INTEGRATED IN VITRO – IN SILICO SCREENING STRATEGY FOR DISCOVERING ANTIBACTERIAL COMPOUNDS

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BACKGROUND

Cell-based antimicrobial HTS assay using recombinant luminescent bacterial strain (*E. coli* K-12/pTetLux1)

Aiming to improve antimicrobial screening

- Antimicrobial assay time reduction from 24 to 2 hours
- Higher-throughput: miniaturization feasible to 384-format using automated liquid handling
**ASSAY PRINCIPLE**

*E. coli* K12 (pTetLux1)
HTS ASSAY DEVELOPMENT

**Preparations**
- Optimize conditions for sensor bacteria storage
- Determination of CFU count in overnight cultures

**Assay optimization 1**
- Optimize the amount of bacteria and assay temp.
- Determine luminescence time point measurements

**Assay optimization 2**
- DMSO tolerability, pre-incubation time testing
- Creating SOP

**Assay miniaturization**
- Miniaturization from 96 to 384-well plate format
- Optimising automated liquid handling, HTS SOP

**Assay validation**
- Known antibiotics
- Screen of known bioactives, 2000 compounds
ASSAY OPTIMIZATION & VALIDATION

Comparison of assay quality parameters and day-to-day variations in the 384-well assay.

<table>
<thead>
<tr>
<th>Antibiotic (µg/ml)</th>
<th>Inhibition %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 µl</td>
</tr>
<tr>
<td>Erythromycin (50)</td>
<td>99.2 ± 0.0</td>
</tr>
<tr>
<td>Erythromycin (2.5)</td>
<td>63.9 ± 2.5</td>
</tr>
<tr>
<td>Rifampicin (5)</td>
<td>95.9 ± 1.1</td>
</tr>
<tr>
<td>Rifampicin (0.625)</td>
<td>47.8 ± 2.6</td>
</tr>
<tr>
<td>Kanamycin (10)</td>
<td>99.0 ± 0.5</td>
</tr>
<tr>
<td>S/B</td>
<td>127 ± 9.1</td>
</tr>
<tr>
<td>S/N</td>
<td>15 ± 10.4</td>
</tr>
<tr>
<td>Z'</td>
<td>0.73 ± 0.2</td>
</tr>
</tbody>
</table>

Plate uniformity test

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PILOT SCREEN: SPECTRUM BIOACTIVES LIBRARY (TOTAL 2000 COMPOUNDS)

Inhibition category 1 (>90 %) n=34
- Antibacterials: 91.2%
- Antifungal & antiparasitic: 5.9%
- Unknown activity: 2.9%
- Other classifications: 0.7%

Inhibition category 2 (60-90 %) n=20
- Antibacterials: 85.0%
- Antifungal & antiparasitic: 10.0%
- Other classifications: 5.0%

Inhibition category 3 (30-60 %) n=35
- Antibacterials: 31.4%
- Antifungal & antiparasitic: 14.3%
- Unknown activity: 14.3%
- Other classifications: 40.0%

Inhibition category 4 (10-30 %) n=148
- Antibacterials: 56.1%
- Antifungal & antiparasitic: 18.2%
- Antiviral: 12.2%
- Unknown activity: 12.8%
- Other classifications: 0.7%
DOSE-RESPONSE RESULTS FOR SELECTED HITS

Azithromycin

Chloramphenicol

Doxycycline

Meclocycline

Minocycline

Rifaximin
Cell-based primary HTS

Hit identification

Hit expansion by virtual screening

Secondary testing

Compounds confirmed active

Growth inhibition against standard bacterial ATCC strains

Preliminary human cell cytotoxicity

Compound solubility in assay medium

Novelty assessment, promiscuity
PRIMARY HTS

- 10,240 compounds from the ChemBridge DIVERset collection
- Compounds pre-plated into 384-well plates (30 nL volumes) using Echo® liquid transfer system
- Screened in singles (320 compounds per plate) at a final concentration of 10 µM (0.1% DMSO). Positive controls: erytromycin and rifampicin
- Bacterial culturing & HTS protocol:
  
