Programa Cooperación Farma-Biotech

9º encuentro (4 de julio de 2013)

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

Barcelona, 4 de julio de 2013

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.

Multidisciplinar Research Line: National collaboration

Biological evaluation

Universitat Autònoma de Barcelona

Irene Bolea

Mar Hernández

Elisenda Sanz

Montse Solé

Laura Fernández

Gerard Esteban

Ping Sun

INc Institut de Neurociències

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

GOBIERNO DE ESPAÑA MINISTERIO DE CIENCIA E INNOVACIÓN

SAF 2003-02725

SAF 2006-08764-C02-02

SAF 2009-07271

SAF 2012-33304

CENIT MET-DEV-FUN 2006

Multidisciplinar 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 multidisciplinar framework : Synthesis, modelling, tridimensional structure studies, receptors binding, enzyme kinetics, animal models of neurological disorders etc,.

GOBIERNO DE ESPAÑA MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD

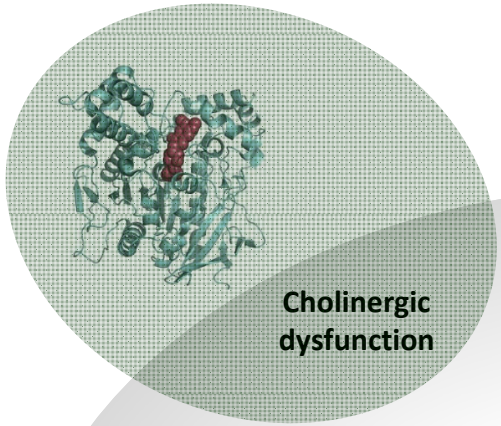
MEDICAMENTOS INNOVADORES Plataforma Tecnológica Española

UAB Universitat Autònoma de Barcelona

INCs Institut de Neurociències

farmaindustria

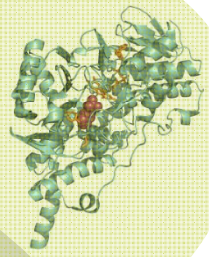
AD: A MULTIFACTORIAL DISORDER



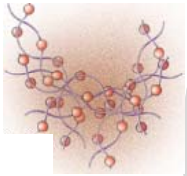
Cholinergic dysfunction

Zn^{2+} Mg^{2+} Fe^{2+} Cu^{2+}

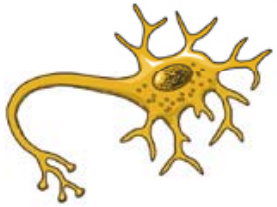
Metal dyshomeostasis



Monoaminergic disturbances

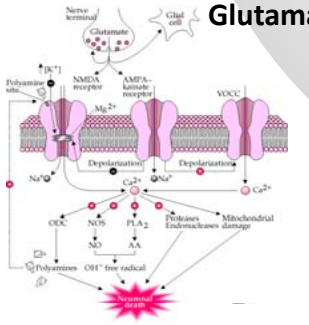


tau hyperphosphorilation



NEURONAL DEATH

Mitochondrial dysfunction

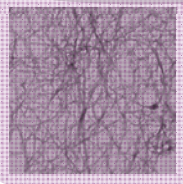


Glutamatergic dysfunction

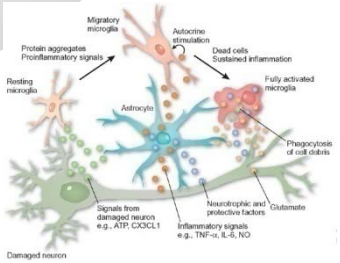
$OH^{\cdot}$ $^{\cdot}O_2^{-}$ $ONOO^{-}$

Oxidative stress

Aβ aggregation



Inflammation



Novel drugs for the Alzheimer´s disease therapy

Name	Company	Therapy/Target	Phase	Potential Launch
- Beta-Amyloid Focused Immunotherapy				
Passive				
Gammagard	Baxter	Intravenous Immunoglobulin (aimed at Beta Amyloid)	III	2015
RG7412	Roche/AC Immune	Beta Amyloid Monoclonal Antibody	II	2017
Gantenerumab	Roche/Morphosys	Beta Amyloid Monoclonal Antibody	II	2019
Active				
ACC-001	PFE/JNJ/ELN	Active Immunotherapy - Beta Amyloid	II	2016
CAD106	NVS/Cytos	Active Immunotherapy - Beta Amyloid	II	2016
AD02	Affiris/GSK	Active Immunotherapy - Beta Amyloid	II	2017
- Gamma Secretase Inhibitors				
BMS-708163	BMY	Gamma Secretase Inhibitor - Beta Amyloid	II	2016
- Beta Secretase Inhibitors				
MK-8931	Merck	Beta Secretase Inhibitor	II	2018
AC1 91	AC Immune	Beta Secretase Inhibitor	II	2018
LY-2434074	Eli Lilly	Beta Secretase Inhibitor	I	-
E2609	Eisai	Beta Secretase Inhibitor	I	-
- Amyloid Aggregation Inhibitor				
ELND005	Elan/Transition	Amyloid Aggregation Inhibitor	II	2016
PBT2	Prana Biotechnology	Metal Ion Therapy/Amyloid Aggregation Inhibitor	II	2017
- Nerve Growth				
CERE-110	Ceregene	Nerver Growth Factor	II	2017

