Uptake of new technology in lead optimization for drug discovery

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The arrival of combinatorial chemistry in pharmaceutical lead optimization has usually been greeted with reservation by chemists at the bench – it isn't what medicinal chemists have trained for years to do. The apparent mindless application of automated synthesis seems to have the potential to convert the demand for skilled chemists to one of machine operators. At Glaxo Wellcome, this initial concern has been converted to a widespread embrace of newer technologies and techniques in the ever-increasing race for new drugs. The author describes how this process has evolved over the past five years, and how the experiences, both successful and otherwise, provide both a salutary lesson and a useful reference for the changes that are undoubtedly yet to come.

The introduction of automation and the increasing levels of miniaturization in the high-throughput screening (HTS) arena at the start of the 1990s provided the impetus for the development of combinatorial chemistry in drug discovery[1]. Put simply, there wasn't enough compounds in the corporate collection to meet the needs of screening. At Glaxo (now Glaxo Wellcome) in the UK, as in many other pharmaceutical companies, an investment was made into combinatorial chemistry techniques, equipment and the generation of large libraries to meet this need. The belief was that large diversity libraries would give excellent coverage of pharmacological 'space' and hence provide active compounds against most, if not all, new targets being developed for HTS. Moreover, these compounds would be provided by combinatorial chemistry, and as such would contain many attributes sought after for lead compounds[2]. This might include a desirable molecular weight, good hydrophobicity profile and pharmacophorically biased functionality (so called privileged structures based on past experience, for example benzdiazepines), which could all be delivered by careful selection of the monomers and chemistry. Additionally, the fact that the compounds were generated as part of a combinatorial library would mean good tractability as lead compounds. The chemistry to explore structure–activity relationships (SAR) around the lead would already have been extensively developed during the library exploration phase. These underlying principles behind combinatorial chemistry for lead discovery libraries still operate today, although experience has shown that the design of meaningfully diverse libraries is not a simple process.

At Glaxo in the UK we chose a slightly different approach to most groups – development of robotics and processes to support solution-phase combinatorial synthesis of large libraries for HTS (Ref. 3). The choice of solution chemistry was driven by several factors. Other groups within the international Glaxo research organization were actively pursuing solid-phase chemistry routes to libraries for screening. Also the small group established to explore the new approach had limited experience in solid-phase chemistry, but several years' experience of traditional solution

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Box 1. Solution- or solid-phase chemistry?

Much of combinatorial chemistry has been driven using solid-phase chemistry. This has been used extensively in the peptide field, and it is from this area that combinatorial chemistry first significantly developed. The ability to drive reactions to completion using large excesses of removable soluble reagents is the reason behind this use of solid-phase chemistry. The approach is well suited for long linear syntheses where high purity is required at each step to avoid compromising product purity.

When we started on our libraries we were targeting small-molecule, low molecular weight compounds, with few reactions required to create these. In such cases the costs (in terms of time) of carrying out reactions to attach and detach from a support seemed greater than the potential advantages of product purity offered by solid phase. Moreover, we were intending to use expensive, proprietary compounds that would not be available in significant amounts. Finally, we were preparing samples directly in a format analogous to that required for screening, using spatial addressing to identify the compounds, and as such wished to avoid any potential translation errors during a cleavage and reformatting approach. Since that original approach, we have developed an understanding of both solid and solution combinatorial chemistries, and it is the ability to apply either, when appropriate to the target, that gives the greatest potential to drive lead discovery and optimization forward.

chemistry. Finally the overall advantages of solid-phase chemistry did not, to us, seem to outweigh the advantages that a solution-phase approach could offer (Box 1).

