Published in final edited form as:

Org Lett. 2009 July 2; 11(13): 2936–2939. doi:10.1021/ol901054r.

Synthesis of the Isoxazolo[4,3,2-*de*]phenanthridinone Moiety of the Parnafungins

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Abstract

A practical route to the labile tetracyclic isoxazolo[4,3,2-*de*]phenanthridinone moiety of the antifungal parnafungins has been developed. Zinc reduction of a methyl 2'-hydroxymethyl-2-nitro-3-biphenylcarboxylate, which was prepared by a Suzuki coupling, afforded a benzisoxazolone that was treated with MsCl and base to generate the labile tetracyclic ring system in 37–47% yield. This compound decomposes to the phenanthridine in CDCl_3 and the phenanthridine *N*-oxide in aqueous base.

A Merck group recently reported the isolation of parnafungins A1 (**1a**), A2 (**1b**), B1 (**2a**) and B2 (**2b**) that interconvert readily by an elimination that opens the xanthone ring to form an enone and a phenol and then a conjugate addition from either phenol OH group to either face of the enone to reform the xanthone (see Scheme 1).¹ The parnafungins demonstrate broad spectrum antifungal activity with no antibacterial activity by inhibiting fungal polyadenosine polymerase (PAP) and show *in vivo* efficacy against *Candida albicans* in a mouse model.^{1b} Affinity selection/mass spectrometry demonstrated that the linear parnafungin A binds preferentially to PAP.^{1c} The *O*-methylated parnafungins C and D were recently isolated.^{1d}

The isoxazolone ring of **1** and **2** is very labile. Treatment of **1** and **2** at neutral or basic pH generated phenanthridines **3** and **4**, respectively, in less than 1 hour. The same compounds were

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Supporting Information Available Complete experimental procedures, copies of ^1H and ^{13}C NMR spectral data. This material is available free of charge via the Internet at <http://pubs.acs.org>.

generated over 10–20 hours at pH 3. Unfortunately, phenanthridines **3** and **4** are biologically inactive.^{1a}

The parnafungins might be biosynthesized by oxidative coupling of **5** with anthranilic acid to give **6** (see Scheme 2). Further oxidation of the benzylic methyl group and aniline could give parnafungins A1 (**1a**) and A2 (**1b**). Blenolide C (**5b**) was recently isolated² and numerous natural products are known that are derived from blenolide C by oxidative dimerization or coupling at the carbon marked by an asterisk. Bräse³ and Nicolaou⁴ have recently reported syntheses of blenolide C (**5b**).

We therefore decided to start our synthetic studies by developing a route to the tetracyclic isoxazolo[4,3,2-*de*]phenanthridinone moiety **10** that can then be extended to the synthesis of the parnafungins by starting with blenolide C or an analog. We were guided by the studies of Wierenga⁵ that were based on earlier work of Bamberger⁶ and Cohen.⁷ Wierenga reported that reduction of methyl *o*-nitrobenzoate (**7**) with zinc, NH₄Cl, and Na₂CO₃ in MeOH containing formaldehyde afforded hydroxymethylbenzisoxazolone (**9**) in 44% yield (see Scheme 3). A similar reduction without formaldehyde gave **8** in low yield, which could be converted to **9** by reaction with basic formaldehyde.

Our retrosynthesis of **10** is shown in Scheme 4. An intramolecular Friedel-Crafts alkylation of **11** could give **10**. Hydroxymethylbenzisoxazolone **11** should be accessible by Wierenga's procedure from biphenylcarboxylate **12**, which has been prepared by Liu from methyl 3-chloro-2-nitrobenzoate (**16**) by Suzuki coupling with phenylboronic acid (**15**, R = H).⁸ However, the Friedel-Crafts reaction may not work well with an unactivated aromatic ring and this route differs from the proposed biosynthesis in which the ring is closed by C-N rather than C-C bond formation. We therefore considered an alternate approach in which the C-N bond of **10** is formed from **13** by an intramolecular S_N2 reaction. Suzuki coupling of **16** with the appropriate boronic acid **15**, R = CH₂OH, should provide **14**, which should form **13** by zinc reduction without formaldehyde.

