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Evaluation of hadacidin analogues

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ABSTRACT

Several derivatives of hadacidin have been developed and evaluated for activity against adenylosuccinate synthetase.

Hadacidin (**1**, *N*-formyl-*N*-hydroxyglycine) was originally isolated from a fermentation broth of *Penicillium frequentans*. It was later identified as a potent anti tumor agent.[1] In 1962, Gordon and co-workers reported the inhibitory effect of hadacidin on the de-novo biosynthesis of adenylic acid in Ehrlich ascites tumor and rat liver cells.[2,3] Hadacidin is a known inhibitor of adenylosuccinate synthetase (AdSS). Structurally, hadacidin resembles aspartic acid (Fig. 1). Our group is investigating a series of newly developed hadacidin analogues and testing the inhibitory effects on adenylosuccinate synthetase.

Cells in approximately 25% of many common human cancers are deficient in the enzyme methylthioadenosine phosphorylase (MTAP)[4] and rely entirely on the de-novo biosynthesis of adenine nucleotides. Adenylosuccinate synthetase catalyses the first committed step in the de-novo biosynthesis of adenosine monophosphate (AMP). The enzyme catalyses the reaction in two steps; first, transfer of γ-phosphate group of GTP to O-6 of IMP forming a highly reactive 6-phosphoryl IMP derivative along with GDP. This step is facilitated by Mg^{2+} ion. Second, the amino group of aspartic acid displaces the 6-phosphoryl group of 6-phosphoryl-IMP to form adenylosuccinate monophosphate (ASMP) (Fig. 1).[5] The enzyme adenylosuccinate lyase then converts ASMP into AMP.

In 1984, Jahngen and Rossomondo evaluated hadacidin analogues that were functionalized at both terminal ends.[6] In this Letter, we decided to examine the compounds that would probe the aspartic acid active site by increasing the chain length of carbon atoms in between the terminal ends and by adding a branched α-chain. Herein, we describe our syntheses and evaluation of these hadacidin analogues.

The synthesis of branched hadacidin compounds began with commercially available α-keto acids (**3**).[7,8] The α-keto acids were

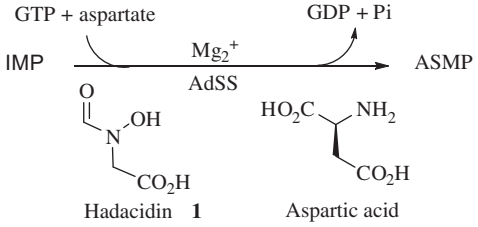

Figure 1. The conversion of IMP into ASMP.

treated with hydroxylamine hydrochloride in aqueous NaOH which provided the corresponding oximes **4a**–**f** in 80–90% yield. Reduction of the oximes **4a**–**f** with $NaBH_3CN$ in acetic acid provided the racemic branched hydoxylamino acids **5a**–**f** in moderate yield. Installation of the required formyl group by treatment of **5a**–**f** with a mixture of formic acid in acetic anhydride provides the α-substituted hadacidin analogues **6a**–**f** in excellent yields (Scheme 1).

A similar method, shown in Scheme 2, was used for the synthesis of compound **9**, which is a one carbon extension of **6a**. Commercially available 4-ketopentanoic acid (**7**) was treated with hydroxyl amine in acetic acid; this was followed by $NaCNBH_3$ reduction of the oxime and treatment with concentrated hydrochloric acid to provide compound **8**. Formylation of **8** with formic acid in acetic anhydride provided compound **9** in 56% yield.

Compound **12**, a one carbon homolagation of hadacidin, was synthesized in four steps. This was acquired through a Michael type addition of *N*-benzyloxyamine onto ethyl acrylate which made available compound **10** in 40% yield.[9] The β-benzylhydroxylamino ester was hydrolyzed with lithium hydroxide providing the intermediate acid that was debenzylated with palladium on carbon. This two-step sequence supplied compound **11** in 90% yield. Formylation of **11** with formic acid in acetic anhydride

