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

Christian C. Fritz, Jie Ge, Nafeeza Hafeez, Bonnie Tillotson, Kris Depew, John Coco, Johan Basuki, Alice Lim, Jon Patterson, James R. Porter, Vito Palombella and Emmanuel Normant

Infinity Pharmaceuticals Inc., 780 Memorial Drive, Cambridge, Massachusetts, USA

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 including ansamycin derivatives that are semi-synthetic derivatives of the natural product geldanamycin (e.g. 17-AAG, IPI-504, 17-DMAG) or synthetic small molecules (e.g. purine derivatives, isoxazoles, pyrazoles). Ansamycin derivatives are potent Hsp90 inhibitors that demonstrate selective cell 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 natural product derived and synthetic compounds.

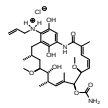
Materials and methods: The biochemical affinity of inhibitors to Hsp90 was determined using a competition binding assay using radioactively labeled competitor compound and Hsp90 purified from Hela cells. The growth inhibition induced by Hsp90 inhibitors was evaluated in human normal and cancer cells by measuring cell growth in the presence of varying concentrations of compounds using the Alamar Blue assay after 72h of compound addition.

Results: The biochemical affinities to purified Hsp90 for the inhibitors tested range from 0.1 to 500 nM. There is an approximate correlation between biochemical affinity and cell growth inhibition of cancer cells with a relative activity ranking of isoxazoles > ansamycins > purines. While ansamycins demonstrate selective growth inhibition of cancer cells compared to normal cells as previously described in the literature, the more potent isoxazole derivative also potently inhibits the growth of some normal cell types. Three of the most potent compounds on cancer cells were also tested *in vivo* for their ability to induce the degradation of a Hsp90 client protein (mutEGFR) in a xenograft model of human NSCLC. In this model, we observe similar suppression of mutant EGFR and similar induction of cleaved caspase 3 for all three compounds, independent of their biochemical affinity for purified Hsp90. One possible explanation for this observation is provided by our measurements of off-rates of inhibitors from Hsp90.

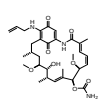
Conclusion: The experiments presented above raise the question of whether synthetic Hsp90 inhibitors with high affinity for Hsp90 have lost some of the in vitro therapeutic window between cancer and normal cells that makes Hsp90 inhibitors such attractive candidates for cancer therapeutics.

Compounds studied

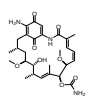
IPI-504



17-AAG



IPI-493



NVP-AUY922

SNX-2112

BIIB-021

Biochemical activity of Hsp90 inhibitors

Inhibitor	K_i (nM)	
	Reducing conditions (+ TCEP)	Non-reducing conditions (- TCEP)
IPI-504	9.0 ± 4.7 (IPI-504)	530 ± 170 (17-AAG)
IPI-493	3.0 ± 1.8 (17-AG HQ)	21 ± 7.5 (IPI-493)
NVP-AUY922	0.064 ± 0.058	0.069 ± 0.047
SNX-2112	1.1 ± 0.41	2.8 ± 2.1
BIIB-021	13 ± 3.5	26 ± 18

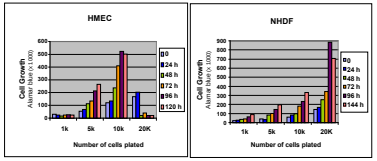
Figure 1: Binding affinities of ansamycin related and synthetic Hsp90 inhibitors
Affinities were determined using a competitive binding assay with ^{3}H-labeled Hsp90 inhibitors and Hsp90 purified from Hela cells. Affinities were measured both under reducing (+ 2.0 mM TCEP) or non-reducing (- TCEP) conditions. Under non-reducing conditions, IPI-504 is oxidized to 17-AAG; under reducing conditions, IPI-493 is converted to the 17-AG hydroquinone. Note that IPI-504 (17-AAG hydroquinone) is ~50 times more potent than 17-AAG.

Activity of Hsp90 inhibitors on cancer cells

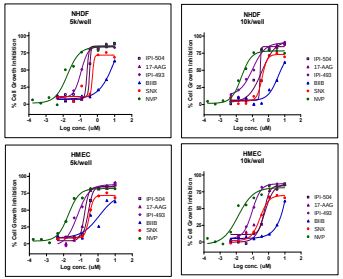
Cells	Cell type	IPI-504	17-AAG	IPI-493	NVP-AUY922	BIIB-021	SNX-2122
1650	NSCLC	35	31	24	12	321	118
1975	NSCLC	28	25	17	7	137	67
HCC-827	NSCLC	44	70	38	12	194	89
H460	NSCLC	16	16	9	9	67	32
H4006	NSCLC	63	46	32	5	135	48
A549	NSCLC	13	15	11	9	128	59
HCT-116	CRC	88	78	34	4	151	51
H729	CRC	6	10	2	42	88	28
SW480	CRC	42	49	30	5	256	71
L3.5s1	PaCa	10	10	2	6	119	34
L3.6p1	PaCa	50	64	7	7	213	57
AsPC1	PaCa	10	20	4	41	147	55
BT-474	BrCa	16	12	9	2	31	17
JIMT-1	BrCa	19	27	13	2	31	67
SKBr3	BrCa	17	21	17	5	109	23
MBA-MB-231	BrCa	939	1000	29	9	264	64
A2780	Ovarian	33	34	30	4	190	35
SKOV3	Ovarian	45	51	24	3	224	39
IGROV-1	Ovarian	310	430	235	11	1712	144
RPMI-8226	MultMyeloma	193	170	55	13	88	53
KMS-12	MultMyeloma	206	222	29	3	95	28
H929	MultMyeloma	22	40	13	1	95	31
MV4-11	AML	18	12	11	1	42	9
K562	CML	68	68	7	3	73	13
DaudI	Lymphoma	412	131	218	17	304	34
RL	Lymphoma	626	1449	56	7	245	47
Toledo	Lymphoma	811	604	84	17	329	73

