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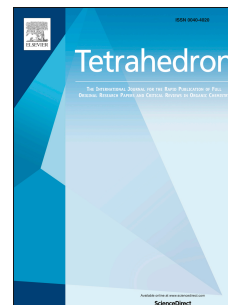
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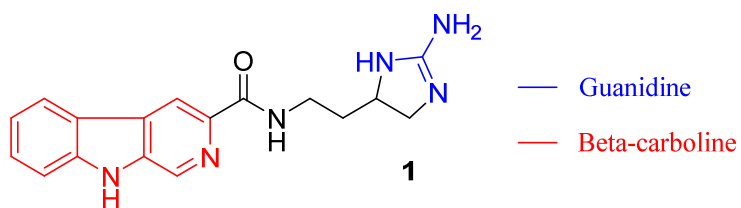


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The alkaloid tiruchanduramine **1** was prepared in racemic form from 3-aminopropan-1-ol and tryptophan using a convergent strategy with the longest linear sequence being eight steps.



A synthesis of tiruchanduramine and a reinvestigation of its glycosidase inhibitory activity

Matthew L. Buck^a, Daniel M. Evans^a, Victoria A. Evans^a, Dominik Herkt^a, Hazel Sharp^b, Jackie Hollinshead^b, Fabian Mitschang^a, Patrick J. Murphy^{*a}, Robert J. Nash^b, Jack Wenbourne^a.

Abstract: Tiruchanduramine **1** was prepared using a convergent strategy with the longest linear sequence being eight steps. Synthetic **1**, displayed a broader range of inhibition than reported previously and, in addition to α -glucosidases, **1** also inhibits almond β -glucosidase, β -galactosidase and β -N-acetylglucosaminidase from jack bean.

Keywords: Sinoicum macroglossum; tiruchanduramine; β -carboline; guanidines; Glycosidase inhibition

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Introduction

The alkaloid tiruchanduramine **1** (Fig. 1.) was isolated by Ravinder et al.¹ from the ascidian *Sinoicum macroglossum* and was shown to consist of a β -carboline core, substituted with a side chain which contains a cyclic 5-membered guanidine. Tiruchanduramine **1** was found to be a potent α -glucosidase inhibitor² when compared with acarbose, a drug commonly used to treat type 2 diabetes with an IC₅₀ of 100 μ g/mL compared with tiruchanduramine 78.2 μ g/mL.

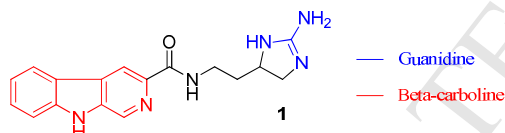
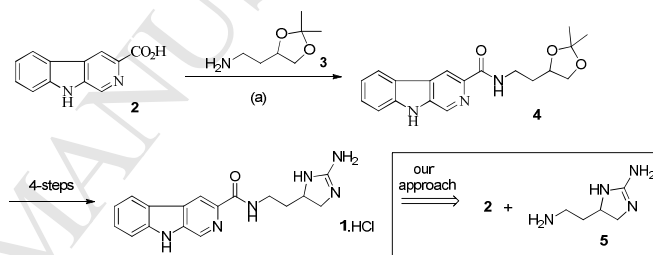


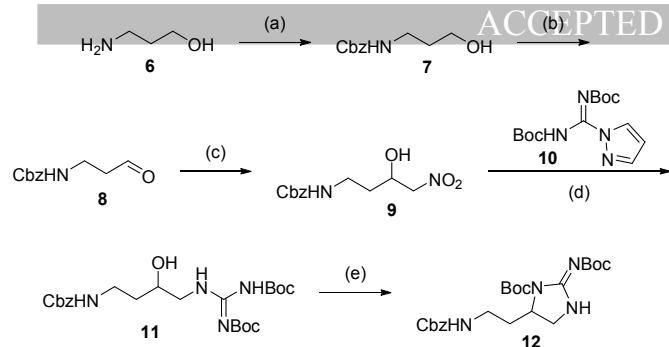
Fig. 1. Structure of tiruchanduramine (**1**)

Ravinder et al.¹ also reported the synthesis of the natural product in racemic form over 10 steps in 3.2 % overall yield from tryptophan and but-3-en-1-ol. Their linear strategy began with the β -carboline-3-carboxylic acid **2**,³ easily prepared in 4 steps from tryptophan, which was coupled with the amine **3**, prepared in 5 steps from but-3-en-1-ol, to give the amide **4**. This was converted into the hydrochloride salt of **1** via a 4-step protocol involving the introduction of the guanidine under Mitsunobu conditions (Scheme 1). In our synthesis it was hoped to adopt a more convergent approach in which the guanidine heterocycle **5** is prepared separately then coupled to the β -carboline-3-carboxylic acid **2**.



