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Antischistosomal Activity of Pyrido[1,2-*a*]benzimidazole Derivatives and Correlation with Inhibition of β-haematin Formation

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ABSTRACT

The extensive use of praziquantel against schistosomiasis raises concerns about drug resistance. New therapeutic alternatives targeting critical pathways within the parasite are therefore urgently needed. Haemozoin formation in *Schistosoma* presents one such target. We assessed in vitro antischistosomal activity of pyrido[1,2-*a*]benzimidazoles (PBIs), and investigated correlations with their ability to inhibit β-haematin formation. We further evaluated the in vivo efficacy of representative compounds in experimental mice and conducted pharmacokinetic analysis on the most potent. At 10 μM, 48/57 compounds resulted in >70% mortality of newly transformed schistosomula, while 37 of these maintained > 60% mortality of adult *S. mansoni*. No correlations were observed between β-haematin inhibitory and antischistosomal activities against both larval and adult parasites, suggesting possible presence of other target(s) or a mode of inhibition of crystal formation that is not adequately modelled by the assay. The most active compound in vivo showed 58.7% and 61.3% total and female worm burden reduction, respectively, and further pharmacokinetic analysis hinted at solubility-limited absorption and high hepatic clearance as possible contributors to the modest efficacy despite good in vitro activity. The PBIs evaluated in this report thus merit further optimization to improve their efficacy and to elucidate their possible mode of action.

Keywords

Schistosoma mansoni, Pyrido[1,2-*a*]benzimidazoles, Praziquantel, Pharmacokinetics, Haemozoin, Beta-haematin

Schistosomiasis is a major public health concern, with ~700 million people at risk in 74 countries and over 200 million infected.[1] One of the three main etiological agents of this infection is the parasitic digenetic trematode *Schistosoma mansoni,* known to digest large amounts of host blood, in its adult stage, for nutritional supplementation and completion of its sexual development.[2] This process is characterised by multi-enzymatic degradation of host-derived haemoglobin,[3] leading to formation of peptides, essential amino acids and haem.[4]

Haem is an amphiphilic molecule that serves as the prosthetic group of numerous haemoproteins and is essential in such biological processes as control of protein synthesis, cell differentiation and respiration as well as drug detoxification.[5] In its free state, however, haem acts as a pro-oxidant able to induce free radical formation [6] as well as compromise phospholipid membrane integrity.[7] Blood-feeding parasites, such as *S. mansoni* and *Plasmodium falciparum*, have consequently evolved various detoxification mechanisms to rid themselves of the toxicity of free haem, one of the most efficient of which involves biocrystallization of haem molecules into inert and insoluble haemozoin (Hz).[8-10] Indeed, in *S. mansoni*, haem crystallization as a preventive antioxidant defence has been documented [11] and evidence of large amounts of Hz within the gut of adult female worms has been reported.[12] Furthermore, earlier findings where treatment of *Schistosoma*-infected mice with chloroquine (CQ) inhibited Hz formation and significantly reduced both worm burden and egg deposition in the mouse liver [13] hint at Hz formation as the main haem detoxification pathway. As in *P. falciparum*, Hz formation therefore presents a vulnerable druggable target in *S. mansoni* since it is exclusive to the parasite, indispensable for its survival and not genome-encoded (hence immutable). This concept has been extensively explored against *P. falciparum* through classical aminoquinoline compounds known to interact with free haem molecules, consequently impeding their crystallization into Hz.[14]

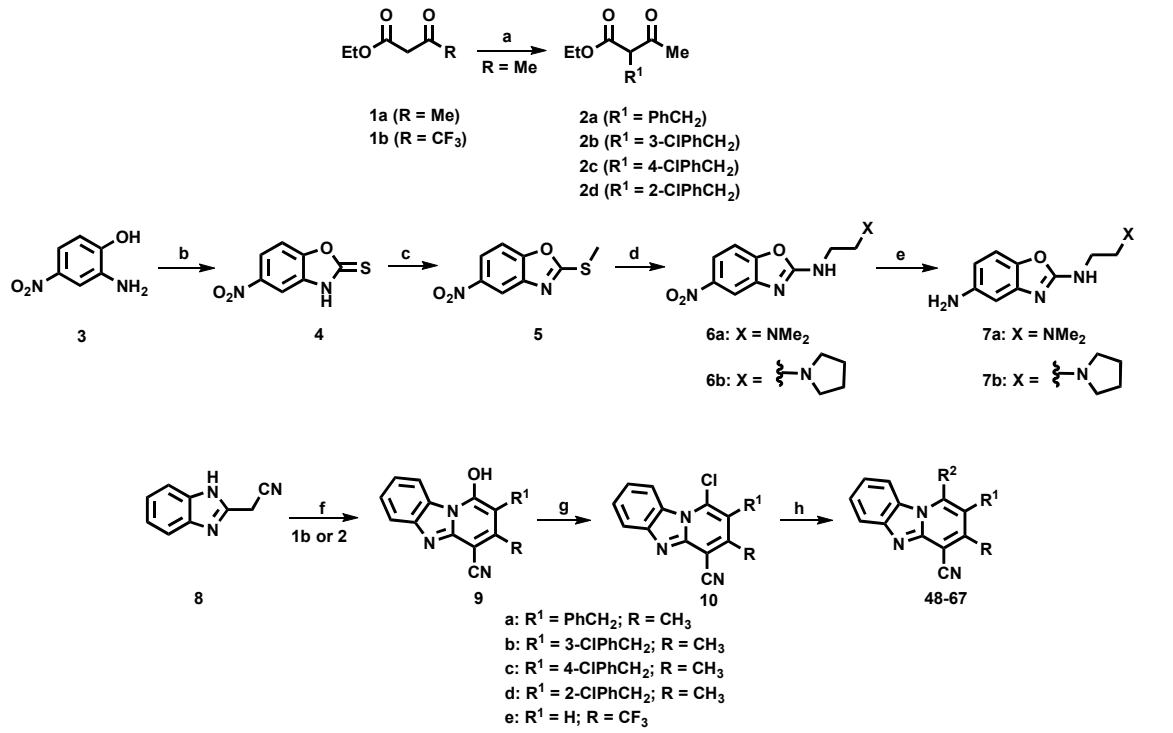
While literature is scant on the process of Hz formation in *S. mansoni*, a recent study reported fundamental differences to that in *P. falciparum*, in particular with respect to the extracellular localization of the process and structural organization of the subsequent Hz crystals.[15] In spite of these contrasts, the resulting Hz crystals themselves are structurally identical and therefore it would not be unreasonable to postulate similar inhibition of crystal formation in the two organisms. In malaria parasites, this is thought to involve docking of drugs or of the haem-drug complexes onto a growing crystal face through π-π stacking and specific weak hydrogen bonding thus blocking its growth [16] and allowing for interaction of the unbound monomeric/dimeric haem molecules with membranes leading to severe toxicity, ultimately halting parasite proliferation through oxidative stress.[17] However, Xue et al. recently showed that though the canonical Hz inhibitors CQ, mefloquine (MQ) and quinine (QN)

exhibited excellent antischistosomal activity, this did not correlate with their Hz inhibition potencies,[18] possibly suggesting a unique mechanism in *S. mansoni* of Hz inhibition dissimilar to that in *Plasmodium* or differences in haem-drug complex formation in the two parasite species. Praziquantel (PZQ), which constitutes the current mainstay of schistosomiasis control and therapy, is thought to act through disruption of Ca^{2+} ion homeostasis by modification of membrane fluidity and composition thus altering its permeability to ions.[19] Though safe, well-tolerated and effective in a single oral dose against the adult stages of all forms of schistosomiasis, the lack of more therapeutic alternatives to PZQ presents a genuine concern and warrants the search for more potent and novel antischistosomal drugs. Towards this aim, organometallic complexes, natural products and a number of repositioned antimalarial agents have variously been explored as antischistosomal agents and recently reviewed.[20-22]

