Challenges in Agrochemicals Design

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Computational Chemistry & Biology
BASF SE, Ludwigshafen

BASF – *The* Chemical Company

104.779 employees worldwide, 33.000 at Ludwigshafen and > 8.000 in R&D

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BASF´s Portfolio

Agricultural Solutions

Crop Protection

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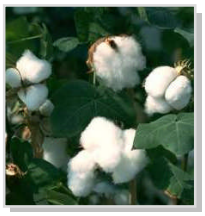
Agricultural Solutions

Crop protection

Herbicides
against weeds

Insecticides
against harmful insect pests

Fungicides
against harmful diseases



Others
e.g. growth regulators

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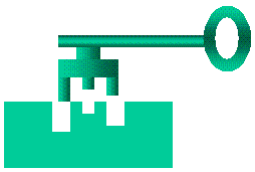
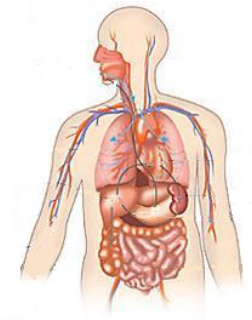
Drugs & Agrochemicals

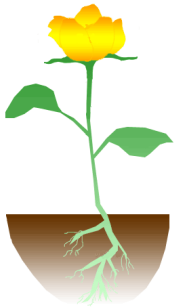




Efficacy

&

Bioavailability



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Mode of Action of Agrochemicals

Herbicides

Lipid Synthesis
- Acetyl CoA carboxylase

Branched Chain aa Synthase
- Acetolactate synthase

Photosynthesis PS I / II

Protoporphyrinogen Oxidase

Pigment Synthesis
- PDS
- HPPD

EPSP Synthase (*Glyphosate*)

Microtubule Assembly

Cell Division

Cell Wall (cellulose) Synthesis

Auxin Transport

Fungicides

Nucleic Acid Synthesis
- RNA polymerase I
- Adenosin-deaminase

Mitosis and Cell Devision
- β-tubulin assembly

Respiration
- Succinate-dehydrogenase
- Cytochrome bc1 (Q_o & Q_i)

AA and Protein Synthesis

Signal Transduction
-MAP/Histidine kinase

Lipid and Membrane Synthesis
- Methyltransferase

Sterol Biosynthesis
- C14 demethylase
- Δ^{14} reductase

Insecticides

Nervous System
Acetylcholinesterase

Ion Channels
GABA-gated Cl-channels
Sodium channel modulators
nAChR agonists
nAChR allosteric modulators
nAChR channel blocker
VGSC blocker
Ryanodine receptor modulators
Chloride channel activators

Respiration Chain
Mitochondrial cplx. I-V inhibitors

Growth Regulators
Chitin biosynthesis

Nuclear Receptor
Ecdysone receptor agonists

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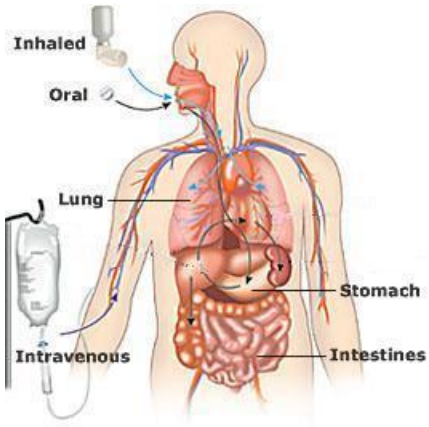
Bioavailability of Drugs
Absorption



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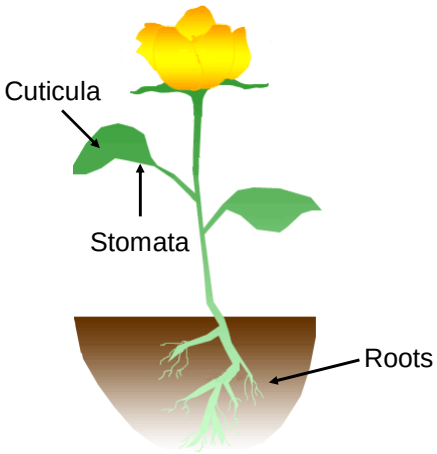
Bioavailability of Agrochemicals
Absorption – Plants



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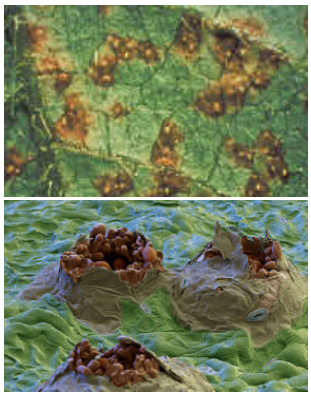
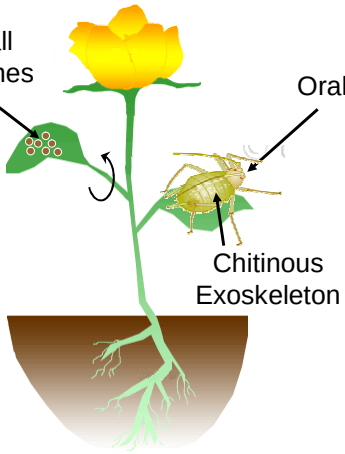
Bioavailability of Agrochemicals
Absorption – Plants, Fungi and Insects





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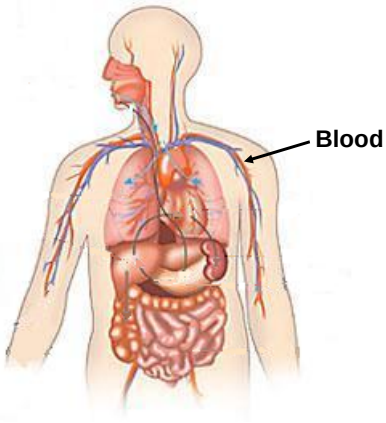
Bioavailability of Drugs
Distribution



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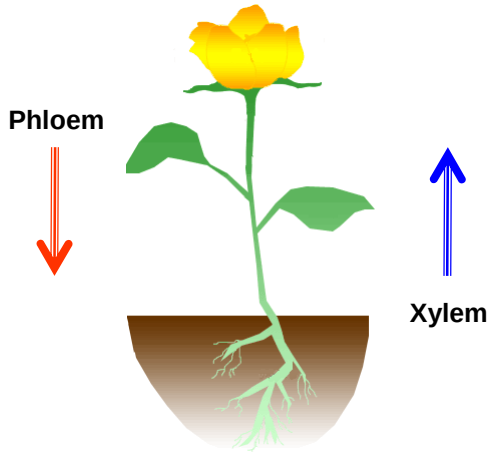
Bioavailability of Agrochemicals
Distribution/Translocation - Plants



