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Multi-substituted 8-aminoimidazo[1,2-*a*]pyrazines by Groebke–Blackburn–Bienaymé reaction and their Hsp90 inhibitory activity†

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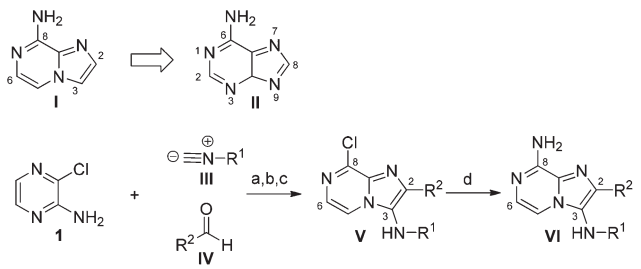
Using a 2,3-diamino pyrazine substrate and yttrium triflate catalyst, various 2-alkyl and aryl substituted 3,8-diaminoimidazo[1,2-*a*]pyrazines were efficiently prepared through Groebke–Blackburn–Bienaymé MCR. In particular, a novel 2-piperonyl 3,8-diaminoimidazo[1,2-*a*]pyrazine structure was prepared exclusively with this new method and was found to have moderate Hsp90 inhibitory activity. A crystalline complex with N-terminus ATP domain of Hsp90 and one of the new Hsp90 inhibitors was also obtained to elucidate the origin of activity of 2-piperonyl 3,8-diaminoimidazo[1,2-*a*]pyrazines.

Introduction

8-Aminoimidazo[1,2-*a*]pyrazines **I** are highly useful in medicinal chemistry research for their evident similarity with adenine **II**, which is ubiquitous in bioactive structures. Consequently, installation of different substituents on the 8-aminoimidazo[1,2-*a*]pyrazine ring system has become an intriguing topic, and can usually be achieved *via* certain ring formation reactions from substituted fragments.[1] Preparation of a multi-substituted 8-aminoimidazo[1,2-*a*]pyrazine ring system is more demanding and less reported. It is known that multicomponent reactions (MCRs) are advantageous in the syntheses of heavily substituted heterocycles. However, syntheses of multi-substituted 8-aminoimidazo[1,2-*a*]pyrazines through the MCR pathway are rarely discussed and seem to be less efficient. For example, David *et al.*[2] attempted the preparation of 2-substituted 3,8-diaminoimidazo[1,2-*a*]pyrazines through Groebke–Blackburn–Bienaymé MCR[3] and obtained poor yields. Interestingly, one recent article by Pirali *et al.*[4] described more efficient syntheses of 2-substituted 3,8-diaminoimidazo[1,2-*a*]-pyrazines **VI** starting from chloropyrazine **1**, isocyanides **III** and aldehydes **IV** *via* two step sequences, including Groebke–Blackburn–Bienaymé MCR and the following aminolysis of intermediate **V**. Nevertheless, it was found that only aryl carboxaldehydes were produced under the reported conditions, and this meant that the R^2 on the 2-substituted 3,8-diaminoimidazo[1,2-*a*]pyrazine products **VI** could only be aryl groups (Scheme 1).



Scheme 1 A comparison of imidazo[1,2-*a*]pyrazines **I** and adenine **II** and one of the known synthetic methods[4] to 8-aminoimidazo[1,2-*a*]pyrazines **VI**. Reaction conditions: (a) compound **1** (1 equiv.), R^2CHO (1.2 equiv.), MeCN, reflux, 2 h; (b) TMSCl (1.2 equiv.), MeCN/DCM, 30 min; (c) R^1NC (1.2 equiv.), reflux, overnight; (d) aq. NH_4OH/dioxane, 100 °C, overnight.

