Bioisosteres of Piperazine & Related Diamines in the Design of Biologically Active Compounds

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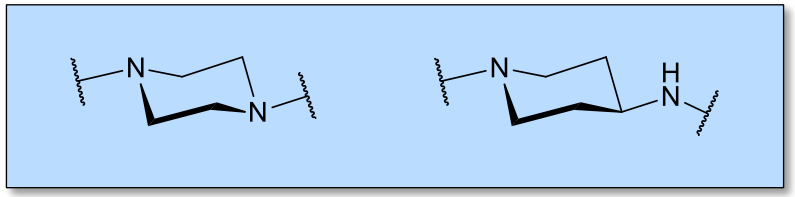
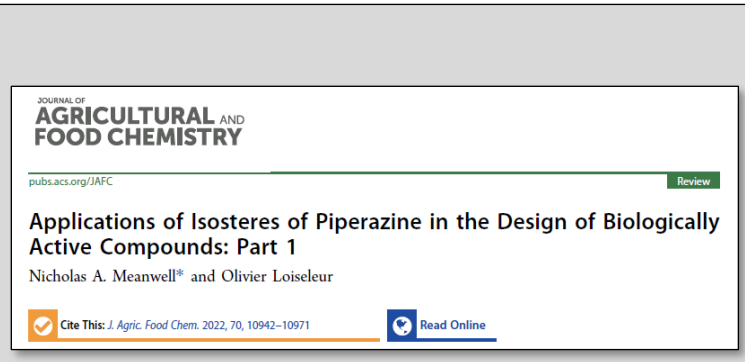
Outline

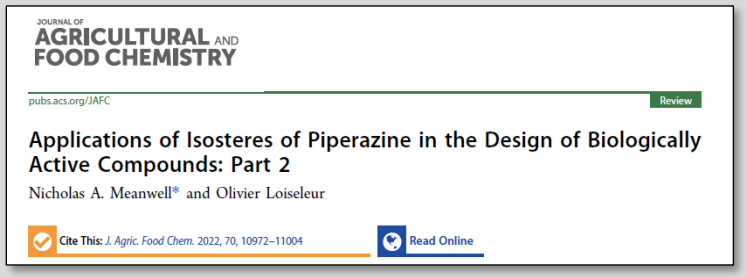
- Background
  - applications & prevalence of piperazines
- Piperazine & bioisosteres
  - geometrical parameters
  - pK_a data
  - conformation
- Applications of piperazines & bioisosteres
  - solubility enhancement
  - conformational mimicry
- Piperazines with fused rings
  - carbocyclic, heterocyclic
  - amine-containing rings
- Azetidine derivatives
  - linear, spiro
- Pyrrolidine derivatives
  - saturated, partially saturated
- Isosteres of piperazines
  - non-diamines
- Conclusion

Applications of Piperazines

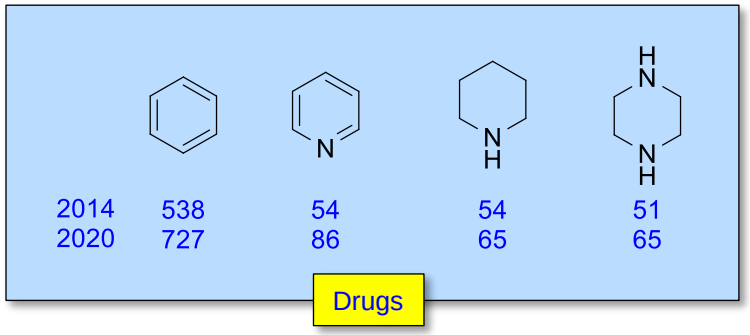


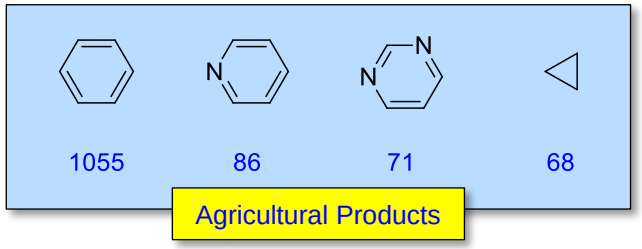
- Piperazines are widely used in drug design
  - common structural element in CNS-active compounds
  - not heavily utilized in agricultural chemistry
  - (4-NH_2)-piperidine a commonly-used homologue
- The 2 N atoms provide convenient handles for functionalization
  - basicity can be retained, modulated or sacrificed
- Versatile scaffolding element
  - defined exit vectors from N & C atoms
  - deployed to project pharmacophoric elements
- 3-Dimensional
  - equatorial or axial disposition of substituents
  - modulation of exit vectors by bridging elements
- Pharmacophoric element
  - typically relies upon protonation of the piperazine ring
  - most prominently for CNS applications
- Solubilizing element
  - relies upon a protonated basic N atom
  - common application in kinase inhibitors





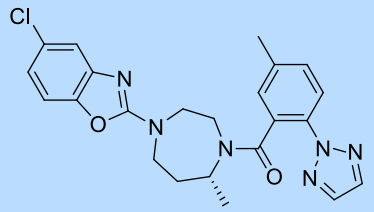
Prevalence of Piperazines: Drugs & Agricultural Products





- Rings are common in marketed drugs & agricultural products
  - phenyl & pyridine rings most prevalent in both fields
- Piperidine & piperazine have a similar prevalence to pyridine
  - 1/10th that of the phenyl ring in marketed drugs
- Piperazines & piperidines not common in agricultural chemistry
  - basic amines exhibit poor properties for agricultural products
- One marketed agricultural product contains a piperazine ring
  - the antifungal triforine: non-basic aminal
  - circumstance may be beginning to change

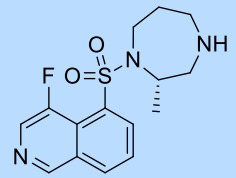
Drugs with homo-piperazine rings



suvorexant
dual orexin antagonist
insomnia

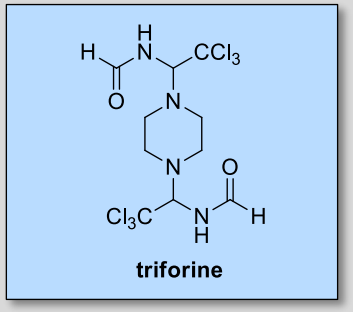
emedastine
H_1 antagonist
allergic conjunctivitis

fasudil
Rho-kinase inhibitor
vasodilator
pulmonary hypertension

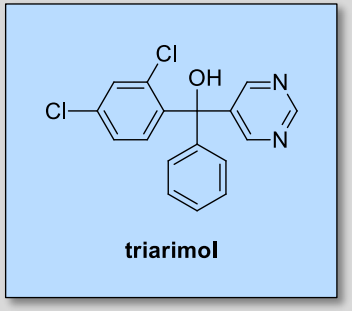


ripasudil
Rho-kinase inhibitor
glaucoma & ocular hypertension

- 4 Marketed drugs incorporate homo-piperazine rings



triforine



triarimol

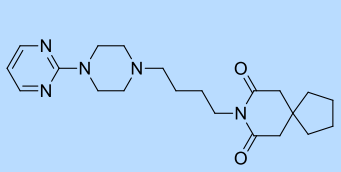
- Triforine marketed as a fungicide
  - symmetrically-substituted piperazine
  - interesting aminal derivative: non-basic
- Similar spectrum & mechanism to triarimol
  - inhibitors of ergosterol biosynthesis
  - steroid 14α demethylase inhibitor

Agricultural Products

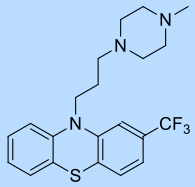
- 1 Marketed agricultural product incorporates a piperazine ring

R.D. Taylor *et al.*, *J. Med. Chem.,* 2014, **57**, 5845-5859; *J. Med. Chem.,* 2022, **65**, 8699-8712
N.A. Meanwell & O. Loiseleur, *J. Agric. Food Chem.,* 2022, **70**, 10942-10971; *J. Agric. Food Chem.,* 2022, **70**, 10972-11004

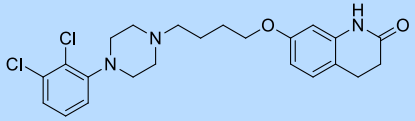
Drugs Incorporating a Piperazine Ring



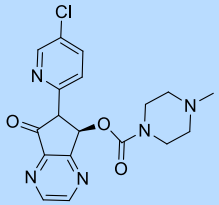
busprione
5-HT$_{1A}$ agonist
anxiolytic



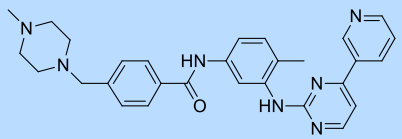
trifluoperazine
central dopamine &
adrenergic modulator
typical anti-psychotic



aripiprazole
dopamine receptor modulator
atypical anti-psychotic

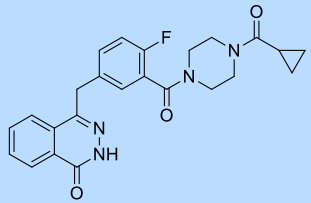


eszopicione
GABA$_A$ PAM
insomnia

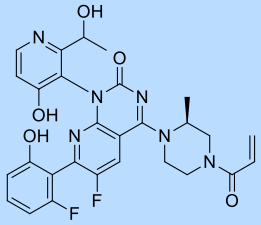


imatinib
BCR/Abl kinase inhibitor
chronic myelogenous leukemia

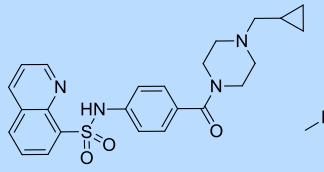
dasatinib
BCR/Abl kinase inhibitor
chronic myelogenous leukemia



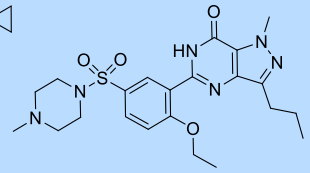
olaparib
PARP inhibitor
cancer



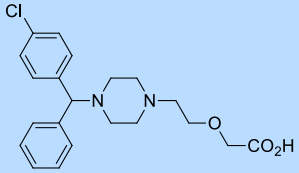
sotorasib
G12C K-Ras inhibitor
NSCLC



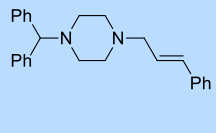
mitapivat
pyruvate kinase activator
hemolytic anemia



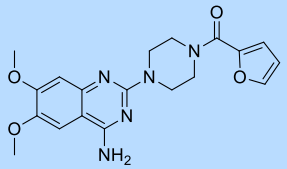
sildenafil
PDE5 inhibitor
erectile dysfunction



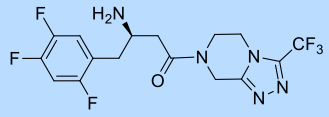
cetirizine
histamine H$_1$ antagonist
allergic rhinitis



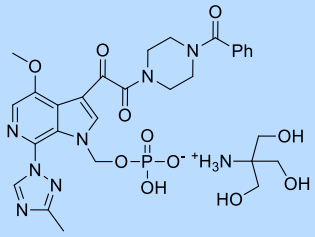
cinnarizine
anti-histamine/Ca^{2+}-blocker
asthma



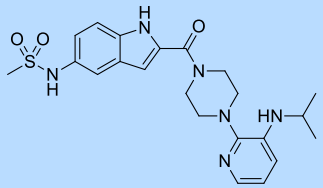
prazosin
α_1-adrenergic blocker
hypertension



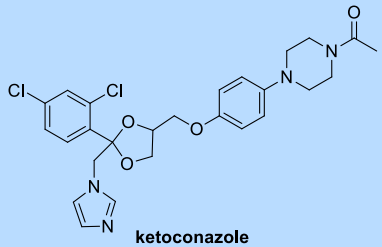
sitagliptin
DPP-4 inhibitor
diabetes



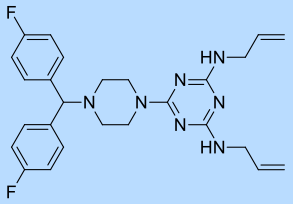
fostemsavir
HIV-1 attachment inhibitor
HIV-1 infection



delavirdine
HIV-1 NNRTI
HIV-1 infection



ketoconazole
ergosterol synthesis inhibitor
anti-fungal

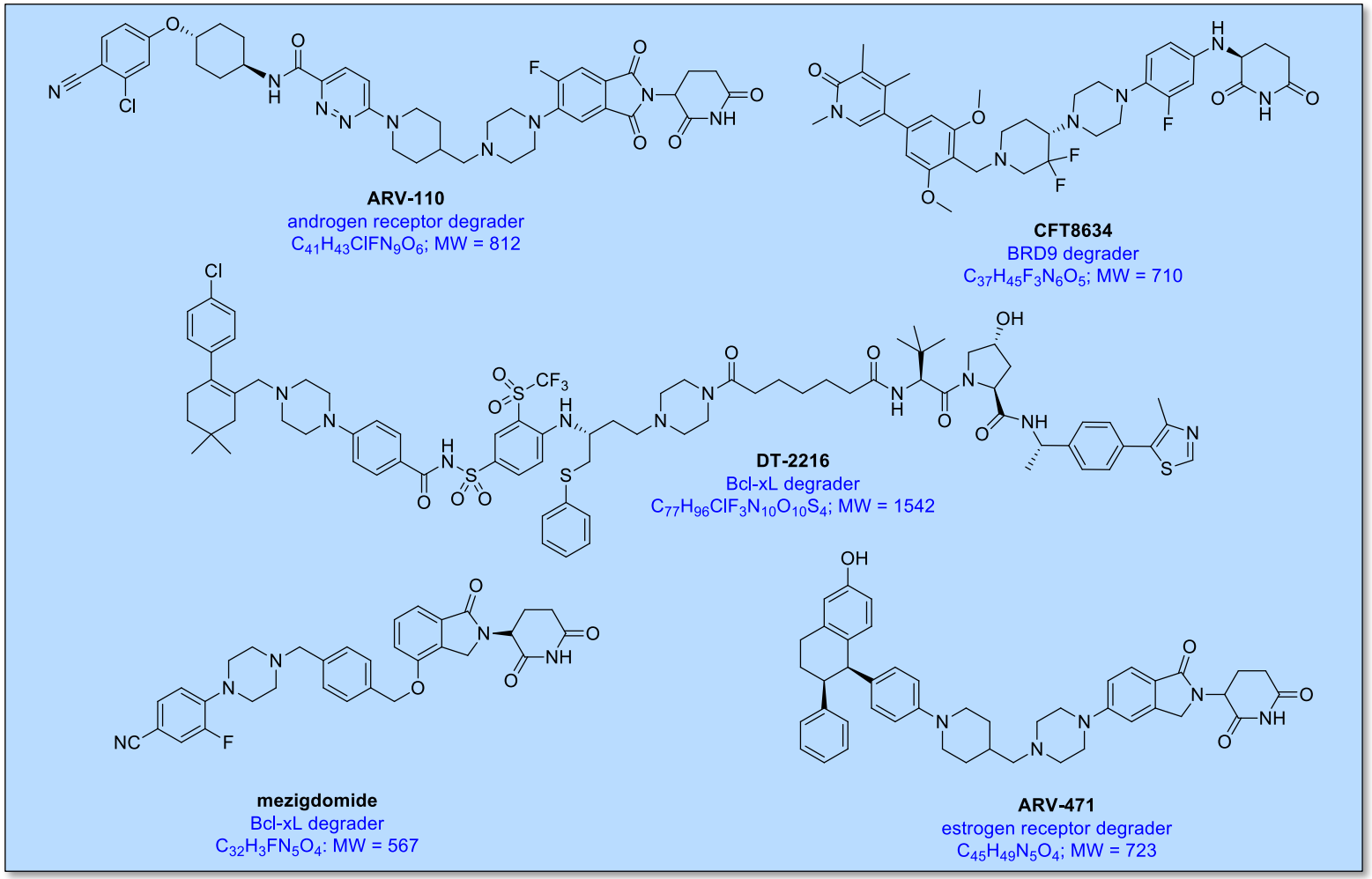


almitrine
respiratory stimulant
chronic obstructive pulmonary disease (COPD)

- Piperazine is a component of or embedded in a wide range of marketed drugs across all therapeutic areas
  - pharmacophore & scaffolding elements

M. Novella Romanelli *et al., Exp. Opin. Drug Discov*., 2022, **17**, 969-984; *Molecules*, 2024, **29**, 68; R.V. Patel *et al*., *Mini-Rev. Med. Chem.,* 2013, **13**, 1579-1601
M. Shaquiquzzaman *et al., Eur. J. Med. Chem*., 2015, **102**, 487-529

Piperazines & Piperidines are Prevalent in Degrader Linkers



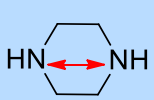
- Piperazines & piperidines are prominent linker elements in PROTAC & molecular glue protein degrader design
  - selectivity, basicity, solubility, PK property modulation

M.N. O'Brien Laramy *et al., Nat. Rev. Drug Discov.*, 2023, **22**, 410-427; L. Goracci *et al., RSC Adv.,* 2022, **12**, 21968

Piperazine & Piperazine Mimics

Geometries & *N*-*N* Distances

Geometries & *N*-*N* Distances

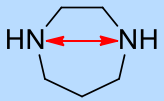


2.92 Å

piperazine

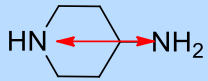
2.87 Å

2,5-diazabicyclo[2.2.1]heptane



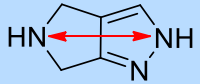
2.75-3.30 Å

homopiperazine



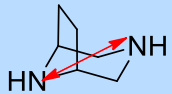
4.28 Å

4-aminopiperidine



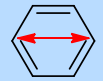
4.29 Å

2,4,5,6-tetrahydropyrrolo [3,4-*c*]pyrazole



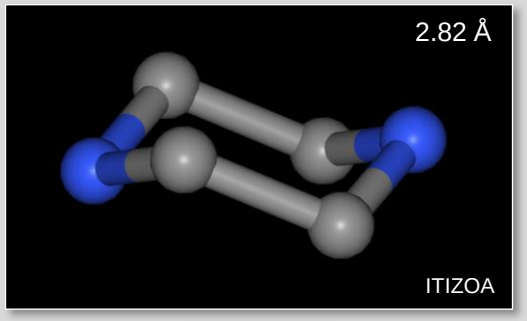
2.77-2.88 Å

3,8-diazabicyclo [3.2.1]octane

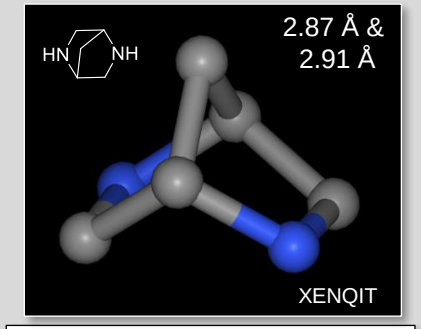


2.79 Å

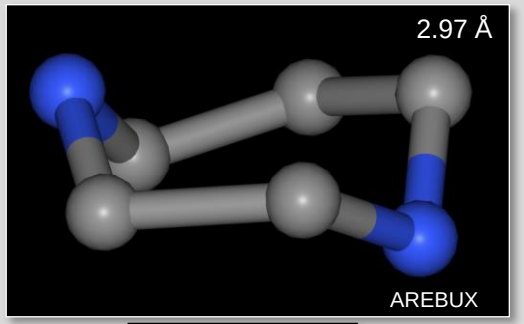
phenyl ring



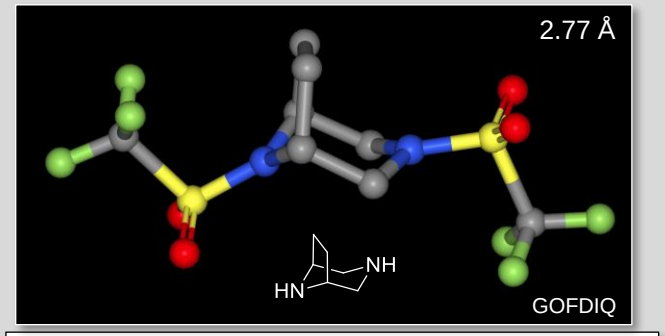
piperazine



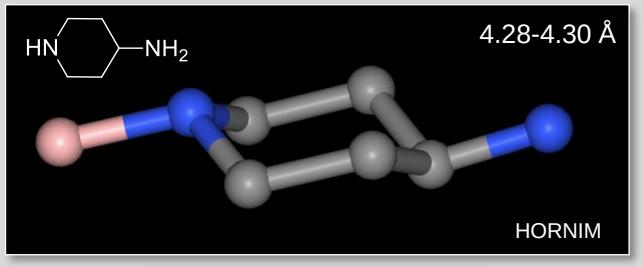
2,5-diazabicyclo[2.2.1]heptane



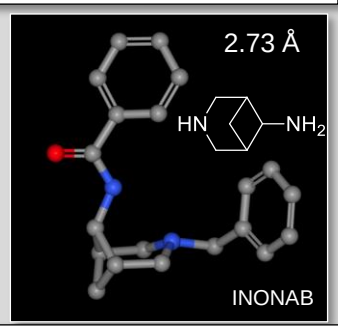
homopiperazine



3,8-bis((CF$_3$)sulfonyl)-3,8-diazabicyclo[3.2.1]octane



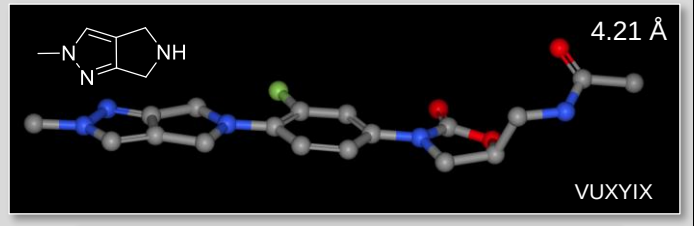
4-aminopiperidine borane



3-azabicyclo[3.1.1]heptan-6-amine

Key dimensions of piperazine & select bioisosteres

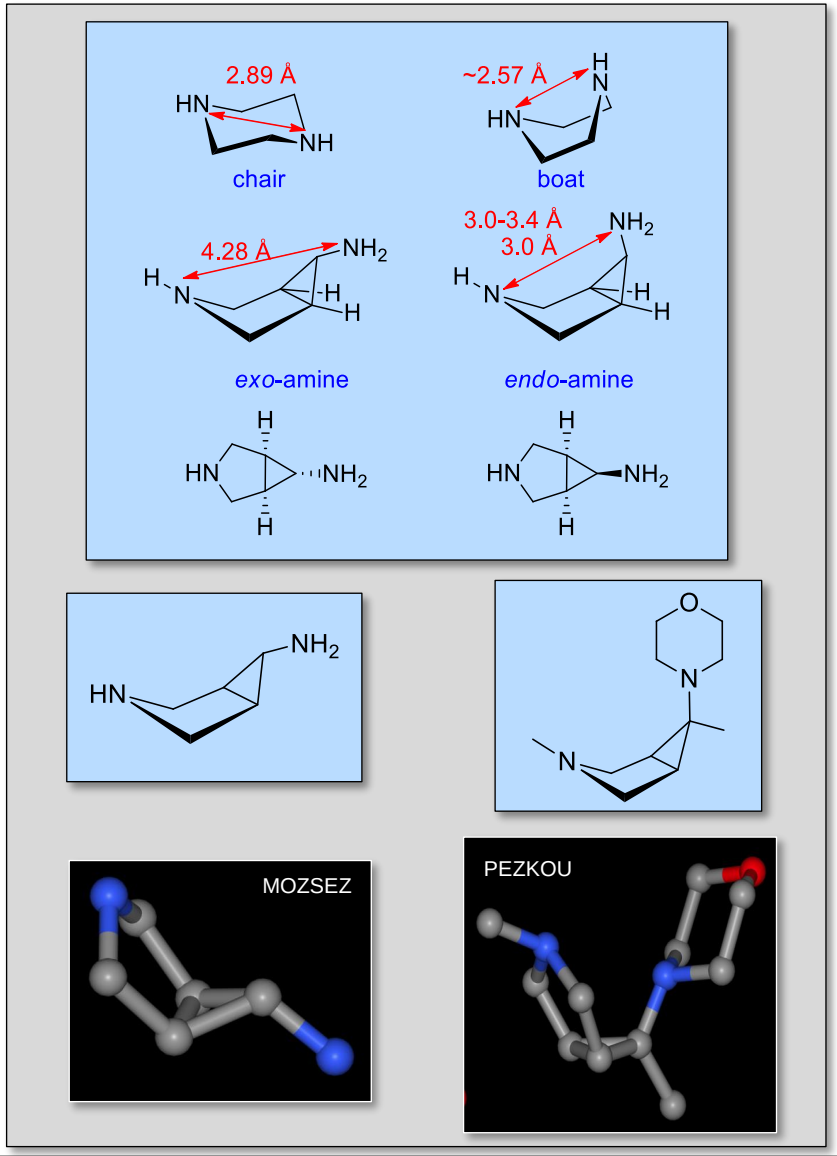
- *N*-*N* distances from X-ray structures

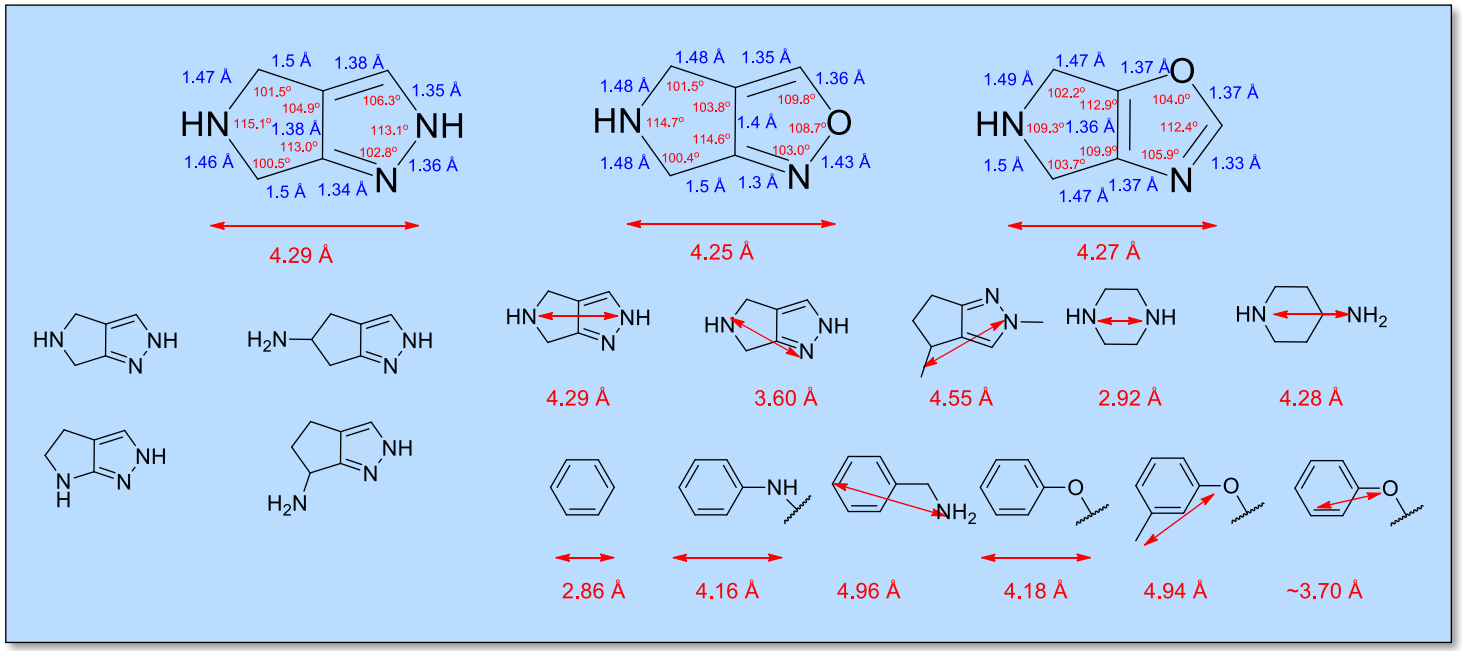


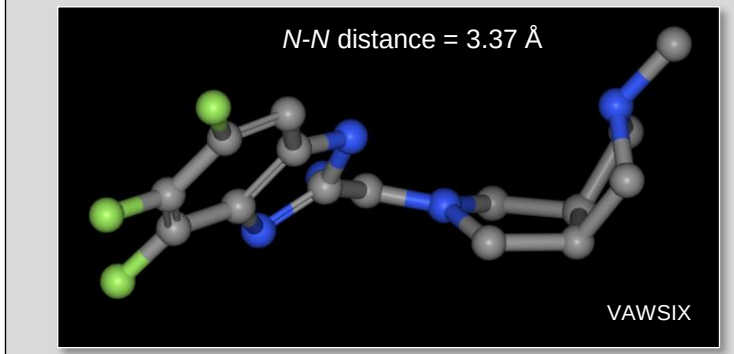
RWJ-416457
(2,4,5,6-tetrahydropyrrolo[3,4-*c*]pyrazole)

A. Parkin *et al., Acta Crystallogr., Sect. B: Struct. Sci.*, 2004, **60**, 219-227; S.N. Britvin *et al., Acta Crystallogr., Sect. E: Crystallogr. Commun.*, 2017, **73**, 1861-1865
R.A. Aitken *et al., Molbank*, 2021, **2021**, M1200; B.A. Shainyan *et al*., *Tetrahedron*, 2014, **70**, 4547-4551; A.V. Denisenko *et al*., *Org. Lett.*, 2010, **12**, 4372-4375

Pyrrolidine Derivatives: Some Key Dimensions



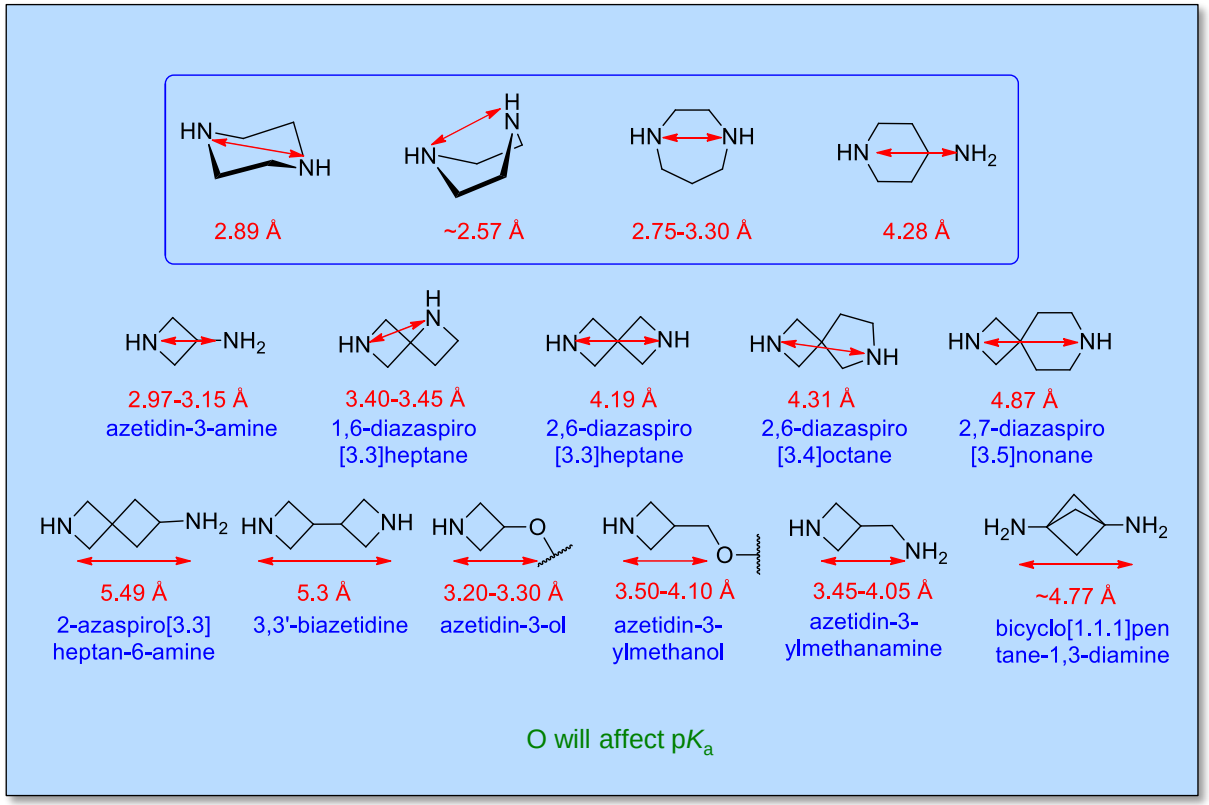




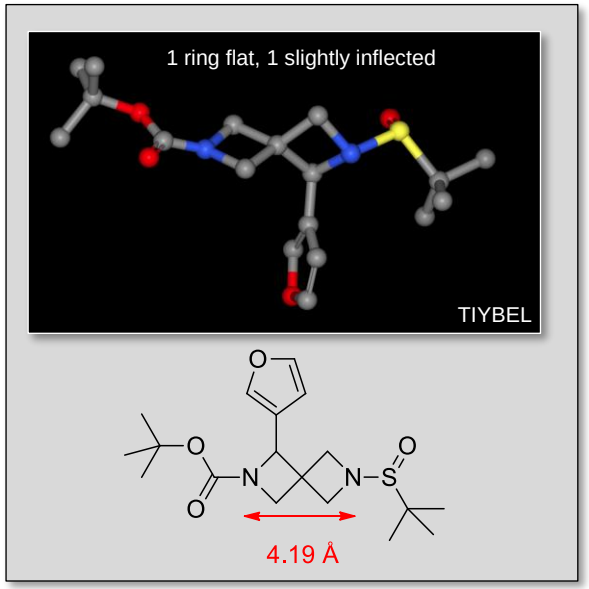
Not quite a full *endo-endo:* one ring is flattened: reflects conformational flexibility

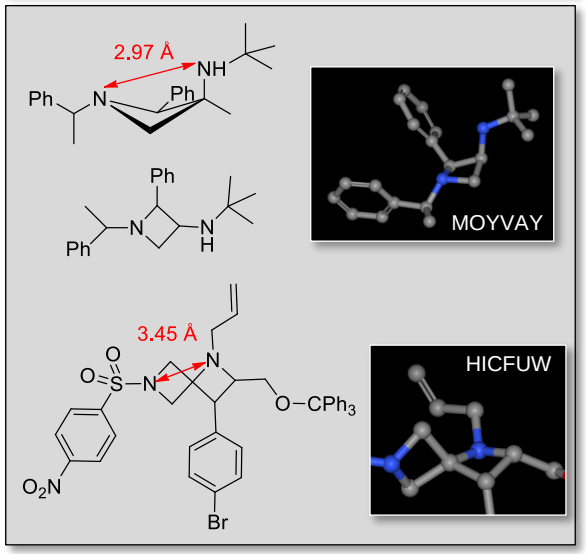
A. de Meijere *et al*., *Chem. Eur. J.,* 2002, **8**, 3789-3801; V. Butz *et al., J. Chem. Soc. Perkin Trans. 2,* 1993, 1907-1913; A. Osipova *et al., Synthesis,* 2007, 131-139
C.A.L. Lane *et al., Bioorg. Med. Chem. Lett.,* 2012, **22**, 1156-1159; N.A. Meanwell & R. Sistla, *Adv. Het. Chem.,* 2021, **134**, 31-100

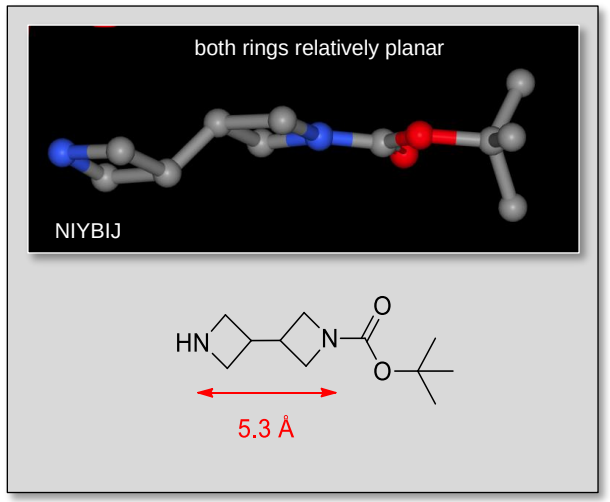
Azetidine Derivatives - Geometries & *N-N* Distances

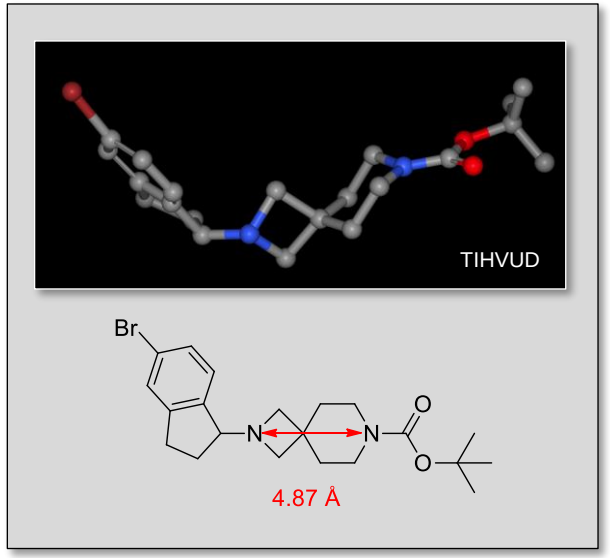


- Key dimensions of azetidine-based piperazine bioisosteres
  - 3-amino-azetidine offers a similar *N-N* bond distance to piperazine
- Spiro[3.3]heptane & spiro[3.4]octane comparable to 4-NH_2-piperidine
  - azetidine-3-ylmethanamine similar
- Offer unique conformational presentations of the 2 N atoms
  - bespoke exit vectors from N & ring atoms