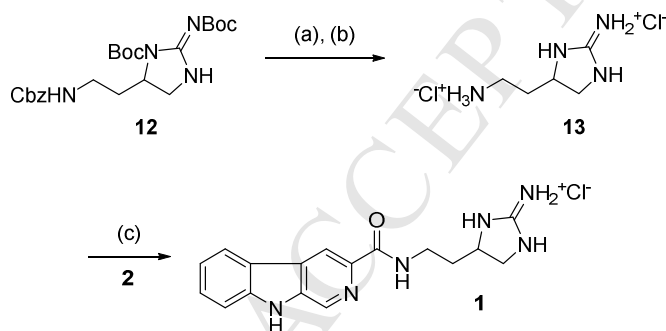
Scheme 1: Ravinder's synthesis of **1** (a) DCC, DMAP, CH₂Cl₂, 62%, or (EDCI, HOBT, DMF) 65%.

Initial attempts at the synthesis of **5** began with the Cbz-protection of 3-aminopropan-1-ol **6** to give **7** in 88% yield⁴ which was oxidized to the aldehyde **8** using PCC in 60% yield.⁵ The nitro-aldol reaction of **8** with nitromethane catalysed by DIPEA gave **9** in 90% yield. Reduction of **9** with nickel boride and sodium borohydride gave an intermediate amine which was guanidinylation *in situ* with **10** to give the guanidine **11** in 81%. Cyclisation of **11** was effected under conditions similar to those previously employed in our work on cylindrospermopsin.^{6,7} Thus treatment of **9** with a combination of triphenylphosphine, iodine and imidazole at -20 °C resulted in the complete disappearance of the starting material and the formation of **12** as indicated by ¹H NMR. (Scheme 2)



Scheme 2: synthesis of guanidine **11** (a) CbzCl, NaOH, 0 °C, 18 h, 88%. (b) PCC, CH₂Cl₂, 5 h, 60%. (c) Nitromethane, DIPEA, CH₂Cl₂, 5 days, 90%. (d) i) Ni(II)Cl₂·6H₂O, NaBH₄, MeOH, 4 h. ii) **10** (1.2 equiv.), NEt₃, 3 days, 81%. (e) Dppe, imidazole, I₂, CH₂Cl₂, -20 °C, 2 h, quantitative.

Attempted purification of **12** was difficult as the product co-eluted with triphenylphosphine oxide and was also unstable to silica gel as had been previously observed with similar structures.⁸ We thus switched to using dppe as a substitute for triphenylphosphine and found that the cyclisation reaction proceeded in a similar fashion. Purification of **11** was easily achieved in high yield and good purity (>95%) by trituration/precipitation of the crude reaction product with diethyl ether. With **12** in hand we next attempted to selectively deprotect the terminal amine under hydrogenation conditions and thus treated it with H₂ over Pd/C. This resulted in the loss of the Cbz-protecting group, however it was apparent from ¹H NMR analysis that a mixture of products had been formed. This mixture might possibly have arisen from the intermediate amine undergoing protecting group migration as has been observed previously in related systems.⁸ We thus took this mixture and removed the Boc-protecting groups by treatment with aqueous 3 M HCl to give the crude guanidine **13** as its dihydrochloride salt. The coupling of **13** with the acid **2** proved capricious but was eventually achieved by treatment of **2** with 1,1'-carbonyldiimidazole (CDI) in a 1:1 mixture of THF and DMF⁹ followed addition of the free base of **13**. After purification by HPLC, tiruchanduramine **1** was obtained as its hydrochloride salt in 11.5% yield over three steps.



Scheme 3: Synthesis of tiruchanduramine **1**. (a) Pd/C 10%, MeOH, 24 h. (b) HCl (3 M), 24 h. (c) i) **2**, CDI, THF:DMF (1:1), ii) **13**, NEt₃, 1 week, 11.5%.