During our study of Groebke–Blackburn–Bienaymé MCR,[5] a typical experiment indicated that 2,3-diaminopyrazine was a superior substrate for this MCR, leading to 2-aryl, 2-benzyl, and 2-alkyl 3,8-diaminoimidazo[1,2-*a*]pyrazines. Using this efficient methodology, a panel of 2-piperonyl substituted 3,8-diaminoimidazo[1,2-*a*]pyrazines were prepared for the first time. Some of the 2-piperonyl substituted 3,8-diaminoimidazo-[1,2-*a*]pyrazines were found to have moderate heat shock

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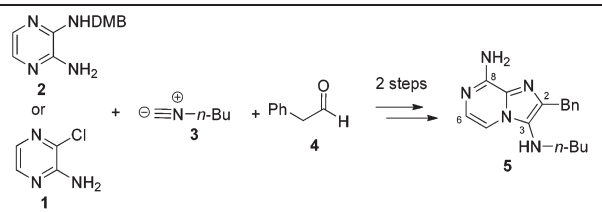
protein 90 (Hsp90) inhibitory activity and could serve as leads for further optimization. These results are described herein.

Results and discussion

Initially, using yttrium triflate catalyst, it was found that the *N*-2,4-dimethoxybenzyl (*N*-DMB) protected diamino pyrazine **2** reacted with isocyanide **3** and phenyl acetaldehyde **4** to give the desired product **5** with good yield, after removing the N-protection with acid (Table 1, entry 1). This was unique because in comparison experiments, TMSCl[4] failed to give the conversion and TfOH only gave low yield (Table 1, entries 2 and 3). Changing from diamino pyrazine **2** back to chloropyrazine **1** again failed to give the reaction with TMSCl, and this was consistent with the findings by Pirali *et al.*[4] (Table 1, entry 4). With TfOH or rare earth-metal triflate catalysts, the product **5** was observed after aminolysis, however, the yield was low (Table 1, entries 5–7). As phenyl acetaldehyde **4** is a special type of alkyl aldehyde, the abovementioned discovery served as a successful example of a Groebke–Blackburn–Bienaymé MCR leading to 2-alkyl 3,8-diaminoimidazo[1,2-*a*]pyrazines.

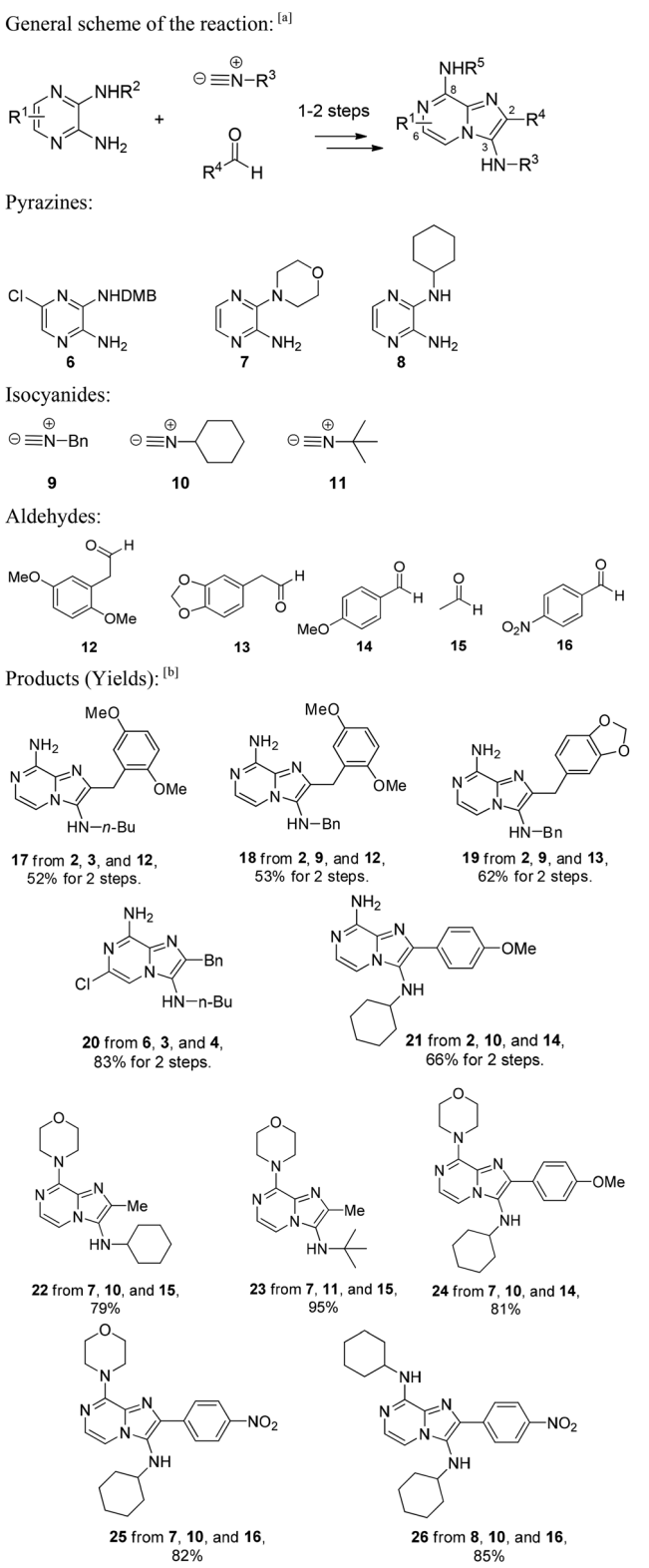
To further explore the scope of the yttrium triflate catalyzed synthesis of 2-substituted 3,8-diaminoimidazo[1,2-*a*]pyrazines, different substrates, including pyrazines **6–8**, isocyanides **9–11**, as well as aldehydes **4** and **12–16**, were subjected to the new reaction conditions described in Table 1, entry 1 (Scheme 2). For common electron rich substituted phenyl acetaldehydes, the yields were moderate to good with different isocyanides. Because the yields were still lower than that with phenyl

