Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 13, pp. 1747-1752, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)00307-7

SYNTHESIS AND IN VITRO ANTIBACTERIAL ACTIVITIES OF NOVEL CONFORMATIONALLY RESTRICTED HYGROMYCIN A ANALOGUES[1]

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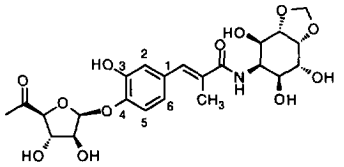
Abstract: The preparation of semisynthetic conformationally restricted hygromycin A analogues are described. Antibacterial results from these compounds suggest active conformations for this class of agents.

Hygromycin A is a fermentation derived natural product first isolated from a *Streptomyces hygroscopicus* culture in 1953.[2] While known to possess moderate antibacterial activity, it has only recently demonstrated excellent activity in vitro against the bacterium *Serpulina* (*Treponema*) *hyodysenteriae*, the causative agent of swine dysentery.[3] Furthermore, oral therapeutic efficacy has been observed for this antibacterial in induced swine dysentery studies at doses of 5 to 20 grams/ton feed.[4]

Over the past five years, several research groups have reported their examinations of synthetic approaches to hygromycin A and related semi-synthetic analogues. In 1991, Chida and coworkers disclosed a total synthesis of this natural product,[5] and more recently, our laboratories have detailed synthetic contributions to the structure-activity relationship (SAR) of sugar,[6] aromatic ring,[7] enamide,[8] and aminocyclitol analogues.[9] This communication details the SAR from unusual substituted aromatic analogues, and further describes the synthesis and biological activities of novel, conformationally restricted hygromycin A analogues.

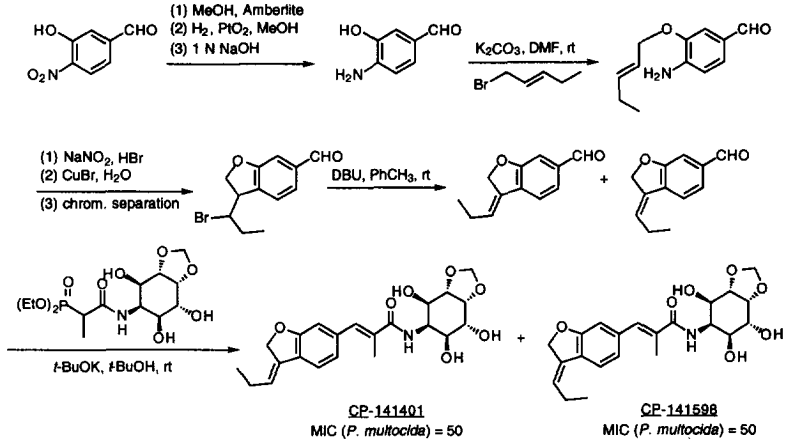


Hygromycin A

As previously reported, a number of hygromycin A analogues bearing small, electron-rich substituents at C3 confer antibacterial activity in vitro. Somewhat surprising, however, is the lack of biological activity for several hydroxyl and amine bioisosteres that were evaluated (ethers, substituted amines, and amides). We theorized that steric constraints at or near the C3-C4 molecular binding domain for these analogues might diminish their inhibitory activity. This hypothesis was also consistent with the apparent correlation between van der Waal radius and biological activity observed for the C3-halogen subseries.[7] We elected to prepare several C3-C4 bicyclic analogues to determine if synthetically "tethering" the C3 substituent would restore antibacterial activity. The synthesis and biological[10] activities for these derivatives are presented in Figures 1 and 2 on the following page. Alkylation of 3-hydroxy-4-aminobenzaldehyde proceeded smoothly to provide the O-alkylated aniline only. Conversion of this material to the diazonium bromide, followed by cuprous bromide-mediated decomposition lead to the intramolecular trapping of the radicaloid species and production of both diastereomeric pairs of 3-(1-bromopropyl)dihydrobenzofurans.[11] These racemates could be separated by silica gel chromatography and

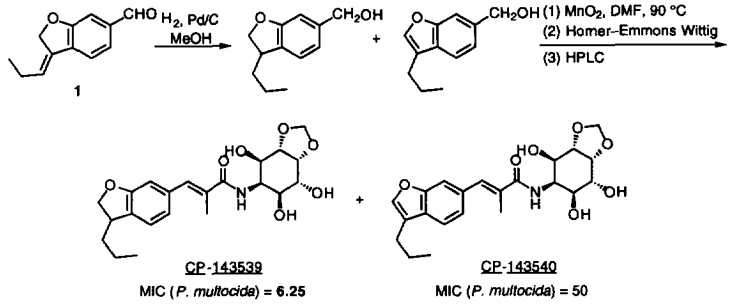
independently subjected to dehydrobromination conditions. Horner–Emmons Wittig olefination of the two 3-propylidenyldihydrobenzofuran aldehydes provided CP-141401 and CP-141598.

