ЕВРОПЕЙСКИ СЪЮЗ
ЕВРОПЕЙСКИ ФОНД ЗА РЕГИОНАЛНО РАЗВИТИЕ

ОПЕРАТИВНА ПРОГРАМА НАУКА И ОБРАЗОВАНИЕ ЗА ИНТЕЛИГЕНТЕН РАСТЕЖ


ФАРМАЦЕВТИЧЕН ФАКУЛТЕТ
МЕДИЦИНСКИ УНИВЕРСИТЕТ - СОФИЯ
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# Acetylcholinesterase Inhibitors Designed in the Drug Design and Bioinformatics Lab at the Medical University of Sofia

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Drug Design and Bioinformatics Lab
Faculty of Pharmacy, Medical University of Sofia

ИКТ



*Проект BG05M2OP001-1.001-0003 „Център за върхови постижения по Информатика и информационни и комуникационни технологии“, финансиран от Оперативна програма „Наука и образование за интелигентен растеж“, съфинансирана от Европейския съюз чрез Европейските структурни и инвестиционни фондове.*

# Alzheimer's Disease

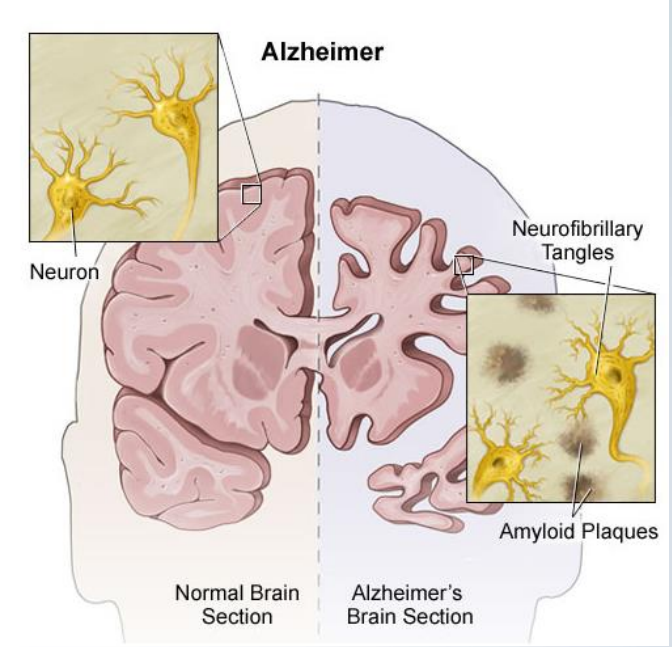


Neurodegenerative disorder, characterized with progressive and irreversible loss of neurons

Pathological hallmarks:

- extracellular β-amyloid (Aβ) plaques

- intracellular neurofibrillary tangles (NFTs) of hyperphosphorylated τ protein



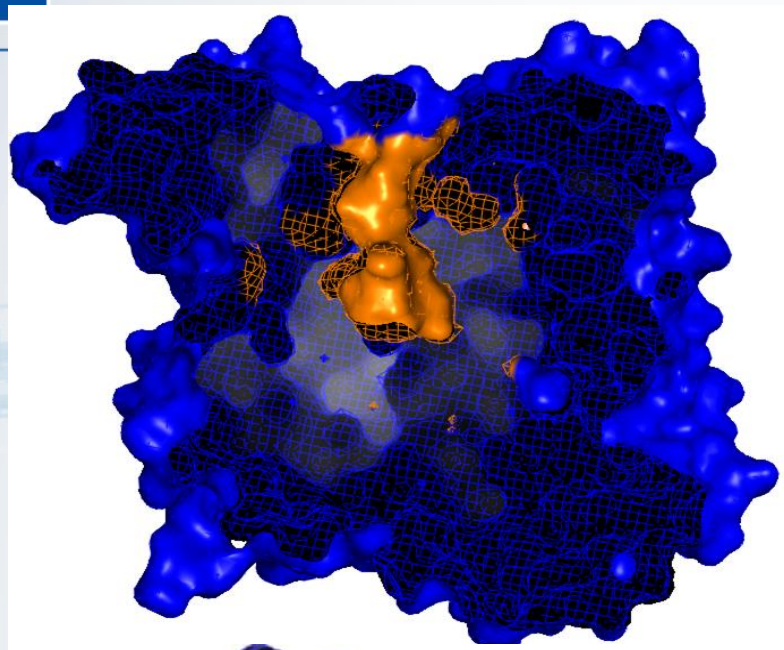

This leads to:

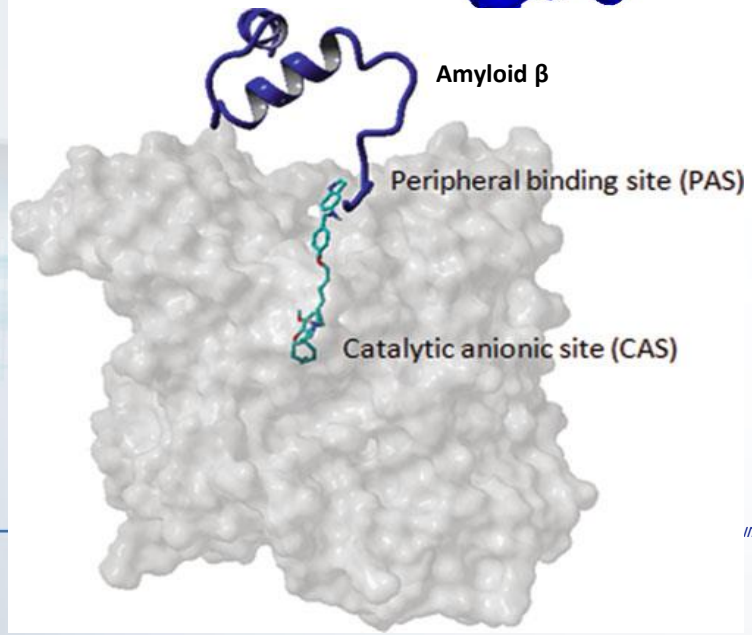
- short-term memory loss

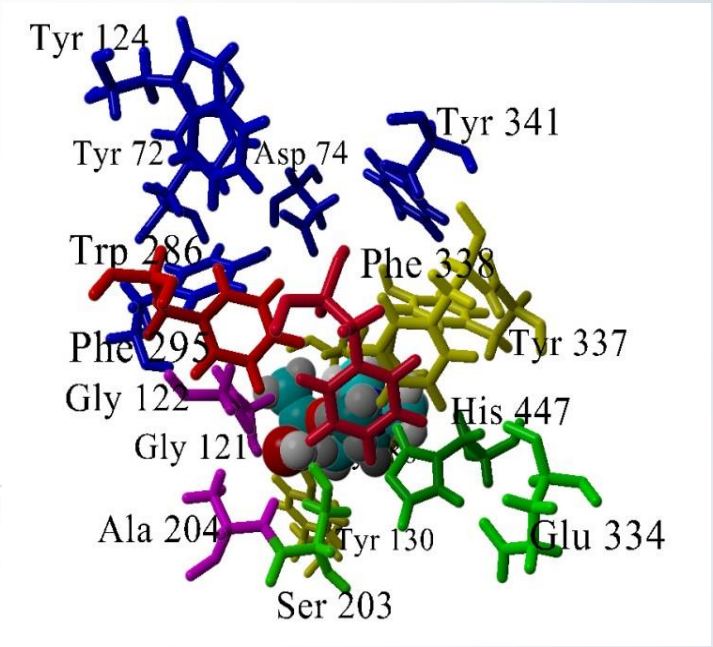
- impairment in cognitive function as thinking, speaking, learning, orientation, judgment, communication etc.