Our compound gave identical data to those reported in the literature¹ with the exception of one aromatic CH signal in the ¹H NMR, which was reported at δ 8.20 (1H, d, *J* 8.0 Hz) ppm that we observed at δ 8.37 (1H, d, *J* 7.8 Hz) ppm. We were

unable to obtain an original sample of **1** or original copies of original NMR spectra.

Synthetic **1**, the known carboxylic acid **2** and the guanidine **13** were submitted to assays on a panel of glycosidases at 143 μ g/ml and **1** was more inhibitory (over 50%) to Bacillus α -glucosidase than **2** (31%) and **13** (11%). Compound **1** was also more inhibitory to yeast α -glucosidase (26%) than **2** (9%) and **13** gave no inhibition. Synthetic **1** gave over 40% inhibition of β -glucosidase whereas **2** was a much weak inhibitor (14%). Heterocycle **2** was not inhibitory to jack bean hexosaminidase but **1** gave 37% inhibition. Both **1** (20%) and **2** (37%) inhibited bovine hexosaminidase. β -Galactosidase was strongly inhibited (80%) by **1** but **2** and **13** were not inhibitory to this enzyme. Compound **13** in fact weakly increased the activity of the bovine (11%) and jack bean (15%) hexosaminidases at the top concentration used. α -Galactosidase and α -mannosidase were not inhibited by any of the compounds. Our results confirm the α -glucosidase inhibition reported by Ravinder¹ although the compound is only a weak to moderate inhibitor of the two α -glucosidases tested here. It should be noted that acarbose used as the comparator for **1** by Ravinder is not a particularly potent inhibitor of glucosidases.¹ Compound **1** does, however, show a broad range of inhibition and also inhibits almond β -glucosidase, β -galactosidase and β -N-acetylglucosaminidase from jack bean. For **1** to be suitable as an alternative to acarbose for diabetes, further modifications would be needed to make it more specific. However, hexosaminidase activity is elevated in many diseases including Alzheimer's¹⁰ and so perhaps this inhibition is of more interest if selectivity can be improved. There are also indications that azasugars can improve the folding and function of glucosylhydrolases which can become aberrant in disease states including Alzheimer's.^{10,12}

Conclusions

We have succeeded in the second racemic synthesis of tiruchanduramine and are currently investigating an asymmetric Henry reaction to effect a stereoselective synthesis of tiruchanduramine.¹³ Synthetic **1**, displayed a broader range of inhibition than reported previously and, in addition to α -glucosidases, **1** also inhibits almond β -glucosidase, β -galactosidase and β -N-acetylglucosaminidase from jack bean.

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Experimental

Column chromatography was carried out on silica gel (60Å, 40-63 μ m) and TLCs were conducted on precoated Kieselgel 60 F254 (Art. 5554; Merck) with the eluent specified in each case. All non-aqueous reactions were conducted in oven-dried apparatus under a static atmosphere of argon. Diethyl ether, THF and dichloromethane were dried by a Pure Solv MD-3 solvent purification system. Dry methanol and DMF was purchased from Aldrich. Chemical shifts are reported in δ values relative to residual chloroform (7.26/77.16 ppm), methanol (3.31/49.0 ppm) and DMSO (2.50/39.52 ppm) as internal standards. Proton and carbon NMR spectra were

recorded in CDCl₃ on a Bruker AC250/400/500 spectrometer unless otherwise stated. Mass spectra data were obtained at the EPSRC Mass Spectrometry Service Centre at the University of Wales, Swansea. Infrared spectra were recorded as thin films (oils) on a Bruker Tensor 27 series instrument. Melting points were performed on a Stuart SMP10 apparatus and are uncorrected.

Benzyl (3-hydroxypropyl)carbamate **7**⁴

A stirred solution of 1-aminopropan-3-ol **6** (12.0 g, 133 mmol) and NaOH (aqueous, 1 M) (40 mL, 3.0 equiv.) was cooled (0 °C) and benzyl chloroformate (22.5 g, 133 mmol, 1 equiv.) was added drop-wise over 10 min. After warming to rt the mixture was stirred for 1 h and CH₂Cl₂ (100 mL) was added and stirring continued for 16 h. The layers were separated and the aqueous layer extracted with CH₂Cl₂ (2 x 50 mL) and EtOAc (2 x 50 mL). The combined organic layers were dried (MgSO₄) and evaporated under reduced pressure to yield the crude product as a pale yellow solid. The solid was dissolved in warm EtOAc (ca. 50 mL) and cooled (−20 °C) overnight to give crystals which were filtered and washed with petrol to give **7** as white crystals (22.5 g, 117 mmol) 88% yield.

