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Structure–activity relationships

126 POSTER

Development of a new series of tricyclic pyrimido-indole inhibitors targeting Aurora kinases

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The Aurora kinase family of proteins are serine/threonine kinases that regulate the processes of centrosome separation and duplication in preparation for mitotic spindle formation and chromosome separation. Aurora-A is overexpressed in several solid tumor types, including breast, ovary, prostate, pancreas and colorectal cancers and its overexpression is thought to contribute to tumor progression by increasing genomic instability and altering cell cycle checkpoints. Because of its role in the process of tumorigenesis, Aurora-A has been reported to be an attractive target for anti-cancer drug development. We have initiated a drug development program to identify specific inhibitors of Aurora kinase activity. This program is based on a combination of rational design, synthesis and screening. We have developed a novel series of potent and selective ATP-competitive Aurora kinase inhibitors utilizing tricyclic pyrimido-indole core, which is structurally distinct from other reported kinase inhibitors. Such tricyclic compounds modeled into the ATP-binding site of Aurora kinase in such a way that the tricyclic pyrimidine ring orients into the hydrophobic adenine-binding site to form backbone H-bonds with the E211, Y212 and A213 residues of the hinge region. Several leads from this series have emerged from SAR studies around 4, 6 and 7th position of pyrimido-indole moiety. A lead compound from this series, MP-235, has been shown to inhibit the Aurora kinases at nanomolar concentrations (IC50 = 90nM). This lead has been further modified to identify analogues with more potent activity and greater selectivity towards the Aurora kinases. Cell growth studies in the human pancreatic cell lines MiaPaCa-2 and Panc-1 as well as other cancer cell lines show that these novel Aurora kinase inhibitors can result in antiproliferative effects in tumor cells. (Supported by NIH Grant CA 95031-01)

127 POSTER

Integrin receptor binding and cytotoxicity of cyclopeptides and their Chlorambucil conjugates containing RGD or NGR sequence

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Introduction: The RGD peptide sequence found in most ECM component is the general recognition site for the integrin receptor family like vitronectin ($\alpha_v \beta_3$) and fibronectin ($\alpha_5 \beta_1$) receptor, however other adhesion sequences, like the NGR came into focus as well. Selective $\alpha_v \beta_3$ ligands are suitable for vasculature targeted cancer therapy and also serve as tools for targeted drug delivery into the tumor vasculature. For this purpose we have synthesized and investigated peptide derivatives (single capital letters for *L*-amino, small letters for *D*-amino acids; pF-F for *p*-fluoro-phenylalanine; pNH_2-F for *p*-amino-phenylalanine) and their Chlorambucil (Clb) conjugates.
Methods: Linear peptides were prepared by solid phase method, cyclisations were performed in solution. For fluorescent labeling 5(6)-carboxyfluorescein was used. Receptor recognizing ability of the peptide derivatives was checked in a competitive displacement assay using a ^{125}I-radiolabeled multivalent ligand for $\alpha_v \alpha_3$ integrin (RGD-protein conjugate). For *i n vitro* cytotoxicity assay HUVEC, human HBL and LND1 melanoma cells and fibroblasts were used.
Results: c(VRGDf) **1**, c(VRGDpFf) **2**, c(DapRGDf) **3**, c[Dap(Clb)RGDf] **4**, c[K(Clb)RGDf] **5**, c(VRGDpNH $_2$f) **6** show equally high affinity for $\alpha_v \beta_3$ receptor, while H-CNGRCV-NH_2 **7**, c(LNGRV) and c(LNGRv) do not bind to it, according to the radioactive displacement assay.
All cell types used in the cytotoxicity assay show different fibronectin and vitronectin receptor expression. Except for HUVEC, chlorambucil-coupled peptides show significantly less toxicity than Chlorambucil alone in all cell types tested, suggesting a compromised ability to cross the cell membrane. In addition, free peptides show by themselves some cytotoxicity to most cell types used, compound **3** being by far the most toxic to HUVEC.
Conclusions: All the cyclopeptide derivatives and their alkylating conjugates containing the RGD sequence preserve the selective $\alpha_v \beta_3$ integrin receptor binding affinity of the reference peptide **1**, while cyclopeptides with the NGR motif do not bind to this receptor. Cell adhesion kinetics on fibronectin matrix appears to be correlated with the level of expression of the corresponding receptors. The higher toxicity observed on HUVEC can be explained by a possible initiation of the apoptotic pathway.

