Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 2427–2432

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

SYNTHESIS OF 6-*O*-METHYL-AZITHROMYCIN AND ITS KETOLIDE ANALOGUE *VIA* BECKMANN REARRANGEMENT OF 9(E)-6-*O*-METHYL-ERYTHROMYCIN OXIME

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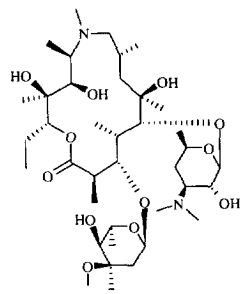
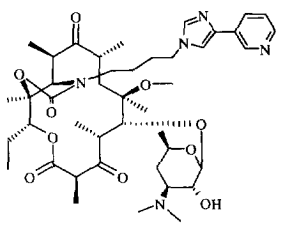
Received 15 May 1998; accepted 17 July 1998

Abstract : The synthesis of 6-*O*-methyl-azithromycin and its aza-ketolide analogue have been achieved by carrying out the Beckmann rearrangement of the readily available 9(E)-6-*O*-methyl-erythromycin oxime **1**. In contrast to the C14 ketolides like HMR 3647, the aza-ketolide turns out to be inactive, thus demonstrating that the addition of a 3 keto function and ring expension, from 14 to 15 membered ring, could be deleterious for the antibacterial activity.

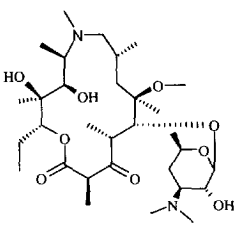
Renewal of interest in macrolides was triggered by roxithromycin[1] in the early 1980s. This new macrolide was later challenged by clarithromycin[2] and azithromycin[3] with regard to improved pharmacokinetic properties in comparison to those of erythromycin. The antibacterial spectra of all these drugs typically includes respiratory pathogens; however, there are several drawbacks, such as a lack of efficacy against macrolide-lincosamide-streptogramin B (MLS_B)-resistant pneumococci and, with the exception of azithromycin, only modest activity against *Haemophilus influenzae*. In the search of compounds likely to overcome the problem of pneumococcal resistance, a new class of 14-membered-ring macrolide antibacterial agents so called ketolides has been generated[4]. Ketolides are characterized by a ketone group at position 3 of the macrolactone ring, which replaces the L-cladinose moiety, a neutral sugar long thought to be essential for antibacterial activity.



