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Lipid and sulfur substituted prenylcysteine analogs as human Icmt inhibitors

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ABSTRACT

Inhibition of isoprenylcysteine carboxyl methyltransferase (Icmt) offers a promising strategy for K-Ras driven cancers. We describe the synthesis and inhibitory activity of substrate-based analogs derived from several novel scaffolds. Modifications of both the prenyl group and thioether of *N*-acetyl-*S*-farnesyl-L-cysteine (AFC), a substrate for human Icmt (hIcmt), have resulted in low micromolar inhibitors of Icmt and have given insights into the nature of the prenyl binding site of hIcmt.

Proteins possessing a -CaaX motif at their C-terminus undergo three sequential post-translational modifications that are necessary for their proper cellular localization and function.[1] It is thought that more than 120 human proteins utilize this three-step modification process, including the small GTPases.[2] The first step in the sequential processing pathway involves attachment of a C_{15}-farnesyl or C_{20}-geranylgeranyl isoprenoid to the free thiol of the cysteine in the -CaaX motif. This prenylation event is catalyzed by either farnesyl transferase (FTase) or geranylgeranyl transferase-I (GGTase-I), respectively.[3] The next step involves the removal of the -aaX residue by the endoprotease Ras-converting enzyme-1 (Rce1).[4,5] The newly exposed carboxylate of the prenylcysteine is then methylated by human isoprenylcysteine carboxyl methyltransferase (hIcmt; Fig. 1).[6] The summation of these steps generates increased hydrophobicity that is necessary for association of the protein to the target membrane and its function.[7,8]

This processing pathway has been investigated for potential cancer therapeutics because approximately 20% of human cancers, including 90% of pancreatic cancers, result from mutated Ras proteins.[9] Potent FTase inhibitors have been discovered, but unfortunately are ineffective against K-Ras driven tumors[10] due to alternative prenylation by GGTase-I, which allows the Ras protein to properly localize.[11] However, hIcmt is the only known enzyme capable of methylating the prenylcysteine substrate[12] and is thus an intriguing putative therapeutic target.[13]

Preliminary work on hIcmt inhibitors focused on modifications to *N*-acetyl-*S*-farnesyl-L-cysteine (AFC),[14] a minimal substrate for human Icmt (hIcmt) that contains a peptide bond. Amide-modified farnesyl cysteine analogs (AMFCs)[15,16] were synthesized and studied in our laboratories to investigate tolerances and specific requirements upstream of the prenylcysteine. The culmination of this work resulted in low micromolar inhibitors of hIcmt (**A** and **B**; Fig. 2) that are based on a prenylcysteine scaffold. In contrast, our initial studies to investigate substitutions in the isoprene region led to poor hIcmt inhibitors.[17]

Other synthetic and screening approaches have also resulted in inhibitors of hIcmt (Fig. 2). A small-molecule library screening effort led to cysmethynil, an indole based inhibitor.[18] A small molecule prenylcysteine analog, *S*-farnesyl-thiosalicylic acid (FTS), is both an hIcmt inhibitor as well as an inhibitor of H-Ras driven cell growth.[19]

Despite some initial success, there is still much room for improvement of the characteristics of substrate-based (AFC) inhibitors of hIcmt. Current successful inhibitors of this nature have thus far included the less than ideal characteristics existing in AFC: the isoprenoid tail, the allylic thioether, and the highly lipidic disposition. Herein, we report the investigation into lipid and thioether replacements leading to structurally novel AFC-based inhibitors of hIcmt. This study has been approached in two parts: (1) C_{15}-lipid replacement with diverse aryl motifs and (2) thioether replacement with 1,2,3-triazoles.

