A Perspective on Applications of Fluorine in Drug Design Through the Lens of Bioisosterism

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Fluorine in Medicinal Chemistry - Outline

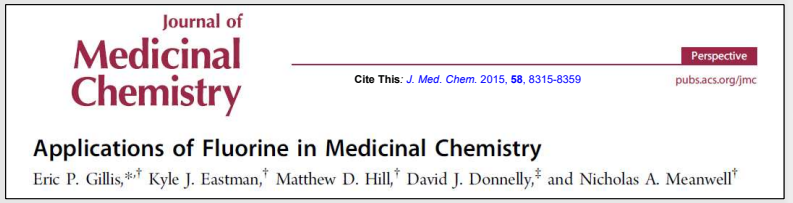
- The presence of fluorine in marketed drugs & agricultural chemicals
  - fludrocortisone in 1955
  - pressure on the deployment of fluorine - PFAS
- Synopsis of key properties of F & the C-F bond
  - the versatility of fluorine in medicinal chemistry
- C-F as a bioisostere of C-H: the most prevalent application
  - the design of alkyl, aryl bioisosteres
  - drug-target interactions
  - conformation
  - metabolism & pharmacokinetics
  - modulating amine basicity
- Fluorine in the design of amide mimetics
  - CF_3-C-NH, vinyl fluorides

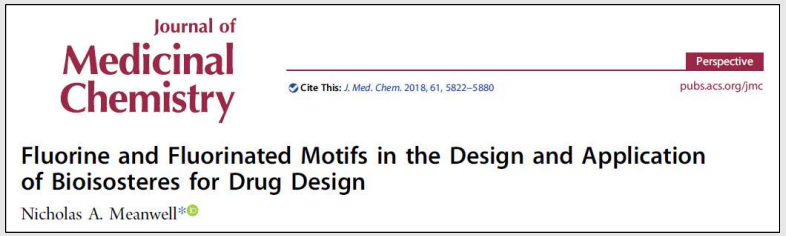
Part 1

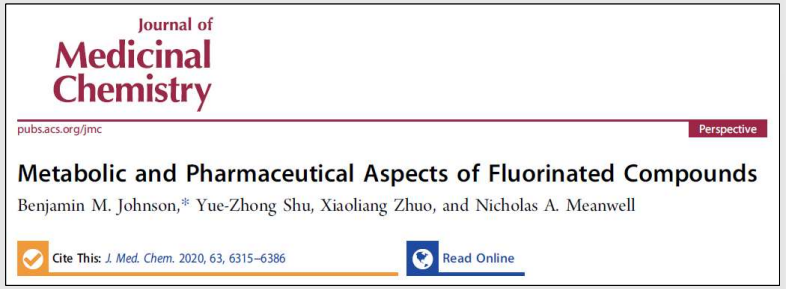
Today

- Fluorine & C=O, S=O bioisosterism
  - C-F, CF_2, $C=CF_2$
- Fluorine & the design of C-OH or C-SH mimetics
  - C-F, CHF_2
- Fluorine & amine mimesis
  - C-F for C≡N, amine, azine N
- Fluorine & O mimesis
  - CF_2 to mimic O, S, SO_2
- Emerging fluorinated motifs
  - $CO.NCHF_2$, SO_2NCHF_2, CF_2X, N-CF_3, SF_5
- Metabolic activation of fluorinated compounds
  - the dark side of fluorine
- Conclusion

Part 2

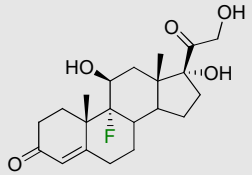




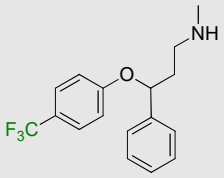


Prevalence of Fluorine in Marketed Drugs & Agricultural Products

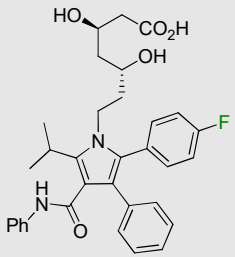
Presence of Fluorine in Approved Drugs



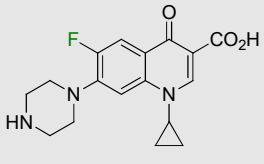
fludrocortisone
1955



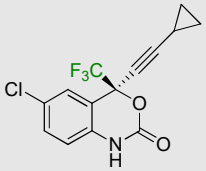
fluoxetine
1987



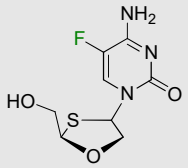
atorvastatin
1996



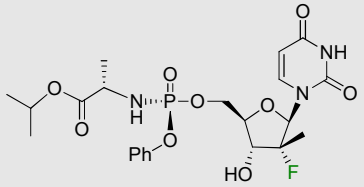
ciprofloxacin
1987



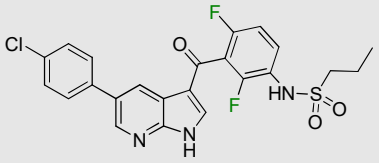
efavirenz
1998



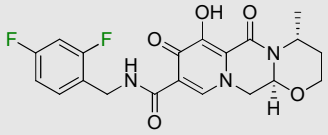
emtricitabine (FTC)
2003



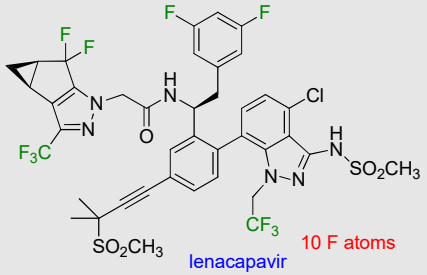
sofosbuvir
2013



vemurafenib
B-raf kinase inhibitor
2017

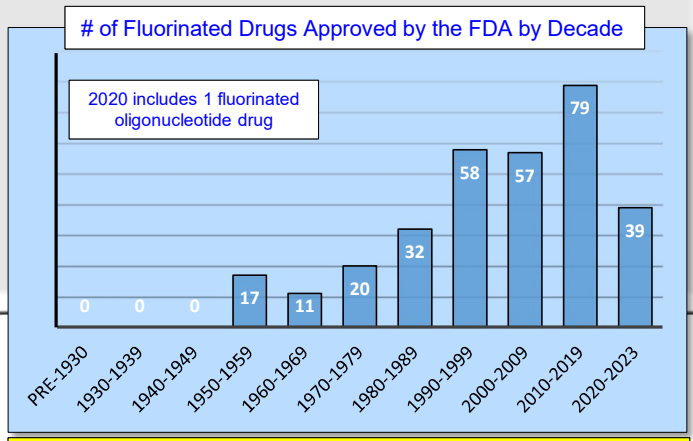


dolutegravir
2013



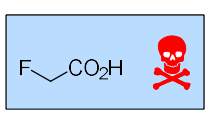
lenacapavir
2022

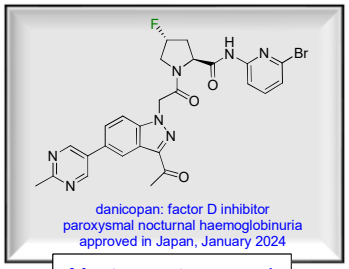
10 F atoms



45% of small molecule drugs approved in 2018 & 2019 contained F; 33% in 2020; 25% in 2021; 11% in 2022
12 fluorinated drugs approved by the FDA in 2023
5 (of 38 total, 23 small molecules) approved in 2024

- Fluorine is present in a wide range of important pharmaceuticals
  - fludrocortisone the first fluorinated drug: approved August 18th, 1955
    - 10x more potent than H prototype; Cl & Br weaker
  - 16 additional fluorinated drugs introduced in the 1950s
  - ~20% prevalence in the pharmacopeia
- Fluoroacetic acid occurs naturally
  - a potent mitochondrial toxin
  - taken up into the Krebs (citric acid) cycle
  - metabolized to a reversible but very tight binding inhibitor of aconitase



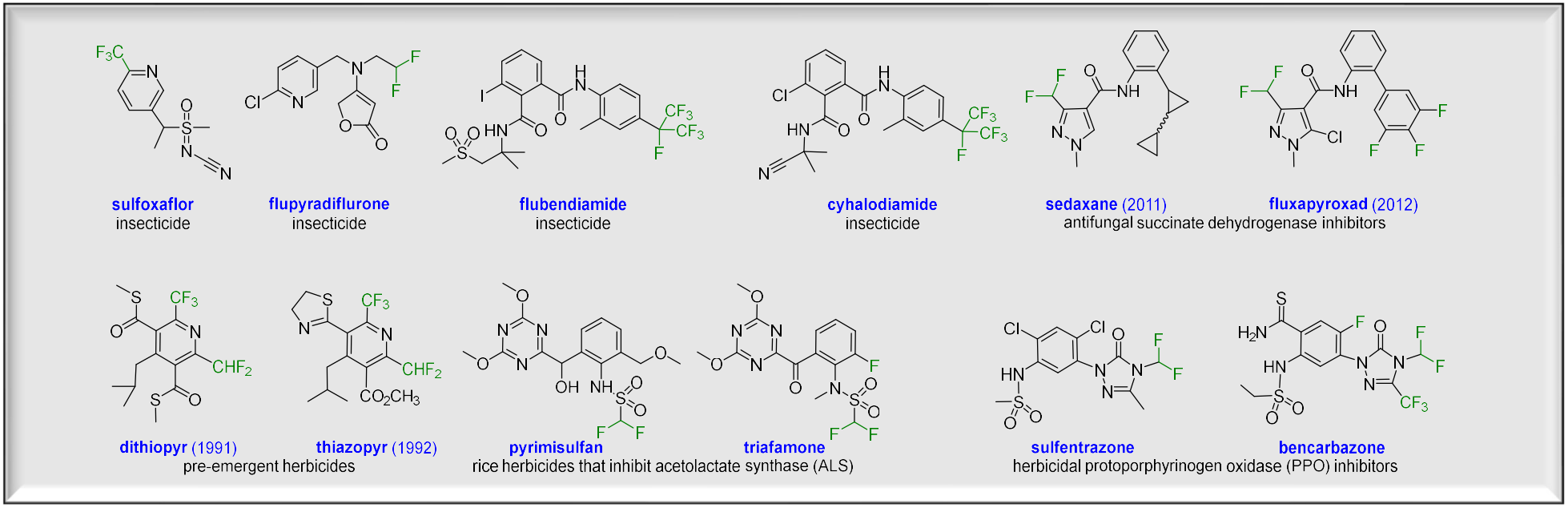


danicopan: factor D inhibitor
paroxysmal nocturnal haemoglobinuria
approved in Japan, January 2024

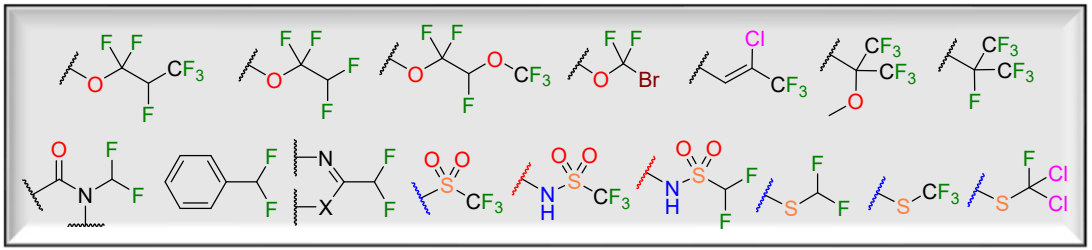
Most recent approval

H. Liu *et al., Chem. Rev.,* 2014, **114**, 2432-2506; J.T. Njardarson *et al., J. Med. Chem.*, 2014, **57**, 2832−2842; D. O'Hagan, *J. F. Chem.*, 2010, **131**, 1071-1081; P. Jeschke, *ChemBioChem,* 2004, **5**, 570-589; F. Viani *et al., ChemBioChem,* 2004, **5**, 590-613; N.A. Meanwell, *J. Med. Chem.,* 2018, **61**, 5822-5880; S. Ali & J. Zhou., *Eur. J. Med. Chem.*, 2023, **260**, 115476

Presence of Fluorine in Agricultural Products



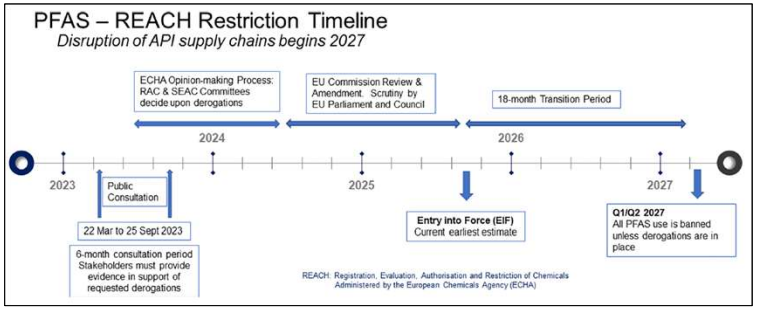
- Fluorinated motifs heavily exploited in agricultural products
  - 52% of pesticides 2010-2017 contained F
  - 25% of 229 herbicides launched before 2014 contained F
- 238 Agrochemicals with ISO names assigned 1998-2020
  - 127 (53%) contained F
- Many fluorinated motifs have originated in ag. chem.
  - adopted by medicinal chemistry a decade later



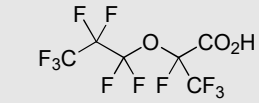
P. Jeschke, *ChemBioChem,* 2004, **5**, 570-589; P. Jeschke, *Pest Manag. Sci.* 2017, **73**, 1053-1066; T. Fujiwara & D. O'Hagan, *J. Fluorine Chem.*, 2014, **167**, 16-29
N. Shibata *et al., iScience*, 2020, **23**, 101467. Thanks to Olivier Loiseleur & Torsten Luksch (Syngenta AG) for details on fluorinated motifs used in agricultural chemistry

Fluorine & PFAS

- PFAS - polyfluoroalkyl substances
  - fluoropolymers, polyfluorinated surfactants
  - agricultural products, drug substances
- Many are not biodegradable
  - accumulate in the environment
- Fluoxetine is metabolized to CF_3CO_2H
  - CF_3CO_2H stable towards degradation
  - accumulates in the environment
- Initiative in Europe to restrict PFAS to essential products
  - does not distinguish between fluoropolymers
  - ongoing debate
- Will likely restrict some fluorinated motifs
  - may ensnare CF_3 substituents
- Will require disciplined exploitation of fluorine in drug & agricultural substances
  - harmonize fluorinated motifs with evolving environmental concerns & legislation
  - a source of creativity in drug design & synthesis?

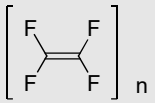


PFAS - polyfluoroalkyl substances

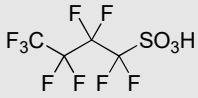


hexafluoropropylene oxide dimer carboxylate

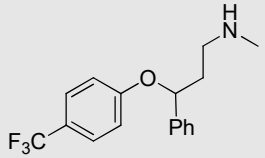
perfluorooctanoic acid



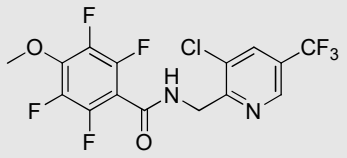
Teflon®



perfluorobutyl sulfonic acid



fluoxetine



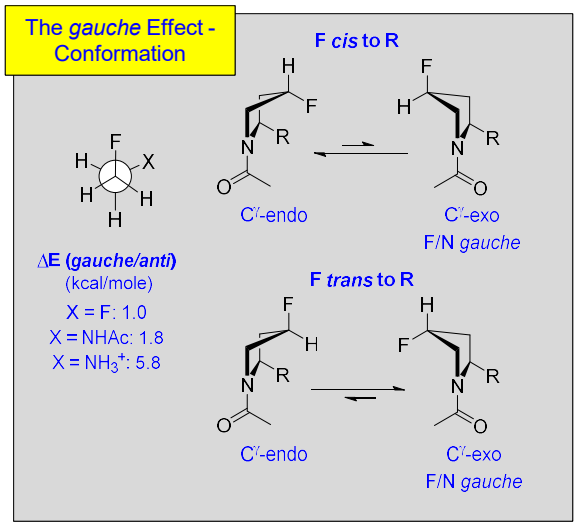
fluopimomide agricultural fungicide

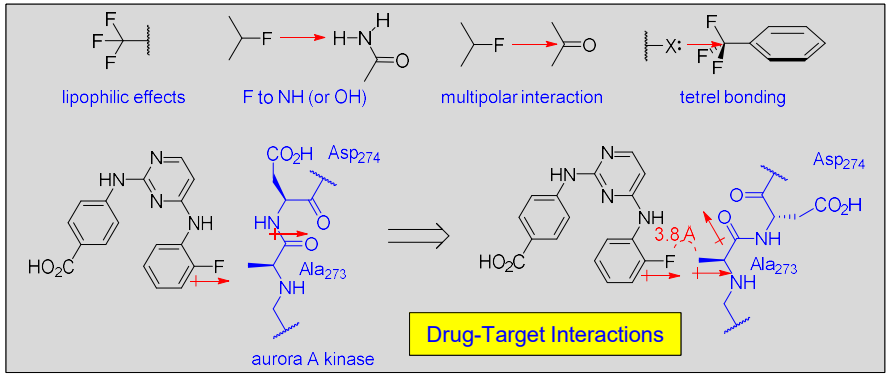
CF_3CO_2H

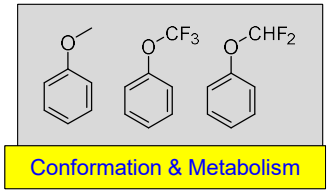
trifluoroacetic acid

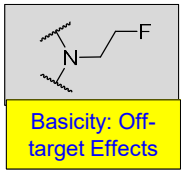
N.D. Tyrrell, *Org. Proc. Res. Devel.*, 2023, **27**, 1422-1426; D. O'Hagan & R.J. Young, *Med. Chem. Res.*, 2023, **32**, 1231-1234
M.F. Khan *et al., Appl. Microbiol. Biotechnol.*, 2021, **105**, 9359-9369 ; M. Sun *et al., Chemosphere*, 2020, **254**, 126894; L.P. Wackett, *Microb. Biotechnol.*, 2022, **15**, 773-792

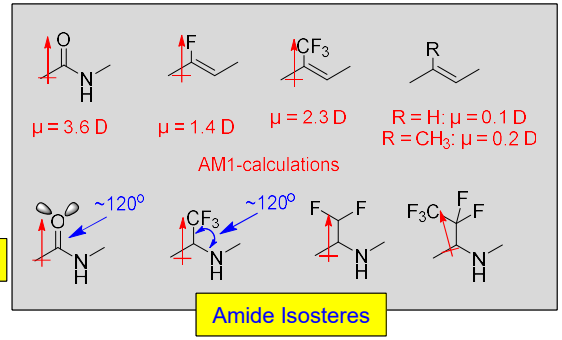
The Versatility of Fluorine in Drug Design

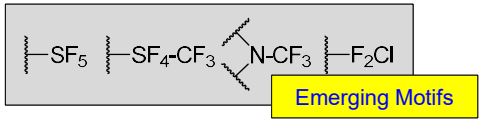


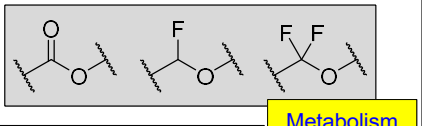


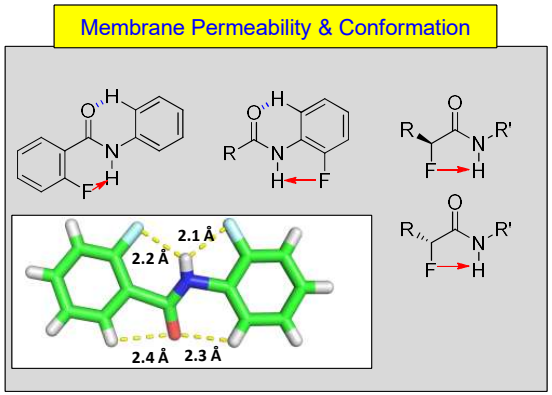


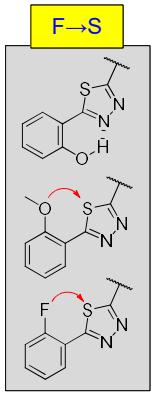


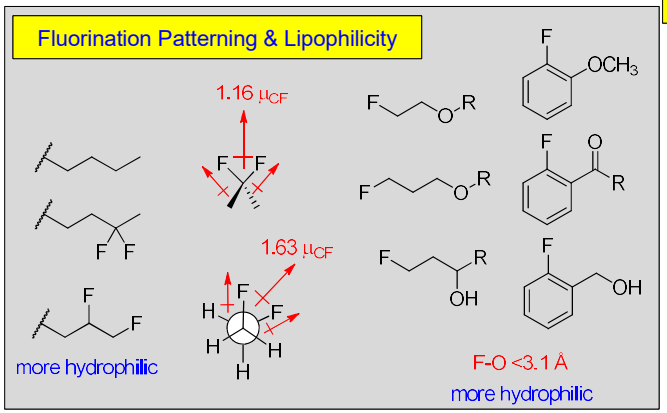


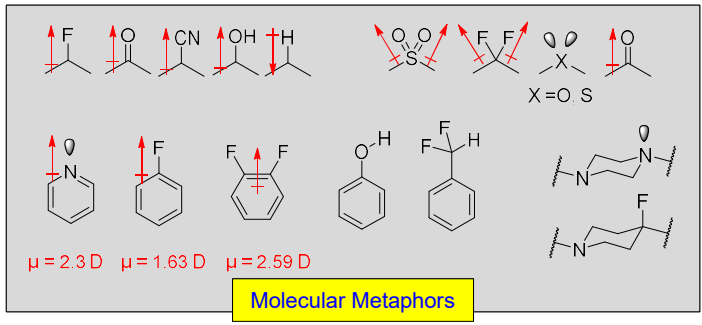












E.P. Gillis, N.A. Meanwell *et al., J. Med. Chem.,* 2015, **58**, 8315-8359; N.A. Meanwell, *J. Med. Chem.,* 2018, **61**, 5822−5880

