Novel strategies for solid-phase construction of small-molecule combinatorial libraries

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During the past decade we witnessed a rapid advance in the new field of chemical science, combinatorial chemistry. The pharmaceutical industries invested heavily in accelerating the development of this new technology. As a result, it has become an extremely important tool in lead identification and optimization in current pharmaceutical research. It also quickly crossed the boundaries of the original chemical discipline and demonstrated great potential in many other important areas, such as searching for novel and highly efficient catalysts and superconductive material. Researchers from both academic and industrial laboratories have directed great effort towards the development of novel strategies for combinatorial synthesis.

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▼ The field of combinatorial chemistry has received a great deal of interest for the rapid generation of novel and drug-like heterocyclic scaffolds. Today, chemists face increasingly high demands for novel compound libraries with drug-like properties for screening against a rapidly growing range of therapeutic targets resulting from genomics research. Combinatorial libraries, depending on their utility, can be categorized into several classes, including broad and diverse discovery libraries, targeted libraries and focused libraries. Therefore, the strategies for design and synthesis of these libraries are implemented in different ways[1]. However, the following challenges and problems in generating a small-molecule combinatorial library are always encountered regardless of whether it is a mixture- or single-compound-based library:

- Solid-phase vs solution-phase synthesis;
- Drug-like properties;
- Novelty and patentability;
- Molecular diversity;
- Efficiency of synthesis;
- Purity of compound libraries;
- Cost of production; and
- Compatibility with automation.

Although there has been a rapid growth of literature dealing with the development of novel strategies for addressing these issues, the scope of this article is limited. Thus, this review can only highlight the recent and representative examples with a combination of the author's own work and current interests, primarily focused on novel synthetic strategies for rapidly generating heterocyclic scaffolds and combinatorial libraries on a solid support. The strategies relating to the development of novel linkers for solid-phase synthesis[2], high-throughput purification of combinatorial libraries[3] and rational approaches in library design[4] are not reviewed here.

Template-based approach

The concept of 'privileged structures' was introduced by Evans and coworkers in 1988 (Ref. 5), and recently updated by Patchett[6]. A privileged structure is 'a single molecular framework able to provide ligands for diverse receptors'. A classic example of privileged structure is the benzodiazepine moiety that is found in a variety of therapeutic agents. To exploit molecular diversity and generate drug-like combinatorial libraries for various biological targets, these privileged structures have been used as templates from which functional variations can be achieved at multiple diversification positions using combinatorial approaches. These libraries are used for screening in a variety of biological assays and exploring their ability to affect particular pathways in cells or organisms, hopefully leading to the discovery of highly specific and effective molecules.

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The purine structure can be found in many agonists, antagonists and substrates[7,8], and it plays a crucial role in many cellular processes. Therefore, the exploration of chemical diversity based on the purine template and the development of practical synthetic protocols was an attractive objective; there are several approaches reported in the literature (Fig. 1)[9–16]. The most impressive was the solid- and solution-phase combination strategy developed by Schultz and coworkers[9,10,12,14], which enables the complete diversification at the C2, C6 and N9 positions on the purine template. From the libraries, the same group then identified a series of highly potent and selective CDK2-kinase inhibitors. The most potent inhibitor has an IC_{50} value against CDK2-cyclin A of 6 nM (Ref. 9).

The common features in both the solid- and solution-phase syntheses shown in Fig. 1[9–15] are the Mitsunobu alkylation at the N9 position, the nucleophilic amination at the C6 position and the Mitsunobu alkylation or nucleophilic amination at the C2 position. However, the order of these reactions might vary in the different protocols.

More recently, 2,6,8-trisubstituted purines were synthesized from 2,6-dichloropurine bound to Rink resin at the N9 position[16]. Following conventional aminations at the C2 and C6 positions, the C8 substitutes were then introduced by bromination at the C8 position and a palladium (Pd)-mediated cross-coupling reaction.