Table 1 MCR of pyrazines **1** or **2**, isocyanide **3**, and aldehyde **4**[a]



No.	Pyrazine	Catalyst	Yield (for 2 steps)[b]
1	**2**	$Yb(OTf)_3$	75%
2	**2**	TMSCl	n.d.
3	**2**	TfOH	28%
4	**1**	TMSCl	n.d.
5	**1**	TfOH	4%
6	**1**	$Sc(OTf)_3$	8%
7	**1**	$Yb(OTf)_3$	11%

[a] Reaction conditions: for entries 1 and 3: (a) compound **2** (1.0 equiv.), compound **3** (1.2 equiv.), compound **4** (1.2 equiv.), catalyst (10 mol%), MeOH, 70 °C, 5 h; (b) TFA, DCM, 50 °C, 1 h. For entries 2 and 4: (a) compound **4** (1.2 equiv.), MeCN, 100 °C, 2 h; (b) TMSCl (1.2 equiv.), MeCN/DCM, rt, 30 min; (c) compound **3** (1.2 equiv.), 100 °C, 12 h. For entries 5–7: (a) compound **1** (1.0 equiv.), compound **3** (1.2 equiv.), compound **4** (1.2 equiv.), catalyst (10 mol%), MeOH, 100 °C, 12 h; (b) aq. NH_4OH/dioxane, sealed tube, 120 °C, 24 h. [b] Yield was determined after flash chromatography.



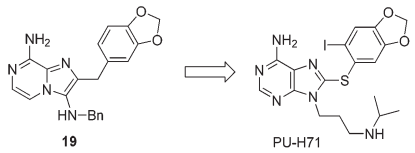
Scheme 2 New conditions for Groebke–Blackburn–Bienaymé MCR led to various multisubstituted 3,8-diaminoimidazo[1,2-*a*]pyrazines. [a] Reaction conditions: for products **17–21**: (a) pyrazines (1.0 equiv.), isocyanides (1.2 equiv.), aldehydes (1.2 equiv.), $Yb(OTf)_3$ (10 mol%), MeOH, 70 °C, 5 h; (b) TFA, DCM, 50 °C, 1 h. For products **22–26**: pyrazines (1.0 equiv.), isocyanides (1.2 equiv.), aldehydes (1.2 equiv.), $Yb(OTf)_3$ (10 mol%), MeOH, 70 °C, 5 h. [b] Yield was determined after flash chromatography.

