Masaryk University

http://orgsyn.sci.muni.cz/

CZECH REPUBLIC

Chemistry site

ECBS2017
BUDAPEST

a nominated partner site of eu openscreen

Our organic chemistry laboratory focuses on the development of synthetic methods and strategies for preparation of structurally non-trivial small molecules for applications in biomedical research.

THE PEOPLE

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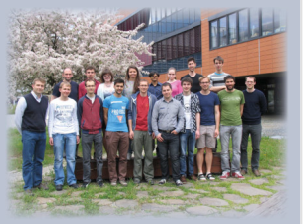
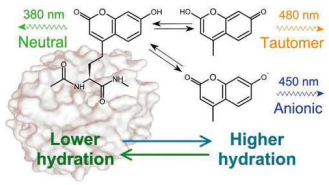
paruch@chemi.muni.cz



THE PROJECTS

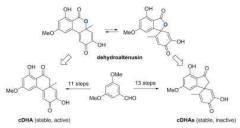
1. Synthesis of new fluorescent probes and unnatural aminoacid for in vivo labelling of proteins and monitoring of their hydration.



Hydration of proteins profoundly affects their functions. We describe a simple and general method for site-specific analysis of protein hydration based on the in vivo incorporation of fluorescent unnatural amino acids and their analysis by steady-state fluorescence spectroscopy. Using this method, we investigate the hydration of functionally important regions of dehalogenases. The experimental results are compared to findings from molecular dynamics simulations.

2. Synthesis of new inhibitors of mammalian DNA polymerase alpha.

Syntheses of carbocyclic analogs of dehydroaltenusin tautomers are reported. Both target compounds, cDHA (2,8-dihydroxy-6-methoxy-10a-methyl-10,10a-dihydrophenanthrene-3,9-dione) and cDHAs (4',5-dihydroxy-6'-methoxy-2-methylspiro[cyclohexa[2,5]diene-1,1'-indene]-3',4(2'H)-dione), were prepared from 3,5-dimethoxybenzaldehyde in 11 and 13 steps, respectively. Unlike dehydroaltenusin, both cDHA and cDHAs are stable and their structures were confirmed by X-ray crystallography. Compound cDHA was found to be active against calf DNA polymerase α but not related isozymes, while the spirocyclic analog cDHAs was inactive.



3. Preparation of potent and selective inhibitors of CHK1 kinase for synthetic lethal treatments in modern oncology.

Treatment options for TP53-mutated lymphoid tumors are very limited. In experimental models, TP53-mutated lymphomas were sensitive to direct inhibition of checkpoint kinase 1 (Chk1), a pivotal regulator of replication. We initially tested the potential of the highly specific Chk1 inhibitor SCH900776 to synergize with nucleoside analogs (NAs) fludarabine, cytarabine and gemcitabine in cell lines derived from B-cell malignancies. In p53-proficient NALM-6 cells, SCH900776 added to NAs enhanced signaling towards Chk1 (pSer317/pSer345), effectively blocked Chk1 activation (Ser296 autophosphorylation), increased replication stress (p53 and γ-H2AX accumulation) and temporarily potentiated apoptosis. In p53-defective MEC-1 cell line representing adverse chronic lymphocytic leukemia (CLL), Chk1 inhibition together with NAs led to enhanced and sustained replication stress and significantly potentiated apoptosis. Altogether, among 17 tested cell lines SCH900776 sensitized four of them to all three NAs. Focusing further on MEC-1 and co-treatment of SCH900776 with fludarabine, we disclosed chromosome pulverization in cells undergoing aberrant mitoses. SCH900776 also increased the effect of fludarabine in a proportion of primary CLL samples treated with pro-proliferative stimuli, including those with TP53 disruption. Finally, we observed a fludarabine potentiation by SCH900776 in a T-cell leukemia 1 (TCL1)-driven mouse model of CLL. Collectively, we have substantiated the significant potential of Chk1 inhibition in B-lymphoid cells.

THE EQUIPMENT

- *in-house 500 MHz NMR*
- *MS-TOF*
- *autopurification LC/MS*
- *semi-prep. chiral HPLC*
- *SciFinder and reaxys . . .*

THE MEDCHEM

- chemical synthesis (wet chemistry, work in inert atmosphere (glovebox))
- microwave chemistry
- high pressure chemistry (up to 200 bar)

associated core facility:
X-ray Diffraction Core Facility

THE OUTPUT

1. Hylsová, M. et al., 2016, Eur. J. Med. Chem, DOI: 10.1016/j.ejmech.2016.12.023
2. Amaro, M. et al., 2013, J. Phys. Chem. B, DOI: 10.1021/jp403708c
3. Amaro, M. et al., 2015, J. Am. Chem. Soc., DOI: 10.1021/jacs.5b01681
4. Kováčová, S. et al., 2015, Tetrahedron, DOI: 1016/j.tet.2015.08.005
5. Zemanová, J. et al., 2016, Oncotarget, DOI: 10.18632/oncotarget.11388

15 international (US) patents e.g. WO 2015/165428 A1, WO 2015/192817 A1.

Funding is provided through national and international resources: FP7, EU-Structural Funds, Grant Agency of the Czech Republic, Grant Agency for Health Research of the Czech Republic, Alfred Bader (private support) and Masaryk University.

THE FUTURE

capacity:
2 medium-size medicinal chemistry projects

Masaryk University - Department of Chemistry, CZ Openscreen,
Faculty of Science, Kamenice 5/A8, CZ - 625 00 Brno
International Center for Clinical Research, St. Anne's University Hospital Brno,
Pekařská 53, CZ - 656 91 Brno

MASARYK UNIVERSITY

Institute of Molecular Genetics

www.img.cas.cz

Czech Republic

high-capacity screening site

ECBS2017 BUDAPEST

a nominated partner site of eu:openscreen

THE PEOPLE

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Šárka Šímová, PhD., simova@img.cas.cz

THE PROJECTS

Small molecules as modulators of Wnt pathway

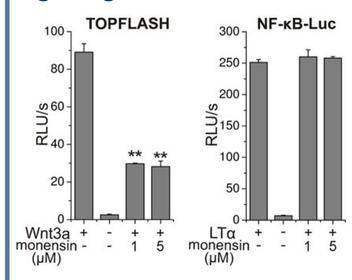
Monensin inhibits canonical Wnt signaling in human colorectal cancer cells

- luciferase reporter gene HTS assay was used to search for novel inhibitors of Wnt/β-catenin signalling

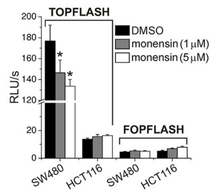
- the HTS included 15 000 compounds

- polyether antibiotic **monensin** identified as one of the inhibitors

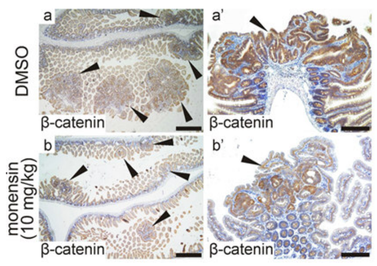
The inhibitory effect of monensin is specific for Wnt signaling

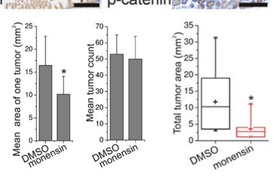


Differential sensitivity of SW480 and HCT116 colorectal carcinoma sells to monensin

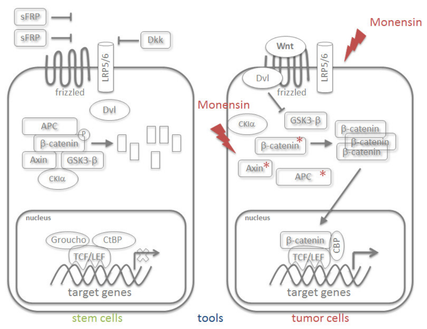


Monensin treatment dereases the size of adenomsa in APC+/Min mice

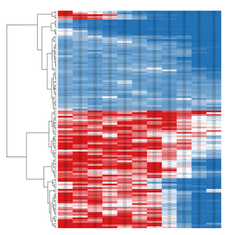




Monensin specifically antagonized the Wnt signaling pathway at the LRP and β-catenin levels



Monensin analogs could be developed as anticancer treatment in neoplasias with deregulated Wnt pathway



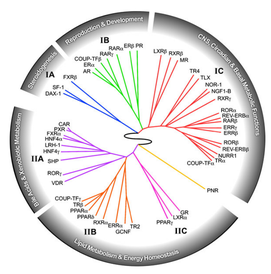
Collaboration with APIGENEX s r.o. on the development of monensim analogs

APIGENEX

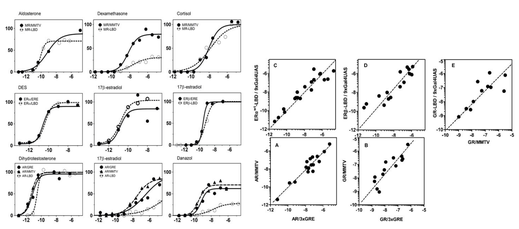
Novel steroid receptor modulators

ReceptorX - multi-level platform for profiling of nuclear receptors

Nuclear receptor family



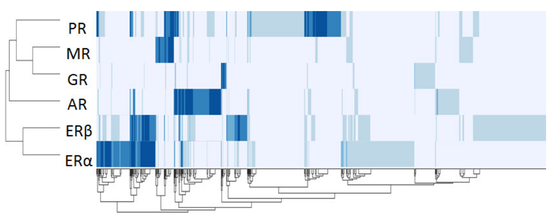
Collection of selective luciferase reporter U2OS - based cell lines

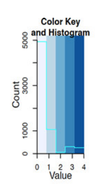


48 human nuclear receptors (Gal4-LBD) for HTS (clonal cell lines) and NR profiling

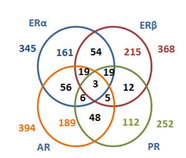
ERα	RARα	PPARα
ERβ	RARβ	PPARβ/δ
AR	RARγ	PPARγ
GR	RXRα	RORα
MR	RXRβ	RORβ
PR	RXRγ	RORγ
PXR	TRα	TR4
ERRα	TRβ	TLX
ERRβ	LXRα	NURR1
ERRγ	LXRβ	SF-1
	VDR	LRH-1

HTS of 3000+ steroid compound library using steroid hormone receptors (SR) reporter cell lines

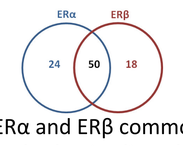




Hierarchical clustering of active compounds in the agonist mode using InCHlib

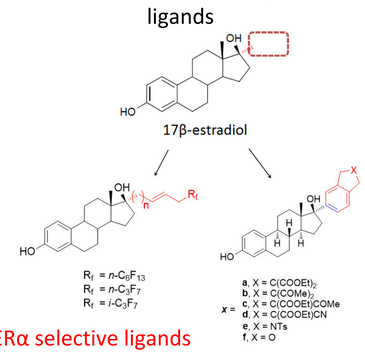


Ligands acitve in the sex steroid receptors ERα, ERβ, AR and PR



ERα and ERβ common and selective ligands

Novel classes of ERα and ERβ selective ligands



ERα selective ligands

ERβ selective ligands

THE HARDWARE

CZ-OPENSCREEN is equipped with state-of-the-art technologies for high-throughput screening (HTS) and compound storage and logistics:

- integrated robotic HTS station for screening chemical compounds (cell:explorer - PerkinElmer)
- robotic integrated system for automated microscopic analysis and label-free technology
- integrated system for compound storage and sample preparation including robotic liquid handling workstation used for dispensing, copying and reformating of microplates in 96/384/1536 formats (currently storing > 100 000 compounds)

THE SOFTWARE

Probes & Drugs portal (www.probes-drugs.com)

- in-house developed software tool
- public source of bioactive compound libraries, their annotatioins and comprehensive information about the probes

Laboratory Management System (LIMS)

- in-house developed system for compound management,
- for storage, analysis, data mining and reporting of HTS data

InCHlib

- open-source Javascript library that provides an easy way to display and analyze the hierarchically clustered data and heatmaps

THE OUTPUT

Publications

1. Skuta C, Popr M, Muller T, Jindrich J, Kahle M, Sedlak D, Svozil D, Bartunek P. (2017) Probes & Drugs Portal: an interactive, open data resource for chemical biology. Nat Methods 14,8 in press.

2. Kralova J, Kolar M, **Kahle M**, Truksa J, Lettlova S, Balusikova K, **Bartunek P**. (2017) Glycol porphyrin derivatives and temoporfin elicit resistance to photodynamic therapy by different mechanisms. Sci Rep. 2017 Mar 15;7:44497

3. Škuta C, Bartůněk P, Svozil D. (2014) InCHlib - interactive cluster heatmap for web applications. J Cheminform. 6:44

4. Tumova L, **Pombinho AR**, Vojtechova M, Stancikova J, Gradl D, Krausova M, Sloncova E, Horazna M, Kriz V, Machonova O, **Jindrich J**, Zdrahal Z, **Bartunek P**, Korinek V. (2014) Monensin inhibits canonical Wnt signaling in human colorectal cancer cells and suppresses tumor growth in multiple intestinal neoplasia mice. Mol Cancer Ther. 13:812-22.

5. Beranova L, **Pombinho AR**, Spegarova J, Koc M, Klanova M, Molinsky J, Klener P, **Bartunek P**, Andera L. (2013) The plant alkaloid and anti-leukemia drug homoharringtonine sensitizes resistant human colorectal carcinoma cells to TRAIL-induced apoptosis via multiple mechanisms. Apoptosis.

Patents

Patent: CZ 306011
Bartůněk P, Kořínek V, Pombinho A, Tůmová L. Monesin-containing pharmaceutical composition for treating diseases associated with deregulated Wnt pathway
Patent: EP3054941
Grekov I, Pombinho A, Šíma M, Kobets T, Bartůněk P, Lipoldová M. Pharmaceutical composition comprising diphenyleneiodonium for treating diseases caused by the parasites belonging to the family Trypanosomatidae
Patent: EP 2527351 B1
Rejman D, Pohl R, Bartůněk P, Pombinho AR, Krásný L, Látal T. Lipophosphonoxins, method of their preparation and use

We collaborate with:

- 9 institutes of the Czech Academy of Sciences
- 5 faculties of Charles University, Prague
- 2 faculties of Masaryk University, Brno
- 2 faculties of Palacký University, Olomouc
- a nubmer of SMEs

The Czech Academy of Sciences · Charles University · APIGENEX

CZ-OPENSCREEN partner sites:

- Department of Chemistry, Masaryk University
- Laboratory of Informatics and Chemistry, University of Chemistry and Technology
- Institute of Molecular and Translational Medicine, Palacky University

Institute of Molecular Genetics of the ASCR, v. v. i.
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CZ OPENSCREEN National Infrastructure for Chemical Biology · IMG Institute of Molecular Genetics of the ASCR, v. v. i.

www.openscreen.cz

SCREENING UNIT FMP

http://www.leibniz-fmp.de/core-facilities/screening-unit/

GERMANY

high-capacity screening site

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Jens von Kries


Carola Seyffarth


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Martin Neuenschwander


Romy Leu


Andreas Oder


Silke Radetzki

THE PEOPLE

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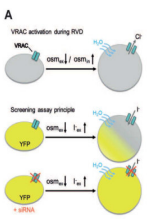
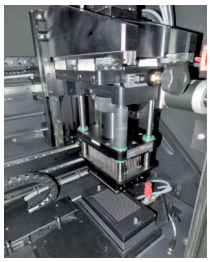
Dr. Katina Lazarow (Functional genomics)
Dr. Silke Radetzki (High-content screening)
Dr. Martin Neuenschwander (Data analysis, lab automation)
M. Sc. Marc Wippich (Image data analysis, IT)
Carola Seyffarth (Biochemical screening)
Sabrina Kleissle (Cell-based screening)
Romy Leu (Cell-based screening)
Andreas Oder (Biacore & biochemical screening)

THE PROJECTS

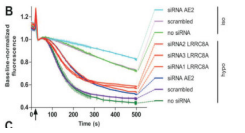
Reference Project 1

F. K. Voss et al., Identification of LRRC8 heteromers as an essential component of the volume-regulated anion channel VRAC. Science 344, 634-638 (2014)





to be able to track the fast fluorescence changes immediately after hypotonic buffer addition, a real time fluorescence imager equipped with a 384-channel pipetting head was used (FLIPR TETRA)

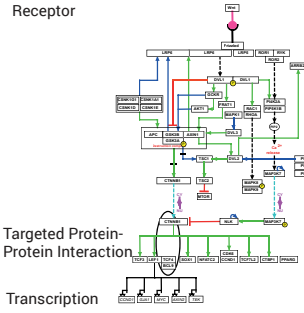


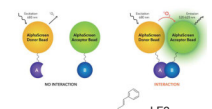
Suppression of LRRC8A expression led to lower activity of VRAC, visible as delayed fluorescence decrease compared to scrambled/no siRNA controls

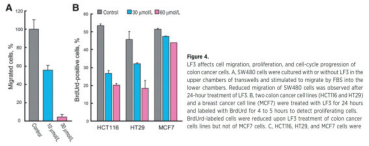
Regulation of cell volume is critical for many cellular functions, yet the molecular identity of the key player VRAC (volume-regulated anion channel) remained unknown despite decades of efforts. VRAC is nearly inactive under resting conditions, but opens upon hypotonic swelling. Activity was assayed by using HEK cells constitutively expressing YFP, upon hypotonic swelling induced by addition of a iodine-containing low salt buffer, iodine influx could be observed indirectly by YFP fluorescence quenching. By transfecting cells with a human RNAi library that uses three separate RNAi per gene, one RNAi probe succesfully identified the LRRC8A genes as being the responsible entity for VRAC activity.

Reference Project 2

L. Fang et al., A small-molecule antagonist of the beta-Catenin/TCF4 interaction blocks the self-renewal of cancer stem cells and suppresses tumorigenesis. Cancer Res 76, 891-901 (2016).







Wnt/beta-Catenin signalling contributes to high cell motility, high cell proliferation, and cell-cycle progression in colon cancer cells. Activating mutations of Wnt signalling are often found in downstream components of the pathway. Using AlphaScreen technology, a small molecule library (ChemBioNet collection with a diversity set of 16'671 compounds) was screened for molecules that disrupt the beta-Catenin/TCF4 protein interaction, leading to the successful identification of LF3. LF3 is able to reduce cell motility and proliferation specifically in colon cancer cell lines, and furthermore induces cell differentiation of stem cells, thereby sensitizing them for therapy.

THE HARDWARE

- 3 Tecan Freedom EVO and 1 Beckman FxP liquid handling workstation equipped with fixed tip and disposable tip 384-channel pipetting heads and integrated cell incubators
- Arrayscan high-content fluorescence microscopes
  EZReader II capillary electrophoresis system for kinases/phosphatases
  MACSQuant flow cytometer for epitopes and genetic reporters
  FLIPR TETRA for ion-channels
  Plate readers equipped for HTRF, luminescence, and fluorescence based readouts
- The small-molecule library consists of a diversity set (33'088), academic compounds collected from multiple groups across germany (6'424), and a reference set with FDA approved drugs, drug candidates and LOPAC (3'168), and is managed in a fully automated tube store at -20°C. The functional genomics libraries consist of a human and mouse RNAi library (with subsets available for kinases) and is currently expanded to CRISPR/CAS9 libraries.

