Comparison of the cellular and biochemical properties of ansamycin and non-ansamycin based Hsp90 inhibitors

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Abstract

Background: Heat shock protein 90 (Hsp90) has emerged as an important target for the treatment of cancer due to its essential role in several key oncogenic signaling pathways. Several classes of Hsp90 inhibitors have recently advanced into clinical trials. Several ansamycin derivatives have been synthesized and tested in cell lines and xenografts. However, these studies have not demonstrated selective growth inhibition toward cancer cells as compared to normal cells. We have determined the biochemical and cellular properties of a group of published Hsp90 inhibitors, including both reduced protect and synthetic analogs.

Methods: A battery of Hsp90 inhibitors was tested for their biochemical affinity for purified Hsp90 under non-reducing and reducing conditions. The growth inhibition of cancer and normal cells was measured using Alamar blue dye. Apoptosis was measured using the Annexin V assay and the cleavage of caspase-3 was measured by ELISA.

Results: The biochemical affinities to purified Hsp90 for the inhibitors tested varied from 0.0 to 530 nM. Under non-reducing conditions, IPI-493 is oxidized to 17-AAG and IPI-504 is converted to the 17-AG hydroquinone. Note that IPI-504 (17-AAG) is dramatically more potent than 17-AAG.

Conclusions: The experiments presented above raise the question of whether the Hsp90 inhibitors with high affinity for Hsp90 have lost some of the in vivo therapeutic window between cancer and normal cells that makes Hsp90 inhibitors such attractive candidates for cancer therapeutics.

Figure 1: Binding affinities of ansamycin related and synthetic Hsp90 inhibitors

Figure 2: Growth inhibition of cancer cell lines by ansamycin and non-ansamycin Hsp90 inhibitors

Figure 3: Growth inhibition of normal cells by ansamycin and non-ansamycin Hsp90 inhibitors

Figure 4: Client protein suppression (mutant EGFR) and cleaved caspase 3 activity by IPI-504, IPI-493 and NVP-AUY922 in vivo

Figure 5: Dissociation rates of Hsp90 inhibitors

Summary

- We find the order of biochemical potency to be NVP-AUY922 > BIIB-021 > IPI-493 > IPI-504 > SNX-2112 and the dissociation rates are ranked as follows: IPI-493 ~ IPI-504 ~ NVP-AUY922 ~ BIIB-021 > SNX-2112.
- A similar order of potency is found in two normal human cell types (NHDF and HMEC) under conditions where these cells proliferate. The average GI50 values for these two cell lines are 15 nM (NVP-AUY922), 100 nM (IPI-493), 345 nM (SNX-2112) and 570 nM (IPI-504).
- The ability of IPI-504, IPI-493 and NVP-AUY922 to suppress the abundance of a client protein (mutEGFR) and to induce activated caspase 3 was determined in vivo in their published xenografts. No significant differences between these compounds were detected.
- Off-rate measurements show that although NVP-AUY922 has a ~50-fold lower Ki than the other inhibitors, it is only three- to seven-fold slower than IPI-493 and IPI-504.