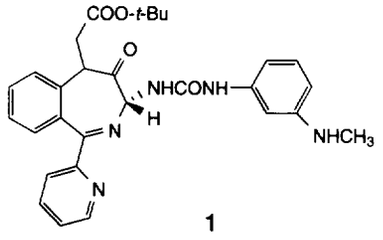
Monitor: molecules and profiles

Monitor provides an insight into the latest developments in drug discovery through brief synopses of recent presentations and publications together with expert commentaries on the latest technologies. There are two sections: *Molecules* summarizes the chemistry and the pharmacological significance and biological relevance of new molecules reported in the literature and on the conference scene; *Profiles* offers commentary on promising lines of research, emerging molecular targets, novel technology, advances in synthetic and separation techniques and legislative issues.

Orally active CCK_B antagonist

Recent evidence has suggested that gastrin/CCK_B antagonists may be useful in the treatment of gastro-oesophageal reflux and other gastrointestinal disorders. Semple, G. and coworkers [*J. Med. Chem.* (1997) 40, 331–341] have prepared and evaluated a series of new 1,4-benzodiazepin-2-one-based compounds as potential CCK_B antagonists. The selectivity of the compounds for the CCK_B and CCK_A receptors was assessed by measuring the ability of these compounds to inhibit binding of [^{125}I]CCK-8 to rat brain CCK_B receptors and [^{3}H]L364718 to rat pancreatic CCK_A receptors. Initial *in vivo* studies demonstrated that these types of compound were able to inhibit pentagastrin-induced gastric acid secretion in anaesthetized rats. Further *in vivo* assessment of the ability of selected compounds to inhibit gastric acid secretion in Heidenhain pouch dogs following intravenous and oral administration identified (3*R*)-*N*-[1-(*tert*-butylcarbonylmethyl)-2,3-dihydro-2-oxo-5-(2-pyridyl)-1*H*-1,4-benzodiazepin-3-yl]-*N*′-[3-(methylamino)phenyl]urea **1** as a



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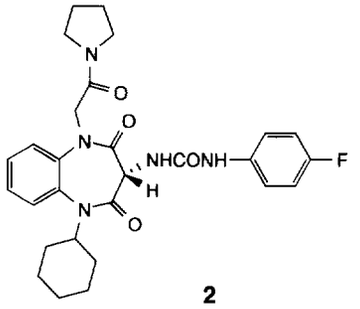
suitable candidate for further clinical evaluation.

In another paper, workers from Glaxo Wellcome (Stevenage, UK) have reported the synthesis and evaluation of an alternative series of gastrin/CCK_B antagonists based on 1,5-benzodiazepindione [Bailey, N. *et al. Bioorg. Med. Chem. Lett.* (1997) 7, 281–286]. This has led to the identification of potent, selective and orally bioavailable candidate **2** for further evaluation.



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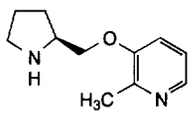
Nicotinic acetylcholine receptor ligand

Neuronal nicotinic acetylcholine receptor ligands with improved safety and pharmokinetic profiles over nicotine have application in the treatment of CNS disorders. A group from Abbott Laboratories (Abbott Park, IL, USA) have demonstrated that 2-methyl-3-[2(*S*)-pyrrolidinylmethoxy]pyridine **3** has cognition-enhancing and anxiolytic activities in animal models with a reduced propensity, relative to (*S*)-nicotine, to activate peripheral ganglionic type receptors and cause seizures or effects on homothermic control or locomotive activity [Lin, N-H. *et al. J. Med. Chem.* (1997) 40, 385–390]. In addition, this compound has oral bioavailability and is therefore a suitable candidate for further evaluation as a treatment for cognitive disorders.



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Muscarinic analgesics

The treatment of abdominal pain associated with irritable bowel syndrome (IBS) with analgesic therapy is limited by the side effects associated with the use of opioid and nonsteroidal anti-inflammatory agents. Furthermore, the use of atropine-like muscarinic antagonists as antispasmodics to reduce the high-amplitude gastrointestinal contractions associated with IBS is also restricted by the typical muscarinic side effects. Workers from Lilly Research Laboratories (Indianapolis, IN, USA) and Novo Nordisk (Bagsvaerd, Denmark) have evaluated a series of new 1,2,5-thiadiazole-based muscarinic antagonists for analgesic activity using the mouse writhing assay and ability to normalize spontaneous cluster contractions in the ferret jejunum as a model of IBS in humans [Mitch, C.H. *et al. J. Med. Chem.* (1997) 40, 538–546]. These

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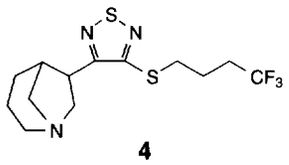
studies have identified (5*R*,6*R*)-*exo*-6-[4-[(4,4,4-trifluorobutyl)thio]-1,2,5-thiadiazol-3-yl]-1-azabicyclo[3.2.1]octane, **4**, as a candidate for further evaluation as a potential agent to treat IBS.