THE SOFTWARE

- Konstanz Information Miner (KNIME) for data analysis and reporting
- R statistics framework for IC_{50} plot generation (using automated outlier detection after Motulsky et al.)
- MySQL for frequent hitter database
- DACS, database of accessible chemical substances, in-house software for compound search and library design (AG Kühne)

http://www.leibniz-fmp.de/de/ssfa0/database-dacs.html

THE OUTPUT

1. Du J, et al. (2017) Pharmacological restoration and therapeutic targeting of the B-cell phenotype in classical Hodgkin lymphoma. Blood 129, 71–81.
2. Wetzel C, et al. (2017) Small-molecule inhibition of STOML3 oligomerization reverses pathological mechanical hypersensitivity. Nat. Neurosci 20, 209-218.
3. Chenge JT, et al. (2016) Structural characterization and ligand/inhibitor identification provide functional insights into the Mycobacterium Tuberculosis Cytochrome P450 CYP126A1. J. Biol. Chem. 292, 1310-1329.
4. Khatri Y, Ret al. (2016) Substrate hunting for the myxobacterial CYP260A1 revealed new 1α-hydroxylated products from C-19 steroids. Chembiochem 17, 90–101.

Collaborations:
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Thomas Jentsch (FMP Berlin)
Claus Scheidereit (MDC Berlin)
Karoline Krause (Charité Berlin)
Gary Lewin (MDC Berlin)
Walter Birchmeier (MDC Berlin)
Clemens Schmitt (Charité Berlin)
Andrew Munro (Univ. of Manchester)

THE FUTURE

- Upgrade to acoustic dispensing technology
- Installation of BSL2 HTS lab

Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP), & Max-Delbrück-Centrum in der Helmholtz-Gemeinschaft (MDC) Robert-Rössle Str. 10, D - 13125 BERLIN

LEIBNIZ FORSCHUNGSINSTITUT FÜR MOLEKULARE PHARMAKOLOGIE

MDC MAX-DELBRÜCK-CENTRUM FÜR MOLEKULARE MEDIZIN IN DER HELMHOLTZ-GEMEINSCHAFT

Research Group Medicinal Chemistry

Leibniz-Forschungsinstitut für Molekulare Pharmakologie

www.fmp-berlin.de/nazare

GERMANY

Chemistry site

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The medicinal chemistry group optimizes small molecule hits emerging from screening or from rational drug design approaches by iterative chemical optimization cycles, consisting of molecular modeling, chemical synthesis and biological testing.

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THE PEOPLE

THE PROJECTS

Specifc inhibition of clathrin-mediated endocytosis...

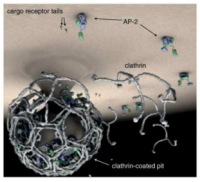
André Horatscheck, Haydar Bulut, Sandra Miksche, Mark J Robertson, Lisa von Kleist, Andreas Oder, Adam McCluskey, Jens Peter von Kries, Volker Haucke, Marc Nazaré

...by disruption of clathrin-endocytic protein interactions with Pitstop derivatives

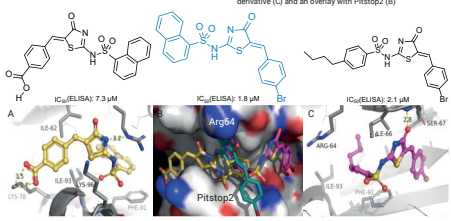
Background: Clathrin-mediated endocytosis (CME) regulates many key physiological processes for the internalization of growth factors and receptors, entry of pathogens (e. g. HIV-1), and synaptic transmission by formation of so-called 'clathrin-coated vesicles'.

Approach: An ELISA-based high-throughput screen (HTS) using the 17K ChemBioNet library resulted in the identification of Pitstop compounds that inhibit complex formation between the clathrin terminal domain (TD) and amphiphysin B. We synthesized focused libraries of around 150 compounds, providing a first SAR.

Results: X-ray co-crystallization gave insight into the key interactions between these Pitstop ligands and the clathrin TD. Surprisingly, six nearly equipotent inhibitors showed four different binding modes. These non-canonical binding modes of the novel Pitstop analogues revealed the structural basis for the disruption of clathrin TD endocytic protein interactions.



Upper row. Schematic representation of clathrin mediated endocytosis (CME). **Lower row.** X-ray co-crystal structures for Pitstop2 derivatives/clathrin illustrating the reversed, non-canonical binding modes within the clathrin box. New binding modes of 4-carboxy derivative (A) and 4-n-butyl derivative (C) and an overlay with Pitstop2 (B)



Cryo-electron microscopy image of a clathrin-coated vesicle

Schematic representation of a clathrin triskelion

Discovery and Optimization...

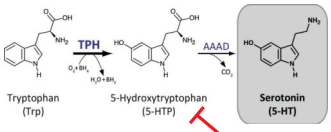
Edgar Specker, Susann Matthes, Anja Schütz, Martin Neuenschwander, Udo Heinemann, Jens von Kries, Michael Bader, Marc Nazaré

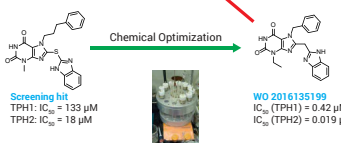
...of Tryptophan hydroxylase (TPH) inhibitors

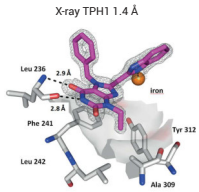
Background: Serotonin [5-hydroxytryptamine (5-HT)] is causally involved in multiple aspects of mood control in the central nervous system. In peripheral tissues, serotonin regulates vascular tone, gut motility, primary hemostasis, and cell-mediated immune responses, and is associated with diseases like irritable bowel syndrome and carcinoid syndrome.

Approach: The biosynthesis of serotonin is a highly regulated two-step process, starting with the essential amino acid L-tryptophan (Trp), while tryptophan hydroxylase (TPH) is the initial and rate-limiting enzyme in the biosynthesis of serotonin.

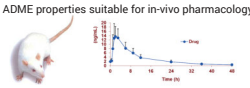
Results: We have identified and further developed highly active TPH inhibitors that are able to modulate physiological serotonin levels. The X-ray co-crystal structures obtained with our inhibitors allowed us to elucidate the binding mode and to reveal the structural determinants for for efficient protein-ligand interaction. Several inhibitors are currently undergoing in vivo efficacy studies in mice.







ADME properties suitable for in-vivo pharmacology



THE EQUIPMENT

- *Kiwi Store (LiCONiC AG) automated compound store and FreedomEvo Tecan, pipetting workstation*
- *LCMS- SQ and LCMS-TOF*
- *NMR Bruker AV300 and AV600*
- *Automated purification prep HPLC and*
- *Biotage systems*
- *Rayonet photoreactor*
- *Microwave Biotage Initiator*
- *Parallel synthesis equipment*
- *Mnova NMR software, Reaxys database, Chemaxxon, Pipeline-Pilot, MOE and PyMol software.*

THE MEDCHEM

The chemical tools of interest cover a broad range of applications from modulation of protein-ligand and protein-protein interactions, SAR studies, labeling studies for target deconvolution, and in-vivo proof of concept studies.

Each research project is guided by at least one of the following principles:

- New chemical structures of the small molecule modulator
- New unexplored mechanisms of action for a given biological protein target
- New unexplored biological targets or pharmacological applications/ therapeutic concept

- Hit optimization is enabled by state-of-the-art laboratory equipment for solution-phase chemistry, parallel synthesis and automated purification systems
- Rescaffolding, fragment-based approaches and structure-based design by computer modeling and X-ray crystallography
- Compound management of a 66.000 Drug-Like Small Molecule Library
- Co-localized support and collaboration at FMP. Screening Unit, FMP, Jens Peter von Kries and Drug Design group, FMP, Ronald Kühne.

Collaborations (academic/industrial):

international: Uwe Grether, F.Hoffmann-La Roche, Basel, Switzerland; Haiyu Hu, Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College; Stefan Krauss, University Hospital, Oslo, Norway;

national: Michael Bader, MDC, Berlin; Walter Birchmeier, MDC, Berlin; Udo Heinemann, MDC, Berlin; Hans-Jürgen Holdt, University of Potsdam, Potsdam; Christoph Rademacher, MPI, Potsdam; K. Lenhard Rudolph, FLI, Jena; Claus Scheidereit, MDC, Berlin; David W. Will, EMBL, Heidelberg

Networks:

Helmholtz Drug Research, ChemBioNet, Berlin-Institute of Health

THE FUTURE

Capacity:
2-3
EU-OPENSCREEN chemical tool optimization projects

THE OUTPUT

1. *Griger, J.; Schneider, R.; Lahmann, I.; Schowel, V.; Keller, C.; Spuler, S.; Nazare, M.; Birchmeier, C., Loss of Ptpn11 (Shp2) drives satellite cells into quiescence. Elife 2017, 6.*
2. *Sun, H.; Horatscheck, A.; Martos, V.; Bartetzko, M.; Uhrig, U.; Lentz, D.; Schmieder, P.; Nazaré, M.; Direct Experimental Evidence for Halogen-Aryl π Interactions in Solution from Molecular Torsion Balances, Angew. Chem., Int. Ed., 2017, 129, 6554-6558.*
3. *Bel Abed, H.; Schoene, J.; Christmann, M.; Nazare, M.; Organophosphorus-mediated N-N bond formation: facile access to 3-amino-2H-indazoles. Org. Biomol. Chem. 2016, 14, 8520-8528.*
4. *Kozian, D. H.; von Haeften, E.; Joho, S.; Czechtizky, W.; Anumala, U. R.; Roux, P.; Dudda, A.; Evers, A.; Nazare, M.; Modulation of Hexadecyl-LPA-Mediated Activation of Mast Cells and Microglia by a Chemical Probe for LPA5. ChemBioChem 2016, 17, 861-865.*
5. *Aretz, J.; Kondoh, Y.; Honda, K.; Anumala, U. R.; Nazaré, M.; Watanabe, N.; Osada, H.; Rademacher, C.; Chemical fragment arrays for rapid druggability assessment. Chem. Commun. 2016, 52, 9067-9070.*
6. *Hu, H.-Y.; Lim, N.-H.; Juretschke, H.-P.; Ding-Pfennigdorff, D.; Florian, P.; Kohlmann, M.; Kandira, A.; Peter von Kries, J.; Saas, J.; Rudolphi, K. A.; Wendt, K. U.; Nagase, H.; Plettenburg, O.; Nazare, M.; Schultz, C.; In vivo visualization of osteoarthritic hypertrophic lesions. Chem.Sci. 2015, 6, 6256-6261.*
7. *Halland, N.; Schmidt, F.; Weiss, T.; Saas, J.; Li, Z.; Czech, J.; Dreyer, M.; Hofmeister, A.; Mertsch, K.; Dietz, U.; Strübing, C.; Nazaré, M.; Discovery of N-[4-(1H-Pyrazolo[3,4-b]pyrazin-6-yl)-phenyl]-sulfonamides as Highly Active and Selective SGK1 Inhibitors. ACS Med. Chem. Lett., 2015, 6 (1), 73-78.*

Patents:

1- Bader, M.; Specker, E.; Matthes, S.; Schuetz, A.; Mallow, K.; Grohmann, M.; Nazaré, M. Xanthine derivatives, their use as a medicament, and pharmaceutical preparations comprising the same. WO 2016135199, 2- Hu, H.-Y; Nazaré, M.; Han Lim, N.; Ding-Pfennigdorff, D.; Plettenburg, O.; Ritzeler, O. Juretschke, H.-P.; Saas, J.; Bartnik, E.; Florian, P.; Wendt, U.; Schultz, C.; Nagase, H. DOTAM derivatives for therapeutic use. WO 2015075699, 3- Nazaré, M.; Halland, N.; Schmidt, F.; Kleemann, H.-W.; Weiss, T.; Saas, J.; Struebing, C.; N-(4-(Azaindazol-5-yl)-phenyl)-sulfonamides and their use as pharmaceuticals. WO 2014140055; 4- Petry, S.; Nazaré, M.; Schmidt, T.; Matter, H.; Fluorescence-labelled fatty acids binding to fatty acid-binding compd. to elicit FRET (Foerster resonance energy transfer) and uses thereof. WO 2014037394

Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP),
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Robert-Roessle Str. 10, D - 13125 Berlin, Germany

LEIBNIZ FORSCHUNGSINSTITUT FÜR MOLEKULARE PHARMAKOLOGIE

Leibniz-Gemeinschaft

Fraunhofer Institute for Molecular Biology & Applied Ecology – ScreeningPort

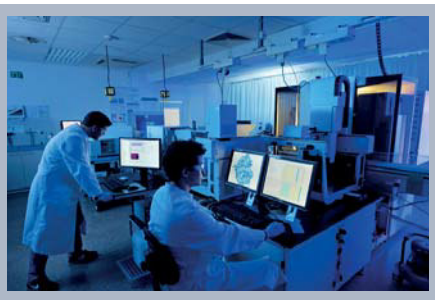
http://www.ime.fraunhofer.de/en/businessareasMB/screeningport.html

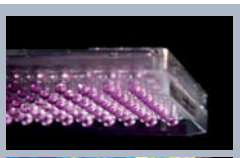
Germany

high-capacity screening site

ECBS2017 BUDAPEST

a nominated partner site of eu openscreen





THE PEOPLE

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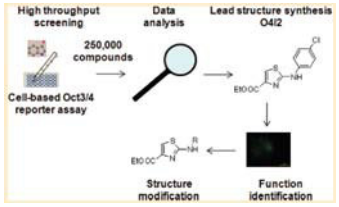
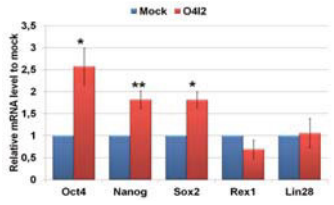
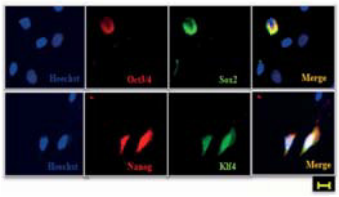
Dr. Björn Windshügel
(bjoern.windshuegel@ime.fraunhofer.de)

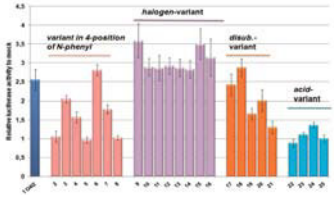
THE PROJECTS

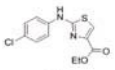
drug-iPS (BMBF Systems Biology, MedSys)



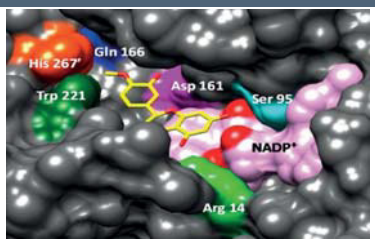


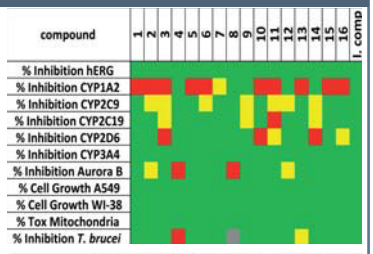


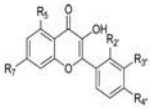




NMTrypI (EU FP7)







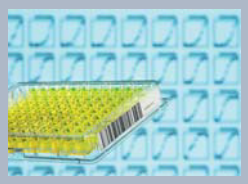
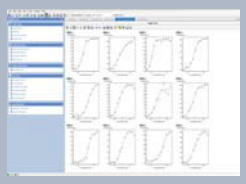
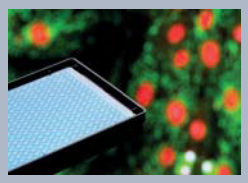
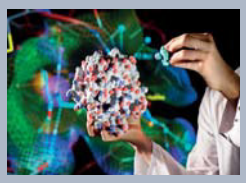
compd	R_5	R_7	R_2'	R_3'	R_4'	IC_{50} TbPTR1 (μM)	IC_{50} *T. brucei* (μM)
NP-27	H	H	H	H	H	12.8	9.1
NP-28	OH	OH	H	H	OH	28.0	9.0
NP-29	OH	OH	OH	H	H	76.9	27.0
NP-31	OH	OH	H	OCH_3	OH	13.9	11.2

compd	EC_{50} *T. brucei* (μM) ± SD	CC_{50} ± SD or NOAEL	selectivity index (CC_{50}/EC_{50})
1	5.18 ± 1.10	20	4
2	7.56 ± 0.51	53 ± 2	7
3	12.29 ± 2.82	80 ± 2	6
4	18.04 ± 0.50	100	5
5	2.32 ± 0.42	20	7
6	4.29 ± 0.71	20	4
7	1.36 ± 0.57	10	6
9	2.32 ± 1.01	10	4
10	1.43 ± 0.42	10	7
11	1.14 ± 0.24	10	8
12	1.17 ± 1.07	20	17
13	20.12 ± 2.27	20	1
14	1.10 ± 0.47	10	9
15	2.02 ± 0.51	10	5
16	2.99 ± 1.86	25	8
pentamidine	0.00155 ± 0.00024	10	6440

THE HARDWARE

1. Label free screening including multimode capacity (Enspire EPIC technology, PerkinElmer Inc), SPR screening (384 well, MASS-1, Sierra Sensors), live cell imaging (IncuCyte ZOOM, Essen Instruments), single molecule spectroscopy diffusion and fluorescence intensity analysis (Clarina, Evotec AG), ion channel and transporter protein screening in cell free formats (Iongate , Nanion), brightfield and fluorescence cell imaging in multimode format (EnSight, PerkinElmer), multimode capacity (EnVision, PerkinElmer Inc) and HCS (Opera, PerkinElmer Inc).
2. AlphaScreen, TR-FRET, fluorescence intensity, confocal high content imaging, absorbance, luminescence, reporter gene, cellular biosensors, caspase induction, cAMP, immunocytochemistry, cell migration, translocation, ion sensing (FluxOR, Fluo-4 and Fura-2), ELISA, Proximity Ligation Analysis, cell free electrophysiology and fluorescence polarisation.
3. Kinase, GPCR, ion channel, HDAC, PDE, deubiquitinylase, PPI, transporter, efflux pump, protease, nuclear receptor, DNA binding protein and toxicity (CYP450, gene toxicity, hERG, mitochondrial toxicity).
4. LOPAC, ENZO, Enamine, Evotec, MRCT, HYPHA Discovery, CHEMBRIDGE, Sanofi, MMV and Kailash.

THE SOFTWARE

ActivityBase XE , IDBS.
Spotfire, PerkinElmer.
Columbus, PerkinElmer.
GOLD, CCDC.
AutoDock, Scripps.
AutoDock Vina, Scripps.
KNIME, KNIME.
GROMACS, Groningen University.
MOE, CCG.
SMDDS, Schrödinger.

THE OUTPUT

1. ETHYL 2-((4-CHLOROPHENYL)AMINO)THIAZOLE-4-CARBOXYLATE (O4I2) AND DERIVATIVES ARE POTENT INDUCERS OF OCT3/4. Cheng, et al. Journal of Medicinal Chemistry, 2015, 58, 5742-5750.
2. IDENTIFICATION OF 2-[4-[(4-METHOXYPHENYL)METHOXY]-PHENYL]ACETONITRILE (O4I1) AND DERIVATIVES AS POTENT OCT3/4 INDUCERS. Cheng, et al, Journal of Medicinal Chemistry, 2015, 58, 4976-4983.
3. PROFILING OF FLAVONOL DERIVATIVES FOR THE DEVELOPMENT OF ANTI-TRYPANOSOMATIDIC DRUGS. Borsari, et al. Journal of Medicinal Chemistry, 2016, 59, 7598-7616.
4. ETHOXYLATED 2'-HYDROXYCHALCONES AS ANTIPARASITIC HIT COMPOUNDS. Borsari, et al. European Journal of Medicinal Chemistry, 2017, 126, 1129-1135.
5. CHROMAN-4-ONE DERIVATIVES TARGETING PTERIDINE REDUCTASE 1 AND SHOWING ANTI-PARASITIC ACTIVITY. Di Pisa, et al. Molecules, 2017, 22, E426.

