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Review

Combinatorial chemistry in the agrosciences

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ABSTRACT

Combinatorial chemistry and high throughput screening have had a profound effect upon the way in which agrochemical companies conduct their lead discovery research. The article reviews recent applications of combinatorial synthesis in the lead discovery process for new fungicides, herbicides and insecticides. The role and importance of bioavailability guidelines, natural products, privileged structures, virtual screening and X-ray crystallographic protein structures on the design of solid- and solution-phase compound libraries is discussed and illustrated.

Contents

1. Introduction

In the 1980s it was estimated that an agrochemical company needed to synthesise and screen between twelve and twenty thousand new compounds in order to discover one possessing the attributes needed for commercialisation.[1,2] However, it was already predicted that this number would increase by four to fivefold during the next decade.[2] The reasons for this predicted increase were partly due to more stringent toxicological and environmental safety factors and partly due to the success of the industry in delivering solutions for the problems faced by farmers. The remaining problems were more difficult to solve and their solution required greater resources. One of the ways in which the agrochemical industry sought to address these challenges was to increase the number of compounds screened each year. During the 1990s companies invested heavily in developing high throughput in vivo screens capable of testing hundreds of thousands of compounds

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each year.[3,4] The same period witnessed very significant advancements in the area of combinatorial chemistry.[5] Whereas previously a chemist had been able to make between 100 and 300 novel compounds each year,[1,4] it now became possible to prepare literally thousands. The new synthesis technology fitted well with the newly developed high capacity screens and was enthusiastically adopted within the agrochemical industry. The hope was that by screening large numbers of compounds, there would be a corresponding increase in the number of biologically active leads discovered. However, it was not long before it became clear that the observed hit rate was much lower than had been expected.[6] The design of early combinatorial libraries was heavily influenced by a desire to achieve high structural diversity and by what was technically achievable in the laboratory, meaning that structural compromises were often made. Since that time combinatorial technology has evolved enormously allowing much greater emphasis to be placed upon biology-oriented library design. This has certainly led to improvements in the overall success, to the extent that reports describing commercial pharmaceuticals discovered with the help of combinatorial approaches are now starting to appear.[7]

The extensive use of combinatorial chemistry and high throughput screening (HTS) have significantly increased productivity and decreased the cost per compound associated with synthesis and screening. Nonetheless, the total costs for synthesising and screening a hundred thousand compounds are still very significant. There is a widespread desire to reduce these overall costs by increasing the number of biological hits and leads coming from compound libraries. Virtual compounds are cheap to generate and virtual screens much cheaper than the real thing, once the one-off cost for hardware has been paid. Consequently, there is a growing interest in the use of virtual screening techniques in order to analyse large virtual libraries of 100,000 compounds or more in order to select those compounds which are worthy of synthesis.

The aim of the current article is to review research papers relevant to the application of combinatorial chemistry in the agrosciences published since the area was last reviewed in 2005.[8] In addition the use of bioavailability criteria, virtual screening methodologies and X-ray protein crystallography in the design of libraries for agrochemical in vivo screening will be discussed and illustrated.

2. Bioavailability guidelines

Whereas in the pharmaceutical industry high throughput screening is limited to in vitro assays, the agrochemical industry has the luxury of being able to conduct high throughput in vivo screening directly upon target organisms. In order to take full advantage of this, it makes sense that a screening library should contain compounds selected for their good bioavailability. Commercial agrochemicals and drugs clearly have to fulfil certain bioavailability criteria and a number of researchers have analysed their physical properties and applied their findings to the lead discovery process. This has led to the development and proposal of a series of relatively simple rules to help ensure good absorption and distribution within an organism. The first and most famous of these are the 'Rule of Five' for drug discovery published by Lipinski and co-workers in 1997.[1] Poor absorption or permeation were predicted to be more likely for compounds with physical properties lying outside the limits set by Lipinski's Rules (Table 1).

Optimal bioavailability parameters for pharmaceuticals need not be the same as for agrochemicals, which for example cannot be administered by injection. Consequently, Briggs[10] conducted an analysis of commercial agrochemicals and proposed a 'Rule of Three' for the selection of compounds for high throughput in vivo screening. According to Briggs, a compound is more likely to be active in an in vivo agrochemical screen when MWt = 300 ± 100, $\log P = 3 \pm 3$, $\Delta\log P \leqslant 3$, number of H-bond donors $\leqslant 3$, mp $\leqslant 300$ °C and equivalent hydrocarbon (EH) number = 30 ± 5 (Table 1). Three of the Briggs criteria are similar to those used by Lipinski (MWt, $\log P$, H-bond donors) and three new factors were introduced, namely $\Delta\log P$, mp and EH. Whereas $\log P$ is a good measure of lipophilicity, $\Delta\log P$ ($\log P_{\text{octanol/water}} - \log P_{\text{hexane/water}}$) is a measure of polarity and the likelihood of cell penetration.[11] As a general rule of thumb, polar compounds with $\Delta\log P > 3$ do not efficiently penetrate cell membranes. The melting point (mp) was included due to its correlation with water solubility.[12] Synthetic molecules with mp > 300 °C tend to be insoluble in water and are, therefore, less bioavailable. Since the melting points of library members are rarely determined, Briggs proposed that very approximate melting points for the evaluation of compound libraries could be estimated using Eq. 1.[10] The most difficult part of this equation is estimating the number of rotatable bonds since steric restraints may result in restricted rotation for some bonds (e.g., Strobilurins).

$$\text{mp}\ (^\circ\text{C}) \approx 30\Delta\log P + 60 \times \text{No. of rigid rings} - 20 \times \text{No. of rotatable bonds} \quad (1)$$

The third new criterion is the equivalent hydrocarbon (EH) number which is used to estimate the vapour pressure of a compound.[13] The idea here is that the vapour pressure and boiling points of the simple hydrocarbons are all known, so that converting other molecules containing heteroatoms to the appropriate 'equivalent hydrocarbon' allows their vapour pressure to be estimated. In order to do this, the number of carbon atoms in a molecule are counted and then increments derived from the average boiling point increases for each element are added: 0 for H or F, 1 for Si, 2 for Cl or O, 3 for N, 4 for Br, P or S and 6 for I.[10] In addition 1 EH unit is added for each 35 °C increment in melting point above 25 °C (e.g., Add 2 for a mp of 100 °C). The EH value is important because compounds with EH $\leqslant$ 24 may be lost during evaporation from DMSO when preparing samples for high throughput screening and may be too volatile for field testing

Table 1
Physical chemistry based bioavailability guidelines

	Lipinski 'Rule of 5' for oral absorption (1997)	Briggs 'Rule of 3' for agrochemical in vivo HTS (1997)	Clarke-Delaney 'Guide of 2' for lead progression (2001)
MWt	<500	300 ± 100	200–400
$\log P_{\text{octanol/water}}$	<5	3 ± 3	⩽4
H-Bond donors (sum OH + NH)	<5	⩽3[a]	⩽2
H-Bond acceptors (sum O + N)	<10	–	–
$\Delta\log P$	–	⩽3[a]	<2
mp (°C)	–	⩽300	⩽200
EH	–	30 ± 5	–

[a] Unless water solubility >3 g/l.

