Published in final edited form as:
Bioorg Med Chem Lett. 2011 May 1; 21(9): 2611–2615. doi:10.1016/j.bmcl.2011.01.084.

Syntheses and Studies of Amamistatin B Analogs Reveals that Anticancer Activity is Relatively Independent of Stereochemistry, Ester or Amide linkage and Select Replacement of one of the Metal Chelating Groups

Chunrui Wu, **Patricia A. Miller**, and **Marvin J. Miller**
Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556

Abstract

A series of analogs of the amamistatin natural products was designed and synthesized to facilitate additional anticancer structure-activity-relationships. The results indicate that the anticancer activity is relatively independent of stereochemistry, ester or amide linkage and replacement of the oxazoline/oxazole based iron-binding group with a catechol.

Amamistatins A (**1**) and B (**2**) are natural products isolated from the actinomycete *Nocardia asteroides* (Figure 1).1–3 Amamistatin A (**1**) exhibits anti-proliferative activity against a variety of cancer cell lines, including MCF-7 breast, A549 lung, and MKN45 stomach cancer. Amamistatins A and B also show cytotoxicity against mouse lymphocytic leukemia cells P388 (IC_{50} 15 and 16 ng/mL, respectively).1 In addition, these compounds structurally resemble mycobactins,4 analogs of which exhibit anti-tuberculosis activity and have other therapeutic potential due to the chelation of iron (III),4–7 and other more recently reported siderophores including the nocardimicins,8,9 nocobactin NA,10,11 formobactin,12 and brasilibactin A.13–15 Iron chelation by these similar siderophores arises from three coordination sites, a hydroxyphenyl oxazoline or oxazole and two hydroxamic acids. The origin of the anticancer activity of amamistatins is not yet known, but because of they are natural metal ligands, they are capable of chelating metals essential for enzymatic activities. It was previously proposed that the anticancer activity of amamistatins was due to HDAC inhibition by the *N*-formyl hydroxylamine ligand.5 However, recent studies showed that the synthetic amamistatin B and an analog lacking the *N*-hydroxy group on the cyclic hydroxamate displayed no HDAC activity at the concentration of 100 μM.6

Herein, we wish to report the design and syntheses of new analogs of amamistatins and their activity against MCF-7 and PC-3 human cancer cell lines. The first series is represented by analogs **3–6**, with variation of the oxazole moiety and the ester linkage as well as controlled stereochemical modification. The second compound type involves change of the hydroxyphenyl oxazole to a catechol (**7**) since catechols are frequent ligands in other natural siderophores.16 (Figure1).

Correspondence to: Marvin J. Miller.

The retro-synthetic analysis of amamistatin B analogs is shown in Figure 1. Disconnection of the ester reveals the constituent mycobactic acid and cobactin and further simplification gives four fragments: a hydroxyphenyl oxazole or its analog (**A**), a linear hydroxamic acid (**B**), a β-hydroxy or β-amino acid (**C**), and a lysine-based cyclic hydroxamic acid, *N*-hydroxycaprolactam (**D**). Syntheses of fragments **A** (compounds **8** to **10**), **B** (compound **11**), and **D** (compound **12**) (Scheme 2) were carried out according to published procedures.[5–7] The oxazole, and oxazoline were prepared from salicylic acid and the corresponding amino acid, serine (R^1 = H) or threonine (R^1 = Me). Both the linear and cyclic hydroxamic acid were prepared from Cbz-protected lysine.

The β-hydroxy carboxylic acid **16** (fragment **C**) was derived from Meldrum's acid (**13**) and octanoyl chloride using an acylation, decarboxylation, and asymmetric reduction protocol (Scheme 1). Thus, treatment of Meldrum's acid with octanoyl chloride in the presence of pyridine followed by refluxing in MeOH afforded β-keto ester **14** in 77% yield.[17] Asymmetric hydrogenation of **14** with a catalyst generated *in situ* from (cod)Ru(2-methylallyl)$_2$ and (*R*)- or (*S*)-BINAP worked very efficiently under one atmosphere of hydrogen to provide the (*R*)- or (*S*)-β-hydroxy ester **15R/15S** in excellent optical (*ee*) and chemical yields.[18] Saponification of these β-hydroxy esters with LiOH and subsequent acidification gave the corresponding β-hydroxy carboxylic acids **16R/16S**.

