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Conjugation with Angiopep2 as a strategy for the brain delivery of a mitochondriotropic inhibitor of the potassium channel Kv1.3

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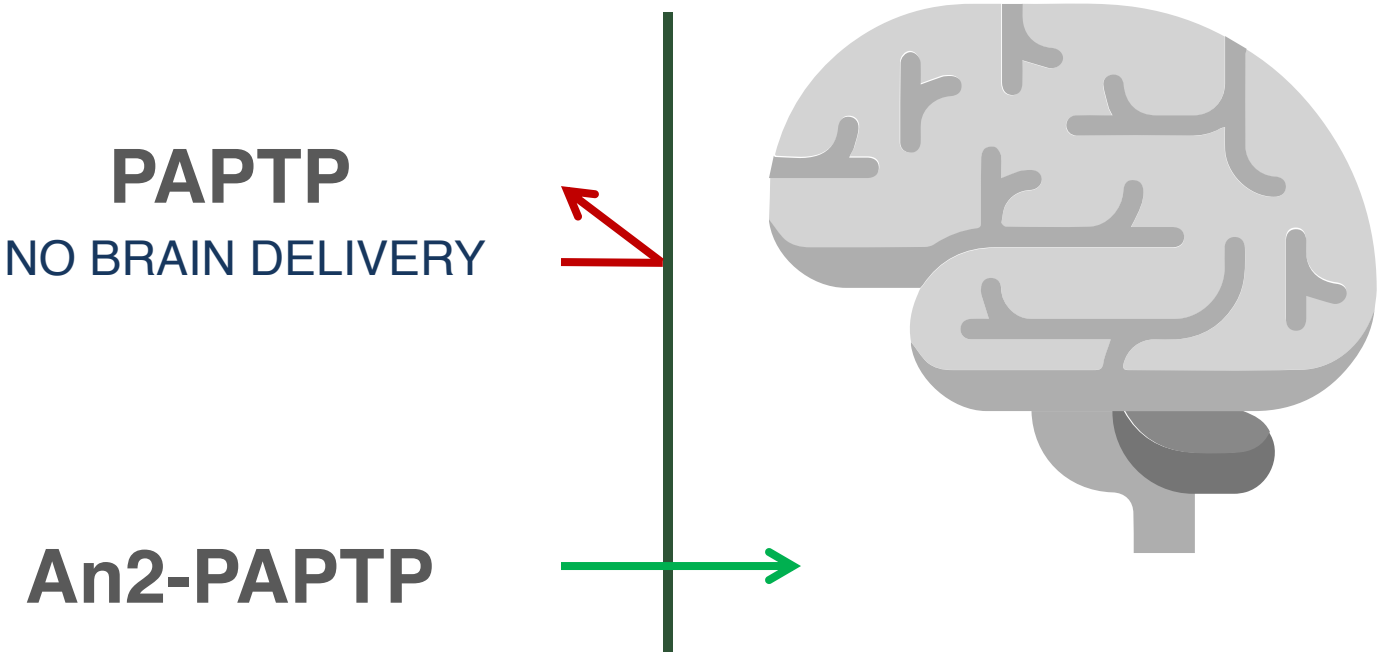
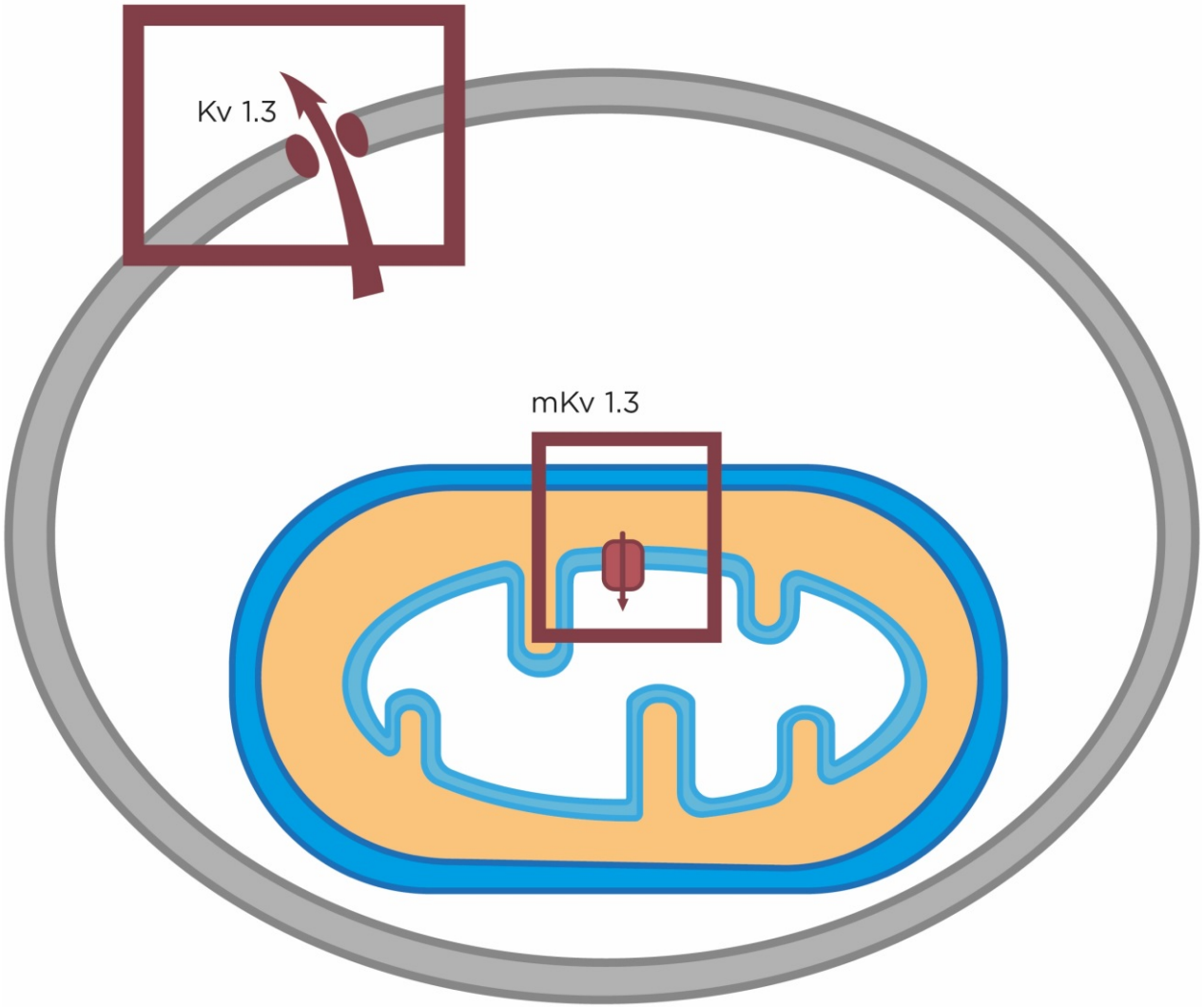
Conjugation with Angiopep2 (An2) as a strategy for the brain delivery of a mitochondriotropic inhibitor of the potassium channel Kv1.3



Abstract: The voltage-gated potassium channels Kv1.3, expressed by the cells at the plasma membrane and mitochondria level, is highly expressed in several cancers such as melanoma, pancreatic cancer (PDAC), glioblastoma and neuroblastoma, thus turning out an interesting pharmacological target against cancer. Inhibition of the mitochondrial population of the channel leads to pro-apoptotic processes. PAPTP, one of the novel inhibitors of the mitoKv1.3, turned out to be highly effective against melanoma and PDAC in vivo and against glioblastoma cell lines in vitro. It cannot be exploited in orthotopic models of glioma because it is completely unable to cross the blood brain barrier (BBB). A possible approach to enhance the BBB permeability of drugs relies on the use of brain penetrating peptides. The aim of this study is to synthesize angiopep2-PAPTP and to evaluate its absorption into the brain in vivo. To design the conjugate as a pro-drug, the triphenylphosphonium (TPP+) moiety of PAPTP was modified adding a linker to one of the phenyl groups (PAPTPL), conjugated to angiopep2 through a bio-reversible carbamate bond. Angiopep2-PAPTP was administered to C57CL/6 mice (5 μmol/kg b.w.), which were sacrificed after 15 (n=5), 30 (n=4) and 60 (n=6) minutes. The results show that angiopep2-PAPTP is present in the brain at 15 and 30 minutes after the injection. The analysis of the liver showed that Angiopep2-PAPTP is mainly metabolized through the cleavage of the peptide chain. Summarizing, conjugation of PAPTP to angiopep2 represents a promising strategy to deliver PAPTP to the brain.