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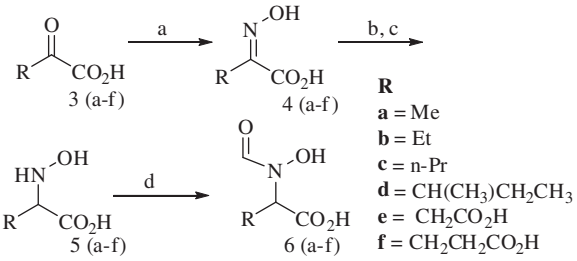
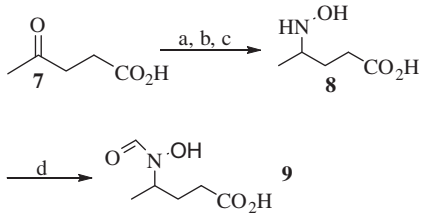


Scheme 1. Synthesis of hadacidin analogues with different R groups at α-carbon. Reagents and conditions: (a) hydroxylamine hydrochloride, NaOH; 80–90%; (b) $NaBH_3CN$, 50% acetic anhydride; 40–50%; (c) concd HCl; (d) acetic anhydride, formic acid, 70–80%.

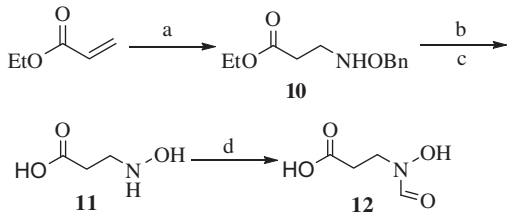


Scheme 2. Reagents and conditions: (a) hydroxylamine hydrochloride, NaOH; 52%; (b) $NaBH_3CN$, 50% acetic anhydride; 18 h; (c) concd HCl; 92%; (d) acetic anhydride, formic acid, 56%.

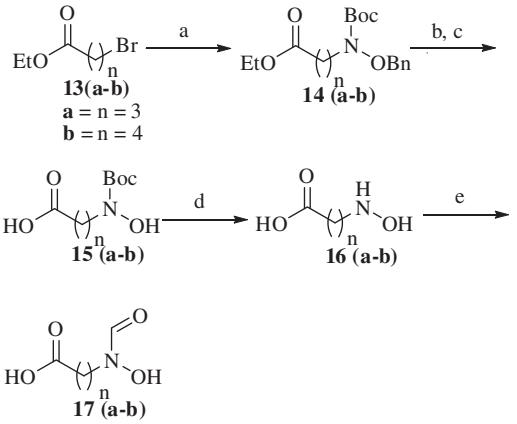
proceeded smoothly and provided *N*-formyl-*N*-hydroxy-β-aminopropionic acid (**12**; Scheme 3).

The extended derivatives, compounds **17a** and **17b**, of hadacidin were available through the reaction of *N*-benzyloxy-*N*-*tert*-butoxycarbonylamino[10] with either 3- or 4-bromoesters (**13a** or **13b**) providing compound 1**4a** or **14b**. The yield for **14a** was 70%. Compound **14b** was carried forward without purification when 0.9 equiv of sodium hydride was utilized. The hydrolysis of the ester was accomplished with LiOH. For either substrate the yields were excellent. The deprotection of the benzyl hydroxamate with hydrogenolysis afforded compounds **15a** and **15b**. The overall yield of compound **16** after hydrolysis, benzyl deprotection and treatment with TFA for the removal of the Boc group was 90%. Introduction of a formyl group on nitrogen provided the analogues with either 3 or 4 carbon linkers between the *N*- and *C*-terminus of hadacidin (Scheme 4).

For AdSS production, pMOB45[11] was introduced into *Escherichia coli* BL21(DE3), and positive transformants were selected on LB agar supplemented with 30 μg/mL kanamycin. A single colony was transferred to 3 mL LB supplemented with 30 μg/mL kanamycin and grown at 37 °C and 250 rpm for 10 h. A 0.5 mL aliquot was transferred to 50 mL LB supplemented with 30 μg/mL kanamycin and incubated at 37 °C and 250 rpm overnight. Subsequently, 5 mL of the culture was used to inoculate 500 mL LB supplemented with 30 μg/mL kanamycin. The 500 mL culture was incubated at



Scheme 3. Reagents and conditions: (a) $NH_2OBn{\cdot}HCl$, Et_3N, EtOH, −80 °C, 3 days, 40%; (b) 1 N LiOH, THF/MeOH/H_2O, 80%; (c) H_2/Pd-C, quant.; (d) acetic anhydride, formic acid, 80%.



