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N-Myristoyltransferase: a Prospective Drug Target for Protozoan Parasites

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The development of new drugs targeted at parasite pathogens represents an important step towards decreasing the morbidity and mortality caused by these organisms, which affect hundreds of millions of people worldwide. Many of the drugs used today to treat diseases such as malaria, leishmaniasis, and African sleeping sickness were discovered decades ago and are often ineffective against resistant organisms, or possess toxic side effects. As a result an increasing amount of fundamental research is being directed towards identifying and validating new drug targets.[1] Identification of suitable macromolecular targets that can be exploited for therapeutic development is challenging, as physiological knowledge and tools for genetically manipulating these organisms are somewhat limited. Numerous biochemical pathways are being assessed as drug targets and include post-translational modification mechanisms such as phosphorylation in kinase pathways[2,3] and lipidation.[4] For the latter category, the inhibition of prenylation processes with farnesyltransferase inhibitors has shown potential for treating malaria and African trypanosomiasis.[5] However, as discussed below, more recent data are emerging on the suitability of another type of lipid-modification pathway involving the enzyme myristoyl-CoA:protein *N*-myristoyltransferase (NMT).

NMT is a monomeric enzyme, ubiquitous in eukaryotes, that catalyses the co-translational transfer of the saturated 14-carbon fatty acid, myristate, from myristoyl-CoA (14:0 CoA) to the N-terminal glycine residue of susceptible proteins by an amide bond. The co-translational nature of this modification was demonstrated by showing that the nascent polypeptide is labelled whilst still attached to the ribosome,[6] and this is thought to be the normal sequence of events for the majority of the *N*-myristoylation modifications of a range of proteins with a variety of cellular roles. One exception is a subset of proteins that have been shown to be post-translationally modified by NMT after caspase-mediated cleavage of a mature protein.[7–9] Post-translational modification of proteins from the bacterial type III secretion system by NMT has also been predicted[10] and confirmed.[11,12]

NMTs have been identified and characterised from a range of eukaryotic organisms including human,[13,14] mouse, rat, and cow,[15] as well as plant and insect species.[16,17] NMT was first purified from *Saccharomyces cerevisiae* (ScNMT), yielding a protein of ~50 kDa,[18] and extensive studies have also been conducted on fungal NMTs, from *Candida albicans*,[19] *Cryptococcus neoformans*, and *Histoplasma capsulatum*.[20] More recently, NMTs have also been characterised from the parasitic protozoa *Leishmania major*, *Trypanosoma brucei*,[21] and *Plasmodium falciparum*.[22] Alignments of NMT sequences from many species can be obtained from http://mendel.imp.ac.at/myristate/SUPLalignment.htm (last access: February 1, 2008).[23] There are two sequence motifs characteristic for all NMTs: EINFLCxHK and KFGxGDG; these are thus presumably of functional necessity to this enzyme.

N-Myristoylation by NMT has been demonstrated, by product inhibition profiles, to proceed via an ordered Bi–Bi reaction mechanism in which binding of myristoyl-CoA generates a second binding pocket for the docking of the substrate protein, as summarised in Figure 1.[24,25] The myristate group from myristoyl-CoA is then transferred to the N-terminal glycine of the bound protein in a nucleophilic addition–elimination reaction. This is followed by stepwise release, first of the free CoA and then the *N*-myristoylated protein.[24,26,27] The catalytic mechanism requires the presence of an N-terminal glycine on the substrate protein exposed by the action of methionine aminopeptidase.[28] Initial studies indicated the presence of an acyl intermediate formed as a result of physical transfer of the myristate group to the enzyme,[29] and have led to the proposal of an acyl intermediate in the reaction mechanism,[30] although this has yet to be observed. Crystallographic studies of ScNMT in binary complex with myristoyl-CoA and ternary complex with *S*-(2-oxo)pentadecyl-CoA and the peptide substrate GLYASKLA has allowed the residues involved in the active site to be identified.[31] The availability of this X-ray crystal structure