Figure 2: Growth inhibition of cancer cell lines by ansamycin and non-ansamycin Hsp90 inhibitors
Cells were plated subconfluently according to ATCC conditions, exposed to various compound concentrations, and the GI_{50} (concentration of compound that inhibits cellular growth by 50%) was determined using Alamar Blue after 72h. The median GI50 values are 7 nM (NVP-AUY922), 24 nM (IPI-493), 43 nM (IPI-504), 48 nM (17-AAG), 50 nM (SNX-2112) and 149 nM (BIIB-021).

Normal cells proliferate under conditions tested



Activity of Hsp90 inhibitors on normal cells



GI_{50} values (nM) for normal cells

Cells	Cell type	IPI-504	17-AAG	IPI-493	NVP-AUY922	BIIB-021	SNX-2112
HMEC	Normal breast epithelial cells	560	550	100	10	3700	290
NHDF	Normal dermal fibroblasts	580	500	100	20	2200	400

Figure 3: Growth inhibition of normal cells by ansamycin and non-ansamycin Hsp90 inhibitors
To determine at which plating densities HMEC (Human mammary epithelial cells) and NHDF (Normal human dermal fibroblasts) are able to replicate in tissue culture, freshly thawed cells were plated according to suppliers instruction and the growth was measured using Alamar Blue (Figure 3A). Both cell types show robust growth at 5k and 10k plating densities and these densities were used to determine GI_{50} values (Figure 3B, results for 10k densities summarized in Figure 3C). Note that the Hsp90 inhibitor that is most potent on cancer cells (NVP-AUY922) also has the biggest effect on normal cells.

In vivo activity of Hsp90 inhibitors

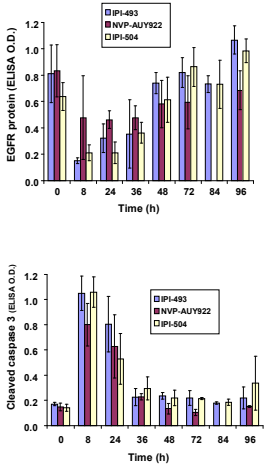
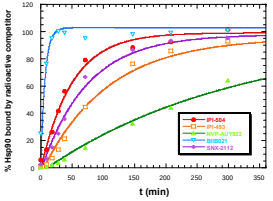


Figure 4: Client protein suppression (mutant EGFR) and cleaved caspase 3 induction by IPI-504, IPI-493 and NVP-AUY922 *in vivo*
Subcutaneous xenografts of the NSCLC cell line H1650 [EGFR (del E746-A750)] were established in athymic mice and the effect of different Hsp90 inhibitors on EGFR abundance and induction of cleaved caspase 3 were measured over time. Compounds were given as a single dose at their published MTD (IPI-493 and IPI-504 at 100 mg/kg, NVP at 50 mg/kg).

Off rates of Hsp90 inhibitors



Compound	k_{off} (min^{-1})	$t_{1/2}$ (min)	K_i (nM)
IPI-504	0.021 ± 0.009	33	9.0 ± 4.7
IPI-493	0.010 ± 0.003	68	3.0 ± 1.8
NVP-AUY922	0.0033 ± 0.0002	212	0.066 ± 0.049
BIIB-021	0.14	5	18 ± 12
SNX-2112	0.014 ± 0.001	48	2.0 ± 1.6

Figure 5: Dissociation rates of Hsp90 inhibitors
Hsp90 was pre-bound with individual inhibitors under reducing conditions and the dissociation rates were measured by incubation with an excess of radiolabeled competitor and separation of bound compound through a 96-well spin plate.
Note that the off-rate of NVP-AUY922 is 3 to 7 times slower than the off-rate of IPI-493 and IPI-504

Summary

- **We find the order of biochemical potency to be NVP-AUY922 >> SNX-2112 ≥ IPI-493 ≥ IPI-504 ≥ BIIB-021 (Fig.1)**
- **In a panel of cancer cells, the order of activity for cell growth inhibition is NVP-AUY922 > IPI-493 > IPI-504 ≈ SNX-2112 > BIIB-021 with median GI_{50} values of 7, 24, 43, 50 and 149 nM, respectively (Fig. 2)**
- **A similar order of potency is found in two normal human cell types (NHDF and HMEC) under conditions where these cells proliferate. The average GI_{50} for these two cells are 15 nM (NVP-AUY922), 100 nM (IPI-493), 345 nM (SNX-2112) and 570 nM (IPI-504) (Fig. 3)**
- **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* at their published MTDs. No significant differences between these compounds were detected (Fig.4)**
- **Off-rate measurements show that although NVP-AUY922 has a ~50-fold lower K_i, the off-rate is only three- to seven-fold slower than that of IPI-493 and IPI-504 (Fig. 5)**