Beyond the Genomes: Small Molecules for Biology

Biological processes are dynamic!

- ⇨ Dissection: must be time-resolved!
- ⇨ Small molecule modulators:
  - Acute not chronic: rapid, reversible, conditional.
  - ⇨ Which small molecule modulators?
  - ⇨ "Chemical space" problem!

Beyond the Genomes: Small Molecules for Biology

The „Space" problem:

Chemical space occupied by small bioactive molecules?

⇨Cannot be covered by synthesis: Not enough matter/time!

⇨Does not have to be covered: Nature did not explore it!

The Decisive Question

How to identify biologically relevant and pre-validated starting points in chemical structure space for compound development?

Natural Product Chemical Space

Analyze the structures of the small molecules created by nature in evolution!

- ⇨ **Map natural product chemical space!**
- ⇨ **Intuitively accessible classification/logic?**
- ⇨ **Reduction of structural complexity retaining kind of bioactivity?**
- ⇨ **Synthesis and supply problem?**

Research Strategy

Is there a logic to reduce NP complexity at retained biological relevance? → Hierarchical, intuitively accessible classification of NP structure!

↓

Design of compound collections approaching NP structure/complexity? → Synthesis of NP-inspired compound collections!

↓

Evaluate NP-inspired compound collections in phenotypic/cell-based assays! → Will the collections approach the biological performance of NPs?

↓

Identify the target(s)/MOA! → Novel biological insight?

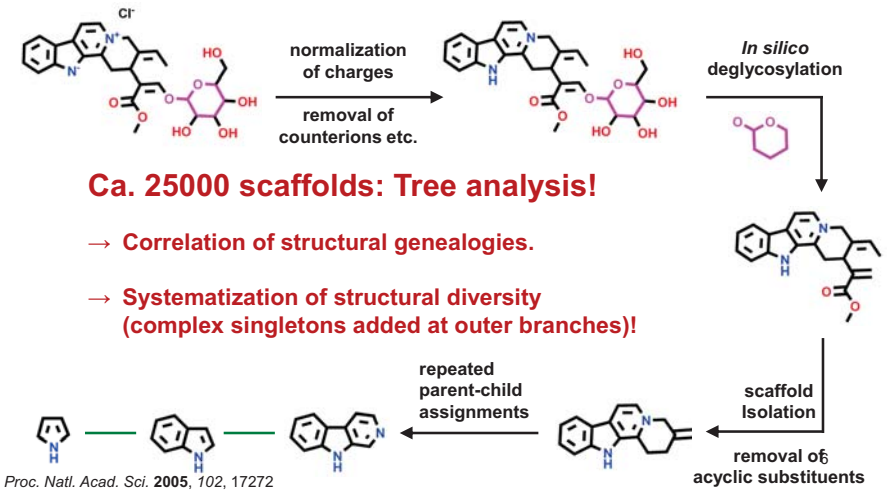
↓

Inspire drug discovery?! → Relevant to disease?

Structural Classification of Natural Products (SCONP)

(Collaboration with P. Ertl, A. Schuffenhauer, Novartis)

Data: CRC Dictionary of Natural Products (02/05) → ca. 190 000 entries.



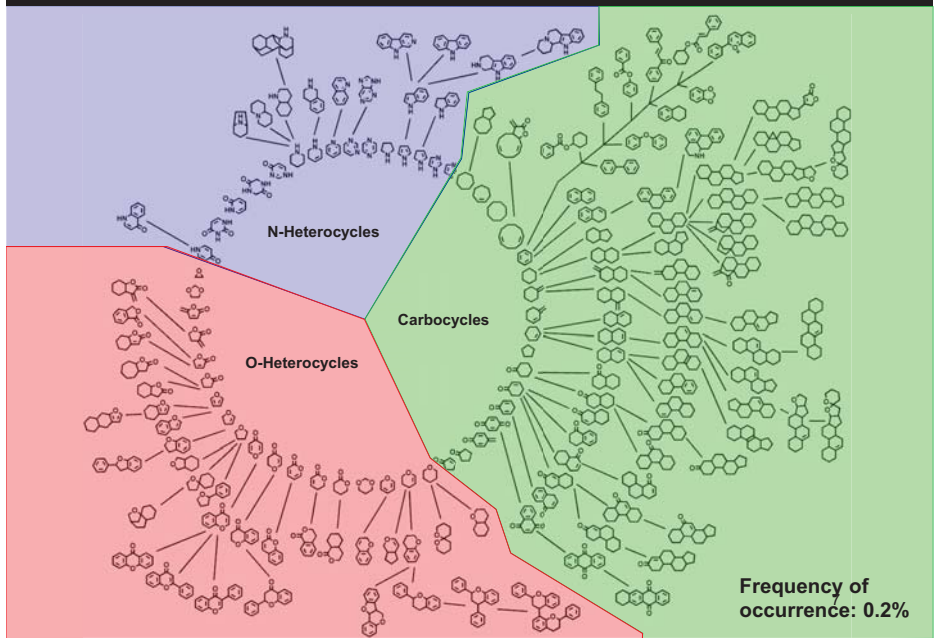
Ca. 25000 scaffolds: Tree analysis!

→ Correlation of structural genealogies.

→ Systematization of structural diversity (complex singletons added at outer branches)!

Proc. Natl. Acad. Sci. **2005**, *102*, 17272

A "Periodic Table of Natural Products" (FAZ)



A "Periodic Table of Natural Products" (FAZ)

⇨Biology Oriented Synthesis(BIOS)

Biologically validated/evolutionary selected scaffolds.

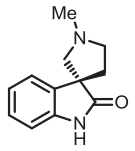
Synthesis and biological evaluation of NP-inspired compound collections!

Non-NP biologically relevant classes!

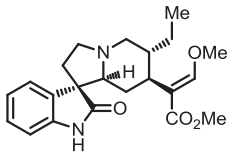
Reviews: *Angew. Chem. Int. Ed.* **2011**, *50*, 10800
J. Am. Chem. Soc. **2014**, *136*, 11853

Proc. Natl. Acad. Sci. **2005**, *102*, 17272

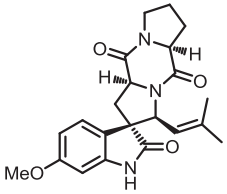
Pyrrolidinyl-Spirooxindole Natural Products



