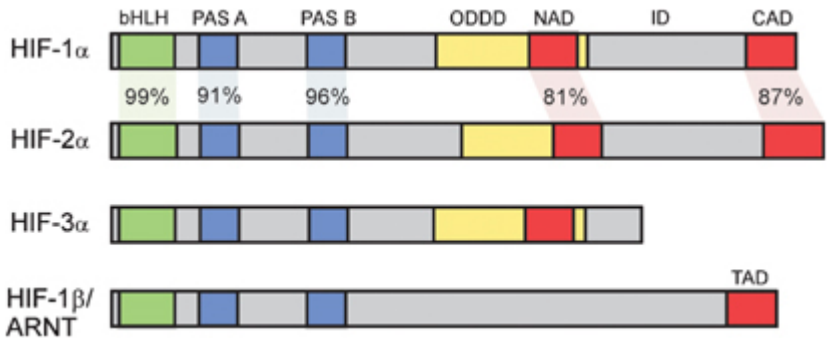
Isoform-Selective and Stereoselective Inhibition of Hypoxia Inducible Factor-2

SCHEUERMANN, T. H.; STROUD, D.; SLEET, C. E.; BAYEH, L.; SHOKRI, C.; WANG, H.; CALDWELL, C. G.; LONGGOOD, J.; MACMILLAN, J. B.; BRUICK, R. K.; GARDNER, K. H.; TAMBAR, U. K.

J. Med. Chem. **ASAP**

Celeste Alverez
Current Literature
August 8, 2015

HIFs



HIF: Hypoxia Inducible Factors

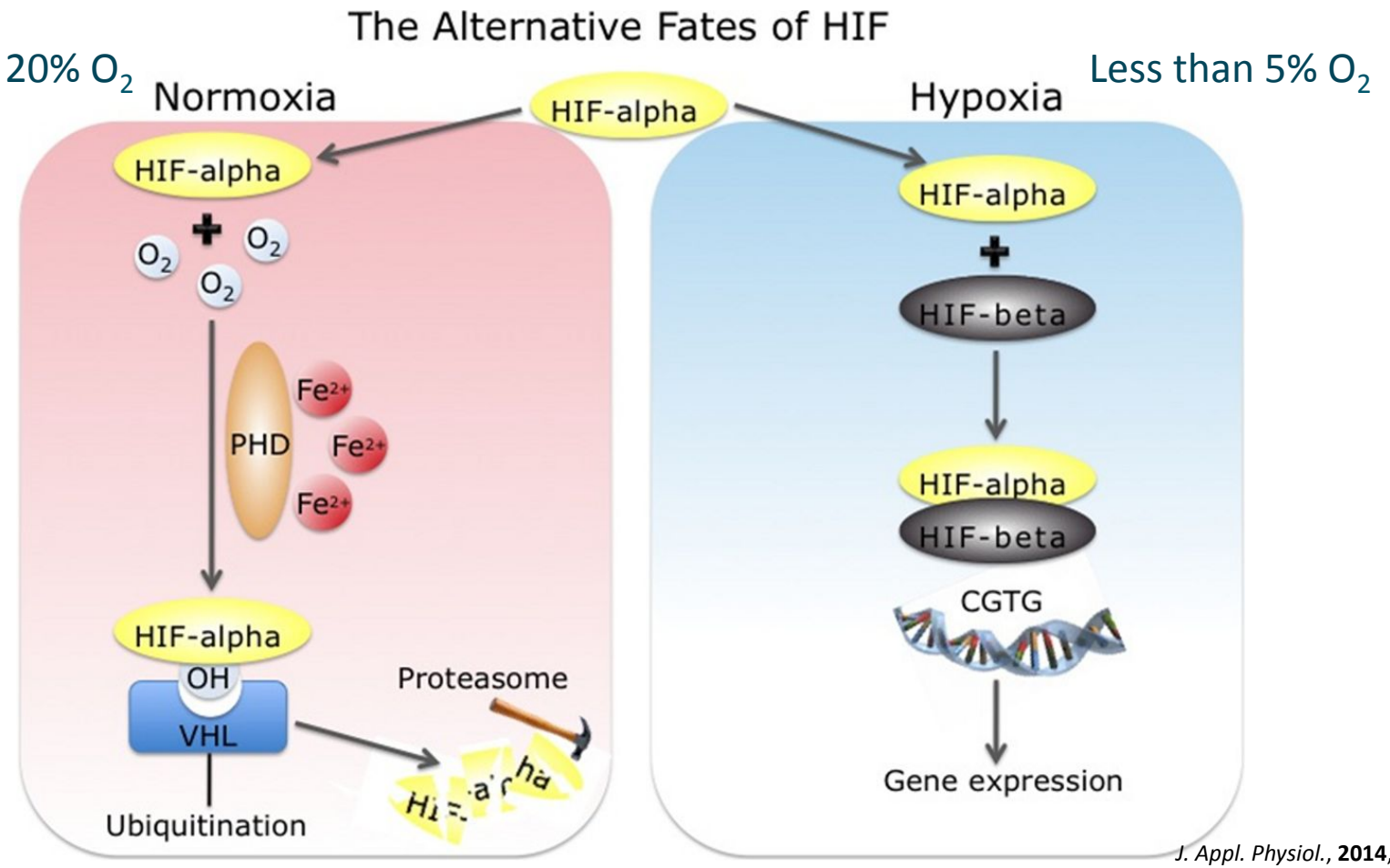
Transcription factors that trigger cellular responses to low oxygen levels in the cell

Structure:

- Heterodimeric complex composed of an α and a β subunit
- 3 isoforms of α subunit:
  - HIF-1α, HIF-2α, HIF-3α
- 3 isoforms of β subunit (aryl hydrocarbon receptor nuclear translocator):
  - HIF-1β (ARNT), HIF-2β (ARNT2), HIF-3β (ARNTL)
- bHLH domain: DNA binding domain
- PAS (Per-ARNT-Sim) domain: facilitates/stabilizes heterodimerization
- C-terminal: recruits transcriptional coactivator proteins, site of hydroxylation leading to degradation

