8th Congress of Pharmacists of Serbia
October 15th, 2022
Belgrade, Crown Plaza

Neuroimmune aspects of mood, anxiety and cognitive effects of leads/drug candidates acting at $GABA_A$ and/or sigma-2 receptors: *In vitro/in vivo* delineation by nano- and hiPSC-based platforms

Program IDEAS - Science Fund of the Republic of Serbia

- **Project acronym:** *NanoCellEmoCog*
- **Sub-program**: (Bio)medical sciences
- **Participating Scientific and Research Organization:** *University of Belgrade – Faculty of Pharmacy (FPUB)*
- **Principal investigator (PI):** Miroslav Savić
- **14 participants with different research backgrounds**

Background

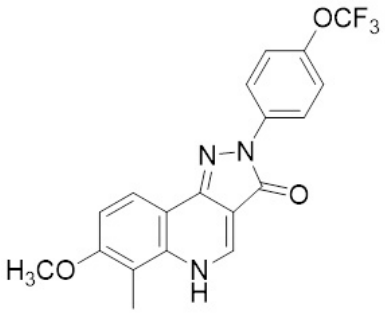
- Significant alterations in **mood, anxiety, and cognitive symptom** domains are common findings in neurodegenerative and psychiatric disorders.
- **Pharmacotherapeutic options** for such symptoms are valuable but **limited,** and often rely on off-label use of psychotropic drugs, which is additionally hampered by their safety issues.
- **Discovery** of novel psychotropic drugs meets many **difficulties**, related to: selection of an appropriate drug target, drug delivery to the site of action, disease modeling, and **gaps in *in vitro* to *in vivo* extrapolation.**
- Disbalance in **neuroimmune-mediated crosstalk** between neurons, microglia and astrocytes, observed in mood, anxiety, and cognitive disorders, reflects a common theme that is still insufficiently exploited in drug discovery.
- General focus on modulation of **cell membrane targets** (such are **$GABA_A$ receptors**), rather than specific interaction with **intracellular signaling**, although showed to be successful, has begun to plateau in recent years.
- The **σ2 receptor,** recently evolved 'from obscure binding site to bona fide therapeutic target' (Adv Exp Med Biol 2017;964:49–61), is one of rare **intracellular targets** implicated in the whole range of manifestations of CNS disorders, from anxiety and depression to cognitive impairment seen in Alzheimer's disease (eNeuro 2020;7(6)), with evidence of its **immunomodulatory roles** (Front Pharmacol 2013;4:23).

Background

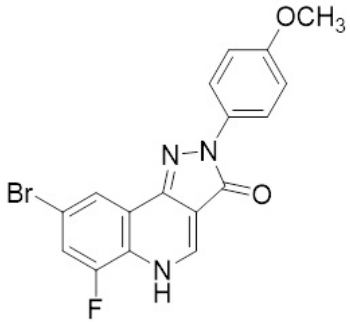
- Analyzing PDSP affinity data of numerous pyrazoloquinolinone (PQ) and benzodiazepine (BZ) ligands* revealed that two PQ ligands (MM-I-03 and CW-02-079) have potent and selective affinity to σ2 receptors, with no (CW-02-079) or mild (MM-I-03) binding and activity via GABAA receptors.

- Two other GABAA ligands, DK-I-56-1 (a PQ; J Med Chem 2018;61:2422-46) and GL-II-73 (a BZ; Mol Neuropsychiatry 2019;5:84-97) are drug candidates notable for their selective positive modulation of GABAA receptors that contain the α6 and α5 subunit, respectively, and are aimed to be tested in parallel to our novel (MM-I-03 and CW-02-079), and experimental reference (siramesine and RHM-1) σ2 ligands.

- Both chemotypes, BZ and PQ, are known for their tolerability and safety, if sedation and cognitive impairment are precluded, which is the case with selected ligands (lack of substantial activation of α1GABAA receptors).



