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9-Dihydroerythromycin ethers as motilin agonists—Developing structure–activity relationships for potency and safety

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ABSTRACT

A series of derivatives of the amine of 9-dihydro-9-*O*-ethylamino-*N*-desmethyl-*N*-isopropyl erythromycin A derivatives were synthesized as motilin agonists. The compounds were developed for potency without showing antibacterial activity and inhibition of the hERG potassium channel. The formamide of the amide series was found to show the optimal combination of properties relative to carbamates, ureas, thioureas, and amines. This prompted an investigation of heterocyclic isosteres for the amide. In this series the triazole had the optimal combination of properties. From the study, two compounds met the criteria for detailed pharmacokinetic studies.

1. Introduction

Motilin is a 22-amino acid hormone, responsible for normal regulation of gastrointestinal (GI) motility.[1,2] Motilin's activity is mediated through a G-protein coupled receptor found on smooth muscle and enteric neurons of the GI tract.[3–6] Erythromycin A **1**, a 14-membered macrolide antibiotic, also acts as an agonist of the motilin receptor (EC_{50} ~1 μM).[1,7] Indeed it has been shown to compete with labeled motilin for receptor sites in membrane preparations,[8] causes Ca^{2+} currents in whole cell systems expressing the receptor,[9,10] induces contractions in isolated GI smooth muscle[8,11] and is an effective prokinetic in animal models which measure gastric emptying[12] and motility.[13] Further, it has been proven clinically to stimulate gastric motility in patients with gastroparesis[14,15] and is able to provide symptomatic relief to patients with gastroparisis.[16,17] However, in a prokinetic that may be required chronically the antibiotic activity of **1** is undesirable. Nonantibiotic erythromycin derived motilin agonists, often referred to as motilides, have therefore been proposed for the treatment of GI motility disorders such as gastroesophageal reflux disease (GERD) and gastroparesis.[18]

Several groups have investigated erythromycin based macrolides as motilin receptor agonists. These motilides have been tuned for improved acid stability and potency in conjunction with reduced antibiotic activity.[19–21] Of these, ABT-229 **2** and GM-611 **3** showed the desired in vitro potency but failed to demonstrate long-term efficacy in clinical studies, due to receptor desensitization (Fig. 1). This phenomenon—known as tachyphylaxis—is mediated through a receptor desensitization mechanism.[22,23] While **2** was effective in in vitro muscle contractility assays and promoted gastric emptying following a single administration,[24] it failed to show efficacy upon repeated doses.[25–28] In vitro assays for tachyphylaxis have been developed, which indicate that agonist potency and tachyphylaxis are separable,[22,29,30] validating the potential to find motilides that retain activity over continued administration.

Our own studies had identified the 9-dihydroerythromycin as a scaffold with improved potency and acid stability as compared to erythromycin **1**. Further, by replacing the *N*,*N*-dimethylamine of the desosamine with an *N*-methyl-*N*-isopropylamine as in **4** the antibacterial activity was significantly reduced while showing nanomolar potency in a muscle strip assay.[31] It is noteworthy that this molecule shows little tachyphylaxis relative to both **2** and **3**. However, as is common with macrolides, **4** still exhibits inhibition of the hERG channel (showing 80% inhibition at 30 μM). hERG is a potassium ion channel found in the heart, which if inhibited can lead to prolongation of the QT interval and potentially fatal cardiac arrhythmia.[32–35]

By alkylation to form 9-*O*-acetamides the potency was improved and the antibacterial activity reduced.[36] Further the acetamides also reduced the ability of the molecules to inhibit hERG.

Abbreviations: GI, gastrointestinal; GERD, gastroesophageal reflux disease; hERG, human ether-a-go-go related gene; SAR, Structure–activity relationship; EDCI, ethyl-3-(3-dimethylaminopropyl)carbodiimide; Cbz, carboxybenzyl; ELSD, evaporative light scattering detection; MIC, minimum inhibitory concentration.

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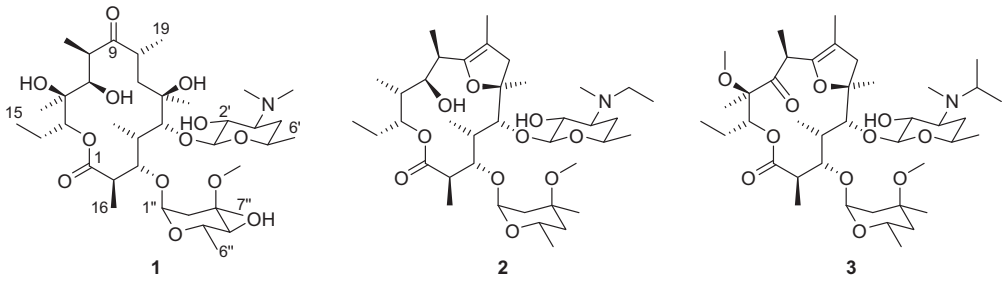


Figure 1. Structures of **1**, **2**, and **3**.

The *N*-methyl acetamide **5** had the optimal balance in vitro characteristics—high potency, low hERG inhibition, little tachyphylaxis and minimal antibacterial activity (Fig. 2).

In this study we wanted to investigate the nature of the substituent at the 9-position further, by introducing a series of alternative functional groups in an attempt to expand our knowledge of the structure–activity relationships (SAR) at this position. As in our previous study we wanted to find compounds that were highly potent without causing tachyphylaxis. The compounds also needed to be effectively inactive against an erythromycin-sensitive strain of *Streptococcus pneumoniae* (ATCC 6301) and be weak inhibitors of hERG at 300 μM.

2. Results and discussion

To begin the study it was decided to investigate switching the orientation of the amide relative to the acetamide series. In order to do this it was first necessary to install an alkyl amino group onto the 9-hydroxyl of **4**. Using a similar procedure to that used in our previous study, an ethylamino group was installed using bromoethylamine hydrobromide in the presence of sodium hydroxide to generate **6**.[36]

From this amine, a series of amides **7a**–**7c** was synthesized (Scheme 1) using ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) as used in our previous studies.[36,37]

We were conscious of the versatility of the free amine **6** as an intermediate, which allows for the chemoselective reaction with chloroformates, isocyanates, thioisocyanates, and sulfonyl chlorides. In this way it was possible to rapidly generate and screen carbamates **8**, a urea **9**, thioureas **10**, and the methylsulfonate **11** (Scheme 2).

