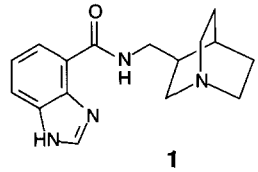
Monitor: molecules, synthesis and profiles

Monitor provides an insight into the latest developments in drug discovery through brief synopses of recent presentations and publications together with expert commentaries on the latest technologies. There are three sections: *Molecules* summarizes the chemistry and the pharmacological significance and biological relevance of new molecules reported in the literature and on the conference scene; *Synthesis* outlines the latest advances in synthetic and separation techniques, approaches to the total synthesis of natural products of pharmaceutical relevance and the screening of new chemical entities; *Profiles* offers commentary on promising lines of research, emerging molecular targets, novel technology and legislative issues.

Selective 5-HT_3 receptor ligands

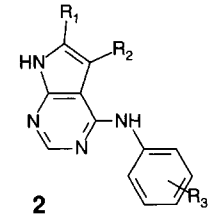
5-HT_3 receptor antagonists are now clinically used for the treatment of chemotherapy-induced emesis, and are being evaluated for use in the treatment of other therapeutic indications, such as anxiety and cognitive disorders. However, many of the 5-HT_3 receptor antagonists also have affinity for the 5-HT_4 receptor. This has recently led to the need to identify more potent and selective ligands for both these subclasses. López-Rodriguez, M.L. and coworkers [*Bioorg. Med. Chem. Lett.* (1996) 6, 1195–1198] have reported the synthesis and evaluation of a series of novel benzimidazole-4-carboxylic acid derivatives as potential 5-HT_3 receptor ligands. These studies identified **1** as having high affinity for the 5-HT_3 receptor (K_i = 3.7 nM) with minimal affinities for the 5-HT_4 (K_i > 1,000 nM) and 5-HT_{1A} (K_i > 10,000 nM) receptors. Preliminary studies were also undertaken on this compound using the two-compartment (light–dark) behavioural test, which suggested that the compound is a 5-HT_3 antagonist.



1

EGF-receptor protein kinase inhibitors

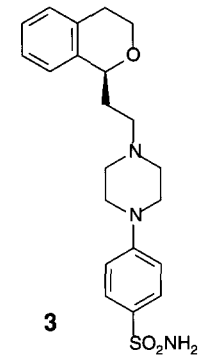
The epidermal growth factor (EGF) receptor protein tyrosine kinases have been suggested as potential therapeutic targets for the treatment of various epithelial diseases. A group from Ciba Pharmaceuticals [Traxler, P.M. *et al. J. Med. Chem.* (1996) 39, 2285–2292] have identified the 4-(phenylamino)pyrrolopyrimidines, such as **2**, as a novel class of potent tyrosine kinase inhibitors that selectively inhibit the EGF-mediated signal transduction pathway. Such compounds may have use as potential anticancer agents.



2

Selective D_4 antagonist

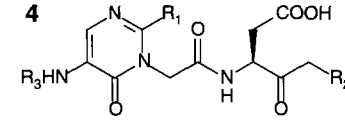
Selective dopamine D_4-receptor antagonists are widely sought after as potential therapeutic agents for the treatment of schizophrenia and other psychotic disease states. TenBrink, R.E. and coworkers [*J. Med. Chem.* (1996) 39, 2435–2437] have identified (*S*)-(–)-4-{4-[2-(isochroman-1-yl)ethyl]-piperazin-1-yl}benzene sulphonamide (**3**) as a potent, selective dopamine D_4 antagonist with an oral bioavailability of 76% in the monkey and a half-life of 13.6 hours. This compound has been progressed into phase II clinical trials for the treatment of schizophrenia, and will also serve as a useful tool for the evaluation of the role of D_4 receptors under normal and pathological conditions.



3

Peptidomimetic inhibitors of ICE

Interleukin-1β converting enzyme (ICE) offers potential as a therapeutic target for the control of inflammation elicited by the interleukin-1β (IL-1β) cytokine. Workers from Sanofi Winthrop Inc. [Dolle, R.E. *et al. J. Med. Chem.* (1996) 39, 2438–2440] have reported the first examples of peptidomimetic inhibitors of ICE, all derivatives of amide **4**. Some of these compounds were found to have second-order rate constants comparable with peptide-based ICE inhibitors, which have been reported previously [Dolle, R.E. *et al. J. Med. Chem.* (1995) 38, 220–222]. These compounds may form the basis for the development of some orally active agents to evaluate the therapeutic potential of ICE inhibitors for the treatment of inflammation in the clinic.