Adapted from *Cell Death Differ.*, **2008**, *15*, 642

Hypoxia and HIFs



J. Appl. Physiol., **2014**, *116*, 875

HIF Misuse in Cancer

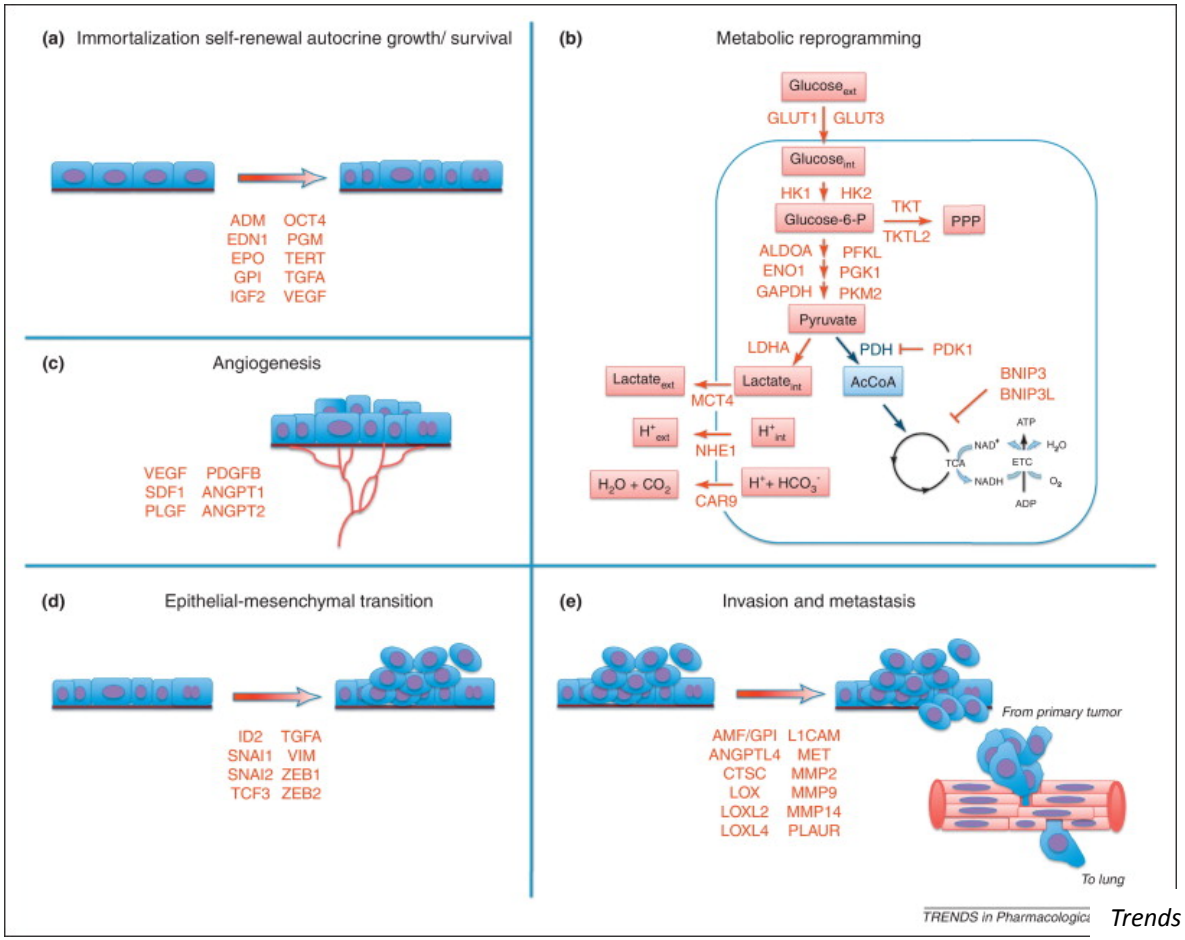
Under hypoxic conditions: HIFs dimerize, accumulate in the nucleus, bind the DNA, start gene transcription, deal with/survive hypoxic conditions

- Good in normal cases of hypoxia: embryonic development, metabolism, angiogenesis
- Bad in cancer

Supports growth and metastasis of solid tumors

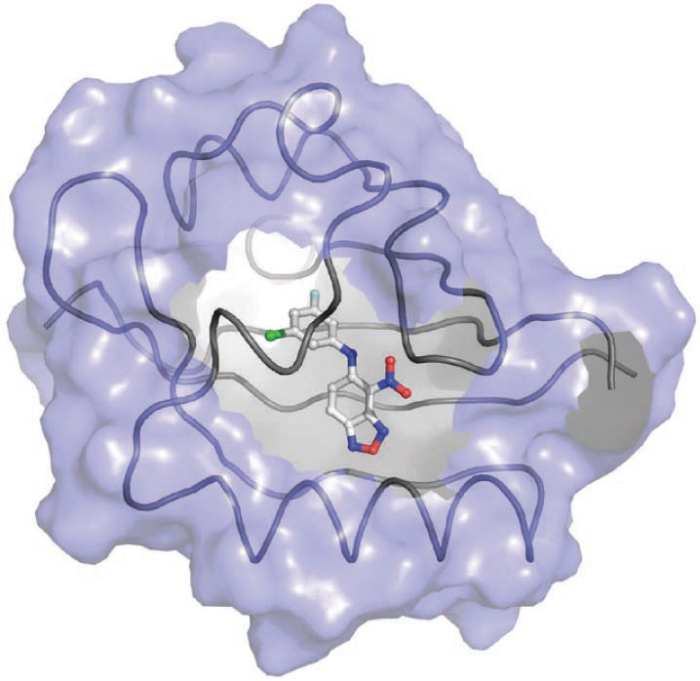
- Would not necessarily progress due to oxygen deprivation otherwise
- Also promotes resistance to chemotherapy and radiation therapy

HIF and Related Pathway Misuse in Cancer



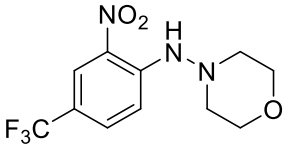
Trends Pharmacol. Sci., **2013**, *33*, 207

