ChemComm

COMMUNICATION

View Article Online
View Journal | View Issue

Cite this: *Chem. Commun.*, 2020, **56**, 8047

Received 1st April 2020,
Accepted 9th June 2020

DOI: 10.1039/d0cc02361b

rsc.li/chemcomm

Activity-directed expansion of a series of antibacterial agents†

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The feasibility of using activity-directed synthesis to drive antibacterial discovery was investigated. An array of 220 Pd-catalysed microscale reactions was executed, and the crude product mixtures were evaluated for activity against *Staphylococcus aureus*. Scale-up of the hit reactions, purification and evaluation, enabled expansion of a class of antibacterial quinazolinones. The novel antibacterials had MICs from 0.016 μg mL^{-1} (*i.e.* 38 nM) to 2–4 μg mL^{-1} against *S. aureus* ATCC29213.

The discovery of bioactive small molecules is generally driven through iterative design-make-purify-test cycles. The process is generally underpinned by a remarkably narrow toolkit[1] of reliable reaction classes which limits the diversity of explored chemical space.[2] We have recently introduced activity-directed synthesis[3] (ADS), a structure-blind and function-driven discovery approach. In ADS, inherently promiscuous chemistry is harnessed in which arrays of reactions are performed, and each reaction has multiple possible outcomes. The crude reaction mixtures are then directly screened for biological activity. We have previously harnessed metal carbenoid chemistry to drive the activity-directed discovery of diverse series of androgen receptor agonists and protein–protein interaction inhibitors.[3]

Here, we demonstrate the generality of ADS by showing that other reaction classes (here, Pd-catalysed carbonylation[4]) and assay types (here, whole-cell antibacterial activity against the ESKAPE[5] pathogen *S. aureus*) may be harnessed. We show that the approach may be used to expand a series of quinazolinones[6] that was developed to target penicillin-binding proteins, and displays promising activity (minimum inhibitory concentrations [MICs] as low as 0.003 μg mL^{-1}) against multiple strains of *S. aureus*.[6a]

We envisaged that Pd-catalysed reactions may complement the metal carbenoid chemistry that we have previously harnessed in ADS (Fig. 1). Specifically, we noted its potential to yield alternative scaffolds from a common substrate (such as **1**). For example, Pd-catalysed carbonylation, followed by reaction with an amine and cyclisation, could yield 2,3-disubstituted quinazolinones such as **2**. Alternatively, Pd-catalysed amination, without carbonylation, could yield anilines (*e.g.* **3**) or, with subsequent cyclisation, benzimidazoles (*e.g.* **4**). Finally, intramolecular Heck reaction could yield quinolinones (*e.g.* **5**).

Initially, we adapted a Pd-catalysed carbonylation reaction[7] for execution on a 300 μl scale (100 μmol substrate) in microscale vials in 96-well plate format (Scheme 1). Accordingly, the *o*-iodo anilide **6a** (final concentration: 333 mM), the aniline (1.2 eq.), $Mo(CO)_6$ (1 eq.), $Pd_2(dba)_3$ (0.1 mol%), $P(^tBu_3)\cdot HBF_4$ (0.6 mol%) and NEt_3 (2.5 eq.) were dissolved in *o*-xylene; after

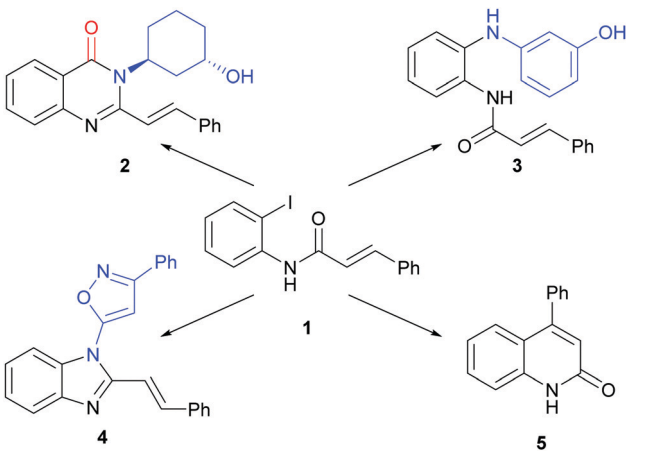

Fig. 1 Potential of Pd-catalysed chemistry to yield alternative scaffolds from common substrates (such as **1**), amines and, in some cases, a carbon monoxide source. Groups derived from an amine co-substrate (blue) and carbon monoxide (red) are indicated.