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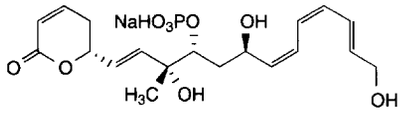
Absolute stereochemistry of fostriecin (CI920)

The structurally novel phosphate ester fostriecin (**5**, CI920) obtained from *Streptomyces pulveraceus* is active *in vitro* against leukaemia, lung, breast and ovarian cancers, has been shown to have *in vivo* activity against L1210 leukaemia in mice [Jackson, R.C. *et al. Adv. Enzyme Regul.* (1985) 23, 193] and is presently undergoing phase I clinical trials at the US National Cancer Institute. Boger, D.L., Hikota, M. and Lewis, B.M. [*J. Org. Chem.* (1997), in press] determined the absolute stereochemistry of this potent antitumour antibiotic to be 5*R*, 8*R*, 9*R*, 11*R* using a combination of methods including 2D ^{1}H-^{1}H NMR NOE experiments on the cyclic phosphate and acetonide derivatives.



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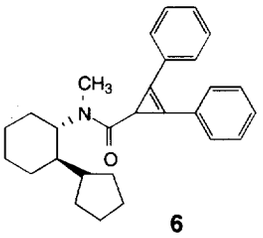
Lipophilic kappa opioid agonists

In recent years, considerable research efforts have been focused towards the development of selective kappa opioid receptor agonists with potent analgesic activity without the side effects of constipation, respiratory depression and dependence associated with activation of the mu subtype. In addition, kappa opioid receptor agonists that are unable to penetrate the CNS may reduce the centrally-associated side effects of sedation, diuresis and dysphoria. Sabin, V. and coworkers [*Bioorg. Med. Chem. Lett.* (1997) 7, 291–296] have described the synthesis and evaluation of some lipophilic analogues of the kappa opioid agonist U50488, and have identified the lipophilic, chemically novel *trans*-2,3-diphenyl-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl]-2-cyclopropene-1-carboxamide (**6**) as a potent, selective kappa opioid agonist.



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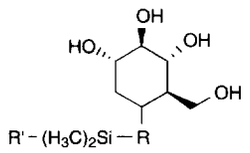
α-glucohydrolase inhibitors

Intestinal α-glucohydrolase inhibitors may have application as oral antihyperglycaemic agents in the treatment of diabetes mellitus by slowing down carbohydrate digestion, thereby delaying gastrointestinal absorption of sugars following food intake. Lesur, B. and coworkers [*Bioorg. Med. Chem. Lett.* (1997) 7, 355–360] have reported the synthesis and evaluation of a series of new *N*-alkyl-, alkenyl- and benzyl-substituted, silicone-containing deoxynojirimycin derivatives, **7**, as potential inhibitors of intestinal α-glucohydrolases. These compounds were shown to be potent inhibitors of intestinal disaccharidases with better selectivity than deoxynojirimycin and *N*-methyldeoxynojirimycin.



R = alkyl, alkenyl or aryl
R' = alkyl or aryl

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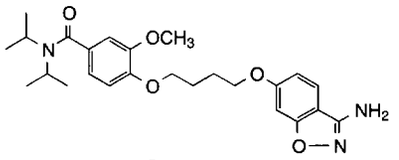
LTB_4 receptor antagonist

The leukotriene LTB_4 is released from a wide range of inflammatory cell types in response to various stimuli. This agent stimulates aggregation and degranulation of neutrophils, chemotaxis of leukocytes and generation of superoxide. Elevated levels of LTB_4 have been associated with numerous inflammatory diseases including irritable bowel syndrome, bronchial asthma and rheumatoid arthritis. Inhibitors of LTB_4 binding would therefore be useful for treating inflammation associated with these diseases. Suh, H. and coworkers [*Bioorg. Med. Chem. Lett.* (1997) 7, 389–392] have described the synthesis and evaluation of a series of 3-amino-1,2-benzisoxazoles as inhibitors of LTB_4 binding to human neutrophils. HS1141 (**8**) was found to be one of the most potent inhibitors of LTB_4 receptor binding reported (IC_{50} = 7 nM).