An alternative strategy to the construction of the diverse combinatorial libraries is based on natural-product-like templates. These templates have rigid conformations and the potential for multisite functionalization. They are usually assembled by highly efficient solid-phase chemistry as reported by Schreiber and coworkers (Fig. 2)[17,18]. The same group proposed the diversity-oriented organic synthesis concept

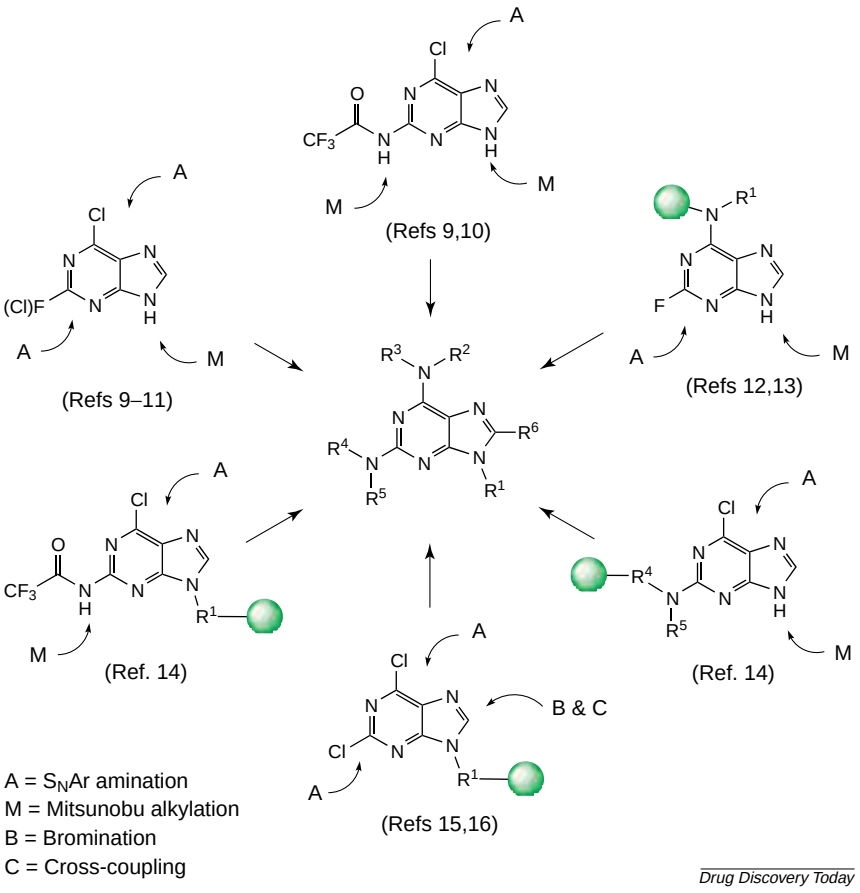

Figure 1. Solution- and solid-phase syntheses of combinatorial libraries based on the purine template.