Figure 1. Synthesis and biological activities of 3'-vinyldihydrobenzofuran hygromycin A analogues.



The activities observed for these analogues, while modest, were nevertheless intriguing. As both double bond isomers demonstrated identical potencies in vitro, the enzyme target appears somewhat tolerant of variations in steric bulk within the subdomain associated with the tether portion of these compounds. This molecular "fit", however, was presumed to be poorer than that of the more potent C4 alkyl ethers, such as the C4 allyl ethers—present in a number of active analogues—which can enjoy free rotation about the three carbon sidechain. We therefore sought to examine the effect of saturation of the dihydrobenzofuran side chain, allowing free rotation about all three tether carbons to occur. Two representative saturated analogues that could potentially be approached by our synthetic route to CP-141401 and CP-141598 were the 3-propylbenzofuran and the 3-propyldihydrobenzofuran. Serendipitously, in our attempt to fully saturate the *exo*-vinyl aldehyde **1**, we obtained a small percentage of isomerized, aromatized material as well.[12] This inseparable mixture of alcohols was oxidized, olefinated, and ultimately separated by HPLC to give CP-143539 and CP-143540 as shown in Figure 2 below.

Figure 2. Preparation and biological activities of 3-propyl- and 3-propyldihydrobenzofurans.

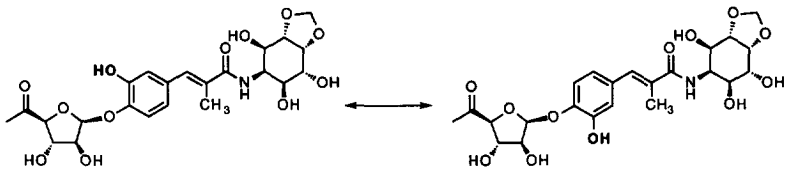


As anticipated, the improved antibacterial activity observed for CP-143539 mirrors that noted for the C3-hydroxy-C4-propyl ether analogue, thus confirming the hypothesis that steric hindrance at or near the C3-C4 binding subdomain is responsible for the loss of activity with certain C3-substituted aromatic analogues.

α-Methylcaffeic Acid Binding Orientation:

In 1980, Guerrero and Modolell proposed a mechanism of action for the antibiotic activity of hygromycin A. Through enzyme competition experiments involving the RNA pentamer CACCA-[^{3}H]Leu, as well as the known antibacterials [^{14}C]chloramphenicol, and [^{14}C]lincomycin, these workers concluded that hygromycin A inhibited protein synthesis through competitive inhibition of prokaryotic ribosomyl peptidyl transferase, preferentially binding at the P site for this enzyme.[13] Furthermore, the unsymmetrical aromatic substitution pattern observed for hygromycin A suggested that optimum enzyme inhibition, and hence, antibacterial activity, may be confined to a preferred rotational isomer. In general terms, the two rotational extremes ($\varphi = 0°$, 180°) are depicted in Figure 3 below.

Figure 3. Possible hygromycin A/analogue "preferred" enzyme binding rotational isomers.



We elected to synthesize a selected series of conformationally restricted analogues to examine the rotational requirements, if any, of hygromycin A/analogue antibacterial activity. The synthetic routes to our initial pair of conformers are described in Figures 4 and 5 below; the in vitro antibacterial activities for these species are presented in Table 1 following.

Figure 4. Preparation of 6,7-disubstituted-3,4-dihydro-naphthoic acid analogue.