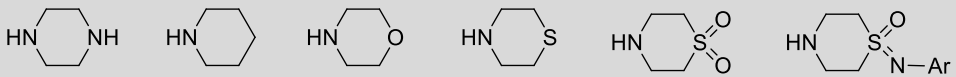






L.R. Reddy *et al., Org. Lett.* 2019, **21**, 3481–3484; O. Feskov *et al., J. Org. Chem.,* 2019, **84**, 1363-1371; J.T. Lowe *et al.*, *J. Org. Chem.*, 2012, **77**, 7187-7211

pK_a Data



pK_a = 9.7
pK_a = 5.4

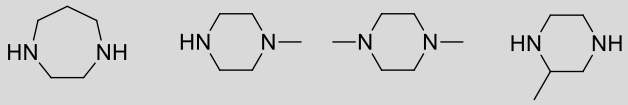
pK_a = 11.1

pK_a = 8.5

pK_a = 9.0

pK_a = 5.4

pK_a = 5.2

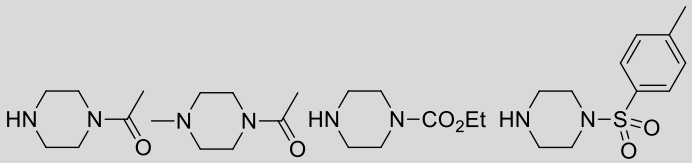


pK_a = 10.4

pK_a = 9.1
pK_a = 4.6

pK_a = 8.4
pK_a = 3.8

pK_a = 9.6
pK_a = 3.8

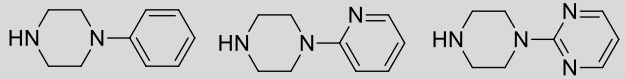


pK_a = 7.9

pK_a = 7.1

pK_a = 8.2

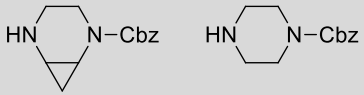
pK_a = 7.4



pK_a = 9.0

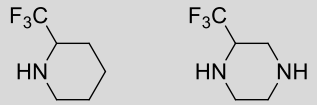
pK_a = 8.9

pK_a = 8.9



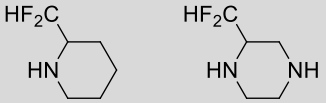
pK_a = 6.7

pK_a = 8.1



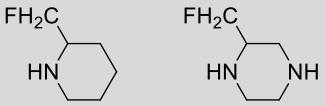
pK_a = 6.41
Δ = 4.69

cpK_a = 5.1



pK_a = 7.99
Δ = 3.11

cpK_a = 6.59



pK_a = 9.52
Δ = 1.59

cpK_a = 8.11

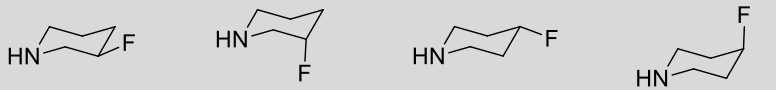
Fluorination modulates pK_a

pK_a = 11.1

pK_a = 9.4

pK_a = 8.5

pK_a = 7.4

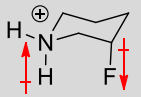


cpK_a = 8.8
pK_a exp = 7.4

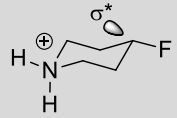
cpK_a = 9.7

cpK_a = 9.4

cpK_a = 10.2
pK_a exp = 8.5

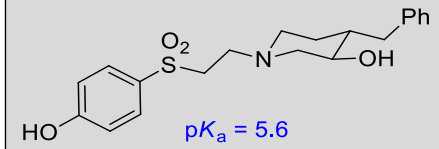


stabilizes protonated state
pK_a higher than anticipated

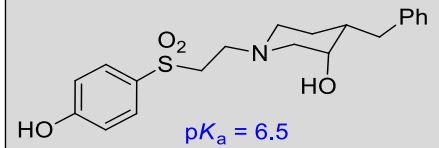


better aligned C-X bond dipole
or pronounced through-bond σ-conjugative transmission
through properly aligned σ*C-X
*intervening σC-C orbitals

- pK_a values in piperazines sensitive to substitution
  - modulated by both ring & N substituents
- pK_a values in piperidines modulated by substitution
  - predictable based on electron withdrawing effects
    - relationship with N
- Effects of ring substituents on pK_a depend on disposition
  - axial substituent effects less pronounced for F & OH

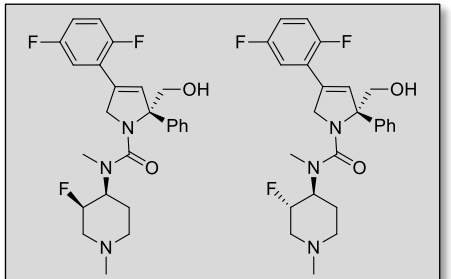


pK_a = 5.6



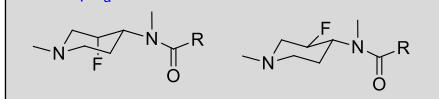
pK_a = 6.5

axial OH disposition exerts
lower effect on pK_a value



Axial F
pK_a 1 log unit lower
pK_a = 7.6

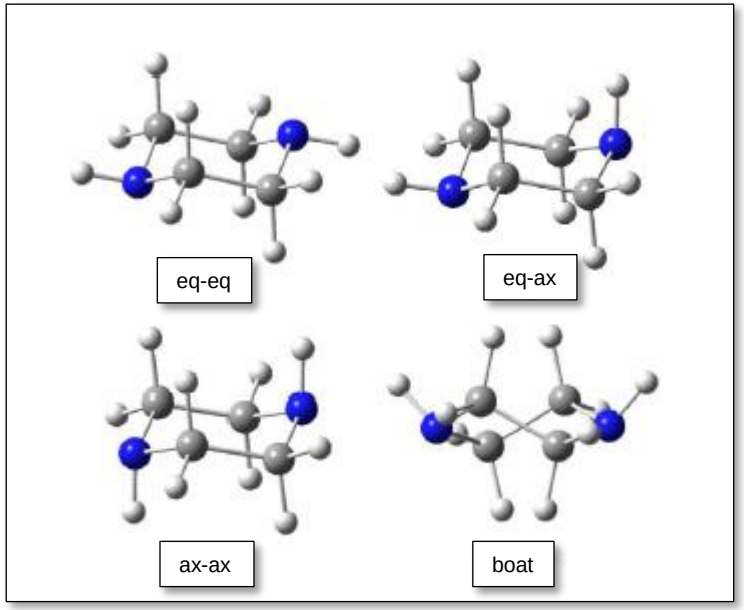
Equatorial F
pK_a 2 log units lower
pK_a = 6.6



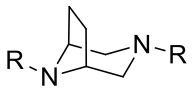
Equatorial F & OH lower pK_a
more than axial isomers

A. Henni *et al., J. Chem. Eng. Data,* 2009, **54**, 2914-2917; K. Müller *et al., ChemMedChem*, 2007, **2**, 1100-1115; K.P. Melnykov *et al., Chem. Eur. J.*, 2023, **28**, e202201601
C. Gnamm *et al., Eur. J. Med. Chem.*, 2017, **126**, 225-245; C.D. Cox *et al., J. Med. Chem.,* 2008, **51**, 4239-4252; *Bioorg. Med. Chem. Lett.,* 2007, **17**, 2697-2702

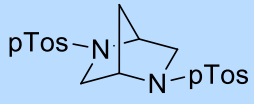
Piperazine Conformation

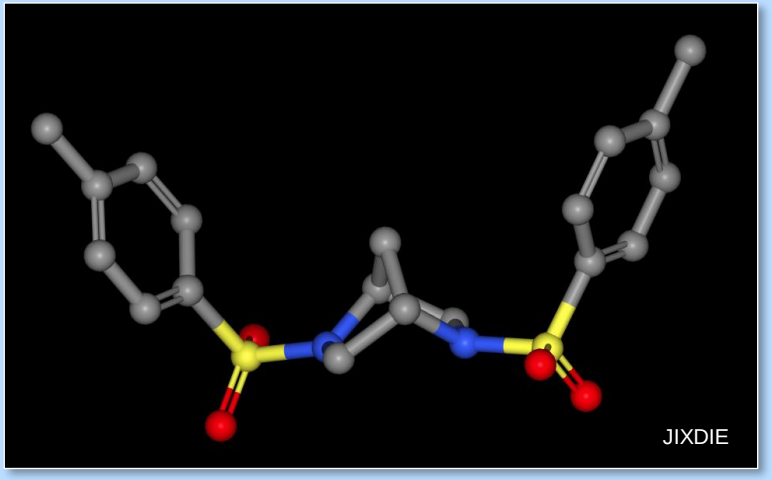


- Chair, boat, twist boat, half boat conformations
  - modulated by both ring & N substituents
- For chair, N-substituents can be axial or equatorial
  - eq-eq, eq-ax, ax-ax combinations
- Conformation modulated by bridging rings, fused rings
  - 1C Cα-Cα bridge to different N affords norbornane topography
  - 1C Cα-Cα bridge to same N retains chair conformation
- Fusing a cyclopropane promotes half-chair conformation
- Substituents can affect conformational preferences
  - reciprocal effects

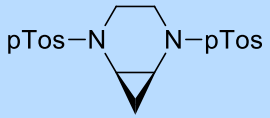


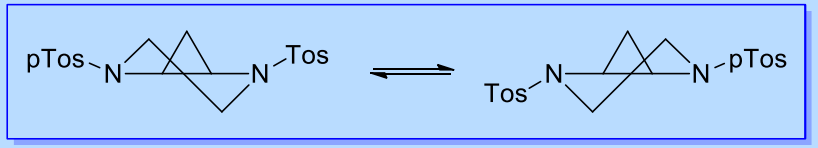
Norbornane conformation
Bridged boat

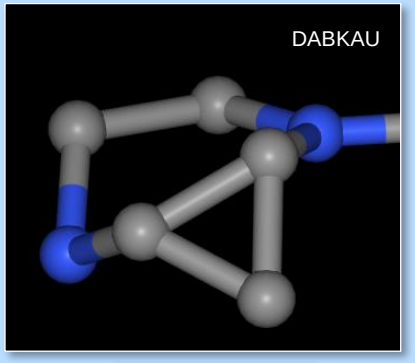




Half-chair conformation favored



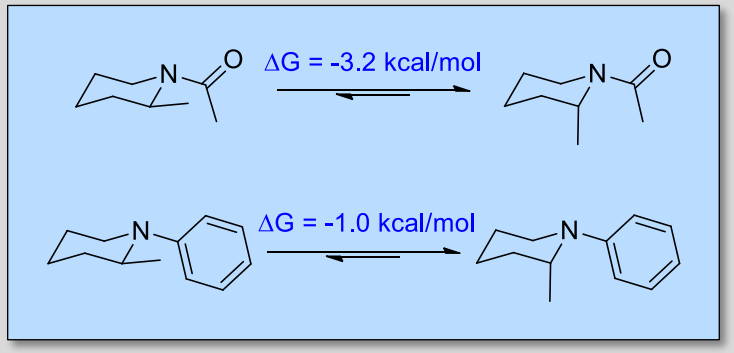


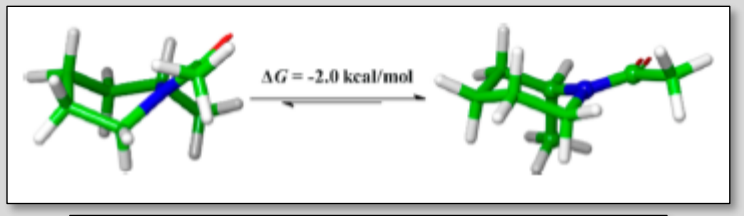


in a quinolone

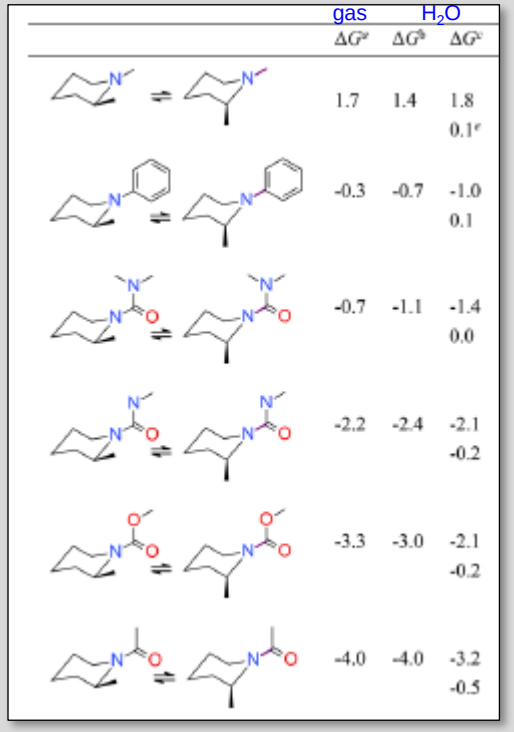
S. SenGupta *et al., Chem. Phys*. 2014, **436-437**, 55-62; R. Melgar-Fernández *et al., Eur. J. Org. Chem*., 2008, 655-672
R.R.R. Taylor *et al., Tetrahedron,* 2010, **66**, 3370-3377; M.W. Majchrzak *et al., J. Het. Chem.,* 1983, **20**, 815-817

2-Substituted Piperidine/Piperazine Conformation

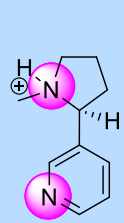




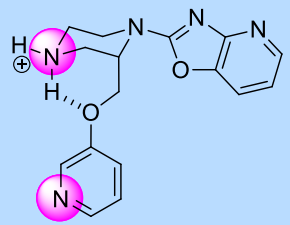
Twist boat is 2.0 kcal/mol less stable than chair



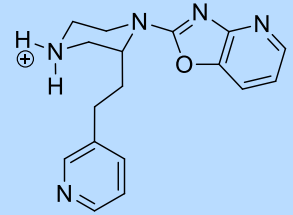
α-7 nicotinic ACh receptor agonists



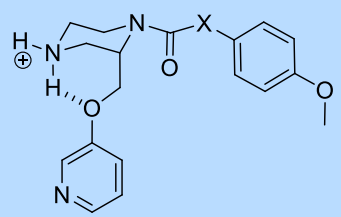
pharmacophoric elements



α7 IC_{50} = 6 nM

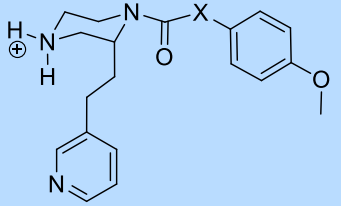


α7 IC_{50} ND



X = NH: α7 IC_{50} = 19 nM
X = O: α7 IC_{50} = 11 nM

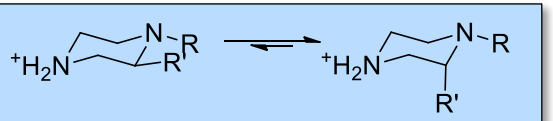
>250x
>450x



X = NH: α7 IC_{50} >5,000 nM
X = O: α7 IC_{50} >5,000 nM

- 2-Substituent prefers an axial orientation
  - piperazines & piperidines
  - ΔG varies, dependent on substituent identity
    - more pronounced in the gas phase
- Pseudo-allylic-1,3-strain favors axial in N-acyl piperidines
  - intramolecular H-bonding to N-H can play a role in piperazines

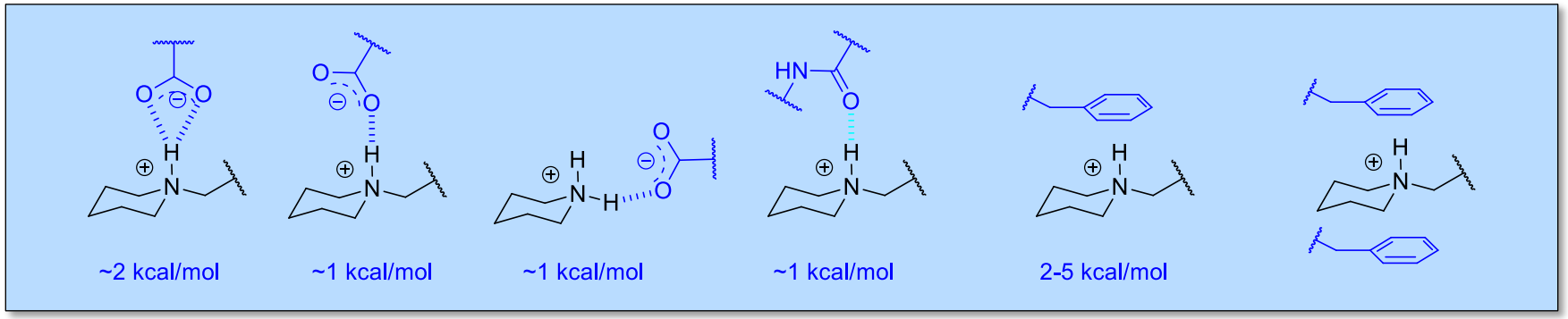
- Axial alkoxy-CH_2 favored over equatorial
  - in gas phase & solvent
- Stabilized by intramolecular H-bond
  - to protonated N atom
- Conformation affects potency at α7
  - axial disposition a potent ligand



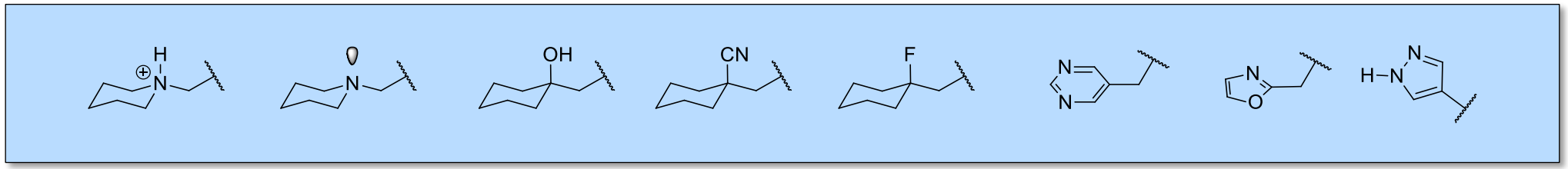
R' = alkyl	1.0-2.4 kcal/mol in gas phase 0.32-3.4 kcal/mol in solvent
R' = alkoxy-CH_2	5.7-9.6 kcal/mol in gas phase 2.5-6.4 kcal/mol in solvent

H. Zhao, *ACS Omega*, 2022, **7**, 9080-9085; E.A. Kallel *et al., Bioorg. Med. Chem. Lett.*, 2016, **26**, 3010-3013

Amine-Protein Interactions



- Salt bridge amounts to ~2 kcal/mol
  - H-bonds are ~1 kcal/mol
- π-cation interaction can be a strong contributor
  - 2-5 kcal/mol
- Many π-cation interactions engage 2 ring systems
  - histone deacetylases, cholinesterases

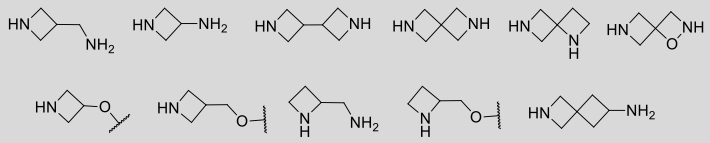


- Established & potential amine mimics

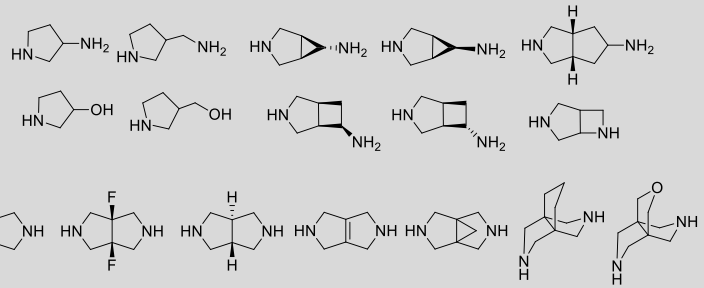
R. Pellicciari *et al., J. Chem. Inf. Model.,* 2009, **49**, 900-912; D.A. Dougherty, *Acc. Chem. Res.,* 2013, **46**, 885-893; F. Diederich *et al., Angew. Chem. Int. Ed*., 2011, **50**, 4808-4842

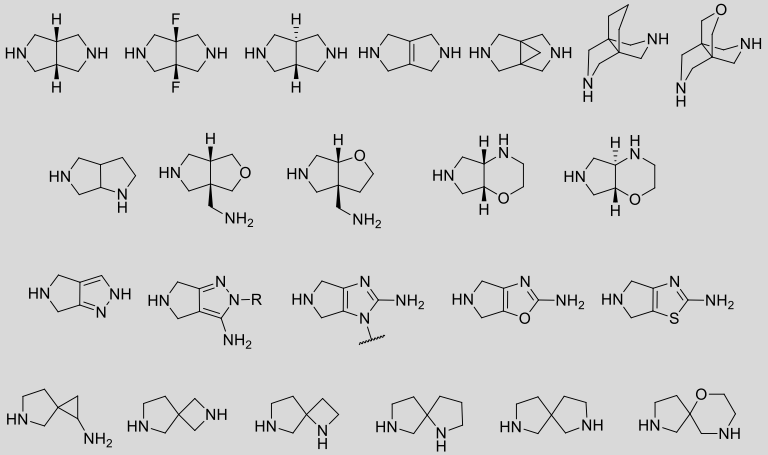
A Synopsis of Piperazine Mimics

azetidine-based piperazine mimics

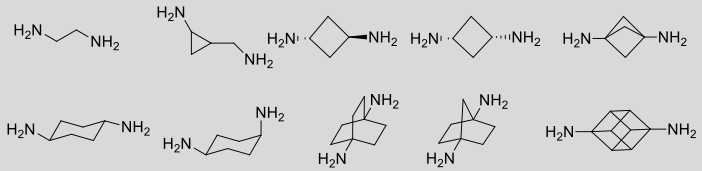


pyrrolidine-based piperazine mimics

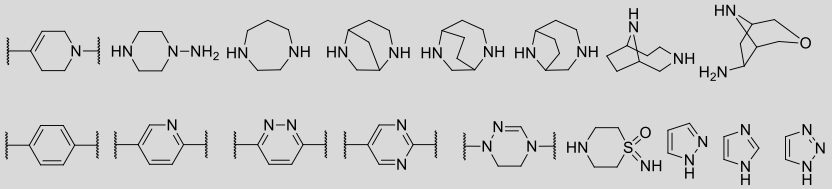




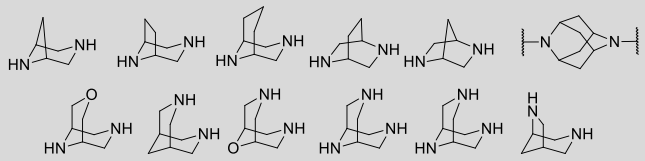
diamino alkanes and cycloalkanes



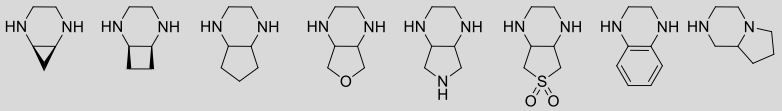
miscellaneous



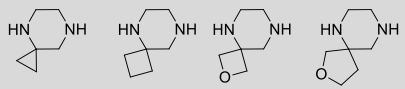
bridged piperazines



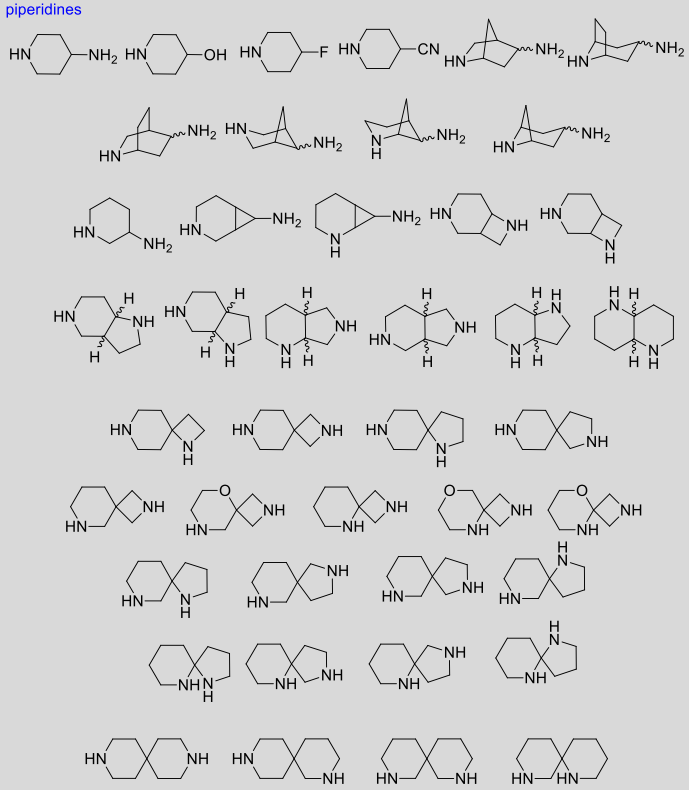
fused piperazines

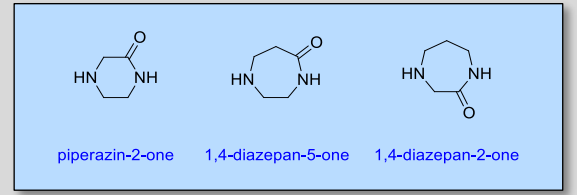


spiro piperazines



piperidines





The mimicry between a basic N atom, OH, F & C≡N substituents in the context of piperidine derivatives can, in principle, be exported to other motifs.

◆ Application will be context-dependent
- pharmacophore or scaffold

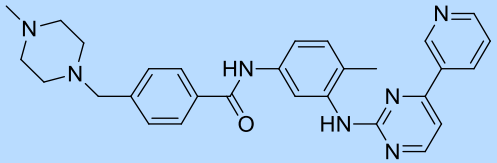
Amide homologues add to diversity & property modulation

N.A. Meanwell & O. Loiseleur, *J. Agric. Food Chem.*, 2022, **70**, 10942-10971; *J. Agric. Food Chem.*, 2022, **70**, 10972-11004

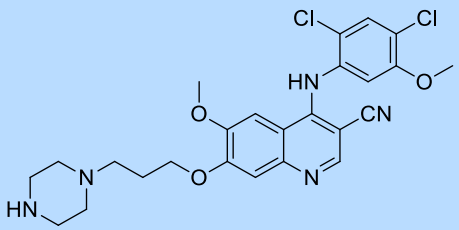
Applications of Piperazines & Piperazine Mimics in Drug Design

Applications of Piperazines: Solubilizing Element

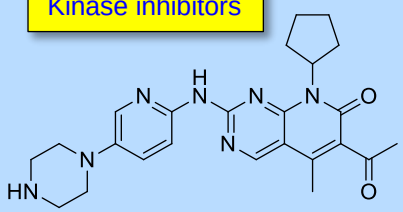
Kinase inhibitors



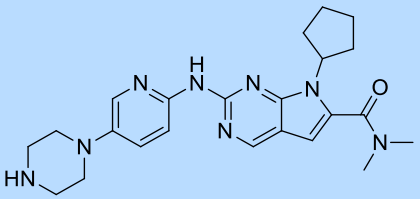
imatinib
IC_{50} = 370 nM
Sol. = 30.7 μM at pH = 7.4



bosutinib
Sol. = 9.4 μg/mL at pH = 8
Sol. = 53 mg/mL at pH = 2

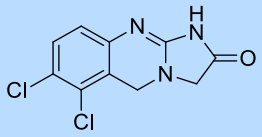


palbociclib
Sol. >0.7 mg/mL at pH = 4
Sol. <0.002 mg/mL at pH = 9

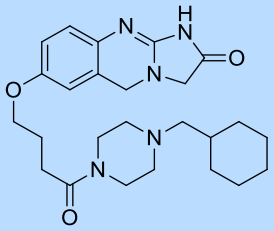


ribociclib
Sol. >2.4 mg/mL at pH = 4
Sol. = 0.08 mg/mL at pH = 6.8

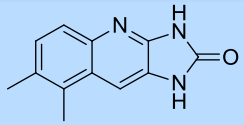
cAMP PDE 3 inhibitors



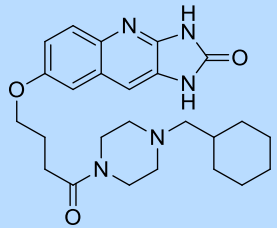
anagrelide
slightly soluble in H_2O
sparingly soluble in DMSO
mp indistinct
IC_{50} ADP-induced platelet aggregation = 1.05 μM



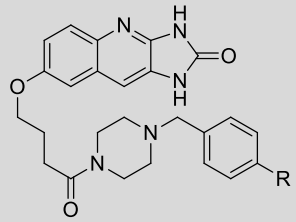
solubility of diHCl salt >5 mg/mL
mp = indistinct
IC_{50} ADP-induced platelet aggregation = 7.2 μM



BMY-20844
slightly soluble in H_2O
mp ~415 °C
IC_{50} ADP-induced platelet aggregation = 200 nM



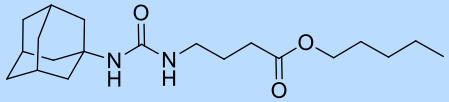
BMY-43351
solubility of diHCl salt >10 mg/mL
mp 258-260 °C
IC_{50} ADP-induced platelet aggregation = 510 nM



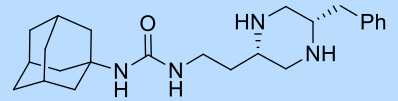
R	H	F	Cl
ADP IC_{50} (nM)	170	530	170
Sol. (mg/mL)	>10	>10	<10
π	-	0.14	0.71

Single Cl atom reduced solubility

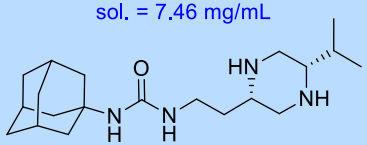
- Urea-based sEH inhibitors
  - high melting solids
  - poor solubility
- Explored 2º pharmacophore
  - introduced piperazines
  - solubility increased 3-4x
  - potency fell by 10x



hsEH IC_{50} = 0.17 μM
mp = 114 °C
sol. = 1.69 mg/mL



hsEH IC_{50} = 1.37 μM
mp = 77-78 °C
sol. = 7.46 mg/mL

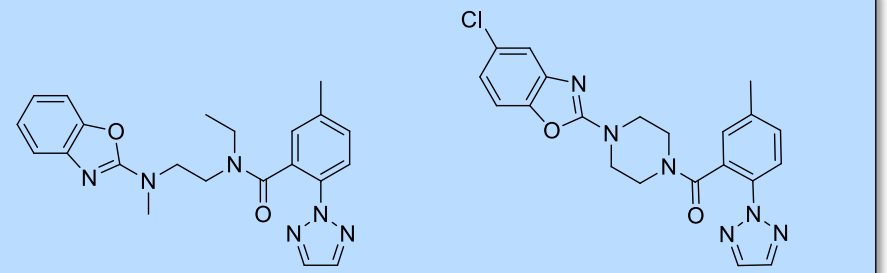


hsEH IC_{50} = 12.6 μM
mp = 69-70 °C
sol. = 8.11 mg/mL

- Solubilizing element
  - relies upon a protonated basic N atom
  - common application in kinase inhibitors
- Effective in PDE-3 inhibitors
  - high melting solids: brick dust
  - inherently poorly soluble

M. Vieth *et al.*, *J. Med. Chem.,* 2009, **52**, 6456-6466; J. Green *et al., Bioorg. Med. Chem. Lett.,* 2018, **28**, 2616-2621
N.A. Meanwell *et al., J. Med. Chem.,* 1992, **35**, 2688-2696; Y.-Q. Long *et al, Bioorg. Med. Chem.,* 2006, **14**, 6586-6592

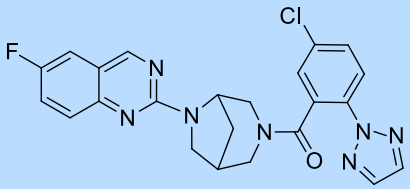
Orexin Antagonists for Insomnia - Conformation



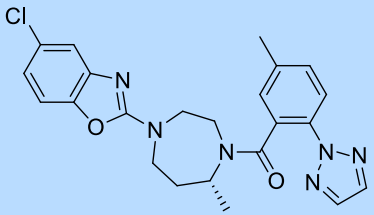
hOX_1 IC_{50} = 13 nM
hOX_2 IC_{50} = 18 nM

hOX_1 IC_{50} = 1,651 nM
hOX_2 IC_{50} = 1,831 nM

OX_1R K_i = 1.2 nM
OX_2R K_i = 0.6 nM
YOYJEC

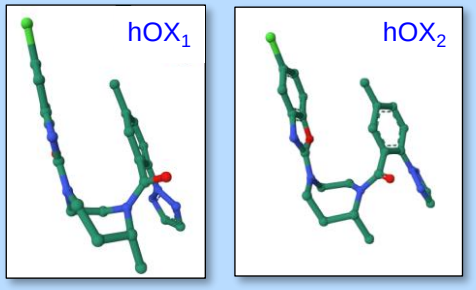


OX_1R K_i = 0.4 nM
OX_2R K_i = 0.6 nM
WUPJUN02

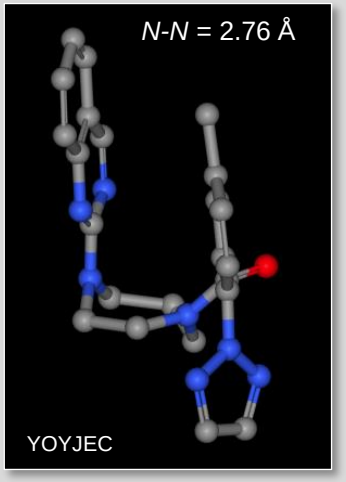


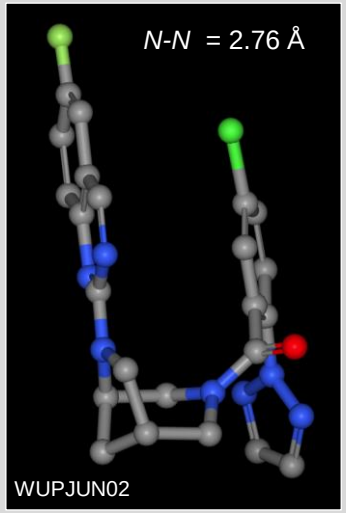
suvorexant
OX_1R K_i = 0.55 nM
OX_2R K_i = 0.35 nM

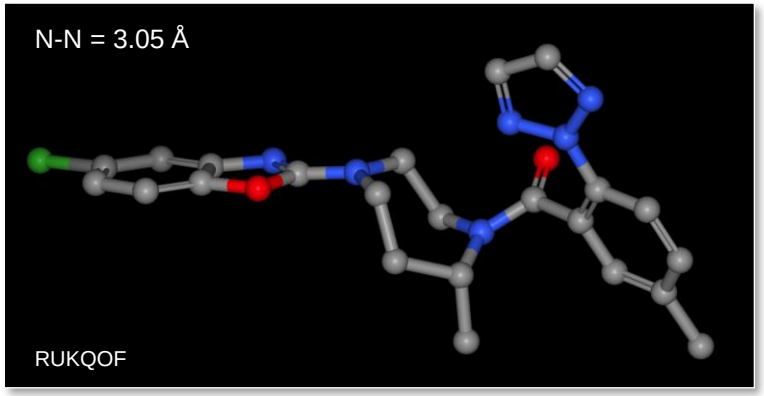
3000x piperazine

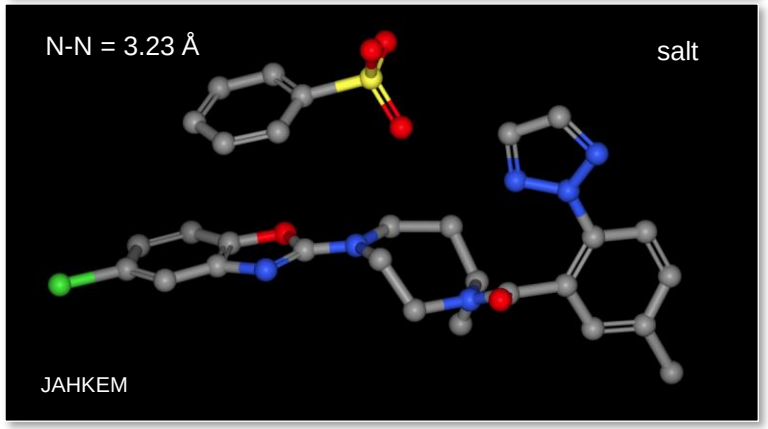


Suvorexant in cocrystals







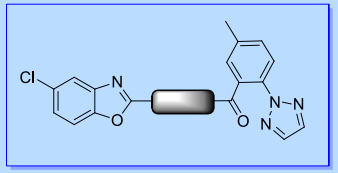


- Conformational analysis of orexin antagonists
  - homopiperazine derivatives
  - U-shaped topography recognized by the receptor
    - N-N distance of 2.76 Å
  - can crystallize in more planar conformation
- Confirmed with 3,6-diazabicyclo[3.2.1]octane
  - set the stage for the discovery of suvorexant

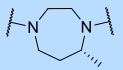
C.D. Cox *et al., Bioorg. Med. Chem. Lett.*, 2009, **19**, 2997-3001; P.J. Coleman *et al.*, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 2311-2315
S. Gundlapalli *et al.*, *CrystEngComm*, 2021, **23**, 7739-7749; G. McGaughey *et al.*, *J. Comput.-Aided Molec. Des.*, 2014, **28**, 5-12

