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Bioorganic & Medicinal Chemistry Letters 18 (2008) 2645–2648
Bioorganic & Medicinal Chemistry Letters

# Novel antibacterial azetidine lincosamides☆

Hardwin O'Dowd,* Jason G. Lewis, Joaquim Trias, Rumi Asano, Johanne Blais, Sara L. Lopez, Craig K. Park, Charlotte Wu, Wen Wang and Mikhail F. Gordeev

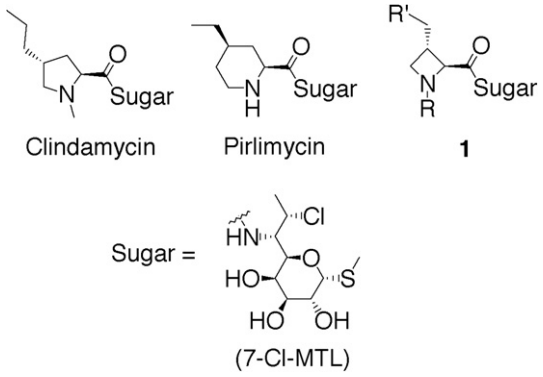
*Pfizer Global Research & Development, Fremont, Formerly Vicuron Pharmaceuticals, Inc., CA 94555, USA*

Received 23 January 2008; accepted 7 March 2008
Available online 14 March 2008

**Abstract**—The synthesis and evaluation of novel azetidine lincosamides **1** are described. Eleven new (3-*trans*-alkyl)azetidine-2-carboxylic acids were synthesized via alkylation of *N*-TBS-4-oxo-azetidine-2-carboxylic acid and subsequent elaboration then coupled to 7-chloro-1-methylthio-lincosamine. The resulting lincosamides differ from the drug clindamycin in both the size of the ring and the position/structure of the alkyl side-chain. SAR within the series was explored with attention to alkyl variants in positions 1 and 3 of the azetidine ring.


Of the lincosamide class of antibacterial protein synthesis inhibitors, clindamycin (CLI) is the most widely used in human medicine. The clinical importance of clindamycin has increased with the emergence of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) infections, where it offers an effective therapeutic alternative to β-lactam antibiotics.[1,2] We have chosen to explore this class further, with the goal of discovering novel antibacterial agents with improved potency, spectrum and ADME/toxicity profile. In terms of chemical structure clindamycin consists of two subunits: an amino acid moiety (*N*-methyl-4′-(*R*)-propyl-L-proline, or 4-*n*-propylhygric acid) and an amino sugar moiety (7-chloro-1-methylthio-lincosamine, 7-Cl-MTL). Increasing the amino acid ring-size from pyrrolidine (five-membered) to piperidine (six-membered) has resulted in potent synthetic lincosamide derivatives (e.g., the veterinary antibiotic pirlimycin[3] and the recently disclosed VIC-105555).[4] This report describes our investigation of azetidine (four-membered ring) lincosamides.

The unsubstituted azetidine-2-carboxamide derivative of 7-Cl-MTL has previously been reported to display only




mediocre activity (MIC vs *S. aureus* = 25 μg/mL).[3] We have reasoned that it would be possible to improve the activity of azetidine lincosamides by incorporating an alkyl side-chain on the ring. Molecular modeling of the conformation of substituted azetidine, pyrrolidine and piperidine amino acids indicated that the 3-*trans*-alkyl azetidine amino acid structure would be a close mimic for both the clindamycin and pirlimycin amino acid moieties. Twelve new (3′-*trans*-alkyl)azetidine lincosamides (**1**) were synthesized with variation at the 3′ and 1′ positions; 7-chloro-1-methylthio-lincosamine was used as the sugar moiety in all cases.