With all fragments in hand, analogs **3**, with either the (*R*) or (*S*) stereochemistry of the β-hydroxy amide and the unnatural (for amamistatins) L-(*S*)-configuration of the linear lysine components were first targeted for syntheses, because we had previously shown that the diastereomer of amamistatin B with the (*S*) configuration of the linear lysine had anticancer activity similar to amamistatin B, itself, which has a D-(*R*)-linear lysine component. Thus, additional changes of stereochemistry at other stereogenic centers including the β-hydroxy amide were anticipated to help elaborate structure-activity-relationship (SAR) studies. A convergent approach was designed to couple oxazole **A** with lysine derivative **B** to give the corresponding mycobactic acid and couple separate enantiomers of β-hydroxy acids **C** (Y = O) with cyclic hydroxamate **D** to generate diastereomeric cobactins. Final coupling of the mycobactic acid with the individual diastereomers of the cobactins would complete the syntheses.

As anticipated, DCC-mediated coupling of β-hydroxy carboxylic acids **16R** and **16S** with hydroxamate **12** provided diastereomerically pure cobactins **17R** and **17S** in high yields (Scheme 2). Interestingly, HOAt/EDC-mediated coupling of **16R** and **12** was much less effective and gave only a 34% yield of **17R**. However, use of HOAt/EDC was more effective than a DCC-mediated processes for coupling of oxazole **8** and hydroxamic acid **18** to give protected mycobactic acid **19**, especially with addition of DMAP. LiOH-mediated saponification of methyl ester **19** gave mycobactic acid **20** cleanly.

The direct coupling of mycobactic acid **20** and cobactins **17R** and **17S** was anticipated to provide the dibenzyl-protected forms of diastereomeric amamistatin analogs **3**. However, all attempts at esterification were unsuccessful, including use of EDC/4-PPY, HOAt/EDC or Yamaguchi coupling conditions. Since we had previously found that coupling of simple protected forms of linear lysine derivatives (fragment **B**) with other cobactins, including those containing a tertiary alcohol instead of the secondary alcohol of **18**, was effective,[5] we attempted the same method on separate substrates **17R/S**. Again, to our surprise, these less hindered alcohols (**17R/S**) did not react with the linear lysine component **11** under the same conditions. A variety of other conditions, including 1-methylimidazole/TsCl mediated coupling,[19,20] Mitsunobu reaction,[21] EDC/4-PPY all failed to generate **21**. Fortunately, in this case, Yamaguchi conditions provided the desired individual diastereomers of **21** in good yields.[22] HOAt/EDC-mediated coupling of acid **8** and the individual diastereomers of

dibenzyl protected amines **22R/S**, derived from treatment of the Cbz-protected derivatives **21R/S** with HBr/HOAc provided both isomers (**23R/S**) of the benzyl protected amamistatin analogs in approximately 80% yield. Separate hydrogenolysis of **23R** and **23S** generated analogs **3R** and **3S**, resepectively, in about 70% yield each (Scheme 3).

The des-methyl oxazole analogs **5R** and **5S** were also synthesized using a similar strategy. Starting from individual diastereomers of **21**, removal of the Cbz-protecting groups with HBr/HOAc, followed by EDC/HOAt-mediated couplings with oxazole acid **9** afforded the protected analogs **24**, which were debenzylated by hydrogenolysis to give analogs **5** (Table 1).