Suzuki coupling of **16** and phenylboronic acid (**17a**) as described by Liu afforded **12** in 65% yield (see Scheme 5). The yield was improved to 99% using SPhos, Pd(OAc)₂ and K₃PO₄ in wet THF.⁹ Reduction of **12** with Zn and NH₄Cl in THF/MeOH/H₂O with sonication for 30 minutes afforded a 3:1 mixture of the desired benzisoxazolone **19a** and amino ester **20a**. Benzisoxazolone **19a** decomposed on chromatography, but it could be obtained in 60% yield by washing the mixture with 9:1 hexanes/Et₂O to remove **20a**. Reaction of **19a** with formaldehyde and Na₂CO₃ in aqueous THF afforded the desired hydroxymethylbenzisoxazolone **11** in 61% yield. This two-step sequence gave pure **11**; impure **11** was obtained in one step by reduction of **12** with Zn and formaldehyde. Unfortunately, all attempts to form **10** by intramolecular Friedel-Crafts alkylation on the phenyl ring of **11** were unsuccessful.

We then turned to a more electron rich aromatic ring to see if the Friedel-Crafts alkylation would work with an optimized substrate. The analogous Suzuki coupling of **16** with 3,5-dimethoxyphenylboronic acid (**17b**) gave **18b** in 99% yield. Zinc reduction gave **19b** in 66% yield, which was treated with formaldehyde to give **21b** in 76% yield. We were delighted to find that treatment of **21b** with 10% TFA in CH₂Cl₂ generated an iminium cation that added to the aromatic ring to give **22b** in 27% yield. Although this route to **22b** provided the first synthesis of an isoxazolo[4,3,2-*de*]phenanthridinone, it is not applicable to the synthesis of the parnafungins because the Friedel-Crafts reaction would have to occur meta to the oxygen substituents and para to the carbonyl group. We therefore turned our attention to the alternate route using C-N bond formation.

The analogous Suzuki coupling of **16** with 2-hydroxymethylphenylboronic acid (**23a**) afforded biphenyl **24a** in 95% yield (see Scheme 6). Zinc reduction afforded a mixture of benzisoxazolone **25a** and the amino ester analogous to **20**. Treatment of this mixture with MsCl and Et₃N in CH₂Cl₂ for 15 min at 0 °C afforded the mesylate of **25a**, which was treated with Na₂CO₃ in 1:1 THF/H₂O for 40 minutes to give the desired tetracyclic isoxazolo[4,3,2-*de*] phenanthridinone moiety **10** in 37% yield from **24a**.

Application of this route to a coupling product of blenolide C (**5b**) would require functionalization of a benzylic methyl group. We therefore prepared **24b** in 95% yield by Suzuki coupling of **16** with *o*-tolylboronic acid (**23b**). Bromination of **24b** with NBS and (BzO)₂ in CCl₄ at reflux gave **24d**, R = H, X = Br. Reaction of **24d** with KOAc in DMF afforded **24e**, R = H, X = OAc, which was hydrolyzed with K₂CO₃ in MeOH to give **24a** in 79% overall yield from **24b**. Other approaches were unsuccessful. Reduction of **24d** with Zn gave **25b** resulting from reduction of both the nitro and bromomethyl groups. Functionalization of the methyl group must be carried out before formation of the benzisoxazolone ring. Zn reduction of **24b** gave **25b** in 62% yield, which decomposed on treatment with NBS.

Suzuki coupling of **16** with boronic acid **23c**¹⁰ provided biphenyl **24c** (83%) with the oxygen substituents on the same carbons as in the parnafungins. Zinc reduction gave benzisoxazolone **25c**, which was treated with MsCl and Et₃N to give the mesylate. Treatment of the mesylate with Na₂CO₃ in 1:1 THF/H₂O provided **26c** in 47% overall yield from **24c**. This sequence provides a very short and high yield route to the labile tetracyclic isoxazolo[4,3,2-*de*] phenanthridinone moiety of the parnafungins that should be applicable to the synthesis of the natural product.