EU FP7: MarineFungi, PDE4NPD, NMTRYPI, CVgenes@Target, EURhythDia and DropTech.
Innovative Medicines Initiative - K4DD, ND4BB-TRANSLOCATION and EBISC.
Marie Curie Skłodowska/COST Actions - TRANSLOCATION, INTEGRATE, CM1406, CM1307.
German National Programs: Neu2 (>10 Multiple Sclerosis drug discovery projects), DZHK, DrugiPS, MINAC, NanoCancer, Hepachip, Virtual Liver, NanoUmwelt, Synergime and HUMIT, Pluritest 2 (CIRM/Scripps), Risk-It, NEMBO
Industrial collaborations: Qiagen GmbH, Bayer Technologies, Evotec AG, Promega Inc., PerkinElmer Inc., Tecan AG, Eppendorf AG, SigmaAldrich Co. LLC

THE FUTURE

2017: 3 small, 2 medium and 2 large screens.

2022: 4-5 small, 4-5 medium and 3 large screens.

Invest in technologies and provide state-of-the-art solutions for:

1. iPS cell technologies for drug discovery, including access to patient derived cell lines for in-vitro disease models.
2. Structure based drug design & virtual screening.
3. Anti-bacterial drug discovery for gram negative pathogens.

Fraunhofer Institute for Molecular Biology & Applied Ecology – ScreeningPort, Schnackenburgallee 114, D-22525 Hamburg, Germany

Fraunhofer IME

Translational Medicinal and Biological Chemistry Laboratory (CIB-CSIC)

https://www.cib.csic.es/research/chemical-and-physical-biology/translational-medicinal-and-biological-chemistry

SPAIN

Chemistry site

ECBS2017 BUDAPEST

a nominated partner site of eu openscreen

THE PEOPLE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Dr. Carmen Gil (carmen.gil@csic.es)


Dr. Ruth Perez (ruth.perez@csic.es)

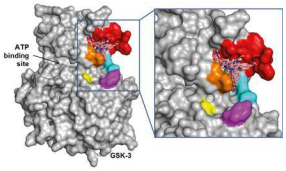
THE PROJECTS

VP-3.15, A neuroprotective, remyelinating agent...

...for multiple sclerosis

Project scope: Design allosteric modulators of GSK-3, synthesis, evaluation and development as neuroprotective and neurogenic drug candidates for multiple sclerosis.

Scientific features: Rational drug design based in target structure. Optimization of the synthetic pathway to a two steps procedure (to be able to scale up in further development). In vitro GSK-3 evaluation and kinetic studies. BBB penetration determination by PAMPA methodology. More than 70 compounds synthesized to optimize biological potency and ADME properties.



Key outcomes:

8 published scientific papers (JMC 2012, 55(4):1645-61; Eur J Pharm Sci. 2012, 5(5):677-84; ACS Chem Neurosci. 2012, 3(11):963-71; J Neurochem. 2012,122(6):1193-202; J Immunol. 2013,190(10):5000-11; J Chem Neuroanat. 2017, 80:27-36 and Sci Rep 2017, 7:43545)

1 granted patent in Europe (EP2484670 (B1), USA (US9604947 (B2) and Australia (AU2010302536 (B2) in explotation by ANKAR Pharma.

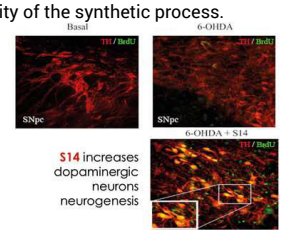
1 Spin-off founded: ANKAR PHARMA (www.ankarpharma.com): Today VP3.15 is AP-1, an oral drug candida-

S14, an innovative drug candidate...

...for Parkinson disease therapy

Project scope: Design, synthesis, evaluation and development of PDE7 inhibitors as innovative drugs for neurological disorders.

Scientific features: Discovery of quinazoline family of PDE7 inhibitors by virtual screening based in similarity index. Hit-to-lead expansion based in classical medchem approaches. Synthesis of more than 80 quinazolines. Selection of S14 based on biological profile, ADME properties and scalability of the synthetic process.



S14 increases dopaminergic neurons neurogenesis

Key outcomes:

7 Scientific papers (ChemMedChem. 2009, 4(5):866-76; PLoS One. 2011 Feb 24;6(2):e17240; Neurobiol Aging. 2013, 34(9):2133-45; Expert Opin Ther Pat. 2014, 24(12):1311-21; Neurobiol Aging. 2015, 36(2):1160-73; Stem Cells Transl Med. 2015, 4(6):564-75; Stem Cells. 2016. doi: 10.1002/stem.2480).

1 Granted patent in Europe (EP2433637 (B1), USA (US9192610 (B2), Japan (JP5563068 (B2)) in explotation by ARACLON biotech.
IND-filled. Presented in Spanish Medicines Agency. Approval for starting phase I pending

THE EQUIPMENT

- Synthetic and Analytical Capacity: Hoods, Lab Space, Microwave, NMR, LC-MS, Prep. HPLC, Chiral HPLC, Elemental analysis
- Automated column chromatography, circular centrifuge chromatography.
- ADME profile portfolio: Solubility, PAMPA (BBB and oral absorption), Microsomal stability, plasma protein binding.
- Binding Kinetics: Standard Assays for protein kinases, X-Ray, SAR by NMR
- Chemoinformatics: Work stations (Silicon Graphics, Linux and GPU GTX 980Ti), access to CSIC supercomputer TRUENO (230 nodes, 1900 cores, over 20Tflops os processing power), Scientific software (Sybyl, Schrodinger, Discovery Studio, Bioeclipse, etc.)

THE MEDCHEM

- Therapeutic Area, Target classes
  CNS diseases (mainly neurodegenerative diseases: AD, PD, ALS, MS) and neglected diseases (leishmaniasis, schistosomiasis)
  Our targets: Protein kinases, cAMP Phosphodiesterases, Cholinesterases, Proteases
- Structured based drug design (SBDD) is used frequently in the lab. We used either the receptor or substrate structure for our drug design programs, Some examples are NP-61, a dual binding site AChE inhibitor able to modulate the beta-amyloid pathology (JMC 2005, 48:7223) or our PDE7 inhibitors discovered using our pharmacophore model for this target (JMC 2012, 55:3274)
- Forward and reverse chemical genetics approaches for drug discovery
- Use of CODES programs to codify molecules in mathematical models
- A unique in-house chemical library of diverse small molecules with drug like properties. Compounds are storage in powder. HPLC control quality periodically
- Virtual screening, computational chemistry and QSAR analysis are ever present in our programs. Some examples include VP0.7, as an allosteric GSK-3 modulator (JMC 2011, 54:8461), development of neural networks for discovery new PDE7 inhibitors (ACS Chem Neurosci 2012, 3:793), ITDZs as substrate competitive GSK-3 inhibitors (JMC 2012, 55:1645)

THE OUTPUT

1. Palomo V et al. Subtly Modulating Glycogen Synthase Kinase 3 β: Allosteric Inhibitor Development and Their Potential for the Treatment of Chronic Diseases. JMC 2017 doi: 10.1021/acs.jmedchem.7b00395.
2. Mansilla A.et al. Interference of the complex between NCS-1 and Ric8a with phenothiazines regulates synaptic function and is an approach for fragile X syndrome. Proc Natl Acad Sci U S A. 2017, 114(6):E999-E1008.
3. Alquezar C et al. Targeting TDP-43 phosphorylation by Casein Kinase-1δ inhibitors: a novel strategy for the treatment of frontotemporal dementia.Mol Neurodegener. 2016, 11(1):36
4. García AM et al Modulation of cAMP-specific PDE without emetogenic activity: new sulfide-like PDE7 inhibitors. J Med Chem. 2014, 57(20):8590-607
5. Salado IG et al. Protein kinase CK-1 inhibitors as new potential drugs for amyotrophic lateral sclerosis. J Med Chem. 2014, 57(6):2755-72.

Collaborations (academic/industrial): AMO Pharma, ARACLON Biotech

Networks: PDE4NPD (FP7 project), DRIVE (ITN), MuTaLig (COST action)

Training capacities: Graduate, master and PhD students together with Post doc researchers have a place in our laboratories. Doctorate programs in collaboration with different Universities. Every year we receive several ERASMUS students and visitors from countries all over the world.

Patents: P201730399 (22/03/17) Purine derivatives for the treatment of neurodegenerative diseases. OPEN to Licence, WO2017051046 (23/09/15) Aminophenothiazines for modulating the number of synapsis. OPEN to Licence, US9592224 (B2) (22/01/13) Substituted benzothiazoles and therapeutic uses thereof for the treatment of human diseases . OPEN to Licence, ES2544519 (B1) (22/05/15) S-substituted quinazolines and their therapeutic applications for the treatment of diseases mediated by PDE7. Worldwide licencesed to ARACLON Biotech, WO2011039403 (02/09/09) Substituted 5-imino-1,2,4-thiadiazoles that can be used to treat neurodegenerative diseases. Worldwide licencesed to ANKAR PHARMA, EP2769720 (B1) (30/09/11) Heterocyclic GSK-3 allosteric modullators. Worldwide licenced to AMO PHARMA.

Spin-outs: ANKAR PHARMA

THE FUTURE

Plans:

- Licence our patents and be involved in the development to clinical trials
- Discover new innovative drugs for CNS and neglected diseases

Added value:

- Technology transfer know-how
- Experience in pharmaceutical management
- Our spin-off is focused in cover the gap from the lab to the clinical setting

Centro de Investigaciones Biologicas-CSIC
Ramiro de Maeztu 9
ES - 28040 Madrid (Spain)

University of Santiago de compostela
Innopharma

innopharmaplatform.com/

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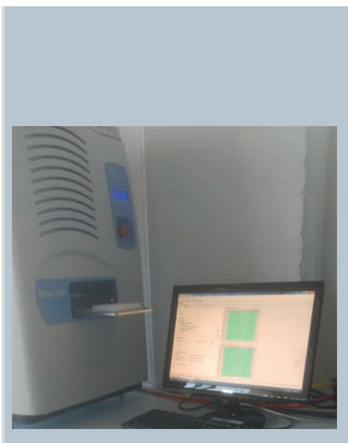
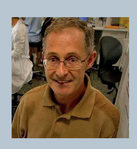


THE PEOPLE

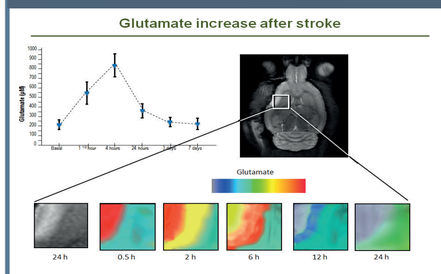
María Isabel Loza García

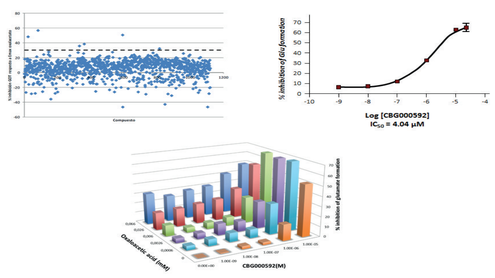
Ángel Carracedo Álvarez

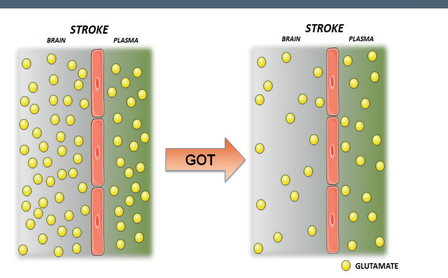


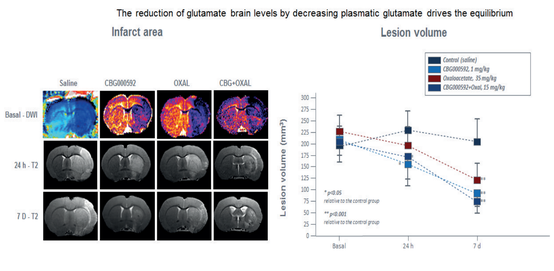
THE PROJECTS

Blood glutamate grabbing in stroke: A proof of concept

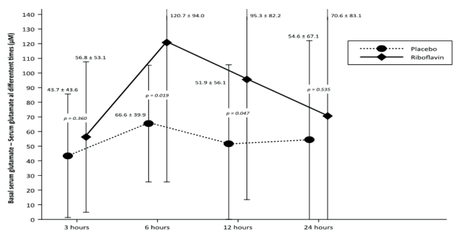






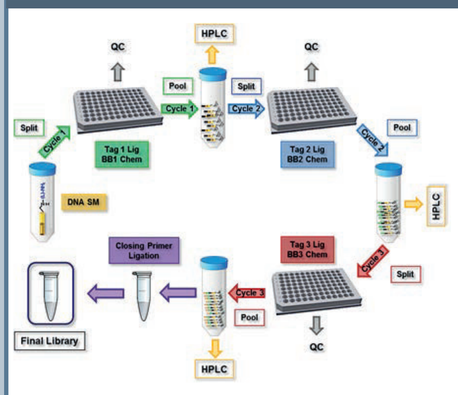


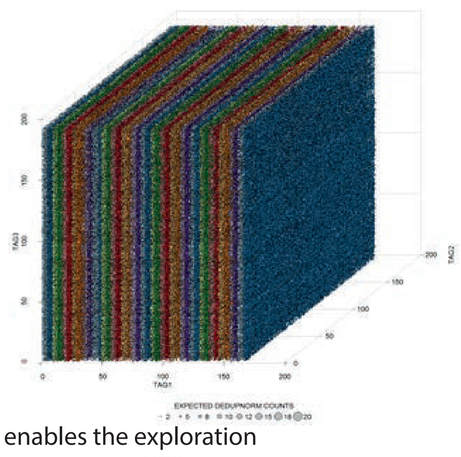
CBG000592 was identified from HTS. It synergistically enhanced the oxaloacetate activity at non-toxic doses of both compounds in a synergistic way



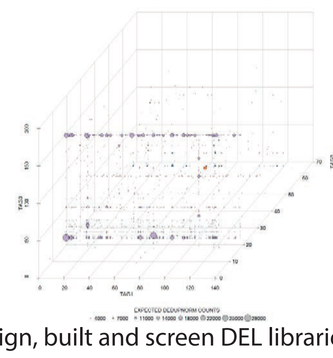
Clinical proof of concept showed both a significant reduction of glutamate plasmatic levels after 6 hours of drug administration and a significant reduction of average hospital stay

DNA encoded libraries for undruggable targets





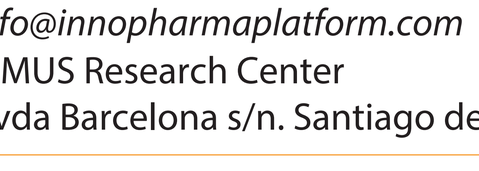
DNA-encoded chemical libraries (DEL) technology enables the exploration of chemical spaces up to five orders of magnitude higher than by HTS methods



We have developed novel tools to design, built and screen DEL libraries to efficiently identify active biomolecules

THE HARDWARE

- Liquid handling: acoustic liquid handling (Echo) and tip-based liquid handling (Freedom Evo, Janus)
- Automated HTS: Thermo Orbitor integrating Cytomats, Multidrops, plate washers , fluorescence imaging plate reader (Hamamatsu FDSS 7000) and multilabel plate readers (Tecan M1000 Pro, Perkin Elmer Enspire)
- Microfluidics mobility shift assays: Labchip EZ Reader II (Caliper)
- Fluorescence imaging plate reader: Hamamatsu FDSS 7000
- High Content Screening: Operetta automated microscope
- Automated Patch-clamp: Ionflux HT (Fluxion)
- Chemical analysis and separation unit: UPLC MS/MS (Waters Xevo TQD)
- Chemical library of 60000 compounds including repurposing libraries (Prestwick and Microsource) completed by *in silico* screening in order to cover the higher chemical and biological diversity.



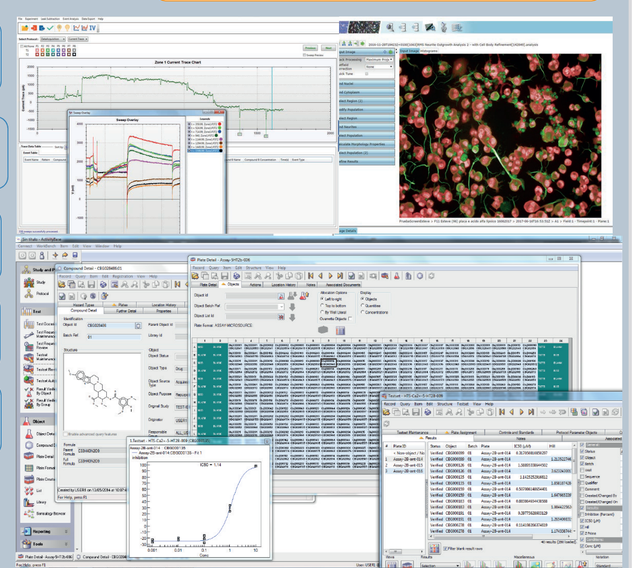
Chemical space covered by Innopharma chemical library

Chemical space covered by the representative subset

In silico predictions of diversity subset

THE SOFTWARE

GPCRs	Radioligand binding, second messenger assays, beta-arrestin translocation, DMR label-free assays
Nuclear receptors	AR, ER, PPAR and prostanoid receptors
Enzymes	Kynases (Automated microfluidic mobility shift assays), phosphodiesterases, beta-secretase, acetylcholinesterase, epigenetic enzymes
Ion channels	Automated patch clamp for both voltage and ligand-gated ion channels. Fluorescence assays
Transporters	DAT, NET, SERT, Pgp ...

