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SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF HMR 3647 A NEW KETOLIDE HIGHLY POTENT AGAINST ERYTHROMYCIN-RESISTANT AND SUSCEPTIBLE PATHOGENS[1]

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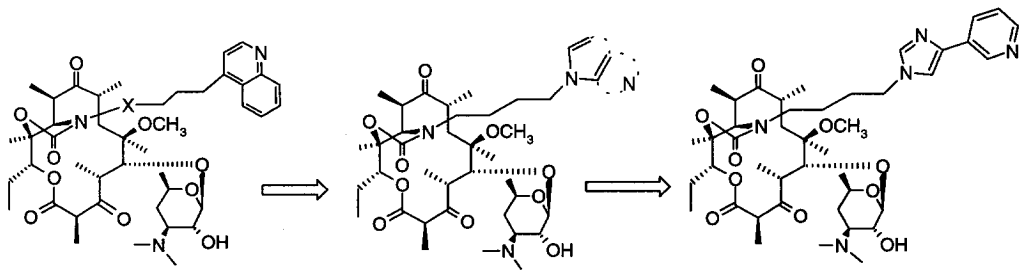
Abstract: In the search for new ketolides with improved activities against erythromycin-resistant *S. pneumoniae* and *H. influenzae* we synthesized a new 11,12 carbamate ketolide substituted by an imidazo-pyridyl side chain: HMR 3647. This compound demonstrated a potent activity against erythromycin susceptible and resistant pathogens, including penicillin G/erythromycin A-resistant *S. pneumoniae* and *H. influenzae*. *In vivo*, HMR 3647 displayed good pharmacokinetic parameters (Cmax = 2.9 µg/ml, bioavailability= 49%, AUC_{0-8} = 17.2 µg.h/l, $t_{1/2}$= 1h) and was shown to have a high therapeutic efficacy in mice infected by various respiratory pathogens, including multi-resistant *S. pneumoniae* and Gram negative bacteria such as *H. influenzae*. HMR 3647 appears to be a very promising agent for the treatment of respiratory infections and is currently in clinical trials.

The extensive clinical application of macrolide antibiotics has resulted during the last decades in an increasing emergence of macrolide resistance in Gram positive cocci[2], especially *S. pneumoniae*. At the same time, the resistance of *S. pneumoniae* to penicillin, frequently associated with a a high percentage of cross resistance to macrolides, has continuously grown[2b,d,e]. This situation has driven researches towards new generation of macrolides that can overcome the problem of macrolide resistance while maintaining the usually excellent safety profile of this class of antibiotics. The ketolides are a newer major class of semisynthetic erythromycin derivatives discovered in the search for new antibiotics active against erythromycin-resistant *S. pneumoniae* and *H. influenzae*[3,4]. They are characterized by a 3-keto function instead of the L-cladinose sugar residue which has long been thought essential for the antibacterial activity of erythromycin. The ketolides were found to be very active against penicillin G/erythromycin A-resistant *S. pneumoniae* and non inducers of MLS_B resistance. They were effective against respiratory pathogens with a spectrum that covers multiresistant *S. pneumoniae*, *H. influenzae*, group A streptococci, intracellular bacteria: *Legionella* spp. and *Chlamydia* spp., and atypical microorganisms such as *Mycoplasma pneumoniae*[3,4].



HMR 3004 X=NH
1 X=CH_2

HMR 3647

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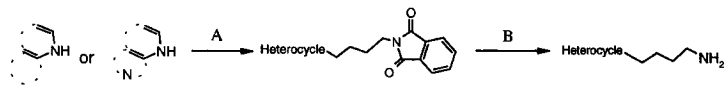
We recently reported that in addition to the 3-keto function, further modifications of the macrolactone backbone allowed the discovrey of some highly potent 11,12-carbamate and 11,12-hydrazonocarbamate ketolides. The structure activity relationships (SAR) generated by this series of molecules, clearly demonstrated the importance of the hetero-aryl side chain for the *in vitro and in vivo* activity[4]. This was also later demonstrated in different series synthesized at Abbott Laboratories[5] (e.g. 6-O-substituted ketolides[5b] and aza-imino tricyclic ketolides[5c]). The very good results obtained with the hydrazono-carbamate series, prompted us to reinvestigate the role of the heterocyclic moiety in the carbamate series. According to the results previously obtained with the quinoline carbamate **1** structure that generated the optimized hydrazono ketolide HMR 3004[4], we speculated that additional heterocyclic nitrogen would be beneficial for the antibacterial activity.