Orexin Antagonists – An Extensive Survey of Diamine Mimics

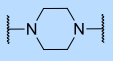
- 3,8-Diazabicyclo[4.2.0]octane optimal for dual inhibition
  - active in both topologies
- hOX_1 selectivity achieved with bicycles
  - 3,7-diazabicyclo[3.3.1]nonane
  - 9-oxa-3,7-diazabicyclo[3.3.1]nonane



- Spiro bicycles also afforded hOX_1 selectivity
  - 2,7-diazaspiro[4.5]decane
  - 2,6-diazaspiro[4.5]decane

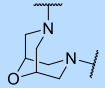


hOX_1 IC$_{50}$ = 1.0 nM
hOX_2 IC$_{50}$ = 2.8 nM
suvorexant



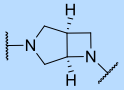
hOX_1 IC$_{50}$ = 1,651 nM
hOX_2 IC$_{50}$ = 1,831 nM

hOX_1 IC$_{50}$ = 6.5 nM
hOX_2 IC$_{50}$ = 542 nM

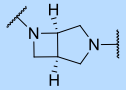


hOX_1 IC$_{50}$ = 163 nM
hOX_2 IC$_{50}$ = >6,460 nM

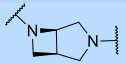
hOX_1 selective



hOX_1 IC$_{50}$ = 1,742 nM
hOX_2 IC$_{50}$ = 3,100 nM
racemic

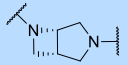


hOX_1 IC$_{50}$ = 13 nM
hOX_2 IC$_{50}$ = 25 nM
racemic

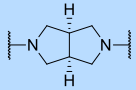


hOX_1 IC$_{50}$ = 5.1 nM
hOX_2 IC$_{50}$ = 17 nM

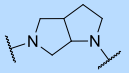
potent



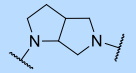
hOX_1 IC$_{50}$ = 1,880 nM
hOX_2 IC$_{50}$ = 2,935 nM



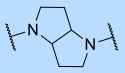
hOX_1 IC$_{50}$ = 71 nM
hOX_2 IC$_{50}$ = 64 nM



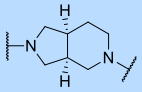
hOX_1 IC$_{50}$ = 1,030 nM
hOX_2 IC$_{50}$ = 544 nM



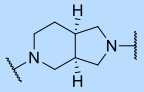
hOX_1 IC$_{50}$ = 118 nM
hOX_2 IC$_{50}$ = 119 nM



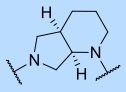
hOX_1 IC$_{50}$ = 1,556 nM
hOX_2 IC$_{50}$ = 2,375 nM



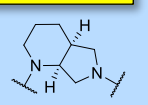
hOX_1 IC$_{50}$ = 386 nM
hOX_2 IC$_{50}$ = 746 nM



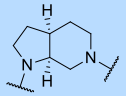
hOX_1 IC$_{50}$ = 125 nM
hOX_2 IC$_{50}$ = 137 nM



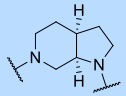
hOX_1 IC$_{50}$ = 1,548 nM
hOX_2 IC$_{50}$ = 528 nM



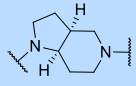
hOX_1 IC$_{50}$ = 2,249 nM
hOX_2 IC$_{50}$ = 1,118 nM



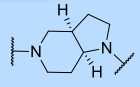
hOX_1 IC$_{50}$ = 26 nM
hOX_2 IC$_{50}$ = 45 nM



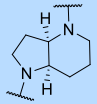
hOX_1 IC$_{50}$ = 204 nM
hOX_2 IC$_{50}$ = 290 nM



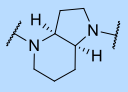
hOX_1 IC$_{50}$ = 669 nM
hOX_2 IC$_{50}$ = 1,048 nM



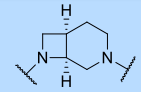
hOX_1 IC$_{50}$ = 1,925 nM
hOX_2 IC$_{50}$ = 1,315 nM



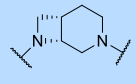
hOX_1 IC$_{50}$ = 1,343 nM
hOX_2 IC$_{50}$ = 915 nM



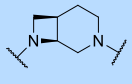
hOX_1 IC$_{50}$ = 2,242 nM
hOX_2 IC$_{50}$ = 912 nM



hOX_1 IC$_{50}$ = 1.7 nM
hOX_2 IC$_{50}$ = 7.5 nM
racemic

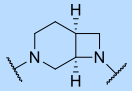


hOX_1 IC$_{50}$ = 0.9 nM
hOX_2 IC$_{50}$ = 2.9 nM

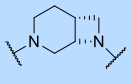


hOX_1 IC$_{50}$ = 474 nM
hOX_2 IC$_{50}$ = 1251 nM

potent

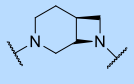


hOX_1 IC$_{50}$ = 22 nM
hOX_2 IC$_{50}$ = 31 nM

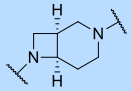


hOX_1 IC$_{50}$ = 12 nM
hOX_2 IC$_{50}$ = 17 nM

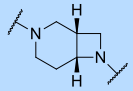
potent



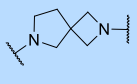
hOX_1 IC$_{50}$ = 668 nM
hOX_2 IC$_{50}$ = 3,240 nM



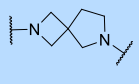
hOX_1 IC$_{50}$ = 163 nM
hOX_2 IC$_{50}$ = 2,080 nM



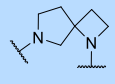
hOX_1 IC$_{50}$ = 359 nM
hOX_2 IC$_{50}$ = 995 nM



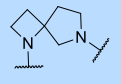
hOX_1 IC$_{50}$ = 1,643 nM
hOX_2 IC$_{50}$ = 1,415 nM



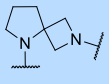
hOX_1 IC$_{50}$ = 464 nM
hOX_2 IC$_{50}$ = 722 nM



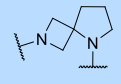
hOX_1 IC$_{50}$ = 127 nM
hOX_2 IC$_{50}$ = 1,253 nM



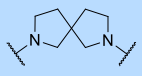
hOX_1 IC$_{50}$ = 1,403 nM
hOX_2 IC$_{50}$ = 1,008 nM



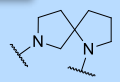
hOX_1 IC$_{50}$ = 281 nM
hOX_2 IC$_{50}$ = 117 nM



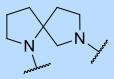
hOX_1 IC$_{50}$ = 291 nM
hOX_2 IC$_{50}$ = 528 nM



hOX_1 IC$_{50}$ = 494 nM
hOX_2 IC$_{50}$ = 995 nM



hOX_1 IC$_{50}$ = 1,270 nM
hOX_2 IC$_{50}$ = 392 nM



hOX_1 IC$_{50}$ = 892 nM
hOX_2 IC$_{50}$ = 895 nM

hOX_1 IC$_{50}$ = 1,270 nM
hOX_2 IC$_{50}$ = 2,350 nM

hOX_1 IC$_{50}$ = 1,190 nM
hOX_2 IC$_{50}$ = 960 nM

hOX_1 IC$_{50}$ = 8.6 nM
hOX_2 IC$_{50}$ = 514 nM

hOX_1 selective

hOX_1 IC$_{50}$ = 212 nM
hOX_2 IC$_{50}$ = 1,039 nM

hOX_1 IC$_{50}$ = 1.4 nM
hOX_2 IC$_{50}$ = 47 nM

hOX_1 selective

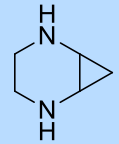
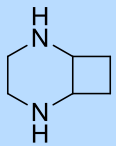
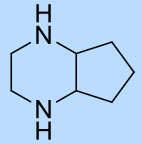
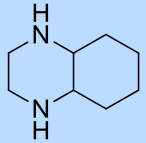
hOX_1 IC$_{50}$ = 292 nM
hOX_2 IC$_{50}$ = 1,200 nM

hOX_1 IC$_{50}$ = 2,223 nM
hOX_2 IC$_{50}$ = 1,858 nM

hOX_1 IC$_{50}$ = 1,087 nM
hOX_2 IC$_{50}$ = 1,451 nM

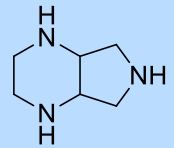
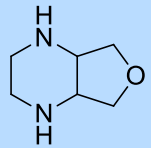
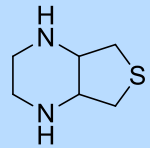
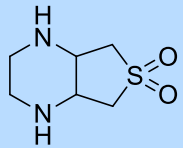
B. Heidmann *et al., ChemMedChem,* 2016, **11**, 2132-2146

Piperazines With Fused Rings – Carbon & Heterocycles

			
2,5-diazabicyclo [4.1.0]heptane	2,5-diazabicyclo [4.2.0]octane	octahydro-1*H*-cyclopenta [*b*]pyrazine	decahydroquinoxaline
92 references 11 journal articles	5 references – all patent applications	32 references, 18 with biological application – all patent applications	60 references in SciFinder,34 journals - ~1/2 with biological application

- Piperazines with fused carbocycles have a reasonable literature presence
  - examined in the context of biologically active compounds
  - many exemplified in patent applications

- Piperazines with fused heterocycles have been studied only sparsely
  - limited presence in SciFinder
- Disposition of O & SO_2 will affect basicity of N atoms
  - similar effect due to symmetry

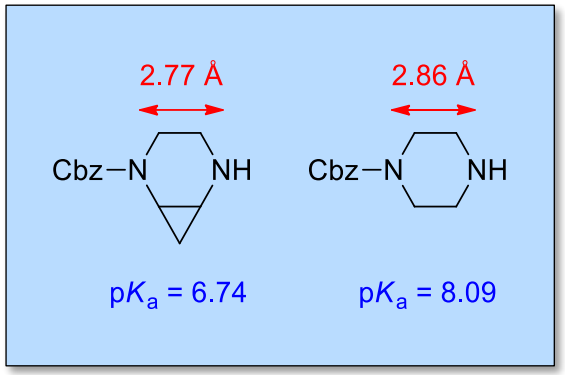
			
octahydro-1*H*-pyrrolo [3,4-*b*]pyrazine	octahydrofuro[3,4-*b*] pyrazine	octahydrothieno [3,4-*b*]pyrazine	octahydrothieno[3,4-*b*] pyrazine 6,6-dioxide
9 references – all patent applications	5 references – all patent applications	3 references – all patent applications	9 references – all patent applications

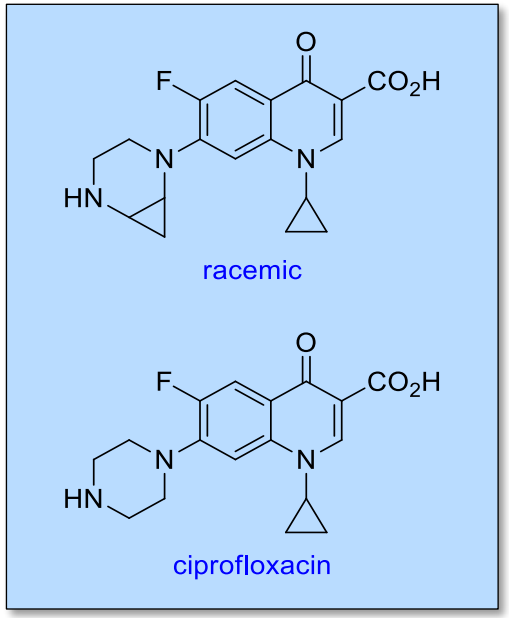
Data analysis from early 2024

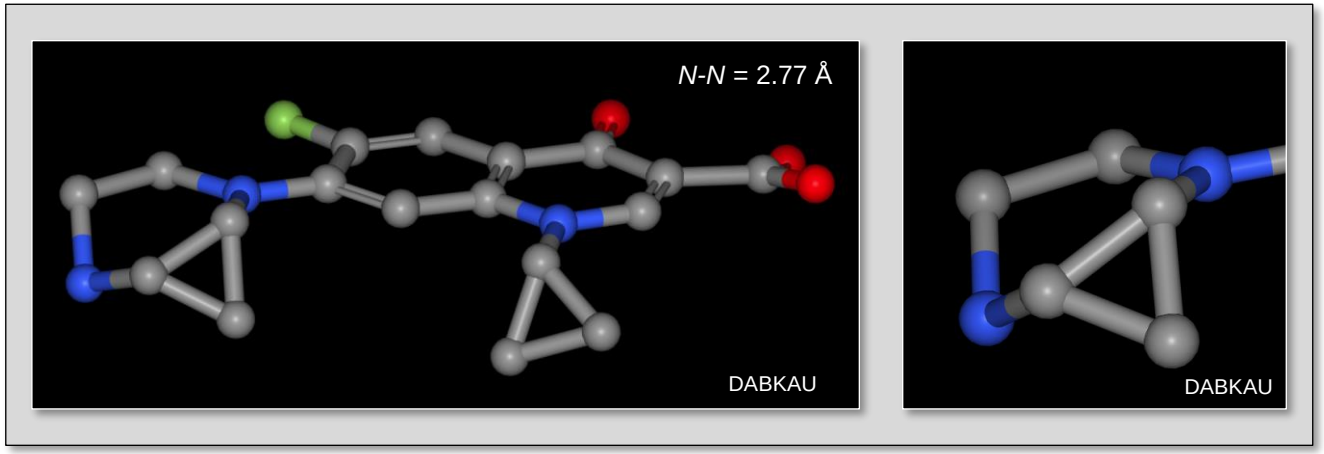
N.A. Meanwell & O. Loiseleur, *J. Agric. Food Chem.,* 2022, **70**, 10942-10971*; J. Agric. Food Chem.,* 2022, **70**, 10972-11004

2,5-Diazabicyclo[4.1.0]heptane in a Quinolone

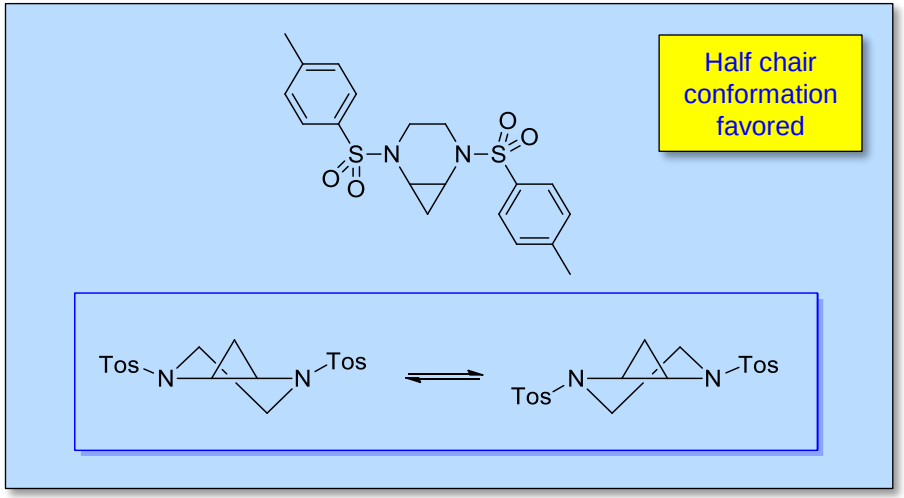
Piperazines with fused carbocyclic rings







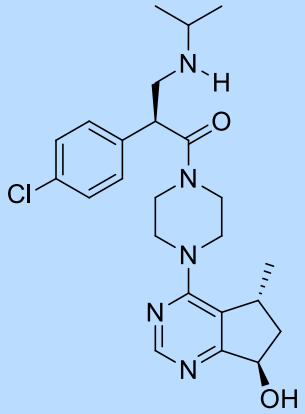
- Analogue of ciprofloxacin synthesized
  - prepared as the racemate
  - cyclopropane ring reduces pK_a
- Comparable potency toward *N. gonorrheae, N. meningitis, H. influenzae*
  - *S. pneumoniae* & *E. coli* less sensitive: MICs 4x higher
  - attributed to reduced basicity
  - no PK data generated



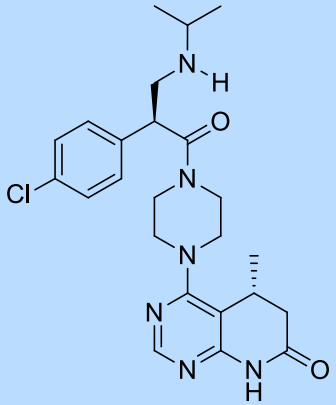
R.R.R. Taylor *et al., Tetrahedron,* 2010, **66**, 3370-3377; M.W. Majchrzak *et al., J. Het. Chem.,* 1983, **20**, 815-817

2,5-Diazabicyclo[4.1.0]heptane: pan-Akt Inhibitors

Piperazines with fused carbocyclic rings

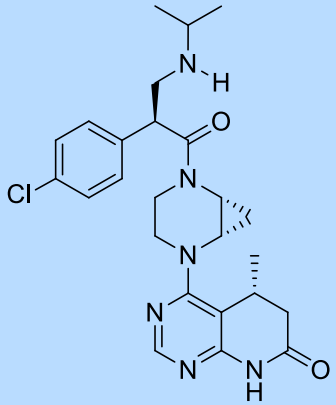


ipatasertib (GDC-0068)
Akt1 IC_{50} = 3.8 nM
Akt2 IC_{50} = 24 nM
Akt3 IC_{50} = 0.3 nM



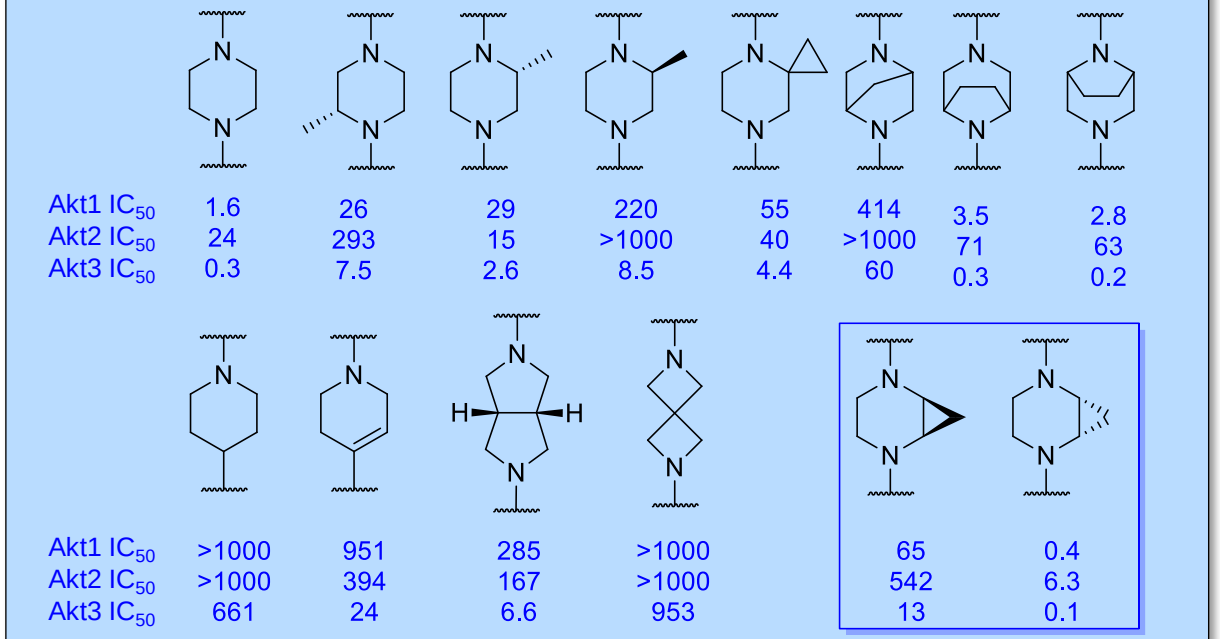
lead
Akt1 IC_{50} = 1.6 nM
Akt2 IC_{50} = 24 nM
Akt3 IC_{50} = 0.3 nM
$AUC_{0\text{-inf}}$ = 241 ng.h/mL

AUC data mouse 10 mpk PO



NTQ1062
Akt1 IC_{50} = 0.4 nM
Akt2 IC_{50} = 6.3 nM
Akt3 IC_{50} = 0.1 nM
$AUC_{0\text{-inf}}$ = 2170 ng.h/mL

AUC increased by 9x

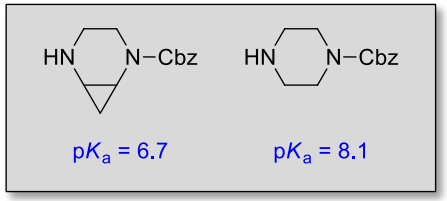


Data are IC_{50} values vs Akt 1, 2, & 3 (nM)

Potency depends on the absolute configuration

- Ipatasertib (GDC-0068): a pan-Akt inhibitor competitive with ATP
  - advanced into clinical study
  - sub-optimal PK profile
- Lead compound with modified hinge binder
  - potent pan-Akt inhibitor: poor PK profile in mice
- Examined hinge binder & piperazine modifications
  - 2,5-diazabicyclo[4.1.0]heptane most effective
    - 4x potency advantage
    - 100x sensitivity to the absolute configuration
- NTQ1062 identified as a clinical candidate

1 C atom increases potency by 4x
in vivo AUC increased by 9x
"magic methylene"

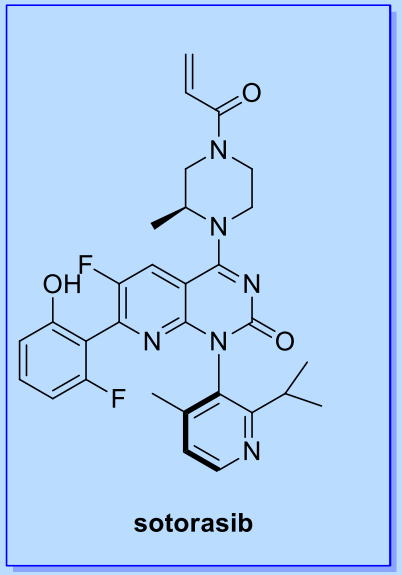


C. Ma *et al., J. Med. Chem.*, 2022, **65**, 8144-8168

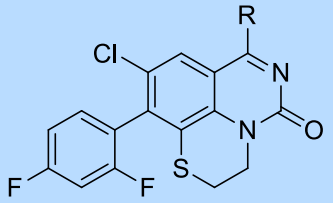
Covalent Inhibitors of KRAS G12C

Piperazines with fused heterocyclic rings

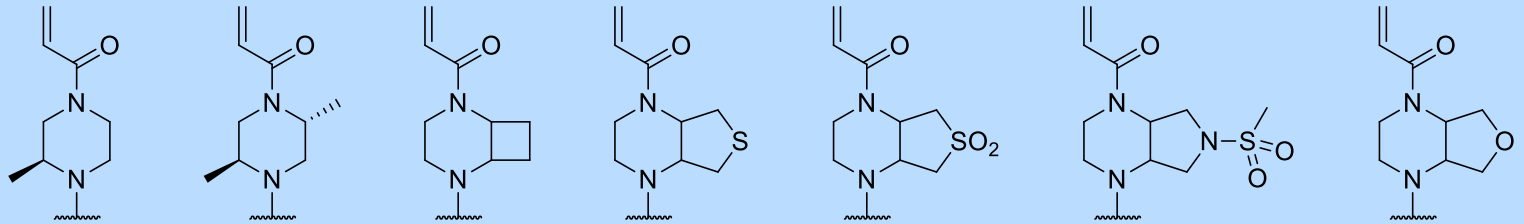
- Sotorasib approved by the FDA to treat KRAS G12C mutant
  - covalent inhibitor
- Me-piperazine an effective scaffold to project the acrylamide toward Cys_{12}
  - covalent interaction via Michael addition
- Activity preserved in many homologues
  - 3,6-diazabicyclo[3.1.1]heptane an exception



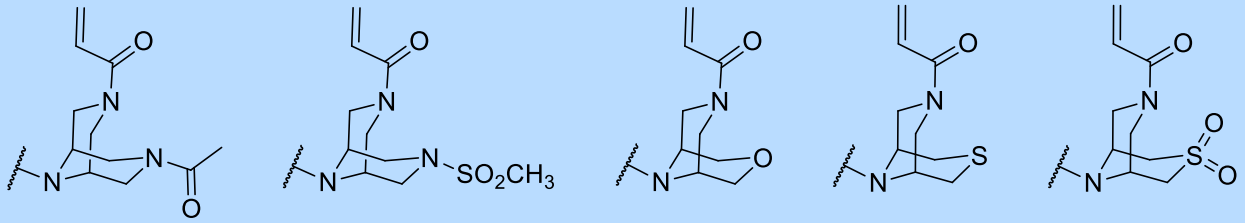
sotorasib



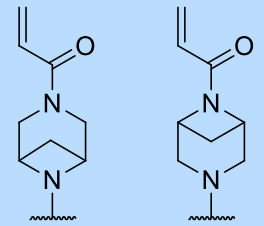
Effect of electron withdrawal on acrylamide electrophilicity?



70% 93% 58% 64.9% 82.2% 48.3% 85%



80.5% 70.5% 94.4% 95.3% 43.8%



0% 16.9%

3,6-diazabicyclo[3.1.1]heptane

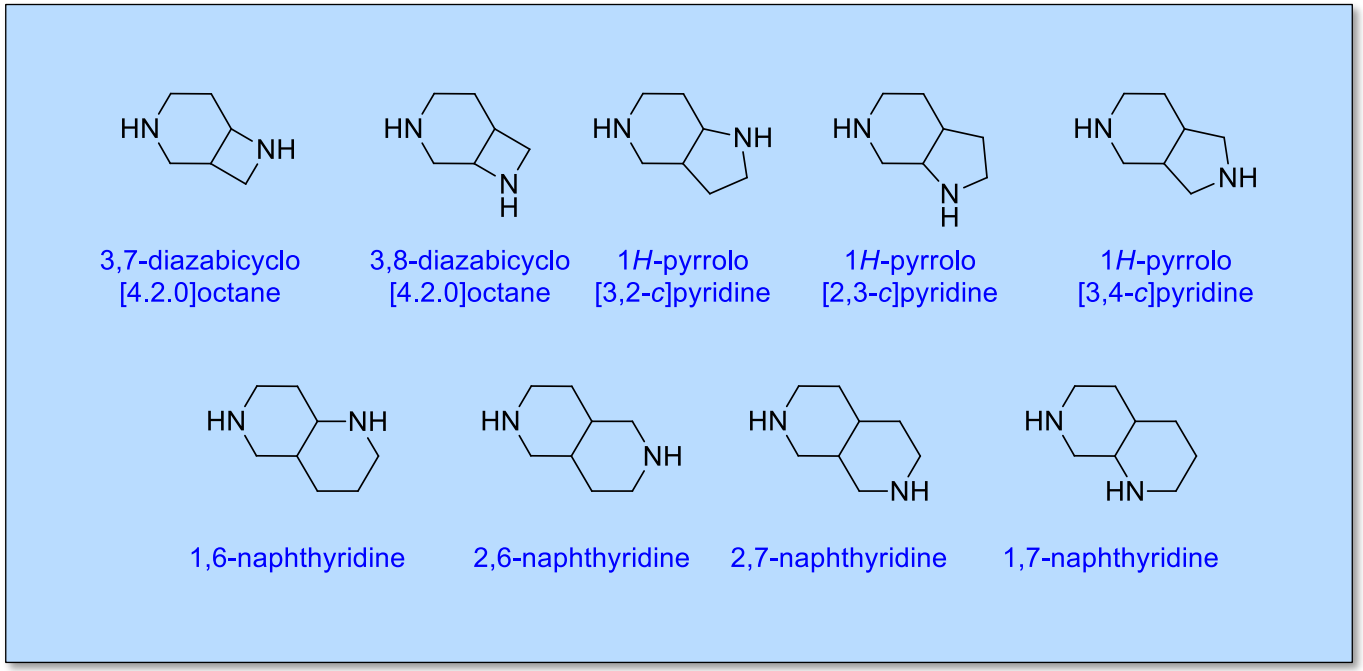
% covalent binding after 60 minutes incubation at 10 µM

presumably geometrical constraints

J. Feng *et al., World Patent Application,* WO 2021/127404

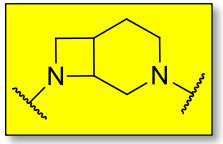
Piperidines with Fused Amine-Containing Rings

Piperidines with fused heterocyclic rings

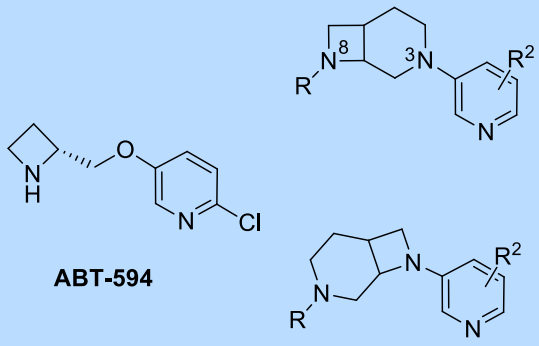


- Large family of fused heterocyclic diamines
  - well represented in the literature
- Topological relationship between N atoms can be varied
  - confer conformational constraint
  - axial/equatorial disposition of substituents adds to topographical diversity

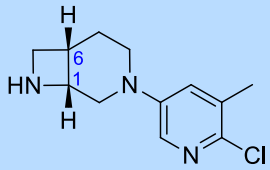
Diazabicyclo[4.2.0]octane Derivatives



Piperidines with fused heterocyclic rings

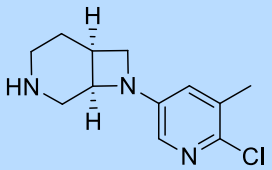


3,8-diazabicyclo[4.2.0]octane



K_i rat α4β2 = 0.031 nM
hα4β2 EC_{50} = 7.2 nM
E_{max} = 207%
hα3β4 EC_{50} = 7.2 nM
E_{max} = 123%

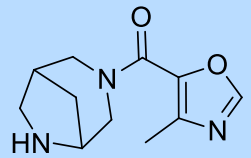
≡ 10x



K_i rat α4β2 = 0.043 nM
hα4β2 EC_{50} = 76.1 nM
E_{max} = 103%
hα3β4 EC_{50} = 1910 nM
E_{max} = 91%

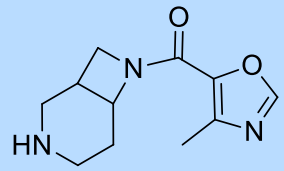
- α4β2 nicotinic receptor agonists derived from ABT-594
  - designed to overlay with azetidine & pyridine ring *N* atoms
- Highly potent ligands with good efficacy
  - both topologies comparably potent at rat receptor
    - potency differed at human receptors by 10x; efficacy 2x
  - analgesic effect in rat formalin model of persistent pain

- Ghrelin full agonists
  - stimulate growth hormone & insulin-like growth factor-1 release
- 3-Amino-piperidine exhibited poor oral absorption in rodents
  - low membrane permeability, P-gp efflux in rat & human jejunum tissue
  - reduce NH count to enhance membrane permeability
- Amide methylation reduced potency, lowered permeability but increased efflux
  - annealing to a ring improved potency & permeability

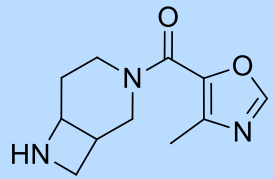


hα4β2 K_i = 4.6 nM

3,6-diazabicyclo[3.2.1]octane



hα4β2 K_i = 515 nM

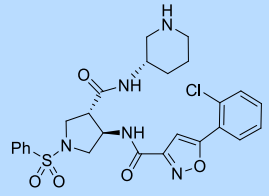
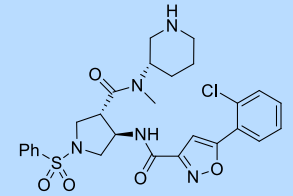
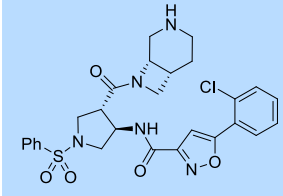


hα4β2 K_i = 792 nM

3,7-diazabicyclo[4.2.0]octane

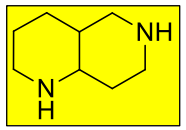
- Nicotinic agonists at hα4β2 receptor
  - 3,6-diazabicyclo[3.2.1]octane potent – bridged homopiperazine
  - 3,7-diazabicyclo[4.2.0]octane a poor mimic in either topology

Cyclization enhances potency 6x; reduces P-gp recognition

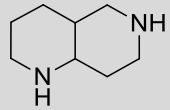
Ghrelin Agonists			
EC_{50} agonism (nM)	0.442	12.37	2.24
Log *D*	1.9	1.7	1.7
Solubility (μM)	12	113	265
Caco-2 P_{app} A to B (ER)	0.31 (34)	0.11 (220)	0.53 (32)
Cl_{int} rat hepatocytes	8.3	5.8	4.7
Cl_{int} HLM	<3	4.2	7.3

J. Frost *et al., J. Med. Chem.,* 2006, **49**, 7843-7853; M. Ye *et al*., *Bioorg. Med. Chem. Lett.,* 2009, **19**, 127-131; P.S. Hammond *et al*., *World Patent Application*, WO2008/112734
M. Cooper *et al., J. Med. Chem.,* 2020, **63**, 9705-9730

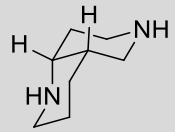
Decahydro-1,6-Naphthyridine Derivatives



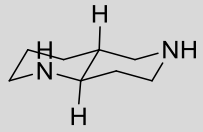
Piperidines with fused heterocyclic rings



decahydro-1,6-naphthyridine

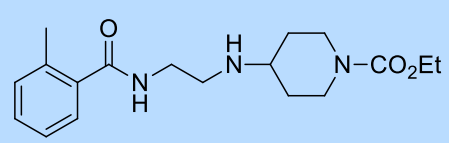


cis ring junction

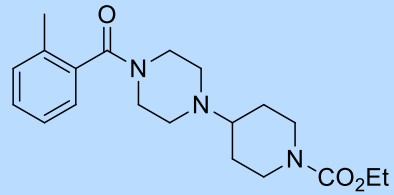


trans ring junction

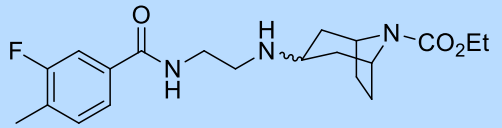
- *cis*- & *trans*- ring junctions
  - explore axial, equatorial amine disposition
- Examined in muscarinic M_1 agonists
  - *cis*- more potent than *trans*- ring junction
  - axial amine preferred
- Facilitated tropane design
  - geometrical preference reproduced
  - axial 10x more potent than equatorial amine
- Tropane topology important
  - 10x difference in potency
  - axial isomer of more potent tropane not accessible



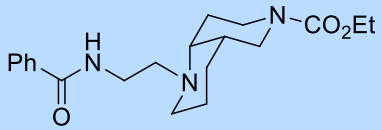
ML071
hM_1 EC_{50} = 1,600 nM
(35% ACh_{max})



hM_1 <30% ACh_{max} at 10 μM

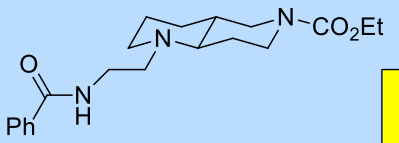


hM_1 EC_{50} = 470 nM
(40% ACh_{max})
70:30 endo:exo inseparable mixture



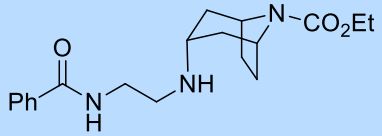
hM_1 EC_{50} = 1,200 nM
(13% ACh_{max})

3x

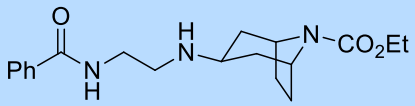


hM_1 EC_{50} = 3,200 nM
(13% ACh_{max})

Axial 3x more potent

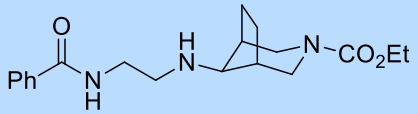


hM_1 EC_{50} = 110 nM
(34% ACh_{max})



hM_1 EC_{50} = 970 nM
(49% ACh_{max})
tropane

10x



hM_1 EC_{50} = 92 nM
(53% ACh_{max})
Only a single product from the reductive amination

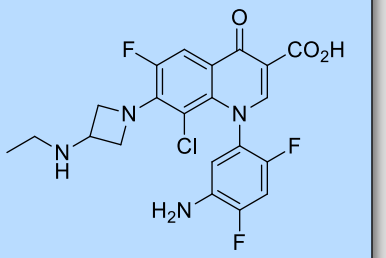
10x Δ in potency based on bridge disposition

E.P. Lebois *et al., Bioorg. Med. Chem. Lett.,* 2011, **21**, 6451-6455; B.J. Melancon *et al., Bioorg. Med. Chem. Lett.,* 2012, **22**, 3467-3472

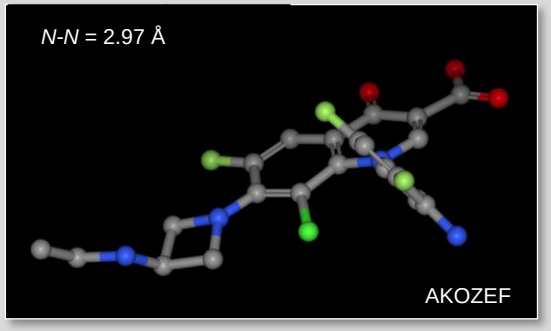
Azetidine-Based Isosteres of Piperazine & Homo-piperazine

4-Amino-Azetidines

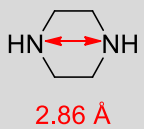
Azetidine-based piperazine mimics



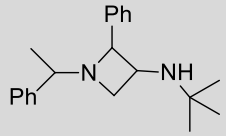
MIC Gram +ve = 0.07 μg/mL
MIC Gram +ve = 0.03 μg/mL