In this report, we sought to examine the in vitro and in vivo antischistosomal activity of pyrido[1,2-*a*]benzimidazoles (PBIs), a chemotype that has previously been investigated for antibacterial, antifungal, antiviral and antitumor activity.[23] The antimalarial efficacy of PBIs was first reported by our group in 2011,[24] and recently confirmed with a set of derivatives exhibiting a more diverse range in scaffold substitutions.[25] Though the exact mechanism of action of PBIs remains unknown, inhibition of β-1, 6-glucan synthase in fungi,[26] the lytic activity of pore-forming perforin,[27] and cytochrome *b* in trypanosomes,[28] by various PBIs has been reported. Earlier, we hypothesised that PBIs can potentially inhibit formation of β-haematin/Hz due to their planar heterocyclic architecture, which would make them candidates for π-π interactions, a hallmark feature of haem-drug complex formation in inhibition of Hz crystal growth. This hypothesis was corroborated by our recent data in support of inhibition of Hz formation as a contributing mechanism to the antimalarial effect of PBIs.[25] In the current study, we present the in vitro and in vivo activity of 57 PBIs. While some members of the library have already been reported in a previous paper,[25] a hitherto unexplored new set of 3-trifluoromethyl pyrido[1, 2-*a*]benzimidazole derivatives was synthesised in order to increase the number and diversity of derivatives for structure-activity relationship (SAR) studies (**Scheme 1**). Furthermore, we have examined the metabolic profiles of the compounds in liver microsomes and

present pharmacokinetic analysis of the most active analogues in the in vivo set up. Finally, as a mechanistic evaluation, we report analyses on the correlations between the antischistosomal activities of these compounds and their ability to inhibit formation of β-haematin (βH) in a pyridine-based detergent-mediated assay.

Chemistry: The synthesis of target pyrido[1,2-*a*]benzimidazole derivatives[a] is shown in **Scheme 1**



[a] Reagents and reaction conditions: (a) appropriate benzyl halide, *t*BuOK, *t*BuOH, THF, 70°C, 8-24 h; (b) CS_2, KOH, EtOH, 80°C, 48 h; (c) MeI, CH_3CN, 25°C, 2 h; (d) $XCH_2CH_2NH_2$, CH_3CN, MW, 120°C, 20-30 min; (e) $SnCl_2$, HCl, THF, 60°C, 24 h; (f) NH_4OAc, 150°C, 1 h; (g) $POCl_3$, 130°C, 2 h; (h) **7a** – **b** or amine, Et_3N, THF, 80°C, MW, 20-40 min (**48** - **59**, **61** - **62**) and $Pd_2(dba)_3$, BINAP, K_2CO_3, Toluene, 120˚C, 12-16 h (**60**, **63** - **67**).

Compound **2a** – **d** were synthesised by reacting methyl acetoacetate with corresponding benzyl bromide.[29] Benzoxazole analogues **7a** – **b** were obtained from 2-amino-4-nitro phenol using literature a method,[30] while the target PBI derivatives **48 - 67** were synthesised as previously described.[25]

RESULTS AND DISCUSSION

Effect of Pyrido[1,2-a]benzimidazoles (PBIs) on Newly Transformed Schistosomula (NTS) and Adult S. mansoni

Of the 57 compounds screened against newly transformed schistosomula (NTS) at an initial concentration of 10 μM, 48 showed >70% inhibition of worm viability - a threshold that was selected for subsequent investigation against adult *S. mansoni* at the same drug concentration where 37 of them caused >60% worm mortality (Supporting Information Table S1 and S2). IC_{50} determination against adult worms was also carried out for this set of compounds (exhibiting >60% worm mortality) and ranged between 0.21 - 39.4 μM while that of PZQ was 0.1 μM (Table 1 and Supplementary Information Table S1). Compounds **14**, **30**, **36**, **38** and **41** were the most potent (IC_{50} range: 1.20 - 2.08 μM) against the adult worms, which could suggest that the presence of chloro- group(s) on either side of the ring system, as seen in four of these five compounds, is favoured for activity. Interestingly, **14,** which bears the same side chain as CQ, was one of the most potent against adult worms (IC_{50}: 1.20 μM). Among the aniline-type series, with no modifications except on the aromatic side chain, the cyano- (**56**) and sulfonylmethyl-substituted (**60**) derivatives were the most active (IC_{50} = 0.21 μM and 0.97 μM, respectively). Additionally, replacement with heteroaromatic side chains also seemed to preserve antischistosomal activity as exemplified by **63, 65, 66** and **67** (IC_{50} range 0.4 – 3.14 μM). Notably, derivatives based on piperidine (**15**, **17**, **19**, **20**) and sulphonamide substitution (**24)** in place of the ethylamine group were not active against adult *S. mansoni* (< 60% at 10 μM), implying these modifications are not favoured for antischistosomal activity (Supporting Information Table 1 and 2). Basicity of the nitrogen linker in the aniline series appeared necessary for activity as potency was lost with the N-methylated **62** (26.1% NTS mortality) compared to the non-methylated analogue **57** (95.7% NTS mortality). Some compounds (**19, 20, 24**, **47, 50** and **55**) seemed very potent against the juvenile parasites (73.9 – 100% NTS mortality range) but activity significantly diminished (0 - 42%) against adult *S. mansoni* parasites (Supporting Information Table S2).

Incubation of the compounds with Chinese hamster ovarian (CHO) cells helped ascertain their potential in vitro cytotoxicity. Activity against *S. mansoni* was greater than in CHO cells, with the

aniline-based analogues exhibiting better selectivity in general. Emetine, a potent inhibitor of protein synthesis in eukaryotic cells was used as control and as expected, exhibited cellular toxicity at extremely low concentrations (Table 1and Supplementary Information Table S2)

Beta-haematin Inhibition Activity (BHIA) and Correlation with Antischistosomal Activity

In this assay an IC_{50} cut-off of < 100 μM was invoked to discriminate active from inactive compounds, and 27/57 compounds were classified as active against formation of βH. There was no clear trend in activity that could be correlated to the substituents on the PBI core. Thus the abilities of these compounds to inhibit Hz formation appear to depend on the presence of a planar ring system, aliphatic hydrophobicity and the availability of a protonation site. However, the aniline-substituted derivatives were generally poor βH inhibitors (Table 1 and Supplementary Information Table S2).

We further attempted to correlate in vitro βH inhibition activity with antischistosomal activity and observed no associations in either the NTS or the adult *S. mansoni* (Figure 1). The lack of association in the juvenile worms is likely reflective of the observation that blood ingestion and consequent detoxification of haemoglobin degradation products predominantly occurs in the mature *S. mansoni* worms.[2] The absence of correlation in the adult worms seems driven, at least in part, by the inordinate βH inhibition IC_{50}s of the aniline-substituted derivatives (Table 1 and Supplementary Information Table S2). Expectedly, a winnowed analysis excluding these analogues (n = 9) yields a weak but significant positive correlation between βH inhibition and activity against adult worms (R^2 = 0.1388; p < 0.05; n = 29). This weak correlation implies that inhibition of Hz formation cannot be discounted as a contributing mechanism in the activity of these compounds, and that there could be additional (and possibly more dominant) mechanism(s) involved in the antischistosomal activity of these PBIs, or factors influencing the localisation of these compounds in the worm gut. Also, the lack of association is consistent with recent findings involving the antimalarials CQ, MQ and QN, which reported a lack of correlation between antischistosomal activity and their Hz inhibition properties,[18] with MQ in fact demonstrated to interfere with glycolysis in NTS.[31] It is noteworthy that unlike in *P. falciparum*, Hz formation proceeds extracellularly in *S. mansoni* and is thought to be possibly initiated by, or involve

proteins, lipids and other hydrophobic components in the gut of the parasite.[15] It is therefore conceivable that these PBIs inhibit Hz formation in *Schistosoma* in a manner dissimilar to *Plasmodium* by interacting with any of these targets, resulting in the perturbation of the crystal growth microenvironment. It is instructive to note that the βH inhibition assay discussed in this report constitutes an in vitro cell-free setup that can only capture to a limited extent the physiological landscape involved in intracellular drug activity. Therefore, in-organism mechanistic investigations that take into account the localization of Hz formation in lipid-like droplet particles [15] would be more informative. Indeed, using a pyridine-labelled haem fractionation assay that spectroscopically determines the amounts of haemoglobin, "free haem" (which can be associated with neutral lipids) and Hz present in trophozoites treated with increasing doses of a drug, we recently showed that inhibition of Hz formation could be a contributing mechanism in the activity of these compounds, albeit in *P. falciparum.*[25] Though no similar assay presently exists for *S. mansoni*, such a model would be invaluable for investigating drug action in this parasite.