Further a reductive amination was carried out to obtain the secondary amine **12**, while the guanidine **13** was generated by reaction with *N*,*N*′-di-Cbz-*S*-methylisothiourea followed by hydrogenation to remove the carboxybenzyl (Cbz) groups (Scheme 3).[38]

The compounds were initially tested for their potency and antimicrobial activity. Many of the compounds were taken on to investigate their inhibition of hERG and the extent to which they exhibited tachyphylaxis (Table 1).

The data showed that the nature of the substituent on the amine can have a profound effect on the potency of the compound. In particular, having a protonatible amine (e.g., **6** and **12**) or guanidine **13**, results in a significant drop in potency. However, acylation has a positive effect with the amides **7a**–**7c** being the most potent. The carbamates **8a**–**8b** and urea **9** show similar 100 nM potency while there is an approximate two fold drop in potency for the thioureas **10a**–**10b** and sulfonamide **11**. The compounds show an improvement of at least 1–2 dilutions over **4** in terms of antibacterial activity with none of those tested showing any tachyphylaxis (in comparison to both **2** and **3**).

The hERG data shows several trends. As the lipophilicity of the side chain increases the hERG inhibition increases, for example, **7a** versus **7b**, **8a** versus **8b**, **10a** versus **10b**, and **6** versus **12**. This is particularly striking in the case of the amides, where moving from the formamide **7a** to the acetamide **7b** increases the hERG inhibition at 30 μM by 40%. It is interesting to note that the benzimidazole amide **7c** does not show any improvement in hERG inhibition in spite of the polar nature of this group. The hERG inhibition increases from urea to thiourea to carbamate.

The activity of the amides was particularly striking and similar to that observed in the acetamide series.[36] It was considered that it may be possible to replace the amide with an aromatic group, which may influence the physiochemical properties of the molecules. Thus, a series of arylmethyl ethers **14a**–**14f** was generated from **4** (Scheme 4).

Again the compounds were tested in the standard in vitro screens (Table 2). Of this group the triazole **14e** shows the best combination of in vitro parameters, with excellent potency, no tachyphylaxis and low inhibition of hERG. Moving from **14e** to imidazole **14f** does not impact potency but increases the hERG inhibition significantly. Indeed hERG inhibition is a significant problem for this class of compounds. The free NH found in **14e** and **14f** appears to be responsible for the strongest potency. All compounds show only little antibiotic activity.

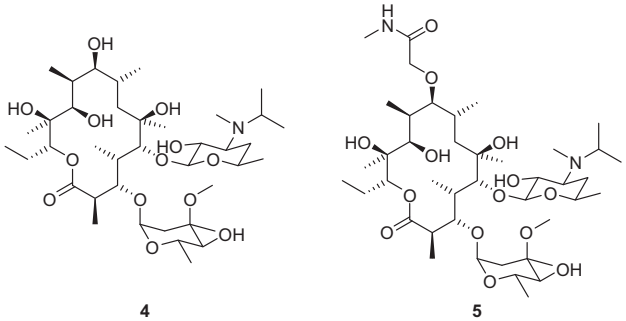
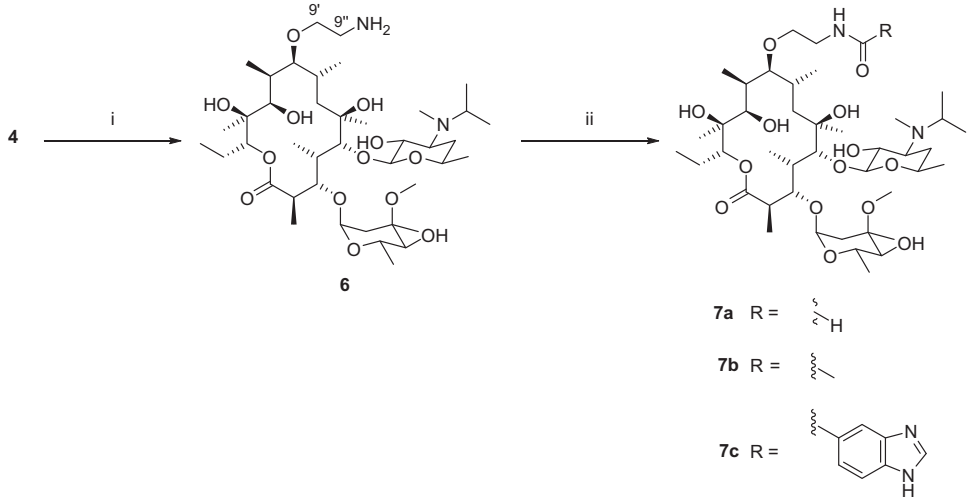


Figure 2. Structures of **4** and **5**.