acetaldehyde **4** (Table 1, entry 1), it might be speculated that the electron donating groups on the phenyl ring of the aldehyde component reduced the yield. Retaining phenyl acetaldehyde **4**, chloro-substituted pyrazine **6** gave product **20** again with good yield. The above examples (for products **17–21**) were all based on *N*-DMB protected pyrazines **6**, and several points are noteworthy: (1) compared to chloropyrazine **1** starting material, *vic*-diamino substitution on diamino pyrazine **2** could significantly improve the MCR yield with various isocyanide and aldehyde components; (2) as illustrated in the general reaction schemes in Scheme 2 and Table 1, deprotection of N^8-DMB on 3,8-diaminoimidazo[1,2-*a*]pyrazine intermediate (formed from pyrazine **2**) gave better yield than aminolysis of 8-Cl on 3,8-diaminoimidazo[1,2-*a*]pyrazine intermediate (formed from chloropyrazine **1**), rendering the syntheses of 2-substituted 3,8-diaminoimidazo[1,2-*a*]pyrazines more efficient.

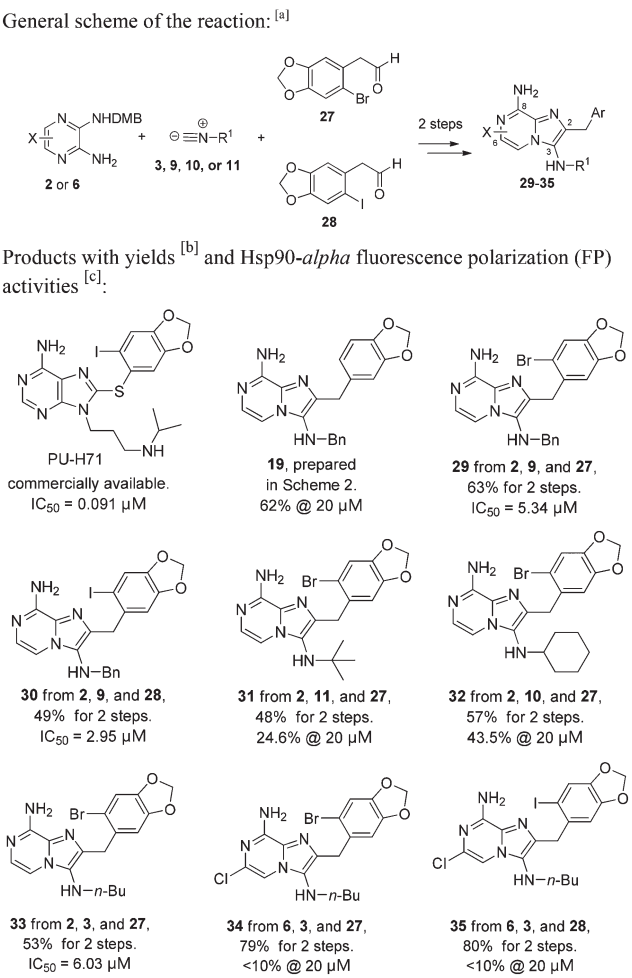
Subsequently, morpholine substituted pyrazine **7** reacted with acetaldehyde **15** and different isocyanides to give products **22** and **23** with good yields (Scheme 2). This is not trivial, because simple aldehydes, such as **15**, were poor partners for this type of reaction. Moreover, pyrazines **7** and **8** could react with aryl carboxaldehydes **14** and **16** as well to give the desired 2-substituted 3,8-diaminoimidazo[1,2-*a*]pyrazines **24–26** (Scheme 2). The reactions with morpholine substituted pyrazine **7** and *N*-cyclohexyl pyrazine **8** were highly practical for the following reasons: (1) the diamino substituted pyrazine substrates improved the MCR yield substantially, with both alkyl aldehyde and aryl carboxaldehyde; (2) they eliminated the necessity of N-deprotection and yielded more interesting *N*-alkylated 2-substituted 3,8-diaminoimidazo[1,2-*a*]pyrazines; (3) morpholine or alkylated amino groups are widely used in medicinal chemistry.

