# Conference proceedings

# INTERDISCIPLINARY CHEMICAL APPROACHES FOR NEUROPATHOLOGY CM1103

## "4th Neuroscience Day University of Malta"

| 22nd Tuesday | Speaker | Topic // Title |
|---|---|---|
| 14.00-14.30 | UoM - **Richard Muscat**<br>MT COST - **Janet Mifsud** UoM-St Andrews, UK- **Rona Ramsay**<br>UoM - **Giuseppe Di Giovanni** | Welcome //<br>Introduction |
| **Session 1** | **Chair: Rona Ramsay** | |
| 14.30-15.00 | Di Giovanni Giuseppe (MT, WG4) | GPCRs modulation of extrasynaptic GABAARs |
| 15.00-15.30 | Peter Gmeiner (DE, guest speaker) | GPCR ligands probing structure and controlling function |
| 15.30-16.00 | Tipton Keith (CR, WG4) | From magic bullet to scatter-gun; is there a viable alternative? |
| 16.00-16.30 | Mavri Janez (CR, WG4) | How Enzymes Work? QM/MM Simulation of MAO |
| **Session 2** | **Chair: Mück-šeler Dorotea** | |
| 17.00-17.30 | Crespi Francesco (IT, guest speaker) | Non-invasive analysis of brain penetration of chemicals: concomitant Near-Infrared Spectroscopy [NIRS] and pharmacokinetic - pharmacodynamic [PK/PD] study |
| 17.30-18.00 | Magdalena Majekova (SK, WG4) | Bioactivity parameters of indole - type compounds and their possible relevance to treatment of neurological diseases |

| 23rd Wednesday | Speaker | Topic // Title |
|---|---|---|
| **Session 3** | **Chairs: Giuseppe Di Giovanni/Richard Muscat** | 4th Neuroscience @ University of Malta |
| 9.00-9.30 | Valentino Mario (MT, No COST) | Two-photon imaging of cortical microvessels and astrocytic interactions in live mouse brain |
| 9.30-10.00 | Ruben Cauchi (MT, No COST) | Modelling Spinal Muscular Atrophy in Drosophila: a Fruitful Approach? |
| 10.00-10.30 | Neville Vassallo (MT, No COST) | Lipid Membranes - a new target for neurodegeneration |
| 10.30-11.00 | Zammit Christian (MT, No COST) | Immature axons: a new therapeutic target for neonatal white matter ischaemia? |
| **Session 4** | **Chair: Marco-Contelles José** | |
| 11.30-12.00 | Simic Goran (HR, WG4) | The necessity of reliable biomarkers for monitoring potential treatments in Alzheimer's disease |
| 12.00-12.30 | Roberto Di Maio (USA, NoCOST) | Muscarinic stimulation elicits abnormal GABA-ergic differentiation in Mouse-derived stem cells |
| 12.30-13.00 | Philippe De Deurwaerdére (FR, WG3) | 5-HT2C receptors: a G-protein coupled receptor involved in opposite and distributed controls in basal ganglia |
| 13.00-13.30 | Mauro Pessia, (IT, guest speaker) | Potassium channels as target of CNS disorders |
| **Session 5** | **Chair: Di Giovanni/Muscat** | 4th Neuroscience @ University of Malta |
| 14.30-14.50 | Massimo Pierucci (MT, No COST) | Nicotine Addiction and Lateral Habenula |
| 14.50-15.10 | Gabriella Andrina Mifsud(MT, No COST) | Oligodendrocyte pathophysiology and treatment strategies in ischemia |
| 15.10-15.30 | Stephanie Ghio and Michelle Briffa. (MT, No COST) | Amyloid neurodegeneration: from electrophysiology to flies |
| 15.50-16.10 | Frau Robert (IT, WG4) | Positive allosteric modulation of GABA-B receptors: a novel therapeutic approach for schizophrenia |
| 16.10-16.30 | Esteban Gerard (ES, WG1) | 'Effect of new MTDL hybrids based on donepezil, pyridyl and indolyl moieties on Monoaminergic and Cholinergic systems: An HPLC metabolic approach'. |

| 24th Thursday | Speaker | Topic // Title |
|---|---|---|
| **Session 6** | **Chair: Valoti Massimo** | |
| 9.00-9.30 | Musilek Kamil (CZ WG2) | Design, synthesis and evaluation of modulators counteracting ABAD Aβinteraction |
| 9.30-10.00 | Unzeta Mercedes (ES WG3) | In vivo and in vitro biological assessment of ASS234, a novel Donepezil-indolpropargylamine, as a multifunctional molecule with a potential therapeutic profile for Alzheimer's disease |
| 10.00-10.30 | Mück-šeler Dorotea (HR WG4) | Serotonergic receptors, the new targets in the treatment of Alzheimer's disease |

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| 10.30-11.00 | Marco-Contelles José (ES WG2) | The Revisited MAO Inhibition by N-(Furan-2-ylmethyl)-N-prop-2-yn-1-amine Derivatives as Potential Drugs for the Treatment of Alzheimer's Disease |
| **Session 7** | **Chair: Dr Maria Carreiras** | |
| 11.30-12.00 | Najat Aourz (BE, WG3) | Sst2 and sst3 - but not GHS-R1a- receptors are involved in the anticonvulsant effects of cortistatin-14 |
| 12.00-12.30 | Stark Holger (DE, WG1, 2) | Bioisosteric Replacement in Dopamine D2-like Receptor Agonists |
| 12.30-13.00 | Carreiras Maria (PT, WG1,2) | Synthesis, pharmacological assessment, and molecular modeling of AChE/BuChE inhibitors: effect against amyloid-β |
| 13.00-13.30 | Marcello Leopoldo (IT, guest speaker) | Recent Advances in the Study of 5HT7 Receptor Pharmacology: Focus on the Selective Agonist LP-211 |
| **Session 8** | **Chair: K. Tipton** | |
| 14.30-15.00 | Ponimaskin Evgeni (DE, No COST) | Interplay between serotonin receptors 5-HT1A and 5-HT7 in regulation of receptor functions in the brain |
| 15.00-15.30 | Nikolic Katarina (RS WG1) | Pharmacophore Modeling of Novel Non-imidazole Histamine H3 Receptor Ligands with Inhibitory Histamine N-Methyltransferase Activity |
| 15.30-16.00 | Valoti Massimo (IT, WG3) | CYP-dependent metabolism and vascular effects of ASS234, a novel multitarget-directed ligand |
| **Session 10** | **Chair: Simic Goran** | |
| 16.30-17.00 | Vianello Robert (HR WG1) | Recent progress in understanding the catalytic activity of monoamine oxidases |
| 17.00-17.30 | Yelekci Kemal (TR WG1) | In silico design of novel and selective neuronal nitric oxide synthase (nNOS) inhibitors |
| 17.30-18.00 | Butini Stefania (IT, Guest Speaker) | Novel Tools for Disease Modifyng anti-Alzheimer's Drugs: hChEs and b-Amyloid Aggregation Inhibitors |

# ABSTRACTS

## P1.1
## MODULATION OF EXTRASYNAPITIC GABAA RECEPTORS BY G-PROTEIN-COUPLED RECEPTORS

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$GABA_A$ receptors ($GABA_ARs$), the main inhibitory neurotransmitter-gated ion channels in the central nervous system, are finely tuned by other neurotransmitters and endogenous ligands. The regulation of synaptic $GABA_ARs$ ($sGABA_ARs$) by G protein-coupled receptors (GPCRs) has been well characterized and is known to occur either through the conventional activation of second-messenger signalling cascades by G proteins or directly by protein-protein coupling. In contrast, research on the modulation of extrasynaptic $GABA_AR$ (eGABAARs) is still in its infancy and it remains to be determined whether both of the above mechanisms are capable of controlling $eGABA_AR$ function. In this talk, I will summarize the available literature on $eGABA_AR$ modulation by GPCRs, including $GABA_B$, dopamine (DA) and serotonin (5-HT) 2A/2C (5–$HT_{2A/2C}$). Although at present these GPCRs−$eGABA_ARs$ cross-talks have been investigated in a limited number of brain areas (i.e. thalamus, cerebellum, hippocampus, striatum), it is already evident that $eGABA_ARs$ show nucleus and neuronal type-selective regulation by $GPCR_s$ that differs from that of $sGABA_ARs$. This distinct regulation of $eGABA_ARs$ versus $sGABA_ARs$ by GPCRs provides mechanisms for receptor adaptation in response to a variety of physiological stimuli and under different pathophysiological conditions. Further research will advance our understanding of $eGABA_ARs$ and GPCR signalling and offer novel targets for the treatment of many neurological and neuropsychiatric disorders where abnormalities in $eGABA_ARs$ have been suggested to exist.

