**ABSTRACT:** N-Myristoyltransferase (NMT) represents a promising drug target within the parasitic protozoa *Trypanosoma brucei* (*T. brucei*), the causative agent for human African trypanosomiasis (HAT) or sleeping sickness. We have previously validated *T. brucei* NMT as a promising druggable target for the treatment of HAT in both stages 1 and 2 of the disease. We report on the use of the previously reported DDD85646 (1) as a starting point for the design of a class of potent, brain penetrant inhibitors of *T. brucei* NMT.

**INTRODUCTION**

Human African trypanosomiasis (HAT) or sleeping sickness is prevalent in sub-Saharan Africa1 with an estimated “at risk” population of 65 million.2 The causative agents of HAT are the protozoan parasites *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense*3,4 transmitted through the bite of an infected tsetse fly. HAT progresses through two stages. In the first stage (stage 1), the parasites proliferate solely within the bloodstream. In the second, late stage (stage 2), the parasite infects the central nervous system (CNS) causing the symptoms characteristic of the disease, such as disturbed sleep patterns and often death.5 Currently, there are a number of treatments available for HAT, though none are without issues, including toxicity and inappropriate routes of administration for a disease of rural Africa.6

Research has revealed enzymes and pathways that are crucial for the survival of *T. brucei*, and based on these studies, a number of antiparasitic drug targets have been proposed.7–10 *T. brucei* N-myristoyltransferase (*TbNMT*) is one of the few *T. brucei* druggable targets to be genetically and chemically validated in both *in vitro* and in rodent models of HAT.7,11,12 NMT is a ubiquitous essential enzyme in all eukaryotic cells. It catalyzes the co- and post-translational transfer of myristic acid from myristoyl-CoA to the N-terminal glycine of a variety of peptides. Protein N-myristoylation facilitates membrane localization and biological activity of many important proteins.11,13

NMT has been extensively investigated as a potential target for the treatment of other parasitic diseases including malaria,14 leishmaniasis,15 and Chagas’ disease16,17 resulting in the identification of multiple chemically distinct small molecule inhibitors.18 NMT has also been shown to be a potential therapeutic target for human diseases such as autoimmune disorders19 and cancer.20,21

Previously we have reported the discovery of compound 1 (Figure 1),7,22,23 which showed excellent levels of inhibitory potency for *TbNMT* and *T. brucei brucei* (*T. br. brucei*) proliferation *in vitro* and was used as a model compound to validate *TbNMT* as a druggable target for stage 1 HAT.7,22 However, 1 is not blood–brain barrier penetrant, a requirement for stage 2 activity. Two approaches were taken to increase the brain penetration of 1. A classical lead optimization approach is described elsewhere.24 This article describes a second approach that used a minimum pharmacophore of 1 aiming to derive a structurally distinct series of potent *TbNMT* inhibitors with brain penetration, as leads for the identification of suitable candidates for the treatment of stage 2 HAT.

**COMPOUND RATIONALE AND DESIGN**

To aid compound design, and to significantly lower molecular weight and polar surface area (PSA), the chlorines and the
sulfonamide moieties of 1 were removed to define a minimum pharmacophoric scaffold (Figure 2A). This scaffold was chosen because the piperidine makes a key interaction through the formation of a salt bridge with NMT’s terminal carboxylate.\textsuperscript{10} This interaction is highly conserved across the binding modes of NMT inhibitors covering multiple chemotypes including 1 (Figure 2B); known antifungal NMT inhibitors such as Roche’s (2-benzofurancarboxylic acid, 3-methyl-4-[[3-(3-pyridinylmethyl)amino]propoxy]-ethyl ester (RO-09-4609),\textsuperscript{26,27} Searle’s N-[2-4-[4-(2-methyl-1H-imidazol-1-yl)-butyl]phenyl][acetyl]-i-seryl-N-(2-cyclohexylethyl)-i-lysineamide (SC-58272)\textsuperscript{28} (Figure 2C), and Pfizer’s 2-((1R,4R)-4-(aminomethyl)cyclohexanecarboxamido)-N,N-dimethylbenzofuran-6-carboxamide (UK-370,485).\textsuperscript{29} Attempts to crystallize ThNMT had proved to be unsuccessful; therefore, the fungal Aspergillus fumigatus NMT (AfNMT)\textsuperscript{24,30} was used as a surrogate model for ThNMT in this study. AfNMT is 42% identical to ThNMT; however, within the peptide binding groove the level of identity is 92%. Previously, a selection of molecules from series 1 were assayed against AfNMT and ThNMT using the SPA biochemical assay and pIC\textsubscript{50} values compared using linear regression analysis. The pIC\textsubscript{50} values were shown to be correlated with an \textit{R}-squared value of 0.73 suggesting that AfNMT is a suitable surrogate system for study within this chemical series (see Supporting Information).

This minimum pharmacophoric scaffold had low molecular weight (237) and low PSA (12 Å\textsuperscript{2} to maximize the potential for CNS penetration) from which we could design varied chemistry (Figure 2A) to either access the serine pocket (occupied by the pyrazole moiety in 1) or the peptide recognition region, as seen in the peptomimetic compound highlighted in red (Figure 2C).

**Compound Design.** The adopted compound design strategy covered both compounds based on 1 (where common sulfonamide bioisosteres\textsuperscript{31} and pyrazole mimics were included) and compounds based on the binding pocket structural features, probing these with diverse hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD) groups. We employed high throughput chemistry, using technologies and techniques such as scavengers and solid supported reagents enabling arrays to be made in parallel. Three different but complementary chemistries

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**Figure 1.** Compound 1. \*Potencies were determined against recombinant ThNMT and HsNMT,\textsuperscript{11} and against bloodstream form \textit{T. brucei} (T. br. brucei) and MRC-5 proliferation studies \textit{in vitro} using 10 point curves replicated \textgeq 2. \*Calculated using Optibrium STARDROP software. \*Ligand efficiency (LE), calculated as 0.6-\textit{ln}(IC\textsubscript{50})/(heavy atom count) using \textit{T. brucei} NMT IC\textsubscript{50} potency.\textsuperscript{25} IC\textsubscript{50} values are shown as mean values of two or more determinations. Standard deviation was typically within 2-fold from the IC\textsubscript{50}. \*Enzyme selectivity calculated as HsNMT IC\textsubscript{50} (μM)/ThNMT IC\textsubscript{50} (μM).

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**Figure 2.** Development of the chemistry scaffold. (A) Two-dimensional interaction map of 1 bound to AfNMT leading to the design of the minimal scaffold. (B) Crystal structure of 1 bound; key recognition residues are highlighted and labeled. (C) Proposed minimal scaffold (C atoms gold) docked into the crystal structure of AfNMT overlaid with peptomimetic compound PDB 2NMT (C atoms cyan); the key S/T K peptide recognition region is highlighted red.
of Suzuki couplings, amidations, and Mitsunobu reactions were chosen to explore all positions around ring A (Figure 3).

Crossing the blood–brain barrier (BBB) was an essential part of our chemistry design and presented its own challenges. Improving the BBB permeation of molecules has been widely studied and \textit{in silico} prediction methods developed based on known CNS penetrant and nonpenetrant compounds.\textsuperscript{32,33} Examination of the physicochemical properties of molecules and their influence on affecting BBB permeability has suggested some guiding principles and a physicochemical property range to increase the probability of improving the BBB permeability.\textsuperscript{33} The top 25\% CNS penetrant drugs sold in 2004 were found to have mean values of PSA (Å\textsuperscript{2}) 47, HBD 0.8, cLogP 2.8, cLogD (pH 7.4) 2.1, and MW 293. They suggested the following maximum limits when designing compounds: PSA < 90 Å\textsuperscript{2}, HBD < 3, cLogP 2\textsuperscript{−} 4.5, cLogD (pH 7.4) 2\textsuperscript{−} 5, MW < 500. As this was the first round of compound design, we restricted the compounds to the following parameters: PSA 40\textsuperscript{−} 70 Å\textsuperscript{2}, HBD < 3, cLogP 2\textsuperscript{−} 4.5, MW 250\textsuperscript{−} 400.

Virtual libraries of all possible compounds that could be constructed from our in-house chemical inventory were constructed and minimized to ensure that a wide region of chemical space was explored, and structures were not biased to one region. Reaction schemes, intermediates, and examples of compounds made are described in the Supporting Information.