# Acetylcholinesterase (AChE)








**Catalytic site - in green**

**Anionic site – in yellow**

**Acyl pocket – in red**

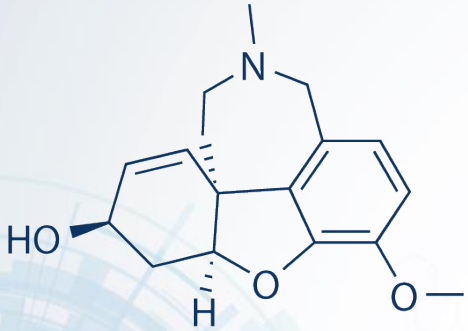
**Oxyanion hole – in magenta**

**Peripheral anionic site (PAS) – in dark blue**

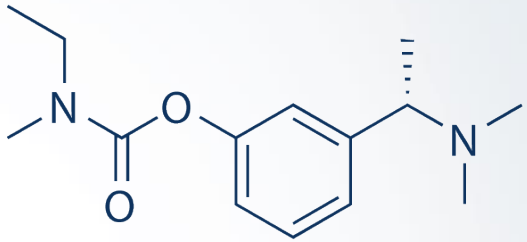
**ACh – in element color balls**

# AChE Inhibitors (AChEIs)

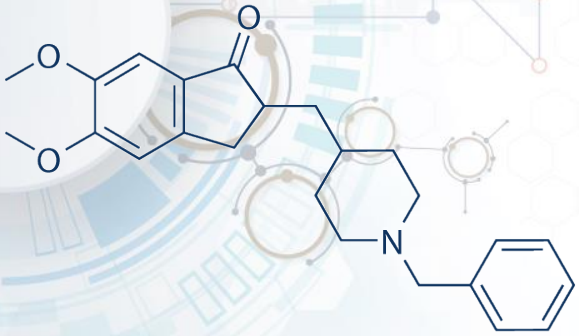




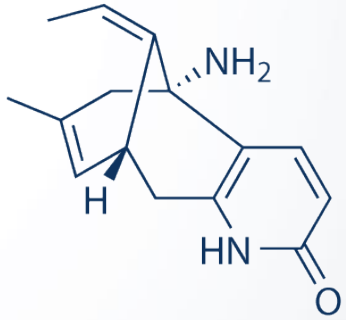

Galantamine - alkaloid from *Galanthus and Lycoris sp.*




Rivastigmine - designed from the lead compound of natural AChEI alkaloid, physostigmine




Donepezil – synthetic drug

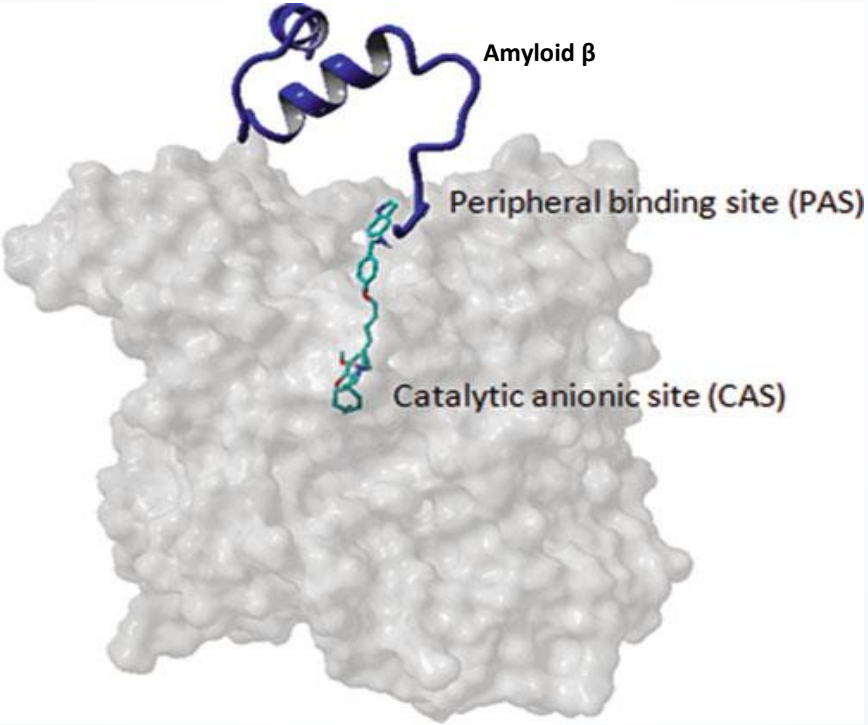



Huperizin A - natural compound from Chinese plant Huperzia serrate; drug in China, dietary supplement in USA

