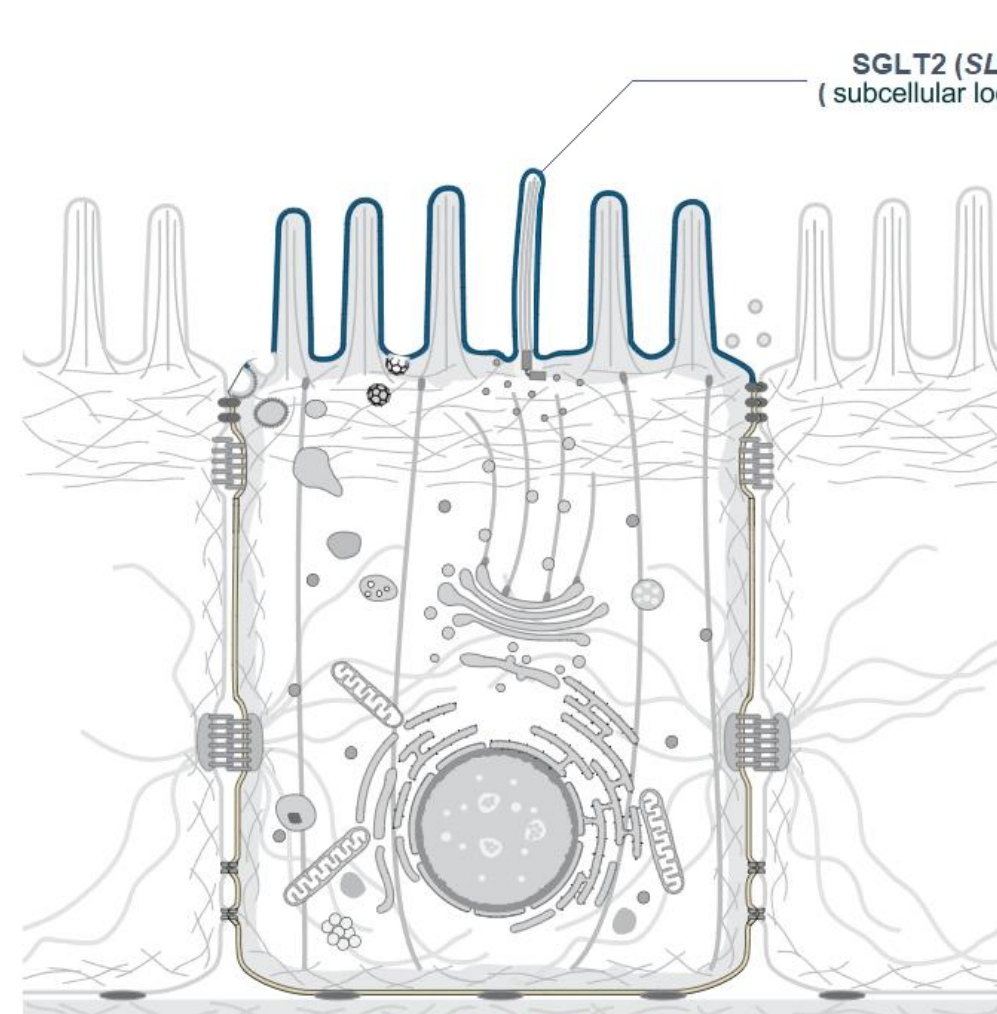


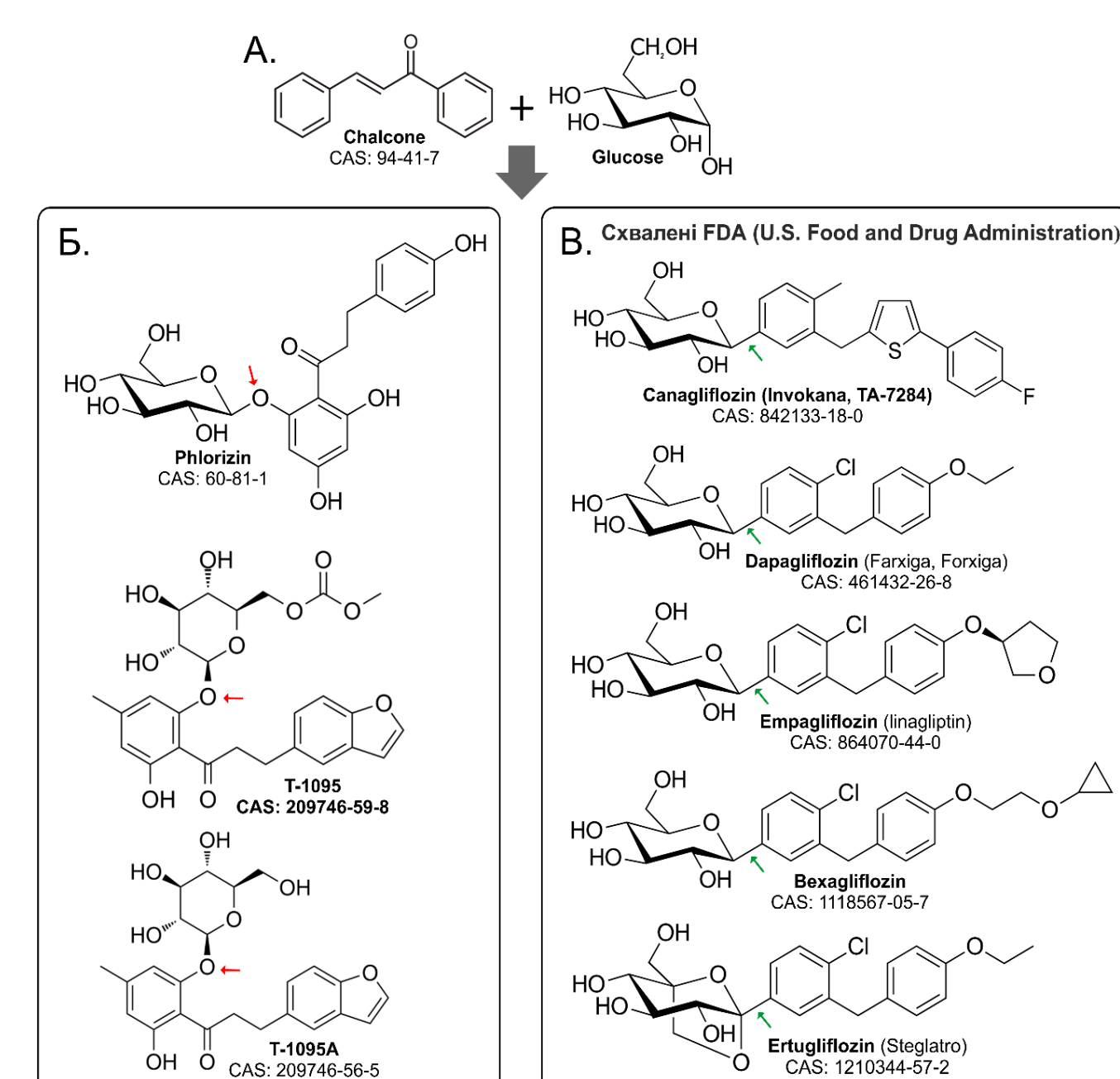


Blood glucose control remains one of the most critical factors in diabetes therapy. Major progress in the development of antihyperglycemic agents was marked by the discovery of gliflozins — selective inhibitors of sodium-glucose cotransporter 2 (SGLT2), encoded by the *SLC5A2* gene. By inhibiting SGLT2, these agents promote urinary glucose excretion and have proven effective for the management of type 2 diabetes mellitus (T2DM).

