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15-Amido Erythromycins: Synthesis and *in Vitro* Activity of a New Class of Macrolide Antibiotics

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Abstract An array of 15-amido substituted erythromycin A compounds was synthesized using a chemobiosynthesis approach. It was found that while the *in vitro* antibacterial activities of aryl amides were inferior to erythromycin A, substituted benzylamides showed equivalent and in some cases improved activity against the macrolide-resistant strains. The 15-amidoerythromycins represent a new class of antibacterial macrolides.

Keywords chemobiosynthesis, erythromycin, antibacterial, diketide

Introduction

The macrolide antibiotic erythromycin A (**1**, Figure 1) introduced in the 1950's, continues to be a widely used orally administered antibiotic for community-acquired infections. It suffers several drawbacks, such as sensitivity to acidic conditions in the stomach resulting in formation of a prokinetic 8,9-anhydro-6,9-hemiketal and loss of activity due to bacterial resistance through multiple mechanisms, especially methylation of the ribosome (*erm*) or efflux (*mef*) [1]. To overcome acidic instability, a second generation of macrolides was generated in which formation of a 6,9-hemiketal was prevented, for example by blocking the 6-hydroxyl as in clarithromycin (**2**) [2]. These compounds do not overcome the current major forms of bacterial resistance, however. Ketolides, derivatives of erythromycin in which the C-3 cladinosyloxy group is replaced by a ketone, were first reported in 1995 [3]. These improved macrolides show good activity even against most relevant erythromycin A-resistant organisms and one of these, telithromycin **3**, is now approved for use in the United States and is on the market in Europe [4].

The ketolide skeleton itself possesses poor antibacterial activity, but introduction of an aromatic group linked to the macrolide ring through either the 6-position (as in cethromycin) or through attachment to an 11,12-carbamate (as in telithromycin) has been found to greatly enhance potency [5]. These compounds have the aromatic group attached to opposite sides of the macrolide, yet they seem to make similar contacts with domain II of the ribosome, resulting in improved binding [6, 7]. Removal of the cladinosyloxy group also appears to minimize induction of *erm*(B) resistance and efflux-based resistance [8]. Thus, ketolides such as **3** retain activity against both *erm*(B)- and *mef*-containing *Streptococcus pneumoniae*. Activity of ketolides against staphylococci and *Haemophilus influenzae* is modest, and staphylococci constitutively expressing the *erm* methylase are typically fully resistant.

We have previously reported the production of novel erythromycins through chemobiosynthesis [9]. In this process, synthetic starter units are introduced into the erythromycin polyketide by feeding a diketide analogue to an engineered strain in which the ketosynthase domain of the polyketide synthase module 1 has been inactivated. We recognized that it might be possible to use this technology to introduce aromatic side-chains that might provide similar improvements in activity against resistant organisms to that

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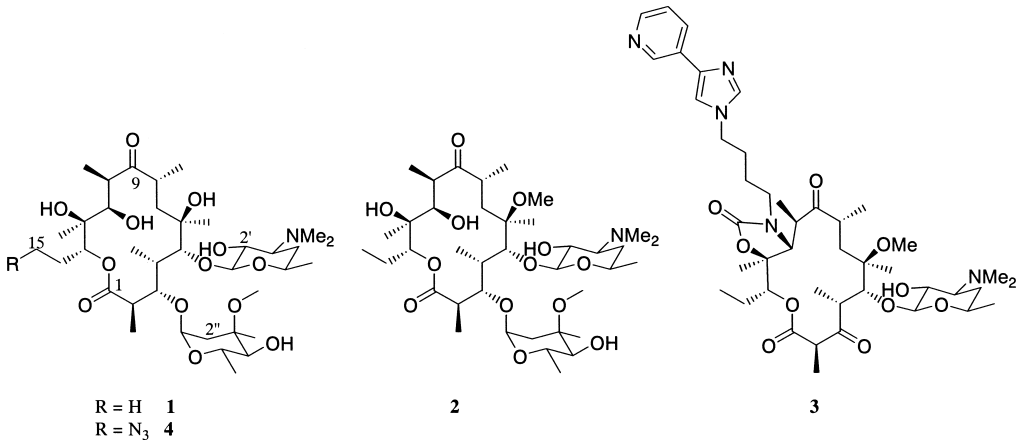


Fig. 1

obtained in the case of **3**.

As an initial test of this theory, we have introduced a range of aromatic groups onto the 15-position of erythromycin through an amide linkage. Many of these compounds maintain activity or in some cases show improved activity against both sensitive and resistant organisms. We have further developed a synthetic strategy amenable to combinatorial production of analogues, which provided an initial structure-activity relationship for this new class of antibacterial agent.

Materials and Methods

Antibiotics and Microorganisms

Erythromycin was obtained from Sigma. Bacterial strains were from ATCC as indicated, or were clinical isolates.

Bacterial Susceptibility Testing

MIC values for reference and test compounds were determined in Mueller-Hinton broth (BBL) by the broth microdilution method according to NCCLS guidelines [10].

Compound Characterization

NMR spectra were obtained in $CDCl_3$ at ambient using a Bruker DRX-400 spectrometer fitted with a 3-mm proton-carbon probe (Nalorac). ^1H spectra were obtained at 400 MHz and ^{13}C spectra were obtained at 100 MHz. Exact masses were obtained using a Mariner time-of-flight mass spectrometer (Applied Biosystems) with electrospray ionization. Samples were introduced by flow injection using 4 : 1 CH_3CN/CH_3OH containing 5 mM NH_4OAc and three standard compounds whose *m/z* ratios were used to calibrate the exact mass.

Preparation of 15-Azido-2′-acetyl-erythromycin A (5)

To a solution of 15-azido-erythromycin A [11] (2.00 g, 2.58 mmol, 1.0 eq) in dichloromethane (20 ml) was added acetic anhydride (0.48 ml, 5.16 mmol, 2.0 eq) and the solution stirred at room temperature for 1.5 hours. The solution was partitioned between EtOAc (40 ml) and $NaHCO_3$ (40 ml) and the organics further washed with brine (30 ml) before drying ($MgSO_4$) and concentrating under reduced pressure. MPLC (0 → 20 minutes 15 → 40% acetone - hexane, 1% Et_3N; 20 → 40 minutes 40% acetone - hexane, 1% Et_3N) yielded the title compound as a white solid (1.60 g, 76%); NMR data is given in Tables 1 and 3.