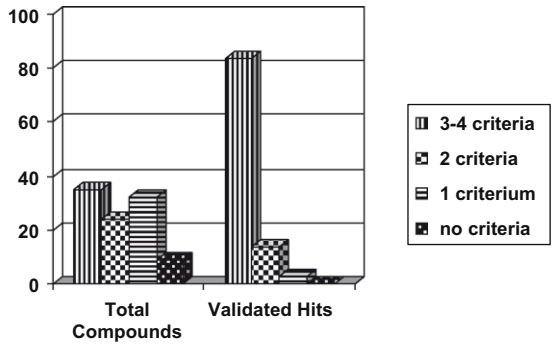


Figure 1. Fulfilment of modified Briggs F-criteria (MWt 300 ± 100; log*P* 3 ± 3; PSA <75; EH 35 ± 10) by HTS F-hits in comparison to total compounds screened.

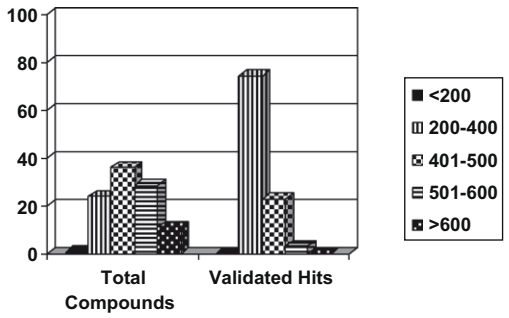
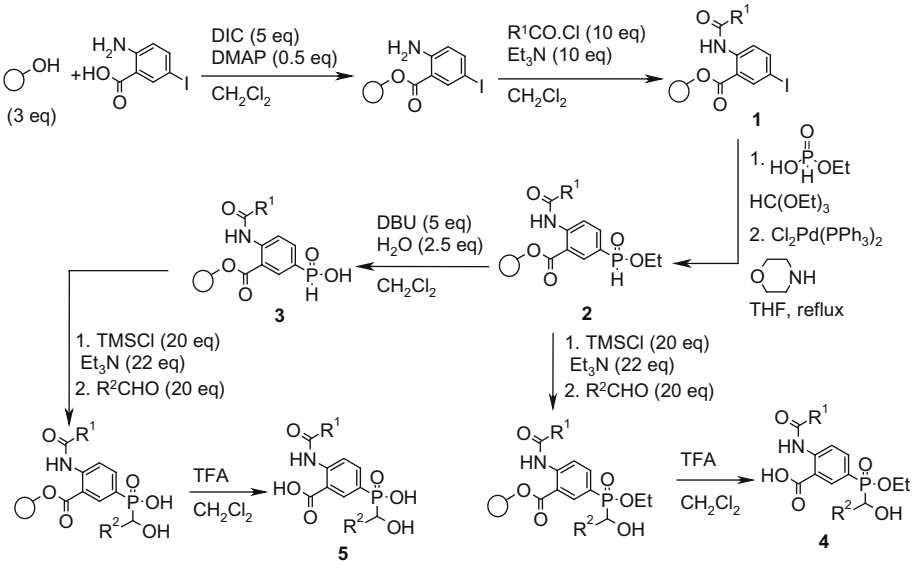


Figure 2. MWt distribution of HTS F-hits in comparison to total compounds screened.

(potential loss of ⩾ 500 g/ha/day at 25 °C). An EH value of 35 is the upper limit for vapour activity which can be a very desirable property (e.g., for soil insecticides).[10]

A comparison of the properties of commercial pharmaceuticals[14] and agrochemicals[15] with those of lead compounds has shown that their properties are not radically different. This shows that the Lipinski and Briggs rules can be applied for the selection of potential lead structures even though they were derived using commercial products. Nonetheless, optimisation is most often achieved by adding additional substituents and increasing the complexity of a lead molecule. For this reason, Clarke and Delaney[16] have proposed a 'Guide of 2' in which the probability of successful lead progression is increased when: MWt 200–400, log*P* ⩽ 4, Δlog*P* < 2, H-bond donors < 2, mp ⩽ 200 °C (Table 1). It is important to keep in mind that all of the criteria listed in Table 1 are best regarded as flexible guidelines which can and should be adjusted to meet specific requirements. For example, Tice[17] has proposed modified guidelines for the selection of post- and pre-emergence herbicides and insecticides.

A retrospective statistical analysis of compounds subjected to high throughput screening has been undertaken at Bayer CropScience, in which an attempt was made to assess the impact of bioavailability guidelines upon the compound selection process.[18] Some of the results are summarised in Figures 1 and 2 which shows high throughput fungicidal screening results from the year 2000. The tested compounds were selected without the use of strict physical chemistry guidelines and show a good mixture of physical properties. In Figure 1, the distribution of compounds with properties obeying four modified Briggs rules, chosen to better reflect the physical properties of fungicides, is shown.[19] The MWt and log*P* criteria are the same as given in Table 1 but the EH range of 25–45 is broader and Δlog*P* has been replaced by polar surface area (PSA). Both Δlog*P* and PSA attempt to reflect the polarity of a molecule but PSA has the advantage that it can be more readily calculated.[20] Figure 1 shows that 83% of the validated biological hits, as compared with only 35% of the total compounds tested, matched 3 or 4 of the modified Briggs criteria. Of the four criteria selected, the molecular weight appears to have the largest effect as shown in Figure 2. In this case, 74% of the validated hits but only 24% of the total compounds tested had a MWt between 200 and 400. Desbordes and co-workers showed that MWt plays such a key role because as molecular size increases it becomes increasingly difficult to avoid compounds which are either too lipophilic (log*P* > 6) or too polar (PSA > 75).[19] For example, a hydrocarbon containing 30 carbons will have a log*P* of around 15. The addition of sufficient oxygen or nitrogen atoms to bring the log*P* down below 6, inevitably takes the polarity up above the PSA bioavailability limit, especially if NH or OH groups are present. The results discussed above, suggest that the application of physical chemistry based bioavailability rules can increase the hit rate by a factor of 2 or 3, over a more diversity based compound selection process. However, the overall effect is likely to be greater



Scheme 1.

