New non-L-subtypes Calcium channels blockers for applications on Nervous System Pathologies

Barcelona, October 20th, 2015

GOBIERNO DE ESPAÑA
MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD
MEDICAMENTOS INNOVADORES
Plataforma Tecnológica Española
farmaindustria

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The Health Research Institute, La Princesa Hospital Madrid

UAM UNIVERSIDAD AUTONOMA DE MADRID

ith Instituto Teófilo Hernando

Hospital Universitario de La Princesa

Universidad Autónoma de Madrid

INSTITUTO DE INVESTIGACIÓN SANITARIA HOSPITAL UNIVERSITARIO DE LA PRINCESA

TECHNOLOGICAL PLATFORMS AT LA PRINCESA

- Unit of Methods
- Confocal Microscope
- HPLC/Mass spectrometry
- Biobank
- Clinical trials unit
- Innovation Platform

TECHNOLOGICAL PLATFORMS AT UAM

- Animal House
- Interdepartamental Research Service (NMR, HPLC, IR, X-ray…)

ITH-5
Medicinal Chemistry and Pharmacological Screening

Chemical Library at ITH: 800 compounds

2008 (First Medicinal Chemistry Laboratory in a School of Medicine in Spain)

Medicinal Chemistry at ITH/IIS

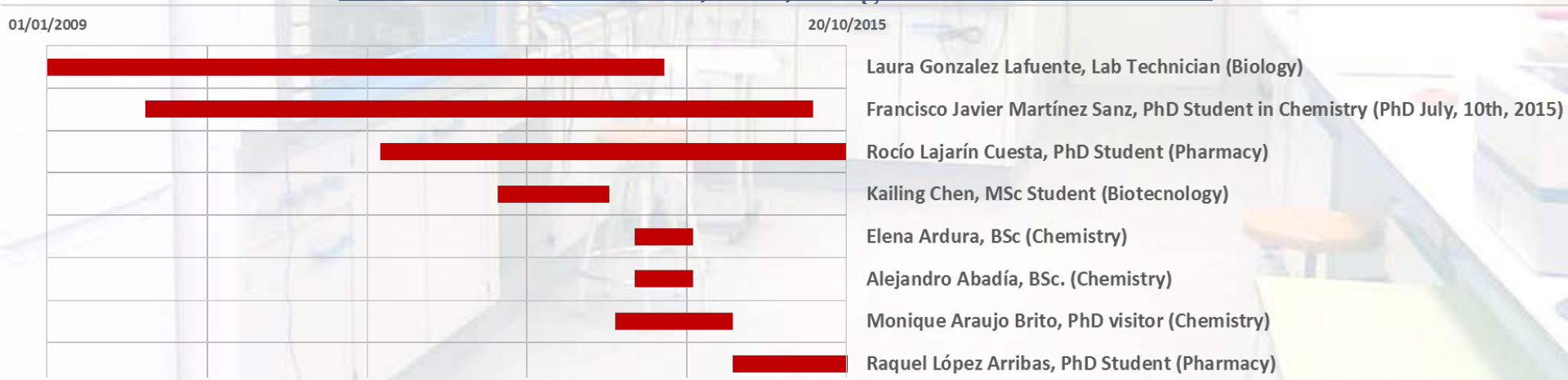
IIS Area 2. Traslational Neuroscience.

Line 1. Neuropharmacology and Neuroprotection.

Coordinator: Prof. Antonio G. García, M.D., Ph.D.

Research Group of Medicinal Chemistry and Pharmacological Screening

P.I. Cristóbal de los Ríos, PhD, "Miguel Servet" Researcher



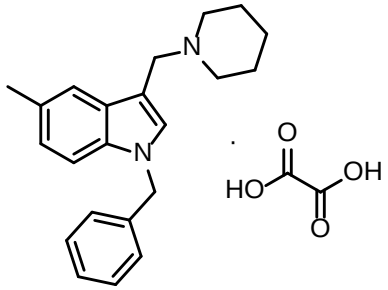
Research Lines at Cris' Group

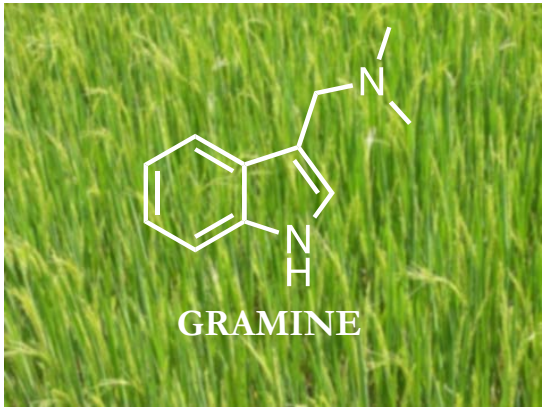
- Protein phosphatase 2A as therapeutic target for the treatment of neurodegenerative diseases and cancer