General Procedure for Amide Formation and Deprotection

To a solution of 15-azido-2′-acetyl-erythromycin A (0.050 g, 0.062 mmol, 1.0 eq) in dichloromethane or THF (1.0 ml) was added a 1.0 M solution of trimethylphosphine in THF (0.31 ml, 0.31 mmol, 5.0 eq). The solution was stirred at room temperature for 45 minutes before transferring to a solution of the carboxylic acid (0.092 mmol, 1.5 eq), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (0.019 g, 0.099 mmol, 1.6 eq) and 1-hydroxybenzotriazole (0.017 g, 0.124 mmol, 2.0 eq) in dichloromethane or THF (1.0 ml) also stirred at room temperature for 45 minutes. The resulting solution was stirred at room temperature for 14 hours before partitioning between EtOAc (10 ml) and $NaHCO_3$ (10 ml). The aqueous phase was extracted with EtOAc (3×10 ml) and the combined organics further washed with brine (25 ml) before drying (Na_2SO_4) and concentrating under reduced pressure. The residue was dissolved in methanol (2 ml) and stirred at 50°C for 14 hours before cooling and concentrating under reduced pressure. MPLC (0 → 10 minutes 50% acetone - hexane, 1% Et_3N; 10 → 20 minutes 60% acetone - hexane, 1% Et_3N;

Table 1 Proton shifts for the macrolide protons (Me2, Me4, Me6, Me8, Me10, and Me12 are between 1.30 and 1.10 ppm for all compounds)

Proton	**4**	**5**	**7a**	**7b**	**7c**	**7d**	**7e**	**7f**	**7g**	**7h**	**7i**
H-2	2.87	2.82	2.94	2.91	2.87	2.89	2.77	2.82	2.81	2.82	2.85
H-3	4.00	3.95	4.09	4.07	3.96	4.02	3.93	4.00	3.94	4.12	3.97
H-4	1.96	1.90	1.96	2.02	1.83	2.01	1.91	1.95	1.89	1.94	1.99
H-5	3.58	3.50	3.61	3.57	3.52	3.54	3.51	3.54	3.52	3.53	3.54
H-7a	1.90	1.78	1.96	1.92	1.94	1.90	1.86	1.87	1.88	1.90	1.81
H-7b	1.71	1.64	1.74	1.73	1.70	1.71	1.70	1.70	1.69	1.72	1.74
H-8	2.69	2.67	2.66	3.10	3.88	2.68	2.63	2.69	2.59	2.68	2.65
H-10	3.08	3.06	3.10	3.09	3.04	3.07	3.03	3.03	3.02	3.05	3.06
H-11	3.84	3.81	3.98	3.92	3.86	3.90	3.75	3.82	3.75	3.81	3.83
H-13	5.17	5.17	5.27	5.27	5.24	5.27	4.93	5.06	5.04	5.05	5.07
H-14a	2.20	2.20	2.04	2.25	2.05	2.24	2.04	2.07	2.04	1.94	1.98
H-14b	1.72	1.71	1.54	1.79	1.63	1.99	1.59	1.64	1.59	1.58	1.64
H-15a	3.32	3.32	3.93	3.92	3.46	3.84	3.85	3.52	3.57	3.47	3.60
H-15b	3.24	3.22	3.08	3.05	3.07	3.01	2.82	2.92	2.86	2.85	2.76
NH	na	na	7.58	7.18	7.11	6.82	6.02	6.75	6.98	5.86	6.02
H-1′	4.40	4.53	4.42	4.41	4.37	4.41	4.38	4.38	4.39	4.39	4.39
H-2′	3.22	4.75	3.22	3.21	3.20	3.20	3.22	3.20	3.19	3.21	3.21
H-3′	2.44	2.61	2.43	2.42	2.40	2.48	2.45	2.43	2.42	2.44	2.44
H-4′a	1.68	1.72	1.67	1.66	1.66	1.68	1.66	1.64	1.64	1.69	1.64
H-4′b	1.24	1.27	1.26	1.24	1.23	1.24	1.25	1.22	1.23	1.24	1.24
H-5′	3.49	3.47	3.47	3.47	3.45	3.46	3.46	3.48	3.47	3.48	3.47
H-6′	1.22	1.27	1.25	1.22	1.22	1.28	1.25	1.23	1.23	1.24	1.24
NMe	2.27	2.26	2.28	2.28	2.26	2.30	2.29	2.27	2.27	2.28	2.28
H-1″	4.88	4.88	4.84	4.88	4.82	4.85	4.80	4.82	4.79	4.85	4.86
H-2″a	2.35	2.36	2.34	2.36	2.32	2.33	2.32	2.31	2.35	2.35	2.35
H-2″b	1.58	1.60	1.54	1.55	1.51	1.53	1.54	1.55	1.5	1.58	1.53
H-4″	3.02	3.02	2.98	3.05	2.99	2.99	3.01	2.98	3.00	3.00	2.99
H-5″	3.98	3.98	4.02	4.00	3.97	3.99	4.00	3.98	3.98	4.10	4.00
H-6″′	1.28	1.22	1.27	1.28	1.23	1.25	1.28	1.23	1.25	1.25	1.25
OMe	3.31	3.35	3.31	3.32	3.29	3.30	3.30	3.29	3.29	3.30	3.31

20→30 minutes 60% acetone - hexane, 1% Et_3N; 30+ minutes 80% acetone - hexane, 1% Et_3N) yielded a 15-amido compound as a white solid after lyophilization from benzene.

15-(3-Quinolylcarboxyamido)erythromycin A (7a)

NMR data: see Tables 1 and 3 for macrolide assignments. Side-chain assignments: ^1H (400 MHz, $CDCl_3$) δ 9.20 (1H, s, ArH-2), 8.66 (1H, s, ArH-4), 8.12 (1H, d, J=8.5 Hz, ArH-8 or ArH-5), 7.90 (1H, d, J=8.0 Hz, ArH-8 or ArH-5), 7.77 (1H, t, J=8.0 Hz, ArH-7 or ArH-6), 7.58 (1H, t, J=8.0 Hz, ArH-7 or ArH-6). ^{13}C (100 MHz, $CDCl_3$) δ 148.9, 148.3, 136.1, 133.8, 131.0 (2C), 129.7, 128.9, 127.2. MS m/z 906 $(M+H)^+$ (Found: $(M+H)^+$, 904.5116. $C_{47}H_{73}N_3O_{14}$ requires $(M+H)^+$, 904.5163).

15-(6-Quinolylcarboxyamido)erythromycin A (7b)

NMR data: see Tables 1 and 3 for macrolide assignments. Side-chain assignments: ^1H (400 MHz, $CDCl_3$) δ 8.97 (1H, dd, J=1.5, 4.0 Hz, ArH-2), 8.33 (1H, d, J=2.0 Hz, ArH-5), 8.25 (1H, dd, J=1.5, 8.0 Hz, ArH-4), 8.14 (1H, d, J=9.0 Hz, ArH-8), 8.08 (1H, dd, J=2.0, 9.0 Hz, ArH-7), 7.45 (1H, dd, J=4.0, 8.0 Hz, ArH-3). ^{13}C (100 MHz, $CDCl_3$) δ 151.8 (ArC-2), 149.3 (ArC-8a), 137.2 (ArC-4), 132.6 (ArC-6), 129.9 (ArC-8), 127.7 (ArC-5), 127.6 (ArC-4a), 127.3 (ArC-7), 121.8 (ArC-3). MS m/z 905 $(M+H)^+$, 747 $(M+H\text{-cladinose})^+$, 453 $(M/2+H)^+$ (Found: $(M+H)^+$, 904.5164. $C_{47}H_{73}N_3O_{14}$ requires $(M+H)^+$, 904.5165).

