Demonstrated improved bioavailability
  - promotes absorption in lower GI tract: colon
  - rapidly metabolized *in vivo*:
    - no prodrug in circulation
  - dose-linear exposure 10-400 mg
- Potential for less frequent dosing
  - with an immediate/controlled release formulation

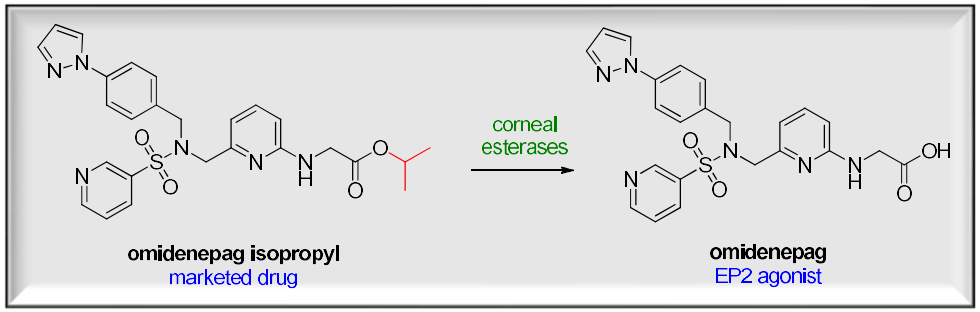


- 4,4'-Dihydroxydiphenyl-(2-pyridyl)-methane is a laxative
  - stimulates colonic peristalsis
- Sodium picosulfate was designed as a colon-targeted prodrug
  - sulfates enhance solubility
  - sulfates cleaved by bacterial enzymes in the colon

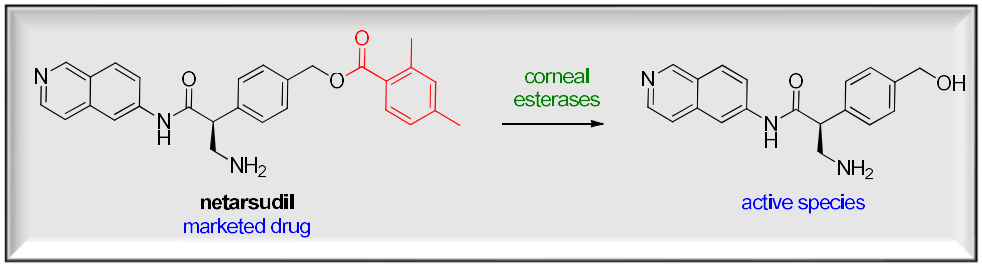
A.M. Walji *et al.*, *ChemMedChem*, 2015, **10**, 245-252; S.M. Hoy *et al.*, *Drugs*, 2009, **69**, 123-136