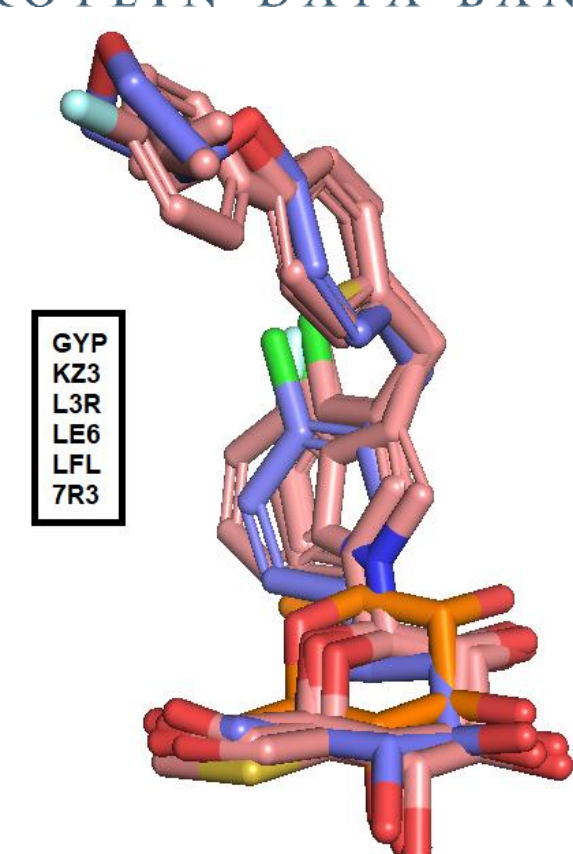
In 2022, the first cryo-EM structures of the human SGLT2 transporter were deposited in the RCSB Protein Data Bank, opening new opportunities for rational design and virtual screening of renal glucose reabsorption inhibitors.

The objective of this work was to perform a structural-biological analysis and virtual screening of the Enamine Ltd. chemspace for new potential SGLT2 inhibitors.

Can SGLT2 be specifically inhibited?

