Steering Clear of the Drug Discovery Black Hole

Mark O’Neil-Johnson
How to Survive for 11 Years as a Natural Products Drug Discovery Company

Mark O’Neil-Johnson
NEW DRUGS FROM THE PHARMACEUTICAL INDUSTRY?

“...nothing that drug companies have done in the past 60 years has succeeded in raising the mean annual NME output...”

Bernard Munos, Corporate Strategy, Eli Lilly & Company
*Nature Reviews Drug Discovery*, **2009**, 8, 959-968
NEW DRUGS FROM THE PHARMACEUTICAL INDUSTRY?

“...the number of new drugs that are approved annually is no greater now than it was 50 years ago.”

Bernard Munos, Corporate Strategy, Eli Lilly & Company
Nature Reviews Drug Discovery, 2009, 8, 959-968
"Opportunity in the Unknown Universe of Chemistry?"

24 Million Compounds CAS DB
836,708 different Frameworks in CAS
0.2% of Frameworks represent >70% compounds (~150 scaffolds)

Zoloft

Zoloft Framework

Known Universe of Chemistry (0.2%)?

Unknown Universe of Chemistry?

Natural Products?

Taxonomically Diverse Plant Collection

Parallel Purification Technologies

State-of-the-art NMR Probe Technology
Plants to Drug-Like Compounds

Sequoia Sciences’ Plant Collection
Sequoia Sciences’ Plant Collection Team
Preferred, Novel and Neglected Scaffolds

SEQUOIA 2008

SEQUOIA 2005
Preferred, Novel and Neglected Scaffolds

HIMBACINE

RAPAMUNE

PHLORIZIN

Cytisine
Ground Plant

Extract

Flash Chromatography

C18 SPE to remove lipophilics

LC/MS analysis, and removal of tannins with polyamide SPE

Preparative HPLC
5 x 40 20 mL chromatographic fractions

40 prep fractions from Flash fraction 5. Prep tubes 1 to 40 beginning at 60% organic on a C18 Prep Column to 85% at tube 36 to tube 40.

Extremely lipophilic fraction is discarded

A - 50:50 ethyl acetate : ethanol
B - 100 methanol
C - 75:25 hexane : ethyl acetate
D - 25:75 hexane : ethyl acetate
E - 100 ethyl acetate
F - 70:30 ethyl acetate : methanol
G - 50:50 ethyl acetate : methanol
H - 100 methanol : 5% NH₄OH

“Flash fractions”
PLANT EXTRACTION

NORMAL PHASE FLASH CHROMATOGRAPHY

REVERSE PHASE SOLID PHASE EXTRACTION

PREPARATIVE HPLC GENERATES
200 CHROMATOGRAPHIC FRACTIONS

ANALYSIS BY
8-channel
HPLC/MS/ELSD

Discarded Compounds From Flash Fractions

FF-6

FF-6

FF-6

FF-6

FF-8

FF-7,8

FF-7,8
Flash Chromatography

5

6

7

8

9

“Flash fractions”

PAPAVERINE

RESERPINE

PHLORIZIN

BRUCINE

QUINIDINE
1. 1 to 5 compounds per sample
2. Samples of known quantities
3. Drug-like compounds
   a. Average MW < 400 daltons
   b. Log P from 0 to 5

~ 110 samples / plant

Analysis performed on each sample

Sequoia Sciences, Inc.
High-Throughput Natural Products Chemistry
1. Normal Phase / Reverse Phase Chromatography (1 g. on column)

**4 to 20 fold increase in concentrations (250-50 mg)**

2. Parallel Preparative HPLC (50 mg. on column)

**50 to 250 fold increase in concentrations (1 mg-200 μg)**

3. HPLC/MS/ELSD Analysis

**200 to 4,000 fold increase in concentrations**
Antibiotic Indole Sesquiterpene Alkaloids
Natural Product Framework

Russell H. Williams,2 Jim Feng Ho, Krista M. Olson, Gary R. Eldridge, and Cowartney M. Stowe
Academic Research and Rapid Innovation: A Multidisciplinary Group, School of Pharmacy, University of Wisconsin,
Received March 8, 2018

High-throughput natural product chemistry on indole sesquiterpene alkaloids (4, 5) from Geotrichum candidum and M. penniculata (4) was determined in this study. The framework defined by all-reduced ester and the halogen thiol system found in these alkaloids are representative of the known indole sesquiterpenes. The structures of the compounds were identified by 1D and 2D NMR spectroscopy. The compounds were purified by HPLC and characterized by MS and NMR spectroscopy.

Recent publications have detailed the lack of diversity in synthetic organic compounds.1,2 One of the reasons for this is the lack of diversity in the natural products that are isolated from microorganisms.3,4 The framework defined by all-reduced ester and the halogen thiol system found in these alkaloids is representative of the known indole sesquiterpenes. The structures of the compounds were identified by 1D and 2D NMR spectroscopy. The compounds were purified by HPLC and characterized by MS and NMR spectroscopy.


What can I do with only micrograms of a sample?
Move to Miniaturize
Protasis CapNMR Probe

5 mm std. NMR tube

5 mm Rf Coil

270 μlit. (~17 mm)

1.5 μlit. (~1 mm)

5 μlit.
2 μg Quinidine in MeOH,d₄

5mm TXI probe @ 900 MHz
2 μg in 550 μL MeOH,d₄

CapNMR probe @ 600 MHz
2 μg in 8 μL MeOH,d₄; Infuse 5 μL
Sample Preparation Hardware and NMR Solvent

- 96-well plate
- 25 μl gas tight syringe
- mini-tube
- NMR Solvent
High-Throughput Natural Products Chemistry

Protasis Gradient Dual Coil Probe

Simultaneous Multiplexed Mode NMR Data Acquisition
Sequoia Sciences, Inc.

