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Synthesis of a new trifluoromethylketone analogue of L-arginine and contrasting inhibitory activity against human arginase I and histone deacetylase 8

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ABSTRACT

As part of our continuing search for new amino acid inhibitors of metalloenzymes, we now report the synthesis and biological evaluation of the trifluoromethylketone analogue of L-arginine, (*S*)-2-amino-8,8,8-trifluoro-7-oxo-octanoic acid (**10**). While this novel amino acid was initially designed as a potential inhibitor of human arginase I, it exhibits no measurable inhibitory activity against this enzyme. Surprisingly, however, **10** is a potent inhibitor of human histone deacetylase 8, with $IC_{50} = 1.5 \pm 0.2$ μM. Additionally, **10** weakly inhibits the related bacterial enzyme, acetylpolyamine amidohydrolase, with $IC_{50} = 110 \pm 30$ μM. The lack of inhibitory activity against human arginase I may result from unfavorable interactions of the bulky trifluoromethyl group of **10** in the constricted active site. Since the active site of histone deacetylase 8 is less constricted, we hypothesize that it accommodates **10** as the *gem*-diol, which mimics the tetrahedral intermediate and its flanking transition states in catalysis. Therefore, we suggest that **10** represents a new lead in the design of an amino acid or peptide-based inhibitor of histone deacetylases with simpler structure than previously studied trifluoromethylketones.

Arginase is a binuclear manganese metalloenzyme that catalyzes the hydrolysis of L-arginine to form L-ornithine and urea through a metal-activated hydroxide mechanism.[1–3] Two isozymes are present in humans;[4,5] arginase I is predominantly found in the liver, where it catalyzes the final cytosolic step of the urea cycle,[6,7] but it is also found in extrahepatic tissues where it functions to regulate cellular concentrations of L-arginine and L-ornithine for subsequent biosynthetic transformations, for example, nitric oxide biosynthesis or polyamine biosynthesis.[8–10] The highest concentrations of arginase II are found in the kidney, but this isozyme, too, functions in L-arginine homeostasis in various tissues and cell types.[11,12] Both arginases are increasingly prominent as drug targets due to the fact that arginase inhibitors enhance L-arginine bioavailability to nitric oxide (NO) synthase, and thereby amplify endogenous NO levels and NO-dependent processes such as vasodilation in penile erection[13–17] or bronchodilation in the asthmatic airway.[18–20] Thus, the exploration of new L-arginine analogues for evaluation as potentially therapeutically useful arginase inhibitors continues to challenge the field of medicinal chemistry.

Interestingly, the arginases and the metal-dependent histone deacetylases (HDACs) share a common α/β protein fold despite exhibiting insignificant amino acid sequence identity.[21–23] The conservation of a metal binding site between these two enzyme families despite substantial evolutionary drift further indicates that they divergently evolved from a common metalloprotein ancestor.[3] HDACs catalyze the hydrolysis of acetyl-L-lysine residues to yield acetate and L-lysine in histone and non-histone protein substrates. HDAC8 is the most studied isozyme in terms of structure–function relationships,[24–28] although numerous structures of other isozymes[29–31] and HDAC-related deacetylases[22,32–34] such as acetylpolyamine amidohydrolase (APAH)[33] are available to guide inhibitor design efforts. Given that HDAC activity facilitates cancer cell proliferation, HDAC inhibitors are rapidly emerging as chemopreventive and chemotherapeutic drugs for cancer treatment. To date, two HDAC inhibitors are approved for cancer chemotherapy and several more are in clinical trials, so there is a great deal of current effort focused on the discovery of new inhibitor designs.[35–37]

In order to assess structure–function relationships between the arginases and the HDACs, we now report the synthesis of a new trifluoromethylketone analogue of L-arginine, (*S*)-2-amino-8,8,8-trifluoro-7-oxo-octanoic acid (**10**) (Scheme 1). As initially outlined by Gelb et al.[38] the introduction of fluorine atoms adjacent to a ketone carbonyl group of an enzyme inhibitor enhances the