Solution-phase chemistry equipment

Having chosen a solution-chemistry process, we then needed to develop equipment capable of supporting solution synthesis on large numbers in a parallel format. Commercial robotics to handle solutions were readily available and, thus, the means to move reactants and reagents around was straightforward (although, allowing for the need to ensure chemical compatibility between robotic equipment and chemical vapours – the effect of TFA vapours on aluminium robotic housings or brass drive shafts can be both impressive and expensive). The initial system was developed around a liquid-handling system (Tecan 5072 Robotic Sample Processor). However, (organic) solvent removal prior to preparing samples for (aqueous-based) screening needed to be addressed. A system to remove solvent by using nitrogen streams blown

Figure 1. *ARNIE, the first solution-phase robotic synthesizer at Glaxo, capable of 480 parallel reactions and solvent removals.*

across the samples, 480 reaction vessels at a time, was developed in collaboration with an engineering consultancy firm (Cambridge Consultants, Cambridge, UK). This led to the development of ARNIE (Fig. 1), the first solution-phase organic synthesizer within Glaxo, capable of 480 parallel reactions (equivalent to six microtitre plates of test compounds assuming 80 wells used for test samples). Although initially developed for amide chemistry, it quickly became evident that a wider range of chemistries could be developed and applied[4], and over a period of two years the equipment was used to prepare about one million compounds for assay. Preparing both pooled samples and discrete entities, this output led to many significant results in our lead discovery programme (manuscripts under preparation).

As an aside, the apparent acronym ARNIE has puzzled many people inside and outside Glaxo, however, the letters do not stand for anything; ARNIE was named by a medicinal chemist in reference to Arnold Schwarzenneger and the Terminator series of films – typically black humour at its finest.

The fact that the group had developed a piece of automated equipment capable of performing solution-phase chemistry did not pass unnoticed by some of the surrounding project groups. Within a few weeks of the systems becoming operational we were preparing 'small' arrays of compounds for a select few lead-optimization projects. Thus in 1993, in a small way, the process of technology uptake in medicinal chemistry working practices had begun.

But is it real chemistry?

Within a year of establishing ARNIE as a viable solution-phase synthesis robot and with a range of chemistries developed, several projects had used the system to prepare analogues for screening. The number of compounds varied from a modest 50–100 for targets aimed at specific lead optimization targets[5] through to several hundreds of compounds for directed screening approaches used to identify novel series in established projects. However, although the approach had been demonstrated to be useful, and occasionally highly successful, there were few medicinal chemists involved in the work.

Even more interesting was that for those projects using the system, the support for the approach often resided in a single individual in that group. For most chemists, the automated synthesis approach was simply an example of increased mindless handle turning, not appropriate to the craftsman style of medicinal chemistry. Nowhere better was this illustrated than in the attitude of many of the project chemists who found themselves transferred to the combinatorial teams as the project grew. Unclear at the outset on the objectives of the group (due usually to not wanting to know until it was 'too late'), and often surrounded by colleagues with a 'glad it's not me' attitude, it was not surprising that the initial enthusiasm levels of new appointees to the project were often at a low ebb. It is a tribute to the resourcefulness and adaptability of chemists that this low period was usually short lived, often a matter of one or two days. But once the realization that the combinatorial and parallel route was an opportunity to obtain, for example, SAR data far more rapidly than previously possible, and that the handle-turning aspects were mostly taken care of by either the machinery itself or support mechanisms (Box 2), the avenues for project advancement using the new approaches were wide open. Moreover, newer technical and scientific challenges became driving forces to enthuse and interest the chemist – such as how to select a good set of compounds or how to optimize a piece of chemistry for generic reactants.

The original UK Glaxo team grew in a matter of two years from the founding two-man operation to a full six-man team, with second and third teams following as the range of chemistries and need for compounds developed. By the time of the merger between Glaxo and Wellcome in 1995 there were 18 people directly working in the combinatorial group in the UK, focusing on a range of chemistry approaches for lead-identification targets as well as supporting the array needs of several lead-optimization projects. An indication of the commitment to the new approaches and techniques that this group developed can be derived from the Glaxo Wellcome merger process: every long-term member of the combinatorial group elected to remain in one of the two new combinatorial groups developed in the merged company rather than move 'back' into a mainstream project area. Since the merger, combinatorial chemistry in Glaxo Wellcome research in the UK has continued to involve the two groups working in close collaboration, with the Discovery Chemistry Group having the role of supporting lead-identification approaches and the Core Combinatorial Group supporting lead optimization and technology transfer approaches.

