Leads from Crop Protection against Neglected Diseases

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Why do we need unorthodox approaches to find new tools against Neglected Diseases?

- Most important non-viral infectious diseases worldwide:

Malaria	(*Plasmodium falciparum*)
Tuberculosis	(*Mycobacterium tuberculosis*)
Chagas disease	(*Trypanosoma cruzi*)
Leishmaniasis	(*Leishmania donovani*)
Sleeping sickness	(*Trypanosoma brucei*)
Buruli ulcer	(*Mycobacterium ulcerans*)

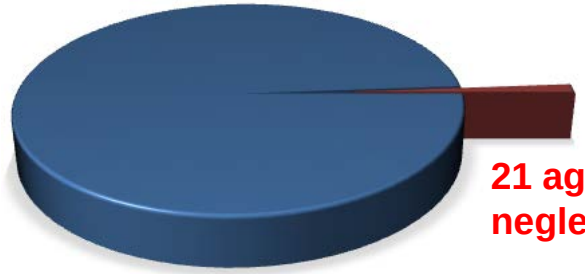
- causing 11% of DALYs* and >>1.000.000 deaths/year

⇒ UN millennium goal to reduce these “neglected” diseases

⇒ Development of new drugs key long term success factor!

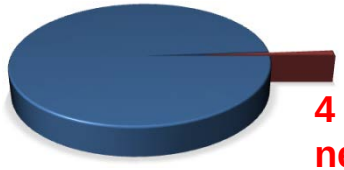
⇒ <u>But:</u> disappointing progress in providing more drugs

1974-2004: 1535 new drugs (NCEs)



21 against neglected diseases

2000-2012: 336 new drugs (NCEs)**



4 against neglected diseases

- Hardly addressed by pharma industry in last decades, as deemed commercially unattractive
- Research mainly done by academic sector, supported by PPPs like MMV or DNDi
- No commercial target for BASF, but history in supporting global health projects (Interceptor®, Fendora®, Abate®)

➔ **Sabbatical in 2009 at ETH: could AgChem knowhow help to find innovative solutions?**

*DALY: disability-adjusted life years (WHO)

B. Pedrique et al., Lancet **2013, e371.

Why agrochemicals?

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- Most important parasitic pathogens are eukaryotes, like e.g. protozoans, worms or helminths
- Agrochemicals are designed to efficiently control eukaryotic organisms, while remaining non-toxic to mammals
- Biocidal Pharma indications (antibiotics, oncology,…) not well suited for serendipitous identification of leads/drugs against eukaryotic neglected disease pathogens

- Since end of the “life science concept” in the late 90's, most agro and pharma companies have separated

⇒ As a result very limited crosstalk between agro and pharma

⇒ Concepts examined to establish link of agrochemistry and antiparasitic research:

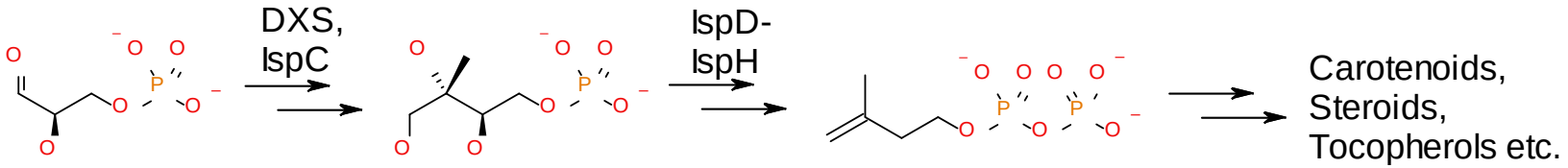
- Inhibitors of herbicidal pathways against malaria
- Commercial agrochemicals against protozoan disease pathogens
- Agrochemically-active natural products against neglected disease pathogens
- Commercial antiparasitic drugs/published leads against agronomic pests

➔ **So far almost* no examples linking agchem and parasitic disease research!**

*Swanton CJ, Mashhadi HR, Solomon KR, Afifi MM, Duke SO. Pest Manag Sci. 2011 ;67(7):790.