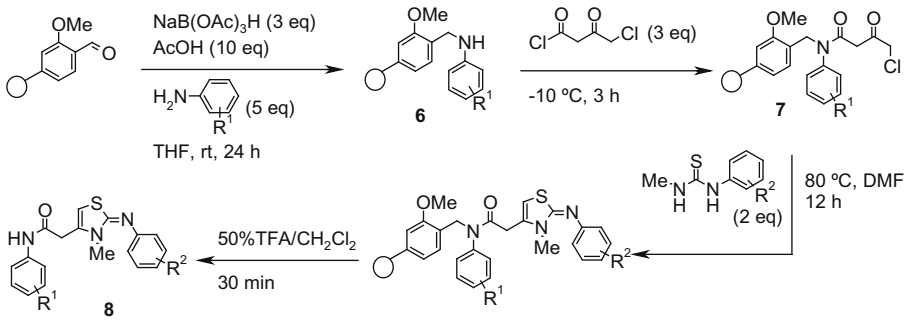
than this because low MWt bioavailable hits have a greater chance of progressing through the discovery process to give useful leads and development compounds.

3. Solid-phase library synthesis

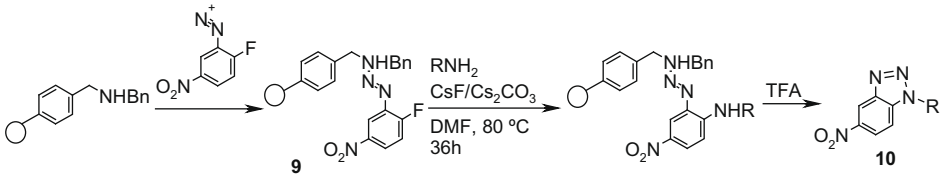
The synthesis of a compound library on the solid-phase is advantageous when variables are introduced early on in a multi-step synthetic sequence, especially when large libraries are required. A good agrochemically relevant example has been reported by Haaf who prepared a library of arylphosphinates as potential herbicidal inhibitors of the enzyme dehydropicolinate synthase for biological screening (Scheme 1).[21] The key step involved a palladium catalysed coupling reaction of ethylphosphinate with the resin-bound aryl iodides **1** yielding the resin-bound phosphinates **2** in high yield and purity. The ethyl esters **2** could be cleanly converted to the free phosphinic acids **3** by treatment with diazabicycloundecene (DBU). The intermediates **2** and **3** were silylated and then reacted with aldehydes to yield resin-bound α-hydroxyphosphinic esters and acids. Cleavage from the resin with trifluoroacetic acid (TFA), yielded a library of 2000 α-hydroxyphosphinates **4** and **5** with purities generally greater than 85% and in near quantitative yields. The fact that the phosphinic acids were released into solution only during the final cleavage step reduced the handling and purification problems commonly associated with the synthesis of polar phosphorus acids in solution.

A small solid-phase library synthesis of twenty 2-imino-1,3-thiazolines **8** designed to have anti-fungal properties against *pyricularia oryzae* was synthesised by Hahn and co-workers using a CHEMSPEED ASW 2000 automated synthesiser (Scheme 2).[22] Reductive amination of BAL (backbone amide linker) resin gave the resin-bound amines **6** which were acylated with chloroaceoacetyl chloride to give the amides **7**. Condensation with various thioureas followed by cleavage from the resin with TFA gave a library of 20 target thiazolines **8**, with an average purity of 83% and yield of 25%. Even though the acylation step to give **7** did not go to completion, due to a competing HCl salt formation reaction of amines **6**, the final purity was still high since only the amide products were cleaved from the resin upon acid treatment. Half of the thiazolines **8** showed anti-fungal activity at 250 ppm and 20% also showed good activity at a lower dosage level of 100 ppm.

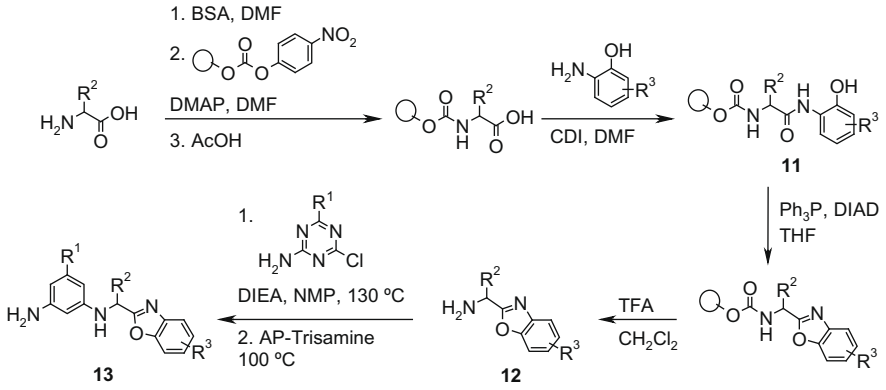
An efficient solid-phase synthesis of a library of 130 benzotriazoles has been reported by Es-Sayed et al. (Scheme 3).[23] A coupling reaction between benzylamino-substituted Merrifield resin and a diazonium salt yielded the resin-bound triazine **9**. Subsequent



Scheme 2.



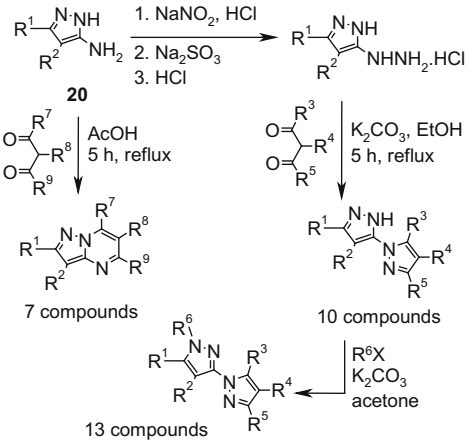
Scheme 3.



