Emerging molecular targets

Cystic fibrosis and airway mucin expression

Lung disease is the primary life-threatening complication of cystic fibrosis (CF). Individuals with this most common genetic disease suffer from airway obstruction by excess mucus, a decreased hydration and altered electrolyte composition of airway secretions, an unusual profile of bacterial infections, and chronic airway inflammation. Together, these aberrations promote a complex downward spiral of ever more serious successions of infection and inflammation of the airways. Today, more than 95% of the deaths attributed to CF are due to chronic lung disease.

The cause of the excessive mucus secretion in CF has long been sought. Now, Dr Jian-Dong Li and coworkers at the University of California (San Francisco, CA, USA) and Columbia University (New York, NY, USA) have found that the culture supernatant of *Pseudomonas aeruginosa*, a common organism infecting the lungs of CF patients, contains substances that trigger transcription of the mucin gene *MUC2* [*Proc. Natl. Acad. Sci. U. S. A.* (1997) 94, 967–972].

CF is caused by a host of different mutations in the gene that encodes a protein called the cystic fibrosis transmembrane conductance regulator (CFTR). This protein is normally found on the apical surface of epithelial cells, where it functions as a chloride ion channel. In CF, mutations of the CFTR gene cause the protein to either fail to be inserted into the membrane of the epithelial cell or, if it is inserted into the apical membrane, to be defective in transporting chloride ions. In the airways, the decreased hydration and the altered ionic properties of the airway secretions that arise as a consequence of the defective CFTR are believed to promote infection by *P. aeruginosa*. The findings of Li and coworkers now suggest that excess mucin production is a tertiary effect of the CFTR gene mutation that occurs as a direct result of colonization by *P. aeruginosa*.

Li and colleagues conclude that the substances in the culture supernatant responsible for activation of the *MUC2* gene are bacterial polysaccharides such as lipolysaccaride (LPS), which are common mediators of inflammation derived from Gram-negative bacteria. Their conclusion is based upon an examination of the chemical and physical properties of the activating agents in the culture medium, and the observation that purified LPS from *P. aeruginosa* was capable of activating the *MUC2* gene in a manner similar to that observed in the culture supernatants. They also report that two tyrosine kinase inhibitors, tyrophostin AG126 and genistein, block the activation of the *MUC2* gene by the components of the culture supernatant.

Future work on the details of LPS signaling leading to the activation of the *MUC2* gene in epithelial cells is likely to yield novel targets for the discovery of drugs to block the excess mucin secretion characteristic of CF. Such drugs could be highly useful in reversing the extremely complex path of lung disease that so often ends the life of the CF patient.

A membrane-bound chemokine with a CX_3 motif

Protein chemokines control the migration of T cells, monocytes, neutrophils and other cellular components of the immune system. The repertoire of proteins that make up the chemokine system is complex, and new components are still being discovered. Until now, all the known chemokines were relatively small proteins that fell into one of three structural categories based upon specific cysteine-rich motifs: CXC, CC and C, where C is a cysteine and X is any amino acid. Now, Dr J. Fernando-Bazan and coworkers at the DNAX Research Institute (Palo Alto, CA, USA) and the University of Oxford (UK) have identified a new chemokine with unusual characteristics including a distinct signature motif of CXXXC [*Nature* (1997) 385, 640–644].

The new chemokine was discovered by searching databases of DNA sequences for those genes that encode proteins with structures similar to known chemokines. Using this approach, the authors of the study found a most unusual DNA sequence that codes for a 397-amino-acid protein, of which only a portion, the 76-amino-acid N-terminus of the mature protein, contains a chemokine-like structure. The remainder of the molecule is more analogous to a mucin structure with an uninterrupted stretch of 18 hydrophobic amino acids, the hallmark of a transmembrane domain, close to the C-terminus. The newly discovered protein appears to consist of a chemokine that is dangled into the extracellular milieu at the end of a long mucin-like stalk that is anchored to the plasma membrane.

