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Total Synthesis and Semi-Synthetic Approaches to Analogues of Antibacterial Natural Product Althiomycin

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Abstract—Numerous analogues of the naturally occurring antibiotic althiomycin have been synthesised exploiting both total- and semi-synthetic methodologies. The antibacterial activity of these derivatives has been determined in whole cell assays and indicates the natural product exhibits a restricted SAR.

Althiomycin (**1**) is an antibiotic, isolated in 1957 from *Streptomyces althioticus*,[1] whose biological action is believed to derive from its ability to inhibit protein synthesis at the peptidyltransferase stage.[2] It has been characterised as a broad spectrum agent endowed with low cytotoxicity and good selectivity towards prokaryots.[3] The structure of althiomycin was determined independently by various groups[4] and has been confirmed by total synthesis.[5] As depicted in Figure 1, the cysteine embedded in the thiazoline moiety has an (*S*)-configuration whereas the stereochemistry of the asymmetric centre on the serine residue is still ambiguous: it may either exist in racemic form or it might undergo racemisation during the isolation procedure.

The potency and selectivity profile of althiomycin along with the relative simplicity of its structure makes it an attractive medicinal chemistry target. The molecule, which can be retrosynthetically disconnected at both amide bonds, appears well suited to a parallel synthesis strategy based around a functionalised thiazoline core **2** (Fig. 2).

In this letter, we wish to present the results of our initial explorations directed towards establishing chemical routes to althiomycin analogues, as well as preliminary SAR that has emerged. A small number of analogues have already been reported,[6] but in some cases antimicrobial activities were not evaluated and there is, however, insufficient information to define a usable SAR.

We simplified chemistry by starting with the core **2** prepared from glycine (Fig. 2, X = H), which also allowed us to eliminate ambiguities with respect to the stereochemistry. This modification was not expected to have a detrimental effect on activity as dehydroxymethylalthiomycin is reported to be as potent an antibiotic as althiomycin itself.[6a]

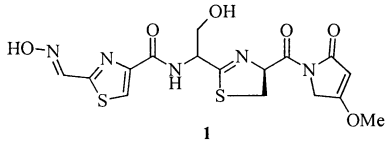

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Figure 1. Structure of althiomycin.

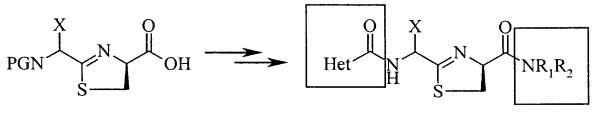

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Figure 2. Envisaged synthetic pathway.

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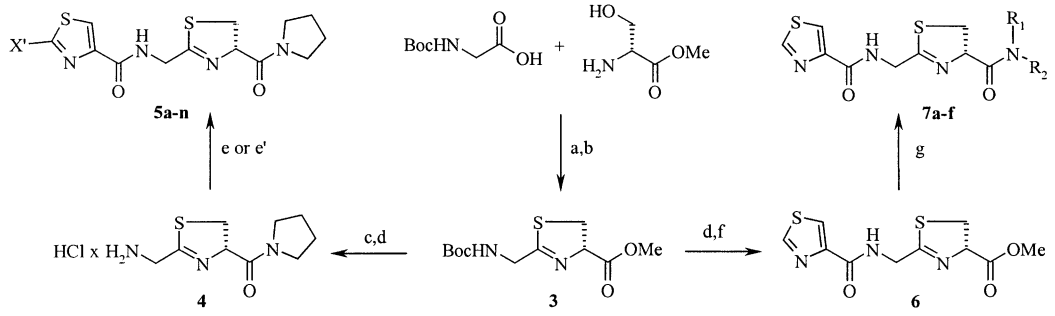


Scheme 1. (a) DCC, TEA, DCM, rt, 70–80%; (b) Lawesson's reagent, xylene, reflux, 40–50%; (c) pyrrolidine neat, rt, 1 h, 90%; (d) 4 N HCl/dioxane, rt, 2 h, quantitative yield; (e) **8a–e**, PyBOP, TEA, DCM, rt, 3–18 h, 50% to quantitative yield; (e′) **8f–n**, EDC, HOBT, TEA, DCM, rt, 18 h, yield >70%; (f) TEA, PyBOP, DMAP, rt, thiazole-2-carboxylic acid, 18 h, 50%; (g) HNR_1R_2 **9a–f**, neat, 50–75%.

