III SEQT Summer School

MEDICINAL CHEMISTRY IN DRUG DISCOVERY THE PHARMA PERSPECTIVE

SEQT

Tres Cantos (Madrid)
June 25-27, 2013

SCIENTIFIC PROGRAM

Tuesday ,June 25 2013

11:30-12:00	Registration
12:00-12:15	Opening (José Mª Fiandor -GlaxoSmithKline- and Antoni Torrens–SEQT-)
12:15-13:30	Inaugural Lecture: Needles in a Haystack: Methodologies and Challenges of Early Drug Discovery in Pharma. (Julio Martín-GlaxoSmithKline)
13:30-14:30	Lunch

Afternoon Session

15:00-16:30	Case Study 1: PDE10 inhibitors as potential novel treatment for schizophrenia. (José Manuel Bartolome-Janssen)

Bus to hotel (45')

19:00-20:00	Poster Session (Poster 1-20
20:30	Introductions (Teachers) & Dinner

Wednesday, June 26 2013

9:00 Bus to GSK (45')

Morning Session

10:00-11:30	Case Study 2: Residence Time in Medicinal Chemistry (Rick Roberts-Almirall)
11:30-11:45	Coffee Break
12:00-13:30	Case Study 3: Fat is not a moral problem. It's an oral problem. Influence of lipophilicity in drug discovery (María Marco-GlaxoSmithKline)
13:30-14:30	Lunch

Afternoon Session

14:30-16:00	Case Study 4: Safety Assessment in Drug Discovery and Development (Antonio Guzmán-Esteve)
16:00-17:30	Case Study 5: Scale-up: Pharmaceuticals, from mg to kg (Victor Rubio-Faes)

Bus to hotel (45')

19:00-20:00	Poster Session (Poster 21-41)
20:30-21:00	Selection of the oral communications (Meeting–Organizing Committee and Workshop Speakers)
21:00	Announcement of the selected posters & Dinner

Thursday, June 27 2013

9:00 Bus to GSK (45')

Morning Session

10:00-11:15	Selected oral communications (C1-C4)
11:15-11:45	Coffee Break
11:45-12:30	Selected oral communications (C5-C7)
12:30-13:30	Tour to GSK facilities
13:30-14:30	Lunch
14:30-14:45	Concluding remarks (José Mª Fiandor -GlaxoSmithKline- and Antoni Torrens–SEQT-)

Inaugural Lecture

JulioMartín
GlaxoSmithKline

I am currently working at the Molecular Discovery Research automation facility of GlaxoSmithKline in Tres Cantos (Spain). This R&D centre is devoted to the industrialisation of HTS operations. I have been responsible of ultra-HTS campaigns from screen development to dose-response and preliminary SAR. Likewise, I have been engaged in the development and implementation of new statistical tools and assay technologies for the improvement of HTS efficiency. I have explored label-free technologies for the screening of small molecules in protein and cellular assays in a joint collaboration with external companies. More recently, I have been involved in the implementation of the strategy for drug discovery against tryponosomatids based on open innovation and collaborative research.

I hold a PhD degree in Biochemistry from University of Madrid, where I acquired background and expertise on protein chemistry and enzymology. Prior to my current position, I worked at the R&D Department of Glaxo, where I managed programmes for discovery of new antimicrobial leads.

Selected Publications

- Dominguez, JM and Martin, JJ (2001) "Identification of a putative sordarin binding site in Candida albicans elongation factor 2 by photoaffinity labeling". Journal of Biological Chemistry 276 (33), 31402–31407
- Cameron A., Martin JJ et al. (2004) "Identification and activity of a series of azole-based compounds with lactate dehydrogenase-directed anti-malarial activity". Journal of Biological Chemistry 279 (30), 31429–31439.
- Vazquez, M.J., Martin, J.J. et al. (2006) "Utilization of substrate induced quenching for screening targets promoting NADH and NADPH consumption", J. Biomol. Screening 11 (1), 75–81.
- Wood E., Martin JJ et al. (2009) "Discovery of an Inhibitor of Insulin-Like Growth Factor 1 Receptor Activation: Implications for Cellular Potency and Selectivity over Insulin Receptor". Biochemical Pharmacology 78(12), 1438–47
- Isabel Coma, Jesus Herranz, Julio Martin (2009) Statistics and Decision Making in High–Throughput Screening, in "High Throughput Screening: Methods and Protocols", Second Edition, Editor(s): William P. Janzen, Paul Bernasconi (Springer), pp. 69–106.
- Martin, J.J. (2010) "Label-free Imaging and Temporal Signature in Phenotypic Cellular Assays: A New Approach to High Content Screening", in Currents Protocols of Pharmacology editor Terry Kenakin (Wiley-Blackwell)
- Laura Vela, Peter N. Lowe, John Gerstenmaier, Lance G. Laing, Julie B. Stimmel, Lisa A. Orband-Miller and Julio J. Martin (2011) "Validation of an Optical Microplate Label-Free Platform in the Screening of Chemical Libraries for Direct Binding to a Nuclear Receptor" ASSAY and Drug Development Technologies 9 (5): 532–548

NEEDLES IN A HAYSTACK: METHODOLOGIES AND CHALLENGES OF EARLY DRUG DISCOVERY IN PHARMA.

JulioMartín

GlaxoSmithKline. Screening and Compound Profiling, Director. Tres Cantos, Spain

Nowadays, the pharmaceutical industry is immersed inside the perfect storm: patients are still in need of effective drugs for many diseases, payers are increasingly only prepared to pay for innovative rather than derivative drugs, investors believe they can get better return on investment elsewhere and legislators are demanding that only the very safest possible drugs are licensed. Furthermore, researchers are equally frustrated. Despite all the accumulated knowledge from the new technologies, the challenge of successfully navigating through everything to find novel drugs seems to get harder rather than easier. The fruit is possibly not as low hanging as before, and the pharmaceutical R&D efficiency has been declining over the last decades. The consequence of all this is that the average cost for getting a medicine in the market is now $1.8bn. The improvement of R&D productivity has emerged as the pharmaceutical industry´s grand challenge.

Amongst the major advances in science and technology produced in drug discovery over the past 60 years, high-throughput screening (HTS) has resulted in a drastic reduction in the cost of testing compound libraries against therapeutic targets, as well as in a substantial shortening in time. Now, testing a large library of 2 million compounds in a few days is almost routine in specialized HTS facilities. In the mid–1990s, pharma R&D scientists and managers envisaged the realization of a dream: the experiment of facing all possible therapeutic targets identified from genome sequencing with the huge chemical diversity from combinatorial and parallel synthesis. Expectations were raised high as the solution to the R&D productivity. Seeking and finding the golden needles in the haystack seemed feasible. Unfortunately, the outcome has not lived up to expectations. Despite the success in the development and implementation of fit-for-purpose technologies and processes, HTS has struggled significant challenges, such as translational chemical biology. That is, how to interpret the signals from reductionist assays and models in order to navigate throughout the chemical space and decrease the attrition towards drug candidate selection?

In this lecture we will review the current methodologies employed in HTS and we will address the challenges that may contribute to increase the pharmaceutical R&D efficiency in early drug discovery.

Case Study 1

José Manuel Bartolome
Janssen Research and Development

Jose Manuel graduated in Organic Chemistry from Autónoma University (Madrid, Spain) in 1993, before obtaining his M.S. (Summa Cum Laude) in 1994 from the same University. In 1998, after a 3 year stay at the Medicinal Chemistry Institute of the Spanish Research Council (C.S.I.C.), Jose Manuel received his Ph.D. (Summa Cum Laude), also from Autónoma University for his work on the field of CCK_A antagonists.

In 1997, Jose Manuel joined Janssen Research & Development as a Scientist in the Medicinal Chemistry department at the Toledo (Spain) site. In 2002 he was promoted to Senior Scientist and then in 2008 to Principal Scientist within the Neuroscience Medicinal Chemistry department. Over more than 15 years Jose Manuel has been involved in more than 15 Medicinal Chemistry Program in the areas of Gastrointestinal, Neurology and Psychiatry and has contributed to the successful delivery of 5 NME candidates. He is currently the Team Leader of a Medicinal Chemistry Team focussed on neurology and psychiatry targets.

Jose Manuel is co-author of 17 peer reviewed papers, is co-inventor of 33 international patent applications and has more than 20 communications (oral and poster) to international conferences. He has also been awarded with the Janssen-Cilag Award (VII Summon of the Spanish Medicinal Chemistry Society Awards for Novel Researchers, 1997). Jose Manuel is also member of the Spanish Medicinal Chemistry Society.

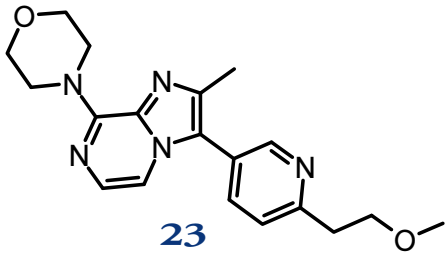
PDE10 INHIBITORS AS POTENTIAL NOVEL TREATMENT FOR SCHIZOPHRENIA

José Manuel Bartolome

Principal Scientist. Neuroscience Medicinal Chemistry.Janssen Research and Development

Schizophrenia is a severe mental disorder characterized by a combination of positive (e.g., hallucinations and delusions), negative (e.g., anhedonia and poverty of speech) and cognitive symptoms.[1] All currently available antipsychotic therapies rely on dopamine D_2 receptor antagonism to exert their action and are highly efficacious addressing positive symptoms but ineffective for the other core symptoms of the disease.[2] Furthermore, they are associated with severe side-effects (e.g., Parkinson-like extrapyramidal (EPS) symptoms, prolactin release, weight gain or cardiac risk) which limit patient compliance.[2] As a result, during the past 10–15 years, several alternative mechanisms of action have been investigated[3] aiming for improved antipsychotic medications. Among these different approaches, inhibition of phosphodiesterase 10A (PDE10A) has been proposed as a new approach for the treatment of schizophrenia supported by the activity of PDE10A inhibitors in preclinical models of positive, cognitive and negative symptoms.

Starting from a HTS campaign, a focused medicinal chemistry optimization has lead us to the identification of a series of imidazopyrazine derivatives as a novel class of PDE10A inhibitors. These compounds inhibit PDE10A mediated c-AMP hydrolysis *in vitro* and have also proven to be efficacious in preclinical models of schizophrenia. Evolution of our medicinal chemistry program, SAR and SPR analysis as well as a detailed profile for optimized PDE10A inhibitor 23 will be described.



23

1. Tandon, R.; Nasrallah, H.A.; Keshavan, M.S. *Schizophr. Res.* 2009, *110*, 1–23.

2. Conn, J. P.; Lindsley, C. W.; Jones, C. K. *Trends Pharmacol Sci.* 2009, *30*, 148–155.

3. Macdonald, G.J.; Bartolome J.M. *Progress in Medicinal Chemistry* 2010, *49*, 37-80.

PDE10 Inhibitors as Potential Novel Treatment for Schizophrenia

José Manuel Bartolomé Nebreda, PhD

Neuroscience Therapeutic Area

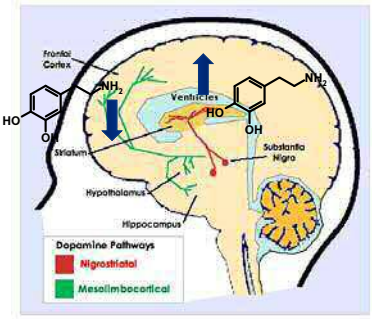
janssen PHARMACEUTICAL COMPANIES OF Johnson & Johnson

Defining schizophrenia

Persistent mental and behavioral symptoms that cannot be explained as secondary to another medical or psychiatric condition

Positive Symptoms	*Delusions, hallucinations, disorganized speech, agitated behavior, no comprehension of behavior by patient*
Negative Symptoms	*Lack of emotional expression, speech, drive, motivation, social withdrawal*
Cognitive Dysfunction	*Verbal memory, executive function, visuospatial capacity, verbal fluency, attention*

Dopamine hypothesis of schizophrenia



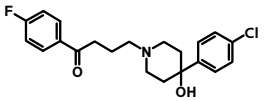
Schizophrenia is characterized by a dysregulation in the dopaminergic circuitry:

- Dopamine releasing agents (amphetamine, cocaine) induce psychotic–like symptoms
- The nigrostriatal (substantia nigra to striatum) and mesolimbic (VTA to Nacc) pathways are sensitized and show hyperdopaminergia
- The corticolimbic pathway shows hypodopaminergia

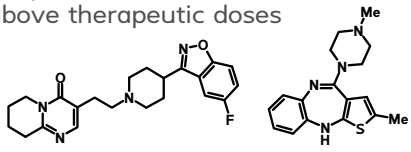
The dopamine hypothesis of schizophrenia proposes that increased levels of dopamine (or dopamine receptors) in the striatum underlie the positive symptoms of the disorder, whereas loss of dopamine regulation in the prefrontal cortex has been associated with cognitive deficits

Current treatments for schizophrenia

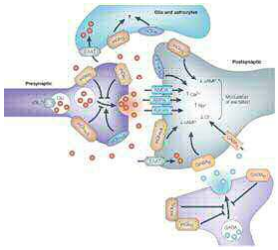
- *Older 'Typical' anti-psychotic agents (mid-1950's)*
  - Haloperidol, chlorpromazine….
  - Potent and relatively selective ***D_2 blockade***
  - Highly efficacious against Positive Symptoms
  - High incidence of prolactin release, EPS, restlessness and sexual difficulties when administered at or above therapeutic doses



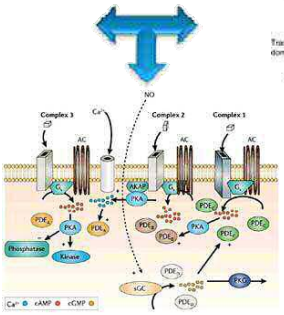
- *Newer 'Atypical' anti-psychotic agents*
  - Risperidone, Paliperidone Olanzapine, Aripiprazole, Quetiapine….
  - ***Blockade of D_2*** and 5-HT_{2A} receptors at therapeutic doses
  - Moderate claims of improvements in Negative Symptoms
  - Lower incident of EPS
  - Associated with side effects such as prolactin release and metabolic problems



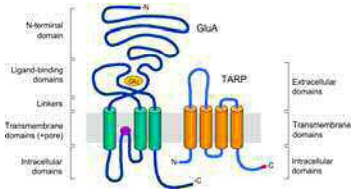
In search of the next generation therapeutics



mGluR Modulators

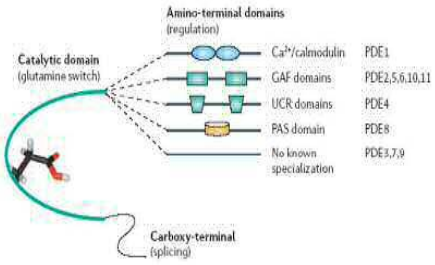


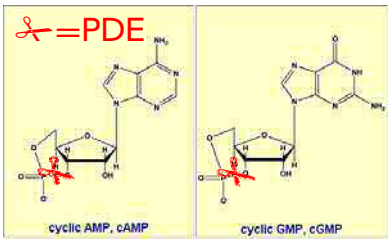
PDE inhibitors



iGluR Modulators

Cyclic nucleotide phosphodiesterases (PDE's)





cAMP specific: PDE4, PDE7, PDE8
cGMP specific: PDE5, pDE6, PDE9
Dual : PDE1, PDE2, PDE3, PDE10, PDE11

- Intracellular enzymes which metabolically inactivate the key intracellular second messengers cAMP and cGMP
- 11 different families (21 genes)
- Specific distribution throught the body

PDEs are key regulators of signal transduction mediated by cAMP and cGMP (strength, duration and place of signaling)

PDE's: broad function and druggable target class

PDE 3
Milrinone

Neuronal function
Apoptosis
Cell proliferation/ differentiation/migration
Cardiac myocyte contraction
Relaxation of blood vessels
GI mobility
Insulin response
Bone formation
Immune Response
Airway distension
Inhibition of platelet aggregation

PDE 10/4/3
Papaverine

PDE 4
Roflumilast

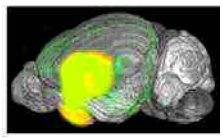
PDE 10/4/3
Ibudilast

PDE 3
Cilostazol

PDE 5
Sildenafil
Tadalafil
Vardenafil

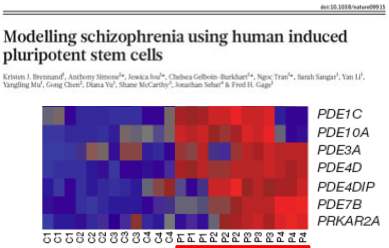
PDE's and the CNS

- High brain expression
- Expression profile in diseased states
- Profile of KO animals
- Genetic association
- Effect of inhibitors



Allen's Brain Atlas

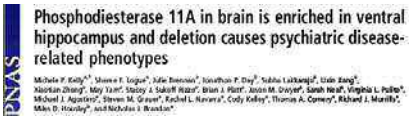
Striatal expression of PDE10A



Modelling schizophrenia using human induced pluripotent stem cells

PDE1C
PDE10A
PDE3A
PDE4D
PDE4DIP
PDE7B
PRKAR2A

schizophrenic patients

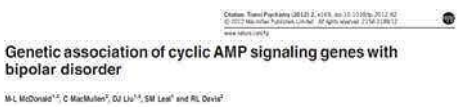


Phosphodiesterase 11A in brain is enriched in ventral hippocampus and deletion causes psychiatric disease-related phenotypes

J Neuropsychiatry Clin Neurosci 19:471-472, November 2007

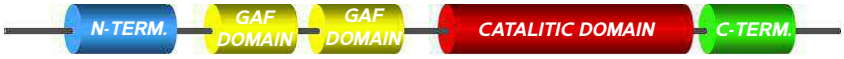
Cilostazol, a cAMP Phosphodiesterase 3 Inhibitor, in the Treatment of Poststroke Depression

Katsuji Nishimura, M.D., Department of Psychiatry, School of Medicine, Tokyo Women's Medical University, Tokyo, Japan,

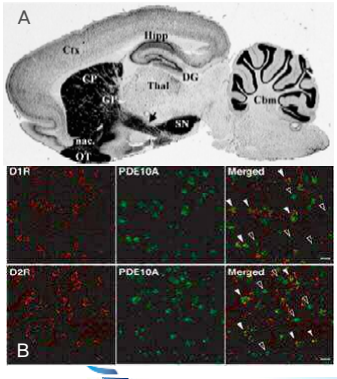


Genetic association of cyclic AMP signaling genes with bipolar disorder

PDE10A characteristics



- Discovered in 1999
- 11 known protein variants (PDE10A1 & A2 main human variants)
- Hydrolyses both cAMP (K_M = 0,05 μM) and cGMP (K_M = 3,0 μM)
- Contains two cGMP modulatory binding domains (GAF domains)

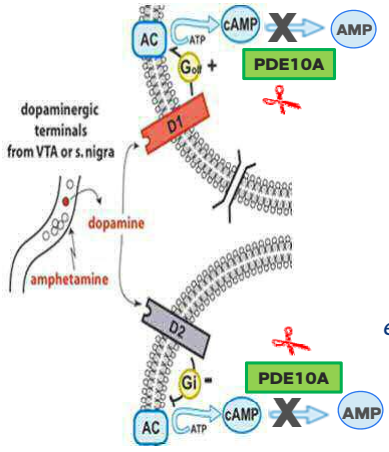


PDE10A Expression

- PDE10A is a brain-specific PDE (apart from expression in testis)
- Co-incidence of high PDE10A and high dopamine receptor D_1 and D_2 expression in striatum in medium spiny neurons (direct and indirect pathway) an area in the brain that is implicated in psychiatric diseases

[A]Seeger et al., 2003; IHC **rat brain**
[B]Sano et al., 2008; Double ISH in CPU **mouse brain**

PDE10A inhibition: hypothesis



↑ **cAMP**

D_1 (AC+) agonist : cAMP increase

D_2 (AC-) antagonist : cAMP increase

PDE10A: cAMP decrease

A PDE10A inhibitor is expected to increase cAMP levels mimicking the effects of antagonism at the D_2, and agonism at the D_1 receptor

↑ **cAMP**

Inhibition of PDE10A has emerged as a new target to control dopamine transmission, that is not based on direct agonism or antagonism at the receptor level

Therapeutic potential of PDE10A inhibition

PDE10A inhibitors may be useful to treat positive, negative and cognitive symptoms in psychosis:

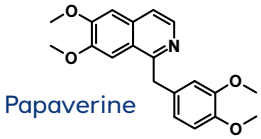
- Positive symptoms (*mimick D_2 antagonism*)
- Cognitive deficits (*mimick D_1 agonism*)
- Negative symptoms (*mimick D_1 agonism*)

PDE10A inhibitors are supposed to be devoid of some of the side effects of D_2 antagonists:

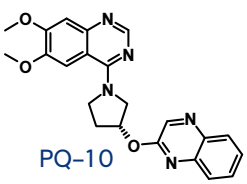
- No induction of consistent catalepsy (*mimick D_1 agonism*)
- No stimulation of prolactin release (*no peripheral expression*)
- No induction of weight gain (*selectivity vs. H_1, 5-HT_{2C}*)
- No interaction with monoamine receptors responsible for the side effects with currently available antipsychotics (*selectivity vs. H_1, adrenergic receptors, muscarinic receptors….*)

PDE10A inhibition: preclinical validation

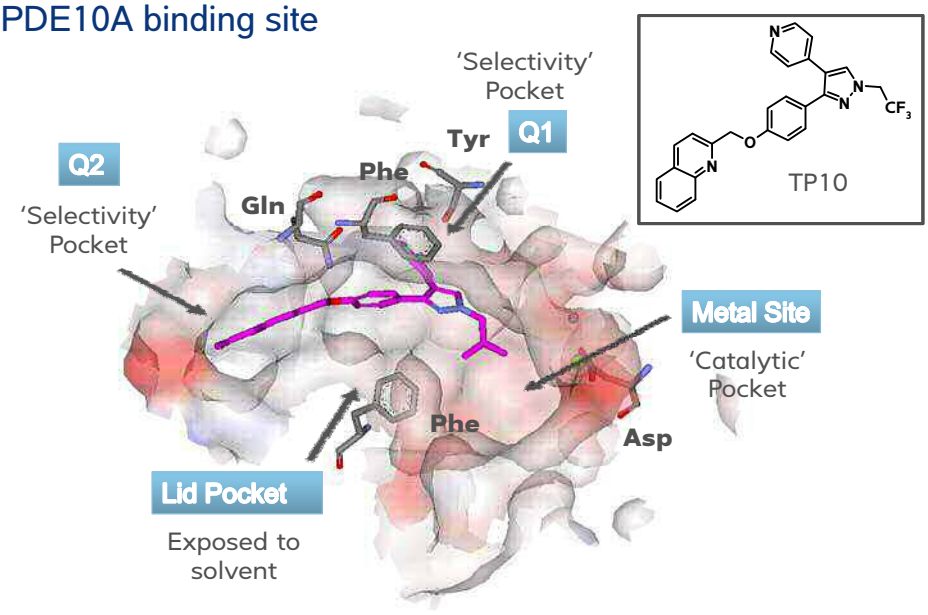
- Psychosis:
  - Reverse apomorphine-induced hyperactivity
  - Decrease PCP-, amphetamine and novelty-induced locomotor behaviour (*papaverine*, *PQ10*, TP10)
  - Inhibit conditioned avoidance responding


Papaverine

- Cognition:
  - Reverse MK801- and scopolamine-induced object recognition deficit (*PQ10*)
  - Reduce the effect of amphetamine on auditory gating in auditory evoked potential (TP10)
  - Reverse the PCP-induced deficits in attentional set shifting (*papaverine*)
  - Increase object recognition memory in rat and attenuated ketamine-induced deficit in object retrieval detour task in rhesus monkey (*THPP1*)
- Biochemical Markers:
  - Increase striatal cAMP and cGMP levels as well as increase pGluR1 and pCREB (*Papaverine*, *TP10* and *MP10*)

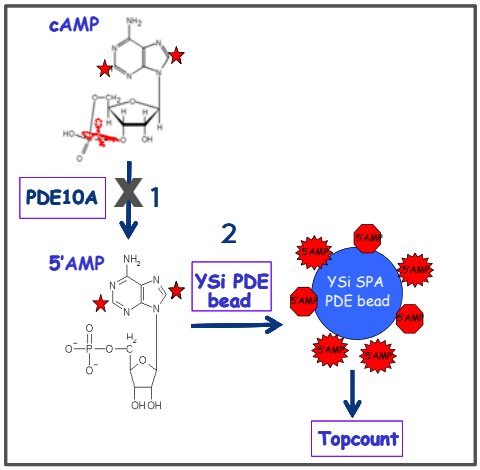

PQ-10

PDE10A binding site



X-Ray structures obtained in collaboration with BioFocus (Saerum)

Primary Screening Assay - Rat Recombinant PDE10A Enzymatic Activity



1) Hydrolysis reaction

– Phosphodiesterase PDE10A catalyzes the hydrolysis of cAMP to 5'–AMP

2) Detection reaction

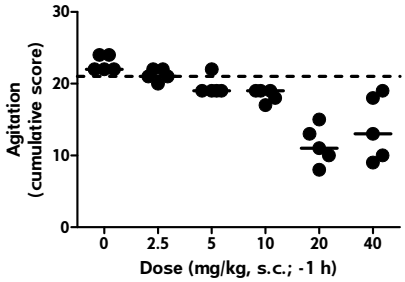
– PDE YSi SPA beads allow PDE activity to be measured by direct binding of the primary phosphate groups of tritiated non–cyclic AMP to the beads.