Therefore the strategy was to introduce several new heterocyclic groups e.g. aryl-imidazoles, benzimidazoles, triazoles in position 4 of the butyl side chain, with the aim of finding new carbamate ketolides with higher antibacterial activities and /or improved pharmacokinetics.

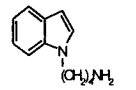
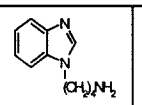
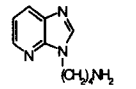
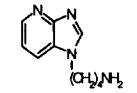
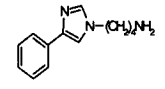
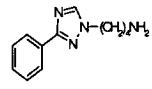
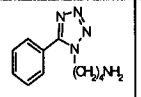
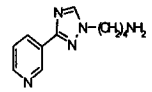
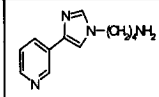
Chemistry

According to our previous report[4], the desired 11, 12-cyclocarbamate ketolides were all synthesized by stirring the 12 acyl-imidazolyl ketolide **2** in CH_3CN/H_2O with the corresponding amines at 60°C (Scheme 2, Table 1). The synthesis of the amino butyl heterocyclic amines was achieved by alkylation of the appropriate heterocycles with 4-bromophtalimide in the presence of NaH or K_2CO_3 in DMF, followed by hydrazinolysis of the phtalimido intermediates (Scheme 1). The overall yield for the two steps ranged between 24 to 64%. In the case of the imidazo(4,5-b)pyridine, the alkylation using K_2CO_3 in DMF afforded the two alkylated isomers A and B in a 60/40 ratio. Most of the heterocycles were commercially available with the exception of 3-phenyl triazole[6], 3-(3-pyridyl)-1H-triazole[7] and 4-(3-pyridyl)-1H-imidazole[8] that were prepared according to the literature procedures.

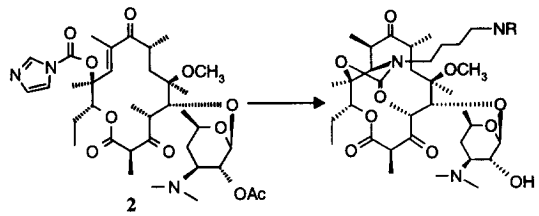
Scheme 1: Synthesis of 4-aminobutyl heterocycles



A: NaH or K_2CO_3, 4-Bromophtalimide, DMF; B: NH_2NH_2-H_2O, EtOH.

Compd			 iso A	 iso B	
yields	49%	64%	29%	24%	45%
Compd					
yields	44%	52%	49%	36%	

Scheme 2: Synthesis carbamate ketolides



1- $RN(CH_2)_4NH_2$, CH_3CN, H_2O, 60°C; 2-MeOH, r.t.

Compd	3	4	5a	5b	6	7	8	9	HMR 3647[9]
NR									
yields	24%	28%	42%	40%	33%	32%	49%	30%	50%

Results and discussion

All the ketolides were tested *in vitro* by standard agar dilution method against both erythromycin-susceptible and erythromycin-resistant staphylococci, streptococci and *S. pneumoniae* including constitutive (EryRc) and inducible (EryRi) phenotype. In addition one strain of *Haemophilus influenzae* and *E. coli* were also tested.

