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A novel ketolide class: Synthesis and antibacterial activity of a lead compound

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Abstract—Synthesis and antibacterial activity of a new class of ketolide antibiotics, exemplified by the prototype GW680788X (**1**), are described. The structure of (**1**) has been elucidated by spectroscopic analysis. The good antibacterial activity shown by (**1**) in comparison with clarithromycin prompted us to consider this compound as a lead molecule for further exploration.

Macrolide antibiotics represent an established class of antibacterial compounds effective for the treatment of respiratory infections.[1–4] Erythromycin, which belongs to the 14-membered ring macrolides, was the first marketed compound of this class.

An undesirable feature of erythromycin is its instability in the acidic stomach environment, causing intramolecular reactions, such as ketalisation of the C-9 carbonyl by the 6-OH and 12-OH groups, which decreases the oral absorption of the drug and produces unpleasant gastrointestinal irritation.[5,6]

Several erythromycin derivatives have been prepared[7–9] to overcome this undesired intramolecular ketalisation. Effective modifications were obtained by multi-step methylation of erythromycin at 6-OH to give clarithromycin, and by ring expansion of erythromycin with the introduction of a basic nitrogen to give azithromycin (Fig. 1).

Nevertheless, further improvements on this class of antibacterial agents are required to overcome relatively recent issues associated with the development of bacterial resistance.[10–13] Ketolides HMR3647 (Aventis, Telithromycin™) and ABT773 (Abbott, Cethromycin™) represent the first potential solutions to this problem.

These compounds showed an antibacterial profile better than that of clarithromycin, including activity against macrolide-resistant strains of pneumococci with good oral bioavailability.

The new ketolides (Fig. 2) share two distinguishing structural features: the first one is the presence of a keto group at position 3, the second one is the presence of an alkyl-heteroaryl side chain. Although structurally different and linked to different positions of the macrolide scaffold, the side chains in HMR3647 and ABT773 engender a remarkable improvement of the antimicrobial property.[14–17]

On the basis of NOE data on ABT 773 and HMR 3647 and of molecular modelling work, we have concluded that these two molecules could place their heteroaromatic ring in a 'common' region of space (Fig. 3).

The effect of the side chains is to enhance the binding affinity to both methylated and unmodified domain II of the bacterial ribosomes.[18,19] Furthermore, the keto-function at position C-3 of the macrolide ring—replacing the L-cladinose sugar—avoids, as demonstrated by Le Mahieu et al.[20–22], induction of MLSB resistance in streptococci and staphylococci and it also confers acid stability to the molecule itself. Moreover, it is reported[23]

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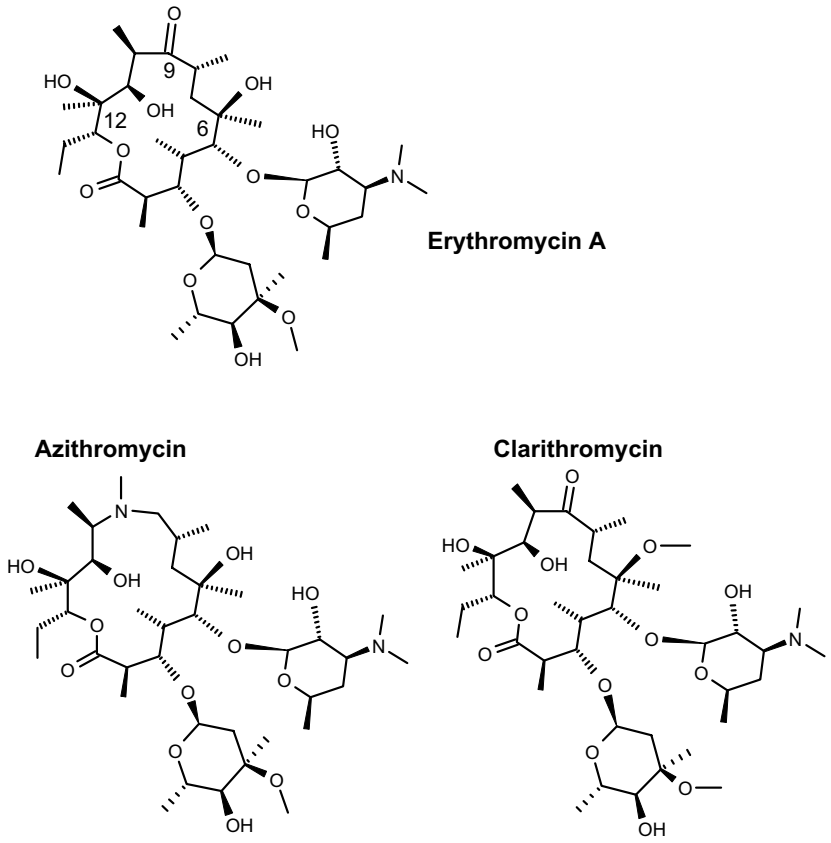


