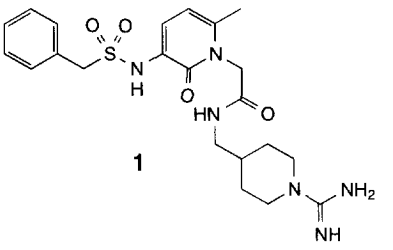
Monitor: molecules and profiles

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.

Thrombin inhibitors

The serine protease thrombin is the primary enzymatic mediator in the blood coagulation process in response to vascular injury. The enzyme catalyses the conversion of fibrinogen to fibrin and is a potent activator of platelets and other coagulation factors. Inhibitors of this enzyme have potential uses as anticoagulants in the treatment of such conditions as pulmonary embolism, thrombolysis and deep vein thrombosis. A number of previous studies have shown that the glycylproline amide backbone of peptide inhibitors of human leukocyte elastase, interleukin 1β converting enzyme and thrombin inhibitors may be effectively replaced by a pyridinone acetamide template. Using this approach and a combination of X-ray crystallography, molecular modelling and empirical structure optimization, a group from Merck Research Laboratories (West Point, PA, USA) have reported the first achiral, noncovalent, subnanomolar [K_i (thrombin) = 0.5 nM], peptidomimetic thrombin inhibitor, L373890 (**1**) [Sanderson, P.E. *et al. Bioorg. Med. Chem. Lett.* (1997) 7, 1497–1500]. This compound was found to show good selectivity for thrombin over trypsin [K_i (trypsin) = 570 nM] and was inactive against the serine proteases plasmin, tissue-type plasminogen activator, activated protein C, plasma kallikrein and chymotrypsin. In the *in vivo* rat ferric chloride model of arterial thrombosis, occlusion was prevented in all test animals at an intravenous infusion rate of 10 μg/kg/min.



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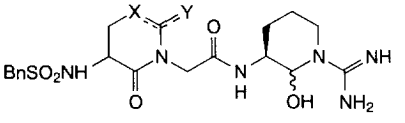

2 X = CH, Y = H
3 X = CH, Y = CH_3
4 X = NCH_3, Y = O
5 X = N, Y = CH_3

In another recent report, workers from Corvas International (San Diego, CA, USA) have described a similar approach in which a range of novel heterocyclic peptide surrogates based on pyridones, uracils and pyrimidinones, have been used as P3-P2 mimics [Tamura, S.Y. *et al. Bioorg. Med. Chem. Lett.* (1997) 7, 1543–1548]. These compounds (**2–5**) were shown to be potent, selective thrombin inhibitors (Table 1). Compound **2** was shown to have an ED_{50} of 2.9 mg/kg on oral administration in a rat arteriovenous shunt model of thrombosis and an absolute oral bioavailability of 31% in conscious dogs. A third recent publication, from Dupont Merck (Wilmington, DE, USA), has described a series of novel biaryl-substituted alkylboronate esters as potent thrombin inhibitors [Quan, M.L. *et al. Bioorg. Med. Chem. Lett.* (1997) 7, 1595–1600]. These compounds were designed to reduce the peptidic nature of the highly effective thrombin inhibitor DuP714 (**6**). The most potent compound **7** had a K_i (thrombin) of 0.21 nM and K_i (trypsin) of 0.7 nM compared with a K_i (thrombin) of 0.04 nM and K_i (trypsin) of 0.045 obtained for the parent compound.