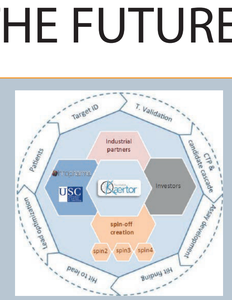


THE OUTPUT

1. PLoS Pathog. 2016;12(7):e1005763.
2. J Biomol Screen. 2016;21(8):816-23
3. Nature. 2016;535(7613):561-5
4. Science. 2013;340(6140):1567-70
5. Future Med Chem. 2017;9(8):731-748

- Involved in 10 NCE that entered into clinical trials
- 3 companies created (Pharmatools, Allelyus, Oncostellae)
- Authorship in 14 patents
- More than 80 pHD Theses supervised
- Members of the Coordination Committee for Education and Training topics of the Spanish Technological Platform for Innovative Medicines
- Coordinator of the Drug Discovery Galician Network (2007-onwards) and the Drug Discovery Spanish Network of Excellence (REDEFAR) and contributors to many national and international networks
- 25 collaboration agreements with Academic institutions for technology transference.
- 5 master agreements with pharmaceutical companies
- A Joint Research Unit with Laboratorios Esteve

THE FUTURE



Kaertor: a drug discovery hub to discover better drugs by generating preclinical and clinical Proof-of-Concept data. Incubation, strategic partnerships, co-development and spin-out strategies constitute the core of our open innovation ecosystem

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CIMUS Research Center
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USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

Innopharma

Screening Platform CIPF

www.cipf.es

SPAIN

specialist

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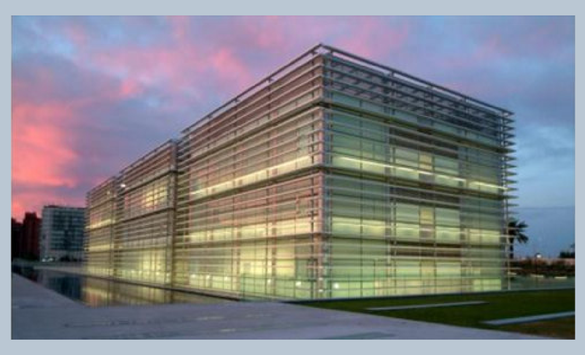
THE PEOPLE



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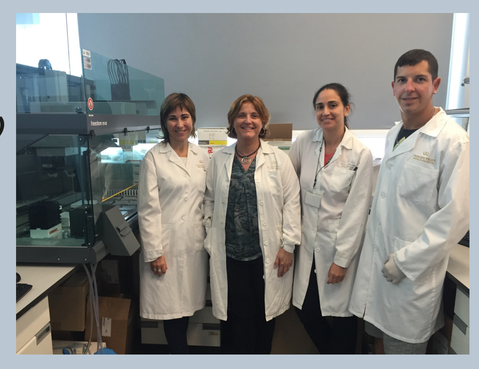
Esther Masia (emasia@cipf.es)

David Charbonnier (dcharbonnier@cipf.es)



Dr. Antonio Pineda (apinedalucena@gmail.com)

Dr. María Jesús Sanz (Maria.J.Sanz@uv.es)

THE PROJECTS

Towards the Design of Personalised Polymer-based Combination Nanomedicines for Advanced Stage Breast Cancer Parients. MyNano

The main objective of this project is to develop a nanomedicine-based combination therapy in a personalized manner, not only to treat the primary tumor, but also to prevent metastasis (by inhibiting tumor-associated exosome release). This study will be carried out in clinically-relevant breast cancer patient-derived models.

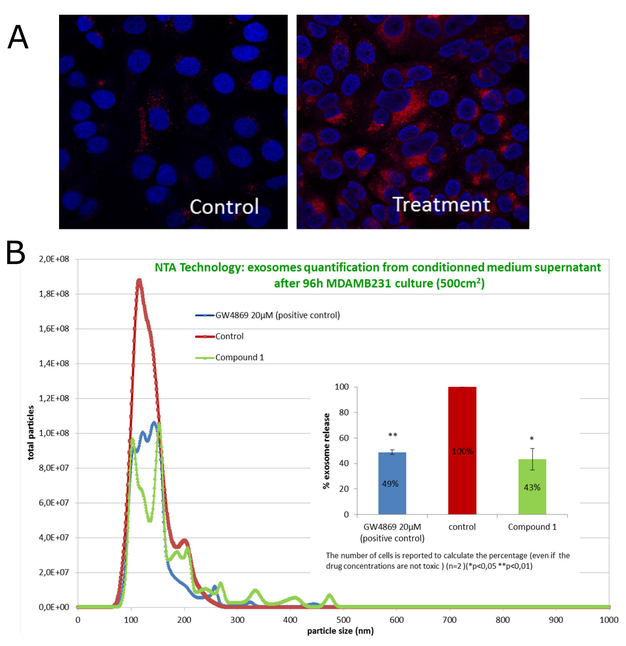


Fig 1. A. Confocal Images from BC cells treated with an exosome inhibitor, compound 1 (right), exosome are trapped inside cells (red marker). B. Exosome quantification by NTA technology.

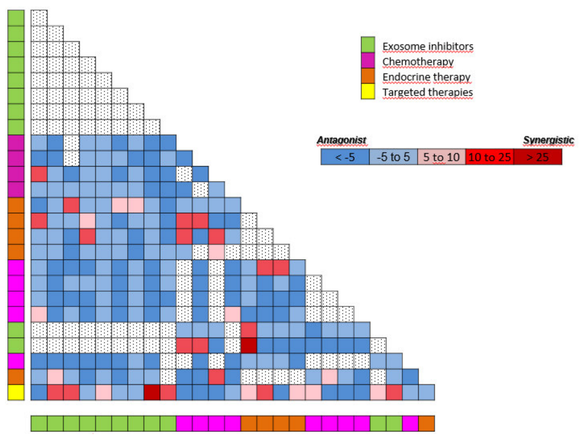


Fig 2. Cell viability diagram showing the screening of Drug combination in triple negative BC cells, MTS assay.

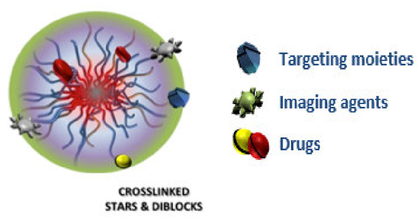
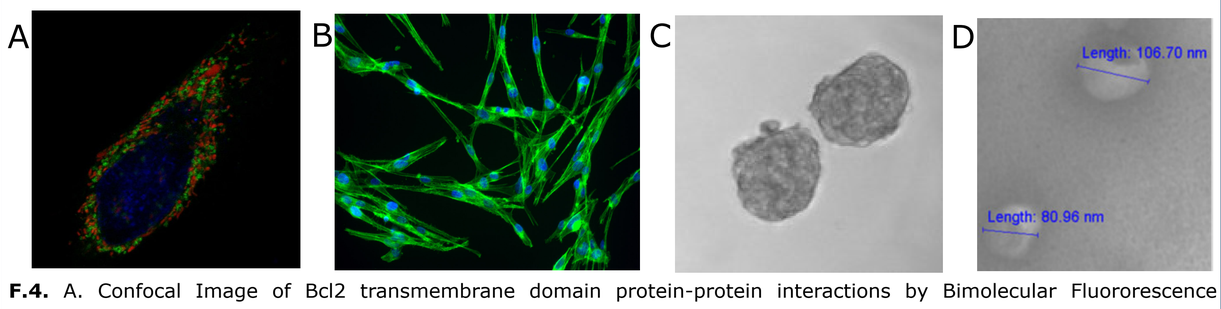


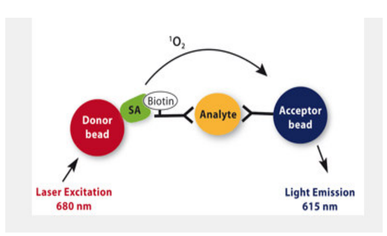
Fig 3. Schematic representation of a design polymer-based combination nanoconjugate.

Others

In many neurodegenerative disorders or aggressive pathological processes such as cancer, pharmacological high throughput screening (HTS) approaches are required to identify novel effective and safe therapeutics. Our screening site, using complementary advanced technologies including label-free, alpha-screen, and imaging tools among others, can rapidly and reliably identify novel hits. The CIPF is a specialized site offering a diversity of cell-based assays, from commercially available cell lines to patient derived 3D models. Assays on organotypic cultures are also available for screens.



F.4. A. Confocal Image of Bcl2 transmembrane domain protein-protein interactions by Bimolecular Fluororescence complementation (BiFC). B. InCell Image of Hunan melanoma cell line. Nuclear staining with DAPI and an antibody against actin filaments (green marker). C. Brightfield of Confocal Image of lung tumorspheres. D. BC cell exosomes, TEM Image.



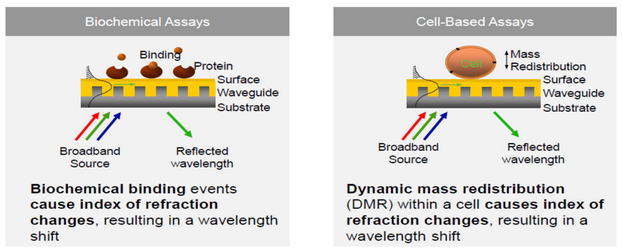


Fig 5. Some examples of the advanced technologies available on our screening platform. A. AlphaScreen/AlphaLisa technology (Drawing courtesy of BMG LABTECH). B. Label-free technology (Drawing courtesy of Perkin Elmer)

THE HARDWARE

ClarioStar BMG LABTECH
Ensight Label-free PerkinElmer
Leica SP8 HyVolution2
TECAN Li-Ha MCA96
Incell 1000/ Incell 6000
Luminex 200
NanoSight-NTA NS300
Myriad and Prestwick Libraries

THE SOFTWARE

Mars / Spotfire
Workout Plus MMD / Kaleido
Image J / LAS X / Metamorph
EVOware Standard
Incell Image Analysis Software

THE OUTPUT

1. *Duro-Castano A. 2017. Adv materials; 2015 Mol Pharm.*
2. *Azarin S.M. 2015 Nature comunications.*
3. *Kowal J. 2016. PNAS.*
4. *Lehar J. 2009. Nature biotechnology.*
5. *M.J. Vicent, A. Duro-Castaño, V.J. Nebot, WO2017025298A1.*

Collaborations

Academic

Industrial

Networks

THE FUTURE

To reinforce our capacity towards analysis in 3D patient-derived cell models and in vivo models.

Personalised medicine to assist clinical trials.

Centro de Investigación Príncipe Felipe
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PRINCIPE FELIPE
CENTRO DE INVESTIGACION

FUNDACIÓN MEDINA

www.medinadiscovery.com

SPAIN

high-capacity screening site

ECBS2017 BUDAPEST

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THE PEOPLE

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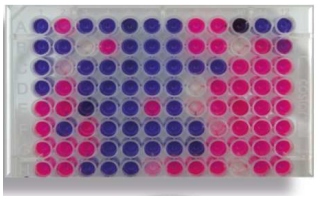
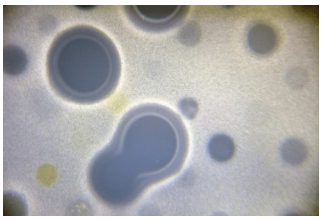
Francisca Vicente (francisca.vicente@medinaandalucia.es)

THE PROJECTS

Novel Anti-infectives

Antibacterial and antifungals discovery programs are focused on the phenotypic screening of our Natural Products libraries against panels of Gram positive and Gram negative bacteria and fungal human pathogens. Validation of hit compounds on a panel of clinical MDR strains, preliminar MOA studies and preclinical safety and ADME evaluation has allowed to identify 16 novel families of molecules, including a new chemical class of broad spectrum antibiotics against Gram negative pathogens (MDN-0057/-0060), selected for preclinical development in the IMI-ND4BB ENABLE program.

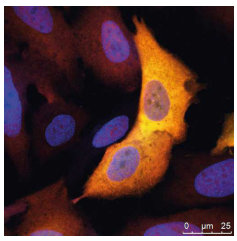


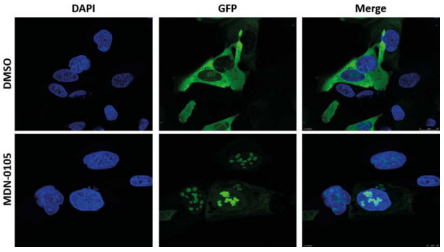


Discovery of novel anticancer compounds

Natural products research in cancer involves: 1) a combination of phenotypic assays targeting tumor specific compounds on a panel of human solid tumor cancer cell lines (liver, breast, pancreas, colon, and melanoma cancer), and 2) whole-cell target-based HTS assays looking for novel inhibitors with specific mode of action in the VHL/HIF pathway and the FOXO nuclear-cytoplasmic translocation.

Two families of compounds including the novel chemical class of PDK1 inhibitors MDN-0088 for cancers overexpressing PI3K (breast, colon, etc..) and the pancreatic cancer specific compound MDN-0090 targeting the MAPK pathway and with efficacy in in vivo animal models (including pancreas cancer stem cells) are in preclinical development.





THE HARDWARE

HTS screening systems

HTS Liquid Handlers and workstations (96/384/1536 wells): **Acoustic nanodispensing Echo 550** Labcyte; Tecan **Aquarius**; **Thermo Multidrop**; FlexDrop; **Beckman Biomek FX** (Beckman; microdispensing; 96/384 wells; Stackers); **Packard EP3**.

Compound libraries

- MEDINA Natural Products libraries: 135.000 extracts and fractions derived from microbial sources
- Future allocation of one copy of EU-OPENSCREEN compound library

Readouts/ Screening technologies

Readers: **High Content BD Pathway**; Perkin Elmer **EnVision; FLIPR**; **VIPR-II**; Perkin Elmer **Victor II**; Tecan **Ultra**; Tecan **Spectrafluor; Typhoon; Image analyzers; Odyssey**

Screening technologies: fluorescence, colorimetry, luminescence, polarisation, HTRF, FRET, FLIPR, ELISA, AlphaLISA, Alphascreen, Thermofluor, High Content Screening, cellular reporter assays, cytometry, WB, PCR, agar-diffusion, LC-HRMS, LC-MS/MS

Phenotypic and target-based screens:

- **Human targets:** cell viability, cell cycle, NO levels, ATP levels, nuclear translocation, PPi, immunomodulation, ion channels, mitochondrial membrane depolarization, kinases, caspases, CYP450 inhibition and induction
- **Antimicrobial targets:** bacterial and fungal pathogen viability, synergism, biofilm inhibition, essential viability genes; tropical parasites (kinetoplastids and P. falciparum)

THE SOFTWARE

Software tools:

Commercial Software: Genedata Screener 14.0.0, Dotmatics 5.0 Studies/Browser, IDBS XLfit; EnVisison Tools 1.13; Odyssey 3.0

In-house developments: Image Analyser, Data Browser 1.0; Activity Browser 1.0, Patterns-Matching 1.2; Regression v1.0; WIFF Converter

Compound logistics software: Nautilus LIMS 9.0 (Thermo); Inventory (Dotmatics)

Bioinformatics software tools: Bionumerics 6.6 (Applied Maths); FermInfo 1.0 (Internal)

In silico screening and rational drug discovery tools: Open Babel, Vina & Tools (AutoDock), Pymol v0.99, HyperChem 8.0

Chemoinformatics & metabolomics:

Commercial Software: ACD Labs ChemSketch and Suite; DNP (Dictionary of Natural Products); Chem Office Ultra 12.0 Suite; NIST 14; Metabolite Pilot 2.0; Alpha Markerview 1.2.1.1; Peak view 1.2.0.3

In House developments: HPLC Studio 2.0; MASS Studio 1.0

Screening informatics & data analysis:

- **Input and processing tools:** a) QC statistical parameters (signal to noise ratio, Z Factor); b) noise reduction procedures; c) outlier automated detection; d) systematic non-random patterns automated detection (side-edge effects and gradients).
- **Data Management tools** for data integrity and consistency, traceability procedures, and data redundancy and archiving.
- **Oracle Data Base tools**. Data referential integrity and consistency; data archiving and backup procedures.
- **Hardware tools**. Redundant units (RAID). Backup and archiving procedures. Virtualization of servers and equipment computers; centralized maintenance.

THE OUTPUT

1. *Pérez-del Palacio et al. (2017). Exploring the Role of CYP3A4 Mediated Drug Metabolism in the Pharmacological Modulation of Nitric Oxide Production. Front. Pharmacol. 8: 1-14.*
2. *Cautain et al. (2016). Discovery of a Novel Isothiazolonaphthoquinone-Based Small Molecule Activator of FOXO Nuclear-Cytoplasmic Shuttling. PLoS ONE 11: e0167491.*
3. *de Pedro et al. (2016) Protective Effects of Isolecanoric Acid on Neurodegenerative Diseases. Neuropharmacology, 101, 538-548.*
4. *Cautain et al., (2013). HCS Strategy Targeting Dysregulation Of The VHL/HIF Pathway For Drug Discovery. Adv Biosci and Biotechnol, 4: 398-405*
5. *Genilloud and Vicente (2012) Novel approaches to exploit natural products from microbial resources. In Drug Discovery from Natural Products, Eds. O. Genilloud and F. Vicente, RSC series. Ch. 11, pp. 221-248*

- Key Collaborations: CMerck Sharp & Dohme • CUBIST Pharmaceuticals • MICROBIOPHARM • WarpDrive Bio • Cyclenium Pharma • CrossBeta • DTU Biosustain • SYNBIOCHEM • Natural Products Discovery Institute • CSIC • CNM – Institute of Health Carlos III • Vall d'Hebron Institute of Oncology • CNIO • CNB • University of Granada • Complutense University • IBIS •
- Patents: 5 (ES2433142 A1, 2012; WPO2014/170295 A1., 2013; WO 2015/000825 A1., 2013; WO 2016/128401 A1., 2015; EP11559EP00, 2015)
- Training capacities: EU Innovative Training Networks (ITN) Marie Curie actions; University Granada Master Programs: Regenerative Medicine, Molecular Biology Applied to Biotechnological Bioenterprises, Biotechnology; Mobility European Projects: Leonardo Da Vinci Projects
- Networks: EU-OPENSCREEN; ORPHANET; BEAM ALLIANCE; EUROPEAN BIOTECHNOLOGY NETWORK; SPANISH DRUG DISCOVERY NETWORK; REDEFAR (RED DESCUBRIMIENTO TEMPRANO DE FARMACOS); RICET (RED DE INVESTIGACION COLABORATIVA EN ENFERMEDADES TROPICALES)

THE FUTURE

Future plans: expand core capacities plans to include 1) 3D cell based assays; 2) medicinal chemistry and chemical synthesis capacities.

Added value: Access to a world reference multidisciplinary HTS platform with core expertise in natural products drug discovery , enabling the full integration of discovery programs from the target validation to the preclinical evaluation of early drug candidates.

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ChemBio Lab (University of Barcelona)

http://www.ub.edu/chembiolab/htm/home.htm

SPAIN

Chemistry site

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BUDAPEST

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THE PEOPLE

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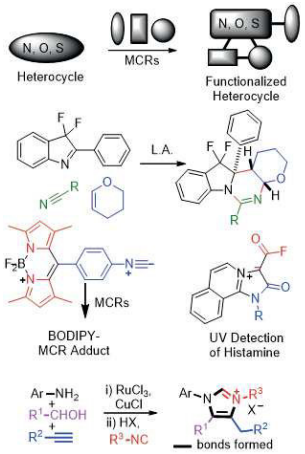
E-mail: rlavilla@ub.edu

THE PROJECTS

Development of New Multicomponent Reactions...

...with Heterocycles

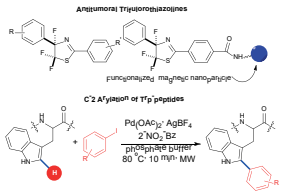
We have introduced the concept of heterocyclic MCRs as a new working area. We have started to systematically explore heterocyclic variants of many well stated MCR processes. Also we have focused our attention in non classical MCRs based on isocyanides to expand the synthetical uses of this methodology. We have described new ways for reaction discovery based on the exploration of formally forbidden processes (anti-Bredt cyclizations) or unknown reactivity (indoles with isocyanides). We have described the unexpected formation of a heteroaromatic dipolar acid fluoride from a new MCR. This has allowed the description of a completely new reaction mechanism and the development of biosensors for the selective detection of histamine. We have also described the preparation and uses of fluorescent isocyanides.



