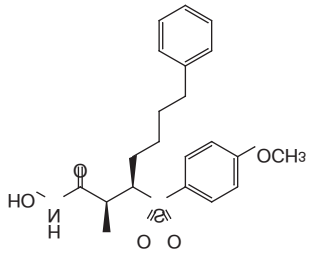


**8**

A recent communication from workers at Rhône-Poulenc Rorer (Collegeville, PA, USA) describes the first compounds, exemplified by β-(arylsulfonyl)hydroamic acid **8**, which simultaneously inhibits both $PDE_4$ and the MMPs gelatinase, collagenase and stromelysin [Groneberg, R.D. *et al.* (1999) *J. Med. Chem.* 42, 541–544]. These compounds may be of use in the treatment of a wide range of inflammatory disorders.

### Emerging therapeutic targets

*Asthma treatments*

The ultimate goal in the treatment of asthma is the development of orally active agents that will modify the underlying disease processes of asthma and reduce airway inflammation, hyperreactivity and bronchoconstriction. Current therapies tend to treat the symptoms of the disease rather than the disease processes, resulting in a polypharmacy approach to modern day asthma treatment. Although leukotrienes are important in the pathophysiology of asthma, the administration of leukotriene receptor antagonists alone fails to provide a single means of treating all of the symptoms of this multifaceted disease.

Recent research has provided a greater insight into the underlying mechanisms of asthma. This research indicates that asthma may be driven by dysregulation of T-lymphocyte function and a Th2-like (helper T2-lymphocyte) response. This dysregulation process may be controlled by the administration of specific immunomodulatory cytokines. Such an approach offers the potential advantage that the problems of generalized immunosuppression or hormonal dysregulation, which occur upon administration of steroids, may be avoided. However, given the pleiotropic effects of many cytokines, this may require specific delivery to the site of action.

A recent review by Cerasoli, F. discusses this potential use of immunomodulatory cytokines for the treatment of asthma and describes the application of regulated gene therapy as a means of delivering cytokines directly to the affected airway [*Emerging Therapeutic Targets* (1999) 3, 27–39]. The paper highlights the advantages of gene therapy for overcoming the problems normally associated with conventional cytokine therapy, such as poor oral bioavailability and expensive protein manufacture.

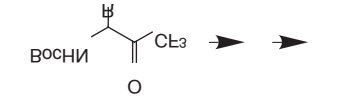
Another recent paper, from Levitt, R.C. and Nicolaides, N.C., highlights the important role that interleukin-9 plays in the pathogenesis of asthma [*Emerging Therapeutic Targets* (1999) 3, 41–51]. The paper also describes other approaches to suppressing the asthma-associated inflammatory cascade, including the use of immunoglobulin antagonists, adhesion molecule antagonists and cytokine antagonists. All of these therapeutic strategies have been shown in animal models to be effective in preventing or treating the underlying asthma-associated inflammation.

*Andrew Lloyd*

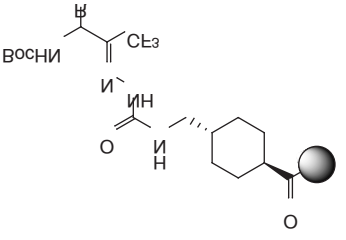
## Combinatorial chemistry

### Peptidyl trifluoromethyl ketones

Several serine proteases are attractive targets for pharmacological intervention. Consequently, there has been a concerted effort to find compounds that have an affinity for these enzymes, and the trifluoromethyl ketone group has been a key structural feature of substrate-based inhibitors of elastase, chymotrypsin and CMV protease. In order to accelerate the discovery of novel serine protease inhibitors, a solid-phase approach to the preparation of peptidic trifluoromethyl ketones has recently been disclosed [Poupart, M-A. *et al.* (1999) *J. Org. Chem.* 64, 1356–1361].



**1**



**5**

This approach relies on the use of a semicarbazone linker that can act as a reversible protecting group for the ketone. Various trifluoromethyl ketone synthons (**1**) were prepared and used to generate semicarbazides, which were in turn tethered to BHA polystyrene resin through an ester linkage (**2**). The linkage was stable under the conditions used to deprotect the amine and extend the peptide chain. At the end of the synthesis, the ketones could be regenerated from the resin by heating in aqueous HCl and acetic acid. Following semi-preparative HPLC, the desired products were isolated in 15–40% yields. The method has been used to prepare more than 100 trifluoromethyl ketones as potential HCMV protease inhibitors.

### Tetanus toxin inhibitors

The bacterial protein, tetanus neurotoxin (TeNt) is a zinc endopeptidase that cleaves synaptobrevin selectively at the single peptide bond, $Gln^{76}$-$Phe^{77}$. As the substrate protein is an essential part of the neurotransmitter exocytosis apparatus, inhibition of the metallopeptidase would be a possible target for the treatment of tetanus. A recent study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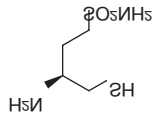
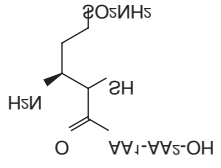
has described the synthesis of a library of pseudotripeptides that have micromolar affinity for TeNt [Martin, L. *et al.* (1999) *J. Med. Chem.* 42, 515–525].