Properties of Fluorine

Bond	Length (Å)	van der Waals Radius (Å)	Total Size (Å)	Electro-negativity	Dipole Moment μ (D)
C-H	1.09	1.20	2.29	2.20	~-0.4
C-F	1.35	1.47	2.82	3.98	1.41
C=O	1.23	1.50	2.73	3.44	2.33 (H_2C=O)
C-OH	1.43	1.52		3.44	2.87 (CH_3OH)
C-CN	2.22 (HCN H-N)				3.92 (CH_3CN)
S=O	1.44 ($MeSO_2Me$)	1.52		3.44	4.44 ($MeSO_2Me$)

Amine	pK_a
$CH_3CH_2NH_2$	10.7
$FCH_2CH_2NH_2$	8.97
$F_2CHCH_2NH_2$	7.52
$CF_3CH_2NH_2$	5.7

Acid	pK_a
CH_3CO_2H	4.76
FCH_2CO_2H	2.59
F_2CHCO_2H	1.24
CF_3CO_2H	0.23

- F affects pK_a values of proximal functionality
  - reduces basicity of amines
  - increases acidity of acids & alcohols
  - effects are additive in nature

- F is 20% larger than H,
  - closer in size to C=O
- F behaves more like H in P-gp, met. stab. & permeability assays
  - use F-corrected MW (up to 5 F atoms)
    - (MW_{FC}): total MW – MW derived from F
- C-F bond is the most polarized in organic chemistry
  - large dipole interacts electronically with polar substituents
  - C-F dipole is in the same direction as C=O
    - …but the reverse of C-H
- F is the most electronegative atom
  - 3.98 vs 2.20 for H
- C-F bond dissociation energy is high
  - 105.4 kcal/mole;
  - compare to C-H: 98.8; C-Cl: 78.5 kcal/mole

But… there are many examples where bioactivation results in loss of F as a leaving group

Atom	σ_p	σ_m	π
F	0.06	0.34	0.14
Cl	0.23	0.37	0.71
Br	0.23	0.39	0.86
I	0.18	0.35	1.12

- Modest EWG at *para*-position
  - stronger effect when *meta*-
  - Cl & Br are stronger EWGs
  - σ- vs. mesomeric effects
- F is the smallest & least lipophilic
- C-F does not have a low lying σ*
  - poor halogen bond donor

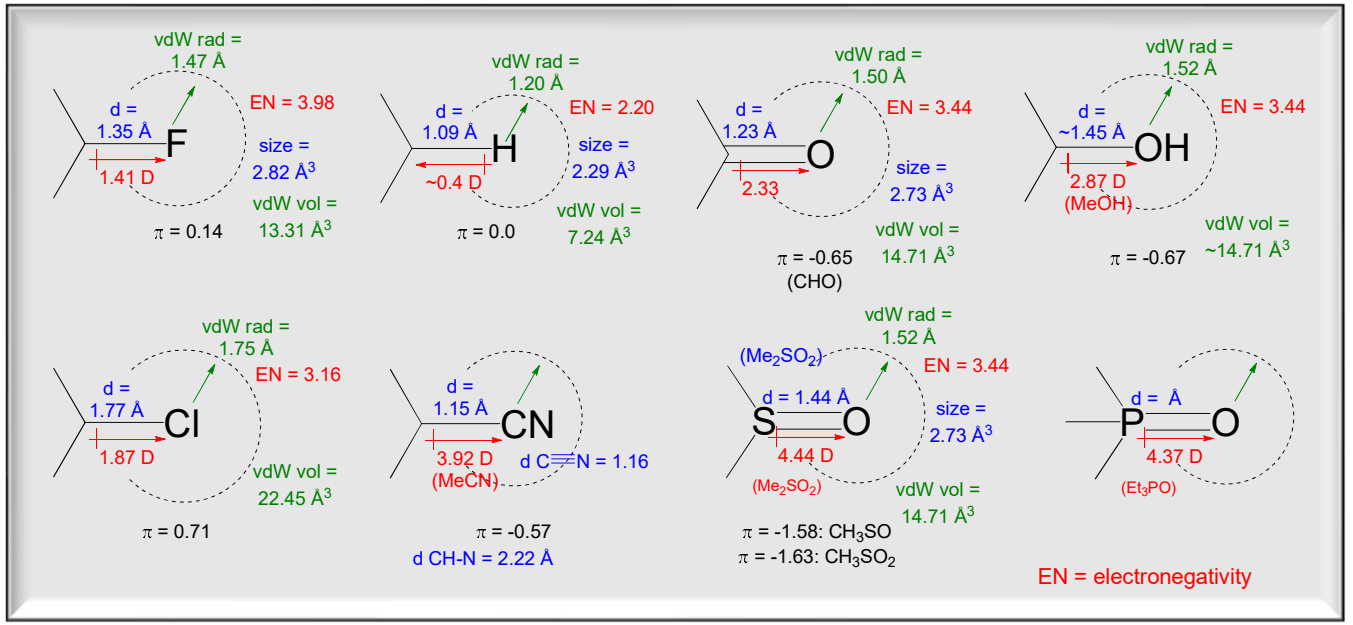
- ^{19}F-isotope useful for NMR analysis
  - assess drug-target interactions
  - fragment screening
- ^{18}F-isotope is a positron emitter
  - positron emitting tomography (PET)
  - imaging, receptor occupancy studies
  - useful pre-clinically & clinically
  - $t_{1/2}$ = 109 min

E. Gillis, N.A. Meanwell *et al., J. Med. Chem.,* 2015, **58**, 8315−8359; N.A. Meanwell, *J. Med. Chem.,* 2018, **61**, 5822-5880
H.-J. Böhm *et al., ChemBioChem,* 2004, **5**, 637-643; M. Pettersson *et al., J. Med. Chem.,* 2016, **59**, 5284-5296; B.M. Johnson *et al., J. Med. Chem.,* 2020, **63**, 6315-6386

Applications of Fluorine in Isosterism

Fluorine to Replace Hydrogen
Fluorine & the Design of Higher Order Bioisosteres

Fluorine & Bioisosterism



- F is 20% larger than H
  - closer in size to C=O, C-OH based on van der Waals radii
- C-F bond is the most polarized in organic chemistry
  - C-F dipole is same directions as C=O, C-OH, S= O but the reverse of C-H
  - large dipole interacts electronically with polar substituents
- F is the most electronegative atom
  - 3.98 vs 2.20 for H & 3.44 for O, 3.16 for Cl
- C-F bond dissociation energy
  - 105.4 kcal/mole; C-H: 98.8; C-Cl: 78.5

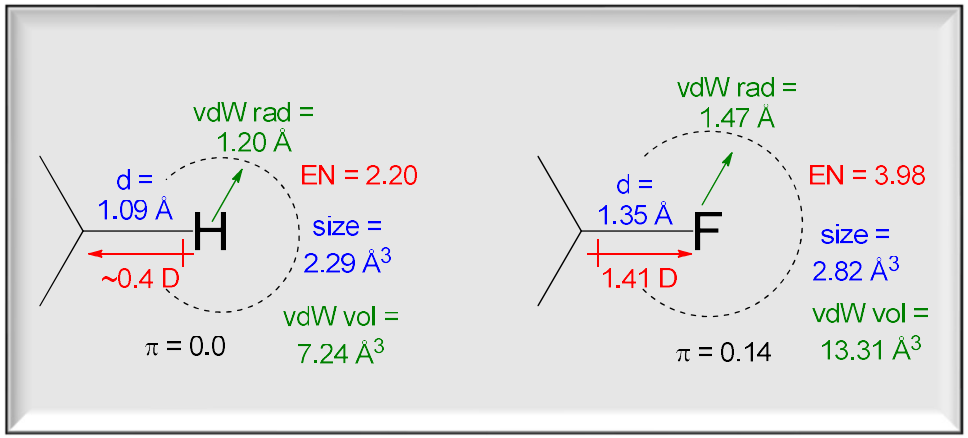
McConnell´s MedChem Atoms (by Size)

Atom	H	F	O	N	C	Cl	S	Br	I
Atom #	1	9	8	7	6	17	16	35	53
MW	1.008	18.998	15.999	14.007	12.011	35.45	32.06	79.904	126.9
vDW radii	1.2	1.5	1.5	1.6	1.7	1.8	1.8	1.9	2.0
vDW volume	7.2	13.3	14.7	15.6	20.6	22.5	24.4	26.5	32.5
Surface Area	18.1	27.2	29.0	30.2	36.3	38.5	40.7	43.0	49.3
%SA versus H	100%	150%	160%	167%	201%	213%	225%	238%	272%
%SA versus C	50%	75%	80%	83%	100%	106%	112%	118%	136%
Atoms depicted with relative SA	H	F	O	N	C	Cl	S	Br	I

https://mcconnellsmedchem.com/2024/10/07/how-big-are-your-medchem-atoms/

NuArq MedChem Consulting LLC

Fluorine-Hydrogen Bioisosterism



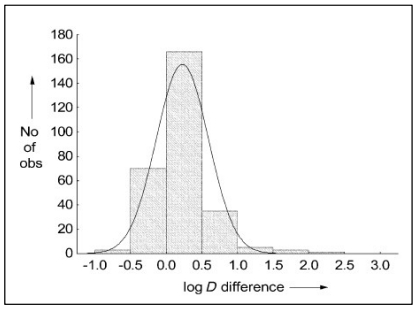
Atom	H	F
Atom #	1	9
MW	1.008	18.998
vDW radii	1.2	1.5
vDW volume	7.2	13.3
Surface Area	18.1	27.2
%SA versus H	100%	150%
%SA versus C	50%	75%
Atoms depicted with relative SA	H	F

- The most common application
  - highly versatile element
    - enchanted?
- Exerts a range of effects on compound properties
  - conformation
  - potency
  - dissociation $t_{1/2}$
  - solubility
  - membrane permeability
  - basicity of amines

https://mcconnellsmedchem.com/2024/10/07/how-big-are-your-medchem-atoms/

Fluorination Patterning & Lipophilicity

- Studied 293 matched pairs (MMPs) of molecules in the Roche compound collection
  - only a F for H change; all had measured Log *D* values
- Log *D* (lipophilicity) increased by an average of 0.25 log units
  - π for F = 0.14
- Histogram shows Gaussian distribution
  - tail of plot extends below 0
  - F for H *reduces* lipophilicity in some settings
- Observed recurring structural patterns
  - low energy conformation with F proximal to O
    - distance is <3.1 Å
- Origin unknown - attributed to:
  - overall increase in polarity leading to gain in solvation energy in polar *vs* non-polar medium
  - F polarizes proximal O leading to stronger H-bonds in polar medium
  - dipole effects?

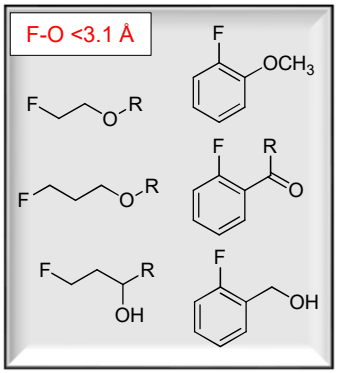


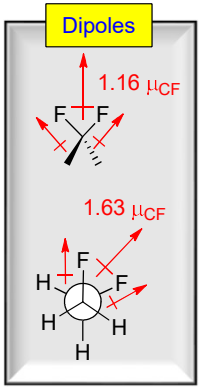
propyl series					
exp. Log *P*	3.3	2.8	2.9	3.1	2.5
Soly (μM)	200			30	>1720

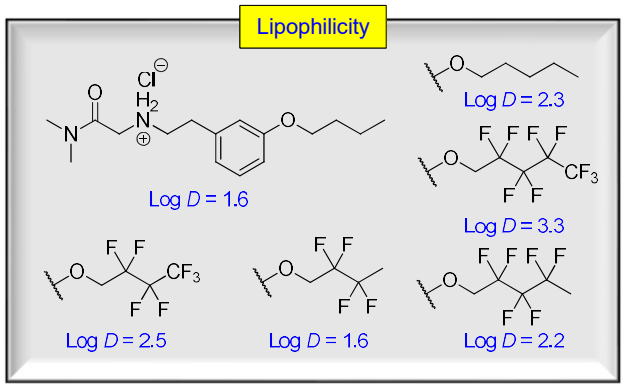
butyl series				
exp. Log *P*	2.8	3.1	3.0	2.8
Soly (μM)	820	182	115	277

- Experimental Log *P* data for fluorinated alkyl indoles
  - fluorination lowers Log *P* in the propyl series
- Vicinal di-F derivatives differ from geminal isomers
  - less lipophilic & more soluble than gem-di-F
  - observed in nPr & nBu series
- Fluorination pattern modulated Log *D* in a series of ethers
  - lipophilicity of $CH_3(CF_2)_n$-X similar to $CH_3(CH_2)_n$-X
  - conformation in H_2O & octanol differ
- Attributed to dipole effects
  - higher in internally per-F derivatives
  - higher in polar (H_2O) than non-polar (octanol) solvent
    - chameleon behavior

Dipole alignment important for polarity & solubility
Vicinal difluoroalkanes stabilized by a *gauche* interaction



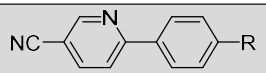


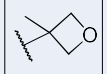
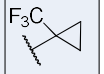


H.-J. Böhm *et al., ChemBioChem,* 2004, **5**, 637-643; K. Müller *et al.*, *J. Med. Chem.,* 2015, **58**, 9041-9060; B. Linclau *et al., J. Med. Chem.*, 2018, **61**, 10602-10618

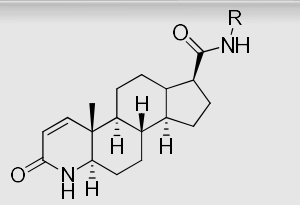
F & Alkyl Bioisostere Design

Fluorine & Bioisosterism



									
RLM $t_{1/2}$ (min)	30	125	70	135	37	7	25	11	>400
HLM $t_{1/2}$ (min)	51	202	122	274	38	9	66	35	150

- Systematic study of metabolic stability of *tert*-butyl replacements
  - 6-(4-(tert-butyl)phenyl)nicotinonitrile as structural background
- CF_3-substituted cyclopropyl moiety emerged as optimal
  - effective in the context of the steroid 5α-reductase inhibitor finasteride
  - t-Bu moiety is metabolically labile
  - HLM $t_{½}$ ↑ from 63 to 114 minutes

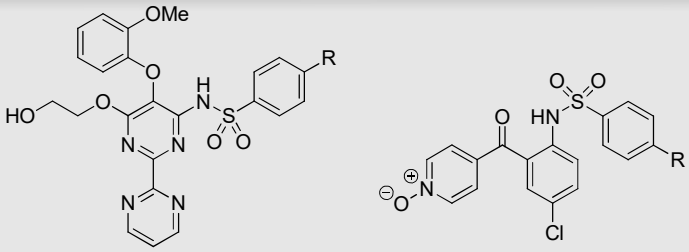


finasteride

R = *t*Bu — HLM $t_{1/2}$ = 63 min

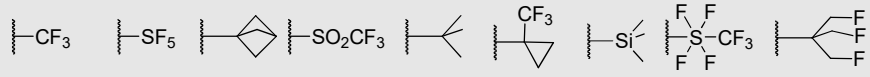
R = Cp-CF_3 — HLM $t_{1/2}$ = 114 min

- tBu mimics surveyed in the context of bosentan & vercirnon
  - all except Cp-CF_3 are smaller than tBu based on calculated volumes
- Bosentan: Cp-CF_3 & BCP performed similarly to tBu at endothelin receptors
  - CF_3 & SF_5 10-fold less potent
- Vercirnon: all performed similarly in a CCR9 functional assay
- All showed a trend towards enhanced metabolic stability over tBu
  - CF_3 & SF_5 most effective
- No significant CYP inhibitory effects observed
- Log *D* measurements: CF_3 < SF_5 < Cp-CF_3 < tBu < BCP
- Effects on solubility varied in bosentan: reduced solubility in vercirnon
- N-H pK_a increased in the order: SF_5 < CF_3 < Cp-CF_3 < tBu ≈ BCP



bosentan: R = tBu

vercirnon: R = tBu



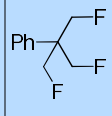
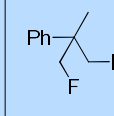
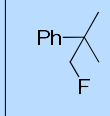
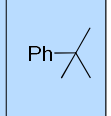
44.3 Å^3 | 66.4 Å^3 | 75.0 Å^3 | 78.0 Å^3 | 79.2 Å^3 | 83.5 Å^3 | 92.0 Å^3 | 92.8 Å^3 | 94.7 Å^3

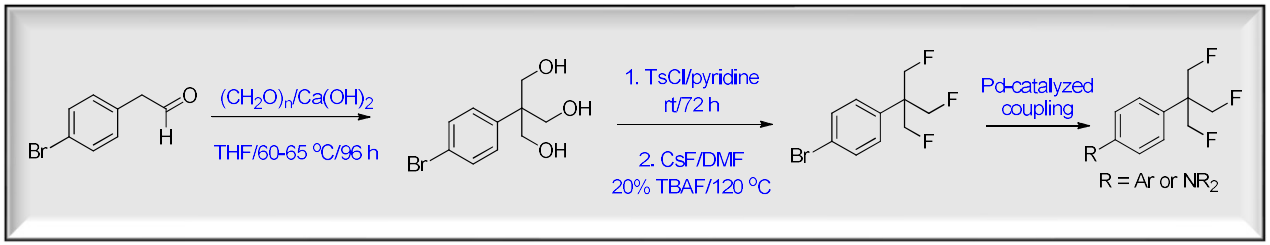
calculated volumes (www.molinspiration.com)

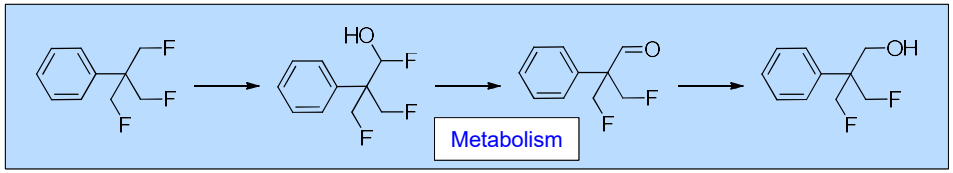
D. Barnes-Seeman *et al., ACS Med. Chem. Lett.*, 2013, **4**, 514-516; *Curr. Topics Med. Chem.*, 2014, **14**, 855-864; E. Carreira *et al., ChemMedChem.*, 2015, **10**, 461-469

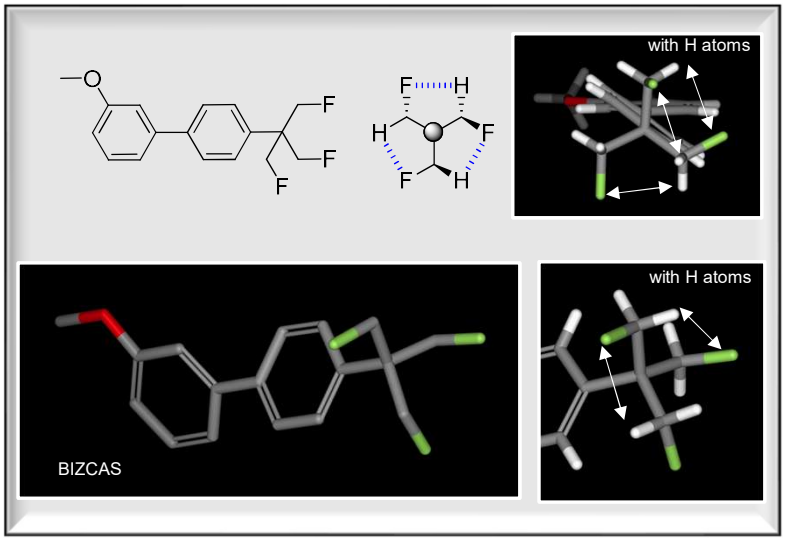
β,β′,β″-Trifluoro-*tert*-butyl to Optimize a tBu Moiety

Fluorine & Bioisosterism

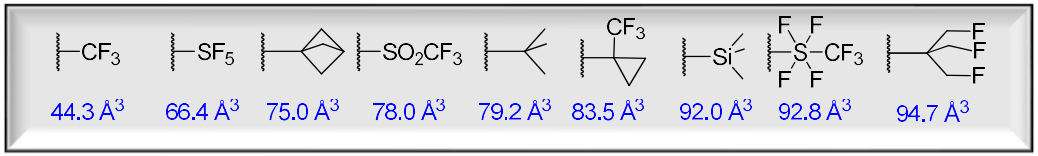
				
Log *P*	2.42	2.62	2.93	4.10
μ (D)	1.97	2.30	1.64	0.36







- Limited study in medicinal chemistry
  - synthetic access optimized
- Reduces lipophilicity of tBu moiety
  - dipole effects which are maximal at 2 F atoms
  - trifluoro has lower dipole but still has lowest Log *P* value
- Preference for topographical arrangement in which F point away from each other
  - stabilized by F to H electrostatic interaction
- Metabolic stability enhanced over tBu
  - metabolized by α-hydroxylation to release F^-

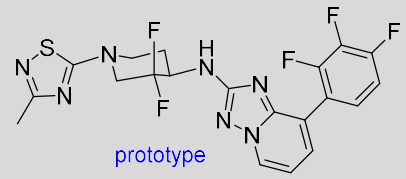


D. O'Hagan *et al., Org. Lett.*, 2023, **25**, 6802-6807

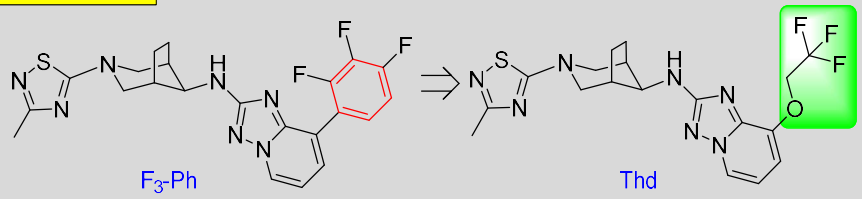
Terminal Phenyl Mimics: γ-Secretase & PKC-θ Inhibitors

