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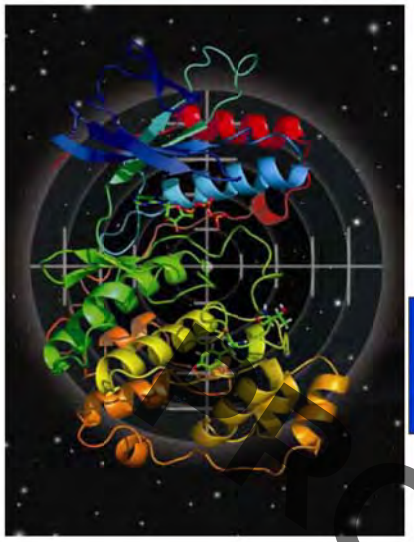
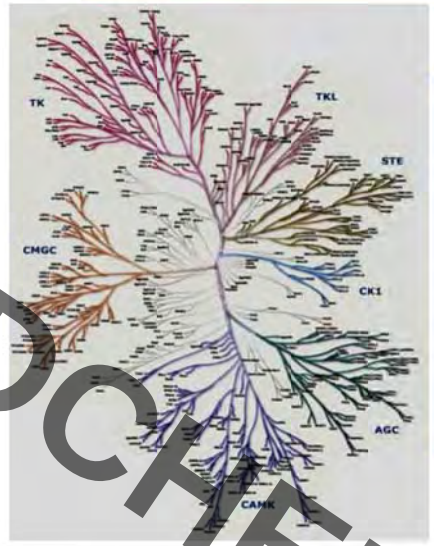


Targeting Protein-Kinases
-
The Selectivity Problem

Prof. Dr. Stefan Laufer

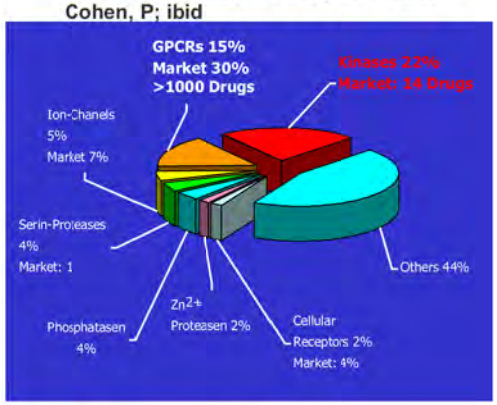
THE 6TH BRAZILIAN SYMPOSIUM ON MEDICINAL CHEMISTRY
BRAZMEDCHEM 2012

Protein Kinases: Drug Targets for the 21st Century?



The Druggable Genome

Hopkins, A.; Nat. Rev. Drug Disc. 2002
Cohen, P; ibid



518 Proteinkinase Genes,
244 Kinase-Genes are disease-related

Hopkins, A.; NDD 2002, Cohen, P, NDD 2002

TIME: A special Report: May18, 1998

- Targeted Cancer Therapy
- „The dream of the Magic Bullet“
- Side Effect-Free Cure of Cancer

Cancer knocked out?

Today: Facts and Fantasy ?

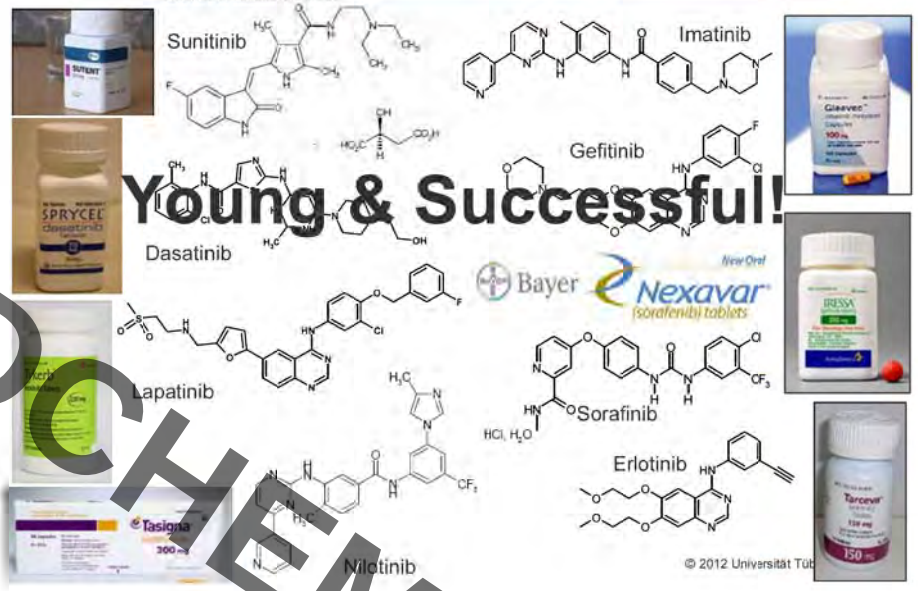
- Targeted Cancer Therapy ?
- „The dream of the Magic Bullet“ ?
- Side Effect-Free Cure of Cancer ?

“It’s an award for a cancer cure, but it only works on mice.”

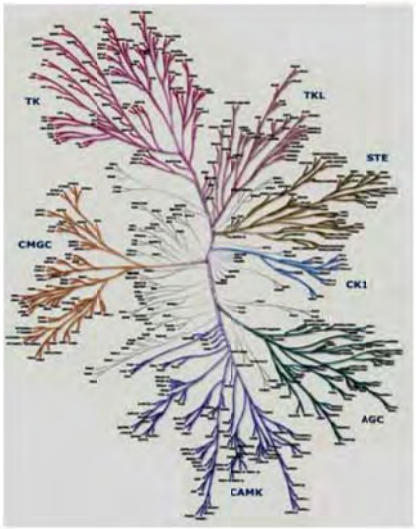
New Ammunition in the War against Cancer

May 2001

Sales 2010: 10 Bil. USD



The Human Kinome



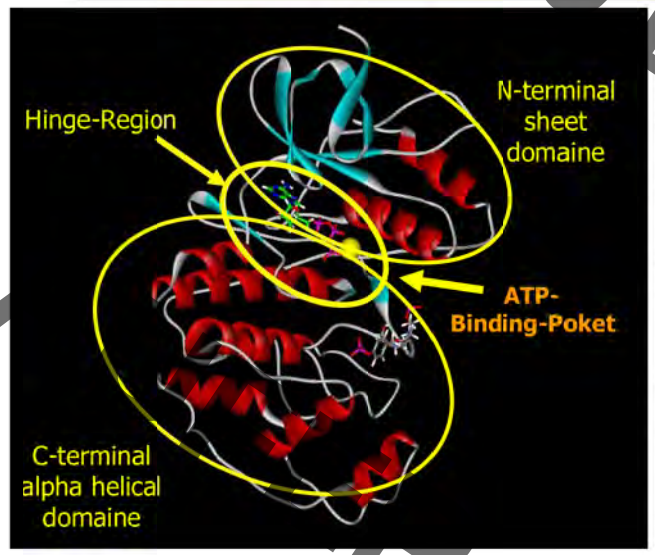
- 518 Proteinkinase Genes in human Kinome (~ 5 splice variants each)
- ~ 2500 Kinase Proteins
- >1000 somatic mutations in coding exons (of 518 PK genes) found in 210 diverse cancers

Greenman: Nature 2007; 446: 153

Major challenge:

SELECTIVITY !

