Química Medicinal

HTS & HCS

Prof. Dr. Andrei Leitão

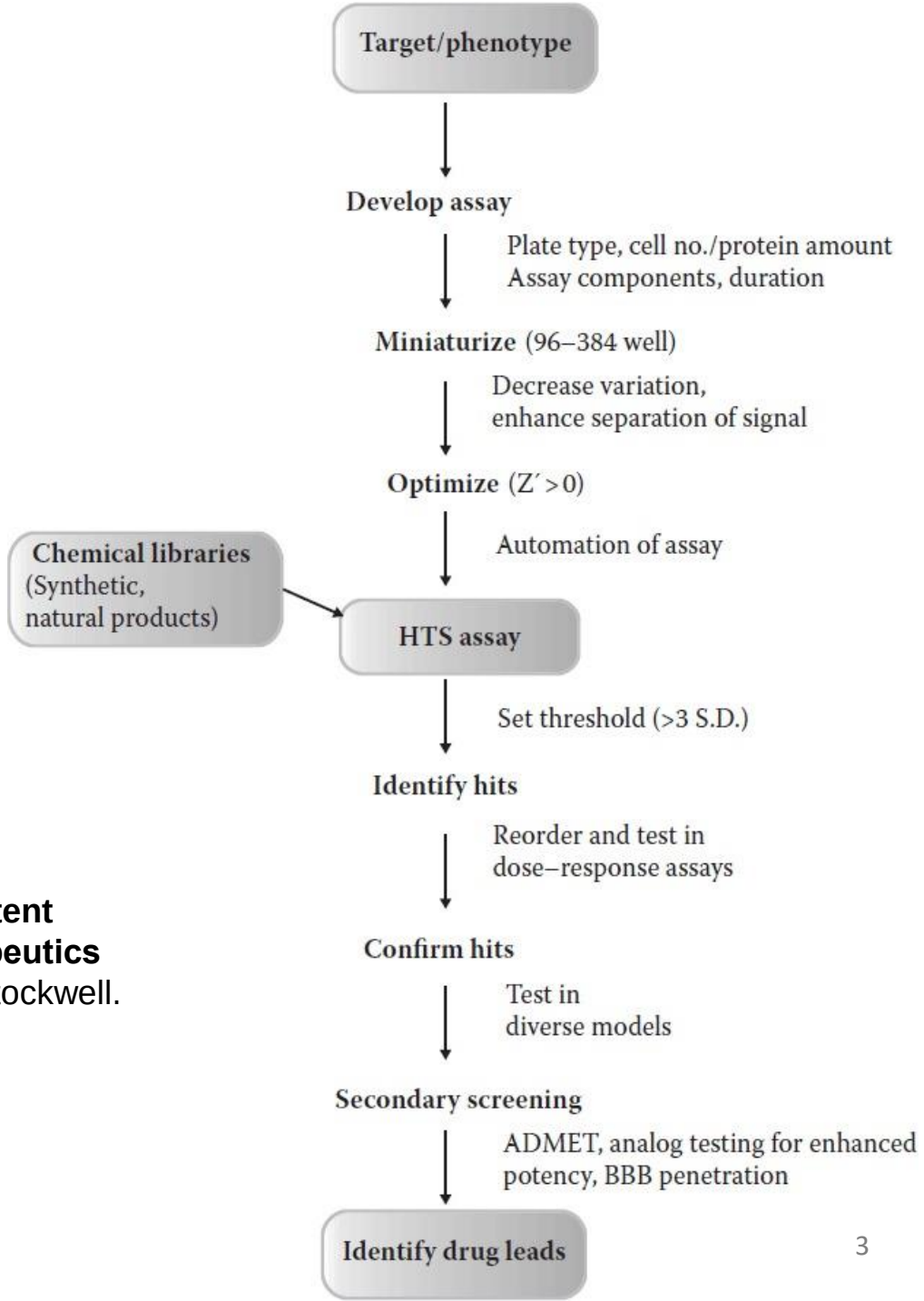
HTS & HCS

HTS: ensaio em massa (High-throughput assay)

HCS: ensaio de elevado conteúdo (High-content screening), também denominado HCA (High-content analysis) → baseado em imagem

https://www.genengnews.com/insights/drug-discovery-approaches-utilizing-three-dimensional-cell-culture/

HTS setup



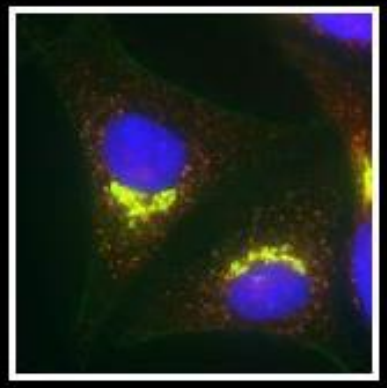
Chapter 5. High-Throughput and High-Content Screening for Huntington's Disease Therapeutics
Hemant Varma, Donald C. Lo, and Brent R. Stockwell.

In: Neurobiology of Huntington's Disease: Applications to Drug Discovery.

HTS setup

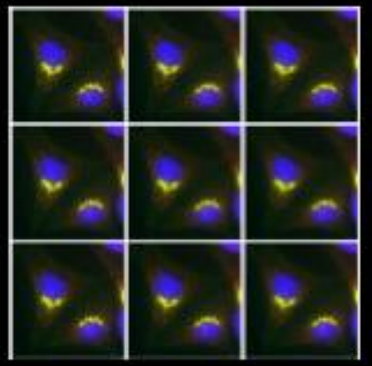
https://www.gesundheitsindustrie-bw.de/en/article/press-release/hit-discovery-constance-gmbh-a-new-hub-for-hts-and-compound-management

The need for high content screening and analysis



Number of genes / compounds studied

Experimental detail obtained



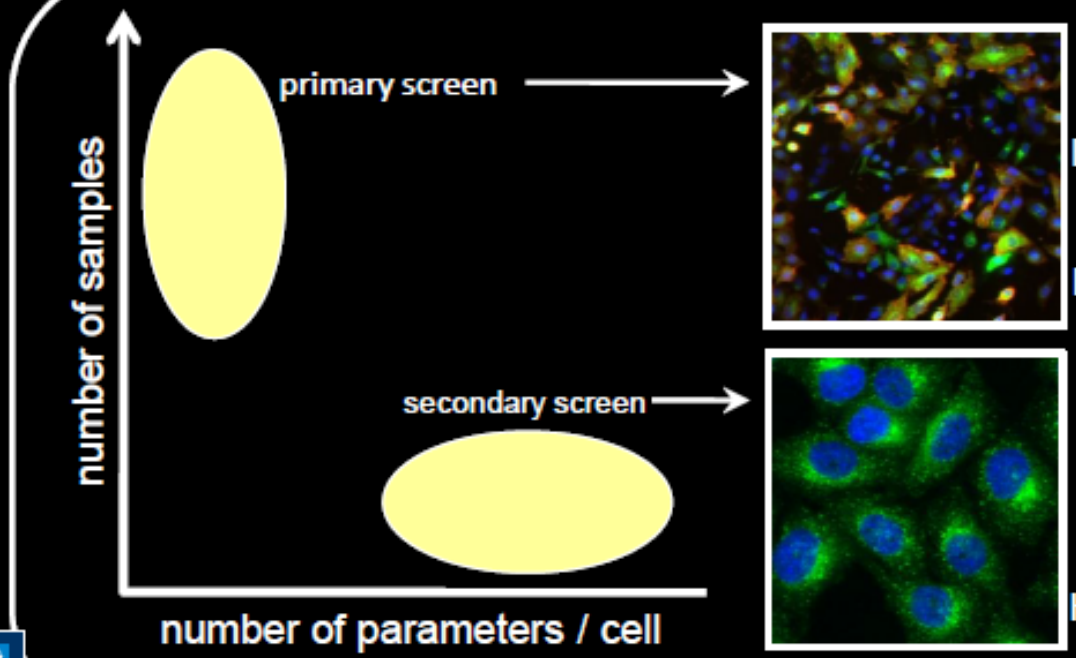
Human genome sequencing

- comprehensive cDNA libraries
- genome-wide RNAi reagents
- novel compound libraries

Jeremy Simpson, Ph.D.
University College Dublin
Dublin, Ireland

HCS experiments - so many decisions to make!

