Consiglio Nazionale delle Ricerche

Institute of Biostructures and Bioimaging

Exploring the neuroprotective effects of synthetic compounds for new neurodrug development

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1st International Conference on Modern research trends in biomedical sciences (MRTBS 2024), *Opole, Poland 18 April 2024*

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Graduated cum laude in Chemistry (thesis in organic chemistry), Federico II University, Naples (2002)

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Researcher at IBB CNR (2006-today)*permanent position in 2012

H-index (Scopus) **28**

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1 patent

Project leader for IBB-CNR:
NoBiasFluors, H2020, 872331

POR-FESR 2014-2020 project 'PON03F

European Commission
Horizon 2020
European Union funding
for Research & Innovation

PI:
Industrial Research Project (Altergon-IBB agreement 2014)
Lr n. 5 2007 (Regione Campania) 2015
Bilateral project (SRNSF-CNR): Nucleopeptides for biomedic

Prizes and other awards:
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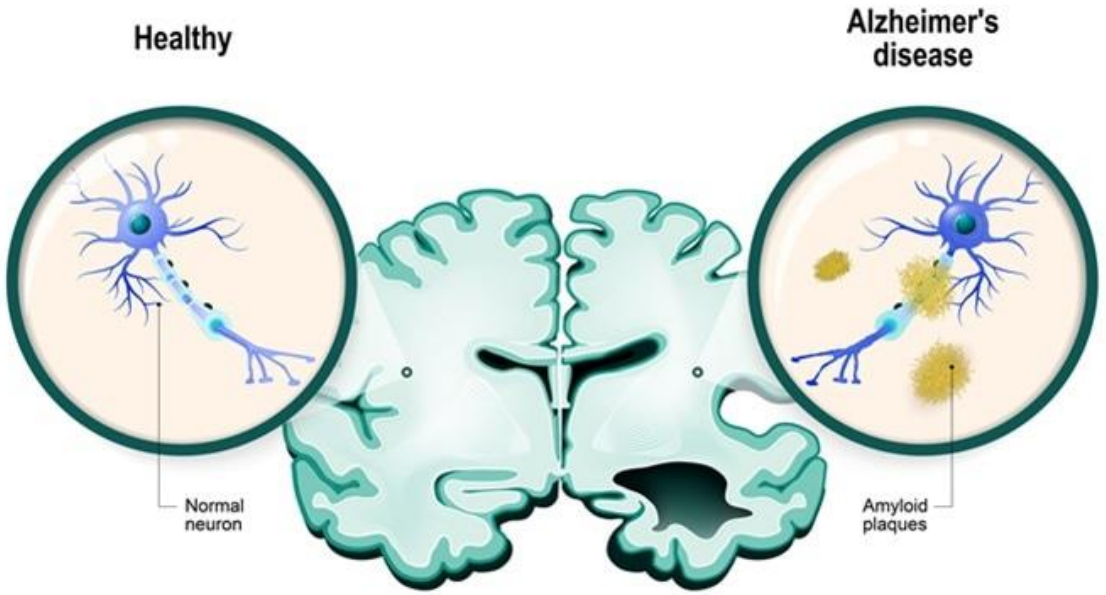
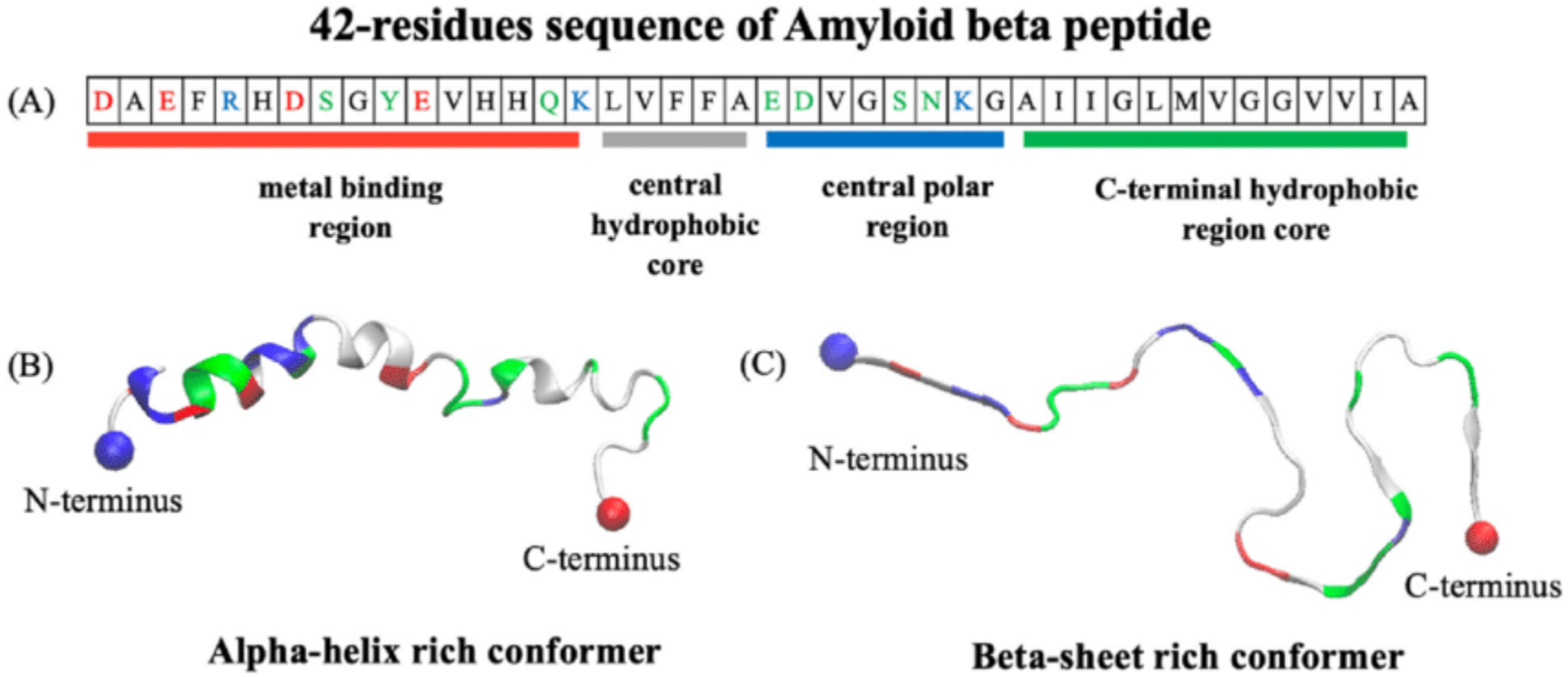
-Professor honoris causa Medicinal Chemistry - Geomedi University, Tbilisi Georgia, 2012
-Bursary for 'Corso di Management e Valorizzazione della Ricerca: Come va la ricerca' - CNR, ufficio PSC – promozione e sviluppo di collaborazioni 2012
-Short Term Mobility 2018 – CNR => FAU University, Germany =>EU project NoBiasFluors, H2020, 872331



- Amyloid plaques: Characterized by accumulation of misfolded proteins in brain tissue.
- Amyloid: Integral to neurodegenerative disorders; hallmark of conditions like Alzheimer's.
- Neurodegeneration: Linked to cognitive decline, synaptic dysfunction, and memory impairment.
- Structure of Aβ42: Crucial in Alzheimer's pathology; forms toxic aggregates in brain.



- Aβ42 structure: Comprises 42 amino acids, prone to aggregation into β-sheet-rich fibrils.
- Composition properties: β-amyloid peptides derived from amyloid precursor protein (APP) cleavage.
- Pathology: Aβ42 aggregation leads to formation of insoluble plaques, contributing to neurodegeneration.

Importance of Preventing Amyloid Beta 1-42 Aggregation and Disaggregation for Neurodrug Development:

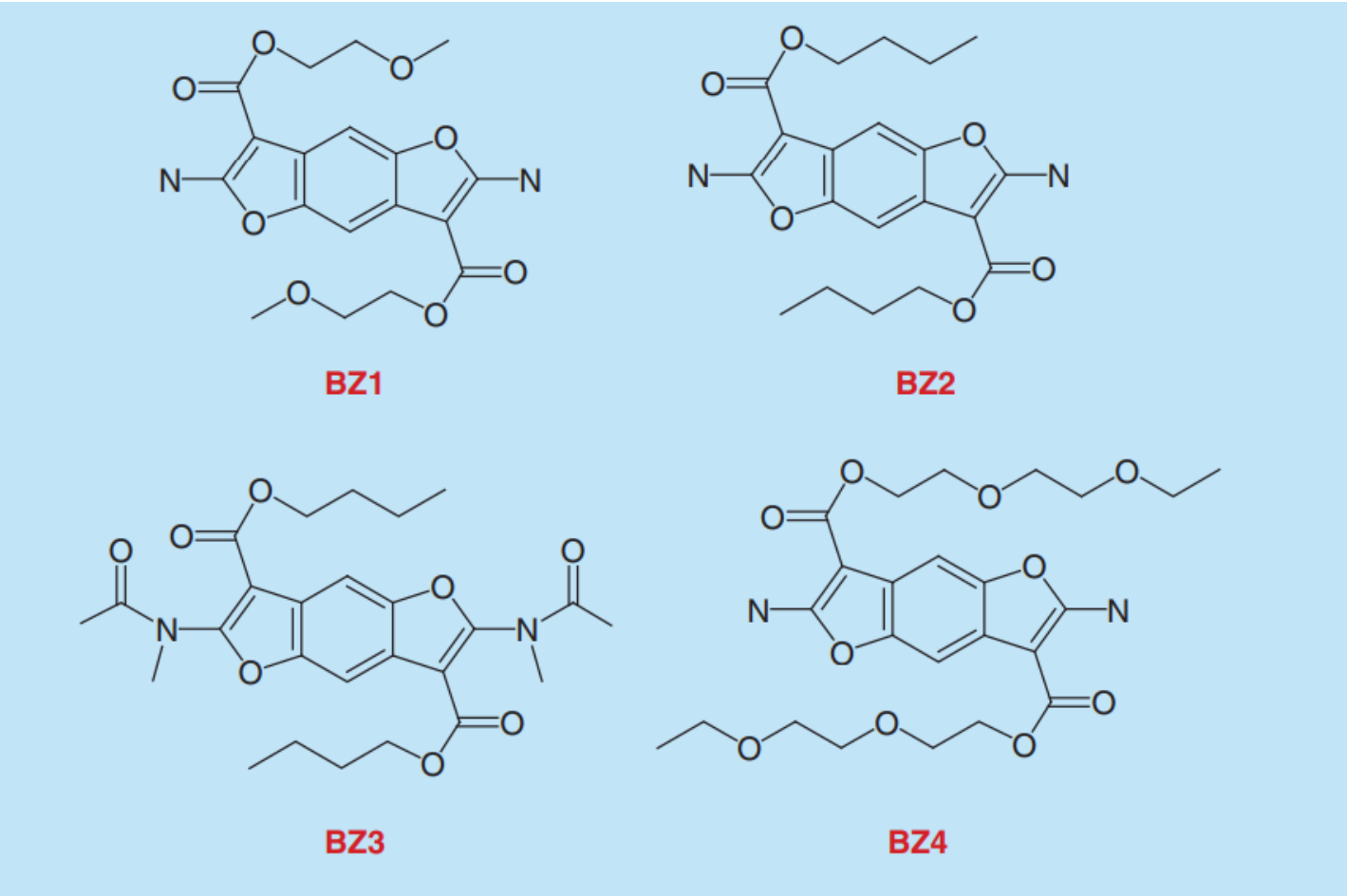
- Neurodegenerative Diseases: Strongly associated with the pathogenesis of neurodegenerative diseases, particularly Alzheimer's disease (AD).
- Toxicity Reduction: Implicated in neuronal toxicity and synaptic dysfunction, leading to cognitive decline and memory impairment.
- Disease Modification: Offers a promising approach for disease modification in neurodegenerative disorders.
- Therapeutic Intervention: Potential to slow disease progression, improve cognitive function, and enhance quality of life.
- Biomarker Identification: Helps identify biomarkers for early disease detection and monitoring treatment efficacy.

Synthetic compounds:

Benzodifurans (BZ1, BZ2, BZ3 and BZ4).

Isoquinoline alkaloids (Coralyne vs. Sanguinarine and Chelerithrine)

ISOAC1 3-(3-oxoisoindolin-1-yl)pentane-2,4-dione (ISOAC1)



BZ1, BZ2, BZ3 and BZ4 derivatives.

Benzodifurans

Vicidomini, C., Cioffi, F., Broersen, K., Roviello, V., Riccardi, C., Montesarchio, D., ... & **Roviello, G. N.** (2019). Benzodifurans for biomedical applications: BZ4, a selective anti-proliferative and anti-amyloid lead compound. *Future Medicinal Chemistry*, *11*(4), 285-302.

Nanostructures formed by benzodifurans

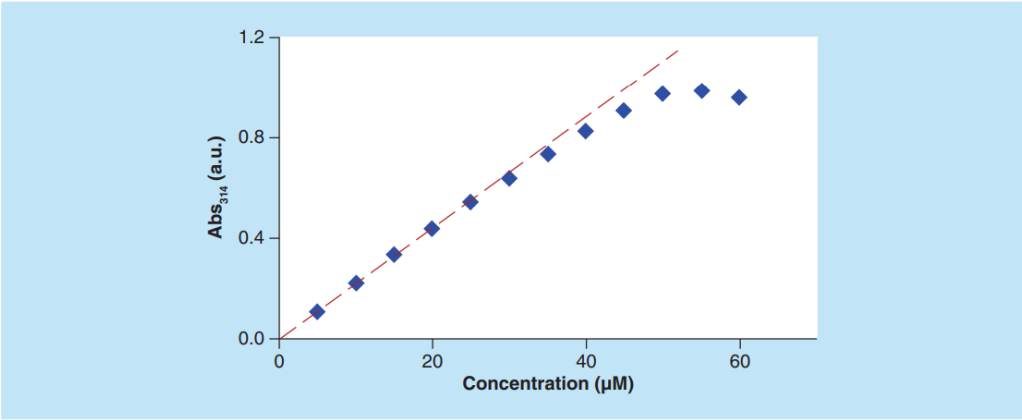


Figure 5. UV absorbance of BZ4 monitoring at 314 nm at different concentrations (5–60 μM) in phosphate-buffered saline (pH 7.2) at 25°C. The measurements were performed on freshly prepared solutions obtained by diluting **BZ4** in phosphate-buffered saline from concentrated stock solutions in DMSO (from 0.75 to a maximum of 9 mM), maintaining a constant DMSO concentration (0.67%), necessary to dissolve the samples.

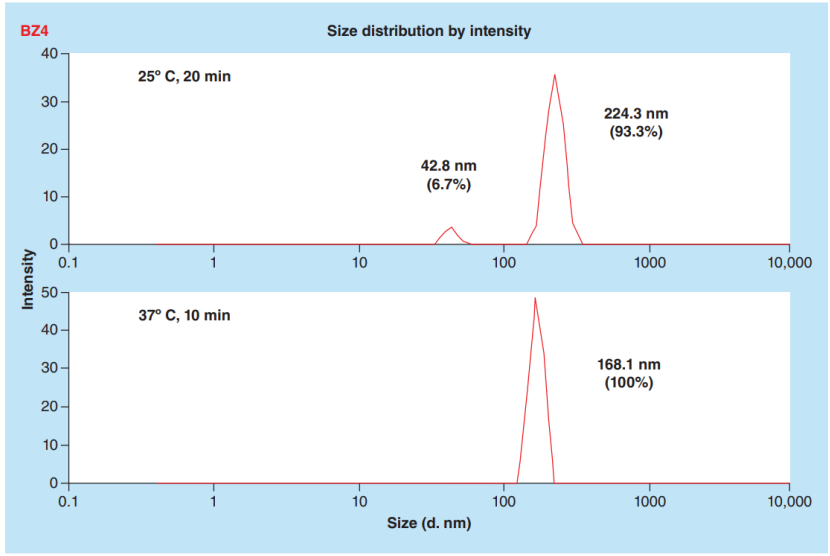


Figure 6. Representative dynamic light scattering spectra of BZ4 (at 10 μM concentration in phosphate-buffered saline solution) at different temperatures (25°C and 37°C). The time required for signal stabilization at each temperature investigated was also reported.