Coerulescine

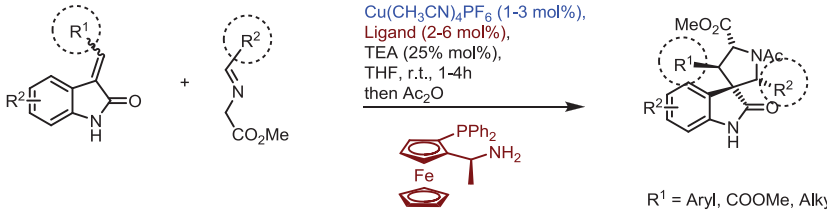


Rhynchophylline



Spirotryprostatin A

See e. g.:
Williams et al. J. Am. Chem. Soc. **2000**, *122*, 5666

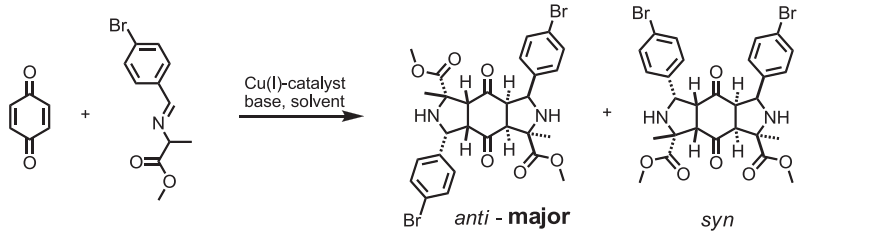


R^1 = Aryl, COOMe, Alkyl
R^2 = Aryl
58-97%, e.e. = 84-98%

Previous and related work, see e.g.:
Carretero et al., *J. Am. Chem. Soc.* **2005**, *127*, 16394
Gong et al., *J. Am. Chem. Soc.* **2009**, *131*, 12891
Gao et al., *J. Am. Chem. Soc.* **2008**, *130*, 17250

Nature Chemistry **2010**,*2*, 735

One-pot Two Step Enantioselective Catalytic 1,3-Dipolar Cycloaddition

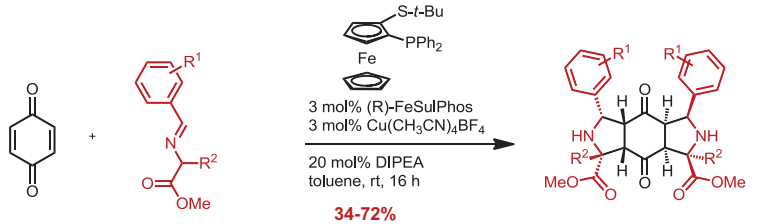


anti - **major**

syn

point symmetry - achiral

No point symmetry - **chiral**

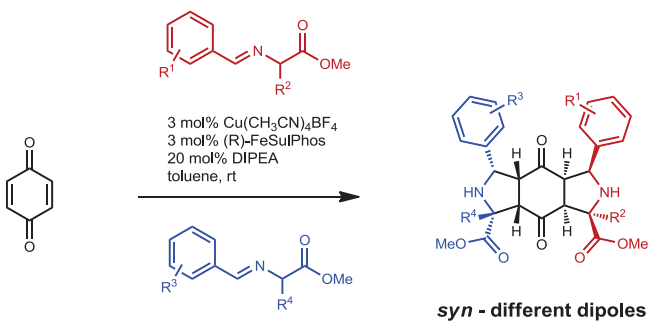


34-72%
r.r. = 94:6
d.r. >95:%
e.r. = 98:2

***syn* - major**

One-pot Two Step Enantioselective Catalytic 1,3-Dipolar Cycloaddition

1 equiv., 1h



1.2 equiv. , 15h

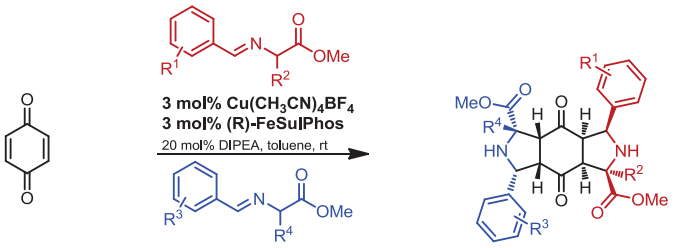
***syn* - different dipoles**

31-73%;
r.r. = 94:6;
d.r. >95;
e.r. = 98:2

Antonchick, Waldmann et al., *Nat. Chem. Biol.* **2012**, *8*, 428

One-pot Two Step Enantioselective Catalytic 1,3-Dipolar Cycloaddition

1 equiv., 1h



1.2 equiv. , 15h

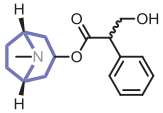
***anti* - different dipoles**

34-71%;
r.r. = 94:6;
d.r. >95;
e.r. = 98:2

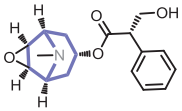
- ⇨ **ligand-controlled regio-, diastereo- and enantioselectivity**
- ⇨ **8 stereogenic centers set in one transformation**
- ⇨ **Selective formation of 1 stereoisomer out of 512 possible isomers**
- ⇨ **Programmable ⇨ enantiomers : change order of addition!**

Antonchick, Waldmann et al., *Nat. Chem. Biol.* **2012**, *8*, 428

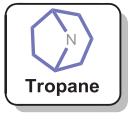
Natural Products with Tropane Scaffold



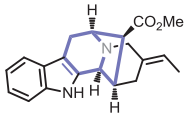
Atropine
anticholinergic,
anti-Parkinson



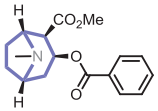
Scopolamine
autism, cancer,
Down syndrome



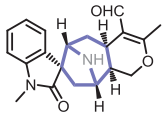
> 650 compounds known



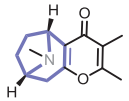
Periclivine



Cocaine

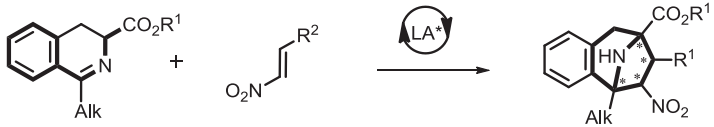


Alstonal



(-)-Darlingine

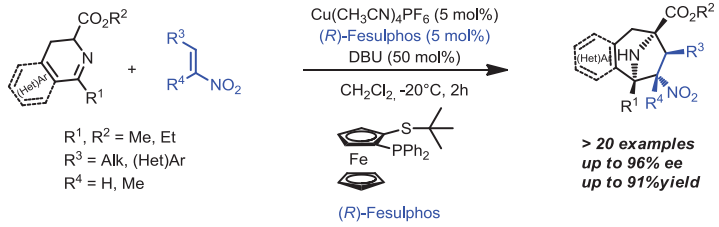
Dipolar Cycloaddition with Azomethine-Ylides for Tropane Synthesis



R^2 = Alk or Ar

⇨ **S-shaped-1,3-fused azomethine ylide**
⇨ **Unsymmetrical ketimines**
⇨ **Up to 3 quaternary stereocenters in one-step**

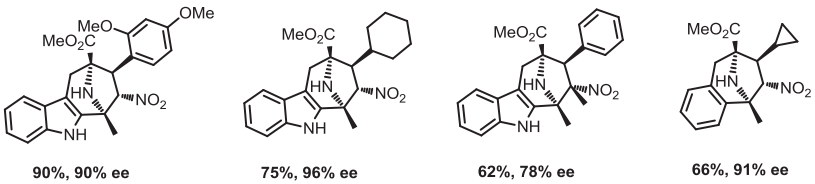
Enantioselective Cu(I) *Catalyzed* 1,3-Dipolar Cycloaddition