Kinase Structures



All Ser/Thr/Tyr Kinasen have similar structures

> 175 X-ray structures

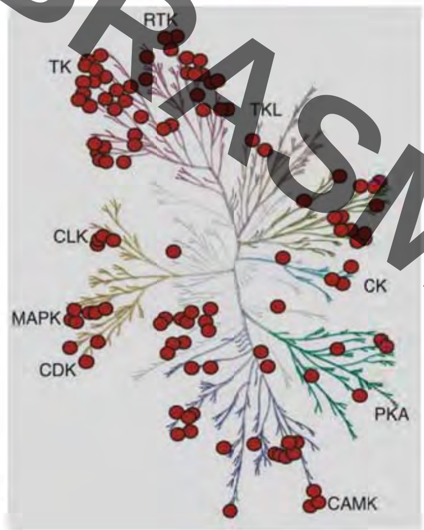
ATP-Binding site is highly conserved

➢ATP 2-10 mM !!!

DiscoverRX KINOMEscan: scanMAX

451 Kinases,

covering most families of Kinase-Genome



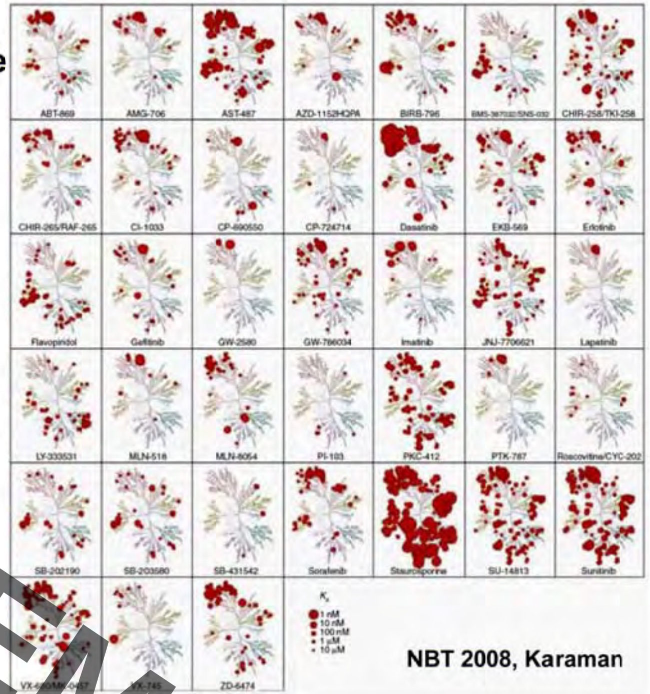
Karaman et al., Nature Biotechnology 26, p.127 (2008)

Selectivity Profile

Kinase Inhibitors Clinical Trials or Launched

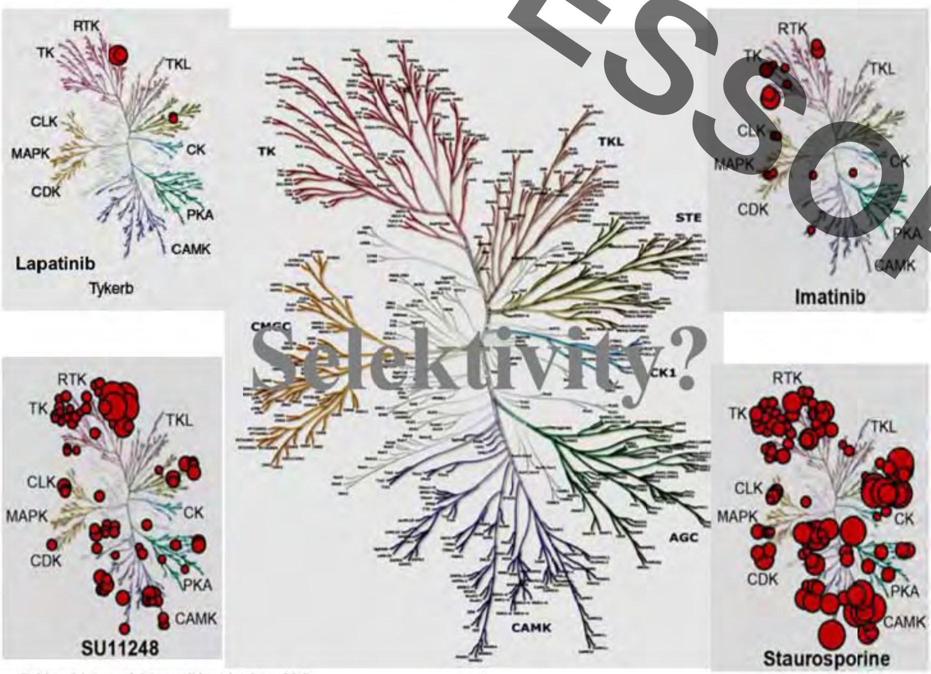
Selectivity ???

- Pan Kinase Inhibitors
- Multi Kinase Inhibitors
- Selective Multi Kinase Inhibitors
- Selective Kinase Inhibitors



ABT-869
AMG-706
AST-487
AZD-1152HQPA
BIRB-796
CHIR-258/TKI-258
CHIR-265/RAF-265
CI-1033
CP-690550
CP-724714
Dasatinib
EKB-569
Erlotinib
Flavopiridol
Gefitinib
GW-2580
GW-786034
Imatinib
JNJ-7706621
Lapatinib
LY-333531
MLN-518
MLN-8054
PI-103
PKC-412
PTK-787
Roscovitine/CYC-202
SB-202190
SB-203580
SB-431542
Sorafenib
Staurosporine
SU-14813
Sunitinib
VX-745
ZD-6474

NBT 2008, Karaman



Fabian, M.A. et al. Nature Biotechnology 23,3, 2005

One Drug, one Target, one Disease ?