1st Concept: Herbicidal inhibitors of the non-mevalonate pathway against *P. falciparum*

- Essential pathway in plants
- Plasmodium incorporated a red algae during evolution, resulting in the apicoplast organelle
- Main function of the apicoplast: synthesis of IPP via the non-mevalonate pathway (NMP)
- NMP is not present in mammals



G3P MEP IPP

- HTS-screens on several plant-enzymes of NMP at BASF for herbicide lead identification*,**,***

⇒ Test of plant HTS hits on *P. falciparum* at SwissTPH (Prof. R. Brun) and further follow-up with groups of Profs. Diederich (ETH), Fischer (Hamburg), Groll (TU Munich)

➔ **New concept to generate leads for neglected disease research**

*Pest Manag Sci. 2013;69(5):559. **Angew Chem Int Ed 2011 ;50(34):7931. ***PLoS One 2014, accepted.

Herbicidal NMP-inhibitors with activity against *P. falciparum*

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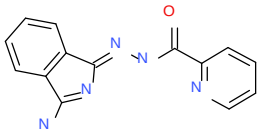
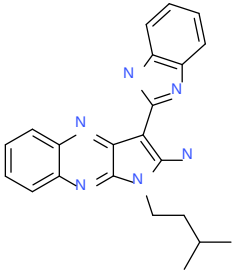
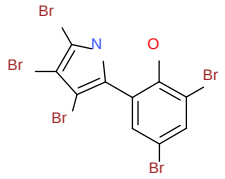
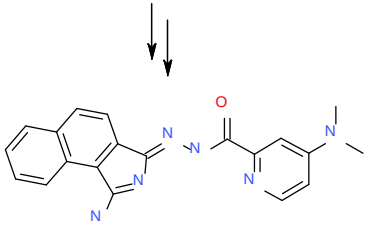
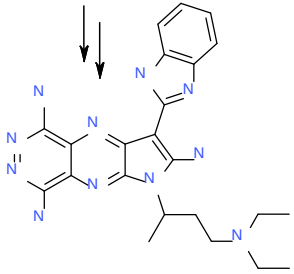
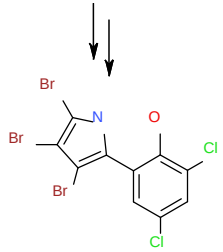
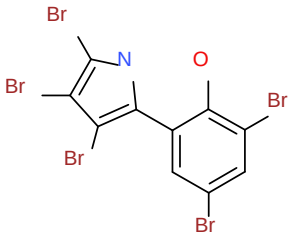
Hits from HTS-screens:			
Plant target activity:	13 µg/ml (IspE)	2.4 µg/ml (IspD)	0.70 µg/ml (IspD)
Duckweed activity:			
Pf-activity (cell based):	16 ng/ml	69 ng/ml	704 ng/ml
Optimization:			
Pf-activity (cell based):	6 ng/ml	16 ng/ml	499 ng/ml

➔ **Identification of potent inhibitors of *P. falciparum*; different primary targets than NMP?**

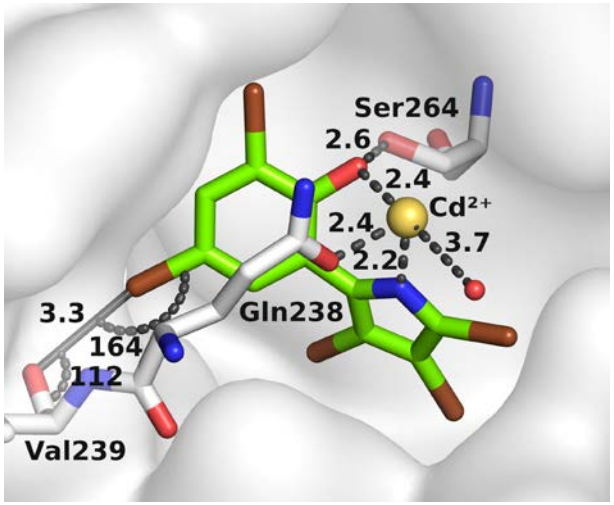
*ChemMedChem 2012 ;7(1):151. **Angew Chem Int Ed 2014 ;DOI 10.1002. ***Angew Chem Int Ed 2014;53(8):2235.