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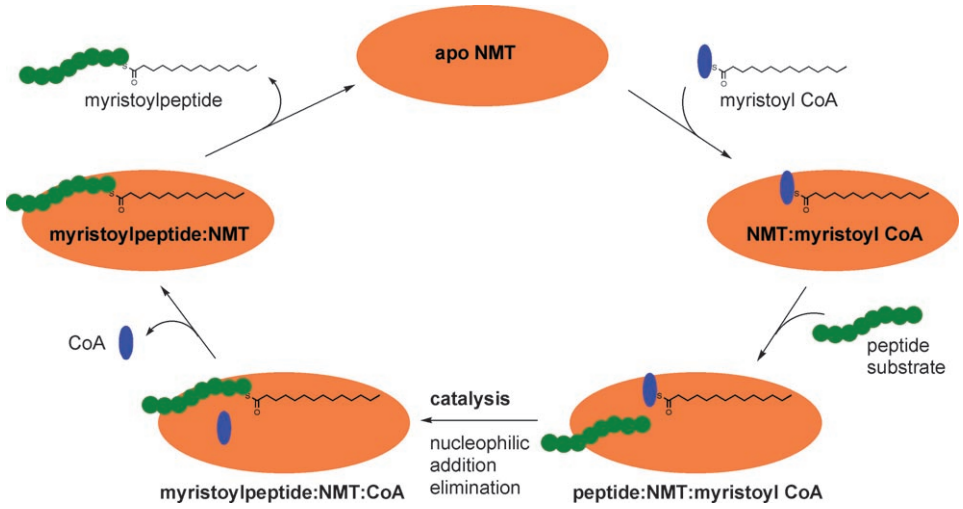

Figure 1. Proposed Bi–Bi mechanism of NMT action: The catalytic cycle for *N*-myristoylation is depicted. The enzyme first binds myristoyl-CoA to cause a structural rearrangement sufficient to allow binding of the N terminus of the peptide/protein substrate. The nucleophilic addition–elimination reaction is catalysed by stabilising interactions from the protein leading to formation of products that then dissociate in a stepwise manner.[24]

has also provided additional important insight into: the enzyme-catalysed mechanism including conformational changes associated with substrate binding, key residues involved in stabilization of intermediates along the reaction pathway, the role of the oxyanion hole in the enzyme, and the requirement of an N-terminal glycine residue on the protein target that is myristoylated. The importance of the C-terminal NMT residue, as well as Tyr 203, Asn 169, and the residues of the oxyanion hole in ScNMT was confirmed by mutagenesis and pre-steady-state kinetics studies.[32] More recently, analysis of crystal structures of ScNMT in complex with myristoyl-CoA and various nonpeptidic inhibitors in combination with site-directed mutagenesis studies have also identified Asp 22 and Asp 23 as playing a key role in binding the peptide substrate but not in catalysis. Furthermore, this work also demonstrated that the N-terminal region of the protein is important for the molecular recognition and binding of myristoyl-CoA.[33] The conservation of the critical residues across the NMTs suggests that the mechanism will also be conserved.

The many cellular functions of NMT have led to numerous investigations of this protein as a target for the development of anticancer, antiviral, and antifungal compounds (discussed in refs. [34–37]); however, investigations of NMT as an antiparasitic target are just emerging. Initial studies have shown that through inhibition of either substrate binding pocket, it was possible to decrease NMT activity. Blockade of the myristoyl-CoA binding site was demonstrated by using acyl-CoA-based inhibitors obtaining K_i values in the micromolar range.[38] Further potent inhibitors of this site include *S*-(2-oxo)pentadecyl-CoA and 2-substituted acyl-CoA analogues.[39,40] The myristoyl-CoA binding site, in comparative studies between human NMT1 (HsNMT1) and ScNMT, was found to be well conserved, leading to the suggestion that selective inhibitor development should focus on the peptide binding pocket.[41] The distinct yet overlapping nature of the peptide binding sites of *C. albicans* NMT (CaNMT) with HsNMT1, and ScNMT with CaNMT[13,19] has led to research focussed on creating selective NMT inhibitors that would bind to the peptide binding pockets. In addition, there is potential to target the NMTs of protozoan parasites.[23] A hypothesis behind the research in this area is that there may be exploitable selectivity for the peptide binding pocket of the parasite NMT over the two known HsNMTs (forms 1 and 2).

