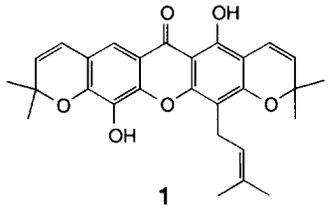
Monitor: molecules and profiles

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.

Novel pyranoxanthones

Following a study of sixteen xanthones and two coumarin derivatives isolated from the stem bark and root of *Calophyllum apetalum* (Guttiferae), Iinuma, M. and coworkers [*Heterocycles* (1997) 45, 299–307] have reported the identification of two new linear pyranoxanthones [caxoxanthone I (**1**) and caloxanthone J (**2**)] from the stem bark and two xanthones [caxoxanthone K (**3**) and 1,3,6,8-trihydroxy-2-methoxyxanthone (**4**)] from the roots. The biological activities of these compounds have yet to be investigated.



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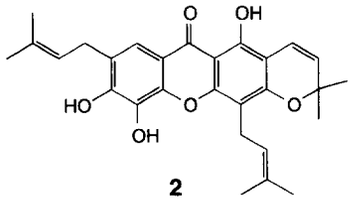

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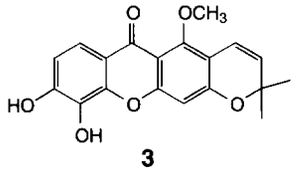

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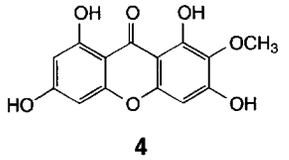

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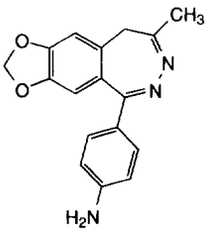
Novel AMPA receptor antagonists

2-Amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA) excitatory amino acid receptor antagonists may have use in the treatment of epilepsy, cerebral ischaemia and various neurodegenerative disorders. Previous studies have shown that 1-(4-aminophenyl)-4-methyl-7,8-(methylenedioxy)-5*H*-2,3-benzodiazepine (**5**, GYKI 52466) does not bind to the benzodiazepine receptors and therefore lacks the sedative-hypnotic effects associated with other 1,4-benzodiazepines. This compound is, however, a highly selective AMPA/kainate receptor antagonist and has anticonvulsant and muscle relaxant activity. Recently, 2,3-benzodiazepine analogues of this compound were shown to be effective anticonvulsants [De Sarro, G. *et al. Eur. J. Pharmacol.* (1995) 294, 411–422].

This group have now extended this work to the synthesis and anticonvulsant activities of a series of new 1-aryl-3,5-dihydro-4*H*-2,3-benzodiazepin-4-ones in an attempt to identify further compounds as potent, selective AMPA receptor antagonists [Chimirri, A. *et al. J. Med. Chem.* (1997) 40, 1258–1269]. On evaluation of anticonvulsant activity following seizure stimulation in DBA/2 mice and by pentylenetetrazole or maximal electroshock in Swiss mice, compounds **6** and **7** were found to be the most active of the series and were more potent, longer lasting and less toxic than **5**. Ligand binding studies demonstrated that these compounds do not bind to the benzodiazepine, NMDA or metabotropic glutamate receptors.



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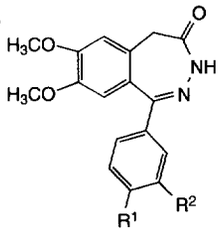

6 R^1=NH_2, R^2=H
7 R^1=H, R^2=NH_2

Growth hormone secretagogues

Growth hormone therapy is used clinically for the treatment of growth hormone deficiency and may be beneficial in various other cases including preventing osteoporosis, promoting wound healing and reducing the effects of ageing. Growth homone secretagogues offer the advantage over bolus growth hormone therapy in allowing a more natural pulsatile release of growth

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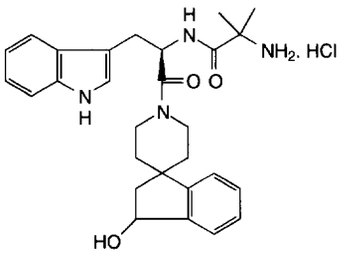
  1359-6446/97/$17.00. PII: S1359-6446(97)01056-8

hormone. A group from Merck Research Laboratories (Rahway, NJ, USA) have reported the synthesis and evaluation of a series of modified spiroindane growth factor secretagogues [Tata, J.R. *et al. Bioorg. Med. Chem. Lett.* (1997) 663–668]. These studies demonstrated that the incorporation of polar functionalities into the benzylic position of the spiroindane secretagogues, exemplified by **8**, yields peptidomimetic growth hormone secretagogues with subnanomolar activity. These compounds also show enhanced intravenous and oral activity in the dog model compared with the parent spiroindane molecules.



