^{125}I-labelling and biological evaluation of a *closo*-borane containing Tyr^3-octreotate derivative

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Introduction: Despite the improvements in cancer therapy during the last years, high-grade gliomas and many other types of cancer are still extremely resistant to all current forms of therapy. Boron neutron capture therapy (BNCT) provides a way to destroy cancer cells without damaging healthy tissue. However, BNCT in practice is still limited because of the lack of boron containing compounds which selectively deliver boron to cancer cells [1]. Since many neuroendocrine tumours show an overexpression of the somatostatin receptor, it was our aim to synthesize compounds, which contain a large number of boron atoms which are still highly affine towards this transmembrane receptor. The synthetic peptide Tyr^3-octreotate (Tyr^3-TATE) was chosen as highly affine tumour targeting vector (TTV) which was coupled with a (sarcosine)$_5$-(glycine)$_1$-spacer and linked with a *closo*-borane conjugated compound. The obtained affinity data (1.8 ± 0.3 nM) demonstrate that the use of a spacer between TTV and *closo*-borane conjugated compound is an option to avoid a loss of the biological affinity of *closo*-borane conjugated peptides [2]. Due to these promising results biological studies were performed by the use of a [^{125}I]-labelled *closo*-borane conjugated Gly-(Sar)$_5$-Tyr^3-TATE.

Experimental: Peptide synthesis has been achieved on a fully automated synthesizer using Fmoc/^{t}Bu-building blocks. On-resin disulfide formation were performed by simultaneous deprotection and oxidation of the Cys(Acm) protecting groups. Afterwards, the *closo*-borane compound was conjugated. Purification of the final product was achieved by RP-HPLC after cleavage from the solid support.

[^{125}I]-labelling of the tyrosine moiety was achieved in a buffered DMSO solution. The product was purified by RP-HPLC [3]. Serum stability of the labelled compound was determined in human serum incubated at 37 °C and analysed by radio-RP-HPLC. For binding studies, 700000 cells (AR42J) were seeded into 6-well plates and cultivated at 37 °C for 24 h. ^{125}I-labelled peptide was added to the cells (1×10^6 cpm/well) and incubated for appropriate times. Radioactivity was determined with a γ-counter and calculated as percentage applied dose per 10^6 cells.

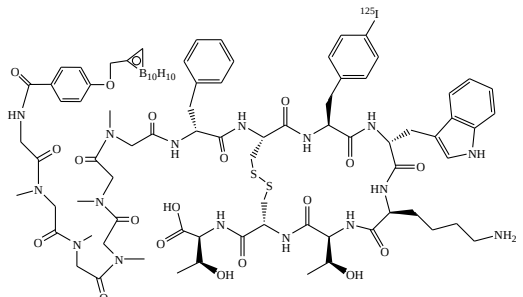

Figure 1: [^{125}I]-labelled *closo*-borane conjugated Gly-(Sar)$_5$-Tyr^3-TATE.

Results: Radioiodination of the tyrosine moiety of the *closo*-borane conjugated Tyr^3-TATE gave a labelling yield of 88 ± 15 % and an overall radiochemical yield (RCY) of 45 ± 15 % after HPLC-purification. HPLC quality controls of the labelled compound verified a radiochemical purity of > 99 %. The incubation in human serum demonstrated a biological half life of 31 h. The AR42J-cell assay showed a binding of the *closo*-borane conjugate of up to 12 % compared to the Tyr^3-octreotate with 44 % after 4 h.

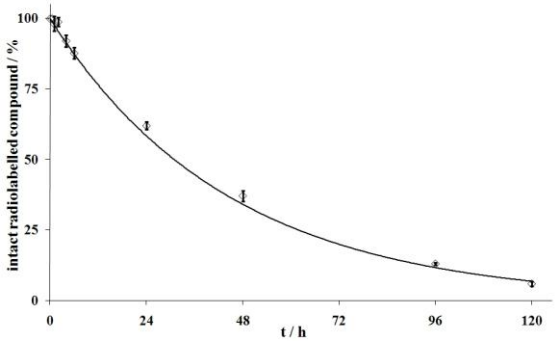

Figure 2: Stability of the [^{125}I]-labelled *closo*-borane conjugated Tyr^3-TATE in human serum incubated at 37 °C.

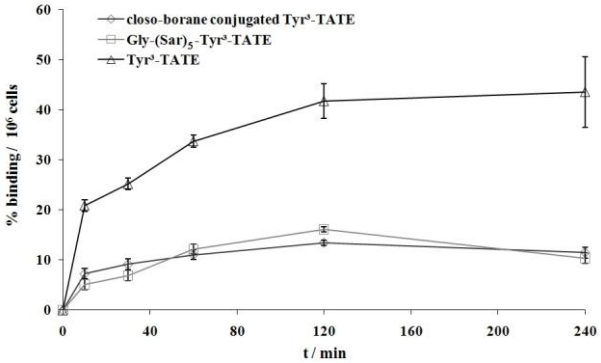

Figure 3: Cellular binding of the [^{125}I]-labelled *closo*-borane conjugated Tyr^3-TATE and its substructures.

Conclusions: A *closo*-borane Tyr^3-TATE derivative has been successfully labelled in good RCY and very high radiochemical purity, ready for its usage in biological evaluations. The serum stability and cellular binding properties reveal the potential application in BNCT.

Further evaluations such as organ distribution, pharmacokinetics and molecular imaging studies are in progress.

References

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