- Innovative approaches in the Cell Calcium signal as therapeutic target

  - CALHM1 (Moreno, A.J. et al., *Neuropharmacology,* 2015)
  - mNCX (Martínez-Sanz, F.J. et al., *ACS Chem. Neurosci.* 2015)
  - **Non-L-VDCC (Lajarín-Cuesta et al., P201500354, May, 15th, 2015)**

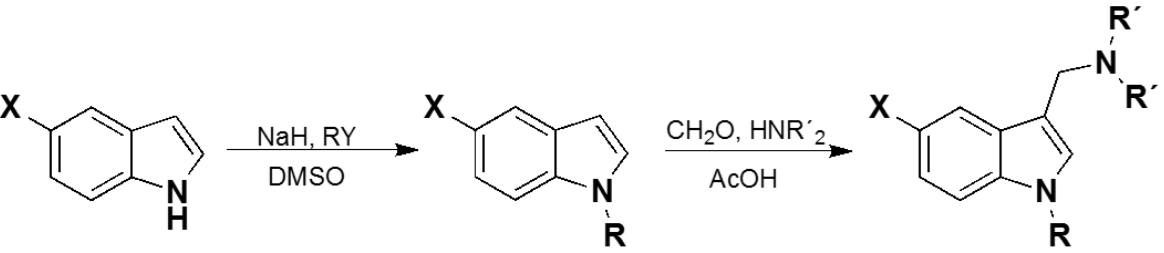
The Product: The gramine analogue ITH12657

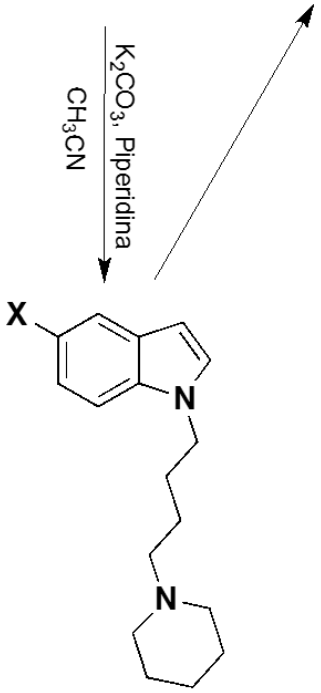




- Indole alkaloid produced by plants
- Some derivatives block voltage-dependent Ca^{2+} channels (Iwata et al, *Eur. J. Pharmacol.* 2001)
- Gramine protected neuroblastoma cells against several toxic stimuli (*not published results*)

Our Gramine analogues are easily-prepared under multigram-scalable chemical procedures





Compound	X	R'	R
Ia	Br	CH_3	CH_2Ph
Ib	Br	$-(CH_2)_5-$	CH_2Ph
Ic	Br	CH_3	$CH_2CH_2CH_2CH_3$
Id	Br	$-(CH_2)_5-$	$CH_2CH_2CH_2CH_3$
Ie	Br	CH_3	$(CH_2)_3COOCH_2CH_3$
If	Br	$-(CH_2)_5-$	$(CH_2)_3COOCH_2CH_3$
Ig	Br	$-(CH_2)_5-$	$CH_2CH_2CH_2CH_2Cl$
Ih	Br	CH_3	$CH_2C\equiv CH$
Ii	Br	$-(CH_2)_5-$	$CH_2C\equiv CH$
Ij	Br	CH_3	$CH_2(CH_2)_3$-N
Ik	Br	$-(CH_2)_5-$	$CH_2(CH_2)_3$-N
Il	CH_3	CH_3	CH_2Ph
Im (ITH12657)	CH_3	$-(CH_2)_5-$	CH_2Ph
In	CH_3	$-(CH_2)_5-$	$CH_2CH_2CH_2CH_3$
Io	CH_3	$-(CH_2)_5-$	$CH_2CH_2CH_2CH_2Cl$
Ip	CH_3	CH_3	$CH_2C\equiv CH$
Iq	CH_3	$-(CH_2)_5-$	$CH_2C\equiv CH$
Ir	CH_3	CH_3	$CH_2(CH_2)_3$-N
Is	CH_3	$-(CH_2)_5-$	$CH_2(CH_2)_3$-N