All the compounds were inactive against *E.coli* and erythromycin-resistant (MLS_B constitutive type) strains of *S. aureus* (MIC >40 μg/ml). They were very effective against inducibly erythromycin-resistant staphylococci and *S. pneumoniae* as well as constitutively resistant *S. pneumoniae*. With the exception of the indole derivative, they were as potent as azithromycin against *H. influenzae*. (Table 1). Without exception all tested macrolides (clarithromycin and azithromycin) were inactive (MICs > 40 μg/ml) against erythromycin resistant strains whatever the phenotype. The fused bicyclo-heterocyclic compounds (**3, 4, 5**) were less active against inducibly resistant *S. pneumoniae* than the parent compound **1**. However, they were slightly more active against *Haemophilus influenzae*. Within the non fused bicyclo-heterocyclic compounds, the tetrazole **8** was shown to be poorly active while the triazole and imidazole derivatives **6** and **7** were equivalent to **1** . Finally, introduction of an heteroatom in the aryl group, as demonstrated by the two pyridyl derivatives **9** and HMR 3647, allowed us to obtain the desired profile of antibacterial activity. The introduction of an imidazolo-pyridyl group in the side chain resulted in a dramatic increase of activity against erythromycin-resistant (MLS_B constitutive type) *S. pneumoniae*. HMR 3647 was very potent against both resistant and sensitive Gram positive pathogens (MICs between 0.15-0.02 μg/ml) and comparable to azithromycin against *H. influenzae*.

Due to its very promising profile HMR 3647 was tested against several respiratory pathogens including many clinically isolated *S. pneumoniae* (erythromycin A and penicillin G resistant) and also against *Staphylococcus* spp. and *H. influenzae*. As shown in Table 2 the MIC_{50} and MIC_{90} of HMR 3647 against most resistant and sensitive pathogens indicated the potential therapeutic value of this ketolide. With the exception of EryRc *S. aureus* the growth of 90% of *Staphylococcus* and *Streptococcus* spp. and *H. influenzae* was inhibited within a range of concentration of 0.02 to 1.2 μg/ml (Table 2).

Table 1 . *In vitro* evaluation of 11,12-carbamate ketolides (MIC, µg/ml)

Compd	*S. a.*[a] EryS[b] 011UC4	*S. a.* EryRi 011GO25i	*S. a.* EryRc 011CB20	*S. pyo.* EryS 02A1UC1	*S. p.* EryS 032UC1	*S. p.* EryRc 030PW23c	*S .p.* EryRc 030CR18c	*S. p.* EryRc 030SJ1	*S. p.* EryRi 030SJ5i	*H. i.* 351HT3	*E. c.* 250 UC5
CLA[c]	0.3	40	40	0.08	0.04	40	40	40	40	5	40
AZI[d]	0.3	40	40	0.6	0.15	40	40	40	40	1.2	20
1	0.04	0.08	40	0.02	0.02	1.2	1.2	1.2	0.02	1.2	40
2	0.08	0.15	40	0.02	0.02	10	10	10	0.08	2.5	20
3	0.04	0.08	40	0.02	0.02	2.5	2.5	2.5	0.08	0.6	10
4a	0.04	0.15	40	0.02	0.08	2.5	2.5	1.2	0.04	0.6	10
4b	0.08	0.15	40	0.02	0.02	5	2.5	2.5	0.15	0.6	10
5	0.04	0.08	40	0.02	0.02	2.5	2.5	5	0.04	1.2	20
6	0.08	0.3	40	0.02	0.02	20	1.2	2.5	2.5	2.5	40
7	0.02	0.15	40	0.02	0.04	1.2	1.2	0.6	0.3	1.2	40
8	0.02	0.6	40	0.02	0.02	0.6	1.2	0.3	0.02	1.2	20
HMR 3647	0.04	0.08	40	0.02	0.02	0.08	0.15	0.08	0.02	1.2	10

[a] *S. a.: Staphylococcus aureus; S. p.: Streptococcus pneumoniae; S. pyo.: Streptococcus pyogenes; H. i.: Haemophilus influenza; E. c.: Escherichia.coli.* [b] EryS= erythromycin susceptible, EryRc= constitutively erythromycin resistant, EryRi= inducibly erythromycin resistant; [c] CLA: clarithromycin; [d] AZI: azithromycin.

