

# The impact of microwave synthesis on drug discovery

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**Abstract** | In the past few years, using microwave energy to heat and drive chemical reactions has become increasingly popular in the medicinal chemistry community. First described 20 years ago, this non-classical heating method has matured from a laboratory curiosity to an established technique that is heavily used in academia and industry. One of the many advantages of using rapid 'microwave flash heating' for chemical synthesis is the dramatic reduction in reaction times — from days and hours to minutes and seconds. As will be discussed here, there are good reasons why many pharmaceutical companies are incorporating microwave chemistry into their drug discovery efforts.

## Combinatorial chemistry

The generation of large collections, or 'libraries', of compounds by synthesizing all possible combinations of a set of smaller chemical structures.

## Compound libraries

Large collections of compounds (hundreds to millions), often synthesized through reactions performed simultaneously (in parallel) or by using combinatorial chemistry principles.

## Chemical space

The space spanned by all possible (energetically stable) combinations of atoms and topologies in molecules.

Improving research and development (R&D) productivity is one of the biggest tasks facing the pharmaceutical industry. Advances in genomics and proteomics in recent years have led to an explosion in the number of possible drug targets, and so pharmaceutical companies have made major investments in high-throughput screening and combinatorial chemistry to identify more potential drug candidates for these novel targets. However, lead compound optimization and traditional medicinal chemistry remain the bottlenecks in the drug discovery process. Developing chemical compounds with the desired biological properties is time-consuming and expensive. Consequently, there is increased interest in technologies and concepts that facilitate more rapid synthesis and screening of chemical substances to identify compounds with appropriate qualities. One such high-speed technology is microwave-assisted organic synthesis (MAOS).

Microwave-assisted heating under controlled conditions is an invaluable technology for medicinal chemistry and drug discovery applications because it often dramatically reduces reaction times, typically from days or hours to minutes or even seconds<sup>1</sup>. Many reaction parameters, such as reaction temperature and time, variations in solvents, additives and catalysts, or the molar ratios of the substrates, can be evaluated in a few hours to optimize the desired chemistry. Compound libraries can then be rapidly synthesized either in a parallel or sequential (automated) format. In addition, MAOS technology often facilitates the discovery of novel reaction pathways, because the extreme reaction conditions attainable by microwave heating sometimes lead to unusual reactivity that cannot always be duplicated by conventional heating. This serves to expand chemical space in general, and 'biologically relevant, medicinal chemistry space' in particular.

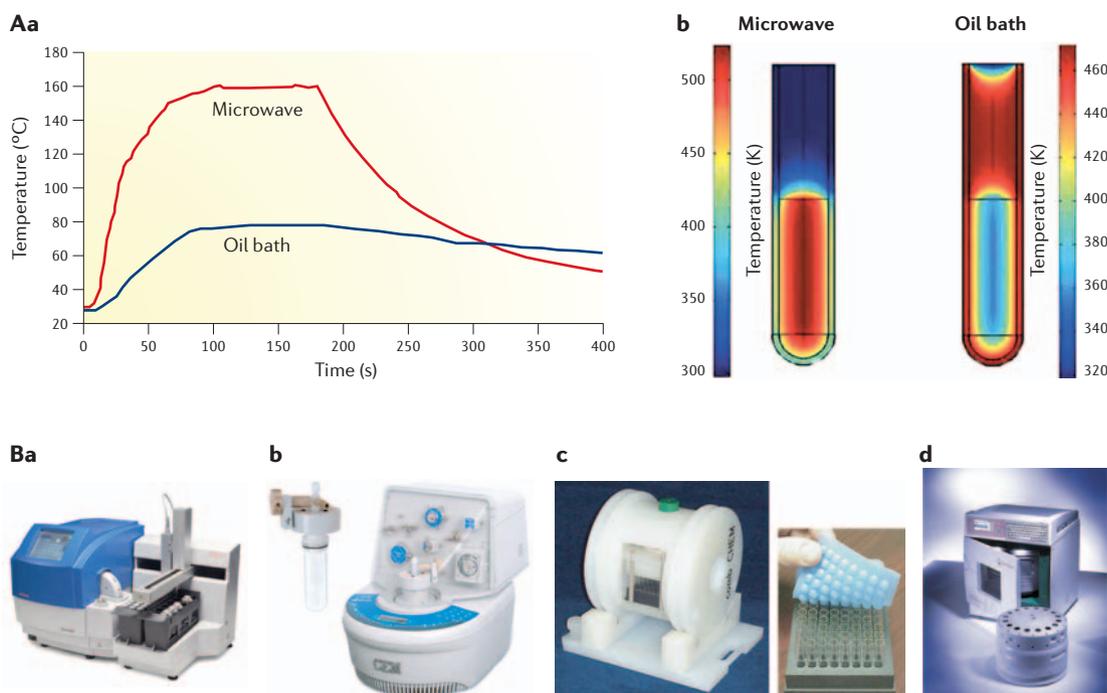
Microwave synthesis has the potential to influence medicinal chemistry efforts in at least three major phases of the drug discovery process: generation of a discovery library; hit-to-lead efforts; and lead optimization. A common theme throughout all these processes is speed. Greater speed provides a competitive advantage, and allows for more efficient use of expensive and limited resources, faster exploration of structure–activity relationships (SARs), enhanced delineation of intellectual property and more timely delivery of crucially needed medicines. Ultimately, these factors can determine company position in the marketplace. To the drug discovery industry, time truly does equal money, and microwave chemistry has become a central tool in this fast-paced, time-sensitive field. Not surprisingly, most pharmaceutical and biotechnology companies are already heavily using MAOS as frontline methodology in their chemistry programmes, both for lead generation and lead optimization, because they recognise the capacity of this technology to speed chemical reactions and, therefore, ultimately the drug discovery process<sup>1</sup>.

Here we outline some of the fundamental principles, concepts, technologies and future directions of MAOS applied to the drug discovery industry. This review is not a comprehensive guide, but a general overview of the field (for more detailed coverage of the subject see REFS 1–7).

## Background to microwave chemistry

Traditionally, organic reactions are heated using an external heat source (such as an oil bath), and therefore heat is transferred by conductance. This is a comparatively slow and inefficient method for transferring energy into the system because it depends on the thermal conductivity

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**Figure 1 | Differences between conventional and microwave heating, and examples of microwave reactor technology.** **Aa** | Difference in temperature profiles for a 5-ml sample of ethanol (boiling point 78 °C) heated under single-mode, sealed-vessel microwave irradiation (maximum set temperature 160 °C) and open-vessel oil-bath conditions (oil-bath temperature 100 °C) for 3 minutes. Dielectric heating with microwave energy is significantly more rapid than heating in an oil-bath by convection currents. Both experiments were carried out in the same reaction vessel with stirring using an internal fibre-optic temperature-monitoring device. Using sealed-vessel microwave irradiation, a significantly higher temperature can rapidly be reached, compared with the oil-bath experiment, which was carried out under standard open-vessel reflux conditions. After the set temperature of 160 °C is reached in the microwave experiment (~100 s), an algorithm controlled by feedback with the temperature-monitoring device adjusts the microwave power to maintain the set temperature. Active gas-jet cooling (180–400 s) then rapidly cools the reaction mixture after microwave irradiation. **Ab** | Inverted temperature gradients in microwave versus oil-bath heating. Temperature profiles (modelling) 1 minute after heating by microwave irradiation (left) compared with treatment in an oil-bath (right). Microwave irradiation raises the temperature of the whole volume simultaneously (bulk heating), whereas in the oil-heated tube the reaction mixture in contact with the vessel wall is heated first. Temperature scale in Kelvin. **B** | Microwave reactor technology for high-throughput synthesis and scale-up. **Ba** | Automated single-mode microwave synthesizer (Initiator 60; Biotage AB). A robotic gripper device moves the sealed reaction vessels (0.2–20 ml) in and out of the microwave cavity. Up to 60 reactions can be processed in an automated sequential fashion. **Bb** | Continuous-flow single-mode microwave reactor (Voyager; CEM Corp.). Scale-up is achieved by pumping reaction mixtures in and out of an 80-ml sealed reaction vessel following a stop-flow processing regime. **Bc** | Set-up for parallel microwave synthesis (CombiCHEM system; Milestone Inc.). The barrel-type overhead rotor system (left) can hold up to two 96-deep-well microtitre plates (right) for parallel synthesis on a 0.5–4-ml scale. The set-up is irradiated in a multimode microwave reactor (not shown). **Bd** | Multimode microwave scale-up system for parallel batch processing (Synthos 3000; Anton Paar GmbH). Microwave synthesis is performed in multivessel rotors (8 or 16 vessels) with reaction volumes of up to one litre. Part **Ab** reproduced, with permission, from REF. 5 © Wiley-VCH (2004).