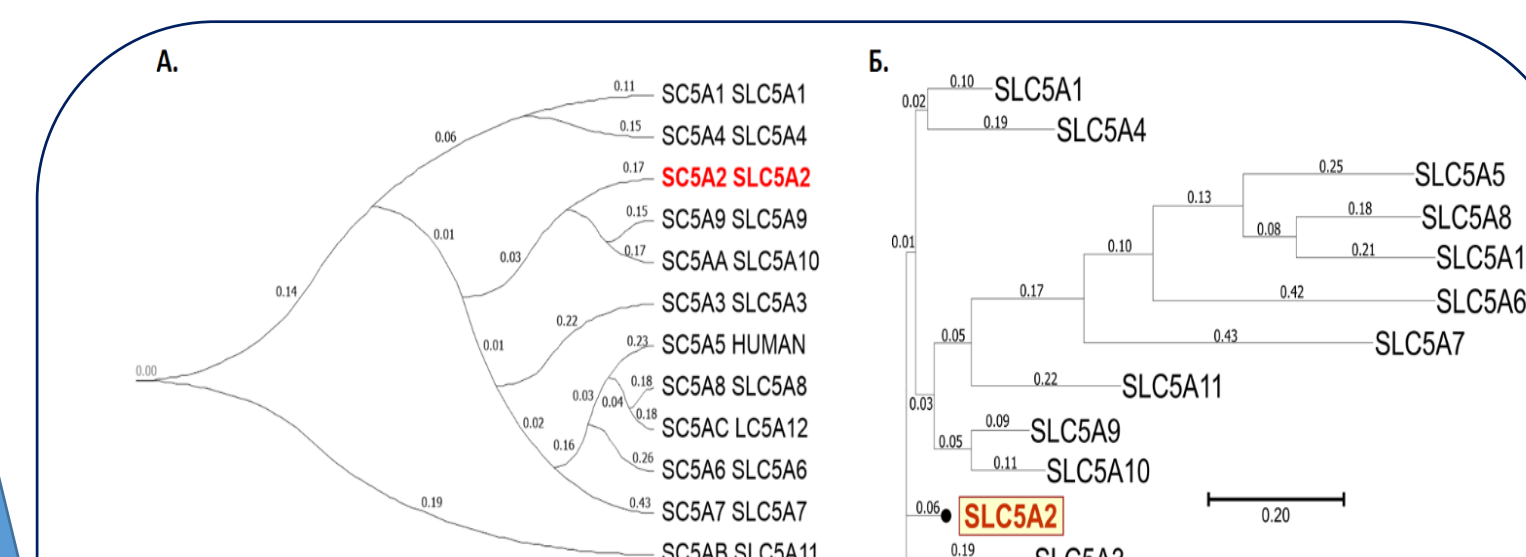


RCSB PDB PROTEIN DATA BANK



AF_SLC5A1_P13866 NIGGHxGLxTxIFxExATxLxMxLxTxWxWxKxQLFDYIxQTxYx
AF_SLC5A2_P31639 NIGGHxGLxTxVxExAxVxLxLxVxSxWxWxKxQLFDYIxQTxYx
AF_SLC5A3_P53794 NIGGHxGLxTxVxExAxVxLxLxVxSxWxWxKxQLFDYIxQTxYx
AF_SLC5A4_Q9NY91 NIGGHxGLxTxTxTxExSxLxLxMxTxWxWxKxQLIHYTxESxYx
AF_SLC5A5_Q92911 FMSVQxGVxExMxQxYxYxGxVxWxMxVxLxQxSxFMGxYL
AF_SLC5A6_Q9Y289 FQSVxVxGVxExYxYxYxYxHxGxMxSxLYQDVLQAAIxFGYx
AF_SLC5A7_Q9GZV3 FVGVYxGTxWxQxQxYxSxWxLxMxLxGxIwYxWxSxYx
AF_SLC5A8_Q8N695 FMSVTxGTxExFxExYxYxHxGxTWTxSxIYxALLQxALFGxYL
AF_SLC5A9_Q2M3M2 NxGLxGLxTxVxExAxTxLxLxLxWxWxKxQLFDYIxQTxYx
AF_SLC5A10_A0PJK1 SExGLxGLxSxVxExAxLxLxLxMxMxWxWxKxQLFIYxMxQTxYx
AF_SLC5A11_Q8WVX8 NxGHxGLxSxVxExGxTxVxDLxPxSxYxWxKxQLFIYIxQTxYx
AF_SLC5A12_Q1EBH4 FxSVTxGTxExFxExYxYxHxGxTWTxSxIYxVxVxQLxHxGxYL

5 Å The binding site fragments defined by a 5 Å cutoff from the reference ligand cluster (PDB-derived).