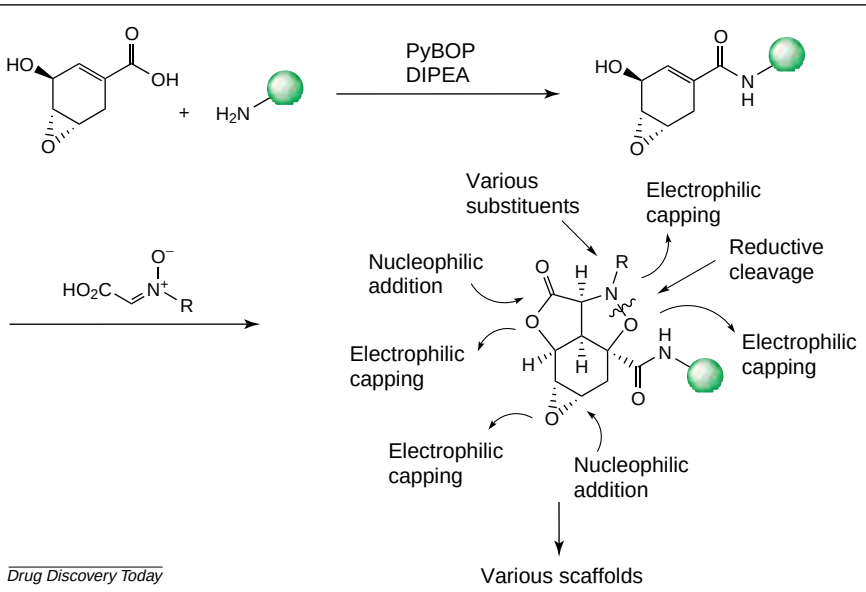

Figure 2. Combinatorial synthesis based on a synthetic polycyclic template[17,18]. Abbreviations: DIPEA, *N*,*N*,diisopropylethylamine; PyBOP, benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate.

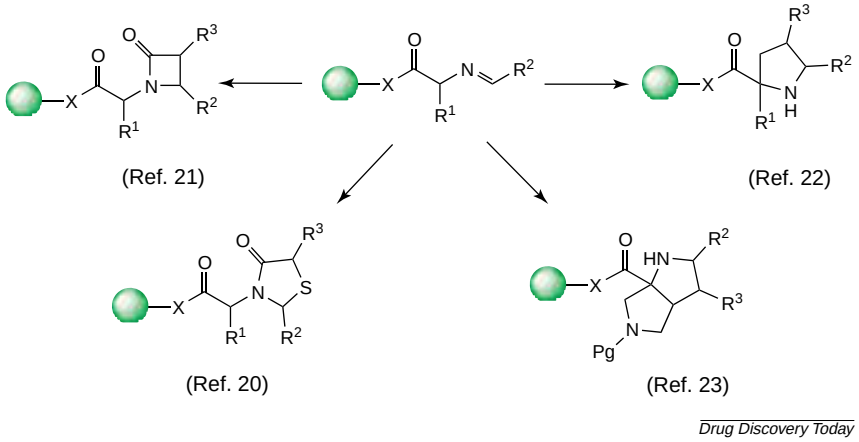

Figure 3. Resin-immobilized imines as precursors for rapid generation of multiple heterocyclic scaffolds.

and demonstrated the importance of novel and structurally complex templates for generating the small-molecule libraries that are useful for exploiting chemical genomics[19].

Versatile precursor-based approach

The use of highly versatile polymer-bound precursors for the rapid generation of multiple scaffolds became an effective method for quick access to diversity combinatorial libraries. These polymer-bound building blocks are usually readily prepared from commercially available starting material, and contain multiple functional group(s) and/or reactive sites suitable for exploring structure diversification. Unlike the template-based approach, these precursors undergo higher degrees of structure variations through a series of chemical reactions and proliferate structurally diverse and biologically interesting heterocyclic scaffolds.

These polymer-bound versatile precursors were often developed based on classic organic synthesis. For example, in addition to its utility in reductive alkylation, the imine moiety has been widely used in the formation of diverse nitrogen-containing heterocyclic rings through cycloadditions, condensation reactions and other nucleophilic additions. Several laboratories then adapted these conventional transformations to solid-phase synthesis using resin-immobilized imines via the condensation of tethered amino acids with aldehydes, generating 4-thiazolidinone, β-lactam, pyrrolidine and polycyclic scaffolds (Fig. 3)[20–23].

Armstrong[24] proposed that a multiple core structure library (MCSL) generated from a common precursor

