Organic &
Biomolecular
Chemistry

Cite this: *Org. Biomol. Chem.*, 2011, **9**, 659

www.rsc.org/obc

COMMUNICATION

New synthetic strategies towards psammaplin A, access to natural product analogues for biological evaluation†

Matthias G. J. Baud,[a] Thomas Leiser,[b] Franz-Josef Meyer-Almes[b] and Matthew J. Fuchter*[a]

Received 4th October 2010, Accepted 12th November 2010
DOI: 10.1039/c0ob00824a

New synthetic routes towards the natural product psammaplin A were developed with the particular view to preparing diverse analogues for biological assessment. These routes utilize cheap and commercially available starting materials, and allowed access to psammaplin A analogues not accessible *via* currently reported methods. Preliminary biological studies revealed these compounds to be the most potent non peptidic inhibitors of the enzyme histone deacetylase 1 (HDAC1, class I) discovered so far. Interestingly, psammaplin A and our synthetic analogues show class I selectivity *in vitro*, an important feature for the design and synthesis of future isoform selective inhibitors.

Psammaplin A (**1**, Fig. 1), is a member of a family of natural products isolated from several marine sponges including *Pseudoceratina purpurea*.[1a]

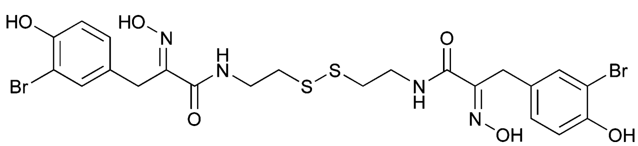


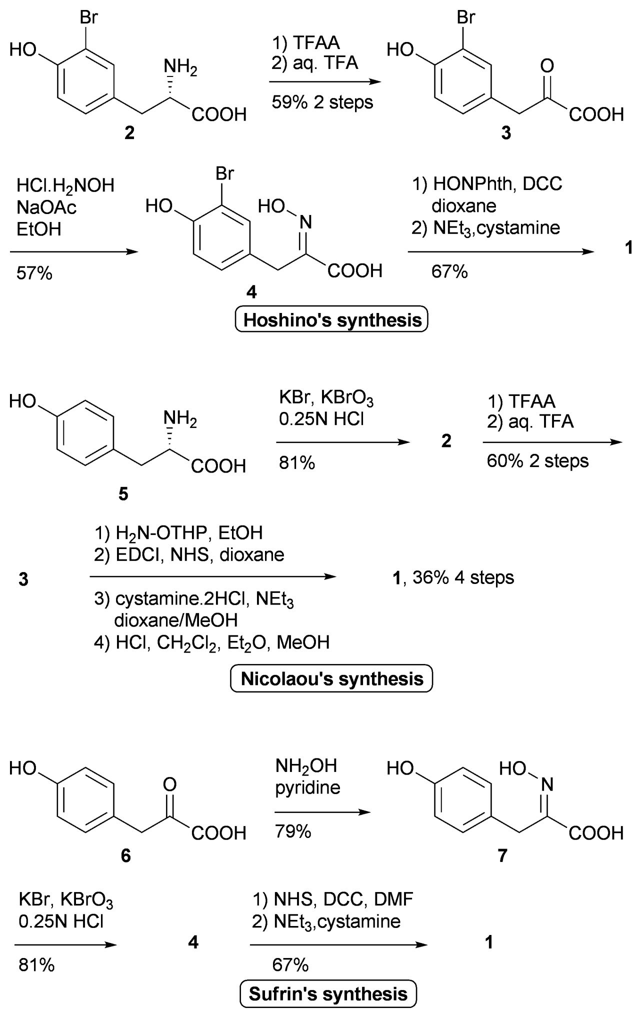
Fig. 1 Psammaplin A (**1**).