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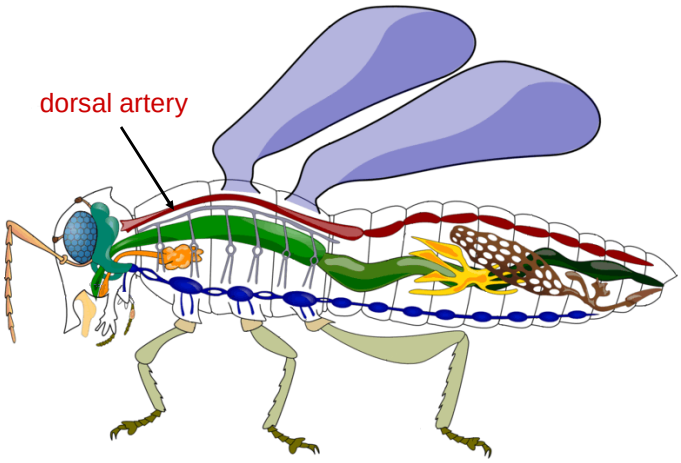
Bioavailability of Agrochemicals
Distribution - Insects



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Bioavailability of Drugs
Metabolism

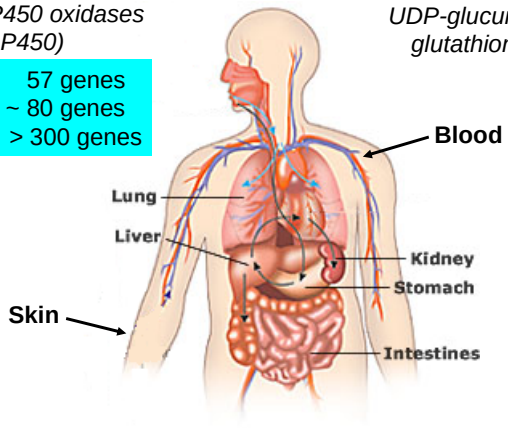
Phase I Reaction
Oxidation, Reduction, Hydrolysis

cytochrome P450 oxidases (Cyp P450)

Humans	57 genes
Drosophila	~ 80 genes
Arabidopsis	> 300 genes

Phase II Reaction
Conjugation of water-soluble groups

UDP-glucuronosyltransferases
glutathione S-transferases



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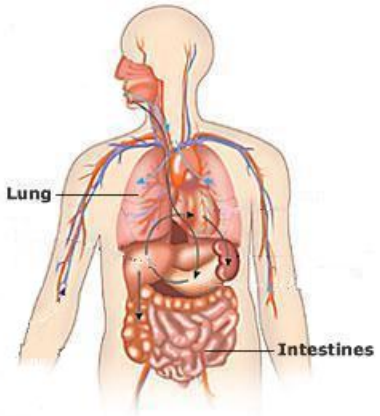
Bioavailability of Drugs
Excretion



Urine & Feces

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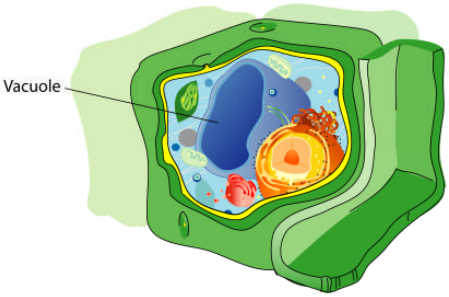
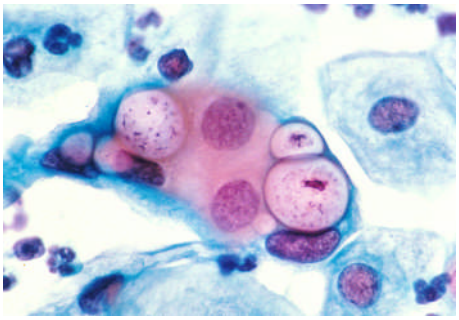
Bioavailability of Agrochemicals
Excretion - Plants





inclusion of waste products

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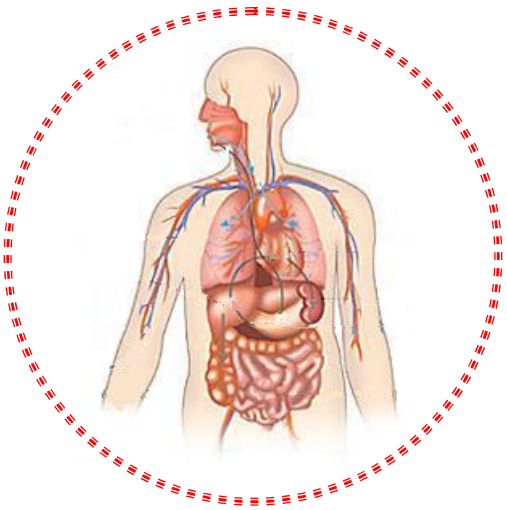
Toxicology of Drugs



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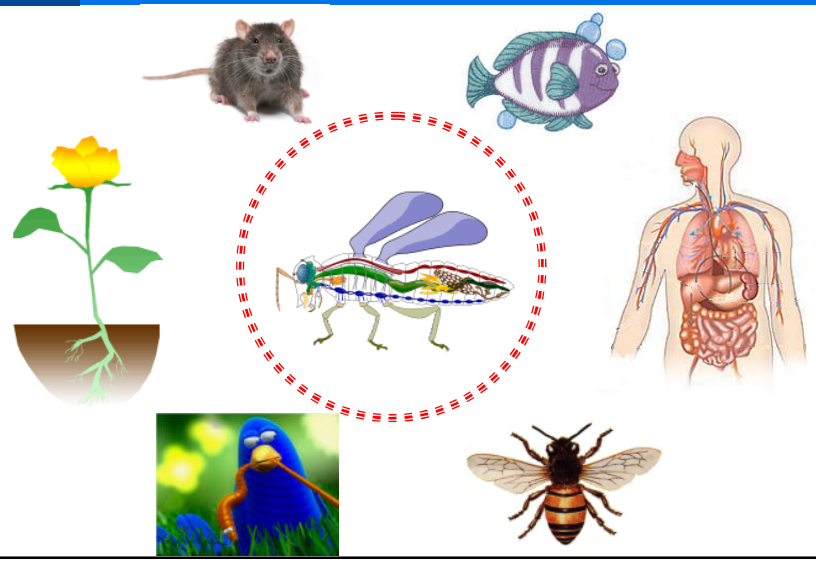
Toxicology of an Insectizide