15-(4-Quinolylcarboxyamido)erythromycin A (7c)

NMR data: see Tables 1 and 3 for macrolide assignments.

Table 2 Proton shifts for the macrolide protons (Me2, Me4, Me6, Me8, Me10, and Me12 are between 1.30 and 1.10 ppm for all compounds)

Proton	7j	7k	7l	7m	7n	7o	7p	7q	7r	7s
H-2	2.70	2.86	2.78	2.80	2.80	2.81	2.80	2.83	2.78	2.88
H-3	4.04	4.02	3.95	3.97	3.98	3.96	3.97	4.01	3.95	3.93
H-4	1.89	2.00	1.93	1.93	1.94	1.93	1.82	1.97	1.98	2.02
H-5	3.90	3.57	3.54	3.57	3.55	3.53	3.49	3.55	3.57	3.57
H-7a	1.96	1.96	1.86	1.89	1.92	1.87	1.90	1.89	1.86	1.85
H-7b	1.68	1.72	1.70	1.72	1.72	1.73	1.68	1.74	1.70	1.58
H-8	2.61	2.71	2.67	2.62	2.69	2.62	2.69	2.70	2.68	2.80
H-10	3.05	3.08	3.04	3.03	3.02	3.05	3.09	3.08	3.01	3.08
H-11	4.10	3.84	3.77	3.79	3.80	3.84	3.79	3.83	3.79	3.79
H-13	5.07	5.16	4.99	5.02	5.02	5.06	5.02	5.05	5.03	5.27
H-14a	2.17	2.10	1.89	2.00	2.00	2.04	2.02	2.03	1.96	2.08
H-14b	1.65	1.67	1.53	1.58	1.59	1.58	1.57	1.55	1.56	1.68
H-15a	3.64	3.49	3.44	3.51	3.48	3.53	3.53	3.6	3.52	3.25
H-15b	2.80	2.99	2.85	2.82	2.84	2.83	2.84	2.81	3.02	3.06
NH	6.13	6.84	5.83	5.93	5.86	5.97	5.95	5.86	6.00	—
H-1′	4.43	4.40	4.39	4.39	4.39	4.39	4.40	4.40	4.38	4.57
H-2′	3.20	3.22	3.21	3.20	3.20	3.21	3.23	3.22	3.19	3.37
H-3′	2.44	2.43	2.45	2.42	2.44	2.41	2.48	2.47	2.44	2.14
H-4′a	1.65	1.71	1.62	1.66	1.68	1.68	1.72	1.64	1.56	1.91
H-4′b	1.24	1.25	1.31	1.25	1.23	1.21	1.28	1.26	1.25	1.42
H-5′	3.46	3.48	3.47	3.48	3.46	3.47	3.47	3.49	3.49	3.38
H-6′	1.24	1.22	1.22	1.25	1.24	1.23	1.24	1.26	1.23	1.30
NMe	2.28	2.80	2.29	2.28	2.28	2.28	2.31	2.31	2.28	2.67
H-1″	4.87	4.85	4.82	4.82	4.81	4.80	4.82	4.83	4.80	4.87
H-2″a	2.32	2.33	2.34	2.30	2.33	2.32	2.36	2.36	2.33	2.42
H-2″b	1.54	1.55	1.55	1.54	1.52	1.50	1.53	1.55	1.52	1.56
H-4″	3.03	3.02	3.00	3.00	3.01	2.98	2.98	3.04	3.02	3.04
H-5″	4.11	3.99	4.00	3.99	4.00	3.98	4.01	3.98	3.98	4.13
H-6″	1.24	1.24	1.25	1.27	1.25	1.27	1.25	1.26	1.24	1.24
OMe	3.35	3.30	3.30	3.33	3.30	3.29	3.30	3.31	3.29	3.36

Side-chain assignments: ^{1}H (400 MHz, $CDCl_3$) δ 8.89 (1H, d, J=4.5 Hz, ArH-2), 8.20 (1H, d, J=8.5 Hz, 1H of ArH-8 or ArH-5), 8.10 (1H, d, J=8.5 Hz, 1H or ArH-8, ArH-5), 7.71 (1H, t, J=8.0 Hz, 1H of ArH-7 or ArH-6), 7.56 (1H, t, J=8.0 Hz, 1H of ArH-6 or ArH-5), 7.43 (1H, d, J=4.5 Hz, ArH-3). ^{13}C (100 MHz, $CDCl_3$) δ 149.9, 148.5, 142.2, 129.9, 129.7, 127.6, 125.4, 124.6, 118.6. MS *m/z* 905 $(M+H)^+$, 747 $(M+H\text{-cladinose})^+$, 453 $(M/2+H)^+$ (Found: $(M+H)^+$, 904.5164. $C_{47}H_{73}N_3O_{14}$ requires $(M+H)^+$, 904.5165).

15-(5-Indolylcarboxamido)erythromycin A (7d)

NMR data: see Tables 1 and 3 for macrolide assignments. Side-chain assignments: ^{1}H (400 MHz, $CDCl_3$) δ 8.86 (1H, br s, indole NH), 8.12 (1H, s, ArH-3 or ArH-2), 8.11 (1H, s, ArH-4), 7.61 (1H, d, J=8.5 Hz, ArH-7 or ArH-6), 7.37 (1H, d, J=8.5 Hz, ArH-7 or ArH-6), 6.59 (1H, s, ArH-3 or ArH-2). ^{13}C (100 MHz, $CDCl_3$) δ 137.6, 133.8, 127.5, 125.5, 120.9, 120.4, 111.0, 103.6. MS *m/z* 893 $(M+H)^+$, 735 $(M+H\text{-cladinose})^+$, 447 $(M/2+H)^+$ (Found: $(M+H)^+$, 892.5217. $C_{46}H_{73}N_3O_{14}$ requires $(M+H)^+$, 892.5165).

15-(3-Indolylacetamido)erythromycin A (7e)

NMR data: see Tables 1 and 3 for macrolide assignments. Side-chain assignments: ^{1}H (400 MHz, $CDCl_3$) δ 8.68 (1H, br s, indole NH), 7.55 (1H, d, J=8.0 Hz, ArH-7 or ArH-4), 7.38 (1H, d, J=8.5 Hz, ArH-7 or ArH-4), 7.22~7.08 (3H, ArH-6, ArH-5, ArH-2), 3.70 (2H, s, $ArCH_2CO$). ^{13}C (100 MHz, $CDCl_3$) δ 136.5, 127.1, 124.1, 122.3, 119.8, 118.7, 111.4, 108.9, 36.0. MS *m/z* 907 $(M+H)^+$, 749 $(M+H\text{-cladinose})^+$, 454 $(M/2+H)^+$ (Found: $(M+H)^+$, 906.5374. $C_{47}H_{75}N_3O_{14}$ requires $(M+H)^+$, 906.5322).