– The signal is measured by scintillation counting

– Inhibitory potency is measured as IC_{50} (or pIC_{50})

Inhibition of apomorphine stereotypy

- *Apomorphine* is a direct dopamine receptor agonist
- *Apomorphine* induces stereotyped behavior (repetitive, abnormal behaviors) in rats
- *D_2 antagonists* reverse stereotyped behavior



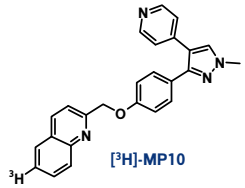
PDE10A inhibitors resemble D_2 antagonists inhibition of apomorphine induced stereotypy

Development of an *in vivo* occupancy assay

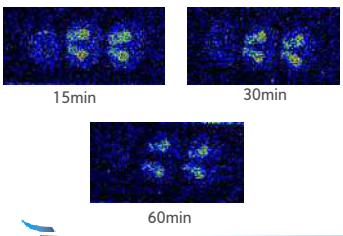
Occupancy studies are key to confirm the interaction of drug candidates with their targets in the brain

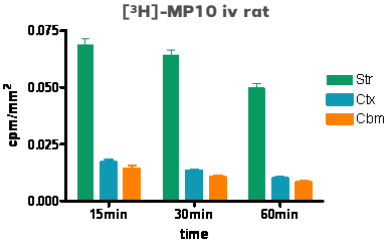
~6 months

- Precursor synthesis
- Tritiation of [^{3}H]ref. compound
- Autoradiographic characterization of [^{3}H]ref. compound: identification of the first radioligand
- First in vivo occupancy assay



[^{3}H]-MP10 in vivo binding in rat 10µCi iv rat

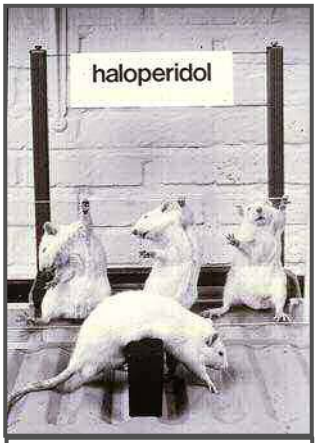




D_2 antagonists and extrapyramidal symptoms (EPS)

- Parkinson's disease:
  - Symptoms: tremor, rigidity, slowness of movement and difficulty with walking and gait.
  - Cause: *suppression of nigrostriatal dopaminergic neurotransmission* due to *neuronal degeneration of nigrostriatal DA neurons*
- D_2 receptor blockers:
  - Side effects: Parkinson-like extrapyramidal symptoms (EPS)
  - Cause: *suppression of nigrostriatal dopaminergic neurotransmission* due to *excessive blockade of striatal D_2 receptors*

Catalepsy in rats: thought to be a rodent correlate of EPS

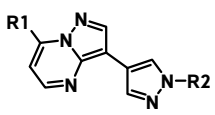


• Wax-like rigidity

PDE10A inhibitors Hit to Lead

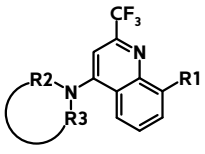
- Five chemical series selected from HTS
- Good in vitro potency. Strong focus on *in vitro* ADME profile
- All series originated from external libraries acquired by ECOS

Cluster 3



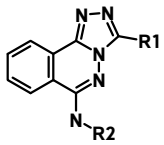
Pyrazolopyrimidines

Cluster 5



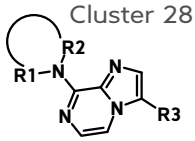
CF_3-Quinolines

Cluster 14



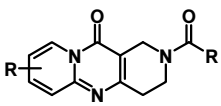
Triazolophthalazines

Cluster 28



Imidazopyrazines

Cluster 16

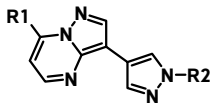


Tricyclic pyrimidones

PDE10A inhibitors Hit to Lead overview

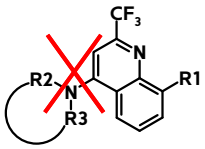
- Three series transferred into LO
- Two chemotypes prioritized (cluster 3 and 28)
- Third chemotype (cluster 16) selected as a potential back-up series

Cluster 3



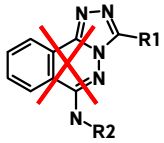
Pyrazolopyrimidines

Cluster 5



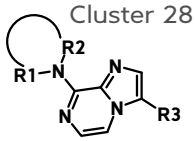
CF_3-Quinolines

Cluster 14



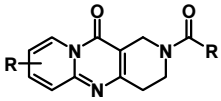
Triazolophthalazines

Cluster 28



Imidazopyrazines

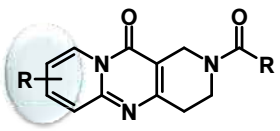
Cluster 16



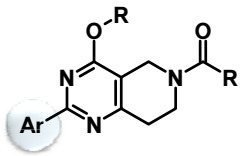
Tricyclic pyrimidones

PDE10A Inhibitors: Cluster 16, tricyclic pirimidones

- Series evolved from tricyclic to bicyclic systems



PDE10A pIC_{50} ~ 6.5



PDE10A pIC_{50} ~ 7

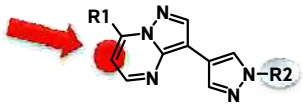
Moderate *in vitro* potency
Good selectivity *vs* other PDEs
Poor solubility
Good oral exposure in rat
Moderate brain exposure in rat
Low in vivo activity

In vitro potency modest increase
Good selectivity *vs* other PDEs
Improved solubility
In vivo activity modest increase
Flat SAR

Cluster 16 put on hold based on moderate in vitro & in vivo activity & flat SAR

PDE10A Inhibitors: Cluster-3, pyrazolopyrimidines

Metabolic hot-spot: oxidation

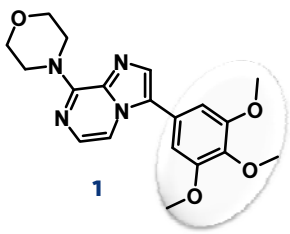


PDE10A pIC_{50} ~ 6.5

– Good Selectivity *vs* other PDEs
– Good *in vitro* ADME profile (solubility, CYPs, hERG, PAMPA)
– Potent *in vivo* activity (APO LAD 1.25 mpk (sc))
– Moderate *in vivo* occupancy (ED_{50} 13 mpk (sc))
– Good brain exposure. B/P = 0.8
– Very poor oral exposure. High and Rapid clearance

– All efforts focussed on metabolic stability increase

PDE10A Inhibitors: Cluster-28, imidazopyrazines

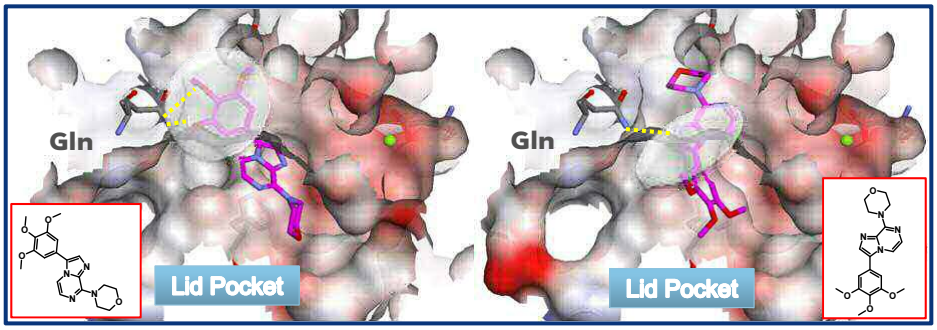


1

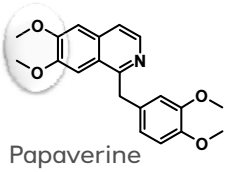
– PDE10A pIC_{50} = 6.8
– Poor selectivity vs. other PDEs
– Good *in vitro* ADME profile
– Moderate *in vivo* activity (APO LAD 10 mpk (sc))
– Good oral and brain exposure in rat
– Low solubility

– Overall promising profile
– First efforts devoted to improve selectivity

PDE10A Inhibitors: Cluster-28, understanding selectivity



- *Catechol-like motifs are common to PDEs ligands*
- ***1** can bind to preserved Gln726 in two ways:*
  - *Through the catechol (like other PDE ligands)*
  - *Through the central core*

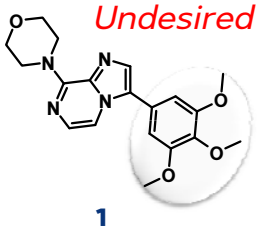


Papaverine

PDE 10/4/3

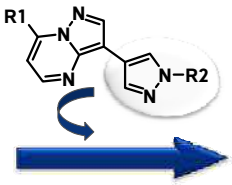
PDE10A Inhibitors: Cluster-28, SAR transfer

Undesired



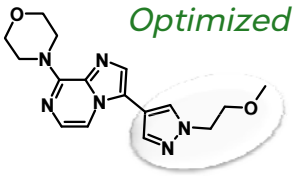
1

PDE10A pIC_{50} = 6.8



Cluster 3 SAR transfer

Optimized



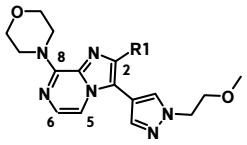
2

PDE10A pIC_{50} = 6.0

- Moderate in vitro potency
- Good selectivity vs other PDEs
- Good AMET profile (solubility, CYPs, hERG, PAMPA)
- Good metabolic stability

***2** selected as viable Lead for Lead Optimization*

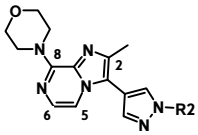
PDE10A Inhibitors: Cluster-28, 2-position SAR



Compound	R^1	PDE10A pIC_{50}	APO ED_{50} (mg/kg)	rLM (% met, 15 min)
2	H	6.0	5.0	1
3	Me	**6.85**	**3.6**	**26**
4	Et	**6.81**	**5.0**	41
5	cyclopropyl	**7.27**	**5.0**	42
6	CN	**7.07**	**5.0**	n.t.
7	CF_3	**6.51**	**>10**	n.t.
8	MeO	6.20	n.t.	n.t.

n.t. = not tested

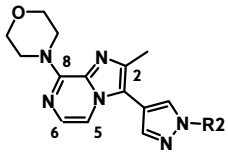
PDE10A Inhibitors: Cluster-28, pyrazole substitient SAR



Compound	R^2	PDE10A pIC_{50}	APO ED_{50} (mg/kg)	rLM (% met, 15 min)
3		**6.85**	**3.6**	26
9	Et	6.63	7.9	n.t.
10		7.10	>10	n.t.
11	*iso*propyl	6.95	>10	n.t.
12	*iso*butyl	**6.84**	**2.0**	**50**
13	CH_2CF_3	7.33	5.0	61
14	CH_2CH_2F	6.85	5.0	66
15		7.02	10	34
16		**7.22**	**1.2**	25
17		**7.02**	**1.2**	24

n.t. = not tested

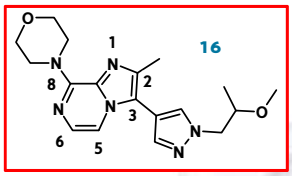
PDE10A Inhibitors: Leads comparative profiles

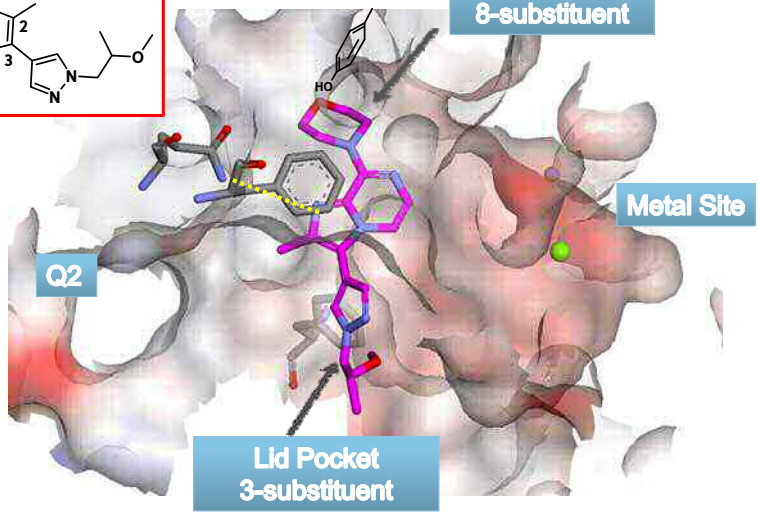


						Rat, 10 mg/kg, PO				
Cpd	R2	PDE10 pIC_{50}	Occ. ED_{50} (mg/kg)	APO ED_{50} (mg/kg)	PCP ED_{50} (mg/kg)	C_{max} (ng/mL)	AUC 0-inf (ng·h/mL)	$t_{1/2}$ (h)	F (%)	Cl (L/h/kg)
3		6.9	4.5	3.6	3.1	1980	8487	3.5	52	1.7
12	*isobutyl*	7.2	8.0	1.2	5.0	320	616	2.8	22	3.6
16		7.0	6.4	1.2	2.5	201	1066	1.3	26	2.4
17		6.8	5.6	2.0	5.4	295	297	1.0	8	2.8

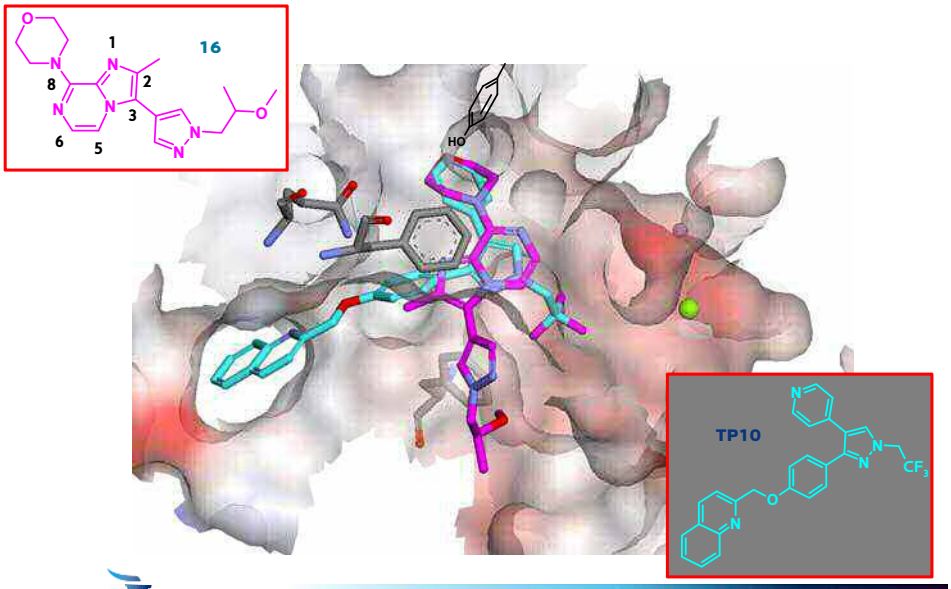
- ***3*** *was the first compound combining interesting in vivo activity, occupancy and good PK*
- *Main goal was to improve potency*

PDE10A Inhibitors: understanding the binding mode



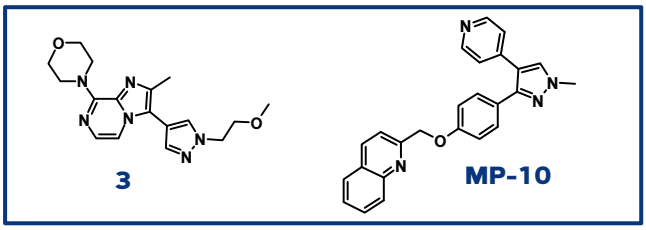


PDE10A inhibitors: different binding modes



PDE10A inhibitors: understanding *in vivo* efficacy

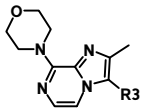
Free drug hypothesis: Drug action is dependent on availability of unbound drug at the site of action ($C_{u,brain}$)

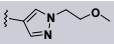
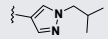
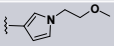
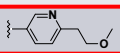
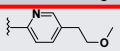
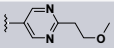


				Rat, 10 mg/kg, PO, 1h				
Cpd	PDE10 IC_{50} (nM)	Occ. ED_{50} (mg/kg)	APO ED_{50} (mg/kg)	BTB rat (%)	C_{brain} (ng/gr)	$C_{u,brain}$ (ng/gr)	$C_{u,brain}$ (nM)	$C_{u,brain}/IC_{50}$
3	140	4.5	3.6	76.2	1200	286	83	0.59
MP10	1.2	1.6	0.67	99.8	867	1.73	0.44	0.37

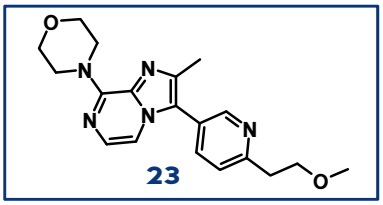
Lower potency at PDE10A enzyme is compensated by larger free brain concentration ($C_{u,brain}$)

PDE10A Inhibitors: Cluster-28, pyrazole replacements



Compound	R^3	PDE10 pIC_{50}	APO LAD (mg/kg)	rLM (% met, 15 min)
3		6.85	3.6	26
11		6.95	> 10	n.t.
18		6.30	n.t.	n.t.
19		6.80	10	40
20		6.60	10	23
21		6.60	> 10	0.1
23		6.72	1.25	24
24		6.66	> 2.5	32
25		6.83	2.5	14

PDE10A Inhibitors: **23** *in vitro* profile



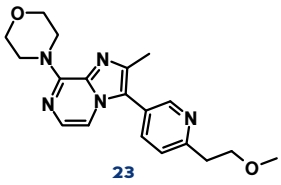
	Ki (μM)	window vs PDE10A
PDE1B	4.70	133
PDE2A	37.80	1074
PDE3A	>67	1903
PDE4D	18.10	514
PDE5A	28.90	821
PDE6AB	64.40	1829
PDE7A	>33	937
PDE8A	>50	1420
PDE9A	>33	937
PDE10A	0.035	1
PDE11A	43.30	1230

- K_i hPDE10A = 0.035 μM
- No relevant species differences in affinity for PDE10A
- Selectivity versus other PDE family members > 133 fold
- No affinity for CEREP receptor panel (50 receptors) or enzyme panel at 10 μM
- No inhibition of kinases (230 kinases) in Milipore panel:< 50 % inhibition at 10 μM

PDE10A Inhibitors: **23** ADMET profile

- *Permeability:*
  - Good permeability through gut lumen (PAMPA/Caco-2)
  - No indication of PgP efflux mechanism
- *Protein binding:*
  - Moderate plasma protein binding in all species (< 74 %)
  - Medium rat homogenate brain binding (~ 77 %)
- *DDI:*
  - Low potential as an inhibitor of major CYP450's (> 30 µM)
  - Limited potential as a victim drug of CYP450 inhibitors (polyzymic CYP450 metabolism)
  - No potential to induce CYP3A4 and 1A2.
  - Low potential as a mechanism-based inhibitor of CYP450 3A4
- *No genotox (AMES II) / cytotox alerts*
- *Low inhibition potential of hERG channel in vitro and in vivo (guinea pig)*
- *No prolactin release*

PDE10A Inhibitors: NME identification



23
PDE10A pIC_{50} = 6.7

- Good Selectivity vs other PDEs, FRP and Cerep & kinase panel
- Good *in vitro* ADMET profile
- Good *in vivo* pharmacology & occupancy
- Acceptable preclinical PK profile
- No hERG blockade alerts
- **Anesthetized Dog: Effect on LVEDP**

Key Core Modifications

- *In vitro* potency increased
- Comparable *in vitro* pharmacology & ADME
- Comparable *in vivo* pharmacology & PK
- Clean anesthetized dog

NME Candidate

PDE10A Inhibitors: Team Members

Chemistry :
Susana Conde
Gregor Macdonald
Sergio Alonso
Marta Artola
Alcira del Cerro
Óscar Delgado
Paqui Delgado
Arantxa García
Lourdes Linares
Encarna Matesanz
Luz Martín
Carlos Martínez
Miguel A. Pena
Michiel Van Gool
Han Min Tong

Molecular Informatics:
Wendy Sanderson
Carola Wassvik

X-Ray Crystallography:
Sareum

Biology :
Greet Vanhoof (BTL)
Marijke Somers (ADME)
Pim Drinkenburg
Xavier Langlois
Anton Megens,
Luc Ver Donck

PET :
Ignacio Andrés
Jesús Álcazar
Meri De Angelis
Xavier Langlois
Mark Schmidt
Guy Bormans (KUL)
Stefanie Dedeurwaerdere (KUL)

ADMET:
Claire Mackie
Marijke Somers

Clinical Development :
Luc Tritsmans (CDTL)
Marc Ceusters
Ilse Van de Velde
Peter De Boer
Joris Berwaerts
Eric De Waal
Kristof Dubois
Laurent Leclercq
Sivi Ouwerkerk-Mahadevan
Serge Van Der Geyten
Bart Remmerie

CV Safety
David Gallacher
Ard Teismans

Legal:
Luc Quaghebeur
María García

Case Study 2

Rick Roberts

Almirall S.A.

Background

1990-1993	Degree in Natural Sciences, University of Cambridge, U.K
1993-1997	PhD in Organic Chemistry, University of Cambridge, U.K
1997-2000	Post doc at Imperial College, London, U.K.
2000-2002	Team Leader, Syngenta (formerly Zeneca Agrochemicals), U.K
2002-	Senior Scientist, Almirall, Spain

Interests and Experiences

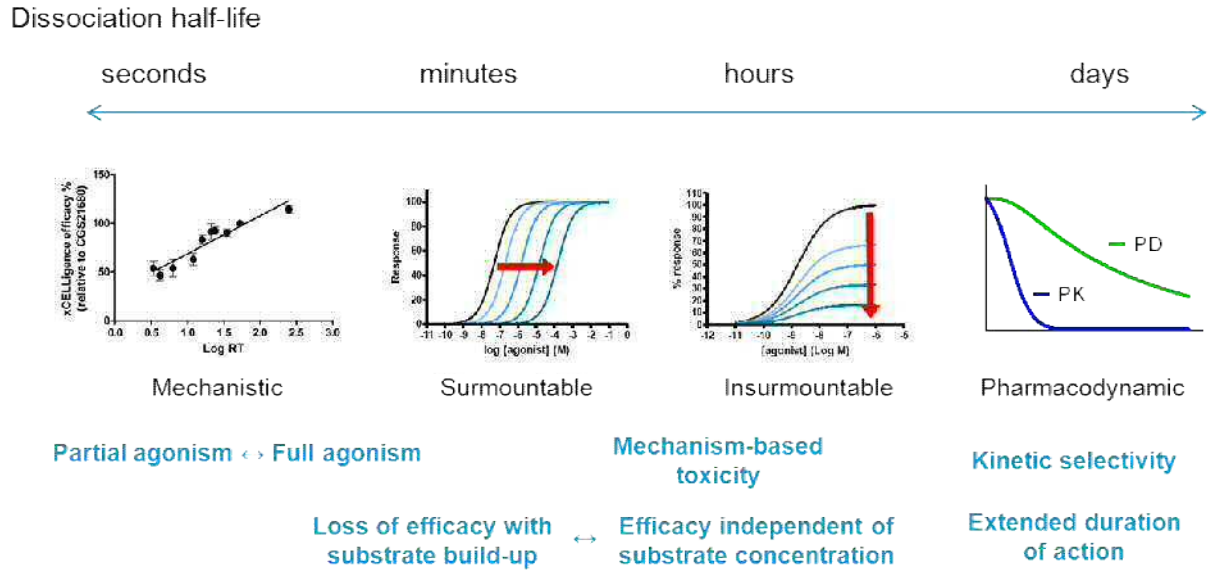
- A Degree not just in chemistry, but with many hours of biology, physics and rock guitar. Here, I learnt chemistry.
- A PhD in organosilicon chemistry and synthetic organic chemistry methodologies. Here, I learnt to think.
- A postdoc spread between unnatural amino acid synthesis, parallel synthesis methods on solid phase, ring opening metathesis polymerization (ROMP) and managing collaborations. Here, I learnt to work.
- A first job spread between herbicides and insecticides, taking a compound to the fields of Taiwan and discovering a new mechanism of action along the way. Here I learnt about working.
- A current job which has mixed plenty of medicinal chemistry with parallel synthesis, running external collaborations, programme leadership, compound management IT systems and a new language. I am still learning.

RESIDENCE TIME IN MEDICINAL CHEMISTRY

Rick Roberts

Senior Scientist, Medicinal Chemistry Department. Almirall S.A.

The measurement of ligand binding potency is fundamental to Medicinal Chemistry. However simple potency data gives no indication of the underlying kinetics of the binding (and unbinding) processes. Binding events can occur over seconds, minutes, hours or days, and these differences can give rise to both desirable and undesirable consequences. The medicinal chemistry community is now embracing the phenomenon of residence time, not only by unraveling the possible consequences of fast and slow kinetics, but by taking advantage of this extra dimension of ligand-target binding. This talk will start from the basics of the binding event, moving through examples of the role played by residence time and finishing with a case study from a recent research program.



Residence Time in Medicinal Chemistry

SEQT Summer School
Tres Cantos

25-27 June 2013

Rick Roberts
Almirall

Medicinal Chemistry in a nutshell ….

Drug + target $\underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}}$ Drug : target → Response

Drug availability →	Binding kinetics →	Occupancy →	Response
DMPK • absorption • clearance • protein-binding	Association rate Dissociation rate	Fraction bound Residence time	Coupling factors • conformation • equilibrium • non-equilibrium
Quantitation: C_{max}, C_{min} V_d $t_{½}$ clearance %f	k_{on} k_{off} $t_{½}$ dissociation	% bound K_i	IC_{50}, EC_{50} Maximum effect

"Applications of Binding Kinetics to Drug Discovery"

Swinney
Pharm. Med. (2008), *22*, 23-34

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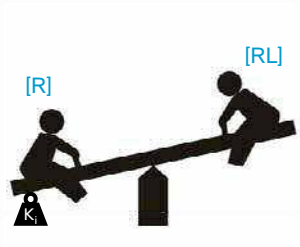
Does a compound bind?

$$[R] + [L] \underset{K_{off}}{\overset{K_{on}}{\rightleftharpoons}} [RL]$$

Receptor Occupancy

Fraction of Receptor occupied

$$RO = \frac{[RL]}{[\text{Total R}]} \qquad \boxed{RO = \frac{[L]}{[L] + K_i}}$$

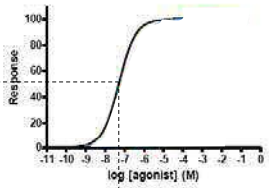


The standard dose-response curve

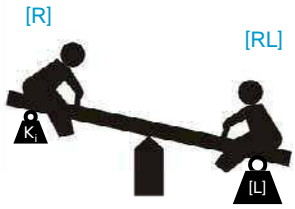
RO = 1

RO = 0.5

RO = 0



[L] = K_i



RO: How far does the seesaw tip?

Applications:

[L] = K_i is a reference point for all subsequent considerations of ligand concentrations

[L] is greater than K_i, more than half of receptors bound and effect likely

[L] is lower than K_i, less than half of receptors bound and effect unlikely

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3

Typically we only measure potency of compounds

Potency (nM) vs k_{on} vs k_{off}

k_{on} M^{-1} s^{-1}	k_{off} s^{-1}								
Very fast association	10^8	10	1	0.1	0.01	0.001			
	10^7	100	10	1	0.1	0.01	0.001		
fast association	10^6	1000	100	10	1	0.1	0.01	0.001	
	10^5	10 µM	1000	100	10	1	0.1	0.01	0.001
slow association	10^4		10 µM	1000	100	10	1	0.1	0.01
	10^3	"inactive"		10 µM	1000	100	10	1	0.1
Very slow association	10^2				10 µM	1000	100	10	1
		1	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}
Dissociation half-lives			7 s	11 min		19 h		80 days	

A simple binding measurement gives no clue to how fast the association and dissociation are

Very fast associating compounds that are very very dissociating

Medium associating compounds that are medium dissociating

Very slow associating compounds that are very very dissociating

} Are all equipotent

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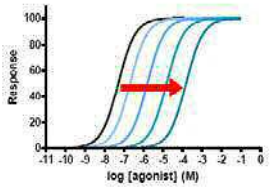
4

Competitive (surmountable) vs Non competitive (unsurmountable)

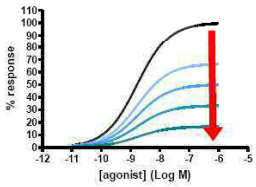


Competitive (surmountable) inhibition

- Normal agonist binding is reduced in the presence of an antagonist
- However this effect can be overcome by larger concentrations of agonist

Black line – normal dose response for agonist
Red arrow – increasing concentrations of antagonist

Blue curves – new dose response curves shifted to the right



Non-competitive (unsurmountable) inhibition

- Normal agonist binding is reduced in the presence of an inhibitor
- Increasing agonist concentration cannot overcome the inhibitor effect

Black line – normal dose response for agonist
Red arrow – increasing concentrations of antagonist

Blue curves – new dose response curves shifted down

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5

The Venture Capital Challenge

- There are 3 Biotech companies
- Each Biotech has identified a biological target which they think are going to provide a breakthough in medicine
- These projects need funding to be able to investigate properly
- The three Biotechs must compete between themselves to win the approval and financial support of the Venture Capitalist

Biotech 1 has identified an over-active enzyme ENZ1 which converts PIM into potentially harmful PAM.
You want to identify inhibitors of this enzyme ENZ1.

Biotech 2 has found a signalling receptor SIG1 which when activated leads to the disease. SIG1 is closely related to SIG2 which is necessary for good health.
You want to identify selective blockers of SIG1.

Biotech 3 has identified a membrane-bound channel signalling protein MEM1. Some patients with incorrect MEM1 function suffer from an embarrassing problem.
You want to identify a easy-to-use treatment which modulates MEM1 activity

- Choose 3 of the strategies listed and think how they might apply to your project.
- Your business case will be built on these 3 strategies
- (If necessary, you can invent whatever other details of the business case you need)
- Each Biotech will select a CEO to present their case to the Venture Capitalist.
- Explain your choice of the 3 strategies and why you think these will be useful to your project.

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The Venture Capital Challenge

Num	Strategy	Notes
1	Set up an X-ray crystallography collaboration to observe compounds bound into the active site of the target	
2	Set up a collaboration to measure ligand binding kinetics at an early stage	
3	Set up an computational chemistry group to model ligand-target transition states in silico	
4	Look for a kinetic selectivity approach based upon long residence time in the target	
5	Look for long resident ligands to overcome any pharmacokinetic deficiencies of your molecules	
6	Look for short resident – purely competitive (surmountable) ligands	
7	Look for ligands with a very clean profile, i.e. very selective potency for the desired target	
8	Look for non-competitive (unsurmountable) ligands to overcome high concentration of natural substrate	
9	Look for the most potent ligands possible (at least sub nanomolar)	
10	Look for compounds with an excellent oral pharmacokinetic profile to give a once-daily tablet	
11	Look to make a degradable drug which lasts a very short time in the body	
12	Look to give multiple daily intravenous injections as the patients' disease is life-threatening	
13	Plan to give high doses of your drug to ensure its effects last all day long	

Case Study 3

María Marco

GlaxoSmithKline

Dr. Maria Marco was born in Madrid, Spain, and obtained her B. Sc. in Organic Chemistry from the Complutense University of Madrid in 1999. She then spent a year working for GlaxoWellcome in Madrid as a graduate trainee, working a protein kinase C project.

After that, she moved to King's College London to carry out her Ph.D. on the development of new synthetic methodologies for metal–mediated organic synthesis under the supervision of Dr. Nicholas E. Leadbeater.