γ-Secretase Modulators

note: the piperidine & bicyclo piperidine are phenyl replacements



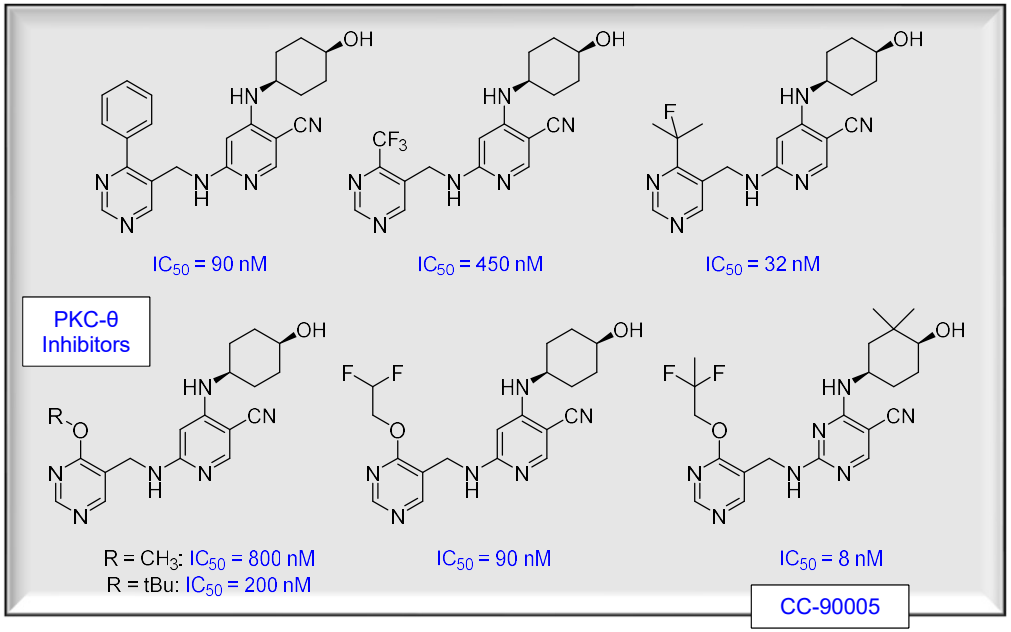
prototype associated with phototoxicity risk



	Lead	Bioisosteric analogue
	F_3-Ph	Thd
h IC_{50} Aβ42 total/free (nM)	4.5/1	21/12
Mouse IC_{50} total/free (nM)	4/0.9	9/5
in vivo remaining %Aβ42 @ 1/3/10 mg/kg PO	56/27/22	59/33/15
Log D	4.14	3.17
Solubility (μg/mL)	3	198
PPB f.u. (h/m) (%)	0.4/0.3	2.8/10.1
IC_{50} UVA (μM)	1.67	443
Cl heps (h/m): (μL/min/Mcells)	6.6/5.4	1.3/1.4
hERG IC_{50} (μM)	<2	>10

IMPACT
reduced phototoxicity risk; lower lipophilicity; enhanced solubility; reduced plasma protein binding; reduced protein covalent binding; reduced hERG inhibition

- γ-Secretase modulators: phototoxicity issue
  - trifluorophenyl moiety replaced by a CF_3-CH_2-O
  - reduced Log *D*, phototoxicity, hERG, PCB risks



- PKC-θ inhibitors
  - CF_3, OCH_3, O-tBu poor substitutes for phenyl
  - $CF(CH_3)_2$ 3-fold more potent than Ph
- O-CH_2-CHF_2 exhibits comparable potency to phenyl
  - potency enhanced 10x by methylation
- CC-90005 selected for clinical evaluation
  - highly selective over PKC-δ & 456 other kinases

R.M. Rodríguez Sarmiento *et al., J. Med. Chem.,* 2020, **63**, 8534-8553; D.S. Mortensen *et al., J. Med. Chem.,* 2021, **64**, 11886-11903

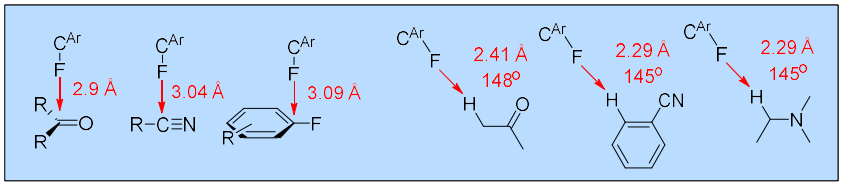
Applications of Fluorine in Drug Design

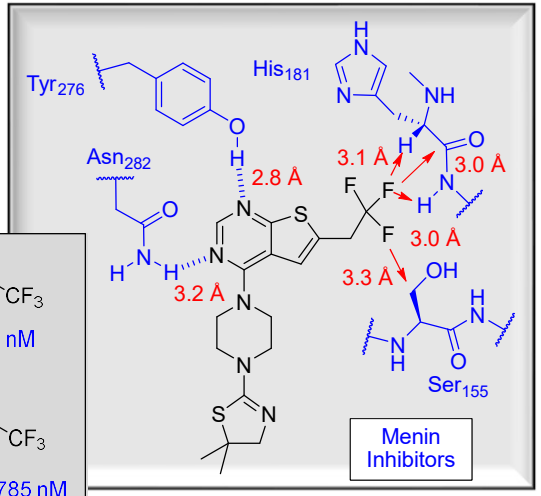
Drug-Target Interactions

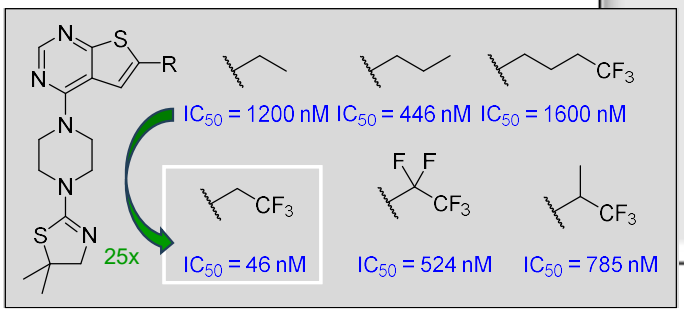
Intermolecular F to C=O & F to H Interactions

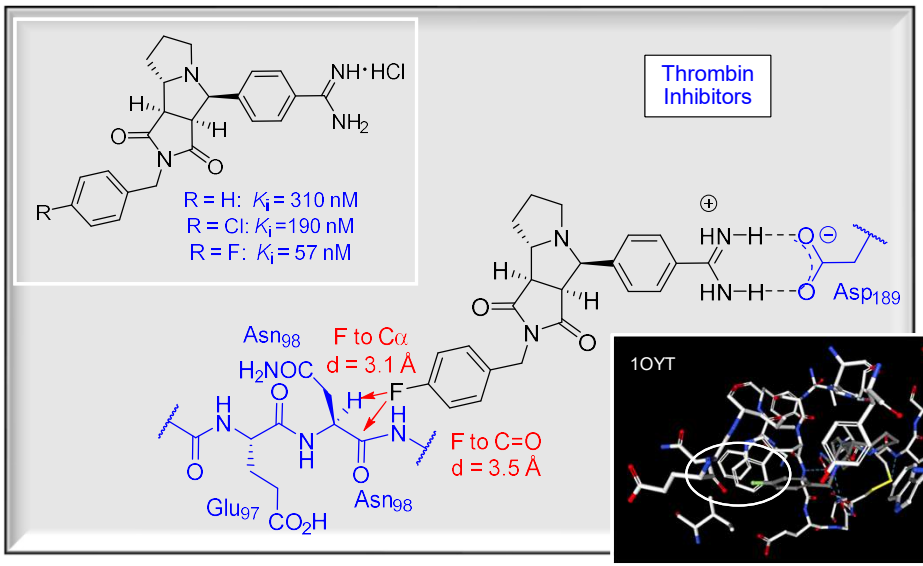
Multipolar Interactions

- Benzamidine-based thrombin inhibitors
- 4-F more potent than 4-H (6x) & 4-Cl (4x)
  - 5-10x better than other F patterns
- X-ray cocrystal revealed F interactions
  - F atom proximal to Asn_{98} C=O C atom
  - F close to N-H of Asn_{98}
- Search of CSD identified close Ar-F contacts
  - dipolar interactions with e-deficient C atoms
  - close contacts with several C-H elements







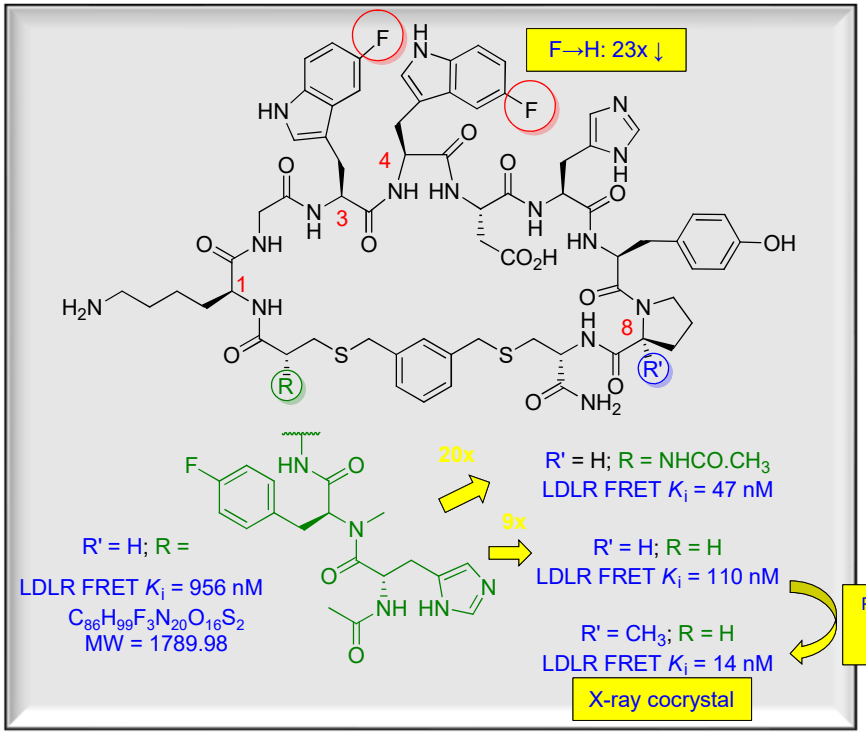


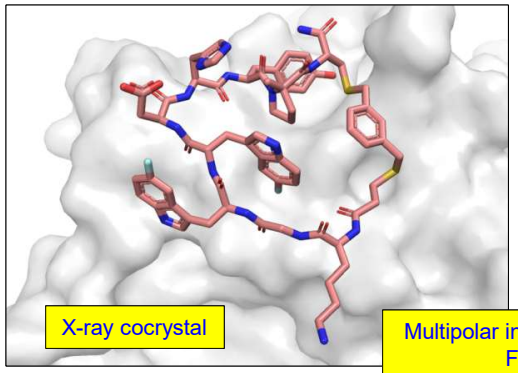
- Binds to menin, an oncogenic cofactor in acute leukemias
- Blocks interaction with mixed lineage leukemia (MLL) cofactor
  - X-ray cocrystal structure - binds in the MLL pocket
- Further optimization focused on nPr substituent
  - CF_3CH_2 the most potent analogue: 10x over nPr; 26x over Et
- X-ray cocrystal structure revealed close F interactions with menin
  - F atom proximal to His_{181} C=O carbon atom - 3.0 Å
  - same F atom close to α-C-H & N-H - 3.1 Å
  - 2nd F atom close to Ser_{155} side chain - 3.3 Å

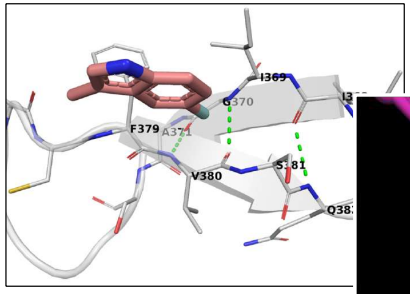
J.A. Olsen *et al., ChemBioChem* 2004, **5**, 666-675; A. Shi *et al., Blood,* 2012, **120**, 4461-4469; J. Grembecka *et al., Nature Chem. Biol.*, 2012, **8**, 277-284
T. Cierpicki *et al., J. Med. Chem.*, 2015, **58**, 7465-7474; F. Diederich *et al., Angew. Chem. Int. Ed.*, 2005, **44**, 1788-1805

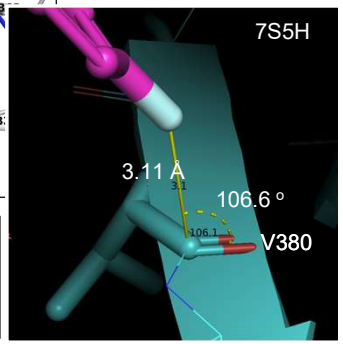
The Importance of Fluorine in PCSK9 Inhibitors

Multipolar Interactions









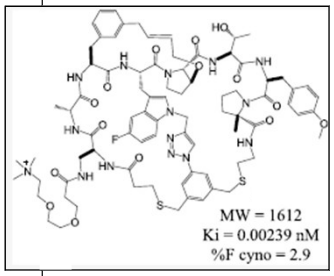
Multipolar interaction between F & V380 C=O
F-C = 3.10 Å; θ = 106.1°
vdW radius: C = 1.7 Å ; F = 1.47 Å; Σ = 3.17 Å
n→π* *d* <3.22 Å; Bürgi-Dunitz θ = 109° ± 10°

- Cyclic peptide PCSK9 inhibitor discovered by mRNA display screening
  - truncation enhanced potency 20x ($COCH_3$); 9x (H)
  - CH_3 at position 8 enhanced potency by an additional 8-fold
- X-ray cocrystal structure revealed donut-shaped molecule
  - 5-F-Trp at position 4 projected into binding site

- 5-F-Trp at positions 3 & 4 replaced by Trp (R= H)
  - 3-Trp K_i = 103 nM (2x Δ)
  - 4-Trp K_i = 1070 nM (23x Δ)
- 5-F at residue 4 confers 23-fold potency advantage
  - remarkable effect for a single F atom (~1.3% of MW)
  - only a 2x reduction at 3-Trp
- X-Ray cocrystal structure suggests F engages:
  - NH of Gly_{370} ($d_{F\rightarrow N}$ = 4.91 Å)
  - C=O of Val_{380} ($d_{F\text{-}C}$ = 3.11 Å)
  - C=O of Ile_{369} ($d_{F\rightarrow C}$ = 5.10 Å)
- Use of 5-F-Trp in selection process crucial to success of screen
  - MK-0616 in Phase 2 clinical trials by PO dosing
  - Phase 3 trials initiated 3Q 2023

$C_{68}H_{79}F_2N_{15}O_{13}S_2$
MW = 1416

multi-polar F to C=O interactions

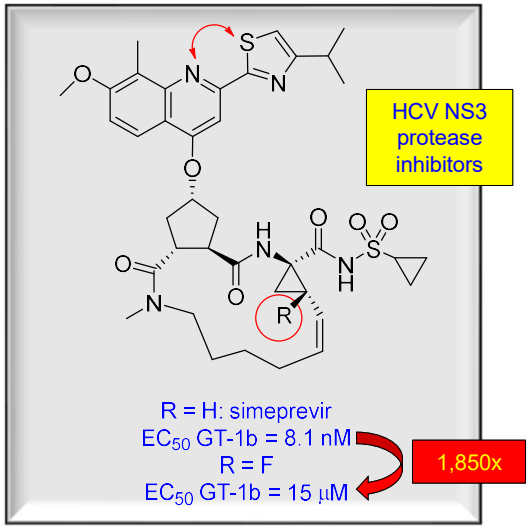


T.J. Tucker *et al., J. Med. Chem.*, 2020, **63**, 13796-13824; 2022, **64**, 16770-16800
R.W. Newberry & R.T. Raines, *Acc. Chem. Res.*, 2017, **50**, 1838-1846; F. Diederich *et al., Angew. Chem. Int. Ed.*, 2005, **44**, 1788-1805

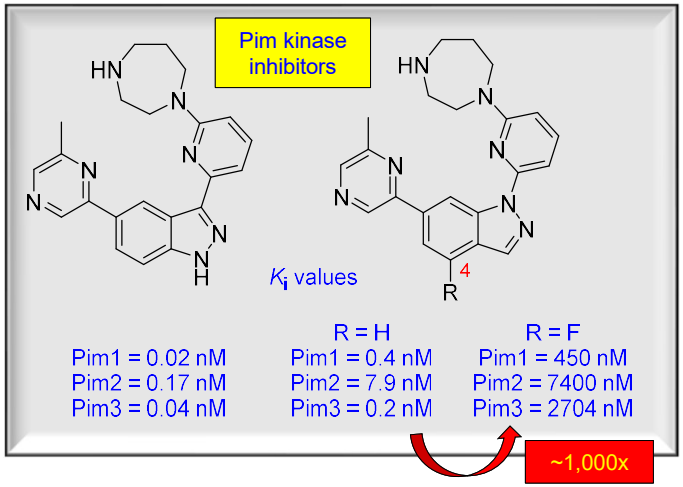
NuArq MedChem Consulting LLC

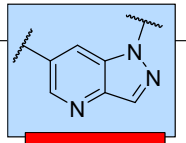
The Effects of Fluorine: Reduced Potency

Fluorine & Potency



- Simeprevir a potent inhibitor of HCV NS3 protease
  - EC_{50} = 8.1 nM in GT-1b replicon
- Fluorination of the cyclopropane probed
  - (*Z*)-isomer EC_{50} = 15 μM
- Modeling studies
  - P1-P2 backbone peptide may be rotated slightly
    - repulsive F to P2 C=O interactions?
  - may also disturb location of the acylsulfonamide



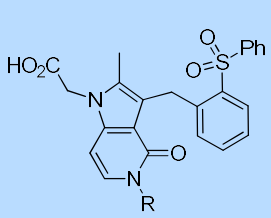


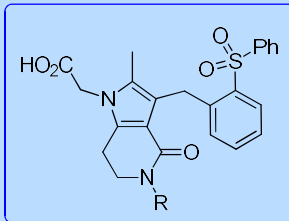
- Pim kinase inhibitors
- Potent indazole lead
  - N-H engages hinge C=O
  - indazole glucuronidated *in vivo*
- Indazole isomer avoids N-H
  - C-H engages hinge C=O
- F at C-4 reduced potency 1000x
  - C-4 to N: 10-40x lower potency
  - electronic or steric clash with gatekeeper Leu_{120}?

C-F ≡ azine N

G. Milanole *et al., Org. Lett.*, 2015, **17**, 2968-2971; X. Wang *et al.*, *J. Med. Chem.* 2017, **60**, 4458-4473

The Effects of Fluorine: Dissociation $t_{1/2}$ in PGD_2





R = CH_3
IC_{50} = 7 nM
$t_{1/2}$ = 5.3 h

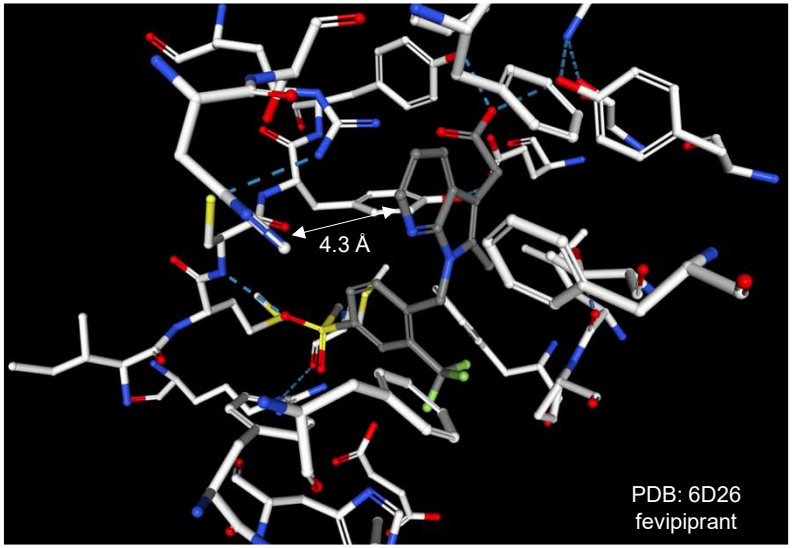
R = $PhCH_2$
IC_{50} = 1.5 nM
$t_{1/2}$ = 6.7 h

R	GTPγS binding IC_{50}	Dissoc. $t_{1/2}$	tPSA	Caco-2
H	8.6 nM	1.3 h	114	0.1
CH_3	4.9 nM	8 h	105	0.1
CHF_2	2.2 nM	21 h	105	0.4

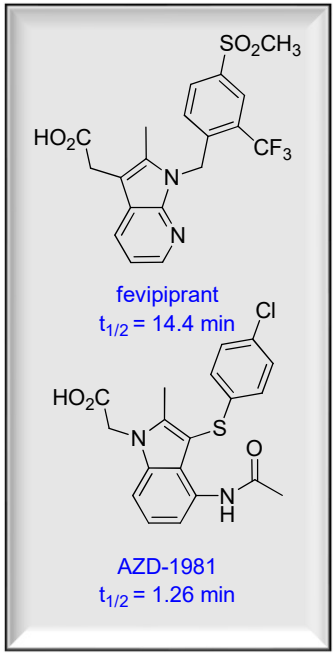
Caco-2: A→B direction P_{app} x 10^{-6} cm/s

- CRTH2 (PGD_2) antagonists
  - GPCR involved in chemotaxis of Th2 lymphocytes, eosinophils & basophils
- Potency enhanced by amide N-substitution
  - N-CHF_2 ~ 4x N-H
- Focus on residence time
  - N-CHF_2 > N-CH_3 > H
- Low membrane permeability
  - solved by ester prodrugs

CRTH2 = chemoattractant receptor-homologous molecule expressed on T_H2 cells



- Fevipiprant a potent PGD2 antagonist
  - K_d= 1.14 nM
  - dissociation $t_{1/2}$ = 14.4 minutes
  - considered to be slow binding
  - AZD-1981 $t_{1/2}$ = 1.26 minutes
- No overt interaction with C-6 substituents
  - His_{107} close but vectors may not be optimal



