The Role of the Pyridazine Ring in Molecular Recognition & Drug Discovery

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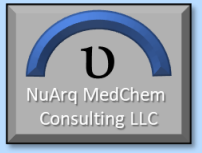
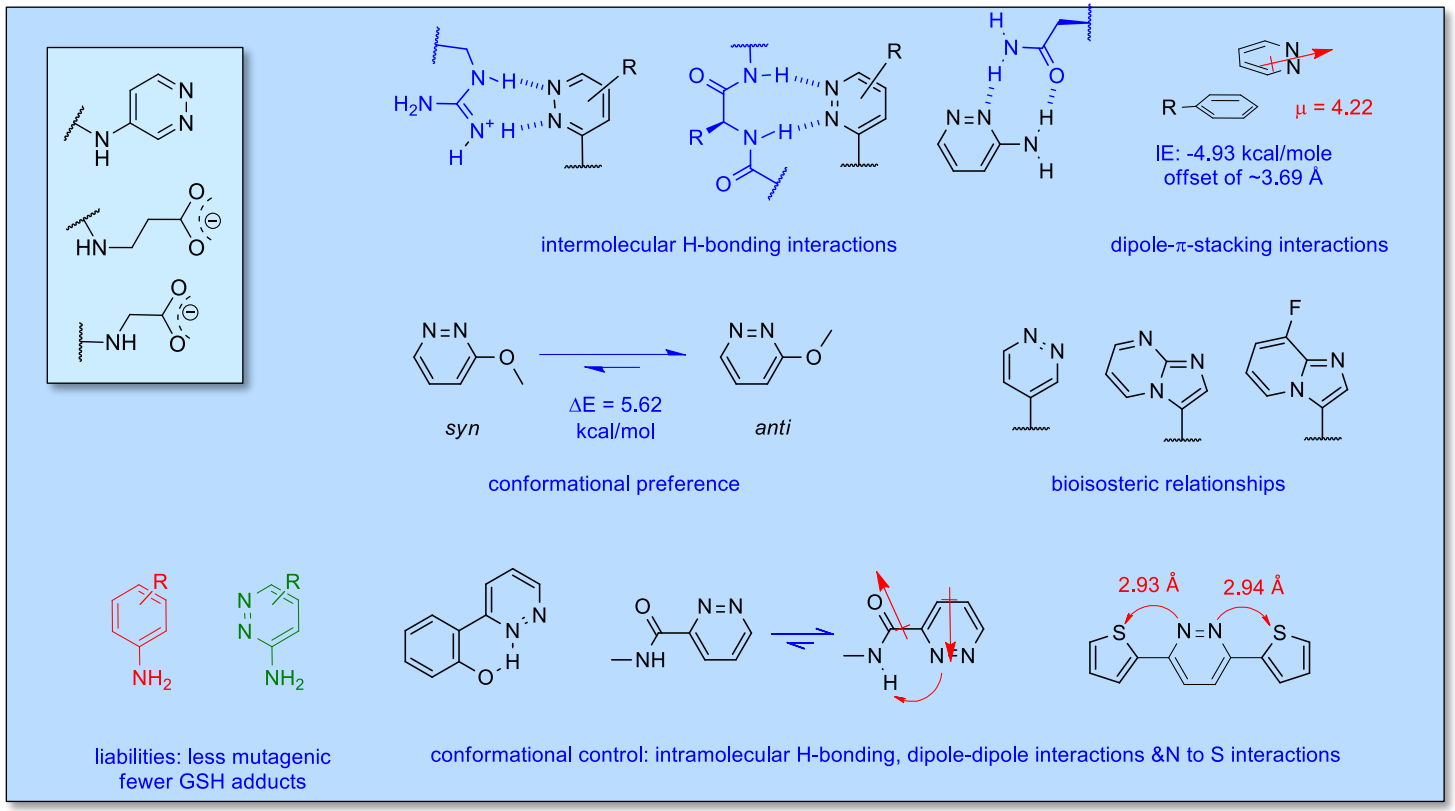


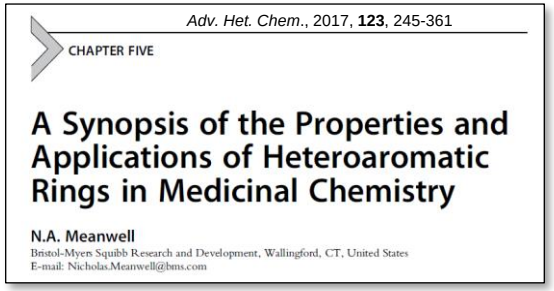
Baruch S. Blumberg Institute

Monday, November 6th, 2023

Pyridazines in Drug Design - Outline

- Prevalence of pyridazines in marketed drugs
  - candidates in development
- Physicochemical properties of pyridazines
  - lipophilicity
  - dipole interactions
  - electron withdrawing properties
- Conformational aspects of pyridazines
  - pyridazine-3-ethers
  - pyridazine-3-CH_2-OR
  - sulfur interactions
- Pyridazines & potency
  - increases, decreases
- Pyridazines & H-bonding
  - drug-target interactions
  - CO_2^- mimicry?
- Pyridazines & electrophilicity
  - activation of C≡N, acidification of NH
- Bioisosteres of pyridazines
  - fluorobenzenes, fused heterocycles
- Pyridazines & liability issues
  - can mitigate hERG, aniline problems
- Pyridazine-3-CO.NHR derivatives
  - interplay of substituent & ring
- Pyridazine-3-ones
  - molecular glues
- Conclusion




Adv. Het. Chem., 2017, **123**, 245-361

CHAPTER FIVE

A Synopsis of the Properties and Applications of Heteroaromatic Rings in Medicinal Chemistry

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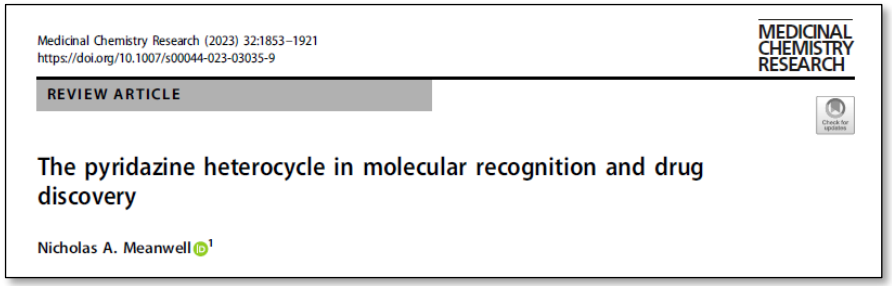
Medicinal Chemistry Research (2023) 32:1853–1921
https://doi.org/10.1007/s00044-023-03035-9

MEDICINAL CHEMISTRY RESEARCH

REVIEW ARTICLE

The pyridazine heterocycle in molecular recognition and drug discovery

Nicholas A. Meanwell[1]

N.A. Meanwell, *Adv. Het. Chem.*, 2017, **123**, 245-361; *Med. Chem. Res.*, 2023, **32**, 1853-1921

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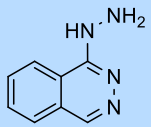
Heterocycles in Drug Design

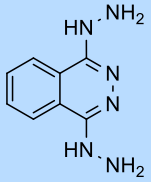
- Heterocycles are the mainstay of medicinal chemistry
  - ubiquitous as drug scaffolds, structural elements, appendages and pharmacophores
- Heterocycles play a prominent role in the design of molecular metaphors (bioisosteres)
  - e.g. azoles as amide isosteres; tetrazoles as acid isosteres
- Almost infinite opportunity for structural variation – highly plastic in nature
  - electronic and steric effects of substituents add to the rich panoply of properties
- Properties of heterocycles:
  - can be basic or acidic: may depend on substitution pattern
  - unique vectors for deploying critical drug functionality
  - tautomeric nature provides additional opportunities for structural variation
  - heterocycle properties can be modulated by substituents
    - properties of substituents can be modulated by the heterocycle
  - H-bond donor: N-H, O-H, C-H
  - H-bond acceptor: predominantly *N* atoms, but *O* also can engage H-bond donors
  - engage in π-π (dipole) interactions with amides, aromatic rings
  - non-bonded interactions *via* σ* effects in *S*-containing heterocycles
  - tautomerism adds to the diversity of effects
- The pyridazine ring has unique physicochemical properties of value in the design of bioactive compounds
  - extends to diazoles

Pyridazines in Marketed Drugs & Drugs in Development

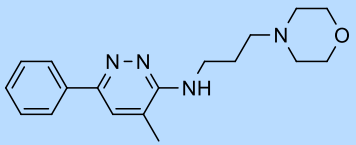
Marketed or Advanced Pyridazine-Containing Drugs



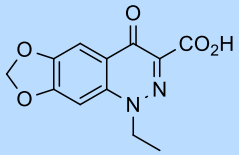
hydralazine
vasodilator
approved 1953



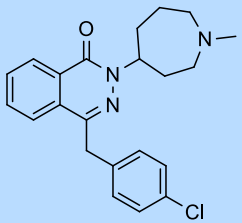
dihydralazine
vasodilator
not approved in US



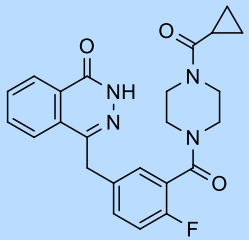
minaprine
MAO inhibitor
approved France 1972
withdrawn 1996: convulsions
not approved in US



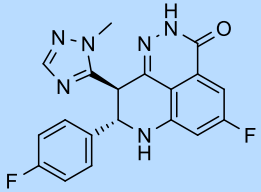
cinoxacin
quinolone antibiotic
approved 1980



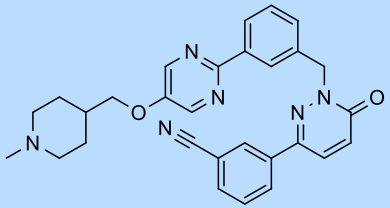
azelastine
allergic rhinitis
approved 2008



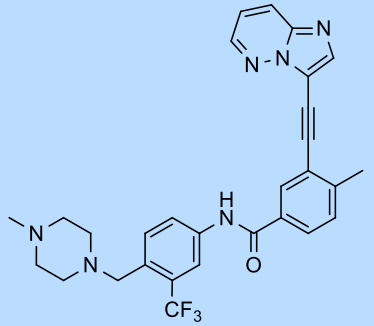
olaparib
PARP inhibitor
approved 2014



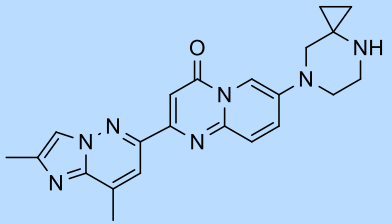
talazoparib
PARP inhibitor
approved 2018



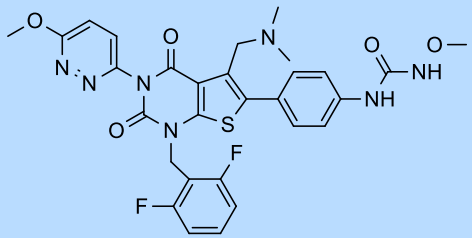
tepotinib
MET inhibitor NSCLC
approved 2021



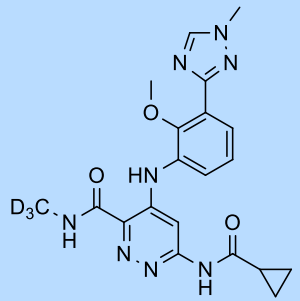
ponatinib
multi-target kinase inhibtior
approved 2012



risdiplam
SMN - RNA splicing modulator
approved 2020

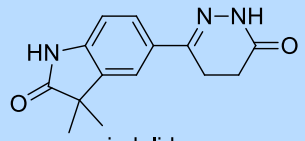


relugolix
GnRH receptor antagonist
prostate cancer
approved 2020

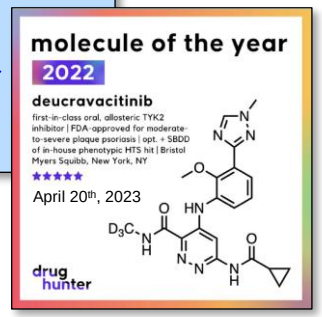


deucravacitinib
allosteric TYK2 inhibitor
approved 2022

- 3 Marketed drugs incorporate a pyridazine ring: all 3-amino derivatives
  - phthalazine & cinnoline represented
- Pyridazine-3-one embedded in 4 marketed drugs
  - prevalent chemotype in 1980s: PDE3 inhibiting cardiac stimulants
- Pyridazine ring embedded in bicyclic structures in 2 drugs
  - ponatinib & risdiplam

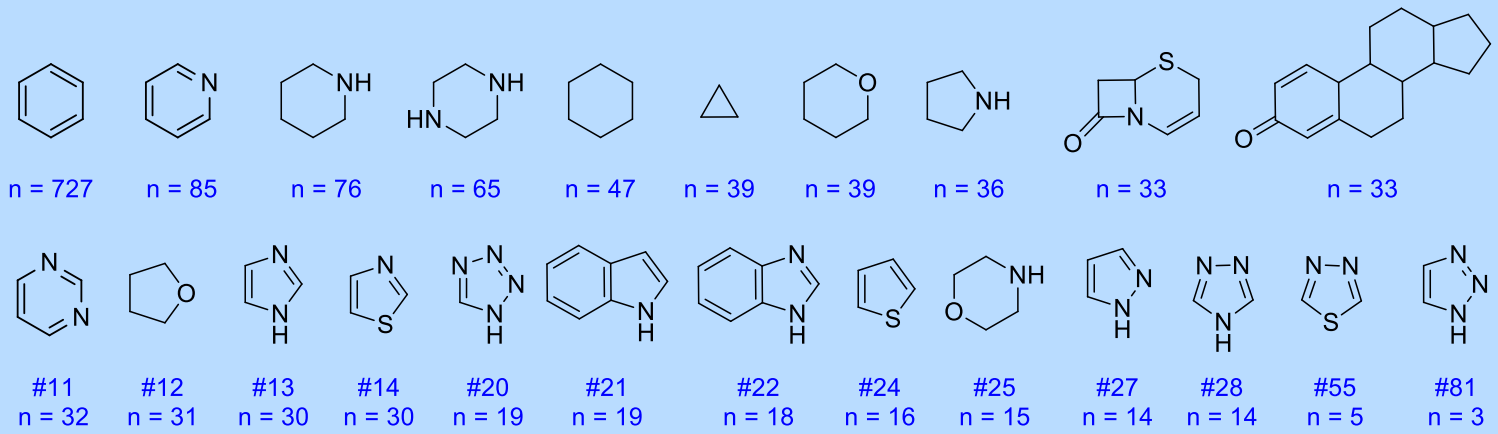


indolidan
PDE3 IC_{50} = 80 nM

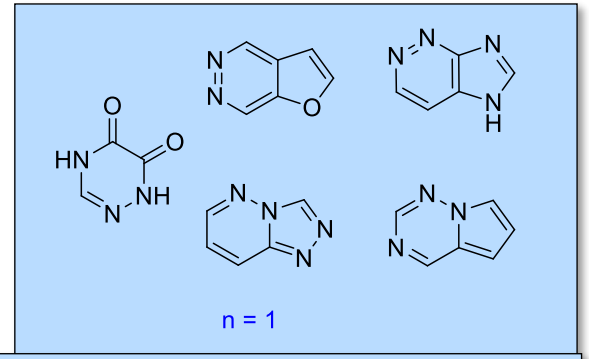


N.A. Meanwell, *Med. Chem. Res.*, 2023, **32**, 1853-1921; *Adv. Het. Chem.*, 2017, **123**, 245-361

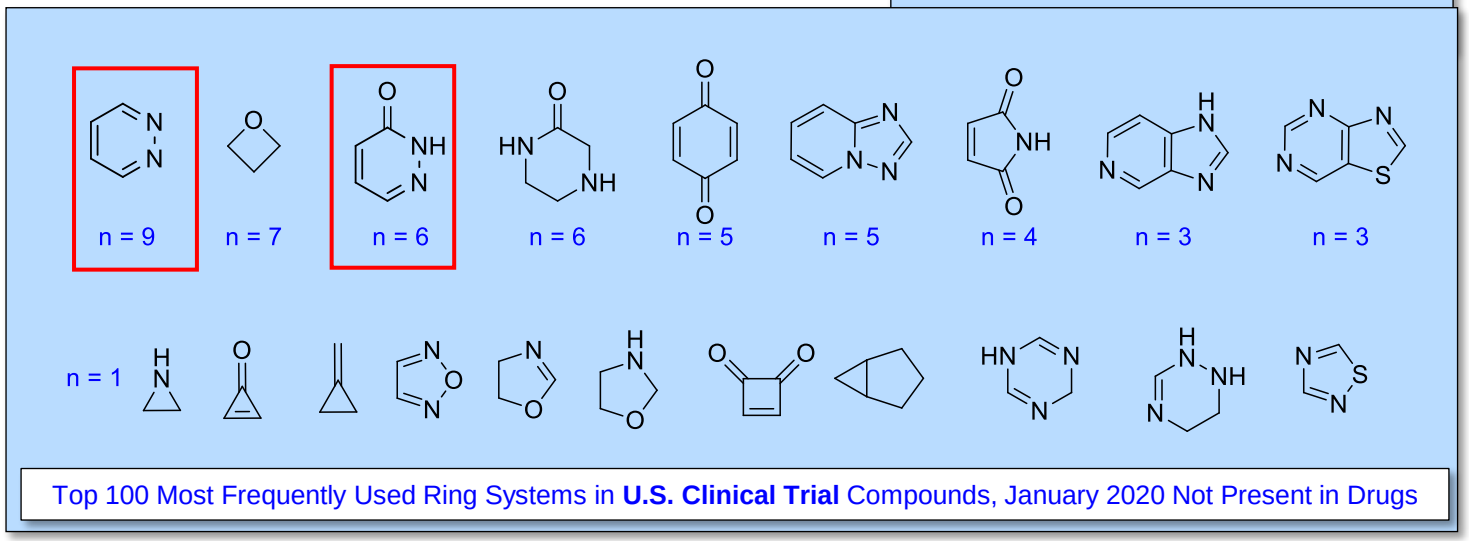
Pyridazine-Containing Drugs & Candidates in Development



Top 100 Most Frequently Used Ring Systems from **Small Molecule Drugs** Listed in the FDA Orange Book before January 2020



- Pyridazine rings absent from top 100 marketed drugs
  - most prevalent rings system in clinical trials
  - pyridazin-2-one 3rd most prevalent in this set
- Only 9 ring systems with n >1 representation
  - 15/48 (30%) are pyridazine-based
- Embedded pyridazines appearing in clinical trials
  - 5 with low frequency: n = 1
- Pyridazine homologues in marketed drugs
  - triazole #28, thiadiazole #55



Top 100 Most Frequently Used Ring Systems in **U.S. Clinical Trial** Compounds, January 2020 Not Present in Drugs

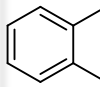
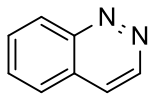
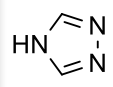
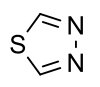
R.D. Taylor *et al., J. Med. Chem.* 2022, **65**, 8699-8712

n = 1 arranged in order of ascending MW

Physicochemical Properties of Heterocycles

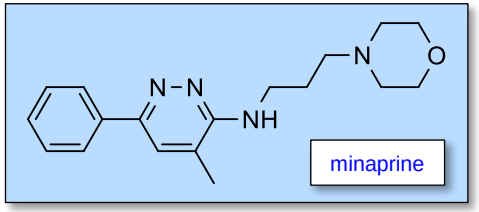
Where Pyridazines Appear in The Landscape

Physicochemical Properties

						
pK_a	2.0	3.17	2.5-2.7	2.45		
pK_{BHX}	1.65	1.97	~1.65	2.6	1.3	~2.51
Dipole (*D*)	4.22	4.88	4.41	5.74	3.04	3.28
cLog *P*	-0.51	0.68	1.14	-0.89	-0.69	-0.2
cLog $D_{pH=1}$	-2.5	-1.51	-0.58	-2.85	-0.69	-0.22
TPSA (Å^2)	25.8	25.8	25.8	36.75	33.95	54
C^{Ph}_X	0.417					

				
pK_a	5.2	0.93	-1.7	0.37
pK_{BHX}	1.86	1.07	0.32	0.92
Dipole (*D*)	2.22	2.33	0	0
cLog *P*	0.84	0.26	-0.73	-0.002
cLog $D_{pH=1}$	-1.66	-0.58	-1.82	-0.43
TPSA (Å^2)	12.9	25.8	38.7	25.8
C^{Ph}_X	0.41 (C2 & C4)	0.43 (C2); 0.5 (C4)		0.47

- Pyridazine is a strong H-bond acceptor
  - approaching that of pyridine but much less basic
  - not associated with CYP inhibition
  - de-symmetrized by substitution
- Pyridazine has the largest dipole amongst azines
  - reflected in polarity (cLog *P* & cLog *D*)
    - -1.35 unit Δ from pyridine
  - higher TPSA than pyridine
- Pyridazine C-3 is electron deficient
  - comparable to pyridine; less than pyrimidine
  - affects properties of substituents
- 3-C-H is a H-bond donor
  - stronger than pyridine 2-C-H

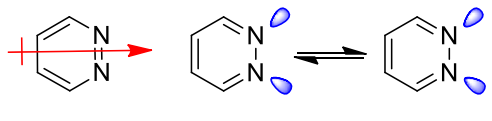


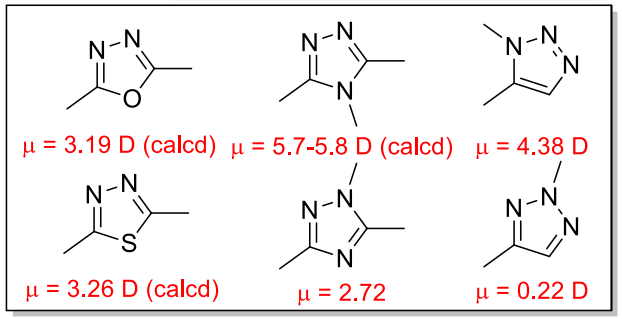
3-amino-pyridazines are more basic

minaprine supports salt formation - stable di-HCl salt

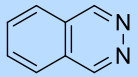
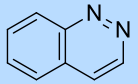
aromaticity index (AI) = 79 compared to 100 for phenyl

Triazole dipole varies based on identity & substitution pattern

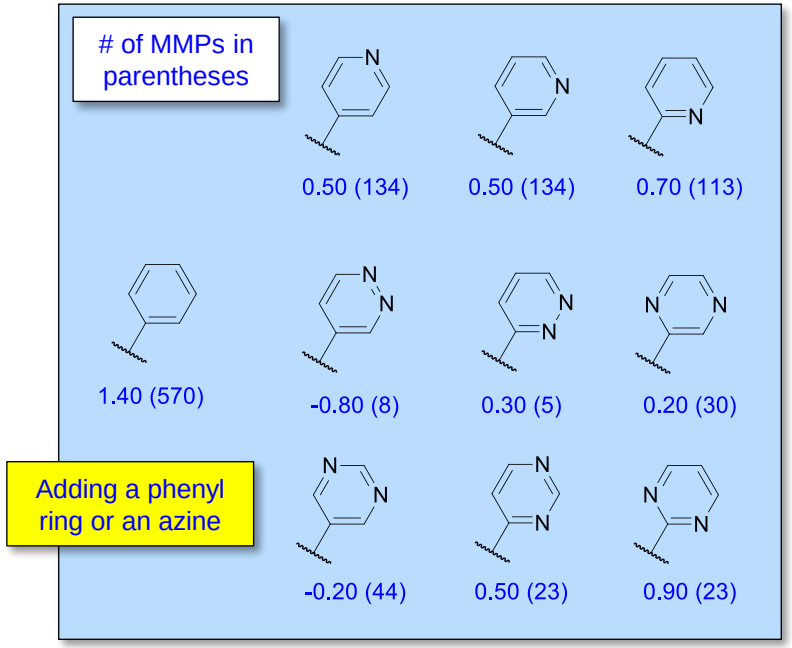


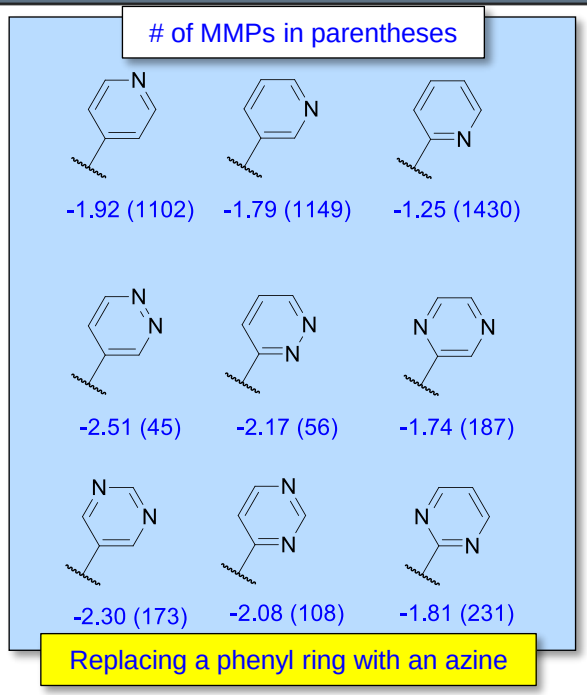


Physical Properties & Lipophilicity

	pK_{BHX}	pK_a	μ	cLog *P*
	1.86	5.2	2.3 *D*	0.84
	1.65	2.0	3.9 *D*	-0.51
	1.07	0.93	2.4 *D*	0.26
	0.92	0.37	0.0 *D*	0
	0.32	-1.7	0.0 *D*	-0.73
	1.97	3.17	4.88 *D*	0.68
	~1.65	2.5-2.7	4.41 *D*	1.14

- Pyridazine - strong H-bond acceptor
  - less basic than pyridine
  - more basic than pyrazine
- The largest dipole moment in the azine series
  - can be modulated by substituents
- Limited impact of benzo-fusion on H-bonding
  - pK_a for phthalazine & cinnoline increased
  - reduction in pK_a is more typical



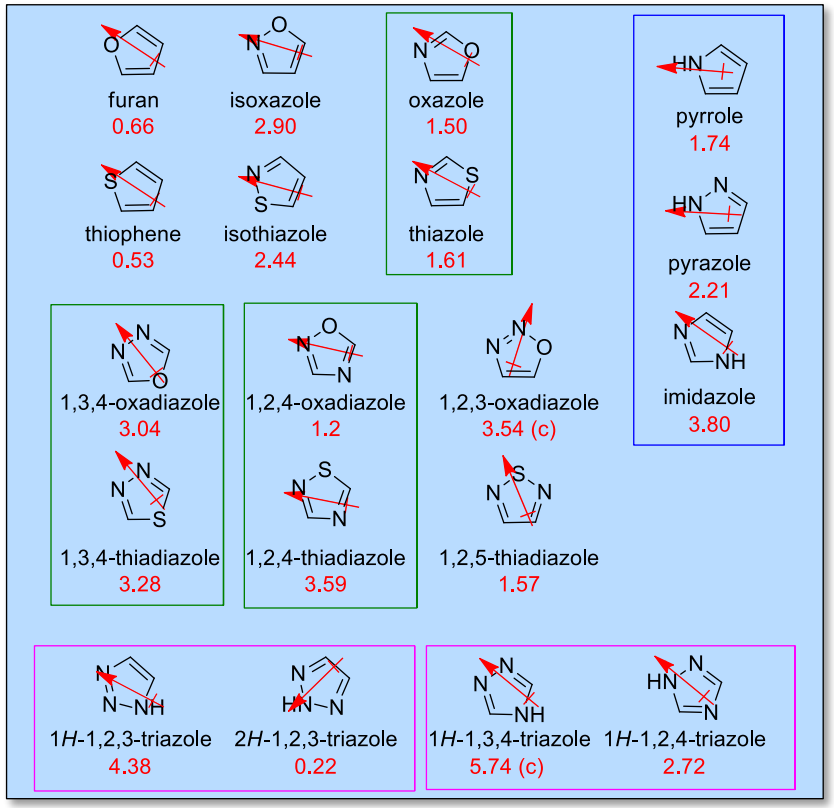


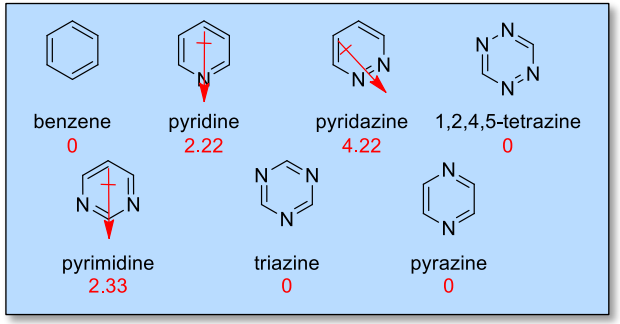
Effect on Log *D*

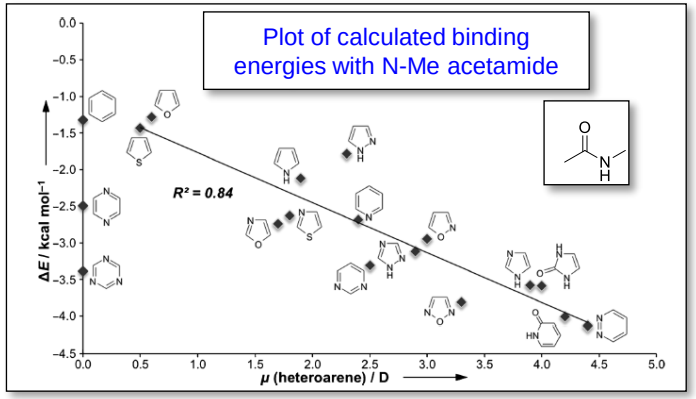
- MMP analysis:
  - context not disclosed: adds an element of variability
- Adding a phenyl ring increases Log *D*
  - azine addition associated with lower cLog *P* increases
  - extent varies based on ring identity & topology
- Replacing a phenyl ring with an azine lowers Log *D*
  - pyridazine ring more effective than other azines
- Heteroatoms adjacent to attachment sit more lipophilic
  - shield the polarity