In vivo Activity against adult S. mansoni

We further examined the efficacy, in experimental mice, of 10 compounds (**11, 28, 30, 38, 39, 56, 60, 63, 66** and **67**) which had shown the best in vitro activity against NTS and adult *S. mansoni* (IC_{50} range: 0.21 – 2.44 μM), moderate to excellent metabolic stability and favourable cytotoxicity profiles. Treatment of mice with 400 mg/kg of **11** failed to induce any in vivo activity (Table 2) as there was no observed reduction in total worm burden, and one of the mice died 10 days post-treatment. Compounds **30**, **38**, **39, 60, 63, 66** and **67** only showed modest activity with < 50% reduction in both total and female worm burden. Significant reduction in worm burden was, however, observed following treatment with **28** and **56,** and although comparatively lower than parasite burden reduction reported for PZQ,[32] these were superior to previously reported ferroquine-derivatives,[33] repurposed anticancer drugs,[34] PZQ - endoperoxide conjugates [35] and some Malaria Box Medicine for Malaria Venture (MMV) compounds.[36] There was a discernible trend of increasing worm burden reduction with compound metabolic stability in mouse liver microsomes (MLMs) and in vitro potency in adult worms. This suggests that poor metabolic stability most likely contributed to the low in vivo efficacy

of **63** and **66** while the role of solubility, which would result in low systemic exposure due to limited absorption, could explain the average in vivo activities observed with **56** and **60** despite their excellent in vitro potencies and stability in MLMs.

Pharmacokinetic Analysis in mouse

To obtain more insight on the efficacy of **28**, we investigated its pharmacokinetic (PK) properties by dosing the compound orally (20 mg/kg) and intravenously (2 mg/kg) in mice. Blood samples were collected at various time points over 24 hours and the compound concentrations measured by LC-MS/MS. The PK parameters derived from non-compartmental analysis of the data are summarized in Table 3 and the PK profiles shown in Figure 2.

Compound **28** was slowly and incompletely absorbed, with large inter-individual variations in oral bioavailability. This observation is likely due to poor solubility of the compound and suggests a likelihood of solubility-limited absorption. This would result in plateauing of the C_{max} and consequently efficacy despite an increase in dose, and has previously been observed with this series.[24] The oral half-life of this compound was also lower than that of **11**, **38** and **39** reported in our previous analyses[24-25] and is, at least in part, due to a relatively higher clearance. This is further supported by the observation of the *N*-deethylation metabolite in blood for this compound, but not for **11**, **38** or **39**,[24-25] suggesting a higher in vivo hepatic clearance. The in vivo efficacy of **28** is therefore tied to its low exposure on oral dosing, due to solubility-limited absorption, and a relatively short half-life due to high hepatic clearance.

In summary, our results reveal an antischistosomal property of PBIs that further complements the broad antimicrobial potency of this class of compounds. Though their activity in *S. mansoni*-infected mice was generally low, encouraging efficacy was noted in two of the compounds suggesting further lead optimization efforts can afford derivatives with improved in vivo activity. In addition, our preliminary data suggests that inhibition of Hz formation cannot be discounted as a contributing mechanism through which these compounds exert their antischistosomal effect, especially in the adult parasites. Evaluation of more PBI derivatives is needed in order to delineate meaningful SARs. Furthermore, direct correlation of antiparasitic activity and inhibition of Hz formation in the worm needs to be established as do any other additional mechanisms involved.

Experimental Procedures

General Method. All commercially available chemicals were purchased from either Sigma-Aldrich or Combi Blocks. All solvents were dried by appropriate techniques. Unless otherwise stated, all solvents used were anhydrous. Purification of compounds was carried out by either column chromatography on silica gel 60 (Fluka), particle size = 0.063−0.2 mm (70−230 mesh), as the stationary phase. All target compounds and intermediates were characterized by ^{1}H NMR and LC-

MS. NMR spectra were recorded on either a Varian Mercury-300 (^{1}H 300.1 MHz, ^{13}C 75.5 MHz) or a Bruker-400 (^{1}H 400.2 MHz, ^{13}C 100.6 MHz) instrument using $CDCl_3$, CD_3OD, and DMSO-d6 as solvents. Liquid chromatograph with mass spectrometer (LC-MS) analysis was performed using an Agilent 1260 Infinity binary pump, an Agilent 1260 Infinity diode array detector (DAD), an Agilent 1290 Infinity column compartment, an Agilent 1260 Infinity standard autosampler, and an Agilent 6120 quadrupole (single) mass spectrometer, equipped with APCI and ESI multimode ionization source. Purities were determined by Agilent LC-MS using a Kinetex Core C18 2.6 μm column (50 mm × 3 mm). Mobile phase B consisted of 0.4% acetic acid, 10 mM ammonium acetate in a 9:1 ratio of HPLC grade methanol, and type 1 water. Mobile phase A consisted of 0.4% acetic acid in 10 mM ammonium acetate in HPLC grade (type 1) water, with flow rate = 0.9 mL/min; detection was by diode array (DAD), and all final compounds were confirmed to have ≥95% purity.

General procedure for the synthesis of compound 48-55, 57-59, 61-62 and 64

Respective amine (2.0 equiv.) [N-ethyl ethylenediamine (**48-51**), **7a** (**52**), **7b** (**53**), aniline (**54**), 4-methylaniline (**55**), 4-methoxyaniline (**57**), *N*,*N*-dimethylbenzene-1,4-diamine (**58**), 4-amino-N-methylbenzamide (**59**), 4-(morpholinomethyl)aniline (**61**), 4-methoxy-N-methylaniline (**62**), pyrimidin-2-amine (**64**)] was added to a stirred mixture of compound **10** (1.0 equiv.) and triethylamine (2.0 equiv.) in THF (10 mL). The mixture was irradiated in microwave reactor at 80^{o}C for 20 min., filtered hot, and allowed to cool. The solvent was removed in vacuo, and the residue washed with minimum amount of ice-cold ethanol. The resulting solid was recrystallized from acetone or ethanol to afford the target compound.

2-benzyl-1-((2-(ethylamino)ethyl)amino)-3-methylbenzo[4,5]imidazo-[1,2-*a*]pyridine-4 carbonitrile (**48**). Yield: 0.0023 g, 7%, as an orange solid. ^{1}H NMR (400 MHz, DMSO-*d*6) δ 8.28 (dt, *J* = 8.4, 1.0 Hz, 1H), 7.83 (dt, *J* = 8.2, 1.0 Hz, 1H), 7.53 (ddd, *J* = 8.2, 7.1, 1.1 Hz, 1H), 7.33 (m, 3H), 7.20 (m, 3H), 4.27 (s, 2H), 3.21 (t, *J* = 5.9 Hz, 2H), 2.66 (t, *J* = 5.9 Hz, 2H), 2.49 (s, 3H), 2.45 (q, *J* = 7.1 Hz, 2H), 0.97 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-*d*6) δ

151.0, 149.0, 148.3, 145.1, 139.7, 129.3, 129.1 (3C), 128.1, 126.7, 126.0, 120.8, 119.0, 116.9, 116.5, 110.3, 55.3, 48.9, 47.1, 43.4, 32.0, 19.8, 15.6. MS (EI+) *m/z* calculated for $C_{24}H_{25}N_5$: 383.21; found, 384.2 (M+1). HPLC purity: 99%.

