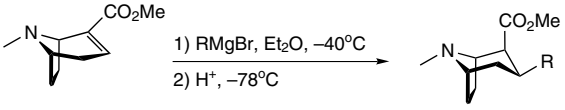
Dopamine transporter

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.

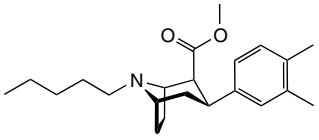
To obviate the need to synthesize inactive compounds, a method has been developed allowing the facile identification of highly active compounds from libraries generated from multicomponent Grignard reagents [2]. This method is illustrated by the search and discovery of potential cocaine antagonists by 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

Neuroscience

A gallant way to attenuate seizures and pain via galanin receptors

Galanin is a 29–30 amino acids-long neuropeptide that has been shown to affect seizure and pain threshold as well as feeding, cognitive, and sexual behavior. Three G-protein-coupled galanin receptors can be found in the brain, of which GalR1 seems to be most important in seizure regulation. GalR1-deficient mice display spontaneous seizures, whereas transgenic mice overexpressing galanin show a higher threshold for epileptic behavior. Despite remarkable efforts to create selective, stable and easily administered drugs targeting GalR1 (4 million compounds have been screened by Johnson & Johnson and Schering), up to now only one nonpeptide agonist, Galnon, with low affinity and a non receptor subtype specificity, has been found.

Recently, Bartfai *et al.* discovered a non-peptide, subtype specific and systemically active GalR1 agonist called Galmic [4]. They used the known active side chains of galanin and added them to a rigid triacid platform resulting in a drug with anticonvulsant effects in experimental self-sustained status epilepticus in rats. When administered systemically, it reduced the duration and number of seizures significantly. Besides these effects on neuronal excitability involved in seizure generation, Galmic also showed a clear diminution in flinch responses after formalin paw injection in mice, indicating its antinociceptive potency, a property that could not be detected for Galnon. Galmic applied i.p. shows antidepressant-like effects in the forced-swim test.

Two conclusions can be drawn from this study: first, galmic is a promising new GalR1 agonist with a potential role in the treatment of neurological disorders such as epilepsy, inflammatory pain and anxiety; second, the method of using a rigid scaffold and adding active side chains to it can be a very useful tool for developing future peptidomimetic drugs.

4 Bartfai, T. *et al.* (2004) Galmic,a nonpeptide galanin receptor agonist, affects behaviours in seizure, pain and forced-swim tests. *Proc. Natl. Acad. Sci. U. S. A.* 101, 10470–10475

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the underlying molecular mechanism of cancer development. Mounting evidence has demonstrated that the ability of cancer cells to evade apoptosis (programmed cell death) contributes to tumourigenesis and resistance to chemotherapeutic treatments.

The Inhibitors of Apoptosis (IAPs) inhibit caspases, a family of cysteine proteases that constitute the core of the apoptotic machinery. The anti-caspase activity of IAPs is attributed to their characteristic baculovirus inhibitory repeat (BIR) domains that bind directly to and inhibit caspases. During apoptosis, second mitochondria-derived activator of caspases (Smac) is proteolytically processed and released from the mitochondria into the cytosol, where its exposed N-terminal tetrapeptide sequence interacts with BIR domains of IAPs, thereby allowing caspases to execute the apoptotic program. This tetrapeptide has been shown to sensitize cancer cells to apoptosis induced by TNF-related apoptosis inducing ligand (TRAIL) or chemotherapeutic drug treatments in both human tissue culture and xenograft models in mice. However, the use of this tetrapeptide as a therapeutic is hindered by its low bioavailability, mostly because of its inefficient crossing of cell membranes. To circumvent this problem, researchers are exploring the possibility of using 'peptidomimetics'.

Li *et al.* now report the generation and characterization of a Smac mimetic [3]. Based on computer-simulated conformations using N-terminal Smac tetrapeptide as the starting point, 180 compounds that harbour non-natural amino acid replacements were synthesized. This peptidomimetic library was screened to find molecules that most effectively compete at the Smac-binding site on BIR3 domain of X-linked IAP (XIAP). A candidate was selected for further chemical modifications, which eventually yielded the very promising 'compound 3'. In cancer cell culture, Compound 3 can penetrate cell membranes, block the caspase inhibition function of XIAP, bind and eliminate cIAP-1 and cIAP-2 activities and promote both TRAIL- and TNFα-induced apoptosis at low nanomolar concentrations, demonstrating it as a true Smac mimetic.

These exciting results have positioned Compound 3 as a lead structure for the development of IAP antagonists potentially useful as therapy for cancer. However promising the prototype Compound 3 might appear to be as an anti-cancer agent, future *in vivo* studies are essential for the assessment of its pharmacokinetics and potential therapeutic value. Regardless, the development of Compound 3 perhaps epitomizes a new era in which the guiding principles on the development and design of anti-cancer treatments are based on insight into the molecular mechanism of tumourigenesis.

3 Li, L. *et al.* (2004) A small molecule Smac mimic potentiates TRAIL- and TNF-α-mediated cell death. *Science* 305, 1471–1474

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Virology

DNA immunization might offer protection against canine distemper virus (CDV)

Dahl and colleagues have recently investigated a new alternative vaccine candidates against canine distemper [5]. Using a highly susceptible host of canine distemper virus (CDV), the mink (*Mustela vison*), the authors demonstrate a new DNA vaccine can induce high levels of virus-neutralizing (VN) antibodies, which offers future possibility of a complete

protection against canine distemper in its natural host.