J.J. Crawford *et al., ACS Med. Chem. Lett.*, 2020, **11**, 72-76; T.J. Ritchie *et al., Eur. J. Med. Chem.*, 2016, **124**, 1057-1068

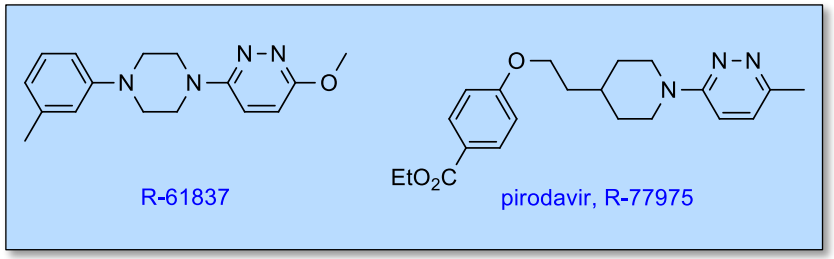
Heterocycles & Dipole Interactions

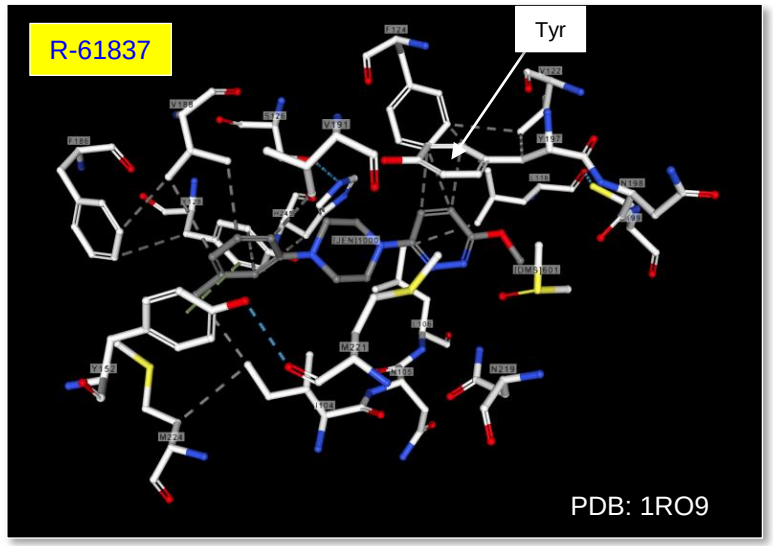


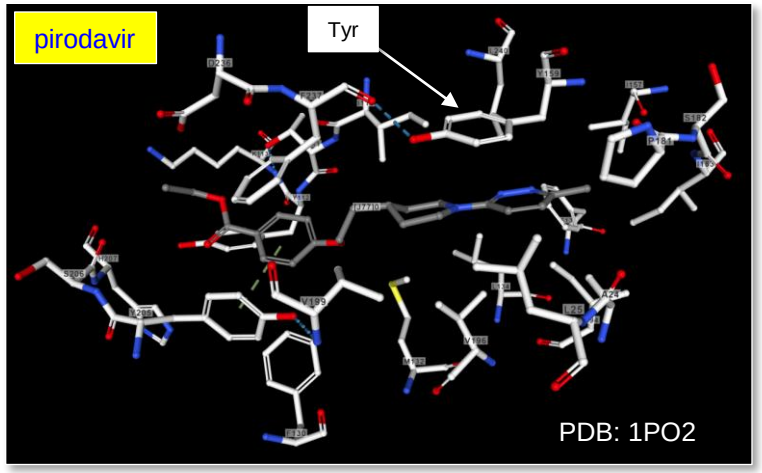




- Calculated energies & heterocycle dipoles
  - good correlation with amide association
  - some circumstances where this is not evident
- For heterocycles with no dipole moment
  - interaction E equates with ring electron density
  - stronger for electron deficient rings
- π-π interaction important in HRV capsid inhibitors
  - pyridazine ring to Tyr (& Phe in 1 polio variant)
  - H-bond to protein *via* H_2O may contribute

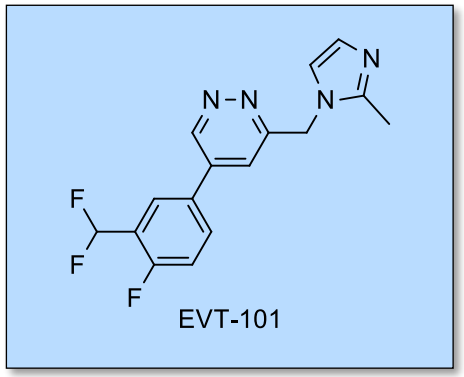




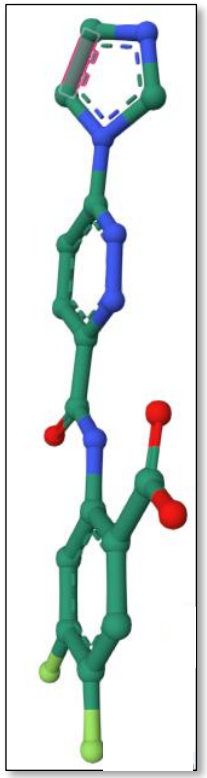


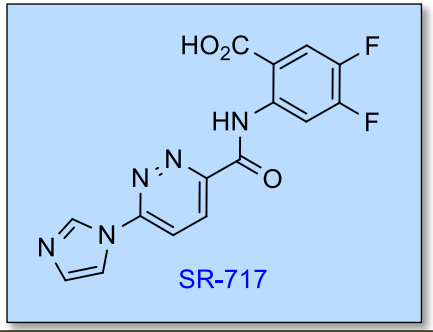
L.M. Salonen *et al., Chem. Eur. J.*, 2012, **18**, 213-222; M. Harder *et al., ChemMedChem*, 2013, **8**, 397-404; M.S. Chapman *et al., J. Mol. Biol.,* 1991, **217**, 455-463
For anticorrelation of dipole effects see: M.L. Waters *et al., J. Am. Chem. Soc.*, 2020, **142**, 17048-17056; *Protein Sci*., 2023, **32**, e4533

Pyridazines & Dipole Interactions

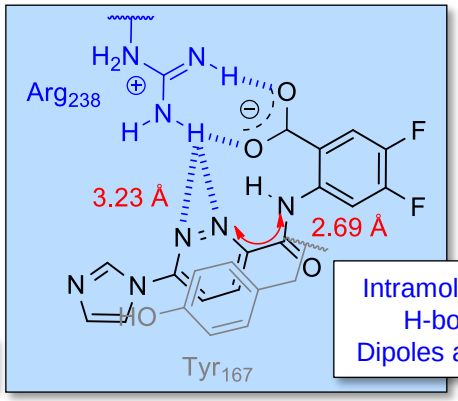


allosteric glutamate N2B antagonist

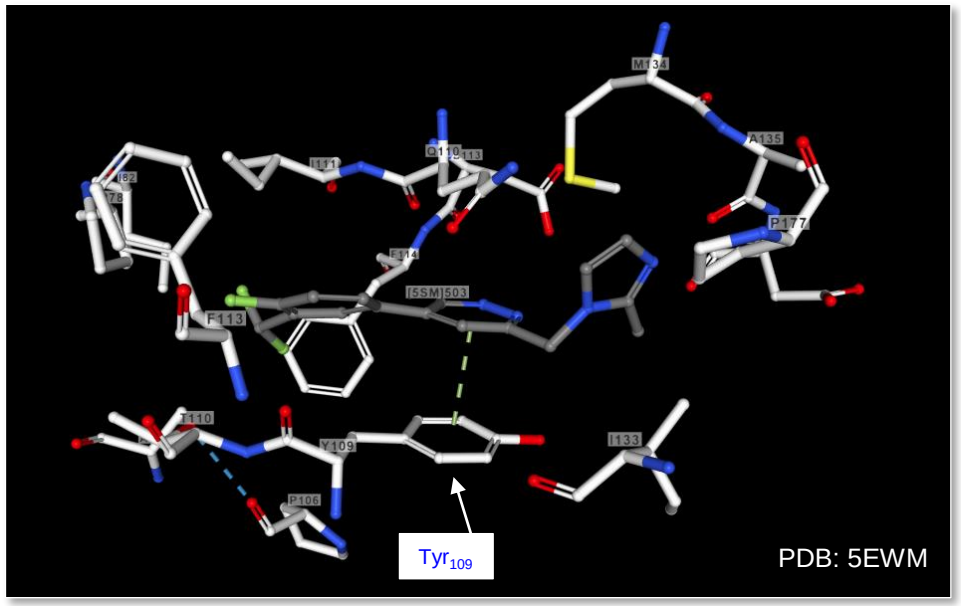




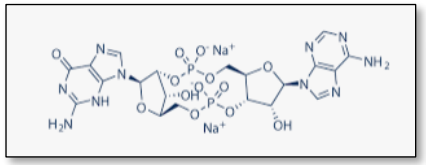
STING-activating cGAMP mimetic

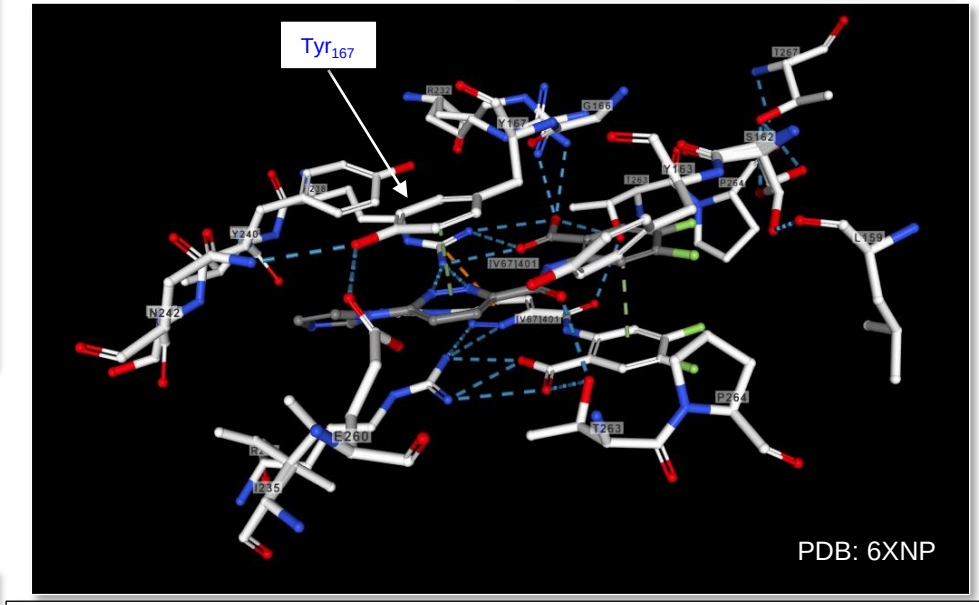


Intramolecular H-bond Dipoles aligned



π-π stacking interactions with Tyr





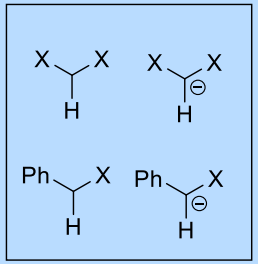
- 2 Molecules bind at the base of the STING dimer intersubunit cleft
  - mimics the binding mode of cGAMP
  - pyridazine overlays bases; acid overlays phosphate

D. Stroebel *et al., Molec. Pharmacol*., 2016, **89**, 541-551; E.N. Chin *et al., Science,* 2020, **369**, 993-999

NuArq MedChem Consulting LLC

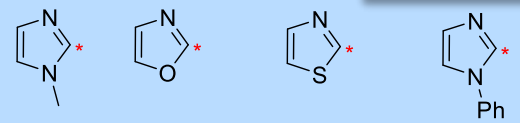
Electron Withdrawing Properties of Heterocycles

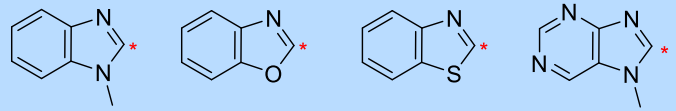
C_X^{Ph} value = charge demand = the fraction of π-charge withdrawn from an adjacent charged trigonal carbon atom

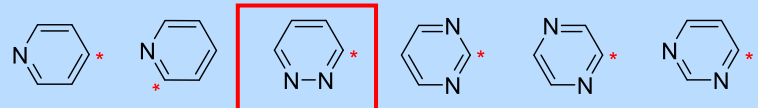


C_X^{Ph} values

Significant effect of Ph substitution







$PhSO_2$ *	0.28
N≡ *	0.28
Ph *	0.29
MeO_2C *	0.40
Me_2NCO. *	0.42
MeCO. *	0.51
PhCO. *	0.56

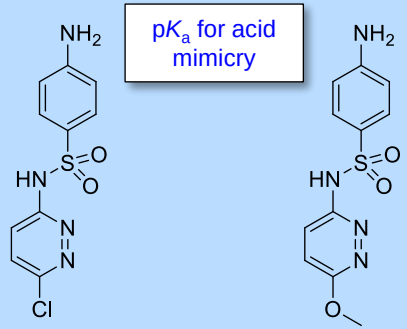
more electron withdrawing

Pyridazine comparable to $CONMe_2$

Electron withdrawing effects depend on the heterocycle, site on ring, & substitution pattern

- Electron withdrawing properties calibrated by NMR analysis
  - ^{13}C NMR shift differences between neutral & anionic species
- 2-Position of azoles is the most electron deficient
  - enhanced by benzo fusion
  - 7-methyl-7*H*-purine the most electron deficient ring
- Azines are more electrophilic
  - pyrimidine 4-position more electron deficient than the 2-position
- Properties can be modulated by substituents
  - EWGs will enhance electrophilicity; EDGs reduce

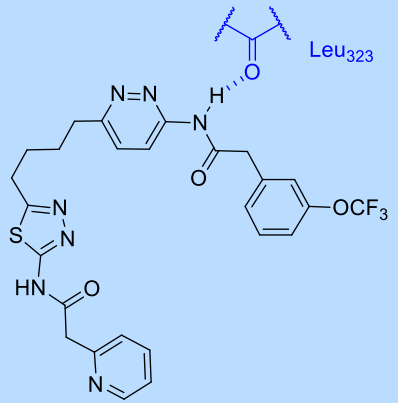
pK_a for acid mimicry



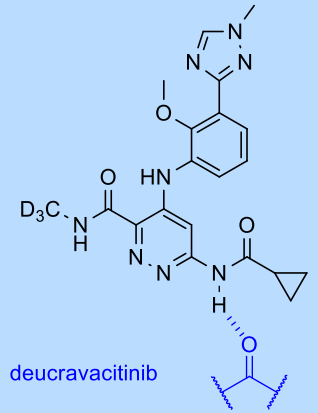
sulfachlorpyridazine

sulfamethoxypyridazine

Practical Manifestations

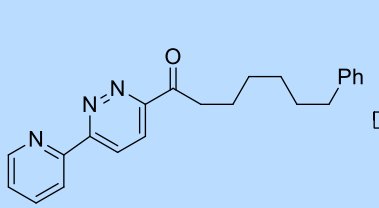


telaglenastat, CB-839

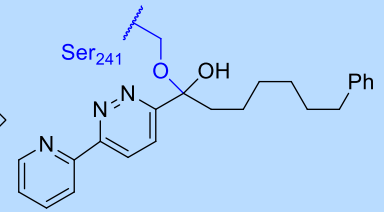


deucravacitinib

H-bond donation



FAAH inhibitor

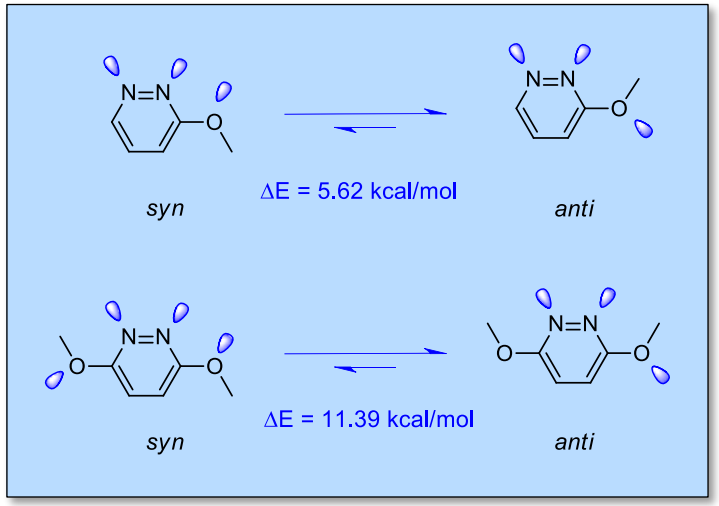


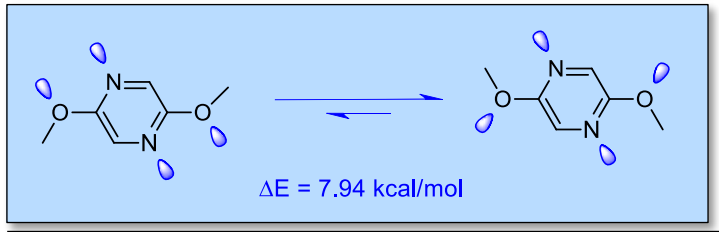
C=O activation

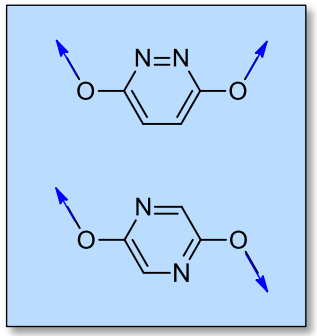
G.A. Pagani *et al., J. Org. Chem.,* 1998, **63**, 436-444; 1996, **61**, 1761-1769; 2002, **67**, 5753-5772; N.A. Meanwell, *Adv. Het. Chem.*, 2017, **123**, 245-361

Conformational Aspects of Pyridazines

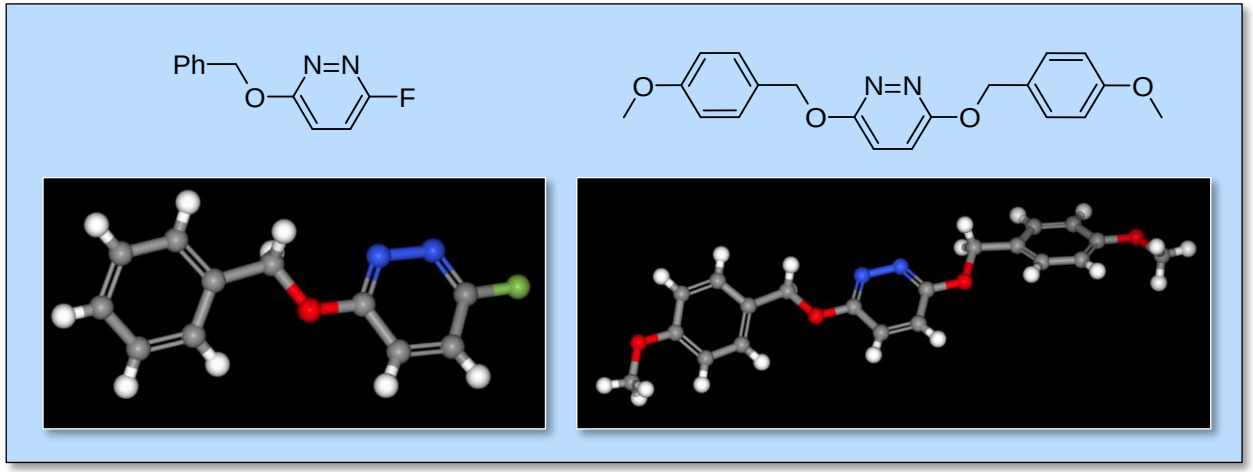
Conformation: Pyridazine-OR

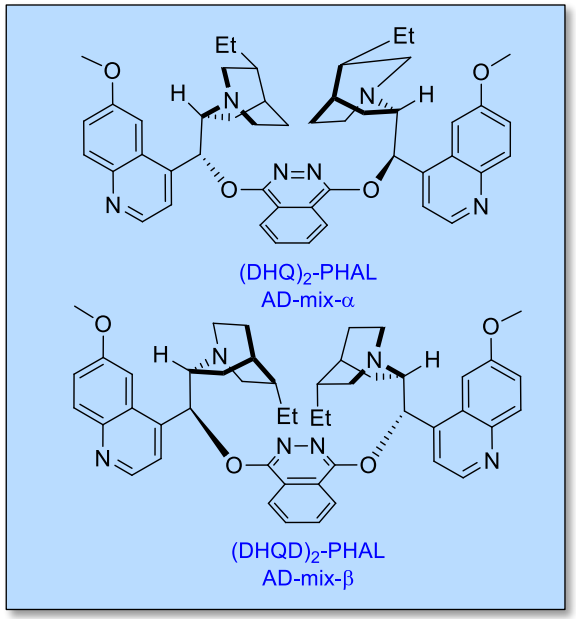


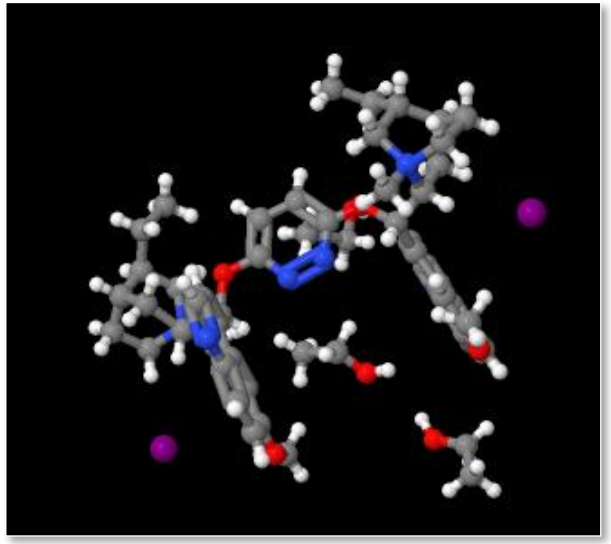




pyridazine & pyrazine offer complementary preferred exit vectors



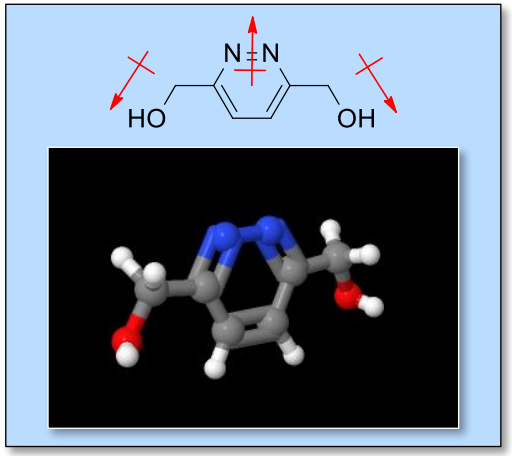


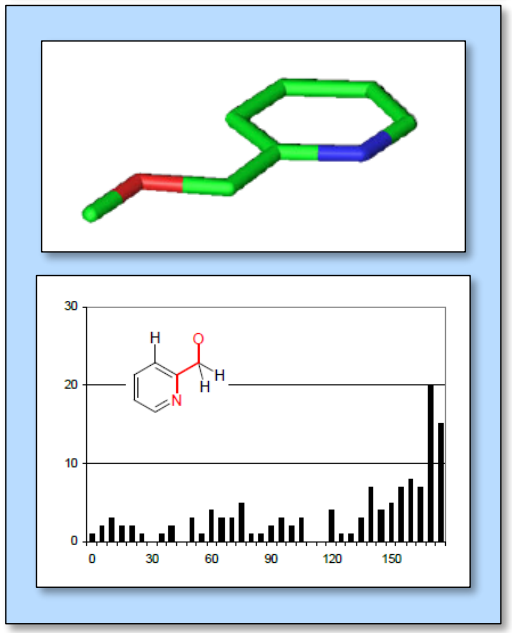


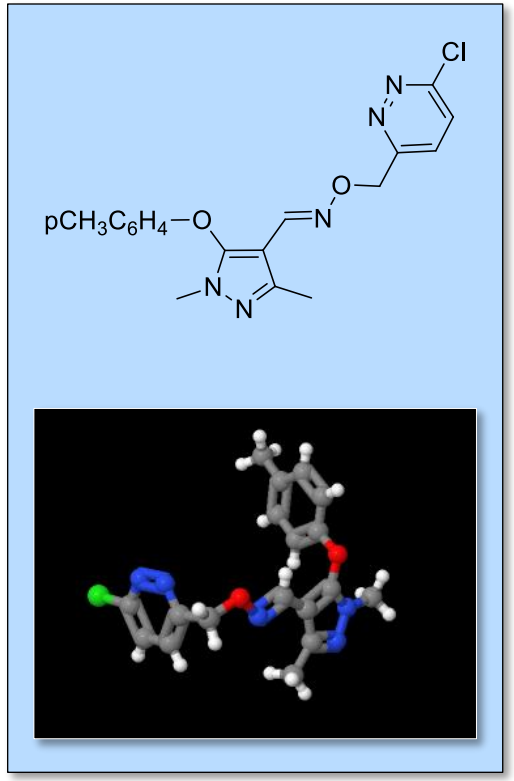
- Heteroaryl ether topology depends on non-bonded interactions
  - observed in single crystal X-ray structures
  - provides a measure of control over exit vectors
- Catalysts for Sharpless asymmetric dihydroxylation of olefins
- Phthalazine moiety an essential scaffold for projecting alkaloid element
  - creates enzyme-like binding pocket to orient olefin
- Conformation depends on N, O lone pair-lone pair repulsion
  - confirmed by X-ray crystallography of the pyridazine analogue

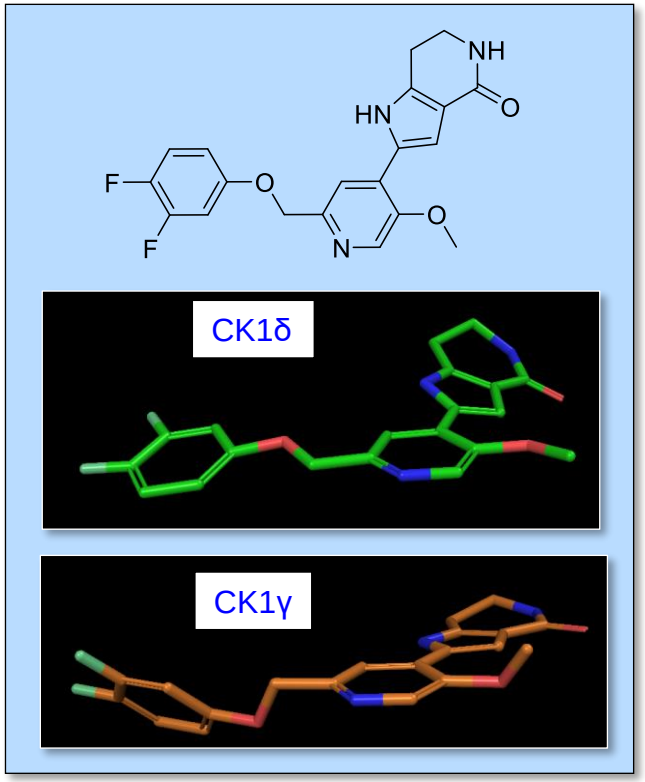
R.J Chien & E.J. Corey, *Org Lett*. 2010, **12**, 132-135; M. Stahl *et al*., *J. Chem. Inf. Model.,* 2008, **48**, 1-24
K.B. Sharpless *et al*., *Chem. Rev.,* 1994, **94**, 2483-2547; E.J. Corey *et al., Tet. Lett*., 1994, **35**, 2861-2864