Previously, our group demonstrated that a biphenyl group replacing the second and third isoprene positions was accepted as a substrate for FTase.[20] It was also shown that the third isoprene

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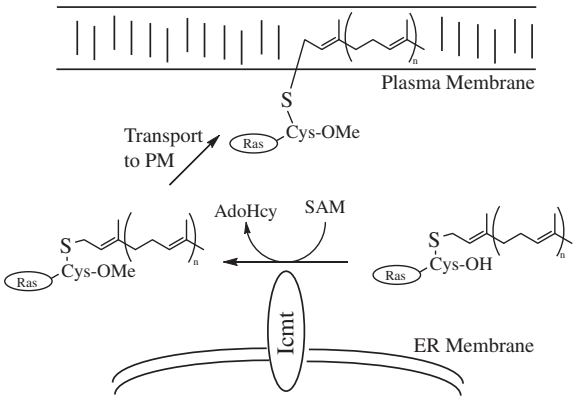


Figure 1. Methylation of Ras by hIcmt is required for proper localization to the plasma membrane and downstream signaling.

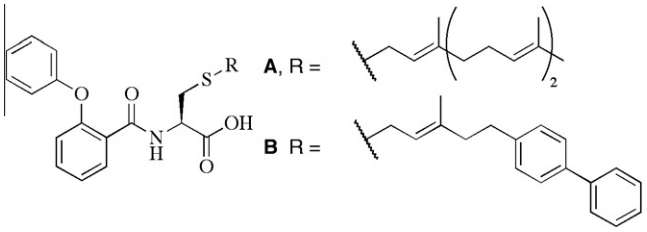


Figure 2. Current inhibitors of hIcmt developed from amide- and prenyl-modified AFC.

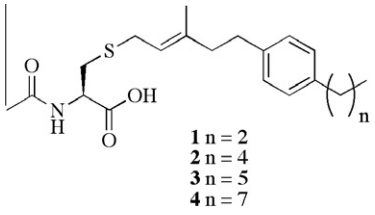


Figure 3. Depiction of aryl–alkyl analogs.

position is flexible in accepting aryl substitutions, as modified anilinogeranyl analogs are high-affinity substrates for FTase.[21] Early evidence from the design of squalene synthase inhibitors also demonstrated the ability for isoprenoid binding pockets to tolerate aryl ligands.[22] More recently, Boger and co-workers inserted aryl motifs in lieu of unsaturated fatty acid chains in the development of fatty acid amide hydroxylase inhibitors.[23]

Using these studies as a guide, our first series of compounds included an aryl-alkyl tail that was designed to investigate tolerances of a prenyl binding pocket for hIcmt (Fig. 3, see Supplementary data for synthesis). Two key reactions were utilized in the synthesis of this class of molecules. A Kumada coupling[24] installed the required alkyl chain to the aryl core representing a modified tail region. This reaction was followed by a zirconium-assisted carboalumination of the terminal alkyne[25,26] to establish the required first isoprene group in a stereocontrolled manner.

This series of compounds (Fig. 3) explored the general ability of an aromatic residue to mimic the central isoprene unit of the farnesyl chain. Evaluation of **1–4** revealed an interesting trend: inhibitory activity increased with elongation of terminal alkyl-chain, with the exception of **3** (Table 1). The replacement of the central isoprene in conjunction with n-alkyl group tails led to modest inhibition of hIcmt despite a deviation from an isoprene-based structure. The overall length of **1** is closest to that of AFC, and **4** more

Table 1

Compd	Percent Inhibition of specific activity[a,b]	IC_{50}[a,c], μM
AFC	100 ± 1.2	
1	20.7 ± 3.3	
2	23.1 ± 1.5	
3	18.8 ± 1.7	
4	31.1 ± 1.4	34.6 ± 2.4
5	9.8 ± 1.7	
6	15.5 ± 3.3	
7	14.5 ± 1.8	
8	19.4 ± 4.8	
9	22.4 ± 2.1	
10	15.7 ± 1.4	
11	26.6 ± 8.1	
12	25.3 ± 1.5	
16a	19.5 ± 3.1	
16b	35.9 ± 2.4	19.4 ± 1.0
16c	17.0 ± 2.5	
16d	10.1 ± 3.6	
16e	22.7 ± 2.9	

[a] Values represent mean of three experiments.
[b] Values determined at 10 μM analog and 25 μM AFC by VDA assay previously described[38].
[c] Calculated by GraphPad Prizm 5.0.