R^1, R^2 = Me, Et
R^3 = Alk, (Het)Ar
R^4 = H, Me

(*R*)-Fesulphos

> 20 examples
up to 96% ee
up to 91%yield



90%, 90% ee

75%, 96% ee

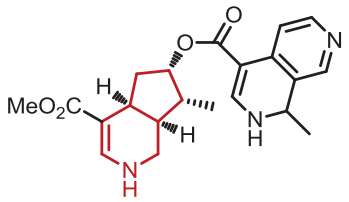
62%, 78% ee

66%, 91% ee

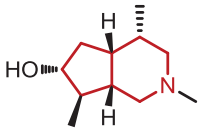
⇨ **First general catalytic enantioselective synthesis of tropane scaffold**
⇨ **Complete exo'-selectivity**

Antonchick, Waldmann et al., *Angew. Chem. Int. Ed.*, **2013**, *52*, 12892

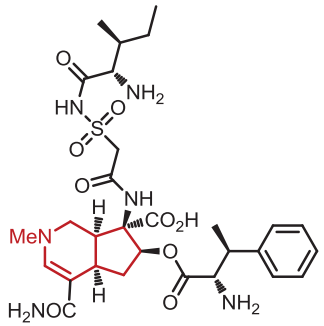
Natural Products with Annulated Piperidine Scaffold



Scaevodimerine A

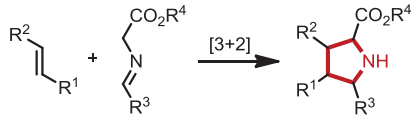


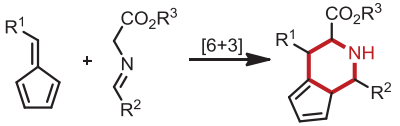
Incarvilline

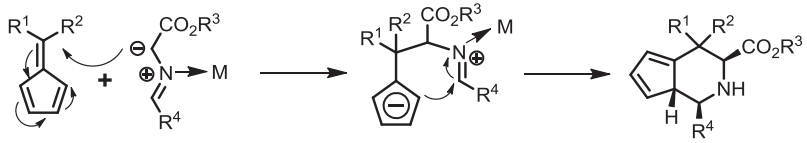


SB-203208

Enantioselective Catalytic [6+3] Cycloaddition

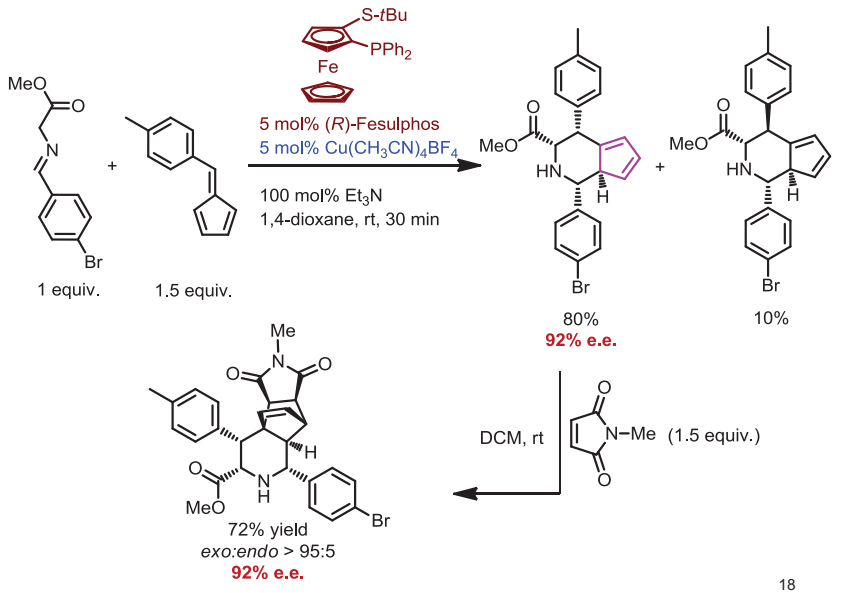






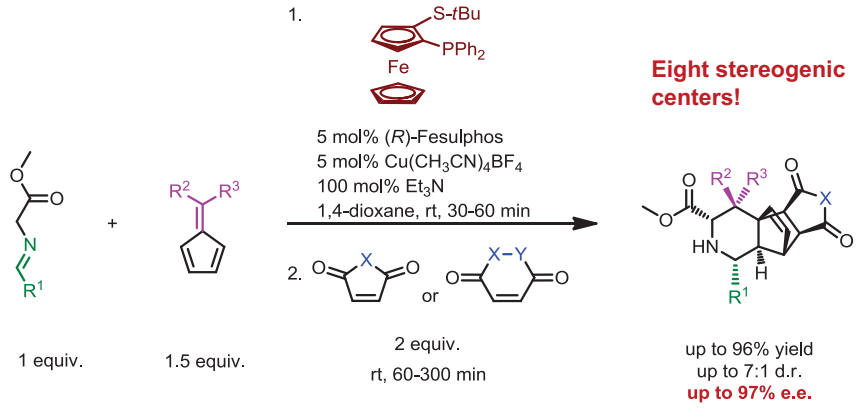
B.-C. Hong et al., *Org. Lett.* **2003**, *5*, *10*, 1689-1692.

Enantioselective Catalytic [6+3] Cycloaddition



Antonchik, Waldmann, et al., *Angew. Chem. Int. Ed.* **2012**, *51*, 9512; *Chem. Commun.*, **2013,** *49*, 7800

One-Pot, Two-Step Cycloaddition Sequence

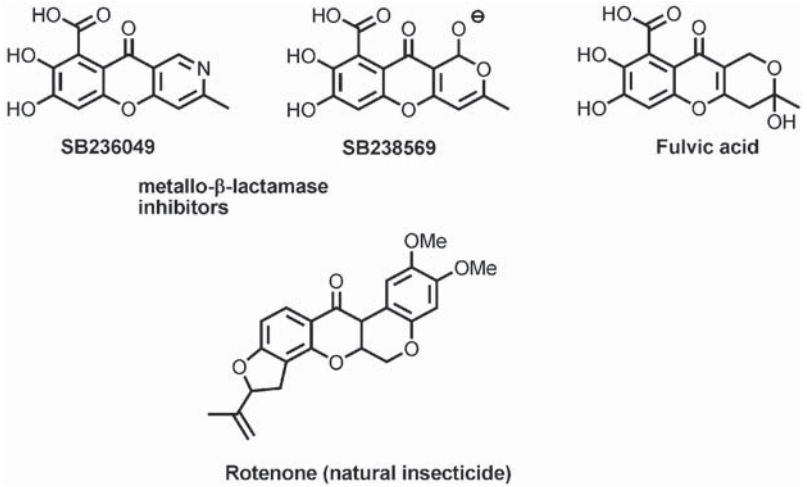


R^1 = (hetero)aromatic; R^2, R^3 = H, aromatic: 62-80%; e.e. = 92-97%,
R^1 = (hetero)aromatic; R^2, R^3 = H, aliphatic: 73-96%; e.e. = 36-61%
R^1 = aliphatic < 5%

