HETEROCYCLES, Vol. 100, No. 4, 2020, pp. 609 - 621. © 2020 The Japan Institute of Heterocyclic Chemistry
Received, 14th February, 2020, Accepted, 10th March, 2020, Published online, 19th March, 2020
DOI: 10.3987/COM-20-14236

THE SYNTHESIS AND GLYCOSIDASE INHIBITORY ACTIVITY OF ANALOGUES OF TIRUCHANDURAMINE

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Abstract – Five analogues (**7a-e)** of the metabolite tiruchanduramine **1** were prepared. Compounds **7d** and **7e** were specific inhibitors of yeast α-glucosidase, whilst **7e** specifically inhibited *Bacillus* α-glucosidase. Compounds **7b** and **7c** were the best inhibitors of β-glucosidase. All 5 compounds inhibited β-galactosidase, but once again **7e** was the best inhibitor. It was apparent that different chain lengths in the compounds **7a-e** affect the degree of inhibition. Some of the synthetic analogues show significantly improved inhibition of α-glucosidases when compared to tiruchanduramine **1** and also improved specificity.

INTRODUCTION

The alkaloid tiruchanduramine **1** (Figure 1) was isolated by Ravinder *et al*.[1] from the ascidian *Synoicum 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 reported to be a potent α-glucosidase inhibitor with an IC_{50} of 78.2 μg/mL which compares well with acarbose (IC_{50} of 100 μg/mL), a drug commonly used to treat type 2 diabetes. However, neither compounds can really be considered potent inhibitors of the α-glucosidase enzyme as reported. Both Ravinder *et al*.[1] and our own group[2] reported syntheses of **1** in racemic form. We also reinvestigated the activity of racemic **1** and found it to be not a very specific inhibitor. It was inhibitory to *Bacillus* α-glucosidase, yeast α-glucosidase, jack bean hexosaminidase, bovine hexosaminidase, and

β-galactosidase, whereas α-galactosidase and α-mannosidase were not inhibited.[2] Our results confirmed the α-glucosidase inhibition reported by Ravinder *et al*[1] although racemic **1** was only a weak to moderate inhibitor of the two α-glucosidases tested. The hexosaminidase activity was of interest as it is elevated in many diseases including Alzheimer's disease.[3] Specific α-glucosidase inhibitors have a wide range of potential health applications[4] and have been shown to be inhibitory to distinctive glucosidases of haematophagous ecto-parasites (e.g. *Rhodnius prolixus*) which can utilize α-glucosidase to detoxify haemoglobin-derived heme.[5]

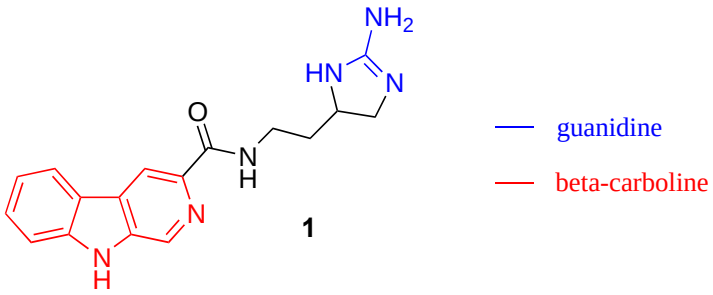
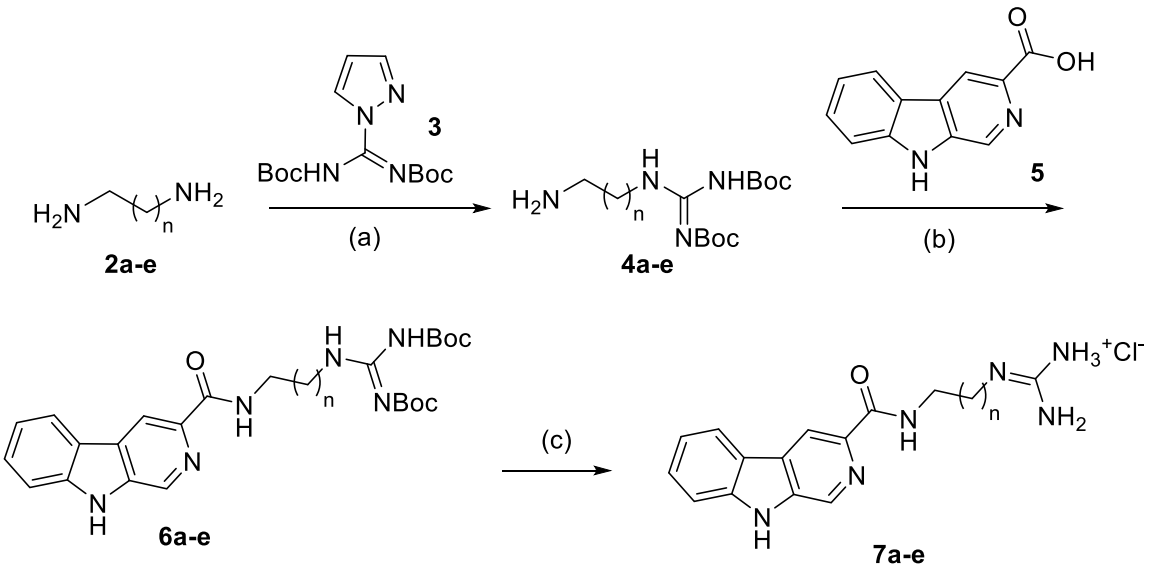


