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Synthesis of 3-acyltetramates by side chain manipulation and their antibacterial activity†

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An efficient approach for the introduction of 3-acyl side chain groups onto a core tetramate system, which are suitable for further manipulation by nucleophilic displacement or Horner–Wadsworth–Emmons coupling, provides access to a diverse library of substituted tetramates related to two distinct classes of natural products, equisetin and pramanicin. Assessment against *S. aureus* and *E. coli* indicated that some compounds exhibit significant antibacterial activity, providing unusual leads for further optimisation in the drug discovery process.

3-Acyltetramic acids **1** are core structural skeletons found in a wide range of natural products, exhibiting diverse biological activity.[1–3] Several are of interest for their antibacterial activity, notable examples being equisetin,[4] reutericyclin,[5] kibdelomycin,[6] and streptolodygin,[7] and significant progress in the development of synthetic routes to such systems[8] and their analogues has recently been made.[3] Interestingly, methodology for the general preparation of β-tricarbonyl systems is scarce,[9] and we have recently reported methodology providing access to highly substituted 3-acyltetramates, which relies upon Dieckmann cyclisation of templates **2** derived from serine,[10] threonine[11] or cysteine,[12] the chemoselectivity of which can be controlled by judicious use of reaction conditions and substituents to give products **3** and/or **4** (Scheme 1).[13] Moreover, we have established a reliable approach for the introduction of diverse 3-acyl groups into either of **3** or **4**,[14] and this permits rapid generation of chemical diversity around the core tetramate scaffold. In some cases, these compounds possess potent antibacterial activity,[15,16] even though the core tetramate system itself is generally devoid of such activity.[17] Aiming to further extend this approach, we report here a modification of the strategy which permits selective and efficient 3-acyl side chain manipulation in tetramate systems, thereby permitting wider access to libraries of modified derivatives. Interest in the total synthesis of naturally occurring antibiotics and their analogues is increasing given the recognition of the urgent need for new generations of antibiotics.[18–23]

Key to the approach is the successful extension of our *O*-acylation/rearrangement procedure, which had been found to be most effective for aromatic carboxylic acids, for the introduction of substituted acyl derivatives;[14] the successful rearrangement is readily seen by disappearance of the enol acetate signal at about δ 6.3 once the *C*-acyl product is formed. Thus, treatment of tetramate **3** (R^1 = H; X = O) with any of acetic acid, 4-pentenoic acid, chloroacetic acid and bromoacetic acid with DCC–DMAP gave the products **5a–d** in good yield (Scheme 2), although reaction with vinylacetic acid gave a complex product mixture. Assignment of **5a** existing in fact as the major tautomer **6b** was made by ^{13}C NMR analysis in which the respective tautomers could be readily identified by down-shifted carbonyl signals (**6a**, C(6) 195 ppm, C(8) 172 ppm; **6b**, C(6) 188 ppm, C(8) 180 ppm, assigned by analogy to earlier work[14]) as a result of deshielding from H-bonding and we assume that the other analogues are the same; detailed analysis of related systems has indicated that such *exo*-enol forms generally predominate in these tetramate systems.[14,16] Successful access to these systems provided opportunity for side chain extension, and by way of exemplification, **5a** when treated with aromatic aldehydes in the presence of mild base (piperidine) gave the enamine products **7a–c** in good yields. The newly formed *trans*-double bond geometry was evident from the large coupling constant (*ca.* 16 Hz) and the *exo*-enamine tautomeric form is assumed from the above analysis, although the geometry about this double bond was not determined. Of interest is that these enamines could not be hydrolysed under acidic conditions (aq. HCl), as might have been expected, but instead required alkaline conditions (LiOH,