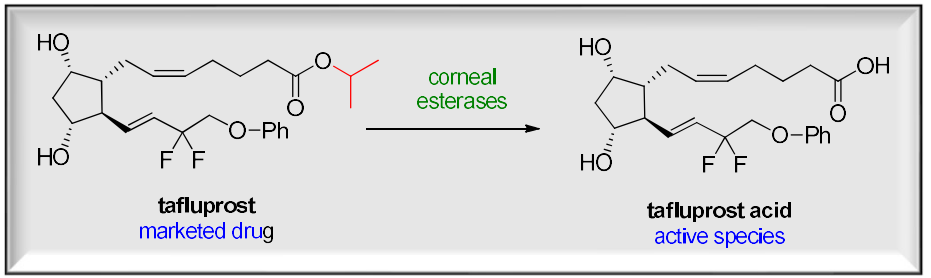
Design of Prodrugs for Ocular Delivery

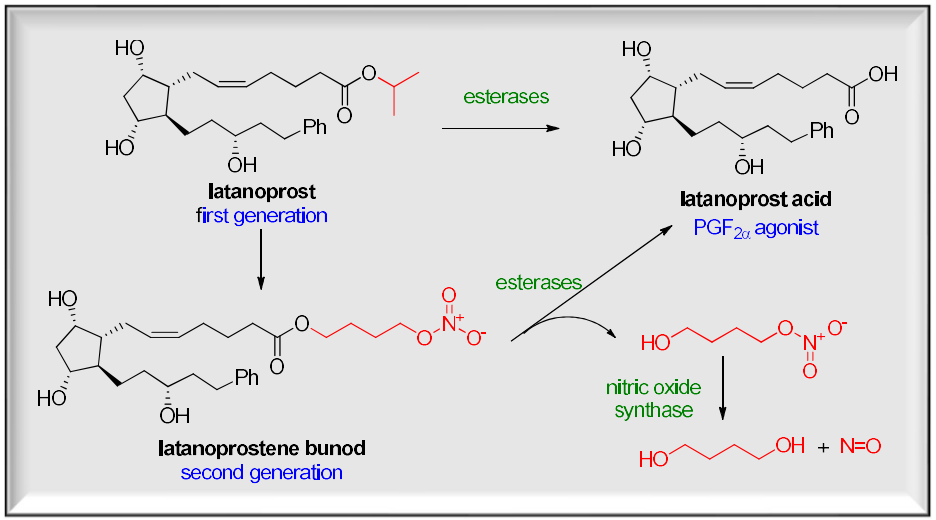
Membrane Permeability



	Caco-2 P_{app} A-B cm/s	PAMPA (cm/s)
acid	1×10^{-7}	0.9×10^{-6}
ester	-	2.8×10^{-5}





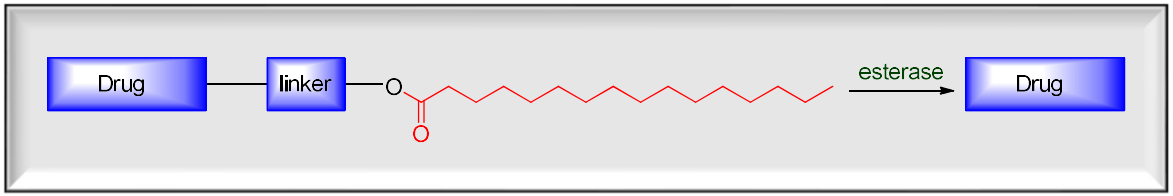


G. Vrindra *et al., J. Pharmacol. Exp. Ther.*, 2019, **370**, 602; R. Iwamura *et al., J. Med. Chem.*, 2018, **61**, 6869-6891; M. Fingeret *et al., Clin. Exp. Optom.*, 2019, **102**, 541-550
S.M. Hoy *et al., Drugs*, 2015, **75**, 1807-1813; *Drugs*, 2018, **78**, 773-780; A. Hammid & P. Honkakoski, *Drug Metab. Rev.*, 2024, DOI: 10.1080/03602532.2024.2368247