Chemistry

The key thiazoline intermediate **3** (Scheme 1) was initially prepared by condensation of Cys-OMe with the ethyl imidate of Boc-Gly, adapting the chemistry used by Shiba in his total synthesis of althiomycin,[5] but we found that this procedure did not readily lend itself to large scale preparations.[7] The thiazoline formation strategy utilised in the only other reported synthesis of althiomycin[3a] was deemed to be unacceptably low yielding for our purposes, so an alternative methodology was sought. After consulting the literature we decided that the dehydration of a thioacyl serine using Burgess' reagent[8] was the most promising alternative for merit of its mildness and high yield. To this end Boc-Gly-Ser-OMe was prepared and heated with Lawesson's reagent. Fortuitously we found that the thioacyl serine formed in situ cyclised directly under the reaction conditions to give the thiazoline, providing a short and extremely convenient protocol for the preparation of **3**. Subsequent hydrolysis of the methyl ester proved capricious under various conditions and the desired acid, when detected, was always accompanied by substantial amounts of a thiazoline ring opened product. This unforeseen setback restricted the possibilities for scaffold decoration, but nonetheless direct amidation with pyrrolidine proved successful, furnishing the desired amide in 90% yield. Amine deprotection using HCl gave **4**, which was coupled to a series of substituted thiazole carboxylic acids **8a–n** (Fig. 3) to produce a small array of dehydroxymethylalthiomycin analogues **5a–n**.

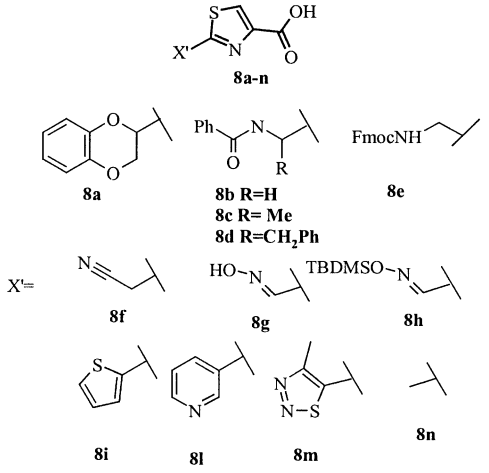


Figure 3. Thiazole acids **8a–n** used in the synthesis of amides **5a–n** (Scheme 1).

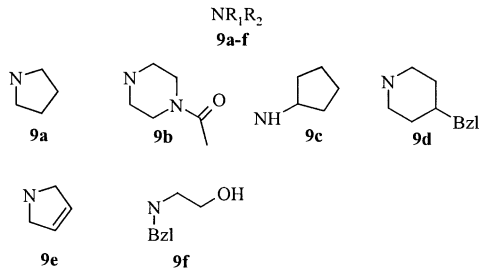
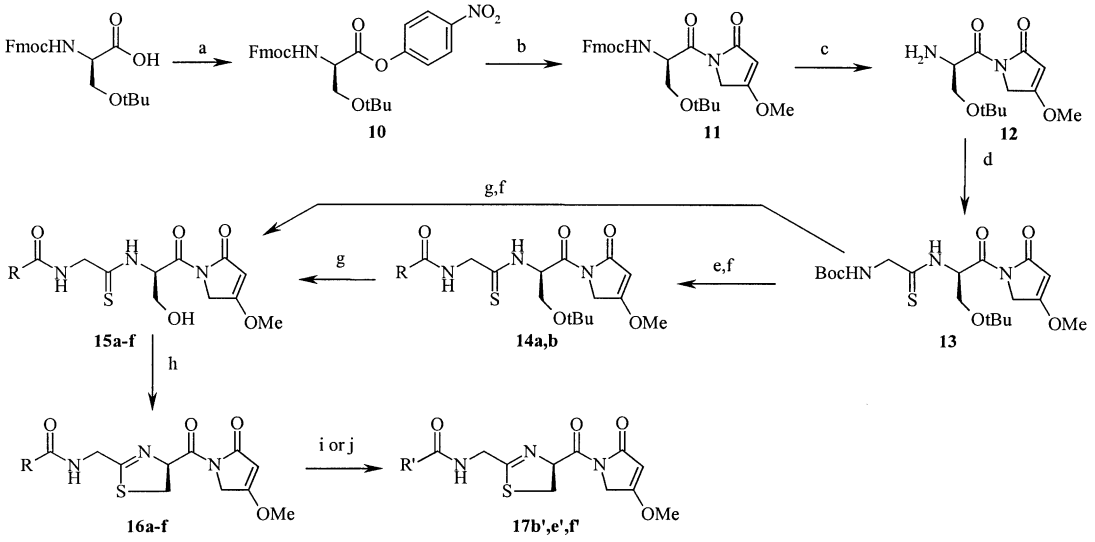