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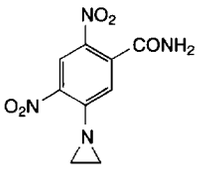
Mustard prodrugs

Gene-directed enzyme prodrug therapy (GDEPT), in which a gene encoding a foreign enzyme that can activate a non-cytotoxic prodrug, is expressed within the target cells, is an attractive concept for improving the selectivity of cancer chemotherapy because it would activate an antitumour agent at the required site of action only. Friedlos, F. and coworkers [*J. Med. Chem.* (1997) 40, 1270–1275] have described the evaluation of twenty nitrogen mustard analogues derived from 5-(aziridin-1-yl)-2,4-dinitrobenzamide (**9**, CB1954) as potential prodrugs for GDEPT in Chinese hamster V79 cell lines genetically engineered to express *Escherichia coli* nitroreductase. The compounds were assessed for toxicity (IC_{50}) and for the ability to cause cytotoxicity against non-enzyme expressing cells in a culture with enzyme expressing cells (TE_{50}). Four compounds were found to be more potent than **9** with higher IC_{50} values and lower TE_{50}s. The most effective of these compounds (**10**) only required 0.1% of the cells in the culture to be expressing the *E. coli* nitroreductase to cause 50% cytotoxicity against the nonexpressing cells.



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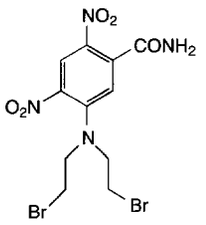

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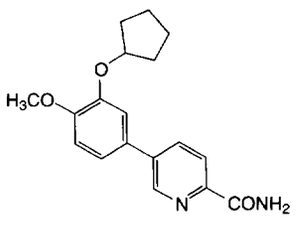
Phosphodiesterase 4 inhibitor

The clinical efficacy of chimeric monoclonal tumour necrosis factor (TNF-α) antibody in the treatment of rheumatoid arthritis has recently confirmed the role of this inflammatory cytokine as a key mediator in the progression of this disease [Elliott, M.J. *et al. Lancet* (1994) 344, 1125–1127]. Phosphodiesterase 4 (PDE 4) inhibitors have been shown to inhibit the production of TNF-α by increasing intracellular cyclic adenosine monophosphate (cAMP). The synthesis and evaluation of a series of biarylcarboxamides by workers from Pfizer (Groton, CT, USA) has led to the identification of **11** (CP353164) as a potent inhibitor of PDE 4 and TNF-α production.



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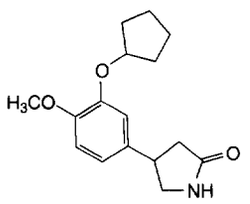

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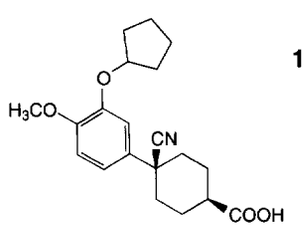

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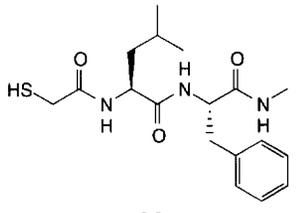
This compound was found to be more effective at blocking the release of TNF-α *in vitro* using isolated human monocytes and human whole blood, and *in vivo* in a murine TNF-α production model, than the existing PDE 4 inhibitors, rolipram (**12**) and SB207499 (**13**). Compound **11** may therefore be useful for the treatment of rheumatoid arthritis.