2-(4-chlorobenzyl)-1-((2-(ethylamino)ethyl)amino)-3-methylbenzo-[4,5]imidazo-[1,2-*a*]pyridine-4-carbonitrile (**50**). Yield: 0.137 g, 20 %, as a yellow solid. ^{1}H NMR (400 MHz, DMSO-*d*6) δ 8.30 (d, *J* = 8.4 Hz, 1H), 7.83 (d, *J* = 8.1 Hz, 1H), 7.53 (t, *J* = 7.7 Hz, 1H), 7.33 (m, 3H), 7.20 (d, *J* = 8.4 Hz, 2H), 4.26 (s, 2H), 3.20 (m, 2H), 2.67 (t, *J* = 5.9 Hz, 2H), 2.45 (m, 5H), 0.97 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-*d*6) δ 155.9, 150.8, 149.6, 148.1, 138.6, 131.6, 131.2, 129.8, 129.1, 127.3, 122.2, 120.9, 119.1, 115.8, 115.7, 102.4, 101.0, 89.6, 48.7, 45.9, 44.1, 31.2, 18.7, 13.7. MS (EI+) *m/z* calculated for $C_{24}H_{24}ClN_5$: 417.17; found, 418.1 (M+1). HPLC purity: 99%.

1-((2-((2-(dimethylamino)ethyl)amino)benzo[d]oxazol-6-yl)amino)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-*a*]pyridine-4-carbonitrile **(52)**. Yield: 0.07 g, 23%, as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.81 (d, *J* = 8.1 Hz, 1H), 8.03 (br t, *J* = 5.6 Hz, 1H), 7.57 (d, *J* = 8.0 Hz, 1H), 7.35 (m, 2H), 7.13 (t, *J* = 7.1 Hz, 1H), 6.87 (d, *J* = 2.0 Hz, 1H), 6.62 (dd, *J* = 8.3, 2.1 Hz, 1H), 5.78 (s, 1H), 3.66 (q, *J* = 10.4 Hz, 2H), 3.29 (t, *J* = 7.2 Hz, 2H), 2.82 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.9 (2C), 151.7, 150.6, 147.3, 144.5, 144.2 (2C), 130.8 (2C), 124.6, 122.4, 119.3, 118.5, 117.0, 116.0, 114.9, 109.5, 109.4, 89.2, 56.4, 43.3, 38.3 (2C). MS (EI+) *m/z* calculated for $C_{24}H_{20}F_3N_7O$: 479.47; found, 480.2 (M+1). HPLC purity: 99%.

1-((2-((2-(Pyrrolidin-1-yl)ethyl)amino)benzo[d]oxazol-6-yl)amino)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-*a*]pyridine-4-carbonitrile **(53)**. Yield: 0.13 g, 14%, as

a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.81 (d, J = 7.7 Hz, 1H), 8.03 (br t, J = 5.0 Hz, 1H), 7.59 (d, J = 7.9 Hz, 1H), 7.38 (m, 2H), 7.17 (t, J = 8.3 Hz, 1H), 6.90 (d, J = 2.0 Hz, 1H), 6.65 (dd, J = 8.3, 2.1 Hz, 1H), 5.84 (s, 1H), 3.68 (q, J = 10.4 Hz, 2H), 3.42 (t, J = 5.9 Hz, 2H), 3.30 (br s, 4H), 1.95 (br s, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.9 (2C), 151.3, 150.4, 146.4, 144.7, 144.3, 134.5, 130.5, 125.1, 124.9, 122.2, 119.7, 118.1, 117.1, 115.8, 115.0, 109.6, 109.5, 89.8, 55.3 (2C), 53.9, 53.4, 23.0 (2C). MS (EI+) *m/z* calculated for $C_{26}H_{22}F_3N_7O$: 505.51; found, 506.2 (M+1). HPLC purity: 99%.

1-(phenylamino)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-*a*]pyridine-4-carbonitrile **(54)**. Yield: 0.08 g, 23%, as a yellow powder. ^{1}H NMR (300 MHz, DMSO-*d*6) δ 8.80 (d, J = 8.2 Hz, 1H), 7.70 (d, J = 7.0 Hz, 1H), 7.59 (t, J = 7.3 Hz, 1H), 7.45 (m, 3H), 7.15(m, 3H), 6.22 (s, 1H). ^{13}C NMR (101 MHz, DMSO-*d*6) δ 148.5, 148.4, 148.2, 130.1 (4C), 128.7, 127.1 (2C), 124.5, 124.0, 122.7 (2C), 122.5, 117.5 (2C), 115.0, 114.4. MS (EI+) *m/z* calculated for $C_{19}H_{11}F_3N_4$: 352.09; found, 353.1 (M+1). HPLC Purity: 99%.

General method for the synthesis of 56, 60, 63 and 65-67

Compound **10** (1.0 equiv.), the appropriate amine (1.2 equiv.) [4-cyanoaniline (**56**), 4-(methylsulfonyl)aniline (**60**), 2-fluoropyridin-4-amine (**63**), 5,6-dimethoxypyrimidin-4-amine (**65**), pyrazin-2-amine (**66**), 4-methylthiazol-2-amine (**67**)], $Pd_2(dba)_3$ (0.03 equiv.), BINAP (0.06 equiv.) and K_2CO_3 (20 equiv.) in toluene (5ml) were heated in a sealed tube at 120˚C for 4-17 hours. The cooled reaction mixture was stirred in ethyl acetate (50ml) and water (100ml) for 30 minutes. The separated organic layer was washed with water (2 x 50ml), followed by saturated NaCl (2 × 50ml), dried over magnesium sulphate and filtered over celite. The organic fraction was concentrated in vacuo and ethanol used to precipitate the

crude product which was filtered to furnish the final product. Occasionally, purification by recrystallization from ethanol was done.

1-((4-cyanophenyl)amino)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-*a*]pyridine-4-carbonitrile **(56).** Yield: 0.102g, 27%, as a pale yellow powder. ^{1}H NMR (300 MHz, DMSO-*d*6) δ 8.80 (d, *J* = 7.9 Hz, 1H), 7.84 (d, *J* = 8.7 Hz, 2H), 7.66 (d, *J* = 7.3 Hz, 1H), 7.59 (m, 1H), 7.41 (m, 1H), 7.21 (d, *J* = 8.5 Hz, 2H), 6.22 (s, 1H). ^{13}C NMR (101 MHz, DMSO-*d*6) δ 148.3, 148.1, 135.3, 135.0, 134.4 (3C), 128.6, 127.4 (2C), 123.1 (2C), 122.9 (2C), 119.8, 117.9 (2C), 115.2, 105.5, 96.6. MS (EI+) *m/z* calculated for $C_{20}H_{10}F_3N_5$: 377.09; found, 378.1 (M+1). HPLC Purity: 99%.

1-((4-(methylsulfonyl) phenyl) amino)-3- (trifluoromethyl) benzo[4,5]imidazo [1,2-*a*]pyridine-4-carbonitrile **(60)**. Yield: 0.175 g, 40%, as a yellow powder. ^{1}H NMR (300 MHz, DMSO-*d*6) δ 8.79 (d, *J* = 8.0 Hz, 1H), 7.88 (d, *J* = 8.6 Hz, 2H), 7.62 (d, *J* = 7.9 Hz, 1H), 7.46 (m, 1H), 7.25 (m, 3H), 6.00 (s, 1H), 3.21 (s, 3H). ^{13}C NMR (101 MHz, DMSO-*d*6) δ 155.1, 149.3, 133.8, 132.8, 130.0, 129.6, 129.0 (2C), 125.5, 122.7 (2C), 121.4, 120.4, 117.2 (2C), 115.0, 106.7, 91.4, 86.0, 44.0. MS (EI+) *m/z* calculated for $C_{20}H_{13}F_3N_4O_2S$: 430.07; found, 431.0 (M+1). HPLC Purity: 99%.

