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REPLACEMENTS FOR LYSINE IN L-SERYL-L-LYSYL DIPEPTIDE AMIDE INHIBITORS OF *CANDIDA ALBICANS* MYRISTOYL-COA:PROTEIN *N*-MYRISTOYLTRANSFERASE

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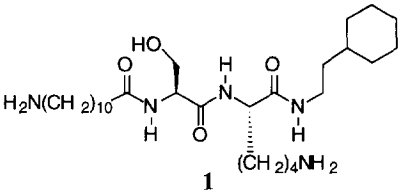
Abstract: A survey of potential cyclic and acyclic lysine replacements in known L-seryl-L-lysyl dipeptide inhibitors of *C. albicans* NMT identified the thioether **16** and glycinamide **18** as submicromolar inhibitors of *C. albicans* NMT, which retained good selectivity over the human enzyme. All of the heterocyclic lysine mimetics that were examined exhibited dramatically weaker affinity with the fungal enzyme.

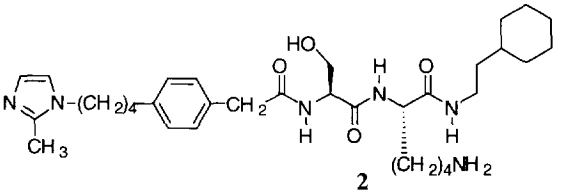
The monomeric enzyme myristoyl-CoA:protein *N*-myristoyltransferase (NMT, EC 2.1.3.97), catalyzes a cotranslational transfer of myristate (C14:0) from myristoyl-CoA to the *N*-terminal glycine residue of certain eukaryotic cellular proteins.[1,2] The resulting *N*-myristoylated proteins regulate numerous important cellular functions including protein-protein and protein-lipid interactions.[3] Recently, the introduction of temperature-sensitive NMT alleles into *Cryptococcus neoformans* and *Candida albicans* demonstrated that these pathogenic fungi require NMT for their viability.[4] This led to the hypothesis that an NMT inhibitor might arrest fungal growth and thus provide an exciting new approach for the treatment of systemic fungal infections.[5]

The acyl-CoA binding sites of NMTs are known to be highly conserved[6] while the peptide substrate specificities are widely varied.[2,7,8] This difference in peptide binding affinity has been exploited to provide the L-seryl-L-lysyl dipeptides **1**,**2** as the first reported[9] submicromolar inhibitors that were competitive with the peptide binding site in *C. albicans* NMT. Compound **2** represents the first known NMT inhibitor lacking an *N*-terminal primary amino group to have specificity and potency for this peptide binding site. The potency of **1** is believed to arise partly from the basic *N*-terminal amino group (pK_a = 10.1 ± 0.5). In contrast, the pK_a of the 2-methyl-imidazole moiety (pK_a = 7.1 ± 0.5) in **2** is significantly lower and more closely matches the basicity of the *N*-terminal glycine amino ($pK_{a(est.)}$ ~ 8.0) group in a typical substrate. The overall rigidity of the *p*-phenacetyl moiety in **2** compared to the flexible 11-aminoundecanoyl moiety in **1** also enhances selectivity for *C. albicans* NMT. Here we extend these studies and describe our efforts to replace the L-lysine (pK_a = 9.8 ± 0.5) sidechain in **1** and **2** with less basic functionalities.



