Chemical Matter - the Good, the Bad and the Ugly

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Medicinal Chemistry and Organic Synthesis play a very important role in modern drug discovery

What is Medicinal Chemistry?

What is Organic Synthesis?
A Medicinal Chemist is not the same as a Synthetic Chemist

• For a medicinal chemist, synthesis is a tool, not a goal

• A medicinal chemist integrates data from a variety of sources, and uses this information to design new generations of compounds

• Knowledge of synthetic chemistry comes in choosing these new targets, in designing and implementing the syntheses
Many disciplines play critical roles... but chemistry is central...
The role of the medicinal chemist is unique in the drug discovery process... because everything starts with the molecule; and with the iterative design process that optimizes the properties of the molecule.
Your Partners Provide Key Data

**BIOLOGICAL SCREENING**

*Do my molecules interact with the desired targets?*

**MODELLING**

*Hypothesis generation, selection/triage of new targets*

**STRUCTURAL BIOLOGY**

*Identification of binding modes*

**DRUG METABOLISM**

*What removes my compound from the body?*

**MEDICINAL CHEMISTRY AND ORGANIC SYNTHESIS**

**PHARMACOLOGY**

*Do my molecules work in disease models?*

**PHARMACEUTICS**

*Are my molecules stable? soluble? crystalline?*

**TOXICOLOGY**

*What properties are impeding the ability of my drug to get where it needs to go, and to do its job once it’s there?*

*What are the adverse interactions I need to avoid?*
Responding to metabolism data

- **BIOLOGICAL SCREENING**
- **STRUCTURAL BIOLOGY**
- **DRUG METABOLISM**
- **PHARMACEUTICS**
- **MODELLING**
- **MEDICINAL CHEMISTRY AND ORGANIC SYNTHESIS**
- **PHARMACOLOGY**
- **PHARMACOKINETICS**
- **TOXICOLOGY**
Responding to metabolism data

Active OT receptor antagonist
Rapidly metabolized
Low bioavailability

Both metabolites are active OT antagonists; the N-Me is also metabolized rapidly, but the -NH$_2$ analog is stable, with improved bioavailability.

Plasma drug, ng/mL
Responding to safety data

BIOLOGICAL SCREENING

STRUCTURAL BIOLOGY

DRUG METABOLISM

PHARMACEUTICS

MODELLING

PHARMACOLOGY

PHARMACO-KINETICS

TOXICOLOGY

MEDICINAL CHEMISTRY AND ORGANIC SYNTHESIS
Responding to safety data

Inhibitor of cell adhesion
Potent, good PK profile
Ames positive

From DEREK analysis:
Aryl-NO₂ can be genotoxic

Potent, good PK profile
Ames negative

The Ames test is a widely employed method that uses bacteria to test whether a given chemical can cause mutations in the DNA of the test organism.

It is a biological assay to assess the mutagenic potential of chemical compounds.
The Complex Reality

• If you want to make a drug, you have to solve all of these challenges simultaneously, in one molecule

• It isn’t sufficient to take them on one at a time; it doesn’t help to make 500 analogs of a lead structure...
  • if they all have the same metabolism problem...
  • or the same safety issue...
  • or they are all highly bound to plasma proteins...
  • or none of them can cross membranes to get to the target

• You (with your partners) need to identify critical issues ASAP, and focus your attention on addressing these
  • Your testing strategy needs to adjust so that you can get rapid feedback on key challenges
  • Be aware, that solutions to one problem, can introduce another!
Designing New Targets

• It’s not enough to make new compounds because they look like your current leads

• At the *beginning* of a program, you need to be thinking about the *end* of the program
  
  • What is the target profile (TPP) for your ideal compound?
  
  • How does your current lead fall short of this target?
  
  • What hypotheses do you have, for how to address these shortcomings?
  
  • What compounds can you design (and make) to test these hypotheses?
An Evolving Role

- Medicinal chemists have a role in
  - Hit-To-Lead (HTL) Evaluation
    - Structure alerts
      - Toxicity “flags”
    - False hits, PAINS
  - Homology searching to probe SAR
  - Lead Optimization (LO)
    - Multi-property optimization through SAR studies
  - Candidate selection
    - “Tight SAR” for final optimization of properties
    - Early scale-up to support advanced characterization
...but...I’m a *REAL* chemist...