Matrix metalloproteinase inhibitors

The matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases that have an important role in the degradation and remodelling of extracellular matrix. The overexpression of MMPs in a variety of disease states has driven the search for MMP inhibitors to treat inflammatory disorders such as rheumatoid arthritis, ulcerative colitis, psoriasis and multiple sclerosis. Many of the existing MMP inhibitors utilize a hydroxamic acid as the zinc binding moiety, and they often have poor oral bioavailability and physicochemical properties. Other zinc binding ligands such as phosphinates, aminocarboxylates and thiols have generally been found to be less potent. Researchers from Chiroscience (Cambridge, UK) have described the synthesis and evaluation of a series of novel MMP inhibitors, exemplified by **14**, that utilize a mercaptoacyl group as the zinc binding ligand [Baxter, A.D. *et al. Bioorg. Med. Chem. Lett.* (1997) 7, 897–902]. Compound **14** was shown to be a modest, broad-spectrum inhibitor of the MMP enzymes *in vitro*. Although the potency of **14** was reduced compared with the corresponding hydroxamic acid, the compound was found to be orally active in an adjuvant arthritic rat model of rheumatoid arthritis. Further work is ongoing in order to



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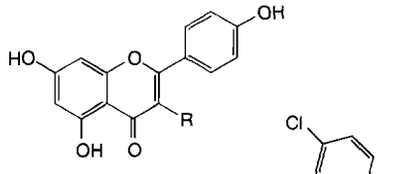
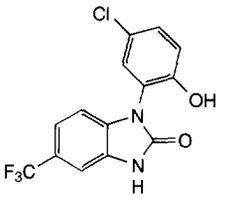
improve the potency and oral activity of this class of compounds.

Calcium channel openers

Calcium-dependent, large conductance potassium channels are believed to play an important role in cellular excitation and function in a variety of cell types including neurones and muscles. Specific channel opening modulators are potential therapeutic targets for a number of disease states. Li, Y. and coworkers [*Bioorg. Med. Chem. Lett.* (1997) 7, 759–762] have reported the identification of two flavonoids (**15**, **16**) following a three-dimensional database search based on the pharmacophore model of the known calcium-dependent, large conductance potassium channel opener NS004 (**17**) and a subsequent electrophysiological evaluation of candidate molecules using the cloned calcium-dependent, large conductance potassium channel *m*Slo expressed in *Xenopus laevis* oocytes.



15 R = H
16 R = OH



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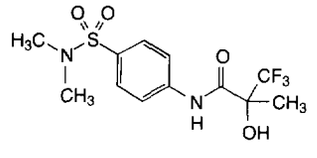
In another recent article, workers from Zeneca Pharmaceuticals (Wilmington, DE, USA) have described the synthesis and evaluation of 4-sulphonamidoanilide tertiary carbinols as a novel series of ATP-dependent potassium channel openers [Empfield, J.R. *et al. Bioorg. Med. Chem. Lett.* (1997) 7, 775–778]. Compound **18** was found to be the most active of these compounds (IC_{50} = 0.48 μM).



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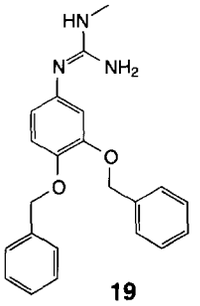
Nonpeptidic C5a receptor antagonists

Complement component C5a is generated on activation of the complement system and stimulates the migration of leukocytes to sites of inflammation. It also causes degranulation and release of respiratory burst products from neutrophils, and stimulates the expression of adhesion molecules on leukocytes and endothelial cells. C5a antagonists are therefore a potential therapeutic target for the treatment of pathological conditions involving the recruitment and activation of leukocytes. Although a number of peptide C5a antagonists have been identified, only a limited number of nonpeptidic agents have been reported. A group from Rhône-Poulenc Rorer (Dagenham, UK) recently described the identification of a series of phenylguanidine-based compounds that bind to the C5a receptor [Astles, P.C. *et al. Bioorg. Med. Chem. Lett.* (1997) 7, 913–918]. The lead compound **19** (IC_{50} = 30 μM) was identified through a random screening programme. The lead compound was systematically modified to yield the competitive antagonist **20** with submicromolar activity, which was shown to be a functional antagonist of C5a using the respiratory burst assay.