Knowledge derived from the development of fungal NMT inhibitors targeted to the peptide binding site can be used to accelerate the development of inhibitors of parasitic NMTs, a process termed piggybacking.[4] Most published work on NMT as an antifungal target has focussed on the pathogens *C. albicans*, *Cryptococcus neoformans*, and to a lesser extent, *Aspergillus fumigatus* using different development routes and both peptidomimetic and nonpeptidic inhibitors of the peptide binding pocket.[34] For example, the development of peptidomimetic inhibitors (reviewed in ref. [42]) has resulted in compounds with fungicidal effects against *C. albicans* and *C. neoformans* that have been shown to be selective for the pathogen NMTs.[26,43,44] Inhibitors against CaNMT were optimised by starting from the competitive octapeptide inhibitor ALYASKLS-NH_2. This molecule was based on the recognised substrate GLYASKLS-NH_2 with alanine replacing the glycine that is essential for *N*-myristoylation. Scanning alanine mutagenesis of this octapeptide demonstrated that residues 1, 5, and 6 were most important for binding affinity.[45] The amino acids were selectively replaced whilst retaining the essential components for high-affinity binding. This depeptidisation resulted in compound SC-58272 (**1**; Figure 2), with $IC_{50} = 56$ nM and $K_i = 30$ μM against CaNMT. The serine and lysine residues, two of the critical features determined by scanning alanine mutagenesis to be important for substrate binding, are still retained in this molecule.[46,47] However, because of poor in vivo performance, further depeptidisation of this compound was conducted to create a nonpeptidic inhibitor that would be less readily broken down in vivo.[48] The result of this process was compound **2**, shown in Figure 2. The strategy employed in the design of this compound was to create molecular structures absent of peptide bonds but that maintain the conformation of the hydroxy and primary amino functionalities provided respectively by the critical serine and lysine residues. The resulting nonpeptidic inhibitor maintained an affinity for CaNMT similar to that of SC-58272 and also showed fungicidal activity against both *C. albicans* and *C. neoformans*.[49,50] Impressively, this nonpeptidic inhibitor was created by the stepwise modifi-

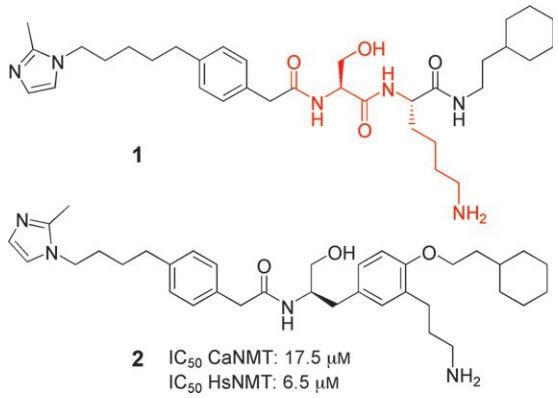

Figure 2. Development of *C. albicans* NMT inhibitor SC-58272:[45] Compound **1** was created by the selective replacement of residues from the peptidic inhibitor ALYASKLS-NH_2. The lysine and serine residues (shown in red) are critical for high-affinity binding of the parent peptide and remain a feature in the depeptidised compound. Replacement of the serine and lysine residues with peptide mimics in an attempt to maintain the orientation of the serine hydroxy group and lysine amine yielded SC-58272 (**2**). Specificity and antifungal activity were maintained, but no selectivity was achieved with this molecule.

cation of a peptidomimetic inhibitor without the benefit of crystal structure information.

Two other series of nonpeptidic inhibitors have been documented using either a benzofuran or benzothiazole scaffold. The benzofuran series was developed after the discovery of an inhibitor from screening a random chemical library at Roche.[47,48] Compound **3** (Figure 3) was then modified based on the predicted crystal contacts established from the CaNMT structure[49] and structure–activity relationship (SAR) analysis.[50]

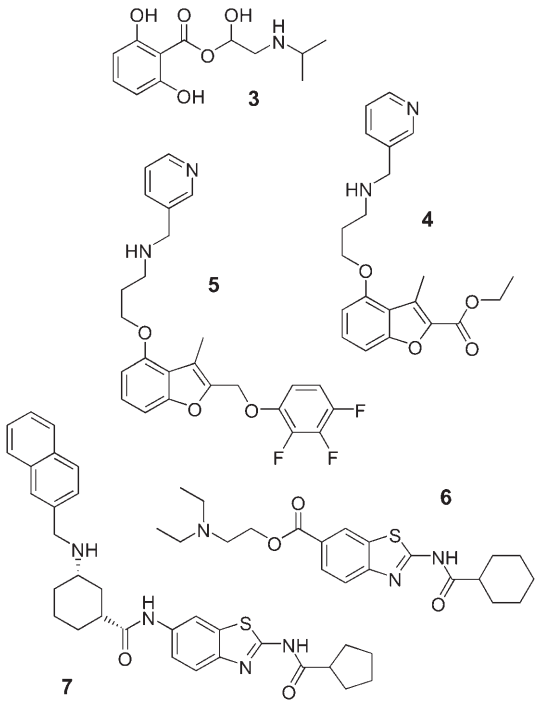

Figure 3. Comparison of benzofuran[48–52] and benzothiazole[53,54] *C. albicans* NMT inhibitors.