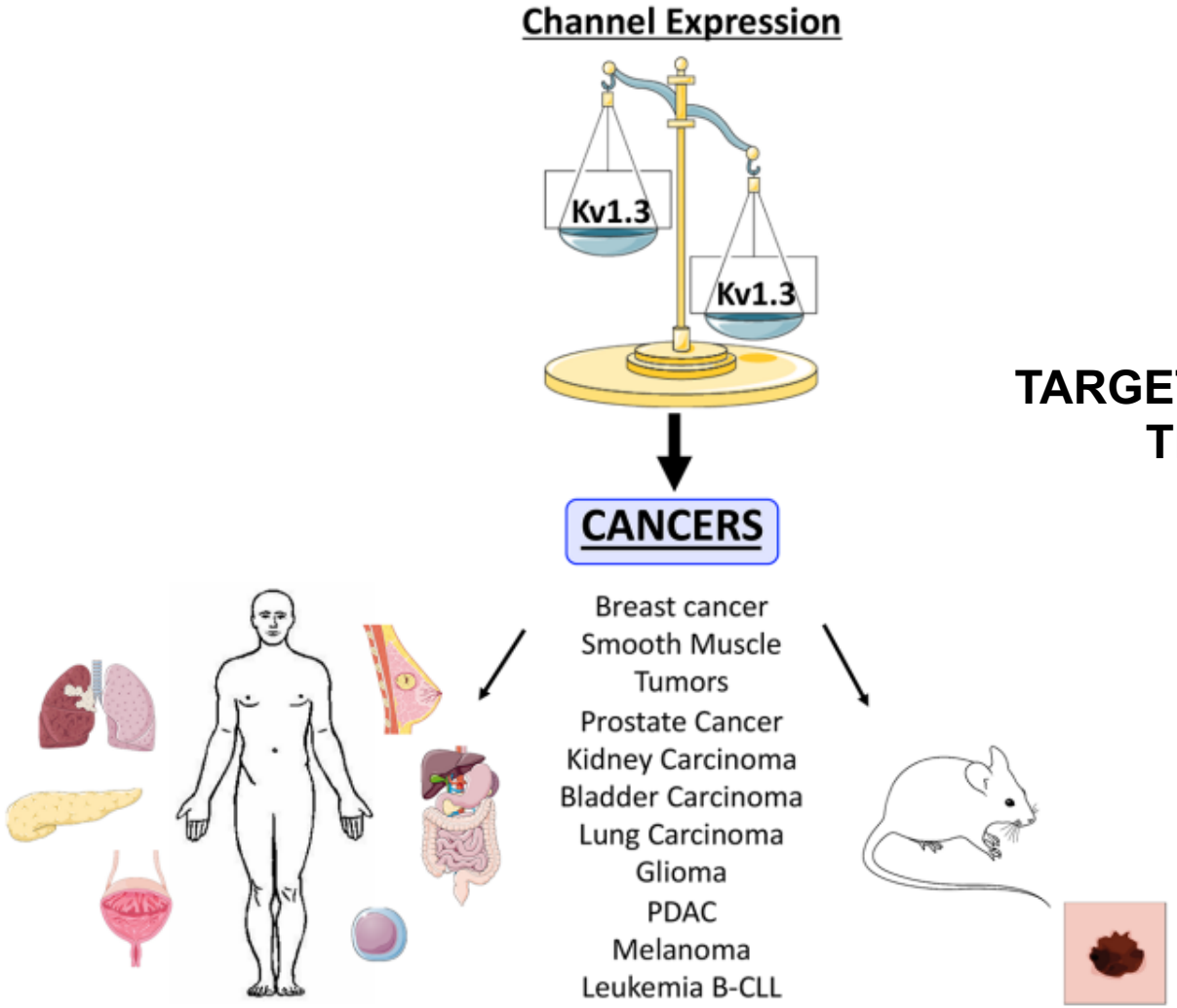
Keywords: Angiopep2; blood brain barrier; brain delivery; cancer

Kv1.3 CHANNEL



- **Plasma membrane Kv1.3:** cell proliferation, migration and metastasis
- **Mitochondrial Kv1.3:** apoptosis

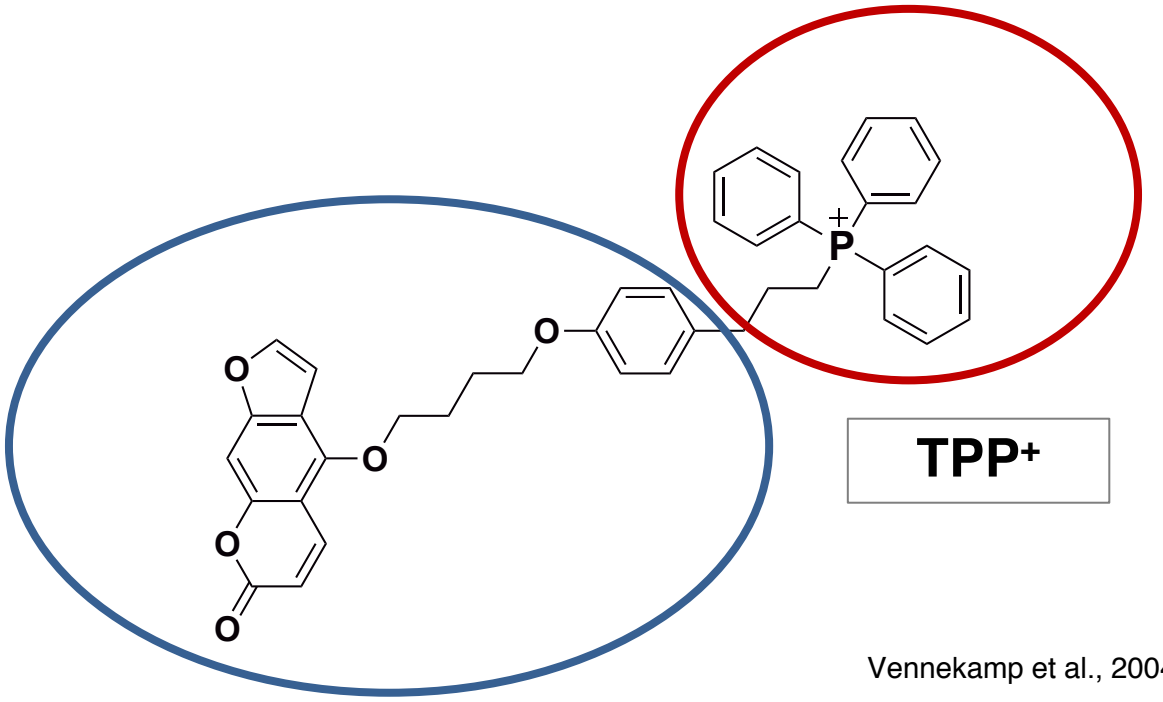
Kv1.3 CHANNEL



TARGET FOR CANCER THERAPY?