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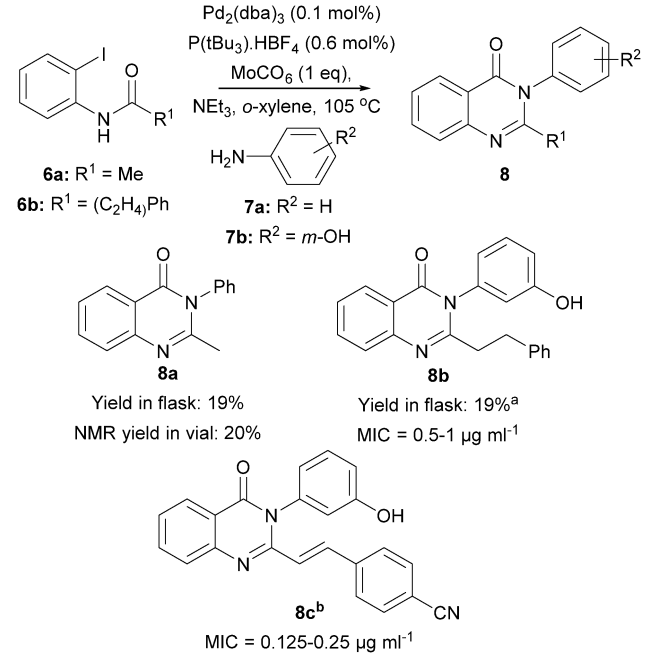
† Electronic supplementary information (ESI) available: Experimental procedures and NMR spectra. CCDC 1991665. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/d0cc02361b



Scheme 1 Configuration of Pd-catalysed chemistry for use in ADS. [a] LC-MS analysis of the reaction performed in a microscale vial revealed that the product had been formed successfully. [b] Prepared using a precedented method (ref. 6*a*).

Table 1 Configuration of antibacterial assay to be suitable for ADS

Entry[a]	Substrate	Co-Substrate[b]	Reagents[c]	Conc.[d]/μM	Activity[e]
1	**6b**	**7b**	All	50	√[f]
2	**6a**	**7b**	All	50	✗[f]
3	**6b**	—	All	50	✗
4	**6a**	—	All	50	✗
5	—	**7b**	All	50	✗
6	—	—	$P(^tBu_3)\cdot HBF_4$	50	✗
7	—	—	$Mo(CO)_6$	50	✗
8	—	—	$Pd_2(dba)_3$	50	✗
9	**6b**	**7b**	All	100	√[f]
10	**6b**	**7b**	All	25	✗[f]
11	**6b**	**7b**	All	10	✗[f]

[a] Reactions performed in microscale vials at 105 °C; crude reaction mixtures were filtered through silica, evaporated and dissolved in DMSO (total product concentration: 333 mM) before evaluation. [b] 1.2 eq. [c] $Mo(CO)_6$ (1 eq.), 0.1 mol% Pd_2dba_3, 0.6 mol% $P(^tBu_3)\cdot HBF_4$, NEt_3 (2.5 eq.), *o*-xylene. [d] Total product concentration based on limiting substrate. [e] Activity against *S. aureus* ATCC29213 (see Fig. 2 for colour scale); a tick indicates visual growth inhibition (see ESI). [f] LC-MS revealed that a quinazolinone had been formed.

sealing, the vial was heated at 105 °C for 48 h. It was shown that the yield of **8a**, determined by NMR spectroscopy using an internal standard, was broadly similar to that obtained on a preparative scale in a crimp-top vial. At this stage, the quinazolinones **8b** and **8c**[6a] were also prepared. Crucially, we had shown that Pd-catalysed chemistry was viable in a format suitable for ADS, and that stirring and an inert atmosphere were not necessary.

