Intracellular binding of Fluorinated compounds to carbonic anhydrase isoforms explored by In-Cell ^{19}F NMR

Azzurra Costantino [a], *Letizia Barbieri* [a,b], *Simone Giovannuzzi* [c], *Alessio Nocentini* [c], *Claudio T. Supuran* [c], *Enrico Luchinat* [a,b,d], *Lucia Banci* [a,b,d]

[a] *CERM – Magnetic Resonance Center, University of Florence, via Luigi Sacconi, 6, 50019, Sesto Fiorentino, Italy 1;* [b] *Consorzio Interuniversitario Risonanze Magnetiche di Metallo Proteine – CIRMMP, via Luigi Sacconi, 6, 50019, Sesto Fiorentino, Italy;* [c] *NEUROFARBA Department, Section of Pharmaceutical and Nutraceutical Sciences, University of Florence, Sesto Fiorentino, Italy;* [d] *Chemistry Department, University of Florence, Via della Lastruccia, 3, 50019, Sesto Fiorentino, Italy; e-mail: azzurra.costantino@unifi.it*

Drug development is a complex and time-consuming process, largely due to the challenge of predicting key properties of candidate molecules within the cellular environment, such as cellular uptake, target engagement, and selectivity (1). In this context, in-cell nuclear magnetic resonance (NMR) spectroscopy has emerged as a powerful technique to study molecular interactions directly within living cells, providing atomic-level insight under physiologically relevant conditions (2,3). In particular, fluorine-19 (^{19}F) in-cell NMR is ideally suited for this purpose, offering high sensitivity, broad chemical shift dispersion, and no background signal from endogenous metabolites (4).

In this study, we developed and characterized a panel of novel fluorinated benzenesulfonamide derivatives as inhibitors of human cytosolic carbonic anhydrase (CA) isoforms, a widely studied family of zinc enzymes implicated in diseases such as glaucoma and epilepsy (5). The interactions between these fluorinated ligands and overexpressed CA isoforms (CA1, CA2, CA3, CA7, and CA13) in HEK293T cells were investigated using ^{19}F in-cell NMR spectroscopy. The resulting spectra showed distinct binding profiles for all CA isoforms except CA3, which displayed no binding, consistent with its known resistance to sulfonamide inhibition. To qualitatively assess relative binding affinities, cells were treated with two different fluorinated compounds. Comparative analysis of ^{19}F signal intensities from the resulting cell samples enabled affinity ranking for each isoform. Finally, real-time ^{19}F NMR measurements in a NMR bioreactor enabled the evaluation of uptake kinetics, highlighting compound-specific differences in membrane permeability.

These results demonstrate the power of ^{19}F in-cell NMR as a versatile tool for characterizing protein-ligand interactions and monitoring cellular uptake in real time. The identified compounds may serve both as starting points for the development of selective CA inhibitors and as fluorinated probes for competitive binding assays in drug screening applications.

References: 1. Dowden, H.; Munro, J. Trends in Clinical Success Rates and Therapeutic Focus. Nat Rev Drug Discov 2019, 18 (7), 495–496. 2. Siegal, G.; Selenko, P. Cells, Drugs and NMR. Journal of Magnetic Resonance 2019, 306, 202–212. 3. Luchinat, E.; Barbieri, L.; Cremonini, M.; Nocentini, A.; Supuran, C. T.; Banci, L. Drug Screening in Human Cells by NMR Spectroscopy Allows the Early Assessment of Drug Potency. Angew. Chem. Int. Ed. 2020, 59 (16), 6535–6539. 4. Chen, H.; Viel, S.; Ziarelli, F.; Peng, L. 19F NMR: A Valuable Tool for Studying Biological Events. Chem. Soc. Rev. 2013, 42 (20), 7971. 5. Imtaiyaz Hassan, Md.; Shajee, B.; Waheed, A.; Ahmad, F.; Sly, W. S. Structure, Function and Applications of Carbonic Anhydrase Isozymes. Bioorganic & Medicinal Chemistry 2013, 21 (6), 1570–1582.