Figure 4. Amines **9a–f** used in the synthesis of amides **7a–f** (Scheme 1).

Couplings were initially effected using PyBOP, which gave the desired amides in excellent yield. Unfortunately, purification was complicated due to the presence of byproducts arising from the reagent itself that proved difficult to separate chromatographically. Coupling conditions were therefore revised to use EDC in order to facilitate product isolation. Following this synthetic route the oxime-substituted thiazole moiety of althiomycin can be introduced in either protected or non-protected form (Fig. 3).

The key intermediate **3** was also deprotected and coupled with thiazole-2-carboxylic acid to afford compound **6**. Selective hydrolysis of the methyl ester was again found to be impractical, but an additional small array of dehydroxymethylalthiomycin analogues **7a–f** was obtained by direct treatment of the ester with different commercially available amines **9a–f** (Fig. 4). Some more functionalised and more hindered amines failed in this direct amidation procedure due to decomposition and unreactivity, respectively.

As mentioned above, the lability of the thiazoline ring in methyl esters **3** and **6** towards hydrolysis prevents access to a more activated acylating species, thereby seriously limiting the derivatives that may be prepared via our original reaction pathway — in particular, the poorly nucleophilic pyrrolinone present in althiomycin cannot be introduced by direct displacement of the methyl ester. To circumvent these limitations we turned to an



Scheme 2. (a) (i) *p*-nitrophenylchloroformate, TEA, CH_2Cl_2, 0°C, 15 min; (ii) DMAP, 0°C, 30 min, 100%; (b) BuLi, 4-methoxy-3-pyrrolin-2-one, THF, rt, 2 h, 89%; (c) DBU, MeCN, 40°C, 1 h, 97%; (d) [2-(6-nitro-benzotriazol-1-yl)-2-thioxo-ethyl]-carbamic acid *tert*-butyl ester, THF, rt, 24 h, 61%; (e) 1 M HCl/Et_2O, DMS, 100%; (f) **18a–f**, EDC, HOBt, K_2CO_3, 35–90%; (g) TFA, DMS; (h) Burgess' reagent, THF, rt to 50°C, 30–90%; (i) TBAF, THF, rt, 1 h (56%, **17b**); (j) $H_2NOH{\cdot}HCl$, TEA, MeOH, rt, 1 h (72%, **17e**; 48%, **17f**).

alternative pathway in which the sensitive thiazoline ring is constructed at the last possible moment. In this case it is not possible to exploit the 'one-pot' thiazoline formation used previously due to the presence of a second amide group, which would react with Lawesson's reagent. Hence, we reverted to our original plan, preparing the thioacyl serine, via coupling of serine to an activated thioacyl species, and subsequently dehydrating with Burgess' reagent. As hoped this sequence turned out to be simple and high yielding with the only drawback of having to protect other moieties, such as an oxime, that are subject to dehydration.

This second route proved considerably more versatile and should be suitable for the preparation of almost any desired derivative but it is penalised by the number of chemical transformations involved, meaning that the time required to accumulate enough data for an SAR is greatly extended.