For the Groebke–Blackburn–Bienaymé MCR, there might be several reasons accounting for the yield improvement using diamino substituted pyrazine substrates and yttrium triflate catalyst: (1) *vic*-diamino substitution could enhance the nucleophilicity of pyrazine substrates and result in an improved condensation reaction; (2) *vic*-diamino substitution could provide a better chelation environment for yttrium metal,[6] which is an essential catalyst for Groebke–Blackburn–Bienaymé MCR. This might also explain why TMSCl failed to work with diamino substituted pyrazine substrates.

On the basis of the abovementioned discovery, we looked for applications in medicinal chemistry. It is known that Hsp90 belongs to a large family of heat shock proteins, which are evolutionarily conserved and required for essential housekeeping functions such as protein folding, assembly, and transportation.[7] Discovering new Hsp90 inhibitors is an important approach in combating cancer.[8] We noticed that some ATP-competitive Hsp90 inhibitors contain the 8,9-disubstituted adenine motif,[9] which might be replaced with the 2,3-disubstituted 8-aminoimidazo[1,2-*a*]pyrazine scaffold, according to the structural similarity between adenine and 8-aminoimidazo[1,2-*a*]pyrazine. Furthermore, computational study using the scaffold hopping concept also suggested that our



Scheme 3 The structure similarity between compound **19** and a known Hsp90 inhibitor PU-H71.



Scheme 4 Synthesis and activity study of a Hsp90 targeted, focused library. [a] Reaction conditions: (a) pyrazines (1.0 equiv.), isocyanides (1.2 equiv.), aldehydes (1.2 equiv.), $Yb(OTf)_3$ (10 mol%), MeOH, 70 °C, 5 h; (b) TFA, DCM, 50 °C, 1 h. [b] Yield was determined after flash chromatography. [c] See ESI† for details.

new product **19** or similarly substituted 8-aminoimidazo[1,2-*a*]-pyrazines might possess Hsp90 inhibitory activity due to the remarkable resemblance to PU-H71,[10] an Hsp90 inhibitor in Phase I clinical trial (Scheme 3).

To test the hypothesis, a focused library of 8-aminoimidazo-[1,2-*a*]pyrazines bearing 2-piperonyl and 3-alkyl amino substitutions was prepared using the new methodology (Scheme 4).