Mp. 49–50 °C (Lit 50–51 °C); R_f 0.13 (50 % EtOAc in PE, PMA); δ_H 1.68–1.74 (2H, m, CH₂), 2.59 (1H, br. s, OH), 3.34 (2H, apparent q, *J* 6.2, CH₂), 3.67 (2H, t, *J* 5.8 Hz, CH₂), 5.08 (1H, br. s, 1H, NH), 5.11 (2H, s, CH₂), 7.30–7.39 (5H, m, Ph); δ_C 32.6, 37.7, 59.6, 66.9, 128.1, 128.2, 128.6, 136.5, 157.3.

Benzyl (3-oxopropyl)carbamate **8**⁵

PCC (8.75 g, 40.6 mmol, 1.7 equiv.) and Celite® (9 g) were stirred in CH₂Cl₂ (50 mL) for 5 min whereupon alcohol **7** (5.0 g, 24 mmol) dissolved in CH₂Cl₂ (30 mL) was added. After 4 h the reaction was diluted with Et₂O (100 mL) and passed through a short pad of layered silica and Celite®. The remaining solids were suspended in CH₂Cl₂ (25 mL) and precipitated with Et₂O (50 mL) and this mixture passed through the same pad. This process was repeated a further three times and the filtrate evaporated under reduced pressure to give a yellow oil (5.0 g). Column chromatography (40% EtOAc in PE) gave **8** as a colourless viscous oil (3.0 g, 14.5 mmol) in 60% yield.

R_f 0.4 (50% EtOAc in petrol, PMA); δ_H 2.75 (2H, t, *J* 5.7 Hz, CH₂), 3.49 (2H, apparent q, *J* 6.0 Hz, CH₂), 5.09 (2H, s, CH₂), 5.15 (1H, br s, HN), 7.30–7.39 (5H, m, Ar), 9.81 (1H, s, CHO); δ_C 34.6, 44.2, 66.9, 128.2, 128.3, 128.7, 136.5, 165.4, 201.3; ν_{max} 3445, 1704, 1645 cm^{−1}.

Benzyl (3-hydroxy-4-nitrobutyl)carbamate **9**

Aldehyde **8** (2.2 g, 10.2 mmol) and nitromethane (18.7 g, 16.5 mL, 0.31 mol, 30 equiv.) were dissolved in CH₂Cl₂ (20 mL) and cooled (0 °C). DIPEA (3.7 g, 5 mL, 28.6 mmol, 2.8 equiv.) was added to the solution in a drop-wise manner and the mixture stirred for 5 days. NH₄Cl solution (aqueous, saturated, 250 mL) was added and stirring continued for 10 min. The organic layer was separated and the aqueous layer extracted with CH₂Cl₂ (3 x 100 mL). The combined organic layers were dried (MgSO₄), evaporated under reduced pressure and the crude product (4.8 g) purified by column chromatography in 50% EtOAc in petrol to give **9** (2.5 g, 9.2 mmol) in 90% yield as a clear colourless oil.

R_f 0.29 (50% EtOAc in PE, PMA); δ_H 1.56–1.73 (2H, m, CH₂), 3.22–3.29 (1H, m, CH), 3.54–3.66 (1H, m, CH), 3.85 (1H, br s, OH), 4.36–4.47 (3H, m, CH₂ CH), 5.05 (1H, br s, NH), 5.12 (2H, s, CH₂), 7.30–7.40 (5H, m, Ph); δ_C 34.3, 37.1, 66.0, 67.4, 80.4, 128.3, 128.5, 128.8, 136.3, 157.7; ν_{max} 3404,

3034, 1692, 1553 cm^{−1}; HRMS(CI) found 269.1134, C₁₂H₁₇N₂O₅ ([M+H]⁺) requires 269.1132.

