Amido-1,2,3-thiadiazole derivatives as novel S1P$_1$ selective agonists

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Nuria Aguilar
Multiple Sclerosis

- Multiple Sclerosis is a chronic, inflammatory, demyelinating disease that affects the CNS.

- **Symptoms** can vary widely depending on the part of the brain that is more affected. In general: muscle weakness, abnormal muscle spasms, problems to speech, visual problems, fatigue, chronic pain, changes in sensation…

- It is considered as an **autoimmune** disease in which **lymphocytes** recognize myelin as a foreign and attack it as if it were an invading virus.
Multiple Sclerosis

- No definitive cause has been found. MS likely occurs as a result of some combination of both environmental and genetic factors.

- In Europe, north of the 46th degree of latitude, MS, with an average of approximately 60 affected per 100,000 inhabitants, belongs to the most frequent neurological diseases.

- MS primarily affects adults, with an age of onset typically between 20 and 40 years, and is more common in women than in men.
In October 2010, Fingolimod, developed by Novartis, was approved by the FDA and in most European countries like the first oral treatment of MS under the tradename of Gylenia.
Fingolimod Mode of Action (I)

Fingolimod-phosphate

Sphingosine-1-phosphate

IC$_{50}$ (nM) competition S1$^{33}$P binding

<table>
<thead>
<tr>
<th></th>
<th>S1P$_1$</th>
<th>S1P$_2$</th>
<th>S1P$_3$</th>
<th>S1P$_4$</th>
<th>S1P$_5$</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1P</td>
<td>0.47</td>
<td>0.31</td>
<td>0.17</td>
<td>95</td>
<td>0.61</td>
</tr>
<tr>
<td>metabolite</td>
<td>0.21</td>
<td>&gt;10$^5$</td>
<td>5.0</td>
<td>5.9</td>
<td>0.59</td>
</tr>
<tr>
<td>FTY720</td>
<td>300</td>
<td>&gt;10$^5$</td>
<td>&gt;10$^5$</td>
<td>&gt;5000</td>
<td>2623</td>
</tr>
</tbody>
</table>

Fingolimod-P is a non selective full agonists of S1P receptors

S. Mandala et al. Science 2002 296, 346
Fingolimod Mode of Action (II)

- S1P, the endogenous ligand of S1P₁ receptor, is involved in the migration of naïve lymphocytes from lymph nodes (LN) to blood during immunosurveillance. Cells exit LN following the gradient of S1P that exists between LN, lymph and blood. This effect is mediated by S1P₁.

- Fingolimod-P and all synthetic S1P₁ agonists cause S1P₁ receptor internalization, leading to disappearance of the receptor from the cellular membrane and making cells unable to respond to the endogenous ligand.

- Thus, synthetic S1P₁ agonists behave as functional antagonists of the S1P₁ receptor and cause the retention of lymphocytes in lymph nodes resulting in peripheral blood lymphopenia.

- The retention of cells in the LN prevents autoreactive lymphocytes from migrating to the disease target tissues (i.e., CNS) and this results in clinical efficacy.
Almirall S1P₁ Agonists Program Objectives

- Identification of a compound that does not require phosphorylation to be active

- Agonist of S1P₁ and selective versus S1P₃
  S1P₃: potential contribution to bradycardia, respiratory issues and blood pressure, based on receptor expression pattern.

- Once a day oral compound with a DMPK profile able to:
  - Prevent the exit of lymphocytes from LN to blood between administrations
  - Permit fast recovery of lymphopenia after treatment discontinuation in case of adverse events
  - Direct PK/PD relationship (no hysteresis)
**HTS Campaign: Identification of Amidothiadiazoles**

![Structural analysis of hit compounds](image)

- Amidothiadiazole core forms a pseudo bicyclic system thanks to a S-O interaction

**Structural analysis of hit compounds:**

- **EC$_{50}$ S$_1$P$_1$(GTP$_{\gamma}$S) = 4.5 µM**
- **EC$_{50}$ S$_1$P$_1$(GTP$_{\gamma}$S) = 9 µM**

**S—O distance = 2.65 Å**

**O=C-N-C diedral angle = 2.5°**
Comparison of ATDAs with known S1P₁ agonists

- The additional lipophilic tail identified in our series resulted to be key for activity. Its removal accounted for a complete loss in activity.