AZITHROMYCIN



HMR 3647



AZA-KETOLIDE

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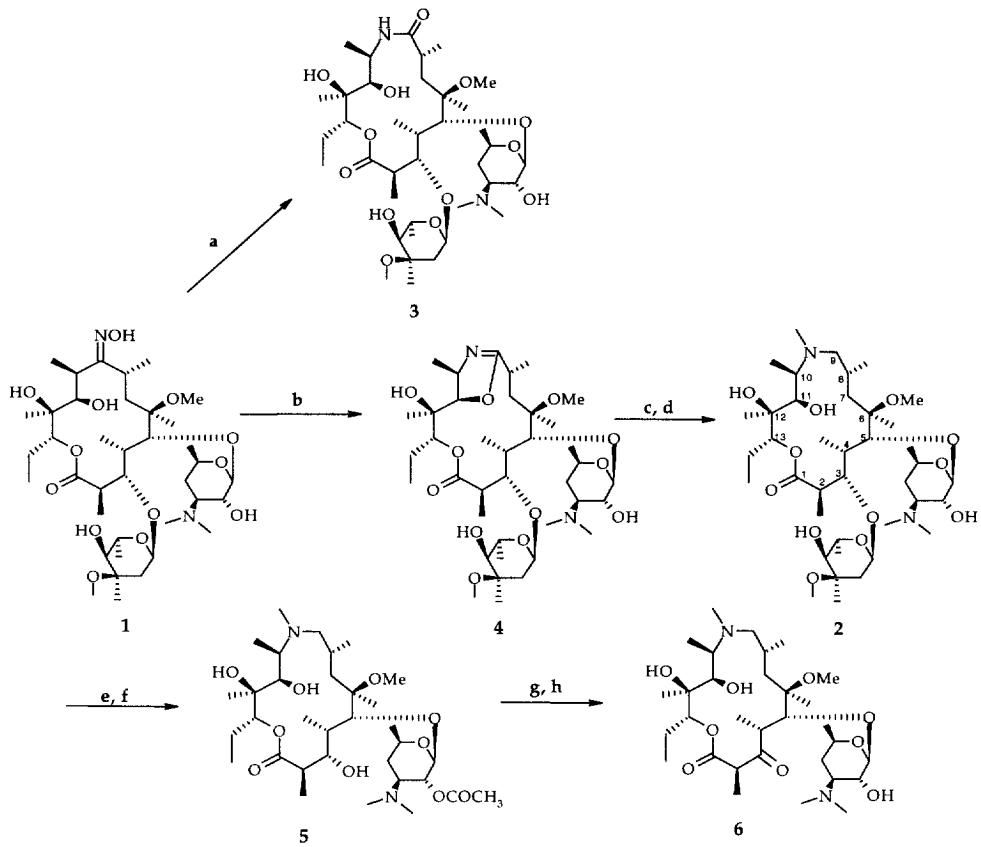
PII: S0960-894X(98)00402-8

Although it was concluded from the azithromycin antibacterial spectrum that the introduction of a basic nitrogen in the lactone ring was beneficial for the activity against Gram negative bacteria, the level of activity of two ketolides under clinical development against *Haemophilus influenzae* demonstrated that despite a non azalide structure, this class of compound could reach a significant activity against this pathogen in addition to strong activity against MLS_B pneumococci as demonstrated by the clinical candidate HMR 3647[5]. However, considering the two structures: azalides and ketolides, we wondered if combining the structural elements of the azalides and ketolides to produce the aza-ketolides, would incorporate the beneficial antibacterial activities of both classes.

The synthesis of 6-*O*-methyl-azithromycin **2** was previously described by direct methylation of azithromycin[6]. However, it has recently turned out that in fact the methylation of hydroxy group of azithromycin can only afford 12, 11 and 4'' O-methylated derivatives and that the structure of **2** was incorrect[7]. To address this question we devised a straightforward synthesis of **2** and of its 3-keto-6-*O*-methyl analogue based on the Beckmann rearrangement of 9(E)-6-*O*-methyl-erythromycin oxime[2] **1**. Thus the already described 6-*O*-methylated oxime was choosen as a precursor of the azalide skeleton. Moreover, we anticipated from the different studies concerning the Beckmann rearrangement in erythromycin series[8], that depending on the conditions, the reaction of **1** with tosyl chloride would give rise to the formation of an internal cyclic 9,11-iminoether **4**[9].

