Structural and biochemical characterization of novel Heme Oxygenase 1 (HO-1) inhibitors

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Heme oxygenase (HO) enzymes catalyze heme degradation into biliverdin, bilirubin, iron, and carbon monoxide, which have anti-inflammatory and antioxidant properties.HO1 is widely expressed in various cancers, whereas HO2 is constitutively expressed in the brain and testes(1).The scientific community has focused on developing non-porphyrinic HO1 inhibitors with a non-competitive mechanism(2).In collaboration with the University of Catania, this study presents two crystallographic structures of HO1 and HO2 in complex with the same imidazole-based inhibitor, paving the way for the rational design of HO1 selective inhibitors. Selectivity is crucial because HO2 plays a key role in heme homeostasis, acting as a heme-buffering factor that controls heme bioavailability(3).These structures reveal the binding poses of the inhibitor in both enzymatic isoforms. The active site is highly conserved, with identical hydrophobic and polar interactions in both proteins. The imidazole portion interacts directly with the iron ion of heme, while the central region ensures hydrophobic contact with L147.The northeastern region forms polar interactions with F167, F37, F47, and M34. A key difference is in the western region: the bromine atom interacts with L217 in HO1, while in HO2, it is oriented towards Y134. The aromatic ring enables π-π stacking with F214 and hydrophobic contacts with L20. We developed a biochemical assay to characterize the uncompetitive inhibition of the two recombinant proteins and observed nonspecific binding between hemin and the imidazole derivative.To confirm this biochemical mechanism, kinetic biophysical techniques such as SPR and MST will be required. Finally, a study will investigate the complex formation between NADPH reductase and HO1,with and without specific inhibitors, as these proteins physiologically interact, leading to high enzymatic activity.(1) Consonni et al. (2024)Front.Immunol(2)Fallica et al.(2021)J. Med. Chem.(3)Hanna et al.(2022 J.Biol.Chem.