GSK1120212 is a result of the potent and selective inhibition of MEK1/2, and support its advancement for the treatment of cancer in humans.

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Design, synthesis, biochemical and biological evaluations of novel and potent small-molecule inhibitors of STAT3

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Constitutive activation of the Signal Transducers and Activators of Transcription 3 (STAT3) is frequently detected in human cancer specimens from patients with advanced diseases and cancer cell lines, but not in normal epithelial cells. Persistent activation of STAT3 signaling has been demonstrated to directly contribute to oncogenesis by stimulating cell proliferation and preventing apoptosis in human cancer cells. STAT3 activation may not only provide a growth advantage, allowing accumulation of tumor cells, but also confer resistance to conventional therapies that rely on apoptotic machinery to eliminate tumor cells. STAT3 represents an important and specific molecular target for designing an entirely new molecularly targeted therapy for human cancer with constitutively active STAT3 with potentially low toxicity to the normal cells without constitutive STAT3 signaling.
STAT3 is recruited from cytosol and makes specific interactions through its SH2 domain with different cytokine receptor with phosphotyrosine docking sites on the receptors. STAT3 then becomes phosphorylated on a carbonyl terminal tyrosine (Tyr705). Tyrosine physphorylation of STAT3 causes it to dimerize and translocate to the nucleus and bind to specific promoter sequences on its target genes. Dimerization of STAT3 is a decisive event for its activation. Thereby, blocking the dimerization of STAT3 using a small molecule antagonist is a very attractive therapeutic approach for developing a molecularly targeted therapy for the treatment of human cancer in which STAT3 is constitutively activated. Herein, we wish to report the design, synthesis, biochemical and biological evaluations of novel and potent small-molecule inhibitors of STAT3. Our most potent inhibitors bind to Stat3 with low nanomolar affinities and display excellent selectivity over Stat1 and Stat5. These compounds are excellent biochemical and pharmacological tools to further elucidate the role of Stat3 in cancer and promising lead compounds for the development of potent and specific Stat3 inhibitors for the treatment of human cancer.

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Enhanced drug delivery to brain tumors with a new paclitaxel-peptide conjugate

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Background: The main limiting factor in the treatment of brain tumors or metastasis is the low accessibility of the central nervous system (CNS) to drugs due to the blood–brain barrier. In the present study, the utilization of a new strategy based on a peptidic vector (Angiopep) capable of delivering drugs into the CNS in non-invasive manner was evaluated. Paclitaxel which accumulation into the CNS is hindered due to the P-glycoprotein efflux pump, was conjugated to the peptidic vector. The in-vitro and in-vivo properties of this conjugate (ANG1005) were characterized using different approaches.
Material and Methods: The sensitivity of a panel of cancer cell lines to ANG1005 was evaluated in vitro. The pharmacokinetic behavior of ANG1005 in plasma after IP, IV injection or IV infusion and its toxicity were determined in vivo on healthy Nude rats. The antitumor activity of ANG1005 was evaluated by MRI in a model of Nude rats bearing NCI H460 lung tumor implanted in the brain.
Results: Among all tumor cell lines tested in vitro, ANG1005 displayed an IC50 (concentration inducing a 50% cell death) in the nanomolar range for the NCI H460 and U 87 MG cell lines. These IC50 were of the same order of magnitude than for paclitaxel. Toxicity experiments showed that the maximal total treatment dose (MTTD) using a Q3Dx5 schedule was 6 mg/kg/inj when ANG1005 was injected IV. With the same schedule, IV infusion enabled to increase treatment doses as the MTTD reached 15 mg/kg. Pharmacokinetic studies indicated that maximal ANG1005 plasma concentrations were similar after a single IV injection at 11.25 mg/kg or an IV infusion at 15 mg/kg. However, the area under the time-concentration curve (AUC) was slightly higher for rats receiving ANG1005 via IV infusion as compared to rats dosed via IV injection. After a single IP injection at 75 mg/kg, ANG1005 plasma concentrations and AUC remained lower. A preliminary in vivo experiment was performed in a model of Nude rats bearing NCI H460 tumors. Magnetic resonance imaging revealed a reduction of tumor growth early after the start of treatments for rats treated IP with ANG1005 at 75 mg/kg as compared to rats receiving the vehicle or paclitaxel.
Conclusions: These results demonstrate that ANG1005 delivers paclitaxel into the CNS and enhances its activity in an aggressive model. ANG1005 is currently under evaluation in phase I clinical trials for the treatment of glioma and brain metastases in human.