Table 1. Selective thrombin inhibitors

Compound	IC_{50} (thrombin) (nM)	IC_{50} (trypsin) (nM)
2	0.505	26.2
3	0.467	10.9
4	141	396
5	2.32	160

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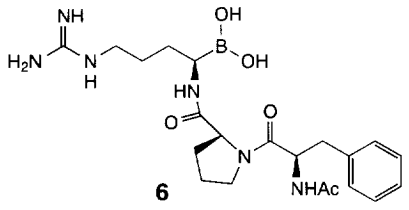

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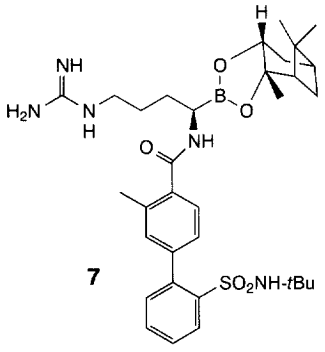

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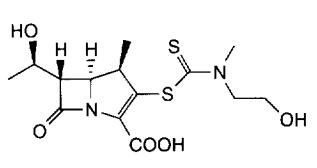
Anti-MRSA agents

The escalation of incidences of methicillin-resistant *Staphylococcus aureus* (MRSA) infections in hospitals is presently causing serious concern because there are still few anti-MRSA agents that are clinically effective. Although vancomycin has potent anti-MRSA activity, its clinical use is restricted by its side effects. A group from Banya Pharmaceutical Company (Okubo, Tsukuba, Japan) have recently reported the synthesis and evaluation of a new series of carbapenems in which a disubstituted dithiocarbamate moiety is directly attached to the C-2 position [Ohtake, N. *et. al. Bioorg. Med. Chem. Lett.* (1997) 7, 1617-1622]. This series of compounds was found to have potent *in vitro* activity against high-level MRSA. Compounds **8**



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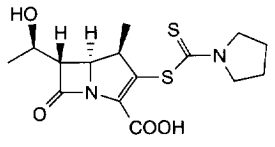

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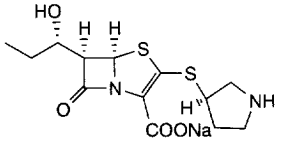
and **9** were also shown to be effective against high-level MRSA in the *in vivo* mouse septicaemia model.

Of the known mechanisms of resistance of bacteria to antibiotics the modification of the active site of β-lactamases – leading to more efficient degradation of β-lactams and the active site – and penicillin-binding proteins – reducing the affinity of the target site to β-lactams – are probably of greatest concern. Ishiguro, M. and coworkers [*J. Med. Chem.* (1997) 7, 2126–2132] have described the synthesis and evaluation of a series of 5,6-*cis*-penem derivatives designed to have high affinity for the penicillin-binding protein 2a of MRSA and to form stable acyl intermediates with β-lactamases thereby blocking the action of this enzyme. The *cis*-penems **10** and **11** were shown to be particularly potent anti-MRSA antibiotics with broad spectrum activity against a wide variety of β-lactamase producing microorganisms.



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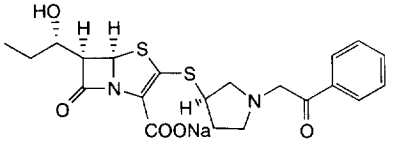

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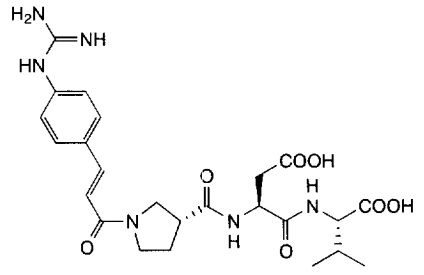
GPIIb–IIIa antagonists

Platelet aggregation is dependent on the binding of fibrinogen to the platelet membrane-bound receptor glycoprotein IIb–IIIa (GPIIb–IIIa). As GPIIb–IIIa is expressed in response to a variety of stimuli, agents that block this receptor may have use in the treatment of various thrombotic disorders. Although the binding of fibrinogen to this receptor is primarily mediated by the γ-chain C-terminal dodecapeptide, small synthetic peptides containing the tripeptide integrin receptor recognition sequence Arg-Gly-Asp (RGD), found in the α-chain, have been shown to block the GPIIb–IIIa receptor and thereby inhibit platelet aggregation. A number of nonpeptide GPIIb–IIIa receptor antagonists contain rigid central constraints to hold the key structural functional groups in their correct spatial orientation. Klein, S.I. and coworkers [*Bioorg. Med. Chem. Lett.* (1997) 7, 1773–1778] have extended this approach to the synthesis of a novel series of GPIIb–IIIa receptor antagonists, based on the peptidic fibrinogen receptor antagonists, which include the natural Asp-Val terminal dipeptide. The most potent of these compounds was **12**, which showed potent inhibition of both platelet aggregation (IC_{50} = 65 nM) and fibrinogen binding (IC_{50} = 3 nM).