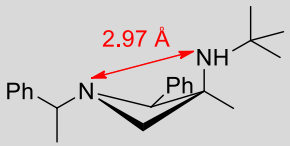


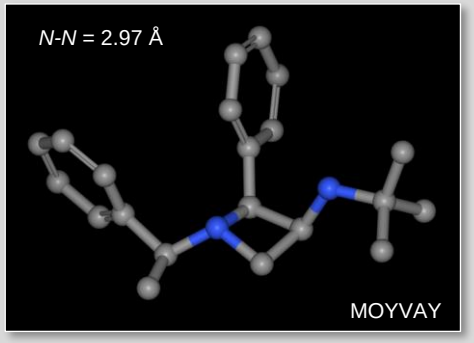
MIC (μg/mL)	*S. pneumoniae*	MRSA	*P. aeruginosa*	*E. coli*
ciprofloxacin	1	128	8	128
trovafloxacin	0.25	128	16	>128
azetidine	0.008	1	1	1

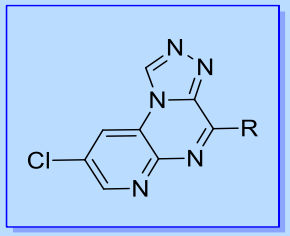


2.86 Å

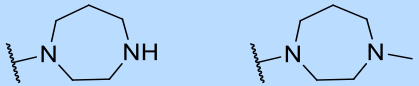




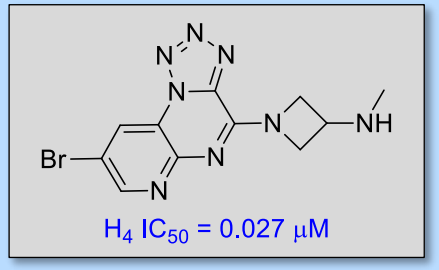




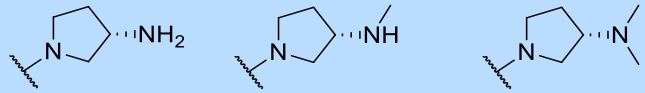
H_4 IC_{50} = 0.13 μM H_4 IC_{50} = 0.077 μM
Me effect = 2x



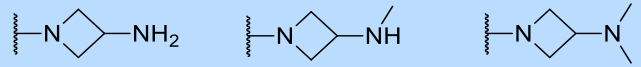
H_4 IC_{50} >10 μM H_4 IC_{50} = 2.9 μM
Me effect >3x



H_4 IC_{50} = 0.027 μM



H_4 IC_{50} = 6.3 μM H_4 IC_{50} = 4.1 μM H_4 IC_{50} = 4.8 μM
Me effect = 1.5x



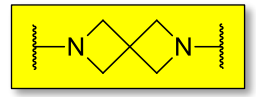
H_4 IC_{50} = 5.2 μM H_4 IC_{50} = 0.065 μM H_4 IC_{50} = 1.2 μM
Me effect = 80x

Methyl effect amplified

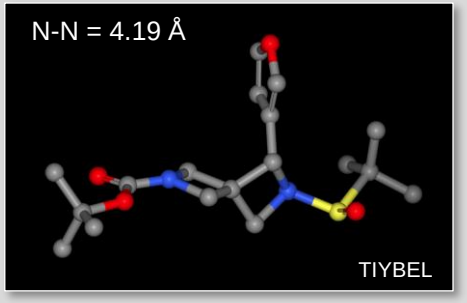
- 3-EtNH-azetidine examined in quinolone antibiotics
  - advantageous potency compared to ciprofloxacin & trovafloxacin
  - azetidine ring adopts planar topography in single crystal X-ray structure
  - N-N distance approximates piperazine
- Histamine H_4 antagonists for the treatment of atopic dermatitis
  - Me-3-amino azetidine uniquely potent; retained in optimized analogue
  - 80x Me effect: muted in piperazine, homopiperazine, pyrrolidines

Z. Zhang *et al., Inorganica Chim. Acta,* 2009, **362**, 1687-1691; Y. Kuramoto *et al., J. Med. Chem.*, 2003, **46**, 1905-1917; K. Ko *et al., J. Med. Chem.,* 2018, **61**, 2949-2961

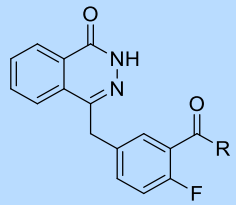
2,6-Diazaspiro[3.3]heptane

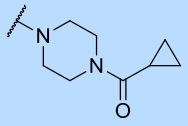


Azetidine-based piperazine mimics

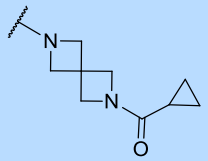


PARP-1 Inhibitors



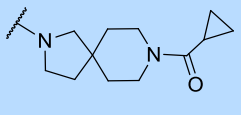


olaparib
PARP-1 IC_{50} = 6.0 nM
EC_{50} = 14.6 μM

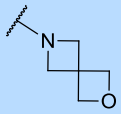


PARP-1 IC_{50} = 12.6 nM
EC_{50} = 1,500 nM
no DNA damage at 10 μM

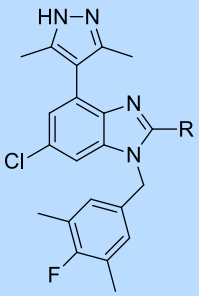
PARP-1 IC_{50} = 44.3 nM
EC_{50} = 24.8 μM

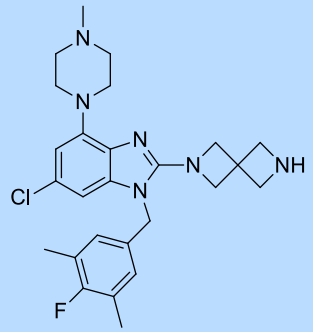


PARP-1 IC_{50} = 224.9 nM
EC_{50} = 41.6 μM



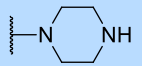
PARP-1 IC_{50} = 24.9 nM
EC_{50} = 52.2 μM



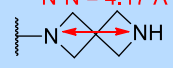


K_d = 44 nM

N-N = 4.17 Å

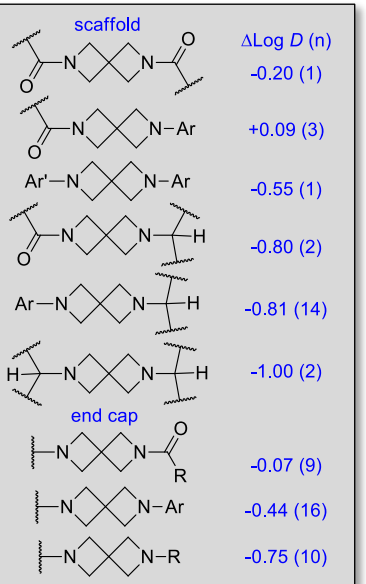


K_d = <100 nM
ALog *P* = 5.49



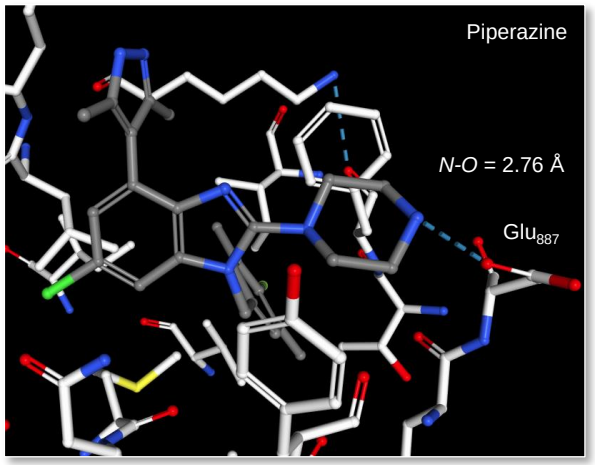
K_d = 9 nM
ALog *P* = 5.12
LipE = 2.91

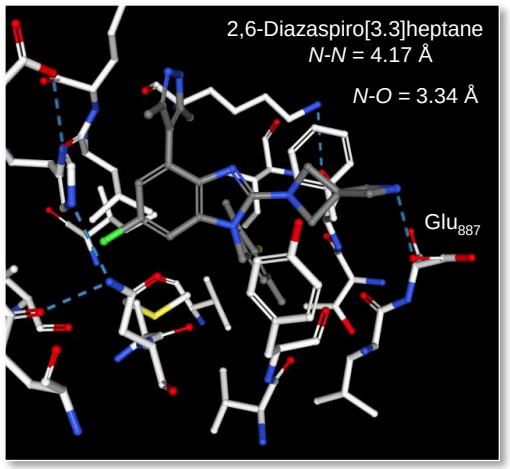
SOS-1 agonists



Δ relative to piperazine

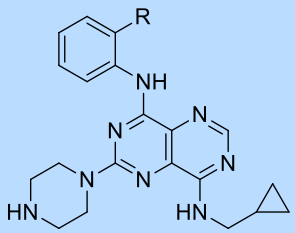
- 2,6-Diazaspiro[3.3]heptane is a stretched piperazine mimic
  - N-N distance is 50% longer
  - as scaffold, reduces lipophilicity relative to piperazine
  - as endcap: -0.17 to -0.75
- PARP-1 inhibitors that demonstrated cytotoxicity
  - 2,6-diazaspiro[3.3]heptane retained potency
  - showed reduced DNA damaging properties
    - potential in inflammation & neurodegeneration
- Son-of-sevenless homologue 1 (SOS-1) agonists
  - SOS-1 exchanges GDP for GTP on RAS
    - piperazine engages Glu_{887}
  - spiro azetidine exhibits enhanced affinity
    - engages Glu_{887} with different geometry
  - attributed to reduced flexibility compared to piperazine



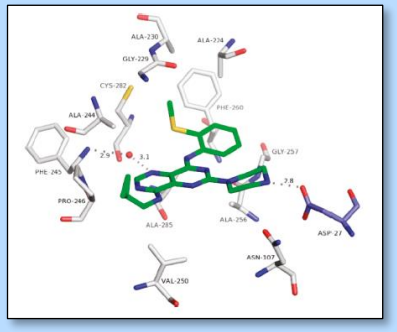


S.L. Degorce *et al., ACS Med. Chem. Lett.*, 2019, **10**, 1198-1204; S.W. Reilly *et al., J. Med. Chem.*, 2018, **61**, 5367-5379; T.R. Hodges *et al., J. Med. Chem.*, 2018, **61**, 8875-8894

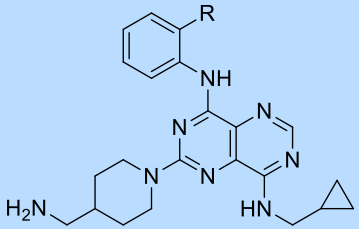
2,6-Diazaspiro[3.3]heptane in Ketohexokinase Inhibitors



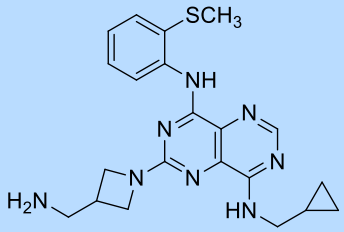
R = CH_3: KHK-C IC_{50} = 210 nM
R = SCH_3: KHK-C IC_{50} = 12 nM



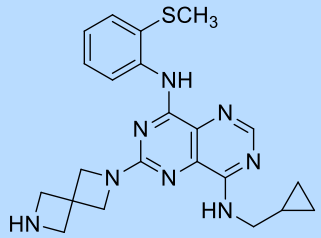
KHK-C IC_{50} = 200 nM



R = CH_3: KHK-C IC_{50} = 70 nM
R = SCH_3: KHK-C IC_{50} = 10 nM

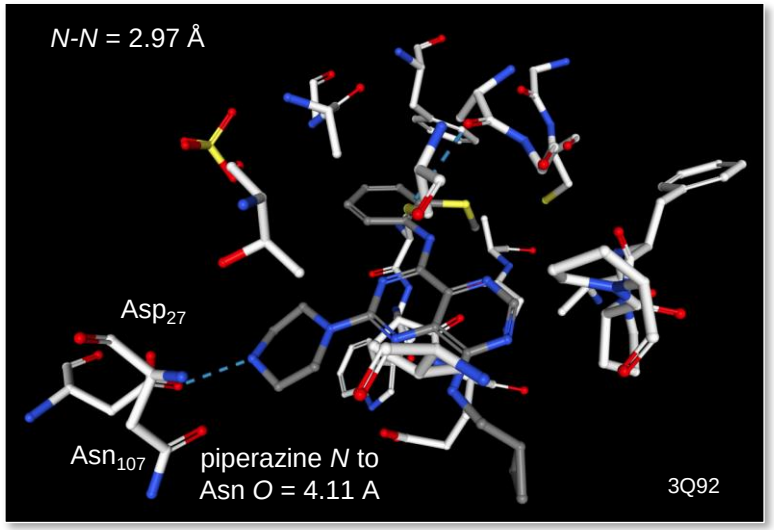


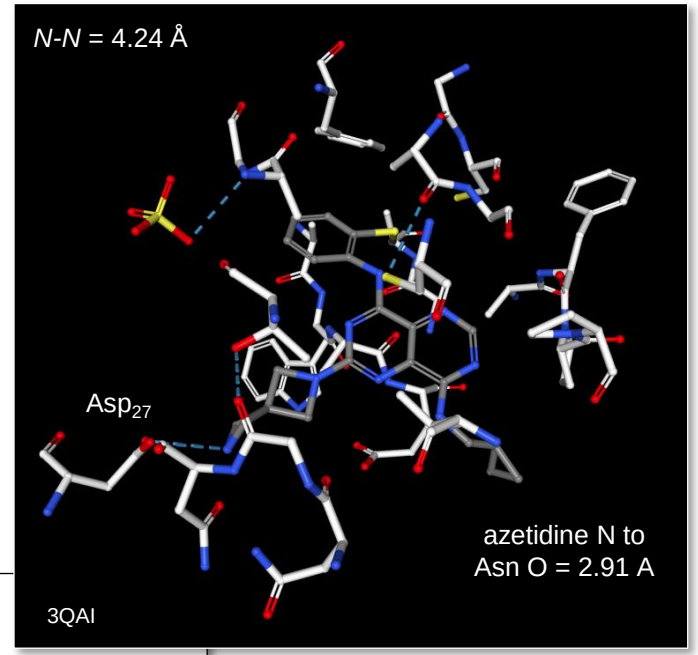
KHK-C IC_{50} = 30 nM



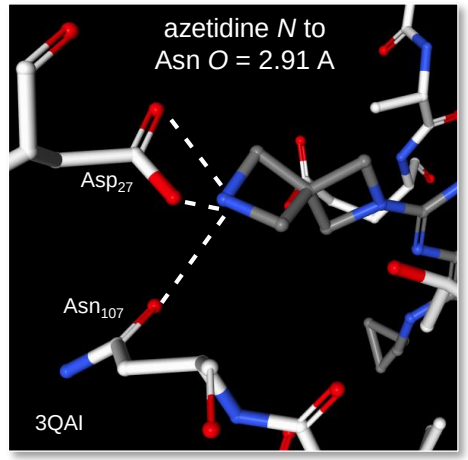
KHK-C IC_{50} = 8 nM

Ketohexokinase Inhibitors





- Ketohexokinase (fructokinase) inhibitors for obesity
  - inhibition of fructose phosphorylation
  - inhibitors prevent fructose from entering the glycolytic pathway
- Lead discovered by HTS
  - piperazine derivative: SCH_3 enhanced potency 15x
- X-ray cocrystal revealed drug target interactions
  - piperazine engaged Asp_{27}
- 4-NH_2-piperidine comparable to piperazine
  - piperidine-4-CH_2-NH_2-NH_2 more potent: SCH_3 7x CH_3
- 2,6-Diazaspiro[3.3]heptane optimal potency
  - 4x azetidine-3-CH_2-NH_2; 1.5x piperazine
  - engages Asp_{27} & Asn_{107} in dual H-bonding interaction
  - closer to Asn_{107} than piperazine

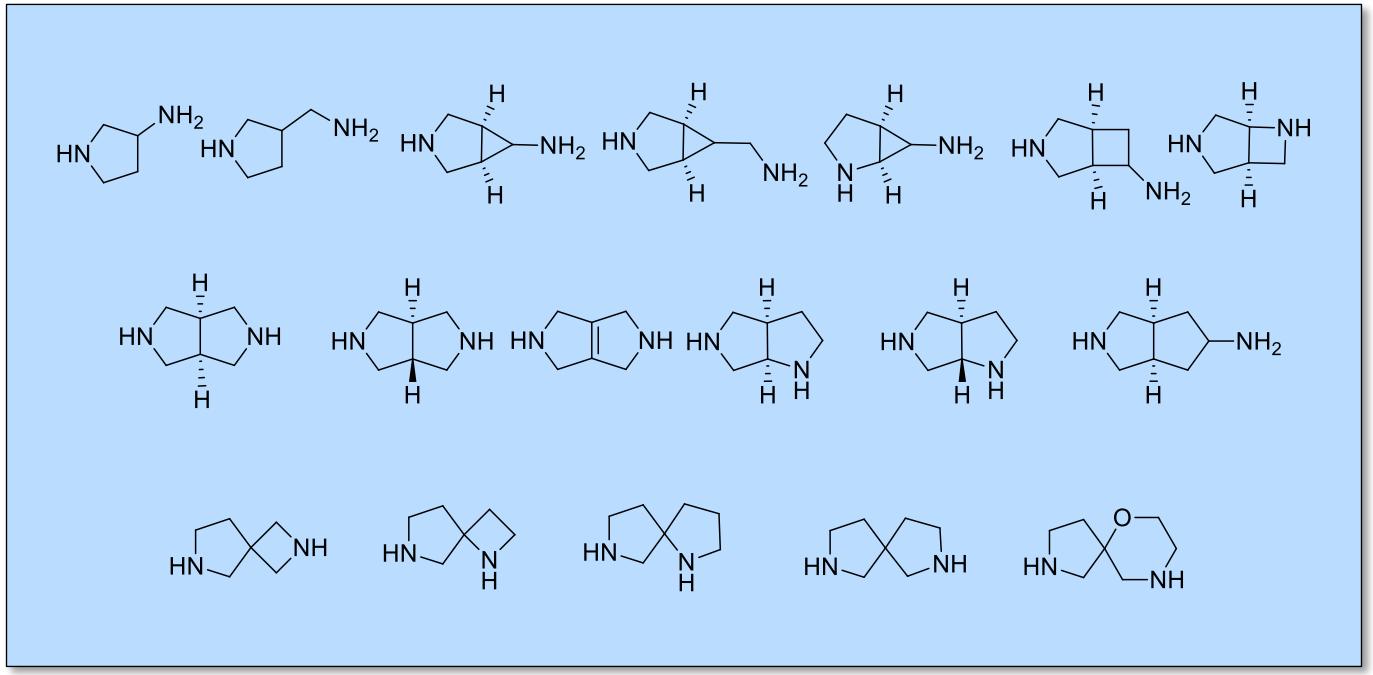


D.C. Ebner *et al., Med, Chem. Rev.*, 2023, **58**,135-153; B.E. Maryanoff *et al.*, *ACS Med. Chem. Lett.*, 2011, **2**, 538-543

NuArq MedChem Consulting LLC

Pyrrolidine-Based Isosteres of Piperazine & Homo-piperazine

Pyrrolidine-Based Piperazine & Homo-Piperazine Isosteres



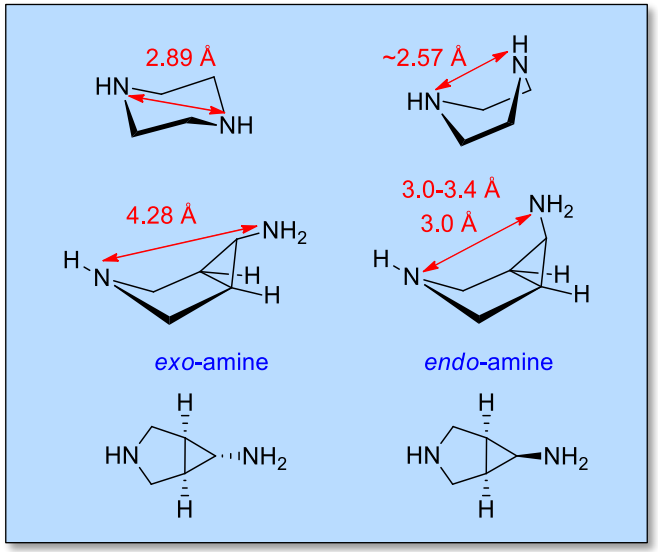
- Large family of fused & spirocyclic heterocyclic diamines
  - confer conformational constraint
  - exit vectors vary
  - ring junctions can be *cis, trans*, or unsaturated for larger rings
    - modulates exit vector topography
  - axial/equatorial disposition of substituents adds to topographical diversity

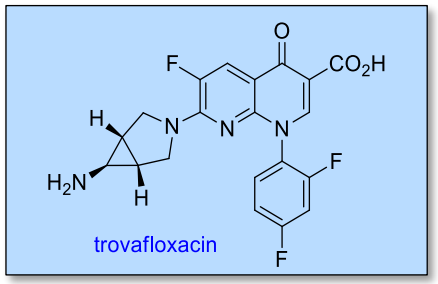
N.A. Meanwell & O. Loiseleur, *J. Agric. Food Chem.,* 2022, **70**, 10942-10971*; J. Agric. Food Chem.,* 2022, **70**, 10972-11004

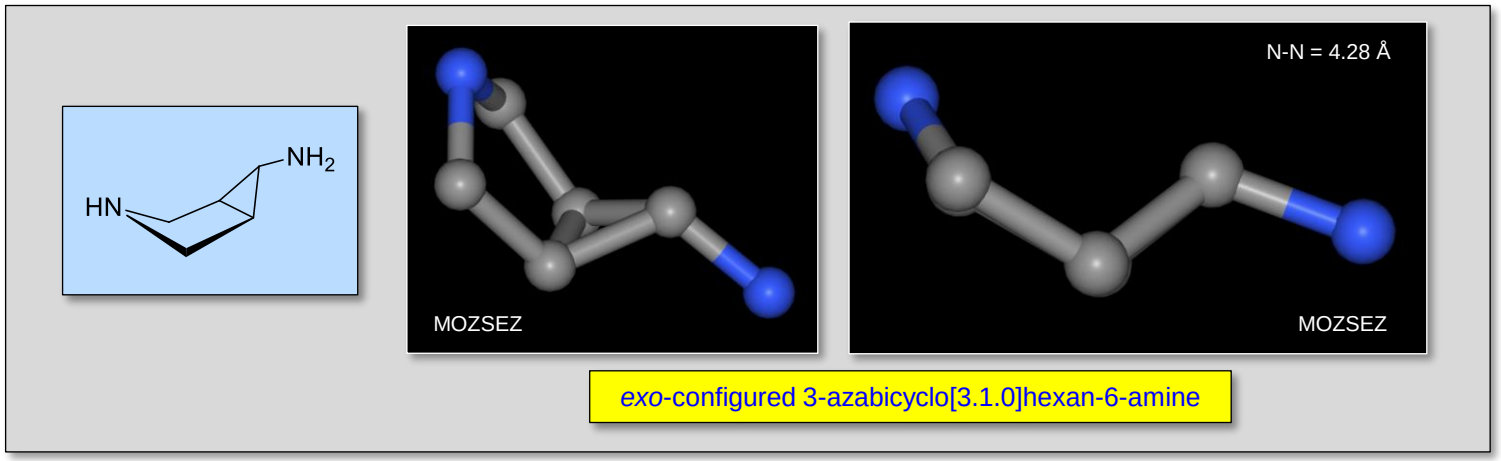
3-Azabicyclo[3.1.0]hexan-6-amine Derivatives

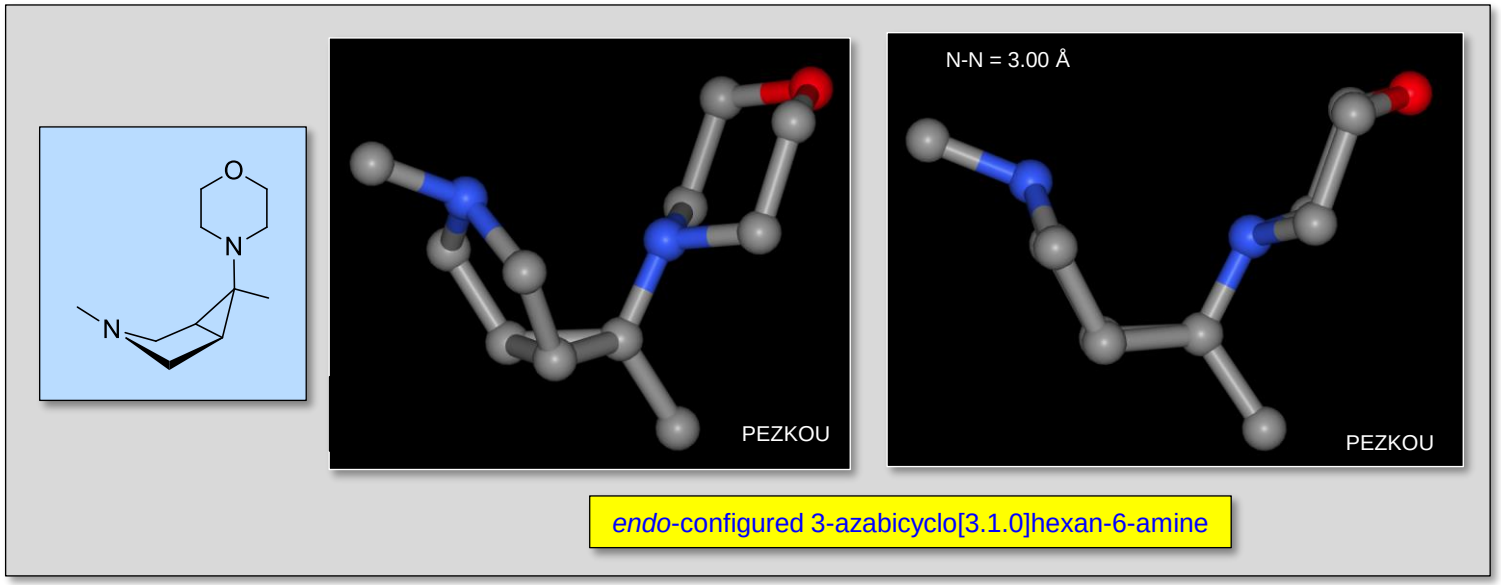
Pyrrolidines with fused carbocyclic rings

- 3-Azabicyclo[3.1.0]hexan-6-amine derivatives
  - *exo*- & *endo* configurations add diversity
  - geometries & distances from single crystal X-rays
- Structural feature of trovafloxacin
  - withdrawn by FDA in May 2000 - hepatotoxicity

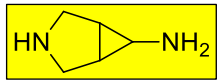








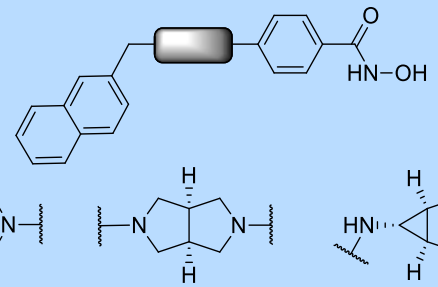
A. de Meijere *et al.*, *Chem. Eur. J.,* 2002, **8**, 3789-3801; V. Butz *et al., J. Chem. Soc. Perkin Trans. 2,* 1993, 1907-1913; A. Osipova *et al., Synthesis,* 2007, 131-139



3-Azabicyclo[3.1.0]hexan-6-amine Derivatives

Pyrrolidines with fused carbocyclic rings

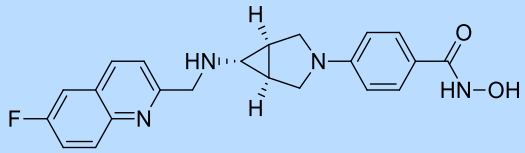
HDAC inhibitors



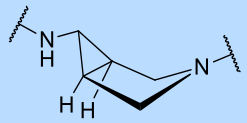
HDAC IC_{50} = 1.5 nM

HDAC IC_{50} = 13 nM

HDAC IC_{50} = 12.4 nM



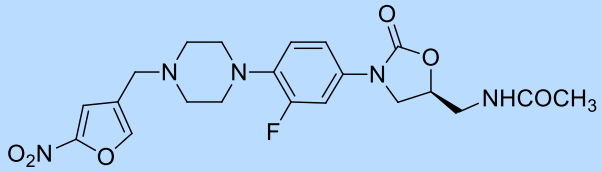
nanatinostat (CHR-3996)
HDAC IC_{50} = 8 nM



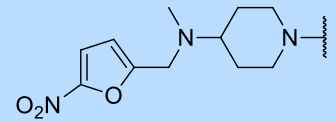
exo-amine

- Class I selective orally active histone deacetylase inhibitor
  - octahydropyrrolo[3,4-*c*]pyrrole promising lead series
- 3-Azabicyclo[3.1.0]hexan-6-amine provided clinical candidate
  - exo-amine disposition
  - topological isomer not explored in this series
- Nanatinostat (CHR-3996) nominated for clinical development
  - combination with valganciclovir in Phase 2 trials
  - treatment of R/R EBV-positive lymphomas
  - accorded Fast Track status in 2019 & orphan drug 2023

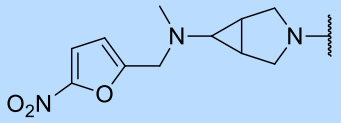
Antibacterials



ranbezolid
S.a. = 1
MRSA = 1
E.fa = 2
VRE = 2
S.py = 0.125



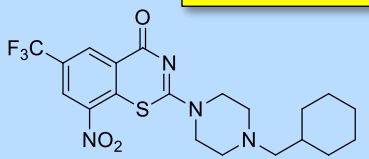
S.a. = 1
MRSA = 0.5
E.fa = 0.5
VRE = 0.25
S.py = 0.125



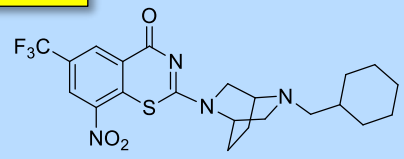
S.a. = 2
MRSA = NT
E.fa = 2
VRE = 1
S.py = 0.06

Less potent

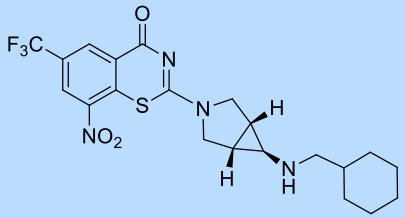
TB DprE1 inhibitors



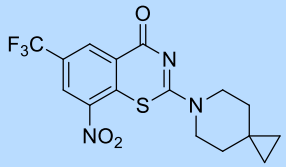
MIC = 2 nM
soly. <0.01 μg/mL



MIC = 32 nM
soly. <0.03 μg/mL



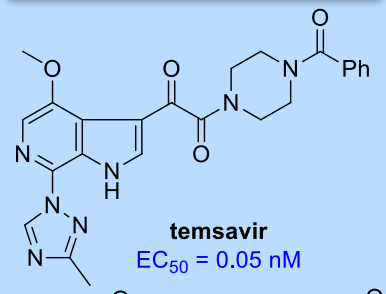
MIC = 32 nM
soly. = 4.8 μg/mL



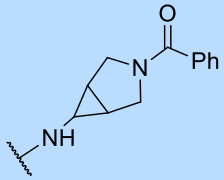
MIC = 16 nM
soly. = 0.3 μg/mL

16x less potent but 500x enhanced aqueous solubility

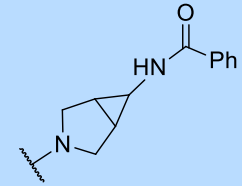
HIV-1 attachment inhibitors



temsavir
EC_{50} = 0.05 nM



EC_{50} = 35 nM

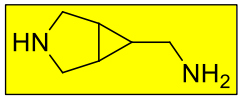


EC_{50} = 91.5 nM

Less potent

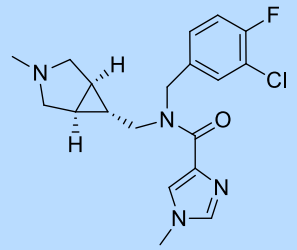
- Mixed success in anti-infectives

D. Moffatt *et al., J. Med. Chem.,* 2010, **53**, 8663-8678; B. Haverkos *et al., Blood Adv*., 2023, **7**, 6339-6350; B. Das *et al., Bioorg. Med. Chem. Lett*., 2005, **15**, 4261-4267; C.A. Aldrich *et al., ACS Med. Chem. Lett*., 2019, **10**, 348-351; S. Cocklin *et al., Bioorg. Med. Chem. Lett.,* 2014, **24**, 5439-5445; *Molecules,* 2018, **23**, 1940

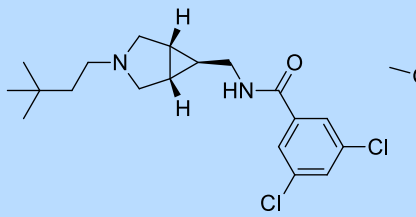


3-Azabicyclo[3.1.0]hexan-6-ylmethanamine

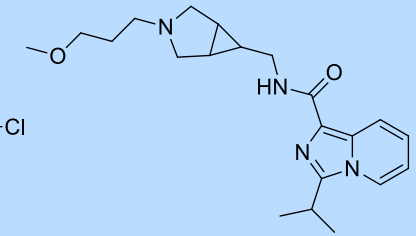
Pyrrolidines with fused carbocyclic rings



PF-03463275
Gly-T inhibitor
Clinical study but abandoned



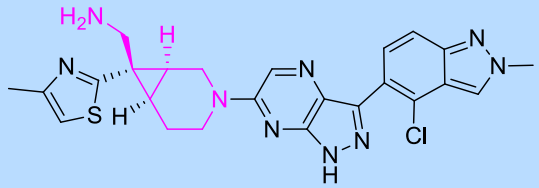
MIL 218
T-type calcium channel inhibitor



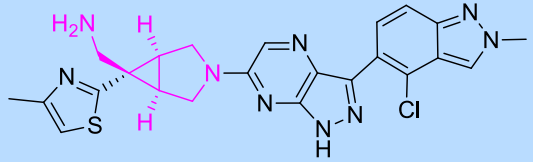
5HT$_4$ partial agonist

- Homologue 3-azabicyclo[3.1.0]hexan-6-ylmethanamine has attracted attention

Allosteric SHP2 phosphatase inhibitors



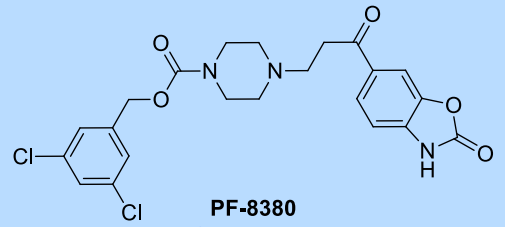
SHP2 IC_{50} = 6 nM
pERK EC_{50} = 14 nM
hERG IC_{50} = 107 nM



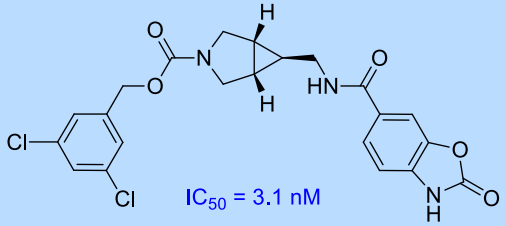
SHP2 IC_{50} = 5 nM
pERK EC_{50} = 24 nM
hERG IC_{50} >30 μM

Much reduced hERG inhibition

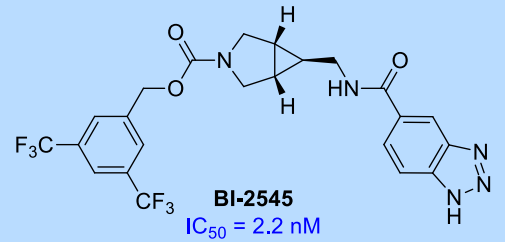
Autoaxin inhibitors



PF-8380
IC_{50} = 1.7 nM



IC_{50} = 3.1 nM

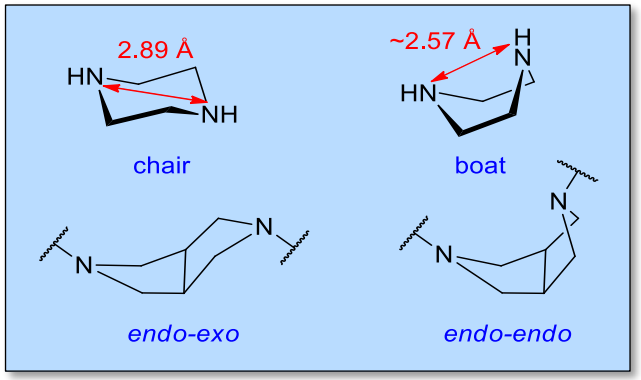


BI-2545
IC_{50} = 2.2 nM

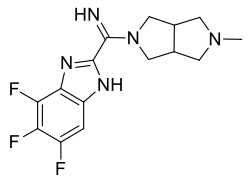
B. Das *et al., Bioorg. Med. Chem. Lett.*, 2005, **15**, 4261-4267; J.A. Lowe III *et al., Bioorg. Med. Chem. Lett.*, 2009, **19**, 2974-2976; A. Petrocchi *et al.*, *ACS Med. Chem. Lett.*, 2023, **14**, 645-651; Z. Xiang *et al., ACS Chem. Neurosci.*, 2011, **2**, 730-742; C.A. Kuttruf *et al., ACS Med. Chem. Lett.*, 2017, **8**, 1252-1257