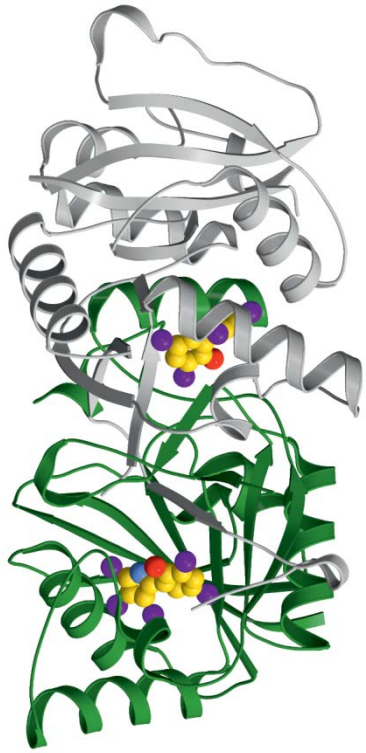
Herbicidal marine natural product inhibiting IspD and *P. falciparum*

- Pentabromopseudilin natural product from a marine sponge, isolated in 1950s
- Activity on plants with bleaching symptomology published by BASF 1995



plant-IspD	7.2 µg/ml
Pv-IspD	26 µg/ml
Pf cell	0.7 µg/ml





- Co-crystallisation with plant IspD (M. Groll)

⇒ Binding in an allosteric pocket next to active site

⇒ Unusual halogen-bonding interactions

⇒ Very few examples of *in vivo* active *Plasmodium* inhibitors in enzyme crystal structures

⇒ Even fewer with antiplasmodial natural products!

➔ **Proof of concept for identification of malaria-leads from target based herbicide research!**

Angew Chem Int Ed 2014;53(8):2235.

Serine-hydroxymethyl-transferase (SHMT): Pyrazolopyrans

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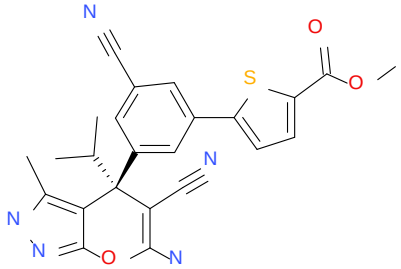
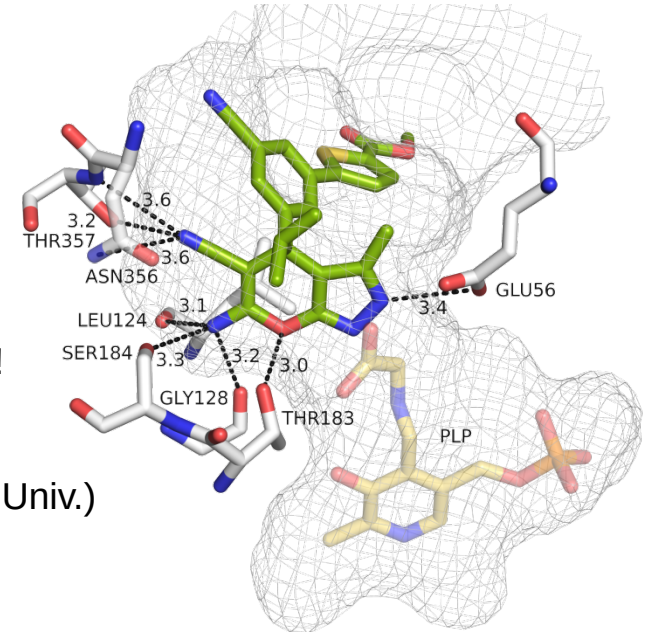
- Pyrazolopyran SHMT inhibitors *in vitro*-based herbicide project at BASF
  - ⇒ SHMT proposed malaria-target; high sequence homology between plants and *Plasmodium*
  - ⇒ Testing of plant SHMT leads in *P. falciparum* cell based assay at SwissTPH
  - ⇒ Several hits with <100ng/ml; best: **0.7 ng/ml** ➔ better than best standard (Artesunate, 1.6 ng/ml)!