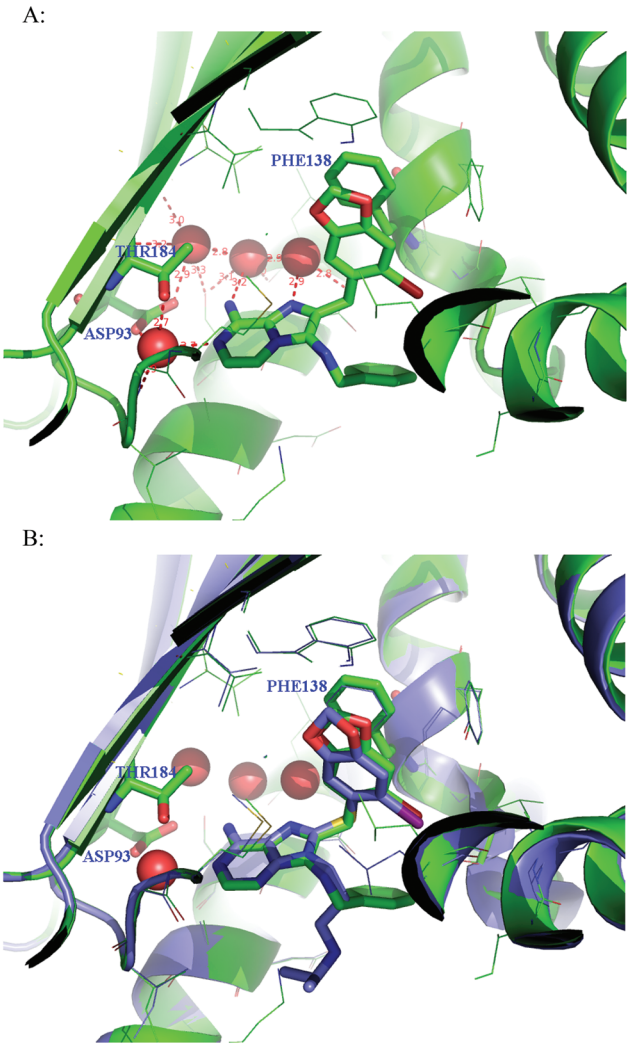


Fig. 1 (A) Co-crystal structure of Hsp90 with compound **29** (pdb entry: 4R3M). (B) Superimposition of crystal structures **29**-Hsp90[11] and PU-H71-Hsp90 (pdb entry: 2FWZ). The protein is shown in cartoon style, and the crystal water molecules are shown as red spheres. The carbon atoms of compound **29** are in green, and those of PU-H71 in blue.

With diamino pyrazine **2** and piperonals **27** and **28**, moderate to good yields were documented for products **29–32**. When chloro-substituted diamino pyrazine **6** was used, the yields were again good for products **34** and **35**, and this was consistent with the observations in Scheme 2, conversions from **6**, **3**, and **4** to **22**.

The inhibitory activity of the library against Hsp90 was measured and analyzed (Scheme 4): (1) the halogenated 2-piperonyl substitution was found to be essential for Hsp90 binding, as suggested by the activity comparison of compounds **19**, **29**, and **30**. Compared to compound **19**, the bromine atom on compound **29** significantly increased the activity to 5.34 μM, whereas the iodine on compound **30** further improved the value to 2.95 μM. (2) The substitution on C3 of the 8-aminoimidazo[1,2-*a*]pyrazine scaffold affected the Hsp90 affinity, as demonstrated by the activities of compounds **29**, **31**, **32**, and **33**. The 2-*tert*-butyl amine group on compound **31** or the 2-cyclohexyl amino group on compound **32** may not be long enough to reach the van der Waals surface of the Hsp90 protein, and resulted in decreased activities. (3) The 6-chloro substitution on 8-aminoimidazo[1,2-*a*]pyrazine was found to be destructive to Hsp90 inhibitory activity, and this was demonstrated by the data of compounds **34** and **35**. Generally, the moderate inhibitory activity of 2-piperonyl and 3-alkyl amino 8-aminoimidazo[1,2-*a*]-pyrazines suggests that this new scaffold could serve as a promising starting point for further structural optimization.

For a better understanding of the interactions between 2,3-disubstituted 8-aminoimidazo[1,2-*a*]pyrazine and Hsp90 binding site, compound **29** was cocrystallized with the N-terminus ATP domain of Hsp90 and the co-crystal structure was successfully determined (pdb entry: 4R3M).[11] As shown in Fig. 1, in the Hsp90 N-terminus ATP domain, the 8-aminoimidazo[1,2-*a*]pyrazine ring was situated at the same position as the adenine ring of PU-H71 (Fig. 1B). The 8-aminoimidazo [1,2-*a*]pyrazine ring system formed several indirect hydrogen bonds with the binding site of Hsp90 through four conserved water molecules (red spheres in Fig. 1) and two direct hydrogen bonds with ASP93 and THR184 residues. The 2-piperonyl substitution showed typical π–π stacking interaction with the PHE138 residue, and the bromine on 2-piperonyl substitution was close to GLY135 residue on the Hsp90 backbone. A halogen bond with the oxygen atom of the GLY135 residue was thus speculated for the short Br–O distance of 3.4 Å. In addition, compared to the PU-H71 structure, a discrepancy between the orientations of the 3-benzyl amino group on compound **29** and the 9-alkyl amino group on PU-H71 was observed. This might account partially for the activity difference between these two compounds.