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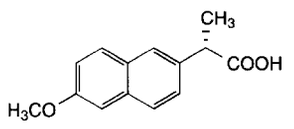
5-lipoxygenase inhibitors

Cycloxygenases, COX-1 and COX-2, and 5-lipoxygenase are involved in the biochemical oxidation of arachidonic acid resulting in the ultimate production of prostaglandins, prostacyclin, thromboxane and leukotrienes. The nonsteroidal anti-inflammatory agents that inhibit the cycloxygenases are well established for the treatment of a wide range of inflammatory conditions. More recently it has been shown that inhibitors of 5-lipoxygenase may be used for the treatment of asthma [Israel, E. *Ann. Allergy* (1994) 72, 279–284]. A group from Abbott Laboratories (Abbott Park, IL, USA) has described the use of nonsteroidal anti-inflammatory cyclooxygenase inhibitors such as ibuprofen, naproxen and indomethacin as orally bioavailable scaffolds



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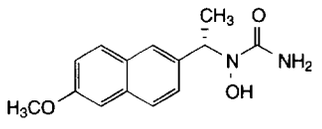

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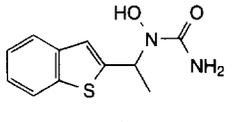

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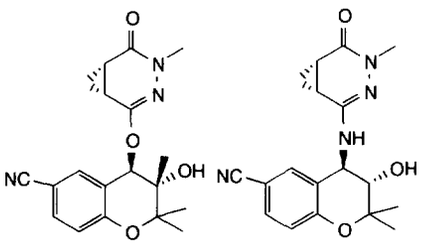
for the design of 5-lipoxygenase inhibitors by replacing the carboxylic acid group with a *N*-hydroxyurea group [Kolasa, T. *et al. J. Med. Chem.* (1997) 40, 819–824]. The effectiveness of this approach was exemplified by the conversion of naproxen **9** into a selective, orally active 5-lipoxygenase inhibitor **10** with comparable biochemical activity to zileuton **11**.

ATP-sensitive potassium channel openers

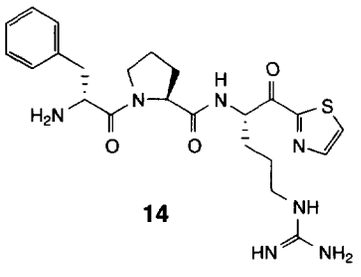
ATP-sensitive potassium channel openers cause peripheral vasodilation and therefore have application as antihypertensive agents. Horino, W. and coworkers [*Bioorg. Med. Chem. Lett.* (1997) 7, 437–442] have described the synthesis and hypotensive activity of *trans*-3,4-dihydro-3-hydroxy-4-[(5-oxo-3,4-diazabicyclo[4.1.0]hept-2-en-2-yl)oxy]-2*H*-1-benzopyrans and their congeners. Potassium channel opening activity, measured in terms of ^{86}Rb efflux, and antihypertensive activity in spontaneous hypertensive rats after oral administration showed **12** and **13** to be potent antihypertensives.



12 **13**

Thrombin inhibitors

The trypsin-like serine protease thrombin has an essential role in the blood coagulation cascade by mediating the conversion of fibrinogen into fibrin and stimulating platelet aggregation. In recent years this enzyme has been targeted in an attempt to produce novel antithrombotic agents. Akiyama, Y. and coworkers [*Bioorg. Med. Chem. Lett.* (1997) 7, 533–538] have reported the synthesis and evaluation of α-ketothiazole derivatives as novel thrombin inhibitors. D-Phe-Pro-Arg-thiazole **14** was shown to be a potent thrombin inhibitor (IC_{50} = 1.5 nM) and to have a greater effect on clotting time than argatroban at a dose of 3 mg/kg in *ex vivo* anticoagulation studies.



