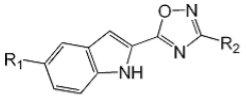
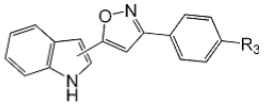
targets. Among the oxadiazole series, compound 21 showed the best activity. The pro-apoptotic activity testing of all novel compounds is in progress. Based of the docking studies and biological data, SAR analysis and structural modifications could result in better selective pro-apoptotic leads.

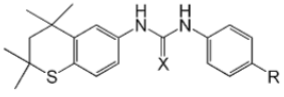


Indole-based 1,2,4-oxadiazoles

21: R_1= OCH_3, R_2= 4-$OCH_3C_6H_4$



Indole-based isoxazoles



Flex-Hets

X= O, S

R= NO_2, $COOCH_3$, SO_2NH_2

References

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Interaction of chlorambucil and intercalating aniline mustards with defined DNA sequences using MALDI and ESI mass spectrometry

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Chlorambucil, like all nitrogen mustards, is prone to hydrolysis and is inclined to alkylate proteins in addition to DNA. This results in low dose-potency in the clinical setting. An additional practical difficulty is that the crosslinking effectiveness of nitrogen mustards is limited, so that the majority of adducts are monofunctionally bound to DNA, which provides a platform for mutagenesis and the later induction of tumours in long-term cancer survivors. As with the minor groove-directed alkylating agents, one way to overcome these deficiencies, so as to augment the specificity for alkylating DNA *per se*, is to navigate aniline mustards to DNA by appending them to a reversible-binding carrier such as an intercalating agent. We have explored the use of matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF) and electrospray ionisation time-of-flight mass spectrometry (ESI-TOF) to study the DNA complexes of DNA-directed alkylating cytotoxins. We have investigated the binding of intercalator-directed acridine mustards, and chlorambucil to the 2 dodecanucleotides CGCGAATTCGCG (A2T2) and ATATGGCCATAT (G2C2). Alkylation of purines at the N3 and N7 positions quaternises the base, imparting a positive charge and weakening the glycosidic bond to hydrolysis. As a result, apurinic sites are generated which lead to phosphate hydrolysis and breakage of the DNA backbone at the alkylated base. For the intercalating acridine mustards binding to A2T2 and G2C2, we find that they alkylate purines surrounding their intercalation sites with enhanced potency compared to chlorambucil, but, unlike chlorambucil, they are unable to form crosslinks. Directing the alkylating group to DNA with an intercalating moiety enhances the reactivity of the alkylating agent by some 100-fold. Both chlorambucil and the acridine mustards alkylate the same adenines and guanines on A2T2 and G2C2, but, whereas chlorambucil forms a variety of inter-strand and intra-strand crosslinks involving both adenine-guanine and guanine-guanine linkages, the bifunctional intercalating mustard failed to form crosslinks of any variety.

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Ex-vivo plasma protein binding and in vitro evaluation of AP5346 (ProLindac TM; PL), a novel polymer-bound platinum: Evidence showing that >72 h DACH-platinum (Pt) release may play a major role in cytotoxicity

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Background: PL displays activity in a wide variety of solid tumors in preclinical models and clinical trials. PL is designed to selectively release DACH Pt into the acidic tumor environment. PL has a cytotoxic profile similar to that of oxaliplatin in our human cancer cell line panel. We investigated PL binding to plasma proteins and the kinetics of Pt release.
Materials and Methods: Plasma protein binding and Pt release were evaluated ex-vivo in plasma at 300 and 30 μg/mL (concentrations representing the therapeutic range, Cmax and Cmin) at 37ºC with adjusted pH (7.35–7.4) using Ultra-4 filters (Amicon) with 50 and 3 kDa cut-off. Reversibility of binding was investigated by protein precipitation with acetonitrile. Pt levels were measured by atomic absorption. Antiproliferative effects were evaluated in HT29 and HCT116 human cancer cell lines by MTT assay after 1−72 h of exposure.
Results: Both PL and oxaliplatin bind plasma proteins. PL induces non-covalent protein binding: addition of acetonitrile caused dissociation of all weakly bound ligands. PL binding to proteins was sustained for up to >144 h (6 days). In these experiments, PL protein binding was about 94% immediately after PL addition. Unbound Pt was 2.96% (6.3 μg/mL) and 5.73% (1.7 μg/mL) for Cmax and Cmin, respectively. Interestingly, Pt release from plasma-protein bound PL-polymer increased progressively over time reaching a steady-state at >72−96 h. This slow Pt release was consistent with exposure cytotoxicity kinetics. In vitro, PL also displayed time-dependent cytotoxicity in HT29 and HCT116 colon cancer cells, PL exposure >72 h showing higher antiproliferative effects than shorter exposures (<24 h). At equimolar concentrations, oxaliplatin was slightly more active than PL for short exposure durations (<48 h). Conversely, for duration of exposure >72 h, PL displayed IC50 ranging from 0.3–0.5 μM in colon cancer cells while oxaliplatin IC50 ranged from 0.5–0.9 μM. Similarly, PL-induced Pt DNA incorporation was time-dependent, with a higher level of Pt bound to DNA observed for exposure >72 h in human cancer cells.
Conclusions: Together, our data strongly suggest that protein-bound PL polymers progressively release free-Pt in plasma, reaching a sustained steady state after >72 h, resulting in sustained exposure to Pt. Considering that extended duration of exposure is essential for PL cytotoxicity, our data may help optimize dosing schedules in the design of future combination clinical trials.

Heat shock proteins

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XL888, a novel, synthetic, orally bioavailable inhibitor of Hsp90

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Background: Hsp90 is a component of a molecular chaperone complex that promotes the conformational maturation and stabilization of many tumor-promoting oncoproteins. A hallmark of Hsp90 inhibition is the corresponding degradation of client proteins and loss of tumor cell growth and survival. XL888 is a novel, synthetic small molecule inhibitor of Hsp90 identified from a biochemical library screen coupled with extensive medicinal chemistry to optimize its drug like properties. We describe key aspects of its preclinical activity profile here.
Materials and Methods: Biochemical and x-ray crystallographic methods were used to determine the binding characteristics of XL888 to Hsp90. Proliferation IC50s were performed using a BrdU-incorporation ELISA. Client protein degradation, pathway inhibition, and heat shock induction responses in tumor cell lines and xenograft tumors were determined by Western blot. Human tumor xenografts were grown in nude mice for PD and efficacy studies.
Results: XL888 is a potent and selective ATP-competitive inhibitor of Hsp90. It binds in a manner that is structurally distinct from 17-AAG and other small molecule Hsp90 inhibitors. XL888 treatment inhibited the proliferation of a broad panel of human tumor cell lines with IC50 values ranging from 0.1 to 45 nM, and resulted in marked degradation of client proteins including HER2, MET, mutant BRAF and mutant EGFR. Client protein degradation correlated with attenuation of receptor signaling, with significant loss of phospho-receptor, phospho-S6 and phospho-ERK