128 POSTER

Sapphyrins: structure-activity relationships in a novel series of potential anti-cancer agents

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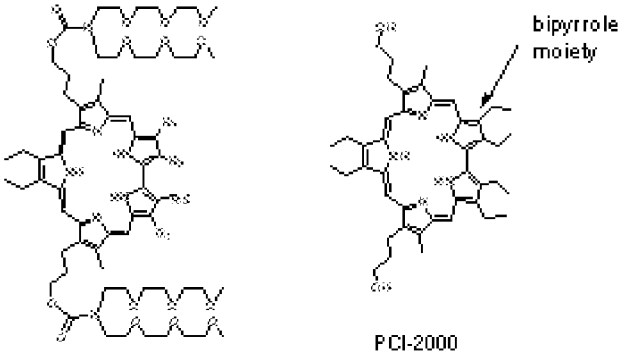
Sapphyrins are pentapyrrolic metal-free expanded porphyrins. We have previously shown that the first generation sapphyrin compound, PCI-2000, induces apoptosis in a variety of hematologic tumor cell lines. PCI-2000 triggered an apoptotic pathway as demonstrated by apoptotic morphology, annexin V binding, release of cytochrome C from mitochondria, activation of caspases 9, 8, and 3 and cleavage of the caspase 3 substrate PARP. To investigate structure activity relationships among sapphyrin derivatives, we focused on four 2nd generation derivatives, PCI-2050, PCI-2051, PCI-2052 and PCI-2053 where polyethylene glycol groups were introduced to increase their water solubility. Structurally, these four compounds differ by their alkyl substituents on the bipyrrole moiety. Treatment of Ramos cells in tissue culture with these derivatives (1 uM for 16 hrs) showed the following activity profile as assessed by annexin V binding and caspase activity: PCI-2050> PCI-2051> PCI-2052> PCI-2053.Interestingly, treatment of Ramos cells with 0.5 uM of each sapphyrin for 48 hrs showed a slightly different activity profile: PCI-2050> PCI-2052> PCI-2051> PCI-2053. Drug uptake, measured as fluorescence emission >650 nm after excitation at 488 nm correlated with drug activity (except for PCI-2053, which is not fluorescent under the experimental conditions). To explore in vivo biological activity, we treated CD-1 nude mice bearing Ramos xenografts with each of the sapphyrins (10 umol/kg). Animals were sacrificed 48 hrs after treatment and analyzed for drug uptake in the tumor using flow cytometry. The relative order of uptake into tumor cells was PCI-2050> PCI-2052> PCI-2051. Tumor cells from sapphyrin-treated animals grew less well in culture and had more apoptotic cells than those derived from control animals in proportion to the drug uptake in tumor cells. Inhibition of sapphyrin treated tumor cell growth relative to control tumor cell growth was: 91% for PCI-2050, 79% for PCI-2052, 20% for PCI-2051 and 16% for PCI-2053. PCI-2050 showed anti-tumor activity in a Ramos xenograft model with minimal toxicity when given at 10 umol/kg × 2 days. Our work demonstrates that sapphyrins induce apoptosis both in tissue culture and



PCI-2000

PCI-2050, R_1 = Et, R_2 = Me
PCI-2051, R_1 = Et, R_2 = Et
PCI-2052, R_1 = Me, R_2 = Me
PCI-2053, R_1 = H, R_2 = H

in an animal tumor model. Subtle changes of the alkyl substituents on the bipyrrole moiety result in significant changes in activity. PCI-2050 and other derivatives that show in vivo efficacy will be further evaluated as possible anti-cancer agents.