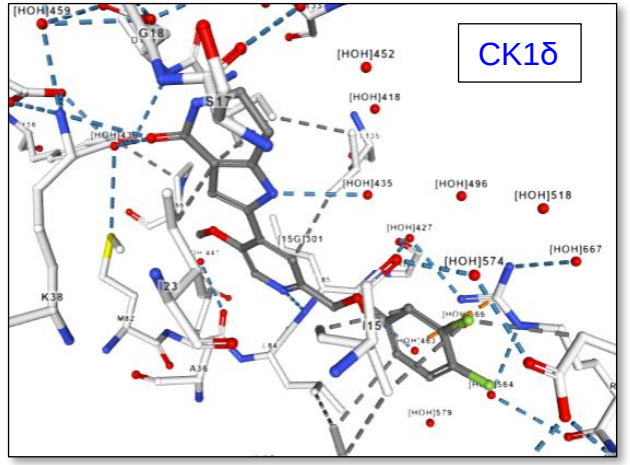
Conformation: Pyridazine-CH_2OH

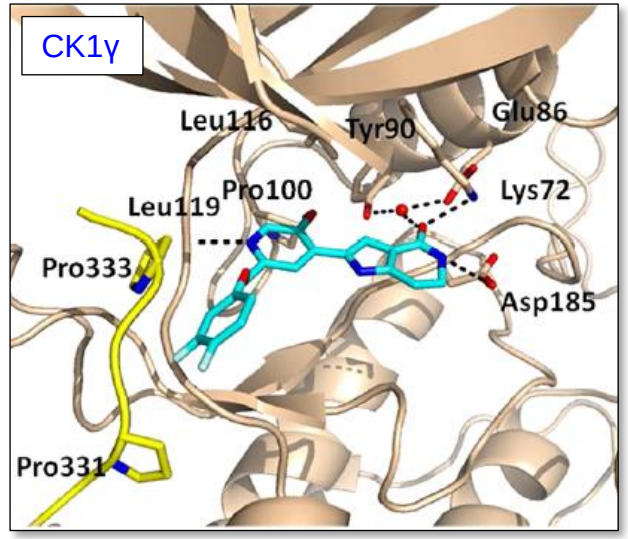








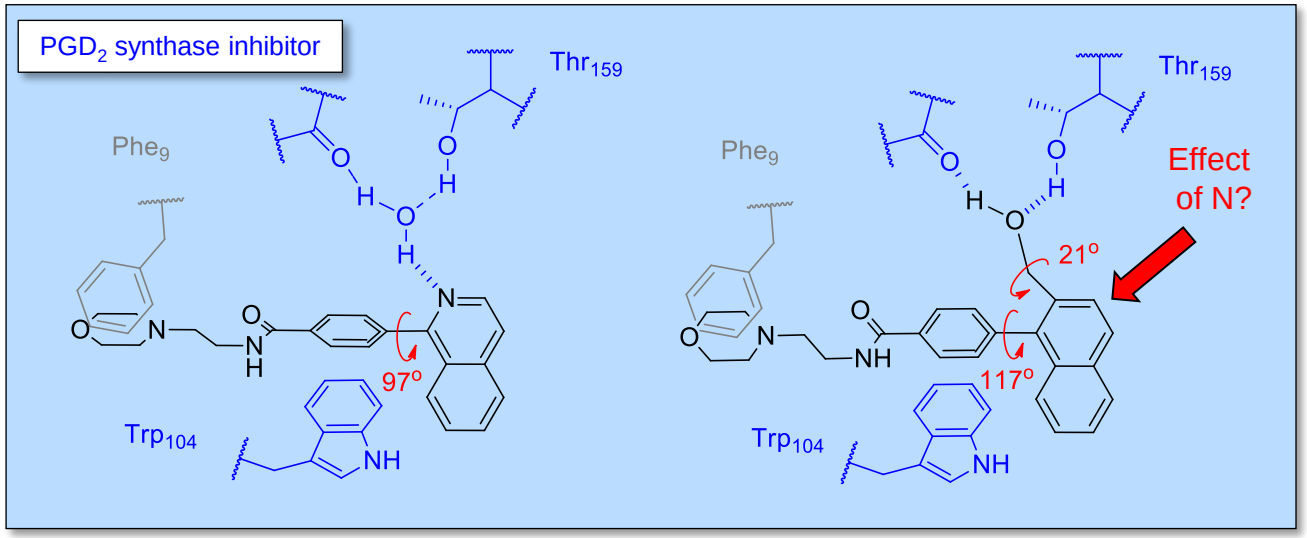


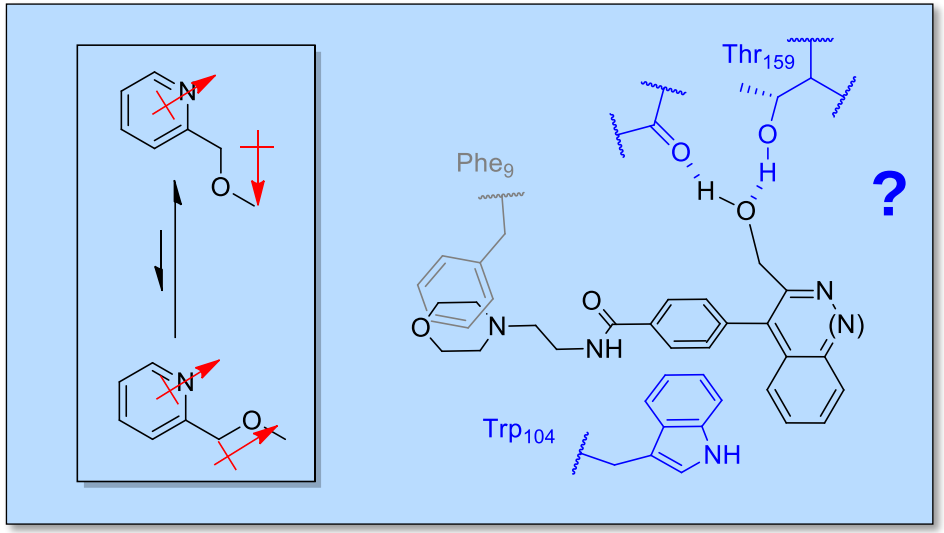


- Pyridazinyl-CH_2OH derivatives adopt a planar structure
  - observed in single crystal X-ray structure
- Common to heteroaryl-CH_2-OR derivatives
  - dipole-dipole interactions drive conformational preferences
- Flexible conformation
  - can readily access orthogonal conformation
- Exemplified by pyridine-CH_2-OR CK1 inhibitors
  - same inhibitor adopts different conformations in CK1δ & CK1γ

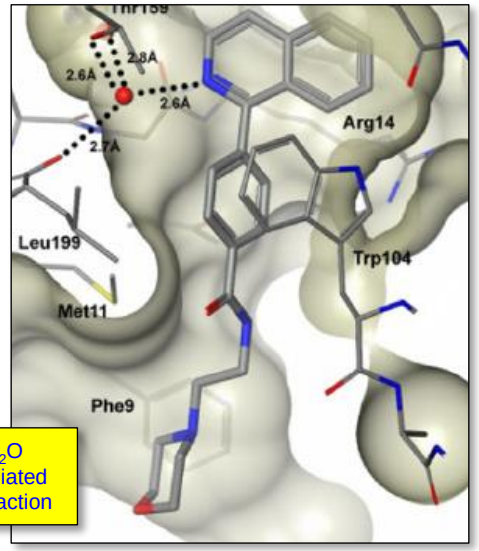
F. Abraham *et al., Acta Crystallogr.,Sect. C: Crystal Structure Commun.,* 1988, **44**, 1267-1269; F.Z. Hu *et al., Acta Crystallogr., Sect. E: Structure Reports Online,* 2006, **62**, o3676-o3677
M. Stahl *et al*., *J. Chem. Inf. Model.,* 2008, **48**, 1-24; *http://infochim.u-strasbg.fr/CS3/program/material/Stahl.pdf*; H. Huang *et al., ACS Med. Chem. Lett*., 2012, **3**, 1059-1064

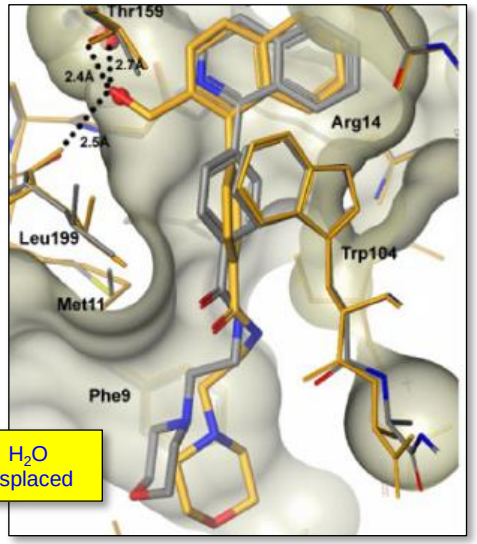
Displacing H_2O in PGD_2 Synthase: Azine-CH_2OH?





- Isoquinoline-based **hPGDS inhibitor**: IC_{50} = 2.34 nM
  - X-ray co-crystal structure isoquinoline interacting with a bound H_2O
- Attempted to displace bound H_2O by incorporating into inhibitor
  - naphthyl-CH_2OH: IC_{50} = 1480 nM; naphthyl-CH_2NH_2: IC_{50} = 845 nM
- X-ray co-crystal showed successful H_2O displacement **but** altered inhibitor geometry
  - naphthyl-CH_2OH dihedral Φ 21° & 27° vs preferred 90°
  - angle between naphthyl and phenyl = 117° rather than the low energy 97°
- Energy required for structural distortion offsets entropic advantage
- 2-$ROCH_2$-pyridines & pyridazines prefer a coplanar conformation
  - can readily access orthogonal conformation: flexible motif
- Topology influenced by:
  - dipole-dipole & lone pair-lone pair interactions
  - reduced allylic 1,3-strain compared to phenyl

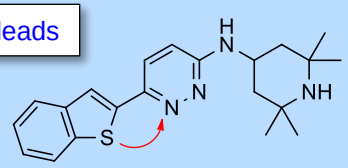




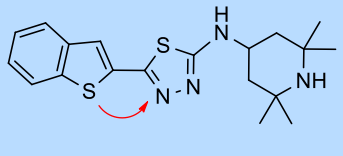
J.I. Trujillo *et al., Bioorg Med. Chem. Lett.*, 2012, **22**, 3795-3799; M. Stahl *et al.*, *J. Chem. Inf. Model.,* 2008, **48**, 1-24
M. Stahl, *http://infochim.u-strasbg.fr/CS3/program/material/Stahl.pdf*

Pyridazine, Thiadiazole & Intramolecular Interactions

leads



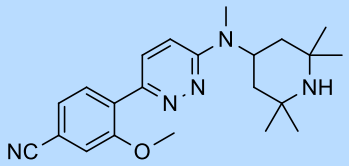
EC_{50} = 0.6 μM
SMN protein: 2.5x increase



EC_{50} = 0.02-0.1 μM

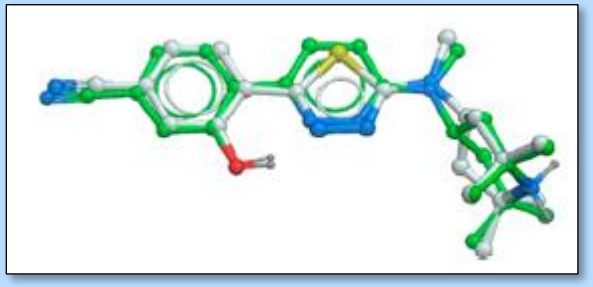
S ≡ OH

EC_{50} = 0.031 μM
SMN protein: 3.1x increase

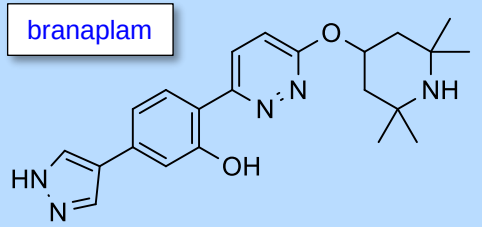


EC_{50} = 1.69 μM
SMN protein: 2.3x increase

Planarity important – intramolecular H-bond
Confirmed by single crystal X-ray structures



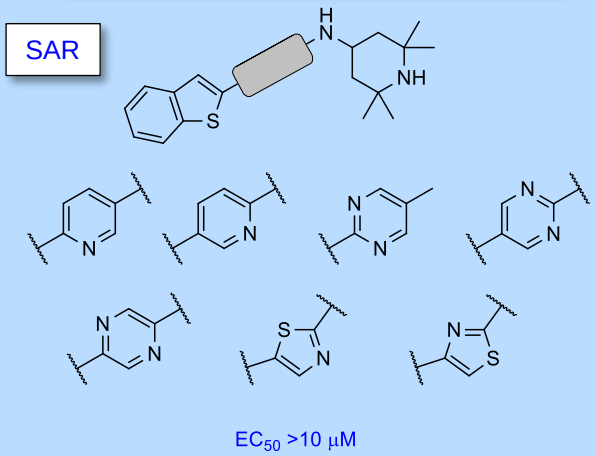
branaplam



EC_{50} = 0.02 μM
SMN protein: 3.6x increase

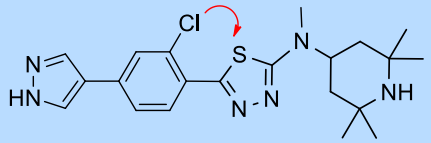
Pyridazine & thiadiazole uniquely active

SAR

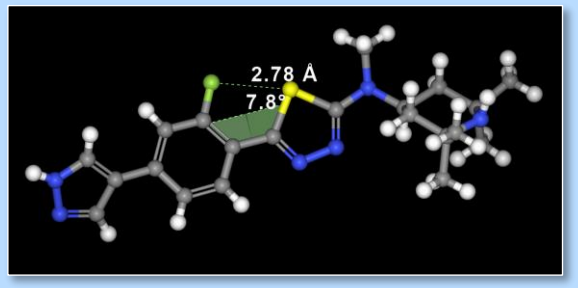


EC_{50} >10 μM

X-ray shows Cl & S proximity

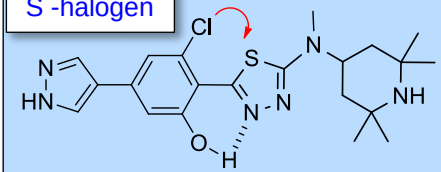


EC_{50} = 0.034 μM
SMN protein: 2.5x increase

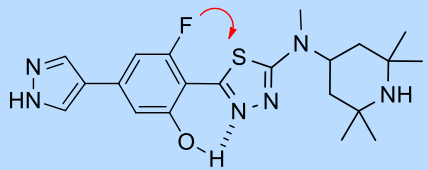


vdW radius S = 1.8 Å; Cl = 1.75 Å Σ = 3.55 Å

S -halogen



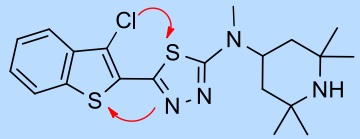
EC_{50} = 0.003 μM
SMN protein: 2.5x increase



EC_{50} = 0.006 μM
SMN protein: 2.6x increase

Intramolecular H-bond; halogen to S interaction

EC_{50} = 4.47 μM
SMN protein: 1.7x increase



EC_{50} = 0.53 μM
SMN protein: 2.8x increase

N-S favors planarity; augmented by Cl to S interaction

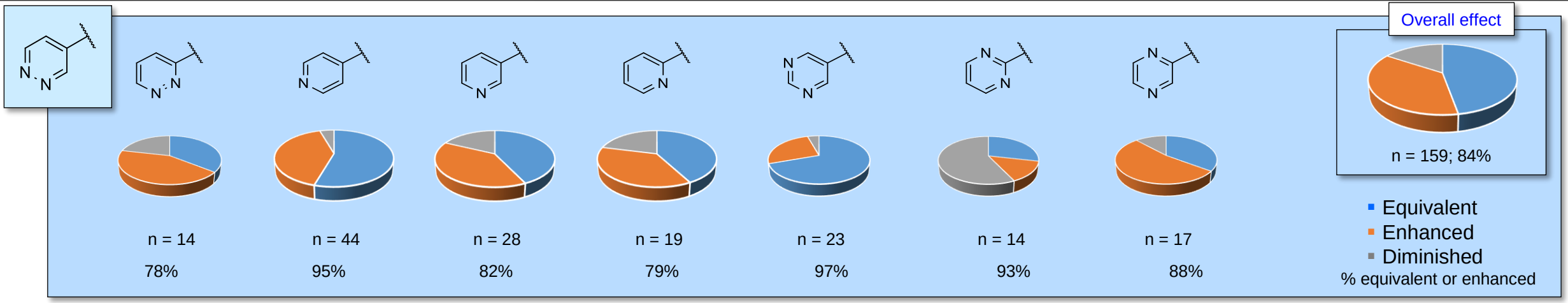
◆ SMN2 splicing modulators

Stabilize transient DS RNA structure formed by the *SMN2* pre-mRNA & U1 small nuclear ribonucleic protein (snRNP) complex

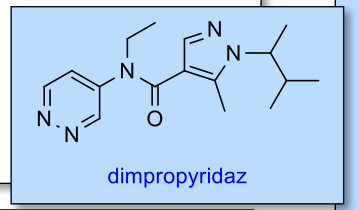
A.T. Cheung *et al., J. Med. Chem.*, 2018, **61**, 11021-11036; J. Axford *et al., J. Med. Chem.*, 2021, **64**, 4744-4761; J. Palacino *et al., Nature Chem. Biol.*, 2015, **11**, 511-517

Pyridazines & Potency

Pyridazines & Potency

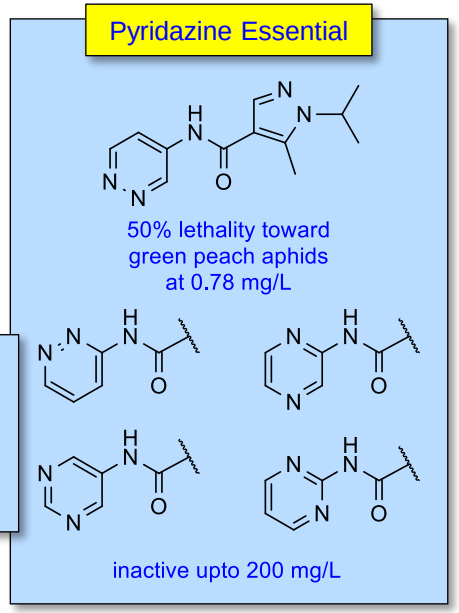


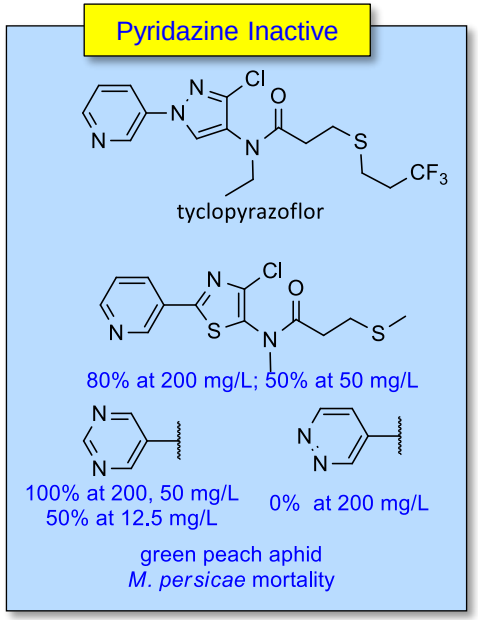
- Study of analogues of the insecticide dimpropyridaz
  - unknown mechanism of action
- Evaluated replacements of the 4-pyridazine ring
  - all analogues studied were **inactive**
- Evaluated effect of azine replacements for 4-pyridazine ring in the SwissBioisostere database
  - overall 84% of the time the molecular edit will be equivalent or beneficial
  - conversely, switching to a 4-pyridazine will be equivalent or deleterious 52% of the time
- Did not translate to dimpropyridaz
  - mammalian vs. insect targets?
  - a unique circumstance
- Analogues of tyclopyrazoflor
  - pyrimidine enhances potency; pyridazine impotent



- Emphasizes unique nature of pyridazine

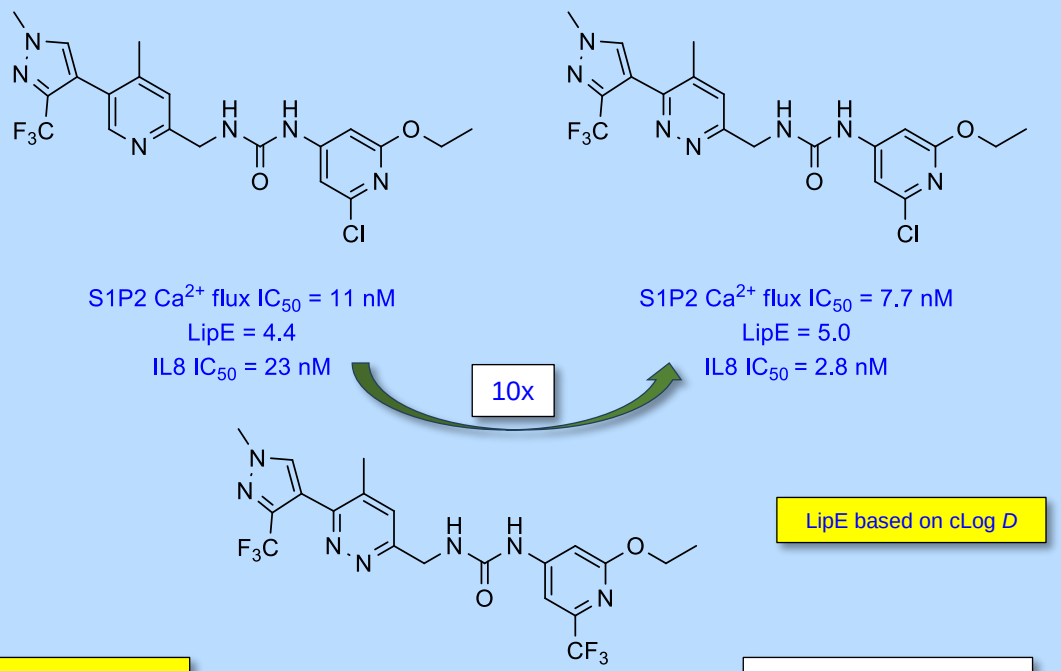
- but … properties that can be effective in one context can be deleterious in another setting

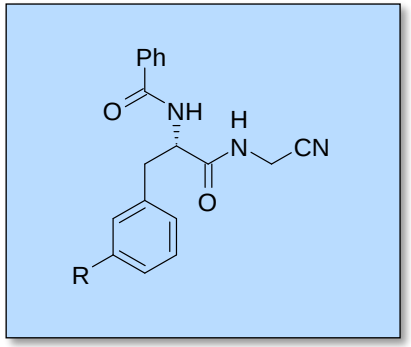


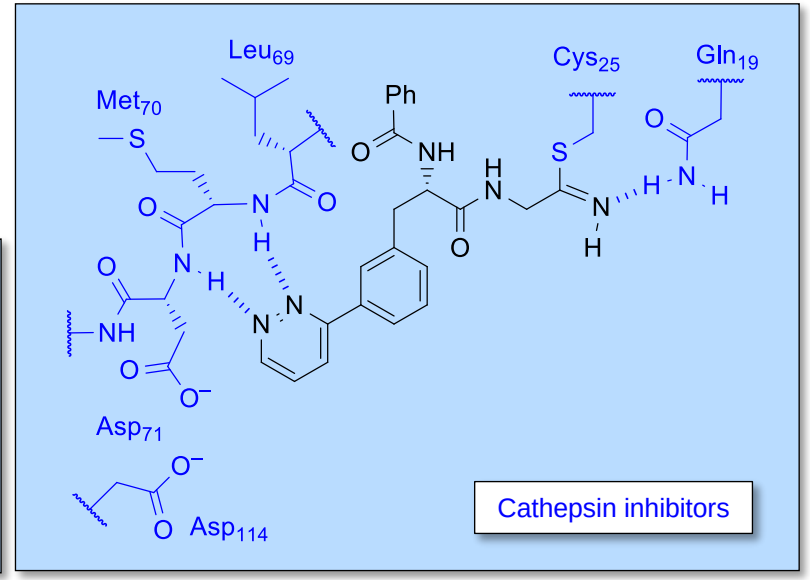


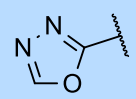
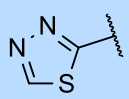
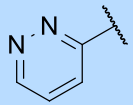
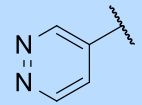
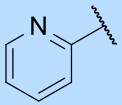
M. Chen, P. Maienfisch, *et al., J. Agric. Food. Chem.,* 2022, **70**, 11109-11122; 2022, **70**, 11123-11137

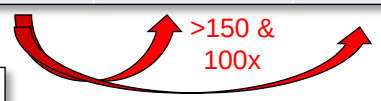
Pyridazines that Increase Potency







IC_{50} (nM)					
Cat L2	160	25	65	>10,000	>10,000
Cat S	20	2.5	8	790	625



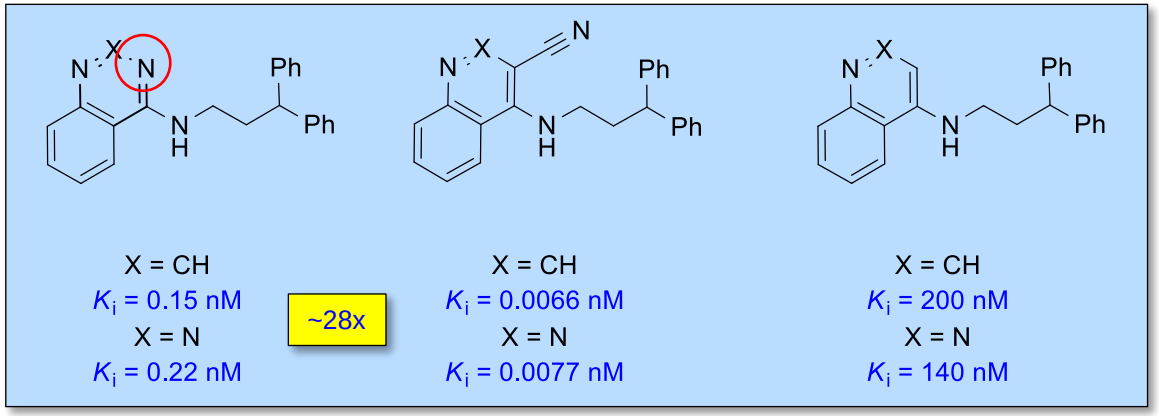
- LipE-guided optimization
  - pyridazine 10x more potent than pyridine in cell-based assay
  - reduced CYP inhibition
- Good PK, active in a bleomycin-induced model of pulmonary fibrosis

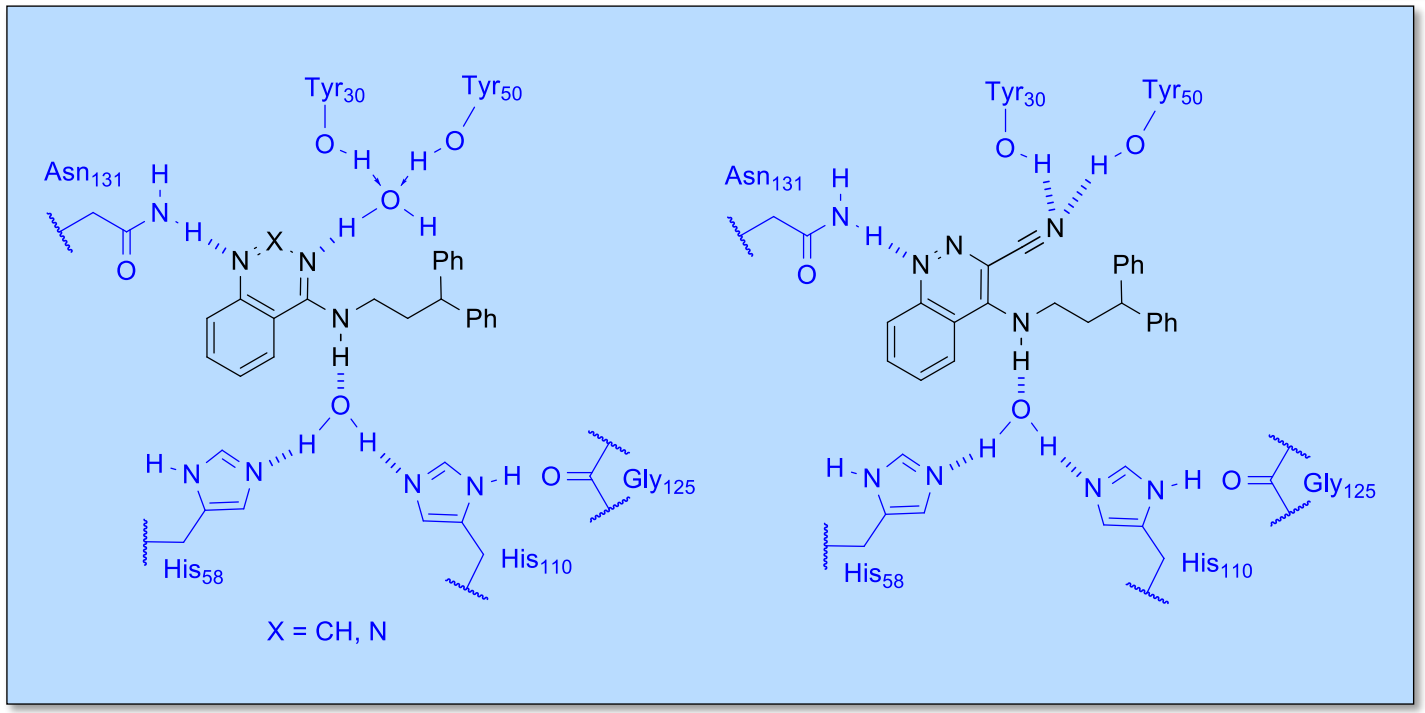
- Pyridazine potency a function of H-bonding
  - mimicked by oxadiazole & thiadiazole
    - excellent bioisosteres
  - isomeric pyridazine & pyridine poor