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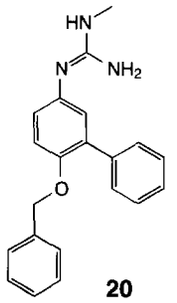

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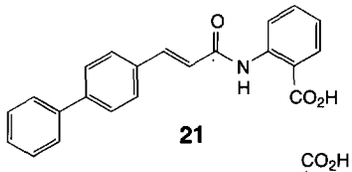
Leukotriene B_4 receptor antagonists

Leukotriene B_4 is another inflammatory mediator that stimulates neutrophil recruitment and activation, and it has been implicated in a broad spectrum of inflammatory diseases including rheumatoid arthritis, psoriasis, asthma and inflammatory bowel disease. In an attempt to identify potential antagonists that may be useful therapeutic agents, a group at Novartis Pharmaceuticals [Greenspan, P.D. *et al. Bioorg. Med. Chem. Lett.* (1997) 7, 949–954] has prepared a series of *N*-(carboxyaryl)-phenylcinnamides based on the lead compound **21**. Lead optimization afforded **22**, which demonstrated potent *in vitro* activity in a whole-cell binding assay (IC_{50} = 15.9 nM) and a neutrophil-aggregation assay (IC_{50} = 42 nM).



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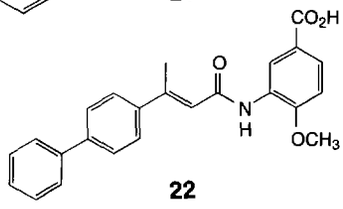

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News on HTS deals

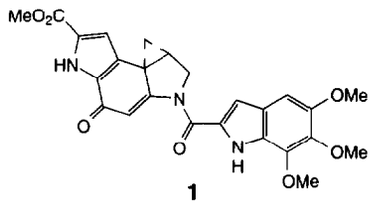
Bristol-Myers Squibb and **Cubist** have reached the point in their collaboration (research and licensing agreement valued at $56 million) where the second and third milestone payments have been made under their agreement to target pathogen-specific aminoacyl-tRNA syntheses required for protein translation. Cubist is developing and transferring technology to Bristol-Myers Squibb to conduct high-throughput screening for the identification of novel lead compounds.

Trophix Pharmaceuticals, a US-based neurotherapeutics company, announced (*Scrip* 12 March 1997) that from their high-throughput screening programme they have identified and are developing two novel chemical compound classes (termed GRI-1 and GRI-2). Both classes of compound are glycine re-uptake inhibitors, GRI-1 being brain-selective with potential for the treatment of schizophrenia, whereas GRI-2 is spinal cord-selective and will be developed as a therapy for spasticity. Phase I trials with the GR-2 compound will start in the first quarter of 1998. GR-2 will be developed and marketed in collaboration with a pharmaceutical company yet to be announced.

Mark V. Rogers

Mechanism of DNA binding of duocarmycins and CC1065

Although the potent antitumour antibiotics duocarmycin SA (**1**), duocarmycin A (**2**) and CC1065 (**3**) are known to act through the sequence-selective alkylation of duplex DNA, the structural characteristics defining the basis of the selectivity and mechanism of the DNA alkylation have been the subject of contention. Boger, D.L. and coworkers from The Scripps Research Institute (La Jolla, CA, USA) have reported some elegant chemistry in two back-to-back articles that both unambiguously establishes the origin of the DNA alkylation selectivity of CC1065 and the duocarmycins, and identifies a previously unrealized source of catalysis for the DNA alkylation reaction [*J. Am. Chem. Soc.*, in press]. Analogues of the natural enantiomers of duocarmycin SA, in which the orientation of the DNA binding subunits was reversed, were found to alkylate DNA with the selectivity typically observed for the unnatural enantiomers and vice versa. This is only consistent with the non-covalent binding model and incompatible with the alkylation site models of the origin of the DNA alkylation selectivity. Using this result as a basis for further design, the group was able to identify a set of enantiomeric agents that alkylate the same sites regardless of their absolute configuration.



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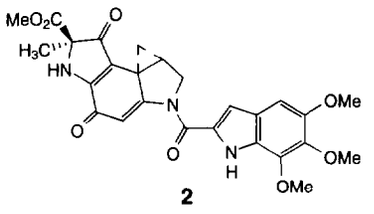

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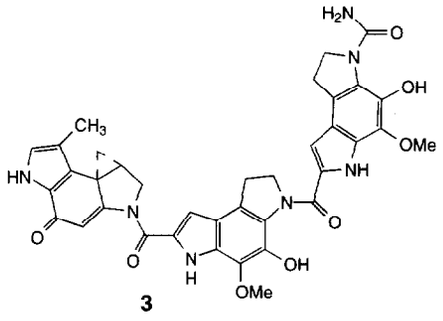