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Regulatory relevant issues of Agchems

Enviromental Fate

Soil dissipation/accumulation
Bound residues
Ground water
Surface water
Air
Relevant metabolites

Ecotoxicology

Aquatic
Honey bee, non-target arthropods
Non-target plants
Soil organisms
Wildlife (birds, mammals)
Buffer zones
Recovery of biocoenoses

Residues

Residues level
Toxic metabolites

Toxicology

CMR properties
Endocrine disruptors
Immuno toxicity
Acute/Chronic toxicity
Risk for consumer, worker, resident
Selected formulants

Biology

Efficacy
Crop tolerance
Resistance risk
Impact on food quality
Impact on succeeding & adjacent crops

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Bioavailability of Agrochemicals
Specificity of Herbicides

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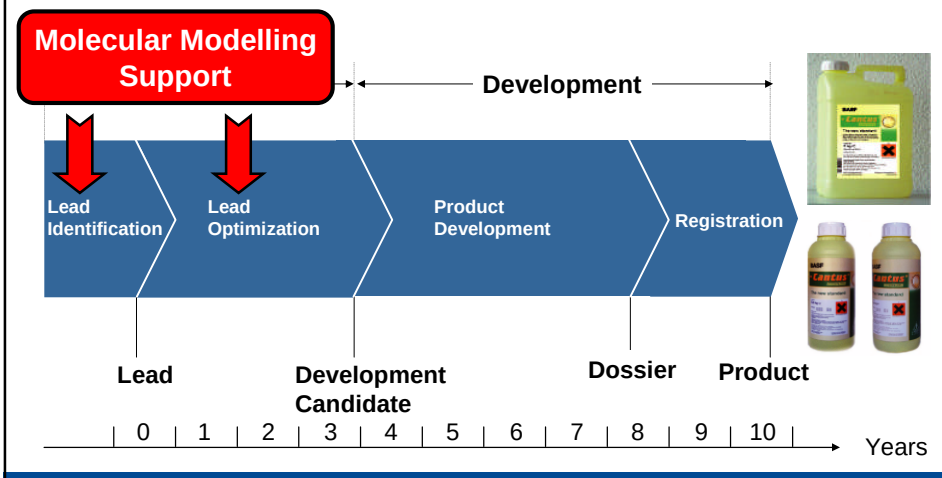
R&D Process



➔ R&D cost per product around $ 250 million (industry average)

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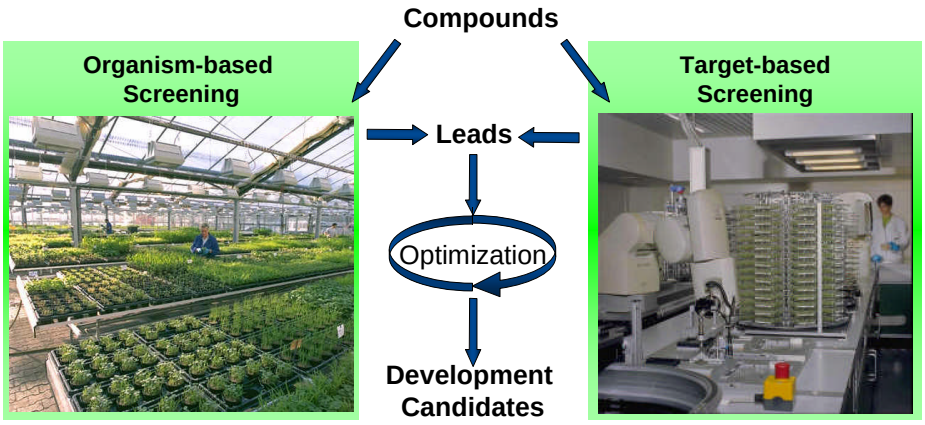
New Lead Identification in Crop Protection



➔ Output is complementary!

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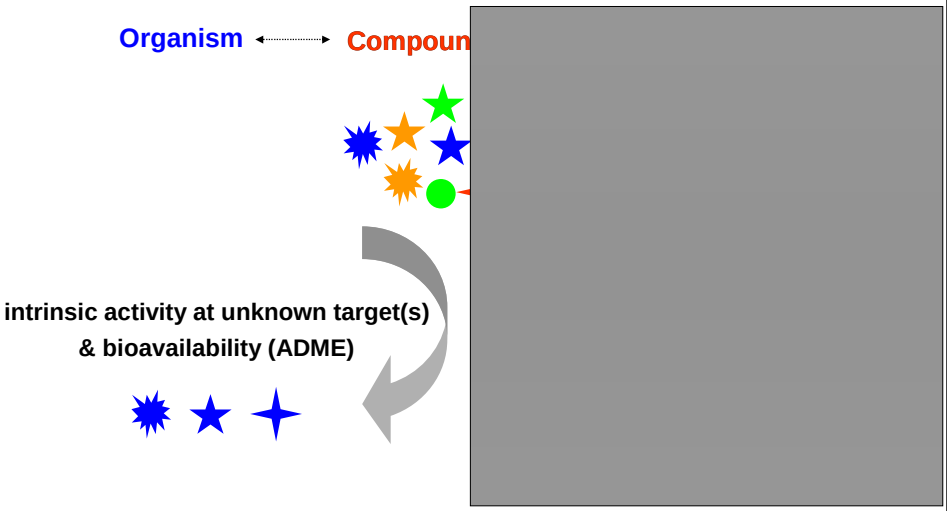
Requirements of a Compound Library



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Organism-based Lead Identification

PreScreen

HIT ID

HIT TO LEAD

PreScreen

Leadfinder

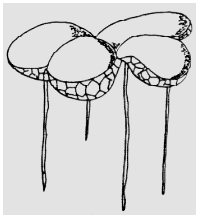
Primary Screening Greenhouse

Hit-validation

Analog Syntheses

Greenhouse Physico-Chemistry

Lead-identification



Lemna (Duckweed)