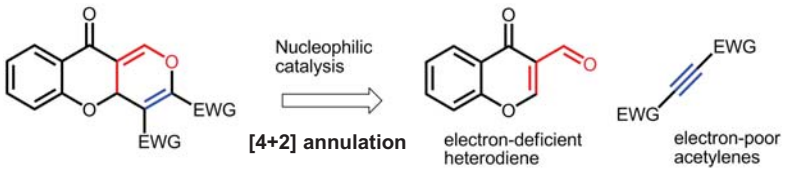
X, X-Y = maleimide, maleic anhydride, p-quinones

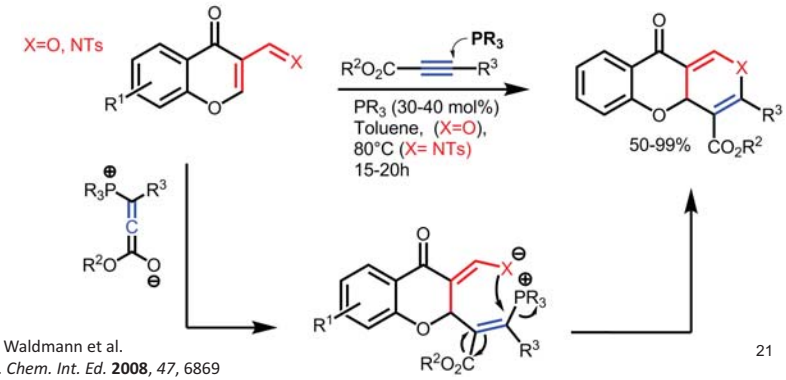
Antonchik, Waldmann, et al., *Angew. Chem. Int. Ed.* **2012**, *51*, 9512; *Chem. Commun.*, **2013,** *49*, 7800

Natural Products With Benzopyrone Scaffold



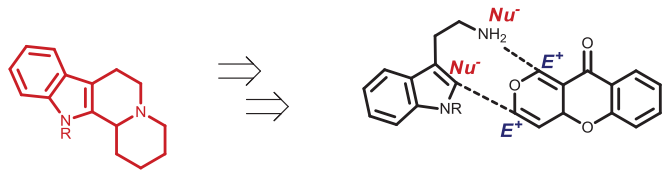
An Organocatalyzed Benzopyrone Synthesis



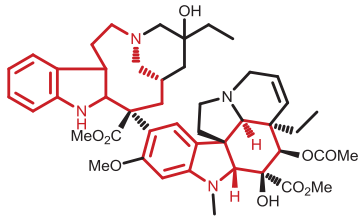


Kumar, Waldmann et al.
Angew. Chem. Int. Ed. **2008**, *47*, 6869

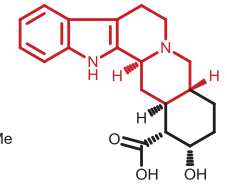
Extension of the Sequence to NP Scaffolds Related to Mitosis/Cancer



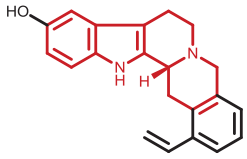
Tetrahydroindolo[2,3-*a*]quinolizines **Tryptamine** **Benzopyrone**



