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Automated medicinal chemistry

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With the advent of high throughput technologies in biological screening in the 1980s, providing sufficient numbers of small molecules for screening became a bottleneck in the drug discovery process. Combinatorial chemistry was the first attempt by chemists to address this issue. However, since its first applications, combinatorial chemistry has evolved rapidly into diverse fields. This review will focus on the evolution and the current status of what we refer to today as automated medicinal chemistry.

The increasing demand for new small molecules (for use in biological screening campaigns in the pharmaceutical industry) led to the invention of new technologies in the field of medicinal chemistry. Originally invented in the context of peptide chemistry as solid-phase peptide synthesis (SPPS) [1], solid-phase organic synthesis (SPOS) was developed by chemists addressing the need for larger compound numbers [2]. Split-and-mix synthesis, as a new tool in chemistry, in combination with SPOS [3] was applied to generate millions of compounds with a synthetic effort comparable with more-traditional chemistry that generates dozens of compounds. However, the drawback of these early approaches was that although large numbers of compounds were accessible in theory, as a result of the split-and-mix synthesis approach, mixtures of compounds were obtained. Even worse, there were no methods of purification and quality control available, resulting in frustrating screening results with regard to false-positives and problems in the deconvolution of active ingredients in the screened mixtures. In addition to the difficulties in identifying the active compounds, only a very limited chemistry repertoire was available to combinatorial chemistry. Therefore, the resultant library compounds had a tendency towards an unfavourable physicochemical (and thus unfavourable pharmacokinetic) profile (i.e. high molecular weight and high logP values). The high expectations placed upon early combinatorial libraries remained largely unfulfilled [4–6].

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This review will describe current tasks and workflows in automated medicinal chemistry (AMC). It will also highlight the current status of library design, available equipment for synthesis, purification and logistics [7–10]. Finally, the impact AMC has on the drug discovery process, as well as future trends for the field, will be discussed.

Tasks for AMC

The main task of AMC is not necessarily to provide large numbers of diverse compound sets for corporate screening decks. Rather, it is the generation and assembly of various focused libraries (Figure 1) [3]. The term focused libraries is not clearly defined and comprises several subcategories. One subcategory is target-family-oriented libraries, consisting of compounds specifically designed to fit certain target-protein families [11], such as kinases [12] or G-protein-coupled receptors (GPCRs) [13]. Such libraries, typically containing 10,000–50,000 compounds derived from several dozen scaffolds and chemistries, are used to supplement (or sometimes even replace) the corporate HTS collections that can consist of millions of compounds. The idea here is to establish compound sets with enriched hit rates. More-specific focused libraries address a certain target protein. This is a typical application within hit-to-lead or lead-optimization stages of drug discovery projects.

Synthesized libraries in a target-family-oriented library setting typically consist of up to 2000 compounds designed around a common scaffold class. In a hit-to-lead or lead-optimization setting the compound number is smaller, resulting in a set of ~20–

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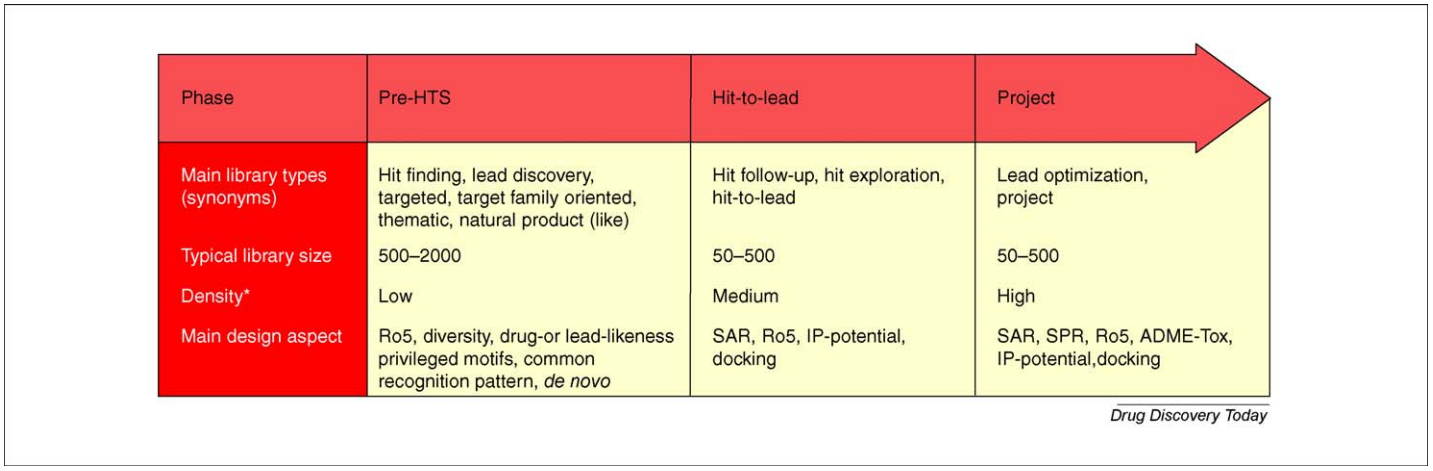