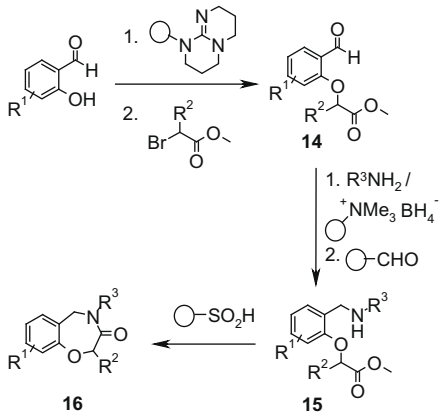
Scheme 4.

nucleophilic displacement of fluorine with an amine, followed by acid catalysed cleavage from the resin with concomitant cyclisation yielded the target benzotriazoles **10** in good purities (70–90%). The majority of the compounds had physical properties in accordance with the modified Briggs rules for fungicides (Fig. 1).[19] Biological screening revealed fungicidal activity and a follow up chemistry program was started in order to investigate the structure–activity relationships within the class.

Starting from the commercial herbicide triaziflam, Giencke and co-workers designed and synthesised a library of 300 benzoxazolyl triazines **13** using a combination of solid- and solution-phase synthesis techniques (Scheme 4).[24] Detailed physico-chemical property profiling was conducted in order to ensure that the library compounds fitted the desired herbicidal profile. Starting from *p*-nitrophenyl carbonate Wang resin, successive reaction with 5 different aminoacids then 12 different aminophenols yielded 60 resin-bound amidophenols **11**. Cyclisation under Mitsunobu conditions, followed by cleavage with TFA yielded the desired amines **12** in an average yield and purity of 76% and 72%, respectively. In the final step, amines **12** were reacted with an excess of 5 different chlorotriazines in the solution-phase. A trisamine resin was used to scavenge the excess chlorotriazine, yielding 300 benzoxazolyl triazines **13** in 50–95% purity. All of the compounds had a molecular weight under 500 and a *clogP* value smaller than 6. The hit rate of the library in the herbicidal primary screen was an impressive 28%.[25]



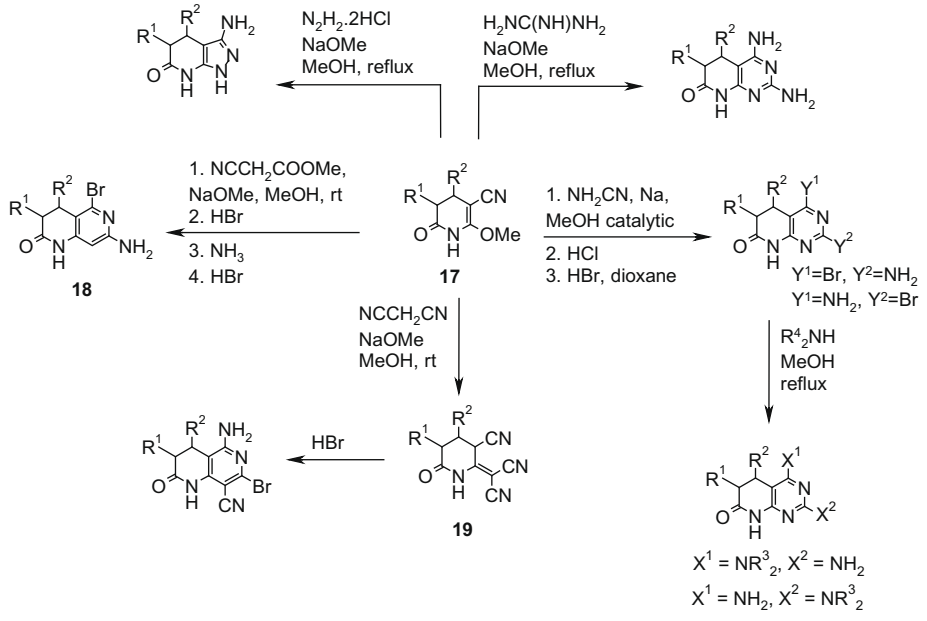
Scheme 7.



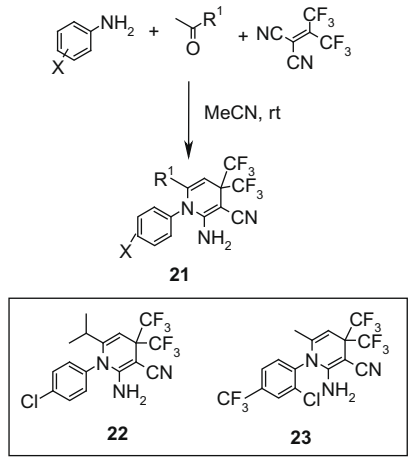
Scheme 5.

4. Solution-phase library synthesis

When synthesising solution-phase compound libraries it is common to use excesses of polymer-bound reagents to drive reactions to completion and scavenger resins to remove excess starting materials or reagents.[5d] A good example illustrating the use of both has been published by Paulitz and co-workers who used polymer-assisted solution-phase (PASP) synthesis to prepare a library of 4,5-dihydro-1,4-benzoxazepin-3(2*H*)-ones (Scheme 5).[26] In the first step they used a polymer-bound base to facilitate alkylation of different phenols to give benzaldehydes **14**. Reductive amination using polymer-bound borohydride, followed by scavenging of excess amine with a resin-bound aldehyde, yielded the amines **15**. In some cases these had cyclised to the final products **16** but in most cases a separate cyclisation using Amberlyst A-15 was necessary to effect this transformation. In total, 47 compounds were prepared (average purity 58%), purified and sent for biological testing.



Scheme 6.



Scheme 8.

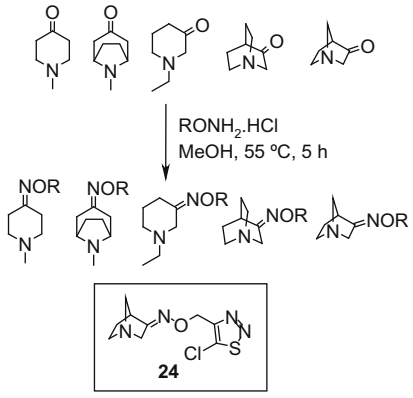
Martinez-Teipel et al. synthesised 18 small screening libraries comprising a total of 116 compounds, all starting from the key pyridone intermediate **17**.[27] A selection of some of the key transformations and compound types synthesised is shown in Scheme 6. The libraries were designed to comply with different bioavailability rules[9,17] and tested for agrochemically relevant activity. Several compounds were reported to show fungicidal activity against various plant pathogens, for example the 1,6-napthyridines **18** and cyanomethylene derivatives **19**.