Basic assay → Primary screen → Secondary screen → Identify candidates → Characterise candidates



Primary screen

High sample number: eg: 80,000 siRNAs
High image number: eg: >1m images
High cell number: eg: >10m cells
Low parameter number: eg: 3 values/cell

Secondary screen

Low sample number: eg: 2,000 siRNAs
Low image number: eg: 20,000 images
Low cell number: eg: 0.5m cells
High parameter number: eg: >50 values/cell

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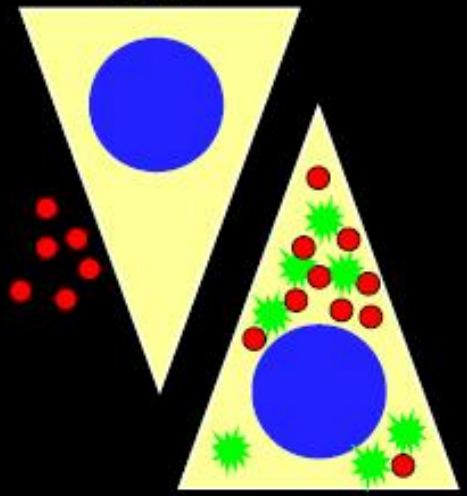
Dublin, Ireland

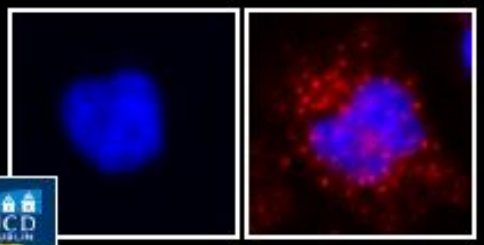
What can HCS / HCA tell us?

How much / how many ?

None

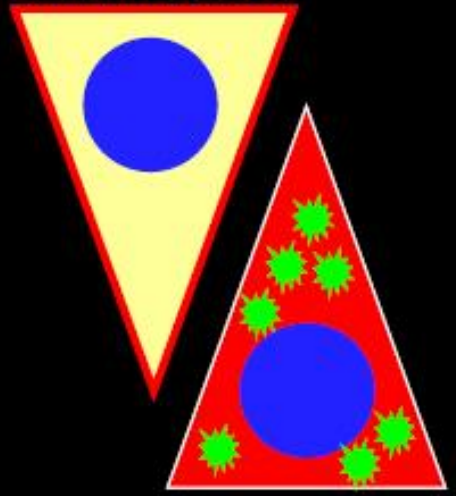


Many

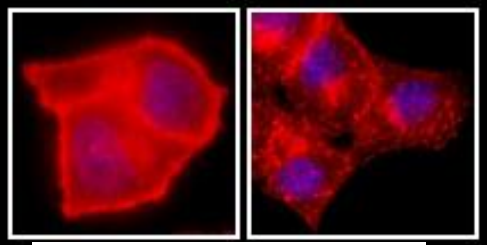


Where ?

Not internalised

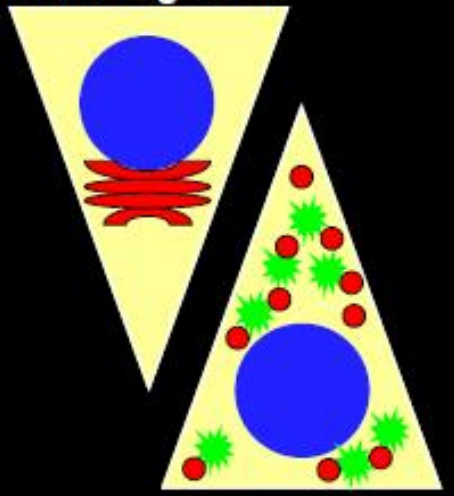


Internalised

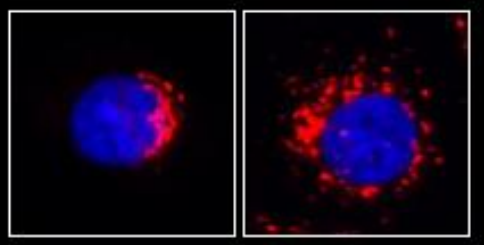


What effects ?

Intact organelle



Disrupted



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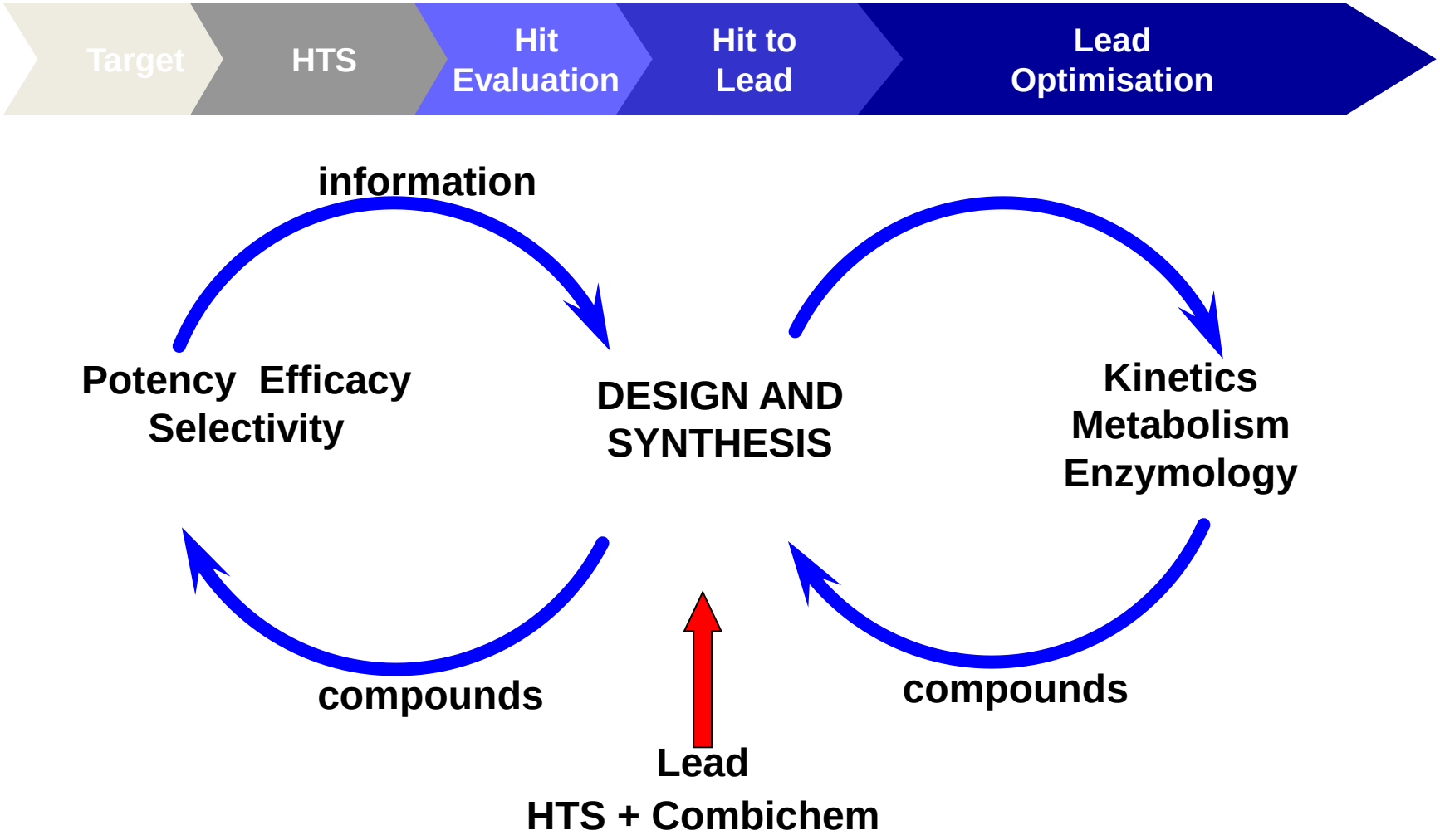
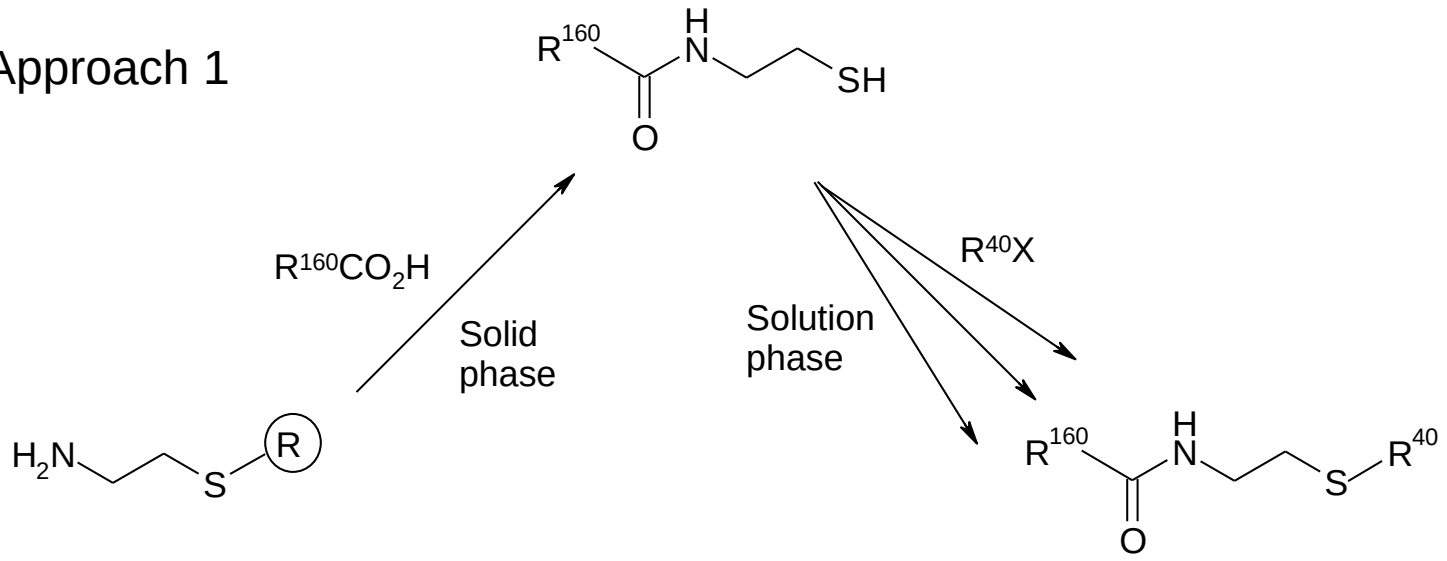
Fastest - first and best