# Aim

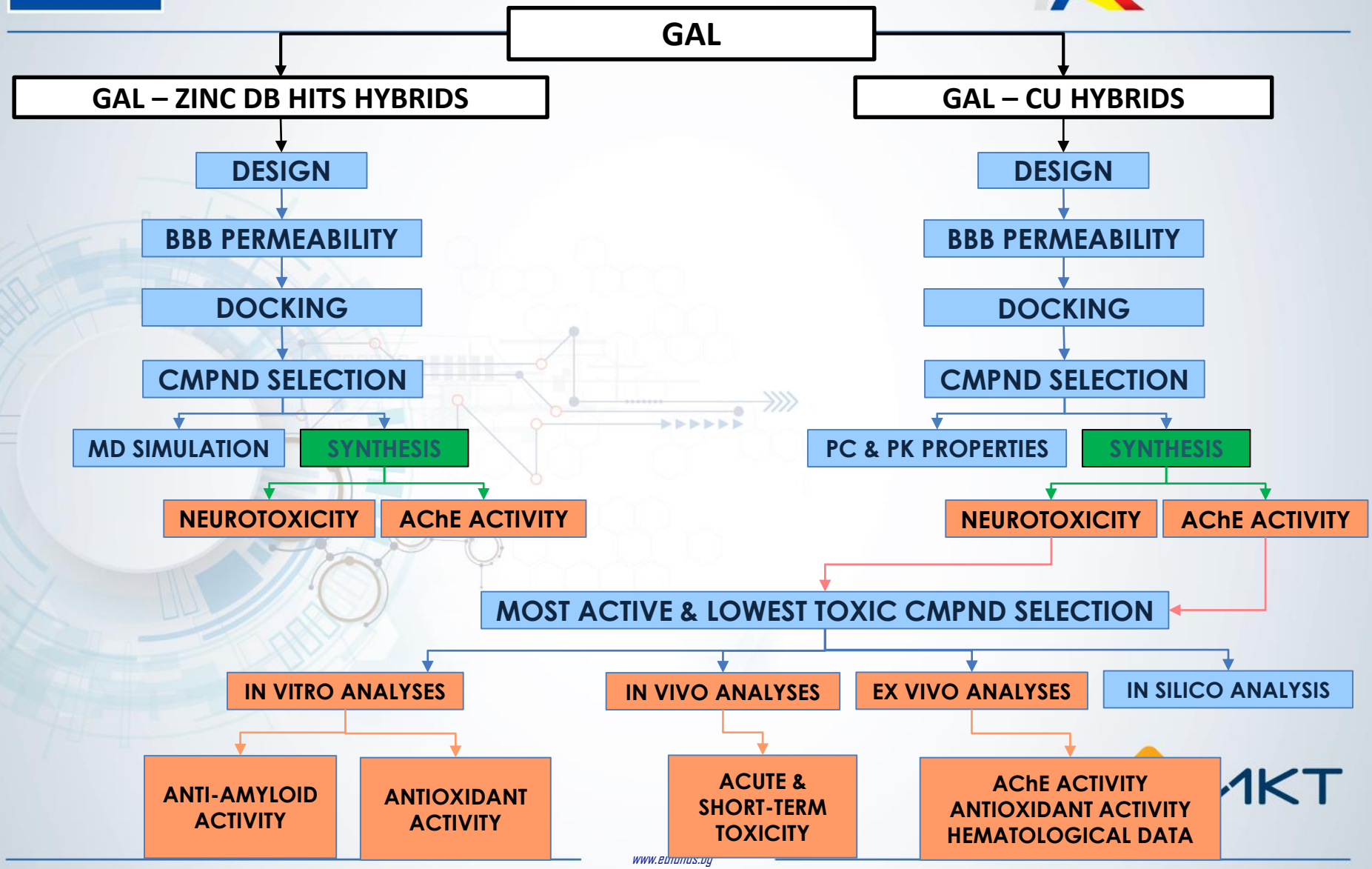


**To discover new dual-site binding AChEIs as multi-target agents**



# Workflow

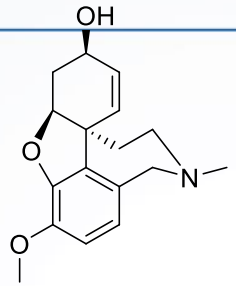



GAL
GAL – ZINC DB HITS HYBRIDS
GAL – CU HYBRIDS
DESIGN
BBB PERMEABILITY
DOCKING
CMPND SELECTION
MD SIMULATION
SYNTHESIS
PC & PK PROPERTIES
SYNTHESIS
NEUROTOXICITY
AChE ACTIVITY
NEUROTOXICITY
AChE ACTIVITY
MOST ACTIVE & LOWEST TOXIC CMPND SELECTION
IN VITRO ANALYSES
IN VIVO ANALYSES
EX VIVO ANALYSES
IN SILICO ANALYSIS
ANTI-AMYLOID ACTIVITY
ANTIOXIDANT ACTIVITY
ACUTE & SHORT-TERM TOXICITY
AChE ACTIVITY
ANTIOXIDANT ACTIVITY
HEMATOLOGICAL DATA

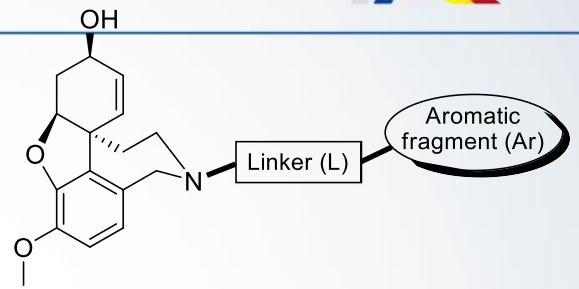
# GAL – ZINC Hits Hybrids



Fragment-based Combinatorial library

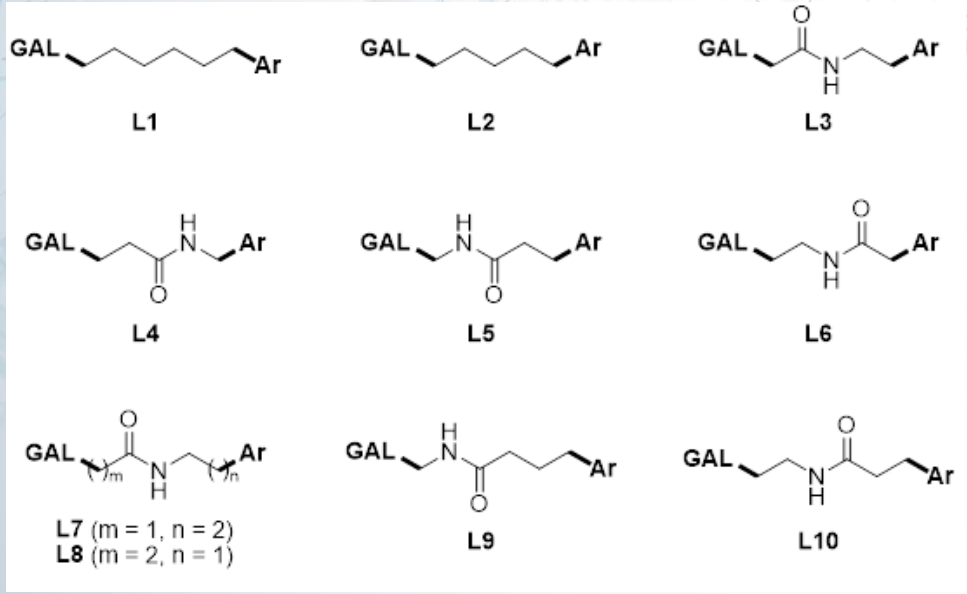



**Galantamine (GAL)**
core structure

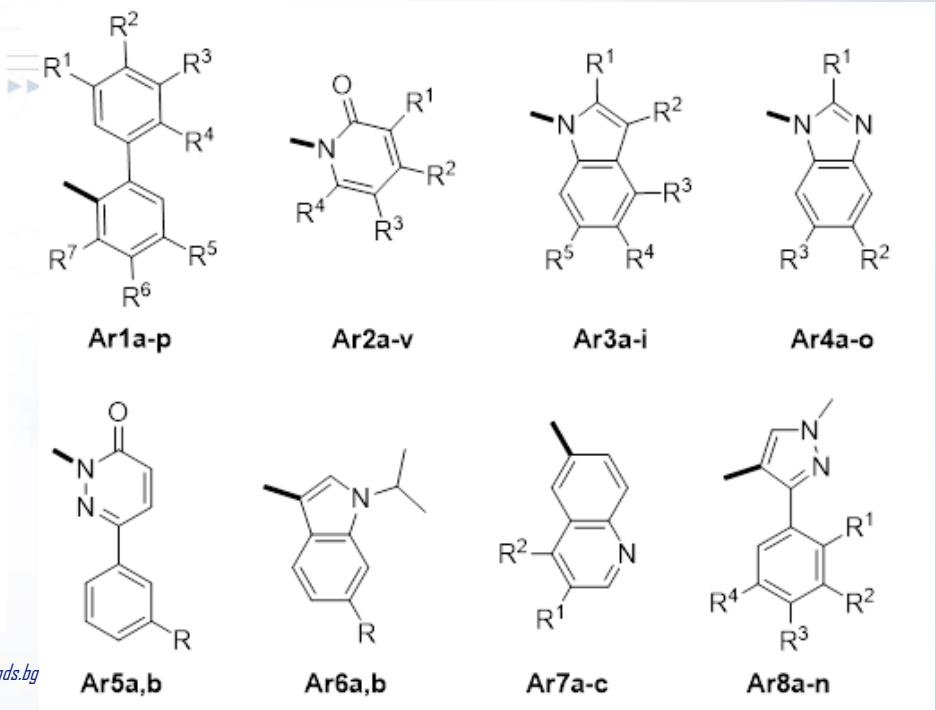



**GAL-L-Ar**
designed structures

## Linkers




## Ar fragments






*Stavrakov et al, Molecules, 2021;26:2058*

# GAL – ZINC Hits Hybrids



**GAL – ZINC DB HITS HYBRIDS**

**DESIGN**

**18** Likers + **180** Ar fragments

**1220** designed compounds

**BBB PERMEABILITY**

**Step 1:** 1 filter via SwissADME - **381** passed it

http://www.swissadme.ch

**Step 2:** 8 filters via BBB Predictor – **199** passed them

https://www.cbligand.org/BBB/predictor.php

**DOCKING**

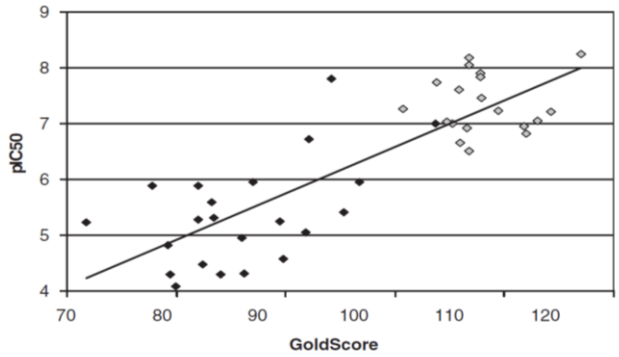
Previously optimized and validated protocol –

