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| Title | SLOW AND TIGHT BINDING INHIBITION KINETICS OF CYP17A1 BY ABIRATERONE AND ITS ACTIVE METABOLITE |
| Keywords (up to 5) | Enzyme Inhibition |
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| Abstract | Cytochrome P450 17A1 (CYP17A1) is a critically important bifunctional enzyme in humans that catalyzes the formation of all endogenous androgens. With substantial evidence alluding to the reactivation of the androgen signaling axis in metastatic castration resistant prostate cancer, abiraterone was developed as a first in class CYP17A1 inhibitor. Additionally, a downstream steroidal metabolite of abiraterone (∆4 abiraterone or D4A), has also been shown to possess comparable CYP17A1 inhibitory activity.1 However, previous structural analysis and *in vitro* assays investigating the nature and potency of CYP17A1 inhibition by abiraterone and D4A have reported considerable incongruities. This underscores the essentiality of conducting detailed biochemical characterization of the drug-target interactions.2–4 We hypothesized that abiraterone and D4A are slow, tight-binding inhibitors of CYP17A1. Pre-incubation time-dependent inhibition assays using progesterone and 17α-hydroxypregnenolone as probe substrates for the 17α-hydroxylase and C17,20-lyase reactions were conducted to determine the first-order rate constants for onset of enzyme inhibition (kobs). Next, concentration-response plots of fractional velocity against inhibitor concentration allowed derivation of the apparent dissociation constants (*Ki,app*) for the initial CYP17A1-inhibitor complexes, which was subsequently corrected for the mode of inhibition to obtain *Ki*. Finally, regression analyses were performed to elucidate the true inhibitory potential (Ki\*) and dissociation half-life of CYP17A1-inhibitor complexes. The observed hyperbolic dependence of *kobs* on abiraterone/D4A concentration affirmed the slow onset of inhibition, with initial weak binding (EI) preceding the subsequent slow isomerization to a final high affinity EI\* complex. Assessment of the overall Ki\* revealed significant (>50-fold) increases in inhibitory potencies compared to *Ki*, demonstrating that assumptions of rapid equilibrium may often preclude detection of time dependent effects crucial for the holistic assessment of the true inhibitory potencies of non-classical inhibitors. Furthermore, the long dissociation half-lives (>40 h) observed reflect protracted target occupancy (i.e. tight binding) and corresponding kinetic selectivity. This could eventually support prospective studies exploring lower dosing requirements involving abiraterone, resulting in enhanced safety while preserving the desired pharmacodynamic effect.  |
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