Monitor

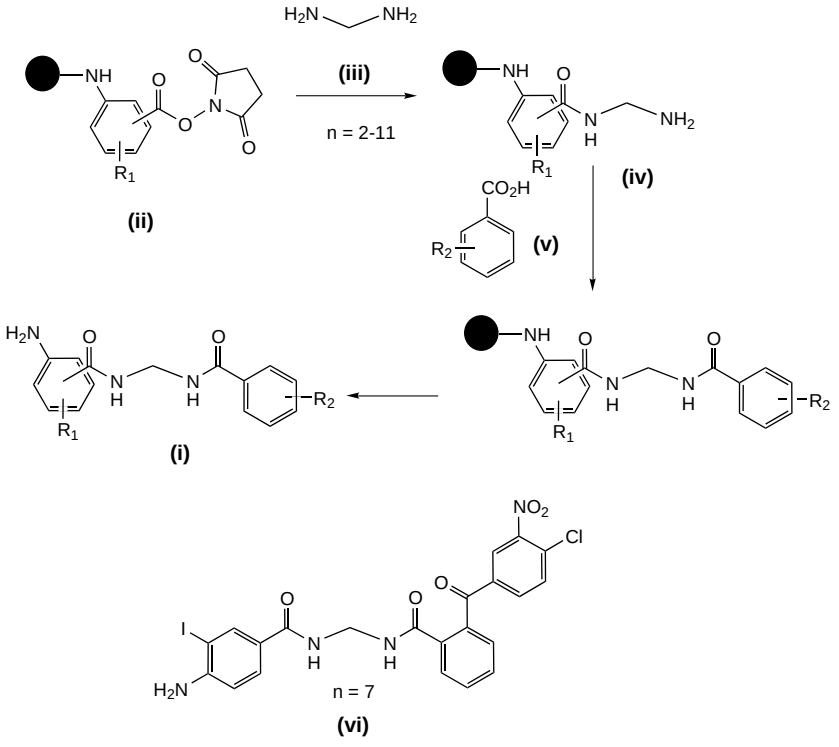
Monitor **provides an insight into the latest developments in the pharmaceutical and biotechnology industries.** ***Chemistry*** **examines and summarises recent presentations and publications in medicinal chemistry in the form of expert overviews of their biological and chemical significance, while** ***Profiles*** **provides commentaries on promising lines of research, new molecular targets and technologies.** ***Biology*** **reports on new significant breakthroughs in the field of biology and their relevance to drug discovery.** ***Business*** **reports on the latest patents and collaborations, and** ***People*** **provides information on the most recent personnel changes within the drug discovery industry.**

Chemistry

Antitumour molecules

Farnesyltransferase inhibitors

Farnesyltransferase (FT) is a potential anticancer target. FT inhibitors act by inhibiting tumour cell proliferation without substantial interference with normal cell growth. A library of diamides (**i**) as potential FTIs was designed and synthesized [1]. These 144 discrete compounds were synthesised on solid phase chlorotrityl resin by displacing a solid phase succinimidyl active ester (**ii**) with diamines (**iii**), followed by capping of the resultant solid phase amine (**iv**) with carboxylic acids (**v**) according to the general scheme below. Cleavage under acidic conditions generated the diamides (**i**). Compounds were then screened in an in vitro FT inhibitory assay. One of the most potent compounds was (**vi**).



Accordingly this work is of interest as it provides valuable tool compounds with inhibitory properties against farnesyltransferase and further work in this area is warranted.

1 Park, J.G. *et al.* (2004) Improved loading and cleavage methods for solid-phase synthesis using chlorotrityl resins: synthesis and testing of a library of 144 discrete chemicals as potential farnesyltransferase inhibitors. *J. Comb. Chem.* 6, 407–413

Cardiovascular disease therapy

Cysteine protease inhibitors

Many cysteine proteases are essential in regulation of physiological processes and disease propagation. These proteases are potentially important targets for the treatment of cardiovascular diseases, oncology and arthritis, for example.

Worldwide, more than 12 million people are infected with leishmaniasis. This disease is caused by the protozoal parasite *Leishmania*, and many more people are exposed to the risk of infection. This parasite causes cutaneous or visceral lesions, many of which are difficult to treat. A tandem array of 19 genes express a cysteine protease, CPB, which has been identified as an important virulence factor in *Leishmania mexicana*. Inhibition of CPB would therefore appear to be a promising approach for the development of drugs to treat this disease. The protease, termed *L. mexicana* CPB2.8ΔCTE has been cloned and isolated in a recombinant form lacking

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the C-terminal extension and used for substrate specificity studies. Protease inhibitors should be potent and selective for this enzyme to avoid side effects occurring through the inhibition of other physiologically relevant proteases.