Vinblastin
antimitotic, anticancer drug

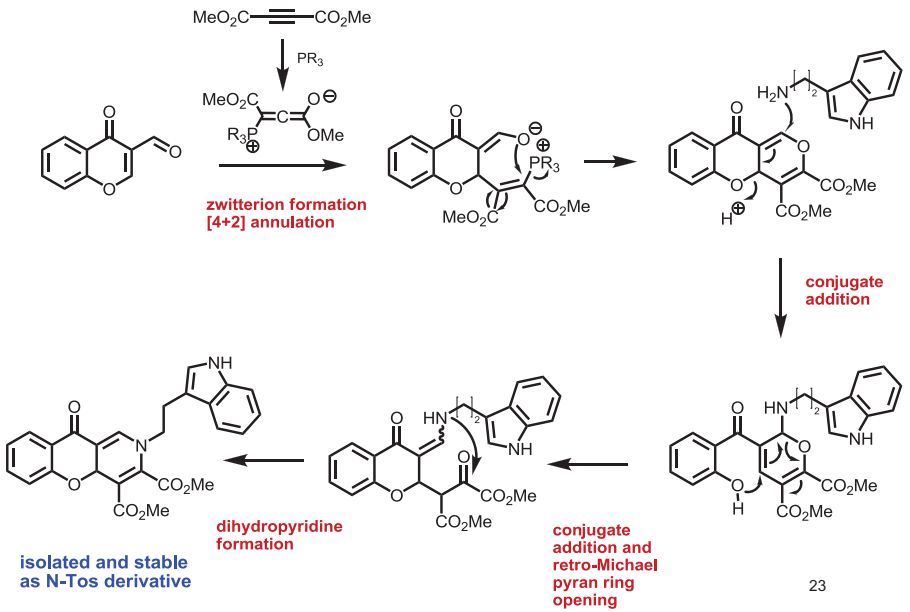


Yohimbine
anticancer activity

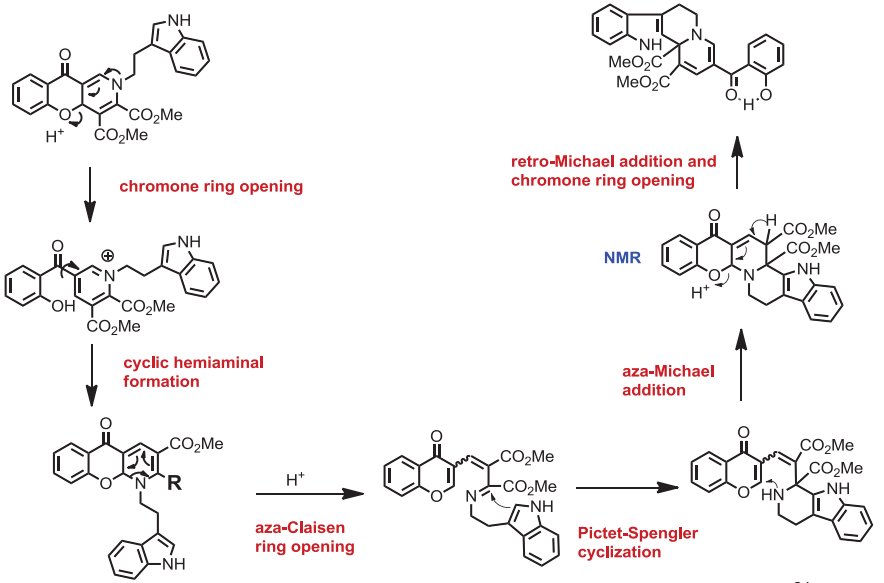


10-Hydroxyaugustine
cytotoxix to human T14 bladder-cancer cell line

Extension of the Sequence



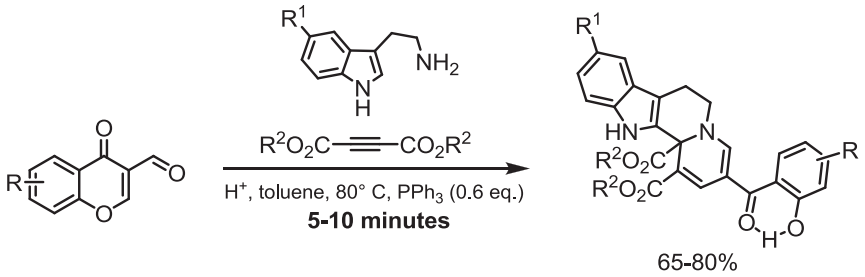
Extension of the Sequence



R = CO_2Me; **R = H/COOMe: crystal structure/NMR**

Kumar, Waldmann et al., *Nat. Chem. Biol.* **2012**, *8*, 179

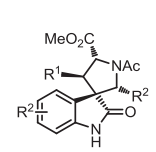
One-pot Cascade Synthesis of Indoloquinolizines



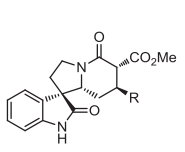
- ⇨ **One-pot synthesis.**
- ⇨ **Twelve consecutive transformations including nine different reactions.**
- ⇨ **Longest cascade sequence known.**
- ⇨ **Enantioselective catalysis.**

Kumar, Waldmann et al., *Nat. Chem. Biol.* **2012**, *8*, 179

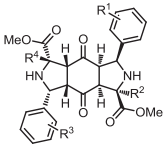
Synthesis of NP-Inspired Compound Collections



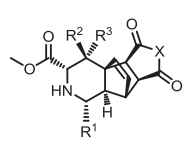
Nature Chemistry **2010**,*2*, 735



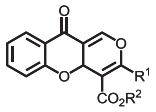
Chem. Biol. **2013**, *20*, 500.



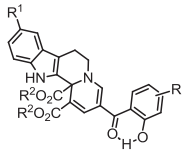
Nat. Chem. Biol. **2012**, *8*, 428



Angew. Chem. Int. Ed. **2012**, *51*, 9512



Angew. Chem. Int. Ed. **2008**, *47*, 6869



Nat. Chem. Biol. **2012**, *8*, 179
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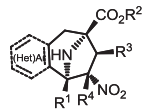
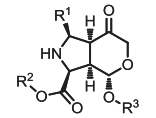


Angew. Chem. Int. Ed., **2013**, *52*, 12892



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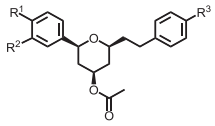

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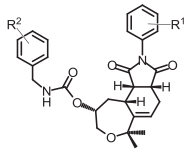
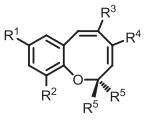
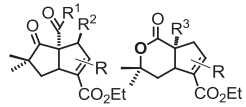


Proc. Natl. Acad. Sci.. **2011**, *108*, 6805

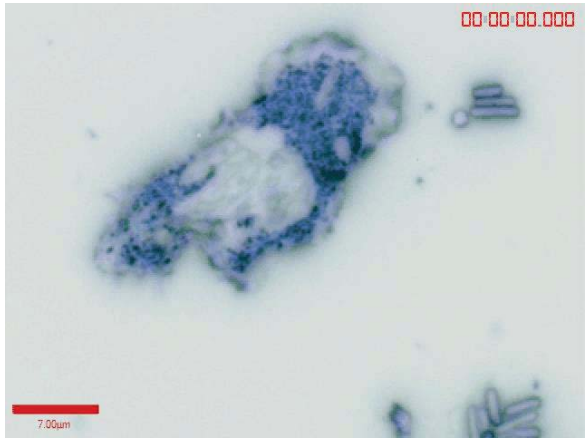


Angew. Chem. Int. Ed. **2011**, *50*, 9076



Angew. Chem. Int. Ed., **2013**, *52*, 9576

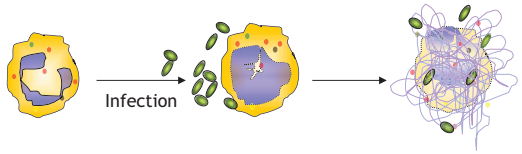
Neutrophil Chase

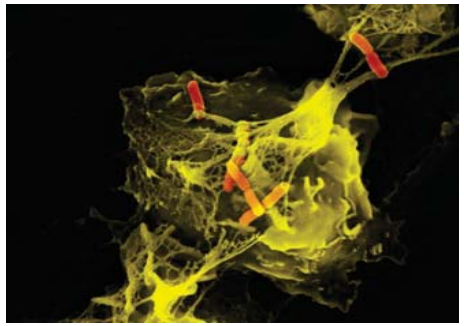


Movie:
V. Brinkmann,
MPIIB, Berlin

- ⇨ **Uptake of pathogen by the phagolysosome**
- ⇨ **Formation of reactive oxygen species (ROS)**
- ⇨ **Neutrophilic enzymes from granules (elastase, defensins)**
- ⇨ **Antimicrobial activity.**

New Mechanism: Neutrophil Extracellular Traps

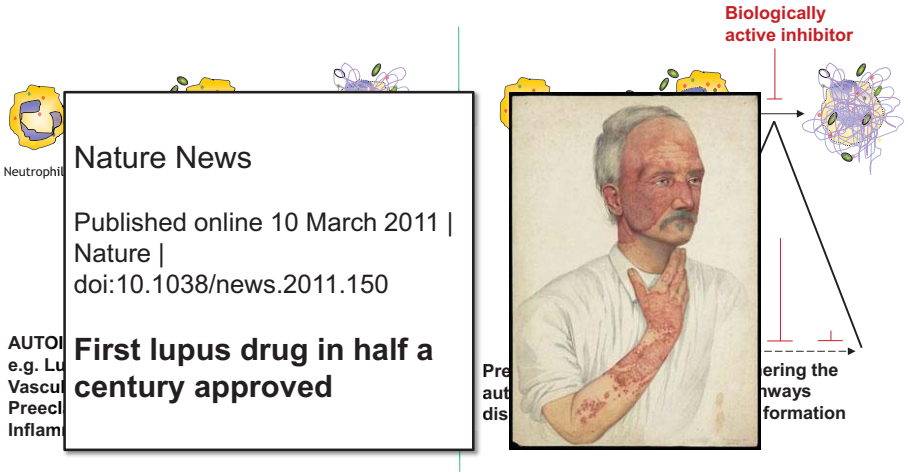




- **NETs: formed via „netosis“:**
  - ⇨**Activation of NADPH oxidase**
  - ⇨**ROS formation**
  - ⇨**Lobules of neutrophils disappear**
  - ⇨**Chromatin expands**
- **Extracellular structures that are composed of chromatin, with specific proteins from the neutrophilic granules attached, e. g. proteases.**
- **NETs bind to gram-positive & gram-negative bacteria as well as fungi**