Prosdocimi et al., 2019,
Cell Physiol Biochem

PAPTP



TPP+

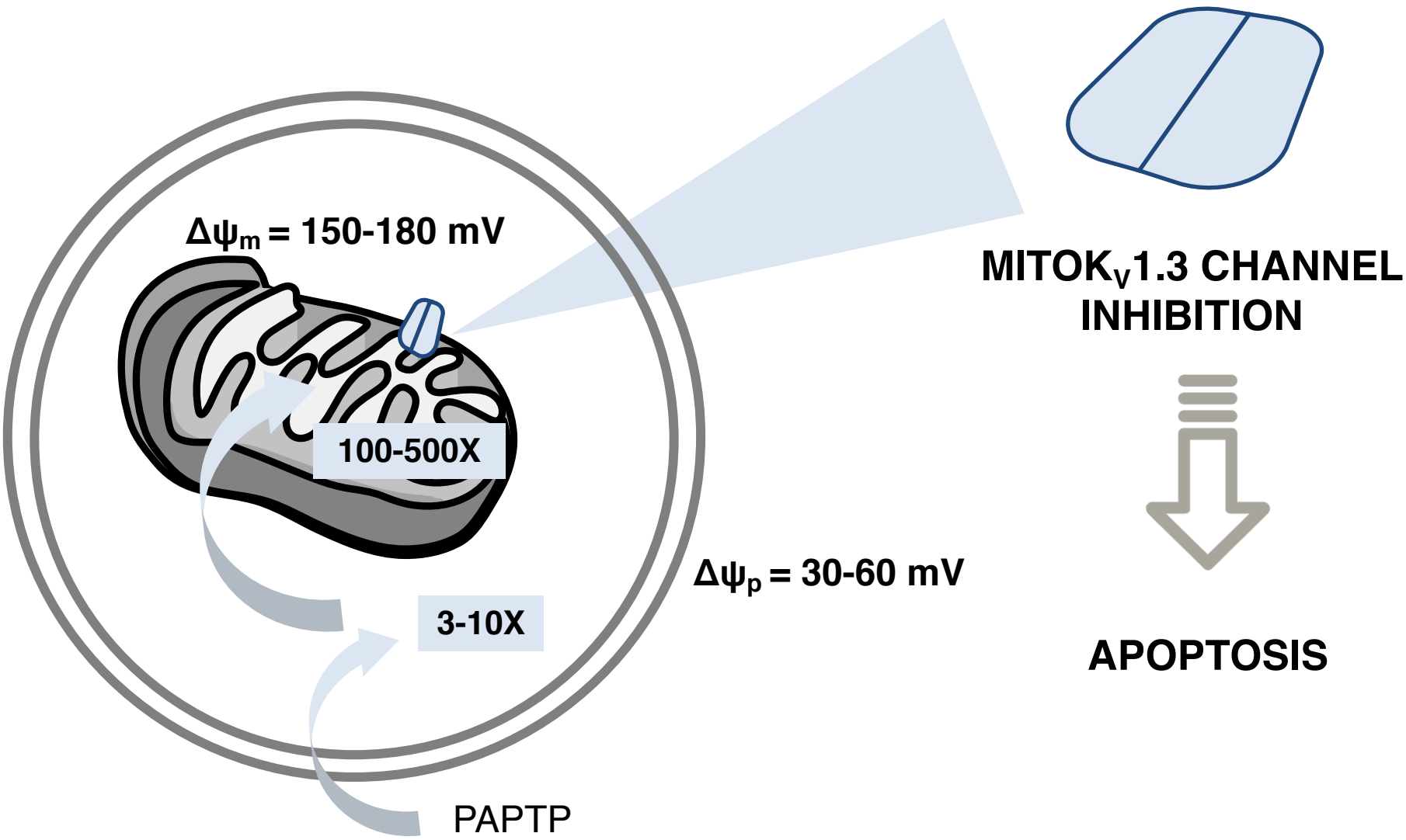
PAP-1

Vennekamp et al., 2004 *Mol Pharmacol*

PMK$_V$1.3 channel inhibition
REDUCTION OF PROLIFERATION

mitoK$_V$1.3 channel inhibition
APOPTOSIS

PAPTP

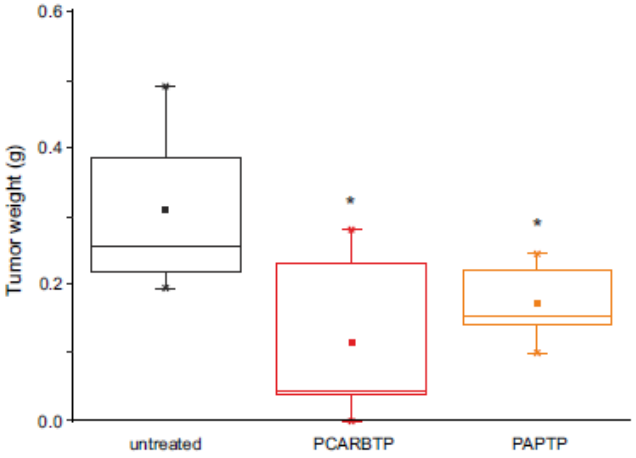

$\Delta\psi_m$ = 150-180 mV
100-500X
3-10X
PAPTP
$\Delta\psi_p$ = 30-60 mV
MITOK$_V$1.3 CHANNEL INHIBITION
APOPTOSIS

Article

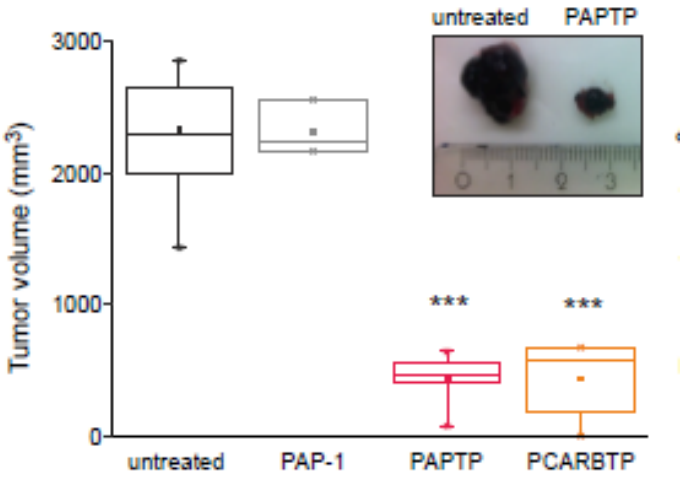
Cancer Cell

Direct Pharmacological Targeting of a Mitochondrial Ion Channel Selectively Kills Tumor Cells In Vivo

Luigi Leanza,[1,9] Matteo Romio,[2,9] Katrin Anne Becker,[3] Michele Azzolini,[4,5] Livio Trentin,[6] Antonella Managò,[1] Elisa Venturini,[3] Angela Zaccagnino,[7] Andrea Mattarei,[2] Luca Carraretto,[1] Andrea Urbani,[1] Stephanie Kadow,[3] Lucia Biasutto,[4,5] Veronica Martini,[6] Filippo Severin,[6] Roberta Peruzzo,[1] Valentina Trimarco,[6] Jan-Hendrik Egberts,[7] Charlotte Hauser,[7] Andrea Visentin,[6] Gianpietro Semenzato,[6] Holger Kalthoff,[7] Mario Zoratti,[4,5] Erich Gulbins,[3,8,*] Cristina Paradisi,[2,*] and Ildiko Szabo[1,5,10,*]



PDAC in vivo model
PAPTP 5 μmol/kg, i.p.

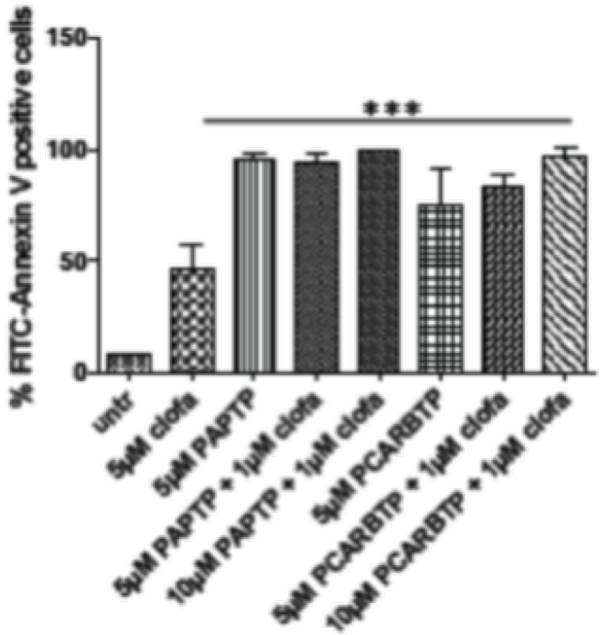


Melanoma in vivo model
PAPTP 5 μmol/kg, i.p.

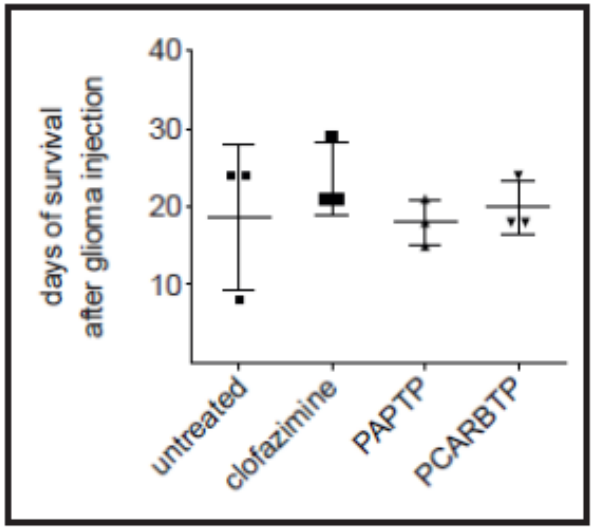
Targeting the Potassium Channel Kv1.3 Kills Glioblastoma Cells

2017

Elisa Venturini[a] Luigi Leanza[b] Michele Azzolini[c] Stephanie Kadow[a]
Andrea Mattarei[d] Michael Weller[e] Ghazaleh Tabatabai[f] Michael J. Edwards[g]
Mario Zoratti[c] Cristina Paradisi[d] Ildikò Szabò[b,c] Erich Gulbins[a,g]
Katrin Anne Becker[a]