[1]
Aim several targets at once

[2]
Case history: Gleevec

[3]
Promiscuous Drugs

Playing dirty
Forget drugs carefully designed to hit one particular molecule — a better way of treating complex diseases such as cancer may be to aim for several targets at once, says Simon Frantz.
[1]
[2]
[3]
Take aim
"The idea of magic bullets is great, but in practice it's probably not going to be the right approach for complex diseases." — Bryan Roth

S. Frantz, Nature, (2005), 437, 942-943

Kinase Selectivity, is it necessary ?

It Depends !

- Cancer
- Inflammation/Autoimmune

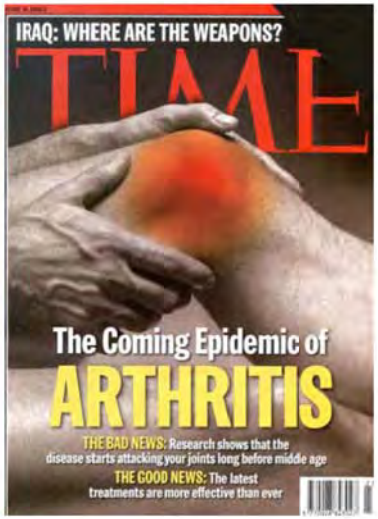
- Acute vs. Chronic
- Application route

Which are the „untouchable“ Kinases ?

Even for (selective) Multi Kinase Inhibitors, we have to understand the mechanisms to build in selectivity in the molecules !

Need for highly selective probes and tools

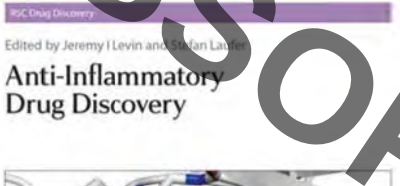
Inflammation: The Secret Killer

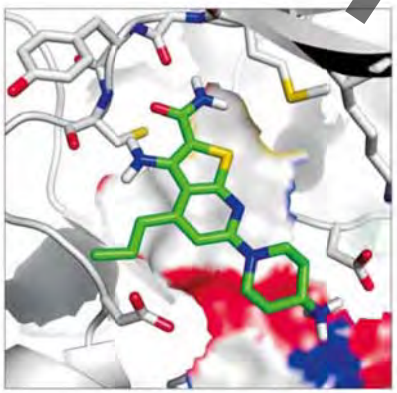


Molecular Target in Inflammation

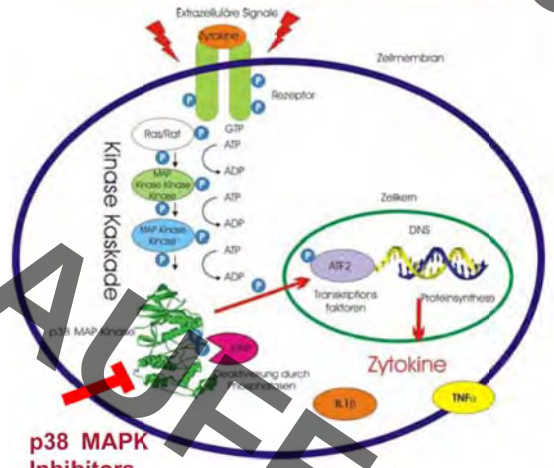
Eicosanoids, AA:	mPGES-1
	$cPLA_2$
	LTA4-Hydrolase
	CRTH2-Antagonists
Kinases:	p38 MAPK
	MAPKAP K2 (MK2)
	Syk Kinase
	Janus Kinases (JAKs)
	IKKß
	Bruton‘s Tyrosin Kinase (Btk)
GPCRs:	CCR1
	CCR2
	CB_2 Agonists
Sphingolipids:	S1P Receptor-Agonists
	Sphingosine Kinase / ~Lyase

Non-steroidal Glucocorticoid- Receptor-Agonists





p38 MAPK Signalling – TNFα, IL1, IL6



p38 MAPK Inhibitors

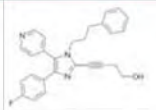
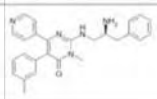
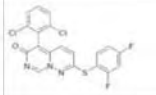
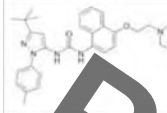
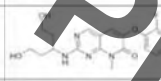
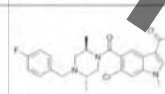
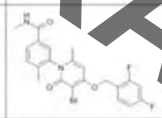
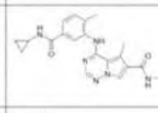
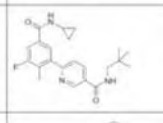
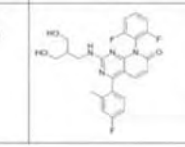
p38 MAPK controls proinflam. CK-synthesis on transscriptional and translational level

(TNFa, IL-1ß, IL-6)

- Rheumatoid Arthritis
- Bone Resorption (Osteoarthritis, Osteoporosis)
- IBD
- Psoriasis
- Alzheimers Disease
- Artherosclerosis
- Restenosis
- Stroke
- COPD
- Septic S.

p38 Inhibitors are validated small molecule anti cytokine drug candidates

p38 MAPK – Clinical Candidates

RWJ-67657		discontinued; no Phase II data reported; efficacy demonstrated in the human endotoxaemia pharmacodynamic model demonstrated [29];
AMG-548		discontinued after Phase I; elevated liver enzymes reported;
VX-745		discontinued after a Phase II trial in RA; primary endpoints were met but CNS adverse events expected based on non-clinical data;
BIRB-0796 (Doramapimod)		discontinued after Phase II; no efficacy in RA, CD and psoriasis; elevated liver enzymes after repeated administration;
Pamapimod		discontinued based on failure in Phase II trials in RA patients (+/- co-medication with MTX)
SCIO-469 (Talmapimod)		discontinued based on failure in Phase II trials in RA; outcome of studies in multiple myeloma unknown
VX-702		discontinued based on failure in Phase II trials in RA patients (+/- co-medication with MTX)
PH-797804		Phase II; RA study completed but results not disclosed; Cinical trials for treatment of COPD and neuropathic pain ongoing;
BMS-582949		Phase II; Cinical trials for treatment of RA, plaque psoriasis and atherosclerosis completed; results not reported;
Losmapimod (GW-856553)		Phase II; Cinical trials for treatment of RA, COPD and depression completed; results not reported;
Dilmapimod (SB-681323)		Phase II; Cinical trials for treatment of RA, COPD and neuropathic pain completed; results not reported;