V. Brinkmann und A. Zychlinsky,
Nature Reviews Microbiology, **2007**, 5, 577 - 582

Need for Modulator Screen



Nature News

Published online 10 March 2011 | Nature | doi:10.1038/news.2011.150

First lupus drug in half a century approved

Neutrophils:

⇨ Develop in the bone marrow

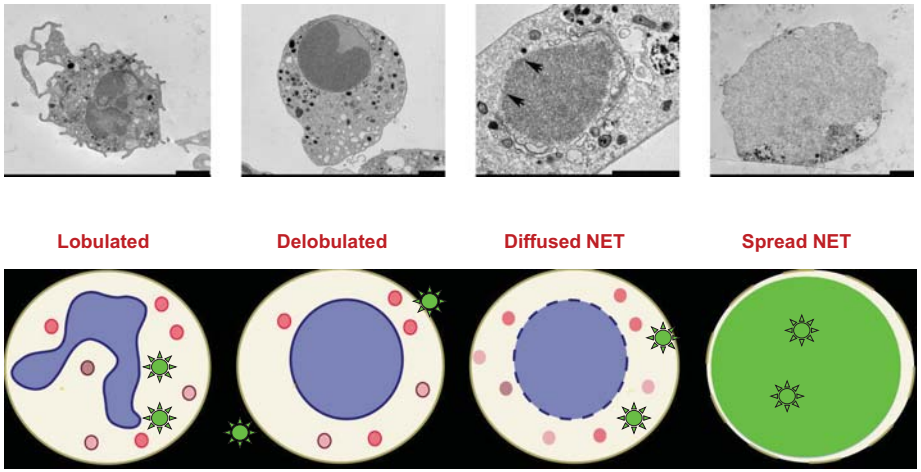
⇨ Reach circulation when they are terminally differentiated: **half life of six hours**

⇨ Due to short half life and developmental state: **cannot be transfected or transduced.**

⇨ **No available cell lines** that faithfully mimic the characteristics of neutrophils.

Phenotypic NET Formation Modulator Screen

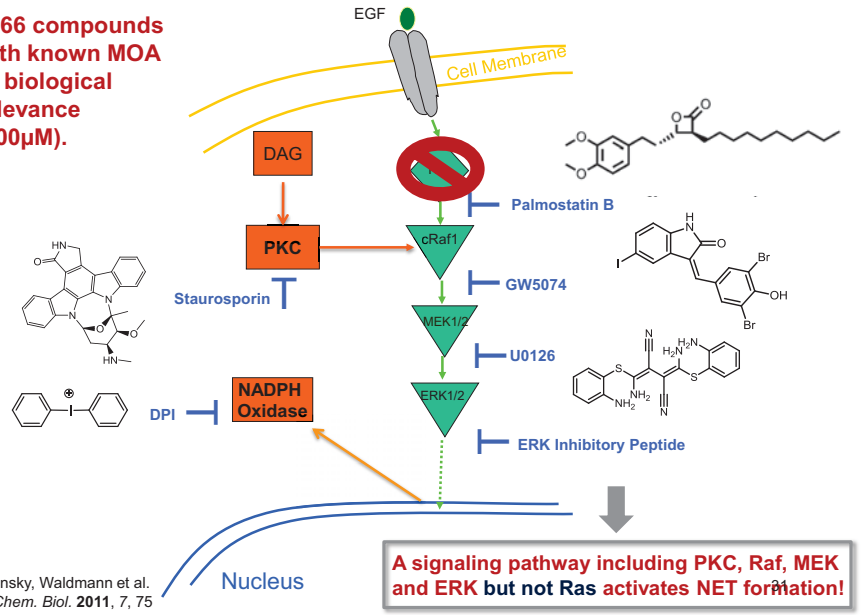
Automated microscopy and phenotype scoring!



Detection of cell death by Sytox Green incorporation into DNA of dead cells

The Raf/MEK/ERK Pathway in NET Formation

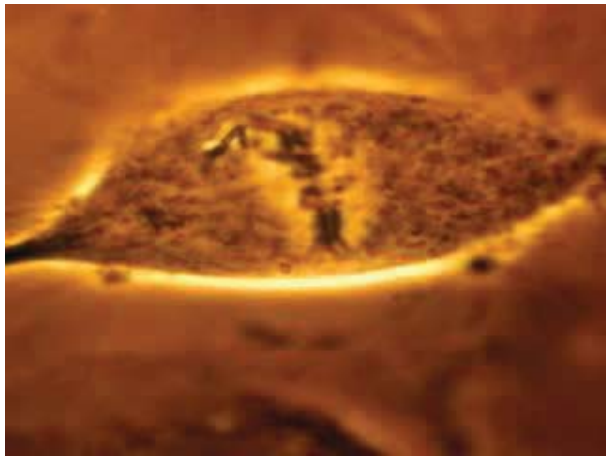
1266 compounds with known MOA or biological relevance (100µM).



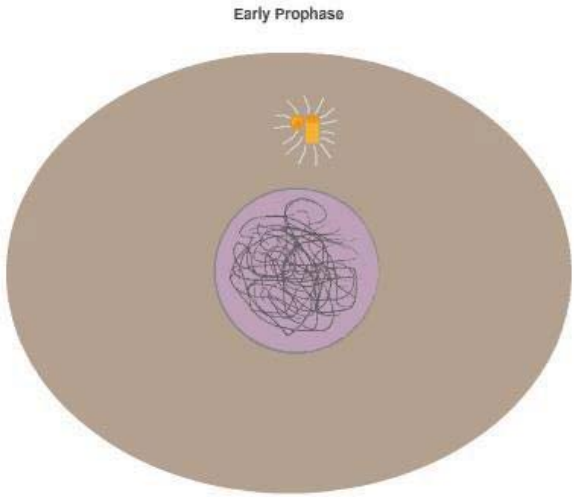
Zychlinsky, Waldmann et al. *Nat. Chem. Biol.* **2011**, *7*, 75

A signaling pathway including PKC, Raf, MEK and ERK but not Ras activates NET formation!