\section*{RESULTS AND DISCUSSION}

\textbf{Scaffold Array Results.} No compounds made in the Suzuki chemistry (1, Figure 3) derived series had a potency <10 μM against \textit{TbNMT} (see Supporting Information for compounds made). Table 1 shows the potency against \textit{TbNMT} for selected examples from the amide (3–7), homologated amide (8–12), and ether series (17–24). The most potent compound in the amide series was 3 (TbNMT IC\textsubscript{50} 1.7 μM). Amides directly linked to the phenyl ring in the 3-position were found to be more potent than the corresponding 4-substituted analogues (5 vs 4 and 7 vs 6). The homologated amide series in comparison to the amide series were on the whole >3-fold more potent (6 vs 12) with the most potent compound achieving a TbNMT IC\textsubscript{50} value of 0.07 μM (10). In the homologated amides series the 4-position amides showed greater potency than the corresponding 3-position analogues (the opposite trend to the amide series). Further optimization of both the directly linked and homologated amide series failed to improve the potency or the pharmacokinetic properties.

The ether array produced compounds with good levels of activity against \textit{TbNMT}, the most active of these achieved an IC\textsubscript{50} value of 0.5 μM (24). The more potent compounds were substituted in the 4-position, on average showing around 10-fold greater potency over their 3-position analogues, e.g., 3-position compound 22 (35 μM) vs 4-position compound 23 (1.2 μM) or 3-position compound 20 (13 μM) vs 4-position compound 21 (1.4 μM). Interestingly, the replacement of the sulfonamide in structure 1 with an ether linkage (17) was completely inactive against \textit{TbNMT} (IC\textsubscript{50} > 100 μM). This was surprising, as methyl ethers are considered possible sulfonamide bioisosteres.\textsuperscript{31} Compound 24 was not selective over human NMT (HsNMT1) but exhibited an EC\textsubscript{50} of 2 μM in the \textit{T. br. brucei} proliferation assay, with good microsomal stability and moderate levels of selectivity against proliferating human MRC-5 cells (Figure 4).

The crystal structure of 24 bound to \textit{AfNMT} (Figure 4A) shows the ligand binds in the peptide binding groove in an overall U-shaped conformation, with the ligand wrapping round the side chain of Phe157. The central aryl rings of 24 lie perpendicular to each other allowing the ligand to sit in the cleft formed by the side
chain of Tyr263, Tyr393, and Leu436. The cleft is formed by the movement of the side chain of Tyr273; a feature observed in the binding mode of benzofuran ligands\textsuperscript{26,27} and subsequent derivatives.\textsuperscript{34}

The pyridyl nitrogen of \textsuperscript{24} forms an interaction with Ser378 in a similar orientation as the trimethyl-pyrazole group of \textsuperscript{1}, and the piperidine moiety interacts directly with the C-terminal carboxyl group of Leu492.

Compound \textsuperscript{24} does not interact with His265, an interaction formed by the sulfonamide in \textsuperscript{1} (overlaid with \textsuperscript{24}, Figure 4B), which potentially explained the drop in potency between \textsuperscript{1} (TbNMT 0.002 μM) and \textsuperscript{24} (0.5 μM). Despite this loss of activity, \textsuperscript{24} had comparable ligand efficiency (LE)\textsuperscript{35} of 0.33 to \textsuperscript{1}, LE = 0.36, and in combination with the observed binding mode, gave us confidence that the design strategy was valid.

Optimization of Compound 24. With the aim of increasing potency against TbNMT, the diphenyl piperidine ring was replaced with the dichlorophenyl-pyridyl-piperidine moiety of \textsuperscript{1}. This change reduced the logP by ~1 log unit from 4.3 for \textsuperscript{24}, with an increase in PSA from 34 Å\textsuperscript{2} (19) to 50 Å\textsuperscript{2}, which was within the acceptable guidance limits for BBB permeability\textsuperscript{32,33} to give \textsuperscript{29} (synthesis shown in Scheme 1).

Compound \textsuperscript{29} (Figure 5) exhibited a 4-fold improvement in potency against TbNMT (IC\textsubscript{50} 0.1 μM) and improved efficacy in the T. br. brucei proliferation assay (EC\textsubscript{50} 0.7 μM), while retaining good microsomal stability (1.4 mL/min/g) and LE (0.33). Encouragingly, \textsuperscript{29} showed good levels of brain penetration (brain–blood = 0.4), a significant improvement over \textsuperscript{1} (brain–blood < 0.1),\textsuperscript{32} indicating that the strategy of reducing MW and PSA was a valid approach (\textsuperscript{1}, PSA 101 Å, MW 495). The crystal structure of \textsuperscript{29} bound to AfNMT (Figure 6) was determined.

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Table 1. Array Chemistry Selected Results for the Amide, Homologated Amides, and Ether Series

| Amides | TbNMT IC\textsubscript{50} (μM)\textsuperscript{a} | Homologated Amides | TbNMT IC\textsubscript{50} (μM)\textsuperscript{b} | Ether | TbNMT IC\textsubscript{50} (μM)\textsuperscript{b} |
|--------|------------------|-------------------|------------------|--------|------------------|
| 3\textsuperscript{a} | 1.7               | 8\textsuperscript{a} | 0.4               | 17\textsuperscript{a} | >100              |
| 4\textsuperscript{a} | >100              | 9\textsuperscript{a} | 0.60              | 18\textsuperscript{a} | 9.5               |
| 5\textsuperscript{a} | 15                | 10\textsuperscript{a} | 0.07              | 19\textsuperscript{a} | 1.6               |
| 6\textsuperscript{a} | >100              | 11\textsuperscript{a} | 13                | 20\textsuperscript{a} | 13                |
| 7\textsuperscript{a} | 2.9               | 12\textsuperscript{a} | 0.60              | 21\textsuperscript{a} | 1.4               |
| 1\textsuperscript{a} | 0.002             |                   |                   |        | 35                |
| 22\textsuperscript{a} |                   |                   |                   |        | 35                |
| 23\textsuperscript{a} |                   |                   |                   |        | 1.2               |
| 24\textsuperscript{a} |                   |                   |                   |        | 0.5               |

\textsuperscript{a} Compounds greater than 90% pure. \textsuperscript{b} Compounds >95% pure. \textsuperscript{c} IC\textsubscript{50} values are shown as mean values of two or more determinations. Standard deviation was typically within 2-fold from the IC\textsubscript{50}. nd = not determined.
showing the ligand adopted a conformation similar to 1 with the biaryl system sitting in plane with the 2,6-dichlorophenyl ring stacking in plane with the side chain of Tyr273. Key interactions between the piperidine N to Ser378 and the piperazine to the C-terminal carboxyl group are retained from 24.

Replacement of the 2,6-Dichlorophenyl Ring. Optimization of 29 focused on modifications to the central 2,6-dichlorophenyl ring to increase enzymatic selectivity relative to HsNMT1 (0.3 μM, 3-fold compared to TbNMT IC₅₀). These modifications were made employing the same chemistry as outlined in Scheme 1, by varying the starting substituted bromophenol used in the Mitsunobu step. These 2,6-dichlorophenyl modifications are detailed in Table 2.

None of the core modifications improved potency against TbNMT when compared to 29 (Table 2) nor LE and enzyme selectivity, although some demonstrated increased levels of potency against HsNMT1 (37, HsNMT1, IC₅₀ 0.01 μM). The reason for this increase in HsNMT1 activity was not explained using the available crystal structure data. Certainly inhibitors of human NMT such as 37 are of potential interest in the treatment of cancer, and further elaboration of the core could be explored.

Pyridyl Headgroup SAR. The next phase of optimization focused on modifications to the ether pyridyl ring of 29 shown in Table 3. These compounds were made using the same common phenol intermediate (Scheme 2), applying solid phase reagents such as polystyrene bound triphenylphosphine, and running reactions and purifications in parallel using commercially available alcohols or alcohols derived from commercially available carboxylic acids or esters after reduction with borane or lithium aluminum hydride (see Supporting Information).

Modifications to the pyridyl headgroup showed encouraging results with 47 equipotent to 29 (IC₅₀ ≈ 0.1 μM) but with ~65-fold selectivity over HsNMT1, equivalent activity in the T. br. brucei proliferation assay, and promising microsomal stability (Cₐst 4.2 mL/min/g). Compound 30, though, had equivalent activity to 29 in the MRC-5 counter screen, indicating that HsNMT1 activity may not have been driving the MRC-5 toxicity.

Homologation of the linker to the pyridyl group did not improve potency, as did groups on the pyridyl ring at the 6- (48) or 4-positions (49), though both 48 and 49 showed equivalent activity in the T. br. brucei proliferation assay to 29. The crystal structure of 29 overlaid with the trimethylpyrazole of 1 suggested that additions of methyl substitution may have been beneficial to potency (Figure 7A) because the trimethyl substitution of pyrrole in 1 was essential for activity. Subsequent crystal structures of 48 showed that the binding pocket the pyridyl headgroup accesses is small and that these substituents in the case of 49 forced the ether pyridyl ring to twist in the pocket to avoid steric clashes with its dichlorophenyl ring, and for 48, the 4-methyl forces the pyridyl ring out of the pocket. In both cases, the direct hydrogen bond from the pyridyl nitrogen to the serine was broken, but 48 still formed an interaction, though this was now water mediated (Figure 7B).