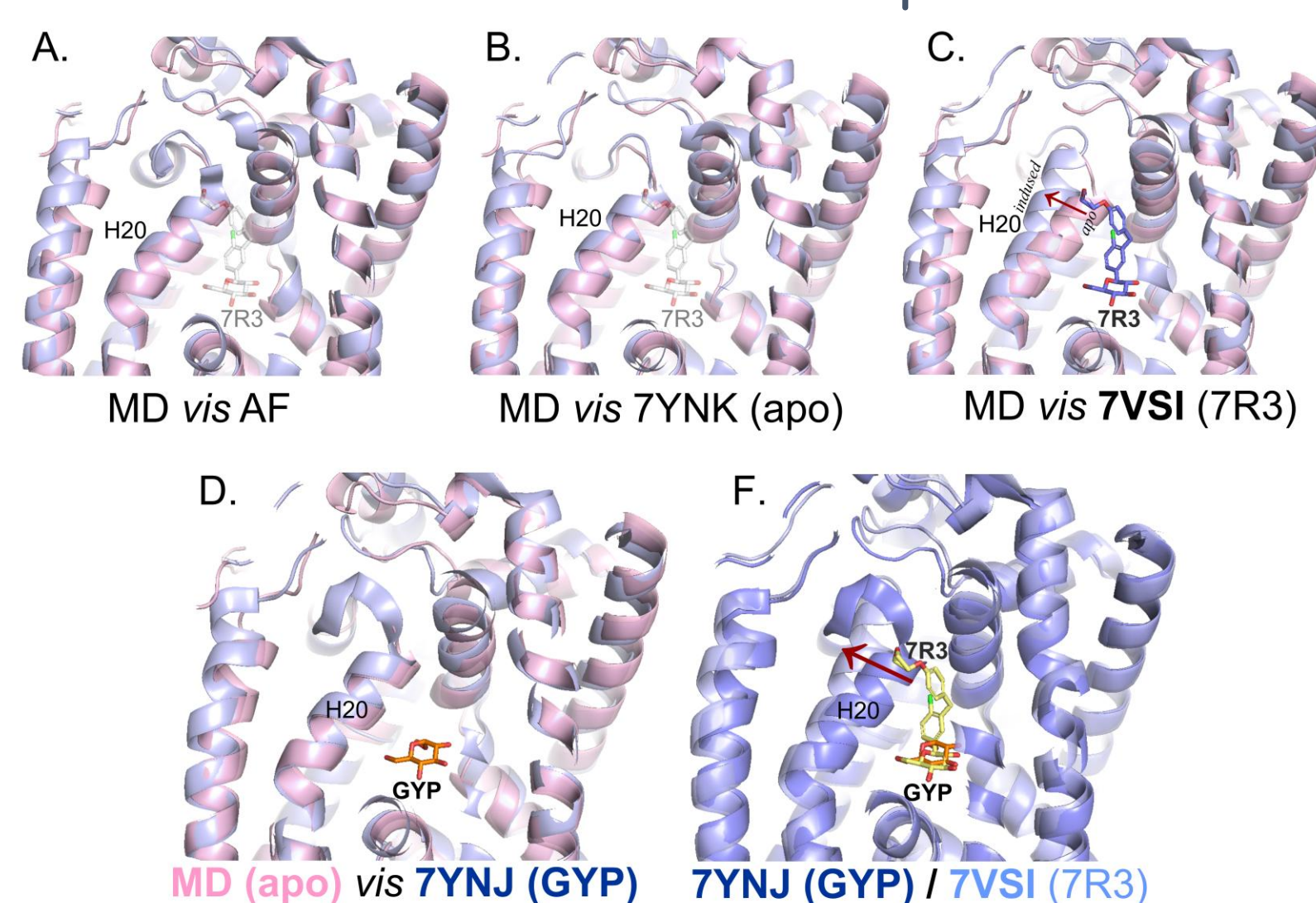


The clustering of SLC5 a.a. sequences (A) and binding site pockets (B). The unique a.a. composition of the pocket confirms the potential for SGLT2-specific inhibition.

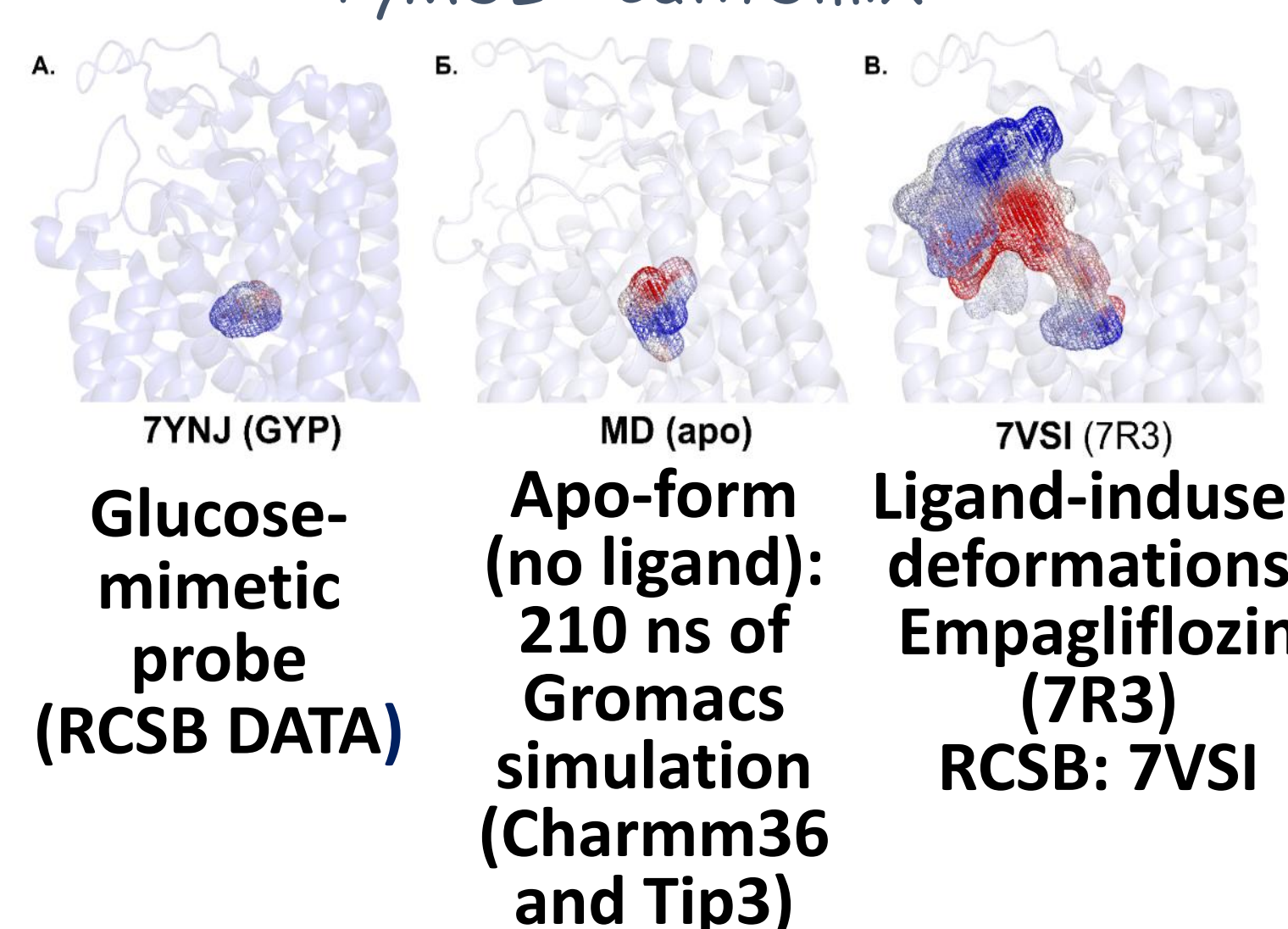
Clustering of the ligand-binding pockets across 12 SLC5 cotransporters confirmed that SGLT2-specific inhibition is achievable. Furthermore, the analysis of 3D models, PDB structures, and molecular dynamics simulations confirmed that an induced-fit mechanism occurs during the formation of SGLT2-inhibitor complexes.