1-((2-fluoropyridin-4-yl)amino)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-*a*]pyridine-4-carbonitrile **(63).** Yield: 0.021g, 6% as a yellow powder. ^{1}H NMR (400 MHz, DMSO-*d*6) δ 8.78 (d, *J* = 8.3 Hz, 1H), 8.13 (d, *J* = 5.4 Hz, 1H), 7.68 (d, *J* = 8.1 Hz, 1H), 7.57 (t, *J* = 7.6 Hz, 1H), 7.38 (t, *J* = 7.9 Hz, 1H), 6.98 (dt, *J* = 5.4, 1.7 Hz, 1H), 6.72 (s, 1H), 6.23 (s, 1H). ^{13}C NMR (101 MHz, DMSO-*d*6) δ 166.3, 164.0, 161.0, 148.6 (2C), 135.8, 128.7, 127.2, 124.1,

122.7, 121.4, 117.9, 116.1, 115.3, 113.3, 101.7, 101.3, 95.7. MS (EI+) *m/z* calculated for $C_{18}H_9F_4N_5$: 371.08; found, 372.1 (M+1). HPLC Purity: 99%.

1-((5,6-dimethoxypyrimidin-4-yl)amino)-3-(trifluoromethyl) benzo[4,5] imidazo[1,2-*a*]pyridine-4-carbonitrile **(65).** Yield: 0.073g, 17%, as a yellow fluffy powder. ^{1}H NMR (300 MHz, DMSO-*d*6) δ 9.08 (d, *J* = 8.58 Hz, 1H), 8.36 (s, 1H), 7.69 (d, *J* = 8.7 Hz, 1H), 7.63 (m, 1H), 7.47 (m, 1H), 7.40 (s, 1H), 4.00 (s, 3H), 3.80 (s, 3H). ^{13}C NMR (101 MHz, DMSO-*d*6) δ 163.2, 162.7, 151.3, 147.5, 141.4, 135.4, 135.0, 128.7, 127.7(2C), 124.1, 123.1 (2C), 121.4, 118.5, 114.9, 100.0, 60.6, 54.0. MS (EI+) *m/z* calculated for $C_{19}H_{13}F_3N_6O_2$: 414.11; found, 415.1 (M+1). HPLC Purity: 98%.

1-(pyrazin-2-ylamino)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-*a*]pyridine-4-carbonitrile **(66).** Yield: 0.041g, 23%, as an orange powder. ^{1}H NMR (400 MHz, DMSO-*d*6) δ 9.08 (d, *J* = 8.4 Hz, 1H), 8.54 (d, *J* = 1.4 Hz, 1H), 8.41 (dd, *J* = 2.7, 1.4 Hz, 1H), 8.25 (d, *J* = 2.7 Hz, 1H), 7.69 (ddd, *J* = 8.1, 1.2, 0.7 Hz, 1H), 7.63 (ddd, *J* = 8.2, 7.3, 1.1 Hz, 1H), 7.57 (s, 1H), 7.47 (ddd, *J* = 8.5, 7.3, 1.3 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-*d*6) δ 161.2, 156.4, 149.8, 147.9, 147.3, 142.5 (2C), 138.5, 135.5, 128.8, 127.5, 123.1, 118.9, 118.5, 114.8, 112.8, 99.3. MS (EI+) *m/z* calculated for $C_{17}H_9F_3N_6$: 354.08; found, 355.1 (M+1). HPLC Purity: 98%.

1-((4-methylthiazol-2-yl)amino)-3-(trifluoromethyl)benzo[4,5]imidazo[1,2-*a*]pyridine-4-carbonitrile **(67)**. Yield: 0.138g, 37% as a brown powder. ^{1}H NMR (400 MHz, DMSO-*d*6) δ 9.04 (d, *J* = 8.0 Hz, 1H), 7.83 (d, *J* = 8.1 Hz, 1H), 7.59 (ddd, *J* = 8.3, 7.2, 1.2 Hz, 1H), 7.41 (ddd, *J* = 8.4, 7.2, 1.2 Hz, 1H), 6.97 (s, 1H), 6.84 (d, *J* = 1.2 Hz, 1H), 2.33 (d, *J* = 1.2 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-*d*6) δ 150.9, 148.0, 140.9, 137.5, 136.7, 136.3, 131.5,

129.8, 126.7, 121.8, 118.9, 118.1, 117.8, 114.9, 104.8, 100.0, 14.6. MS (EI+) *m/z* calculated for $C_{17}H_{10}F_3N_5S$: 373.06; found, 374.1 (M+1). HPLC Purity: 99%.

MATERIALS AND METHODS

Compounds

For in vitro studies, compounds were dissolved in DMSO (Fluka, Buchs, Switzerland) to obtain stock solutions of 10 mg/ml while in vivo analyses involved suspension of compounds in 7% (v/v) Tween 80 and 3% (v/v) ethanol shortly before oral treatment (10 ml/kg) of mice.

Animals and Parasites

Animal studies were carried out in accordance with Swiss national and cantonal regulations on animal welfare at the Swiss Tropical and Public Health Institute (Basel, Switzerland). This current study was approved by the local veterinary agency, based on Swiss cantonal and national regulations (permission no. 2070). Female mice (NMRI strain; age 3 weeks; weight ca. 14 g)) were purchased from Charles River, Germany, kept under environmentally controlled conditions (temperature ~ 25°C; humidity ~70%; 12-hour light and 12-hour dark cycle) with free access to water and rodent diet and acclimatized for one week before infection. Cercariae of *S. mansoni* (Liberian strain) were obtained from infected intermediate host snails, *Biomphalaria glabrata*, as previously described.[37]

In Vitro Studies

a) Screening on newly transformed schistosomula (NTS)

S. mansoni cercariae were mechanically transformed to newly transformed schistosomula (NTS) as described elsewhere.[38] Briefly, snails were placed under light to stimulate cercarial shedding and cercarial suspension collected. The tails were separated from the heads by rinsing three times with cold HBSS. NTS were then incubated overnight in culture medium and used the next day.

Test compounds and controls were dissolved in DMSO (Fluka, Buchs, Switzerland) to a concentration of 10 mM. A hundred NTS were then incubated in each well of a 96-well plate with culture medium

and the test compound for a final well volume of 250 µL. Culture medium was composed of Medium 199 (Invitrogen, Carlsbad, CA) supplemented with 5% foetal calf serum (Lucerne, Switzerland) and 1% penicillin/streptomycin mixture (Lucerne, Switzerland). Compounds were tested at 10 µM in triplicate with NTS incubated in no more than 1% DMSO serving as control. NTS were kept in an incubator at 37°C and 5% CO_2 for up to 72 hours after which the condition of the NTS was microscopically evaluated using a scale from 3 (normal activity and morphological alteration) to 0 (dead). IC_{50} determination assays for the compounds were subsequently performed in the same manner.

b) Adult S. mansoni worms

To obtain adult schistosomes, mice were infected subcutaneously with 80 - 100 cercariae. The mice were then euthanized after 7 - 8 weeks with CO_2 and the worms collected from the hepatic portal and mesenteric veins. Two pairs of adult worms were incubated in each well of a 24-well plate with 2 ml culture medium and the test compound. Culture medium was composed of RPMI 1640 (Invitrogen, Carlsbad, CA) supplemented with 5% foetal calf serum (Lucerne, Switzerland) and 1% penicillin/streptomycin mixture (Lucerne, Switzerland). Only compounds with > 70% activity against NTS were evaluated against adult worms at 10 µM with incubation at < 1% DMSO used as control. Worms were kept in an incubator at 37°C and 5% CO_2 for up to 72 hours after which their condition was microscopically evaluated using a scale from 3 (normal activity and no morphological alterations) to 0 (dead). IC_{50} determination was conducted for compounds with > 60% activity. For the *in vitro* drug sensitivity assays, all viability scores were averaged across replicates and normalized to the average viability scores of the control wells. IC_{50} values were calculated using CompuSyn2 (ComboSyn Inc., Paramus, NJ).