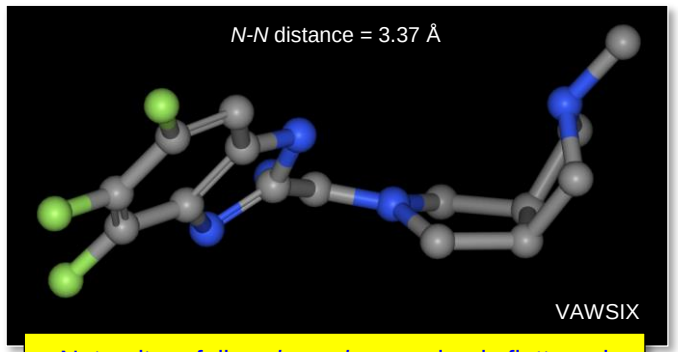
Octahydropyrrolo[3,4-*c*]pyrrole Derivatives

Pyrrolidines with fused heterocyclic rings

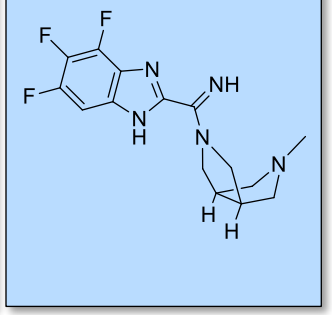


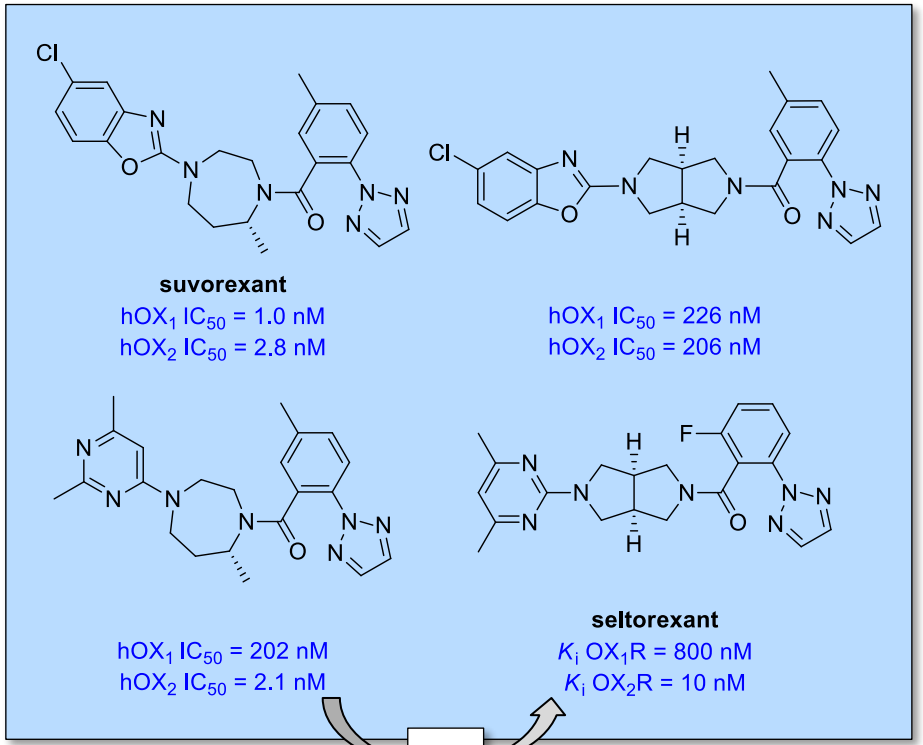
- 3-Azabicyclo[3.1.0]hexan-6-amine derivatives
  - *endo-exo-* & *endo-endo* configurations add diversity
  - *endo-endo* mimics boat conformation of piperazine
- More basic than piperazine
  - pK_a = 8.28 vs 6.86 (calculated)
- One single crystal X-ray structure
  - 1 ring *endo*, other planarized
  - *N-N* distance in X-ray = 3.37 Å

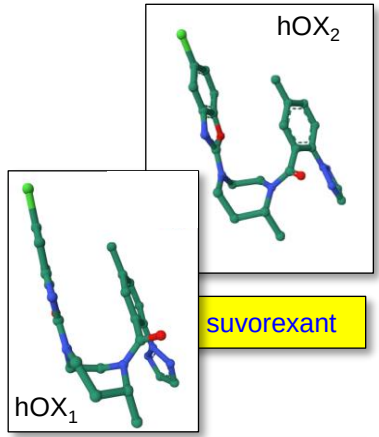


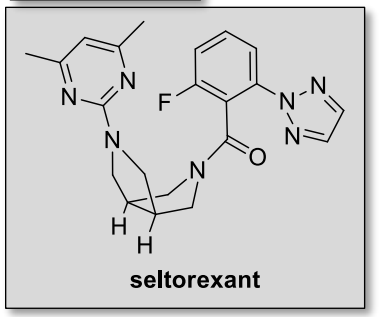


Not quite a full *endo-endo:* one ring is flattened



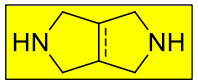






- Suvorexant adopts the same conformation in hOX$_1$ & hOX$_2$
  - *N-N* distance 2.63-2.68 Å
- Homopiperazine replaced by pyrrolo[3,4-*c*]pyrrole
  - 200x ↓ at hOX$_1$; 100x ↓ at hOX$_2$
- Dimethyl pyrimidine mediates hOX$_2$ selectivity
  - isomer enhances hOX$_2$ binding in bicyclic core
  - seltorexant in clinical trials for insomnia & depression

C.A.L. Lane *et al., Bioorg. Med. Chem. Lett.,* 2012, **22**, 1156-1159

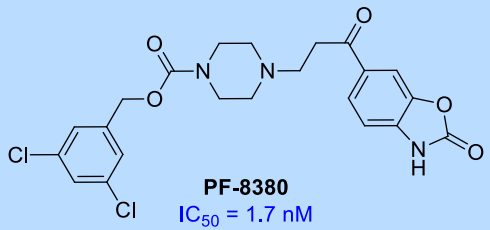


Pyrrolidino[3,4-c]pyrrolidines

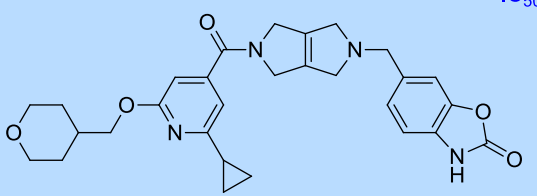
Pyrrolidines with fused heterocyclic rings

Autotaxin inhibitors

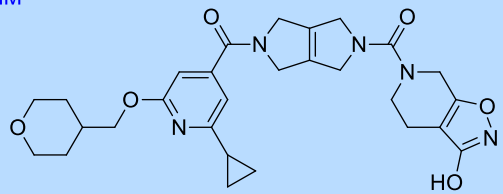
Piperazine is a scaffold
Good tolerance for diamines



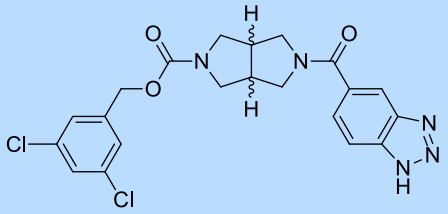
PF-8380
IC_{50} = 1.7 nM



IC_{50} = 1 nM

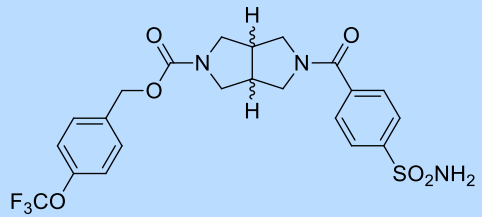


IC_{50} = 1 nM



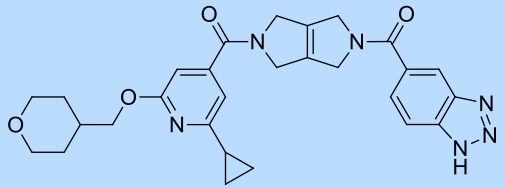
cis IC_{50} = 25 nM

trans IC_{50} = 11 nM

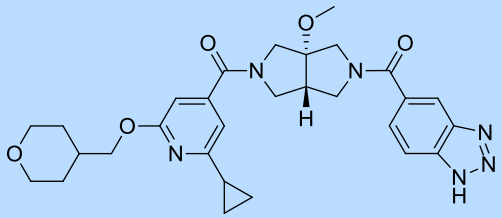


cis IC_{50} = 3 nM

trans IC_{50} = 4 nM

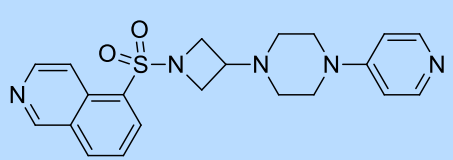


IC_{50} = 5 nM



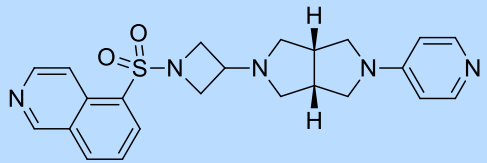
IC_{50} = 13 nM

Scaffolding elements



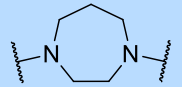
hM_1 IC_{50} = 0.11 μM

≡

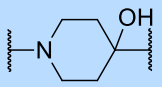


hM_1 IC_{50} = 0.23 μM

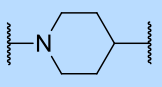
Muscarinic M1 allosteric antagonists



inactive

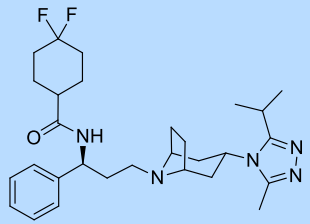


inactive

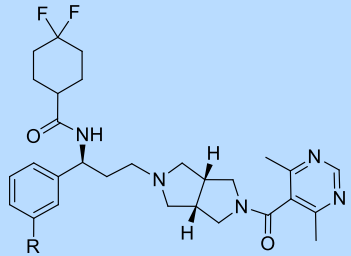


inactive

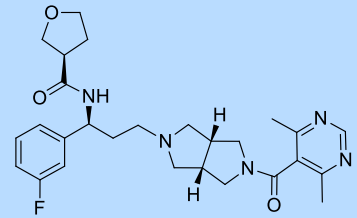
CCR5 antagonists for HIV-1



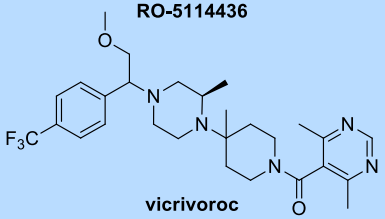
maraviroc
IC_{50} = 14 nM; EC_{50} = 3 nM



R = H: IC_{50} = 27 nM; EC_{50} = 6 nM
R = F: IC_{50} = 12 nM; EC_{50} = 1.1 nM



RO-5114436

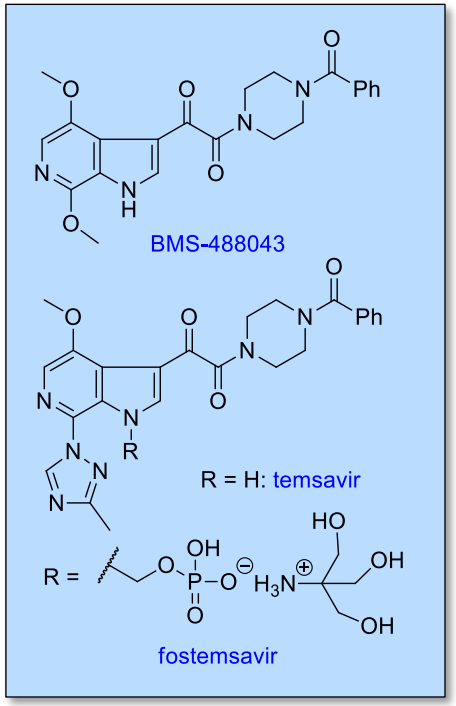


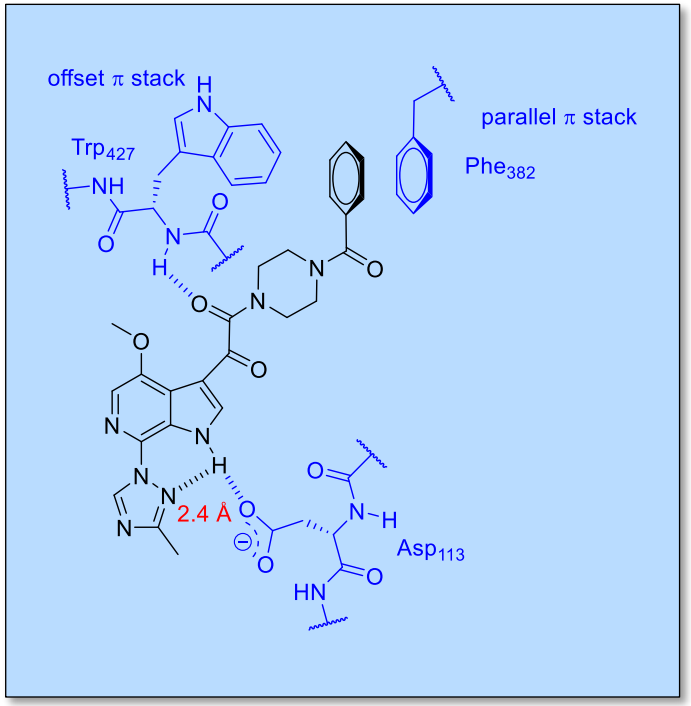
vicrivoroc

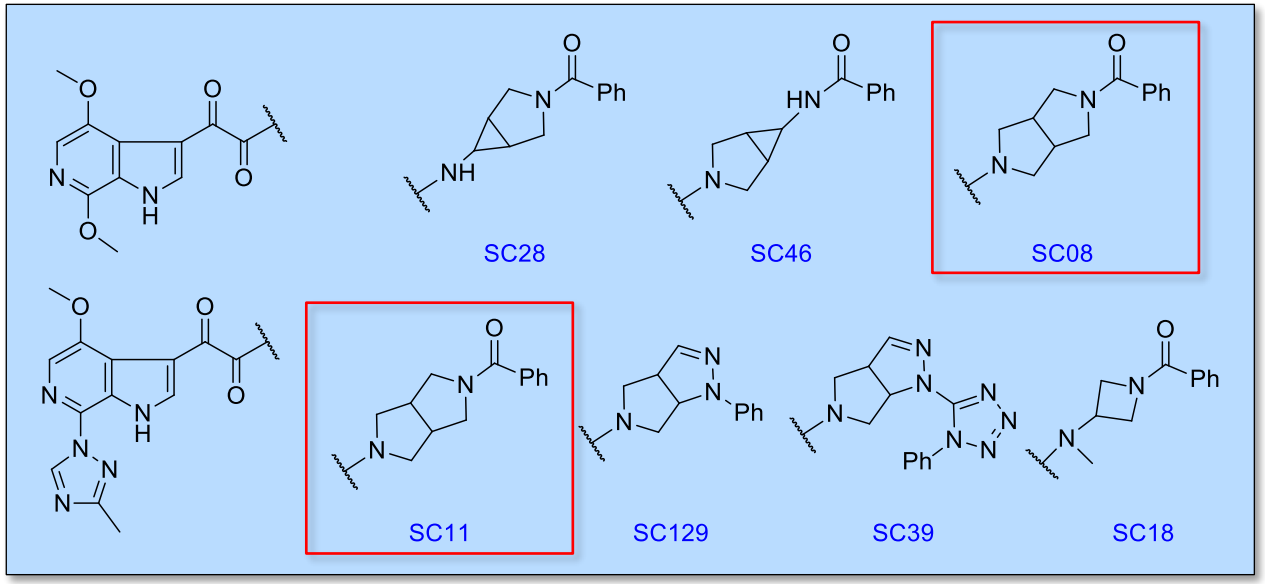
D. Castagna *et al*., *J. Med. Chem*., 2016, **59**, 5604-5621; J. Hert *et al. World Patent Appl*. WO 2014/139978; P. Di Giorgio et al., *World Patent Appl.,* WO 2017/050732; B.J. Melancon *et al., Bioorg. Med. Chem. Lett*., 2012, **22**, 5035-5040; D.M. Rotstein *et al., Bioorg. Med. Chem. Lett*., 2010, **20**, 3116-3119; X. Huang *et al.*, *Org. Process Res. Dev.,* 2010, **14**, 592-599

Piperazine Mimics in HIV-1 Attachment Inhibitors

Pyrrolidines with fused carbocyclic & heterocyclic rings







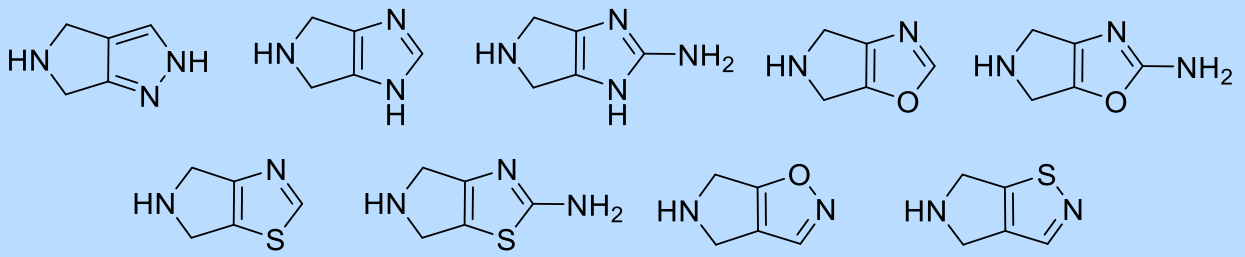
- Indole glyoxamide inhibitors of HIV-1 attachment
  - prevent HIV-1 gp120 from binding to host cell CD4
- Piperazine a critical scaffold
  - projects PhCO moiety to π-stack with Phe_{382}
- Difficult to replace
  - SC11: octahydropyrrolo[3,4-*c*]pyrrole performed best
    - 10x less potent than temsavir
  - SC08: active in the BMS-488043 series
    - lower potency

10x less potent

	EC_{50} (nM)		Soluble gp140 binding kinetics & affinity		
	HIV-1 JR-CSF	B41	k_a (M^{-1} s^{-1})	k_d (Ms^{-1})	K_D (nM)
temsavir	0.06	0.05	3.89×10^4	5.9×10^{-4}	15.2
SC28	NT	35	NT	NT	NT
SC46	NT	91.5	NT	NT	NT
SC08	90	NT	NT	NT	NT
SC11	0.6-0.8	2	4.33×10^3	2.87×10^{-4}	66
SC129	NT	6	NT	NT	NT
SC39	143	5320	1.39×10^3	3.81×10^{-2}	2740
SC18	132	360	1.59×10^4	2.48×10^{-2}	1560

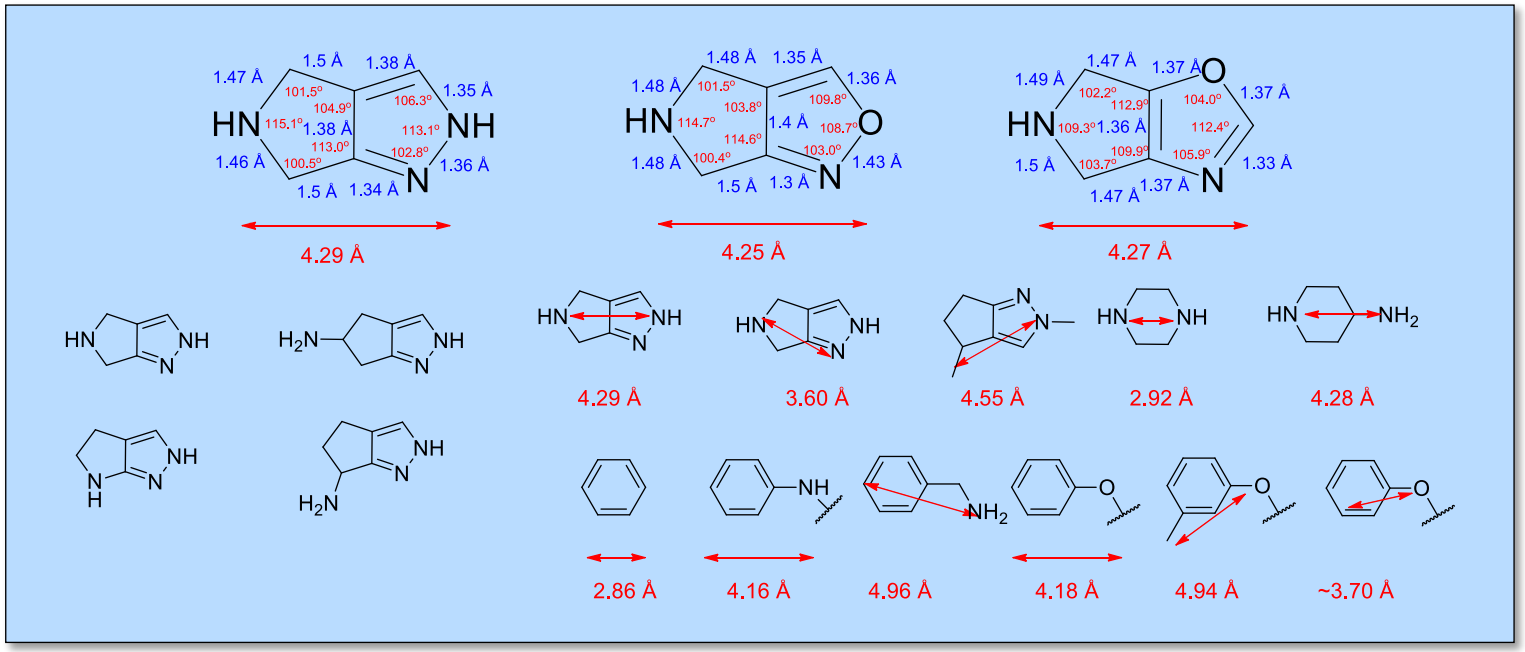
N.A. Meanwell *et al.*, *J. Med. Chem.,* 2018, **61**, 62-80; T. Wang *et al., Bioorg. Med. Chem. Lett.,* 2009, **19**, 5140-5145
S. Cocklin *et al., Bioorg. Med. Chem. Lett.,* 2014, **24**, 5439-5445; *Molecules,* 2018, **23**, 1940; *Molecules,* 2020, **25**, 1430

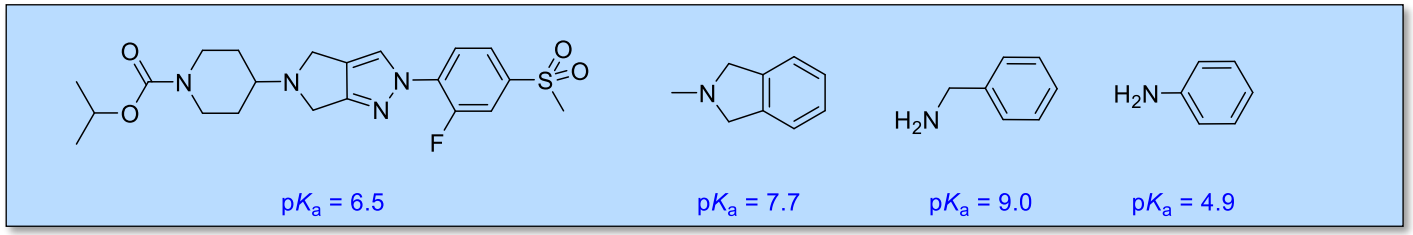
Pyrrolidino Azoles as Piperazine Bioisosteres



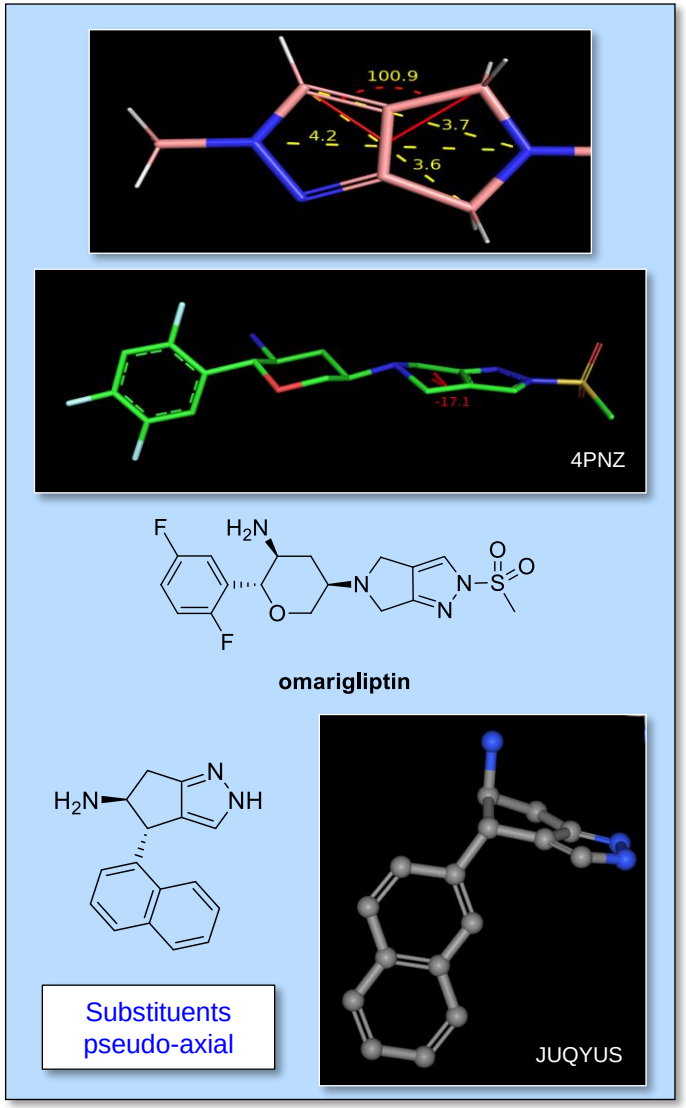
Geometries, X-Ray & pK_a Data

Pyrrolidines with fused heterocyclic rings





- Pyrrolidines & cyclopentanes adopt a planar topography
  - sp^3 centers allow flexibility for deviation from planarity
  - topographical preferences can be modulated by judicious substitution
- Nitrogen pK_a modulated by azole heterocycle identity & topology
  - affected by topology, electron withdrawing properties

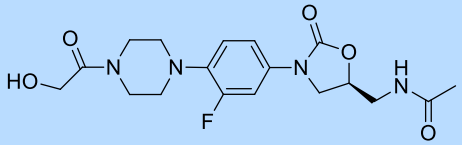


N.A. Meanwell & R. Sistla, *Adv. Het. Chem.,* 2021, **134**, 31-100; T. Biftu *et al., J. Med. Chem.* 2014, **57**, 3205-3212; E.M. Carreira *et al., Helv. Chim. Acta,* 2020, **103**, e2000058

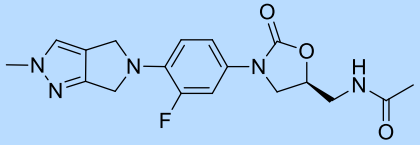
Pyrrolidino-Pyrazoles as Piperazine Bioisosteres

Pyrrolidines with fused heterocyclic rings

linezolid



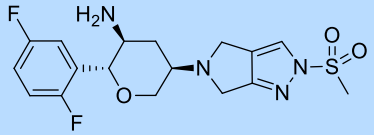
eperezolid



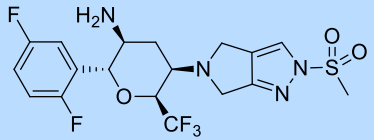
RWJ-416457

antibacterial agents

sitagliptin
DPP-4 inhibitor

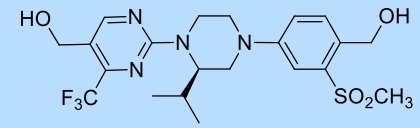


omarigliptin
long-acting DPP-4 inhibitor

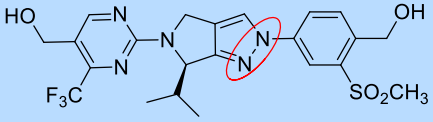


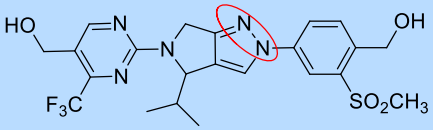
HSK7653
DPP-4 inhibitor

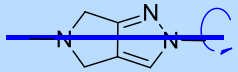
DPP-4 inhibitors



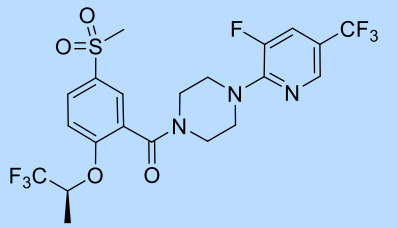
VTP-766



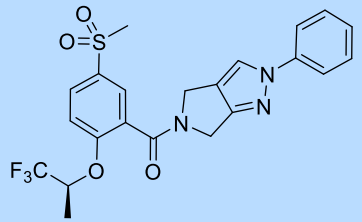




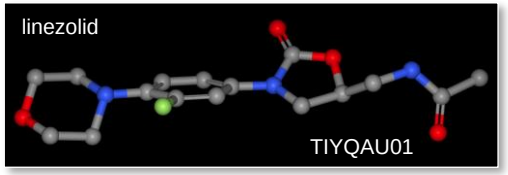
brain penetrant LXR modulators

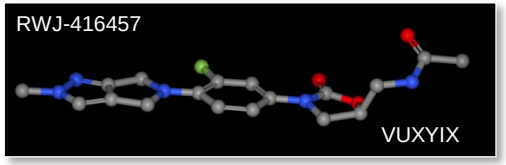


bitopertin
hGlyT IC_{50} = 30 nM

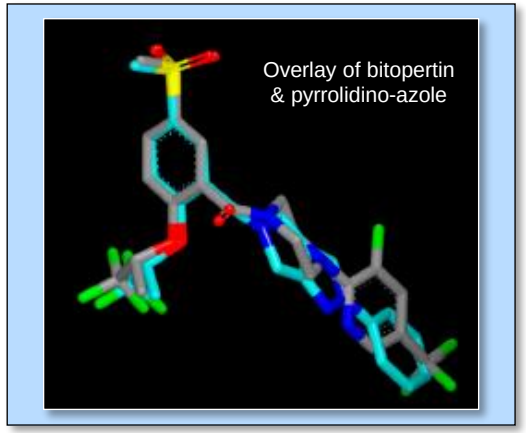


rGlyT IC_{50} = 4 nM
hGlyT IC_{50} = 15 nM
rat CL_{int} = 2 µL/min/mg
human CL_{int} = 0 µL/min/mg





- 2,4,5,6-Tetrahydropyrrolo[3,4-*c*]pyrazole the most exploited ring system
  - facile synthetic accessibility
- Applications predominantly as mimic of morpholine or piperazine
  - RWJ-416457 an analogue of linezolid
- DPP-4 inhibitors - sitagliptin
  - mimic of 5,6,7,8-tetrahydro-[1,2,4]triazolo[4,3-*a*]pyrazine
  - omarigliptin a long-acting (QW) drug approved in Japan
- Piperazine mimic in brain penetrant LXR modulators
  - recognized 1 aspect of topological asymmetry



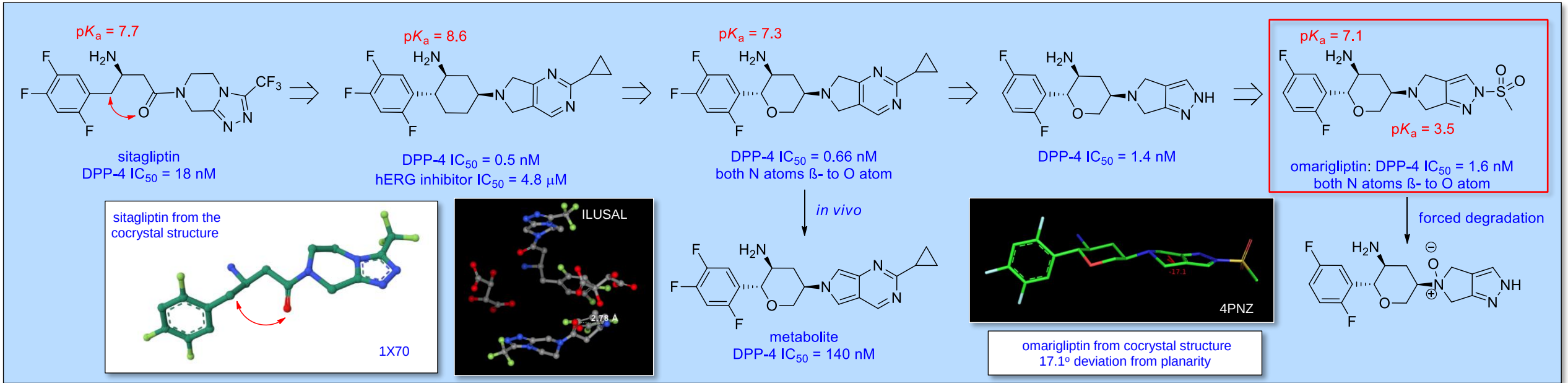
- Piperazine mimic in glycine trasport (GlyT) inhibitors
  - analogues of bitopertin
  - potency 2x ↑; low Cl

B.D. Foleno *et al., Antimicrob. Agents Chemother*., 2007, **51**, 361-365; T. Biftu *et al., J. Med. Chem.,* 2015, **57**, 3205-3212; L. Dong *et al., J. Med. Chem*., 2023, **66**, 11593-11631
C.J. Tice *et al., Bioorg. Med. Chem. Lett.*, 2016, **26**, 5044-5050; J. Hall *et al., Cell Chem. Biol*., 2021, **28**, 1221-1234; V.J. Santora *et al., J. Med. Chem.,* 2018, **61**, 6018-6033

NuArq MedChem Consulting LLC

Omarigliptin - A Long-Acting DPP-4 Inhibitor

Pyrrolidines with fused heterocyclic rings



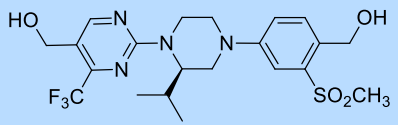
- Optimization of sitagliptin focused on conformational constraint
  - cyclohexane designed to pre-organize into bound conformation
  - 5 single crystal X-rays in CSD; 2 show NH_2 proximal to C=O
- Absence of C=O increased pK_a of NH_2 to 8.6
  - associated with hERG inhibition
- Pyran deploys O β- to NH_2
  - lowered pK_a of NH_2 to 7.3
  - reduced hERG inhibition & QTc prolongation in dogs
- Facile oxidation of the pyrrolo[3,4-*d*]pyrimidine ring observed *in vivo*
  - 50% in rats, 30% in dogs
  - 200x less potent DPP-4 inhibitor

- Pyrrolo[3,4-*c*]pyrazole ring addressed the problem
  - poor PK profile abrogated by $MeSO_2$ substituent
  - F = 100% in rats & dogs
- Forced degradation examined
  - acidic, basic, oxidative (H_2O_2), UV-C light
  - saw desulfonylation & *N*-oxide formation
- High bioavailability in humans (>75%) with $t_{1/2}$ = 82.5 h
  - 90% of drug excreted as parent
- Omarigliptin approved in Japan in September 2015
  - once-weekly dosing regimen for diabetes

T. Biftu *et al., J. Med. Chem.,* 2015, **57**, 3205-3212; C.B. Burness, *Drugs*, 2015, **75**, 1947-1952

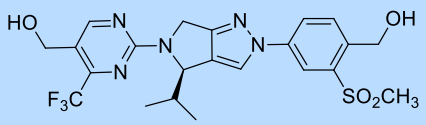
Piperazine Bioisostere in LXR Agonists

Pyrrolidines with fused heterocyclic rings



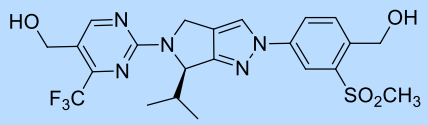
VTP-766

LXRα EC_{50} = 244 nM, Y_{max} = 60%
LXRβ EC_{50} = 21 nM, Y_{max} = 56%



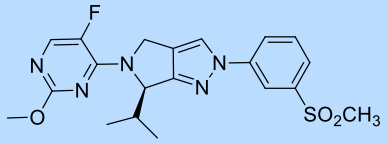
LXRα EC_{50} = 1376 nM, Y_{max} = 74%
LXRβ EC_{50} = 273 nM, Y_{max} = 56%

7x

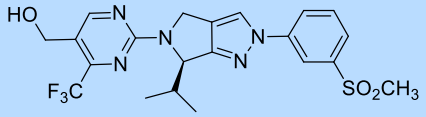


LXRα EC_{50} = 214 nM, Y_{max} = 79%
LXRβ EC_{50} = 36 nM, Y_{max} = 56%

More potent

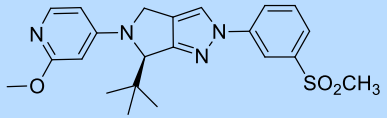


LXRα EC_{50} = 491 nM, Y_{max} = 45%
LXRβ EC_{50} = 15 nM, Y_{max} = 29%



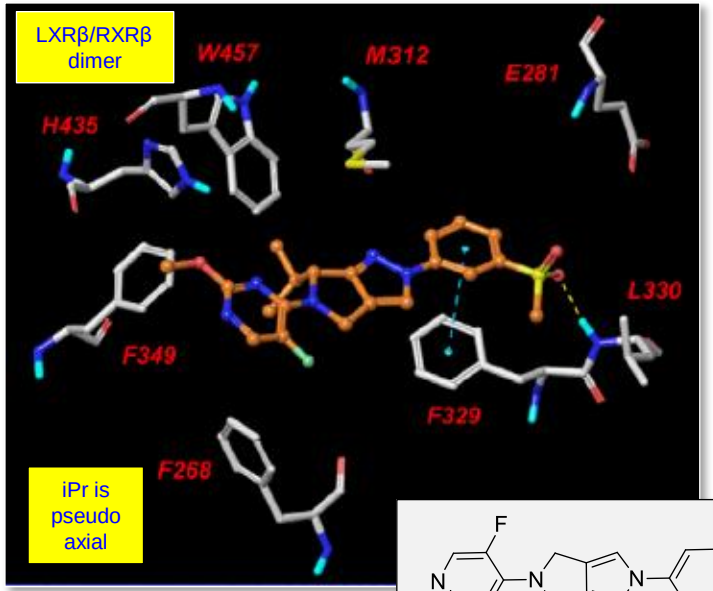
LXRα EC_{50} = 249 nM, Y_{max} = 67%
LXRβ EC_{50} = 46 nM, Y_{max} = 43%

