that there could also be more side effects,' says Eardley.

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Algal compound could reverse multidrug resistance in cancer

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A total synthesis of a naturally occurring compound has been devised that could lead to a way of reversing drug resistance in tumour cells[1]. The development of multidrug resistance (MDR) in tumour cells is commonly observed in cell cultures and, explains pharmacologist Charles Smith of Pennsylvania State University (Hershey, PA, USA), is likely to be a major factor in limiting the clinical success of many anticancer drugs. A major mechanism through which MDR develops is the overexpression of membrane transport proteins: this process simply removes the anticancer drug from the tumour cell, preventing it from accumulating in the cell and so ultimately rendering the therapy ineffective despite its cytotoxicity.

Transporter proteins

Perhaps the most well known transporter is the transmembrane protein P-glycoprotein (P-gp), which controls the traffic of a diverse range of compounds. It is a plasma-membrane-associated, energy-dependent efflux pump and among the compounds transported are the anthracyclines, the vinca alkaloids, paclitaxel (Taxol) and certain antibiotics.

A second transporter, MDR-related protein 1 (MRP1), is also involved in the emergence of resistance to anticancer drugs and both P-gp and MRP1 can be overexpressed in the tumour cells of chemotherapy patients. 'P-gp is actually commonly overexpressed in tumours of patients after chemotherapy,' explains Smith. 'However, the data on expression of MRP1 are much more ambiguous. Although many tumours do express MRP1, there is usually no overexpression of this transporter.' The development of adjuncts to cancer treatments that can side-step these transporter proteins and enable anticancer agents to accumulate at their target sites are being keenly sought. One such compound that could lead to a solution is the natural product dendroamide A (Fig. 1).

Dendroamide A is one of three cyclic hexapeptides discovered by Smith's team in 1996 from the terrestrial blue-green alga (cyanobacterium) *Stigonema dendroideum* Fremy. It has the ability to reverse multidrug resistance in several cancers *in vitro*, such as leukaemias and breast and kidney carcinomas, at non-cytotoxic doses. Smith, who holds a patent on the compound, and his team determined the gross structures of dendroamides A–C using NMR and MS analyses[2]. The absolute stereochemistry was determined by Marfey analysis and chiral GC–MS of the derivatives.

Totally synthesized

Smith believes that the fact that dendroamide is a modified cyclic peptide makes it an amenable starting point for the synthesis of a variety of analogues, which might be further investigated for structure–activity relationships. He and his post-doctoral research colleague, chemist Zuping Xia, have now developed a total synthesis that will facilitate this process.

The total synthesis of most natural products generally involves a reverse

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jigsaw puzzle approach, known as retrosynthetic analysis, in which the completed puzzle (the target compound) is dismantled hypothetically to provide fragments that might be synthesized from off-the-shelf ingredients or other readily accessible starting materials.

Retrosynthetic analysis of dendroamide A by the team[1] has revealed it to be composed of one methyloxazole- and two thiazole-containing subunits connected by peptide bonds. According to Smith, procedures for the synthesis of other cyclic compounds containing either the oxazole or thiazole heterocyclic group have been developed, which he explains provided him and his colleagues with a methodological insight into determining the synthesis of dendroamide A. Computer modelling also assisted them in finding the best starting materials for approaching the synthesis.

Coupling amino acids

They found that coupling of D-alanine and L-threonine with dicyclohexylcarbodiimide followed by reaction with Burgess reagent and oxidation produced a D-Ala-oxazole. Next, they were able to form D-Val-thiazole and D-Ala-thiazole by exploiting a modified version of the commonly used Hantzsch reaction. Finally, the molecular model pointed to the best precursor for the cyclization of the linear peptide chain to form the cyclic product.

A spectroscopic comparison of the synthetic material with the natural product revealed them to be identical. Initial tests of the effect of the synthetic material on P-gp- and MRP1-mediated drug resistance revealed similar activity. Smith points out that several of the precursor compounds also had some biological activity, which, he says, hints that cell permeability – determined by their small size and lack of ionic charge – and the peptide cyclization step are essential for an optimum effect. He adds that the presence of the thiazole and oxazole chemical groups essentially protects dendroamide from protease enzymes that would otherwise cleave them under physiological conditions.

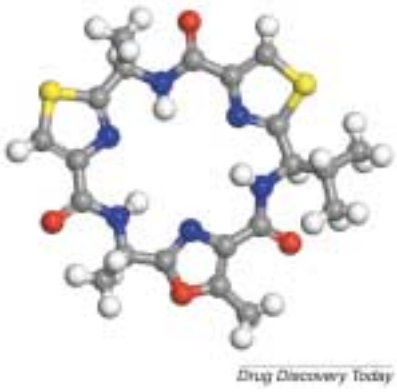

Figure 1. Dendroamide A could ring the changes in multidrug resistance.

Active structures

Further clinical and structural investigations are under way to determine the substrate-binding sites of the drug transport proteins and to define the crucial properties for activity in reversing MDR. Mapping the drug-binding sites of these proteins using structural approaches has not been successful because of their size, glycosylation and hydrophobic properties. A ligand-based approach is more likely to dissect pharmacological differences among the transporters, suggests Smith. To this end, his group has recently published structure–activity studies on quinoxalinone compounds that selectively antagonize P-gp (Ref. 3).

The group is now developing additional classes of P-gp- and MRP1-selective compounds. Smith believes that these selective compounds will have fewer adverse effects on the pharmacodynamics and pharmacokinetics of concurrently administered cytotoxic drugs than the previously tested non-selective antagonists. 'In all, these compounds provide further insight into the pharmacology of MDR, which will ultimately help in the search for potential agents for the treatment of this important clinical problem,' he adds.

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