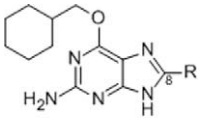
quantum mechanical energy calculations to identify suitable C-8 groups. Addition of a 2-methylphenyl or 2-chlorophenyl group at C-8 restored the potency of this series of 'reverse' binding mode compounds to that of NU2058, providing a novel starting point for inhibitor design. The synthesis, biological evaluation and structural biology of these CDK2 inhibitors will be discussed.



1; R = H
2; R = $CH(Me)_2$

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446 POSTER

Optimisation of tetrahydroisoquinoline based microtubule disruptors as anti-cancer agents

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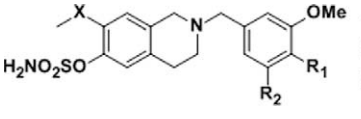
We outline the discovery and optimisation of new microtubule disruptors with *in vivo* anti-tumor activity. Translation of the SAR from a steroidal series of microtubule disruptors led us to identify a series of tetrahydroisoquinoline (THIQ) based systems which exhibit a similar activity profile. Of this new series, 2-(3′,4′,5′-Trimethoxybenzyl)-7-methoxy-6-*O*-sulfamoyl THIQ 1 proved especially potent in both *in vitro* (GI_{50} [DU-145] 297 nM) and *in vivo* experiments. Herein, we describe the results of optimisation at C-6 and C-7 of the THIQ core and assessment of other polymethoxylated *N*-benzyl systems.
Variations at the N-2 and C-6 positions were achieved by alkylation, esterification and etherification. Friedel Crafts acylation of C-7 and functional group interconversion allowed access to various C-7 alkyl and alkoxy derivatives. The various dimethoxybenzyl compounds proved similar in activity to the lead compound 2 (GI_{50} 2.1 mM) in the *N*-mono-methoxybenzyl series while, apart from the 3′,4′,5′-trimethoxybenzyl compound 1, only 2′,4′,5′-trimethoxybenzyl substitution delivered sub-micromolar activity. Investigations of the effect of C-6 substitution proved more fruitful. In contrast to the SAR observed for 2 where the sulfamate group is essential for activity, the 6-OH, with the 6-*O*-acyl and 6-*O*-mesyl derivatives of 1 displayed similar or improved activity to the parent compound (GI_{50}s range from 650 to 220 nM). The 6-*O*-methyl derivative, in contrast, proved completely inactive, highlighting the importance of a H-bond donor directly attached to C-6 or a H-bond acceptor projecting further out from this position. The most pronounced improvement in activity was obtained from exploration of C-7 substitution. In the 3′,4′,5′-trimethoxybenzyl series isosteric replacement of methoxy with ethyl delivered a 7-fold improvement in activity (3 GI_{50} 41 nM). Intriguingly, the corresponding phenol proved significantly active suggesting different binding modes operate for the phenol and sulfamate derivatives since the H-bond acceptor properties of the C-7 substituent of the former are clearly important. Incorporation of a C-7 ethoxy group meanwhile proved detrimental for both sulfamate and phenol derivatives. The same transformations were made to 2, though no improvement in activity was obtained.
In order to establish the potential of these compounds as anti-tumor agents their activity in the RPMI-8226 multiple myeloma xenograft model was assessed. The >75% inhibition of tumor growth observed (3 p.o. 40 mg/kg, 28 d) in this preliminary study augers well for the development of this class of anti-cancer agents.



1 X = O, R_1 = R_2 =OMe
2 X = O, R_1 = R_2 = H
3 X = CH_2, R_1 = R_2 =OMe

447 POSTER

Stereoisomerism significantly impacts on the anticancer activity of novel oxaliplatin analogues in vitro and in vivo