p38 MAPK – clinical candidates

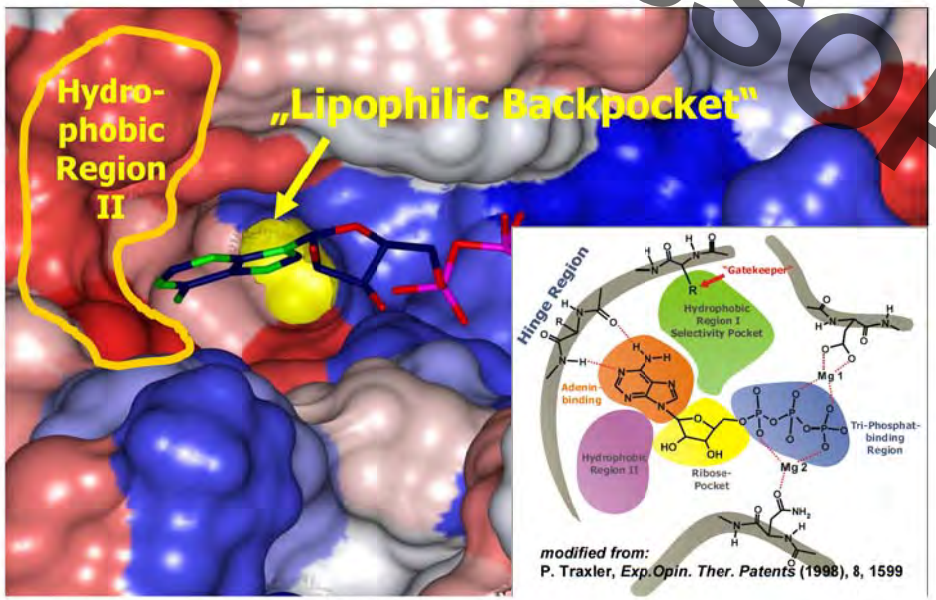
1. Generation: failed due to structure related liver toxicity

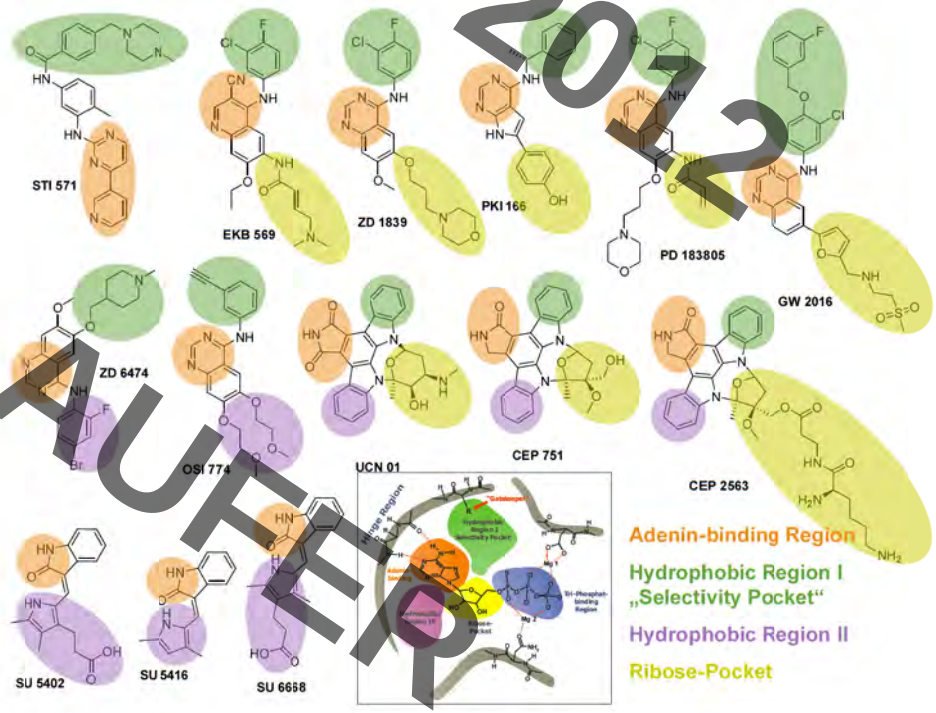
2. Generation: failed in RA, only transient efficacy over 3 months treatment
successful in other inflammatory indications (COPD)

3. Generation: dual inhibitors of inactive and active form of p38
first proof of concept in RA achieved (BMS-582949)
no phase III, hERK-canal inhibition

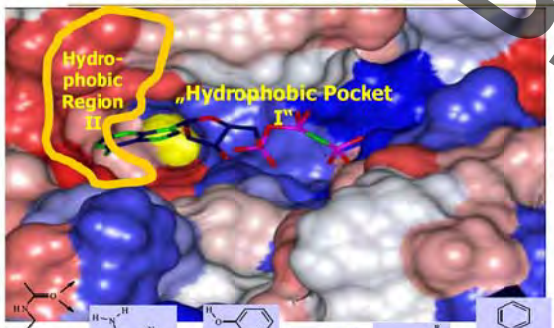
Status quo: target validated, however drug-candidate still missing!

p38 MAPK: ATP Binding Site
Hydrophobic Regions I & II

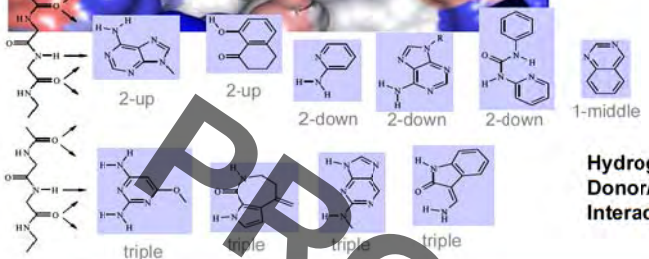




Binding Model Hydrophobic Region I & II



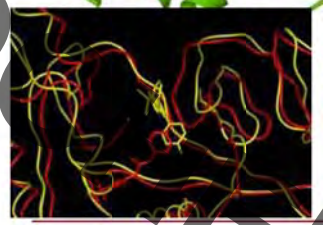
End of the Story ?

