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HOTSPOT MAPPING AND THERMOSTABILITY ANALYSIS OF 17-DMAG BINDING TO *LEISHMANIA BRAZILIENSIS* HSP83

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Introduction: Previously, we have shown that human molecular chaperone heat shock protein 90 (*Hs*Hsp90) inhibitors, including 17-DMAG, exhibit both *in vitro* and *in vivo* leishmanicidal activity. It is reasonable to assume that 17-DMAG exerts its effects by inhibiting the parasite's Hsp (Hsp83), a key regulator of phase transitions in its life cycle. However, direct experimental evidence supporting this hypothesis is still lacking. This knowledge gap hinders the molecular simplification of 17-DMAG to reduce synthesis costs, limits the evaluation of critical hotspot residues (e.g., Asp90 in Hsp90), and the efforts to enhance its selectivity profile. **Hypothesis:** Evolutionary constraints have differently shaped the hotspot regions of *Hs*Hsp90 and *L. braziliensis* Hsp83 (*Lb*Hsp83). **Objective:** To confirm that 17-DMAG binds to *Lb*Hsp83 and to identify key hotspot residues involved in this interaction. **Methods:** Hotspot analysis was performed using the DRUGpy plugin along with FTmap and e-FTmap servers. Recombinant N-terminal *L. braziliensis* Hsp83 was expressed, purified via chromatographic methods, and analyzed in differential scanning fluorescence assays in the presence or absence of 17-DMAG. **Results:** The binding of 17-DMAG to *Lb*Hsp83 was confirmed by its large increase in melting temperature (ΔTm = 10.4°C) upon 17-DMAG addition (500 μM), which was consistent with high-affinity binding, further validated by concentration-response assays (Kd = 0.32μM). After establishing *Lb*Hsp83 as the molecular target of 17-DMAG, the hotspot profiles of *Hs*Hsp90 and *L. major* Hsp83 (*Lm*Hsp83), the closest homolog with 3D structure available were compared. The analysis revealed differences in hotspot strength (*druggable vs. borderline-druggable*), suggesting that inhibiting *Lm*Hsp83 may be more challenging than targeting *Hs*Hsp90. Additionally, this analysis shows a fraction of the *Lm*Hsp83 hotspot remained unoccupied by 17-DMAG, indicating potential for affinity enhancement. A comparison of atomic consensus sites from these structures highlighted that fine-tuning apolar and hydrogen donor interactions is crucial for designing selective inhibitors. **Conclusion:** Identifying *Lb*Hsp83 as the molecular target of 17-DMAG was paramount to carrying out hotspot analysis that offers valuable insights to guide potency and affinity optimization.

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