DDT Vol. 6, No. 5 March 2001

New tools for parallel automated chemistry

A. Ganesan, Department of Chemistry, University of Southampton, Southampton, UK SO17 1BJ. tel: +44 (0)2380 593897, fax: +44 (0)2380 596805, e-mail: ganesan@soton.ac.uk

Nobody can point to a single drug on the marketplace discovered by combinatorial chemistry. Nevertheless, there is universal acceptance that the technology accelerates drug discovery. This sentiment was echoed by the attendees of IIR's 3rd annual conference on *Embracing New Tools for Parallel Automated Chemistry* (29–30 November 2000, London, UK). Although specific details vary, many of the presentations also highlighted the convergence in thinking on how libraries should be created and purified within the pharmaceutical industry.

Computational methods

The modelling of libraries *in silico* is increasingly important prior to synthesis. Richard Lewis (Eli Lilly, Windlesham, UK) described the use of 'negative selection' filters such as Lipinski rules and chemical reactivity to guide the selection of library building blocks. One caveat is that drug profiles might vary according to the therapeutic area. In-house virtual screening has also been used for examining cytochrome P450 activity, protein binding and Ames mutagenicity. For structure–activity relationship (SAR) libraries, a diverse set of compounds that efficiently tests the SAR hypothesis and provides new information is preferable to focusing on the best initial lead. As an illustrative example, 18 Eli Lilly biaryl compounds with known efficacy for the nicotinic receptor were used to build a pharmacophore. Out of 24 potential agonists modeled, nine were synthesized by Suzuki-coupling of aryl halides and boronic acids, and four had micromolar activity.

The retrospective analysis of the huge quantities of information generated by HTS campaigns was tackled by Paul Labute (Chemical Computing Grouping, Montreal, Canada) using probability modeling with binary quantitative SAR (QSAR). One example[1], using an oestrogen receptor binding assay, featured a training set of 62 actives and 348 inactives (15% of the total screened), and was used to predict the activity of 53 randomly selected compounds. The actives were predicted with 78% accuracy, and the inactives with 98% accuracy. Most errors occurred near the activity threshold. Binary QSAR was also used to select optimum building blocks for combinatorial library design.

Automated synthesis and purification

Steve Jordan (Roche, Welwyn Garden City, UK) emphasized the need to make reasonably high quantities (10–20 mg) of library compounds that can then be analyzed by a bank of detectors rather than only being able to produce a single measure of yield and purity. At Roche, scavenger resins and liquid–liquid extraction are employed for solution-phase synthesis, and purification effected with high-throughput HPLC using UV- and MS-controlled fraction collectors. On a semiprep scale (10–15 mg), the throughput was ~300 compounds per week, and ~1000 compounds per month for larger quantities (30–40 mg). The more recent developments of supercritical fluid chromatography and chemiluminescent nitrogen detection for sample quantitation were found to be promising alternatives to traditional methods. The latter is generally reliable, although it is unsuited for certain classes of compounds that can readily lose nitrogen such as hydrazides and triazoles. A 'fantasy' prediction for the future was the 8-channel MUX for parallel MS analysis.

David Hunter (GlaxoSmithKline, Harlow, UK) presented the present status of automation at the Harlow site. The Myriad Personal Synthesiser is now standard equipment in medicinal chemistry laboratories, and array synthesis for lead optimization is widely practised. In the combinatorial chemistry group, the Zymark and Argonaut Qwest are largely used for the large-scale synthesis of proprietary building blocks. For lead generation, the goal is to prepare many relatively small libraries with different core scaffolds rather than large arrays. The company uses the Irori MicroKans and the Myriad Core System for synthesis, and automated liquid–liquid extraction and high-throughput flash chromatography through the Biotage systems for compound purification.

Some statistics revealed the general impact of high-throughput technologies at Harlow. In 1999, ~60% of leads came from the archived file collection, ~30% from arrays, and the rest from other sources such as arrayed beads, solution mixtures and purchased compounds. This year, ~60% of hits were from arrays and ~30% from the archives, with an average IC_{50} below 0.3 μmol. The archives and combinatorial arrays now constitute equal proportions (~30% each) of the total compound collection.