On completion of her Ph. D., she started working for GlaxoSmithKline in Tres Cantos, Spain in the antimalarial research field as a medicinal chemist. She has worked on Falcipains and *P. falciparum* DHODH targets as well as phenotypic approaches.

Recently, the focus of her research interests has moved to other Neglected Diseases, such as Human African Trypanosomiasis, Chagas and Leishmaniasis working in collaboration with other institutions in the discovery of novel antikinetoplastida drugs.

Fat is not a moral problem. It's an oral problem. Influence of lipophilicity in drug discovery

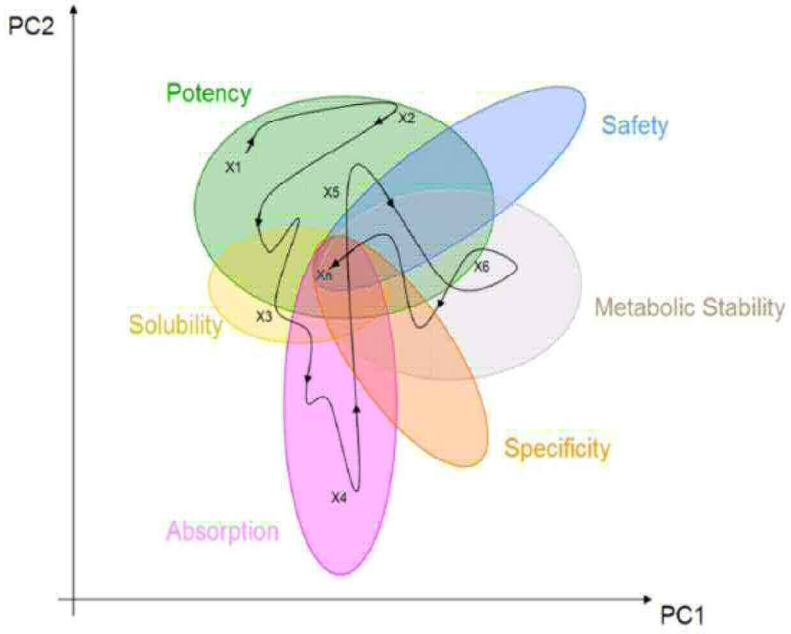
Maria Marco
Principal Scientist. Diseases of the Developing World. GlaxoSmithKline

What characteristics should an oral drug have? In these days where development of medicines suffer from extremely high attrition rates, tools are needed to select the best possible compounds before entering clinical phases in where no further modifications on the molecule take place.

Over the past decades, potency was one of the principal drivers during the drug discovery process. Despite an overall high number of clinical candidates during these years, failure at clinical stages has been also very high and the number on NCEs reaching the market has decreased.

Successful and failed drug candidates and discovery compounds have extensively been analyzed resulting in a better understanding of the role of physicochemical properties in drug attrition.

Measured and calculated physicochemical properties can be used early in the lead optimisation process to drive their chemistry to a successful outcome. Impact of these properties on solubility and other DMPK attributes will be exemplified through representative case studies.



Case Study 4

Antonio Guzmán Esteve

Graduate in Biological Sciences (1990) and Ph.D. in Genetics (2008) from the Autonomous University of Barcelona, and EUROTOX registered Toxicologist. After a short stay in a preclinical CRO, he joined ESTEVE in 1991 as Toxicology Department Scientist, being involved in all aspects of the preclinical safety assessment of new drug candidates from early candidate selection to regulatory toxicology studies. In 2002 was appointed Head of the Toxicology Department, being responsible for all preclinical safety assessment activities conducted during the drug discovery and development phases and post-approval activities, preparation of technical and regulatory documents, and interacting with regulatory agencies. He is member of the Spanish Toxicology (AETOX), Environmental Mutagenicity (SEMA) and Laboratory Animal Sciences (SECAL) Societies, invited speaker as Preclinical Toxicology expert in several University Master Degrees and Scientific Meetings and author of several publications on the preclinical safety assessment of drug candidates in peer reviewed journals.

SAFETY ASSESSMENT IN DRUG DISCOVERY AND DEVELOPMENT

Antonio Guzman

Head of the Toxicology Department. Esteve

Toxicology testing is a pivotal component of the drug discovery and development process. It is a complex research field that implies a wide number of experimental disciplines aiming to characterize the toxicological properties of a potential drug candidate. During the early phases of lead optimization and candidate selection, the conducted toxicology screening studies should allow to screen out those compounds with toxicological properties precluding further development, or to prioritize follow-up confirmatory studies. Regulatory toxicology studies are conducted both in advance and in parallel to the conduct of clinical studies, and their aim is to characterize the toxicological properties of the drug candidate, to estimate potential risk for human toxicities and to identify parameters for clinical monitoring of potential adverse effects. The conducted toxicology studies should allow to identify the inherent toxicological properties of the test substance (hazard identification), and provide sufficient safety data for estimating potential risk to the targeted clinical population (i.e., the likelihood that a toxic effect can be produced at an expected exposure level and condition). Consequently, potential adverse effects are assessed on aspects such as organ toxicity, developmental and reproductive toxicity, safety pharmacology, genotoxicity, carcinogenicity, etc. In this context, it is of outmost importance that the conducted studies are supported by adequate chemical characterization of the test substance, so that the potential contribution of drug substance related impurities can be taken into account.

(Dybing E. (2002) Hazard characterisation of chemicals in food and diet: dose response, mechanisms and extrapolation issues. Food and Chemical Toxicology 40, 237–282)

(Lutz Müller (2006). A rationale for determining, testing, and controlling specific impurities in pharmaceuticals that possess potential for genotoxicity. Regulatory Tox. and Pharm. 44, 198–211)

(Snodin DJ (2010). Genotoxic Impurities: From Structural Alerts to Qualification. Organic Process Research & Development, *14,* 960)

Case Study 5

Victor Rubio

Faes Farma S.A.

Academic Background

- MSc in Chemistry (Universidad de Zaragoza)
- PhD (Chemistry, Universidad de Oviedo).

Professional Experience

- 1975–1981: Assistant Professor (Organic Chemisty Department, Universidad de Oviedo)
- 1981–1991: Faes Farma Synthesis Department
- 1992–2008: Area Responsible at Faes Farma Synthesis Department
- 2004–2005: Associate Professor (Organic Chemistry II Department, Universidad del Pais Vasco).
- 2009–Current: Section Chief at Faes Synthesis Department

Teaching Experiencie

- 2004–2005: Associate Professor (Organic Chemistry II Department, Pais Vasco University)
- Participation at numerous postgraduate programs.
- Coordinator of the Master Program (Industrial Section)

Other

He is author of numerous patents, he has published seven research articles, and he has presented many oral and poster communication in congresses.

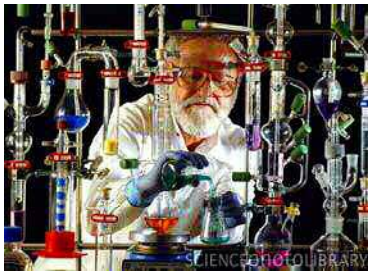
SCALE-UP: PHARMACEUTICALS, FROM MG TO KG

Victor Rubio

Section Chief, Synthesis Department, Faes Farma S.A.

The purpose of a Pharmaceutical company is to discover new chemical entities, NCE, and to launch them into the market. These molecules are first synthesized in the medicinal chemistry laboratory. However, more than 95% of these syntheses cannot be scaled-up to a production plant. For this purpose, the development chemist must design new and safer synthetic routes using low-cost raw materials with a minimal waste output, suitable for scale-up.

"Scale-up: Pharmaceuticals, from mg to kg"



Víctor Rubio, Faes Farma S.A.

Tres Cantos, June 2013

The estimated cost of taking a drug from discovery to market is about $ 750 million and can take between 10 and 12 years ($350 in 1997, $500 in 1999)

"The cost of bringing a drug to market is over a billion dollars"Per Lindberg , 2006

B.L.Robeson, Business Briefing: Pharmatech 2002, April, 72-77(2002)

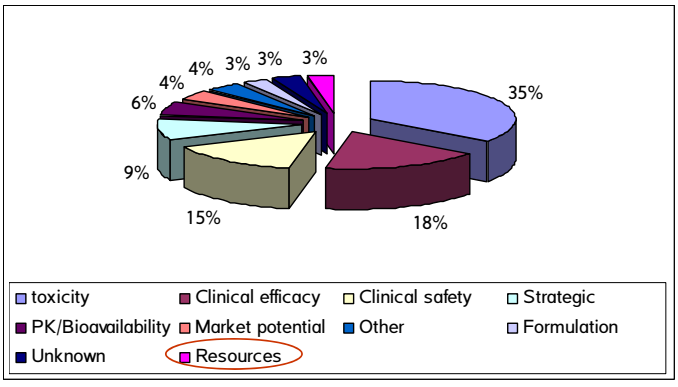
NCE introduction

No compounds	> 3000	100	10	1 or 2
Cost	20%	20%	60%	
Phase	Discovery	Pre-Clinic	Clinic	
Amount	10 mg – 1 g	10 g – 1 kg	10 – 100 kg	
Site	Laboratory	Kilolab	Pilot plant	Plant
Type of synthesis	Expedient	Practical	Efficient	Optimal

David Newman, National Cancer Institute

The need for Chemical Development

Terminations

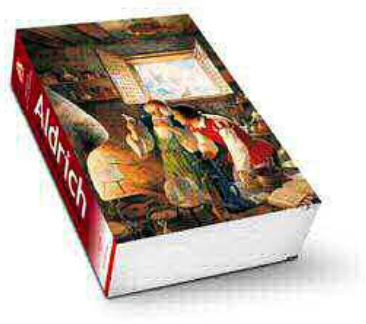


Causes for attrition. Primary reasons for New Chemical Entity (NCE) termination in 2002–2006 (average across all phases of R&D)

Objective: **Convergent and safe synthetic routes using low cost raw materials,with a minimal waste output, suitable for scale up must be designed**

"If we can make 1 g how come you have so much trouble producing 1 Kg"

Most research procedures cannot be scaled up **!!!**



Operations that cannot be readily used on scale

Forbidden for safety

Forbidden solvent

Drying over solid desiccants

Concentrating to dryness

Flash chromatography

To a suspension of **13c** (4.02 g, 20 mmoles) and potassium carbonate (2.76 g, 20 mmoles) in dimethylformamide (20 ml), 4-fluorobenzyl chloride (2.88 g, 20 mmoles) was added. The mixture was heated at 80° for 16 hours and then allowed to cool and poured into ice-cooled water (300 ml). The precipitate was filtered off, washed with water, and dried. The dry solid was dissolved in dimethylformamide (40 ml) and added to a stirred slurry of hexane-washed sodium hydride (0.96 g, 20 mmoles) in dimethylformamide (40 ml) at 0°. The mixture was stirred at 0° for 20 minutes and at 25° for 30 minutes and then treated with a solution of 2-chloroethyl methyl ether (1.88 g, 20 mmoles) in dimethylformamide (10 ml). The mixture was heated at 60° for 8 hours, allowed to cool, poured into water, and extracted with ether. The ethereal extract was washed with water, dried, and concentrated. The residue was subjected to flash chromatography (chloroform/methanol, 95:5) to give **58** as an oil which crystallized as the hydrogen fumarate from 2-propanol (6.91 g, 72%), mp 176-178°; ^{1}H nmr (DMSO d_6): δ 1.9-2.0 (m, 4H), 3.0-3.1 (m,

The ideal process

- ✓ **be safe: safety is always the number one priority**
- ✓ **be eco-friendly**
- ✓ **be reproducible**
- ✓ **fit the plant physically**
- ✓ **produce product (quality and specifications)**
- ✓ **be economical**

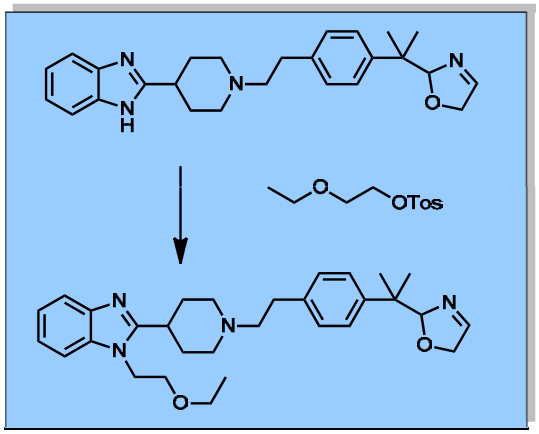
Safety hazards

- ✓ **Thermal instability**
  - Stay below decomposition T of reagents, intermediates
  - Calorimetry of reactions
- ✓ **Toxicity**
  - LD_{50}
  - Carcinogenicity
- ✓ **Flammability**
  - Flash point
  - Vapor pressure

Thermal instability

DMF + **HNa** and HEAT

Safety



16 *batches* of 25 kg

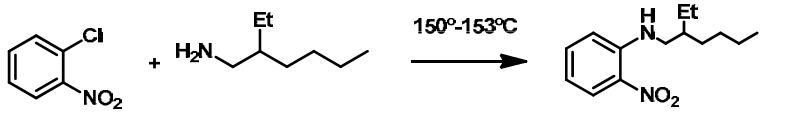
Solvent	Bazse	Temp. (°C)	Yield (%)	Purity (%)
DMF	HNa	65	92	87-96

Thermal instability

WHAT IS A RUNAWAY REACTION?

Many industrially useful reactions are heat-producing or "exothermic." A runaway can occur when a chemical reaction produces heat more rapidly than it can be removed from the system. Excess heating further accelerates the reaction, causing the temperature to skyrocket. Components of the mixture may then boil violently or decompose to form gases, and the resulting pressure may cause a vessel rupture or explosion. Generally the larger a reaction vessel is, the more difficult it is to cool effectively, and the greater the risk of a runaway. Even reactions that require heating for initiation are frequently exothermic and may be susceptible to a runaway. Runaway reactions have been responsible for a number of catastrophic chemicals accidents, including those in Seveso, Italy (1976); Bhopal, India (1984); and Lodi, New Jersey (1995).

Thermal instability



Automate Yellow 96 Dye

In 4 portions
3500 L reactor

All at once
7500 L reactor

Temperature-control problems occurred in eight of the 32 previous batches

Morton International Inc. 1998

Toxicity

"Nothing is poison, everything is poison:
the difference is in the dose" (Paracelso, XVI)

LD_{50} (mg/kg)	Toxic	Human Lethal Dose
< 1 (dioxin, 0.020)	Dangerously	A taste
1–50 (NaCN, 6.4)	Seriously	5 mL
50–500 (aspirin, 200)	Highly	29 mL
500–5,000 (NaCl, 3000)	Moderately	0.5 L
5,000–15,000 (vit. C, 11900)	Slightly	1L
> 15,000 (sugar, 29700)	Low	1L

Solvents Limited For Pharmaceutical Use

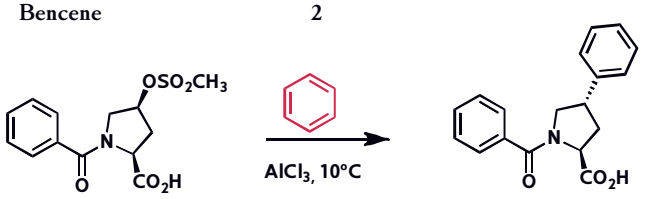
CLASS 1 - Solvents to be avoided (known or suspected carcinogens)		
Benzene	1,2-Dichloroethane	Carbon tetrachloride
1,1-Dichloroethane	1,1,1-Trichloroethane	

CLASS 2 - Solvents to be limited (neurotoxins or teratogens)		
Acetonitrile	1,4-Dioxane	Nitromethane
Chlorobenzene	2-Ethoxyethanol	Pyridine
Chloroform	Ethylene glycol	Sulfolane
Cyclohexane	Formamide	Tetralin
1,2-Dichloroethene	Hexane	Toluene
Dichloromethane	Methanol	1,1,2-Trichloroethene
1,2-Dimethoxyethane	2-Methoxyethanol	Xylene
N,N-Dimethylacetamide	Methylbutyl ketone	
N,N-Dimethylformamide	Methylcyclohexane	

CLASS 3 - Solvents considered to have low toxic potential at normal pharmaceutical levels		
Acetic Acid	Ethyl Ether	Methyl-t-butyl ether
Acetone	Ethyl Formate	Methylethyl ketone
Anisole	Formic Acid	Methylisobutyl ketone
1-Butanol	Heptane	Pentane
2-Butanol	Isobutanol	1-Pentanol
Cumene	Isobutyl acetate	1-Propanol
Dimethylsulfoxide	Isopropyl acetate	2-Propanol
Ethanol	Methyl acetate	Tetrahydrofuran
Ethyl acetate	3-Methyl-1-butanol	

Toxicity

Class 2 solvents	Limit(ppm)	PDE(mg/día)
Chloroform	60	0,6
Pyridine	200	2,0
1,4-Dioxane	380	3,8
Acetonitrile	410	4,1
Dichlorometane	600	6,0
Methanol	3000	30.0
Bencene	2	



Flammability

solvent	Flash point	Ignition Temperature
Dichlorometane	—	615
Acetone	-18	465
Carbon disulfide	-30	90
Ethyl acetate	-4	427
Ethanol	13	365
Diethyléther	-45	160
Heptane	-4	225
Hexane	-22	260

Safety and flammability

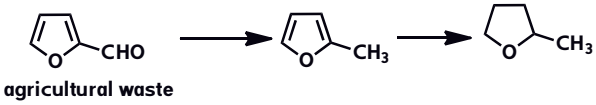
Solvents rarely used on scale

Solvent	Undesirable characteristic	Alternative
Diethylether	Flammability	**MTBE**
Diisopropylether	Peroxide formation	**MTBE**
HMPA	Toxicity	**NMP; NEP**
Pentane	Flammablility	**Heptane**
Hexane	Electrostatic discharges; neurotoxicity	**Heptane**
Benzene	Toxicity	**Toluene**
Chloroform	Mutagenicity; environmental	**Diclhorometane**
CCl_4	Mutagenicity; environmental	**Diclhorometane**
CS_2	Flammability; toxicity	**?**
1,2-Dichloroetane	Cancer suspect agent	**Dichlorometane**
Ethylene glycol	Toxicity	**1,2-Propanodiol**
$HOCH_2CH_2OR$	Toxicity	**1,2-Propanodiol**
1,2-dimethoxyethane	Teratogenicity	**Diethoxymethane**
Dioxane	Cancer suspect agent	**Diethoxymethane**

Useful solvents on scale-up

	Dielectric Constant ε_r	P.E. (ºC)	P.E water in azeotrope (ºC)		Dielectric Constant ε_r	P.E. (ºC)	P.E water in azeotrope (ºC)
Agua	80,1	100		Acetato metilo	7,1	56	56(5%)
MeOH	33,0	65	N.A.	MIBK *	13,1	117	88(24,3%)
1,2 propanodiol *	27,5	188	N.A.	DEM *	7,3	85	76(10,5%)
EtOH	25,3	78	78(4%)	Acetato etilo	6,1	77	70(8,5%)
AcOH	6,2	118	N.A.	THF	7,5	66	64(5,3%)
n-BuOH	17,8	118	93(43,5%)	2-MeTHF *	7,0	77	71(10,6%)
i-PrOH	20,2	82	80(12,6%)	Acetato isopropilo *	5,7	89	77(10,6)
CH_3NO_2	38,3	101	86(23,6%)	PhCl	5,7	132	90(28,4%)
CH_3CN	36,6	81	76(14,2%)	Acetato isobutilo	5,1	117	71(16,5%)
DMSO	47,2	189	N.A.	Dioxano	2,2	101	87(17,6%)
DMF	38,3	152	N.A.	MTBE	4,5	55	88(4%)
t-BuOH	12,5	83	80(11,8%)	$(EtO)_2CH_2$	2,5	88	53(10%)
NMP *	32,6	204	N.A.	Tolueno	2,4	111	75(13,5%)
Acetona	21,1	56	N.A.	Et_3N	2,4	89	75(10%)
t-AmOH	5,8	102	87(27,5%)	Xilenos	2,0	137-144	93(45%)
CH_2Cl_2	8,9	40	38(1,5%)	Heptano	1,9	98	79(12,9%)
Piridina	13,3	115	94(43%)	Ciclohexano	2,0	81	69(9%)

2-Me-THF



agricultural waste

Penn Specialty Chemicals. Bulk

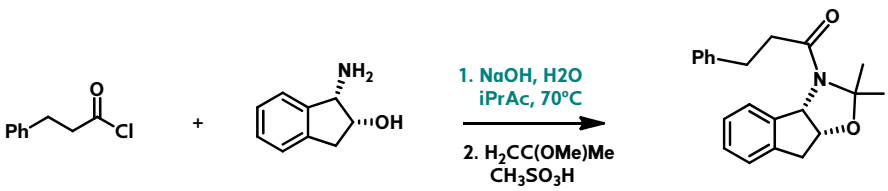
	MeTHF	THF	Éter
Cte. dielectric	6,97	7,5	4,4
mom. dipolar	1,38	1,69	1,11
sol. H_2O (g/100g)	4	mis.	1,2

P.Eb.	80,2°C
density	0,854
F.P.	–11
sol. in water (20°C)	14
sol. water in MTHF	4
azeotrope P.Eb	71
azeotrope % water	10,6

GRIGNARD	electrof.	THF	MeTHF
BrPh	butanal	73	75
ClPh	butanal	76	71
1-Cl-But	MEK	72	73
$BrCH_2Ph$	MEK	20	76
2BrFurano	$BOEt_3$	78	75

Grignard
Reformatky
$AlLiH_4$
Couplings
Biphasic (CH_2Cl_2 sustitute)
Useful for extraction.

isopropyl acetate



Schottten-Baumann in IPA at 70°C

CRIXIVAN intermediate

Chimia **1997**,51, 306

ACS Publications

Organic Process Research & Development

"The journal encourages researchers to consider the environmental consequences of the way in which they perform their experiments and to minimize waste."

and

"From 2012 the policy on use of organic solvents has been changed to discourage scientists from using particular solvents and to encourage them to seek alternatives wherever possible; papers containing strongly undesirable solvents (e.g., benzene, carbon tetrachloride, chloroform, HMPA, carbon disulfide, etc.) will only be considered if accompanied by an analysis of alternatives or if a convincing justification for such use is presented."

2012 Scope and Editorial Policy, found in Information for Authors under Submission & Review at the OPRD home page, http://pubs.acs.org/journal/ oprdfk

The ideal process: be safe

Safety hazards

- ✓ **Thermal instability**
  - Stay below decomposition T of reagents, intermediates
  - Calorimetry of reactions
- ✓ **Toxicity**
  - LD_{50}, carcinogenicity
- ✓ **Flammability**
  - Flash point
  - Vapour pressure
- ✓ **Explosiveness**
  - dust explosion test, hammer test
  - weak bonds between heteroatoms O-O, N-N, N-O
  - compounds that can eliminate N_2, O_2, NO or NO_2

The ideal process

- ✓ be safe:
- ✓ be eco-friendly
- ✓ be reproducible
- ✓ fit the plant physically
- ✓ produce product (quality and specifications)
- ✓ be economical

"One of the main criteria of any chemical activity must be respect for human health and the environment "

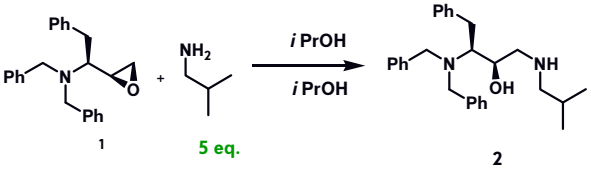
eco-friendly

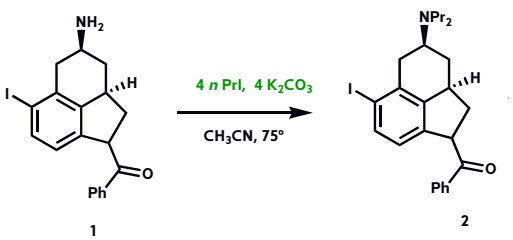
Improving the "greeness" of the process

Reduce waste at the end of the pipeline	Minimize waste at the source
✓ Recycling solvents and reagents	✓ Using "clean" chemistry
✓ Minimize the amount of solvents and reagents	✓ Using water as solvent
✓ Avoiding toxic solvents and reagents	✓ Using unconventional energy sources
✓ Avoiding solvents with low boiling points	✓ Using catalytic process

No. of equivalents

an excess of 2-10% over the limiting reactant is generally used but..





Yield
Work-up
Impurities

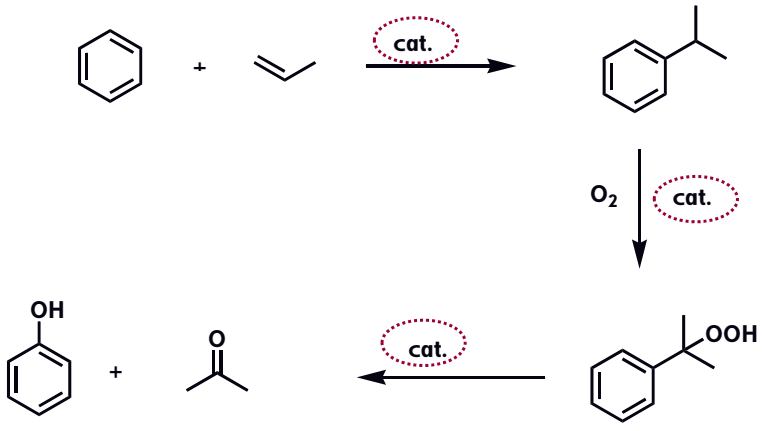
eco-friendly

WASTE GENERATION

Industrial sector	Production metric tones	Waste kg/ kg product
Refinery	$10^6 - 10^8$	0,1
"Bulk chemicals"	$10^4 - 10^6$	< 1–5
"Fine chemicals"	$10^2 - 10^4$	5–50
Pharmaceuticals	$10 - 10^3$	25 –100

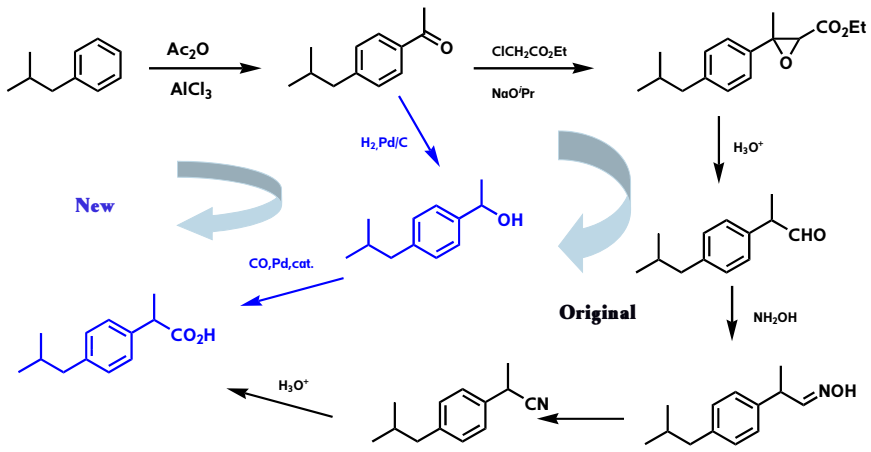
eco-friendly

WASTE GENERATION
BP process for PHENOL



eco-friendly

IBUPROFEN SYNTHESIS

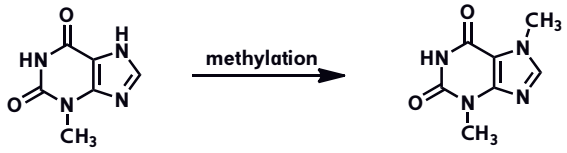


This improved synthesis won the Presidential Green Chemistry Challenge: 1997 Greener Synthetic Pathways Award

eco-friendly

THEOBROMINE SYNTHESIS

PTC



3-methylxantine — theobromine

Original process

DMS in ethanol
Cooling water : 6,300m³/Tm
Energy : 44 kw/hr
Incineration : 7,400 kg/Tm
Yield : 81.5%

New process

MeCl in PTC
Cooling water : 1.340m³/Tm
Energy : 4 kw/hr
Incineration : 50 kg/Tm
Yield : 88%

Production Integrated Environmental Prtection and Waste Management in the Chemical Industry, Wiley. **1999, pp 57** **HOECHST**

The ideal process

- ✓ be safe
- ✓ be eco-friendly
- ✓ be reproducible
- ✓ fit the plant physically
- ✓ produce product (quality and specifications)
- ✓ be economical

Identify and define all critical process parameters

Some plant conditions are difficult to simulate in the laboratory (stirring efficiency, rate of heat transfer, T gradient, surface area/volume)