FIGURE 1

Features of the main library types along the drug discovery process. *Density describes the similarity of these library compounds to other library compounds.

500 (max) compounds per library. In project-related work, sometimes we experience higher synthesis failure rates than for other libraries (~70–80% successfully synthesized compounds). Generally, this observation can be related to two project-specific challenges. On the one hand, one might have chosen building blocks that are of low reactivity but that are highly relevant for SAR purposes. On the other hand, time pressure is usually higher within a given project. Therefore, a conflict could arise between meeting the deadline for the delivery of compounds and the time required to optimize the chemistry.

In this context, it should not be forgotten that a good, lively interaction between traditional medicinal chemistry laboratories and AMC laboratories is vital for successful collaborative project work. Our observation is that the initial scepticism of more-traditionally oriented chemists towards the new technologies is constantly decreasing. This decrease is mainly triggered by regular and frequent interactions (i.e. in project teams), as well as by gaining knowledge with regard to the scope and limitations of each approach.

Further activities within AMC include the generation of natural-product-derived libraries [14,15], diversity-oriented synthesis-related approaches [16] and dynamic combinatorial libraries [17]. Although natural-product-derived libraries have received more attention recently, diversity-oriented synthesis-related approaches and dynamic combinatorial libraries are less common in practice and could have limited prospects for the future.

Another important task of AMC is the implementation of software and adaption of hardware for automation equipment. The financial and human resources needed to implement and maintain the sophisticated automation equipment must not be underestimated. In our hands, it takes anywhere from a few weeks to several months (depending on the complexity of the system) until a piece of equipment can be used in production. Once the initial installation is finished, the optimization and refinement of the overall workflow is a constantly ongoing effort.

Workflow options in AMC

A typical workflow for the production of compounds in our department is shown in Figure 2. This procedure is performed in many other companies in similar ways. The process begins with the selection of a library core-structure, which might originate from a HTS exercise (Figure 2). After establishing the appropriate library chemistry, enumeration of virtual libraries and library design is done, often in close collaboration with computational chemists.

Especially for timely delivery of hit-to-lead and lead-optimization libraries, the availability of required building blocks and scaffolds for library synthesis is crucial. Therefore, most pharmaceutical companies have implemented (and maintain) internal building-block repositories. Another option consists of outsourcing the building-block logistics to service providers such as ASDI (www.ASDI.net), which does not require so many internal resources but certainly requires an adequate budget.

Once the selection and compilation of a final set of building blocks is finished, the actual library production begins, using dedicated equipment. After automated synthesis, the collected crude products go to a high-throughput purification and analysis laboratory. Here, the crude compounds are analyzed and purified by liquid chromatography–mass spectroscopy (LC–MS). After purification of the crude products, the fractions obtained are pooled into glass tubes. The solvents used during the purification process are then removed and the dry, purified compounds are subsequently reformatted on a dedicated system that we call the high-throughput reformatting unit (HTR). Here, the yields of the purified products are determined by weighing them, and multiple copies of each compound (for reanalysis and compound logistics) are prepared (deep-well plates and 4 ml vials are used for storage of the solid compounds). After reanalysis, compounds obtained in sufficient amounts and purity are registered to our corporate compound database and are forwarded to the compound logistics laboratory, which is responsible for the preparation of screening plates and the storage of compounds. For library submission, compounds must be >85% pure according to UV at 220 nm and the minimum amount required is 4.5 μmoles. The average purity obtained in our department is >95% by UV. Currently, >80% of the compounds produced in our unit are isolated in quantities >10 mg. The equipment used throughout the production process is sophisticated and requires special user expertise.

