COMBINATORIAL CHEMISTRY OF SMALL ORGANIC MOLECULES

Angel Messeguer
IQAC (CSIC)
Barcelona
Introduction

Combinatorial libraries and drug discovery

Libraries of controlled mixtures of peptoids

Neuroprotection

Modulation of protein-protein interactions

Business opportunities
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COMBINATORIAL CHEMISTRY

METHODS FOR THE MULTIPLE SIMULTANEOUS OR PARALLEL SYNTHESIS OF A SIGNIFICANT NUMBER OF COMPOUNDS WITH WELL DEFINED LEVELS OF DIVERSITY.
COMBINATORIAL CHEMISTRY LEVELS

NATURE

Amino acids and nucleic acids as building blocks

Combinatorial strategies

LIFE

IMMUNE SYSTEM

Combinatorial production of antibodies

Production of specific antibodies after recognition

COMBINATORIAL CHEMISTRY

Peptide and oligonucleotide libraries

Libraries of small organic molecules

ORGANIC CHEMISTRY

INORGANIC CHEMISTRY

Catalysts and materials
COMBINATORIAL CHEMISTRY AND ORGANIC CHEMISTRY

POTENTIAL FOR NEW DISCOVERIES               (Bohacek et al., 1996)

★ Stable structures from 30 elements: C, O, N, S, H  \(10^{63}\)

★ With 1 mg available  \(10^{60}\) g

★ Mass of the sun  \(2 \times 10^{33}\) g

★ Mass of the universe  \(10^{63}\) g

★ The organic library  \(10^{27}\) suns

Not enough suns
Not enough carbon

★ Current chemical universe: 50 million compounds.
1 mg available represent 50 Kg
(or the ratio of the mass of one proton to the mass of the sun)

NEGLIGIBLE SMALL COMPARED TO THE POSSIBLE DIVERSITY
COMBINATORIAL CHEMISTRY

HISTORY:

1984 Parallel synthesis or "pines" Geysen
Late 80s Combinatorial concept (Furka)
Early 90s Methods development, peptide synthesis, solid phase

92 Entry of major pharmaceutical companies
95 World wide interest

2000 4 Publications per day
       over 1 billion dollars in business
COMBINATORIAL CHEMISTRY LIBRARIES

- Agronomics
- Electrochemistry
- HPLC Chiral Ligands
- Materials Science
- Catalysis
- Medicinal Chemistry
COMBINATORIAL CHEMISTRY AND MEDICINAL CHEMISTRY

Use of high advanced concepts of ORGANIC SYNTHESIS → Well-defined building blocks → LIBRARIES OF ANY KIND

Well-defined building blocks → Synthetic strategies

LIMITATION IN DRUG DEVELOPMENT → Access to structural diversity within the limits of known chemistry
THE DRUG DISCOVERY ADVENTURE

2-4 years  DISCOVERY  10,000 compounds
7-12      DEVELOPMENT  10
9-16      MARKETED DRUG  1

COST AND TIME MINIMISATION

600-800 MILLION EUROS

EARLY PHASES

BOTTLENECKS

* TARGET IDENTIFICATION
* LEAD IDENTIFICATION
* LEAD OPTIMISATION
THE DRUG DISCOVERY ADVENTURE

FDA APPROVED NEW MOLECULAR ENTITIES

THE DRUG DISCOVERY ADVENTURE

THE DRUG DISCOVERY ADVENTURE

THE DRUG HUNTING PROCESS

Target Selection  Lead Identification  Lead Optimization

Identification of potential targets  Screen development  Lead optimization
Target verification  High-throughput screening  Potency in disease
Target selection  Secondary assays / Mechanism of action  Pharmacokinetics
Target validation  Hits → Leads  Early toxicology

Drug Candidates
HISTORICAL APPROACHES TO DRUG DISCOVERY

- Recombinant Proteins
- Rational Drug Design
- Existing Individual Collections
- Chemical Synthesis SAR
- Ferment & Find (Bacterial Broth)
- Grind & Find (Natural Sources)
- Potential Therapeutical Compounds