*Atanasova et al. Mol Inf, 2015;34:394-403.*
*Atanasova et al. Bioorg Med Chem, 2015;23:5382-5389.*

**CMPND SELECTION**

**MD SIMULATION** | **SYNTHESIS**

**NEUROTOXICITY** | **AChE ACTIVITY**




**41** Gal derivatives

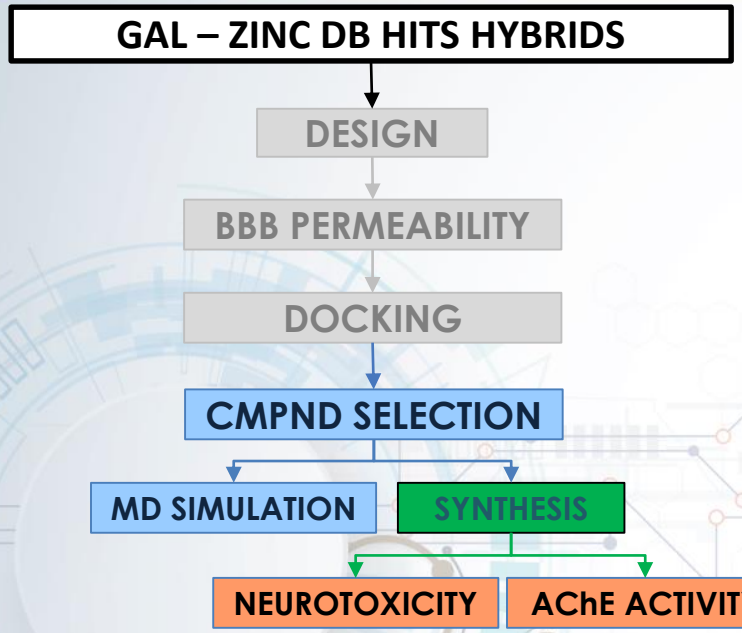
r = 0.826

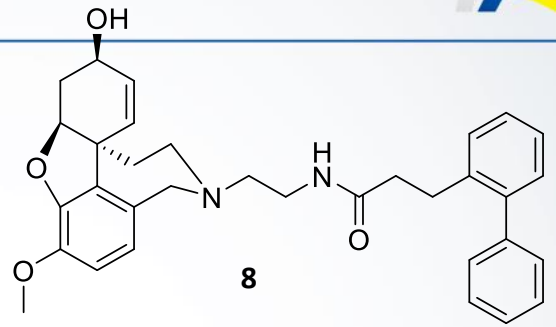


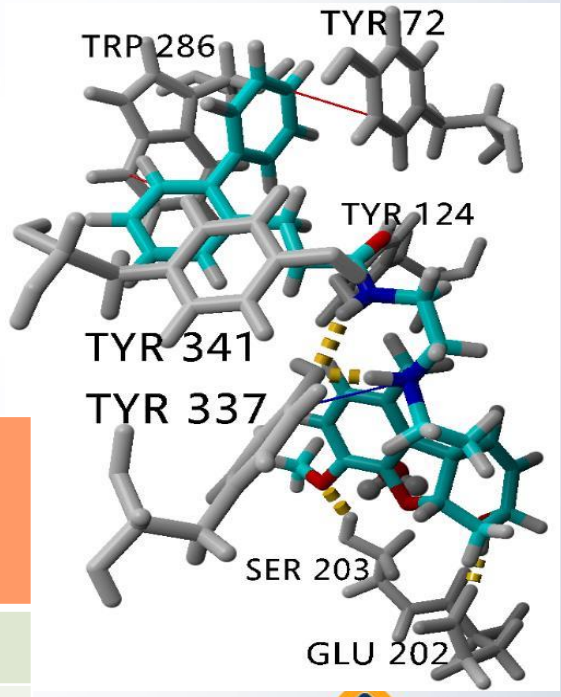
*Stavrakov et al, Molecules, 2021;26:2058*

# GAL – ZINC Hits Hybrids





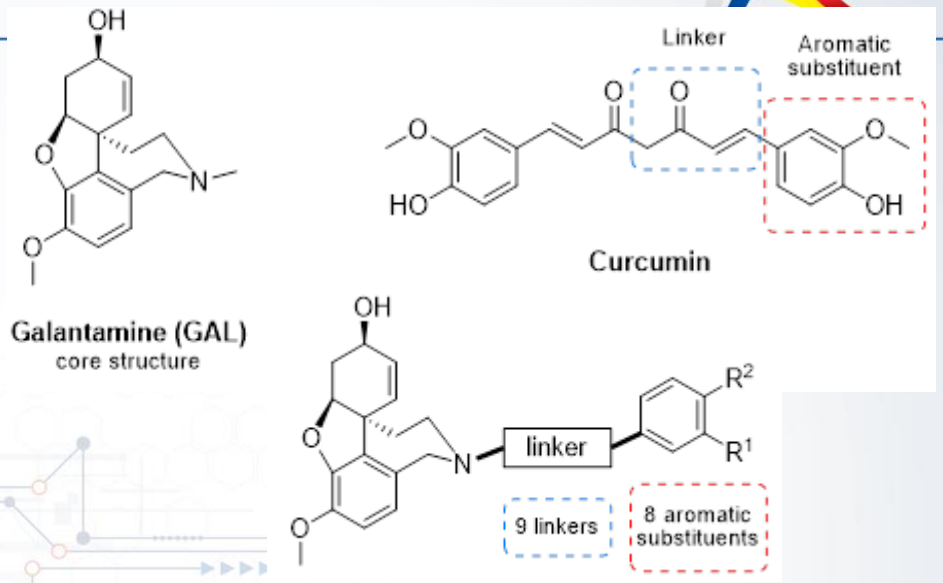

| Compound | Docking Score | $IC_{50}$, µm | Times more active than GAL | $IC_{50}$, µM Neuro 2A |
|---|---|---|---|---|
| **8** | 105.56 | 0.028 | 68 | >100 |
| **GAL** | 73.59 | 1.070 | 1 | >50 |

Hydrogen bonds - yellow dashes
π-π interactions - red lines
Cation-π interactions - blue lines

