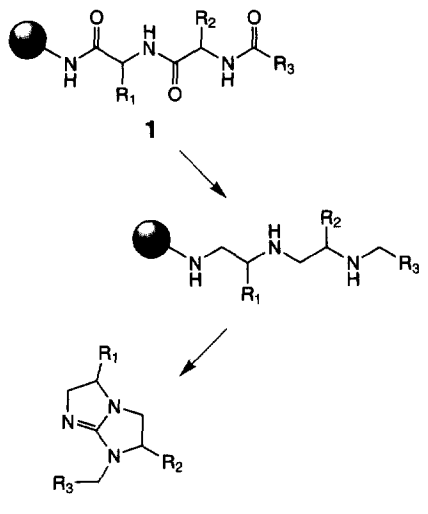
Combinatorial chemistry

Bicyclic guanidine libraries

A novel method for the solid-phase synthesis of bicyclic guanidines has been described in a recent publication [Ostresh, J.M. *et al.* (1998) *J. Org. Chem.* 63, 8622–8623]. Following the preparation of acylated dipeptides (**1**) on methylbenzhydrylamine-derivatize polystyrene resin, exhaustive reduction using borane-THF yielded triamines that could be cyclized with thiocarbonyldiimidazole. The bicyclic guanidine products (**2**) could be cleaved from the resin beads by treatment with hydrogen fluoride, although there was evidence that the products could also be cleaved with 100% trifluoroacetic acid. Steric factors had a key effect on the cyclization step: aminoisobutyric acid in the R_1 position led to complete cyclization, while in the R_2 position, cyclization was inhibited.



1

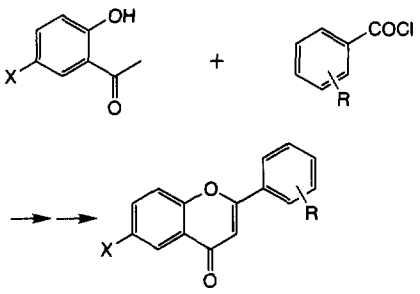
2

Guanidines are a common functionality in pharmacologically active compounds where their cationic nature is responsible for strong binding to negatively charged regions of target proteins. With three positions of variation, the synthetic approach above can generate large numbers of library products. The methodology was used to generate a combinatorial library using the positional-scanning format and using predetermined isokinetic ratios of the amino acid and carboxylic acid monomers. The products are currently being used to identify antibacterial and opioid receptor ligands.

Flavone benzodiazepine receptor ligands

Several naturally occurring flavonoids, in addition to the flavone nucleus itself, are known to be ligands of the central benzodiazepine receptors (BDZ-Rs). Synthetically modified derivatives with electron-deficient groups exhibit even higher affinity for the BDZ-Rs and have potent anxiolytic activity. An approach to small solution-phase libraries of flavone derivatives has been described [Marder, M. *et al.* (1998) *Biochem. Biophys. Res. Commun.* 249, 481–485].

The library of 36 flavone derivatives (**3**) was made by the solution reaction of mixtures of four 2′-hydroxyacetophenones with each of nine benzoyl chlorides. The mixtures of four products generated were separated by HPLC before screening against the BDZ-Rs.



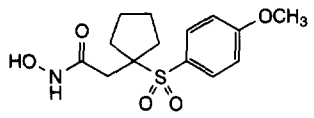
3

The compounds were screened against BDZ-Rs in cerebral cortex, which contains approximately equal proportions of both type I and II receptors. More active compounds were also tested against cerebellar membranes, which are enriched in the type I BDZ-R. Of the library screened, one compound (R = 3-Br, X = Br) had a K_i of 19 nM. Preliminary pharmacological experiments in mice demonstrated that this compound had anxiolytic activity without depressant effects following intraperitoneal administration at 0.1 and 0.3 mg kg^{-1}.

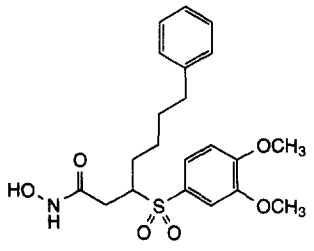
Multi-target libraries

Increasingly the design of combinatorial libraries has attempted to generate as diverse a set of compounds as possible with the intention of maximizing drug discovery success against a range of targets. Although the truly universal library may not be readily achievable, recent libraries have attempted to target several biological targets simultaneously. A recent contribution to this strategy is the preparation of a library that produced inhibitors of both matrix metalloproteinases (MMPs) and phosphodiesterases (PDEs) [Burns, C.J. *et al.* (1998) *Angew. Chem. Int. Ed.* 37, 2848–2850].

Using either solution- or solid-phase chemistry, two structurally related but functionally distinct β-sulphonyl hydroxamic acid libraries were prepared using as a key reaction the Michael addition of an aromatic thiol onto an α,β-unsaturated ester. Variation of three regions in the products allowed the synthesis of an initial library of >300 products tested against MMP-1, MMP-2, MMP-3 and PDE4. As an illustration of the selectivity of the products discovered, the cyclopentane derivative **4** was a selective MMP inhibitor (MMP-2 K_i = 10 nM),



4



5

1359-6446/99/$ – see front matter PII: S1359-6446(99)01327-6

while the phenylbutyl derivative **5** selectively inhibited PDE4 (IC_{50} = 1 nM).