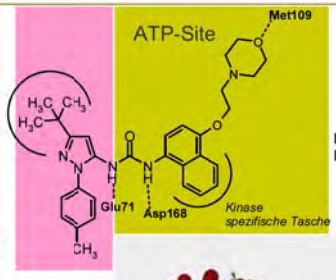


Hydrogen-Bond Donor/Acceptor Systems Interaction with the Hinge

Adapted from Axxima

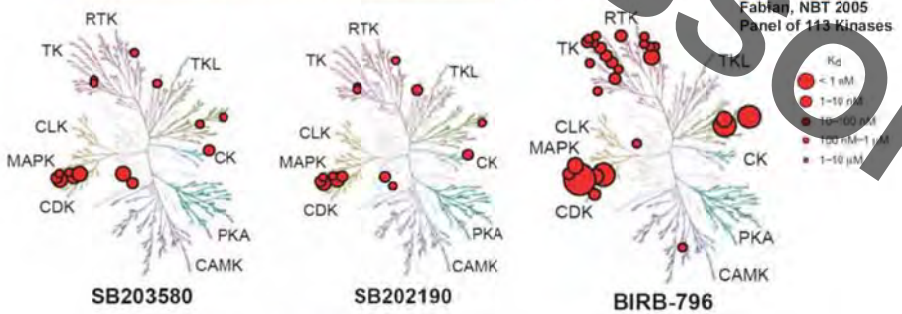
„Allosteric" (Type II) Inhibitors



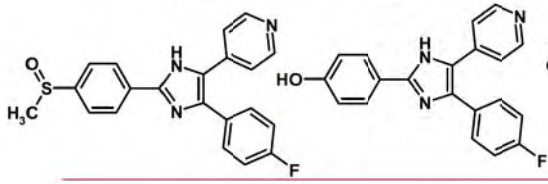


Boehringer Ingelheim BIRB 796

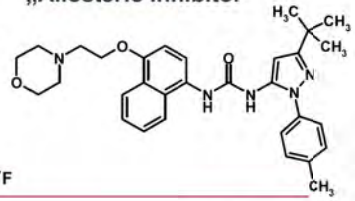
Selectivity of p38 MAPK Inhibitors Imidazole-based Inhibitors are Selective !



„ATP – competitive Inhibitors"



„Allosteric Inhibitor"



Selectivity by Glycin-Flip ?

C.E. Fitzgerald, Nature Structural Biology, September 2003, 764-9
Inhibitor-induced Peptide Flip at the Hinge Region (Met 109 – Gly 110)

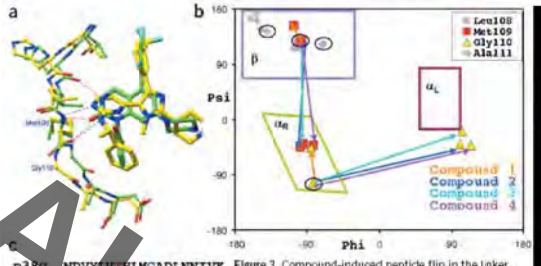
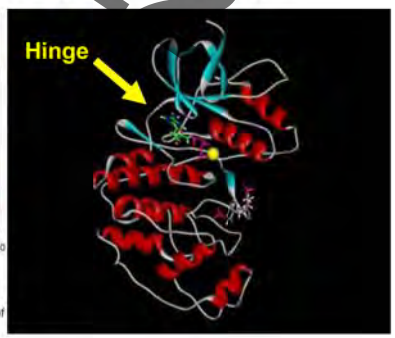
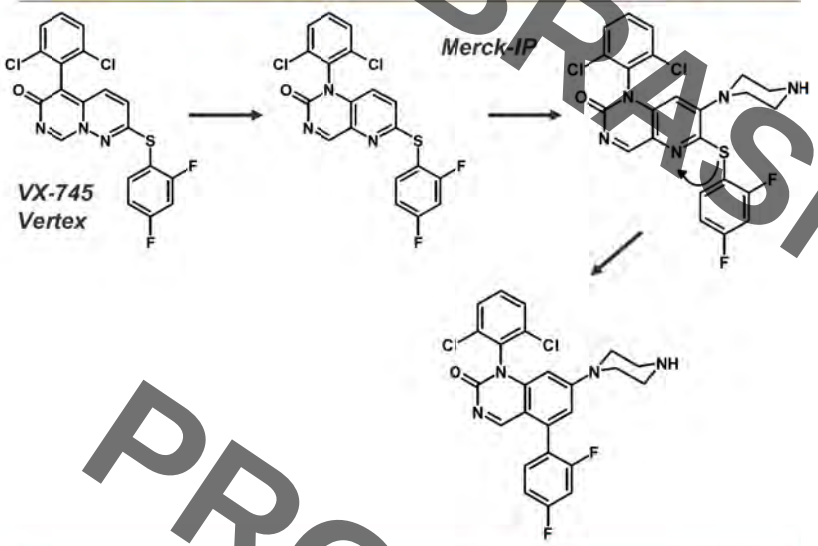


Figure 3 Compound-induced peptide flip in the linker region. (a) Overlay of p38α-bound compounds 1 (yellow) and 2 (green)[24]. (b) Schematic diagram of peptide flip in the Ramachandran plot. Black circles indicate positions of residues Leu108–Ala111 in the WT enzyme. Arrows indicate the shifts associated with binding of compounds 1–4. (c) Alignment of sequences of human MAP kinases (residues 100–118, p38α numbering): this region includes part of hydrophobic pocket I and the linker region. Thr106 (red) and Gly110 (blue) are unique to p38α and β, and are the residues responsible for the p38 selectivity of the pyridinylimidazole, quinazolinone and pyridol-pyrimidine inhibitors.



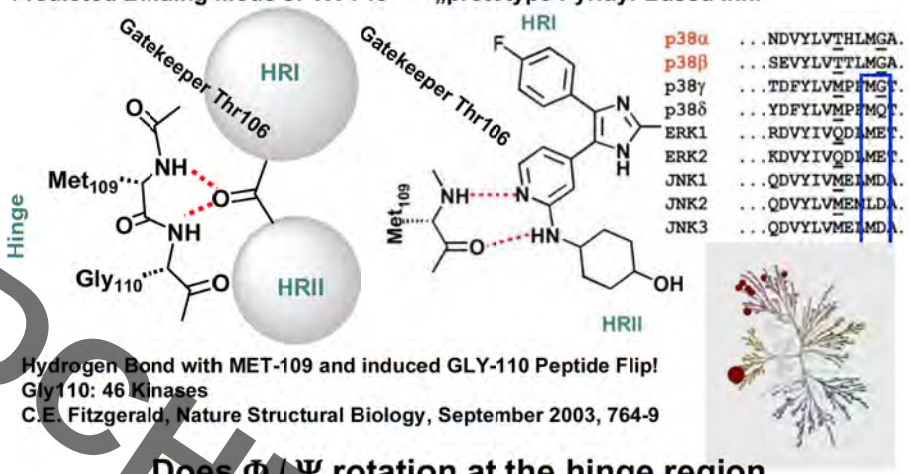
Does of Φ / Ψ rotation at the hinge region contribute to selectivity ?