#### Microwave irradiation

Electromagnetic irradiation in the frequency range of 0.3–300 GHz, corresponding to wavelengths of 1 cm–1 m. All microwave reactors for chemical synthesis operate at a frequency of 2.45 GHz (corresponding to a wavelength of 12.25 cm) to avoid interference with telecommunication and cellular phone frequencies.

of the various materials that must be penetrated, and results in the temperature of the reaction vessel being higher than that of the reaction mixture. By contrast, microwave irradiation produces efficient internal heating by direct coupling of microwave energy with the polar molecules (for example, solvents, reagents and catalysts) that are present in the reaction mixture.

**Theory.** MAOS is mainly based on the efficient heating of materials by ‘microwave dielectric heating’ effects<sup>8,9</sup>. Microwave dielectric heating is dependent on the ability of a specific material to absorb microwave energy and convert it to heat. Microwave irradiation triggers heating

by two main mechanisms: dipolar polarization and ionic conduction. Whereas the dipoles in the reaction mixture (for example, the polar solvent molecules) are involved in the dipolar polarization effect, the charged particles in a sample (usually ions) are affected by ionic conduction. When irradiated at microwave frequencies, the dipoles or ions of the sample align in the applied electric field. As the applied field oscillates, the dipole or ion field attempts to realign itself with the alternating electric field and, in the process, energy is lost in the form of heat through molecular friction and dielectric loss. The amount of heat generated by this process is directly related to the capacity of the matrix to align itself with the frequency of

## Box 1 | Molecular magic with microwaves?

Since the early days of microwave-assisted organic synthesis, the observed accelerations in rate, and sometimes discrepancies in product distributions compared with oil-bath experiments have led to speculations on the existence of 'specific' or 'non-thermal' microwave effects. Historically, it was claimed that these effects occurred when the outcome of a synthesis carried out under microwave conditions was different from the conventionally heated counterpart at the same apparent temperature. The literature is full of examples of conflicting reports on the involvement or non-involvement of 'microwave effects' for various different types of chemical reactions.

'Specific' microwave effects are defined as accelerations in rate that cannot be achieved or duplicated by conventional heating, but that are essentially still attributed to thermal effects. For example, the selective heating of strongly microwave-absorbing heterogeneous catalysts or reagents in a less polar reaction medium.

In addition, so-called 'non-thermal' effects (accelerations that cannot be rationalized by either purely thermal/kinetic or specific effects) have been proposed to result from a direct interaction of the electric field with specific molecules or intermediates in the reaction medium (orientation effects). For example, it has been argued that the presence of an electric field leads to a lowering of the activation energy for reactions with a polar mechanism, where the polarity is increased going from the ground state to the transition state. In particular, the area of 'non-thermal' microwave effects is highly controversial (for more detailed surveys and reports see REFS 11, 12).

the applied field. If the dipole does not have enough time to realign, or reorients too quickly with the applied field, no heating occurs. The allocated frequency of 2.45 GHz used in all commercial systems lies between these two extremes, and gives the molecular dipole time to align in the field but not to follow the alternating field precisely. Under such conditions, rapid heating of chemical reaction mixtures to high temperatures will be observed, particularly if a sealed vessel system is used (FIG. 1Aa).

The reaction vessels used are typically made out of microwave-transparent materials, which results in an inverted temperature gradient in the bulk reaction mixture compared with that generated by conventional thermal heating (FIG. 1Ab). A recent study comparing the energy efficiency of conventional oil-bath synthesis and MAOS demonstrated that for most chemical transformations significant energy savings (up to 85-fold) are experienced using microwaves as the energy source on a laboratory scale<sup>10</sup>.

In MAOS, reactions frequently occur much faster than under conventional oil-bath conditions. On reviewing the present literature, it seems that in most of these cases the rate enhancements observed in MAOS are the result of a purely thermal/kinetic effect (applying the Arrhenius law). This means they are a consequence of the higher reaction temperatures that can rapidly be attained when irradiating polar materials in a microwave field (FIG. 1Aa). In addition, so-called 'microwave effects' are also thought to contribute to the frequent discrepancies in reaction rate between conventional and microwave heating<sup>11,12</sup> (BOX 1).

**Microwave reactors.** Although many of the early pioneering experiments in MAOS were carried out in domestic kitchen-type microwave ovens<sup>13,14</sup>, the current trend is undoubtedly to use dedicated instruments for chemical synthesis. All the specialized microwave reactors commercially available today feature built-in magnetic stirrers, direct temperature control of the reaction mixture

with the aid of fibre-optic probes or infrared sensors, and software that enables on-line temperature and pressure control by regulation of microwave power output<sup>1</sup>. Currently, two different microwave reactor designs are emerging: multimode and monomode (also referred to as single mode) reactors. In the multimode instruments (conceptually similar to a domestic oven), the microwaves that enter the typically large cavity (~40–50 litre) are reflected by the walls of the cavity, and therefore interact with the sample in a chaotic manner. In the much smaller monomode cavities, only one mode is present and the electromagnetic irradiation is directed through an accurately designed rectangular or circular wave guide onto the reaction vessel that is mounted at a fixed distance from the radiation source, creating a standing wave. In the context of drug discovery, there is a key difference between the two types of reactor systems: in multimode cavities several reaction vessels can be irradiated simultaneously in multi-vessel rotors (FIG. 1Ba) or deep-well microtitre plates (FIG. 1Bc); in monomode systems, by contrast, only one vessel can be irradiated at any time. However, high throughput can be achieved in monomode systems by using integrated robotics that move individual reaction vessels in and out of the microwave cavity (FIG. 1Ba). Most companies developing microwave instruments for commercial applications offer a variety of diverse reactor platforms with different degrees of sophistication with respect to automation, database capabilities, safety features, temperature and pressure monitoring, and vessel design<sup>1</sup> (FIG. 1B).

### High-throughput microwave synthesis

High-speed MAOS is applicable to a wide variety of synthetic transformations<sup>1–7</sup>. It is becoming evident that microwave approaches — often providing faster reactions and improved yields (BOX 2) — can probably be developed for most chemical transformations that require heat. Whereas in the past microwave chemistry was often applied only to a difficult or slow reaction step, today the number of examples in which microwave heating is used for many, if not all, of the reactions in a multistep synthesis is rapidly growing<sup>15–18</sup>. Microwave reactors are no longer used as the 'last resort' when all other means to drive a reaction to a synthetically useful conversion fail, but instead are often viewed as a 'first choice' for performing chemical synthesis on a laboratory scale. Therefore, the number of published applications of high-speed microwave synthesis is burgeoning (see REFS 1–7 for several thousand examples of MAOS).

**Automated sequential library synthesis.** In drug discovery, it is often important to rapidly and efficiently generate collections of compounds (compound libraries) for the testing of biological function. Integrating robotic devices with a single-mode microwave cavity (as shown in FIG. 1Ba) can lead to a substantially higher synthetic throughput than that afforded by conventional synthesis<sup>19,20</sup>. The high throughput afforded by microwave technology is exemplified by the successful synthesis of a small, focused library of multifunctionalized dihydropyrimidine (DHPM) derivatives<sup>21</sup> via the Biginelli

#### Arrhenius law

The relationship between reaction rate and temperature. The rate of a chemical reaction increases when the temperature is raised according to:  $k = A \exp(-E_a/RT)$ .

#### Microtitre plates

Sample holders used for synthesis, storage, analysis and screening of compound libraries. The plates typically have 6, 24, 96, 384 or even 1,536 sample wells arranged in a 2:3 rectangular matrix.

