ASS234: a new multipotent cholinesterase/monoamine oxidase inhibitor with antioxidant properties and anti-Aβ aggregating profile for the therapeutic use in Alzheimer's disease
Main Research Lines

Design, synthesis and biological evaluation of novel multipotent molecules as enhancers of the cholinergic and monoaminergic transmission for its therapeutical use in Parkinson and Alzheimer’s diseases.
Multidisciplinary Research Line: National collaboration

Biological evaluation

Universitat Autònoma de Barcelona

Irene Bolea
Mar Hernández
Elisenda Sanz
Montse Solé
Laura Fernández
Gerard Esteban
Ping Sun

Cristina Gutiérrez

Organic Chemistry synthesis

CSIC

Prof. José Luis Marco
Abdelouahid Samadi
Cristóbal de los Ríos

Modelling

UNIVERSITAT DE BARCELONA

Prof. F. Javier Luque
Jordi Juárez

SAF 2003-02725
SAF 2006-08764-C02-02
SAF 2009-07271
SAF 2012-33304
CENIT MET-DEV-FUN 2006
Multidisciplinary Research Line: International collaboration

Projects:

- COST CHEMISTRY GROUP, UE. **Working Group: D13/018/01** Title: New monoamine oxidase inhibitors as cytoprotective and neuroprotective drugs (2001-2005) (Six european universities involved)

- COST CHEMISTRY GROUP, UE, **Working group : D34/003/05**. **Title**: Molecular targeting and drug design and neurological and bacterial diseases. *(2005-2010)* (Eight european universities involved)

- COST CHEMISTRY GROUP, UE, **Working group : MC 1103**. **Title**: Structure -based drug design for diagnosis and treatment of neurological diseases: dissecting and modulating complex function in the monoamiergic systems of the brain (2011-2014)

20 European research groups, collaborating in a multidisciplinary framework: Synthesis, modelling, tridimensional structure studies, receptors binding, enzyme kinetics, animal models of neurological disorders etc..
AD: A MULTIFACTORIAL DISORDER

- Cholinergic dysfunction
- Metal dyshomeostasis
- Monoaminergic disturbances
- Mitochondrial dysfunction
- Oxidative stress
- Inflammation
- Glutamatergic dysfunction
- tau hyperphosphorilation
- Aβ aggregation

NEURONAL DEATH

Zn$^{2+}$, Mg$^{2+}$, Fe$^{2+}$, Cu$^{2+}$
# Novel drugs for the Alzheimer’s disease therapy

<table>
<thead>
<tr>
<th>Name</th>
<th>Company</th>
<th>Therapy/Target</th>
<th>Phase</th>
<th>Potential Launch</th>
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<tbody>
<tr>
<td><strong>Beta-Amyloid Focused Immunotherapy</strong></td>
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<tr>
<td><strong>Passive</strong></td>
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<td>Gammagard</td>
<td>Baxter</td>
<td>Intravenous Immunoglobulin (aimed at Beta Amyloid)</td>
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<td>Roche/AC Immune</td>
<td>Beta Amyloid Monoclonal Antibody</td>
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<td>Gantenerumab</td>
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<td>Beta Amyloid Monoclonal Antibody</td>
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<td><strong>Gamma Secretase Inhibitors</strong></td>
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<td>BMS-708163</td>
<td>BMY</td>
<td>Gamma Secretase Inhibitor - Beta Amyloid</td>
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<td>MK-8931</td>
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<td>AC1 91</td>
<td>AC Immune</td>
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<td>LY-2434074</td>
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<td>I</td>
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<td><strong>Amyloid Aggregation Inhibitor</strong></td>
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<td><strong>Nerve Growth</strong></td>
<td>Ceregene</td>
<td>Nerver Growth Factor</td>
<td>II</td>
<td>2017</td>
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</tbody>
</table>
Recent AD Trials: Mostly Negative Trials

Negative Phase III:

- Xaliproden (neuroprotection)
- Tramiprosa te (amyloid anti-aggregation)
- Tarenflurbil (gamma secretase inhibitor)
- Rosiglitazone (metabolic, ant-inflammatory)
- Leuprolide (endocrine)
- Dimebon (mitochondrial)
- Semagacestat (γ-secretase inhibitor)
Programa Cooperación Farma-Biotech
9º encuentro (4 de julio de 2013)

Recent AD Trials: Promising Targets

Phase III in progress:

Monoclonal anti-amyloid Aβ antibodies:
- Gantenezumab, Phase III
- Crenezumab, Phase III

Anti-Aβ aggregating
- PTB2, in progress (anti-Aβ aggregating)
- Souvenaid, approved 2013.
Cholinergic Hypothesis: AChE/BuChE inhibitors

1: Rivastigmine  
(AChE - BuChE)

2: Donepezil  
(AChE)

3: Galantamine  
(AChE - nAChR modulator)

4: Tacrine  
(AChE - BuChE)
MULTI-TARGET DIRECTED-LIGANDS (MTDLS)

ASS234

DRUG
Donepezil

MTDL

TARGET
AChE
ChEs
Aβ
MAOs

Therapeutic effect
limited
enhanced

Buccafusco & Terry (2000)
Youdim & Buccafusco (2005)
Cavalli et al (2008)
In AD, multiple neurotransmitter systems are affected

- Disturbances in other neurotransmitter systems have been found to account for AD symptoms
- An interplay between neurotransmitter systems is necessary to induce the loss of cognition in AD.

**COGNITIVE DECLINE**
- Loss of cholinergic, monoaminergic and glutamatergic neurons

**BEHAVIOURAL CHANGES**
- Serotonergic and noradrenergic systems

References:
Perry et al, (1999)
Francis et al, (1993)
Snyder et al, (2005)
MAO-B inhibition as potential target in AD

- **L-Deprenyl**
  - Knoll et al. (1965)
  - More potent than deprenyl

- **Rasagiline**
  - Finberg et al. (1965)
  - More selective than deprenyl
  - Does not produce amphetamine-like derivatives

**Collaboration with PRODESFARMA Laboratory.**
Title: Study of the involvement of indolalkylamine derivatives on neuroprotection and neuroregeneration of the toxic effects caused by the MPTP toxin.
(1995-1998) (Total cost: **179.593 euros**).
Neuroprotective effect of the monoamine oxidase inhibitor PF 9601N [N-(2-propynyl)-2-(5-benzoxo-indoly]-methylamine on rat nigral neurons after 6-hydroxydopamine-striatal lesion
Blanca Cutillas*, Santiago Ambrosio*, Mercedes Unzeta**

APK

Antioxidant properties of PF9601N, a novel MAO-B inhibitor: assessment of its ability to interact with reactive nitrogen species
Lydia Bellik1, Stefania Dragoni1, Federica Pessina1, Elisenda Sanz1, Mercedes Unzeta1 and Massimo Valoti1

Journal of Neurochemistry

Anti-apoptotic effect of MAO-B inhibitor PF9601N [N-(2-propynyl)-2-(5-benzoxo-indoly]-methylamine is mediated by p38 pathway inhibition in MPP+-treated SH-SY5Y human dopaminergic cells
Elisenda Sanz1, Albert Quintana1, Valentina Battaglia1, Antonio Tosinello1, Juan Hidalgo1, Santiago Ambrosio1, Massimo Valoti1, Jose Luis Marco, Keith F. Tipton*1 and Mercedes Unzeta1

PF9601N [N-(2-propynyl)-2-(5-benzoxo-indoly]-methylamine] attenuates MPTP-induced depletion of striatal dopamine levels in C57BL/6 mice
Vasili Perez, Mercedes Unzeta*

Research Article
Protective effect of N-(2-propynyl)-2-(5-benzoxo-indoly]-methylamine in PF9601N on mitochondrial permeability transition
V. Battaglia1, E. Sanz1, M. Sahib1, M. I. Unzeta1 and A. Tosinello1