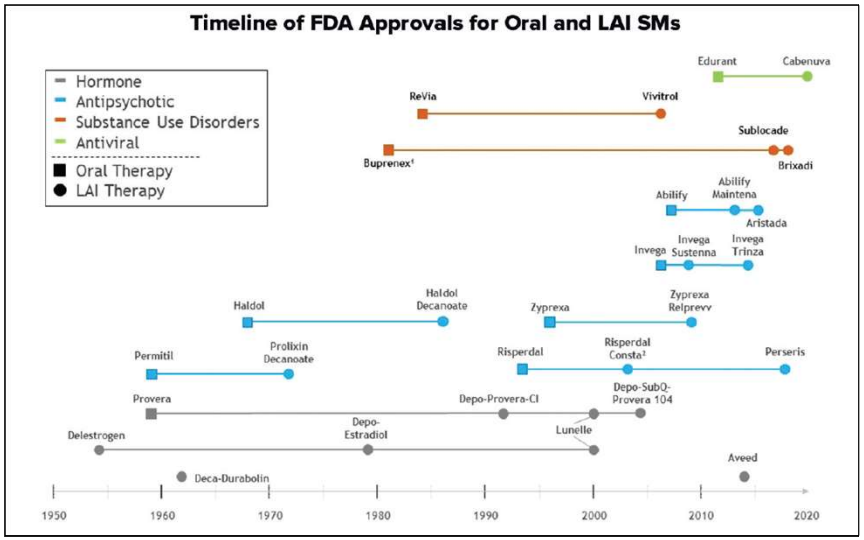
Prodrugs & Long-Acting Parenteral Drugs

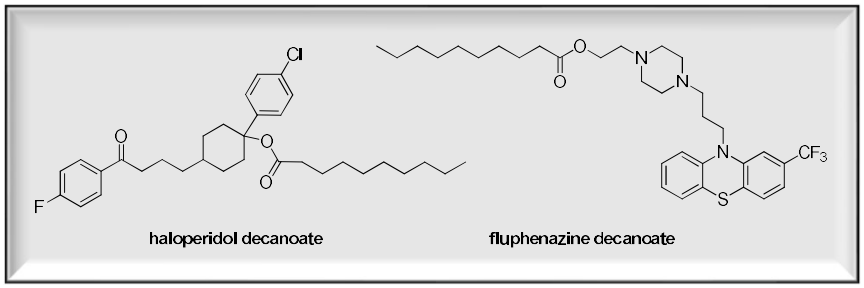
Long-Acting Injectable (LAI) Drugs

Drug Disposition



- Subcutaneous (SC) or intramuscular (IM) delivery
  - depot deposited in tissue from which drug diffuses slowly
- Solubility modulates release kinetics
  - low drug solubility preferable
- Lipophilic prodrugs confer targeted physicochemical properties
  - slowly leach into circulation where they are cleaved to release parent drug
  - dissolution rate is typically controlled by intrinsic solubility properties, formulation
- Blunts C_{max}, prolongs $t_{1/2}$ of parent drug: "flip-flop" kinetics
  - several antipsychotic drugs are marketed
  - HIV-1 inhibitors are in development
- Injection site reactions common
  - an inflammatory response but generally well-tolerated
- Approved long-acting HIV-1 drugs have had slow uptake in first 2.5 years on market
  - none are prodrugs
  - attributed to differences in clinic capabilities to support implementation

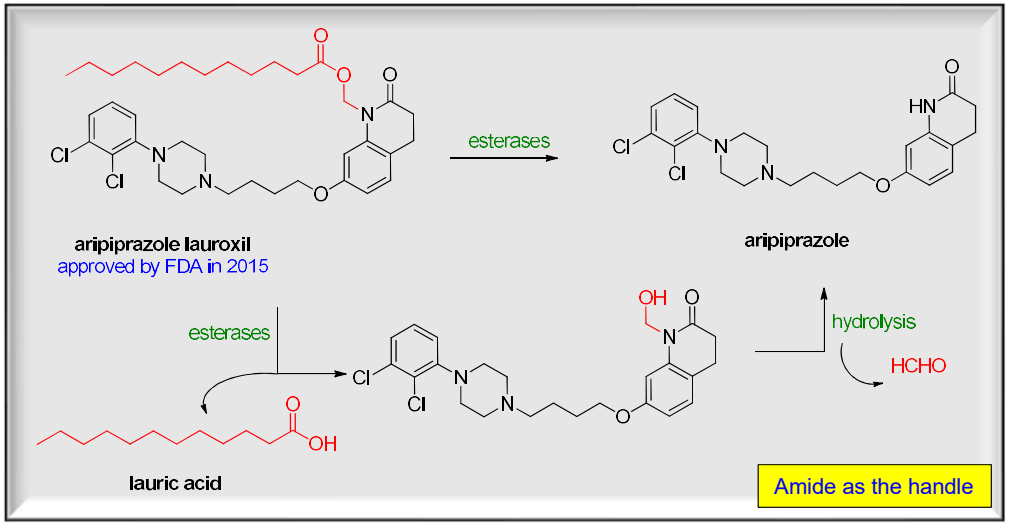


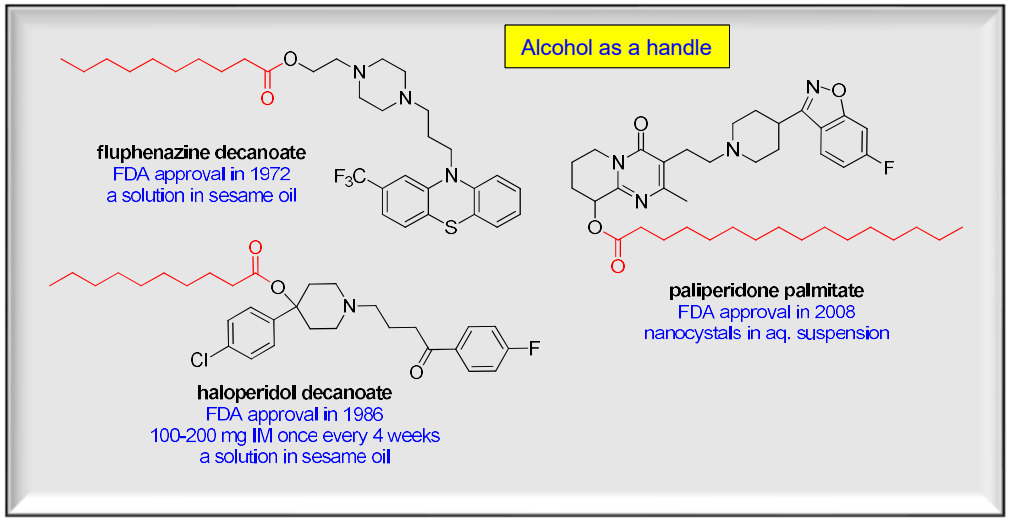


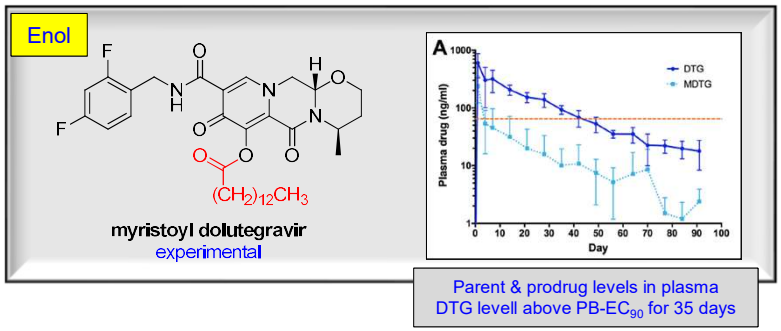
A.Z. Gonzales *et al., Med. Chem. Rev.*, 2020, **55**, 383-406; J.F. Remenar, *Mol. Pharmaceutics,* 2014, **11**, 1739-1749; H.E. Gendelman *et al., Trends Microbiol.*, 2019, **27**, 593-606

