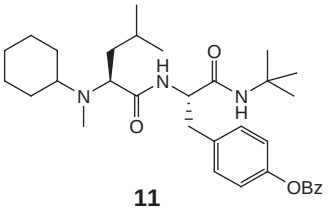

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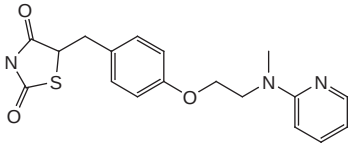
treatment of stroke and pain, Hu, L-Y. and coworkers have identified a series of *N,N*-dialkyl-dipeptidylamines, exemplified by (**11**), with potent functional activity at N-type voltage-sensitive calcium channels. These compounds have also been shown to possess efficacy in the audiogenic-seizure mouse model. Structure–activity studies have demonstrated that the N-type calcium channel blocking activity correlates with lipophilicity and the molecular size of the *N*-substituents.

Combinatorial chemistry

Solid-phase synthesis of BRL49653

Obesity is an increasingly common disease in the western world and incidence has been associated with a range of other diseases including hypertension, atherosclerosis and type 2 (non-insulin dependent) diabetes. However, little is known about the proteins that mediate gene control of lipid metabolism. One possible clue is the class of peroxisome proliferator activated receptors (PPARs), a family of nuclear receptors that respond to long-chain fatty acids and prostaglandins and that have been a subject of this column in previous months. Further study of this family of receptors might yield vital clues in the understanding of the signalling pathways that regulate energy balance.

One agent known to bind to the mPPARg is the antidiabetic thiazolidinedione, BRL49653 (**1**), and it was judged that a common route to the synthesis of analogues might help clarify an understanding of the role of PPARs. A recent paper describes the solid-phase synthesis of BRL49653 and indicates how this route might be used for the preparation of other analogues [Brummond, K.M. and Lu, J. (1999) *J. Org. Chem.* 64, 1723–1726].



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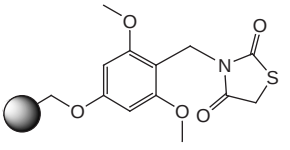

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The key to the synthesis of BRL49653 was the discovery that the 2,4-thiazolidinedione moiety could be attached to a polystyrene support through the 3,5-dimethoxyphenol linker (**2**), thus allowing final cleavage under mildly acidic conditions. The molecule was built up by Knoevenagel condensation, Mitsunobu chemistry and nucleophilic aromatic substitution. The authors are currently using this synthetic route in the preparation of structurally diversified analogues of BRL49653.

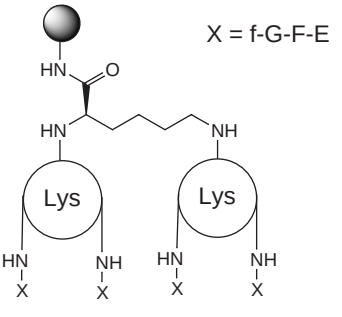
Novel protein kinase inhibitors

Calcium is key to the regulation of a diverse range of cellular functions, often through a calcium receptor protein such as the well-characterized calmodulin (CaM). However, the ubiquity with which CaM functions as a calcium receptor makes it an unattractive molecular target for drug design. A recent paper describes the use of combinatorial chemistry to optimize peptide inhibitors of a protein kinase that clearly do not function through the calcium receptor [Lukas T.J. *et al.* (1999) *J. Med. Chem.* 42, 910–919].

Although myosin light-chain kinase (MLCK) possesses a CaM–calcium-binding region, functional genomic studies were used to identify a lead peptide inhibitor based on a core autoinhibitory sequence. The lead peptide, Arg-Lys-Lys-Tyr-Met-Ala-Arg-Arg-Lys-NH_2, with an IC_{50} value of 1.2 μM, was modified using the mix-and-split library method. As the basic amino acid residues were important for selective inhibition of MLCK, these were held constant and the library focused on variation of the central three residues. Using 18 amino acid residues, the first round of synthesis generated 18 pools, each of 5832 possible peptide sequences. Recursive deconvolution eventually revealed a peptide sequence (Arg-Lys-Lys-Tyr-Lys-Tyr-Arg-Arg-Lys-NH_2) with an IC_{50} value of 50 nM, and 4000-fold selectivity over CaM kinase II, thus demonstrating that this inhibitor has little affinity for the calcium-binding protein. Such inhibitors add to the tools available for the deconvolution of complex signal-transduction pathways.

Inhibition of RAS farnesylation

Much is known about the structure and function of RAS proteins. Although they have a central function in normal cell processes, point mutations activate the oncogenic potential of RAS genes. The proteins undergo a series of post-translational modifications at their carboxy terminals, including farnesylation and subsequent terminal tripeptide cleavage. Oncogenic RAS proteins have been considered as reasonable targets for the discovery of cancer therapeutics, often using the inhibition of farnesyl transferase as a pharmacological



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mechanism. Recently, an alternative strategy of finding compounds that bind to the carboxy terminus of the RAS protein has been described [Dong D.L. *et al*. (1999) *Chem. Biol.* 6, 133–141].

A combinatorial library of molecular forceps consisting of short peptide chains attached to both rigid (chenodeoxycholic acid) and flexible (lysine) scaffolds were prepared using a set of 15 L- and D-amino acids. The total library of 151,875 molecules was prepared on TentaGel resin using mix-and-split synthesis, with the synthetic, history of each bead being recorded using molecular catechol tags. Analysis of beads that bound to the RAS carboxy-terminal decapeptide revealed a number of active structures. Of these, compound (**3**) was found to bind to the carboxy terminus of RAS and inhibited the farnesylation with an IC_{50} value of 100 μM.

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