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Organism-based Lead Identification

Leadfinder

HIT ID

HIT TO LEAD

PreScreen

Leadfinder

Primary Screening Greenhouse

Hit-validation

Analog Syntheses

Greenhouse Physico-Chemistry

Lead-identification

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Organism-based Lead Identification
Greenhouse

HIT ID

HIT TO LEAD

PreScreen

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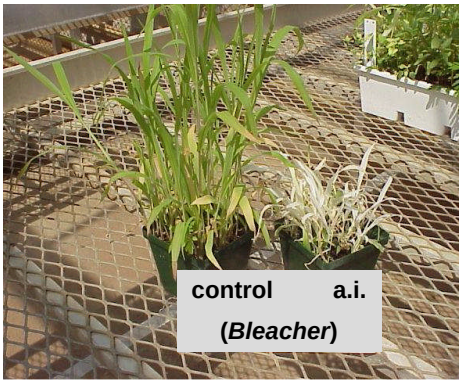
Primary Screening Greenhouse

Hit-validation

Analog Syntheses

Greenhouse Physico-Chemistry

Lead-identification



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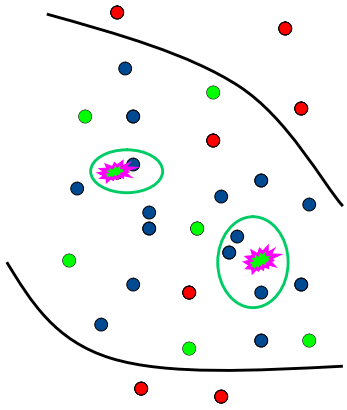
Compound Selection for Random Screening

1) Relevant chemical space
   remove reactive & toxic fragments
   choose only agro-like substances

2) Select a diverse subset for screening

3) Look for similar compounds close to screening hits ⇨ *hit validation*



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Descriptors for Filtering

1) remove reactive & toxic fragments

➢ substructures defined by chemist's knowledge

2) choose only agro-like substances

➢ 1-D descriptors and pharmacophore points

3) Select a diverse subset for screening

4) Look for similar compounds

➢ MACCS keys and topological descriptors

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PhysChem Properties of Agrochemicals

Pesticide Manual 11th Ed. 1997 (~ 700 cpds)

- ***Molecular Weight***
  - 200 to 500 range (86%); < 200 (11%); > 500 (3%)
- ***Melting Point*** (°C)
  - 50 - 200 (60%); < 50 (30%); > 200 (10%)
- ***pKa*** (acid)
  - ~10% pKa < 5
- ***pKa*** (base)
  - ~1% pKa > 5

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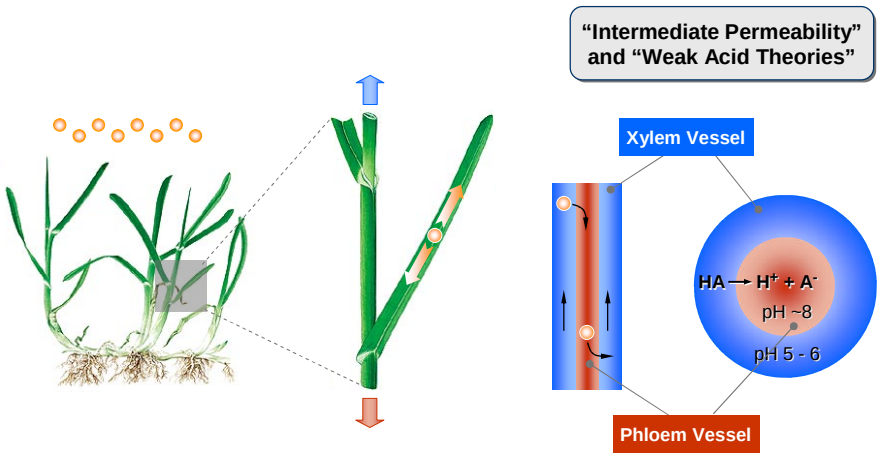
Long-Distance Transport

Translocation in Xylem and Phloem



modified, from D.A. Kleier et al. (1998)

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Bioavailability Rules: Agro *vs.* Drugs

Authors / Properties	Briggs	Tice	Clarke-Delaney	Lipinsky
molecular weight	~ 300	150 – 500	200 – 400 500 (I)	≤ 500
$logP_{OW}$	≤ 3 (Δ logP < 3)	≤ 3.5 (H) 0 - 5 (I)	1 – 5 7 (I)	≤ 5
HB-donors	≤ 3	≤ 3 (H) ≤ 2 (I)	0 - 1	≤ 5
HB-acceptors	-	2-12 (H) 1-8 (I)	0.7 - 2	≤ 10
melting point	< 300° C	-	< 200° C	-
	"Rule of 3"	I & post-emergence H activity more likely	„Guide of 2"	"Rule of 5"

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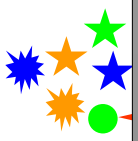
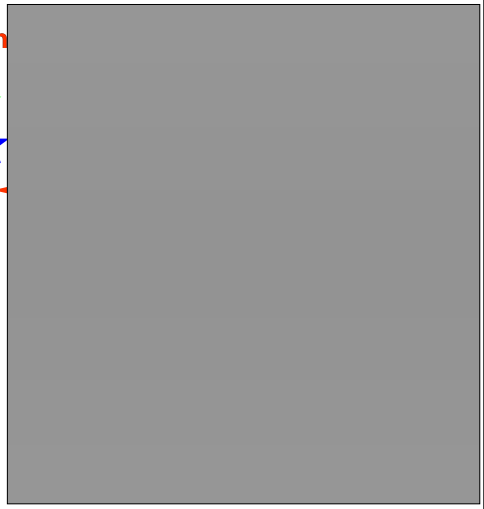
Screening Strategies

Organism ⟷ Compoun



intrinsic activity at unknown target(s)
& bioavailability (ADME)



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Target-Identification
Antisense Technology