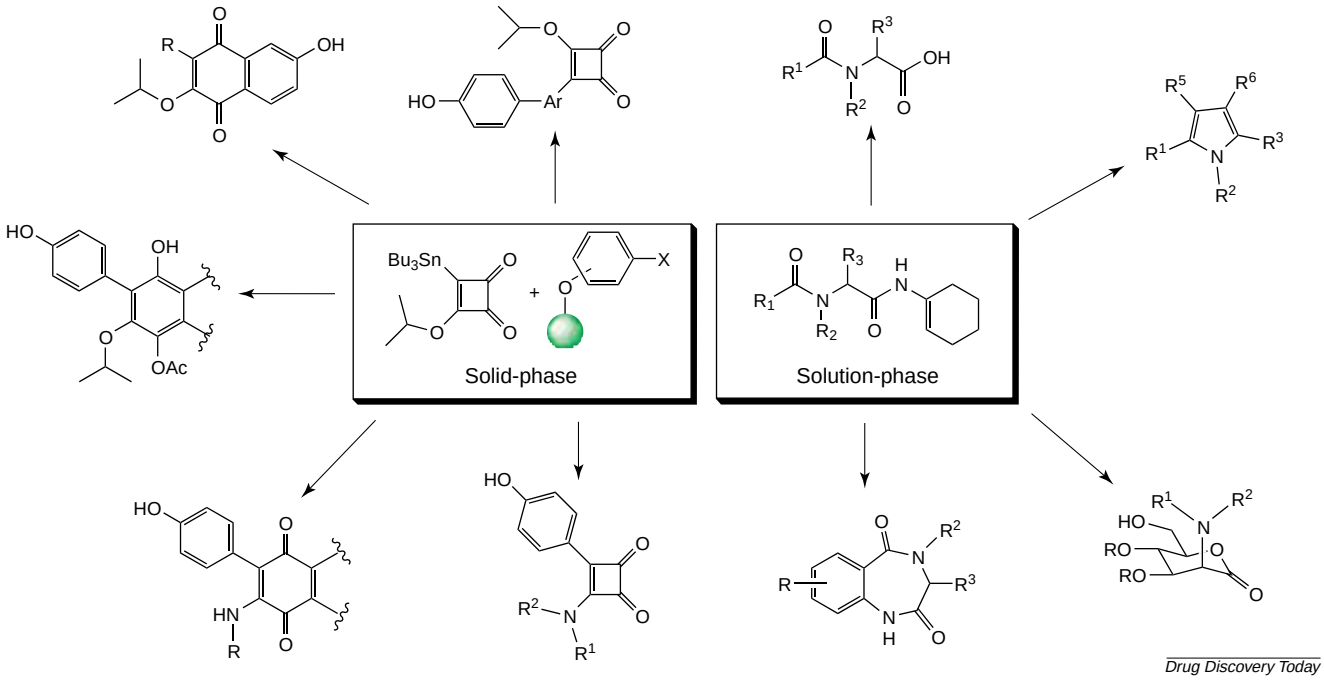

Figure 4. Armstrong's solid- and solution-phase strategies for the rapid generation of Multiple Core Structure Libraries (MCSL)[24,25].

possesses a higher degree of diversity because of the structure variations not only from the functionalities but also from the orientation of the display. A single-core structure-based library generates different compounds by changing only the functionalities that are displayed. Squaric acid was identified as a versatile precursor and then attached to a solid support via an aryl ether linkage. Transformations involving the polymer-bound squarates gave a variety of fused aromatic and heteroaromatic scaffolds as shown in Fig. 4.

The same research group also applied a similar strategy to solution-phase combinatorial synthesis. By using a 'universal isocyanide', 1-isocyanocyclohexene, in the Ugi reaction, the product cyclohexenamides can serve as a powerful precursor to a variety of scaffolds[25]. An oxazolinium-5-one (münchnone) was proposed as an active intermediate that reacts with many nucleophiles to yield the products illustrated in Fig. 4.

