Synthesis of novel selective histone deacetylase inhibitors for the development of a suitable ^{18}F-labelled radiotracer for the molecular imaging of HDAC1 in brain tumours

Oliver Clauß[1], Magali Toussaint[1], Linda Schäker-Hübner[2], Barbara Wenzel[1], Winnie Deuther-Conrad[1], Sladjana Dukic-Stefanovic[1], Friedrich-Alexander Ludwig[1], Daniel Gündel[1], Rodrigo Teodoro[1], Klaus Kopka[1], Peter Brust[1], Finn K. Hansen[2] and Matthias Scheunemann[1]

[1] *Helmholtz-Zentrum Dresden-Rossendorf, Institute of Radiopharmaceutical Cancer Research, Department of Neuroradiopharmaceuticals, Research site Leipzig, Germany*

[2] *Bonn University, Pharmaceutical Institute, Pharmaceutical and Cell biological Chemistry, 53121 Bonn, Germany*

Objectives: Epigenetic mechanisms like methylation and acetylation of histones regulate the gene expression on the chromatin level. Thus, the degree of acetylation of lysine residues on histones influences the accessibility of DNA and furthermore the gene expression. Histone deacetylases (HDACs) are overexpressed in various tumour diseases, resulting in the interest in HDAC inhibitors (HDACi) for cancer therapy. The aim of this work is the development of a novel ^{18}F-labelled HDAC1-selective inhibitor with an *ortho*-aminoanilide zinc-binding group (ZBG) to visualize this enzyme in brain tumours by positron emission tomography (PET).

Methods: Based on the selective HDAC1-3 inhibitors tacedinaline and entinostat, a series of fluorine-containing derivatives was synthesized and the IC_{50} values were determined by an in-house biochemical enzyme assay. Out of several ligands with high inhibitory potency and selectivity for HDAC1, *N*-(2-amino-5-(thiophen-3-yl)phenyl)-4-((2-fluoropropanamido)methyl)benzamide (**BA3**, **Figure 1A**) was selected for radiofluorination. The two-step one-pot radiosynthesis of [^{18}F]**BA3** was performed by a nucleophilic aliphatic substitution reaction of the protected 2-bromopropionyl precursor **2** and subsequent deprotection. The process was successfully transferred to a TRACERlab FX2 N radiosynthesizer (**Figure 1B**). For the characterization of **BA3**, the *in vitro* stability in mouse and human liver microsomes and the cell toxicity in glioblastoma cell lines (U251-MG, F98) were assessed. In parallel, the *in vivo* metabolism of [^{18}F]**BA3** was investigated (mouse plasma and brain samples, 30 min p.i.) as well as PET studies in mice were carried out.

Results: BA3, containing a PAMBA linker (*para*-aminomethylbenzoic acid), shows a high inhibitory activity against HDAC1 and high selectivity towards HDAC3 and HDAC6 (see **Table 1**). The cell viability of U251-MG and F98 cells after incubation with 50 µM **BA3** for 72h was only 64% and 36%, respectively. The automated radiosynthesis of [^{18}F]**BA3** resulted in a radiochemical yield of 1%, a radiochemical purity of > 96% and a molar activity between 21 and 51 GBq/µmol (n = 5, EOS). The PET studies in mice showed a low [^{18}F]**BA3** accumulation in the brain, suggesting a low blood-brain barrier penetration ($SUV_{5\ min}$: 0.24). Furthermore, the amount of intact radiotracer in the brain and plasma at 30 min p.i. was only 25% and 7%, respectively.

Conclusion: Due to the low blood-brain barrier penetration and the high amount of brain-penetrable radiometabolites, [^{18}F]**BA3** is classified as unsuitable for further PET-related investigations. The obtained results will be used in the design of metabolically more stable HDAC inhibitors.

Reference: [1] Krieger et al., *J. Med. Chem.* **2019,** *62*(24), 11260-11279.

Table 1: IC_{50} values of the lead structures tacedinaline and entinostat and the synthesized compound **BA3** towards HDAC1-3 and HDAC6

	IC_{50} [nM]			
Compound	**HDAC1**	**HDAC2**	**HDAC3**	**HDAC6**
BA3	4.8 ± 0.6	39.9 ± 3.2	> 1110[a]	> 10 000[b]
Tacedinaline	636 ± 114	696 ± 11	263 ± 31	> 10 000[b]
Entinostat[c]	519 ± 63	505 ± 28	2850 ± 220	> 10 000

[a] < 30% inhibition at 1110 nM; [b] < 10% inhibition at 10 000 nM; [c] data taken from reference [1]

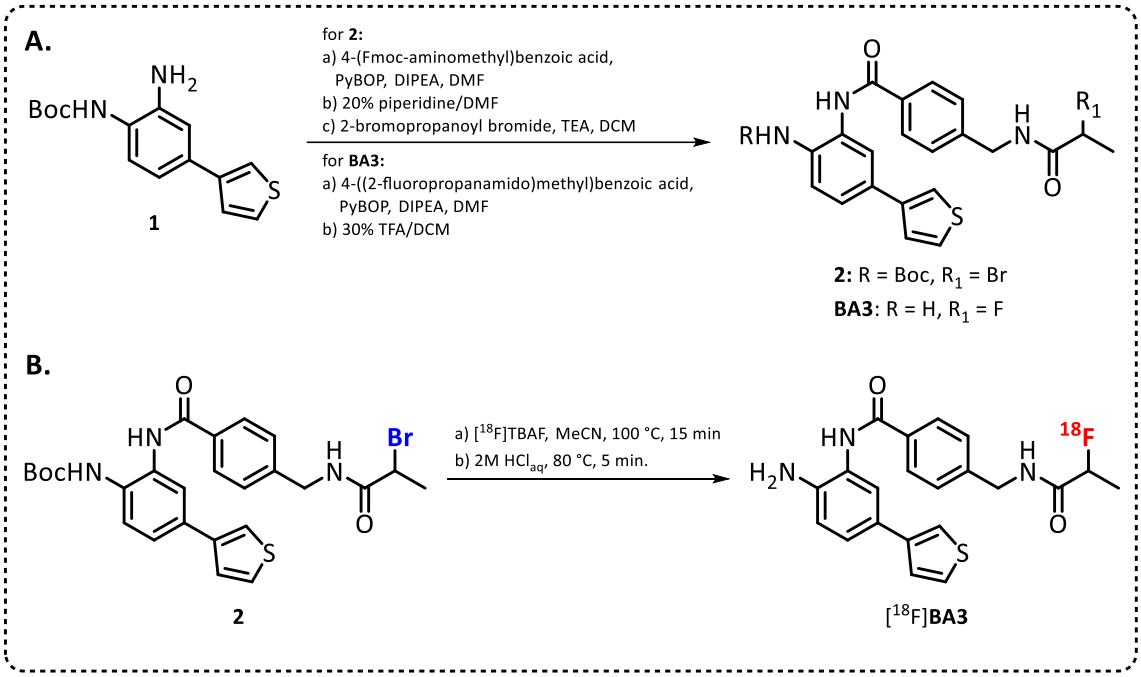

Figure 1: **A.** Strategy for the synthesis of **BA3** and **2**; **B.** Radiosynthesis of [^{18}F]**BA3**.