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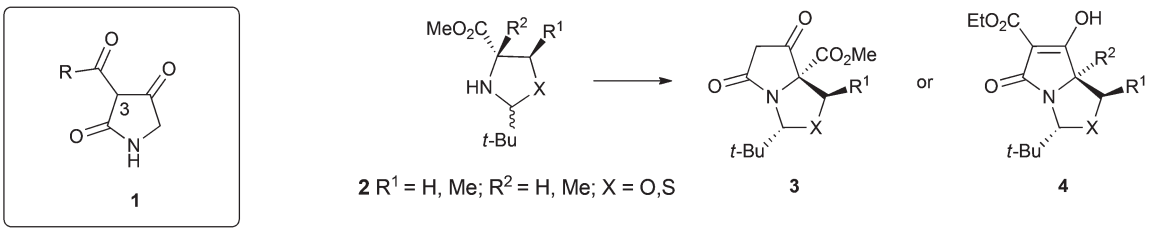
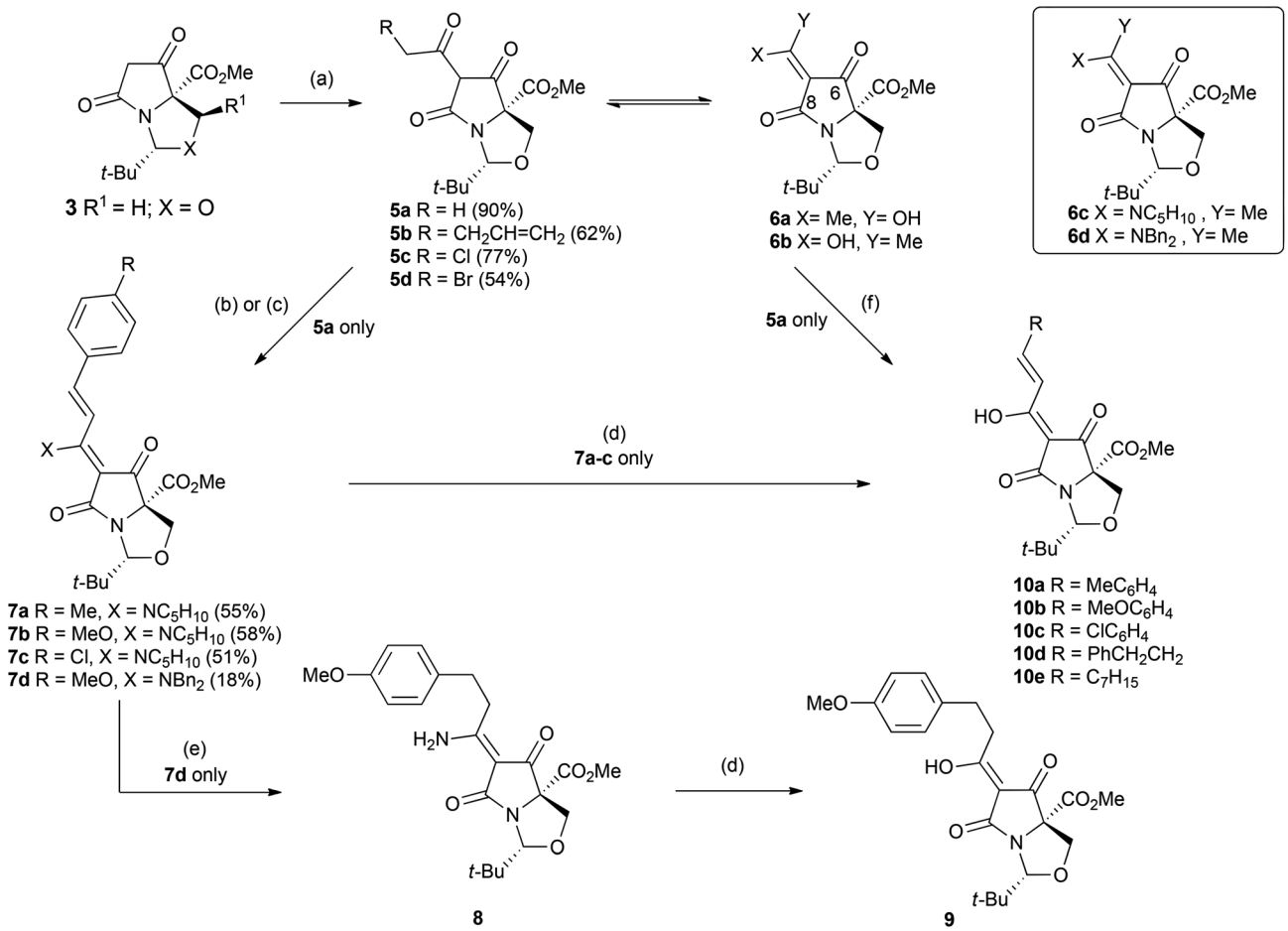
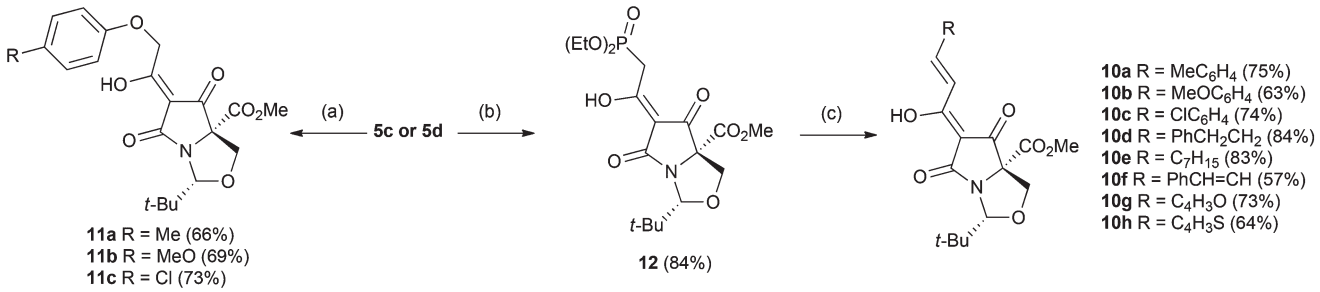


