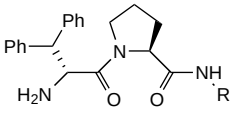
Combinatorial chemistry

Thrombin inhibitors with nonbasic P1 side-chains

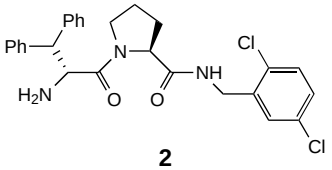
Last month a paper from Merck Research Laboratories [Brady, S.F. *et al. J. Med. Chem.* (1998) 41, 401–406] was featured, in which combinatorial chemistry had been used to find thrombin inhibitors with improved pharmacokinetics. More recently, the same team have published another approach to thrombin inhibitors seeking nonbasic residues in the P1 position [Lumma, W.C. *et al. J. Med. Chem.* (1998) 41, 1011–1013].

Inhibition of the serine protease, thrombin, has been selected as a method for the treatment of diseases characterised by inappropriate thrombus formation, such as deep vein thrombosis and stroke. Many inhibitors found so far, contain a basic P1 side-chain that interacts with an aspartic acid residue at the bottom of the enzyme recognition pocket. As inhibitors with guanidine and amidine functionality have poor oral absorption, medicinal chemists have focused on the search for non-basic P1 residues.

Solid-phase parallel synthesis was used to prepare several analogues of D-diphenyl-Ala-Pro (**1**). The Boc-protected dipeptide was synthesized on Kaiser resin and cleaved from the support by reaction with a diverse range of amines. SAR studies indicated that 2,5-lipophilic substituents were preferred and that 2,5-dichlorobenzylamide (**2**), incidentally synthesized using a traditional solution-phase route, was found to be the most potent (K_i = 3 nM).



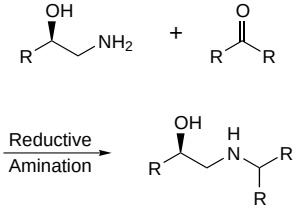
1



2

Library of adrenergic agents

Ligands that selectively modulate the response of β-adrenergic receptors have been successfully used to treat a range of medical conditions including angina, asthma and hypertension. It has been found that even slight modification of the central ethanolamine pharmacophore can have a dramatic effect on ligand potency or selectivity. With the intention of finding novel adrenergic ligands, an array of ethanolamine compounds (**3**) has been prepared using high-throughput solution-phase parallel synthesis [Siegel, M.G. *et al. Mol. Diversity* (1998) 3, 113–116].



3

A 96-compound array was synthesized in glass vials by reductive amination of 8 ketones with 12 ethanolamines. The reactions were driven to completion by the addition of excess ketone, and a key step in the process was the removal of all non-basic products by ion exchange chromatography. This purification step led to the majority of products subsequently being obtained in good yield and near-analytical purity. The methodology is currently being used for ethanolamine SAR studies.

Simultaneous measurement of binding constants

As combinatorial chemistry methods become more effective at the rapid generation of large compound libraries, there is a need to achieve a commensurate increase in screening throughput. Various methods have been proposed for the screening of mixtures, and one method, affinity capillary electrophoresis-electrospray ionization-mass spectrometry (ACE-MS) offers the promise of simultaneous measurement of multiple binding constants [Dunayevskiy, Y.M. *et al. J. Med. Chem.* (1998) 41, 1201–1204].

Affinity capillary electrophoresis (ACE) depends on the measurement of the mobility change of a ligand when eluted with the receptor present in the electrophoretic buffer. The technique offers the opportunity to examine several substances at the same time, provided that the analytes are separated from each other, and can be unambiguously identified. Furthermore, the technique does not require an accurate knowledge of ligand concentration, nor high purity of compounds or receptor.

This recent report describes the use of ACE-MS to examine the affinity of tetrapeptides for vancomycin, measuring the dissociation constants of Fmoc-DXYA, where X is any of the 19 common amino acids (excluding Cys). The K_d values determined, whilst not greatly differing from each other, were in close agreement with binding affinities determined with individual compounds, demonstrating the advantages of a method that can simultaneously analyse compounds in mixtures.

Nick Terrett
Discovery Chemistry
Pfizer Central Research
Sandwich, Kent, UK
fax: +44 1304 618422
e-mail: nick_terrett@sandwich.pfizer.com

Emerging molecular targets

Selective CB_2 cannabinoid antagonists – potential new immune modulators?

The enigmatic failure of the immune system to attack and destroy tumor cells is a major dilemma in cancer therapy. Examples for the critical role of the immune system in cancer are the increased likelihood of cancer occurring in individuals undergoing immuno-

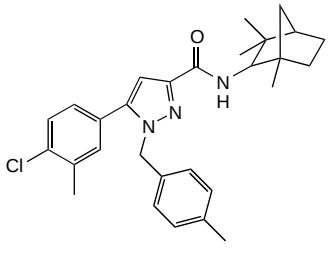
  1359-6446/98/$19.00. PII: S1359-6446(98)01214-8

suppression following organ transplantation, and in AIDS patients. Several innovative cancer therapies target the immune system; a notable current drug candidate being the cytokine interleukin-2 (IL-2), which is also a potential therapeutic target for treating AIDS and other retroviral diseases.

