Pigments from sea urchins: chemistry and pharmacology

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Despite the fact that oceans cover more than 70% of the earth’s surface, the intensive exploration of marine ecosystems has only began in XX century, with the emergence of snorkeling, and around 1970, with the use of remotely operated vehicles. The marine environment harbors a number of macro and micro organisms that have developed unique metabolic abilities to ensure their survival in diverse and hostile habitats, resulting in the biosynthesis of an array of secondary metabolites with specific activities.
Marine natural products

John W. Blunt,*a Brent R. Copp, b Robert A. Keyzers, c Murray H. G. Munroa and Michèle R. Prinsep d


This review covers the literature published in 2013 for marine natural products (MNPs), with 982 citations (644 for the period January to December 2013) referring to compounds isolated from marine microorganisms and phytoplankton, green, brown and red algae, sponges, cnidarians, bryozoans, molluscs, tunicates, echinoderms, mangroves and other intertidal plants and microorganisms. The emphasis is on new compounds (1163 for 2013) together with the relevant biological activities, source organisms and country of origin. Reviews, biosynthetic studies, first syntheses, and syntheses that lead to the revision of structures or stereochemistries, have been included.
The phylum-preferences of the marine natural product research community across a 50-year period from 1963
Russia has an extensive coastline of over 37,000 km along the Arctic and Pacific Oceans, as well as along the Baltic, Azov, Black and Caspian Seas.
Marine pigments constitute a group of chemically heterogeneous and biosynthetically unrelated molecules that are united by a common feature: their electronic structure contains a chromophore that is responsible for the characteristic colors of these compounds.

From an evolutionary point of view, the presence of pigments has empowered organisms with the ability to develop new and diversified strategies of survival, including concealment in the environment, mimicry, advertisement and warning coloration.
Pigments are of remarkable economical relevance due to their wide use across several industries, from foodstuffs to cosmetics and pharmaceuticals. In fact, latest reports show this as a multi-billion dollar industry with a staggering annual growth around 10-15% and many of the top molecules used, such as astaxanthin or cantaxanthin, are already of marine origin*

Naphthoquinone pigments occur widely in nature among higher plants (*Juglans nigra*) and microorganisms.

In the animal kingdom this class of compounds has been encountered only in echinoderms. Even within this phylum only the sea urchins have been the prime producer of these substances.
The presence of naphthaquinone pigments in echinoids has been recorded intermittently since 1883 when MacMunn first reported red pigment “echinochrome” in the perivisceral fluid of *Echinus esculentus.*

Crystalline echinochrome A was isolated by McClendon (1912), but a pure sample was not obtained until 1934, and the structure was determined subsequently by Kuhn & Wallenfels (1939) and confirmed by synthesis*.

The polyhydroxynaphthaquinone pigments occurring in spines, shells, and tests of sea urchins and are mainly known as **spinochromes**.

Different spinochromes have been distinguished by adding a suffix letter to the name, e.g. spinochrome B.

Polyhydroxynaphthoquinone pigments are generated via a series of enzymatic, oxidative and photochemical reactions from shikimic acid - a precursor of naphthoquinone pigments.

Common spinochromes

Spinochromes
A = B_3 = M = M_1 = Aka_2 = P
B = B_1 = M_2 = N = P_1
C = B_2 = F = F_1 = M_3 = spinone A = isoechinochrome
D = Aka = Aka_1
Naphtazarins
Naphtazarins

Echinamines
Bisnaphtazarins

Ethylidene-6,6-bis(2,3,7-trihydroxynaphthazarin)

Anhydroethylidene-6,6-bis(2,3,7-trihydroxynaphthazarin)

Mirabiquinone
*Strongylocentrotus droebachiensis* is commonly known as the green sea urchin because of its characteristic green color.