Mitosis

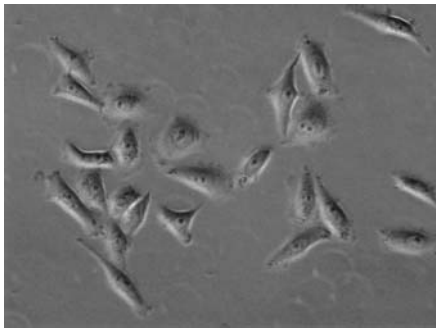


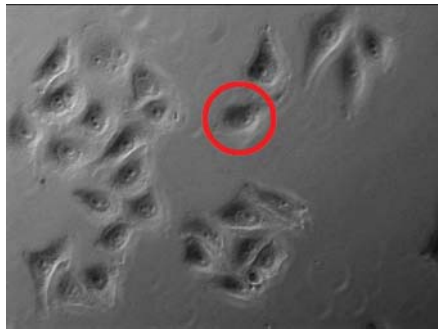
Mitosis



Cell Division

HeLa cells (cervical cancer)



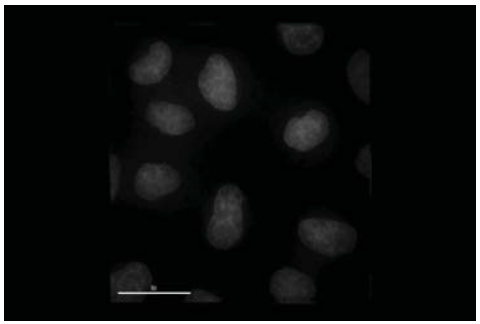


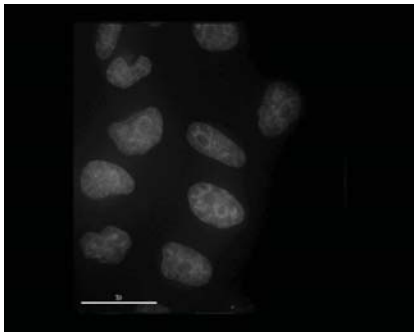
Control **30 µM compound**

Kumar, Waldmann et al., *Nat. Chem. Biol.* **2012**, *8*, 179

Chromosome Misalignment

HeLa cells with histone H2B-GFP



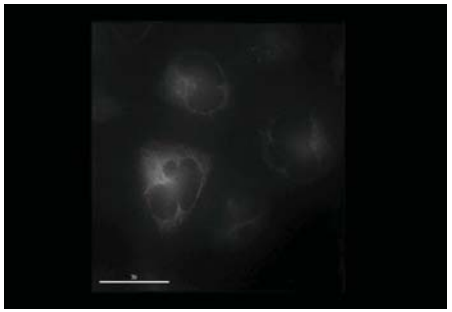


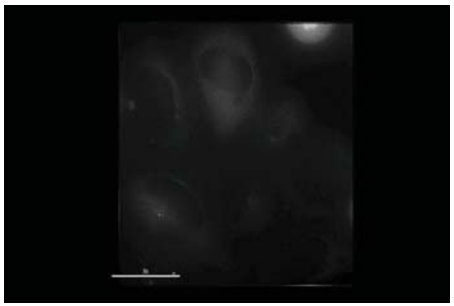
Control **30 µM compound**

Kumar, Waldmann et al., *Nat. Chem. Biol.* **2012**, *8*, 179

Spindle Formation During Mitosis

U2OS (osteosarcoma cells) with Cherry tubulin





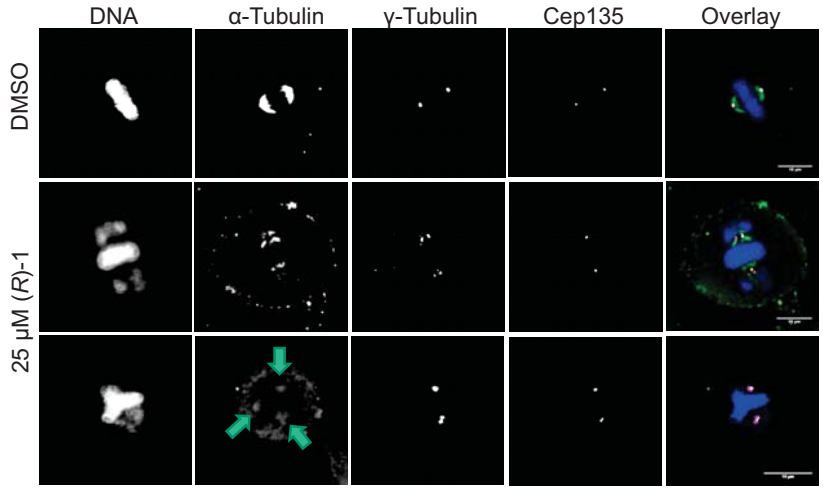
Control **30 µM compound**

Additional cell lines: COS-7, SW 480, MCF-7

Kumar, Waldmann et al., *Nat. Chem. Biol.* **2012**, *8*, 179

Phenotype: Impaired Centrosome Integrity

Hela cells – phenotypes observable to 1µM

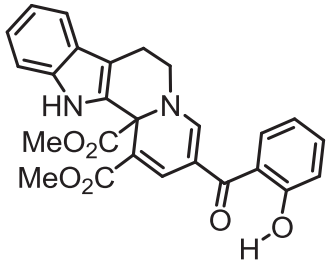


Chromosome misalignment. Metaphase plate impaired.

Multipolar spindles – aberrant centrosome numbers.

Centrosome integrity impaired!

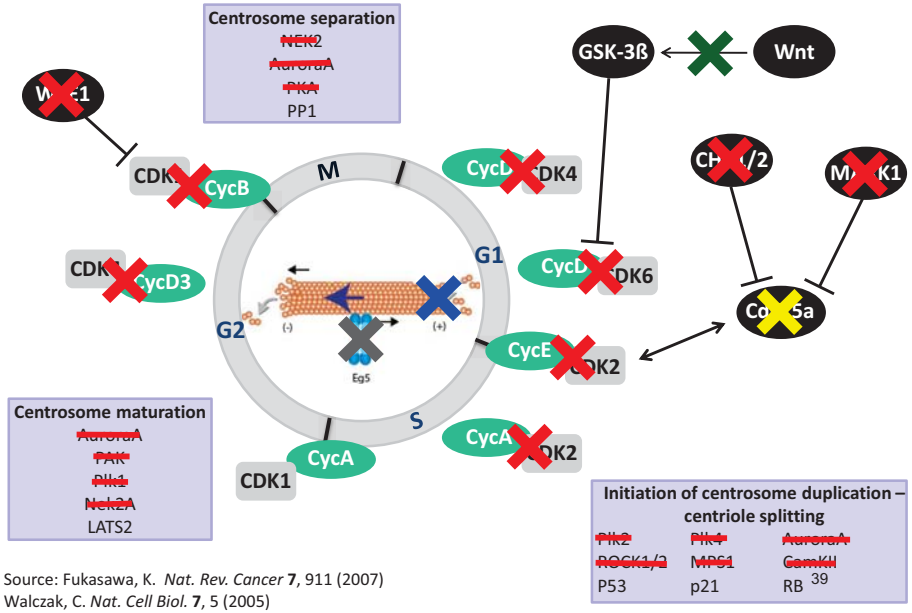
Parent Indoloquinolizine Obtained from the One-pot Cascade