Figure 1. Structures of erythromycin A, azithromycin, and clarithromycin.

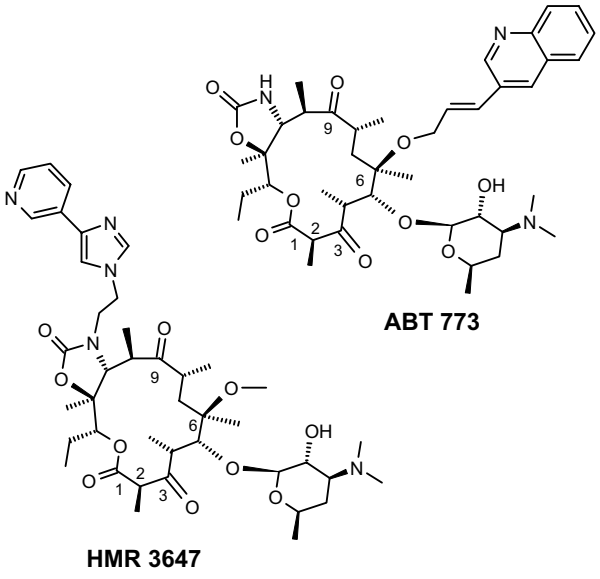


Figure 2. Structures of HMR3647 and ABT773.

that the 11,12-oxazolidinone ring plays a key role in enhancing the binding to the bacterial ribosome, likely through one or both oxygen atoms.

In our search for novel and more efficacious ketolides we turned our attention to the modification of the oxazolidinone ring fused at positions 11 and 12.

Replacement of this moiety with a γ-lactone (**1**), as depicted in Figure 4, was thought to offer advantages over the currently available ketolides. In particular, the presence of a C-sp^3 instead of a nitrogen atom was deemed very helpful in positioning of the side chain towards the common region occupied by the aromatic moiety present in ABT773 and HMR3647. This was predicted to result in a stronger interaction with the bacterial ribosome, hence in more potent antibacterial activity.

The antimicrobial activity of the parent compound **1** was determined in order to ensure that the activity against erythromycin sensitive bacterial strains was close to the antibacterial activity shown by **2**, a mandatory requirement to support further medicinal chemistry exploration. In this paper, we report the preparation of **1** and its antimicrobial activity in comparison with **2** and clarithromycin. The chemistry setup to prepare **1** was designed in order to permit the introduction of a side chain and an aromatic moiety, at position 21, that should result in a further enhancement of the antibacterial activity particularly against resistant bacterial strains.[24,25]

At the onset of this work, though intramolecular Michael additions of nucleophilic nitrogen of carbamate on intermediates of type 2, assisted by the 12-OH, were known,[26–29] no examples of such reaction using carbon as nucleophile were reported.