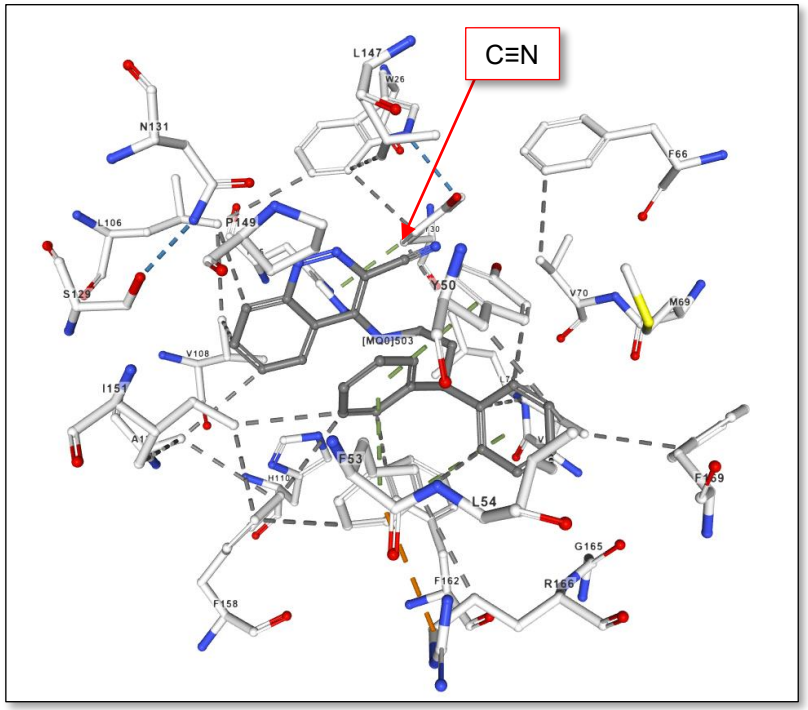
O. Mammoliti *et al., J. Med. Chem.,* 2021, **64**, 6037-6058; P.A. Bethel *et al., Bioorg. Med. Chem. Lett.*, 2009, **19**, 4622-4625

Cinnarizine & H_2O Displacement

- Scytalone dehydratase
  - enzyme in the plant fungal pathogen *Magnaporthe grisea*
  - catalyzes 2 steps in the melanin biosynthesis pathway
- Potent inhibitors identified:
  - quinazoline: K_i = 0.15 nM; - benzotriazine: K_i = 0.22 nM
- Modeling in active site recognized potential to replace H_2O to Tyr_{30} & Tyr_{50}
- Nitriles prepared and evaluated – exhibited ~28x improved potency:
  - cinnoline: K_i = 0.0077 nM; - quinoline: K_i = 0.0066 nM



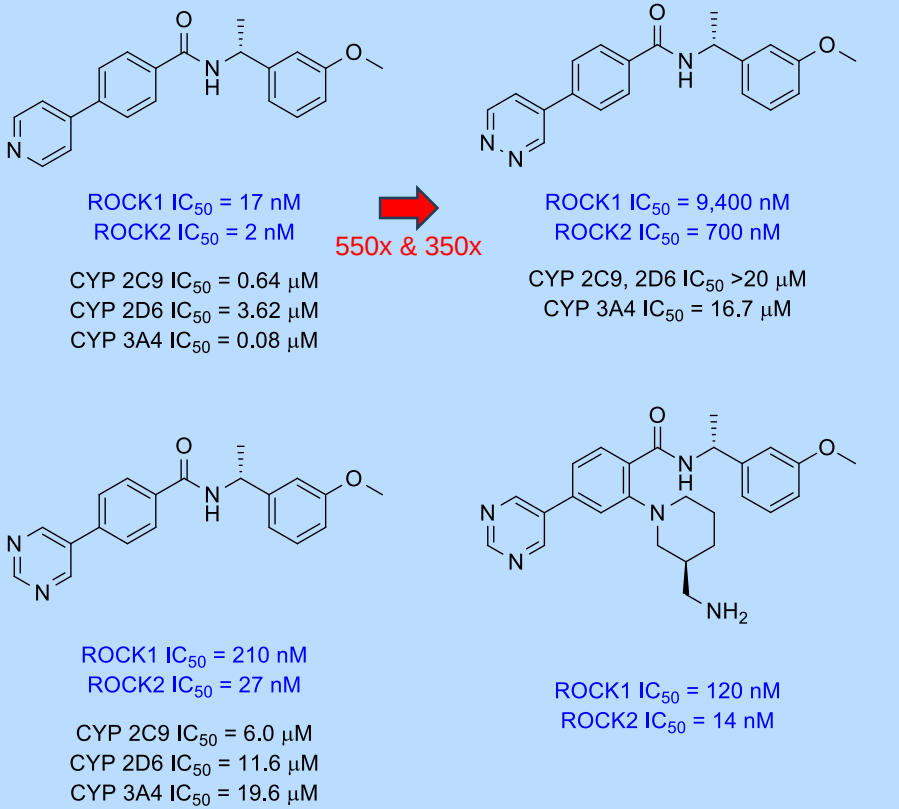




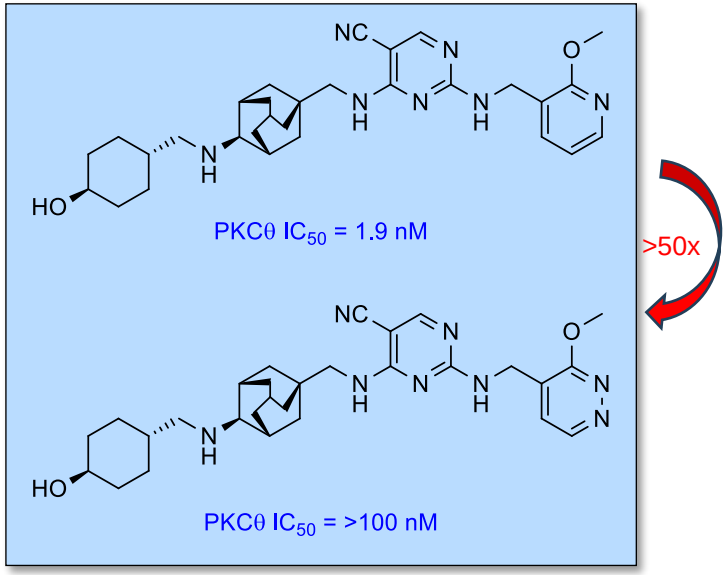
J.M. Chen *et al., Biochemistry* 1998, **37**, 17735-17744

NuArq MedChem Consulting LLC

Pyridazines Associated with Reduced Biological Activity

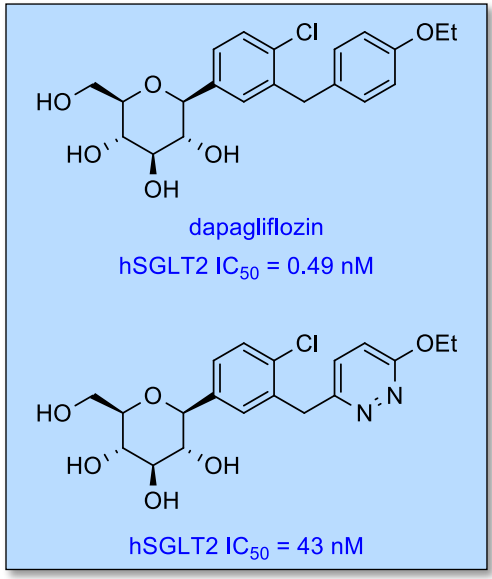


- Pyridine a potent ROCK1/ROCK2 dual inhibitor
  - also a potent CYP inhibitor
- Pyridazine poorly active: 350-550x ↓
  - reduced CYP inhibition
- Pyrimidine the compromise



>50x

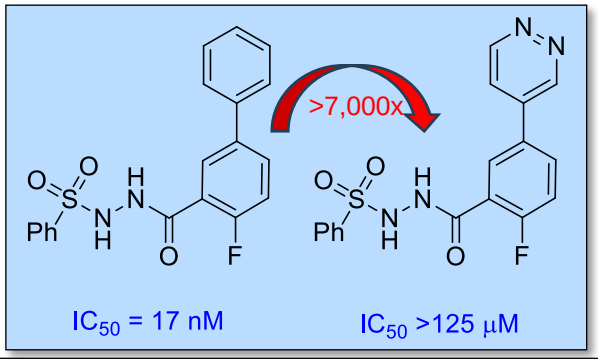
- PKCϴ inhibitors
  - MeO-pyridine a potent inhibitor
  - enhanced solubility of OCF_3
- Pyridazine >50x weaker
  - no explanation



100x

- hSGLT2 inhibitors
  - hypoglycemic agents
  - dapagliflozin marketed
- Pyridazine 100x weaker

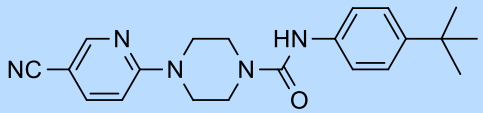
- KAT6A histone acetylase inhibitor
  - antitumor agent
  - mimics diphosphate of AcCoA
- Pyridazine >7,000x weaker
  - 2-pyridyl potent



A.D. Hobson *et al., J. Med. Chem.*, 2018, **61**, 11074-11100; S. Kunikawa *et al., Bioorg. Med. Chem.*, 2019, **27**, 790-799; M.J. Kim *et al., Bioorg. Med. Chem. Lett.*, 2010, **20**, 3420-3425
J.B. Baell *et al., J. Med. Chem.*, 2020, **63**, 4655-4684; *Nature*, 2018, **560**, 253-257

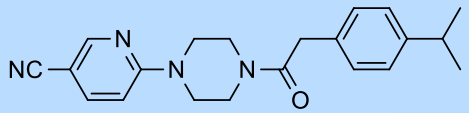
Pyridazines & Intermolecular H-Bonding in Drug-Target Interactions

Pyridazine & Intermolecular H-Bonds



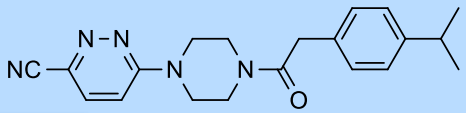
PanK3 IC_{50} = 510 nM

urea → amide: 9x

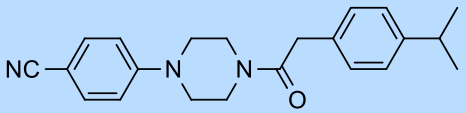


PanK3 IC_{50} = 60 nM

pyridine → pyridazine: 60x

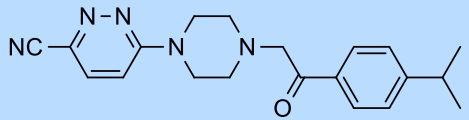


PanK3 IC_{50} = 1 nM



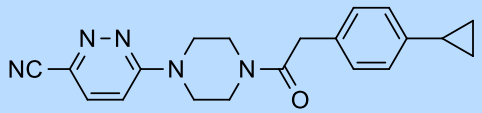
PanK3 IC_{50} = 580 nM

pyridazine → phenyl: 580x↓



PanK3 IC_{50} >100 μM

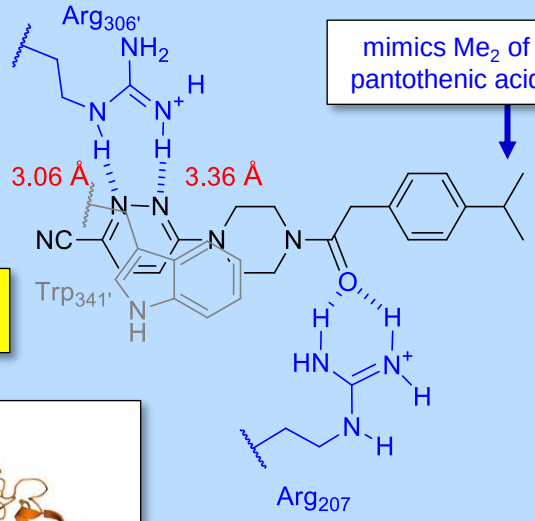
amide topology: 5x↓



PanK3 IC_{50} = 5.3 nM

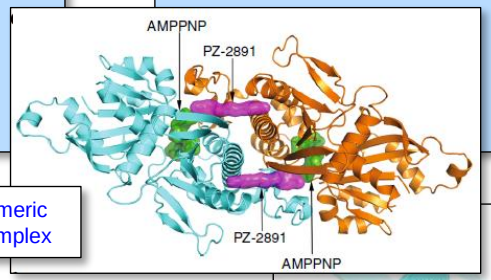
iPr → cPr: 5x↓

$Arg_{306'}$ in other protomer - not engaged by substrate

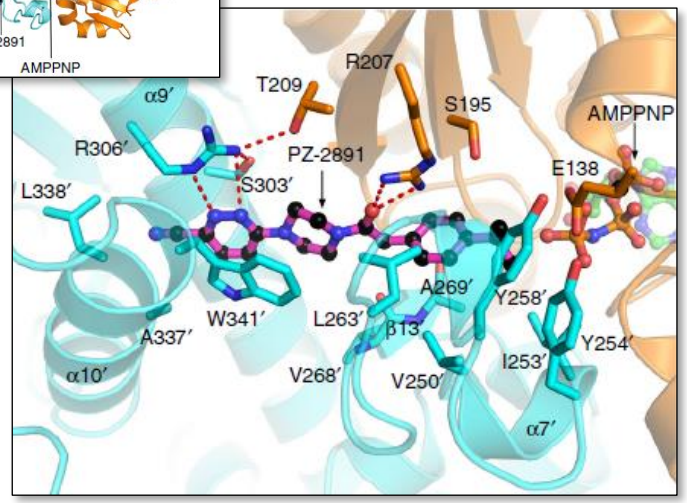


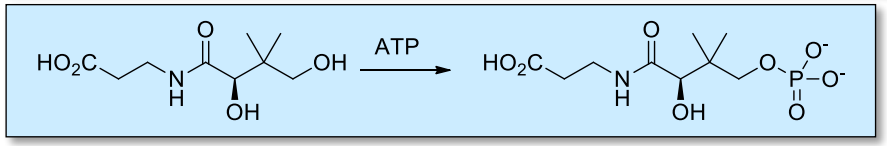
mimics Me_2 of pantothenic acid

Pyridazine a CO_2^- bioisostere?



Dimeric complex





ATP

Concept: activate PANK3 to substitute for PANK2

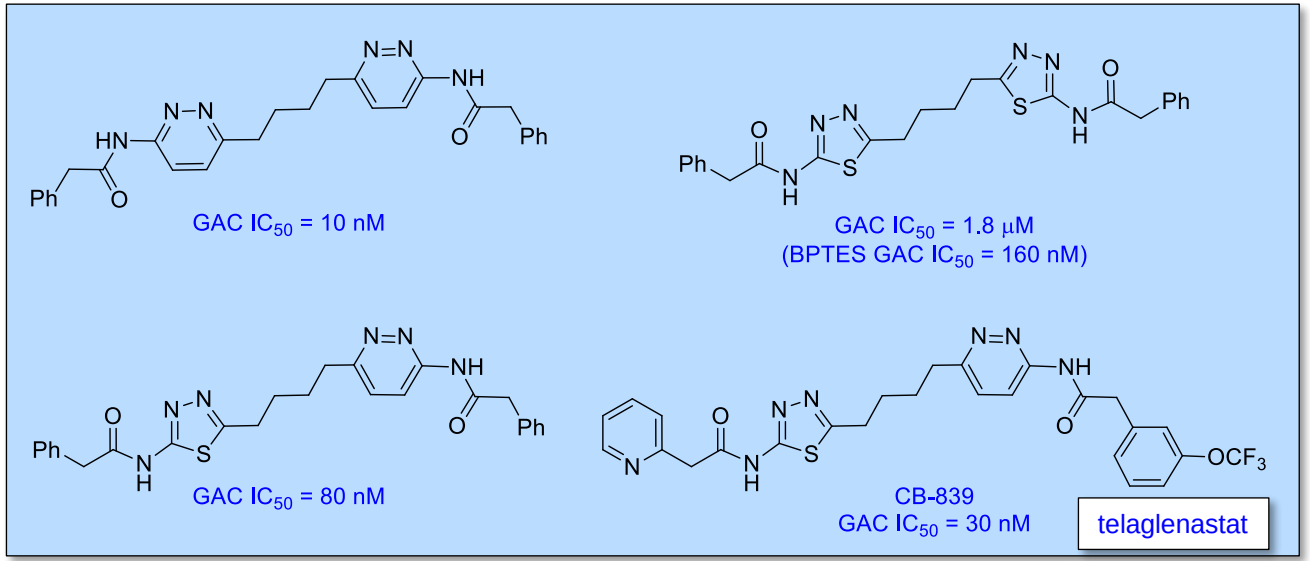
- Pantothenate kinase catalyzes 1st & RDS in Coenzyme (CoA) a synthesis
  - regulates levels of an important cellular cofactor
- 4 Isoforms in humans: mutations in neuronal PanK2 reduce levels of CoA
  - rare disease that leads to neurological movement disorder
    - Hallervorden-Spatz syndrome
- Enzyme is a dimer & subject to feedback inhibition
  - HTS identified allosteric modulators of PANK3
- Bind to one active site across dimer interface
  - locks other active site in the catalytically active conformation
  - resistant to feedback inhibition

Complex mechanism: orthosteric inhibitor allosteric activator

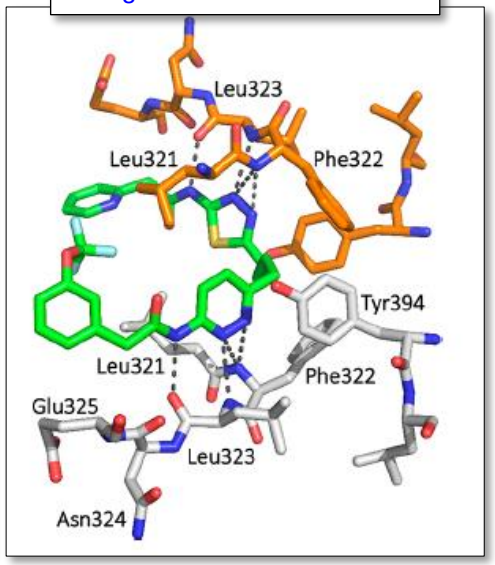
- CNS penetration of inhibitors important
  - cLog *P* & LLE (LipE) monitored
- C≡N not essential; Cl similarly active
- X-ray cocrystal structure
  - binds to enzyme + Mg^{2+} ATP
    - induced fit mechanism
    - amide C=O engages Arg_{207}
- Pyridazine N atoms engage $Arg_{306'}$
  - suggests pyridazine as CO_2^- isostere
  - would extend to diazoles

S. Jackowski *et al.*, *Nature Commun.*, 2028, **9**, 4399; R.E. Lee *et al., Bioorg. Med. Chem.,* 2021, **52**, 116504; C. Subramanian *et al., Sci. Transl. Med.*, 2021, **13**, eabf5965

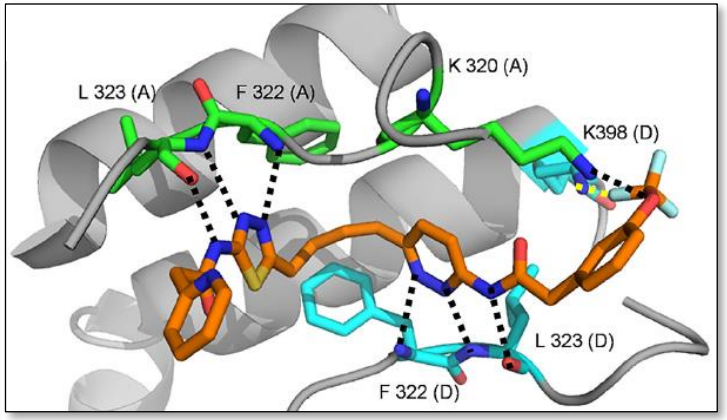
Pyridazines & Isosterism in Glutaminase Inhibitors



Telaglenastat bound to hKGA



Telaglenastat bound primarily to the A and D chains of GAC in crystal structure 5HL1



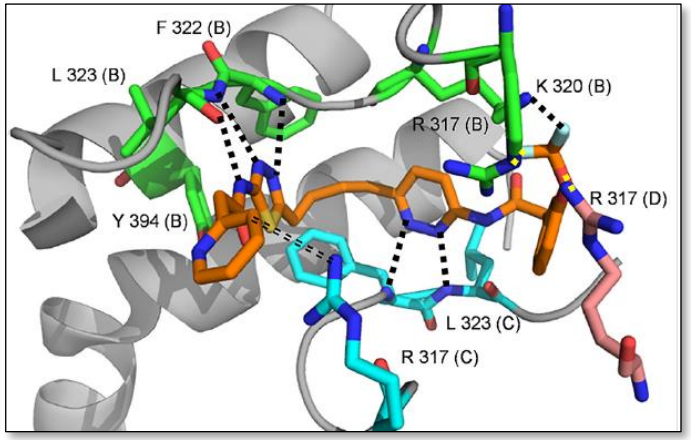
- Glutaminase inhibitors
  - *GLS1*: kidney-type glutaminase (KGA) & glutaminase C (GAC)
  - have identical catalytic domains
  - *GLS-2*: liver-type glutaminase (LGA)
- Block conversion of glutamine to glutamate
  - c-Myc, Raf, Ras, Rho-GTPase oncogenes cause upregulation
- CB-839 (telaglenastat) in clinical trials
  - cervical cancer, myelodysplastic syndromes
- X-ray cocrystal data identified drug-target interactions
  - allosteric inhibitors
  - tetrameric enzyme binds 2 molecules across dimer interface
- 8 & 9 High strength H-bond interactions: reflected in potency
  - all pyridazine & thiadiazole *N* atoms engage the protein

Inventory of H-bond interactions with heterocycles

CB-839-L1	thiadiazole S	Tyr 394 (A)	>= 2.6	n.a.
CB-839-L1	thiadiazole N1	Phe 322 (A)	2.1	14.6
CB-839-L1	thiadiazole N2	Leu 323 (A)	2.1	6.0
CB-839-L1	thiadiazole amide NH	Leu 323 (A)	1.6	5.9
CB-839-L1	Pyridazine N1	Phe 322 (D)	2.1	17.9
CB-839-L1	Pyridazine N2	Leu 323 (D)	2.2	18.8
CB-839-L1	Pyridazine amide NH	Leu 323 (D)	2.1	20.4
CB-839-L1	OCF_3 "O"	Lys 320 (D)	>= 1.6	n.a.
CB-839-L1	OCF_3 "CF_3"	Lys 398 (A)	>= 2.3	n.a.
CB-839-L2	thiadiazole S	Tyr 394 (B)	>= 2.4	n.a.
CB-839-L2	thiadiazole N1	Phe 322 (B)	2.1	12.5
CB-839-L2	thiadiazole N2	Leu 323 (B)	2.2	15
CB-839-L2	thiadiazole amide NH	Leu 323 (B)	1.8	5.7
CB-839-L2	Pyridazine N1	Phe 322 (C)	2.2	20.5
CB-839-L2	Pyridazine N2	Leu 323 (C)	2.4	11.7
CB-839-L2	Pyridazine amide NH	Leu 323 (C)	2.7	56
CB-839-L2	OCF_3 "O"	Arg 317 (D)	2.1	38.3
CB-839-L2	OCF_3 "O"	Arg 317 (D)	>= 1.8	n.a.
CB-839-L2	OCF_3 "CF_3"	Arg 317 (D)	>= 1.8	n.a.
CB-839-L2	OCF_3 "CF_3"	Lys 320 (B)	>= 2.0	n.a.

Red fall just outside the Berndt criteria

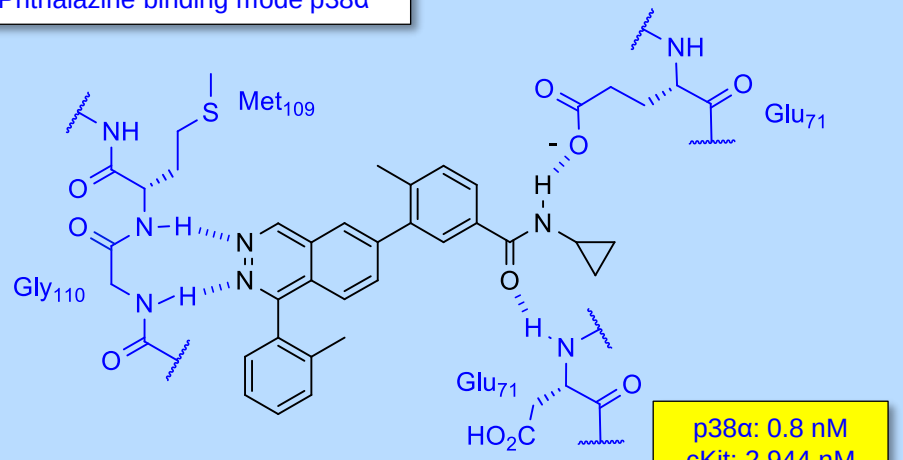
Telaglenastat bound primarily to the B and C chains of GAC in crystal structure 5HL1



J. Sivaraman *et al., Oncotarget*, 2016, **7**, 57943-57954; L.A. McDermott *et al., Bioorg. Med. Chem.,* 2016, **24**, 1819-1839
T. Tsukamoto *et al., J. Med. Chem*., 2018, **62**, 46-59; R. Cerione *et al., J. Biol. Chem.,* 2018, **293**, 3535-3545

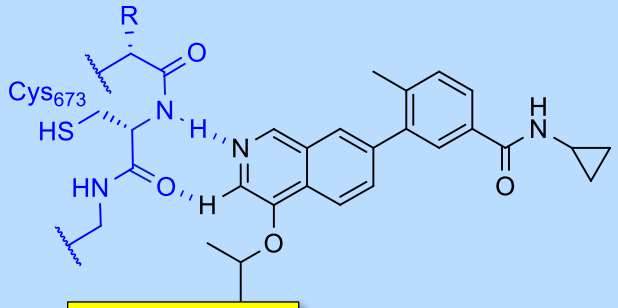
Heterocycles: H-Bonding & Selectivity

Phthalazine binding mode p38α



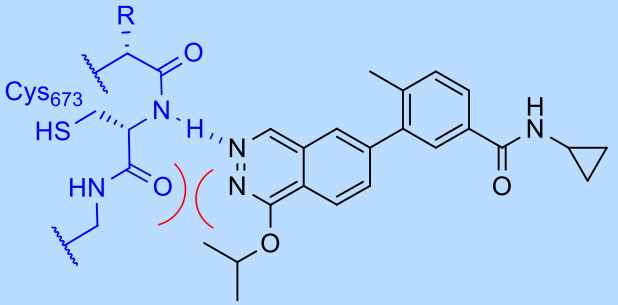
p38α: 0.8 nM
cKit: 2,944 nM

isoquinoline: proposed binding mode in p38α & cKit

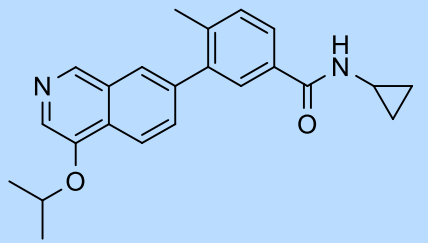


p38α: 0.6 nM
cKit: 41 nM

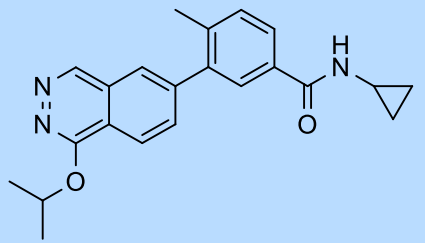
phthalazine binding mode in cKit



- Phthalazine-based p38α MAP kinase inhibitors
  - bind to ATP pocket, IC_{50} = 0.8 nM
  - high selectivity over Kdr, Lck, cKit, JNK1-3
- X-ray co-crystal with p38α
  - H-bonds from protein to both pyridazine *N* atoms
  - NH of Met_{109} and NH of Gly_{110}
- In p38α, Gly_{110} is flipped to project NH to inhibitor
  - hinge residue in cKit is substituted: Cys_{673}
  - higher energy required to flip conformation
  - accounts for high specificity



p38α IC_{50} = 0.6 nM
cKit IC_{50} = 41 nM



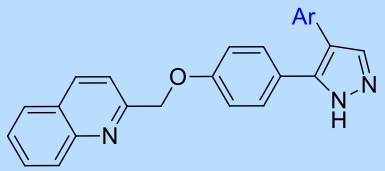
p38α IC_{50} = 0.6 nM
cKit IC_{50} = 421 nM

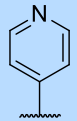
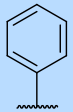
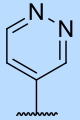
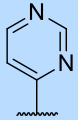
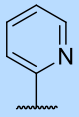
Effect of pyridazine/phthalazine on potency is manifested as selectivity

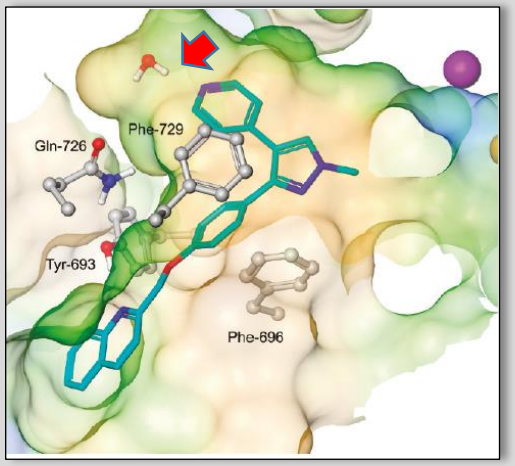
B. Herberich *et al., J. Med. Chem.*, 2008, **51**, 6271-6279; L.H. Pettus *et al., J. Med. Chem.*, 2008, **51**, 6280-6292; B. Wu *et al., J. Med. Chem.*, 2010, **53**, 6398-6411

H-Bonding in PDE 10A & PI3Kδ Inhibitors

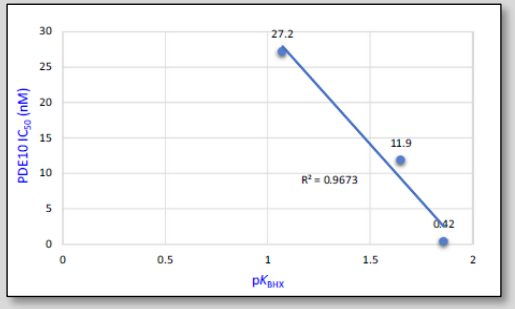
PDE 10A



Ar					
IC_{50} (nM)	0.42	258	11.9	27.2	371
calc. H-bond energy (kcal/mol)	-6.68		-6.08	-5.88	
pK_{BHX}	1.86		1.65	1.07	

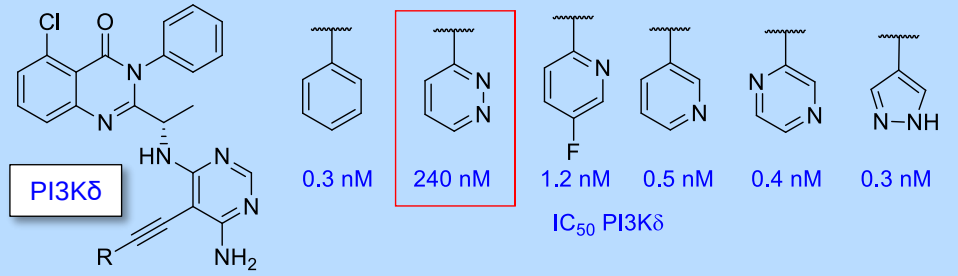


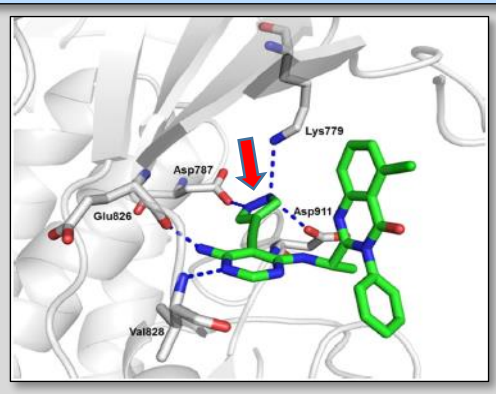
- Potent series of PDE10A inhibitors
  - schizophrenia
- Pyridine *N* involved in H-bond interaction
  - engages backbone C=O of Gln_{726} *via* a H_2O
- Pyridazine 25x less potent than pyridine
  - a function of H-bonding potential
- Calculated H-bonding potential predicts rank order
  - plot of pK_{BHX} vs IC_{50} shows excellent correlation
- Nice calibration of H-bonding effects of pyridazine
  - presumably would reduce CYP inhibition potential
  - useful compromise?