Numerous bromotyrosine derivatives have been isolated from these marine sponges,[1a–h] notably from sponges of the order Verongida, known to be a rich source of such metabolites.[2] Psammaplin A was structurally characterized in 1987,[1b–d] and represents the first example of a disulfide-containing metabolite isolated from a marine sponge. While it has been implicated as an inhibitor of numerous targets such as topoisomerase II,[3] DNA gyrase,[4] leucine aminopeptidase,[1g] farnesyl protein transferase,[1g] chitinase,[1f] mycothiol-S-conjugate amidase,[5] aminopeptidase N[6] and DNA polymerase α-primase,[7] studies by Crews and co-workers showed it to be an extremely potent enzymatic inhibitor of both histone deacetylases (HDACs) and DNA-methyltransferases (DNMTs).[1a] These enzymes play a crucial role in the epigenetic regulation of gene expression, and misregulation of their activity has been found to be involved in cancer pathogenesis.[8a–c] HDAC and DNMT enzymes therefore represent promising targets for the development of anticancer therapies. Indeed, there is increasing interest in epigenetic therapies, in part due to the success of DNMT and HDAC inhibitors, such as decitabine and vorinostat, in the clinic and their recent FDA approval for use in certain tumour types. *In vitro*, psammaplin A (and several other psammaplins), displayed potent activity against an HDAC cell extract (IC_{50} = 4.2 nM) and DNMT1 (IC_{50} = 18.6 nM). Subsequently, studies on its anti-proliferative properties have shown it to have significant cytotoxicity (ED_{50}, μg mL^{-1}) against human lung (A549, 0.57), ovarian (SK-OV-3, 0.14), skin (SK-MEL-2, 0.13), CNS (XF498, 0.57), and colon (HCT15, 0.68) cancer cell lines.[9] *In vivo*, it inhibited tumour growth in the A549 lung xenograph mouse model while maintaining low toxicity.[1a]

Our laboratory is currently involved in several medicinal chemistry projects, focusing on the modulation of enzymes involved in the epigenetic regulation of gene expression. We considered that access to diverse psammaplin A analogues would enable the establishment of structure–activity relationships (SARs) to explore its reported potency against HDAC and DNMT enzymes. To date, several total syntheses of psammaplin A have been reported[10–12] (Scheme 1), starting from tyrosine or phenylpyruvic acid derivatives, with minimal analogues reported. These syntheses suffer from the low commercial availability of tyrosine and phenypyruvic acid derivatives, notably with diverse aromatic substitution patterns. We therefore considered the development of alternative synthetic routes with improved substrate scope to allow the synthesis of biologically interesting analogues. In general, bromotyrosine derivatives represent a diverse class of marine natural products structurally related to psammaplin A (Fig. 2) and such synthetic procedures should facilitate their preparation and further study.[13,14]

To overcome the shortcomings of prior routes we considered the well known 2 step Erlenmeyer oxazolone synthesis–hydrolysis sequence as a viable route to a variety of arylpyruvic acids, which utilizes cheap and commercially available substituted benzaldehydes **11** as substrates (Scheme 2). Upon exposure to *N*-acetyl glycine and acetic anhydride in the presence of sodium acetate, aldehydes **11** were converted to oxazolones **12**. Further treatment with aqueous HCl afforded arylpyruvic acids **13**. The structurally diverse acids **13** generated were used as precursors to psammaplin A analogues following the previously reported

[a]*Imperial College London, South Kensington Campus, Department of Chemistry, London, UK SW7 2AZ. E-mail: m.fuchter@imperial.ac.uk*
[b]*University of Applied Sciences, Department of Chemical Engineering and Biotechnology, Schnittspahnstraβe 12, 64287 Darmstadt, Germany*
† Electronic supplementary information (ESI) available: Experimental procedures, biological assay procedures and full characterization for all new compounds. See DOI: 10.1039/c0ob00824a



Scheme 1 Reported syntheses of psammaplin A.