**KEY WORDS:** Absence epilepsy, metabotropic receptors, monoamines, phosphorylation, tonic $GABA_A$ inhibition.

## P1.2
## GPCR LIGANDS PROBING STRUCTURE AND CONTROLLING FUNCTION

**Peter Gmeiner**[1]
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GPCRs constitute a large superfamily of target proteins (nearly 800 different human genes encode for GPCRs) and each of them can adopt functionally distinct conformations. The first X-ray crystal structures of druggable GPCRs in complex with ligands provide a basis for the investigation of molecular determinants responsible for affinity and selectivity of ligands. Moreover, the structures of different activity states of GPCRs allow us to identify molecular interactions discriminating between inverse agonists, antagonists and agonists. These fundamental results also contribute to the rational discovery of drugs selectively binding to particular conformational states. Thus, there is growing evidence that homo- and heterodimers effect and diversify G-protein coupling. Besides this, the concept of functional selectivity (biased signaling) owing to ligand-specific GPCR conformations has been corroborated. Although GPCR-binding drugs could be evolved for a number of target GPCRs, the rational development of drugs with beneficial selectivity patterns between structurally related GPCRs and functionally relevant GPCR conformations, controlling intrinsic activity profiles, requires a better understanding for GPCR ligand interactions. We have developed GPCR ligands as molecular probes for structural investigations and structure-function relationship studies. Probing the molecular determinants of GPCR function, we designed functionally selective dopamine D2 receptor agonists that are able to differentiate between the activation of two relevant G-proteins, $G_o$ and $G_i$.

**KEY WORDS:** GPCR, molecular probe, functional selectivity.

## P1.3
## FROM MAGIC BULLET TO SCATTERGUN: IS THERE A VIABLE ALTERNATIVE?

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It is over 100 years since the Ehrlich concept of the magic bullet, but, leaving aside monoclonal antibodies,

very few drugs have achieved the selectivity and specificity that he had hoped for. In many cases multi-targeted drugs have proven advantageous. However, the complexities of drug actions and interactions in the tissues make it difficult to envisage likely responses without time-consuming experimentation and testing. Systems biological approaches may help to shorten the time and expense of drug development and assessment. The approach described here involves deconstruction of the putative drug molecule into component structures that can then be used to predict its metabolic fate in the tissues and the metabolic products that might influence its actions. Extensions also allow the possibility of predicting receptor interactions and groups on the molecule that may impede such interactions, which may then assist rational drug design. Finally, *in silico* approaches to investigate tissue and species differences in the metabolism of drugs will be outlined.

**KEY WORDS:** *in silico* drug development, drug metabolism, systems biology.

## P1.4
## HOW ENZYMES WORK? QM/MM STIMULATION OF MAO

**Janez Mavri[1,2], Matej Repič[1], Rok Borštnar[1], Miha Purg[1], Fernanda Duarte[3], S.C. Lynn Kamerlin[3] and Robert Vianello[4]**
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Understanding of biological processes at the molecular level is one of the greatest challenges in biomedical research and the key to understanding how biomolecules, biomolecular systems, cells, and ultimately, living organisms function. Molecular dynamics simulations of hydrated enzymes provide rate constants for enzymatic reactions.

In this talk I will give an overview of this simulation of hydrated enzymes. The choice of initial state, effectively polarized vs. polarizable force fields, proper treatment of long-range electrostatics, protonation states of ionizable residues and associated pKa values, inclusion of explicit water molecules and necessity for hierarchical treatment of enzymes will be discussed. We will touch the ideas behind treatment of chemically reactive systems using QM/MM approach and quantization of the nuclear motion allowing for treatment of tunneling. As a case study I will use monoamine oxidase B (MAO B), an enzyme that catalytically decomposes dopamine and to a lesser extent serotonin. For this enzyme we suggested the mechanism that is consistent with all available experimental data and we performed a series of biomolecular simulations.

**KEY WORDS:** Biomolecular simulation, hydrated enzymes, electrostatics, QM/MM, MAO B, enzyme, dopamine, serotonin.

## P1.5
## NON INVASIVE ANALYSIS OF BRAIN PENETRATION OF CHEMICALS: CONCOMITANT NEAR-INFRARED SPECTROSCOPY [NIRS] AND PHARMACODYNAMIC [PK/PD] STUDY

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Near-infrared spectroscopy (NIRS) selectively monitors non-invasively the absorption spectra of the oxygenation - deoxygenation states of haemoglobin ($HbO_2$/Hb, respectively). These measurements and the total haemoglobin concentration ($HbO_2$ + Hb) considered as total blood volume are indicative of the state of vascular activity, the level of oxygen saturation, and therefore the state of the metabolism in the living tissue. This study proposes that changes in brain metabolism measured by NIRS are a useful index of brain penetration and therefore brain activity of chemical entities. Compounds from different chemical classes were selected on the basis of their known brain penetration and pharmacokinetic profile. In particular, two NK1-SSRI receptor antagonists (GSK135... and GSK189...) having similar molecular characteristics and two glycine-1 transporter inhibitors (GSK270... and GSK267...) were chosen based on in vitro high or low rat brain penetration (B/B) ratio, respectively. It appears that treatment with GSK135 (B/B ratio: 2.70:1) modifies the NIRS parameters while GSK189 (B/B ratio: 0.22:1) does not significantly alter $HbO_2$ - Hb levels when comparing to vehicle treated rats. Similar results are obtained using GSK270 or GSK267 (brain concentration 1hr post treatment: 388 or 13ng/g, respectively).

These results indicate a direct relationship between brain penetration (and possibly efficacy) of drugs and brain metabolism. Thus, they support that *in vivo* non-invasive NIRS contributes to assess brain penetration of chemicals, i.e. significant changes in

NIRS parameters could be related to brain exposure, or vice versa the lack of significant changes in NIRS $HbO_2$/Hb could be indicative of low brain exposure and indeed low efficacy.

**KEY WORDS:** *in vivo* non-invasive NIRS, HbO2/Hb, rat brain, blood brain barrier.

## P1.6
## BIOACTIVITY PARAMETERS OF INDOLE-TYPE COMPOUNDS AND THEIR RELEVANCE TO TREATMENT OF NEUROLOGICAL DISEASES

**Magdalena Majekova[1], Milan Stefek[1], Marta Soltesova Prnova[1], Ivana Milackova[1], Jana Ballekova[1], Zdenka Gasparova[1], Pavol Janega[2] and Pavol Majek[3]**

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Compounds with indolic moiety are known for their manifold potential in biological activities. In the perspective of an intervention against neurological diseases several activities are coming into focus, as the ability to prevent oxidation stress, the preservation of the monoamine neurotransmitter signal (e.g. by the inhibition of MAO enzymes), the anti-inflammation properties, etc. The summary of our recent knowledge in this field is the goal of the presentation.

The hexahydropyridoindoles derived from their parent structure stobadine ((-)-cis-2,8-dimethyl-2,3,4,4a,5,9b-hexahydro-1H-pyrido[4,3-b]indole) exhibited neuroprotective properties manifested in hypoxia-reoxygenation treated brain tissues and slices (in vitro) and ischemia/reperfusion brain (in vivo). From the compounds studied, the derivatives with R8 methoxy substitution exceeded others in the antioxidant and neuroprotective activities. Studies in vivo established anxiolytic effect for the methoxy derivative SMe1EC2 (±)-8-methoxy-1,3,4,4a,5,9b-hexahydro-pyrido[4,3-b]indole-2-carboxylic acid ethyl ester. The derivative SMe1EC2 was found to protect the hippocampus of rats exposed to trimethyltin (a model of Alzheimer-like neurodegenerative disorder) from cell death and damage. For further study, the elaborated model of MAO-B inhibition based on 2v5z complex with safinamide with YAMBER3 force field was used. The key interactions for methoxy substituted derivatives were determined.

The derivatives of 1-indole acetic acid were found to be efficient inhibitors of aldo-keto reductases (AKR). The role of the AKR enzymes in the development of neurodegenerative disorders and a possible intervention via AKR inhibition are brought for discussion.

**KEY WORDS:** Indole-type compounds, MAO-B inhibition, molecular modeling, trimethyltin, neuroprotection.

Acknowledgement: supported by COST-CM1103, VEGA 2/0067/11, VEGA 2/0048/11 and VEGA 2/0030/11.

