Fighting the Resistance: Controlling Antibacterial Activity with Visible Light

Lluita contra les resistències: control de l'activitat antibacteriana amb llum visible

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28 d'abril de 2022

Seminaris de Recerca de la Facultat de Farmàcia i Ciències de l’Alimentació
More than 750,000 deaths a year worldwide caused by resistant bacteria. Estimated to raise to 10,000,000 deaths by 2050.
Appearance of Resistances

- Non-complete treatment
- Accumulation in the environment
- Proliferation
- Drug Has Become Inefficient
- Evolution
- Proliferation + Evolution
Estimated annual deaths by AMR in 2050 vs. Other major causes of death.


The Lancet, 2022; DOI: 10.1016/S0140-6736(21)02724-0
On the Hunt for New Antibiotics

“Peripheral Modifications with Added Synergistic Mechanisms of Action Provide Durable and Potent Antibiotics”
Engineered Antibiotics

Peripheral Modifications with Added Synergistic Mechanisms of Action Provide Durable and Potent Antibiotics


VanA VRE MIC = 0.01–0.005 μg/mL

- 3 mechanisms of action
- potent and durable activity

Total synthesis of the aglycon (efficient access to engineered antibiotics?)

<table>
<thead>
<tr>
<th>Total Syntheses</th>
<th>LL6</th>
<th>Overall Yield</th>
<th>Atroposelectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>AB</td>
</tr>
<tr>
<td>Boger (1999)</td>
<td>25</td>
<td>0.2%</td>
<td>1:1</td>
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<tr>
<td>Boger (2009)</td>
<td>24</td>
<td>0.05%</td>
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<tr>
<td>Boger (2017)</td>
<td>32</td>
<td>0.4%</td>
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</tr>
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</table>

Boger (2020) 17  2%  >20:1  8:1  14:1
Paradigm Change: Self-Immolative Antimicrobials

“Cationic Polybenzyl Ethers as Self-Immolative Antimicrobial Polymers”
Palermo and co-workers
Biomacromolecules 2017, 18, 3400.

“Built in Deactivation Switch”
Liang and co-workers
Biomacromolecules 2020, 21, 2187.
Photopharmacology

Szymanski, Feringa and co-workers: 
## Azobenzenes and Other Photoswitches

### cis/trans isomerisations

**Azobenzenes**
- cis: Structure 1
- trans: Structure 2
  - 365 nm → trans
  - 405 nm → cis
  - 430 nm → trans
  - 520 nm → cis

**Stylenes**
- cis: Structure 3
- trans: Structure 4
  - 300 nm → cis
  - 280 nm → trans

**Hemithioindigo**
- trans: Structure 5
- cis: Structure 6
  - 406 nm → cis
  - 480 nm or Δ → trans

**Other**
- cis: Structure 7
- trans: Structure 8
  - 313 nm → trans
  - Vis light → cis

### open/closed isomerisations

**Diarylethanes**
- open: Structure 9
  - 256 nm → closed
  - 530 nm → open

**Spiropyrans**
- closed: Structure 10
  - 365 nm → open
  - > 400 nm or Δ → closed

**Fulgimide**
- open: Structure 11
  - 365 nm → closed
  - 528 nm → open
Photoswitchable Antibiotics

Photoswitchable Quinolones: the First Light-Regulated Antibiotics:

- Up to 3-fold increase in potency upon irradiation.

Engineered Antibiotic:

- Low isomerisation ratios.
- Only up to 1-fold change in potency between irradiated and not-irradiated compound.
- An azobenzene photoswitch did not allow control of antibacterial activity in this case.
Photoswitchable Antibiotics

Photoswitchable Carbohydrate-Based Surfactants:

Biofilm growth modulation (strain dependent).

Limited impact of isomerisation.

Molecules stack when azobenzene is in trans configuration.

Light-mediated disassembly triggers generation of ROS (reactive oxygen species, such as $^{1}\text{O}_2$).
Photoswitchable Antibiotics

Quorum sensing regulation:

- **Light-induced configurational change** swaps between inhibitory or agonist activity.
- **700-fold change in activity.**

**Photoswitchable short peptide antibiotics:**

- **3-fold potency increase upon activation.**


Photoswitchable Gramicidins

- UV light needed for activation.
- Great loss of activity and low trans/cis discrimination.
- Deactivates within minutes on its own.

- Non-Reversible Isomerisation under Thermal Conditions
- Requires UV irradiation for deactivation.

