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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

Molecules

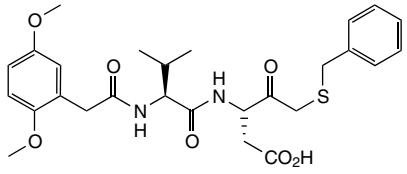
Caspase-3 inhibitors

The molecular mechanisms of apoptosis have begun to unravel with study, and the importance of a group of cysteinyl aspartate specific proteases (caspases) has become apparent in the programmed cell death process. To date, 13 human caspases have been identified as inactive proenzymes, which are subsequently activated to their catalytically active forms by proteolytic cleavage.

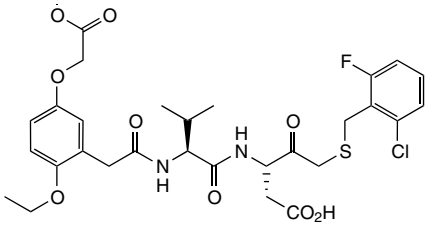
Caspases can be categorized into three subfamilies based upon their substrate specificity and cellular functions. Group I (1,4 and 5) caspases are believed to be involved primarily in inflammation, whereas Group II (2,3,6 and 7) and Group III (2,6,8, 9 and 10) caspases are key in triggering apoptosis. Caspase-3 (casp-3) has been shown to be instrumental in cleaving a range of proteins significant in apoptosis, including cytoskeletal proteins, kinases and DNA repair enzymes during apoptosis. Casp-3 knockout murine phenotype suggested the necessity of this enzyme during brain development and recent studies revealed its activation in many models of apoptosis.

Thus, the development of potent and selective casp-3 inhibitors has emerged as an attractive therapeutic target. Recent reports have also indicated that caspase inhibitors were effective in animal models of ischemia injury, burns, endotoximia and neonatal hypoxia. These inhibitors, however, are either irreversible pan-caspase inhibitors, inhibit other cysteine proteases, or have poor whole-cell activity and *in vivo* stability. In order to assess the importance of casp-3 activation in apoptosis, potent selective and reversible inhibitors are needed. Recently, such inhibitors have been discovered [1]. A library of 100 compounds was synthesised employing a split and pool protocol using the IRORI MacroKan® technology. Four of the most potent compounds identified at this stage were re-synthesised and assayed against casp-1, -3, -7, and -8. All of these inhibitors were found to be selective, fully reversible and competitive against rh-casp-3.

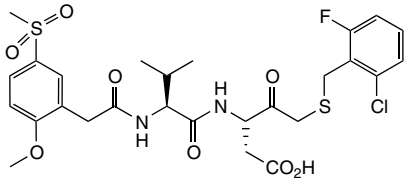
One compound (**i**) was active in an NT2 whole-cell assay with an IC_{50} of 10 μM. Here, the potency of compounds to inhibit camptothecin-induced apoptotic cell death in the neuronal precursor (NT2) cells was determined. Compound (**i**) was thus the starting point for a further two rounds of optimization, with a further 600 compounds synthesized. This led to the discovery of (**ii**), which was potent (IC_{50} 53 nM) and also not significantly shifted (intrinsic to whole cell) in comparison with other compounds tested. It was shown that compound (**ii**), because it is an ester group, is acting as a prodrug, leading to enhanced whole-cell activity because of improved cell permeability. Further medicinal chemistry design led to (**iii**) with excellent intrinsic and whole-cell potency with IC_{50}s of 5 nM and 1000 nM,



(i)



(ii)



(iii)

respectively, and selectivity against other caspases tested. Accordingly, this work is of interest as it provides valuable tools for studying the importance of casp-3 activation in cell-based systems.

1 Han, Y. *et. al.* (2004) Discovery of novel aspartyl ketone dipeptides as potent and selective caspase-3 inhibitors. *Bioorg. Med. Chem. Lett.* 14, 805–808

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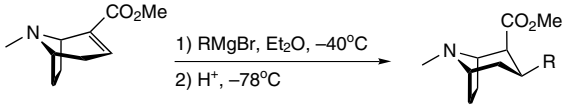
Dopamine transporter

In order to determine the usefulness of combinatorial chemistry methods when compared to traditional synthetic methods producing single compounds at a time, one could measure the combinatorial procedure against criteria such as the ease of synthetic execution, and whether the protocols are reliable. Often these demands are difficult to satisfy. However, recently it has been found that multicomponent Grignard reagents can be prepared and reacted with differing electrophiles simultaneously, to generate uniform mixtures of products. The identification of biologically active library members from within these mixtures normally requires re-synthesis of many inactive compounds as well as active library members.

