DOI: 10.1002/cmdc.200600152

# Investigating Carboxylic Acid Analogues of Ambruticin through Semi-Synthesis

Yuan Xu, Zhan Wang, Zong-Qiang Tian, Yong Li, and Simon J. Shaw*[a]

Systemic fungal infections have increased significantly in recent years, principally in immunocompromised patients. This is particularly an issue for those with HIV/AIDS and patients undergoing cancer or organ transplant therapy. There are only a small number of drugs available to treat such infections including Amphotericin B, which has considerable side effects.[1] Therefore, new antifungal agents are needed.

The polyketide antifungal antibiotic ambruticin was first isolated in the late 1970s by Warner-Lambert from the myxobacteria *Polyangium cellulosum* var. *flavum*.[2] The molecule comes in two series characterised by the functional group found at the 5-position (ambruticin numbering, see Figure 1); the S series in which there is a hydroxyl group **1** and the more active VS series with an amino group at this site, either as the free amine (VS-5) **2**, monomethylamine (VS-4) **3**, or dimethylamine (VS-3) **4** (Figure 1). The molecule is thought to exert its antifungal activity through interference with osmoregulation in a similar manner to pyrrolnitrin.[3]

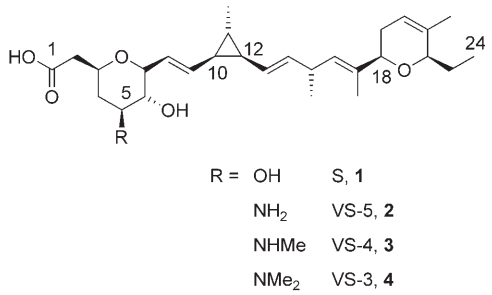
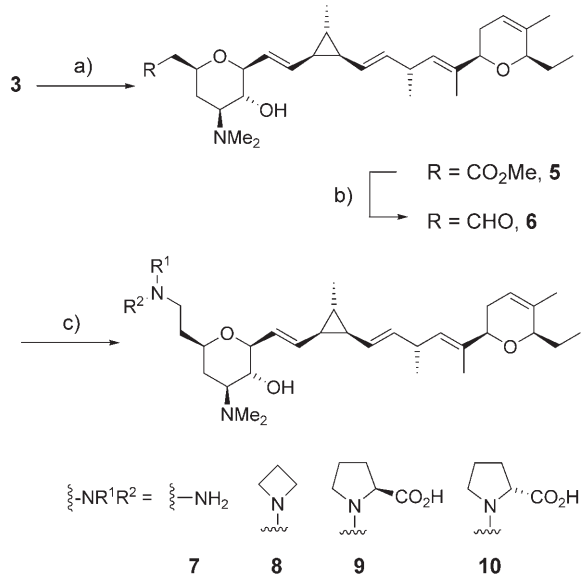



**Figure 1.** The S **1** and VS series **2**–**4** ambruticins.

Recently, there has been considerable synthetic interest in this molecule. Following Kende's initial report[4] in 1990 there have been a total of three syntheses reported[5] and several partial syntheses. However, the only structure–activity relationship (SAR) work reported was carried out with the S series in the late 1970s at Warner-Lambert.[6,7] A group of amide, ester, and ketone analogues of the carboxylic acid, and secondary and tertiary alcohols at this position suggested that the carboxylic acid was not critical for activity provided that the steric bulk was not significantly increased.[6] There have been no reports of ambruticin analogues since this time. In this communication we report our own investigations into analogues of the carboxylic acid in the VS series. In particular, the carboxylic acid has been replaced either by an amine, oxime, nitrile, tetrazole, or has been deleted. The antifungal activities of these compounds suggest that some polarity at this position is necessary for antifungal activity, and as was reported in the S series, increasing the steric bulk at this position reduces potency.

We chose to concentrate on the amine containing VS series because of its superior antifungal activity. VS-3 **4** was our initial starting material since the 5-amino group required no further protection. Studies began by investigating the effect of amines and oximes at the 1-position. Thus, conversion to the methyl ester **5** followed by controlled reduction yielded the aldehyde **6**, a versatile intermediate, which was immediately used in the subsequent reactions because of its instability.

Treatment of **6** with a selection of amines under reductive conditions resulted in the corresponding amino compounds **7**–**10** (Scheme 1) in low to moderate yields. The free amine and azetidine derivatives probe the effect of placing a small amino group at this position while the two proline derivatives probe the effects of repositioning the carboxylic acid.