Figure 1. Structure of tiruchanduramine **1**

We were interested in investigating the glycosidase inhibitory and potential anti-parasitic (endo-parasite *Schistosoma mansoni*) activities further and embarked on the preparation of a range of analogues **7a-e** of **1**. We envisaged these as containing the key structural motifs found in the natural material namely the *β*-carboline core and a guanidine separated by a side chain amide linker. The synthesis of these analogues should be possible via the coupling of the protected guanidines **4a-e** with the carboxylic acid **5** leading to **6a-e** using the strategy adopted in our total synthesis of **1**. Guanidines **4a-e** should be easily prepared from commercially available diamines **2a-e** and the guanidinylating agent **3** and acidic deprotection of **6a-e** will lead to the desired compounds **7a-e**. (Scheme 1)



Scheme 1. Synthesis of **7a-e**: Reagents and conditions: (a) **2a-e**, CH_2Cl_2 or MeCN, 0 °C-rt, **3**, 2-22 h; 35-68%. (b) (i) CDI, DMF, 30 min; (ii) **4a-e**, 20-36 h; 17-66%. (c) 2M HCl/MeOH, 3-4 h; 77-96%. For structures **2**, **4**, **6** and **7**: **a**, n = 1; **b**, n = 2; **c**, n = 3; **d**, n = 4; **e**, n = 5.

RESULTS AND DISCUSSION

The mono-guanidinylated amines **4a-e** are known compounds, prepared previously using *N*,*N*'-di-Boc-*S*-methylisothiourea, which generates methane thiol during the reaction.[6] However we wished to use the more convenient reagent **3** which had only previously been used in the preparation of compound **4c**[7] and in our hands treatment of amines **2a-e** with **3** in CH_2Cl_2 or MeCN gave **4a-e** in 35-68% yield. These compounds were then coupled with the known[2] carboxylic acid **5** using 1,1'-carbonyldiimidazole (CDI) in DMF as the coupling agent. After an extractive work-up, chromatography in ethyl acetate and chloroform gave **6a-e** in 17-66% yield. The deprotection of the Boc-protecting groups in **6a** was initially attempted using aqueous 3 M HCl. Unfortunately, this led to the decomposition of the substrate and the carboxylic acid **5** was the only identifiable product isolated. The use of trifluoroacetic acid in dichloromethane was also investigated and again this led to a mixture of products which appeared to contain an inseparable impurity in which the ^{13}C signal for the guanidinium carbon was absent. This hydrolysis of the guanidine to the corresponding amine under acidic conditions had been observed in related compounds.[8] Ravinder had reported[1] a similar deprotection in the synthesis of tiruchanduramine **1** using a 2 M solution of HCl in methanol over 4 hours. We employed these conditions and obtained **7a** in 93% yield and similarly prepared **7b-e** in 77-96% yield after purification.

ASSAYS

The analogues of tiruchanduramine, **7a-e**, were submitted to assays on a panel of glycosidases at 143 μg/mL; results are summarised in Table 1. Whereas **7a-c** gave 30 to 50% inhibition of yeast α-glucosidase, **7d** and **7e** inhibited significantly more (87% and 94%, respectively). However, only **7e** inhibited *Bacillus* α-glucosidase more than 50%, at 72%, whilst with **7d** inhibition was only 30%. Thus, it appears that the different chain lengths affect the degree of inhibition. Indeed, **7e** was the best inhibitor of yeast α-glucosidase, with an IC_{50} of 7.5 μM followed by **7d** with an IC_{50} of 12 μM. Interestingly, the best inhibitors of β-glucosidase were **7b** and **7c**, both with about 60-65% inhibition. IC_{50} determination showed **7b**, at 54 μM, to be a better inhibitor of β-glucosidase than **7c**, at 119 μM. The other enzymes assayed were bovine β-galactosidase, hexosaminidase and β-glucuronidase. Of these, only the β-galactosidase was inhibited and this was over 80% by all of the compounds, **7a-e**. Once again, **7e** was the best inhibitor with an IC_{50} of 2.6 μM, but this was closely followed by **7c** with a value of 6.4 μM. Compounds **7a**, **7b** and **7d** had IC_{50} values of 53 μM, 25 μM and 12 μM, respectively.