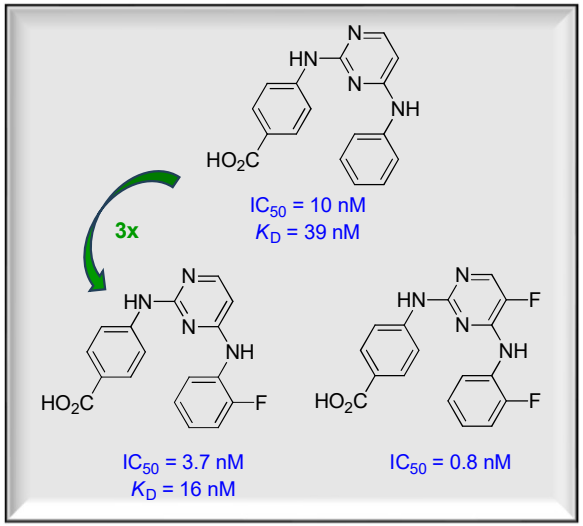
fevipiprant
$t_{1/2}$ = 14.4 min

AZD-1981
$t_{1/2}$ = 1.26 min

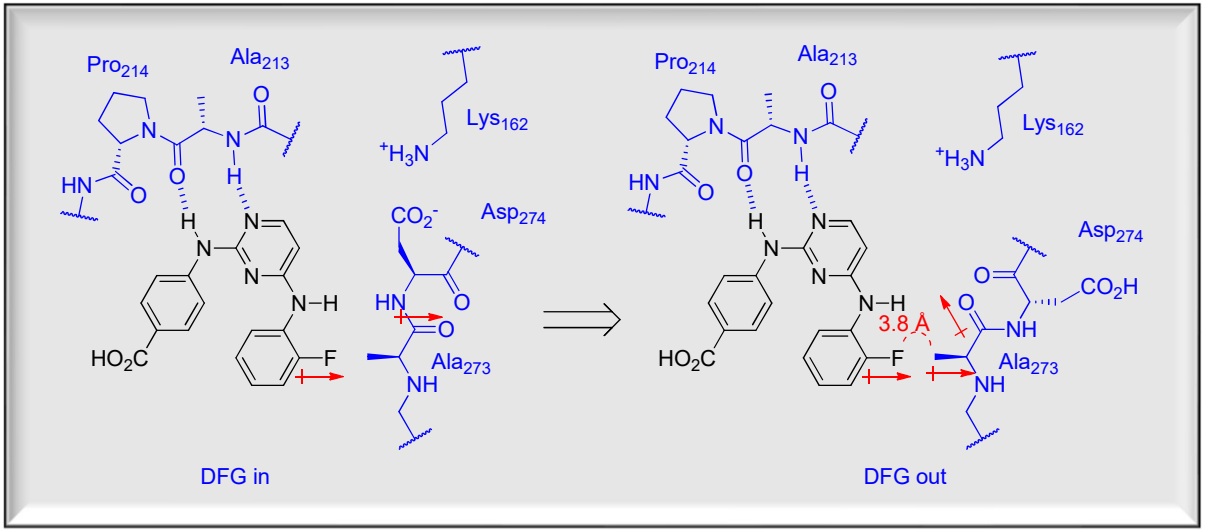
PGD_2/CRTH2 antagonists

M. Andrés *et al., Bioorg. Med. Chem. Lett.,* 2014, **24**, 5111-5117; D.A. Sykes *et al., Mol. Pharmacol.*, 2016, **89**, 593-605; L. Wang *et al., Mol .Cell*, 2018, **72**, 48-59

Fluorine & DFG Loop in Aurora Kinase Inhibitors



R	IC_{50} (nM)
H	10
F	3.7
Cl	2.5
Br	2.1
CN	43
CF_3	35
OCF_3	28
Ph	149
CO_2H	6.1

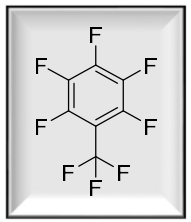


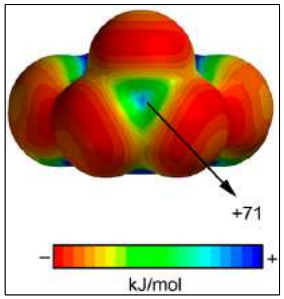
- Aurora A kinase inhibitors
  - NH-pyrimidine hinge binder
- F-phenyl homologue 2x more potent than prototype
  - potency further enhanced by F-pyrimidine
- X-ray cocrystal structure revealed different binding modes
  - DFG loop in the active "in" conformation for prototype
- DFG loop flips to the inactive "out" conformation in F derivatives
  - 100° rotation around the Ala_{273} amide bond
  - also seen with Cl, Br, C≡N

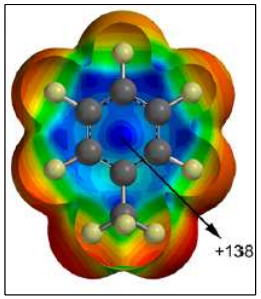
- Cl, Br, CN substituents had same effect
  - CF_3, CF_3O behaved like prototype: not steric in origin
- Attributed to an effect of the C-X dipole
  - F is 3.8 Å away from Ala_{273} CH_3
    - colinear alignment
- Interpretation: induces a dipole in the Cα-Cβ bond
  - transmits to amide C=O
  - facilitates rotation to align dipoles in favorable fashion

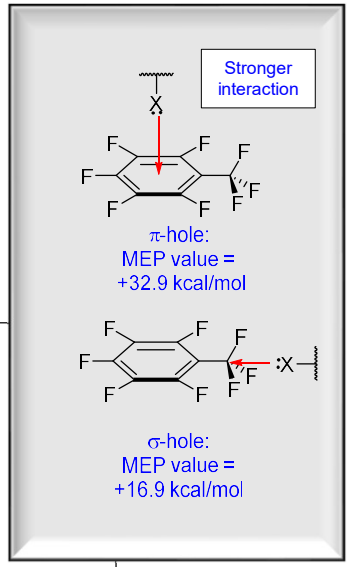
M.P. Martin *et al., ACS Chem. Biol.*, 2012, **7**, 698-706

Ar-CF_3 & Tetrel Bonding



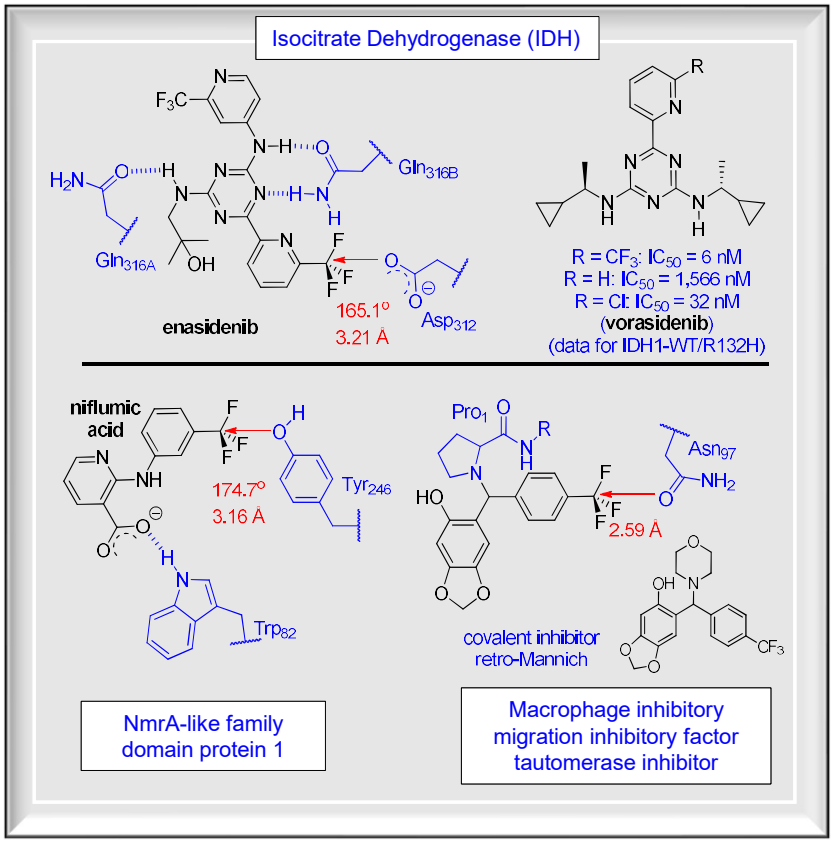






- σ-hole associated with the CF_3 substituent
  - modest MEP; sensitive to geometry: optimal at 180°
  - π-hole associated with penta-F ring is larger & dominates
- Examples of tetrel bonding identified by survey of PDB
  - electron rich atom within 3.37 Å (C: 1.7 Å: O: 1.52 Å)
  - X-C···O angle of >180°: CF_3-C σ*
  - resolution of ≤3 Å
- 8 Complexes identified
  - C-O distances ranged from 2.59 Å to 3.35 Å
  - shortest in macrophage migration inhibitory factor tautomerase inhibitor
- Examined two cases closely
  - isocitrate dehydrogenase-inhibitor complex: Asp_{312}
    - CF_3 important: H-analogue in related series 260x weaker
    - CF_3 can be replaced by Cl – halogen bond with Asp_{273}
  - niflumic acid with NMRAL1
    - Tyr_{246} O donates to the CF_3-C σ*
  - MIF inhibitor (covalent mechanism *via* retro-Mannich)
    - side chain C=O of Asn_{97} donates to the CF_3-C σ*

Cl in vorasidenib is 2.73/2.79 Å away from O of Asp_{312}
vdW radii: Cl = 1.75 Å; O = 1.52 Å; Σ = 3.27 Å



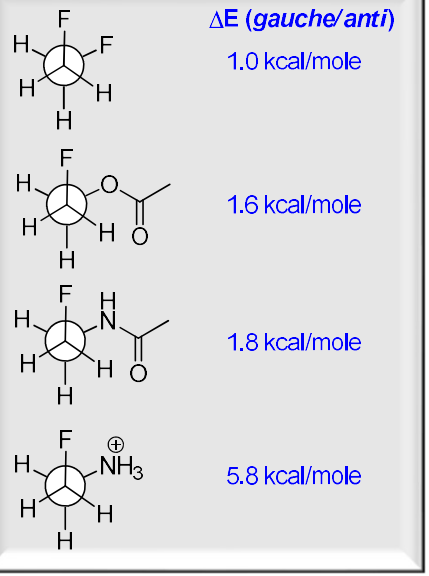
X. García-LLinás *et al.*, *J. Phys. Chem. A,* 2017, **121**, 5371-5376; Z. Konteatis *et al., ACS Med. Chem. Lett.,* 2020, **11**, 101-107
W.L. Jorgensen *et al., Bioorg. Med. Chem. Lett*., 2016, **26**, 2764-2767

MEP = molecular electrostatic potential

Fluorine for Hydrogen: Conformation

The Gauche Effect: Proline/Pyrrolidine Conformation

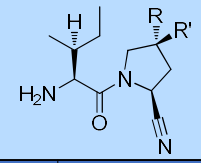
Fluorine & Conformation



Hyperconjugation
σC-H donates to σ*C-F
Electrostatic Effect
C-Fδ⁻ to C-N δ⁺ in F-NH_3^+
Dipole Alignment
strong C-F dipole

DPP4 Inhibitor

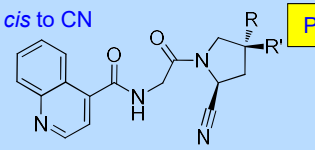
F *cis* to CN



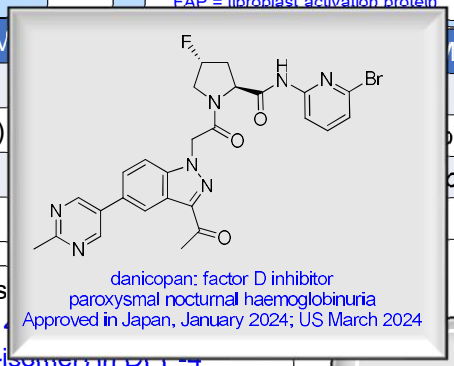
R	R'	DPP-4 IC_{50} (nM
H	H	1.5
H	F	0.6 (Cγ-*endo*)
F	H	290 (Cγ-*exo*)
F	F	0.8

FAP Inhibitor

F *cis* to CN



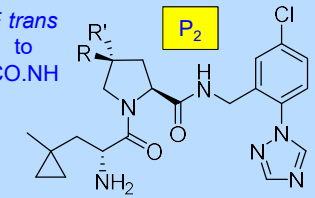
FAP = fibroblast activation protein



danicopan: factor D inhibitor
paroxysmal nocturnal haemoglobinuria
Approved in Japan, January 2024; US March 2024

Thrombin Inhibitor

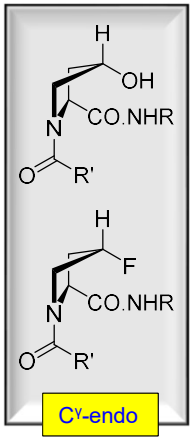
F *trans* to CO.NH



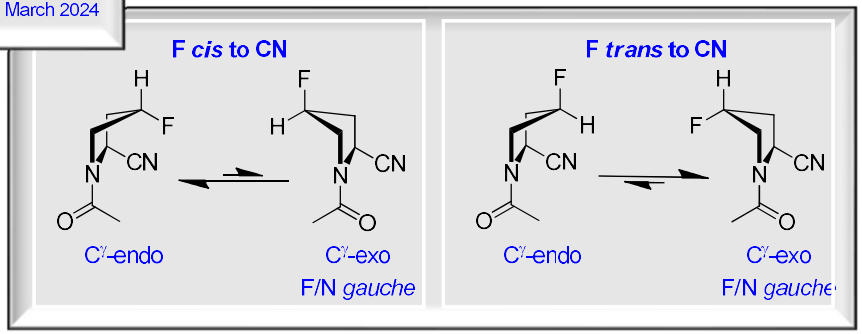
R	R'	thrombin K_i (nM)
H	H	0.6
H	F	0.37 (Cγ-*exo*)
F	H	110 (Cγ-*endo*)
F	F	3.6

Stereochemical preference inverts at P_2

F ≡ OH



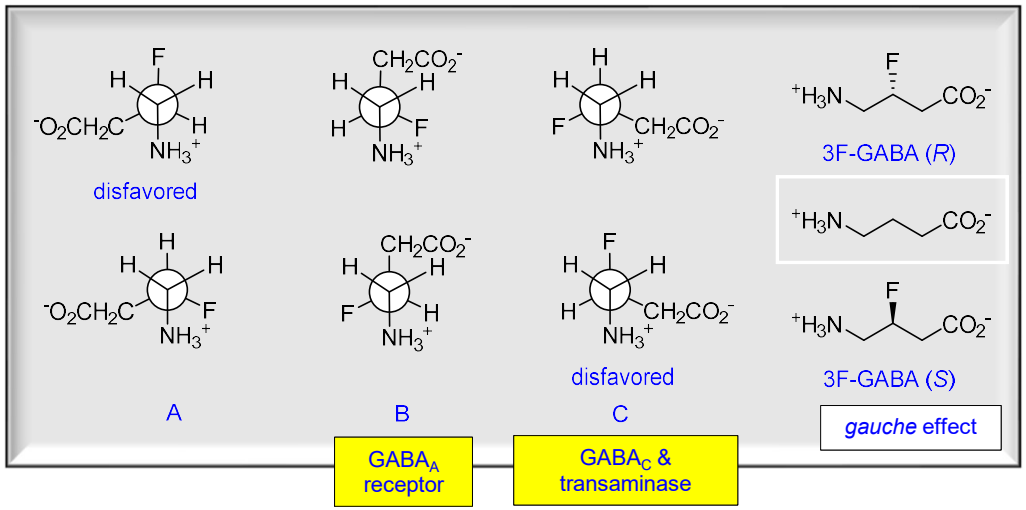
- C-4 Fluorination increases
  - *cis*-4-F (*(S)*-isomer)
    than *trans*-4-F *((R)*-isomer) in DPP-4
  - SAR reproduced in FAP inhibitors
- Thrombin SAR is the inverse
  - P_2 not P_1? – inverted binding topology?
- Not a steric effect: 4,4-diF retains potency
  - *cis-(S)*-4-F stabilizes Cγ-endo pucker
  - *trans*-(*R*)-4-F stabilizes Cγ-exo pucker
- F mimics effect of OH in collagen
  - originally considered to be H-bond effect
  - electronic effect on conformation of Pro ring



H. Fukushima *et al., Bioorg. Med. Chem.*, 2004, **12**, 6053-6061; K. Jansen *et al., J. Med. Chem.*, 2014, **57**, 3053-3074; D.D. Staas *et al., Bioorg. Med. Chem.*, 2006, **14**, 6900-6916
R.T. Raines *et al., Protein Sci.*, 2003, **12**, 1188-1194; *J. Am. Chem. Soc.*, 2001, **123**, 777-778; 2003, **125**, 9262-9263

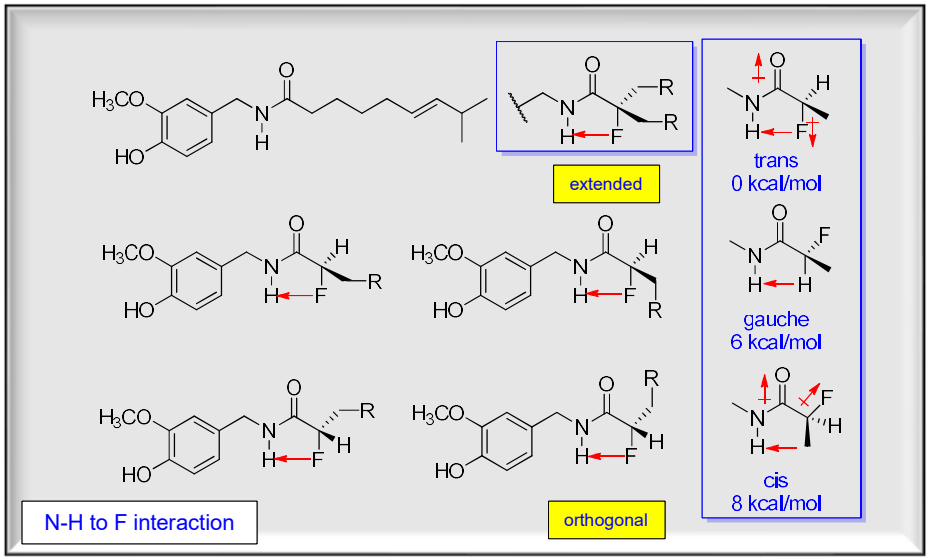
F & Conformation – GABA & Capsaicin

Fuorine & Conformation



- Both enantiomers of 3-F GABA synthesized
  - pK_a = 8.95 & 3.30; pK_a for GABA = 10.35 & 4.05
  - preserves the zwitterionic nature of GABA
- F-NH_3^+ *gauche* interaction favored
  - extended conformation predominates in solution (^{1}H-NMR)
- Each enantiomer interacted similarly with GABA$_A$ receptor
  - extended conformer B recognized by GABA$_A$
- (*R*)-isomer exhibited higher affinity for GABA$_C$ & transaminase
  - conformer C recognized by GABA$_C$ & transaminase

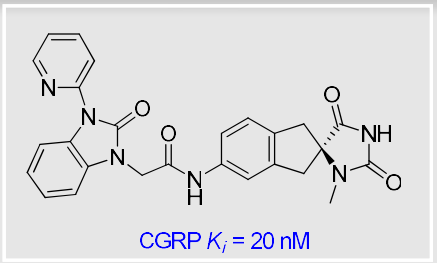
- α-Fluoro capsaicin isomers synthesized in optically pure form
- *trans* conformation favored:
  - 6 kcal/mol over *gauche* & 8 kcal/mole over *cis*
- Stabilized by intramolecular interactions
  - C-F/C=O dipole alignment
  - electrostatic interaction between F(δ$^-$) & NH (δ$^+$)
- Both enantiomers performed similarly as agonists at the TRPV1 receptor
  - suggests extended bound conformation accessible to both



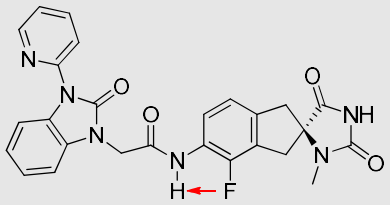
D. O'Hagan *et al., ChemBioChem,* 2007, **8**, 2265-2274; *Chem. Commun.,* 2011, **47**, 7956-7958; D. O'Hagan *et al., ChemBioChem,* 2009, **10**, 823-828

F to NH & Conformation – CGRP & GPR119

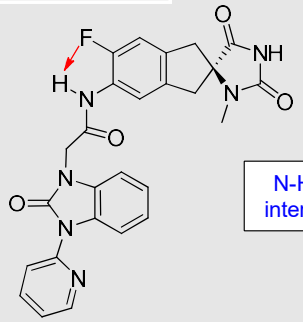
Fluorine & Conformation



CGRP K_i = 20 nM

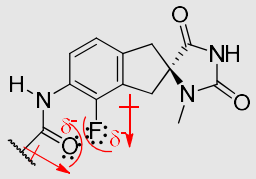


CGRP K_i = 55 nM

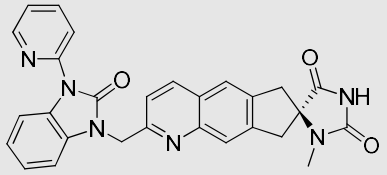


CGRP K_i = 720 nM

N-H to F interaction

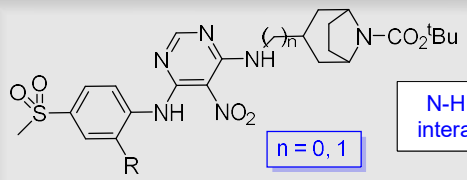


allylic 1,3-strain



CGRP K_i = 17 nM

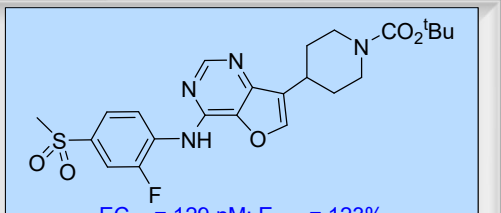
- Potent calcitonin gene-related peptide (CGRP) receptor antagonist
  - treatment & prevention of migraine
- Amide isosteres sought to reduce PSA & increase oral absorption
- Torsion plot indicated preference for amide & phenyl to be coplanar
  - lowest energies at 0° & 180°
- F introduced *ortho*- to NH to bias conformation
  - stabilized by F to NH electrostatic interaction
  - favored by steric/electronic interactions between C=O & F
  - more potent isomer suggested an extended conformation
- Confirmed with the synthesis of the fused pyridine