Dual anticancer and anti-amyloid activities of benzodifurans

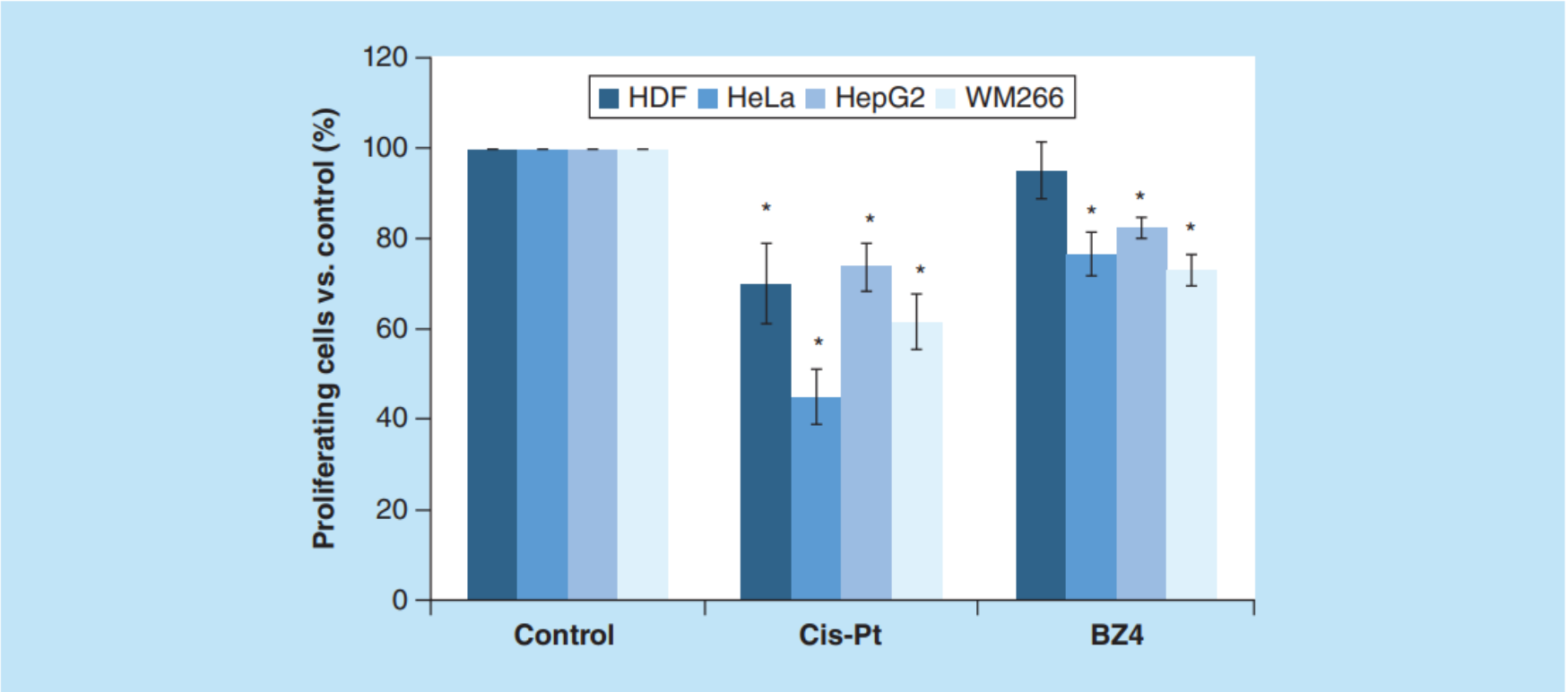


Figure 7. Cytotoxic effects of BZ4 on different cell lines in comparison with cisplatin. Cells were incubated with the tested compounds at 10 μM concentration at 37°C for 24 h. Proliferating cells are reported as percentage respect to the control (vehicle-treated cells) and are expressed as means ± SE. All the cell experiments on the antiproliferative activity of **BZ4** were carried out at least in triplicate. Statistical significance was analyzed using Student's t-test, unpaired and two-sided.
*$p < 0.05$.
HDF: Human dermal fibroblast; HeLa: Human cervix adenocarcinoma cells; HepG2: Liver hepatocellular cells; WM266: Malignant melanoma.

DNA binding activity

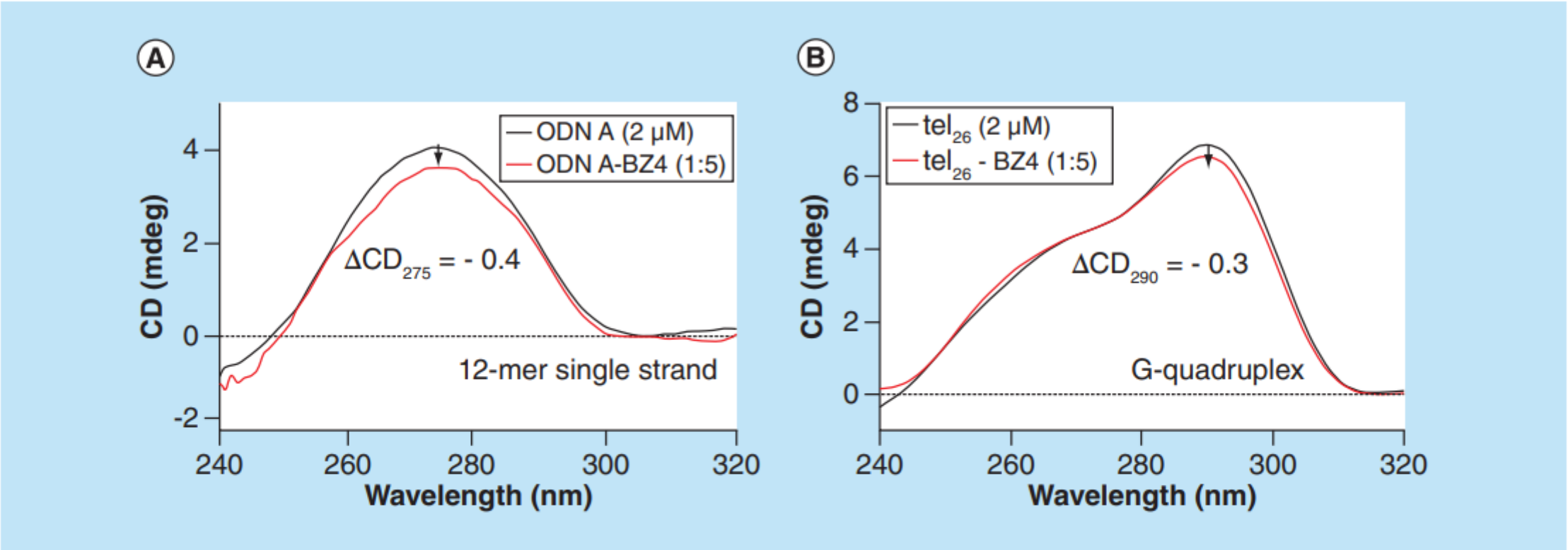


Figure 8. DNA binding spectroscopic studies. (A) Overlapping CD spectra of the single strand ODN and **(B)** the G4 tel_{26} (2 μM each) in the absence (black lines) and presence (red lines) of 5 equiv. of **BZ4**, in a 100 mM KCl-phosphate buffer solution (pH 7.2). Each titration was repeated three-times (the error on the calculated ΔCD value was ± 0.07 mdeg): in the figure a representative example for each system was reported.
CD: Circular dichroism; ODN: Oligodeoxyribonucleotide.

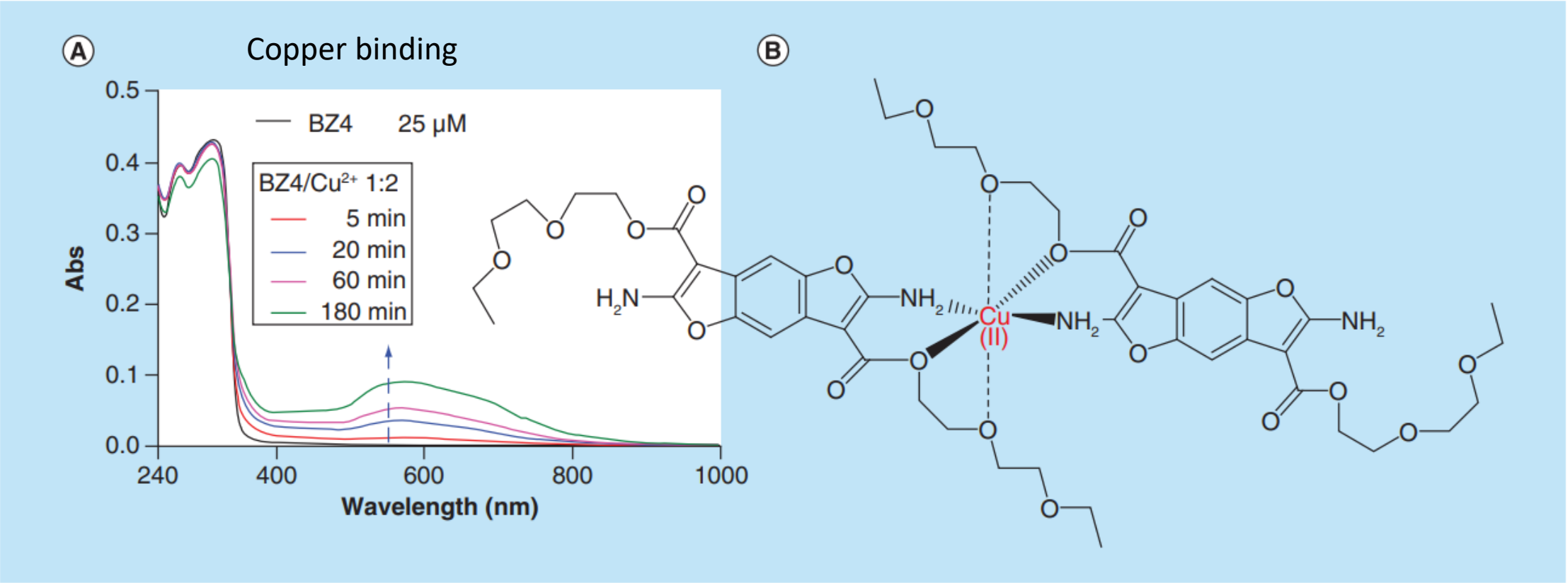


Figure 9. Copper (II) binding studies. (A) Overlapped UV spectra of a **BZ4** solution (25 μM) in PBS after signal stabilization (black line), and a solution containing both **BZ4** (25 μM) and $CuCl_2$ (50 μM) in phosphate-buffered saline at different time periods (5 min after Cu^{2+} addition = red line, 20 min = blue, 1 h = magenta, 3 h = green); **(B)** hypothesis of copper(II)-binding by **BZ4**.