A172 cell line

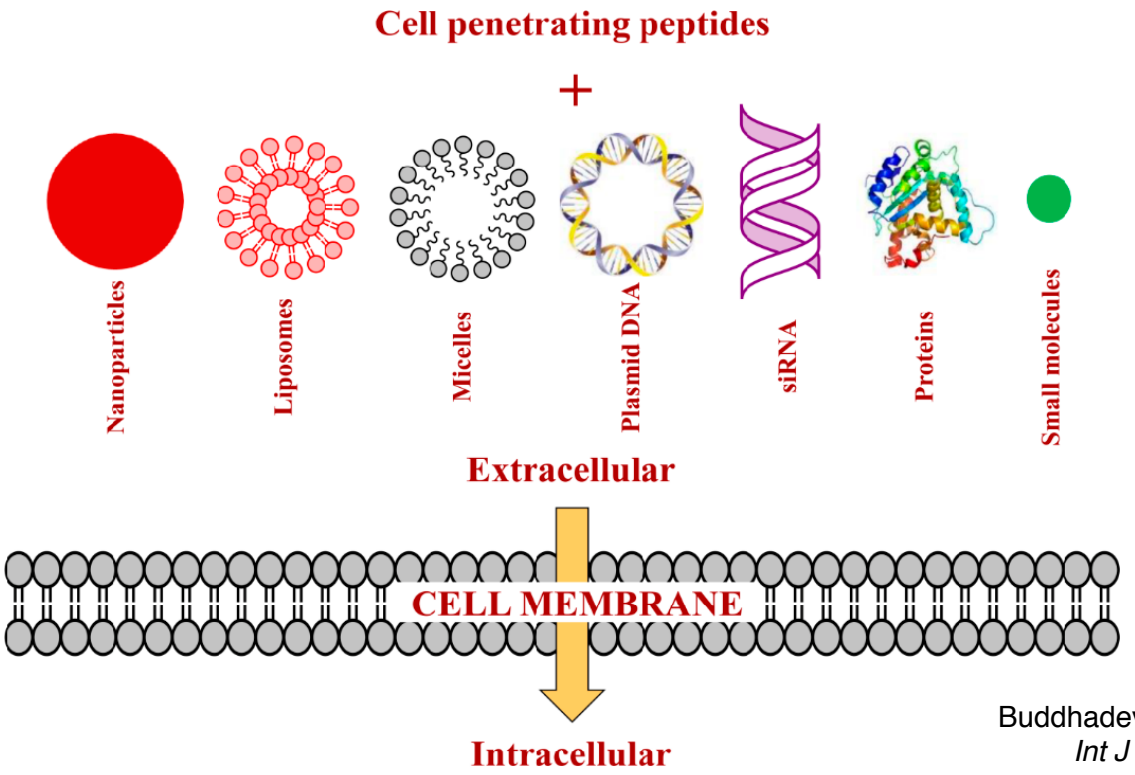


NO ACTIVITY IN AN ORTHOTOPIC ANIMAL MODEL OF GLIOBLASTOMA

Venturini et al., 2017, *Neurosignals*

PHARMACOKINETICS: NO BRAIN UPTAKE

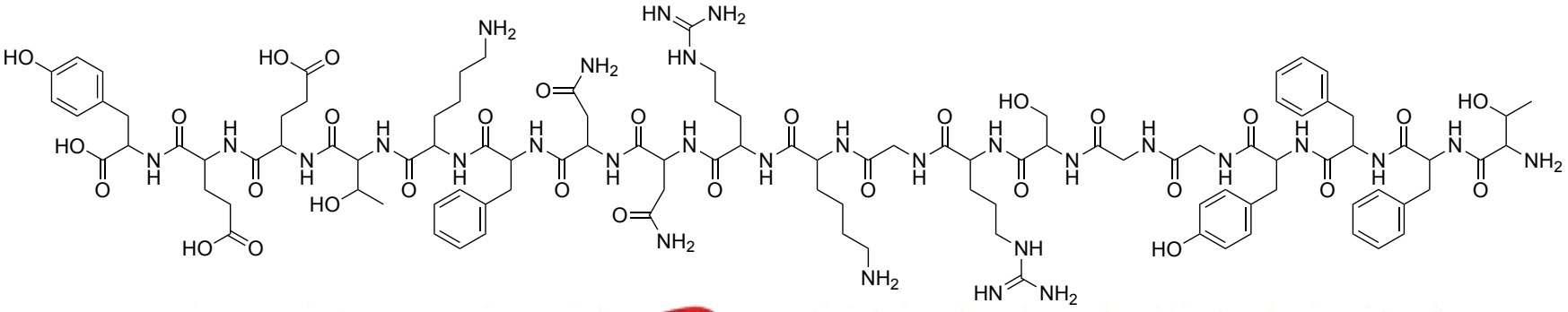
PEPTIDES AS A STRATEGY FOR BRAIN DELIVERY

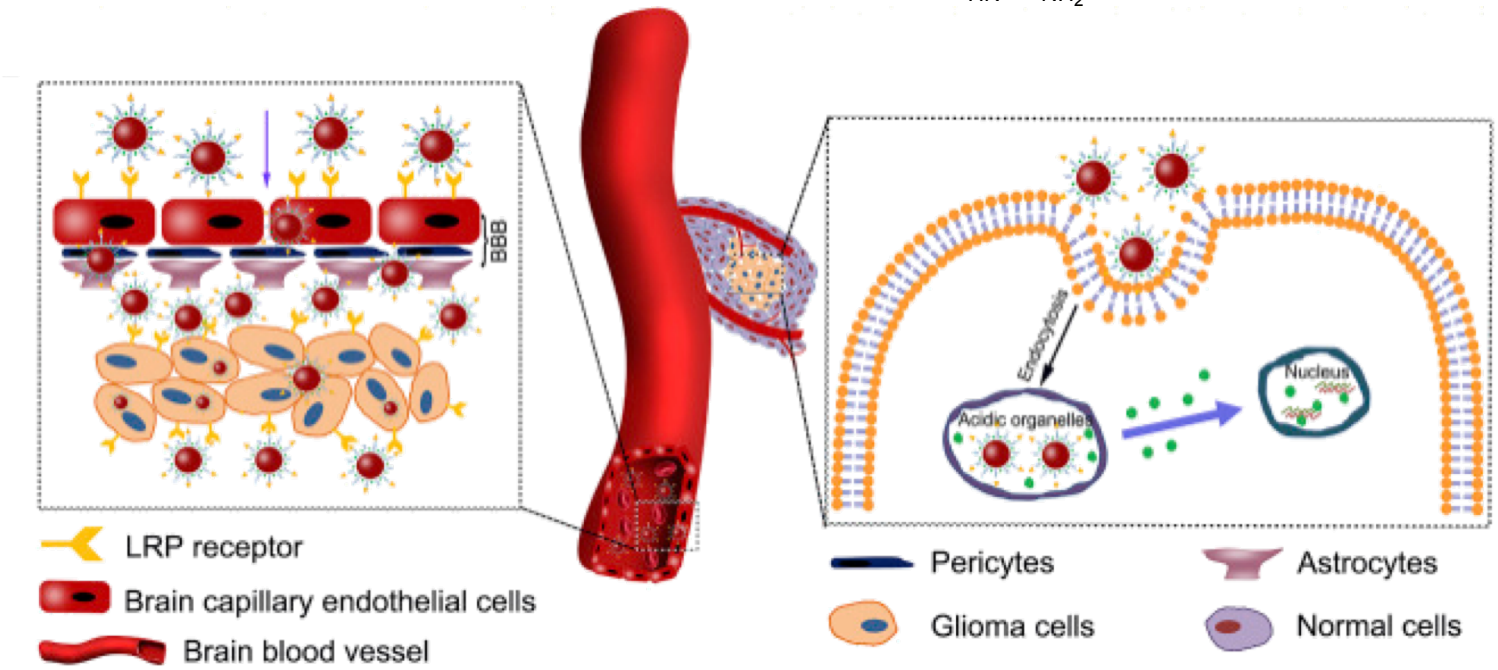


Buddhadev et al., 2015,
Int J Mol Sci

TRANSPORTER | **RECEPTOR MEDIATED** | **ABSORPTIVE** | **DIFFUSION**

Angiopep2 (An2)





Ruan et al., 2015, *Biomaterials*

Angiopep2 (An2) AS A BBB PENETRATING PEPTIDE

Molecular Cancer Therapeutics

Therapeutic Discovery

Safety, Pharmacokinetics, and Activity of GRN1005, a Novel Conjugate of Angiopep-2, a Peptide Facilitating Brain Penetration, and Paclitaxel, in Patients with Advanced Solid Tumors

Razelle Kurzrock[1], Nash Gabrail[4], Chandtip Chandhasin[1], Stacy Moulder[2], Carrie Smith[4], Andrew Brenner[3], Kamalesh Sankhala[3], Alain Mita[3], Kelly Elian[5], Danielle Bouchard[5], and John Sarantopoulos[3]

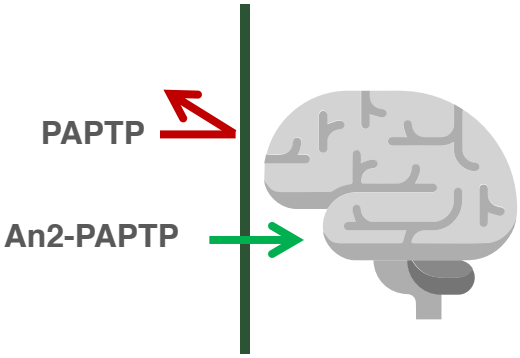
Clinical Cancer Research

Cancer Therapy: Clinical

Phase I Study of GRN1005 in Recurrent Malignant Glioma

Jan Drappatz[1,2,5,6], Andrew Brenner[7], Eric T. Wong[3,5], April Eichler[4,5], David Schiff[9], Morris D. Groves[8], Tom Mikkelsen[10], Steve Rosenfeld[11], John Sarantopoulos[7], Christina A. Meyers[8], Robert M. Fielding[12], Kelly Elian[13], Xiaolin Wang[14], Betty Lawrence[13], Mona Shing[14], Stephen Kelsey[14], Jean Paul Castaigne[13], and Patrick Y. Wen[1,2,5]