VX as lead for Merck and others



Selectivity by Glycin-Flip: Lessons from VX

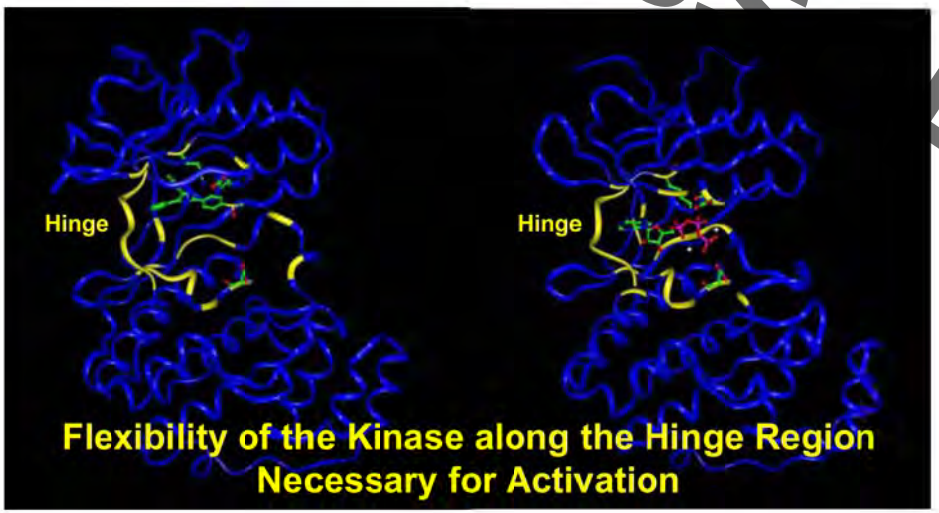
Predicted Binding Mode of VX-745 - „prototype Pyridyl-Based Inh.



Hydrogen Bond with MET-109 and induced GLY-110 Peptide Flip!
Gly110: 46 Kinases
C.E. Fitzgerald, Nature Structural Biology, September 2003, 764-9

Does Φ / Ψ rotation at the hinge region contribute to selectivity ?

Activation of p38 MAP Kinase



Selectivity by Glycin-Flip and Beyond Design Hypothesis

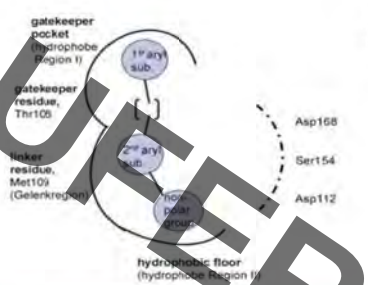
Minimal Pharmacophore: C=O

„Small is beautyful“: Linear Binders

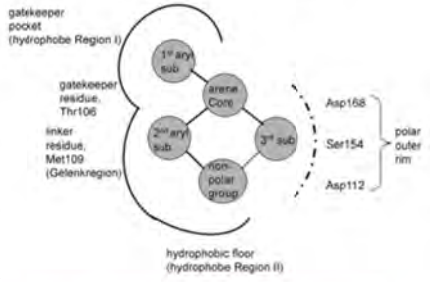
Flexible Target - Flexible Ligand: Unselective Inhibitor

Flexible Target - Rigid Ligand: Selective Inhibitor?

Linear Binder



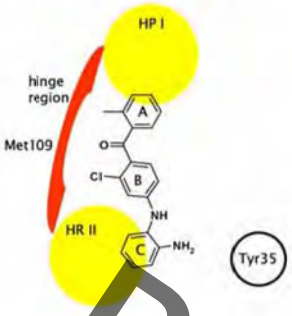
Teardrop Binder



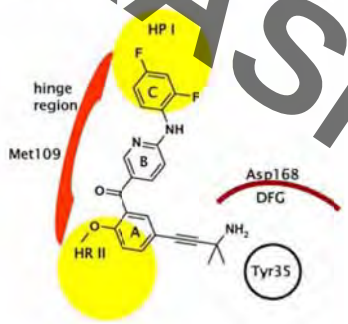
Sketch from: Lee et al., Curr.Med.Chem. 2005, 12, 2979-94

Carbonyl-based Linear Binders

Examples of Carbonyl-based Linear Binders

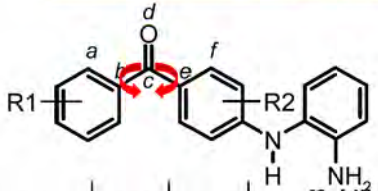


Ottosen et al. (2003)
J Med Chem 46:5651



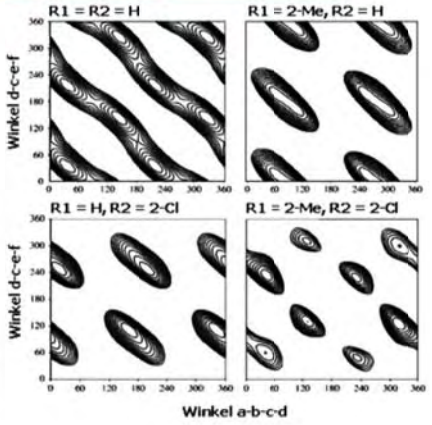
Revesz et al. (2004)
Bioorg Med Chem Lett 14:3601

Towards more Rigid Inhibitors
SAR of LEO-Type Compounds



Code	R1	R2	IC_{50} [nM] p38*	IC_{50} [nM] TNF-α§
(3)	H	H	145 ± 62	159
(40)	2-CH_3	H	29 ± 9	47
(42)	2-Br	H	54 ± 17	34
(41)	3-CH_3	H	574 ± 16	467
E	2-CH_3	2-Cl	10 ± 3	6
F	2-CH_3	2-CH_3	13 ± 3	8
(47)	2-OCH_3	2-Cl	5 ± 2	20
(46)	2-CH_3	3-CH_3	143 ± 26	127
(44)	H	2-Cl	139 ± 9	214

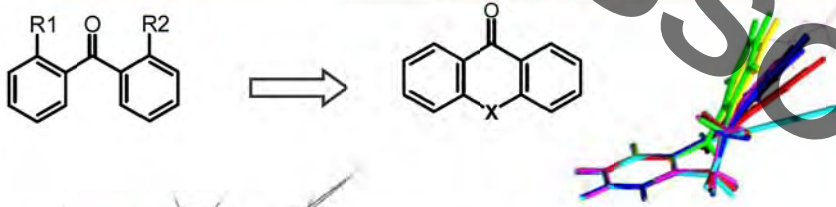
* humane rekombinante aktivierte p38α
§ LPS-induzierte TNF-α-Freisetzung aus humanen PBMCs

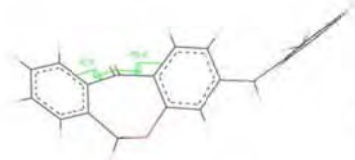


Ottosen et al. (2003)
JMC 46:5651

Torsion Angles Determines Activity ?