Table 1. Glycosidase assays on compound **7a** - **7e**

Compound	% Inhibition(i)	IC_{50} (μM)	% Inhibition(i)	% Inhibition(i)	IC_{50} (μM)
Enzyme	α-D-glucosidase	α-D-glucosidase	α-D-glucosidase	β-D-glucosidase	β-D-glucosidase
Source	Yeast	Yeast	*Bacillus*	almond	almond
7a	38		31	15	
7b	45		46	66	54
7c	36		26	62	119
7d	87	12	30	44	
7e	94	7.5	72	52	

Compound	% Inhibition(i)	IC_{50} (μM)	% Inhibition(i)	% Inhibition(i)
Enzyme	β-D-galactosidase	β-D-galactosidase	N-acetyl-β-D-glucosaminidase	β-glucuronidase
Source	bovine liver	bovine liver	bovine kidney	bovine liver
7a	82	53	<5	<5
7b	87	25	<5	<5
7c	92	6.4	<5	<5
7d	85	12	21	<5
7e	89	2.6	<5	<5

(i) Measured at 143 μg/mL.

With glucosidase inhibitors being reported to be inhibitory to distinctive glucosidases of haematophagous parasites, compounds **7a-e** were also screened against *S. mansoni* schistosomula parasites at a final concentration of 10 μM for a period of 72 hours using an automated high throughput anthelmintic platform.[9] While these immature *S. mansoni* do not feed on blood, we hypothesised that distinctive parasite glucosidases may have other important functions in the parasite. The motility and phenotype scores were evaluated, and any compounds that fell within the 'hit zone' (whereby motility values were below -0.35 and phenotype values lower than -0.15) were considered active. Of the five compounds screened, none fell within the hit zone and did not affect either motility or phenotype of the schistosomula (See SI; Figure 2). As expected, Praziquantel (PZQ) fell within the hit region after 72 hours as did the other positive control Auranofin (AUR), while the negative control (DMSO) fell outside this hit region. The phenotype of each of the tested compounds were compared visually to that of the positive and negative controls (See SI; Figure 3). The phenotype of the five tested compounds were similar to that observed in the DMSO control, which is located in the non-hit region, as opposed to the positive hit controls AUR and PZQ.

CONCLUSION

Although tiruchanduramine **1** is an inhibitor of α-glucosidases, it is not very specific, not very potent and also inhibits other glycosidases.[1,2] We have previously shown that both the racemic and non-racemic compound have similar activities.[2] This lack of specificity probably helps to give broad spectrum protection of the producing organism from predators but makes the molecule less good as a potential pharmaceutical compound. The synthetic analogues **7a-e** have significantly improved inhibition of α-glucosidases in some case and have improved specificity over racemic tiruchanduramine **1**. It was shown[2] that the yeast α-glucosidase inhibition of racemic **1** (not reaching 50% inhibition at 0.4 mM) could be improved by extending the chain length as in **7e** to achieve an IC_{50} of 7.5 μM. Compound **7e** was also weakly inhibitory to the β-glucosidase used here and so more selectively inhibitory to α-glucosidase than **1**.[2] However, all compounds were potent inhibitors of the β-galactosidase with **7e** having an IC_{50} of 2.6 μM. The compounds did not appear to affect immature stages of *S. mansoni*.

EXPERIMENTAL

Column chromatography was carried out on silica gel (60Å, 40-63 μm) 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.

Et_2O, THF and CH_2Cl_2 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 $CHCl_3$ (7.26/77.16 ppm), MeOH (3.31/49.0 ppm) and DMSO (2.50/39.52 ppm) as internal standards. Proton and carbon NMR spectra were recorded in $CDCl_3$ 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.

Preparation of guanidines 4a-e.

General details: Diamine **2a-e** (5 equiv.) dissolved in the reaction solvent (50 mL) was cooled (0 °C) and *N,N'*-di-Boc-1*H*-pyrazole-1-carboxamidine **3** (1 equiv.) was added in small portions over 5 min. The mixture was stirred for 2-22 h and followed by TLC until pyrazole **3** (Rf 0.31 in 25% EtOAc in PE) was consumed. The reaction was diluted with water (50 mL), then extracted with CH_2Cl_2 (2 x 50 mL). The combined organic phases were washed with water (5 x 500 mL) to remove unreacted diamine **2a-e**; back washing of each aqueous layer with a small volume of DCM was performed). Drying ($MgSO_4$) and evaporated *in vacuo* gave the amines **4a-e** which were used without further purification, data was in agreement with the literature.[6]

***N,N'*-Bis-Boc-*N''*-(2-aminoethyl)guanidine 4a:** Diamine **2a** (2.9 g, 48.3 mmol, 5.0 equiv.), CH_2Cl_2, *N,N'*-di-Boc-1*H*-pyrazole-1-carboxamidine **3** (3.0 g, 9.67 mmol, 1.0 equiv.), 22 h; **4a** (1.24 g, 4.10 mmol, 43%).

***N,N'*-Bis-Boc-*N''*-(3-aminopropyl)guanidine 4b:** Diamine **2b** (7.17 g, 96.7 mmol, 10.0 equiv.), MeCN, *N,N'*-di-Boc-1*H*-pyrazole-1-carboxamidine **3** (3.0 g, 9.67 mmol, 1.0 equiv.), 18 h; amine **4b** (1.04 g, 1.4 mmol, 35%).