- Properties of pyridazine can present challenges
  - exercise care when deploying

PI3Kδ





- PI3Kδ inhibitors
  - pyridazine uniquely impotent
- Hypothesize introduction of unfavorable drug-target interactions
  - 2nd N atom proximal to Asp_{787} or Asp_{911}
- Pyrazole optimal
  - dual H-bond donor/acceptor interaction with Asp_{787} & Lys_{779}
  - pyridazin-3-one??

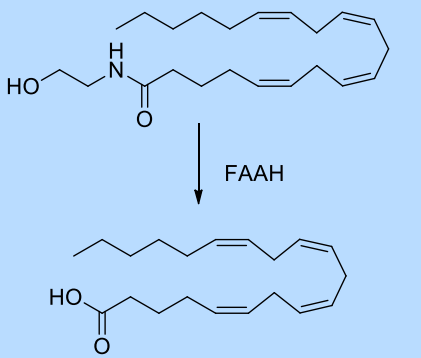
L. Patel *et al., J. Med. Chem.,* 2016, **59**, 3532-3548; P.R. Verhoest *et al., J. Med. Chem.,* 2009, **52**, 5188-5196

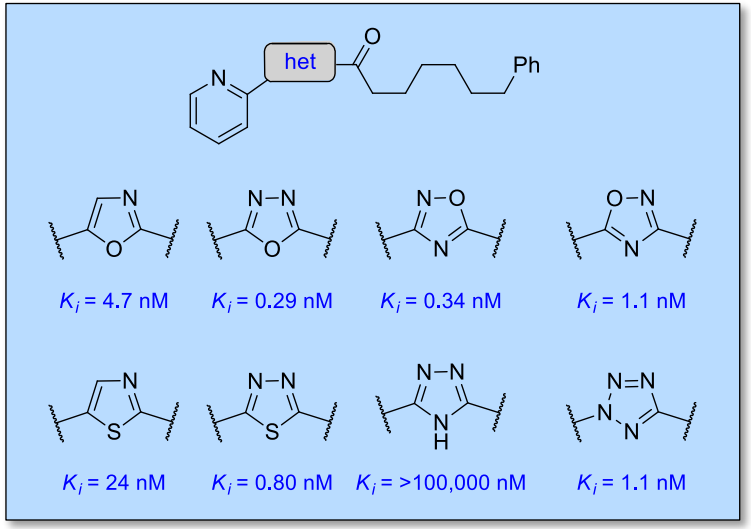
NuArq MedChem Consulting LLC

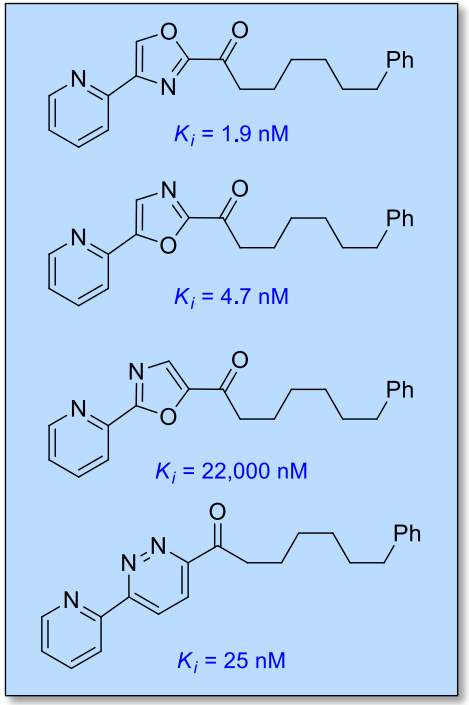
Pyridazines & Electrophilicity

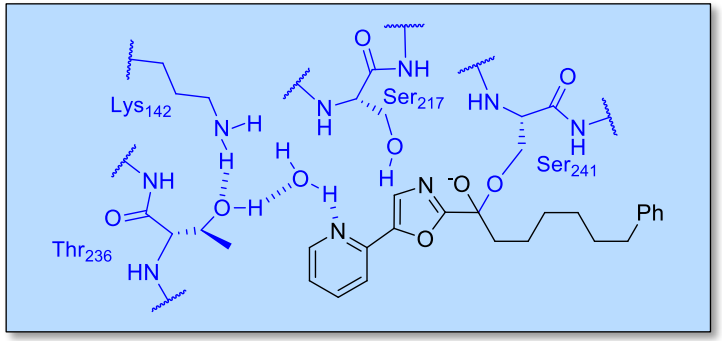
FAAH Inhibitors – Core Heterocycle

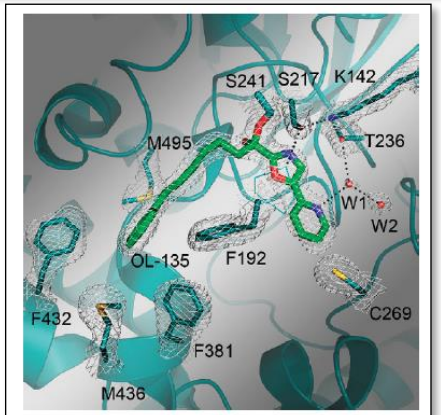
FAAH = fatty acid amide hydrolase





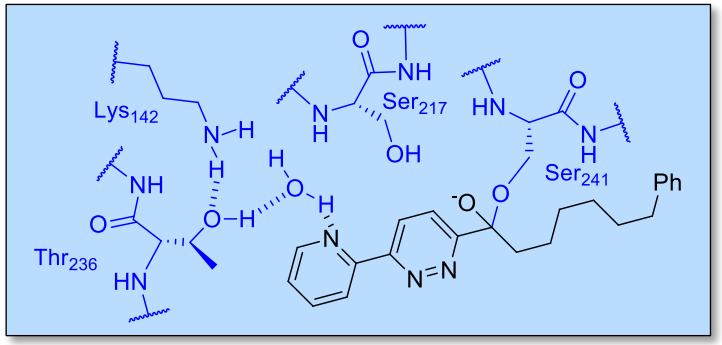






- Mechanism-based inhibitors of fatty acid amide hydrolase
  - a serine hydrolase that converts amides to arachidonic acid
  - uses a Ser-Ser-Lys catalytic triad (Ser_{241}-Ser_{217}-Lys_{142})
- Variation of core ring shows dependence on e-withdrawing properties
  - potency correlates with electron withdrawing effect
- Introduction of N atom at the 4-position of oxazole and thiazole increases potency
  - 4-15-fold in the oxazole to 1,3,4-oxadiazole & 1,2,4-oxadiazoles
  - 30-fold for the thiazole to 1,3,4-thiadiazole change
- Tetrazole & **pyridazine** rings afford good potency
- Triazole poor - attributed to presence of acidic NH
- Oxazole topology only important in the context of electron withdrawing effects
  - C-2 electron withdrawing; C-5 much less so
  - no H-bond with Ser_{217}, as anticipated

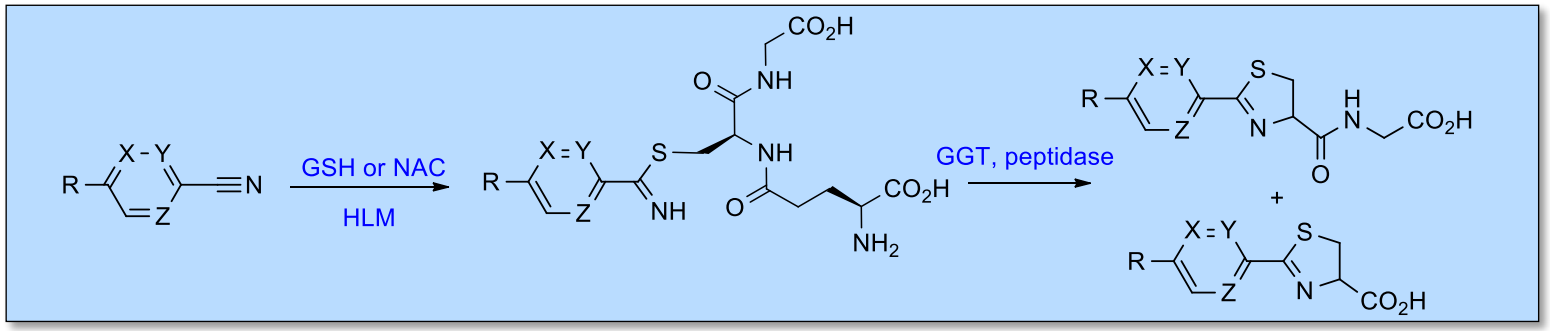
- Oxadiazole & thiadiazole superior to pyridazine
  - exit vectors?

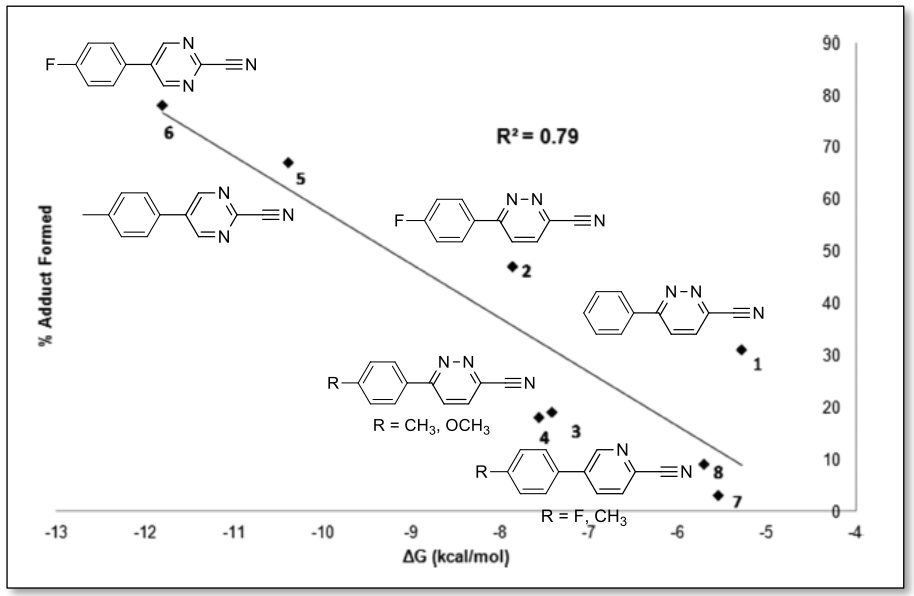


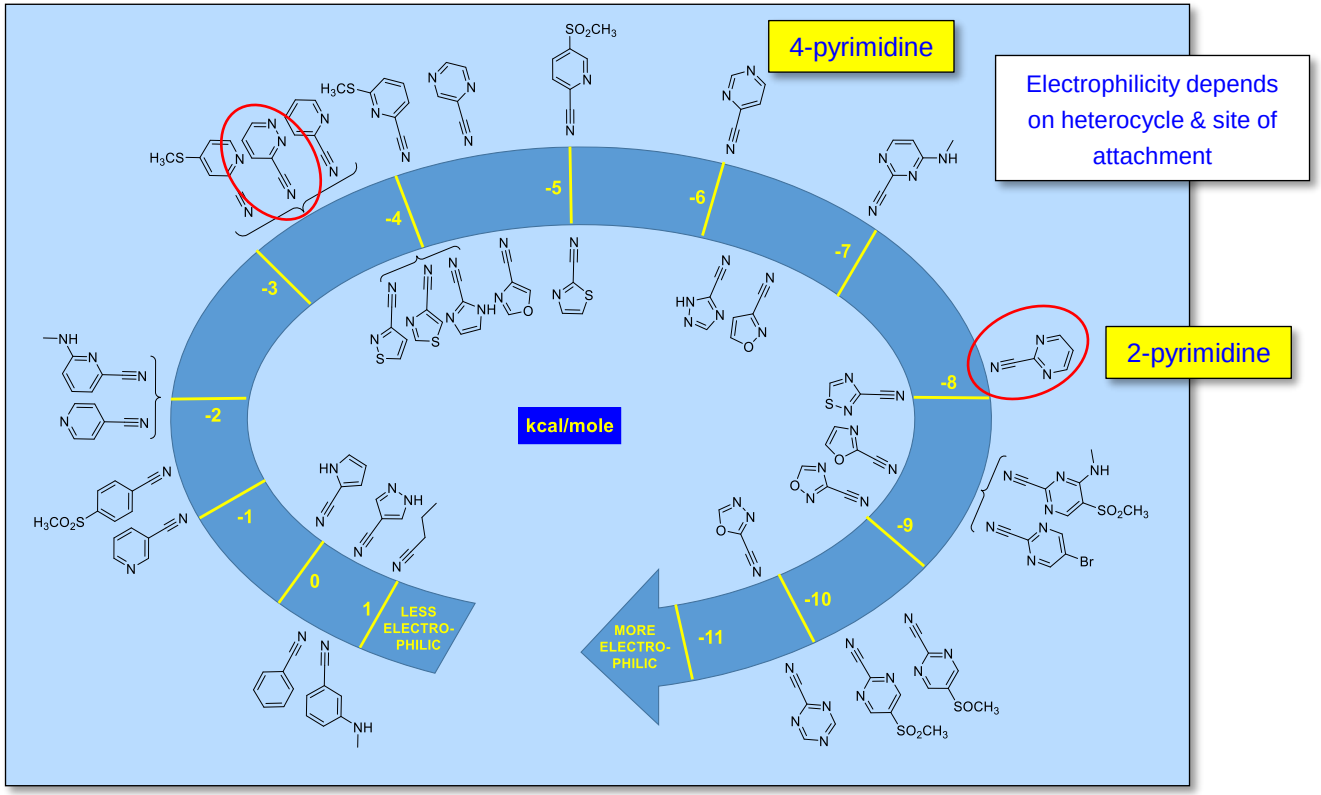
D.L. Boger *et al., J. Med. Chem.*, 2005, **48**, 1849-1856; *J. Am. Chem. Soc*, 2009, **131**, 10497-10506

Reactivity of Pyridazine-3-Nitriles

- Pyridazine nitriles react with thiols
  - catalyzed by GSH in HLM
- Reactivity correlates with EWG effects
  - 3-pyridazine less reactive than 2-pyrimidine
    - 2-pyrimidine >4-pyrimidine
  - more reactive than 2-pyridine





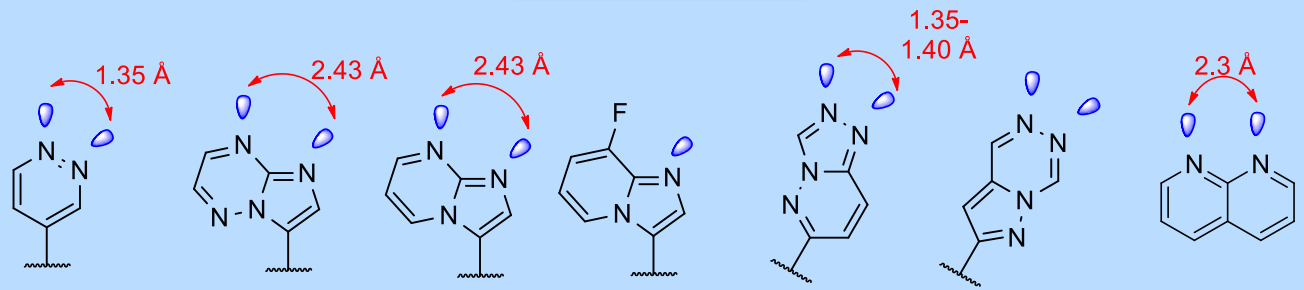


B.M. Johnson *et al., Chem. Res. Toxicol.*, 2014, **27**, 2052-2061; R.M. Oballa *et al., Bioorg. Med. Chem. Lett.*, 2007, **17**, 998-1002

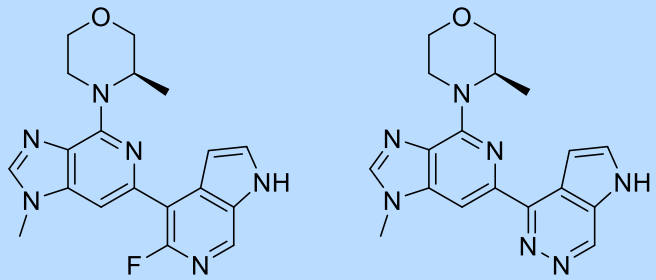
Bioisosteres of Pyridazines

Bioisosteres of Pyridazines

Lone Pair Mimicry



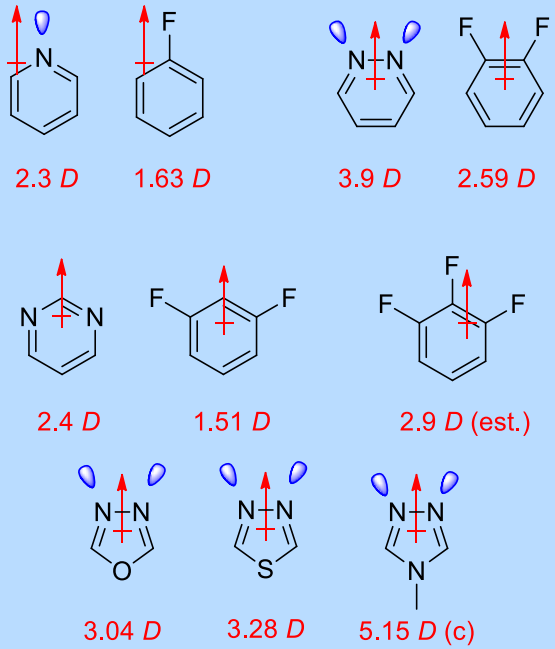
ATR Kinase Inhibitors



IC_{50} = 0.1 nM

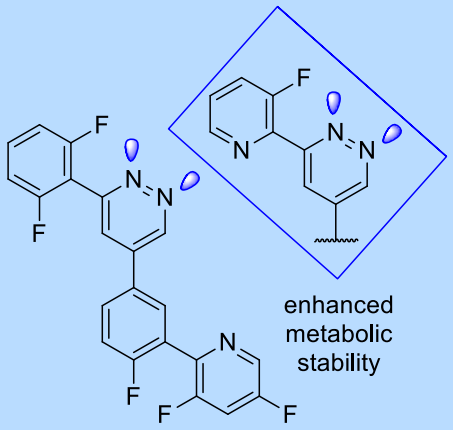
IC_{50} = 0.5 nM

Dipole Mimicry

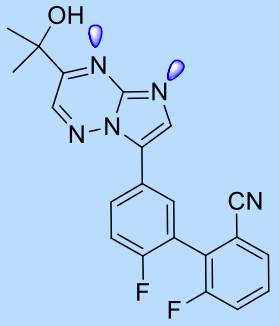


N.A. Meanwell, *Med. Chem. Res.*, 2023, **32**, 1853-1921; P.A. Barsanti *et al., ACS Med. Chem. Lett.*, 2015, **6**, 42-46

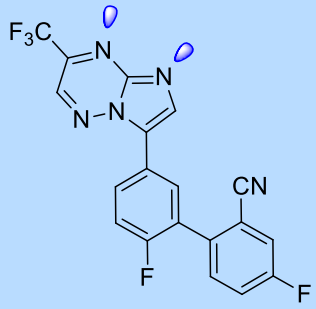
Pyridazine Bioisosterism in $GABA_A$



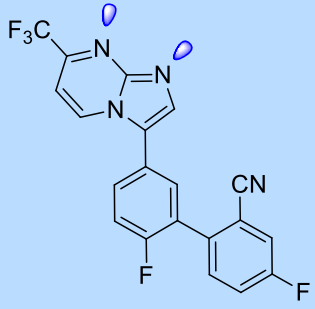
K_i α1 = 0.1 nM
(antagonist)
K_i α3 = 0.4 nM
(partial agonist: 24%)



K_i α1 = 2.7 nM
(partial agonist: 45%)
K_i α3 = 1.3 nM
(partial agonist: 45%)

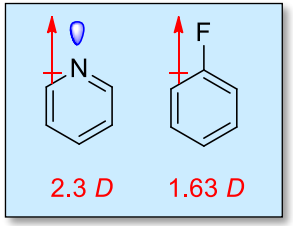


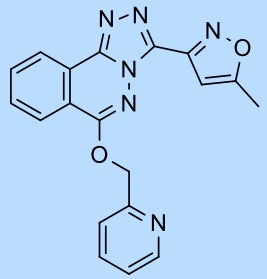
K_i α1 = 0.25 nM
(antagonist)
K_i α3 = 0.8 nM
(antagonist)



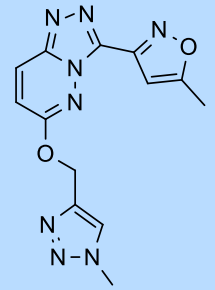
K_i α1 = 1.2 nM
(antagonist)
K_i α3 = 0.73 nM
(partial agonist 25%)

- $GABA_A$ ligands
  - α1 antagonist combined with varying degrees of α2 and α3 agonism
- Pyridazine identified by screening
  - isosterism with imidazotriazine & imidazopyrimidine series recognized
- Replaced diF phenyl in pyridazine with F-pyridine to reduce metabolic turnover
  - C-F isosterism with N
- Evolution to other fused ring systems mimicking pyridazines
  - embedded triazoles & fused pyridazines



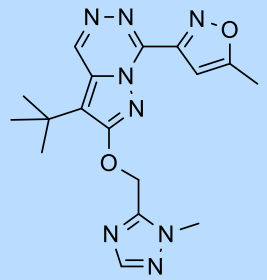


$GABA_A$ α1 K_i = 1.4 nM; eff. -0.02
$GABA_A$ α2 K_i = 2.7 nM; eff. 0.25
$GABA_A$ α3 K_i = 1.4 nM; eff. -0.04
$GABA_A$ α5 K_i = 0.80 nM; eff. -0.46



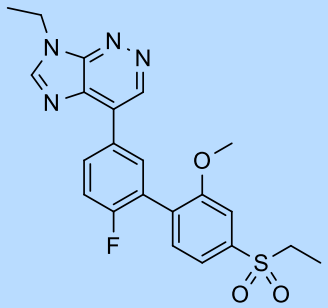
α51A

$GABA_A$ α1 K_i = 0.88 nM; eff. -0.25
$GABA_A$ α2 K_i = 0.58 nM; eff. 0.10
$GABA_A$ α3 K_i = 0.61 nM; eff. -0.11
$GABA_A$ α5 K_i = 0.66 nM; eff. -0.70



MRK-016

$GABA_A$ α1 K_i = 0.83 nM; eff. -0.16
$GABA_A$ α2 K_i = 0.85 nM; eff. 0.06
$GABA_A$ α3 K_i = 0.77 nM; eff. -0.09
$GABA_A$ α5 K_i = 1.4 nM; eff. -0.55



PF-06372865, CVL-865

$GABA_A$ α1 K_i = 0.18 nM; eff. 0.11
$GABA_A$ α2 K_i = 2.92 nM; eff. 0.35
$GABA_A$ α3 K_i = 1.06 nM; eff. 0.49
$GABA_A$ α5 K_i = 18.0 nM; eff. 0.70

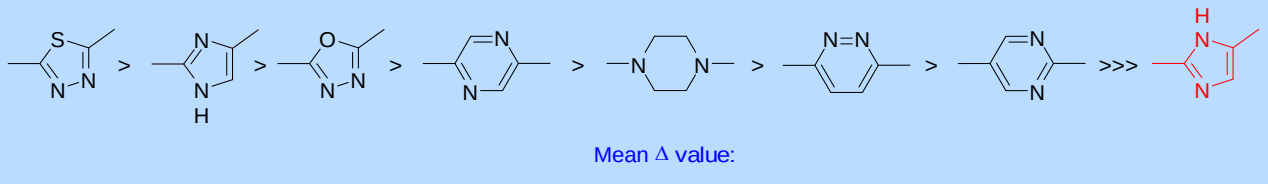
All are effective pyridazine mimics in this context

R.T. Lewis *et al., J. Med. Chem.,* 2006, **49**, 2600-2610; S.C. Goodacre *et al., J. Med. Chem.*, 2006, **49**, 35-38
L.J. Street et al., *J. Med. Chem.*, 2004, **47**, 3642-3657; M.S. Chambers *et al., J. Med. Chem.*, 2004, **47**, 5829-5832

Solving Liability Issues

Pyridazines & Solving Liabilities

Metabolic stability in LMs

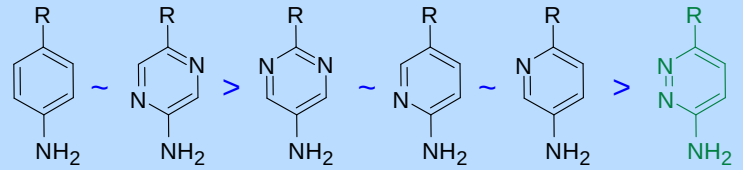


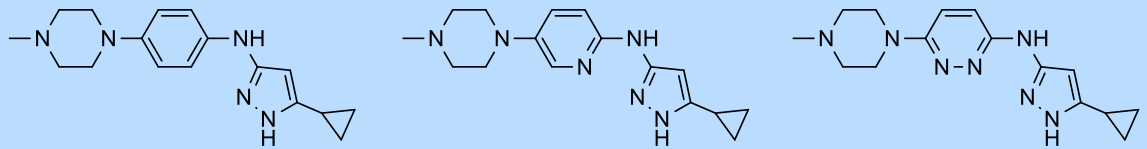
Mean Δ value:

0.65	0.64	0.45	0.46	0.30	0.25	0.19	-0.33

Effect on met. stab. in LMs of replacement of a *para*-substituted benzene ring with a heterocycle

GSH adduct formation



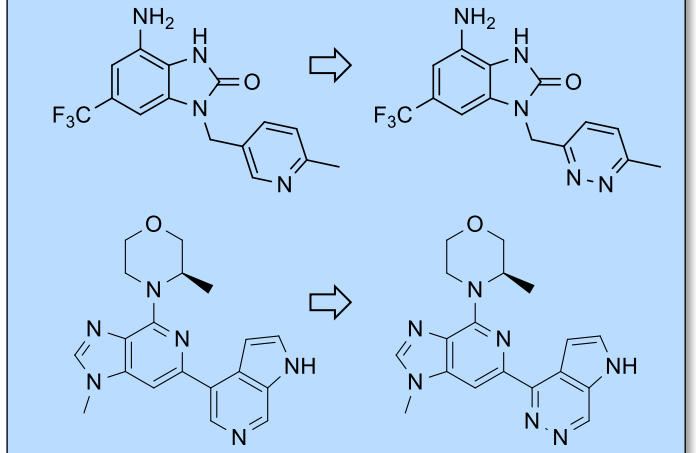


92% remaining
1 GSH adduct at 100% formation
relative to parent

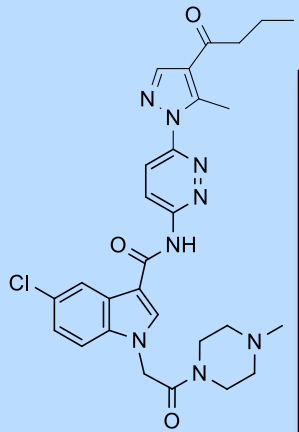
73% remaining
1 GSH adduct at 4% formation
relative to parent