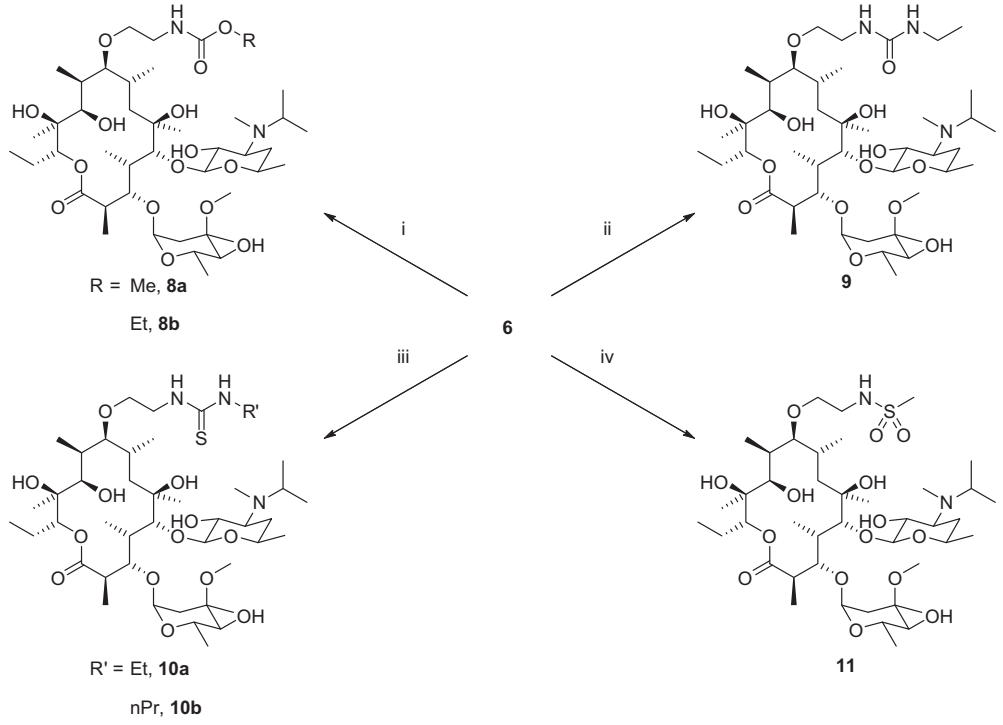
3. Conclusion

The compounds presented build on the results obtained in the 9-*O*-acetamide series.[36] Reversing the orientation of the amide does not effect potency, with the amides formed **7a**–**7c** being potent motilin agonists. These compounds meet the targeted profile in terms of tachyphylaxis and minimal antibiotic activity. In the case of the formamide **7a**, the hERG potency was also acceptable, meaning that this compound met our criteria for the project. Indeed this compound is similar in profile to the methylacetamide **5** indentified in our previous study.

In order to more fully investigate the SAR of both the motilin agonist potency and hERG inhibition a series of compounds was generated from the installed amino ethyl group. The compounds



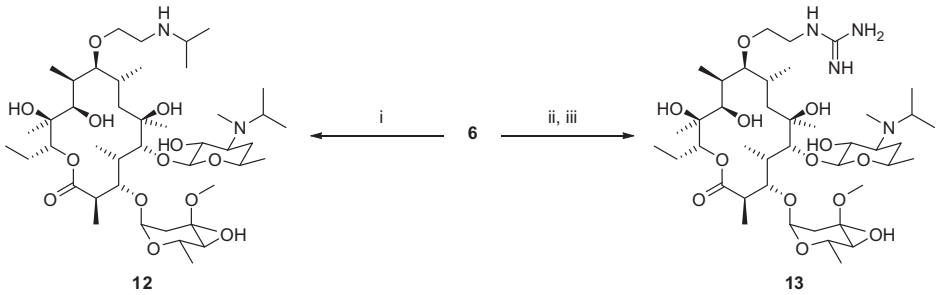
Scheme 1. Reagents and conditions: (i) $BrCH_2CH_2N^+H_3Br^-$ (2.9 equiv), NaOH (9.5 equiv), THF, rt, 20 h; (ii) RCO_2H (2.0 equiv), EDCI (2.2 equiv), HOBt (2.0 equiv), DMF, 0 °C, 3 h.



Scheme 2. Reagents and conditions: (i) ROCOCl (1.2 equiv), pyridine (2.0 equiv), CH_2Cl_2, rt, 4 h; (ii) EtNCO (2.0 equiv), CH_2Cl_2, rt, 4 h; (iii) R′NCS (1.5 equiv), CH_2Cl_2, rt, 18 h; (iv) $MeSO_2Cl$, pyridine, CH_2Cl_2, rt, 2 h.

with protonatible groups (the free amine **6**, isopropylamine **12** and the guanidine **13**) show no agonist activity. By contrast, all the compounds containing a similar N–H to that in the amides—carbamates, urea, thioureas, and sulfonamide—show moderate to good agonist potency, with the carbamate moiety being the most potent. None of the compounds displayed antibiotic activity and in these compounds tested tachyphylaxis was not of issue. The carbamates were strong inhibitors of the hERG channel but switching to the urea moiety reduced this inhibition, demonstrating a clear correlation between the polarity of the moiety and hERG inhibition.

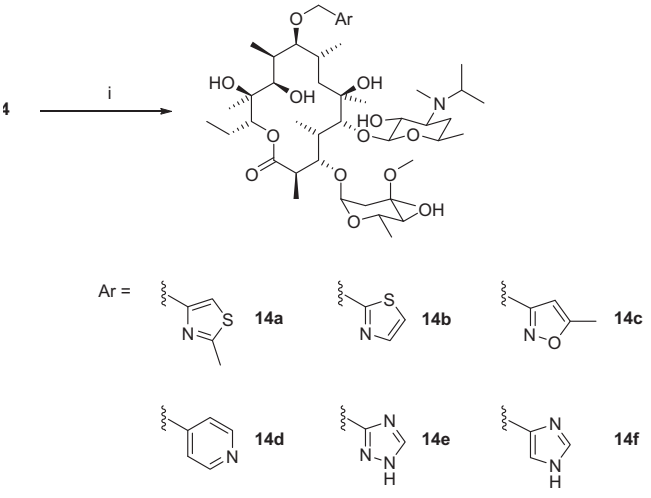
The better potency of the amides led us to the aromatic ethers as amide surrogates. It is notable that the two compounds containing a hydrogen bond donor (an N–H bond similar to that found in the amide derivatives), the triazole **14e** and imidazole **14f**, have the best potency, while the thiazoles **14a** and **14b**, isoxazole **14c** and pyridine **14d** compounds that have only hydrogen bond acceptors are not as potent. The optimal profile in this series is found with the triazole **14e** which as well as being potent does not show tachyphylaxis and meets our requirement in terms of hERG inhibition. Through this study we have been able to identify two compounds, the formamide **7a** and the triazole **14e** that met the project criteria. These two compounds were taken on further into pharmacokinetic studies as well as models of gastric emptying, which will be reported in due course.