CW-02-079 (14d)

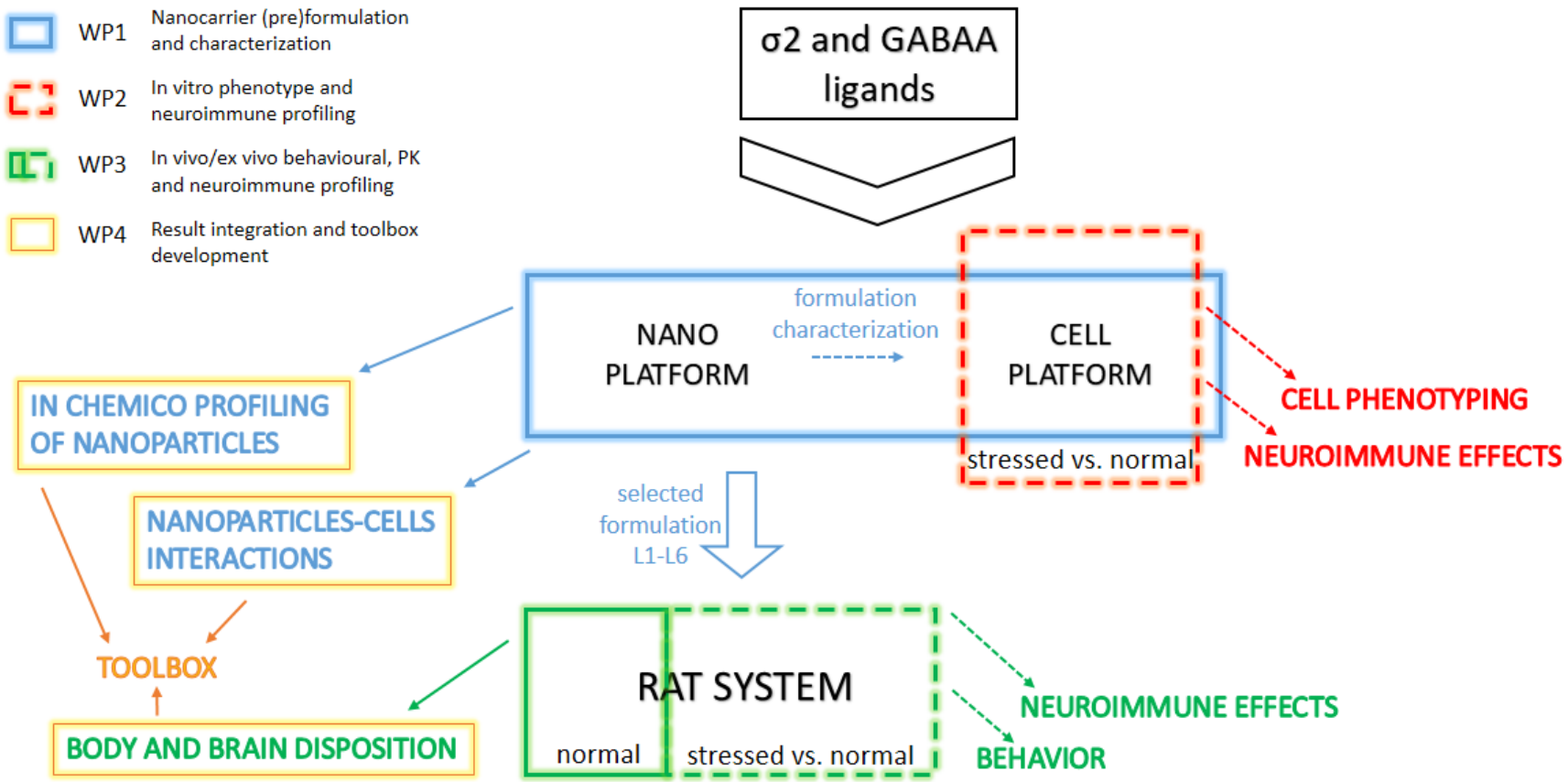


MM-I-03 (14f)

** Patently protected and co-owned by the consortium involving Faculty of Pharmacy*

Project goal

The main project goal is to test the hypothesis that the **selected BZ and PQ ligands may substantially improve neuroimmune and/or behavioral outputs assessed in *in vitro* and *in vivo* systems** made to mimic a compromised neuroimmune status. Affirmative results obtained with any of four novel ligands would give rise to filing the use patent as a prerequisite for their drug development for treatment of **anxiety, mood and cognition symptom domains**



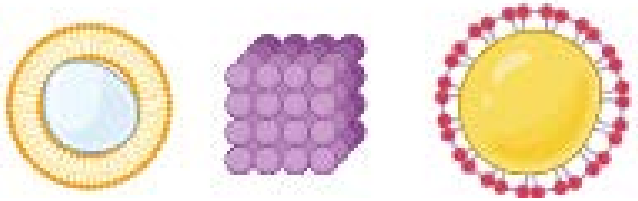
Project realization: WP1 – NANO-PLATFORMS

L/DC-tailored nano-platform design: in chemico/in vitro/in vivo characterization for GABAA and/or sigma 2 receptors targeting