Recent AD Trials: Mostly Negative Trials

Negative Phase III:

- Xaliproden (neuroprotection)
- Tramiprosate (amyloid anti-aggregation)
- Tarenflurbil (gamma secretase inhibitor)
- Rosiglitazone (metabolic, ant-inflammatory)
- Leuprolide (endocrine)
- Dimebon (mitochondrial)
- Semagacestat (γ-secretase inhibitor)

Recent AD Trials: Promising Targets

Phase III in progress:

Monoclonal anti-amyloid Aß antibodies:

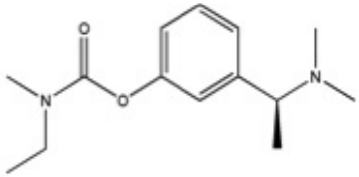
- Gantenezumab ,Phase III

  Crenezumab, Phase III

Anti –Aß aggregating

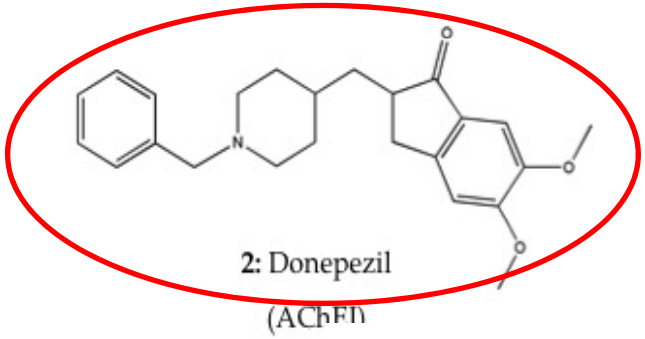
- PTB2, in progres (anti –Aß aggregating)
- Souvenaid, aproved 2013.

Cholinergic Hypothesis: AChE/BuChE inhibitors



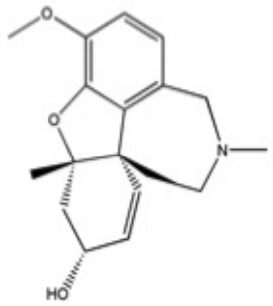
1: Rivastigmine

(AChEI - BuChEI)



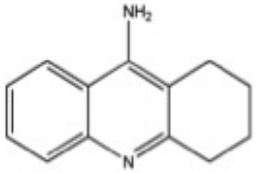
2: Donepezil

(AChEI)



3: Galantamine

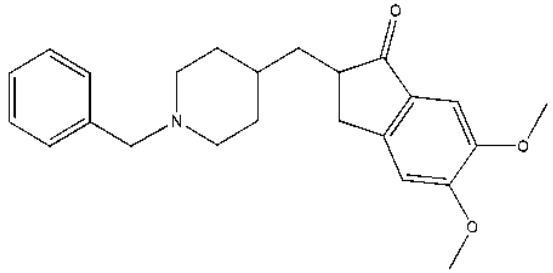
(AChEI - nAChR modulator)