Benzyl (4-di-Boc-guanidino-3-hydroxybutyl)carbamate **11**

NiCl₂·6H₂O (5.3 g, 149 mmol, 3 equiv.) dissolved in methanol (400 mL) was cooled (0 °C) and NaBH₄ (2.5 g, 67 mmol, 9 equiv.) was added slowly in small portions in such a way as to prevent excessive foaming and following this the mixture was stirred for 45 mins. Alcohol **9** (2.00 g, 7.45 mmol) dissolved in methanol (25 mL), was then added followed by the careful addition of further NaBH₄ (5.6 g, 149 mmol, 20 equiv.) in small portions over 30 mins. After 2 h the mixture was filtered through a pad of Celite® which was washed with methanol (2 x 80 mL). Triethylamine (66 g, 92 mL, 0.65 mol, 88 equiv.) was added to the filtrate and the mixture stirred for 1 h. *N,N'*-Di-Boc-1*H*-pyrazole-1-carboxamidine **10** (2.77 g, 8.94 mmol, 1.2 equiv.) was then added and the mixture stirred for 3 days. The methanol was evaporated under reduced pressure and the resulting viscous oil dissolved in water (400 mL), which was then extracted with EtOAc (3 x 100 mL). The combined organic extracts were dried (MgSO₄) and evaporated under reduced pressure to give crude **9** (4.6 g) as a viscous purple oil. Purification by column chromatography (35% EtOAc in PE) gave **11** (2.9 g, 6.0 mmol) in an 81% yield as a glassy solid containing trace amounts of EtOAc.

R_f 0.19 (40% EtOAc in PE, PMA); δ_H 1.46 (9H, s, 3 x Me), 1.48 (9H, s, 3 x Me), 1.53–1.66 (2H, m, CH₂), 3.20–3.39 (2H, m, 2 x CH), 3.40–3.60 (2H, m, 2 x CH), 3.75–3.85 (1H, m, CH), 5.08 (2H, s, CH₂), 5.41 (1H, br s, NH), 7.27–7.34 (5H, m, Ph), 8.68 (1H, br s, NH), 11.44 (1H, br s, NH); δ_C 28.1, 28.3, 34.9, 38.0, 47.6, 66.8, 69.5, 79.7, 83.6, 128.2, 128.2, 128.6, 136.7, 153.1, 157.1, 157.3, 162.9; ν_{max} 3681, 3415, 3331, 2984, 2935, 1725, 1638 cm^{−1}; HRMS(CI) found 481.2644, C₂₃H₃₇N₄O₇ ([M+H]⁺) requires 481.2657.

(*E*)-*tert*-butyl-5-(2-(((benzyloxy)carbonyl)amino)ethyl)-2-(((*tert*-butoxycarbonyl)imino)imidazolidine-1-carboxylate **12**

Dppe (1.24 g, 3.12 mmol, 1.5 equiv.) and imidazole (0.5 g, 7.3 mmol, 3.5 equiv.) were added to a cooled (−20 °C) solution of **11** (1.00 g, 2.08 mmol) in anhydrous CH₂Cl₂ (20 mL) and the mixture stirred to ensure dissolution. At this point finely powdered iodine (0.8 g, 3.1 mmol, 1.5 equiv.) was added and the mixture stirred for 2 h. The reaction mixture was diluted with CHCl₃ (200 mL) then washed with NH₄Cl solution (aqueous, saturated, 200 mL) and brine (200 mL). The organic layer was dried (MgSO₄) and evaporated under reduced pressure to give the crude product (2.7 g). This was dissolved in CH₂Cl₂ (10 mL) and diethyl ether (100 mL) was added which effected the precipitation of phosphine oxide by products. After storage at −20 °C for 12 h, the solution was filtered and the filtrate evaporated to give crude **12** (1.06 g) which was used in the next step without further purification.

R_f 0.18 (40% EtOAc in PE, PMA); δ_H (partial data, NH not observed) 1.46 (9H, s, Boc), 1.49 (9H, s, Boc), 1.71–1.84 (1H, m, CH), 1.85–1.95 (1H, m, CH), 3.15–3.29 (2H, m, CH₂), 3.51 (1H, br d, *J* 12.3 Hz, CH), 3.88 (1H, dd, *J* 8.9, 12.3 Hz, CH), 4.19–4.29 (1H, m, CH), 5.06 (2H, s, CH₂), 5.25 (1H, br s, NH) 7.25–7.35 (5H, m, Ph); δ_C (partial data, 2 x quaternary C not observed) 28.1, 34.3, 36.9, 51.2, 54.5, 66.7, 81.4, 84.0, 128.1, 128.1, 128.5, 136.5, 150.7, 156.5, 166.1; ν_{max} 3332, 2982, 2933, 1712, 1645 cm^{−1}; HRMS(CI) found 463.2545, C₂₃H₃₅N₄O₆ ([M+H]⁺) requires 463.2551.