***N,N'*-Bis-Boc-*N''*-(4-aminobutyl)guanidine 4c:** Diamine **2c** (4.26 g, 48.3 mmol, 5.0 equiv.), CH_2Cl_2, *N,N'*-di-Boc-1*H*-pyrazole-1-carboxamidine **3** (3.0 g, 9.67 mmol, 1.0 equiv.), 22 h; amine **4c** (3.10 g, 9.38 mmol, 68%).

***N,N'*-Bis-Boc-*N''*-(5-aminopentyl)guanidine 4d:** Diamine **2d** (5.00 g, 48.9 mmol, 4.8 equiv.), CH_2Cl_2, *N,N'*-di-Boc-1*H*-pyrazole-1-carboxamidine **3** (3.15 g, 10.2 mmol, 1.0 equiv.), 2 h; amine **4d** (1.26 g, 3.66 mmol, 38%).

***N,N'*-Bis-Boc-*N''*-(6-aminohexyl)guanidine 4e:** Diamine **2e** (5.82 g, 50.1 mmol, 5.18 equiv.), CH_2Cl_2, *N,N'*-Di-Boc-1*H*-pyrazole-1-carboxamidine **3** (2.99 g, 9.63 mmol, 1.0 equiv.), 2 h; amine **4e** (2.08 g, 5.80 mmol, 60%).

Preparation of amides 6a-e

General details: 9*H*-Pyrido[3,4-*b*]indole-3-carboxylic acid **5** (1.1 equiv.) was suspended in dry DMF (ca. 3 mL per mmol of **5**), CDI (1.78 equiv.) was added and the mixture stirred until the solids dissolved (with gentle warming if needed and if precipitation occurs). A solution of amine **4a-e** in DMF (2-3 mL per mmol) was added and the mixture stirred for 20-36 h. Water (1-2 mL) was added and the mixture stirred for 30 min. Further water (50 mL) and EtOAc (200 mL) were added, and the resultant layers separated. After extraction of the aqueous layer with EtOAc (2 x 100 mL), the combined organic phases were washed with water (3 x 200 mL) and then brine (200 mL). After drying ($MgSO_4$) and evaporation in *vacuo,* silica gel column chromatography gave **6a-e** as solids.

***N*-(2-(*N',N''*-Bis-Boc-guanidino)ethyl)-9*H*-pyrido[3,4-*b*]indole-3-carboxamide 6a:** Carboxylic acid **3** (0.811 g, 3.82 mmol, 1.1 equiv.), CDI (1.00 g, 6.18 mmol, 1.78 equiv.), **4a** (1.06 g, 3.51 mmol, 1.0 equiv.): SGCC gradient elution with 10-75% EtOAc in $CHCl_3$ gave **6a** (0.293 g, 0.527 mmol, 17% yield). Mp 252 °C (dec); **Rf** 0.19 (40% EtOAc in $CHCl_3$); **δ_H** 11.50 (1H, s, NH), 9.38-9.60 (1H, br m, NH), 8.84 (1H, s, CH), 8.75 (1H, s, CH), 8.62 (1H, s, NH), 8.29 (1H, s, NH), 8.10 (1H, d, *J* 7.7 Hz, CH), 7.51-7.61 (2H, m, 2 x CH), 7.23-7.35 (1H, m, CH), 3.63-3.74 (4H, m, 2 x CH_2), 1.48 (9H, s, 3 x Me), 1.45 (9H, s, 3 x Me); **δ_C** (101 MHz, $CDCl_3$) 166.3, 163.6, 156.8, 153.2, 141.0, 140.0, 137.4, 131.9, 129.5, 128.9, 122.2, 121.9, 120.8, 114.6, 112.0, 83.4, 79.6, 40.6, 39.1, 28.4, 28.1; ν_{max} (solid) 3314, 3286, 3272, 3152, 2977, 2932, 1977, 1721, 1613, 1530, 1460, 1411, 1365, 1330, 1246, 1228, 1130, 1048, 1018, 900, 875, 849, 807, 774, 747, 730, 664, 631, 607, 593, 480, 424; **MS(CI)** 1015.5 (40% $[2M+Na]^+$), 519.2 (100% $[M+Na]^+$), 497.3 (50% $[M+H]^+$), 397.2 (48% $[M-Boc+H]^+$), 297.1 (93% $[M-2Boc+H]^+$); **HRMS(CI)** found 497.2501; $C_{25}H_{33}N_6O_5$ ($[M+H]^+$) requires 497.2507.