Compound **4** (Figure 3) was the product of the development process, but was inactive in a rat candidiasis model despite showing antifungal activity against cultured *C. albicans*. Further structure-aided redesign resulted in removal of the ester functionality to improve in vivo properties and yielded compound **5** (Figure 3), which was found to be active in the in vivo models as well as in vitro.[47,49–51] The bioavailability of these compounds was limited in oral administration, and it had been shown that compound **5** was unstable in artificial gastric fluid at pH 1.2. Redesign was conducted to remove the cause of this lability. The benzofuranylmethyl aryl ether was replaced by a range of benzofuranyl heteroaromatic ketones.[52] Furthermore, inhibition of CaNMT has been reported with the use of compounds based around a benzothiazole core structure after isolation of a weak hit through in silico screening (compound **6**; Figure 3).[53] Structure-guided design, using information from crystal structures of inhibitor complexes with CaNMT, has led to the development of compounds such as FTR1335 (**7**; Figure 3) that are able to potently inhibit CaNMT with high selectivity.[53,54] The potential of the benzothiazole scaffold in developing antiparasitic compounds targeted at NMT is discussed below.

NMT has been identified as a drug target in protozoan parasites. Comparative biochemical studies of NMT from *P. falciparum* (a principal causative agent of malaria) with human NMT highlighted the potential of the enzyme for the development of antiparasitic compounds.[22] Subsequently, genomic searching and comparisons of NMT protein sequences and other genetic and functional data have led to the proposal that NMTs from other protozoan parasites such as *Entamoeba hystolytica* and *Giardia intestinalis*, along with the TriTrp group *L. major*, *T. brucei*, and *T. cruzi* (causative vectors of cutaneous leishmaniasis, African sleeping sickness, and Chagas' disease, respectively), would also be good drug targets.[21,23] NMT has been demonstrated as a single-copy gene in protozoan parasites, and gene targeting and RNA interference studies have shown that NMT is essential for viability in both *L. major* and *T. brucei*, respectively.[21] This essentiality is likely related to a requirement for *N*-myristoylation of a number of important cellular proteins to permit correct localisation to membranes and organelles. A number of proteins have been identified to be *N*-myristoylated in protozoan parasites and include ADP ribosylation factors (ARF and ARF-like proteins),[21,55–57] hydrophilic acylated surface proteins (HASPs in *L. major*),[58–60] a calpain-type protease (CAP5.5 in *T. brucei*),[61] and calcium-dependent protein kinase 1 and the 45-kDa gliding association protein (PfCDPK1 and PfGAP45 in *P. falciparum*).[62,63] Studies of the ARF and ARL proteins of *T. brucei* have demonstrated the importance of these proteins in cellular function,[21,56,64,65] paralleling work in *S. cerevisiae* that linked growth arrest with failure to *N*-myristoylate ARF proteins.[66]

The availability of genomic information has enabled further studies into the identification and cellular importance of NMT substrates. A recent bioinformatic comparison was carried out with the TriTrp genomes (*L. major*, *T. brucei*, and *T. cruzi*)[57] using a relaxed version of the Prosite motif predictor derived from the *NMT Predictor* program[23,67] for NMT substrates, G-

{EDRKHPFYW}-x(2)-[STAGCNDEF]-{P} (see ref. [63] for a full description) in combination with the *Myristoylator* program.[68] As shown in Table 1, which includes a similar prediction for NMT substrates in the *P. falciparum* genome[69] for comparison, at least 0.54% of each parasite genome is predicted with high confidence to encode proteins that are either known or novel NMT substrates. The majority of these proteins do not have an annotated function. However, there is also evidence that some putative kinases and phosphatases may also be NMT substrates, suggesting that *N*-myristoylation can influence signal-transduction processes.[57,62] In particular, novel protein phosphatases (PPEF) were identified in *T. brucei* and *L. major*, and the leishmanial protein was subsequently shown to be myristoylated and localised to the endocytotic system. However, given that the most numerous annotations for putative NMT substrates are those for hypothetical and hypothetical conserved proteins, it is fair to say that there is a great deal more to understand about *N*-myristoylation and the processes involving *N*-myristoylated proteins.

