[^{18}F]ALX5406: A Brain-Penetrating Prodrug for GlyT1-Specific PET Imaging

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Objectives ALX5407 (**1**) is a potent and selective inhibitor of glycine transporter type 1 (GlyT1) originally developed for the treatment of certain neurologic disorders like cognitive decline or schizophrenia. While it did not reach clinical trials, ALX5407 could provide a starting point for development of GlyT1-selective PET tracers and was previously radiolabeled with carbon-11, but no preclinical studies have been published so far. The aim of the present work was to prepare the ^{18}F-labeled counterpart [^{18}F]ALX5407 ([^{18}F]**1**) as well as its methyl ester [^{18}F]ALX5406 ([^{18}F]**2**), and to subject both candidate tracers to a preclinical evaluation.

Methods The radiolabeling precursor was prepared by asymmetric reduction of 4'-bromo-3-chloropropiophenone into the respective (*R*)-alcohol (97% *ee*), followed by etherification via Mitsunobu reaction with 4-phenylphenol (>95% *ee*), amination with sarcosine methyl ester and finally Miyaura borylation. The radiosynthesis was performed using the protocol for alcohol-enhanced Cu-mediated radiofluorination. To this end, a solution of Et_4NHCO_3 in *n*BuOH (400 µL) was used to elute $^{18}F^-$ from a QMA anion exchange cartridge into a solution of the radiolabeling precursor and $Cu(py)_4(OTf)_2$ (30 µmol of each) in DMA (800 µL) and the reaction mixture was heated at 110 °C for 10 min under air to afford [^{18}F]**2**. The latter was hydrolyzed with 6 M NaOH to give [^{18}F]**1**. Both tracers were evaluated by *in vitro* autoradiography in rat brain slices, *in vivo* µPET imaging in healthy rats, and *ex vivo* radiometabolite analysis in rat brain tissue and blood.

Results The precursor was obtained in 15% yield over four steps. [^{18}F]**1** and [^{18}F]**2** were prepared in a ready-to-use form in radiochemical yields of 55±7% (n=8) and 62±5% (n=4) within 90–120 min, respectively, with molar activities of 14–137 GBq/µmol. *In vitro* evaluations showed accumulation of [^{18}F]**1** in brain regions consistent with the distribution pattern of GlyT1, but *in vivo* brain uptake of the probe was very low. In contrast, [^{18}F]**2** showed no specific binding in brain slices, but rapidly crossed the blood brain barrier (BBB) and showed an *in vivo* brain distribution pattern consistent with GlyT1 specific binding. Metabolite studies demonstrated rapid hydrolysis of [^{18}F]**2** to [^{18}F]**1** in rat brain tissue and blood ($t_{1/2}$=12 min), confirming that it acts as a BBB-penetrating prodrug.

Conclusion [^{18}F]**2** is a promising and readily available prodrug for preclinical PET imaging of GlyT1 in the brain.

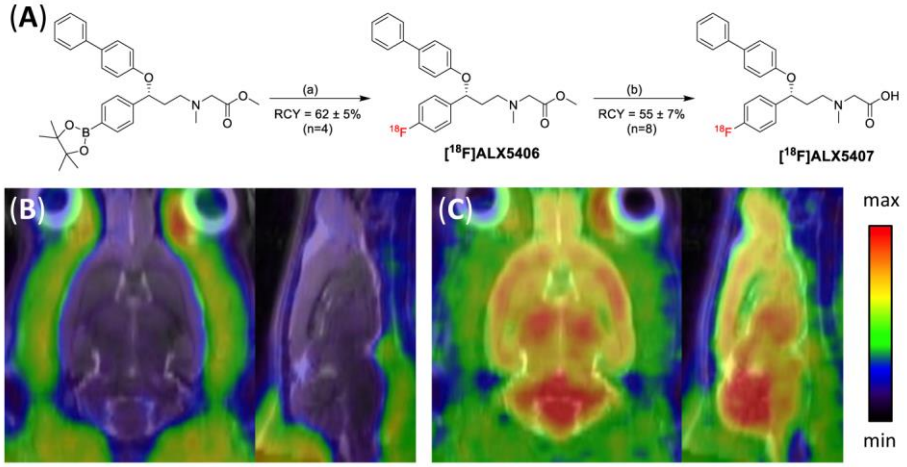

Figure 1: (A) Conditions: (a) elution of $^{18}F^-$ with Et_4NHCO_3 in *n*BuOH into a solution of precursor and $[Cu(OTf)_2(py)_4]$ in dimethylacetamide (DMA), then 110 °C, 10 min; (b) 6 M NaOH, 110 °C, 10 min. Isolated radiochemical yields (RCYs) after HPLC purification are provided. *In vivo* µPET images of [^{18}F]ALX5407 (B) and [^{18}F]ALX5406 (C) in healthy rats, 180–240 min after tracer administration. Average µPET images (n=3 per tracer; summed images over 60 min each) were projected onto an MRI template in the horizontal plane.

Acknowledgments This work was financially supported by DFG (Zl 65/1-1). The authors thank Prof. S. M. Ametamey (ETH Zürich, Switzerland) for his suggestion to apply the methyl ester of [^{18}F]ALX5407 as a prodrug.

References

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