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Background: Aim of this study was to compare the anticancer properties of two oxaliplatin derivatives (KP1537, KP1691) in vitro and in vivo which differ only in the orientation of the methyl substituent at the cyclohexane ring (equatorial and axial, respectively).
Methods: Cytotoxic/antiproliferative effects were tested against selected human cancer cell lines by MTT analysis. Platinum accumulation was determined by ICP-MS. For in vivo experiments, cells were transplanted i.p. (leukemic) into immuno-competent or immuno-deficient mice.
Results: In vitro both oxaliplatin analogues exerted cytotoxic/cytostatic activity with IC_{50} values in the low μM range, with those for KP1691 beeing significantly lower in comparison to oxalipaltin. In contrast, KP1537 was moderately less active. KP1691 accumulated in tumour cells to the same extend as oxaliplatin. Surprisingly, KP1537 was taken up more rapidly and accumulated over time to 3-fold higher intracellular concentrations. However, the distribution between nuclear and cytosolic compartments was similar between all three platinum drugs. Remarkably, first in vivo experiments demonstrated that both novel substances were less toxic than oxaliplatin resulting in an altered therapeutic window. Generally, all compounds tested prolonged the survival of leukemia-bearing mice, but to different extents. KP1691 was least active, whereas oxaliplatin treatment resulted in an increase in life-span (ILS) by about 100% and 1/5 long-term survivor (LTS). Unexpectedly, the in vitro less active compound KP1537 induced a stronger ILS (>300%) and 3/5 LTS. Furthermore, the impact of the immune system was tested. As known for oxaliplatin, the novel compounds were more active in an immuno-competent background.
Conclusion: Taken together, these findings demonstrate that small sterical changes can have major impacts on the activity of anticancer metal complexes. Thus, the axial methylated KP1691 is more active in vitro but obviously does not efficiently reach its molecular target in vivo. In contrast, the equatorial methylated KP1537, which is less active in vitro, exerts very promising anticancer properties in vivo. Several aspects, including the higher accumulation rates, less adverse effects and the higher in vivo anticancer activity of KP1537 as compared to oxaliplatin, suggests further (pre)clinical development of this novel oxaliplatin analogue.
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448 POSTER

Regulation of uridine phosphorylase-2 redox-control mechanism to improve capecitabine selectivity

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The key for a successful chemotherapy agent is the selectivity and specificity for tumor tissue sparing the surrounding normal tissues from toxic anti-proliferative effects. Few agents have been designed to be selectively activated in tumor tissue, one of them is the 5-fluorouracil (5-FU) pro-drug Capecitabine. Capecitabine undergoes a 3-step activation process, initially hydrolysis of the carbamate side-chain by the hepatic carboxylesterases and eventually metabolized from 5′-deoxy-fluorouridine to 5-FU mainly in the tumor tissue that presents an increased phosphorolytic activity due to an elevated presence of the two phosphorolytic enzymes: uridine and thymidine phosphorylases. Several organs and tissues, including liver, express the same two phosphorolytic enzymes resulting in the activation to 5-FU with consequent toxic effects.
In mammalians Uridine Phosphorylase (UPP) is present in two isoforms: UPP1 and the more recently characterized UPP2. Human UPP2, a 317 aa. protein of 35 kD molecular mass, is 60% identical to human UPP1, while murine UPP2 is 85% identical to human UPP2. UPP-2 has broader substrate specificity than UPP1. In addition to uridine and deoxyuridine, UPP-2 utilizes thymidine as substrate. However, no phosphorolytic activity was detected when the enzyme was incubated with adenosine, cytidine, guanosine, deoxyadenosine, deoxycytidine or guanosine. In humans the protein is expressed in kidney, liver and spleen while in mouse UPP2 is present in liver and in much less amount in kidney and brain.
We have completed the crystallographic structure determination of hUPP2, having collected a 1.5Å dataset at SSRL and phased the data using Molecular Replacement, searching with a homology model of hUPP2 constructed

from the known hUPP1 structure. This high resolution structure revealed unequivocally the presence of an intramolecular disulfide bridge that repositions a critical, active-site, phosphate-coordinating arginine residue (Arg100) to a location that does not support catalysis of the enzyme's phosphorolytic activity. Consistent with this structural finding, in vitro comparison of mammalian UPP1 and UPP2 activity reveals a substantial sensitivity to oxidative inactivation in the latter isoform. Together these results demonstrate that UPP2 is intracellularly controlled by a redox mechanism that could be exploited to inactivate the enzyme and therefore limit the activation of Capecitabine in the liver and other organs expressing this UPP isoform.