We adapted standard susceptibility testing methodology[8] to assess antibacterial activity against *S. aureus* ATCC29213. Quinazolinone solubility was improved by using Iso-Sensitest Broth (ISB)[9] as the growth medium, and MICs were determined for **8b–c** [**8b**: 0.5–1 μg mL^{-1} (1.45–2.90 μM); **8c**: 0.125–0.25 μg mL^{-1} (0.34–0.68 μM)]. The reaction of **6b** and **7b** (which would yield the antibacterial **8b**) was then repeated in a microscale vial, the reaction filtered through silica, evaporated, and the crude reaction mixture dissolved in DMSO (total product concentration: 333 mM). The stock solution of the crude product was screened at multiple total product concentrations (100 μM, 50 μM, 25 μM and 10 μM; entries 1 and 9–11, Table 1): visual growth inhibition was only detected at the two highest concentrations. Crucially, the reaction of **6a** and **7b** did not yield an active crude product at 50 μM total product concentration (entry 2). In addition, the reactions of the individual reactants **6a**, **6b** and **7b** (entries 3–5), as well as the individual components $P(^tBu_3)\cdot HBF_4$, $Mo(CO)_6$ and $Pd_2(dba)_3$ (entries 6–8), also did not result in detectable activity. The assay was deemed suitable for activity-directed antibacterial discovery since activity was only observed for reactions that could have yielded the positive control **8b**.

Having established appropriate synthetic and biological methods for ADS, we designed a reaction array based on ten substrates **S1–S10** (and no substrate) and nineteen co-substrates **C1–C19** (and no co-substrate). The array of 220 reactions was designed to enable formation of a diverse range of quinazolinones, some related to known analogues,[6] as well as other products of Pd-catalysed processes. The substrates were mainly analogues of **S1** (*e.g.* dehomologated, constrained), whilst the aniline and amine co-substrates bore structurally-diverse aryl, hetaryl and other substituents. To expand the possible reaction outcomes, some substrates and co-substrates bore alternative (*e.g.* **S2** and **S9**) or additional (*e.g.* **C6**, **C12** and **C16**) nucleophiles. The substrates and co-substrates were all inactive against *S. aureus* ATCC29213 at 50 μM. The array was performed in a 96-well OptiBlock and assembled from stock solutions using multi-channel pipettes. After 48 h at 105 °C, the crude reaction mixtures were filtered through silica, evaporated and dissolved in DMSO (total product concentration: 333 μM). Randomly-selected product mixtures (n = 23) were analysed by LC/MS; 16 of these contained carbonylated intermolecular products, 13 of which had been dehydrated. In addition, one product mixture (**S3** + **C1**) contained the product of an intramolecular reaction of **S3**, later shown to stem from a reductive Heck reaction[10] (see ESI†).

The crude reaction mixtures were screened in duplicate against *S. aureus* ATCC29213 (final total product concentration of 50 μM in 1% DMSO in ISB) (Fig. 2). Six of the 220 reactions displayed significant antibacterial activity in both replicate cultures: substrate **S1** (with **C1** or **C18**); substrate **S7** (with **C18**); and substrate **S10** (with **C1**, **C6** or **C18**). Reassuringly, the combination of **S1** and **C1** had been previously validated in our method development work (Table 1). The remaining five hit reactions were scaled fifty-fold, and the products purified by mass-directed HPLC (Table 2). In each case, quinazolinones with an extended hydrophobic substituent in the 2-position, and a *meta*-substituted phenyl group in the 3-position, were obtained. The products had MIC values