Conclusions

In conclusion, with 2,3-diamino pyrazine substrate and yttrium triflate catalyst, various 2-alkyl and aryl substituted 3,8-diaminoimidazo[1,2-*a*]pyrazines could be efficiently prepared through Groebke–Blackburn–Bienaymé MCR. Elimination of the chloride aminolysis step made this method more favorable compared to previous synthetic approaches. We further demonstrated that 2-piperonyl 3,8-diaminoimidazo [1,2-*a*]pyrazine structures, prepared exclusively with our new synthetic method, were potential Hsp90 inhibitors. A crystalline complex with N-terminus ATP domain of Hsp90 and one of the new Hsp90 inhibitors were also obtained to elucidate the origin of activity of the 2-piperonyl 3,8-diaminoimidazo [1,2-*a*]pyrazines. The new scaffold, moderate preliminary biological activity, and crystal structure details together suggested that 2-piperonyl 3,8-diaminoimidazo[1,2-*a*]pyrazines were promising leads for Hsp90 inhibitor discovery.

Acknowledgements

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Notes and references

1 (*a*) T. L. Gilchrist, *Heterocyclic Chemistry*, Prentice Hall, 3rd edn, 1997; (*b*) J. A. Joule and K. Mills, *Heterocyclic Chemistry*, Blackwell Science Ltd., 4th edn, 2000 For some recent examples, see: (*c*) H. Zeng, D. B. Belanger, P. J. Curran, G. W. Shipps Jr., H. Miao, J. B. Bracken, M. A. Siddiqui, M. Malkowski and Y. Wang, *Bioorg. Med. Chem. Lett.*, 2011, **21**, 5870–5875; (*d*) S. Martinez Gonzalez, A. I. Hernandez, C. Varela, M. Lorenzo, F. Ramos-Lima, E. Cendon, D. Cebrian, E. Aguirre, E. Gomez-Casero, M. I. Albarran, P. Alfonso, B. Garcia-Serelde, G. Mateos, J. Oyarzabal, O. Rabal, F. Mulero, T. Gonzalez-Granda, W. Link, J. Fominaya, M. Barbacid, J. R. Bischoff, P. Pizcueta, C. Blanco-Aparicio and J. Pastor, *Bioorg. Med. Chem. Lett.*, 2012, **22**, 5208–5214; (*e*) S. Martinez Gonzalez, A. I. Hernandez, C. Varela, S. Rodriguez-Aristegui, R. M. Alvarez, A. B. Garcia, M. Lorenzo, V. Rivero, J. Oyarzabal, O. Rabal, J. R. Bischoff, M. Albarran, A. Cebria, P. Alfonso, W. Link, J. Fominaya and J. Pastor, *Bioorg. Med. Chem. Lett.*, 2012, **22**, 1874–1878; (*f*) S. Martinez Gonzalez, A. I. Hernandez, C. Varela, S. Rodriguez-Aristegui, M. Lorenzo, A. Rodriguez, V. Rivero, J. I. Martin, C. G. Saluste, F. Ramos-Lima, E. Cendon, D. Cebrian, E. Aguirre, E. Gomez-Casero, M. Albarran, P. Alfonso, B. Garcia-Serelde, J. Oyarzabal, O. Rabal, F. Mulero, T. Gonzalez-Granda, W. Link, J. Fominaya, M. Barbacid, J. R. Bischoff, P. Pizcueta and J. Pastor, *Bioorg. Med. Chem. Lett.*, 2012, **22**, 3460–3466; (*g*) J. M. Bartolome-Nebreda, F. Delgado, M. L. Martin-Martin, C. M. Martinez-Viturro, J. Pastor, H. M. Tong, L. Iturrino, G. J. Macdonald, W. Sanderson, A. Megens, X. Langlois, M. Somers, G. Vanhoof and S. Conde-Ceide, *J. Med. Chem.*, 2014, **57**, 4196–4212.