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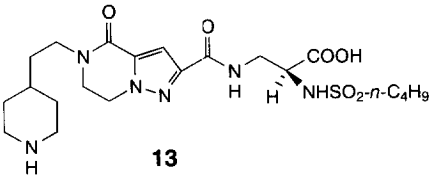
In a recent paper, workers from the Merck Research Laboratories (West Point, PA, USA) reported the synthesis and antiplatelet activity of a series of pyrazolopiperazinone nonpeptide fibrinogen receptor antagonists [Askew, B.C. *et al. Bioorg. Med. Chem. Lett.* (1997) 7, 1531–1536]. The sulphonamide analogue **13** (L734115) showed potent *in vitro* inhibition of platelet aggregation (IC_{50} = 9 nM) and significantly inhibited *ex vivo* platelet aggregation 24 h after oral administration to dogs and rhesus monkeys.

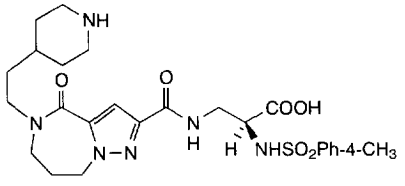
The specificity of GPIIb–IIIa antagonists over other RGB recognition integrins is regarded as particularly important in the development of oral anti-platelet



13

agents. A comparison of the ability of **13** to inhibit platelet aggregation with its ability to reduce the binding of human umbilical vein endothelial cells to surfaces precoated with fibrinogen, vitronectin and fibronectin indicated that **13** had greater than 33,000-fold selectivity for the GPIIb–IIIa receptor.

Another recent paper by this same group [Askew, B.C. *et al. J. Med. Chem.* (1997) 40, 1779–1788] describes an extension of this work that has led to the identification of the pyrazolodiazepinone **14** (L738167) as another potent, selective nonpeptide fibrinogen receptor antagonist. This compound was found to have similar *in vitro* selectivity and potency as **13** but displayed an extended pharmacodynamic profile following intravenous or oral administration to dogs. This pharmacodynamic profile appears to be a consequence of the high-affinity binding of **14** to the GPIIb–IIIa receptor on circulating platelets. The authors suggest that this compound may therefore be suitable for once-a-day dosing.



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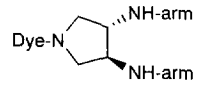
Combinatorial chemistry

Water-soluble synthetic receptors

Over the last few years, Still's group at Columbia University has demonstrated the considerable power of combinatorial chemistry to aid exploration of supramolecular chemistry. They have demonstrated the discovery of optimized ligands for synthetic receptors and the isolation of optimized receptors for specific peptide sequences. The latest paper from this group [Torneiro, M. and Still, W.C. *Tetrahedron* (1997) 53, 8739–8750] describes the design and synthesis of new water-soluble synthetic receptors (**1**,**2**). These compounds, made soluble by the introduction of



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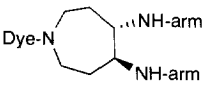

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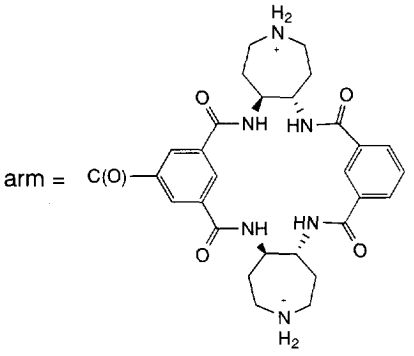


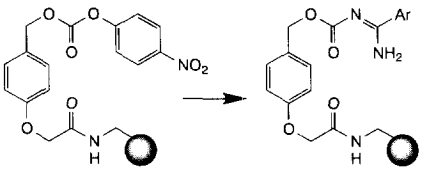
azepinoid groups, are tagged with a dye to aid the library-screening step.