## P2.1
## TWO- PHOTON IMAGING OF CORTICAL MICROVESSELS AND ASTROCYTIC INTERACTIONS IN LIVE MOUSE BRAIN

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In vivo imaging with two-photon microscopy is becoming an indispensable technique to investigate cellular and subcellular phenomenon in living tissues including the central nervous system. This microscopy enables us to image the dynamics of molecules, morphology, and excitability with minimal invasion to tissues and with unsurpassed spatial and temporal resolution. Two-photon microscopy provides a number of advantages that aid the study of the mechanisms underlying neurovascular coupling and cerebrovascular disease in animal models, including: (i) the resolution needed to visualize single cortical vessels and their surrounding cells; (ii) penetration depths of 250μm through a PoRTS (polished and reinforced thin skull) window and 500μm with dura-removed craniotomies, and even deeper imaging with longer excitation wavelengths; (iii) reduced photodamage and photobleaching; (iv) high-speed user-defined line scans for near-simultaneous measurement of RBC velocity, lumen diameter, and local cellular activity; (v) longitudinal imaging over several months; and (vi) the ability to image vascular dynamics deep in the cortex of awake mice. This two-photon imaging method allows extremely high spatial and temporal resolution for studying pathological mechanisms that underlie ischemic injury.

We will provide examples on how we apply these techniques to the study of local blood flow regulation and vascular pathologies such as small-scale stroke including abnormal changes in calcium cell signalling, vascular dysfunction following photothrombosis, and inflammation.

**KEY WORDS:** Two-photon microscopy, cranial window, neurovascular coupling, cerebrovascular disease, vascular dynamics, photothrombosis, calcium signalling.

## P2.2
## MODELLING SPINAL MUSCULAR ATROPHY IN DROSOPHILA: A FRUITFUL APPROACH?

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Spinal muscular atrophy (SMA) is the most common genetic killer of new-borns. The cause of this devastating neuromuscular disorder has been pinned on very low levels of the survival motor neuron (SMN) protein. SMN partners with the Gemin proteins to form a highly ordered complex. The best-characterised function of the SMN-Gemin complex involves assembly of the basic units that form the spliceosome or the chief editor of RNA messenger molecules that instruct cells how to fabricate proteins. Flies have a minimalistic complex that is amenable to genetic manipulation. We describe the phenotypes resulting from disruption of the *Drosophila* SMN complex. Our findings inform on the molecular pathway that might be negatively impacted in SMA.

**KEY WORDS:** Spinal muscular atrophy, *Drosophila*, survival motor neuron, SMN-Gemin complex, gemins, motor neuron degeneration.

## P2.3
## LIPID MEMBRANES- A NEW TARGET FOR NEURODEGENERATION

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Alzheimer's disease (AD) and Parkinson's disease (PD) are neurodegenerative disorders characterised by the misfolding of proteins into soluble prefibrillar aggregates. In our work, we have demonstrated that amyloid aggregates of recombinant amyloid-β(1-42) peptide, tau-441 and α-synuclein proteins, robustly compromised the membrane integrity of model liposomes. Interestingly, such liposome permeabilisation mimicked that of the pore-forming bacterial peptides gramicidin. Also, we screened 11 natural polyphenolic compounds, 8 synthetic N'-benzylidene-benzohydrazide compounds and black tea extract for protection against membrane damage by the amyloid aggregates. We therefore identified a select group of potent inhibitory compounds which include baicalein, morin, nordihydroguaiaretic acid and black tea extract. Since mitochondria are intimately involved in the pathophysiological cascades of both AD and PD, we further explored the interaction of soluble amyloid aggregates with mitochondrial membranes. Here, we made use of two *in vitro* model systems, namely: (i) lipid vesicles with defined membrane compositions that mimic those of mitochondrial membranes, and (ii) respiring mitochondria isolated from neuronal SH-SY5Y cells. Briefly, it was found that aggregates, but not monomers, induced a robust permeabilisation of mitochondrial-like vesicles, and triggered cytochrome c release from isolated mitochondrial organelles. Importantly, the effect on mitochondria was shown to be dependent upon cardiolipin, an anionic phospholipid unique to mitochondria and a well-known key player in mitochondrial apoptosis. Thus, we propose a generic mechanism of thrilling mitochondria in which soluble amyloid aggregates have the intrinsic capacity to permeabilise mitochondrial membranes, without the need of any other protein.

**KEY WORDS:** Alzheimer disease, Parkinson disease, amyloid aggregate, amyloid-β, tau-441, α-synuclein, mitochondria, baicalein, morin, nordihydroguaiaretic acid.

## P2.4
## IMMATURE AXONS: A NEW THERAPEUTIC TARGET FOR NEONATAL WHITE MATTER ISCHAEMIA?

**Christian Zammit**[1]
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Brain injury in the premature infant, especially in very low birth weight infants, is a problem of major importance in our society. Recent advances in neonatal intensive care have dramatically increased the survival rate of such infants. Premature infants are at a great risk of developing cerebral palsy together with cognitive, attentional, behavioural, and socialization deficits that significantly impair their quality of life. Cerebral white matter injury is increasingly recognised as a common form of perinatal brain injury that predisposes to such neurological defects. Extensive studies point to the premyelinating oligodendrocyte to be the key

cellular target involved in neonatal cerebral white matter injury, due to a series of maturation-dependent events. However, the premyelinating oligodendrocyte must not be regarded as the sole target.

By imaging GFP-M expression in neonatal mice optic nerves, we found highly selective injury to ischaemia of the small-diameter fluorescent axons that corresponded to the larger pre-myelinated axons. These axons, after having initiated diameter expansion and expression of functional voltage-gated calcium channels, are exquisitely sensitive to ischemic injury. Moreover, pharmacological treatment with a combination of glutamate receptor blockers and voltage-gated calcium channel blockers offered a high degree of protection following an ischaemic insult. This elevated susceptibility of early maturing axons to ischemic injury may significantly contribute to selective white matter pathology and places these axons alongside pre-oligodendrocytes, previously regarded as the most ischemia-sensitive element within immature white matter. Therefore, future therapeutic strategies must include protection to both of these white matter elements.

**KEY WORDS:** Perinatal brain injury, white matter ischaemia, large pre-myelinated axons, optic nerve, voltage-gated calcium channels.

## P2.5
## THE NEED FOR RELIABLE BIOMARKERS FOR MONITORING POTENTIAL TREATMENTS IN ALZHEIMERS DISEASE

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The search for predictive biomarkers for Alzheimer's disease (AD) is of high priority in neurodegenerative disease research underlined by the lack of significant progress in identifying new treatments for the past 12 years. Despite major efforts and considerable investments, the treatments approved for AD are only palliative. They include cholinesterase inhibitors (donepezil, galantamine, and rivastigmine) that act on the cholinergic deficit, and the NMDA receptor antagonist, memantine, which has neuroprotective effects. These agents are generally considered to have marginal efficacy. As it can be logically assumed that a late therapeutic intervention would be less efficient than an early one, development of biomarkers for AD both to diagnose the disease early and to follow-up its progression, remains a major challenge. Currently, it is comprised of 6 main approaches: 1) behavioral assessment, including measurement of cognitive status using various neuropsychological scales (MMSE, ADAS-Cog, etc.); 2) changes in brain structure (mainly volume of the cerebral cortex, particularly entorhinal cortex and hippocampus); 3) alterations in brain metabolism (most notably within the default mode network) by using FDG-PET; 4) measurement of β-amyloid load within the brain by PIB-PET; 5) cerebrospinal fluid (CSF) biomarker profiles (the three main CSF biomarkers of AD being β-amyloid, total tau, and phosphorylated forms of tau proteins); and 6) post-mortem confirmation of characteristic AD histopathology. In my talk I will attempt to describe new developments within each of these biomarker approaches, analyzing their pathological specificity, early diagnostic sensitivity, and correlation with AD progression. Finally, I will argue that, despite numerous publications and recommendation criteria, the predictive usefulness of these various biomarker approaches, individually or collectively, has yet to be established.