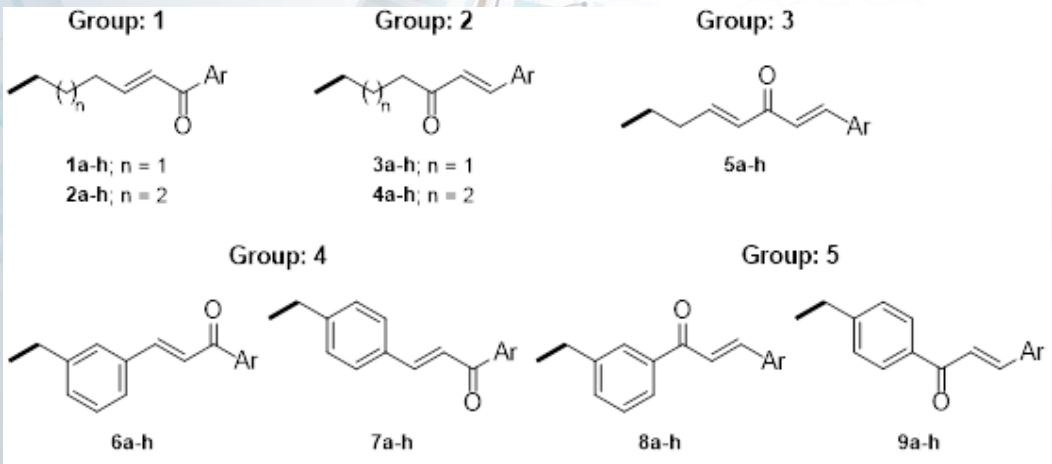
# GAL – CU Hybrids



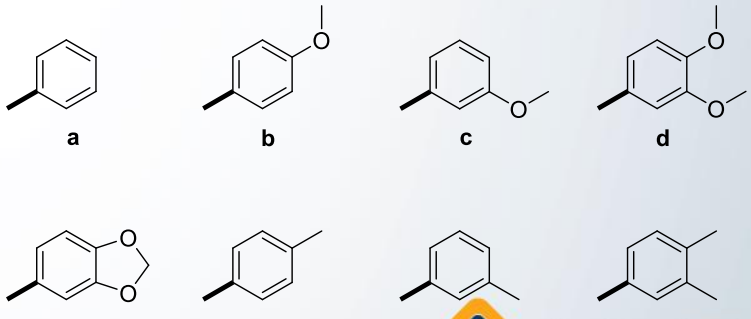
Fragment-based
Combinatorial library




Linkers




Ar fragments



# GAL – CU Hybrids



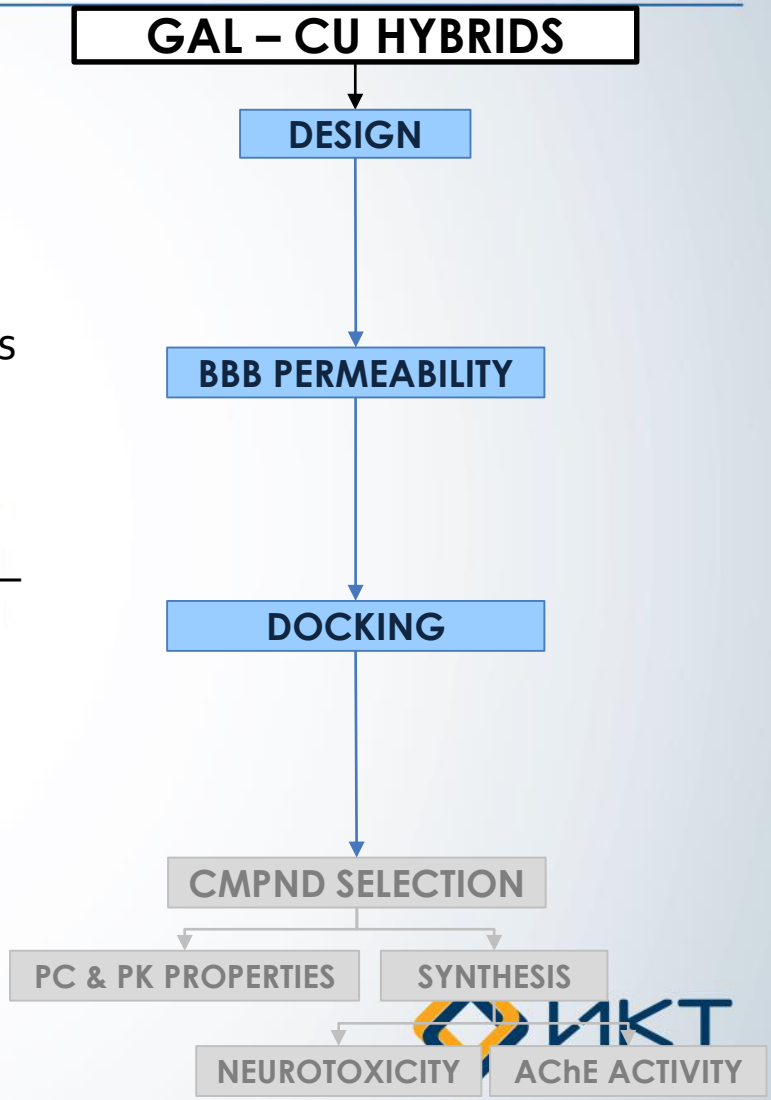
**9** Likers + **8** Ar fragments

**72** designed compounds

**11 filters**: 9 filters for BBB permeability
GIT permeability + PAINS filters

**44** compounds passed all tests

Previously optimized and validated protocol –
*Atanasova et al. Mol Inf, 2015;34:394-403.*
*Atanasova et al. Bioorg Med Chem, 2015;23:5382-5389.*

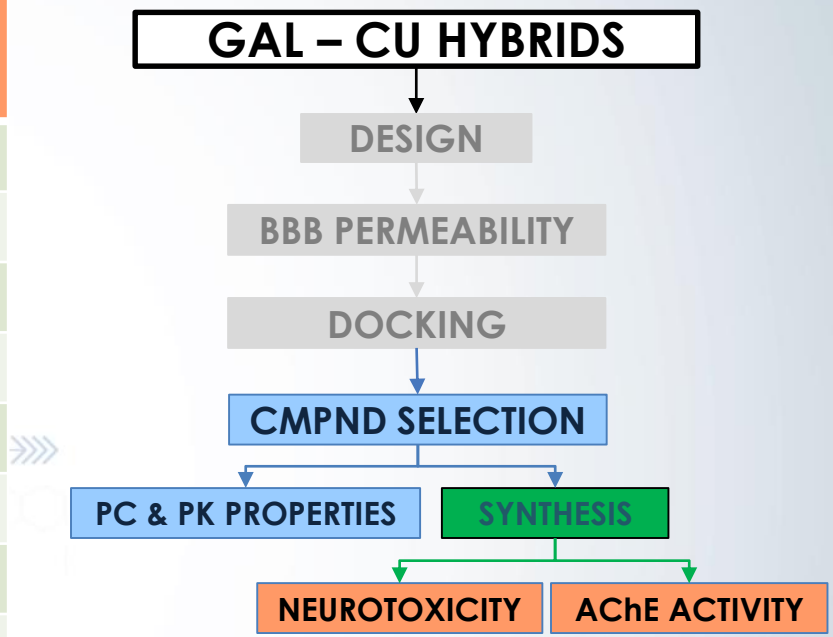


ЕВРОПЕЙСКИ СЪЮЗ
ЕВРОПЕЙСКИ ФОНД ЗА РЕГИОНАЛНО РАЗВИТИЕ

# GAL – CU Hybrids

ОПЕРАТИВНА ПРОГРАМА НАУКА И ОБРАЗОВАНИЕ ЗА ИНТЕЛИГЕНТЕН РАСТЕЖ

| ID | Docking Score | $IC_{50}$, μM Neuro 2A | $IC_{50}$, μM eeAChE | Times more active than Gal |
|---|---|---|---|---|
| 8c | 114.54 | 25.55 | - | - |
| 6a | 114.50 | 12.14 | - | - |
| 8g | 111.92 | 17.80 | - | - |
| 6b | 111.15 | 7.91 | - | - |
| 8f | 109.22 | 24.32 | - | - |
| 8h | 108.95 | 25.00 | - | - |
| **8b** | **104.01** | **28.87** | **0.086** | **41** |
| 4h | 96.98 | 21.93 | - | - |
| **4e** | **96.07** | **42.91** | **0.036** | **98** |
| **4f** | **95.05** | **34.35** | **0.033** | **110** |
| **4b** | **94.31** | **52.53** | **0.020** | **186** |
| **4a** | **93.74** | **30.65** | **0.046** | **75** |
| 4g | 90.71 | 23.68 | - | - |
| 4c | 88.28 | 24.11 | - | - |
| GAL | 74.56[a] | >50[a] | 3.520 | 1 |
| CU | 88.93 | 26.30 | 67.69[b] | |



