# Supporting Information

# Evaluation of Free Energy Calculations for the Prioritization of Macrocycle Synthesis

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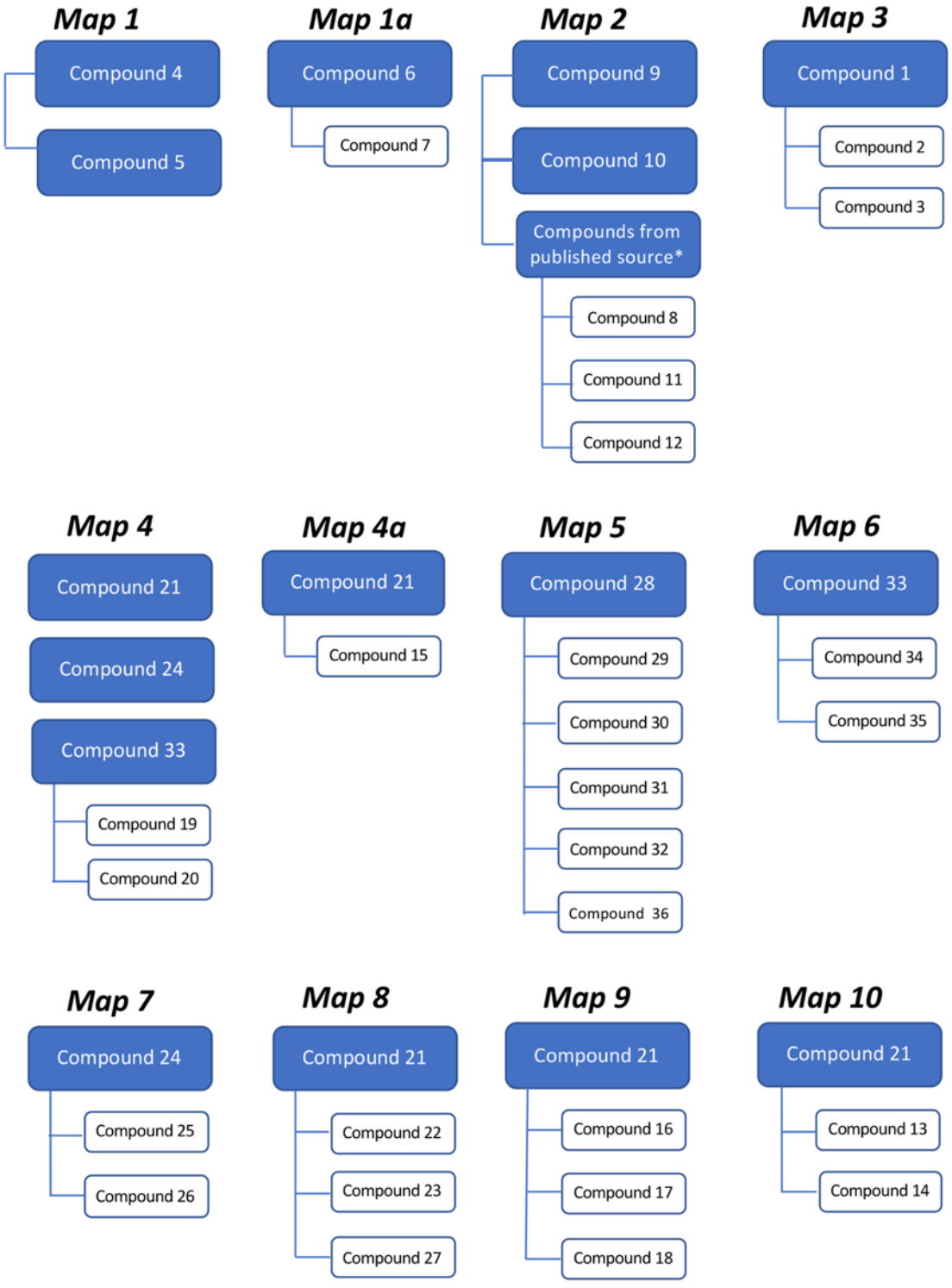
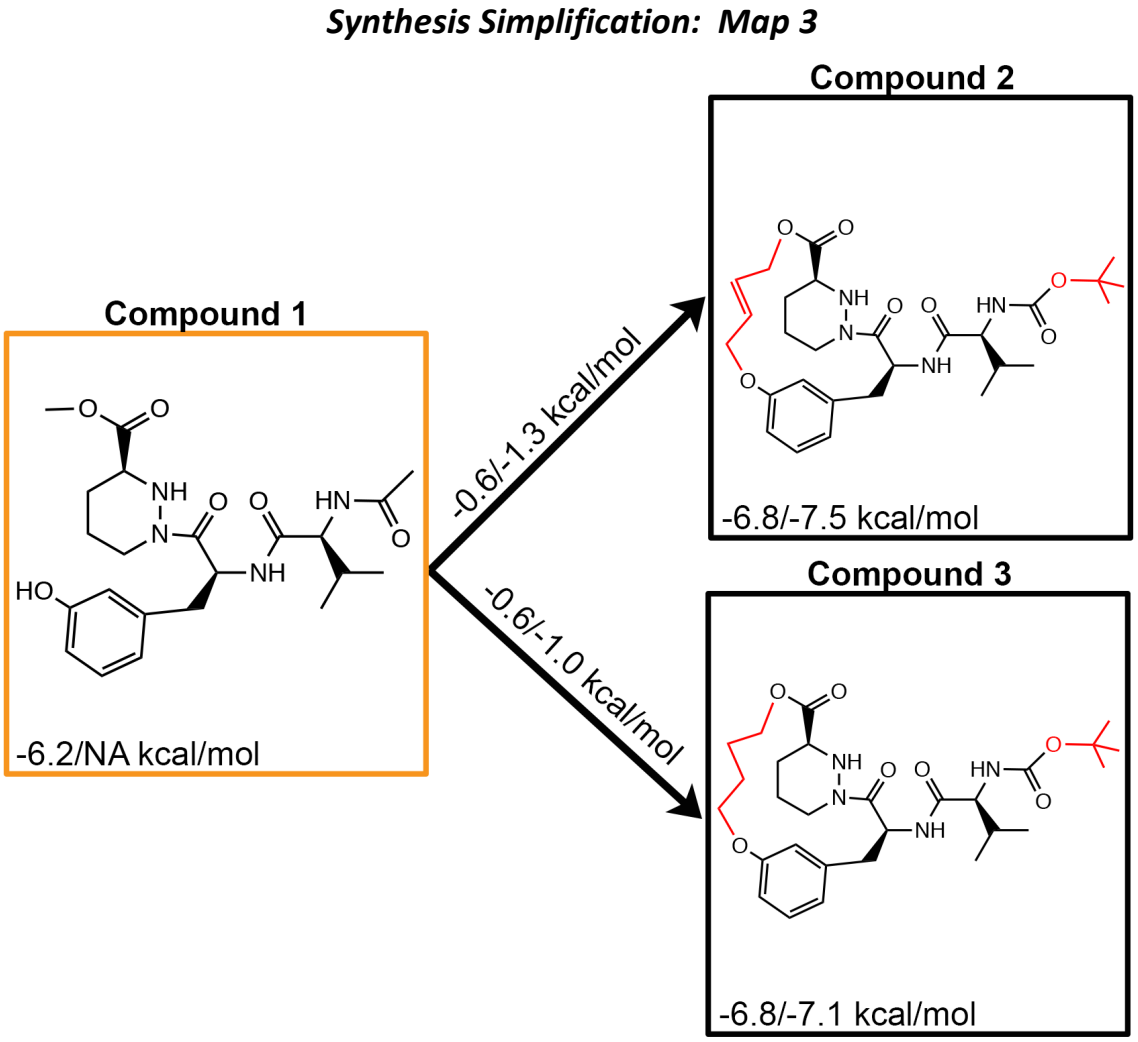
**Figure S1**. Reference compounds used for each FEP map are shown in a solid blue box with white text. Predicted compounds shown in white boxes.