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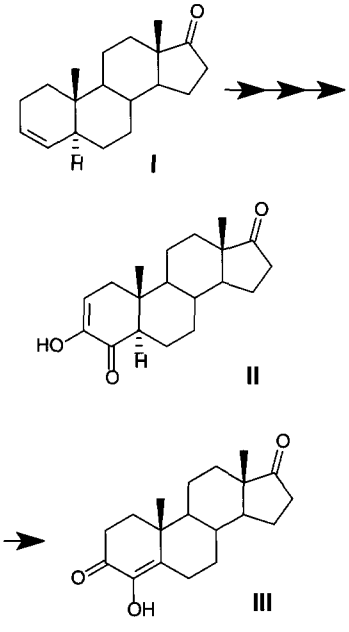
Novel approach to the synthesis of 4-hydroxyandrost-4-ene-3,17-dione

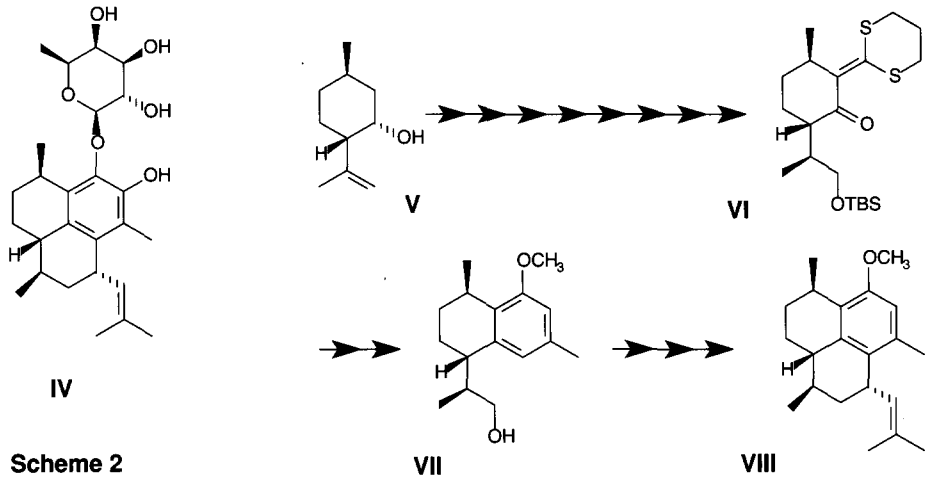
The inhibition of oestrogen biosynthesis by the use of cytochrome P450 aromatase inhibitors has application in the treatment of hormone-dependent diseases. In particular, the steroid inhibitor 4-hydroxyandrost-4-ene-3,17-dione **III** has been shown to be effective against breast cancer. Tavares de Silva, E.J. and coworkers [*J. Chem. Soc., Perkin Trans. 1* (1996) 1649–1650] have reported a novel, efficient approach to the synthesis of this aromatase inhibitor through the oxidation of the available 5α-androst-3-en-17-dione **I** to the diosphenol **II**, which yields the required inhibitor **III** on base-catalysed isomerization (Scheme 1).

Scheme 1



Pseudopterosins

The pseudopterosins, which include **IV**, are a family of diterepene pentose glycosides, which have been shown to have potent anti-inflammatory and analgesic activity. The potential medicinal use of these compounds, which were originally isolated from the Caribbean sea whip *Pseudoterogorgia elisabethae*, has driven the search for approaches to their total synthesis. Gill, S. and coworkers [*Chem. Commun.* (1996) 1743–1744] have reported an elegant synthesis of the tricyclic core (**VIII**) of the pseudopterosins from readily available (1*S*,2*S*,5*R*)-neoisopulegol **V**, using the hitherto unreported cyclization of a dithioacetal **VI** to an arene **VII** (Scheme 2).