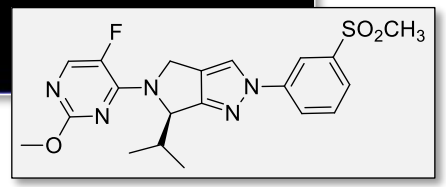
X-ray cocrystal



LXRα EC_{50} = 166 nM, Y_{max} = 83%
LXRβ EC_{50} = 38 nM, Y_{max} = 77%

upregulated ABCA1 *in vivo* in mice





- VTP-766 identified as a LXR NHR agonist
  - explored for potential to treat Alzheimer's disease
  - upregulation of ApoE
- VTP-766 exhibited modest bias for LXRβ over LXRα
  - LXRα EC_{50} = 244 nM, K_i = 81 nM, Y_{max} = 60%
  - LXRβ EC_{50} = 21 nM, K_i = 3 nM, Y_{max} = 56%
- VTP-766 exhibited several liabilities
  - low CNS exposure in mice
  - CYP 2C9 inhibitor
  - poor stability in LMs with species dependence
  - potential for release of an aniline *in vivo*

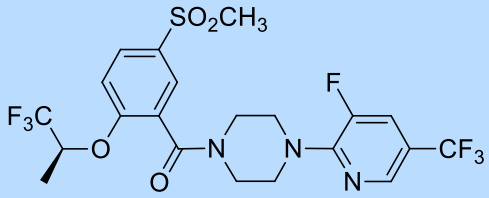
- Sought piperazine replacements
  - pyrrolidino[3,4-*c*]pyrazole explored
  - activity sensitive to heterocycle topology
    - 7x difference
- Further optimization focused on phenyl ring substitution
  - SO_2Me preserved: π stacking; H-bond to NH of Leu_{330}
  - removal of CH_2OH enhanced LXRβ potency
- Oral administration of MeO-pyridine/tBu to mice
  - upregulated LXR target gene ABCA1
  - no effect on Aβ levels
  - approach abandoned

C. Tice *et al., J. Med. Chem.,* 2014, **57**, 7182-7205; Y.-J. Zheng *et al., J. Med. Chem.,* 2016, **59**, 3264-3271

GlyT1 Inhibitors for Cognition Enhancement

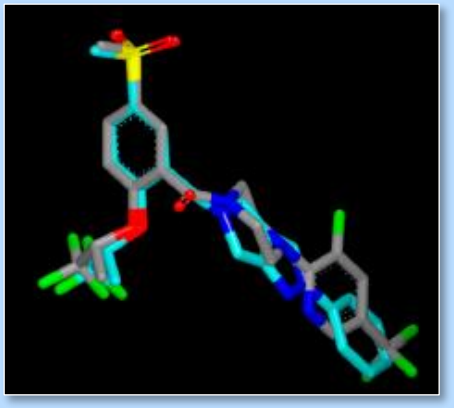
Pyrrolidines with fused heterocyclic rings

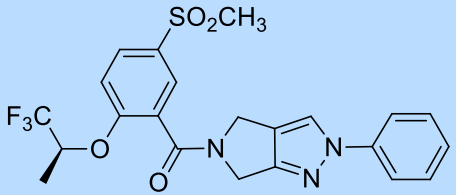
- Glycine transporter-1 (GlyT1)
  - expressed in glial & neuronal cells in hippocampus & cortex
  - regulates synaptic levels of glycine, a co-agonist at the NMDA receptor
- Inhibitors of GlyT1 explored for multiple indications
  - pain, OCD, Parkinson's disease, enhancing cognition
  - treatment of anxiety, depression & the -ve & +ve symptoms of schizophrenia
- Bitopertin a lead GlyT1 inhibitor
  - replacing piperazine with a pyrrolidino-pyrazole explored
  - modeling studies indicated potential for good structural overlap
    - recognized that phenyl would be projected further from amide
- Prototype exhibited excellent potency
  - good selectivity over GlyT2 IC_{50} = >10 µM
  - stable in HLM & RLM
  - no hERG inhibition at ≤10 µM
  - good permeability in a PAMPA assay
- Replacing $MeSO_2$ with C≡N reduced efflux in MDCK cells
  - enhanced CNS exposure
  - F = 81%, rapid absorption, slow clearance from plasma
- Optimized compound enhanced Gly levels in rat CSF after PO dosing
  - active in novel object recognition (NOR) model at oral doses of 0.1, 1 & 3 mg/kg



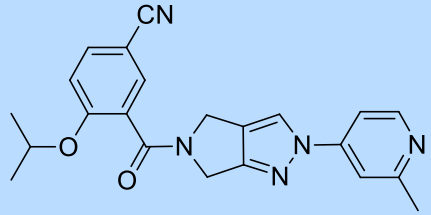
bitopertin

hGlyT1 IC_{50} = 30 nM
hGlyT2 IC_{50} >30 µM
hERG IC_{50} = 17 µM
mouse B/P = 0.5





rGlyT IC_{50} = 4 nM
hGlyT1 IC_{50} = 15 nM
rat CL_{int} = 2 µL/min/mg
human CL_{int} = 0 µL/min/mg



rGlyT1 IC_{50} = 38 nM
hGlyT1 IC_{50} = 21 nM
rat CL_{int} = 7.2 µL/min/mg
human CL_{int} = 3.7 µL/min/mg
rat $t_{1/2}$ = 2.4 h; F = 81%

Piperazine bioisostere

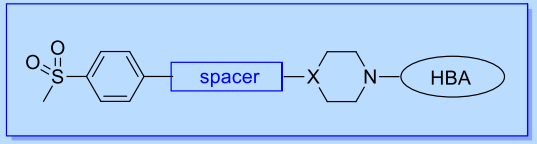
- Bitopertin recently designated as an orphan drug
  - erythropoietic protoporphyria

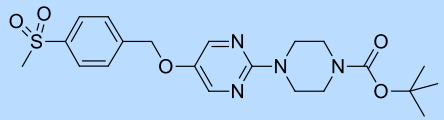
OCD: obsessive-compulsive disorder

V.J. Santora *et al., J. Med. Chem.,* 2018, **61**, 6018-6033

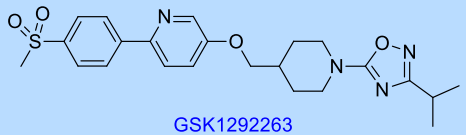
GPR119 Agonists

Pyrrolidines with fused heterocyclic rings

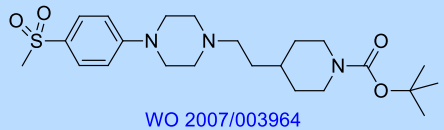




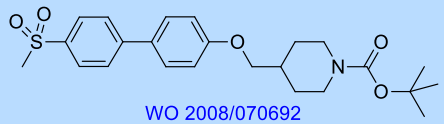
cAMP IC_{50} = 65 nM
IA = 83%



GSK1292263

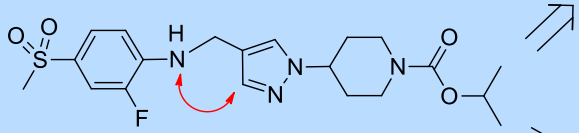


WO 2007/003964



WO 2008/070692

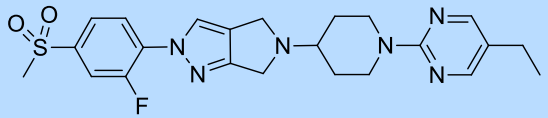
- GPR119 agonists
  - stimulate GLP-1 secretion
- Pharmacophore mapped
  - spacer element variable
  - piperazines claimed by Prosidion
  - phenoxy derivatives claimed by GSK



cAMP IC_{50} = 181 nM
IA = 41%
CL_{int} = 9.7 μL/min/mg

cAMP IC_{50} = 864 nM
IA = 105%
CL_{int} = 10.5 μL/min/mg

cAMP IC_{50} = 260 nM
IA = 48%
CL_{int} = 36.1 μL/min/mg



cAMP IC_{50} = 83 nM
IA = 118%
CL_{int} = 23 μL/min/mg

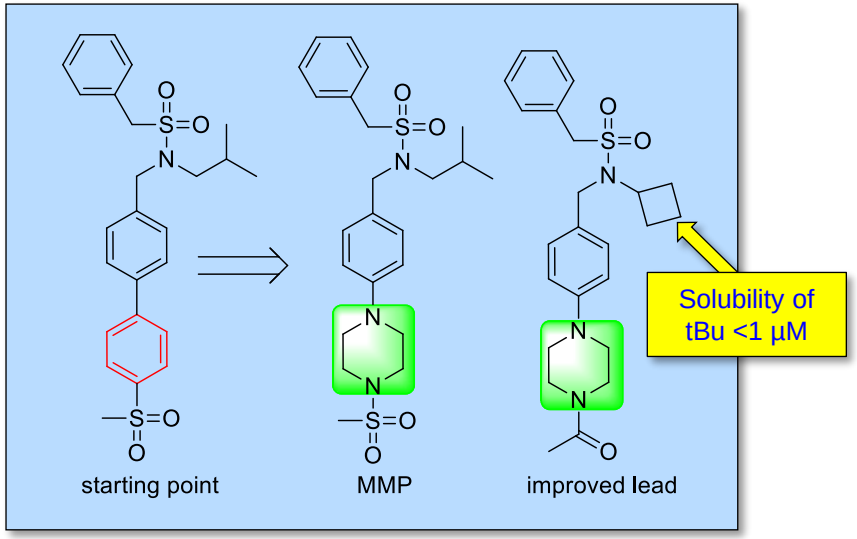
- Optimization of lead GPR119 agonist explored conformational constraint
  - pre-organize for binding
  - reduce potential for aniline release *in vivo*
- Recognized topological pseudo-symmetry
  - modeled & explored both topological variants
- Potency & efficacy dependent on structure
  - more efficacious isomer had the more basic pyrrolidine
  - piperidine conformers more readily able to sample axial topography
- Optimization enhanced potency 10-fold while maintaining efficacy
  - abandoned since inferior to existing GPR119 agonists

Phenyl, azine and piperidine/piperazine bioisostere

K. Futatsugi *et al*., *Bioorg. Med. Chem. Lett.,* 2013, **23**, 194-197; J.S. Scott *et al*., *Bioorg. Med. Chem. Lett.,* 2013, **23**, 3175-3179; K. Ritter *et al., J. Med. Chem*., 2016, **59**, 3579-3592

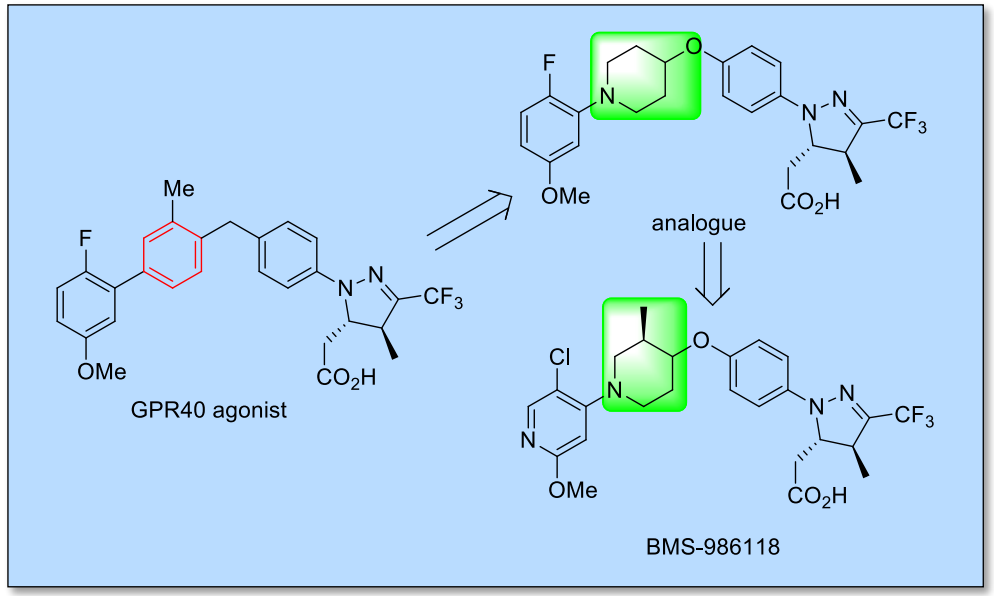
Isosteres of Piperazine

Phenyl Bioisosterism in RORγ, GPR40, PHD-1 HIF



	Lead	Bioisosteric analogue		Impact
	Start	MMP	lead	
RORγ SRC1 EC_{50} (nM)	25	21	30	reduced lipophilicity; improved solubility; lower protein binding
RORγ Cell EC_{50} (nM)	450	NT	120	
cLog *P*	4.5	2.9	3.2	
Sol. (µM)	<1	<1	37	
Human PPB	99%		94%	
MDCK P_{app} A-B ($*10^{-6}$ cm/s)	3		24	

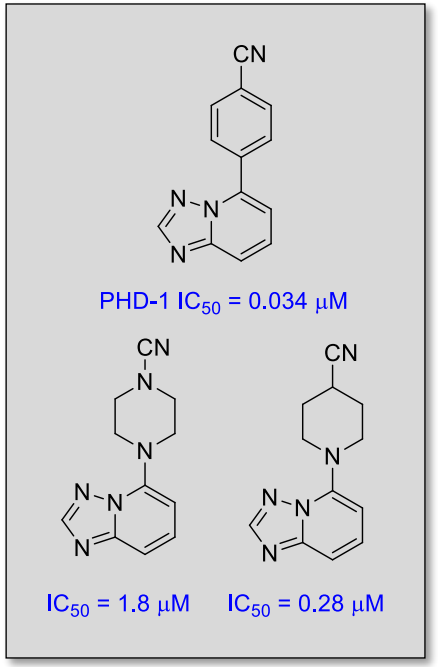
- Replaced a phenyl ring with piperazine
  - potency retained; cLog *P* reduced
  - optimized compound: higher solubility & permeability



	Lead	Bioisosteric analogue		Rationale/impact
	lead	analogue	BMS-986118	
hGPR40 EC_{50} (nM)	33	10	70	increased sp^3 content; lower cLog *P*; reduced metabolism
mGPR40 EC_{50} (nM)	31	70	63	
cLog *P*	7.7	5	5.9	
PPARγ EC_{50} (µM)	2.6	>50	>47	
HLM % rem.		42	89	

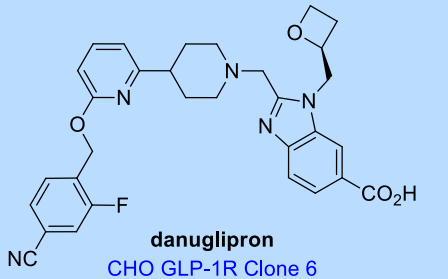
- Replaced a scaffolding phenyl ring with piperidine
  - potency retained; cLog *P* reduced
- Optimized compound exhibited higher metabolic stability
  - improved selectivity over PPARγ

- PHD-1 HIF inhibitors
  - piperazine poorly effective
  - cyano-piperidine better
  - still 10 less potent

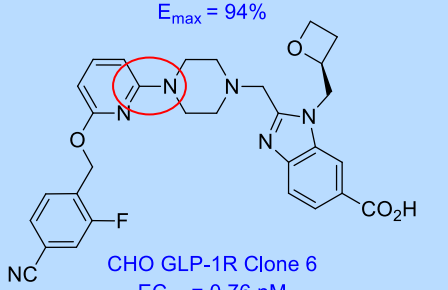


B.P. Fauber *et al., Bioorg. Med. Chem. Lett.,* 2014, **24**, 3891-3897; J. Shi *et al., J. Med. Chem.,* 2018, **61**, 681-694; S. Ahmed *et al., J. Med. Chem*., 2017, **60**, 5663-5672

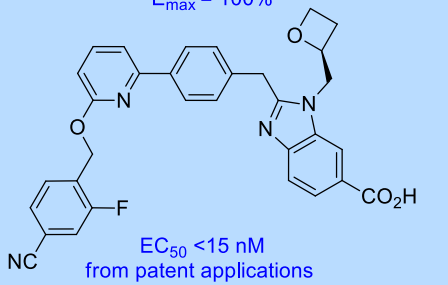
Glucagon-like Peptide (GLP-1) Agonists



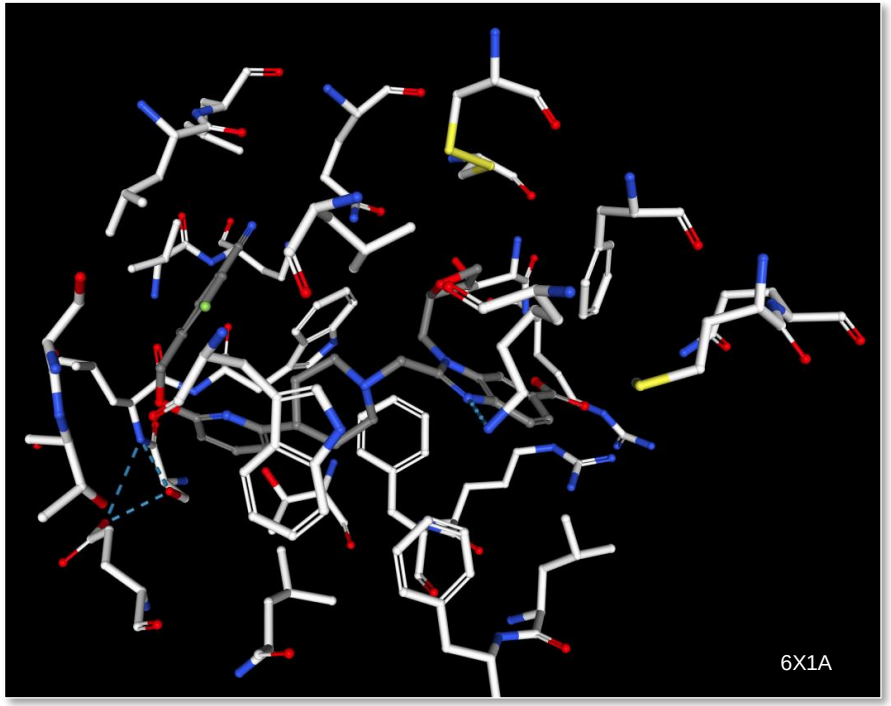
danuglipron
CHO GLP-1R Clone 6
EC_{50} = 0.34 nM
E_{max} = 78%
EC_{50} = 7.4 nM
E_{max} = 94%

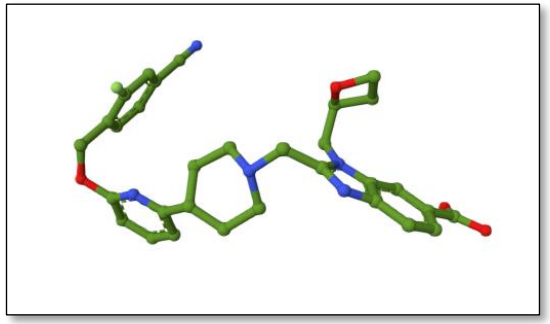


CHO GLP-1R Clone 6
EC_{50} = 0.76 nM
E_{max} = 88%
EC_{50} = 8.8 nM
E_{max} = 100%



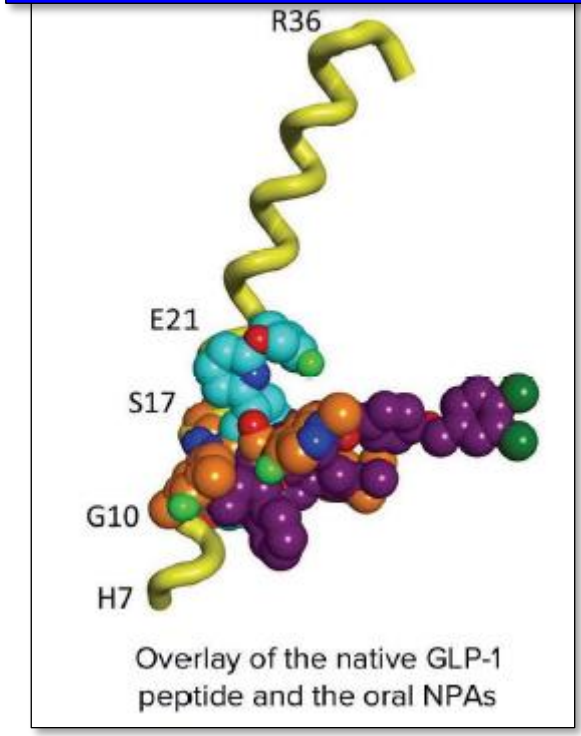
EC_{50} <15 nM
from patent applications





- GLP-1 agonists
  - bind to & activate a GPCR
  - mimic GLP-1, a 30 (7-36) or 31 (7-37) residue peptide incretin
- Danuglipron - small molecule GLP-1 agonist
  - completed P2a study as BID formulation:
    - lower efficacy, higher side effects than competitors
  - QD formulation to be studied
- Piperazine equivalent to piperidine
  - scaffolding element
  - phenyl also potently active: several patent applications

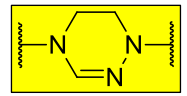
HAEGTFTSDVSSYLEGQAAKEFIAWLVKGR

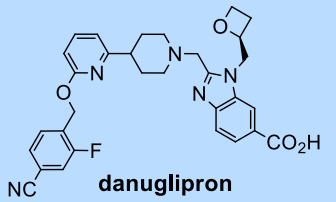


Overlay of the native GLP-1 peptide and the oral NPAs

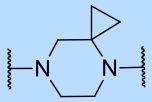
T. Fields *et al., Med. Chem. Rev*., 2023, **58**, 107-132; G.E. Aspnes *et al., World Patent Application,* WO 2018/109607;
X. Zhang *et al., Molec. Cell*, 2020, **80**, 485-500; D.A. Griffith *et al., J. Med. Chem*., 2022, **65**, 8208-8226; Z. Wenge *et al.,* US 2020/0325121

1,4,5,6-Tetrahydro-1,2,3-triazine in GLP-1 Agonists

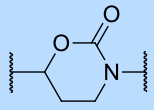




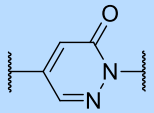
danuglipron
EC_{50} = 0.04 nM



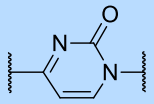
EC_{50} = 1 nM



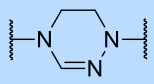
EC_{50} = 7 nM



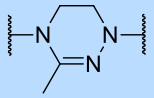
EC_{50} = 0.5 nM



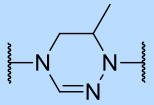
EC_{50} = >10,000 nM



EC_{50} = 0.1 nM

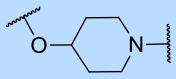


EC_{50} = 33 nM

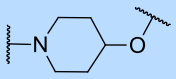


EC_{50} = 0.6 nM

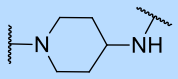
1,4,5,6-tetrahydro-1,2,4-triazine



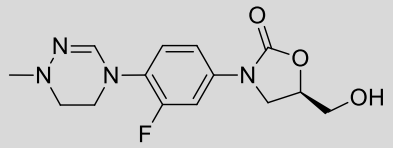
EC_{50} = 0.7 nM



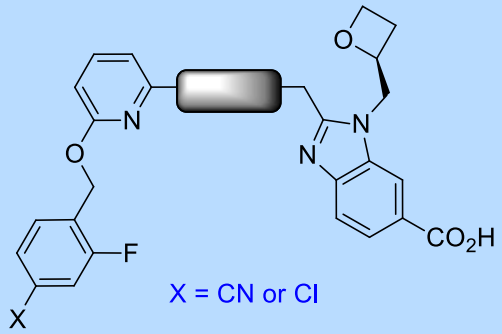
EC_{50} = 13 nM



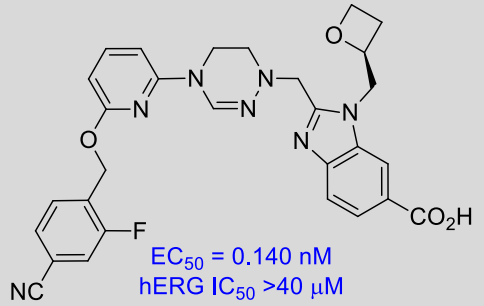
EC_{50} = 153 nM



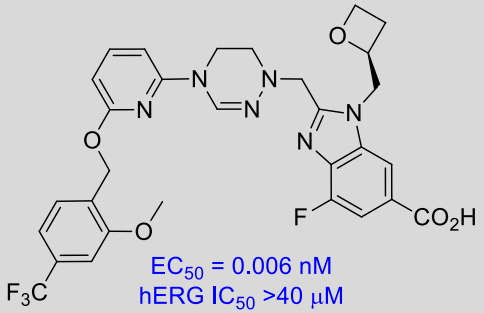
delpazolid



X = CN or Cl

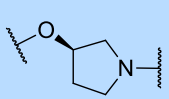


EC_{50} = 0.140 nM
hERG IC_{50} >40 μM

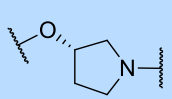


EC_{50} = 0.006 nM
hERG IC_{50} >40 μM

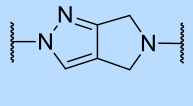
danuglipron hERG IC_{50} = 2.6 μM



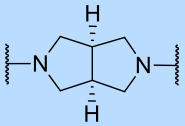
EC_{50} = 1.6 nM



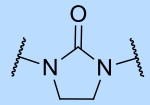
EC_{50} = 77 nM



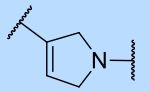
EC_{50} = 96 nM



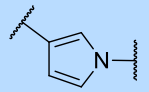
EC_{50} = >10,000 nM



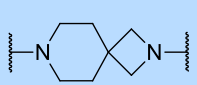
EC_{50} = 134 nM



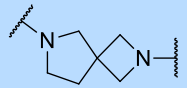
EC_{50} = 1 nM



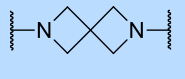
EC_{50} = 37 nM



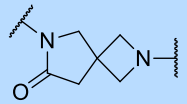
EC_{50} = 3,517 nM



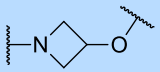
EC_{50} = 5,005 nM



EC_{50} = 7,372 nM



EC_{50} = >10,000 nM

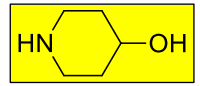


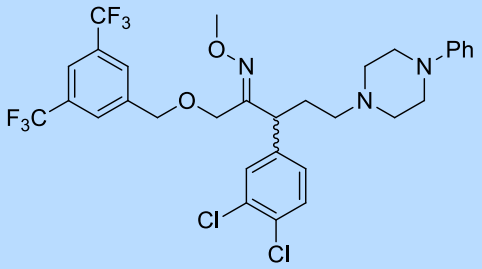
EC_{50} = 25 nM

- Wide range of scaffolding elements explored
  - variable potency
- 1,2,3-Triazine retains GLP-1 agonism
  - reduced hERG inhibition compared to danuglipron in MMP
- 1,2,3-Triazine exploited previously in delpazolid
  - replacement for morpholine & piperazine

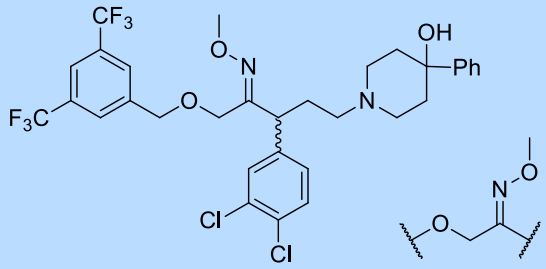
L. Chen *et al*., *J. Med. Chem*., 2023, **66**, 7988-8010; J.-W. Jeong *et al., Antimicrob. Agents. Chemother.,* 2010, **54**, 5359-5362; Y.L. Cho *et al., Appl. Sci*., 2020, **10**, 2211

C-OH for N in Piperazines



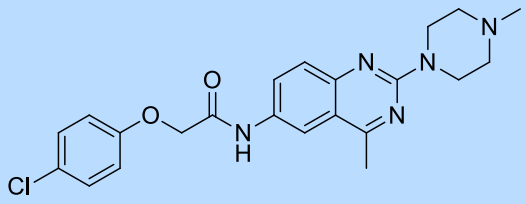


NK_1: K_i = 181 nM
NK_2: K_i = 978 nM

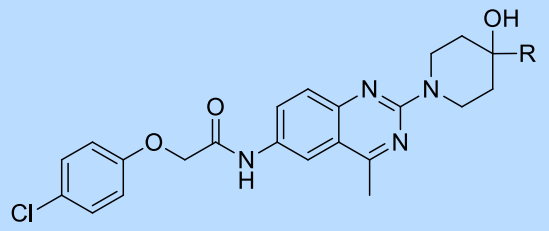


NK_1: K_i = 25 nM
NK_2: K_i - 21 nM

NK_1: K_i = 21 nM
NK_2: 35% @ 1 μM



hMCHR1 IC_{50} = 220 nM
IP3 IC_{50} = 3,800 nM

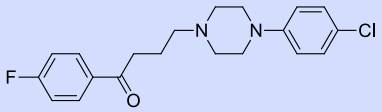
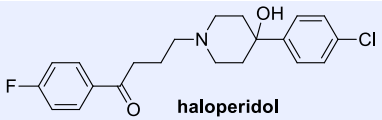


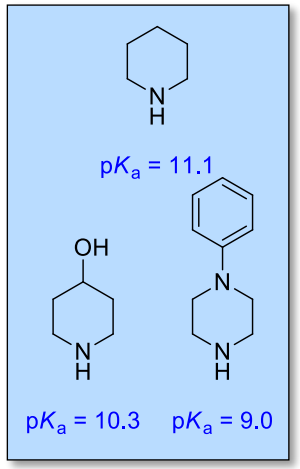
R = H
hMCHR1 IC_{50} = 150 nM
IP3 IC_{50} = 163 nM

R = CH_3
hMCHR1 IC_{50} = 237 nM
IP3 IC_{50} = 827 nM

- Dual NK1/NK2 antagonists with potential as asthma therapeutics
  - piperidinol more potent than piperazine at both receptors
  - confers more balanced antagonism
  - oxime geometry important
- Piperazines could be optimized to more potent, dual, balanced antagonists

- Melanin concentrating hormone receptor 1 (MCHR1) antagonists
  - treatment of obesity – induce weight loss
- 4-OH piperidine studied based on prevalence in GPCR ligands
  - 803 molecules in ChEMBL in 153 chemotype clusters
- Modest potency with some advantage in hERG assays

K_i (nM)	D_2	D_4	$5HT_{1A}$	$5HT_{2A}$	$5HT_{2C}$	H-1	D_2/D_4	D_{2L} pK_d	D_{2L} k_{on} (M^{-1} min^{-1})	D_{2L} k_{off} (min^{-1})	$t_{1/2}$ (min)
	253.5	17.5	90.9	109.6	3,552	157.6	14.5	7.55	2.86 x10^7	0.80 x10^9	0.89
 haloperidol	0.89	10	3,600	120	4,700	440	0.09	9.31	1.29 x10^9	0.61 x10^9	1.15

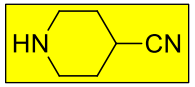


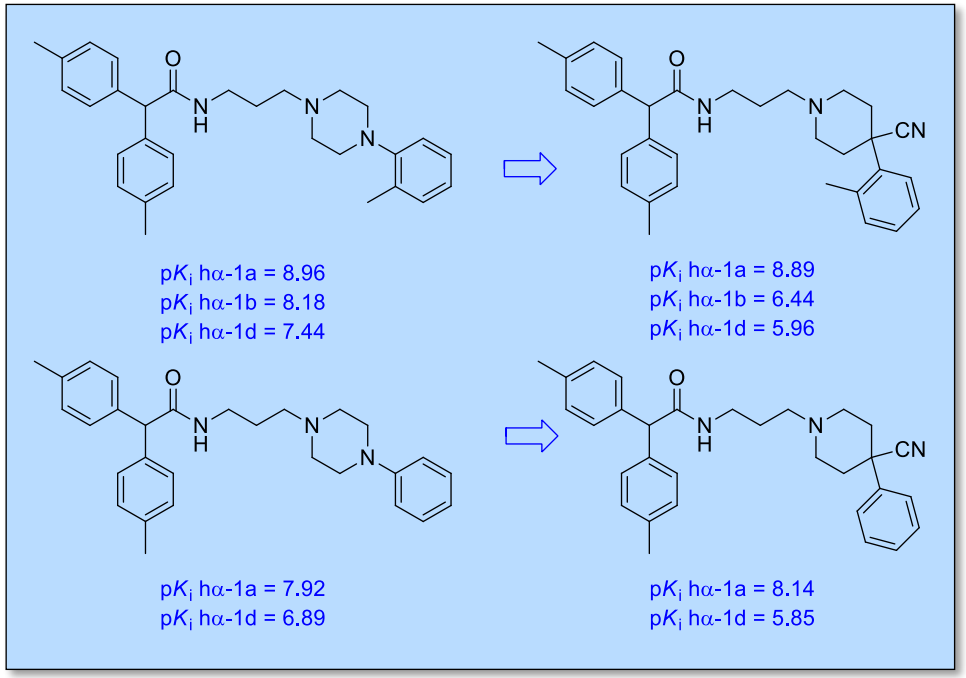
pK_a = 11.1
pK_a = 10.3
pK_a = 9.0

- Haloperidol subject to dehydration & oxidation to a pyridinium species that may be associated with toxicity toward dopaminergic neurons
- N to C-OH edit reduces D_2 binding but preserves affinity for D_4; reflected in slower D_2 binding kinetics; $5HT_{1A}$ binding enhanced by 40x

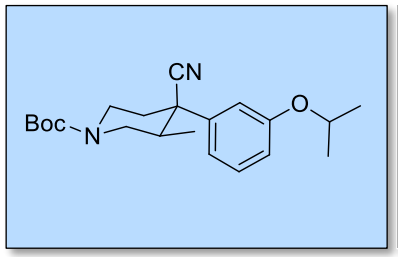
G.A. Reichard *et al., Bioorg. Med. Chem. Lett.,* 2000, **10**, 2329-2332; P.C. Ting *et al., Bioorg. Med. Chem. Lett.,* 2000, **10**, 2333-2335; S. Sasmal *et al., Bioorg. Med. Chem. Lett.,* 2012, **22**, 3157-3162; S.Y. Ablordeppey *et al., Bioorg. Med. Chem.,* 2008, **16**, 7291-7301; T.J. Fyfe *et al., J. Med. Chem.,* 2019, **62**, 9488-9520

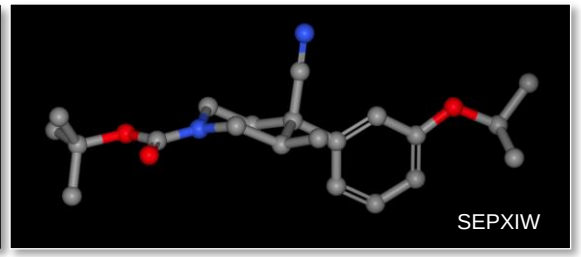
C-CN for N in Piperazines

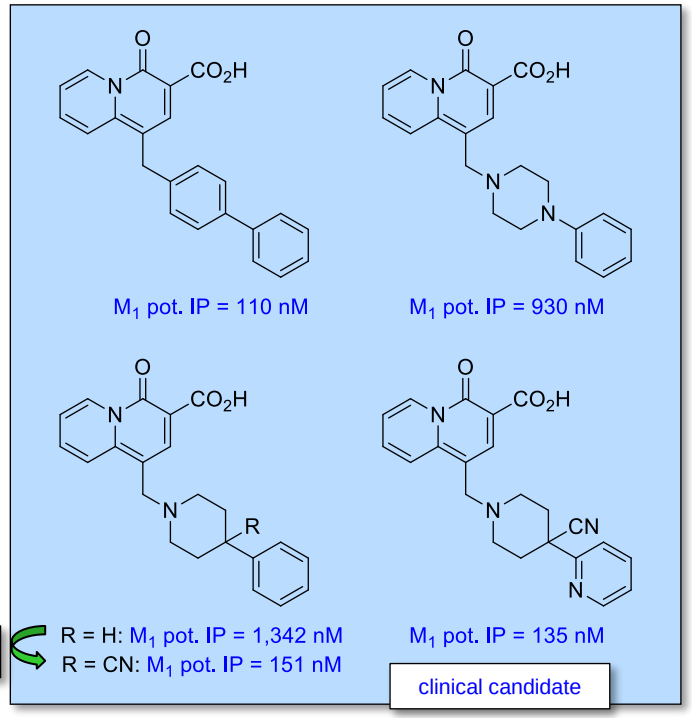




- Human α_{1a} adrenergic ligands
  - CN-piperidine maintains α_{1a} receptor affinity
- Enhances receptor selectivity
  - reduces affinity for α_{1b} & α_{1d} receptors