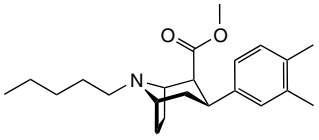
To obviate the need to synthesize inactive compounds, a method has been developed allowing the facile identification of highly active compounds from libraries generated from multicomponent Grignard reagents [2]. This method is illustrated by the search and discovery of potential cocaine antagonists by synthesizing an under-represented class of aliphatic substituted tropane derivatives from 1,4-conjugate addition of Grignard reagents to methyl ecgonidine (**iv**), see Scheme 1.

A total of 16 libraries, each containing 25 compounds, were synthesized and several active compounds re-synthesized. Compounds were screened for inhibition of the monoamine transporters hDAT, hSERT and hNET in a competitive-binding assay, and also for the monoamine uptake using cells expressing the three transporters and radiolabelled dopamine or serotonin. Several potent mixtures were obtained from these assays and, where individual compounds were resynthesised, this led to



Scheme 1

(iv)



(v)

(**v**), which was one of the most potent compounds discovered, with a K_i (binding) of 14 nM against hDAT and 15-fold selectivity over hSERT.

This work provides a reliable and powerful method for rapid synthesis of homologous compounds and the approach can be extended to other libraries, with the caveat that for vastly larger libraries, the method does rely on very large differences in biological activity between exceptional and unexceptional library members.

2 Bülow, A. *et. al.* (2004) Two- and three dimensional combinatorial chemistry from multicomponent Grignard reagents. *J. Comb. Chem.* 6, 509–519)

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Biology

Physiology

Cherish your cholesterol

The atheroprotective role of high-density lipoproteins (HDL) is due to their capacity to either absorb diffusible cholesterol present in the plasma or to extract cholesterol from the cell via an active transport. The latter process involves the ATP-binding cassette transporter ABCA1. In patient suffering from cardiovascular disease (CVD), the atheroprotective role of HDL is impaired, in part due to the level of HDL oxidation within the human artery wall.

Two independent studies have now shown the direct implication of myeloperoxidase (MPO) and its oxidation product hypochlorous acid in the oxidation of Apolipoprotein 1 (ApoA1), which is the major component of HDL [1,2]. MPO can oxidize other enzymes or proteins implicated in atheroprotection, either by nitration or chlorination of tyrosines in their active site, thereby inactivating or interfering with their normal function.

HDL isolated from human atherosclerotic lesions showed an increased level of chlorotyrosine [1]. Further analysis via liquid chromatography coupled to mass spectrometry confirmed the presence of MPO in HDL complexes. Looking at ApoA1 nitration, Zheng *et al.* showed that, in HDL lesions, ApoA1 nitration level is increased [2]. Performing coimmunoprecipitations on plasma of healthy donor supplemented with MPO, these authors demonstrated the direct interaction of MPO with ApoA1.

Both groups took their studies further and addressed the effect of oxidized ApoA1 and HDL on cholesterol transport *in vitro*. They concluded that, whether ApoA1 and HDL are chlorinated or nitrated, the action of the ABCA1 transporter is impaired, and active cholesterol transport is considerably slowed. Conversely, the oxidation level of ApoA1 or HDL has no effect on the passive absorption of cholesterol. These two studies are the first to demonstrate the direct implication of MPO in the oxidation of artery walls and its effect via HDL and ApoA1 on active cholesterol transport.

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1 Bergt, C. *et al.* (2004) The myeloperoxidase product hypochlorous acid oxidizes HDL in the human artery wall and impairs ABCA1-dependent cholesterol transport. *Proc. Natl. Acad. Sci. U. S. A.* 101, 13032–13037
2 Zheng, L. *et al.* (2004) Apolipoprotein A-I is a selective target for myeloperoxidase-catalyzed oxidation and functional impairment in subjects with cardiovascular disease. *J. Clin. Invest.* 114, 529–541

Cancer Biology

Coaxing tumour cells to commit suicide

The World Health Organization estimates that new global cases of cancer will increase sharply from 10 million in 2000 to 15 million in 2020. The pursuit for effective anti-cancer treatment regimens remains a priority for cancer researchers, and it is becoming clear that this process will be greatly facilitated by a rational molecular approach that depends on understanding

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