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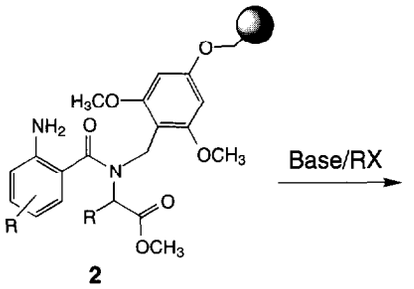
The group also demonstrated that an extended rigid N^2 amide substituent is required for the DNA alkylation reaction and identified a true source of catalysis. The catalysis is a conformational change in the bound agents, induced by DNA binding, that disrupts the stabilization the vinylogous amide provides to the alkylation subunit, and activates the agents for nucleophilic attack. The authors have proposed the concept of 'shape-selective catalysis' in which the narrower, deeper AT-rich minor groove induces the greatest conformational change in the agent, preferentially activating it for nucleophilic attack. Thus, the 'shape-selective recognition' (preferential AT-rich noncovalent minor groove binding) and 'shape-dependent catalysis' (AT-rich > GC-rich minor groove induced twist in N^2 amide) combine to restrict the S_N2 nucelophilic addition to accessible N3 sites.

Combinatorial chemistry

Solid-phase synthesis of benzodiazepinediones

The 1,4-benzodiazepine-2,5-dione (**1**) is a privileged structure that occurs in a number of pharmacologically significant molecules. Analogues have shown utility as antithrombotics, antitumour agents and antibiotics, and have potential applications as ethanol-intoxication antagonists. Ellman's group has now published a description of a solid-phase library synthesis of these compounds [Boojamra, C.G. *et al. J. Org. Chem.* (1997) 62, 1240–1256].

The library was designed to be constructed from several different building blocks, using commercially available monomers where possible, and using synthetic transformations that are compatible with a range of functionalities. In fact, the library employed 11 alkylating agents, 12 anthranilic acids and 19 α-amino acids (nine sets of enantiomeric pairs and glycine) to give a total of 2,508 compounds.



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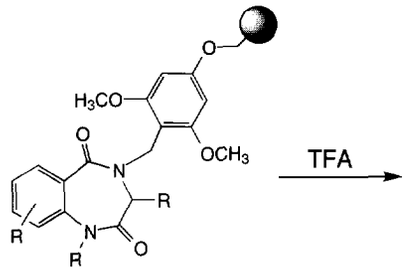

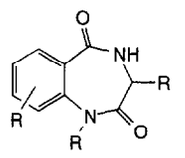

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The synthesis was initially attempted on Multipins, but more consistent results were obtained by loading the precursors onto Merrifield chloromethyl resin beads, and using a simple 96-well based parallel synthesis apparatus. A resin-bound aldehyde was reductively aminated with an amino acid and then acylated with an anthranilic acid to give intermediate **2**. Cyclization was effected using conditions basic enough to provide an anilide ion for subsequent alkylation. The final benzodiazepinediones were cleaved from the resin with trifluoroacetic acid, and the integrity of the compounds ascertained using HPLC on all samples and proton NMR of 36 randomly chosen compounds.

Interleukin 1β converting enzyme substrate specificities

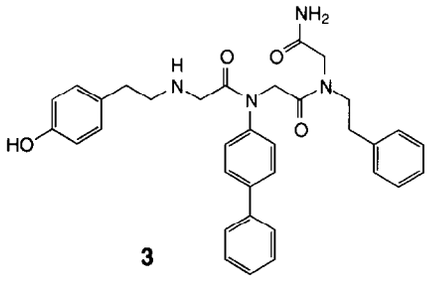
Interleukin 1β converting enzyme (ICE) is the protease responsible for the generation of interleukin 1β in monocytes; inhibitors of this enzyme may have utility for the treatment of inflammatory diseases. Using a combinatorial library approach it has been possible to determine the substrate amino acid preferences of the enzyme [Rano, T.A. *et al. Chem. Biol.* (1997) 4, 149–155].

A positional scanning peptide library was constructed and it was ascertained that the preferred substrate is WEHD and not YVAD, the sequence found in human pro-interleukin 1β and previously believed to be the optimal sequence. Knowledge of this amino acid sequence has led to the design of potent aldehyde-based inhibitors including Ac-WEHD-CHO, which has an IC_{50} of 56 pM. X-ray studies have revealed how this binding potency was achieved.