The synthesis of 3-*trans*-alkyl-L-azetidine carboxylic acids **2** is depicted in Scheme 1. *N*-TBS-4-oxo-azetidine-2*S*-carboxylic acid was doubly deprotonated with LDA then alkylated with either an alkyl or allyl electrophile with high stereoselectivity.[5,6] Conversion of the

*Keywords*: Antibacterial; Antibiotic; Protein synthesis inhibitor; Clindamycin; Lincosamide; Azetidine; Unnatural amino acid.
☆ This work was presented in part at the 45th Interscience Conference on Antimicrobial Agents and Chemotherapy, 2005, Washington, DC, Poster F-2037.
* Corresponding author. E-mail: hardwin@hotmail.com

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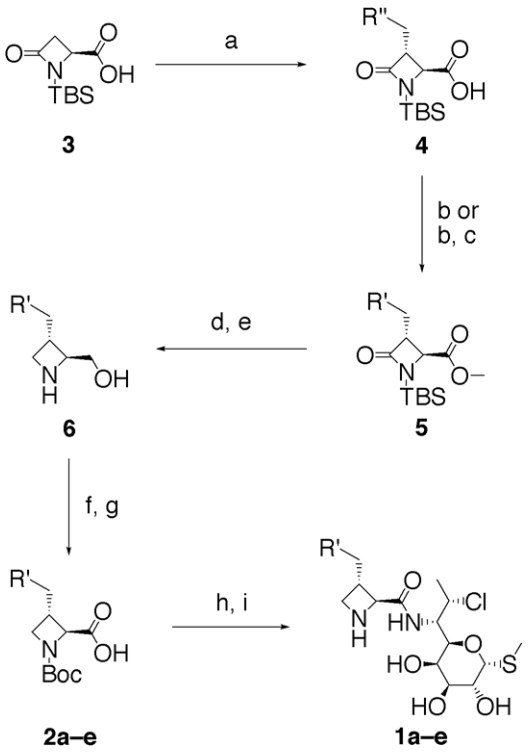
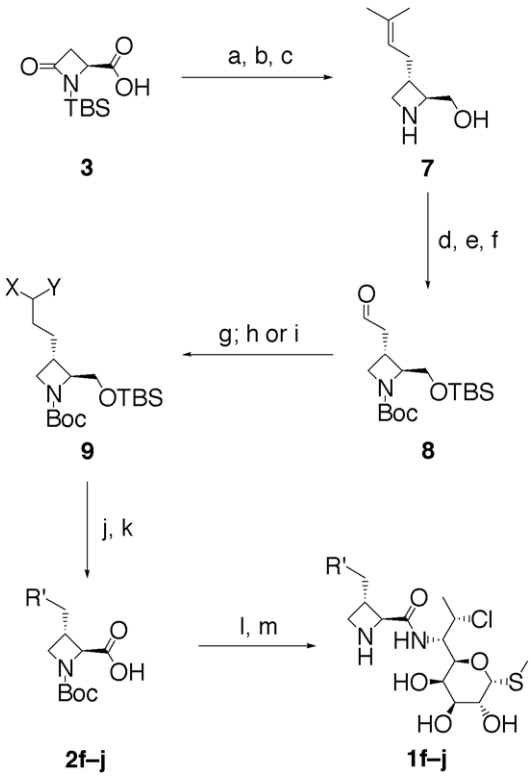
doi:10.1016/j.bmcl.2008.03.032




**Scheme 1.** Reagents: (a) LDA, R″$CH_2$Br, THF; (b) $TMSCHN_2$, MeOH; (c) $H_2$, Pd/C, EtOAc; (d) $Et_3N{\cdot}3HF$, THF; (e) $LiAlH_4$, THF; (f) $Boc_2O$, $CH_2Cl_2$; (g) $RuCl_3{\cdot}H_2O$, $NaIO_4$, aq acetone; (h) 7-Cl-MTL, HBTU, DIPEA, DMF; (i) TFA, $H_2O$, DCE.



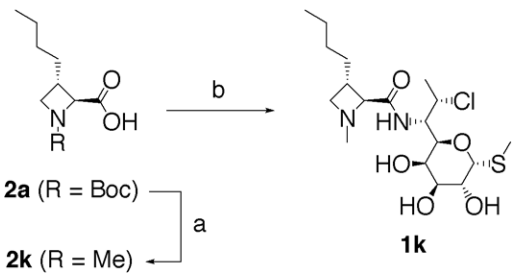

**Scheme 2.** Reagents: (a) LDA, THF, $Me_2C{=}CHCH_2Br$; (b) $Et_3N{\cdot}3HF$, THF; (c) $LiAlH_4$, THF; (d) $Boc_2O$, $CH_2Cl_2$; (e) TBSCl, imidazole, DMF; (f) $O_3$, $PPh_3$, $CH_2Cl_2$; (g) $XYC{=}PPh_3$, THF; (h) $H_2$, Pd/C, EtOAc; (i) $KO_2CN{=}NCO_2K$, AcOH, dioxane; (j) TBAF, THF; (k) $RuCl_3H_2O$, $NaIO_4$, aq acetone; (l) 7-Cl-MTL, HBTU, DIPEA, DMF; (m) TFA, $H_2O$, DCE.