HIF Inhibitors

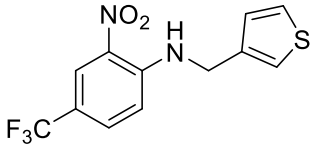


Previously ran HTS and performed NMR-based screening of drug-like fragments against HIF-2α PAS-B

3 hits/leads emerged:



1



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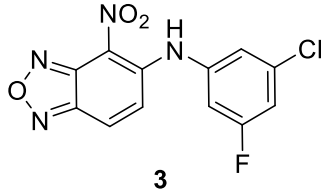


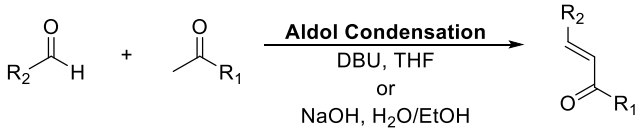
3

Nitro groups found to necessary for activity– metabolic/tox liability

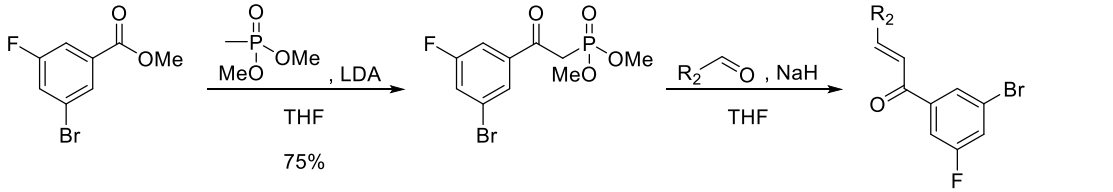
Large cavity in the HIF-2α PAS-B domain where these bind– potentially room for a more 3 dimensional inhibitor