Novel Scaffolds in MedChem

i) Antitumoral Trifluorothiazolines. The propaoptotic activity of these compounds in a p53-independent manner has opened new expectations in the field. These structures are prepared in an accessible chemistry leading to this selective scaffold for new antitumoral therapies. In collaboration with Profs Gil (U. Barcelona) and Handa (Tokyo Institute of Technology), we have identified prohibitins as the molecular target.

ii) Postsynthetic Modification of Peptides. The synthetic modification of biologics, can be done either by preparing new modified monomers, or by site-selective transformation of biopolymers. We have developed a method for "post-synthetic modification of peptides" based in the chemoselective C-arylation of tryptophan residues via Pd-catalyzed C-H activation protocol. We have performed also the intramolecular arylation, which constitutes a new stapling technique for peptides.



THE EQUIPMENT

- *Synthetic and Analytical Capacity:*

Hoods, Lab Space, NMR, LC-MS, Prep. HPLC, Chiral HPLC, Microwave, Reactors for scale up, Solid Phase equipment for peptides, Synthesizers for Parallel Synthesis, Autoclaves, Hydrogenation Laboratory

THE OUTPUT

1. *Modern Synthetic Avenues for the Preparation of Functional Fluorophores. F. de Moliner, N. Kielland, R. Lavilla, M. Vendrell; Angew. Chem. Int. Ed. 2017, 56, 3758–3769.*
2. *Insertion of Isocyanides into N-Si bonds: Azine MultiComponent Reactions Leading to Potent Anti-parasitic Compounds. K. G. Kishore, O. Ghashghaei, C. Estarellas, M. M. Mestre, C. Monturiol, N. Kielland, J. M. Kelly, A. F. Francisco, S. Jayawardhana, D. Muñoz-Torrero, B. Pérez, F. J. Luque, R. Gámez-Montaño, R. Lavilla; Angew. Chem. Int. Ed. 2016,55, 8994-8998.*
3. *Spacer-free BODIPY fluorogens in antimicrobial peptides for direct imaging of fungal infection in human tissue. L. Mendive-Tapia, C. Zhao, A. R. Akram, S. Preciado, F. Albericio, M. Lee, A. Serrels, N. Kielland, N. D. Read, R. Lavilla, M. Vendrell; Nature Commun. 2016, 7, 10940*
4. *New Peptide Architectures through C-H Activation Stapling between Tryptophan -Phenylalanine/Tyrosine Residues. L. Mendive-Tapia, S. Preciado, J. García, R. Ramón, N. Kielland, F. Albericio, R. Lavilla; Nature Commun. 2015, 6: 7160.*
5. *Novel trifluorinated thiazoline scaffold leading to pro-apoptotic agents targeting prohibitins. A. Pérez-Perarnau, S. Preciado, C. M. Palmeri, C. Moncunill-Massaguer, D. Iglesias-Serret, D. M. González-Gironès, M. Miguel, S. Karasawa, S. Sakamoto, A. M. Cosialls, C. Rubio-Patiño, J. Saura-Esteller, R. Ramón, L. Caja, I. Fabregat, G. Pons, H. Handa, F. Albericio, J. Gil R. Lavilla; Angew. Chem. Int. Ed. 2014, 53, 10150-10154.*

THE MEDCHEM

Synthesis of Privileged and Novel enriched Scaffolds, Development of New Synthetic Methodologies, Unique Chemical Skills

Collaborations (academic/industrial)
Prof. Juan Gil (U. Barcelona, Cancer); Prof. Marc Vendrell (U. Edinburgh, Bioimaging); Prof. J. F. Luque (Computational Chemistry); Prof. J. Sánchez-Céspedes (U. Sevilla, Antiviral compounds); Prof. R. Eritja (CSIC, Barcelona, DNA chemistry), Minoryx (Pharma, rare diseases)

Networks: CIBER-BBN(network for Bioengineering, Biomaterials and Nanomedicine); Spanish Society of Medicinal Chemistry (SEQT), Spanish Royal Society o Chemistry (Real Sociedad Española de Química)

Training capacities:
Master and Ph. D students are tutored in financed projects. The courses are integrated in the programs of the Faculties of Chemistry and Pharmacy of the University of Barcelona. We are training scientists in Organic and Medicinal Chemistry. Especially in the areas of Heterocycles, Peptides and Combinatorial Chemistry (Multicomponent Reactions)

Patents: 1-Marc Vendrell Escobar, Ramón Subiros Funosas, Lorena Mendive Tapia, Fernando Albericio Palomera, Rodolfo Lavilla Grifols, Rodolfo; Nick D. Read, Nick D. "Fluorogenic compounds, process of preparation thereof and methods of use". PCT Int. Appl. (2016), WO 2016207626. 2-Joan Gil Santano, Rodolfo Lavilla Grifols, Fernando Albericio Palomera, Alba Pérez Perarnau, Sara Preciado Gallego, Diana Mª González Girones, Daniel Iglesias Serret, Rosario Ramon Albalate. "Fluorinated thiazoles for use in treating cancer". PCT Int. Appl. (2012), WO 2012028757 A1 20120308. 3-Francesc Mitjans Prat, Jaume Adan Plana, Carme Calvis Calpe, Fernando Albericio Palomera, Rodolfo Lavilla Grifols, Javier Ruiz Rodriguez. "Cyclic rgd peptides of amino acids based on thiazoles or oxazoles as selective antagonists of the alfa agb 3 integrin". PCT Int. Appl. (2012), WO 2012062777 A1 20120518. 4-Sara Preciado Gallego, Alba Pérez Perarnau, Joan Gil Santano, Fernando Albericio Palomera, Rodolfo Lavilla Grifols. "Fluorinated Indolenines for Use in the Treatment of Cancer". PCT Int. Appl. (2013), WO 2013079754 A1 20130606

THE FUTURE

Plans:
- Developing novel preparative processes based on novel multicomponent reactions upon common heterocycles
- Novel methods for specific modification of biomolecules through non-standard chemistry
- Discovery of novel scaffolds for MedChem and application to selected programs

Added value:
- Access to non-trivial molecular connectivities through short synthetic sequences (and related optimization of bioactivity
- Interdisciplinary collaborations with experts in fields related to MedChem (Computationnal, Physicochemical, Pharmacology, Chemistry, Biological, Bioimaging, etc.)

Prof. Rodolfo Lavilla, ChemBio Lab., University of Barcelona (UB) - Barcelona Science Park (PCB), C/Baldiri Reixac, 10-12, 08028 Barcelona (SPAIN)

UNIVERSITAT DE BARCELONA

University of Helsinki, Faculty of Pharmacy (UH-PHAR)

http://www.helsinki.fi/pharmacy/bio/en/Research/index.html

Check out the University of Helsinki Youtube Video

FINLAND

specialist screening site

ECBS2017 BUDAPEST

a nominated partner site of eu openscreen

This partner site provides specialized screening and supporting chemoinformatics facility that complements the high throughput site (UH-FIMM) in Helsinki. In addition, to the generic skills, equipment and expertise the UH-PHAR partner site hosts specific fields of expertise in the fields of antimicrobial screens, natural product screening and pharmacokinetic extension of ADME profiles.

THE PEOPLE

Prof. Arto Urtti (arto.urtti@helsinki.fi)

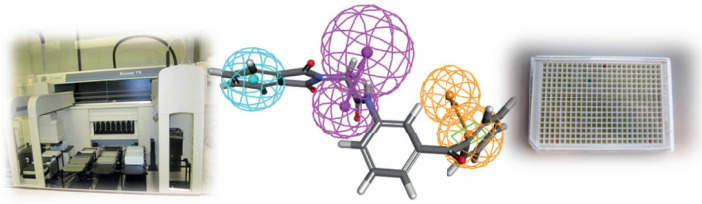
Dr. Päivi Tammela *Dr. Henri Xhaard* *Dr. Heidi Kidron*

THE PROJECTS

Integrated in vitro – in silico screening approach...

...in discovering novel antibacterials

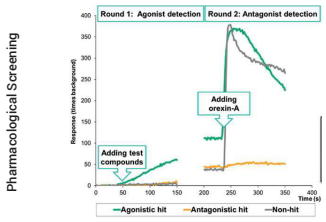
Multidrug-resistant bacterial infections are an increasing source of healthcare problems, and in this project, we developed a screening strategy that integrates cell-based HTS with in silico analogue search for antimicrobial small molecule drug discovery. We performed a HTS on a diverse chemical library by using an assay based on a bioluminescent E. coli K-12 (pTetLux1) strain. The HTS yielded eight hit compounds with >50% inhibition. These hits were then used for structural similarity- based virtual screening, and out of 29 analogues selected for in vitro testing, four compounds displayed potential activity in the pTetLux assay. The 11 most active compounds from combined HTS and analogue search were further assessed for antimicrobial activity against clinically important strains of E. coli and S. aureus and for in vitro cytotoxicity against human cells.



Pharmacophore model...

...to discover orexin receptor agonists

Orexin receptors are G protein-coupled receptors involved in sleep/wake regulation as well as other physiological functions such as metabolic regulation. Up to recently, only antagonists of these receptors were known and developed as a new class of compounds to treat insomnia. Agonists, useful as attention-altering agents or potentially to treat narcolepsy, had proven difficult to develop. Using a pharmacophore in silico screening combined with the testing of 400 compounds from the FIMM library obtained through DDCB, we discovered weak agonists of the Orexin receptors. We are now conducting follow-up studies searching for analogues of our hit compounds to test. In addition we are involved in discussions with the pharmaceutical industry about compound development.



Pharmacological testing data for 393 virtual hits. (A) Correlation of the agonist activity (ability to elevate Ca2+) between OX1 and OX2 receptor-expressing cells measured within 2 min. The activities of compounds 4–7 are shown as plain circles, and those of the rest of the 24 compounds presented for agonist validation are shown as gray squares.

THE HARDWARE

HTS screening systems:

- *Automated liquid handling (Biomek FX, Nano-Plotter)*
- *Plate readers/imagers: Cytation 5, Varioskan LUX, Victor, Multiskan GO*

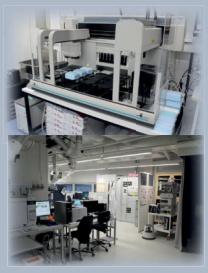
Readouts/ Screening technologies:

- *LUM, FI, TRF, ABS, HCS/Imaging*

Other instruments and infrastructure:

- *Biosafety level 2 microbiology and cell culture facilities*
- *96-well zeta sizer for particle size analyser (detection of precipitates)*
- *Analytical services: triple quadruple LC/MS instrument and Q-TOF MS*

Specialised on antimicrobial targets, efflux transporters and ADME profiling



THE SOFTWARE

Software tools:
We have access to academic licences to commercial software such as Scrödinger Maestro, Accelrys Discovery Studio, Volsurf+. Computational services include include: 1) Chemical library design; 2) Predictive computational ADME profiling; 3) Virtual screens, follow-up screens including both ligand- and structure- based methods; 4) Hit-to-lead compound design, bioisosteric replacements; 5) Data integration, KNIME workflows. Computational tools are being developed and made freely available to the scientific community; for example, predictive ADME models, and drug data mining tools. A web site has been developed in 2015 and published in 2016 and has already received considerable visibility (ADME/Tox and Adverse effects Predictive modelling, http://idaapm.helsinki.fi.

Data analysis tools:
Chem-/bioinformatics resources high performance supercomputing facilities are accessible both through the Finnish IT Center for Science. CSC-IT furthermore organize access to commercial software licences as a national consortia https://www.csc.fi/home.

THE OUTPUT

1. Nybond S, Ghemtio L, Nawrot D, Karp M, Xhaard H, Tammela P. Integrated in vitro – in silico screening strategy for the discovery of antibacterial compounds. Assay Drug. Dev. Tech., 2015, 13(1): 25-33. http://dx.doi.org/10.1089/adt.2014.625
2. Wissel G, Kudryavtsev P, Ghemtio L, Tammela P, Wipf P, Yliperttula M, Finel M, Urtti A, Kidron H, Xhaard H. Exploring the structure-activity relationships of ABCC2 modulators using a screening approach. Bioorg. Med. Chem., 2015, 23:3513-25. http://dx.doi.org/10.1016/j.bmc.2015.04.029
3. Turku A, Borrel A, Leino TO, Karhu L, Kukkonen JP, Xhaard H. Pharmacophore model to discover OX1 and OX2 orexin receptor ligands. J. Med. Chem., 2016, 59:8263-75. doi: 10.1021/acs.jmedchem.6b00333
4. Legehar A, Xhaard H, Ghemtio L. IDAAPM: integrated database of ADMET and adverse effects of predictive modeling based on FDA approved drug data. J. Cheminform., 2016, 8, 33. doi: 10.1186/s13321-016-0141-7. eCollection 2016.
5. Sjöstedt N, Holvikari K, Tammela P, Kidron H. Inhibition of breast cancer resistance protein and multidrug resistance associated protein 2 by natural compounds and their derivatives. Mol. Pharm., 2017, 14(1):135-146. http://dx.doi.org/10.1021/acs.molpharmaceut.6b00754

Collaborations (academic/industrial):
FIMM, Institute for Molecular Medicine Finland, Institute of Biotechnology, University of Helsinki, Neuroscience Centre, University of Helsinki, University of Eastern Finland

Networks:
Drug Discovery and Chemical Biology Network (national)
Nordic Chemical Biology Consortium

Training capacities:
Training for BSc, MSc, and PhD students in screening technologies and computational methods

THE FUTURE

- Development of phenotypic 3D-cell culture models and multispecies co-culture assays, and tools for their analyses
- Aiming to develop our computational activities as web-based services that use our own data and tools and will both provides visibility and access to customers

Biocenter 2, Viikki Campus, Viikinkaari 5E (P.O. Box 56), FI-00014 Helsinki

UNIVERSITY OF HELSINKI
FACULTY OF PHARMACY

FIMM High Throughput Biomedicine unit

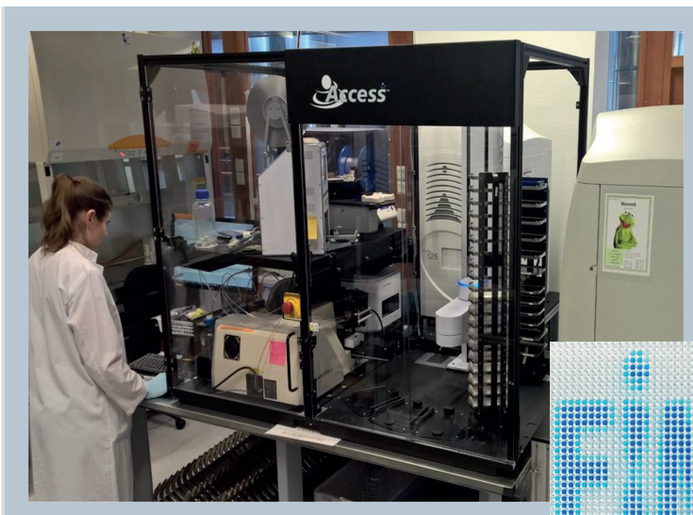
www.fimm.fi/en/services/technology-centre/high-throughput-biomedicine

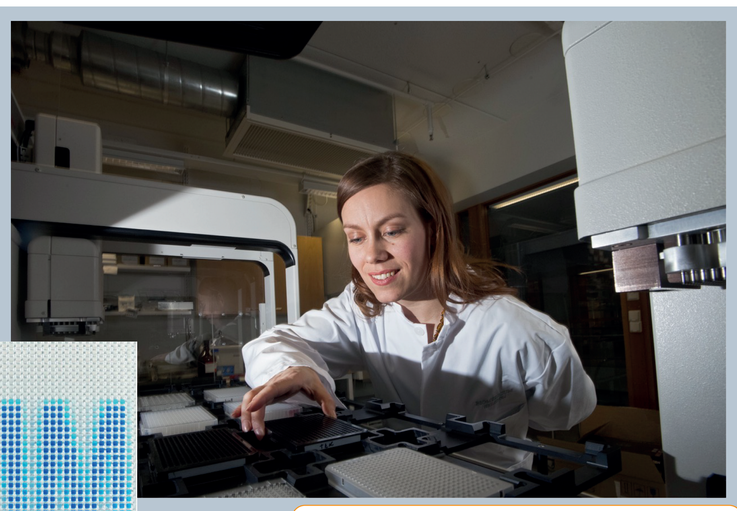
FINLAND

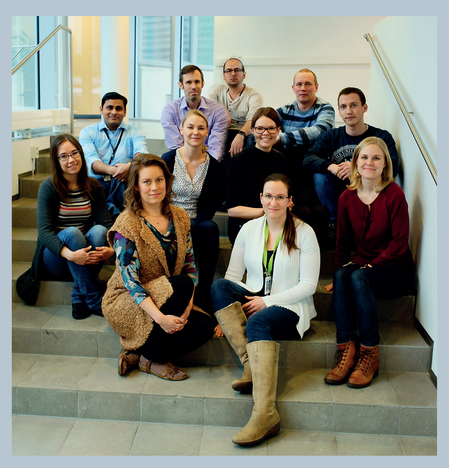
high-capacity screening site

ECBS2017 BUDAPEST

a nominated partner site of eu:::openscreen







THE PEOPLE

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Krister Wennerberg
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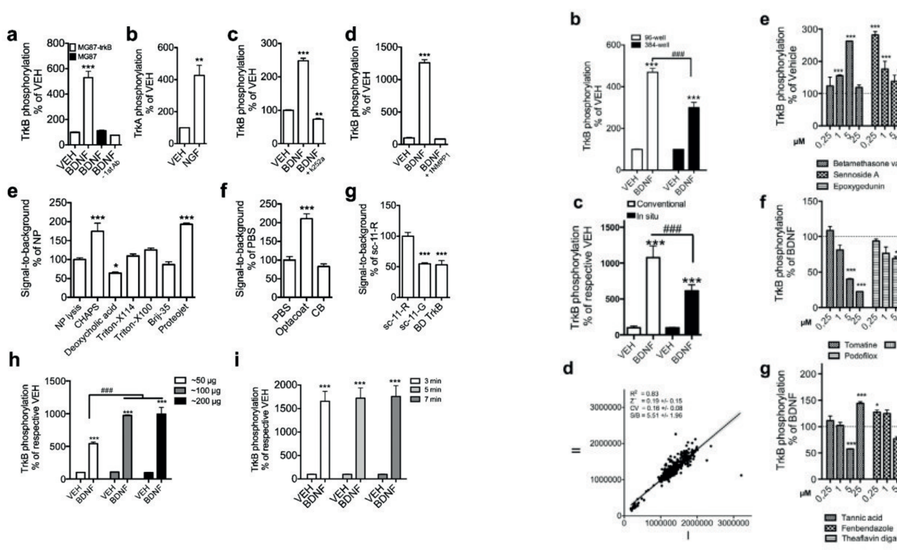
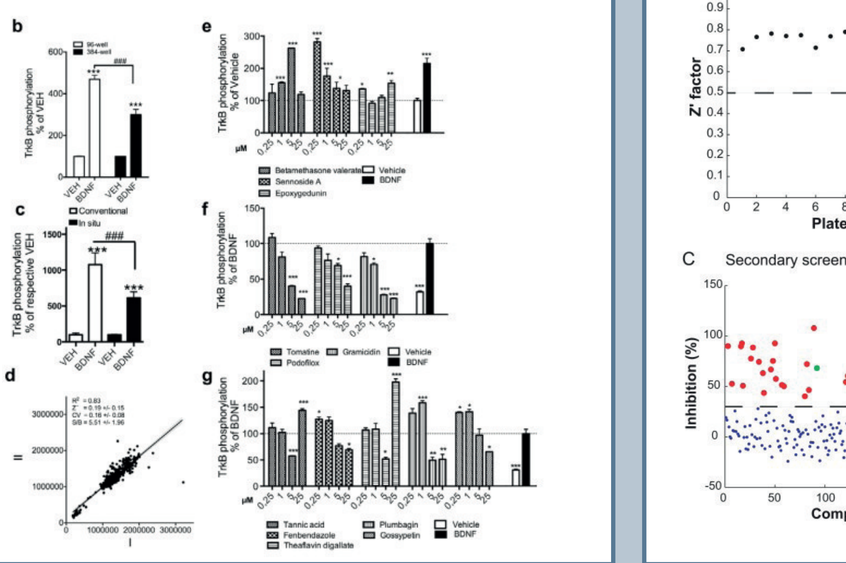
THE PROJECTS