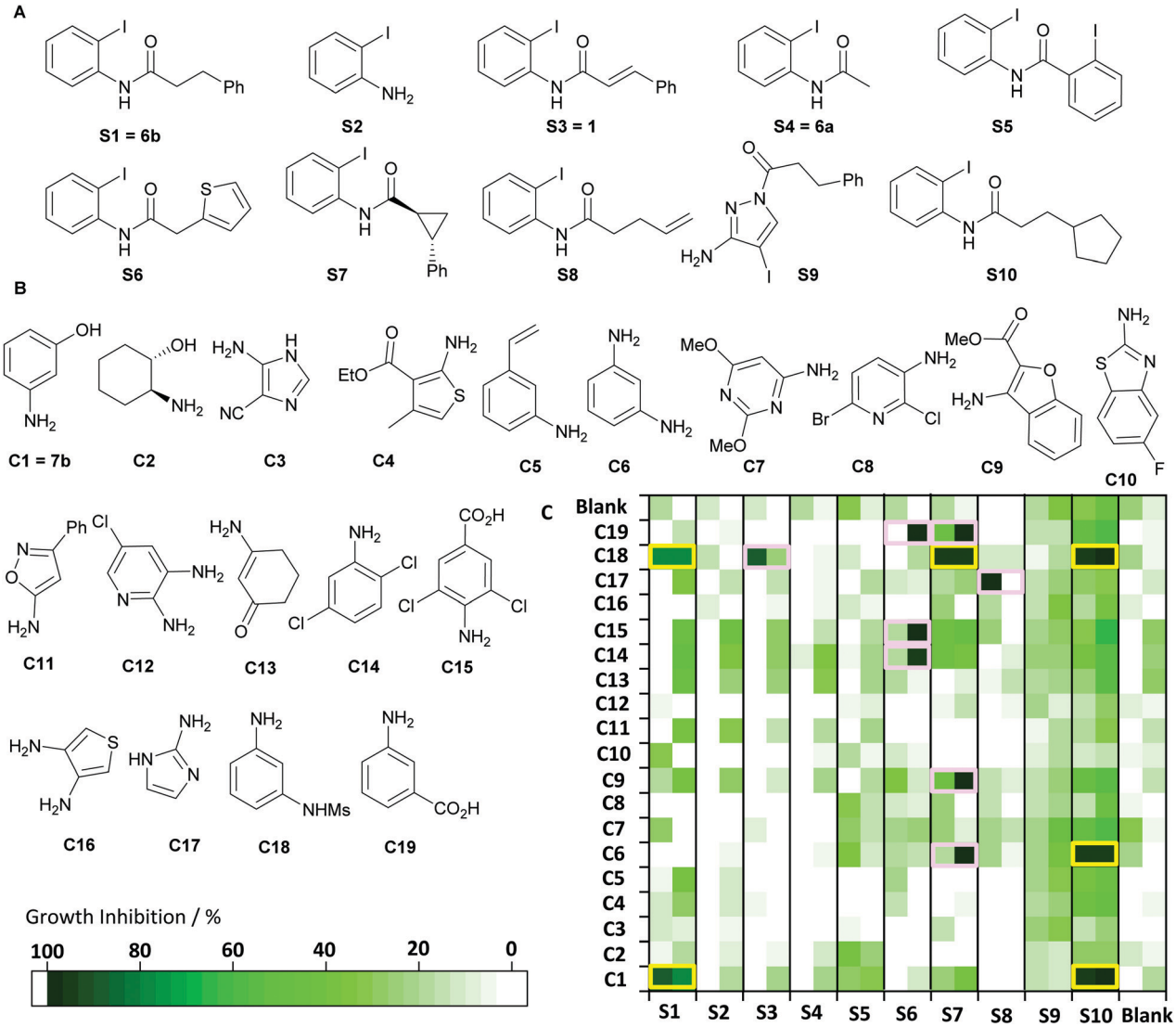


Fig. 2 Activity-directed antibacterial discovery (see Table 1 for reaction conditions). Panels A and B: Structures of substrates (A) and co-substrates (B). Panel C: Activity against *S. aureus* ATCC29213 (total product concentration: 50 μM), with active combinations that were scaled up shown (yellow). Product mixtures that displayed activity against only one colony (pink) were re-assayed, but none of these combinations were validated as hits (see ESI†).

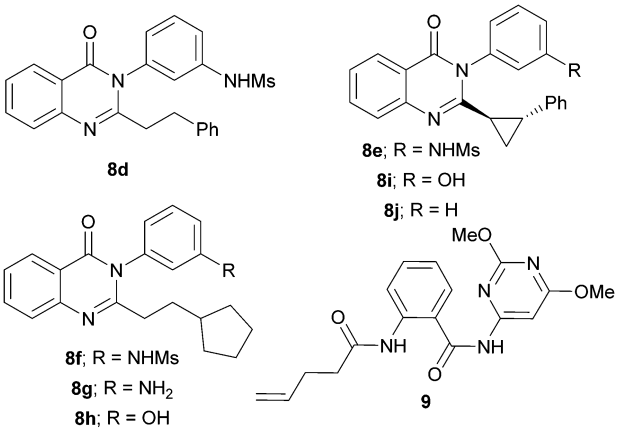
against *S. aureus* ATCC29213 ranging from 0.016 μg mL^{-1} (*i.e.* 38 nM) for **8d** to 2–4 μg mL^{-1} (*i.e.* 6–12 μM) for **8g**. Notably, **8d** was ~10-fold more active than the positive control[6a] **8c**. The products **8d–h** had comparable activity against the methicillin-resistant USA300 JE2 strain, but were significantly less active against laboratory strain SH1000. In addition, we purified the products of three reactions that were not identified as hits: **S7** + **C1**; **S7** + aniline; and **S4** + **C9**; two of these reactions also yielded quinazolinones, and the third an uncyclised amide **9**, and, crucially, all of these products displayed significantly lower (or no detectable) antibacterial activity.