Alternative Nonpyridyl Head Group SARs. To advance the series, two regions within the structure were modified with the aim to improve potency, first examining pyridyl replacements and modifications to the pyridyl ring and replacement of the piperazino-pyridine moiety. First, the pyridyl ring was replaced with a range of five-membered heterocycles, mainly thiazoles, with various substitutions; see Table 4. The most potent of these showed levels of promising activity against TbNMT (IC₅₀ ≈ 0.05−0.06 μM; 58 and 57). The SAR around 57 was tight. The removal of either methyl groups (60 and 65) lost activity against TbNMT; in addition, substitution of the 2-methyl group with either ethyl (64) or isopropyl (66) lost all activity in the T. br. brucei proliferation assay. Compound 57 showed good stability to microsomal turnover (Cₐst 2.4 mL/min/g) but also improved selectivity over MRC-5 cytotoxicity. Both 58 and 57 showed equivalent levels of potency against HsNMT1 (IC₅₀ ≈ 0.03−0.08 μM) and again showed very different MRC-5 activities, indicating that MRC-5 toxicity may not be entirely driven by HsNMT1 activity.

Replacement of the Piperazino-Pyridine Moiety. We had previously validated TbNMT as a druggable target in the...
determined. Enzyme selectivity calculated as \( \frac{\ln(\text{IC}_{50})}{\text{heavy atom count}} \) using \( \text{Tb} \) HCl in diethyl ether.

Stage 2 model for HAT in mice using Figure 6.

Standard deviation was typically within 2-fold from the IC\(_{50}\). nd = not determined. *Values are shown as mean values of two or more determinations. Stdev.

Figure 5. Compound 29 profile. *Values calculated using the Optibrium STARDOCK software. 1Ligand efficiency (LE), calculated as 0.6-\( \ln(\text{IC}_{50})/(\text{heavy atom count}) \) using \( \text{T. brucei} \) NMT IC\(_{50}\) potency. \( \text{IC}_{50} \) values are shown as mean values of two or more determinations. Standard deviation was typically within 2-fold from the IC\(_{50}\); nd = not determined. *Enzyme selectivity calculated as \( \frac{\text{HsNMT1 IC}_{50} (\mu M)}{\text{TbNMT IC}_{50} (\mu M)} \).

Figure 6. Binding mode of 29 (C atoms gold) bound to A\( \text{fNMT} \) (PDB 5T6C). Binding mode of 1 (C atoms cyan) is shown for comparison.

Scheme 1*

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25 + HO\( \text{-} \)N   a)   Cl + Cl   b) c) d) N
26               27                      28
               + Cl

29

Reagents and conditions: (a) polymer supported-PPh\(_3\), DIAD, alcohol, THF; (b) dioxane/1 M aq K\(_3\)PO\(_4\), Pd(PPh\(_3\))\(_4\); (c) TFA, DCM; (d) 2 M HCl in diethyl ether.
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*pConclusions*

By using 1 as a starting point to identify alternative TbNMT inhibitor scaffolds with physicochemical properties suitable for penetration into the brain to treat stage 2 HAT, we identified an ether linker as a replacement of the sulfonamide of 1. This modification reduced molecular weight and polar surface area, producing a viable alternative series with excellent levels of brain penetration. This work highlights the importance of decreasing the PSA as a way of increasing the probability of brain penetration. Further optimization identified compounds with good levels of TbNMT and \( \text{T. brucei} \) antiproliferative activity and microsomal stability. Though in comparison with the original structure 1, further potency gains against the enzyme and in the parasite proliferation assay are required. This series
**EXPERIMENTAL SECTION**

**Synthetic Materials and Methods.** Chemicals and solvents were purchased from the Aldrich Chemical Co., Fluka, ABCR, VWR, Acros, Fisher Chemicals, and Alfa Aesar and were used as received unless otherwise stated. Air- and moisture-sensitive reactions were carried out under an inert atmosphere of argon in oven-dried glassware. Analytical thin-layer chromatography (TLC) was performed on precoated TLC plates (layer 0.20 mm silica gel 60 with fluorescent indicator UV254, from Merck). Developed plates were air-dried and analyzed under a UV lamp (UV254/365 nm). Flash column chromatography was performed using prepacked silica gel cartridges (230−400 mesh, 40−63 μm, from SiliCycle) using a Teledyne Presearch ISCO CombiFlash 4X or CombiFlash Retrieve. 1H NMR and 13C NMR spectra were recorded on a Bruker Avance II 500 spectrometer (1H at 500.1 MHz, 13C at 125.8 MHz) or a Bruker DPX300 spectrometer (1H at 300.1 MHz). Chemical shifts (δ) are expressed in ppm recorded using the residual solvent as the internal reference in all cases. Signal splitting patterns are described as singlet (s), doublet (d), triplet (t), quartet (q), pentet (p), multiplet (m), broad (br), or a combination thereof. Coupling constants (J) are quoted to the nearest 0.1 Hz. LC−MS analyses were performed with either an Agilent HPLC 1100 series connected to a Bruker Daltonics micrOTOF or an Agilent Technologies 1200 series HPLC connected to an Agilent Technologies 6130 quadrupole LC−MS, where both instruments were connected to an Agilent diode array detector. LC−MS chromatographic separations were conducted with a Waters Xbridge C18 column, 50 mm × 2.1 mm, 3.5 μm particle size; mobile phase, water/acetonitrile + 0.1% HCOOH, or water/acetonitrile + 0.1% NH3; linear gradient 80:20 to 5:95 over 3.5 min, and then held for 1.5 min; flow rate 0.5 mL min⁻¹. All assay compounds had a measured purity of ≥95% (by TIC and UV) as determined using this analytical LC−MS system; a lower purity level is indicated. High-resolution electrospray measurements were performed on a Bruker Daltonics MicrOTOF mass spectrometer. Microwave-assisted chemistry was performed using a Biotage Initiator Microwave Synthesizer.

**tert-Butyl-4-(3-Bromophenyl)piperidine-1-carboxylate (14).** A solution of 4-(3-bromophenyl)piperidine·hydrochloride (13) (5.1 g, 18.4 mmol, 1 equiv), Boc₂O (4.4 g, 20.2 mmol, 1.1 equiv), and triethylamine (3.87 mL, 27.8 mmol, 1.5 equiv) in THF (50 mL) was stirred at room temperature for 16 h. The reaction was filtered, and the filtrate was washed with dilute 10% citric acid and extracted into ethyl acetate. The ethyl acetate layer was washed with water, then dried over MgSO₄, filtered, and evaporated to give an off-white solid (14) (6.13 g, 98% yield). 1H NMR, 500 MHz, CDCl₃ δ 1.51 (s, 9H), 1.57−1.66 (m, 2H), 1.81−1.86 (m, 2H), 2.64 (tt, J = 3.70, 12.21, 1H), 2.77−2.85 (m, 2H), 4.22−4.32 (m, 2H), 7.14−7.22 (m, 2H), 7.35−7.39 (m, 2H). [M + H]+ = 388.4.

**tert-Butyl 4-(3′-Hydroxy-[1,1′-biphenyl]-3-yl)piperidine-1-carboxylate (15).** tert-Butyl 4-(3-bromophenyl)piperidine-1-carboxylate (14) (2 g, 5.88 mmol, 1 equiv), 3-hydroxyphenyl boronic acid (974 mg 7.06 mmol, 1.2 equiv), anhydrous dioxane (10 mL), and 1 M aq K₂CO₃ (6 mL) were combined in a microwave vessel and argon bubbled through the mixture for 5 min. Pd[PPh₃]₄ (136 mg 0.118 mmol, 2%), was added, and the reaction was degassed again for a further 5 min before microwaving at 140 °C for 15 min. The resulting solution was extracted into dichloromethane, washed with sat. aq NaHCO₃, and passed...
through a phase separation cartridge. The organic layer was then absorbed onto silica and purified by flash column chromatography running a gradient from 0% ethyl acetate/hexane to 50% ethyl acetate/hexane to give 15 as a colorless oil (1.76 g, 85% yield).