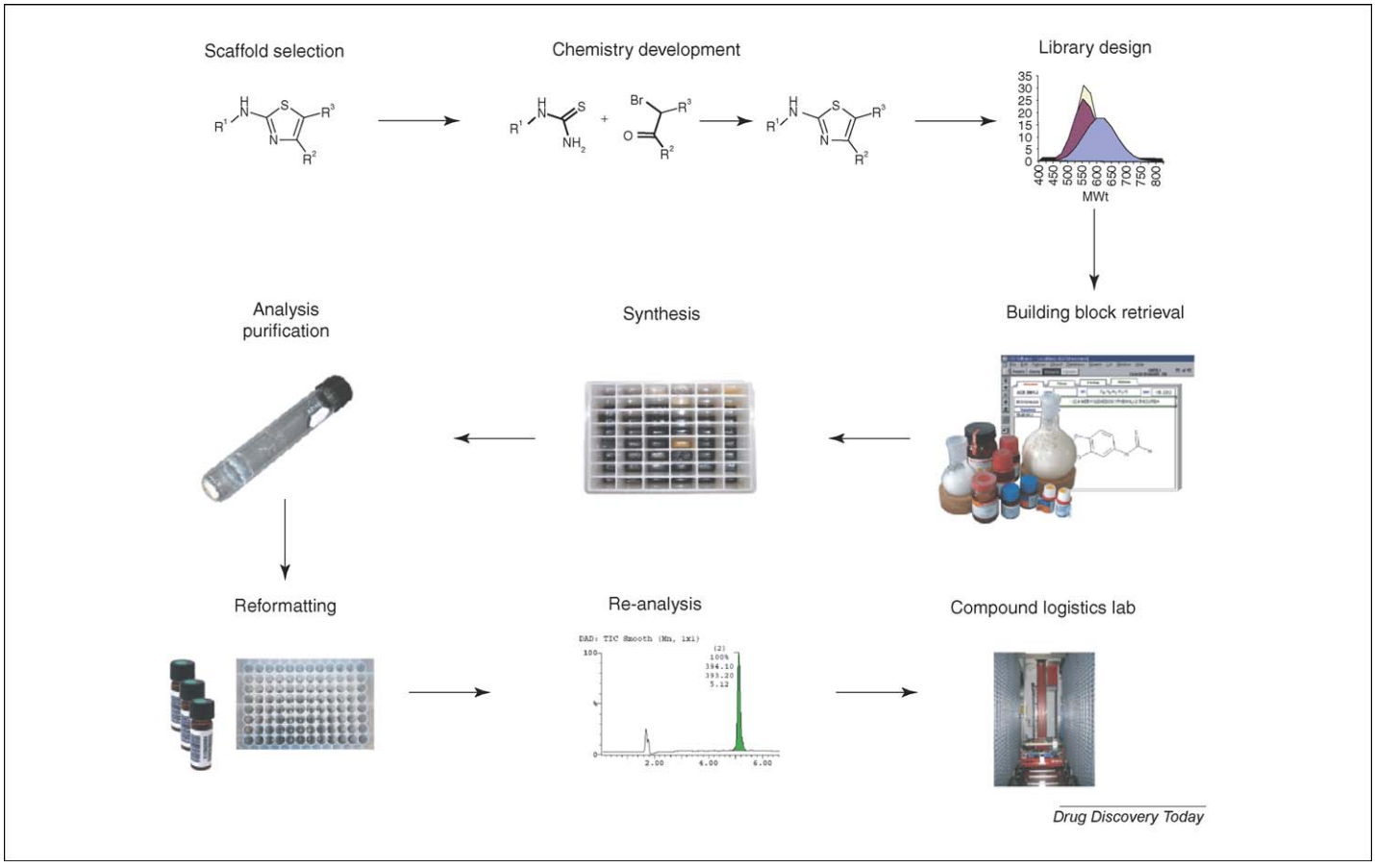

FIGURE 2

Typical workflow of the library production process within automated medicinal chemistry.