It has recently been suggested that endogenously produced cannabinoids, such as anandamide (arachidonylethanolamide) and 2-arachidonylglycerol, might participate in immune modulation via specific type 2 cannabinoid receptors (CB_2) located on B and T cells [Kaminski, N.E., *Biochem. Pharmacol.* (1996) 52, 1133–1140]. Activation of such CB_2 receptors on lymphocytes has been associated with decreased cyclic AMP production, and an associated reduced expression of IL-2, by lymphocytes.

The cannabinoid receptors, therefore, represent logical therapeutic targets for modulating immune response in cancer and AIDS patients. However, type 1 cannabinoid receptors (CB_1) are ubiquitously expressed in brain neurons and glia, heart muscle cells and organ associated endothelium. In addition, endogenous cannabinoids seem to be implicated in many physiological functions including cognition and blood pressure. Thus, blocking the action of endogenous cannabinoids without causing major side-effects is not a simple task.

However, Sanofi Recherché (Montpellier, France), which has pioneered cannabinoid drug research, has recently reported the discovery of SR144528 (**1**), the first selective CB_2 cannabinoid antagonist [Rinaldi-Carmona M. *et al. J. Pharmacol. Exp. Ther.* (1998) 284, 644–650]. This recent study showed SR144528 to be a highly potent [K_d for the cloned human CB_2 = 0.6 nM] and selective CB_2 antagonists, being 700-fold less potent for the cloned human CB_1 receptor. Moreover, it did not seem to interact with other identified receptors or ion channels. In addition, SR144528 was shown to be orally active in mice [ED_{50} = 0.35 mg kg^{-1}], blocking the binding of the tritium-labelled synthetic cannabinoid [^{3}H]-CP55940 in mouse spleen, but not in brain membranes. No doubt, this new drug will be a very valuable tool for clarifying the role of the endogenous cannabinoids in controlling the immune system, and may soon emerge as a prototype drug for modulating immune function.



1

David Gurwitz
National Laboratory for the Genetics of Israeli Populations
Sackler Faculty of Medicine
Tel-Aviv University
Tel-Aviv 69978, Israel
fax: +972 3 640 7611
e-mail: gurwitz@post.tau.ac.il

Development of new cholesterol-lowering drugs

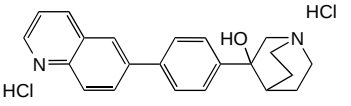
Cholesterol biosynthesis control

The inhibition of cholesterol biosynthesis constitutes an important approach to the reduction of LDL cholesterol, a key risk factor in coronary heart disease. The 'statins', a family of HMG-CoA reductase inhibitors, that are currently clinically used as cholesterol-lowering drugs, could in principle suppress all post-mevalonate biosynthetic pathways. Thus, the supplies of biologically important nonsteroidal isoprenoids (e.g. dolichol, ubiquinone, isopentenyl tRNA and prenylated proteins), which play important roles in regulation of normal cellular processes, would be compromised. Paradoxically, however, levels of HMG-CoA reductase tend to increase because of up-regulation of gene transcription and translation; clinically, this is counterbalanced by up-regulation of LDL-receptor levels leading to a net lowering of serum cholesterol. Nonetheless, selective inhibition of cholesterol biosynthesis is a desirable pharmaceutical goal, and enzyme inhibitors for three enzymes – squalene synthase (SS), squalene epoxidase (SE) and oxidosqualene cyclase (OSC), which are unique to cholesterol biogenesis – have been potential targets for the design of such therapeutic agents [Abe I. *et al. Natural Products Report* (1994) 11, 279–302].

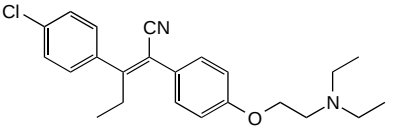
Squalene synthase inhibitors

SS catalyzes the first committed step in the *de novo* biosynthesis of cholesterol – the reductive dimerization of farnesyl diphosphate with NADPH to form squalene at the final branch-point of the isoprenoid biosynthetic pathway. Several compounds designed as substrate analogues or high-energy intermediate analogues and novel classes of natural products that inhibit SS have been reported. Many of these compounds have been shown to be potent and effective cholesterol-lowering agents in animal models. However, they could induce a marked increase in HMG-CoA reductase activity, resulting in an accumulation of a toxic farnesol-derived dicarboxylic acid. This was also the case for the recently disclosed RPR107393 (**1**), an orally effective and extremely potent SS inhibitor from Rhône-Poulenc Rorer (IC_{50} = 0.6 nM for rat liver SS) [Amin, D. *et al. J. Pharmacol. Exp. Therap.* (1997) 281, 746–752].

By contrast, P3622 (**2**), a diethylaminoethoxystilbene derivative from



1



2

  1359-6446/98/$19.00 PII: S1359-6446(98)01206-9