Biological studies of the newly discovered protein show that it is found on the plasma membrane of endothelial cells, where it promotes the adhesion of leukocytes. The N-terminus CXXXC chemokine portion of the molecule may be shed, and the soluble form of the chemokine acts as a chemoattractant for T cells and monocytes. A detailed search for cell-surface receptors for the soluble chemokine and an understanding of the presumed signaling activity of the membrane-bound form of the protein are likely to lead to interesting new molecular targets for the discovery of drugs to modulate immune function.

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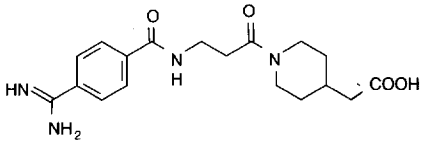
Combinatorial chemistry

A library of GPIIb/IIIa antagonists

Nonpeptide antagonists of the platelet fibrinogen receptor (GPIIb/IIIa) are useful inhibitors of platelet aggregation. Consequently they are a focus of much current medicinal chemistry research. Earlier this year, I highlighted the use of combinatorial chemistry in the optimization of an active GPIIb/IIIa antagonist [Hoekstra, W.J. *et al. Bioorg. Med. Chem. Lett.* (1996) 6, 2371–2376]. Harada, T. and coworkers [*Bioorg. Med. Chem. Lett.* (1997) 7, 209–212] have now published their own



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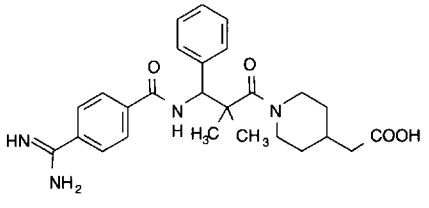

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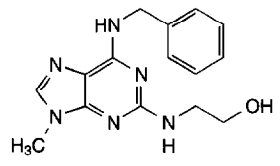
use of combinatorial chemistry in the discovery and optimization of novel nonpeptide GPIIb/IIIa antagonists. Working on the premise that a highly basic group and a free carboxyl group suitably separated are essential for binding, a library of 27 individual trimeric compounds were prepared on solid-phase from a set of three amidines/guanidines, three spacer groups and three carboxyl-containing units.

The best compound from this set (**1**, IC_{50} = 2.5 μM), was optimized by the systematic replacement of the central spacer group to give racemic NSL95301 (**2**, IC_{50} = 0.19 μM). Resolution of the racemate resulted in the discovery of (+)-NSL95301, which had an IC_{50} value for the GPIIb/IIIa receptor of 0.092 μM.

Purine synthesis

Various pharmacological actions have been observed from purine analogues. For example, the purine derivative olomoucine (**3**) is a potent and selective inhibitor of a cyclin-dependent kinase complex $p33^{cdk2}$/cyclin A. A recent paper from DuPont Merck (Wilmington, DE, USA) describes a solid-phase synthesis of purines, including olomoucine, that offers potential for the preparation of combinatorial libraries of these molecules [Nugiel, D.A. *et al. J. Org. Chem.* (1997) 62, 201–203]. The work was based on a combination of two literature observations; (i) that the tetrahydropyranyl (THP) group has been used as a purine protecting group, and (ii) that Ellman has described the use of a THP solid-phase linker.



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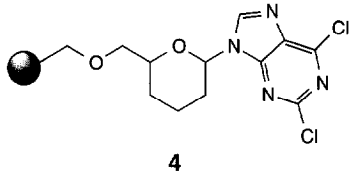

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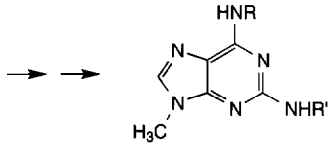

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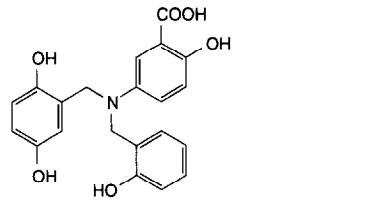
Thus the synthesis commenced with the THP linker being used to tether 2,6-dichloropurine to resin beads (**4**). Sequential displacement of the two chlorines with amine nucleophiles gave an intermediate that could be cleaved from the resin using a standard acid cleavage protocol. In solution, the N-9 imidazole position could be alkylated to give a range of different purine derivatives (**5**) in good yield. For example, the synthesis of olomoucine gave the desired product in 70% yield after chromatography.