We, therefore, decided to establish dedicated subgroups that perform synthesis, purification or reformatting procedures for the whole department.

It is important to note that the production workflow is often barcode-supported to allow unambiguous tracking of compounds at every stage of the process. Equally, data management and archiving in a high-throughput laboratory is of considerable importance. Small errors in the data and sample management can lead to losses of complete production runs. The need for tracking and retrieval of data throughout the whole process requires a large amount of software. Usually, data generated are obtained in several files and file formats. Because these formats are often very different in different companies, it can be assumed that each AMC group within the pharmaceutical industry has to come up with its own individual sample- and data-workflow. The individual workflows then require customized solutions from equipment vendors. No matter how an individual workflow is organized, the following chapters will provide an overview of technologies frequently used.

Library design

In silico design of small-molecule structures has become an important point of consideration, not only in AMC [18,19]. As proven in the past, many early combinatorial libraries had little useful physicochemical properties and, therefore, careful and smart design of libraries is of the utmost importance for success in AMC. Depending on the purpose of a library, the applied design principles can vary dramatically (Figure 1). Generally speaking, it can be assumed that for early-stage (pre-HTS) libraries (e.g. hit-finding libraries, target-family-oriented libraries) one will choose more-basic design tools, such as Lipinski's rule of five [20], diversity or selection of compounds filling voids in a certain screening set [21–23]. Care has to be taken for the selection of privileged structures, substructural elements that show effects on more than one target protein, irrespective of the corresponding target family they might belong to [24]. These design elements allow a high probability for biological activity, but recently it has been shown that certain biological effects of these elements are caused by nonspecific inhibition phenomena like aggregation (promiscuous binders, frequent hitters). Unfortunately, from a structural point of view these elements are not easily identifiable by medicinal chemists [25–30].

Additionally, neural nets, providing scores for drug- or lead-likeness, [31] or machine learning approaches [32] might be used to assess virtual compounds with regard to their target-family-fit or their probability to overcome certain hurdles, such as poor solubility or poor bioavailability. For kinases, we have developed an all-kinase pocket-homology model, which is used to assess proposals for new scaffolds with regard to their fit into the ATP-binding pocket.

In more-advanced stages of hit-to-lead or lead-optimization projects, more-sophisticated tools, such as docking, pharmaco-

phore- or QSAR-based models, or even highly individualized designs for a certain project, might be used in conjunction with (or instead of) the other tools previously mentioned [33].

Synthetic equipment and strategies

A plethora of synthesis equipment is available for the production of compounds in a library format. This can be roughly divided into two categories: independent modular equipment, which splits the synthesis into subprocedures (mainly the addition of reagents and incubation with reactants); and fully automated units, which perform the whole synthesis without manual interference. Clearly, on the one hand, fully automated systems are more complex, require more expertise for use, are less flexible and are more prone to instrument breakdown. On the other hand, they normally do not require manual interference and should, therefore, save valuable resources. Both approaches are currently followed in the pharmaceutical community. However, there appears to be a trend to the semiautomated modular approach, because it offers more possibilities with respect to flexibility and throughput, and the dependability of fully automated systems sometimes has been below expectations. Typical production scales range between 1 and 100 mg [34], with a tendency to higher amounts.

Beyond the degree of automation, the synthetic strategy also has an influence on the choice of the synthetic equipment. For parallel chemistry in solution, stock solutions of reactants are usually dispensed by a liquid-handling unit into reactors of various formats, basically simulating the flask used in single-synthesis approaches. With classical solid-phase chemistry, the products are assembled by temporarily anchoring one of the reactants to a resin and, after a series of transformations has been executed, obtained by a final cleavage step that releases them into solution. Consequently, solid-phase equipment has to have additional filtration and resin-wash capabilities. Of course, this equipment is also useful for solid-supported solution-phase synthesis approaches [35,36]. Zinsser Analytics has developed a more automated solution by integrating a monomode system into their Sophas platform. More recently, Chemspeed released a similar system called Swave.