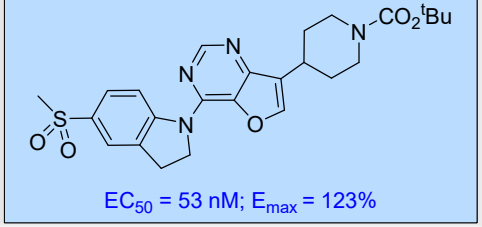
n = 0, 1

N-H to F interaction

R	n	EC_{50} (nM)	
H	0	11.5	
F	0	1.5	10x
H	1	>10,000	
F	1	6.7	1500x



EC_{50} = 129 nM; E_{max} = 123%



EC_{50} = 53 nM; E_{max} = 123%

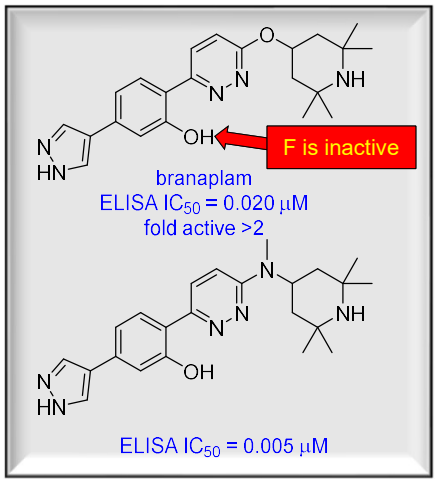
- Similar observations with GPR119 agonists: inspired ring design to mimic topology

C.A. Stump *et al., Bioorg. Med. Chem. Lett.*, 2010, **20**, 2572-2576

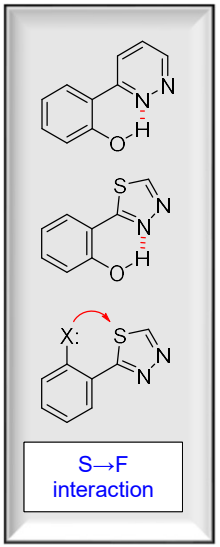
Z.Yang *et al., Bioorg Med. Chem. Lett.*, 2013, **23**, 1519-1521

T. Koshizawa *et al., Bioorg Med. Chem. Lett.*, 2017, **27**, 3249-3253

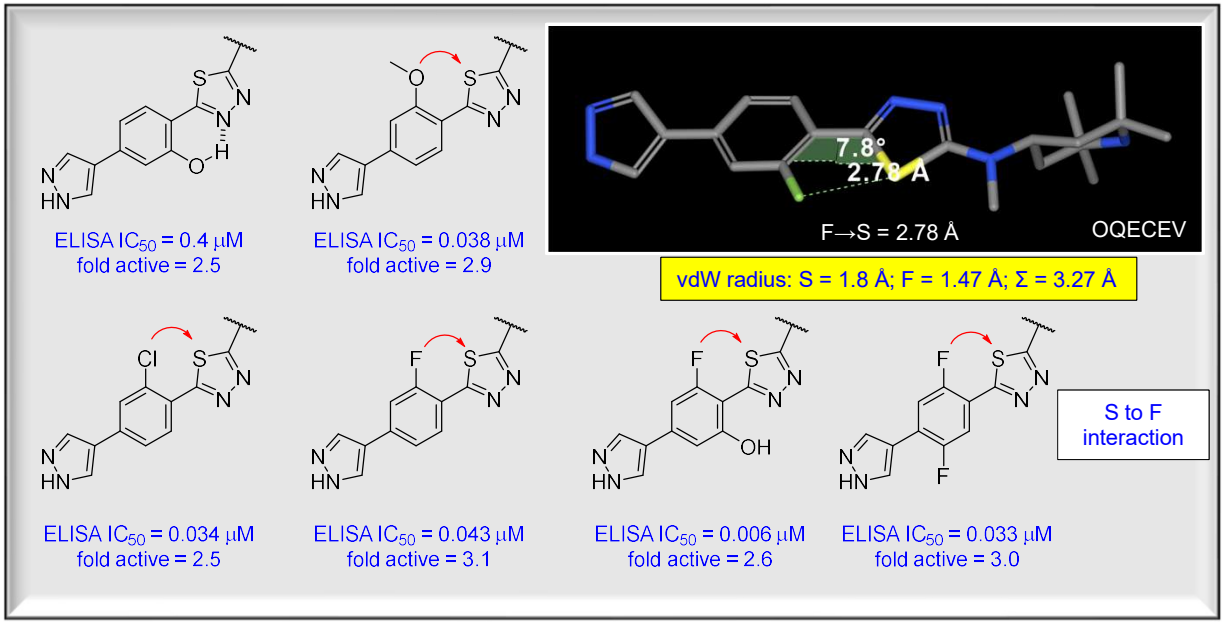
Fluorine-Sulfur Interactions in SMN2 Splicing Modulators



ELISA measures SMN protein elevation: gives potency & fold-activation



SMN1 = survival motor neuron 1

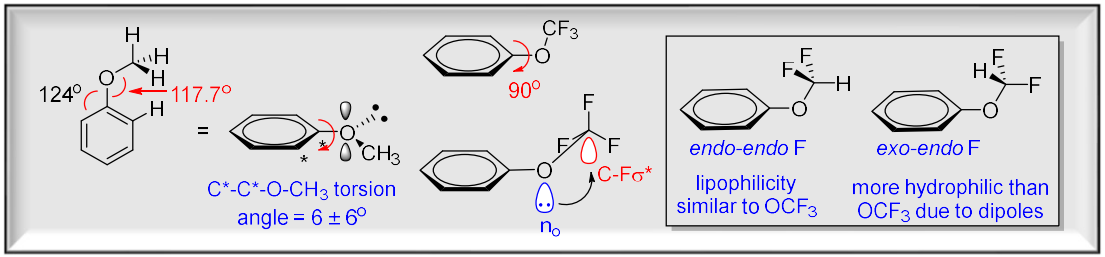


- Branaplam enhances levels of full length SMN2 gene
  - modulates splicing of SMN2 to enhance exon-7 inclusion
  - therapy for spinal muscular atrophy (SMA) & Huntington's
    - compensates for genetic homozygous inactivation of the SMN1 gene
- Biaryl moiety favors a planar topography
  - stabilized by an intramolecular H-bond
- Explored replacing the pyridazine with 1,3,4-thiadiazole
  - offers potential for H-bond & S σ* interactions to modulate planarity

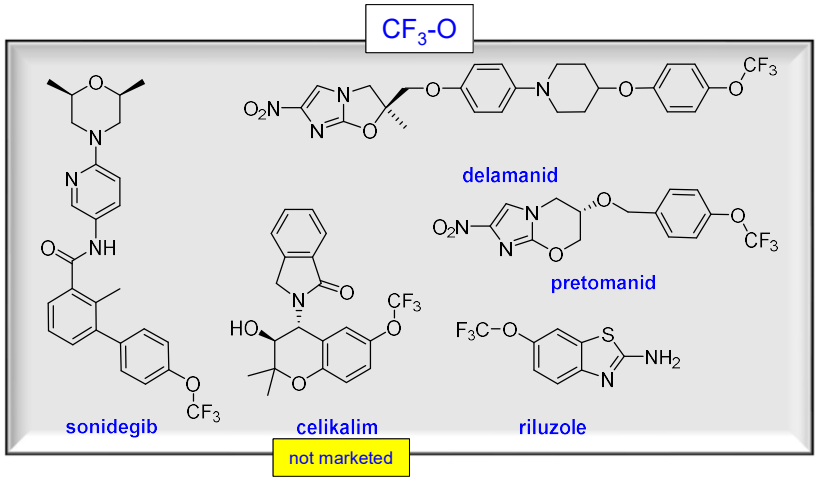
- OH & OMe gave good potency & efficacy but poor PK properties
  - replaced with F & Cl
- X-ray of F derivative revealed planar topography
  - 7.8° twist; at 2.78 Å
  - F & S within the sum of van der Waals radii
- F & Cl derivatives exhibited improved plasma exposure PO dosing
  - brain concentration highest with di-F analogue
  - active in a mouse model of SMA

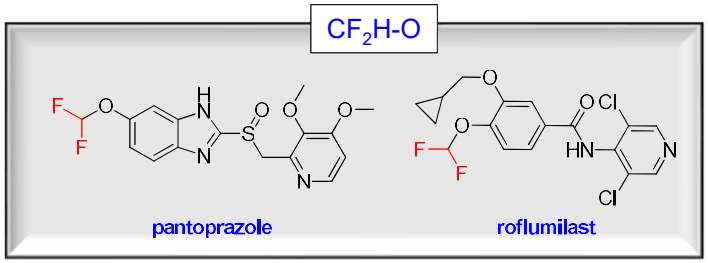
B. Hurley *et al., J. Med. Chem.,* 2021, **64**, 4744-4761; N.A. Meanwell *et al., J. Med. Chem.,* 2015, **58**, 4383-4438

F & Conformation – Aryl Ethers



- Anisole conformation with OCH_3 coplanar with ring favored by ~3 kcal/mol
- *Ortho* substituents distort OCH_3 from coplanarity
  - allylic 1,3-strain
- α-Fluorination of the CH_3 favors an orthogonal conformation
  - OCF_3 prefers 90° conformation by ~0.5 kcal/mol; O lone pair – CF-σ* hyperconjugation
    - reduced lone pair donation to π orbitals
  - $OCHF_2$ exhibits no strong preference for either conformation based on 22 examples in CSD
    - 2 examples examined closely adopted 90° conformation: 1 *endo-endo*, 1 *exo-endo*
- Matched pairs analysis of developability issues with Ar-OCH_3 vs Ar-OCF_3 & Ar-$OCHF_2$
  - 4 marketed drugs incorporate an Ar-OCF_3 substituent
  - 2 marketed drugs incorporate an Ar-$OCHF_2$ moiety
- H to F substitution
  - non-linear increase in Log *P*: 0.17, 0.27, 0.66 for a total = 1.1 units
- 439 MMPs & HLM stability
  - no difference between Ar-OCH_3 & Ar-OCF_3
- OCH_3 substitution improved permeability in 70% of the MMPs
  - Ar-OCH_3 permeability > Ar-$OCHF_2$ > Ar-OCF_3
- Concluded that Ar-$OCHF_2$ offers advantage over Ar-OCH_3 & Ar-OCF_3 during optimization
  - improves metabolic stability without compromising permeability





W.J. Hehre *et al.*, *JACS*, 1972, **94**, 1496-1504; K.A. Brameld *et al., J. Chem. Inf. Model.*, 2008, **48**, 1-24; D.B. Horne *et al., Tet. Letts.*, 2009, **50**, 5452-5455; L. Xing *et al., ChemMedChem,* 2015, **10**, 715-726; M.A Massa *et al., BMCL*, 2001, **11**, 1625-1628; E.J. Reinhard *et al., J. Med. Chem.*, 2003, **46**, 2152-2168; J. Liu *et al., J. F. Chem.*, 2022, **257-258**, 109978

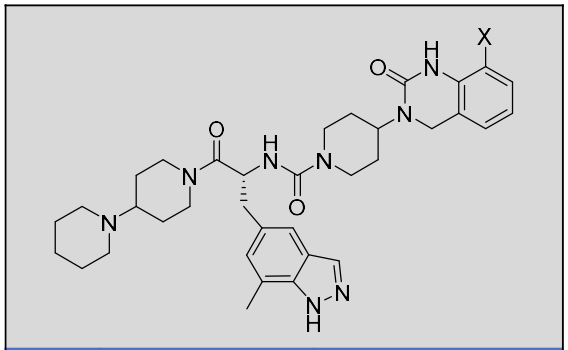
Fluorine for Hydrogen: Compound Developability

Effects on Solubility, Membrane Permeability,

CYP Inhibition, Metabolism & Pharmacokinetic Properties

The Effects of Fluorine: Solubility

Fluorine & Solubility

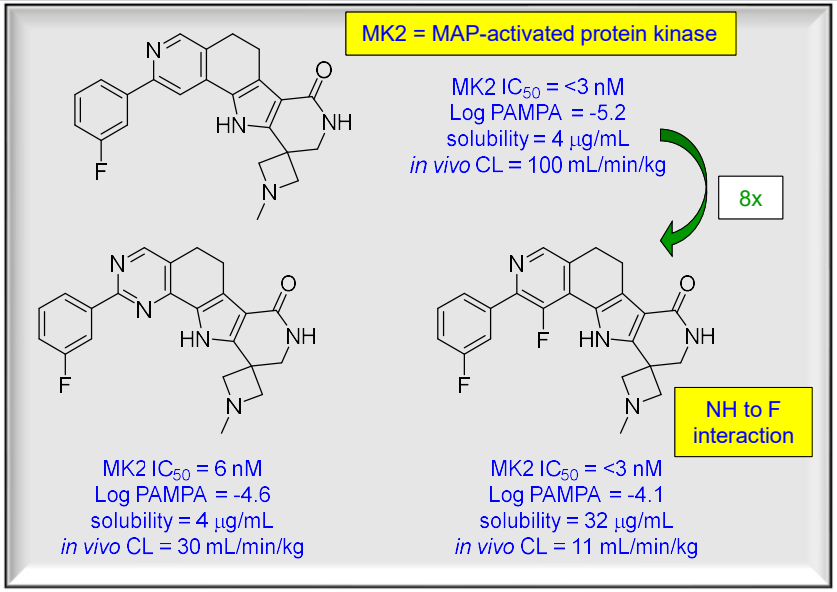


X	hCGRP K_i (nM)	Solubility (mg/mL)
H	0.010	15
F	0.013	>500

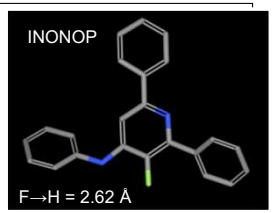
>33x

- CGRP receptor antagonists
  - potential for the treatment of migraine
- F *ortho*- to NH
  - leads to increased aqueous solubility
- F may polarize NH:
  - a more powerful H-bond donor
  - enhances solvation by H_2O?
  - effect on melting point?

MK2 = MAP-activated protein kinase

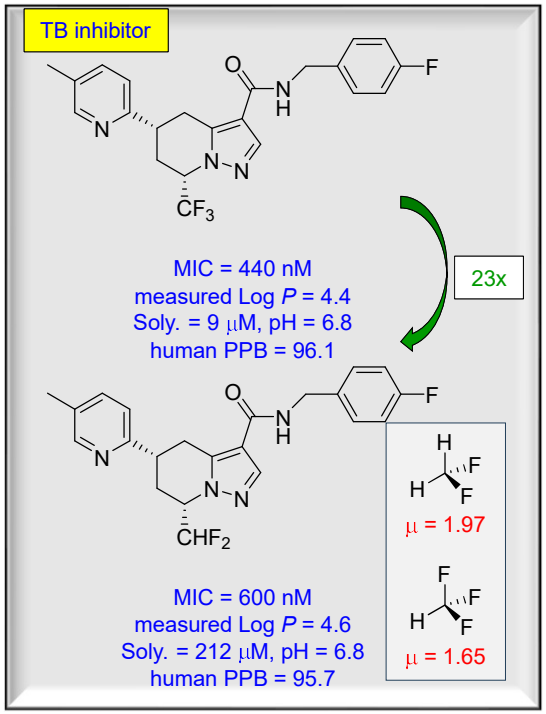


- Potent MK2 inhibitors
  - block TNFα production
- Pyridine active in cell-based assays
  - membrane permeability poor
- Pyrimidine: improved membrane permeability
  - intramolecular N-H to N
  - cell-based activity poor
- Fluoropyridine offered compromise
  - increased solubility: reduces planarity of biaryl
  - lower clearance



vdW radii:
F = 1.47 Å
H = 1.20 Å
Σ = 2.67 Å

TB inhibitor

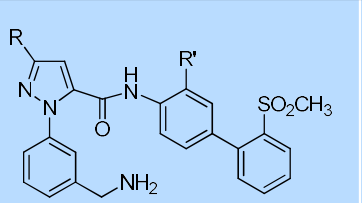


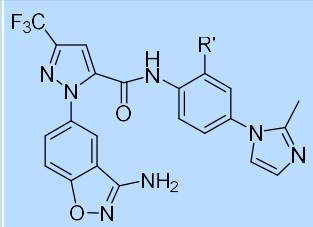
- TB inhibitors discovered by HTS
  - modestly potent *in vitro*
- CF_3 to CHF_2
  - increased solubility by 23-fold
  - increased Log *P* by 0.2
  - dipole moment of CF_2 > CF_3

A.P. Degnan *et al., J. Med. Chem.,* 2008, **51**, 4858-4861; J. Velcicky *et al., ACS Med. Chem. Lett.*, 2018, **9**, 392-396; F. Yokokawa *et al., ACS Med. Chem. Lett.*, 2013, **4**, 451-455
Z. Chen *et al., Org. Lett.*, 2010, **12**, 4376-4379

F for H: Membrane Permeability

Fluorine & Membrane Permeability





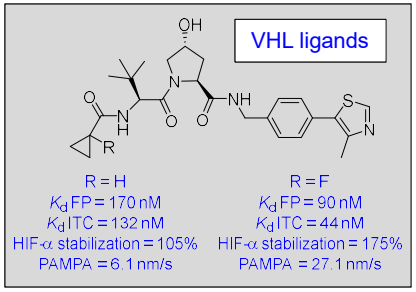
R	R'	Caco-2 perm.	R'	Caco-2 perm.
CH_3	H	1.20 x 10^{-6} cm/s	CN	<0.1 x 10^{-6} cm/s
CH_3	F	3.14 x 10^{-6} cm/s	H	0.82 x 10^{-6} cm/s
CF_3	H	3.38 x 10^{-6} cm/s	F	7.41 x 10^{-6} cm/s
CF_3	F	4.86 x 10^{-6} cm/s	Factor Xa Inhibitors	

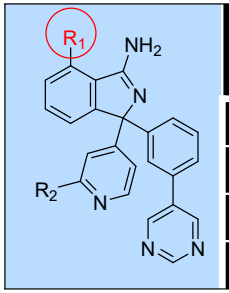
- Inhibitors of coagulation enzyme Factor Xa
- F *ortho*- to N-H increased Caco-2 permeability
  - effect demonstrated in 2 different series
- Electrostatic effect between F & H
  - shields H-bond donor
  - not mimicked by linear CN
- Prevalent motif in kinase inhibitors
  - extended to other motifs

VHL = von Hippel–Lindau

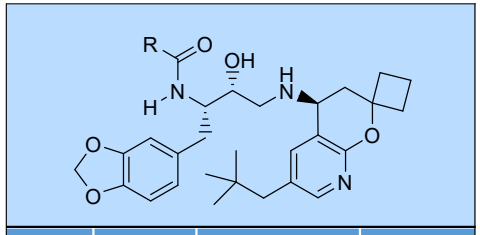
- Two series of BACE inhibitors
  - P-gp efflux an issue for CNS penetration
- β-OH ethylamine-based inhibitors
  - F to engage N-H across the C=O
  - comparable effect to MeO
- Amidine-based inhibitors of the Asp protease BACE
  - F *ortho* to amidine reduces pK_a by 1.3
  - also forms a weak interaction with N-H
- Solvation energy of F analogue is less –ve than for H
  - electronic & steric effects shield the polar N atom
  - less than 2 H-bond donors seen by environment
- Both permeability & efflux ratios are improved
  - BACE inhibitory potency retained
- Effect exploited in VHL E3 ligase ligands
  - F-cyclopropyl amide enhances PAMPA permeability

N-H to F interaction





R_1	R_2	IC_{50} (nM)	P_{app} (10^{-6} cm/s)	Efflux Ratio	pK_a
H	H	500	3.4	12	8.4
			4x	4x	
F	H	158	12	3.1	7.1
H	CF_3	134	0.13	>10	n.d.
			300x	16x	
F	CF_3	241	39	0.6	6.9

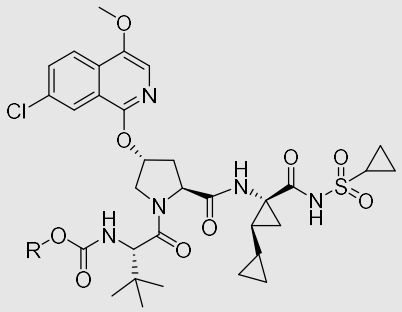


R	IC_{50} (nM)	P_{app} (10^{-6} cm/s)	Efflux Ratio
CH_3	8	11	19
	17	23	4
	29	16	2
	76	11	1

BACE Inhibitors

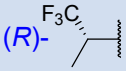
D.J.P. Pinto *et al., J. Med. Chem.,* 2001, **44**, 566-578; B.-M. Swahn *et al., J. Med. Chem.,* 2012, **55**, 9346–9361; M.W. Weiss *et al., J. Med. Chem.*, 2012, **55**, 9009-9024
A. Ciulli *et al., J. Med. Chem.,* 2018, **61**, 599-618

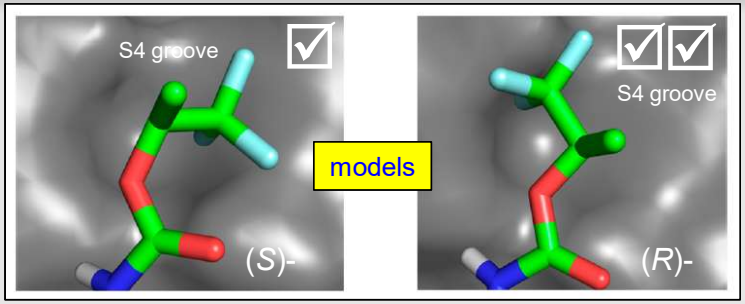
CF_3 Moiety at P4 in HCV NS3 Protease Inhibitors