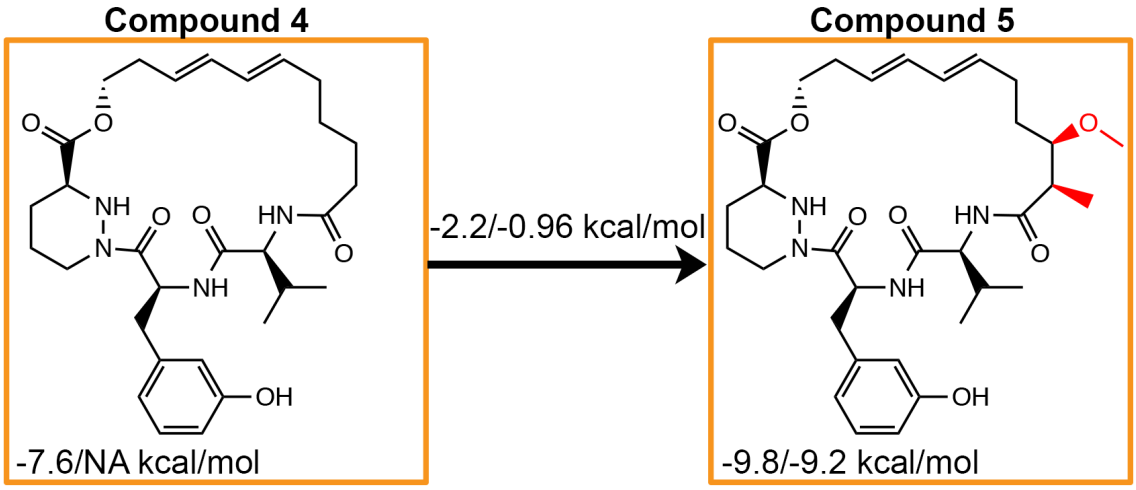



* reference compounds can be found in ref. 13

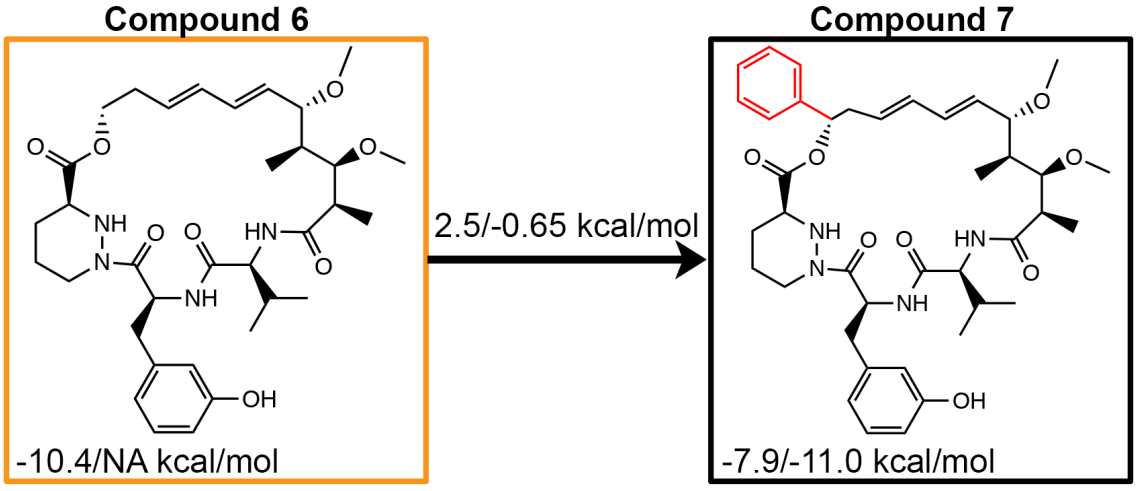
**Figure S2**. Key free energy perturbation (FEP) predictions shown for each FEP map. Experimental and predicted free energies (ΔG) are represented as experimental/predicted values in kcal/mol. Values displayed over the arrows are the experimental and predicted ΔΔG respectively. An orange outlined box indicated a co-crystalized protein:ligand complex was provided.