Fmoc-Ser(tBu)-OH was activated as its *p*-nitrophenyl ester **10** and coupled to 4-methoxy-3-pyrrolin-2-one to furnish **11**. Amine deprotection and subsequent thioamide formation using Rapoport's thioacylbenzotriazole procedure[9] gave thioamide **13**. The procedure developed to complete the synthesis consisted of selective deprotection of the Boc group using HCl followed by coupling to an activated acid to give **14**. TFA deprotection of the ^{t}Bu ether and final thiazoline formation using Burgess' reagent then gave dehydroxymethylalthiomycin analogues **16**.

This procedure was subsequently changed after encountering problems of stability of the protected oxime **14b** towards TFA treatment. The revised procedure calls for global deprotection of the Boc and ^{t}Bu ether groups of **13**, followed by amide coupling and cyclisation; the presence of an unprotected serine residue during the amide coupling turned out to be inconsequential.

A small array of derivatives was prepared using the acids **18a–f** depicted in Figure 5.

TBAF deprotection of **16b** gave synthetic dehydroxymethylalthiomycin **17b′** and aldehydes **16e**,**f** were converted into their oximes **17e′**,**f′**.

Semi-synthetic approach

Concurrently with the work described above, fermentations were set up to provide a supply of althiomycin to allow both further biological characterisation and the preparation of analogues via semi-synthetic strategy. The range of potential products available from such a strategy is somewhat limited, but those that are may be of particular importance in determining the roles played by the oxime and hydroxyl groups.

Initial experiments reacting **1** (Scheme 3) with amines, which were expected to substitute the pyrrolinone, were successful using pyrrolidine and phenylpiperazine

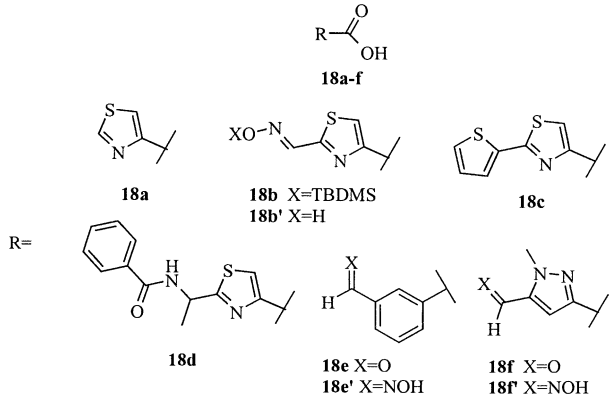
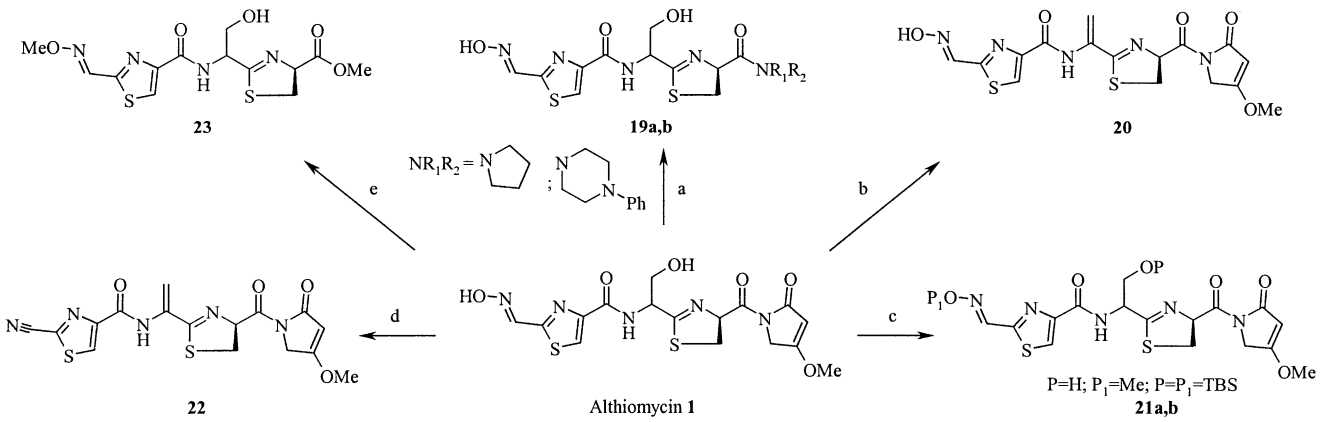


Figure 5. Acids used in the synthesis of amides **14a,b** and **15b–f** (Scheme 2).