129 POSTER

Biarylalanine inhibitors of histone deacetylase enhance radiation sensitivity in cancer cells

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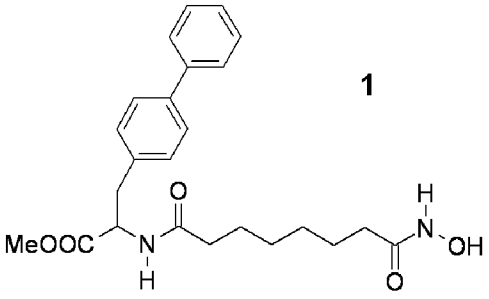
Background: We wanted to investigate the enhancement of radiation sensitivity in cancer cells by biarylalanine containing histone deacetylase inhibitors.
Material and Methods: The compounds are obtained from a suitably protected hydroxamic acid derivative of 4-bromophenylalanine by microwave assisted Suzuki coupling with arylboronic acids in reaction times of 5–10 minutes in good yields. Rat liver histone deacetylase and a fluorescent substrate are used for the determination of the IC_{50} values concerning in-vitro enzyme inhibition[1]. Human squamous carcinoma cells SQ-20B which had been shown previously to be intrinsically resistent to radiation were used for the investigation of the enhancement potential. Trichostatin A (TSA) and SAHA were used for comparison. IC_{50}-values for inhibition of proliferation were obtained and then cells were exposed to the compounds at their IC_{50}-value and graded doses of γ radiation according to standard protocols[2]. D_0-values as a measurement of the extent of enhancement were obtained for each compound.



Results: The parent biphenylalanine **1** which was reported previously as an HDAC inhibitor (IC_{50} = 290 nM) showed an IC_{50}-value in the SQ cells around 1 μM (TSA 200 nM, SAHA 3 μM). It proved to be an excellent enhancer of radiosensitivity with a D_0 of 1.45 at 1 μM. Control D_0 is 2.65, for TSA D_0 is 1.65 at 200 nM and for SAHA D_0 is 1.88 at 3 μM. We have synthesized several new substituted biphenylalanines as well as 4-heteroaryl phenylalanines. The most potent enzyme inhibitor so far is the 4-thienyl-phenylalanine analogue of **1** (IC_{50} = 190 nM, TSA: 10 nM, SAHA: 170 nM). The cellular investigation of the new analogues is currently under way.
Conclusion: Exchange of the anilide moiety of the histone deaceylase inhibitor SAHA that is currently undergoing clinical trials for the treatment of cancer by biarylalanines leads to compounds with similar enzyme inhibitory properties but an increased potency to enhance radiation sensitivity of cancer cells.

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130 POSTER

Structural features of texaphyrin metal complexes leading to altered metal cation response in cancer cells

