In silico identification of small molecule agonist binding sites on KCC2

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Background

- Potassium-Chloride Cotransporter 2 (KCC2) is a **neuronal membrane protein** specific to the central nervous system.
- It is responsible for removing Cl^- ions from the intracellular space, **maintaining a normal Cl^- gradient**. This is critical to the function of certain inhibitory synapses.
- Dysregulation causes an **upward shift in the Cl^- reversal potential** resulting in a hyperexcitable state of the postsynaptic neuron.
- KCC2 has also been previously implicated in EtOH dependance.
- Several novel direct KCC2 agonists have been discovered [4]
- VU0500469, one of the recently identified agonists, was used for *in silico* modeling to identify possible KCC2 binding sites

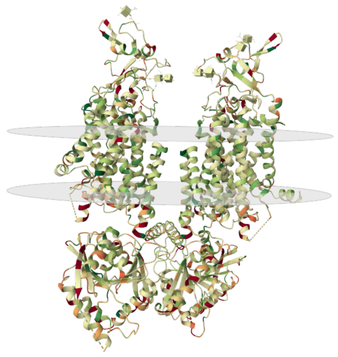


Figure 1. *3-D structure of human KCC2. Accessed from RCSB Protein Databank, including membrane prediction [1, 2, 3]*

Methods

Preparation and Visualization

Software: AutoDock Tools, PyMOL

- 3-D structures of human KCC2 were obtained from RCSB Protein Databank.
- VU0500469 was recreated manually (Figure 2).
- PDB files were loaded into AutoDock tools and converted into .pdbqt files.

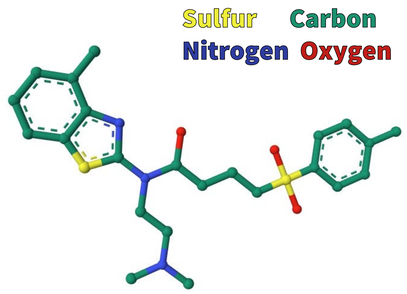


Figure 2. *VU0500469.* N-(2-(Dimethylamino)Ethyl)-N-(4-Methylbenzo[d]Thiazol-2-yl)-4-Tosylbutanamide. Formula: $C_{21}H_{26}C_lN_3O_3S_2$