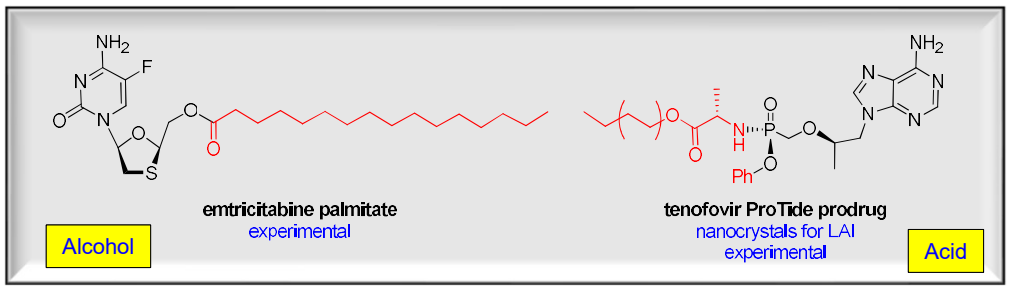
Design of Prodrugs as Long-Acting Injectables (LAIs)

Drug Disposition





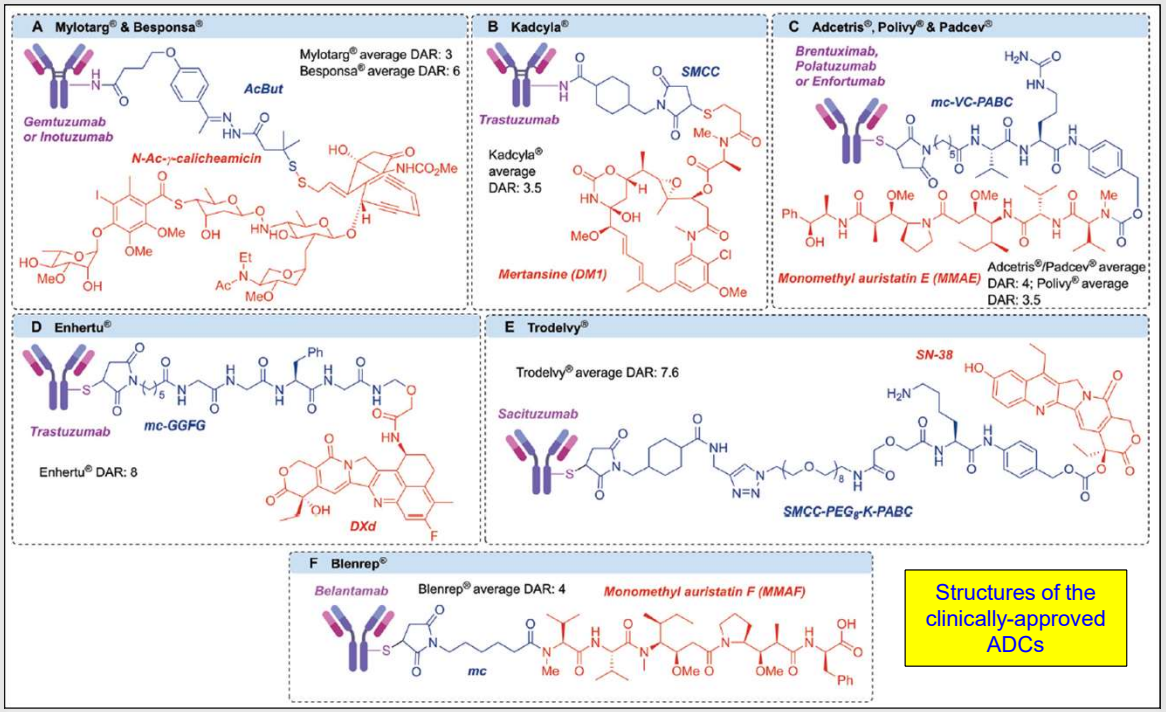




A.Z. Gonzales *et al., Med. Chem. Rev.*, 2020, **55**, 383-406; J.F. Remenar, *Mol. Pharmaceutics,* 2014, **11**, 1739-1749; H.E. Gendelman *et al., Trends Microbiol.*, 2019, **27**, 593-606
J. McMillan *et al., Antimicrob. Agents. Chemother.*, 2018, **62**, e01316-17; I.M. Ibrahim *et al., Int. J. Nanomed.*, 2019, **14**, 6231-6247; D.A. Cobb *et al., Nat. Commun.*, 2021, **12**, 5458