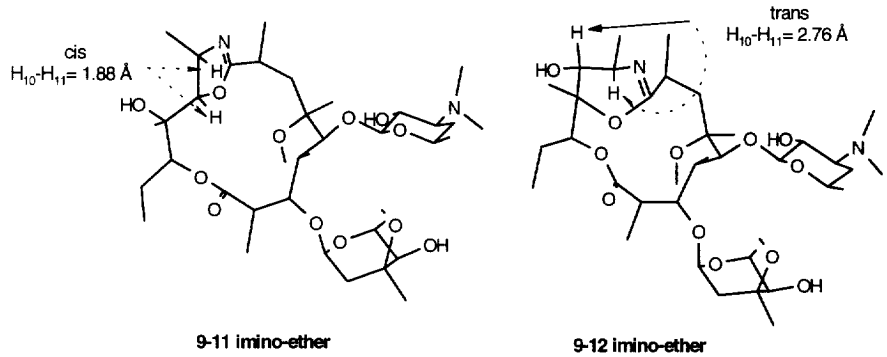
When **1** was reacted in the standard conditions of azithromycin synthesis with TsCl and $NaHCO_3$ in aqueous acetone, we obtained the undesired lactam **3** instead of the desired 9,11-iminoether **4**. However, when the reaction was carried out in ether with pyridine, the reaction of 11 hydroxyl group with the nitrilium intermediate occured as expected in 40% yield. Similar differences in chemical behavior of hydroxyl groups during Beckmann rearrangement in erythromycin series have already been described[10]. The iminoether **4** was reduced using a high pressure hydrogenation with PtO_2 in acetic acid to afford the intermediate amine which was methylated under Eschweiler-Clarke conditions to give **2** in 58% overall yield. Next, the cladinose sugar was removed by treatment with aqueous HCl and the 2'-OH group protected by acetylation prior to oxidation of 3-OH group to yield **5**. The oxidation of the 3-hydroxyl group was carried out using Pfizner-Moffat modified conditons: 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC,HCl)/DMSO/ Pyridinium trifluoroacetate in methylene chloride, the 2' acetyl group was removed by methanolysis to give **6** in 68% yield.



(a) TsCl, $NaHCO_3$, acetone/H_2O (70%); (b) TsCl, pyridine, ether, 0° to r.t. (40%); (c) H_2, PtO_2, AcOH; (d) HCO_2H, $CHCl_3$, reflux (58% c+d); (e) HCl 1.2 N; (f) Ac_2O, K_2CO_3, acetone (60% e+f); (g) EDC, DMSO, Pyridinium trifluoroacetate, CH_2Cl_2, r.t.; (h) MeOH, r.t. (68% g+h)

The chemical structure of **2**, **3**, **4** and **6** were determined by NMR using NOE experiments, MS and elemental analysis[12]. For **4**, a strong NOE effect was observed between the two proton H_{10} and H_{11}, suggesting, as predicted by molecular modelling[11] (Figure 1), a cis stereochemistry in the cyclic 9,11-iminoether rather than a trans stereochemistry of a 9,12-iminoether. Furthermore, the preferential traping of the nitrilium intermediate by the 11-OH group leading to the formation of **4** was in good agreement with the previously mentioned formation of an 9,11 iminoether structure in the 6-OH series[8b].

Figure 1 : H_{10}-H_{11} distances calculated by molecular modelling of the two isomeric iminoethers



The antibacterial activities of **2**, **3**, **4** and **6** were determined against both erythromycin sensitive and resistant bacteria using azithromycin and HMR 3647 as references. The iminoether **4** displayed a very weak activity whereas the lactam **3** and the azalide **2** were still antibacterial but less active than azithromycin. In contrast to HMR 3647 that was active against all strains including the resistant pneumococci, the aza-ketolide **6** was essentially inactive. This unexpected result reveals that the addition of two major changes in the erythromycin nucleus: 3 ketone and ring expansion, from a 14- to 15-membered ring, are deleterious to the antibacterial activities of these analogues.

Table 1 : **MIC's (µg/ml)**

compd	*S. aureus* 011UC4	*S. pneumoniae* 030SJ1 EryRc	*S. pneumoniae* 030SJ5i EryRi	*S. pneumoniae* 032UC1	*S. pyogenes* 02A1UC1	*H. influenzae* 351HT3	*E. coli* 250 UC5
2	1.2	>40	>40	0.15	0.15	2.5	20
3	1.2	40	10	1.2	0.6	2.5	40
4	20	40	40	40	40	10	40
6	40	40	40	40	40	40	40
AZI	0.3	40	40	0.15	0.6	1.2	20
HMR3647	0.04	0.02	0.02	0.02	0.02	1.2	10

Antibacterial activities were determined by standard broth microdilution assay.