Scheme 3. Reagents and conditions: (i) acetone, AcOH (2.0 equiv), $Na(CN)BH_3$ (2.0 equiv), MeOH, rt, 12 h; (ii) CbzNHC(SMe)NCbz (1.5 equiv), Et_3N (1.5 equiv), THF, rt, 14 h; (iii) H_2, Pd–C, MeOH, rt, 2 h.

Table 1
In vitro data for compounds **6**–**13** and standard compounds

Compound	Potency EC_{50} (nM)	ATCC 6301 MIC (μg/mL)	Tachyphylaxis % @ 4th dose	hERG %inhibition	
				@ 30 μM	@ 300 μM
1	1200	0.0025	97 ± 1	27	90
2	7	64	22 ± 10	98	100
3	11	128	9 ± 4	84	100
4	260	32	85 ± 3	80	100
5	58	128	89 ± 8	7	37
6	2300	128	nd	25	76
7a	31	>128	100 ± 1	8	47
7b	48	>128	94 ± 6	47	73
7c	37	128	94 ± 10	71	100
8a	130	128	nd	40	95
8b	100	64	nd	67	100
9	100	>128	88 ± 3	20	57
10a	220	>120	nd	36	88
10b	210	128	nd	53	100
11	240	>128	94 ± 7	12	79
12	2000	64	nd	40	93
13	3600	nd	nd	nd	nd



Scheme 4. Reagents and conditions: (i) KO^{t}Bu, $ArCH_2Cl$, THF, rt, 4 h.

4. Experimental

Unless otherwise noted, ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ at 300 K using a Bruker DRX 400 spectrometer, where possible spectra were assigned using COSY, HSQC, and HMBC experiments. Scanned copies of the proton and carbon spectra are included in the Supplementary data. Infra-red spectra were obtained using a Perkin–Elmer Spectrum One FTIR with an attenuated total reflectance accessory containing a zinc selenide plate. High resolution mass spectra were obtained by flow injection with manual peak-matching using an Applied Biosystems Mariner TOF spectrometer with a turbo-ion spray source. All final compounds were obtained as solids after lyophilization from benzene and were >95% pure by LC–MS when detecting by evaporative light scattering detection (ELSD) using a linear gradient of 15% (5 mM NH_4OAc in CH_3CN–MeOH [4:1]) in 5 mM NH_4OAc in water to 100% (5 mM NH_4OAc in CH_3CN–MeOH [4:1]) over 10 min using a 3 μm, 4.6 × 150 mm Varian Metasil Basic or Phenomenex Luna C-18(2) column. All numbering is consistent with that shown in Figure 1 and Scheme 1.

Table 2
In vitro data for compounds **14a**–**14f**

Compound	Potency EC_{50} (nM)	ATCC 6301 MIC (μg/mL)	Tachyphylaxis % @ 4th dose	hERG %inhibition	
				@ 30 μM	@ 300 μM
14a	480	128	nd	63	100
14b	290	128	nd	61	100
14c	110	128	78 ± 1	45	90
14d	420	128	nd	68	100
14e	54	128	97 ± 8	8	40
14f	63	128	nd	48	98

4.1. Synthesis of 9-*O*-(2-amino)ethyl-9-dihydro-*N*-des-methyl-*N*-isopropylerythromycin 6

To a solution of 9-dihydro-*N*-des-methyl-*N*-isopropylerythromycin A **4** (0.055 g, 0.072 mmol, 1.0 equiv) in tetrahydrofuran (2.4 mL), was added bromoethylamine hydrobromide (0.043 g, 0.209 mmol, 2.9 equiv) followed by potassium hydroxide (0.038 g, 0.684 mmol, 9.5 equiv). The solution was stirred at room temperature for 20 h before diluting with EtOAc (15 mL) and washing with $NaHCO_3$ (15 mL). The aqueous phase was extracted with EtOAc (3 × 15 mL) and the combined organics dried ($MgSO_4$) before concentrating under reduced pressure. Column chromatography (silica, 35% acetone—hexane, 1% triethylamine) yielded **6** (0.023 g, 40%) as a white solid; IR (film) 3477, 2970, 2935, 1731, 1453, 1378, 1178, 1086, 1053, 999, 900 cm^{-1}; ^1H NMR (400 MHz) δ (Table 3); ^{13}C NMR (100 MHz) δ (Table 4); *m*/*z*: 808 $[M+H]^+$, 649 (found $[M+H]^+$, 807.5550, $C_{41}H_{78}N_2O_{13}$ requires $[M+H]^+$ 807.5577).

4.2. General procedure for the synthesis of amides

To a solution of **6** (0.150 g, 0.186 mmol, 1.0 equiv) in dimethylformamide (2.0 mL) at 0 °C was added EDCI (0.079 g, 0.409 mmol, 2.2 equiv) and hydroxybenzotriazole (0.050 g, 0.372 mmol, 2.0 equiv) followed by the corresponding acid (0.372 mmol,

2.0 equiv). The solution was stirred at 0 °C for 30 min and room temperature for 3 h before partitioning between EtOAc (25 mL), and $NaHCO_3$ (25 mL). The aqueous phase was extracted with EtOAc (25 mL). The combined organics were washed with water (35 mL), $NaHCO_3$ (35 mL) and brine (40 mL) before drying (Na_2SO_4) and concentrating under reduced pressure. Column chromatography (silica, 40% acetone—hexane, 1% triethylamine) yielded the corresponding acetamide **7**.

4.2.1. Compound 7a

Using the general procedure on a 0.186 mmol scale yielded **7a** (0.072 g, 46%) as a white solid; IR (film) 3468, 2971, 2936, 1732, 1678, 1452, 1381, 1179, 1085, 1056, 999, 900 cm^{-1}; ^1H NMR (400 MHz) δ (Table 3) 8.13 (1H, s, CHO); ^{13}C NMR (100 MHz) δ (Table 4) 161.8; *m/z*: 836 $[M+H]^+$, 678 (found $[M+H]^+$, 835.5501, $C_{42}H_{78}N_2O_{14}$ requires $[M+H]^+$ 835.5526).