Nat. Chem. Bio, **2013**, *9*, 271

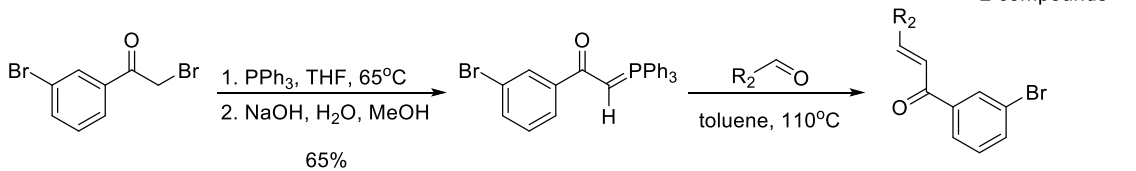
Synthesis



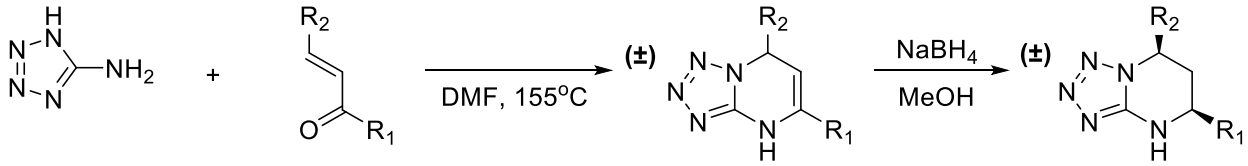
17 compounds



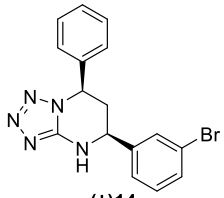
2 compounds



4 compounds



Racemic Analogs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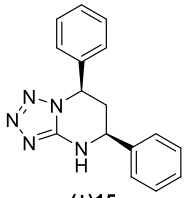
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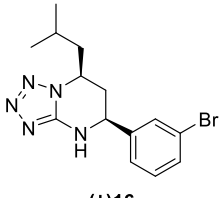
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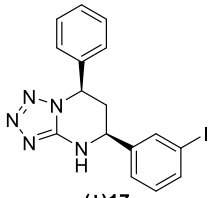
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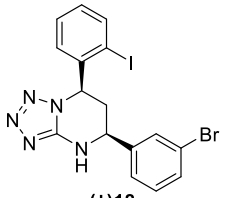
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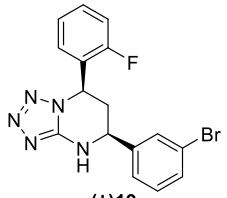
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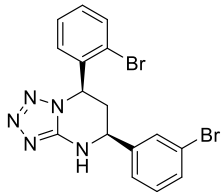
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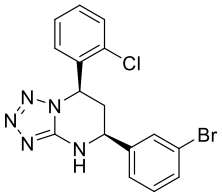
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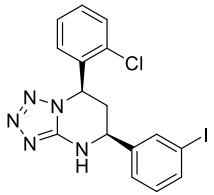
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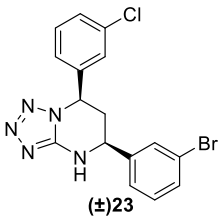
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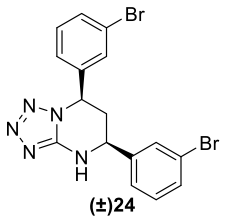
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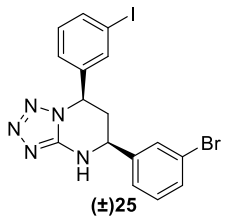
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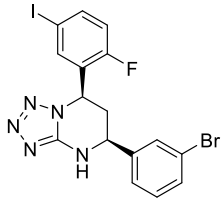
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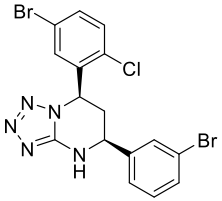
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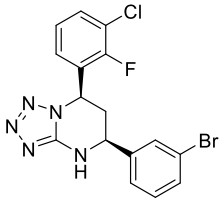
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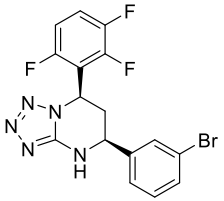
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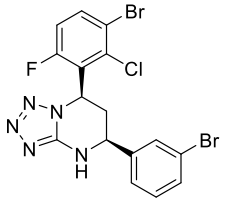
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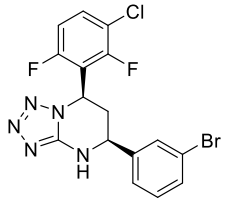
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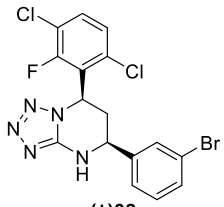
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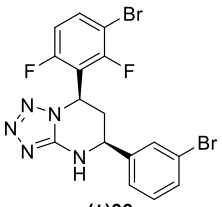
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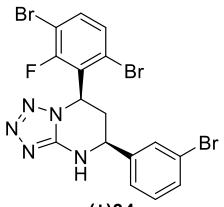
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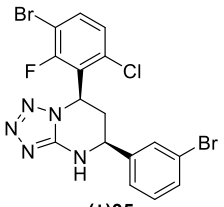
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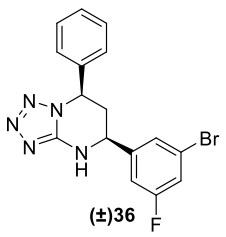
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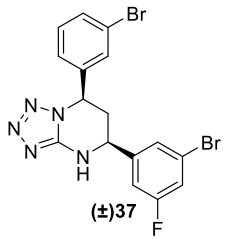
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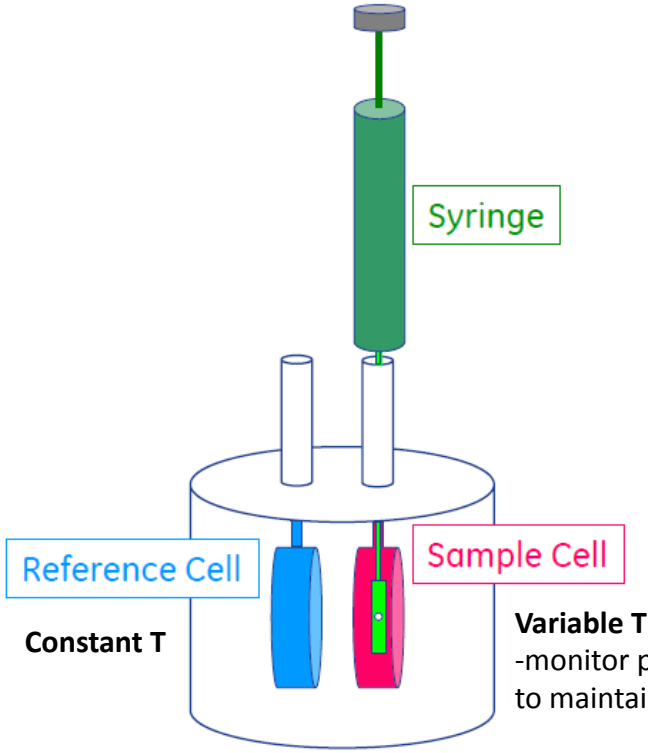
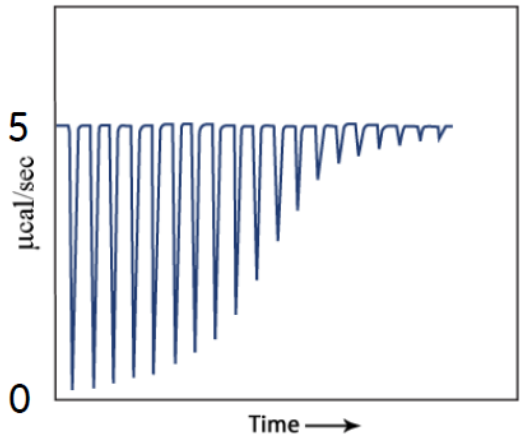
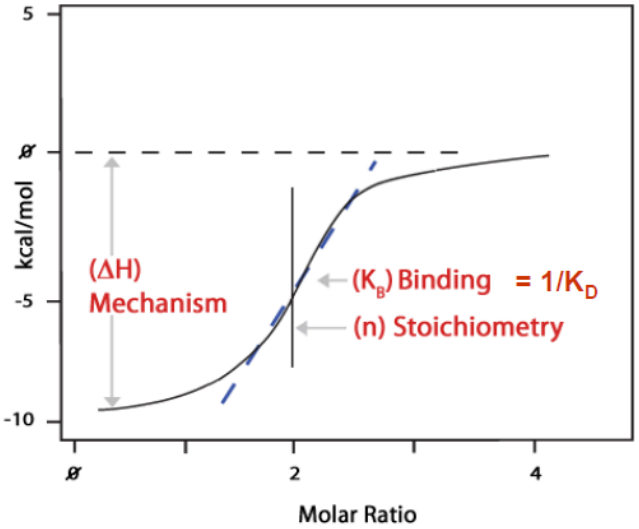
ITC



Constant T

Variable T
-monitor power
to maintain set T





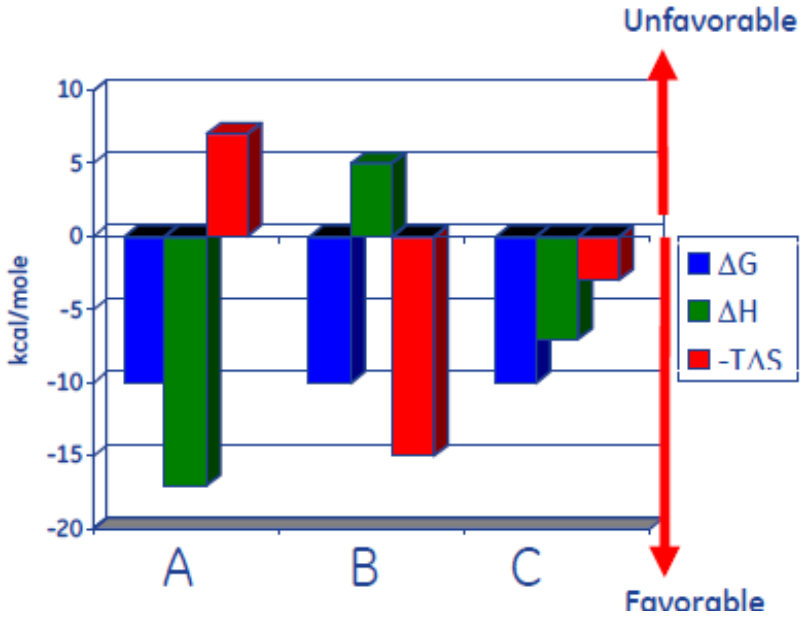
http://www.malvern.com/en/products/technology/isothermal-titration-calorimetry/

