Leads from Crop Protection against Neglected Diseases

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Most important non-viral infectious diseases worldwide:

- Malaria \((\text{Plasmodium falciparum})\)
- Tuberculosis \((\text{Mycobacterium tuberculosis})\)
- Chagas disease \((\text{Trypanosoma cruzi})\)
- Leishmaniasis \((\text{Leishmania donovani})\)
- Sleeping sickness \((\text{Trypanosoma brucei})\)
- Buruli ulcer \((\text{Mycobacterium ulcerans})\)

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!

⇒ But: disappointing progress in providing more drugs

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®)

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

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

1974-2004: 1535 new drugs (NCEs)

4 against neglected diseases

21 against neglected diseases

*DALY: disability-adjusted life years (WHO)
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 agrochem and parasitic disease research!

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

\[
\begin{align*}
\text{G3P} & \xrightarrow{\text{DXS, IspC}} \text{MEP} & \xrightarrow{\text{IspD, IspH}} \text{IPP} & \rightarrow \text{Carotenoids, Steroids, Tocopherols etc.}
\end{align*}
\]

- 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

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

- **Hits from HTS-screens:**
  - Plant target activity: 13 µg/ml (IspE)  
  - Duckweed activity: 
  - *Pf*-activity (cell based): 16 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?

**Angew Chem Int Ed 2014;DOI 10.1002.*  
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

<table>
<thead>
<tr>
<th>Source</th>
<th>Activity (µg/ml)</th>
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<tbody>
<tr>
<td>Plant-IspD</td>
<td>7.2</td>
</tr>
<tr>
<td><em>Pv</em>-IspD</td>
<td>26</td>
</tr>
<tr>
<td><em>Pf</em> cell</td>
<td>0.7</td>
</tr>
</tbody>
</table>

- 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!**

Serine-hydroxymethyl-transferase (SHMT): Pyrazolopyrans

- 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)!

- 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!

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!

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

**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)??

**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

⇒ 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!

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

⇒ 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**

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

- Current treatments against Leishmaniasis with severe side effects: Miltefosine; Antimony complexes
- Most interesting hit Zoxamide (IC₅₀ 84 ng/ml)
- Access to animal models difficult
- Further selected analogues in testing

- Current treatments for sleeping sickness: Eflornitin (expensive; dose 400 mg/kg/d!); Arsenic derivatives
- Most interesting hit also Zoxamide (IC₅₀ 2 ng/ml)
- Zoxamide (LD₅₀>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 most interesting candidate, but likely too labile for curative action

First interesting hit: **Viniconazole** (= Croconazole, fungicidal drug)
- Cell based *M. ulcerans* assay IC\textsubscript{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??

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

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

⇒ Further follow-up supported using BASF compound base

Further studies with Viniconazole and new hits ongoing

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

- Pseudilin (H): 700 ng/ml (Pf)
- Mycophenolic acid (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)

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$_{50}$

<table>
<thead>
<tr>
<th>Compound</th>
<th>Pf IC$_{50}$</th>
<th>POST</th>
<th>PRE</th>
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<tbody>
<tr>
<td>Benzochinon GNF-Pf-3600</td>
<td>24 nM</td>
<td>77</td>
<td>0</td>
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<tr>
<td>Nitropyrimidon CHEMBL598903</td>
<td>358 nM</td>
<td>37</td>
<td>8.9</td>
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<tr>
<td>Benzpyrimidon CHEMBL585425</td>
<td>1016 nM</td>
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<tr>
<td>Triazolopyrimidin CHEMBL475813</td>
<td>79 nM</td>
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<tr>
<td>Salicylamide TCMDC-124051</td>
<td>1141 nM</td>
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Insecticidal activity of hits from published antimalarial screens

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<th>Structure</th>
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<tr>
<td>Imidazolium CHEMBL603013</td>
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<tr>
<td>Urea CHEMBL10835</td>
<td>1160 nM</td>
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<tr>
<td>Coumaron CHEMBL532382</td>
<td>265 nM</td>
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<tr>
<td>Benzamide CHEMBL601789</td>
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<td>Pyridonimine CHEMBL568092</td>
<td>189 nM</td>
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<tr>
<td>Compound</td>
<td>Pf IC&lt;sub&gt;50&lt;/sub&gt;</td>
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<tr>
<td>Imidazolium CHEMBL603519</td>
<td>319 nM</td>
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<tr>
<td>Pyridinium CHEMBL1482585</td>
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<tr>
<td>Pyrimidine CHEMBL528809</td>
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<td>Indol CHEMBL285157</td>
<td>1045 nM</td>
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<tr>
<td>Carbazole CHEMBL 537336</td>
<td>97 nM</td>
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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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• M. Rottmann
• M. Kaiser
• Prof. G. Pluschke
• N. Scherr

University of Hamburg:
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• B. Illarionov
• Prof. A. Bacher

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• P. Chitnumsub

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• T. Mietzner
Thank You!!
Non-mevalonate pathway

\[
\text{pyruvate} + \text{G3P} \xrightarrow{\text{dxs}} \text{CO}_2 \xrightarrow{\text{IspC}} \text{DXP} \xrightarrow{\text{dxr}} \text{NADPH} \xrightarrow{\text{IspD}} \text{CTP} \xrightarrow{\text{PP}_i} \text{CDP-ME} \xrightarrow{\text{IspF}} \text{MECDP} \]

\[
\text{IspE} \xrightarrow{\text{ATP}} \text{ADP} \xrightarrow{\text{CDP-ME2P}} \text{IspG} \xrightarrow{\text{HMBPP}} \text{IspH} \xrightarrow{\text{IPP-Isomerase}} \text{IPP} \xrightarrow{\text{DMAPP}}
\]
Folate cycle

Tetrahydrofolate (THF)

Gly

Ser

SHMT

5,10-CH₂-THF

SHMT

TS

5,10-CH₂-THF

dUMP

dTMP

THF

DHFR

NADPH

DHF

DHFS

DHP

DHFS

DHP

Pteridine diphosphate + PABA

NADP⁺