Based upon the structures of known herbicidal inhibitors of protoporphyrinogen oxidase (PPO), a small targeted library of herbicidal pyrazolylpyrazoles and pyrazolopyrimidines has been reported by Yang and co-workers (Scheme 7).[28] They also used a common heterocyclic intermediate, in this case the aminopyrazole **20**, for the synthesis of both compound types. The library was prepared using a mixture of traditional and parallel solution-phase synthesis methods.

Multi-component reactions provide a powerful methodology for the efficient and rapid synthesis of modern solution-phase combinatorial libraries.[29] A good agrochemically based example has been reported by Cottrell et al. in their synthesis of a library of miticidal dihydropyridines **21** (Scheme 8).[30] A computer-based positional scanning approach was used to test the steric, electronic and lipophilic properties of potential substituents prior to library synthesis. In the final library, 8 ketones and 18 anilines were reacted with 1,1-dicyano-2,2-bis(trifluoromethyl)ethylene to give a library of 144 dihydropyridines **21**. The synthesis was carried out in parallel using solution phase liquid handling methods and filtration blocks to collect the precipitated products. The product purities were estimated to be >90% and yields were mostly in the 50–80% range. Following biological testing, two field candidates, **22** and **23**, were identified from the library. These not only had better activity than the lead compound but also an improved toxicological profile. A further two field candidates were identified following derivatisation work and further exploration of the chemistry.

Benting et al. have reported on the synthesis and screening of a library of oxime ethers designed as insecticidal agonists of the muscarinic acetylcholine receptor (Scheme 9).[31] A liquid handling robot was used to mix stock solutions of five cyclic aminoketones with 45 hydroxylamine hydrochlorides to produce, after heating for 5 h at 55 °C, a library of 225 oxime ethers. The average purity was 78% and 4.4% of the oxime ethers were shown to be muscarinic agonists, including the insecticidally active thiadiazole **24**.[25]

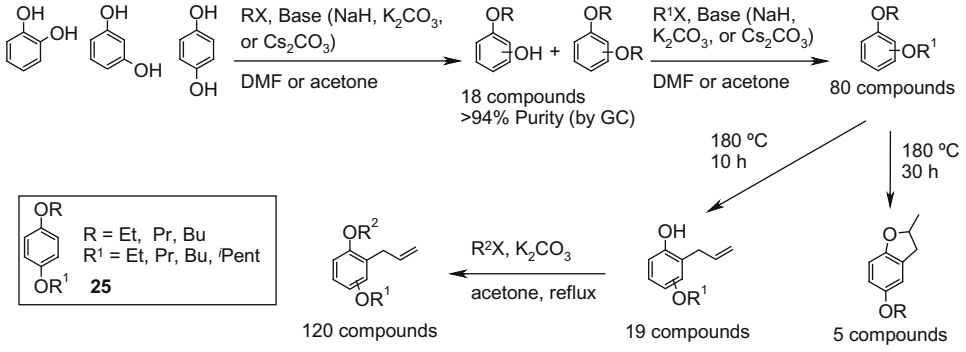
A series of small libraries designed to interfere with insect olfaction or gustation have recently been reported by Padrurau et al.[32] The libraries were made up of variously substituted alkoxy benzenes, some of which were synthesised via a Claisen rearrangement (Scheme 10). Analogues **25** showed significant inhibition of the antennal response to the gypsy moth sex attractant pheromone (+)-disparlure at high doses.



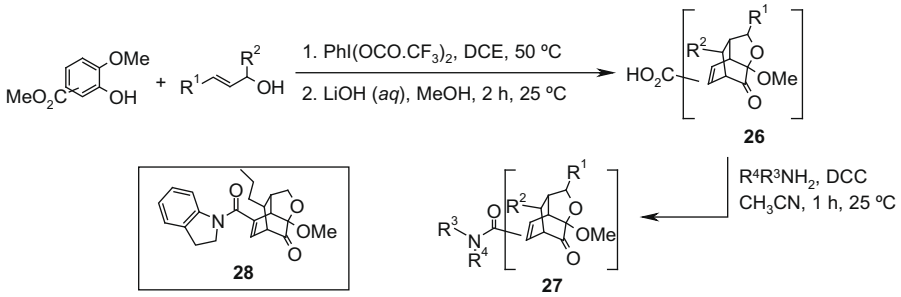
Scheme 9.

5. Libraries based upon natural products or privileged structures

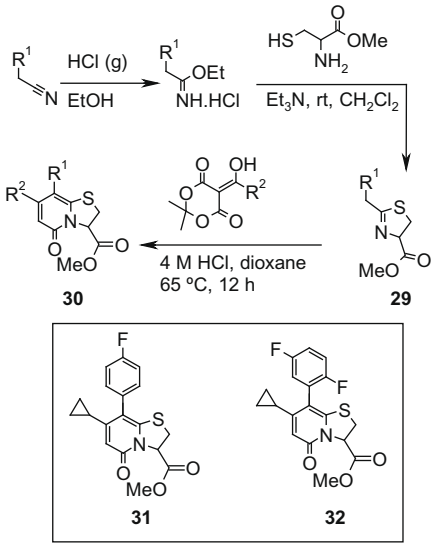
In recent years there has been an increasing trend for agrochemical and pharmaceutical companies to use natural products and 'privileged structures' as a basis for designing new libraries.[33] The term privileged structure was first introduced by Evans et al. and was defined as 'a single molecular framework able to provide ligands for diverse receptors'.[34] Since Evans and co-workers described the benzodiazepine scaffold as a privileged structure, many others have been identified including benzoazapines, spiropiperidines, benzamides, biphenyltetrazoles, indoles, benzopyrans, benzylpiperidines, β-glu-



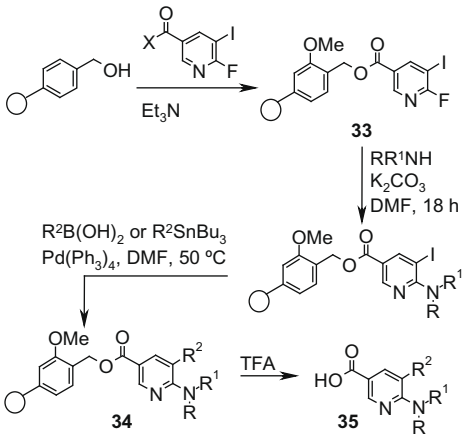
Scheme 10.



Scheme 11.



Scheme 12.