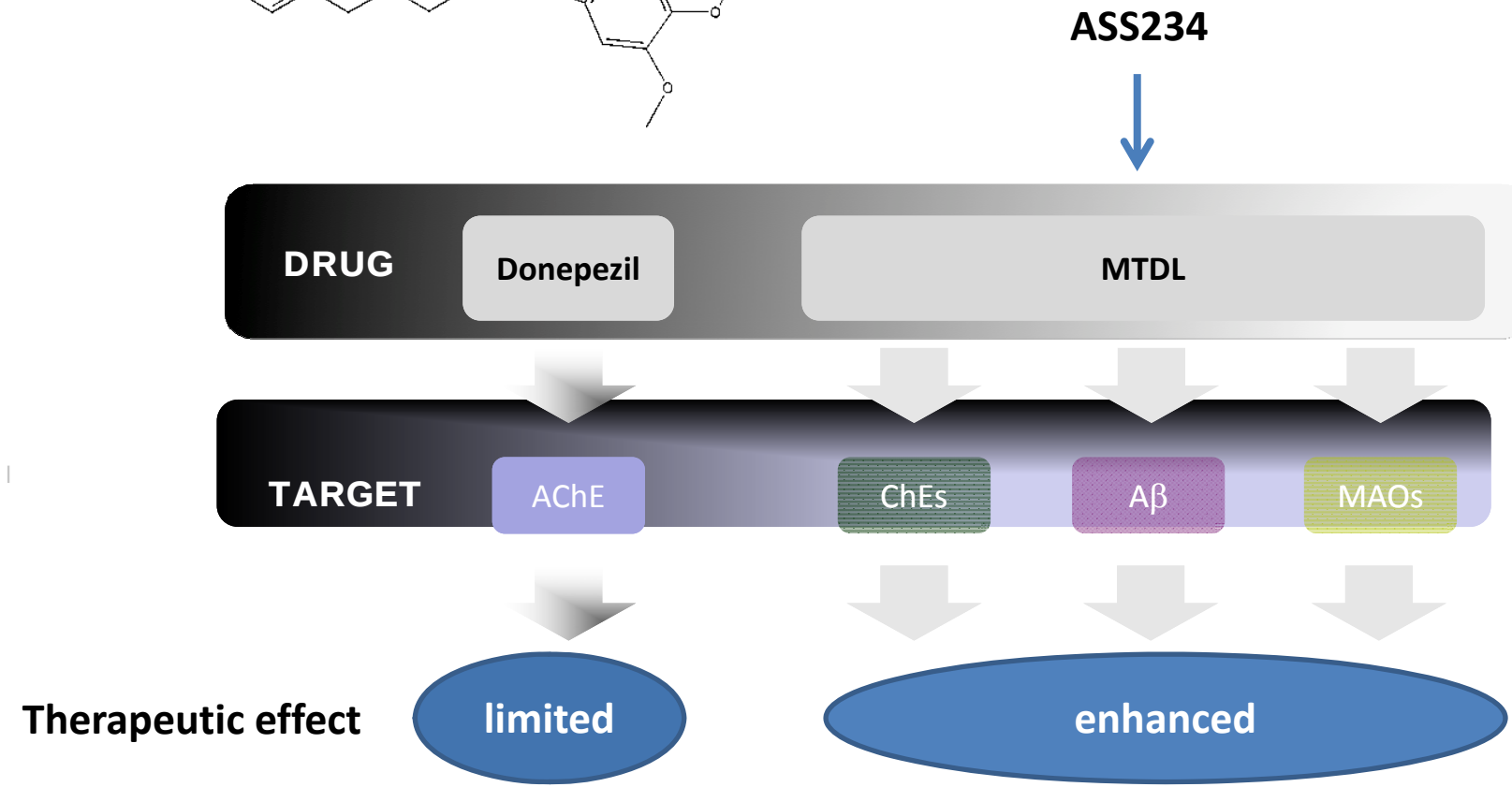


4: Tacrine

(AChEI - BuChEI)

MULTI-TARGET DIRECTED-LIGANDS (MTDLS)





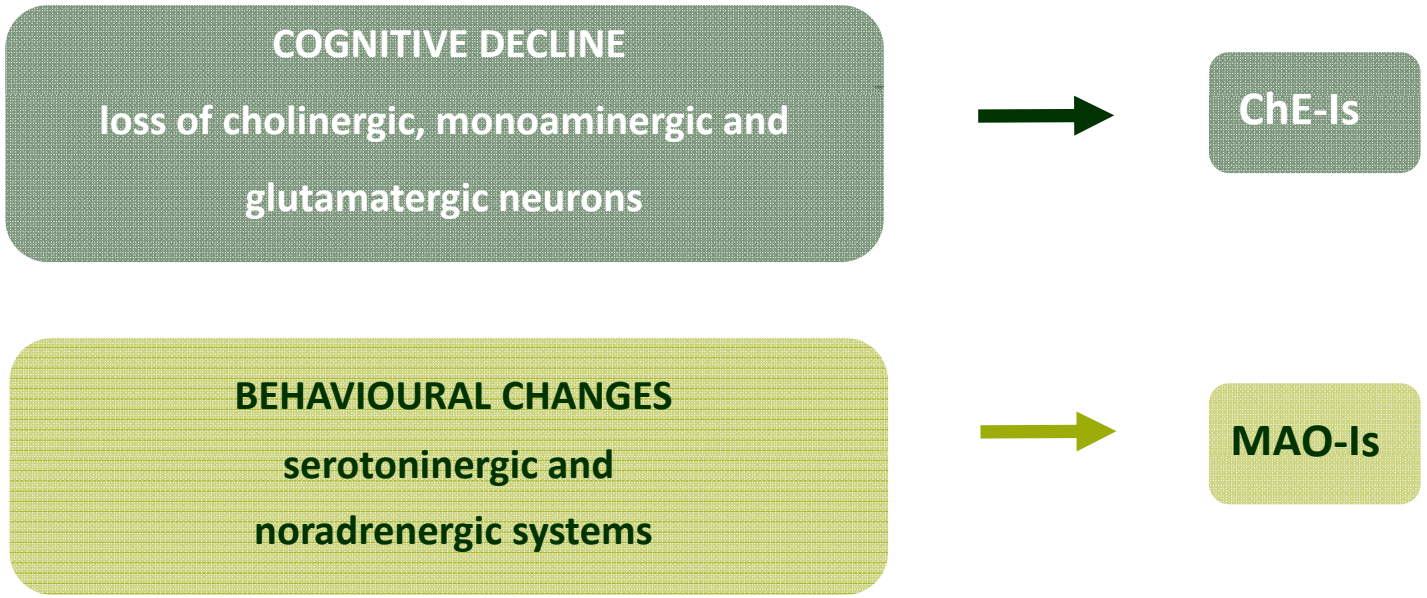
Buccafusco & Terry (2000)

Youdim & Buccafusco (2005)

Cavalli et al (2008)

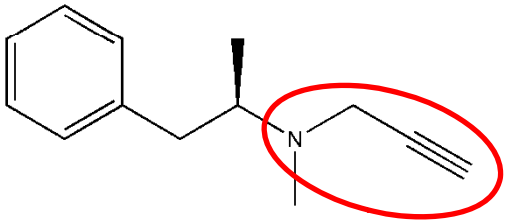
In AD, multiple neurotransmitter systems are affected

- 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.