  Automated liquid handling using Biomek FX:
  - 30 µl total well volume
  - Bacteria: $5 \times 10^4$ CFU/well
  - Pre-incubation 30 min 37 °C
  - Luminescence induction by addition of tetracycline

  ![Diagram of the automated liquid handling process]
HTS RESULTS

• Assay performance quality (32 plates): $Z' = 0.7\pm0.2$, $S/B = 122\pm11$  $S/N = 14\pm12$

<table>
<thead>
<tr>
<th>Inhibition</th>
<th>Number of compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>50-100%</td>
<td>8</td>
</tr>
<tr>
<td>40 - 50 %</td>
<td>22</td>
</tr>
<tr>
<td>30 - 40 %</td>
<td>189</td>
</tr>
<tr>
<td>20 – 30 %</td>
<td>907</td>
</tr>
<tr>
<td>10 – 20 %</td>
<td>734</td>
</tr>
</tbody>
</table>

• 8 hit compounds above 50% inhibition
• Hit rate (50% cutoff) : 0.08%
• Hit inhibitions ranged between 52% and 87%
HIT EXPANSION BY VIRTUAL SCREENING

• Identification of maximum common substructure was performed based on the HTS hit structures. The active compounds were also used to develop two 3D pharmacophore models.

![MCS](image1)

3D pharmacophores

• Ligand-based virtual screening was performed on a FIMM library consisting of 119,027 compounds.

• A set of 29 virtually selected compounds (plus 7 original HTS hits) were followed up with secondary screens.
HIT CONFIRMATION

Performed in 384-format, 10 μM using the *E.coli/*pTetlux assay

![Graph showing inhibition % for compounds 1 to 11. HTS hits and 4 most active from compound set chosen by virtual screening.]

HTS hits

4 most active from compound set chosen by virtual screening
SECONDARY SCREENING RESULTS

Compounds followed up by dose-response with *E. coli/pTetlux1*

<table>
<thead>
<tr>
<th>Compound number</th>
<th>IC_{50} (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.2±0.2</td>
</tr>
<tr>
<td>2</td>
<td>3.7±0.5</td>
</tr>
<tr>
<td>3</td>
<td>3.6±1.3</td>
</tr>
<tr>
<td>4</td>
<td>5.4±0.5</td>
</tr>
<tr>
<td>5</td>
<td>2.7±0.8</td>
</tr>
<tr>
<td>6</td>
<td>0.5±0.0</td>
</tr>
<tr>
<td>7</td>
<td>0.5±0.1</td>
</tr>
<tr>
<td>8</td>
<td>11.3±0.5</td>
</tr>
<tr>
<td>9</td>
<td>8.2±1.4</td>
</tr>
<tr>
<td>10</td>
<td>7.7±0.6</td>
</tr>
<tr>
<td>11</td>
<td>6.7±0.5</td>
</tr>
</tbody>
</table>
ANTIMICROBIAL GROWTH INHIBITION AGAINST STANDARD STRAINS

Standard absorbance-based assay (96-well, initial inoculum: 5×10^4 CFU/well)

**E. coli**
ATCC 25922
4 h (A) & 24h (B)

**S. aureus**
ATCC 25923
7 h (C) and (D) 24 h
PRELIMINARY CYTOTOXICITY AGAINST HUMAN CELLS & DETERMINATION OF COMPOUND SOLUBILITY

Cytotoxicity towards Huh-7 (human hepatocellular carcinoma derived) cells (%±SD at 100 µM) using ATP cell viability assay