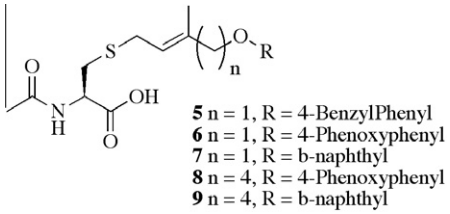


Figure 4. Depiction of ether and extended ether compounds.

closely mimics the C_{20} geranylgeranyl-cysteine, the product from prenylation by GGTase-I, which is an equivalent substrate for carboxyl methylation.[27] Analog **4** is the most potent of this series (IC_{50} = 34 μM).

To further remove isoprene character from the prenyl region of AFC, a second class of AFC analogs was prepared with an aryl ether group. Both the central and terminal isoprene units were replaced with aromatic moieties (**5–7,** Fig. 4; see Supplementary data for synthesis). Selenium dioxide catalyzed allylic oxidation[28] of prenyl alcohol followed by bromination resulted in the first isoprene synthon necessary for ether synthesis.

None of these compounds were substrates for hIcmt, nor were they efficient inhibitors (Table 1). Despite differences in flexibility and length, these three compounds possessed similar inhibitory potency. The trend of **1–4** prompted us to introduce flexibility and increased lipid resemblance by introducing a methylene bridge between the first isoprene and ether (**8–9**). This modification resulted in an approximate 2-fold increase in enzyme inhibition, (Table 1) but did not surpass that of **4**. These compounds were also poor Icmt substrates. From this limited set of compounds we learned that specific spatial organization and positioning of aryl substituted isoprene groups may be crucial for their ability to bind to the hIcmt active site.

The generation of the vinyl iodide first-isoprene synthon allowed us to generate a third set of analogs (**C**, Fig. 5a). Substituting the flexible farnesyl group with highly rigid and bulky replacements was thus investigated (**10–12**, Fig. 5b, see Supplementary Content data for synthesis). A Suzuki coupling was utilized to install the phenyl ring in the first isoprene.[29] To add the tail of the isoprene mimetic, a copper-mediated allylic –OTHP displace-

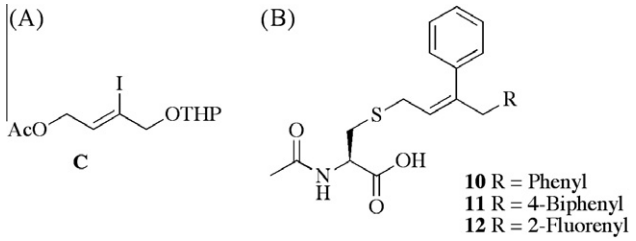


Figure 5. (A) New vinyl first isoprene synthon;(B) Compounds containing highly rigid and bulky prenyl groups.

ment[30] with Grignard reagents gave the desired redesigned isoprene.

The more effective analogs contained aryl groups that could possibly extend deeper into a prenyl binding site (**11–12**). Despite the lack of the flexibility found in the other compounds, these molecules exhibited similar inhibition of hIcmt (Table 1). These results highlight the need to further investigate structural motifs that can mimic the necessary isoprenoid characteristics for Icmt binding. Our previous work demonstrated inhibitory potency of hIcmt by lipid-modified prenylcysteines containing a 4-biphenyl group in the isoprene moiety.[16,17] Our future work will aim to elucidate other specific motifs for hIcmt inhibition.