Antibody-Drug Conjugates – A Unique Prodrug Format

- Typically exploited for tumor cell targeting
  - Ab recognizes a tumor-specific antigen
- Ab-receptor complex is endocytosed
  - degraded in lysosomes
- Payload released by proteolysis
  - typically a cytotoxic agent
  - can diffuse into adjacent cells
    - bystander effect
- Complex drug delivery technology
  - vibrant area of drug discovery
  - >100 ADCs in development
- Key issues:
  - site/mode of attachment
  - drug-antibody ration (DAR)
  - premature release of payload
  - solubility & *in vivo* $t_{1/2}$ of ADC
- Only 1% of ADC finds its way to tumor cells



Structures of the clinically-approved ADCs

Conclusion & Acknowledgements

- Prodrugs provide potential solutions to a range of problems associated with drug delivery
  - enhanced membrane permeability
  - increased solubility: PO & IV delivery
  - drug disposition & tissue targeting
- Optimally deployed by anticipating problems as early as possible
  - integrate with the drug design/discovery process
- Not without challenges
  - synthetic challenges
  - prodrug stability challenges
  - more complex analytical profiling in PK studies
  - species differences in prodrug conversion
  - potential for toxicity with some promoiety fragments
- Can markedly change the PK profile of parent molecule
  - full toxicological work up due to changes in *in vivo* disposition
- Many successful prodrugs in the Top 200 marketed drugs
  - an important drug delivery technology
- Opportunity for additional innovation
  - ProTide phosphoramidate delivery technology more effective than simple esters
  - complex unmasking process

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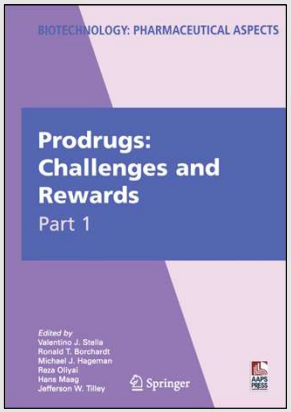
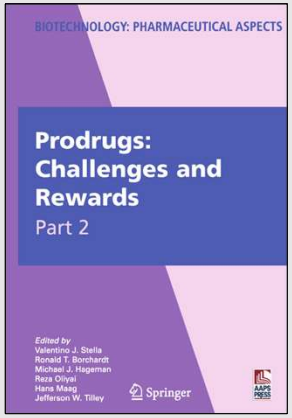
M.A.M. Subbaiah, J. Rautio & N.A. Meanwell, *Chem. Soc. Rev.*, 2024, **53**, 2099-2210

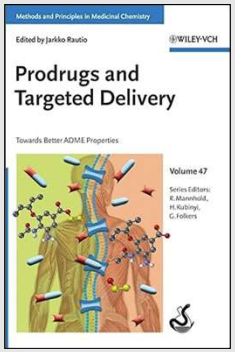
Additional Slides

Some Useful Literature

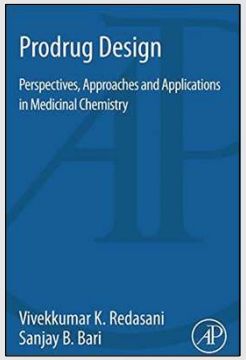


2007

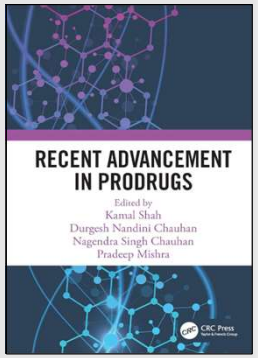




2011



2015



2020

Nature Rev. Drug Discov., 2008, **7**, 255-270

Prodrugs: design and clinical applications

Jarkko Rautio, Hanna Kumpulainen*, Tijcho Heimbach[†], Reza Oliyai[§], Dooman Oh[||], Tomi Järvinen* and Jouko Savolainen[†]*

Abstract | Prodrugs are bioreversible derivatives of drug molecules that undergo an enzymatic and/or chemical transformation *in vivo* to release the active parent drug, which can then exert the desired pharmacological effect. In both drug discovery and development, prodrugs have become an established tool for improving physicochemical, biopharmaceutical or pharmacokinetic properties of pharmacologically active agents. About 5–7% of drugs approved worldwide can be classified as prodrugs, and the implementation of a prodrug approach in the early stages of drug discovery is a growing trend. To illustrate the applicability of the prodrug strategy, this article describes the most common functional groups that are amenable to prodrug design, and highlights examples of prodrugs that are either launched or are undergoing human trials.

