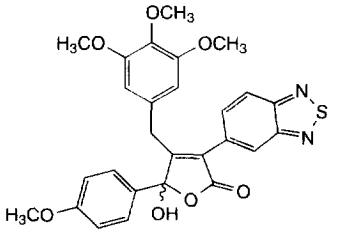
was **9**, which was found to have potent activity against Ras-activated tumour cells in culture and to inhibit tumour growth in a nude mouse xenograft model. This compound was also shown to have high selectivity for the Ras farnesyltransferase, only weakly inhibiting other enzymes that utilize prenyldiphosphate as a substrate, and it showed no toxicity against 3T3 mouse fibroblast cells at 100 μM.

Endothelin antagonists

The endothelins are a family of potent endogenous peptidic vasoconstrictor and pressor agents that appear to play important roles in several disease states including hypertension and heart failure. Over recent years a wide range of non-peptide endothelin antagonists have been reported by various groups. In general, the most potent of these compounds contain a methylendioxyphenyl group. This group is common in natural and synthetic medicinal compounds providing an electronegative function that is non-polar and relatively unreactive. However, this functional group undergoes cytochrome P450-mediated metabolism resulting in the irreversible binding of the substrate to haem iron of cytochrome P450. This metabolism may cause drug–drug interactions or nonlinear pharmacokinetics.

In an attempt to overcome this problem a group from Merck (Darmstadt, Germany) have used self-organizing neural networks to analyse the molecular electrostatic potentials of existing endothelin receptor ligands in order to identify a suitable bioisosteric group for methylendioxyphenyl [Anzali, A. *et al. Bioorg. Med. Chem. Lett.* (1998) 8, 11–16]. Using the Kohonen neural network to generate Kohonen maps of bioisosteric candidates, the group identified benzothiadiazole as a potential bioisosteric substitute.

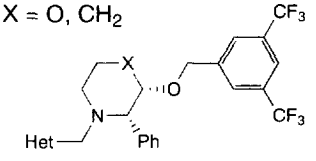
In a subsequent paper the group describes the synthesis and biological properties of a series of different methylendioxyphenyl, benzothiadiazole and benzofurazan derivatives [Mederski, W.W.K.R. *et al. Bioorg. Med. Chem. Lett.* (1998) 8, 117–122]. These studies confirmed the hypothesis of the use of a benzothiadiazole as a bioisoster of methylendioxyphenyl in the development of endothelin receptor antagonists and led to the discovery of EMD122801, the sodium salt of **10**, as a potent selective ET_A receptor antagonist (IC_{50} = 0.3 nM).



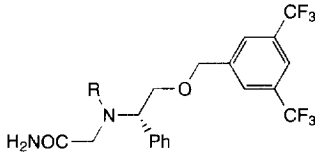
10

NK_1 receptor antagonists

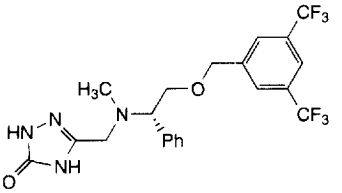
Recent studies have shown that the introduction of heterocyclic moieties into piperidine- and morpholine-derived human NK_1 receptor antagonists, to give compounds of type **11**, leads to improved potency *in vivo* and *in vitro*. Owens, A.P. and coworkers have extended this approach by investigating the effects of heterocyclic replacement of the carboxamido group in phenylglycinol-derived human NK_1 receptor antagonists (**12**) [*Bioorg. Med. Chem. Lett.* (1998) 8, 51–56].



11



12



13

The group substituted the carboxamido group with triazole, triazolinone and tetrazole heterocycles to give a series of compounds. Compound **13** was found to be the most potent (IC_{50} = 430 pM) and was shown to have excellent selectivity for the NK_1 receptor over the other neurokinin receptors (NK_2, NK_3; IC_{50} > 1 mM) and low-affinity binding to the calcium channel (IC_{50} > 1 mM). Studies in rats demonstrated that the compound has modest oral bioavailability on dosing at 3 mg kg^{-1} and has a plasma elimination half-life of 0.8 h following *in vivo* administration of the same dose. Studies *in vitro* have demonstrated that the major metabolic pathway involves *N*-demethylation, suggesting that oral bioavailability may be improved by replacing the *N*-methyl group with less metabolically labile substituents.

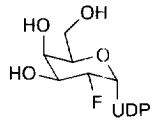
Combinatorial chemistry

Library analysis by mass spectrometry

Electrospray mass spectrometry (ESMS) has been successfully employed in the rapid analysis of combinatorial libraries of enzyme inhibitors [Wu, J. *et al. Chem. Biol.* (1997) 4, 653–657]. The enzyme in question was β-1,4-galactosyltransferase, responsible for the production of *N*-acetyllactosamine (LacNAc), a major component of glycoconjugates that function as cell-recognition molecules. All 20 compounds of an initial library were individually incubated with the enzyme, reactants and a standard. The ability of each compound to inhibit the enzyme was assessed by using MS to measure the ratio of the intensities of the product and reference ions.