1H NMR 500 MHz, CDCl₃  δ 1.53 (s, 9H), 1.60-1.76 (m, 2H), 1.83-1.91 (m, 2H), 2.71 (tt, J= 3.68, 12.33, 1H), 2.70-2.91 (m, 2H), 4.24-4.35 (m, 2H), 6.36 (s, 1H), 6.88 (dd, J = 2.50, 8.11, 1H), 7.10-7.20 (m, 3H), 7.28-7.46 (m, 4H).

tert-Butyl 4-(4′-Hydroxy-[1,1′-biphenyl]-3-yl)piperidine-1-carboxylate (16). A mixture of 4-hydroxyphenylboronic acid (183 mg, 1.32 mmol, 1 equiv), tert-butyl 4-(3-bromophenyl)piperidine-1-carboxylate (14) (450 mg, 1.32 mmol, 1 equiv), Pd(dppf)Cl₂, (c) PS-PPh₃, DIAD, alcohol, THF; (d) TFA, DCM.

Table 3. Pyridyl Head Group SAR of Compound 29

| R | TbNMT IC₅₀ (μM) | HsNMT1 IC₅₀ (μM) | T. brucei Tryps EC₅₀ (μM) | MRC-5 EC₅₀ (μM) | LE⁹ | Enzyme Selectivity⁹ |
|---|---|---|---|---|---|---|
| 29 | 0.1 | 0.3 | 0.7 | 3.7 | 0.33 | 3 |
| 45 | 5.4 | >1 | >25 | >1 | 0.26 | nd |
| 46 | 1.6 | 0.1 | >1 | >1 | 0.28 | 0.67 |
| 47 | 0.1 | 6.5 | 0.4 | 4.6 | 0.34 | 65 |
| 48 | 0.8 | 1.3 | 0.6 | 2.8 | 0.28 | 1.6 |
| 49 | 0.3 | 0.2 | 0.5 | 2.3 | 0.31 | 0.7 |
| 50 | 0.8 | >1 | >1 | >1 | 0.29 | nd |
| 51 | 1.3 | 0.5 | >10 | >1 | 0.28 | 0.4 |
| 52 | 4.6 | 0.7 | >10 | >1 | 0.25 | 0.15 |

“IC₅₀ values are shown as mean values of two or more determinations. Standard deviation was typically within 2-fold from the IC₅₀. nd = not determined. " Ligand efficiency (LE), calculated as 0.6·ln(IC₅₀)/(heavy atom count) using T. brucei NMT IC₅₀ potency.” Enzyme selectivity calculated as HsNMT1 IC₅₀ (μM)/TbNMT IC₅₀ (μM).

**Scheme 2**

**Reagents and conditions:** (a) Boc₂O, NEt₃, THF; (b) 4-bromo-2,6-dichlorophenol, MeCN/1 M aq K₃PO₄, Pd(dppf)Cl₂; (c) PS-PPh₃, DIAD, alcohol, THF; (d) TFA, DCM.

4-(3′-(1,3,5-Trimethyl-1H-pyrazol-4-yl)methoxy)-[1,1′-biphenyl]-3-yl)piperidine (17). tert-Butyl 4-(3′-hydroxy-[1,1′-biphenyl]-3-yl)piperidine-1-carboxylate (15) (100 mg, 0.28 mmol, 1 equiv), (1,3,5-trimethylpyrazole)methanol (44 mg, 0.31 mmol, 1.1 equiv), polystyrene bound-PPh₃ (PPh₃ = triphenylphosphine, 1.84 mmol/g loading, 228
DIAD (diisopropyl azodicarboxylate, 5 mL, 24.8 mmol, 1.2 equiv) was added to a suspension of 4-bromo-2,6-dichlorophenol (25) (5.0 g, 20.7 mmol, 1 equiv), and polystyrene bound-PPh3 (1.84 mmol/g loading, 0.92 mmol, 1.2 equiv), and stirred at RT overnight. The resulting reaction was evaporated in vacuo before dissolving in dichloromethane (10 mL) and then washed with 10% citric acid (2 × 10 mL) and MeOH (3 × 10 mL) before eluting the product with 7 N ammonia in methanol. This was evaporated to give 17 as a white solid (4 g, 71% yield).1H NMR 500 MHz, CDCl3 δ 1.68, 7.68 Hz, 1H), 8.50 (dd, J = 1.68, 4.90 Hz, 1H), LC–MS [M + H]+ = 361.2. HRMS [M + H]+ calculated for C22H25N4O1 = 361.2023, found = 361.2033.

For compounds 29–36, the resulting residue was triturated, the beads filtered, and washed with ether (104 mg, 71% yield).1H NMR 500 MHz, DMSO-δ6 δ 3.23 (m, 4H), 3.85 (m, 4H), 5.48 (s, 2H), 7.30 (m, 1H), 7.78 (d, J = 7.45 Hz, 1H), 8.74 (d, J = 5.75 Hz, 1H), 9.39 (br s, 2H). LC–MS [M + H]+ = 361.2. HRMS [M + H]+ calculated for C22H23Cl2N4O1 = 429.1243, found = 429.1240.

For compounds 37–48, the filtrate concentrated in vacuo, extracted into dichloromethane, and then washed with aq NaHCO3. The two-phase system was passed through a phase separation cartridge, the filtrate concentrated in vacuo, and the title compound purified by flash column chromatography using 8% MeOH/ethyl acetate + 1% aq NH3 as the eluent. The residue was taken up in dichloromethane, ethereal HCl was added (2 M, 2 mL) and concentrated, and the dihydrochloride salt of 29 was triturated with ether, filtered, and washed with ether (104 mg, 71% yield).1H NMR 500 MHz, DMSO-δ6 δ 2.82 (s, 3H), 3.17–3.23 (m, 4H), 3.85–3.90 (m, 4H), 5.29 (s, 2H), 7.16 (d, J = 5.20 Hz, 1H), 7.27–7.30 (m, 1H), 7.78–7.86 (m, 1H), 8.06 (s, 2H), 8.22 (d, J = 5.20 Hz, 1H), 8.41–8.51 (m, 1H), 9.87–10.77 (m, 1H), 9.15 (br s, 2H). LC–MS [M + H]+ calculated for C16H13ClN4O1 = 353.0913, found = 353.0886.

For compounds 49–50, the reaction was evaporated in vacuo before dissolving in dichloromethane and loading onto a prewashed SCX cartridge. The SCX cartridge was washed with dichloromethane (3 × 10 mL) and MeOH (3 × 10 mL) before eluting the product with 7 N ammonia in methanol. This was evaporated to give 17 (64 mg, 61% yield).1H NMR 500 MHz, CDCl3 δ 1.66 (s, 3H), 1.67–1.75 (m, 2H), 1.87–1.92 (m, 2H), 2.70–2.77 (m, 1H), 2.80–2.89 (m, 2H), 3.76 (s, 3H), 4.22–4.35 (m, 2H), 4.90 (s, 2H), 6.39 (br s, 1H), 6.98–7.01 (m, 1H), 7.20–7.23 (m, 3H), 7.37–7.47 (m, 4H), [M + H]+ = 427.2.

Compounds 14–20 were made in an analogous manner to 17 from see Supporting Information for analytical data.

Prototypical Mitsunobu Reaction of a Pyridyl Alcohol and a Substituted Phenol (Scheme 1). See Supporting Information for the synthesis of intermediates 30–35 for compounds 36–41 (Table 2). 3-(4-Bromo-2,6-dichlorophenoxymethyl)-2-methylpyridine (27). DIAD (diisopropyl azodicarboxylate, 5 mL, 24.8 mmol, 1.2 equiv) was added to a suspension of 4-bromo-2,6-dichlorophenol (25) (5.0 g, 20.7 mmol, 1 equiv), and polystyrene bound-PPh3 (1.84 mmol/g loading, 0.92 mmol, 1.2 equiv), and pyridin-2-yl)piperazine-1-carboxylate (30) (150 mg, 0.54 mmol, 1 equiv) and 3-((4-bromo-2,5-dichlorophenoxy)methyl)-2-methylpyridine (31) (106 mg, 0.34 mmol, 1 equiv) and di-tert-butyl-diisocarbonate (BocO, 2.5 g, 11.3 mmol, 1.1 equiv), in THF (20 mL) and triethylamine (2.1 mL, 15.4 mmol, 1.5 equiv) was stirred at RT overnight. The resulting reaction was extracted into dichloromethane, and then washed with 10% citric acid and then water. The dichloromethane layer was dried over MgSO4, filtered and evaporated to give 28 (as a white solid (4 g, 100% yield).1H NMR 500 MHz, CDCl3 δ 1.28 (s, 12H), 1.42 (s, 9H), 3.45–3.50 (m, 8H), 6.90 (d, J = 4.91, 1H), 6.97 (s, 1H), 8.14 (dd, J = 1.02, 4.89, 1H), [M + H]+ = 389.45.