In our experiments sea urchins from Barents Sea were used.
Shikov et al. (2011). The offline combination of thin-layer chromatography and high-performance liquid chromatography with diode array detection and micrOTOF-Q mass spectrometry for the separation and identification of spinochromes from sea urchin (Strongylocentrotus droebachiensis) shells. *Journal of Chromatography A*. 1218(50), 9111-9114
Shikov et al. (2011). The offline combination of thin-layer chromatography and high-performance liquid chromatography with diode array detection and micrOTOF-Q mass spectrometry for the separation and identification of spinochromes from sea urchin (Strongylocentrotus droebachiensis) shells. *Journal of Chromatography A*. 1218(50), 9111-9114
Free radical scavenging activity after TLC separation and DPPH• derivatization

Ascorbic acid
$\text{ID}_{50} = 0.542 \, \mu g$

Echinochrome A
$\text{ID}_{50} = 0.134 \, \mu g$

Spinochromes B and D
$\text{ID}_{50} = 0.057 \, \mu g$

Dimers
$\text{ID}_{50} = 0.043 \, \mu g$


Radical scavenging activity


### Anti-allergic effect

Hyperemia rabbit eye after Compound 48/80

<table>
<thead>
<tr>
<th>Time</th>
<th>Clinical score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>After 5 minutes (n=6)</td>
<td>8.66±2.52</td>
</tr>
<tr>
<td>After 15 minutes (n=6)</td>
<td>9.67±1.76</td>
</tr>
<tr>
<td>After 1 hour (n=6)</td>
<td>9.50±2.64</td>
</tr>
<tr>
<td>After 2 hours (n=6)</td>
<td>10.16±1.64</td>
</tr>
<tr>
<td>After 24 hours (n=6)</td>
<td>5.53±2.31</td>
</tr>
</tbody>
</table>

Effect on histamine-induced contraction of the isolated guinea pig ileum

Docking poses of individual spinochrome ligands (orange sticks) superimposed onto H₁R crystal structure (3RZE)

Dimers did not fully fit the binding site and remained partially interacting also with the loops shielding binding side of the protein, because they are too bulky.
Autodock Vina results from docking of spinochromes into the set of H\textsubscript{1}R structures obtained from molecular dynamics.

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Binding energy [kcal/mol]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spinochrome B</td>
<td>-8,7 ± 0,3</td>
</tr>
<tr>
<td></td>
<td>-9,4 - -7,9</td>
</tr>
<tr>
<td>Spinochrome D</td>
<td>-8,8 ± 0,4</td>
</tr>
<tr>
<td></td>
<td>-9,6 - -8,1</td>
</tr>
<tr>
<td>Ethylidene-6,6''-bis(2,3,7-trihydroxynaphthazarin)</td>
<td>-10,6 ± 0,9</td>
</tr>
<tr>
<td></td>
<td>-12,4 - -9,2</td>
</tr>
<tr>
<td>Anhydroethylidene-6,6''-bis (2,3,7-trihydroxynaphthazarin)</td>
<td>-10,8 ± 1,0</td>
</tr>
<tr>
<td></td>
<td>-13,0 - -8,9</td>
</tr>
</tbody>
</table>

The binding energies of spinochrome dimers are higher than the binding energies of monomers, however, the dimers do not exactly complement the ligand binding site and bind further from the site up to the flexible loops region. As monomers fit better to the active site, they may compete with histamine more effectively than dimers and therefore may be more effective inhibitors than the dimers.
## Antidiabetic effect

### Blood glucose level in STZ/NA diabetic mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Day of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Intact</td>
<td>4.7±0.3</td>
</tr>
<tr>
<td>Control (diabetes + placebo)</td>
<td>6.9±0.5*</td>
</tr>
<tr>
<td>Pigments, 1.8 mg/kg</td>
<td>5.9±0.5*</td>
</tr>
</tbody>
</table>

### Effect of treatment on GSH and MDA

<table>
<thead>
<tr>
<th>Group</th>
<th>GSH (mMol/mL)</th>
<th>MDA (μMol/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td>0.60±0.04</td>
<td>11.1±0.4</td>
</tr>
<tr>
<td>Control (diabetes + placebo)</td>
<td>0.26±0.06*</td>
<td>19.0±1.3*</td>
</tr>
<tr>
<td>Pigments, 1.8 mg/kg</td>
<td>0.66±0.05×</td>
<td>9.4±0.6**×</td>
</tr>
</tbody>
</table>