Abell and co-workers:

Ulrich, Komarov et al.
Advantages of Visible Light Photoswitchable Drugs

- **Non-Harmful** (as opposite to UV).
- **Red Light and IR** penetrate tissue without harming it.
- **Sunlight** is thoroughly available (can we use it?).
Use of red light is thoroughly used in cosmetic and therapeutic devices.
Visible Light Photoswitchable Antibiotics

- Breakthrough in tetra-ortho-substituted azobenzenes.
- Highly limited functional group tolerance.

Feringa and co-workers:
Engineered Antibiotic

Tyrocidine A

Backbone of Tyrocidine A from crystal structure

Engineered Antibiotic: Hypothesis

trans-Tyrocidine PS

cis-Tyrocidine PS

Visible Light Photoswitchable Amino Acid
Visible Light-Operated Azobenzene Amino Acid(s)

Only previous Visible Light-Operated Azobenzene Amino Acid:

Visible Light-Operated Azobenzene Amino Acid(s)

Only previous Visible Light-Operated Azobenzene Amino Acid:

\[
\begin{array}{c}
\text{Br} & \text{F} & \text{NH}_2 \\
\end{array}
\xrightarrow{6 \text{ steps}}
\begin{array}{c}
\text{MttHN} & \text{N} & \text{N} & \text{F} & \text{F} & \text{OH} \\
\end{array}
\text{12\% yield}


Our new Photoswitch:

\[
\begin{array}{c}
\text{F}_3\text{CO} & \text{N} & \text{H} & \text{NH}_2 \\
\end{array}
+ \begin{array}{c}
\text{O}_2\text{N} & \text{O} & \text{H} \\
\end{array}
\xrightarrow{\text{reaction}}
\begin{array}{c}
\text{F}_3\text{COCHN} & \text{N} & \text{N} & \text{Cl} & \text{Cl} & \text{CO}_2\text{H} \\
\end{array}
\text{Cl} & \text{Cl} & \text{Cl} & \text{Cl}
\]
Visible Light-Operated Azobenzene Amino Acid(s)

Only previous Visible Light-Operated Azobenzene Amino Acid:


Our new Photoswitch:

2.0 g scale - 46% overall yield (3 steps)
Only 1 chromatographic step

Zn powder; then FeCl₃
[2 g scale]

Pd(OAc)₂ NCS
85%
[2 g scale]

AcOH
54% (2 steps)
Solid Phase Peptide Synthesis

- **REACTION** (solvent and reagents)
- **WASH** (solvent)
- **CLEAVAGE** (solvent and reagent)

- Evacuate
- Filter
- Evacuate
- Collect Product

**ITERATIVE SYNTHESIS**
(repeat for each reaction)

- Functionalised resin bead

* = Functionalised resin bead
Automated Solid Phase Peptide Synthesis

Manual Synthesis
~2 h per amino acid (coupling + deprotection)
10-Aa peptide in ~3 days

Automated Synthesis
~2 h per amino acid (coupling + deprotection)
10-Aa peptide in ~1 day

Microwave-Assisted Synthesis
~20 min per amino acid (coupling + deprotection)
10-Aa peptide in ~3 hours
Custom Made Strategy (AKA Wisdom through Challenge)

Rinkamide: Acidic cleavage (TFA).
- Unstable under very mild aqueous basic conditions (NEED TO CYCLISE AFTER CLEAVAGE).

Allyl ester:
- Basic hydrolysis (NaOH).
- Pd(0) catalysed deallylation (Pd(PPh₃)₄ + PhSiH₃).

No problems detected so far....

Azide: Mild Staudinger reduction (H₂O and PMe₃).
- Cannot use most common protecting group (Boc) if cyclisation on resin is not possible.
- No precedents of base-free Staudinger reduction on resin (and it didn’t work!).
- Need to deprotect after cleavage and cyclisation.

Trifluoroacetamide: Basic hydrolysis (NaOH).
- Cannot use Fmoc group (PIPERIDINE ATTACKS PHOTOSWITCH).
- Deprotection conditions incompatible with Rinkamide resin (NEED TO CYCLISE AFTER CLEAVAGE).
Synthesis of Photoswitchable Tyrocidine A

H₂N –> H₂N

a) i. Fmoc-Aa-OH
    DIC, oxtyma
ii. piperidine

b) Tfa-CEBA-OH
   DIC, oxyma

c) TFA

---

[Chemical structures]
By-Product Formed During Basic Hydrolysis
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MS of cyclic product detected instead of the expected hydrolysis product.
By-Product Formed During Basic Hydrolysis