An2-PAPTP MODEL



BIO-REVERSIBLE CARBAMATE BOND

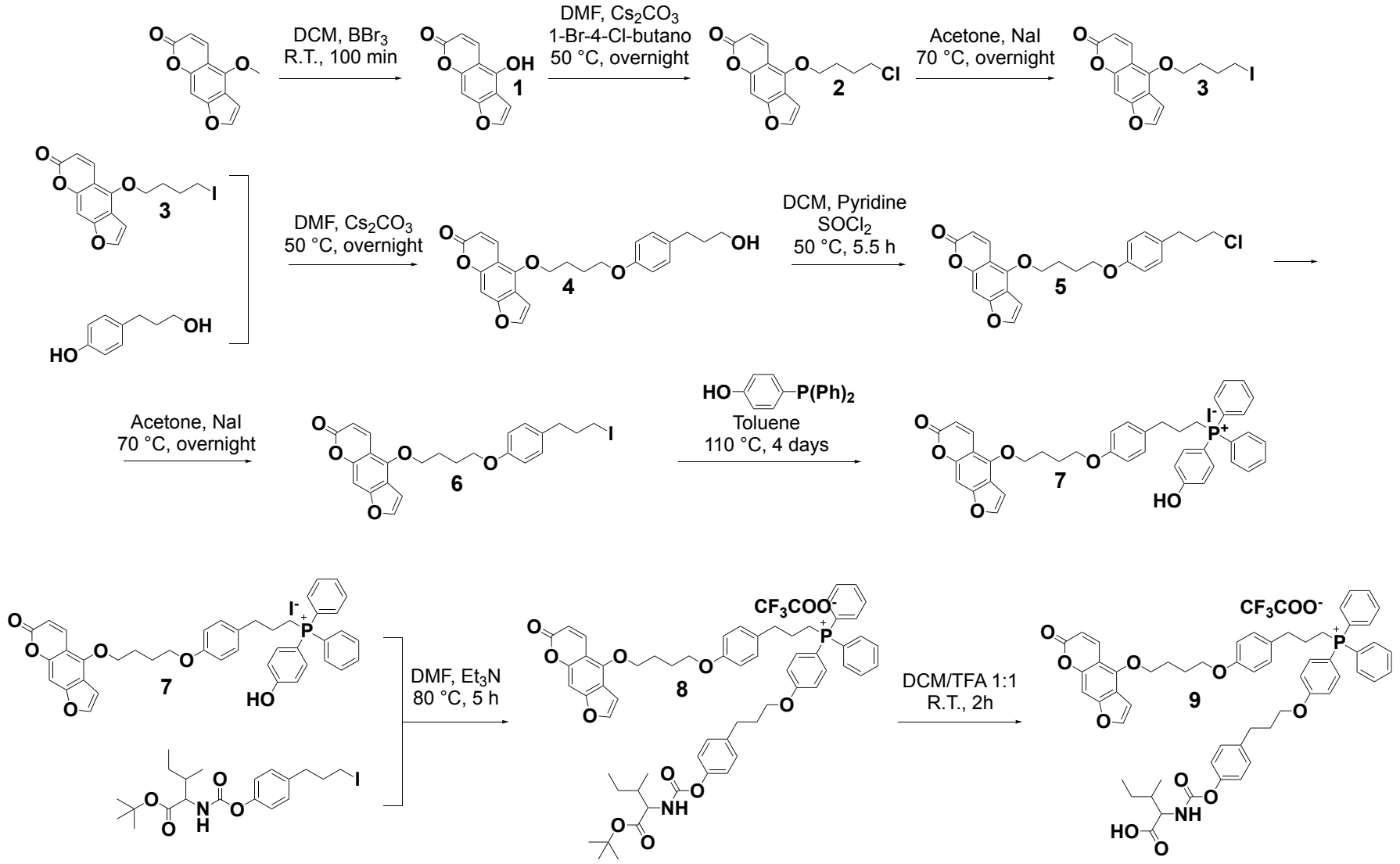
ANGIOPEP2

LINKER

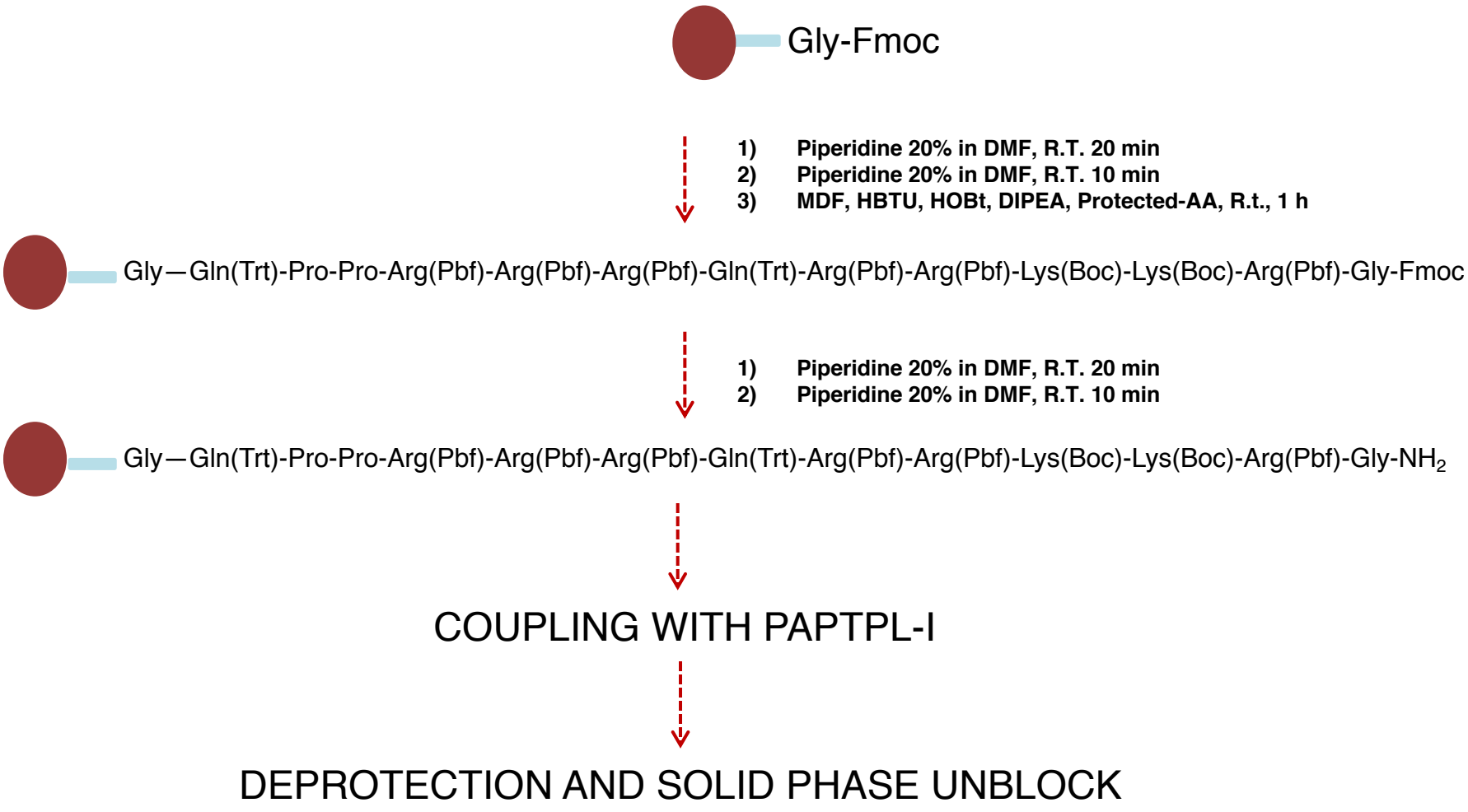
PAPTP

ISOLEUCINE

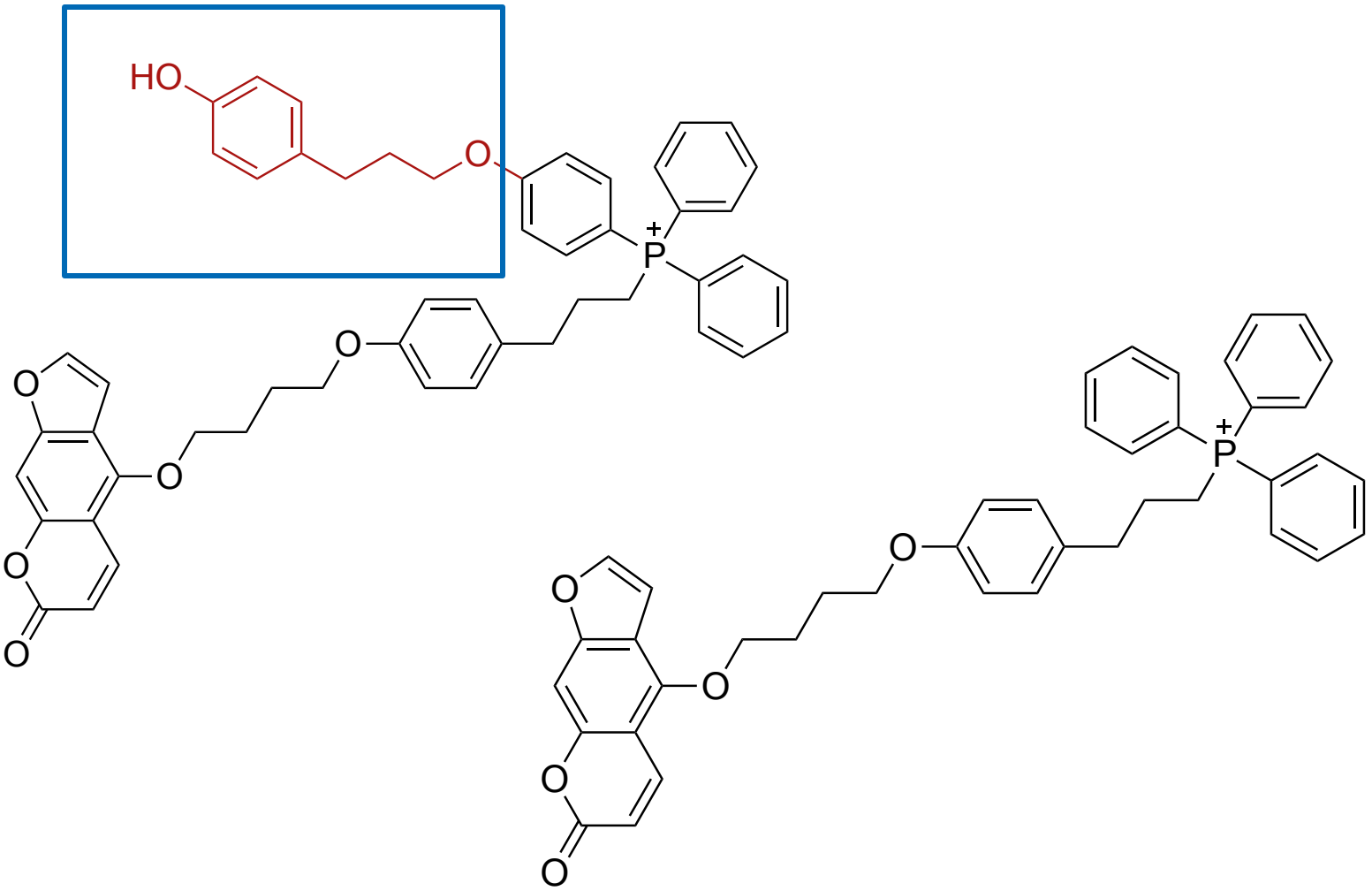
PAPTPL-I SYNTHESIS



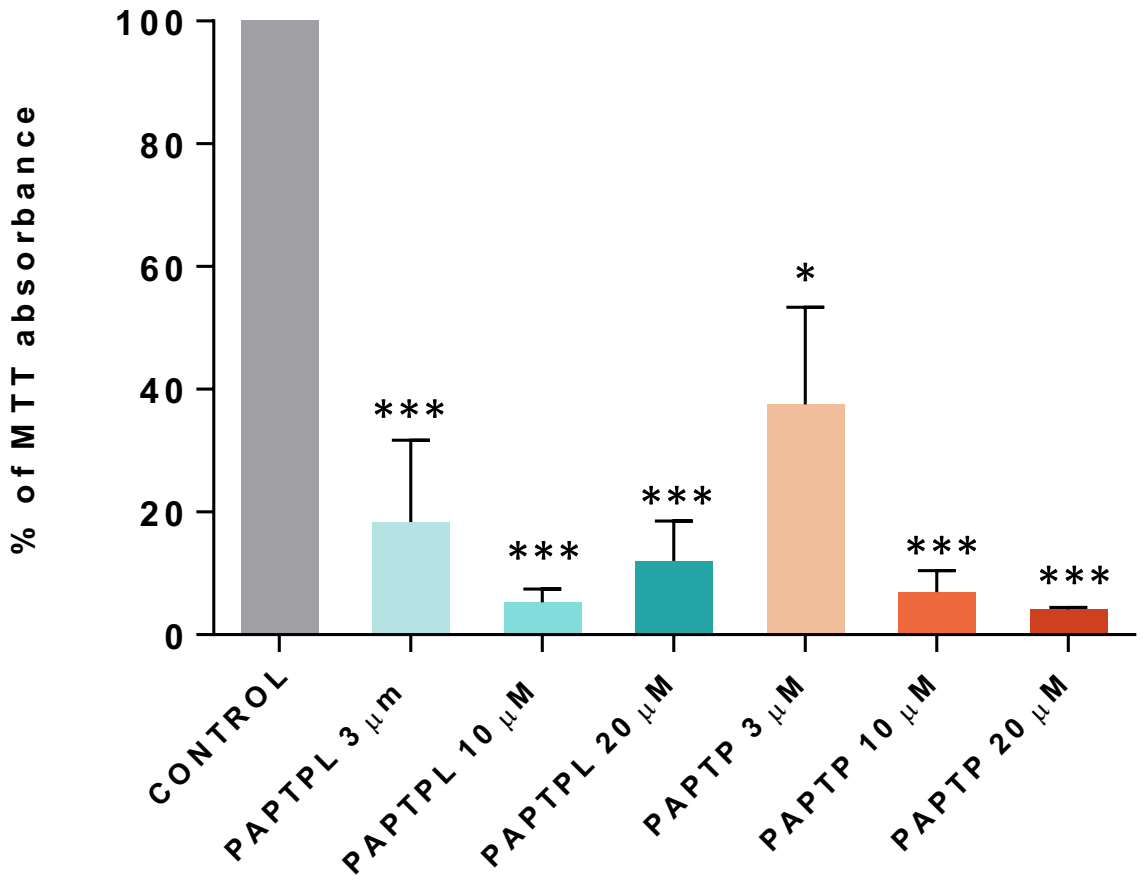
Angiopep2 SYNTHESIS



PAPTPL vs PAPTP

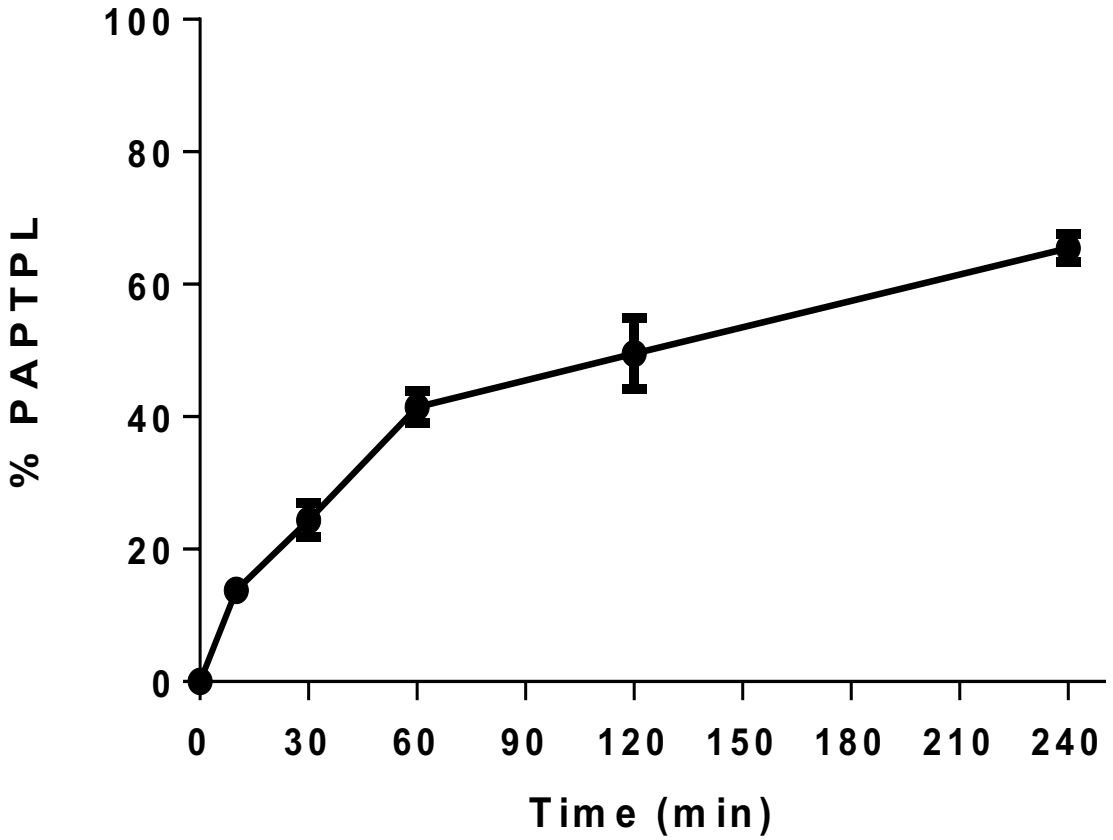


VIABILITY ASSAY



Cell viability of SHSY-5Y cells following treatment for 24 hr with the indicated compounds (MTS assay, 540 nm). Values are reported as mean percentage of viable cells normalized with respect to untreated cells (n = 3); each condition was significantly different from control: *$p < 0.05$, ***$p < 0.001$, One-way ANOVA.