Solid-phase synthesis of lavendustin A



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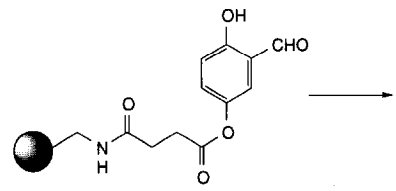

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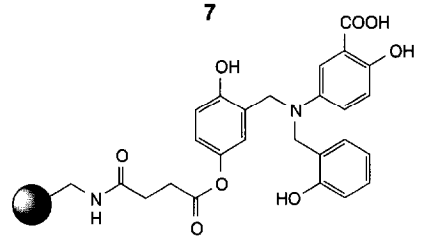

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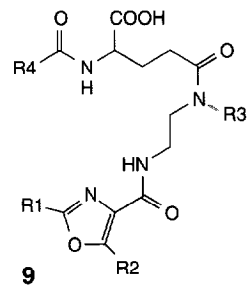
First isolated from *Streptomyces griseolavendus*, the natural product, lavendustin A (**6**) is a potent protein-tyrosine kinase (PTK) inhibitor. Chemists from Purdue University (West Lafayette, IN, USA) have demonstrated that this compound and related analogues may be synthesized on solid-phase, and that combinatorial chemistry or parallel synthesis may be used for the preparation of a wide variety of analogues [Devraj, R. and Cushman, M. *J. Org. Chem.* (1996) 61, 9368–9373].

Initial attempts to prepare lavendustin A on Wang resin were frustrated by decomposition of the product during acid cleavage from the solid-phase. Thus an alternative approach using aminomethyl polystyrene resin was investigated. The resin was derivatized with succinic anhydride to which was linked 2,5-dihydroxybenzaldehyde (**7**). This ester bond was labile under basic conditions, using a cleavage cocktail of triethylamine and dimethyl sulphide in methanol. Lavendustin A and analogues were prepared by a reductive amination with an aniline followed by reductive alkylation with an aldehyde to give **8**. The range of available substituted amines, aldehydes or benzyl halides offers enormous potential for large libraries of structurally related PTK inhibitors.

Protein phosphatase inhibitor library



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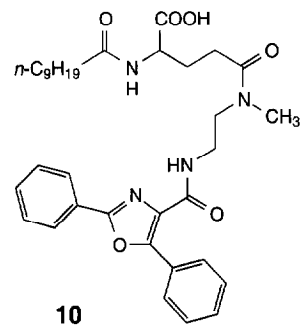

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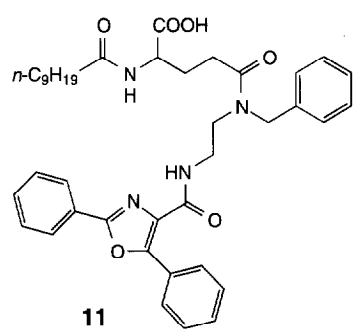

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A combinatorial library of 18 compounds has been prepared in the search for inhibitors of serine/threonine protein phosphatases (PSTPase) [Wipf, P. *et al. Bioorg. Med. Chem.* (1997) 5, 165–177]. By examining the available natural product inhibitors of these enzymes, a parent pharmacophore model (**9**) was devised and this provided the platform for combinatorial library design. The synthesis was devised in solution and then transferred to Wang resin commencing with the allyl ester of Fmoc-glutamic acid.

Of the 18 derivatives prepared, one compound (**10**) demonstrated *in vitro* inhibition of the PSTPase PP2A, and another (**11**) exhibited a concentration-dependent inhibition of MDA-MB-231 breast cancer cell growth.