- 2001 – MRM selects Sequoia Sciences for test program to evaluate first commercially available CapNMR probe
- 2002 – Sequoia receives the first gradient 1H/13C CapNMR probe
- 2004 – Sequoia receives the first gradient 1H/13C/15N CapNMR probe
- 2010 – Bruker Instruments and Sequoia Sciences sign strategic collaboration to promote and develop 1.7mm MicroCryoProbe and software for natural products discovery research
High-Throughput Natural Products Chemistry

Bruker 1.7 MicroCryoProbe

1.7 mm MicroCryoProbe
2 µg 13 µl MeOH,d4 in 1 mm Tube
64 scans

CapNMR Probe
2 µg in 6 µl MeOH,d4
1024 scans
10 μg in 13 μl MeOH,d4

1 hr Acquisition

Bruker 1.7 MicroCryoProbe

High-Throughput Natural Products Chemistry
15 μg, 2 HPLC collections before screening

To NMR
Sequoia’s First Sample Acquired TCI 1.7 MicroCryoProbe
Sequoia’s First Sample Acquired TCI 1.7 MicroCryoProbe

1 hr. COSY

3 hr. HSQC

36 hr. HMBC
1.7mm MicroCryoProbe™

**g-HMBC**

(Long Range Proton-Carbon Correlation)

1.7 mm MicroCryoProbe

(Acquisition Time = 13 hrs.)

CapNMR Probe

(Acquisition Time = 20.5 hrs.)
Combine tubes C7, D7, E7 and F7

SepFraction 15827
9 μg total (2 HPLC Collections)
Original Sequoia SepFraction Generation Protocol

To NMR
New Sequoia SepFraction Generation Protocol
With TCI 1.7 MicroCryoProbe
Bruker TCI 1.7 MicroCryoProbe

SepFraction 18517 in MeOD (1 mm Tube)

SepFraction 18511 in MeOD (1 mm Tube)
Plant Family: Asteraceae
Species: *Layia platyglossa*

Combine tubes F10, G10 and H10

SepFraction 15509
6 ug total (2 HPLC runs)

To NMR
High-Throughput Natural Products Chemistry

ACD Software

64 Scans

SepFraction 15064 in MeOD

512 Scans

SepFraction 15509 in MeOD

Data base peak search
ACD Software
Sequoia’s Structure Elucidators

Courtney Starks

Russell Williams
High-Throughput Natural Products Chemistry

ACD Labs Structure Elucidator
ACD Labs Structure Elucidator

The Best Structure

High-Throughput Natural Products Chemistry
Programs at Sequoia Sciences

1) **Chronic UTI vaccine** (Phase 1 clinical trial to commence, Nov. 2011)
2) **Biofilm Inhibitor**
3) **Oncology lead compound**
4) **Natural product library**
5) **Gram positive antibacterial potentiator lead compound**
Sequoia Sciences Biofilm Inhibitor

High-Throughput Natural Products Chemistry

CONTROL    SEQUOIA’S COMPOUND
Pseudomonas Swarming

Control

0.5 µg/ml SQC
Pseudomonas Swarming

Control / Control  0.5 µg/ml SQC / Control
Biofilms / Swarming / Twitching

• More than 10 academic laboratories
  ❖ Greenberg, Wozniak, Mattick, Tolker-Nielsen, O’Toole, Singh, Kolter, Parsek, etc.

Cystic Fibrosis Patients

• Matsui, et al. *PNAS*, 2006, 103(48), 18131-6

Chronic Gram-negative Bacterial Infections
Expanding Demand for Antibiotics

1) ANTIBIOTIC RESISTANCE

2) LACK OF NEW GRAM-NEGATIVE ANTIBIOTICS

3) RESTRICTED ANTIBIOTICS

4) BLACK BOX WARNINGS
Control

4 ug/ml tobramycin

2 ug/ml tobramycin

0.5 ug/ml SQC
N = 520 CF patients - inhaled tobramycin versus inhaled placebo

## Sequoia Sciences Oncology Lead Program – Lead 8

<table>
<thead>
<tr>
<th>Tumor Cell Line</th>
<th>IC$_{50}$ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A375</td>
<td>73</td>
</tr>
<tr>
<td>M14</td>
<td>64</td>
</tr>
<tr>
<td>UACC – 62</td>
<td>44</td>
</tr>
<tr>
<td>SK-MEL-2</td>
<td>94</td>
</tr>
<tr>
<td>H460</td>
<td>220</td>
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<tr>
<td>SF-295</td>
<td>250</td>
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<tr>
<td>ACHN</td>
<td>360</td>
</tr>
<tr>
<td>PC-3</td>
<td>490</td>
</tr>
</tbody>
</table>

- No growth inhibition of mouse macrophages at 1 uM
- Demonstrates caspase 3/7 induction in the H460 tumor cell line in 24 hours using Promega Caspase-Glo 3/7 Assay System
- LOX-IMVI cell line established and tested by August 2011
- 60 tumor cell line panel tested in July / August 2011
1) Be a scrapper
2) Stay small
3) Stay focused
4) Only take the money that you need
5) Forget the fancy building, desks and chairs
6) Excellent analytical technology
7) Luck
Sequoia Sciences, Inc.

Courtney Starks, Russell Williams, Jin-Feng Hu, and Gary Eldridge

Protasis
Magnetic Resonance Microsensors

James Norcross
Tim Peck
Dean Olson
(National Institute of Health #RR16387)

Oskar Schett
Werner Maas

Jim Miller
Very Special Thanks to Ryan Sasaki of ACD
Thank You