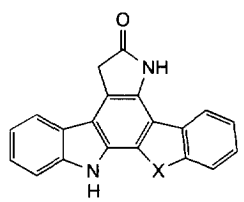
enhance the immune response. Interferon-γ (IFN-γ) is a potent immune activator but has dose-related side effects that limit its effectiveness. It has been suggested previously that molecules that enhance the activity of IFN-γ might allow clinicians to prescribe lower doses of IFN-γ while retaining therapeutic efficacy. A group from Cephalon (West Chester, PA, USA) has reported a series of fused pyrrolo[2,3,*c*]carbazol-6-ones (**12**) that potentiate the IFN-γ induction of major histocompatability complex class II molecules in a human monocyte-derived cell line [Hudkins, R.L. *et al. J. Med. Chem.* (1997) 40, 2994–2996]. These compounds may provide an alternative approach, through the enhancement of the natural immune system, for the treatment of viral infections and cancer growth.



X = O, S, NH or CH_2

12

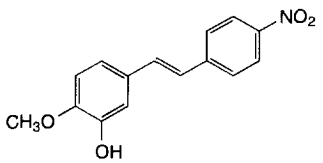
Combinatorial chemistry

Hydroxystilbene kinase inhibitor library

Protein kinases are attractive drug targets because protein phosphorylation catalysed by these enzymes is a key mechanism in signal transduction pathways. However, a major obstacle to the discovery of selective inhibitors is the considerable number of known protein kinases and the degree of homology between the family members. To overcome these difficulties, a combinatorial library of stilbenes has been tested against B cell cytoplasmic tyrosine kinases in the search for inhibitors of receptor-mediated phosphorylation [Bishop, A.C. *et al. Tetrahedron* (1997) 53, 11995–12004].

The assay was based on the use of an anti-phosphotyrosine that binds almost any phosphotyrosine-containing protein. Thus it was possible to examine the extent of phosphorylation of many different cellular tyrosine kinase substrates simultaneously using a protein blotting assay. The combinatorial library products, based on 3,4,3′,5′-tetrahydroxy-*trans*-stilbene, were individually tested against the B cell antigen receptor-initiated signalling cascade, and several compounds were found to disrupt kinase activity.

One compound in particular (**1**), when tested at 250 μM, inhibited the phosphorylation of four of the cellular proteins, thus revealing a modest level of enzyme selectivity and providing a potential starting point for further libraries of kinase inhibitors.



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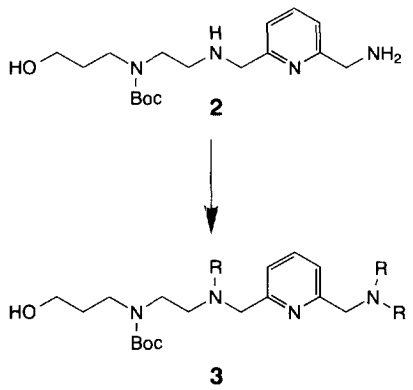
Antibacterial solution libraries

Solution methods for combinatorial chemistry are effective approaches to libraries when the route is a high-yielding one-step synthesis. Several libraries based on linear diaminopyridines have been prepared and screened for antibacterial activity [An, H. *et al. J. Org. Chem.* (1997) 62, 5156–5164]. Templates such as the diamine (**2**) were prepared using a cyclic nickel complex, and were then reacted in the library-forming step with equimolar amounts of six different benzylic bromides. Although there are three sites of derivatization, the symmetry of the primary amine led to a final library size of 126 components. The *tert*-butoxycarbonyl (Boc) protecting group in (**3**) could be removed to allow the formation of further sublibraries.

Testing the libraries as mixtures against *Streptococcus pyogenes* and *Escherichia coli* revealed several with



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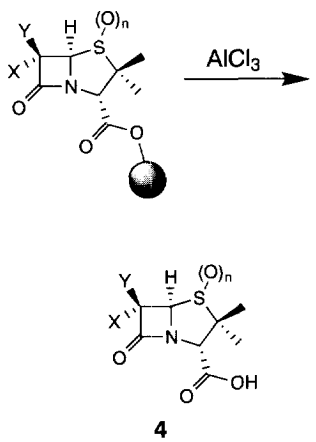
3

MIC values in the range of 1 to 5 μM. Some libraries also showed antifungal activity by inhibiting the growth of *Candida albicans*.

Synthesis of penicillins on solid phase

As the solid-phase preparations of pharmacologically significant molecules gain greater importance, methods to accommodate the synthesis of sensitive structures are rapidly being determined. The β-lactam structure, which occurs in penicillins and cephalosporins, is readily degraded under strongly acidic conditions, and thus the resin-cleavage conditions often used with Merrifield or Wang resins are unsuitable for its preparation. A recent paper discloses the development of an alternative cleavage method that avoids exposure of these labile molecules to acidic conditions [Mata, E.G. *Tetrahedron Lett.* (1997) 38, 6335–6338].