TrkB activator screening

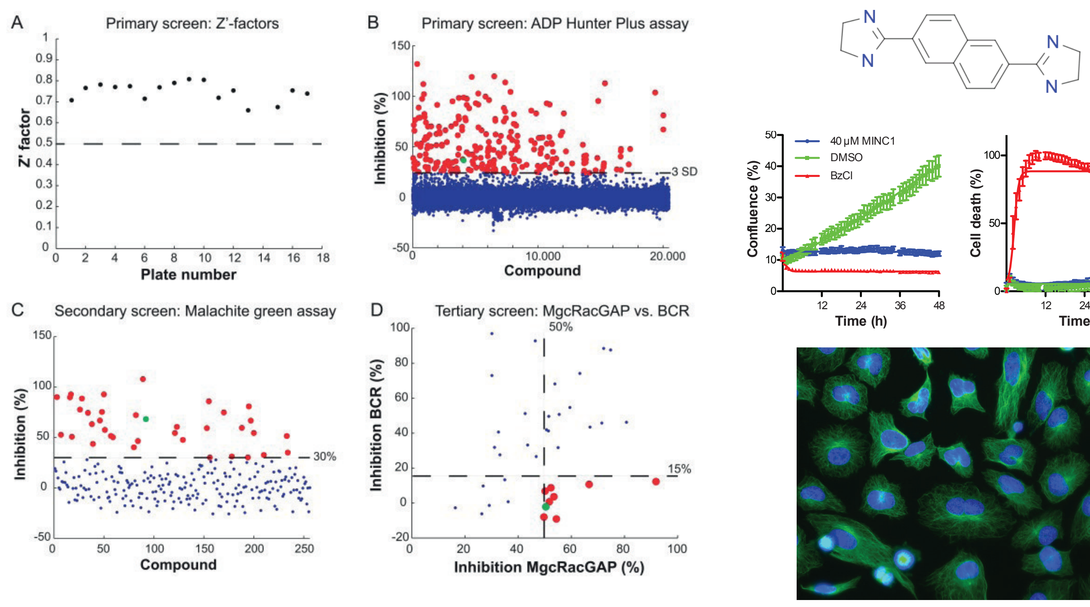
Trk receptor tyrosine kinases regulate multiple important neuronal processes during development and in the adulthood. Small molecules activating Trk receptors could therefore serve as molecules promoting peripheral nerve regeneration. However, methods allowing simple and large-scale Trk phosphorylation analyses in cultured cells were previously lacking. To address this, we developed an in situ phospho-TrkB ELISA assay where cell culture, receptor stimulation and Trk phosphorylation analysis were all performed on the same multiwell plate. The whole assay was very complex (total assay time 120 h) and absolutely required the systematic assay handling by the FIMM HTB main robotic automation system for reproducible results. The assay readily and specifically detects TrkB phosphorylation in cells. A 100 000 compound screen was run with this method.
Reference: Antila et al. Journal of Neuroscience Methods, 2014, 222, 142–146.





Identification of MgcRacGAP inhibitors

MgcRacGAP, a Rho family GTPase activating protein involved in cytokinesis, is upregulated in many cancers and associated with poor clinical prognosis. Therefore, we designed and performed a biochemical and high-throughput screen of over 20 000 compounds in 1536-well format to identify small molecule inhibitors of MgcRacGAP. Through follow-up assays we finally identified the compound MINC1. Sequential cell-based assays showed that MINC1 induces an increased rate of cytokinetic failure and multinucleation in addition to other cell division defects.
Reference: van Adrichem et al., Comb Chem High Throughput Screen, 2015, 18, 3-17



THE HARDWARE

-Labcyte Access robotic system
(including Labcyte Echo 550 and 525 -acoustic dispensers, Nexus X-peel plate peeler and Agilent PlateLoc plate sealer and V-Spin centrifuge, Thermo Scientific Cytomat 10 -plate hotel, Labyte Deerac LX –bulk filler) to make assay ready compound plates

-BeckmanCoulter integrated robotic system
(including Agilent PlateLoc plate sealer and V-Spin centrifuge, BMG Pherastar FS plate reader, two ThermoFisherScientific Multidrop Combis, one Multidrop combi nl, Cytomat 24MPH plate hotel and Cytomat 10C plate incubator, Beckman Coulter Biomek FXp pipetting robot) for running HTS assays

-Biotek EL406 plate washer/dispenser with plate stacker

- Stand alone BMG Pherastar FS -plate reader with plate stackers
  BioTek Cytation 5 -plate reader and microscope with plate stackers

-StoragePod chemical storage system

-Aushon Biosystems 2470 –Array printer
- Innoscan 710 AL – slide scanner

THE EXPERTISE

Absorbance
Acoustic Dispensing
AlphaScreen
BSL II
Fluorescence
Fluorescence Polarization/anisotrophy
FRET, BRET
Luminescence
Time-resolved fluorescence, TR-FRET
Plate-based cytometry
High content imaging

- Cell-based and biochemical screens using targeted or large chemically diverse libraries
- Molecular probe discovery
- Biological profiling using libraries of known bioactives
- Drug repositioning
- Personalized medicine screening (drug resistance and sensitivity) using approved and investigational drugs

THE SOFTWARE

Dotmatics Software
- Browser
- Studies
- Vortex
- Register
- Pinpoint
- Nucleus

Breeze - web-based in-house developed analysis, database and visualization software

SynergyFinder - web-based software for drug combination analyses

THE COMPOUNDS

>130 000 chemically diverse screening compounds (Specs, Chembridge, ChemDiv, Tripos)

>5 000 known bioactive and clinically investigated compounds (various sources)

THE OUTPUT

-Kulesskiy E et al. 2016. Precision Cancer Medicine in the Acoustic Dispensing Era: Ex Vivo Primary Cell Drug Sensitivity Testing. Journal of Laboratory Automation, 21, p. 27-36
-Pemovska, T., et al. 2015. Axitinib effectively inhibits BCR-ABL1(T315I) with a distinct binding conformation. Nature. 519, 7541, p. 102-225
-Pemovska T. et al. 2013. Individualized Systems Medicine (ISM) strategy to tailor treatments for patients with chemorefractory acute myeloid leukemia. Cancer Discovery. 3, p. 1416-29.
-van Adrichem, A. J., et al. 2015. Discovery of MINC1, a GTPase-Activating Protein Small Molecule Inhibitor, Targeting MgcRacGAP. Combinatorial Chemistry & High Throughput Screening. 18, 1, p. 3-17

- 50 academic and 7 industrial (including SMEs) collaborations in 2016
- Member of:
  EU-LIFE,
  Nordic EMBL Partnership for Molecular Medicine
  Nordic Chemical Biology Consortium
  European Cell-based Assays Interest group

eulife

THE FUTURE

Automation upgrade in 2018
Dynamic robotics with capability of reconstructing screening system according to assay

Adding high throughput flow cytometry screening capacity

Institute for Molecular Medicine Finland (FIMM)
University of Helsinki, Tukholmankatu 8
Helsinki, Finland

FIMM UNIVERSITY OF HELSINKI

NOR-Openscreen, Bergen Node

http://www.openscreen.no/
http://www.uib.no/en/rg/biss
http://www.uib.no/rg/biorec

NORWAY

Specialist screening site
Biophysical screens

ECBS2017
BUDAPEST

a nominated partner site of eu-openscreen

THE PEOPLE

Ruth Brenk

Aurora Martinez

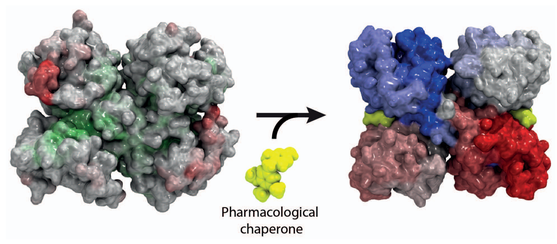
Jarl Underhaug

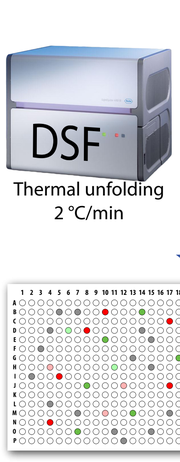
THE PROJECTS

Reference Project 1

Discovery of pharmacological chaperones

Small and selective molecules that aid in the renaturation of unstable, misfolded conformations of a targeted protein, recovering (totally or partially) the original structure and function.





Primary hit list:

Plate 1, D06: ΔT_m=2.0
Plate 1, E10: ΔT_m=5.1
Plate 2, F02: ΔT_m=2.4
Plate 2, E09: ΔT_m=4.5
Plate 2, H11: ΔT_m=3.3
Plate 3, B03: ΔT_m=2.6
Plate 3, B04: ΔT_m=8.5
Plate 3, B05: ΔT_m=3.2
Plate 4, F10: ΔT_m=2.1
Plate 6, A07: ΔT_m=5.0

1) High-throughput screening (HTS) of a chemical library
Based thermal stabilization induzed by ligand binding
(by differential scanning fluorimetry; DSF)

2) In vitro and in cell validation studies
WT and mutant binding and stabilization by SPR, DLS, and ITC
Enzyme kinetics to investigate possible inhibitory effects.
Transient expression of mutants in eukaryote cells (protein and activity)

3) Hit-to-lead expansion and optimization

4) Mice studies

Reference Project 2

Virtual screening for ligands binding to the FMN riboswitch

In house databse ≈ 4.8x10⁶ curated purchasable compounds

Filtering for desired properties

- SlogP ≥ -3.5 & ≤ 3.5
- HAC ≥ 12 & ≤ 30
- HBD ≥ 1
- HBA ≥ 1
- HBD+ HBA ≥ 2 & ≤ 12
- MW ≤ 500
- rot. bonds ≤ 7
- no unwanted groups
- net charge: -1;0;+1
- aromatic rings ≥ 1
- fused rings ≥ 1

Calculation of isomers ("prot, taut, stereo")

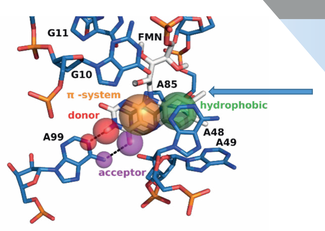
Pharmacophore search

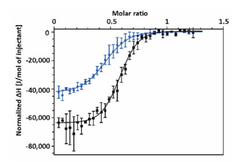
Conversion to dockable format

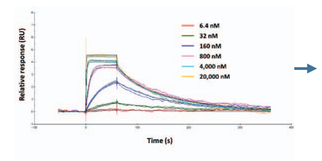
Molecular docking

VS hits

Extensive hit validation using biophysical methods (ITC and SPR)







One confirmed hit series

THE HARDWARE

HTS screening systems
Bravo (Agilent), Mosquito

Readouts/Screening technologies
DSF, Octet Red 96, SPR, Multimode plate readers, NMR, virtual screening, fragment screening

Compound libraries
Fragment (700 cps), Diversity (10 000 cps), Prestwick (1280 cps)

Target classes
RNA, Molecular chaperones, enzymes associated with genetic disorders, protein-protein interactions

THE SOFTWARE

Data analysis tools
In house software

Software tools
Schrödinger package, Bio3D, DrugPred, BioSolveIT package

THE OUTPUT

1. Jorge-Finnigan et al. (2013) Hum Mol Genet. 22, 3680-9
2. Aubi et al. (2015) J Med Chem. 58, 8402-12
3. Yuste-Checa et al. (2017) Hum Mutat. 38:160-168
4. Krasowski et al. (2011) J Chem Inf Model. 51:2829-42
5. Ulrich et al. (2013) ACS Chem Biol. 8:1044-52

Collaborations — (Several universitites: Oslo, Oulu, Basque Country, Autnoma of Madrid)
Networks — (NOR-Openscreen, NNP, KG Jebsen, Toppforsk, Biss)
Training capacities — (Liquid handling robotics, DSF, Octet)
Patents — (5, among others patent for new treatment for PKU)

THE FUTURE

Self-sustained core facility
http://www.uib.no/en/rg/biss

Department of Biomedicine, UiB
Jonas Lies vei 91
N-5009 Bergen

ruth.brenk@uib.no

NOR-openscreen

UiT- The Arctic University of Norway / Marbio

www.uit.no
https://en.uit.no/forskning/forskningsgrupper/gruppe?p_document_id=380005

NORWAY

specialist screening site

ECBS2017 BUDAPEST

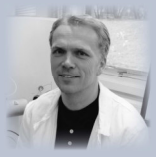
a nominated partner site of eu openscreen

Photo : UiT

Photo : UiT

THE PEOPLE

Jeanette H. Andersen (jeanette.h.andersen@uit.no)



Espen Hansen (espen.hansen@uit.no)

THE PROJECTS

MabCent-SFI Centre for research-based innovation

The overall scope of MabCent was to find and develop high-value bioactive products through screening of Arctic organisms for future commercial exploitation.
In this project:

- Marbio performed more than 20 various bioassays and approximately 300 000 screening events. This was to support the bioassay-guided isolation of marine natural products.
- Marbio isolated 150 bioactive compounds from crude extracts of marine invertebrates.

Photo: Adnan Icagic

KinSea - Kinase inhibitors from the sea

The Kinsea project aims at advancing the hits from the anticancer screening program of marine invertebrates through the biodiscovery pipeline. This will be achieved through optimization of biological activity through chemical synthesis and the key outcome is to nominate lead compounds.

Photo: Erling Svensen

THE HARDWARE

- Flash chromatography systems (prefractionation of extracts)
- Autopurification HPLC systems (isolation of natural products)
- High-resolution MS-MS with ion mobility (identification of natural products)
- Biomek minicore system (liquid handler Biomek NX, CO2 incubator, plate washer, barcode reader, plate reader, shakers, integrated with SCARA robotic arm)
- Biomek3000 liquid handler
- Biomek NXp liquid handler
- Precison liquid handler
- Envision Alphascreen multimode detector
- Flow cytometer

THE EXPERTISE AND CAPABILITIES

- Experience in screening complex mixtures (i.e. natural product extracts and fractions) for biological activity in biochemical and cell based assays.
- Dereplicating (identifying) known compounds in complex mixtures using chromatography and high-resolution mass spectrometry.
- Isolation of bioactive natural products from crude extracts using a combination of classic techniques such as liquid-liquid extractions and Flash chromatography, as well as state of the art mass guided fractionation based on HPLC.
- Structural elucidation of complex natural products using several types of high resolution MS as well as infrared and NMR spectroscopy.
- Data management of datasets for natural product drug discovery, including geographical sample information, taxonomical data, sample tracking through complex extraction and fractionation schemes, analytical chemistry data and extensive bioactivity profiles of extracts, fractions and pure compounds.

THE OUTPUT

1. *Metabolomic Profiling Reveals the N-Acyl-Taurine Geodiataurine in Extracts from the Marine Sponge Geodia macandrewii (Bowerbank). J Nat Prod. 2016 May 27;79(5):1285-91 doi: 10.1021/acs.jnat prod.5b00966.*
2. *Screening for marine natural products with potential as chemotherapeutics for acute myeloid leukemia Current Pharmaceutical Biotechnology 2016;17(1):71-7. PMID: 26278527 doi: 10.2174/1389201016666150817095537*
3. *Antioxidant and anti-inflammatory activities of barettin, Mar. Drugs 2013, 11, 2655-2666 doi: 10.3390/md11072655.*
4. *A combined atomic force microscopy and computational approach for structural elucidation of breit fussin A and B, highly modified halogenated dipeptides from the Arctic hydrozoan Thuiaria breitfussi Angew. Chem. Int. Ed. 2012, 51, 1 – 6 doi: 10.1002/anie.201203960*
5. *Synoxazolidinones A and B; novel bioactive alkaloids from the ascidian Synoicum pulmonaria. Organic Letters 2010; Volum 12 (21). ISSN 1523-7060.s 4752 - 4755.s doi: 10.1021/ol101707u.*

- NOR-OPENSCREEN: Marbio is one the 4 nodes in the Norwegian network NOR-OPENSCREEN, that offers advanced scientific equipment/facilities within chemical biology. The expert areas in NOR-OPENSCREEN are marine bioprospecting, microbiology screening and metabolomics as well as cell-based HTS flow cytometry.
- Marbio has a unique opportunity to train both master,- PhD students as well as technical personnel in bioassay screening and identification of natural products.
- International collaborators: Lead Discovery Centre, Germany, Medina, Spain, University of Aberdeen, UK, University College of Cork, Ireland, Technical university of Denmark (DTU), Sea4us, Portugal

THE FUTURE

The strategy of Marbio is to continuously set-up additional bioassays to identify novel chemistry in our unique marine extract library.

UiT-The Arctic University of Norway / Marbio, Siva innovation centre, 9037 Tromsø, Norway

Instagram: @marbio_uit

Chemical Biology Platform, NCMM

http://www.med.uio.no/english/research/core-facilities/chemical-biology-screening/

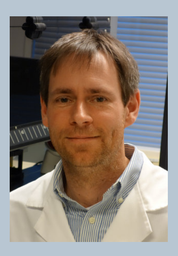
NORWAY

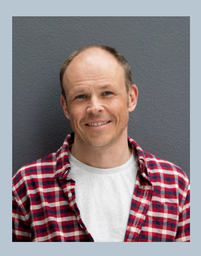
assay adation screening site

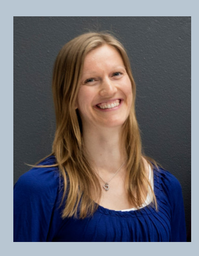
ECBS2017 BUDAPEST

a nominated partner site of eu:openscreen

THE PEOPLE







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THE PROJECTS

Targeting protein-protein interactions

Regulating adrenergic Ca^{2+} reabsorption into sarcoplasmic reticulum by targeting the AKAP18δ-PLB interaction in the heart

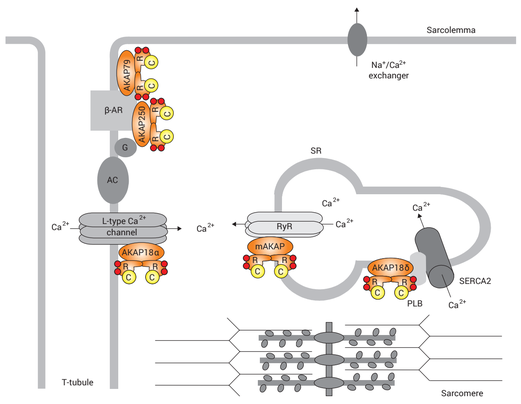


Figure 1 . Schematic illustration of a cardiac myocyte and Ca^{2+} handling. Major components in the excitaion–contraction coupling in the myocyte are shown. The cycling of Ca^{2+} is indicated by arrows and the different protein kinase A (PKA)–A-kinase anchoring protein (AKAP) complexes that provide adrenergic regulation of various components of the Ca^{2+} handling machinery are indicated.
AC: Adenylyl cyclase; β-AR: β-adrenergic receptor; C: Catalytic subunit of PKA; mAKAP. Muscle-specifi cAKAP; PLB: Phospholamban; R: Regulatory subunit of PKA; RyR: Ryanodine receptor; SERCA: Sarcoplasmic reticulum Ca^{2+}-ATPase; SR: Sarcoplasmic reticulum; Red circle: cAMP.