ITC

Parameters Determined:

- N (stoichiometry)
  - Determined from the inflection point of graph
- K_D (binding affinity)
  - Determined from the linear portion of graph
- ΔH (enthalpy)
  - Determined from the magnitude and direction of graph
  - Mainly from H-bonds and Van der Waals
- ΔG (Gibbs free energy)
  - Calculated from above parameters via: $\Delta G = -RT\ln K_D$
- ΔS (entropy)
  - Calculated from above parameters via: $\Delta G = \Delta H - T\Delta S$
  - From hydrophobic interactions, release of coordinated waters, conformational changes

ITC



ITC can indicate binding mechanism

A. favorable binding composed of favorable H-bond formation and unfavorable organization of the complex

B. favorable binding composed of unfavorable enthalpic contributions and favorable hydrophobic interactions

C. favorable binding composed of favorable H-bond formation and favorable hydrophobic interactions

http://bcmp.med.harvard.edu/sites/bcmp.med.harvard.edu/files/facilities/ITC200%20training_pdf.pdf

Use of ITC in Medicinal Chemistry

Generally can determine the heat of binding/interaction between 2 binding partners

- Can specifically be used in medicinal chemistry for determining the heat of binding between a small molecule and a target protein

Can be used as a screening method for selection of initial hit compounds

At advanced stages in drug development many similarly potent compounds can be found

- Instead of just simply looking at classic physical characteristics (sol., stability, ect.) can also consider ΔH (though more difficult)
- Improving ΔH indicates improving number and strength of protein-ligand interactions

It has been found that in 2 important and well studied class of targets (statins, HIV protease inhibitors), the best in class compounds had better enthalpic contributions vs. the first in class compounds

Nat. Rev. Drug Discov. **2010**, *9*, 23

Racemic K_D values

Compound	B ring	C ring	K_D (nM)	Δ*H* (kcal/mol)	incA
(±) **14**	3-BrPh	Ph	883	-16.2	0.65
(±) **15**	Ph	Ph	>2000		
(±) **16**	3-BrPh	*i*-Bu	1170	-14.8	0.55
(±) **17**	3-IPh	Ph	1410	-15.5	0.66
(±) **18**	3-BrPh	2-IPh	776	-10.7	0.5
(±) **19**	3-BrPh	2-FPh	327	-16.2	0.52
(±) **20**	3-BrPh	2-BrPh	400	-15.5	0.49
(±) **21**	3-BrPh	2-ClPh	361	-15.3	0.56
(±) **22**	3-IPh	2-ClPh	837	-15.7	0.65

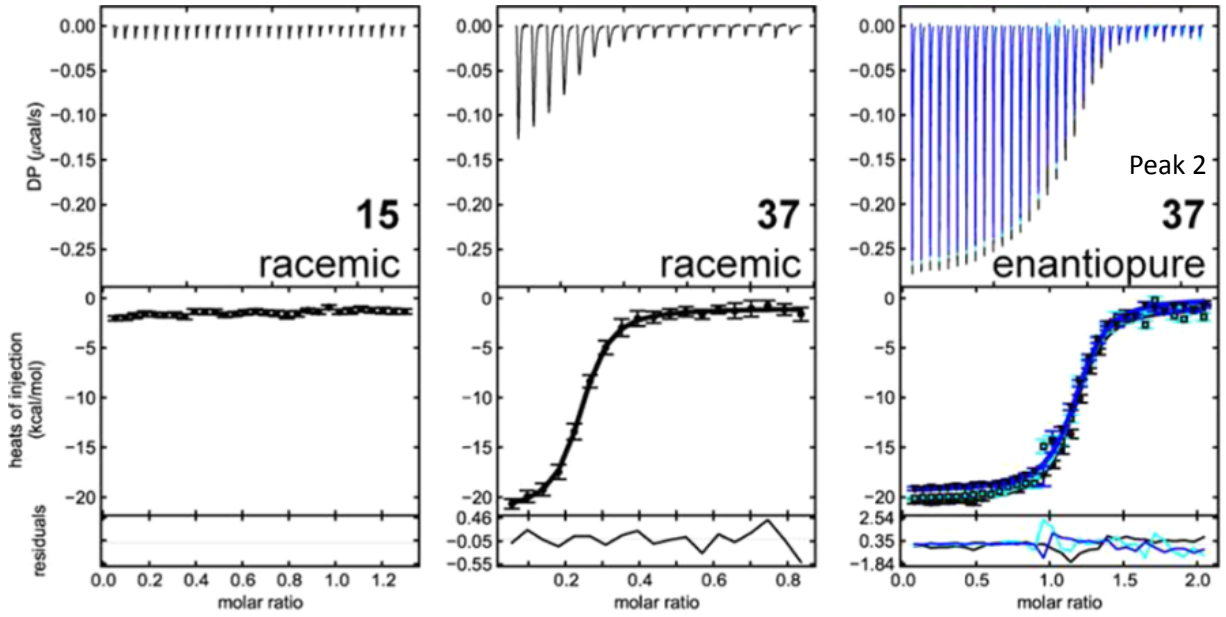
Racemic K_D values

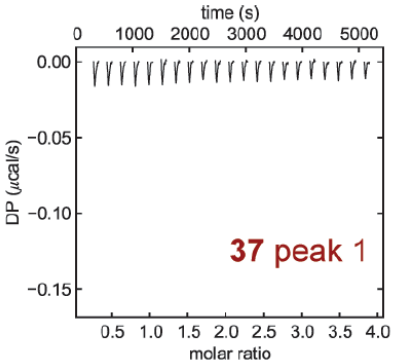
Compound	B ring	C ring	K_D (nM)	ΔH (kcal/mol)	incA
(±) **23**	3-BrPh	3-ClPh	116	-18.6	0.51
(±) **24**	3-BrPh	3-BrPh	70	-18.8	0.56
(±) **25**	3-BrPh	3-IPh	103	-20.4	0.55
(±) **26**	3-BrPh	2-F-5-IPh	159	-12.9	0.67
(±) **27**	3-BrPh	2-Cl-5-BrPh	126	-18.4	0.63
(±) **28**	3-BrPh	2-F-3-ClPh	38	-16.3	0.49