2-(2-iminoimidazolidin-4-yl)ethanamine hydrochloride **13**

A solution of crude **12** (0.60 g) in anhydrous methanol (15 mL) together with 10% Pd/C (0.50 g) were stirred under an atmosphere of H₂ (1 atm) gas for 24 h. The reaction mixture was then filtered through a pad of Celite® which was washed with further methanol. Evaporation of the filtrate under reduced pressure gave a yellow viscous oil (0.42 g) to which aqueous HCl (3 M, 10 mL) was added, and the mixture stirred for 24 h. At this point the solution was filtered through a small pad of Celite® (to remove final traces of phosphine oxide impurities) and evaporated under reduced pressure. The product was dried under reduced pressure (P₂O₅) to give crude **13** (0.26 g) which was used in the next reaction without further purification.

δ_{H} (DMSO *d*₆) 1.80–1.89 (2H, m, CH₂), 2.75–2.91 (2H, m, CH₂), 3.23 (1H, dd *J* 6.5, 9.5 Hz, CH), 3.69 (1H, dd *J* 9.5, 9.5 Hz, CH), 3.99–4.07 (1H, m, CH), 7.89 (2H, s, NH₂), 8.05 (1H, s, NH), 8.29 (3H, br s, NH₃⁺), 8.49 (1H, s, NH); δ_{C} (DMSO *d*₆) 32.4, 35.3, 47.7, 52.3, 159.2; ν_{max} 3685, 3375, 1687, 1520, 1477, 1438, 1334, 1215, 1122, 1027 cm⁻¹; HRMS(CI) found 129.1131, C₅H₁₃N₄ ([M+H]⁺) requires 129.1135.

Tiruchanduramine 1

Carboxylic acid **2** (250.0 mg, 1.17 mmol, 1 equiv.) was suspended in anhydrous DMF:THF (1:1, 30 mL) and stirred for 1 h. CDI (220.0 mg, 1.29 mmol, 1.1 equiv.) was added and the mixture stirred for 7 h. This solution was then added via cannula to a cooled (0 °C) solution of crude **12** (0.26 g) and triethylamine (263 mg, 0.38 mL, 2.6 mmol, 2.2 equiv.) in DMF:THF (1:1, 30 mL). The resulting mixture was warmed slowly to rt and stirred for 7 days, during which time the solution became orange in colour. Methanol (20 mL) was added and the mixture stirred for 45 min. After evaporation under reduced pressure (freeze-dried) a crude product (0.56 g) was obtained as a dark brown solid. A sample (144.0 mg) of this was purified on an ACE 10 C18 250 x 21.8 mm column (HiChrom) using 80:20 water:acetonitrile with 0.01% TFA for 2 min increasing to 50:50 over 13 min with a flow rate of 15 mL/min (monitored at 250 nm) to give tiruchanduramine hydrochloride **1** (10.0 mg) in 11.5% yield over three steps. Preparative TLC on a sample (205 mg) gave tiruchanduramine hydrochloride **1** (13.2 mg) as a solid in 8.5% yield over three steps (approx. 95% purity by ¹H NMR).