- Characterisation discarded the cyclic product.
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By-Product Formed During Basic Hydrolysis

- MS of cyclic product detected instead of the expected hydrolysis product.
- Characterisation discarded the cyclic product.
By-Product Formed During Basic Hydrolysis

- Same MW as cyclic product.
- Ninhydrin test positive.
- Mechanistic studies confirm reaction of Asp side chain.

\[
\text{succinimide formation} \quad \text{——FAST——}
\]

\[
\text{trifluoroacetamide hydrolysis} \quad \text{——slow——}
\]

\[m/z \text{ of intermediate detected by UPLC-MS}\]
Use of PMe₃ allows mild conditions for the Staudinger reaction (RT and no NaOH).

~20% isolated yield after the whole synthetic route.
Custom Made Strategy (AKA Wisdom through Challenge)

Rinkamide: Acidic cleavage (TFA).

Alloc: Pd(0) catalysed deallylation (Pd(PPh₃)₄ + PhSiH₃).

Boc: Acidic deprotection (TFA).

Alloc: Pd(0) catalysed deallylation (Pd(PPh₃)₄ + PhSiH₃).

Cyclisation on resin doesn’t work! Need to change for a group that avoids Pd(0), acids, amine nucleophiles or aqueous base.

Need to change PG on photoswitch.
Custom Made Strategy (AKA Wisdom through Challenge)

**Rinkamide**: Acidic cleavage (TFA).

**Allyl ester**: Pd(0) catalysed deallylation ($\text{Pd(PPh}_3\text{)}_4 + \text{PhSiH}_3$).

**Azide**: Mild Staudinger reduction ($\text{H}_2\text{O and PMe}_3$).

**Alloc**: Pd(0) catalysed deallylation ($\text{Pd(PPh}_3\text{)}_4 + \text{PhSiH}_3$).
Alternative Sequence Towards Cyclic Photoswitchable Tyrocidine A

One-pot and Quantitative Protecting Group Exchange:

Tfa-CEBA-OH $\xrightarrow{\text{NHTfa, NaOH, H}_2\text{O, THF, ii. Alloc-OCOCl}}$ Alloc-CEBA-OH
Alternative Sequence Towards Cyclic Photoswitchable Tyrocidine A

One-pot and Quantitative Protecting Group Exchange:

\[ \text{Tfa-CEBA-OH} \xrightarrow{\text{i. NaOH, H}_2\text{O, THF}} \text{NHTfa} \xrightarrow{\text{ii. All-OCOCI}} \text{Alloc-CEBA-OH} \]
Synthesis of Cyclic Photoswitchable Tyrocidine A

- Reactions have been optimised to be compatible with a one-pot procedure.
- Irradiaion of the cyclisation reaction mixture did not affect the outcome.
Visible Light Isomerisation – Cyclic Compound

650 nm, 369 W·m⁻²

Cyclic Photoswitchable Tyrocidine trans

650 nm

Cyclic Photoswitchable Tyrocidine cis

White Light

Visible Light Isomerisation – Linear Compounds

Linear Photoswitchable Tyrocidine \textit{trans}

Linear Photoswitchable Tyrocidine \textit{cis}

\begin{align*}
\text{trans} / \text{cis} & : 88 : 12 && \text{trans} / \text{cis} & : 18 : 82 \\
650 \text{ nm} & \quad \downarrow \quad 380 \text{ nm} && \text{white light} & \quad \downarrow \quad \text{daylight} \\
\text{after isolation} & \quad \downarrow & \quad \text{white light} & \quad \downarrow \quad \text{daylight} \\
\text{trans} / \text{cis} & : 84 : 16 && \text{trans} / \text{cis} & : 83 : 17 \\
\end{align*}

Azobenzenes

trans 10% trans to 90% cis  

10% trans to 90% cis
Thermal trans isomer (most stable)

Photo-excite trans isomer (most stable)

Photo-excite cis isomer

Photo-isomerisation (FAST)

Excitation breaks double bond.

Double bond cannot rotate.