Biological Assays
HISTORICAL APPROACHES TO DRUG DISCOVERY

- Recombinant Proteins
- Rational Drug Design
- Combinatorial Libraries
- Potential Therapeutical Compounds
- Biological Assays
  - Existing Individual Collections
  - Traditional Chemical Synthesis
  - SAR
  - Ferment & Find (Bacterial Broth)
  - Grind & Find (Natural Sources)
Introduction

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Business opportunities
THE DRUG DISCOVERY IN THE NEW CENTURY

- Genomics
- Proteomics
- Combinatorial Chemistry
- Drug Discovery
- High Throughput Screening
- Bioinformatics
COMBINATORIAL CHEMISTRY AND DRUG DISCOVERY

Target structural requirements

THERAPEUTIC TARGET
COMBINATORIAL CHEMISTRY AND DRUG DISCOVERY

Target structural requirements

Restrictions:
- Pharmacodynamic
- Pharmacokinetic
- Pharmaceutic

VIRTUAL LIBRARY

DIVERSITY FILTRATION

FOCUSED LIBRARY

THERAPEUTIC TARGET

PRECLINICAL PERIOD

Individuals
COMBINATORIAL CHEMISTRY AND DRUG DISCOVERY

VIRTUAL LIBRARY

DIVERSITY FILTRATION

FOCUSED LIBRARY

Activity assays (HTS)

Optimisation (Combichem)

LEADS

HITS

TARGET

Restrictions:
Pharmacodynamic
Pharmacokinetic
Pharmaceutic

Target structural requirements

PRECLINICAL PERIOD
1992-2005:

OVER 60 COMPOUNDS IN HUMAN TRIALS FROM HIGH THROUGHPUT SCREENING OF LIBRARIES

Antagonist of 5-HT$_6$ receptor

STI571 (Gleevec)

AMN107 (Tasigna)
COMBINATORIAL CHEMISTRY OF SMALL ORGANIC MOLECULES

LIBRARY FORMATS

INDIVIDUAL COMPOUNDS
- Spatially addressed chemical synthesis
- Multiple parallel organic synthesis

CONTROLLED MIXTURES
- Split and mix
- Positional scanning
COMBINATORIAL CHEMISTRY OF SMALL ORGANIC MOLECULES

LIBRARY FORMATS: PROS AND CONS

Libraries of discretes

- Easy development of library chemistry
- Automatisation of synthesis
- Direct individual screening for biological activity
- Demands high capacity of screening
- Only small libraries made by hand
- Optimize leads
- Focused libraries
- Increasingly preferred by companies
LIBRARY FORMATS: Positional Scanning

$3 \times 3 \times 3 = 27$ compounds

$Rm = \text{random mixture}$

A

B

C

\[\begin{array}{l}
ALS \\
ALT \\
ALU \\
AMS \\
AMT \\
AMU \\
ANS \\
ANT \\
ANU
\end{array}\]

\[\begin{array}{l}
BLS \\
BLT \\
BLU \\
BMS \\
BMT \\
BMU \\
BNS \\
BNT \\
BNU
\end{array}\]

\[\begin{array}{l}
CLS \\
CLT \\
CLU \\
CMS \\
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CNU
\end{array}\]
LIBRARY FORMATS: Positional Scanning

3 x 3 x 3 = 27 compounds

Rm = random mixture

A → ALS
ALT
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C → CLS
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CLU
CMS
CMT
CMU
CNS
CNT
CNU

Kn → Rm
Rm
Rm

OXX
**LIBRARY FORMATS: Positional Scanning**

3 x 3 x 3 = 27 compounds

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</table>

Kn   Rm   Rm
OXX

Rm   Kn   Rm
XOX
LIBRARY FORMATS: Positional Scanning

3 x 3 x 3 = 27 compounds

Rm = random mixture

Kn Rm Rm
OXX

Rm Kn Rm
XOX

Rm Rm Kn
XXO
LIBRARY FORMATS: Positional Scanning

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Rm = random mixture

3 x 3 x 3 = 27 compounds

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AMT
BMT
CMU
AMU
BMT
BMU
ANU
BNU