Journal of Medicinal Chemistry

J. Med. Chem. 2018, **61**, 2211-2226

Perspective

pubs.acs.org/jmc

The ProTide Prodrug Technology: From the Concept to the Clinic

Miniperspective

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The expanding role of prodrugs in contemporary drug design and development

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Abstract | Prodrugs are molecules with little or no pharmacological activity that are converted to the active parent drug in vivo by enzymatic or chemical reactions or by a combination of the two. Prodrugs have evolved from being serendipitously discovered or used as a salvage effort to being intentionally designed. Such efforts can avoid drug development challenges that limit formulation options or result in unacceptable biopharmaceutical or pharmacokinetic performance, or poor targeting. In the past 10 years, the US Food and Drug Administration has approved at least 30 prodrugs, which accounts for more than 12% of all approved small-molecule new chemical entities. In this Review, we highlight prodrug design strategies for improved formulation and pharmacokinetic and targeting properties, with a focus on the most recently marketed prodrugs. We also discuss preclinical and clinical challenges and considerations in prodrug design and development.

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Prodrugs as empowering tools in drug discovery and development: recent strategic applications of drug delivery solutions to mitigate challenges associated with lead compounds and drug candidates

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The delivery of a drug to a specific organ or tissue at an efficacious concentration is the pharmacokinetic (PK) hallmark of promoting effective pharmacological action at a target site with an acceptable safety profile. Sub-optimal pharmaceutical or ADME profiles of drug candidates, which can often be a function of inherently poor physicochemical properties, pose significant challenges to drug discovery and development teams and may contribute to high compound attrition rates. Medicinal chemists have exploited prodrugs as an informed strategy to productively enhance the profiles of new chemical entities by optimizing the physicochemical, biopharmaceutical, and pharmacokinetic properties as well as selectively delivering a molecule to the site of action as a means of addressing a range of limitations. While discovery scientists have traditionally employed prodrugs to improve solubility and membrane permeability, the growing sophistication of prodrug technologies has enabled a significant expansion of their scope and applications as an empowering tool to mitigate a broad range of drug delivery challenges. Prodrugs have emerged as successful solutions to resolve non-linear exposure, inadequate exposure to support toxicological studies, pH-dependent absorption, high pill burden, formulation challenges, lack of feasibility of developing solid and liquid dosage forms, first-pass metabolism, high dosing frequency translating to reduced patient compliance and poor site-specific drug delivery. During the period 2012–2022, the US Food and Drug Administration (FDA) approved 50 prodrugs, which amounts to 13% of approved small molecule drugs, reflecting both the importance and success of implementing prodrug approaches in the pursuit of developing safe and effective drugs to address unmet medical needs.

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Review article

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The landscape of small-molecule prodrugs