It was found that the presence of a diphosphate was essential for inhibitory activity, with uridine derivatives being especially active. From the initial set of compounds, uridine-5′-diphospho-(2-deoxy-2-fluoro)galactose (UDP-2F-Gal) (**1**) was identified as the most potent.

1359-6446/98/$19.00. PII: S1359-6446(98)01173-8



1

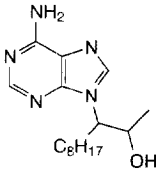
Varying the concentration of the inhibitors in the same MS experiment allowed the IC_{50} value to be determined as 119 μM.

Other experiments have demonstrated that mixtures of compounds can be simultaneously assessed against the enzyme using the ESMS method. Inactive mixtures appear as being totally inactive, while mixtures containing active components can be analysed by individual assessment of constituent compounds.

Adenosine deaminase inhibitor library

Solution-phase combinatorial libraries have been screened using a combination of pulsed ultrafiltration (PUF) and ESMS [Zhao, Y-Z. *et al. J. Med. Chem.* (1997) 40, 4006–4012]. PUF is an effective way of identifying ligands for a macromolecular target by pumping library compounds past a protein trapped by an ultrafiltration membrane in a flow-through cell. Compounds with high affinity for the protein target have their elution profile perturbed, and by coupling the cell to an ESMS the identity of the active ligand can be determined. Elution characteristics have also been used to calculate a range of classical binding parameters.

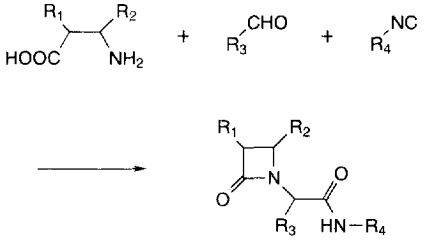
This approach has been used for the analysis of the binding of erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA) analogues to adenosine deaminase, an important enzyme in the inactivation of adenosine analogues used for the treatment of cancer and AIDS. From a mixture of eight analogues prepared without purification it was ascertained that three of the compounds bound to adenosine deaminase. Of these, one analogue (**2**) with an octyl side-chain had more than twofold greater affinity for the enzyme than EHNA itself.



2

β-Lactam library

Human leukocyte elastase (HLE) has degradative effects on lung elastin and has been implicated as a causative factor in several respiratory diseases including emphysema, bronchitis and cystic fibrosis. In the search for novel inhibitors of this enzyme, a library of β-lactams (**3**) has been prepared using a solution-phase combinatorial approach [Pitlik, J. and Townsend, C.A. *Bioorg. Med. Chem. Lett.* (1997) 7, 3129–3134]. The rationale for this library design is that the activated carbonyl of the β-lactam will acylate the key active-site serine residue resulting in enzyme inactivation.



3

A total of 126 compounds were prepared using the Ugi reaction, an approach that permits complex structures to be synthesized in one step. Three monomer sets consisting of seven β-amino acids, six isocyanides and three aldehydes were used to give library products as individual compounds analysed using electron ionization MS. Biological evaluation of the compounds against both HLE and chymotrypsin is ongoing.

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

High-throughput screening

Accessible UHTS?

Screening-based drug discovery is a numbers game; according to current doctrine, the more compounds examined, the more a company is likely to succeed. The definition of high-throughput screening currently hovers between 100 and 1,000 data points per day, and increasingly there is discussion of ultra-high-throughput screening (UHTS), in which up to 100,000 data points would be generated per day. So far, this level of screening has not been achieved for most companies. However, Zymark (Hopkinton, MA, USA) is beta-testing its new Allegro™ system, which it claims will conduct automated assays at a rate of 1,000 microtitre plates per 24 h period. Assuming the use of 96-well plates and the ability to run the system around the clock, this new technology may allow more companies to contemplate the UHTS format.

According to Zymark, the Allegro™ is designed to be an open architecture system that will facilitate 96- or 386-well microtitre plates, and will allow the insertion of new instrumentation, such as nanoliter dispensing equipment, as it is developed. This flexibility should allow the system to keep up with the rapidly developing technology in areas such as liquid handling and new assay design. The Allegro™ is composed of a series of interlinked modular workstations and includes a transfer station, 8-channel dispensers, storage, source, and final destination carousels, plate washers, 96-well dispensers and plate readers. The system is claimed to be extremely flexible and fully compatible with all commonly used assay technologies including ELISA and SPA.

Zymark highlights the increased level of UHTS that can be achieved using the Allegro™ system when used with 386-well (or higher density) microplates. However, the most compelling reason for looking at the system for many companies may be the ability to enter the world of UHTS without completely redesigning their cherished 96-well plate assays.

Robert W. Wallace

1359-6446/98/$19.00. PII: S1359-6446(98)01171-4