Box 2. Parallel processes

The ability to perform hundreds of reactions in parallel is not itself enough to enhance the processes of discovery or optimization. The whole of the process needs to be geared up to match the throughput. Supply of building blocks for the library in a pre-weighed format, typically to a given molar amount, and in the correct vessels for a synthesis can rapidly enhance the processing time. Any purification and analysis steps should also be carried out using equipment capable of handling the format that the parallel reactions have been performed in. Needless to say, the actual manipulation of the samples before assay and the test results themselves also need to be at the same scale. If any one of these requirements is not at the same level there will be significant interruptions to the process and at worst this will lead to the process itself being abandoned as unworkable. The final element in this parallel jigsaw is data handling. Synthesis data, spectroscopic quality control data, compound registration data and biological results all need to be handled in a format suitable for the number of compounds.

Who needs a computer?

By the start of 1995 several projects had used the solution-phase array equipment to further SAR studies or in new lead identification programmes. Also, by this stage we had developed a wider range of equipment to support parallel solution-chemistry. Integral to this had been a decision to change the philosophy of the technical engineering, such that instead of developing a single piece of equipment to perform all the functions for the synthetic process we had separated the functions into stand-alone modules. This allowed more-flexible equipment scheduling and use, and

still left the potential to fully automate reaction block transfers in the future if deemed necessary. It meant that the chemistry stage could be performed using a wide range of solution-handling robotics, with the solvent-removal steps separated from the synthesis robotics on stand-alone units.

To the group there seemed a logical further step to take. Although the flexibility of separate pieces of synthesis equipment allowed greater access to the technology for the project teams, we were still performing the array processes for those teams ourselves. Why should a project team need to come to a specialist laboratory and team when for a modest outlay we could equip project laboratories themselves with the synthesizing liquid-handling robotics? Consequently, liquid-handling robots were placed in a selection of medicinal chemistry laboratories. To our disappointment, the use of these robots was very limited and we found that we were still serving the needs of projects by carrying out parallel-array syntheses on their behalf, using the same equipment. So why was this the case?

It became clear that we had not fully understood the processes that were being applied in project chemistry at the time, and what part the parallel processes were playing in the optimization arena. The parallel arrays were not being used to perform traditional medicinal chemistry optimization, but rather used as a means to expand exploration around the edges of a project in the search for novelty. The core structure of the project remained around traditional single-compound synthesis. As is usually the case, exploration around the edges of a project can be justified as long as the effort is not too large in relation to the potential for success. If the combinatorial group was performing the operation then there was limited investment of effort from the project and, thus, it was a justifiable approach. If the process involved project members having to become familiar with novel, computationally driven synthesis robotics, then the investment was seen to be too high for the potential return. For many, the new techniques were a step too far to take, and even viewed as a threat by some, as well as an opportunity. The placement of computer-driven equipment into the laboratories was, in fact, the least likely approach to lead to the take-up of novel technologies and techniques required for a fundamental change in the processes of lead optimization in drug discovery.

So how can successful technology take-up occur?

Today every project in medicinal chemistry optimization at Glaxo Wellcome uses a wide range of combinatorial and array approaches, allied to more-traditional SAR programmes. Solid-phase chemistry, solution-phase chemistry, manual, semi-automated and fully automated synthesis, parallel purifications, automatic preparative chromatography and high-throughput analyses all are being applied as and when appropriate. So how in the space of three years has this radical shift in the processes of optimization occurred?

One factor that has been instrumental to the increase in general use of the wide range of techniques is the active support of all layers of the management structure. Individual project groups continue to be challenged by their own leaders to consider the use of the new technologies. Even more importantly, senior management has demonstrated a strong visible commitment to using the newer techniques wherever appropriate. This support has been expressed in many ways: publicly expressed encouragement, an active role challenging the justification for not pursuing new approaches, and the incorporation into reward processes of an element of new technology take-up. Although, there has been no 'blanket' order of 'it must be done in the new way' as there is recognition that scientific research cannot be prescribed in such a way. Confidence in the scientific judgement of the project scientists as to when to apply the techniques must remain and be clearly expressed. To support this there has been a drive for scientists to acquire a sound practical working knowledge of the range of techniques, thus allowing informed scientific judgement to be applied in each case.