Scheme 4. Reagents and conditions: (a) BocNHOBn, NaH, NaI, dry DMF, 60–70%; (b) 1 N LiOH, THF/MeOH/H_2O, 90%; (c) H_2/Pd-C, quant.; (d) TFA, DCM, 0 °C, quant.; (e) acetic anhydride, formic acid, 50–80%.

18 °C and 250 rpm for ~9 h (or until OD_{600} = 0.5), and gene expression was induced with 0.1 mM IPTG. Cells were harvested by centrifugation after incubation at 18 °C and 250 rpm for 14 h post-induction. Pelleted cells were resuspended in 100 mM Tris–HCl pH 8.0 and 300 mM KCl and lysed using a French Press using one pass at 15,000 psi. Following high-speed centrifugation, the soluble extract was loaded onto a Profinia™ protein purification system equipped with a 1 mL Bio-Scale™ Mini Profinity IMAC cartridge and a 10 mL Bio-Scale™ Mini Bio-Gel® P-6 desalting cartridge (Bio-Rad). The purified protein was concentrated using an Amicon Ultra 10,000 MWCO centrifugal filter (Millipore Corp.) and stored as 50% glycerol stocks at −20 °C. Under these conditions, no loss in activity of recombinant AdSS was observed for minimally 6 months.

The ability of hadacidin derivatives to inhibit AdSS was demonstrated by HPLC detection. Adenylosuccinate synthetase catalyses the condensation of IMP with aspartate forming adenylosuccinate monophosphate. The enzymatic reaction also requires the conversion of GTP to GDP. The mixture (100 μl) contained, 20 mM Hepes (pH 7.5), 50 μM IMP, 25 μM GTP, 500 μM $MgCl_2$, 10 mM test compound and 2.0 μg of purified AdSS enzyme. The assay was initiated at room temperature with the addition of either 100 or 500 μM of aspartate. After 5 min, the solution was stopped by freezing in a methanol/dry ice bath. The solution was allowed to reach room temperature before 20 μl of reaction mixture was injected onto the HPLC. The mobile phase for the assay contained 65 mM potassium phosphate, 1 mM PIC A, and 10% methanol. The buffer has a pH of 4.4. The HPLC assay procedure allows separation of all UV

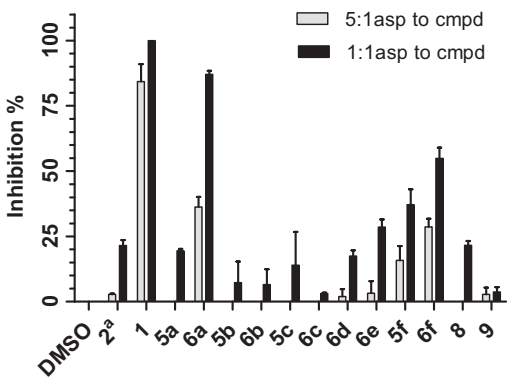


Figure 2. Inhibition of adenylosuccinate synthetase activity by hadacidin analogues with different R groups at α-C. [a]*N*-hydroxy glycine.

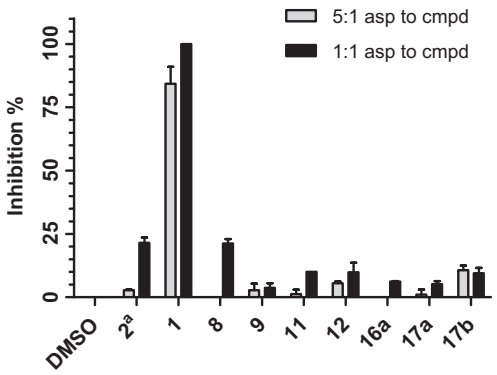


Figure 3. Inhibition of AdSS by hadacidin analogues with varying carbon linker between *N*-hydroxy and carboxylic acid. [a]*N*-hydroxy glycine.

active components (GDP, GTP, and ASMP) of the mixture at 266 nm. The amount of adenylosuccinate monophosphate formed was quantified by the integration of peak areas over three runs.