The Merck group suggested that the rearrangement of parnafungins **1** and **2** to **3** and **4**, respectively, could occur by either an E2 reaction or by hydrolysis of the isoxazolone followed by loss of water. We explored the stability of **10**, **22b** and **26c** in CDCl₃. All three compounds rearranged cleanly to the respective phenanthridine **27a**,¹¹ **27b** and **27c** without any evidence for the formation of an intermediate (see Scheme 7). This indicates either that the reaction proceeds by an E2 elimination or that loss of water is much faster than hydrolysis of the isoxazolone. Tetracycle **26c** with the oxygen substituents in the same position as the parnafungins rearranges fastest with a half life of 6 days. Tetracycle **10** rearranges with a half life of 10 days and tetracycle **22b** rearranges slowest with a half life of 27 days. The C-methyl group of *m*-methoxytoluene is deprotonated twice as fast as that of toluene by lithium amide bases, whereas the C-methyl group of *o*-methoxytoluene is deprotonated ten times slower than that of toluene.¹² Deprotonation occurs in the rate determining step of an E2 elimination even if protonation of the carbonyl group by adventitious HCl is the initial step. Therefore, the two methoxy groups meta to the methylene group of **26c** should accelerate deprotonation and E2 elimination, whereas the methoxy groups ortho and para to the methylene group of **22b** should retard deprotonation and elimination. The parnafungins have oxygen substituents meta to the methylene group as in **26c** and a carbonyl group para to the methylene group, which should further increase its acidity and accelerate the E2 elimination.

We also explored the decomposition of **10** under basic condition with NaOD in CD₃CN/D₂O. Under these conditions, we detected an intermediate with the CH₂ group shifted upfield to δ 4.32 from δ 4.72 in **10**. We tentatively assigned structure **28** to this intermediate (see Scheme 8). To our surprise, the final product is not **27a**, but a 1:10 mixture of **27a** and the *N*-oxide **29** resulting from oxidation of **28**, presumably by air. The autoxidation of hydroxylamine in aqueous base has been extensively studied¹³ and the oxidation of alkylhydroxylamines to oximes in MeOH has been reported.¹⁴ The formation of **29** by this decomposition pathway is noteworthy because we were unable to prepare **29** directly by oxidation of **27a** using procedures

that work well on phenanthridine itself. Both electron withdrawing groups and peri substituents are known to retard the *N*-oxidation of heterocycles.¹⁵

In conclusion, a practical route to the labile tetracyclic isoxazolo[4,3,2-*de*]phenanthridinone moiety (**10** and **26c**) of the antifungal parnafungins has been developed. Zinc reduction of methyl 2'-hydroxymethyl-2-nitro-3-biphenylcarboxylates **24a** and **24c**, which were prepared by Suzuki couplings, afforded benzisoxazolones **25a** and **25c** that were treated with MsCl and then base to generate the labile tetracyclic ring systems **10** (37%) and **26c** (47%). These compounds rearrange to phenanthridines **27a** and **27c** in CDCl₃ and **10** decomposes to phenanthridine *N*-oxide **29** in aqueous base.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgment

We are grateful to the National Institutes of Health (GM-50151) for support of this work. We thank Professor Stephen Buchwald, Massachusetts Institute of Technology for advice on Suzuki couplings.

