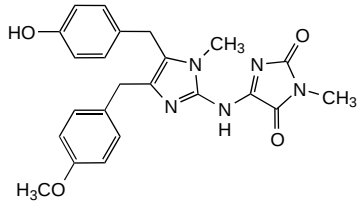
Monitor: molecules and profiles

Monitor provides an insight into the latest developments in drug discovery through brief synopses of recent presentations and publications together with expert commentaries on the latest technologies. There are two sections: *Molecules* summarizes the chemistry and the pharmacological significance and biological relevance of new molecules reported in the literature and on the conference scene; *Profiles* offers commentary on promising lines of research, emerging molecular targets, novel technology, advances in synthetic and separation techniques and legislative issues.

Natural antitumour agent

The overexpression of epidermal growth factor (EGF) receptors by certain cell types has been previously linked to tumorigenicity. In tumours dependent on EGF for growth, EGF receptor antagonists and agents that interfere with the EGF signalling pathway have been shown to be effective antiproliferative agents. Copp, B.R. and coworkers have recently reported the isolation of a known 2-aminoimidazole alkaloid, Naamidine A (**1**), from a Fijian *Leucetta* sp. sponge as an EGF receptor inhibitor [*J. Med. Chem.* (1998) 41, 3909–3911].



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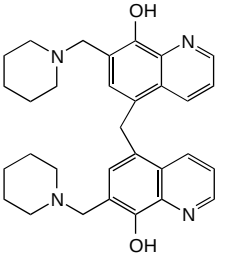
The compound was found to be a potent inhibitor of the EGF signalling pathway and shown to be more specific for the EGF-mediated mitogenic response than the insulin-mediated mitogenic response. Evaluation of this inhibitor *in vivo*, against EGF-dependent A431 tumours in athymic mice, showed >85% growth inhibition at the maximally tolerated dose (25 mg kg^{-1}). Initial mechanistic studies have shown that the compound does not inhibit the binding of EGF to the receptor and has no effect on the catalytic activity of *c-src* tyrosine kinase, which is involved in the signalling pathway.

N-type calcium channel blockers

A range of voltage-sensitive calcium channels regulate intracellular calcium concentrations in neurones. The intracellular calcium levels control various neuronal functions including homone secretion, neurotransmitter release and metabolism. As elevated calcium levels have been associated with tissue damage following ischaemic or traumatic events, N-type voltage-sensitive calcium channel blockers have recently been investigated as potential therapeutic agents in the treatment of traumatic brain injury, focal cerebral ischaemia and pain.

Yen, P-W. and coworkers have described the identification of a series of aminomethyl-substituted isoquinolinol derivatives with potent functional activity for N-type voltage-activated calcium channels following high-volume screening of the Parke-Davis Pharmaceutical Research compound library and optimization of the lead compound PD029361 (**2**) [*Bioorg. Med. Chem. Lett.* (1998) 8, 2415–2418]. The most potent compound was the diphenylbutyl-substituted analogue **3** with an IC_{50} of 0.46 μM.



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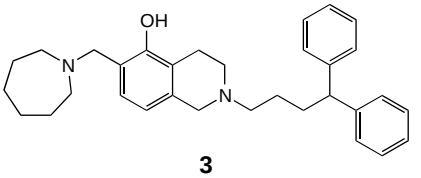

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Leukotriene D_4 receptor antagonists

Leukotriene D_4 plays a major role in the pathogenesis of asthma through its ability to cause bronchoconstriction and increase vascular permeability. As part of a study of the indole nitrogen region of Zafirlukast (**4**) – an orally active

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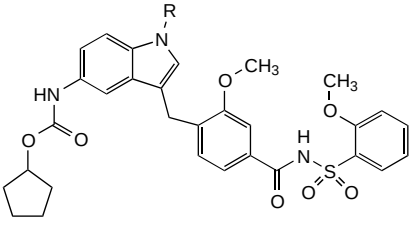
leukotriene D_4 antagonist presently in clinical trials – workers from Pfizer Central Research (Groton, CT, USA) have identified a series of potent cysteinyl leukotriene D_4 antagonists exemplified by **5** (IC_{50} = 0.5 nM) [Brown, M.F. *et al. Bioorg. Med. Chem. Lett.* (1998) 8, 2451–2456].



