De novo design and virtual high-throughput screening to identify novel inhibitors of membrane proteins

K. Simmons, A. Johnson and C. Fishwick

Introduction

Membrane proteins are intrinsically involved in both human and pathogen physiology, and are the target of 60% of all marketed drugs. During the past decade, advances in the studies of membrane proteins using x-ray crystallography, electron microscopy and NMR-based techniques led to the elucidation of over 250 unique membrane protein crystal structures. The aim of the European Drug Initiative for Channels and Transporter (EDICT) project is to use the structures of clinically significant membrane proteins for the development of lead molecules. One of the approaches used to achieve this is a virtual high-throughput screening (vHTS) technique initially developed for soluble proteins, another uses iterative rounds of ligand design based on newly available crystal structures to develop new ligands. The application of these methods to three targets as part of the EDICT project is discussed below.

Results

1. Identification of selective inhibitors of the potassium channel Kv1.1-1.2((3)) by high-throughput virtual screening

Two voltage-dependent potassium channels, Kv1.1 (KCNA1) and Kv1.2 (KCNA2), are found to co-localize at the juxtaparanodal region of axons throughout the nervous system and are known to co-assemble in heteromultimeric channels, most likely in the form of the concatemer Kv1.1-1.2((3)) . Loss of the myelin sheath, as is observed in multiple sclerosis, uncovers the juxtaparanodal region of nodes of Ranvier in myelinated axons leading to potassium conductance, resulting in loss of nerve conduction. The selective blocking of these Kv channels is therefore a promising approach to restore nerve conduction and function. The combined use of four popular virtual screening approaches (eHiTS, FlexX, Glide, and Autodock-Vina) led to the identification of several compounds as potential inhibitors of the Kv1.1-1.2((3)) channel. From 89 electrophysiologically evaluated compounds, 14 novel compounds were found to inhibit the current carried by Kv1.1-1.2((3)) channels by more than 80 % at 10 μM. Accordingly, the IC50 values calculated from concentration-response curve titrations ranged from 0.6 to 6 μM. Two of these compounds exhibited at least 30-fold higher potency in inhibition of Kv1.1-1.2((3)) than they showed in inhibition of a set of cardiac ion channels (hERG, Nav1.5, and Cav1.2), resulting in a profile of selectivity and cardiac safety.

2. A virtual high-throughput screening approach to the discovery of novel inhibitors of the bacterial leucine transporter, LeuT

A suitable protein for conducting a vHTS is LeuT, a bacterial homologue of the NSS family characterized by their ability to use the sodium gradient across the plasma membrane to drive the transport of its solute against its concentration gradient. From screening a library of commercially available molecules, 1000 compounds were selected for further evaluation and consensus scoring using the *de novo* design program SPROUT. These 1000 compounds were selected based on their predicted binding to the protein as predicted using the eHiTS scoring algorithm. From this set, 11 molecules were selected for purchase. These 11 compounds were chosen based upon their predicted binding affinity to the protein as determined using the eHiTS and SPROUT scoring functions and also their predicted binding pose. From an initial purchase set of 11 compounds, five compounds were found to exhibit activity against the protein when tested at 1 mM, a hit rate of 45%. The four most active compounds were tested for their dose-response inhibition of the [^{3}H]-leucine binding with their IC50 values being ~ 40 μM (Figure 1).