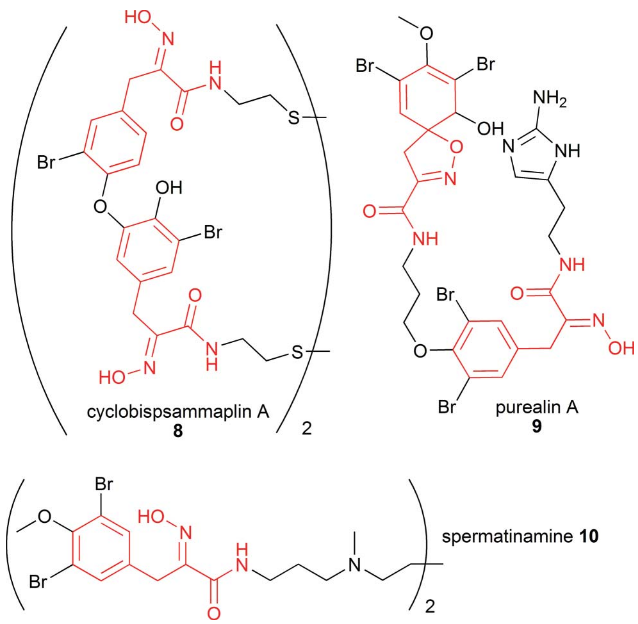
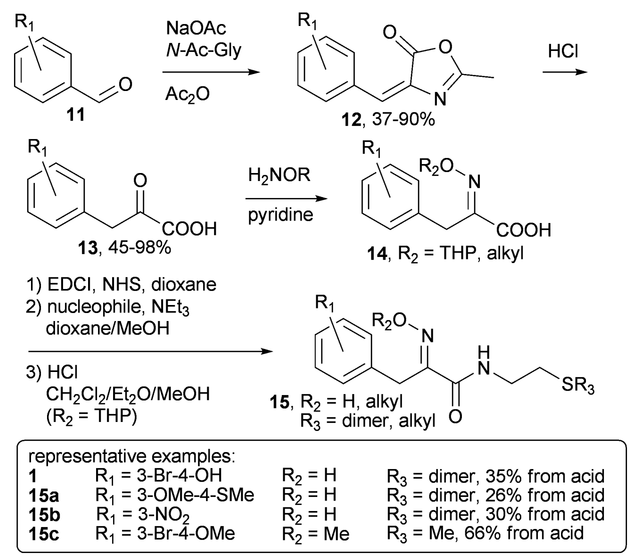


Fig. 2 Examples of bromotyrosine based natural products.



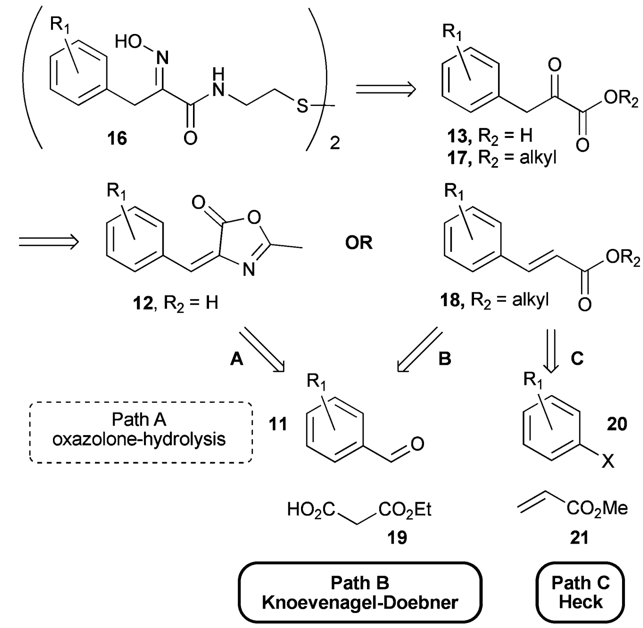
Scheme 2 Erlenmeyer oxazolone synthesis–hydrolysis path.

routes.[10–12] This sequence allowed us to synthesise psammaplin A and a collection of more than 70 psammaplin A analogues. Representative examples are given in Scheme 2.