**KEY WORDS:** Alzheimer's disease, biomarkers, default mode network, DTI, fMRI, PET.

## P2.6
## 5-HT2C RECEPTORS: A G-PROTEIN COUPLED RECEPTOR INVOLVED IN OPPOSITE AND DISTRIBUTED CONTROLS IN BASAL GANGLIA

**Philippe De Deurwaerdère**[1], **Mélanie Lagière**[1], **Marion Bosc**[1], **Sylvia Navailles**[1]
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5-$HT_{2C}$ receptors, one of the seven-transmembrane G-protein coupled receptors for serotonin (5-HT), is a potential therapeutic target of numerous diseases, such as Parkinson's disease and schizophrenia, that involve combined dysfunctions of dopamine (DA) transmission and basal ganglia, a group of subcortical structures involved in motor behaviours. 5-$HT_{2C}$ receptors, present in the whole basal ganglia, would exert tonic, phasic and constitutive controls, the latter being independent of the presence of 5-HT. Using appropriate 5-$HT_{2C}$ receptor pharmacological tools (agonists for phasic, antagonists for tonic, inverse agonists for constitutive control), we have addressed in rats the organisation of these different controls on a motor behaviour, the purposeless orofacial movements, on the expression of the proto-oncogene c-Fos, a marker of change of neuronal activity, and on the electrophysiological

responses of neurons located in the output structures of basal ganglia, namely the entopeduncular nucleus (EPN) or the substantia nigra *pars reticulata*.

Both 5-$HT_{2C}$ agonists and inverse agonists increased abnormal orofacial movements via 5-$HT_{2C}$ receptors. c-Fos imaging studies indicated that different 5-$HT_{2C}$ controls are expressed in the input structures of the basal ganglia, the striatum and the subthalamic nucleus. In addition, agonists and inverse agonists altered neuronal activity in the output structures which could be associated with the emergence of orofacial movements. 5-$HT_{2C}$ controls are influenced by the level of DA transmission. Indeed, DA neurons lesion potentiated behavioural and electrophysiological responses induced by a 5-$HT_{2C}$ agonist by acting in the EPN. The stimulation of D2 receptors enhanced oral dyskinesia and electrophysiological responses of the cortico-subthalamonigral pathway; these effects were suppressed by selective 5-$HT_{2C}$ antagonists. This work illustrates the complexity of the controls exerted by 5-$HT_{2C}$ receptors and their outcome with respect to central DA transmission. A better understanding of the controls in these regions would permit to apprehend possible treatments using $5-HT$ and/or 5-$HT_{2C}$ agents.

**KEY WORDS:** Serotonin 2C receptor, basal ganglia, dopamine, dyskinesia, parkinson's disease, entopeduncular nucleus, subthalamic nucleus, substantia nigra pars reticulata, striatum.

## P2.7
## POTASSIUM CHANNELS AS A TARGET OF CNS DISORDERS

**D'Adamo Maria Cristina[1], Servettini Ilenio[1], Guglielmi Luca[1] and Pessia Mauro[1]**
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$K^+$ channels are critical for neuronal excitability and they are essential effectors of neurotransmitter-mediated signaling. They are distinguished by being the largest and most diverse class of ion channels, being encoded by more than 70 genes. In the past decades several types of human diseases have been associated to dysfunction of $K^+$ channels, resulting from mutations in their encoding genes. Indeed, $K^+$ channels defects underlie a number of distinct forms of epilepsies that have been named "$K^+$ *channelepsies*". Also different types of ataxias have been associated with altered $K^+$ channels function. In particular we have shown that episodic ataxia type 1 (EA1), a $K^+$ channelopathy, which manifests with short attacks of cerebellar ataxia, is caused by *loss-of-function* mutations in Kv1.1 (KCNA1) channels. The direct and indirect involvement of $K^+$ channels in a number of psychiatric disorders including autism spectrum disorders (ASDs), schizophrenia, and mental retardation has been reported. ASDs are characterized by impaired ability to properly implement environmental stimuli that are essential to achieve a state of cultural and social inter-relationships. The main features of this disease are marked impairments of verbal and non-verbal communication with restricted and repetitive behaviors. We have performed the genetic analysis of individuals affected by autism and epilepsy and identified new heterozygous point mutations in the KCNJ10 gene that encodes the inwardly-rectifying $K^+$ channel Kir4.1, expressed predominantly, but not exclusively, in astrocytes. Functionally, the mutated channels exhibited a phenotype consistent with *gain-of-function* defects. These new findings highlight the emerging role of inwardly-rectifying $K^+$ channels and astrocyte dysfunction in autism spectrum disorders associated with epilepsy.

**KEY WORDS:** Potassium channels, mutation, epilepsy, ataxia, $K^+$ channelopathy, ASD, astrocyte dysfunction, inward- rectifying $K^+$ channels.

## P2.8
## EFFECT OF ACUTE AND REPEATED NICOTINE ADMINISTRATION ON THE ELECTRICAL ACTIVITY OF THE LATERAL HABENULAR NEURONS IN RATS

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Tobacco smoking represents a well-known risk factor for health that still accounts for a high number of deaths. So far, existing smoking cessation therapies have not been proven very successful at quitting this habit and a better undrstanding of the neurobiology of tobacco dependence is still needed. Nicotine is the neuroactive compound contained in tobacco that is responsible for its rewarding and reinforcing properties by acting on the midbrain dopaminergic system. The lateral habenula (LHb) is an epithalamic structure involved in pain, stress, depression and in encoding aversive stimuli. This structure is known to indirectly

inhibit the DA system through the activation of the RMTg, a GABA-ergic area located at the back of the VTA. The RMTg receives a strong glutamatergic imput from the LHb and is activated by the systemic injection of nicotine in rats. Thus the LHb might represent a possible target for the action of nicotine. Our data shows that systemic administration of nicotine dose-dependently increases the activity of single LHb neurons recorded extracellularly in vivo in rats, particularly at high doses. Following two weeks of nicotine chronic treatment, this response is drastically decreased while after 1 day of withdrawal only low doses of nicotine are again able to significantly increase the firing activity of the LHb neurons compared to the control group. These evidences strongly suggest that the LHb might play an important role in mediating the effects of nicotine on the midbrain DA system thus participating to the mechanism of addiction to this drug.

**KEY WORDS:** Drug of addiction, extracellular recording, serotonin, dopamine.

## P2.9
## OLIGODENDROCYTE PATHOPHYSIOLOGY AND TREATMENT STRATEGIES IN ISCHEMIA

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Oligodendroglia, the myelin-forming cells of the CNS, form a functional unit with axons and play a crucial role in axonal integrity. An episode of hypoxia-ischemia causes rapid and severe damage to these particularly vulnerable cells via the overactivation of glutamate and ATP receptors (excitotoxicity), oxidative stress and mitochondrial disruption. Oligodendrocytes appear to be more vulnerable to HI than other CNS glia, and in certain brain regions and stages of development, more vulnerable than neurons, due to the possession of numerous features, which predispose them to injury. The cardinal effect of oligodendrocyte pathology is demyelination and dysmyelination, and has profound effects on axonal function, transport, structure, metabolism and survival. The oligodendrocyte is a primary ischemic target, in adult-onset stroke and especially in periventricular leukomalacia, and should therefore also be considered a primary therapeutic target. Further emphasis is required on therapeutic strategies targeting oligodendroglia, myelin and their receptors, as these have the potential to significantly attenuate white-matter injury in hypoxia-ischemia.

**KEY WORDS:** Excitotoxicity, hypoxia-ischemia, oligodendrocyte, oxidative stress, stroke.

## P2.10
## AMYLOID NEURODEGENERATION: FROM ELECTROPHYSIOLOGY TO FLIES

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Alzheimer's, Parkinson's and Motor Neuron disease are characterized by the deposition of abnormally aggregated forms of Aβ1-42, α-synuclein and TDP-43, respectively. An intriguing possibility that is being investigated, is the possibility of pore formation in mitochondrial membranes by aggregates of these proteins. Such pores can have deleterious consequences on the electrical physiology of a neuron.
Electrophysiology studies are performed using a lipid bilayer workstation, which allows detailed electrophysiological characterisation upon incubation of amyloid aggregates with mitochondrial membranes. Electrical currents at the level of a single channel are recorded, and changes in membrane permeability can be correlated to toxic channel activity. The potential of natural polyphenols and bioactive extracts to block amyloid pores will be assessed, thereby preventing disruption of neuronal ion homeostasis.
Currently there are no drugs or clear-cut pathogenic mechanisms that do more than improve the symptoms associated with these diseases. Identification of compounds that lead to a marked and consistent recovery, will be a great asset to developing new therapeutic approaches.
Drosophila models of neurodegenerative disease have been successfully used in whole-genome screens aimed at identifying genetic modifiers, which can lead to the discovery of drug targets. The disease fly models are being generated by the overexpression of the respective human transgene in the wild-type fly brain.
A graded dose of a select group of test drugs are being tested and adult flies monitored for survival and climbing ability using well-established protocols. Data will be analysed to determine whether the drug-supplemented diet markedly, and consistently ameliorates the phenotypic defects intrinsic to the disease fly models.