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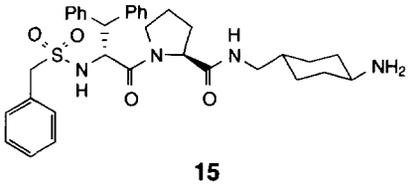
Another recent paper, from a group at Merck Research Laboratories (West Point, PA, USA), reports the discovery of a novel lipophilic binding pocket in the thrombin active site [Tucker, T.J. *et al. J. Med. Chem.* (1997) 40, 830–832]. By optimizing the lipophilic interactions in both the S3 binding pocket of thrombin and the novel binding pocket the group was able to design a novel, extremely potent, selective thrombin inhibitor **15** (K_i = 2.5 pM).



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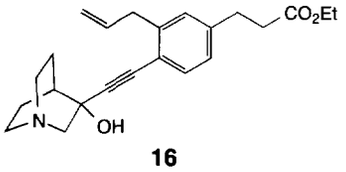
Squalene synthase inhibitors

Inhibitors of squalene synthase may be used to treat hypercholesterolaemic patients and those with existing coronary heart disease by inhibiting cholesterol biosynthesis. A group from Zeneca Phamaceuticals (Macclesfield, UK) recently reported the optimization of quinuclidine squalene synthase inhibitors [Brown, G.R. *et al. Bioorg. Med. Chem. Lett.* (1997) 7, 597–600]. 3-[2-[2-allyl-4-(2-ethoxycarbonylethyl)-phenyl]ethynyl]quinuclidin-3-ol (**16**) was shown to be a potent inhibitor of both rat (K_i = 6 nM) and human (K_i = 43 nM) squalene microsomal synthases. The *R*-enantiomer of this compound and the corresponding carboxylic acid afforded ED_{50} values for the inhibition of rat cholesterol biosynthesis of less than 1 mg/kg on oral administration.



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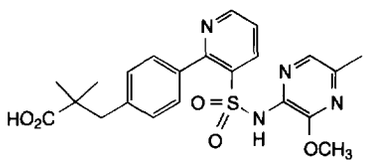
Selective ET_A antagonist

At the *Pharmacology '97* conference held in March in San Diego (CA, USA) Wilson, C. and coworkers from Zeneca (Macclesfield, UK) presented a novel, endothelin A (ET_A)-selective antagonist, ZD1611 (**17**). This compound has a similar potency to that of previously published ET_A-selective antagonists, but an improved duration of action. In cloned human endothelin receptors a pIC_{50} of 8.6 was measured for **17** and the compound displays a 1,000-fold selectivity for the ET_A over the ET_B receptor. The threshold dose for oral activity is 0.3 mg/kg, and at this dose the compound is effective in conscious rats for over 4 h.



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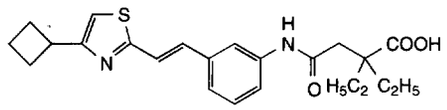
Contributions to *Profiles*

Profiles offers commentary on promising lines of research, new technologies and progress in therapeutic areas. We welcome offers for this series. Articles should provide an accurate summary of the essential facts together with an expert commentary to provide a perspective. Brief outlines of proposed articles should be directed to the *Monitor* Editor (see below). Articles for publication in *Monitor* are subject to peer-review and occasionally may be rejected or, as is more often the case, authors may be asked to revise their contribution. The *Monitor* Editor also reserves the right to edit articles after acceptance.

All suggestions or queries relating to *Monitor* should be directed to: Dr Andrew W. Lloyd, *Monitor* Editor, Department of Pharmacy, University of Brighton, Moulsecoomb, Brighton, UK BN2 4GL. tel: +44 1273 642049, fax: +44 1273 679333, e-mail: a.w.lloyd@brighton.ac.uk.

Combinatorial chemistry

Mathematical description of combinatorial chemistry



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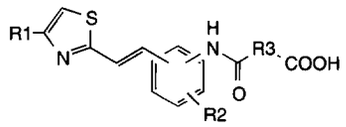

A B C

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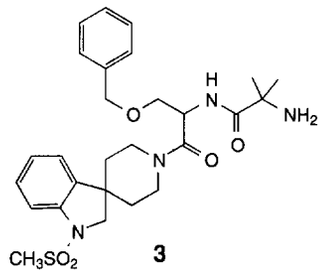
A recent publication has applied mathematical set theory to describe the processes of combinatorial chemistry. Maehr applies mathematical set theory to produce succinct and accurate descriptions of library structure, and then proceeds to use this approach to define methods for the identification of the bioactive component [Maehr, H. *Bioorg. Med. Chem.* (1997) 5, 473–491]. This approach is complex for the non-mathematical chemist, but it allows a ready assessment of the number of synthetic operations required to prepare a library and also permits a determination of the effectiveness of different library approaches.