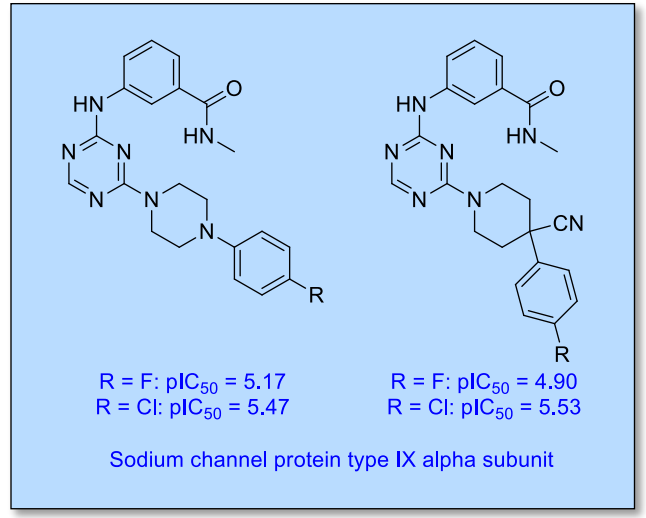


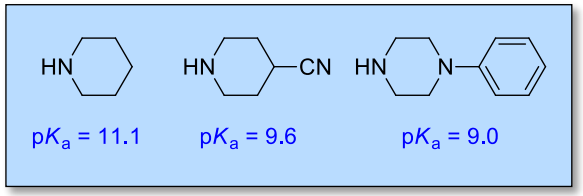




- Muscarinic M_1 positive allosteric modulator (PAM)
- Biphenyl prototype potent
  - poorly soluble; presumed high protein binding
- Phenyl piperazine 9x less potent: lower PPB (63/74% r/h)
- Phenyl piperidine a weak PAM
  - nitrile enhanced potency almost 10x; 86/89% PB in r/h
- Pyridine optimal compound
  - high free fraction; good physical properties
  - good CNS penetrance; active in models of cognition
- Selected for clinical evaluation

- Na channel IX subunit inhibitors
  - data from SwissBioisostere
- N to C-CN is a neutral edit

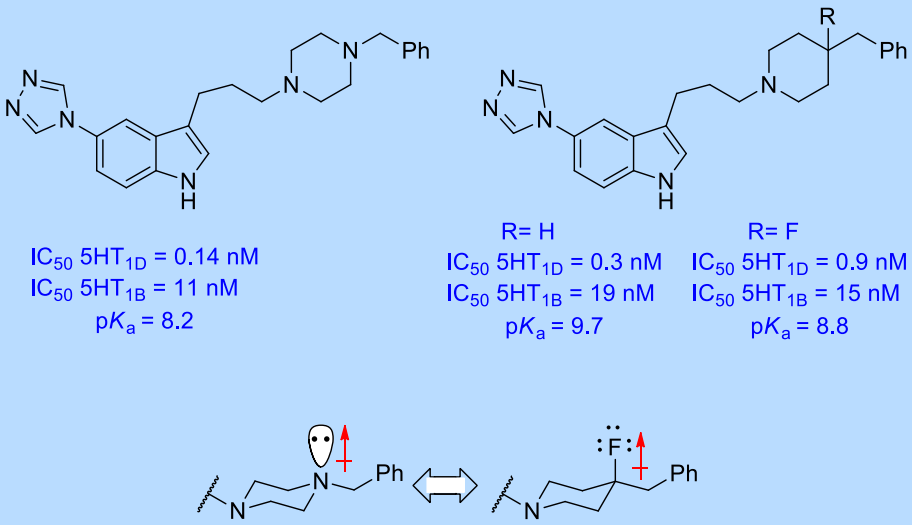




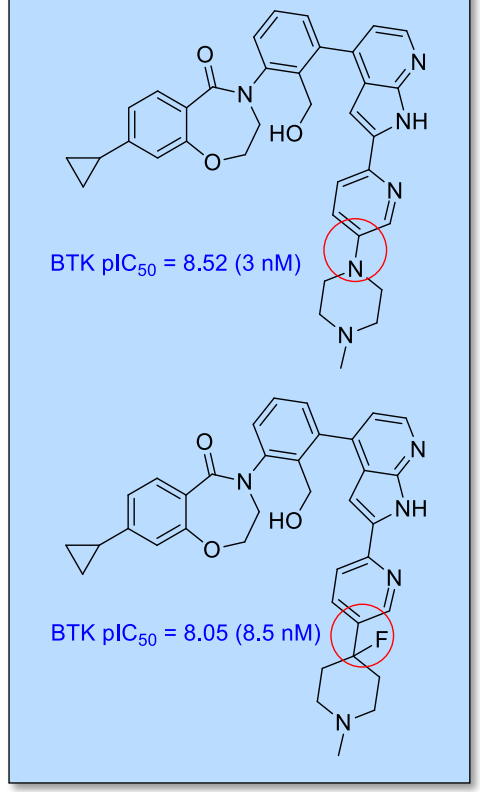
Effect of C-CN for N substitution in piperazines depends on context

S. Kuduk *et al., ACS Med. Chem. Lett.*, 2010, **1**, 263-267; *J. Med. Chem.*, 2011, **13**, 4773-4780; M.A. Patane et al., *Bioorg. Med. Chem. Lett.*, 2000, **10**, 1621-1624
S. Schmitt *et al., J. Org. Chem.* 2013, **78**, 1222-1229 (X-ray)

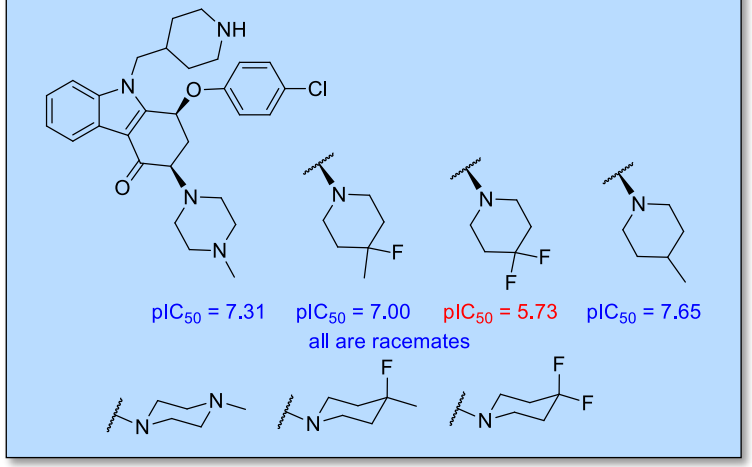
C-F for N in Piperazines



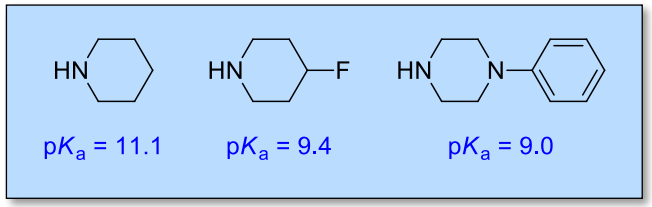
- $5HT_{1D}$ agonists with potential as migraine therapy
- Piperidine & 4-F-piperidine effective mimics of piperazine
  - 4-H: partial agonist at $5HT_{1D}$ receptor: Y_{max} = 63%
  - 4-F: partial agonist at $5HT_{1D}$ receptor: Y_{max} = 58%
- pK_a of 4-F piperidine similar to piperazine
  - less basic than piperidine
  - isosterism based on electron density of F mimicking lone pair
- 4-F-piperidine exhibited much higher absorption in rat than piperidine
  - 4-F-piperidine > piperazine >> piperidine



- BTK inhibitors
  - patent application
  - data from SwissBioisostere
- N to C-F is a neutral molecular edit
  - in this context

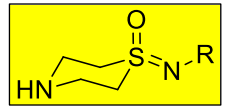


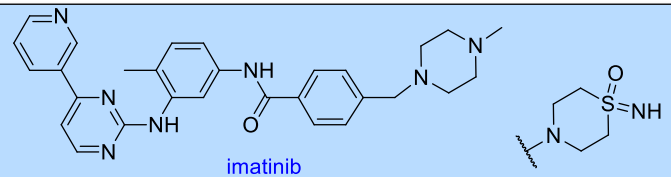
- NPY-1 antagonists
  - F-piperidine a neutral edit
  - F_2 piperidine poor
- Not clear that N is pharmacophoric
  - Me-piperidine fully active



M.B. van Neil *et al., J. Med. Chem.,* 1999, **42**, 2087-2104; M. Thakkur *et al.*, *World Patent Application,* WO2013/157022
R. Di Fabio *et al., Bioorg. Med. Chem. Lett.,* 2006, **16**, 1749-1752

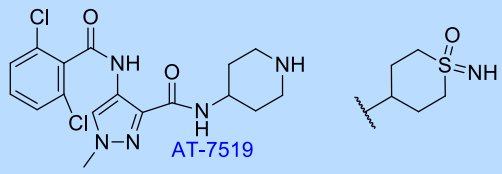
A Cyclic Sulfoximine as a Piperazine Bioisostere





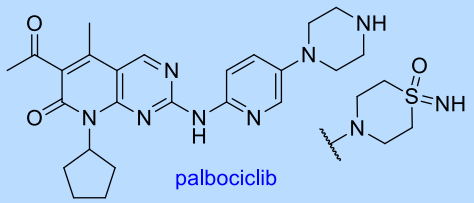
Less potent vs ABL1
Comparable vs KIT, PDGFRβ
Less permeable

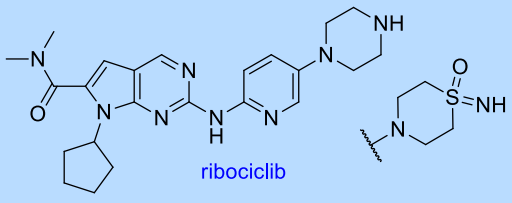
K_d (nM)			Phys. chem. properties		Cl (L/h/kg)		Permeability	
ABL1	KIT	PGDFRβ	Sol. pH 6.5 (µg/Ml)	Log *D* pH 7.5	rHep	HLM	Caco 2 P_{app} A→B (nm/s)	ER
1.1	13	14	112	1.9	2.3	0.48	39	2.7
79	11	19	54	2.0	1.9	0.34	<2	134



Less potent
Less permeable
Less soluble
Lower Cl

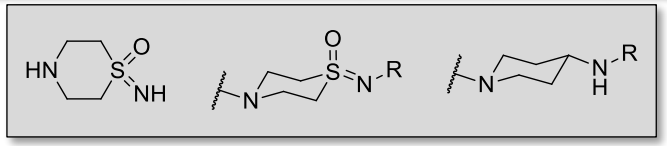
IC_{50} (nM)			Phys. chem. properties		Cl (L/h/kg)		Permeability	
CDK2	CDK9	A2780 (cell assay)	Sol. pH 6.5 (µg/mL)	Log *D* pH 7.5	rHep	HLM	Caco 2 P_{app} A→B (nm/s)	ER
96	6	131	1524	1.3	1.7	0.24	1.0	92
522	124	351	52	1.6	0.06	0.06	1.4	37

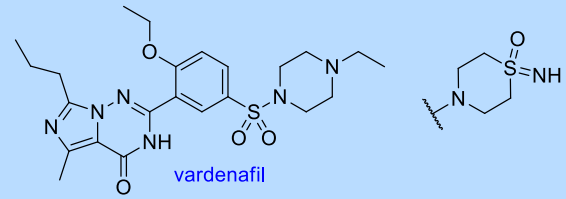




Less potent,
Less permeable
Lower Cl

IC_{50} (nM)			Phys. chem. properties		Cl (L/h/kg)		Permeability	
CDK4	CDK6	MOLM-13 (cell assay)	Sol. pH 6.5 (µg/mL)	Log *D* pH 7.5	rHep	HLM	Caco 2 P_{app} A→B (nm/s)	ER
7	57	41	34	1.9	1.3	0.45	70	2.6
101	240	128	30	2.0	1.1	0.24	25	9.1
67	803	89	334	1.7	2.3	0.52	135	1.3
216	>1000	1150	22	1.8	1.1	0.21	22	11

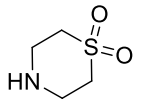


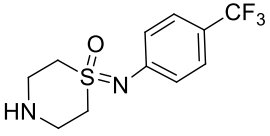


Potency retained
Less permeable
Less soluble
Lower Cl

PDE5 IC_{50} (nM)	Sol. pH 6.5	Log *D* pH 7.5	Cl (L/h/kg)		Caco 2 P_{app} A→B (nm/s)	ER
			rHep	HLM		
0.029	220 µg/mL	2.6	3.0	1.1	206	0.87
0.025	52 µg/mL	2.0	2.1	0.43	0.71	258

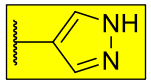
- 1λ⁶-Thiomorpholine-1-imine 1-oxide explored as a potential piperazine mimetic
  - installed in tyrosine kinase inhibitors & a PDE5 inhibitor
- Weakly basic at both *N* atoms
  - sulfoximine N pK_a <2; ring N ~5.2
- Maybe useful as a scaffolding element
  - geometry better as a 4-NH_2-piperidine mimic?

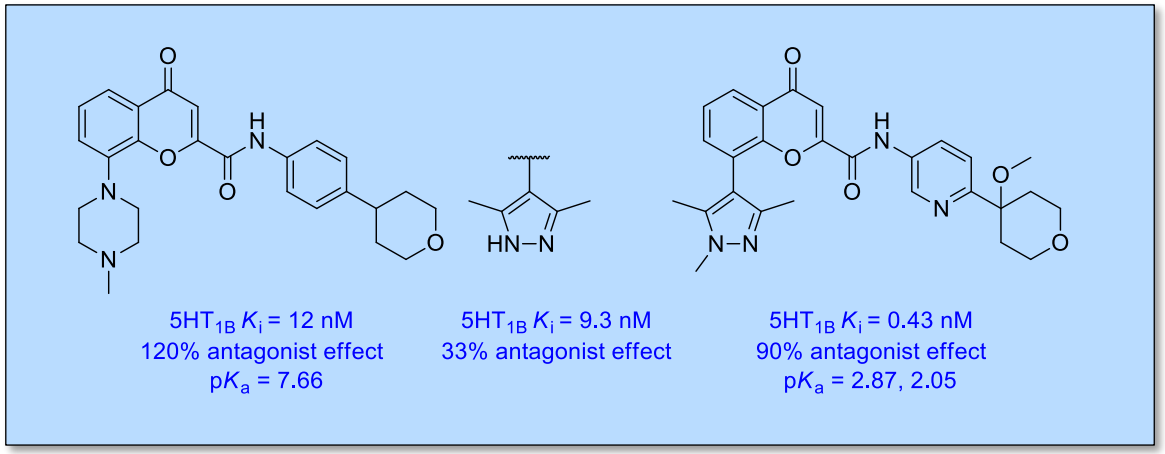

pK_a = 5.4


pK_a = 5.2

U. Lücking *et al*., *ChemMedChem,* 2017, **12**, 487-501; C. Gnamm *et al., Eur. J. Med. Chem*., 2017, **126**, 225-245

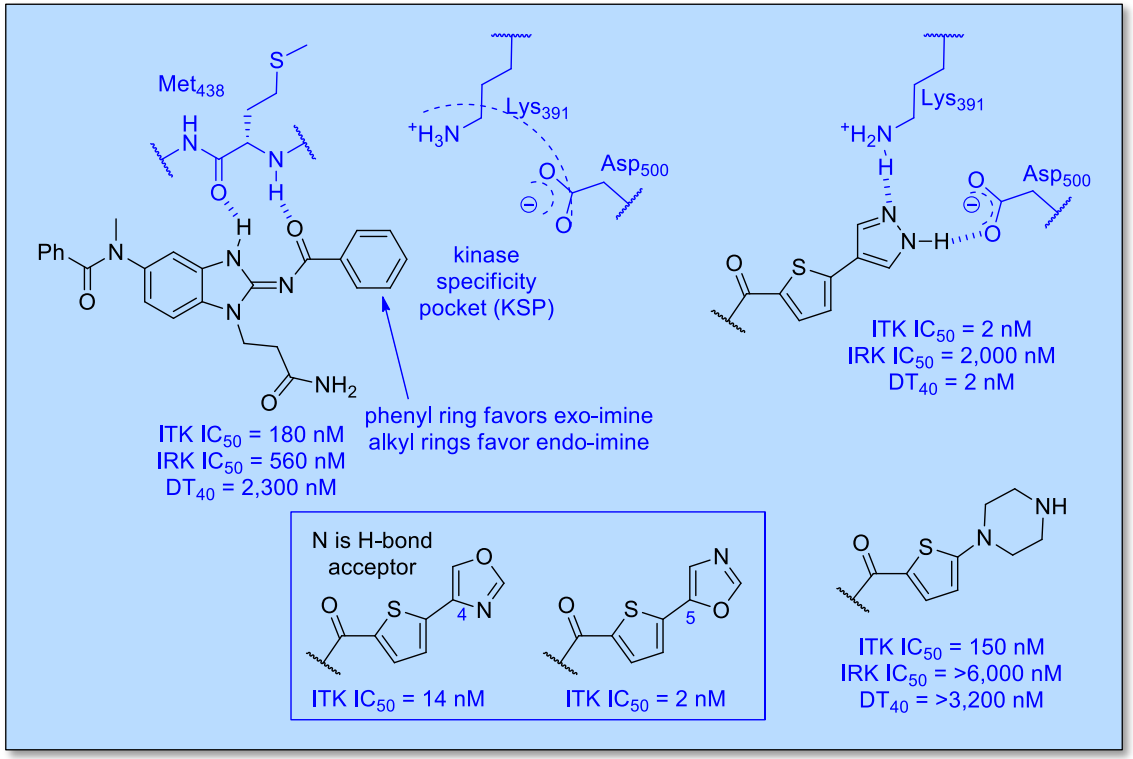
Pyrazole as a Piperazine Mimetic





- Design premise:
  - pyrazole N-H can mimic protonated piperazine
  - X-ray of TGFβ kiinase inhibitor showed pyrazole N-H engaging CO_2^-
- Prototype exhibited high affinity
  - antagonitstic efficacy compromised
- Optimization enhanced affinity & antagonism
  - N-H not essential
- Questions fundamental design concept in this context

- Interesting concept: where protonated piperazine acts as H-bond donor, other H-bond donors may be effective



- Interleukin-2-inducible kinase (ITK) inhibitor
  - amino benzimidazole is hinge binder: exo-imine essential
  - build specifity over insulin receptor kinase (IRK) by accessing KSP
- Thiophene provided optimal exit vector
  - NH pyrazole optimal substuent:
  - dual H-bonds with ITK
- 5-Oxazole 10x less potent: can only engage Lys$_{391}$
  - 4-oxazole 7x less potent: reflects H-bonding preference for N
- Piperazine 75x less potent: can only engage Asp$_{500}$

D.A. Nugiel *et al.*, *J. Med. Chem.*, 2010, **53**, 1876-1880; B.N. Cook *et al., Bioorg. Med. Chem. Lett.*, 2009, **19**, 773-777

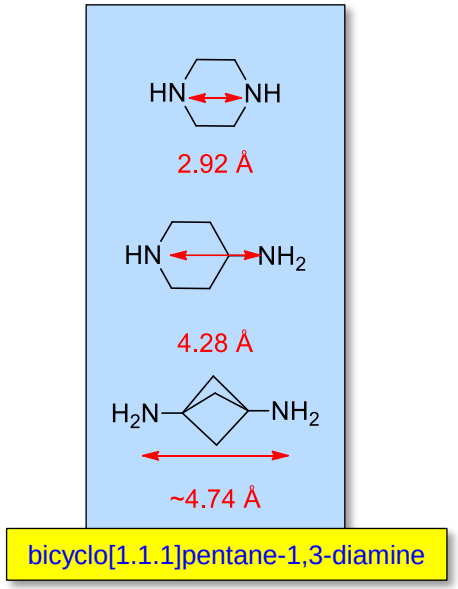
Conclusion

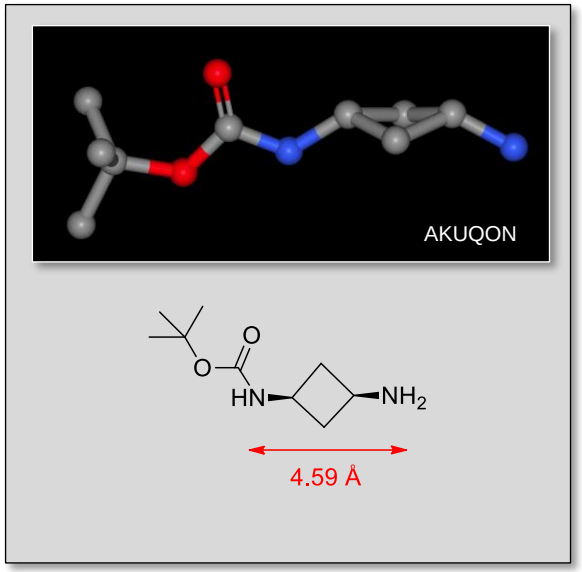
Conclusion

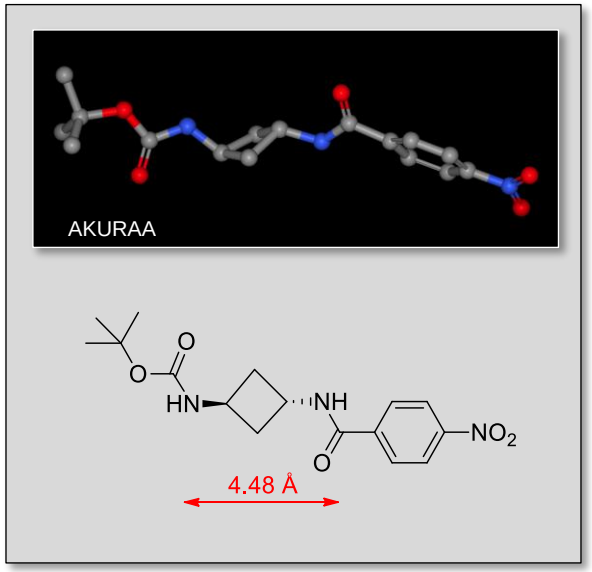
- Piperazine rings offer properties of value in drug design
  - basicity: pharmacophore, solubility
- Many circumstances where piperazine is inadequate
  - conformation, exit vectors
- Many piperazine bioisosteres have been explored to solve a range of problems
  - improve potency
  - to enhance or modulate selectivity
  - to alter physical properties
- The successful exploitation of bioisosterism is typically dependent upon context
  - tailor/optimize a bioisostere to specific application

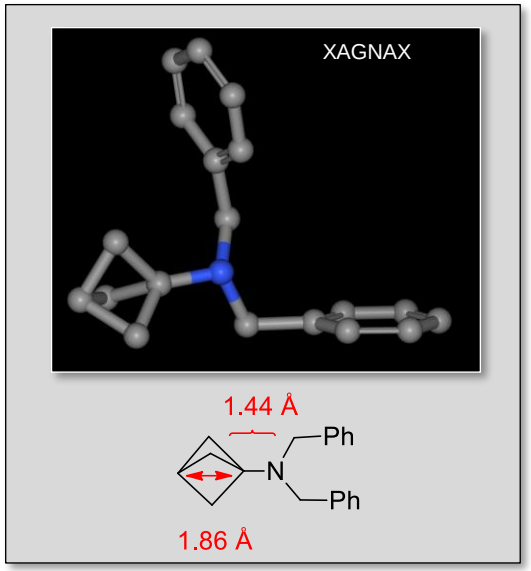
Additional Slides

Diamino Cyclobutane, BCP & Cubane

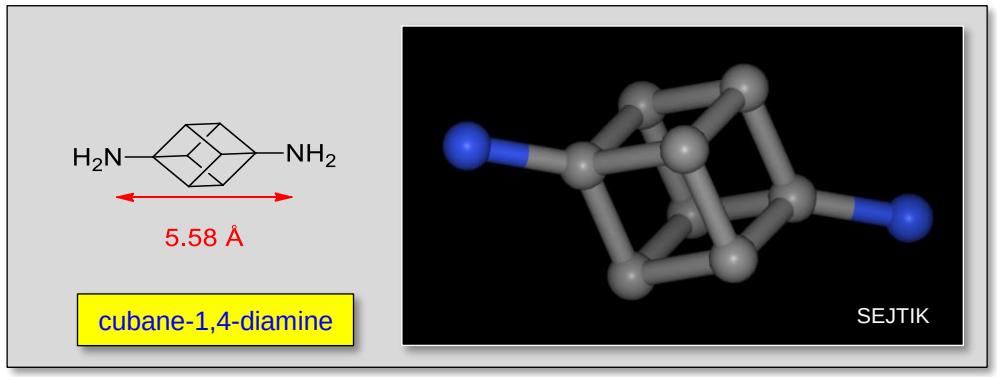






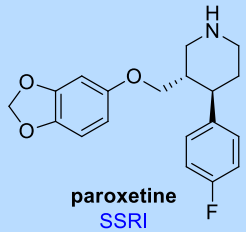


- 1,3-Diamino cyclobutane offers potential as a piperazine mimic
  - *N-N* bond distance is ~1.6 Å longer
  - closer to 4-amino-piperidine
- Diamino bicyclo[1.1.0]pentane confers linearity
  - *N-N* bond distance is ~1.8 Å longer
- Diamino cubane is 2.66 Å longer
  - well-stretched compared to piperazine, 4-NH_2-piperidine

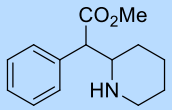


D.S. Radchenko *et al., J. Org. Chem.*, 2010, **75**, 5941-5952; P.S. Baran *et al.*, *J. Am. Chem. Soc.*, 2017, **139**, 3209-3226; *Science*, 2016, **351**, 241-246
H.L. Ammon *et al.*, *Acta Crystallogr., Sect. C: Crystal Struct. Commun.*, 1990, **46**, 295-298

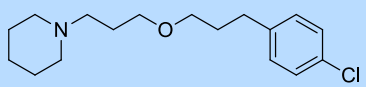
Drugs Incorporating Piperidine & 4-Aminopiperidine



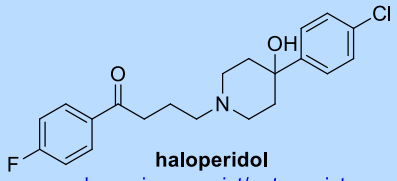
paroxetine
SSRI
depression



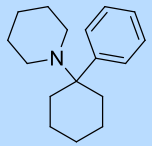
methylphenidate (ritalin)
CNS stimulant
ADHD



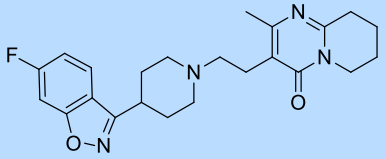
pitolisant
histamine H_3 antagonist/inverse agonist
narcolepsy



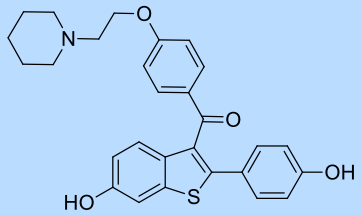
haloperidol
dopamine agonist/antagonist
antipsychotic



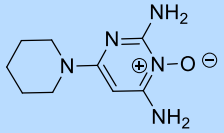
phencyclidine
NMDA antagonist
dissociative anesthetic



risperidone
dopamine, adrenergic, histamine receptors
atypical antipsychotic- schizophrenia

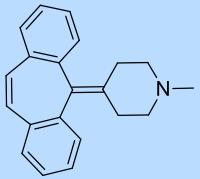


raloxifene
SERM
osteoporosis, breast cancer

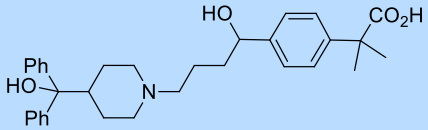


minoxidil
vasodilator
hair loss

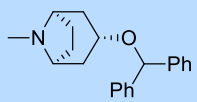
Piperidines



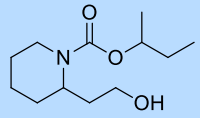
cyproheptadine
anti-histamine
allergies



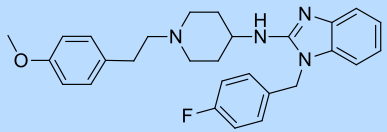
fexofenadine
anti-histamine
allergies



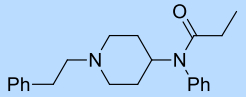
benzatropine
anticholinergic/antihistamine
movement disorders, Parkinson's



icaridin
odorant binding protein 1 ligand
topical insect repellant

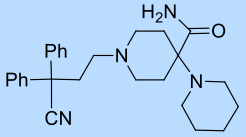


astemizole
2nd generation anti-histamine
withdrawn due to arrhythmia - hERG

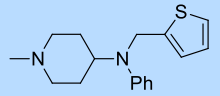


fentanyl
opioid analgesic
Schedule 1 narcotic
approved in US in 1968

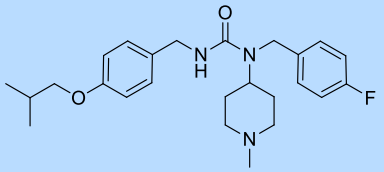
mizolastine
H_1 histamine antagonist



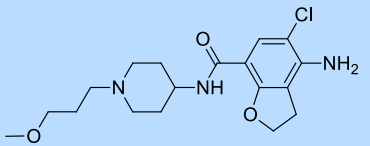
b
opioid analgesic
Schedule 1 narcotic



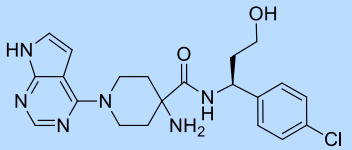
thenalidine
anti-histamine, cholinergic
withdrawn due to neutropenia



pimavanserin
atypical antipsychotic



prucalopride
$5HT_4$ agonist
promotes gut motility - constipation



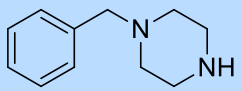
capivasertib
pan-AKT inhibitor
cancer

4-Aminopiperidines

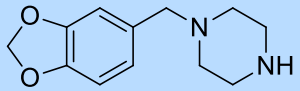
- Piperidine is a component of or embedded in a wide range of marketed drugs
  - pharmacophore or scaffolding element
  - 4-amino piperidine has application as a piperazine mimetic

A.N. Vereshchagin *et al., Int. J. Mol. Sci.*, 2023, **24**, 2937; K.M. Youssef *et al.*, *Future J. Pharm. Sci.,* 2021, **7**, 188; P. O'Brien *et al., RSC Med. Chem.,* 2022, **13**, 1614-1620

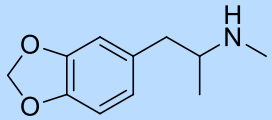
Simply-Substituted Piperazines: CNS Active Drugs



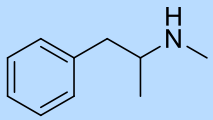
benzyl piperazine (BZP)
Burroughs-Wellcome 1944
amphetamine-like but 10x weaker *in vivo*
legal - but baned in many countries



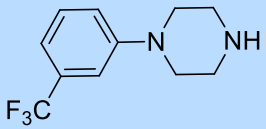
MDBZP
designer drug



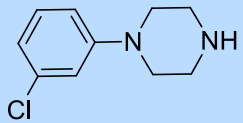
MDMA (ecstasy, molly or mandy)
Merck 1912
used to enhance psychotherapy in 1970s
drug of abuse 1980- to date
amphetamine & monoamine relasing agent
illegal - but shows efficacy in PTSD



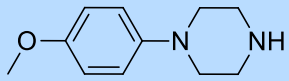
ampehetamine
1887: stimulant effects 1927
CNS stimulant
ADHD, narcolepsy
Schedule II, heavily regulated



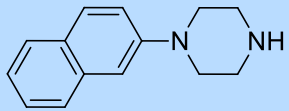
TFMPP
serotinin receptor agonist
SERT inhibitor
used in combination with BZP
not a scheduled substance
but baned in some jurisdictions



mCPP
1970s
psychostimulant,
anxiety-provoking,
hallucinogenic
5HT receptors, SERT
not scheduled



MeOPP
relaxant, not a stimulant
5HT receptors
monoamine reuptake inhibitor
not scheduled



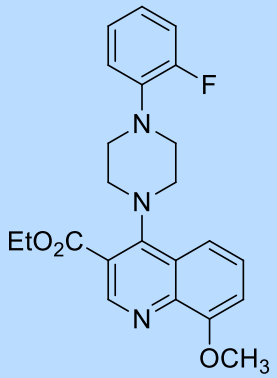
quipazine
5HT, SERT
psychedelic

- Piperazines are prominent pharmacophores CNS-active drugs
  - active at mono-amine neurotransmitter sites, transporters
- Drugs of abuse
  - many are regulated

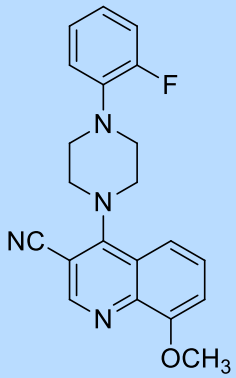
R.R. Kumar *et al., ChemMedChem*, 2021, **16**, 1878-1901; M. Novella Romanelli *et al.*, *Exp. Opin. Drug Discov.*, 2022, **17**, 969-984; P.A. Magriotis, *RSC Med. Chem.*, 2020, **11**, 745-759

Piperazine Replacements in $mGlu_7$ PAMs

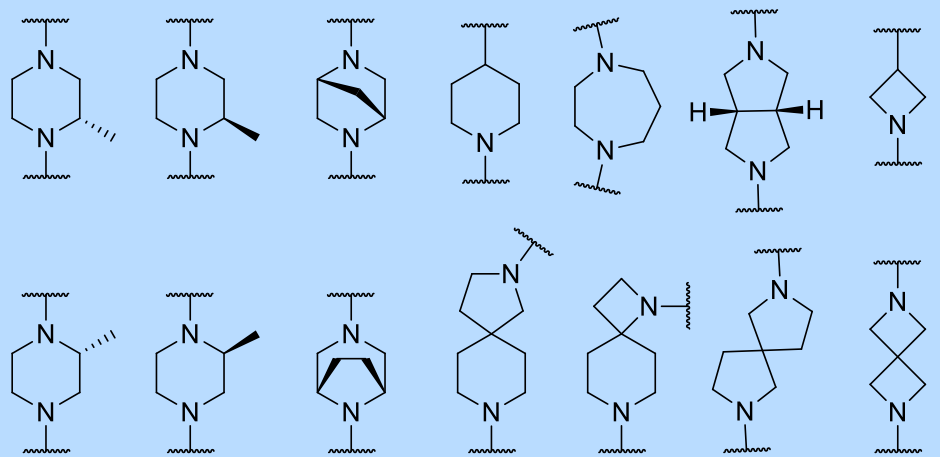
Examples where piperazine is optimal



$mGlu_7$ NAM
IC_{50} = 6.1 μM
12% L-AP_4 min



$mGlu_7$ PAM
IC_{50} = 0.88 μM
51% L-AP_4 min



variation of the piperazine moiety: all inactive at $mGlu_7$

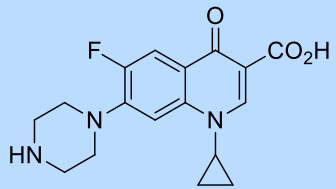
- Ester is an $mGlu_7$ NAM
  - nitrile exhibits inverted pharmacology: functions as a PAM
- Useful tool compound but …
  - also a dopamine transporter (DAT) inhibitor
- Wide range of piperazine bioisosteres explored to resolve DAT
  - conservative replacements, more radical scaffolds
  - **all were inactive**

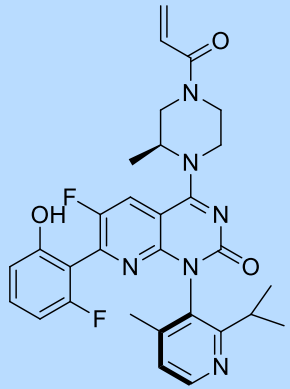
NAM: negative allosteric modulator
PAM: positive allosteric modulator

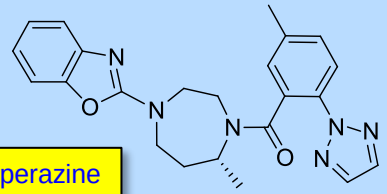
Z. Orgovan *et al., ChemMedChem,* 2021, **16**, 81-93; F. Yang *et al., ACS Med. Chem. Lett.,* 2016, **7**, 289-293; C.W. Reed *et al.*, *ACS Med. Chem. Lett.,* 2020, **11**, 1773-1779

Applications of Piperazines: Key Chemotypes

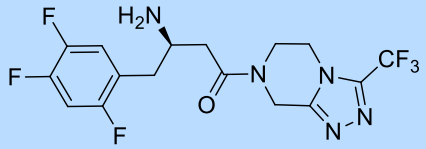
- Prominent piperazine-containing drugs
  - scaffolding elements & pharmacophores

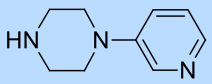

ciprofloxacin

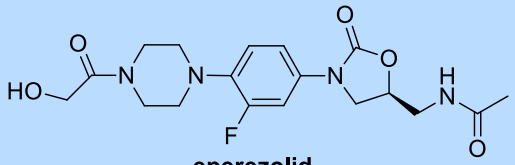

sotorasib


suvorexant

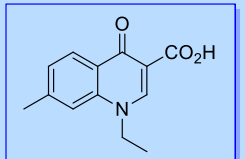
homopiperazine


sitagliptin

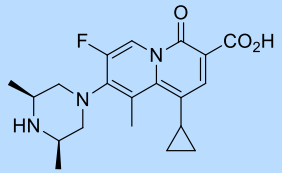

nicotinics

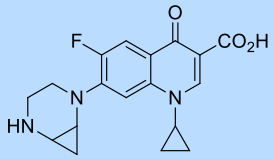

eperezolid

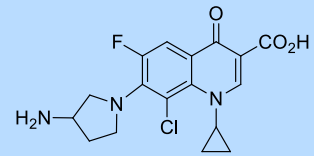
- Quinolones have inspired piperazine mimic design
  - nalidixic acid the prototype
- Wide range of cyclic & bicyclic amines explored
  - several in marketed drugs