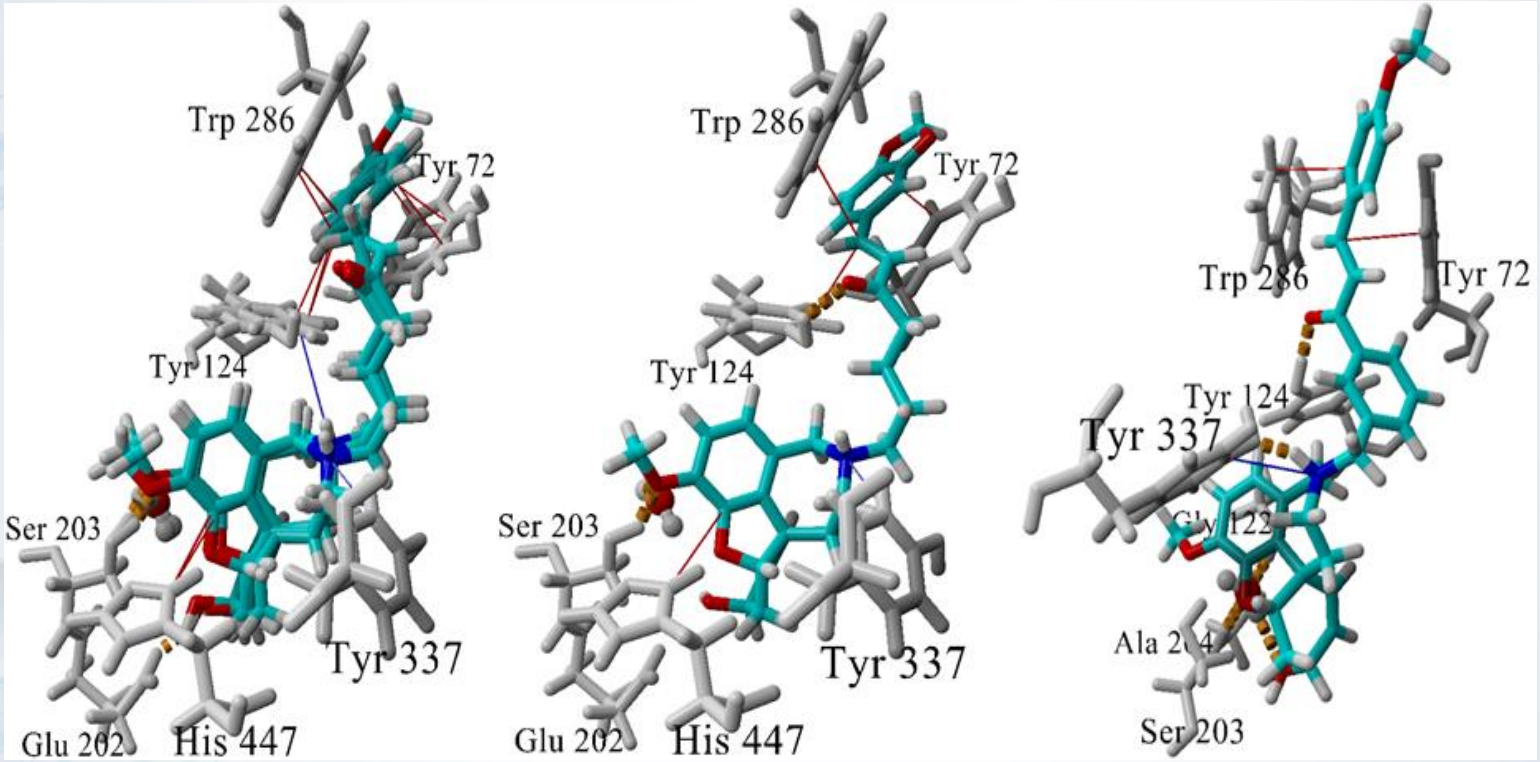

ИКТ

# GAL – CU Hybrids



Docking Poses & Intermolecular Interactions between AChE and

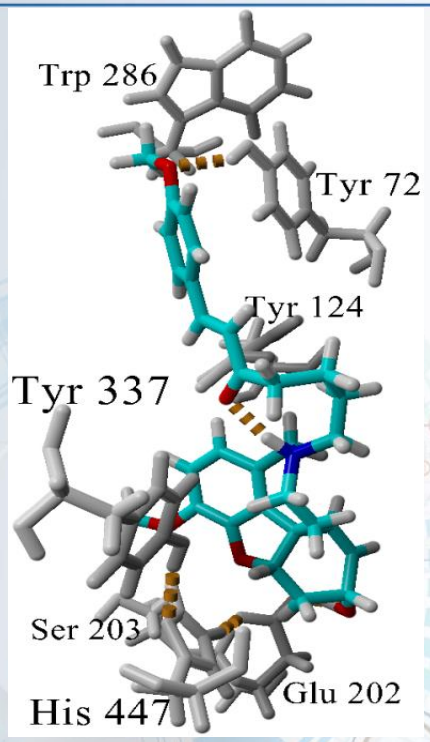
**4a, b, f** **4e** **8f**

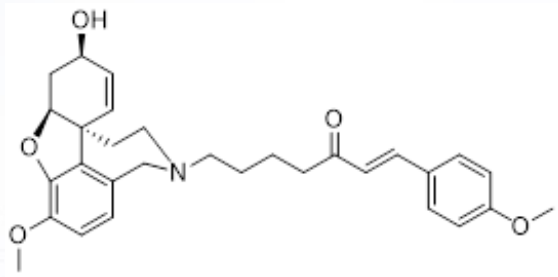



Hydrogen bonds - yellow dashes π-π interactions - red lines
Cation-π interactions - blue lines

# GAL – CU Hybrids






4b

**GAL – CU HYBRIDS**

- DESIGN
- BBB PERMEABILITY
- DOCKING
- CMPND SELECTION
  - PC & PK PROPERTIES
  - SYNTHESIS
    - NEUROTOXICITY
    - AChE ACTIVITY

**MOST ACTIVE & LOWEST TOXIC CMPND SELECTION**

- IN VITRO ANALYSES
  - ANTI-AMYLOID ACTIVITY
  - ANTIOXIDANT ACTIVITY
- IN VIVO ANALYSES
  - SHARP & SHORT-TERM TOXICITY
- EX VIVO ANALYSES
  - AChE ACTIVITY
  - ANTIOXIDANT ACTIVITY
  - HEMATOLOGICAL DATA
- **IN SILICO ANALYSIS**



