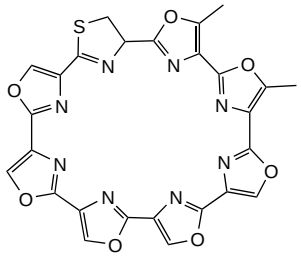
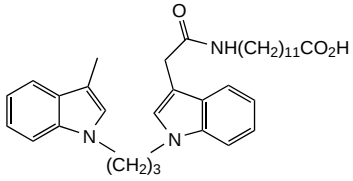
inhibitor designated as telomestatin **(vi)**, from *Streptomyces anulatus* 3533-SV4 (Ref. 6). Inhibitory effects against telomerase, which was semi-purified from the cell lysates of human B lymphoma Namalwa cells, were estimated using a modified telomerase repeat amplification protocol (TRAP) assay and **(vi)** was found to have an IC_{50} value of 0.005 μM with no activity against *Taq* polymerase.



(vi)

In another recent report, Sasaki and coworkers at Kyushu University (Fukuoka, Japan) and Mitsubishi-Tokyo Pharmaceuticals (Yokohama, Japan) have described the synthesis and evaluation of a series of bisindole derivatives as telomerase inhibitors[7]. Inhibitors with IC_{50} values in the submicromolar range were reported by the stretch PCR assay (e.g. compound **vii**, IC_{50} = 20 μM).



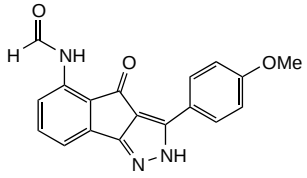
(vii)

6 Shin-ya, K. *et al.* (2001) Telomestatin, a novel telomerase inhibitor from *Streptomyces annulatus*. *J. Am. Chem. Soc.* 123, 1262–1263

7 Sasaki, S. *et al.* (2001) Development of novel telomerase inhibitors based on a bisindole unit. *Bioorg. Med. Chem. Lett.* 11, 583–585

Novel cyclin-dependent kinase inhibitors

Cyclin-dependent kinases (CDKs), in association with regulatory cyclins, have a key role in regulating the cell cycle machinery, and a growing body of research has shown a link between tumour development and CDK-related malfunctions. An intensive search for CDK inhibitors as cancer chemotherapeutics has been undertaken and two molecules, flavopiridol and UCN01, have entered into clinical trials based on this mechanism of action. Nugiel and coworkers at DuPont Pharmaceuticals (Wilmington, DE, USA) have disclosed indenopyrazoles, such as **(viii),** as a new structural class of CDK inhibitors (IC_{50} vs CDK4/D1 = 0.2 μM), which are selective for CDKs compared with other kinases[8]. Compound **(viii)** is also active in cell culture against a transformed colon cell line (HCT 116; IC_{50} = 0.69 μM) and demonstrates *in vivo* activity by reducing tumour growth in a human xenograft mouse model, in a dose-dependent manner.



(viii)

8 Nugiel, D.A. *et al.* (2001) Indenopyrazoles as novel cyclin-dependent kinase (CDK) inhibitors. *J. Med. Chem.* 44, 1334–1336

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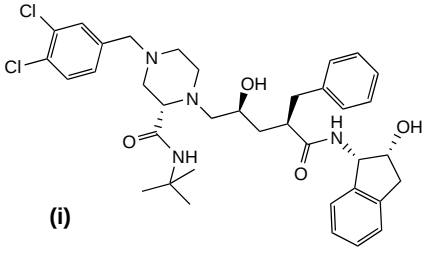
Combinatorial chemistry

HIV protease inhibitors

The discovery of clinically effective HIV protease inhibitors (PI) has significantly improved the lifestyles of many individuals afflicted with the virus. Nevertheless, current protease inhibitors suffer to some extent from issues that are not limited to first-pass metabolism, toxicities and food restrictions, which often contribute to patient non-compliance. Recently, the emergence of multi-drug resistant viral variants has been confirmed, further compromising the effectiveness of current PI therapy. In an effort to produce compounds possessing improved pharmacokinetic and potency profiles, a combinatorial library of compounds targeting HIV inhibition was synthesized[1]. A library of 60 compounds was synthesized in mixtures of 20 on Rapp TentaGel-S CO_2H resin.

These compounds were tested in mixtures of 20, using *in vivo* dosing of multiple component mixtures, for their ability to prevent cleavage of a substrate by the protease enzyme (IC_{50}) and to inhibit the spread of viral infection in MT4 human T-lymphoid cells infected with the IIIb isolate (CIC_{95}). Of those mixtures tested, the most active **(i)** gave an enzyme inhibition IC_{50} value of 1.1 nM, and a value for the inhibition of viral spread (IIIb) CIC_{95} of 200 nM. This library is successful because it provides a step forward towards the identification of a second generation HIV-PI possessing improved metabolic and potency profiles.



