References

[1] Johnstone R.W. (2002) Histone-deacetylase inhibitors: Novel Drugs for the Treatment of Cancer. Nature Reviews 1, 287–291.
[2] Marks P.A., Rifkind. R.A., Richon V.M, Breslow R., Miller T. and. Kelly W.K (2001) Histone Deacetylases and Cancer: Causes and Therapies. Nature Reviews 1, 194–202.
[3] Arts, J., de Schepper, S., Van Emelen, K. (2003) Histone deacetylase inhibitors: From chromatin remodeling to experimental cancer therapeutics. Current Medicinal Chemistry, 10(22), 2343–2350.
[4] Van Emelen et al. Discovery of a Novel Class of Aromatic Hydroxamic Acids as potent HDAC Inhibitors. AACR-NCI-EORTC International Conference on "Molecular Targets and Cancer Therapeutics", Boston, 2003, Abstract Nr C-39.

Structure–activity relationships

126 POSTER

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

S.L. Warner[1,2], H. Vankayalapati[3], S. Bashyam[1,2], C.L. Grand[3], H. Han[1], D.D. Von Hoff[1], L.H. Hurley[1,2], D.J. Bearss[3]. *[1]University of Arizona, Arizona Cancer Center, Tucson, USA; [2]University of Arizona, College of Pharmacy, Tucson, USA; [3]Montigen Pharmaceuticals, Inc., Salt Lake City, USA*

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

H. Süli-Vargha[1], N. Mihala[1], R. Morandini[2], R.J. Kok[3], G. Ghanem[2].
[1]Hungarian Academy of Sciences, Research Group of Peptide Chemistry, Budapest, Hungary; [2]Free University of Brussels, LOCE Institute J. Bordet, Brussels, Belgium; [3]Groningen University, Institute for Drug Exploration, Groningen, The Netherlands

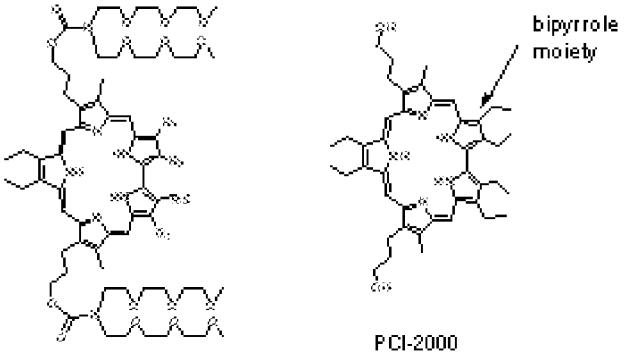
Introduction: The RGD peptide sequence found in most ECM component is the general recognition site for the integrin receptor family like vitronectin ($\alpha_v \beta_3$) and fibronectin ($\alpha_5 \beta_1$) receptor, however other adhesion sequences, like the NGR came into focus as well. Selective $\alpha_v \beta_3$ 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

L. Naumovski, M. Sirisawad, P. Lecane, Z. Wang, L. Fu, D. Magda, P. Thiemann, G. Boswell, J. Ramos, R. Miller. *Pharmacyclics, Inc., Sunnyvale, CA, USA*

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