81% remaining
0 GSH adducts
(levels below detectable)

Blocking aldehyde oxidase

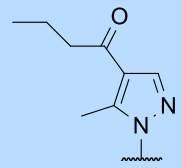


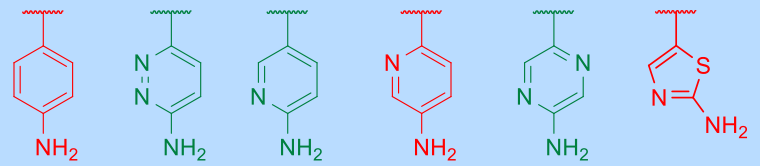
Mutagenic potential



SAR216471
binding IC_{50} = 17 nM
AA rat PRP EC_{50} = 60 nM

Direct acting P2Y12 antagonist

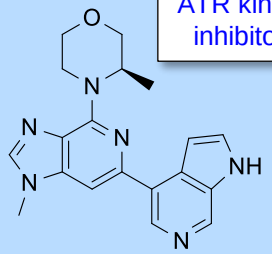




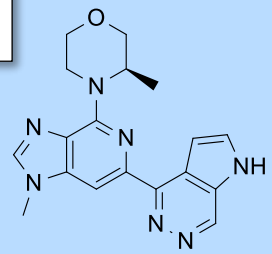
C. Boldron *et al., J. Med. Chem*., 2014, **57**, 7293-7316; C. Zhang *et al., Chem. Res. Toxicol.,* 2020, **33**, 1950-1959

Pyridazine: hERG, CYP 3A4 TDI & AO

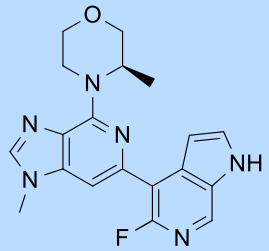
ATR kinase inhibitors



ATR IC_{50} = 0.4 nM
Soly. = >1,700 μM
Caco2 AB/BA = 6/44
hERG IC_{50} = 13 μM
CYP 3A4 TDI: +ve

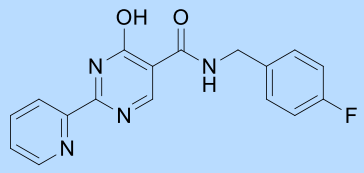


ATR IC_{50} = 0.4 nM
Soly. = >2,400 μM
Caco2 AB/BA = 3.6/38
hERG IC_{50} >30 μM
CYP 3A4 TDI: >24 μM

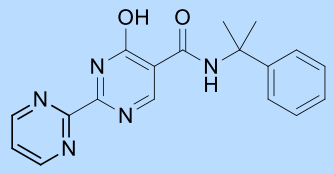


ATR IC_{50} = 0.1 nM
Soly. = >2,300 μM
Caco2 AB/BA = 38/38
hERG IC_{50} >30 μM
CYP 3A4 TDI: +ve

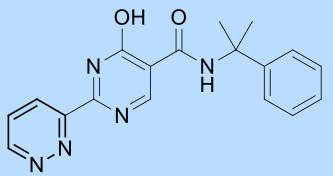
- Ataxia telangiectasia & Rad-3 related protein (ATR)
  - regulates S & G2 checkpoints; sensitizes cancer cells to cytotoxics
- Azaindole introduced to take advantage of H-bond to Lys_{2327}
  - improved potency; hERG & CYP 3A4 TDI, AO & P-gp substrate
- 2-Fluoro substituent reduced basicity
  - abrogated hERG, AO & P-gp but not TDI CYP 3A4
- Pyridazine addressed hERG, P-gp, AO and TDI CYP 3A4 inhibition
  - lower basicity believed to reduce hERG & P-gp recognition
  - exhibited moderate F in rats but low Cl
  - useful tool molecule



PHD2 IC_{50} = 110 nM



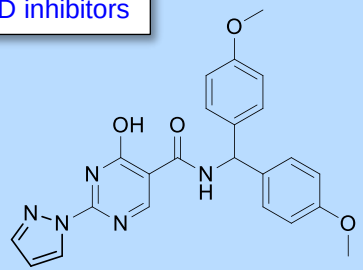
PHD2 IC_{50} = 390 nM



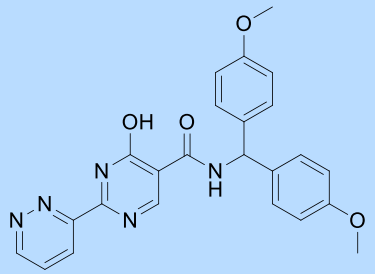
PHD2 IC_{50} = 12 nM

PHD inhibitors

10-30x more potent

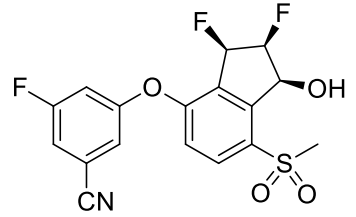


PHD2 IC_{50} = 1.1 nM
hERG IC_{50} = 900 nM



MK-8617
PHD2 IC_{50} = 1 nM
hERG IC_{50} = >30 μM
$t_{1/2}$ in dog, monkey = 10-19 h

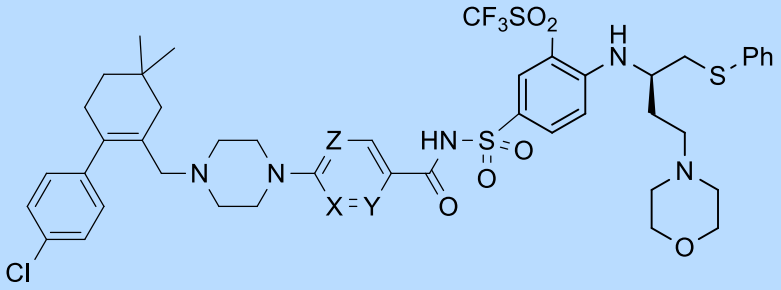
- Pan-inhibitors of hypoxia-inducible factor prolyl hydroxylase 1-3 (HIF PHD1-3)
  - stimulate erythropoiesis
- Pyridazine superior potency to pyridine & pyrimidine
  - early compounds had very long $t_{1/2}$ in vivo
- Pyrazole potent but hERG inhibitor
  - pyridazine clean
- Supplanted by belzutifan



belzutifan

P.A. Barsanti *et al., ACS Med. Chem. Lett.*, 2015, **6**, 42-46; J.S. Debenham *et al., J. Med. Chem.*, 2016, **59**, 11039-11049

Pyridazines & Solubility Properties

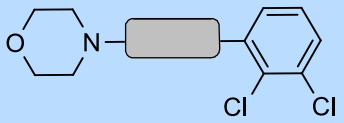


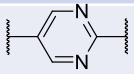
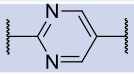
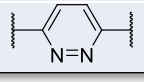
	X	Y	Z	Bcl-2 FP IC_{50} (nM)	Bcl-$_{XL}$ FP IC_{50} (nM)	RS4;11P EC_{50} (nM)	Sol. (µM)
phenyl	C-H	C-H	C-H	11	16	11	14
pyridazine	N	N	C-H	270	378	563	38
pyrimidine	N	C-H	N	18	39	177	3

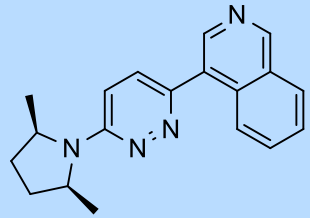
- Bcl protein-protein interaction inhibitors - large molecules
- Pyridazine 3x more soluble than phenyl - potency reduced 20-50x
- Pyrimidine more potent - 4x less soluble than phenyl

Y	Z	CK2α IC_{50} (nM)	CK2α' IC_{50} (nM)	CC_{50} (µM)	Sol. (µg/mL)
C-H	C-H	20	11	8.5	2.7
N	C-H	17	4.6	>30	14
C-H	N	14	10	>30	90
N	N	14	9.6	>30	1,025

- CK2α inhibitors
- Pyridazine 400x more soluble than phenyl - pyridines 4-30x
- Enzyme inhibitory potency maintained - cell potency poor



	CB_2 pEC_{50} (efficacy)	Rat Cl (mL/min/kg)	Sol. (mg/mL)	cLog $D_{pH7.4}$
phenyl	7.4 (86%)	20	NA	4.0
	7.3 (92%)	2.8	<1	2.3
	6.7 (67%)	NA	NA	3.4
	7.1 (76%)	2.8	6	2.1

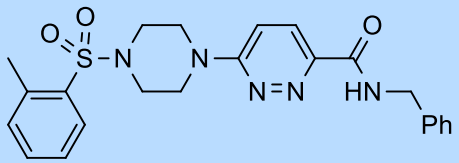


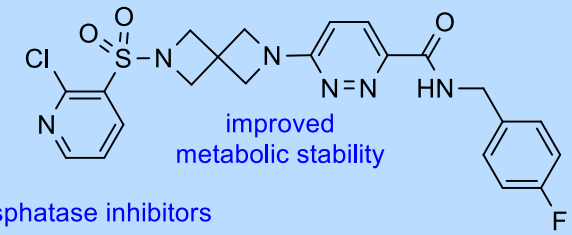
CB_2 pIC_{50} = 8.0 (85%)
CB_1 pIC_{50} = 5.1 (71%)
sol. of di-HCl salt >1 mg/mL

J.G. Varnes *et al., Bioorg. Med. Chem. Lett.*, 2014, **24**, 3026-3033; H. Ohno *et al., Bioorg. Med. Chem.,* 2016, **24**, 1136-1141; R.J. Gleave *et al., Bioorg. Med. Chem. Lett.*, 2010, **20**, 465-468

Pyridazine-3-CO.NHR Derivatives

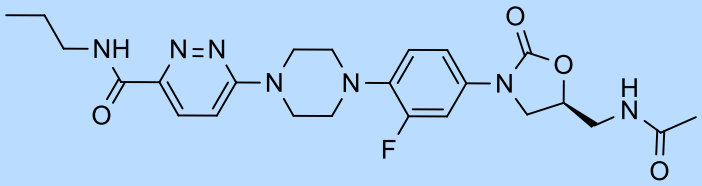
Recent Pyridazine 3-CO.NHR Derivatives



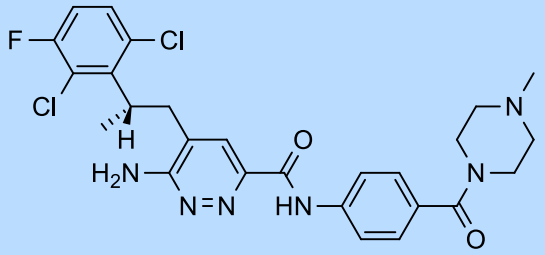


improved
metabolic stability

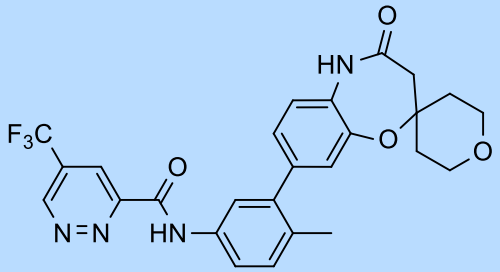
dCTP pyrophosphatase inhibitors



oxazolidinone antibacterial agent

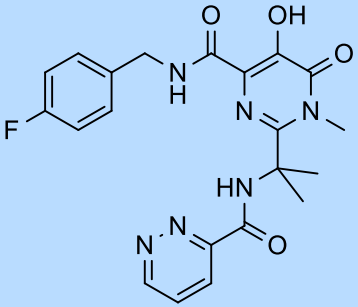


X-376, ensartinib
2nd gen. ALK & ROS1 tyrosine kinase inhibitor

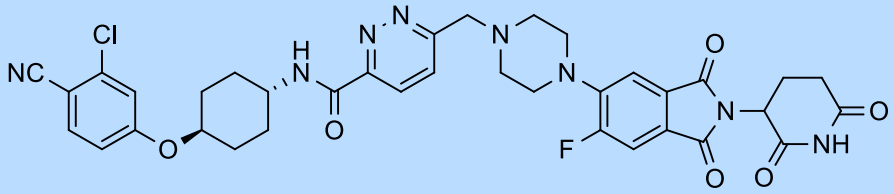


SHR902275
RAF inhibitor targeting RAS mutant cancers

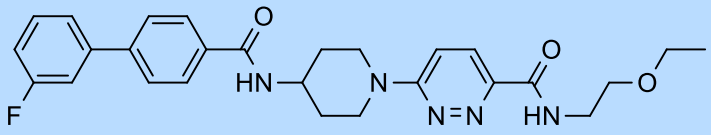
histone deacetylase (HDAC) inhibitor



HIV-1 integrase inhibitor



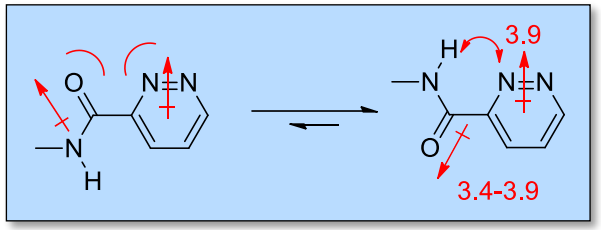
ARV-110
androgen receptor degrader

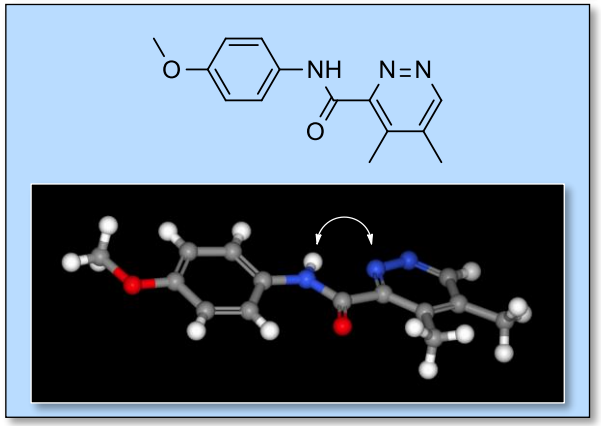


hematopoietic prostaglandin D synthase (HPGDS) inhibitor

◆ Prevalence in investigational drugs increasing

Pyridazine-3-CO.NHR: Intramolecular H-Bonds & Potency

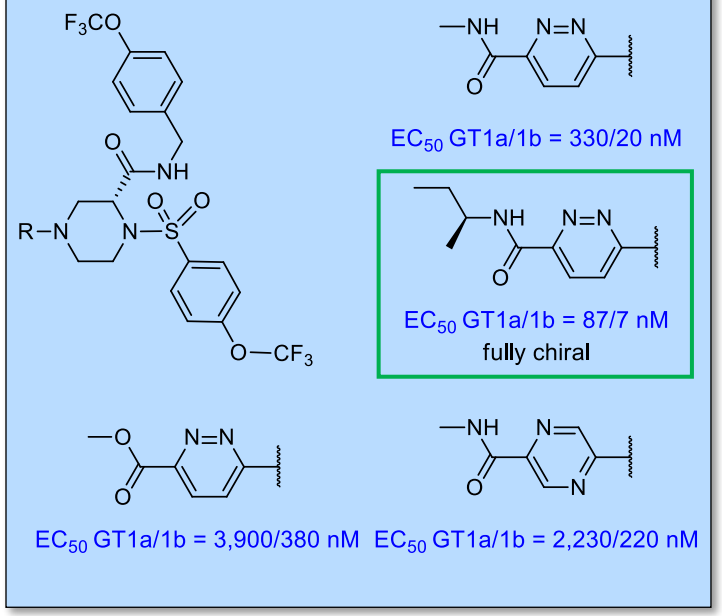


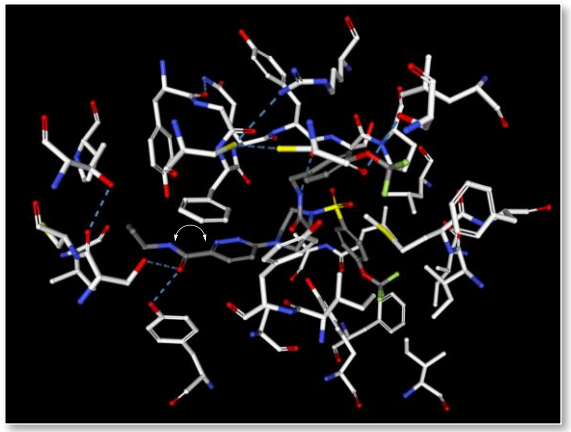


- Topology & planarity favored by:
  - intramolecular H-bond
  - non-bonded interactions
  - dipole alignment

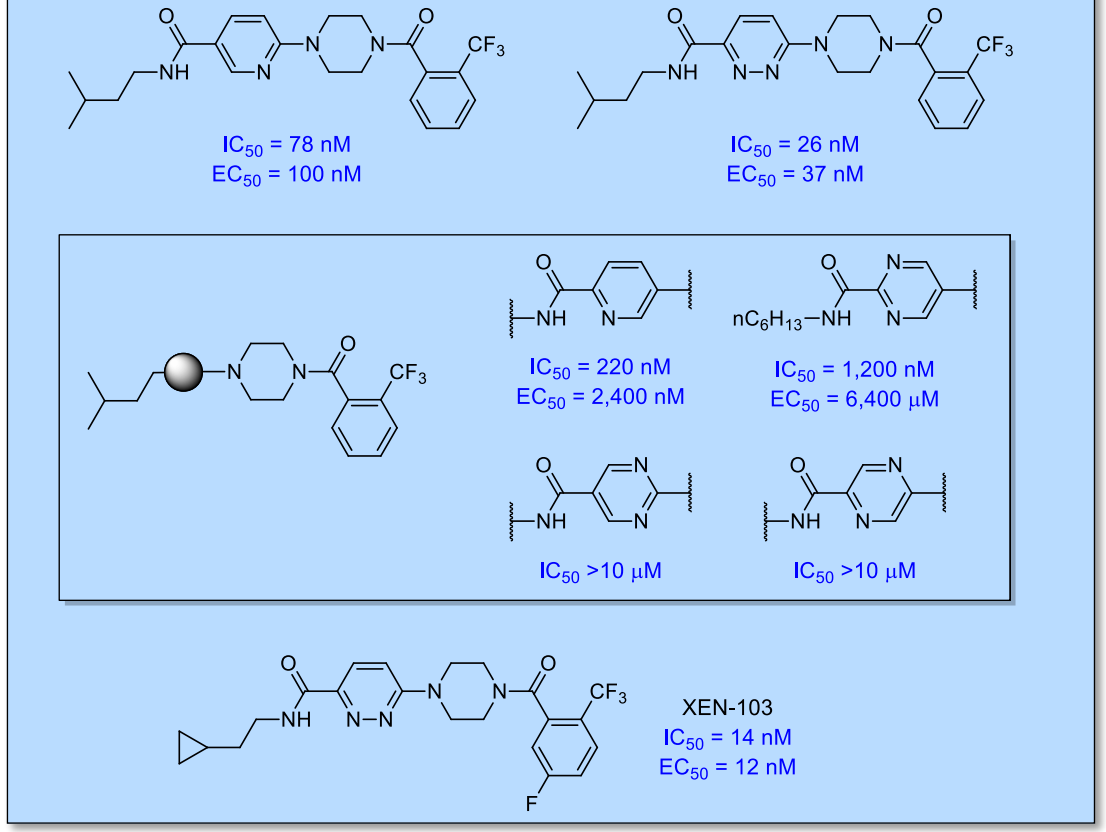
- HCV NS5B inhibitors
  - potency favored by topology of azine
  - dipole alignment; intramolecular H-bond
  - pyrazine places N in unacceptable place
- Topology observed in cocrystal structure

HCV NS5B RdRp Inhibitors





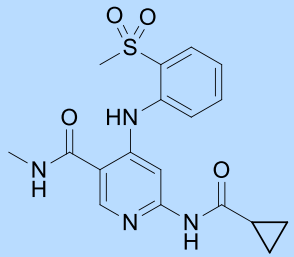
Stearoyl-Coenzyme A Desaturase-1 Inhibitors



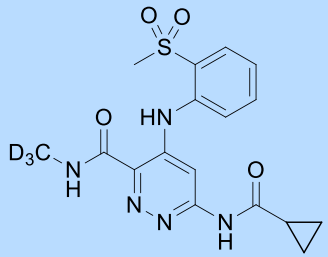
- SCD-1 inhibitors: lipid metabolism, metabolic disorders
  - converts stearoyl-CoA to oleoyl-CoA
- Potency dependent on azine identity
  - pyridazine optimal
  - pyridine analogues 3-10x less potent

A.V. Komkov *et al., Org. Lett.,* 2015, **17**, 3734-3737; R.G. Gentles *et al., Bioorg. Med. Chem. Lett*., 2011, **21**, 2212-2215; 2011, **21**, 3142-3147
Z. Zhang *et al., J Med Chem*., 2014, **57**, 5039-5056 (review); 2013, **56**, 568-583

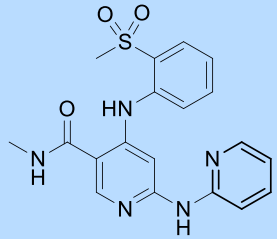
Pyridazines: Intramolecular H-Bonds & Permeability



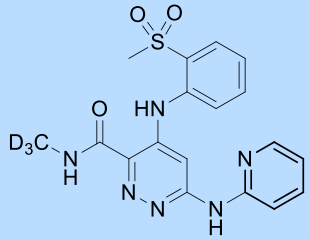
Caco-2 Pc AB/BA = 17/29 nm/s
HPLC Log *P* = 1.89



Caco-2 Pc AB/BA = 69/7.7 nm/s
HPLC Log *P* = 2.03

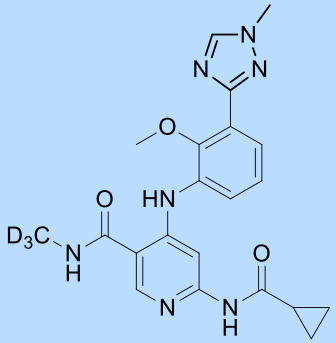


Caco-2 Pc AB/BA = 102/9.8 nm/s
HPLC Log *P* = 2.24

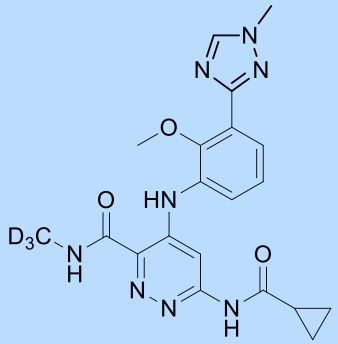


Caco-2 Pc AB/BA = 280/2.1 nm/s
HPLC Log *P* = 2.45

- Pyridine → pyridazine increases Log *P*
  - intramolecular H-bond
  - offsetting dipole effects



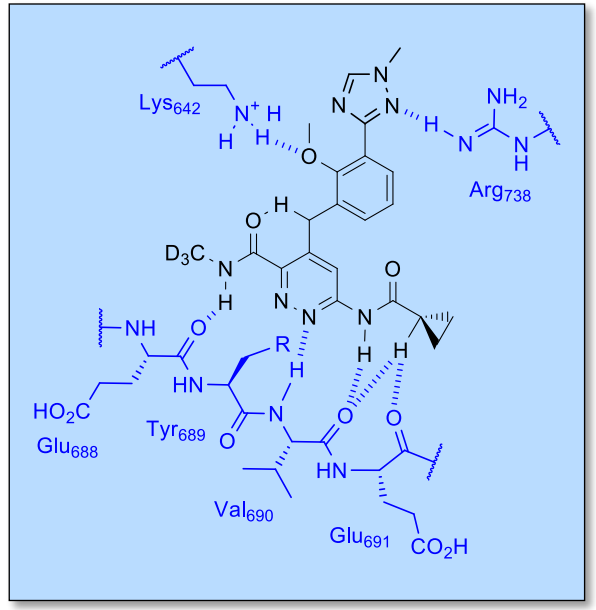
TYK2 JH2 IC_{50} = 0.30 nM
cell IFNα EC_{50} = 8 nM
whole blood $EC5_0$ = 13 nM
Caco-2 Pc AB/BA = <15 nm/s

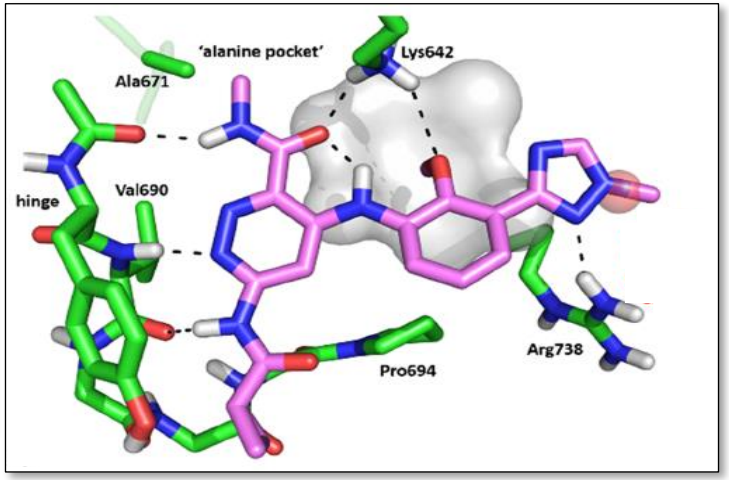


TYK2 JH2 IC_{50} = 0.20 nM
cell IFNα EC_{50} = 5 nM
whole blood $EC5_0$ = 13 nM
Caco-2 Pc AB/BA = 73/740 nm/s

deucravacitinib

- TYK2 kinase inhibitors
  - bind to an allosteric pseudokinase site
- Membrane permeability enhanced by intramolecular H-bond
  - consistent structure-property effect
- Deuterium introduced to slow CYP-mediated demethylation
  - primary amide a less selective kinase inhibitor
- Deucravacitinib approved December 2022
  - treatment of moderate-to-severe plaque psoriasis
  - 1st de novo deuterated drug to be approved



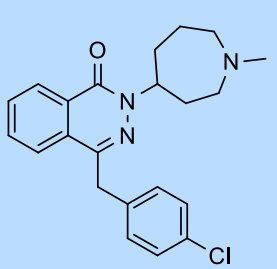


R. Moslin *et al., J. Med. Chem.*, 2019, **62**, 8953-8972; S.T. Wrobleski *et al., J. Med. Chem.,* 2019, **62**, 8973-8995

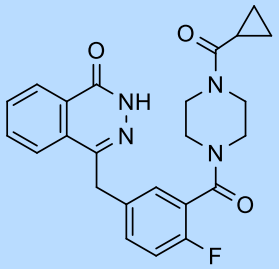
NuArq MedChem Consulting LLC

Pyridazin-3-ones

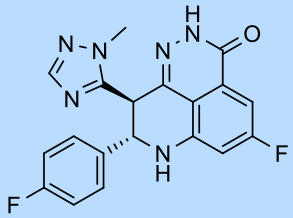
Pyridazin-3-ones – PARP, cAMP PDE3 Inhibitors



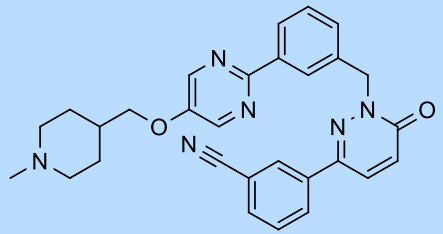
azelastine
allergic rhinitis
approved 2008



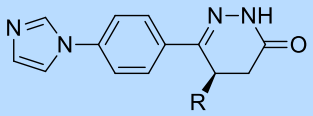
olaparib
PARP inhibitor
approved 2014



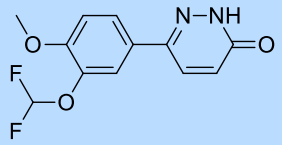
talazoparib
PARP inhibitor
approved 2018



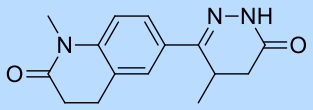
tepotinib
MET inhibitor NSCLC
approved 2021



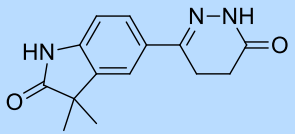
R = H: CI-914
PDE3 IC_{50} = 6,100 nM
R = CH_3: CI-930
PDE3 IC_{50} = 600 nM



zardaverine
PDE3 IC_{50} = 580 nM

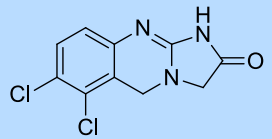


Y-590
PDE3 IC_{50} = 90 nM



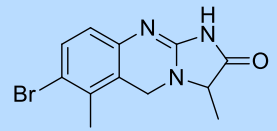
indolidan
PDE3 IC_{50} = 80 nM

- cAMP PDE3 inhibitors
- positive inotropic agents
- blood platelet aggregation inhibitors