Table 1. Predicted NMT substrates for several human parasites.

Species	Predicted number of proteins encoded in the genome	Number of high-confidence *N*-myristoylation predictions	Predicted *N*-myristoylated proteins [%]
L. major	8233[78]	62[57]	0.75
T. brucei	8164[79]	62[57]	0.76
T. cruzi	22570[80][a]	123[57]	0.54
P. falciparum	5268	43	0.68

[a] Genomic sequence contains two different haplotypes.[77]

In the case of protozoan parasites, the "piggyback" approach,[4] which involves screening of potential antifungal NMT inhibitors, has benefited greatly from the establishment of recombinant protein production,[69,70] and an assay format that allows rapid assessment of the inhibitory properties of compounds.[69–71] Heterologous expression systems using *Escherichia coli* as a host and purification procedures have been established for the production of soluble forms of NMT in milligram quantities from *L. major*[70] (LmNMT, containing an N-terminal fusion to glutathione-*S*-transferase), and *T. brucei*[70] (TbNMT) and *P. falciparum*[69] (PfNMT), both containing N-terminal histidine tags. The successful production of milligram amounts of PfNMT, compared with an earlier study in which microgram quantities of this protein were produced,[22] was facilitated by using a synthetic gene optimised for the purpose of increasing protein expression in bacteria by decreasing the high AT content (from 73 to 60%) and the elimination of rare codons.[69] All three proteins were shown to be active using synthetic peptide substrates with micromolar affinities[69,70] similar to those reported for human and *S. cerevisiae* NMTs.[26]

The requirement for a convenient and simple assay of NMT activity is clearly important for evaluation of the protozoan parasite NMTs as potential targets for drug development. There are many published reports related to NMT assays. The majority of these assays make use of the high sensitivity that can be obtained by detecting the incorporation of a radiolabelled material into the reaction product.[39,71–75] However, this incorporation always occurs in a manner that requires multiple individual reactions and/or sample extractions to perform a kinetic analysis. A medium-throughput discontinuous radiolabelling assay was recently reported.[76] This assay uses a biotinylated peptide as a substrate for HsNMT1. The product could be isolated on streptavidin-coated 96-well plates, and after extensive washing, scintillant fluid was applied to measure the amount of product formed. This is a successful assay for NMT but requires extensive handling of radiolabelled components in the absence of suitable automation. More recently, a scintillation proximity assay (SPA; Figure 4) was investigated as an alternative for compound screening.[69,70] The basis of the SPA is to monitor the transfer of one radiolabelled substrate to a second substrate with an affinity tag attached to it. Subsequent capture of the affinity tag (in this case biotin) by a streptavidin-coated bead that incorporates a scintillant ensures that the radiolabel on the reaction product is sufficiently close to