Lygren & Taskén 2008, Expert Opinion on Biological Therapy

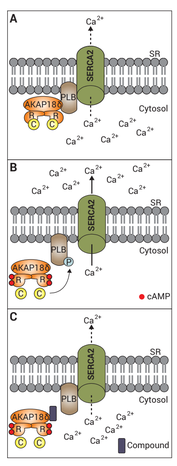
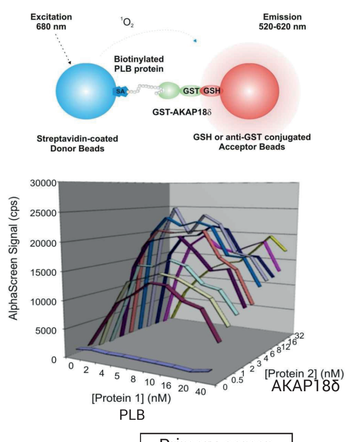


Figure 2. AKAP18δ–PLB–sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA)2 complex. A. Resting situation, no adrenergic drive, low heart rate, SERCA2 inhibited by PLB with low ATP- and energy consumption. B. Adrenergic stimulation paces the heart, increased heart rate. SERCA2 released from PLB inhibition by PKA phosphorylation. Fast Ca^{2+} reabsorption, high ATP- and energy consumption. C. Disruption of AKAP18δ–PLB complex prevents PKA phosphorylation and dissociation of PLB and thereby SERCA2 activation. Low ATP- and energy consumption.



Primary screen
79 000 comp.
51 hits

↓

Secondary screen
344 comp.
62 hits

↓

Cell assay
10 comp.

↓

Tertiary screen
80 comp.

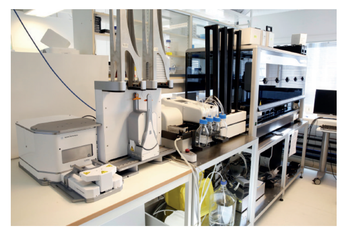
↓

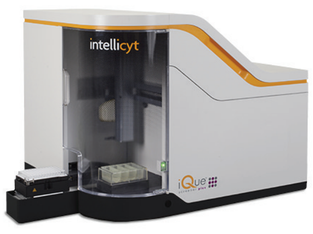
45 compounds

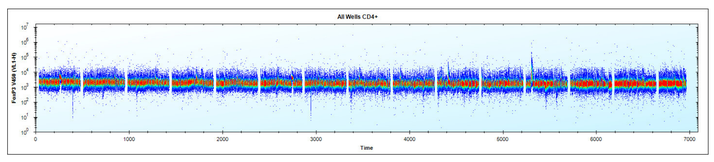
High-througput flow cytometry

Analysis of FoxP3 expression on $CD4^+$ T cells

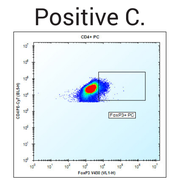
Fully automated sample preparation in 384-well format followed by sample analysis on the iQue screener PLUS.



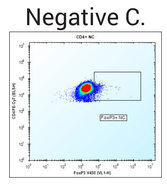


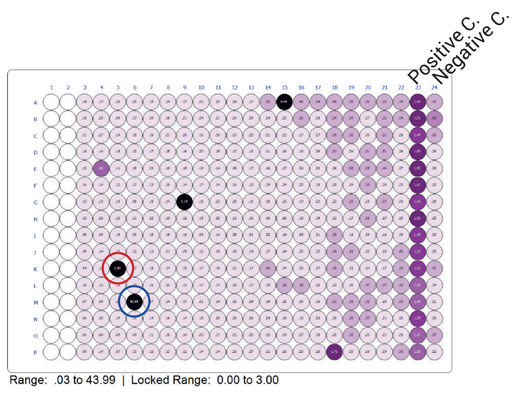


Positive C.

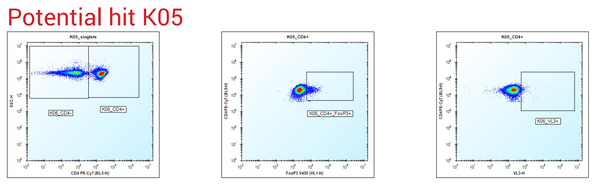


Negative C.

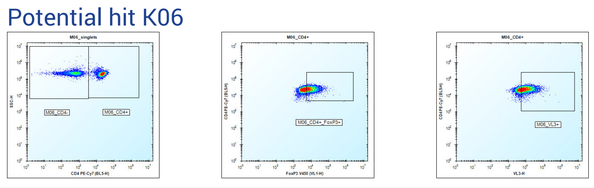




Potential hit K05



Potential hit K06



THE HARDWARE

liquid handling: *Access Workstation with Echo550, Hamilton microlab star*

readers: *BioTek Synergy Neo2, PerkinElmer EnVision, Molecular Devices FLIPR384, IntelliCyt iQue Screener PLUS, BD LSRFortessa*

assays: *AlphaScreen, Lum, FI, FP, TRF, UV-visible Abs, Ca^{2+}-flux, HT flow cytometry*

compound storage: *Roylan StoragePod (humidity controlled N_2 atmosphere)*

compound libraries: *Enamine, ChemBridge, ChemDiv, Prestwick, Enzo Target/Pathway, SelleckChem Cancer/Kinase, Tocriscreen mini, BioMol Kinase*

THE SOFTWARE

software tools:
- *Dotmatics Studies*
- *Dotmatics Vortex*
- *ChemAxon JChem*
- *Prism Graphpad*

THE OUTPUT

1. *Dukic et al. (2017) SLAS Discov.*
2. *Calejo & Taskén (2015) Front. Pharmacol.*
3. *Bach et al. (2014) Naunyn-Schmiedeberg's Arch Pharmacol.*
4. *Ellinger et al. (2014) Assay Drug Dev. Technol.*
5. *Vang et al. (2012) Nature Chem. Biol.*

collaborations: *GSK, Seald AS, Rheumatech AS, Various groups from University / U. Hospital Oslo*

networks: *NOR-OPENSCREEN, Nordic Chemical Biology Network*

patents: *Taskén et al. WO 2013171332*

THE FUTURE

future plans:
expanding the compound collections
attraction of international users

added value:
expertise in targetting protein-protein interactions and HT flow cytometry

NCMM, University of Oslo, Norway
Forskningsparken, Gaustadalléen 21, 0349 Oslo

NCMM
Centre for Molecular Medicine Norway
Nordic EMBL Partnership for Molecular Medicine

Screening Laboratory of Anticancer Compounds

Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences.

https://www.cbmm.lodz.pl/

POLAND

specialist screening site

ECBS2017 BUDAPEST

a nominated partner site of eu openscreen

THE PEOPLE

Prof. Barbara Nawrot
tel. (+42) 680-32-18
e-mail: cbmm@cbmm.lodz.pl

Dr. Julia Kaźmierczak-Barańska

Dr. Marcin Cieślak

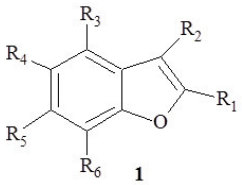
Dr. Karolina Królewska-Golińska

THE PROJECTS

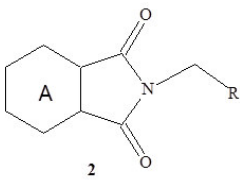
New benzo[b]furan and dicarboximide derivatives...

...with anticancer activity - mechanism of action in human cells

Anticancer properties of 80 derivatives have been evaluated. 9 compounds were cytotoxic against several tumor cell lines and non-toxic to normal human cells. The identified compounds induced apoptosis in leukemic cells and showed selective toxicity toward different types of leukemia cells. These derivatives exhibited higher toxicity and selectivity to leukemic cells than reference drugs such as cytarabine, bortezomib, sorafenib, CPT-11 and doxorubicin. Screening of the initial pool of benzo[b]furans (1) and dicarboximides (2) allowed to identify two hit compounds that are candidates for the development of new anticancer drugs used in the treatment of leukemia or other proliferative diseases.



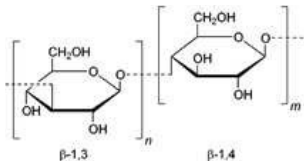
1



2

Evaluation ...

...of cytotoxic and immunomodulatory properties of high quality beta-glucan isolated from oats



The project was aimed to identify cancer cell lines susceptible for beta glucan. 8 cancer cell lines were tested using MTT assay. The immunostimulatory potential of beta glucan was examined by measuring the mRNA levels of 5 cytokines: TNFa, IL-1b, IL-3, IL-6, IL-12 in immune cells.

(http://betaglukan-bio.com/obetabio_en.html)

Project supported by Beta Bio Technology sp.z o.o. (SME)

THE HARDWARE

Screening Lab

- Readout and screening technologies are based on UV/Vis
- Fluorescence and luminescence readout with FLUOStar Omega plate reader (BMG Labtech)
- Fluorescence activated cell sorting (FACS) with Excalibur instrument (Becton-Dickinson)

- Organic synthesis Labs
- Molecular biology Labs
- Cell culture Labs (3 laminar hoods, CO2-incubators, liquid nitrogen storage system)
- G-Box, Nano-Drops, UV-VIS spectrometer with Peltier heater
- Two DNA/RNA synthesizers, several HPLC & PREP-HPLC systems
- Cool room, gel electrophoresis , capillary electrophoresis
- PCR, Roche LightCycler qPCR, Plate qPCR, 3 autoclaves
- Radioisotope Lab, MST

OTHER

Institute instruments:

- NMR (200, 300, 500, 600 MHz) + 700 MHz (TUL)
- Mass spectrometers (2 MAL DI-TOF, FAB, ESI MS)
- AFM, DSC, FTIR

Collaborations:

Ca. 15 bilateral collaboration projects with the research groups affiliated in Warsaw Medical University, Lodz Medical University, Lublin University, Ludwig Maximilian University Munich, Institute of Bioorganic Chemistry PAS Poznan.

THE OUTPUT

1. *Thiosemicarbazone-derived copper(II), cobalt(II) and nickel(II) complexes as potential anticancer agents: nuclease activity, cytotoxicity and apoptosis studies. New J. Chem., 2016, 40, 9761-9767.*
2. *New, Substituted Derivatives of Dicarboximides and their Cytotoxic Properties. Anti-cancer Agents Med. Chem. 2016, 16(7): 852-864.*
3. *Phosphorothioate analogs of P1,P3-di(nucleosid-50-yl) triphosphates: Synthesis, assignment of the absolute configuration at P-atoms and P-stereodependent recognition by Fhit hydrolase. Bioorg. Med. Chem. 2016, 24: 5068-5075.*
4. *Synthesis and Cytotoxic Properties of Halogen and Aryl-/Heteroarylpiperazinyl Derivatives of Benzofurans. Anti-Cancer Agents in Medicinal Chemistry, 15, 115-121 (2015)*
5. *Synthesis and in Vitro Cytotoxicity of Cross-Conjugated Prostaglandin A and J Series and Their Hydroxy Derivatives. Org. Biomol. Chem., 13, 7000-7012 (2015)*

Patents:

1. Halogen derivatives of benzo[b]furans useful as anti-neoplastic or anti-proliferative drugs. EP2631232B1 European patent.
2. Pochodne alkilowe 7-hydroksy-15-deoksy-13,14-dihydro-19,20-dinor-18-(4-metylofenylo)-ent-PGA1 sposób ich wytwarzania oraz ich zastosowanie. P.401115 Polish patent.
3. Zastosowanie soli fosfoniowych przeciw komórkom nowotworowym raka szyjki macicy HeLa lub komórkom przewlekłej białaczki szpikowej. Polish patent no. 220707.
4. Halogenopochodne benzo[b]furanów, ewentualnie w postaci farmaceutycznie dopuszczalnej soli oraz zastosowanie halogenopochodnych benzo[b]furanów. P.398193 Polish patent.

THE FUTURE

Development of approach based on biotinylated anticancer agents to identify molecular targets

Centre of Molecular and Macromolecular Studies,
Polish Academy of Sciences
Sienkiewicza 112, PL - 90-363 Łódź, POLAND

(CMMS PAS, LODZ, POLAND)

High-throughput Screening Laboratory
Institute of Bioorganic Chemistry, PAS

Noskowskiego 12/14, 61-704 Poznan, Poland
www.ibch.poznan.pl

POLAND

specialist screening site

ECBS2017
BUDAPEST

a nominated partner site of eu openscreen

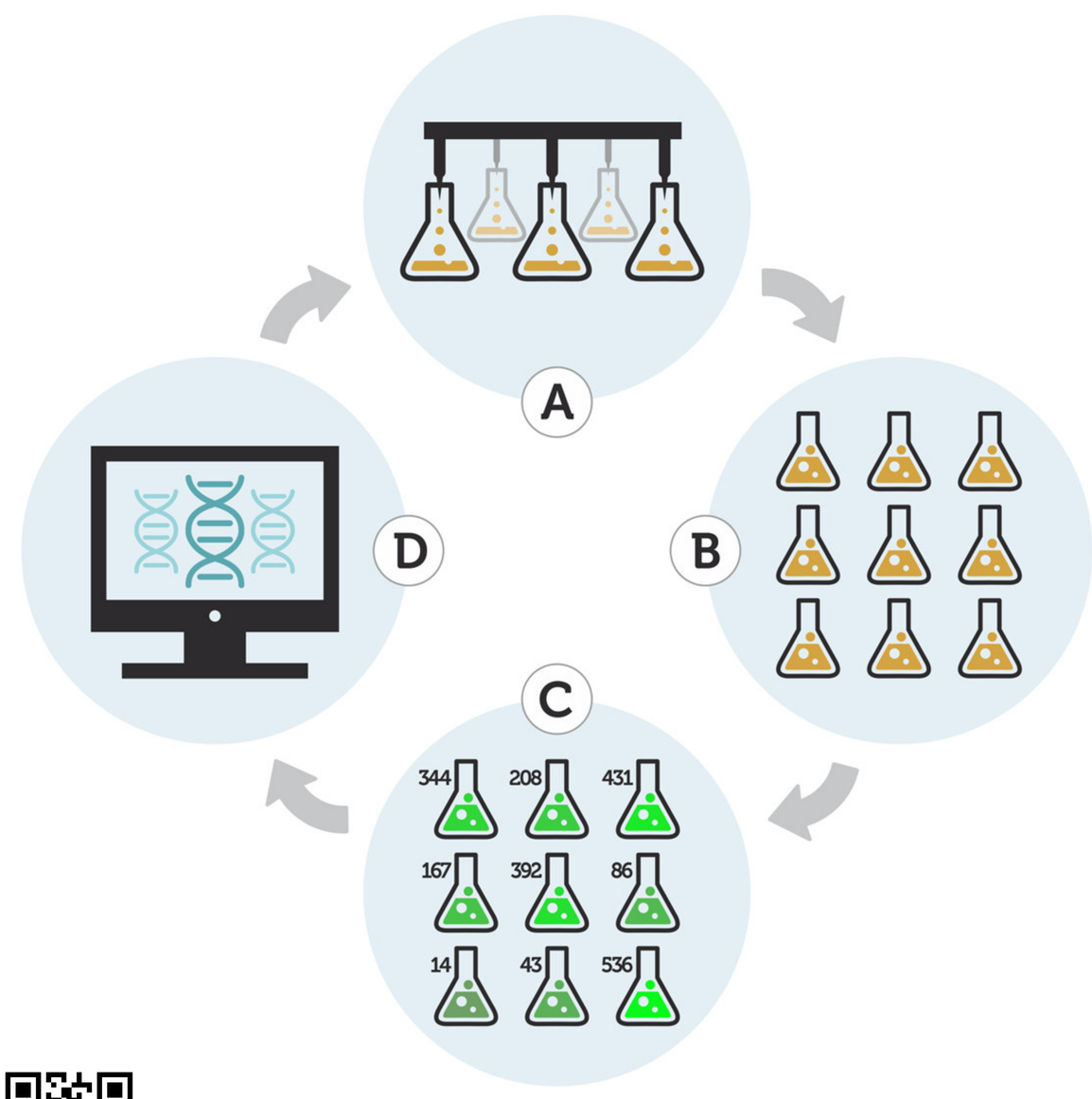
PROJECTS

Reference Project 1

The EPICELL project is carried out within the framework of a strategic Polish governmental program for finding new opportunities in regenerative medicine (STRATEGMED program). The main scope of EPICELL is determination of the epigenetic small molecule cocktails (cell media formulations) for reprogramming the myoblasts derived from myocardial post-infarction patients into IPSC and next toward cardiomyocytes. The cardiomyocytes obtained via small molecules will be surgically introduced into the patients' myocardium for their better recovery

Reference Project 2

PLANTVIR project is aimed at the plant biotechnology data mining and optimization of the bioprocesses by automatic search for optimal physicochemical conditions for transient viral expression in liquid bioreactor plant cultures. The obtained models will find application in molecular research on viral infection for using Agrobacterium, expression systems as well as in the production of proteins in bioreactor plant cultures (monoclonal antibodies, hormones, vaccines, enzymes, interferons, interleukins, metabolites).



THE SOFTWARE

We developed closed-loop high-throughput combinatorial system for solving different biological problems. HTS robotics supported by genetic algorithms have been used, as they offer efficient exploring multidimensional search space and a realistic possibility of determining key factors and exploring their mutual interactions.

THE FUTURE

We will provide unique technologies and expertise concerning closed-loop, high-throughput combinatorial screening driven by the artificial intelligence system and based on animal and plant cell models.

Institute of Bioorganic Chemistry
Polish Academy of Sciences

The Institute of Biochemistry and Biophysics (IBB)
of the Polish Academy of Sciences

www.ibb.waw.pl

POLAND

Chemistry site

ECBS2017
BUDAPEST

a nominated partner site of eu openscreen

THE PEOPLE

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tel: +48 22 592 2145
fax: +48 22 592 2190

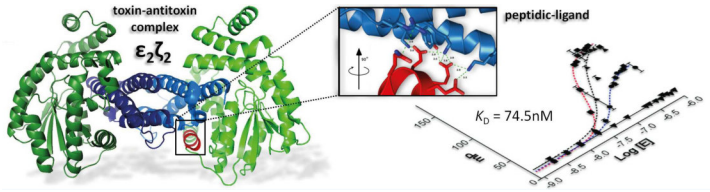
Dr. Pawel Siedlecki (pawel@ibb.waw.pl)

THE PROJECTS

Mapping Protein-Protein Interactions...