## Box 2 | Advantages of microwave synthesis

- Higher reaction temperatures can be obtained by combining rapid microwave heating with sealed-vessel (autoclave) technology (FIG. 1Aa).
- In many instances significantly reduced reaction times, higher yields and cleaner reaction profiles will be experienced, allowing for more rapid reaction optimization and library synthesis.
- Solvents with lower boiling points can be used under pressure (closed vessel conditions) and be heated at temperatures considerably higher than their boiling point.
- Microwave heating allows direct 'in core' heating of the reaction mixture, which results in a faster and more even heating of the reaction mixture (FIG. 1Ab).
- Specific microwave effects (BOX 1) that cannot be reproduced by conventional heating can be exploited — for example, the selective heating of strongly microwave-absorbing catalysts.
- Easy on-line control of temperature and pressure profiles is possible, which leads to more reproducible reaction conditions.
- Microwave heating is more energy efficient than classical oil-bath heating because of direct molecular heating and inverted temperature gradients.
- Can easily be adapted to automated sequential or parallel synthesis.

multicomponent reaction<sup>22</sup> (FIG. 2a). This condensation reaction is particularly attractive from the drug discovery point of view because the resulting privileged DHPM scaffold has a wide range of biological effects and so a number of lead compounds have been developed based on the DHPM structural core<sup>23</sup>. For example, functionalized DHPMs have emerged as orally active antihypertensive agents,  $\alpha_{1A}$  adrenoceptor-selective antagonists or mitotic kinesin Eg5 inhibitors<sup>23</sup>.

The standard synthetic procedure for the Biginelli reaction involves a one-pot condensation of the three building blocks shown in FIG. 2a in a suitable solvent, such as ethanol, using a strongly acidic catalyst<sup>22</sup>. One major drawback of the original method, apart from the long reaction times involving reflux temperatures (2–12 hours, 80 °C), is that, when using more complex building blocks, the product yield is frequently only moderate (20–60%). Implementing sealed-vessel microwave irradiation at 120 °C in the Biginelli protocol provides significant rate-enhancements and higher product yields (30–90%), and the reaction is complete in 10–20 minutes<sup>19</sup>.

When the reaction conditions had been optimized, a small DHPM library was produced. As a collection of structurally diverse representative building blocks, 17 individual CH-acidic carbonyl compounds, 25 aldehydes and 8 ureas/thioureas were chosen<sup>19</sup> (FIG. 2a). Combination of all these building blocks would generate a library of 3,400 individual DHPMs. An instrument with automation features similar to those of the one shown in FIG. 1Ba, but also incorporating a liquid handler for dispensing stock solutions of building blocks, was used to generate a representative sub-library of 48 DHPM analogues, involving all of the building blocks mentioned above<sup>21</sup>. With an average processing time of ~15 minutes (including the time needed for dispensing reagents and moving vials in and out of the cavity), the generation of the 48-member library could be achieved within 12 hours. Most microwave synthesizers are

designed for fully automated unattended operation, and so a library of this size can conveniently be prepared overnight. The final products were obtained in a high state of purity in 52% average isolated yield and typically in >100-mg quantity.

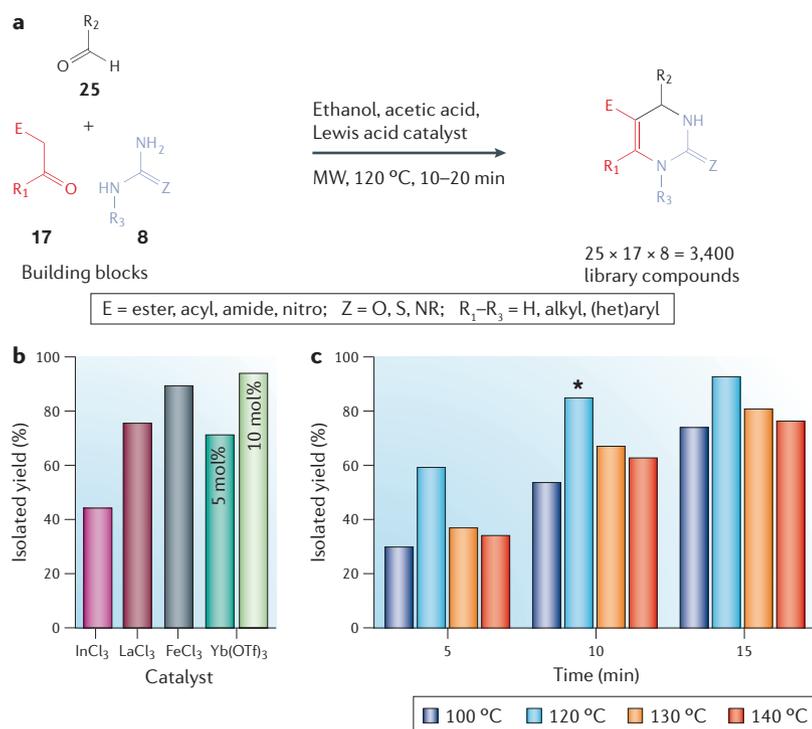
As shown in the synthesis of the DHPM library outlined above, there are numerous advantages associated with the application of high-speed microwave synthesis. Reaction conditions can be optimized in a few hours rather than in days or weeks, as is the case with conventional chemistry. In addition, a larger number of reaction parameters (choice of solvent and catalysts, molar ratio and concentration of building blocks/catalysts, reaction temperature and time) can be optimized in a relatively short time-frame compared with a conventional-type synthesis (FIG. 2b). Importantly, optimizations are carried out in an automated sequential fashion and not in parallel, which means that the result of one single run (or of a few runs) can be immediately fed into the design of the next experiment. Therefore, non-productive combinations leading to a waste of chemicals, time and resources can often be avoided. Because a significant number of reaction parameters can be considered, microwave-assisted reaction optimization today is often accompanied by a statistic-based design of experiment (DoE) approach, which can readily gauge interaction effects between the limiting factors of a reaction<sup>24–27</sup>.

Another advantage of the sequential microwave approach is that troublesome building block combinations can be individually optimized to maximize product yields and therefore minimize subsequent purification issues. In the Biginelli reaction (FIG. 2a), for example, certain building blocks or combinations of building blocks are known to require a specific set of conditions (different catalyst or solvent, lower reaction temperatures and so on). These issues can be readily addressed in the automated sequential format, if several sets of optimized reaction conditions (rather than only one set of conditions) are used for the subsequent automated library production<sup>19</sup>.

**Conventional versus microwave-assisted library synthesis.** Depending on the molecular complexity and the method of synthesis of a given compound library, the time required from the design of the library to the production stage can often be anywhere from 15 to 22 weeks (historical in-house data from Boehringer Ingelheim)<sup>28</sup> (FIG. 3a). The largest proportion of time spent in library development and production is generally devoted to the proof-of-principle stage (FIG. 3a), in which experimental designs and potential synthetic directions are evaluated. In this phase, chemical yields and purities are optimized for different strategies to determine the most feasible route to the final product. The proof-of-principle stage is followed by library validation, during which reagent compatibility and diversity are analysed before the library is produced. Based on the results presented for the DHPM case study described above it is clear that microwave synthesis will have the largest impact on the proof-of-principle and library-validation phases of the production timeline (FIG. 3a).

## Design of experiment

(DoE). The use of factorial experiments instead of the one-factor-at-a-time method for the optimization of reaction conditions. This allows studying the effect of each factor on the response variable, while requiring fewer observations than by conducting separate experiments for each factor independently.



**Figure 2 | Dihydropyrimidine library synthesis and reaction optimization using automated sequential microwave synthesis.** **a** | Biginelli three-component reaction for the generation of multifunctionalized dihydropyrimidine libraries. The combination of 17 CH-acidic carbonyl compounds, 25 aldehydes and 8 urea/thioureas as building blocks potentially provides 3,400 dihydropyrimidines. Microwave heating in sequential format allows the preparation of a sub-set of 48 derivatives within 12 hours. **b** | Rapid optimization of the Lewis acid catalyst under microwave conditions. The highest isolated yield (92%) is achieved with 10 mol% (relative to the building blocks) of Yb(OTf)<sub>3</sub> using microwave heating at 120 °C for 10 minutes in a 3/1 acetic acid/ethanol solvent mixture. **c** | Fine tuning of reaction time and temperature using 10 mol% of Yb(OTf)<sub>3</sub> as catalyst. The highest yields are obtained for runs performed at 120 °C. The conditions indicated by the asterisk (microwave, 120 °C, 10 minutes) were chosen for library synthesis. These optimized conditions can be obtained in a few hours using microwave synthesis.

#### Dielectric properties

The ability of a specific substance to convert electromagnetic energy into heat at a given frequency and temperature is determined by the 'loss tangent',  $\tan \delta$ . A reaction medium with a high (>0.5)  $\tan \delta$  value is required for efficient absorption and rapid heating.