Racemic K_D values

Compound	B ring	C ring	K_D (nM)	Δ*H* (kcal/mol)	IncA
(±) **29**	3-BrPh	2,3,6-F_3Ph	393	-15.5	0.54
(±) **30**	3-BrPh	2-Cl-3-Br-6-FPh	411	-20.3	0.9
(±) **31**	3-BrPh	2,6-F_2-3-ClPh	85	-16.8	0.61
(±) **32**	3-BrPh	2,5-Cl_2-6-FPh	194	-16.9	0.55
(±) **33**	3-BrPh	2,6-F_2-3-BrPh	49	-18.2	0.61
(±) **34**	3-BrPh	2,5-Br_2-6-FPh	240	-16.9	0.74
(±) **35**	3-BrPh	2-Cl-5-Br-6-FPh	85	-16.6	0.74
(±) **36**	3-Br-5-FPh	Ph	216	-15.9	0.53
(±) **37**	3-Br-5-FPh	3-BrPh	28	-20.2	0.78

Effect of Enantiopurity



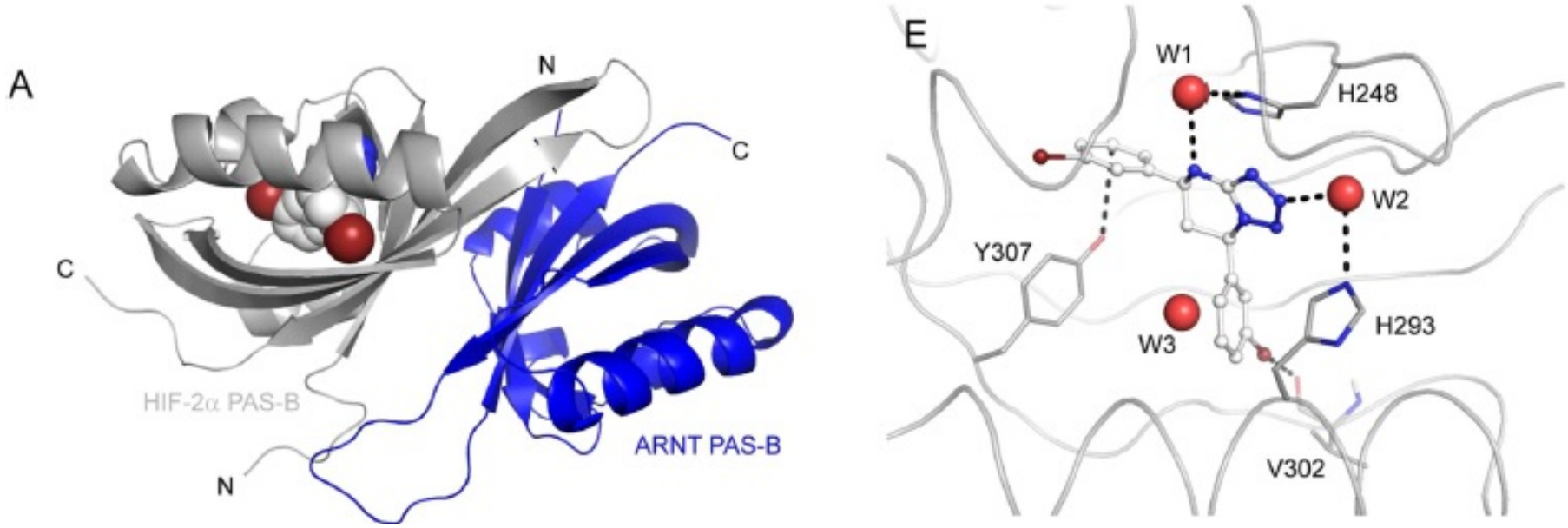


syringe (µM):	40	40	50
cell (µM):	4	4	5
K_D (nM):	--	28 (23, 34)	40 (32, 49)
ΔH (kcal/mol):	--	-20.2 (-20.7, -19.7)	-22.5 (-23.2, -21.8)
inc fraction:	--	A: 0.78	B(ave): 0.13