Scheme 1



Scheme 2 (a) $RCH_2C(O)OH$ (1.1 eq.), DCC (1.1 eq.), DMAP (0.05 eq.), CH_2Cl_2, r.t.; (b) RC_6H_4CHO, $C_5H_{10}NH$, toluene, reflux; (c) $MeOC_6H_4CHO$, Bn_2NH, toluene, reflux; (d) LiOH (1.0 eq.), THF–H_2O (1 : 1), r.t., 1.5 h; (e) NH_4CO_2H, 10% Pd/C, MeOH, reflux, 28 h; (f) LDA (2.1 eq.) then RCHO.

THF, H_2O), giving the enone products **10a–c** in excellent yield (93, 100 and 89%, respectively); presumably this process occurs by an addition-elimination reaction. In an alternative process, condensation of acetyl derivative **5a** with *p*-methoxybenzaldehyde in the presence of dibenzylamine gave a mixture of enamines **7d** and **6d** (each 18%), the former of which could be converted to enone product **9** in a two-stage process consisting of hydrogenolysis to **8** and subsequent alkaline hydrolysis (Scheme 2, enamine and enol geometry of **7**, **8**, **9** and **10** not unequivocally assigned); the isolated product was consistent with structure **9**, as indicated by LRMS and ^{1}H NMR spectrum (absence of enamine signals near 10 ppm). The identity of intermediate **8** was made from the presence of a peak at 415 *m*/*z* (ESI^-; corresponding to $[M - H]^-$) by LRMS analysis, and critically the ^{1}H NMR showed broad signals near 10 ppm, assumed to be the enamine $-NH_2$ protons. Of interest was that enamine **6d** isolated from this process could be further condensed with more *p*-methoxybenzaldehyde, to give enamine **7d**, in a conversion of 71% (based on ^{1}H NMR analysis), suggesting that the earlier reactions leading to **7a–c** proceeded by the initial formation of enamine **6c**, rather than by formation of the enolate of **5a**. Although this process is only applicable to non-enolisable aldehydes, it was nonetheless more efficient than a single-step one involving formation of the dianion by deprotonation of acetyl **5a** with excess LDA to generate the dianion and reaction with aldehydes, which