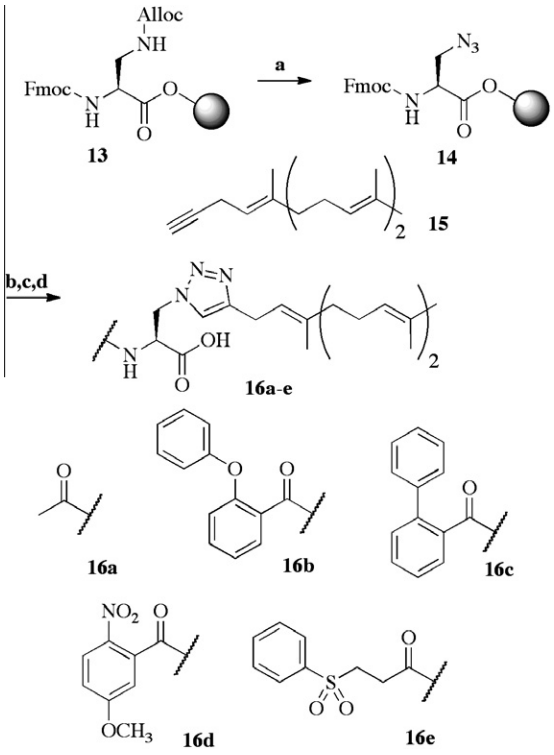
In previous reports by the Rando laboratory, the heteroatom requirements for prenylcysteine recognition by AFC were investigated. They found that a nucleophilic sulfur was crucial for efficient carboxylate methylation. Sulfoxide-, seleno-, amine-, and ether-, prenylcysteines maintained the ability to bind to the enzyme; however, deviation from sulfur decreased enzymatic activity.[31] Our groups furthered this effort through the synthesis of an 'all-carbon' prenylcysteine and found that complete removal of the thioether ablated hIcmt substrate and inhibitor activity.[32]

Despite the apparent need for a linking heteroatom, preferably sulfur, between the peptide and farnesyl group of the substrate, it would be beneficial to find a suitable replacement that would not be turned over as substrate but would still bind to and thus inhibit hIcmt. The presence of the allylic thioether is also undesirable as they are chemically and enzymatically labile.[33,34]

We introduced a heteroaryl motif that could act as a surrogate for the thioether. Utilizing solid-phase chemistry previously developed in our laboratory[16] and copper-assisted cycloaddition,[35] 1,4-substituted 1,2,3-triazole prenyl-'cysteines' were generated. Maintaining a heteroatom proximal to the natural position of sulfur in AFC was paramount in our design, based on the work of Rando and co-workers.[31]

A solid-phase synthetic route was used for the synthesis of **16a–e**, which began by loading orthogonally protected diamine onto 2-chlorotrityl resin generating starting material **13** (Scheme 1, detailed procedure in Supplementary data). Cleavage of the alloc group followed by diazo transfer with trifyl azide generated in situ[36] afforded azidocysteine analog **14**. Cycloaddition[37] with **15** followed by standard Fmoc peptide coupling afforded dually modified derivatives **16a–e**. Following cleavage from the resin, HPLC (C_8 column) was used to purify these dually modified compounds. This solid phase approach allowed us to investigate the effects of prenylcysteines substituted at two important locations: the thioether and the amide. We have previously reported on the SAR of amide modified prenylcysteine (AMFC) compounds[15,16] and in this study we selected amide modifications that previously resulted in substrates and inhibitors of hIcmt to highlight the effect of the triazole-for-sulfur substitution.

When evaluated as substrates of hIcmt, none of the triazole-for-sulfur compounds exhibited any activity (data not shown). These



Scheme 1. Solid phase synthesis of triazole-for-sulfur substituted analogs of AMFCs on 2-chlorotrityl resin. Reagents and conditions: (a) $PhSiH_3$, $Pd(PPh_3)_4$, DCM; (b) (i) NaN_3, Tf_2O, Ph-H; (ii) $ZnCl_2$, Et_3N, MeOH; (c) **15**, CuI, Ascorbic Acid, DIEA, DMF; (d) (i) HOBT, HBTU, DIEA, DCM/DMF; (ii) 0.5% TFA/DCM.