- there’s a very important role for a Chemist in pharma
  ...in process research

- In process chemistry, you have a single synthetic target
  (the drug candidate)

  - Scale up (mg -> g -> kg and beyond)
  - Synthetic efficiency
  - Minimizing waste
  - Co$t
How med-chemists make Emend$^\text{®}$

11 steps, < 20% overall yield
Toxic/reactive reagents
Low temperatures/inert atmosphere

Anti-emetic
Merck
The commercial process

- **6 STEPS, 55% OVERALL YIELD**
- **Total production waste reduced by 85%**
Chemical & Engineering News looks at 46 drugs that have had a major impact on human health and society.
ALPHABETICAL INDEX

• Allegra
• Aspirin
• AZT
• Botox
• Cisplatin
• Crixivan
• Cyclosporine
• Digoxin
• Erythropoietin
• Ether
• Fentanyl
• Fluoride
• Fosamax
• Hydrocortisone
• Insulin
• Isonizid

• Ivermectin
• Librium
• Lovastatin
• Medical marijuana
• 6-Mercaptopurine
• Methadone
• Morphine
• Oral contraceptives
• Oxytocin
• Penicillin
• Phenobarbital
• Premarin
• Prontosil
• Prozac

• Quinine
• Ritalin
• Rituxan
• RU-486
• Salbutamol
• Salvarsan
• Tagamet
• Taxol
• Thalidomide
• Thorazine
• Thyroxine
• Vaccines
• Viagra
• Vioxx
• Vitamins

~80% synthetic compounds!!!

Félix Hoffmann (1898)

Sir Simon Campbell
Source of new drugs

Until the beginning of twentieth century, the substances used for the treatment of diseases were obtained from natural sources.

Natural sources include plants, animals, and minerals.

Among the natural sources, plants were mainly used.

Sometimes minerals and occasionally animals were used for the same purpose.

Nowadays most of the drugs are manufactured in the laboratory, i.e. synthetic drugs.

Microorganisms also serve as a source of a large number of drugs.
The major categories used are as follows:

“B” Biological; usually a large (>45 residues) peptide or protein either isolated from an organism/cell line or produced by biotechnological means in a surrogate host.

“N” Natural product.

“NB” Natural product “Botanical” (in general these have been recently approved).

“ND” Derived from a natural product and is usually a semi-synthetic modification.

“S” Totally synthetic drug, often found by random screening/modification of an existing agent.

“S*” Made by total synthesis, but the pharmacophore is/was from a natural product.

“V” Vaccine.
Natural Products as Sources of New Drugs from 1981 to 2014
David J. Newman*† and Gordon M. Cragg*

DOI: 10.1021/acs.jnatprod.5b01055

All New Approved Drugs; n = 1355

"B" Biological;
"N" Natural product.
"NB" Natural product “Botanical”
"ND" Derived from a natural product and is usually a semi-synthetic modification.
"S" Totally synthetic drug, often found by random screening/modification of an existing agent.
"S*“ Made by total synthesis, but the pharmacophore is/was from a natural product.
"V" Vaccine.
"NM" Natural product mimic”

Synthetic – 53%
Source of Small Molecule Approved Drugs; n = 1073

- "B" Biological
- "N" Natural product
- "NB" Natural product “Botanical”
- "ND" Derived from a natural product and is usually a semi-synthetic modification
- "S" Totally synthetic drug, often found by random screening/modification of an existing agent
- "S*" Made by total synthesis, but the pharmacophore is/was from a natural product
- "V" Vaccine
- "NM" Natural product mimic

Synthetic – 66%
William C. Campbell, Satoshi Ōmura and Youyou Tu Win 2015 Nobel Prize for Physiology or Medicine

Awards: Researchers' work led to drugs against roundworm diseases and malaria

**Artemisinin: Discovery from the Chinese Herbal Garden**

Louis H. Miller* and Xinzhuang Su†

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†Correspondence: lmiller@niaid.nih.gov

DOI 10.1016/j.cell.2011.08.024

This year’s Lasker DeBakey Clinical Research Award goes to Youyou Tu for the discovery of artemisinin and its use in the treatment of malaria—a medical advance that has saved millions of lives across the globe, especially in the developing world.