ANU
BNU
CNU

Kn
Rm
Rm
OXX

3 Libraries with different structure of mixtures
Easy automatisation
Direct deconvolution

Library chemistry (relative kinetics)
COMBINATORIAL LIBRARIES

POSITIONAL SCANNING

Deconvolution:

\[
\begin{align*}
\text{OXX} & \quad \checkmark \quad \checkmark \\
A & \quad B & \quad C \\
\text{XOX} & \quad \checkmark \\
L & \quad M & \quad N \\
\text{XXO} & \quad \checkmark \\
S & \quad T & \quad U
\end{align*}
\]

\[\checkmark = \text{diversity element}\]
\[\times = \text{aleatory position}\]
\[\bigcirc = \text{defined position}\]

Synthesis of:

\[
\{ B, C \quad \text{L, N} \quad \text{T} \}
\]

\[B-L-T, B-N-T, C-L-T, C-N-T\]
LIBRARY FORMATS: PROS AND CONS

**Positional scanning:**

- Easy synthesis automatisation
- Direct deconvolution with several possible hits
- Low number of biological assays required
- Biological testing of mixtures (non independent activities)
- Complex development of library chemistry (relative kinetics)
- Random screening for new leads
**Dynamic library**: set of molecules resulting from the reversible formation of covalent bonds or molecular interactions

**Deconvolution**: The interaction with the target shifts the equilibrium thus making possible the identification of the active components

\[ A_{1,i} + B_{1,j} \rightleftharpoons A_{1,i}B_{1,j} \]

Virtual library

**Components**

**Available diversity**
DYNAMIC COMBINATORIAL LIBRARIES

Imine formation
\[ \text{O} \quad + \quad \text{H}_2\text{N-R} \quad \rightleftharpoons \quad \text{N_R} \]

Aldol formation
\[ \text{O} \quad + \quad \text{O} \quad \rightleftharpoons \quad \text{OH-O} \]

Disulfide formation
\[ \text{SH} \quad + \quad \text{HS} \quad \rightleftharpoons \quad \text{S-S} \]

Metal coordination
\[ M^{m+} \quad + \quad nL \quad \rightleftharpoons \quad [ML_n]^{m+} \]

Cis-trans isomerisation
\[ \equiv \quad \rightleftharpoons \quad \equiv \]

Tautomerism
\[ \text{NH} \quad \rightleftharpoons \quad \text{O} \quad \equiv \quad \text{NHOH} \]

THE CHEMICAL GENETICS CONCEPT

S. Schreiber
(Harvard)
Introduction

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Libraries of controlled mixtures of peptoids

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Libraries of controlled mixtures of peptoids

**PEPTOIDS**: Oligomers of \(N\)-Alkylglycines

**PEPTIDE**

\[
\begin{align*}
R_1 & \quad \text{H} \\
R_2 & \quad \text{H} \\
R_3 & \quad \text{H}
\end{align*}
\]

**PEPTOID**

\[
\begin{align*}
R_1 & \quad \text{H} \\
R_2 & \quad \text{H} \\
R_3 & \quad \text{H}
\end{align*}
\]

**PROS:**
- Metabolic stability
- Wide chemical diversity
- Less steric restrictions
Libraries of controlled mixtures of peptoids

Great conformational mobility

PROS
Wide optimisation range (opposite to a rigid hit)

CONS
More than one active conformation (undesired side effects)

HIT TO LEAD
Libraries of controlled mixtures of peptoids

LIBRARY OF PEPTOIDS

- Format: positional scanning
- Number of compounds: \(20 \times 16 \times 16 = 5120\)
- Solid phase synthesis