<table>
<thead>
<tr>
<th>Compound number</th>
<th>Solubility [µM]</th>
<th>Human cell Cytotoxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100±50</td>
<td>7.8 ± 0.9</td>
</tr>
<tr>
<td>2</td>
<td>100±50</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>25±5</td>
<td>10.3±3.3</td>
</tr>
<tr>
<td>4</td>
<td>≥200</td>
<td>61.0±1.0</td>
</tr>
<tr>
<td>5</td>
<td>62.5±12.5</td>
<td>98.6±0.6</td>
</tr>
<tr>
<td>6</td>
<td>25±5</td>
<td>15.0±0.9</td>
</tr>
<tr>
<td>7</td>
<td>25±5</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>87.5±12.5</td>
<td>28.4±2.5</td>
</tr>
<tr>
<td>9</td>
<td>≥200</td>
<td>8.4±2.1</td>
</tr>
<tr>
<td>10</td>
<td>40±10</td>
<td>18.8±5.4</td>
</tr>
<tr>
<td>11</td>
<td>87.5±12.5</td>
<td>12.7±5.5</td>
</tr>
</tbody>
</table>

IC$_{50}$ determination of human cell toxicity:

55 µM (compound 4)
14 µM (compound 5)
ADMET AND ADVERSE EFFECT PREDICTIVE MODELLING BASED ON FDA-APPROVED DRUGS

• Background

- ADMET and adverse effects (ADMET-A) are crucial for failing of compounds and withdrawal of approved drugs.

- Overall attrition rates remain high, despite effort to improve pharmacokinetic profiles of small-molecules.

- Computational predictions of ADMET-A are effective approaches to minimize the risk of late-stage attrition.

• Aims

- Build a relational database to link FDA-approved drugs properties to known druggable/toxic targets and ADMET-A data.

- Coupled the database with a data analytics platform (KNIME) to allow access, analysis and predictive modeling.
ADMET-A PREDICTIVE MODELING BASED ON FDA APPROVED DRUG DATA (IDAAPM)
IDAAPM: UTILITY

Virtual Screening Docking

Predictive Modeling

Drugs Molecular Descriptors

Target

Adverse Effects

ADME-T
IDAAPM: FREQUENCY OF ADVERSE EFFECTS BY THEIR CORRESPONDING DRUG AREA
IDA2PM: KNIME WORKFLOWS

A) IDA2PM access
1. Right Click -> Execute (upload the database)
2. Right Click -> Configure
3. Choose data you want to visualize or export
4. Click Apply -> Ok
5. Right Click -> Execute

D) Structure similarity
Structure similarity search, view and export
Draw/Upload compound structure

B) Export dataset
CDK to Molecule
Column Filter
SDF Writer

E) Predictive classification
SDF Reader
Normalization
Partitioning
LIBSVM.Learner
LIBSVM.Predictor
Scorer

C) Chemical space and Clustering
Similarities or dissimilarities between compounds

HeatMap
(jFreeChart)
Image to Report

Similarity Viewer

Column Filter
Similarity Search
Distance Matrix Calculator
Hierarchical Clustering (DistMatrix)
Hierarchical Cluster Viewer

2D/3D Scatterplot
MDet (DistMatrix)
IDAAPM: MAIN
KNIME NODE

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IDAAPM MAIN
GRAPHICAL USER INTERFACE
USE CASE 1: OCULAR ADVERSE EFFECTS FOR LUMIGAN AT 0.1 MG/ML AND (0.3 MG/ML)
USE CASE EXP 2: OCULAR ADVERSE EFFECT FOR DRUGS USED IN CANCER TREATMENT.

ocular adverse effects frequency > 0.02
CONCLUSIONS

- 2 HTS hit compounds (4&5) exhibited very good bacterial growth inhibitory activities against both standard *E. coli* and *S. aureus*
- However, the compounds were also found to display high human cell toxicity
- Interesting compounds: 9 -11
- Low human cell cytotoxicity & good solubility in assay media
- Showed growth inhibitory activities against standard strains (most active at logarithmic growth phases)
- IDAAPM offers integrated data on approved drugs (physicochemical properties, ADMET-A, targets, bioactivities)
- IDA2PM enables researchers to perform data analysis and data mining from safe drugs data and to identify the space of similar compounds and similar targets.
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  Dr. Kristian Wennerberg
  Dr. Laura Turunen
  Ida Lindenschmidt
REFERENCES