SHMT-inhibitor		
SHMT (plant)	IC_{50}:	4 ng/ml
Pf (in blood cell)	IC_{50}:	0.7 ng/ml
Cytotox (L6)	IC_{50}:	7800 ng/ml
SHMT (*Pf*)	IC_{50}:	36 ng/ml



- One of the most active *Pf* cell-based hits from target based approaches!
- Good cytotox selectivity *Pf* vs. mammalian cells (>5000x)
- Activity on *Pf*-SHMT and *Pv* co-crystalstructure (Prof. Chaiyen, Mahidol Univ.)
- In first animal model no significant activity due to ester instability
  - ⇒ Follow-up in Diederich group to improve pharmacokinetics

➔ **Step forward for target-based approach in malaria-research!**

PNAS 2014, submitted.

2nd Concept: Commercial agrochemicals as leads against neglected diseases

- Commercial agrochemicals are some of the best studied existing chemicals
- But: No systematic examination of activity against neglected disease pathogens done so far

⇒ Test of 700 commercial agrochemicals against major neglected disease pathogens at SwissTPH*

⇒ Several interesting leads against all tested pathogens

⇒ Especially against malaria several a.i. with nanomolar activity

⇒ Some activity also in animal model; but inferior to new pipeline candidates

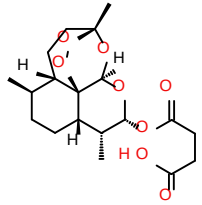
⇒ Further follow-up with analogues with expired IP from BASF compound library

➔ **Innovative source of potential new leads against neglected diseases!**

*PLoS Negl Trop Dis. 2012;6(10):e1805.

Malaria (*P. falciparum, P. vivax*)

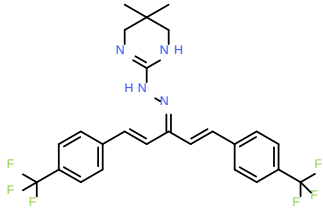
- Resistance against almost all available drugs ➔ high reliance on Artesunate
- Recently candidates with new MOA (GNF156, KAE609) promoted in clinical phases
- 10 agrochemicals with <100 ng/ml in cell-based *P. falciparum* assay



Artesunate 2 ng/ml

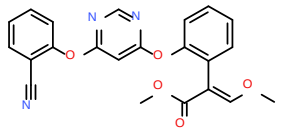
- **Hydramethylnon** in *Pf* cell based assay IC_{50}: **23 ng/ml**
  - In *P. berghei*-mouse with 4x100 mg/kg oral increase of survival from 3 to 16 days
  - Interesting PK-properties (t1/2: 79/133h), C_{max} 10000 ng/ml!

⇒ Additional transmission control *via* insecticidal activity (like Ivermectin)??



Hydramethylnon 23 ng/ml

- **Azoxystrobin** in *Pf* cell based assay IC_{50}: **6 ng/ml**
  - In *P. berghei*-mouse with 4x100 mg/kg *s.c.* increase of survival from 4 to 13.3 days
  - Excellent activity on liver stage *P. berghei* **(<2.6 ng/ml**), comparable to best drugs



Azoxystrobin 6 ng/ml

⇒ Selection of pre-described, patent-expired BASF-strobilurins from 1990's

⇒ Strobilurins identified with IC_{50} **<< 1 ng/ml**; activity at 2x50 mg/kg in mouse; but tight safety margin

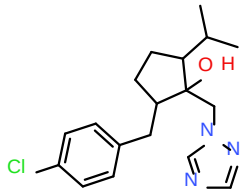
➔ **Transmission control and/or broad activity on many stages of *Pf* essential!**

PLoS Negl Trop Dis. 2012;6(10):e1805.