acid to a methyl ester was followed by hydrogenation (if required) to give the saturated *N*-TBS-3-*trans*-alkyl-4-oxo-azetidine-2-methyl ester. The TBS-group was removed, and then both the amide and ester were reduced using $LiAlH_4$. The resulting free amine was Boc-protected, then the primary alcohol was oxidized to provide the desired Boc-protected 3-*trans*-alkyl-L-azetidine carboxylic acids **2**.[6,7] The Boc-protected azetidine acids **2** were coupled with 7-Cl-MTL, then deprotected to furnish the 3′-*trans*-alkyl azetidine lincosamides **1**.[8]

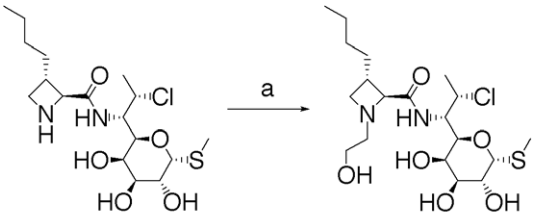
An alternative approach that allowed for diversification of the amino acid side-chain at a later stage was also developed, as shown in Scheme 2. *N*-TBS-4-oxo-azetidine-2*S*-carboxylic acid was alkylated with 3,3-dimethylallyl bromide and elaborated in a similar manner as before to the Boc-amino alcohol. The alcohol was TBS-protected, then the side-chain alkene was oxidatively cleaved to provide an aldehyde 'handle' for further modification. Olefination of aldehyde **8** followed by hydrogenation gave access to a variety of cycloalkyl-alkyl side-chains, in the case of cyclopropyl analogs diimide reduction was used in order to achieve selective saturation of the alkene. TBS-deprotection was followed by oxidation, coupling and Boc-deprotection as before.

*N*-Methyl azetidine amino acid **2k** was synthesized via a combined one-pot deprotection/Eschweiler–Clark reaction of **2a** with formic acid and coupled directly with 7-Cl-MTL to give **1k** (Scheme 3). 1′-(2-Hydroxyethyl)azetidine lincosamide, **1l**, was obtained via treatment of **1a** with ethylene oxide (Scheme 4). Each final compound was purified to >95% via HPLC.

Novel azetidine lincosamides **1a**–**l** were assayed against a panel of bacterial isolates, using standard broth microdilution techniques,[9,10] and the resulting minimum inhibitory concentration (MIC) data are summarized in Table 1. Select compounds were also screened for inhibition of the bacterial protein synthesis pathway in a cell-free *Escherichia coli* transcription/translation (TT) inhibition assay[11] (Table 1). Five of the new azetidine analogs were tested for in vivo efficacy against *S. aureus* (Smith strain) in a murine septicemia protection model,[12,13] Table 2.




**Scheme 3.** Reagents: (a) $CH_2O$ aq, $CHO_2H$; (b) 7-Cl-MTL, HBTU, DIPEA, DMF.




**Scheme 4.** Reagents: (a) ethylene oxide, MeOH.

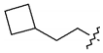
*Results:* The 3-*trans*-substituted-2*S*-azetidine moiety is well-tolerated as a mimic of the 4-*n*-propylhygric acid portion of clindamycin. Cell-free transcription/translation assay data and the lack of susceptibility of *S. aureus* cMLSB strain confirm that azetidine lincosamides share the same protein synthesis inhibitory mode of action and bacterial 50S RNA target with clindamycin.[14,15] *E. coli* transcription/translation inhibition data for the novel compounds were proportional to the observed MIC in most cases, with linear R′ side-chains affording superior activity. Increasing the length of the side-chain

**Table 2.** In vivo efficacy

| | CLI | 1a | 1d | 1f | 1h | 1j |
|---|---|---|---|---|---|---|
| MIC (μg/mL) | 0.125 | 0.25 | 0.06 | 0.03 | 0.25 | 0.125 |
| $ED_{50}$, IV (mg/kg) | 2.8 | 1.1 | 1.7 | >10 | 3.0 | 3.1 |
| $ED_{50}$, PO (mg/kg)[a] | 19.9 | 10.8 | 28.0 | NT | NT | NT |

[a] NT, not tested.