**KEY WORDS:** Amyloid, neurodegeneration, Drosophila, mitochondria, aggregates, drugs.

## P2.11
## POSITIVE ALLOSTERIC MODULATION OF GABA-B RECEPTORS: A NOVEL THERAPEUTIC APPROACH FOR SCHIZOPHRENIA?

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Preclinical and clinical investigations have suggested that Gamma-amino-butyric acid $(GABA)_B$ receptors may play a key role in the pathophysiology of psychiatric disorders. We previously reported that baclofen, the prototypical $GABA_B$ agonist, exerts antipsychotic-like properties in two well-validated rodent models of schizophrenia, the prepulse inhibition (PPI) deficits produced by dizocilpine (MK-801) and the genetically low PPI displayed by DBA/2 mice. However, the adverse side effects elicit by Baclofen, point to develop alternative therapeutic tools for regulating $GABA_B$ in schizophrenia.

Thus, we investigated the impact of a new allosteric enhancers of $GABA_B$, *rac-BHFF* (RAC), on the MK-801 mediated-PPI disruption in Sprague-Dawley (SD) rats and C57/BL mice, two of the most used rodent species in PPI with high baseline of PPI and susceptibility to the NMDA receptor manipulations. Furthermore, we evaluated the properties of RAC in ameliorating the naturally low PPI performance displayed by DBA/2J, in comparison with the positive control antipsychotic, clozapine. RAC did not produce any effects on PPI per se and dose-dependently counteracted the PPI impairments produced by MK-801 in both SD and C57. Notably, dissimilar to Baclofen, these effects were not accompanied with significant alterations of startle parameters. Moreover, RAC was able to restore PPI deficits in DBA/2J, akin to the atypical antipsychotic clozapine.

Our data strengthen previous evidence of $GABA_B$ receptors as an important biological target for the modulation of PPI and suggest a new potential therapeutic application in neuropsychiatric disorders related to sensorimotor gating dysfunctions, without exerting the side effects share by the putative $GABA_B$ agonists.

**KEY WORDS:** $GABA_B$, PAM, pre-pulse inhibition, sensorimotor gating, schizophrenia, NMDA receptors.

## P2.12
## EFFECT OF NEW MULTITARGET-DIRECTED LIGANDS BASED ON DONEPEZIL, PYRIDYL AND INDOLYL HYBRIDS ON MONOAMINERGIC AND CHOLINERGIC SYSTEMS: AN HPLC METABOLIC APPROACH

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The multifactorial nature of Alzheimer's disease (AD) has prompted the search for new Multitarget-Directed Ligands (MTDL) able to simultaneously bind both cholinesterases and monoamine oxidases. We have developed and assessed novel series of MTDLs based on Donepezil-Indolyl hybrids [**MBA98F1** ($IC_{50}$); AChE= 0.19μM; BuChE= 0.83μM; MAO A= 5.5nM; MAO B= 0.15μM], Donepezil-Pyridyl hybrids [**MBA115** ($IC_{50}$); AChE= 1.4nM; BuChE= 0.51μM; MAO A= 53.3μM; MAO B= 10.2μM] or α-Aminonitriles hybrids [DHP6 ($IC_{50}$); AChE= 1.8μM; BuChE= 1.6μM; MAO A= 6.2μM; MAO B= 10.2μM; with metal-chelating properties] for their potential pharmacological use in AD.

The effect of the MAO A-selective inhibitors clorgyline and the multipotent **ASS234** on the monoaminergic system, was also evaluated on human neuroblastoma SHSY-5Y and undifferentiated pC12 cell lines. High activity levels of MAO A were determined in both cell lines; this activity was fully inhibited after treating the cells with 1μM of clorgyline or **ASS234** for 24 hours.

Both inhibitors were able to modulate the levels of monoamines by HPLC after treatments. Levels of serotonin (5-HT) and 3-metoxytryptamine (3-MT) were significantly increased while those of dopamine, DOPAC, 5HIIA and homovanillic acid (HVA) decreased. The levels of noradrenaline and L-DOPA remained unaltered. These results suggest that novel multipotent inhibitors herein presented deserve further investigation for their potential pharmacological treatment of Alzheimer's disease.

**KEY WORDS:** Multipotent drugs, Fe/Cu Chelators, ChE/MAO inhibitors, monoamines, Alzheimer's disease.

## P3.1
## DESIGN, SYNTHESIS AND EVALUATION OF MODULATORS COUNTERACTING ABAD-AβINTERACTION

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Although the aetiology of AD is still unknown, the build-up of amyloid β-peptide (Aβ) is considered to play a central role in the pathogenesis of the disease. It is well established that the intracellular accumulation of Aβis associated with AD and increasing evidence suggests that mitochondria may be an important target for intracellular Aβto exert its neurotoxic effects.

Amyloid-binding alcohol dehydrogenase (ABAD) is to date the most characterized Aβ-binding intracellular protein. Direct interaction of this mitochondrial enzyme with Aβwas confirmed by many different methods. Aβbinding to ABAD triggers a series of events leading to mitochondrial dysfunction characteristic for AD. Thus this interaction may represent a novel target for treatment strategy against AD.

The benzothiazole urea analogues related to known immunosuppressant frentizole was synthesized and in vitro evaluated for its capability to inhibit interaction between ABAD and Aβ. Several prepared compounds showed ability to inhibit ABAD *in vitro*. These promising compounds are going to be further tested on living cells.

**KEY WORDS:** Mitochondria, ABAD, β-amyloid, inhibitor, benzothiazole.

## P3.2
## *In vivo* AND *in vitro* BIOLOGICAL ASSESSMENT OF ASS234, A NOVEL DONEPEZILINDOLPROPARGYLAMINE, AS A MULTIFUNCTIONAL MOLECULE WITH A POTENTIAL THERAPEUTIC PROFILE FOR ALZHEIMER'S DISEASE

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A key pathological hallmark of AD is amyloid beta (A$\beta$ aggregation and deposition. Growing evidence suggest that the neurotoxicity of these peptide is related to the formation of toxic oligomeric aggregates. Thus, a deeply investigated therapeutic strategy comes at present from blocking the formation of these species to non-toxic aggregates. Nevertheless, clinical trials evaluating anti-A$\beta$ drugs are not giving conclusive results and brain penetration of these molecules is also an important challenge to be solved. The multifactorial nature of Alzheimer's disease (AD) supports the most current innovative therapeutic approach, which proposes that single molecules acting on multiple targets might be more suitable for the treatment. Thus, molecules possessing a rich pharmacology are of great interest. In this context, we recently identified ASS234, a new multipotent drug showing an interesting inhibitory profile towards cholinesterase and monoamine oxidase enzymes, possessing also a significant anti-A$\beta$ aggregation activity. In this work, we explore more in detail its anti-A$\beta$ activity and show that ASS234 reduces A$\beta_{1-42}$ aggregation more efficiently than that of A$\beta_{1-42}$, as well as completely blocks the AChE-induced A$\beta_{1-42}$ and A$\beta_{1-42}$ aggregation. We also describe that ASS234 is able to limit the A$\beta_{1-42}$-mediated cytotoxicity, by preventing the activation of the mitochondrial pathway of apoptosis. Moreover, we demonstrate a significant ability of ASS234 to reduce oxidative stress and the finding of its capability to cross the blood-brain barrier. Overall, our results demonstrate that ASS234 is able to bind to multiple targets and suggest that it might be considered for therapeutic development against AD.

**KEY WORDS:** Alzheimer's disease therapy, amyloid cholinesterase inhibitors, multi-target directed ligand, neuroprotection, propargylamines.

## P3.3
## SEROTONERGIC RECEPTORS: THE NEW TARGETS IN THE TREATMENT OF ALZHEIMERS DISEASE

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Serotonergic neurotransmission is implicated in the modulation of many physiological (sleep, sexuality, appetite), behavioural (aggression, mood) and cognitive (learning, memory) functions, which change in aging related disorders. *In vivo* and *in vitro* evidence suggest neuroprotective and pro-cognitive effect of serotonin in Alzheimer's disease (AD). Serotonin also plays a crucial role in the development of behavioural and psychological symptoms of dementia (BPSD) which are present in up to 90% of patients with AD.