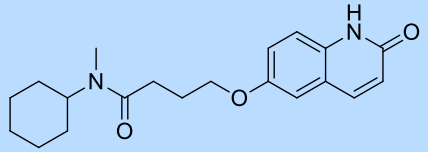


anagrelide
PDE3 IC_{50} = 32 nM

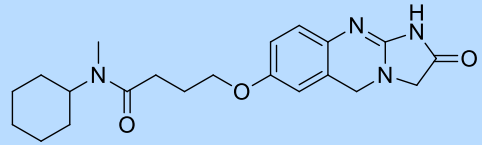
quazinone (Ro 13-6438)
PDE3 IC_{50} = 580 nM



Ro 15-2041
PDE3 IC_{50} = 70 nM



cilostamide
PDE3 IC_{50} = 27 nM



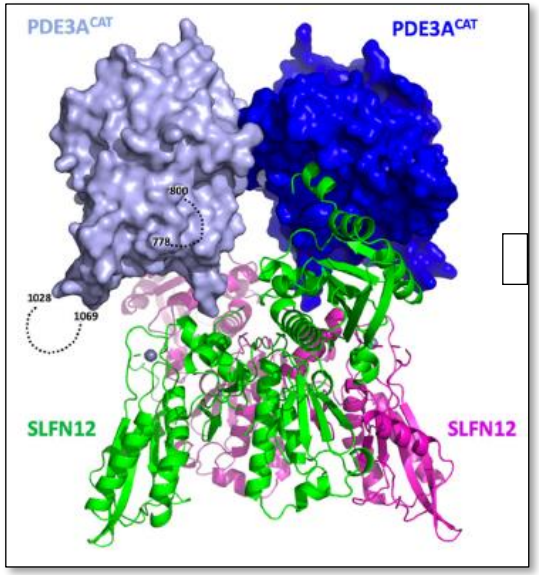
lixazinone
PDE3 IC_{50} = 10 nM

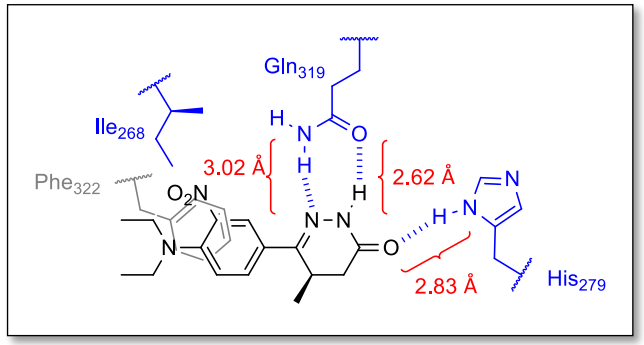
cAMP PDE3 Inhibitors & Cytotoxicity

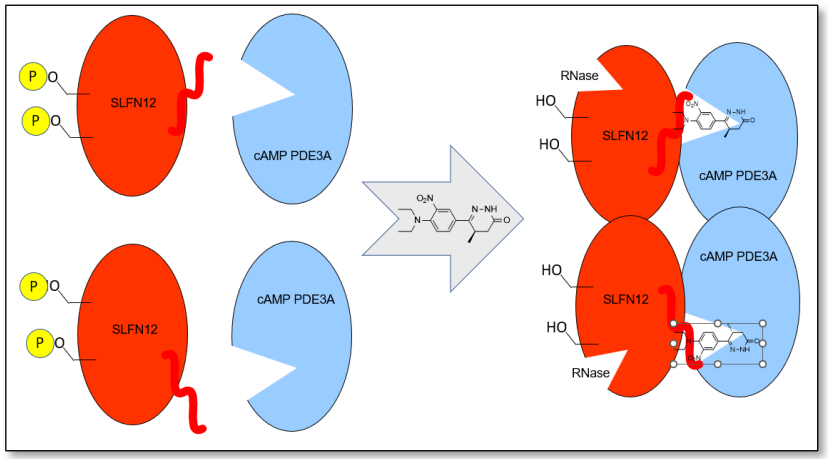
- Recent studies to identify selective tumor cell cytotoxins
  - **phenotypic** screening approach using p53 WT & mutant cell lines
  - selected compounds active **only toward p53** mutant cell lines
- Chemogenomic analysis of 766 cell lines with differential response
  - identified dependence on cAMP PDE3A
- Immunoprecipitation experiments with/without inhibitor
  - identified **Schlafen12** as a partner
    - SLFN12 is an RNase: 1 of 6 with a range of functions in cells
- Hydrocarbon receptor-interacting protein (AIP) also required
  - required for PDE/SLFN12 complex assembly: may be a chaperone
- X-ray and cryo-EM structures of inhibitors bound to cAMP PDE3A
  - **first structural data** for PDE3A inhibitors
- Cocrystal structures of PDE3A/SFLN12/inhibitor
  - tetrameric complex with 2 inhibitors bound
- Some PDE3A inhibitors act as **molecular glues**
  - stabilize a complex between PDE3A & SLFN12
  - other PDE3A inhibitors can block the effect
- Prolongs half life of SLFN12 & activates its RNase activity
  - stimulates dephosphorylation of SLFN12
  - selectively degrades tRNALeu (TAA): spares tRNALeu (TAG)
  - **story still developing**: BAY 2666605 in clinic (Bayer-Broad)

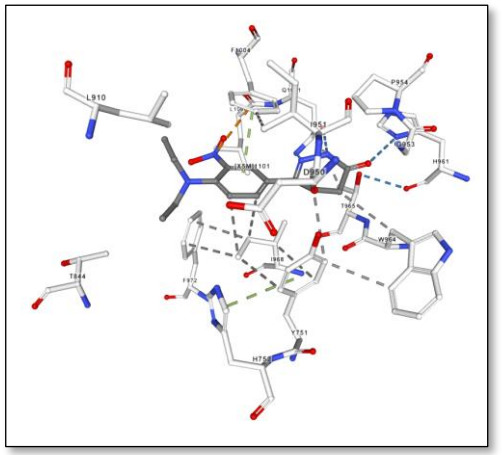
velcrins

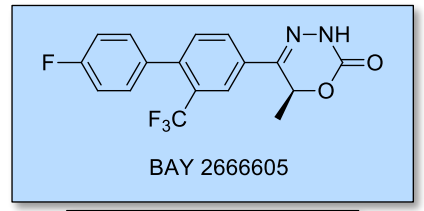
- The power of phenotypic screening











pyridazinone isostere

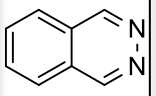
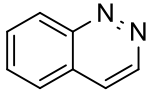
H. Greulich *et al., Nat. Chem. Biol.*, 2016, **12**, 102-108; *Nat. Commun.,* 2021, **12**, 4375; C.W. Garvie *et al., Nat. Commun.,* 2021, **12**, 4375
N.A. Meanwell, *ACS Med. Chem. Lett*., 2023, **14**, 350-361

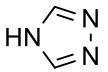
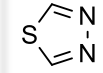
Conclusion

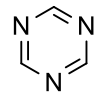
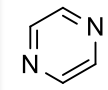
- The pyridazine ring has unique physicochemical properties
  - these can be gainfully exploited in bioactive compound design
- The key properties are:
  - effective, dual H-bonding properties in the absence of overt basicity
    - inter- & intra-molecular H-bonding effects
  - a large dipole moment that can play a role in π-π and π-amide stacking
  - modulation of ether & CH_2-O conformation
  - ring nitrogen interactions with proximal electron deficient S atoms
    - influence on conformation
- Properties are useful in solving liability problems
  - reduced CYP 450 inhibition
  - can lower susceptibility to AO
  - reduced hERG inhibition
  - lower susceptibility to metabolic activation
  - 3-amino derivatives less likely to be mutagenic
  - lower Log *P*; enhanced solubility
- Many of these properties extend to diazoles
  - bioisosteric relationship

Back-up Slides

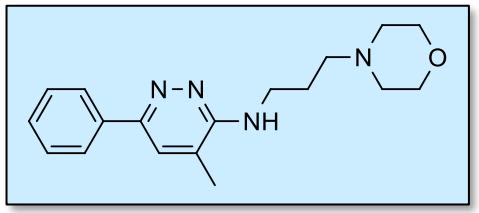
Physicochemical Properties

			
pK_a	2.0	3.17	2.5-2.7
pK_{BHX}	1.65	1.97	
Dipole (*D*)	4.22	4.88	4.41
cLog *P*	-0.51	0.68	1.14
cLog $D_{pH=1}$	-2.5	-1.51	-0.58
TPSA (Å^2)	25.8	25.8	25.8
C_X^{Ph}	0.417		

				
pK_a	5.2	2.45		
pK_{BHX}	1.86	2.6	1.3	
Dipole (*D*)	2.22	5.74	3.04	3.28
cLog *P*	0.84	-0.89	-0.69	-0.2
cLog $D_{pH=1}$	-1.66	-2.85	-0.69	-0.22
TPSA (Å^2)	12.9	36.75	33.95	54
C_X^{Ph}	0.41 (C2 & C4)			

			
pK_a	0.93	-1.7	0.37
pK_{BHX}	1.07	0.32	0.92
Dipole (*D*)	2.33	0	0
cLog *P*	0.26	-0.73	-0.002
cLog $D_{pH=1}$	-0.58	-1.82	-0.43
TPSA (Å^2)	25.8	38.7	25.8
C_X^{Ph}	0.43 (C2); 0.5 (C4)		0.47

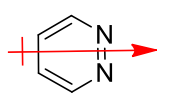
- Pyridazine is a strong H-bond acceptor
  - approaching that of pyridine but much less basic
  - not associated with CYP inhibition
  - de-symmetrized by substitution
- Pyridazine has the largest dipole amongst azines
  - reflected in polarity (cLog *P* & cLog *D*)
  - higher TPSA than pyridine
- Pyridazine C-3 is electron deficient
  - comparable to pyridine; lest than pyrimidine
  - affects properties of substituents
- 3-C-H is a H-bond donor
  - stronger than pyridine



3-amino-pyridazines are more basic

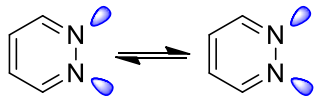
minaprine supports salt formation
- stable di-HCl salt

aromaticity index (AI) = 79
compared to 100 for phenyl

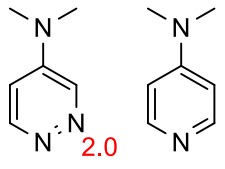


μ = 3.9 *D*

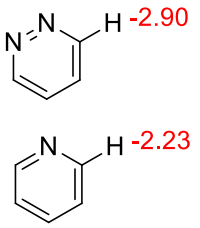
strongest dipole
in the azine series



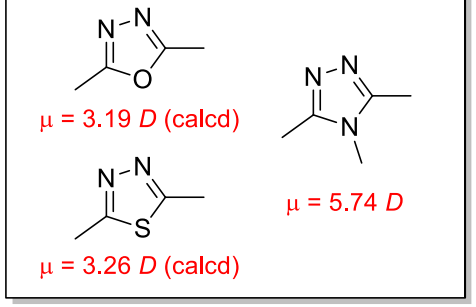
pK_{BHX} = 1.65
pK_a = 2.0
strong H-bond acceptor
weak base
poor CYP inhibitor



2.6 2.0 2.80
pK_{BHX}



strongest C-H H-bond
donor in the azine series

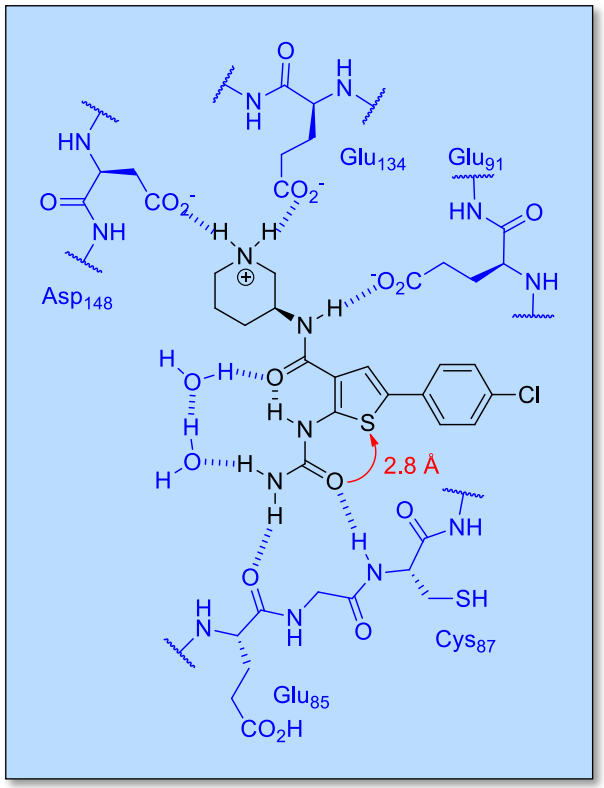


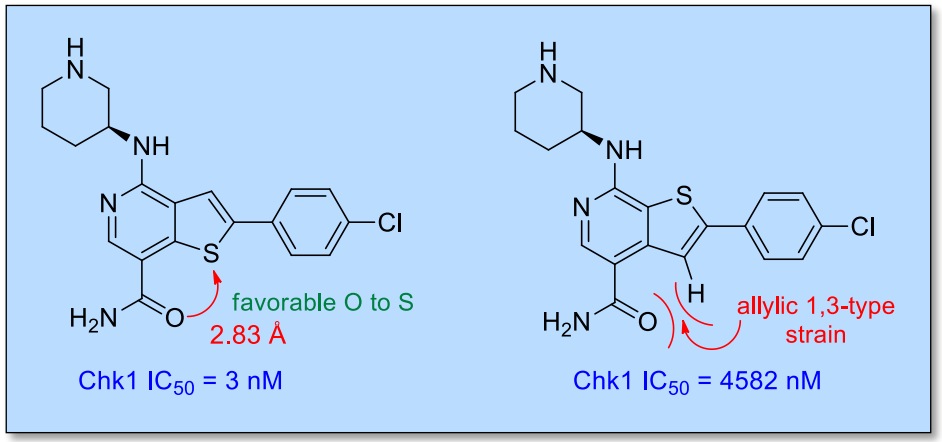
μ = 3.19 *D* (calcd)

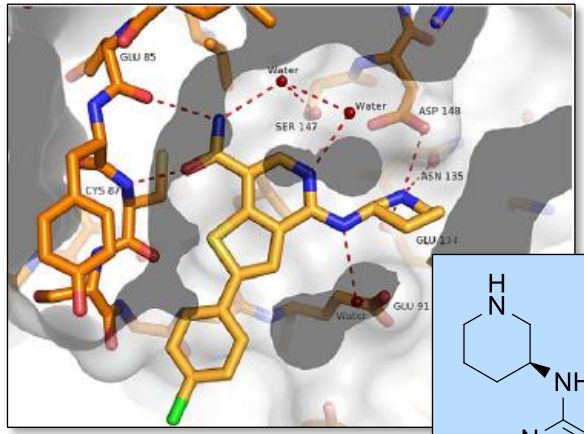
μ = 5.74 *D*

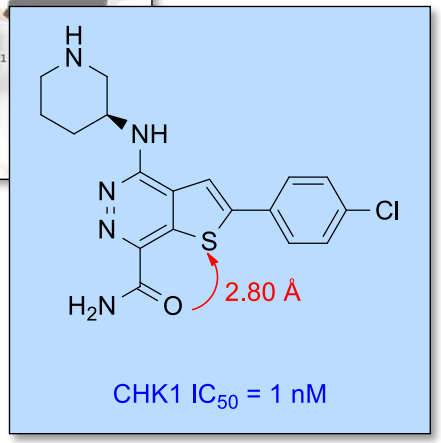
μ = 3.26 *D* (calcd)

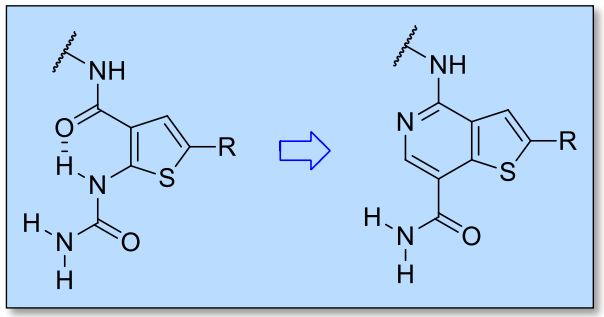
O to S Interactions & Activity – Chk1 Kinase



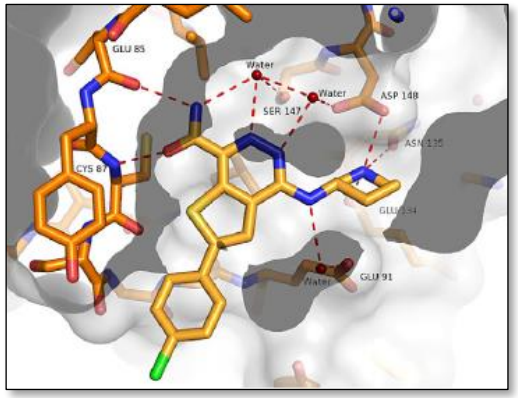






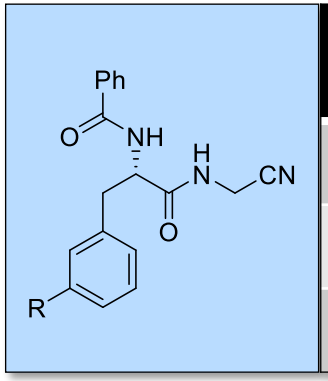


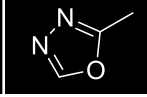
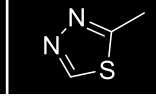
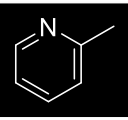
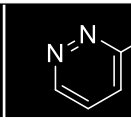
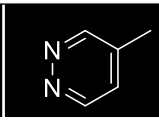
- Intramolecular H-bonded *bis*-amide moiety orients hinge interaction
  - close O to S contact in cocrystal: 2.8 Å (ΣvdW = 3.32 Å)
- Pyridine ring designed to mimic topology & H-bond to H_2O
  - potency maintained: O to S distance 2.83 Å
- Thiophene isomer 1,000x less potent
  - $CONH_2$ distorted from planarity; interferes with hinge binding interactions
- Pyridazine active
  - planar topology maintained
  - O to S of 2.8 Å in cocrystal
- Amide NH aligned with pyridazine N atom
  - no data on membrane permeability or cell-based activity
- Striking & powerful sensitivity to thiophene topology
  - in the absence of other effects



L. Zhao *et al., Bioorg. Med. Chem. Lett.,* 2010, **20**, 7216-7221; D. Shen *et al., Bioorg. Chem*. 2022, **121**, 105704

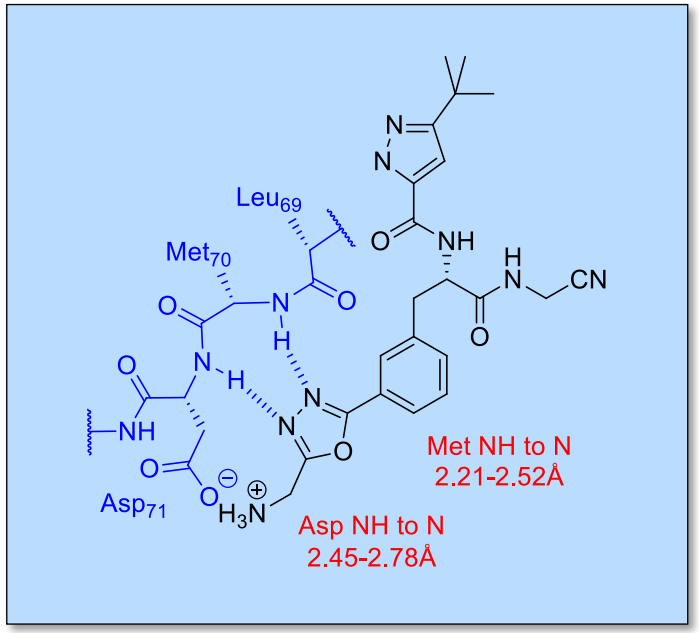
Dual H-Bonding in Cathepsin Inhibitors

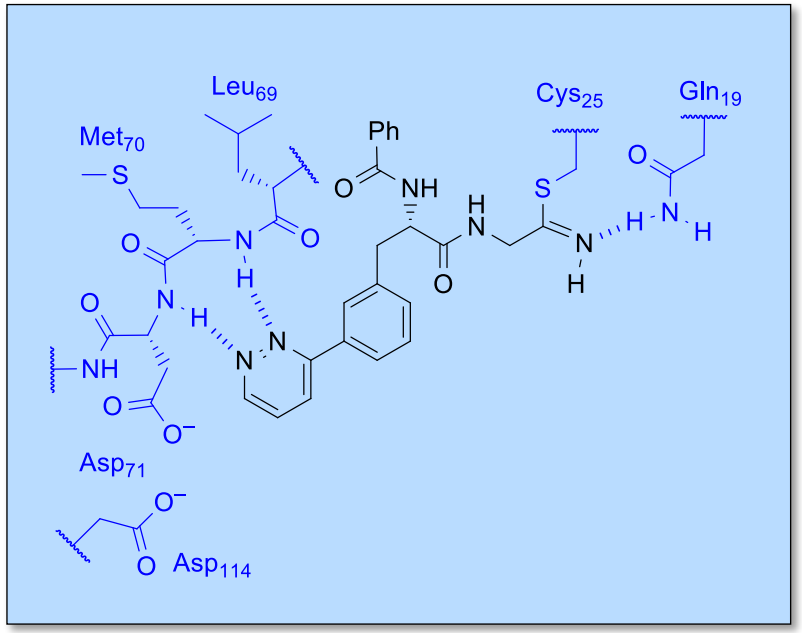


R	Cl						pyrazole
Cat L	6.6	6.3	6.7	5.5	5.9	<5.1	8.7
Cat L2	6.1	6.8	7.6	<5	7.2	<5	7.4
Cat S	6.9	7.7	8.6	6.2	8.1	6.1	6.7

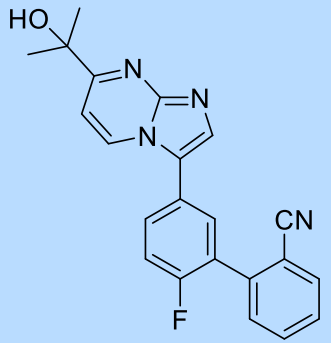
pIC_{50} values

- Cathepsin inhibitors – lead has modest selectivity
  - sought to improve selectivity by engaging Asp_{71}
- Use scaffolds capable of engaging Met_{70} & Asp_{71} NHs
  - oxadiazole, thiadiazole
  - increased Cat L2 & S potency
- 3-Pyridazine highly potent vs Cat L2 and Cat S
  - pyridine, isomeric 4-pyridazine much poorer
  - attributed to H-bond interaction with pyridazine
  - much larger than the typical 15x (1.2 log)
- Pyrazole increases pIC_{50} by 0.8 over Cl ($\Delta LE = 0.16$)
  - X-ray revealed H-bonds to Met_{70} & Asp_{71} NH
  - distances relatively long; better in Cat S?

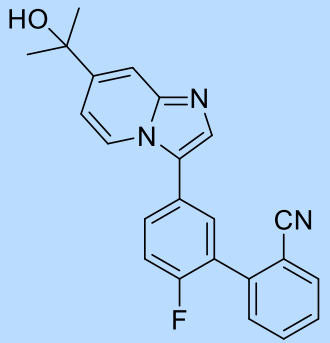




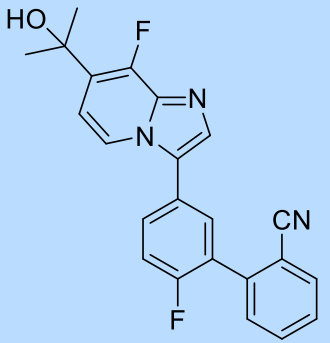
F-Phenyl as Azine Bioisostere: $GABA_A$



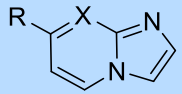
$GABA_A$ α1 K_i = 0.71 nM
$GABA_A$ α3 K_i = 0.47 nM
μ of core: 5.10 D



$GABA_A$ α1 K_i = 4.35 nM
$GABA_A$ α3 K_i = 5.16 nM
μ of core: 3.37 D



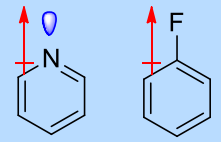
$GABA_A$ α1 K_i = 0.20 nM
$GABA_A$ α3 K_i = 0.32 nM
μ of core: 4.52 D



X	Calc. dipole (*D*)	exp. pK_a	exp. Log $D_{7.4}$
N	3.37 (R = CH_3)	4.9 (R =H)	-0.2 (R =H)
C-H	5.10 (R = CH_3)	6.9 (R =H)	0.8 (R =H)
C-F	4.52 (R = CH_3)	4.9 (R =H)	0.9 (R =H)

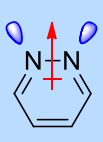
- Dipoles of F-benzenes mimic azine heterocycles
  - mono-F weaker dipole than pyridine
  - 1,2-di-F & 1,2,3-tri-F benzene stronger
- C-F mimicked N analogue in $GABA_A$ agonists
  - C-H analogue 6-10x less potent
  - C-F analogue fully restored potency
- Analyzed aspects of the core for mimicry
  - dipole resembles that of the N analogue
  - pK_a values of F & N similar
  - pK_a value of H 2 units higher than F, N
  - Log *D* of F closer to H than N

- C-F has higher lipophilicity, lower dipole, reduced pK_a
  - improved CNS exposure



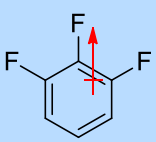
2.3 *D*

1.63 *D*



3.9 *D*

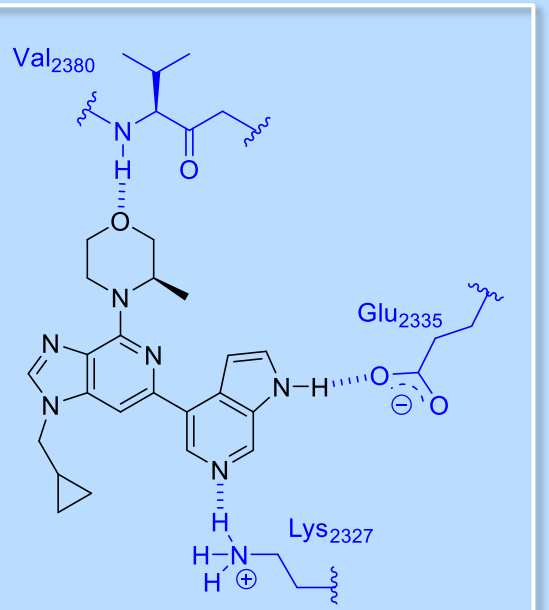
2.59 *D*



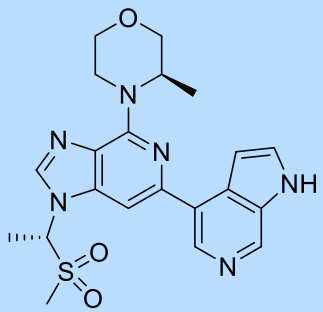
2.9 *D* (est.)