Keep within specified limits

Write master procedures

Reproducible

on repetition & on scale up

Validation

The ideal process

- ✓ **be safe**
- ✓ **be eco-friendly**
- ✓ **be reproducible**
- ✓ **fit the plant physically**
- ✓ **produce product (quality and specifications)**
- ✓ **be economical**

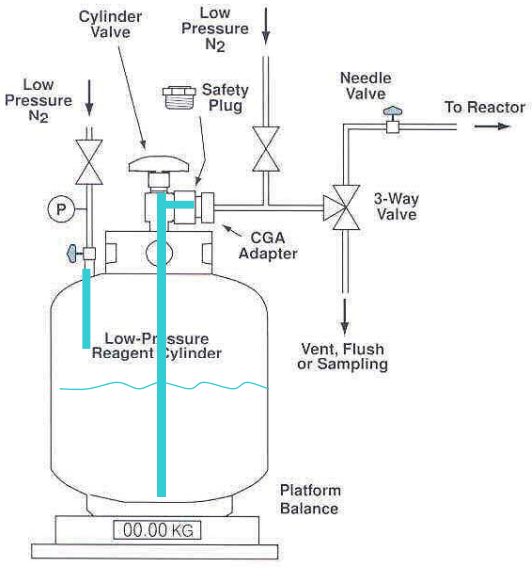
- ❒ **Reactants:** Must flow in and out of reactors
- ❒ **Volumes:** Solvent/solute 5:1
  Avoid extreme total volume changes
- ❒ **Temperatures:** Adjusted so that the plant can achieve them in a specific reactor
- ❒ **Process timing:** Most operations take longer on a plant scale (not reactions)
  Reactions and workup must fit into the workday of the plant
- ❒ **Distillations:** Temperature and vacuum must be adjusted to plant capabilities
  Avoid evaporating to dryness
- ❒ **Crystallizations:** Define cooling rate
  Define stirring rates and seeding
- ❒ **Filtrations:** Avoid hot filtrations
  Eliminate pastes or fine crystals
- ❒ **Extractions:** Emulsions must be avoided
  Separation times and no. of extractions minimized

fit the plant physically

	Laboratory		Scale-up	
	Common	Easy	Common	Easy
Rotavapor	X	X		
Concentrate to dryness	X	X		
Use of flammables solvents	X	X		
Column chromatografy	X	X		X
Solid dessicants (Na_2SO_4)	X	X		X
Azeotropic drying		X	X	X
Controlled additions		X	X	X
Use of dangereous reactives (BuLi, ICH_3)				

fit the plant physically

Setup for Charging Hazardous Reagents



fit the plant physically

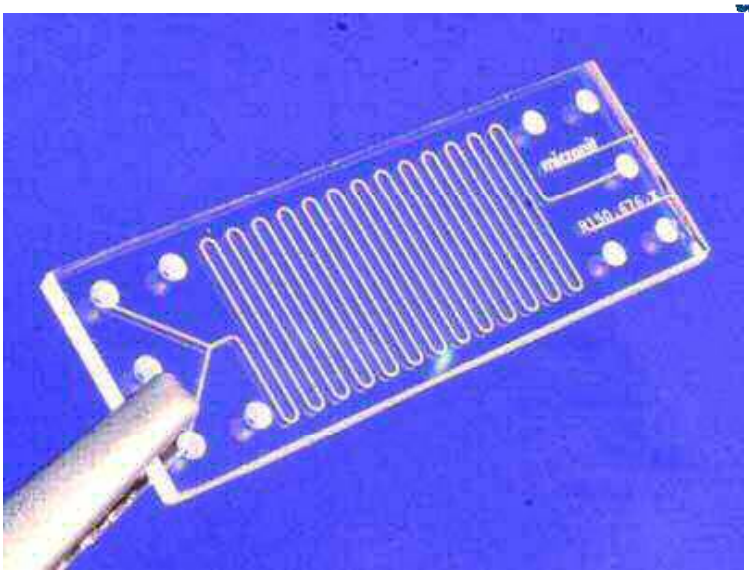
Reactor size and area/volume relationship

Reactor	radius	height	Transfer area	Area/volume (cm^2/cm^3)
100 mL	2,9 cm		104 cm^2	1.042
5 L	10,6 cm		1412 cm^2	0,282
20 L	16,8 cm		3564 cm^2	0,178
100 L	28,8 cm		10416 cm^2	0.104
1140 L	64 cm	3,5 m	70,4 ft^2	0.058

18 times more difficult

Datos de Pfaudler, Inc.

fit the plant physically



Glass Microreactor. The channels of the chip in the picture are 150 µm wide and 150 µm deep.

fit the plant physically

Reactor size and area/volume relationship

Reactor	radius	height	Transfer area	Area/volume (cm^2/cm^3)
100 mL	2,9 cm		104 cm^2	1,042
5 L	10,6 cm		1412 cm^2	0,282
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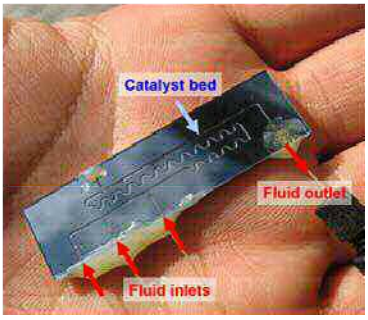
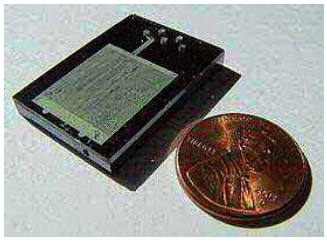
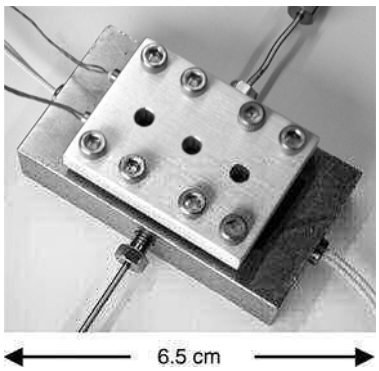
100 L	28,8 cm		10416 cm^2	0,104
1140 L	64 cm	3,5 m	70,4 ft^2	0,058
Microreactors				**200**

Datos de Pfaudler, Inc.

4. ADAPTABLE

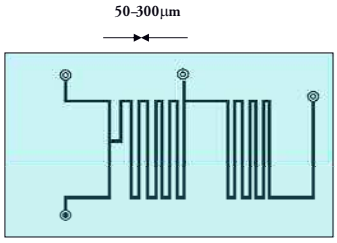






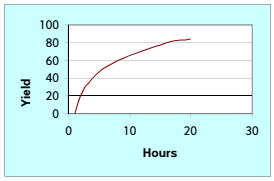
fit the plant physically

MICROREACTORS



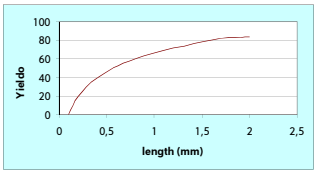
Nº Reynolds		
Turbulent regime	> 3000	→ Mixing by stirring
Laminar regime	< 2000	
Microreactores	2-200	→ Mixing by diffusion

Area/volume cm^2/cm^3	
1000 L	0,05
100 mL	1
Microreactor	200



Batch

Time controlled reaction



Microreactor

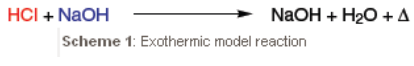
Length controlled reaction

P.Wattsand C.Wiles, Chem.C0mmun., **2007, 443-467**

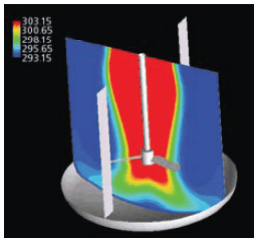
fit the plant physically

MICROREACTORS

- Exothermic control
- Impurity control
- Better selectivity
- Use of dangerous substances
- Management of unstable intermediates
- "numbering-up" instead of "scale-up"
- No hot spots

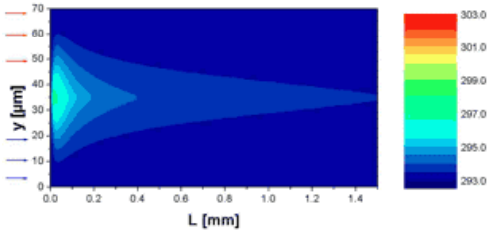


Scheme 1: Exothermic model reaction



Heat distribution in a batch synthesis reactor

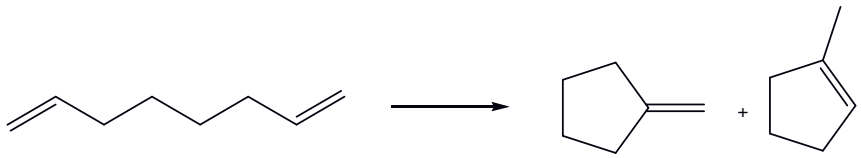
3ºK versus 60ºK



Heat distribution in a microreactor

fit the plant physically

MICROREACTORS



ALDRICH 4 kg/ day

5 %
batch

Impurity control

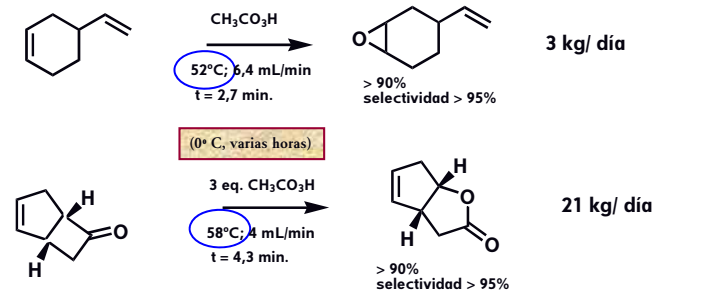
Diazonium salts ⟶ Dyes

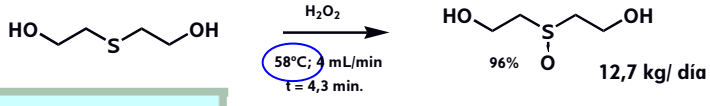
CLARIANT 80 Tonnes/ year

Use of dangerous substances

fit the plant physically

MICROREACTORS

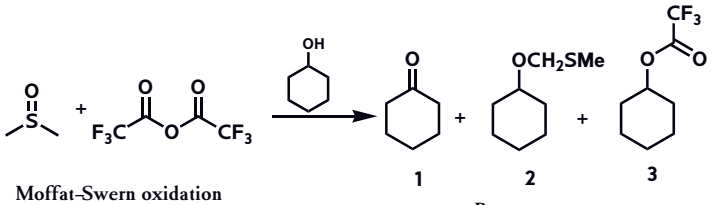




Exotherm control

SYNTHACON (founded in 2003) (synthacon.biz), plant for 20 Tm/year

MICROREACTORS



Moffat-Swern oxidation

Pumerer reagroupment

	Batch		Microreactor		
T°	-70°	-20°	-20°	0°	20°
1	85	19	88	89	88
2	10	2	6	7	5
3	5	70	5	1	2

Exotherm control

P.Wattts et al.Org.Biomol.Chem. ***2007****, 5, 727*

The ideal process

- ✓ be safe
- ✓ be eco-friendly
- ✓ be reproducible
- ✓ fit the plant physically
- ✓ produce product (quality and specifications)
- ✓ be economical

The ideal process: Produce product that meets specifications

Specifications

- Melting point
- Color of solution
- Loss on drying
- Residue of ignition
- Particle size
- Polymorphism
- Solvates
- Chemical purity
- Sterochemical purity
- by-product content
- heavy-metal content
- pH

Ensure that final product meets quality specifications

MINIMIZE IMPURITIES

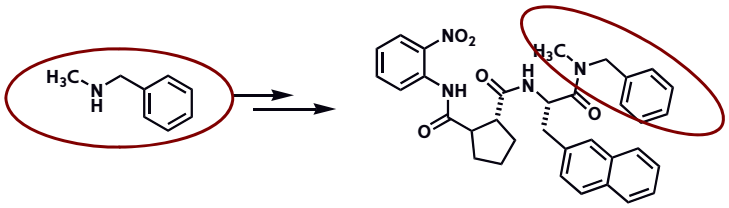
quality

Meet specifications

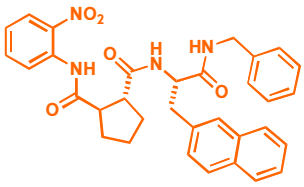
Unknown impurity (> 0,1%)

STRATEGY

- Isolate the by-product by prep. HPLC
- Determine its structure by NMR and MS
- Propose a mechanism for its formation
- Identify the synthetic step in wich it forms
- Alter the reaction conditions to minimize the undesirable by-product

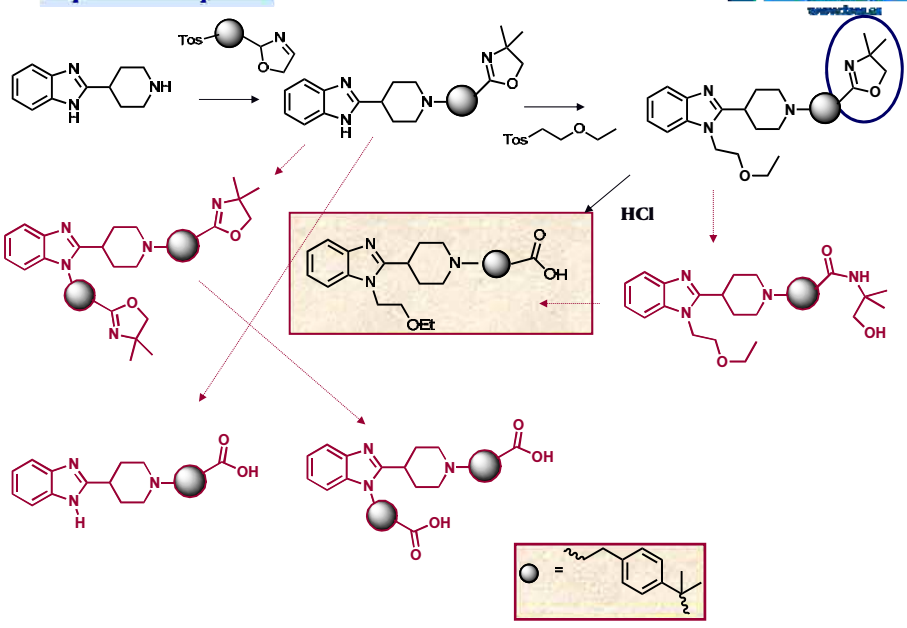


SDZ NKT343 NOVARTIS



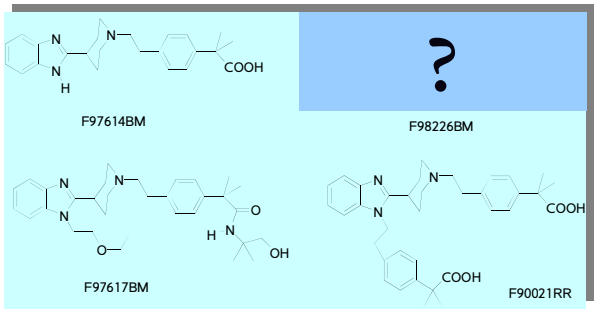
M.Prashad et al Org.Process Res.Dev., **1999, 3**, 409

Impurities development

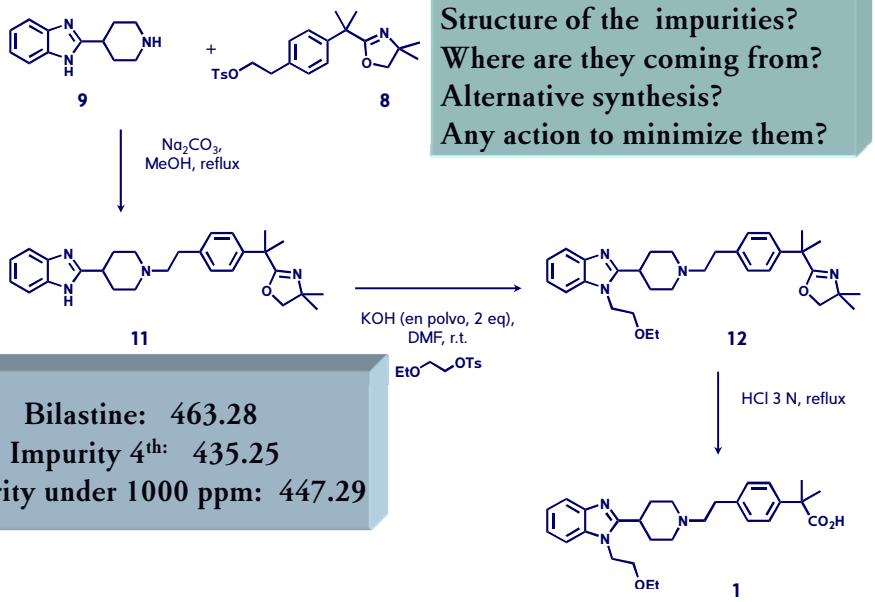


Impurities development

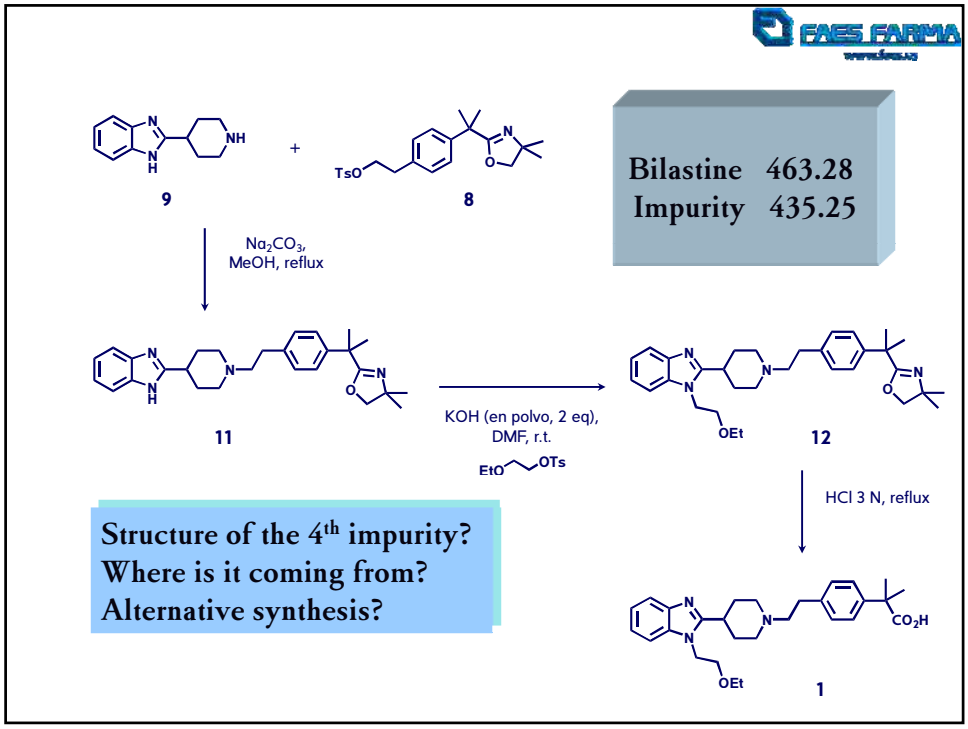
Four impurities > 0.10%
Seven impurities between 10–1000 ppm

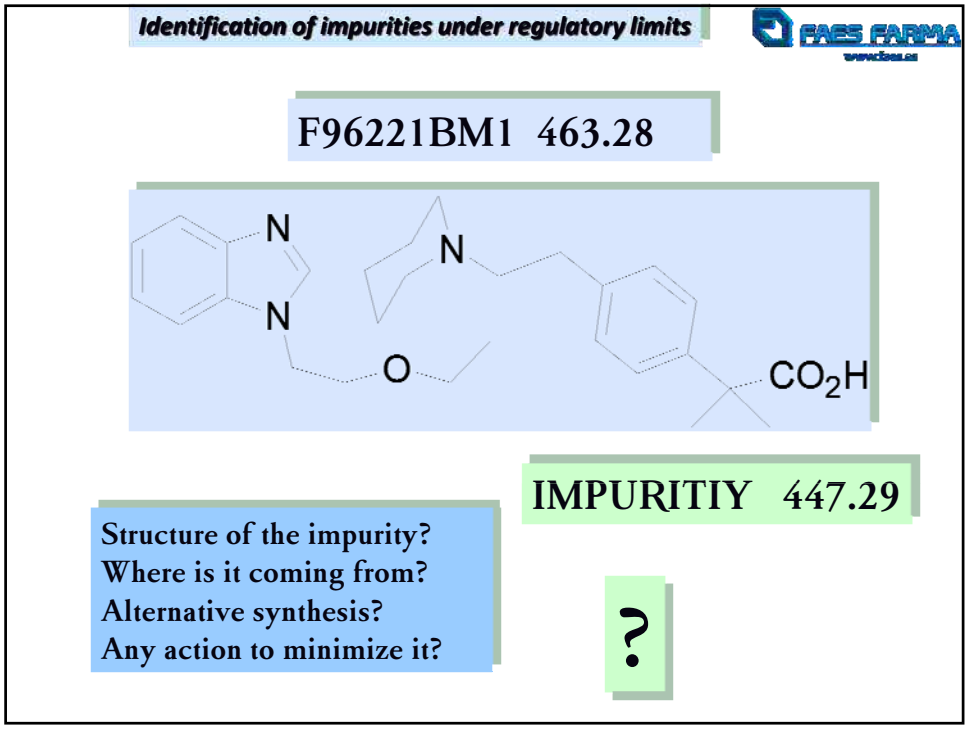


Structure of the impurities?
Where are they coming from?
Alternative synthesis?
Any action to minimize them?



Bilastine: 463.28
Impurity 4th: 435.25
Impurity under 1000 ppm: 447.29


9
8
Na_2CO_3, MeOH, reflux
11
KOH (en polvo, 2 eq), DMF, r.t.
EtO OTs
12
HCl 3 N, reflux
1
Bilastine 463.28
Impurity 435.25
Structure of the 4th impurity?
Where is it coming from?
Alternative synthesis?


Identification of impurities under regulatory limits
F96221BM1 463.28
IMPURITIY 447.29
Structure of the impurity?
Where is it coming from?
Alternative synthesis?
Any action to minimize it?
?

The ideal process

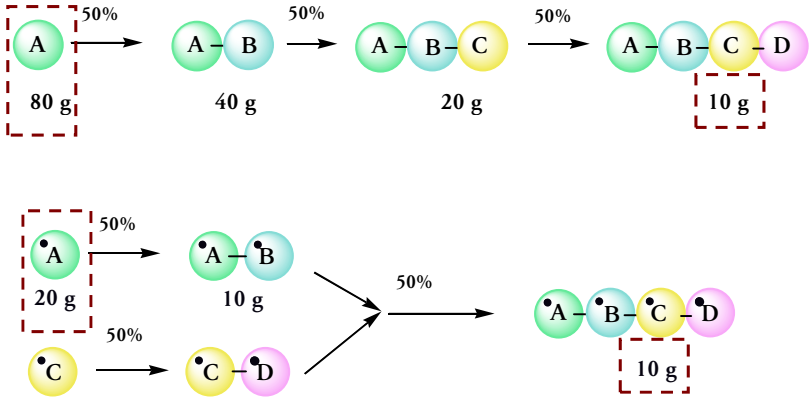
- ✓ **be safe**
- ✓ **be eco-friendly**
- ✓ **be reproducible**
- ✓ **fit the plant physically**
- ✓ **produce product (quality and specifications)**
- ✓ **be economical**

How to make a process less expensive ???

- ❒ Improving reaction yields
- ❒ Lowering the cost of raw materials and reagents
- ❒ Looking for convergent synthesis

economical

CONVERGENT SHYNTHESIS

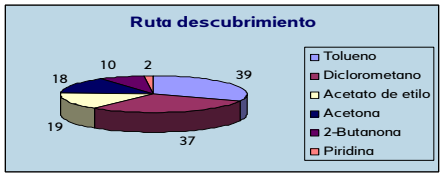


economical

Commercial Route to Sildenafil

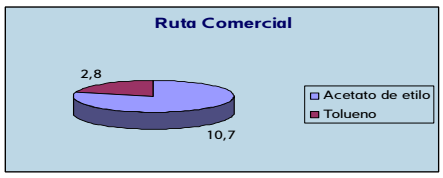
⇨ **Medicinal Chemistry Route**

- 9 linear steps
- Low yield: 7.5%
- Potentially toxic materials in the last step
- 125 L of solvents/Kg of product
- Large amounts of waste streams generated



⇨ **Commercial Route**

- ✓ Convergent synthesis
- ✓ High yielding process 75.8%
- ✓ Safe and robust route
- ✓ Volume of solvents minimized 13.5 L/Kg
- ✓ Excepcionally low environmental impact



Org. Proc. Res. & Dev. **2000**, *4*, 17-22

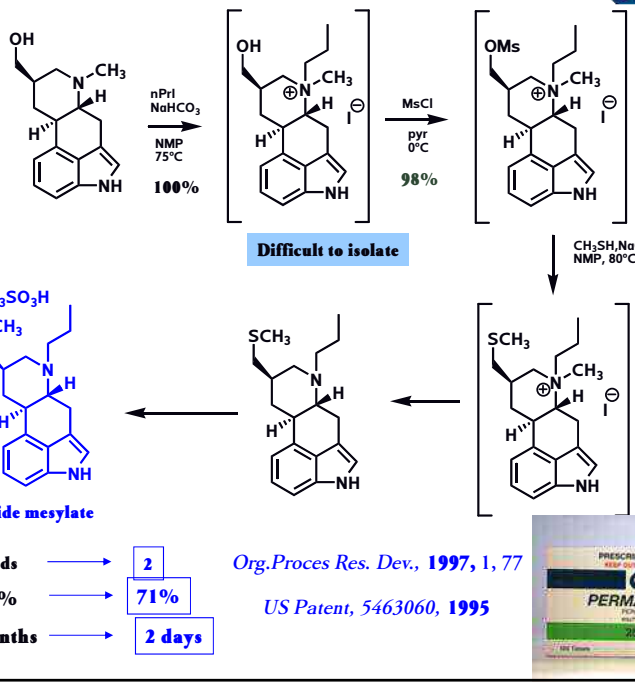
The ideal process: be economical

How to make a process less expensive ???

- Improving reaction yields
- Lowering the cost of raw materials and reagents
- Looking for convergent synthesis
- Telescoping reactions

telescoping

PERGOLIDE MESYLATE



18 solids → 2

15-23% → 71%

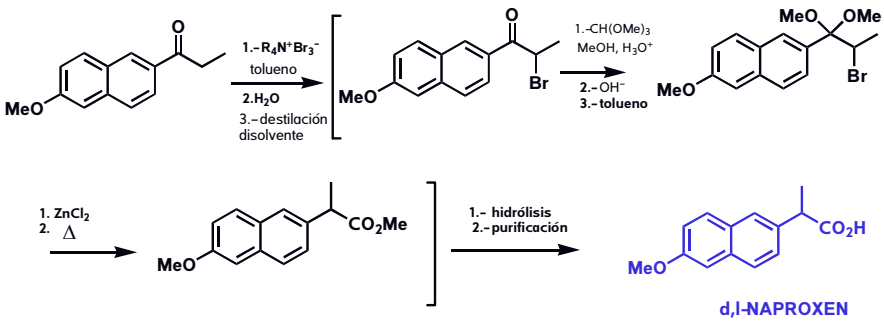
4-6 months → 2 days

Org.Proces Res. Dev., **1997**, 1, 77

US Patent, 5463060, **1995**

telescoping

NAPROXEN



d,l-NAPROXEN

50% ⟶ 90% (10 años)

The ideal process: be economical

How to make a process less expensive ???