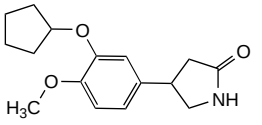
4, R = CH_3
5, R = $CONH(CH_2)_3Ph$

Although these compounds were found to be more potent than Zafirlukast *in vitro*, the compounds were generally found to be less potent *in vivo*. Pharmacokinetic studies in monkeys suggest that the reduced efficacy *in vivo* may be attributed to poor absorption of these compounds. Although attempts to identify compounds in this series with acceptable pharmacokinetic profiles were unsuccessful, further exploration of the indole N-substitution may identify more efficacious compounds.

Phosphodiesterase inhibitors

An alternative strategy for asthma treatment is to inhibit cell function and cytokine liberation in inflammatory cells through the administration of phosphodiesterase 4 (PDE4) inhibitors. As PDE enzymes inactivate cAMP, inhibition of these enzymes causes elevation of cAMP levels, which in turn activates various protein kinases responsible for decreasing inflammatory cell activity and airway smooth muscle tone. On the basis of their anti-inflammatory activities, PDE inhibitors have also been suggested as possible therapeutic agents for the treatment of rheumatoid arthritis.

Of the seven known PDEs, PDE4 is known to be cAMP specific and found in airway smooth muscle. The side effects associated with existing PDE4 inhibitors such as Rolipram (**6**) have led various groups to investigate alternative orally active PDE4 inhibitors with improved therapeutic profiles in man.



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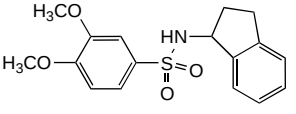
In the first of several recent papers describing PDE4 inhibitors, Montana, J.G. and coworkers have reported the development of a series of aryl sulphonamides as selective PDE4 inhibitors (IC_{50} = 6 μM) [*Bioorg. Med. Chem. Lett.* (1998) 8, 2635–2640].

These compounds showed activity both *in vivo* and *in vitro*, with compound **7** showing no emetic side effects in a model of ferret emesis at doses efficacious in a guinea pig skin eosinophil model. The group's data also support the hypothesis that the side effects associated with existing PDE4 inhibitors are caused by the binding of PDE4 inhibitors to both the PDE4 catalytic binding site and a high affinity binding site, because compound **7** binds 175-times less strongly to the high affinity binding site than Rolipram.



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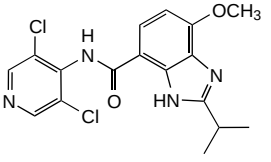
Another recent paper has described a new family of PDE4 inhibitors based on a benzimidazole framework [Regan, J. *et al. Bioorg. Med. Chem. Lett.* (1998) 8, 2737–2742].

Many of these compounds, such as **8** (IC_{50} = 27 nM), were found to be more potent inhibitors of the PDE4 than Rolipram (IC_{50} = 300 nM) *in vitro* whilst binding less strongly to the high affinity binding site (**8**, K_i = 24 nM; Rolipram, K_i = 5 nM). Several of these compounds, including **8**, had good oral bioavailability, and inhibited both tumour necrosis factor α (TNF-α) release in mice challenged with lipopolysaccharide and streptococcal cell wall-induced arthritis in rats.



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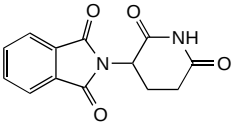
Although thalidomide (**9**) is known to inhibit TNF-α, the mechanism of this inhibition is unknown. Various groups have attempted to enhance the TNF-α inhibitory potency of thalidomide and eliminate/decrease its teratogenic properties. Celgene Corporation (Warren, NJ, USA) has previously reported a series of thalidomide-based analogues based on 3-amino-3-arylpropionic acids with enhanced TNF-α inhibitory potency [Muller, G.W. *et al. J. Med. Chem.* (1996) 39, 3238–3240].

A recent paper from this group has reported an investigation into the ability of these compounds to inhibit PDE4 [Muller, G.W. *et al. Bioorg. Med. Chem. Lett.* (1998) 8, 2669–2674]. These studies show that these compounds, exemplified by **10**, are potent inhibitors of PDE4 (**10**, IC_{50} = 130 nM).



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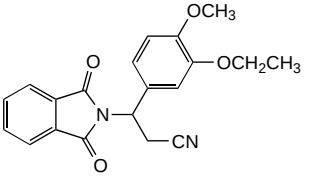

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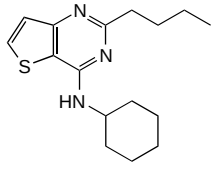
Although thalidomide was not shown to inhibit PDE4, it is possible that the TNF-α activity is a consequence of the inhibition of PDE4 by a thalidomide metabolite.

