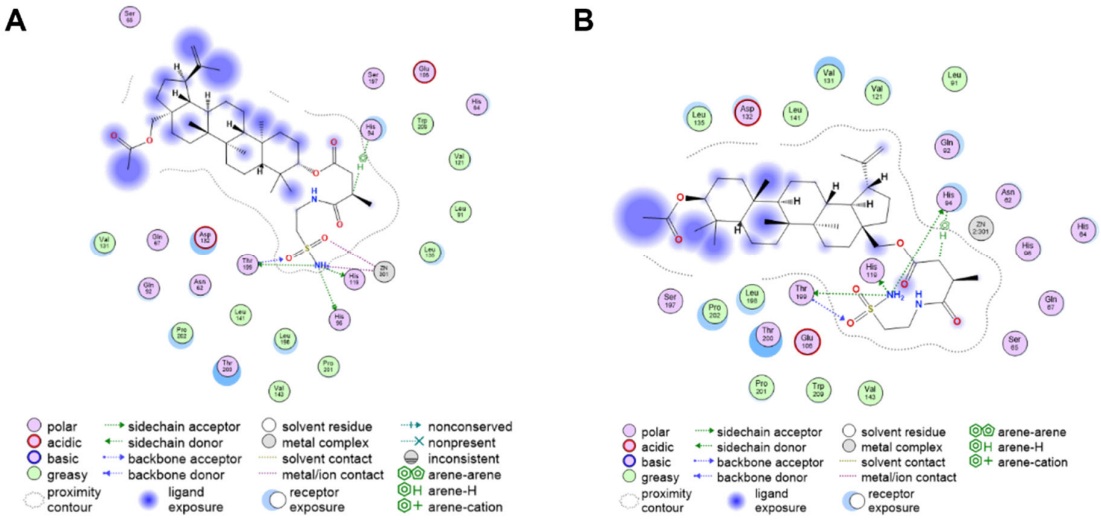
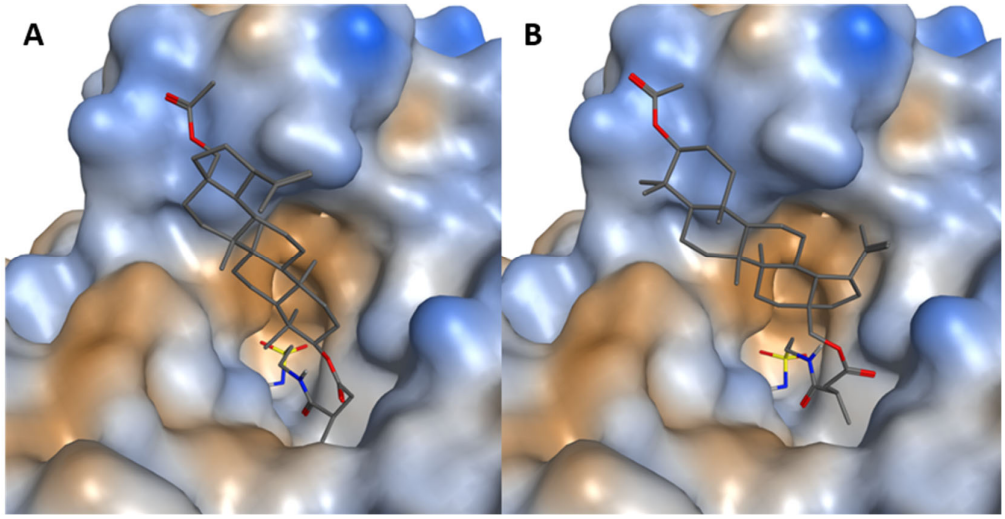
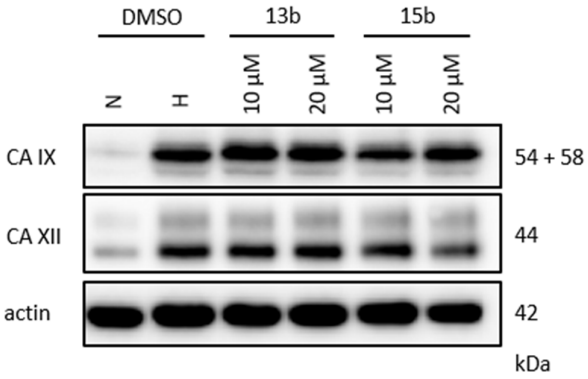
Supplementary Files



Supplementary Figure S1. Ligand interaction diagram [1]. It was generated with MOE 2020 (Molecular Operating Environment, 2018; Chemical Computing Group ULC, 1010 Sherbrooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7) illustrating the interactions of the betulin derivatives 13b **(A)** and 15b **(B)** with the active site residues of CA IX.



Supplementary Figure S2. Illustration of possible docking poses for 13b (**A**) and 15b (**B**) in the binding pocket CA XII (PDB code: 1jd0). The orientation of the enzyme is consistent with that of CA IX in Figure 7 of the main section. It can be seen that the orientation of the two inhibitors in the binding pockets is very similar. The binding pocket of CA XII is colored as a molecular surface according to lipophilicity (brown: lipophilic, blue: hydrophilic, white: neutral).



Supplementary Figure S3. Western blots of CA IX and CA XII. HS578T breast cancer cells were cultured under hypoxic conditions (0.1 % O_2) and were subsequently treated with 10 µM and 20 µM 13b and 15b for 3 h. The cell lysates were analyzed by Western blot and the protein levels of CA IX and CA XII were examined. Actin was used as a loading control. The figure shows one representative Western blots of four independent experiments.

Supplementary Table S1. Total ChemPLP fitness values of the docked ligands 13b and 15b [2]**.** The score values represent an empirical fitness function optimized for pose predictions of the GOLD molecular docking program. The values given are dimensionless and higher values indicate higher predictive power.

	13b	**15b**
Gold.PLP.Fitness	65.5	82.7

References

1. Clark, A.M.; Labute, P. 2D depiction of protein-ligand complexes. *Journal of Chemical Information and Modeling* **2007**, *47*, 1933–1944, doi:10.1021/ci7001473.
2. Korb, O.; Stützle, T.; Exner, T.E. Empirical scoring functions for advanced protein-ligand docking with PLANTS. *Journal of Chemical Information and Modeling* **2009**, *49*, 84–96, doi:10.1021/ci800298z.