Target Gen

⇩

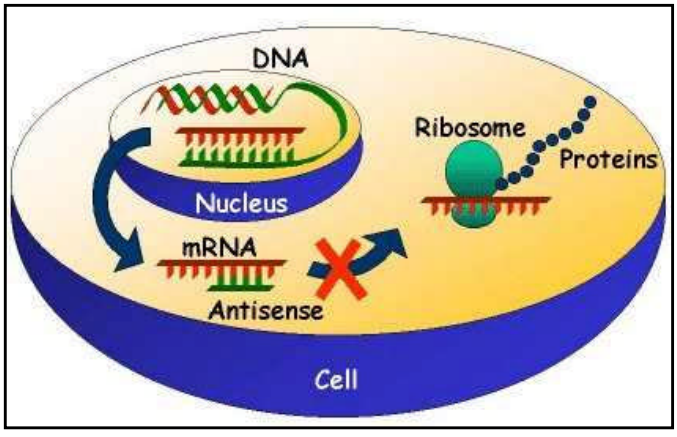
Reduce
Genexpression

⇩

Rate
of Vitality

⇩

Target



Antisense-Technology

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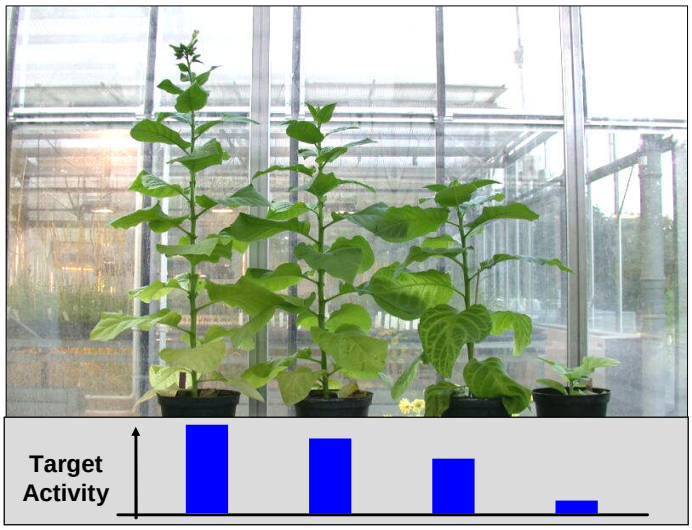
Effect in the Plant (*Phenotype*)



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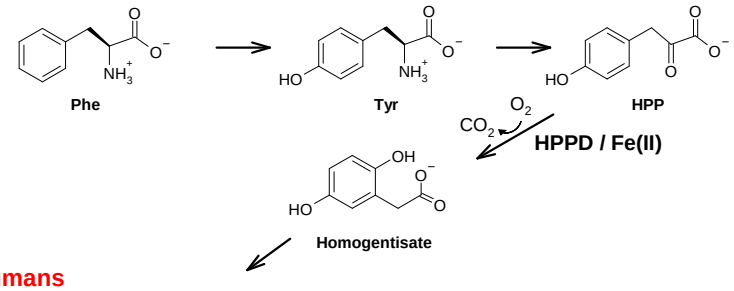
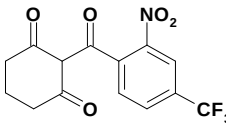
Plant specific Targets?

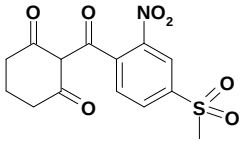
Example: HPPD Inhibitors



Humans



Nitisone (Orfadin®)



Mesotrione (Callisto®)

Basis for Screening Strategy

- validated target
- clear *in vitro – in vivo* correlation
- biochemical assay
- lots of active compounds
- several X-ray co-crystallized structures

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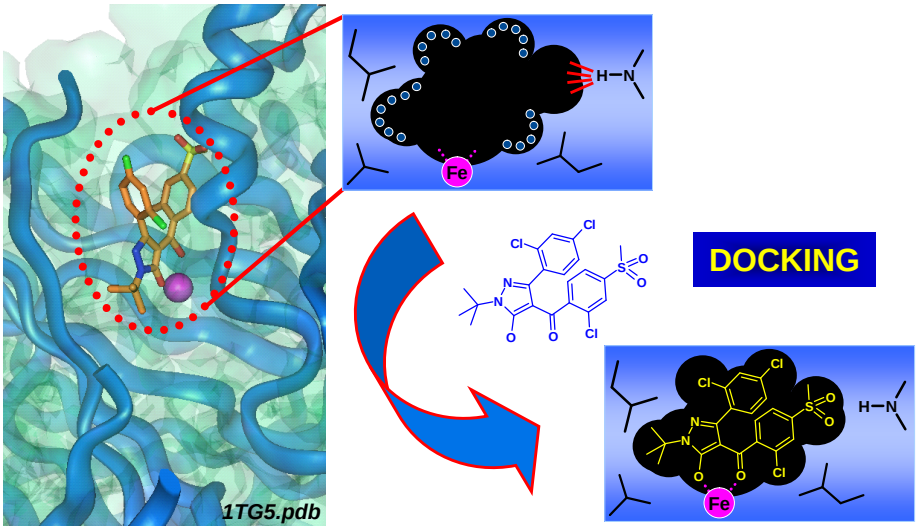
Structure-based Screening



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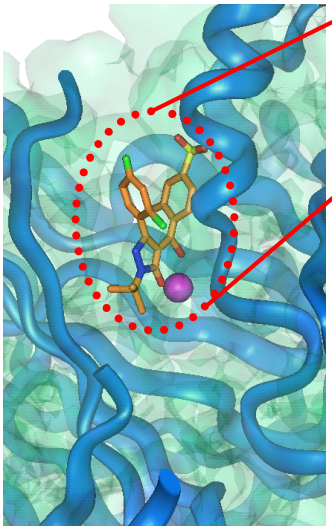
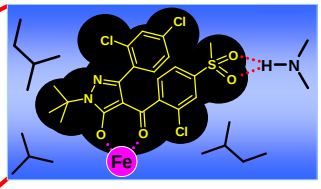
Structure-based Screening





Estimation of

- H-bonds
- Salt-bridges
- vdW-contacts
- electrostatic interactions
- etc.