In an accompanying paper Maehr uses his terminology to describe the construction and analysis of a 700-component, nonpeptide, solution-phase library based on a known leukotriene D_4 antagonist, Ro24-5913 (**1**) [Maehr, H. and Yang, R. *Bioorg. Med. Chem.* (1997) 5, 493–496]. Using an iterative mix and split approach, the library (**2**) was constructed from ten thiazoles (A), seven anilines (B) and ten acyl groups (C). A final set of 42 potentially active compounds was identified and synthesized. Of these, however, the most active was the original lead compound, although replacing the cyclobutyl group with a 4-fluorophenyl or *tert*-butyl group gave compounds with similar bioactivity.

Solid-phase synthesis of spiroindolines

The spiroindoline group is an important pharmacophore capable of interacting with a number of diverse receptors. For example, it occurs in the growth hormone secretagogue, L163191 (**3**). Workers from Merck have now published a solid-phase synthesis of this system [Cheng, Y. and Chapman, K.T. *Tetrahedron Lett.* (1997) 38, 1497–1500]. TentaGel resin was used as the solid-phase support for piperidine-4-carboxaldehyde linked through a succinic anhydride linker (**4**). Reaction with aryl hydrazines using the Fischer indole synthesis was found to proceed under trifluoroacetic acid catalysis, although the use of methylene chloride as solvent was essential for forming the desired products. Reduction of the intermediate indolenine intermediate with sodium triacetoxyborohydride and acylation gave products **5** in good yield and good to excellent purity.



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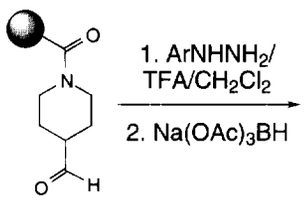

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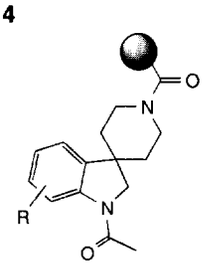

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Emerging molecular targets

Ubiquitin-specific protease and herpes simplex virus type 1 expression

Just like an extremely patient assassin, the herpes simplex virus sits inactive in its host for long periods until the right conditions arise to trigger the production of new viral particles, which then go on to decimate the surrounding cells. Although the specific conditions needed for the emergence of herpes viral particles remain poorly defined, studies of mutant viruses have shown that the herpes simplex virus type 1 immediate-early protein (Vmw 110), an activator of gene expression, is one of the components that controls the transition of the virus from a latent to an active state.

Recent work by Dr Roger D. Everett and coworkers at the Medical Research Council (Glasgow, UK) has now further defined the function of the essential Vmw 110 protein. Using a glutathione-S-transferase fusion protein with the C-terminal 180 residues of the Vmw 110 protein as bait, they found that Vmw 110 protein binds tightly to a 135 kD protein that is a unique ubiquitin-specific protease [*EMBO J.* (1997) 3, 566–576]. The authors believe that the protease is an essential component in the control of viral gene expression. If they are correct, it may also turn out to be an interesting target for the discovery of new chemical compounds that will maintain the herpes virus in its latent state.

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HTS features

The September issue of *Drug Discovery Today* will focus on high-throughput screening, and will include a special report on the development of the new automated system installed at Glaxo Wellcome (UK).

High-throughput screening

Robotic systems for HTS

In the first part of this two-part report on HTS automation the three main systems approaches were outlined [Rogers, M.V. *Drug Discovery Today* (1997) 2, 209]. In this second part, some specific systems now in operation are described.

Several groups have recently reported the development of dedicated and integrated robotic HTS systems utilizing linear-track-based ORCA or CRS robotic arms. Most HTS robots have been designed to run either defined biochemical assays, such as scintillation proximity assays (SPAs), or certain cell-based assays, such as those based on the expression of reporter proteins such as luciferase. Few systems have the versatility to perform a variety of assay types.

In vitro/biochemical assays. McCaffrey, C., Powers, G. and Kelly-Talbot, M. [*J. Biomol. Screening* (1996) 1, 187–190] report the development of an integrated robot to perform SPAs at Hoffman-La Roche (Nutley, NJ, USA). According to the authors, this is the first venture of Roche (USA) into automated HTS. The system was built by Sagian and uses their ORCA arm. It also includes a Hamilton MPH2200 liquid-handling device, a bar code reader, a Beckman Biomek 2000, static plate hotels and a Packard Topcount liquid scintillation counter. The system has obtained a throughput of 2,000 compounds in 8 h.