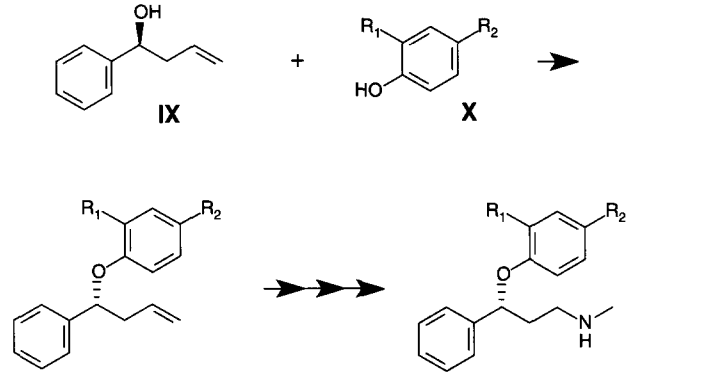


Scheme 2

Chemoenzymatic synthesis of (*R*)-fluoxetine

Fluoxetine (Prozac®) is a selective serotonin re-uptake inhibitor widely used for the treatment of depressive disorders. Although the fluoxetine is marketed as a racemate, studies have indicated that the (*R*)-enantiomer may be more effective than the (*S*)-enantiomer [Robertson, D.W. *et al. J. Med. Chem.* (1988) 31, 1412–1417]. Enantioselective syntheses using various strategies have therefore recently been proposed for the preparation of the enantiomerically pure (*R*)-fluoxetine **XI**. Bracher, F. and Litz, T. [*Bioorg. Med. Chem.* (1996) 4, 877–880] have described the use of (*S*)-1-phenyl-3-buten-1-ol (**IX**) as a building block for the synthesis of (*R*)-fluoxetine **XI** and the related (*R*)-tomoxetine **XII**. The butenol **IX** was obtained in high optical purity using a lipase-catalysed enantio-selective transesterification to acetylate the unwanted enantiomer using vinyl acetate as the acetate donor. (*R*)-Fluoxetine **XI** and (*R*)-tomoxetine **XII** were obtained by treatment of the butenol **IX** with the appropriate phenols **X** under Mitsunobu conditions, ozonolysis of the terminal alkene, mesylation of the resultant alcohol and substitution of the mesylate with methyl amine (Scheme 3). This synthesis provides a relatively simple, cost-effective route for the preparation of these drugs in an enantiomerically pure form.

Scheme 3

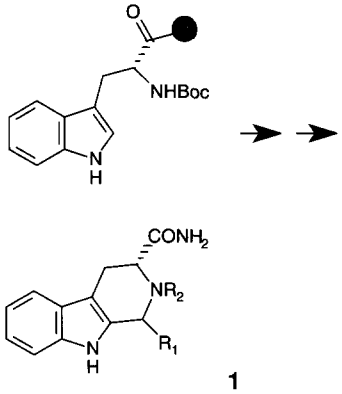


XI R_1=H, R_2=CF_3
XII R_1=CH_3, R_2=H

Combinatorial chemistry

1,2,3,4-Tetrahydro-β-carboline systems

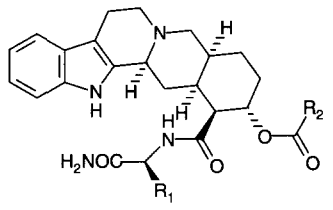
Early descriptions of library synthesis invariably focused on the preparation of linear oligomers. However, so many drug molecules contain heterocycles that it is no surprise that there is now a veritable flurry of papers describing the synthesis of these molecules. The 1,2,3,4-tetrahydro-β-carboline system **1** has been prepared on solid-phase using the Pictet–Spengler reaction [Mohan, R. *et al. Tetrahedron Lett.* (1996) 37, 3963–3966]. Reaction of tryptophan, attached to Kaiser oxime resin, with aldehydes gave the β-carboline product. Further diversity in these compounds was obtained by the derivatization of the amine with acid chlorides, isocyanates or sulphonyl chlorides. Ammonia-induced cleavage gave these useful heterocycles as the carboxamide derivatives.



1

Combinatorial libraries of Rauwolfia *alkaloids*

To increase the chances of finding active compounds in biological assays, combinatorial chemists are continually seeking novel templates upon which to build libraries. An original idea from Jeffrey Jacobs' group at Affymax is the synthesis of library compounds derived from the *Rauwolfia* alkaloids [Atuegbu, A. *et al. Bioorg. Med. Chem.* (1996) 4, 1097–1106]. Two libraries were prepared using the mix-and-split method, generating 792 analogues of both yohimbine and rauwolscine through derivatizing the E-ring carboxylate with amino carboxamides and acylating the E-ring hydroxyl. The rauwolscinamide library **2** was prepared using an encoding method, employing the simultaneous attachment of dialkylamine tags to allow ready identification of the most active library constituents.