## Initial analogues

<table>
<thead>
<tr>
<th>Compound</th>
<th>S1P₁ EC₅₀</th>
<th>S1P₃ %E@40µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>&gt;10µM</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>1.5µM</td>
<td>31%</td>
</tr>
</tbody>
</table>

**Chemical Structures:**

- **Compound 4:**
  - ![Chemical Structure 4](image1.png)

- **Compound 5:**
  - ![Chemical Structure 5](image2.png)
## Initial analogues

<table>
<thead>
<tr>
<th>Compound</th>
<th>S1P&lt;sub&gt;1&lt;/sub&gt; EC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>S1P&lt;sub&gt;3&lt;/sub&gt;&lt;br&gt;%E@40µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>&gt;10µM</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>1.5µM</td>
<td>31%</td>
</tr>
<tr>
<td>6</td>
<td>0.3 nM</td>
<td>300 nM</td>
</tr>
</tbody>
</table>
Docking of the initial analogues in an in-house generated homology model based on bovine Rhodopsine
SAR conclusions

- All analogues kept a reasonable S1P₃ selectivity (>100 fold with examples in the 1000 fold selectivity range) and were stable in both human and rat liver microsomes.

- H, Me low active
- Propyl, CyMe potent
- Bu optimal

- o-substitution preferred
- Benzylic amides also allowed

- Me led to increased S1P₃ selectivity

*Bioorg Med Chem Lett accepted for publication*
Compound 6: Lead profile

<table>
<thead>
<tr>
<th>Compound</th>
<th>S1P1 EC&lt;sub&gt;50&lt;/sub&gt; (nM)</th>
<th>S1P3 EC&lt;sub&gt;50&lt;/sub&gt; (nM)</th>
<th>S1P4 EC&lt;sub&gt;50&lt;/sub&gt; (nM)</th>
<th>S1P5 EC&lt;sub&gt;50&lt;/sub&gt; (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>0.3</td>
<td>300</td>
<td>106</td>
<td>62</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Rat microsomes</th>
<th>Human microsomes (%) dgr.</th>
<th>H. hepatocytes Cl pred (ml/min/Kg)</th>
<th>Vss (l/Kg)</th>
<th>CI (ml/min/Kg)</th>
<th>t&lt;sub&gt;1/2&lt;/sub&gt; (h)</th>
<th>F (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Degr.</td>
<td>12%</td>
<td>8.2</td>
<td>2.0</td>
<td>16.6</td>
<td>1.8</td>
<td>50</td>
</tr>
</tbody>
</table>

Lymphopenia in Wistar rats

Efficacy in the EAE rat model

Rat microsomes

H. hepatocytes Cl pred (ml/min/Kg)

Vss (l/Kg)

Cl (ml/min/Kg)

t<sub>1/2</sub> (h)

F (%)
Compound 6 elimination pathways

1mg/kg iv single administration

Compound 6 is eliminated in rats mostly unaltered and/or as a taurine conjugate.

Amide hydrolisis is also observed but to a minor extent.
### Compound 6 close analogues rat PK profile

<table>
<thead>
<tr>
<th>Compound</th>
<th>S1P1 EC&lt;sub&gt;50&lt;/sub&gt; (nM)</th>
<th>S1P3 EC&lt;sub&gt;50&lt;/sub&gt; (nM)</th>
<th>Vss (l/Kg)</th>
<th>Cl (ml/min/Kg)</th>
<th>t&lt;sub&gt;1/2&lt;/sub&gt; (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>2.3</td>
<td>900</td>
<td>3</td>
<td>49</td>
<td>1.4</td>
</tr>
<tr>
<td>8</td>
<td>0.9</td>
<td>860</td>
<td>0.7</td>
<td>4.8</td>
<td>2.2</td>
</tr>
<tr>
<td>9</td>
<td>1.4</td>
<td>1300</td>
<td>1</td>
<td>11.6</td>
<td>1.6</td>
</tr>
<tr>
<td>10</td>
<td>44</td>
<td>24%E at 10μM</td>
<td>1.4</td>
<td>10</td>
<td>2.4</td>
</tr>
</tbody>
</table>
Fenoxycetyl derivatives