Gramine analogues protected against various in vitro models of neuronal damage

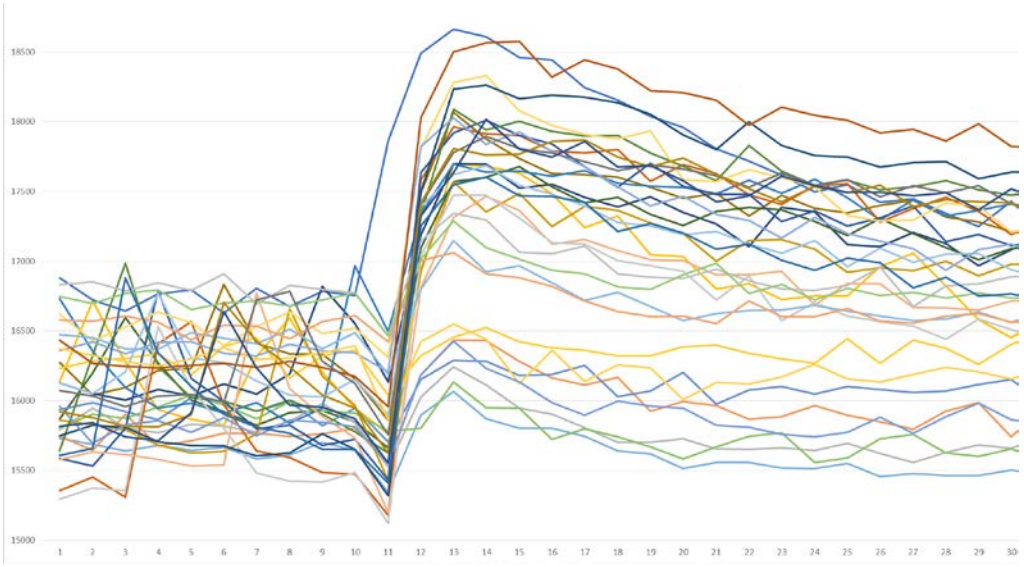
- **Cell viability measured by the method of the MTT reduction**

- **Neuronal models: SH-SY5Y neuroblastoma cells, embrionary rat cortical neurons, rat hippocampal slices**

- **Toxic stimuli:**
  - Tau hyperphosphorylation (in SH-SY5Y cells, okadaic acid): 70% of the compounds protected (**ITH12657**, 47%)
  - Ca^{2+} overload
    - In embryonary rat cortical neurons (Veratridine): 92% of the compounds protected (**ITH12657,** 52%)
    - In rat hippocampal slices (Glutamate): 78% of the compounds protected (**ITH12657,** 46%)
  - Oxidative stress (in SH-SY5Y cells, rotenone plus oligomycin A): 15% of the compounds protected (**ITH12657,** 20%)

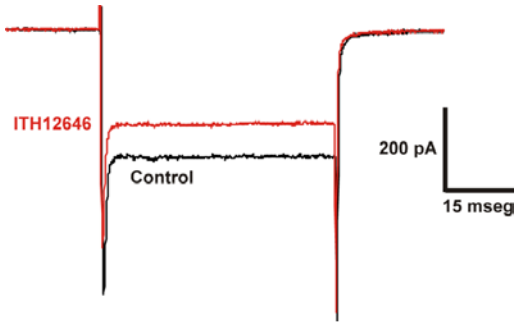
Gramine analogues reduced Cell Ca^{2+} increase exerted by depolarization

- SH-SY5Y neuroblastoma cells stimulated with 70 mM of K^+, measured with the fluorescent dye Fluo-4/AM



	% Blockade
Ia	38
Ib	45
Ic	-
Id	63
Ie	-
If	16
Ig	57
Ih	-
Ii	42
Ij	-
Ik	18
Il	17
Im	**26**
In	21
Io	35
Ip	-
Iq	30
Ir	-
Is	-

Selected derivatives reduced Ca^{2+} currents induced by depolarizing pulses



- Bovine chromaffin cells rested at -80 mV and subjected to 0 mV pulses under the whole-cell configuration patch-clamp experiments

Compound	X	R_1,R_2	R_3	% Blockade
Ia	Br	$-CH_3$	CH_2Ph	9
Ib	Br	$-(CH_2)_5-$	Ph	33
Id	Br	$-(CH_2)_5-$	$CH_2CH_2CH_3$	15
Ig	Br	$-(CH_2)_5-$	$CH_2CH_2CH_2CH_2Cl$	10
Ih	Br	$-CH_2C{\equiv}CH$	$CH_2CH_2CH_2CH_2Cl$	10
Im (ITH12657)	**CH_3**	**$-(CH_2)_5-$**	**Ph**	**40**
In	CH_3	$-(CH_2)_5-$	$CH_2CH_2CH_3$	20