#### Diversity-oriented synthesis

(DOS). Efficient synthesis of a collection (combinatorial library) of structurally diverse and complex small molecules that differ in stereochemistry, functional groups and molecular framework. Rather than being directed toward a single biological target, DOS libraries can be used to identify new ligands for a variety of targets.

In an attempt to estimate the time savings associated with the use of microwave-assisted library synthesis, two independent researchers were given the same target library to synthesize<sup>28</sup> (FIG. 3b). The two 'contestants' could use any route of synthesis, but only one of the chemists had access to an automated microwave synthesizer similar to the instrument displayed in FIG. 1Ba. As shown in FIG. 3b, both scientists — based on available literature precedent — arrived at the same three-step synthetic strategy for the construction of the desired 4-acyl-1,2,3,4-tetrahydroquinoxalin-2-one library. For all three steps, the conventionally heated reactions were sluggish, providing only moderate product yields, even after extensive optimization attempts. In total, the time spent on the non-microwave method was 37 working days with an overall yield of 4%. Because of the low efficiency of the process, the chemist determined that a synthetic route for this scaffold that would be intended for parallel synthesis would not be feasible<sup>28</sup>.

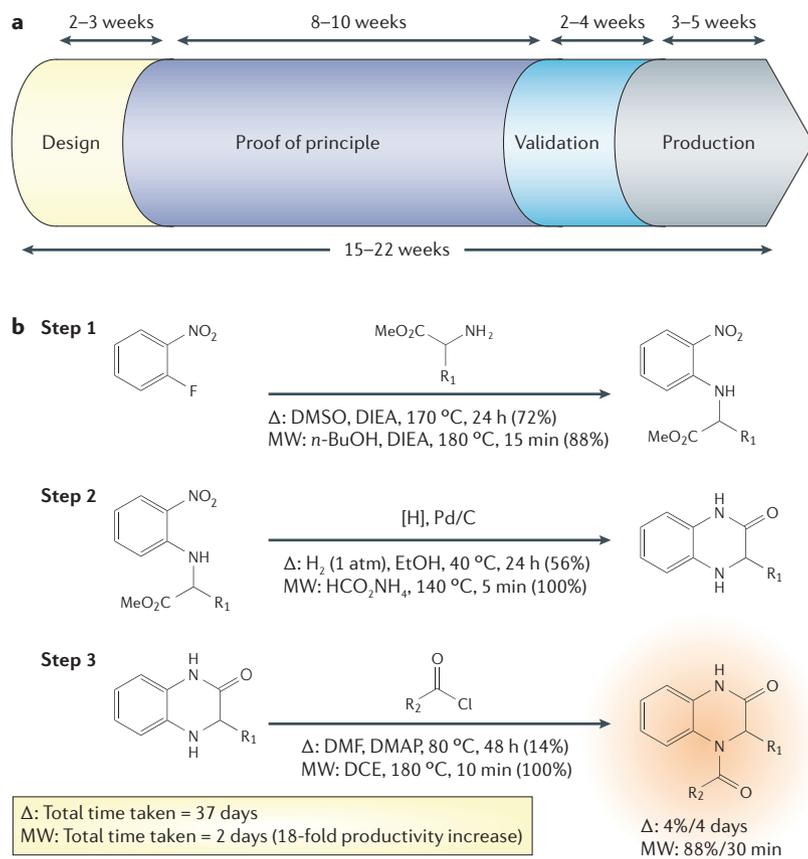
By contrast, the microwave approach allowed rapid optimization of the reaction conditions for all three steps, and afforded products in short reaction times and in excellent yields. The desired target compound was produced in 88% overall yield in only 2 working days, representing an 18-fold increase in productivity (FIG. 3b).

**Parallel microwave library synthesis.** The current trend in the pharmaceutical industry is to generate comparatively small, focused libraries containing ~30–300 compounds for a typical drug discovery project. Automated sequential library synthesis utilizing robotized microwave reactors (FIG. 1Ba) is a useful tool in this context. Given the short reaction times in microwave synthesis, an automated sequential approach is almost as effective as a conventional parallel synthesis (if not more), if the number of compounds to be prepared is relatively small (<300). However, there are times when it could be desirable to generate a large compound library with 1,000–10,000 substances using a standard solution-phase synthetic method. A simple calculation, taking into account the average processing time of a typical robotized microwave synthesizer, indicates that this approach is generally not feasible. To synthesize the full set of 3,400 DHPMs (FIG. 2a) would require more than 1 month of continuous instrument operation, despite an average processing time per sample of only 15 minutes.

In an effort to increase the throughput in microwave synthesis, several research groups have experimented with parallel-based approaches using a multimode instrument, typically carried out in 96-well microtitre plates<sup>28–33</sup>. These plates are routinely used in conventional (thermal) parallel chemistry throughout the drug discovery industry, but have limitations when used in a microwave field. This is mainly because the electromagnetic field inside a multimode microwave reactor is not fully homogeneous, and therefore all the wells are not heated evenly<sup>28,32</sup>. These problems can be overcome to some extent by using plates consisting of a base of carbon-doped Teflon (Weflon) for better heat distribution, and utilizing glass inserts as reaction vessels<sup>28,30,33</sup>. The material used for the preparation of the plates efficiently absorbs microwave energy, which means that the sealed glass vials will be heated regardless of the dielectric properties of the reaction mixtures. Such a system is commercially available (CombiCHEM system; Milestone Inc.) (FIG. 1Bc) and the use of this equipment for synthetic applications has been described<sup>30,33</sup>, albeit not in the context of a large library synthesis. Several plates can be mounted in an overhead rotor system, and so several hundred reactions could potentially be carried out in one irradiation cycle.

#### Drug discovery applications

**Lead generation and optimization.** With the competitive nature of the drug discovery industry, lead discovery groups are challenged to develop promising programmes rapidly and secure a strong intellectual property position early. A recent concept in drug discovery is diversity-oriented synthesis (DOS)<sup>34</sup>. The DOS strategy stands in



**Figure 3 | Time savings associated with microwave-assisted library synthesis.**

**a** | Typical library production timelines based on in-house data from Boehringer Ingelheim<sup>28</sup>. **b** | Direct comparison of thermal ( $\Delta$ ) and microwave (MW) results for the synthesis of a 4-acyl-1,2,3,4-tetrahydroquinoxalin-2-one library. For all three steps (1, nucleophilic aromatic substitution; 2, nitro group reduction; and 3, *N*-acylation), microwave synthesis provided significantly higher yields in dramatically reduced reaction times. Optimization experiments (not shown) were carried out for both methods evaluating different types of reaction conditions. Because of the much faster reaction times in the microwave runs a higher degree of optimization could be achieved. Whereas the thermal approach required 37 working days for optimization and validation to reach the final target structure (with steps 1, 2 and 3 taking 15, 10 and 12 days, respectively), this could be achieved within 2 working days with microwaves.

sharp contrast to a more typical library design, in which a common scaffold is 'decorated' with various monomer units. As many leads in pharmaceutical research identified from high-throughput screening are small-molecule heterocycles, efficient strategies to produce diverse collections of such compounds are of particular relevance. Protocols for the rapid preparation of diverse collections of heterocyclic scaffolds from common 1,2-diketone intermediates (FIG. 4a) have been developed<sup>35–40</sup> and simple, high-yielding microwave-assisted procedures have been reported for the preparation of diversely substituted 1,2,4-triazines<sup>35</sup>, imidazoles<sup>36</sup>, fused pyrazines (for example, quinoxalines)<sup>37</sup>, pyrazin-2(1*H*)-ones<sup>38</sup> and canthine derivatives<sup>39</sup> (FIG. 4a). In most cases, condensation of a suitable 1,2-diketone building block, which serve as diversification elements, with the appropriate reaction partner(s) required only 5–10 minutes of microwave irradiation and resulted in

a high yield of the corresponding nitrogen heterocycle. Compared with classical thermal heating (8–24 hours, 30–65% yields), there is a marked acceleration in the rate of the reaction.

Based on the highly efficient microwave chemistry displayed in FIG. 4a, a series of potent and selective allosteric AKT (also known as protein kinase B (PKB)) kinase inhibitors were developed. These inhibitors were derived from a 2,3-diphenylquinoxaline core and have an unprecedented level of selectivity for both **AKT1** and **AKT2**<sup>38</sup>. AKT is a serine/threonine kinase that has attracted a great deal of attention as a promising molecular target for cancer therapy because of its crucial role as a regulator of the apoptotic machinery of cells<sup>38</sup>. Both isozymes AKT1 and AKT2 are commonly overexpressed or constitutively active in a large number of human cancers, including brain, gastric, colon, breast, lung and prostate carcinomas, and their activation correlates with cancer progression<sup>38</sup>.