Copper ions have been implicated in promoting the aggregation of amyloid-beta (Aβ) peptides, which are a hallmark of Alzheimer's disease.

By chelating copper ions, a molecule can prevent them from interacting with Aβ peptides, potentially inhibiting the formation of amyloid plaques. This could slow down the progression of amyloid diseases and reduce neurotoxicity associated with copper-mediated amyloid aggregation.

Circular Dichroism studies: amyloid peptide spectra at 24 and 48 h at 37°C without and with BZ4

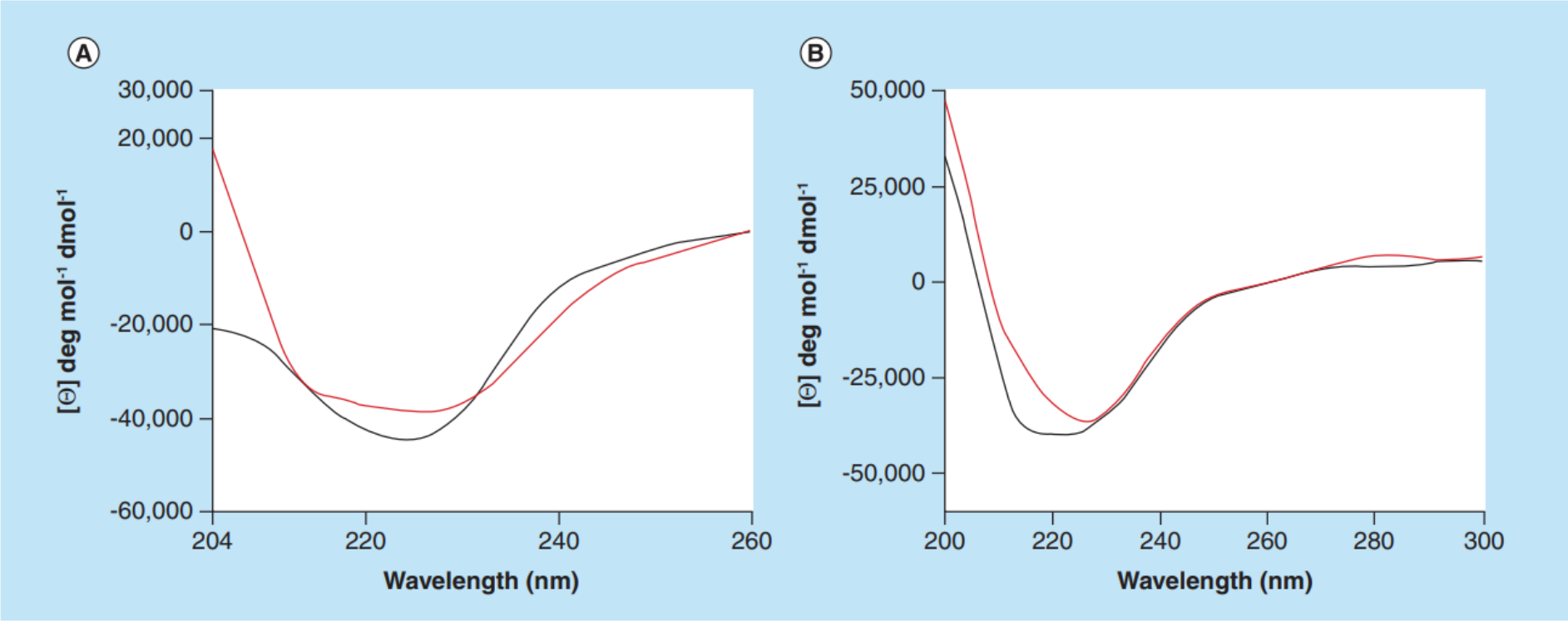


Figure 10. Circular dichroism studies on the interaction of amyloid peptide with BZ4. (A) Circular dichroism spectra of $A\beta_{42}$ (5 μM concentration in phosphate-buffered saline, black line) and $A\beta_{42}$ + **BZ4** (2 equiv., red line) after 24 h and **(B)** 48 h of incubation at 37°C.

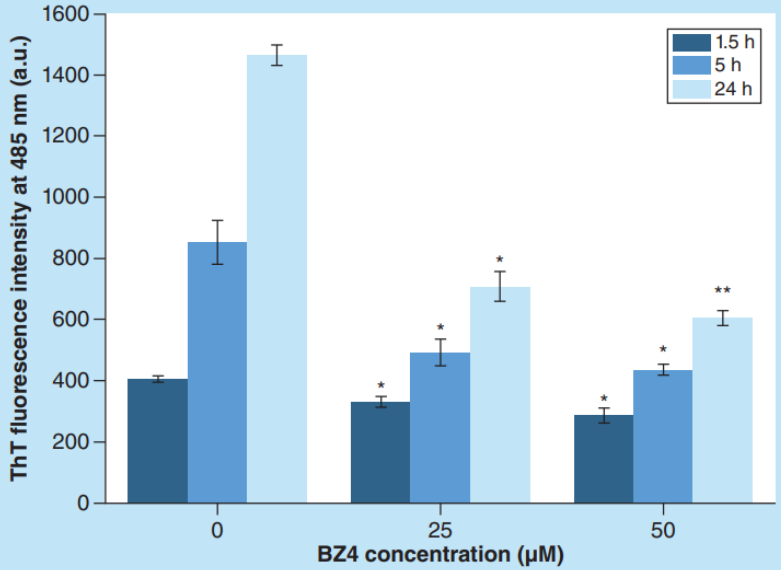


Figure 11. BZ4 exerts a dose-dependent inhibiting effect on the amyloid fibril formation of $A\beta_{42}$. Solutions containing $A\beta_{42}$ at a concentration of 25 μM were incubated in the presence and absence of **BZ4** at 37°C for 1.5, 5 and 24 h. Amyloid fibril formation was detected using ThT fluorescence intensity measurements at a detection wavelength of 485 nm. The reported values represent the results obtained from three independent experiments. The statistical significance of the replicates was assessed by p-values using paired two-tailed t-tests (GraphPad Software). *$p < 0.05$; **$p < 0.005$.

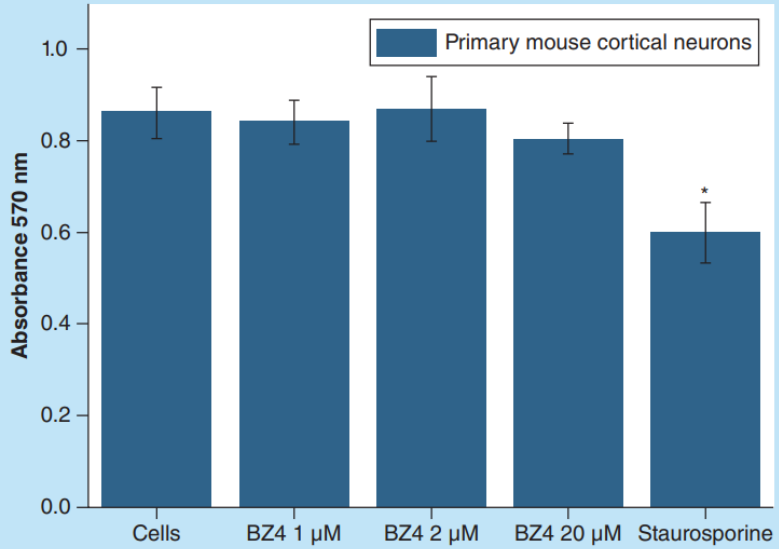
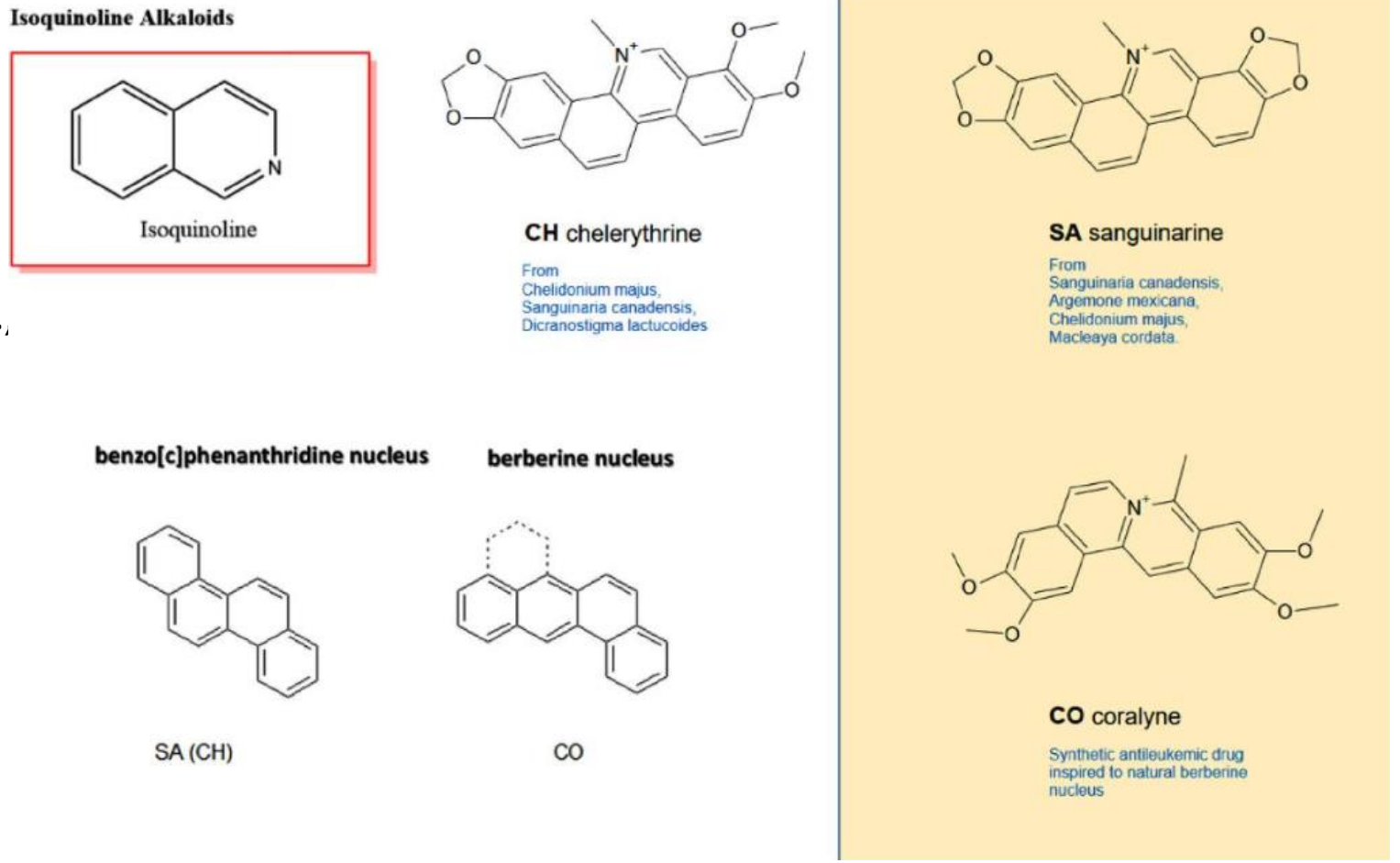