Cytotoxicity Testing

Compounds were screened for *in vitro* cytotoxicity against Chinese Hamster Ovarian (CHO) mammalian cell-lines, using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT)-assay. The reference standard, emetine, was prepared to 2 mg/ml in distilled water while stock solutions of test compounds were prepared to 20 mg/ml in 100% DMSO with the highest

concentration of solvent to which the cells were exposed having no measurable effect on the cell viability. The initial concentration of the drugs and control was 100 μg/mL, which was serially diluted in complete medium with 10-fold dilutions to give 6 concentrations, the lowest being 0.001 μg/mL. Plates were incubated for 48 h with 100 μL of drug and 100 μL of cell suspension in each well and developed afterwards by adding 25 μL of sterile MTT (Thermo Fisher Scientific) to each well and followed by 4 h incubation in the dark. The plates were then centrifuged, medium aspirated off and 100 μL of DMSO added to dissolve crystals before reading absorbance at 540 nm. IC_{50} values were then obtained from dose-response curves, using a nonlinear dose-response curve fitting analysis via GraphPad Prism v.4.0 software (La Jolla, USA). The assay was conducted in triplicate and conducted on two separate occasions

Beta-haematin Inhibition Activity (BHIA)

Stock solutions of controls and test compounds were made to 20 mM in DMSO (Fluka, Buchs, Switzerland). A solution containing water/305.5 μM NP40/DMSO at a v/v ratio of 70%/20%/10%, respectively was added to columns 1-11 in a 96-well plate while 140 μL of water and 40 μL of 305.5 μM NP40 were added to column 12 to mediate the formation of β-haematin. Twenty μL of drug (20 mM) was added to column 12 and 100 μL of this solution serially diluted to column 2, with column 1 left as a blank (0 μM compound). In case the compound was coloured (for instance AQ), a pre-reading of the plate was done by measuring absorbance at 405 nm on a SpectraMax plate reader. A 178.8 μL aliquot of hematin stock was suspended in 20 ml of a 1 M acetate buffer, pH 4.9 and 100 μL of this hematin suspension added into each well. Plates were then incubated for ~5 h at 37 °C after which 32 μL of pyridine solution (20% water, 20% acetone, 10% 2M HEPES buffer (pH 7.4), 50% pyridine) was added followed by addition of 60 μL of acetone to all wells. Plates were again read at 405 nm and IC_{50}s plotted in GraphPad Prism v.4.0 software (La Jolla, USA).

In vivo studies in S. mansoni-infected mice

Groups of 3– 4 NMRI mice harbouring chronic schistosomal infection were treated 49 days post-infection with single oral doses of 400 mg/kg of compounds **11, 28, 30, 38, 39, 56, 60, 63, 66** and **67**

dissolved in 7% Tween 80 and 3% ethanol in water (v/v/v) while untreated mice served as controls. The drug vehicle (7% Tween 80 and 3% ethanol in water) has no antischistosomal properties in vitro and in vivo (unpublished observation). At 21 days post-treatment, animals were euthanized by the CO_2 method and dissected. Surviving schistosomes residing in the mesenteric veins and the liver were counted and sexed as previously described elsewhere.[39]

Single point in vitro metabolic stability assay

The metabolic stability assay was performed in duplicate in a 96-well micro titre plate. The test compounds (0.1 μM) were incubated individually at 37 °C in a solution containing 0.35 mg/ml mouse liver microsomes (MLM; male Mouse BALB/c, Xenotech) and metabolic reactions initiated by the addition of NADPH (1 mM) in phosphate buffer (100 mM, pH 7.4) and incubated for 30 min. Reactions were quenched by adding 300 μL of ice cold acetonitrile containing internal standard (carbamazepine, 0.0236 μg/mL). Test compounds in the supernatant were centrifuged, filtered and analysed by means of LC-MS/MS (Agilent Rapid Resolution HPLC, AB SCIEX 4000 QTRAP MS). The incubations of compounds and controls (midazolam and MMV390048) were performed in triplicates. The relative disappearance of parent compound over the course of the incubation was monitored by LC-MS/MS and results reported as % remaining after 30 min incubation. Metabolite searches were not conducted during the metabolic stability assay.

Mouse pharmacokinetic (PK) Analysis

Compound **28** was weighed out and prepared freshly immediately prior to dosing. The compound was prepared in dimethylacetamide, polyethylene glycol, and propylene glycol/ethanol mixture 4:1 at a ratio of 1:3:6 for intravenous dosing (n = 3) and administered in a volume of 50 μL via the tail vein after anaesthetising the animals. For the orally dosed group (n = 3), compound **28** was formulated as suspension in 100% hydroxypropyl methylcellulose and the dose administered by gavage. Approximately 20μL of blood was drawn from the tail of each animal at a series of predetermined time-points and placed into a microcentrifuge tube coated with lithium heparin S12 to prevent clotting. Samples were kept on ice and transferred to storage at -80 °C within one hour. Quantitation

of the levels of the compounds was carried out using high-performance liquid chromatography and mass spectrometry (LC-MS/MS) method developed in-house. Animals are housed in temperature-controlled rooms and fed a standard diet with water provided ad libitum. The study was conducted using three animals per dosing route; all animals were killed by inhalation of isoflurane to knock them out followed by cervical dislocation. Authorisation for the study was obtained from the University of Cape Town Research Ethics Committee protocol number AEC 013/028.

Statistics

In vitro activities of the compounds against NTS and adult *S. mansoni* were calculated from the mean viability values in relation to the control value using the formula:

Drug effect = $1 - (\text{score}^{\text{drug}} \div \text{score}^{\text{control}})$

From the obtained drug effects, IC_{50} values were calculated using CompuSyn software (version 3.0.1; ComboSyn) with the linear correlation coefficient (*r* value) reflecting the conformity of the experimental data The non-parametric Mann-Whitney test was applied for in vivo studies, comparing the medians of the worm counts in the treatment and control groups. A difference in median was considered to be significant if the p-value < 0.05 (GraphPad Prism, version 4.0, La Jolla, USA).

Supporting Information

Additional details of the structures of all derivatives assessed as well as tabulated biological activity data of all compounds evaluated against larval and adult parasites, β-haematin formation, mammalian cells and mouse liver microsomes. Excel file with the compounds SMILES format is also provided

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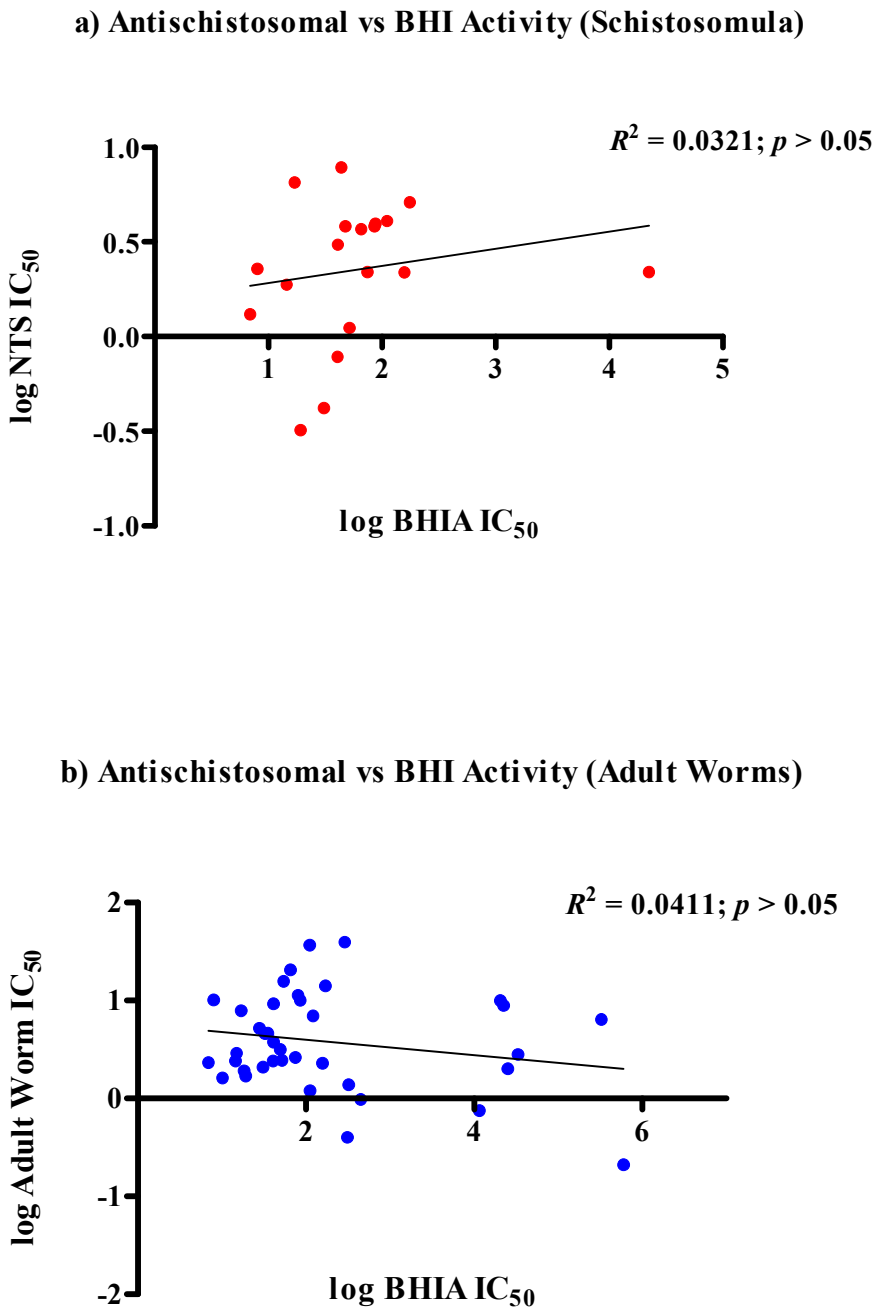