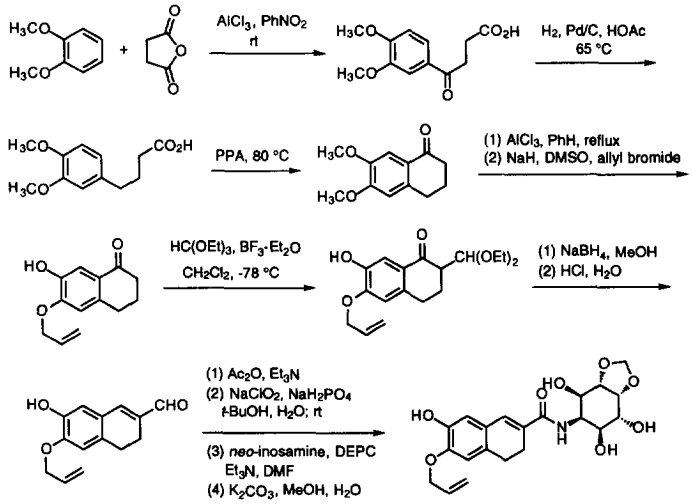


Figure 5. Preparation of 5,6-disubstituted-3,4-dihydro-naphthoic acid analogue.

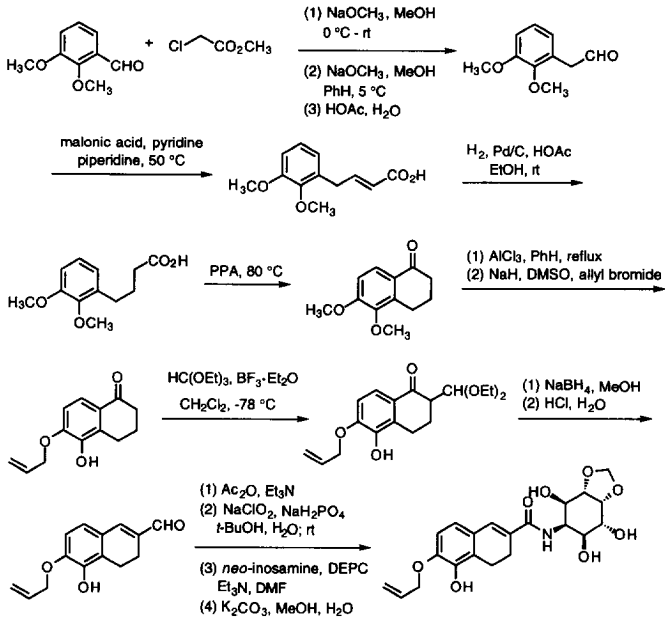
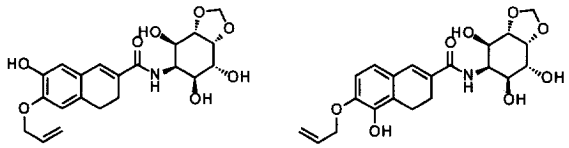


Table 1. In vitro antibacterial activity: 3,4-dihydronaphthoic acid amides.



S. hyodysenteriae **MIC (μg/mL)**	**6.25-12.5**	**>200**
P. multocida **MIC (μg/mL)**	**12.5**	**>200**

The antibacterial activity data described above strongly suggests a preferred enzyme binding conformation for hygromycin A and related analogues in vitro. Several additional fused analogues were prepared to examine the synthetic scope of this analogue subseries. The conformationally restricted derivatives, while active, did not demonstrate improved biological activity in vitro relative to the natural product. The syntheses and biological activities for these agents are detailed in Figure 6 and Table 2 below.

Figure 6. Preparation of additional bicyclic hygromycin A analogues.