Modulator of centrosome duplication: Centrocountin 1

Kumar, Waldmann et al., *Nat. Chem. Biol.* **2012**, *8*, 179

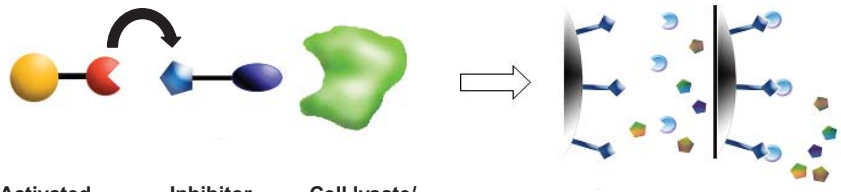
Potential Targets: Educated Guesses



Source: Fukasawa, K. *Nat. Rev. Cancer* **7**, 911 (2007)
Walczak, C. *Nat. Cell Biol.* **7**, 5 (2005)

Target Identification: Chemical Proteomics

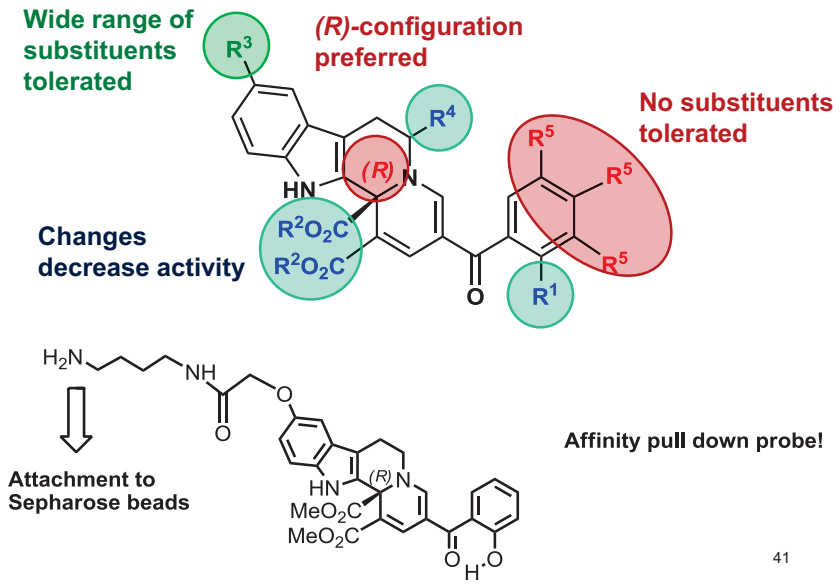
- Pulldown from HeLa cell lysate



Activated sepharose beads | **Inhibitor with linker** | **Cell lysate/ Protein mixture** | **Release**

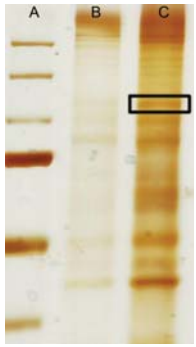
⇒ Identification of bound proteins

Structure Activity Relationship (SAR) Analysis



Target Identification: Mass Spectrometry

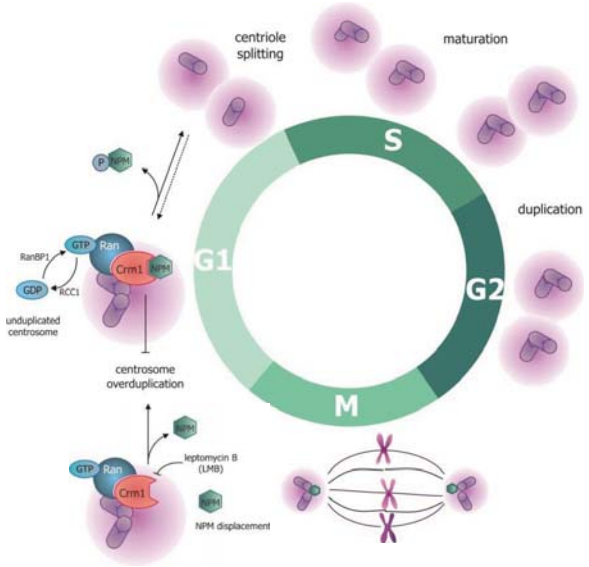
Separation on 1D-SDS-Gel



Nucleophosmin (NPM)-Crm1-Complex

- **Release of bound proteins from beads with free ligand**
- **Analysis of tryptic peptides by Nano-LC-MS/MS**
- **Identification of proteins by MS/MS ion search**

Cellular Centrosome Counting



NPM and Crm1 Validation

Molecular Biology: immunoblot, competitive blot

NPM, Crm1 confirmed with specific antibodies
Competition with free ligand

Biophysics: fluorescence polarization, surface plasmon resonance, isothermal titration calorimetry, T_m-shift, ELISA

Concentration dependent polarization signal increase upon binding of fluorescent-tagged Centrocountin to NPM, Crm1

Genetics: RNAi knockdown

NPM, Crm1 knockdown: centrosome amplification (n>2); impaired
Wang et al., *Nat.Cell Biol.* **2005**, *7*, 823
Zhang, C. et al., *Biochem. Biophys. Res. Commun.* ***2009***, *384*, 383.

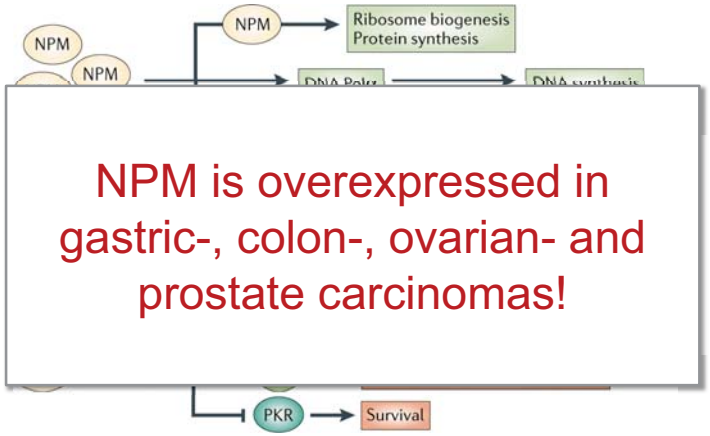
Cell biology/imaging: Interaction in cells

Fluorescence lifetime reduction for Citrine-NMP, YFP-Crm1 upon binding of fluorescent-tagged Centrocountin

Kumar, Waldmann et al., *Nat. Chem. Biol.* **2012**, *8*, 179

NPM Overexpression in Tumour Cells: Increased Proliferation and Inhibition of Apoptosis

S. Grisendi et al., *Nature Reviews Cancer*, **2006,** *6*, 493



NPM is overexpressed in gastric-, colon-, ovarian- and prostate carcinomas!

Opposes the apoptosis machinery be different mechanisms, e. g.:
⇨ Interacts with and inhibits apoptosis initiation by p53
⇨ Opposes DNA-fragmentation by caspase-activated DNAse (CAD)

Acknowledgements

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