Prototypical Suzuki Reaction of an Aryl Bromide and a Boronate Ester (Compounds 29–35). 1-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2-yl)piperazine-1-carboxylate (28). A solution of 1-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2-yl)piperazine (2.97 g, 10.27 mmol, 1 equiv), di-tert-butyl-diisocarbonate (BocO, 2.5 g, 11.3 mmol, 1.1 equiv), in THF (20 mL) and triethylamine (2.1 mL, 15.4 mmol, 1.5 equiv) was stirred at RT overnight. The resulting reaction was extracted into dichloromethane, and then washed with 10% citric acid and then water. The dichloromethane layer was dried over MgSO4, filtered and evaporated to give 28 (as a white solid (4 g, 100% yield).1H NMR 500 MHz, CDCl3 δ 1.18 (s, 12H), 1.42 (s, 9H), 3.45–3.50 (m, 8H), 6.90 (d, J = 4.91, 1H), 6.97 (s, 1H), 8.14 (dd, J = 1.02, 4.89, 1H), [M + H]+ = 389.45.

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Figure 7. Binding mode of pyridyl headgroup modifications. (A) Binding mode of 49 (C atoms aquamarine; PDB ST6H) compared with 29 (C atoms gold). The side chain of Phe278 rotates to accommodate the 4-methyl group. (B) Binding mode of 49 (C atoms aquamarine; PDB ST6E); the interaction with Ser378 is now a water bridged interaction. (C) Compound 48 compared with 29.
Table 4. Pyridyl Head Group Replacements

|   | R       | TbNMT IC₅₀ (µM)ᵃ | HsNMTI IC₅₀ (µM)ᵇ | T. brucei Tryps EC₅₀ (µM)ᶜ | MRC-5 EC₅₀ (µM)ᶜ | LEᵈ | Enzyme Selectivity⁺ |
|---|---------|------------------|-------------------|-----------------|-----------------|-----|---------------------|
| 29| ![Chemical Structure](image) | 0.1              | 0.3               | 0.7             | 3.7             | 0.33 | 3                   |
| 56| ![Chemical Structure](image) | 0.3              | 4.4               | 1.1             | 3.5             | 0.32 | 15                  |
| 57| ![Chemical Structure](image) | 0.05             | 0.08              | 0.33            | 2.2             | 0.34 | 2                   |
| 58| ![Chemical Structure](image) | 0.06             | 0.03              | 0.8             | >10             | 0.34 | 0.5                 |
| 59| ![Chemical Structure](image) | 2.9              | >100              | >100            | >100            | 0.34 | >34                 |
| 60| ![Chemical Structure](image) | 1.3              | 15                | 28              | 3.1             | 0.26 | 11                  |
| 61| ![Chemical Structure](image) | 0.3              | 14                | >10             | >10             | 0.29 | 46                  |
| 62| ![Chemical Structure](image) | 0.5              | 9.9               | >100            | >1             | 0.33 | 20                  |
| 63| ![Chemical Structure](image) | 0.2              | 0.9               | >1              | >100            | 0.33 | 5                   |
| 64| ![Chemical Structure](image) | 0.3              | 0.3               | >100            | >1              | 0.30 | 1                   |
| 65| ![Chemical Structure](image) | 2.0              | 0.9               | >100            | >1              | 0.29 | 0.5                 |
| 66| ![Chemical Structure](image) | 3.5              | 3.8               | >15             | 2.0             | 0.24 | 1                   |
| 67| ![Chemical Structure](image) | 4.2              | 12                | >10             | >10             | 0.24 | 3                   |

ᵃIC₅₀ values are shown as mean values of two or more determinations. Standard deviation was typically within 2-fold from the IC₅₀. nd = not determined. ᵇLigand efficiency (LE), calculated as 0.6·ln(IC₅₀/(heavy atom count)) using *T. brucei* NMT IC₅₀ potency. ćEnzyme selectivity calculated as HsNMT1 IC₅₀ (µM)/TbNMT IC₅₀ (µM).

[1-(4-(2,6-Difluoro-4-((2-methylpyridin-3-yl)methoxy)phenyl)pyridin-2-yl)piperazine Dihydrochloride (39). Prepared from 3-((4-bromo-3,5-difluorophenoxy)methyl)-2-methylpyridine (33) (106 mg, 0.34 mmol, 1 equiv) and 28 (98 mg, 0.34 mmol, 1 equiv), according to the method outlined for the synthesis of 29, to give 39 as a dihydrochloride salt (109 mg, 69% yield).] ¹H NMR 500 MHz, DMSO δ 2.77 (s, 3H), 3.16–3.21 (m, 4H), 3.77–3.82 (m, 4H), 5.40 (s, 2H), 6.82 (d, J = 4.95 Hz, 1H), 7.03–7.07 (m, 1H), 7.14 (d, J = 9.90 Hz, 2H), 7.86–7.91 (m, 1H), 8.23 (d, J = 5.20 Hz, 1H), 8.49 (d, J = 6.30 Hz, 1H), 8.75 (d, J = 5.45 Hz, 1H), 9.20 (br s, 2H). LC–MS [M + H]+ = 397.2. HRMS [M + H]+ calculated for C₂₂H₂₃F₂N₄O₁ = 397.1834, found = 397.1852.

[1-(4-(2-Methyl-4-((2-methylpyridin-3-yl)methoxy)phenyl)pyridin-2-yl)piperazine Dihydrochloride (40). Prepared from 3-((4-bromo-3-methylphenoxy)methyl)-2-methylpyridine (34) (150 mg, 0.51 mmol, 1 equiv) and 28 (148 mg, 0.51 mmol, 1 equiv), according to the method outlined for the synthesis of 29, to give 40 as a dihydrochloride salt (110 mg, 48% yield).] ¹H NMR 500 MHz, DMSO δ 2.29 (s, 3H), 2.77 (s, 3H), 3.17–3.23 (m, 4H), 3.83–3.88 (m, 4H), 5.34 (s, 2H), 6.80–6.84 (m, 1H), 6.96–7.00 (m, 1H), 7.04 (dd, J = 2.58, 8.43 Hz, 1H), 7.10 (d, J = 2.50 Hz, 1H), 7.25 (d, J = 8.45 Hz, 1H), 7.85 (t, J = 6.63 Hz, 1H), 8.15 (d, J = 5.80 Hz, 1H), 8.47 (d, J = 7.25 Hz, 1H), 8.72 (d, J = 5.20 Hz, 1H), 9.799
9.27 \( (\text{br s, 2H}) \). LC−MS \([\text{M + H}]^+ = 375.2\). HRMS \([\text{M + H}]^+\) calculated for C\(_{23}\)H\(_{27}\)N\(_4\)O\(_1\) = 375.2179, found = 375.2191.

Scheme 3\( ^a \)

Reagents and conditions: (a) 9-BBN, THF; (b) Pd(PPh\(_3\))\(_3\), K\(_3\)PO\(_4\), H\(_2\)O, DMF; (c) TFA, DCM; (d) CH\(_2\)O, Na(OAc)\(_3\), BH, CHCl\(_3\).

Scheme 4\( ^a \)

Reagents and conditions: (a) H\(_2\)SO\(_4\), EtOH; (b) phenylboronic acid, 1 M K\(_3\)PO\(_4\)/dioxane, Pd(PPh\(_3\))\(_4\); (c) 2 M LiAlH\(_4\) in THF, 0 °C; (d) Pd(t BuP)\(_2\) 0.5 M isobutylzinc bromide, anhydrous THF.