4.2.2. Compound 7b

Using the general procedure on a 0.062 mmol scale yielded **7b** (0.030 g, 57%) as a white solid; ^1H NMR (400 MHz) δ (Table 3) 1.96 (3H, s, $NCOCH_3$); ^{13}C NMR (100 MHz) δ (Table 4) 170.9, 23.0; *m/z*: 850 $[M+H]^+$, 691 (found $[M+H]^+$, 849.5682, $C_{43}H_{80}N_2O_{14}$ requires $[M+H]^+$ 849.5682).

4.2.3. Compound 7c

Using the general procedure on a 0.121 mmol scale yielded **7c** (0.042 g, 48%) as a white solid; IR (film) 3453, 2971, 2932, 1730, 1642, 1535, 1453, 1366, 1179, 1083, 1054, 1083, 998 cm^{-1}; ^1H NMR (400 MHz) δ (Table 3) 8.30 (1H, s, ArH-2), 8.16 (1H, s, ArH-4), 7.78 (1H, dd, *J* 8.5, 1.5 Hz, ArH-6), 7.67 (1H, d, *J* 8.5 Hz, ArH-7; ^{13}C NMR (100 MHz) δ (Table 4) 169.5, 143.2, 129.1, 121.8; *m/z*: 952 $[M+H]^+$, 794 (found $[M+H]^+$, 951.5898, $C_{49}H_{82}N_4O_{14}$ requires $[M+H]^+$ 951.5900).

4.3. General procedure for the synthesis of carbamates 8

To a solution of **6** (0.050 g, 0.062 mmol, 1.0 equiv) in dichloromethane (1.0 mL) was added pyridine (0.010 g, 0.010 mL, 0.124 mmol, 2.0 equiv) followed by the chloroformate (0.074 mmol, 1.2 equiv). The solution was stirred at room temperature for 4 h before adding $NaHCO_3$ (15 mL). The organics were extracted with CH_2Cl_2 (3 × 15 mL), combined, dried (Na_2SO_4) and concentrated under reduced pressure. Column chromatography (silica, 33% acetone—hexane, 1% triethylamine) yielded the carbamate **8**.

4.3.1. Compound 8a

Using the general procedure on a 0.062 mmol scale yielded **8a** (0.025 g, 47%) as a white solid; IR (film) 3468, 2971, 1731, 1536, 1453, 1379, 1262, 1179, 1262, 1179, 1087, 1054, 999, 900 cm^{-1}; ^1H NMR (400 MHz) δ (Table 3) 3.70 (3H, s, OCH_3); ^{13}C NMR (100 MHz) δ (Table 4) 157.7, 52.2; *m/z*: 866 $[M+H]^+$ (found $[M+H]^+$, 865.5630, $C_{43}H_{80}N_2O_{15}$ requires $[M+H]^+$ 856.5632).

4.3.2. Compound 8b

Using the general procedure on a 0.062 mmol scale yielded **8b** (0.029 g, 53%) as a white solid; IR (film) 3475, 2972, 2934, 1722, 1453, 1379, 1260, 1178, 1087, 1038, 999 cm^{-1}; ^1H NMR (400 MHz) δ (Table 3) 4.15 (2H, m, OCH_2), 1.08 (3H, m, CH_3); ^{13}C NMR (100 MHz) δ (Table 4) 156.5, 60.8; *m/z*: 880 $[M+H]^+$ (found $[M+H]^+$, 879.5796, $C_{44}H_{82}N_2O_{15}$ requires $[M+H]^+$ 879.5788).

4.4. Synthesis of the ethyl urea 9

To a solution of **6** (0.080 g, 0.099 mmol, 1.0 equiv) in dichloromethane (1.0 mL) at room temperature was added ethyl isocyanate (0.014 g, 0.016 mL, 0.198 mmol, 2.0 equiv). The solution was stirred at room temperature for 16 h before adding further ethyl isocy-

Table 3
^1H NMR resonances for macrolide portion of compounds **6**, **7a–7c**, **8a–8b**, **9**, **10a–10b** (H-16, H-17, H-18, H-19, H-20, H-21, H-6′, $NCH(CH_3)_2$, H-6″, and H-7″ are in the range 1.25–1.00 ppm). Resonances for the substituents appear in Section 4