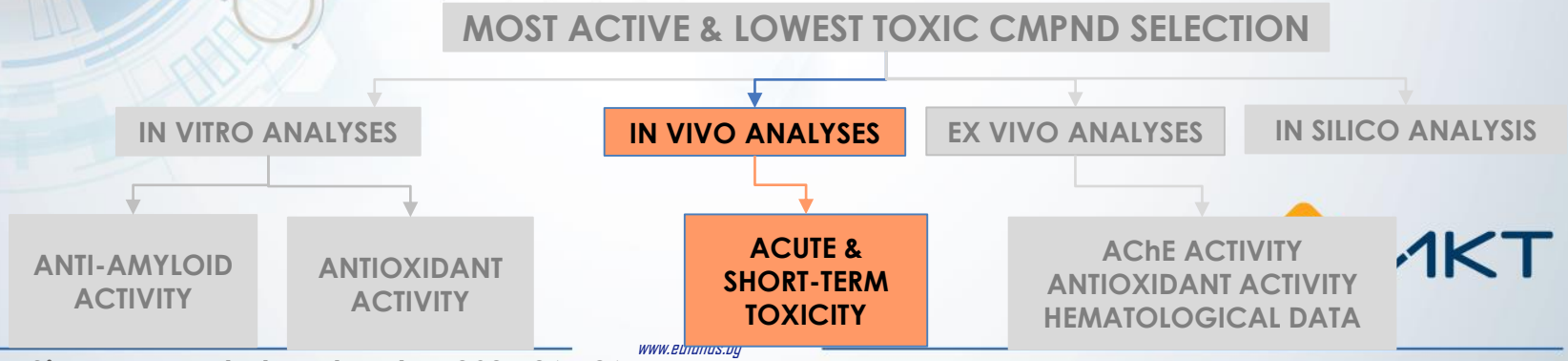
*Stavrakov et al, Molecules, 2020;25:3341*

# GAL – CU Hybrids



| ID | ACUTE TOX $LD_{50}$, mg/kg |
|---|---|
| 4b, p.o. | 49 |
| GAL, i.p. | 10 |
| GAL, p.o. | 15-45 (med. 30) |
| CU, p.o. | > 2000 |

| ID | SHORT-TERM mg/kg for 14 days |
|---|---|
| 4b | 2.5 (1/20 $LD_{50}$) |
| 4b | 5 (1/10 $LD_{50}$) |
| GAL | 3 (1/10 $LD_{50}$) |
| CU | 25 (1/10 $LD_{50}$) |