Combinatorial chemistry libraries offer the potential to generate large numbers of compounds and screen for biological activity against the target of interest. Screening of these libraries and selecting the best hits can provide for new structural motifs to base further design strategies around. The development of one-bead-two-compound libraries allow the screening of millions of compounds in a competitive fashion in which each inhibitor in a single bead competes with a fluorogenic quenched substrate for binding to the protease. Because the synthetic strategy (split-and-mix) initially produce a unique structure on each bead to which a common fluorescence quenched substrate is attached, the library can be viewed as a collection of microreactors (with a volume of ~50 nL) that illuminates upon cleavage of the substrate when containing poor inhibitors. Potent inhibitors, in turn, can be identified by selecting the darkest beads where the substrate is intact due to high protease affinity for the inhibitor.

Recently the synthesis and screening of a combinatorial library of peptidotriazoles has been reported [2]. A library consisting of about half of 800,000 possible peptidotriazoles was synthesised on 450,000 beads. The library was incubated with *L. mexicana* CPB2.8ΔCTE. The fluorescence intensity was monitored and, after 24 hours, only a few beads remained dark. The long exposure to enzyme ensured only the most potent inhibitors were selected. After a sorting procedure, 48 hits (very dark beads) were selected. The inhibitors were re-synthesised in solution as C-terminal carboxamide with a free N terminus to mimic the inhibitors present in the solid phase library. Of these compounds, one of the most potent was (**vii**) with a Ki of 76 nM against against *L. mexicana* CPB2.8DCTE. This work has produced novel peptidotriazole compounds as promising inhibitors of *L. mexicana* cysteine protease and further work in this area is warranted.

H-Gly-RTr-ClF-Leu-Thr-Ile-Ser-Arg-Gly-NH_2

(vii)

2 Tornoe, C.W. *et al.* (2004) Combinatorial library of peptidotriazoles: identification of [1,2,3]-triazole inhibitors against a recombinant *L. mexicana* cysteine protease. *J. Comb. Chem.* 6, 312–324

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Biology

Neuroscience

Sour solution: postischemic neuroprotection via ASICs

Rising levels of intracellular calcium ions (Ca^{++}) in neurons after ischemia are believed to be responsible for the resulting brain damage. Up to now, NMDA receptors were thought to provide the main Ca^{++} entry pathway into the cell, and some clinical trails are currently underway or already completed, testing NMDA receptor antagonists for their neuroprotective effects after ischemia. Unfortunately, theses studies have shown rather disappointing results in protecting against brain damage after stroke in humans. Therefore, the question arises again: how do calcium ions enter the neurons after ischemia?

A recent paper published in *Cell* concentrated on this problem by considering the acidic environment that occurs during brain ischemia due to anaerobic glycolysis [1]. Increasing concentration of H^+ activates the acid-sensing ion channels (ASICs), which are known to be permeable to Na^+ and Ca^{++}. The subunits ASIC1a and ASIC2a are abundant in the brain and could provide a way of calcium ion entry that is independent of glutamate receptors. ASICs are sensitive to the sodium channel blocker Amiloride, whereas Psalmotoxin 1, a substance from venom of tarantula, has been shown to be a specific blocker of ASIC1a.

Xiong *et al.* exposed cultured mice cortical neurons to a pH of 6 and showed, by calcium imaging with fura-2, that the level of intracellular calcium is increased. The same effect occurs by depriving the cells of oxygen and glucose, which is an *in vitro* model for ischemia. Both methods led to increased cell death, which could effectively be inhibited by Amiloride and Psalmotoxin 1. Interestingly, neurons from ASIC1a-knockout mice displayed no increase in intracellular calcium ions when exposed to pH 6, and they seemed to be resistant against acid-induced cell death.

The authors induced a focal transient ischemia by occluding the middle cerebral artery in rats and mice. The evoked infarct volume was significant smaller in rats treated with Amiloride or Psalmotoxin 1. No treatment was necessary for the ASIC1-/- mice to show a strongly reduced infarct volume compared with wild-type mice. When combining Psalmotoxin 1 in wild-type mice with memantine, an uncompetitive NMDA receptor antagonist used in clinical trials, the authors could detect an additive effect. Thus, this study reveals the importance of the acid-sensing ion channel 1 in the development of brain injury after ischemia. These findings could help in the design of novel therapeutic neuroprotective strategies for brain ischemia.

1 Xiong, Z.G. *et al.* (2004) Neuroprotection in ischemia; blocking calcium-permeable Acid-sensing ion channels. *Cell* 118, 687–698

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Molecular Biology

It's a SNP

Variability within disease genes can influence the aetiology of resultant disorders and their amenability to treatment; with high-throughput genetic