Applying medicinal chemistry principles to solve environmental problems

Marta Correia-da-Silva 1,2*

1 Laboratory of Organic and Pharmaceutical Chemistry (LQOF), Faculty of Pharmacy, University of Porto, Rua Jorge Viterbo Ferreira, 228, 4050-313, Porto, Portugal;
2 Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), Terminal de Cruzeiros do Porto de Leixões, Avenida General, Norton de Matos S/N, 4450-208, Matosinhos, Portugal.

* m_correiasilva@ff.up.pt
Applying medicinal chemistry principles to solve environmental problems

Graphical Abstract

HTS  MOLECULAR TARGETS  LEAD

SAR  HIT

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Abstract: Ocean-related activities are increasing every day and the successive build-up of organisms on the ship hulls (marine biofouling) leads to higher heavy oil consumption which incurs in increased costs and emissions of polluting gases, contributing to acceleration of greenhouse and acid rain effects. Other major ecological consequence of marine biofouling on the ship hulls is the marine biodiversity decline due to the trans-global contamination of ecosystems with non-indigenous species. On the other hand, antifouling paints in use are continuing leaching persistent, bioaccumulative and toxic substances to the oceans and the marine industry is facing the phase-out of most of the current biocide-based coatings, shortening the available alternatives. Climate changes and oceans temperature raising are changing the dynamic of species, also creating new challenges to combat marine biofouling. Therefore, the development of harmless and effective antifouling systems to prevent the marine biofouling is an urgent demand. The Natural Products and Medicinal Chemistry Group of CIIMAR has been synthesizing several antifouling (AF) compounds and applying the same principles of Drug Discovery process: high throughput screening to discover hits, lead generation after some optimization of hit compounds, and finally lead optimization. If this approach is followed, it is highly probable that the necessary balance between the feasible synthesis, antifouling potency, low toxicity and bioaccumulation will be found. From this work, it is possible to conclude that the application of Medicinal Chemistry principles to solve environmental problems becomes an extremely useful tool and fulfills the broad sense of the concept of this scientific area.

Keywords: antifouling, eco-friendly, proteomics, SAR, xanthones
Introduction: marine biofouling

Deposition of organic material
Attachment of microorganisms
Attachment of macroorganisms

Minutes
Hours
Days
Introduction: the problem

- **Loss of speed**
  - Increase in propulsive energy
  - More fuel
  - Increased costs and emissions of polluting gases

- **Corrosion**
  - Increased costs

- **Transport of non-indigenous species**
  - Biodiversity reduction and public health risks

- **Fuel consumption**
- **Greenhouse gases**
- **Coating deterioration**
- **Antifouling maintenance**
- **Legionnaire’s Disease**

Introduction:

- Loss of speed:
  - Increased propulsive energy
  - More fuel
  - Increased costs and emissions of polluting gases

- Corrosion:
  - Increased costs

- Transport of non-indigenous species:
  - Biodiversity reduction and public health risks
Nucella lapillus extinction

Imposex in female gastropods

Oysters malformations

Tin-based antifouling coatings

Chemical control

Introduction: the problem

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Copper-based antifouling coatings + booster biocides (herbicides/pesticides)

1 million tonnes of copper per year

Chemical control

Introduction: the problem

Persistent and Toxic

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Restrictions on copper-based coatings? Alternatives are needed!
Our strategy: synthesize Nature-inspired antifouling compounds
Four series of synthetic Nature-inspired antifouling compounds

Cinnamic and gallates

Seagrass

Marine-derived fungus

Starfish

Sponge

Flavonoids

Xanthones

Steroids


Applying the same principles of the Drug Discovery process
Evaluation of the settlement of *Mytilus galloprovincialis* larvae

1. Collect *Mytilus galloprovincialis*

2. Select small and viable larvae

3. Incubate larvae with compounds solution

4. Count larvae adherence

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Non-toxic antifouling agents

EC$_{50}$ < 25 µM  \quad \text{LC$_{50}$} > 200 µM

Efficacy versus toxicity

U.S. Navy recommendations:
\[ \text{LC}_{50}/\text{EC}_{50} > 15 \]

LC$_{50}$, the median lethal dose
EC$_{50}$, concentration that inhibited 50% of larval settlement
LC$_{50}$/EC$_{50}$, therapeutic ratio
One step synthesis from natural raw materials

**EC$_{50}$ < 25 µM**

**LC$_{50}$ > 500 µM**

**Efficacy versus toxicity**

1 flavonoid

1 xanthone

1 gallate

Less toxic than Econea ($LC_{50} > 500$ µM)

**Econea®**

$EC_{50} = 4$ µM

$LC_{50} = 108$ µM

**Most potent ($EC_{50} = 17$ µM)**

Scientific reports, 2017, 7, 42424.
SERIES II

EC_{50} < 25 \mu M
LC_{50} > 500 \mu M

Efficacy versus toxicity

4 oxygenated xanthones at 3 and/or 4 positions
1 thioxanthone

Less toxic than Econea (LC_{50} > 500 \mu M)

Econea®
EC_{50}=4 \mu M
LC_{50}=108 \mu M

Oxygenated xanthones and thioxanthones

International patent PCT/IB2019/059886; Biomolecules, 2020, 10, 1126.