**Ivermectin**

Ivermectin: C&EN’s Top Pharmaceuticals That Changed the World:
http://pubs.acs.org/cen/coverstory/83/8325/8325ivermectin.html
DNDi 2016
Innovation & Access - Partners’ Meeting
RIO DE JANEIRO • 6-8 JUNE
The Lead Optimization Latin America (LOLA) consortium: collaborative drug discovery for Neglected Tropical Diseases (NTDs)

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³AbbVie Inc., Chicago, USA
⁴Drugs for Neglected Diseases initiative (DNDi), Geneva, Switzerland
⁵Independent consultant
Origins of leads against *T. cruzi*
Early leads for new drugs for Chagas disease

**Monocyclic series**

![Chemical structure of TDR30139]

**TDR30139**

$IC_{50} = 0.34 \ \mu M \ (in \ vitro)$

- TDR screening campaign
- TDR optimisation project

**Bicyclic series**

![Chemical structure of LOLA4]

**LOLA4**

$IC_{50} = 0.03 \ \mu M \ (in \ vitro)$

- NIH funded screen of the Broad Institute compound collection

**Medicinal Chemistry Centre for Chagas Disease in Brazil**

**World Health Organization**

New Medicinal Chemistry Centers to Join Drug Discovery Networks

T24/181/136 ID No. A80141

The Special Program for Research and Training in Tropical Diseases

TDR/UNICEF/UNDP/WB/WHO

**PRINCIPAL INVESTIGATORS**

- **ADRIANO D. ANDRICOPULO**
  University of Sao Paulo
  MEDICINAL CHEMISTRY AND DRUG DESIGN

- **GLAUCIUS OLIVA**
  University of Sao Paulo
  STRUCTURAL BIOLOGY AND STRATEGIC PLANNING

- **LUÍZ CARLOS DIAS**
  UNICAMP
  ORGANIC SYNTHESIS
### Project Collaborators in Alphabetical Order and Their Contributions

- **Antwerp University, Laboratory of Microbiology, Parasitology and Hygiene (LMPH, Belgium)**
  The LMPH (Laboratory of Microbiology, Parasitology and Hygiene) conduct in vitro testing of new compounds against *T. cruzi*, *L. infantum*, *T. brucei*, *T.b. rhodesiense* and parallel assessments of cytotoxicity against MRC-5 (human fibroblast) cells and PMMs (primary mouse macrophages). The same lab can test any active compounds in mouse and hamster animal models of VL.

- **GSK, Tres Cantos (Spain)**
  Test compounds for CYP51 inhibition to rule out this mode of action.

- **London School of Hygiene and Tropical Medicine (LSHTM)**
  World leading centre for research and education in public and global health. Providing testing for compounds in an acute mouse model of Chagas disease as a proof of concept.

- **Sandexis LLP (UK)**
  Provide expert medicinal and computational chemistry support to DNDi, and have been supporting the optimization of the new series from the Pfizer collection.

- **Swiss Tropical and Public Health Institute (Switzerland)**
  Public organization which runs, *in vitro* drug action studies in *T. cruzi* on 2 leading compounds from the cyanopyridine series.

- **Swiss TPH**
  Prof. L.C. Dias lab runs the project at UNICAMP. Selection of targets provided by DNDi. Planning and synthesis of derivatives. Data evaluation and decision on course of the series.

- **University of Dundee – Drug Discovery Unit (UK)**
  Compounds tested in a CYP51 assay to evaluate the primary mechanism of action of the cyanopyridines against *T. cruzi*.

- **University of Sao Paulo at Sao Carlos, Centre for Research and Innovation in Biodiversity and New Drugs-CIBFAR, IFSC-USP**
  The LQMC (Laboratory of Medicinal and Computational Chemistry) conduct medicinal chemistry studies including *in vitro* testing of new compounds against *T. cruzi* and *L. donovani*, and parallel assessments of cytotoxicity against MRC-5 (human fibroblast) cells and PMMs (primary mouse macrophages). The same lab is establishing validated assays to test promising active compounds in animal models of VL and *T. cruzi*.