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Motexafin gadolinium (MGd, Xcytrin®) selectively localizes in tumors and promotes stress by oxidizing intracellular reducing species. We recently showed by microarray analysis that treatment of A549 human lung carcinoma cells with MGd led to induction of metallothioneins (MT) and zinc transporter 1 (Hacia, Proc. AACR 43:3211, 2002). We have also reported that MGd at low concentrations modulates the cytotoxicity of the transition metal cations cadmium and zinc in cancer cells (Proc. AACR 45:1226, 2004). In the present study, we describe the effect of MGd and other metallotexaphyrins on the response of cancer cell lines to treatment with these ions. Human lymphoma (Ramos, DHL-4), lung carcinoma (A549), or prostate cancer cells (PC3) were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum. Zinc (0–100 μM) or cadmium (0–50 μM) and 0–25 μM MGd or texaphyrin congeners (**1-6**; M = Gd, Nd, Sm, Eu, Dy, Lu; R = OH; n = 2; **7-10**, M = Cd, Mn, Co, $FeO_{1/2}$; R = OH; n = 1) were added for 24 hr. Medium was exchanged and proliferation was assessed using a tetrazole (MTT) reduction assay at the end of 3 days. In other experiments, cells were treated with MGd and zinc or cadmium, and analyzed by flow cytometry using propidium iodide. RNA from treated cultures was harvested and metallothionein induction assessed by Northern blotting. Treatment with 6.25 μM or higher MGd raised the IC50 of cadmium, but lowered that of zinc, in all cell lines tested. Treatment with transition metal texaphyrins **7-10** at concentrations up to 25 μM did not alter the cytotoxic effect of zinc or cadmium, whereas early lanthanide series texaphyrin complexes **2-5** were as active as MGd. Late lanthanide series texaphyrin MLu, **6**, was inactive. This order of activity was found to correlate with MT induction. In order to evaluate whether the absence of activity of MLu was due to the lower solubility of this analogue, the more water-soluble diamine derivatives (**11-12**; M = Gd, Lu; R = NH_2; n = 2) were tested, and both found to be active. In summary, our findings suggest that texaphyrin lanthanide, but not transition metal complexes sensitize cancer cell lines to zinc and antagonize response to cadmium, provided these are sufficiently hydrophilic. These observations support the characterization of texaphyrins as a redox cycling agents that alter metal cation response by inducing the expression of metallothioneins and related genes.

Angiogenesis and metastasis inhibitors

131 POSTER

Pharmacodynamic analysis of apoptosis and anti-vascular activity in GIST patients treated with Imatinib

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Background: Most gastrointestinal stromal tumors (GISTs) contain activating mutations in the receptor tyrosine kinases c-Kit or platelet-derived growth factor-α (PDGFR-α) ε Imatinib mesylate (Gleevec) is a potent inhibitor of the c-Kit receptor tyrosine kinase. However, the mechanism(s) underlying its anti-tumor activity remains unknown. In an ongoing study we are investigating the mechanisms of early anti-tumor activity in GIST patients that achieve a response as measured by 18-FDG PET imaging. We hypothesize that imatinib's efficacy is due to both induction of GIST tumor cell apoptosis and anti-vascular activity via induction of tumor-associated endothelial cell apoptosis.
Material and Methods: We developed a clinical trial whereby patients with potentially resectable GIST were treated with imatinib (600 mg/day) for 3, 5, or 7 days before surgery. Perfusion CT and 18-FDG PET scans were performed before and after the initiation of imatinib therapy for 3, 5, or 7 days. All patients underwent pre-imatinib biopsy followed by surgical resection within 24 hours after completion of induction therapy. CT perfusion parameters acquired included blood flow (BF) and blood volume (BV). PET imaging was used to assess the standard uptake value (SUV) of FDG. Paired tumor biopsies and surgically resected tumors were examined using immunofluorescence coupled with laser laser scanning cytometry to quantify endothelial and tumor cell apoptosis, microvessel density (MVD), phosphorylated-c-Kit and phosphorylated-PDGR-α expression.
Results: Four out of five treated patients had a decrease in BF (avg. 40%, SD ±22.3, P=0.04) and BV (avg. 31%, SD ±22.8; P=0.05) in the solid portion of tumors corresponding to areas demonstrating a decrease in SUV (avg. 63%, SD ±19, P=0.05). One patient had little FDG uptake and displayed a 20% increase in BF/BV. The four responders to imatinib displayed a substantial decrease in phosphorylated-c-Kit expression in the tumor-associated endothelium (avg. 45%, SD ±12, P=0.07) and tumor cell compartment (avg. 52%, SD ±42%, P=0.13). These tumors displayed a 7-fold (P=0.08) and 2.8 fold (P=0.23) increase in endothelial and tumor cell death, respectively, and a 36% reduction in MVD. The tumor with the greatest reduction in BF (74%)/BV (61%) displayed the greatest increase in endothelial cell death (0.05% to 11%, $p<0.05$) after 3 days. The most significant reduction in MVD (78%, $P<0.05$) was observed in the tumor with the greatest reduction in FDG uptake (85%) after 7 days. Constitutive