Structural studies on second generation soluble adenylyl cyclase inhibitors and characterization of a putative ATPase domain

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Cyclic AMP is an important second messenger that plays a key role in numerous signal transduction pathways. In mammals, cAMP is produced from ATP by either G-protein regulated transmembrane adenylyl cyclases or bicarbonate-regulated soluble adenylyl cyclase. The soluble isoform is for example associated with skin and prostate cancer, sperm capacitation and motility, offering a target for pharmaceutical drug and male contraceptive development [1]. In contrast to the catalytic domain, little is known about the C-terminal region of sAC. Based on a prediction of the domain composition, mammalian soluble adenylyl cyclase belongs to the "signal transduction ATPases with numerous domains (STAND) family. A sAC ortholog was discovered in *Mycobacterium tuberculosis*, comprising the consecutive genes Rv0891c and Rv0890c, serving as model for further analysis. For future characterization of the predicted NTPase domain and its partner domains, first experiments were performed using the C-terminal region of the orthologous enzyme. Initial enzyme activity assays using an UPLC system confirmed its ATPase activity and will allow its further characterization.

Recent pharmacologic evaluation of sAC inhibitors for usage as on-demand, non-hormonal male contraceptives suggested that both, high intrinsic potency and long residence times are essential design elements for successful contraceptive applications. We focused on purification, crystallization and structure determination of novel sAC inhibitor complexes by protein X-ray crystallography and obtained multiple datasets yielding excellent density for the inhibitors and indicating molecular features that might cause their differences in potency and other drug properties [2].

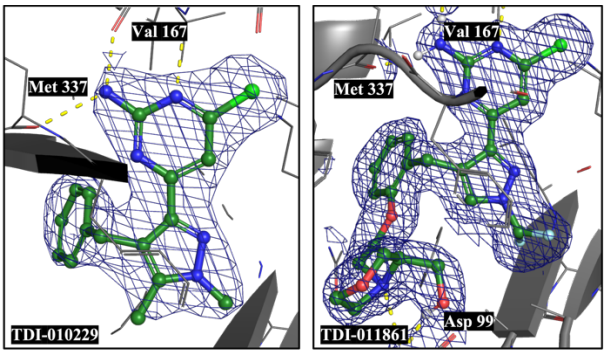

Figure 1: Crystal structure of hsAC in complex with TDI-010229 (left) and TDI-011861 (right). $2F_o$-F_c map contoured at 1σ.

1. Steegborn, C. Structure, mechanism, and regulation of soluble adenylyl cyclases - similarities and differences to transmembrane adenylyl cyclases. *Biochim. Biophys. Acta - Mol. Basis Dis.* **1842**, 2535–2547 (2014).

2. Fushimi, M. *et al.* Discovery of TDI-10229: A Potent and Orally Bioavailable Inhibitor of Soluble Adenylyl Cyclase. *ACS Med. Chem. Lett.* **12**, 1283–1287 (2021).