***N*-(3-(*N',N''*-Bis-Boc-guanidino)propyl)-9*H*-pyrido[3,4-*b*]indole-3-carboxamide 6b:** Carboxylic acid **5** (0.923 g, 4.35 mmol, 1.0 equiv.), CDI (1.26 g, 7.77 mmol, 1.78 equiv.), DMF, **4b** (1.51 g, 4.78 mmol, 1.1 equiv.), SGCC gradient elution 10-30% EtOAc in $CHCl_3$ gave **6b** (0.800 g, 1.57 mmol, 36% yield). Mp 226 °C (dec); **Rf** 0.21 (40% EtOAc in $CHCl_3$); **δ_H** 11.51 (1H, s, NH), 9.28 (1H, br s, NH), 8.87 (1H, s, CH), 8.76 (1H, s, CH), 8.56 (1H, t, *J* 5.0 Hz, NH),

8.27 (1H, t, *J* 6.0 Hz, NH), 8.13 (1H, d, *J* 7.9 Hz, CH), 7.50-7.61 (2H, m, 2 x CH), 7.28-7.37 (1H, m, CH), 3.48-3.59 (4H, m, 2 x CH_2), 1.85-1.94 (2H, m, CH_2) 1.47 (9H, s, 3 x Me), 1.45 (9H, s, 3 x Me); **δC** (101 MHz, $CDCl_3$) 166.1, 163.7, 156.6, 153.3, 140.9, 140.4, 137.3, 131.9, 129.6, 129.0, 122.3, 122.0, 120.9, 114.6, 111.9, 83.3, 79.4, 38.6, 37.1, 29.8, 28.4, 28.2; ν_{max} (solid) 3372, 3326, 3141, 2978, 2937, 2871, 2056, 2012, 1951, 1720, 1640, 1614, 1592, 1575, 1536, 1499, 1485, 1460, 1418, 1386, 1361,1328, 1295, 1251, 1230, 1135, 1110, 1051, 1019, 922, 890, 878, 861, 850, 814, 775, 758, 742, 696, 662, 631, 621, 586, 504, 486, 462, 440, 425, 407; **MS(CI)** 511.3 (100%, $[M+H]^+$), 411.2 (16%, $[M+H\text{-}Boc]^+$), 311.2 (50%, $[M+H\text{-}2Boc]^+$); **HRMS(CI)** found 511.2654, $C_{26}H_{35}N_6O_5$ ($[M+H]^+$) requires 511.2663.

***N*-(4-(*N',N''*-Bis-Boc-guanidino)butyl)-9*H*-pyrido[3,4-*b*]indole-3-carboxamide 6c:** Carboxylic acid **5** (0.742 g, 3.50 mmol, 1.0 equiv.), CDI (1.01 g, 6.22 mmol, 1.78 equiv.), DMF, **4c** (1.38 g, 4.18 mmol, 1.0 equiv.); SGCC, gradient elution in 10-30% EtOAc in $CHCl_3$ gave **6c** (1.04 g, 1.98 mmol, 57%). Mp 148-50 °C (dec); **Rf** 0.28 (40% EtOAc in $CHCl_3$); **δH** 11.50 (1H, s, NH), 10.22 (1H, s, NH), 8.90 (1H, s, CH), 8.80 (1H, s, CH), 8.37 (1H, t, *J* 5.2 Hz, NH), 8.18 (1H, t, *J* 6.1 Hz, NH), 8.09 (1H, d, *J* 7.9 Hz, CH), 7.45-7.60 (2H, m, 2 x CH), 7.24-7.29 (1H, m, CH), 3.45 (2H, dt, *J* 6.1, 6.4 Hz, CH_2), 3.37 (2H, dt, *J* 5.1, 7.9 Hz, CH_2), 1.50-1.67 (4H, m, 2 x CH_2), 1.45 (18H, s, 6 x Me); **δC** 165.9, 163.6, 156.3, 153.3, 141.2, 139.9, 137.5, 132.1, 129.4, 128.8, 122.1, 121.8, 120.6, 114.5, 112.1, 83.3, 79.5, 40.7, 39.1, 28.3, 28.1, 27.2, 26.7. ν_{max} (thin film) 3328, 3287, 3212, 3170, 3097, 2980, 2936, 2869, 1722, 1651, 1645, 1622, 1579, 1537, 1500, 1463, 1416, 1368, 1336, 1254, 1230, 1158, 1136, 1053, 1027, 911, 732, 646, 611; **MS(CI)** 1071.5 (20% $[2M+Na]^+$), 525.3 (55% $[M+H]^+$), 425.2 (24% $[M\text{-}Boc+H]^+$), 325.2 (100% $[M\text{-}2Boc+H]^+$); **HRMS(CI)** found 525.2809, $C_{27}H_{37}N_6O_5$ ($[M+H]^+$) requires 525.2820.

***N*-(5-(*N',N''*-Bis-Boc-guanidino)pentyl)-9*H*-pyrido[3,4-*b*]indole-3-carboxamide 6d:** Carboxylic acid **5** (0.200 g, 0.94 mmol, 1.0 equiv.), CDI (0.286 g, 1.76 mmol, 1.8 equiv.), DMF, **4d** (0.35 g, 1.02 mmol, 1.1 equiv.); SGCC gradient elution 10-30% EtOAc in $CHCl_3$ gave **6d** (0.337 g, 0.63 mmols, 66%). Mp 148-50 °C (dec); **Rf** 0.25 (40% EtOAc in $CHCl_3$); **δH** 11.50 (1H, s, NH), 9.22-947 (1H, br m, NH), 8.91 (1H, s, CH), 8.79 (1H, s, CH), 8.34 (1H, t, *J* 5.7 Hz, NH), 8.13-8.19 (2H, m, NH and CH), 7.49-7.60 (2H, m, 2 x CH), 7.36 (1H, br t, *J* 6.0 Hz, CH), 3.47 (2H, app. q, *J* 6.4 Hz, CH_2), 3.37 (2H, dt, *J* 5.7, 5.9 Hz, CH_2), 1.55-1.65 (4H, m, 2 x CH_2), 1.48 (9H, s, 3 x Me), 1.47 (9H, s, 3 x Me), 1.32-1.44 (2H, m, CH_2); **δC** 165.7, 163.7, 156.3, 153.4, 141.0, 140.5, 137.3, 131.9, 129.7, 129.0, 122.3, 122.0, 120.8, 114.5, 111.9, 83.2, 79.5, 40.9, 39.4, 29.6, 28.9, 28.4, 28.2, 24.4; ν_{max} (solid) cm^{-1}: 3327, 3286, 3208, 3144, 2978, 2933, 2866, 2182,