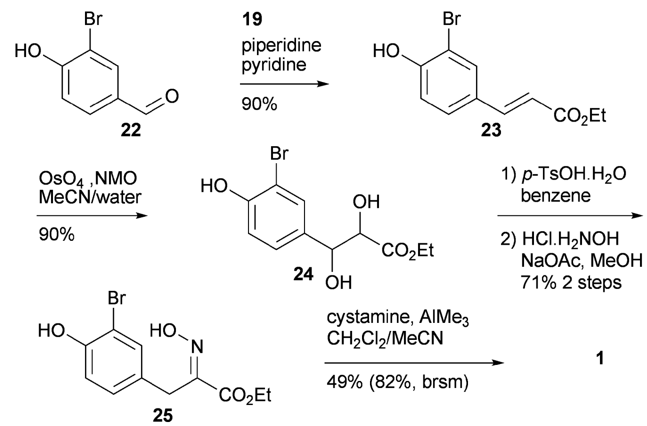
We faced several synthetic issues however when starting with electron rich benzaldehydes, such as *p*-dimethylaminobenzaldehyde. While the Erlenmeyer oxazolone synthesis is efficient in the case of electron poor aromatic aldehydes, low yields are generally obtained for electron rich substrates. Moreover, adjustment of the pH and isolation of the subsequent arylpyruvic acids can become difficult when the aromatic ring bears basic (*e.g.* amino) functionality. Finally, the strong acidic work-ups involved in the condensation and coupling steps of reported syntheses make these routes unsuitable in the case of compounds containing basic and/or acid-sensitive functionality.

To overcome these problems, we developed alternative routes to psammaplin A and analogues non-accessible *via* this procedure. Our retrosynthetic analysis is given in Scheme 3. Product **16** would be accessible by double amidation of ester **17** and condensation with hydroxylamine to introduce the oxime unit. Ester **17** would be accessible from unsaturated ester **18**, *via* dihydroxylation and regioselective dehydration. Unsaturated ester **18** would be obtained from either Knoevenagel–Doebner condensation between aromatic aldehyde **11** and 3-ethoxy-3-oxopropanoic acid **19**, or Heck coupling between aromatic halide **20** and methyl acrylate **21**. Such substrates are commercially available and cheap reagents.

Knoevenagel–Doebner condensation between **22** and 3-ethoxy-3-oxopropanoic acid **19** afforded unsaturated ester **23** in excellent yield (Scheme 4). The latter was converted in high yield to diol **24** with osmium tetroxide in a water–acetonitrile mixture. Regioselective dehydration of diol **24** with catalytic amounts of *p*-TsOH in refluxing benzene, followed by condensation with hydroxylamine afforded ester **25** in good yield after 2 steps, as a single isomer. Compound **25** has been previously reported in the literature during the synthesis of the natural product verongamine by Spilling *et al.*,[15] and its structure confirmed by X-ray crystallography.[16] Comparison of our data with that reported matched perfectly. This protecting-group free sequence employs mild reaction conditions, and represents a considerable advantage compared to the use Nakamura's α-OTBS-protected



Scheme 3 Retrosynthetic analysis.