SCORING

→ **relative binding affinities**

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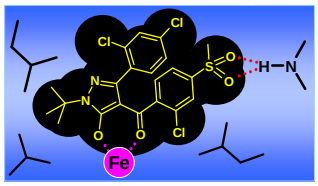
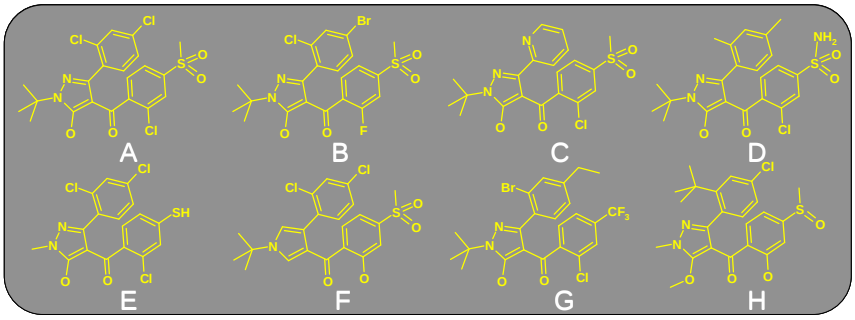
Structure-based virtual screening



Compound library



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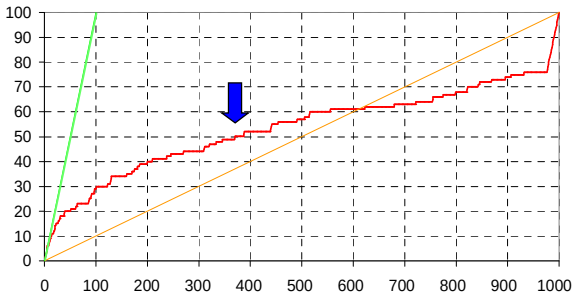
Structure-based Virtual Screening
Scoring with default Parameters

100 *HPPD* Inhibitors and 900 Chemicals from ACD



Default parameters

$$EF_{50\%} = \frac{tested\ by\ chance}{tested\ by\ VS} = \frac{500}{375} \approx 1.3$$

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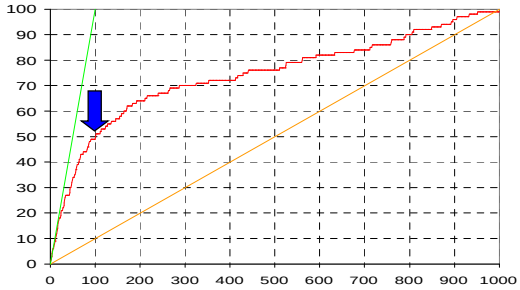
Structure-based Virtual Screening
Scoring with optimized Parameters

100 *HPPD* Inhibitors and 900 Chemicals from ACD



Optimized parameters
(e.g. clash factors)

$$EF_{50\%} = \frac{tested\ by\ chance}{tested\ by\ VS} = \frac{500}{100} = 5$$

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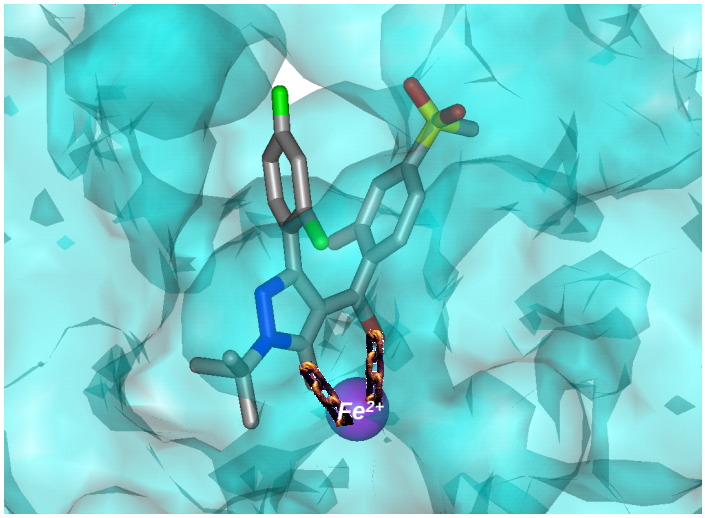
Structure-based Virtual Screening
Crucial Ligand-Protein Interaction Pattern



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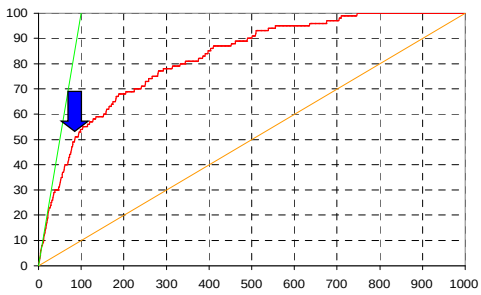
Structure-based Virtual Screening
Scoring with Pharmacophore Constraints

100 *HPPD* Inhibitors and 900 Chemicals from ACD



Default parameters
$EF_{50\%}$= 1.3

Optimized parameters
$EF_{50\%}$= 5

Optimized parameters + Binding mode
$EF_{50\%}$= 6.3

You only have to test 8% of the library to find 50% of the actives!

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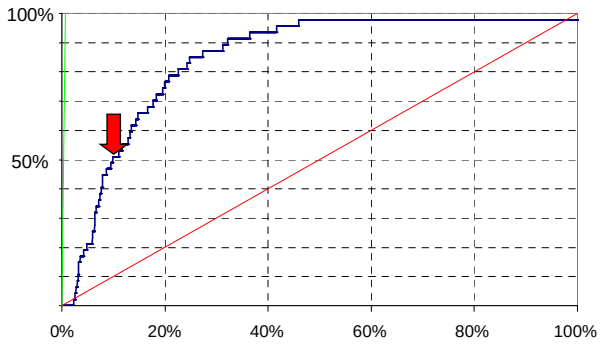
Structure-based Virtual Screening

Test: 41 Actives and > 6.000 HTS non-Actives

Enrichment Factor ($EF_{50\%}$) = 5.0



Score/sqrt(molwgt) ➔ Enrichment Factor = 7.1

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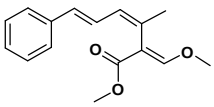
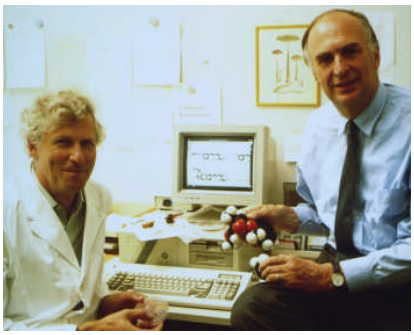
Strobilurins - Fungicides from Fungi

Defensive chemicals isolated from fungi (mid 70th):

Kiefernzapfenrübling
(Strobilurus tenacellus)