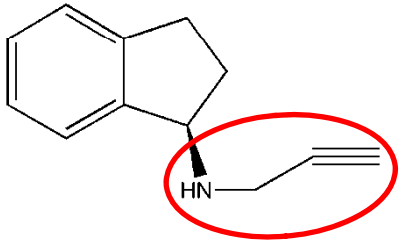
Perry et al, (1999)
Francis et al, (1993)
Ballard et al, (2008)
Snyder et al, (2005)

MAO-B inhibition as potential target in AD



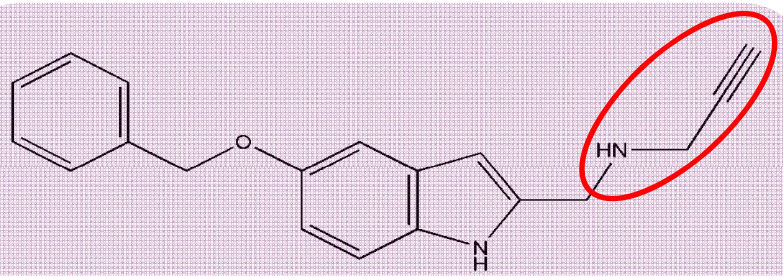
L-Deprenyl

Knoll *et al*, (1965)



Rasagiline

Finberg *et al*, (1965)



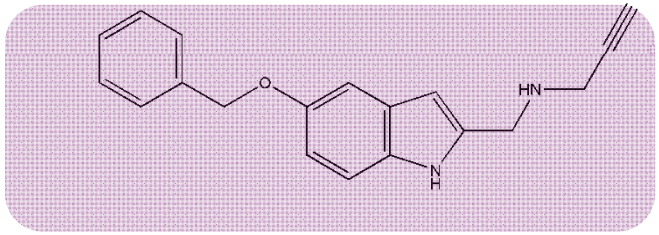
PF9601N

Pérez *et al*, (1999)

- More potent than deprenyl
- 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).*

PF9601N



ELSEVIER

Neuroscience Letters 329 (2002) 165–168

Neuroscience Letters

www.elsevier.com/locate/neulet

Neuroprotective effect of the monoamine oxidase inhibitor PF 9601N [*N*-(2-propynyl)-2-(5-benzyloxy-indolyl) methylamine] on rat nigral neurons after 6-hydroxydopamine-striatal lesion

Blanca Cutillas[a], Santiago Ambrosio[a], Mercedes Unzeta[b,*]

ELSEVIER

Neurochemistry International 42 (2003) 221–229

NEUROCHEMISTRY International

www.elsevier.com/locate/neuint

PF 9601N [*N*-(2-propynyl)-2-(5-benzyloxy-indolyl) methylamine], a new MAO-B inhibitor, attenuates MPTP-induced depletion of striatal dopamine levels in C57/BL 6 mice

Virgili Perez, Mercedes Unzeta*

ACTA ABP BIOCHIMICA POLONICA

Vol. 57, No. 2/2010
235–239
on-line at: www.actabp.pl

Regular paper

Antioxidant properties of PF9601N, a novel MAO-B inhibitor: assessment of its ability to interact with reactive nitrogen species

Lydia Bellik[1], Stefania Dragoni[1], Federica Pessina[1], Elisenda Sanz[2], Mercedes Unzeta[2] and Massimo Valoti[1]

Cell. Mol. Life Sci. 63 (2006) 1440–1448
1420-682X/06/121440-9
DOI 10.1007/s00018-006-6105-8
© Birkhäuser Verlag, Basel, 2006

Cellular and Molecular Life Sciences

Research Article

Protective effect of *N*-(2-propynyl)-2-(5-benzyloxy-indolyl) methylamine (PF9601N) on mitochondrial permeability transition

V. Battaglia[a], E. Sanz[b], M. Salvi[a], M. Unzeta[b] and A. Toninello[a,*]

Journal of Neurochemistry JNC

JOURNAL OF NEUROCHEMISTRY | 2008 | 105 | 2404–2417 doi: 10.1111/j.1471-4159.2008.05326.x

Anti-apoptotic effect of MAO-B inhibitor PF9601N [*N*-(2-propynyl)-2-(5-benzyloxy-indolyl) methylamine] is mediated by p53 pathway inhibition in MPP^+-treated SH-SY5Y human dopaminergic cells

Elisenda Sanz,* Albert Quintana,† Valentina Battaglia,‡ Antonio Toninello,‡ Juan Hidalgo,† Santiago Ambrosio,§ Massimo Valoti,¶ Jose Luis Marco,** Keith F. Tipton†† and Mercedes Unzeta*

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Molecular and Cellular Neuroscience