David Casebier (Arqule, Woburn, MA, USA) outlined the Arqule strategy to

 PII: S1359-6446(01)01699-3

library synthesis. Following initial optimization of reaction and workup conditions for a small set of compounds, a process development approach was taken to validate the building blocks. This phase was found to be the bottleneck in the overall process, although compounds produced actually account for ~30% of the final library. The company has now automated over 170 synthetic protocols, including a variety of carbon–carbon bond-forming reactions and the use of organometallic reagents. Their HPLC purification facility is currently not operating at full capacity, which would be 5000 compounds per month for small-scale (5–50 mg) and 500 compounds per month for large-scale (50–200 mg) production. The total collection is now 740,000 compounds in over 250 chemotypes. Arqule is aware of two leads that have led to development candidates from client screening, and there was some debate from participants whether this was a reasonable rate of return.

Solution-phase synthesis

Solution-phase methods for library synthesis are now firmly entrenched in combinatorial chemistry. Steven Ley and his colleagues' efforts in this area were covered by Ian Baxendale (University of Cambridge, Cambridge, UK). Multistep sequences using polymer-supported reagents and scavengers included routes to the analgesic natural product epibatidine[2] and sildenafil[3] (Viagra™). In-house preparation of immobilized resins was recommended, as the quality of the commercial materials is both batch- and manufacturer-dependent. To ensure fast reaction kinetics, 3–5 equivalents of resin are required. Andrew Coffey (Polymer Laboratories, Church Stretton, UK) compared microporous with macroporous scavenger resins. The macroporous resins tend to have a lower loading, as ~50% of the final resin volume is occupied by the pores. However, the highly crosslinked macroporous resins have a fixed size that is not solvent-dependent, resulting in rapid diffusion of reagents.

Neerja Bhatnagar (Aventis, Romainville, France) presented industrial examples (Fig. 1) of solution-phase libraries. Dimethylaminomethyl polystyrene was a useful polymer-supported reagent for alcohol carbamoylation and mesylation. A 1500-compound 4-thioalkylimidazole library of >90% purity was prepared for the optimization of a G protein-coupled receptor (GPCR) lead. The methodology was also adapted to solid-phase chemistry for a 5000-compound library, illustrating the advantages of the latter for very large arrays. A β-amino ketone library was used to probe for potential cysteine protease inhibitors, while a 1,2-benzimidazole synthesis began with Marko-oxidation of benzyl alcohols and subsequent condensation with 1,2-diaminobenzenes.