The change in management support has not been the only factor behind the successful uptake of new technologies. When the liquid-handling robots were placed in laboratories three years ago there was no track record of such approaches proving successful except in the hands of the 'expert' groups. More importantly, there was limited general experience of these methods. It was clearly important to the success of technology take-up that the belief in the techniques that had developed in the expert user was conveyed to the project teams, along with the experiences. It is an easy job to transfer the knowledge of technological changes: a simple report, memo, or highlight document can each ensure that all chemists would rapidly know of a new technology, and it could be concluded that the job of technology transfer had been done. However, experience, both in this field and in 'real life', readily demonstrates that information so rapidly transferred will be just as likely to be rapidly forgotten by all but a small minority. 'Cold' demonstrations of techniques and technologies have more impact,

Box 3. Aqueous separations

An aqueous work-up process is one that all organic chemists learn to use, and yet often, little thought is given to the power of the process. Judicious choice of the organic and aqueous phase combination and contents can have a dramatic effect on the purity of a sample (who can forget the visual impact of a sodium sulphite aqueous work-up of a reaction involving iodine). However, separating funnels, traditionally used in the process, are not amenable to parallel processing, and automatic techniques for detecting liquid–liquid phase transitions are generally too expensive or complex for simple laboratory use.

However, a serendipitous observation of the behaviour of an aqueous organic system in a Teflon-based reaction block led us to explore the use of hydrophobic materials as an effective means of performing such separations in parallel. A hydrophobic membrane will allow an organic solvent to pass through yet retain an aqueous phase. If the organic layer is denser than water then gravity will perform the separation through such a filter. Even difficult-to-separate emulsions can often be successfully separated in this manner. This can be easily extended to parallel approaches, providing an aqueous work-up procedure to match the throughput of the reaction block systems.

but it is the timely demonstration of the suitability and power of an approach to a current problem that has the real impact. It is still relatively easy to transfer understanding of the potential of a new approach (winning the minds), but it is in the real application of the approach to a personal situation that will ensure that the new technique or technology is truly taken on board (winning the hearts). A process of proving success by working with a partnership between experienced practitioners and the project groups has, thus, efficiently provided this element of the growth in the use of the newer techniques and equipment.

Scale of operation

Another significant problem with the original robotic introduction was that the equipment was not targeted at the correct scale of operation. Several projects had chosen to use the services of a 'production environment' (i.e. the combinatorial group of the time) to prepare significant numbers of compounds. These preparations were part of overall project plans that ensured the availability of suitable assays for the numbers, the format and the quality of the samples. A standard project preparing and assaying single purified compounds was unlikely to embrace the technologies to prepare hundreds of crude samples, when the mechanisms to handle, test and make use of the resulting data were all absent. As described earlier (Box 2), the overall parallel planning would not be in place. A typical project might have been capable of handling an increase in productivity of one order of magnitude (10–20 compounds instead of singles), but the level of automation supplied went well beyond this scale.

In this light a review of the type of equipment being evaluated and supplied to projects was taken, and the process of targeting of this smaller increase in magnitude began. Working in close collaboration with several manufacturers, several new pieces of equipment were brought in for evaluation and subsequent use. Multiple position parallel reaction blocks for solution-phase and parallel-position vacuum blocks for solid-phase chemistry in polypropylene-fritted tubes readily gave access to 20 or more compounds in either parallel arrays or true combinatorial chemistry format. Solvent removal was enabled using vacuum centrifugation, and spectroscopic analysis of products was readily carried out using rapid automated (though serial) techniques such as HPLC–MS. If purity of the samples needed to be improved for assay purposes then parallel techniques such as aqueous organic separation approaches (Box 3) or solid-phase extraction could be applied. Finally, if high purity levels were needed, serial purification using automated HPLC could be used to provide highly purified samples on the same scale of numbers. It has been the uptake of these technologies, readily accessible to all chemists, easily placed in laboratories, and yet providing a significant increase in the productivity of the laboratories that has been the final factor to revolutionize lead optimization.

