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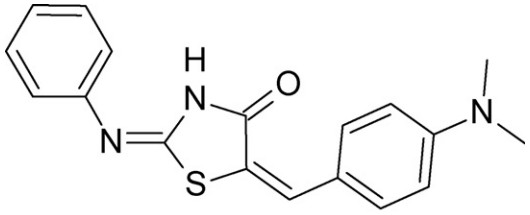
Combinatorial library methodology applied to cancer and protease targets

Thiazolidinone derivatives targeting drug-resistant lung cancer cells

The number one cause of cancer-related deaths worldwide is lung cancer. Despite advances in cancer research, the overall five-year survival of lung cancer patients remains low. More than 80% of bronchogenic malignancy arises from non-small cell lung cancer. Inherent and acquired resistance to treatment [1] and the dose-limiting toxicity caused by the narrow therapeutic window of many cancer drugs [2] are recognized as obstacles for effective treatment of cancer. Resistance to treatment may arise through multidrug resistance (MDR), which is a phenotype of cross-resistance to multiple drugs. One mechanism underpinning MDR is the overexpression of the MDR-1 gene that encodes the transmembrane, ATP-dependent, drug efflux transporter P-glycoprotein (P-gp) in response to chemotherapy [3]. Attempts to coadminister P-gp modulators or inhibitors to increase cellular availability by blocking the actions of P-gp have met with limited success [3]. A more promising approach lies in the design of compounds that are not substrates of P-gp and, thus, remain effective against drug-resistant cancer. Thiazolidinone derivatives have been investigated for a range of pharmacologic indications, such as anti-inflammatory [4] and antibacterial activities [5]. Their effects as anticancer agents have, however, been less widely documented. To search for more selective and novel compounds with an acceptable therapeutic window, thus helping to reveal structure–activity relationship for cytoselective anticancer activity, a series of thiazolidinone derivatives were designed [6]. The 216 singleton library compounds thus prepared all met Lipinski's "rule of five" [7] and were screened against paclitaxel-sensitive and -resistant NSCLC cell lines H460 and $H460_{taxR}$. Additionally, hit follow-up approaches led to the discovery of novel thiazolidinone-related compounds that were highly toxic to NSCLC H460 cells and their paclitaxel-resistant variant $H460_{taxR}$. Compounds that exhibited toxicity to both cancer cell lines, but not to normal cells, were selected for secondary confirmation assays. From these compounds synthesized, eleven were identified as potent agents for inducing cytoselective toxicity. One of the most potent was (i), which possessed IC_{50}s against the following cell lines: IC_{50} H460 500 nM; IC_{50} $H460_{taxR}$ 210 nM. This work is of interest because it has provided a new series of thiazolidinone-derived compounds that possess a useful therapeutic window for their cytoselective toxicity for drug-resistant cancer cells, when tested against the drug-sensitive and drug-resistant nonsmall cell lung cancer cell lines H460 and $H460_{taxR}$. Because $H460_{taxR}$ expresses excessive amounts of P-gp, these anticancer compounds were evidently not P-gp substrates on the basis of their cytotoxicity. Thus, this anticancer compound pharmacophore may be useful in further database searching to scaffold-hop to find novel anticancer molecules. Further studies to determine the mechanism of these anticancer compounds, as well as to optimize anticancer activity within this series of compounds, will be desirable.



(i)

Identification of protease substrates by combinatorial profiling on TentaGel beads

Proteases are involved in several biological processes. Protease substrates themselves are often used for tracking and studying proteases in relation to this biological role, as well as for screening protease inhibitors in drug discovery programs [8]. Profiling studies are thus used to discover protease substrates. Specific modalities used for the discovery of protease substrates include MS-based methods of identifying cleavage sites in reference proteins and peptides [9], activity measurements with synthetic fluorogenic peptide substrates, including combinatorial libraries of FRET substrates on a solid support [10] and active site directed probes [11]. All of these methodologies suffer from the need to perform complex experiments (synthesis and analysis). Thus, there remains a need for a simpler methodology for the discovery of protease substrates. A recent report [12] has disclosed

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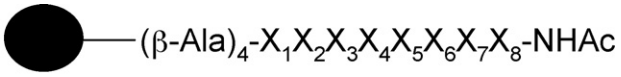
protease profiling experiments based on the direct reaction of the protease with a combinatorial library of peptides on TentaGel beads (www.rapp-polymere.com) to simplify this discovery process. The methodology involves the use of a simple assay in which protease substrates are identified by surface proteolysis (bead shaving) [13] of synthesis beads in a mix-and-split combinatorial peptide library, followed by selective staining of beads carrying a proteolyzed peptide with an amine-selective reagent, and bead decoding. A typical assay with 50 mg of solid support, corresponding to approximately 65,000 beads, is deemed sufficient for these experiments. Thus, using this approach, a combinatorial library of octapeptides, was prepared by split-and-mix synthesis, giving 65,536 octapeptides of general structure (ii). Sixteen different amino acids were used at the X position, including hydrophobic (Val, Leu, Ile, Met, Pro and Hyp), aromatic (Phe and Tyr), positively charged (Arg and His), negatively charged (Asp and Glu) and small and polar amino acids (Gly, Ala, Ser and Thr). Each amino acid appeared at only two variable positions in the library, thus facilitating sequence determination. After library synthesis was accomplished, on the bead, the staining of the free N-terminus liberated by proteolysis was tested by comparing the acylated library (general structure (ii); negative control) with the nonacylated (free-amine) version of the same library. The protease profiling experiment was carried out for the serine proteases trypsin, chymotrypsin and subtilisin and the aspartic protease pepsin. The analysis of stained beads gave octapeptide sequences consistent with known specificity of the proteases. For example, in the case of trypsin, all stained beads contained at least one arginine residue in their sequence, while nonstained beads had arginine only at Arg-Pro and Arg-Hyp dyads. The data from the on-bead cleavage assays were confirmed by resynthesis of several selected octapeptides and determination of their cleavage pattern in solution against each of the four proteases. The cleavage sites were determined using MS analysis of the protease digest mixture, confirming the results of the on-bead assays with cleavage sites corresponding to known protease specificities. Thus, these experiments demonstrate that direct proteolysis of combinatorial peptide libraries on a solid support followed by selective tagging of free N-termini and decoding by amino acid analysis provides a rapid methodology for the identification of protease substrates. It would be expected that the library composition could be adapted for specific needs depending on the targeted protease family, and this could provide a basis for future exploration.



TentaGel resin;
0.63 mmol/g (ii)

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