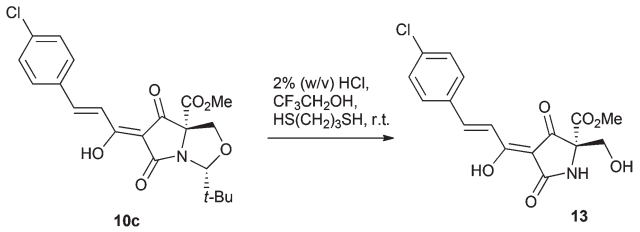


Scheme 3 (a) RC_6H_4OH, Cs_2CO_3, MeCN, r.t.; (b) NaH, then $(EtO)_2P(O)H$, THF; (c) *t*BuOK (2.1 eq.), THF, 0 °C, RCHO.

returned only 12, 14, 31, 11 and 18% yield of enones **10a–e**, respectively (Scheme 2).

Moreover, side chain manipulation of chloro- and bromo-derivatives by nucleophilic displacement also proved to be readily feasible. Thus, displacement of the halogen in chloroacetyl **5c** with phenols gave ethers **11a–c** very efficiently (Scheme 3) and this approach was readily extendible to reaction using bromoacetyl **5d** with diethyl phosphite, to give phosphorane **12** in excellent yield. For this compound **11c**, poor NMR spectral resolution was found in both $CDCl_3$ and CD_2Cl_2 as solvents, but detailed analysis by COSY-EXSY demonstrated that this was due to the presence of a tautomeric mixture. Condensation of the anion of phosphonate **12**, generated by treatment with *t*-butoxide, with a series of aldehydes (Scheme 3), gave excellent yields of the enone products **10a–h**, a process which was significantly more efficient than those involving direct condensation and shown in Scheme 2. Attempted deprotection of these systems, however, *via* Corey–Reichard protocol[24] proved to be problematic; for example, reaction of **10c** under conditions which we have previously found to be universally successful in related substrates,[25–27] gave a low and irreproducible yield of the expected tetramate **13** (Scheme 4). In addition, use of other acidic conditions such as THF–H_2O–formic acid (3 : 1 : 1)[28] or $BiBr_3$–MeCN with catalytic H_2O[29] resulted in no conversion of the oxazolidine. We believe that this unexpected behaviour is due to unusual structural features in these rigid and highly functional systems,[30] and it is also worth highlighting that all tetramic acids with the exocyclic enol isolated *via* silica gel column chromatography are subsequently washed with dilute HCl to remove chelated metal cations in order to give a well resolved ^1H NMR spectra with distinct resonance signals; that this is necessary to obtain pure samples has been reported previously.[3,9,31]

These compounds could be considered to be hybrid mimics of pramanicin and equisetin, and when assayed against *S. aureus* and *E. coli*, the antibacterial profile shown in Table 1 was obtained. The phenotypic assay used here automatically selects for cell penetration activity, a property now recognised as being difficult to re-introduce into candidates identified by target screening approaches, for example.[32] Although the parent systems **5a–d** and ether derivatives **11a–c** showed no activity of any significance, some of the enamines (**7a** and **7c**) and most of the enones (**10a**, **10c–f** and **10h**), and