Figure 12. Cell viability assay using the MTT protocol on primary mouse cortical neurons exposed to 1, 2 and 20 μM concentrations of BZ4. A 2 μM solution of staurosporine and nontreated cells were used, respectively, as negative and positive controls. Statistical significance was analyzed using student's t-test, unpaired and two-sided. All the experiments were performed in triplicate
*$p < 0.01$

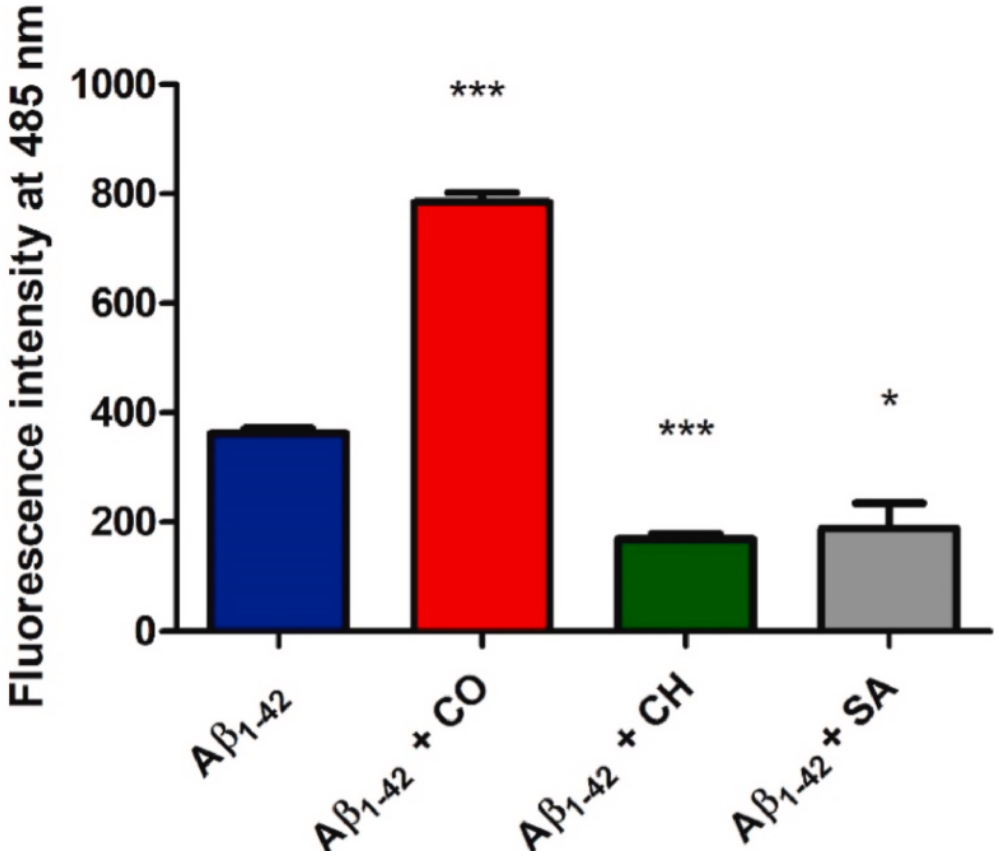
- Synthesis and characterization of a new benzodifuran derivative, BZ4, are reported.
- Biological properties, including anticancer and anti-amyloid activities, as well as interactions with DNA model systems, are evaluated.
- BZ4 is designed from the previously reported BZ1, with elongated oligoethylene glycol chains to enhance solubility in aqueous solutions and improve hydrophilic-lipophilic balance.
- Significant antiproliferative activity is observed on various human cancer cells, with no impact on normal HDF or primary neuronal cells.
- Antiproliferative activity is linked to the formation of nanoaggregates, as evidenced by UV-vis spectroscopy and DLS analysis.
- BZ4 acts as a copper (II) ion chelator, demonstrated by UV spectroscopy.
- Inhibitory effects on Aβ peptide aggregation, a key target in Alzheimer's disease therapy, are observed in a dose-dependent manner.
- Interaction with Aβ is confirmed by CD spectroscopy and SEM microscopy.
- BZ4 exhibits no toxicity towards primary neuronal cells.
- BZ4 represents a valuable core scaffold for generating optimized analogs targeting cancer and Alzheimer's disease.

Isoquinoline alkaloids (Coralyne (CO) vs. Sanguinarine (SA) and Chelethrine (CH))

Marasco, D., Vicidomini, C., Krupa, P., Cioffi, F., Huy, P. D. Q., Li, M. S., Florio, D., Broersen, K., De Pandis, M. F., & **Roviello, G. N.*** (2021) *Chemico-biological interactions*, *334*, 109300. https://doi.org/10.1016/j.cbi.2020.109300