Towards more Rigid Inhibitors
Fixing the Angle



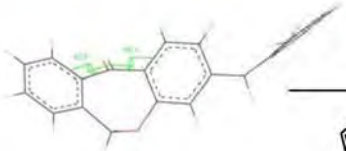


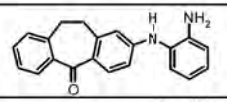
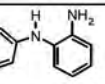
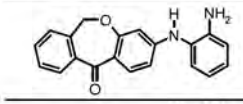
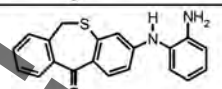
X: CH_2-CH_2, CH_2-O, CH_2-S, CH=CH, $O-CH_2$, $S-CH_2$

	[α]	[β]	[α]-[β]
Thiepinon	55,6°	69,7°	14,1
Oxepinon	42,6°	55,4°	12,8°
$-CH_2-CH_2-$	47,5°	63,9°	16,4°
-CH=CH-	58,1°	57,8°	0,3°

Laufer et al.: JMC 2006

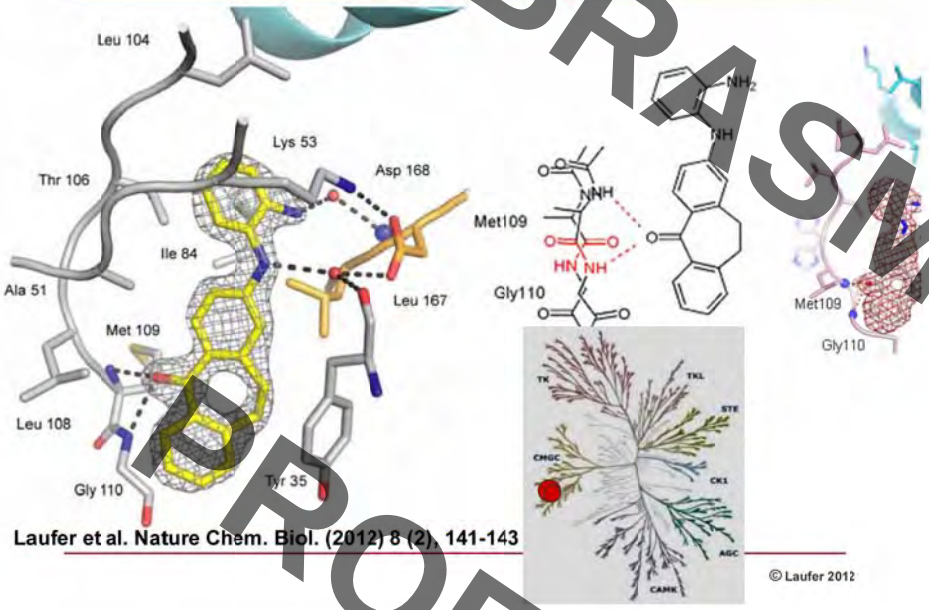
Towards more Rigid Inhibitors
Fixing the Angle: SAR



	Torsion	IC_{50} (p38α) [μM]
	16,4°	0,087
	-0,3°	1,06
	12,8°	0,303
	14,1°	0,196

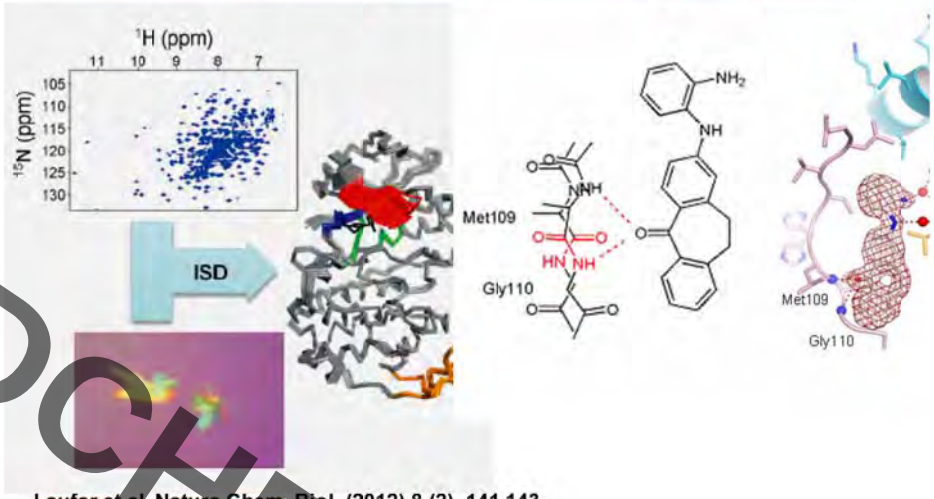
Torsion Angle Describes Activity !

Rigid Inhibitors: General Structure and Binding Mode



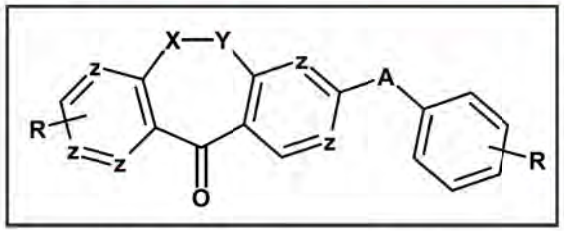
Laufer et al. Nature Chem. Biol. (2012) 8 (2), 141-143