Scheme 4

especially those with long hydrophobic side chains, showed potent activity against *S. aureus*, although not against *E. coli*; this Gram-positive selectivity appears to be typical for tetramate systems.[15,17] Correlation with cheminformatic descriptors[33,34] suggests that enamine compounds with *c* log *P* values in the range 4.1–4.8 and %PSA values of about 11%, and enones with values of 2.3–4.7 and 14–20% exhibit optimal bioactivity, and this is similar behaviour to other tetramate libraries.[12,15,35] Although members of the library have a low hydrogen bond donor count (HBD), many of the most active (*e.g.* **5b**, **7c**, **10a**, **10c–f**, **10h**) have high numbers of rotatable bonds (5–10). These calculated physicochemical properties[36] are acceptable for good oral absorption as described by Lipinski,[37] although they do not match the physicochemical characteristics of existing antibacterial classes.[19]

The antibacterial activity observed for tetramic acids **10a–h** is consistent with the known antibacterial activity of natural products possessing a tetramic acid skeletal core. However, the target responsible for the observed phenotypic activity still remains to be elucidated; our recent work has established that similar tetramic acids with pendant 3-acyl groups act as bacterial RNA polymerase (RNAP) and/or UPPS inhibitors,[15] while similar systems have been reported either to have quorum sensing activity,[38] interfering with the bacterial proton gradient and membrane potential *via* non-specific interactions, or siderophoric activity.[39] Noteworthy, though, is that some 3-acyltetramic acids (analogous to **10**) reported in these studies showed selectivity for bacterial cells over mammalian cells, suggesting the potential of such compounds to be developed for use as therapeutic agents for human use.

This work has shown that hybrid mimics of two related but distinct antimicrobial natural products, equisetin and the pramanicin (Fig. 1), comprising the tetramate core of the former[4]

Table 1 Antibacterials bioactivity and cheminformatic parameters for selected compounds

Compound	Conc. (mg ml^{-1})	Bioactivity (mm)		*c* log *P*	*c* log $D_{7.4}$	PSA	MSA	CMR	%PSA	HBD	rotB
		SA	EC								
5a	4	NA	NA	1.22	0.32	93.1	649.9	110.1	14.3	1	3
	2	NA	NA								
	1	NA	NA								
5b	4	20	13	2.33	1.29	93.1	496.6	86.2	18.8	1	6
	2	19	NA								
	1	18	NA								
5c	2	NA	NA	1.53	−1.11	93.1	448.2	77.0	20.8	1	4
	1	NA	NA								
	0.5	NA	NA								
5d	2	NA	NA	1.71	−0.64	93.1	451.7	80.0	20.6	1	4
	1	NA	NA								
	0.5	NA	NA								
7a	4	19	16	4.74	4.74	76.2	712.5	131.5	10.7	0	6
7b	4	NA	14	4.07	4.07	85.4	729.4	133.0	11.7	0	7
7c	4	28	15	4.83	4.82	76.2	697.7	131.3	10.9	0	6
7d	4	NA	NA	6.67	6.67	85.4	879.6	170.0	9.7	0	11
6c	4	11	NA	2.25	2.25	76.2	570.2	96.3	13.3	0	4
10a	4	25	NA	3.71	3.01	93.1	574.1	107.7	16.2	1	5
	2	23	—								
10b	2	NA	NA	3.04	2.20	102.4	589.6	109.1	17.4	1	6
	1	NA	NA								
	0.5	NA	NA								
10c	4	33	15	3.80	2.76	93.1	558.4	107.4	16.7	1	5
	1	29	13								
	0.5	19	NA								
10d	2	30	14	4.00	3.52	93.1	603.5	111.8	15.4	1	7
	1	28.5	NA								
	0.5	25	NA								
10e	2	35	NA	4.65	4.16	93.1	650.0	110.1	14.3	1	10
	1	35	NA								
	0.5	33	NA								
10f	2	30.5	14	3.72	3.08	93.1	574.2	112.9	16.2	1	6
	1	28	NA								
	0.5	25	NA								
10g	2	13	NA	2.26	0.97	106.3	513.4	95.0	20.7	1	5
	1	NA	NA								
	0.5	NA	NA								
10h	2	25	13.5	3.11	2.10	93.1	521.8	99.5	17.8	1	5
	1	20	NA								
	0.5	13.5	NA								
11a	2	NA	NA	3.02	0.13	102.4	588.7	103.3	17.4	1	6
	1	NA	NA								
	0.5	NA	NA								
11b	2	NA	13.5	2.35	−0.56	111.6	604.6	104.7	18.5	1	7
	1	NA	12.5								
	0.5	NA	NA								
11c	2	NA	14	3.11	−0.04	102.4	572.5	103.1	17.9	1	6
	1	NA	NA								
	0.5	NA	NA								
12	2	NA	NA	0.83	−2.0	128.7	641.3	101.5	20.1	1	9
	1	NA	NA								
	0.5	NA	NA								