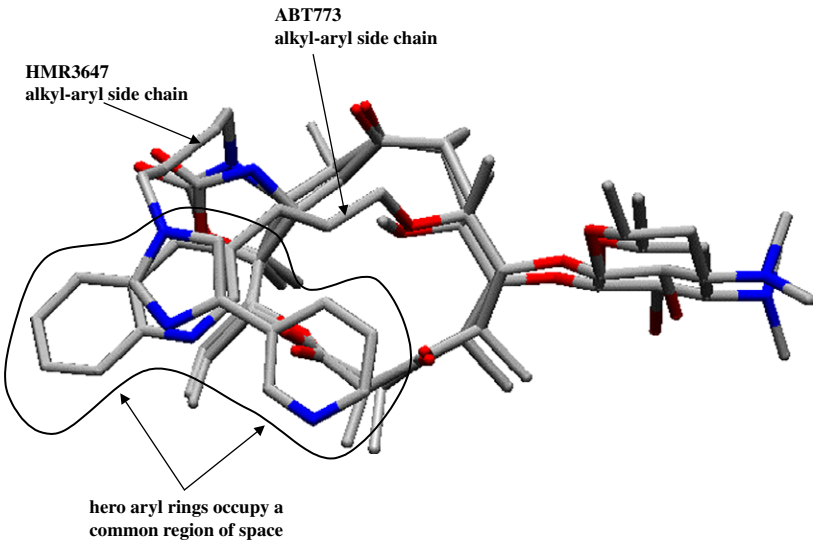


Figure 3. ABT773 and HMR3647 superimposition. The 'common' region of space occupied by the two different hetero-aryl rings is depicted with the curved solid line.

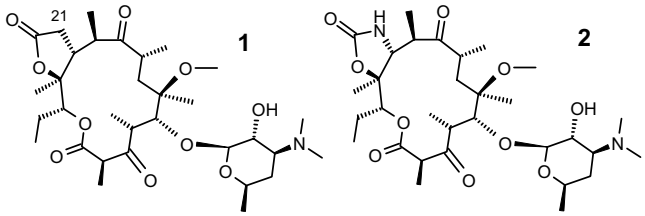


Figure 4. Structures of the ketolide prototype **1** where the nitrogen of the oxazolidinone ring of the ketolide **2** has been replaced with a C-sp^3.

We envisaged using a malonate moiety to form a carbon–carbon bond at position 11, and then exploiting the second malonate ester group to introduce a side chain (Scheme 1).

The L-cladinose sugar at position 3 of the macrolide was perceived as a protective group for the 3-OH, therefore acylation of the 12-OH was carried out directly on **2** using methylmalonyl chloride/pyridine in toluene to give intermediate **3**. Removal of the L-cladinose sugar under acidic condition turned out to be a slower reaction than expected. However, a mixture of compounds **4** and **5** was ultimately obtained by adding intermediate **3** into a 2-M HCl aqueous solution. The ratio of **4** and **5** was shown to be reaction-time-dependent.

The methylmalonate **4** was isolated by flash chromatography in yields ranging from 40% to 60%.

Cyclisation of **4** to give **6** was carried out in satisfactory yield (53%) in water–acetonitrile (9:1) using DBU as a promoter, however, poor diastereocontrol was observed at positions C-10 and C-21 (three main peaks in a ratio 1:1:3 were observed). Decarboxylation of the methyl ester was carried out heating intermediate **6** in DMSO in presence of LiCl[30] to give **7** in moderate yield (46%). Spectroscopic investigation of intermediate **7** showed only the presence of the depicted C-10 (*R*)-diastereomer (Fig. 5), H-10 at 3.00 ppm with NOE enhancement of the Me at C-8), suggesting that the C10 (*S*)-epimer was either lost during the purification process or it converts to **7** during the decarboxylation reaction. Oxidation of the 3-OH of intermediate **7** under Pfitzner–Moffat condition (EDC, TFAA, DMSO) gave the corresponding 3-keto derivative **8**. Overnight treatment with methanol cleaved the acetyl protective group on the D-desosamine moiety and compound **1** was isolated in 33% yield. Its absolute stereochemistry has been further confirmed by NOE studies (Fig. 5).