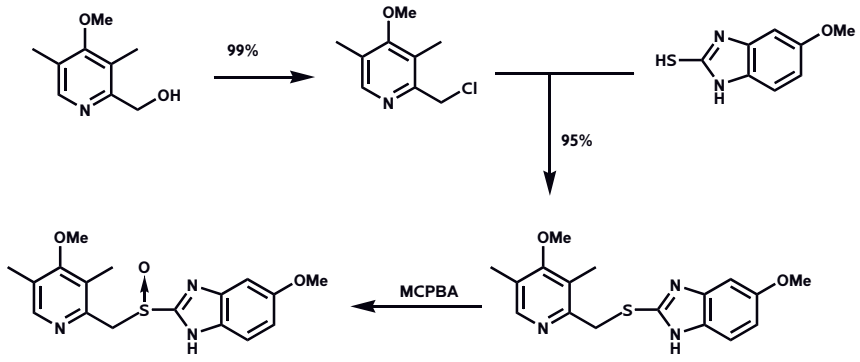
- ❒ Improving reaction yields
- ❒ Lowering the cost of raw materials and reagents
- ❒ Looking for convergent synthesis
- ❒ Telescoping reactions
- ❒ Reducing the no. of synthetic steps ⇒ Most effective

Yield = f (1/e $^{\text{no. steps}}$)

ESOMEPRAZOL

ASTRA ZENECA

OMEPRAZOL ⟶ ESOMEPRAZOL

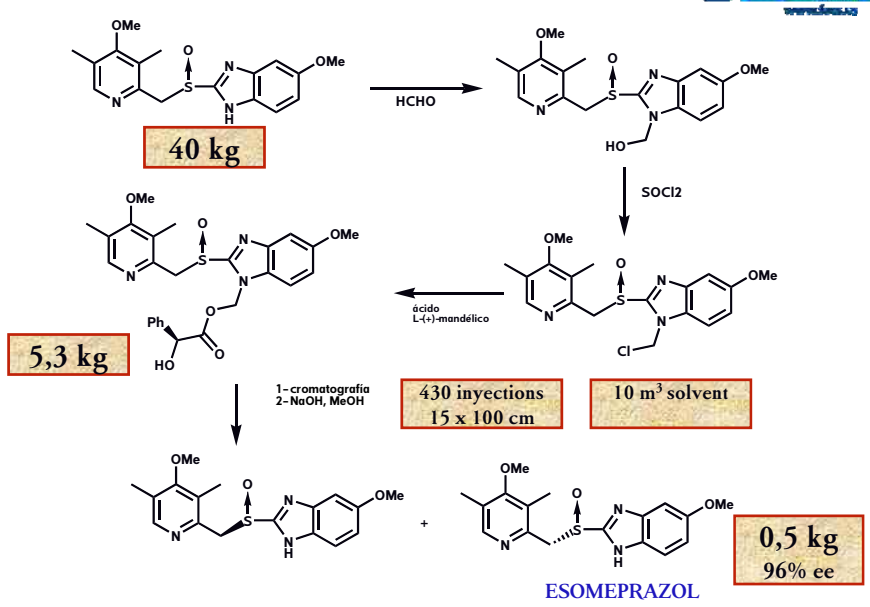


Omeprazol

1960 beginning of the project
1979 1ª synthesis
1988 launch of omeprazol (Losec®)
1990 s 6000 milliones $
2000 launchof esomeprazol (Nexium ®)

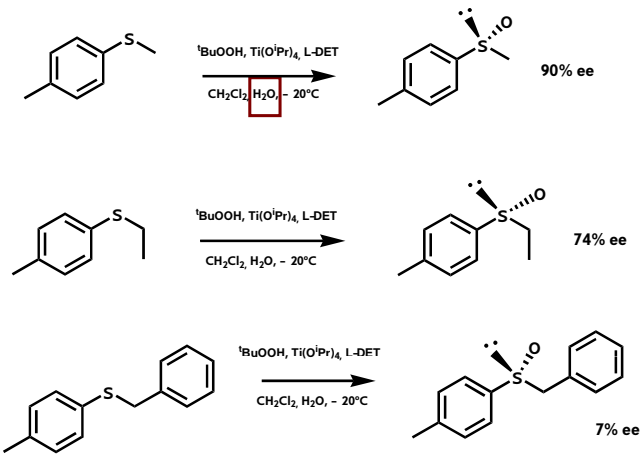
H-J. Federsel, presented at Chiral Europe, Malta, October 2000 **ASTRA ZENECA**

OMEPRAZOL → ESOMEPRAZOL



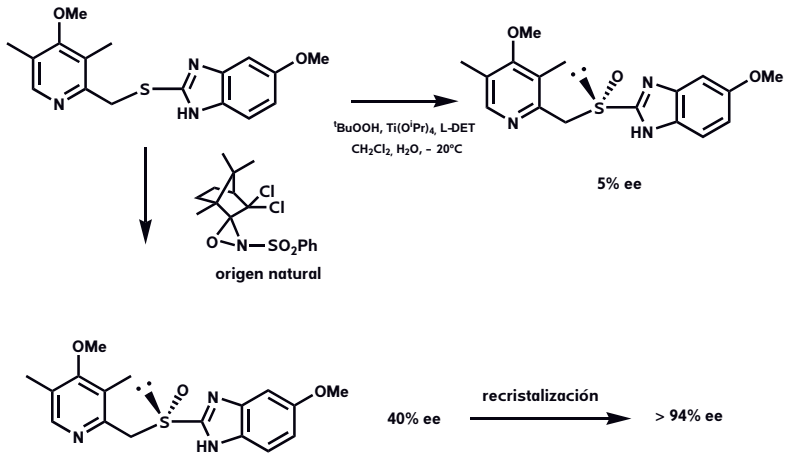
H-J. Federsel, Chirality, ***2003*** **ASTRA ZENECA**

OMEPRAZOL → ESOMEPRAZOL

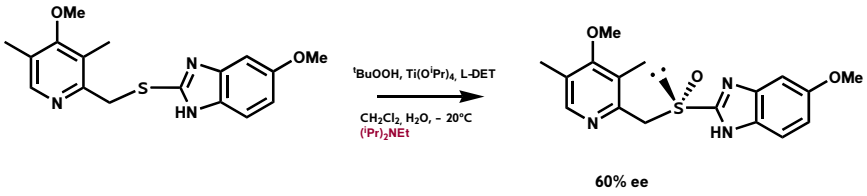


Kagan et al. J.Am.Chem.Soc. ***1984,*** *106, 8188*

OMEPRAZOL ⟶ ESOMEPRAZOL
Primeros estudios clínicos



OMEPRAZOL ⟶ ESOMEPRAZOL
Method of Kagan modified

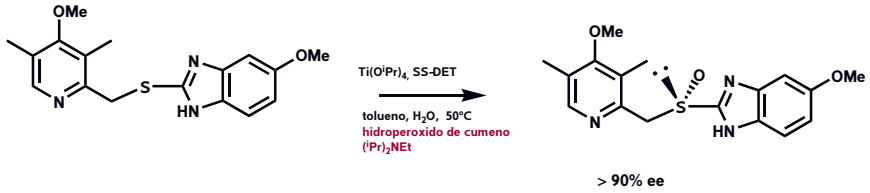


3 months to improve 5%

- The adition of a base improved significantly the ee (Hünigs)
- The amount of water played an important role (0,3–1,0 eq H_2O/Ti)
- The solvent also influenced significantly in the ee(toluene)
- Ti/tartrate relationship must be 1/2

H-J.Federsel en "Asymmetric catalysis on industrial scale" pp 413-436

OMEPRAZOL ⟶ ESOMEPRAZOL
Method of Kagan modified



> 90% ee

1–Heat a solution of sulfide, titanium isopropoxide (0,04 eq), diethyltartrate and water, in toluene, at 50-55ºC for one hour

2–Add ethyldiisopropylamine and cumene hydroperoxide, at 30ºC.

Yield: >90%

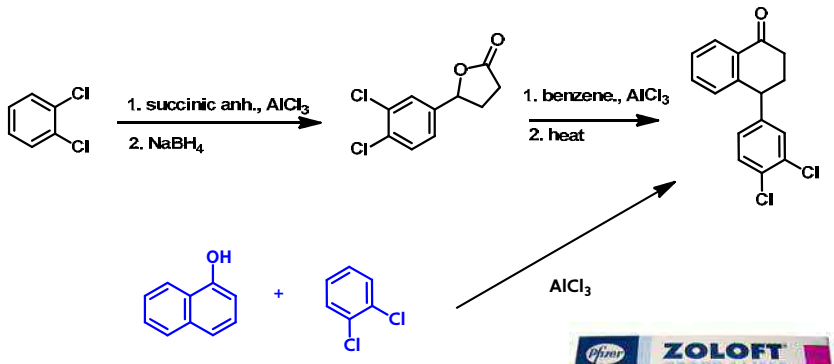
ee: >94%

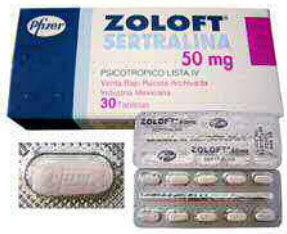
After Mg salt crystallisation: 100% ee

Sales: 6.000 million $

H-J.Federsel en "Asymmetric catalysis on industrial scale" pp 413-436
H.Cotton et al. Asymmetry **2000**, *11, 3819*

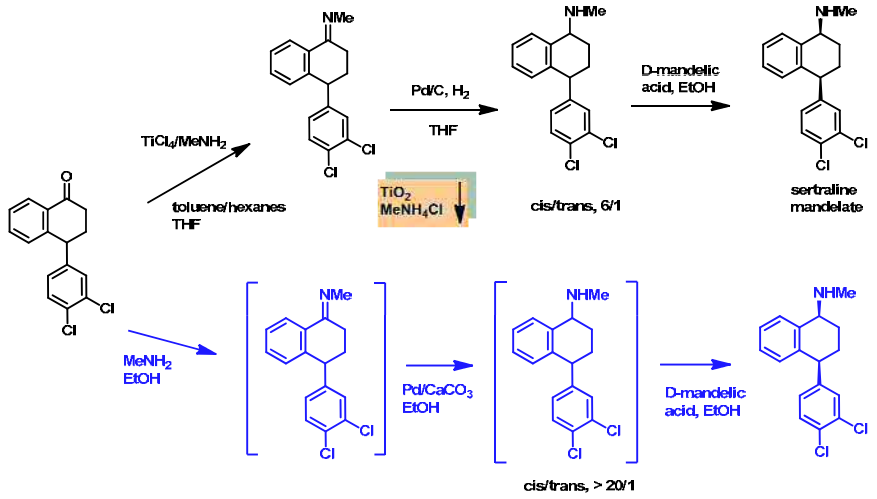
SERTRALINE





J.C.Colberg et al., Org.Process Res.Dev., 8, 385, ***2004*** **PFIZER**

SERTRALINE



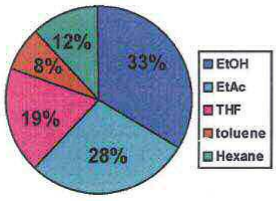
*J.C.Colberg et al., Org.Process Res.Dev., 8, 385, **2004*** **PFIZER**

SERTRALINE

Comparison of solvent utilization

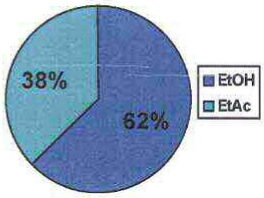
Solvents in L/tons Sertraline-HCl of the old and new process:

Sertraline Hydrochloride First Commercial Route



*EtOH	34,000 L
*EtAc	28,400 L
*THF	19,000 L
*Toluene	8,000 L
*Hexane	12,000 L
Total	101,400 L

Sertraline Hydrochloride New Route



*EtOH	15,000 L
*EtAc	9,000 L
Total	24,000 L

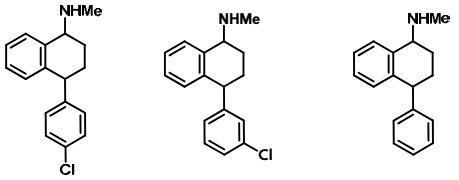
G. P. Taber, D. M. Pfisterer, J. C. Colberg, *Org. Proc. Res. Develop.* **2004**, *8*, 385-388.

SERTRALINE

100 Tm/year

Inhibitor of serotonin uptake
Treatment of depression and other anxiety-related disorders

- Elimination of 440 tons/year of TiO_2-$MeNH_2$.HCl wet-cake waste
- Elimination of 40 tons/year of unwanted trans isomer
- Elimination of 140 tons/year of Ti/Cl_4
- Elimination of 90 tons/year of $MeNH_2$
- Reduction from 5 to 2 solvents
- Reduction from 101,000 L/Tm to 24,000 L/Tm solvents
- Elimination of impurities of over-reduction



Abstracts

P1. EXPLORING THE PHOSPHOTYROSINE PHOSPHATASES PTPA AND PTPB FROM MYCOBACTERIUM TUBERCULOSIS AS TARGETS FOR NOVEL DRUGS TO COMBAT TUBERCULOSIS

Priscila G. Alves Martins [1; 2], Ruben Gonzalez del Rio [2], Pedro A. Torres-Gomez [2], Eva M. Lopez-Roman [2], Louise D. Chiaradia-Delatorre [1], Maria Esther Perez-Herran [2], Alfonso Mendoza-Losana [2], Hernán Terenzi [1]

[1] *Centro de Biologia Estrutural Molecular (CEBIME) – Universidade Federal de Santa Catarina (UFSC) – Florianópolis, Santa Catarina, Brazil*

[2] *GlaxoSmithKline – Diseases of the Developing World (DDW)– Tuberculosis– Tres Cantos, Madrid, Spain*

Tuberculosis (TB) is an airborne infection disease that affects mainly the lungs, it is caused by the bacillus *Mycobacterium tuberculosis* (Mtb). There were 9 million new cases and 1.4 million estimated TB deaths in 2011 (WHO, 2012). During the infection Mtb secretes two proteins tyrosine phosphatases (PTP), PtpA and PtpB, which are reported to be fundamental to its pathogenicity (BACH et al., 2008; ZHOU et al., 2010). Due to the marked importance of both PTPs for the survival of Mtb within the macrophage, these two proteins appear as promising targets of new drugs against TB. Recently, six new potent inhibitors demonstrated to have competitive inhibition and low-micromolar range IC_{50} activity against these Mtb enzymes *in vitro* (CHIARADIA et al., 2011). The evaluation of activity and toxicity of those inhibitors in *in vitro* assays is the main objective of this work. To investigate the toxicity a serial dilution of the compounds (ranging from 0.1 to 50 micromolar) was assayed in HepG2 and THP1 cell cultures. The minimum inhibitory concentrations (MICs) for the six compounds were also measured in *Mycobacterium bovis* BCG and Mtb H37Rv using concentrations ranging from 0.23 to 500 micromolar. The evaluation of activity and toxicity of the PtpA and PtpB inhibitors showed that two compounds out of the six (43 and 95) presented reasonable therapeutic window, and one (96) showed low micromolar antitubercular activity (MIC values: 6.8 μM towards *Mycobacterium bovis* BCG and 7.8 μM towards Mtb H37Rv). Further analyses to address their activity in activated macrophages infected with *Mycobacterium bovis* BCG are in progress.

Global Tuberculosis Report 2012, World Health Organization. Available in http://apps.who.int/iris/bitstream/10665/75938/1/9789241564502_eng.pdf (acessed April 28 2013).

Bach, H.; Papavinasasundaram, K. G.; Wong, D.; Hmama, Z.; Av-Gay, Y. Mycobacterium tuberculosis Virulence is mediated by PtpA dephosphorylation of human vacuolar protein sorting 33B. Cell Host Microbe 2008, 3 (5), 316-322)

Zhou, B.; He, Y.; Zhang, X.; Xu, J.; Luo, Y.; Wang, Y.; Franzblau, S. G.; Yang, Z.; Chan, R. J.; Liu, Y.; Zheng, J.; Zhang, Z. Y. Targeting mycobacterium protein tyrosine phosphatise B for antituberculosis agents. Proc. Natl. Acad. Sci. U.S.A. 2010, 107 (10), 4573-4578.

Chiaradia, L. D.; Alves Martins, P. G.; Cordeiro, M. N. S.; Guido, R. V. C.; Ecco, G.; Andricopulo, A. D.; Yunes, R. A.; Vernal, J.; Nunes, R. J.; Terenzi, H. Synthesis, biological evaluation, and molecular modelling of chalcone derivatives as potent inhibitors of Mycobacterium tuberculosis protein tyrosine phosphatases (PtpA and PtpB). Journal of Medicinal Chemistry. 2011,55, 390-402.

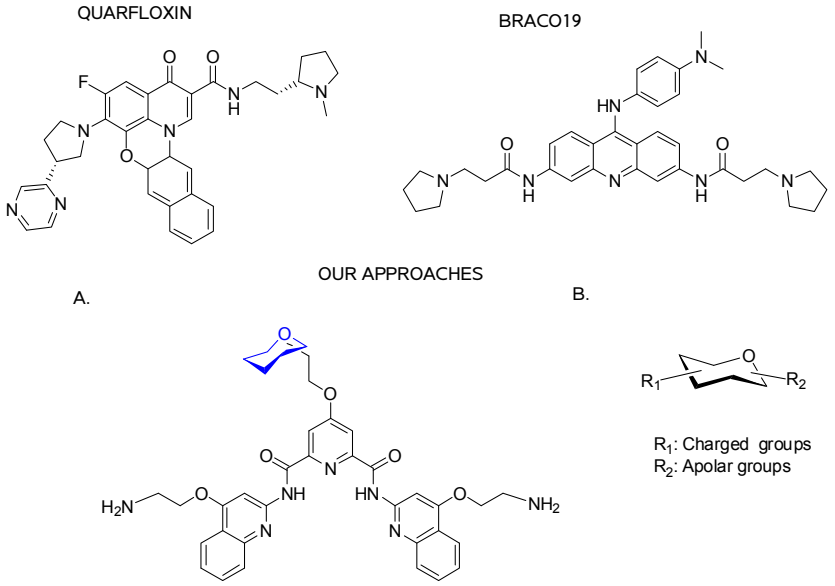
P2. G-QUADRUPLEX LIGANDS BASED ON CARBOHYDRATES AS ANTICANCER AGENTS

Matilde Arévalo-Ruiz, Juan Carlos Morales

Department of Bioorganic Chemistry, Institute of Chemical Research, CSIC – University of Seville, 49 Americo Vespucio, 41092 Seville, Spain

G-quadruplex are tertiary structures of DNA, which have raised an increasing interest, according to their biological importance [1]. They are found in the human telomeric sequence and other proto-oncogens expression regulation areas, as in *c-myc* [2] and c-kit [3], so their regulation can be associated with control of cancer processes.

A variety of molecules have been developed to stabilize those structures, acting as anticancer drugs, such as quarfloxin or BRACO-19 [4]. This stabilization takes place through aromatic stacking between aromatic areas and G-quartet planar surface (π-π interactions) and through electronic interactions with the phosphate groups in DNA. All these structures have in common their high molecular weight and hydrophobicity, features inadequate for pharmacologic applications. We propose designing new structures in which pharmacokinetic features are improved trying to respect Lipinski's rule of five. We will follow two strategies: a) incorporation of carbohydrate motifs into several aromatic scaffolds in order to improve solubility and binding selectivity and b) use of carbohydrate units as the central scaffold of the drug. Our group has shown how natural and apolar carbohydrates can stack onto DNA duplex and G-quadruplex structures via CH-π interactions [5-7]. Therefore we proposed that carbohydrate scaffolds could be used for drug design. So far, only glucose and galactose have been attached to pyridostatin aromatic derivatives [8-9].



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(6) R. Lucas, E. Vengut-Climent, I. Gómez-Pinto, A. Aviñó , R. Eritja, C. González and J. C. Morales. *Chem. Commun.*, 2012, 48, 2991–2993.
(7) I. Gómez-Pinto, E. Vengut, R. Lucas, A. Aviñó, R. Eritja, C. Gonzalez, J. C. Morales, *Chem. Eur. J.* 2013*, 19,* 1920-1927.
(8) S. Müller, D. A. Sanders, M. Di Antonio, S. Matsis, J.-F. Riou, R. Rodrigueza and S. Balasubramanian, *Org. Biomol. Chem.*, 2012, 10, 6537–6546;
(9) M.D. Antonio, G. Biffi, A. Mariani, E-A. Raiber, R.I Rodriguez, and S. Balasubramanian. *Angew. Chem. Int. Ed.* 2012, 51, 1–7.

P3. DE NOVO DESING, SYNTHESIS AND VALIDATION OF A HELICAL PEPTIDE LIBRARY OF INTEREST IN THE SEARCH FOR PROTEIN–PROTEIN MODULATORS

Beatriz Balsera, Mª Ángeles Bonache, Mª Jesús Pérez de Vega and Rosario González Muñiz.

Instituto de Química Médica, Juan de la Cierva 3, 28006-Madrid.

The modulation of protein-protein interactions (PPIs) is a very attractive and challenging approach for the discovery of new drugs, due to the relevant role that they play in a number of biological processes of therapeutic relevance, like cell growth and differentiation.1 When the structure of the targeted proteins is unknown, a valid approach to identify new modulators is the evaluation of diverse compounds.

The α-helix motif is very often implicated in the intercommunication of associated proteins, playing a pivotal role in many PPIs. Quite frequently the key contacts between these helical motifs and the complementary helix-binding sites are mediated by hydrophobic residues.2-4

A collection of 81 linear peptides (13-mer), designed to stabilize α-helices and bearing hydrophobic residues at 5, 9 and 12 positions, have been synthesized. Conformational studies have confirmed their tendency to adopt the expected helical structures. This collection has been validated using well known PPIs such as p53-HDM22 and VEGF-VEGFR-13, and led to the identification of compounds able to modulate some TRP channels.

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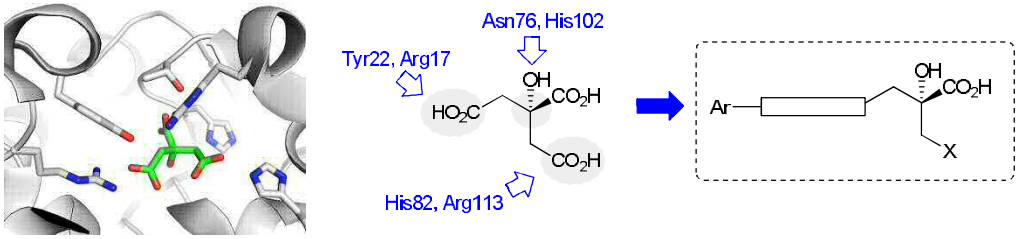
P4. CITRIC ACID DERIVATIVES TARGETING AN ESSENTIAL ENZYME IN HELICOBACTER PYLORI

Beatriz Blanco, Adrián Robles, Antonio Peón and Concepción González-Bello*

Centro Singular de Investigación en Química Biológica y Materiales Moleculares (CIQUS), Universidad de Santiago de Compostela, c/ Jenaro de la Fuente s/n, 15782 Santiago de Compostela, Spain.
www.gonzalezbello.com; *e-mail: concepcion.gonzalez.bello@usc.es

Helicobacter pylori is a major cause of gastric and duodenal ulcers and it has been classified as class I carcinogen. Recent studies have revealed that eradication rates are at the lowest levels seen in the past decade and are likely to fall further as antimicrobial resistance becomes more prevalent worldwide.[1] Therefore, the development of new agents that are able to overcome existing resistance mechanisms or that have novel mechanisms of action is much needed. In the past few years, our research group has been studying the possible development of new antibiotics whose mode is based on the selective and effective inhibition of the shikimic acid pathway, in particular by the inhibition of the third enzyme of the pathway, the dehydroquinase enzyme (DHQ2), which is essential in *H. pylori*. We became interested in finding new inhibitors structurally different from those developed to date, specifically, substrate analogs and mimetics of the reaction intermediate.[2] Our starting point was citrate, which proved to be a poor competitive inhibitor of the enzyme with a K_i value of 2.5 mM.[3] Based on our previously reported crystal structure of DHQ2 from *H. pylori* at 2.75 Å (PDB code 2XB9)[4] and recently comparative binding energy analysis,[5] we have developed new citrate analogs of improved activity. In this communication, we will present our latest results in this project.



Acknowledgements
Financial support from the Xunta de Galicia (10PXIB2200122PR and GRC2010/12) and the Spanish Ministry of Science and Innovation (SAF2010-15076) is gratefully acknowledged. BB and AP thank the Spanish Ministry of Science and Innovation for their respective FPU fellowships.

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P5. DRUGS4RARE: DRUG DISCOVERY FOR RARE DISEASES

Daniel Blasi[1], Mercè Reig[1], Nikita Remez[2], José Manuel Brea[3], Maria Isable Loza[3], Jordi Mestres[2], Jordi Quintana[1]

1Drug Discovery Platform (PDD), Parc Cientific de Barcelona, Barcelona, Spain.
2Chemogenomics Laboratory, Research Program on Biomedical Informatics (GRIB), IMIM Hospital del Mar Research Institute – Universitat Pompeu Fabra, Barcelona, Spain.
3USEF Screening Platform. Centre of Research on Molecular Medicine and Chronic Diseases (CIMUS), Universidad de Santiago de Compostela (USC), Santiago de Compostela, Spain.

We have developed a platform for Drug Discovery for Rare Diseases, Drugs4Rare, with three main actions: a) to gather information on the chemical structures, published mechanisms of action (including biological targets), and orphan diseases, associated to chemical compounds of therapeutic interest; b) to carry out a virtual polypharmacology analysis for each chemical compound of therapeutic interest, predicting its interactions with a large number of biological targets, thus defining a virtual chemical biology matrix for each compound; and c) to validate experimentally the highest affinity predicted chemico-biological interactions.

The results of these three consecutive actions are deployed in an integrated chemical biology annotated Drugs4Rare database, which contains key virtual and experimental information in the drug discovery process for new orphan drugs for rare diseases, or for repurposing of therapeutically active compounds towards rare diseases.

In order to generate the Drugs4Rare Drug Discovery platform, we have analyzed 49 chemical compounds which have been approved in Europe for the treatment of certain rare diseases. After gathering information about these compounds and associated rare diseases from the Orphanet database (www.orpha.net), we analyzed the information on their chemical structures, biological targets, and mechanisms of action in public (Pubchem, ChEMBL) and private access (SciFinder, Integrity) databases. Then we used the virtual polypharmacology predictive platform developed in the Chemogenomics Laboratory at IMIM, which allowed us to generate a profiling of the 49 compounds in front of around 4500 biological targets. Analysis and prioritization of this virtual chemical biology matrix, based on the commercial availability of the selected compounds, and on the availability of biochemical assays for the highest affinity predictions, led us to the selection of three compounds, for which experimental validation of new biological targets was determined.

We are currently expanding this initial “proof of concept” of our Drugs4Rare Drug Discovery Platform to additional compounds from the Orphanet database, as well as to other compounds of therapeutic interest.

P6.NOVEL 5-HT6 RECEPTOR ANTAGONISTS/D2 RECEPTOR PARTIAL AGONISTS TARGETING BEHAVIORAL AND PSYCHOLOGICAL SYMPTOMS OF DEMENTIA

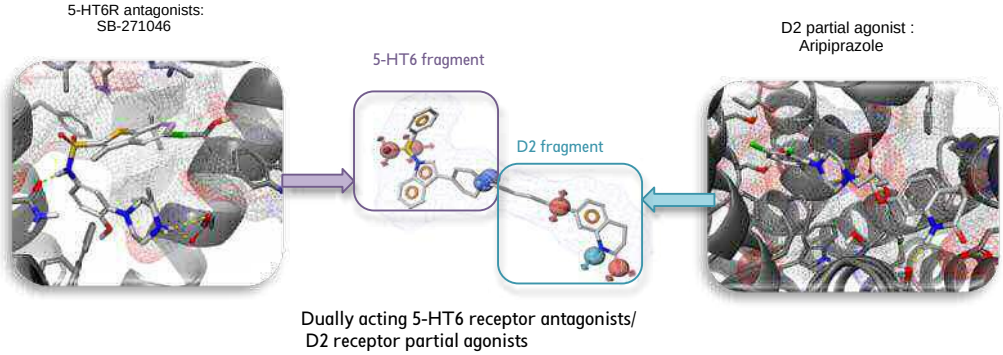
Marcinkowska Monika,[1] Bucki Adam,[1] Pawłowski Maciej,[1] Wesołowska Anna,[1] Mierzejewski Paweł,[2] Bieńkowski Przemysław[2] and Kołaczkowski Marcin[1,3]

[1]Jagiellonian University, Medical College, Cracow, Poland; [2]Institute of Psychiatry and Neurology, Warsaw, Poland. [3]Adamed Ltd., Pieńków, Poland;

All dementia patients suffer from impairment of cognitive functions and up to 90% of them show also behavioral and psychological symptoms (BPSD) such as: depression, anxiety, agitation, aggression, irritability or psychosis. Those symptoms were found to be even more disturbing than cognitive decline and are the most common cause of patient's institutionalization. In view of lack of specific treatments, BPSD have been commonly treated using antipsychotic drugs, which display only partial efficacy. Moreover, they were found to exacerbate preexisting cognitive deficits, as well as cause serious cardiovascular and motor side effects, and thus are not approved for the treatment of BPSD [1]. Therefore, development of an effective and safe therapy of BPSD remains an increasing clinical and social unmet need.

Pharmacological studies revealed procognitive role of 5-HT6 receptor (5-HT6R) antagonists, and indicated their potential anxiolytic and antidepressant-like activity [2,3] Moreover recent clinical findings confirm their utility in treatment of Alzheimer's disease [4]. Similarly, a growing body of evidence suggests the high therapeutical potential of D2 receptor (D2R) partial agonists as both antipsychotic and antidepressant agents, with a favorable safety profile [5].