Nick Terrett
Discovery Chemistry
Pfizer Central Research
Sandwich, Kent, UK
fax: +44 1304 655419
e-mail: nick_terrett@sandwich.pfizer.com

Inhibition of the proteasome as a therapeutic approach

In the late 1970s, research into proteolytic enzymes was considered one of the most uninteresting fields of research. Some of these enzymes worked with amazing specificity, but the destruction of the proteins appeared childishly simple compared with the complexity of other metabolic pathways.

The hydrolysis of a peptide bond is a thermodynamically favored reaction, even though proteolytic reactions requiring ATP hydrolysis have been known for 40 years [Hershko, A. (1996) *Trends Biochem. Sci.* 21, 445–449]. A major breakthrough was the discovery that the energy-dependent proteolytic system required the presence of a small, 76 amino acids long polypeptide, later named ubiquitin [Hershko, A. (1996) *Trends Biochem. Sci.* 21, 445–449; Ciechanover, A., Hod, Y. and Hershko, A. (1978) *Biochem. Biophys. Res. Commun.* 81, 1100–1105]. In 1980, Wilk and Orlowski reported the existence of a pituitary protease of unexpectedly high molecular mass and multiple activities [*J. Neurochem.* (1980) 35, 1172–1182], and that was linked to the ubiquitin system [Eytan, E. *et al.* (1989) *Proc. Natl. Acad. Sci. U. S. A.* 86, 7751–7755]. Following years have brought the dissection of this proteolytic pathway: it is characterized by energy consumption, it requires ubiquitin, ubiquitin activating and conjugating enzymes, and also the multicatalytic proteinase complex – which has been named the proteasome in 1988 [Arrigo, A.P. *et al.* (1988) *Nature* 331, 192–194].

The 1990s could as well have been named the decade of the proteasome, as it brought a boom of papers regarding the ubiquitin system. Scientists working in different fields, for instance antigen presentation [Tanaka, K. *et al.* (1997) *Adv. Immunol.* 64, 1–38], cell-cycle regulation [Pagano, M. *et al.* (1995) *Science* 269, 682–685; Glotzer, M., Murray, A.W. and Kirschner, M.W. (1991) *Nature* 349, 132–138; Hershko, A. (1997) *Curr. Opin. Cell Biol.* 9, 788–799], signal transduction [Pahl, H.L. and Bauerle, P.A. (1996) *Curr. Opin. Cell Biol.* 8, 340–347] and apoptosis [Wójcik, C. *et al.* (1997) *Apoptosis* 2, 455–462], discovered that they were all studying the same proteolytic machinery.

Proteasomes are present in all studied eukaryotic cells and account for up to 1% of total cell protein [Hendil, K.B. (1988) *Biochem. Int.* 17, 471–477]. Their structure was revealed through the study of archeabacterial enzymes. Similar complexes have also been discovered in eubacteria [Tamura, T. *et al.* (1995) *Curr. Biol.* 5, 766–774]. Much of our knowledge of the ubiquitin–proteasome system has been elucidated through the discovery and use of various inhibitors. Proscript (Cambridge, MA, USA) will shortly be initiating clinical trials of proteasome inhibitors for the treatment of malaria and cancer [Featherstone, C. (1997) *Mol. Med. Today* 3, 367; Adams, J. *et al.* (1998) *Bioorg. Med. Chem. Lett.* 8, 333–338]. Proteasome inhibitors are apparently effective anticancer drugs against mouse colon carcinoma cells (T. Stoklosa *et al.*, unpublished) and human Burkitt's lymphoma in nude mice [Orlowski, R.Z. *et al.* (1998) *Cancer Res.* 58, 4342–4348]. A new class of drugs is therefore appearing on the therapeutic horizon.

The ubiquitin–proteasome pathway has recently received attention in several excellent reviews by leading experts in the field [Coux, O., Tanaka, K. and Goldberg, A.L. (1996) *Annu. Rev. Biochem.* 65, 801–847; Varshavsky, A. (1997) *Trends Biochem. Sci.* 22, 383–387; Ciechanover, A. and Schwartz, A.L. (1998) *Proc. Natl. Acad. Sci. U. S. A.* 95, 2727–2730, (1998) *J. Biochem.* 123, 195–204]. Therefore, only the essentials will be discussed here.

Proteasomes – multicatalytic protease complexes

The 20S proteasome is an ~700 kDa complex, formed by four stacked rings: two inner β rings and two outer α rings, with a set of inner cavities (Fig. 1). The rings are arranged according to a C2 symmetry, so the proteasomes can be divided into two identical halves, each consisting of one α and one β ring. The rings of each type are composed of seven different subunits of the α- and β-family respectively. The yeast 20S proteasome has been crystallized and analysed at a 2.4 Å resolution [Groll, M. *et al.* (1997) *Nature* 386, 463–471].

The proteasome was characterized by the presence of different proteolytic activities, defined against small synthetic peptides as the chymotrypsin-like (ChTL), trypsin-like (TL), peptidyl-glutamylpeptide-hydrolyzing (PGPH), small neutral amino acids-preferring (SNAAP), and branched chain amino acid-preferring activity (BrAAP) [Orlowski, M. (1990) *Biochemistry* 29, 10289–10297]. These activities depend on the presence of a free N-terminal Thr residue on three of seven β-type subunits. This Thr acts as a nucleophile and is essential in the mechanism of the catalytic activity. In contrast with lower eukaryotes, mammalian genomes encode three more active β subunits, which can be expressed and incorporated into the 20S proteasomes after interferon γ (IFN-γ) stimulation.

1359-6446/99/$ – see front matter PII: S1359-6446(99)01324-0