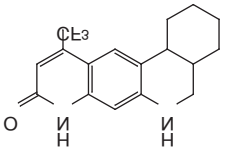
Nonsteroidal androgen receptor agonists

The androgens testosterone and dihydrotestosterone are important in male sexual and musculo-skeletal development. However, as the oral bioavailability of these compounds is poor, androgen deficiency is normally treated using transdermal patches or intramuscular injection. A recent paper from Ligand Pharmaceuticals Inc. (San Diego, CA, USA) describes the synthesis and evaluation of a series of androgen receptor agonists based on 4-alkyl-, 4,4-dialkyl- and 3,4-dialkyl-1,2,3,4-tetrahydro-8-pyridono[5,6-*g*]quinoline [Higuchi, R.I. *et al.* (1999) *Bioorg. Med. Chem. Lett.* 9, 1340–1355]. A number of compounds, exemplified by (**9**), were found to be as effective as agonists as dihydrotestosterone in both competitive receptor binding and androgen receptor cotransfection assays.



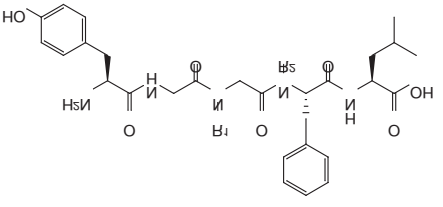
9

Profiles

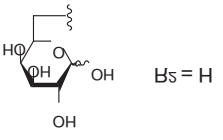
Sugar-based peptidomimetics

The opioid receptors, μ, δ and κ, and their subtypes, are involved in the control of various aspects of the perception of pain, pleasure and mood as well as the regulation of immune function. The development of selective opioid receptor ligands offers the potential for improving clinical treatments involving these systems. In the search for potent opioid ligands, the two endogenous opioid peptides, Leu- and Met-enkephalin (H-Tyr-Gly-Gly-Phe-Leu/Met-OH) make an ideal template and many selective and conformationally restricted analogues of those peptides have been prepared.

In the course of such studies, Horvat, Š. and coworkers [*J. Chem. Soc. Perkin Trans.* 1 (1998) 1789–1795] produced novel types of sugar-based peptidomimetics (**10,11**) related to the pentapeptide Leu-enkephalin, in which Gly^2 (**10**), or both Gly^2 and Gly^3 residues (**11**), were replaced by an *N*-alkylated glycine residue bearing a 6-deoxy-D-galactose moiety. The synthesis of the mono- and the bis-glycated pentapeptide were performed in a stepwise manner in solution by employing *N*-glycated glycine as the building block. The incorporated carbohydrate element in (**10,11**) offers applications in molecular recognition studies and might serve as a point of attachment (through the unsubstituted anomeric centre) for amino groups of proteins and other biologically active amines.

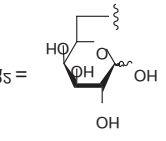


10 R_1 =



R_2 = H

11 $R_1 = R_2$ =



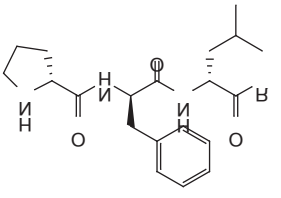
Jaroslav Horvat
Rudjer Boskovic Institute
Zagreb
Croatia
tel: +385 1 45 61 040
fax: +385 1 46 80 195
e-mail: jhorvat@rudjer.irb.hr

Combinatorial chemistry

A new fibrinogen receptor motif

The Arg-Gly-Asp tripeptide motif is well known to bind to the platelet gpIIbIIIa fibrinogen receptor and has been the basis for the design of a large number of novel non-peptidic inhibitors. Using combinatorial chemistry, a novel motif of unnatural amino acids has been discovered [Thorpe, D.S. *et al.* (1999) *Biochem. Biophys. Res. Commun.* 256, 537–541].

Having demonstrated that a bead-based library of pentapeptides of the structure Tyr-X-X-Asp-Val (where X is 1 of 19 L-amino acids) could be used to reveal the Arg-Gly-Asp motif through the staining of beads containing active sequences, the project moved on to explore unnatural peptide sequences. Using 18 D-amino acids plus glycine to generate an on-bead library of pentapeptides, the motif D-Pro(D-Phe/D-Tyr)D-Leu (**1**) was identified. The most active compound detected had an IC_{50} value of 14 μM.



1

Intriguingly, these compounds lacked the carboxylic acid of the Arg-Gly-Asp sequence that is presumed to bind calcium, and molecular modelling was employed to suggest a mode of molecular recognition. A reversed binding mechanism was noted, which is often observed with D-amino acid mimetics, and the model also proposed that π-electrons substituted for the carboxylic acid of Arg-Gly-Asp. This library discovery offers a number of new opportunities for the design and synthesis of novel integrin inhibitors.