52 controlled mixtures

Libraries of controlled mixtures of peptoids

**LIBRARY OF PEPTOIDS**

- **Biological screening**
  - Septic shock
    - LPS
    - J. Med. Chem., 2005
  - Neuronal regeneration
  - Cancer
    - MDR
    - JPET, 2005
    - Serine proteases
  - Apoptosis
    - APAF
    - (Cell Death Differ, 2006)
  - Neuroprotection
    - JPET, 2002
    - ??
  - Analgesia
    - TRPV1
    - PNAS, 2002
  - Antibiotics
    - J. Comb. Chem., 2003
  - NMDA Antagonists
    - JPET, 2002
  - Cancer
    - UBC13-UEV
    - (2007)
Libraries of controlled mixtures of peptoids

LIBRARY OF PEPTOIDS

- **Biological screening**

  - **Septic shock**
    - LPS
    - J. Med. Chem., 2005

  - **Neuronal regeneration**
    - Semaphorins
    - (In preparation)

  - **Antibiotics**
    - J. Comb. Chem., 2003

  - **NMDA Antagonists**
    - JPET, 2002

  - **Cancer**
    - MDR
    - (JPET, 2005)

  - **Serine proteases**

  - **Apoptosis**
    - APAF
    - (Cell Death Differ, 2006)

  - **Neuroprotection**
    - ??
    - JPET, 2002

  - **Analgesia**
    - TRPV1
    - PNAS, 2002

  - **Cancer**
    - UBC13-UEV
    - (2007)
Libraries of controlled mixtures of peptoids

**PEPTOIDS IDENTIFIED EXHIBITING IN VITRO AND IN VIVO ACTIVITIES**

**Analgesia TRPV1 antagonists**

N16-15-15C
MW 643

N19-15-15C
MW 645

PNAS, 2002, 99, 2374

Unknown topology of the receptor and of its interaction with these antagonists

Use of conformationally restricted analogues of these peptoids for reducing undesired side-effects

obtaining structural information about TRPV1 target
Libraries of controlled mixtures of peptoids

PEPTOIDS IDENTIFIED EXHIBITING IN VITRO AND IN VIVO ACTIVITIES

Neuroprotection

Target unknown

Use of conformationally restricted analogues of these peptoids for obtaining structural information about the target

Use of peptoids attached to polymer supports (Sepharose) for capturing potential targets

JPET, 2002, 301, 29
JPET, 2002, 302, 163
Scope

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Modulation of UBC13-UEV1 interaction

Development of new inhibitors of polyubiquitylation through disruption of the UBC13-UEV1 complex.

UBC13-UEV complex is responsible of the formation of non-canonical (Lys63) polyubiquitin chains.

PUq chains modify substrate proteins that participate in signaling pathways, activation of NF-κB, and other essential cell cycle regulation processes.

UBC13-UEV regulates processes that generally enhance the survival of cells in response to certain forms of stress.
Modulation of UBC13-UEV1 interaction

Yeast two-hybrid assay as a screen tool to identify small molecule protein-protein interaction antagonists

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</tr>
<tr>
<td>YES</td>
<td>YES (HIGH)</td>
</tr>
<tr>
<td>NO</td>
<td>NO (LOW)</td>
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</table>
Modulation of UBC13-UEV1 interaction

LIBRARY OF PEPTOIDS (5.120)

Screening

IDENTIFICATION OF 4 ACTIVE COMPOUNDS

COMPUTATIONAL STUDIES: DOCKING OF CONFORMATIONALLY RESTRICTED ANALOGUES

Synthesis and biological evaluation

LEAD CANDIDATES
Modulation of UBC13-UEV1 interaction

Library deconvolution: identification of hits

N37-37-9C

N37-37-13C

N15-37-9C

N15-37-13C
Modulation of UBC13-UEV1 interaction

Design of conformationally restricted analogs through docking analysis (CDOCK) againsts UBC13

Best compounds determined by virtual screening: both from peptoid N37-37-9C

Scheper, J. et al, submitted for publication
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Scientific and technologic research has grown significantly in recent years

PUBLICATIONS:

Quantity  0.97% in 1981-85 to 2.37% in 1993-1997

Quality  Impact factor multiplied per 2.2 within the 1981-1997 period

THS INCREASE IS THE RESULT OF THE INVESTMENT IN INFRASTRUCTURE AND PERSONNEL TRAINING.
Technology Transfer: from Academy to Business