- **Wuxi AppTec (China)**
  CRO based in Shanghai, China, providing DMPK services to the project. This will mainly be *in vivo* rodent (mouse, hamster & rat) study to provide PK results for novel compounds. These results will be used to set appropriate dosing regimens for testing in subsequent animal models of Chagas and/or VL, and to understand general DMPK properties for further optimization.
General Synthesis

**monocyclic cyanopyridines**

\[
\text{MeOCOCMe} + \text{NC\textsubscript{2}S\textsubscript{2}NH\textsubscript{2}} \xrightarrow{\text{Et}_3\text{N, ethanol, reflux, 30 min}} \text{thiopyridone}
\]


**bicyclic cyanopyridines**

\[
\text{H-O-Ar} + \text{NC\textsubscript{2}S\textsubscript{2}NH\textsubscript{2}} \xrightarrow{\text{Et}_3\text{N, ethanol, reflux, 30 min}} \xrightarrow{\text{then piperidine, reflux, 18 h}} \text{thiopyridone}
\]

Synthesis of TDR30139 derivatives

- TDR30139
  - IC₅₀ = 0.34 µM

- LOLA4
  - IC₅₀ = 0.03 µM
  - Monocyclic

- LOLA3
  - IC₅₀ = 0.31 µM
  - Monocyclic

- TDR91228
  - IC₅₀ = 1.2 µM
  - Monocyclic

- TDR100524
  - IC₅₀ = 26 µM
  - Monocyclic

- TDR100612
  - IC₅₀ = 70 µM

- TDR95696
  - IC₅₀ = 2.0 µM

- LOLA48
  - IC₅₀ = 7.9 µM
  - Bicyclic

- LOLA67
  - IC₅₀ = 0.58 µM
  - Bicyclic

- MAD328
  - IC₅₀ > 100 µM
  - Bicyclic

- LOLA67
  - IC₅₀ = 0.03 µM
MOA is not CYP51 inhibition

- TDR30139 & TDR91219 have promising *in vitro* activity against *T. cruzi*
- Hit to lead chemistry in progress at University of Campinas
- Check for CYP51 inhibition before investing too much effort:

  ![Chemical Structures]

  TDR30139
  *T. cruzi* IC₅₀ = 0.34 µM
  CYP51 IC₅₀ > 10 µM

  TDR91219
  *T. cruzi* IC₅₀ = 0.7 µM
  CYP51 IC₅₀ > 10 µM

- Experiment kindly carried out by collaborators at GSK, Tres Cantos, and Dundee Drug Discovery Unit
Deeper characterisation of *in vitro* activity

Recovery of *T. cruzi* amastigotes: Standard assay vs. wash-out

- **TDR30139**
  - ![Chemical Structure](image1.png)
  - **Graph**
    - % infected cells vs. log(cpd concentration) in µg/ml
    - Graphs for L6 CPRG 96h, PMM Giemsa 96h, PMM Giemsa 24 + 72h, PMM Giemsa 96 + 168h
  - **Table**
    - | Concentration (µg/ml) | L6 96h CPRG IC50 | L6 96h CPRG IC90 | PMM 96h Giemsa IC50 | PMM 96h Giemsa IC90 | PMM 24h + 72h Giemsa IC50 | PMM 24h + 72h Giemsa IC90 | PMM 96h +168h Giemsa IC50 | PMM 96h +168h Giemsa IC90 |
    - | | | | | | | |
    - | TDR91219 | 0.166 | 3.23 | 0.536 | 1.97 | 1.66 | na | 2.40 | na |
    - | TDR30139 | 0.263 | 2.65 | 0.624 | 8.61 | 2.21 | na | 2.40 | na |

- Further confirmation of good *in vitro* activity
- Aim to test relevance of residual parasites in an *in vivo* assay
Good potency and SI possible within the series
Metabolic Stability

Incubation in human and rat liver microsomes (60 min, 10 µM) (AbbVie).