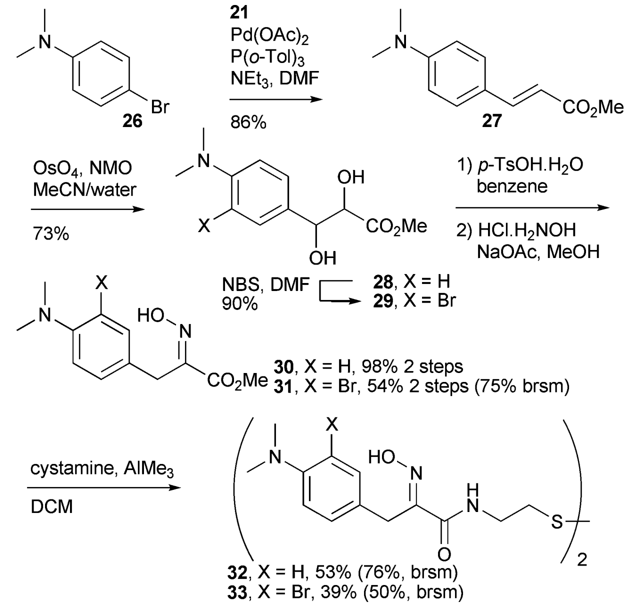


Scheme 4 Newly developed synthetic route.

dimethylphosphonate in a Horner–Wadsworth–Emmons reaction with aromatic aldehydes.[14,17,18]

Heating ester **25** in the presence of 0.5 equivalents of cystamine in methanol led to no reaction after extended periods of time, despite the successful application of these conditions to the synthesis of the natural product verongamine.[15] Instead the best results were obtained in the presence of trimethylaluminium, however the choice of the solvent system, reaction time and quantity of aluminium reagent were found to be crucial. Indeed, relatively apolar organic solvents, such as CH_2Cl_2 and $CHCl_3$, led to no reaction or very low yields respectively. This was principally attributed to low substrate solubility. Solvent polarity was therefore varied and the use of a CH_2Cl_2–MeCN mixture allowed the formation of psammaplin A in good yield.

This synthetic route was successfully employed for the synthesis of analogues **32** and **33** (Scheme 5). Unsaturated ester **27** was prepared *via* a Heck reaction between 4-bromo-*N*,*N*-dimethylbenzenamine **26** and methyl acrylate **21**. Dihydroxylation afforded diol **28** in good yield. We were pleased to observe quantitative formation of ester **30** in 2 steps. The regioselective



Scheme 5 Synthesis of **32** and **33**.

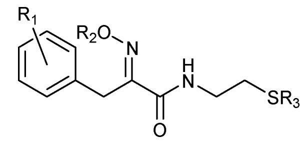
dehydration was thought to be particularly efficient in the case of electron-rich aromatics, due to increased stabilization of the positively charged benzylic position involved in the elimination process. Amidation as before afforded **32** in good yield.

Analogue **33** was obtained following a similar sequence (Scheme 5). Bromination of diol **28**, followed by dehydration–condensation and final amidation afforded **33** in moderate yield. The reaction conditions for the syntheses of **1**, **32** and **33** were not significantly optimized, suggesting that higher yields are potentially achievable, notably for the dehydration and coupling steps.

In light of their potent reported activity against Class I HDACs, psammaplin A and our synthetic analogues were evaluated in *in vitro* assays against HDAC1 and HDAC6. Psammaplin A is thought to act as a prodrug, inhibiting HDAC activity following intracellular reduction of the disulfide moiety, generating the corresponding thiol.[19] Indeed, in our assay, the reduced form was found to be more potent in each case. IC_{50} values for reduced forms are given in Table 1. Both psammaplin A and our synthetic analogues were found to be extremely potent and selective against HDAC1, with IC_{50} values ranging from low nM to pM in their reduced form, therefore more potent than reference compounds trichostatin A[20a–c] or the FDA approved compound SAHA (vorinostat).[21a–d] Moreover, comparison of HDAC1 (class I) and HDAC6 (class II) data showed an interesting selectivity for class I HDACs over class II. Further studies are underway in order to understand the observed selectivity towards HDAC1 and HDAC6. Interestingly, methylthioether **15c** was found to be completely inactive, which is in full agreement with the thiol hypothesis for the active species. Full biological assessment of our library against both HDAC and DNMT enzymes is underway and will be the subject of a future manuscript.