SCIENTIFIC AND TECHNOLOGIC RESEARCH IN CHEMISTRY AND LIFE SCIENCES

ACADEMIA: UNIVERSITIES, SCIENCE PARKS, CSIC

HIGH LEVEL SCIENTISTS
(risks of specialisation in poor market areas)

EXCESS OF TECHNOLOGY AND KNOWLEDGE
(Academy is not ready to absorb this excess)
Technology Transfer: from Academy to Business

TRANSFER OF TECHNOLOGY OR KNOWLEDGE

ACADEMIA ----> COMPANY

CONTRACTS BETWEEN COMPANIES AND INSTITUTIONS

CONTRACTS BETWEEN SCIENTISTS AND COMPANIES
Technology Transfer: from Academy to Business

TRANSFER OF TECHNOLOGY OR KNOWLEDGE

OTHER POSSIBILITIES

ACADEMIA → INSTITUTE → COMPANY → SPIN-OFF
CREATION OF A SPIN-OFF
IN BIOTECHNOLOGY OR IN THE PHARMACEUTICAL AREA
THE DRUG DISCOVERY ADVENTURE

2-4 years DISCOVERY 10,000 compounds
7-12 DEVELOPMENT 10
9-16 MARKETED DRUG 1

COST AND TIME MINIMISATION

400-800 MILLION EURO

TARGET IDENTIFICATION AND VALIDATION
HIT IDENTIFICATION AND LEAD OPTIMISATION

OUTSOURCING

BOTTLENECKS (EARLY PHASES)

Technology Transfer: from Academy to Business
Technology Transfer: from Academy to Business

DRUG DEVELOPMENT PROCESS

**Preclinical trials:**
- Chemistry and synthesis
- Efficacy in animals
- Bioavailability
- Pharmacokinetics
- Toxicology

**Clinical trials:**
- Phase 1
- Phase 2
- Phase 3

0 2 4 6 8 10 12
Years

Register

BIG PHARMA

SPIN-OFF  A SMALL PIECE OF THE CAKE
CREATION OF A SPIN-OFF
IN BIOTECHNOLOGY OR IN THE PHARMACEUTICAL AREA
Technology Transfer: from Academy to Business

CREATION OF A SPIN-OFF
IN BIOTECHNOLOGY OR IN THE PHARMACEUTICAL AREA

Initial requirements:

- A TECHNOLOGY OR A PRODUCT INTERESTING TO MARKET
- WELL DEFINED INTELLECTUAL PROPERTY
- HUMAN CAPITAL
- BUSINESS PLAN
- INVESTORS
Technology Transfer: from Academy to Business

A TECHNOLOGY OR A PRODUCT INTERESTING TO MARKET

⭐ SERVICES

⭐ RESEARCH AND DEVELOPMENT (INNOVATION)

PRODUCT

KNOW-HOW
WELL DEFINED INTELLECTUAL PROPERTY

PATENT OR PUBLISH \rightarrow \text{PATENT AND PUBLISH}

INTELLECTUAL PROPERTY

ROLE OF PUBLIC INSTITUTIONS

TRAINING OF SPECIALISED PERSONNEL

FACILITATING TECHNOLOGY TRANSFER
Technology Transfer: from Academy to Business

HUMAN CAPITAL

✓ TRAINED SCIENTISTS (PhDs + postdoc periods)
✓ TECHNICIANS
  University degree
  Technical degree

? INCORPORATION OF UNIVERSITY OR PUBLIC CENTRES STAFF
? CEO, CSO AND CONSULTORS MARKET
Technology Transfer: from Academy to Business

BUSINESS PLAN ← BIOINCUBATORS → INVESTORS

Support From Public Institutions
- Subventions
- Financing

Support From Private Companies of the Area

Capital Risk
- Spain
- Europe
Technology Transfer: from Academy to Business

PharmaMar

Lipotec

DiverDrugs

Crystax

Advancell

........
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