	6	7a	7b	7c	8a	8b	9	10a	10b	11	12	13
H-2	2.68	2.63	2.64	2.71	2.62	2.63	2.65	2.64	2.63	2.65	2.69	2.78
H-3	4.00	3.94	3.91	3.96	3.94	3.95	3.96	3.95	3.99	3.94	4.01	3.93
H-4	1.83	1.81	1.89	1.90	1.80	1.84	1.84	1.82	1.82	1.80	1.85	1.90
H-5	3.53	3.50	3.50	3.62	3.54	3.56	3.51	3.51	3.50	3.52	3.56	3.63
H-7a	1.76	1.94	1.63	1.89	1.87	1.80	1.64	1.98	1.98	1.64	1.81	1.78
H-7b	1.17	1.16	1.24	1.34	1.28	1.18	1.28	1.26	1.23	1.39	1.27	1.26
H-8	2.38	2.45	2.42	2.45	2.50	2.51	2.43	2.37	2.37	2.47	2.37	2.39
H-9	3.06	3.17	3.11	3.22	3.13	3.12	3.17	3.17	3.17	3.18	3.03	3.17
H-10	1.96	2.09	2.05	2.14	2.08	2.07	2.08	2.08	2.07	2.10	1.98	2.17
H-11	3.63	3.78	3.70	3.78	3.74	3.67	3.80	3.81	3.81	3.75	3.65	3.70
H-13	4.97	4.91	4.95	5.10	4.97	4.96	4.90	4.87	4.86	4.93	4.98	5.10
H-14a	1.92	1.87	1.88	1.87	1.91	1.90	1.93	1.87	1.84	1.89	1.94	1.92
H-14b	1.42	1.47	1.41	1.45	1.44	1.42	1.48	1.47	1.47	1.46	1.42	1.46
H-15	0.85	0.89	0.86	0.90	0.89	0.86	0.88	0.89	0.88	0.88	0.87	0.90
H-1′	4.57	4.61	4.60	4.69	4.59	6.61	4.63	4.62	4.62	4.57	4.59	4.78
H-2′	3.21	3.23	3.25	3.23	3.23	3.23	3.19	3.23	3.22	3.28	3.22	3.57
H-3′	2.60	2.60	2.64	2.96	2.62	2.63	2.62	2.62	2.61	2.64	2.61	2.80
H-4′a	1.61	1.64	1.79	1.75	1.64	1.80	1.63	1.64	1.63	1.89	1.62	1.89
H-4′b	1.39	1.42	1.36	1.42	1.42	1.40	1.42	1.35	1.38	1.46	1.40	1.58
H-5′	3.57	3.60	3.63	3.83	3.59	3.62	3.60	3.60	3.61	3.61	3.61	3.88
NCH_3	2.20	2.21	2.22	2.30	2.22	2.22	2.23	2.21	2.20	2.22	2.21	2.15
NCH	2.89	2.89	2.91	3.04	2.90	2.90	2.90	2.89	2.87	2.91	2.87	2.78
H-1″	5.01	5.12	5.12	4.92	5.07	5.07	5.13	5.12	5.12	5.06	5.05	5.04
H-2″a	2.40	2.43	2.42	2.41	2.44	2.44	2.43	2.42	2.41	2.42	2.40	2.49
H-2″b	1.53	1.59	1.56	1.41	1.61	1.62	1.58	1.58	1.56	1.63	1.57	1.61
H-4″	3.00	3.05	3.04	2.94	3.06	3.07	3.06	3.05	3.04	3.08	3.03	3.10
H-5″	4.03	4.04	4.03	4.11	4.04	4.07	4.03	4.00	4.01	4.06	4.03	4.10
NH	na	7.44	6.87	na	5.83	5.58	5.64	7.07	6.87	6.06	na	na
OCH_3	3.34	3.36	3.34	3.34	3.36	3.36	3.36	3.35	3.35	3.45	3.35	3.39
H-9′a	3.76	3.72	3.57	3.98	3.48	3.78	3.79	3.91	3.99	3.80	3.75	3.93
H-9′b	3.42	3.60	3.26	3.63	3.23	3.76	3.58	3.68	3.58	3.63	3.61	3.78
H-9″a	2.87	3.54	3.58	3.63	3.47	3.45	3.40	4.05	3.92	3.29	2.88	3.37
H-9″b	2.70	3.41	3.58	3.63	3.47	3.23	3.26	3.50	3.92	3.29	2.57	3.37

Table 4
^{13}C NMR resonances for macrolide portion of compounds **6**, **7a–7c**, **8a–8b**, **9**, **10a–10b** (C-18, C-21, C-6′, $NCH(CH_3)_2$ and C-7″ are in the range 22.0–19.0 ppm). Resonances for the substituents appear in Section 4

	6	7a	7b	7c	8a	8b	9	10a	10b	11	12	13
C-1	176.8	177.2	177.2	177.0	176.8	176.8	177.3	177.4	177.3	177.2	177.8	177.1
C-2	44.3	44.2	44.2	44.9	44.1	44.1	44.2	44.2	44.0	44.4	44.2	44.1
C-3	78.0	77.3	77.8	77.6	78.2	78.1	77.7	77.5	77.3	78.2	77.9	77.8
C-4	42.9	44.0	43.8	43.5	44.1	44.0	43.7	43.9	43.8	43.8	43.9	43.0
C-5	84.3	85.6	85.1	83.6	85.3	84.7	85.1	85.4	85.4	85.6	84.6	84.0
C-6	75.3	75.5	75.3	75.3	75.4	75.2	75.5	75.6	75.6	75.4	75.3	75.4
C-7	37.0	37.8	37.6	37.8	37.7	37.7	37.9	38.2	38.3	37.5	37.0	37.4
C-8	32.5	31.2	31.2	30.9	30.8	30.9	31.7	31.8	31.8	32.2	33.1	31.5
C-9	91.1	92.5	92.9	92.6	92.5	92.6	93.7	94.5	94.5	92.4	90.8	92.1
C-10	32.2	32.4	32.2	32.1	32.2	32.1	32.3	32.5	32.5	32.9	32.3	32.2
C-11	69.1	70.1	70.1	70.0	69.5	70.4	70.3	70.5	70.5	69.9	68.8	70.0
C-12	73.8	74.6	74.2	74.3	74.1	74.0	74.8	75.4	75.5	74.4	73.7	74.3
C-13	77.1	77.5	77.0	76.4	77.3	77.1	77.3	77.3	77.3	77.3	76.7	76.6
C-14	22.0	22.3	22.1	21.9	22.3	22.2	22.2	22.4	22.4	22.2	23.4	21.7
C-15	11.3	11.4	11.3	10.5	11.5	11.5	11.4	11.5	11.5	11.3	11.4	10.3
C-16	13.7	13.0	13.2	12.4	13.1	13.1	13.1	13.0	12.9	13.3	13.5	12.8
C-17	9.1	9.3	9.1	8.5	9.3	9.2	9.2	9.3	9.3	9.3	9.1	8.4
C-19	16.7	16.7	16.6	16.3	16.7	16.5	16.6	16.7	16.7	16.7	17.0	16.5
C-20	16.1	14.8	15.1	14.3	15.1	14.6	15.0	14.5	14.6	15.2	16.6	14.4
C-1′	102.2	102.2	102.1	101.5	102.4	102.2	102.1	102.2	102.1	102.6	102.2	101.0
C-2′	70.1	70.0	70.1	70.3	70.1	69.9	70.0	70.0	69.9	69.5	70.9	69.0
C-3′	62.2	62.0	62.1	61.0	62.1	62.0	62.0	62.1	61.9	62.1	62.3	63.0
C-4′	32.9	32.8	32.9	33.1	33.0	32.9	32.8	32.9	32.8	32.9	33.0	32.2
C-5′	69.2	69.6	69.3	69.7	70.0	69.4	69.5	69.7	69.6	69.5	69.3	67.3
NCH_3	31.0	31.1	31.2	30.3	31.1	31.2	31.1	31.2	31.1	31.2	31.1	32.4
NCH	52.6	52.7	53.1	52.8	52.7	52.9	52.1	52.7	52.7	52.7	52.7	50.8
C-1″	95.1	94.8	94.9	95.4	95.5	95.3	94.7	94.7	94.5	95.5	95.0	95.4
C-2″	34.7	34.6	34.6	34.4	34.7	34.6	34.6	34.6	34.5	34.5	34.8	34.4
C-3″	72.8	72.8	72.8	72.9	72.9	72.8	72.8	72.8	72.8	72.8	73.0	73.2
C-4″	77.8	77.6	77.7	76.4	77.7	77.7	77.3	77.7	77.6	77.6	78.1	77.5
C-5″	65.5	65.7	65.7	65.3	65.8	65.8	65.7	65.7	65.6	65.8	65.6	65.4
C-6″	18.2	17.8	17.8	17.5	17.8	17.8	17.7	17.8	17.8	17.9	18.2	17.7
OCH_3	49.3	49.3	49.3	48.4	49.4	49.3	49.3	49.3	49.3	49.3	49.3	48.4
C-9′	72.8	71.3	70.8	70.0	69.9	70.4	73.4	73.5	72.8	70.1	70.9	69.4
C-9″	41.0	37.6	39.4	40.1	41.1	41.0	40.3	44.9	44.9	43.2	46.7	41.6