Spatially arrayed mixture technology

New methods for the construction of combinatorial libraries continue to emerge. A recent development is an approach entitled SpAM (Spatially Arrayed Mixture) technology [Berk, S.C. and Chapman, K.T. *Bioorg. Med. Chem. Lett.* (1997) 7, 837–842]. This approach is a variant of the indexed library method and depends on the same compounds being synthesized in two differently arranged sets of mixtures. These mixtures are constructed so that only one compound is common to any particular pair of mixtures, and biological activity observed in two mixtures will automatically indicate which active compound is present.

A library of 9,216 peptoids [tri(N-substituted glycines)] was prepared using the SpAM approach and tested against the α_{1a} receptor. The library was designed to contain a known active receptor ligand and this compound (**3**) was duly identified following screening.



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Emerging molecular targets

c-fos-Mediated apoptosis of retinal photoreceptor cells

Retinitis pigmentosa is a genetic disease characterized by a gradual atrophy of the retina with a concomitant contraction of the field of vision. In animal models it has been shown that the loss of retinal photoreceptor cells associated with the disease occurs by apoptosis, or programmed cell death. The proto-oncogene *c-fos* is known to trigger apoptosis in various cell types, and it is induced in retina cells upon exposure to light. Farhad Hafezi and coworkers at the University Clinic (Zürich, Switzerland) and the Institute of Molecular Pathology (Vienna, Austria) have now investigated the connection between the induced *c-fos* and apoptosis of retinal cells in retinitis pigmentosa [*Nat. Med.* (1997) 3, 346–349].

Using a mouse model, the investigators found that mice with the *c-fos* gene began to undergo apoptosis of retina cells after 6 hours of exposure to bright light and had extensive damage after 12 and 24 hours of exposure. Genetically altered mice that did not have the *c-fos* gene, however, showed no apoptosis of retina cells even after 24 hours of continuous exposure to light. Moreover, they showed that *c-fos* is up-regulated at the time of cell death. They concluded that the product of the *c-fos* gene is a constituent of the signaling pathway leading to the degeneration of retinal cells in retinitis pigmentosa, and that targeting the *c-fos* gene with drugs to block its expression in retinal cells may be an effective therapy for the disease.

New protease-activated thrombin receptor

A thrombin receptor present on platelets and other cell types, the 7-transmembrane, G-protein-coupled PAR1 receptor, has been known for some time and is well characterized. But the suspicion of many investigators in the field is that other receptors for thrombin must exist to account for its diverse thrombotic, inflammatory and proliferation activities. One candidate is the PAR2 receptor, the gene for which was discovered in a mouse DNA library [Nystedt, S. *et al., Proc. Natl. Acad. Sci. U. S. A.* (1994) 91, 9208–9812]. Like PAR1, PAR2 is also a 7-transmembrane receptor that is cleaved by trypsin, a proteolytic event that is believed to be part of a unique activation mechanism for thrombin receptors. PAR2 is expressed in highly vascularized tissues such as kidney, small intestine and the stomach.

Now, a third protease-activated thrombin receptor (PAR3) has been cloned by Hiroaki Ishihara and coworkers from the University of California (San Francisco, CA, USA). The amino acid sequence of PAR3 reveals that it is also a 7-transmembrane, G-protein-coupled receptor. It has a sequence homology of 27% to PAR1 and of 28% to PAR2. The characteristic thrombin cleavage site is also present in PAR3, and similar to its PAR1 and PAR2 cousins, it is proteolyzed when expressed on Cos 7 cells and exposed to thrombin. The newly discovered receptor is expressed in high levels in megakaryocytes, spleen and bone marrow cells in the mouse, where it appears to be the major thrombin receptor on platelets; PAR1 is the major thrombin receptor on human platelets. Functional studies in Cos 7 cells transfected with the PAR3 cDNA revealed the coupling of the receptor to phosphoinositide hydrolysis, which led to calcium ion mobilization within the Cos 7 cells upon thrombin stimulation [*Nature* (1997) 386, 502–505].

These two new receptors for thrombin provide an opportunity for the discovery of therapeutic agents that will differentially regulate the many physiological functions of this important extracellular signaling agent.

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HTS features

The September issue of *Drug Discovery Today* will focus on high-throughput screening, and will include reviews on time-resolved fluorimetry, adapting biochemical and cell-based assays for HTS, and a special report on the development of the new automated system installed at Glaxo Wellcome (UK).

