CHEMICAL APPROACHES TO SPHINGOLIPID RESEARCH

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Lipids: structure and functions

Lipids are a large and diverse group of naturally occurring molecules that are related by their solubility in nonpolar organic solvents and general insolubility in water.
Lipid molecules contain a hydrophilic region or (polar head), and a hydrophobic region (nonpolar tail).

Functions of lipids include:

- Structure of biological membranes
- Energy storage
- Cell signalling
Lipids: structure and functions

Fatty acid (saturated or insaturated) -> Glycerol (1,2,3-propanetriol) -> Oleic acid (C₁₈)

Cholesterol

Glycerophospholipid

Sphingolipids

phosphate
phosphoethanolamine
phosphocholine

Glucose, galactose, lactose,
Complex polysaccharides

Sulfatides
Lactoseries
Neolactoseries
Globoseries
Ganglioseries

Glycerolipids
Sphingolipids

http://www.lipidmaps.org

J. Lipid Res. (2009) 50: S9-S14
Sphingolipids are a family of lipids that play essential roles both as structural cell membrane components and in cell signalling. The cellular contents of the various sphingolipid species are controlled by enzymes involved in their metabolic pathways.

Sphingolipid metabolism

Sphingolipid and glycosphingolipid functions

**Structural role**

- **CERAMIDE**
  - Growth inhibition
  - Cell cycle arrest
  - Cell differentiation
  - Cell senescence
  - Apoptosis
  - Autophagy

- **SPHINGOSINE**
  - Growth inhibition
  - Cell cycle arrest
  - Cell differentiation
  - Cell senescence
  - Apoptosis
  - Autophagy

- **DIHYDROSPHINGANINE**
  - Growth inhibition
  - Cell cycle arrest
  - Cell differentiation
  - Cell senescence
  - Apoptosis
  - Autophagy

- **SPHINGOSINE-1-PHOSPHATE**
  - Cell growth stimulation
  - Cell proliferation
  - Angiogenesis
  - Cell migration
  - Calcium mobilization
  - Autophagy

- **CERAMIDE-1-PHOSPHATE**
  - Cell growth stimulation
  - Cell proliferation
  - Angiogenesis
  - Cell migration
  - Calcium mobilization
  - Autophagy

- **GLYCOSPHINGOLIPIDS**
  - Signal transduction
  - Cell proliferation and differentiation
  - Cell-cell recognition and adhesion

SPHINGOLIPIDS AND GLYCOSPHINGOLIPIDS IN DISEASE

- SPHINGOLIPIDOSES
- LIPOTOXIC DISEASES: DIABETES 2
- ATHEROSCLEROSIS
- CANCER
  - MULTIDRUG RESISTANCE
  - METASTASIS
- AIDS
- NEURODEGENERATIVE DISEASES
- SYSTEMIC MYCOSES: FUNGAL METABOLISM

Chemical tools for the study and modulation of sphingolipid metabolism

Sphingolipid analogues
Enzyme inhibitors

Probes to study SL metabolism
Fluorogenic substrates for enzyme activity
Azidosphingolipids (click chemistry)
Chemical tools for the study and modulation of sphingolipid metabolism

Sphingolipid analogues
Enzyme inhibitors

Selected targets

Dihydroceramide desaturase (Des1)
Sphingosine 1P-lyase (S1PL)
Acid ceramidase (ASAH1)

Acid ceramidase is a Cys protease

Acid ceramidase in disease

Table 3. Cancer Types in which a Role for AC has been Reported

<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>Cell Lines</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostate</td>
<td>DU145, LaCaP, PC3, PPC1</td>
<td>[52a, 52e, 51]</td>
</tr>
<tr>
<td>Breast</td>
<td>Samples from patients</td>
<td>[52c, 58]</td>
</tr>
<tr>
<td>Fibrosarcoma (murine)</td>
<td>L929 (parental and sublines)</td>
<td>[59]</td>
</tr>
<tr>
<td>Head and neck squamous cell</td>
<td>SCC1</td>
<td>[29]</td>
</tr>
<tr>
<td>Liver</td>
<td>HepG2, Hep-3B, SK-Hep1, HepG2 &amp; Hep3;</td>
<td>[56]</td>
</tr>
<tr>
<td>T-cell large granular lymphocyte leukemia</td>
<td>Samples from patients</td>
<td>[52b]</td>
</tr>
<tr>
<td>Glioblastoma</td>
<td>U-87 MG, U87-W E6</td>
<td>[55]</td>
</tr>
<tr>
<td>Melanoma</td>
<td>Samples from patients</td>
<td>[60]</td>
</tr>
</tbody>
</table>