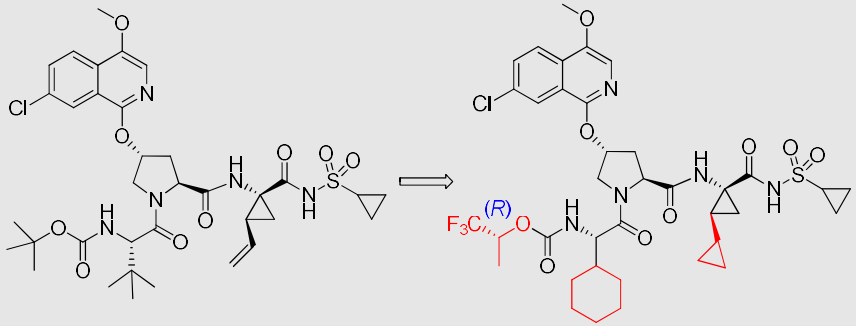


R	GT-1a IC_{50}	GT-1b EC_{50}	HLM $t_{1/2}$
	6 nM	18 nM	83 min
	10 nM	67 nM	35 min
	9 nM	1.9 nM	128 min
(*S*)- 	8 nM	146 nM	>200 min
(*R*)- 	1.3 nM	4.7 nM	127 min

Cardiac effects

R	Change HR	Change SNRT
(*R*)- 	-13%	16%
	-14.3%	50.4%





asunaprevir BMS-890068

Rat PK Profile Comparison (PO 15 MPK)

	ASV	'068
$t_{1/2}$ (hr)	4.2	8.6
F (%)	14	86
AUC (μM*h)	1.26	21.1
PO Liver @ 24h (μM)	15.2	51.0

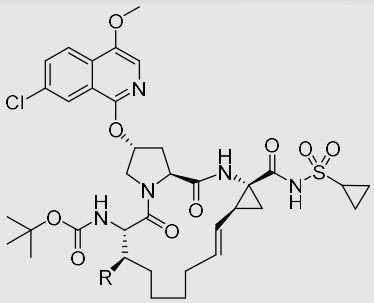
- Sought a back-up to asunaprevir: cyclopropyl to replace olefin
- CF_3-t-BuO potent but exerted significant cardiac effects in rabbit Langendorff model
  - CF_3-*iso*-propyl carbamate offered improved profile
  - (*R*)-isomer allows optimal van der Waals contact with S4 groove (model)

- Improved PK properties
  - enhanced metabolic stability with fluorinated P4
  - higher %F, higher AUC
  - reduced liver:plasma ratio

L.-Q. Sun *et al., J. Med. Chem.,* 2016, **59**, 8042-8060; A. Akbar *et al., J. Med. Chem.*, 2021, **64**, 11972−11989; J. Zephyr *et al., J. Mol. Biol.,* 2022, **434**, 167503

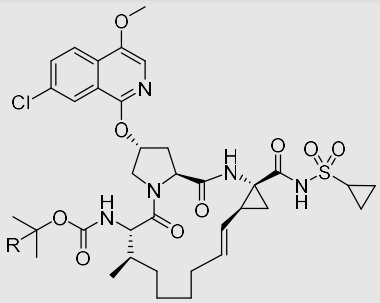
CF_3 Moiety at P4 in HCV NS3 Protease Inhibitors

Pan-genotype inhibitors



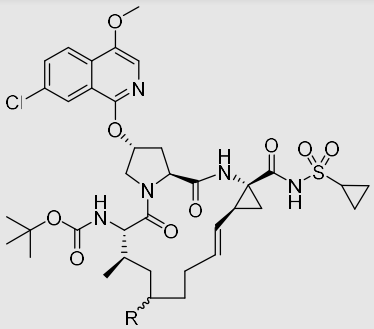
GT3a R = H: IC_{50} = 51 nM
R = CH_3: IC_{50} = 11 nM

Me on tether enhances potency



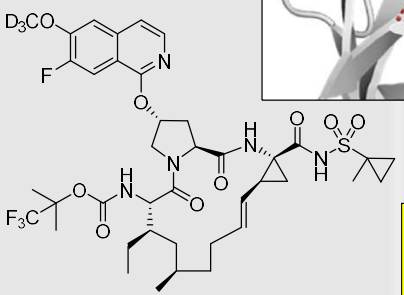
R = CH_3: IC_{50} = 11 nM
HLM $t_{1/2}$ = 11.5 min
R = CF_3: IC_{50} = 12.8 nM
HLM $t_{1/2}$ = 30.3 min

CF_3 enhances metabolic stability



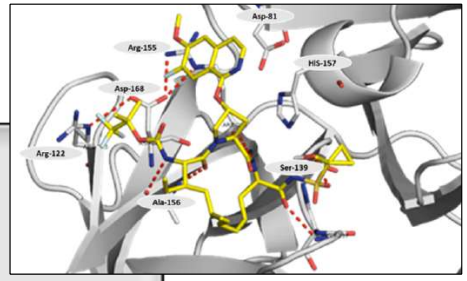
GT3a (*R*)-CH_3: IC_{50} = 4.4 nM
(*S*)-CH_3: IC_{50} = 50 nM

2nd tether substituent enhanced potency



BMS-986144
GT3a IC_{50} = 4.8 nM
HLM $t_{1/2}$ = 33 min

optimal P2* moiety



X-ray cocrystal data (22 compounds): Fluorinated P4 caps can sample alternate binding conformations that enable adapting to structural changes induced by mutations

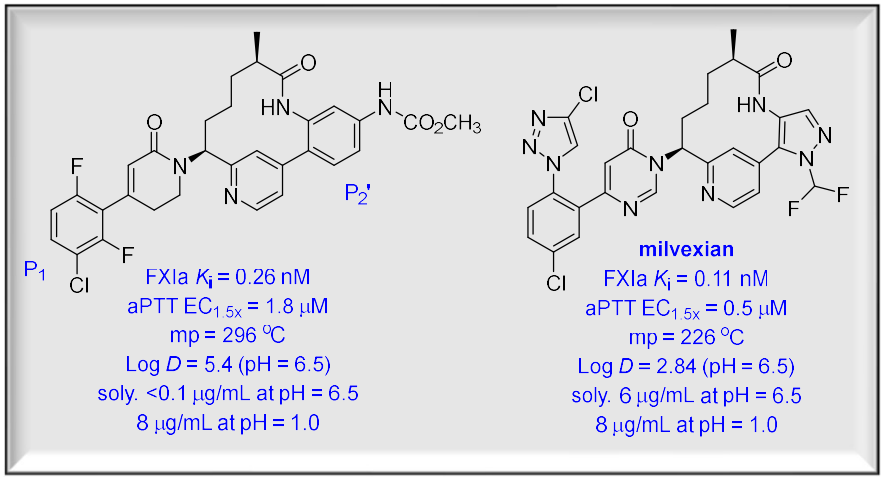
- Sought pan-genotype HCV NS3 inhibitors
  - retain activity toward viruses resistant to 2nd generation inhibitors
  - introducing substituents to the macrocycle tether a unique approach
- CH_3 at P3 enhanced potency toward sentinel GT3a virus by 5x
  - asunaprevir IC_{50} = 395 nM
- CF_3-substituted carbamate preserved antiviral activity
  - favorable interactions with D168, R123, & V158 in the S4 pocket
  - enhanced metabolic stability in HLM

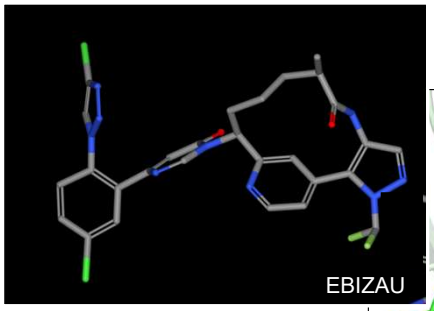
- 2nd CH_3 in tether further enhanced potency 3x
  - (*R*)-isomer 10x (*S*)-isomer
- Achiral CF_3-t-BuO did not present CV problems
  - macrocyclic background
- BMS-986144 the optimal compound
  - C7-F, C-6-CD_3 at P2* essential for balanced properties
  - CD_3 abrogated TDI of CYP 3A4
  - CH_3 at P1' enhanced plasma levels at C24

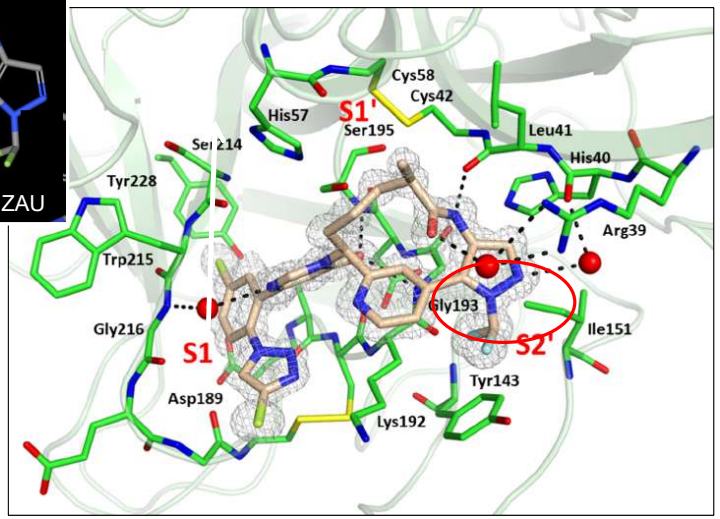
No cardiac effects in this background

L.-Q. Sun *et al., J. Med. Chem.,* 2020, **63**, 14740-14760; A. Akbar *et al., J. Med. Chem.*, 2021, **64**, 11972−11989; J. Zephyr *et al., J. Mol. Biol.,* 2022, **434**, 167503

Fluorination to Reduce Metabolism - Milvexian





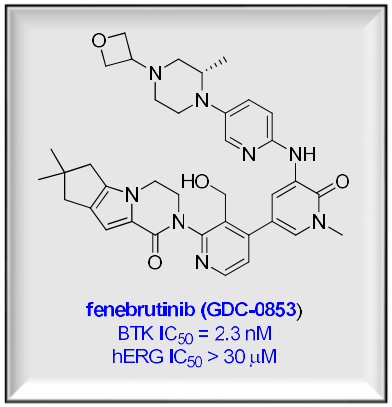


- Factor XIa inhibitors as antithrombotic agents
  - macrocycle a potent FXIa inhibitor
  - promising PK properties
- Poor aqueous solubility due to crystal packing
  - intermolecular H-bonding with carbamate NH & C=O
- Carbamate moiety engages FXIa *via* 2 H-bonds
  - H-bond donor to His_{40}; via H_2O to Ile_{151}
- Replaced phenyl ring with azole heterocycles
  - *N*-Me pyrazole emerged as optimal from survey of isomers
  - further optimization of P_1 required to restore potency

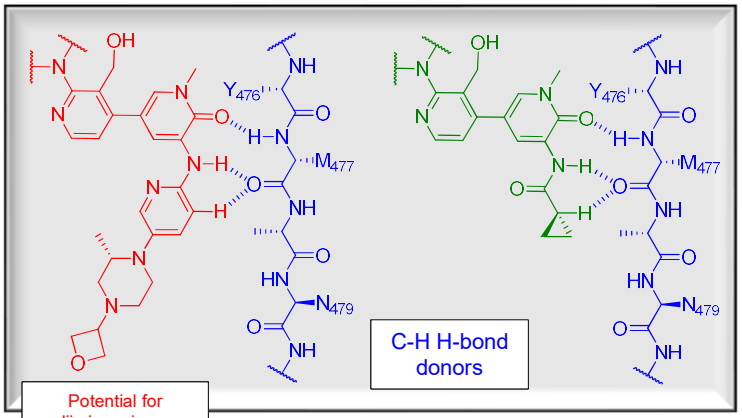
- Pyrazole *N*-Me modified to CHF_2
  - enhanced metabolic stability in HLM
    - demethylation one of the pathways
  - $t_{1/2}$ 100 min vs 68 min for Me in prototype
- Milvexian offered best overall profile
  - doses of 25-200 mg BID & QD in clinical trials
  - efficacy observed at doses of >100 mg

W. Yang *et al., J. Med. Chem.*, 2020, **63**, 7226-7242; A.K. Dilger, J.R. Corte, W.R. Ewing *et al., J. Med. Chem.*, 2022, **65**, 1770-1785; J.I. Weitz *et al., N. Engl. J. Med.*, 2021, **385**, 2161-2172

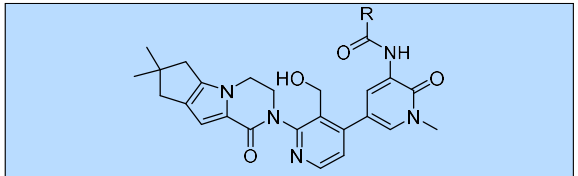
Fluorinated Cyclopropyl Carboxamides – BTK



fenebrutinib (GDC-0853)
BTK IC_{50} = 2.3 nM
hERG IC_{50} > 30 μM



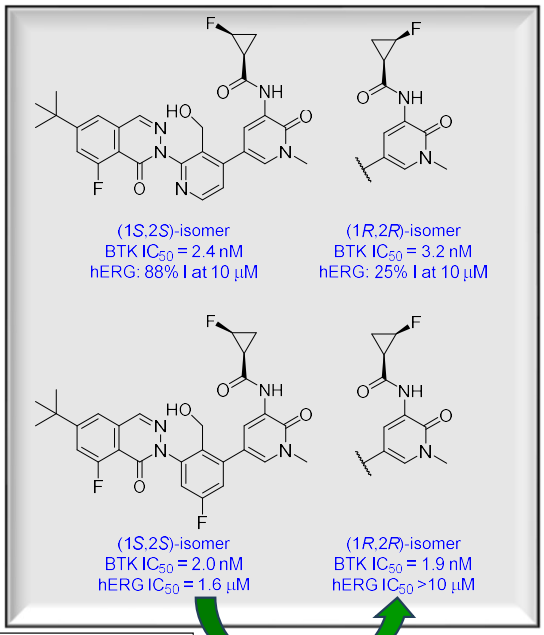
Cyclopropyl amide ≡ 2-aminopyridine



R	BTK IC_{50} (nM)	Log $D_{7.4}$	Soly. (μM)
	7.1	3.0	9
	200	2.6	85
	2.3	2.5	93
	3.8	2.3	95

2x

10x

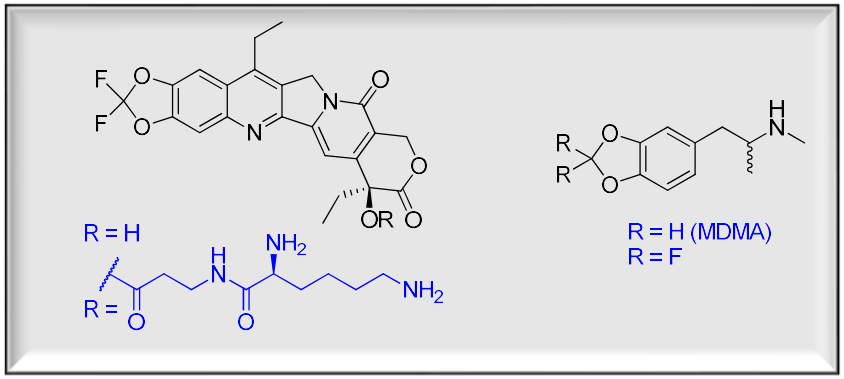


- Molecular editing of the hinge binding & H2 pocket elements
  - reduce size of molecule
  - remove 1,4-diamino pyridine: bioactivation concerns
- Replaced pyridine C-H donor with smaller cyclopropylamide moiety
  - uniquely potent among series of homologues
  - fluorination enhances potency 2x & solubility 10x
- Introduced hERG inhibition
  - dependent on the absolute configuration: 6x Δ
  - unusual observation

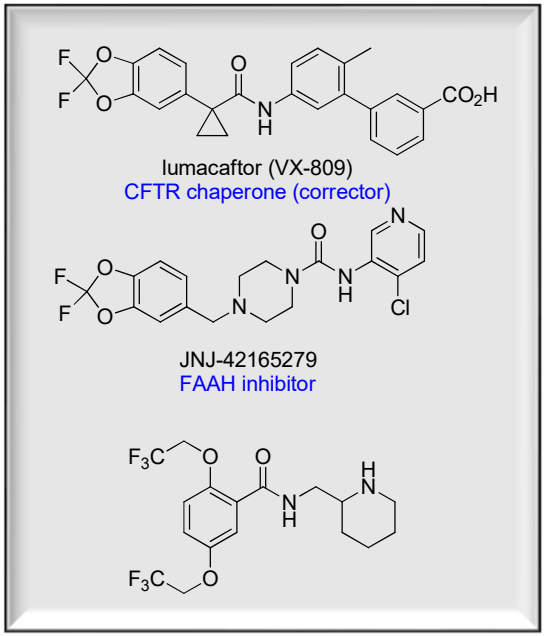
(*R*,*R*)-isomer shows reduced hERG inhibition

J.J. Crawford *et al.*, *ACS Med. Chem. Lett.*, 2020, **11**, 1588-1597

F for H to Modulate Metabolism



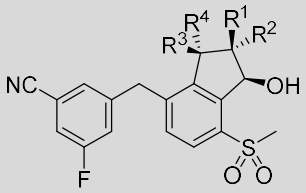
- Methylenedioxy moiety problematic
  - metabolism-based CYP 450 inhibition
- Block by fluorination
  - diF-methylenedioxy moiety not widely exploited in drug design
- F's introduced into camptothecin analogue to increase metabolic stability
  - resulted in improved oral exposure
- MDMA (ecstasy) is metabolized to a catechol derivative
  - diF analogue prepared to use as a probe
  - examine role of metabolites in the psychopharmacology & toxicity:
    - no data published
- Lumacaftor (VX-809) approved for the treatment of cystic fibrosis
  - improves processing of the ΔF508 mutant: corrector
- JNJ-42165279 clinically evaluated
  - mechanism-based FAAH inhibitor
- Applicable to other ethers



FAAH = fatty acid amide hydrolase

W.C. Rose *et al., Cancer Chemother. Pharmacol.*, 2006, **58**, 73-85; J.M. Keith *et al., ACS Med. Chem. Lett.*, 2015, **6**, 1204-1208
D. Traschel *et al., Chem. Biodivers.*, 2006, **3**, 326-336; F. Van Goor *et al., Proc. Natl. Acad. Sci. USA*, 2011, **108**, 18843-18848

Fluorination Patterning in an Inhibitor of HIF-2α



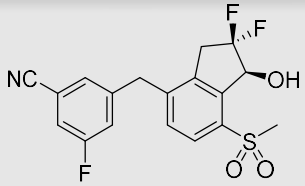
V_{max} (pM/min/mg)

R^1	R^2	R^3	R^4	SPA IC_{50} nM)	EC_{50} (nM)	UGT2B17
F	F	H	H	12	27	280
H	H	H	H	160	930	3.8
F	H	H	H	41	39	14
H	F	H	H	350	490	50
H	H	F	H	210	460	1.4
H	H	H	F	>1,000	NT	NT
F	H	F	H	9	11	4.7
F	H	H	F	1,900	1,900	NT
H	H	F	F	6,500	NT	NT
F	F	F	H	9	4	440

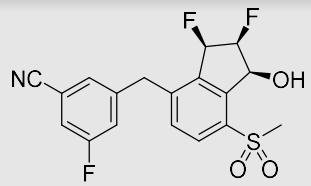
PT-2385

PT-2977

PT-2385 cocrystal



PT-2385
Log $D_{7.4}$ = 2.2
VEGFA EC_{50} = 46 nM
82% protein bound (human)
UGT2B17 = 280 pM/min/mg



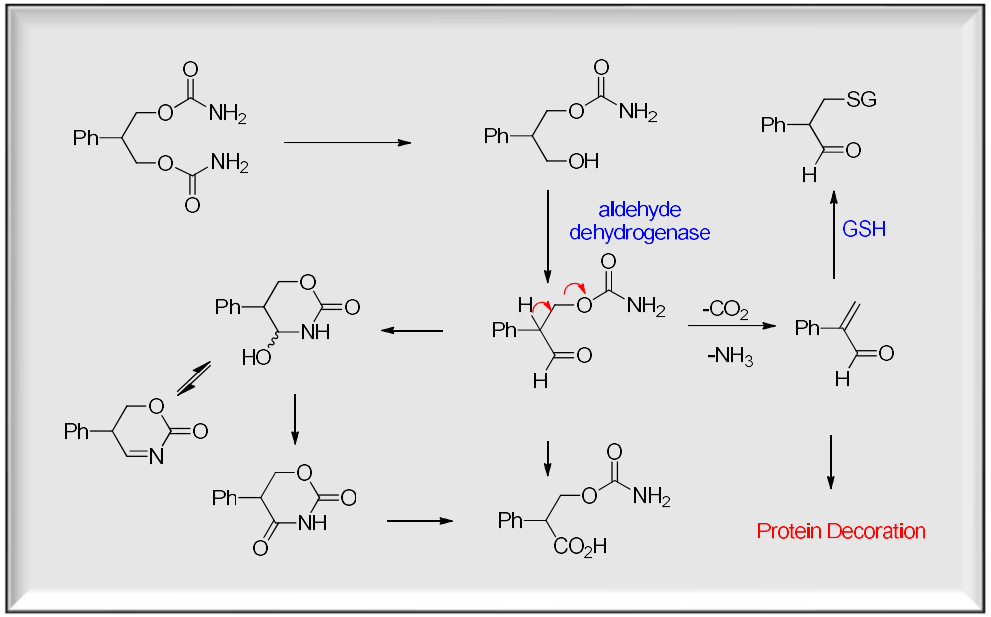
PT-2977
Log $D_{7.4}$ = 1.2
VEGFA EC_{50} = 17 nM
52% protein bound (human)
UGT2B17 = 4.7 pM/min/mg