Because of the different biological activities exhibited by mycobactins S and macobactin T which differ in their growth promotion or inhibition of *Mycobacterium smegmatis* and *Mycobacterium tuberculosis* only on the basis of their diastereomeric relationship,23 individual amamistatin diastereomers **6**, each with varied configuration at the stereogenic center of the oxazoline and the β-hydroxy ester components, were also prepared from **21R** and **21S**. The yields for the four isomers of **6** and **25** are also summarized in Table 1.

We next turned our attention to analogs **4R** and **4S** in which the normal ester was replaced by an amide. This required preparation of the corresponding diastereomeric amines, **25** which was accomplished by reaction of the individual diastereomeric alcohols **17R** and **17S** with diphenyl phosphoryl azide under Mitsunobu-like conditions and subsequent reduction of the resulting azides (Scheme 4). To complete the syntheses of the amides, we again used a sequential process in which compounds **26R/S** were synthesized first before the final coupling to the oxazole. Diastereomers **26** were produced by coupling individual diastereomers of **25** with the NHS activated ester of **11**. Removal of the Cbz-protecting groups followed by coupling with the NHS-activated ester **27** of oxazole **8** provided the benzyl-protected analogs **28R/S**. Hydrogenolytic deprotection of diastereomeric forms of **28** afforded the desired amide-linked analogs **4** in excellent yields.

Alteration of iron binding moieties of amamistatins was also considered. Thus, in a second series of analogs, we replaced the oxazoline/oxazole group with a catechol to form the separate diastereomers **7R** and **7S**. The syntheses, summarized in Scheme 5, are similar to those used to prepare **4** and **6**.

The analogs of amamistatin B were submitted for biological studies using MCF-7 and PC-3 tumor cellular assays. All of the analogs were found to have moderate to good activity. As shown in Table 2, the anticancer activity varied only slightly among all of the analogs, including diastereomers, with the catechol (**7**), being only slightly less active overall.

Conclusion

The syntheses of a series of amamistatin analogs was accomplished and all of the analogs were found to be comparably active against MCF-7 and PC-3 human cancer cell lines indicating that metal chelation might be more important than stereochemistry or the specific metal ligand. Further studies to allow detailed elaboration of the mode of action of these interesting natural iron chelators as anticancer agents are under consideration.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We gratefully acknowledge support from the National Institutes of Health (AI 054193).

References

1. Kokubo S, Suenaga K, Shinohara C, Tsuji T, Uemura D. Tetrahedron. 2000; 56:6435.
2. Suenaga K, Kokubo S, Shinohara C, Tsuji T, Uemura D. Tetrahedron Lett. 1999; 40:1945.
3. Yokokawa F, Izumi K, Omata J, et al. Tetrahedron. 200; 56:3027.
4. Vergne AF, Walz AJ, Miller MJ. Nat. Prod. Rep. 2000; 17:99. [PubMed: 10714901]
5. Fennell KA, Miller MJ. Org. Lett. 2007; 9:1683. [PubMed: 17397173]
6. Fennell KA, Moellmann U, Miller MJ. J. Org. Chem. 2008; 73:1018. [PubMed: 18173285]
7. Xu Y, Miller MJ. J. Org. Chem. 1998; 63:4314.
8. Banks JC, Moody CJ. Tet. Lett. 2009; 26:3371.
9. Ikeda Y, Nonaka H, Furumai T, et al. J. Nat. Prod. 2005; 68:1061. [PubMed: 16038549]
10. Ratledge C, Snow GA. Biochem. J. 1974; 139:407. [PubMed: 4614794]
11. Sakagami H, Ishihara M, Hoshino Y, et al. In Vivo. 2005; 19:277. [PubMed: 15796187]
12. Murakami Y, Kato S, Nakajima M, et al. J. Antibiotics. 1996; 49:839. [PubMed: 8931715]
13. Tsuda M, Yamakawa M, Oka S, et al. J. Natl Prod. 2005; 68:462.
14. Mitchell JM, Shaw JT. Org. Lett. 2007; 9:1679. [PubMed: 17397172]
15. Ying Y, Hong J. Tet. Lett. 2007; 48:8104.
16. Hider RC, Kong X. Natl. Prod. Reports. 2010; 27:637.
17. Kocienski PJ, Pelotier B, Pons J-M, Prideaux H. J. Chem. Soc., Perkin Trans. 1998; 1:1373.
18. Ratovelomanana-Vidal V, Girard C, Touati R, Tranchier JP, Ben Hassine B, Genet JP. Adv. Synth. Catal. 2003; 345:261.
19. Wakasugi K, Iida A, Misaki T, Nishii Y, Tanabe Y. Adv. Synth. Catal. 2003; 345:1209.
20. Nakatsuji H, Morimoto M, Misaki T, Tanabe Y. Tetrahedron. 2007; 63:12071.
21. Dembinski R. Eur. J. Org. Chem. 2004:2763.
22. Inanaga J, Hirata K, Saeki H, Katsuki T, Yamaguchi M. Bull. Chem. Soc. Jpn. 1979; 52:1989.
23. Hu J, Miller MJ. J. Am. Chem. Soc. 1997; 119:3462.
24. Experimental details and characterization data for new compounds are provided in the Supporting Information.