Table 2. Diseases in which a Role for Ceramidases has been Reported

<table>
<thead>
<tr>
<th>Disease</th>
<th>Ceramidase</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farber disease</td>
<td>AC</td>
<td>[38]</td>
</tr>
<tr>
<td>Atopic dermatitis</td>
<td>bacterial</td>
<td>[39]</td>
</tr>
<tr>
<td>Harlequin ichthyosis</td>
<td>n.a.</td>
<td>[40]</td>
</tr>
<tr>
<td>Alzheimer’s disease</td>
<td>AC</td>
<td>[42]</td>
</tr>
<tr>
<td>Cystic fibrosis</td>
<td>AC</td>
<td>[43]</td>
</tr>
<tr>
<td>Myocardial ischemia-reperfusion</td>
<td>n.a.</td>
<td>[44]</td>
</tr>
<tr>
<td>Diabetic nephropathy</td>
<td>NC</td>
<td>[45]</td>
</tr>
<tr>
<td>Type 2 diabetes, insulin resistance and metabolic syndrome</td>
<td>AC</td>
<td>[46b, 46e]</td>
</tr>
<tr>
<td>AC</td>
<td>NC</td>
<td>[46a]</td>
</tr>
<tr>
<td>AC</td>
<td>ACER</td>
<td>[46c]</td>
</tr>
<tr>
<td>AC</td>
<td>n.a.</td>
<td>[46f]</td>
</tr>
<tr>
<td>Pulmonary fibrosis</td>
<td>AC</td>
<td>[48]</td>
</tr>
<tr>
<td>Retinal degeneration</td>
<td>ACER</td>
<td>[49]</td>
</tr>
<tr>
<td>Retinal degeneration</td>
<td>Drosophila</td>
<td>[12b]</td>
</tr>
</tbody>
</table>

Anticancer Agents Med Chem 2011, 11, 830-843
Prog Lipid Res 2010, 49, 316-34


Irreversible inhibitor
IC_{50} 52 nM

No activity on neutral CDase

Dosis-response curve
Chemical tools for the study and modulation of sphingolipid metabolism

Sphingolipid analogues
Enzyme inhibitors

Probes to study SL metabolism
Fluorogenic substrates for enzyme activity
Azidosphingolipids (click chemistry)

Library synthesis and screening

Fluorogenic assay for ceramidase activity


Excellent performance in both intacts cells & cell lysates
Synthesis of a Fluorogenic analogue of Sphingosine-1-Phosphate and its use to determine Sphingosine-1-Phosphate Lyase activity.

Chemical tools for the study and modulation of sphingolipid metabolism

- Sphingolipid analogues
- Enzyme inhibitors
- Probes to study SL metabolism
  - Fluorogenic substrates for enzyme activity
  - Azidosphingolipids (click chemistry)
ω-Azidosphingolipids

Probes for sphingolipid metabolism, sphingolipid targets, sphingolipid trafficking and cell population tagging for LC/MS analysis.

- Non cytotoxic
- Same metabolic pattern as natural SL's

General approach to “clickable” azidosphingolipids

ω-Azidosphingolipids

Garner’s aldehyde:

- Reduction (for ωN₃kSa and ωN₃dhSo)
- Oxidation (for ωN₃kSa)
- Cross-metathesis
- Replacement with N₃⁻
- Deprotection (for ωN₃So and ωN₃dhSo) and acylation (for ωN₃Cer and ωN₃dhCer)

ωN₃kSa  ωN₃dhCer’s  ωN₃So  ωN₃Cer’s  ωN₃dhSo
Click Chemistry Reactions

“A click chemistry reaction must be modular, wide in scope, give very high yields, generate only inoffensive byproducts that are easily separated and be stereospecific. The process must include simple reaction conditions, readily available starting materials and reagents, the use of no solvent or a solvent that is benign or easily removed, and simple product isolation.”

C=C Additions
Nucleophilic opening of strained rings
Cycloadditions
'Special' carbonyl chemistry

Efficient and selective bioorthogonal reaction to link molecules rapidly and in high yield.
Bioorthogonal reaction: any chemical reaction that can occur inside of living systems without interfering with native biochemical processes.

Most popular click reactions in chemical biology

Thiol-ene Reaction
Staudinger Ligation
Huisgen 1,3-Dipolar Cycloaddition
Click Chemistry Reactions

**Huisgen 1,3-Dipolar Cycloaddition**

\[
\begin{align*}
R^1 & \quad + \quad R^2 \\
\text{Cu free} & \quad \text{“Strain-promoted” (SPAAC)}
\end{align*}
\]

**APPLICATIONS OF AZIDOSPHINGOLIPIDS**

- Fluorescent membrane lipids
- Cell trafficking and localization studies
- Quantitative cell metabolism
Fluorescent membrane lipids ("in situ" fluorogenic click chemistry)

Cell trafficking and localization studies
"strain promoted" click chemistry ("SPAAC")
Cell trafficking and localization studies
“strain promoted” click chemistry (“SPAAC”) -

Fluorescence analysis by confocal laser scanning microscopy. A549 cells without (Az-) or with (Az+) the azide probe. Propidium iodide (in red) was used for nuclei staining.

“Azide + click” vs fluorophores for sphingolipid labeling

Sphingosine-NBD

Good SL mimick
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