In this study we present design, synthesis and pharmacological evaluation of a series of innovative hybrid molecules acting as 5HT6R antagonists and D2R partial agonists. Based on molecular modeling studies we combined indoleamine moieties characteristic for 5-HT6 antagonists with aryloxy fragments providing D2 partial agonism [6].



A series of molecules was synthesized and characterized for affinity towards 5-HT6, D2 and M3 receptors as well as hERG channels. The most promising compounds displayed a desired profile of 5-HT6/D2 activity with only a negligible affinity for antitargets. Lead molecules were characterized in rodent models of anxiety and depression and possessed a more favourable activity then the selective 5-HT6R antagonist SB-271046. Pharmacological profile of novel indoleamine-based hybrid molecules indicates their relevance for BPSD drug discovery.

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P7. New Improved BM212 MmpL3 Inhibitor Analogues: In Vitro and in Vivo Biological Evaluation against Mycobacterium Tuberculosis

Giovanna Poce,[1] Robert H. Bates,[2] Salvatore Alfonso,[1] Martina Cocozza,[1] Giulio Cesare Porretta,[1] Lluis Ballel,[2] Joaquin Rullas,[2] Fatima Ortega,[2] Alessandro De Logu,[3] Emanuela Agus,[3] Baojie Wae,[4] Scott G. Franzblau,[4] Fabrizio Manetti, [5] Maurizio Botta, [5] and Mariangela Biava [1]

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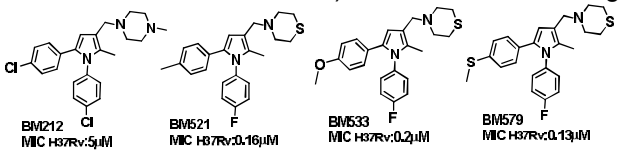
[2]*Desease of Developing World; Tres Cantos Medicines Development Campus GlaxoSmithKline, Severo Ochoa 2, 28760 Tres Cantos, Madrid, Spain;*

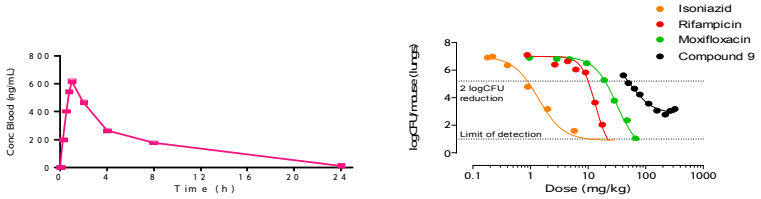
[3]*Dipartimento di Scienze della Vita e dell'Ambiente, Università degli Studi di Cagliari, via Porcell 4, 09124 Cagliari, Italy;*

[4]*Institute for Tuberculosis Research, University of Illinois at Chicago, Chicago, IL 60612, USA;* [5]*Dipartimento Farmaco Chimico e Tecnologico, Università degli Studi di Siena, via Alcide de Gasperi 2, 53100 Siena, Italy.*

Mycobacterium tuberculosis, the causative agent of tuberculosis (TB), infects one third of the world's population and is the second leading cause of mortality worldwide. The number of multidrug-resistant cases (MDR-TB) is continuously increasing, thus a new shorter and simpler drug regimens is needed. In this context, a class of 1,5-diphenyl-pyrroles compounds endowed with high *in vitro* potency against *M. tuberculosis* was identified at Sapienza[1]. Moreover, this family of compounds also showed moderate activity against *M. tuberculosis* in the Low Oxigen Recovery Assay (LORA, MICs ranging from 6.74 to 24.74 μM) that simulates anaerobic conditions of mycobacterium growth. First, a panel of four hit compounds (BM212, BM521, BM533, BM579) was evaluated for drug-like properties.



After identifying the microsomal stability as the mayor issue, morpholine analogues of BM521, BM533, BM579 were synthesized. The replacement of the sulfur atom with oxygen led, in general, to an improvement in the physicochemical properties keeping potency. Therefore, a new set of morpholine derivatives has been synthesised by maintaining the substitution patterns decorating the phenyl rings at both N1 and C5 of the most active previously synthesized derivatives. This new set of compounds was tested for evaluating their *in vitro* potencies (aerobic and anaerobic conditions) and their cytotoxicities against Vero and HepG2 cell lines. Furthermore, drug-like parameters such as lipophilicity, Human Serum Albumine (HSA) binding as well as microsomal stability were evaluated. In general all the new synthesised compounds showed very good potencies under both aerobic and anaerobic conditions. Compounds showed high values of HSA binding across the series with a clear correlation between these values and lipophicities Above all, the introduction of the morpholine moiety led to an improvement of the microsomal stability, with some compounds showing promising results (<2mL/min/g). Finally, to better characterize the potentialy of this family of compounds, the best derivatives in terms of both potency and stability (BM635) was selected for *in vivo* pharmacokinetic and efficacy[2] studies.



This encouraging *in vivo* efficacy data provides an increasing interest for this class of derivatives for further optimisation.

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P8. NEW ANTICANCER AGENTS WITH HYBRID STRUCTURE PYRIDAZINONE DITHIOCARBAMATE

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Toxicity and side effects of anticancer drugs currently on the market justify the need to develop more potent and selective agents against neoplastic cells.
In the search for new drugs, pyridazine ring can be considered a privileged structure because the remarkable spectrum of pharmacological activities (cardiotonic, antihypertensive, antiplatelet, antidepressant, anxiolytic, antiinfective, anticancer etc.) showed by pyridazine derivatives (1). Many of pyridazine analogues are 6-aryl-pyridazin-3(2H)-one derivatives, a structural pattern also common in analogues with anticancer properties. However, pyridazinones with antiproliferative effects have great structural diversity involving the different ring positions (N2, C4, C5 and C6).
Dithiocarbamates also show a variety of biological effects including antiproliferative properties; moreover, they are good coadjuvants for chemotherapy, in particular to prevent renal toxicity or myelosuppression. This has promoted the incorporation of dithiocarbamate fragment into different pharmacophores for anticancer activity to give a number of hybrid structures (2). However, there are no previous data in literature about hybrid compounds pyridazinone dithiocarbamate.
Considering the interesting biological properties of both moieties, a series of poly-substituted pyridazin-3(2H)-ones including different dithiocarbamate groups linked at C6 by an alkyl chain ranged from one to three carbons has been designed as potential anticancer agents (Figure 1).

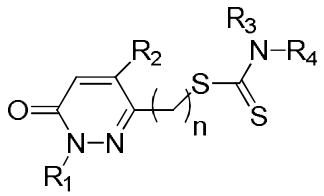

Figure 1. General structure of target compounds

The proposed compounds were synthesized following a multi-step strategy, based on alkylfurans oxidation by singlet oxygen, in which the appropriate 6-hydroxyalkylpyridazin-3(2H)-ones are the key intermediates (3). The hydroxylpyridazinones were converted into the corresponding 6-bromo derivatives, by reaction with CBr4 and PPh3, that finally by a one-pot reaction with different secondary amines and CS2 in the presence of K3PO4 provided the new hybrid analogues in good yield.
The antiproliferative activity of the synthesized compounds was evaluated in vitro against five human cancer cell lines, lung cancer (NCI-H460), cervical cancer (Hela 229), ovarian cancer (A 2789), breast cancer (MCF-7) and promyelocytic leukemia (HL-60). The studied compounds showed a significant and selective anticancer activity against the cell lines corresponding to human solid tumors, with IC50 values in the M range. The results of this study will be discussed.

Acknowledgements
We acknowledge the Xunta de Galicia and the Universidade de Vigo for the financial support.

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P9. MECHANISM FOR VPg INHIBITION BY FUTP IN FOOT–ANDMOUTH DISEASE VIRUS

Sonia de Castro,[1] Cristina Ferrer-Orta[2], Gloria Fernández Cureses,[1] Nuria Verdaguer,[2] Esteban Domingo[3], María-José Camarasa[1]

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Foot-and-mounth disease (FMD) is a highly contagious disease that affects cloven-hoofed animals, including domestic and wild bovids. The virus responsible for the disease is foot-and-mouth disease virus (FMDV) that belongs to the picornavirus family. This kind of viruses use a protein of 20-24 aminoacids, termed viral protein genome-linked (VPg), to initiate viral RNA synthesis. During replication initiation, the first step is the linkage of a UMP to the Tyr3 hydroxyl group of the VPg protein. Thus, virally encoded RNA-dependent RNA polymerase (3D) requires the uridilylated form VPg to act as the primer for both positive- and negative-strand synthesis.

Recent studies in FMDV[1] showed that 5-Fluorouridine triphosphate (FUTP) may act as a potent competitive inhibitor of VPg uridylation. In this way, peptide analysis by mass spectrometry has identified a VPg fragment containing FUMP covalently attached to Tyr, but the molecular basis of this block is still unknown.

In order to investigate this possible novel role for FUMP, the synthesis and X-ray studies of two models of VPg1 that contain U or FU in a 15 mer peptide linked through the hydroxyl group of Tyr3 we will be presented. Interestingly, X-ray co-crystal structure of 3D-pol FMDV/VPg-FU showed a significant conformational change at the β9-α11 loop, protruding into the active site of the polymerase, thus, blocking the access of the template and of the incoming nucleotides.

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P10. EXPLORING THE ORTHOSTERIC nACh RECEPTOR BIDING SITE BY CONFORMATIONAL RESTRICTION OF THE n-ACh AGONIST DMABC

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If we are to talk about classic approaches in Medicinal Chemistry conformational restriction and controlled geometry of ligands is a must. Old but never outdated, this approach is being used for a better understanding of the topography and interactions that occurs at the orthosteric binding site of nicotinic acetylcholine receptors (nAChR). The orthosteric binding site of these ligand-gated ion channels is located in the interface of α-β or α-α subunits. The amino-acid sequences that form this binding site are highly conserved among the different receptor subtypes, therefore achieving a high degree of selectivity turns out to be a challenge that requires a fine tuning in the design of potential selective ligands in order to exploit the small differences present in the receptor cavity.

DMABC is a small synthetic agonist related to acetycholine (ACh) that exhibits a significant selectivity towards the α4β2 subtype. The predicted linear binding conformation of this molecule, similar to that of ACh or epibatidine, was shown to be in disagreement with a recent X-ray crystallography study that revealed a folded conformation of DMABC to ACh-binding protein1. Based on these new findings four series of DMABC analogues, cyclopropane, piperazine/piperidine, diazepane and aminopirrolidine containing derivatives, were designed, synthesized and pharmacologically characterized in a [3H]epibatidine binding assay at the α4β2, α3β2 and α4β4 subtypes and a FLIPR Membrane Potential Blue assay at the α4β2 and α3β4 subtypes

The synthesized compounds represent different degrees of conformational restriction of DMABC, and in general the results reveal strict structural requirements regarding stereochemistry and conformation for activating the nAChRs.

P11. NITROGEN HETEROCYCLES AS POTENTIAL AGENTS ANTI ALZHEIMER

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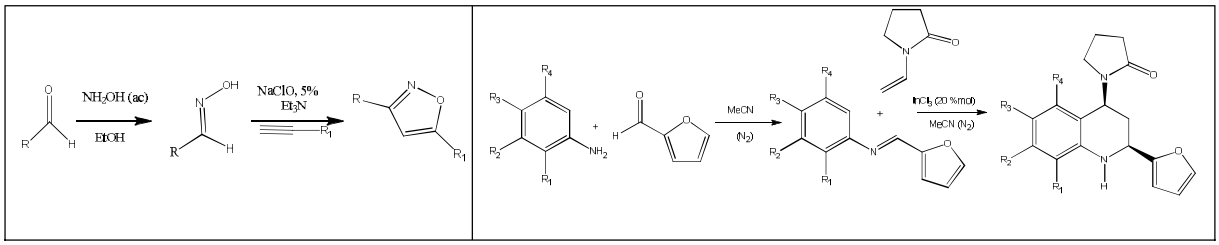
Nitrogen heterocycles are a part of a special group of organic substances due to its many applications in the pharmaceutical market, exist a large number of structures carrying nitrogenous substances. Among these heterocycles the isoxazoles and tetrahydroquinolines[1] have shown significant biological activity on different therapeutic targets.

Alzheimer's disease is a neurodegenerative disease, being the most common form of irreversible dementia[2]. We now know that over 25 million people worldwide suffer from, and annual socioeconomic costs over U.S. $ 200 billion. The current treatments are palliative focusing mainly on the restoration of the levels of acetylcholine, the cholinergic hypothesis is considered as the main therapeutic target for the control of this disease.

In the search for inhibitors of acetylcholinesterase, 9 isoxazoles derivatives were synthesized by 1,3 dipolar cycloaddition and 8 tetrahydroquinolines derivatives were synthesized by Povarov reaction, these compounds were purified by conventional chromatographic techniques and characterized spectroscopically. Anticholinesterase activity was assessed by spectrophotometric measurements, determining the IC_{50} for each compound against the enzyme. For each series was selected the most active compound and determined its molecular interactions with the active site of the enzyme by molecular docking. In order to improve the activity obtained for each series, the pharmacophore of each one of them was subjected to *de novo design* giving new chemical substituents capable of generating improved interactions with the active site, which will be reflected in a diminution IC_{50} of new molecules to be synthesized.

The use of bioinformatics tools helps establish an accurate way binding energies generated between the active site and the inhibitor and it is an additional aid to organic synthesis for drug design.



1,3 dipolar cicloadition Povarov reaction

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P12. AMIDE SUBSTITUTED COUMARINS AS DUAL INHIBITORS OF ACHE AND MAO FOR THE TREATMENT OF NEURODEGENERATIVE DISEASES

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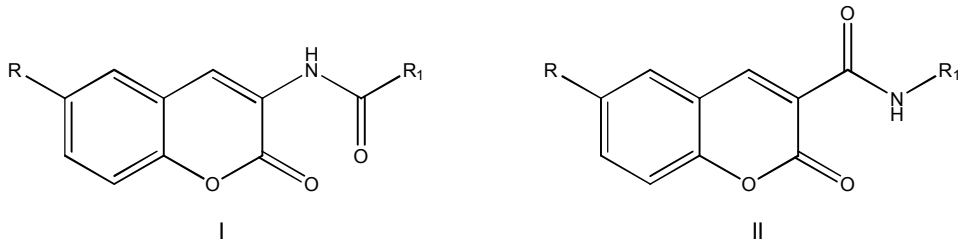
The discovery of new drugs for neurodegenerative diseases is a growing and demanding area. Within this field are described different types and intervention processes, which include the development of monoaminoxidase (MAO) and acetylcholinesterase (AChE) inhibitors, which combined with another drugs are used on therapy for Parkinson's and Alzheimer's diseases. [1,2] These inhibitors allow dopamine and acetylcholine levels in the brain to stabilize or even enhance them, since it's established that both enzymes are responsible for the metabolism of these important neurotransmitters. [1-3]

Coumarins are a large family of compounds of natural and/or synthetic origin that proved to have numerous pharmacological properties. [4] In our group, we have already synthesised multiple novel compounds incorporating the coumarin moiety with remarkable activity towards MAO [3] and/or AChE. [5] In this work, we continue to exploit this scaffold by creating new synthetic methodologies to construct novel *multi-target* inhibitors focused on the treatment of neurodegenerative diseases. As it is shown on fig.1, in this work we centre our attention on amides incorporated at position 3 of the coumarin nucleus.

Some preliminary biological activity results are presented in this communication, while docking studies and further completion of the substituted series are currently in progress.



I II

R= H, -CH_3;
R1= C_6H_6, C_6H_{12}, *p*-$CH_3(C_6H_6)$, *p*-$Cl(C_6H_6)$, *p*-$OCH_3(C_6H_6)$

Figure 1 – 3,6-disubsituted coumarins series.

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P13. NEW INHIBITORS OF FATTY ACID SYNTHASE: VALIDATION AS A THERAPEUTIC TARGET FOR BREAST CANCER TREATMENT

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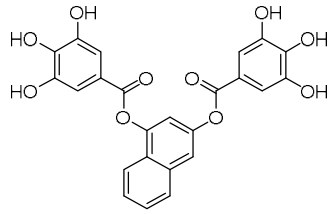
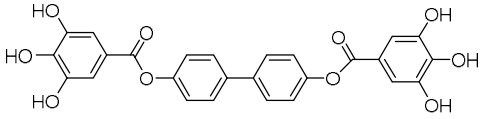
Fatty acid synthase (FASN) is overexpressed in human breast carcinoma and other human cancers.[1] However, the pharmacological use of well-characterized inhibitors of FASN –natural compounds cerulenin and (-)-epigallocatechin-3-gallate (EGCG), and synthetic analogue C75– has been limited due to their chemical instability, poor bioavailability and/or undesirable body weight loss. In a project aimed at the development of new FASN inhibitors with the objective of advancing toward the validation of FASN as a new therapeutic target for the treatment of cancer, compounds UCM028 and UCM056 were identified as hits endowed with antitumor properties.[2]

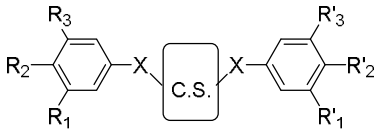


UCM028



UCM056

In the hit to lead process new compounds I were synthesized, maintaining the cyclic subunit (C.S.) present in the identified hits, and considering structural modifications of the spacer (X) and the number and position of the hydroxy groups (R_1-R_3).



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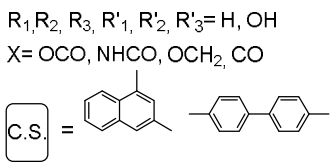

Data obtained from cytotoxicity assays show that the replacement of the ester for an amide, ether or ketone group in the spacer results in a complete loss of cytotoxic activity. Therefore, in the next optimization step the influence of the number and position of the hydroxy groups (R_1-R_3) was studied for ester derivatives (X = OCO). Although some of the compounds exhibited good cytotoxicity values, they were not able to inhibit FASN activity. Therefore, the optimizedmoiety in the new class of FASN inhibitors was found to be a gallate subunit, and previously identified compound UCM028 was selected for further pharmacological characterization. Notably, *in vitro* and *in vivo* PK properties determined for UCM028 indicate a higher metabolic stability than that of natural inhibitor EGCG. Altogether, these results support the suitability of the new FASN inhibitor UCM028 for efficacy studies in breast cancer models. We are currently exploring its full potential in two models of breast cancer: *in vivo* in a xenograft model and *in vitro* in tumor cells with acquired resistance to anti-HER2 drugs.[3] These results will enable the definitive validation of FASN as a therapeutically useful option for cancer treatment.

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P14. 3-ARYLMETHYLENE-2,5-PIPERAZINEDIONES, VERSATILE SCAFFOLDS FOR THE SYNTHESIS OF NITROGEN HETEROCYCLES

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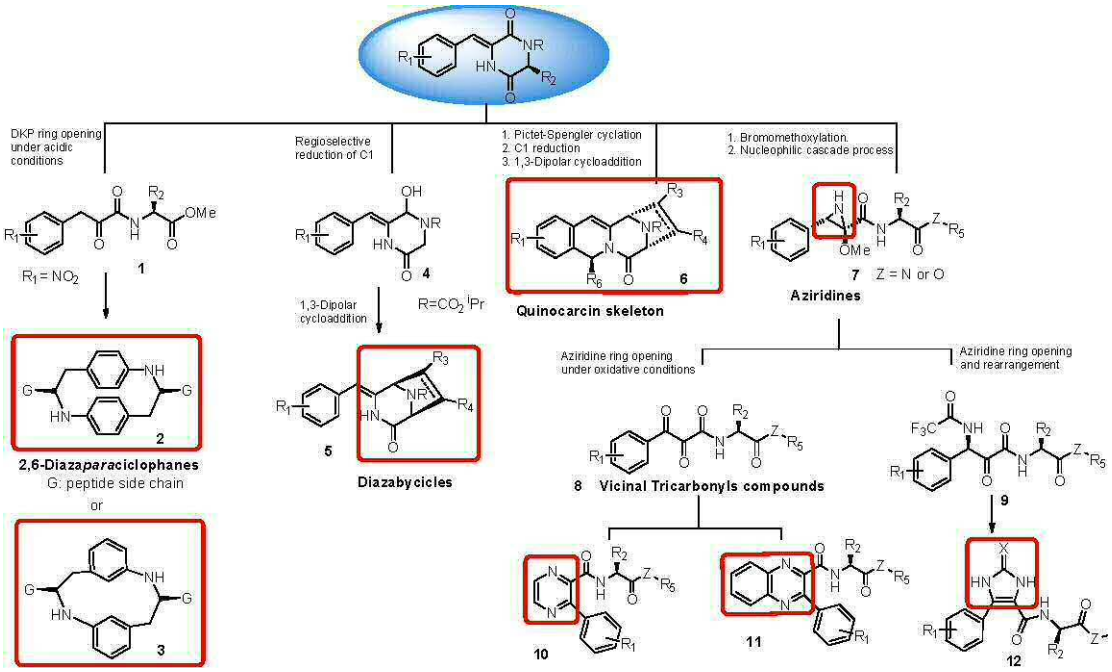
Nitrogen heterocycles are arguably the most important class of bioactive compounds and have an essential role in the medicinal chemistry field. Among them, arylmethylenepiperazine-2,5-diones can be considered as privileged structures in drug discovery[1] and are also important synthetic starting materials.[2]

We describe in this communication a number of strategies (shown in the scheme), to transform 3-arylmethylene-2,5-piperazinediones into a variety of biologically relevant heterocyclic scaffolds such as 2,6-diazaciclophanes 2 and 3,[3] diazabicycles 5, bridged compounds related to the quinocarcin core 6[4] and aziridines 7. We also took advantage of the reactivity of the highly functionalized aziridines 7 to obtain pyrazines 10, quinazolines 11 and imidazole derivatives 12.



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P15. PP2A LIGAND ITH12246 PROTECTS AGAINST MEMORY IMPAIRMENT IN MICE

Silvia Lorrio, [1] Alejandro Romero, [2] Laura Gonzalez-Lafuente, [1] Rocio Lajarín-Cuesta, [1] Francisco J. Martínez-Sanz, [1] Mercedes Villarroya, [1] Manuela G. López, [1] Cristobal de los Ríos. [1]

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The present study is relevant in the context of the emerging view that the phosphatase PP2A may become an attractive target in the field of neuroprotection in Alzheimer´s disease (AD),[1] as this enzyme is the main responsible for dephosphorylation of the protein τ.[2] Briefly, τ hyperphosphorylation leads to microtubules disassembly and self-aggregation, forming the socalled neurofibrillary tangles (NFT),[3] due to an imbalance between protein kinases and protein phosphatases, as a result of either overactivity of the former or underactivity of the latter

In the search of new drugs for the treatment of neurodegenerative diseases, we have recently described the synthesis and biological evaluation of ITH12246, a 1,8-naphthyridine with an interesting neuroprotective profile in in vitro models of Alzheimer´s disease (AD).[4] ITH12246 showed neuroprotective properties against in vitro models of neurodegeneration related to amyloidogenesis and τ protein hyperphosphorylation.[1] Besides, it inhibits acetylcholinesterase (CI50= 60 ⌈M).[4] These effects were proposed to be due in part to a regulatory action on PP2A phosphatase inhibition, as it prevented binding of the inhibitor okadaic acid to PP2A.

In order to deep into the pharmacological properties of ITH12246, we have studied its neuroprotective effect in new in vitro and in vivo models of Alzheimer´s disease. Thus, subjecting SH-SY5Y neuroblastoma cells to the oxidative stimulus elicited by the cocktail of rotenone and oligomycin A (O/R), ITH12246 protected the cells against loss of cell viability. Also, ITH12246 mitigated the glutamate-exerted excitotoxicity in rat hippocampal slices from.

To evaluate its ability to counteract the memory impairment, evoked by scopolamine, we used the object placement test in mice. In these experiments, the decrease in the memory index carried out by scopolamine was partially reversed by ITH12246 administered at 10 mg/kg.

Thus, ITH12246 can be considered as a wide-spectrum neuroprotectant, an interesting profile taking into account the multifactorial nature of neurodegenerative diseases.

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P16. TCAMS Triazole Series as Potential Serine Protease Inhibitors

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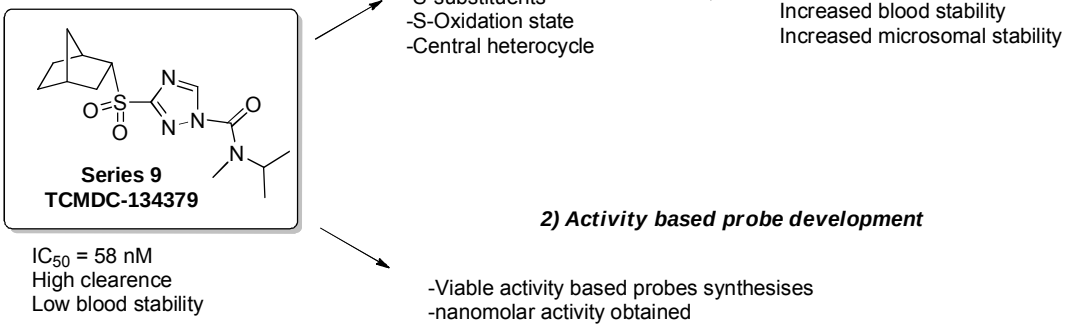
The triazole series identified by the TCAMS[1,2] study shows excellent in vitro anti-Plasmodial activity.This class of compound are known to be selective, irreversible inhibitors of serine proteases[3], a relatively unexplored target class for malaria. However, issues with microsomal and plasma stability as well as a lack of oral efficacy in an in vivo mouse model of malaria provide significant challenges. The project is, therefore, working towards two goals:

1) To develop the series into compounds with lead-like properties for anti-Malarial agents.

2) Design, synthesis and utilisation of activity based chemical probes for the identification of potentially novel serine protease targets in Plasmodia.

A range of analogues have been synthesised to obtain SAR data on the series and to date several compounds have displayed interesting properties. Current work is focussing on development of this series. In addition we have developed viable activity based probes that are active against Plasmodium falciparum in vitro suitable for target identification.



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P17. STUDIES ON THE ENANTIOMERS OF RC-33, PROMISING NEUROPROTECTIVE AGENTS ACTING AS SIGMA$_1$ RECEPTOR AGONISTS. ISOLATION, CONFIGURATIONAL ASSIGNMENT AND BIOLOGICAL PROFILE.

Daniela Rossi,[1] Raffaella Gaggeri,[1] Annamaria Marra,[1] Luca Pignataro,[2] Dirk Schepmann,[3] Bernhard Wuensch,[3] Marco Peviani,[4] Daniela Curti,[4] Ornella Azzolina,[1] Simona Collina[1]

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Our project is based on the assumption that the sigma$_1$ receptor (σ_1-R) may represent a novel therapeutic target for amyotrophic lateral sclerosis (ALS).[1,2] In our recent researches racemic RC-33 was identified as a potent and metabolically stable σ_1-R agonist (Figure 1).[3]

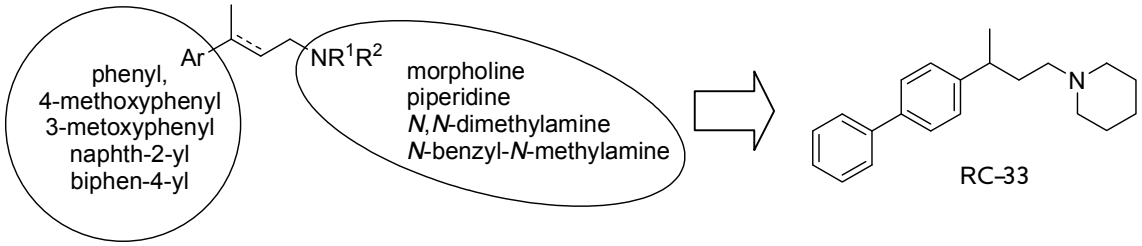

Figure 1

Since RC-33 has a chiral centre, and considering that the enantiomers of a chiral drug may behave differently in a physiological environment, herein we describe the isolation of RC-33 pure enantiomers, their absolute configuration assignment, and *in vitro* biological study, in order to address the role of chirality in their biological activity and metabolic processes. To this aim, an integrated strategy combining chiral HPLC, asymmetric synthesis and CD analysis was applied. Overall, results of the biological investigation led us to select (*R*)-RC-33 as the optimal candidate for further *in vivo* studies in animal model of ALS.

