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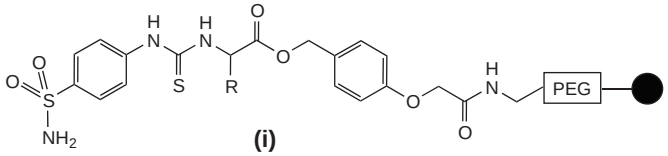
monitor

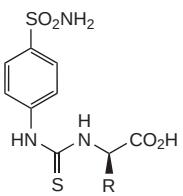
COMBINATORIAL CHEMISTRY

Carbonic anhydrase inhibitors

Sulfonamide inhibitors of the metalloenzyme carbonic anhydrase have been used in clinical medicine and as diagnostic tools for the treatment of diseases, such as glaucoma and diverse neuromuscular disorders, and as antitumour drugs [1]. Many of these drugs act systemically and, unfortunately, can show undesirable side-effects as a result of non-selective inhibition of carbonic anhydrase isozymes in the target tissue or organ. To address the issue of selectivity, attempts have been made to design and synthesize new sulfonamides that are free from crossreactivity. The most successful approaches to date have focused on the use of hydrophilic 'tails' attached to a scaffold of aromatic or heterocyclic sulfonamides that have additional amino, imino or hydroxy moieties [2]. A search for new lead structures acting as carbonic anhydrase inhibitors was initiated [3]. Compounds identified as inhibitors had different 'tails', which enabled modulation of the physiochemical properties of the resulting sulfonamides. The synthesis of these inhibitors necessitated the development of a solid-phase library protocol that minimized formation of side-products, thus reducing time-consuming and expensive purification steps. A small library of compounds was synthesized on NovaSyn® Tentagel resin functionalized with an amino acid starting material that was orthogonally protected for solid-phase peptide synthesis. Next, the resulting library compounds were screened on the bead for their inhibitory activity against human carbonic anhydrase I, II and IX isozymes, with compound activity being determined by a spectrophotometric assay that evaluated the esterase activity of the selected enzymes. These resin-bound esters (**i**) were compared with the analogous free (acid) sulfonamides (**ii**) afforded by solution-phase synthesis. Comparable results were obtained, which indicates that on-bead testing of carbonic anhydrase inhibitors in a library of thioureido-benzene-sulfonamides can be performed successfully. One of the most active compounds was (**iii**), which had a K_i value of 5 nM for human carbonic anhydrase II, both as the resin bound ester and the corresponding free acid. This research has demonstrated that it is possible to use on-resin assay methodology to screen a selection of carbonic anhydrase inhibitors rapidly, providing for potent inhibitors and further work in this area is warranted.



(i)



(ii)

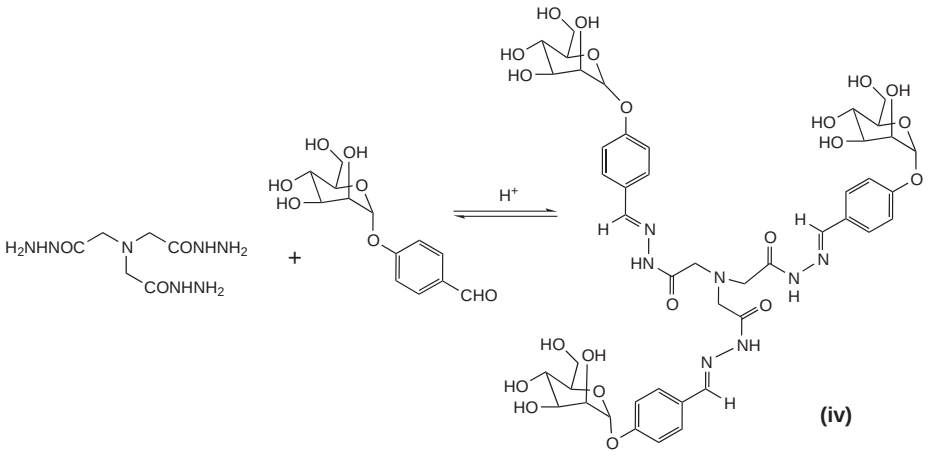
(iii) R = isopropyl

1 Supuran, C.T. and Scozzafava, A. (2000) Carbonic anhydrase inhibitors and their therapeutic potential. *Exp. Opin. Ther. Patents 10*, 575–600
2 Scozzafava, A. *et al.* (1999) Carbonic anhydrase inhibitors: synthesis of water-soluble, topically effective, intraocular pressure-lowering aromatic/heterocyclic sulfonamides containing cationic or anionic moieties: is the tail more important than the ring? *J. Med. Chem.* 42, 2641–2650
3 Innocenti, A. *et al.* (2004) Carbonic anhydrase inhibitors: the first on-resin screening of a 4-sulfamoylphenylthiourea library. *J. Med. Chem.* 47, 5224–5229

Concanavalin A lectin

Dynamic combinatorial chemistry (DCC) is a new approach to rapid ligand or receptor identification based on the implementation of dynamic assembly and recognition processes [4]. The concept is founded on formation of reversible connections between suitable building blocks, leading to the spontaneous assembly of all possible combinations. This methodology also facilitates simple one-step generation of extended libraries. Because the libraries produced are dynamic, they enable target-driven and self-screening processes that could lead to the preferential expression of the active species, thus presenting the strongest binding to the target entity. Molecular recognition of carbohydrates is an area of research with potential bearing on drug discovery [5]. Carbohydrates have central roles in many biological processes, for example, cell–cell interactions and communication, and are therefore highly attractive tools for generating mimics and analogues of such recognition processes. The synthesis of combinatorial carbohydrate libraries is a challenging task and the application of DCC could partly alleviate