Figure 1: Correlation between antischistosomal and βH inhibition activity of the PBI compounds. This analysis was performed on **a**) 19 PBIs exhibiting a broad spectrum of activity against schistosomula and **b**) 38 compounds with IC_{50} data against adult *S. mansoni*.

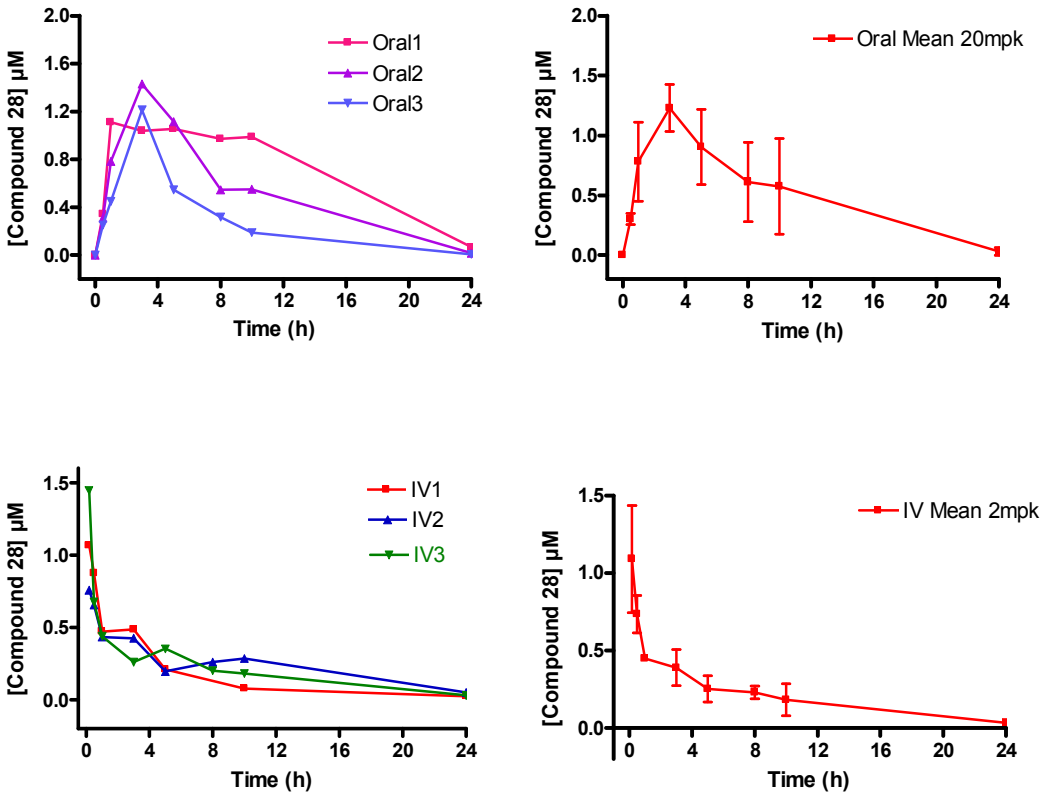
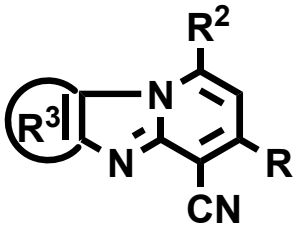
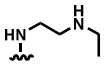
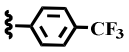
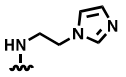
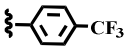
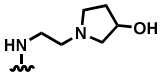
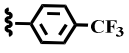
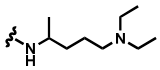
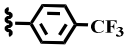
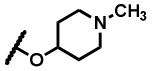
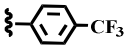
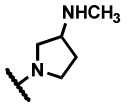
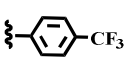
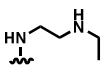
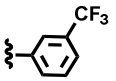
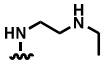
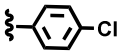
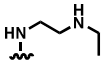
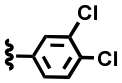
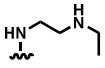
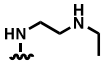
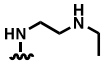
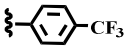
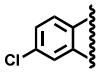
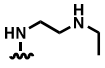
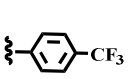
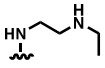


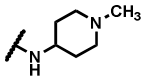
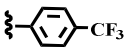
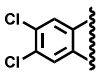
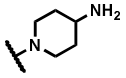
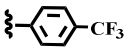
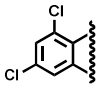
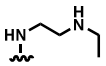
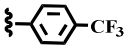
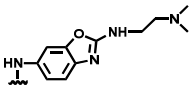
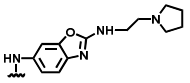
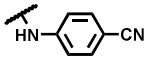
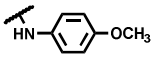
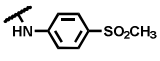
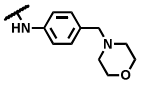
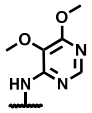
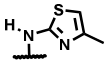
Figure 2: Systemic exposure of compound **28** following single intravenous and oral dose administration to healthy mice (n = 3).

Table 1: In vitro effect against adult stages of *S. mansoni,* β-haematin and CHO cells of PBIs that were active against both NTS (>70 mortality) and adult parasites ($IC_{50} \leq 10$ µM)



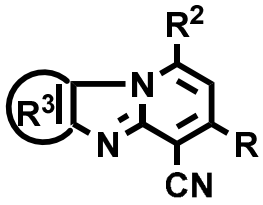
Compound	R^2	R	R^3	Antischistosomal Activity	Cytotoxicity

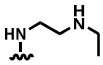
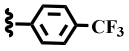
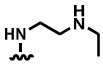
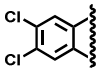
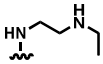
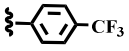
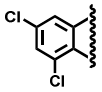
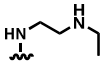
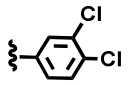
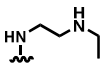
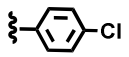
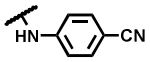
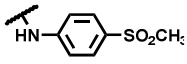
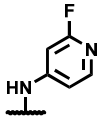
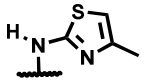
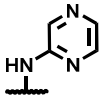
				IC$_{50}$ Adult *S.mansoni* (r value) µM	IC$_{50}$ BHIA (µM)	IC$_{50}$ CHO (µM)	Selective Index
11				2.41 (0.88)	40.5	1.56 ± 0.03	0.6
12				10.14 (0.88)	8.0	10.5 ± 2.93	1.0
13				10.02 (0.99)	85.3	6.12 ± 1.20	0.6
14				1.20 (0.93)	87.1	2.91 ± 0.5	2.4
16				9.20 (0.95)	40.8	4.07 ± 0.21	0.4
22				6.96 (0.95)	121		
27				2.28 (0.90)	157.1	5.92 ± 1.84	2.6
28				2.44 (0.88)	51.5	9.60 ± 1.58	3.9
30				1.69 (0.92)	19.2	1.55 ± 0.44	0.9
33				2.62 (0.99)	74.5	3.19 ± 0.42	1.2
34				8.86 (0.92)	22,380	3.77 ± 0.50	0.4
35				2.41 (0.85)	14.5	8.71 ± 0.68	3.6
36				1.62 (0.99)	10.1	1.63 ± 0.24	1.0
37				2.89 (0.94)	14.9	1.55 ± 0.60	0.5
38				2.08 (0.90)	30.8	3.39 ± 0.70	1.6
39				2.32 (0.94)	6.9	11.2 ± 1.1	4.8