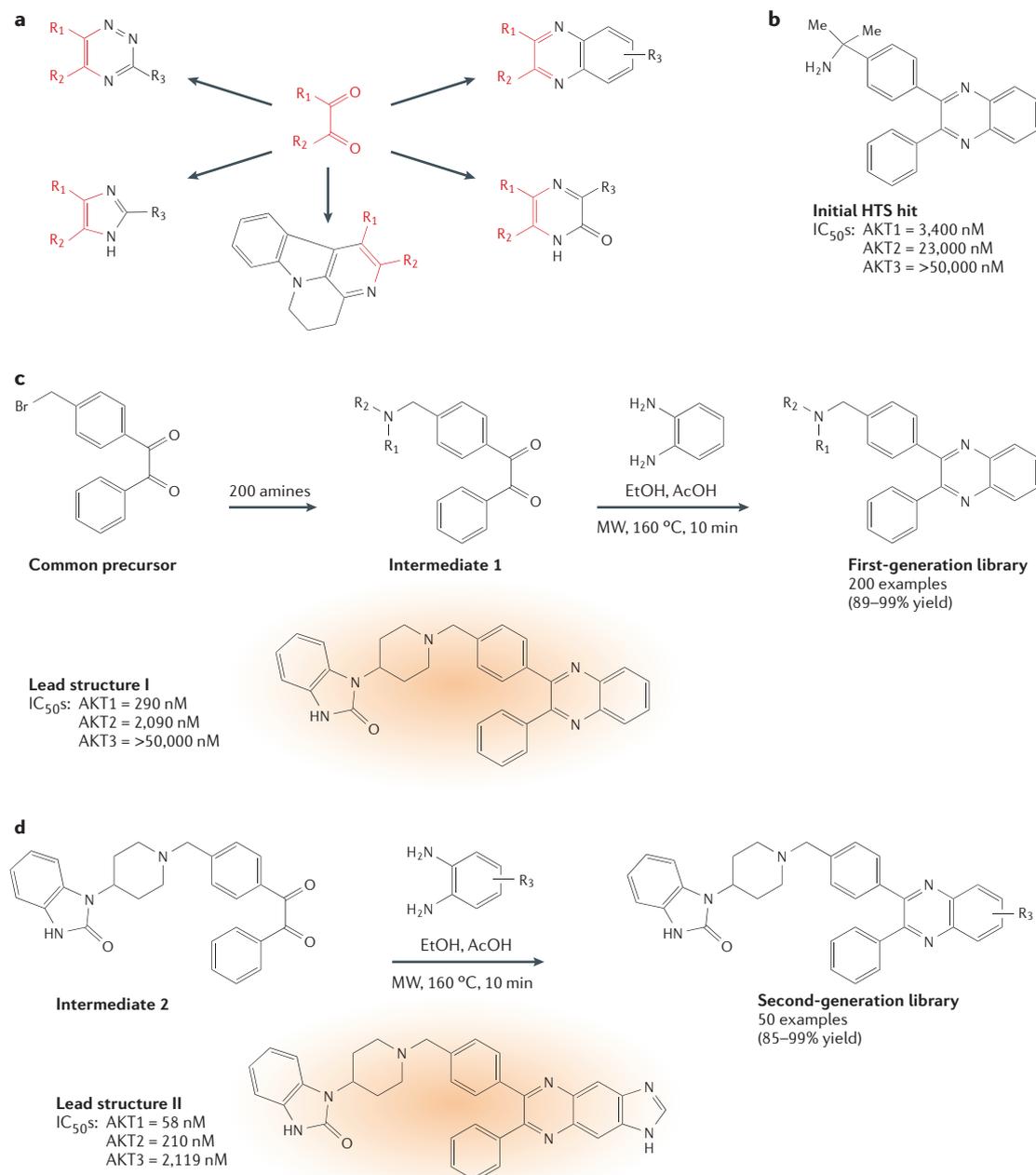
One single hit from a high-throughput screening effort designed to identify compounds capable of inhibiting the three AKT isozymes (AKT1, AKT2 and **AKT3**) provided a 2,3-diphenylquinoxaline structure that had high selectivity for AKT isozymes (FIG. 4b). To develop a SAR for this initial compound rapidly, an iterative analogue library synthesis approach was used<sup>38</sup>. The commercially available bromomethyl functionalized 1,2-dicarbonyl building block was treated with 200 different amines to provide 200 amino-functionalized intermediates. Each of these intermediates was then treated with benzene-1,2-diamine under microwave irradiation (160 °C for 10 minutes) to afford 200 amino-functionalized quinoxalines (FIG. 4c). Among this set of 200 structures, lead structure I was identified with a tenfold increased potency relative to the initial screening hit. However, this compound had poor solubility and lacked cell activity. In a further optimization cycle addressing the quinoxaline ring itself (FIG. 4d), intermediate 2 — containing the key structural element of the previously optimized amine moiety — was treated with 50 different functionalized aryl-1,2-diamines to deliver 50 second-generation analogues. Among them, lead structure II was selected for further evaluation as it had improved potency, in addition to enhanced solubility and cell permeability. This dual AKT1/AKT2 inhibitor was also shown to sensitize tumour cells to apoptotic stimuli and inhibit the phosphorylation of both AKT1 and AKT2 *in vivo*<sup>38</sup>. A structurally related set of AKT1/AKT2 inhibitors with improved physical properties that was also developed using microwave chemistry by the same group has been reported<sup>40</sup>.

This example highlights the potential of microwave-assisted synthesis to derive potent lead structures quickly via rapid generation of SARs. It should be pointed out that the preparation of the 2,3-diphenylquinoxaline ring system by condensation of aryl-1,2-diamines with 1,2-dicarbonyl compounds under conventional thermal conditions in the same solvent system requires long reaction times, provides only moderate yields and leads to dramatic variations in reaction time and yield depending on the incorporated functionality<sup>37</sup>. Although this could be acceptable if only a single compound has to be

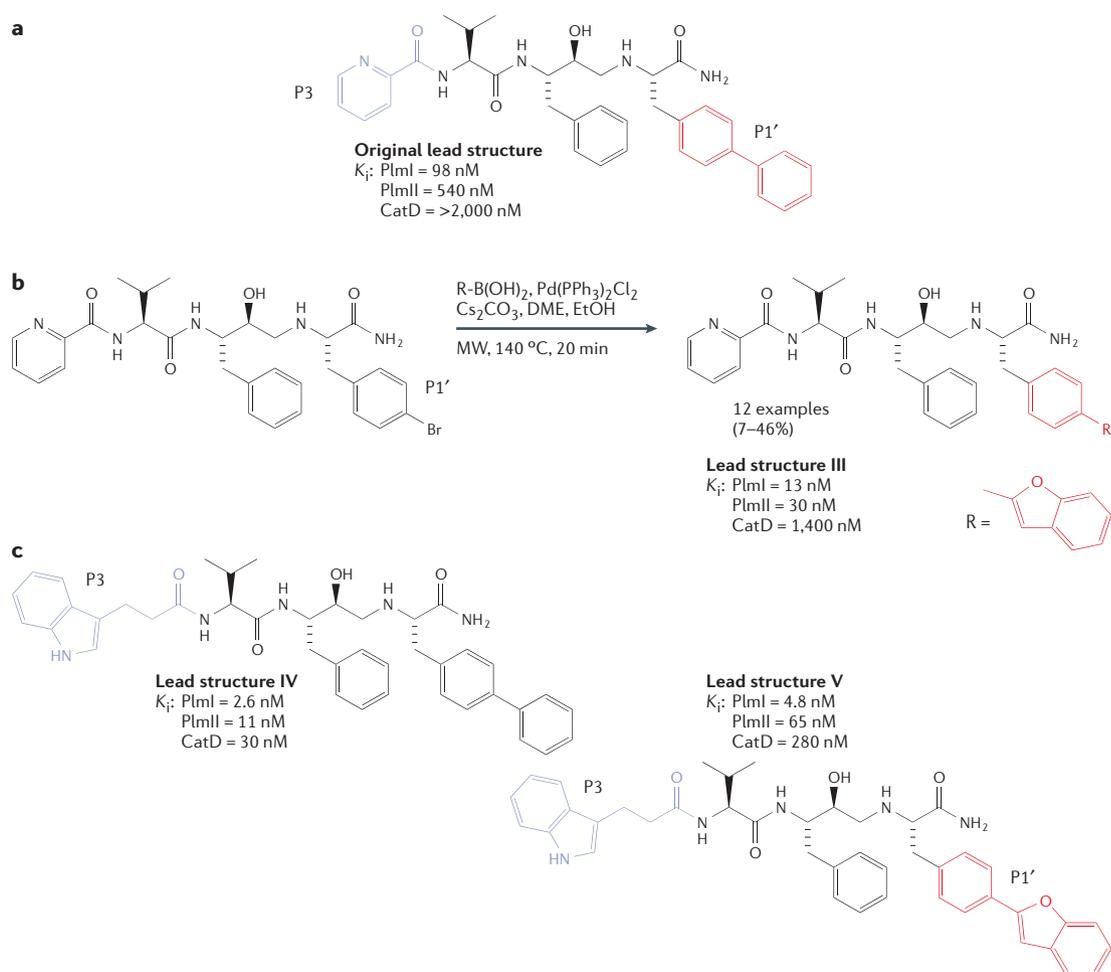
synthesized, a general high-yielding method is required for the generation of larger libraries where structural diversity needs to be maximized.