References

1. (a) Parish CA, Smith SK, Calati K, Zink D, Wilson K, Roemer T, Jiang B, Xu D, Bills G, Platas G, Peláez F, Díez MT, Tsou N, McKeown AE, Ball RG, Powles MA, Yeung L, Liberator P, Harris G. J. Am. Chem. Soc 2008;130:7060–7066. [PubMed: 18461935] (b) Jiang B, Xu D, Allocco J, Parish C, Davison J, Veillette K, Sillaots S, Hu W, Rodriguez-Suarez R, Trosok S, Zhang L, Li Y, Rahkhoodae F, Ransom T, Martel N, Wang H, Gauvin D, Wiltse J, Wisniewski D, Salowe S, Kahn JN, Hsu M-J, Giacobbe R, Abruzzo G, Flattery A, Gill C, Youngman P, Wilson K, Bills G, Platas G, Pelaez F, Diez MT, Kauffman S, Becker J, Harris G, Liberator P, Roemer T. Chem. Biol 2008;15:363–374. [PubMed: 18420143] (c) Adam GC, Parish CA, Wisniewski D, Meng J, Liu M, Calati K, Stein BD, Athanasopoulos J, Liberator P, Roemer T, Harris G, Chapman KT. J. Am. Chem. Soc 2008;130:16704–16710. [PubMed: 19049464] (d) Overy D, Calati K, Kahn JN, Hsu M-J, Martín J, Collado J, Roemer T, Harris G, Parish CA. Bioorg. Med. Chem. Lett 2009;19:1224–1227. [PubMed: 19147347]
2. Zhang W, Krohn K, Zia-Ullah, Flörke U, Pescitelli G, Di Bari L, Antus S, Kurtán T, Rheinheimer J, Draeger S, Schulz B. Chem. Eur. J 2008;14:4913–4923.
3. (a) Nising CF, Ohnemüller (née Schmid) UK, Bräse S. Angew. Chem. Int. Ed 2006;45:307–309. (b) Gérard EMC, Bräse S. Chem. Eur. J 2008;14:8086–8089.
4. Nicolaou KC, Li A. Angew. Chem. Int. Ed 2008;47:6579–6582.
5. (a) Wierenga W, Harrison AW, Evans BR, Chidester CG. J. Org. Chem 1984;49:438–442. (b) Wierenga W, Evans BR, Zurenko GE. J. Med. Chem 1984;27:1212–1215. [PubMed: 6471074]
6. Bamberger E, Pyman FL. Ber 1909;42:2297–2330.
7. Cohen T, Gray WF. J. Org. Chem 1972;37:741–744.
8. Liu B, Moffett KK, Joseph RW, Dorsey BD. Tetrahedron Lett 2005;46:1779–1782.
9. (a) Walker SD, Barder TE, Martinelli JR, Buchwald SL. Angew. Chem. Int. Ed 2004;43:1871–1876. (b) Barder TE, Walker SD, Martinelli JR, Buchwald SL. J. Am. Chem. Soc 2005;127:4685–4696. [PubMed: 15796535]
10. Tan Y-L, White AJP, Widdowson DA, Wilhelm R, Williams DJ. J. Chem. Soc., Perkin Trans. 1 2001:3269–3280.
11. (a) Sengupta D, Anand N. Ind. J. Chem 1985;24B:923–927. (b) Atwell GJ, Baguley BC, Denny WA. J. Med. Chem 1988;31:774–779. [PubMed: 3351855]
12. (a) Streitwieser A Jr, Koch HF. J. Am. Chem. Soc 1964;86:404–409. (b) Schlosser M, Maccaroni P, Marzi E. Tetrahedron 1998;54:2763–2770.
13. Hughes MN, Nicklin HG. J. Chem. Soc. A 1971:164–168.

14. Horiyama S, Suwa K, Yamaki M, Kataoka H, Katagi T, Takayama M, Takeuchi T. Chem. Pharm. Bull 2002;50:996–1000. [PubMed: 12130865]
15. Katritzky, AR.; Lagowski, JM. Chemistry of the Heterocyclic N- Oxides. Academic Press Inc.; New York: 1971. p. 67

Scheme 2.
Possible Biosynthesis of the Parnafungins

Scheme 3.
Wierenga Benzisoxazolone Synthesis

Scheme 4.
Retrosynthesis of Tetracycline **10**

Scheme 5.
Synthesis of Tetracycle **22b**

Scheme 6.
Synthesis of Tetracycles **10** and **26c**

Scheme 7.
Rearrangement of **10**, **22b** and **26c** in CDCl_3

Scheme 8.
Decomposition of **10** in Base