anate (0.022 g, 0.025 mL, 0.316 mmol, 3.2 equiv) and stirring at room temperature for 4 h. The solution was poured into $NaHCO_3$ (15 mL) and the organics extracted with CH_2Cl_2 (3 × 15 mL). The combined organics were dried ($MgSO_4$) and concentrated under reduced pressure. Column chromatography (silica, 35 → 50% acetone—hexane, 1% triethylamine) yielded **9** (0.019 mg, 22%) as a white solid; IR (film) 3393, 2972, 2936, 1732, 1649, 1559, 1452, 1378, 1178, 1083, 1054, 998, 900 cm^{-1}; ^1H NMR (400 MHz) δ (Table 3) 4.99 (1H, t, *J* 5.5 Hz, NH), 3.23 (2H, m, $NHCH_2CH_3$), 1.05 (3H, m, $NHCH_2CH_3$); ^{13}C NMR (100 MHz) δ (Table 4) 158.6, 34.8, 20.3; *m*/*z*: 879 $[M+H]^+$, 721 (found $[M+H]^+$, 878.5954, $C_{44}H_{83}N_3O_{14}$ requires $[M+H]^+$ 878.5948).

4.5. General procedure for the synthesis of thioureas 10

To a solution of **6** (0.075 g, 0.094 mmol, 1.0 equiv) in dichloromethane (1.0 mL) was added the isothiocyanate (0.141 mmol, 1.5 equiv) and the solution stirred at room temperature for 18 h. The solution was poured into $NaHCO_3$ (15 mL) and the organics extracted with CH_2Cl_2 (3 × 15 mL). The combined organics were dried ($MgSO_4$) and concentrated under reduced pressure. Column chromatography (silica, 50% acetone—hexane, 0 → 1% triethylamine) yielded the thiourea **10**.

4.5.1. Compound 10a

Using the general procedure on a 0.055 mmol scale yielded **10a** (0.034 g, 70%) as a white solid; IR (film) 3371, 2971, 2935, 1732, 1548, 1452, 1379, 1452, 1379, 1272, 1179, 1084, 1053, 999, 958, 900 cm^{-1}; ^1H NMR (400 MHz) δ (Table 3) 6.50 (1H, br s, NH), 3.60 (2H, m, $NHCH_2CH_3$), 1.17 (3H, m, $NHCH_2CH_3$); ^{13}C NMR (100 MHz) δ (Table 4) 183.1, 39.0, 14.8; *m*/*z*: 895 $[M+H]^+$ (found 894.5724, $C_{44}H_{83}N_3O_{13}S$ requires $[M+H]^+$ 894.5719).

4.5.2. Compound 10b

Using the general procedure on a 0.094 mmol scale yielded **10b** (0.032 g, 38%) as a white solid; IR (film) 3370, 2968, 2934, 2877, 1732, 1547, 1453, 1378, 1178, 1083, 1055, 1030, 998, 900 cm^{-1}; ^1H NMR (400 MHz) δ (Table 3) 7.20 (1H, br s, NH), 3.95 (2H, m, $NHCH_2CH_2CH_3$), 1.52 (2H, m, $CH_2CH_2CH_3$), 0.94 (3H, m, $NHCH_2CH_2CH_3$); ^{13}C NMR (100 MHz) δ (Table 4) 183.1, 44.8, 22.4, 11.4; *m*/*z*: 909 $[M+H]^+$, 751 (found $[M+H]^+$, 908.5905, $C_{45}H_{85}N_3O_{13}S$ requires $[M+H]^+$ 908.5889).

4.6. Synthesis of the methyl sulfonate 11

To a solution of **6** (0.075 g, 0.093 mmol, 1.0 equiv) in dichloromethane (1.0 mL) at room temperature was added pyridine (0.015 mL, 0.186 mmol, 2.0 equiv) followed by methanesulfonyl chloride (0.009 mL, 0.112 mmol, 1.2 equiv). The solution was stirred at room temperature for 2 h before adding $NaHCO_3$ (20 mL). The organics were extracted with CH_2Cl_2 (3 × 20 mL), combined, dried ($MgSO_4$) and concentrated under reduced pressure. Column chromatography (silica, 30% acetone—hexane, 1% triethylamine) yielded **11** (0.045 mg, 55%) as a white solid; ^1H NMR (400 MHz) δ (Table 3) 2.96 (3H, s, SO_2CH_3); ^{13}C NMR (100 MHz) δ (Table 4) 40.3; *m*/*z*: 886 $[M+H]^+$, 728 (found $[M+H]^+$, 885.5321, $C_{42}H_{80}N_2O_{15}S$ requires $[M+H]^+$ 885.5352).