Effect of Enantiopurity

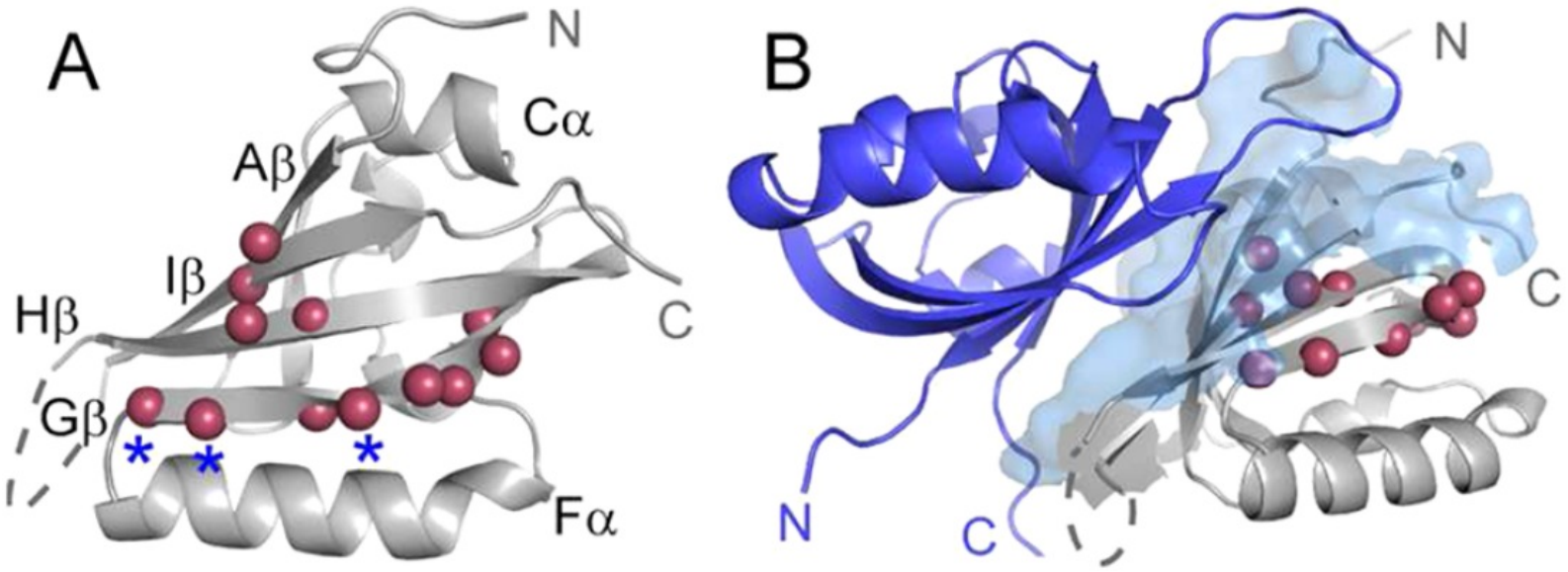
Cmpd	IC_{50} (WT HIF-2α) [nM]	IC_{50} (S304M control) [nM]	ITC K_D (nM)	ITC incA (cmpd)/B (prot)	ITC Δ*H* (kcal/mol)
(*S*,*R*)-**14**	248	>30,000	487	B: 0.24	-18.1
(*R*,*S*)-**14**	>30,000	>30,000	>>2,000	--	--
(*S*,*R*)-**17**	1000	>30,000	1239	A: 0.02	-13.2
(*R*,*S*)-**17**	>30,000	>30,000	>>2,000	--	--
(*S*,*R*)-**20**	314	18,900 µM	360	A: 0.23	-15.1
(*R*,*S*)-**20**	28,300	>30,000	>>2,000	--	--
(*S*,*R*)-**21**	195	15,900	294	A: 0.03	-15.6
(*R*,*S*)-**21**	>30,000	>30,000	>>2,000	--	--
(*S*,*R*)-**24**	112	>30,000	63	B: 0.08	-20.3
(*R*,*S*)-**24**	>30,000	>30,000	>>2,000	--	--
(*S*,*R*)-**25**	108	>30,000	109	B: 0.03	-19.8
(*R*,*S*)-**25**	>30,000	>30,000	>>2,000	--	--
(*S*,*R*)-**27**	87	23,900	125	B: 0.01	-17.2
(*R*,*S*)-**27**	>30,000	>30,000	>>2,000	--	--
(*S*,*R*)-**33**	32	3,800 µM	34	B: 0.09	-20.8
(*R*,*S*)-**33**	22,300	>30,000	>>2,000	--	--
(*S*,*R*)-**36**	97	10,100	165	B: 0.18	-19.1
(*R*,*S*)-**36**	>30,000	>30,000	>>2,000	--	--
(*S*,*R*)-**37**	43	14,500 µM	23	B: 0.14	-21.6
(*R*,*S*)-**37**	>30,000	>30,000	>>2,000	--	--

Crystal Structure



Compounds **1**, **2**, **3**, and (*S,R*)-**24** all have common pharmacophore with (*S,R*)-**24** having the 3-Br that extends beyond **1**, **2**, or **3**