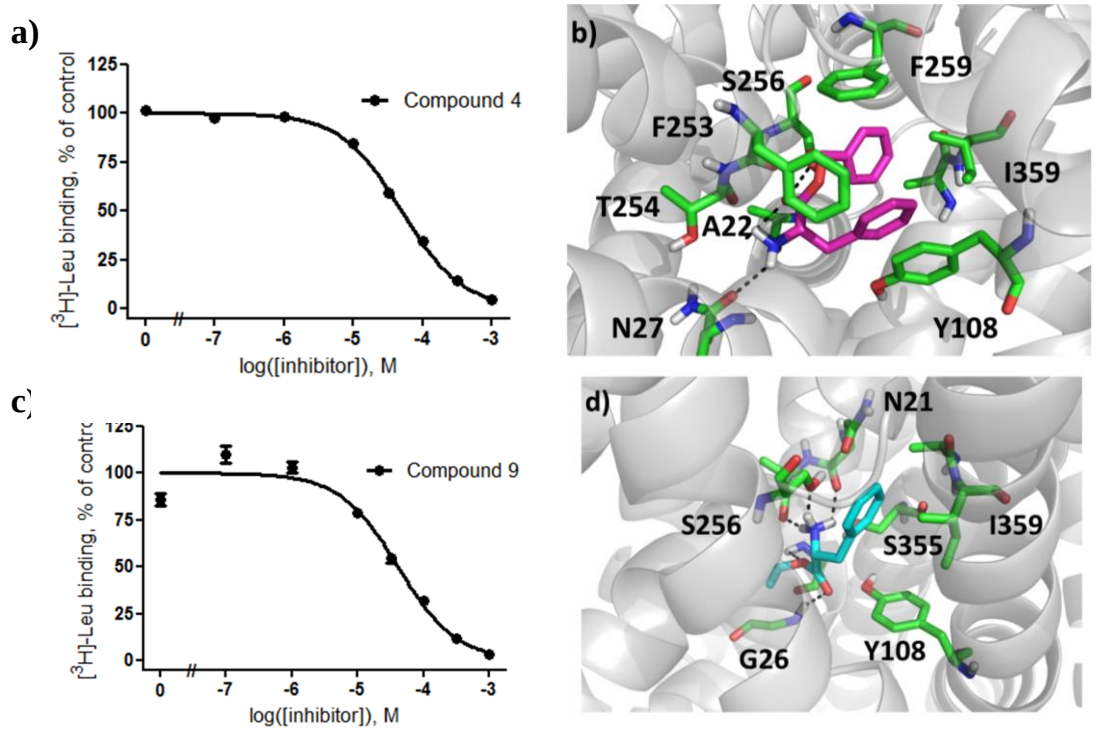

Figure 1: dose-response curves and binding pose of compounds 4 and 9. (a) Dose-response inhibition of [^{3}H]-leucine ([^{3}H]-Leu) binding to LeuT by compound 4. Normalized results are given as mean ± SEM on the basis of four independent experiments. **(b)** Compound 4 (magenta) docked into the crystal structure of the LeuT used for all vHTS (2Q72). The compound makes H-bonding interactions with Ala22, Asn27 and Ser256. **(c)** Dose-response inhibition of [^{3}H]-leucine ([^{3}H]-Leu) binding to LeuT by compound 9. Normalized results are given as mean ± SEM on the basis of four independent experiments. **(d)** Compound 9 (cyan) docked into the crystal structure of the LeuT used for all vHTS (2Q72). The compound makes H-bonding interactions with Asn21, Ala22, Gly24 and 26 and Ser256.

Probing the molecular mechanism of ligand binding by Mhp1

The hydantoin transporter Mhp1 is a sodium-coupled secondary active transport protein and a member of the growing 5-helix inverted repeat superfamily of transporters. The structure of Mhp1 was previously solved in three different conformations revealing the molecular basis of the alternating access mechanism but not the details of substrate binding. We have explored this through a combination of crystallography, ligand design, biochemical assays, molecular dynamics and site-directed mutagenesis. The crystal structure was solved in complex with L-5-indolylmethylhydantoin at 3.5 Å resolution. Using the structure, over 80 new ligands were designed, synthesised or procured and tested for binding to Mhp1 in competitive transport assays. From the deduced structure-activity relationships, roles were inferred for specific Mhp1 residues and tested by site-directed mutagenesis. Two of the new ligands were co-crystallised with Mhp1. Their positions in the protein confirm the poses predicted by docking but also reveal a novel conformational intermediate.

Publications

Wacker, S., Jurkowski, W., Simmons, K., Fishwick, C., Johnson, P., Madge, D., Lindahl, E., Rolland, J-F & de Groot, B. (2012) Identification of selective inhibitors of the potassium channel Kv1.1-1.2(3) by high-throughput virtual screening and automated patch clamp. *ChemMedChem* **7:** 1775-1783.

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