**3**



**4**

The thiol compound (**3**, $K_i$ = 100 μM), recently reported as an inhibitor of TeNt, was the starting point for this study. Making the assumption that the thiol is the zinc-chelating group led to the design of extended analogues that might interact with the S′ subsites of the enzyme's active site. The combinatorial library of 19 mixtures (**4**) were prepared by solid-phase mix and split peptide synthesis on 2-chlorotrityl chloride resin. From the deconvolution of the library it was determined that a preferred TeNt inhibitor had $AA_1$ = Tyr and $AA_2$ = His. Separating the diastereoisomers revealed one compound with $K_i$ = 5 μM. *Ex vivo* and *in vivo* studies of these and other compounds are now in progress.

### D-peptide antigens

Synthetic antigen mimetics, which are recognized by antibodies, are of great interest as they may provide clues to the understanding of antigen recognition, and ultimately lead to the design of more effective immunodiagnostics and synthetic vaccines. Epitope mapping, more recently using combinatorial chemistry, has been invaluable in revealing the peptide sequence of antigens, but a recent study has demonstrated the discovery of novel all D-amino acid peptides that bind an antibody with high affinity [Pinilla, C. *et al.* (1998) *J. Mol. Biol.* 283, 1013–1025].

Positionally scanning libraries of both all-L and all-D hexapeptides were generated and screened for inhibition of monoclonal antibody HGAC 39 G3 that binds to an antigen displaying *N*-acetyl-D-glucosamine residues. It was found that the all-D sequences were the most potent inhibitors with the sequence Ac-yryygl-$NH_2$ recognized with a relative affinity of 300 nM. The study supports the concept that some monoclonal antibodies are functionally polyspecific as they can recognize multiple antigens with distinct chemical characteristics.

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## Contribution of oxidative stress to excitotoxicity-induced deleterious iNOS in the CNS

Nitric oxide (NO) is a gaseous messenger which is involved in several physiological processes in the brain [Dawson, T.D. and Dawson, V.L. (1994) *Neuroscientist* 11, 9–20]. It is synthesized by the NO-synthases (NOS), of which three isoforms have been cloned [Kerwin, J.F. and Heller, M. (1994) *Med. Res. Rev.* 14, 23–74]. The neuronal (NOS-1) and endothelial (NOS-3) isoforms are $Ca^{2+}$-dependent and constitutively expressed whereas NOS-2, also called iNOS, is $Ca^{2+}$-independent and inducible. However, evidence has accumulated during the last decade that demonstrates a deleterious role for NO under pathophysiological conditions in the brain, such as cerebral ischaemia. Nowicki, J.P. and coworkers [*Eur. J. Pharmacol.* (1991) 204, 339–340] first demonstrated the neuroprotective effect of NOS-1 inhibition on mice exposed to cerebral ischaemia. A few years later, a delayed NO production originating from iNOS was described in the formation of ischaemic lesions [Iadecola, C. *et al.* (1995) *Am. J. Physiol.* 268, R286-R292]. Although iNOS has been shown to be induced by multiple mechanisms *in vivo* and *in vitro*, the pathways that lead to iNOS expression during cerebral ischaemia remain unclear.

We have therefore focussed our work in determining the mechanisms responsible for iNOS expression in such a neuropathology. In particular, we were interested in excitotoxicity, a deleterious event that occurs in the very early stages of cerebral ischaemia. Excitotoxicity results from an accumulation in the synaptic cleft of the excitatory amino acid glutamate. Supra-physiological concentrations of glutamate activate post-synaptic glutamate receptors, in particular the NMDA receptor subtype. This NMDA receptor activation has been shown to result in an immediate and detrimental increase in NO production by the activation of NOS-1. Of particular interest was whether this NMDA-induced excitotoxicity is able to trigger delayed NO-synthesis due to its ability to activate iNOS under these conditions.

In a model of excitotoxicity in the rat where NMDA was perfused in the striatum through a microdialysis probe, $Ca^{2+}$-independent NOS activity appeared 48 hours after NMDA exposure [Lecanu, L. *et al.* (1998) *Br. J. Pharmacol.* 125, 584–590]. In addition, this activity was accompanied by an increase in the production of the NO metabolite nitrite, as measured in microdialysate samples. We showed that this NOS activity as well as nitrite production was reduced by dexamethasone, a glucocorticoid known to block iNOS gene expression, and by aminoguanidine, a direct inhibitor of the iNOS enzyme. These results provided the first evidence of an iNOS induction

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