When the synthetic receptors were incubated in water at 5 μM with a library of 24,389 tripeptides attached to tagged resin beads, the beads containing preferred peptide sequences could be identified by the red colour of the rhodamine dye label. Decoding of the tag molecules on the selected beads revealed the sequences of the best receptor ligands. It was found that there was a striking consistency in the peptide structures, with a high preference for a dipeptide sequence containing a carboxamide-bearing amino acid followed by D-Leu. The structures identified in this study also exhibited a close resemblance to the sequences previously shown to have affinity for an analogous receptor in chloroform solution. It is significant that these two-armed receptors are selective enough to preferentially bind peptides with a discrimination equivalent to one tripeptide per thousand sequences.

A linker for amidines

The amidine functional group occurs in a large number of pharmacologically significant molecules including fibrinogen receptor antagonists, antithrombotics and LTB_4 antagonists. However, until recently it has been a difficult group to include in a solid-phase library synthesis because linkers that allowed attachment of the amidine to the solid phase had not been developed. This situation has now changed with the description of a new linker that generates amidines on cleavage [Roussel, P. *et al. Tetrahedron Lett.* (1997) 38, 4861–4864].

The linker relies on the reaction of the amidine precursor with a resin-bound nitrophenylcarbonate (**3**). Following further modification of the compound, the product can be released from the solid phase by treatment with aqueous trifluoroacetic acid. This approach has been used for the solid-phase synthesis of CSG25019C (**4**), a Ciba LTB_4 antagonist currently in phase II clinical trials.



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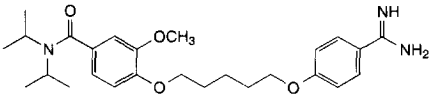

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Nonpeptide stromelysin inhibitors

Matrix metalloproteinases are a class of zinc-dependent proteinases that are involved in matrix degradation and tissue remodelling. Stromelysin is a member of this class, and has been the subject of several inhibitor discovery programmes. In particular, a group from Abbott Laboratories have applied their method of 'SAR by NMR' to the identification of new inhibitor molecules [Hajduk, P.J. *et al. J. Am. Chem. Soc.* (1997) 119, 5818–5827]. As many inhibitors of matrix metalloproteinases contain a hydroxamic acid, acetohydroxamic acid (**5**) (K_d = 17 mM) was used as a starting point for this study.

A range of hydrophobic compounds were incubated with ^{15}N-labelled stromelysin and a saturating amount of

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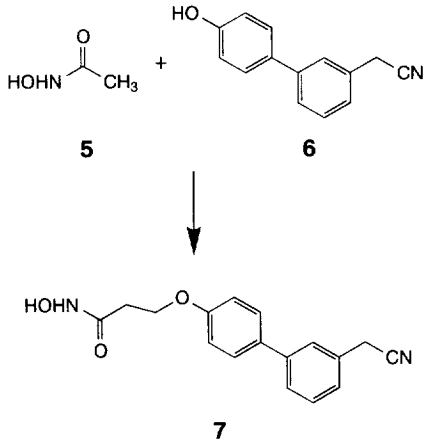
acetohydroxamic acid. By acquiring sensitivity-enhanced ^{15}N-HSQC (heteronuclear single quantum correlation) spectra, compounds that bound to the protein could be identified. After screening just 125 compounds, the biphenyl moiety was identified as being a preferred group, and further studies focused on these derivatives. Ultimately, the biphenyl (**6**) was found to be a preferred ligand (0.02 mM) in the presence of acetohydroxamic acid. NMR studies revealing how the two ligands bound to stromelysin suggested a number of linked compounds. Ultimately, an inhibitor (**7**) constructed from the two precursors was discovered with nanomolar affinity for the enzyme (K_d = 15 nM).