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High-throughput screening

This new monthly section of *Monitor* will focus on a range of topics relating to high-throughput screening (HTS). The main theme of this month's report is screen automation, and much of what appears below and in next month's automation section reflects content from the Lab-Automation '97 conference, held in San Diego, CA, USA in January, the *Journal of Biomolecular Screening* and *Laboratory Robotics and Automation.*

Approaches to HTS automation

Without doubt, automation of HTS allows increased capacity and reliability and, where cost-effective, is being implemented in primary screening programmes. Below are described the three main approaches to automation of HTS.

Integrated, customer-specified, design-and-build systems are the most expensive systems to develop, being built to meet exact customer defined requirements. Most systems are centralized around a robotic arm on a linear track, although other robotic solutions to speed up microtitre plate transfers within these systems are being developed by companies such as SAIC (Seattle, WA, USA). Generally, design-and-build systems are constructed by professional robotics integration companies; examples of vendors include Thurnall (Manchester, UK), SciTec (Lausanne, Switzerland), Sagian (Indianapolis, IN, USA) and Robocon (Vienna, Austria).

Integrated 'off the shelf' systems are marketed as integrated complete robotic systems built by particular equipment suppliers with system configuration for particular assay types and formats. Unlike the design-and-build systems, scope to include user-specified peripherals is limited. Beckman (Fullerton, CA, USA) is now offering an 'off the shelf' HTS screening system which can be configured for receptor binding or cell-based assays.

Workstations. Another approach is to automate labour-intensive parts of assay protocols using individual workstations. Complex assay protocols can be automated by having several workstations in close proximity, each performing a certain part of the protocol, such that an entire assay can be automated. A major limitation of this approach is that a human operator is required to move plates from workstation to workstation and finally to a detector. Throughput is, therefore, dependent on operator availability. This approach has been termed 'hubotic', as opposed to robotic for the integrated systems outlined above.

A wide variety of HTS assay robots is now available; for example, systems based on the optimized robot for chemical analysis (ORCA) offered by Sagian, SciTec and Tecan (Hombrechtikon, Switzerland), or systems based around the CRS robot arm from CRS (Burlington, Ontario, Canada) and TomTec (Hamden, CT, USA), or the workstation-based rotational arm systems offered by Zymark (Hopkinton, MA, USA) and Beckman. For any of these systems to run large batches of plates, they require appropriate software scheduling programmes. Sagian's automated methods integrator (SAMI) software has been reviewed by Murray, C. and Anderson, C. [*Laboratory Robotics and Automation* (1996) 8, 295–305].

As technology advances, and reliability of integrated robotic systems improves, there will be a movement away from the relatively slow, single-grip-arm robots based on linear tracks. In the future, HTS robots will comprise more versatile and complex systems providing closed loop automation of drug screening. At present, the complexity of robotic systems is limited to minimize the error generation and system failures that can occur with large systems with many peripherals and complex controlling software.

In the June issue of *Drug Discovery Today*, some assay-type specific systems will be described.

Ultra-HTS deal for EVOTEC BioSystems

EVOTEC BioSystems (Hamburg, Germany) have announced that they are about to move into the next phase of development of their proprietary ultra-HT fluorescence-based screening technology by signing agreements with Novartis and SmithKline Beecham. The agreements will involve payments to EVOTEC amounting to $30 million and will be for the specific development of nanoscale fluorescence-based technology incorporated into an integrated ultra-HTS system – EVOscreen. For more information on EVOTEC's technology, see Rogers, M.V. *Drug Discovery Today* (1997) 3, 156–160.

Glaxo Wellcome's R2 system operational

Glaxo Wellcome (Stevenage, UK) has installed a state-of-the-art integrated HTS system, R2. The system has been designed to operate with 96- and 384-well microtitre plates. R2 contains two cells that are fully enclosed and run on a continuous production style basis. According to Dr Martyn Banks, Team Leader in Lead Discovery, the drive for efficiency and cost effectiveness in HTS has focused the Glaxo Wellcome team's design ideas on automated solutions adopted in other industries; the R2 robots with their logistical and procurement operation now mirror these systems. The Glaxo system uses Thurnall's proprietary Windows-based scheduling software called SPRINT. For more details on the R2 system, see Hughes, D. *Drug Discovery Today* (1997) 2, 40–43.

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