2067, 1719, 1614, 1533, 1498, 1460, 1413, 1365, 1331, 1288, 1250, 1227, 1155, 1129, 1051, 1019, 899, 876, 851, 807, 789, 747, 729, 664, 630, 607, 593, 481, 460, 425; **MS(CI)** 1099.6 (13% $[2M+Na]^+$), 539.3 (77% $[M+H]^+$), 439.2 (25% $[M\text{-}Boc+H]^+$), 339.2 (100% $[M\text{-}2Boc+H]^+$); **HRMS(CI)** found 539.2967; $C_{28}H_{39}N_6O_5$ ($[M+H]^+$) requires 539.2976.

***N*-(6-(*N'*,*N''*-Bis-Boc-guanidino)hexyl)-9*H*-pyrido[3,4-*b*]indole-3-carboxamide 6e:** Carboxylic acid **3** (0.20 g, 0.94 mmol, 1.0 equiv.), CDI (0.309 g, 1.91 mmol, 2.02 equiv.), DMF, **4e** (0.351 g, 0.98 mmol, 1.04 equiv.); SGCC gradient elution in 10-50% EtOAc in $CHCl_3$ gave **6e** (0.130 g, 0.24 mmol, 25%) as a glass. **Rf** 0.30 (40% EtOAc in $CHCl_3$); **δ_H** 11.50 (1H, s, NH), 10.14 (1H, s, NH), 8.92 (1H, s, CH), 8.80 (1H, s, CH), 8.33 (1H, t, *J* 4.7 Hz, NH), 8.22 (1H, t, *J* 5.8 Hz, NH) 8.12 (1H, d, J 7.9 Hz, CH), 7.50-7.57 (2H, m, 2 x CH), 7.26-7.31 (1H, m, CH), 3.45 (2H, dt, *J* 5.8, 7.1 Hz, CH_2), 3.30 (2H, dt, *J* 4.7, 7.5 Hz, CH_2), 1.53-1.62 (2H, m, CH_2), 1.47 (9H, s, 3 x Me), 1.45 (9H, s, 3 x Me), 1.45-1.52 (2H, m, CH_2), 1.20-1.35 (4H, m, 2 x CH_2); **δ_C** 165.9, 163.6, 156.3, 153.4, 141.2, 140.1, 137.5, 132.1, 129.4, 128.8, 122.2, 121.8, 120.6, 114.5, 112.0, 83.3, 79.5, 41.0, 39.6, 29.7, 29.0, 28.4, 28.2, 26.8, 26.6; ν_{max} (solid) 3325, 3286, 3212, 2981, 2930, 2857, 1721, 1615, 1536, 1500, 1462, 1414, 1367, 1333, 1284, 1251, 1228, 1155, 1131, 1053, 1026, 901, 875, 851, 808, 747, 665, 631, 594, 520, 480, 424; **MS(CI)** 1127.6 (5 % $[2M+Na]^+$), 553.3 (92% $[M+H]^+$), 453.3 (32% $[M\text{-}Boc+H]^+$), 353.2 (100% $[M\text{-}2Boc+H]^+$); **HRMS(CI)** found 553.3125; $C_{29}H_{41}N_6O_5$ ($[M+H]^+$) requires 553.3133.

Preparation of 7a-e

General details: Compound **6a-e** (1 equiv.) was dissolved in a minimum volume of 2 M HCl in MeOH (prepared from conc. HCl 16.7 mL and MeOH 83.3 mL) and stirred at room temperature for 3 h. The mixture was then evaporated in *vacuo* and dried over phosphorus pentoxide to yield the crude product. This was dissolved in a minimum volume of MeOH, filtered to remove any undissolved matter then diluted with Et_2O until the product precipitates as a white/off white solid. After decanting the supernatant liquid then washing/decanting the solids with further Et_2O, the analogue **7a-e**, were obtained after drying under vacuum over P_2O_5.