journal homepage: www.elsevier.com/locate/ymcne

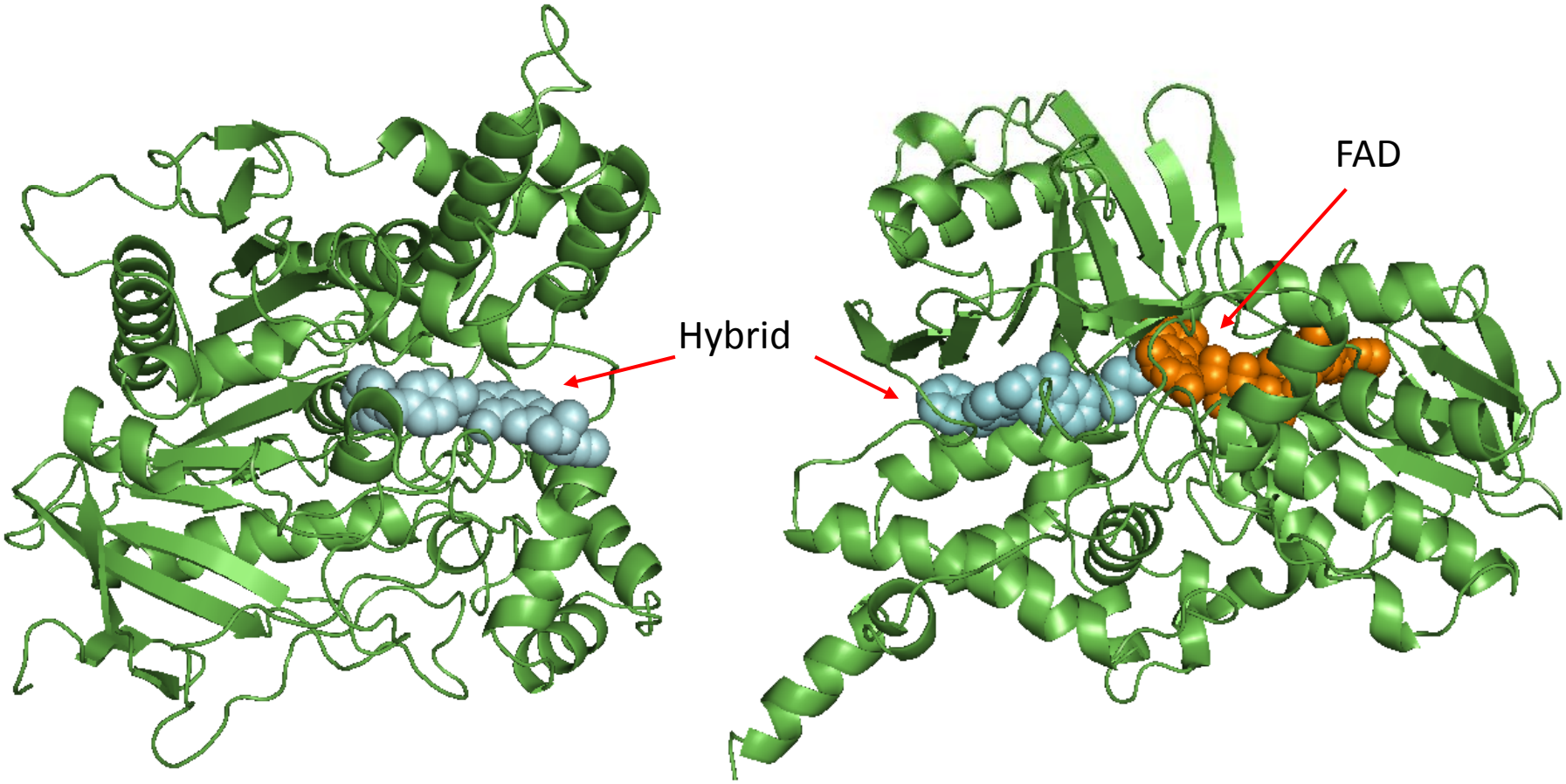
MCN

PF9601N [N-(2-propynyl)-2-(5-benzyloxy-indolyl) methylamine] confers MAO-B independent neuroprotection in ER stress-induced cell death

Elisenda Sanz[a,*], Albert Quintana[b], Juan Hidalgo[b], Jose Luis Marco[c], Mercedes Unzeta[a]

Acetylcholinesterase

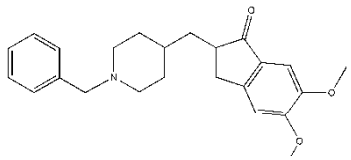
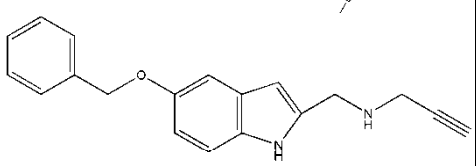
Monoamino Oxidase B