NA = not active; SA = *S. aureus*; EC = *E. coli*; PSA = polar surface area; MSA = molecular surface area; CMR = calculated molar refractivity; %PSA = (PSA/MSA) × 100; HBD = H-bonds donor; rotB = number of rotatable bonds.

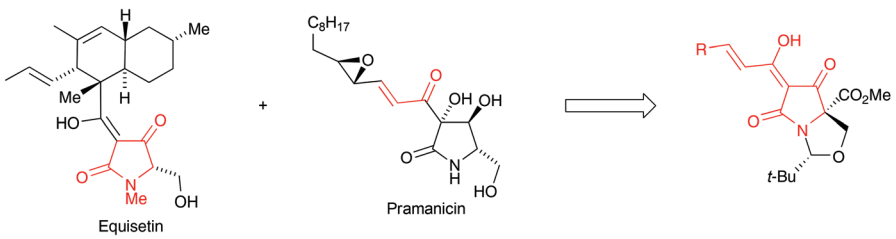


Fig. 1 Relationship of natural products with the tetramate library.

and the enoyl side chain of the latter,[40,41] are readily accessible, and may exhibit high levels of antibacterial activity. It confirms our earlier results which indicate that small ring lactams devoid of side chain functionalization exhibit no or only modest activity, but that the introduction of longer chain appendages can significantly enhance such activity.[17] These results serve to illustrate the validity of a recent call for the greater use of natural products in the drug discovery process,[42,43] particularly of antibiotics,[44] and provide one illustration of non-planar heterocyclic systems[45] suitable for "escaping from flatland"[46,47] of particular relevance to new antibacterial drug candidates.[48]

