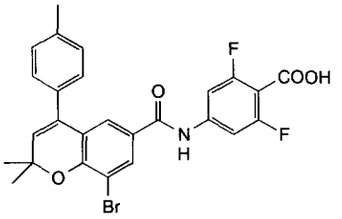
retinoid receptor characteristics of a novel series of α-retinoic acid receptor antagonists, with compound **15** being the most selective in both binding and functional antagonism assays [Teng, M. *et al. J. Med. Chem.* (1997) 40, 2445–2451]. These compounds will have particular use as tools to further our understanding of the physiology associated with this particular RAR subtype and may also serve as useful agents for the treatment of diseases associated with this particular receptor subtype.



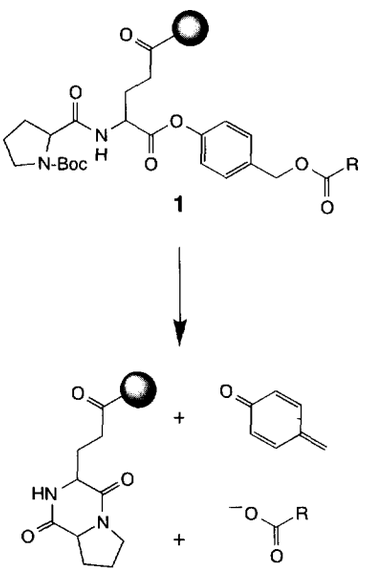
15

Combinatorial chemistry

A pH-cleavable linker for library screening

Linkers play a pivotal role in the solid-phase generation of combinatorial libraries prepared for biological screening. If the library is to be assayed in solution, there is a prerequisite for a step in which the compounds are cleaved from the solid support. However, very few linkers permit cleavage under biological assay-compatible conditions. Many require extremes of pH, and thus the library has to be isolated following cleavage to remove traces of incompatible cleavage reagents. A recent paper describes a new linker that allows the cleavage of library compounds from resin beads at pH 8 [Atrash, B. and Bradley, M. *J. Chem. Soc., Chem. Commun.* (1997) 1397–1398].

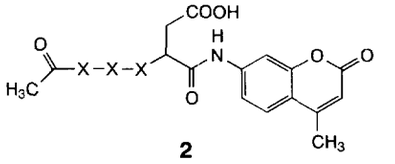
The linker (**1**) contains a key Pro-Glu dipeptide group attached to the solid phase. Removal of the Boc protecting group from this dipeptide reveals the nucleophilic proline amine. Adjusting the solution to pH 8 deprotonates the amine and initiates diketopiperazine formation and product elimination. This linker is especially useful for compound libraries that are tested in zone diffusion assays. The activated linker rapidly cleaves in buffered solution, but at a rate that permits initial distribution of the beads into agarose gel.



Caspase substrate specificities

The caspases are a family of cysteine proteases that include the enzymes interleukin-1β converting enzyme (ICE) and CED-3. These enzymes have been shown to play an essential role in apoptosis, the process of programmed cell death necessary for morphogenesis, tissue homeostasis and host defence. A recent study has used positional scanning combinatorial peptide libraries to determine the preferred substrates and thereby establish functional relationships between the enzymes [Thornberry, N.A. *et al. J. Biol. Chem.* (1997) 272, 17907–17911]. Three sublibraries of 8000 compounds each of the structure **2** were prepared. The libraries were constructed such that in each mixture one of the three amino acid residues was held constant while the others were an equimolar mixture of all of the naturally occurring monomers. By observing which mixtures were the best substrates for each of the enzymes, preferred substrate sequences could be inferred.

It was found that caspases 2, 3 and 7 and CED-3 preferred the tetrapeptide substrate sequence, DEXD (X is a variable amino acid residue), while caspases 6, 8 and 9 and granzyme B had a preference for (I/L/V)EXD. These results suggest functional relationships between the various enzymes, and could lead to the design and synthesis of selective enzyme inhibitors that may further elucidate the enzymes' biological function.

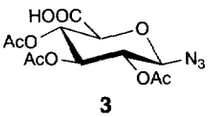


2

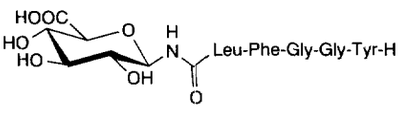
Carbohydrate-modified enkephalins

The addition of glucuronic acid onto morphine has been demonstrated to enhance its analgesic properties 10–50 times. A solid-phase synthetic route has been used to synthesize glucuronic acid enkephalin derivatives with the objective of exploring the effect of this modification on δ-opioid receptor agonist activity [Drouillat, B. *et al. Bioorg. Med. Chem. Lett.* (1997) 7, 2247–2250]. An azidoglucuronic acid (**3**) was used as the starting point for the solid-phase synthesis of these glycopeptides. Following attachment of the glucuronic acid to 2-chlorotrityl resin, reduction of the azide permitted solid-phase peptide synthesis on the free amine leading to the synthesis of C-terminal-modified enkephalins.