In summary, we have developed several novel and expedient routes towards psammaplin A and a variety of synthetic analogues bearing electron rich or electron poor aromatics. The developed strategies allow both aromatic aldehydes and aromatic halides

Table 1 HDAC1 assays, IC_{50} values (μM)



cpd	R_1	R_2	R_3[a]	HDAC1	HDAC6
1	3-Br-4-OH	H	dimer	0.045	1.23
$\mathbf{1}^R$	“	“	H	0.001	0.36
15a	3-OMe-4-SMe	H	dimer	1.67	>50
$\mathbf{15a}^R$	“	“	H	0.0006	1.42
15b	3-NO_2	H	dimer	0.50	7.75
$\mathbf{15b}^R$	“	“	H	0.001	1.21
15c	3-Br-4-OMe	Me	Me	>50	>50
32	4-NMe_2	H	dimer	3.64	>50
$\mathbf{32}^R$	“	“	H	0.001	2.33
33	3-Br-4-NMe_2	H	dimer	0.18	>50
$\mathbf{33}^R$	“	“	H	0.004	0.70

[a] Thiols (X^R) were obtained by *in situ* reduction of the corresponding disulfides, using tris(2-carboxyethyl)phosphine hydrochloride (TCEP)

to be used as substrates. Interestingly, preliminary biological assays have shown our synthetic analogues to be extremely potent HDAC inhibitors, more potent than current inhibitors SAHA,[21a–d] trichostatin A[20a–c] or indeed psammaplin A itself. Further biological studies are ongoing in order to understand the observed selectivity towards HDAC1 over HDAC6.

Acknowledgements

This work was supported by the Association for International Cancer Research (AICR) (08-0407).