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P18. CONVERGENT SYNTHESIS OF GLYCODENDROPEPTIDES BY "CLICK CHEMISTRY" APPROACHES

Mascaraque A.[1], Kowalczyk W.[2], Sánchez-Navarro M.[1], Andreu D.*[2] and Rojo J.*[1]

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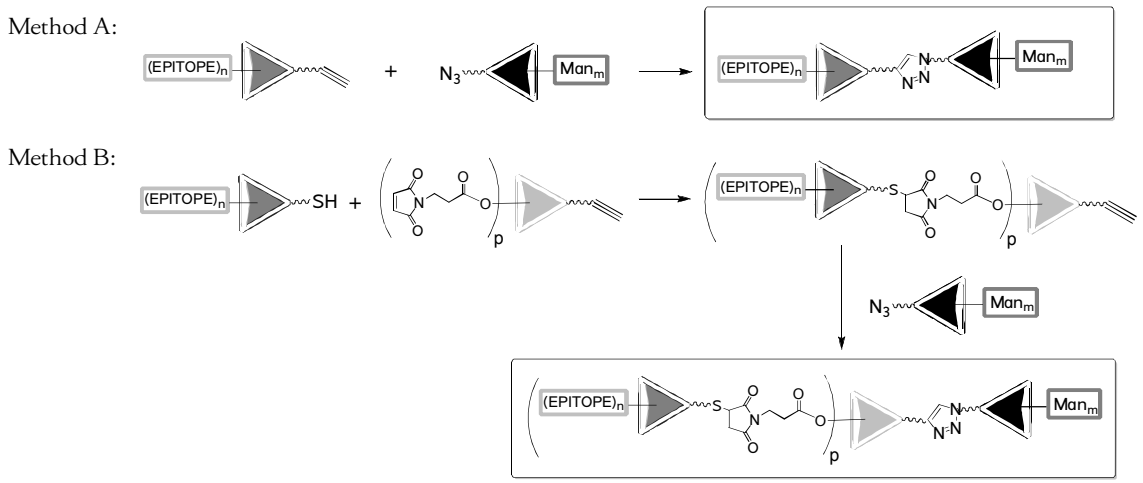
In 1997, Pieters and Koning simultaneously described the biological effects of peptide and protein mannosylation,[1] which included the facilitated uptake of such peptides and proteins through mannose receptors at the cell surface. The carbohydrates induced a receptor-dependent internalization resulting in strong T-cell stimulation. This discovery opened a way to novel vaccine strategies, particularly for those cases (cancer, HIV, etc.) where conventional vaccines had not been successful enough.

Herein, we present two versatile, complementary synthetic strategies converging on glycodendropeptides (GDPs) displaying up to 9 and 16 mannose and peptide copies, respectively. Both approaches (Scheme) rely on Cu(I)-catalyzed azide-alkyne cycloaddition (CuAAC)[2], also known as "click chemistry", for both the synthesis of glycodendron units and their subsequent conjugation to SPPS-made peptides or MAPs. This versatile and straightforward strategy for the preparation of bifunctionalized systems allows a total control on the chemical structure and provides the means to modulate easily the valency.



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P19. Synthesis of new phthalazine derivatives and evaluation of their potential cardiovascular activity

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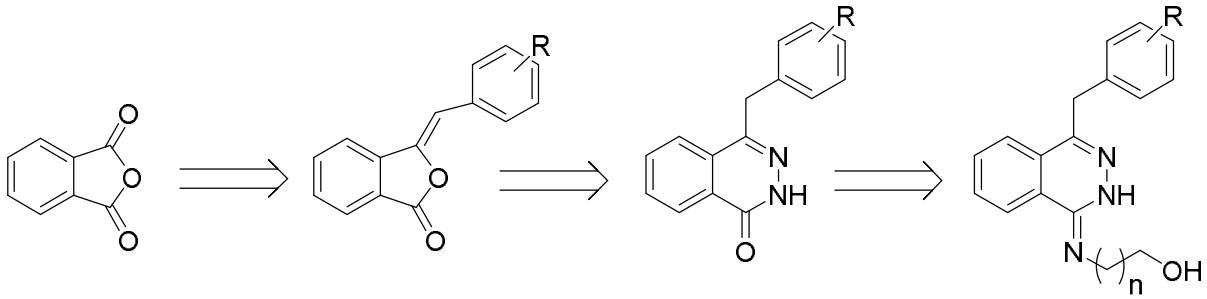
Hypertension is one of the most common cardiovascular diseases that can cause coronary disease, myocardial infarction, stroke and sudden death and is the major contributor to cardiac failure and renal insufficiency. Because of that, great efforts are continuously being made searching novel antihypertensive agents acting through different mechanisms.[1, 2] Studies on the hydralazine group drugs have led to the discovery of some pyridazinones and phthalazine derivatives with broad spectra on the cardiovascular system.[3, 4, 5]

With these precedents and with aim of obtaining new derivatives with potential pharmacological activity, we have synthesized a series of new phthalazine derivatives by introducing of an imine alkyl alcohol group in the position one of the phthalazine moiety. Furthermore a benzyl group with different substituents was introduced in the position four of phthalazine moiety. *In vitro*studies have shown interesting vasorelaxant activity and platelet aggregation inhibition for these new derivatives.



n= 1, 2

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P20. DEVELOPMENT OF A DUAL-TARGET STRATEGY FOR THE DISCOVERY OF NEW ANTI-PARKINSON DRUGS

Alexandra Gaspar[1], Joana Reis[1], Fernando Cagide[1], Maria João Matos[1,2], Eugenio Uriarte[2], Karl Norbert Klotz[3], Stefano Moro[4], Stefano Alcaro[5], Fernanda Borges[1].

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The current pharmacological therapy for Parkinson´s disease (PD) does not stops the neurodegenerative process, which involves a progressive loss of dopaminergic neurons with a subsequent and substantial reduction of dopamine levels, and is mainly focused on the treatment of the motor symptoms that are intrinsically associated. The current therapeutic approach to treat PD is based on dopaminergic drugs that are efficient in the early stages of the disease but not so operative in the long-term treatment.

The multifactorial nature of neurodegenerative diseases have shifted the paradigm of the rational drug discovery and development processes performed so far: from "one molecule-one target" to a multi-target approach. For that reason, a project was outlined to develop new chemical entities, with a potential application as anti-parkinsonian drugs, based on a dual-target approach. Accordingly, a library of chromone derivatives was designed and synthesized and screened towards monoamine oxidase-B (MAO-B), a well-known target for PD, and adenosine A2A receptors, one of most promising non-dopaminergic agents to the treatment of PD motor symptoms. In order to identify the hypothetical binding modes to both targets, and optimize the lead compound, a molecular modelling investigation of the most promising compounds has been carried out. The overall data will be presented in this communication.

This work was supported by the Foundation for Science and Technology (FCT), Portugal (PTDC/QUI-QUI/113687/2009 and Pest/C-QUI/UI0081/2011). A. Gaspar (SFRH/BD/43531/2008) and F. Cagide (SFRH/BPD/74491/2010) M.J. Matos (SFRH/BD/61262/2009) thank FCT grants.

P21. NEW INHIBITORS OF THE ENZYME ISOPRENYLCYSTEINE CARBOXYL METHYLTRANSFERASE (ICMT)

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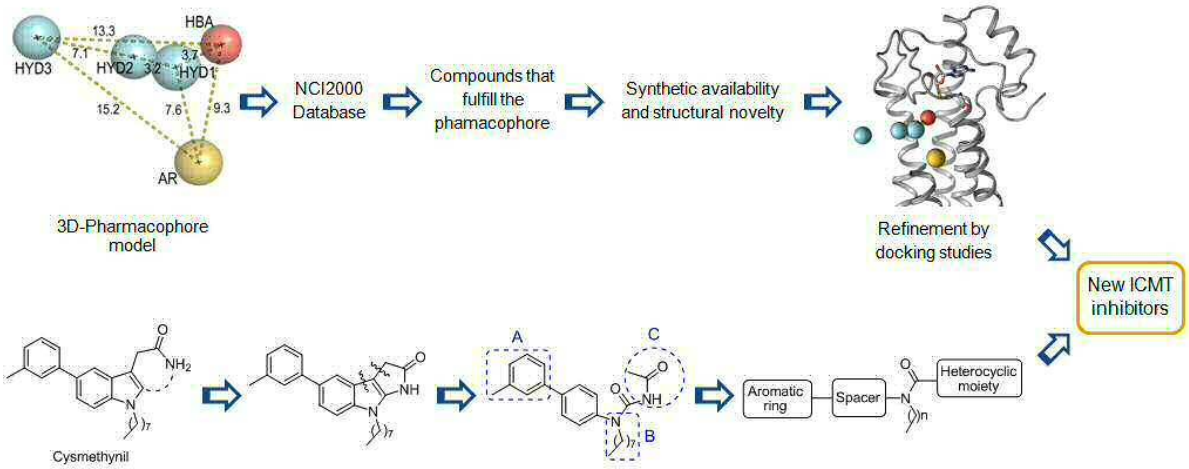
Activating mutations in Ras have been found in almost 30% of all cancers. In absence of its post-translational modifications Ras losses its ability to induce tumor transformation. Therefore, the blockade of the enzymes involved in these modifications represents an attractive strategy to inhibit Ras activity. Among them, isoprenylcysteine carboxyl methyltransferase (ICMT)[1] is receiving an increasing attention. Up to date, very few inhibitors structurally distinct have been disclosed and only one molecule (cysmethynil) has been characterized as an ICMT inhibitor able to block tumor growth.[2] These findings provide a compelling rationale for the development of ICMT inhibitors as another approach to anticancer drug development.

Towards this aim, we have addressed the design of new compounds following two approaches: (a) elaboration of a 3D-pharmacophore model, which has been further refined based on the recently described crystal structure of a prokaryotic ICMT ortholog,[3] and (b) design based on the structure of cysmethynil. To determine the inhibitory activity of the synthesized compounds, we have set up the methodology for the production of recombinant ICMT using a baculovirus expression system and the subsequent fluorimetric assay. From our initial series, we have identified some hits (UCM166 and UCM202, which inhibit a 84% and 93% of the control ICMT activity at 50 μM, respectively) that also show good pharmacokinetic properties.[4] All these results and the ongoing research will be presented.



Acknowledgements. This work has been supported by grants from the Spanish Ministerio de Economía y Competitividad (MINECO, SAF2010-22198) and Comunidad de Madrid (SAL-2010/MBD2353). The authors thank MINECO for predoctoral FPI fellowships to M.B. and F.J.O.

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P22. SYNTHESIS OF DISUBSTITUTED URACIL DERIVATIVES AND THEIR POTENTIONAL APPLICATION IN POSITRON EMISSION TOMOGRAPHY (PET)

Martina Petrović,[1] Tatjana Gazivoda Kraljević,[2] Svjetlana Krištafor,[2] Damjan Makuc,[3,4] Janez Plavec,[3,4,5] Tbbias L. Ross,[6] Simon M. Ametamey[7] and Silvana Raić-Malić[2]

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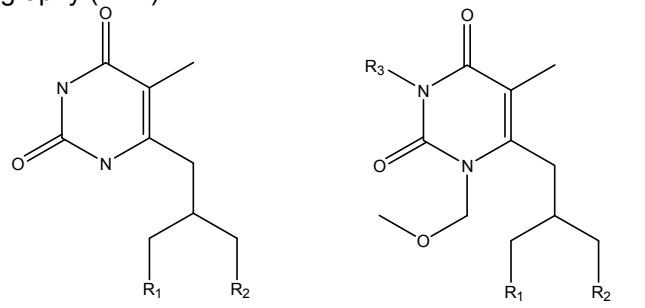
Novel *N*-methoxymethylated (MOM) pyrimidine and pyrimidine-2,4-diones nucleoside mimetics in which an isobutyl side-chain is attached at the C-6 position of the pyrimidine moiety were synthesized. Synthetic methods *via O*-persilylated or *N*-anionic uracil derivatives have been evaluated for the synthesis of *N*-1- and/or *N*-3-MOM pyrimidine derivatives with C-6 acyclic side-chains. A synthetic approach using an activated *N*-anionic pyrimidine derivative afforded the desired *N,N*-1,3-diMOM and *N*-1-MOM pyrimidines in good yield. Introduction of fluorine into the side-chain was performed with DAST as the fluorinating reagent to give a *N,N*-1,3-diMOM pyrimidine with a 1-fluoro-3-hydroxyisobutyl moiety at C-6. Conformational study of the monotritylated *N*-1-MOM pyrimidine by the use of the NOE experiments revealed the predominant conformation of the compound to be one where the hydroxymethyl group in the C-6 side-chain is close to the *N*-1-MOM moiety, while the OMTr is in proximity to the CH_3-5 group. Contrary to this no NOE enhancements between the *N*-1-MOM group and hydroxymethyl or fluoromethyl protons were observed, which suggested a nonrestricted rotation along the C-6 side-chain. Fluorinated *N,N*-1,3-diMOM pyrimidine emerged as a model compound for development of tracer molecules for non-invasive imaging of gene expression using positron emission tomography (PET). [1]



R_1: OBn, OH, OMTr, F
R_2: OBn, OH, OMTr, OMOM

R_1: OBn, OH, OMTr, F
R_2: OBn, OH, OMTr, OMOM
R_3: H, MOM

Scheme 1. General structures of prepared compounds

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P23. MULTI-TRYPTOPHAN MOLECULES AS POTENTIAL GP120-TARGETED ANTI-HIV DRUGS

Eva Rivero-Buceta,[1] Elisa G. Doyagüez,[1] Ernesto Quesada,[1] María-José Camarasa,[1] Jan Balzarini,[2] María-Jesús Pérez-Pérez,[1] Ana San-Félix[1]

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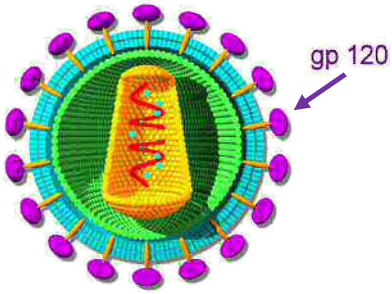
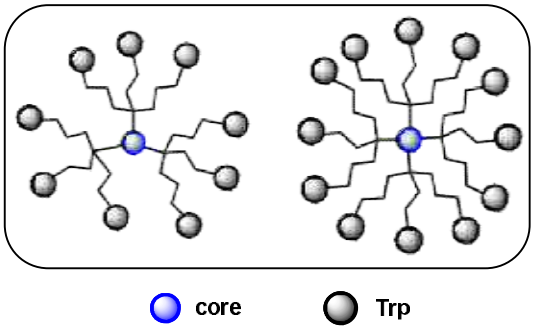
Human Immunodeficiency Virus (HIV) entry into the host cell represents one of the most attractive targets for the development of anti-AIDS therapy.[1] In fact, drugs that interfere with this particular early event of the HIV replication may represent an advantage over other existing therapeutic approaches that target the viral enzymes such as reverse transcriptase or protease, as they may prevent virus entry into target cells and subsequently reduce the number of latent reservoirs for HIV.

HIV entry into host cells is mediated by the viral envelope glycoproteins gp120 and gp41, which interact with the CD4 receptor of the T cell surface. Viral gp120 is of particular importance during viral fusion and entry as it serves as the first point of contact with the host cell.[2] This glycoprotein is extensively glycosylated and approximately 50% of its molecular weight is due to its dense carbohydrate (glycan) shield.

Lectins, i.e. proteins of natural origin that bind carbohydrates, show a potent inhibitory activity against HIV. These lectins exert their anti-HIV activity in the early steps of the replicative cycle by binding to the gp120 carbohydrates.[3]

In the last years our research efforts have been focused on the discovery of synthetic molecules, "lectin mimetics", able to act through a mechanism similar to that of the natural lectins. One of our hits, a molecule with a central core surrounded by six tryptophan residues, significantly inhibits HIV replication and binds gp120 as evident from SPR (Surface Plasmon Resonance) experiments.[4] Moreover, the results obtained so far indicate that multivalency, a typical characteristic of lectin-sugar interactions, is crucial for both anti-HIV activity and gp120 recognition. Based on these results novel tryptophan derivatives, have been prepared. For the design of the novel molecules special attention was paid to multivalency by using multi-branched spacers that connect the central scaffold with the periphery (tryptophans). The synthesis, antiviral evaluation and SPR experiments of these compounds will be presented.





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P24. CHEMICAL PROBES FOR THE STUDY OF CANNABINOID RECEPTORS

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The endogenous cannabinoid system (ECS) is a complex system which regulates a broad number of physiological and physiopathological processes.[1] In spite of all the significant progress made on its study, many aspects remain elusive including the possible existence of new cannabinoid receptors (CBRs) different from the molecularly characterized CB_1 and CB_2 such as GPR55, which has been proposed as a potential CB_3 receptor.[2]

In this context, the development of tagged small-molecule probes would greatly improve our understanding of the ECS, its physiology and its therapeutic potential. Therefore, we are carrying out a project aimed at the development of chemical probes bearing different tags that enable visualization, isolation, enrichment and/or identification of new cannabinoid targets.

Up to this moment, we have synthesized several probes based on the structure of the main endocannabinoids anandamide (AEA) and 2-arachidonoylglycerol (2-AG)[3] as well as on the high-affinity synthetic ligands HU210 and HU308.[4] In addition, we are currently extending this approach to other cannabinoid ligands such as the natural products cannabidiol (CBD) and honokiol (Figure 1). Among the synthesized probes, those showing high affinity for the CBRs and good metabolic stability, have been selected for *in vitro* and *in vivo* experiments.

In parallel, we are setting up a proteomic platform for the identification of new targets, using probes which combine benzophenone and biotin or a fluorophore for their use in the appropriate tissue. These strategies should contribute to optimize the therapeutic exploitation of known or new members of the CBRs by providing valuable information about their location or level of expression.

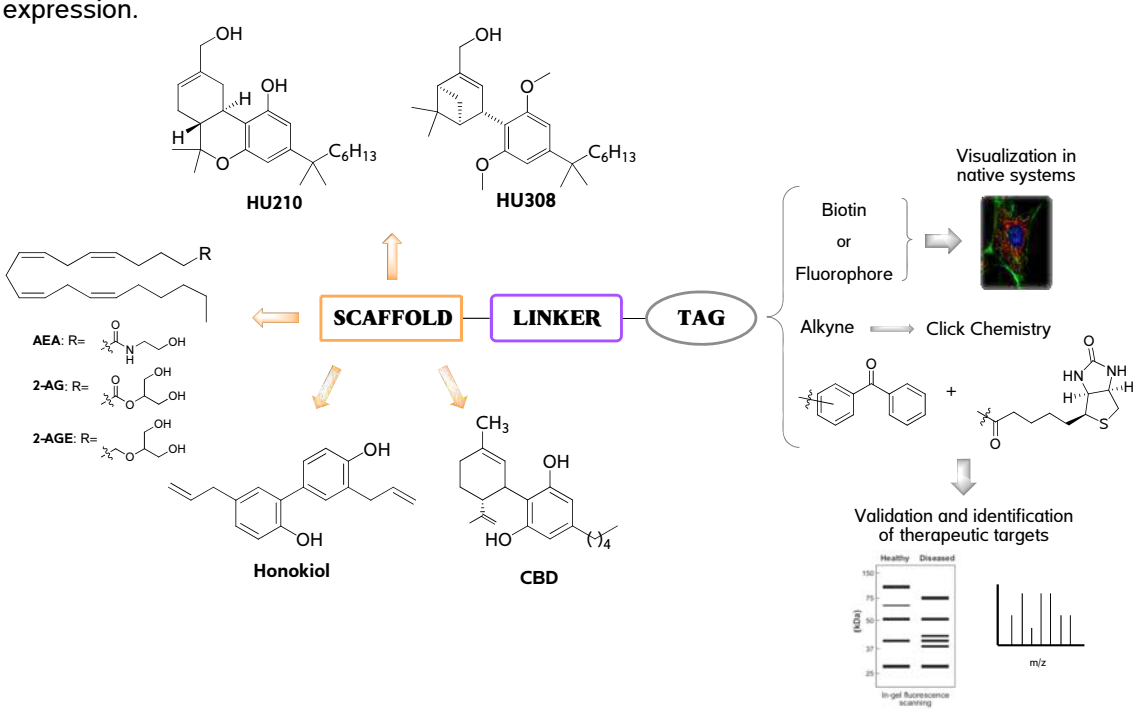

Figure 1. Development of chemical probes for the study of the ECS

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P25. SYNTHESIS AND BIOLOGICAL EVALUATION OF DIMERIZATION INHIBITORS OF TRYPANOTHIONE REDUCTASE OF *LEISHMANIA INFANTUM*.

Marta Ruiz-Santa Quiteria Saavedra,[a] Miguel Toro,[b] Pedro Sánchez-Murcia,[b] Federico Gago,[b] Antonio Jiménez,[b] María José Camarasa,[a] Sonsoles Velázquez.[a]

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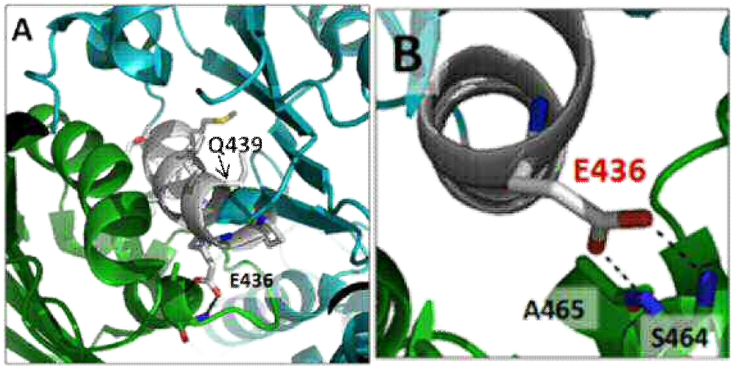
Although leishmaniasis is nowadays a world-broad emerging zoonosis, only a limited and outdated drug arsenal for its treatment is available. Trypanothion Reductase (TryR) is a validated and attractive target in the search for drugs in leishmaniasis treatment. This enzyme is exclusive (does not exist in mammals) and essential for parasites survival.[1] Based on the fact that the biologically functional form of TryR of *Leishmania infantum* (Li-TryR) is a homodimer, [2] we have devised a yet unexplored alternative strategy that attempts to interfere with the dimerization process of the enzyme. Molecular modeling studies and site-directed mutagenesis showed Glu436 and Gln439, contained within a α-helix in the dimerization domain of the enzyme, as two key residues (hot spots) (Figure). From a small library of peptides derived from this interfacial α-helix, linear peptide TRL14 significantly inhibits, both the activity and the dimerization of the enzyme in enzymatic assays.[3]



We herein report the initial steps in the optimization process of prototype TRL14. In order to increase the chemical stability of prototype, Met residues were replaced by Nle. With the aim of studying the minimum length required for activity, a series of peptides truncated at the C-terminal end were prepared and evaluated. Likewise, a second series of conformationally restricted peptides that incorporate a covalent amide bond between the side-chains of two residues of the sequence were investigated. The target peptides have been tested in both enzymatic and dimerization assays. Our results support that targeting the dimerization interface of Li-TryR by rationally designed peptides is a feasible goal.

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P26. DESIGN, SYNTHESIS AND STUDY OF NEW QUINOXALINE 1,4–DI–*N*–OXIDE DERIVATIVES FOR LATENT AND RESISTANT TUBERCULOSIS

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Tuberculosis (TB) is a disease that is contagious via air, caused by *Mycobacterium tuberculosis* (*M.Tb.*). [1,2] It presents a high mortality rate worldwide. Its presence is constantly increasing, leading to growing concern, especially in the year of 2011, when it was reported that 84 countries had diagnosed at least on case of XDR-TB.[3,4] Over the past 50 years, no new drug has been approved; therefore there is a maintained commitment to synthesize new derivatives with potential antituberculos is activity.[5]

The R &D Drug Unit has made structural modulations in differenting positions of the quinoxaline with the aim of improving their antituberculosis activity.[6-10] Maintaining the quinoxaline 1,4-di-N-oxide as a central ring and a methyl group in R3 position, 12 new ketone α,β-unsaturated derivatives has been synthesized and their *in vitro* antimycobacterial activity has been evaluated (Figure 1). At present, compound 1c is the most active and currently in the second stage of *in vitro* evaluation.

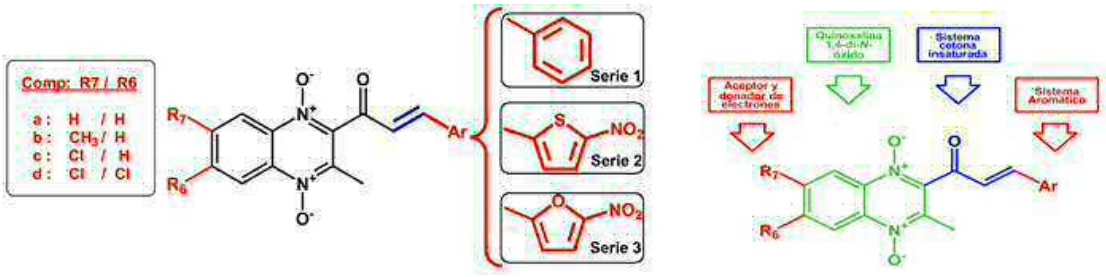

Figure 1. General structure of Quinoxaline 1,4-di—oxide

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P27. Nature-Inspired Artwork: Development of 6-(Heteroaryl/Aryl) Chromones as Novel Adenosine Receptor Ligands

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Adenosine is a purine nucleoside that regulates a wide range of physiological and pathological functions, through interaction with four subtypes of cell-surface G-protein coupled adenosine receptors (ARs), called A_1, A_{2A}, A_{2B} and A_3 receptors. Multiple physiological actions can be associated to adenosine among them are the heart rate, vascular smooth muscle tone, lipolysis, renal, platelet and white blood cell functions [1].

The description of high levels of AR in many cancer cells suggests that they have potential to function either as biomarkers or targets. In fact, it was already shown that the increase of AR expression is correlated with the severity of the disease and in turn, it can act as an important target in anticancer therapy [2]. Evidences have been acquired showing that A_3ARs are abundantly expressed in tumour cells and may be targeted by specific ligands.

Progress in the pursuit of therapeutic adenosine receptor ligands has been performed by using natural flavonoids, and other dietary phytochemicals. Exploring work disclose that in general flavonoids have micromolar affinity at cloned human brain A_3ARs. Based on this information, additional work related with the optimization of flavone nucleus was done and MRS 1088 and MRS 1067 have been identified as the most potent and selective compounds [3]. However, and in accordance with the wide-ranging information in the area, even though a considerable number of selective AR ligands have been discovered, few of them have been clinically evaluated many due to restrictions related with side effects, low absorption, short-half-life and toxicity [3].

On the other hand and keeping in mind that natural products have been historically an important source for drugs, and up to now provide unique lead compounds, a project was designed inspired in the framework of flavonoids. Accordingly, a new library of compounds inspired on the chromone scaffold has been synthesised. The synthetic strategy encloses the use of an acetophenone derivative as starting material and a Suzuki cross-coupling reaction. The screening of the synthesised compounds towards adenosine receptors is under progress.

This work was supported by the Foundation for Science and Technology (FCT), Portugal (PTDC/QUI-QUI/113687/2009 and Pest/C-QUI/UI0081/2011). A. Gaspar (SFRH/BD/43531/2008) F. Cagide (SFRH/BPD/74491/2010) M.J. Matos (SFRH/BD/61262/2009) and F. Borges (SFRH/BSAB/1090/2010) thank FCT grants.

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P28. DESIGN, SYNTHESIS AND ACTIVITY EVALUATION OF ANTIOXIDANT PEPTIDES

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Reactive Oxygen Species (ROS) produced during normal aerobic metabolism, if not promptly removed by the detoxification mechanisms of the cells, can cause direct cell damage or can react with cell lipids producing reactive carbonyl species (RCS). RCS exhibit longer half-lives than radicals but still retain significant chemical reactivity towards proteins' side chains causing proteins' modification and dysfunction. The accumulation of ROS and 4-hydroxynonenal (HNE), a well known RCS, has been associated with a number of diseases and the development of molecules able to react with and inactivate these harmful species represent a promising therapeutic strategy. Caffeic Acid (CA) is a dihydroxycinnamic acid with known antioxidant properties and CA dipeptidyl derivatives have demonstrated an increased radical scavenging activity compared to CA alone[1]. Histidyl Hydrazide (HH), an analogue of the endogenous dipeptide Carnosine, has been studied as reactive carbonyl scavenger[2].

The aim of this study was to examine the possibility of obtaining a molecule being able to act both as HNE and radical scavenger. Since histidine containing dipeptides are more efficient HNE scavengers compared to histidine alone[3], HH analogues modified with natural or non natural amino acids were synthesized. The HNE scavenging activity of each compound was confirmed and most of the compounds were more potent than HH in providing to SH-SY5Y cells a 5-Fold Cell Protection against HNE (5FCP). The coupling of CA to the most active compound in the series resulted in the generation of a new analogue completely retaining the HNE scavenging activity and additionally acting as radical scavenger. Moreover, the concentration needed to provide a 5FCP to cells was reduced of 2/3 compared to HH, probably due to a synergistic effect of the radical scavenging activity. As a parallel investigation, HH was selectively delivered to mitochondria, the main oxidative stress production site, using a known mitochondria penetrating peptide[4]. As a future perspective the two approaches here described could be combined to obtain a multipotent targeted molecule.