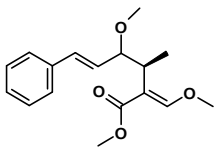


Strobilurin A



Profs. Anke and Steglich

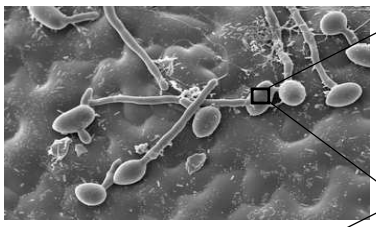
Buchenschleimrübling
(Oudemansiella mucida)



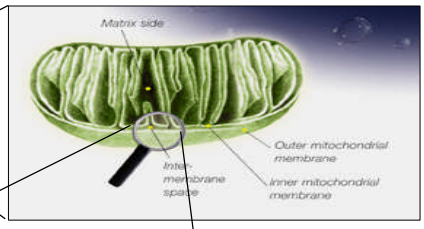
Oudemansin A

University cooperation showed the way to new lead structures

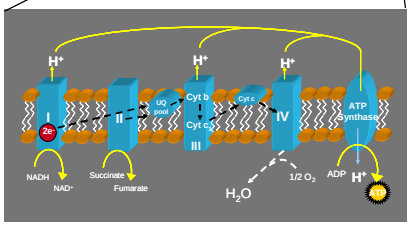
Biochemical site of action: respiratory chain



spore



mitochondria



respiratory chain

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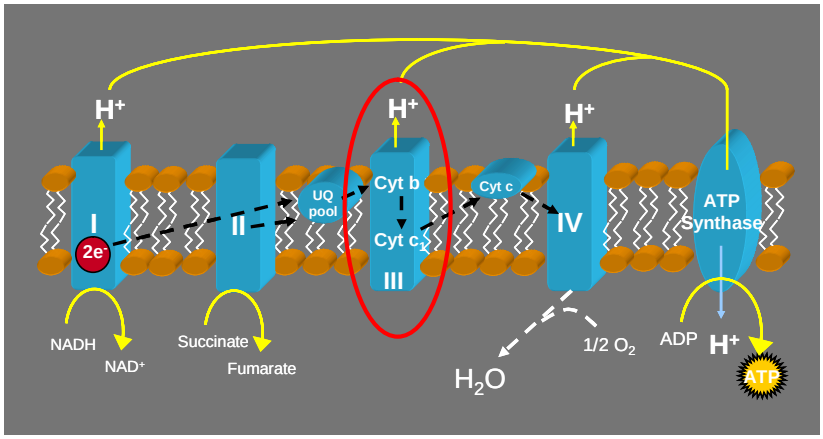
Mode of Action



Strobilurins block the fungal energy production by inhibition of the complex III of the respiratory chain.

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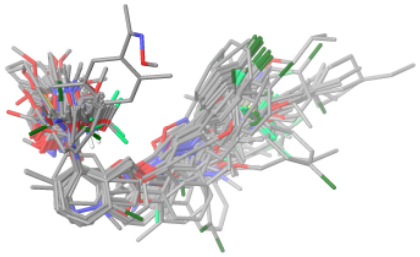
3D-QSAR Model for Strobilurins

BASF The Chemical Company

Input:
Chemically diverse ligands with different activity levels

→ **3D-conformation generation**

↓ **structural alignment**



120 strobilurin analogues
broad activity range ($10^{-10} < IC_{50} < 10^{-5}$)

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3D-QSAR Model for Strobilurins

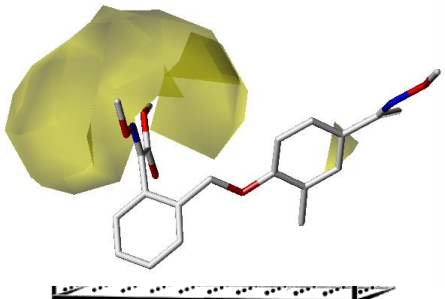
BASF The Chemical Company

Input:
Chemically diverse ligands with different activity levels

→ **3D-conformation generation**

↓ **structural alignment**

↓ **calculate property fields**



property field: steric demand

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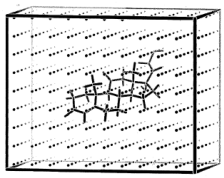
3D-QSAR Model for Strobilurins

Input:
Chemically diverse ligands with different activity levels



Cpd	pIC50	S1	S2	...	Sn	E1	E2	...	En	...
1	7.2									
2	8.5									
3	5.3									
...										
120	9.8									

3D-conformation generation

structural alignment

calculate property fields

correlate property fields with activity

$$-\log(IC_{50}) = y + a \cdot S_1 + b \cdot S_2 + ... + h \cdot S_n + k \cdot E_1 + m \cdot E_2 + ... + z \cdot E_n + ...$$

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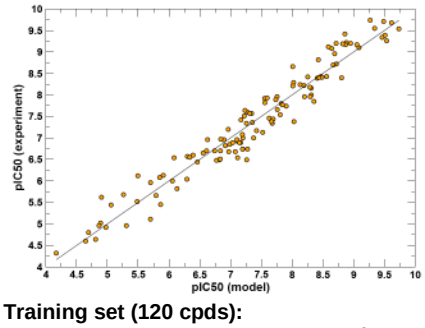
3D-QSAR Model for Strobilurins

Input:
Chemically diverse ligands with different activity levels



Training set (120 cpds):
reproduction of experimental data **r^2 = 0.95**
leave-one-out cross-validation **q^2 = 0.79**

3D-conformation generation

structural alignment

calculate property fields

correlate property fields with activity

3D-QSAR model

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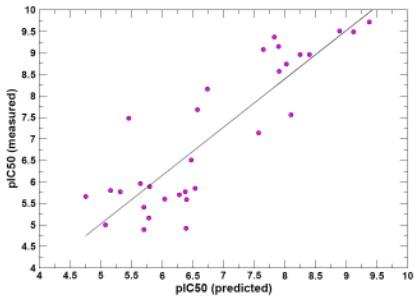
3D-QSAR Model for Strobilurins

Input:
Chemically diverse ligands with different activity levels



Prediction of independent test set: r^2_{pred} = 0.78
(32 compounds)