'SAR by NMR' is a technique that, while obviously derived from combinatorial chemistry, does not depend on the synthesis of large numbers of compounds before potent enzyme inhibitors or receptor ligands can be discovered.

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Emerging molecular targets

Newly discovered human 15-lipoxygenase

Lipoxygenases oxygenate arachidonic acid to hydroperoxyeicosatetraenoic acid (HPETE); this is a key step in the formation of leukotrienes, potent mediators of cellular function and favorite drug discovery targets. Three different human lipoxygenases have been described:

- the 5-lipoxygenase of leukocytes,
- the 12-lipoxygenase from platelets, and
- the 15-lipoxygenase of reticulocytes, eosinophils and macrophages.

Each enzyme oxygenates arachidonic acid at a different position along its carbon backbone. Now, Alan Brash and coworkers at Vanderbilt University School of Medicine (Nashville, TN, USA) have used reverse-transcription PCR to identify a new 15-lipoxygenase in human hair follicles [*Proc. Natl. Acad. Sci. U. S. A.* (1997) 94, 6148–6152].

The newly discovered 15-lipoxygenase was expressed in HEK-293, HeLa and COS cells, and in all cases oxygenated arachidonic acid at C15, with no trace of oxygenation at C12. This is in contrast to the previously described 15-lipoxygenase, which consistently forms a small amount (10–20%) of 12-HPETE from arachidonic acid. The fact that no C12 oxygenation is observed is cited by the investigators as evidence for the uniqueness of the newly described enzyme. In addition, the two 15-lipoxygenases have distinct specificities for arachidonic acid and linoleic acid as substrates. The new 15-lipoxygenase is found in skin, lung, cornea and prostate tissue; it has approximately 40% sequence homology with the well known 15-lipoxygenase from blood cells. Because it has strong structural similarities to the phorbol ester-inducible lipoxygenase found in mouse skin, the investigators suggest that the regulation of expression of the new 15-lipoxygenase may play a key role in its physiology or pathology, but this remains to be determined.

New pathway for regulation of apoptosis

Programmed cell death is an important aspect of development and homeostasis. There is now compelling evidence that defects in apoptosis are directly related to a variety of diseases including cancer, AIDS and various neurodegenerative diseases. Thus, it is not surprising that drug discovery scientists have an interest in understanding the molecular pathways that regulate apoptosis in order to uncover novel targets for new drug discovery.

Several well-characterized pathways for activating apoptosis are known that originate from the occupancy of receptors of the TNF ligand family and Fas. Such receptors contain 'death-domains' capable of initiating a cascade of irreversible signaling reactions within the cell leading to the activation of proteases termed caspases, which irreversibly initiate apoptosis. One of the ligands of the TNF family is termed TRAIL or Apo-2L. It triggers apoptosis of a variety of transformed cells, but TRAIL's cell-surface receptor and mechanism for activation of apoptosis have remained elusive. Now Guohua Pan and coworkers from the University of Michigan Medical School (Ann Arbor, MI, USA) and Human Genome Sciences (Rockville, MD, USA) report that the receptor for TRAIL is a newly recognized member of the TNF-receptor family, which they have termed death receptor-4 or DR4 [*Science* (1997) 276, 111–113]. The death domain of DR4 is significantly different from that of other apoptosis-inducing receptors (sequence homology ranging from 19–30%); however, several key residues that are essential for death-domain activity are conserved in DR4. The DR4 receptor was found to be widely expressed in human tissues.

However, unlike other death-domain receptors, DR4 did not require the common intracellular adaptor molecules FADD, TRADD or RIP to activate the caspase proteases, which are also required for DR4-induced apoptosis. The investigators believe that the lack of involvement of these adaptor molecules is evidence that the DR4 receptor initiates a cascade of molecular events to activate the essential proteases that is different from the other well-characterized death receptors. Understanding this yet to be deciphered cascade may yield productive new targets for drug discovery.

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