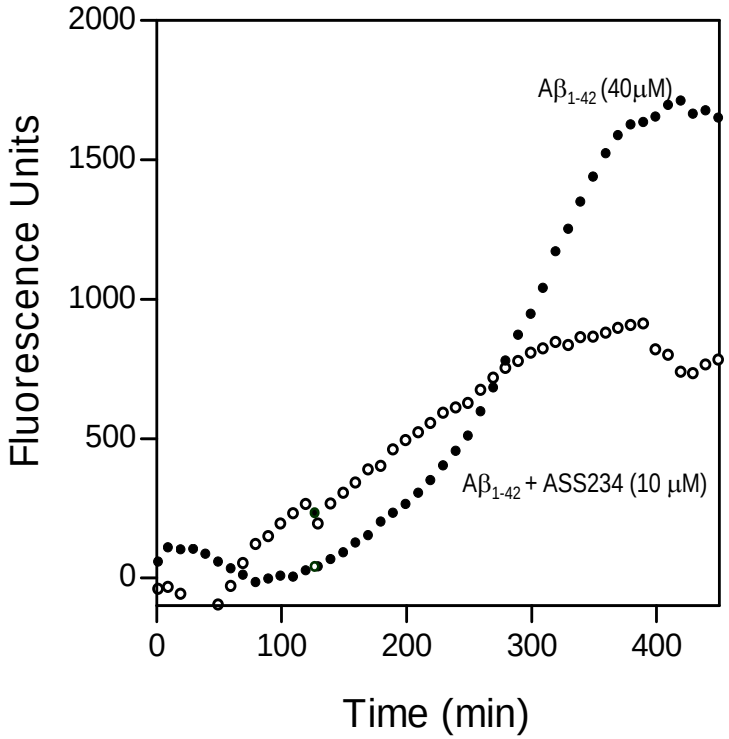
Dr Javier Luque (UB) Spain

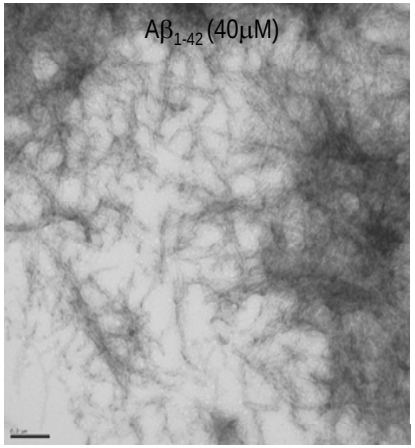
Irene Bolea et al, J Med Chem,2011,54,8251-8270

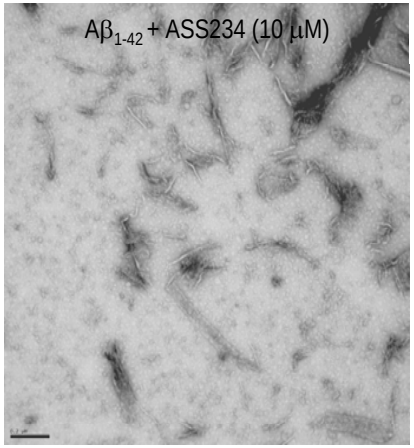
ASS 234 inhibits MAO A and B and AChE and BuChE

Compound	Structure	IC_{50} (nM) MAO-A	IC_{50} (nM) MAO-B	Selectivity MAO-B/MAO-A	IC_{50} (μM) AChE	IC_{50} (μM) BuChE	Selectivity BuChE/AChE
Donepezil		854800 ± 13300	15400 ± 2200	0.02	0.0067± 0.0004	7.4 ± 0.10	1100
PF9601N (FA73)		1250 ± 15	22 ± 1	0.017	>100	43 ± 3	>0.43
ASS200 (n=1)		82.2 ± 3.2	745.4 ± 19.9	9.1	0.31 ± 0.04	1.1 ± 0.2	3.5
ASS188 (n=2)		6.7 ± 1.8	129.6 ± 41.4	19.3	0.42 ± 0.04	2.1 ± 0.2	5.0
ASS234 (n=3)		5.2 ± 1.1	43.1 ± 7.9	8.3	0.35 ± 0.01	0.46 ± 0.06	1.3
ASS251 (n=4)		10.5 ± 4.4	2774 ± 116	264.2	0.26 ± 0.07	0.99 ± 0.08	3.8