Empagliflozin-induced structural rearrangements in SGLT2

RCSB PDB + Gromacs + AlphaFold3

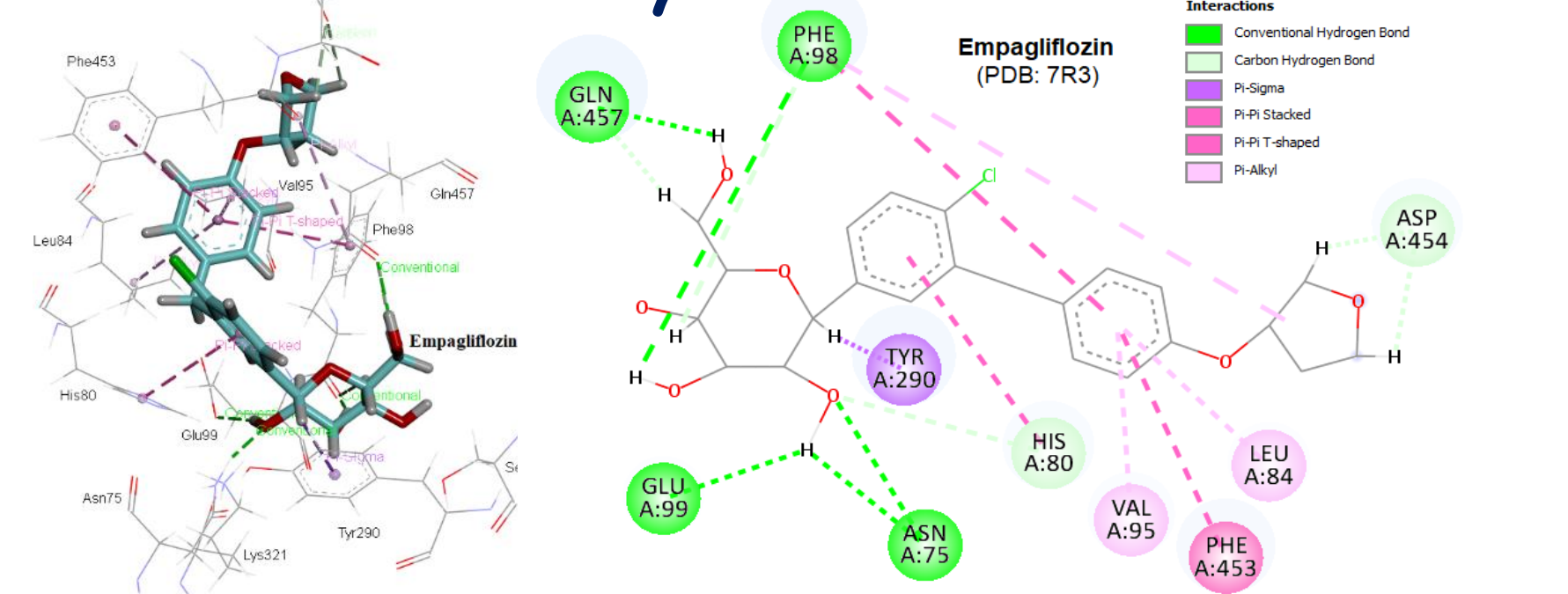


PyMOL + CavitOmiX:

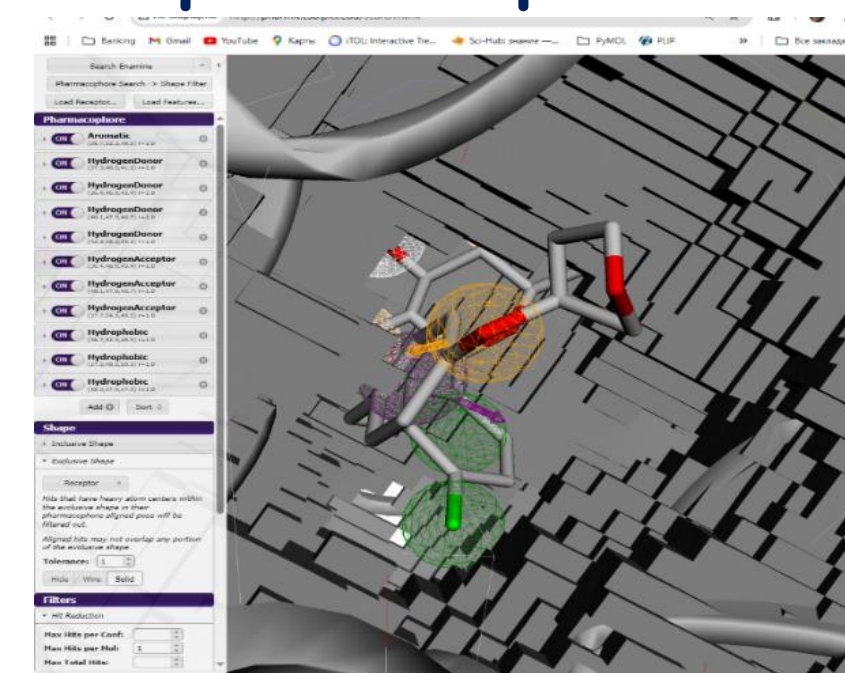


A virtual screening protocol was developed, integrating pharmacophore-based searching and molecular docking using CCDC GOLD and iGEMDOCK. By applying this protocol to the Enamine Ltd. library (4,117,328 unique compounds), 36 potential human SGLT2 inhibitors were identified. Ranking of a combined set of 42 compounds—36 top-scoring hits and 6 reference gliflozins from the RCSB PDB—based on scoring functions and ligand-protein interaction energy, revealed 5 Enamine compounds with predicted activity comparable to the control group. Following a "blind" ranking of the screening results, the selected leads were further characterized using the PubChem, DrugBank, and U.S. FDA databases.