Chagas disease *(T. cruzi)*

- Drugs against Chagas disease with strong side effects: Nifurtimox, Benznidazole
- 10 agrochemicals with <20 ng/ml in cell-based assay

- **Ipconazole** in cell-based *Tc* assay IC_{50}: **1 ng/ml**
  - Racemic product, therefore likely even higher activity for enantiomers
  - In *in-vivo* mouse models so far only weak activity; pharmacokinetic limitations?
  - Related Ravuconazole-prodrug in development by DNDi against Chagas



Ipconazole, 1 ng/ml

⇒ Comparison of Ipconazole and Ravuconazole on Cyp19 (cause for endocrine side effects) and Cyp51 (*Tc* target enzyme)

⇒ Ipconazole more active on Cyp51, 300x-selectivity window; Ravuconazole only 9x!

⇒ Clinical studies from Ravuconazole prodrug not successful due to high recrudescence

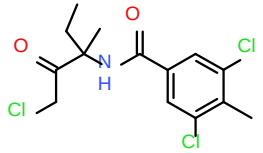
⇒ Deprioritization of Cyp51 inhibitors as Chagas drug candidates

➔ **Ipconazole highly potent cell-based inhibitor, but target likely not suited for cure**

PLoS Negl Trop Dis. 2012;6(10):e1805.

Leishmaniasis (*L. donovani*)
Sleeping sickness (*T. brucei*)

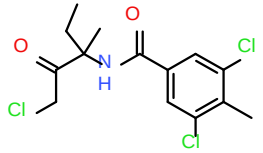
- Current treatments against Leishmaniasis with severe side effects: Miltefosine; Antimony complexes
- Most interesting hit **Zoxamide** (IC_{50} **84 ng/ml**)
- Access to animal models difficult
- Further selected analogues in testing



Zoxamide
84 ng/ml

- Current treatments for sleeping sickness: Eflornitin (expensive; dose 400 mg/kg/d!); Arsenic derivatives
- Most interesting hit also **Zoxamide** (IC_{50} **2 ng/ml**)
- Zoxamide (LD_{50}>2000 mg/kg) in mouse model at 4x50 mg/kg with some activity (no parasites after 7 days, but recurrence after 10 days)
- No improved activity with different dosing

⇒ Zoxamide rapidly metabolized *in vivo*



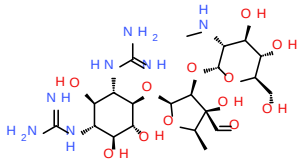
Zoxamide
2 ng/ml

➔ **Zoxamide most interesting candidate, but likely too labile for curative action**

PLoS Negl Trop Dis. 2012;6(10):e1805.

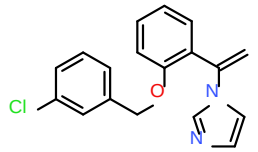
Buruli Ulcer (*M. ulcerans*)

- Only medication Rifampicin+Streptomycin (toxic in chronic application; IC_{50} 300 ng/ml)



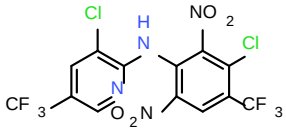
Streptomycin, 300 ng/ml

- First interesting hit: **Viniconazole** (= Croconazole, fungicidal drug)
  - Cell based *M. ulcerans* assay IC_{50} **800 ng/ml***
  - All other 48 commercial azole agrochemicals and drugs inactive in assay
  - Could also treat opportunistic fungal infections; inhibition of Mycolactone synthesis??



Viniconazole, 800 ng/ml

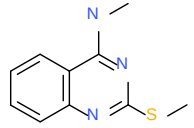
- Most potent hit from screen: **Fluazinam**
  - Cell based *M. ulcerans* IC_{50} **300 ng/ml** (LD_{50} >2000 mg/kg)
  - Likely difficult to use as drug due to short half-life ($t_{1/2}$: 1.3 h)



Fluazinam, 300 ng/ml

- Other hits from published *Mt* screen also highly active on *Mu*

⇒ Further follow-up supported using BASF compound base



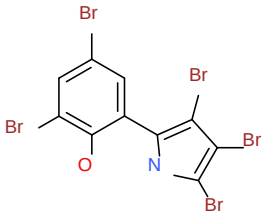
MIC (IC_{90}) 31 ng/ml!