Contents lists available at ScienceDirect
Molecular and Cellular Neuroscience

PF9601N [N-(2-propynyl)-2-(5-benzoxo-indoly]-methylamine] confers MAO-B independent neuroprotection in ER stress-induced cell death
Elisenda Sanz1, Albert Quintana1, Juan Hidalgo1, Jose Luis Marco1, Mercedes Unzeta*
Acetylcholinesterase

Monoamino Oxidase B

Dr Javier Luque (UB) Spain

Irene Bolea et al, J Med Chem, 2011, 54, 8251-8270
### ASS 234 inhibits MAO A and B and AChE and BuChE

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>IC₅₀ (nM)</th>
<th>Selectivity</th>
<th>IC₅₀ (µM)</th>
<th>Selectivity</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MAO-A</td>
<td>MAO-B</td>
<td>MAO-B/MAO-A</td>
<td>AChE</td>
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<tr>
<td>Donepezil</td>
<td><img src="image1.png" alt="Structure" /></td>
<td>854800 ± 13300</td>
<td>15400 ± 2200</td>
<td>0.02</td>
<td>0.0067 ± 0.0004</td>
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<tr>
<td>PF9601N</td>
<td><img src="image2.png" alt="Structure" /></td>
<td>1250 ± 15</td>
<td>22 ± 1</td>
<td>0.017</td>
<td>&gt;100</td>
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<tr>
<td>ASS200</td>
<td></td>
<td>82.2 ± 3.2</td>
<td>745.4 ± 19.9</td>
<td>9.1</td>
<td>0.31 ± 0.04</td>
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<tr>
<td>(n=1)</td>
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<tr>
<td>ASS188</td>
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<td>6.7 ± 1.8</td>
<td>129.6 ± 41.4</td>
<td>19.3</td>
<td>0.42 ± 0.04</td>
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<tr>
<td>(n=2)</td>
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<tr>
<td>ASS234</td>
<td></td>
<td>5.2 ± 1.1</td>
<td>43.1 ± 7.9</td>
<td>8.3</td>
<td>0.35 ± 0.01</td>
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<td>(n=3)</td>
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<tr>
<td>ASS251</td>
<td></td>
<td>10.5 ± 4.4</td>
<td>2774 ± 116</td>
<td>264.2</td>
<td>0.26 ± 0.07</td>
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<tr>
<td>(n=4)</td>
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</table>
ASS234 potently prevents self-induced Aβ1-42 aggregation

• ASS234 prevents the formation of toxic Aβ oligomers and fibers (WB analysis)

• ASS234 is able to interact with the PAS site on AChE, inhibiting the Aβ aggregation

IC50 47.8 ±2.1 %
ASS234 exerts antioxidant properties on PC12 cells

• ASS234 enhances the expression of SOD and Catalase, in SHSY5Y cells lesioned with Aβ1-42
ASS 234 has a neuroprotective effect on SHSY5Y cells lesioned by Aβ1-42

**A**

Control

Aβ1-42

Aβ1-42 + 5 μM ASS234

Aβ1-42 + 10 μM ASS234

**B**

LDH activity (%)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>Aβ1-42 (40 μM)</th>
<th>5</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aβ1-42 (40 μM)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>ASS234 (μM)</td>
<td>-</td>
<td>-</td>
<td>5</td>
<td>10</td>
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</tbody>
</table>

**C**

Control

Aβ1-42

Aβ1-42 + 5 μM ASS234

Aβ1-42 + 10 μM ASS234

**D**

Condensed Nuclei (%)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>Aβ1-42 (40 μM)</th>
<th>5</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aβ1-42 (40 μM)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>ASS234 (μM)</td>
<td>-</td>
<td>-</td>
<td>5</td>
<td>10</td>
</tr>
</tbody>
</table>

•ASS234 shows an antiapoptotic effect on human neuroblastoma cells SHSY5Y, inhibiting the Caspase 3 - cleavage via the mitochondrial pathway
Metabolism of ASS 234 by human Cytochrome P450