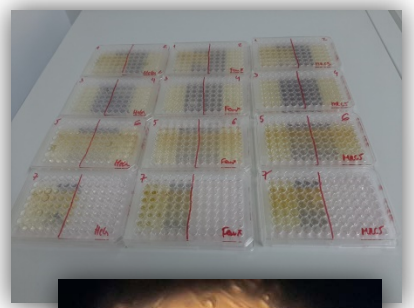
Preformulation screening

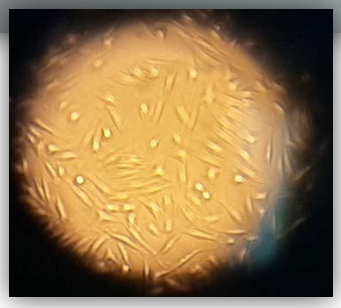
Formulation design, physicochemical and stability testing

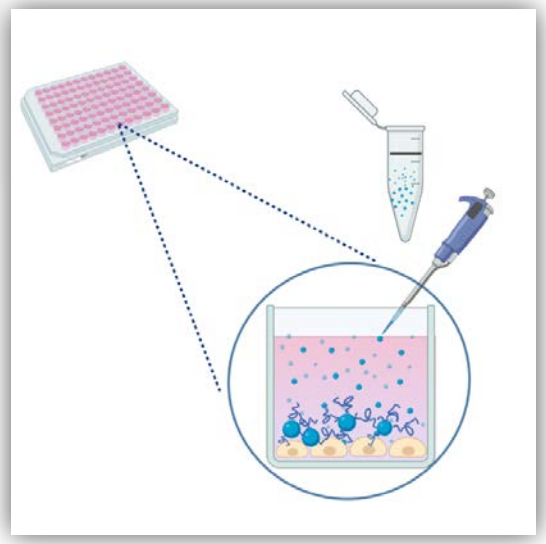
NP-cell interaction assessment and development of custom bioanalytical method for *in vivo* PK and biodistribution studies in rats



liposomes, nanocrystals, nanoemulsions







Project realization: WP1 – NANO-PLATFORMS

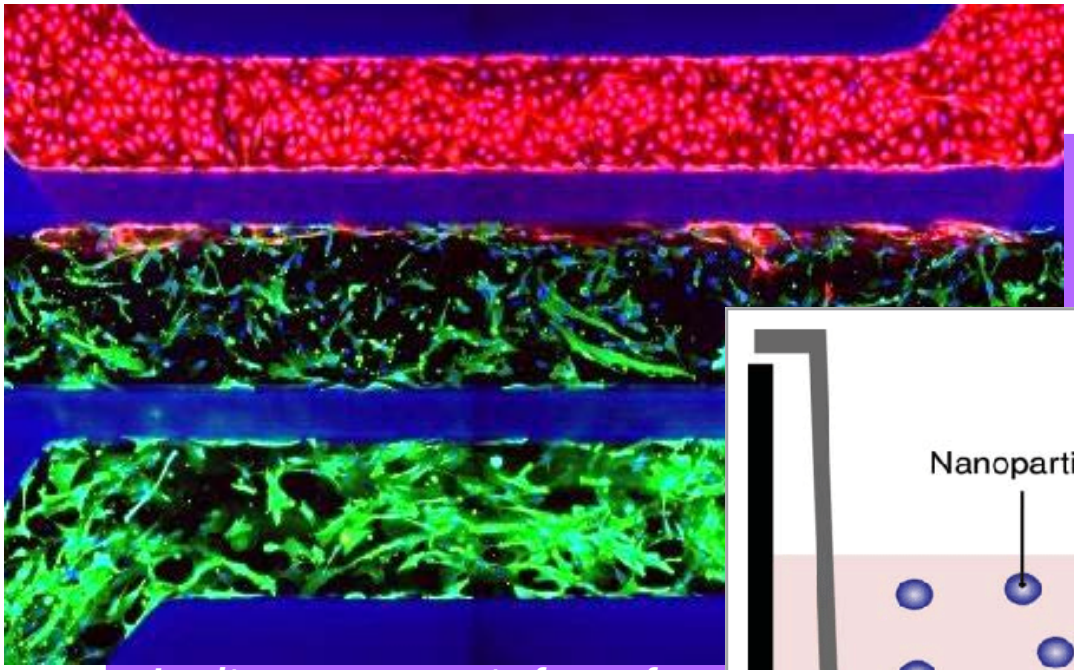


Figure. Fluorescent image showing a BBB culture of endothelial cell
Adapted from MIMETAS Application note "BBB-on-a-chip"