➔ Further studies with Viniconazole and new hits ongoing

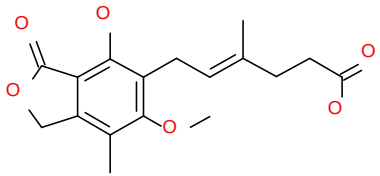
*Antimicrob Agents Chemother. 2012 ; 56(12):6410.

3rd Concept: Agrochemically-active natural products against protozoans

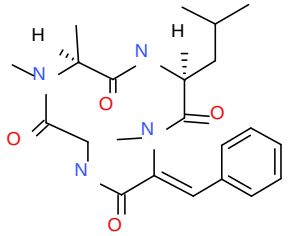
- Several published natural products with agrochemical activity tested on neglected disease pathogens



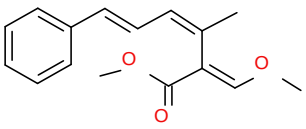
Pseudilin (H)
700 ng/ml (*Pf*)



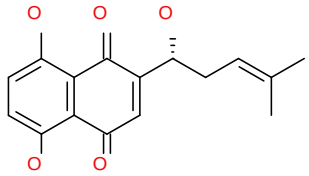
Mycophenolicacid (H)
1613 ng/ml (*Pf*)



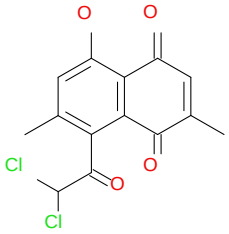
Tentoxin (H)
4973 ng/ml (*Pf*)



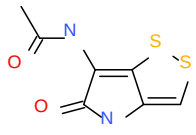
Strobilurin A (F)
336 ng/ml (*Pf*)



Shikonin (F)
14 ng/ml (*T. brucei*)



Mollisin (F)
38 ng/ml (*T. brucei*)



Thiolutin (F)
19 ng/ml (*T. brucei*)

➔ **Several natural products with promising activity against protozoans identified!**

4th Concept: Herbicidal activity of antiparasitic drugs and HTS-hits

Purchase of 126 commercial antiparasitic drugs and test in herbicide screening:

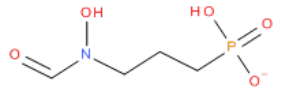
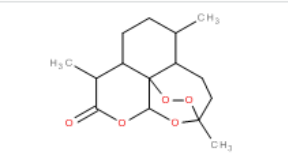
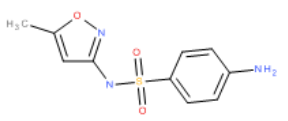
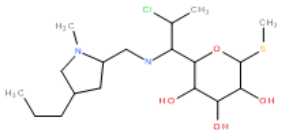
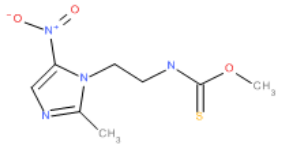
Fosmidomycin inhibitor of non-mevalonate pathway (DXS)

Artemisinin (perturbing redox homeostasis)

Sulfamethoxazole inhibitor of folate synthesis (dihydropteroate synthetase)

Clindamycine inhibitor of protein biosynthesis (50s rRNA inhibitor)

Carnidazole (DNA biosynthesis in anaerobic cells)