ASS234 potently prevents self-induced *AB1-42* aggregation



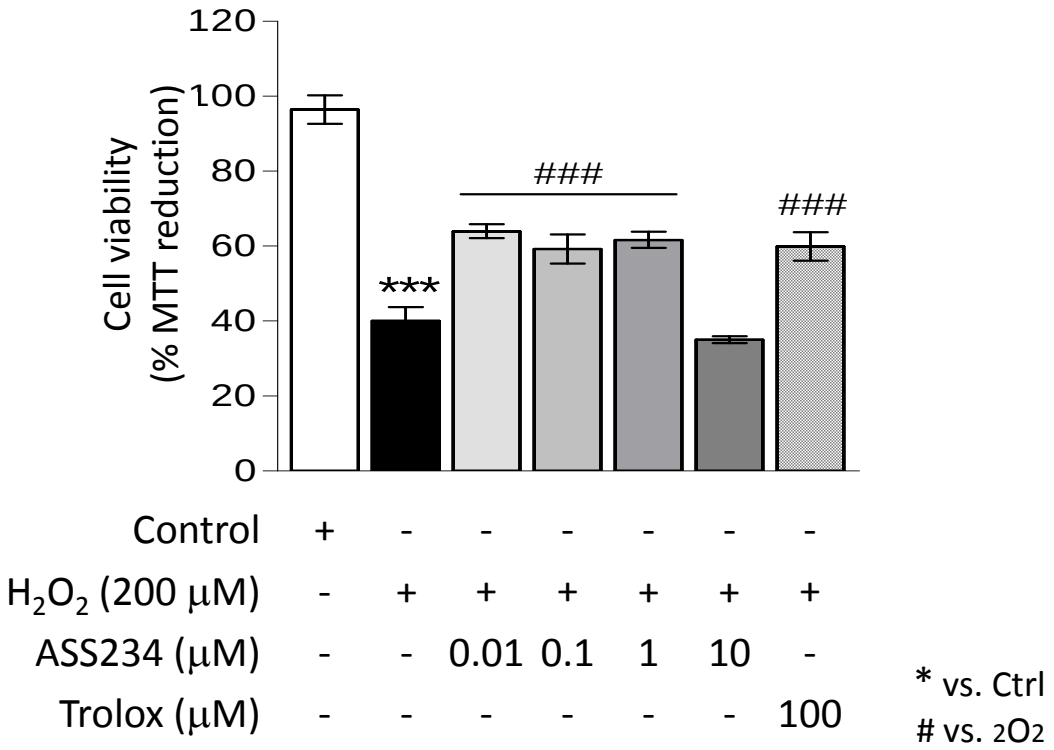




IC50 47.8 ±2.1 %

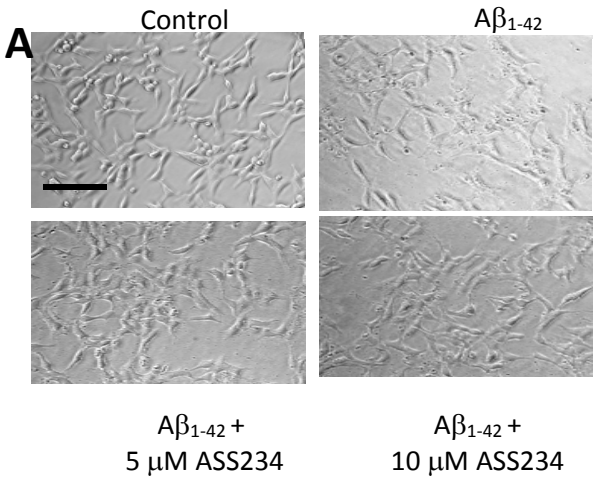
- ASS234 prevents the formation of toxic Aß oligomers and fibers (WB analysis)
- ASS234 is able to interact with the PAS site on AChE, inhibiting the Aß aggregation

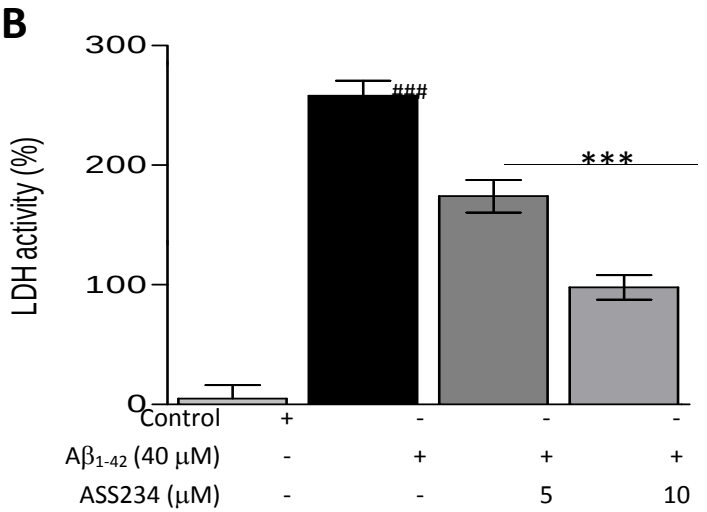
ASS234 exerts antioxidant properties on PC12 cells

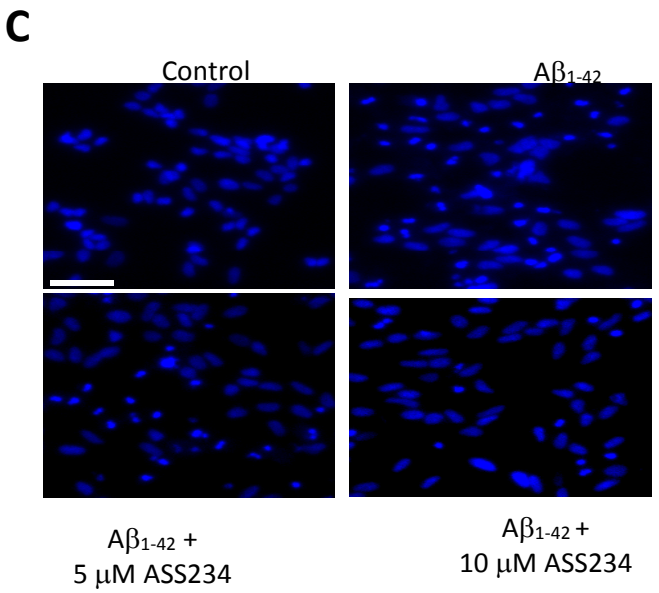


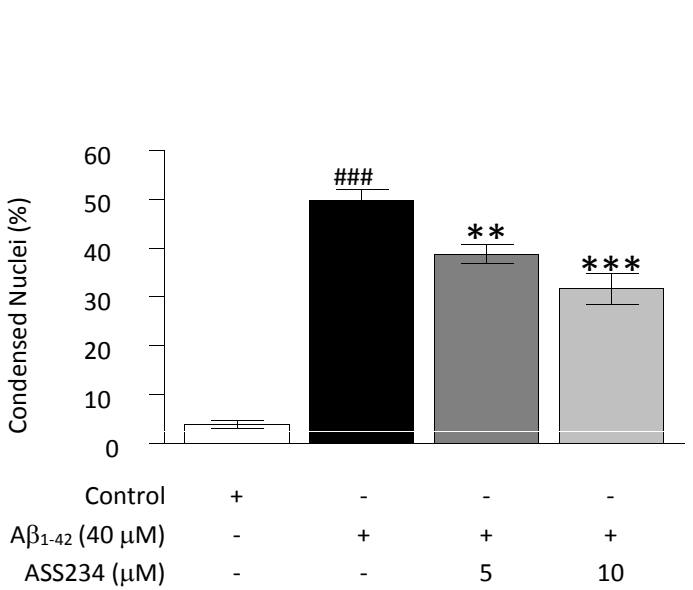
•ASS234 enhances the expression of SOD and Catalase, in SHSY5Y cells lesioned with Aß1-42