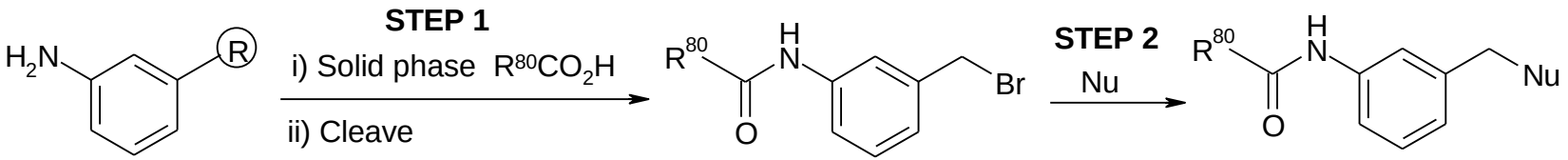
Teague, Leeson, Oprea, Davis, Angew Chem 1999, 38, 3743

“Universal” Library

Approach 1



Approach 2



Walters and Teague Tet Lett. 2000, 41, 2023

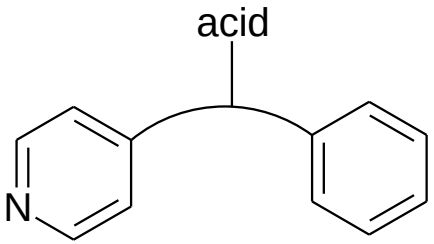
HTS Screening Hits

- Drug-like hits
  - potency of many underperform
  - binding via weak non-specific interactions
  - not all interactions made or very suboptimal
  - *would explain "flat SAR"* in Hit-to-Lead activities
  - *small μM leads easier to optimise than large μM*
- "easy" and "difficult" hit-to-lead projects
  - easy to increase Mwt/logP - increase potency
    - easy to demonstrate SAR, increase potency 10x
  - difficult because of flat SAR
    - difficult to reduce Mwt and logP maintaining potency

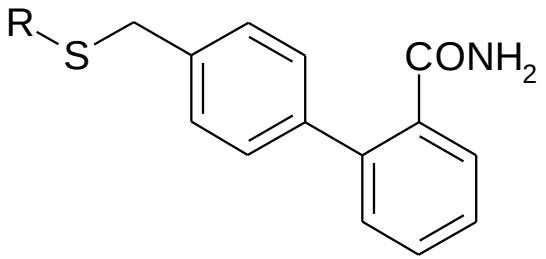
Teague, Leeson, Oprea, Davis, Angew Chem 1999, 38, 3743