Microwave technology has also been applied in transition metal-catalysed reactions for the rapid optimization of inhibitors of the malarial proteases plasmepsin I and II (PlmI and PlmII, respectively)<sup>41</sup>. The recent publication of the genome of *Plasmodium falciparum*<sup>42</sup>,

the most lethal of the protozoan parasites causing malaria, has revealed a number of new targets for drug intervention. Among these are the haemoglobin-degrading aspartic proteases PlmI and PlmII<sup>43</sup>. Several inhibitors for these proteases have been reported in the literature<sup>44,45</sup>. However, selectivity towards the highly homologous human aspartic cathepsin D (CatD) has been a common problem<sup>44</sup>.



**Figure 4 | Lead generation and optimization of allosteric AKT kinase inhibitors derived from a 2,3-diphenyl-quinoxaline core.** **a** | Rapid generation of heterocyclic scaffolds with potential biological activity using 1,2-diketones as common precursors. In most cases, the final products were obtained within 10 minutes of microwave irradiation using suitable reaction partners. **b** | Original lead structure derived from high-throughput screening (HTS) showing modest AKT kinase inhibitory activity. **c** | First cycle of lead generation addressing the amine moiety. Automated sequential microwave synthesis led to 200 analogues in high yields, providing lead structure I with improved inhibitory activity. **d** | Second cycle of lead generation addressing the heterocyclic quinoxaline core. Microwave synthesis delivered 50 second-generation analogues, including a dual AKT1/AKT2 inhibitor with nanomolar activity (lead structure II).



**Figure 5 | Lead optimization of the malarial proteases plasmepsin I and II inhibitors.** **a** | Original hydroxyethylamino-based lead structure derived by rational design<sup>46</sup>. The important amino-acid side-chains for optimization are marked as P1' and P3. **b** | Microwave-assisted Suzuki arylations with sets of boronic acids for the generation of analogues with modified P1' side chain. **c** | Lead structures derived by microwave-assisted Suzuki reactions with modified P3 side chain (lead structure IV) and combined P1'/P3 side chains displaying optimum inhibitory activity (lead structure V).

In an effort to identify potent, selective, orally bio-available and metabolically stable plasmepsin inhibitors as new antimalarial drugs, several focused libraries targeted for the inhibition of PlmI and PlmII were synthesized<sup>41</sup> (FIG. 5). A hydroxyethylamine-based inhibitor (FIG. 5a) — derived from a rational design approach<sup>46</sup> — was used as a starting point. To increase the structural diversity at the important P1' side-chain of the inhibitor, a suitable arylbromide-precursor was subjected to automated microwave-assisted Suzuki cross-coupling chemistry. The reaction with a diverse set of boronic acids under palladium catalysis allowed the production of a set of eight new inhibitors in moderate yields<sup>41</sup>. Among those, a lead structure with significantly improved potency with a benzofuran substructure was identified (lead structure III, FIG. 5b). In addition to the investigation of the P1' side chain, substitution at the P3 position was also studied by reacting a diverse set of carboxylic acids with the primary amine. Next, the same microwave-assisted Suzuki arylation strategy was used to generate a second set of inhibitors that incorporated the biphenyl P1' moiety from the

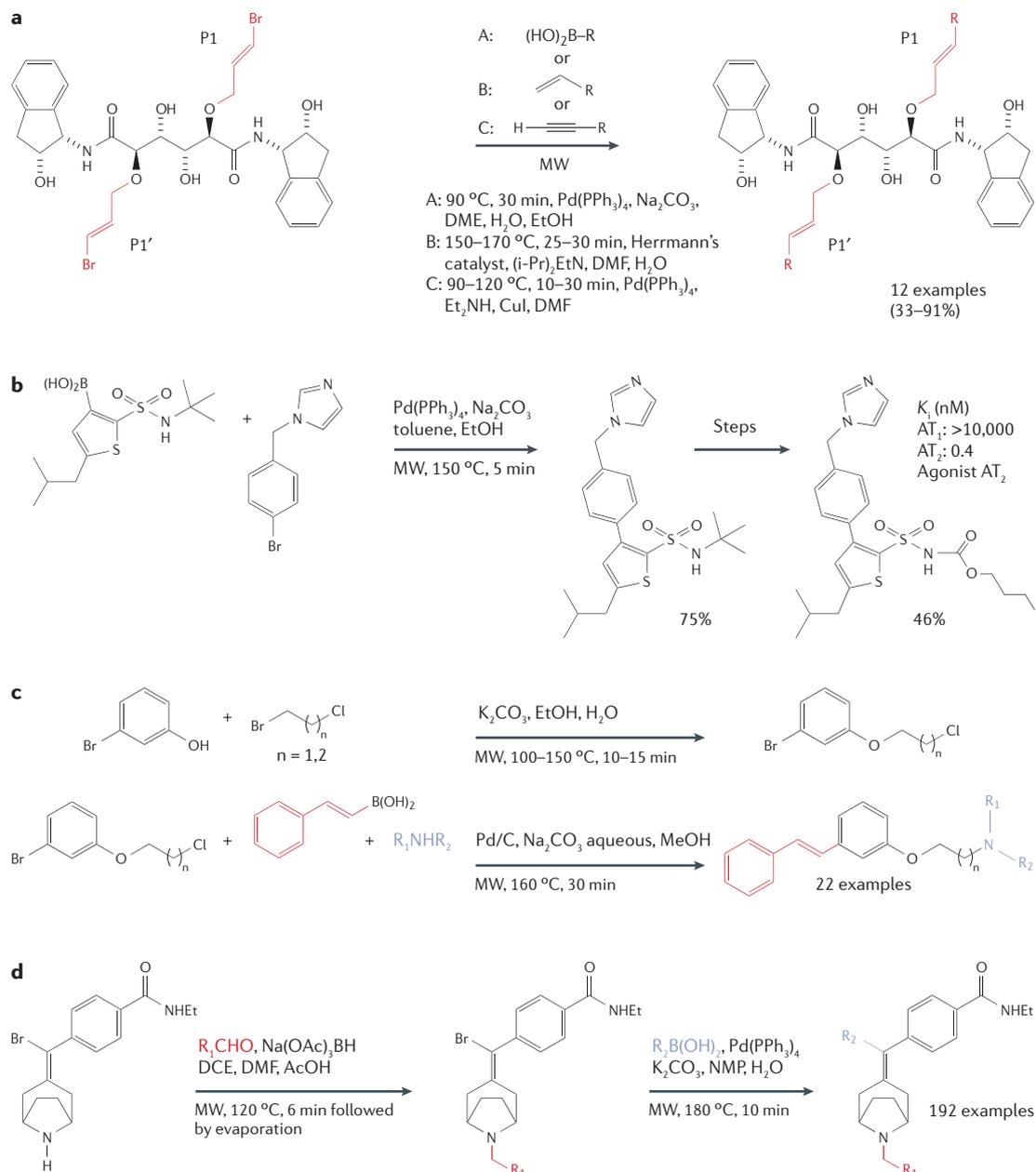
original lead structure. From the nine new substances prepared by this method, lead structure IV was identified as one of the most active and selective inhibitors (FIG. 5c). Finally, a new eight-member library based on a combination of the most active P1' and P3 side chains was prepared. The inhibitor with the highest activity and selectivity from this library was lead structure V, which showed some degree of selectivity for PlmI and PlmII versus CatD<sup>41</sup> (FIG. 5c).