In conclusion we have achieved the first synthesis of 6-*O*-methyl-azithromycin **2** by carrying out the Beckmann rearrangement of the readily available 9(E)-6-*O*-methyl-erythromycin oxime **1**. This has allowed us to generate the first aza-ketolide which, in contrast to the C14 ketolides like HMR 3647, turns out to be inactive.

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[9] In the course of publication of our work on the aza-ketolide synthesis, a communication describing the synthesis of 8a- and 9a-azalides (including **2**) from Beckmann rearrangement of 9(E) or (Z)-6-*O*-methyl-erythromycin oxime was published. Waddell, S. T.; Santorelli, G. M.; Blizzard, T. A.; Graham, A.; Occi, J. *Bioorg. Med. Chem. Lett.*, **1998**, *8*, 1321-1326.

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[11] Molecular Modeling of 4 and its 12-9 isomeric imino-ether were carried out by using Insight II® (MSI corporation, San Diego, CA) software.

[12] Proton assignements for **4** was made using a combination of two-dimensional NMR techniques: COSY (proton-proton correlation) and HMQC (proton-carbon correlation) and COSY only for **2**, **3** and **6**.

Spectral data for **3** : ^{1}H NMR ($CDCl_3$) : **15 Me-** 0.89 (t, 3H), **4Me-** 1.02 (d, 3H), **8 Me-**1.09 (d, 3H), **10Me-** 1.17 (d, 3H), **12Me-** 1.18 (s, 3H), **2Me-** 1.22 (d, 3H), **5'Me-** 1.24 (d, 3H), **3''Me-** 1.26 (s, 3H), **5''Me-** 1.32 (d, 3H), **6Me-** 1.35 (s, 3H), **8H-** 2.21 (m, 1H), **N(Me)$_2$-** 2.34 (s, 6H), **2''H eq**.- 2.34 (m, 1H), **3'H-** 2.48 (m, 1H), **2H-** 2.83 (dq, 1H), **4''H-** 3.04 (d, 1H), **2'H-** 3.22 (m, 1H), **3''OMe-** 3.31 (s, 3H), **6 OMe-** 3.34 (s, 3H), **5'H-** 3.5 (m, 1H), **5H-** 3.76 (d, 1H), **5''H-** 4.06 (dq, 1H), **10H-** 4.17 (q, 1H), **3H-** 4.21 (d, 1H), **1'H-** 4,45 (d, 1H), **13H-** 4.67 (dd, 1H), **1''H-** 4.84 (d, 1H), **NHCO-** 6.12 (d, 1H). FAB-MS : ($M+H^+$)= 763. Anal. Calc. (%) for $C_{38}H_{70}N_2O_{13}$: C 59.81, H 9.25, N 3.67. Found : C 59.7, H 9.4, N 3.4.

Spectral data for **4** : ^{1}H NMR ($CDCl_3$) : **15 Me-** 0.92 (t, 3H), **8Me-** 1.18 (d, 3H), **3''Me-** 1.19 (s, 3H), **4'Hax**-1.24 (m, 1H), **5''**and **5'Me**-1.24 (d, 3 H), **2 Me-**1.25 (d, 3H), **4 Me-**1.26 (d, 3H), **12Me-** 1.28 (s, 3H), **6Me-** 1.43 (s, 3H), **2''H ax**.- 1.48 (m, 1H), **10Me-** 1.49 (d, 3H), **7H eq-** 1.5 (d, 1H), **14H-** 1.61 (m, 2H), **4'H eq.-** 1.71 (m, 1H), **7H ax.-** 2.13 (t, 1H), **4H-** 2.24 (dq, 1H), **N(Me)$_2$-** 2.31 (s, 6H), **2''H eq**.- 2.41 (d, 1H), **2H-** 2.68 (ql, 1H), **3'H-** 2.56 (tl, 1H), **8H-** 2.81 (m, 1H), **4''H-** 2.95 (t, 1H), **6 OMe-** 3.18 (s, 3H), **2'H-** 3.18 (m, 1H), **3''OMe-** 3.34 (s, 3H), **5'H-** 3.5 (m, 1H), **5H-** 3.79 (d, 1H), **5''H-** 4.05 (m, 1H), **10H-** 4.35 (dq, J= 10 and 7 Hz, 1H), **1'H-** 4.48 (d, 1H), **11H-** 4.49 (d, J= 10Hz, 1H), **1''H-** 4.58 (d , 1H), **13H-** 4.64 (dd, 1H), **3H-** 4.82 (sl, 1H). FAB-MS : ($M+H^+$)= 745. Anal. Calc. (%) for $C_{38}H_{68}N_2O_{12}$: C 61.26, H 9.2, N 3.75. Found : C 61.3, H 9.3, N 3.6.