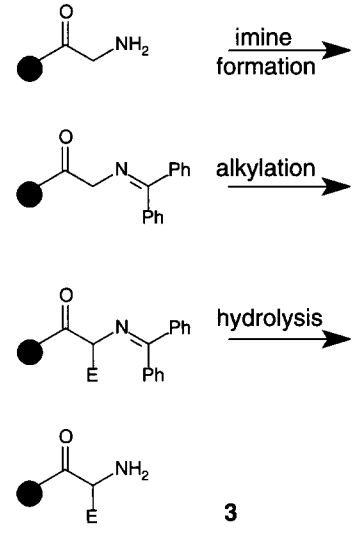
2

Using fluorous solvents to purify solution libraries

The rise in interest in solid-phase chemistry stems from the opportunity it provides to purify by simple filtration. Indeed the whole of combinatorial chemistry is being driven by methodology that permits easy separation of products. A recent article explores the potential for liquid-phase separation techniques to enhance solution library methodology [Curran, D.P. *Chemtracts – Organic Chemistry* (1996) 9, 75–87]. The fact that perfluorinated (fluorous) solvents are generally immiscible with routinely used organic solvents permits the separation of organic library products from specially designed fluorous reagents. This advantageous property is exemplified by the radical debromination of adamantyl bromide using a fluoroalkyl tin hydride. Following reaction, the tin by-products were extracted into a fluorous solvent and inorganics extracted into water, while the product adamantane was left in the methylene chloride fraction. Curran proposes that with a suitable collection of fluorous reagents, much library chemistry could be done in solution with rapid and simple isolation of the products by a liquid–liquid extraction.

Utilization of unusual aminoacids in combinatorial peptide libraries

Peptide synthesis is well-explored on solid-phase, and the diversity of peptide libraries has been limited solely by the available range of amino acids. In a novel twist to the synthesis of unnatural peptides, O'Donnell has transferred his methodology for the synthesis of unusual amino acids to solid-phase [O'Donnell, M.J. *et al. J. Am. Chem. Soc.* (1996) 118, 6070–6071]. Solid-phase bound glycine was activated by the formation of the benzophenone imine **3**. This derivative was deprotonated by iminophosphorane 'Schwesinger bases' and alkylated with a range of electrophiles to give unnatural amino acids. The use of mild reagents and room temperature conditions permits this process to be developed further to allow the automated synthesis of unnatural peptide libraries.



3

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Emerging molecular targets

Proteasomes and inducible nitric oxide synthase

An inducible form of nitric oxide synthase (iNOS) is responsible for production in immune cells of high levels of nitric oxide, a mediator of inflammation. A recent report by Jeanette Griscavage, Sherwin Wilk and Louis Ignarro from the University of California School of Medicine (Los Angeles, CA, USA) suggests that iNOS is induced by a mechanism involving proteolysis of the NF-κB–I-κB complex by the proteasome pathway [*Proc. Natl. Acad. Sci. U. S. A.* (1996) 93, 3308–3312].

Using what are claimed to be relatively selective peptidyl aldehyde protease inhibitors, the investigators found a close correlation between blockage of NF-κB activation, inhibition of iNOS

transcription, and inhibition of nitric oxide generation in the macrophage. Because the most potent protease inhibitor used in the studies, Z-IE(O-t-Bu)A-leucinal, is relatively specific for the proteasome, the investigators propose that activation of NF-κB, leading to nitric oxide production, is triggered by the proteasome. The proteasome is a large extralysosomal, macromolecular complex with proteolytic activity that is found in many cells, plays an important role in the turnover of both normal and abnormal proteins, and has been shown to activate certain transcription factors. If the investigators of this report are proven correct, the proteasome may prove to be a useful target for the discovery of a new class of compounds to control nitric oxide levels.

HIV-1 Nef binding

The HIV-1 *nef* gene codes for a protein that is essential for the progression of HIV-1 infection. The biological function of the Nef protein is unknown; no catalytic activity has been detected. But it binds to the SH3 domain of the Src family of kinases. It seems very likely that this binding is a critical aspect of Nef biochemistry that somehow permits or promotes the normal progression of HIV-1 infection. If this hypothesis is correct, it follows that a compound that blocks the interactions of Nef with SH3 domains might be useful as a therapeutic agent to halt the progression of AIDS.