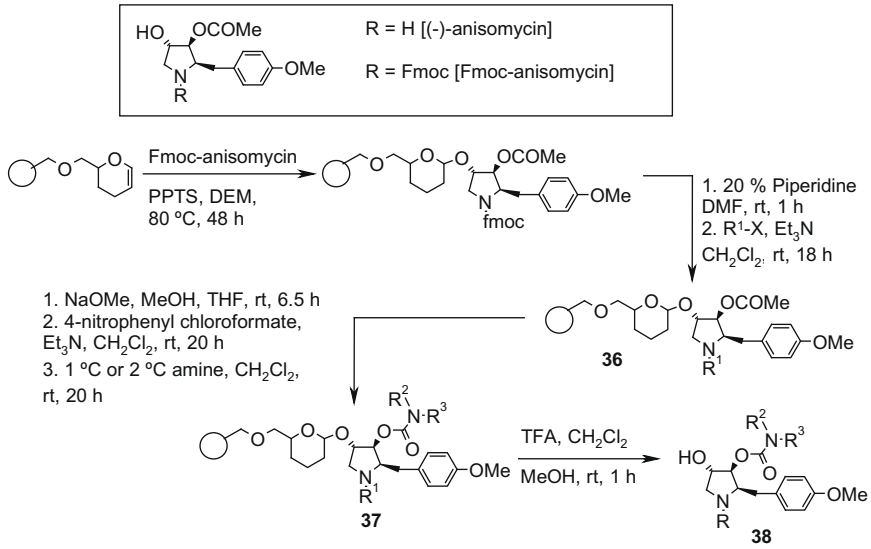
Scheme 13.

cose and monosaccharides.[35] Natural products are also often classified as privileged structures since they populate biologically relevant chemical space and because during their biosynthesis they interact with multiple proteins and often fulfill varying biological functions on different proteins.[36] The term biology oriented synthesis (BIOS) has been coined to describe libraries based upon natural product scaffolds.[37] Basing a library on privileged, natural product, drug-like or agrochemical like structures means that the compounds should have a higher chance of binding to a number of target proteins. In relation to library design this usually means that the core skeletal structure is based upon a natural product or privileged structure.

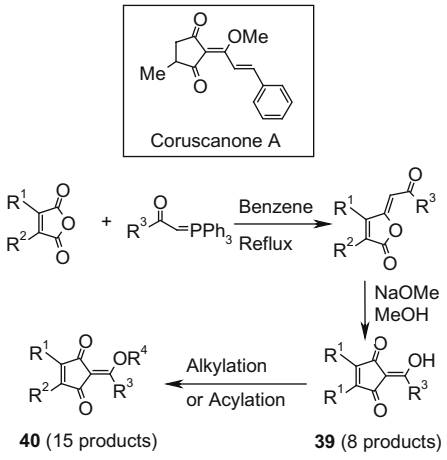
An interesting example of a natural product inspired library has been reported by Smith and co-workers in their synthesis of a library of natural product-like bicycle[2,22]octenones **27** (Scheme 11).[38] In the first two steps, they used Diels–Alder chemistry followed by ester hydrolysis to synthesise a diverse set of 13 key acids **26**. These were reacted further with different amines chosen so as to achieve both structural diversity and agrochemical-like physical properties in the final products. In total, 1126 amides **27**, with an average purity of 63% were synthesised. The library members had an average MWt of 395 and an average clog *P* of 1.8. The library was screened in a 96-well format against a range of insect, fungal and weed model organisms which led to the discovery of the herbicidal lead compound **28**.

Ruiz and Lorsbach[39] proposed that the 2-pyridinone ring system, which is found in many biologically active molecules showing antifungal, antibacterial and anticancer activity,[40] could be considered as a privileged scaffold. They made extensive use of computer-aided design in planning their herbicidally directed library of ring fused 2-pyridinones **30** (Scheme 12). Lipinski's rules[9] and Tice's limits[17] for herbicides and insecticides were adapted to help guide the selection of promising molecules and avoid problematic structures. In addition diversity criteria and 'ag-like' features were used in the design process. Thiazolines **29**, generated using 31 different nitriles, were reacted with 15 different Meldrum's acids to yield 465 ring fused 2-pyridinones **30** in 65–85% purity. Following biological screening, two compounds, **31** and **32**, were found to be sufficiently active as herbicides to warrant follow-up synthesis, which unfortunately, failed to improve the activity.

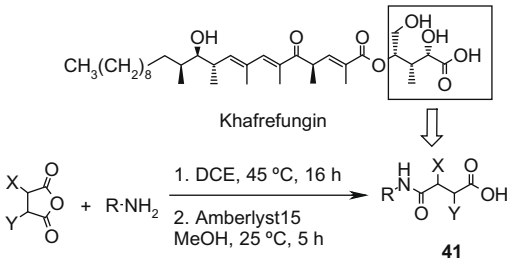
Pyridine carboxylic acids are found in various herbicides possessing different modes of action (e.g., Acecetolactate synthase (ALS) inhibitors (imazapyr, imazaquin), auxins (clopyralid, picloram), auxin transport inhibitor (diflufenzopyr) and microtubulin inhibitors (thiazopyr))[41] and as such can be considered to be privileged structures. Turner et al. have reported the synthesis of a series of ten combinatorial libraries based upon pyridine carboxylic acids.[42] The libraries were synthesised on Wang resin in IRORI minikans and ranged in size from 176 to 2112 compounds. An example is depicted in Scheme 13, in which the resin bound halopyridines **33** were successively reacted with amines and either boronic acids or organostannanes to yield aminopyridines **34**. Cleavage with TFA gave a library of 1320 nicotinic acids **35** which gave an encouragingly high hit rate of 11.6% in the herbicide HTS screen. The average hit rate over all ten libraries was 5.6%, compared with 4.8% for a library of randomly selected acids and 1.2% for a more diverse set of randomly chosen compounds. The authors noted that HTS hit rates are of only limited value in assessing the success of a combinatorial library since a single hit from a library with a low hit rate can result in an important lead. A better indicator of success is to consider compounds that have activity in higher secondary level screens.



Scheme 14.



Scheme 15.

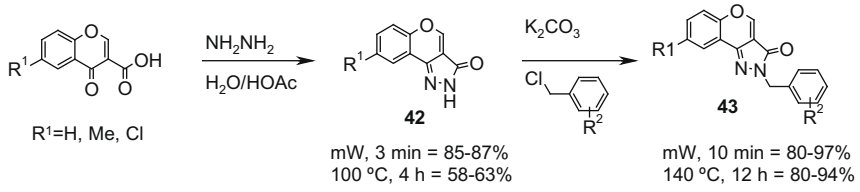


Scheme 16.