In vitro assessment of nanoform
interactions applying hiPSC-deri

cytotoxicity, cellular uptake, BBB-

Evaluation of in vivo PK and bio

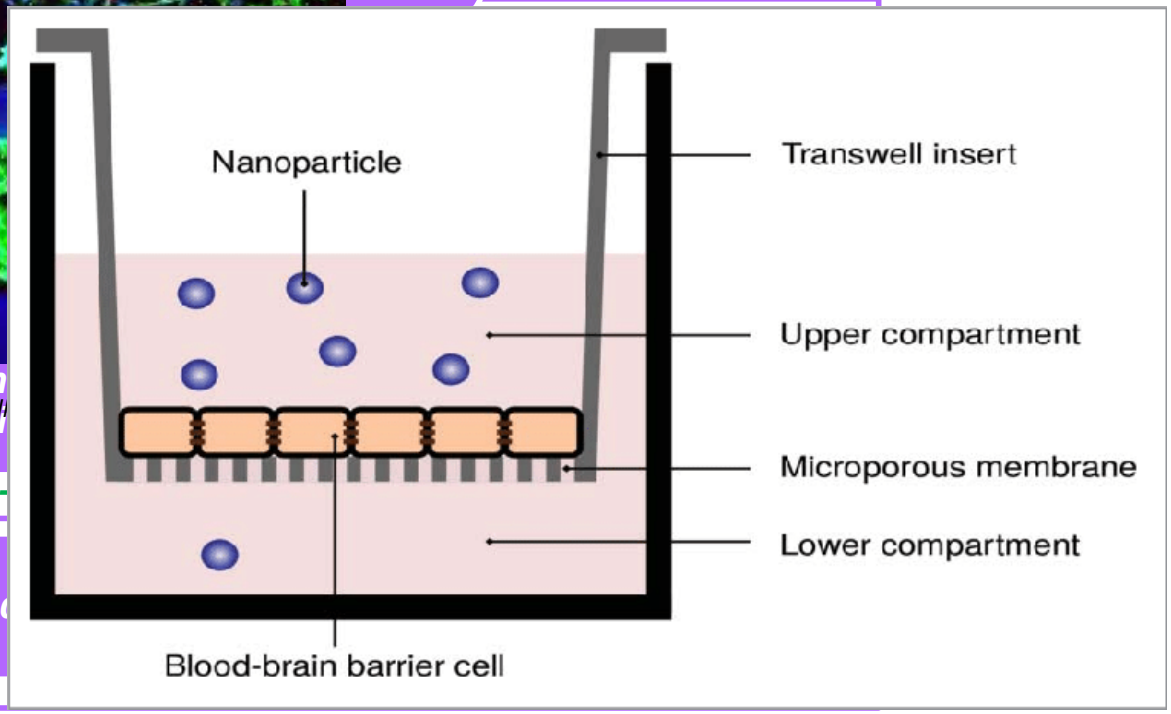


Figure. Transwell system for NP-BBB interaction analysys.,Adapted from Aberg C, 2016. Tissue Barriers

Project realization: WP2 – CELL PLATFORMS

In vitro testing of the potential effect of L/DC on neuroinflammation using human induced pluripotent stem cell (hiPSC)-based tri-culture cell neuroinflammation model

L/DCs cytotoxicity and their effects on morphological phenotype, neurite morphology and synapse formation

Determination of neurotransmitter, kynurenine pathway metabolites, cytokines and chemokines

- A novel *in vitro* **neural tri-culture model** consisting of hiPSC glutamatergic neurons, astrocytes and microglia after LPS/IFN-γ treatment in order to test the potential neuroprotective/antiinflammatory effect of the tested L/DC.

- This model may **reliably mimic neuroinflammatory condition** that accompanies pathological states of the CNS *in vivo,* by providing the microenvironment, the cellular crosstalk and the molecular events that take place during neuroinflammation

Project realization: WP2 – CELL PLATFORMS

Methods that will be used to achieve the goals of this part of the project:

- Potential ***in vitro* cytotoxic effect** of selected ligands on cell viability will be done using the **MTT assay.**
- Potential **antioxidative effect** will be evaluated by determing NO concentration in cell culture supernatants using the **Griess method.**
- **Glial morphology and phenotypic characteristics**, **neurite morphology and synapse formation** will be assessed according to the expression of their specific markers using **immunocytochemical staining** and **fluorescence microscopy.**
- Concentrations of different molecules (cytokines, neurotransmiters, chemokines) enabling **neuron-glia crosstalk in neuroinflammation** in the cell culture supernatant will be done using **multiplex technology** and **ELISA.**