2

EC$_{50}$ S1P$_1$ = 9 μM

11

EC$_{50}$ S1P$_1$ = 11 nM
cLogD(pH7.4) = 5.6

cLogD(pH 7.4) = 2.8

Met R/H: 6% / 11%

$t_{1/2}$ rat = 4 h

12

EC$_{50}$ S1P$_1$ = 2.4 nM
EC$_{50}$ S1P$_3$ = 3.3 μM
cLogD(pH 7.4) = 2.2
Met R/H: 8% / <1%

$t_{1/2}$ rat = 1.6 h

13

EC$_{50}$ S1P$_1$ = 16 nM
EC$_{50}$ S1P$_3$ = 14 μM
cLogD(pH 7.4) = 2.8
Met R/H: 6% / 11%

$t_{1/2}$ rat = 4 h
Compound 13: PK profile in rat and dog

<table>
<thead>
<tr>
<th></th>
<th>AUC$_{0-\infty}$ (ng*h/ml)</th>
<th>Vss (l/kg)</th>
<th>Cl (ml/min/kg)</th>
<th>$t_{1/2}$ (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Compd. 13</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>2653</td>
<td>2.1</td>
<td>6.3</td>
<td>4</td>
</tr>
<tr>
<td>Dog</td>
<td>435</td>
<td>1.5</td>
<td>38</td>
<td>0.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Compd. 13</th>
<th>Human microsomes (% dgr.)</th>
<th>Plasma hydrolisis at 5h (%dgr.)</th>
<th>Hepatocytes pred Cl (ml/min/Kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>6</td>
<td>6%</td>
<td>6.3</td>
</tr>
<tr>
<td>Dog</td>
<td>16</td>
<td>6%</td>
<td>33</td>
</tr>
<tr>
<td>Human</td>
<td>12</td>
<td>2%</td>
<td>19</td>
</tr>
</tbody>
</table>

- **Compound 13** is strongly metabolized in both dog and human hepatocytes, being the amide hydrolisis the major metabolite.

- Calculated Cl in hepatocytes does correlate very well with the observed Cl in both dog and rat.
- A change of a F by a Cl in the benzamide moiety accounts for a compound that is much less metabolized in both human and dog hepatocytes.

- In dog hepatocytes amide hydrolisis is still the main metabolic pathway.

- In human hepatocytes no amide hydrolisis is seen. An oxidation metabolite is the major one.
Compd 14: Pharmacokinetic profile in two species

- Half life prediction to humans with two species indicates that the compound could likely be dosed once a day (22h expected half life).
**Compound 14: Pre-candidate profile**

<table>
<thead>
<tr>
<th>Compound</th>
<th>S1P1 EC50(nM)</th>
<th>S1P3 EC50(nM)</th>
<th>S1P4 EC50(nM)</th>
<th>S1P5 EC50(nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>5.1</td>
<td>4000</td>
<td>120</td>
<td>25</td>
</tr>
</tbody>
</table>

- Rat bradycardia <15% at 100 mg/Kg
- No ADME in vitro liabilities found (CYP450 inhibition nor Glutation adduct formation)
- No hERG activity observed

**Lymphopenia in rat and dog**

**Efficacy in the EAE rat model**

Vehicle

- Compound 14 po
- LAS189255 po
Mechanism of action: Chemotaxis Assay

Chemotaxis induced by S1P in Murine spleen lymphocytes_ Assay principle

A) Migration in presence of Compound

B) 30' Preincubation with Compnd

S1P like  
Fingo like
Chemotaxis assay results and translation *in vivo*

- Fingolimod interaction with S1P₁ induce receptor internalization and slow recycling.
- In Compound 14 the recycling is fast and continuous presence of the compound is needed for efficacy.
- Lymphopenic effect of compound 14 only depends on plasma levels and the time to recover from lymphopenia after treatment stop can be predicted from pharmacokinetics.
Summary and conclusions

- A novel structural class of S1P₁ agonists that do not require phosphorylation to be active has been identified.
- These compounds are potent agonists of S1P₁ and selective versus S1P₃.
- After an intensive optimization process Compound 14 has been identified as a promising pre-candidate suitable for once a day administration.
- Its mechanism of action suggests a direct PK/PD relationship.
The Medicinal Chemistry Team