3D-conformation generation

↓

structural alignment

↓

calculate property fields

↓

correlate property fields with activity

↓

3D-QSAR model

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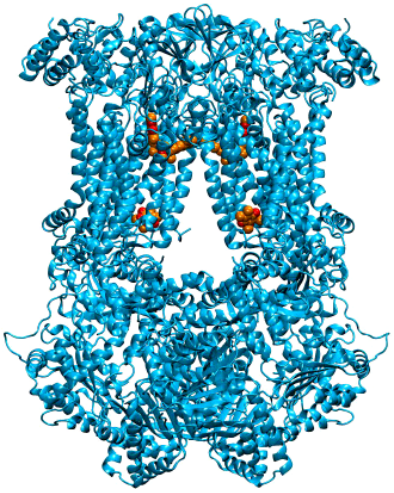
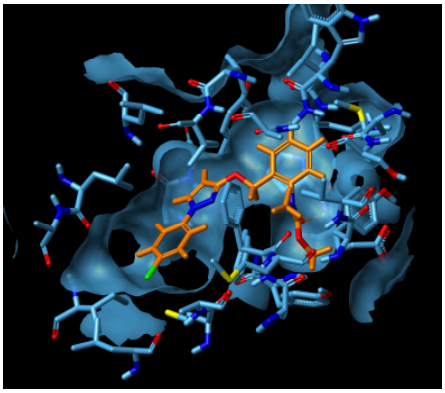
X-ray Structure solved!
The key finds its lock…..





PDB code: 1SQB & 1SQP (2004)

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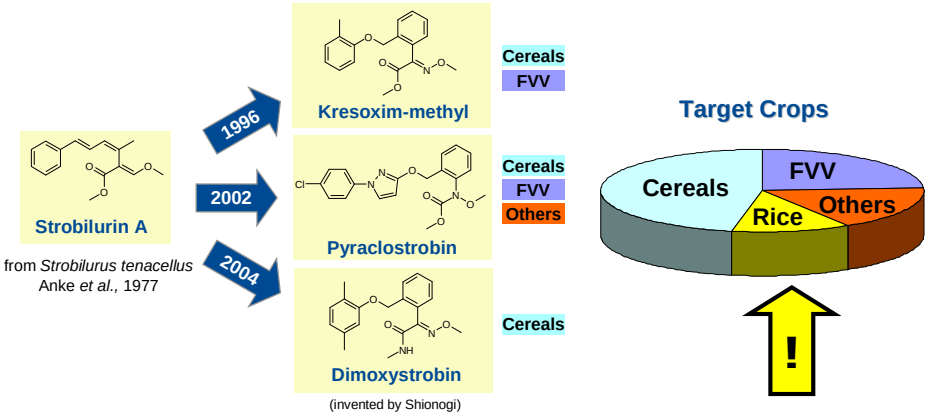
Strobilurin History at BASF

From a Natural Product to Tailor-made Fungicides



➔ Need for a rice fungicide to complete BASF's strobilurin portfolio

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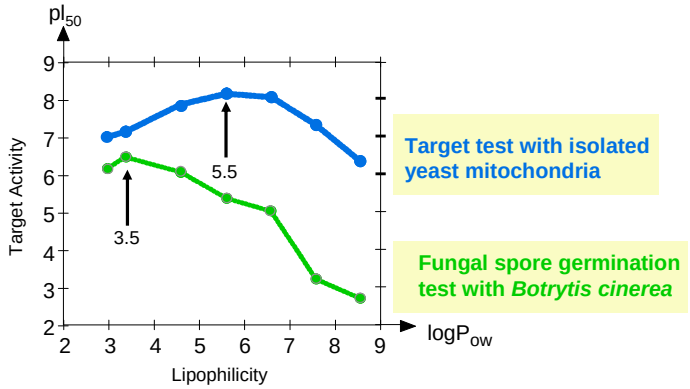
Biological Activity versus Lipophilicity



➔ Optimum $logP_{OW}$ for *in fungus* activity = 3.5 ± 1

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Aquatic Toxicity versus Lipophilicity

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Lipophilicity Optimum

- Lipophilicity Optima — $logP_{OW}$
  - Activity on Target Level — 4.5 ~ 6.5
  - Activity in Whole Fungus — 2.5 ~ 4.5
  - Low Aquatic Toxicity — 0 ~ 3

➔ **Preferred characteristic for a new rice fungicide: $logP_{OW}$ = 2 ~ 3**

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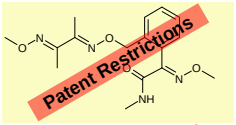
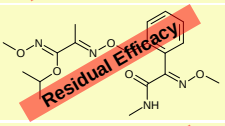
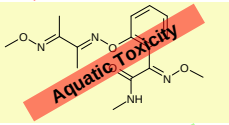
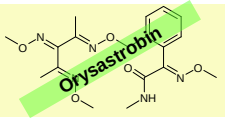
Optimisation Results
Four Potential Candidates Identified

	$logP_{OW}$	IP Rights	Biological Activity *PYRIOR*	Biological Activity *RHIZSO*	Residual Efficacy	Aquatic Toxicology *Fish*	Aquatic Toxicology *Daphnia*	Aquatic Toxicology *Algae*
	2.9	✗ (Ciba-Geigy)						
	2.6	✓	+++	+++	+			
	2.7	✓	+++	+++	+++	+	+	+
	2.4	✓	+++	+++	+++	+++	+++	+++

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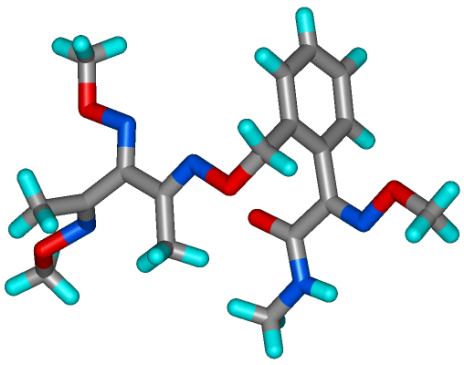
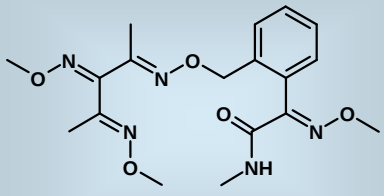
Orysastrobin
Launched as Arashi® in 2007





(E,E,E,E)-isomer

Successfully introduced in the Japanese and Korean markets

嵐

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Acknowledgment

Thomas Mietzner
Gerhard en-Naser
Klaus Kreuz

PhysChem

Orysastrobin

Thomas Grote
Hubert Sauter
Egon Haden
Siegfried Strathmann
Akihide Watanabe

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