For the simpler modular approach, several different parallel reactor blocks are commercially available that can be used alone or with a liquid-handling device (see Table 1 for selected equipment examples). In addition, various pharmaceutical companies have developed customized blocks. The simplest form of reactor blocks for nonsensitive chemistries are micotiter plates, which can be composed of glass, teflon or even simple polypropylene. Common liquid handlers that are often used in conjunction with blocks are provided by Hamilton (www.hamiltoncompany.com), Tecan (www.tecan.com), Gilson (www.gilson.com) or Beckman–Coulter (www.beckman.com).

Well-known automated reaction systems include Chemspeed's Accelerator, Zinsser's Sophas, Mettler–Toledo's Myriad Core System and MultiSyntech's Syro (www.multisyntech.com). Many of the automated systems have solution- and solid-phase synthesis capabilities. An extreme example of how far automation can go is the custom-built SynCar automated synthesis platform (Accelab) recently introduced by SanofiAventis [37].

A unique system developed exclusively for solid-phase applications is IRORI's Kan technology. The method is a more advanced variation of the teabag method [38,39]. Here, the 'teabags' are porous containers called Kans, which contain a machine-readable radio-frequency or 2D barcode tag. This allows automated directed-sorting between the various steps and, therefore, large libraries are possible from relatively little effort. Alternatively, monolithic polymer plugs are commercially available (Mimotopes lanterns).

Microwave synthesizers are firmly established within MedChem laboratories [40,41]. It can be expected that they will replace oil baths for most applications in the near future. The main advantages are that microwave heating provides significant speed gains and that the systems now have dependable sensors for pressure and temperature, thereby reducing the inherent explosion potential. Two systems are available: monomode and multimode ovens. Multimode synthesizers have diffusely distributed microwaves in their cavity, whereas monomode systems deliver a continuous standing-wave with well-defined regions of maximum field strength. The advantage of the monomode systems is the very homogeneous microwave distribution, allowing for optimum reproducibility. The downside is that the cavities hold only single reactors that are limited to ~100 ml in size, whereas the available cavity in multimode systems is about the size of a domestic oven with a working volume of up to ~1000 ml. Consequently, only multimode systems can be used for true parallel synthesis. The current approach for monomode systems is the coupling of the oven with a vial gripper that sequentially feeds the prefilled and sealed vials into the cavity. Zinsser Analytics has developed a more automated solution by integration of a monomode system into their Sophas platform. There have been many discussions as to which system is best-suited for compound production [42,43]. Our experience is, for reactions that require a very clearly defined temperature range for success (±5 °C) it is better to use a monomode system. Other reactions that are less sensitive can be very successfully run in multimode systems. Examples for multimode systems are MLS's Start system and CEM's MarsX. Monomode systems are CEM's Explorer and Biotage's Initiator series, which can be combined with various autosamplers for sequential synthesis. Examples of microwave applications are beyond the scope of this article, but it seems reasonable to suppose that any reaction that does not open up additional pathways upon heating can be speeded up with microwaves.

Often, automated solution-phase library synthesis implies one-step synthesis procedures where two reactants are combined. This allows simultaneous variation of two diversity elements. Frequently, one of the two reactants is not commercially available and needs to be synthesized beforehand as a proprietory intermediate on a larger scale. These reactants are often termed scaffolds or templates and are subsequently reacted with a much larger number of reagents, called building blocks. Easy variation of more diversity elements is only possible by multicomponent reactions, a technique that is, therefore, very popular in solution-phase chemistry. The advantage of solid-phase synthesis is that several consecutive steps can be used to assemble molecules, thereby allowing the introduction of many diversity elements. The biggest limitation here is that all steps need to have high yields to allow the isolation of products in reasonable amounts. Consequently, optimization is more crucial and labour-intensive than in single-step solution-phase synthesis. In practice and on average, chemistry development takes much longer in solid-phase than for solution-