Table 3 Carbon shifts for macrolide carbons

Carbon	4	5	7a	7b	7c	7d	7e	7f	7g	7h	7i
1	175.4	175.3	177.0	177.0	176.7	176.7	176.0	176.3	176.0	176.1	176.5
2	44.7	44.7	44.8	44.8	44.7	44.9	44.7	44.7	44.6	44.7	44.7
3	79.8	79.6	79.6	79.4	79.5	79.4	79.5	79.6	79.5	79.6	79.5
4	39.6	39.4	40.0	40.2	39.9	40.1	39.9	39.9	39.8	39.9	40.0
5	83.3	83.3	83.3	83.1	83.2	83.4	83.2	83.3	83.1	83.2	83.2
6	75.0	75.0	74.8	75.1	74.9	75.0	75.0	74.9	74.9	75.1	75.1
7	38.5	38.1	38.8	38.4	38.4	38.5	38.5	38.5	38.2	38.5	38.4
8	45.2	45.1	45.1	45.1	45.1	45.1	45.1	45.1	45.0	45.2	45.2
9	221.9	222.1	221.6	222.2	221.8	222.1	222.0	222.3	222.1	221.4	222.0
10	37.7	37.7	37.8	37.9	37.9	38.3	37.9	38.0	37.7	37.9	37.9
11	68.6	68.6	69.0	69.1	69.0	69.0	68.8	68.8	68.9	68.8	68.9
12	74.4	74.4	74.4	74.4	74.4	74.4	74.3	74.4	74.1	74.3	74.3
13	73.0	73.1	73.2	73.1	72.9	73.0	72.5	72.9	72.6	72.7	72.6
14	27.8	27.8	28.6	28.4	28.6	28.5	28.6	28.6	28.2	28.7	28.6
15	49.1	49.1	36.8	36.6	36.6	36.4	36.0	36.6	36.0	36.3	35.8
Me2	15.6	15.5	16.3	15.5	15.5	15.6	15.4	15.5	15.5	15.5	15.5
Me4	9.1	9.0	9.1	9.0	9.0	9.1	9.0	9.1	9.0	9.1	9.0
Me6	27.0	27.0	26.8	27.0	26.8	26.8	26.9	26.8	26.9	26.9	26.9
Me8	18.2	18.1	18.1	18.0	18.0	18.1	18.1	18.2	18.0	18.2	18.1
Me10	12.0	12.0	12.0	11.9	11.9	11.9	11.8	11.9	11.8	11.9	11.9
Me12	16.2	16.3	16.3	16.3	16.3	16.3	16.1	16.2	16.1	16.2	16.2
1′	103.2	100.9	103.2	103.2	103.2	103.1	103.2	103.2	103.0	103.2	103.2
2′	70.9	71.7	70.9	70.8	70.8	70.8	70.8	70.8	70.7	70.8	70.8
3′	65.6	63.5	65.6	65.7	65.6	65.7	65.5	65.5	65.5	65.6	65.6
4′	28.6	30.4	29.7	28.6	29.2	28.8	28.2	28.4	28.3	28.4	28.4
5′	69.0	68.4	69.0	69.1	68.9	69.0	69.0	69.0	68.9	69.0	69.0
6′	21.4	21.2	21.5	21.4	21.5	21.3	21.4	21.4	21.4	21.4	21.4
NMe2	40.3	40.7	40.3	40.3	40.3	40.3	40.3	40.3	40.2	40.3	40.3
1″	96.3	96.1	96.3	96.3	96.3	96.3	96.3	96.3	96.2	96.3	96.3
2″	34.9	34.9	34.9	34.9	34.9	34.8	34.9	34.9	34.8	34.9	34.9
3″	72.6	72.7	72.6	72.6	72.6	72.6	72.6	72.6	72.6	72.6	72.8
4″	78.0	77.9	78.0	78.0	78.0	77.9	77.9	78.0	77.9	78.0	78.0
5″	65.6	65.7	65.7	65.7	65.6	65.5	65.7	65.5	65.5	65.6	65.6
6″	18.7	18.6	18.6	18.7	18.7	18.6	18.6	18.6	18.6	18.6	18.6
3″-Me	21.5	21.5	21.5	21.5	21.5	21.4	21.5	21.5	21.4	21.5	21.5
3″-OMe	49.5	49.4	49.5	49.5	49.5	49.5	49.5	49.5	49.5	49.5	49.5
15-NHCO	na	na	165.3	166.7	167.4	168.5	171.6	166.9	169.3	170.9	172.0

15-(3-Benzisoxazolylacetamido)erythromycin A (7f)

NMR data: see Tables 1 and 3 for macrolide assignments. Side-chain assignments: ^{1}H (400 MHz, $CDCl_3$) δ 7.80 (1H, d, J=8.0 Hz, ArH-6), 7.54 (2H, m, ArH-4 and ArH-3), 7.32 (1H, m, ArH-5), 3.91 (2H, s, $ArCH_2CO$). ^{13}C (100 MHz, $CDCl_3$) δ 163.2, 153.7, 130.1, 123.7, 122.1, 121.3, 109.8, 33.6. MS *m/z* 909 $(M+H)^+$ (Found: $(M+H)^+$, 908.5084. $C_{46}H_{73}N_3O_{15}$ requires $(M+H)^+$, 908.5115).

15-(4-[2-Phenyl-5-methyloxazoly1]acetamido)erythromycin A (7g)

NMR data: see Tables 1 and 3 for macrolide assignments. Side-chain assignments: ^{1}H (400 MHz, $CDCl_3$) δ 8.01 (2H, d, J=8.5 Hz, ArH-6, ArH-2), 7.47~7.39 (3H, m, ArH-5, ArH-4, ArH-3), 3.43 (2H, s, $ArCH_2CO$), 2.35 (3H, s, $ArCH_3$). ^{13}C (100 MHz, $CDCl_3$) δ 160.1, 145.4, 130.0, 128.7, 127.9, 127.4, 126.1, 34.8, 10.1. MS *m/z* 949 $(M+H)^+$ (Found: $(M+H)^+$, 948.5461. $C_{49}H_{77}N_3O_{15}$ requires $(M+H)^+$, 948.5428).

15-(Phenylacetamido)erythromycin A (7h)
NMR data: see Tables 1 and 3 for macrolide assignments. Side-chain assignments: ^{1}H (400 MHz, $CDCl_3$) δ 7.33 (2H, m, ArH-5, ArH-3), 7.26 (3H, m, ArH-6, ArH-4, ArH-2), 3.52 (2H, s, $ArCH_2CO$). ^{13}C (100 MHz, $CDCl_3$) δ 135.0, 129.4, 128.9, 127.2, 43.8. MS *m/z* 868 $(M+H)^+$ (Found: $(M+H)^+$, 867.5205. $C_{45}H_{74}N_2O_{14}$ requires $(M+H)^+$, 867.5212).