Rigid Inhibitors Binding Mode confirmed by X-ray and NMR

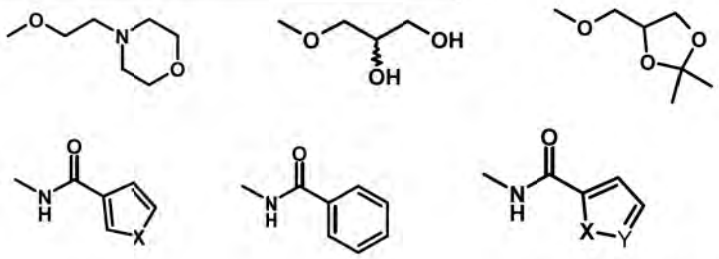


Laufer et al. Nature Chem. Biol. (2012) 8 (2), 141-143
Honndorff, Habeck, Laufer, Griesinger; Angew. Chem. Int. Ed. (2012) 51 (10), 2359-2362

Rigid Inhibitors: ADME Optimization

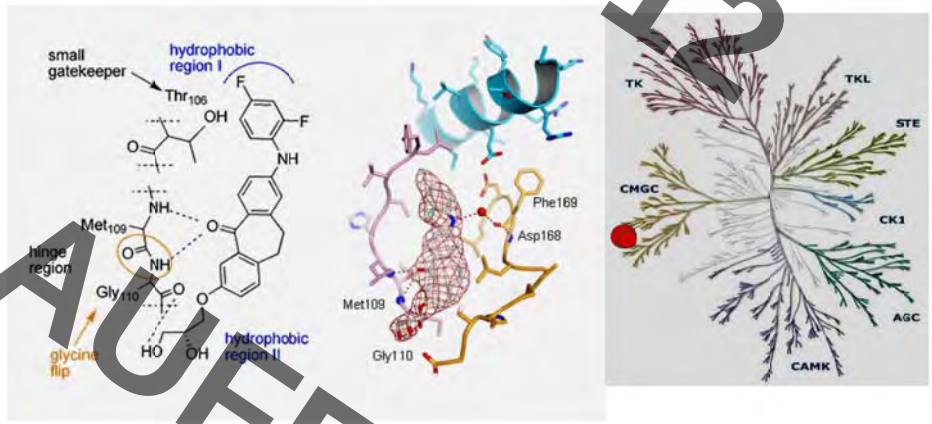


R:



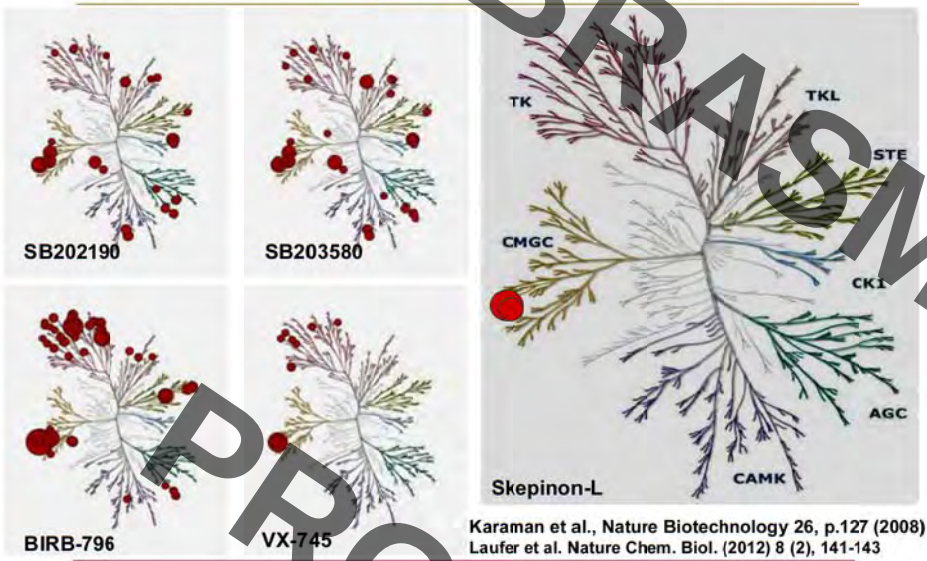
Laufer et al. J. Med. Chem. 2007, 2012, 2012

Highly selective Probe: Skepinone-L



Laufer et al. Nature Chem. Biol. (2012) 8 (2), 141-143

Rigid = Selective !



Karaman et al., Nature Biotechnology 26, p.127 (2008)
Laufer et al. Nature Chem. Biol. (2012) 8 (2), 141-143

Skepinone-L

p38α Kinase Assay (100µM ATP)
IC_{50} = 2 nM, K_d = 1.5 nM

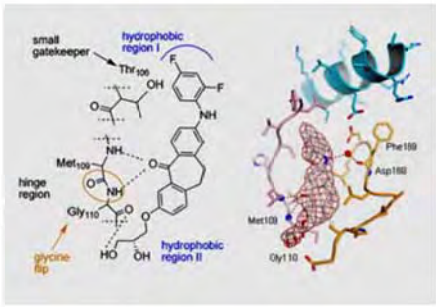
TNFα-release (LPS-stim. hWB)
IC_{50} = 22 nM

Ambit KINOMEscan, 402 Kinases
Except p38β no other Kinase affected
> 1000 fold selectivity

in vivo (Mouse LPS/GalN-induced TNFα-release: ED_{80}=3 mg/kg p.o.)

Orally available, good ADME profile
(3 mg/kg p.o. →C_{max} 200 nM, mice)
Well tollerated up to 6 months (mice)

Selected as „p38 Probe“

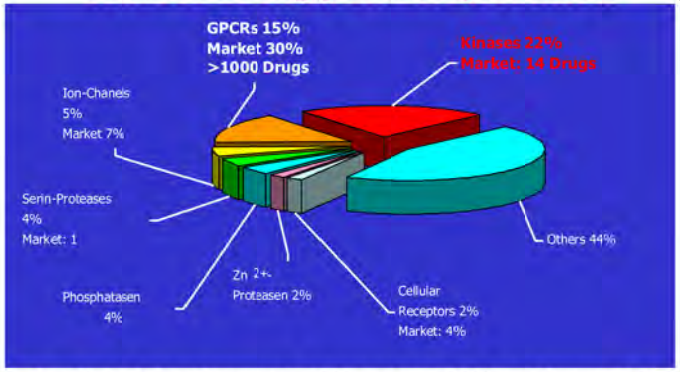


Laufer et al. Nature Chem. Biol. (2012) 8 (2), 141-143

FUTURE? - Druggable Genome!

Currently available drugs only target
15% of the Druggable Genome (450 from 3000 potential Targets)

Kinases strongly underrepresented!



>130 cpds in clinical development, 70% cancer, 30% autoimmune/inflammation
Proof of concept in RA, COPD: JAKs, SYK, (p38) most progressed