Table 2: Extended *in vitro* evaluation of HMR 3647(MIC_{50}/MIC_{90} µg/ml)

Compd	Staphylococci EryRi (66)[a]	EryRc (23)	Streptococci EryR (31)	*S. pneumoniae* EryRi (32)	EryRc (40)	PenR (53)	*H. influenzae* AmpS (47)	AmpR (39)
CLA	40/40	ND	1.2/40	40/40	40/40	0.3/40	1.2/5	2.5/10
AZI	40/40	ND	10/40	40/40	40/40	2.5/40	0.15/1.2	0.3/1.2
Amp[b]	ND	ND	ND	ND	ND	ND	0.3/0.6	1.2/10
HMR 3647	0.08/0.3	40/40	0.02/0.08	0.005/0.02	0.04/0.3	0.005/0.15	0.6/1.2	0.6/0.6

[a] Number of tested strains. [b] Amp: ampicillin

The *in vitro* efficacy of HMR 3647 against EryRi Gram positive isolates and the kinetics of induction carried out in separate experiments[10a] clearly demonstrated that unlike available 14 membered-ring macrolides, HMR 3647 was not able to induce MLS_B resistance. This was in agreement with the results previously obtained with other ketolides[10b].

In vivo evaluation

In acute murine lethal infections models caused by Gram positive cocci susceptible to erythromycin, HMR 3647 exhibited an *in vivo* efficacy equivalent to CLA and better than AZI which was poorly efficient in septicaemia caused by *S. aureus*. In EryRi staphylococcal infections HMR 3647 was about 10 times more active than CLA . Unlike CLA and AZI which showed complete inactivity with PD_{50} up to 100 mg/kg, HMR 3647 displayed a good anti-pneumococcal therapeutic efficacy in infections induced by EryRi or EryRc *S. pneumoniae*. The corresponding effective doses for HMR 3647 ranged between 4 and 15 mg/kg. It should be noted that the protective doses found for HMR 3647 in EryR pneumococcal infections fall within the range of values found with susceptible pathogens (1-16mg/kg).

In infections caused by *H. influenzae* HMR 3647 generally demonstrated a therapeutic activity 2 to 7 times higher than that observed for CLA. Conversely, HMR 3647 displayed an activity comparable to AZI .

Table 3: ***In vivo* evaluation of HMR 3647 against Gram positive pathogens and *H. influenzae***

ED_{50} in mice (mg/kg)[a]

	S. a. 011UC4 EryS	*S. a.* 011GO3 EryRi	*S. p.* 032UC1 EryS	*S. p.* 030RO1 EryRi	*S. p.* 030SJ6 EryRc	*S. p.* 030MV2 EryRc	*S. p.* 030SJ1 EryRc	*S. p.* 030Cr29 EryRc	*S. pyo.* 02A1UC1 EryS	*H.i.* 351GR1	*H.i.* 351TO19 AmpR β(-)	*H.i.* 351RD7 AmpR β(+)
AMP	ND	ND	ND	ND	ND	ND	ND	ND	ND	5.5	39	>600
CLA	6	55	7.5	>50	>50	>50	>50	>50	16	71	>300	120
AZI	30	>100	6	>50	>50	>50	>50	>50	16	56	145	94
HMR 3647	10	4.5	1	4	6.5	4	15	4.5	16	68	40	57

[a] Effective dosage that protect 50% of mice from lethal infection after oral administration.