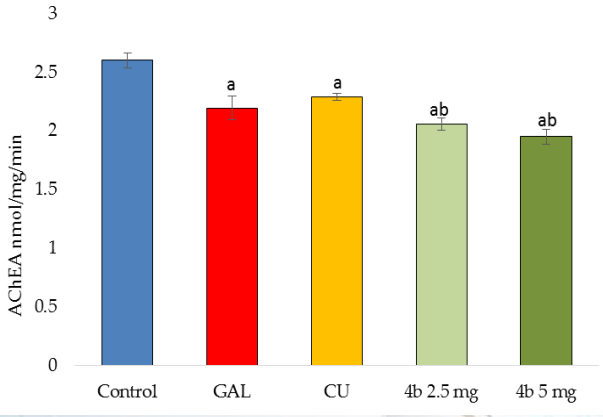


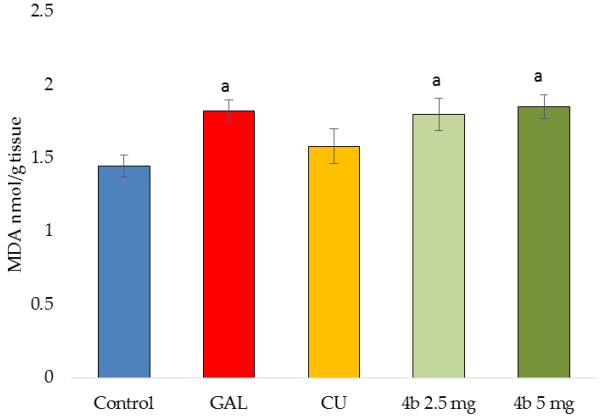

*Simeonova et al, Molecules, 2021;26:1865*

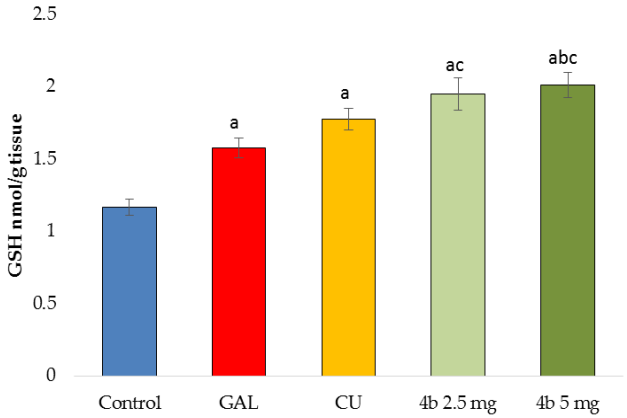
ЕВРОПЕЙСКИ СЪЮЗ
ЕВРОПЕЙСКИ ФОНД ЗА РЕГИОНАЛНО РАЗВИТИЕ

# GAL – CU Hybrids

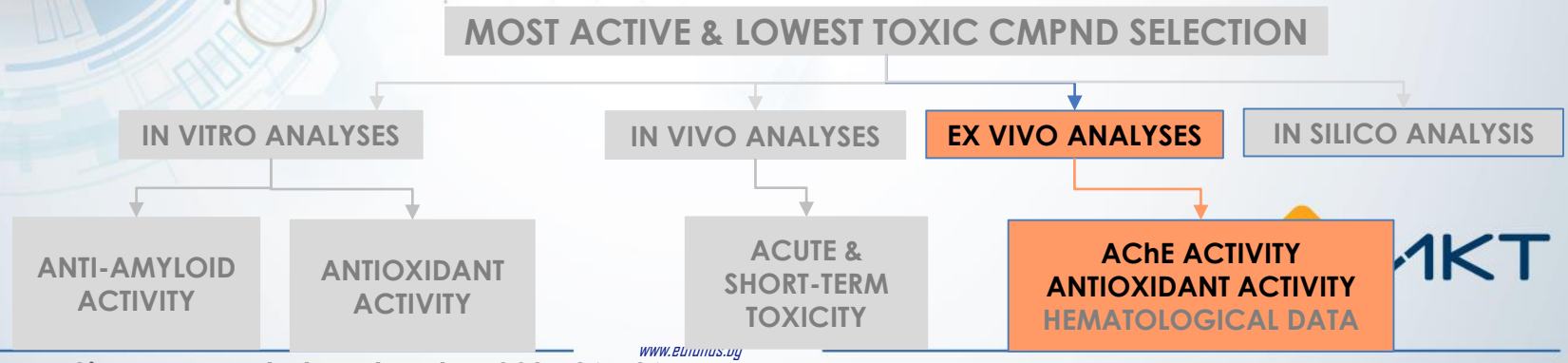
ОПЕРАТИВНА ПРОГРАМА НАУКА И ОБРАЗОВАНИЕ ЗА ИНТЕЛИГЕНТЕН РАСТЕЖ




a - $p < 0.05$ vs. control; b - $p < 0.05$ vs. CU; c - $p < 0.05$ vs. GAL



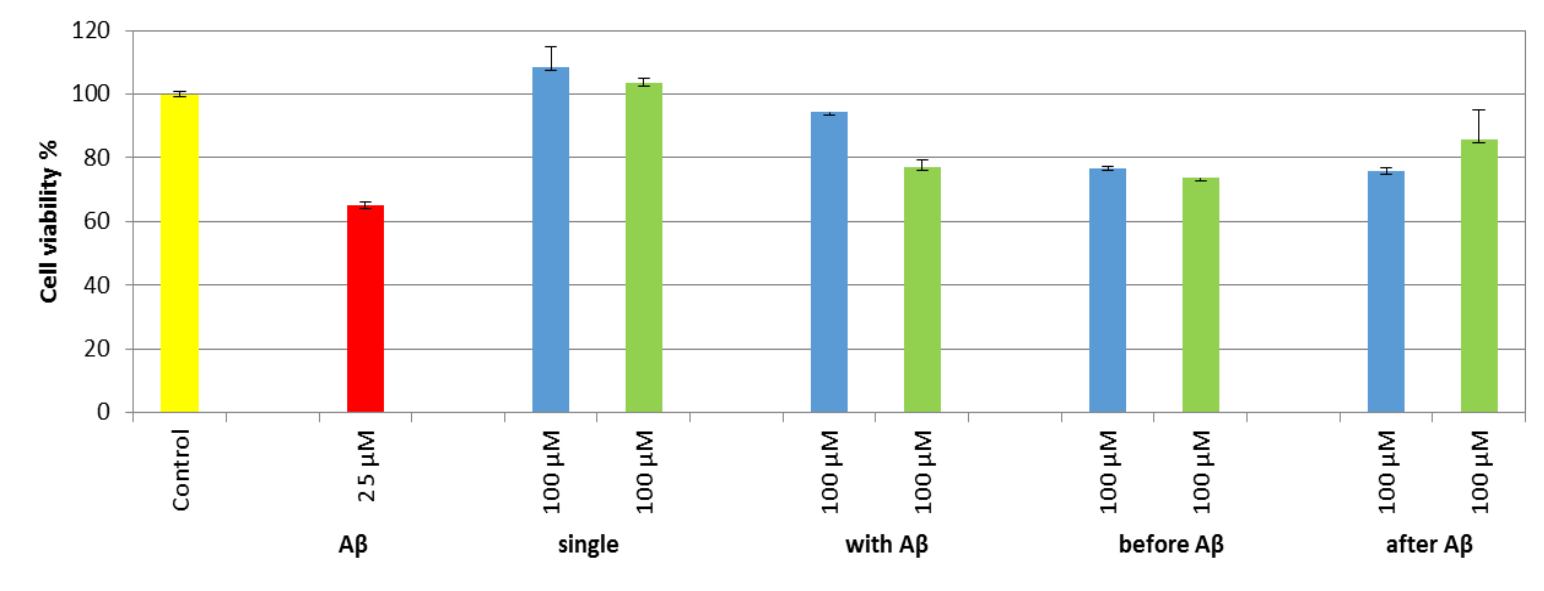


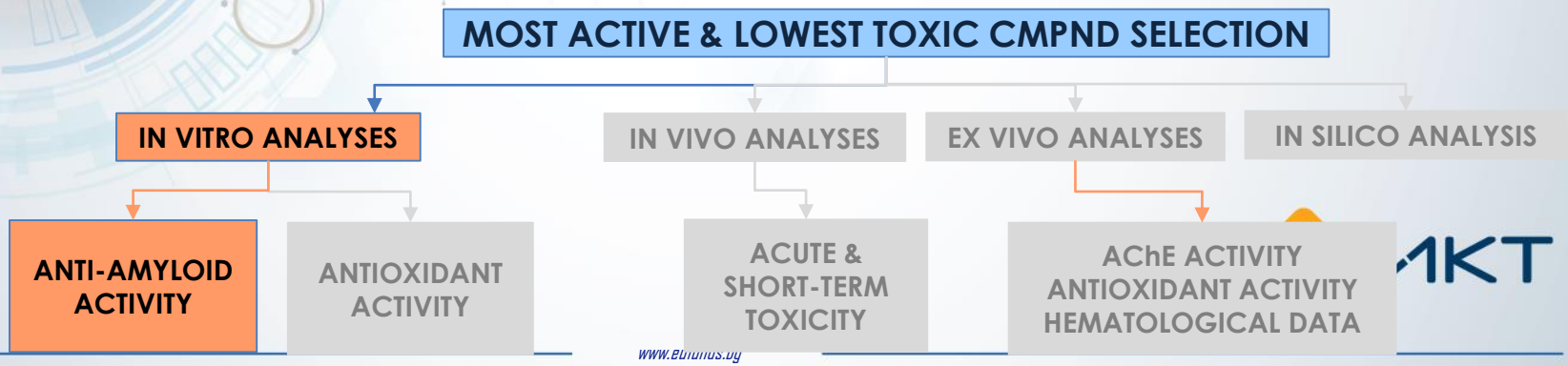

*Simeonova et al, Molecules, 2021;26:1865*

# GAL – CU Hybrids



**4b** (blue bars) and **GAL** (green bars)






*Mladenova et al, Int J Mol Sci, 2021;22:7592*

# Publications



1. Simeonova R, Zheleva D, Valkova I, Stavrakov G, Philipova I, Atanasova M, Doytchinova I. A novel galantamine-curcumin hybrid as a potential multi-target agent against neurodegenerative disorders. Molecules 26, 1865, 2021. IF 4,411, citations: 4
2. Stavrakov G, Philipova I, Lukarski A, Atanasova M, Georgiev B, Atanasova T, Konstantinov S, Doytchinova I. Discovery of a novel acetylcholinesterase inhibitor by fragment-based design and virtual screening. Molecules 26, 2058, 2021. IF 4,411, citations: 1
3. Mladenova K, Stavrakov G, Philipova I, Atanasova M, Petrova S, Doumanov J, Doytchinova I. A galantamine-curcumin hybrid decreases the cytotoxicity of amyloid-beta peptide on SH-SY5Y cells. Int. J. Mol. Sci. 22, 7592, 2021. IF 5,923, citations: 0
4. Berkov S, Atanasova M, Georgiev B, Bastida J, Doytchinova I. The Amaryllidaceae alkaloids: an untapped source of acetylcholinesterase inhibitors. Phytochem. Rev. in press doi: 10.1007/s11101-021-09790-0. IF 5,374, **top 10%** (21/235 in Plant Sciences)

# Acknowledgements

ЕВРОПЕЙСКИ СЪЮЗ
ЕВРОПЕЙСКИ ФОНД ЗА РЕГИОНАЛНО РАЗВИТИЕ

ОПЕРАТИВНА ПРОГРАМА НАУКА И ОБРАЗОВАНИЕ ЗА ИНТЕЛИГЕНТЕН РАСТЕЖ

ЕВРОПЕЙСКИ СЪЮЗ
ЕВРОПЕЙСКИ ФОНД ЗА РЕГИОНАЛНО РАЗВИТИЕ

ИКТ

ОПЕРАТИВНА ПРОГРАМА НАУКА И ОБРАЗОВАНИЕ ЗА ИНТЕЛИГЕНТЕН РАСТЕЖ

**Проект „BG05M2OP001-1.001-0003**
**„Център за върхови постижения по Информатика и информационни и комуникационни технологии“, по Оперативна програма „Наука и образование за интелигентен растеж“ 2014 – 2020”**

ФОНД НАУЧНИ ИЗСЛЕДВАНИЯ
МИНИСТЕРСТВО НА ОБРАЗОВАНИЕТО И НАУКАТА

ИКТ