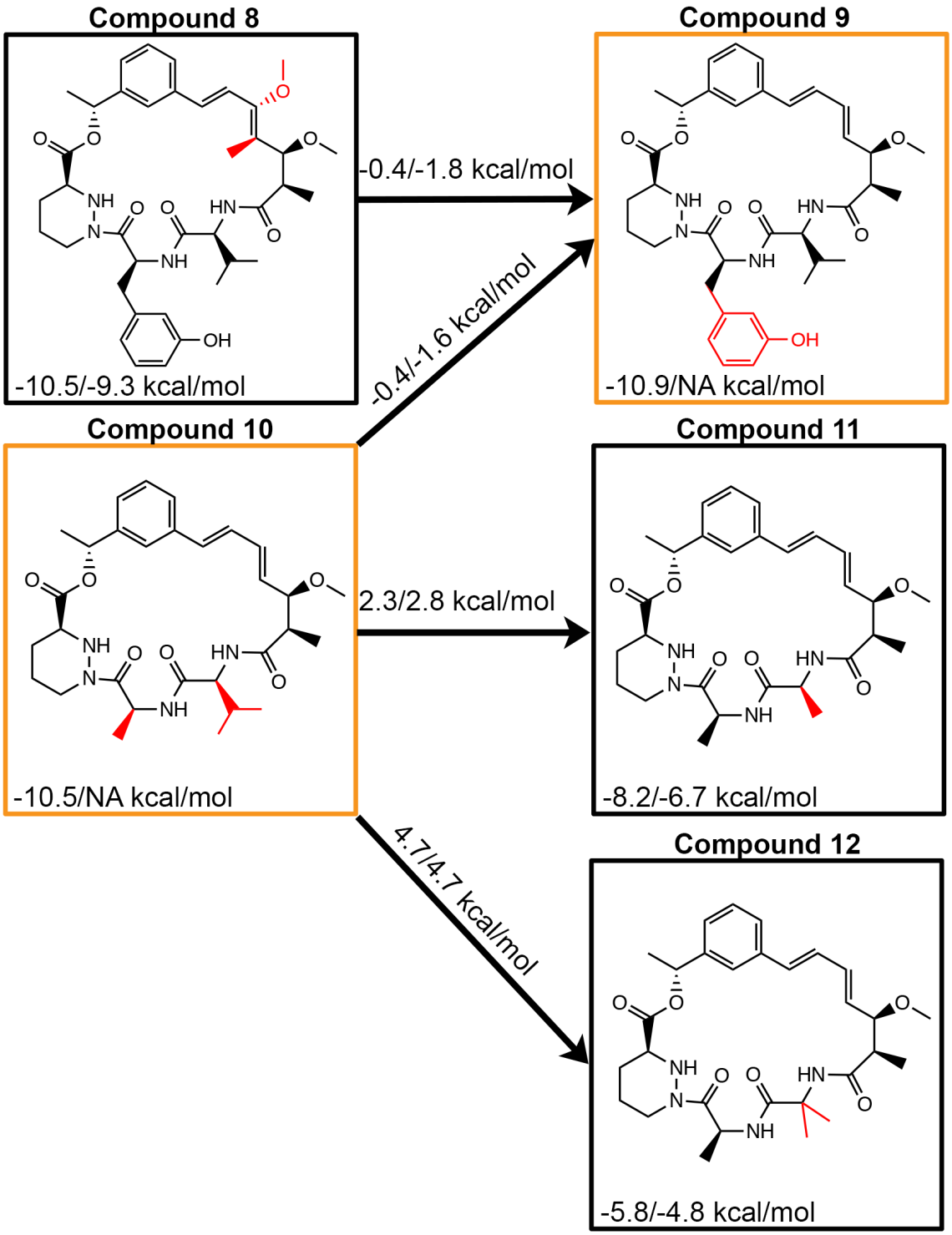
***Essential Core for Potency: Map 1***



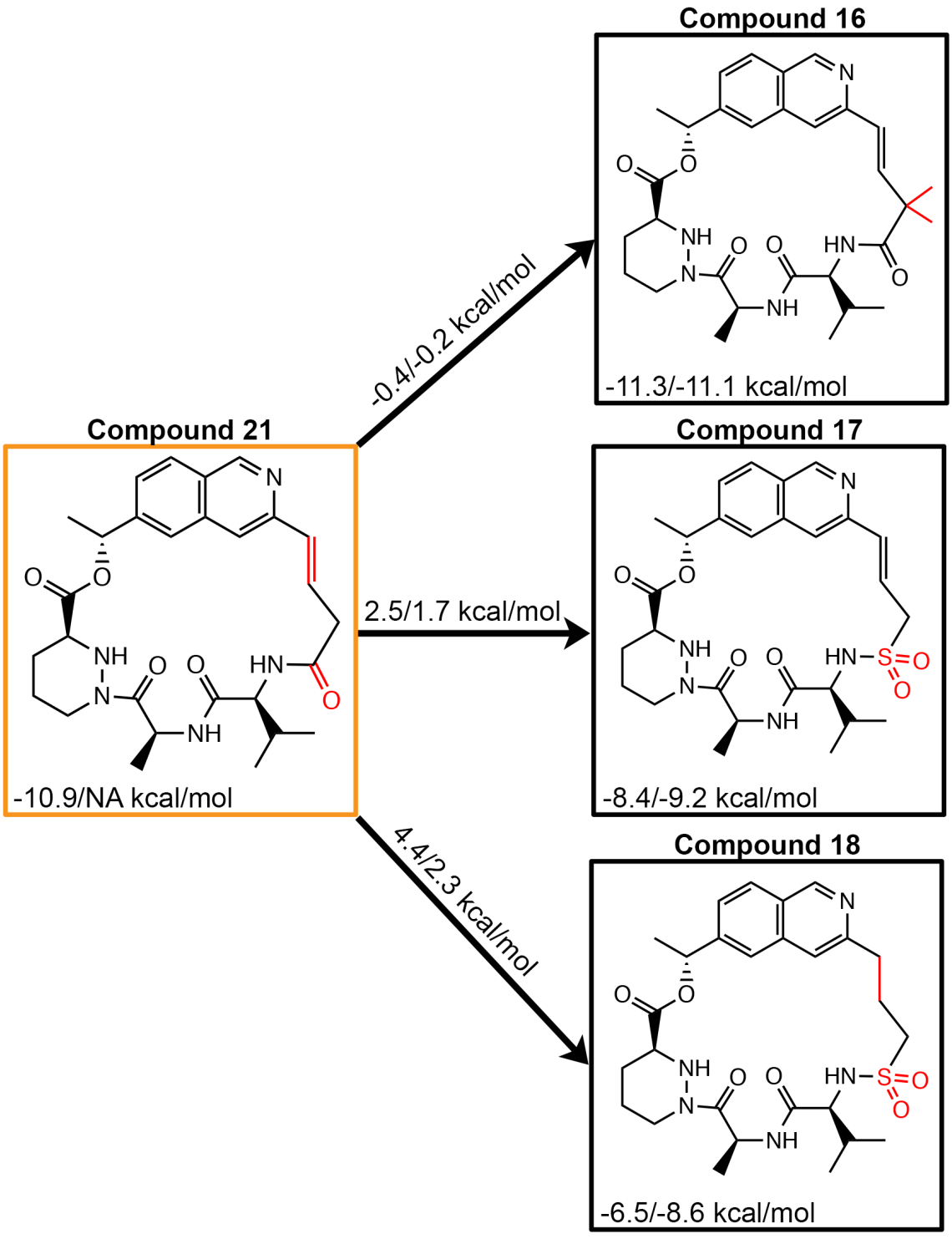