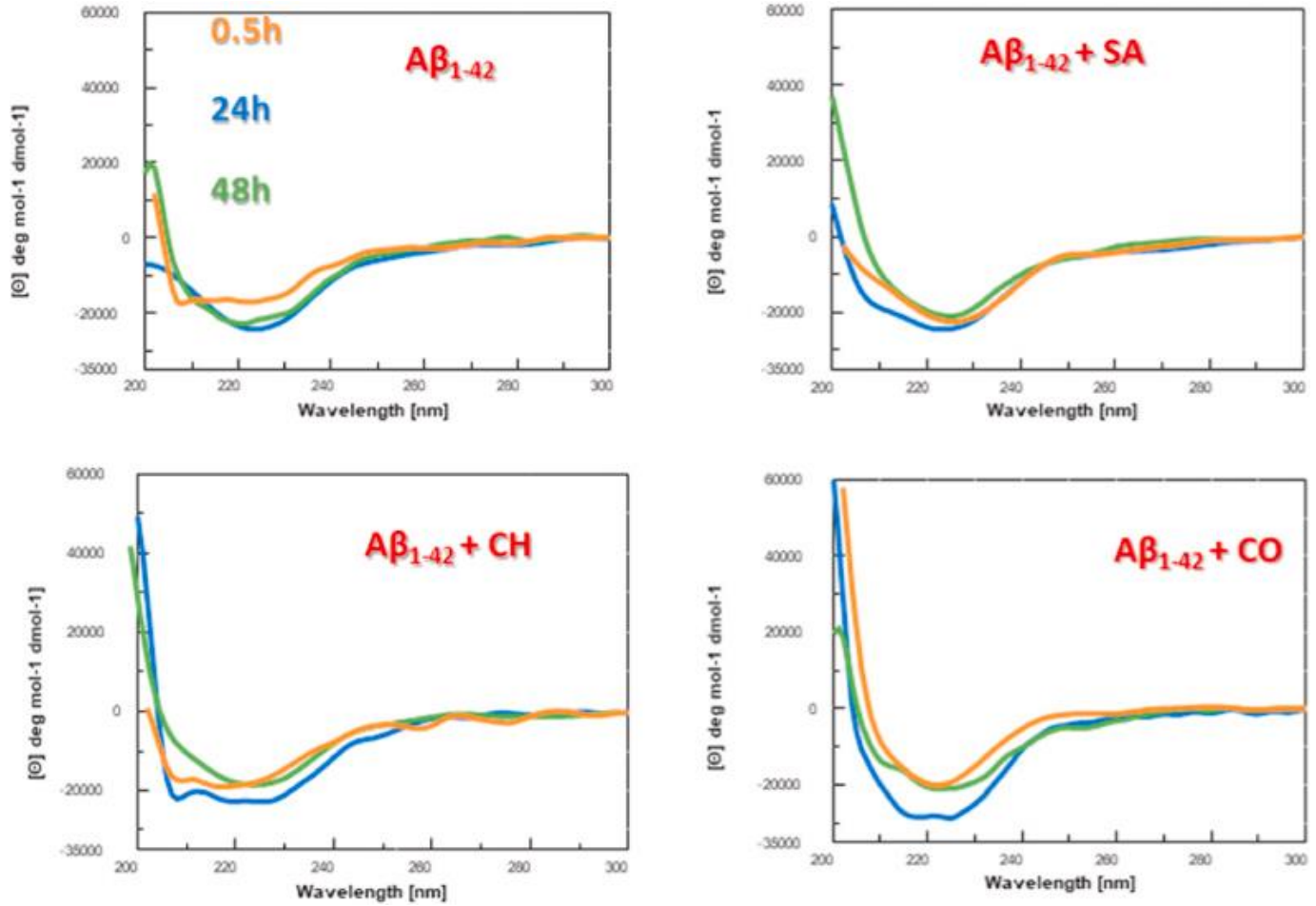


- Comparative SAR (Structure-Activity Relationship) of CO, SA, and CH:
  - Coralyne (CO) induces ThT-positive Aβ1-42 assemblies, promoting aggregation.
  - Sanguinarine (SA) and Cheletrine (CH) inhibit ThT-positive oligomer formation of Aβ1-42.
  - SA and CH show a reduction in ThT fluorescence signal by approximately 40% compared to untreated Aβ1-42.

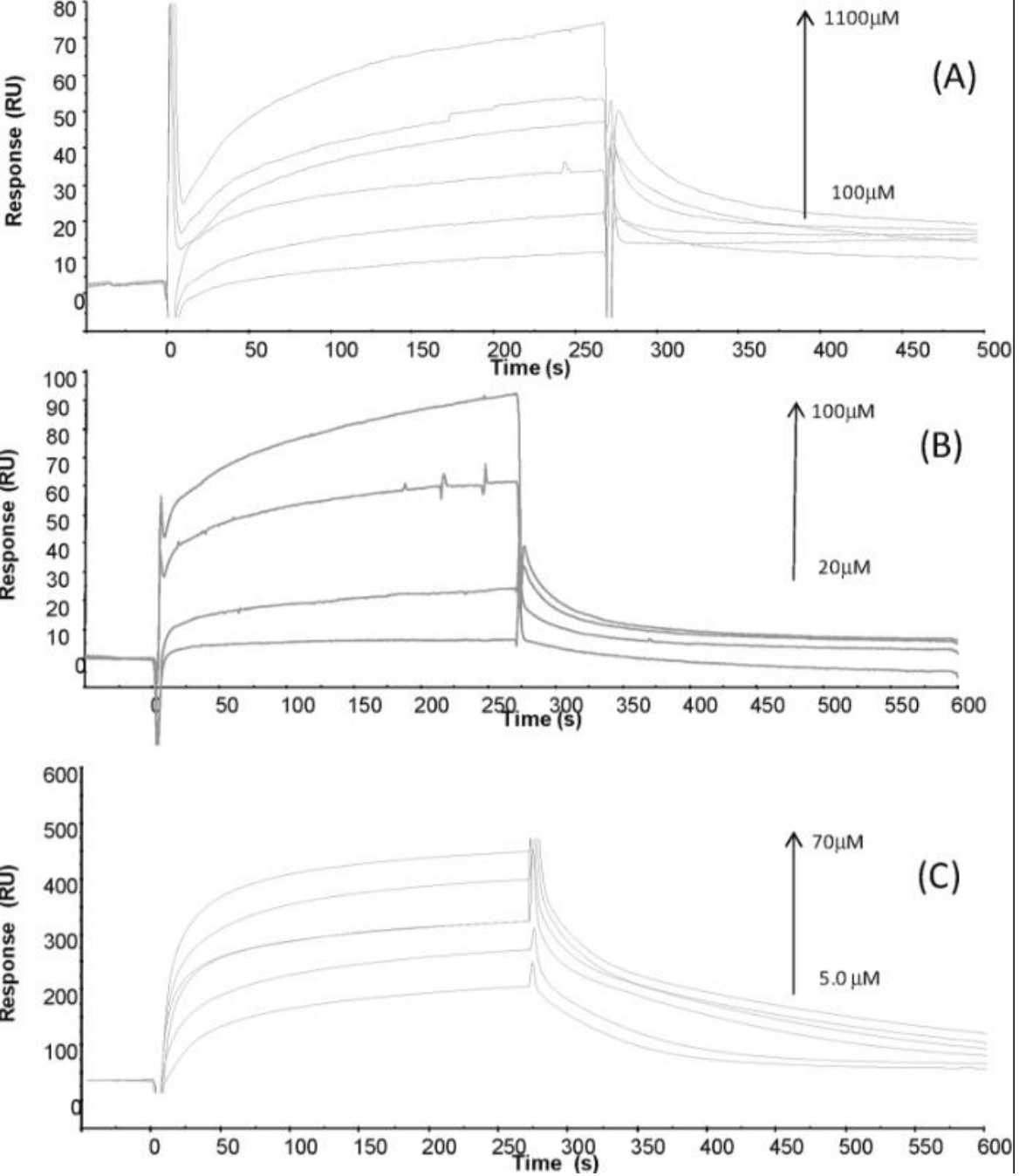


SA and CH inhibit ThT-positive oligomer formation of $A\beta_{1-42}$, whereas CO induces ThT-positive assemblies. Solutions containing $A\beta_{1-42}$ at a concentration of 25 μM were incubated in the presence and absence of **SA, CH and CO (at 1:1 ratio)** at 37 °C for 2 h. ThT-positive aggregate formation was detected using ThT fluorescence intensity measurements at a fluorescence emission wavelength of 485 nm upon excitation at 450 nm. The reported values represent the results obtained from three independent experiments. The statistical significance of the replicates was assessed by p-values using paired two-tailed t-tests (GraphPad Prism). $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ compared with the control ('$A\beta_{1-42}$').

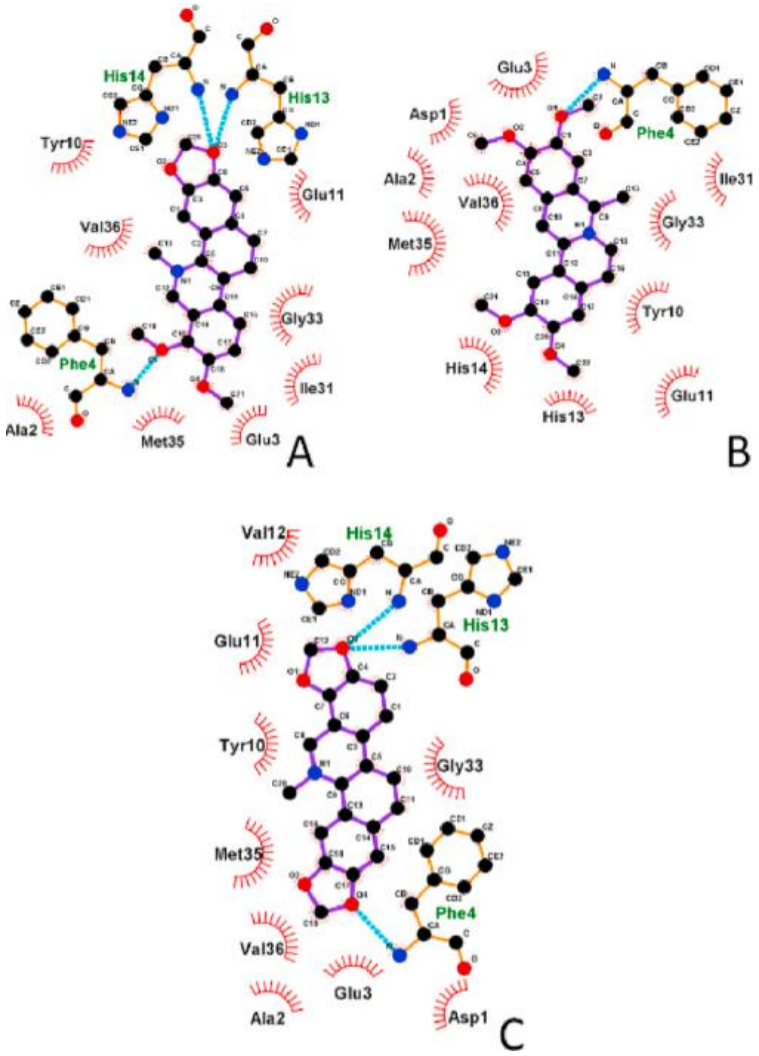
- SA and CH exhibit significant differences in the structural organization of Aβ1-42 compared to CO.
- Isoquinoline alkaloids interact with Aβ1-42, forming complexes in a concentration-dependent manner.
- Thermodynamic dissociation constants suggest varying affinities of CO, SA, and CH for Aβ1-42.