9.27 \( (\text{br s, 2H}) \). LC−MS \([\text{M + H}]^+ = 375.2\). HRMS \([\text{M + H}]^+\) calculated for C\(_{23}\)H\(_{27}\)N\(_4\)O\(_1\) = 375.2179, found = 375.2191.
bromo-3,5-dimethylphenoxy)methyl)-2-methylpyridine (35) (150 mg, 0.49 mmol, 1 equiv) and 28 (142 mg, 0.49 mmol, 1 equiv), according to the method outlined for the synthesis of 29, to give 41 as a dihydrochloride salt (177 mg, 75% yield). \(^\text{*}\)H NMR 500 MHz, DMSO \(\delta\) 2.033 (s, 6H), 2.79 (s, 3H), 3.17–3.23 (m, 4H), 3.84–3.89 (m, 4H), 5.31 (s, 2H), 6.63–6.67 (m, 1H), 6.89–6.93 (m, 3H), 7.89 (t, \(J = 6.70\) Hz, 1H), 8.18 (d, \(J = 5.35\) Hz, 1H), 8.51 (d, \(J = 7.70\) Hz, 1H), 8.73 (dd, \(J = 1.10, 5.65\) Hz, 1H), 9.36 (br s, 2H). LC−MS [M + H]\(^+\) = 389.2. HRMS [M + H]\(^+\) calculated for C\(_{24}\)H\(_{29}\)N\(_4\)O\(_1\) = 389.2336, found = 389.235.

tert-Butyl 4-(4-(3,5-Dichloro-4-hydroxyphenyl)pyridin-2-yl)-piperazine-1-carboxylate (42). As a solution of tert-butyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2-yl)piperazine-1-carboxylate 28 (2.0 g, 5.14 mmol, 1.2 equiv), and 4-bromo-2,6-dichlorophenol (25) (1.04 g, 4.3 mmol, 1 equiv) in acetonitrile (7 mL) and aq 1 M K\(_3\)PO\(_4\) (5 mL) was degassed by bubbling argon through for 5 min; then Pd(dppf)\(_2\)Cl\(_2\) (175 mg, 0.22 mmol, 5%) was added and the reaction degassed again for a further 5 min before microwaving at 100 \(^\circ\)C for 30 min. The cooled solution was diluted with dichloromethane and washed with aq NaHCO\(_3\), the dichloromethane layer was dried over MgSO\(_4\), and the filtrate was evaporated onto silica and purified by flash column chromatography running a gradient from 0% ethyl acetate/hexane to 50% ethyl acetate/hexane to give 42 as a white solid (1.07 g, 49% yield). \(\text{^1H NMR 500 MHz, CDCl}_3 \delta\) 1.52 (s, 9H), 3.58–3.64 (m, 8H), 6.05 (br. s, 1H), 6.72 (s, 1H), 6.79 (d, \(J = 5.30\) Hz, 1H), 7.53 (s, 2H), 8.25 (d, \(J = 5.19\) Hz). [M + H]\(^+\) = 424.2.

1-(4-(3,5-Dichloro-4-(2-(pyridin-3-yl)ethoxy)phenyl)pyridin-2-yl)-piperazine (51). Diisopropyl azodicarboxylate (DIAD, 61 \(\mu\)L, 0.31 mmol, 1.1 equiv) was added to a suspension of 2-(pyridin-3-yl)ethanol (42 mg, 0.34 mmol, 1.2 equiv), polystyrene bound-PPh\(_3\) (1.84 mmol/g loading, 200 mg, 0.37 mmol, 1 equiv), and 42 (120 mg, 0.28 mmol, 1 equiv) in anhydrous THF (20 mL) and then heated at 70 \(^\circ\)C for 4 h. After cooling, the reaction mixture was filtered, the beads washed with

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**Table 5. Pyridyl Substitutions**

| R | T\(_{\text{bNMT}}\) IC\(_{50}\) (\(\mu\)M)\(^a\) | H\(_{\text{NMT1}}\) IC\(_{50}\) (\(\mu\)M)\(^b\) | T. brucei EC\(_{50}\) (\(\mu\)M) | MRC-5 EC\(_{50}\) (\(\mu\)M) | LE\(^b\) | Enzyme Selectivity\(^c\) |
|---|---|---|---|---|---|---|
| 29 | 0.1 | 0.3 | 0.7 | 3.7 | 0.33 | 3 |
| 79 | >50 | >50 | >50 | >50 | 0.28 | 0.4 |
| 80 | 0.1 | 0.04 | >10 | 14 | 0.28 | 0.4 |
| 81 | 0.02 | 0.04 | 0.1 | 1.5 | 0.33 | 2 |
| 82 | 0.08 | 0.2 | 0.1 | 2.1 | 0.28 | 3 |
| 83 | 0.02 | 0.04 | 0.3 | >1 | 0.34 | 2 |
| 84 | 0.06 | 0.18 | 0.5 | 1.4 | 0.29 | 3 |

\(^a\)IC\(_{50}\) values are shown as mean values of two or more determinations. Standard deviation was typically within 2-fold from the IC\(_{50}\). nd = not determined. \(^b\)Ligand efficiency (LE), calculated as 0.6·ln(IC\(_{50}\))/(heavy atom count) using T. brucei NMT IC\(_{50}\) potency. \(^c\)Enzyme selectivity calculated as H\(_{\text{NMT1}}\) IC\(_{50}\) (\(\mu\)M)/T\(_{\text{bNMT}}\) IC\(_{50}\) (\(\mu\)M).

**Figure 9. Comparison of 1 and 81.**
MeOH and dichloromethane, and the filtrate absorbed onto silica and purified by flash column chromatography running a gradient from 0% ethyl acetate/hexane to 100% ethyl acetate. The resulting residue was dissolved in dichloromethane (10 mL), trifluoroacetic acid (10 equiv) was added, and the reaction was stirred at RT for 16 h. The reaction was evaporated in vacuo before loading onto a prewashed SCX cartridge. The cartridge was washed with dichloromethane (3 × 10 mL) and MeOH (5 × 10 mL) before eluting with 7 N ammonium in methanol. This was absorbed onto silica and purified by flash column chromatography running a gradient from 0% MeOH/dichloromethane + 1% NH₄OH to 20% MeOH/dichloromethane + 1% NH₄OH, to give 51 as a white solid (35 mg, 29% yield). H NMR 500 MHz, CDCl₃ δ 3.02–3.05 (m, 4H), 3.22 (t, J = 6.90 Hz, 2H), 3.58–3.61 (m, 4H), 4.30 (t, J = 6.90 Hz, 2H), 6.71 (s, 1H), 6.75 (dd, J = 1.50 Hz, 2H, 1H), 7.18–7.21 (m, 1H), 7.59 (dd, J = 7.93 Hz, 2H, 7.51 (s, 2H), 7.65–7.69 (m, 1H), 8.25 (dd, J = 0.64, 5.25 Hz, 1H), 8.58–8.60 (m, 1H). [M + H]⁺ calculated for C₂₃H₂₅Cl₂N₄O₁ = 429.1243, found = 429.1234, MS [M + H]⁺ = 429.12. 

1-(4-(5-Dichloro-4-(2-(4-hydroxybenzyl)phenyl)ethoxy)phenyl)-2-piperazin-4-yl-piperazine (52). Prepared using 42 (112 mg, 0.28 mmol, 1 equiv) and 2-(4-hydroxybenzyl)piperidine (42 mg, 0.34 mmol, 1.2equiv) according to the Mitsunobu reaction and BOC deprotection procedure outlined in the synthesis of 51, to give 52 as an off-white solid (22 mg, 18% yield). H NMR 500 MHz, CDCl₃ δ 3.02–3.05 (m, 4H), 3.39 (t, J = 6.75 Hz, 2H), 3.83–3.86 (m, 4H), 4.49 (t, J = 6.65 Hz, 2H), 6.71 (s, 1H), 6.75 (dd, J = 1.50 Hz, 2H, 1H), 7.18–7.21 (m, 1H), 7.39 (dd, J = 7.93 Hz, 2H, 7.51 (s, 2H), 7.65–7.69 (m, 1H), 8.25 (dd, J = 0.64, 5.25 Hz, 1H), 8.58–8.60 (m, 1H). [M + H]⁺ calculated for C₂₁H₂₁Cl₂N₁₄O₁ = 429.1243, found = 429.1234, MS [M + H]⁺ = 429.12. 

Compounds 56–67 were synthesized using the standard Mitsunobu coupling conditions followed by BOC deprotection using TFA from 42 according to the procedure outlined in the synthesis of 51 or, in the case of 56, using the methodology used for compound 29. 

3-(4-(2-Dichloro-4-(2-(4-hydroxybenzyl)phenyl)ethoxy)phenyl)-2-methylsulfinyl-2,4-dimethylisoxazole (57). Prepared using 42 (200 mg, 0.47 mmol, 1 equiv) and (2,5-dimethyl-1,3-oxazol-4-yl)methanol (47 mg, 0.42 mmol, 1 equiv) to give 57 as a dihydrochloride salt (70 mg, 36% yield), 1H NMR 500 MHz, DMSO δ 2.45 (s, 3H), 3.17–3.26 (m, 4H), 3.90–3.97 (m, 4H), 5.14 (s, 2H), 6.48–6.50 (m, 1H), 7.24 (d, J = 5.03 Hz, 1H), 7.37–7.40 (m, 1H), 8.07 (s, 2H), 8.20 (d, J = 5.73 Hz, 1H), 9.36 (br s, 2H). LC-MS [M + H]⁺ = 419.1. HRMS [M + H]⁺ calculated for C₂₀H₁₈Cl₂N₂O₁S₁ = 449.0964, found = 449.0949. 