An2-PAPTP BLOOD STABILITY

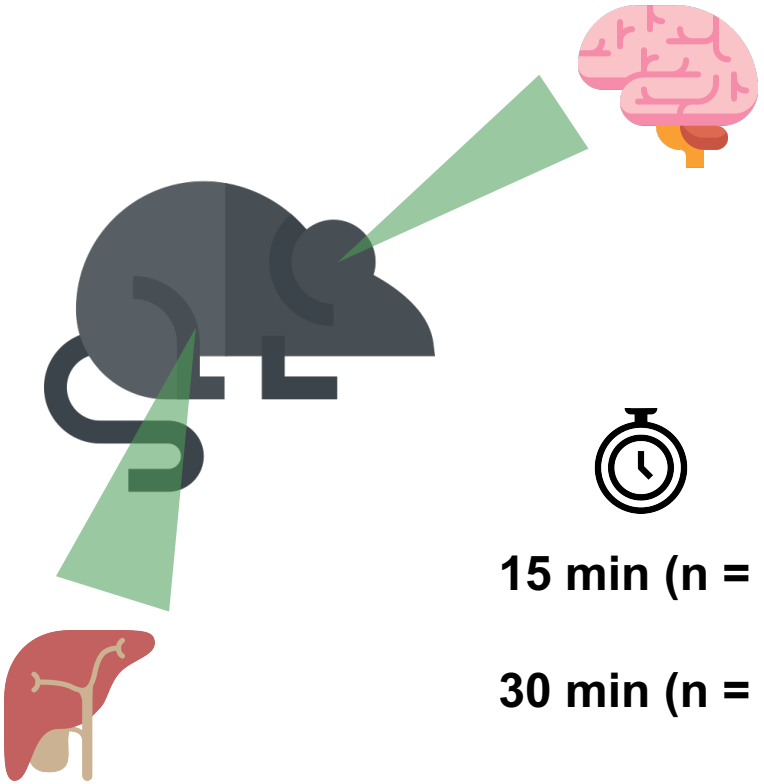


Kinetics of An2-PAPTP hydrolysis in fresh mouse blood. Data refer to the percentage of PAPTPL released and are expressed as mean ± SEM. N = 5.

An2-PAPTP DISTRIBUTION

Angiopep2-PAPTP

5 *μ*mol/kg bw; i.v.



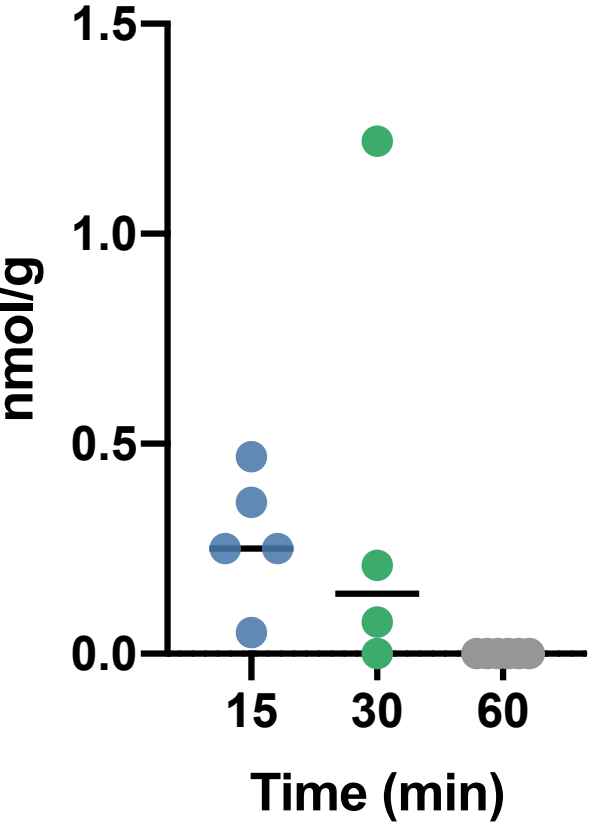
15 min (n = 5)

30 min (n = 4)

60 min (n = 6)

An2-PAPTP DISTRIBUTION

BRAIN

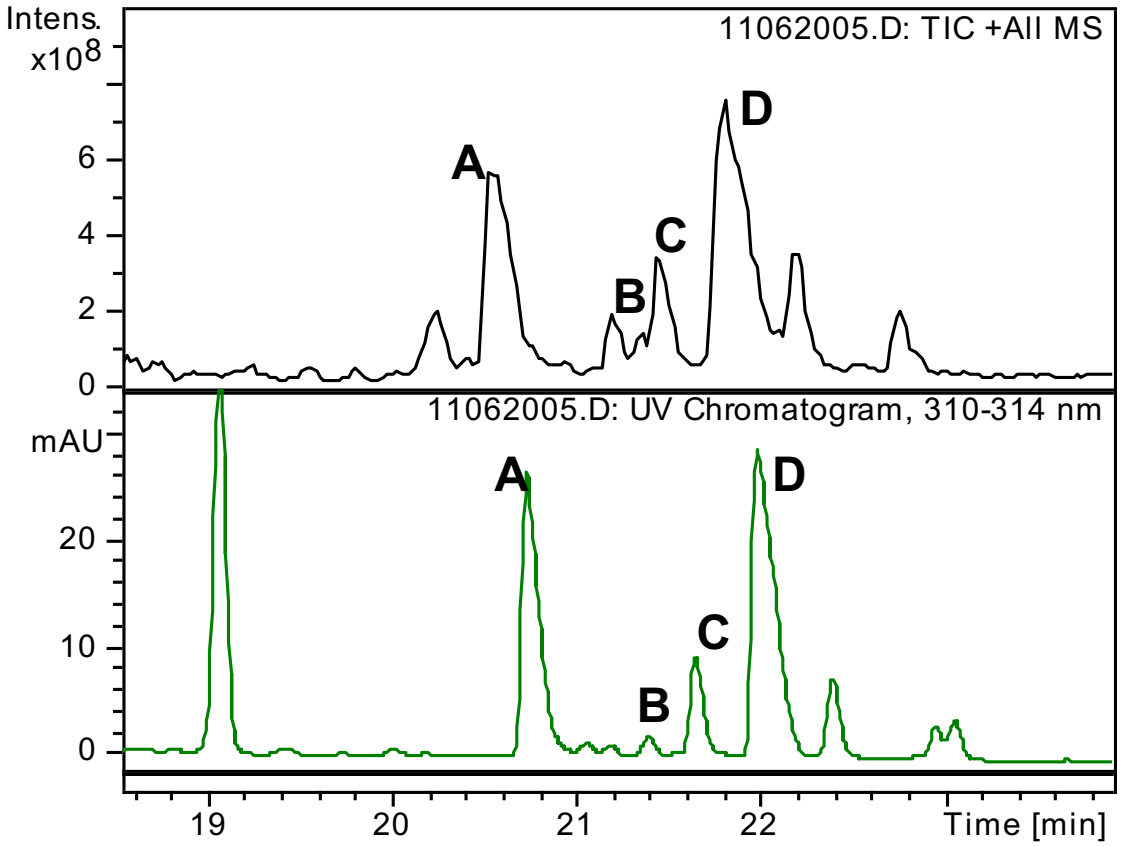


TIME OF TREATMENT (min)	Psoralenic species (nmol/g tissue)	Psoralenic species (% of injected dose)
15	0.3 ± 0.1	0.1 ± 0.04
30	0.4 ± 0.6	0.2 ± 0.2
60	traces	traces

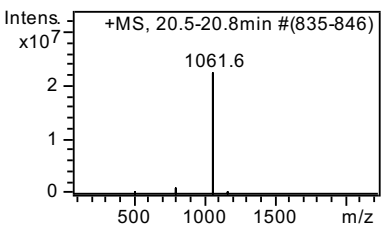
Quantification by HPLC-UV (312 nm). Data in the table are expressed as mean ± SEM (N = 5, 4 and 6 respectively).

