Emerging molecular targets

Exploiting nutraceutical treatments for osteoarthritis and ischaemia

The regulation of the nutraceutical industry has received significant attention from lobbying organizations such as the American Association for Pharmaceutical Scientists over recent years. A recent review on the therapeutic nutraceutical treatments for osteoarthritis and ischaemia by Grant, G.F. and Gracy, R.W. illustrates the potential therapeutic value of these products[1].

The review focusses on nutraceuticals that share common biochemical pathways such as glucosamine, ribose and their derivatives. It would appear that in aged individuals, the cellular regulation of the hexose monophosphate pool limits the production of cellular energy and cartilage in various tissues. The administration of ribose and glucosamine circumvents this regulation through their direct involvement in biochemical pathways for cellular energy maintenance and the repair of cartilage and connective tissues, respectively, in active middle-aged individuals. Oral ribose restores cellular energy lowered by ischaemia that occurs following, for example, myocardial infarction. Meanwhile, oral glucosamine alleviates the symptoms of osteoarthritis by stimulating the synthesis of glycoaminoglycans (GAGs) and thereby facilitating the repair of cartilage and connective tissue. The review concludes by suggesting that, in the future, the success of patented nutraceuticals in the treatment of osteoarthritis and ischaemia will facilitate targeted pharmaceutical intervention to overcome the rate-limiting pathways involved in GAG and ATP synthesis.

The demand for supplements containing these agents will undoubtedly rise with increasing average life expectancy. Furthermore, future evaluation of other biochemical pathways will undeniably lead to the identification of other supplements that might overcome biochemical regulation of cellular pathways in the aged individual, offering improved quality and longevity of life.

1 Grant, G.F. and Gracy, R.W. (2000) Therapeutic nutraceutical treatments for osteoarthritis and ischaemia. *Exp. Opin. Ther. Patents* 10, 39–48

Andrew Lloyd

Combinatorial chemistry

Small-molecule–RNA interactions

The interactions between proteins and nucleic acids are crucial to many biological functions including transcription, RNA splicing and translation. The ability to design selective and potent small compounds that can bind to RNA and DNA is a significant step in the discovery of novel drug molecules. A recent study describes the use of encoded combinatorial libraries in the discovery of novel anti-HIV-1 agents[1].

The transcriptional upregulation of HIV-1 gene expression depends on the binding of the TAT protein to the transactivation response region (TAR) RNA. This is a 59-base stem-loop sequence at the 5′-end of all nascent HIV-1 transcripts. An encoded library of 24,839 possible tripeptide sequences synthesized using all D- and L-alpha amino acids has been prepared on TentaGel resin and incubated with disperse red-labelled TAR. Beads that became red or pink were sequenced by analysis of the encoding molecules by electron-capture gas chromatography.

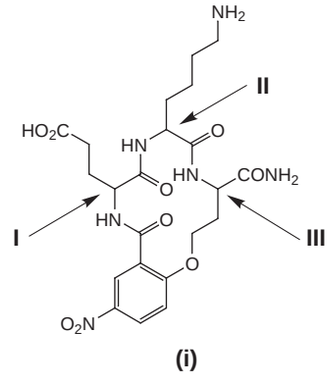
The two most potent tripeptide sequences discovered were (L)Lys-(D)Lys-(L)Asn and (D)Thr-(D)Lys-(L)Asn, suggesting a consensus sequence of X-(D)Lys-(L)Asn. Further analysis revealed that diastereoisomers of the first ligand were much weaker binders indicating that the interaction with TAR is highly stereospecific, and not merely the result of a non-specific Lys– Phosphate interaction. (L)Lys-(D)Lys-(L)Asn was shown to suppress transcriptional activation by the TAT protein in human cells with an IC_{50} value of approximately 50 nM.

1 Hwang, S. *et al.* (1999) Inhibition of gene expression in human cells through small molecule–RNA interactions. *Proc. Natl. Acad. Sci. U. S. A.* 96, 12997–13002

Stereochemical diversity

The focus on diversity in combinatorial libraries is primarily on factors such as MW, lipophilicity, the numbers and types of hydrogen bonds, and the presence of key pharmacophores. However, stereochemistry and conformation also contribute considerably to the assessment of diversity and have been the subject of a recent publication[2].

To prepare libraries of peptidomimetic turn-mimics, targeted for example at nerve growth factor (NGF), it became apparent that conformational diversity would be maximized by the incorporation of D-amino acids. As D-amino acids might be more expensive to use than L-amino acids, compounds that varied the I, II and III positions of compound (**i**) were investigated to explore the impact on conformational diversity. Using circular dichroism and NMR, the overall conclusion was that stereochemical variation of the II position led to the greatest effects



(i)

on conformational diversity.

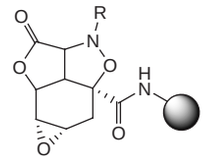
2 Feng, Y. *et al.* (1999) Stereochemical implications on diversity in β-turn peptidomimetic libraries. *J. Org. Chem.*

 1359-6446/00/$ – see front matter PII: S1359-6446(00)01475-6

64, 9175–9177

Chemical genetic assays

The function of proteins in specific biochemical pathways might be explored by genetic experiments that induce mutations in genes. The chemical genetic approach is complementary in that it uses small molecules that directly activate or inactivate specific proteins. In the search for molecules that can bind to target proteins, the synthesis of large libraries of compounds having similar



(ii)

structural features to natural products has been employed[3].

A tetracyclic template (**ii**) was constructed from (−)-shikimic acid attached via a photolabile linker to TentaGel resin, and was used to prepare a binary-encoded library calculated to contain 2.18 million compounds. A trial library set of these compounds was screened in cellular assays and demonstrated significant inhibitory effects on mink lung cell proliferation. Furthermore, a selected combination pool of the compounds activate a TGF-β-responsive reporter gene in a stably transfected mink lung cell line, and the key active compounds were then identified.

3 Tan, D.S. *et al.* (1999) Synthesis and preliminary evaluation of a library of polycyclic small molecules for use in chemical genetic assays. *J. Am. Chem. Soc.* 121, 9073–9087

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

About *Monitor*...

Monitor provides an insight into the latest developments in the fields allied to drug discovery through brief synopses of recent publications and presentations together with expert commentaries on the latest technologies. There are two sections:

Molecules summarizes the chemistry, 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, new technologies, emerging molecular targets, novel strategies and legislative issues. We welcome topical contributions for inclusion as Profiles. Articles of approximately 500–1000 words in length should provide an accurate summary of the essential facts together with an expert commentary to provide a perspective. Authors should be aware that articles for publication in Monitor are subject to peer-review, and occasionally Monitor articles might be rejected or, as is more likely, authors could be asked to revise their contribution. Articles might also be edited after acceptance. Proposals for articles should be directed to: Dr Andrew W. Lloyd, Monitor Editor, Department of Pharmacy, University of Brighton, Moulsecoomb, Brighton, UK BN2 4GJ. tel: +44 1273 642049, fax: +44 1273 679333, e-mail: A.W.Lloyd@brighton.ac.uk

pfizer.com