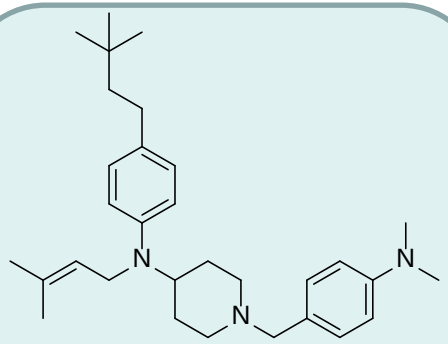
- ITH12657 kept the blockade of Ca^{2+} currents in presence of the L-type VDCC blocker nifedipine, but lost its blocking effect in presence of the N and P/Q-type VDCC blocker ω-conotoxin MVIIC

✓ <u>ITH12657 is a non-L type Ca^{2+} channels blocker</u>

Target Indications: Pain

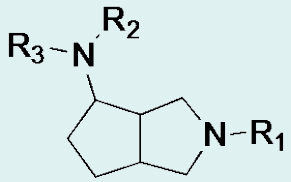
- Preferentially Neuropathic Pain, but without discarding severe chronic pain associated with cancer, AIDS, ischemia, or epilepsy.
  - *Synthetic version of this toxin, Ziconotice, has been approved for the treatment of chronic pain associated with cancer, AIDS, and neuropathies. Efficacious in postsurgery stages. Potent analgesic effects, manifested in patients resistant to opioids*
  - *Blockade of N- and P/Q channels inhibit pronociceptive neurotransmitters and neuromodulators, such as glutamate, substance P, and calcitonin-gene-related peptide*

Differential features facing de Market: The N-type



Parke-Davis/Pfizer

Bioorg. Med. Chem. 2000, 1203



Abbot Laboratories

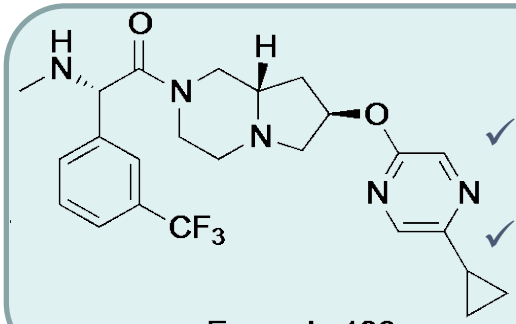
US20110281870

- ✓ 800 compounds assessed in IMR32 cells expressing human N-VDCC (FLIPR)
- ✓ In vivo: Capsaicin (mechanical hiperalgesia); Neuropathic pain (Bennet's)

Convergence Pharmaceuticals

WO2012098400; WO2012004604

- ✓ 300 compounds assessed in HEK-293 cells expressing human N-VDCC (Patch-clamp). No In vivo data



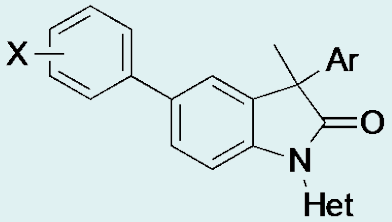
Example 186

Abbvie

WO2013049164; WO2013049174

- ✓ 443 compounds assessed in IMR32 cells expressing human N-VDCC (FLIPR)
- ✓ Neuropathic pain (Bennet's): Example 186 of WO164 exhibited 91% of máximum posible analgesic effect

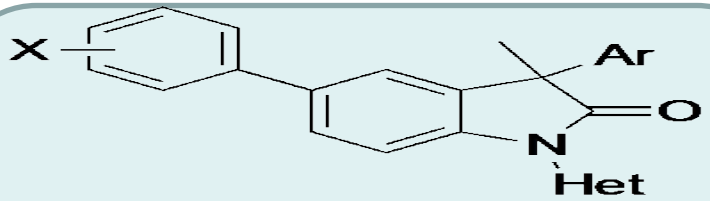
Differential features facing de Market: N- and T-type dual blockers