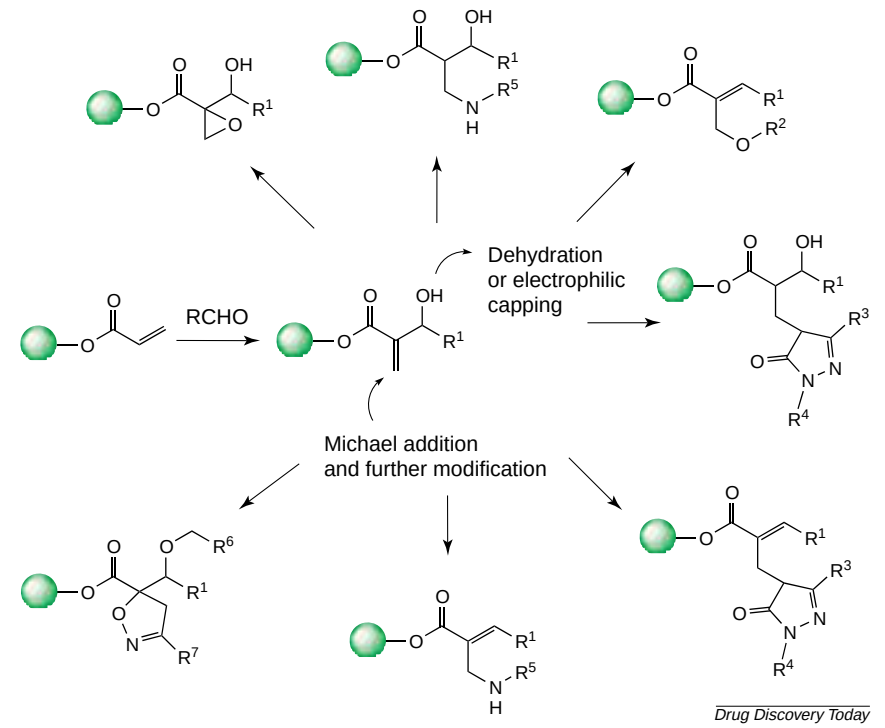

Figure 5. Polymer-bound 3-hydroxy-2-methylidenepropionic acids for synthesis of Multiple Core Structure Libraries (MCSL)[26].

Jung reported a method for the rapid expansion of a scaffold collection based on polymer-bound 3-hydroxy-2-methylidenepropionic acid[26], which was prepared via the Baylis–Hillman reaction of chlorotrityl-resin-bound acrylic ester with aldehydes. The presence of two functional groups of the precursor enabled a wide range of post-modifications, such as the Michael addition, 1,3-dipolar cycloaddition, dehydration and electronic capping resulting in an array of interesting scaffolds (Fig. 5).

During the past several years, many laboratories independently reported the formation of various heterocyclic scaffolds using resin-immobilized 4-fluoro-3-nitrobenzoic acid. A common synthetic strategy adapted in the syntheses is illustrated in Fig. 6 (Refs 27–36). The first step is the displacement of fluoride at position 4 by various nucleophiles, such as amines and thiols. Subsequent reductions of the nitro-intermediates followed by various cyclization protocols led to different heterocyclic structures, such as benzimidazolone, benzimidazole, benzopiperazinone, benzodiazepine, benzothiazepinone and tetrahydroquinoxalinedione. A representative set of scaffolds derived from this building block is shown in Fig. 6.

Recently, our laboratories and others reported a similar approach to constructing biologically important heterocycles using polymer-bound 4-(bromomethyl)-3-nitrobenzoate and the corresponding amides (Fig. 7)[37–39]. The building block undergoes nucleophilic displacement followed by reduction and cyclocondensation reactions to afford 3,4-dihydroquinazolines[37], benzodiazepin-2-one[38], tetrahydroquinazolinone[39] and many other heterocyclic scaffolds (Fig. 7) (Zhang, J. *et al.*, unpublished).

The 'libraries from libraries' concept

Proposed by Houghten in 1994 (Ref. 40), the 'libraries from libraries' concept has been widely practiced in combinatorial synthesis. The central feature of the approach is the conversion of libraries into new libraries while the compounds remain attached to the solid support. The second generation of libraries produced by this process should have markedly different physical, chemical and biological properties compared with the libraries from which they were derived. Houghten demonstrated the concept by converting a peptide library into a permethylated one in which the typical –CONH– peptidic amide bond was modified by *N*-methylation[40].

