Synthesis and effects of Melphalan-Benzylguanidine-Hybrids on neuroblastoma and non-neuroblastoma cells

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Background

Neuroblastoma often requires aggressive treatment strategies, including chemotherapy and radiotherapy. Melphalan, containing alkylating group conjugated to phenylalanine, is a key chemotherapeutic used prior to stem cell transplantation, although its uptake into cells occurs non-specifically via amino acid transporters. In contrast, *meta*-iodobenzyl-guanidine (mIBG), as well as noradrenaline, is more selectively taken up by neuroblastoma cells through the noradrenaline transporter (NAT) and is widely used for both diagnostic ([^{123}I]/[^{124}I]-mIBG) and therapeutic ([^{131}I]-mIBG) applications.

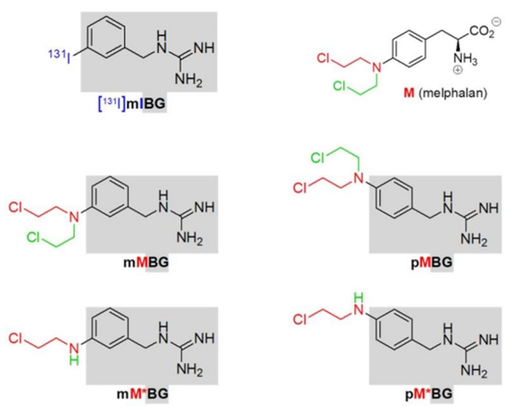

Fig. 1: Structural formulars of the compounds.

To enhance the selectivity and efficacy of chemotherapy in neuroblastoma, this study focuses on the design of hybrid molecules that combine the tumor-targeting benzylguanidine structure of mIBG with the alkylating moiety of melphalan. By leveraging NAT-mediated uptake, these novel compounds aim to improve drug delivery specifically to neuroblastoma cells while reducing off-target toxicity.

Materials & Methods

Human Neuroblastoma Cell Lines: SK-N-BE(2), SH-SY5Y, IMR-32, LS.
Human Non-Neuroblastoma Cells: SK-HEP-1 (liver adenocarcinoma), SNU-475 (liver hepatocellular carcinoma), CAKI-1 (kidney clear cell carcinoma), MRC-5 (normal lung fibroblasts), HFF (normal human foreskin fibroblasts), PBMCs (human primary peripheral blood mononuclear cells).
MTS assays: Substances were tested in the concentration range from 0.01 µM to 100 µM using the CellTiter 96® AQueous One Solution Cell Proliferation Assay (MTS) from Promega (Walldorf, Germany) following the manufacturers instructions. IC50s were calculated using GraphPad Prism 10.1.1 non-linear fit function ([inhibitor] vs. normalized response).
xCELLigence RTCA assays: Agilent (Waldbronn, Germany) xCELLigence Real-Time Cell Analysis (RTCA) technology was used to track proliferation using 0.5 µM compound for 72 h after 24 h untreated cell growth. Cell indices were normalized to last measured timepoint before treatment.
[^{3}H]NA uptake assays: [^{3}H]-Noradrenaline ([^{3}H]NA, 100 nM f.c.) uptake was measured after 15 min incubation at 37 °C in competition to 100x excess (10 µM f.c.) of the respective compound. The percentage uptake was calculated relative to the PBS control.

Results

[^{3}H]NA Uptake (Fig. 2)

- Competitive uptake assays with [^{3}H]NA revealed that both mIBG and pM*BG significantly reduce [^{3}H]NA uptake in the neuroblastoma cell lines SK-N-BE, SH-SY5Y, and IMR-32, while uptake inhibition was markedly lower in most non-neuroblastoma cell lines.
- Notably, LS cells appeared as an exception among the neuroblastoma lines, potentially due to reduced NAT expression levels.

xCELLigence RTCA assays (Fig. 3 right)

- At a compound concentration of 0.5 µM, the proliferation of non-neuroblastoma cells was less affected over time compared to neuroblastoma cell lines.
- LS cells displayed an atypical proliferation pattern under treatment compared to other neuroblastoma cells.
- Interestingly, although CAKI-1 cells are of non-neuroblastoma origin, they showed sensitivity to the hybrids pMBG and mMBG at 0.5 µM.

MTS assays (Fig. 3 left)

- The MBG hybrids mMBG and pMBG, containing two alkylating groups like Melphalan (Fig.1) exhibit cytotoxic effects comparable to melphalan in neuroblastoma cells.
- The IC_{50} values for non-neuroblastoma cells were consistently higher than those observed for the neuroblastoma cell lines SK-N-BE, SH-SY5Y, and IMR-32, indicating greater selectivity towards neuroblastoma cells. LS cells again deviated from this trend, which may be attributed to lower NAT expression.
- Surprisingly, the hybrids mM*BG and pM*BG, containing only one alkylating group, demonstrate a stronger cytotoxic effect than melphalan.