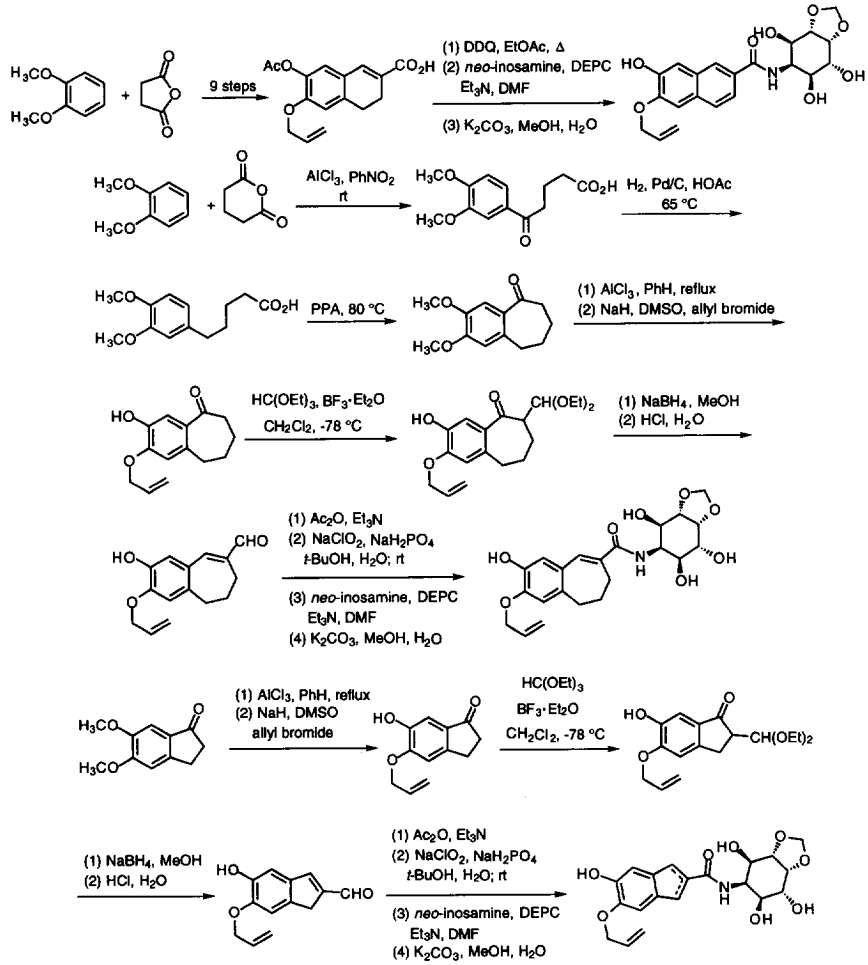
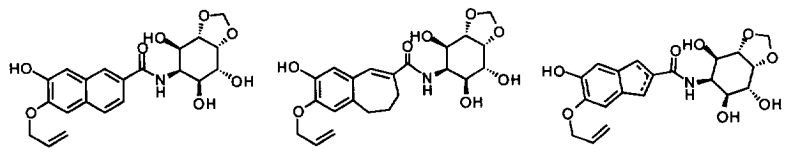


Table 2. In vitro antibacterial activity: additional conformationally restricted analogues.



S. hyodysenteriae **MIC (μg/mL)**	**50**	**25**	**>200**
P. multocida **MIC (μg/mL)**	**50**	**25**	**>200**

Our analogue program indicates that steric hindrance at or near the C3-C4 binding subdomain of hygromycin A appears responsible for the loss of activity noted with certain C3-substituted aromatic analogues.

Although the intrinsic antibacterial activity observed for the "active" dihydronaphthoic acid amide is not superior to the monocyclic C3-hydroxy-C4-allyloxy analogue, the results from both conformationally restricted analogues suggests a pharmacophore model for antibacterial activity within this series. In addition, the activities observed for the naphthalene analogue and the benzocycloheptene derivative serve to reinforce the original premise that free rotation between the arene and the methacrylamide linkage is not required for antibacterial activity within this subseries. Ring expansion of the fused ring system does not appear to offer any advantages over the simpler 6-6 system, and oxidation of the dihydronaphthoamide to the aromatic naphthalene analogue only *marginally* reduces its antibacterial activity, indicating that conjugation of the aromatic system and the amide group is tolerated for this series. Finally, the inactivity of the indenyl analogue[14] is not surprising in view of the drastic changes in the three-dimensional orientation of the aromatic system relative to the aminocyclitol region.

Acknowledgements. HPLC separations were performed by D. A. Koss. In vitro data was provided by Dr. S. F. Hayashi, L. J. LaFleur, and S. B. Seibel. In vivo data was provided by Dr. B. J. Kamicker, and M. A. LeMay (Terre Haute).

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10. *P. multocida* MIC's were measured in a liquid microtiter serial dilution assay; *S. hyodysenteriae* MIC's were determined as in Weber, F. H.; Earley, D. L. *Antimicrob. Agents Chemother.* **1991**, *35*, 2012.
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14. In the course of preparing the indene carboxylic acid, equilibration of the unsaturation resulted in a 1:1 mixture of substituted indenes. The resulting two regioisomeric amide products were virtually inseparable, and thus the mixture was tested as is.

(*Received in USA* 22 *April* 1997; *accepted* 20 *May* 1997)