All scaffold decoration chemistry on this class of transition-state mimetics was carried out by automated microwave-assisted synthesis as described above. Although transition metal-catalysed reactions, such as the Suzuki arylation, are highly chemoselective transformations, these carbon-carbon bond-forming reactions typically require many hours or even days to reach completion under standard reaction conditions. Only through the use of microwave heating can these powerful transformations now be used in high-throughput synthesis. Using similar transition metal-catalysed reactions, efficient microwave-aided lead optimizations

for 1,2-dihydroxyethylene-based plasmepsin inhibitors<sup>47,48</sup> (FIG. 6a), different types of HIV-1 protease inhibitors<sup>47,49,50</sup> and **angiotensin II receptor** antagonists<sup>47,51</sup> (FIG. 6b) have been reported.

Despite MAOS being a relatively new field, there are many additional examples that highlight how this technology can assist lead discovery and optimization in a pharmaceutical setting, including the synthesis

of styrene-based nicotinic acetylcholine receptor antagonists<sup>52</sup> (FIG. 6c), tropanylidene opioid receptor agonists<sup>53</sup> (FIG. 6d), and various discovery libraries based on bicyclic fused azepinones of potential interest as cell-signalling pathway inhibitors<sup>54</sup>. Other examples of the key role of microwave technology in the synthesis of biologically active molecules are found in REFS 55–66.



**Figure 6 | Use of microwave heating in medicinal chemistry.** **a** | Synthesis of plasmepsin I and plasmepsin II (PlmI and PlmII, respectively) inhibitors with extended P1/P1' side chains via microwave-assisted palladium-catalysed coupling reactions<sup>48</sup>. The synthesized compounds showed high inhibitory affinity against both PlmI and PlmII, but no detectable affinity towards human CatD was observed. **b** | High-speed generation of a selective angiotensin II receptor agonist<sup>51</sup>. The reaction time for the Suzuki coupling step could be reduced from several hours to only 5 minutes by applying microwave heating. **c** | Microwave-assisted synthesis of nicotinic acetylcholine receptor (nAChR) antagonists<sup>52</sup>. The styrene-based compounds could be obtained in a two-step reaction sequence, including a one-pot Suzuki coupling/*N*-alkylation reaction. **d** | Parallel solution-phase synthesis of a series of tropanylidene benzamides as opioid agonists in an initial structure–activity exploration study<sup>53</sup>. Of all synthesized compounds, 64% showed a  $K_i$  value less than 10 nM for  $\delta$ - or  $\mu$ -receptors, respectively.

### Integration with other technologies

Not only can MAOS be applied to standard solution-phase chemistry protocols, this technology can also be efficiently integrated into solid-<sup>67</sup> or fluorous-phase<sup>68</sup> organic synthesis. Both of these technologies are designed to simplify product isolation and purification after synthesis. In such a strategy, the substrate is temporarily immobilized on a polymeric or fluorous support, taken through a synthetic sequence and then cleaved from the support into solution. By using excess reagents, reactions can be driven to completion and it is possible to create a high level of molecular diversity. Similarly, microwave synthesis has been used in conjunction with reagents linked to a solid support (the substrates remaining in solution<sup>69</sup>). On completion of the reaction, the excess or spent reagent can then be easily removed by filtration.

One key disadvantage associated with the above strategies is that reactions involving these heterogeneous systems are often slower than their homogeneous solution-phase equivalents. Not surprisingly, microwave heating has provided in many cases a solution to these problems in speeding up otherwise sluggish protocols<sup>1–7</sup>; some examples are summarized in FIG. 7<sup>70–72</sup>.

An important application of microwave-assisted solid-phase synthesis approaches with implications for the drug discovery process is its recent use in the efficient preparation of both peptides and peptide analogues<sup>73–75</sup>, in addition to the generation of combinatorial  $\beta$ -peptide libraries<sup>76</sup>. The therapeutic peptide market is burgeoning and is expected to expand with a growth rate nearly double that of small-molecule drugs. Currently, there are more than 40 marketed peptides worldwide, around 270 peptides in clinical-phase testing and about 400 in advanced preclinical phases. One of the disadvantages associated with peptides is that they are often challenging and costly to synthesize. By using microwave heating<sup>73–76</sup>, most of the investigated peptides could be prepared in significantly higher purity and yield than is possible under conventional conditions. This will aid in the development of new therapeutic peptides and in the larger-scale production of other important peptide products.

In addition to standard crosslinked polystyrene beads used in solid-phase synthesis, planar cellulose membranes have also been used with rapid microwave synthesis<sup>77–79</sup>, allowing the simultaneous, spatially addressed, parallel synthesis of thousands of compounds. This technique allowed, for example, the rapid generation of a diverse 8,000-member library of 1,3,5-triazines on a 18 × 26 cm cellulose membrane, which were screened in parallel directly on the surface of the planar support for binding to the anti-transforming growth factor-R monoclonal antibody Tab2 to identify epitope mimics<sup>77</sup>.

Another recent addition to the powerful toolbox of high-throughput synthesis technologies is the concept of performing reactions in a flow format in micro-channels (microreactors, or lab-on-a-chip)<sup>80,81</sup>. In general, flow-through micro-scale reaction systems have used a sequential-flow approach for the preparation of libraries in which compounds are produced, one after another, through the same reactor channel<sup>80,81</sup>. Virtually all libraries prepared by this approach have been synthesized at

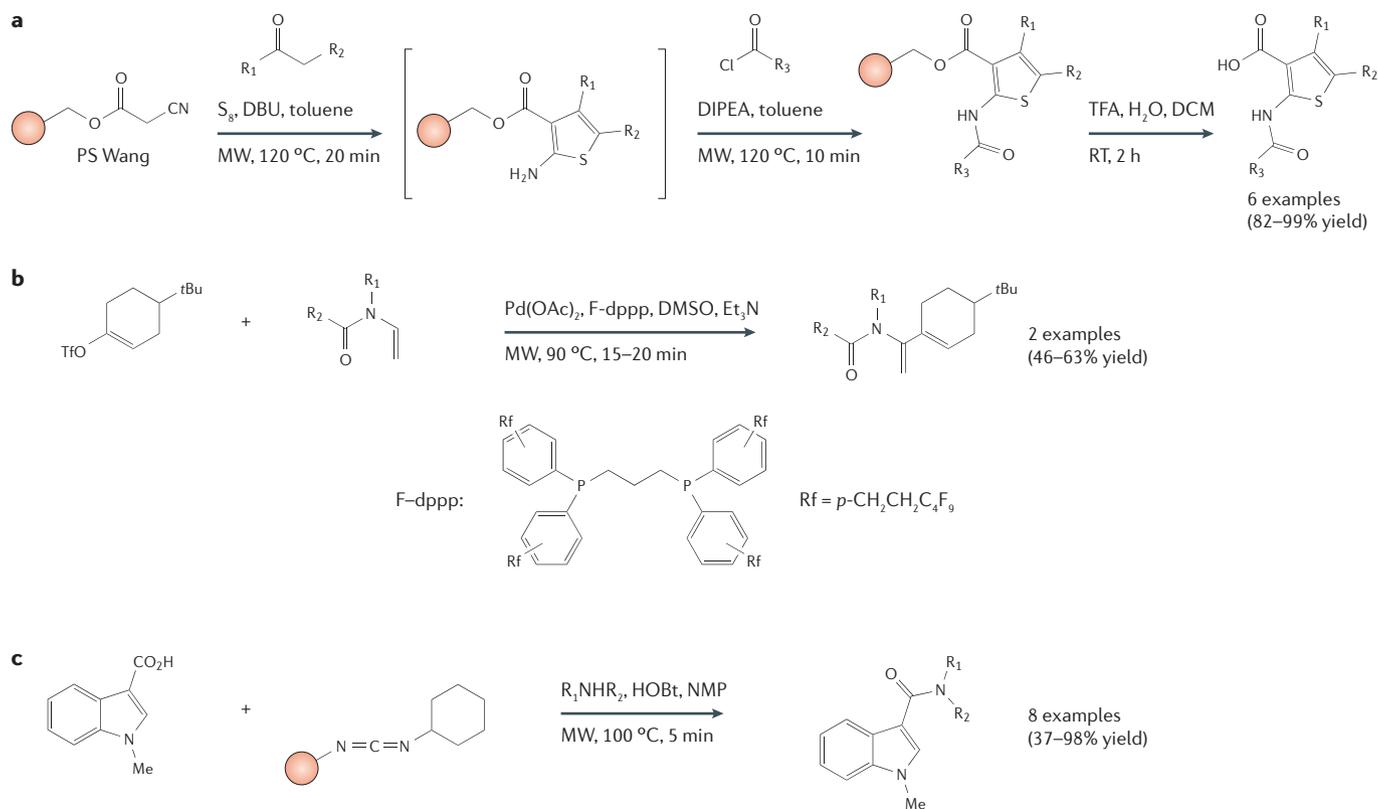
room temperature using glass microchips, because existing microfluidic technology does not fully address the issue of applying, controlling and monitoring heating in these reaction systems, which limits its application<sup>80,81</sup>. Notably, there have been some attempts to apply microwave heating to microreactor technology. Microwave energy was used to deliver heat locally to a heterogeneous palladium-supported catalyst (catalyst channel: 1.5 × 0.08 × 15 mm) located in a microreactor device<sup>82</sup>. A 10–15-nm gold film patch, located on the outside surface of the base of a glass microreactor, assisted in the heating of the catalyst, facilitating effective Suzuki cross-coupling reactions<sup>82–84</sup>. This concept was extended recently in an investigation that used straightforward capillary technology (0.2–1.2-mm inner diameter) inside a commercially available single-mode microwave reactor to perform different chemical transformations in a continuous flow format<sup>85</sup>. The capillaries were coated on the inside with a thin film of strongly microwave-absorbing palladium that aided the heating process and catalysed some of the transformations reported. Notably, this method has also been implemented in the synthesis of combinatorial libraries in a parallel flow format, and a capillary-based approach to parallel synthesis has been developed in which reagents flow in sequence into a multi-capillary reactor device (up to eight inlet ports and four outlet ports) that is heated by microwave irradiation<sup>86</sup>.