McRavey, C.S. and coworkers at Bristol-Myers Squibb (Wallingford, CT, USA) have designed and developed a general purpose enzyme-based HTS system that can handle both heterogeneous and homogeneous assay formats. The system comprises a Zymark stationary XP robot and an XP robot on a linear track around which are a Hamilton MPH2200 liquid handling unit, a bulk reagent addition station, a plate washing/filtration unit and two microplate storage carousels, a custom-built reagent cooling station (TomTec), plate shaker and bar code reader. The system also includes Packard's Discovery fluorimeter for homogeneous time-resolved fluorescence [see Kolb, A., Yamanaka, G. and Manly, S. *J. Biomol. Screening* (1996) 1, 203–210]. The system was designed, built and programmed by the Automation and Robotics Group at Bristol-Myers Squibb.

Laskody and coworkers (Glaxo Wellcome, Research Triangle Park, USA) have reported the automation of multiple kinase assays using an ORCA rail system integrated with a Tecan Genesis, Cavro syringe dispensers, Zymark rapid plate and UV/visual and fluorescence detectors. The system can screen 1,000 compounds in a 16 h period. Three assay formats were examined: radioactive, ELISA and Wallac's dissociation-enhanced lanthanide fluoroimmuno assay (DELFLA) technology. The latter assay type gave the best signal-to-noise ratio (400:1).

Cell-based assays are more difficult to automate than *in vitro* ones because they are generally more labour-intensive, requiring more steps or manipulations.

A group from Ligand Pharmaceuticals (San Diego, CA, USA) has reported the development of a fully automated robot to measure the production of luciferase reporter protein in cellular assays [Reichman, M., Marples, E. and Lenz, S. *Laboratory Robotics and Automation* (1996) 8, 267–276]. The system comprises a CRS robotic arm on a linear track with two CRS C500 controllers (master and slave), a CRS reagent addition station, a Cambridge model 7715 luminometer, a Symbol Technologies bar code scanner, a Biotek EL304 plate washer and three rotating plate carousels. Special features of the system include a modified Robbins Hydra 96-well pipettor with a custom three-position plate shuttle. This allows direct addition of compounds in DMSO to assay plates. There is also a Hot Pack (3103D) tissue culture incubator on the system that has been modified to maintain a stable environment and has 16 pneumatically activated doors for movement of plates by the robot arm. The system has a 120-plate capacity at any one time and a throughput of approximately 4,600 compounds in a 14 h run.

Amgen (Boulder, CO and Thousand Oaks, CA, USA) have developed a versatile system for large-scale cell-based screening for novel therapeutic proteins. They have also developed a second, smaller system for small-molecule screening [Hamilton, S.D. *et al. Laboratory Robotics and Automation* (1996) 8, 287–294]. Both screening systems have ORCA arms on linear tracks. The larger of the two systems has a Sagian tissue culture incubator, a Zymark rapid plate for liquid handling, two Zymark microplate carousels for plate incubation and storage, and a Perspective Biosystems Cytofluor II microplate reader, a Molecular Devices SpectraMax plate reader and pipette storage devices manufactured by Lawrence Berkeley National Laboratories. The smaller system has some of the same equipment, but has been designed to prepare supernatants from small-molecule stimulated cells for ELISA (on another robot) or fluorescence analysis. Both systems are controlled by SAMI scheduler software.

A group from SIBIA Neurosciences (La Jolla, CA, USA) have developed an integrated, fully automated HTS system for the biochemical analysis of fluorescence in mammalian cells loaded with the Ca^{2+} chelator dyes, Fura 2 and Fluo 3. SIBIA have concentrated on cell-based assays where agonist and antagonist read-outs are possible in the same assay, eliminating the need for *in vitro* binding assays. The robotic screening system constructed has performed screens on cells stably expressing voltage-gated ion channels, nicotinic acetylcholine receptors or excitatory amino acid receptors, including NMDA and metabotropic glutamate receptors. The system comprises a Zymark rotational robot arm with a coupled charged device camera for image analysis of 96-well plates illuminated from below. This measuring device needs 60–90 s for real time measurement of Ca^{2+} fluxes in cells.

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Next month in *Update*...
Martin Leach will report on Orchid Biocomputer's credit-card sized drug discovery system