2 (*a*) D. B. Salunke, E. Yoo, N. M. Shukla, R. Balakrishna, S. S. Malladi, K. J. Serafin, V. W. Day, X. Wang and S. A. David, *J. Med. Chem.*, 2012, **55**, 8137–8151; (*b*) N. M. Shukla, D. B. Salunke, E. Yoo, C. A. Mutz, R. Balakrishna and S. A. David, *Bioorg. Med. Chem.*, 2012, **20**, 5850–5863.

3 (*a*) H. Bienaymé and K. Bouzid, *Angew. Chem., Int. Ed.*, 1998, **110**, 2349–2352; (*b*) C. Blackburn, B. Guan, P. Fleming, K. Shiosaki and S. Tsai, *Tetrahedron Lett.*, 1998, **39**, 3635–3638; (*c*) K. Groebke, L. Weber and F. Mehlin, *Synlett*, 1998, 661–663.

4 M. Guasconi, X. Lu, A. Massarotti, A. Caldarelli, E. Ciraolo, G. C. Tron, E. Hirsch, G. Sorba and T. Pirali, *Org. Biomol. Chem.*, 2011, **9**, 4144–4149.

5 (*a*) T. Meng, Z. Zhang, D. Hu, L. Lin, J. Ding, X. Wang and J. Shen, *J. Comb. Chem.*, 2007, **9**, 739–741; (*b*) H. Sun, H. Zhou, O. Khorev, R. Jiang, T. Yu, X. Wang, Y. Du, Y. Ma, T. Meng and J. Shen, *J. Org. Chem.*, 2012, **77**, 10745–10751; (*c*) H. Zhou, W. Wang, O. Khorev, Y. Zhang, Z. Miao, T. Meng and J. Shen, *Eur. J. Org. Chem.*, 2012, 5585–5594.

6 S. Kobayashi, in *Lanthanides: Chemistry and Use in Organic Synthesis*, ed. S. Kobayashi, Springer, Berlin, Heidelberg, 1999, pp. 63–118.

7 (*a*) M. Feder and G. Hofmann, *Annu. Rev. Physiol.*, 1999, **61**, 243–282; (*b*) D. Picard, *Cell. Mol. Life Sci.*, 2002, **59**, 1640–1648; (*c*) C. Garrido, S. Gurbuxani, L. Ravagnan and G. Kroemer, *Biochem. Biophys. Res. Commun.*, 2001, **286**, 433–442.

8 (*a*) M. Hwang, L. Moretti and B. Lu, *Curr. Med. Chem.*, 2009, **16**, 3081–3092; (*b*) W. Xu and L. Neckers, *Clin. Cancer Res.*, 2007, **13**, 1625–1629; (*c*) J. Ren, J. Li, Y. Wang, W. Chen, A. Shen, H. Liu, D. Chen, D. Cao, Y. Li, N. Zhang, Y. Xu, M. Geng, J. He, B. Xiong and J. Shen, *Bioorg. Med. Chem. Lett.*, 2014, **24**, 2525–2529; (*d*) R. Bhat, S. R. Tummalapalli and D. P. Rotella, *J. Med. Chem.*, 2014, **57**, 8718–8728.

9 (*a*) L. Llauger, H. He, J. Kim, J. Aguirre, N. Rosen, U. Peters, P. Davies and G. Chiosis, *J. Med. Chem.*, 2005, **48**, 2892–2905; (*b*) S.-H. Kim, A. Bajji, R. Tangallapally, B. Markovitz, R. Trovato, M. Shenderovich, V. Baichwal, P. Bartel, D. Cimbora, R. McKinnon, R. Robinson, D. Papac, D. Wettstein, R. Carlson and K. M. Yager, *J. Med. Chem.*, 2012, **55**, 7480–7501.

10 H. He, D. Zatorska, J. Kim, J. Aguirre, L. Llauger, Y. She, N. Wu, R. M. Immormino, D. T. Gewirth and G. Chiosis, *J. Med. Chem.*, 2005, **49**, 381–390.

11 See ESI† for additional details.