At 10 mM hadacidin completely inhibited the enzyme (Fig. 2). However, by adjusting the ratio of aspartate to hadacidin to 5:1, the inhibition dropped to 80%. We tested all final compounds at both concentrations. Compound **6a**, having a methyl group at the α-carbon demonstrated approximately 50% reduced ability to inhibit the enzyme when asp:**6a** was 5:1. The inhibition improved to 90% when equimolar concentrations of aspartate and compound **6a** were tested.

Addition of more bulky groups on the α-carbon (**6b–d**) resulted in nearly complete loss of activity at 5:1 ratio of aspartate:test compound. When the concentration of the test compound was increased to 1:1 some inhibition was observed, albeit very low. When the α-chain went from ethyl to propyl, the expected loss of activity was observed. But when the diastereomers of isobutyl **6d** were tested, some activity against the enzyme was retained.

Furthermore, compounds **6e** and **6f** with polar carboxylic groups off the α-carbon showed 30% and 60% inhibition, respectively, with equimolar concentration of aspartate and compounds.

Without the formyl group, *N*-hydroxyglycine showed minimal effect on AdSS activity. With other analogues (**5a–c**, **f**, **8**) inhibition also fell significantly. This confirmed that the formyl group was necessary for inhibition.

In our second series of hadacidin analogues (Fig. 3) the result of increasing the chain length between the *N*-hydroxy-*N*-formyl and the carboxylic acid of hadacidin were as expected. At a concentration of 5:1, all tested compounds showed very weak to nonexistent inhibition. At equimolar concentration to aspartate, compound **11**, with two-carbon linker showed almost negligible inhibition. The formylated derivative, compound **12**, unexpectedly did not show any improvement in activity. There was a similar observed trend of weak inhibition for both the compounds **17a** and **17b**.

In conclusion, several structurally similar hadacidin analogues were tested for possible inhibition of adenylosuccinate synthetase. The introduction of methyl substituent at the α-carbon reduced the ability of the compound to inhibit the enzyme by 50% when tested at aspartate to substrate of 5:1. Since this was a racemic compound, the possibility exists that one isomer may be active. Addition of more bulky groups on the α-carbon further reduced the activity. By increasing the carbon chain length between the terminal ends of the *N*-hydroxy and carboxylic acid resulted in nearly complete loss of activity. None of the analogues tested for activity against adenylosuccinate synthetase showed activity better than hadacidin. We are currently working on the synthesis of optically pure **6a** to further evaluate the active site and to provide compounds with better activity.

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.2010.10.088.

References and notes

1. Kaczka, E. A.; Gitterman, C. O.; Dulaney, E. L.; Folkers, K. *Biochemistry* **1962**, *1*, 340.
2. Shigeura, H. T.; Gordon, C. N. *J. Biol. Chem.* **1962**, *237*, 1932.
3. Shigeura, H. T.; Gordon, C. N. *J. Biol. Chem.* **1962**, *237*, 1937.
4. Kamatani, N.; Nelson-Rees, W. A.; Carson, D. A. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, 1219.
5. Lieberman, I. *J. Biol. Chem.* **1956**, *223*, 327.
6. Jahngen, E. G. E.; Rossomando, E. F. *Arch. Biochem. Biophys.* **1984**, *229*, 145.
7. Kato, Y.; Tsuda, T.; Asano, Y. *Biochem. Biophys. Acta* **2007**, *1774*, 856.
8. Ahmad, A. *Bull. Chem. Soc. Jpn.* **1974**, *47*, 1819.
9. Yakirevitch, P.; Rochel, N.; Albrecht-Gary, A. M.; Libman, L.; Shanzer, A. *Inorg. Chem.* **1993**, *32*, 1779.
10. Kurtz, T.; Geffken, D.; Wackendorff, C. *Z. Naturforsch.* **2003**, *58b*, 457.
11. A gift from Dennis Carson of the Moores Cancer Center at UCSD.