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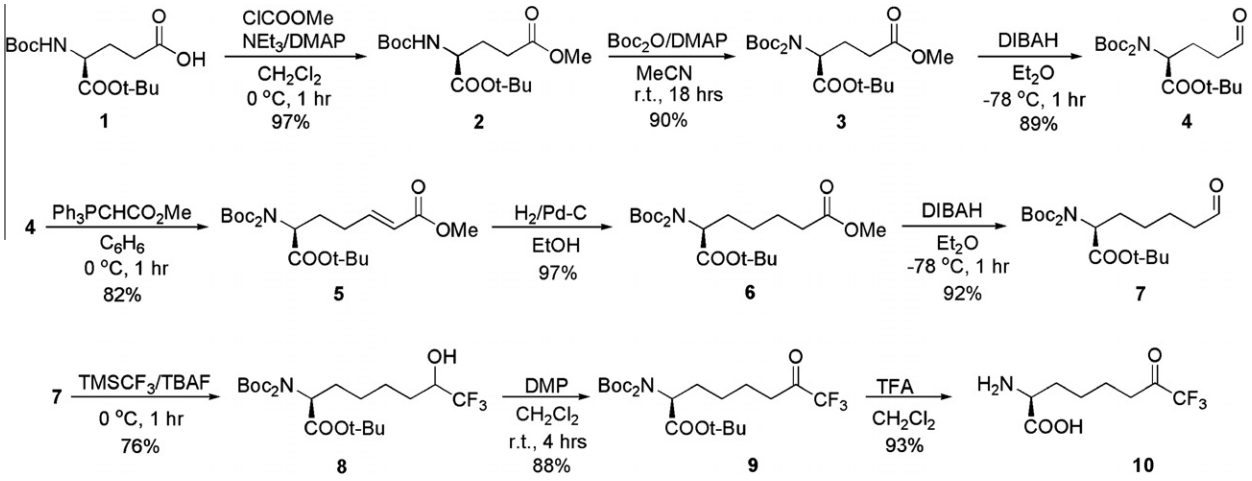

Scheme 1. Synthesis of (*S*)-2-amino-8,8,8-trifluoro-7-oxo-octanoic acid (**10**).

tendency of the ketone to add a nucleophile such as a water molecule, which explains why difluoromethyl and trifluoromethyl ketones exist in aqueous solution predominantly as the *gem*-diol hydrates. As such, appropriately designed trifluoromethyl ketones make effective inhibitors of metallohydrolases because the *gem*-diol hydrate form mimics the tetrahedral intermediate and its flanking transition states in a hydrolytic reaction involving direct attack of a solvent nucleophile at the scissile amide linkage of the substrate. This was first demonstrated in the binding of a trifluoromethyl ketone inhibitor to the prototypical zinc enzyme carboxypeptidase A,[39] leading to the design of trifluoromethylketone inhibitors of other metal-dependent hydrolases such as the HDACs.[29,40,41] However, to date no trifluoromethylketone analogue of L-arginine has been prepared and evaluated against arginase and arginase-related deacetylases, thereby motivating the current study.

The synthesis of **10** is summarized in Scheme 1. The commercially available L-glutamic acid derivative, (*S*)-2-*tert*-butoxycarbonylamino-pentandioic acid 1-*tert*-butyl ester (**1**) was converted into the corresponding methyl ester **2** using methyl chloroformate in methylene chloride. The relatively acidic carbamate proton was replaced by a second *tert*-butoxycarbonyl (Boc) group to allow for the selective reduction of the less sterically hindered methyl ester (intermediate **3)** with diisobutylaluminum hydride (DIBAH), thus yielding the corresponding aldehyde **4**.[20,42] Wittig reaction of **4** with methyl(triphenylphosphoranylidene)acetate, followed by Pd/C-catalyzed hydrogenation of the double bond in intermediate **5** generated ester **6**, which was selectively reduced with DIBAH to form aldehyde **7**.[43,44] Nucleophilic trifluoromethylation of **7** with trifluoromethyltrimethylsilane ($TMSCF_3$) and tetra-*n*-butylammonium fluoride (TBAF) as initiator, using a combination of modified classic procedures,[45–47] afforded (*S*)-*tert*-butyl-2-bis(*tert*-butoxycarbonyl)amino-8,8,8-trifluoro-7-hydroxyoctanoate (**8**) in 76% yield.[48] Dess–Martin periodinane (DMP) alcohol oxidation[49,50] yielded the corresponding ketone derivative **9** as a mixture of the ketone and hydrate forms in a 2:1 molar ratio as determined by ^{19}F NMR,[51] as similarly observed for other trifluoromethyl ketones solubilized in polar solvents.[52] Deprotection with trifluoroacetic acid (TFA) in methylene chloride generated **10** in 93% yield (ketone/hydrate 15:1 molar ratio by ^{19}F NMR).[53]