DOI: 10.1021/acs.jmedchem.7b01255
(s, 2H), 6.77 (s, 1H), 6.78 (dd, J = 1.45, 5.10 Hz, 1H), [M + H]+ = 423.1. HRMS [M + H]+ calculated for C19H19Cl2N4O1S1 = 421.0651, found = 421.064. 4-((2,6-Dichloro-4-(2-(piperazin-1-yl)pyridin-4-yl)phenoxy)methyl)-2-methylpyridine (70). To a solution of tert-butyl (4-bromo-2,6-dichlorophenyl)methyl-2-methylpyridine (0.42 g, 1.2 mmol, 1 equiv) and K2PO4 (1 M in H2O, 2.4 mmol, 2.4 mL, 2 equiv) in anhydrous DMF (2.5 mL) was added and then degassed with argon. (Pd[PPh3]4 (0.024 mmol, 28 mg, 2%) was then added, and the solution was microwaved at 110 °C for 1 h in a microwave. To this crude reaction, 3-((4-bromo-2,6-dichlorophenyl)methyl)-2-methylpyridine (0.42 g, 1.2 mmol, 1 equiv) and K2PO4 (1 M in H2O, 2.4 mmol, 2.4 mL, 2 equiv) in anhydrous DMF (2.5 mL) was added and then degassed with argon. (Pd[PPh3]4 (0.024 mmol, 28 mg, 2%) was then added, and the solution was microwaved at 110 °C for 1 h. The reaction was extracted into dichloromethane, washed with water, and dried over MgSO4. The crude material was purified by flash column chromatography, giving a good room temperature and then partitioned between dichloromethane and sodium bicarbonate solution. The dichloromethane layer was separated and dried over MgSO4 and solvent removed. The crude material was purified by column chromatography, eluting with dichloromethane to dichloromethane–methanol (80:20) with 1% NH4OH, to give 70 (0.105g, 10%) as an oil. 1H NMR 500 MHz, CDCl3 δ 1.05–1.15 (m, 2H), 1.25–1.49 (m, 6H), 1.58–1.64 (m, 2H), 2.57 (dd, J = 7.7, 7.7 Hz, 2H), 2.70 (s, 3H), 4.07–4.10 (m, 2H), 5.05 (s, 2H), 7.16 (s, 2H), 7.21 (dd, J = 4.9, 7.6 Hz, 1H), 7.90 (dd, J = 1.5, 7.6 Hz, 1H), 8.52 (dd, J = 1.7, 4.9 Hz, 1H). HRMS [M + H]+ calculated for C23H21Cl2N4O2S1 = 429.124434, found = 429.124407. 3-((2,6-Dichloro-4-(3-(piperazin-1-yl)propylyphenyl)phenoxy)methyl)-2-methylpyridine (71). 3-((2,6-Dichloro-4-(3-(piperazin-1-yl)propylyphenyl)phenoxy)methyl)-2-methylpyridine (70) (0.07 g, 0.17 mmol, 1 equiv) was taken up in chloroform (10 mL), treated with paraformaldehyde (0.052 g, 1.0 equiv), and heated at 55 °C for 1 h. The reaction mixture was then treated with sodium triacetoxysilane (0.183 g, 0.86 mmol, 5 equiv), and heating continued for 16 h. The reaction mixture was cooled and evaporated to dryness under vacuum, and then absorbed onto silica, and purified by flash chromatography using dichloromethane and sodium bicarbonate solution. The dichloromethane layer was separated and dried over MgSO4 and solvent removed. The crude material was purified by column chromatography, eluting with dichloromethane to dichloromethane–methanol (85:5) with 1% NH4OH, to give 71 (0.30g, 10%) as a white solid (58 mg, 76% yield). 1H NMR 500 MHz, CDCl3 δ 1.34–1.39 (m, 9H), 1.87–1.92 (m, 2H), 2.27 (dd, J = 7.8, 7.8 Hz, 2H), 2.70 (s, 4H), 2.85 (dd, J = 11.3 Hz, 2H), 5.05 (s, 2H), 5.33 (s, 1H), 7.16 (s, 2H), 7.21 (dd, J = 5.1, 7.6 Hz, 1H), 7.90 (dd, J = 1.5, 7.6 Hz, 1H), 8.52 (dd, J = 1.7, 4.8 Hz, 1H). HRMS [M + H]+ calculated for C23H21Cl2N4O2S1 = 421.1889, found = 421.1802. Ethyl 2-Chloronicotinate (72) and Ethyl 2-Ethynonicotinate (75). To a suspension of 2-chloronicotinic acid (4.6 g, 29.2 mmol) in ethanol (50 mL), conc. H2SO4 (2 mL) was added dropwise, and the suspension was heated to reflux for 3 h to form a solution. The reaction was then cooled and evaporated in vacuo, then carefully neutralized with sat. aq NaHCO3 and extracted into ethyl acetate. The organic layer was washed with water and then dried over MgSO4, filtered, absorbed onto silica, and purified using flash column chromatography running a gradient from 0% ethyl acetate/hexane to 100% ethyl acetate/hexane, to give the title compounds (ethyl 2-chloronicotinate 72, bottom spot, 2.33 g, 43% yield; ethyl 2-ethynonicotinate, top spot, 923 mg, 16% yield). Ethyl 2-chloronicotinate (72) 1H NMR 500 MHz, CDCl3 δ 1.45 (t, J = 7.61 Hz, 3H), 4.45 (q, J = 7.07 Hz, 2H), 7.36 (dd, J = 4.76, 7.7 Hz, 1H), 8.19 (d, J = 2.09, 7.87 Hz, 1H), 8.54 (dd, J = 2.09, 4.77 Hz, 1H). [M + H]+ = 183.1.
186.1. Ethyl 2-ethoxy nicotinate (75) \[\text{H NMR 500 MHz, CDCl}_3 \delta 1.14 (t, \text{J} = 7.06 \text{ Hz, 3H}), 1.47 (t, \text{J} = 6.92 \text{ Hz, 3H}), 4.39 (q, \text{J} = 7.20 \text{ Hz, 2H}), 4.50 (q, \text{J} = 7.06 \text{ Hz, 2H}), 6.94 (dd, \text{J} = 4.98, 7.48 \text{ Hz, 1H}), 8.16 (dd, \text{J} = 2.01, 7.48 \text{ Hz, 1H}), 8.30 (dd, \text{J} = 2.01, 4.88 \text{ Hz, 1H}). \text{[M + H]}^+ = 196.1.\]

**Prototypical Negishi Reaction between a Chloropyridine and Alky lboronic Acid. Ethyl 2-phenyl nicotinate (74a).** Anhydrous THF (9 mL) was added to a flame-dried argon flushed flask containing ethyl 2-chloronicotinate (72) (227 mg, 1.2 mmol, 1 equiv) and Pd(But)2 (31 mg, 0.06 mmol, 5%), and the mixture was stirred until clear. To this, isobutylic bromide (0.5 M in THF, 2.6 mL, 1.3 mmol, 1.1 equiv) was added dropwise, and the resulting solution was heated at 60 °C overnight. The reaction was absorbed onto silica and eluted to remove baseline material before purifying again by flash column chromatography using 25% ethyl acetate/hexane as the eluent, to give 74a as a yellow oil (164 mg, 66% yield). \[\text{H NMR 500 MHz, CDCl}_3 \delta 0.95 (d, \text{J} = 6.75 \text{ Hz, 6H}), 1.43 (t, \text{J} = 7.75 \text{ Hz, 3H}), 2.13 (sept, \text{J} = 6.75 \text{ Hz, 1H}), 3.11 (d, \text{J} = 7.25 \text{ Hz, 2H}), 4.41 (q, \text{J} = 7.13 \text{ Hz, 2H}), 8.16 (dd, \text{J} = 1.88, 4.75 \text{ Hz, 1H}), \text{[M + H]}^+ = 208.\]

**Prototypical Suzuki Reaction of a Chloropyridine and Boronic Acid. Ethyl 2-phenyl nicotinate (73).** A solution of ethyl 2-chloronicotinate (72) (793 mg, 4.3 mmol, 1 equiv) and phenylboronic acid (77a) (741 mg, 4.0 mmol, 1 equivalent) in 1 M aq KPO4 (4 mL) and dioxane (6 mL) in a microwave vessel was degassed with argon for 5 min before addition of Pd(PPh3)4 (64 mg, 0.055 mmol, 5%) and degassing again for a further 5 min before microwaving at 140 °C for 15 min. The reaction mixture was partitioned between dichloromethane and sat. aq NaHCO3 and the organic layer was absorbed onto silica and purified by flash column chromatography running a gradient from 0% ethyl acetate/hexane to 25% ethyl acetate/hexane, affording 73 as an oil (927 mg, 95% yield). \[\text{H NMR 500 MHz, CDCl}_3 \delta 1.07 (t, \text{J} = 7.19 \text{ Hz, 3H}), 4.18 (q, \text{J} = 7.19 \text{ Hz, 2H}), 7.37 (dd, \text{J} = 4.91, 7.87 \text{ Hz, 1H}), 7.45–7.48 (m, 3H), 7.55–7.57 (m, 2H), 8.14 (dd, \text{J} = 1.71, 7.76 \text{ Hz, 1H}), 8.79 (dd, \text{J} = 1.83, 4.78 \text{ Hz, 1H}). \text{[M + H]}^+ = 228.2.\]

