401

A novel whole cell histone deactylase (HDAC) activity assay for the identification of small molecule HDAC inhibitors

S. Joel[1], C. Marson[2], C. Mikol[1], P. Savy[2], J. Perry[1], A. Rioja[2], B. Young[1].
[1]St Bartholomew's Hospital, Department of Medical Oncology, London, United Kingdom; [2]University College, Department of Chemistry, London, United Kingdom

Inhibition of HDACs may have therapeutic potential in patients with malignant disease. A rapid *in vitro* HDAC activity assay, based on the deacetylation of an omega-acetylated lysine (MAL), has been described (Hoffmann et al, Nuc Acids Res 1999; 27: 2057-8). We have further developed this assay for use in intact cells with a single time-point reaction. 1×10^6 CEM cells in 1 mL medium were exposed to inhibitors at 6 conc^n for 60 minutes, after which MAL (20 μg/mL) was added for a further 30 minutes, all at 37°C. Cells were then rapidly washed at 4degC, lysed by sonication, the reaction stopped with acetonitrile, and MAL and the deacetylated product ML determined in the supernatant by rapid HPLC analysis. For assays with partially purified rat liver HDAC, enzyme, substrate (5 μg/ml MAL) and inhibitor at 6 conc^n were incubated at 37degC for 60 minutes, after which the reaction was stopped and MAL and ML determined in the supernatant. These assays have been used to investigate the activity of known and novel HDAC inhibitors. The requirement for a hydroxamic acid for potent HDAC inhibition was confirmed in the activity of sodium phenylbutyrate (NaPB)(HDAC activity IC_{50}; rat liver HDAC 153 $\pm$ 51 μM, CEM cells 5572 $\pm$ 2001 μM) and its hydroxamic acid (NaPBHA) derivative (rat liver HDAC 6.2 $\pm$ 2.0 μM, CEM cells 158 $\pm$ 99 μM). IC_{50} values for % viability (3-day exposure) in CEM cells for NaPB (8400 μM) and NaPBHA (244 μM) agreed much more closely with the whole cell HDAC activity values than with rat liver enzyme values, suggesting the whole cell assay gives a much better indication of potential biological activity. Investigation of a number of simple, unsaturated hydroxamic acids found the straight chain sorbohydroxamate to have good HDAC inhibitory activity (rat liver HDAC IC_{50} 0.8 $\pm$ 0.7 μM), but the HDAC inhibitory activity was substantially lower in intact cells (IC_{50} 47 $\pm$ 17 μM). However, increased activity resulted from the novel addition of a phenyl or chlorophenyl group to sorbohydroxamate, (rat liver HDAC IC_{50}, 0.8 $\pm$ 0.3 and 0.4 $\pm$ 0.1 μM respectively), and these compounds retained good HDAC inhibitory activity in intact cells (IC_{50} 3.3 $\pm$ 1.3 and 1.2 $\pm$ 0.4 μM respectively), suggesting improved membrane transport/permeability compared to sorbohydroxamate. Further optimisation of these lead molecules using the assays described is ongoing.

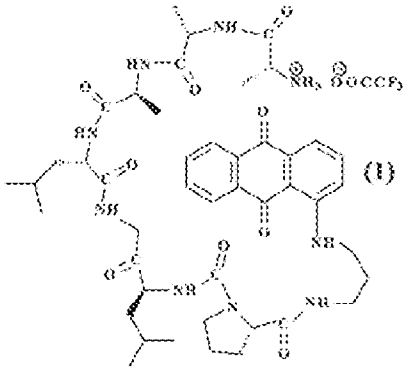
402

Design of tumour-activated oligopeptide prodrugs that exploit the proteolytic activity of matrix metalloproteinases

D.J. Mincher[1], P.M. Loadman[2], J. Lyle[2], A. Di Salvo[1], A. Turnbull[1], M.C. Bibby[2], J.A. Double[2]. *[1]Napier University, School of Life Sciences, Edinburgh, United Kingdom; [2]University of Bradford, Cancer Research Unit, Bradford, United Kingdom*

The ability of cancer cells to metastasise to distant sites is responsible for the majority of cancer related deaths. The matrix metalloproteinases (MMPs) are zinc atom- dependent endopeptidases that are implicated (notably MMP-2 and MMP-9) in the metastatic process; drug design of chemotherapeutics targeted against this family of enzymes to date has thus conventionally been directed towards the synthesis of MMP inhibitors. In contrast to this conventional approach, the aim of this study is to design prodrugs that are converted to active and potent compounds within the tumour environment by the action of overexpressed MMPs. A library of anthracenyl oligopeptide prodrugs [e.g. the D-ala-ala-ala-leu-gly-leu-pro heptapeptide conjugate (1)] has been designed to function as efficient substrates for tumour-associated MMP-9.