15-(Phenylpropanamido)erythromycin A (7i)
NMR data: see Tables 1 and 3 for macrolide assignments. Side-chain assignments: ^{1}H (400 MHz, $CDCl_3$) δ 7.28～7.25 (3H, m, ArH-6, ArH-4, ArH-2), 7.20～7.16 (2H, m, ArH-5, ArH-3), 2.94 (2H, t, *J*=8.0 Hz, 2H of $ArCH_2CH_2CO$), 2.44 (2H, t, *J*=8.0 Hz, 2H of $ArCH_2CH_2CO$). ^{13}C (100 MHz, $CDCl_3$) δ 141.0, 128.5, 128.4, 126.1, 38.4, 31.7. MS *m/z* 882 $(M+H)^+$ (Found: $(M+H)^+$, 881.5357. $C_{46}H_{76}N_2O_{14}$ requires $(M+H)^+$, 881.5369).

15-(Phenylpentanamido)erythromycin A (7j)
NMR data: see Tables 2 and 4 for macrolide assignments. Side-chain assignments: ^{1}H (400 MHz, $CDCl_3$) δ 7.29～7.24 (3H, m, ArH-6, ArH-4, ArH-2), 7.17 (2H, d, *J*=7.0 Hz, ArH-5, ArH-3), 2.60 (2H, m, 2H of $ArCH_2CH_2CH_2CH_2CO$), 2.16 (2H, m, 2H of $ArCH_2CH_2CH_2CH_2CO$), 1.65 (4H, m, $ArCH_2CH_2CH_2CH_2CO$). ^{13}C (100 MHz, $CDCl_3$) δ 151.7, 142.3, 128.3, 125.7, 36.7, 35.7, 31.1, 25.4. MS *m/z* 892 $(M+H)^+$ (Found: $(M+H)^+$, 891.5587. $C_{48}H_{80}N_2O_{13}$ requires $(M+H)^+$, 891.5577).

15-(Benzyloxyacetamido)erythromycin A (7k)
NMR data: see Tables 2 and 4 for macrolide assignments. Side-chain assignments: ^{1}H (400 MHz, $CDCl_3$) δ 7.36～7.31 (5H, m, ArH), 4.57 (2H, s, OCH_2CO), 3.95 (2H, s, $ArCH_2CO$). ^{13}C (100 MHz, $CDCl_3$) δ 136.9, 128.5, 128.1, 127.9, 73.4, 69.4. MS *m/z* 838 $(M+H)^+$ (Found: $(M+H)^+$, 897.5322. $C_{46}H_{76}N_2O_{15}$ requires $(M+H)^+$, 897.5319).

15-[2-(2-Furanyl)phenylacetamido]erythromycin A (7l)
NMR data: see Tables 2 and 4 for macrolide assignments. Side-chain assignments: ^{1}H (400 MHz, $CDCl_3$) δ 7.65 (1H, d, *J*=7.5 Hz, ArH-6 or ArH-3), 7.54 (1H, s, FurH-5), 7.33～7.29 (3H, m, ArH-5, ArH-4 and ArH-6 or ArH-3), 6.55 (1H, d, *J*=3.0 Hz, FurH-3), 6.47 (1H, dd, *J*=3.0, 1.5 Hz, FurH-4), 3.74 (2H, s, $ArCH_2CO$). ^{13}C (100 MHz, $CDCl_3$) δ 152.9, 142.5, 131.9, 131.5, 130.8, 128.3 (2C), 127.7, 111.5, 108.8, 42.7. MS *m/z* 934 $(M+H)^+$ (Found: $(M+H)^+$, 933.5300. $C_{49}H_{76}N_2O_{15}$ requires $(M+H)^+$, 933.5319).

15-[3-(2-Furanyl)phenylacetamido]erythromycin A (7m)
NMR data: see Tables 2 and 4 for macrolide assignments. Side-chain assignments: ^{1}H (400 MHz, $CDCl_3$) δ 7.57 (1H, s, ArH-2), 7.46 (1H, s, FurH-5,), 7.34 (1H, m, ArH-5), 7.21～7.15 (2H, m, ArH-6, ArH-4), 6.67 (1H, d, *J*=3.0 Hz, FurH-3), 6.46 (1H, dd, *J*=3.0, 1.5 Hz, FurH-4), 3.58 (2H, s, $ArCH_2CO$). ^{13}C (100 MHz, $CDCl_3$) δ 153.7, 142.1, 134.0, 131.9, 131.1, 129.8 (2C), 124.3, 111.6, 105.0, 43.6. MS *m/z* 934 $(M+H)^+$ (Found: $(M+H)^+$, 933.5276. $C_{49}H_{76}N_2O_{15}$ requires $(M+H)^+$, 933.5319).

15-[4-(2-Furanyl)phenylacetamido]erythromycin A (7n)
NMR data: see Tables 2 and 4 for macrolide assignments. Side-chain assignments: ^{1}H (400 MHz, $CDCl_3$) δ 7.65 (2H, d, *J*=8.0 Hz, ArH-5, ArH-3), 7.46 (1H, s, ArH-5), 7.28 (2H, d, *J*=8.5 Hz, ArH-4, ArH-2), 6.64 (1H, d, *J*=3.5 Hz, FurH-3), 6.46 (1H, dd, *J*=3.0, 2.0 Hz, FurH-4), 3.53 (2H, s, $ArCH_2CO$). ^{13}C (100 MHz, $CDCl_3$) δ 153.7, 142.1, 134.0, 131.9, 129.8, 124.3, 111.6, 105.0, 43.6. MS *m/z* 934 $(M+H)^+$ (Found: $(M+H)^+$, 933.5294. $C_{49}H_{76}N_2O_{15}$ requires $(M+H)^+$, 933.5319).

15-(4″-Chlorobiphenylacetamido)erythromycin A (7o)
NMR data: see Tables 2 and 4 for macrolide assignments. Side-chain assignments: ^{1}H (400 MHz, $CDCl_3$) δ 7.52～7.49 (4H, m, 4×ArH), 7.38 (2H, d, *J*=8.5 Hz, 2×ArH), 7.33 (2H, d, *J*=8.0 Hz, 2×ArH), 3.55 (2H, s, $ArCH_2CO$). ^{13}C (100 MHz, $CDCl_3$) δ 139.2, 138.8, 134.5, 133.4, 129.9, 128.9, 128.3, 127.4, 43.4. MS *m/z* 978 $(M+H)^+$ (Found: $(M+H)^+$, 977.5138. $C_{51}H_{77}N_2O_{14}Cl$ requires $(M+H)^+$, 977.5136).