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Penicillins, such as **4**, modified on Merrifield resin were found to be readily cleaved using aluminium trichloride in nitromethane instead of the anhydrous hydrogen fluoride usually employed. Similarly, penicillins on Wang resin could be cleaved under the same conditions avoiding the use of trifluoroacetic acid. This approach now provides an opportunity for the solid-phase synthesis of libraries of these important antibiotic compounds.

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High-throughput screening

HTS and gene chip technology

New strategies for synthesizing complex arrays of DNA on small glass surfaces to generate what are termed 'gene chips' are revolutionizing the diagnosis of genetic diseases and basic genomic research. The gene chip technology may also have a major impact on drug discovery, making possible rapid monitoring of the effects of a test compound on the expression of a single gene, a family of related genes or even a large chunk of an organism's entire gene pool.

Transcriptional assays are some of the most difficult to convert to high-throughput format. Certainly, the classic techniques for monitoring gene expression – northern and dot blot strategies – are too cumbersome for high-throughput screening applications. Gene reporter approaches, in which the regulatory region of a particular gene is hybridized to a reporter gene, such as luciferase, is one method that has been successfully converted to a high-throughput screening format, but it requires the isolation and engineering of every gene of interest and its reinsertion into a convenient cell line. Such manipulations are time-consuming and not always possible or desirable.

Glass wafers

Gene chips are small glass wafers, usually about 1–2 cm^2 in area, covered with a lawn of precisely defined oligonucleotide probes. They are used in a manner analogous to classic blotting strategies – the probes hybridize to RNA containing a complementary base sequence and can be used to monitor the level of expression of a gene with the complementary sequence. What differentiates the gene chip from classic blotting techniques is that the single small chip can contain thousands to hundreds of thousands of distinct oligonucleotides, each one representing a different gene or small domain of a single gene, and the hybridization can be done quickly and quantitatively [see *Molecular Medicine Today* (1997) 3, 384–389 for a complete description of the gene chip].

Gene chip technology can be used to resequence a known gene quickly and to check for alterations in its nucleotide sequence, to determine the presence or absence of previously identified gene mutations, or, of greatest interest for drug discovery applications, to measure the level of expression of thousands of different genes simultaneously in response to a chemical perturbation of the cell. Using this technology, a high-throughput screening operation might be imagined in which, with a single assay, the effects of a defined chemical or a natural product extract could be determined simultaneously on the expression of thousands of different genes, without ever having to isolate, engineer or reinsert the genes of interest into a host cell.

On the down side, the gene chip assay has not yet been automated for high-throughput screening operations to provide for the testing of thousands of chemical compounds or extracts on a daily or weekly basis. But it is only a matter of time before such systems become available. An automated gene chip assay system would allow thousands of compounds or extracts to be quickly assayed for their effect on thousands of different genes, providing a new plateau for high-throughput screening in which millions of data points could be generated each day. Indeed, it would not be surprising to learn that proprietary systems are already in development, or even in use, by pharmaceutical laboratories that have embraced the gene chip technology for drug discovery.

Some examples

Affymetrix, Inc. (Santa Clara, CA, USA) is the major company developing the gene chip technology; they currently have a product on the market, the GeneChip HIV PRT Assay, which is used to monitor the specific mutant strains of the HIV protease in an infected individual. Based upon the results of the chip-based diagnostic test, a physician can prescribe a cocktail of anti-HIV drugs tailored to the specific strain of HIV that infects a patient. Although most of Affymetrix's emphasis is on using the new technology for diagnostic applications, they are also collaborating with pharmaceutical companies, including Merck & Co., Inc. (Whitehouse Station, NJ, USA), Hoffman-LaRoche (Basel, Switzerland) and Genetics Institute, Inc. (Cambridge, MA, USA), to use the gene chip technology for drug discovery.

Another company exploiting the new technology for drug discovery is Incyte Pharmaceuticals, Inc. (Palo Alto, CA, USA). Incyte has a Life Chip Array that contains probes for more than 15,000 different human genes on a single chip. These genes have been selected from Incyte's LifeSeq GeneAlbum™, which is a library of approximately 100,000 cDNA probes for expressed human genes.

Synteni (Palo Alto, CA, USA) is producing GEM, a chip with an array of up to 10,000 different cDNA elements that can also be used quantitatively to monitor gene expression. Synteni aims to eventually market chips that can be used to monitor the entire human genome.

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