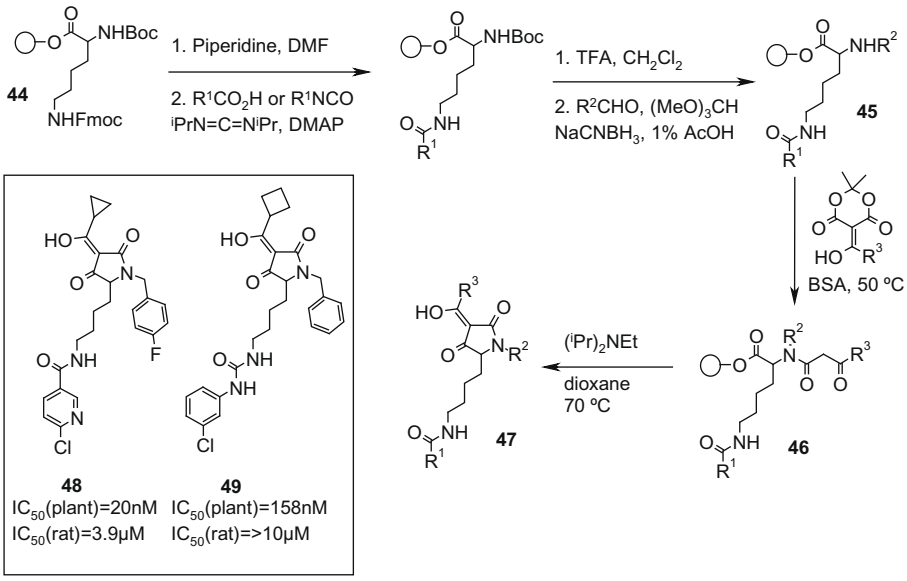
Solid phase synthesis was also used by Shi et al. to synthesise a 10,000 member library based upon the natural product anisomycin (Scheme 14).[43] The natural product based scaffold was attached to Merrifield resin using a dihydropyran linker. In the first step, the Fmoc protecting group was removed and 100 different N-acylating agents were employed to give the esters **36**. Sequential ester hydrolysis, acid activation and addition of 100 diverse amines then yielded the amides **37**, which were cleaved from the resin using TFA to give the final anisomycin analogues **38**. Over 8000 of these had purity >70% and were submitted for screening against bacterial and fungal targets with some samples showing activity against *Staphylococcus aureus* and *Candida albicans*.

Xing-Cong Li and co-workers have reported the synthesis of a focused library based on the plant derived natural product coruscanone A (Scheme 15).[44] They used a Wittig condensation followed by an interesting base-catalysed rearrangement to yield the alcohols **39**. These were alkylated or acylated to give the coruscanone analogues **40**. The library was screened for antifungal activity and several analogues showed in vitro activity against *C. albicans* and *Cryptococcus neoformans*.

Delgado et al. synthesised a library of succinamic acid derivatives as analogues of the antifungal natural product khafrefungin (Scheme 16).[45] Condensation of a series of 8 succinic acid anhydrides with 60 amines, selected based upon structural diversity and modified Lipinski rules, yielded 480 khafrefungin analogues **41**. Excess amine was scavenged from the reaction with the help of an acidic scavenger resin. Five of the succinic acid anhydrides contained acetoxy substituents (X and/or Y) and an additional 300 compounds were synthesised by hydrolysis of the acetoxy derivatives **41** to yield a total of 780 compounds. Around 55% of library members exhibited purities higher than 70% and several



Scheme 17.



Scheme 18.

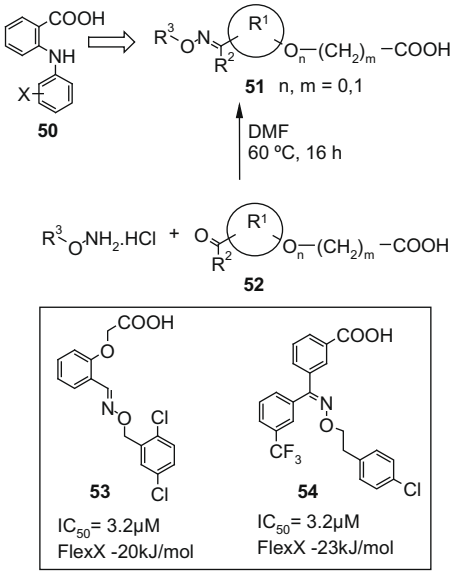
members of the library inhibited growth of *Saccharomyces cerevisiae* at similar levels to the natural product khafrefungin.

Microwave-assisted combinatorial synthesis has emerged as an important methodology for significantly accelerating reaction rates when synthesising combinatorial libraries.[46] Inspired by the insecticidal activity of certain flavenoids (e.g., Rotenone), Yang and co-workers have reported the rapid synthesis of a benzopyranone[4,3-*c*]-pyrazol-3(2*H*)-one library using microwave irradiation in both steps (Scheme 17).[47] The use of microwave irradiation dramatically reduced the reaction times for both steps and improved the yields in the cyclisation step to give **42**. A library of 36 different benzopyranone[4,3-*c*]-pyrazol-3(2*H*)-ones **43** was synthesised, of which 4 exhibited >80% insecticidal activity against both *T. cinnabarinus* at a dosage level of 250 mg L^{-1} and *Nilaparvata legen* at 500 mg L^{-1}.

6. Structure-based library design

The increasing availability of target X-ray crystallographic protein structures coupled with improvements in molecular ligand docking and scoring software means that structure-based design is more feasible than was previously the case.[48] In one approach the members of a library, real or virtual, are sequentially docked into the binding pocket of a target enzyme. The virtual docking begins with the computer generation of plausible 3-D structures for all library members. The next step involves the high throughput docking of these structures into the protein ligand binding site, whereby they are allowed some degree of flexibility in order to obtain a best 'fit'. Finally, the binding affinity of each library member is scored and ranked. The resulting list can be used to 'cherry pick' real substances from a large corporate library or to help select virtual library members for synthesis. Such virtual screening approaches have no bias towards known ligand structures and can, therefore, be a good way of generating new structural types. An interesting variant of this approach uses low molecular weight fragment structures as the starting point for library design.[49] The fragments needed for this approach can be identified using biochemical binding assays, NMR-based screening or X-ray crystallographic methods. Once identified, the fragments can be used for substructure searching within real compound collections or as the basis for designing new virtual libraries.