15-[3-(3-Furanyl)phenylacetamido]erythromycin A (7p)
NMR data: see Tables 2 and 4 for macrolide assignments. Side-chain assignments: ^{1}H (400 MHz, $CDCl_3$) δ 7.76 (1H, s, FurH-5 or FurH-2), 7.47 (1H, s, FurH-5 or FurH-2), 7.40 (2H, m, ArH-4 and ArH-2), 7.34 (1H, m, ArH-5 or ArH-3), 7.16 (1H, d, *J*=7.0 Hz, ArH-5 or ArH-3), 6.71 (1H, s, FurH-4), 3.55 (2H, s, $ArCH_2CO$). ^{13}C (100 MHz, $CDCl_3$) δ 143.6, 138.8, 135.5, 133.0, 129.3, 128.0, 126.9, 126.1, 124.6, 108.8, 43.8. MS *m/z* 934 $(M+H)^+$ (Found: $(M+H)^+$, 933.5293. $C_{49}H_{76}N_2O_{15}$ requires $(M+H)^+$, 933.5319).

15-[3-(3-Furanyl)phenylpropanamido]erythromycin A (7q)
NMR data: see Tables 2 and 4 for macrolide assignments. Side-chain assignments: ^{1}H (400 MHz, $CDCl_3$) δ 7.73 (1H, s, FurH-5 or FurH-2), 7.47 (1H, s, FurH-5 or FurH-2), 7.33～7.28 (3H, m, ArH-6 or ArH-4 and ArH-5, ArH-2), 7.10 (1H, d, *J*=7.0 Hz, ArH-4 or ArH-6), 6.70 (1H, s, FurH-4), 2.97 (2H, d, *J*=8.0 Hz, $ArCH_2CH_2CO$), 2.48 (2H,

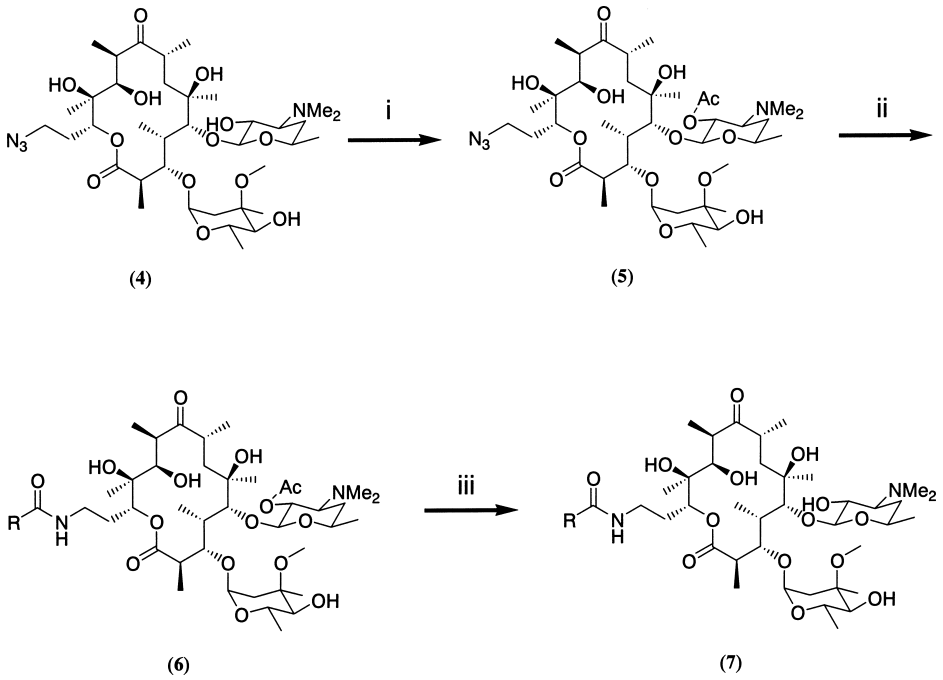
Table 4 Carbon shifts for macrolide carbons

Carbon	7j	7k	7l	7m	7n	7o	7p	7q	7r	7s
1	178.7	175.9	175.8	176.1	176.1	176.2	176.2	176.5	176.2	175.9
2	44.4	44.6	44.6	44.7	44.7	44.7	44.6	44.7	44.6	39.6
3	79.8	79.6	79.5	79.4	79.4	79.5	79.4	79.5	79.5	79.5
4	43.6	39.7	39.9	40.0	40.0	39.9	39.9	39.8	39.7	38.2
5	85.7	83.2	83.2	83.1	83.1	83.2	83.2	83.2	83.2	83.5
6	75.0	74.9	75.1	75.1	75.1	75.0	74.9	75.0	74.9	74.9
7	36.7	38.4	38.5	38.4	38.4	38.4	38.3	38.4	38.3	38.8
8	46.1	45.1	45.1	45.1	45.1	45.1	45.0	45.2	45.1	44.7
9	220.2	222.0	222.1	222.1	222.1	222.8	222.0	221.9	221.9	220.4
10	40.3	37.8	37.9	37.9	37.9	37.9	37.8	37.9	37.8	36.5
11	68.9	68.9	68.8	68.9	68.9	68.9	68.8	68.8	68.8	67.0
12	74.8	74.3	74.2	74.2	74.2	74.3	74.2	74.3	74.2	73.8
13	73.0	72.7	72.7	72.6	72.6	72.6	72.6	72.9	72.5	72.8
14	28.6	28.6	28.4	28.6	28.6	28.3	28.2	29.2	28.3	29.8
15	35.9	35.6	36.3	36.2	36.2	36.2	36.1	35.1	36.1	36.5
Me2	14.7	15.6	15.5	15.4	15.4	15.5	15.4	15.5	15.5	13.0
Me4	8.8	9.0	9.1	9.0	9.0	9.0	9.0	9.0	9.0	8.7
Me6	26.5	26.8	27.0	27.0	27.0	26.9	26.8	26.9	26.8	25.3
Me8	18.2	18.1	18.1	18.1	18.0	18.1	18.0	18.1	18.1	17.6
Me10	11.8	11.9	11.8	11.8	11.8	11.9	11.9	11.9	11.9	10.9
Me12	16.4	16.2	16.1	16.2	16.1	16.2	16.2	16.2	16.2	16.1
1′	102.9	103.1	103.1	103.1	103.1	103.1	103.1	103.1	103	101.9
2′	70.7	70.8	70.8	70.8	70.8	70.8	70.7	70.8	70.7	69.7
3′	65.9	65.6	65.6	65.6	65.6	65.6	65.6	65.6	65.5	65.3
4′	28.2	29.6	29.7	29.7	29.7	28.7	28.7	28.6	28.6	29.3
5′	68.9	68.9	69.0	69.0	69.0	69.0	68.8	69.0	68.9	69.7
6′	21.4	21.4	21.4	21.4	21.4	21.4	21.3	21.4	21.4	20.1
NMe2	40.3	40.2	40.2	40.3	40.3	40.2	40.2	40.3	40.2	38.5
1″	94.5	96.2	96.3	96.3	96.3	96.3	96.2	96.2	96.2	96.3
2″	34.6	34.8	34.9	34.8	34.8	34.8	34.8	34.9	34.8	34.5
3″	70.3	72.5	72.7	72.6	72.6	72.6	72.6	72.6	72.6	72.5
4″	78.1	77.9	77.9	77.9	77.9	78.0	77.9	78.0	77.9	77.5
5″	65.8	65.5	65.7	65.6	65.6	65.6	65.6	65.6	65.5	65.3
6″	18.4	18.6	18.6	18.6	18.6	18.6	18.6	18.6	18.6	17.7
3″-Me	21.6	21.3	21.5	21.5	21.5	21.5	21.4	21.5	21.4	20.3
3″-OMe	49.5	49.5	49.5	49.5	49.5	49.5	49.5	49.5	49.5	48.6
15-NHCO	172.8	169.5	170.9	170.7	170.7	170.8	170.8	172.0	170.8	172.8

