them with penetratin allowing them to enter the cells. Here, we studied the effect of penetratin-peptidimer on HER2 signaling and its synergistic effect with "Docetaxel (Taxotere(R))" on HER2-overexpressing cancer cells in vitro and in vivo. Transformed NIH3T3/HER2 cells and SKBr3 (a human breast cancer line overexpressing HER2) cells were seeded on 6-wells plates and the penetratin-peptidimer was added to the culture medium to test its anti-tumor potential in a clonogenic assay. Treated cell colonies were stained and counted 2 to 3 weeks later. The penetratin-peptidimer inhibited the colony formation with an IC50 of 0.5 μM and 0.05 μM for SKBr3 cells and NIH3T3/HER2 cells, respectively. The levels of phosphorylated AKT and ERK proteins were assessed in order to determine the peptidimer effects in the HER2-dependant signaling pathway. Cells treated with the peptidimer showed a reduction in phosphorylated AKT but ERK phosphorylation remained unchanged. Docetaxel treatment induces overexpression of HER2, in a human prostate adenocarcinoma xenograft established in nude mice. "Trastuzumab (Herceptin(R))", a humanized recombinant monoclonal antibody directed against HER2 was shown to synergize the Docetaxel induced effects. We observed an increased HER2 expression in the two cell lines following Docetaxel administration. The peptidimer significantly enhanced sensitivity to Docetaxel in both NIH3T3/HER2 and SKBr3 cells. This combination was tested in an independent hormone xenograft model, using nude mice, of human prostate cancer overexpressing HER2. A synergistic effect of the peptidimer and Docetaxel was also obtained. These results suggest that the SH3-Grb2 inhibitor has an anti-tumor activity and enhanced cytotoxicity when combined with Docetaxel in HER2-expressing breast cancer cells and in the prostate xenograft.

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Dihydropyrrolopyrazoles as TGF-beta receptor kinase inhibitors for cancer therapy

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TGF-β is a cytokine with diverse biological activities. TGF-β can mediate diametrically opposed activities depending upon the physiological state of a cell. Perhaps the most dramatic example of this phenomenon is TGF-β 's role as a tumor suppressor and tumor promoter. In many cells, TGF-β mediates a growth inhibitory signal via a heteromeric receptor complex composed of two transmembrane serine/threonine kinase receptors, the type I and type II receptors. However, once this inhibitory pathway is disrupted in tumor cells, TGF-β becomes a potent tumor promoter that can be secreted at high levels from the resistant tumor cells. This increased TGF-β expression modulates the extracellular matrix and has angiogenic and immunosuppressive activities. In addition, TGF-β contributes to the epithelial-to-mesenchymal transition of tumor cells, thus creating a more invasive and metastatic phenotype. These tumor-promoting activities of TGF-β provide rationale to target this pathway for therapeutic intervention. A series of orally bioavailable, small molecule kinase inhibitors that are potent and selective for the TGF-β receptors has been identified and characterized in *in vitro* kinase and cell based assays. Direct measures of target modulation in cells involved evaluation of P-Smad2 inhibition. A hallmark of our discovery program has been the characterization of *in vivo* target modulation using a subcutaneous xenograft tumor model to define PK/PD relationships for target modulation in animals. Evaluation of the dihydropyrrolopyrazole SAR *in vivo* yielded compounds with IC_{50} values ranging from 0.020 to 2 μM. In time course experiments, target modulation paralleled plasma exposure with 8–12 hr of measurable activity that necessitated an oral BID schedule in the subsequent anti-tumor efficacy models. A pan-TGF-β neutralizing antibody was used to validate the involvement of TGF-β in the growth of MX1 breast cancer xenografts. Evaluation of dihydropyrrolopyrazole compounds in the MX1 model showed a statistically significant decrease in tumor growth. Anti-tumor efficacy has also been observed for this series of compounds in the Calu6 NSCLC xenograft model. A series of microarray experiments in the Calu6 model has been conducted to evaluate the biological effects of TGF-β in this system *in vitro* and to evaluate the differential sensitivity of TGF-β regulated genes to type I receptor selective or dual type I/type II receptor inhibitors. Extension of this approach to the Calu6 xenograft model will assist in the identification of potential biomarkers for evaluation of on-target compound activity in future clinical trials.