A major puzzle regarding the binding of Nef to SH3-containing kinases is the lack of high-affinity binding of Nef peptides that are known to harbor the SH3-binding site. Intact Nef binds very tightly with a K_d value in the range of 0.25 to 0.38 μM depending upon the particular kinase. The Nef peptides, on the other hand, have affinities that are orders of magnitude lower. One explanation is that other regions of the Nef protein are required to hold its SH3-binding domain in just the right conformation for high-affinity binding. Another more intriguing proposal is that a second domain on the Nef protein also interacts with the kinase and contributes much of the high-affinity binding.

Evidence for the latter explanation has now emerged based on the recently published crystal structure by Chi-Hon Lee and coworkers at The Rockefeller University (New York, NY, USA) of the core of the Nef protein complexed with the Fyn tyrosine kinase [*Cell* (1996) 85, 931–942]. The structure shows that Nef possesses the expected polyproline type II helix with a pxxp sequence that forms an interface with the SH3 domain. Other regions of the Nef protein also interact with the kinase, including a hydrophobic pocket that interfaces with an isoleucine residue of the SH3 domain in Fyn. This residue is known to be variable in different SH3 domains and the crystallographers propose that its interaction with the hydrophobic cleft of the Nef protein provides a large degree of binding specificity.

The large binding domain between Nef and Fyn, estimated to be in the 1200 Å^2 range, may suggest that it will be very difficult to find a small molecule capable of disrupting the interaction. On the other hand, the detailed picture provided by the crystal structure may provide the necessary details to guide rational drug design efforts in discovering such a molecule.

Ezrin and ICAM-2 recognition by NK cells

Blocking the function of adhesion molecules on the surface of lymphocytes is a favorite paradigm for the development of new immunomodulating agents. However, with the exception of antibodies, this approach has had little success so far. Now, a recent paper by Tuula Helander and coworkers at the University of Helsinki (Finland) suggests that the elements of the cytoskeleton that hold the adhesion proteins in a correct orientation for binding may be a viable target for drug discovery [*Nature* (1996) 382, 265–268].

The Helsinki group observed that mouse thymoma BW5147 cells become highly susceptible to attack by natural killer (NK) cells when they are hybridized with cells containing human chromosome 6. The attack by NK cells could be blocked with antibodies against the CD11a/CD18 integrin and ICAM-2. Localization studies revealed that ICAM-2 is on the surface of the mouse thymoma cells, and flow cytometry showed that there is no difference in the absolute amount of ICAM-2 on the surface of the cells resistant or susceptible to NK cell attack. However, the cells resistant to NK attack had ICAM-2 dispersed evenly over their surface, while the cells susceptible to attack by NK cells had ICAM-2 distributed as intense patches on the uropods of the cell. The investigators concluded that the distribution of ICAM-2 in patches on the uropods is the key to triggering attack by NK cells and that some protein encoded by a gene on human chromosome 6 is responsible for the change in distribution of ICAM-2 on the surface of the hybrid mouse thymoma cells.

Checking the identity of the genes known to be present on human chromosome 6 revealed the gene for ezrin, a cytoskeletal protein. When the investigators transfected an antisense DNA for ezrin into the cells, ICAM-2 was redistributed evenly over the cell surface and the mouse cell hybrids were no longer susceptible to attack by NK cells. Their data provide some of the best evidence that cytoskeletal proteins such as ezrin may prove to be fruitful targets for the discovery of new immunomodulating drugs.

Robert W. Wallace

About Bob Wallace...

Bob Wallace, a regular contributor to *Drug Discovery Today*, is a freelance biomedical writer, editor and symposium organizer based in New Milford, CT, USA. He is also the publisher and editor of the *Biomedical Meetings Index*, a comprehensive guide to meetings and short courses in the biomedical sciences. His professional experience includes: Biochemistry Section Leader, Boehringer Ingelheim Pharmaceuticals, Inc., Danbury, CT; Director, Biomedical Marine Research, Harbor Branch Oceanographic Institute, Fort Pierce, FL; and Professor of Pharmacology and Cell Biology at the University of Alabama at Birmingham.

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