Toxicology-side effects

449 POSTER

Increased levels of serum creatine kinase caused by skin toxicity of molecularly targeted anticancer agents in phase 1 clinical trials

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Background: New anticancer agents are often associated with skin toxicity, especially those molecularly targeted agents (MTA) targeting EGFR-RAS-RAF-MEK pathway. Creatine kinase (CK) is an enzyme that catalyses the generation of phosphocreatine from ATP and creatine, and is located in tissues such as brain and muscle, human epidermis and hair follicles. We investigated whether rash caused by MTA could increase serum CK levels.
Material and Methods: Retrospectively reviewed 25 Phase 1 Clinical Trials that included CK measurement in the protocol, conducted in the Drug Development Unit (Royal Marsden Hospital) from June 2002 to May 2010. Trials included MTA directed against EGFR/HER2 (41 pt), mTOR (48 pt), VEGFR (31 pt), Src/Abl (26 pt), Aurora kinases (19 pt), vascular disrupting agents (16 pt), BRAF/MEK (12 pt), PARP (6 pt), CDK (6 pt), A5B1 integrin (3 pt) and other targets (34) or viruses (53 pt). Rash was considered as maculo/papular or papulo/pustular drug induced skin toxicity. Fishers exact test was used to calculate differences in incidence or raised CK between groups who developed rash and those who did not. A Kruskal-Wallis one way analysis of variance test was used to determine differences between CK levels and the grade of rash.
Results: 295 patients were included for analysis. Male/female ratio was 55/45 and median age was 59 years. In 49 pt (17%) an elevated serum CK was found after starting treatment. Overall 20% (58/295) patients developed rash. Patients who developed a rash had a higher incidence of raised CK than those who did not 24/58(41%) Vs 25/237 (10%) ($p < 0.001$). There was an association between the grade of rash and CK levels. The rash was grouped into three cohorts; Group A (No Rash: mean CK = 90 IU/L), Group B (G1 rash: Mean CK IU/ = 138), Group C (G2/3 rash: mean CK = 406 IU/L)($p < 0.001$). When the analysis was limited to patients treated with inhibitors of the EGFR/BRAF or MEK ($n = 53$), the incidence of elevated CK was significantly higher in those who had a rash 16/44 than those who did not 0/9, $p = 0.02$. Rash appeared a median of 11 days before the first increased level of CK (range −18–165). No electrolyte disorder or acute renal failure was associated with the increase of CK.
Conclusions: For the first time we have shown that elevated CK is associated with skin rash caused by MTA. It should be studied further as a surrogate for skin toxicity.

450 POSTER

Glucocorticoids frequently induce survival and growth in tumor cells by activation of classical survival and proliferation pathways which should be avoided during anti-cancer therapy

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Background: Glucocorticoids (GCs) such as Dexamethasone (Dex) are widely used in cancer patients. In the treatment of hematopoietic tumors, GCs are given as cytotoxic drugs to treat the tumor. In solid tumors, GCs are given as adjuvants to alleviate adverse effects such as nausea and headache. Nevertheless, in vitro data, animal trials and clinical showed that the use of GCs was accompanied by inverse prognosis, e.g., in patients with lung cancer. This study aimed to systematically characterize non-apoptotic effects of GCs on tumor cell lines and primary tumor cells together with underlying signaling mechanisms.
Materials and Methods: 16 tumor cell lines from different origins were studied together with 139 primary, patient-derived tumor cells obtained from children with acute leukemia before onset of treatment; one cell line was tested in a preclinical subcutaneous nude-mouse model. To study intracellular signaling mechanisms, cells were transfected using siRNA and subjected to functional assays and Western Blots, including phospho-specific antibodies.
Results: GCs enhanced cell growth in 9 out of 16 solid tumor cell lines in vitro. In one cell line, GCs doubled the growth rate of the tumor cells. When cytotoxic drugs were added, only those drugs inducing significant apoptosis were able to inhibit GC-induced tumor cells growth. In contrast, GCs induced significant proliferation even in the presence of cytotoxic drugs with low or absent potential for induction of apoptosis.
When the lung cancer cell line CALU-6 was transplanted subcutaneously into nude mice, Dexamethasone significantly induced the growth rate leading to increased tumor burden.
On 139 fresh primary, patient-derived tumor cells GCs increased survival in 15% of these cells. 20 samples were tested for GC-induced proliferation. In 1/20 samples, both GCs induced cell growth and the formation of new tumor cells as shown by BrdU incorporation. Thus, GCs induced survival and growth in both tumor cell lines and primary tumor cells freshly from patients.
To characterize signalling mechanisms, we found early and sustained phosphorylation of the glucocorticoid receptor followed by its degradation. Transfection of tumor cells with siRNA directed against the glucocorticoid receptor completely inhibited proliferation by Dexamethasone. Dexamethasone activated the pro-survival and proliferation signaling pathways of protein kinase B/Akt and p38 mitogen-activated protein kinase; inhibitors of these pathways abrogated Dex-induced tumor cell growth.
Conclusion: Translated into clinical praxis, our data argue towards a restricted use of GCs during anti-cancer therapy. Whenever possible, GCs might be replaced by other adjuvant drugs. More effort is required to define biomarkers and/or clinical criteria, how GCs can be used safely in cancer patients. Clinical studies are needed to evaluate, whether inhibitors of Akt and/or p38 MAPK can be used to inhibit GC-induced proliferation in cancer patients.