Exemplos

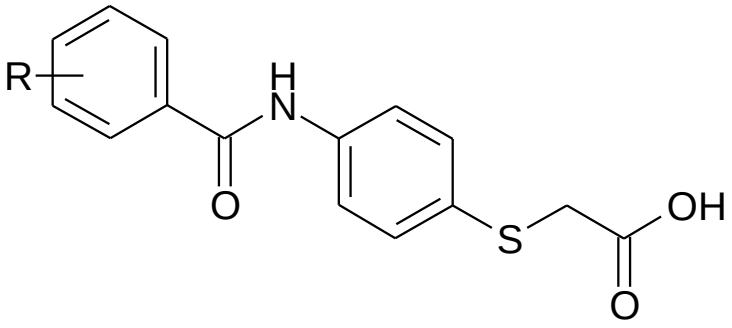
HTS Examples - GPCR Project



IC_{50} = 4.6 μM
Mwt 268
ClogP 3.4

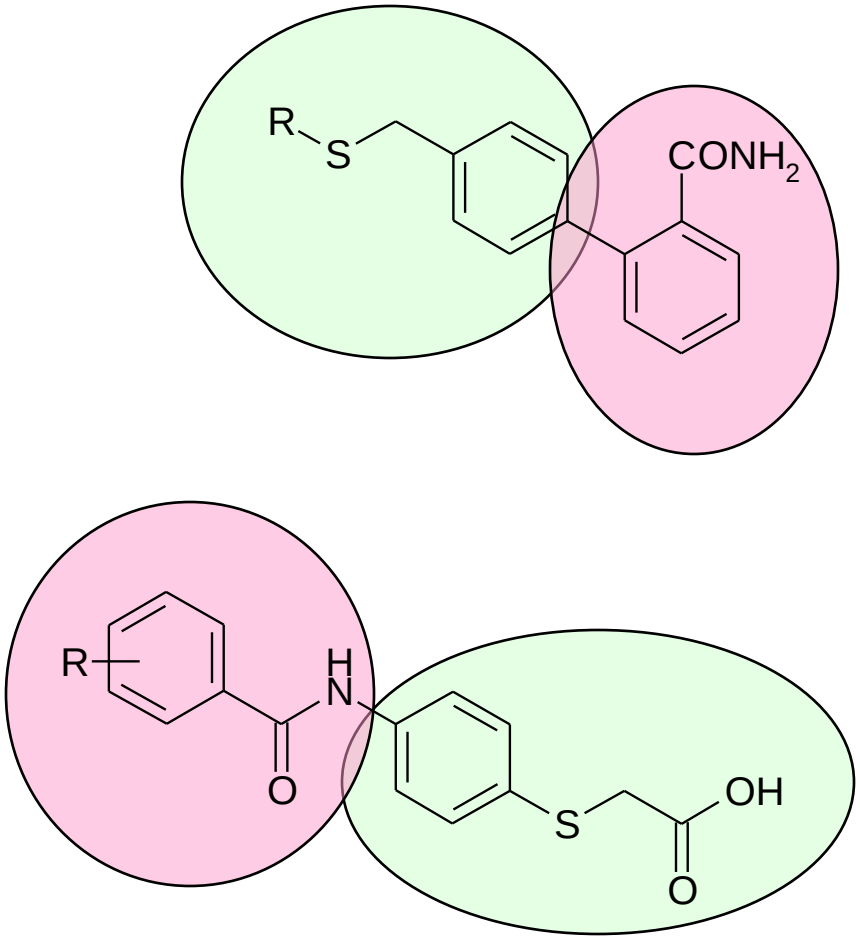


IC_{50} = 0.55 μM
Mwt 350
clogP 3.7



IC_{50} = 0.18 μM
Mwt 380
ClogP = 4.5

GPCR Hit-to-Lead

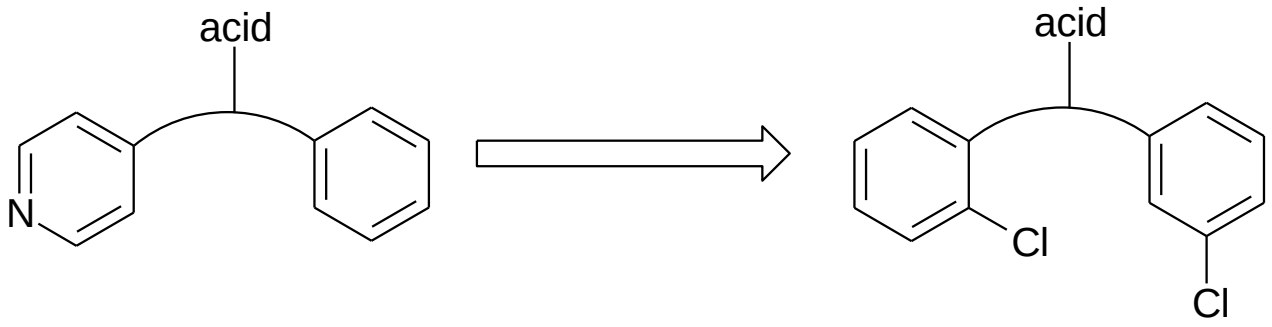


Many analogues
same or loss potency

Many analogues
same potency

- Both series dropped -

GPCR Hit-to-Lead



IC50 = 4.6 μM
Mwt 268
ClogP 3.4

IC50 = 0.02 μM
Mwt 336
ClogP 5.3 (:-<)

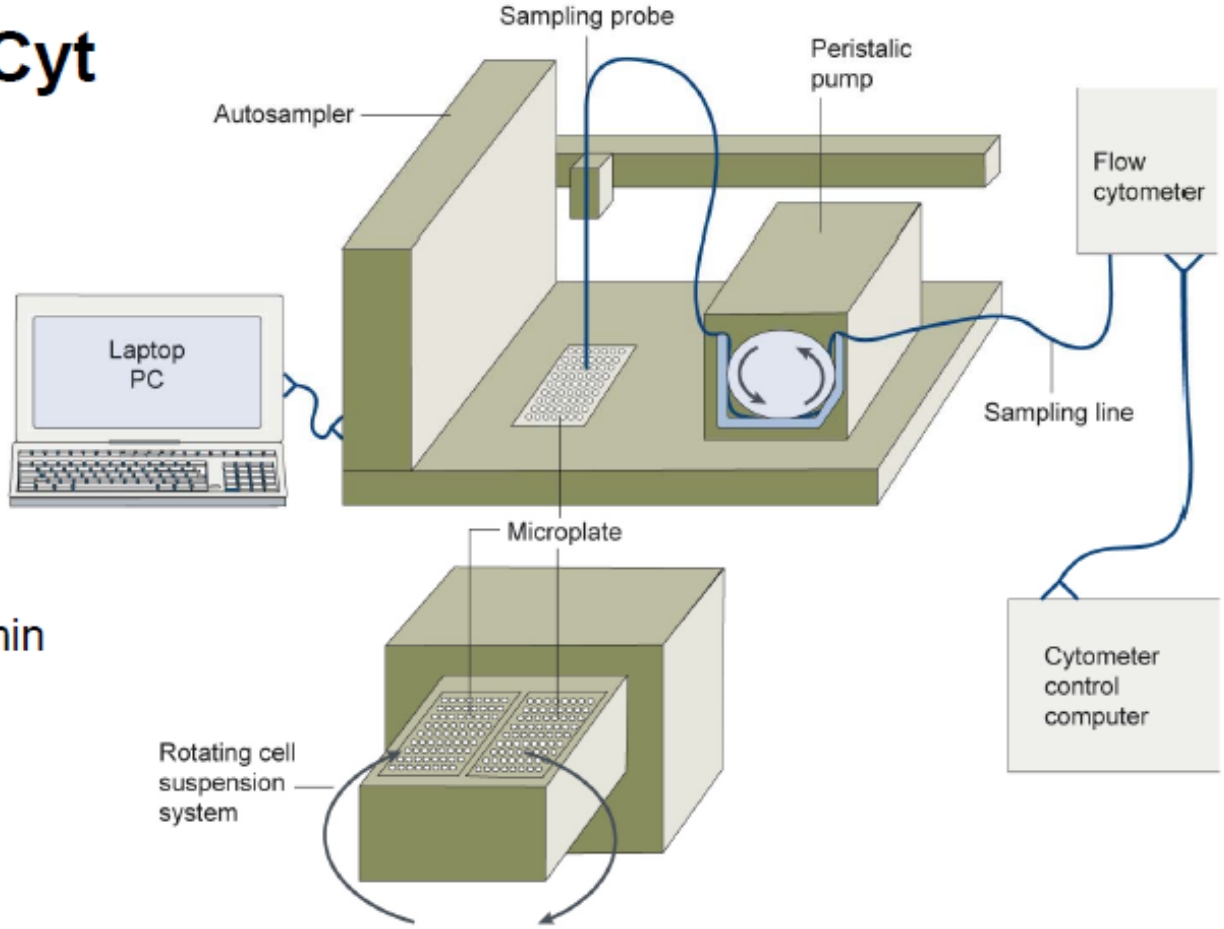
- Rapid Hit-to-Lead optimisation
  - clear SAR
  - drug-like series with good DMPK
  - patentable

Summary

- HTS
  - starting points are crucial to speed throughout process
  - screening file should reflect what chemists can easily work upon
  - ideally we all want to find drugs in our screening file
    - **but generally a HTS finds only leads not drugs**
  - file-size isnt everything = quality is equally important
- Libraries
  - Many approaches - targeted libraries v successful
    - kinase libraries - 4x hit rate - screening file
  - libraries should reflect what you wish to find
    - leads not drugs