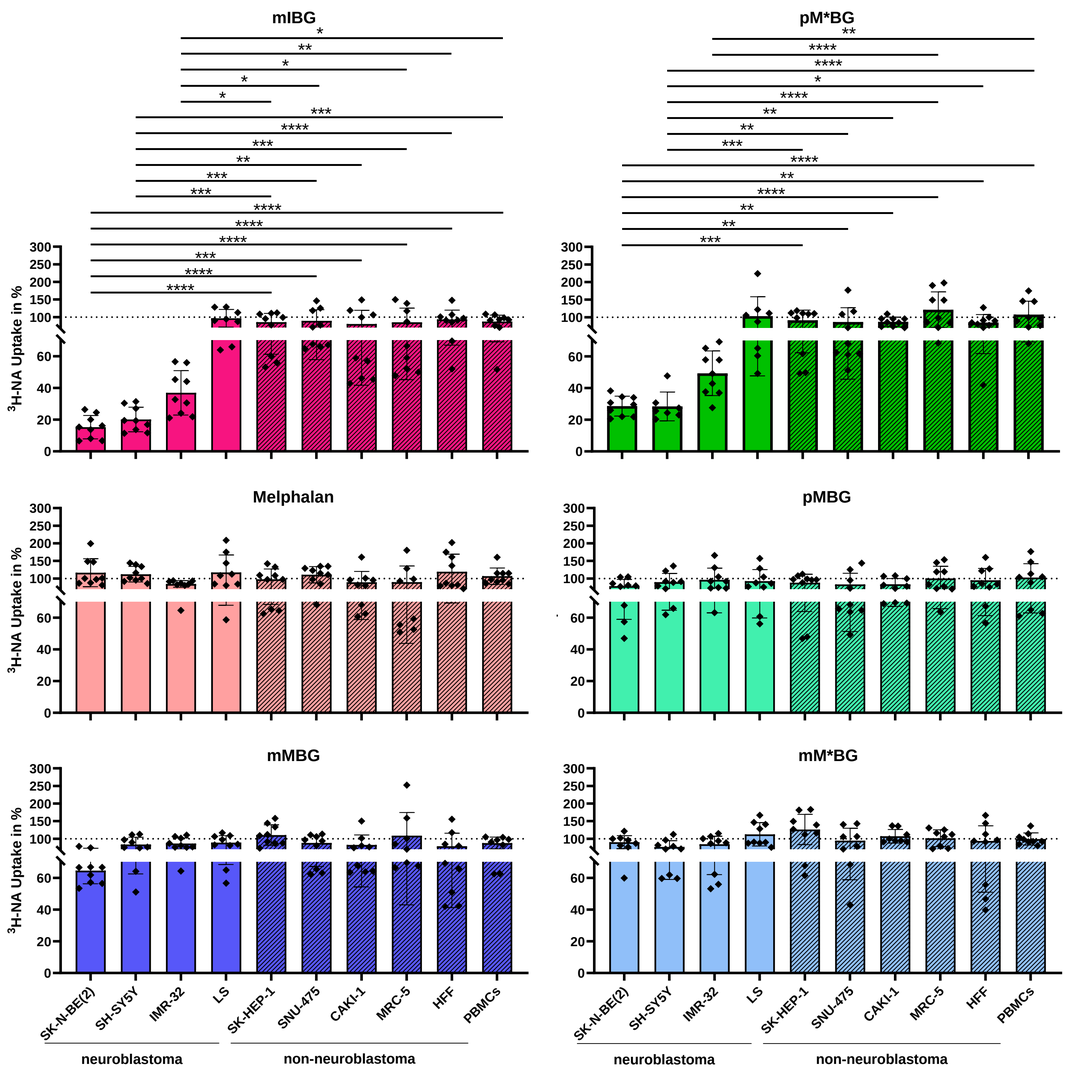

Fig. 2: Uptake of [^{3}H]NA within 15min at 37 °C into cells in a PBS control, designated as 100 % uptake (dotted line), and in the presence of a 100x ecess of the stated compound. Mean value ± SD based on 3 independent experiments, each of which was performed in triplicates. Statistical significance was estimated using a 2way ANOVA and a Turkey's multiple comparisons test. ns: p ≥ 0.05 (not shown), *: p = 0.01 to 0.05, **: p = 0.001 to 0.01, ***: p = 0.0001 to 0.001, ****: p ≤ 0.0001.