1359-6446/05/$ – see front matter PII: S1359-6446(05)03431-8



these problems, particularly in forming libraries of dynamically interchanging carbohydrate clusters. To explore the use of DCC in glycobiology, dynamic libraries of constituents that are susceptible to binding to the plant glycoprotein concanavalin A (con A), using the hydrazidecarbonyl–acylhydrazone interconversion as reversible chemistry, has been undertaken [6]. Acylhydrazone libraries were generated from the dynamic assembly of a series of oligohydrazide core building blocks (used to arrange the interactional components in a given geometry) with a set of aldehyde counterparts capable of interacting with the binding site of the target species: this is exemplified by the synthesis of (**iv**). Six naturally occurring carbohydrates were used to generate a library of 474 constituents. To screen the library against con A, an enzyme-linked lectin assay was adopted based on yeast mannan as the immobilized ligand. Several active components were identified from the screening of the library. To pinpoint the active constituents, a deconvolution process relying on the dynamic features of the library was used. Single building blocks were removed from the complete library, resulting in a redistribution of the remaining compounds incorporating that moiety and suppression from the equilibrating pool of all constituents containing the removed components. For each component of the dynamic combinatorial library, a sublibrary was prepared from which all library constituents based on this element were removed. A decrease in inhibitory effect reveals the importance of the removed component in the generation of active compounds in the dynamic library. From the deconvolution process, several active components were identified, of which (**iv**) was one of the most potent with an IC_{50} of 22 μM in binding to con A. This research has demonstrated that lectin ligands can be generated by acylhydrazone formation and exchange, enabling the efficient generation of dynamic combinatorial libraries in aqueous media. The data also indicate that, in the first stage of component selection for establishing dynamic combinatorial libraries, it is beneficial to introduce flexible components to allow for adaptation to the target of the dynamic combinatorial library constituent generated. This research has shown the potential of DCC to refine the preliminary constituents identified as active to more precisely defined structural components.

4 Ganesan, A. (1998) Strategies for the dynamic integration of combinatorial synthesis and screening. *Angew. Chem. Int. Ed. Engl.* 37, 2828–2831

5 Bertozzi, C.R. and Kiessling, L.L. (2001) Chemical glycobiology. *Science* 291, 2357–2364

6 Ramström, O. *et al.* (2004) Dynamic combinatorial carbohydrate libraries: probing the binding site of the concanavalin A lectin. *Chem. Eur. J.* 10, 1711–1715

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NEUROSCIENCE

A novel single-strand DNA-repair process has implications for neurodegenerative disease

Spinocerebellar ataxia with axonal neuropathy 1 (SCAN1) is a neurodegenerative disease caused by mutations in tyrosyl phophodiesterase 1 (Tdp1). This enzyme removes DNA topoisomerase I peptides from DNA ends during repair, but it is not clear how mutations in this enzyme cause neurodegeneration. El-Khamisy *et al.* have shown that Tdp1 is required in a novel repair process for DNA single-strand breaks (SSBs), which is important in neuronal cells not undergoing DNA repair [1].

Strand breaks were compared in normal and SCAN1 cells after treatment with the topoisomerase I inhibitor, camptothecin (CPT). Unlike normal cells, the SCAN1 cells continued to accumulate strand breaks. These breaks were not repaired even when the cells were moved to a CPT-free medium, showing that SCAN1 cells can't repair CPT-induced DNA breaks.

The authors tested whether strand breaks could occur in cells not undergoing DNA replication. Mimosine was added to the cells to reduce the level of DNA replication. It prevented the accumulation of DNA breaks in normal cells, but only reduced DNA breaks in SCAN1 cells by twofold, showing that half the breaks in SCAN1 cells occur independently of DNA replication. The authors further showed that a large proportion of the breaks were caused in a transcription-dependent manner.

The authors proposed that the SCAN1 cells lacked a Tdp1-dependent SSB repair process. To test this they showed that cell lines with known defects in SSB repair also accumulated CPT-induced strand breaks. The authors also reconstituted Tdp1-dependent SSB repair *in vitro* and showed that extracts prepared from SCAN1 cells were defective in this activity.

It is not clear why defects in single-strand DNA repair only cause phenotypic effects in neurons. It could be due to the high level of oxidative stress in neurons. Consistent with this, the authors also showed that strand breaks persisted in SCAN1 cells in response to oxidative damage, but were repaired in normal cells. Clearly further research is required to fully understand the functions of this pathway.

1 El-Khamisy, S. F. *et al.* (2005) Defective DNA single-strand break repair in spinocerebellar ataxia with axonal neuropathy 1. *Nature* 434, 108–113

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New insight on the acetylcholine receptor

The nicotinic acetylcholine receptor (AChR) is a neurotransmitter-gated ion channel consisting of a ring of five membrane-spanning subunits. It is found in a variety of tissues, including the autonomic nervous system, the neuromuscular junction and the brain in vertebrates. Agonists such as acetylcholine (Ach), carbamylcholine and nicotine produce an influx of sodium through a ligand-gated ion channel that is associated with nicotinic cholinergic activation. A recent atomic model determined by Nigel Unwin [2] via

1359-6446/05/$ – see front matter PII: S1359-6446(05)03444-6