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molecules

Molecules, 2020, **25**, 884

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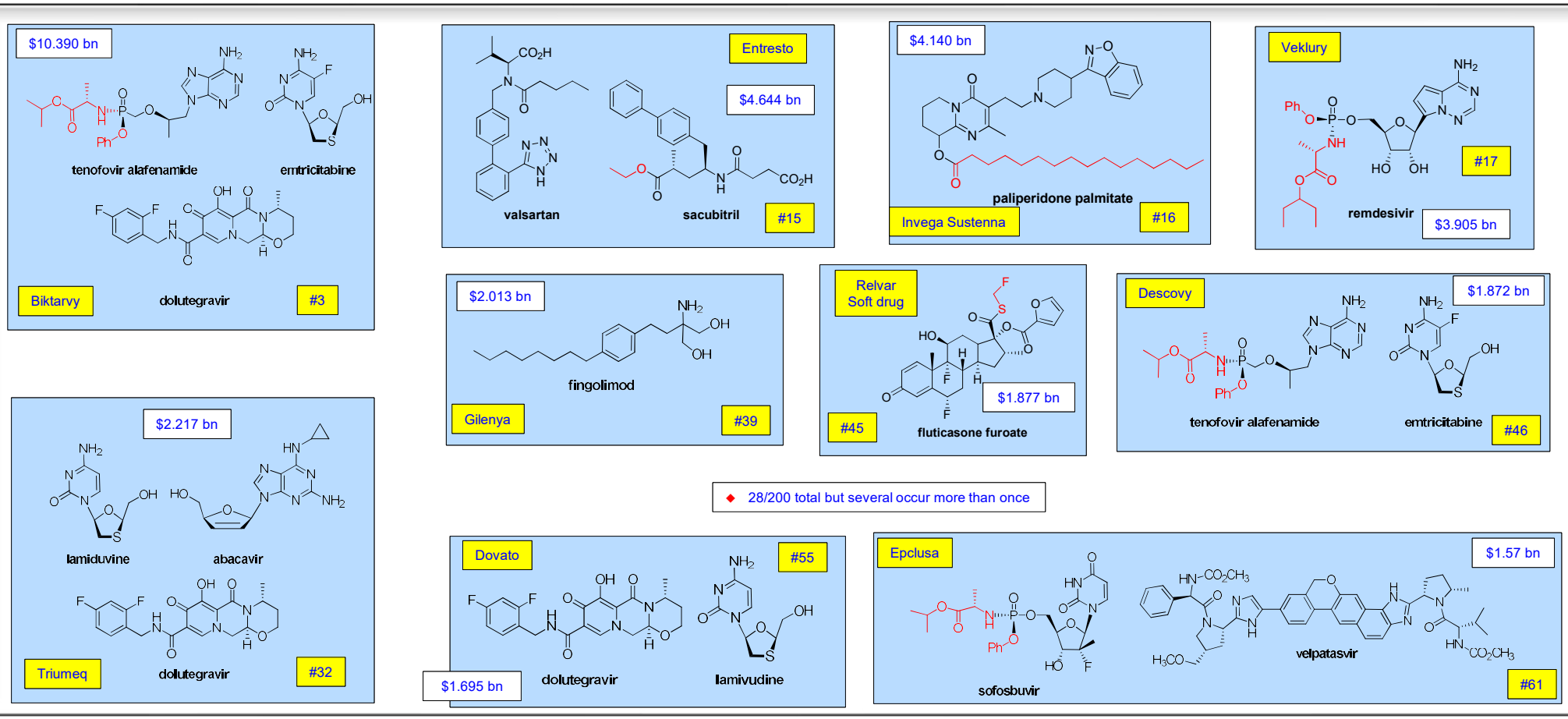
Review

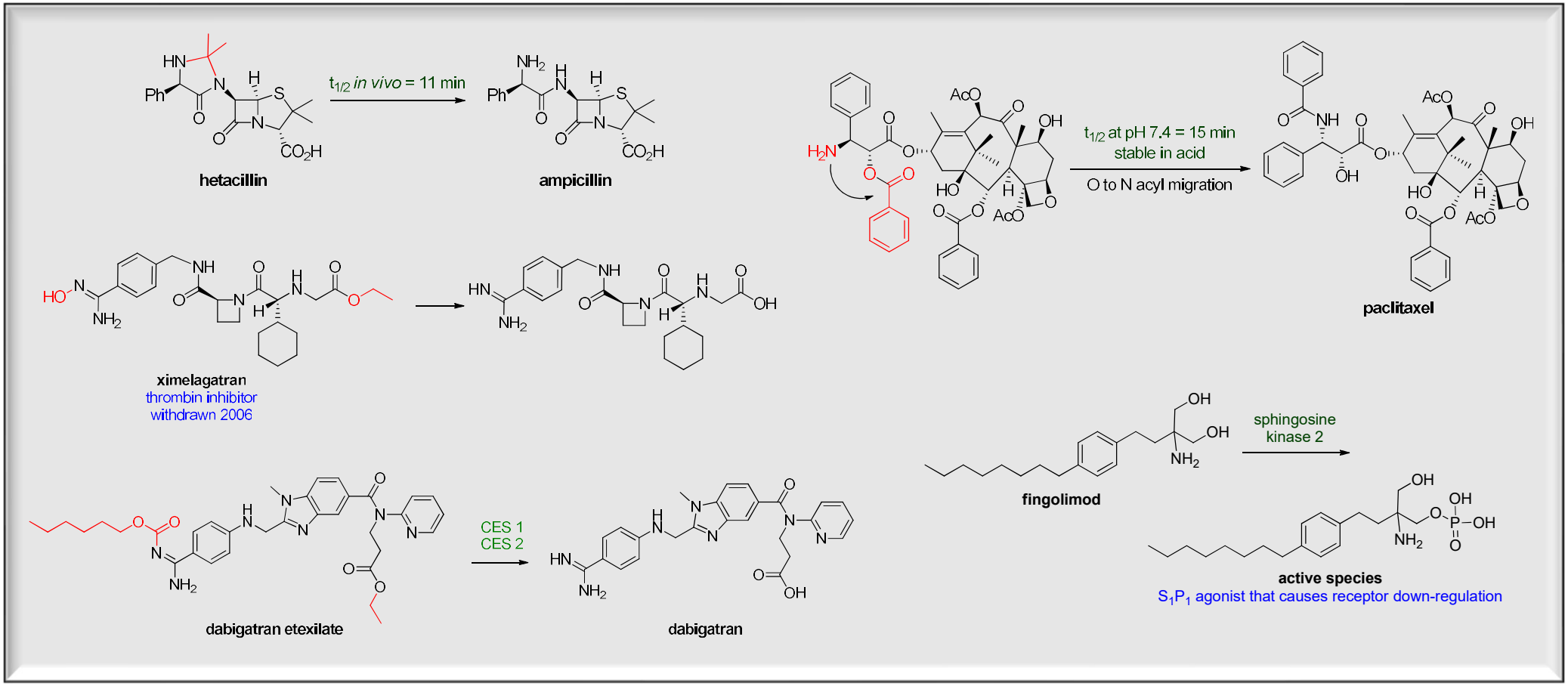
Newly Developed Prodrugs and Prodrugs in Development; an Insight of the Recent Years