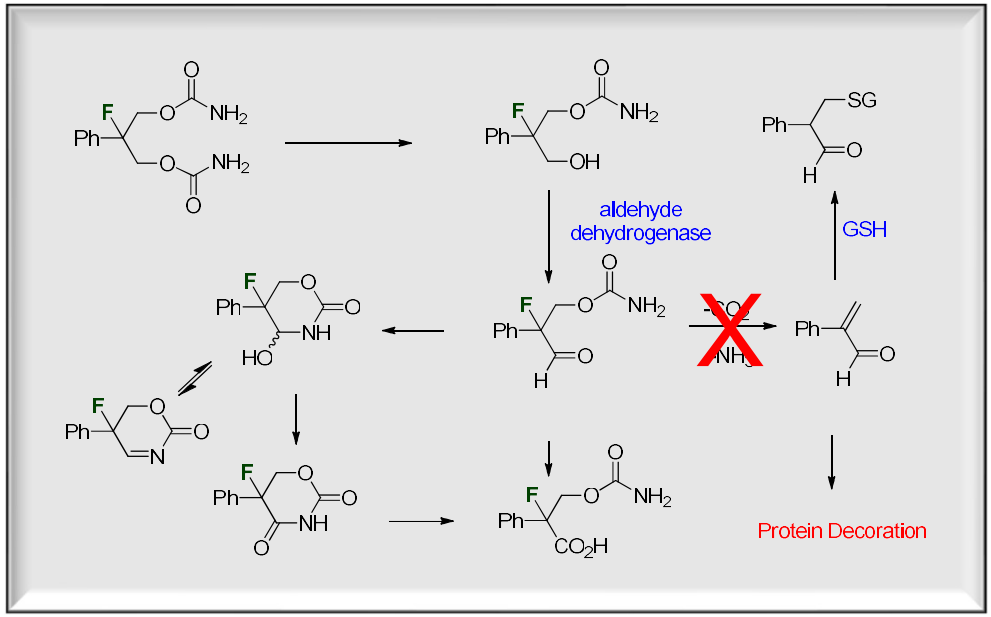
- Systematic study of fluorination
  - potency sensitive to disposition
  - β-F introduces steric clash with His_{248}
- Interesting Tyr O_{lp} to Ar π* interaction
  - O lone pair to sulfone-substituted aryl ring
- Glucuronidation sensitive to fluorination pattern
  - PT-2977 emerged as optimized clinical candidate
  - reduced glucuronidation compared to PT-2385
- Protein binding reduced significantly
  - PB_{adj} EC_{50} = 13 vs 95 ng/mL
- Log $D_{7.4}$ 1 unit lower
  - vicinal di-F associated with higher dipole
- Topology advocated by Klaus Müller & colleagues (Roche)
  - enhanced solubility over CF_2 in test mule study
- Clinically effective exposure at 13-fold lower dose
  - parent/metabolite ratio 18-fold higher than PT-2385

R. Xu *et al., J. Med. Chem.*, 2019, **62**, 6876-6893; C. Xie *et al., Drug Metab. Dispos.*, 2018, **46**, 336-345; K. Müller *et al., J. Med. Chem.,* 2015, **58**, 9041-9060
P.M. Wehen *et al., Med. Chem. Res.* 2023, **32**, 1510- 531

F & Drug Metabolism - Felbamate

Fluorine & Metabolism





- Clinical utility of felbamate limited by aplastic anemia & hepatotoxicity
- Atropaldehyde is potently electrophilic & toxic to fibroblasts
  - thiol adducts found in rat & human urine
- Strategic deployment of F based on detailed understanding of metabolic pathway
  - F atom of fluorofelbamate prevents elimination of carbamate moiety
  - atropaldehyde not formed

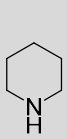
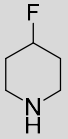
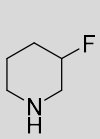
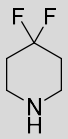
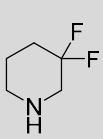
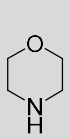
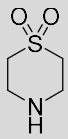
C.M. Dieckhaus *et al., Chem. Biol. Interact.*, 2002, **142**, 99-117; 2002, **142**, 119-1324; R.J. Parker *et al., Chem. Res. Toxicol.*, 2005, **18**, 1842-1848

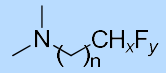
Applications of Fluorine in Drug Design

Modulating Amine Basicity

F & Amine Basicity – Additive Effects

Fluorine & Amine Basicity

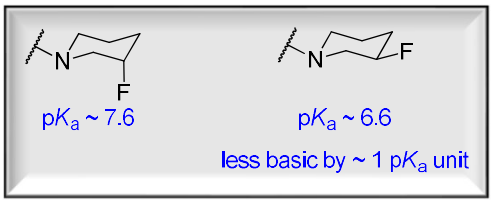
							
σ bond path		γ-F	β-F	γ-F	β-F		
1		-0.7	-1.7	-1.4	-3.4		
σ bond path		γ-F	δ-F	γ-F	δ-F		
2		-0.7	-0.3	-1.4	-0.6		
predicted ΔpK_a		-1.4	-2.0	-2.8	-4.0		
observed pK_a	11.1	9.4	9.3	8.5	7.4	8.5	5.4
observed ΔpK_a		-1.7	-1.8	-2.6	-3.7		



n	Position	ΔpK_a
1	β-F	-1.7
2	γ-F	-0.7
3	δ-F	-0.3
4	ε-F	-0.1

4,4,-diF piperidine ≡ morpholine

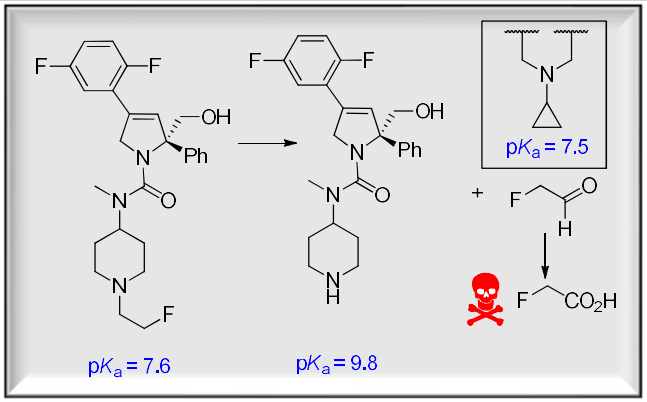
- Effects of F on pK_a of aliphatic amines determined experimentally
  - pK_a varies based on relative position of F, # of F atoms
- In ring systems, F affects pK_a *via* both bond paths
  - add effects from each bond path to calculate ΔpK_a
- Allows reasonable approximation of change in basicity
  - based on F & N relationship
- However, equatorial/axial disposition on cyclohexane ring affects ΔpK_a
  - equatorial F has greater effect than axial F - ~ 1 pK_a unit



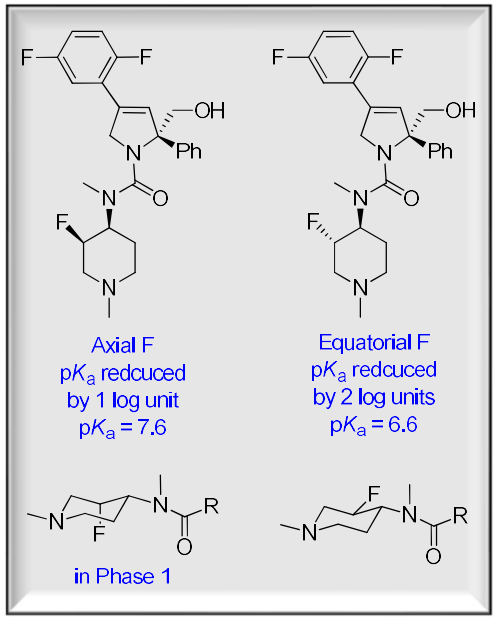
F. Diederich, K. Müller *et al., ChemMedChem.,* 2007, **2**, 1100-1115; K. Müller *et al., ChemMedChem*, 2007, **2**, 285-287

F to Reduce Basicity in KSP Inhibitors – P-gp

Fluorine & Amine Basicity



- Kinesin spindle protein (KSP) inhibitor
  - family of motor proteins
  - novel mechanism for the treatment of
  - taxane-refractory cancer – solid tumors
- CH_2OH moiety reduced hERG binding
- N basicity modulated to reduce PgP efflux
  - pK_a of 6.5-8.0 optimal in tumor cell line
  - pK_a of parent piperidine = 9.8
- N-cyclopropyl moiety reduced basicity
  - pK_a = 7.5
  - but gave time-dependent CYP inhibition
- N-CH_2-CH_2-F dealkylated in rat liver microsomes
  - major metabolite: toxic
- Solution: incorporate F in the piperidine ring
  - effect on pK_a varied
  - axial F more basic than equatorial F



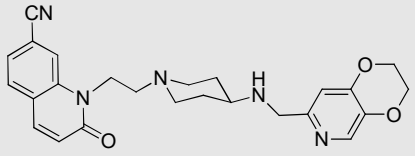
- Modulate amine pK_a
  - basicity attenuated by fluorination
  - pK_a of 7 struck the optimal compromise
- Amine pK_a adjusted to 7 by 2 β-F atoms
  - CHF_2 can be installed at the terminus
    - or branched from the chain

	R			
	NH_2	CHF_2	CF_3	NH_2
KSP IC_{50} (nM)	2.2	12.1	110	5.2
Cell EC_{50} (nM)	6			22.5
hERG IC_{50} (μM)	7.1			15.9
MDR ratio	1200	3	1	5
pK_a	10.3	7.0	5.2	7.0
Log *P*	1.1			3.2
P-gp efflux ratio	19			2.5

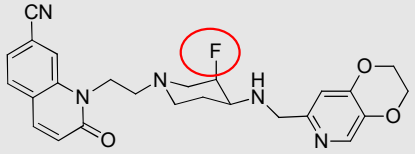
C.D. Cox *et al., J. Med. Chem.*, 2008, **51**, 4239-4252; *Bioorg. Med. Chem. Lett.*, 2007, **17**, 2697-2702

F to Reduce Basicity & hERG

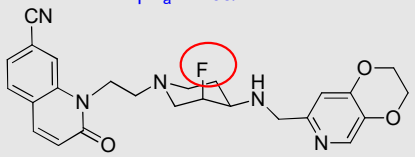
Fluorine & Amine Basicity



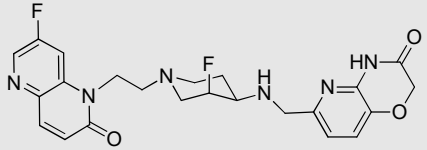
MIC *Staph. aureus* = 0.03 μg/mL
E. coli topo IV IC_{50} = 3.2 nM
Mtb MIC = 2 μg/mL
Mtb Gyr.SC IC_{50} = 1 μM
hERG IC_{50} = 44 μM
pK_a = 8.27/5.75



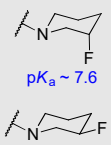
MIC *Staph. aureus* = 0.06 μg/mL
E. coli topo IV IC_{50} = 48 nM
hERG IC_{50} = 233 μM
pK_a = 7.03/4.47



MIC *Staph. aureus* = 0.13 μg/mL
E. coli topo IV IC_{50} = 60 nM
hERG IC_{50} = 199 μM
pK_a = ND



Mtb MIC = 0.13 μg/mL
Mtb Gyr.SC IC_{50} = 0.35 μM
hERG IC_{50} = >100 μM
pK_a = ND



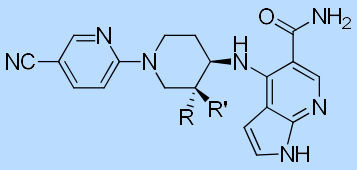
pK_a ~ 7.6

pK_a ~ 6.6

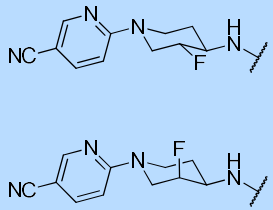
less basic by ~ 1 pK_a unit

- F introduced to modulate basicity of more basic 2° amine
  - attenuated basicity at both *N* atoms
  - hERG inhibition mitigated in both enantiomers
- 3-F-piperidine subsequently exploited in Mtb inhibitor

- JAK3 inhibitors with hERG problem
  - F attenuated basicity at piperidine N atom
  - hERG inhibition mitigated in both enantiomers



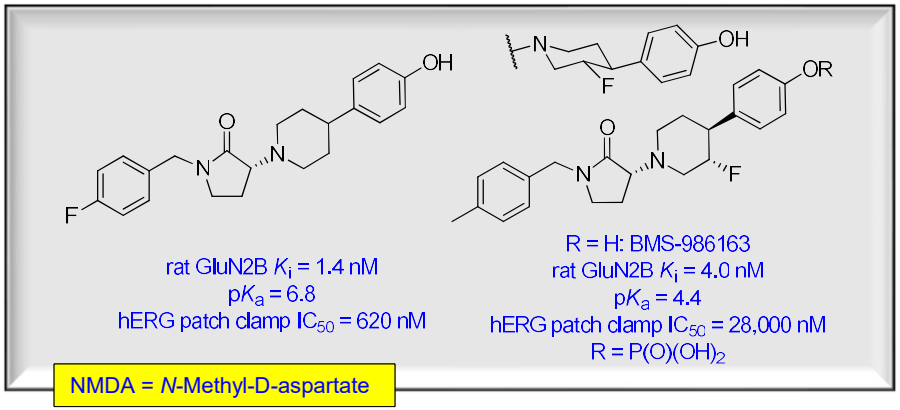
R	R'	JAK3 IC_{50} (nM)	hERG Binding (μM)	cLog *P*
H	H	1.3	13.4	2.8
F	H	1.7	>100	2.7
H	F	0.3	>100	2.7

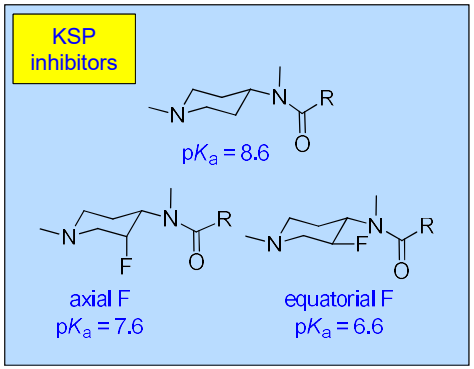


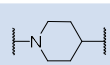
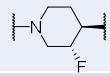
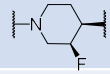
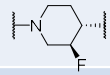
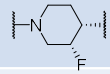
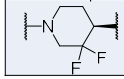
more potent

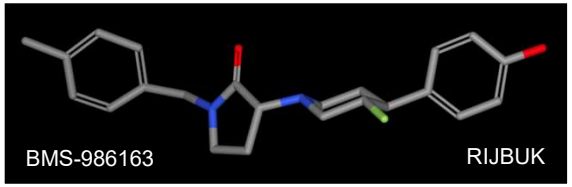
F. Reck *et al., J. Med. Chem.*, 2011, **54**, 7834-7847; 2012, **55**, 6916-6933; P.S. Hameed *et al., J. Med. Chem.*, 2104, **57**, 4889-4905
Y. Nakajima *et al., Bioorg. Med. Chem.,* 2015, **23**, 4871-4883

Fluorination to Reduce hERG in mGluN2B NAMs



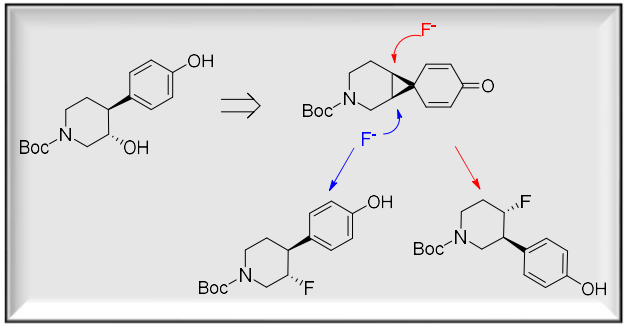


linker		rat GluN2B K_i (nM)	hERG PC IC_{50} (nM)
		1.4	400
	eq	4.0	28,000
	ax	8.4	ND
	eq	4.3	>30,000
	ax	4.4	7,800
		7.7	>30,000



- NMDA GluN2B negative allosteric modulators (NAMs)
  - explored for potential utility as a treatment for depression
- Pharmacophore is 2 non-polar aromatic rings 9-11 Å apart
  - connected by a linker containing a basic N atom
  - overlaps with the hERG inhibitor pharmacophore
- Explored steric effects & electronic modulation of piperidine ring
  - basicity reduction by judicious deployment of F successful
  - measured pK_a fell from 6.8 to 4.4 (equatorial F, larger effect)
  - hERG inhibition mitigated by 45x
- PSA = 44 Å^2; Log *D* = 2.8
  - consistent with good brain penetration: B:P ratio = 2.8
- BMS-986163 selected for clinical development
  - solubility 2 µg/mL; limited IV dose escalation for tox. studies
  - phosphate prodrug: solubility 19.9 mg/mL

- Deoxyfluorination
  - retention of configuration
  - anchimeric assistance by the phenol
- Deoxo-Fluor gave 30:1 isomer ratio
  - 75% isolated yield

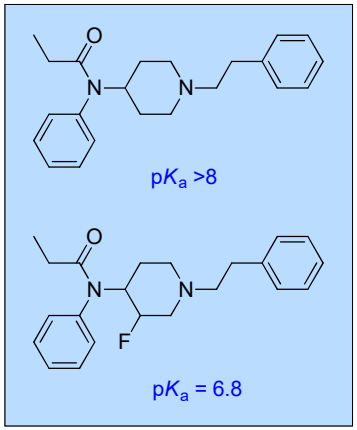


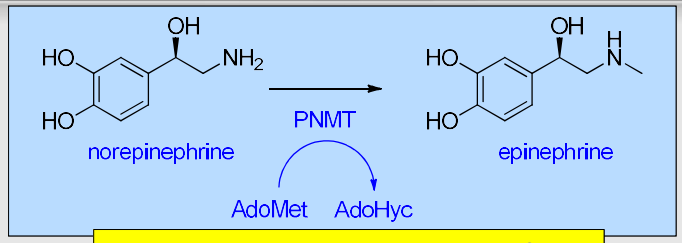
L.R. Marcin *et al., ACS Med. Chem. Lett.*, 2018, **9**, 472-476; L.J. Bristow *et al., J. Pharmacol. Exp. Ther.,* 2017, **363**, 377-393; J. Kempson *et al., Org. Proc. Res. Dev.*, 2018, **22**, 846-855

F to Modulate Basicity & Selectivity

Fluorine & Amine Basicity

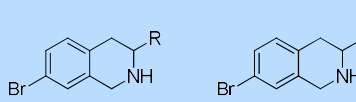
- Fentanyl is a potent μ opioid receptor agonist
  - used to treat pain
  - has central & peripheral activity
  - binds as the salt – engages Asp_{147}
- μ opioid receptors in peripheral sensory neurons
  - drive painful syndromes
  - local tissues are inflamed & acidic in nature
- Explored effect of N-basicity on specificity profile
  - F derivative reduces pK_a by > 1 unit
  - lower propensity to protonated at physiological pH
- Fentanyl binding is not pH-dependent
  - K_i = 1.1 nM at pH = 7
- Binding of the F derivative is pH dependent
  - K_i = 17.9 nM at pH = 7.4 vs 3.7 nM at pH = 6.5
  - binding should be restricted to acidic tissues
- F derivative active in 2 pain models
  - effects reversed by a peripherally restricted μ receptor antagonist
  - fentanyl has peripheral & central effects
- F derivative not associated with respiratory depression, sedation, constipation of fentanyl
  - reduced potential for addiction based on conditioned place preference evaluation
- Nice example of subtle effects on selectivity
  - potential as a non-addictive pain killer with use dependence





PNMT: phenylethanolamine *N*-methyltransferase

- Inhibitors of PNMT
  - the final step in the biosynthesis of epinephrine
- Tetrahydroisoquinolines: potent but non-selective PNMT inhibitors
  - also bind to the α_2 adrenoreceptor
- Explored effect of N-basicity on biological profile
  - CHF_2 moiety gave optimal balance of potency & selectivity
  - "Goldilocks effect"

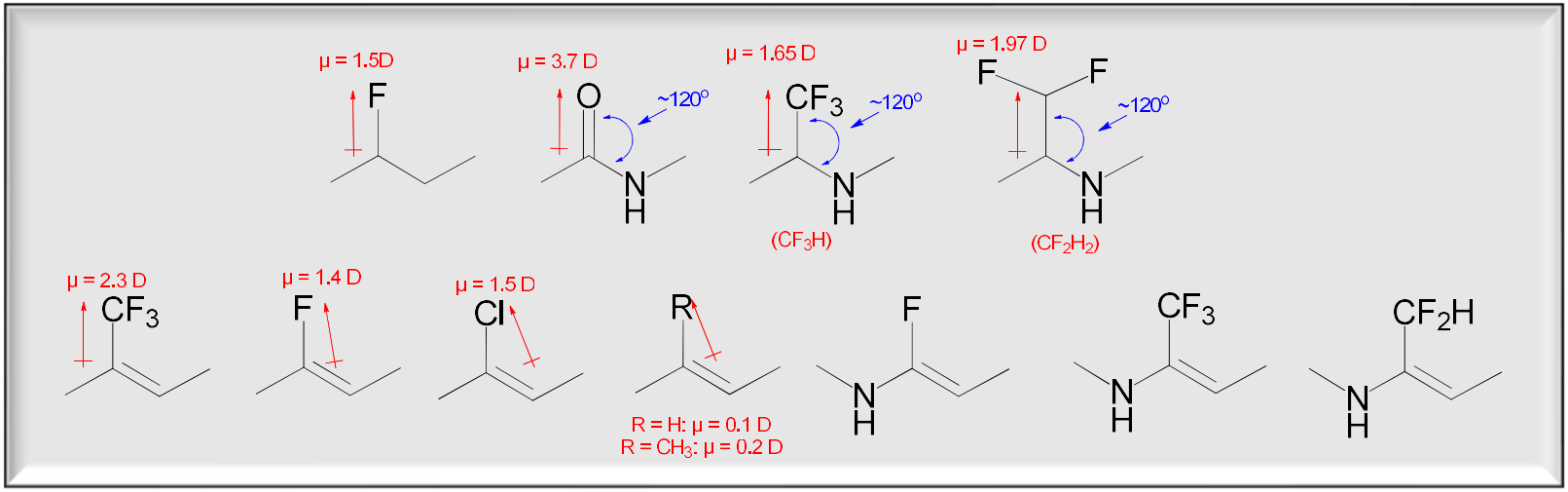


optimal compound

R	pK_a	K_i PNMT(μM)	K_i α_2 (μM)	Selectivity
CH_3	9.29	0.017	1.1	65
CH_2F	7.77	0.023	6.4	280
CHF_2	6.12	0.094	230	2400
CF_3	4.33	3.2	>1000	>310

C. Stein *et al., Science,* 2017, **355**, 966-969; G. Grunewald *et al., J. Med. Chem.,* 2006, **49**, 2939-2952

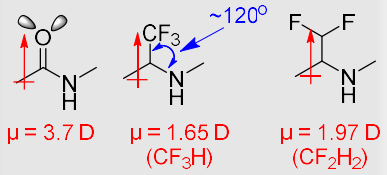
Fluorine & the Design of Amide Bioisosteres



Fluorine & Higher Order Bioisosterism

Fluorine & Amide Mimesis

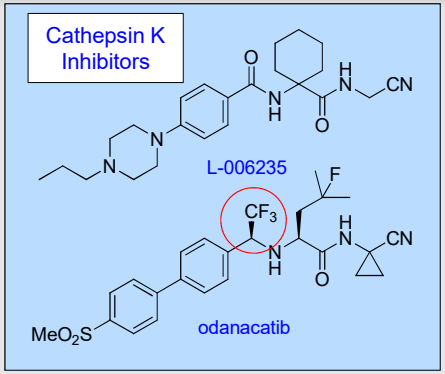
CF_3-C-NH ≡ CO.NH



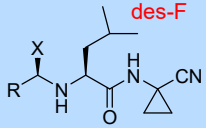
Amine	pK_a
$CH_3CH_2NH_2$	10.70
$F_2CHCH_2NH_2$	7.52
$CF_3CH_2NH_2$	5.70

- CF_3-C-NH developed as amide mimic
  - isosterism established in peptide mimetics
- F β- to amine lowers pK_a progressively with each F
  - CF_3 pK_a = 5.70; CHF_2 = 7.52
  - retains NH H-bond donor
- Offers conformational flexibility
  - potential for a better fit with target
  - but introduces a stereogenic center
- CH(CF_3)NHCH bond is close to 120°
- C-CF_3 bond is isopolar with C=O
  - similar dipole vectors
  - CF_3H dipole is 1.65; CF_2H_2 dipole is 1.97
- CF_3CF_2 & CHF_2 perform in similar fashion
  - electronegative fluorine as mimic of lone pair?