Solid-phase synthesis

Mark Bradley (University of Southampton, Southampton, UK) showed that resin

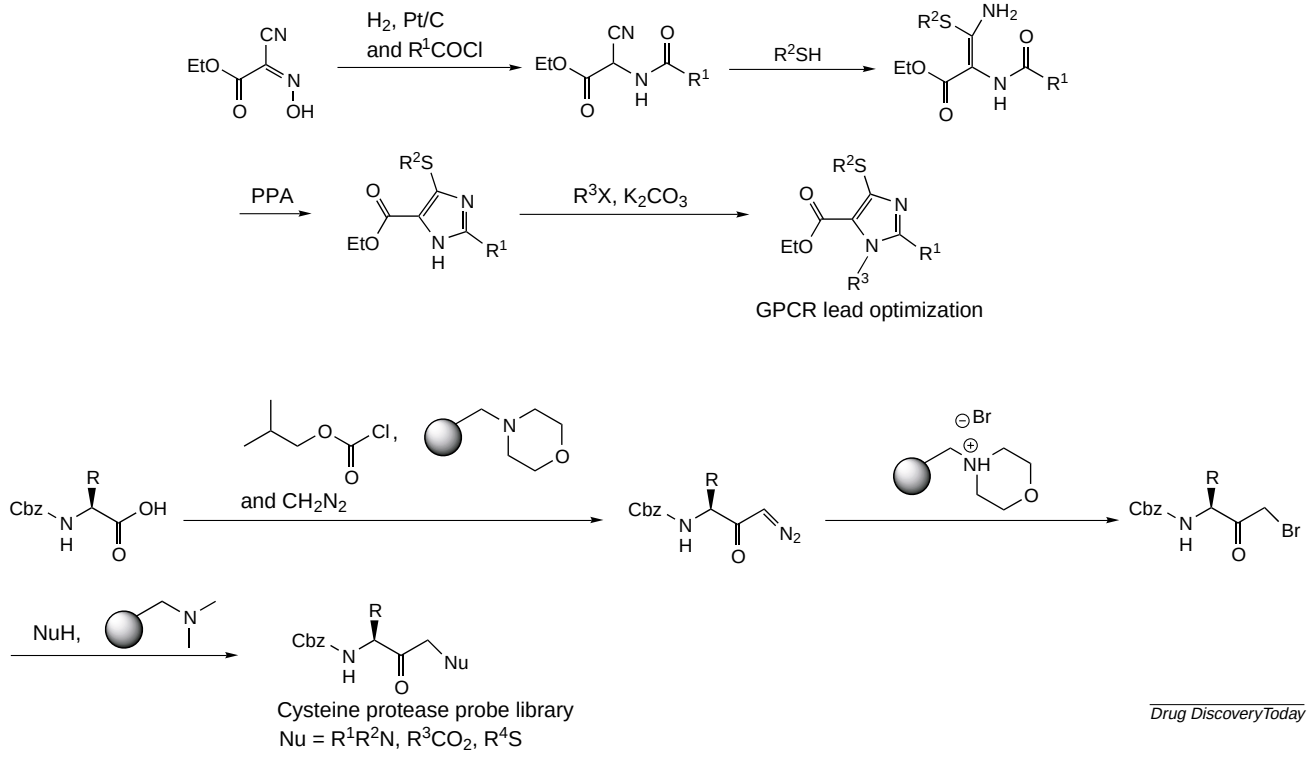

Figure 1. Examples of solution-phase libraries prepared at Aventis, Paris Research Center. Abbreviation: PPA, polyphosphoric acid.