**Prototypical Pyridyl Ester Reduction to an Alcohol. (2-isobutylpyridin-3-yl)methanol (77a).** To a solution of ethyl 2-isobutylnicotinate 74a (774 mg, 3.7 mmol, 1 equiv) in anhydrous THF (5 mL) at 0 °C, 0.5 M LiH2PO4 in THF (5.6 mL, 11.2 mmol, 3 equiv) was added dropwise, and the solution was allowed to warm to room temperature before being stirred at rt for 16 h. Sodium sulfite decachloride was added to the solution, and the reaction was diluted with dichloromethane and allowed to stir for 30 min. The reaction was filtered, the filtered layers separated, and the organic layer dried over MgSO4 and evaporated in vacuo to give 77a as a yellow oil (452 mg, 74% yield). \[\text{H NMR 500 MHz, CDCl}_3 \delta 0.97 (d, \text{J} = 6.67, 6.67, 2.19 (sept, \text{J} = 6.82 \text{ Hz, 1H}), 2.71 (d, \text{J} = 7.42 \text{ Hz, 2H}), 4.79 (d, \text{J} = 5.51 \text{ Hz, 2H}), 7.17 (dd, \text{J} = 4.78, 7.82 \text{ Hz, 1H}), 7.60 (d, \text{J} = 7.68 \text{ Hz, 1H}), 8.50 (dd, \text{J} = 1.74, 4.78 \text{ Hz, 1H}). \text{[M + H]}^+ = 166.2.\]

1-(4-(3,5-Dichloro-4-((2-propylpyridin-3-yl)methoxy)phenyl)pyridin-2-yl)pyperidine (79). Prepared using 42 (200 mg, 0.47 mmol, 1 equiv) and (2-ethoxy pyridin-3-yl)methanol (78) (87 mg, 0.57 mmol, 1.2 equiv), according to the Mitsunobu reaction and BOC deprotection procedure outlined for the synthesis of 51, to give 79 as an off-white solid (30 mg, 33% yield). \[\text{H NMR 500 MHz, CDCl}_3 \delta 1.42 (t, \text{J} = 7.21 \text{ Hz, 3H}), 2.66–2.69 (m, 4H), 3.65–3.68 (m, 4H), 4.41 (q, \text{J} = 7.07 \text{ Hz, 2H}), 6.72 (dd, \text{J} = 1.42, 5.21 \text{ Hz, 1H}), 6.90 (dd, \text{J} = 5.04, 7.20 \text{ Hz, 1H}), 7.53 (s, \text{J} = 1.76, 7.50 \text{ Hz, 1H}), 8.09 (dd, \text{J} = 1.96, 5.04 \text{ Hz, 1H}), 8.24 (ddd, \text{J} = 5.24 \text{ Hz, 1H}). \text{[M + H]}^+ = 459.2. \text{HRMS [M + H]}^+ calculated for C23H16Cl2N3O2 = 459.1349, found = 459.1339.\]

4-(4-(2,6-Dichloro-2-(1-propenyl)-1-yl)pyridin-2-yl)phenoxymethyl pyridin-2-yl)morpholine (80). Prepared using 42 (200 mg, 0.47 mmol, 1 equiv) and (2-morpholinopyridin-3-yl)methanol (110 mg, 0.57 mmol, 1.2 equiv), according to the Mitsunobu reaction and BOC deprotection procedure outlined for the synthesis of 51, to give 80 as an off-white solid (58 mg, 28% yield). \[\text{H NMR 500 MHz, CDCl}_3 \delta 3.04–3.06 (m, 4H), 3.28–3.31 (m, 4H), 3.60–3.63 (m, 4H), 3.88–3.90 (m, 4H), 5.15 (s, 2H), 6.74 (s, 1H), 6.79 (dd, \text{J} = 1.48, 5.29 \text{ Hz, 1H}), 7.60 (s, 2H), 8.07 (dd, \text{J} = 1.93, 7.51 \text{ Hz, 1H}), 8.28 (d, \text{J} = 5.23 \text{ Hz, 1H}), 8.37 (ddd, \text{J} = 1.93, 4.89 \text{ Hz, 1H}). \text{[M + H]}^+ = 500.2. \text{HRMS [M + H]}^+ calculated for C24H21Cl2N3O2 = 500.16457, found = 500.16771.\]
NMT Enzyme Assay. NMT assays44,45 were carried out at room temperature (22–23 °C) in 384-well white optitop plates (PerkinElmer). Each assay was performed in a 40 µL reaction volume containing 30 mM Tris buffer, pH 7.4, 0.5 mM EDTA, 0.5 mM EGTA, 1.25 mM dithiothreitol (DTT), 0.1% (v/v) Triton X-100, 0.125 µM [3H]-myristoyl-CoA (8 Curie (Ci)) mmol−1, 0.5 mM biotinylated CAP5,5, 5 mM NMT, and various concentrations of the test compound. The IC50 values for HsNMT1 and HsNMT2 were essentially identical against 80 compounds tested, and for logistical reasons, only HsNMT1 was used in later studies.

Test compound (0.4 µL in DMSO) was transferred to all assay plates using a Cartesian Hummingbird (Genomics Solution) before 20 µL of enzyme was added to assay plates. The reaction was initiated with 20 nM NMT, and various concentrations of the test compound. The IC50 curve fitting was undertaken using XLFit version 4.2 from IDBS. A four-parameter logistic dose—response curve was used using XFit 4.2 Model 205. All test compound curves had a correlation of 0.99 or better.

ActivityBase from IDBS was used for data processing and analysis. All IC50 curve fitting was undertaken using XLFit version 4.2 from IDBS. A four-parameter logistic dose—response curve was used using XFit 4.2 Model 205. All test compound curves had a floating top and bottom, and profit was used for all four parameters.

Compound Efficacy and Trypanocidal Activity in Cultured T. brucei Parasites. Bloodstream T. brucei 427 was cultured at 37 °C in modified HM9 medium (56 mM 1-thioglycerol was substituted for 200 mM 2-mercaptoethanol) and quantified using a hemocytometer. For the live/dead assay, cells were analyzed using a two-color cell viability assay (Invitrogen) as described previously.46 Cell culture plates were stamped with 1 µL of an appropriate concentration of test compound in DMSO followed by the addition of 200 µL of trypanosome culture (104 cells ml−1) to each well, except for one column, which received media only. MRC-5 cells were cultured in DMEM, seeded at 2000 cells per well, and allowed to adhere overnight. One microliter of test compound (10 point dilutions from 50 µM to 2 nM) was added to each well at the start of the assay. Culture plates of T. brucei and MRC-5 cells were incubated at 37 °C in an atmosphere of 5% CO2 for 69 h, before the addition of 20 µL of resazurin (final concentration, 50 µM). After a further 4 h incubation, fluorescence was measured (excitation 528 nm; emission 590 nm) using a PerkinElmer fluorimeter with a BioTek fluoroscan plate reader.

**ASSOCIATED CONTENT**

Supporting Information
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.7b01255.

Experimental details for compounds 3–12 and 17–24; X-ray data collection and refinement statistics; correlation of enzyme activity data for inhibitors against AfNMT and TbNMT; molecular structures of known NMT inhibitors (PDF)

Molecular formulas strings (CSV)

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Notes
The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

Funding for this work was provided by the Wellcome Trust (Grant ref. WT077705 and Strategic Award WT083481). We would like to thank Gina McKay for performing HRMS analyses and Daniel James for data management. We thank the European Synchrotron Radiation Facility (ESRF) for synchrotron beamtime and support, and Paul Fyfe for supporting the in-house X-ray facility.

**ABBREVIATIONS USED**

NMT, N-myristoyltransferase; T. brucei, Trypanosoma brucei; T. br. brucei, T. brucei brucei; HAT, human African trypanosomiasis or sleeping sickness; CNS, central nervous system; TbNMT, T. brucei N-myristoyltransferase; HsNMT, human NMT; LE, ligand efficiency; PSA, polar surface area; AƒNMT, Aspergillus fumigatus NMT; HBA, hydrogen bond acceptor; HBD, hydrogen bond donor; BBB, blood–brain barrier; MW, molecular weight; cLogP, calculated LogP; LogD, calculated LogD; SAR, structure–activity relationship; nd, not determined

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