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Factors that govern the cell death response induced by inhibition of the molecular chaperone heat shock protein 90

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The molecular chaperone heat shock protein 90 (Hsp90) has emerged as an exciting anticancer drug target due to its role in maintaining the conformation and stability of key oncogenic client proteins. The Hsp90 inhibitor; 17-allylamino, 17-demethoxy geldanamycin (17AAG) binds and inhibits the intrinsic ATPase which is essential for Hsp90 function, and is the first in-class Hsp90 inhibitor to enter and complete a phase 1 clinical trial. *In vitro* 17AAG treatment induces both cytostasis and apoptosis, the extent of which is cell line dependent. The aim of this study was to identify factors that may influence the cell death response following 17AAG treatment. Our laboratory has previously hypothesised that the apoptotic response to 17AAG operates via a Bax-dependent mechanism, based on the absence of apoptosis in KM12 cells that lack Bax, after exposure to 17AAG. This possibility has been explored using an isogenic pair of the human colon cancer cell line HCT116, which differ only in their expression of Bax. We demonstrate that Bax expression is required for apoptosis induced by 17AAG treatment and in its absence necrosis becomes the predominant mechanism of cell death. We also demonstrate that the apoptotic response to Hsp90 inhibition could be further influenced by increased expression of the Hsp70 family, which are inhibitors of the apoptotic pathway. We and others have previously shown that the constitutive (Hsc70), mitochondrial (Mortalin) and inducible (Hsp72) isoforms of Hsp70 are induced in response to 17AAG treatment. Here we use an siRNA approach to show that selectively repressing the induction of Hsp72 in response to 17AAG treatment increased cell death in HCT116 cells after only 24 hours exposure to 17AAG, which is earlier than normally associated with the cell death response in this cell line. The influence of Hsc70 and Mortalin on the cell death response to 17AAG treatment has also been explored using siRNA. In summary, these findings suggest that 17AAG induces apoptotic cell death via the intrinsic pathway mediated by Bax, the degree of which may be impaired by the induction of the anti-apoptotic Hsp70 family. However when Bax is not present 17AAG causes cell death to a lesser extent and via a necrotic mechanism. The anti-apoptotic effect of Hsp72 may begin to explain the predominance of tumour cytostasis versus cytotoxicity, as observed in human tumour xenografts and some patients treated with 17AAG during phase 1 clinical trial.

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Identification of potent, selective, soluble and permeable small molecule PI3 kinase inhibitors for the treatment of cancer

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Phosphatidylinositol-3-kinases (PI3K) are lipid kinases that mediate cell signalling pathways controlling growth, proliferation, survival and motility. There is significant evidence suggesting that deregulation of the PI3K/c-Akt pathway is important in tumour progression, including loss of function of the tumour suppressor PTEN, the phosphatase that counteracts PI3K, and high frequency of mutation of the PI3K p110α isoform in human malignancies.[1]

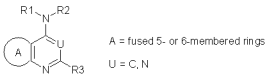

Fig. 1: Generic structure of fused heterocyclic compounds.

Table 1. *In vitro* biochemical and physicochemical properties of PI3K inhibitors

	IC_{50} (μM)		PI3K, SPA		Solubility (μM)	Mouse Microsome Stability[a]
	p110 α	p110 β	p110 δ	p110 γ		
PI103	0.0015	0.003	0.003	0.015	3.5	13
PI509	0.0045	0.037	0.019	0.112	20	5
PI516	0.004	0.045	0.006	0.063	>100	11
PI540	0.010	0.044	0.009	0.321	>100	91

[a] % compound remaining after 30 min.

We have previously reported that PI103, a potent and selective PI3K inhibitor with established *in vivo* efficacy in xenograft models, had been identified as a starting point for the development of a series of novel small molecule therapeutics for the treatment of cancer (Figure 1).[2] However, several features of PI103, including its low aqueous solubility at physiological pH, were subsequently identified as areas for lead optimisation. A medicinal chemistry effort at PIramed has resulted in the discovery of a second generation of PI3K inhibitors with promising biochemical affinity and functional activity, and with improved physicochemical properties. Three such compounds, PI509, PI516, and