High-throughput screening

Miniaturization of high-throughput screening (HTS)

With ever increasing diversity of compound libraries and a requirement to increase throughputs of both compounds and targets, there is a need to develop assays for high-throughput screening in a format that both reduces costs and maximizes throughput.

Significant savings in assay reagents and compound stocks can be achieved by assay miniaturization. The simplest way to do this is to increase the well density of the standard 96-well plate screening format (see Table 1). Many screening programmes are experimenting with assays in the 384-well plate format, although little has been published on this to date. Equipment manufacturers are developing or already marketing machines for liquid handling and for measurement of assays in the 384-well plate format. Several manufacturers supply 384-well plates that are acceptable for HTS. The use of the 384-well plate, coupled with automation, significantly improves the speed and ease of performing HTS screens. It is, therefore, only a matter of time before screening programmes adopt the 384-well plate as the standard screening format.

What is more difficult to predict is whether the 864-well plate will gain acceptance as the next step in a sequential progression to high-density formats (i.e. 96 → 384 → 864 (or 768) → 1536 → ultra-HTS), or whether a direct move to ultra-HTS can proceed without intermediate formats. Dr John Comley (Lead Discovery Unit, Glaxo Wellcome, UK) believes that HTS in 864-well format (volume range: 5–20 µl) represents a realistic and pragmatic 'evolution' from the 96- and 384-well formats, which can be done now with only small modifications to existing technology, whereas higher densities such as 1536 and beyond require 'revolutionary' developments in liquid-handling, automation and reading equipment before these formats will be useful for HTS.

Companies such as Pharmacopeia (Princeton, NJ, USA) are developing higher-density array screening systems, believed to involve 1536-well plates (see Table 1). Alternatively, rapid technological developments may preclude the need to go to further intermediate steps before introducing nano-plate ultra-HTS systems such as those being developed by EVOTEC Biosystems (Hamburg, Germany) and Aurora Biosciences (San Diego, CA, USA).

Alternative technologies are also being developed by other companies. For example, Orchid Biocomputer (Princeton, NJ, USA) is developing 'an advanced system for the massively parallel synthesis and screening of drug leads that will perform conventional chemical reactions in thousands of cells simultaneously, with each cell operating independently'. Their system consists of a multilayered planar structure in which microfabricated components for valving and pumping of liquids are integrated (see page 253 of this issue). Orchid point out that 'a key aspect of the approach is the ability to pump reagents without the need for moving parts, thus enhancing device reliability and ease of chip fabrication'. Another company, ChromaXome Corporation (San Diego, CA, USA), is developing an alternative screening format called 'Virtu-Well' for cell-based assays. This technology allows each biological sample to be encased in a resilient 1–3 mm matrix that remains permeable to both liquids and compounds, but not to cells. Further detail on 'Virtu-Well' technology is available on the network science web page (http://www.awod.com/netsci/Science/Screening/feature04.html).

A change from the 96-well plate format will present a major challenge to screening programmes and will require modification of the entire screening process. Data capture and handling will also need to be improved, and new assay technologies developed to allow assay miniaturization without decreased performance and reliability. Miniaturization of HTS coupled with automation has the potential to significantly improve cost-effectiveness and productivity of HTS programmes and will continue to be a major driving force in this area for some time to come.

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Table 1. Summary of microplate formats within the 96-well plate footprint

Plate density	96-well	384-well	864-well	1536-well
Array format	8 x 12	16 x 24	24 x 36	32 x 48
Increase over 96-well plate	–	Fourfold	Ninefold	16-fold
Centre to centre spacing (pitch)	9 mm	4.5 mm	3 mm	2.25 mm
Well diameter	7 mm	4 mm	2 mm	1.25 mm (?)
Assay volume (range)[a]	300–50 µl	100–20 µl	20–5 µl	2.5–0.5 µl
Potential reagent savings	–	Fourfold	20-fold	At least 40-fold
Availability of different plate types	Numerous	Increasing	1 (Helix)[b]	In development
Average cost/plate	< $1.00	$2.00–$4.00	$5.00–$7.00	?

[a] Excludes non-standard arrays (e.g. 192-wells), deepwell plates and Nanoplates (taken from Comley, J. *et al.*, unpublished).
[b] Helix 864-well plates are available through Vorhies Technologies (Ramona, CA, USA).