Complex functions of the serotonergic system depend on the activity and function of its receptors, classified in seven groups from 5-HT1 to 5-HT7, which differ in terms of structure, action and location. The loss of 5-HT2, 5-HT6 and pre- or post-synaptic 5-HT1A and 5-HT1B receptors were found in patients with AD. It is unclear if these changes are primary or secondary (retrograde), due to the damage of postsynaptic target neurons in regions of the nerve endings. The activation of 5-HT3 and 5-HT4 receptors enhances the acetylcholine release and could induce the pro-cognitive effect. Since preclinical studies have shown that agonists of 5-HT4 and antagonists of 5-HT1A, 5-HT3 and 5-HT6 receptors improve cognitive functions, serotonergic receptors might represent the new pharmacological target for the treatment of AD and BPSD.

**KEY WORDS:** Serotonin, receptor, Alzheimer's disease, medications.

## P3.4
## THE REVISITED MAO INHIBITION BY N-(Furan-2-ylmethyl)-N-prop-2-yn-1-amine DERIVATIVES

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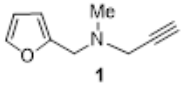

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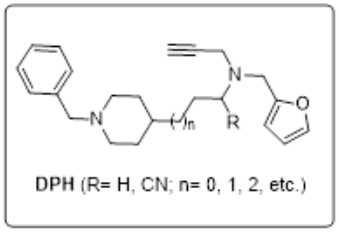


The MAO inhibition analysis of N-(furan-2-ylmethyl)-N-methylprop-2-yn-1-amine (1) has been revisited, showing that this propargylamine is a moderate, but selective, partially reversible and uncompetitive MAO B inhibitor ($IC_{50} = 5.16 \pm 0.86$ μM), whose ADMET properties predict the best profile for acting as CNS drug.

This result paves the way for the projected synthesis and biochemical analysis of new **DPH** ("Donepezil+Propagyl+Hybrid") multipotent molecules, as drugs for the potential treatment of Alzheimer's disease.

**KEY WORDS:** MAO enzymes, inhibitors, propargylamines, kinetics.

## P3.5
## SST2 AND SST3 - BUT NOT GSHR-RECEPTORS ARE INVOLVED IN THE ANTICONVULSANT EFFECTS PF CORTISTATIN-14

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Anticonvulsant and antiepileptic actions of somatostatin-14 have already widely been studied and are thus well known. For the related neuropeptide cortistatin-14 however, only one paper reports on its anticonvulsant effects. Somatostatin-14 and cortistatin-14 are structurally related peptides and have high affinities for the five somatostatin receptor subtypes (sst1-sst5). Despite these homologies, cortistatin-14 seems to act also on other receptors and it has been suggested that the ghrelin receptor (GHSR) may fulfill such a role. Here, we aim to unveil which receptors are involved in the anticonvulsant effects of cortistatin-14 by using in vivo microdialysis and telemetry-based

electrocorticography (ECoG) in rats and mice.
In rats, the involvement of sst2 and sst3 receptors was studied by administering cortistatin-14 (0.1μM - 1μM - 10μM) intrahippocampally, in the presence and absence of sst2 and sst3 receptor antagonists. Seizures were evoked by intrahippocampal pilocarpine perfusion (12mM, 40min) and seizure severity was assessed using a behavioural scoring system and ECoG. Intrahippocampal administration of 1μM and 10μM cortistatin -14 in rats showed clear anticonvulsant actions against pilocarpine-induced seizures. Furthermore, we showed that cortistatin -14 (1μM) - mediated anticonvulsant actions were reversed in the presence of 0.1μM cyanamid, a selective sst2 antagonist or 0.1μM SST3-ODN8, a selective sst3 antagonist. Intrahippocampal perfusion of these antagonists alone did not affect the pilocarpine-induced seizure severity per se.
The involvement of GHSR was tested by administering an anticonvulsant dose (1μM) of cortistatin -14 in both GHSR knock-out (KO) and wild-type (WT) mice. Seizures were evoked by intrahippocampal pilocarpine perfusion (12mM, 40min), and ECoG was used to assess seizure severity, by means of seizure duration. In these mice, both genotype - and treatment dependent alterations in seizure severity were observed by means of two-way ANOVA. Indeed, our results showed that the seizure duration in WT animals was significantly higher, when compared to their KO littermates, and that the seizure duration in the CST- treated animals was significantly lower when compared to the animals receiving only pilocarpine.
In conclusion, our results show that cortistatin -14 prevents seizures in a focal pilocarpine model and that selective sst2 or sst3 receptor antagonism abolishes these anticonvulsant actions in rats. Our findings also demonstrate that the anticonvulsant actions of cortistatin -14 in mice are not mediated via the GHSR receptor.

**KEY WORDS:** Somatostatin, cortistatin, epilepsy, seizures, pilocarpine, ghrelin.

## P3.6
## BIOISOSTERIC REPLACEMENTS FOR OPTIMIZED DOPAMINE RECEPTOR AGONISTS

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LevoDOPA and dopamine agonists have been in therapeutic use for the symptomatic treatment of Parkinson's disease for a long time. Despite the success of this medical approach, numerous unwanted side effects and an unclear receptor-crosstalk raise the need for new and improved therapeutics. Based on the early discovery of Etrabamine and established on the non-ergot dopamine agonist Pramipexole we have developed a series of tetrahydrobenzothiazole derivatives with high receptor affinity, improved receptor subtype selectivity and different efficacy profiles from agonist to antagonist properties. The 2-aminothiazole moiety of Pramipexole has generally been taken as catechol bioisosteric moiety. The replacement of the 2-amino functionality, as well as the modification of the heterocycle, led to novel classes of compounds with moderate to excellent affinity at dopamine D2 and/or D3 receptor subtypes. The synthesis has been performed by reductive amination of cyclohexa-1,4-dione monoketal, followed by deprotection and heterocyclic ring formations by different procedures. Deamination in 2-position could be performed by diazonium formation under reductive conditions.

Some of these derivatives displayed up 400fold binding preference for D3 over D2 receptors, whereas in a functional assay on $[^{35}S]GTP_{\alpha}S$ the binding the preference was less pronounced. Particular compounds showed an impressive biased signaling. The pharmacological *in vivo* profile was assessed for selected compounds in a Parkinsonian model, on 6-OHDA lesioned rats with intraperitoneal (i.p.) and *per os* (p.o.) administration, showing a good potential for further development not only for Parkinson's disease but also for erectile dysfunction.

**KEY WORDS:** Affinity, biased signaling, D2 receptor, D3 receptor, efficacy, pramipexole.

## P3.7
## SYNTHESIS, PHARMACOLOGICAL ASSESSMENT AND MOLECULAR MODELING OF ACETYLCOLINESTERASE/BUTYRYLCHOLINESTERASE INHIBORS: EFFECT AGAINST AMYLOID-BETA - INDUCED NEUROTOXICITY

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Synthesis, molecular modeling, and pharmacological analysis of phenoxyalkylamino-4-phenylnicotinates (**2-7**), phenoxyalkoxybenzylidenemalononitriles (**12-13**), pyridonepezils (**14-18**), quinolinodonepezils (**19-21**), and pyrazolo[3,4-b]quinolines (**35-37**) will be summarized in this talk. The most potent and selective EeAChE inhibitor was ethyl 6-(2-(1-benzylpiperidin-4-yl)ethylamino)-5-cyano-2-methyl-4-phenylnicotinate (**16**) [IC50 (EeAChE)= $0.0167 \pm 0.0002$ μM], which exhibits the same inhibitory potency as donepezil against hAChE. The most potent and selective hAChE inhibitor was ethyl 6-(4-(1-benzylpiperidin-4-yl)butylamino)-5-cyano-2-methyl-4-phenylnicotinate (**18**) [IC50 (hAChE)= $0.25 \pm 0.02$ μM]. Pyridonepezils showed to be selective and moderately potent against hAChE inhibition, whereas quinolinodonepezils showed to be poor hAChE inhibitors. Compounds **2, 7, 13, 17, 18, 35** and **36** significantly prevented the decrease in cell viability caused by $A\beta_{1-42}$. All compounds were effective in preventing the enhancement of AChE activity induced by $A\beta_{1-42}$. Compounds **2-7** caused a significant reduction whereas pyridonepezils **16-18** also showed some activity. The pyrazolo[3,4-b]quinolines **36** and **38** also prevented the upregulation of AChE induced by $A\beta_{1-42}$. Compounds **2, 7, 12, 13, 17, 18** and **36** may act as antagonists of VSCC since they significantly prevented the $Ca^{2+}$ influx evoked by KCl depolarization. Docking studies show that compounds **16** and **18** adopted different orientations and conformations inside the active-site gorges of hAChE and hBuChE. The structural and energetic features of the **16**-AChE and **18**-AChE complexes compared to the **16**-BuChE and **18**-BuChE complexes account for a higher affinity of the ligand toward AChE. Compounds **2, 7, 17, 18** and **36** are attractive multipotent molecules acting in different key pharmacological targets. They may accomplish a potential disease-modifying role in the treatment of Alzheimer's disease.