Teague, Leeson, Oprea, Davis, Angew Chem 1999, 38, 3743

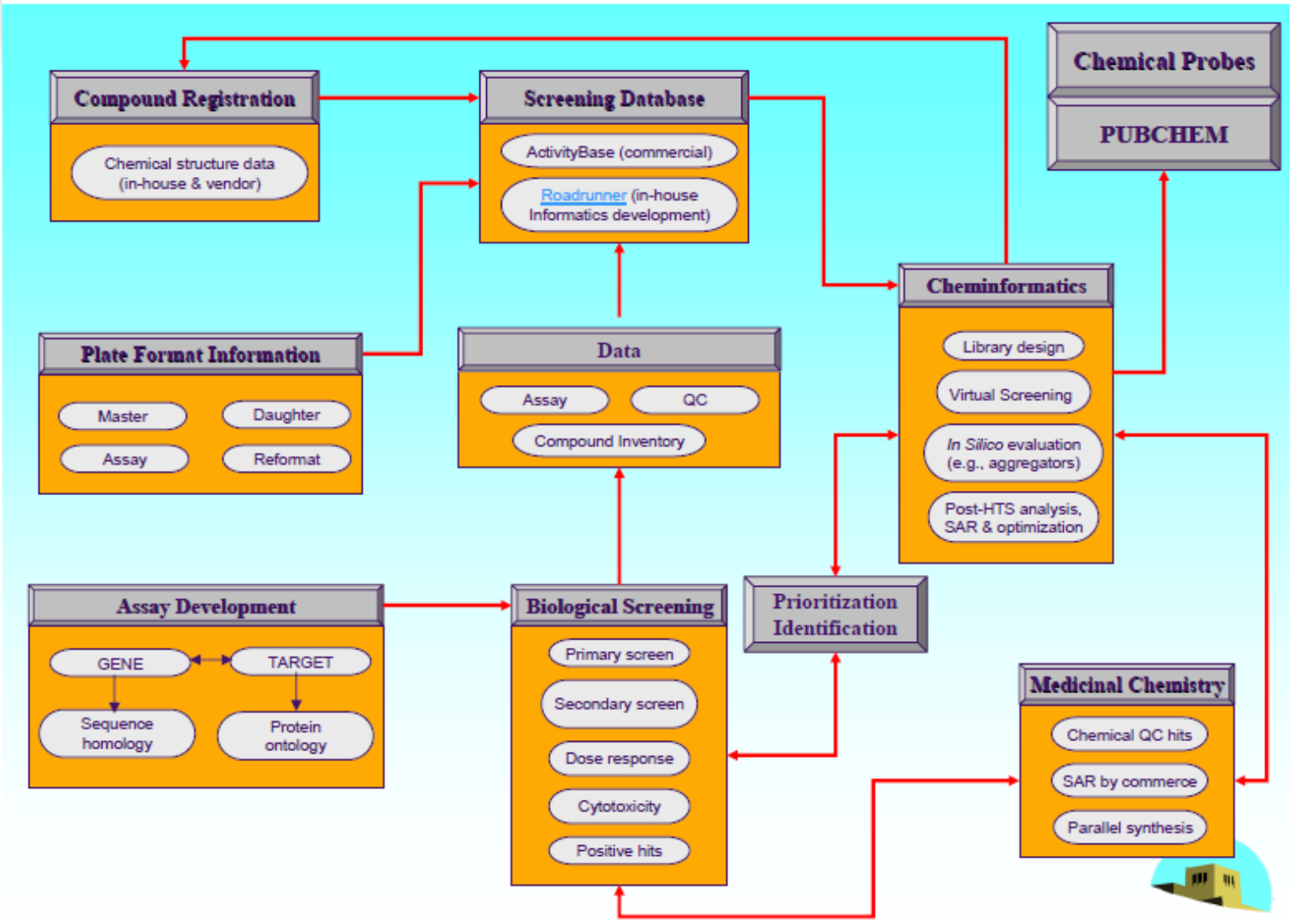
HyperCyt



384 wells/10 min
1 μl/sample

Edwards et al., *Curr. Opin. Chem. Biol.*, **2004** 8: 392-398

Edwards et al., *Nature Protocols*, **2006** 1: 59-66

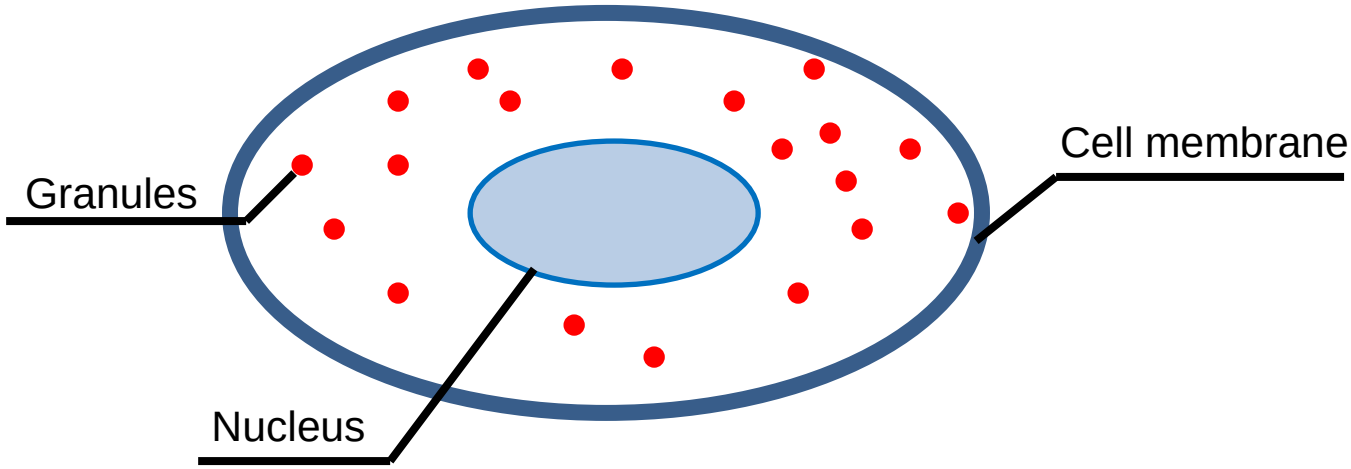


DC Fara et al., DDT Technol 2006, 3:377-385

The University of New Mexico
SCHOOL OF MEDICINE

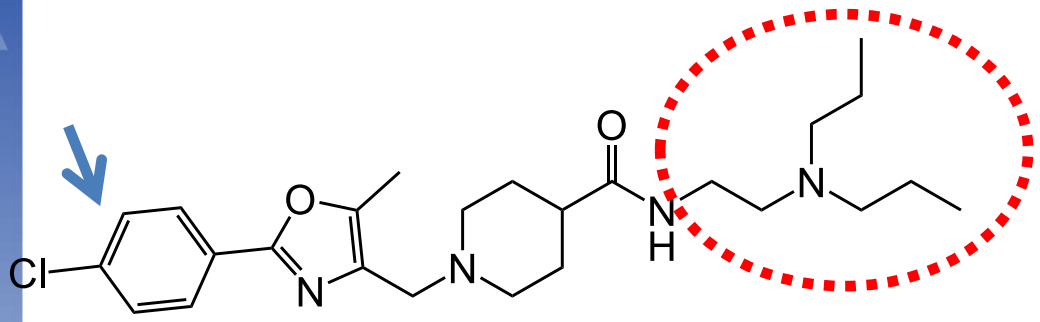
Cell-based assay

- ✓ 25 000 compounds tested
- ✓ Cell lines: AR-dependent (LNCaP) and AR-independent (PC3)
- ✓ Flow cytometry for adherent cells