Anas Najjar [1], Abderrahman Najjar [2] and Rafik Karaman [1,*]

Prodrugs in the Top 200 Small Molecule Drugs 2022

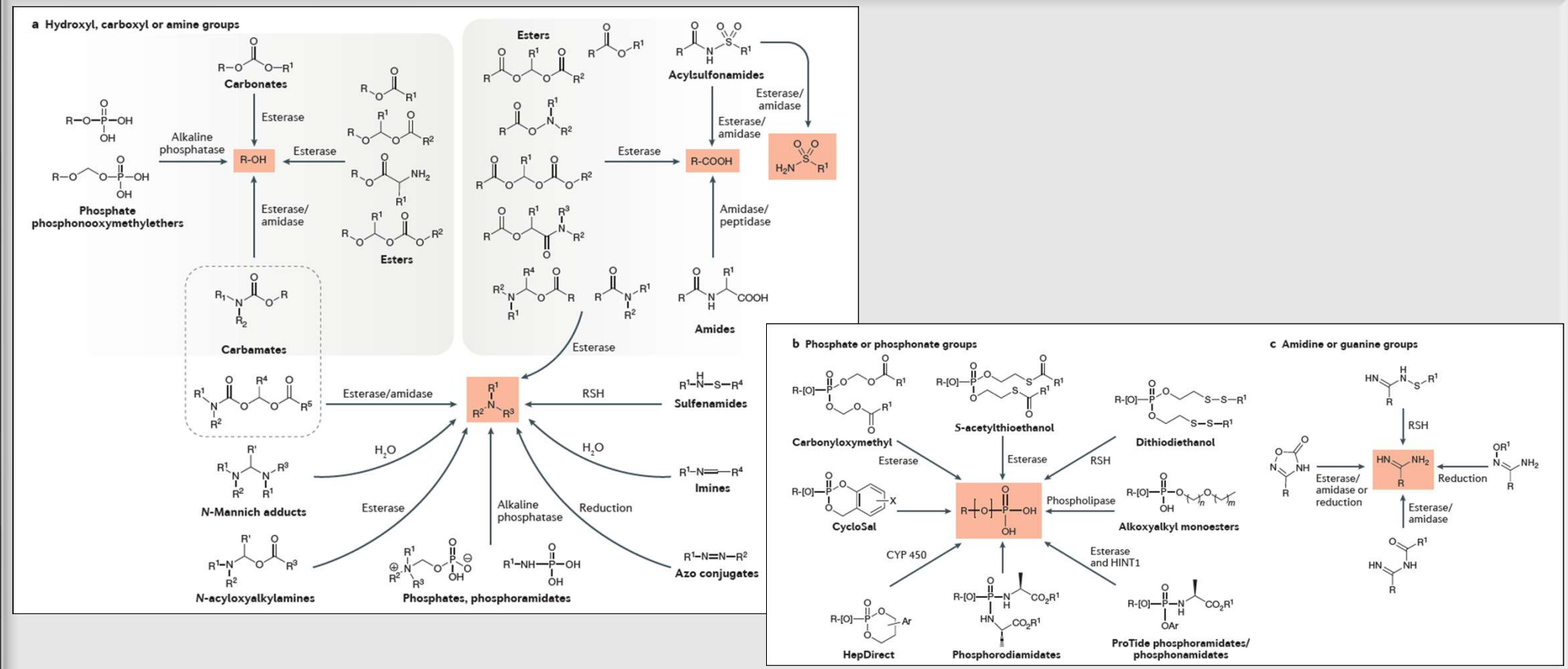


Prodrug Space



M.A.M. Subbaiah, J. Rautio & N.A. Meanwell, *Chem. Soc. Rev.*, 2024, **53**, 2099-2210

Prodrug Strategies for Common Functionalities



J. Rautio *et al., Nature Rev. Drug Discov.,* 2018, **17**, 550-587