(i)

1 Rano, T.A. *et al.* (2000) Combinatorial diversification of indinavir: *in vivo* mixture dosing of an HIV protease inhibitor library. *Bioorg. Med. Chem. Lett.* 10, 1527–1530

Cell-permeable molecules

Small molecules that induce or stabilize the association of macromolecules hold promise as biological effectors. Such chemical inducers of dimerization (CIDs) are well known in nature; anti-cancer agents adriamycin[2] and etoposide[3] act by stabilizing a catalytic intermediate having topoisomerase II bound to its nicked DNA substrate. The immunosuppressive agent FK506 induces the

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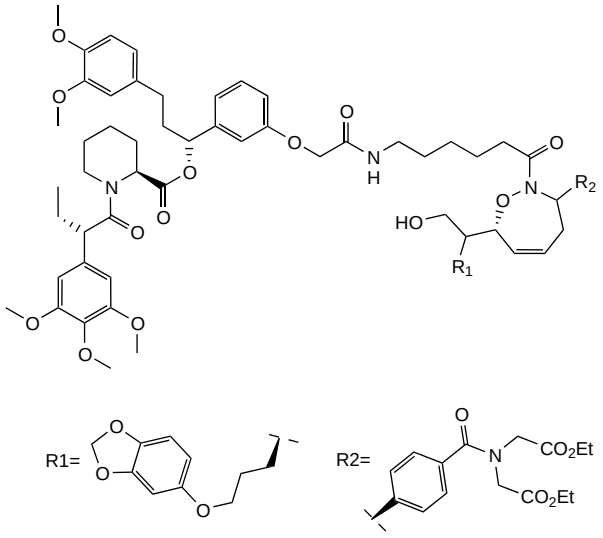

(ii)

dimerization of FK506-binding protein (FKBP) and calcineurin (Cn), thereby cancelling the protein phosphatase activity of calcineurin.

The potential of designed synthetic CIDs to bring together signalling proteins in a spatially and temporally controlled manner enables the engineering of a variety of inducible signal transduction processes into cells. These synthetic 'dimerizers' all involve bifunctional molecules that have two ligand moieties linked together, each of which binds a known receptor. There is also the possibility of inducing the association of one known macromolecule with an unknown one, thereby creating biological relationships that nature might not have explored. Dimerizing two known macromolecules can present a challenge when there is a known ligand for one partner but not the other. Both these lines of research could be pursued by the construction of bifunctional small-molecule libraries, which have an invariant ligand attached to a diversified ligand of which the structure varies among the members of the library.

A combinatorial chemistry approach was used to construct the first such diversified library of synthetic candidate heterodimerizers[4]. A library of 320 individual compounds was synthesized on trityl-chloride solid-phase resin (examples shown in **ii**). For synthetic heterodimerizers to be useful in biological assays with intracellular targets, they must be cell-permeable. To assess the cell-permeability of these library compounds, cells that express two FKBP fusion proteins – one containing the ZFHD1 DNA-binding domain and the other containing the p65 acidic transcriptional activator domain – were used. The small-molecule heterodimer AP1889 brings these two protein components together, thereby activating transcription of the downstream gene encoding secreted alkaline phosphatase (SEAP). A randomly chosen subset of the library comprising 25 heterodimers was screened separately at 500 nM concentration. Each of the compounds tested strongly inhibited SEAP expression indicating cell-permeability and suitability for use in biological screens. Future research is directed at determining whether any of these 320 library members will function as heterodimers, allowing the possibility of engineering a variety of inducible signal transduction processes into cells. Studies are ongoing in a variety of biological assay systems to address these aims.

2 Andoh, T. and Ishida, R. (1998) Catalytic inhibitors of DNA topoisomerase II. *Biochim. Biophys. Acta* 1400, 155–171

3 Burden, D.A. and Osheroff, N. (1998) Mechanism of action of eukaryotic topoisomerase II and drugs targeted to the enzyme. *Biochim. Biophys. Acta* 1400, 139–154

4 Verdine, G.L. *et. al.* (2001) A synthetic library of cell-permeable molecules. *J. Am. Chem. Soc.* 123, 398–408

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Contributions to *Monitor*

We welcome recommendations of papers for review within *Monitor*, in the fields of combinatorial chemistry, pharmacogenomics, pharmacoproteomics, bioinformatics, new therapeutic targets, high throughput screening, new drug delivery technologies and other promising lines of research. Details of recent papers or those *in press* should be directed to Dr Debbie Tranter, Editor, *Drug Discovery Today*, Elsevier Science London, 84 Theobald's Road, London, UK WC1X 8RR. tel: +44 (0) 20 7611 4132, fax: +44 (0) 7611 4485, e-mail: deborah.tranter@drugdiscoverytoday.com