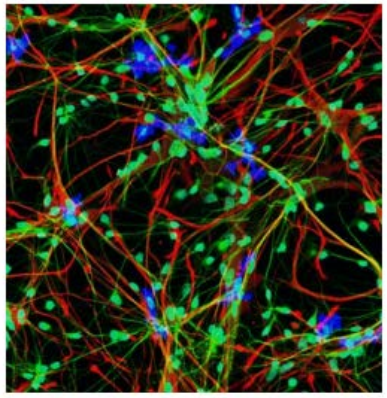


Figure. Imaging a humane iPCS-derived "brain-in-a-dish" – adapted from Application protocol iCell® Microglia Fujifilm Cellular Dynamics

Project realization: WP3 – RAT SYSTEM

The consequences of chronic stress and potential treatment: Mood, anxiety and cognitive effects and neuroimmune status after L/DCs treatment in stressed rats

- ***In vivo*** prolonged exposure of rats to **immunological challenge** (LPS) and **chronic unpredictable mild stress** (CUMS)
- **Pharmacokinetic properties** of L/DCs in Sprague-Dawley rats who underwent LPS/CUMS

PK and PD studies in healthy animals ✓

LPS-CUMS model development — **In progress**

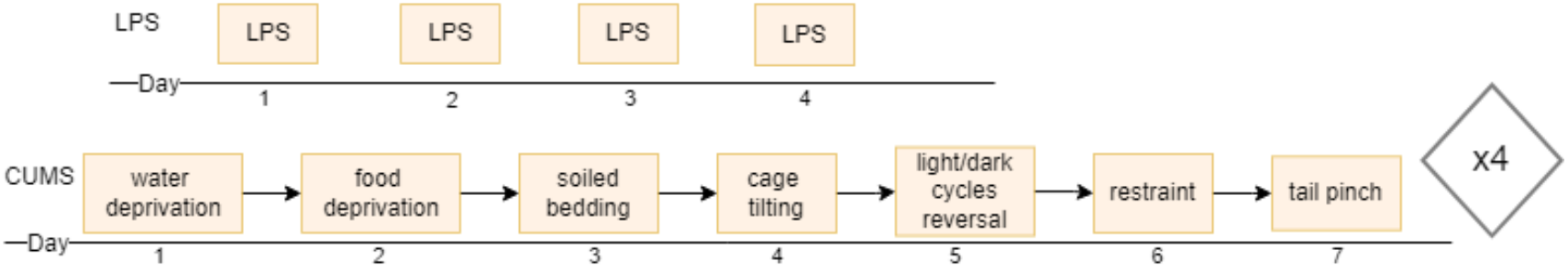
Protracted and acute behavioral effects of L/DCs in LPS-CUMS model — **In progress**

Neuroimmune profiling of L/DCs in LPS-CUMS model (CSF and brain) — **To be done**

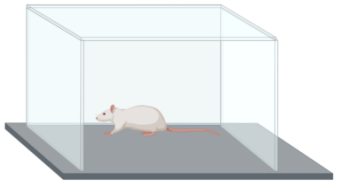
Figure. Sequeira-Cordero A et al. Behavioural characterisation of chronic unpredictable stress based on ethologically relevant paradigms in rats. Sci Rep. 2019.

Project realization: WP3 – RAT SYSTEM

Assessment of potential L/DCs treatment effects in stressed rats



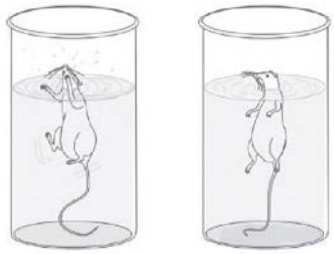
Anhedonia



Anxiety

Anxiety

Memory



Despair

- **Sex differences**
- **Acute vs. protracted** (14 days) i.p. treatment effects on rat behavior
- **Behavioral battery** (sucrose preference test (SPT), open field (OF), elevated plus maze (EPM), two-choice pairwise/visual discrimination paradigm in Bussey-Saksida touch screen operant chambers (BSTS) and forced swim test (FST))