(2-(9*H*-Pyrido[3,4-*b*]indole-3-carboxamido)ethyl)guanidinium chloride dihydrate 7a: Guanidine **6a** (0.074 g, 0.149 mmol) gave **7a** (0.050 g, 0.136 mmol, 91%). Mp 283-5 °C; **δ_H** ($(CD_3)_3SO$) 12.32 (1H, br s, NH), 9.03-9.25 (2H, br m, 2 x NH), 8.95 (1H, s, CH), 8.40 (1H, d, *J* 7.9 Hz, CH), 7.76 (1H, br s, CH), 7.72 (1H, d, *J* 8.4 Hz, CH), 7.66 (1H, app. t, *J* 8.0 Hz, CH), 7.36 (1H, app. t, *J* 7.5 Hz, CH) 6.70-7.93 (4H, br s, 4 x NH), 4.44-6.36 (4H, br s, 2 x H_2O), 3.48-3.56

(2H, m, CH_2), 3.35-3.43 (2H, m, CH_2), (CD_3OD) 9.46 (1H, s, CH), 9.12 (1H, s, CH), 8.45 (1H, d, *J* 7.8 Hz, CH) 7.78-7.86 (2H, m, 2 x CH), 7.68 (1H, br s, NH), 7.52 (1H, t, *J* 6.8 Hz, CH), 3.74 (2H, t, *J* 5.3 Hz, CH_2), 3.53-3.62 (2H, m, CH_2); **δC** (CD_3OD) 162.3, 159.0, 145.9, 136.9, 134.9, 133.7, 132.5, 128.2, 124.2, 123.8, 121.6, 116.9, 114.3, 41.9, 40.4; ν_{max} (solid) 3062, 3011, 1672, 1657, 1631, 1595, 1558, 1515, 1501, 1470, 1430, 1361, 1349, 1308, 1253, 1209, 1149, 854, 758, 725, 688, 654, 583, 538, 482, 470, 417; **MS(CI)** 297.2 (100% $[M+H]^+$); **HRMS(CI)** found 297.1459, $C_{16}H_{19}N_6O$ ($[M+H]^+$) requires 297.1458.

((3-(9*H*-pyrido[3,4-*b*]indole-3-carboxamido)propyl)amino)guanidinium chloride 7b: Guanidine **6b** (0.043 g, 0.084 mmol) gave **7b** (0.027 g, 0.078 mmol, 93%). Mp 227-9 °C; **δH** (CD_3OD) 9.49 (1H, s, CH), 9.13 (1H, s, CH), 8.46 (1H, d, *J* 7.9 Hz, CH) 7.78-7.89 (2H, m, 2 x CH), 7.52 (1H, t, *J* 6.9 Hz, CH), 3.65 (2H, t, *J* 6.2 Hz, CH_2), 3.38 (2H, t, *J* 6.2 Hz, CH_2), 2.03 (2H, pentet, *J* 6.2 Hz, CH_2); **δC** (CD_3OD) 161.7, 158.7, 146.0, 136.8, 135.0, 133.8, 132.5, 128.0, 124.2, 123.8, 121.6, 116.9, 114.3, 40.1, 38.6, 29.7; ν_{max} (solid) 3155, 2921, 1627, 1598, 1555, 1511, 1467, 1429, 1348, 1320, 1256, 1212, 1173, 1144, 1112, 852, 772, 723, 584, 534, 469, 415; **MS(CI)** 311.2 (100% $[M+H]^+$); **HRMS(CI)** found 311.1616; $C_{16}H_{19}N_6O$ ($[M+H]^+$) requires 311.1615.

(4-(9*H*-Pyrido[3,4-*b*]indole-3-carboxamido)butyl)guanidinium chloride 7c: Guanidine **6c** (0.061 g, 0.116 mmol) gave **7c** (0.040 g, 0.094 mmol, 96%). Mp 179-82 °C; **δH** (CD_3OD) 9.45 (1H, s, CH), 9.10 (1H, s, CH), 8.41 (1H, d, *J* 7.4 Hz, CH) 7.74-7.86 (2H, m, 2 x CH), 7.49 (1H, t, *J* 6.7 Hz, CH), 3.60 (2H, t, *J* 6.3 Hz, CH_2), 3.31-3.37 (obscured, 2H, m, CH_2), 1.72-1.92 (4H, m, 2 x CH_2); **δC** (CD_3OD) 161.4, 158.6, 145.9, 136.6, 134.9, 133.7, 132.5, 127.9, 124.2, 123.7, 121.5, 116.7, 114.3, 42.2, 40.8, 27.5, 29.3; ν_{max} (solid) 3312, 3169, 3049, 2936, 1662, 1623, 1595, 1557, 1510, 1471, 1429, 1349, 1324, 1285, 1258, 1230, 1206, 1187, 1112, 1022, 996, 899, 865, 773, 752, 663, 591, 555, 528, 500, 471, 441, 415; **MS(CI)** 325.2 (100% $[M+H]^+$); **HRMS(CI)** found 325.1773; $C_{17}H_{21}N_6O$ ($[M+H]^+$) requires 325.1771.