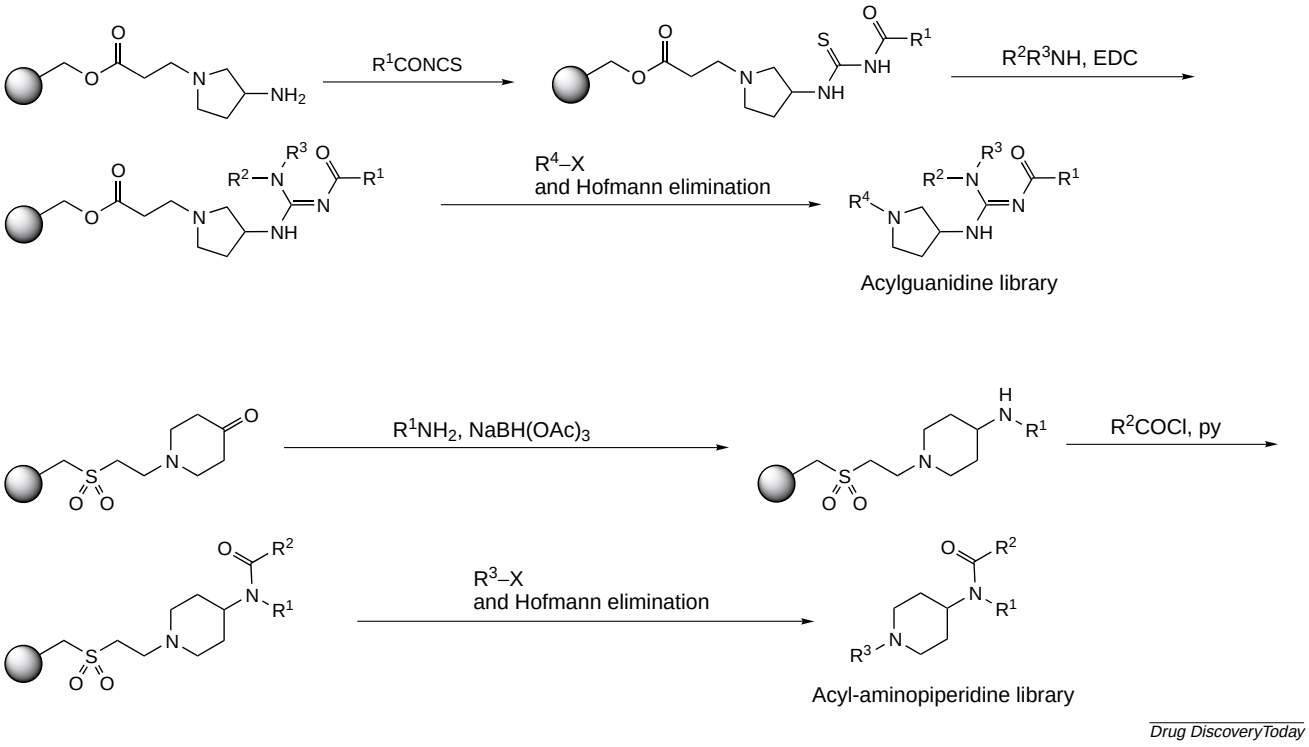

Figure 2. Examples of solid-phase amine synthesis at Organon with the REM (regenerated Michael reaction) linker. Abbreviation: EDC, 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; py, pyridine.

sintering within an inert polyethylene filler provides a new support for solid-phase synthesis that can be fabricated in various shapes. For example, synthesis 'plugs' of ~150 mg contain 60–70 μmoles of resin loading, and show similar reaction kinetics to beads. In another project, a cyclic undecapeptide was prepared[4] on a series of polystyrene resins with varying levels of crosslinking. Although reaction rates were slower with higher (2–6%) crosslinking, as might be expected, the purity of the final peptide was increased relative to the usual 1–2% crosslinked resins.

Applications of the REM (regenerated Michael reaction) linker for solid-phase amine synthesis were presented by Jason Tierney (Organon, Newhouse, Scotland). Using an ACT496 instrument, arrays of 96 compounds were obtained in 24 h containing excess diisopropylethylamine and salts. HPLC desalting then gave products of >95% purity, and automated recycling enabled gram-quantities to be prepared. With larger arrays, the final cleavage was the bottleneck, and a vapour-phase protocol[5] with ammonia gas was developed. Library examples (Fig. 2) included acylguanidines (960 compounds) and 4-aminoacylpiperidines (1040 compounds).

A. Ganesan (University of Southampton, Southampton, UK) discussed experiences with various carbon–carbon bond-forming reactions using solid-phase chemistries ranging from carbanion chemistry to acyliminium, organometallic, radical and multicomponent reactions. Of growing importance were 'strategic' transformations resulting in rapid assembly of complex scaffolds, as illustrated by syntheses of fumitremorgin[6] and quinazoline[7] alkaloids. Miles Congreve (GlaxoSmithKline, Cambridge, UK) outlined the 'analytical construct' concept, in which reporter beads function as the equivalent of TLC for rapid qualitative analysis of solid-phase reactions. The method uses a dual linker that is orthogonally cleavable. For MS analysis, an amine-releasing linker is used that ionizes well under MS. For absolute quantification, a UV chromophore was introduced by an anthracene linker[8]. Use of such methods is likely to increase as it was shown that solid-phase reactions could be carried out using commercial beads mixed with a small quantity of reporter beads (~5%), followed by selective cleavage of the orthogonal linker for MS and UV analysis.

References

1 Gao, H. *et al.* (1999) Binary quantitative structure–activity relationship (QSAR) analysis of estrogen receptor ligands. *J. Chem. Info. Comput. Sci.* 39, 164–168

2 Habermann, J. *et al.* (1999) Synthesis of the potent analgesic compound (+/–)-epibatidine using an orchestrated multi-step sequence of polymer supported reagents. *J. Chem. Soc., Perkin Trans. 1*, 1253–1255

3 Baxendale, I.R. and Ley, S.V. (2000) Polymer-supported reagents for multi-step organic synthesis: application to the synthesis of sildenafil. *Bioorg. Med. Chem. Lett.* 10, 1983–1986