**Table 1.** In vitro antibacterial activity and translation/transcription inhibition activity

| Compound | R | R′ | MIC (μg/mL) | | | | | | TT $IC_{50}$[b] (μM) |
|---|---|---|---|---|---|---|---|---|---|
| | | | *Streptococcus pneumoniae* (*n* = 1) | *S. aureus* (*n* = 3) | *S. aureus* (cMLSB)[a] | *Enterococcus faecium* (*n* = 2) | *Enterococcus faecalis* (*n* = 2) | *Bacteroides fragilis* (*n* = 2) | |
| CLI | — | — | 0.06 | 0.125–0.25 | >8 | 0.125–4 | 0.25–8 | 0.06–1 | 2.70 |
| **1a** | H |  | 0.06 | 0.125–0.25 | >8 | 0.125–1 | 0.125–8 | 0.125–2 | 0.36 |
| **1b** | H |  | 0.125 | 0.5 | >8 | 0.5–4 | 1–>8 | 0.5–4 | 11.06 |
| **1c** | H |  | 0.125 | 0.5 | >8 | 0.5–4 | 0.5–8 | 0.5–4 | 12.85 |
| **1d** | H |  | 0.03 | 0.06–0.125 | >8 | 0.125–0.25 | 0.25–4 | 0.125–0.5 | 1.13 |
| **1e** | H |  | 0.125 | 0.5 | >8 | 0.5–4 | 0.5–16 | 0.5–4 | 15.53 |
| **1f** | H |  | 0.016 | 0.03 | >4 | 0.06–0.25 | 0.06–0.5 | 0.25 | 2.44 |
| **1g** | H |  | 0.125 | 0.25–0.5 | >4 | 0.25–1 | 0.25–>4 | 0.25 | NT |
| **1h** | H |  | 0.125 | 0.25 | >4 | 0.125–1 | 0.25–>4 | 0.25 | 7.80 |
| **1i** | H |  | 0.125 | 1–2 | >4 | 0.5–>4 | 2–>4 | 1–>4 | NT |
| **1j** | H |  | 0.06 | 0.125–0.25 | >4 | 0.125–0.5 | 0.25–2 | 0.25–2 | 2.75 |
| **1k** | Me |  | 0.5 | 1 | >4 | 1–>4 | 2–>4 | 0.125–2 | NT |
| **1l** |  |  | 0.125 | 0.5 | >8 | 0.25–4 | 0.5–>8 | 0.5–8 | NT |

[a] cMLSB: constitutive macrolide, lincosamide and streptogramin B resistance phenotype.
[b] NT, not tested.

was beneficial. Compound **1a** displayed the best overall activity, performing well both in vitro and in vivo, with efficacy ca. 2-fold superior to that for clindamycin for both IV and PO routes of administration. The high in vitro potency of the cyclobutane-containing compound **1f** did not translate into in vivo efficacy, possibly due to inferior PK. N-Alkylated azetidine lincosamides **1k**, **1l** showed lower activity than the parent azetidine lincosamide **1a**.

In summary, an SAR study of novel 3′-*trans*-substituted azetidine lincosamides was conducted. The azetidine is a well-tolerated replacement for the pyrrolidine of clindamycin. Several examples of azetidine lincosamides with similar in vitro potency and spectrum to that of clindamycin were identified. The efficacy of compound **1a** compared favorably to that of clindamycin in a murine septicemia model, via both IV and PO routes of administration. Azetidine lincosamides are promising antimicrobial agents that warrant further study.

## Supplementary data

Copies of $^{1}$H NMR spectra for compounds **1a–l** are available. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.03.032.

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