The non-toxic prodrug (1) was inactive (IC_{50} > 100μM) against the refractory MAC15A murine adenocarcinoma cell line in contrast to its constituent cytotoxic anthracenyl-proline (1:1) truncated conjugate, (IC_{50} 5 μM) after 1h exposure [by MTT assay], which was previously shown to give significant tumour volume reduction and growth delay against MAC15A tumours *in vivo* [Mincher et al, British J. Cancer, 80 (Suppl.2), 50, (1999)]. Incubation of (1) with human recombinant MMP-9 resulted in specific cleavage of the heptapeptide motif at the intended gly-leu MMP-9 cleavage 'hot-spot' to afford the residual anthracenyl-pro-leu-gly tripeptide conjugate (m/z 548), confirmed by LC-MS and MS-MS electrospray spectrometry. Furthermore, incubation of (1) with diluted (1:500) MMP-9-expressing HT1080 human fibrosarcoma tissue homogenates gave rapid initial metabolism (1.8 μmol min-1 g-1) to the same tripeptide conjugate which was sequentially further cleaved to the residual *in vivo*-active anthracenyl-proline metabolite. MMP



The core structure of the C2/C3-unsaturated C2-aryl pyrrolobenzodiazepines.

enzyme substrate-specificity studies have indicated that selective release of the active agent from the parent prodrug may be modulated by rational design of the molecular features of the peptide motif; subverting MMP protease activity to achieve tissue specific drug delivery represents a new strategy to improve therapeutic index.

403

Molecular modelling of quinoxaline derivatives as inhibitors of human telomerase

T. Brown[1], R.J. Anderson[2], D. Cairns[2], S.P. Mackay[3], T.C. Jenkins[1].
[1]University of Bradford, YCR Laboratory of Drug Design, Bradford, United Kingdom; [2]University of Sunderland, Pharmacy, Sunderland, United Kingdom; [3]University of Strathclyde, Pharmacy, Glasgow, United Kingdom

Telomerase is an RNA-dependent DNA polymerase enzyme responsible for the elongation of the telomeric repeat sequences at the 3'-end of eukaryotic chromosomes. Telomerase is not active in healthy somatic cells but is overexpressed in >90% of human cancers. As a result telomerase has attracted considerable attention as a target for anti-cancer chemotherapeutic intervention [1,2]. As part of our drug design programme the molecular modelling of a series of quinoxalines was undertaken and their binding to G-tetraplex DNA examined.3 Dynamic molecular modelling was carried out using the Insight-II 2000 graphics interface and Discover 98.0 simulation software (Accelrys, Cambridge, UK). The cvff force field was used throughout with explicit solvent effects represented by the TIP3P model. Atomic coordinates for human telomeric G-tetraplex DNA were obtained from the Brookhaven Database (PDB ref. 143D) and a model intercalation site generated between the diagonal T2A loop and the first G-tetrad of the tetraplex structure (i.e., the 5'-ApG step). Models for each quinoxaline were constructed (Insight-II) and partial atomic charges approximated from a single-point PM3 calculation using MOPAC. Each DNA-ligand complex was solvated and minimised. The optimised models were subjected to MD simulation (10 ps equilibration and 100 ps production, 1-fs timesteps) with initial atomic velocities taken from a Maxwell-Boltzmann distribution at 300 K. The time-averaged structures from 100 samples taken at 1-ps intervals were minimised. Ligand structures were sampled for conformational averaging in water by immersion in a solvent box of 25 Å3 and MD subject to periodic boundary conditions. Atomic trajectories saved every 1 ps were used for conformational averaging, and these structures were finally minimised to an energy convergence criterion of 0.1 kcal mol-1 Å-1. DNA-ligand binding enthalpies were obtained by subtraction of the potential energy of the DNA and quinoxaline from that of the tetraplex-ligand complex. The computed binding energies obtained ranged from -710 to -815 kcal mol-1. These results suggest that this series of quinoxalines can differentially stabilise tetraplex DNA by intercalation and that the binding is markedly superior to that achieved with earlier anthraquinone ligands [3].

References

[1] Cairns et al., Current Pharm. Design, 2002 - in press.
[2] Perry and Jenkins, Mini Revs. Med. Chem. 2001, 1, 31-41.
[3] Cairns et al., Bioorg. Med. Chem., 2002, 10, 803-807.