**KEY WORDS:** Alzheimer's disease, pyridonepezils, AChE/BuChE inhibitors, Aβpeptide, neuroprotection, $Ca^{2+}$ dyshomeostasis.

## P3.8
## RECENT ADVANCES IN THE STUDY OF 5-HT7 RECEPTOR PHARMACOLOGY: FOCUS ON THE SELECTIVE AGONIST LP- 211

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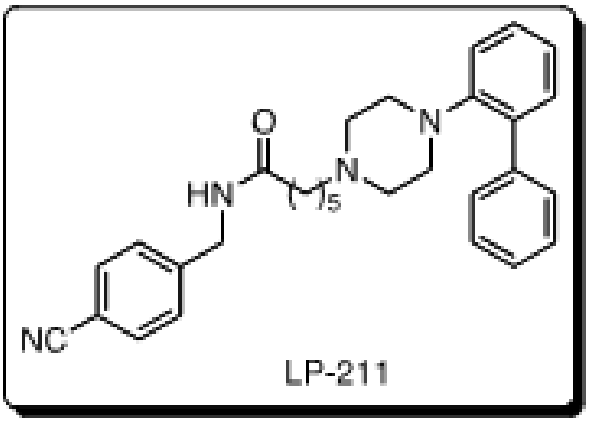
Twenty years after the 5 HT7 receptor was first cloned, there is a large amount of data available in terms of the pathophysiology of this serotonin receptor. Medicinal chemistry efforts have resulted in the identification of 5 $HT_7$ receptor selective agonists and antagonists. While 5 $HT_7$ receptor antagonists have been proposed as antidepressant drugs, the possible therapeutic applications of selective activation of 5 $HT_7$ receptor are emerging in recent years after various selective agonists became available. This lecture will illustrate the process that led to the identification of various selective 5 $HT_7$ receptor agonists in our laboratory, following structure-activity relationship studies on "long-chain" arylpiperazine derivatives. The studies culminated with the discovery of LP-211, a brain-penetrant selective 5 $HT_7$ receptor agonist.




Ki [nM].

| r5-$HT_7$ | h5-$HT_7$ | h5-$HT_{1A}$ | h5-$HT_{1B}$ |
|---|---|---|---|
| 0.58 | 15 | 379 | 215 |
| h5-$HT_{1E}$ | h5-$HT_{2A}$ | h5-$HT_{2B}$ | r5-$HT_{2C}$ |
| > 10000 | 626 | 67 | 91 |
| h5-$HT_3$ | h5-$HT_{5A}$ | h5-$HT_6$ | |
| > 10000 | 178 | 1571 | |

Recent studies conducted with LP-211, have suggested that selective activation of 5 $HT_7$ receptors may represent a novel strategy in the therapy of Fragile-X syndrome; the most common form of inherited intellectual disability and autistic spectrum disorders.

Moreover, treatment of murine striatal and cortical neuronal cultures with LP-211 significantly enhances neurite outgrowth, suggesting the involvement of 5 $HT_7$ receptor in shaping central nervous system connectivity, which may be intimately linked to psychiatric and neurodevelopmental disorders.

**KEY WORDS:** Serotonin 5-$HT_7$ receptor, arylpiperazine, central nervous system.

## P3.9
## ROLE OF HETEROOLIGOMERIZATION BETWEEN SEROTONIN RECEPTORS 5-HT1A AND 5-HT7 IN REGULATION OF RECEPTOR FUNCTIONS

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Serotonin receptors 5-$HT_{1A}$ and 5-$HT_7$ are highly co-expressed in brain regions implicated in depression. However, their functional interaction has not been established. In the present study we show that 5-$HT_{1A}$ and 5-$HT_7$ receptors form heterodimers both in vitro and in vivo. Resonance energy transfer-based assays revealed that, in addition to heterodimers, homodimers composed either by 5-$HT_{1A}$ or 5-$HT_7$ receptors together with monomers co-exist in cells. The highest affinity to form the complex was obtained for the 5-$HT_7$-5-$HT_7$ homodimers, followed by the 5-$HT_7$-5-$HT_{1A}$ heterodimers and 5-$HT_{1A}$-5-$HT_{1A}$ homodimers. Functionally, heterodimerization decreases 5-$HT_{1A}$ receptor-mediated activation of Gi-protein without affecting 5-$HT_7$ receptor-mediated signalling. Moreover, heterodimerization markedly decreases the ability of the 5-$HT_{1A}$ receptor to activate G-protein gated inwardly rectifying potassium channels in a heterologous system. The inhibitory effect on such channels was also preserved in hippocampal neurons, demonstrating a physiological relevance of heteromerization in vivo. In addition, heterodimerization is critically involved in initiation of the serotonin-mediated 5-HT1A receptor internalization and also enhances the ability of the 5-HT1A receptor to activate the mitogen-activated protein kinases. Finally, we found that production of 5-$HT_7$ receptors in hippocampus continuously decreases during postnatal development, indicating that the relative concentration of 5-$HT_{1A}$-5-$HT_7$ heterodimers and, consequently, their functional importance undergoes pronounced developmental changes.

Generally, our data suggest that the regulated and balanced ratio of homo- and heterodimerization on pre- and postsynaptic neurons may be critically involved in both, the onset as well as response to treatment of psychiatric diseases such as depression and anxiety.

**KEY WORDS:** G-protein coupled receptors, serotonin, hetero-oligomerization.

## P3.10
## PHARMACOPHORE MODELING OF NOVEL NONIMIDAZOLE HISTAMINE H3 RECEPTOR LIGANDS WITH INHIBITORY HISTAMINE N-METHYLTRANSFERASE ACTIVITY

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Dual acting compounds able to enhance histaminergic neurotransmission in the central nervous system, are a novel class of nonimidazole histamine $H_3$ receptor ($H_3R$) antagonists, that simultaneously possess strong inhibiting potency on catabolic histamine N-methyltransferase (HMT). The set of thirty-five multipotent $H_3R$/HMT ligands containing a piperidinoalkyl group, are a key structural feature for human H3 receptor ($hH_3R$) antagonism; connected by different spacer lengths to an aminoquinoline moiety, have been studied as a pharmacophoric moiety for HMT inhibiting activity, by the use of 3D-QSAR (Quantitative Structure-Activity Relationship) and pharmacophore study.

In order to better understand the crucial chemical functionalities for combined hH3R/HMT activities, 3D-QSAR pharmacophore models for hH3R antagonistic and HMT inhibiting activities were developed using Pentacle 1.06 program. Created 3D-QSAR models ($hH_3R$: $R^2$ (0.98), $Q^2$ (0.94), RMSE (0.171); and HMT: $R^2$ (0.80), $Q^2$ (0.60), RMSE (0.159)) showed different important DRY, TIP and related variables as essential 3D-pharmacophoric feature for both activities. 3D-Pharmacophoric features for $hH_3R$ antagonistic activity mainly differs from the pharmacophore for HMT inhibiting activity in presence of specific lipophilic/steric components of the $hH_3R$ pharmacophore. The H-bond accepting components of the hH3R pharmacophore, H-bond donating components of the HMT pharmacophore, and a longer optimal distance between H-bond donor and steric

hot spots were observed in the hH3R pharmacophore than in the HMT pharmacophore. Formed 3D-QSAR models were applied for design of novel piperidino-aminoquinoline hybrids, as multitarget hH3R/HMT ligands with a potential therapeutic impact in sleep-wake disorders and cognitive impairment. Designed compounds with 3D-QSAR predicted pKi($hH_3R$)> 9.6 and (pKi($hH_3R$)+$pIC_{50}$(HMT))> 16.8 were selected for further study.