LogP	TPSA
3.27	69.72

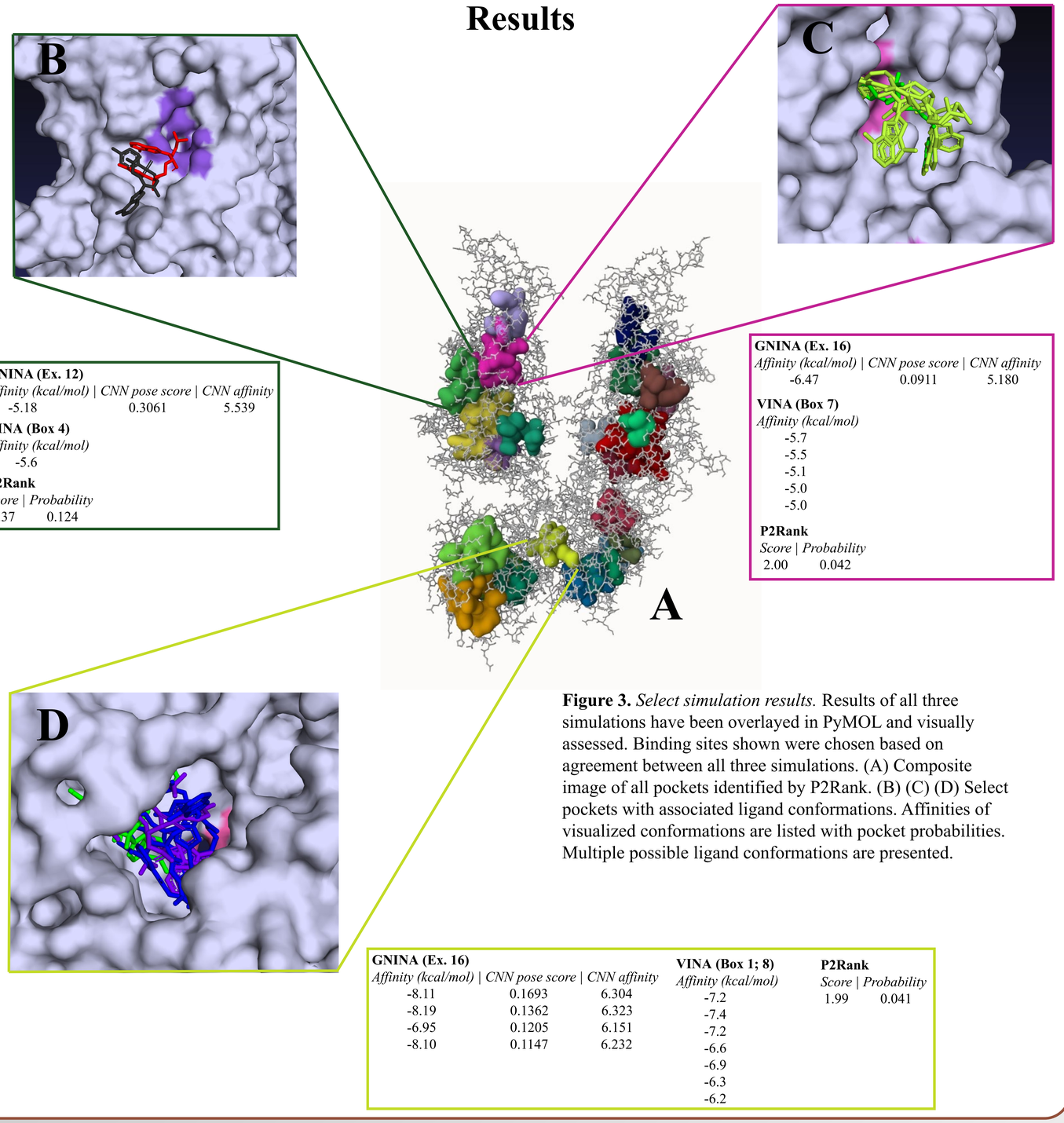
Table 1. *Molecular properties calculations for VU0500469 [8]*

Modeling

Software: AutoDock Vina [5], GNINA [6], P2Rank [7]

- Potential binding pockets for VU0500469 were identified using P2Rank.
- AutoDock Vina and GNINA simulations were used to identify optimal binding site location and conformation.
- Results from all three tools were compared to identify likely binding sites for further investigation.

Results



GNINA (Ex. 12)

Affinity (kcal/mol)	*CNN pose score*	*CNN affinity*
-5.18	0.3061	5.539

VINA (Box 4)

Affinity (kcal/mol)
-5.6

P2Rank

Score	*Probability*
3.37	0.124

GNINA (Ex. 16)

Affinity (kcal/mol)	*CNN pose score*	*CNN affinity*
-6.47	0.0911	5.180

VINA (Box 7)

Affinity (kcal/mol)
-5.7
-5.5
-5.1
-5.0
-5.0

P2Rank

Score	*Probability*
2.00	0.042

GNINA (Ex. 16)

Affinity (kcal/mol)	*CNN pose score*	*CNN affinity*
-8.11	0.1693	6.304
-8.19	0.1362	6.323
-6.95	0.1205	6.151
-8.10	0.1147	6.232

VINA (Box 1; 8)

Affinity (kcal/mol)
-7.2
-7.4
-7.2
-6.6
-6.9
-6.3
-6.2

P2Rank

Score	*Probability*
1.99	0.041

Figure 3. *Select simulation results.* Results of all three simulations have been overlayed in PyMOL and visually assessed. Binding sites shown were chosen based on agreement between all three simulations. (A) Composite image of all pockets identified by P2Rank. (B) (C) (D) Select pockets with associated ligand conformations. Affinities of visualized conformations are listed with pocket probabilities. Multiple possible ligand conformations are presented.

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