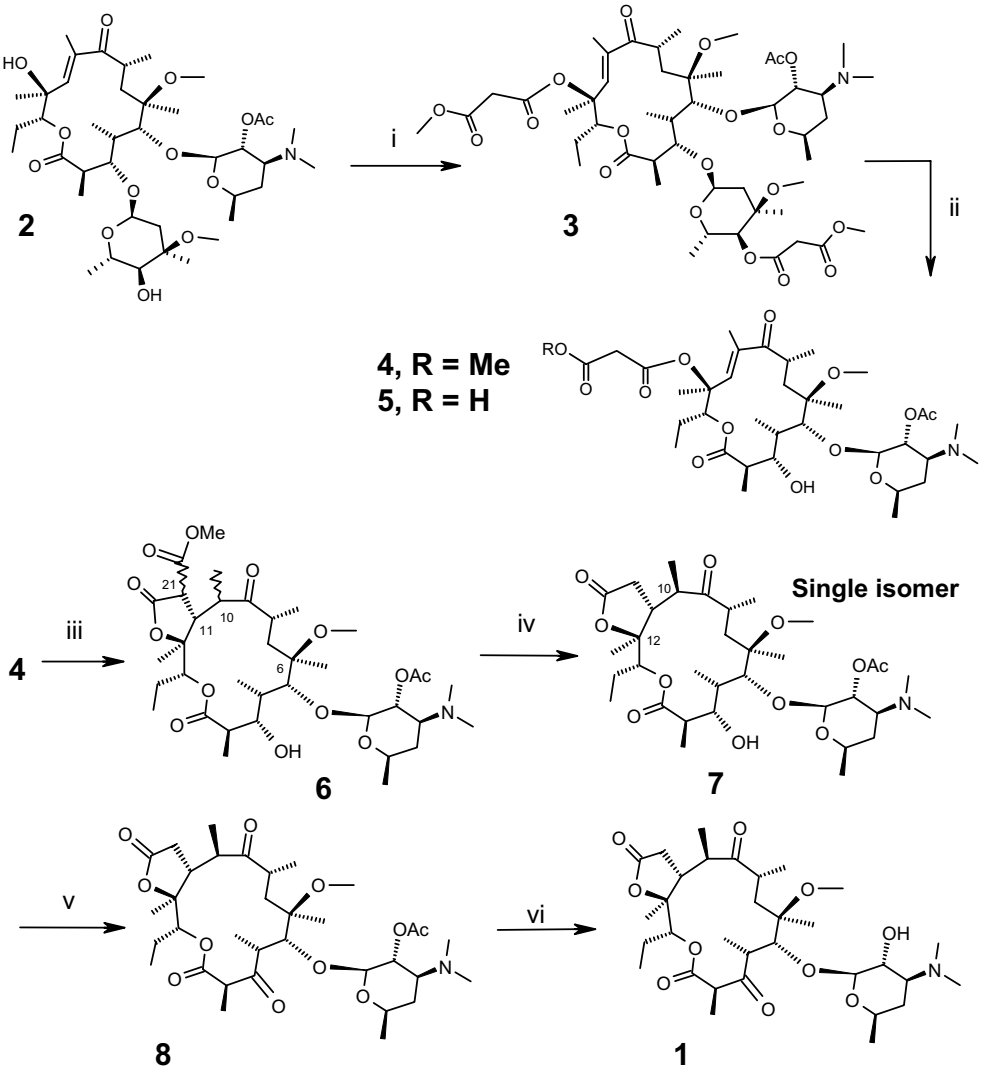
In general ketolides are inactive against *Escherichia coli* strains mainly because of an inherent permeability barrier, and against *Salmonella aureus* strains resistant because of constitutively expressed ribosome methylation.

The antimicrobial activity of compound **1** was determined in comparison with clarithromycin and the unsubstituted 11,12-oxazolidinone **2** (Fig. 4) and it is reported in Table 1.

Interestingly, **1** showed very good antibacterial activity comparable to both clarithromycin and **2**.