Protein-ligand interaction analysis using BIOVIA Discovery Studio 2021

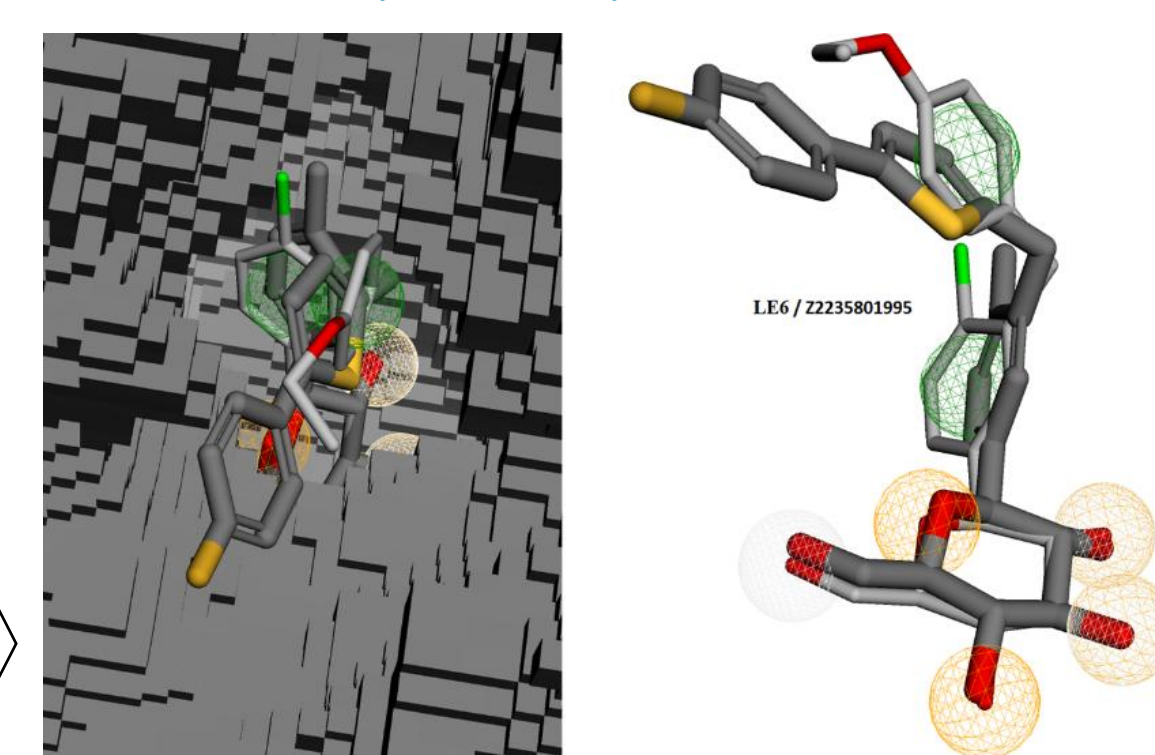


An example of the Pharmit search interface configured for pharmacophore screening