Pharmacokinetics

After oral administration of 10 $mg.kg^{-1}$ in mouse, the pharmacokinetic parameters measured: Cmax = 2.9 µg/ml, bioavailability= 49%, $AUC_{0\text{-}8}$ = 17.2 µg.h/l, $t_{1/2}$= 1h, were in good agreement with the observed *in vivo* activity, the Cmax of 2.9 µg/ml being well above the MICs of targeted pathogens. Furthermore, additional studies have shown that the AUC/MIC ratio has been found to be the factor that best correlated with the *in vivo* efficacy[11].

Conclusion

In summary, the introduction of additional heterocyclic nitrogens into the carbamate ketolide side chain was beneficial with regards to the activity of HMR 3647 towards constitutively resistant to erythromycin *S. pneumoniae*. Moreover, HMR 3647 was shown to have a potent activity against *S. aureus* and *S. pneumoniae* with inducible resistance to erythromycine and against *Haemophilus influenzae*, combined with a well-balanced spectrum regarding its activity against Gram positive resistant and susceptible strains. In addition this compound exhibits a good activity against *Moraxella catarrhalis*[12a] and intracellular bacteria e.g. *Chlamydia*[12b] and *Legionella*[12c,d]. Like all ketolides, HMR 3647 was found to be non inducer of the MLS_B resistance phenotype and to be active against pathogens inducibly resistant to erythromycin. Furthermore, this compound demonstrated a significant activity *in vivo* associated with good pharmacokinetic behavior. Thus, HMR 3647 which is now currently in clinical trials, appears as an innovative and promising new antibacterial agent.

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References and notes

[1] This paper is dedicated to the memory of J.-F. Chantot and J.-M. Auger.

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[9] Spectral data for HMR 3647 : mp: 186-188°C; FAB-MS= 812^+ ($M+H^+$); ^1H NMR ($CDCl_3$): δ 0.83 (t, J= 7 Hz, 3H) $\underline{CH_3}CH_2$, 1.00 (d, J= 7 Hz, 3H) **10-CH_3**, 1.16 (d, J= 7 Hz, 3H) **8-CH_3**, 1.23-1.66 (m, 1H) H_4', 1.24 (d, J= 6 Hz, 3H) **5'-Me**, 1.31 (d, J= 7.5 Hz, 3H) **4-CH_3**, 1.33 (s, 3H) **6-CH_3**, 1.37 (d, J= 7 Hz, 3H) **2-CH_3**, 1.47 (s, 3H) **12-CH_3**, 1.60-1.83 (m, 1H) H_7, 1.66-1.88 (m, 4H) CH_2-CH_2, 1.57-1.94 (m, 2H) H_{14}, 2.26 (s, 6H) **N($\underline{CH_3}$)$_2$**, 2.44 (m, 1H) H_3', 2.59 (m, 1H) H_8, 2.62 (s, 3H) **6-OCH_3**, 3.06 (m, 1H) H_4, 3.14 (q, J= 7 Hz, 1H) H_{10}, 3.18 (dd, J= 7.5 and 10 Hz, 1H) H_2', 3.52 (m, 1H) H_5', 3.56 (s, 1H) H_{11}, 3.65-3.78 (m, 2H) **$\underline{CH_2}$NCO**, 3.87 (q, J= 6.5 Hz, 1H) H_2, 4.01 (m, 2H) **$C\underline{H_2}$N**, 4.23 (d, J= 9 Hz, 1H) H_5, 4.27 (d, J= 7.5 Hz, 1H) H_1', 4.92 (dd, J= 2 and 10.5 Hz, 1H) H_{13}, [7.54 (s, 1H) H_2, 7.34 (s, 1H) H_5] **imidazole**, [8.97 (d, J= 1.5 Hz, 1H) H_2, 8.08 (dt, J= 8 and 1.5 Hz, 1H) H_4, 7.28 (dd, J= 5 and 8 Hz, 1H) H_5, 8.45 (dd, J= 1.5 and 5 Hz, 1H) H_6] **pyridine**. Anal. Calc. (%) for $C_{43}H_{65}N_3O_{10}$: C 63.6, H 8.07, N 8.62. Found : C 63.5, H 8.1, N 8.5.

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