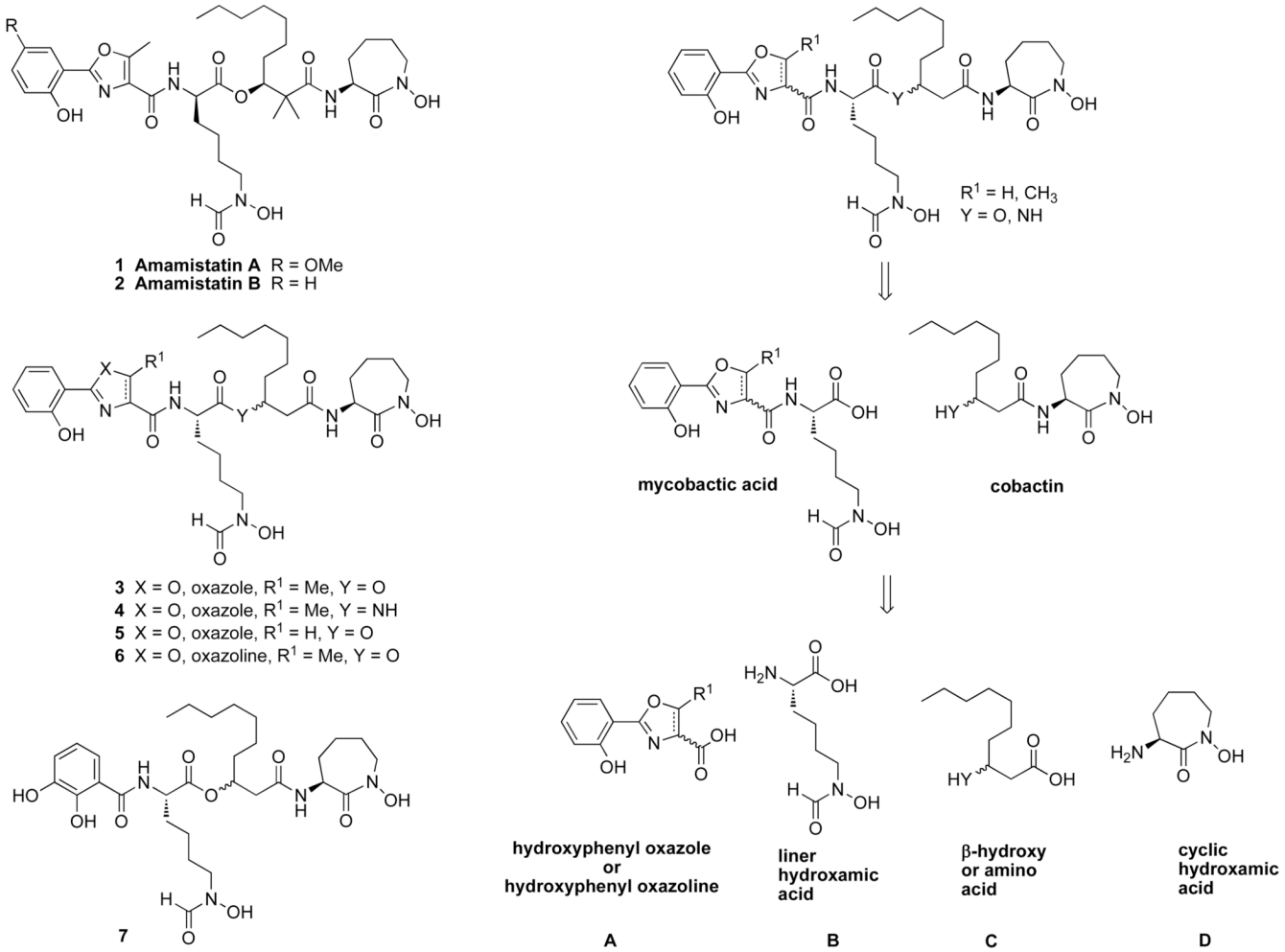
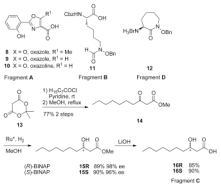