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P29. SYNTHESIS, BIOLOGICAL EVALUATION AND MOLECULAR MODELING STUDIES OF N–SUBSTITUTED PHTHALAZINONES AS INHIBITORS OF ACETYLCHOLINESTERASE

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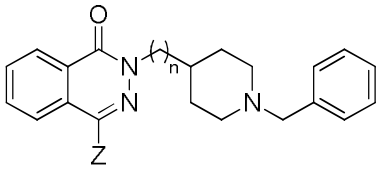
Alzheimer's disease (AD) is a complex and progressive neurodegenerative disorder of the central nervous system that constitutes the most common type of dementia senile worldwide. Pathologically is characterized by extracellular deposits of aberrant proteins, namely β-amyloid (Aβ) and τ-protein, and neuronal loss together with low levels of acetylcholine (ACh)[1]. However, as the etiology of AD is not yet known, the current therapies, including AChE inhibitors (AChEIs) and N-methyl-D-aspartate antagonists, only improve symptoms but they do not have profound disease-modifying effects[2].

The discovery that donepezil not only increases the ACh level in synapses, but also reduces the Aβ aggregation[3], has renewed the interest in AChEIs and in the last few years a number of donepezil analogues have been described. However, none of AChEIs described show in their structure the phthalazinone framework, a possible isosteric group for the indanone system. Bearing this in mind, a series of donepezil analogues based on phthalazin-1(2H)-one scaffold was synthesized and evaluated as AChEIs (Figure 1).



Z= H, Ar; n= 1, 2

Figure 1. General structure of AChEIs proposed

The new donepezil analogues have the N-benzylpiperidine group at N2 of the phthalazinone system including a linking chain between both fragments ranged from one to two carbon atoms. Moreover, these compounds may be substituted or not with a bulky group at C4.

The proposed compounds were synthesized in good yield using the adequate 2H-phthalazin-1-ones and two commercially available N-Boc protected 4-hydroxyalkylpiperidines as starting materials. The strategy followed involved the initial preparation of 2-(N-Boc-4-piperidinylalkyl)phthalazin-1-ones, via the corresponding bromoalkylpiperidine, to then introduce the N-benzyl fragment after removing the protecting group.

The results of AChE inhibition revealed the donepezil homologues (n=2) as the most interesting compounds of this series, with IC_{50} values between 0.90 μM (Z = H) and 6.83 μM (Z = p-tolyl).

Finally, molecular modelling studies were preformed in order to compare the binding mode and ADME properties of novel compounds with donepezil.

Acknowledgements
We acknowledge the Xunta de Galicia and the Universidade de Vigo for the financial support. N.V. thanks the Universidade de Vigo for a pre-doctoral contract.

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P30. LPA1 RECEPTOR AS A NEW THERAPEUTIC TARGET IN THE CENTRAL NERVOUS SYSTEM

Debora Zian, Inés González-Gil, Henar Vázquez-Villa, Silvia Ortega-Gutiérrez, Mª Luz López-Rodríguez

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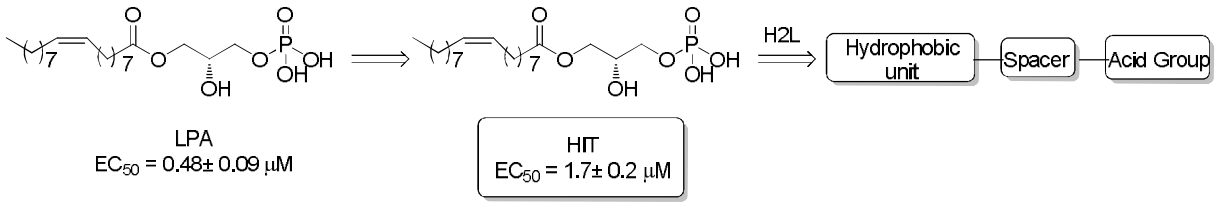
Of all proteins encoded by the human genome, the G protein-coupled receptors (GPCRs) constitute about the 2%. Moreover, its importance as therapeutic targets is clear, since almost half of the drugs on the market act on some type of GPCR. Within these receptors stand out those whose endogenous ligands are lipid molecules and, in particular, lysophospholipids (LPs).[1] Two of the best studied LPs are lysophosphatidic acid (LPA) and sphingosine 1-phosphate (S1P). These LPs are receiving increased attention because, in addition to their structural function in the cell membrane, they are now regarded as important regulators for diverse biological functions through activation of their specific receptors (LPA1-6Rs and S1P1-5Rs respectively).

Given the interest of the LPA signaling in the central nervous system[2] we started a project in our research group aimed at the development of new LPA1R selective ligands. Based on the structure of the LPA we have succeeded in the identification of new LPA1R agonists with EC50 values in the low micromolar range.

These results prompted us to carry out a hit to lead process in order to improve their activity and selectivity for LPA1R versus LPA2-5R, work that is currently underway in our laboratory.



Acknowledgements. This work has been supported by grants from the Spanish Ministerio de Economia y Competitividad (MINECO, SAF2010-22198) and Comunidad de Madrid (S2010/BMD-2353). The author thanks MINECO for predoctoral F.P.U.

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P31. THE UNSATURATED ACYCLIC NUCLEOSIDE ANALOGUES BEARING A STERICALLY CONSTRAINED (Z)-4´-BENZAMIDO-2´-BUTENYL MOIETY: SYNTHESYS, X-RAY CRYSTAL STRUCTURE STUDY AND ANTIVIRAL ACTIVITY EVALUATIONS

Malajka Zlogleda[1], Krešimir Benci[2], Karlo Wittine[2], Mario Cetina[3], Mirela Sedic[4], Sandra Kraljevic Pavelic[5], Krešimir Pavelic[5], Erik De Clercq[6], Mladen Mintas[2]

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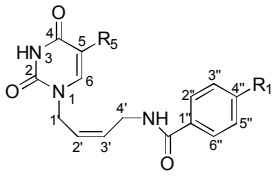
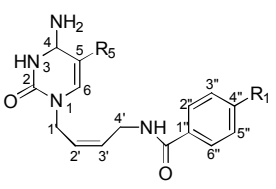
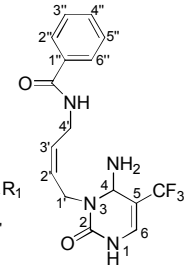
A series of novel acyclic unsaturated pyrimidine (1–12) and adenine (13) nucleoside analogues bearing conformationally restricted (Z)-2´-butenyl moiety were synthesized and evaluated for their potencial antiviral and cytostatic activity against malignant tumor cell lines and normal human fibroblast (WI38). The N-1 and/or N-3 acyclic side chain substitution in pyrimidine ring in N-3 substituted 5-trifluoromethyluracil derivative (11), N-1, N-3 disubstituted 5-fluorouracil derivative (12) and adenine derivative (13) was deduced from their ^{1}H and ^{13}C NMR spectra and confirmed by single crystal X-ray structure analysis.



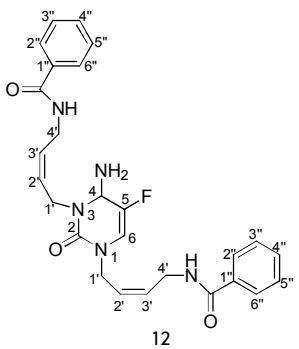
1-4 and 7,8



5, 6 and 7,8

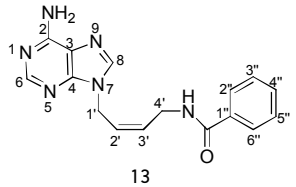


11



12

	1	2	3	4	5	6	7	8	9	10
R5	H	F	CH_3	CF_3	H	F	H	CH_3	H	F
R1	H	H	H	H	H	H	F	F	F	F



13

The X-ray crystal structure analysis 11–13 revealed also supramolecular selfassemblies, in which infinite chains or dimers built two and three-dimensional networks. The results of the in vitro cytostatic activity evaluations of 1–13 indicate that the majority of the compounds tested exhibited a non-specific and moderate antiproliferative effect at the highest concentration (100 IM). Of all evaluated compounds on the tested cell lines only the N-1 4´´-fluoro-substitutedbenzamide uracil derivative (7) showed rather marked and selective inhibitory activity against the growth of MCF-7 cells at a concentration of 2.7 μM and no cytotoxic effect on normal fibroblasts WI38. This compound can be therefore considered as a potential antitumor lead compound for further synthetic structure modification.

Literature: K. Benci et al. / Bioorg. Med. Chem. 18 (2010) 6249-6257

P32 DEVELOPMENT OF TETRAOXANE – PYRIMIDINE NITRILE HYBRIDS, A NOVEL CLASS OF ANTIMALARIALS.

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Artemisinin (ART) is a natural product devoted of potent antimalarial activity.[1] Its endoperoxide core is reductively activated by iron(II)-heme - a byproduct of host hemoglobin degradation - to form carbon-centered radicals capable of reacting with heme and proteins.[1] Many synthetic analogues, such as tetraoxanes, have showed to maintain or even surpass artemisinin's *in vitro* and *in vivo* activity profile.[2] Combination of these potent drugs is yet essential to avoid development of resistance. In this work we address the resistance problem by developing tetraoxane-based hybrid compounds with pyrimidine nitriles (Figure 1). Pyrimidine nitriles are known to be potent antimalarials, acting as inhibitors of falcipain-2 and -3 – essential enzymes for the parasites development and survival.[3] Herein we present the synthesis of the hybrids and preliminary results that show high antimalarial activity against two strains of *Plasmodium falciparum* and low cytotoxicity.

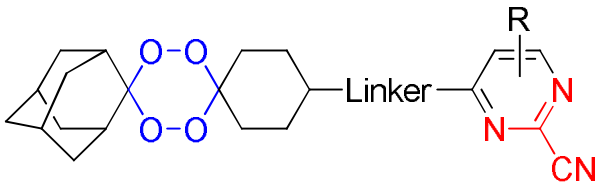

Figure 1: Tetraoxane – pyrimidine nitrile hybrids.

Acknowledgements

This work was supported by the Fundação para a Ciência e Tecnologia (grants PEst-OE/SAU/UI4013/2011 and REDE/1501/REM/2005; fellowship SFRH/BD/63200/2009 to RO) and Fundação Luso-Americana (award to RM).

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P33 SYNTHESIS AND EVALUATION OF NOVEL HYBRID ANTIMALARIALS WITH TETRAOXANE AND 8–AMINOQUINOLINE MOIETIES

Magalhães,Joana[†]; Albuquerque, Inês S.[•]; Gut, Jiri[#]; Rosenthal, Philip J.[#]; Mota, Maria M.[•]; Moreira, Rui[†]; Prudêncio, Miguel[•] and Lopes, Francisca[†].

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In 2010 there were about 219 million cases of malaria and near 660000 people died [1].
The emergence and spread of multidrug-resistant *Plasmodium falciparum* is still the major obstacle in the control of malaria. Elimination of the disease requires compounds that act both in the blood stage and liver stage, including hypnozoites, the reservoirs of the infection [2]. Nowadays there aren't clinically available drugs that are able to kill simultaneously the blood- and the liver-stage of malaria parasites.
Primaquine, a 8-aminoquinoline, is the only antimalarial effective against the liver-stages, hypnozoites. However, primaquine exhibit a poor activity against blood-stage of malaria parasite [3].
Artemisinin-based combination therapy is the best available treatment for malaria [1]. Other endoperoxides have been synthesized and screened for antimalarial activity and among them tetraoxanes have shown great promises. 1,2,4,5-tetraoxanes are very stable compounds with a fast action against the asexual blood stage of the malaria parasite having activity in the same range as artemisinin derivatives [4].
Hybrid molecules offer the possibility to address different targets or different sites of action with a single chemical entity. Two antimalarial drugs combined through a linker may allow not only a more effective way to deliver these agents to the site of action of the parasite but also improve compliance [5].
Herein we report the synthesis of hybrid molecules based in the pharmacophores of 1,2,4,5-tetraoxanes and 8-aminoquinolines in order to have activity against both the liver and blood stage of the malaria parasite. The synthesis and activity of the synthesized compounds will be presented.

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P34 SYNTHESIS OF NEW NUCLEOBASE-FUNCTIONALIZED CYSTEINES TO BE USED AS BUILDING BLOCKS OF α-PEPTIDE NUCLEIC ACIDS (α-PNAS)

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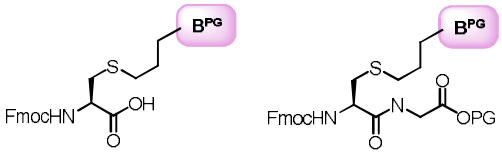
Nucleobase-containing peptides, also known as nucleopeptides, represent a promising class presenting a peptide-like backbone conjugated to nucleobases through different linker moieties.[1]

α-Peptide nucleic acids (α-PNAs) are one class of these of artificial nucleic acids where the sugar-phosphate backbone has been replaced by a peptide made up of α-amino acids some of them carrying a nucleobase in the side chain.[1] α-PNAs are able to interact to complementary RNA or DNA with high affinity while still displaying sufficient specificity to distinguish single-base mismatches.[2] Therefore, α-PNAs can be used as gene therapeutic (antisense and antigene) drugs, in genetic diagnostics, and molecular recognition.[3]

In this work, we synthesise new nucleobase-functionalized cysteine and cysteinyl dipeptides (Scheme 1) that can be used as α-PNAs building blocks. To synthesize these molecules we used thiol-ene radical reaction.[4] Although the formation of the radical can be promoted either by a radical initiator or by UV light, we observed that better yields are obtained when the photochemical approach is used.



Scheme 1: Nucleobase-functionalized cysteines and cysteinyl dipeptide.

Acknowledgments

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P35 MODELLING THE OXIDATION SITE OF *PLASMODIUM FALCIPARUM BC*1 COMPLEX

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The electron transport chain (ETC) in malaria parasites was first recognized as an attractive drug target since the development and clinical use of atovaquone in 1992 [1]. Atovaquone selectively inhibits electron transport by binding to the Qo binding site (oxidation site) of the parasite mitochondrial *bc*1 complex. More specifically, this compound induces the collapse of the mitochondrial membrane potential which results in parasite death [2]. Therefore, being crucial for the survival of *P. falciparum* (*Pf*), the cytochrome *bc*1 complex is currently a validated target for antimalarial drug development [3].
In the absence of a crystallographic structure for the *bc*1 complex of *Pf*, much of the key structural and mechanistic information has been obtained from analogous *bc*1 systems. In particular, the *bc*1 complex of *Saccharomyces cerevisiae* was already chosen to model this pocket and to understand the mechanism of action of potential inhibitors of the *Pf bc*1 complex [4,5]. Nevertheless, a reliable three-dimensional structure of the *Pf* enzymatic complex is essential for successful drug design.
As a result, having in mind the increasing interest in obtaining potential antimalarial drugs that can act in this target, we developed a homology model of cytochrome *bc*1 Qo binding site based on yeast crystallographic structure. Here, we present the methodology followed to obtain the homology model and all the validation procedure employed to verify the reliability of the model generated.

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P36. Endoperoxide-based hybrids with multistage antimalarial activity

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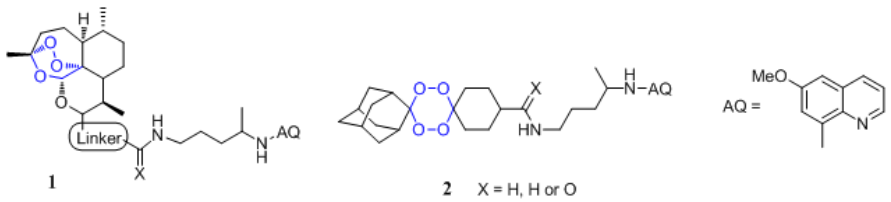
Body Malaria is a potentially life-threatening and one of the world's most prevalent tropical diseases. Is caused by infection with parasites of the genus *Plasmodium* and transmitted to humans through the bite of an infected female *Anopheles* mosquitoes [1]. Malaria parasites undergo an asymptomatic, obligatory developmental phase in the liver, which precedes the formation of red-blood cell-infective forms [2]. The emergence of resistant parasite strains to antimalarial drugs remains a real and ever-present danger. For this reason, WHO recommends artemisinin- based combination therapies (ACT), in which the artemisinin component is combined with a second, longer-acting agent [1,3]. Taking this into consideration, the ultimate goal of eradicating malaria will benefit greatly from a drug that eliminates all life cycle stages of parasites [4]. A great promising approach is to link two pharmacophores, each one targeting a specific stage of the parasite's life cycle, in a single molecule called hybrid drug.

So, in a search for effective compounds against both the blood- and liver-stages of infection by malaria parasites with the ability to block the transmission of the disease to mosquito vectors, and following our initial report on primaquine-artemisinin hybrid compounds [5], we now report on the development of molecules combining either a 1,2,4-trioxane, **1**, or 1,2,4,5-tetraoxane, **2**, and 8-aminoquinoline moieties. This new series of hybrid compounds were screened for their antimalarial activity and stability and in vitro metabolism studies in rat liver microsomes were performed.



FCT is acknowledged for support through the projects PTDC/SAU-FAR/118459/2010, PEst-OE/SAU/UI4013/2011 and *PhD grant SFRH/BD/30418/2006 (RC).*

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P37 STRUCTURAL STUDIES FOR UNDERSTANDING THE INHIBITION OF TYPE I DEHYDROQUINASE ENZYME

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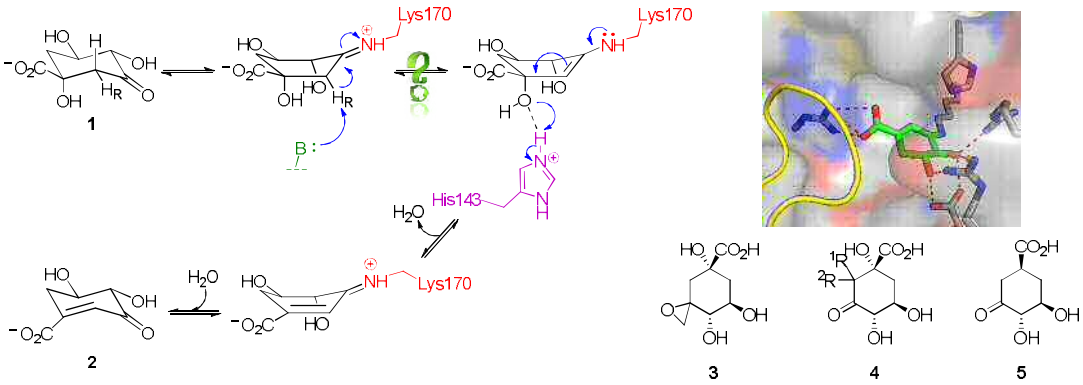
The type I dehydroquinase enzyme (typically *Escherichia coli, Salmonella typhi, Staphylococcus aureus*) catalyzes the reversible dehydration of 3-dehydroquinic acid (1) to form 3-dehydroshikimic acid (2) by an overall *syn* elimination of water with loss of the less acidic *pro*-R hydrogen from C2 of 1 (Scheme 1).[1] The reaction proceeds *via* a multi-step mechanism involving covalent imine intermediates between a conserved Lysine residue and the carbonyl group of the substrate. The enzymatic mechanism also involves an essential Histidine residue that seems to act at different stages of the mechanism.[2] The role of this Histidine residue has been of great controversy. In our research group we have performed structural and computational studies to help clarify the role of the residues involved in the different stages of the process. For the structural studies, compounds 3–5 were designed, tested and crystallized with the enzyme. Our progress in the project will be presented.



Scheme 1. Enzymatic conversion of 3-dehydroquinic acid (1) to 3-dehydroshikimic acid (2) catalyzed by DHQ1 and designed substrate analogs 3–5.

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P38 DISCOVERY OF NEW INHIBITORS OF *HELICOBACTER PYLORI* TYPE II DEHYDROQUINASE ENZYME BY FRAGMENT-BASED HIGH-THROUGHPUT DOCKING

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Fragment screening is a wide accepted technique to identify relatively simple hit-compounds that possess a high binding affinity per heavy atom, and thus are ideal compounds for optimization into clinical candidates with good drug-like properties.[1] This technique has emerged as an important alternative to high-throughput screening, because it has the advantage that incorporates the structural information of the target to preselect the molecules that are most likely to show binding and inhibitory activity. The available experimental knowledge of known inhibitors is incorporated by using pharmacophore constraints to preselect compounds for docking and therefore reducing computation times.

Here, we present the application of the anchor-based library tailoring screening approach (ALTA)[2] to the discovery of new inhibitors of an essential enzyme in *Helicobacter pylori,* the type II dehydroquinase (DHQ2). This bacterium is the major cause of gastric and duodenal ulcers and it has been classified as class I carcinogen. The synthesis of the identified potential inhibitors and their biological activity are also provided.

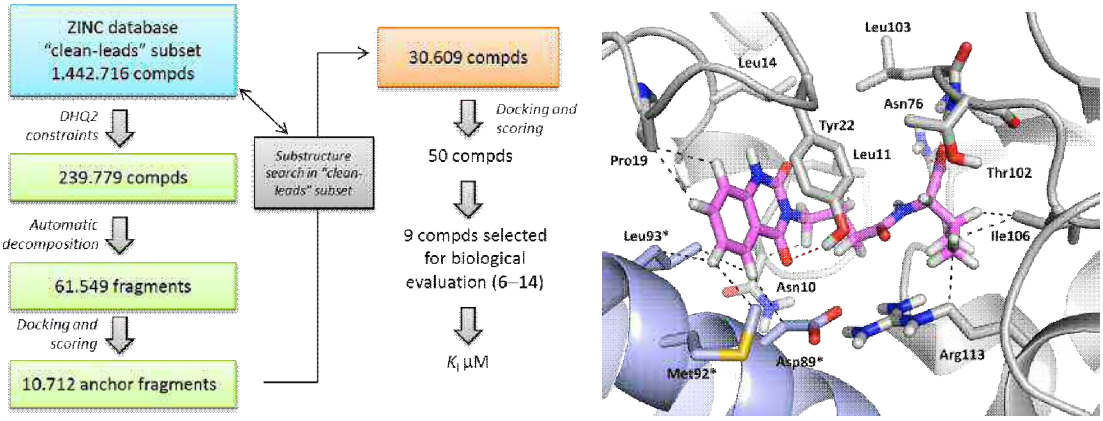

Figure 1. Flow chart of the ALTA virtual screening approach for the *H. pylori* DHQ2 enzyme and predicted binding mode of the most active compound in the enzyme active site.

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P39 DESIGN AND SYNTHESIS OF NEW INHIBITORS OF SHIKIMATE KINASE ENZYME AS NEW ANTIMICROBIAL AND ANTIMALARIAL AGENTS

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Glyphosate [*N*-(phosphomethyl)glycine], the active ingredient in well-known herbicides, inhibits the sixth enzyme of the shikimic acid pathway (EPSP synthase) through which the aromatic amino acids – tyrosine, phenylalanine, and tryptophan – and other important aromatic compounds are biosynthesized. Glyphosate is also effective against several parasitic apicomplexa, including those that cause malaria and toxoplasmosis and it also proved to be effective against multidrug-resistant strains of malaria, such as pyrimethamine.[1] It has been shown recently that the antimicrobial properties of glyphosate are also due to the high concentrations of shikimic acid and protocatechuate that it causes.[2] In our research group, we are studying the possibility that this effect could be also achieved by inhibition of the fifth enzyme of the shikimic acid pathway, shikimate kinase (SK), which transforms shikimic acid (1) into shikimate 3-phosphate (2). This enzyme is essential in several bacteria, such as *M. tuberculosis, H. pylori, A. baylyi, H. influenzae, F. novicida* and *P. aeruginosa.* The absence of the shikimic acid pathway in mammals and its essentiality in those bacteria encourage us to the development of inhibitors of this enzyme as new antimicrobial and antimalarial agents. To this end, several substrate analogs were designed based on the enzymatic mechanism, and by docking and molecular dynamics simulation studies using the crystallographic complexes available.[3] In this communication, our latest results in the project will be presented.

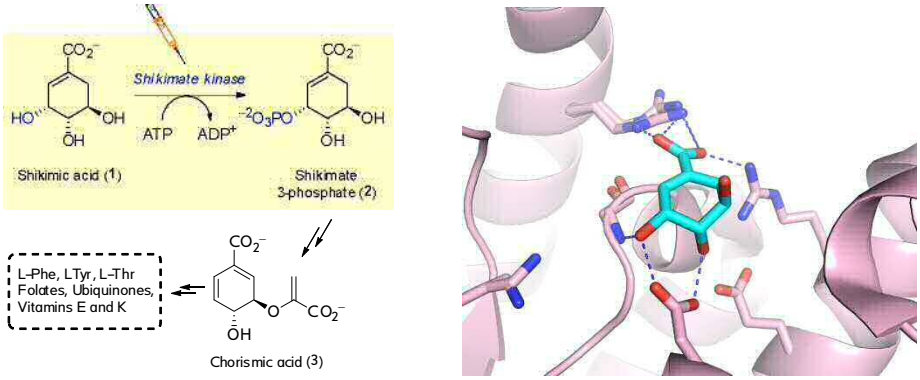

Figure 1. Enzymatic conversion of shikimic acid (1) to shikimate-3-phosphate (2) catalyzed by SK enzyme and section of the active site of the SK enzyme from *M. tuberculosis*.

Acknowledgements

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P40. THIAZOLOPYRIDONE DERIVATIVES: A NOVEL FAMILY OF POSITIVE ALLOSTERIC MODULATORS OF mGlu5 RECEPTOR

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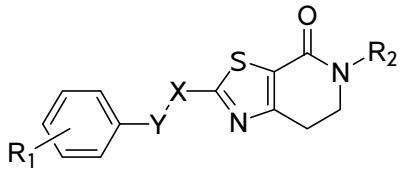
In light of the NMDA receptor hypofunction hypothesis of schizophrenia,[1] metabotropic glutamate 5 (mGlu5) receptor activation has emerged as one of the most appealing non-dopamine based approaches proposed and investigated in recent years for potential therapeutic intervention of schizophrenia.[2] As the development of orthosteric agonists for mGlu5 receptors (as well as for the other mGlu receptors) may be hindered by multiple challenges, (e.g., poor drug-like properties, elusive selectivity or potential tolerance development) current strategies have mainly focused on the identification of positive allosteric modulators (PAMs) instead.[3] This mGlu5 allosteric approach has yielded its first promising results as activity in various preclinical schizophrenia and cognition animal models has already been reported for different mGlu5 receptor PAMs.[3]

Starting from an HTS hit, a focused medicinal chemistry optimization has led us to the identification of a series of thiazolopyridone derivatives as a novel class of mGlu5 receptor PAMs. These compounds potentiate receptor responses in recombinant systems and have also proven to be efficacious in preclinical models of psychosis. Evolution of our medicinal chemistry program, SAR and SPR analysis as well as a detailed profile for an optimized mGlu5 receptor PAM will be described.



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P41. TARGETING THE DREAM PROTEIN: NEW AVENUES FOR THE SEARCH OF DRUGS FOR NEURODEGENERATIVE DISEASES

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Altered neuronal calcium homeostasis and early compensatory changes in transcriptional programs are common features of many neurodegenerative pathologies including Alzheimer's disease (AD), Down syndrome (DS) and Huntington's disease (HD). DREAM (Downstream Regulatory Element Antagonist Modulator), also known as calsenilin or KChIP-3 (potasium channel interacting protein-3), is a multifunctional Ca^{2+} binding protein that controls the expression level and/or the activity of several proteins related to Ca^{2+} homeostasis, neuronal excitability and neuronal survival.[1] This protein is widely expressed in the brain and, depending on the cell type and physiological conditions, shows multiple subcellular localizations, in the nucleus, cytosol or cell membrane.[2]

Initially, the interest in DREAM was based on its key role in the regulation of intracelular Ca^{2+} levels.[1,3] An early reduction in DREAM levels is found in the pre-symptomatic phase of several neurodegenerative mouse models, including AD, DS and HD. These data support the idea that an early down regulation of the DREAM level in neurons during the pre-symptomatic phase of the AD, DS and HD might be part of its neuroprotective mechanism. These findings suggest that DREAM could be a novel and versatile target for therapeutic intervention in neurodegeneration and that molecules able to bind to DREAM and block its physiological functions could be candidates of drugs to treat neurodegenerative diseases. Up to know, low molecular weight molecules have not been described able to interact with DREAM and to modulate its action.

In this communication we report the rational design, the synthesis and the biological evaluation of novel DREAM-binding molecules.

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