***Essential Core for Potency: Map 1a***



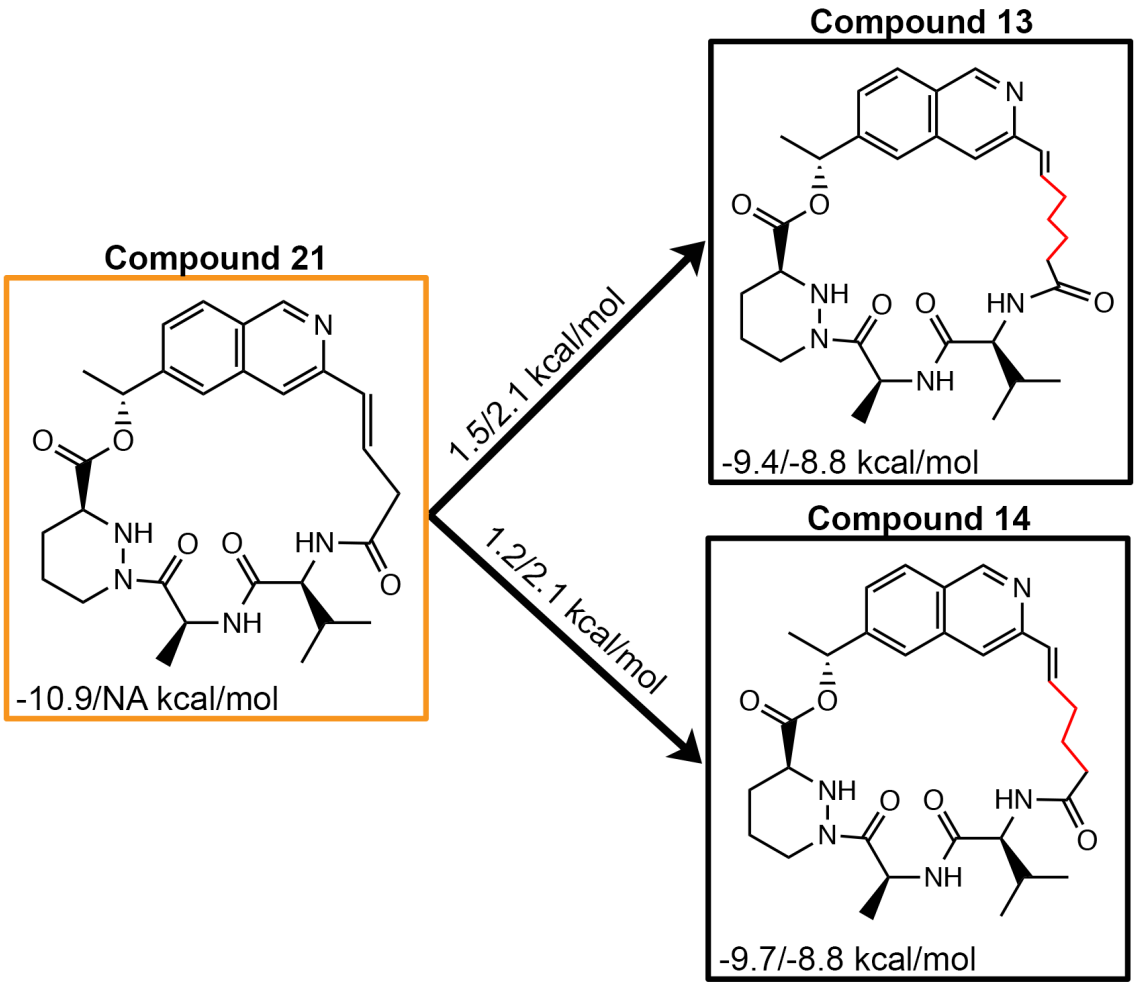
***Discovery of Novel Binding Mode: Map 2***