results were somewhat surprising as the parental AMFC analogs corresponding to **16d,e** were found to be excellent substrates, surpassing that of AFC (unpublished data).[15,16] Evaluation of these five compounds revealed that all are inhibitors of the enzyme. The most potent analog is **16b** (IC_{50} = 19.4 μM, Table 1); note that the parent AMFC has an IC_{50} = 4.6 μM.[16] These data serve as an important comparison, as the transition from thioether to triazole leads to a decrease in potency, but reduces undesirable instability and decreases lipophilicity. Despite the replacement of the thioether with a heterocycle, important structural features of known hIcmt inhibitors can still affect biological function. This result represents an important step forward in the rational design of non-cysteine based inhibitors of hIcmt.

We have synthesized 12 prenylcysteine analogs bearing aromatic isoprenoid replacements and five analogs inserting a triazole replacement for sulfur. The latter series resulted in low micromolar inhibitors of hIcmt with unique and potentially beneficial physical characteristics, including increased rigidity and stability, and decreased hydrophobicity. Spatial organization and positioning of the lipid replacements must be balanced with lipidic character for the design of hIcmt inhibitors. In summary, we have generated interesting results from the introduction of the triazole-for-sulfur substitution leading to a new class of AFC-based hIcmt inhibitors.

Acknowledgements

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.06.053.