* p<0.05 vs Intact,  * p<0.05 vs Control

Anti-inflammatory effect

in rat model of acute formalin-induced rhinosinusitis

<table>
<thead>
<tr>
<th>Group</th>
<th>Leukocytes mucous membrane</th>
<th>Nasal mucosa goblet, cells/mm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td>0</td>
<td>7.30 ± 0.61</td>
</tr>
<tr>
<td>Control</td>
<td>0.90 ± 0.38*</td>
<td>14.67 ± 0.50*</td>
</tr>
<tr>
<td>Aqua-maris</td>
<td>0.40 ± 0.16*</td>
<td>14.10 ± 0.66*</td>
</tr>
<tr>
<td>Pigments (0.25 µg)</td>
<td>0.67 ± 0.17*</td>
<td>9.67 ± 0.91*; x</td>
</tr>
</tbody>
</table>

* p<0.05 vs Intact,  * p<0.05 vs Control
# Anti-microbial effect

<table>
<thead>
<tr>
<th>Samples Code</th>
<th>Final concentration (µg/ml)</th>
<th>E. coli ATCC 25922</th>
<th>S. aureus ATCC 25923</th>
<th>C. albicans ATCC 90028</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ur</td>
<td>100, 200</td>
<td>1.76 ± 2.42, 3.38 ± 1.41</td>
<td>-3.46 ± 1.98, -7.90 ± 2.84</td>
<td>17.43 ± 10.93, 9.47 ± 19.10</td>
</tr>
<tr>
<td>AP</td>
<td>100, 200</td>
<td>3.85 ± 2.40, 3.76 ± 1.43</td>
<td>-1.31 ± 1.53, -4.01 ± 2.91</td>
<td>11.74 ± 9.90, 19.37 ± 22.27</td>
</tr>
<tr>
<td>St</td>
<td>100, 200</td>
<td>4.35 ± 2.53, 4.72 ± 1.01</td>
<td>-9.87 ± 3.31, -15.96 ± 4.05</td>
<td>-2.55 ± 16.37, -7.40 ± 7.01</td>
</tr>
<tr>
<td>AS1</td>
<td>100, 200</td>
<td>4.29 ± 2.56, 4.85 ± 1.27</td>
<td>-0.25 ± 1.31, -2.13 ± 4.06</td>
<td>16.78 ± 11.69, -18.59 ± 16.38</td>
</tr>
<tr>
<td>AS2</td>
<td>100, 200</td>
<td>-7.46 ± 2.08, 15.16 ± 1.97</td>
<td>-34.83 ± 2.81, -26.13 ± 9.22</td>
<td><strong>102.20 ± 1.90</strong>, <strong>105.24 ± 1.34</strong></td>
</tr>
<tr>
<td>AS2.1</td>
<td>100, 200</td>
<td>6.47 ± 2.25, 5.53 ± 2.47</td>
<td>-37.99 ± 5.53, -60.98 ± 3.09</td>
<td>-16.20 ± 5.43, -53.57 ± 15.12</td>
</tr>
<tr>
<td>AS2.2</td>
<td>100, 200</td>
<td>4.41 ± 1.37, 3.79 ± 2.61</td>
<td>-41.79 ± 8.10, -61.16 ± 2.79</td>
<td>-25.64 ± 3.60, -24.99 ± 5.00</td>
</tr>
<tr>
<td>AS2.3 (blank)</td>
<td>100, 200</td>
<td>-1.85 ± 0.76, -75.18 ± 5.73</td>
<td>-7.518 ± 5.73, -44.65 ± 26.84</td>
<td></td>
</tr>
</tbody>
</table>

**Control drugs**
- Gentamicin 8 µg/ml
- Ciprofloxacin 2 µg/ml
- Amphotericin B 2 µg/ml
Pharmacological effects

Antiarrhythmic
Anticancer
Anticoagulant
Antihyperlipidemic
Cardio protective

Application in ophthalmology

Echinochrome A is used for the treatment of:
- proliferative processes,
- degeneration of retina and optic nerve,
- ophthalmic hemorrhage of various genesis,
- glaucoma,
- cataract.