The in vitro inhibitory potency of **10** against recombinant human arginase I was assessed using the fixed point assay with [^{14}C-guanidino]-L-arginine.[54] Briefly, recombinant human arginase I was prepared as previously described.[55] The final working concentrations in the reaction tubes were 1 mM for unlabeled L-arginine and 0.07 μg/μL for human arginase I; the concentration of trifluoromethylketone **10** ranged 0–800 mM. Data analysis was based on the kinetic replot of v_0/v_i as a function of inhibitor concentration, where v_0 and v_i were the observed velocities in the absence and presence of inhibitor, respectively. Since $v_0/v_i \sim 1$ at all inhibitor concentrations tested, no inhibition of human arginase I by **10** was detected.

The inhibitory potency of **10** against HDAC8 and the HDAC-related deacetylase APAH was measured using the commercially available Fluor-de-Lys deacetylase substrates *N*-acetyl-L-Arg-L-His-L-Lys(ε-acetyl)-L-Lys(ε-acetyl)-coumarin or L-Lys(ε-acetyl)-coumarin, respectively (Enzo Life Sciences).[56] The deacetylation of this assay substrate allows for proteolytic cleavage of the C-terminal fluorophore to result in a fluorescence shift. For inhibition of either HDAC8 or APAH, assays were run at 25 °C and each 50 μL reaction sample contained 0.5–1 μM enzyme, 50–150 μM substrate, 25 mM Tris (pH 8.2), 137 mM NaCl, 2.7 mM KCl, and 1 mM $MgCl_2$. For HDAC8 assays, concentrations of trifluoromethylketone inhibitor **10** were 0.1 μM, 0.5 μM, 1.0 μM, 8.0 μM, 10.0 μM, 30.0 μM, 50.0 μM, 75.0 μM, and 500 μM; for APAH assays, inhibitor concentrations were 1 μM, 10 μM, 100 μM, 1 mM and 10 mM. The inhibitor was incubated with the enzyme for 15 min prior to starting the reaction by the addition of substrate. After 20 min (HDAC8) or 30 min (APAH), reactions were stopped by the addition of 100 μM M344 (Sigma) and the appropriate Fluor-de-Lys developer. Fluorescence was measured using a Fluoroskan II plate reader (ex = 355 nm, em = 460 nm). The IC_{50} value was calculated using Prism 5 and plots of inhibitory activity are shown in Figure 1 (GraphPad Software). All measurements were made in triplicate. For HDAC8, these experiments yielded $IC_{50} = 1.5 \pm 0.2$ μM; for APAH, $IC_{50} = 110 \pm 30$ μM.

It is surprising that **10** does not inhibit human arginase I, especially since the corresponding aldehyde, (*S*)-2-amino-7-oxoheptanoic acid, binds as the *gem*-diol hydrate with $K_d = 60 \pm 8$ μM.[44] Possibly, the α-trifluoro moiety of **10** is too bulky to fit in the constricted active site; additionally, the partial negative charge on the fluorine atoms may make an unfavorable electrostatic interaction with the negatively charged side chain of E277 at the base of the active site. This residue accepts a hydrogen bond from the η_1-NH_2 group of the substrate L-arginine, which is isosteric with the η-CF_3 group of **10**. We were unsuccessful in cocrystallizing the human arginase I-**10** complex for structural analysis, presumably due to the relatively weak affinity of inhibitor binding.

It is further surprising that while **10** was designed, but ultimately failed, as an inhibitor of arginase, it exhibits unusually potent, low micromolar activity against HDAC8. It is 73-fold less potent against