- However, 0% remaining in female mouse plasma at rt after 0.5 h (Wuxi).
- Amide likely unstable.
Kinetic Solubility Results

**LOLA67**

- K.S. (pH 2.0) < 1 µM
- K.S. (pH 7.4) < 1 µM

**LOLA2**

- K.S. (pH 2.0) < 1 µM
- K.S. (pH 7.4) < 1 µM

**LOLA3**

- K.S. (pH 2.0) > 200 µM
- K.S. (pH 7.4) = 2.65 µM

**LOLA4**

- K.S. (pH 2.0) > 200 µM
- K.S. (pH 7.4) < 1 µM

Theoretical concentration: 200 µM

K.S. Buffer: 50 µM phosphate buffer, pH 2.0 and 7.4
Formulation studies on LOLA67

In vivo (mouse) PK studies

Acute mouse model of Chagas Disease

Poor plasma solubility

LOLA67

(MAD431)

IC$_{50}$ = 0.58 μM
cLogP = 3.74 ± 0.53

10 mg/mL

10% DMSO,
10% Cremophor EL,
40% PEG400,
40% Water; step by step

SN CN O F
H3C
H3C
IC50 = 0.58 μM
LOLA67
(MAD431)
cLogP = 3.74 ± 0.53
3-cyanopyridines

- Monocyclic and bicyclic subseries
- > 200 analogues synthesized for LOLA
- Sub-μM against *T. Cruzi* (*in vitro*)
- Potency not driven by CYP51 inhibition
- No cytotoxicity issues
- Good stability in human and rat liver microsomes
- Low clearance in human and rat
- *T. Cruzi* amastigote recovery <100% inhibition (limited by solubility)
- CN, C=O, Pyr, side chain, Me groups aryl ring very important

- **Increase solubility**

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
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<tbody>
<tr>
<td><em>T. Cruzi</em> IC(_{50})</td>
<td>0.7 µM</td>
</tr>
<tr>
<td>CYP51 IC(_{50})</td>
<td>&gt; 10 µM</td>
</tr>
<tr>
<td>Cytotox MRC-5 cells IC(_{50})</td>
<td>&gt; 64 µM</td>
</tr>
<tr>
<td>Cytotox PMM IC(_{50})</td>
<td>&gt; 64 µM</td>
</tr>
<tr>
<td>Cl(_{int}) (human mic.)</td>
<td>11.8 L/hr/kg</td>
</tr>
<tr>
<td>Cl(_{int}) (human hep.)</td>
<td>16 L/hr/kg</td>
</tr>
<tr>
<td>Cl(_{int}) (rat mic.)</td>
<td>42 L/hr/kg</td>
</tr>
<tr>
<td>Cl(_{int}) (rat hep.)</td>
<td>45.7 L/hr/kg</td>
</tr>
<tr>
<td>E(<em>{max}) E(</em>{max})</td>
<td>&lt; 100% inhibition</td>
</tr>
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**Solubility**: poor

**IV - Solution** in 60% PEG400, 50 mM sodium citrate, pH 4.5.

**PO- Solution** in 25% hydroxypropyl-β-cyclodextrin, 50 mM sodium citrate, pH 3.3.
Bicyclic series - Issues

- > 40 analogues synthesized for LOLA
- Very variable *in vitro* results
- Low oral bioavailability from mouse PK
- Amides unstable in plasma
- More soluble analogues less active
- Challenging to achieve *in vivo* POC

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<thead>
<tr>
<th>Property</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
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<tbody>
<tr>
<td><em>T. Cruzi</em> (LMPH)</td>
<td>54.86, 0.03, 0.03</td>
</tr>
<tr>
<td></td>
<td>&gt;64 retest</td>
</tr>
<tr>
<td></td>
<td>&gt;64, 26.9 new batch</td>
</tr>
<tr>
<td><em>T. Cruzi</em> (LMQC)</td>
<td>2.01 ± 0.37</td>
</tr>
<tr>
<td>Cytotox MRC-5 cells</td>
<td>&gt;64, 52.9, 30</td>
</tr>
<tr>
<td></td>
<td>25.4, &gt;64 retest</td>
</tr>
<tr>
<td></td>
<td>&gt;64, 34.3 new batch</td>
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DNDi
Drugs for Neglected Diseases initiative

AbbVie

Pfizer