TABLE 1

Typical equipment involved in the various steps along the way to the production of compounds

Step	System	Examples	Website
Synthesis	Manual blocks	Flex Chem Blocks	www.robsci.com
		Calypso Blocks	www.charybtech.com
		MiniBlocks	de.mt.com/home
		Desyre Blocks	www.zinsser-analytic.com
	Automated synthesizer	Accelerator	www.chemspeed.com
		Sophas	www.zinsser-analytic.com
		Myriad Core System	mettler-toledo.mt.com
	Resin containers	IRORI Kans	www.irori.com
		Mimotopes Lanterns	www.mimotopes.com
	Multimode microwave	Start System	www.milestonesrl.com
		MarsX	www.cem.com
	Monomode microwave	Explorer	www.cem.com
		Initiator	www.biotage.com
Analysis and purification	Analytical HPLC–MS	Acquity	www.waters.com
		1200 Series	www.home.agilent.com
	Detectors	Evaporative light scattering detection (ELSD)	www.sedere.com
		Chemiluminescent nitrogen detector (CLND)	www.antek.se
		charged aerosol detector (CAD)	www.coronacad.com
	Preparative HPLC–MS	Purification Factory	www.waters.com
		APS Series	www1.dionex.com
	Supercritical fluid chromatography (SFC)	Berger Analytix MS	de.mt.com/mt
		SFC–MS Resolution	www.thartech.com
Evaporation	Centrifuge	RVC Beta	www.martinchrist.de
		HT-12	www.genevac.com
	Shaker	Combidancer	www.hettich-ag.ch
		Syncore Polyvap	www.buchi.com
	Lyophilizer	EPSILON 2–12D	www.martinchrist.de
		Ultra 35EL	www.virtis.com
Reformatting		Balance Automator	de.mt.com/home
		Accelab HTR	www.accelab.de

phase chemistries where multi-step operations are interrupted by purification of intermediates (scaffolds). The main application of solid-phase is, therefore, the production of larger libraries with many diversity elements, whereas solution-phase is mostly used for smaller libraries and libraries where delivery of compounds is time-dependent (e.g. in the support of projects).

Analysis and work-up (purification) strategies and equipment

Currently, the method of choice for high-throughput analysis of libraries is LC–MS. Mostly, reverse-phase (RP) chromatography is used and the trend here is to maximize throughput by reducing run times as far as possible. An example is outlined in a recent publication by researchers from Arqule, demonstrating that run times of ~1.5 min are possible with conventional LC–MS equipment [44]. Waters has recently introduced their Acquity system for fast RP–LC analysis: run times of below one minute are possible by combining fine 2.5 μm column material with equipment that can handle high back-pressure. The limiting factor here can be the data-acquisition rate of the attached mass spectrometer.

Analysis is performed to assess the purity of samples and also for their quantification [45,46], therefore the detectors play a crucial role. UV detection is well-established and the most popular detection technology; however, it is limited for use with UV-absorbing compounds. In addition, the compound-specific extinction coefficients allow neither absolute purity assessment nor quantification of compounds by integration of peak areas. Evaporative light scattering detection (ELSD) is independent of UV absorption, however the signal depends nonlinearly on various factors like molecular weight, concentration and gradient composition. Therefore, quantitative purity assessment is not possible and compound amounts can only be determined after the establishment of sophisticated calibration curves [47–49]. Chemiluminescent nitrogen detectors (CLNDs) generate a signal by processing the nitrogen contained in the compound. It has been shown that this detector allows relatively accurate quantification of samples.

The downsides are that only nitrogen-containing compounds are detected, only non-nitrogen containing eluents can be used and the signal becomes inaccurate when N–N bonds are present in the molecule [50,51]. A promising new technology is the charged aerosol detector (CAD) from Corona. Little is known about this technology so far, however it shows clear potential to be the most universal of all detectors. Although not as widespread as HPLC, high-throughput NMR has been established in many laboratories for purity analysis and compound quantification. However, no fully automated interpretation of NMR spectra is possible, thereby limiting the usefulness of this technology [22] and only subsets of a library are analyzed and interpreted.