d, J=8.0 Hz, $ArCH_2CH_2CO$). ^{13}C (100 MHz, $CDCl_3$) δ 143.6, 141.6, 138.5, 132.5, 128.9, 127.0, 126.4, 125.9, 123.7, 108.9, 38.4, 31.7. MS *m/z* 948 $(M+H)^+$ (Found: $(M+H)^+$, 947.5477. $C_{50}H_{78}N_2O_{15}$ requires $(M+H)^+$, 947.5475).

15-[3-(3-Thienyl)phenylacetamido]erythromycin A (7r)
NMR data: see Tables 2 and 4 for macrolide assignments. Side-chain assignments: ^1H (400 MHz, $CDCl_3$) δ 7.51~7.48 (3H, m, 3×ArH), 7.39~7.33 (3H, m, 3×ArH), 7.20~7.15 (1H, m, 1×ArH), 3.58 (2H, s, $ArCH_2CO$). ^{13}C (100 MHz, $CDCl_3$) δ 141.9, 136.4, 135.5, 129.3, 128.1, 127.5, 126.4, 126.1, 125.2, 120.5, 43.8. MS *m/z* 950 $(M+H)^+$ (Found: $(M+H)^+$, 949.5078. $C_{49}H_{76}N_2O_{14}S$ requires $(M+H)^+$, 949.5090).

15-[3-(3-Pyrrolyl)phenylacetamido]erythromycin A (7s)
NMR data: see Tables 2 and 4 for macrolide assignments. Side-chain assignments: ^1H (400 MHz, CD_3OD) δ 7.45 (1H, s, ArH-2), 7.39 (1H, d, J=8.0 Hz, ArH-6 or ArH-4), 7.22 (1H, t, J=7.5 Hz, ArH-5), 7.14 (1H, br s, 1×PyrH), 7.02 (1H, d, J=7.0 Hz, ArH-6 or ArH-4), 6.76 (1H, br s,



Scheme 1 Formation of 15-amido erythromycin A compounds. (i) Ac_2O, CH_2Cl_2; (ii) Me_3P, CH_2Cl_2 followed by RCO_2H, EDCI, HOBt; (iii) MeOH, 50°C.

1×PyrH), 6.44 (1H, d, J=1.0 Hz, 1×PyrH), 3.48 (2H, s, $ArCH_2CO$). ^{13}C (100 MHz, CD_3OD) δ 136.9, 135.4, 128.3, 125.1 (2C), 123.7, 123.0, 118.3, 114.4, 105.0, 42.6. MS m/z 933 $(M+H)^+$ (Found: $(M+H)^+$, 932.5505. $C_{49}H_{77}N_3O_{14}$ requires $(M+H)^+$, 932.5478).

Results

Preparation of 15-Amidoerythromycins

The novel erythromycin analogue 15-azidoerythromycin A **4** was chosen as the target of chemobiosynthesis, as the azide provides a versatile functional group readily converted into amines, amides, sulfonamides, carbamates, and ureas. Initial attempts at derivatization investigated hydrogenation of the azide. The free amine was not readily obtained upon hydrogenation over palladium/carbon under 1 atm of hydrogen, the reaction mixture being complicated by partial reduction products. While Staudinger reduction using triphenylphosphine gave no reaction, use of the highly reactive trimethylphosphine resulted in complete consumption of the azide and formation of the phosphazine ylide as determined by LC/mass spectrometry [12]. The ylide itself could be generated and used *in situ* to form an amide bond with carboxylic acids, although use of **4** invariably resulted in formation of products acylated at both the 15-amine and the 2′-hydroxyl groups.

To simplify the product mixture, **4** was first converted into the 2′-*O*-acetyl (**5**) by reaction with acetic anhydride. Subsequent treatment of **5** with trimethylphosphine followed by a carboxylic acid and a coupling reagent then produced the corresponding amide (**6**) in high yields. Removal of the acetate protecting group by heating in methanol finally yielded the desired 15-amido erythromycin (**7**). This route allowed for the preparation of an array of novel 15-amido erythromycins either from commercially available or from readily synthesized carboxylic acids (Figure 2).

Antibacterial Activity

MIC values against a panel of microorganisms are given in Table 5. Analogues with side chains derived from heteroaryl carboxylic acids (Figure 2, **7a**∼**7d**) had higher MIC values than erythromycin A, indicating less antibacterial activity. Adding a spacer between the aryl moiety and the carboxamide linker generally improved activity, particularly against the *erm*(B)-containing *S. pneumoniae* strain OC4444. In particular, the MIC value of the 3-indolylacetamide derivative (**7e**) against OC4444 was 4-fold lower than erythromycin, but the activity against the other strains in the testing panel was poorer. The benzisoxazolylacetamide analogue (**7f**) and phenyl oxazolyl-acetamide (**7g**) had MIC values similar to or lower than those of erythromycin. The MIC values of