A fourth series of PDE4 inhibitors, reported by Crespo, M.I. and coworkers, is based on a nitraquazone pharmacophore [*J. Med. Chem.* (1998) 41, 4021–4035]. Of the compounds investigated, compound **11** has been shown



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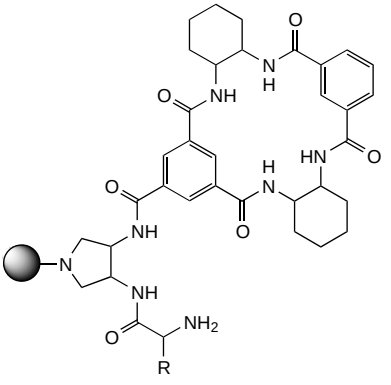
to have an interesting pharmaceutical profile and is presently being evaluated *in vivo* as a potential anti-asthmatic agent.

Combinatorial chemistry

Kinetic resolution by libraries

Ensuring that all chiral molecules are synthesized and tested as single enantiomers in biological systems is an essential requirement of modern drug discovery. Consequently, resolution of racemic materials, possibly using chiral HPLC, is often necessary to separate enantiomers. Combinatorial chemistry has recently been applied in the search for novel chiral selectors that will react enantiospecifically with racemic chemical compounds, specifically in this case, cyclic amino acid derivatives [Weingarten, M.D. *et al. J. Am. Chem. Soc.* (1998) 120, 9112–9113].

Using Still's encoded split synthesis method on resin beads, a small library of 60 selector molecules (**1**) containing a chiral amine were prepared. The beads containing these molecules were incubated with a mixture of L- and D-proline pentafluorophenyl esters to investigate whether the library compounds would selectively react with one of the two enantiomers by the formation of a new amide bond. To permit the recognition of beads that demonstrated such chiral discrimination, the two enantiomeric probe molecules were labelled with either a red or blue dye. Thus, if a bead contained a selector molecule that reacted enantiospecifically, it could be visually identified by a change of colour to red



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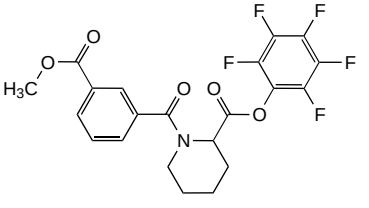
or blue. Beads that exhibited no chiral discrimination would be stained brown as they will have reacted equally with both enantiomers.

Running the reaction and by selecting the reddest and bluest beads, it was determined that resolution with enantiomeric excesses measured at 45–75% had been obtained. Subsequently, the preferred selector molecules were used in a kinetic resolution and filtration process to separate the enantiomers of related cyclic amino acid derivatives. Particular success with the homoproline (**2**) was reported in the paper, and there is every possibility of extending this methodology for the resolution of many other types of chiral molecule.



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High loading single resin beads

The mix-and-split combinatorial method is a highly effective way of generating combinatorial libraries of huge size suitable for the discovery of novel pharmacologically active compounds. As the quantity of material available on each bead is often a limiting factor, considerable effort has been invested into finding novel polymer beads with high loading. One solution to this problem has been Bradley's method by which TentaGel beads can be derivatized with a dendrimer linker that allows significant increases in loading levels.

A recent publication from Bradley's group describes the ability of these high-loading beads to generate sufficient material for full structural analysis [Wells, N.J. *et al. J. Org. Chem.* (1998) 63, 6430–6431]. The peptide Fmoc-Val-Phe-Ala-OH was prepared using standard peptide-coupling conditions on the HMP linker. Sufficient material was available from one resin bead to permit 500 MHz NMR by *in situ* cleavage of the peptide in the NMR tube using 1% F_3CCOOD in deuterochloroform. HPLC analysis revealed that ~32 nmol of essentially pure material had been prepared.

In a separate experiment a small library of 20 Leu-enkephalin analogues were prepared and analysed by HPLC and ESMS, demonstrating the robustness and versatility of these high-loading beads. One additional comment made in the paper was that chemical derivatization of the dendrimer-derivatized material generally proceeded rapidly and under non-forcing conditions.

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