1

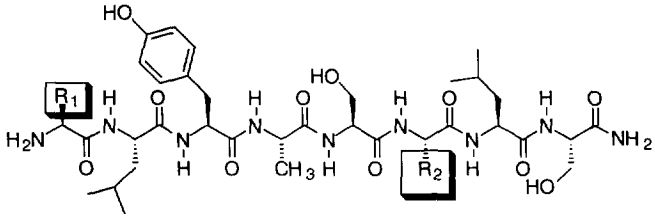
$K_{i(app)}$ = 70 ± 10 nM



2

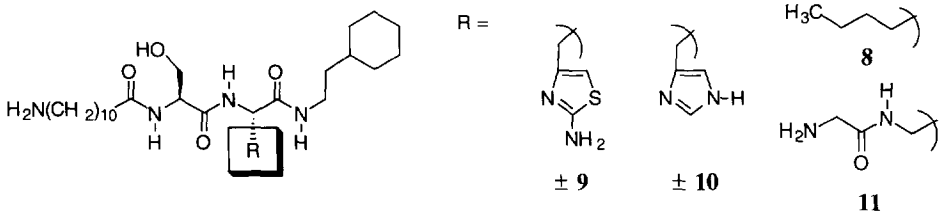
$K_{i(app)}$ = 31 ± 3 nM

Compounds **1** and **2** originated from the high affinity octapeptide substrate GLYASKLS-NH_2 **3** (K_m = 0.6 μM for *C. albicans* NMT), which is derived from the *N*-terminal fragment of Arf2p (ADP ribosylation factor 2), a protein that must be myristoylated by NMT for expression of its essential biological function.[10] Simply replacing the *N*-terminal glycine in **3** with an alanine produces the inhibitor ALYASKLS-NH_2 **4** ($K_{i(app)}$ = 15 μM for *C. albicans* NMT). Systematic replacement of the other amino acid residues in **4** with alanine led to the identification of the *N*-terminal amino group of glycine-1, the hydroxyl group of serine-5, and the ε-amino group of lysine-6 as the three key enzyme recognition elements needed for tight binding in this system. Subsequent incorporation of an L-arginine **5**, L-norleucine **6**, or L-ornithine **7** for L-lysine at position 6 in ALYASKLS gave significantly weaker inhibitors ($IC_{50} \geq 100$ μM for *C. albicans* NMT) and provided evidence that this important interaction at lysine-6 was strongly dependent on the overall basicity and distance out to the ε-amino group.

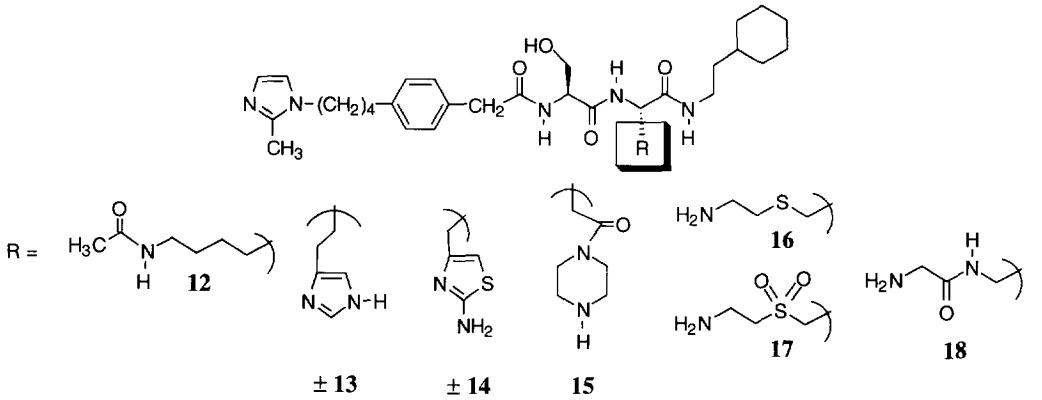


3 GLYASKLS $R_1 = H$, $R_2 = CH_2CH_2CH_2CH_2NH_2$
4 ALYASKLS $R_1 = CH_3$, $R_2 = CH_2CH_2CH_2CH_2NH_2$
5 ALYASRLS $R_1 = CH_3$, $R_2 = CH_2CH_2CH_2NHC{=}NH(NH_2)$
6 ALYAS(nor-Leu)LS $R_1 = CH_3$, $R_2 = CH_2CH_2CH_2CH_3$
7 ALYAS(Orn)LS $R_1 = CH_3$, $R_2 = CH_2CH_2CH_2NH_2$

The lack of significant potency in **7** versus **4** suggests that an extended conformation of the lysine-6 sidechain may be involved. A staggered, extended lysine conformation requires about 6.3-6.4 Å from the alpha-carbon out to the ε-amine nitrogen atom. A variety of acyclic and cyclic amino acids were then evaluated by molecular modeling studies as potential lysine surrogates, which could best maintain this distance. These studies indicated that a flexible acyclic replacement would likely have a better fit with the lysine ε-amino group than would a cyclic mimic. Subsequent replacement of the lysine group in **1** with L-norleucine again produced a significantly weaker inhibitor **8** ($IC_{50} \geq 100$ μM for *C. albicans* NMT) and confirmed the importance of lysine recognition in this inhibitor. Introduction of the known racemic 2-aminothiazol-4-yl-alanyl[11] sidechain in **9** or L-histidine **10** also significantly reduced potency. However, nearly all of the enzyme inhibition in **1** was retained (Table 1) with virtually no change in selectivity by incorporation of the more flexible L-glycinamide ($pK_{a(est.)}$ ~ 8.0) in compound **11**, which can be readily prepared via the known[12] L-α,β-diaminopropionic acid.