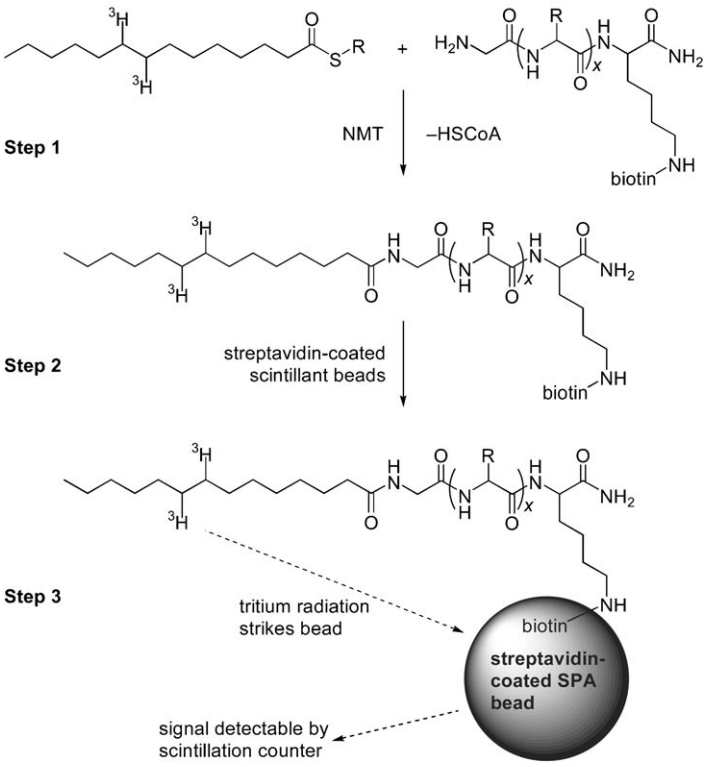

Figure 4. Principle of SPA applied to the reaction of NMT:[69] Step 1) The *N*-myristoylation reaction proceeds as described previously (Figure 1). The tritiated myristoyl-CoA substrate is added to the N-terminal glycine of a synthetic peptide substrate under the action of NMT with HSCoA formed as a by-product. A biotin functional group is present on the peptide substrate; in this case the C-terminal lysine residue is biotinylated. Step 2) The peptide is attached, through the biotin functional group, to a streptavidin-coated bead incorporating a scintillant. Step 3) Radiation from ^3H is sufficiently close to the scintillant to, upon absorption, cause excitation within the bead. This results in emission of a detectable flash that is quantified on a scintillation counter.

the scintillant to allow detection and quantitation of product (reaction progress) on a scintillation counter. The strongly ionising nature of the radiation used prevents nonspecific emissions (from unreacted starting materials) reaching the scintillant bead; the primary requirement from the enzymatic reaction is that the radiolabel is brought sufficiently close to the affinity capture group (biotin) to allow subsequent close proximity to the scintillant. The use of a scintillant immobilised within a solid support, and the affinity localisation of the product obviates the need to separate products from reactants—a prerequisite when scintillant fluids are used. Quantitation can then be conducted in a scintillation counter. The whole reaction can be conducted in 96- or 384-well format as required.

Inhibition studies of recombinant LmNMT, TbNMT, and PfNMT have been reported based on screening a number of potential NMT inhibitors supplied by Pfizer from an earlier antifungal compound development program.[69,70] Initial studies were conducted with a small set of five or six compounds. Although no significant inhibition of LmNMT was observed,[70] as shown in Table 2, compounds **8** and **9** (Figure 5) both demonstrated low-micromolar inhibition in the SPA for both PfNMT and TbNMT, and a respective two- and fivefold decrease in IC_{50} values relative to those obtained for HsNMT1.[69,70] Another compound, **10** (Figure 5) also decreased the activity of TbNMT to 2% as determined in a modified discontinuous assay for NMT activity.[70] Relative inhibition of PfNMT (compared with HsNMT1) was also achieved with UK-370485, although this compound showed no detectable inhibition against TbNMT (Table 2). The study was further extended to screening a larger set of 43 benzothiazole-containing compounds against PfNMT and HsNMT1.[70] Seven compounds demonstrated IC_{50} values of 115 μM or less. Three of these compounds (**11**–**13**; Figure 5) all demonstrated IC_{50} values <50 μM for PfNMT with some selectivity against HsNMT1 (Table 2). Distinct regiochemistries are present in the cyclohexyl moiety linking the benzothiazole and aromatic groups in these compounds, which are 1,4-substituted in UK-370309, and (*R*,*S*)-1,3-substituted in compounds **12** and **13**, relative to the parent compound **14**, which is *trans*-1,4 (Figure 5). Compound **14** failed to show detectable inhibition in the SPA for the TbNMT enzyme, and the IC_{50} value for PfNMT was considerably increased (232 mM), although it had good selectivity against HsNMT1 (Table 2). Interestingly, the dimethylamide moiety at the C6 position of the benzothiazole group is present in the majority of compounds which showed some ability to inhibit PfNMT, including compounds **12** and **13** (Figure 5). This moiety is known to form favourable interactions with the peptide binding site of the fungal CaNMT (Pfizer),[69] and it is therefore possible that the same interactions may also be important for inhibition of PfNMT activity. Notably, the presence of a bulky pyridin-2-yl functionality at the C6 position in compound **15** appears to result in decreased inhibition of PfNMT and enhanced inhibition of HsNMT1 (Table 2 and Figure 5): a reversal of selectivity.[69] Establishment of these initial SARs clearly suggests that there is potential to develop high-affinity selective inhibitors targeted at protozoan NMTs.