An2-PAPTP METABOLISM

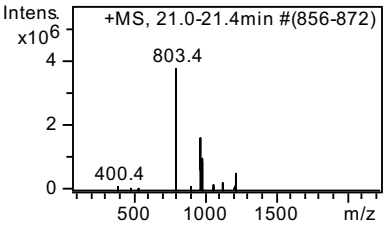
LIVER



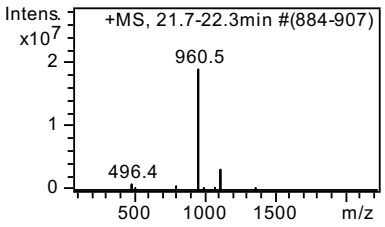
A = PAPTPL-I-T



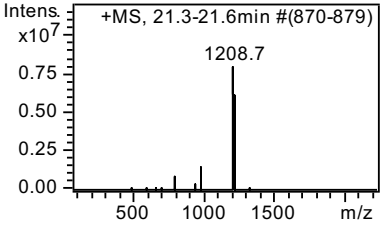
B = PAPTPL



C = PAPTPL-I



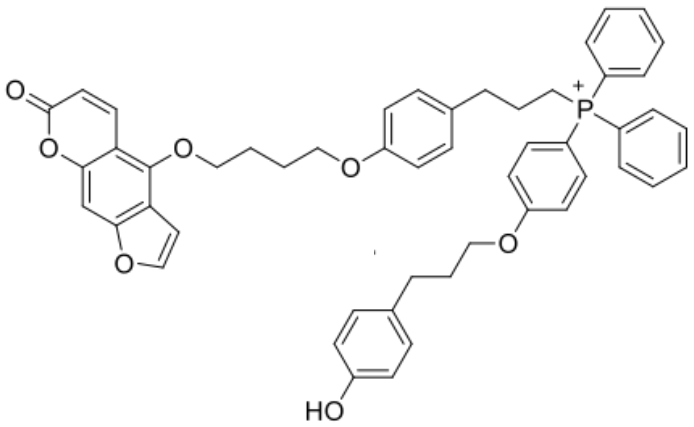
D = PAPTPL-I-TF



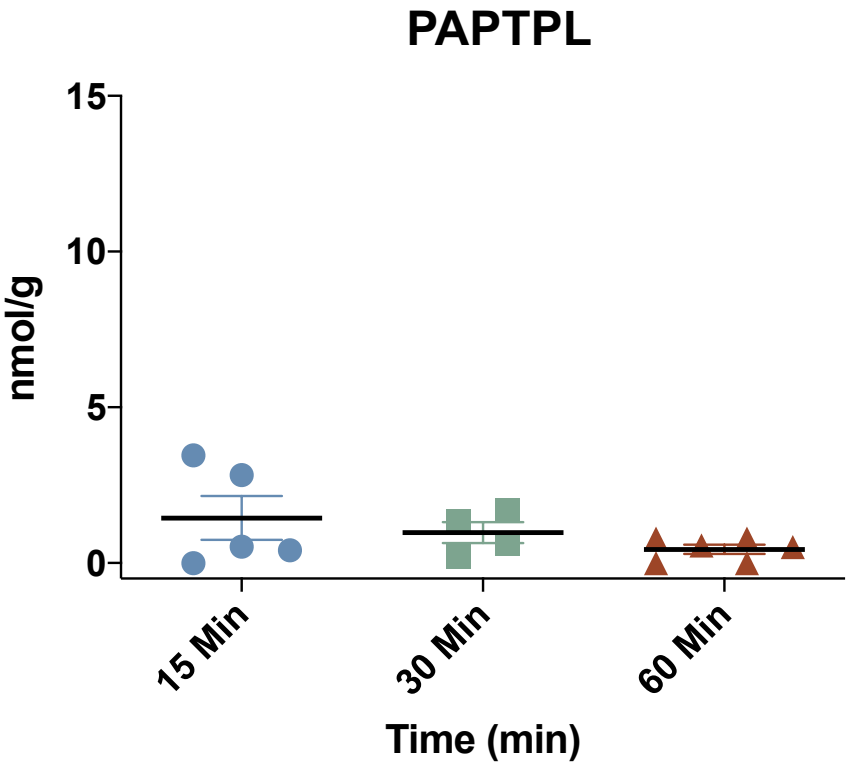
Representative HPLC/MS analysis of a liver extract, 60 minutes after treatment.

An2-PAPTP METABOLISM

LIVER



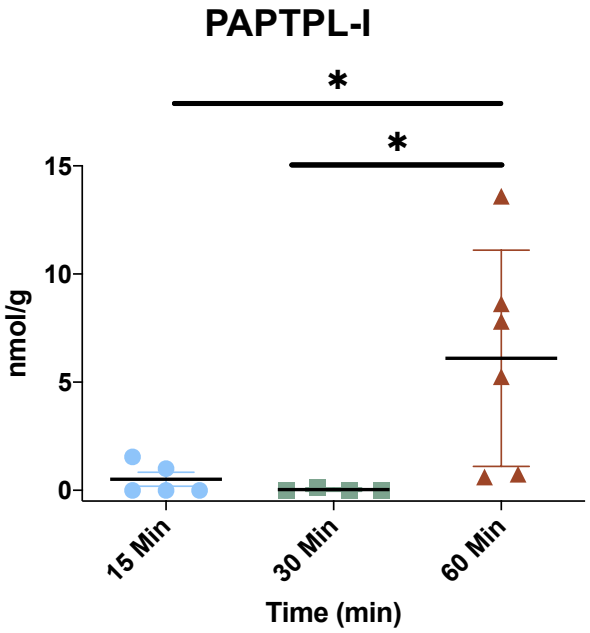
PAPTPL

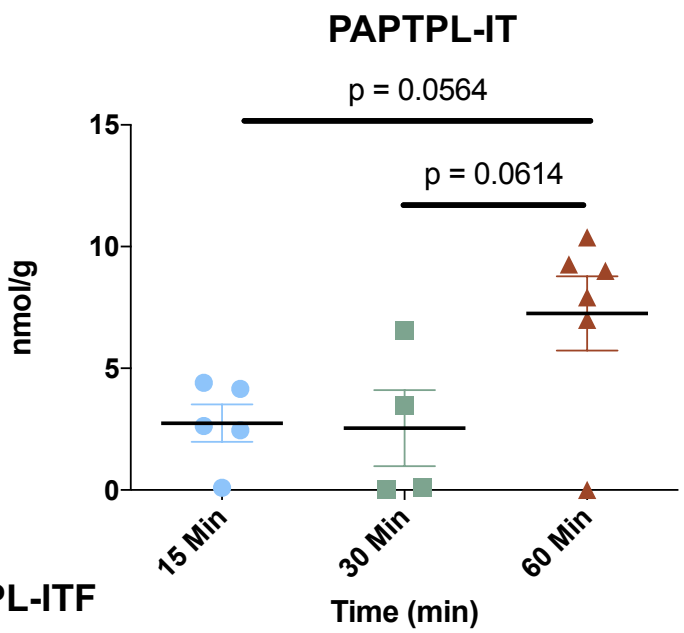


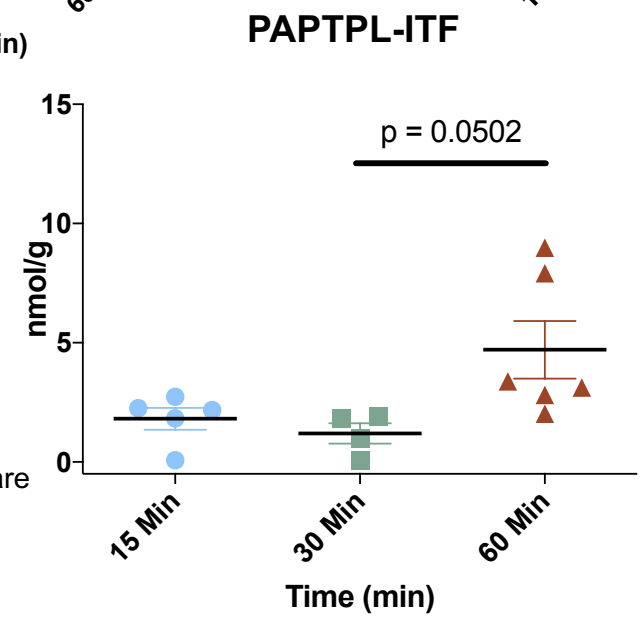
PAPTPL was quantified by HPLC-UV (312 nm). Data are expressed as mean ± SEM (N = 5, 4 and 6 respectively).

An2-PAPTP METABOLISM

LIVER



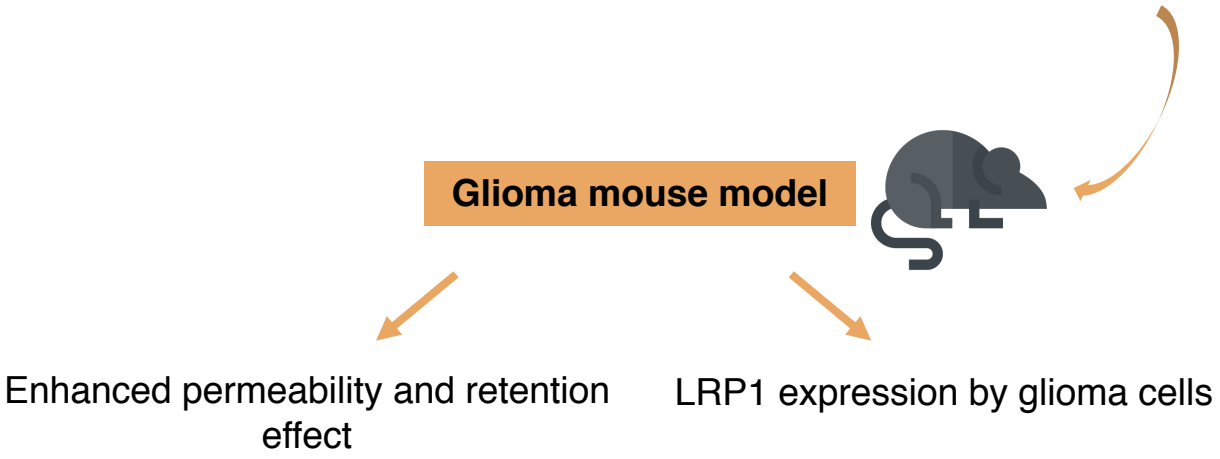




Quantifications by HPLC-UV (312 nm). Data are expressed as mean ± SEM (N = 5, 4 and 6 respectively).

CONCLUSIONS

- First evidence of PAPTP brain delivery
- Angiopep2 is successful for PAPTP brain delivery
- Brain concentrations are still low but may be improved



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Mario Zoratti

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Paolo Ruzza

Riccardo De Lorenzi

Ildikò Szabò

Tatiana Varanita

Daniele Bonesso