140 POSTER

Macrolactone based inhibitors of Heat Shock Protein 90

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Heat shock protein 90 (Hsp90) is an ATP-dependent molecular chaperone for many overexpresed or mutant oncogenic proteins. This enzyme has become an attractive target for chemotherapeutic agents, since its inhibition will disrupt multiple cancer causing pathways simultaneously and hence may address the six hallmarks of cancer. Radicicol (**1**) is a potent natural product inhibitor of Hsp90 in vitro, but does not have any substantial in vivo activity. It has been suggested that this is due to the reactivity and metabolic lability of both the enone and epoxide functionalities. To address this problem, a series of macrocyclic lactones based on radicicol **1** was made in our laboratory. This led to the discovery of compound NP-261 (**2**) which lacks the unwanted functionality present in radicicol **1** but retains nanomolar biochemical activity.
In order to further improve the cellular activity of NP261 **2**, a second generation of analogues was designed **3**–**4**. It was reasoned that cellular activity might be enhanced by increasing the molecular rigidity of the macrocyclic ring. Our strategy was to investigate a number of key analogues which set to achieve this goal. This included a series of triazoles with varying ring sizes **3**, macrolactams and altering the substituents on the macrocyclic ring **4**. We report the synthetic challenges and biological evaluation of these new analogues.

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Design and synthesis of novel indole derivatives as selective apoptosis-inducers

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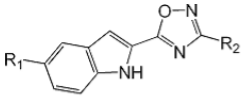
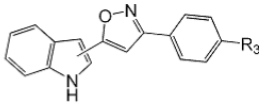
Evasion of apoptosis is one of the hallmarks of cancer [1]. Although the apoptotic pathway contributes to the cytotoxic effect of most cancer chemotherapeutics, selective induction of apoptosis in cancer cells would confer advantages over conventional therapy in terms of efficacy, toxicity and drug resistance.
Different small libraries of novel indole-based heterocyclic systems were designed to act as selective pro-apoptotic agents in cancer cells. Twenty-two compounds of the 5-(2-indolyl)-3-substituted-1,2,4-oxadiazole class were designed, based on a previously reported series of selective proapoptotic 3,5-diaryl-1,2,4-oxadiazoles [2]. The new compounds were prepared from the corresponding indole-2-carboxylate ester and different amidoximes in moderate yields with simple reaction workup. Another library of ten compounds of indole-based 3,5-disubstituted isoxazoles with different indole orientations was prepared by dipolar cycloaddition between terminal alkynes and aldoximes in excellent yields. A third series was designed to act as Bcl-2 inhibitors, based on a series flexible heteroarotenoids [3] with urea or thiourea linkers reported to affect the level of antiapoptotic Bcl-2 proteins in cancer cell lines and have selective apoptosis-inducing activity. Docking of these Flex-Hets at the Bcl-2 surface pocket showed good interaction suggesting the possibility of acting as Bcl-2 inhibitors, but the presence of a deep hydrophobic groove that is not utilized by these Flex-Hets suggested that incorporation of a larger side chain could result in better inhibitors. Further docking studies have revealed possibilities for extension of the Flex-Hets structure to probe further binding interactions with the Bcl-2 domain potentially leading to more potent pro-apoptotic molecules.
Examination of the in vitro cytotoxic effect of the newly prepared compounds of the 1,2,4-oxadiazole series on a panel of human cancer cell lines showed that the COLO 320 (colon) and MIA PaCa-2 (pancreas) were the most chemosensitive cell lines with IC50 mean values in the micromolar range. Moreover, potency and efficacy of compound 21 (5.7 μM and 75.9%, respectively) on the poorly differentiated pancreatic cancer cell line MIA PaCa-2 were almost superimposable to those observed for 5-fluorouracil.
Different novel series of indole-based compounds were designed and synthesized to act as selective pro-apoptotic agents with different molecular

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.

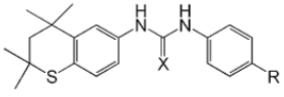


Indole-based 1,2,4-oxadiazoles

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

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142 POSTER

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

144 POSTER

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