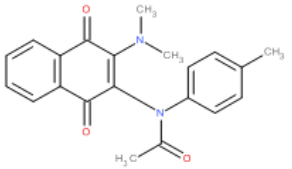
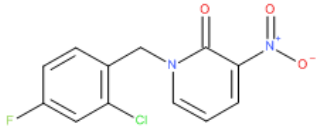
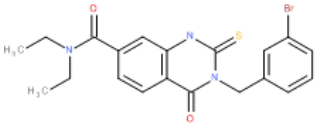
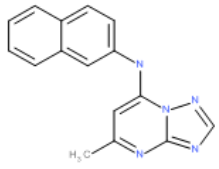
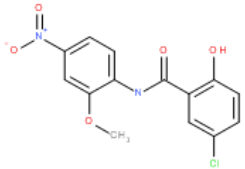
	Monocot.	Dicot.	Monocot.	Dicot.
2kg/ha; % damage	POST	POST	PRE	PRE
	95	58		
	46	100	48	38
	49	5	79	48
	25	70	0	60
	24	50	0	0

➔ **Several herbicidal drugs identified; potential leads with target, SAR and tox-evaluation!**

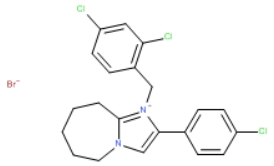
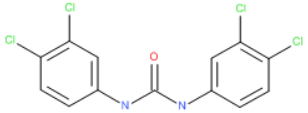
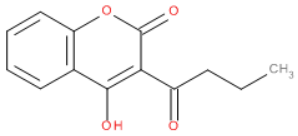
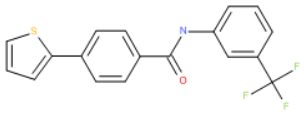
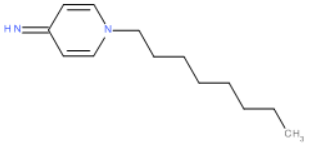
Herbicidal activity of hits from published antimalarial screens

- Commercial hits from HTS-runs against *Pf* have been published by e.g. GSK, Novartis,…

	Pf IC_{50}
Benzochinon GNF-Pf-3600	24 nM
Nitropyrimidon CHEMBL598903	358 nM
Benzpyrimidon CHEMBL585425	1016 nM
Triazolopyrimidin CHEMBL475813	79 nM
Salicylamide TCMDC-124051	1141 nM

2kg/ha; % damage	Monocot. POST	Dicot. POST	Monocot. PRE	Dicot. PRE
	77	78	0	0
	37	66	40	8.9
	43	82	0	0
	30	32	14	0
	39	63	7.1	0

Insecticidal activity of hits from published antimalarial screens

	Pf IC_{50}	Structure	sucking protective	sucking curative	chewing curative
		2500ppm; % control			
Imidazolium CHEMBL603013	459 nM		63	25	100
Urea CHEMBL10835	1160 nM		6	69	100
Coumaron CHEMBL532382	265 nM		0	25	100
Benzamide CHEMBL601789	1935 nM		0	0	81
Pyridonimin CHEMBL568092	189 nM		63	0	19

Fungicidal activity of hits from published antimalarial screens

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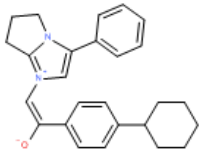
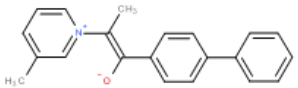
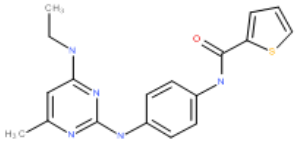
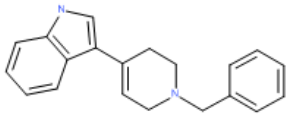
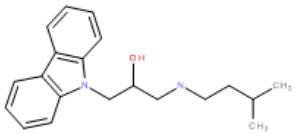
	Pf IC_{50}	100ppm, % infection	Basidomycota curative	Oomycetes protective	Ascomycota protective
Imidazolium CHEMBL603519	319 nM		25	50	54
Pyridinium CHEMBL1482585	2936 nM		33	75	50
Pyrimidine CHEMBL528809	1017 nM		83	17	67
Indol CHEMBL285157	1045 nM		92	17	71
Carbazole CHEMBL 537336	97 nM		75	0	100

Conclusions

- Agrochemical target based research can provide highly potent antimalarial leads
- Commercial agrochemicals can provide interesting leads for neglected disease research
- Natural products identified in agrochemical screens can show high activities also against human pathogens
- Compounds active against human pathogens can also show high activity against agronomic pests

⇒ Based on this project many interesting new leads for neglected disease- as well as for agrochemical research could be identified

⇒ Win-win link for agrochemical- and neglected disease-research!