Conformational response of $A\beta_{1-42}$peptide to SA, CH and CO. Circular dichroism spectra of $A\beta_{1-42}$ (5 μM concentration in PBS, black line) and $A\beta_{1-42}$ in the presence of isoquinoline alkaloids (1:2 M ratio, peptide: small molecule) after 0.5 (orange), 24 (blue), and 48 (green) h of incubation at 37 °C.



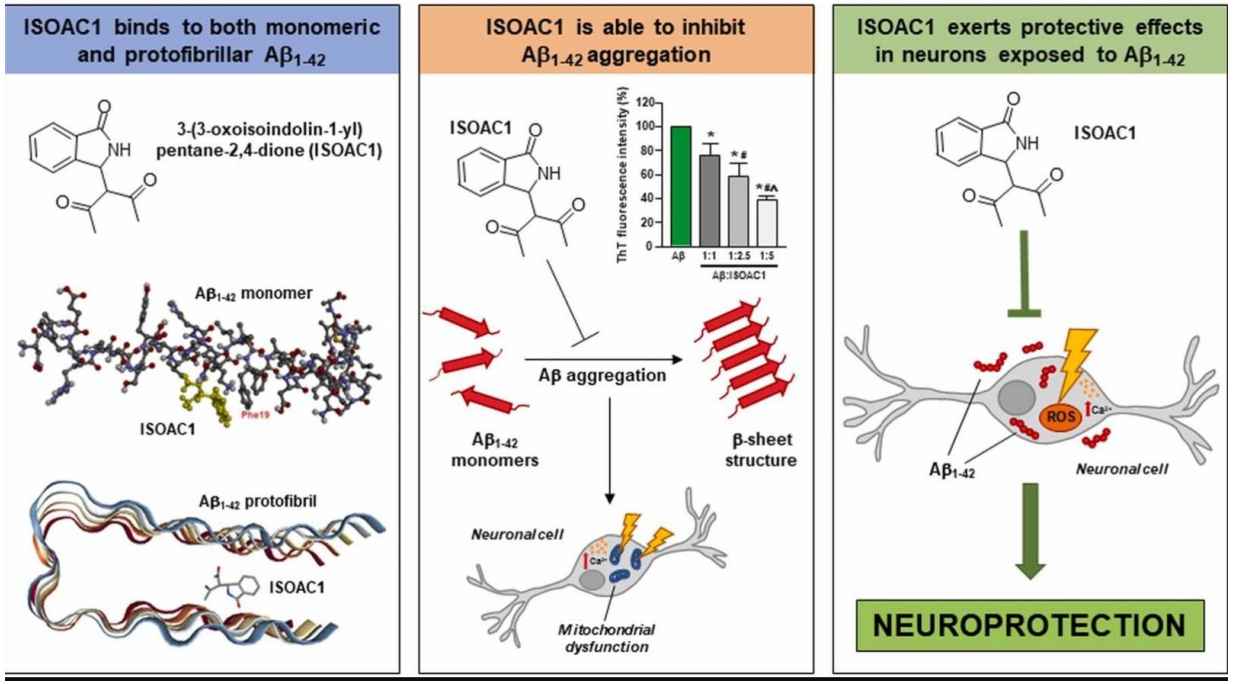
Overlay of sensorgrams for the binding to immobilized $A\beta_{1-42}$ of (**A**) SA, (**B**) CH and (**C**) CO.



Schematic representation of the strongest binding mode of monomeric $A\beta_{1-42}$ to compounds (monomeric model 2, binding mode 1; see Table 2 for more details) showed in 2D form for: A) CH, B) CO, C) SA. $A\beta_{1-42}$ residues involved in hydrophobic interactions with compounds are showed by red lines and black three-letter residue codes, hydrogen bonds are represented by cyan dashed lines and green three-letter residue codes. For clarity, hydrogens are not presented on the plot.

- Future Directions for Synthesis of Neurodrugs:
  - Further exploration of structural modifications of CO, SA, and CH to enhance their efficacy as neurodrugs.
  - Design and synthesis of novel compounds based on the SAR insights obtained from the study.
  - Investigation of additional isoquinoline alkaloid derivatives to expand the pool of potential neurodrug candidates.
  - Evaluation of the pharmacokinetic properties and toxicity profiles of newly synthesized compounds.
  - Screening of synthesized compounds using in vitro and in vivo models to assess their therapeutic potential.
  - Optimization of synthesis routes to improve scalability and cost-effectiveness of neurodrug production.

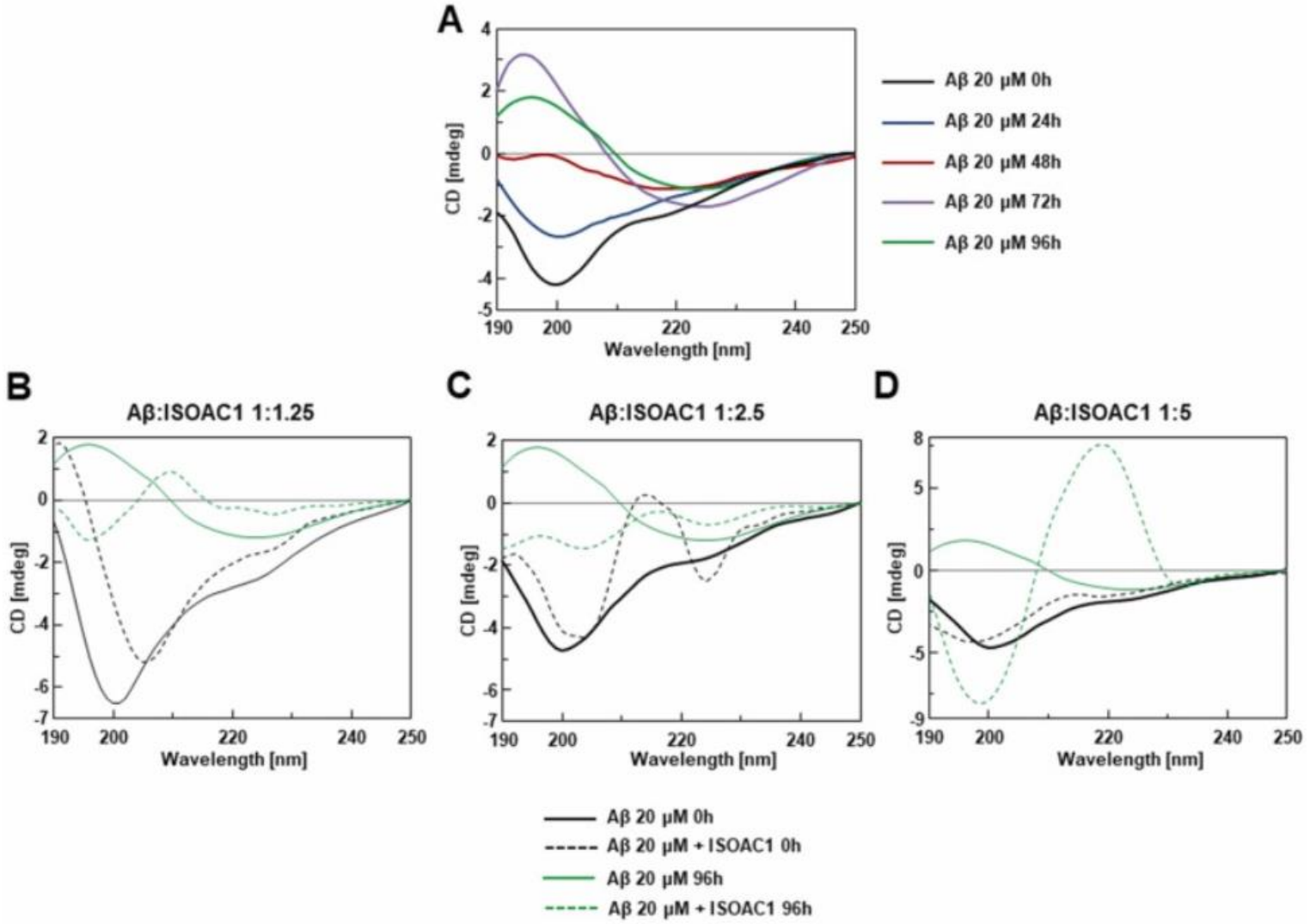
ISOAC1 3-(3-oxoisoindolin-1-yl)pentane-2,4-dione (ISOAC1): Dr. Ilaria Piccialli will give you more details!



Piccialli, I., Greco, F., **Roviello, GN.**, Sisalli, M. J., Tedeschi, V., di Mola, A., ... & Pannaccione, A. (2023). The 3-(3-oxoisoindolin-1-yl) pentane-2, 4-dione (ISOAC1) as a new molecule able to inhibit Amyloid β aggregation and neurotoxicity. *Biomedicine & Pharmacotherapy*, *168*, 115745.