References and notes

1. Gelb, M. H.; Brunsveld, L.; Hrycyna, C. A.; Michaelis, S.; Tamanoi, F.; Van Vooris, W. C.; Waldmann, H. *Nature Chem. Biol.* **2006**, *2*, 518.
2. Reid, T. S.; Terry, K. L.; Casey, P. J.; Beese, L. S. *J. Mol. Biol.* **2004**, *343*, 417.
3. Lane, K. T.; Beese, L. S. *J. Lipid Res.* **2006**, *47*, 681.
4. Bergo, M. O.; Ambroziak, P.; Gregory, C.; George, A.; Otto, J. C.; Kim, E.; Nagase, H.; Casey, P. J.; Balmain, A.; Young, S. G. *Mol. Cell. Biol.* **2002**, *22*, 171.
5. Dolence, J. M.; Steward, L. E.; Dolence, E. K.; Wong, D. H.; Poulter, C. D. *Biochemistry* **2000**, *39*, 4096.
6. Anderson, J. L.; Frase, H.; Michaelis, S.; Hrycyna, C. A. *J. Biol. Chem.* **2005**, *280*, 7336.
7. Michaelson, D.; Ali, W.; Chiu, V. K.; Bergo, M.; Silletti, J.; Wright, L.; Young, S. G.; Philips, M. *Mol. Cell. Biol.* **2005**, *16*, 1606.
8. Michaelson, D.; Silletti, J.; Murphy, G.; D'Eustachio, P.; Rush, M.; Philips, M. *J. Cell Biol.* **2001**, *152*, 111.
9. Downward, J. *Nature Rev. Cancer* **2002**, *3*, 11.
10. Kohl, N. E.; Omer, C. A.; Conner, M. W.; Anthony, N. J.; Davide, J. P.; DeSolms, S. J.; Giuliani, E. A.; Gomez, R. P.; Graham, S. L.; Hamilton, K.; Handt, L. K.; Hartman, G. D.; Koblan, K. S.; Kral, A. M.; Miller, P. J.; Mosser, S. D.; O'Neill, T. J.; Rands, E.; Schaber, M. D.; Gibbs, J. B.; Oliff, A. *Nature Med.* **1995**, *1*, 792.
11. Whyte, D. B.; Kirschmeier, P.; Hockenberry, T. N.; Nunez-Oliva, I.; James, L.; Catino, J. J.; Bishop, W. R.; Pai, J.-K. *J. Biol. Chem.* **1997**, *272*, 14459.
12. Bergo, M. O.; Leung, G. K.; Ambroziak, P.; Otto, J. C.; Casey, P. J.; Young, S. G. *J. Biol. Chem.* **2000**, *275*, 17605.
13. Gelb, M. H.; Brunsveld, L.; Hrycyna, C. A.; Michaelis, S.; Tamanoi, F.; Van Voorhis, W. C.; Waldmann, H. *Nat. Chem. Biol.* **2006**, *2*, 518.
14. Ma, Y. T.; Gilbert, B. A.; Rando, R. R. *Methods Enzymol.* **1995**, *250*, 226.
15. Donelson, J. L.; Hodges, H. B.; MacDougall, D. D.; Henriksen, B. S.; Hrycyna, C. A.; Gibbs, R. A. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4420.
16. Donelson, J. L.; Hodges, H. B.; Henriksen, B. S.; Hrycyna, C. A.; Gibbs, R. A. *J. Org. Chem.* **2009**, *74*, 2975.
17. Anderson, J. L.; Henriksen, B. S.; Gibbs, R. A.; Hrycyna, C. A. *J. Biol. Chem.* **2005**, *280*, 29454.
18. Winter-Vann, A. M.; Baron, R.; Wong, W.; de la Cruz, J.; York, J. D.; Gooden, D. M.; Bergo, M.; Young, S. G.; Toone, E. J.; Casey, P. J. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 4336.
19. Marciano, D.; Ben-Baruch, G.; Marom, M.; Egozi, Y.; Haklai, R.; Kloog, Y. *J. Med. Chem.* **1995**, *38*, 1267.
20. Zhou, C.; Shao, Y.; Gibbs, R. A. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1417.
21. Subramanian, T.; Liu, S.; Troutman, J. M.; Andres, D. A.; Spielmann, H. P. *ChemBioChem* **2008**, *9*, 2872.
22. Biller, S. A.; Abt, J. W.; Pudzianowski, A. T.; Rich, L. C.; Slusarchyk, D. A.; Ciosek, C. P., Jr. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 595.
23. Hardouin, C.; Kelso, M. J.; Romero, F. A.; Rayl, T. J.; Leung, D.; Hwang, I.; Cravatt, B. F.; Boger, D. L. *J. Med Chem* **2007**, *50*, 3359.
24. Hayashi, T.; Konishi, M.; Kobori, M.; Kumada, M.; Higuchi, T.; Hirotsu, K. *J. Am. Chem. Soc.* **1984**, *106*, 158.
25. Rand, C. L.; Horn, D. E. V.; Moore, M. W.; Negishi, E. *J. Org. Chem.* **1981**, *46*, 4093.
26. Negishi, E.; Horn, D. E. V.; Yoshida, T. *J. Am. Chem. Soc.* **1985**, *107*, 6639.
27. Volker, C.; Lane, P.; Kwee, C.; Johnson, M.; Stock, J. *FEBS Lett.* **1991**, *295*, 189.
28. Umbreit, M. A.; Sharpless, K. B. *J. Am. Chem. Soc.* **1977**, *99*, 5526.
29. Miyaura, N.; Suzuki, A. *Chem. Rev.* **1995**, *95*, 2457.
30. Mechelke, M. F.; Wiemer, D. F. *J. Org. Chem.* **1999**, *64*, 4821.
31. Tan, E. W.; Perez-Sala, D.; Rando, R. R. *J. Am. Chem. Soc.* **1991**, *113*, 6299.
32. Henriksen, B. S.; Anderson, J. L.; Hrycyna, C. A.; Gibbs, R. A. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 5080.
33. Gordon, E. M.; Pluscec, J. *J. Am. Chem. Soc.* **1992**, *114*, 1521.
34. Park, S. B.; Howald, W. N.; Cashman, J. R. *Chem Res Toxicol* **1994.**
35. Kolb, H. C.; Sharpless, K. B. *Drug Discovery Today* **2003**, *8*, 1128.
36. Angelo, N. G.; Arora, P. S. *J. Org. Chem.* **2007**, *72*, 7963.
37. Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *33*, 45.
38. Hrycyna, C. A.; Wait, S. J.; Backlund, P. S.; Michaelis, S. *Methods Enzymol.* **1995**, *250*, 251.