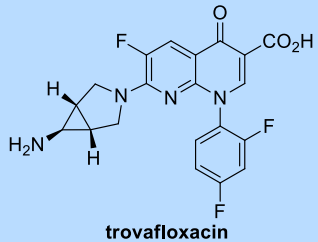

nalidixic acid

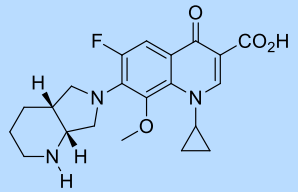

R = C_2H_5: **enoxacin**
R = cyclopropyl: **ciprofloxacin**

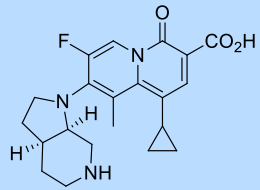


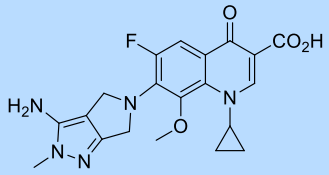



clinafloxacin

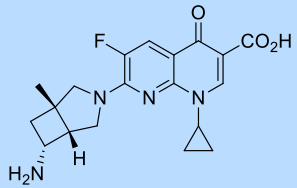

trovafloxacin

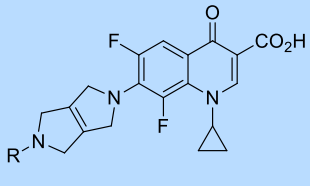


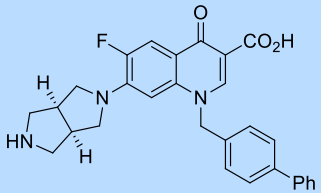

moxifloxacin

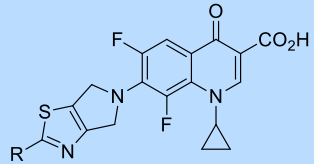


Quinolone Antibiotics


ecenofloxacin


R= H: **KR 10777**
R = CH_3: **KR 10755, HK3140**

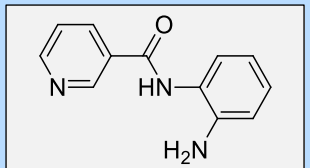



R = CH_3, $NHCH_3$

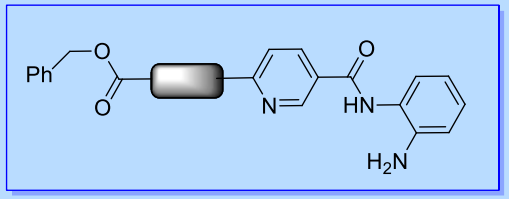
N.A. Meanwell & O. Loiseluer, *J. Agric. Food Chem.,* 2022, **70**, 10942-10971*; J. Agric. Food Chem.,* 2022, **70**, 10972-11004

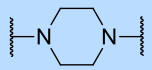
2,5-Diazabicyclo[4.2.0]octanes

Piperazines with fused carbocyclic rings

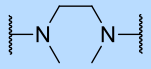


HDAC1 IC_{50} = 12.4 µM

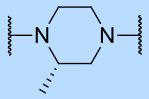




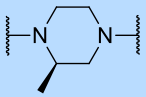
HDAC1 IC_{50} = 168 nM



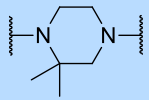
HDAC1 IC_{50} = 963 nM



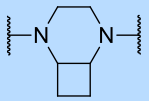
HDAC1 IC_{50} = 73 nM
HCT116-72h GI_{50} = 81 nM



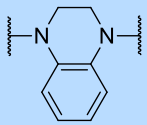
HDAC1 IC_{50} = 40 nM
HCT116-72h GI_{50} = 495 nM



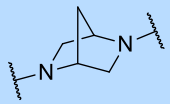
HDAC1 IC_{50} = 169 nM
HCT116-72h GI_{50} = 871 nM



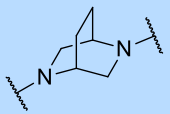
HDAC1 IC_{50} = 140 nM
HCT116-72h GI_{50} = 3,360 nM



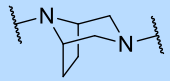
HDAC1 IC_{50} = 92 nM
HCT116-72h GI_{50} = 609 nM



HDAC1 IC_{50} = 68 nM
HCT116-72h GI_{50} = 284 nM

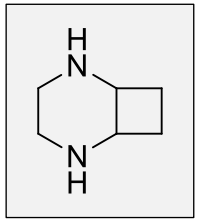


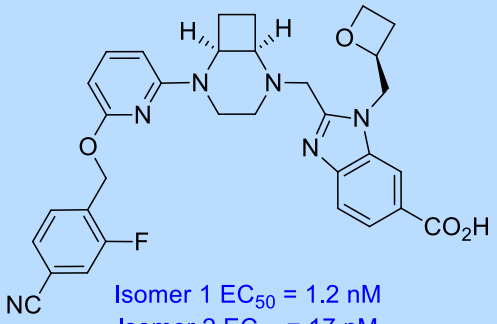
HDAC1 IC_{50} = 67 nM
HCT116-72h GI_{50} = 1,510 nM



HDAC1 IC_{50} = 82 nM
HCT116-72h GI_{50} = 1,034 nM

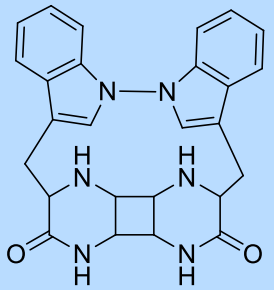
- Limited documentation in drug design
  - properties not fully understood
- Explored as an example in HDAC1 inhibitors
  - potency comparable to piperazine prototype
- Core element of GLP-1 agonists
  - patent applications
- Occurs naturally in an oxidized form
  - photochemical 2+2

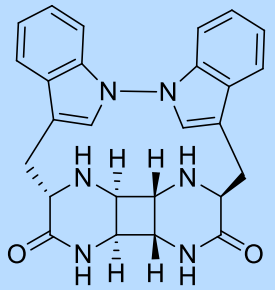




Isomer 1 EC_{50} = 1.2 nM
Isomer 2 EC_{50} = 17 nM

GLP-1 agonist



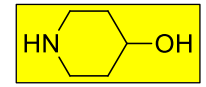


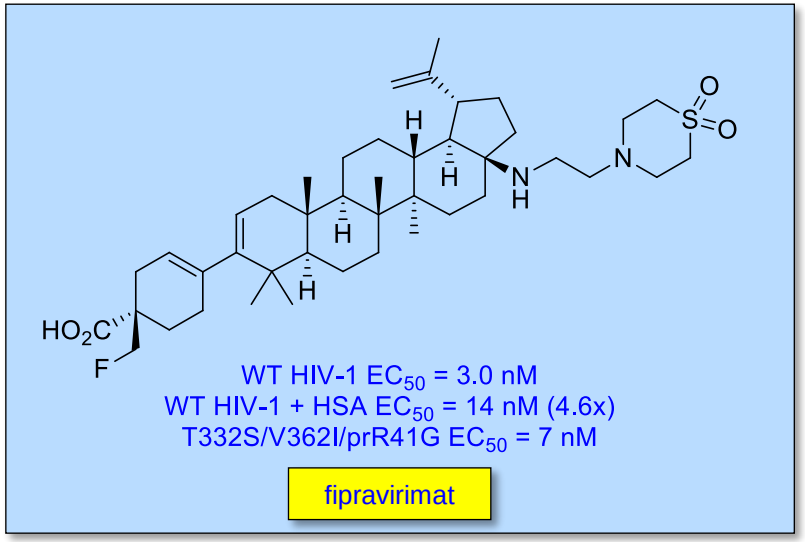
schischkiniin

Natural product in seeds of *Centaurea schischkinii*
Absolute configurations speculative
Antioxidant but weakly cytotoxic: 76 µM

C.L. Hamblett *et al., Bioorg. Med. Chem. Lett*., 2007, **17**, 5300-5309; M. Polla *et al., World Patent Application,* WO 2023/057429
M. Shoeb *et al., Tetrahedron*, 2005, **61**, 9001-9006

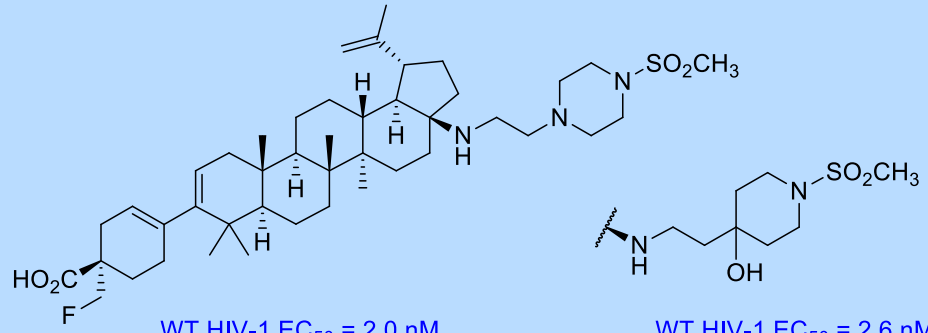
C-OH for N in Piperazines: HIV-1 MAT Inhibitors





WT HIV-1 EC_{50} = 3.0 nM
WT HIV-1 + HSA EC_{50} = 14 nM (4.6x)
T332S/V362I/prR41G EC_{50} = 7 nM

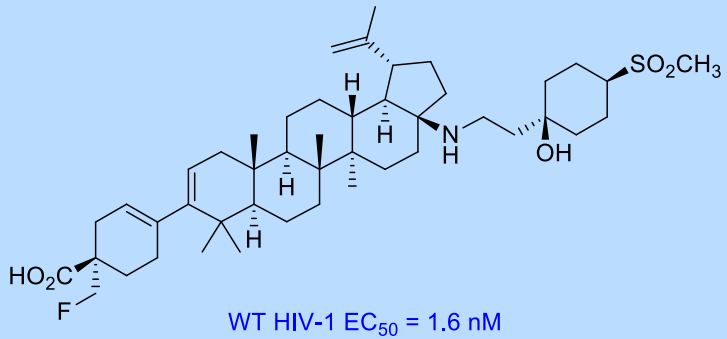
fipravirimat



WT HIV-1 EC_{50} = 2.0 nM
WT HIV-1 + HSA EC_{50} = 18 nM (9x)
T332S/V362I/prR41G EC_{50} = 5.7 nM

WT HIV-1 EC_{50} = 2.6 nM
WT HIV-1 + HSA EC_{50} = 19.8 nM (7.3x)
T332S/V362I/prR41G EC_{50} = 8.3 nM

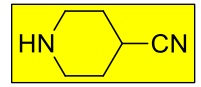
- HIV-1 maturation inhibitors
  - interfere with protease mediated cleavage of capsid protein
  - mildly basic amine side chain preferred
- Piperazine could be replaced by hydroxy piperidine
  - preserved antiviral activity
  - piperazine/piperidine makes intimate contact with target
- Structural element retained in optimized candidate molecule
  - hydroxy cyclohexane replaced piperidine

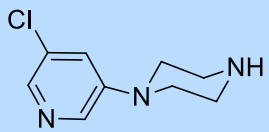


WT HIV-1 EC_{50} = 1.6 nM
WT HIV-1 + HSA EC_{50} = 9.3 nM (5.8x)
T332S/V362I/prR41G EC_{50} = 6.4 nM
V370A EC_{50} = 1.6 nM
V362I/V370A EC_{50} = 2.0 nM

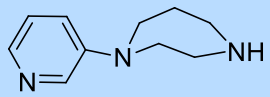
rat F = 24%
dog F = 31%

C-CN for N in Piperazines

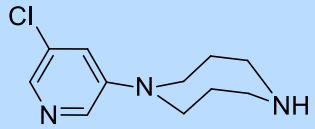




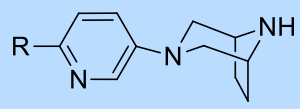
nAChR IC_{50} = 310 nM



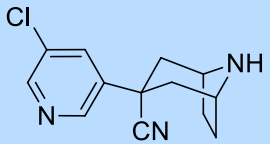
IC_{50} = 1.9 nM



IC_{50} = 2 nM

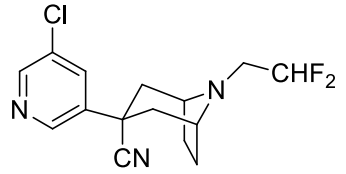


R = Cl
nAChR K_i = 2.7 nM
R = H
nAChR K_i = 0.02 nM

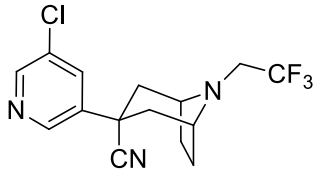


nAChR IC_{50} = 1 nM

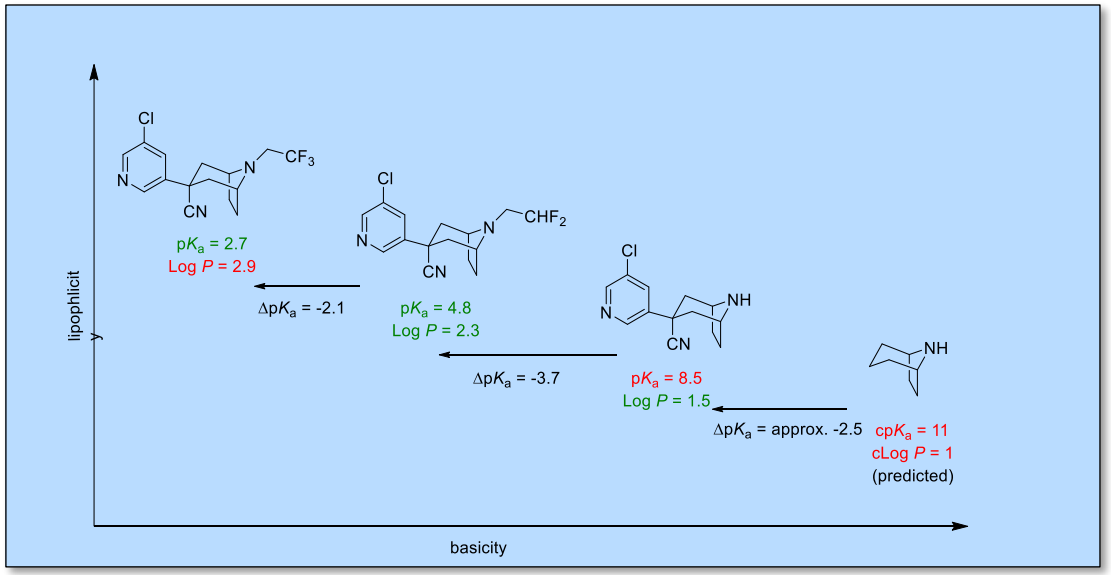
Cl isomer in WO-2000 00447555

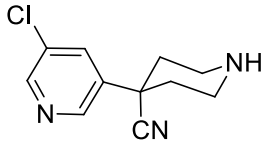


procide

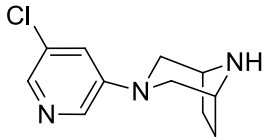


procide
nAChR IC_{50} = 58,900 nM



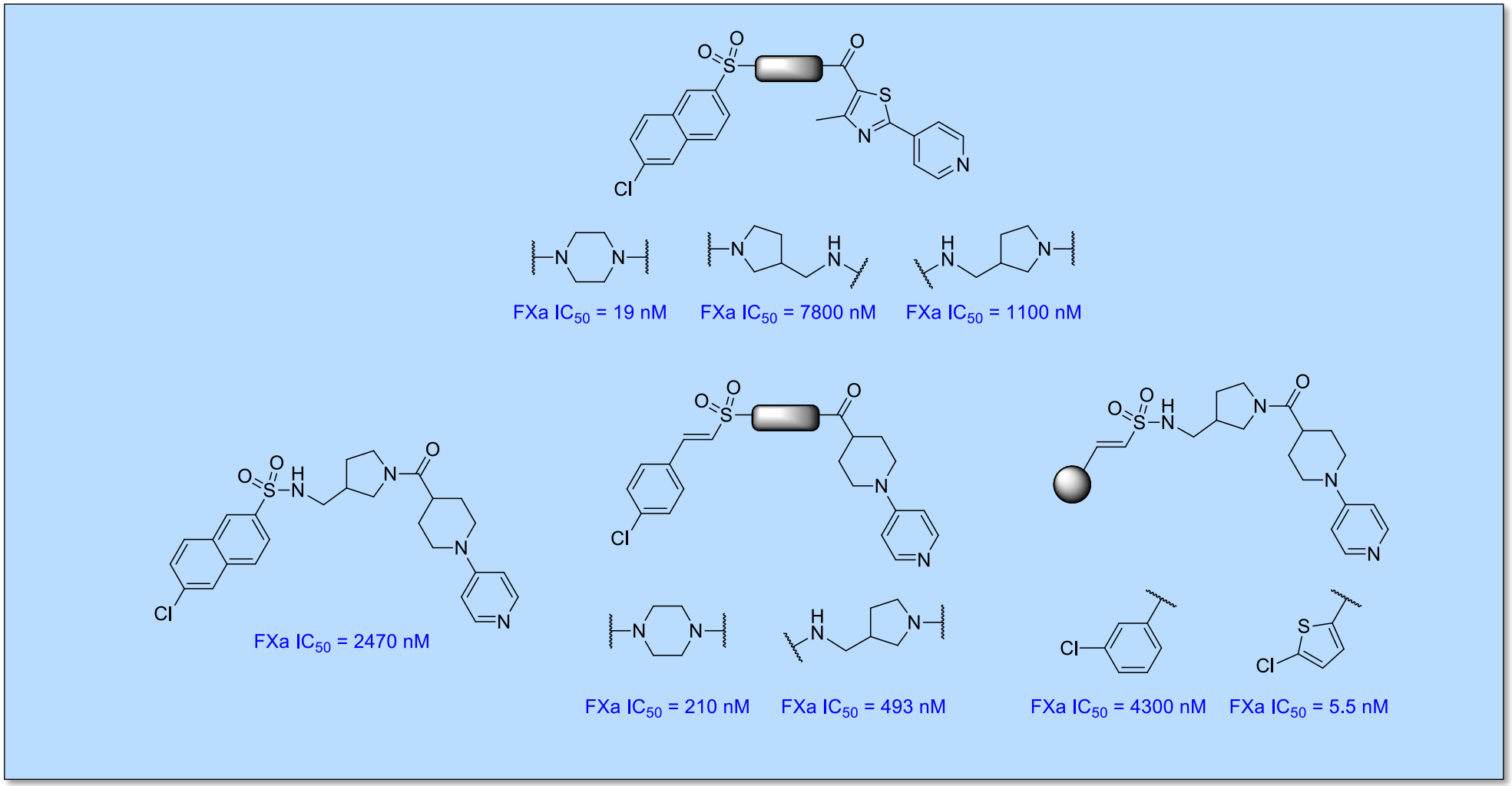


in pesticide patent WO2001017965

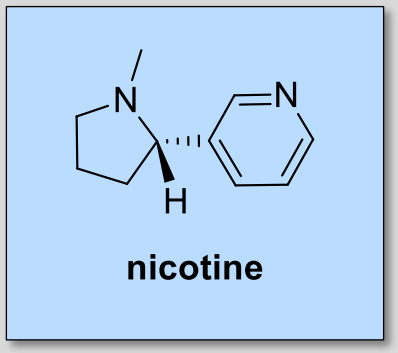


not known except as substructure in 1 patent app WO2000044755

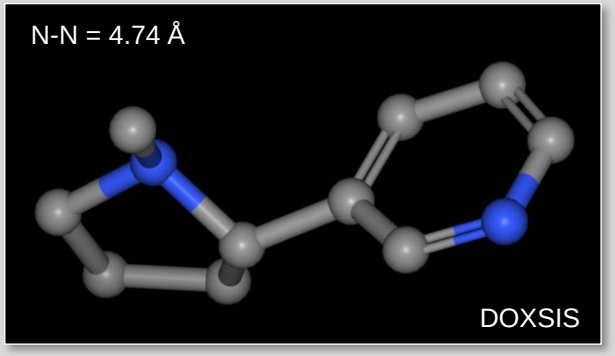
Substituted Pyrrolidines

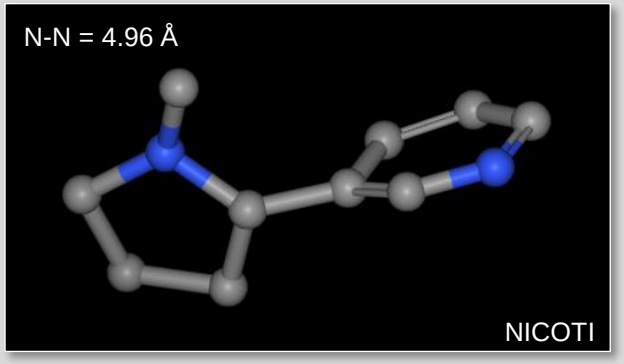


Nicotinic Receptor Ligands



nicotine

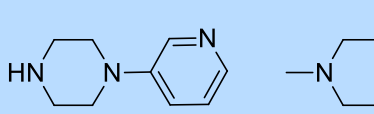




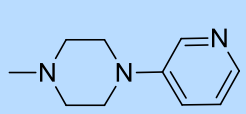
- Pharmacophore is a basic amine with pendent H-bond acceptor
- A wide range of motifs have been explored
  - many based on diamines

R.B. Barlow et al., *Acta Crystallogr.,Sect. C: Crystal Struct. Commun.*, 1986, **42**, 853-856; C.H. Koo, H.S. Kim, *J. Korean Chem. Soc.*, 1965, **9**, 134-141

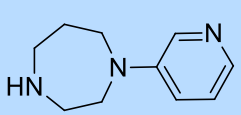
Nicotinic Receptor Ligands



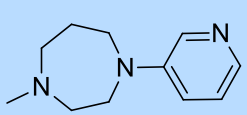
α4ß2 K_i = 90 nM
α7 K_i = N/A



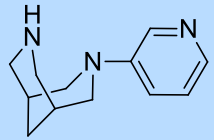
α4ß2 K_i = 90 nM
α7 K_i = N/A



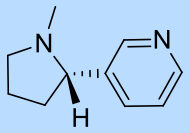
α4ß2 K_i = 1.9 nM
α7 K_i = 150 nM



α4ß2 K_i = 150 nM
α7 K_i = 4,600 nM



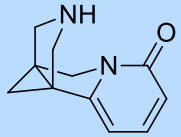
α4ß2 K_i = 0.02 nM
α7 K_i = N/A



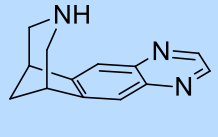
nicotine
α4ß2 K_i = 0.95 nM
α3ß4 K_i = 530 nM
α7 K_i = 6,290 nM

cytisine
α4ß2 K_i = 0.43 nM
α3ß4 K_i = 1,560 nM
α7 K_i = 5,820 nM

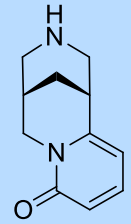
(±)-cyfusine
α4ß2 K_i = 16 nM
α3ß4 K_i >500 nM
α7 K_i >500 nM



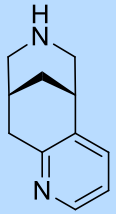
cyclopropylcyfusine
α4ß2 K_i = 144 nM
α3ß4 K_i >500 nM
α7 K_i >500 nM

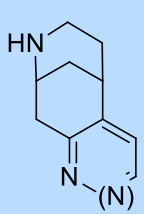


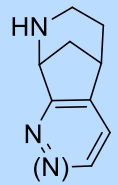
varenicline
α4ß2 K_i = 0.06 nM
α3ß4 K_i = 240 nM
α7 K_i = 322 nM

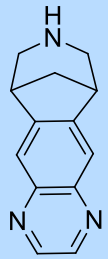


(-)-cytisine

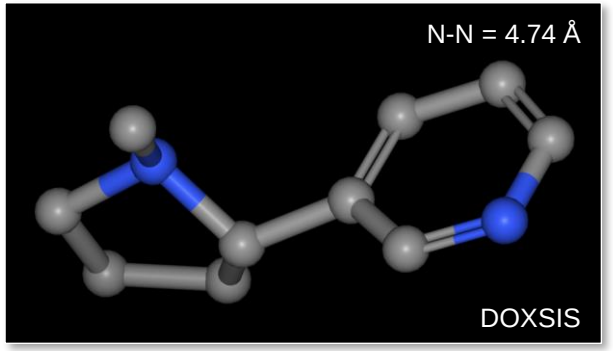


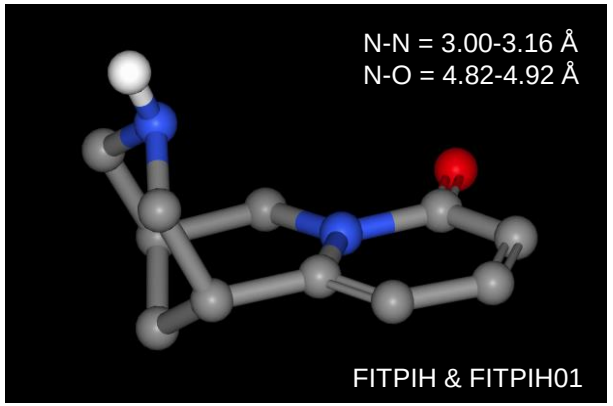


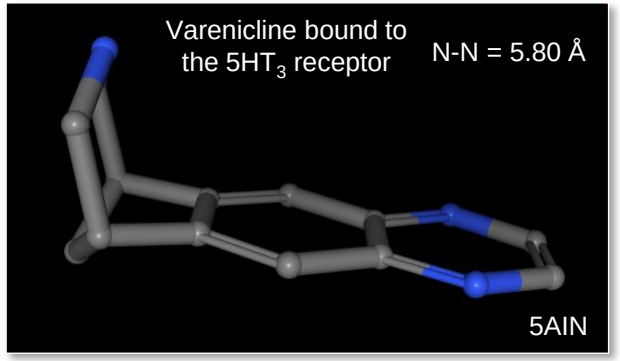




varenicline

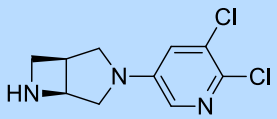




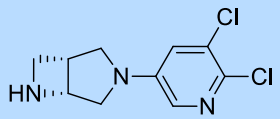


J.W. Coe *et al., J. Med. Chem.,* 2005, **48**, 3474-3477; I. Tomassoli *et al., Bioorg. Med. Chem.,* 2015, **23**, 4375-4389; K.L. Price *et al., ACS Chem. Neurosci*., 2015, **6**, 1151-1157
A.A. Freer *et al., Acta Crystallogr., Sect. C: Crystal Struct. Commun.*, 1987, **43**, 1119-1122; A.K. Przybyl *et al., J. Molec. Struct.*, 2011, **985**, 157-166

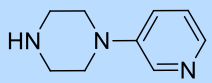
3,6-Diazabicyclo[3.2.0]heptanes: Nicotinic Agonists



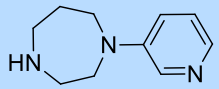
sofinicline (ABT-894)
K_i = 0.3 nM
EC_{50} = 0.4 μM
E_{max} = 110%



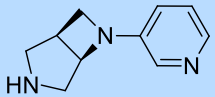
K_i = 0.04 nM
EC_{50} = 0.4 μM
E_{max} = 162%



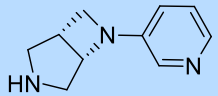
K_i = 90 nM



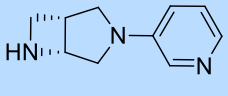
K_i = 1.9 nM



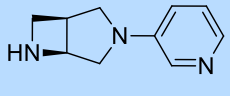
K_i = 0.7 nM
EC_{50} = 2.8 μM
E_{max} = 101%



K_i = 1.3 nM
EC_{50} = 1.9 μM
E_{max} = 116%



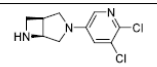
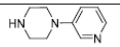
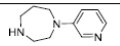
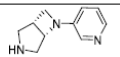
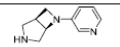
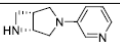
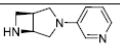
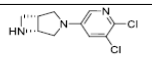
K_i = 0.1 nM
EC_{50} = 0.3 μM
E_{max} = 181%



K_i = 1.2 nM
hEC_{50} = 1.6 μM
E_{max} = 122%

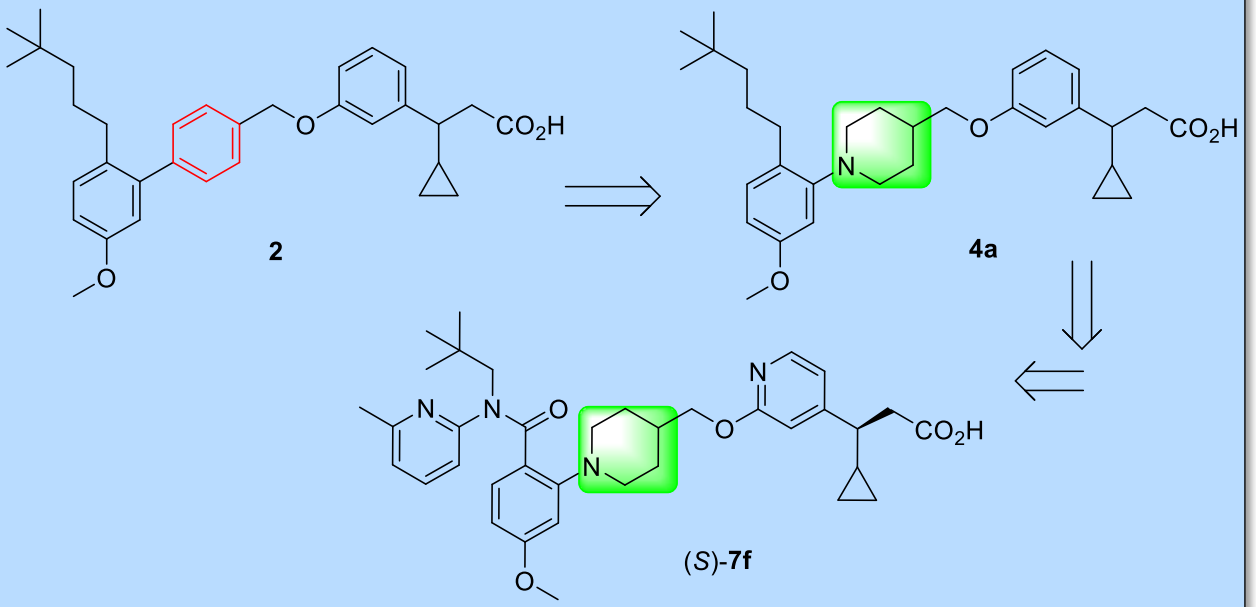
K_i = [^{3}H]-cytosine binding
EC_{50} = hα4β2 Ca^{2+} flux with E_{max} relative to (-)-nictotine

Table 1. Binding Affinities and Efficacy at Nicotinic hα4β2 Receptors for Diamines 26–33

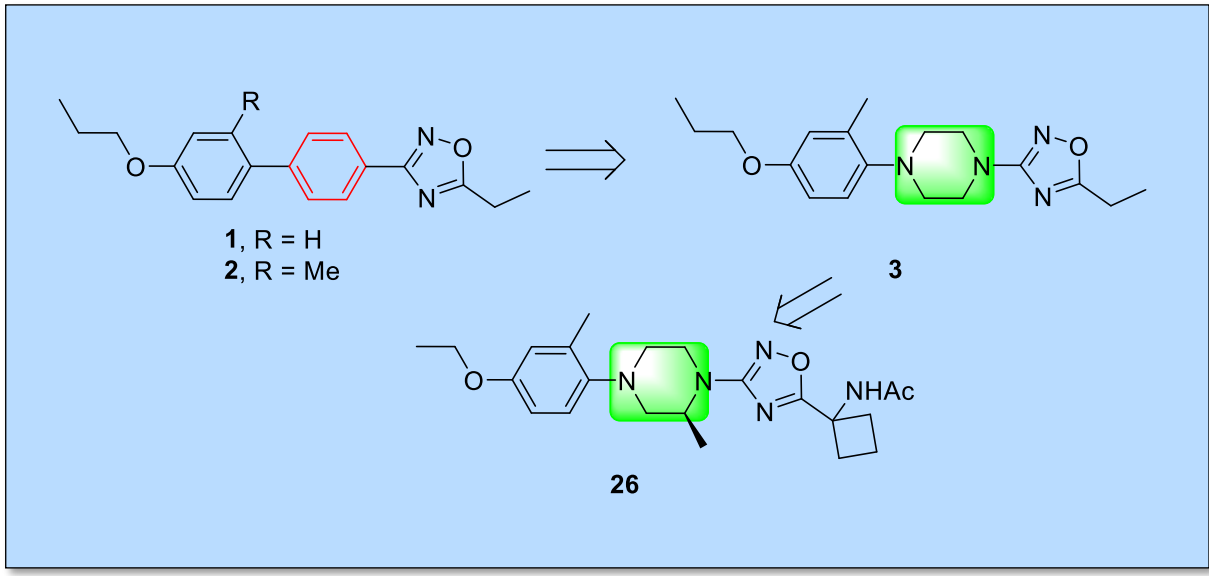
		[^{3}H]-cytisine binding to rat brain homogenates K_i (nM)	hα4β2 Ca^{2+} flux (FLIPR) EC_{50} (μM) (E_{max})
26	 (sofinicline, ABT-894)	0.3	0.4 (110%)
27		90[a]	
28		1.9	
29		1.3	1.9 (116%)
30		0.7	2.8 (101%)
31		0.1	0.3 (181%)
32		1.2	1.6 (122%)
33		0.04	0.4 (162%)

J. Ji *et al., J. Med. Chem.,* 2007, **50**, 5493-5508; M.N. Romanelli *et al., J. Med. Chem.*, 2001, **44**, 3946-3955; I. Tomassoli *et al., Bioorg. Med. Chem.*, 2015, **23**, 4375-4389

GPR40 & hACC1



	Lead	Bioisosteric analogue		Impact
	2	**4a**	**(S)-7f**	reduced aromatic content; lower lipophilicity
hGPR40 EC_{50} (nM)	410	11	12	
E_{max} (%)	105	106	108	
cLog *P*	9.35	8.4	5.8	



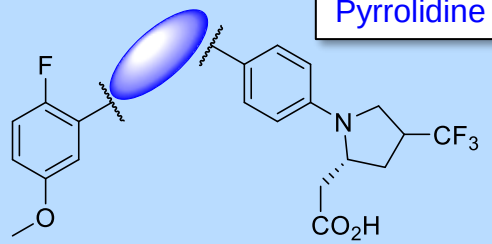
	Lead		Bioisosteric analogue		Impact
	1	**2**	**3**	**26**	improved cell potency; reduced clearance; abrogation of potential safety issues
hACC1 IC_{50} (nM)	4280	1460	4010	193	
hACC2 IC_{50} (nM)	841	126	391	54	
ACC2 cell IC_{50} (nM)	359	103	128	8.2	

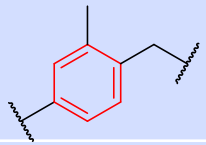
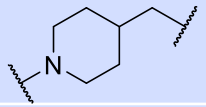
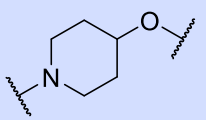
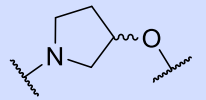
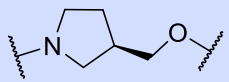
- Lead identified by affinity screening
  - *N*-alkylation
- Analyzed
  -

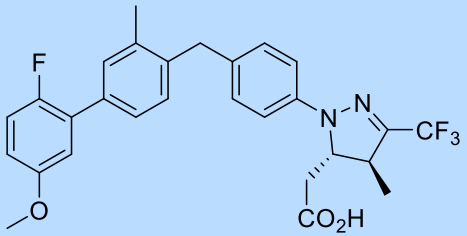
H. Furukawa *et al., J. Med. Chem.,* 2020, **63**, 10352-10379; M.P. Bourbeau *et al., J. Med. Chem.,* 2013, **56**, 10132-10141

Piperidine as Phenyl Mimic in GPR40 Agonists

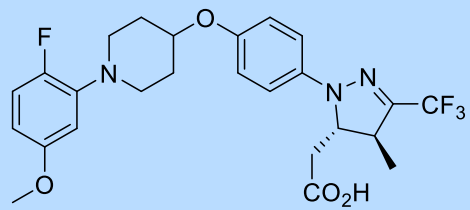
Pyrrolidine series



	FLIPR hGPR40 EC_{50} (nM)	cLog *P*
	210	7
	530	5.73
	210	4.55
	2,830	4.48
	5,360	
	940	4.60
	630	



hGPR40 EC_{50} = 33 nM
mGPR40 EC_{50} = 31 nM
PPARγ EC_{50} = 2.6 μM
cLog *P* = 7.7

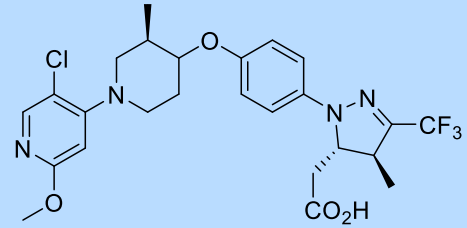


hGPR40 EC_{50} = 110 nM
mGPR40 EC_{50} = 70 nM
PPARγ EC_{50} = 2.6 μM
cLog *P* = 5.39

Impact:
lower Log *P*;
reduced promiscuity

- GPR40 agonists
  - promote glucose-stimulated insulin release
- Saw cardiac (HR, BP) side effects & PPARγ agonism
  - high cLog *P* hypothesized as source of promiscuity
- Sought to lower cLog *P* and increase sp^3 content
  - replaced central phenyl ring with aza heterocycles
- Piperidine offered best *in vitro* performance
  - activity 2.5-3.0 x lower, cLog *P* fell by 1.3-2.5 units
  - saw GSH adducts with anisole moiety
- Optimized by O, CH_3 on piperidine in pyrazole series
  - pyridine ring introduced to reduce GSH adducts

Dihydropyrazole series



hGPR40 EC_{50} = 70 nM
mGPR40 EC_{50} = 63 nM
PPARγ EC_{50} >47 μM
cLog *P* = 5.9
measured Log $D_{6.5}$ = 4.6

J. Shi *et al.*, *J. Med. Chem.*, 2018, **61**, 681-694