ASS 234 has a neuroprotective effect on SHSY5Y cells lesioned by Aβ1-42









•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
- ASS234 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 $A\beta_{1-42}$ 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 $A\beta_{1-42}$
- ASS 234 shows a more potent profile than the donepezilo

Samadi et al.,*Eur J Med Chem. 2012 Jun;52:251-62*
Bolea I et al., *J Med Chem (2011)54,8251-8270]*
Hadjipavlou-Litina D et al., *Eur J Med Chemistry (2013) 63:670-674]*
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

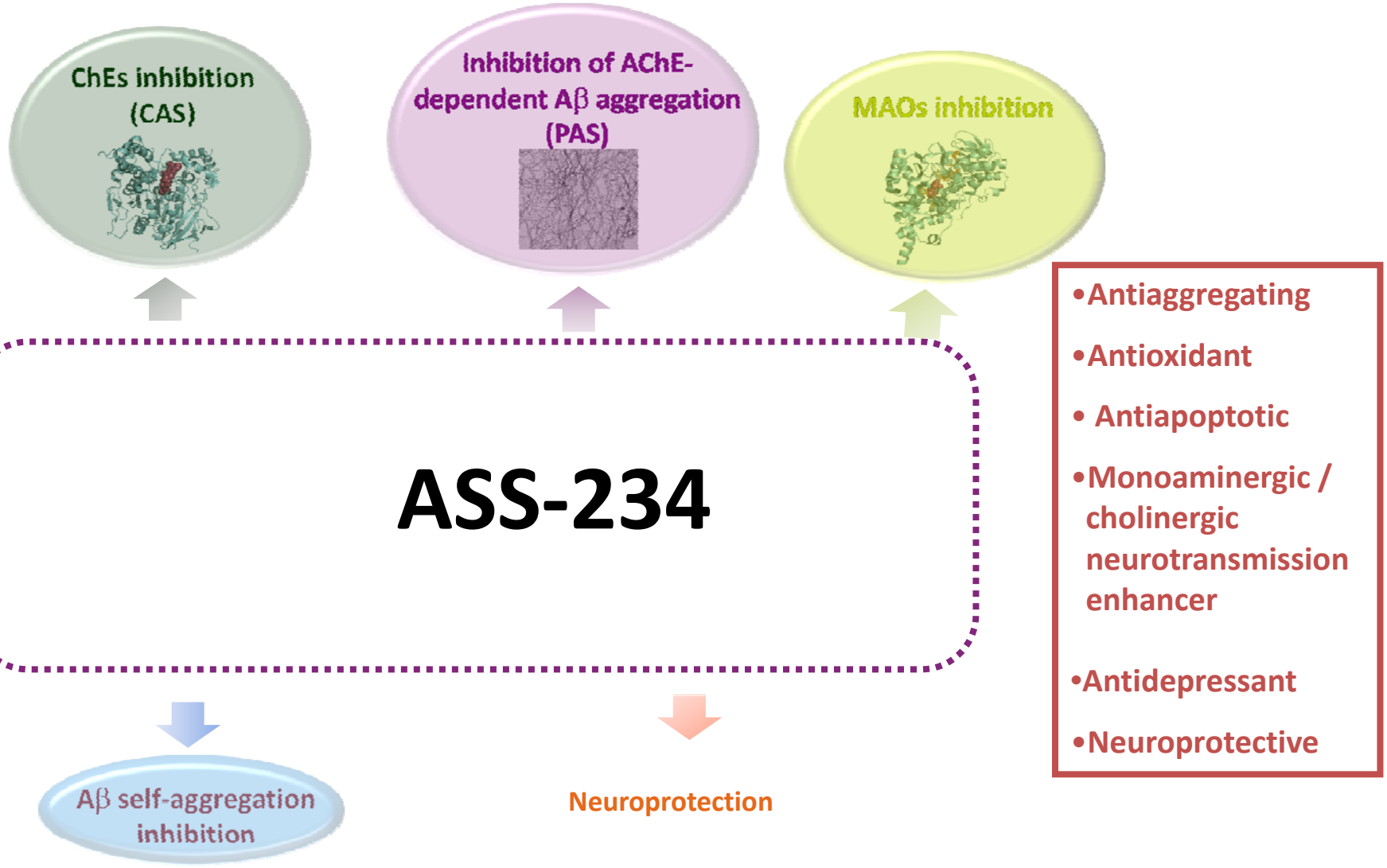
- ASS234 enhances cholinergic and aminergic neurotransmission and has a positive effect on the holeboard memory tests in a rat model of vascular dementia

- 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)



ASS234 is able to modulate several mechanisms relevant to AD

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 Aß $_{25-35}$
2. "*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 *in vitro* assays
   b) Toxicity *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