δ_{H} (DMSO *d*₆) 1.82 (2H, apparent q, *J* 6.6 Hz, CH₂), 3.27 (1H, dd, *J* 7.0, 9.5 Hz, CH), 3.35–3.48 (2H, m, CH₂), 3.73 (1H, apparent t, *J* 9.5 Hz, CH), 3.93–4.01 (1H, m, CH), 7.27–7.33 (1H, m, CH), 7.64 (1H, s, CH), 7.66 (1H, d, *J* 8.6 Hz, CH), 7.84 (2H, br s, 2 x NH), 7.99 (1H, br s, NH), 8.20 (1H, br s, NH), 8.38 (1H, d, *J* 7.9 Hz, CH), 8.83 (1H, d, *J* 1.0 Hz, CH), 8.89 (1H, d, *J* 0.8 Hz, CH), 8.91 (1H, t, *J* 6.2 Hz, NH), 12.05 (1H, br s, NH); δ_{H} (CD₃OD) 1.97–2.01 (2H, m, CH₂), 3.45 (1H, dd, *J* 6.8, 9.5 Hz, CH), 3.56–3.67 (2H, m, CH₂), 3.87 (1H, apparent t, *J* 9.5 Hz, CH), 4.12–4.18 (1H, m, CH), 7.41–7.44 (1H, m, CH), 7.70–7.73 (2H, m, 2 x CH), 8.33 (1H, d, *J* 8.0 Hz, CH), 8.99 (1H, s, CH), 9.01 (1H, s, CH); δ_{C} (DMSO *d*₆) 35.0, 35.3, 48.0, 53.0, 112.3, 114.0, 120.0, 120.9, 122.2, 128.1, 128.6, 132.3, 137.2, 139.6, 141.1, 159.2, 165.2; ν_{max} 3304, 2970, 2919, 1681, 1656, 1648, 1560, 1501, 1462, 1254, 1203, 1124, 1073, 845, 750 cm⁻¹; HRMS(CI) found 323.1609, C₁₇H₁₉N₆O ([M+H]⁺) requires 323.1615.

Method for determining activity¹¹

All enzymes and *para*-nitrophenyl substrates were purchased from Sigma. Enzymes were assayed at 27 °C in 0.1 M citric acid/0.2 M disodium hydrogen phosphate buffers at the optimum pH for the enzyme. The incubation mixture consisted of 10 µL enzyme solution, 10 µL of 1 mg/mL aqueous inhibitor solution and 50 µL of

the appropriate 5 mM *para*-nitrophenyl substrate made up in buffer at the optimum pH for the enzyme. The reactions were stopped by addition of 70 µL 0.4 M glycine (pH 10.4) during the exponential phase of the reaction, which had been determined at the beginning using uninhibited assays in which water replaced inhibitor. Final absorbances were read at 405 nm using a Versamax microplate reader (Molecular Devices). Assays were performed in triplicate.

References

- 1 Ravinder K, Reddy AV, Krishnaiah P, Ramesh P, Ramakrishna S, Laatsch H, Venkateswarlu Y. *Tetrahedron Lett.* 2005;46: 5475–5478.
- 2 Kim J-S, Kwon C-S, Son KH. *Biosci. Biotechnol. Biochem.* 2000;64: 2458–2461.
- 3 Lippke KP, Schunack WG, Wenning W, Müller WE. *J. Med. Chem.* 1983;26: 499–503.
- 4 Geall AJ, Blagbrough IS. *Tetrahedron.* 2000;56: 2449–2460.
- 5 Dickschat JS, Citron CA, Brock NL, Riclea R, Kuhz H. *Eur. J. Org. Chem.* 2011: 3339–3346.
- 6 Evans DM, Murphy PJ. *Chem. Commun.* 2011;47: 3225–3226.
- 7 Evans DM, Horton PN, Hursthouse, MB, Murphy PJ. *RSC Adv.* 2014;4: 20744–20751.
- 8 Al Shuhaib Z, Davies DH, Dennis M, Evans DM, Fletcher MD, Franken H, Hancock P, Hollinshead J, Jones I, Kähm K, Murphy PJ, Nash R, Potter D, Rowles R. *Tetrahedron* 2014;29: 4412–4419.
- 9 Wenyan Y, Sarma PVVS, Ma J, Han D, Chen JL, Cook JM. *Tetrahedron Lett.* 2005;46: 6363–6368.
- 10 Emiliani C, Urbanelli L, Racanicchi L, Orlicchio A, Pelicci G, Sorbu S, Bernardi G, Orlicchio A. *J. Biol. Chem.* 2003;278: 38453–38456.
- 11 Watson AA, Nash RJ, Wormald MR, Harvey DJ, Dealler S, Lees E, Asano N, Kizu H, Kato A, Griffiths RC, Cairns AJ, Fleet GWJ. *Phytochemistry* 1997;46:255–259.
- 12 Nash RJ, Kato A, Yu C-Y, Fleet GWJ. *Future Med. Chem.* 2011;3: 1513–1521.
- 13 A potential synthetic approach to the amine **13** has been reported via an analogous nitrile derivative however the preparation of **1** from this intermediate was not reported: Daniel M, Blanchard F, Nocquet-Thibault S, Cariou K, Dodd RH. *J. Org. Chem.* 2015;80: 10624–10633.