4,117,328 unique compounds

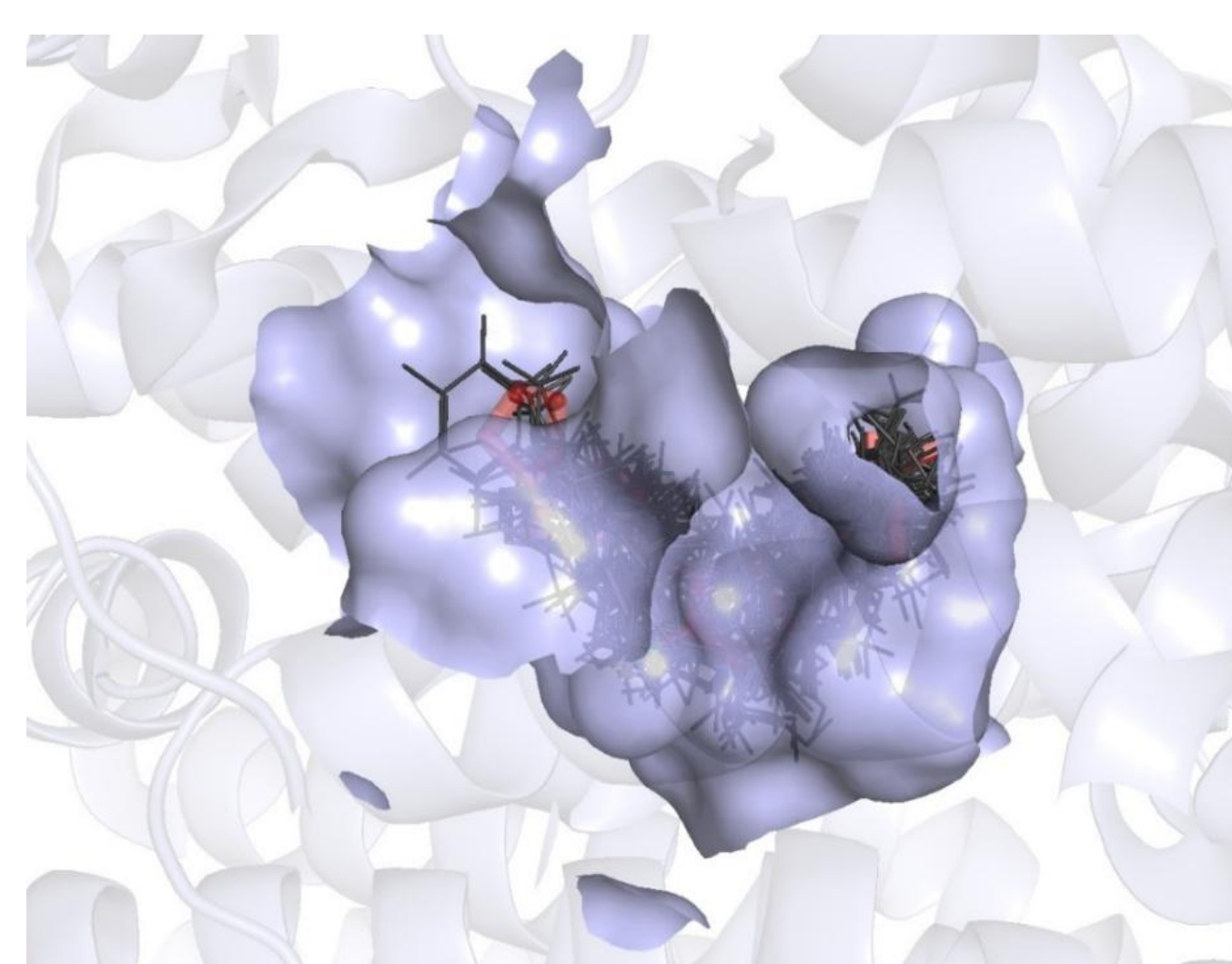
Enamine



Search Enamine	ChEMBL34
Pharmacophore Search -> Shape F	29,963,107 conformers of 2,264,112 molecules Updated: 2024-Jul-19 04:34:16
Load Receptor... Load Feature	ChemDiv
	21,562,497 conformers of 1,456,120 molecules Updated: 2019-Jun-05 06:41:23
Pharmacophore	ChemSpace
ON Aromatic	37,648,042 conformers of 7,659,928 molecules Updated: 2024-Oct-24 05:31:55
ON HydrogenDonor	Enamine
	60,516,302 conformers of 4,117,328 molecules Updated: 2023-Oct-27 11:52:53

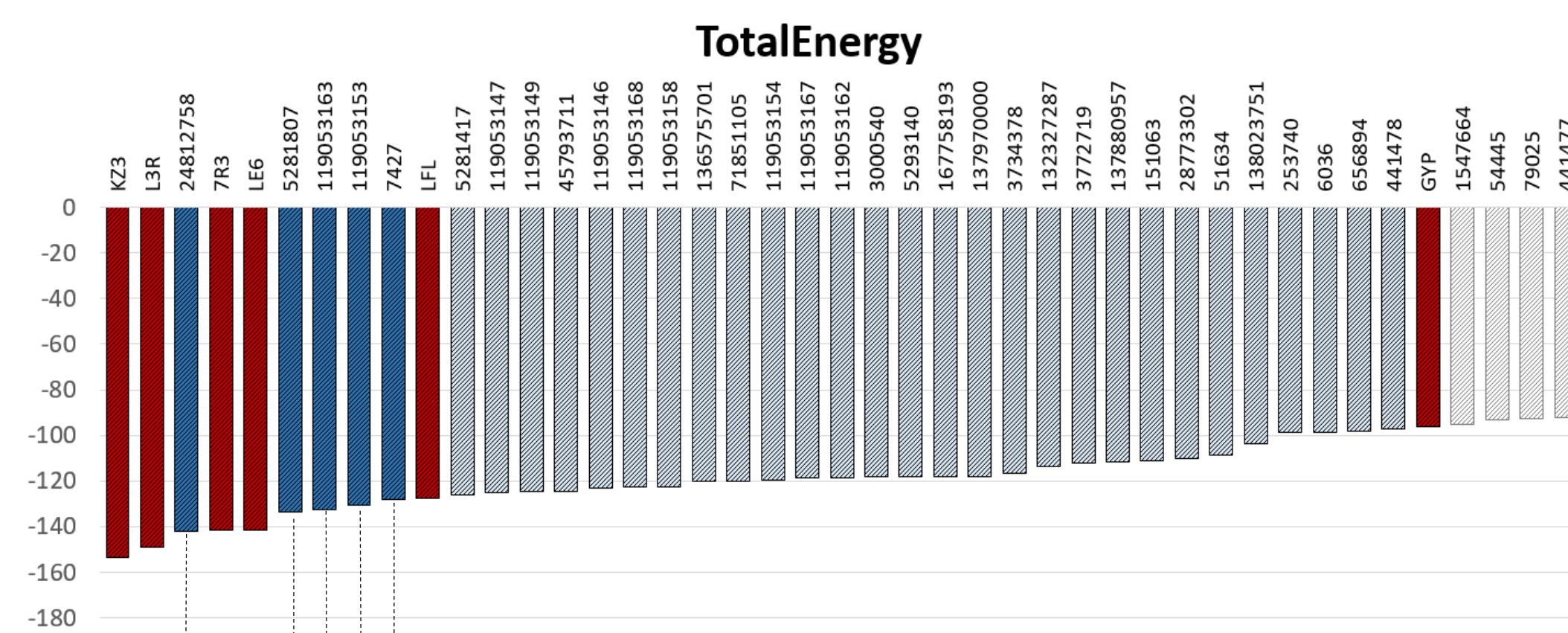
36 hits

CCDC GOLD



iGEMDOCK

TotalEnergy = HBond+VDW+ Elec



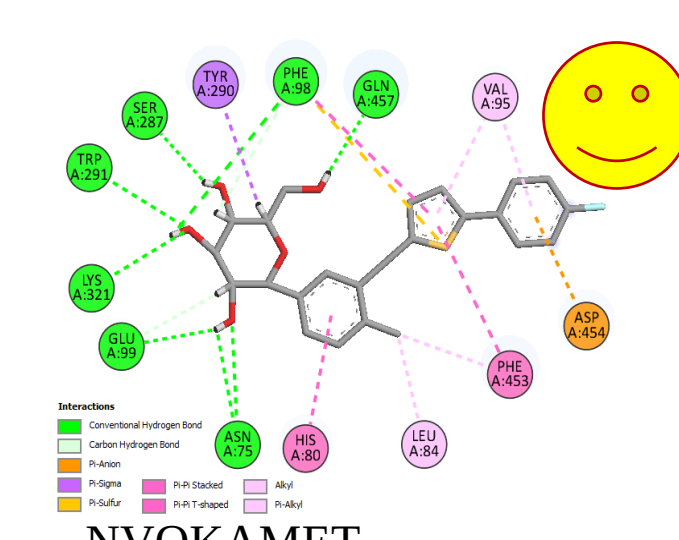
Canagliflozin
Z2235801995
(PubChem CID: 24812758)