Merck Sharp & Dohme

US20128273749

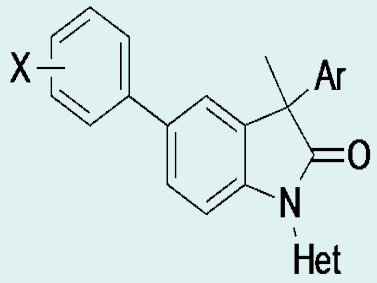
- ✓ 59 compounds assessed in HEK-293 cells expressing human N-and T-VDCC (Patch-clamp)
- ✓ In vivo: Freund's adjuvant (Acute inflammatory pain model



Zalicus Pharmaceuticals

WO2012061926

- ✓ 200 compounds assessed in cells expressing human N and T-VDCC. Some of selectivity towards N-type (FLIPR). No In vivo data



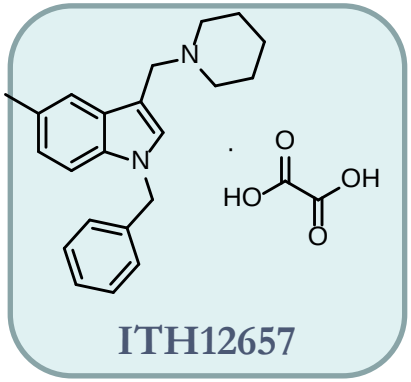
Convergence Pharmaceuticals

WO2013049164; WO2013049174

- ✓ Compounds assessed in HEK-293 cells expressing human N and T-VDCC (Patch-clamp). No In vivo data

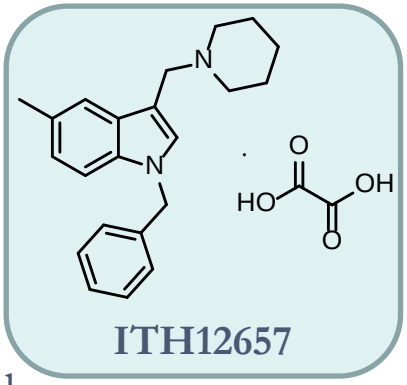
Differential features facing de Market: What is new?

- 3-aminomethylindoles are scarcely studied as drug-able compounds, unlike the extensively studied 3-aminoethylindoles (5-HT, melatonin, tryptophan analogues…), due to chemical instability
  - ➢ *We have designed experimental procedures to increase chemical stability and to ease experimental handling*
- ITH12657 and analogues are synthesized in two straightforward and cheap chemical steps, eligible to proceed in a multi-gram fashion
- Compounds are not highly polar, but they are water-soluble, having potential pharmacokinetic properties to be oral administered
- ITH12657 does not block L-type VDCC (lack of possible cardiovascular side effects)



ITH12657

Current Status



ITH12657

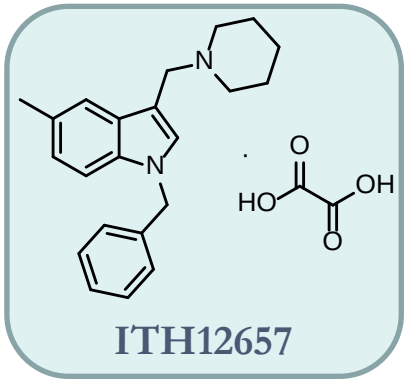
- In vitro experiments
  - Neuroprotection in in vitro models of neuronal damage
  - Fluorescence-based Ca^{2+} levels measurements in SH-SY5Y cells
  - Ca^{2+} currents in bovine chromaffin cells
  - Chemical procedures optimized to be scaled-up

IPR Protection

- **Lajarín-Cuesta et al., P201500354, May, 15th, 2015. "Nuevos derivados de (1*H*-indol-3-ilmetil)dimetilamina con actividad bloqueadora de los canales de Ca^{2+} dependientes de voltaje, preferentemente no-L, y su aplicación en el tratamiento de enfermedades del sistema nervioso**

Pitfalls and risks to be considered

- Although planned (e.g. capsaicin intraplantar injection), no in vivo data are presented
- The pharmacokinetics studies already carried out are only predictive
- Presence of highly metabolizing functional groups (methyl, phenyl), what could lead to short half lives of the active compound
- Toxicity assays are limited to in vitro, well-cultured, MTT assays
- Few compounds evaluated (less than 30)
- Temporary status of the MedChem team



ITH12657

Partnering opportunities

- Companies with interest to license our research work
- Companies with interest to grant in vivo experiments to evaluate ITH12657 in a model of neuropathic pain (intraplantar injection of capsaicin)
- With the goal of extending our research to further optimized compounds, we also search for companies with interest to grant a project centered in the preparation and evaluation of more gramine derivatives

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Thank you

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SaludMadrid