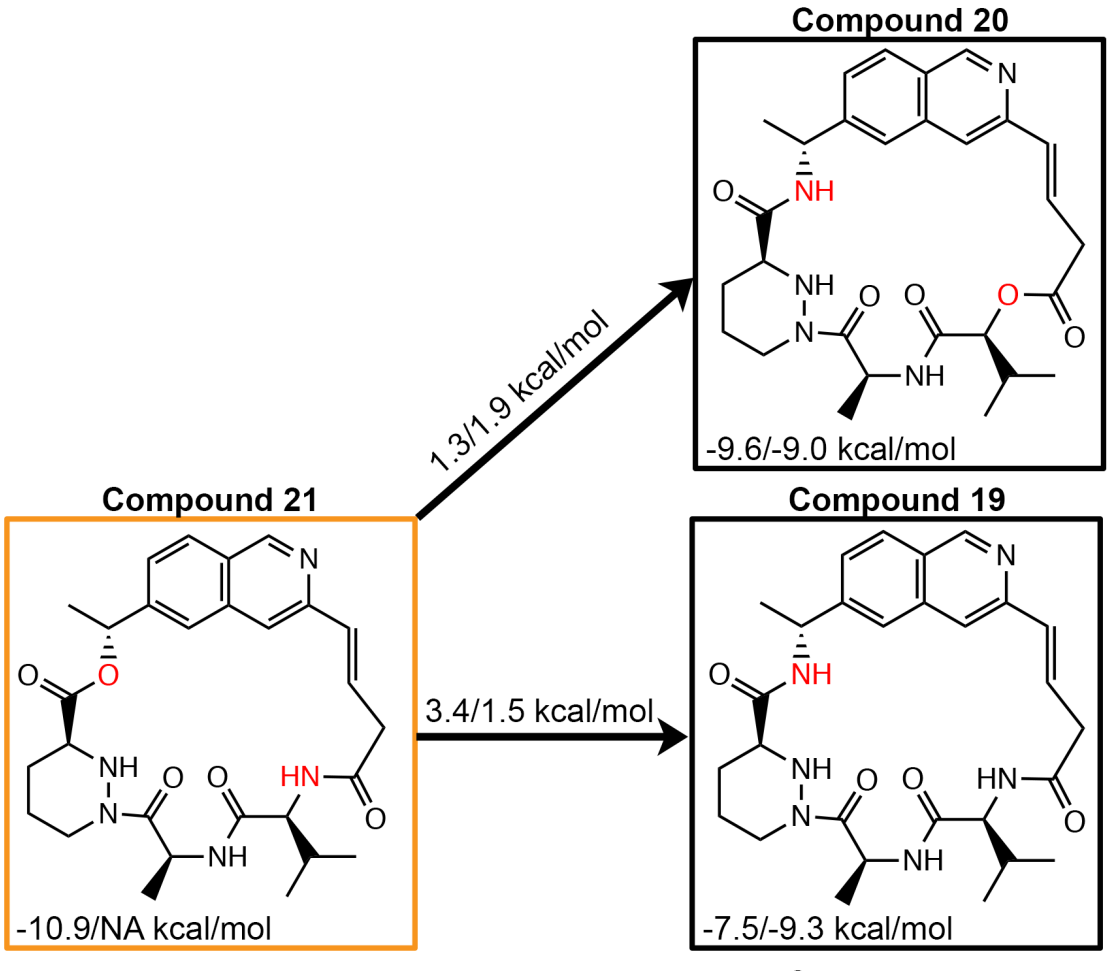
***Rigidification: Map 9***



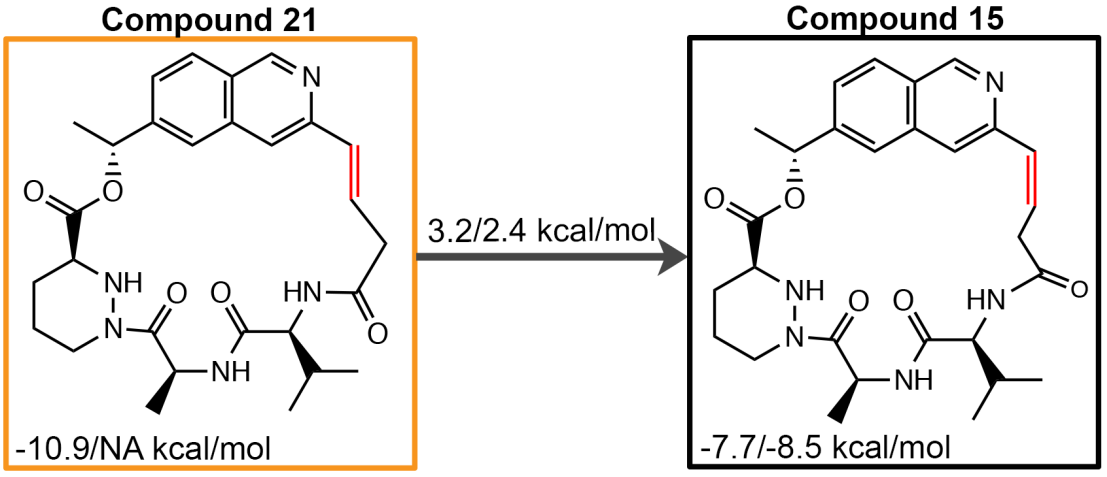
***Rigidification: Map 10***



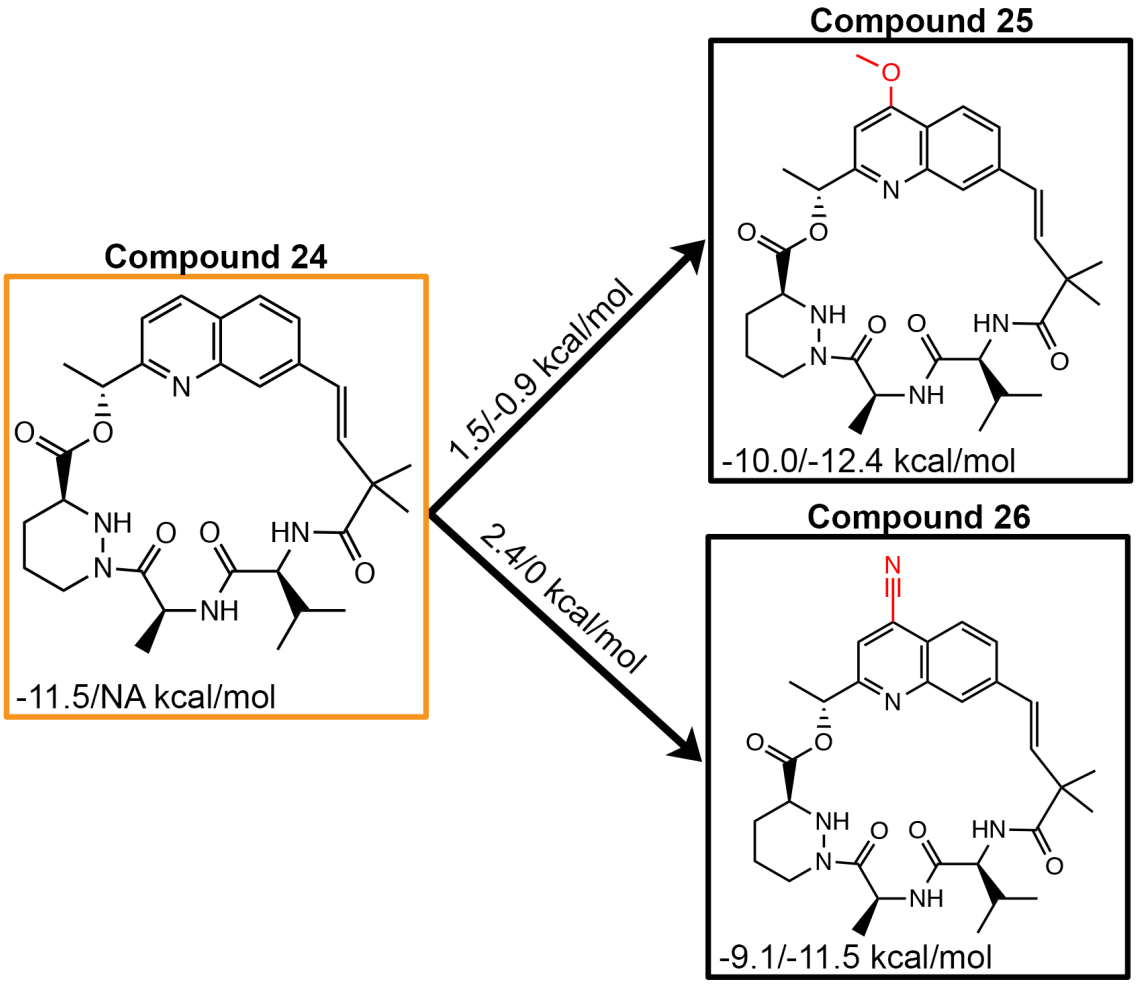
***Increased Permeability: Map 4***



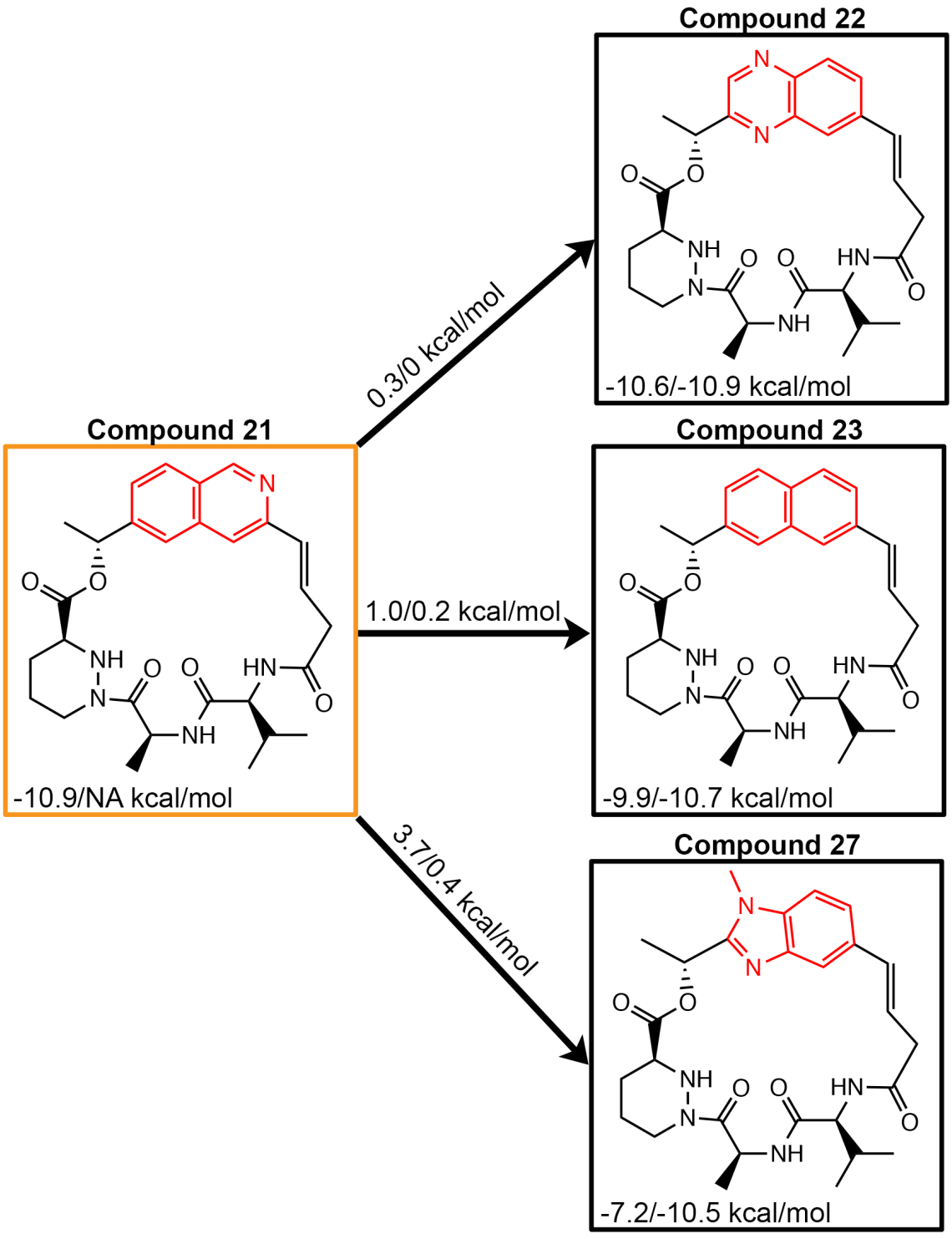

***Increased Permeability: Map 4a***



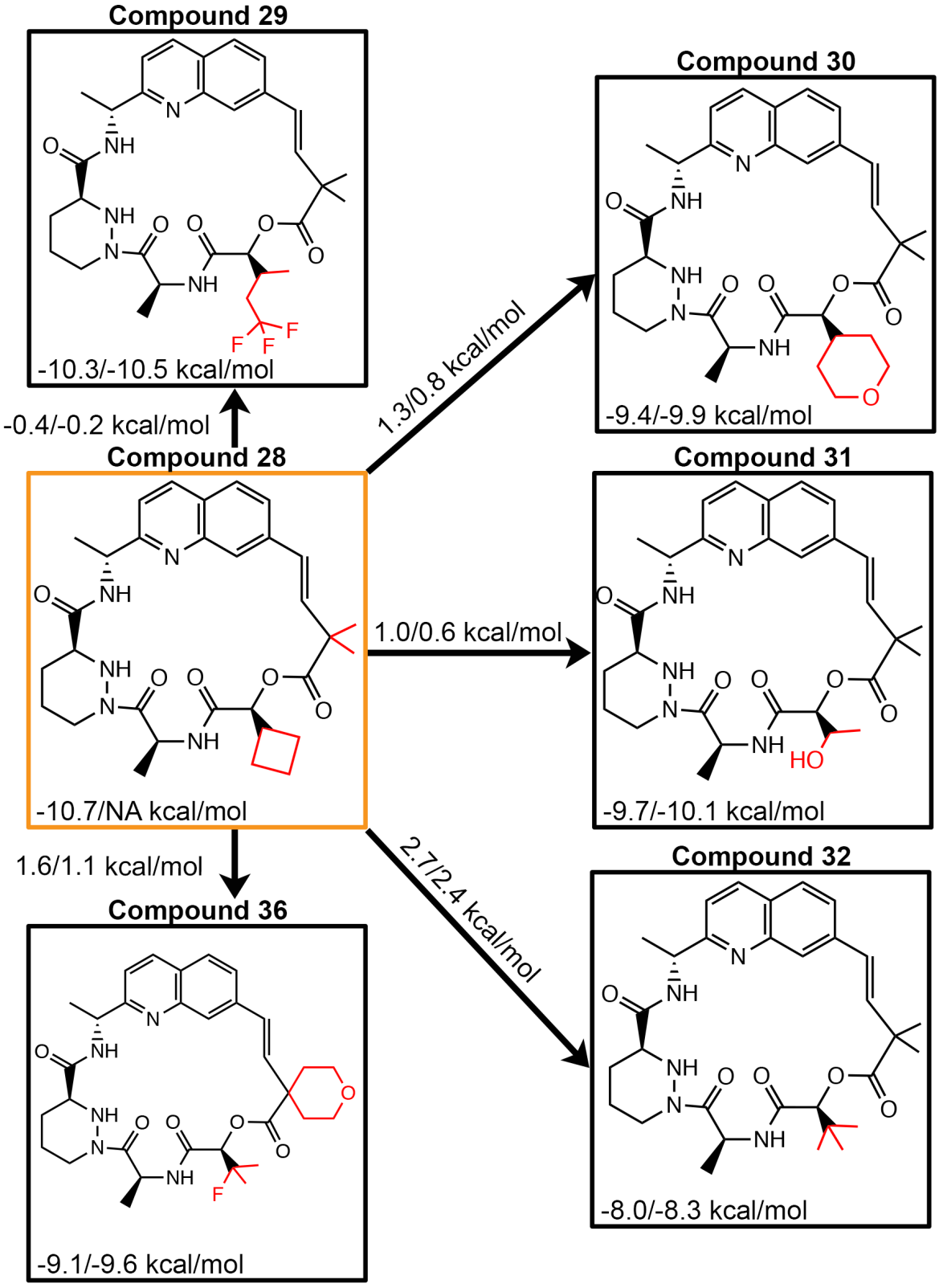