4 Rana, S. *et al*. (2000) The synthesis of the cyclic antibacterial peptide kawaguchipeptin B on 11 different DVB cross-linked PS resins. *Tetrahedron Lett*. 41, 5135–5139
5 Brown, A.R. (1999) Vapor-phase Hofmann elimination: a rapid one-pot method for the cleavage of tertiary amines from radio frequency encoded solid-phase synthesis. *J. Comb. Chem*. 1, 283–285
6 Wang, H. and Ganesan, A. (1999) The *N*-acyliminium Pictet-Spengler condensation as a multicomponent combinatorial reaction on solid phase and its application to the synthesis of demethoxyfumitremorgin C analogues. *Org. Lett*. 1, 1647–1649
7 Wang, H. and Ganesan, A. (2000) Total synthesis of the fumiquinazoline alkaloids: Solid-phase studies. *J. Comb. Chem*. 2, 186–194
8 Williams, G.M. *et al*. (2000) Analysis of solid-phase reactions: product identification and quantification by use of UV–chromophore-containing dual-linker analytical constructs. *Angew. Chem., Int. Ed*. 39, 3293–3296

The Internet in clinical trials: breaking the bottleneck?

Joanna Owens, joanna.owens@current-trends.com

At a recent conference, *Clinical Trials: The Next Phase!* (London, UK, 13–14 December, 2000, organized by Access Conferences International), the use of the Internet in clinical trials, both to provide a global database of high quality clinical trial data and to aid patient recruitment, was debated. The clinical trial stage of drug development is the major bottleneck that prolongs the time between drug discovery and marketing, a fact made worse by an increase in the number of therapeutic targets from the mapping of the human genome, HTS and the advent of combinatorial chemistry. This is resulting in a requirement for larger, more complex trials, to which the access to patients, and investigation site performance cannot be matched.

Potential Internet strategies

C. David Hardison (First Consulting Group, Winston-Salem, NC, USA) started the conference by discussing the current problems of clinical trials in R&D, namely: poor success rate (1 in 5000 NCEs), time taken for drugs to reach the market (8–12 years), finite patent life, costs, and length of FDA submission (>500,000 pages; 0.5–5 years).

With an increasing number of websites being set up in the healthcare arena as online healthcentres, pharmacies and medical databases (e.g. http://www.netdoktor.com,http://www.allcures.com, http://healthwatch.medscape.com), is it possible to apply similar strategies to clinical trials?

The use of the Internet has increased significantly among physicians and by patients finding out more about their illness, potential treatments and support groups. Physicians are already using intranet strategies for medical records and prescriptions, which in itself could provide a basis for interactions between healthcare and clinical trials online. However, large amounts of time and money have been invested into current clinical trial systems, and clinical trial sites might be reluctant to embrace this new technology.

Hardison discussed the stages of e-business maturity, which are:

- publish: to build web awareness by the posting of healthcare and clinical trial information relevant to both patients and physicians;
- interact: encourage chatrooms, personalized information and healthcare communities online to engage the physician and patient;
- transact: incorporate online transactions including randomization, trial data capture, adverse-event reporting;
- integrate: combine data input with the generation of personal health records, trial database management; and
- transform: integration of all web interactions to form an end-to-end system between patients and physicians/trial sites, such that trial participation is a treatment option for all patients.

Hardison gave examples of research institutes that are already using Internet clinical trials in cancer care, namely the National Cancer Institute (Bethesda, MD, USA) and the M.D. Anderson Cancer Center (Houston, TX, USA), and the web-enabled International Verapamil/Trandolapril Study (INVEST; http://invest.biostat.ufl.edu) currently being run by the University of Florida Health Science Center (Gainesville, FL, USA). He continued by highlighting the advantages of a web-enabled trial system, including:

- online enrolment statistics;
- daily patient status reports;
- records of study visit activities;
- standardized letters, invoices, labels merged with patient database;
- automated calendar and resource management; resulting in
- annual cost savings of US$240,000 per site and increased revenue.

Hardison concluded by speculating that investigation sites will start using e-clinical trials to achieve:

- state-of-the-art drug therapies combined with a high level of healthcare for patients;

1359-6446/01/$ – see front matter PII: S1359-6446(01)01698-1