Haynes, M. K.; Leitão, A. et al., *J. Biomol. Screen.* **2009**, 14, 596

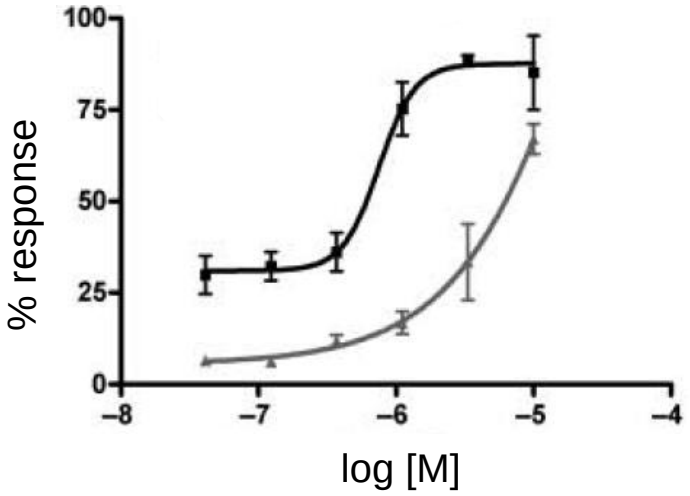
Cell-based assay

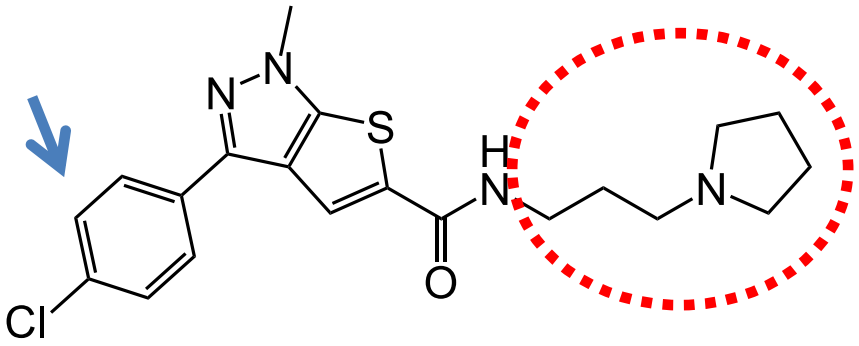


Family 1

■ AR-dependent EC_{50} = 0.77 µM

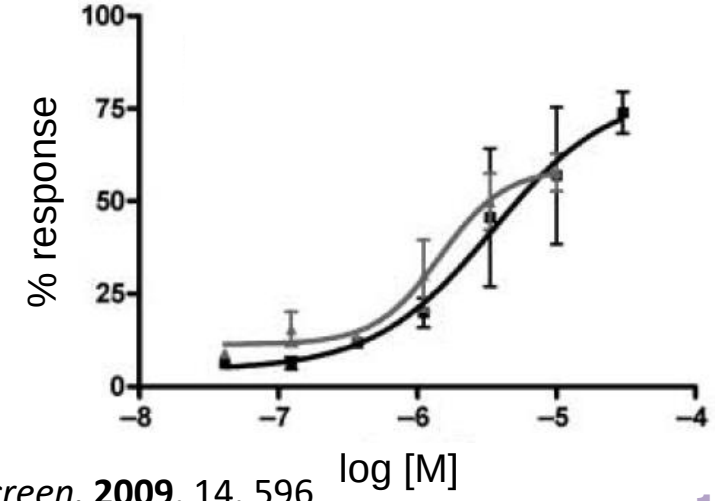
▲ AR-independent EC_{50} = 6.5 µM





Family 2

EC_{50} = 3.35 µM

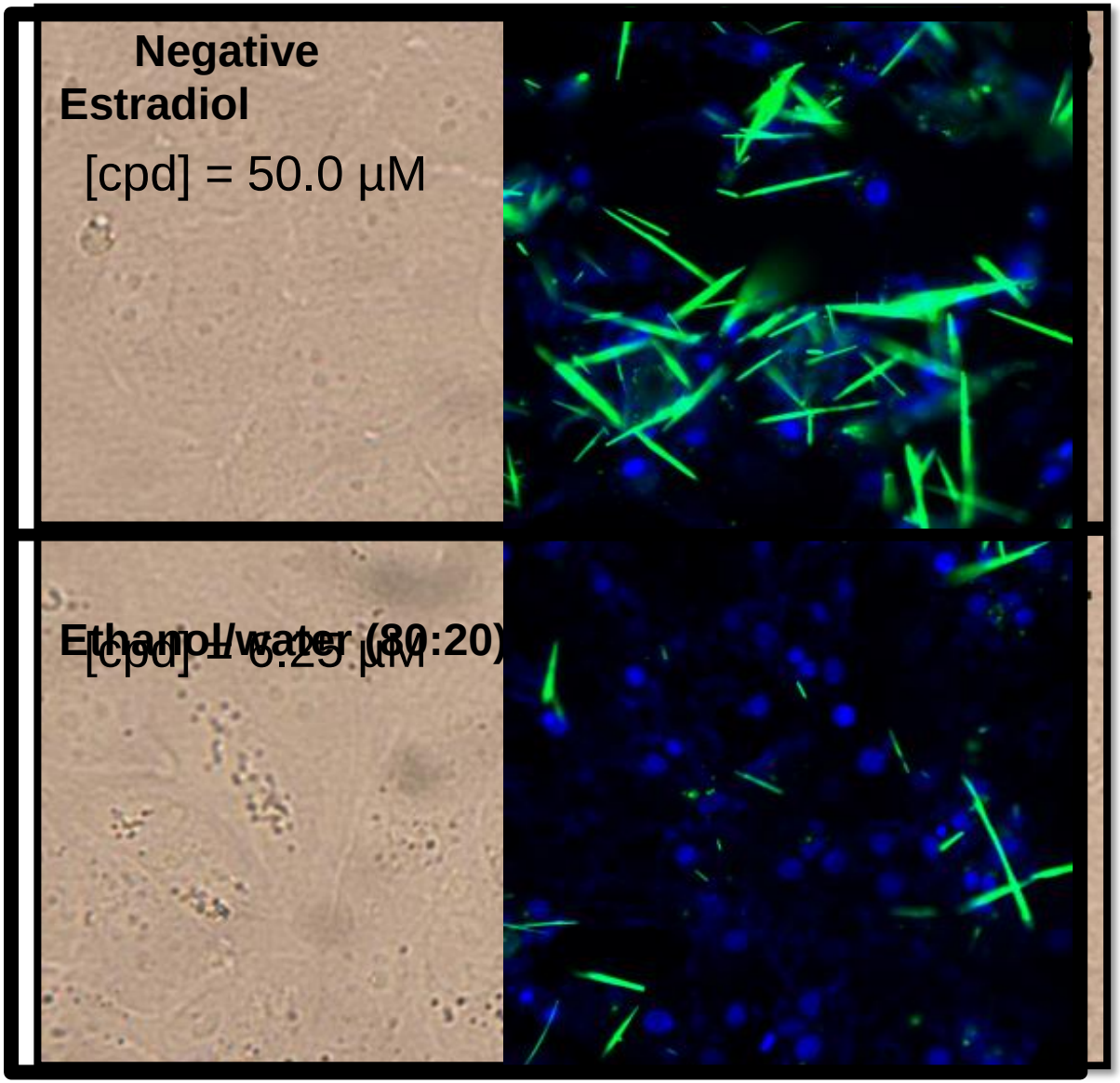


Haynes, M. K.; Leitão, A. et al., *J. Biomol. Screen.* **2009**, 14, 596

Always check your system

Granulation of breast cancer cells (MCF-7) is observed for *Morus alba* extracts high ethanol proportion

Compound precipitation (green and cells (blue)