***Increased Permeability: Map 7***



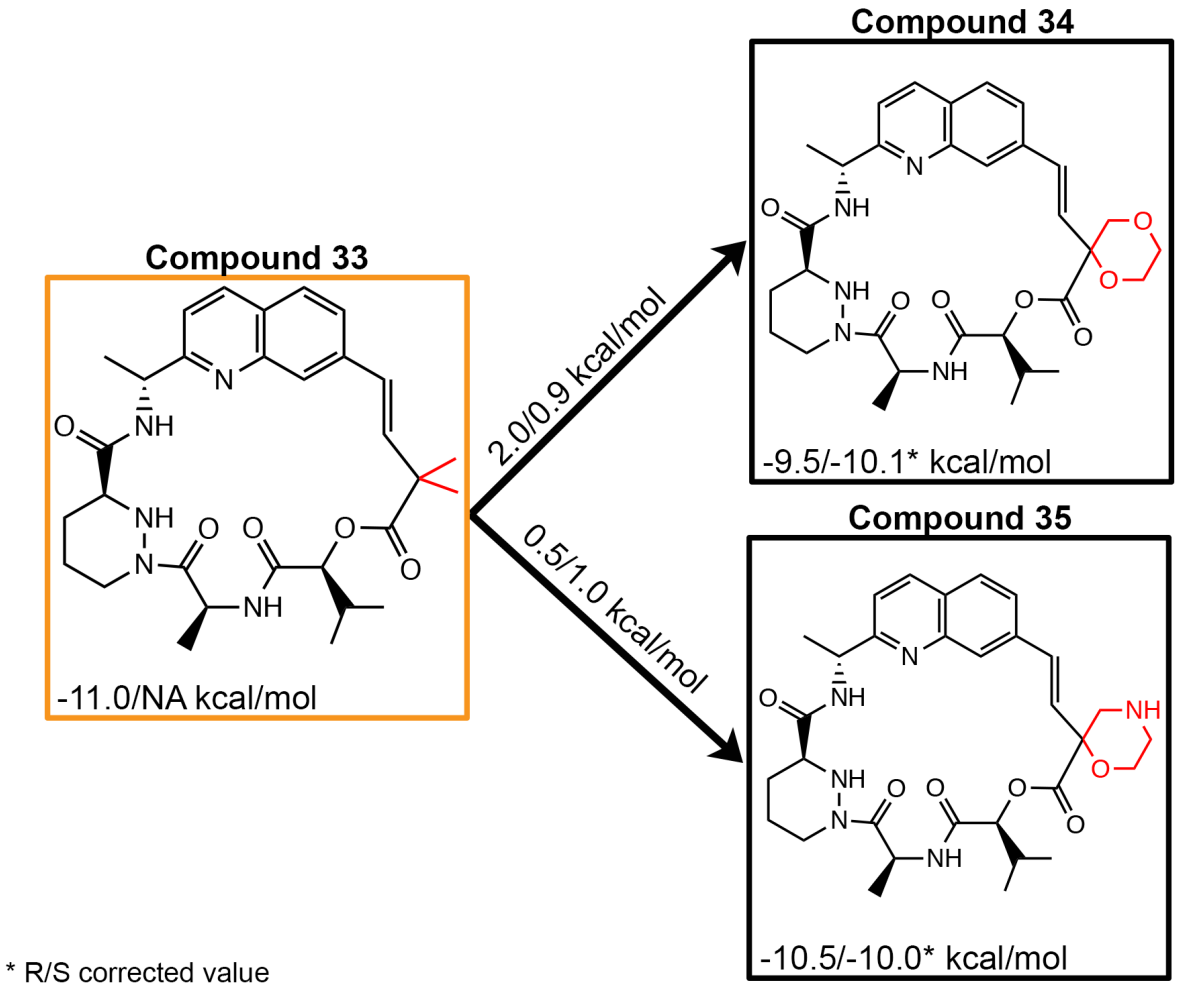
***Increased Permeability: Map 8***



***PK Profile Improvements: Map 5***



## *PK Profile Improvements: Map 6*




* R/S corrected value

**Figure S3.** Example perturbation map showing the full closed