Project realization: WP4 – Data management and toolbox creation

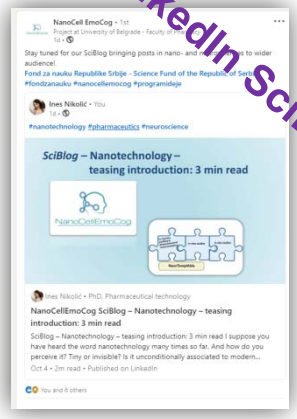
Data usage coordination, **intellectual property rights**, reporting

Communication and dissemination activities for **public outreach**

Preclinical tool development for **selection of optimal L/DCs formulations for *in vivo* studies**

PATENT

LinkedIn SciBlog



open Lab day

Project website:

https://nanocellemocog.github.io/NanoCellEmoCog/index.html

Selected results…

Wiley Online Library

BCPT Basic & Clinical Pharmacology & Toxicology

ORIGINAL ARTICLE

Behavioural interaction of pyrazoloquinolinone positive allosteric modulators at α6GABA$_A$ receptors and diazepam in rats: anti-diazepam-induced ataxia action as a structu… dependent feature

Branka Divović Matović, Dan Knutson, Jelena Mitrović, Vladimir Stevanović, Boban Stan… Snežana Savić, James M. Cook, Miroslav M. Savić

First published: 30 September 2022 | https://doi.org/10.1111/bcpt.13801

pharmaceutics MDPI

Article

The Impact of the Oil Phase Selection on Physicochemical Properties, Long-Term Stability, In Vitro Performance and Injectability of Curcumin-Loaded PEGylated Nanoemulsions

Jelena B. Đoković [1], Sotiria Demisli [2,3], Sanela M. Savić [4], Bojan D. Marković [5], Nebojša D. Cekić [4,6], Danijela V. Randjelovic [7], Jelena R. Mitrović [1], Dominique Jasmin Lunter [8], Vassiliki Papadimitriou [2], Aristotelis Xenakis [2] and Snežana D. Savić [1,*]

Physicochemical/structural investigation of lipid nanoparticles with high lecithin amounts loaded with patent protected pyrazoloquinolinone ligand DK-I-60-3

Jelena Mitrović[1]; Miloš Petković[2]; Danijela Randjelović[3]; Jelena Đoković[1]; Daniel Knutson[4]; James Cook[4]; Vladimir Savić[2]; Miroslav Savić[5]; Snežana Savić[1]

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Article | Published: 14 June 2022

Symptomatic and neurotrophic effects of GABAA receptor positive allosteric modulation in a m… model of chronic stress

Ashley Bernardo, Philip Lee, Michael Marcotte, Md Yeunus Mian, Sepideh Rezvanian, D…
Aleksandra Kovačević, Miroslav M. Savić, James M. Cook, Etienne Sibille & Thomas …

Neuropsychopharmacology **47**, 1608–1619 (2022) | Cite this article

FREEZE-DRIED NANOCRYSTAL DISPERSION OF NOVEL DEUTERATED PYRAZOLOQUINOLINONE LIGAND (DK-I-56-1): PROCESS PARAMETERS AND CRYOPROTECTANT SELECTION THROUGH STABILITY STUDY

Jelena Mitrović[1], Maja Bjelošević[2], Daniel E. Knutson[3], Aleksandar Kremenović[4], Dominique Lunter[5], Pegi Ahlin Grabnar[2], James M. Cook[3], Miroslav M. Savić[6], Snežana D. Savić[1]

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A proposal of innovative injectability assessment method for intravenous formulations – case study on PEGylated nanoemulsions

Jelena Đoković[1], Sanela Savić[2], Nebojša Cekić[2,3], Snežana Savić[1]

[1] Department of Pharmaceutical Technology and Cosmetology, Faculty of pharmacy, University of Belgrade, Serbia
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[3] Department of Pharmaceutical Technology and Cosmetology, Faculty of Technology, University of Niš, Serbia

Science Fund of the Republic of Serbia

NanoCellEmoCog

Partners and collaborators

Signed collaboration commitments of 4 renowned research groups in:

- In-kind contribution of key L/DCs + patent expertise
- In-kind contribution of reference sigma-2 ligands
- Availability of key research equipment for WP1

Team members

Фонд за науку
Републике Србије

Miroslav Savić – PI and WP3 leader
Snežana Savić – WP1 leader
Biljana Bufan – WP2 leader
Ivana Pantelić – WP 4 leader
Ivan Jančić
Branka Divović Matović
Aleksandra Kovačević
Jovana Aranđelović
Tanja Ilić
Ines Nikolić
Danijela Milenković
Jelena Mitrović
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