A.C. Humphries *et al., Bioorg. Med. Chem. Lett.*, 2006, **26**, 1518-1522; N.A. Meanwell, *Adv. Het. Chem.,* 2017, **123**, 245-361

F-Phenyl as Azine Bioisostere: ATR Kinase

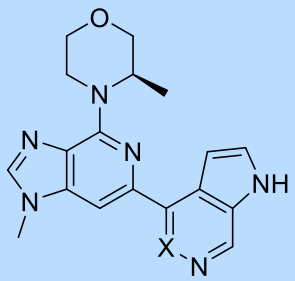


IC_{50} = 26 nM

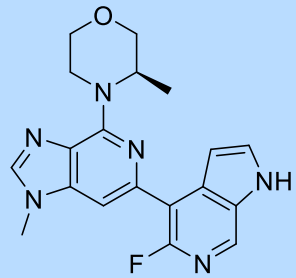


IC_{50} = 0.2 nM

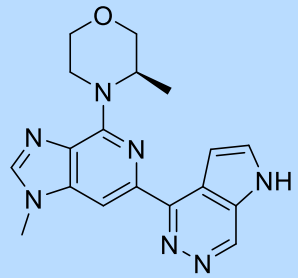
poor PK in rats
low permeability
hERG inhibitor - 9 μM
TDI CYP 3A4 inhibitor



X = CH
IC_{50} = 0.4 nM
X = C-F
IC_{50} = 0.1 nM
X = N
IC_{50} = 0.5 nM



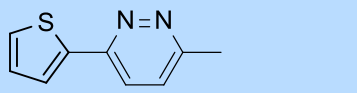
IC_{50} = 0.1 nM

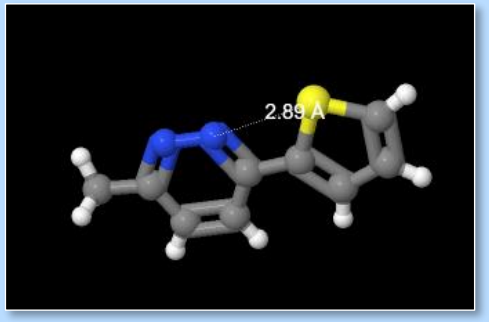


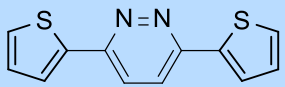
IC_{50} = 0.5 nM

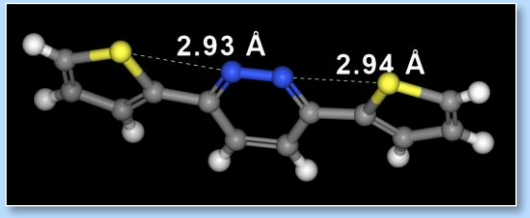
- Potent and selective ATR kinase inhibitor with high cell-based potency
  - proposed binding to ATR based on homology model from PI3Kδ
  - several *in vitro* and *in vivo* liabilities
- Truncating sulfone to a CH_3 improved some properties
  - still hERG, MDR, CYP 3A4 TDI and substrate of aldehyde oxidase
- 5-F eliminated P-gp, reduced hERG inhibition but not CYP 3A4 TDI
- Pyridazine solved problems
  - lower pK_a likely reduces hERG, CYP binding

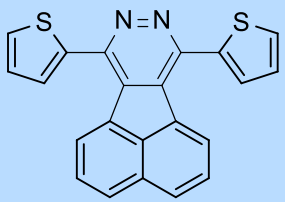
Pyridazine & Sulfur Interactions - Conformation

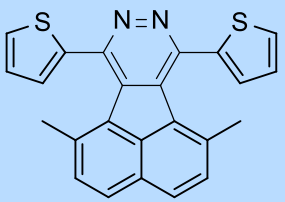


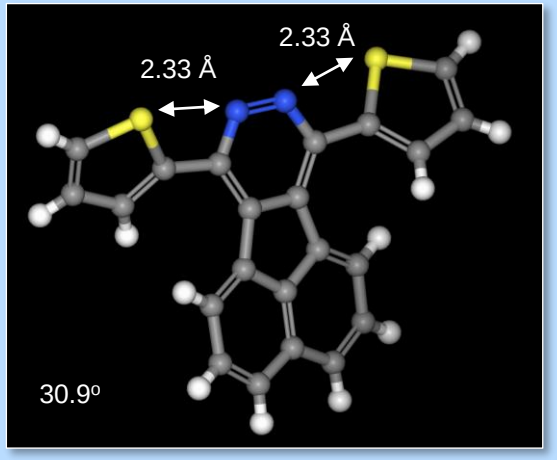


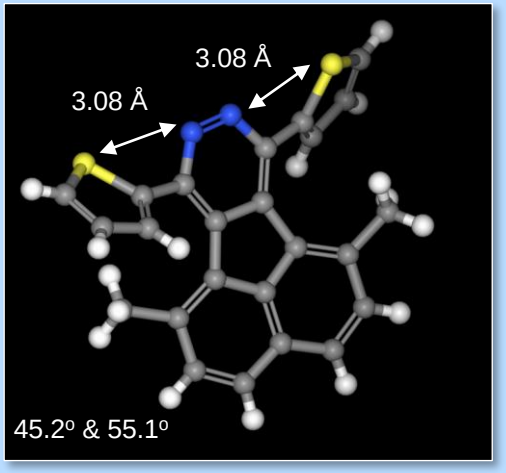




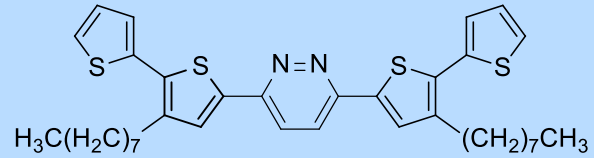


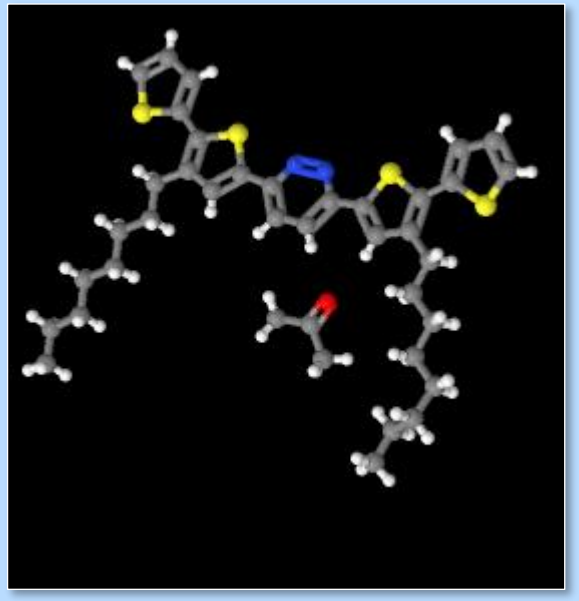






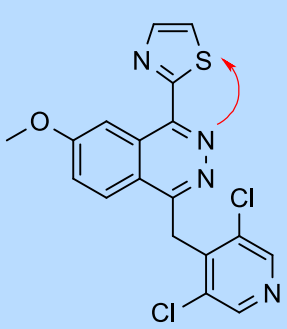
- N to S interactions stabilize a relatively planar conformatin
  - distances less than Σ vdW radii: 3.35 Å (1.55 Å for N; 1.8 Å for S)
- Maintained in the presence of allylic 1,3-strain
  - distorts thiophene ring a further 14-24º



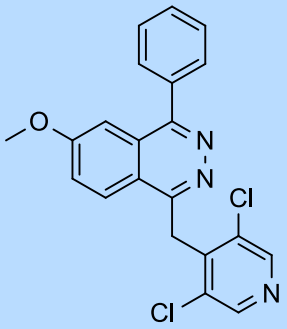


M.-Y. Chang *et al., Tetrahedron* 2015, **71**, 6840-6845; B. Ackers *et al., Acta Cryst.* 2002, **C58**, o640-o641; N. Rahanyan et al.,*Org. Biomol. Chem*. 2009, **7**, 2082-2092

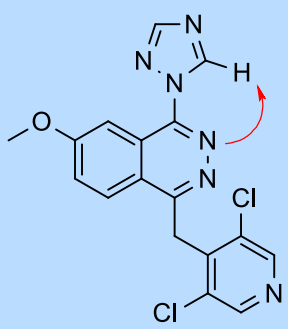
Pyridazine & Sulfur Interactions - Conformation



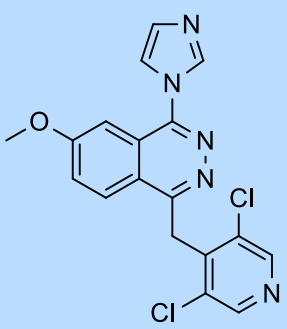
PDE4 IC_{50} = 14 nM
TNF_α IC_{50} = 8 nM



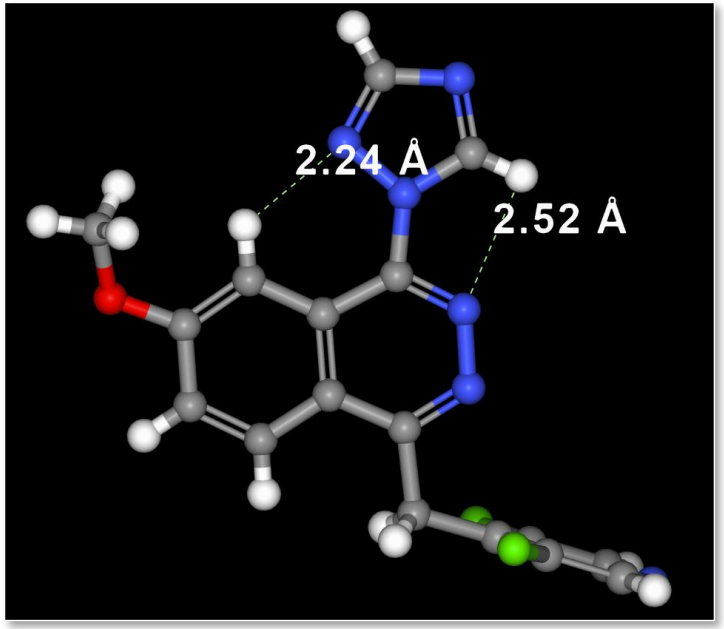
PDE4 IC_{50} = 37 nM
TNF_α IC_{50} = 46 nM



PDE4 IC_{50} = 241 nM
TNF_α IC_{50} = 72 nM



PDE4 = 17% I at 100 nM



- Potency varies dependent upon heterocycle identity
  - planarity between heterocycle & phthalazine core important
- Thiazole is most potent analogue
  - planar topography stabilized by phthalazine N to S interaction
  - absence of unfavorable interaction with peri-H atom
- Phenyl suffers from peri-H interaction
  - triazole stabilized by C-H to N interaction
  - absence of steric clash with peri-H; possible C-H to N
  - imidazole introduces unfavorable interaction with peri-H

M. Napoletano *et al., Bioorg. Med. Chem. Lett.*, 2001, **11**, 33-37

Pedigrees of Heterocycles

- Heterocycles are a mainstay of drug design
  - 5- & 6-membered rings common scaffolds
  - can address a range of problems

Silhouettes between homologues similar (except for S heterocycles) but electronic, physical, biological and developability properties can be very different
  - 1,3,4-oxadiazoles vs 1,2,4-isomers
  - pyridazines vs pyridines

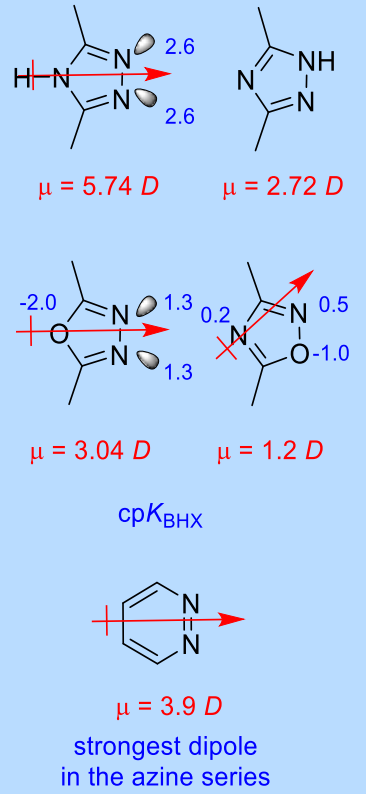
- Key properties:
  - H-bond acceptor; H-bond donor: N-H, O-H, C-H
  - electron withdrawing properties, dipoles

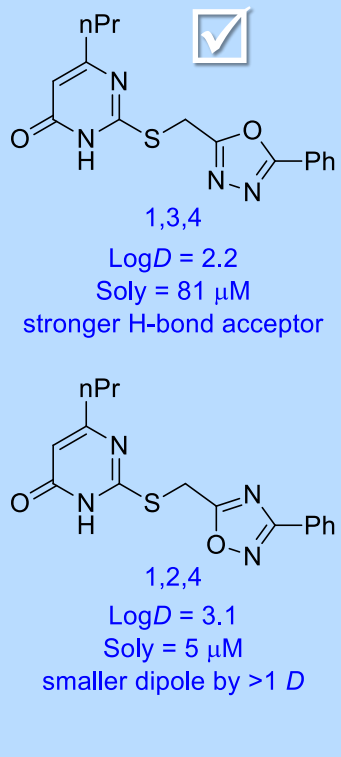
- Properties readily modulated by substituents
  - affect H-bond donor, acceptor; electronics
  - identity, regiochemisty of heterocycle affects substituent properties

- Deployed to modulate potency, geometry, conformation, electronic activation of substituents
  - C=O; C≡N

Heteroaromatic	Solubility	HSA binding	P450 inhib.	Combined score
Pyridazine	3	3	3	3.0
Pyrazine	2	3	3	2.7
Imidazole	3	3	2	2.7
Pyrazole	2	3	3	2.7
1,3,4-Oxadiazole	3	2	2	2.3
1,2,4-Triazole	3	1	2	2.0
Furan	2	2	2	2.0
Pyrimidine	2	2	2	2.0
Oxazole	2	2	2	2.0
Pyrrole	2	2	2	2.0
Pyridine	2	3	1	2.0
1,2,4-Oxadiazole	2	1	3	2.0
1,3,5-Triazine	1	2	2	1.7
1,3,4-Thiadiazole	1	1	3	1.7
Isoxazole	2	2	1	1.7
Tetrazole	3	1	1	1.7
1,2,3-Triazole	1	2	1	1.3
Thiazole	1	1	2	1.3
Thiophene	1	2	1	1.3

DEVELOPABILITY

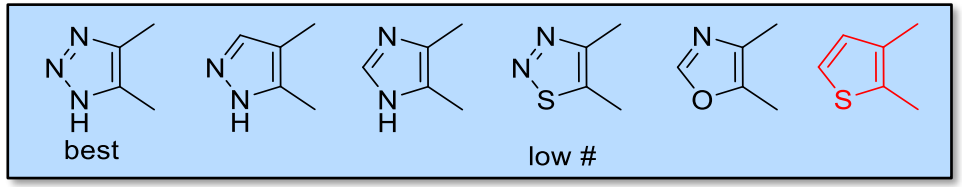




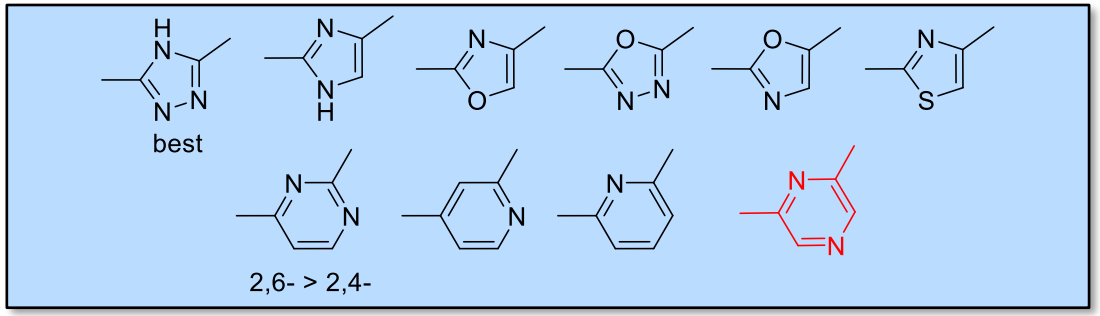
T.R. Ritchie *et al*., *Drug Discov. Today*, 2009, **14**, 1011-1120; 2011, **16**, 164-171; *Med. Chem. Commn*, 2012, **3**, 1062-1069
C. Wermuth, *Med. Chem. Commn*, 2011, **2**, 935-941; J. Boström *et al., J. Med. Chem.*, 2012, **55**, 1817-1830

Phenyl Mimics that can Improve Metabolic Stability

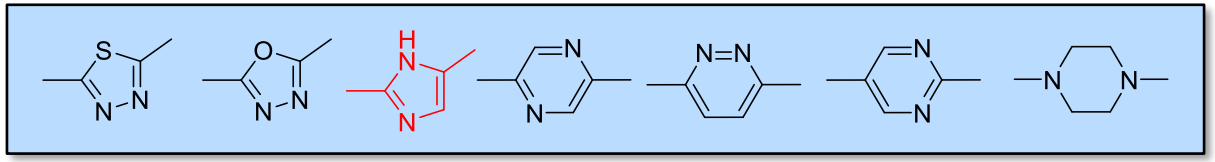
- Heterocycle replacements for a phenyl ring with higher metabolic stability
  - molecular matched pairs analysis
  - 2323 pairs evaluated with 1,2-, 1,3- & 1,4-topologies examined
  - piperazine the sole saturated ring examined in 1,4 relationship
- 1,2-topology: furan, thiophene performed poorly



- 1,3-topology: 5-membered heterocycles generally performed well
  - (3,5)-1*H*-1,2,4,-triazole and (2,4)-1*H*-imidazole the best



- 1,4-topology: 2,5-dipyrazine the best azine; pyridazine & piperazine good
  - (2,5)-1*H*-imidazole poor



1,2-	Mean Δ*
(4,5)-1H-triazole	0.97
(4,5)-1H-pyrazole	0.73
(4,5)-1H-imidazole	0.65
(4,5)-1,2,3-thiadiazole	0.64
2,3-furan	-0.07
2,3-1H-pyrrole	-0.01
2,3-thiophene	-0.12
(2,3)-pyrazine	0.35
(4,5)-pyrimidine	0.33
(2,3)-pyridine	0.20
(3,4)-pyridine	0.15

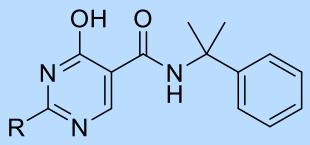
1,4-	Mean Δ*
(2,5)-1,3,4-thiadiazole	0.65
(2,4)-1H-imidazole	0.64
(2,5)-1,3,4-oxadiazole	0.45
(2,5)-1H-imidazole	-0.33
(2,5)-pyrazine	0.46
(3,6)-pyridazine	0.25
(2,5)-pyrimidine	0.19
(2,5)-pyridine	0.11
N,N-piperazine	0.30

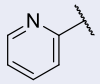
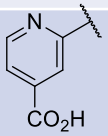
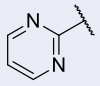
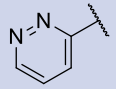
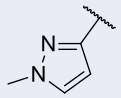
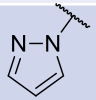
1,3-	Mean Δ*
(3,5)-1H-1,2,4-triazole	0.91
(2,4)-1H-imidazole	0.87
(2,4)-oxazole	0.80
(2,5)-1,3,4-oxadiazole	0.62
(2,5)-oxazole	0.59
(3,5)-isoxazole	0.47
(3,5)-1,2,4-oxadiazole	0.39
(2,5)-oxazole	0.12
(2,4)-thiazole	0.04
(2,6)-pyrimidine	0.43
(2,4)-pyridine	0.23
(2,6)-pyridine	0.17
(3,5)-pyridine	0.14
(2,6)-pyrimidine	0.10
(3,5)-pyrazine	-0.19

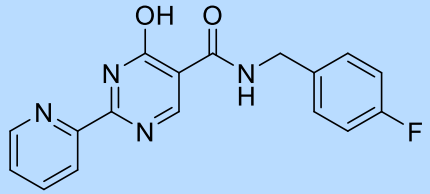
*Mean(Δ($\log_{10}$ Mean Cl_{ints} for transform))

A.G. Dossetter *et al*., *Med. Chem. Commun.,* 2012, **3**, 1164-1169; See T.R. Ritchie & S.J.F. Macdonald, *Eur. J. Med. Chem*., 2016, **124**, 1057-1068 for ADME & terminal phenyl replacements

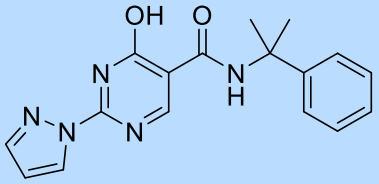
Pyridazine to Solve a hERG Problem in HIF PHD1-3



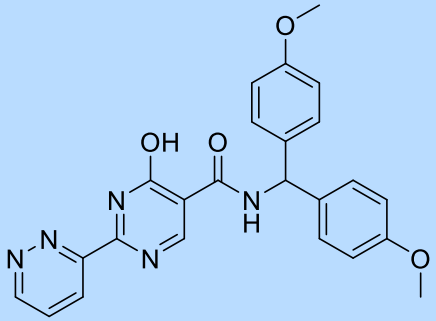
R	PHD2 IC_{50} (nM)
	28
	6
	390
	12
	210
	3.7



PHD2 IC_{50} = 110 nM

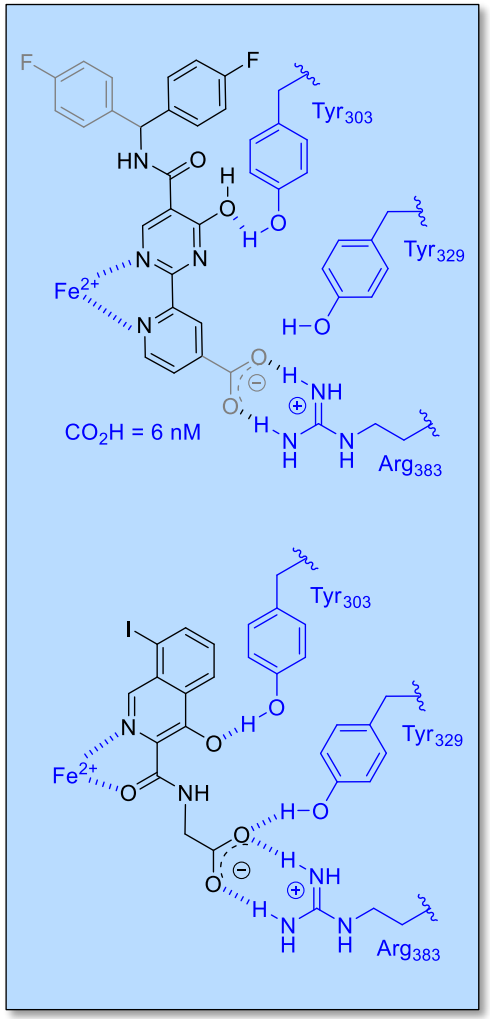


PHD2 IC_{50} = 3.7 nM
hERG IC_{50} = 900 nM
$t_{1/2}$ in dog, monkey: >95 h



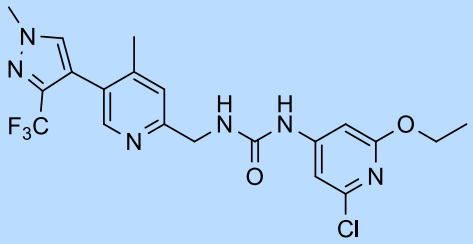
PHD2 IC_{50} = 1 nM
hERG IC_{50} >30 µM
$t_{1/2}$ in dog, monkey: 10-19 h

- Hypoxia-inducible factor (HIF) prolyl hydroxylase 1-3 inhibitor (PHD)
  - treatment of anemia
- Inhibitors stabilize HIF and stimulate RBC production *via* EPO receptor
- PHD is an Fe-containing enzyme
  - Fe chelation by heterocycle interface postulated
  - Arg_{383} interaction for acids: improved potency of pyridine by 5x
- Potent screening lead
  - optimized to pyrazole derivative
  - hERG issue: IC_{50} = 900 nM
- Pyridazine free of hERG problems: IC_{50} >30 µM
  - no inhibition of CYP enzymes: IC_{50} >60 µM except 2C8, 1.6 µM
  - MeO moiety provided metabolic soft spot to reduce *in vivo* half life

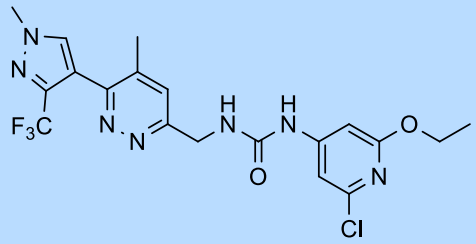


J.S. Debenham *et al., J. Med. Chem.*, 2016, **59**, 11039-11049

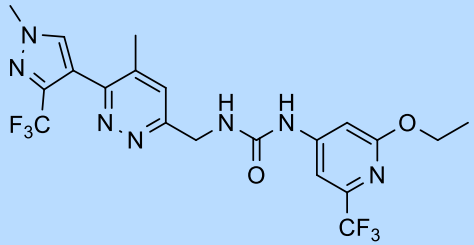
Pyridazines That Increase Potency



S1P2 Ca^{2+} flux IC_{50} = 11 nM
LipE = 4.4
IL8 IC_{50} = 23 nM

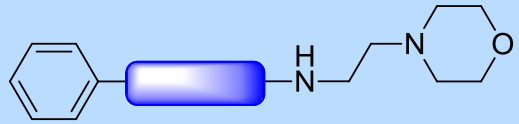


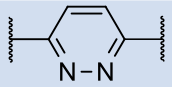
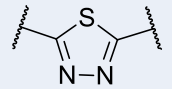
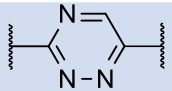
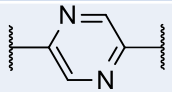
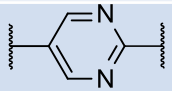
S1P2 Ca^{2+} flux IC_{50} = 7.7 nM
LipE = 5.0
IL8 IC_{50} = 2.8 nM



10x potency increase

GLPG2938
S1P2 Ca^{2+} flux IC_{50} = 8.8 nM
LipE = 4.9
IL8 IC_{50} = 3.8 nM



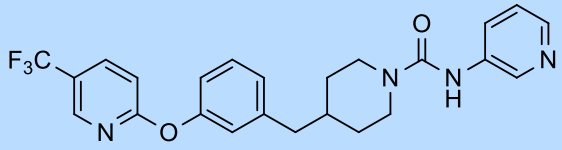
	Reserpine ptosis ED_{50}	5-HT potentiation ED_{50}	Turning behavior MED
	6	3.7	0.5
	4.5	6	0.1
	>10	6	2
	24	30	0.1
	>100	>50	2

- LipE guided optimization
  - pyridazine 10x more potent than pyridine
  - reduced CYP inhibition
- Good PK, active in a bleomycin-induced model of pulmonary fibrosis

- Pyridazine & thiadiazole most potent
  - Reserpine ptosis model, 5-HT potentiation
  -
- Turning behavior model does not differentiate
  -

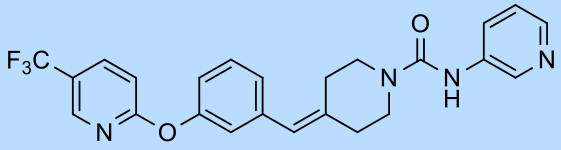
O. Mammoliti *et al., J. Med. Chem.,* 2021, **64**, 6037-6058; C.G. Wermuth, *Med. Chem. Commun.,* 2011, **2**, 935-941

Pyridazine in a FAAH Inhibitors: CYP Inhibition

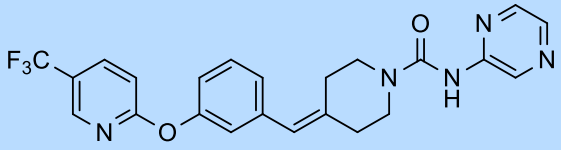


PF-3845

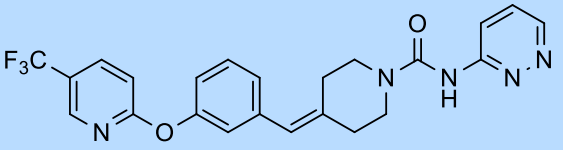
hFAAH k_{inact}/K_i M^{-1} s^{-1} = 12,600
rFAAH k_{inact}/K_i M^{-1} s^{-1} = 3,900
CYP inhibition = NT



hFAAH k_{inact}/K_i M^{-1} s^{-1} = 21,600
rFAAH k_{inact}/K_i M^{-1} s^{-1} = 15,100
CYP 2D6 = 1.4 μM; 3A4 = 0.8-4.3 μM



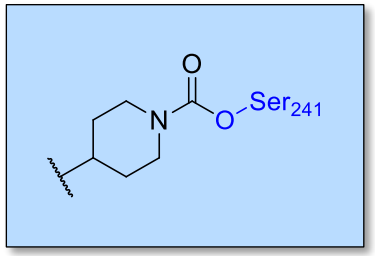
hFAAH k_{inact}/K_i M^{-1} s^{-1} = 42,600
rFAAH k_{inact}/K_i M^{-1} s^{-1} = 23,700
CYP 2D6 = 25.1 μM; 3A4 = 23.5-30 μM

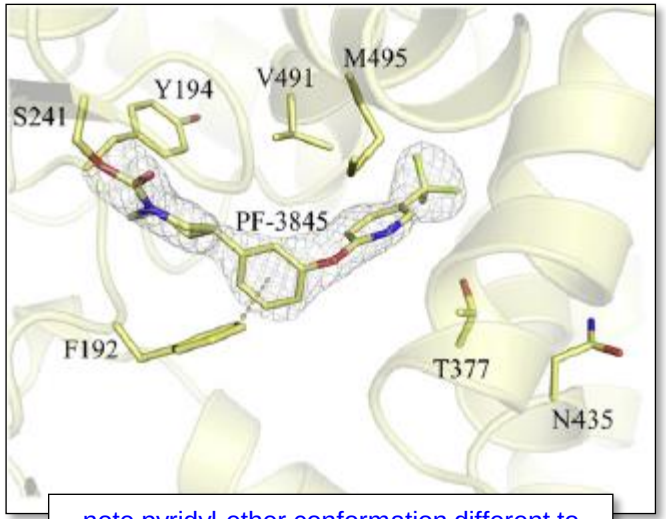


PF-04457845

hFAAH k_{inact}/K_i M^{-1} s^{-1} = 40,300
rFAAH k_{inact}/K_i M^{-1} s^{-1} = 32,400
CYP 2D6 = 14.5 μM; 3A4 = 30 μM

- Time-dependent, mechanism-based fatty acid amide hydrolase inhibitor
  - urea reacts with serine hydroxyl to afford carbamoylated enzyme
  - unique property of FAAH – other hydrolases react with esters/thioesters
  - k_{inact}/K_i = 40,300 $M^{-1}s^{-1}$
- Pyridine inhibited CYPs
  - CYP 2D6 IC_{50} = 1.4 μM
  - CYP 3A4: IC_{50} = 0.8-4.3 μM
- Pyridazine 2-fold more potent FAAH inhibitor
  - 10-fold reduction in CYP 2D6 inhibition: IC_{50} = 15.5 μM
  - CYP 3A4: IC_{50} = 30 μM





note pyridyl ether conformation different to Boger *et al.*, *JACS* 2009, **131**, 10497-10506

K. Ahn *et al., Biochemistry,* 2007, **46**, 13019-13030; *Chem. Biol.*, 2009, **16**, 411-420; D.S. Johnson *et al., ACS Med. Chem. Lett.*, 2011, **2**, 91-96