The same laboratory has extended this approach to the construction of small organic compound libraries. The existing resin-bound peptide libraries have been successfully modified by reduction of amide bonds followed by a series of further chemical manipulations to afford the second generation of libraries. The linear dipeptide libraries were

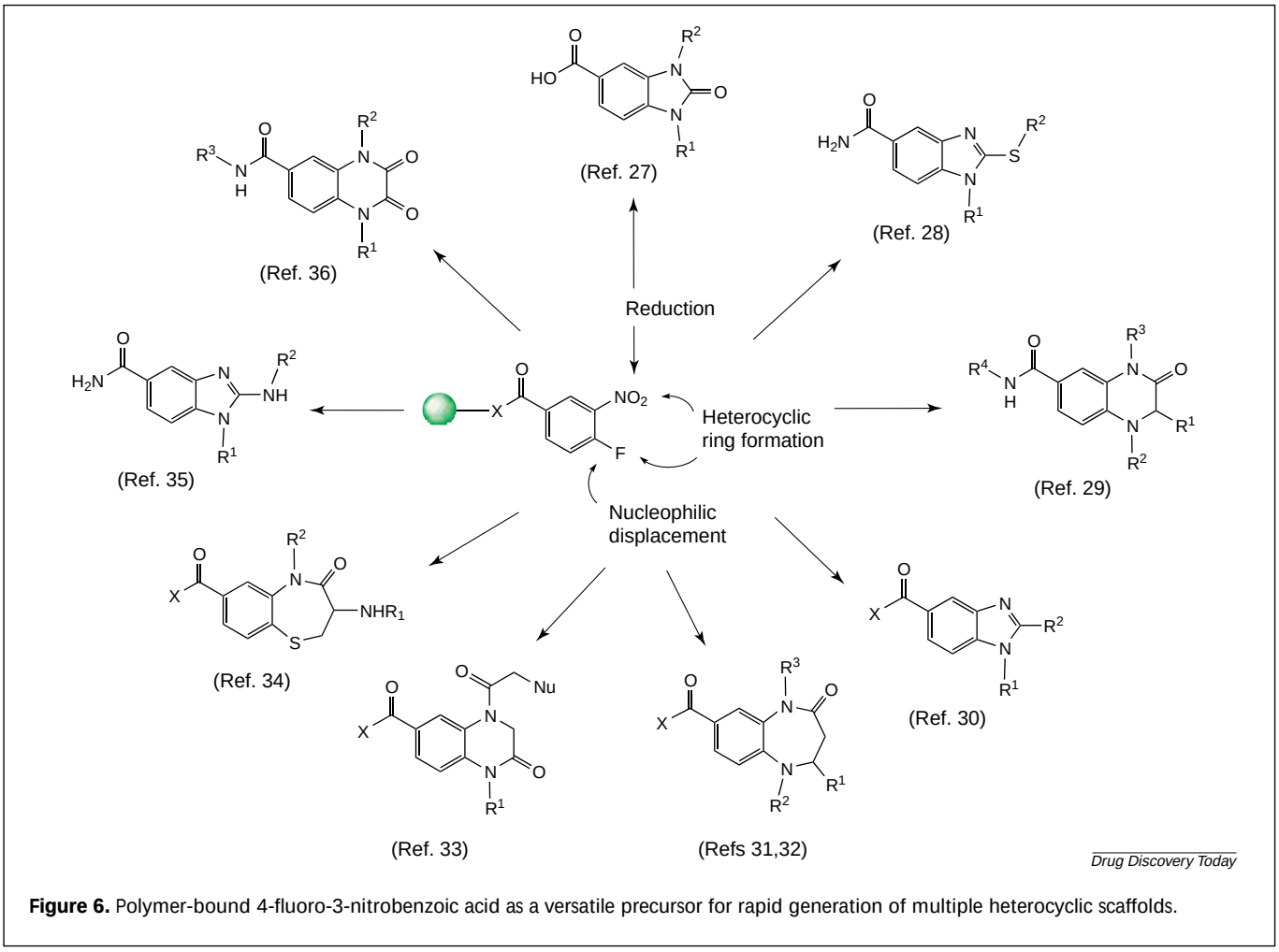

Figure 6. Polymer-bound 4-fluoro-3-nitrobenzoic acid as a versatile precursor for rapid generation of multiple heterocyclic scaffolds.