Notes and references

1 (*a*) I. C. Pina., J. T. Gautschi, G. Y. S. Wang, M. L. Sanders, F. J. Schmitz, D. France, S. Cornell-Kennon, L. C. Sambucetti, S. W. Remiszewski, L. B. Perez, K. W. Bair and P. Crews, *J. Org. Chem.*, 2003, **68**, 3866; (*b*) E. Quinoa and P. Crews, *Tetrahedron Lett.*, 1987, **28**, 3229; (*c*) L. Arabshahi and F. J. Schmitz, *J. Org. Chem.*, 1987, **52**, 3584; (*d*) A. D. Rodriguez, R. K. Akee and P. J. Scheuer, *Tetrahedron Lett.*, 1987, **28**, 4989; (*e*) C. Jimenez and P. Crews, *Tetrahedron*, 1991, **47**, 2097; (*f*) J. N. Tabudravu, V. G. H. Eijsink, G. W. Gooday, M. Jaspars, D. Komander, M. Legg, B. Synstad and D. M. F. van Aalten, *Bioorg. Med. Chem.*, 2002, **10**, 1123; (*g*) J. Shin, H.-S. Lee, Y. Seo, J.-R. Rho, K. W. Cho and V. J. Paul, *Tetrahedron*, 2000, **56**, 9071; (*h*) N. B. Pham, M. S. Butler and R. J. Quinn, *J. Nat. Prod.*, 2000, **63**, 393.
2 P. R. Bergquist and R. J. Wells, *Marine Natural Products*, Academic Press, New York, 1983, vol. 5.
3 D. Kim, I. S. Lee, J. H. Jung, C. O. Lee and S. U. Choi, *Anticancer Res.*, 1999, **19**, 4085.
4 D. Kim, I. S. Lee, J. H. Jung and S. I. Yang, *Arch. Pharm. Res.*, 1999, **22**, 25.
5 G. M. Nicholas, L. L. Eckman, S. Ray, R. O. Hughes, J. A. Pfefferkorn, S. Barluenga, K. C. Nicolaou and C. A. Bewley, *Bioorg. Med. Chem. Lett.*, 2002, **12**, 2487.
6 J. S. Shim, H. S. Lee, J. Shin and H. J. Kwon, *Cancer Lett.*, 2004, **203**, 163.
7 Y. Jiang, E. Y. Ahn, S. H. Ryu, D. K. Kim, J. S. Park, H. J. Yoon, S. You, B. J. Lee, D. S. Lee and J. H. Jung, *BMC Cancer*, 2004, **4**, 70.
8 (*a*) C. B. Yoo and P. A. Jones, *Nat. Rev. Drug Discovery*, 2006, **5**, 37; (*b*) R. W. Johnstone, *Nat. Rev. Drug Discovery*, 2002, **1**, 287; (*c*) M. Rodriguez, M. Aquino, I. Bruno, G. De Martino, M. Taddei and L. Gomez-Paloma, *Curr. Med. Chem.*, 2006, **13**, 1119.
9 Y. Park, Y. Liu, J. Hong, C. O. Lee, H. Cho, D. K. Kim, K. S. Im and J. H. Jung, *J. Nat. Prod.*, 2003, **66**, 1495.
10 O. Hoshino, M. Murakata and K. Yamada, *Bioorg. Med. Chem. Lett.*, 1992, **2**, 1561.
11 K. C. Nicolaou, R. Hugues, J. A. Pfefferkorn, S. Barluenga and A. J. Roecker, *Chem.–Eur. J.*, 2001, **7**, 4280.
12 A. M. Godert, N. Angelino, A. Woloszynska-Read, S. R. Morey, S. R. James, A. R. Karpf and J. R. Sufrin, *Bioorg. Med. Chem. Lett.*, 2006, **16**, 3330.
13 J. Peng, J. Li and M. T. Hamann, *Alkaloids: Chem. Biol.*, 2005, **61**, 59.
14 F. Hentschel and T. Lindel, *Synthesis*, 2010, **2**, 181.
15 T. R. Boehlow, J. J. Harburn and C. D. Spilling, *J. Org. Chem.*, 2001, **66**, 3111.
16 N. P. Rath, T. R. Boehlow and C. D. Spilling, *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.*, 1995, **C51**, 2654.
17 E. Nakamura, *Tetrahedron Lett.*, 1981, **22**, 663.
18 R. Plantier-Royon, D. Anker and J. Robert-Baudouy, *J. Carbohydr. Chem.*, 1991, **10**, 239.
19 D. H. Kim, J. Shin and H. J. Kwon, *Exp. Mol. Med.*, 2007, **39**, 47.
20 (*a*) M. Yoshida, M. Kijima, T. Akita and T. Beppu, *J. Biol. Chem.*, 1990, **265**, 17174; (*b*) M. Yoshida, S. Horinouchi and T. Beppu, *BioEssays*, 1995, **17**, 423; (*c*) L. Qiu, M. J. Kelso, C. Hansen, M. L. West, D. P. Fairlie and P. G. Parsons, *Br. J. Cancer*, 1999, **80**, 1252.
21 (*a*) V. M. Richon, Y. Webb, R. Merger, T. Sheppard, B. Jursic, L. Ngo, F. Civoli, R. Breslow, R. A. Rifkind and P. A. Marks, *Proc. Natl. Acad. Sci. U. S. A.*, 1996, **93**, 5705; (*b*) V. M. Richon, S. Emiliani, E. Verdin, Y. Webb, R. Breslow, R. A. Rifkind and P. A. Marks, *Proc. Natl. Acad. Sci. U. S. A.*, 1998, **95**, 3003; (*c*) L. A. Cohen, S. Amin, P. A. Marks, R. A. Rifkind, D. Desail and V. M. Richon, *Anticancer Res.*, 1999, **19**, 4999; (*d*) B. S. Mann, J. R. Johnson, M. H. Cohen, R. Justice and R. Pazdur, *Oncologist*, 2007, **12**, 1247.