Spectral data for **2** : ^{1}H NMR ($CDCl_3$) : **15 Me-** 0.90 (t, 3H), **8 Me-**0.93 (d, 3H), **4** and **10Me-** 1.08 (d, 6H), **12Me-** 1.11 (s, 3H), **2Me-** 1.22 (d, 3H), **5'Me-** 1.24 (d, 3H), **3''Me-** 1.25 (s, 3H), **5''Me-** 1.29 (d, 3H), **6Me-** 1.35 (s, 3H), **4H-** 2.05 (m, 1H), **9H-** 2.05-2.4 (m, 2H), **N(Me)$_2$-** 2.29 (s, 6H), **NMe-** 2.34 (s, 3H), **2''H-** 2.35 (m, 1H), **3'H-** 2.50 (m, 1H), **10H-** 2.77 (q, 1H), **2H-** 2.87 (m, 1H), **4''H-** 3.03 (d, 1H), **2'H-** 3.23 (dd, 1H), **6 OMe-** 3.28 (s, 3H), **-3''OMe** 3.33 (s, 3H), **5'H-** 3.51 (m, 1H), **11H-** 3.58 (sl, 1H), **5H-** 3.75 (d, J=6.5Hz, 1H), **3H-** 3.96 (dl, J=6.5 Hz, 1H), **5''H-** 4.06 (m, 1H), **1'H-** 4.48 (d, 1H), **13H-** 4.88 (dd, 1H), **1''H-** 4.96 (d, 1H). FAB-MS : ($M+H^+$)= 763. Anal. Calc. (%) for $C_{39}H_{74}N_2O_{12}$: C 61.39, H 9.77, N 3.67. Found : C 61.3, H 9.9, N 3.5.

Spectral data for **6** : ^{1}H NMR ($CDCl_3$) : **15 Me-** 0.90 (t, 3H), **8 Me-**0.95 (d, 3H), **10Me-** 1.08 (d, 3H), **6Me-** 1.20 (s, 3H), **5'Me-** 1.25 (d, 3H), **12Me-** 1.30 (s, 3H), **2Me-** 1.35 (d, 3H), **4Me-** 1.37 (d, 3H), **8H-** 1.76 (m, 1H), **9H-** 1.95 (m, 2H), **N(Me)$_2$-** 2.27 (s, 6H), **NMe-** 2.33 (s, 3H), **3'H-** 2.47 (m, 1H), **10H-** 2.78 (m, 1H), **6 OMe-** 2.93 (s, 3H), **4H-** 3.20 (m, 1H), **2'H-** 3.23 (m, 1H), **5'H-** 3.62 (m, 1H), **11H-** 3.70 (d, 1H), **2H-** 3.84 (q, 1H), **5H-** 4.36 (d, 1H), **1'H-** 4.49 (d, 1H), **13H-** 5.12 (dd, 1H). FAB-MS : ($M+H^+$)= 603. Anal. Calc. (%) for $C_{31}H_{58}N_2O_9$: C 61.76, H 9.69, N 4.64. Found : C 61.8, H 9.8, N 4.9.