Scheme 3. (a) Amine, rt, o/n, 90%; (b) benzyltrichloroacetimidate, TFA, CH_2Cl_2, rt, 70%; (c) **21a**, P = H, P_1 = Me: $TMSCHN_2$, MeOH, rt, 30 min, 48%; **21b**, P = P_1 = TBDMS: TBDMSOTf, DIPEA, DCM, rt, 46%; (d) Ac_2O, Py, DMAP, 80 °C, 30%; (e) $TMSCHN_2$, MeOH, rt, 20 h, 35%.

Table 1. Minimum inhibitory concentration[a] (MIC) results for compounds **1** and **17b′**

Compds	*S. aureus* 853E	*S. aureus* col	*B. subtilis* 6633[b]	*E. faecalis* 850E	*S. pneum* 3512	*E. coli* 1852E PM	*E. coli* 1852E + 3 μg/mL PMBN	*S. cerevisiae* NCYC 81
Ceftriaxone	4	> 32	0.25	16	0.01	0.06	0.01	> 32
1	16	16	16	16	4	1	1	> 32
17b′	32	32	16	32	16	16	8	> 32

[a]Values expressed in μg/mL.

(**1**→**19a,b**), while methylbenzylamine and *m*-anisidine did not react. Attempted benzylation using benzyltrichloroacetimidate in the presence of trifluoroacetic acid induced dehydration without alkylating the oxime (**1**→**20**). Methylation of the oxime was achieved using trimethylsilyldiazomethane in methanol (**1**→**21a**), with subsequent methanolysis of the imide being observed upon extended reaction time (**1**→**23**). The doubly silylated product **21b** was prepared using TBSOTf. Heating the oxime acetate gave the nitrile derivative **22**.

Results and Discussion

All final products and 'full length' precursors, plus a sample of natural althiomycin, were evaluated in a standard MIC assay[10] using a panel of Gram-positive and Gram-negative bacteria. The reported activity of althiomycin was confirmed, although its potency towards a number of clinically significant Gram-positive strains leaves much to be desired.

Unfortunately, only one of the ~50 synthetic molecules tested, dehydroxymethylalthiomycin **17b′**, showed an appreciable (< 32 μg/mL) antibacterial activity (Table 1).

Interestingly, the MICs exhibited by **17b′** in our assay are considerably lower than those reported by others.[6a] Although this result casts a shadow on the validity of our initial decision to work around the dehydroxymethylalthiomycin core, we have succeeded in setting up a versatile synthetic route that opens the way for the preparation of more complex derivatives bearing greater structural similarity to althiomycin itself.

Moreover, the semi-synthetic products have, as hoped, been extremely valuable in helping to determine important pharmacophoric points. The complete loss of antimicrobial activity associated with minor structural changes indicates the essential roles played by the oxime (cf., the methylated derivative **23** to **1**), the hydroxyl group (compare the dehydrated product **20** or the dehydroxymethyl compound **17b′** to **1**) and the methoxypyrrolinone moiety (**19a,b** vs **1**). It is premature to draw conclusions as to the roles that these groups may play, but the next step in defining what appears to be a very restricted SAR could be the preparation of derivatives in which each pharmacophoric element is selectively replaced by carefully chosen alternatives. Metal chelating motifs could be considered as oxime replacements, as H-bond donor/acceptors could be used to substitute the hydroxyl group. Another possibility to further simplify the chemistry and explore the SAR could rest in replacing the chemically labile thiazoline moiety with an alternative heterocycle to determine whether this ring functions as a spacer or if it also plays an important part in the biological action of althiomycin.

Conclusions

In summary, we have described novel synthetic routes to synthesise althiomycin analogues. Products prepared using these approaches, along with those available semi-synthetically, have enabled us to identify several important pharmacophoric points present in althiomycin. Further studies, which are necessary in order to understand the true potential of althiomycin-like compounds as novel antibacterial drugs, will be the subject of future publications.

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