Table 2. In vitro inhibition of purified recombinant NMTs.

Inhibitor	IC_{50} [μM][a]		
	TbNMT[70]	PfNMT[69]	HsNMT[69]
CP-014553 (**8**)	0.77 ± 0.07	0.28 ± 0.12	1.6 ± 0.5
CP-005240 (**9**)	0.74 ± 0.08	0.36 ± 0.04	1.8 ± 0.3
UK-370509 (**11**)	NM[b]	30 ± 3	286 ± 93
UK-370309 (**12**)	NM	17 ± 1	331 ± 29
UK-370713 (**13**)	NM	37 ± 4	329 ± 84
UK-370485 (**14**)	ND[c]	232 ± 46	>1000
UK-370710 (**15**)	NM	115 ± 9	28 ± 9
UK-362799 (**16**)	NM	68 ± 4	309 ± 58

[a] Determined at an inhibitor concentration of 0.05 mM. [b] NM = not measured. [c] ND = not detected.

A number of compounds that demonstrated inhibitory activity against TbNMT and PfNMT were also tested against cultured *T. brucei* and *P. falciparum* parasites. In the case of *T. brucei*, the potent in vitro inhibitors **8** and **10** also demonstrated low ED_{50} values (effective dose that allows 50% parasite growth) of 16 and 66 μM, respectively. CP-030890-27 also showed low levels of toxicity ($ED_{50}>1500$ μM) towards murine macrophages.[70] Cultured asexual blood stages of *P. falciparum*, which are of interest as chemotherapeutic targets because clinical symptoms of malaria are due to these stages of the parasite's life cycle, also proved to be sensitive to **11**–**13** and **16** (Figure 5). At a concentration of 100 μM, a considerable decrease in the number of red blood cells infected by parasites was observed for the four compounds tested, with **13** and **16** demonstrating significant effects at 10 μM.[69] Although there was not a strict correlation between SPA inhibition and parasite death, data from these pilot studies suggest that a number of these compounds show potential as leads for future development as antiparasitic drugs for use in humans.

Another recently published approach to discovering novel peptide-based inhibitors of PfNMT has involved the use of a lipid/combinatorial peptide chimera library in combination with the SPA assay.[76] In this approach a low-affinity small-molecule binding partner of PfNMT, myristate ($IC_{50}\sim30$ μM), was first coupled by an amino-oxy acetic acid group (Aaa) to 130321 (19^4) individual sequences from a peptide library that was generated by a split and mix protocol. The myristate group, in the presence of PfNMT, allows a weak association with the enzyme. Peptides coupled to myristate could extend into, and form favourable interactions with, the peptide binding pocket of PfNMT. Screening this library was performed by first coupling the myristate–peptide aptamers to 90-μm beads, which were then incubated with PfNMT. Beads with the enzyme attached were visualised by immunodetection with anti-His antibodies conjugated to alkaline phosphatase, directed to the His tag attached to recombinant PfNMT. The beads, identified by intense purple staining, were then separated and sequenced. The peptides identified were subsequently resynthesised and tested as inhibitors of PfNMT. A preliminary analysis of five beads was conducted as proof of principle. The sequences were resynthesised and tested in the SPA for inhibitory activity against PfNMT. One compound, Aaa-Val-Leu-Met-Gln-Gly, displayed an IC_{50} value of approximately 65 μM.[76] This inhibitory activity is favourable in comparison with results ob-