• ASS 234 is not a CYP3A4 and CYP1A substrate

• ASS 234 is not an human CYPs inhibitor

• The only metabolite detected is that resulted from the n-depropargylation process

Valoti M, Faculty of Pharmacy, University of Siena (Italy), COST Project UE, Working group : MC 1103
Conclusions of “in vitro” analysis of ASS234

- **ASS234** is able to enhance cholinergic and monoaminergic neurotransmision

- **ASS234** shows anti **Aβ**$_{1-42}$ self-aggregation and anti-aggregation in AChE presence

- **ASS234** shows an antioxidant profile, and enhances catalase and SOD1 expression

- **ASS234** has a neuroprotective and antiapoptotic effect on human neuroblastomes lesioned with **Aβ**$_{1-42}$

- **ASS 234** shows a more potent profile than the donepezilo

---

Bolea I et al., *J Med Chem (2011)54,8251-8270]*
Bolea et al, *Accepted for publication in Current Alzheimer’s Research, 2013*
In vivo analysis of ASS234

• The experimental model of vascular dementia (BCCAO)
  
  • W. Agnieszka Fogel, Medical University of Lodz, Poland. COST Project UE, Working group: MC 1103)

• The experimental model of Scopolamine-induced short-term memory deficit in mice
  
  • Ricardo Martinez Murillo Neurovascular Research Group, Cellular and Developmental Neurobiology, Cajal Institute, Madrid, Spain
Conclusions of “in vivo” analysis of ASS234

- ASS234 enhances cholinergic and aminergic neurotransmission and has a positive effect on the holeboard memory tests in a rat model of vascular dementia.

- ASS234, significantly lowers scopolamine-induced learning deficits in healthy adult C57/Bl6 mice by Object Recognition Task (ORT).

- Toxicity analysis: the cell viability in HepG2 cells at 1mM is of 75.7±1.14% for ASS234 and 34.4±2.73% for Tacrine.

- ASS234 has a low molecular weight, is easy to synthesize, soluble in water and crosses the BBB.
ASS234: A PROMISING MTDL (PCT/ES2011/070186)

ASS-234 is able to modulate several mechanisms relevant to AD

- ChEs inhibition (CAS)
- Inhibition of AChE-dependent Aβ aggregation (PAS)
- MAO inhibition
- Antiaggregating
- Antioxidant
- Antiapoptotic
- Monoaminergic / cholinergic neurotransmission enhancer
- Antidepressant
- Neuroprotective
Programa Cooperación Farma-Biotech
9º encuentro (4 de julio de 2013)

Patents:

• New derivatives of propargylamine having neuroprotective capacity for the treatment of Alzheimer and Parkinson’s disease.

  • Spain ref: PCT/ES2011/070186 extended to Europe and USA.
  • Priority Date: 18/3/2010

• DHP hybrids as Multi-Target-Directed Drugs for the Treatment of Alzheimer’s Disease:

  • Spain ref: T-2012/003
  • Europe ref: EP12190483.3
  • Japan ref: 2012-239025; PCT/ES2011/070186; WO 2011/ 113988
Further Steps

1. Acute Effect of ASS234 on a rat model stereotaxically lesioned with AB\textsubscript{25-35}

2. "\textit{In vivo}" evaluation of ASS234 as cognitive enhancer in mouse models of AD (APP/PS1 transgenic) and of scopolamine-induced cognitive deficits, subjected to object recognition, radial-maze and water maze performance tests.

3. Toxicological analysis:
   a) Toxicity \textit{in vitro} assays
   b) Toxicity \textit{in vivo} assays
   c) Safety Pharmacology

4. Pharmacokinetic studies and bioavailability
Partnering Opportunities

We are looking for a Pharmaceutical Company interested in patent licensing and to develop the new multitarget molecule ASS234 through the preclinical and clinical stages.
Thank you very much for your attention