### Conclusions and perspectives

There are more than 3,000 documented examples of MAOS reported by both academic and industrial laboratories<sup>1–7</sup>, which suggests that most chemical transformations can be carried out successfully under microwave conditions. This does not necessarily imply that dramatic rate-enhancements compared with a classical, thermal process will be observed in all cases, but the simple convenience of using microwave technology (BOX 2) will make this non-classical heating method a standard tool in the laboratory within a few years. In the past, microwaves were often used only when all other options to perform a particular reaction had failed, or when exceedingly long reaction times or high temperatures were required to complete a reaction. This practice is now slowly changing and, because of the growing availability of microwave reactors in many academic and industrial laboratories, routine synthetic transformations are now being carried out by microwave irradiation. In the context of 'reaction scouting', a 'yes' or 'no' answer for a particular chemical transformation can often be obtained within 5–10 minutes (as opposed to several hours in a conventional protocol), which has contributed significantly to the acceptance of microwave chemistry both in industry and academia. The recently reported incorporation of real-time, *in situ* monitoring of microwave-assisted reactions by Raman spectroscopy will facilitate a further increase in efficiency and speed<sup>87</sup>. For the production of new chemical entities in pharmaceutical industry today, microwaves are an essential tool and several laboratories have already incorporated microwave reactors into in-house 'synthesis stations' for producing small- and medium-sized compound libraries in a high-throughput format.

#### Microreactor

Continuous-flow device that consists of a series of interconnecting channels (50–500  $\mu\text{m}$  diameter) and facilitates the performance of chemical reactions on a micro-litre scale. Independent reactants are brought together through different feeder channels to the main channel, where they mix and react as they travel to a reservoir where the products are collected.



**Figure 7 | Integrating microwave heating with other technologies.** **a** | Microwave-assisted solid-phase synthesis<sup>70</sup>. The well-known Gewald synthesis of 2-amino-3-acylthiophenes was carried out as a one-pot synthesis on conventional polystyrene (PS) Wang resin. The overall two-step procedure could be carried out in less than 1 hour, without requiring filtration between the two reaction steps. **b** | Microwave-assisted, palladium (Pd)-catalysed Heck vinylation<sup>71</sup>. The fluorinated bidentate ligand (F-dppp) was easily removed after the reaction by solid-phase extraction with a fluorinated silica gel cartridge (F-SPE). **c** | Microwave-assisted synthesis involving a polymer-supported reagent<sup>72</sup>. A series of amides was prepared utilizing a polystyrene-bound carbodiimide reagent, which was readily removed by filtration after the reaction.

One of the major drawbacks of this relatively new technology is equipment. Although prices for dedicated microwave reactors for organic synthesis (FIG. 1B) have dropped considerably since their first introduction in the late 1990s, the current price range for microwave reactors is still many times higher than that of conventional heating equipment cost, ranging from US\$15,000–100,000. As with any new technology, the current situation is bound to change over the next few years, and less expensive equipment should become available. An even bigger problem, especially for the drug discovery industry, is scalability. It has to be noted that with few exceptions most of the examples of microwave-assisted synthesis published so far were carried out on a small scale (<1 g; typically 1–5-ml reaction volume). This is in part a consequence of the recent availability of single-mode microwave reactors that allow the safe processing of small reaction volumes under sealed-vessel conditions by microwave irradiation (FIG. 1Ba). Although these instruments have been successful in the preparation of new lead compounds and for lead optimization, it is clear that for microwave-assisted synthesis to become a fully accepted

technology in the pharmaceutical industry there is a need to develop larger-scale MAOS techniques that can routinely provide products for lead development and ultimately for production on a large scale (multi-100 kg, or even higher). Two different approaches that address these issues have emerged. Some groups have experimented with larger batch-type reactors<sup>88–92</sup> (FIG. 1Bd), whereas others have used continuous-flow techniques<sup>93–97</sup> (FIG. 1Bb) to overcome the inherent problems associated with MAOS scale-up. Currently, there are no documented published examples of the use of microwave technology for organic synthesis on a production scale level (>1,000 kg), which is a clear limitation of this otherwise so successful technology<sup>98</sup>.

Despite these limitations, microwave chemistry has opened up several new avenues in organic synthesis. Many reactions that previously were not possible, or resulted in a low yield, can now often be performed quickly, safely and efficiently in a few minutes. In summary, MAOS has changed the world of organic chemistry and drug discovery, and it would be wise to embrace this new technology or be left lagging behind with conventional heating methodologies.

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#### Competing interests statement

The authors declare no competing financial interests.

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## Biographies

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Doris Dallinger received her doctoral degree from the University of Graz in 2005, where she worked in the group of C. Oliver Kappe on microwave-assisted synthesis and functionalization of biologically active heterocycles. She is currently involved in research on non-thermal microwave effects and scale-up of microwave-assisted processes in the group of C. Oliver Kappe.

Research in the Kappe laboratories focuses on microwave-assisted organic synthesis (MAOS), covering a wide variety of topics ranging from the investigation of special microwave effects, automated library synthesis, parallel microwave chemistry and scale-up issues associated with microwave technology.

## Online summary

- Lead compound optimization and medicinal chemistry are known to be the bottlenecks in the drug discovery process, and so a need arises for technologies that allow more rapid synthesis of chemical substances. One such high-speed technology is microwave-assisted organic synthesis (MAOS).
- Microwave heating, compared with conventional heating (which means heating with an external source such as an oil-bath) is much more efficient because the reaction mixture is heated internally by direct coupling of microwave energy with polar molecules (solvents, reagents and catalysts, for example). This allows faster heating to higher temperatures using sealed-vessel technology.
- This enabling technology has gained significant influence in the lead optimization and lead generation processes in the pharmaceutical industry because microwave heating dramatically reduces reaction times from days or hours to minutes or seconds.
- Many reaction parameters, such as time, temperature, solvents, concentration or catalysts, can now be evaluated in a fraction of the time, compared with conventional heating, in the optimization step.
- Compound libraries can be rapidly synthesized either in a parallel or sequential automated format for lead discovery or structure-activity studies.
- MAOS can also be combined with solid- or fluorous-phase synthesis or solid-supported solution-phase synthesis, respectively, to achieve simpler product isolation and purification.
- Several examples for high-throughput microwave synthesis using parallel or sequential library production as well as lead optimization and generation studies are covered in this review.

## TOC blurb

Microwave-assisted organic synthesis has considerable potential to accelerate the generation and optimization of lead compounds. Kappe and Dallinger outline the fundamental principles of this technology and discuss its applications in drug discovery.