The microbiological profile observed with the unsubstituted prototype γ-lactone **1** against erythromycin-sensitive strains is of clear interest, showing potent activity against relevant bacteria strains in particular staphylococci and streptococci, with the exception of a borderline activity against *Haemophilus influenzae*. Of course, further studies aimed at the introduction of a suitable side chain at the C-21 position of **1** which might restore activity against both constitutive resistant strains and *H. influenzae*.

In summary, a novel class of ketolides has been identified; the novelty of this class resides in the presence of a γ-lactone between positions 11 and 12 of 6-O alkyl 3-oxoerythromycin derivatives.



Scheme 1. Reagents and conditions: (i) MeO_2CCH_2COCl, Py, toluene, rt, 1 h; (ii) HCl 2 N; (iii) DBU, CH_3CN/water (9/1), reflux, 6 h; (iv) LiCl, DMSO, reflux, 4 h; (v) EDC, DMSO, DCM, 0 °C, Py, TFAA, 3 h; (vi) MeOH.

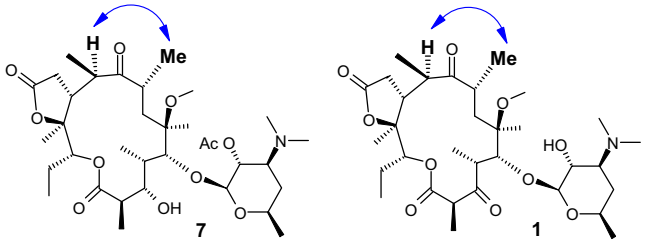


Figure 5. Most significant NOEs observed on compounds **7** and **1**.

Thus this new class appears to constitute an innovative and very promising vehicle for the delivery of novel ketolides with a suitable spectrum of activity for the treatment of infections caused by respiratory pathogens. Compound **1** represents an excellent starting point for a medicinal chemistry lead optimization programme by introduction of a side chain at C-21 to enhance the binding affinity to the ribosomes of resistant bacterial strains.

Table 1. In vitro antibacterial activity MIC (mg/L) of three novel ketolides and clarithromycin against selected aerobic Gram-positive and -negative pathogens

Strains	1	2	Clarithromycin
S. aur. ATCC13709 ery-S	0.5	0.25	0.25
S. aur. PK2 MLSc	>128	>128	>32
S. pne. ery S	⩽0.06	0.06	⩽0.06
S. pne. 4636 MLSc	>128	>128	>128
S. pne. CI137 M	0.12	0.25	8.0
S. pyo. 3565 ery S	0.12	0.06	⩽0.06
S. pyo. MLSi	0.5	0.06	2.0
S. pyo. M	0.12	0.25	2.0
M. cat. ATCC 23246	0.5	0.12	⩽0.25
H. inf. ATCC 49247	16	16	8.0
E. coli ATCC 25922	128	64	64

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.02.019.