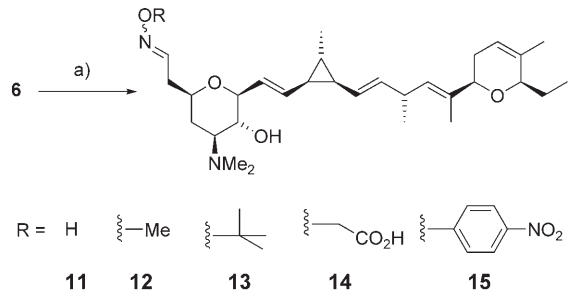



**Scheme 1.** Synthesis of ambruticin VS-3 amines; reagents and conditions: a) $SOCl_2$, MeOH, room temperature, 16 h, 89%; b) DIBAL-H, toluene, −78°C, 5 min, quantitative; c) $R^1R^2NH$, $Na(CN)BH_3$, AcOH, MeOH, room temperature, 16 h, 25-40%. DIBAL-H = diisobutylaluminium hydride, Ac = acetic.

The oxime derivatives **11**–**15** were obtained as mixtures of *E* and *Z* isomers in a single step by reaction with the corresponding hydroxylamines under standard acidic conditions (Scheme 2). The isomers were not separated. The free oxime **11** and alkyl oximes were synthesised along with one aryl oxime.

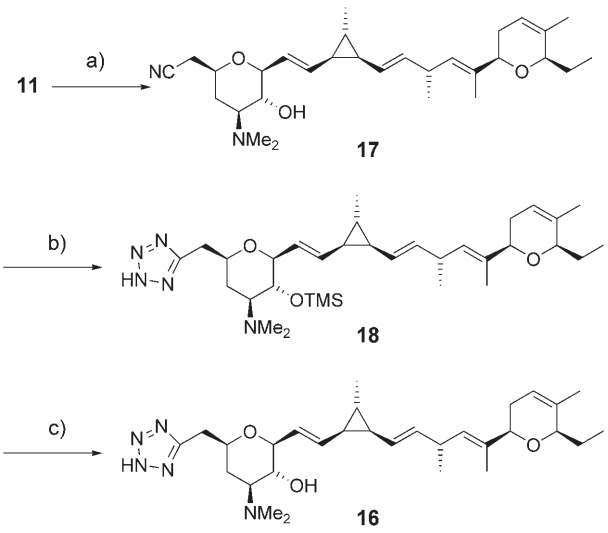
[a] *Y. Xu, Z. Wang, Dr. Z.-Q. Tian, Dr. Y. Li, Dr. S. J. Shaw*
*Department of Chemistry,*
*Kosan Biosciences, Inc.,*
*3832 Bay Center Place,*
*Hayward, California 94545 (USA)*
*Fax: (+1) 510-732-8401*
*E-mail: shaw@kosan.com*



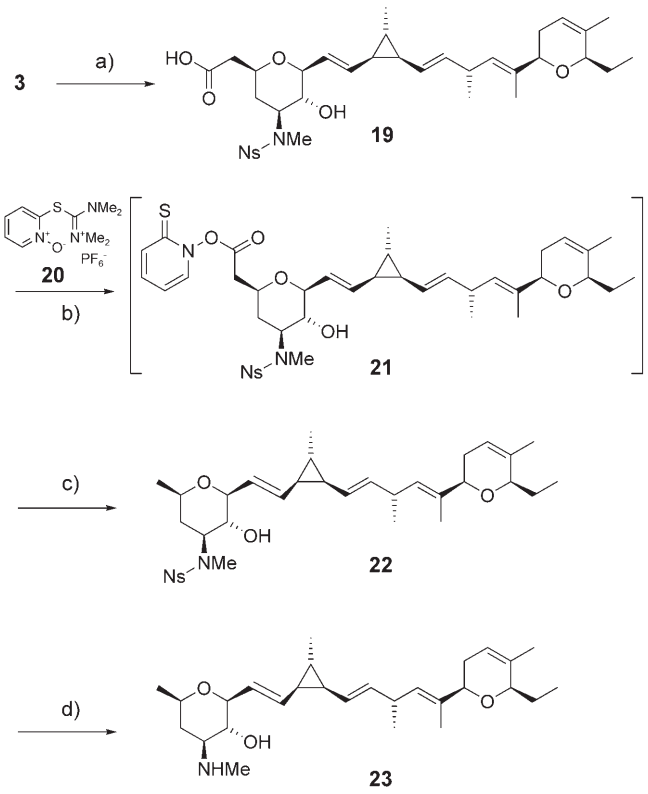

**Scheme 2.** Synthesis of ambruticin VS-3 oximes; reagents and conditions: a) $RONH_2$, AcOH, *i*PrOH, room temperature, 16 h, 28–50%

With the free oxime in hand we generated the tetrazole **16**, a common carboxylic acid isostere. Dehydration of **11** cleanly yielded the nitrile **17**,[8] which did not react with TMS azide alone. However; in the presence of catalytic dibutyl tin oxide, [3+2] dipolar cycloaddition occurs cleanly to yield the silyl protected tetrazole **18**.[9] The silyl group was removed under acidic conditions to obtain **16** (Scheme 3). No reaction with the four double bonds was observed.