40				5.18 (0.94)	27.8	4.19 ± 0.26	0.8
41				1.92 (0.99)	18.3	2.98 ± 0.20	1.6
42				4.60 (0.95)	32.4	4.10 ± 1.7	0.9
52		**CF_3**		3.75 (0.96)	40.8		
53		**CF_3**		4.64 (0.95)	35.1		
54		**CF_3**		2.81 (0.98)	33266		
56		**CF_3**		0.21 (0.92)	596926	7.27 ± 1.7	34.6
57		**CF_3**		6.4 (0.76)	324434		
60		**CF_3**		0.97 (0.97)	445	148.1 ± 11.2	152.7
61		**CF_3**		3.14 (0.90)	50		
63		**CF_3**		0.75 (0.92)	11580	11.2 ± 4.1	14.9
65		**CF_3**		1.38 (0.88)	320	24.0 ± 2.2	17.4
66		**CF_3**		2.01 (0.94)	25130	181.4 ± 9.1	90.2
67		**CF_3**		0.40 (0.91)	309	33.4 ± 5.7	83.5
PZQ				0.021 (0.94)			

Selective Index = IC_{50} CHO/IC_{50} adult *S. mansoni* worm. **PZQ** = Praziquantel

Table 2: Effect on worm burden stratified by sex and worm distribution, following single 400 mg/kg oral doses of selected PBIs administered to mice harbouring a 49-days old adult *S. mansoni* infection.



Compound	R^2	R	R^3	No. mice investigated (n)	Mean number of worms (SD) [a]		TWR [b] (%)	FWR [c] (%)	Met Stability in MLMs
					Males	Females			
Control	-	-		9	17.3 (8.7)	14.2 (8.4)	-	-	
11				3	18.7 (3.1)	14.3 (6.8)	0.0	0.0	97 ± 8.3
38				4	12.8 (5.3)	10.3 (4.8)	26.7	27.8	81 ± 6.1
39				4	12.0 (2.7)	7.5 (3.9)	38.1	47.2	87 ± 8.9
30				4	8.0 (2.6)	8.3 (2.2)	48.3	41.9	65 ± 6.5
28				4	7.5 (2.4)	5.5 (1.3)	58.7**	61.3**	69 ± 10.8
56		CF_3		4	9.0 (1.6)	9.5 (2.5)	54.9**	54.3**	97 ± 6.1
60		CF_3		4	12.8 (5.9)	12.5 (5.8)	38.3	39.8	100 ± 9.4
63		CF_3		4	13.0 (7.6)	12.5 (8.3)	37.8	39.8	56 ± 3.6
67		CF_3		4	12.3 (1.3)	12.0 (1.6)	40.7	42.2	73 ± 6.6
66		CF_3		4	12.0 (1.4)	13.3 (2.8)	38.3	35.9	38 ± 4.3

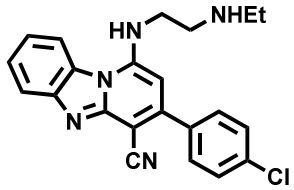
[a] SD = Standard deviation

[b] TWR = Total worm reduction

[c] FWR = Female worm reduction

** Significant ($p < 0.05$) worm burden reduction calculated by comparing TWR or FWR in the drug-treated group to that in the untreated control group

Table 3: Pharmacokinetic parameters of **28** in mice



Parameter	**Intravenous** [a] **2 mg/kg**	**Oral** [b] **20 mg/kg**
$t_{½}$(h)	6.56 (5.41 – 8.75)	3.20 (2.76 – 3.65)
T_{max} (h)	-	2.33 (1-3)
C_{max} (µM)	-	1.23 (1.11 – 1.43)
V_{ss} (L/kg)	10.1 (6.7 – 15.6)	-
Cl_{tot} (mL/min/kg)	17 (14 – 21)	-
AUC (min.µmol/L)	307 (252 – 366)	741 (413 - 1047)
Oral bioavailability (%)	-	25 (14 – 42)

[a]For intravenous dosing (n=3 mice), compounds were formulated in a solution of dimethylacetamide, polyethylene glycol and propylene glycol/ethanol mixture 4:1 at a ratio 1:3:6.

[b] For oral dosing (n=3 mice), compounds were formulated as suspension in 100% HPMC.

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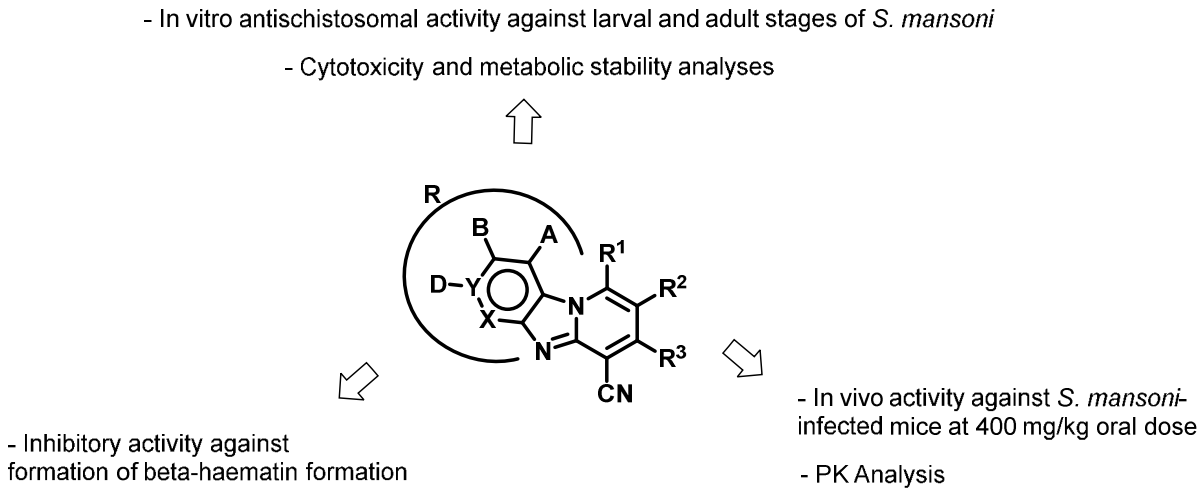
TITLE: Antischistosomal Activity of Pyrido[1,2-*a*]benzimidazole Derivatives and Correlation with Inhibition of β-haematin Formation

AUTHORS: John Okombo, Kawaljit Singh, Godfrey Mayoka, Ferdinand Ndubi, Linley Barnard, Peter M. Njogu, Mathew Njoroge, Liezl Gibhard, Christel Brunschwig, Mireille Vargas, Jennifer Keiser, Timothy J. Egan and Kelly Chibale



SYNOPSIS: The heavy reliance on praziquantel (PZQ) against schistosomiasis raises concerns about potential emergence of drug resistance. Pyrido[1,2-*a*]benzimidazoles (PBIs), which have recently been shown to also have antimalarial efficacy, present a therapeutic alternative that is hypothesised to target haem detoxification, a critical mechanism within the parasite. The graphic describes the identification of PBI derivatives with favourable activity against young and adult worms, good metabolic stability and cytotoxicity profiles, in vivo efficacy and potency against the envisaged target. This chemotype and subsequent data present a starting point towards improving the antischistosomal drug arsenal by designing new drugs to alleviate the extensive use of PZQ against schistosomiasis