References and notes

1. Chu, D. T. W. *Med. Res. Rev.* **1999**, *19*(6), 497.
2. Bryskier, A. *Expert. Opin. Invest. Drugs* **1999**, *8*(8), 1171.
3. Bryskier, A. *Expert. Opin. Invest. Drugs* **1997**, *6*(11), 1697.
4. Chu, D. T. W. *Expert. Opin. Invest. Drugs* **1995**, *4*(2), 65.
5. Kurath, P.; Jones, P.; Egan, R.; Perun, T. *Experientia* **1971**, *27*, 362.
6. Krowicki, K. *J. Antibiot.* **1974**, *26*, 569.
7. Wu, Y. J. *Curr. Pharma. Design* **2000**, *6*, 181.
8. Morimoto, S.; Takahashi, Y.; Watanabe, Y.; Omura, S. *J. Antibiot.* **1984**, *37*, 187.
9. Sakakibara, H.; Omura, S. In *Macrolide Antibiot*; Omura, S., Ed.; Academic: Orlando, Florida, 1984; pp 85–125.
10. Walsh, C. *Nat. Rev. Microbiol.* **2003**, *1*, 65.
11. Morris, A.; Kellner, J. D.; Low, D. E. *Curr. Opin. Microbiol.* **1998**, *1*(5), 524.
12. Neu, H. C. *Science* **1992**, *257*, 1064.
13. Appelbaum, P. C. *Clin. Infect. Dis.* **1992**, *15*, 77.
14. Yassin, H. M.; Dever, L. L. *Expert Opin. Invest. Drugs* **2001**, *10*, 353.
15. Munckhof, W. J.; Borlace, G.; Turmidore, J. D. *Antimicrob. Agents Chemother.* **2000**, *44*, 1749.
16. Dougherty, T. J.; Barrett, J. F. *Expert Opin. Invest. Drugs* **2001**, *10*, 343.
17. Andrews, J. M.; Weller, T. M. A.; Ashby, J. P.; Walker, R. M.; Wise, R. *J. Antimicrob. Chemother.* **2000**, *46*, 1017.
18. Xiong, L.; Sha, S.; Mauvais, P. *Mol. Microbiol.* **1999**, *31*(2), 633.
19. Douthwaite, S.; Mauvais, P.; Hansen, L. H. *Proceedings of the Fifth International Conference on the Macrolides, Azalides, Steptogramins, Ketolides and Oxazolidinones.* Seville, Spain, **2000**, Abstract 02.01.
20. Pestka, S.; Vince, R.; LeMahieu, R.; Weiss, F.; Fern, L.; Unowsky, J. *Antimicrob. Agents Chemother.* **1976**, *9*, 128.
21. LeMahieu, R.; Carson, M.; Kierstead, R. W.; Fern, L. M.; Grunberg, D. E. *J. Med. Chem.* **1974**, *17*, 953.
22. Pestka, S.; LeMahieu, R. *Antimicrob. Agents Chemother.* **1974**, *6*, 39.
23. Champney, W. S.; Tober, C. L. *Curr. Microbiol.* **1999**, *38*, 342.
24. Andreotti, D.; Biondi, S.; Lociuro, S. Patent WO0250092, 2002.
25. Andreotti, D.; Arista, L.; Biondi, S.; Cardullo, F.; Damiani, F.; Lociuro, S.; Marchioro, C.; Merlo, G.; Mingardi, A.; Niccolai, D.; Paio, A.; Piga, E.; Pozzan, A.; Seri, C.; Tarsi, L.; Terreni, S.; Tibasco, J. Patent WO0250091, 2002.
26. Agouridas, C.; Denis, A.; Auger, J. M.; Benedetti, Y.; Bonnefoy, A.; Bretin, F.; Chantot, J. F.; Dussarat, A.; Fromentin, C.; D'Ambrieres, S. G.; Lachaud, S.; Laurin, P.; Le Martret, O.; Loyau, V.; Tessot, N. *J. Med. Chem.* **1998**, *41*(21), 4080.
27. Denis, A.; Agouridas, C.; Auger, J.-M.; Benedetti, Y.; Bonnefoy, A.; Bretin, F.; Chantot, J.-F.; Dussarat, A.; Fromentin, C.; D'Ambrieres, S. G.; Lachaud, S.; Laurin, P. K.; Le Martret, O.; Loyau, V.; Tessot, N.; Pejac, J.-M.; Perron, S. *Bioorg. Med. Chem. Lett.* **1999**, *9*(21), 3075.
28. Or, Y. S.; Clark, R.; Chu, D.; Plattner, J. J. Patent WO 9809978, 1998.
29. Asaka, T.; Kashimura, M.; Manaka, A.; Tanikawa, T.; Sugimoto, T. Patent WO 0110879, 2001.
30. Krapcho, A. P.; Weimaster, J. F.; Eldridge, J. M.; Jahnger, J. r.; Lovely, A. J.; Stephens, W. P. *J. Org. Chem.* **1978**, *43*(1), 138.