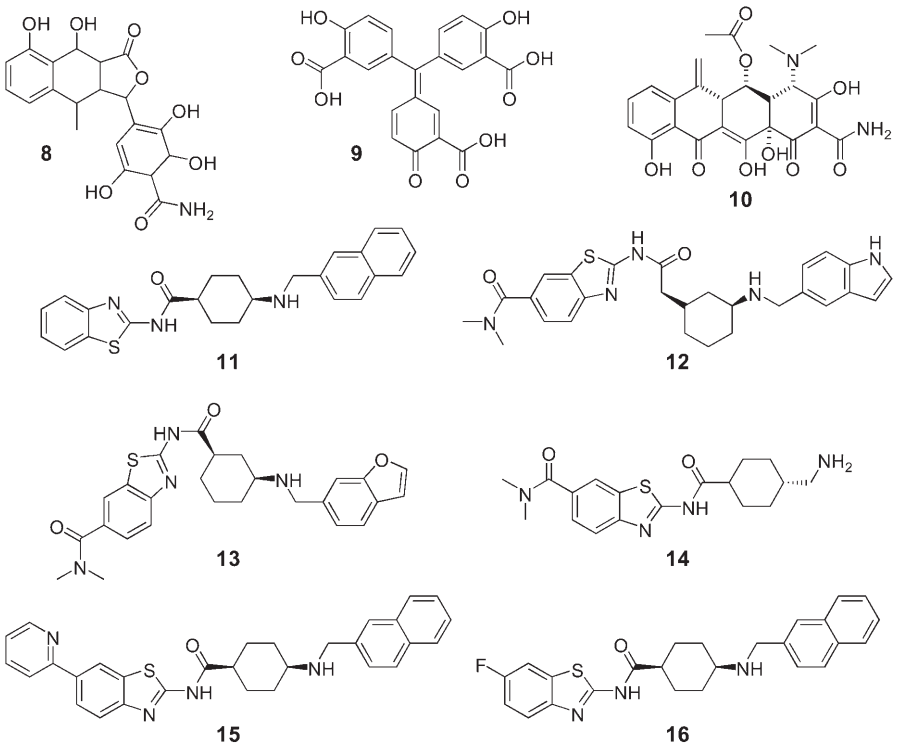

Figure 5. Compounds assessed as parasite NMT inhibitors.[69,70]

tained by the piggyback approach[69,70] and to the values obtained by high-throughput screens against HsNMT1.[77]

The prospects of developing an antiparasitic drug targeted at NMT are not only enhanced by emerging data on the enzymes from these parasites, but are being considerably enhanced by "piggybacking" on existing compounds[69,70] as well as numerous previous studies which have examined the possibility of NMT as an anticancer, antiviral, and antifungal target.[34–37] We are clearly at the first stages in the chemical validation of parasite NMTs as drug targets. However, the availability of active recombinant parasite NMTs and the development of a suitable in vitro assay has allowed the first screening of compounds which were originally developed as inhibitors of fungal NMTs.[69,70] The observations that some of these inhibitors show antiparasitic activity against cultured *P. falciparum* as well as in vitro inhibition using the SPA[69] strongly suggest that PfNMT and possibly NMTs from other parasites represent suitable drug targets worthy of further investigation. The benzothiazole scaffold, which is present in a number of the inhibitory compounds that have been tested so far, is amenable to chemical synthesis with significant diversity. This should provide the opportunity to develop a far wider range of structurally related molecules that could yield inhibitory compounds with higher affinity than lead compounds discovered so far. However, possibly of greater importance is the identification of new chemical scaffolds to be developed in tandem. A library approach to identify peptides capable of binding to NMTs[76] has the potential to discover novel peptide-based scaffolds. Another option is to use the high-throughput nature of the SPA[69] to screen large diverse chemical libraries rather than the small numbers of piggyback fungal NMT inhibitors evaluated so far. Furthermore, improvements in the expression and purification of parasite NMTs offer the possibility of obtaining three-dimensional structures of these enzymes which can also aid in rationalizing structure–activity relationships. If X-ray structures are not successfully obtained, homology modelling of parasite NMT structures represents an alternative, considering that structures of fungal NMTs in a variety of substrate–inhibitor complexes have already been reported.[28,33,49]

Parasitic diseases are a major global health problem, and many of the drugs in current use were introduced decades ago and often display limited efficacy as well as toxic side effects.[1] Coupled with rising levels of resistance and a need for easily administered drugs with rapid effects, the need to identify and explore the potential of new targets is great. The studies described herein on parasite NMTs demonstrate that even at the early stages of this work, compounds exist with inhibition characteristics and selectivity against human NMTs that are capable of leading to parasite death in culture.[69,70] Furthermore, the availability of genomic sequence information[78–80] suggests that the work described herein could be extended to other human parasites. Perhaps even more interesting is the fact that cell death arising from NMT inhibition is most likely due to "downstream effects" related to the loss of myristoylation of a number of different proteins, some of which may prove to be drug targets themselves. Clearly there are many hurdles to overcome before an NMT inhibitor could be available for use in humans; nevertheless NMT clearly has potential as a prospective parasite drug target.

Keywords: antiparasitic agents · drug development · lipids · peptidomimetics · structure–activity relationships

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