Canine distemper virus is an RNA virus from the morbillivirus family, affecting domestic dogs and many other carnivores, including raccoons, skunks and foxes and other wildlife species. It is known to occur worldwide, though often fatal in highly susceptible natural hosts such as ferrets and minks, widespread vaccination programs in young animals have dramatically reduced its incidence, particularly in domestic dogs. Unfortunately, existing attenuated live vaccines have a drawback in their inability to induce protection in the presence of maternally derived antibodies. New incidences of CDV among terrestrial and aquatic mammals have triggered the research group to explore the possibility of alternative non-infectious vaccines.

CDV can be transmitted directly from exposure to affected dogs or ferrets, or by airborne particles in an enclosed environment. The disease is highly infectious, so to mimic natural exposure the group conducted challenge infection by horizontal transmission from infected contact animals. The symptoms of CDV are also quite distinct in the terminal stages, but initially they resemble an upper respiratory disease like influenza. In this study, several animals received a lethal challenge infection of CDV by administration to the mucosae of the respiratory tract and into the muscle.

The DNA plasmids created by the group encoded the virula nucleoprotein (N) and haemagglutinin (H). In the first experiments, vaccination with H plasmid alone appeared to elicit a protective immune response, whereas vaccination with N plasmid alone was probably not sufficient. When mink were challenged with both H and N plasmid vaccine, no virus could be detected in the blood and tissues and there was an increased level of VN antibody in the serum. It appears that DNA immunization by the combined intradermal and intramuscular routes confers a protective immunity, which will encourage future investigations.

5 Dahl L, *et al.* (2004) Immunization with plasmid DNA encoding the hemagglutinin and the nucleoprotein confers robust protection against a lethal canine distemper virus challenge. *Vaccine* 22, 3642–3648

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Business

Collaborations

Intercell AG announces extended collaboration with Aventis on the development of bacterial vaccines

In February 2004, Aventis Pasteur (http://www.aventispasteur.com), the vaccine business of Aventis, and Intercell AG (http://www.intercell.com) announced a research and license agreement for the development of vaccines against bacterial diseases. This collaboration has now been extended, during which Intercell will further develop the antigens that have been successfully identified with its Antigen Identification Program (AIP). This approach uses state-of-the-art molecular and serological methods to identify pathogen structures that are recognized by the human immune system.

Alexander von Gabain, CEO of Intercell, said: 'Aventis Pasteur is a world leader in vaccine research and our ongoing collaboration validates the potential of our antigen identification technology to deliver in a timely fashion and to meet our partners expectations.'

Intercell's strategy and focus centers around the design and development of novel vaccines that combine antigens with immunizers (adjuvants) for prophylactic and therapeutic treatment of diseases with substantial unmet needs. The company has a broad development pipeline, with vaccines for Japanese Encephalitis and Hepatitis C currently in clinical trials.

Blueprint Asia announces collaboration with the Novartis Institute for Tropical Diseases

The Blueprint Initiative Asia, a non-profit company housed at the National University of Singapore and affiliated with the Blueprint Initiative, based in Mount Sinai Hospital, has announced a collaboration with the Novartis Institute for Tropical diseases (NITD) to further the company's research into dengue fever. Blueprint Asia will assemble and curate known protein interactions relevant to the biology of dengue fever and will enter this data into the Biomolecular Interaction Network Database (BIND), a repository of molecular interaction data hosted at Mount Sinai Hospital.

'Our collaboration with NITD is consistent with Blueprint Asia's goal of facilitating research and drug discovery related to neglected diseases that burden the Asia-Pacific region,' said Christopher Hogue, Project Leader and Principal Investigator of The Blueprint Initiative.

Brian Yates, Managing Director of Blueprint Asia, said: 'By examining information about dengue virus alongside other data in the BIND repository, NITD scientists will gain a better understanding of the dengue life cycle and of complex interactions with host proteins…this information can then be used to develop drugs or vaccines to fight the disease.'

Acquisitions

Agilent Technologies to acquire Silicon Genetics

Agilent Technologies (http://www.agilent.com) has announced its acquisition of Silicon Genetics (http://www.silicongenetics.com), a leading software provider for life science discovery. The acquisition, which is subject to closing conditions, will make Agilent a market leader in life science informatics. Financial details of the agreement were not disclosed.

Together, the staffs of Silicon Genetics and Agilent will form a life science informatics team that will be an incubator for the development of Silicon Genetics and Agilent informatics products. Commenting on the acquisition, Fran DiNuzzo, Vice President and General Manager of Agilent's Integrated Biology Solutions business, said: 'Silicon Genetics brings Agilent an outstanding informatics product portfolio and a strong team of people with extensive experience in software development…our combined organizations will offer customers an unparalleled range of informatics solutions.'

Andrew Conway, Silicon Genetics' Founder and Chairman, said: 'This is an extremely exciting opportunity for Silicon Genetics' customers and employees. Our two companies will form a strong team with the opportunity to make significant contributions to an even larger audience of life science researchers.'

Business was written by
Christopher Watson

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