Panorama da descoberta de fármacos

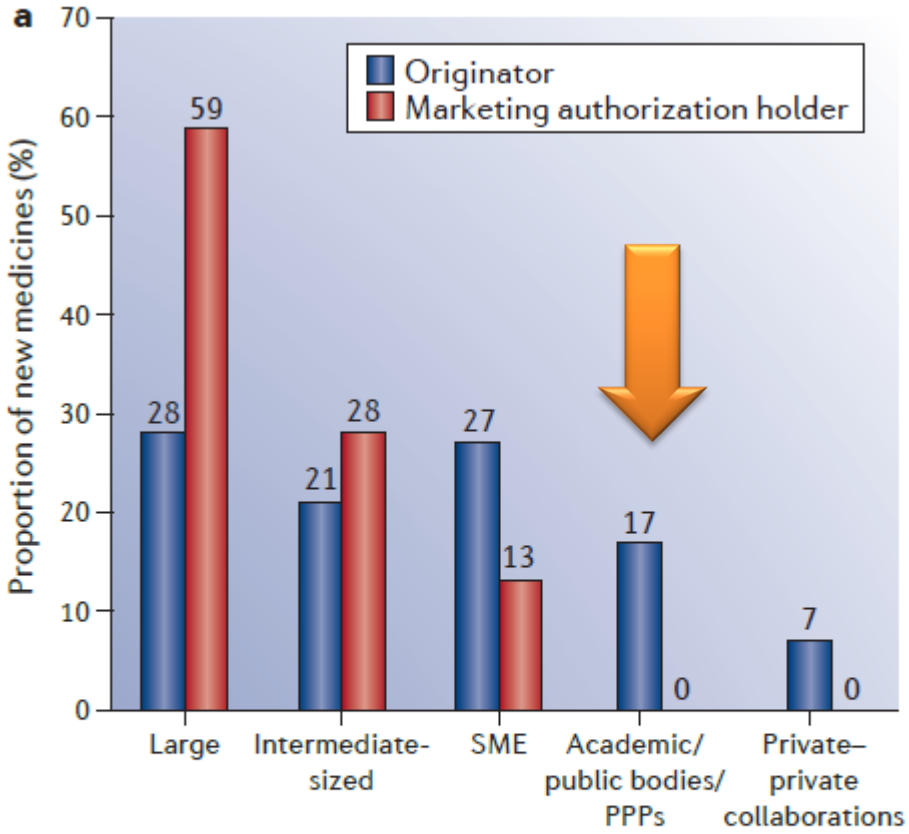
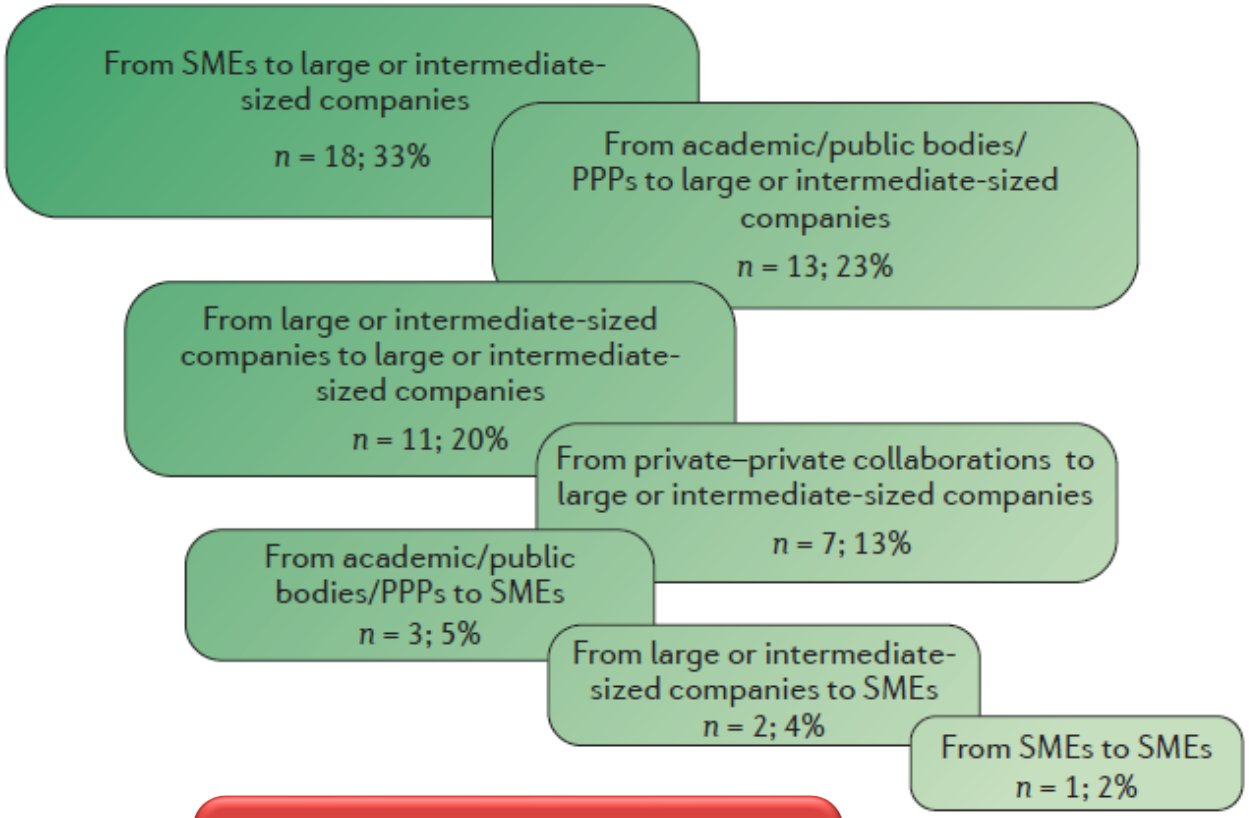


Figure 1 | **Origin of new medicines in the European Union (2010–2012).** **a** | Originator and the marketing authorization holder for all 94 approved products evaluated, divided according to organization type. |

A academia



Parcerias com a academia 28 %

> Although large and intermediate-sized companies still represent the main engine for commercializing new medicines, SMEs, academic institutions, public bodies and PPPs represent an important source of innovation.

Parcerias...

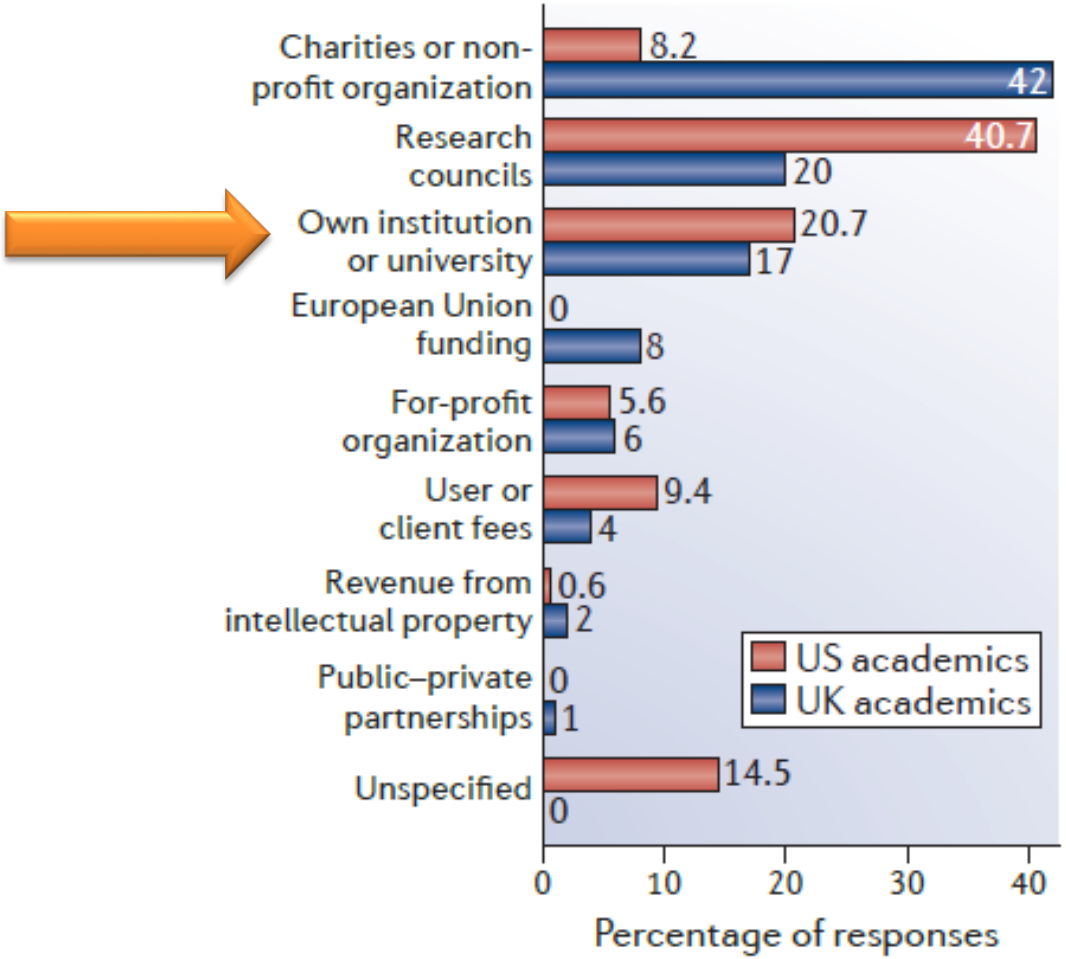


Figure 2 | **Funding sources for academic drug discovery.**

Parcerias...