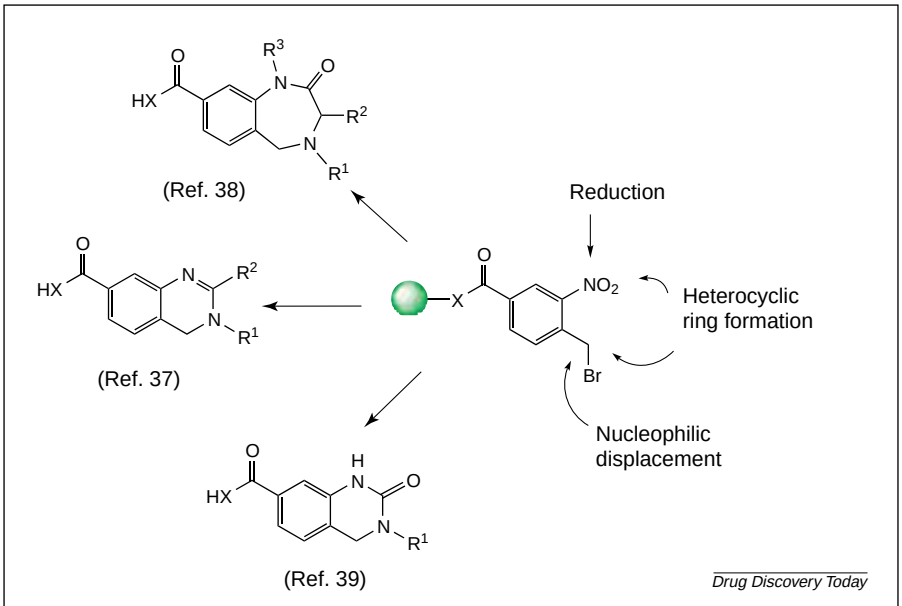

Figure 7. Polymer-bound 4-(bromomethyl)-3-nitrobenzoic acids as a versatile precursor for rapid generation of multiple heterocyclic scaffolds.

used for the generation of small heterocyclic compound libraries, such as cyclic ureas, thioureas, bicyclic guanidines, imidazol-pyrido-indoles, hydantoins and thiohydantoins (Fig. 8)[41–45].

Based on the same concept, a solid-phase synthesis of a piperazine library from the corresponding diketopiperazine library has been recently demonstrated (Fig. 9a)[46]. The reduction of the diketopiperazines by a BH_3–THF complex on solid support was performed in Ares™ reactors on automated synthesizers (Advanced ChemTech, Louisville, KY, USA). A similar approach was undertaken to generate a highly diversified piperazinone library. In this synthesis, the Ugi four-component condensation reaction was carried out on various α-amino acid Wang resins under standard conditions. An array of resin-bound

Ugi-products were then subjected to the reduction by using BH_3–THF at 65°C for 6 h. The piperazinones were released via intramolecular cyclization in the presence of 5% HOAc in dichloromethane (Fig. 9b)[47]. The Ugi reduction–cyclization strategy offers a practical extension of the repertoire of Ugi-chemistry in combinatorial synthesis.