Thermal cis isomer (least stable)

Thermal isomerisation (slow)

R is placed on the left side, Cl on the top right side, and N on the bottom right side of the structures.
Excellent response for photoactivation and deactivation and slow isomerisation in the dark!
### in vitro Results – Antibacterial Activity

<table>
<thead>
<tr>
<th>Gram-positive</th>
<th>TA</th>
<th>NoIr</th>
<th>Irr[^b]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Staphylococcus aureus</strong> ATCC 25923[^c]</td>
<td>8</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td><strong>Bacillus subtilis[^c]</strong></td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td><strong>Staphylococcus epidermidis</strong> F652012[^c]</td>
<td>8</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td><strong>Streptococcus pyogenes</strong> 016[^c]</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td><strong>Enterococcus faecium</strong> VancoR[^c]</td>
<td>4</td>
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<td></td>
</tr>
<tr>
<td><strong>Pseudomonas aeruginosa</strong> ATCC 27853[^d]</td>
<td>&gt;64</td>
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<td><strong>Acinetobacter baumannii</strong> ATCC 19606[^d]</td>
<td>16</td>
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<td></td>
</tr>
<tr>
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<td>32</td>
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</tr>
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<td></td>
</tr>
</tbody>
</table>

Minimum Inhibitory Concentration (µg·mL⁻¹)

[^b]: Cyclic linear amine in PS
[^c]: unsubstituted PS

### in vitro Results – Antibacterial Activity

<table>
<thead>
<tr>
<th>Gram-negative</th>
<th>TA 1</th>
<th>TA 2</th>
</tr>
</thead>
<tbody>
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<td></td>
<td>cyclic</td>
<td>linear</td>
</tr>
<tr>
<td></td>
<td>NoIrr</td>
<td>Irr[b]</td>
</tr>
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</table>

Minimum Inhibitory Concentration ($\mu$g·mL$^{-1}$)

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Analogues to Interrogate N- and C- Termini of Linear Analogues
**Chem. Eur. J. 2021, 27, 12987.**

**in vitro Results – Antibacterial Activity**

<table>
<thead>
<tr>
<th>Gram-positive</th>
<th>TA</th>
<th>1</th>
<th>2</th>
<th>13</th>
<th>14</th>
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<tbody>
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<td>NolIr</td>
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</table>

Minimum Inhibitory Concentration (μg·mL⁻¹)
### in vitro Results – Antibacterial Activity

<table>
<thead>
<tr>
<th>Gram-negative</th>
<th>Gram-positive</th>
<th>cyclic</th>
<th>linear - amine in PS</th>
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<td>2</td>
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</table>

Minimum Inhibitory Concentration (μg·mL⁻¹)

**in vitro Results - Toxicity**

<table>
<thead>
<tr>
<th>IC50 (µg·mL⁻¹)</th>
<th>NoIrr</th>
<th>Irr[b]</th>
</tr>
</thead>
<tbody>
<tr>
<td>TA</td>
<td>25</td>
<td>24</td>
</tr>
<tr>
<td>1</td>
<td>21</td>
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<td>14</td>
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<tr>
<td>15</td>
<td>39</td>
<td>33</td>
</tr>
<tr>
<td>16</td>
<td>23</td>
<td>25</td>
</tr>
<tr>
<td><strong>17</strong></td>
<td><strong>133</strong></td>
<td><strong>173</strong></td>
</tr>
</tbody>
</table>

### Haemolysis (IC50)

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50 (µg·mL⁻¹)</th>
<th>Net Charge</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>123</td>
<td>+2</td>
</tr>
<tr>
<td>13</td>
<td>36</td>
<td>+2</td>
</tr>
<tr>
<td>14</td>
<td>25</td>
<td>+1</td>
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<tr>
<td>15</td>
<td>39</td>
<td>+1</td>
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<tr>
<td>16</td>
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</tr>
<tr>
<td><strong>17</strong></td>
<td><strong>133</strong></td>
<td>0</td>
</tr>
</tbody>
</table>

### Photoswitchable Antimicrobials: Hits

The table below summarizes the antimicrobial activity of compounds 2 and 17 against different strains and IC50 values.

<table>
<thead>
<tr>
<th></th>
<th>NoIr</th>
<th>Ir</th>
<th>NoIr</th>
<th>Ir</th>
</tr>
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<tbody>
<tr>
<td><strong>S. Pyogenes 016</strong></td>
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<td>IC50 (µg·mL⁻¹)</td>
<td>32</td>
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</tr>
</tbody>
</table>

**Chem. Eur. J. 2021, 27, 12987.**
Acknowledgements

Current Team

Prof. Mercedes Amat and all members of Sintefarma

Prof. Ernest Giralt’s group at IRB

Alejandro Yeste

Hospital Clínic de Barcelona / ISGlobal

Javier Moreno  Prof. Jordi Vila  Dr. Clara Ballesté
Group’s website: http://justbaringochem.com  @justbaringochem