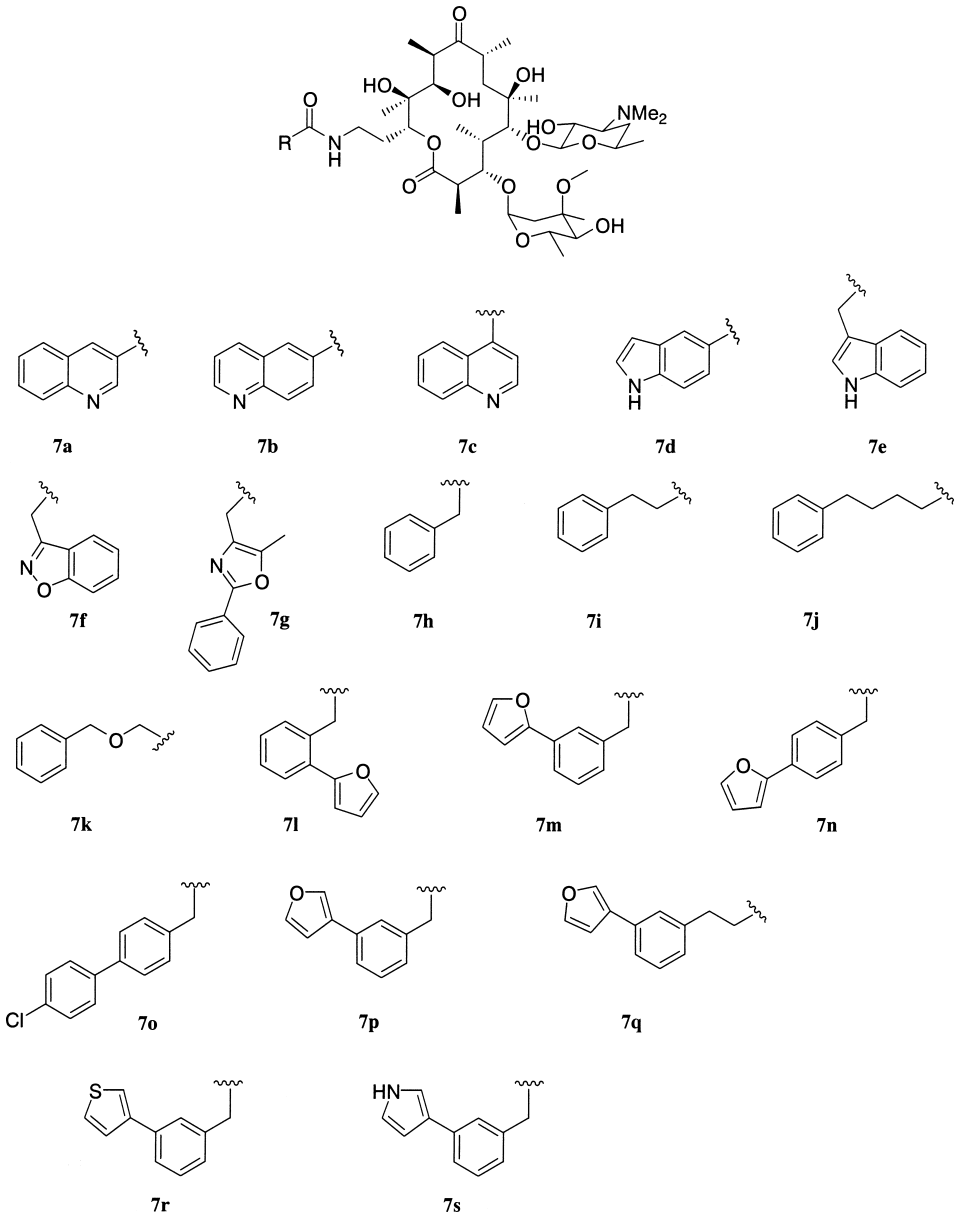


Fig. 2 Compounds in the study.

the phenylalkyl derivatives **7h**∼**7k** were often equivalent to or at least 2-fold higher than those of erythromycin A, although **7h** and **7k** appeared to be 4-fold more active than erythromycin A against the *erm*(B)-containing pneumococcus OC4444. The remaining group of derivatives with biaryl-alkyl R groups (**7l**∼**7s**) had MIC values that were generally equivalent to eight-fold lower than those of erythromycin A against the *H. influenzae* strain and most of the macrolide-susceptible and -resistant pneumococci (note that the MIC values for all compounds were >16 μg/ml against the *erm*(B)-containing *S. pneumoniae* strain OC4051).

Discussion

We have developed a concise route to the preparation of a novel class of antibacterial agent, the 15-amidoerythromycins. Chemobiosynthesis starting from a chlorodiketide analogue readily provides 15-azidoerythromycin A (**4**) [11], a versatile intermediate for the attachment of aromatic groups to the erythromycin scaffold. Staudinger reduction of the azido group and *in situ* acylation of the ylide provides a concise route to 15-amidoerythromycins. The ease of preparation of analogues of this class facilitated determination of a structure-activity relationship for these

Table 5 Minimum inhibitory concentrations for compounds of this study

Compound	MIC (μg/ml) against					
	S. aureus (Smith)	*S. pneumoniae*				*H. influenzae* ATCC49766
		ATCC6301	OC4444 *erm*(B)*	OC4438 *mef*(A)	OC4421 *mef*(A)	
Erythromycin A	0.5	0.06	8	8	4	4
7a	8	2	>16	>16	>16	>16
7b	8	2	16	>16	16	>16
7c	16	1	16	>16	16	>16
7d	16	2	>16	>16	>16	>16
7e	4	0.25	2	>16	16	>16
7f	1	0.12	1	8	8	4
7g	0.5	0.06	1	4	4	2
7h	1	0.25	2	>16	8	8
7i	2	0.25	8	16	8	8
7j	8	0.25	>16	8	4	>16
7k	1	0.12	2	16	8	4
7l	1	0.06	1	4	4	2
7m	0.5	0.06	1	4	2	2
7n	1	0.12	2	4	4	2
7o	1	0.12	2	8	4	4
7p	1	0.12	2	8	4	4
7q	2	0.12	4	8	4	16
7r	0.5	0.06	0.5	4	2	2
7s	4	0.25	4	16	8	8

* MIC values for all compounds were >16 μg/ml against another *erm*(B)-containing *S. pneumoniae* strain (OC4051).

novel antibacterial macrolides.

The aryl carboxamides such as **7a**∼**d** show activity inferior to that of erythromycin (Table 5). Interestingly, the aryl acetamides, such as **7f**∼**g** show activities in a similar range to that of the parent erythromycin and even exceed the activity of erythromycin A against the *erm*(B)-containing strain *S. pneumoniae* OC4444.

Prompted by these observations a study was conducted to establish an optimal chain length, using commercially available *n*-phenylalkyl acids. While activity against *S. pneumoniae* appears generally independent of chain length, with the exception of the poor activity of **7i** against OC4444, there is a steady decrease in activity with increasing chain length in the susceptible *S. aureus* strain as demonstrated by **7h**∼**j**. The one exception to this trend is the benzyloxyacetamido side-chain **7k**. The unexpected superiority of this analogue suggests that much of the decrease in activity with chain length may be due to increasing lipophilicity.

A series of biaryl-acetamide compounds showed the best overall antibacterial activity, with some compounds showing improved MIC values over erythromycin even in the *erm*(B)- and *mef*-containing strains. The *meta*-substituted phenylacetamides **7p** and **7r** show greatly improved activity against OC4444 [*erm*(B)] as well as enhanced activity against the *mef*-containing *S. pneumoniae*, and *H. influenzae*. Homologation to the corresponding propionamido compound **7q** results in decreased activity. Interestingly, the pyrrolyl analogue **7s** shows significantly diminished activity relative to the furyl and thienyl analogues.

The compounds presented show that substitution of erythromycin at the 15-position can be well tolerated and in some cases leads to improvements in the antibacterial activity against resistant strains. In particular aryl acetamides show good activity with biaryl side-chains being the optimal compounds discovered to date. While these studies demonstrate that improved activity against resistant bacteria can be obtained through the introduction of a side-chain at the 15-position, these compounds still clearly

suffer from resistance due to induction of ribosomal methylation and efflux. As both resistance mechanisms are known to be associated with the 3-cladinosyl group in erythromycin, this deficiency could be overcome through conversion of the 15-amidoerythromycins to the corresponding 15-amidoketolides. Work in this area is in progress and will be reported in due course.

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