**Scheme 3.** Synthesis of ambruticin VS-3 nitrile **17** and tetrazole **16**; reagents and conditions: a) $HOReO_3$, toluene, 110 °C, 16 h, 78%; b) $TMSN_3$, $Bu_2SnO$, toluene, 110 °C, 6 h; c) 5% AcOH in $CH_3CN$-$H_2O$, room temperature, 1 h, 80% (over 2 steps). TMS = trimethylsilyl.

The compounds generated probe the effects of converting the carboxylic acid to other functionalities. Strikingly all the compounds contain a functional group at the 1-position. We considered the possibility of removing this group altogether through decarboxylation by the Barton ester.[10] While we were aware that attempting a radical reaction in the presence of many double bonds might be problematic, it was thought that the pyran ring might isolate the carboxylate from the remainder of the molecule. The conversion was attempted using VS-4 **3** which was protected as a nosylate **19** under standard conditions.[11] Barton ester formation was difficult to achieve under standard DCC conditions, however, using the more reactive HOTT reagent **20** developed by Garner,[12] the Barton ester **21** was formed and directly treated with tributyltin hydride and catalytic AIBN to yield decarboxylated **22**, which was deprotected with thiophenol to yield the 1-nor compound **23** (Scheme 4). Although the conversion is poor this appears to be more a result of the poor formation of the Barton ester rather than the radical decarboxylation.




**Scheme 4.** Synthesis of 1-norambruticin VS-4 **23**; reagents and conditions: a) NsCl, $Et_3N$, DMAP, $CH_2Cl_2$, room temperature, 16 h, 85%; b) THF, room temperature, 3 h; c) $Bu_3SnH$, AIBN, $C_6H_6$, 80 °C, 30 min, 24% (over 2 steps); d) PhSH, $Cs_2CO_3$, $CH_3CN$, room temperature, 1 h, 55%. Ns = 2-nitrophenylsulfonyl, DMAP = 4-dimethylaminopyridine, THF = tetrahydrofuran, AIBN = 2,2′-azabisisobutyronitrile.

The molecules were screened for their antifungal activity against two *Aspergillus* strains to determine minimum inhibitory concentrations (MICs) (Table 1).[13] It can be seen that replacing the carboxylic acid with an amino group as either the free amine **7** or azetidine **8** does not result in active compounds. By reintroducing a carboxylic acid in the case of the two proline derivatives **9** and **10** there is an improvement in the potency of the molecule, however, these remain an order of magnitude weaker than the parent ambruticin VS-3 **4**. By contrast conversion of the carboxylic acid to the free oxime **11** maintains good potency. As the size of the substituent on the oxime is increased, the activity decreases suggesting that there is a steric constraint at this position similar to that previously observed. Comparing the free oxime **11** with the oxime acetic acid **14** suggests that the carboxylic acid itself is not required for activity. While the nitrile **17** shows only a weak antifungal

**Table 1.** MIC values in $\mu g\,mL^{-1}$.

| Compd | Description | MIC [$\mu g\,mL^{-1}$] | |
|---|---|---|---|
| | | *A. fumigatus* 204305 | *A. flavus* 204304 |
| **1** | S | 1.0 | 1.0 |
| **2** | VS-5 | 1.15 | 1.69 |
| **3** | VS-4 | 0.47 | 0.60 |
| **4** | VS-3 | 0.80 | 0.50 |
| **5** | VS-3 Me ester | 3 | 2 |
| **7** | VS-3 amine | 16 | 12 |
| **8** | VS-3 azetidine | >32 | >32 |
| **9** | VS-3 L-proline | 8 | 4 |
| **10** | VS-3 D-proline | 12 | 4 |
| **11** | VS-3 oxime | 1.5 | 1.5 |
| **12** | VS-3 Me oxime | 2 | 2 |
| **13** | VS-3 *t*Bu oxime | 8 | 16 |
| **14** | VS-3 oxime acetic acid | 3 | 1.5 |
| **15** | VS-3 aryl oxime | 16 | >32 |
| **17** | VS-3 nitrile | 4 | 3 |
| **16** | VS-3 tetrazole | 1.5 | 1.5 |
| **23** | VS-4 1-nor | 6 | 4 |

activity, the tetrazole **16** is a potent compound showing activity within a dilution of the natural product. The 1-nor compound **23** is weaker than the nitrile **17**, consistent with the requirement for a polar group at this position for good potency.