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References

1 B. J. L. Royles, *Chem. Rev.*, 1995, **95**, 1981–2001.
2 R. Schobert and A. Schlenk, *Bioorg. Med. Chem.*, 2008, **16**, 4203–4221.
3 B. Barnickel, F. Bayliffe, R. Diestel, K. Kempf, S. Laschat, S. Pachali, F. Sassec, A. Schlenk and R. Schobert, *Chem. Biodiversity*, 2010, **7**, 2830–2845.
4 S. B. Singh, D. L. Zink, M. A. Goetz, A. W. Dombrowski, J. D. Polishook and D. J. Hazuda, *Tetrahedron Lett.*, 1998, **39**, 2243–2246.
5 R. Böhme, G. Jung and E. Breitmaier, *Helv. Chim. Acta*, 2005, **88**, 2837–2841.
6 J. Phillips, M. Goetz, S. Smith, D. Zink, J. Polishook, R. Onishi, S. Salowe, J. Wiltsie, J. Allocco, J. Sigmund, K. Dorso, S. Lee, S. Skwish, M. d. l. Cruz, J. Martín, F. Vicente, O. Genilloud, J. Lu, R. Painter, K. Young, K. Overbye, R. Donald and S. Singh, *Chem. Biol.*, 2011, **18**, 955–965.
7 S. Tuske, S. G. Sarafianos, X. Wang, B. Hudson, E. Sineva, J. Mukhopadhyay, J. J. Birktoft, O. Leroy, S. Ismail, A. D. Clark, C. Dharia, A. Napoli, O. Laptenko, J. Lee, S. Borukhov, R. H. Ebright and E. Arnold, *Cell*, 2005, **122**, 541–552.
8 E. V. Prusov, *Appl. Microbiol. Biotechnol.*, 2013, **97**, 2773–2795.
9 E. Teli-Kokalari, V. Stefanou, D. Matiadis, G. Athanasellis, O. Igglessi-Markopoulou, S. Hamilakis and J. Markopoulos, *Fresenius Environ. Bull.*, 2012, **21**, 3215–3223.
10 M. D. Andrews, A. G. Brewster, K. M. Crapnell, A. J. Ibbett, T. Jones, M. G. Moloney, K. Prout and D. Watkin, *J. Chem. Soc., Perkin Trans. 1*, 1998, 223–235.
11 M. Anwar, A. R. Cowley and M. G. Moloney, *Tetrahedron: Asymmetry*, 2010, **21**, 1758–1770.
12 M. Anwar and M. G. Moloney, *Chem. Biol. Drug Des.*, 2013, **81**, 645–649.
13 Y.-C. Jeong, M. Anwar, T. M. Nguyen, B. S. W. Tan, C. L. L. Chai and M. G. Moloney, *Org. Biomol. Chem.*, 2011, **9**, 6663–6669.
14 Y.-C. Jeong and M. G. Moloney, *J. Org. Chem.*, 2011, **76**, 1342–1354.
15 Y.-C. Jeong, M. Anwar, M. G. Moloney, Z. Bikadi and E. Hazai, *Chem. Sci.*, 2013, **4**, 1008–1015.
16 Y.-C. Jeong and M. G. Moloney, *Beilstein J. Org. Chem.*, 2013, **9**, 1899–1906.
17 Y.-C. Jeong and M. G. Moloney, *Synlett*, 2009, 2487–2491.
18 L. L. Silver, *Expert Opin. Drug Discovery*, 2008, **3**, 487–500.
19 R. O'Shea and H. E. Moser, *J. Med. Chem.*, 2008, **51**, 2871–2878.
20 D. J. Payne, M. N. Gwynn, D. J. Holmes and D. L. Pompliano, *Nat. Rev. Drug Discovery*, 2007, **6**, 29–40.
21 F. vonNussbaum, M. Brands, B. Hinzen, S. Weigand and D. Habich, *Angew. Chem., Int. Ed.*, 2006, **45**, 5072–5129.
22 P. Fernandes, *Nat. Biotechnol.*, 2006, **24**, 1497–1503.
23 A. J. O'Neill and I. Chopra, *Expert Opin. Invest. Drugs*, 2004, **13**, 1045–1063.
24 E. J. Corey and G. A. Reichard, *J. Am. Chem. Soc.*, 1992, **114**, 10677–10678.
25 M. Anwar, J. H. Bailey, L. Dickinson, H. Edwards, R. Goswami and M. G. Moloney, *Org. Biomol. Chem.*, 2003, **1**, 2364–2376.
26 P. W. H. Chan, I. F. Cottrell and M. G. Moloney, *J. Chem. Soc., Perkin Trans. 1*, 2001, 3007–3012.
27 J. Dyer, S. Keeling, A. King and M. G. Moloney, *J. Chem. Soc., Perkin Trans. 1*, 2000, 2793–2804.
28 J. S. Panek and C. E. Masse, *J. Org. Chem.*, 1998, **63**, 2382–2384.
29 X. Cong, F. Hu, K.-G. Liu, Q.-J. Liao and Z.-J. Yao, *J. Org. Chem.*, 2005, **70**, 4514–4516.
30 B. S. W. Tan, C. L. L. Chai and M. G. Moloney, *unpublished results*.
31 M. Sodeoka, R. Sampe, S. Kojima, Y. Baba, N. Morisaki and Y. Hashimoto, *Chem. Pharm. Bull.*, 2001, **49**, 206–212.
32 J. A. Lee, M. T. Uhlik, C. M. Moxham, D. Tomandl and D. J. Sall, *J. Med. Chem.*, 2012, **55**, 4527–4538.
33 *Marvin 5.3.8*, ChemAxon, 2010 (http://www.chemaxon.com).
34 A. Ganesan, *Curr. Opin. Biotechnol.*, 2008, **12**, 306–317.
35 C. A. Holloway, C. J. Matthews, M. G. Moloney, C. F. Roberts and M. Yaqoob, *Chem. Biol. Drug Des.*, 2011, **78**, 229–235.
36 Bioassay of products:[49–51] Microbiological assays were performed by the hole-plate method with the test organism *Staphylococcus aureus* N.C.T.C. DS267 or *Escherichia coli* X580. Solutions (100 μl) of the compounds to be tested (4, 2, 1 and 0.5 mg ml^{-1}) were loaded into wells in bioassay plates, and incubated overnight at 37 °C. The diameters of the resultant inhibition zones were measured (±0.5 mm).
37 C. A. Lipinski, F. Lombardo, B. W. Dominy and P. J. Feeney, *Adv. Drug Delivery Rev.*, 2001, **46**, 3–26.
38 C. A. Lowery, J. Park, C. Gloeckner, M. M. Meijler, R. S. Mueller, H. I. Boshoff, R. L. Ulrich, C. E. Barry,