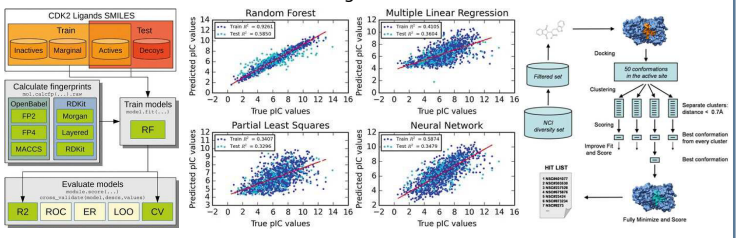
...of the Resistance-Related Bacterial Zeta Toxin-Epsilon Antitoxin Complex ($\varepsilon_2\zeta_2$) with High Affinity Peptide Ligands Using Fluorescence Polarization

In collaboration with Institute of Pharmacy, Pharmaceutical and Medicinal Chemistry on Freie Universität Berlin (prof. dr. Jorg Rademann) we designed the peptidic disruptors of protein-protein interactions between Zeta–Epsilon (toxin–antitoxin) system, which is responsible for the stable maintenance of certain multiresistance plasmids in Gram-positive bacteria. The peptides were used to create a fluorescent competitive binding assay as an excellent tool for the rapid and efficient screening of chemical libraries. The preliminary screening of the library enabled us to find over 150 compounds which are effective inhibitors of protein–protein interactions of the Epsilon–Zeta TA system and that are promising new antibacterial agents.



Novel methods to improve structure based drug design

Bioinformatics Department of IBB PAS is actively developing new cheminformatic methods, which excel the current tools. Last examples include 1) DiSCuS – a virtual screening analysis tool, with novel consensus scoring methodology RankScore; 2) DeCAF – unique method to augment molecule representation with spatial and physicochemical properties to improve the selectivity and sensitivity of ligand-based screening; 3) ODDT – cheminformatic and structural drug discovery toolkit with machine learning models to predict properties of small molecules; 4) RF-Score-VS – innovative scoring function tailored for virtual screening based on Random Forest model and spatial contacts to predict compounds activity. We have shown that RF-Score-VS is at least two times better than other classical scoring functions in terms of early enrichment of active ligands in virtual screening campaigns.



THE EQUIPMENT

- *Mass Spectrometry Laboratory - small molecule analyses for toxicology, metabolomics and quantitative drug analyses (MALDI TOF/TOF, ESI LC/MS/MS Orbitrap Velos, Synapt G2)*
- *Microscale thermophoresis (MST)*
- *One semipreparative and two analytical HPLC purification systems*
- *Two NMR apparatus: 400 MHz and 500 MHz*
- *DNA sequencing (GS FLX (454) Roche, Applied Biosystems 3730 DNA Analyzers and 3730/xl DNA Analyzers, ABI 3900 synthesizer*
- *Microarray laboratory - Affymetrix GeneChip 3000 7G, two GeneChip 450 Fluidics Station oraz and GeneChip 640 Hybridization, Illumina HiScanSQ, IonTorrent PGM, IonTorrent Proton.*
- *HPC cluster with 1152 cores, enterprise level HITACH AMS storage - 1PB. GPU extension cards with Nvidia Tesla K20 and Intel Phi 5110. Classified as the 86th fastest in TOP100 world list.*

THE MEDCHEM

Virtual screening:

- Access to commercial software (Schroedinger, OpenEye).
- Open source tools and our own methodologies, software and services.
- Molecular dynamics simulations: Amber and Gromacs .
- HPC infrastructure to accommodate virtual screening and computational chemistry campaigns

Academic collaborations: DKFZ Heidelberg, DE; MD Anderson Cancer Center Houston, TX; University of Warsaw; INSERM Paris; INSERM Marseille; Freie Universität Berlin

Commercial collaborations: OncoArendi; Olimp Labs

Networks: ELIXIR; CEPT (Center for Preclinical Research); GBIF; EURO-BIOIMAGING

Training capacities: Computer laboratory with dual monitor workstations for 30 participants, HPC cluster infrastructure with dedicated training nodes.

Spin-outs: KF Nicolium Sp. z o. o.

THE OUTPUT

1. *Epigenetic reactivation of tumor suppressor genes by a novel small-molecule inhibitor of human DNA methyltransferases. Cancer Res. 2005 Jul 15;65(14):6305-11.*
2. *Odolczyk N, Fritsch J, Norez C, Servel N, da Cunha MF, Bitam S, Kupniewska A, Wiszniewski L, Colas J, Tarnowski K, Tondelier D, Roldan A, Saussereau EL, Melin-Heschel P, Wieczorek G, Lukacs GL, Dadlez M, Faure G, Herrmann H, Ollero M, Becq F, Zielenkiewicz P, Edelman A. Discovery of novel potent ΔF508-CFTR correctors that target the nucleotide binding domain. EMBO Mol Med. 2013 Oct;5(10):1484-501*
3. *Wójcikowski M, Zielenkiewicz P, Siedlecki P. Performance of machine-learning scoring functions in structure-based virtual screening, Nature Scientific Reports 2017*
4. *Fernández-Bachiller MI, Brzozowska I, Odolczyk N, Zielenkiewicz U, Zielenkiewicz P, Rademann J. Mapping Protein-Protein Interactions of the Resistance-Related Bacterial Zeta Toxin-Epsilon Antitoxin Complex ($\varepsilon_2\zeta_2$) with High Affinity Peptide Ligands Using Fluorescence Polarization. Toxins (Basel). 2016 Jul 16;8(7).*
5. *Wójcikowski M, Zielenkiewicz P, Siedlecki P. Open Drug Discovery Toolkit(ODDT): a new open-source player in the drug discovery field. J Cheminform. 2015 Jun 22;7:26.*

THE FUTURE

Plans: Application for dedicated EU/POL-OPENSCREEN grant to the Polish Ministry of Science and Higher Education; Equipment and personnel upgrade according to the Evaluation Panel comments.

Added value:

- Access to unique bio- and cheminformatics tools, along with expertise in systems biology, machine learning and data exploration.
- Strong HPC cluster infrastructure coupled with large, enterprise level storage and high performance I/O database servers.
- Experienced chemistry department capable of supporting compound development processes.
- Readily available support for additional services, including proteomics, microarrays, DNA sequencing and assay development.

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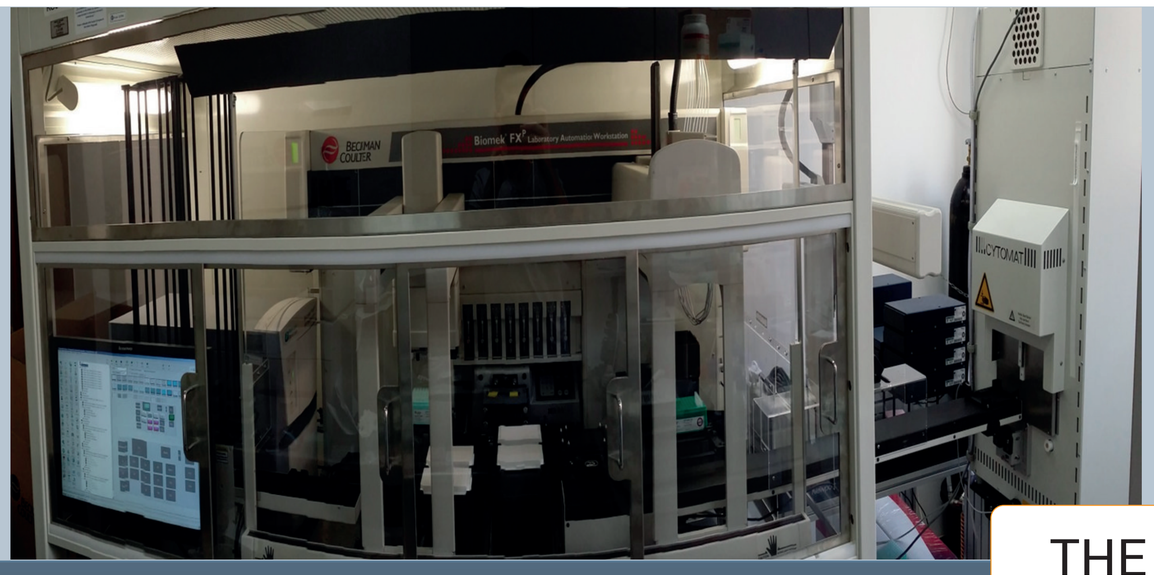
http://www.biochim.ro

România

Assay adaptation site

ECBS2017
BUDAPEST

a nominated partner site of eu openscreen



THE PEOPLE

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THE PROJECT

Identification of first in class chemical scaffolds against Hepatitis C Virus NS2 cysteine protease

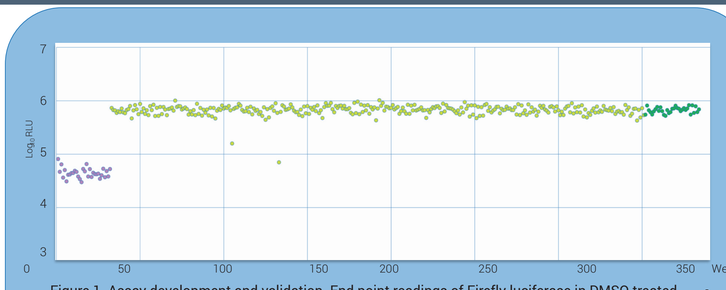

Figure 1. Assay development and validation. End point readings of Firefly luciferase in DMSO treated samples are presented: the negative control (magenta), the positive control (green), the samples (light green). The Z' for the plate is 0.56.

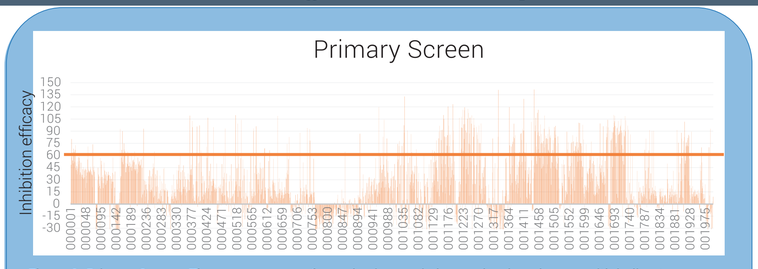

Figure 3. Primary Screen. The screen was performed using our in house developed assay with indirect detection of inhibition by luciferase. After the cells were seeded, the second day they were transfected and after 4 hours inoculated with the selected compounds, and in the third day the cells were lysed and luminescence was measured. The threshold for inhibition was set to 60%.

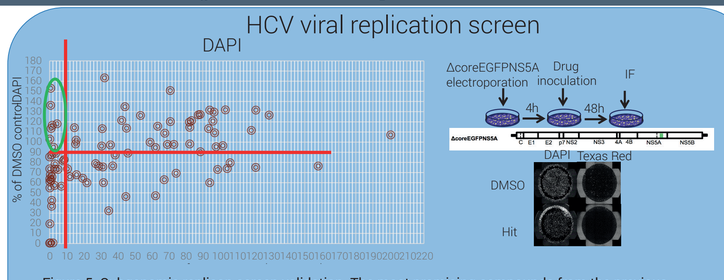

Figure 5. Subgenomic replicon screen validation. The most promising compounds from the previous two screens where selected and tested using the subgenomic replicon system by immunofluorescence.

Assay development and validation → In silico screen (9163 compounds) → Primary screen(Inhibition > 60%) → Counter Screen (Inhibition < 75%) → Viral replication screen (> 90% inhibition; >90% cell viability) → IC 50 (Dose Response Curve)

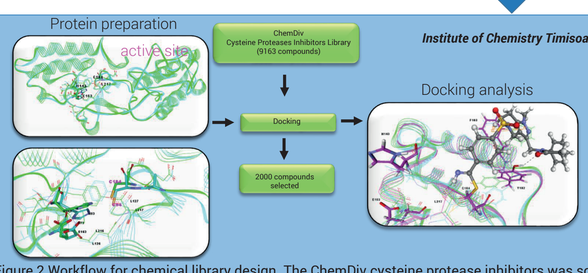

Figure 2 Workflow for chemical library design. The ChemDiv cysteine protease inhibitors was screened „in silico" using the HCV NS22a structure model. The ligand binding site superposition of NS1a (green) with NS2a (turquoise) is presented along with the deep view of the ligand biding site superposition (left). Following „in silico" screening, 2000 compounds were selected. The best docking pose of the active compound at the binding site of the NS2a protein target is presented (right).

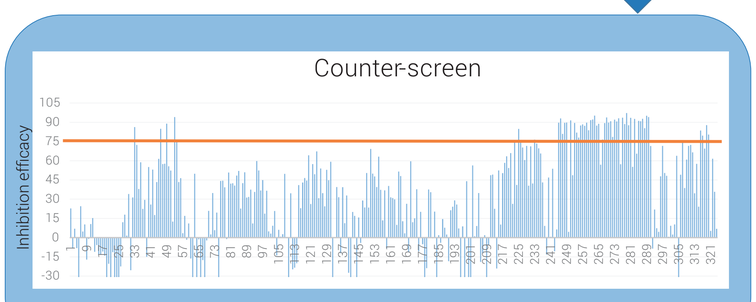

Figure 4. Counter-screen. The second test was set to detect false positives due to non-specific inhibition of luminescence signal. The threshold of this screen was set to < 75%.

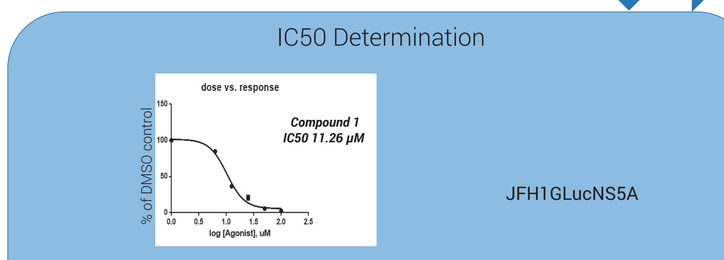

Figure 6. IC50 determination. Dose response curves were performed for selected compounds, using the full length virus JFH1GlucNS5A by luminescence. IC50 was determined by GraphPad Prism software using a normalized response with variable slope curve.

THE HARDWARE

Reagent production and assay development:
- prokaryotic and eukaryotic expression system: cell culture rooms, incubators, FPLC, preparative centrifuges - cell line generation (overexpression, shRNA downregulation, CRISPR/CAS9 knockdown): cell culture room, molecular biology room, cell sorter (BD FACS Aria III – 4 lasers) - assay development: multimodal microplate readers

Target identification and drug selectivity determination:
LC-MS platform HPLC with nanoflow 20-2000 nl/min, (Easy-nLC II) working @ max. pres. of 300 bar MS LTQ Orbitrap Velos Pro equipped with fragmentation techniques like CID, PQD, HCD and ETD (for PTM analysis) working @ max. resolution of 100000 (measured @ m/z 400) Accela HPLC with quaternary gradient solvents working @ max. pres. of 600 bar with flow 1-5000 ul/min equipped with RP-C18 columns (reversed phase) for small molecule/peptide separation and a UV-VIS DAD detector 190-800 nm + fluorescence detector (200-850 nm excitation, 250-900 nm emission) HPLC (ion cromatograph) with quaternary gradient solvents working @ max. pres. of 350 bar (5000 psi) with 1-10000 ul/min equiped with columns for monosaccharides separation coupled with a UVVIS PDA detector 190-900 nm and a ECD (Electrochemical detector) for amperometric electrochemical detection + a AFC 3000 (automated fraction collector) working @ a max. flow of 150 ml/min.

Primary screening: Integrated high throughput screening platform
Liquid handling automation workstation: BiomekFXp (Beckman Coulter) with 96 multichannel pipetting head, Span-8 and a pin-tool system. Microplate washer : ELx405 (Biotek) Cell inculator: Cytomat 48 Hotel (Thermo) Multimodal microplate reader: SpectraMax Paradigm (Molecular Devices): absorbance, luminescence, fluorescence, polarized fluorescence, BRET, FRET, TR-FRET Liquid handling automation workstation: Biomek 3000 (Beckman Coulter)

Chemical libraries:
2000 cmpds – cysteine protease inhibitor trageted library (ChemDiv)
5000 cmpds – diversity library (ChemDiv)

Targets*: anti-viral (Hepatitis C Virus), anti-cancer (melanoma, hepatocellular carcinoma), proteases, phosphatases*

THE SOFTWARE

Data Analysis Tools:
KNIME (Konstanz Information Miner), visual workflow/ data pipelining environments for statistical data analysis and data mining, thus its application is not only restricted to the fields of life science and pharmaceutical research. KNIME possesses various components for data integration, data transformation (filter, converter, combiner), machine learning, data mining and data visualization for library and databasis management.
R package for statistical computing and graphics, data analysis, including linear and nonlinear modelling (supervised classification methods), classical statistical tests, time-series analysis, classification, clustering, user-created packages, specialized statistical techniques, graphical devices, etc.
Computational multiobjective optimization (h-ERG, cytocrome P450, liver/kidney) to identify solutions which comply with multiple criteria, followed by experimental testing. Reducing the errors in lead design by decreasing false positive prediction rates in virtual screening using supervised learning classification methods and/or developing fusion schemes.

Software Tools
Schrödinger package for molecular modeling programs including a comprehensive suite of drug design software such as docking structure-based virtual screening(GLIDE Covalent Docking, and Induced Fit Docking for high-accuracy ligand docking), pharmacophore modelling, 3D ligand-based virtual screening (PHASE, 3D atom-based QSAR , homology modelling (PRIME), molecular dynamics, 2D (3D) QSAR for affinity prediction, quantum mechanics for energy minimization, conformational analysis (MacroModel), prediction of ADMETox properties. Scaffold-hopping using PHASE in case ADMETox properties are not fulfilled.
Gaussian 09 for for quantum chemical calculation including structural optimization, energy and molecular orbital calculation, molecular mechanics, semi-empirical quantum chemistry calculations, self-consistent field, Hartree–Fock, Møller–Plesset perturbation theory, density functional theory (DFT), ONIOM, complete active space, coupled cluster and quadratic configuration interaction (QCI), and quantum chemistry composite methods of high accuracy.
Similarity Search Tool in Cheminformatics (SSTICI) (2011) - 2D similarity search using a wide array of similarity coefficients (correlation, association, distance), sum-rule based fusion of multiple reference molecule scoring relative to large data bases; descriptor binary or continuous, distance or simialrity matrix is generated –available at Institute of Chemistry Timisoara of the Romanian Academy (ICT).
Evaluation Tool in ChemoInformatics (ETICI) (2011) - offers a variety of parameters indicating the performance of virtual screening methods in retrospective benchmarking experiments usually applied in the field of Chemoinformatics and Information Retrieval, early enrichment and overall discriminative power indicators –available at Institute of Chemistry Timisoara of the Romanian Academy

THE OUTPUT

Collaborations*: IBRA is coordinating the ERANET project HCVCYSPROT within the ERARUS.PLUS 2014 call in consortium with Helmholtz Institute for Infectious Research (Dr. Ursula Bilitewski), Institute of Chemistry of the Romanian Academy (Dr. Liliana Pacureanu) and ChemDiv (Dr. Sergey Bugrov).*
Network*: The national network of chemical biology RoChemBioNet was founded and it comprises 27 private and public research organisations which are potential users or collaborators of the IBRA assay adaptation site.*
*IBRA platform was confirmed as an EU-OPENSCREEN **assay adaptation site** following the external technical review.*

THE FUTURE

IBRA participation in EU-OPENSCREEN will bridge the local scientific expertise with the network by developing, validating and transferring assays to interrogate the EU-OPENSCREEN library. As described above, IBRA will be able to offer to the network target identification and connectivity analysis, reagent production and assay development, library selection, in silico screening, primary screening, chemoinformatic assistance in hit to lead optimisation and drug selectivity determination.

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Institute of Biochemistry