Z1494829516
(PubMed CID: 5281807)

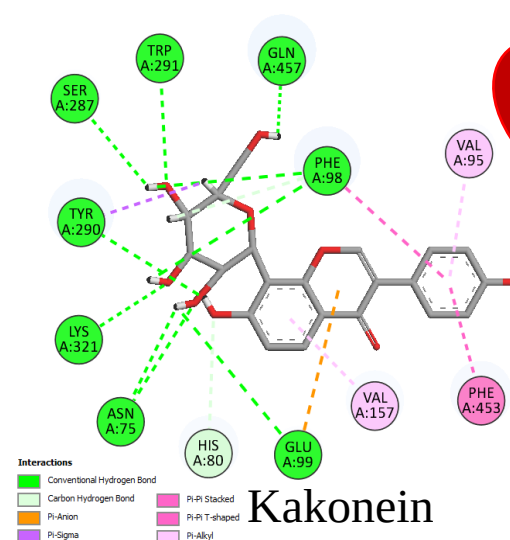
Z2195993226
(PubMed CID: 119053163)

Z2195993230
(PubChem CID: 119053153)

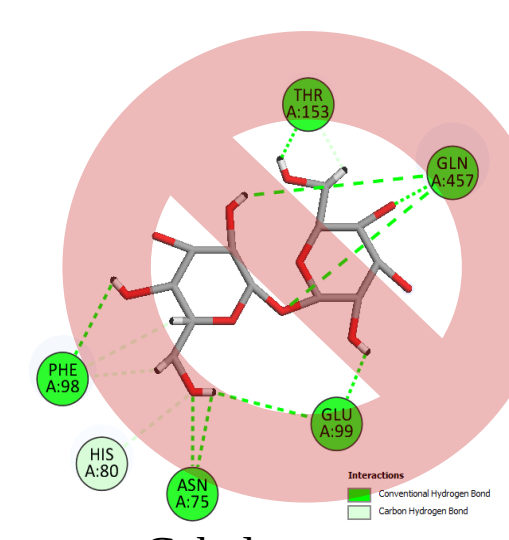
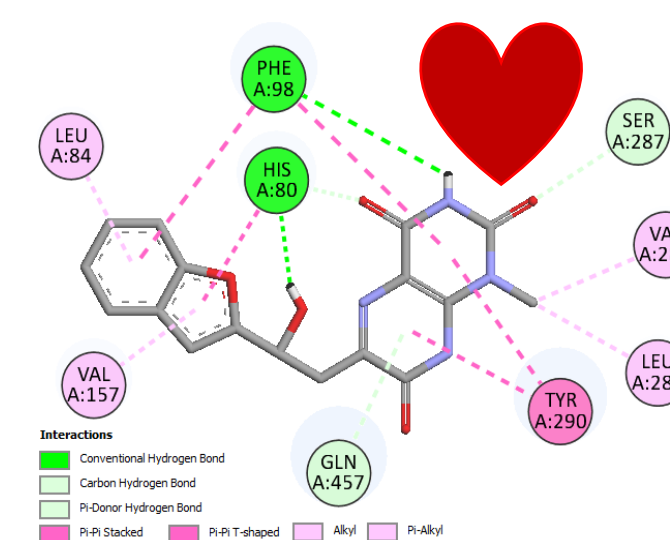
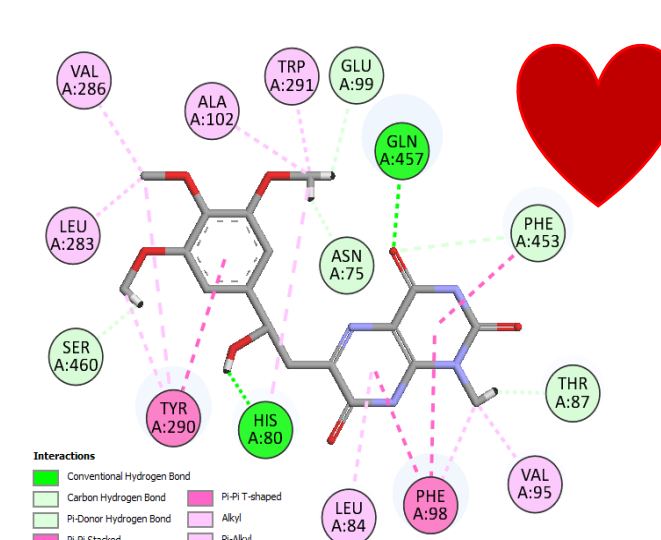
Z2417819595
(PubChem CID: 7427)



Johnson & Johnson, FDA
Reference ID:
4129180



Selected leads for biological evaluation and combinatorial optimization



Excluded following structural expertise.

CONCLUSIONS: Among the three selected leads, two (Z2195993226 and Z2195993230) are reported as potential gliflozins for the first time. Another compound, Z1494829516, was identified as the hydroxyisoflavone puerarin (Kakonein). While puerarin is a known autophagy inducer with established cardioprotective, antioxidant, anti-inflammatory, and antipretic properties, as well as an inhibitor of ferroptosis, its interaction with human SGLT2 and its potential glucose-lowering activity are predicted here for the first time.