(5-(9*H*-Pyrido[3,4-*b*]indole-3-carboxamido)pentyl)guanidinium chloride 7d: Guanidine **6d** (0.048 g, 0.089 mmol) gave **7d** (0.031 g, 0.083 mmol, 93%). Mp 169-71 °C; **δH** (CD_3OD) 9.44 (1H, s, CH), 9.12 (1H, s, CH), 8.46 (1H, d, *J* 7.8 Hz, CH) 7.80-7.90 (2H, m, 2 x CH), 7.53 (1H, t, *J* 6.9 Hz, CH), 3.58 (2H, t, *J* 6.9 Hz, CH_2), 3.23 (2H, t, *J* 6.9 Hz, CH_2), 1.76-1.86 (2H, m, CH_2), 1.66-1.75 (2H, m, CH_2) 1.50-1.62 (2H, m, CH_2); **δC** (CD_3OD) 161.4, 158.6, 146.0, 136.8, 135.1, 133.8, 132.8, 128.0, 124.2, 123.8, 121.6, 116.6, 114.3, 42.4, 41.2, 29.9, 29.5, 25.1; ν_{max} (solid)

3337, 3322, 3241, 3167, 3031, 2931, 2862, 1650, 1634, 1611, 1560, 1512, 1471, 1350, 1321, 1258, 1227, 1213, 1192, 851, 773, 755, 713, 644, 580, 560, 552, 527, 510, 472, 446, 419; **MS(CI)** 339.2 (100% $[M+H]^+$); **HRMS(CI)** found 339.1920; $C_{18}H_{23}N_6O$ ($[M+H]^+$) requires 339.1928.

(6-(9*H*-pyrido[3,4-*b*]indole-3-carboxamido)hexyl)guanidinium chloride 7e: Guanidine **6e** (0.069 g, 0.125 mmol) gave **7e** (0.037 g, 0.095 mmol, 76%). Mp 155-57 °C; **δ_H** (CD_3OD) 9.40 (1H, s, CH), 9.11 (1H, s, CH), 8.45 (1H, d, *J* 7.9 Hz, CH) 7.76-7.89 (2H, m, 2 x CH), 7.53 (1H, t, *J* 6.8 Hz, CH), 3.56 (2H, t, *J* 7.0 Hz, CH_2), 3.21 (2H, t, *J* 7.2 Hz, CH_2), 1.72-1.84 (2H, m, CH_2), 1.59-1.71 (2H, m, CH_2) 1.43-1.57 (4H, m, 2 x CH_2); **δ_C** (CD_3OD) 161.5, 158.6, 146.0, 136.9, 135.0, 133.7, 132.9, 128.1, 124.1, 123.7, 121.7, 116.5, 114.3, 42.4, 41.4, 30.2, 29.8, 29.6, 27.3; ν_{max} (solid) 3136, 2929, 2855, 1654, 1633, 1595, 1556, 1511, 1465, 1424, 1347, 1319, 1258, 1211, 1177, 1112, 851, 750, 722, 667, 629, 589, 559, 474, 419. **MS(CI)** 352.2 (100% $[M+H]^+$); **HRMS(CI)** found 353.2084, $C_{19}H_{25}N_6O$ ($[M+H]^+$) requires 353.2084.

Method for determining glycosidase activity[10]

All enzymes and *para*-nitrophenyl substrates were purchased from Sigma. Enzymes were assayed at 27 ºC 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. An IC_{50} value was calculated using 5 inhibitory concentrations of each compound, assayed together with duplicate water blanks. The mean percentage inhibitions were plotted against compound concentrations (expressed in μM) and the concentration giving 50% inhibition was obtained from the graph.

Screening against *Schistosoma mansoni* schistosomula

All five compounds (**7a-e)** were solubilised in DMSO (Fisher Scientific, UK) to a working concentration of 1.6 mM, and stored at -20 °C until required. Positive controls for *S. mansoni* schistosomula screens included praziquantel (Sigma-Aldrich, UK) and auranofin (Sigma-Aldrich, UK), which were also diluted in DMSO to a working concentration of 1.6 mM. Cercariae were collected from *Biomphalaria glabrata*

(NMRI strain) snails infected with *S. mansoni* (Puerto Rican strain) by shedding for 1 h under light conditions at 26 °C. Cercariae were subsequently mechanically transformed into schistosomula using methods as previously described[11] and then utilised for high throughput screening (HTS) on the Roboworm platform.[9] Schistosomula were added to each well containing each of the five compounds (10 µM in 0.625% DMSO) at a density of 120 parasites per well. Parasites were cultured at 37 °C in an atmosphere containing 5% CO_2 for 72 h with phenotype and motility of each parasite quantified using a previously described image analysis model.[12] Praziquantel (10 µM in 0.625% DMSO) and Auranofin (10 µM in 0.625% DMSO) were used as positive control treatments, whereas DMSO (0.625%) was used as a negative control. The Z' scores obtained for this screen was 0.677 and 0.522 for phenotype and motility respectively.

ACKNOWLEDGEMENTS

Thanks are given to the EPSRC for a fellowship (DE, (EP/J01821X/1) and to BEACON (ERDF) for support (DE, PJM). We also thank the EPSRC National Mass Spectrometry Facility at Swansea for their excellent work and for the Welsh Government funded Life Sciences Research Network Wales for supporting the Roboworm platform used in this study.

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