D. H. Bartlett, V. V. Kravchenko, G. F. Kaufmann and K. D. Janda, *J. Am. Chem. Soc.*, 2009, **131**, 14473–14479.
39 G. F. Kaufmann, R. Sartorio, S.-H. Lee, C. J. Rogers, M. M. Meijler, J. A. Moss, B. Clapham, A. P. Brogan, T. J. Dickerson and K. D. Janda, *Proc. Natl. Acad. Sci. U. S. A.*, 2005, **102**, 309–314.
40 S. Aoki, T. Tsukude, Y. Miyazaki, K. Takao and K. Tadano, *Heterocycles*, 2006, **69**, 49–54.
41 R. E. Schwartz, G. L. Helms, E. A. Bolessa, K. E. Wilson, R. A. Giacobbe, J. S. Tkacz, G. F. Bills, J. M. Liesch, D. L. Zink, J. E. Curotto, B. Pramanik and J. C. Onishi, *Tetrahedron*, 1994, **50**, 1675–1686.
42 S. Danishefsky, *Nat. Prod. Rep.*, 2010, **27**, 1114–1116.
43 A. Ganesan, *Curr. Opin. Biotechnol.*, 2004, **15**, 584–590.
44 D. G. Brown, T. Lister and T. L. May-Dracka, *Bioorg. Med. Chem. Lett.*, 2014, **24**, 413–418.
45 W. R. Pitt, D. M. Parry, B. G. Perry and C. R. Groom, *J. Med. Chem.*, 2009, **52**, 2952–2963.
46 F. Lovering, J. Bikker and C. Humblet, *J. Med. Chem.*, 2009, **52**, 6752–6756.
47 T. J. Ritchie and S. J. F. MacDonald, *Drug Discovery Today*, 2009, **14**, 1011–1020.
48 M. S. Butler, *J. Nat. Prod.*, 2004, **67**, 2141–2153.
49 B. Smith, S. C. Warren, G. G. F. Newton and E. P. Abraham, *Biochem. J.*, 1967, **103**, 877–890.
50 J. E. Baldwin, J. B. Coates, J. Halpern, M. G. Moloney and A. J. Pratt, *Biochem. J.*, 1989, **261**, 197–204.
51 J. E. Baldwin, A. J. Pratt and M. G. Moloney, *Tetrahedron*, 1987, **43**, 2565–2575.