In the 2-methylimidazole series, acetylation of **2** afforded the acetamide **12** which is a poor *C. albicans* NMT inhibitor and confirmed the importance of lysine recognition in this series. Replacement of the lysine in **2** with selected heterocycles: racemic homohistidine[13] **13**, racemic 2-aminothiazol-4-yl-alanyl[11] **14**, or the L-aspartyl-piperidide **15** again significantly reduced enzyme inhibition potency (IC_{50} > 60.0 μM, Table 1). The acyclic thialysine analog **16**, that can be easily prepared from commercially available thialysine, retained most of the enzyme inhibitory (IC_{50} = 0.34 μM) properties of **2**, including selectivity (84-fold) for *C. albicans* NMT versus human enzyme. Interestingly, oxidation to the sulfone **17** afforded a 100-fold loss in enzyme inhibition. As observed previously with **10**, the acyclic glycinamide **18** (IC_{50} = 0.26 μM) exhibited submicromolar inhibition of *C. albicans* NMT and retained much of the selectivity (65-fold) versus the human enzyme. Thus, for the first time, compound **18** demonstrates that potent and selective peptidomimetic inhibitors of NMT can be obtained which contain less basic amine replacements at two of the key recognition sites in **3** and **4** (i.e., lysine-6 as well as the *N*-terminal amino group).



Standard peptide synthetic methodology provided a facile method for the introduction of the various lysine replacements in these inhibitors. The synthesis commenced by formation of the 2-cyclohexylethylamides with a suitably α-*N*-protected L-lysine replacement. Removal of the α-amino protecting group followed by acylation with the activated ester of *N*-BOC-O-benzyl-L-serine provided a protected dipeptide. Anhydrous hydrochloric acid then removed the *N*-BOC of serine, coupling with the desired *N*-terminal sidechain (11-aminoundecanoic acid or *p*-(2-methyl-1H-imidazol-1-ylbutyl)phenylacetic acid[9]), hydrogenolysis of the remaining protecting groups and appropriate purification by reverse-phase HPLC provided the desired inhibitors. All of the resulting products exhibited NMR and high resolution mass spectral data consistent with the assigned structures.

In conclusion, two acyclic lysine replacements, the thioether **16** and glycinamide **18**, were identified that were submicromolar inhibitors of *C. albicans* NMT and exhibited good selectivity versus human enzyme. *C. albicans* NMT can thus recognize amine functionalities with dramatically lower pK_as at the lysine-6 position. These functionalities could be useful as peptidomimetic replacements for lysine in other enzyme inhibitors.

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Table 1. Potencies and Selectivities of Peptidomimetic NMT Inhibitors.[a]

Compound	*C. albicans* NMT IC_{50}, μM	Human NMT IC_{50}, μM	Selectivity[b]
1	0.11 ± 0.03	0.50 ± 0.37	4.5
2	0.056 ± 0.01	14.1 ± 1.3	250
8	74.0	ND	ND
9	10.0	52.0	5.0
10	~100	ND	ND
11	1.0 ± 0.2	1.0 ± 0.2	1.0
12	80	ND	ND
13	9 ± 0.3	≥200	≥25
14	64	>10	ND
15	65	ND	ND
16	0.34 ± 0.05	29 ± 5	84
17	20	ND	ND
18	0.26 ± 0.01	17.2 ± 0.8	66

[a]Potency against the indicated NMT as assessed by IC_{50} using a radiochemical HPLC end-point assay with the peptide GNAASARR-NH_2 at its apparent K_m and [^{3}H]myristoyl-CoA at 1 μM.[9] [b]Selectivity is the ratio of the IC_{50} against human NMT to the IC_{50} against *C. albicans* NMT. ND = not determined.

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