A structure-based design approach to the discovery of new inhibitors of the herbicidal target enzyme 4-hydroxyphenylpyruvate dioxygenase (HPPD) has been described by Camper et al.[50] Molecular modelling studies conducted using the plant (*Arabidopsis*) and rat HPPD protein crystal structures indicated that tetramic acids of general structure **47** had a good chance of binding selectively to the plant enzyme. In particular, examination of the binding pocket suggested that a lysine tail functionalised as an amide or urea would be able to make beneficial hydrogen bonding interactions with a glutamine residue in a previously unexploited binding channel. Consequently, a small solid phase library of lysine tetramic acids **47** was synthesised starting from the bis-protected resin-bound lysine **44** (Scheme 18). A selective deprotection, acyl-



Scheme 19.

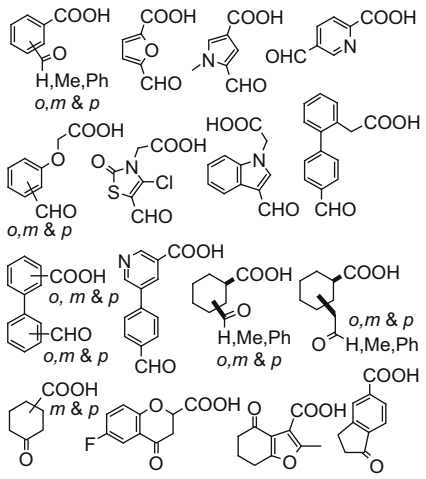


Figure 3. Examples of the precursors **52** from Scheme 19 containing a variable R^1-core.

ation, deprotection, reductive amination sequence yielded the resin-bound amides/ureas **45** which were reacted with acyl Meldrum's acids to give the resin bound β-keto amides **46**. Finally, cleavage from the resin with concomitant ring closure was achieved by heating in the presence of Hünig's base to give the target tetramic acids **47** in good yields (45–90%) and high purities (>85%). Biochemical testing identified the potent inhibitors **48** and **49** which showed excellent selectivity for the plant over the rat HPPD enzyme but the in vivo activity was not compelling.[50]

The use of virtual target-based screening, combined with diversity oriented synthesis,[51] to help design a library of potential herbicidal inhibitors of the enzyme anthranilate synthase has recently been reported.[52] Starting from the known inhibitors **50**,[53] a library based around the general structure **51** was synthesised (Scheme 19). The final step of the library synthesis involved the formation of an oxime ether bond by reacting 80 hydroxylamine hydrochlorides with 60 aldehyde/ketone precursors **52** containing a variable R^1-core (Fig. 3). The incorporation of skeletal diversity within these R^1-cores meant that the spatial orientation of the side chains could be widely varied, thereby enabling a more effective exploration of the enzyme binding pocket. Prior to synthesis, a fully enumerated virtual library was subjected to virtual target based screening using the published X-ray crystal structure of bacterial anthranilate synthase[54] and the commercially available FLEXX docking program.[55] The resulting ranking, based upon the predicted binding score to anthranilate synthase, was used to help select the hydroxylamines and R^1-cores for the library synthesis. Some poorly scoring R^1-cores were included in the final library in order to better evaluate the predictive capability of the FLEXX program. Following synthesis, 4800 target compounds **51** were screened in a high throughput biochemical screen for inhibition of plant anthranilate synthase. The primary hit rate was 10.9% which was ten fold higher than the usual hit rate seen for HTS against this enzyme but the level of in vivo herbicidal activity was disappointingly low. R^1-Cores which had scored badly in the virtual screening step did not yield any inhibitors in the enzyme test. The best inhibitors, as exemplified by compounds **53** and **54**, had FLEXX scores falling within the top 50% of predicted binders but the relative ranking of inhibitors had not been predicted. Two thirds of the library had purity > 70%, MWt < 360, log*P* < 5, PSA < 75 and EH > 31.

The current docking and scoring functions used to calculate and evaluate the binding poses of a ligand in a target protein are still relatively inaccurate. A number of interesting studies comparing different docking and scoring programs have been published.[56] These show that the different programs perform with varying degrees of success against different proteins and that not all protein targets are amenable to the approach. Nonetheless, substantial enrichment was often achieved, even though it was not possible to predict ligand ranking. A major limitation of many current programs is the approximation of the protein as a rigid structure, whereas it is actually flexible and changes shape during complex formation. However, recent publications indicate that progress in accounting for receptor flexibility is being made which should lead to notable improvements in the near future.[57] Another problem is that current high throughput docking and scoring programmes are not able to fully take into account the important role played by water in the overall binding process: Before it can bind, a ligand must first be removed from solvent water (dehydrated) and moved into the protein binding site, from which the water must also be removed. Consequently, any polar groups on the ligand or protein, which are not properly involved in H-bonding interactions within the protein–ligand complex, will have a negative effect on the binding constant. Similarly the removal of apolar groups on the ligand or protein from the solvent water will have a positive effect. The effect on binding of missing H-bonds and the hydrophobic effect in general, have both proven difficult to quantify although recent publications indicate that progress is being made in this area also.[58] It is to be expected that future improvements in docking and scoring programs will increase the chances of successful rational library design and contribute to the overall success of the discovery process in the future.

7. Conclusion and summary

The way in which combinatorial chemistry is practised has changed enormously since the first libraries were produced in the early 1990s. This is partly due to technological improvements and partly due to the realisation that simply increasing the numbers of compounds entering the high throughput screens is not a cost effective way of generating new leads. Recent years have seen an increasing emphasis being placed upon library design and the pre-screening of virtual libraries prior to synthesis. Most simply this means selecting out library members for synthesis which obey a given set of bioavailability guidelines.[9,10,16,17] More recently, attention has turned towards concepts such as diversity oriented synthesis,[51] natural product[33,37] or privileged structure inspired design[35] and virtual target based design.[48] The success rate has improved and although no reports of commercial agrochemicals discovered using combinatorial methods have appeared to date, there are at least reports of field candidates discovered using this approach.[30] In 1999, in an article analysing the impact of combinatorial chemistry on lead finding Roger Lahana wrote, 'It can be surmised that when trying to find a needle in a haystack, the best strategy might not be to increase the size of the haystack'.[6] There is no doubt that since that time, more intelligent library design has reduced the size of the haystack but it is not yet clear if it has been reduced enough to make the search for the needle an economical proposition.

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