In particular, glycopeptide **4** was a potent δ-opioid receptor agonist, showing inhibition of electrically stimulated



3



4

  1359-6446/97/$17.00. PII: S1359-6446(97)01108-2

muscle contractions in both guinea pig ileum and mouse vas deferens several times greater than Leu-enkephalinamide. This solid-phase route to these compounds is leading to the further exploration of combinatorial libraries based on glycopeptides.

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

High-throughput screening

Assay miniaturization for 384-well applications

In the first part of this two-part report on 384-well microtitre-plate-based HTS, liquid-handling systems were outlined [Rogers, M.V. *Drug Discovery Today* (1997) 2, 395–396]. In this second part, two examples of specific HTS assays screened in the 384-well format at Glaxo Wellcome, Stevenage are described.

Based on our experience at Stevenage, we have found that assay miniaturization for HTS can significantly increase screen capacities and throughput and at the same time be very cost-effective. At Stevenage, we now routinely develop cell-based reporter assays and certain biochemical assay types in the 384-well format. Compound supply and screening data handling systems are being upgraded and improved to cope with the movement from the 96- to 384-well plate format. In particular, compound supply will be totally automated and samples held in a high-density store containing 384-well blocks. This will allow direct interfacing with the screening robots.

384-Well biochemical assays on the R2 robotic system

Glaxo Wellcome, like other major pharmaceutical companies, is committed to integrated automation of drug screening. We have several large integrated robots at the research centre in Stevenage. One of these robots, 'R2', built by Thurnall (Manchester, UK), was described in a recent article in *Drug Discovery Today* [Harding, D. *et al. Drug Discovery Today* (1997) 385–390]. One of the two cells on R2 was designed exclusively for running non-radioactive assays in either 96- or 384-well formats.

'Cold R2' consists of a CRS robotic arm on a linear track. Around the track are positioned a Tecan Genesis pipetting station, ambient and 37°C incubators, a plate mixer, colourimeter, fluorimeter, bulk reagent dispenser, delidders and tip carousels. When the 'cold R2' robot became operational earlier in the year, one of the first assays to run on it was designed to detect activators of protein C. The high cost of using protein C at the concentration required for a kinetically valid assay precluded development of a cost-effective screen in the 96-well format. Reducing the volumes of all assay components fourfold for the 384-well format enabled a much more cost-effective screening strategy to be devised. Data relating to throughput of the 'robotized' protein C activator screen was presented at the MIPTEC conference on automation of HTS held in Washington, 23–27 June 1997. This screen was run in transparent, square-well, 384-well Nunc plates. Each sample plate for this screen was prepared from four 96-well plates using a Matrix Technologies PlateMate™, which dispensed 2 μl of compounds diluted in DMSO into dry wells with a high degree of precision.

The primary screen consisted of >100,000 assay points comprising discrete compounds and library samples. "The run took four days to complete on R2, which is almost five times faster than the current Robolab 9600 (Robocon, Austria) system ('R1') maximum for this assay", says David Mobbs of the assay design team. R1 is a robotic system capable of performing the same assay, but only in the 96-well plate format.

In order to achieve maximum sample throughput during an automated HTS campaign, all assay reagents should remain stable in the refrigerated reagent holding vessels on the robots for approximately 24 hours. This is a common problem and an efficient assay validation and robotization process is required in order to ensure optimal use of the robots. Movement to the miniaturized formats reduces reagent consumption and makes it possible to devise better ways of storing the smaller reagent stocks on the robot.

Semi-automated cell-based assay

We are currently building an integrated robot 'R4' specifically designed to perform HTS on a variety of different cell-based assay types in the 96- or 384-well format. At present 384-well cell-based screening is semi-automated and has been successfully used for a variety of assays including a cytokine receptor activation assay. In this case, a cell line was constructed with an episomal reporter construct containing a promoter sequence specific for transcription initiation following selective activation of the cytokine receptor. The reporter gene secreted alkaline phosphatase (SPAP) was positioned downstream of the promoter. Activation of the cytokine receptor leads to the secretion of SPAP into the cell culture medium. The assay was transferred from the project biologists to the assay design team in a 96-well format and was simplified and redeveloped into a 384-well format.

It is not possible to screen 384-well plates manually using hand-held pipetting devices and, therefore, automated liquid dispensing is required. A 'stand alone' Matrix Technologies PlateMate was used for translation of compounds dissolved in DMSO from 96-well stock plates into dry, square-well Nunc 384-well plates for the assay. Assay reagents were added to these plates using a 384-well Labsystems Multidrop™. Both instruments achieved a high degree of accuracy typically returning C.V.'s of <5%. The basic protocol involved addition of compounds using the PlateMate, addition of cells using the Multidrop and then an overnight incubation step at 37°C. Buffer containing a chromogenic substrate for SPAP was then added using the Multidrop and assay plates incubated for 5 hours; wells in which SPAP

  1359-6446/97/$17.00. PII: S1359-6446(97)01101-X