Novel screening methodology

A novel screening method for combinatorial libraries has been employed in the detection of pentapeptides that bind weakly to tryptophan [Sugimoto N. *et al.* (1999) *J. Chem. Soc. Chem. Commun.* 677–678]. This new method

 1359-6446/99/$ – see front matter PII: S1359-6446(99)01381-1

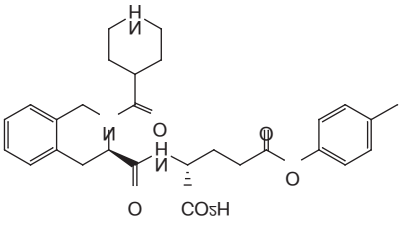
used the very high affinity between avidin and biotin ($K_a = 10^{15}$ M^{-1}) by initially tethering tryptophan to biotin through a hydrophilic linker.

The first step of the two-step screening process involved incubation of the biotinylated tryptophan with beads containing approximately 2.5 million different pentapeptide sequences constructed from a set of 19 amino acids. The second step was incubation of the beads with fluorescein-labelled streptavidin. Only those beads containing peptide sequences that had already bound tryptophan would have biotin exposed, which was therefore available to bind to streptavidin. By pouring the beads onto a plate and examining them under UV light, beads that contained the avidin–biotin complex were detected by their fluorescence and were selected for peptide sequencing. From the beads isolated, peptide sequencing revealed the consensus sequences Tyr/His-Gly-Gly-Tyr and His-Pro-Gly-His.

A protease inhibitor library

The flagellated protozoan, *Trypanosoma cruzi,* is the causative agent of Chagas disease, a debilitating and incurable illness prevalent in Latin America. As the parasite requires access to a broad range of tissue types by penetration through the fibrous extracellular matrix, it has been postulated that specific proteases are expressed to aid this process. Indeed, a 80 kDa protease has been identified, and a recent publication describes the use of combinatorial chemistry in identifying new inhibitors of this protease [Vendeville, S. *et al.* (1999) *Chem. Pharm. Bull.* 47, 194–198].

Two orthoganol, self-deciphering peptide libraries, each of 15625 tripeptides in 125 mixtures, were synthesized in solid-phase from 23 D-amino acids and 2 non-chiral amino acids. Following HF(hydrogen fluoride)-catalyzed cleavage from the solid support, purification and then analysis, the highest level of protease inhibition observed originated from the cleavage by-product, H-Ipe-D-Tic-D-Glu(*S*-paratolyl)-OH (**2**).



5

This compound has an IC_{50} value of 12 μM and introduces a new class of structures for further investigation of the role of this protease in the biology of *T. cruzi.* The compound and close analogues are being studied for their selectivity over other prolyl endopeptidases.

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

Targeting protein–protein interactions: the HIV protease

The retroviral genome is translated as polyprotein fusions that require further processing by proteases. As protease function is necessary for the proper maturation of infectious virions, inhibition of the dimeric HIV-encoded protease immediately offered an attractive therapeutic target. This was aided by the viral enzyme's homology to aspartyl proteases such as renin, while its unique substrate specificity boded well for selective inhibitors. The subsequent development of drugs based on transition-state analogues [reviewed by Vacca, J.P. and Condra, J.H. (1997) *Drug Discovery Today* 2, 261–272] has been a great success for rational and structure-based drug discovery, and has revolutionized the treatment of HIV patients by combination therapy.

Another means of targeting the HIV protease is by disrupting its assembly into a dimer (which occurs with a nanomolar dissociation constant). Because the active site is composed of an aspartate residue from each monomer, dimerization is essential for enzyme activity. The dimerization interface is largely formed by interdigitation of N- and C-terminal residues in a four-stranded, antiparallel β-sheet. Attempts at blocking dimerization are much less developed than active-site occupancy by substrate or transition-state mimetics. One advantage of the dimerization strategy is the heavily conserved nature of the interface, involving cooperative interactions between the N- and C-terminal of each monomer. Thus, viral resistance may be harder to develop by single point mutations relative to active site inhibitors.

J. Chmielewski's group has extensively studied [reviewed in *Synlett* (1998) 1040–1044] tethered peptides based on the protease terminal sequences as mimics of the interface. These peptides are inhibitors of protease activity at micromolar concentrations, and act by affecting the monomer–dimer equilibrium as shown by size-exclusion chromatography, protein crosslinking, and fluorescence spectroscopy. As an alternative approach, H. Schramm and coworkers [*Biochem. Biophys. Res. Commun.* (1996) 227, 484–488; *AIDS* (1998) 12, 682–685] have screened crystallographic databases for potential compounds that bind to the dimerization interface. Several triterpenoids and steroids were identified with micromolar activity.

Extensive screening of natural product extracts was largely unsuccessful in generating non-peptide leads for protease inhibition. A recent paper by Daniel Rich's group [*J. Am. Chem. Soc.* (1998) 120, 8893–8894], however,

 1359-6446/99/$ – see front matter PII: S1359-6446(99)01380-X