Further developments

These changes have since fuelled further developments. Bioassay techniques are now developed with the concept of higher numbers of compounds to be tested, and not only to target potency but also other aspects of drug optimization (manuscript in press). The production (and purification – see Box 4) of arrays and libraries of hundreds of compounds have now become commonplace. Selected projects have pursued their targets using only the array and combinatorial techniques virtually, until development candidates have been identified. Solid-phase and solution-phase approaches are often combined with scavenging and supported reagent approaches[6] to yield high quality compounds for assay that require no further purification.

Box 4. Lollipops

Although approaches such as the hydrophobic frits allow aqueous organic separation in the 10–20 compound numbers range (Box 3), this technique suffers because it is not in a microtitre-plate format and as such is not readily amenable to larger number purifications. We have developed an alternative approach utilizing differentiation between the physical states of the phases. With the exception of a few solvents, it is possible to cool an aqueous organic mixture until the aqueous phase freezes. As long as there is a clean separation between the layers, when a pin is placed into the mixture before cooling, the ice forms a plug around the pin. It is then possible to withdraw the plug by removing the pin (hence the name lollipops). Slightly tapered tubes avoid the dangers of the organic solvent spilling out when the lollipop is removed, and the process works for organic phases both less- and more-dense than the aqueous phase. The procedure has been applied to a 96-well microtitre-plate format (Fig. 2). If the aqueous phase contains the samples of interest then it is possible to place the plugs into a separate plate for thawing and collection. Sequential washes of organic layers (acid, base, etc.) can also be readily performed on a parallel format. This allows a good level of purification to be applied in parallel to larger arrays of compounds.

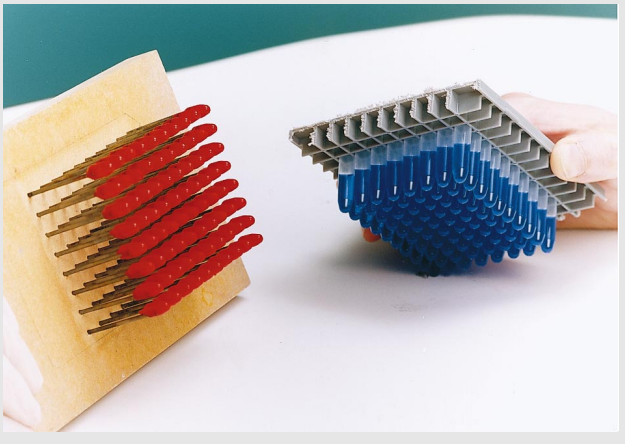

Figure 2. *'Lollipop' separations of 80 frozen aqueous (red) samples from the organic (blue) phase following an aqueous work up.*

In summary, a process that began with limited uptake and success has now revolutionized the processes of lead optimization in drug discovery. These techniques have been embraced by medicinal chemists, and are now applied as part of the wide range of approaches available to tackle the discovery and development of new drugs. Combinatorial chemistry does not threaten to displace the skills of traditional chemistry, but instead to enhance the potentials for our industry. It is correctly viewed as another approach that can be applied to enhance all that we have learnt of medicinal chemistry over the past years. There can be few medicinal chemists who could now debate the question posed in this article – combinatorial chemistry is indeed real chemistry.

The future

The pace of change is still increasing. The economic drive to shorten discovery times is still as great. It is imperative that any pharmaceutical company remains at the leading edge of drug discovery to meet these demands, and this is as important in lead optimization as in the more publicly acknowledged arenas of lead discovery and genetics. The past few years have shown the adaptability of medicinal chemistry, and the willingness to embrace change. Even highly technical and computer controlled equipment is now commonplace in laboratories. So how will medicinal chemistry develop further? Several approaches have recently been described at conferences targeting this area (for example, see recent conference programmes presented by IBC or CHI conference organizers in the USA and Europe). Miniaturization approaches may lead to laboratories on a chip. Full-automation systems capable of performing all types of chemistry are becoming available. The use of radio-frequency tags to follow chemistry is now readily accepted and performed in our laboratories[7]. Many other changes, inconceivable only ten years ago, are now impacting on optimization processes. All we can predict with certainty is that the ability to adapt and change, learnt over the past few years, will be vital to the success of medicinal chemistry in the future.

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