Our experiments enabled the SAR of the quinazolinones to be significantly expanded (Fig. 3).[6] We found that a $-CH_2CH_2-$ linker (as in **8d** and **8f–h**) could be replaced by a *trans*-disubstituted cyclopropane (as in **8e**); however, reactions involving dehomologated (*e.g.* **S6**) or truncated (*e.g.* **S4**) substrates, or those offering alternative reaction pathways (*e.g.* **S2**, **S3**, **S5**, **S8** and **S9**), did not yield antibacterial products. In some cases, the *meta* OH or NHMs group (in **8f** and **8h**) could be replaced by a NH_2 group (in **8g**); however, none of the co-substrates that lacked such a *m*-substituted phenyl group ever yielded antibacterial products. Crucially, the products of reactions that had not been identified as hits were substantially less (or not) active. Although the quinazolinone **8i** is matched pair of **8a**, **8e** and **8h** (MICs: 0.5–2 μg mL^{-1}), it is significantly less active (MIC: 4–8 μg mL^{-1}). The quinazolinones **8j** and the amide **9** were not active, even at 128 μg mL^{-1}.

In conclusion, we have shown that ADS is feasible with a novel reaction class (Pd-catalysed chemistry) and with a phenotypic assay (rather than, as previously,[3] a biophysical assays). An array of 220 reactions enabled efficient exploration of the function of products based on several alternative product scaffolds. Based on our analysis of reaction outcomes by LC-MS, and the range of substrates used, we estimate that most of these reactions productively yielded previously unexplored

Table 2 Scale-up of reactions and evaluation of the activity of products against three *S. aureus* strains (ATCC29213, USA300 JE2, SH1000). The range of MICs observed obtained in duplicate on three different days is shown for each strain. Penicillin G displayed MIC values as expected in all experiments

			MIC (μg mL^{-1})		
Hit?	Substrates	Product[a] (yield[b])	ATCC29213	USA300 JE2	SH1000
✓	**S1, C1**	**8b** (19%)	0.5–1	0.5	4
✓	**S1, C18**	**8d** (4%)	0.016	0.016	0.5–1
✓	**S7, C18**	**8e** (2%)	1–2	2	32–64
✓	**S10, C1**	**8f** (8%)	1	0.5–1	4–8
✓	**S10, C6**	**8g** (8%)	2–4	2–4	16
✓	**S10, C18**	**8h** (14%)	0.5	0.5	1–2
✘	**S7, C1**	**8i** (21%)	4–8	4–8	16
✘	**S4, C9**	**9** (5%)	>128	64	32–64
✘	**S7**, aniline	**8j** (18%)	>128	>128	>128



[a] Co-Substrate (1.2 eq.), $Mo(CO)_6$ (1 eq.), 0.1 mol% Pd_2bda_3, 0.6 mol% $P(^tBu_3)\cdot HBF_4$, NEt_3, *o*-xylene. [b] After mass-directed HPLC. All compounds were also screened against yeast (*Candida albicans*, Ca6) and were found to be inactive at 16 μg mL^{-1}.

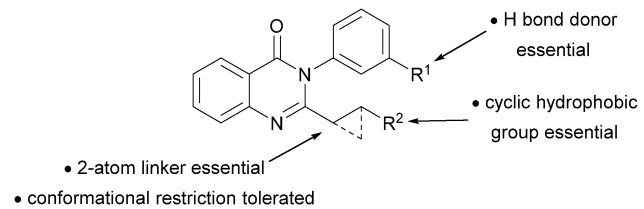


Fig. 3 SAR expanded using ADS.

products. Scale-up of the identified hit reactions, and purification, yielded a range of antibacterial quinazolinones and, thereby, expansion of their SAR. We envisage that, by harnessing alternative reaction classes to explore diverse chemical space, ADS may be exploited in the discovery of novel series of antimicrobials not subject to existing antibiotic resistance mechanisms.[11]

We thank EPSRC (EP/N025652/1) for funding and Luiza Galarion, Merianne Mohamad and Adam Green for useful discussions.

Conflicts of interest

There are no conflicts to declare.

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