It has been postulated that the ambruticins are serum bound in vivo, which reduces their efficacy.[6] Indeed, the addition of 10% serum to our MIC assay resulted in a 6- to 32-fold decrease in potency for the natural ambruticins. It was hoped that the ambruticin analogues discussed above would have different physical properties that would overcome the serum effect. However, it was disappointing to find that in the presence of 10% serum the two most potent analogues, the oxime **11** and tetrazole **16** showed a similar 6- and 8-fold rise in MIC respectively.

In conclusion, we have generated 13 analogues based on the ambruticin VS scaffold to probe the effects of modification of the carboxylic acid found at the 1-position, on antifungal activity and potentially, in vivo efficacy. The results suggest that replacement of the acid with a positively charged amine results in inactive compounds; however, replacement with other polar groups is possible. Indeed the free oxime **11** and tetrazole **16** show similar activities to the parent compound itself. As the polarity is further reduced, as in the cases of the nitrile **17** and 1-nor compound **23,** there is a lowering in antifungal activity compared to the natural products. Consistent with the results of the Warner-Lambert group, there appears to be a steric constraint in this region of the molecule, as increased substituent size corresponds to a decrease in the potency of the molecules. This is best exemplified by the oxime series.

Although the results show that the carboxylic acid is not required for activity and can be replaced with small polar functionalities, these changes have not been able to address the reduction in activity in the presence of serum. The best analogues are still significantly weaker in the presence of serum. Further work will be required to investigate this further.

## *Acknowledgements*

*We would like to thank Leandro Vecher for carrying out antifungal MIC assays, both John R. Carney and Chau Q. Tran for analytical data, and Gary W. Ashley for useful discussions.*

**Keywords:** ambruticin · analogues · antifungal agents · semisynthesis · structure–activity relationships

[1] V. T. Andriole, *J. Antimicrob. Chemother.* **1999**, *44*, 151 – 162.
[2] a) S. M. Ringel, R. C. Greenough, S. Roemer, D. Connor, A. L. Gutt, B. Blair, G. Kanter, M. von Strandtmann, *J. Antibiot.* **1977**, *30*, 371 – 375; b) G. Höfle, H. Steinmetz, K. Gerth, H. Reichbach, *Liebigs Ann. Chem.* **1991**, *91*, 941 – 945.
[3] P. Knauth, H. Reichenbach, *J. Antibiot.* **2000**, *53*, 1182 – 1190.
[4] A. S. Kende, Y. Fujii, J. S. Mendoza, *J. Am. Chem. Soc.* **1990**, *112*, 9645 – 9646.
[5] a) For a review see V. Michelet, J.-P. Genet, *Curr. Org. Chem.* **2005**, *9*, 405 – 418; b) P. Liu, E. N. Jacobsen, *J. Am. Chem. Soc.* **2001**, *123*, 10772 – 10773; c) T. A. Kirkland, J. Colucci, L. S. Geraci, M. A. Marx, M. Schneider, D. E. Kaelin, Jr, S. F. Martin, *J. Am. Chem. Soc.* **2001**, *123*, 12432 – 12433; d) E. Lee, S. J. Choi, H. Kim, H. O. Han, Y. K. Kim, S. J. Min, S. H. Son, S. M. Lim, W. S. Jang, *Angew. Chem.* **2002**, *114*, 184 – 186; *Angew. Chem. Int. Ed.* **2002**, *41*, 176 – 178.
[6] D. T. Connor, M. von Strandtmann, *J. Med. Chem.* **1979**, *22*, 1144 – 1147.
[7] D. T. Connor, M. von Strandtmann, *J. Med. Chem.* **1979**, *22*, 1055 – 1059.
[8] K. Ishihara, Y. Furuya, H. Yamamoto, *Angew. Chem.* **2002**, *114*, 3109 – 3112; *Angew. Chem. Int. Ed.* **2002**, *41*, 2983 – 2986.
[9] S. J. Wittenberger, B. G. Donner, *J. Org. Chem.* **1993**, *58*, 4139 – 4141.
[10] a) D. H. R. Barton, D. Crich, W. B. Motherwell, *Tetrahedron Lett.* **1983**, *24*, 4979 – 4982; b) D. H. R. Barton, D. Crich, W. B. Motherwell, *Tetrahedron* **1985**, *41*, 3901 – 3924.
[11] T. Fukuyama, C.-K. Jow, M. Cheung, *Tetrahedron Lett.* **1995**, *36*, 6373 – 6374.
[12] P. Garner, J. T. Anderson, S. Dey, W. J. Youngs, K. Galat, *J. Org. Chem.* **1998**, *63*, 5732 – 5733.
[13] NCCLS. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi; Approved Standard. Document M38A [ISBN 1-56238-470-8]: National Committee for Clinical Laboratory Standards, Pennsylvania, USA, **2002**.

Received: June 23, 2006
Published online on August 24, 2006