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The design and synthesis of a novel orally available Hsp90 inhibitor CH5164840

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Background: Hsp90 is a constitutively expressed molecular chaperone that governs the maturation and stability of many regulatory factors that are key to cell growth and survival. Since many of the client proteins are implicated in tumor cell growth and survival, Hsp90 inhibitors could induce the simultaneous blocking of multiple signaling pathways. Accordingly, Hsp90 is attracting significant research interest as a target for anticancer agents. Although several Hsp90 inhibitors including geldanamycin derivatives (GM) and non-geldanamaycins are currently under clinical trials, no clear clinical efficacy against solid tumors has been demonstrated yet, probably due to safety, physicochemical properties and/or DMPK issues. We aimed to identify a novel non-geldanamycin type and orally available Hsp90 inhibitor that has better drug-like properties and shows stronger antitumor efficacy than GMs.
Material and Methods: Virtual screening (using docking software FlexX, followed by measurement of ATPase activity) and fragment screening (by surface plasmon resonance (SPR)-based binding assay, followed by hit validation by NMR to confirm their binding to ATP sites) were conducted in parallel to identify lead compounds. X-ray crystal structures of the complexes between the hits and Hsp90 were solved. Structure-guided inhibitor design and optimization were carried out by software Moloc and GRID.
Results: Several weak inhibitors having aminopyrimidine, aminotriazine and indazole moieties as core templates were identified. The X-ray structures of the Hsp90 complex with validated hit compounds revealed important interaction sites in the ATP binding pocket of Hsp90, namely, Asp93, and a hydrophobic pocket formed by Phe138 and Leu107. Starting with these templates, we designed and synthesized a series of simplified macrocyclic analogs with the aim of enhancing both binding affinity and cytotoxicity, in such a way that an inhibitor could form strong hydrogen bonds with crystal water and Lys58. Finally, a potent Hsp90 inhibitor CH5164840 was identified: Kd (Hsp90α) = 0.52 nM, IC_{50} (NCI-N87/Her2$^+$ GC) = 66 nM. Oral CH5164840 had fairly good PK profiles (mice: T1/2 = 1.6 hr, BA = 70.8%), and showed greater antitumor activity than 17-DMAG in various human cancer xenograft models (*e.g.* NCI-N87: TGI 160%; 50 mg/kg, 11 q.d.).
Conclusions: We identified a novel, orally available small molecule Hsp90 inhibitor, CH5164840, which has potent efficacy in human cancer xenograft mouse models.

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Selective inhibition of PI3K alpha using a novel covalent compound

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Background: The PI3K Class I family consists of four isoforms: a, b, g, and d. PI3K a and b are ubiquitously expressed, whereas g and d are more restricted. The PI3K pathway is activated in many cancers through either PTEN inactivation or through activating mutations in the gene that encodes the PI3Ka. Several PI3K inhibitors are currently in clinical development, but most are pan-PI3K inhibitors. Tumor biology data suggests that targeting PI3Ka specifically should be efficacious, and there may be advantages to not disrupting other members of the complex PI3K signaling cascade. Using structure-based drug design (SBDD), we have generated a targeted small molecule inhibitor of PI3Ka that acts through selective and irreversible covalent bond formation. Covalent inhibitors have many advantages including improvements in potency, selectivity, prolonged duration of action, and translational biomarker opportunities.
Materials and Methods: SBDD was used to generate selective small molecule inhibitors of PI3Ka. Mass spectrometry verified covalent bond formation to PI3Ka. PI3K enzyme activity was measured using an HTRF assay. PI3Ka inhibition was evaluated in SKOV-3 cells by measuring P-Akt^{Ser473} levels. Washout experiments were performed to assess prolonged duration of action in cells. SKOV-3 xenograft studies were performed in mice to measure P-Akt^{Ser473} inhibition and tumor growth inhibition *in vivo*. A biotinylated covalent probe molecule specific for PI3Ka was used to verify and quantitate target occupancy by the covalent inhibitor, both *in vitro* and *ex vivo*.
Results: Using mass spectrometry, we verified that CNX-1351 bonds specifically to an amino acid which is structurally unique to PI3Ka, and it does not bond to the other isoforms. CNX-1351 potently inhibits PI3Ka enzyme activity and inhibits P-Akt^{Ser473} in SKOV-3 cells (EC_{50} ~100 nM). PI3Ka activity continued to be inhibited after compound removal, confirming the mechanism of action. CNX-1351 demonstrated *in vivo* inhibition of PI3Ka and tumor growth inhibition in an SKOV-3 xenograft model. A biotinylated covalent probe molecule, which bonds specifically to PI3Ka, was used to verify and quantitate target occupancy, both *in vitro* and *ex vivo*.
Conclusions: CNX-1351 is a specific and potent irreversible inhibitor of PI3Ka that demonstrates prolonged duration of action, and activity *in vivo*. This approach should yield a first-in-class selective covalent PI3Ka inhibitor with numerous clinical advantages.