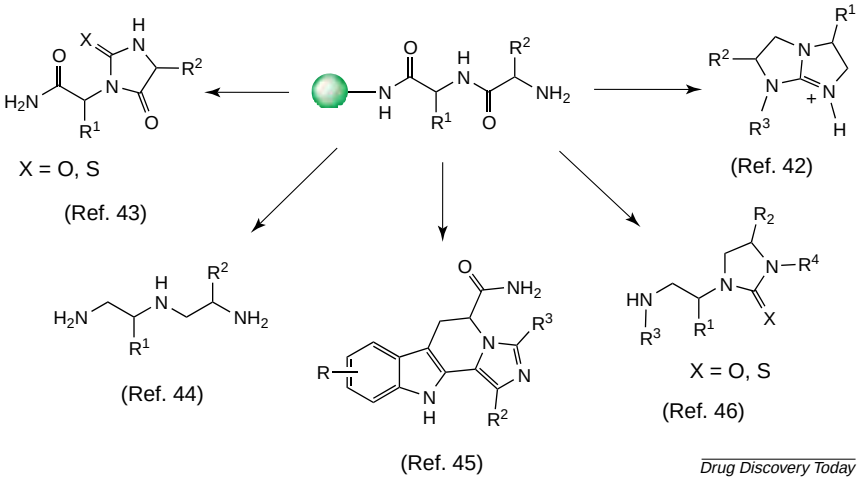

Figure 8. Houghten's 'libraries from libraries' approaches to rapid generation of multiple linear and heterocyclic-based libraries.

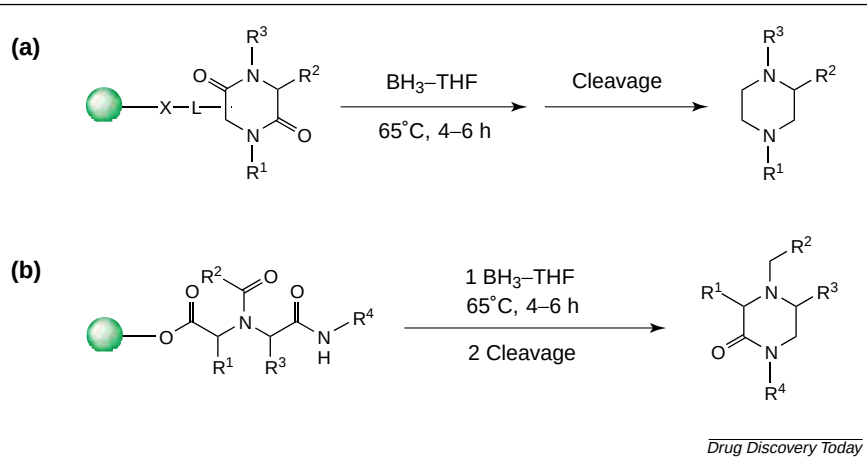

Figure 9. (a) Solid-phase synthesis of piperazine derivatives from the corresponding diketopiperazine library[47]. **(b)** Solid-phase synthesis of piperazinone derivatives based on the Ugi reduction–cyclization strategy[48].

Conclusions

The evolution of combinatorial chemistry has provided access to greatly expanded chemical collections useful for lead identification in pharmaceutical companies. Several new chemical entities generated from combinatorial libraries are currently being evaluated in the clinic for the treatment of various human diseases[48–50]. The current trend to explore chemical diversity has shifted from merely increasing the number of new compounds synthesized, generating huge libraries and exploring relatively simple reactions to creating biologically relevant and information-rich combinatorial libraries in a much more defined and rational fashion.

Although the pharmaceutical industries still continue to mainly adapt the parallel synthesis technologies for the generation of combinatorial libraries, the mix-and-split method devised with ingenious encoding methods, such as radiofrequency, has also demonstrated great value. Recently, several new techniques, such as string synthesis[51] and Euclidean shape-encoded synthesis[52], were reported to combine the high efficiency of the split synthesis with the advantage of the parallel method. However, putting them into real practice in pharmaceutical laboratories remains to be seen.

Developing new concepts to create novel and biologically relevant scaffolds and engineering efficient strategies to accelerate the syntheses of combinatorial libraries are important in the discovery of lead molecules against various new targets validated from genomics and proteomics research. The combination of combinatorial chemistry, rational drug design and classic medicinal chemistry optimization techniques is the key to delivering high-quality drug candidates in the most cost-effective manner. Integration of innovative chemistry technologies and state-of-the-art automation is equally important to achieve these goals.

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