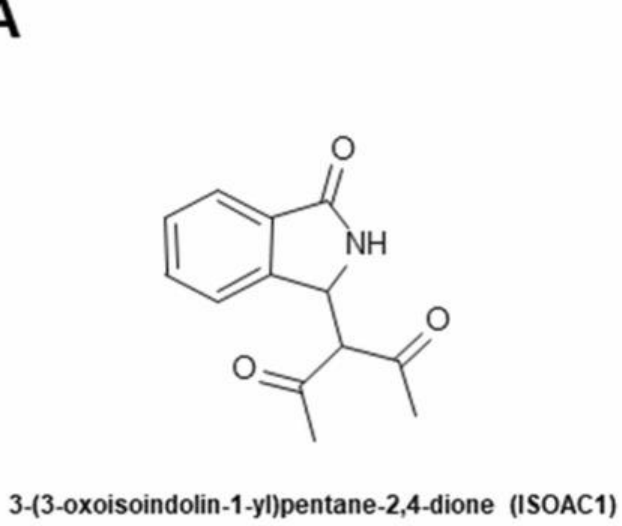
- Anti-aggregating potential of isoindolinone derivative ISOAC1
- Exploration of ISOAC1 inhibitory activity against Aβ aggregation
- Preference for Aβ1–42 peptide due to its relevance in Alzheimer's disease

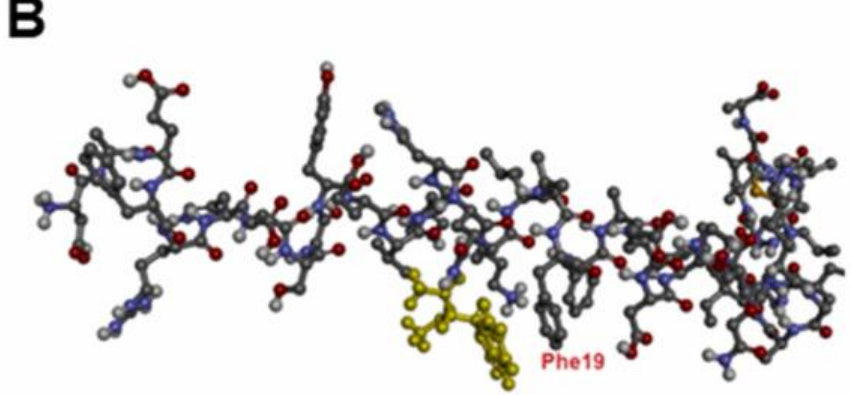
ISOAC1 inhibits the $A\beta_{1-42}$ conformational transition to β-sheet structures. Time-dependent far-UV CD spectra of the freshly prepared 20 μM-$A\beta_{1-42}$ incubated in 10 mM K^+ buffer (9 mM KCl and 1 mM KH_2PO_4, pH 7.4) at 37 °C analyzed after 0, 24, 48, 72, and 96 h (**A**). Far-UV CD spectra of freshly prepared 20 μM-$A\beta_{1-42}$ incubated in the absence (solid lines) and in the presence (dashed lines) of ISOAC1 at the indicated molar ratios analyzed after 0 and 96 h (**B-D**).

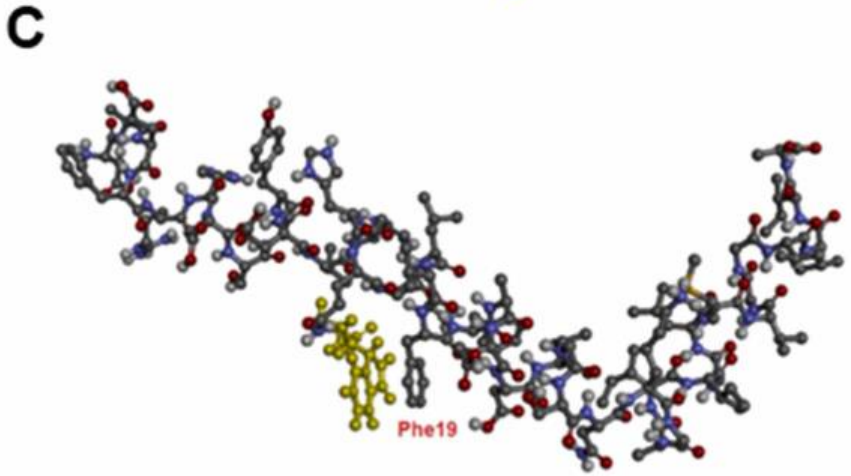


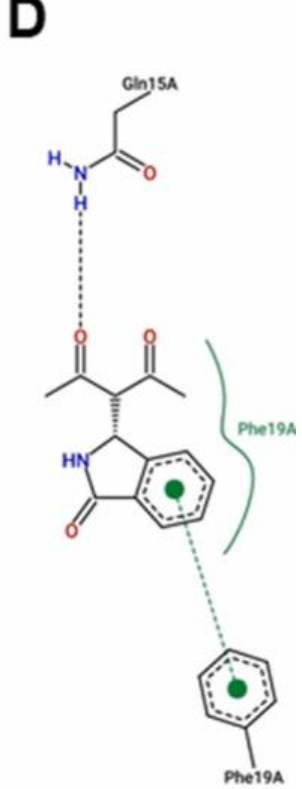
Aβ:ISOAC1 ratio	1:1.25		1:2.5		1:5	
Time/h	0	96	0	96	0	96
Δ_{beta} content	−4.2±0.4	+5.0±0.1	−3.3±0.2	−8.2±0.1	−4.1±0.1	−7.7±0.4
Δ_{turn} content	+4.1±0.4	−2.2±0.1	+1.1±0.2	+3.4±0.1	−0.4±0.1	+4.0±0.4

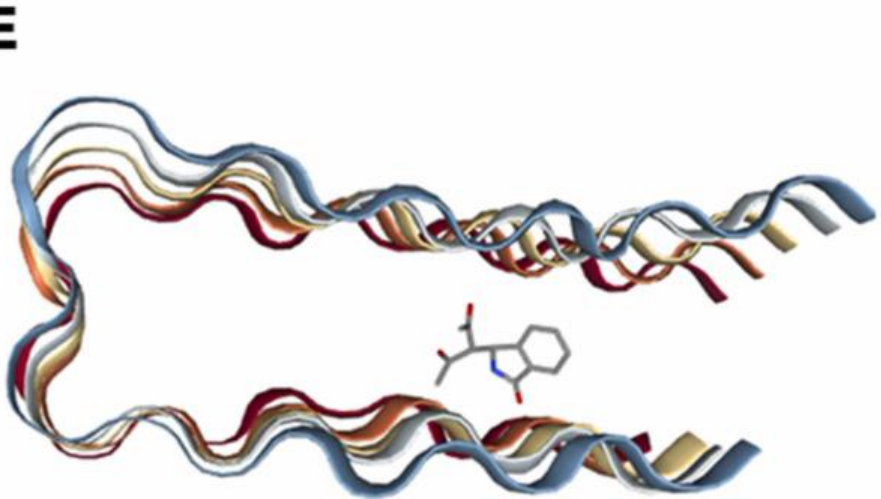
Variation in $A\beta_{42}$ structure content determined by ISOAC1 as calculated by Bestsel algorithm.

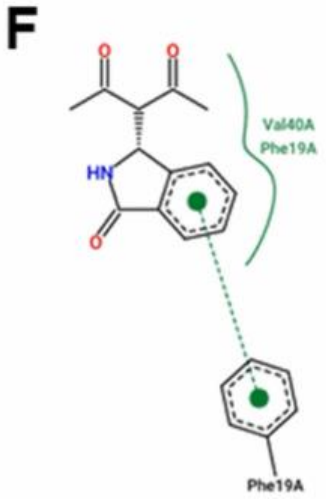












ISOAC1 is able to bind to both monomeric and protofibrillar $A\beta_{1-42}$ structures. Pose views of the complex formed between ISOAC1 and $A\beta_{1-42}$ monomer (PDB ID: 1IYT) as predicted by docking with 1-Click (Mcule) software (**A,B**). 2D peptide-ligand interaction diagram obtained by ProteinPlus with the docked structure of the complex formed by ISOAC1 and $A\beta_{1-42}$ monomer (**C**). Pose view of the complex formed between ISOAC1 and $A\beta_{1-42}$ protofibril (PDB ID:2BEG, represented as cartoon) as predicted by docking with 1-Click (Mcule) software (**D**). Details of the hydrophobic and aromatic binding between ISOAC1 and $A\beta_{1-42}$ protofibril in the 2D peptide-ligand interaction diagram obtained by ProteinPlus (**E**).

Acknowledgements

Division of Pharmacology, Department of Neuroscience, Reproductive and Dentistry Sciences, School of Medicine, Federico II University of Naples, Naples, Italy: Ilaria Piccialli (Speaker Auditorium 1, day 3), Maria Josè Sisalli, Valentina Tedeschi, Agnese Secondo, Anna Pannaccione
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- We express our gratitude to all individuals listed above for their invaluable contributions to this research endeavor.
- Thank you for your kind attention!