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PhD Thesis title: "Structural basis of allosteric $GABA_A$ receptor modulationComputational Studies of SLC Transporters with Special Focus on the GABA Transporter Subfamily

Solute carrier (SLC) transporters facilitate the movement of nutrients, ions, metabolites and drugs across biomembranes. They are therefore essential for physiological processes and cellular homeostasis. There is an ongoing heated debate on how much SLC transporters contribute to the uptake and the disposition of drugs, and more research is needed to clarify drug–transporter associations. Even though SLC transporters are understudied in general, the SLC6 transporter family has been the subject of intensive research due to its crucial role in neurotransmitter signaling. Specifically, the four γ-aminobutyric acid (GABA) transporters (GATs) have emerged as potential new drug targets to treat epilepsy and stroke, as they are involved in the termination of GABAergic signaling. However, even after 20 years of intense research, the mechanisms of ligand recognition and subtype-selectivity are still not fully understood.

The aim of this thesis is twofold: (1) to investigate the extend of SLC-mediated drug uptake of cytotoxic drugs that were associated with a specific SLC transporter in a series of genetic screenings and (2) to elucidate the structural basis for GAT activity as well as subtype-selectivity with emphasis on the GABA transporter hBGT1 by applying structure-based computational methods together with mutagenesis studies. This thesis addresses these aims in five independent studies. Study 1 focuses on the general role of the SLCs in drug uptake, whereas Studies 2 to 5 focus on hBGT1.

In Study 1 we investigate the physicochemical properties of cytotoxic drugs that were associated with an SLC transporter in a genetic screening. By comparing the different screening compound libraries with the drug-like space, we conclude that the cytotoxic drugs show no bias towards specific physicochemical properties. Thus, SLC-mediated drug uptake is most probably the rule and not the exception for compounds with specific properties. In Study 2, we investigate the binding mode of tetrahydropyridine/pyrimidine analogs by means of docking and molecular dynamics (MD) simulations. Subsequent mutagenesis studies identify residues that are relevant for hBGT1 activity and for subtype-selectivity. In Study 3, we suggest

a binding mode for conformationally restricted β-alanine analogs by applying the same computational methods as in Study 2, followed by mutagenesis studies. We identify the same residues as relevant for hBGT1 activity and selectivity as in Study 2. In Study 4, we explore the binding site of noncompetitive hBGT1 inhibitors and postulate that these inhibitors bind to a similar allosteric site already observed in the homologous human serotonin transporter. Based on docking studies, a binding hypothesis is retrieved that can explain the structure–activity relationship of 72 analogs. However, mutational studies did not confirm this site. In Study 5, we investigate the molecular nature of an observed unusual biphasic inhibition profile of different hBGT1 inhibitors. We postulate that this inhibition profile might be the result of additional binding to an allosteric site possibly identical with the site discussed in Study 4. Docking studies in the orthosteric and in the postulated allosteric site identify relevant residues for the observed activity and the biphasic inhibition profile. Subsequent mutagenesis studies confirm one residue in the orthosteric site as relevant for the observed biphasic inhibition profile, while no such residues could be identified in the allosteric site.

Overall, the findings of this thesis support the widespread role of SLC transporters in drug-uptake and distribution. Furthermore, extensive analyses of the binding modes of different BGT1 inhibitors to different binding sites contribute to a better understanding of GAT subtype-selectivity, which will ultimately guide the design of new highly selective and potent tool compounds.