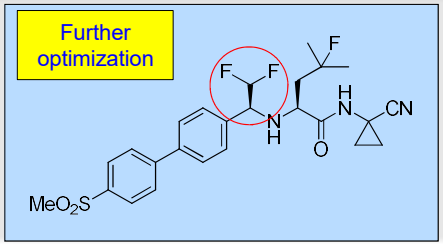
- Cat K inhibitors - lysosomal enzyme
  - treatment of osteoporosis
  - C≡N reacts reversibly with catalytic Cys
- L-006235: good PK, poor selectivity
  - Cat K vs B, L, & S due to lysosomotropic nature
  - strongly basic & lipophilic
    - accumulates in acidic lysozomes
- Odanacatib solved PK & selectivity problems
  - CF_3-C-NH metabolically stable
  - F on Ile blocked hydroxylation
  - cPr reduced amide hydrolysis
- Completed Phase 3 trials
  - NDA not filed due to side effect profile



nM	odanacatib	CHF_2
Cat K	0.2	1
Cat B	1034	2297
Cat L	2995	6326
Cat S	60	210



des-F	odanacatib	CHF_2
Log D_7	3.53	0.11
pK_a	<2.0	2.7



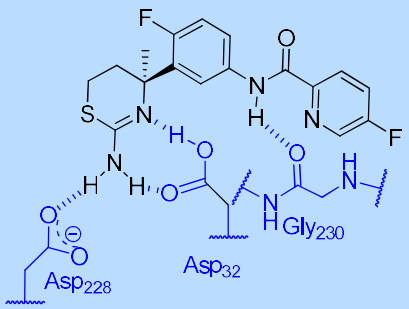
- Highly crystalline, low aqueous solubility
  - ≤10% F from suspension in pre-clinical species
  - dissolution-limited bioavailability
- CHF_2 - Cat K potency & selectivity retained
  - Log *D* decreased by 3 log_{10} units: increased *N* basicity
  - facilitated salt formation with RSO_3H, HCl
  - improved F from 1% methocel suspension

M. Zanda, *J. Fluorine Chem.*, 2012, **143**, 57-93; W.C. Black *et al., Bioorg. Med. Chem. Lett.,* 2005, **15**, 4741-4744; 2008, **18**, 923-928
E. Isabel *et al., Bioorg. Med. Chem. Lett.,* 2011, **21**, 920-923

CF_3-CR_2-N as an Amide Bioisostere: BACE-1 & FXIa

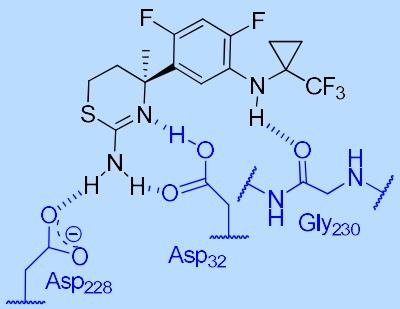
CF_3-C-NH ≡ CO.NH

Model based on close analogue



potency: low nM

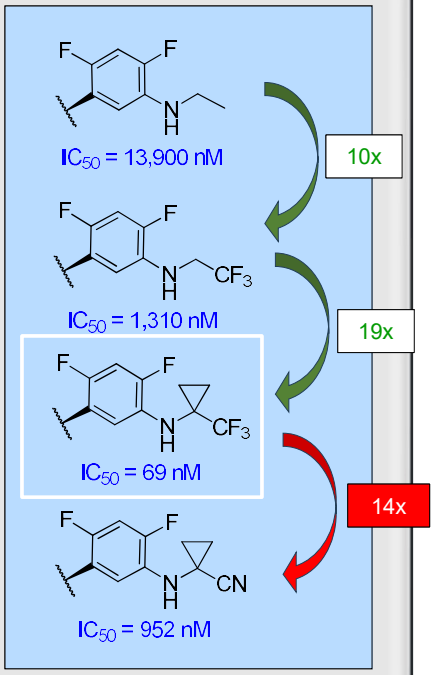
X-ray cocrystal



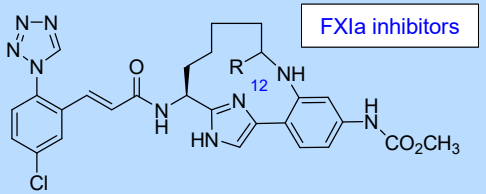
IC_{50} = 69 nM

BACE-1 inhibitors

- BACE-1 inhibitors
  - esterase/amidase release of aniline a concern
- N-H a critical pharmacophore element
  - replacements would need to preserve H-bond donating properties
- Relaxed conformational constraint compared to amide
  - recognized potential improved potency with altered fit
- Basic amines poor in biochemical assay but potent in cell-based assay
  - accumulation in acidic lysozomes where BACE-1 resides
- CF_3-cyclopropane provided best balance of properties
  - bound slightly differently in the active site but retained key interactions
- CN, absence of cyclopropyl & simple ethylamine all poorly active

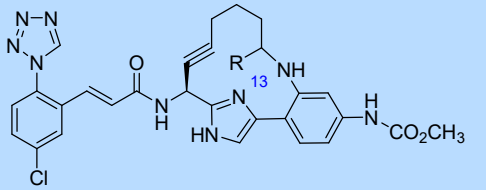


FXIa inhibitors



R = =O: K_i = 1.0 nM
R = (*R*)-CF_3: K_i = 1.1 nM
R = (*S*)-CF_3: K_i = 370 nM

350x



R = =O: K_i = 0.16 nM
R = (*R*)-CF_3: K_i = 53 nM

330x

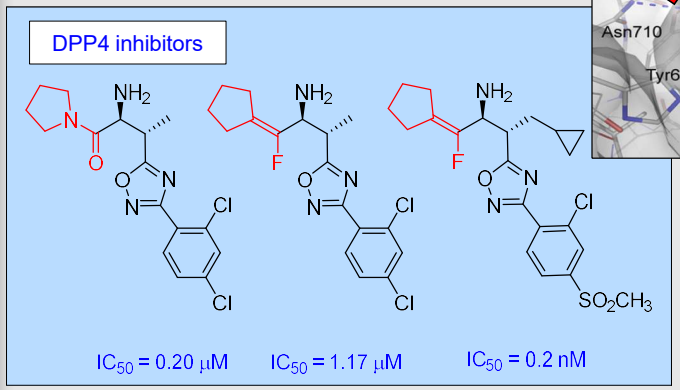
- Factor XIa inhibitors:
  - high PSA:169 Å^2
- Sought bioisosteres with reduced PSA
- CF_3-amine: lower PSA of 152 Å^2
  - (*R*)- isomer 350x (*S*)-isomer
- Less effective edit in 13-ring homologue

C.R Butler *et al., J. Med. Chem.*, 2017, **60**, 386-402; T. Fang *et al., Bioorg. Med. Chem. Lett.*, 2020, **30**, 126949

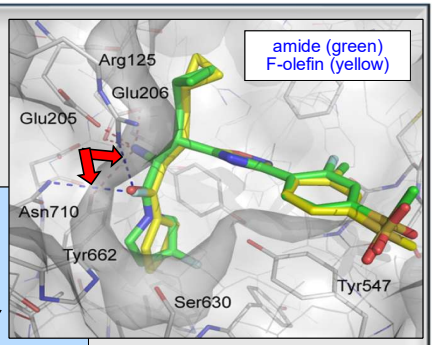
Vinyl-F & Aryl C-F as a C=O Mimic: DPP4 Inhibitors

C-F ≡ C=O

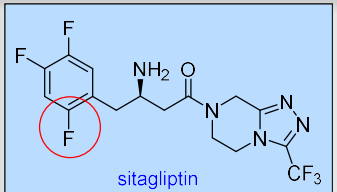
DPP4 inhibitors



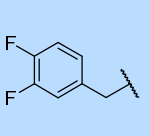
IC_{50} = 0.20 μM IC_{50} = 1.17 μM IC_{50} = 0.2 nM



- Vinyl fluoride reproduces amide topology
  - resistant to cleavage by amidases or protease
- **DPP-4** prototypes similarly potent
  - SO_2CH_3 substituent drives potency
- X-ray of cocrystal revealed similar binding modes
  - F proximal to side chain NHs of Arg_{125} & Asn_{710}
  - described as H-bonding

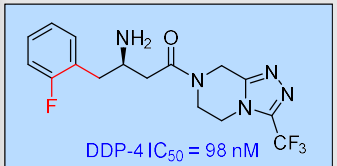


sitagliptin
DPP-4 IC_{50} = 18 nM



DPP-4
IC_{50} = 128 nM

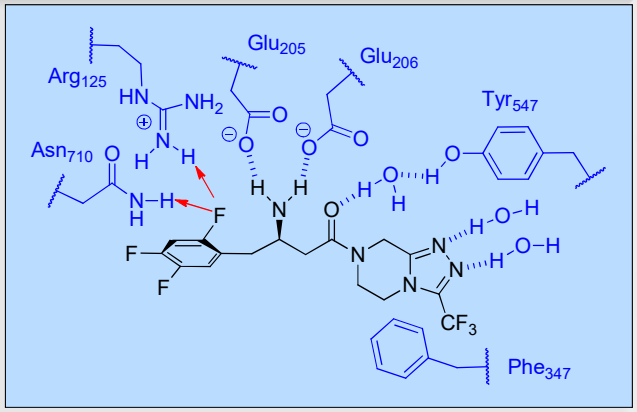
DPP-4
IC_{50} = 27 nM



DDP-4 IC_{50} = 98 nM

DDP-4 IC_{50} = 120 nM

matched molecular pair

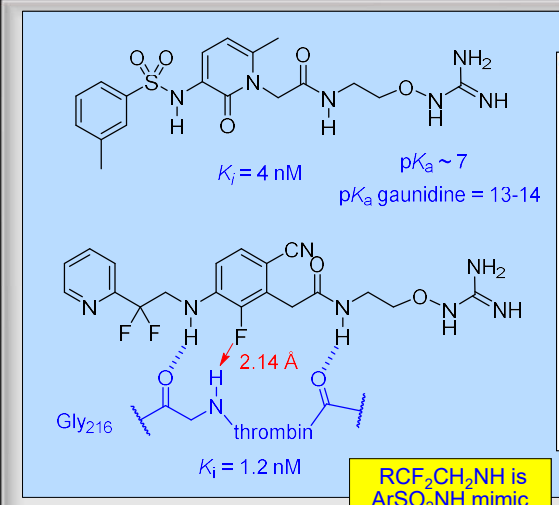


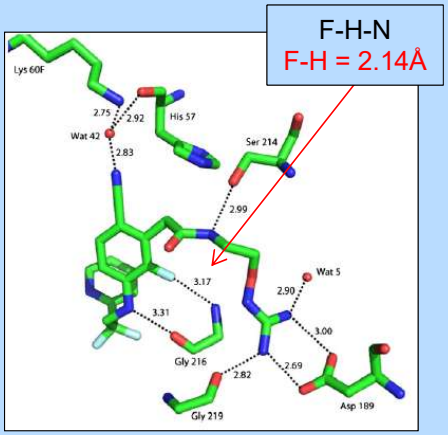
- Fluoroaryl ring of sitagliptin important
  - occupies S_1 subsite of DDP-4
    - enhances potency
    - improves safety profile
- X-ray cocrystal
  - revealed drug-DPP-4 interactions
  - 2-F close to N-Hs of Arg_{125} & Asn_{710}
  - 2,5-isomer 5x over 3,4-isomer
- F mimics an amide C=O in this context
  - exemplified by matched pair comparison

S.D. Edmondson *et al., Bioorg. Med. Chem. Lett.*, 2008, **18**, 2409-2413; D. Kim *et al., J. Med. Chem.*, 2005, **48**, 141-151
G.B. Liang *et al., Bioorg. Med. Chem. Lett.*, 2008, **18**, 3706-3710

Vinyl-F/Amide Bioisosterism: Thrombin & Enkephalins

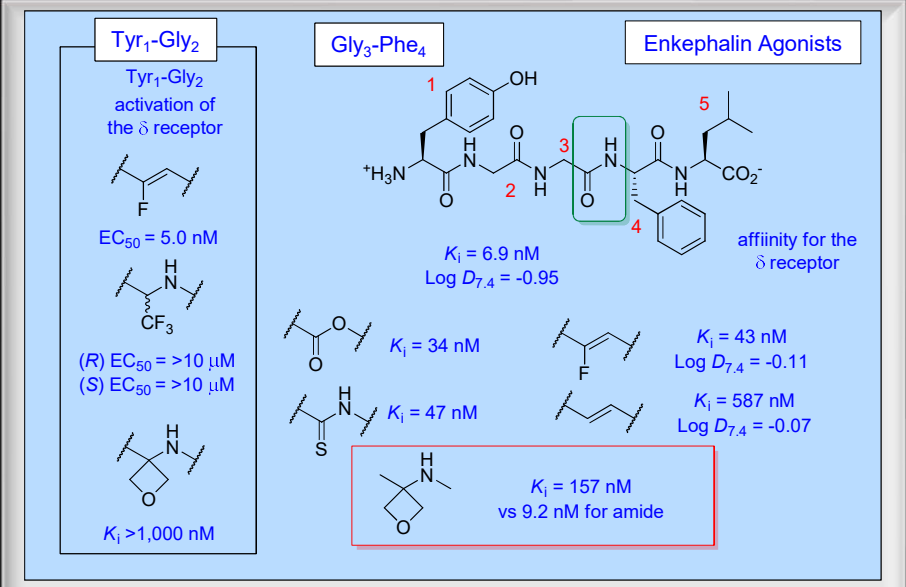
C-F ≡ C=O





RCF_2CH_2NH is $ArSO_2NH$ mimic

- Thrombin inhibitor
  - design relies heavily on isosteres
- Weakly basic oxyguanidine - pK_a ~7
  - isostere of guanidine - pK_a = 13-14
  - improves permeability but sulfonamide showed poor F
- diF-ethylamine moiety acts as lipophilic SO_2 mimic in this context
  - extra CH_2 compensates for longer C-S bonds
    - C-F as S=O mimic
  - reduces basicity of amine, improves H-bond donor
- Pyridone ring replaced with fluoro-benzene
  - close interaction with Gly$_{216}$ NH



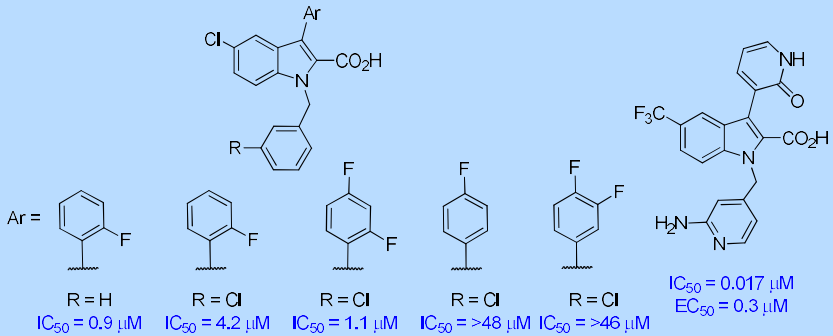
- Vinyl-F effective at Gly$_3$-Phe$_4$ in **enkephalins**
  - 6x weaker than amide
    - comparable to ester, thioamide
  - superior to simple olefin & oxetane
- Vinyl-F effective at Tyr$_1$-Gly$_2$
  - CF_3-CH-N & oxetane ineffective at Tyr$_1$-Gly$_2$

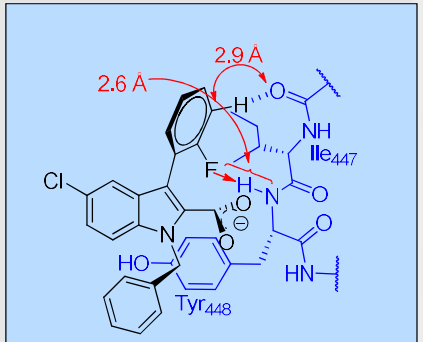
Enkephalin: a brain pentapeptide involved in nociception

M.R. Player *et al., BMCL*, 2007, **17**, 6266-6269; 2008, **18**, 2865-2870; P. Wipf *et al., JOC*, 1998, **63**, 6088-6089; Y.L. Dory *et al., ACS Chem. Neurosci.*, 2017, **8**, 40-49
M. Zanda *et al., ChemMedChem*, 2009, **4**, 1416-1420; G.P. Möller *et al., Org. Lett.,* 2017, **19**, 2510-2513; R.A. Altman *et al., ChemMedChem,* 2017, **12**, 571-576

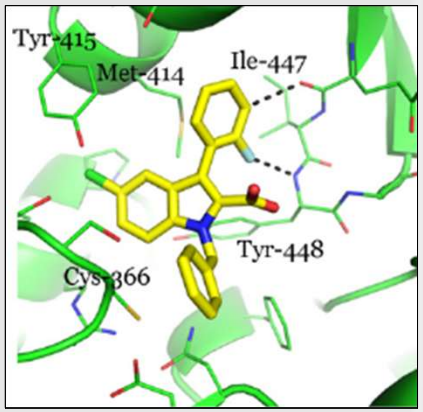
Aromatic F as C=O Mimic in Cyclic Amides

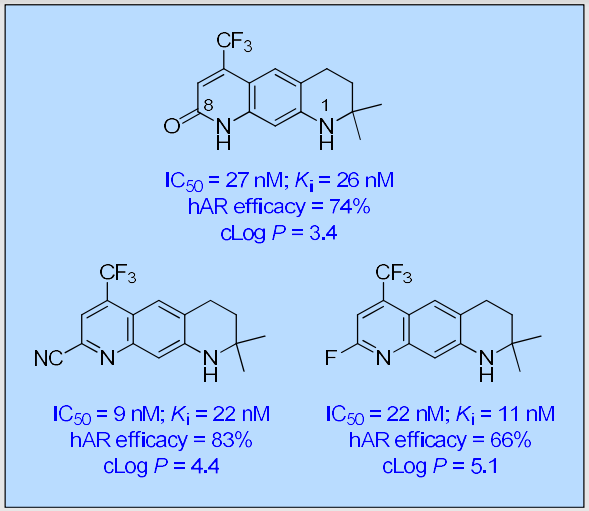
C-F ≡ C=O





- Potent inhibitor of HCV NS5B discovered by HTS
  - X-ray cocrystal structure
  - C-F to NH H-bond with Tyr_{448}: F-N distance = 2.6 Å
  - *ortho*-C-H close to C=O of Ile_{447} C-O distance = 2.9 Å
- Removal of F reduced potency >50x
  - 2-F, 3-pyridyl active
  - 2-OMe, 2-OH retained activity
- SAR evolution replaced the F-benzene with 2-pyridine
  - recognized amide-like interaction of 2-F-phenyl
  - potency enhanced 2-3x
  - further optimization added amnio pyridine
    - active in a GT-1b replicon





- Androgen receptor (AR) antagonist
  - biochemical affinity assay
  - inhibition of AR transcriptional activity
    - efficacy in mammalian CV-1 cells
- Sought to understand bound tautomer of amide
  - small electron withdrawing substituents active
  - C≡N & F optimal
- Concluded that amide C=O is acting as a HBA
  - pyridone rather than pyridine-OH

G.N. Anilkumar *et al., Bioorg. Med. Chem. Lett.*, 2011, **21**, 5336-5341; J.W. Kong *et al., Bioorg. Med. Chem. Lett.*, 2000, **10**, 411-414

End of Part 1