**KEY WORDS:** Histamine H3 receptor, histamine N-methyltransferase, pharmacophore, QSAR, drug design.

## P3.11 CYP-DEPENDENT METABOLISM AND VASCULAR EFFECTS OF ASS234, A NOVEL MULTITARGET DIRECTED LIGAND

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The determination of the metabolic profiles and the safety of a new drug provides information that might be used to guide further modifications of a chemical, in order to obtain favorable therapeutic properties. In this context, cytochrome P450 (CYP) plays a crucial role in metabolism and toxic action of a drug.
Several monoamine oxidase (MAO) inhibitors present a propargylamino moiety. This chemical group confers properties as irreversible inhibitors towards the MAO and could represent a potential molecular site in the formation of suicide substrates toward CYP, which could be the origin of drug-drug interactions. Furthermore, concerning the safety pharmacology, important aspects that have been highlighted are the interactions with the cardiovascular system. For these reasons the metabolic features of a new series of PF9601N derivatives, characterized by MAO and acetylcholine esterase (AChE) inhibiting properties were studied in human liver microsomes, and the vascular effects were studied in the rat aorta rings.
The compounds presented a concentration-dependent inhibition of CYP(s), however this effect resulted in a fully reversible and a competitive fashion. Furthermore the lead compound ASS234, showed an intrinsic clearance value of CLint= $1.7\mu l x min^{-1} \times mg^{-1}$ and CLint= $129.2\mu l x min^{-1} \times mg^{-1} \times mg^{-1}$ in human and rat respectively, indicating that ASS234 is a poor substrate for human CYPs.
In the vascular studies, ASS234 showed to relax phenylephrine-induced contraction at concentrations, either > 3 μM (endothelium denuded) or > 1 μM (endothelium intact rings). The vasodilating effects exhibited by ASS234, however, were at concentrations two orders of magnitude greater than those effective on AChE, and three orders greater than those effective on MAO. These preliminary *in vitro* results, suggest that ASS234 may have a safety vascular profile.

**KEY WORDS:** Multitarget compounds, metabolic stability, cytochrome P450, liver microsomes, aorta rings.

This work was realized in the framework of COST CMST Action CM1103 and working group D34/0003

## P3.12 RECENT PROGRESS IN UNDERSTANDING THE CATALYTIC ACTIVITY OF MONOAMINE OXIDASES

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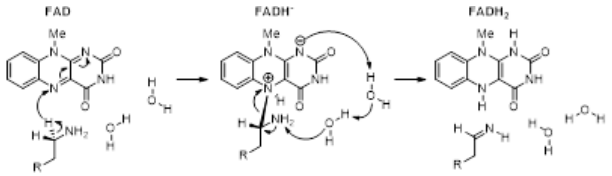
Monoamine oxidase (MAO) is a flavoenzyme responsible for regulating the level of biogenic amines in various parts of brain. Although MAO have been the central pharmacological targets in treating depression and Parkinson's disease for over 60 years, there has been no consensus in the literature about the precise molecular mechanism of its catalytic activity. On the basis of model quantum chemical calculations, we have proposed a new two-step hydride mechanism for the MAO-catalysed oxidative deamination of amines (Scheme 1). In the rate-limiting first step, the flavin N5 atom directly abstracts the hydride anion from the substrate α-carbon atom, and forms a strong covalent adduct intermediate with the thus created cationic substrate. This is subsequently followed by the deprotonation of the substrate amino group to the flavin N1 atom, facilitated with two active-site water molecules, which produces fully reduced flavin, $FADH_2$, and releases neutral imine.

This presentation discusses the significance of the mentioned flavin-substrate adduct formation, since its non-equal feasibility in both MAO isoforms has been suggested, and implied that this feature might play a crucial role in determining differences in catalytic mech-




Scheme 1. Complete two-step mechanism of MAO catalysed amine degradation.

anisms, and substrate selectivities between MAO-A and MAO-B enzymes. Also, we present some results of our preliminary all-atom QM/MM simulations within the Empirical Valence Bond theory that provide further support in favour of the hydride mechanism, particularly in the context of very recent papers by other researchers that do not necessary agree with all aspects of our mechanistic proposal.

**KEY WORDS:** Amine metabolism, computational chemistry, flavoenzymes, monoamine oxidases, neurodegenerative disorders.

## P3.13
## *In silico* DESIGN OF NOVEL AND SELECTIVE NEURONAL NITRIC OXIDE SYNTHASE (nNOS) INHIBTIORS

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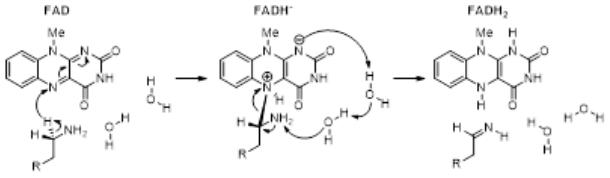
Nitric oxide gaseous free radical molecule (NO) acts as a messenger in various tissues and is responsible for different physiological functions and pathological symptoms. Nitric Oxide synthases (NOS) catalyse the oxidation of L-Arginine to a nitric oxide molecule (NO) and L-citrulline (Figure 1). Mammals contain three different NOS isozymes: Neuronal NOS (nNOS, in the brain), inducible NOS (iNOS, in macrophage cells), endothelial NOS (eNOS, the inner walls of blood vessels). Indeed, NO is a free radical gaseous molecule under normal conditions that is a highly toxic substance to our cells. In our body, it is produced locally at proper concentration and proper time. In endothelial cells, it relaxes smooth muscle causing a decreased blood pressure. Macrophage cells generate NO as an immune defence system to destroy microorganisms and pathogens.

In our brain, after a certain age and under certain pathological conditions, excessive NO is produced, causing tissue damage and oxidative stress. It also reacts with other free radicals to create specific molecular modifications. The overproduction of NO, especially by nNOS (in brain) is implicated in various diseases states such as neurodegeneration, stroke, migraine and chronic headache, Parkinson, Alzheimer, and Huntington diseases, tissue damage, hypotensive crises during septic shock, colitis, arthritis, and various kinds of inflammatory diseases. For this reason, it is important to inhibit nNOS selectively in the brain. Three isozymes show extraordinarily structural similarities hindering the selective inhibitor design. In previous literature there are many outstanding studies, however there has not yet been any drug developed which accomplishes the required affinity and selectivity. In this research, computer-modelling studies were used based on the known crystal structure of three NOS isozymes. The selected scaffolds were used hoping to increase both selectivity and potency toward the nNOS enzyme. Several hundred compounds were screened in *silico* for prioritization of lead candidates. *De novo* design method was used for the modifications of and additions to selected scaffolds within a target-binding site in order to enhance its binding affinity and selectivity to that isozyme. The best candidates showing high activity and selectivity against nNOS over eNOS and iNOS isoforms were determined.




**KEY WORDS:** Nitric oxide synthase, in silico design, selective nNOS inhibitors, de novo design.

## P3.14
## NOVEL TOOLS FOR DISEASE MODIFYING ANTI-ALZHEIMER'S DRUGS; hChEs AND b-AMYLOID INHIBITORS

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Conformational flexibility of AChE active site gorge has been a topic of intense research. We have proposed a thorough structural and bioinformatic analysis of the active site gorge of cholinesterases (ChEs), along with the identification of their fluctuations, which already drew the optimisation of our design strategy to discover extremely potent human Acetylcholinesterase and Butyrylcholinesterase (hAChE and hBuChE) bis-tacrine reversible inhibitors. Starting from these AChE and BuChE ligands, a set of potent multiple binding site homo- and hetero-bivalent inhibitors were designed, aiming to selectively interact with specific protein substructures on the surface of the enzymes

around the peripheral anionic site. Accordingly, functionalised linkers differentially spacing two tricyclic moieties were investigated as molecular yardsticks to probe the finest interactions with specific amino acid residues along ChEs gorge (hot spots). On these molecular supports, and aiming at identifying novel Alzheimer's modifying pharmacological tools, we have more recently developed bis-tacrines functionalized with a specific peptide moiety for interference with the hAChE surface sites which bind amyloid-beta (Aβ) and promote aggregation. These new high molecular weight compounds proved to be inhibitors of hAChE catalytic and non-catalytic functions (binding the catalytic and peripheral sites) can interfere with hAChE-induced Aβaggregation, Aβspontaneous aggregation, and with the Aβself-oligomerization process. Molecular modeling studies for these new ligands in complex with TcAChE confirmed the preliminary results obtained by X-ray and will be presented, highlighting how the bis-tacrine systems span the gorge, while the peptide moieties bulge outside the gorge in proximity of the peripheral site, thus explaining observed activity.

**KEY WORDS:** Cholinesterases, inhibitors, amyloid beta fibrils, amyloid beta oligomers, Alzheimer's disease, anti-Alzheimer's drugs.