Nowadays, many compounds synthesized in a library format are purified. Quality has become a buzzword in this context [42,43]. Extraction and scavenging techniques with appropriate column materials or resins can be performed in parallel and have proven to be efficient in the removal of by-products, reagents and/or starting materials [52].

A silent revolution has occurred in the past couple of years with respect to high-throughput purification using LC–MS. Hundreds of compounds can be efficiently purified per day with minimal manual intervention requirements. Here, the key factors for success were the implementation of combined UV-mass-triggers for fraction collection, establishment of automated software-supported workflows for data-handling and/or evaluation and flow-rates, which were pushed to the limits again – thereby reducing run-times dramatically [53–55]. Previously, it was common to purify 100 mg of compound at flow rates of 20–40 ml/min with run times of ~15–30 min. Now, up to 150 ml/min flow rates are employed with run times of 3–5 min. Another important development for fast and efficient purification was the calculation of optimized gradients derived from analytical pre-screens. Indeed, even complex mixtures can now be easily purified to >95% purity in very short run times [55–58]. A method that has worked well in our laboratories for simultaneously maximizing purity and yield is the purity assessment of collected fractions by post-run analysis of the MS spectra. In addition, the installation of automated solvent- and waste-management systems saves valuable resources and is crucial to enable continuous operation. With all these procedures in hand, purification no longer seems to be a bottleneck in the overall process. A trend is observed where all successfully synthesized compounds from a library are purified regardless of their analytical purity, often without preceding extractive work-up procedures.

Supercritical fluid chromatography (SFC) appears more and more in pharmaceutical laboratories for high-throughput analysis and purification of compounds [59–62]. The advantage of SFC is its speed, possible as a result of the inherent low back-pressure of the eluents (MeOH and CO_2) and also the easy removal of solvents. The biggest issue is that systems for mass-directed fraction collection are still not available.

Auxiliary equipment

Addition of a defined amount of resin to a reactor is mandatory in solid-phase or solid-supported solution-phase synthesis. Volume-based systems include ArgoScoop (www.biotage.com), plates with a trapdoor mechanism for direct-dosing into parallel blocks, Zinsser's DryPette and its automated pendant Redi. Weight-based dosing systems are the Powdernium from Autodose (www.autodose.ch), Mettler's FlexiWeigh and Chemspeed's Accelerator Dosing Station. The novelty of the Chemspeed system is an overhead balance containing the resin to be dosed, which allows dispensing into almost any rack. Also, no reactor grippers are needed, unlike for conventional systems.

Evaporation occurs at various stages in the compound-production process, for example after synthesis, after chromatography and during a reformatting step. Vacuum centrifuges from Christ or Genevac are very popular; however, shaker-based evaporation systems like the IR- or Combi-Dancer and the Büchi Syncore Polyvap can also be used in this process. The advantages of shaker-based systems is that they do not require any sample-balancing and that they are usually faster, however problems can occur with 'easily bumping' liquids (e.g. acetonitrile–water mixtures) coming from preparative HPLC. For HPLC fractions in particular, it is very popular to freeze–dry acetonitrile–water mixtures using cooled storage plates, also DMSO samples can be lyophilized to fluffy powders. Manufacturers include Christ, Hof Sonderanlagenbau (www.hof-sonderanlagen.de) or Virtis.

Reformatting is usually the final step and implies dissolution and transfer of compounds into their final-destination containers [e.g. (barcoded) glass vials or MTPs]. This process is often accompanied by additional weighing and evaporation steps. A commercial system for automated weighing is Mettler–Toledo's balance automator, but companies often utilize their own custom-made systems.