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PDL192, a humanized anti-Tweak receptor monoclonal antibody, mediates antitumor effects in primary human breast carcinoma xenografts

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Background: PDL192 is a humanized IgG1 monoclonal antibody that binds the human TWEAK receptor (TweakR). TweakR, a member of the TNFR (Tumor Necrosis Factor Receptor) superfamily, is overexpressed in several human cancers including breast cancer (BC). In BC, it may also play a role in the invasive and metastatic potential of the disease (Willis et al, Mol Cancer Res 2008). In TweakR-expressing cancer cell lines or mouse xenograft models, PDL192 has a potent antitumor effect (Culp et al, CCR 2010). All these data therefore suggest that anti-TweakR targeting could be a promising new therapeutic approach for human BC patients.
Material and Methods: TweakR expression was assessed by IHC (immunohistochemistry) on 3 Tissue-Micro-Array (TMA) banks of BC samples (basal-like, ERBB2, and luminal A/B), and on 25 primary human BC xenografts (HBC$\times$). The cut-off of positivity was defined as at least 25% cells with membraneous or cytoplasmic staining or by a combined score of percentage of positive staining cells $\times$ intensity $\geqslant$50. The *in vivo* antitumor effect of PDL192 was then assessed on 7 TweakR-positive models (10 mg/kg thrice a week for 3 weeks by intraperitoneal route).
Results: TMA analyses showed that TweakR was expressed in 16/37 basal like BC (43%), 23/37 ERBB2-positive BC (62%), and 38/71 luminal BC (54%). Evaluations of possible correlations between TweakR expression and the clinico-biological characteristics of the tumors, as well as the outcome of the patients, is currently ongoing. Moreover, 13/25 xenografts have been found to be TweakR-positive (52%). Seven human BC models have been treated with PDL192, with 3 models (43%) showing a tumor growth inhibition (TGI) greater than 50%. No correlation has been observed between TweakR expression and *in vivo* TGI.
Conclusions: TweakR is expressed in 77/145 human BC samples (53%). In *in vivo* experiments, PDL192 showed potent TGI in 3/7 models. All these data therefore support the use of anti-TWEAK receptor monoclonal antibodies in the treatment of TweakR-positive BC patients. Further therapeutic combinations should also be evaluated.

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Therapeutic targeting the loss of the Birt–Hogg–Dubé suppresser gene

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Background: Birt–Hogg–Dubé (BHD) disease, an autosomal dominant familial cancer, is associated with increased risk of kidney cancer. BHD syndrome is caused by loss-of-function mutations in the folliculin (FLCN) protein. In order to develop therapeutic approaches for renal cell carcinoma (RCC) in BHD syndrome, we adopted a strategy to identify tumour-selective growth inhibition in a RCC cell line with *FLCN* inactivation.
Material and Methods: The COMPARE algorithm was used to identify candidate anticancer drugs tested against the NCI-60 cell lines that demonstrated preferential toxicity to low *FLCN* expressing cell lines.
Results: Fifteen compounds were selected and detailed growth inhibition (SRB) assays were performed in paired BHD RCC cell lines (UOK257 derived from a patient with BHD). Selective sensitivity of FLCN-null over FLCN-wt UOK257 cells was observed in seven compounds. The most selective growth-inhibitory sensitivity was induced by mithramycin which demonstrated a ~10-fold difference in GI50 values between FLCN-null