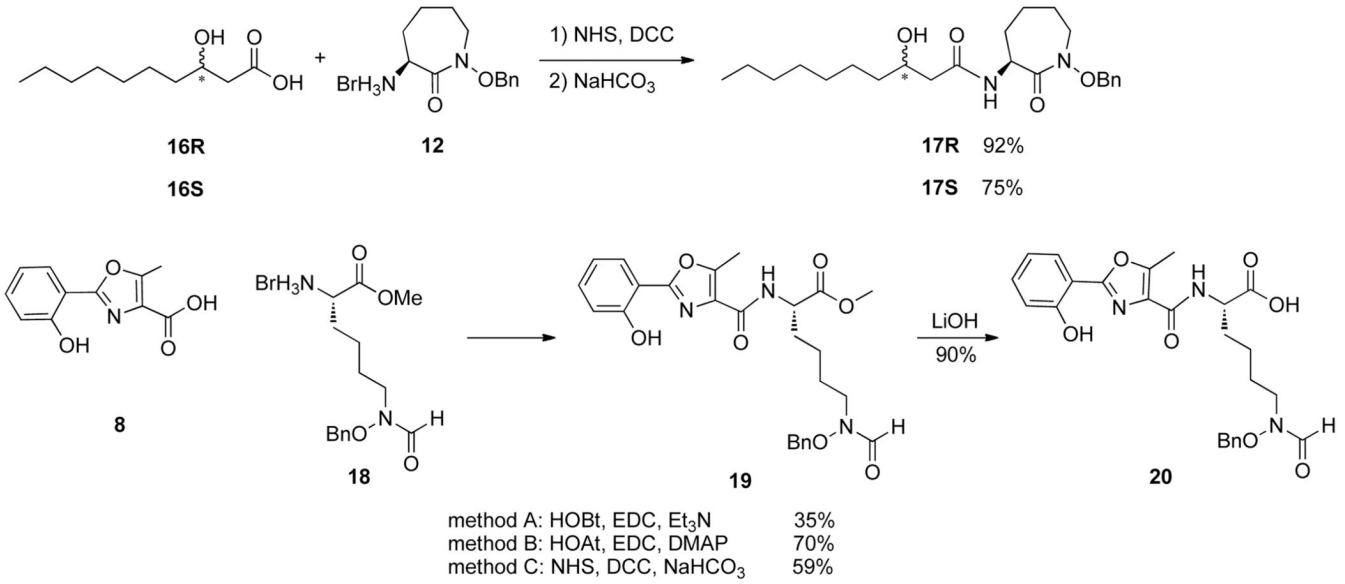