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SERIES III

EC$_{50}$ < 10 µM  
LC$_{50}$ > 200 µM

Efficacy versus toxicity

3 trimethoxyphenyl B ring flavonoids

Less toxic than Econea  
(LC$_{50}$ > 200 µM)

EC$_{50}$ = 4 µM  
LC$_{50}$ = 108 µM

EC$_{50}$ similar to Econea  
(EC$_{50}$ = 3.3-4.01 µM)

Marine Drugs, 2021, 19, 5.
SERIES IV

EC$_{50} < 10$ µM
LC$_{50} > 200$ µM

Efficacy 
versus 
toxicity

4 methyl ester derivatives

Less toxic than Econea
(LC$_{50} > 200$ µM)

Econeа®
EC$_{50} = 4$ µM
LC$_{50} = 108$ µM

One step synthesis from natural raw materials

Bile acids

Efficacy versus toxicity

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HIT selection

15 HIT compounds

- 3 sulfated derivatives
- 4 xanthones
- 4 flavonoids
- 4 bile acids

Efficacy versus toxicity

EC$_{50}$ <25 µM

LC$_{50}$/EC$_{50}$ >20
Ecotoxicity to marine nontarget organisms

**Artemia salina** mortality assay

<table>
<thead>
<tr>
<th>Artemia mortality</th>
<th>No ecotoxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10% (25 µM)</td>
<td></td>
</tr>
</tbody>
</table>

HIT TO LEAD

Bioaccumulation

**Episuite**

Log Kow\(^1\)

\(^1\) Partition coefficient n-octanol/water

<table>
<thead>
<tr>
<th>Log Kow(^1)</th>
<th>No bioaccumulative potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;3</td>
<td></td>
</tr>
</tbody>
</table>
Ecotoxicity to marine nontarget organisms

Marine shrimp
Artemia salina

<10% mortality at 25 µM

>10% mortality at 25 µM

Econea®
100% mortality at 25 µM
Structure-properties relationship for xanthones

Ecotoxicity

Potency

100% mortality at 50 µM
EC$_{50}$ (Mytilus) = 3.53 µM

100% mortality at 50 µM
EC$_{50}$ (Mytilus) = 21.48 µM

100% mortality at 50 µM
EC$_{50}$ (Mytilus) = 15.46 µM

<10% mortality at 50 µM
EC$_{50}$ (Mytilus) = 4.60 µM

<10% mortality at 50 µM
EC$_{50}$ (Mytilus) = 11.53 µM

Potency

No ecotoxicity

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Bioaccumulative potential

Log Kow, partition coefficient n-octanol/water

Log Kow < 3

Log Kow > 3

Econea®

Log Kow = 4.69

www.epa.gov/opt/exposure/pubs/episuite.htm
Structure-properties relationship for xanthones

Log Kow, partition coefficient n-octanol/water

Log Kow = 5.24
EC<sub>50</sub> (Mytilus) = 4.60 µM

Log Kow = 2.88
EC<sub>50</sub> (Mytilus) = 11.53 µM
LEAD selection

- Efficacy versus toxicity
- Eco-friendly profile
- Log Kow <3
- LC50/EC50 >20
- EC50 <25 µM
- Mortality to Artemia <10% (25 µM)

15 HITs
- 8 LEADs
  - 3 sulfated derivatives
  - 2 flavonoids
  - 2 bile acid
  - 1 xanthone
LEAD optimization

Series IIb

EC_{50} = 3.57 \mu M
EC_{50} / EC_{50} > 20

molecular extension strategy

Series II

3,4-dihydroxyxanthone

EC_{50} = 11.53 \mu M
EC_{50} / EC_{50} > 20
Proteome of mussel larvae in response to xanthones

Molecular targets on mussel settlement

Mytilus collagen proteins (PreCols)*

Myosin isoforms from pedal retractor muscle

*protein-2 collagen-like
*precollagen P

Division of Cell Biology, Linköping University, Sweden
LEAD optimization

- Sulfated compounds
- Series IIIb
- Flavonoids
- Bile acids
- Series IVb
- Series Ib
Conclusions

International patent PCT/IB2019/059886: Xanthonic compounds and their use as antifouling agents

Helping Mother Nature through Medicinal Chemistry
Acknowledgments

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FFUP

UIDB/04423/2020
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