cycles that are necessary for predicting the relative binding free energies. Since free energy is a state function we using closed cycles to predict the relative free energies and estimate the error (hysteresis). For illustration purposes, the graph shown below represents the full closed cycles that were calculated for the prediction shown in Figure S2 Rigidification: Map 10. FEP+ maps for all predictions may be downloaded here: [schrodinger.com/paulsen-fep-maps](schrodinger.com/paulsen-fep-maps)

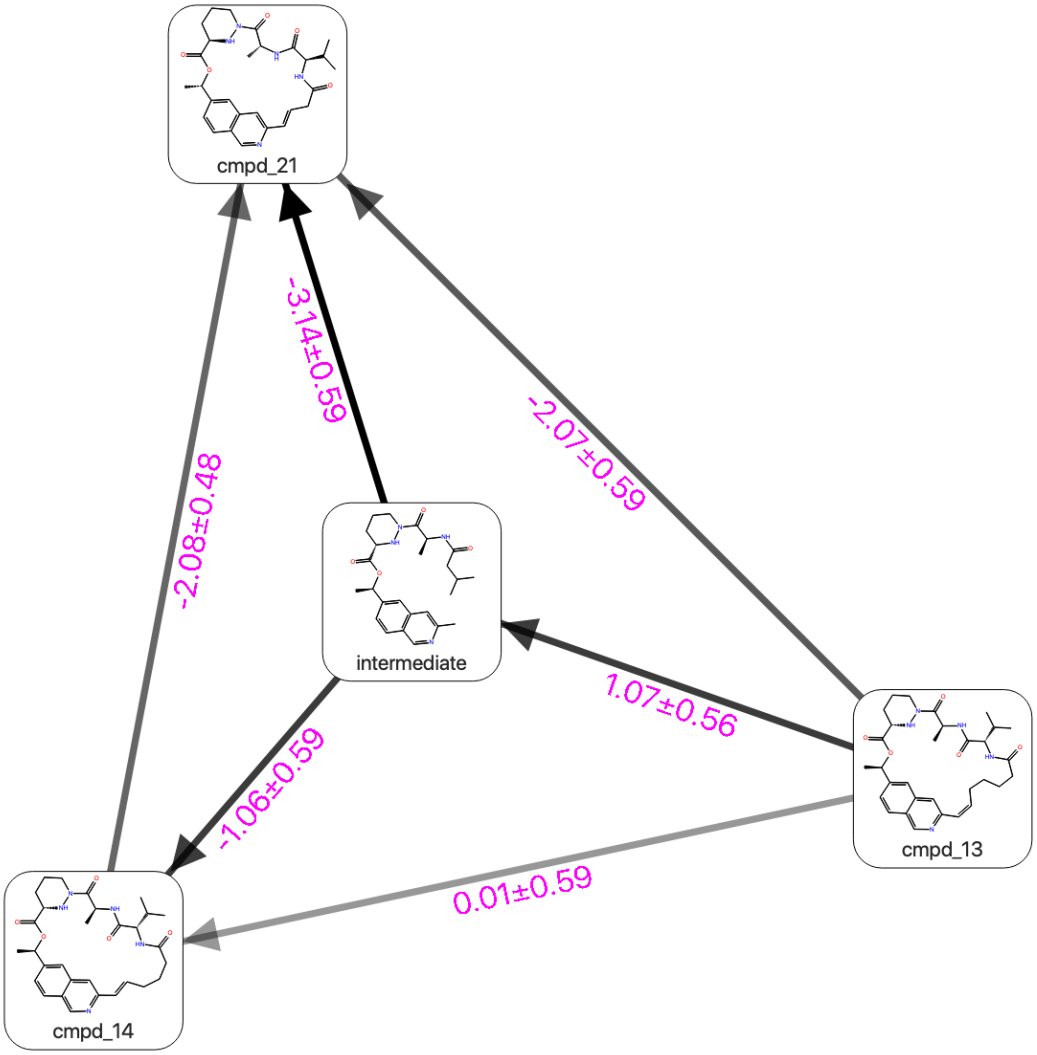


**Figure S4.** Specific dihedral angles scaled for compound **19** and **20** shown in red.