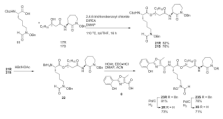
Figure 1.
Amamistatins, analogs and their retrosynthetic analysis.



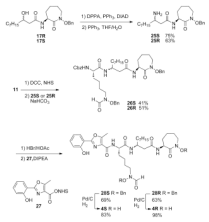
Scheme 1.



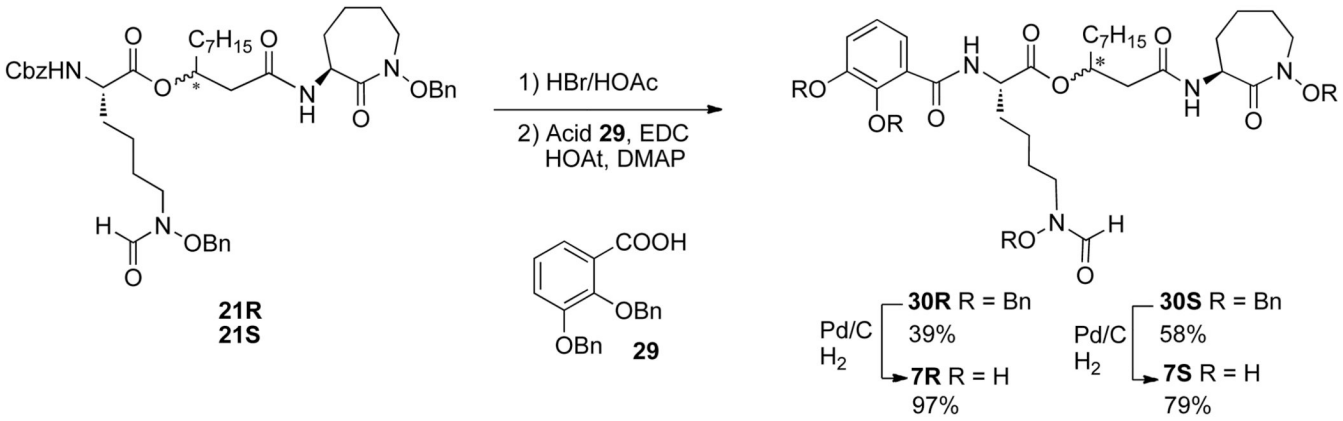
Scheme 2.



Scheme 3.

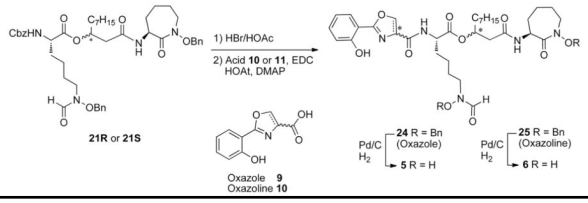


Scheme 4.



Scheme 5.

Table 1



Product	Acid	Compound 21	% Yield of 24 or 25	% Yield of 5 or 6
5R	**9**	**21R**	70	85
5S	**9**	**21S**	73	80
6SR	**10S**	**21R**	36	94
6SS	**10S**	**21S**	52	90
6RR	**10R**	**21R**	59	93
6RS	**10R**	**21S**	51	92

Table 2

Compound	MCF-7		PC-3	
	%inhibition @ 20 μM	IC_{50} (μM)	%inhibition @ 20 μM	IC_{50} (μM)
3R	94	0.2	72	4
3S	91	0.32	62	8
4R	96	0.55	72	9
4S	95	2	68	14
5R	91	0.45	85	3.75
5S	93	0.8	60	9
6SR	87	0.24	83	3
6SS	89	0.5	79	3
6RR	92	0.32	90	2
6RS	90	3	79	7.5
7R	86	6	40	-
7S	81	1	63	7