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Cumulative drug toxicity experience of ARQ 197, a selective c-Met inhibitor, and its correlation with pharmacokinetic (PK) and pharmacogenomic (PG) parameters

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Background: ARQ 197 is an oral, selective, non-ATP competitive inhibitor of c-Met, a receptor tyrosine kinase implicated in cancer cell migration, invasion, and proliferation. Since its first clinical trial in 2006, ARQ 197 has been administered to more than 400 cancer patients (pts) either as monotherapy or in combination. A maximum tolerated dose (MTD)/recommended Phase 2 dose (RP2D) of 360 mg administered orally twice daily (BID) as monotherapy was established previously. Here we summarize cumulative safety data from single-agent clinical trials of ARQ 197 and the correlation among toxicity, PK, and PG parameters.
Methods: Data from four single-agent trials were assessed. Adverse events (AEs) were graded using NCI CTCAE v. 3.0 guidelines and coded using MedDRA terminology. Causalities were assessed by study investigators. For PK analyses, plasma concentrations were determined using LC/MS/MS methodology, and PK parameter estimation was performed using noncompartmental analyses. CYP450 2C19 (2C19) genotyping was tested using a FDA-approved Amplichip CYP450 reagent kit manufactured by Roche Diagnostics.
Results: The most common drug-related AEs (≥5%) in the first 175 pts treated with ARQ 197 monotherapy were fatigue (20.0%), nausea (18.3%), vomiting (9.1%), and diarrhoea (6.3%). Dose-limiting toxicities (DLTs) observed in two Phase 1 monotherapy studies ($n = 120$) mainly consisted of myelosuppression events including 1 Grade (G) 4 and 2 G3 febrile neutropenia, 1 G4 neutropenia, 1 G4 leukopenia, and 1 G4 thrombocytopenia. The remaining DLTs included 1 G3 fatigue, 1 G3 palmar-plantar erythrodysaesthesia (hand-foot) syndrome, 2 G3 stomatitis/oral mucosal inflammation, 1 G3 hypokalaemia, 1 G3 vomiting and 1 G3 dehydration. Preliminary PK data suggested a dose-related increase in exposure, although this increase appeared to be less than dose proportional. PK data in pts with DLTs suggested that in general DLTs were associated with drug accumulation after repeated dosing, a phenomenon likely related to reduced clearance. A high degree of inter-pt variability in C_{max} and $AUC_{(0-12)}$ was observed with coefficients of variation of 75% and 90% respectively. Inter-pt variability appeared to be due in part to 2C19 polymorphism status. One pt was identified as a 2C19 poor metabolizer (PM). Drug exposure in the PM was high in comparison to extensive metabolizers, and this pt experienced G4 febrile neutropenia, G3 stomatitis and other G1/2 drug-related AEs.
Conclusions: ARQ 197 demonstrated a manageable safety profile at treatment doses up to and including the MTD/RP2D and were well tolerated