Impact

There has been a lot of speculation about the putative impact of parallel medicinal chemistry on the development of drugs. Clearly, managers want to know if their investments are justified. Synthesis of analogues has always been an integral part in medicinal chemistry projects but now it can be performed more efficiently in parallel (rather than sequentially, as in the past). Certainly, there is no doubt about the usefulness in this instance. For less project-specific activities, like targeted or more-general lead-generation libraries, the situation is not so clear, partly because pharmaceutical companies do not often reveal the source of their clinical candidates [21,63]. Certainly, appropriate design strategies are crucial for success (see earlier). Our in-house analysis on the performance of our kinase-targeted library (see Figure 3) and our GPCR-targeted library shows that these compilations constitute valuable hit sources. Many other reports support this impression [64,65] and, in most pharmaceutical companies, the scepticism has given way to a firm belief in these approaches.

Future trends

Microwave technology and SFC are areas with significant potential for expansion. For AMC laboratories, parallel monomode microwave systems and the ability to perform mass-directed purification using SFC would be highly appreciated. According to our information, both issues are currently being explored by vendors.

An ever emerging field is that of flow-reactor technology, not only for large-scale synthesis, but also for library production [66–68]. The technology is especially helpful for short and exothermic reactions and it remains to be seen if it will become a standard tool for library synthesis. Meanwhile, the hydrogenation device H-cube is a well-received system (www.thalesnano.com) [69], and

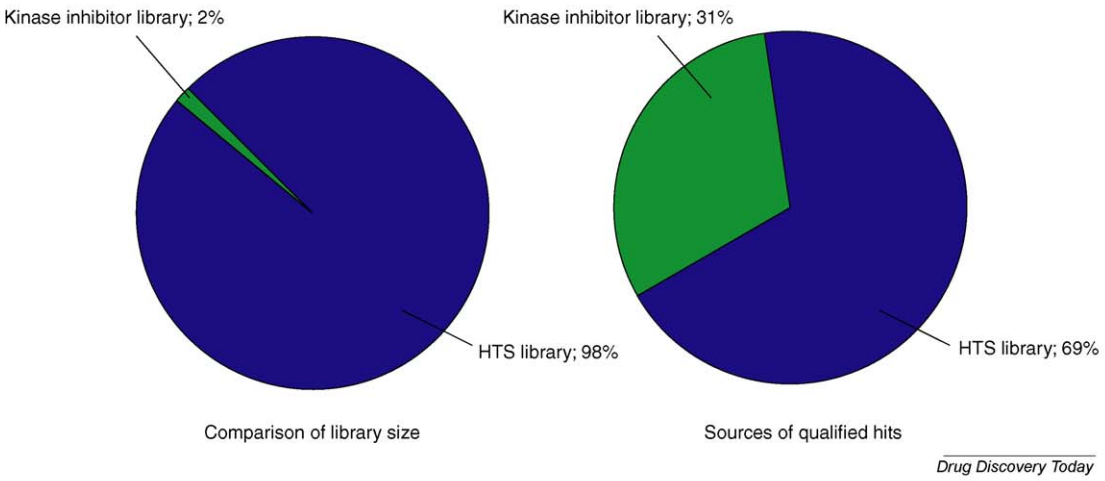

FIGURE 3

Impact of Schering's in-house kinase inhibitor library in recent HTS campaigns against kinase targets. On the left is a comparison of library sizes, Schering's in-house kinase inhibitor library (green) constitutes currently ~2% of Schering's complete HTS library (blue). On the right is the share of qualified hits, Schering's in-house kinase inhibitor library (green) provided 31% of all qualified hits from the 12 kinase HTS campaigns analyzed.

its soon-to-come analogues CO-cube and O-cube will probably follow in its footsteps.

Irrespective of the future technology, it is fair to say that well-considered highly efficient and streamlined workflows (in combination with the appropriate equipment) have been established in many AMC laboratories. Often, the roads to these platforms have been steep and bumpy, requiring tremendous efforts. Although automation clearly has not reached perfection yet, it is improving steadily and can provide significant advantages in the drug discovery process.

Aside from the technology, we are now entering an era in which chemists working in AMC will probably become more chemistry-oriented than they have been in the past decade, when the focus was on developing and implementing a robust and reliable technology platform. In this context, more chemistry-related challenges, such as the discovery and exploitation of new structural motifs in chemical space, development of new chemistries and their application in library synthesis, will hopefully be addressed and solved.

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