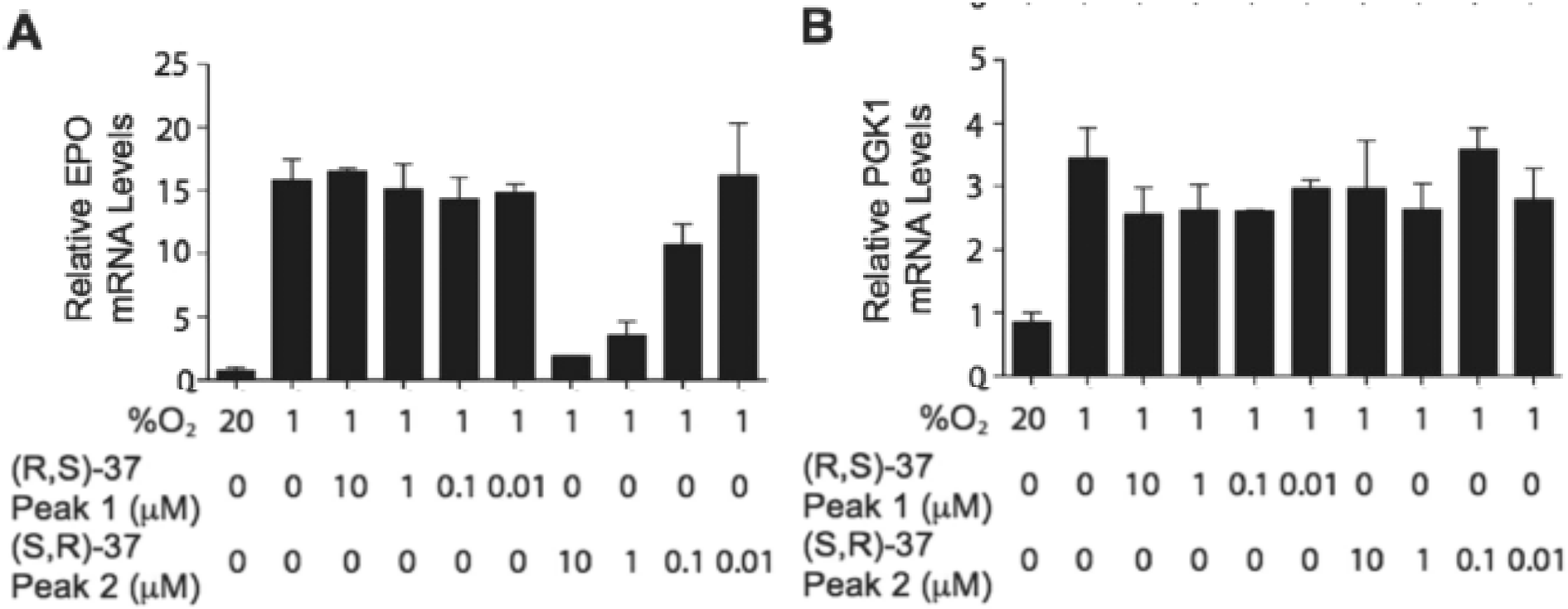
Induced Structural Changes



Structure shown is ligand free protein
Red spheres indicate positions displaced by 0.5 Å or greater by (*S,R*)-**24** binding

* Indicate large conformational differences (1.1, 1.2, and 0.8 Å, from left to right)

Cellular Efficacy/Selectivity



Conclusions/Further Work

Developed chiral ligand of HIF-2α PAS-B domain

- (*S,R*) found to be preferred configuration
- Higher potency
- Induce larger conformation changes in the protein

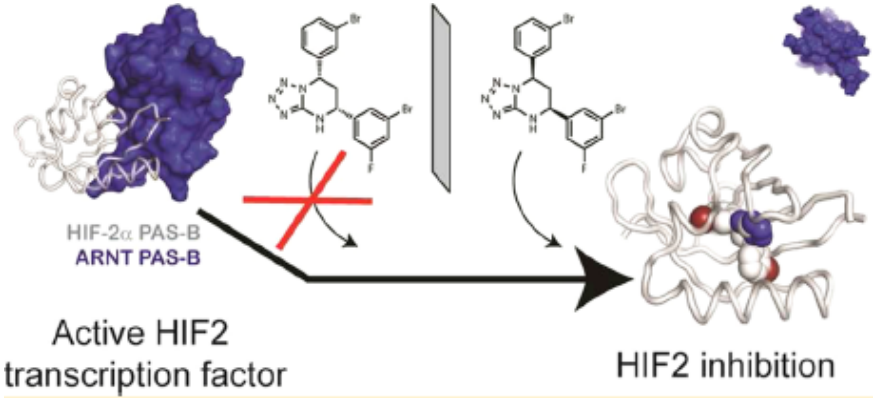
HIF-2α selective

Most effective HIF-2α selective inhibitors

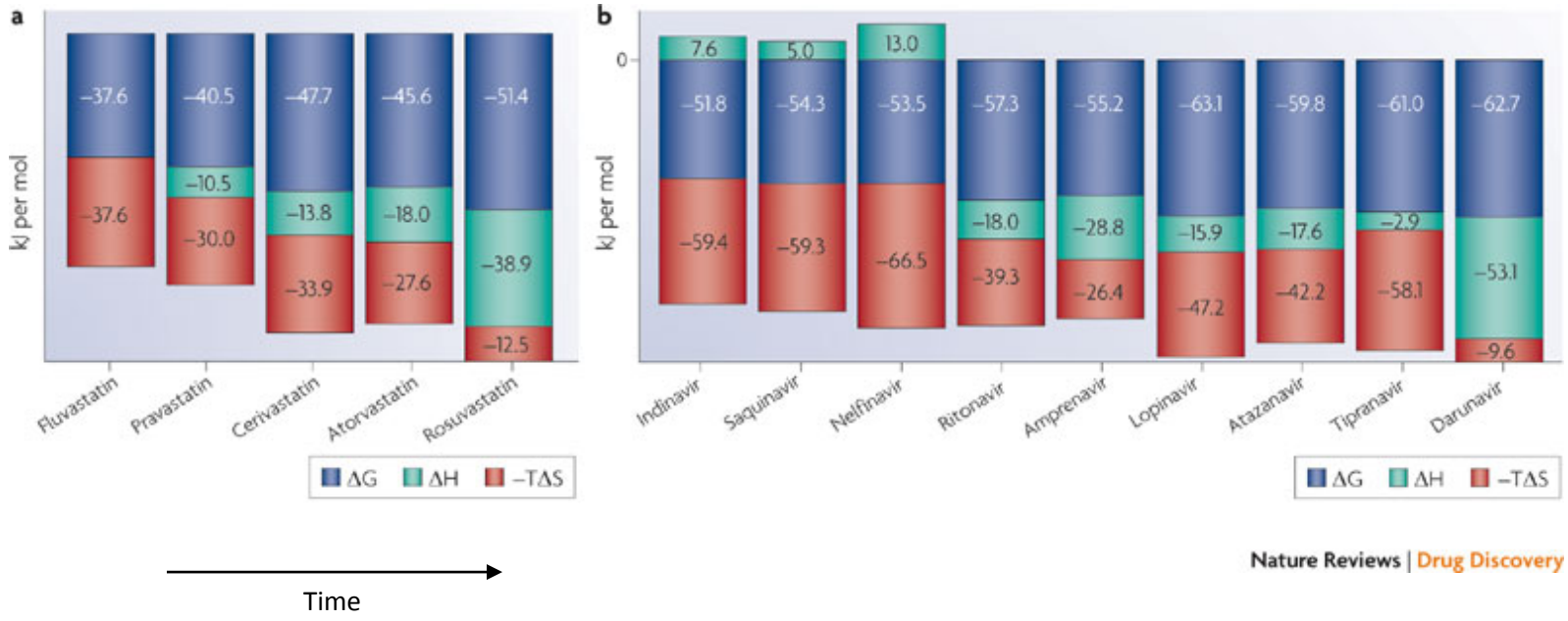
More varied substituents?

In vivo studies?

PK?



ΔH in 1st in class vs Best in class



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Common Pharmacophore