➔ **Proof of concept, that linking AgChem- and Neglected Disease-research makes sense!**

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Non-mevalonate pathway

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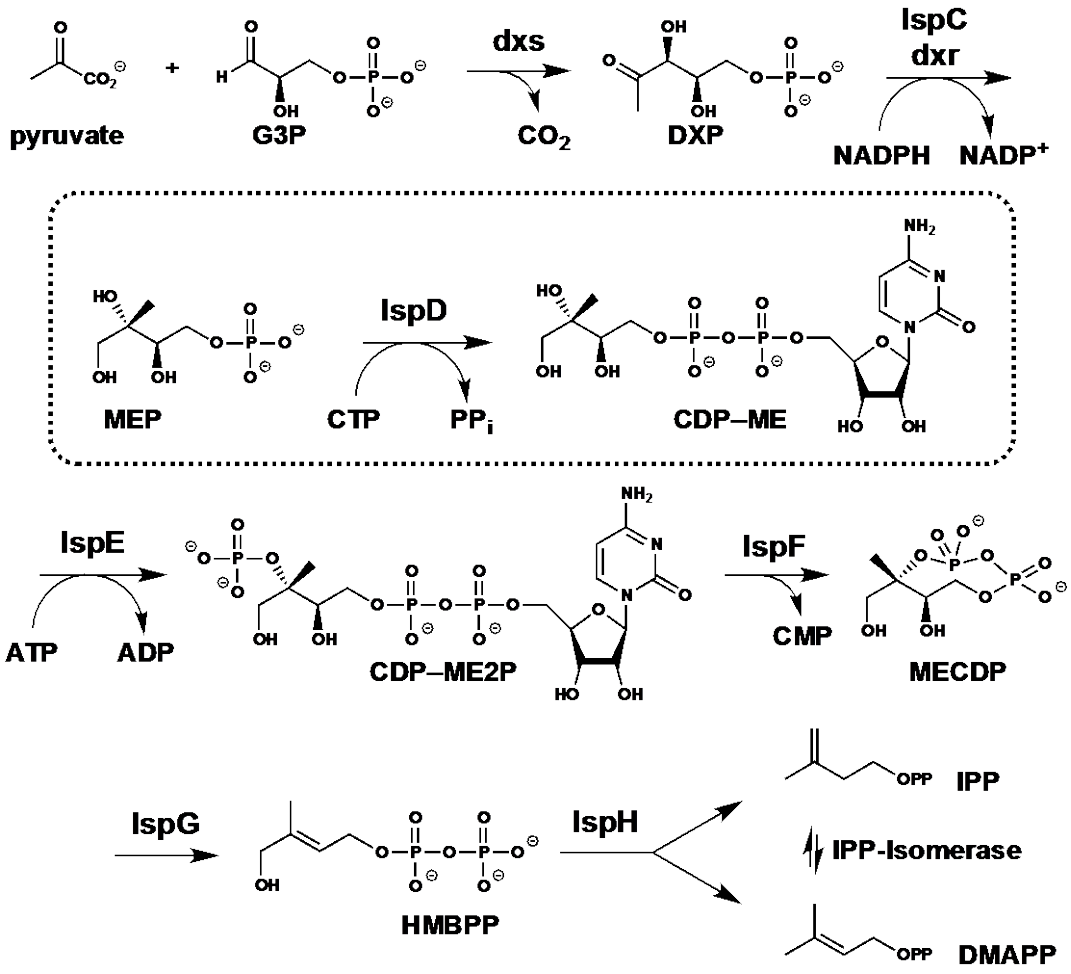

Folate cycle