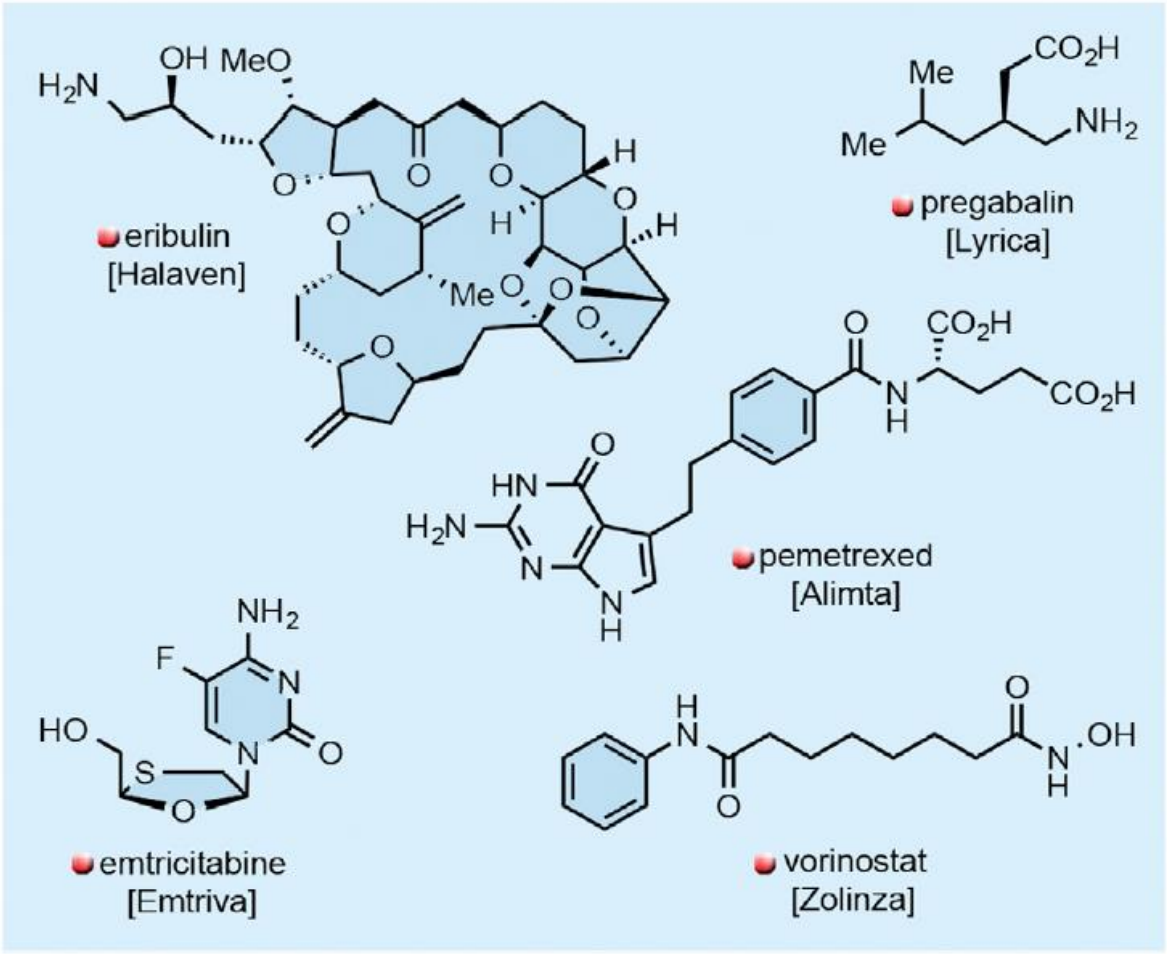
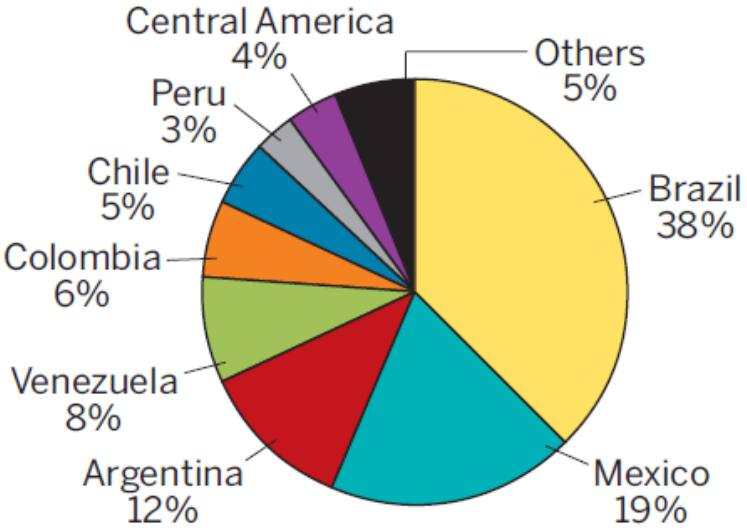


Figure 1. Examples of Drugs Discovered and Developed through Academic-Industrial Partnerships

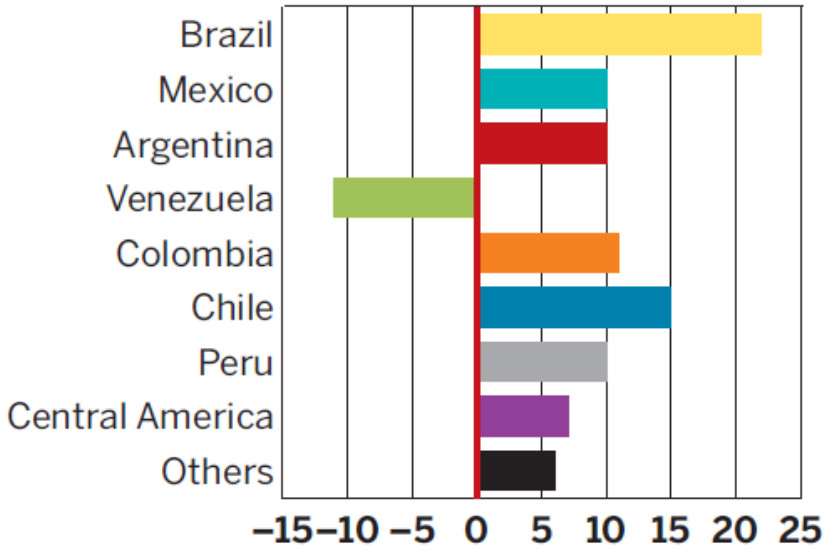
Nicolau, K.C. *Chem. Biol.* **2014**, *21*, 1039

Onde estamos?

LATIN AMERICA Brazil's pharma market is the region's leader, but most countries' markets enjoy double-digit growth.



2011 market = $67.3 billion



Growth rate, 2010–11, %

SOURCE: Deutsche Bank Securities

THE ROAD TO RIO

Multinational drug firms expand through acquisitions in **LATIN AMERICA**

ANN M. THAYER, C&EN HOUSTON

Qual é a estratégia terapêutica?

Fármaco

http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DrugInnovation/ucm429873.htm