Safety

Echinohrome A was not showed significant toxicity on A7r5 cells (rat aortic vascular smooth muscle cell line) and H9c2 cells (rat cardiomyoblasts) up to 100 μM for 24 h\(^1\). The survive of ciliate *Tetraymena pyriformis* was 95.2% after application of 0.001 mg/g of pigment complex isolated from shells of *Strongylocentrotus intermedius* and *Strongylocentrotus nudus*. While after application of 0.003 mg/g of complex the survive was 87.5% only.\(^2\)

Echinohrome exhibits a moderate toxicity; for outbred mouse LD\(_{50}\) = 148 mg/kg and LD\(_{10}\) = 85 mg/kg\(^3\) LD\(_{50}\) = 87 mg/kg body weight (Echinohrome, i/p injection) white mice\(^4\)

No side effects were registered in patients after 10 days of intramuscular injection of Echinohrome A (0.02% solution, 2 ml/day)\(^5\). No changes were observed in the functional parameters of liver and kidneys, no variations were found in blood indexes (hemoglobin and hematocrit), and no allergic reactions were reported.

Clinical application

Randomized clinical trial, 57 teens (14.7±2.4 years, m/f) with erosive gastroduodenitis (*Helicobacter pylori* positive)

Group 1: 35 patients, triple therapies (Omeprazole® 20 mg, Amoxicillin® 1000 mg and Clarithromycin® 500 mg), twice daily for weeks.

Group 2: 22 patients, triple therapies + intramuscular injection of (0.02% of Echinochrome A) 2 ml, 5 injections on alternate days.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healing of erosions</td>
<td>54%</td>
<td>87%</td>
</tr>
<tr>
<td>Decrease of abdominal pain</td>
<td>7.3%</td>
<td>35.3%</td>
</tr>
<tr>
<td>(GSRS scale)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decrease of dyspeptic syndrome</td>
<td>17.0%</td>
<td>35.2%</td>
</tr>
</tbody>
</table>

Clinical application

A group of 554 patients (2 months - 18 years) with traumatic intraocular hemorrhage were treated with 0.02% of Echinochrome A (0.5 ml, subconjunctivally or parabulbarly injection, once daily).

Positive effect was registered in 97.3% of the patients. Clinical recovery was observed in 65.1% of the patients and positive dynamic in 32.2% of patients. In case of hemophthalmia, the transparency of the vitreous was restored on 3-5th day of treatment for most patients. Complete resorption of retinal hemorrhages after traumatic intraocular hemorrhage was registered in 82% of patients after 10-15 days.

Marketed sea urchin pigment in Russia

- as a retina protector for dystrophic damages of retina and diabetic retinopathy;
- for proliferous processes, degeneration and hemophthalmia of various genuses;
- for contusions, burns and penetrating wounds of eyes;
- for cataract, keratitis and uveitis
- for prophylaxis of reperfusion damages after myocardial infarction;
- for treating of ischemia and infarction in acute forms;
Conclusions

☒The investigation of pigments from sea urchin is a rapidly developing scientific field at the intersection of pharmacology and chemistry.

☒Pigments of sea urchin are represented predominantly by polyhydroxynaphthaquinones belongs to spinochromes, naphtazarins, echinamines, bisnaphtazarins.

☒Research have exposed their potential as anti-allergic, antiarrhythmic, anticoagulant, antidiabetic, antiradical, antimicrobial, anti-inflammatory, cardio protective, retina protective agents.

☒The potential of bisnaphtazarins should be explored more detail for development of new medicinal preparations.
Additional information

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