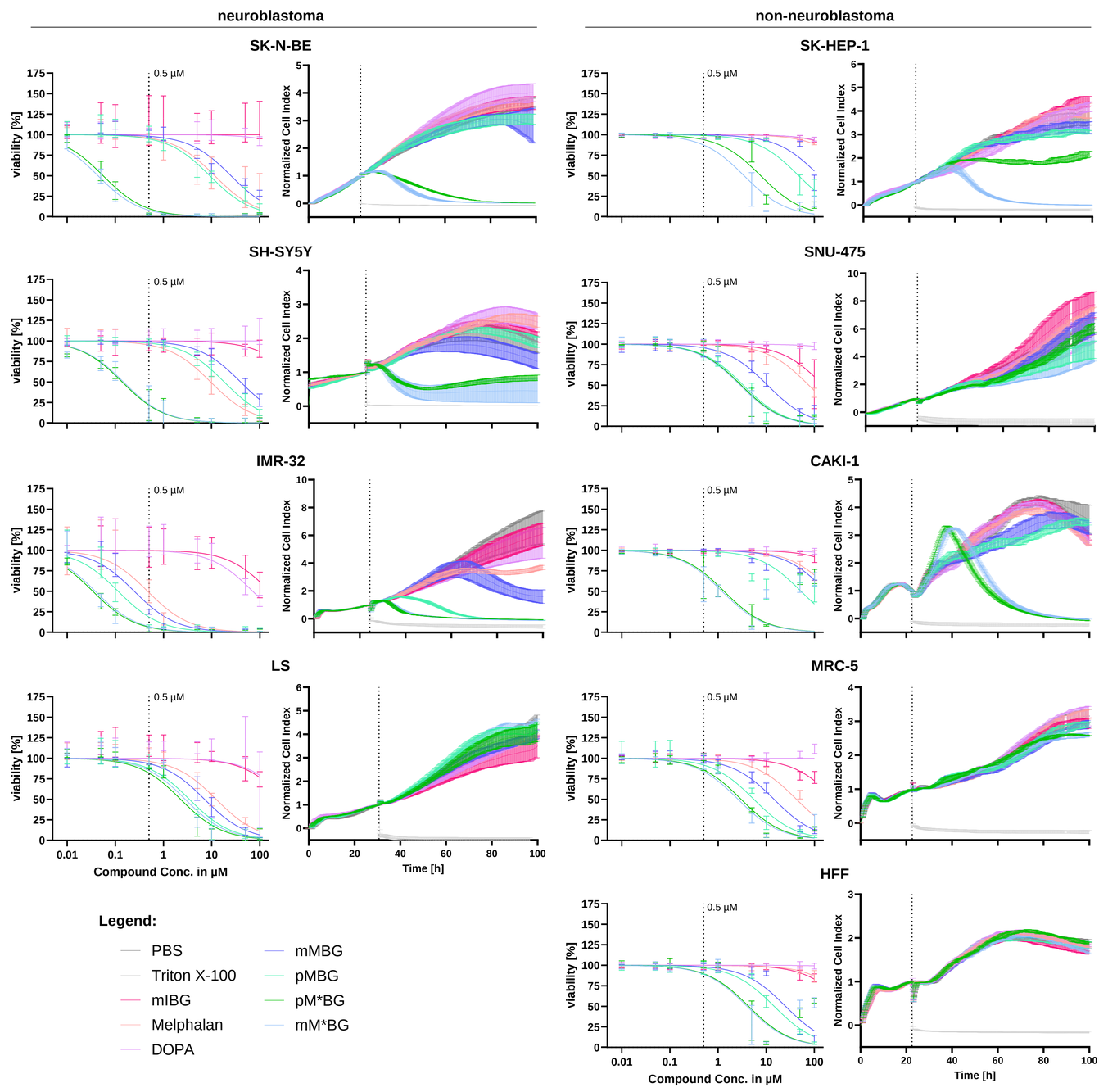

Fig. 3: *Left:* MTS viability assays after incubation with the stated compound for 72 h at 37 °C. Mean value ± SD based on 3 independent experiments, each of which was performed in triplicates. Dotted line indicates 0.5 µM. *Right:* Real-time proliferation monitoring over 72 h in the presence of 0.5 µM of the stated compound after 24h cell seeding. Cell indices were normalized to last measured time point before treatment (dotted line). Triton X-100 (1%) was used as positive control, PBS as negative control. Experiments were performed in triplicates. Mean value ± SD based on triplicates.

Conclusions and Outlook

This study demonstrates that mIBG and the MBG hybrid pM*BG are specifically taken up by neuroblastoma cells via NAT. Among the tested compounds, pM*BG closely mirrors the uptake behavior of mIBG, highlighting its potential as a neuroblastoma-targeted therapeutic agent. In cytotoxicity assays, the MBG hybrids mMBG and pMBG showed comparable effects to melphalan, while mM*BG and pM*BG exhibited even greater cytotoxicity against neuroblastoma cells, indicating a selective toxicity profile favoring neuroblastoma cells. Future studies will focus on *in vivo* evaluation of the pharmacokinetics, biodistribution, and therapeutic efficacy.

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Gesellschaft für KinderKrebsForschung
Mehr Forschung. Mehr Leben.

Reference

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