4.7. Synthesis of the *N*-*i*-propylamine 12

To a solution of **6** (0.050 g, 0.062 mmol, 1.0 equiv) in methanol–acetone (1:1, 2.0 mL) was added acetic acid (0.007 mL,

0.124 mmol, 2.0 equiv). The reaction was stirred at room temperature of 30 min before adding sodium cyanoborohydride (0.008 g, 0.124 mmol, 2.0 equiv) and stirring at room temperature for 12 h. Rochelles' salt (2 mL) was added and the reaction stirred for 15 min before partitioning between EtOAc (30 mL) and Rochelles' salt (30 mL). The organics were washed with brine (25 mL), dried ($MgSO_4$) and concentrated under reduced pressure. Column chromatography (silica, 50% acetone–hexane, 1% triethylamine) yielded **12** (0.033 g, 63%) as a white solid; IR (film) 3470, 2970, 2935, 1729, 1452, 1378, 1167, 1109, 1084, 1053, 998, 899 cm^{-1}; ^1H NMR (400 MHz) δ (Table 3) 2.79 (1H, m, NHC*H*$(CH_3)_2$), 1.05 (6H, m, CH(C*H*$_3)_2$); ^{13}C NMR (100 MHz) δ (Table 4) 49.0, 23–21; *m*/*z*: 850 $[M+H]^+$, 692 (found $[M+H]^+$, 849.6079, $C_{44}H_{84}N_2O_{13}$ requires $[M+H]^+$ 849.6046).

4.8. Synthesis of the guanidine 13

To a solution of **6** (0.075 g, 0.093 mmol, 1.0 equiv) in tetrahydrofuran (1.0 mL) was added triethylamine (0.019 mL, 0.140 mmol, 1.5 equiv) followed by *N*,*N*′-di–Cbz-*S*-methylisothiourea (0.050 g, 0.140 mmol, 1.5 equiv). The reaction was stirred at room temperature for 14 h. The reaction was poured into $NaHCO_3$ (30 mL) and the organics extracted with EtOAc (3 × 30 mL). The combined organics were dried ($MgSO_4$) and concentrated under reduced pressure. Column chromatography (silica, 50% acetone–hexane, 1% triethylamine) yielded the protected guanidine, which was dissolved in MeOH (2.0 mL) and palladium on carbon added. The flask was purged with hydrogen and stirred under an atmosphere of hydrogen for 2 h. The reaction was purged with nitrogen and filtered through Celite®, eluting with MeOH (3 × 10 mL). The filtrate was concentrated under reduced pressure to yield the guanidine **13** 0.043 g, 54% over two steps) as a white solid; IR (film) 3330, 2934, 1729, 1669, 1457, 1377, 1629, 1166, 1077, 1057, 1031, 997, 957, 900 cm^{-1}; ^1H NMR (400 MHz) δ (Table 3); ^{13}C NMR (100 MHz) δ (Table 4) 157.6; *m*/*z*: 850 $[M+H]^+$ (found $[M+H]^+$, 849.5772, $C_{42}H_{80}N_4O_{13}$ requires $[M+H]^+$ 849.5794).

4.9. General procedure for the synthesis of arylmethyl ethers 14

To a solution of **4** (0.120 g, 0.157 mmol, 1.0 equiv) in dimethoxyethane (1.0 mL) was added potassium *tert*-butoxide (0.19 mL of a 1 M solution in THF, 0.188 mmol, 1.2 equiv). After stirring at room temperature for 10 min a solution of the alkylating agent (0.173 mmol, 1.1 equiv) in dimethoxyethane (1.0 mL) was added and the solution stirred at room temperature for 2 h. The reaction was quenched with $NaHCO_3$ (20 mL) and the organics extracted with EtOAc (3 × 20 mL). The combined organics were dried ($MgSO_4$) and concentrated under reduced pressure. Column chromatography (silica, 30 → 60% acetone–hexane, 0.1% triethylamine) yielded the arylmethyl ether **14**.

4.9.1. Compound 14a

Using the general procedure with 4-(iodomethyl)-2-methylthiazole as alkylating agent to yield **14a** (0.077 g, 56%) as a white solid; ^1H NMR (400 MHz) δ (Table 5) 6.95 (1H, br s, ArH), 2.96 (3H, s, $ArCH_3$); ^{13}C NMR (100 MHz) δ 177.2, 166.4, 153.4, 115.4, 102.2, 95.1, 92.0, 84.5, 78.2, 77.8, 77.3, 75.5, 74.1, 73.3, 70.5, 70.1, 69.7, 69.2, 65.9, 62.3, 53.0, 49.6, 46.4, 44.4, 44.2, 38.2, 35.0, 33.3, 32.7, 31.6, 31.5, 23.2, 22.7, 22.0, 21.6, 21.5, 20.7, 19.4, 19.3, 18.0, 17.0, 15.3, 13.5, 11.9, 9.5; *m*/*z*: 876 $[M+H]^+$ (found $[M+H]^+$, 875.5310. $C_{44}H_{78}N_2O_{13}S$ requires $[M+H]^+$ 875.5297).

4.9.2. Compound 14b

Using the general procedure with 2-(iodomethyl)thiazole as alkylating agent yielded **14b** (0.088 g, 65%) as a white solid; *m*/*z*: 862 $[M+H]^+$ (found $[M+H]^+$, 861.5181. $C_{43}H_{76}N_2O_{13}S$ requires $[M+H]^+$ 861.5141).

Table 5
^1H NMR resonances for macrolide portion of compounds **14a**–**14f** (H-16, H-17, H-18, H-19, H-20, H-21, H-6′, NCH$(CH_3)_2$, H-6″, and H-7″ are in the range 1.25–1.00 ppm). Resonances for the substituents appear in Section 4

	14a	14c	14d	14e	14f
H-2	2.48	2.59	2.63	2.66	2.65
H-3	3.90	3.92	3.94	4.06	4.10
H-4	1.99	2.06	1.81	2.20	2.19
H-5	3.51	3.53	3.53	3.62	3.63
H-7a	1.78	1.83	1.89	1.89	1.88
H-7b	1.30	1.35	1.23	1.32	1.31
H-8	2.43	2.56	2.63	2.58	2.61
H-9	2.91	2.97	2.94	2.63	3.06
H-10	2.20	2.15	2.16	2.20	2.19
H-11	3.72	3.78	3.89	3.95	3.94
H-13	4.88	4.88	4.90	4.90	4.90
H-14a	1.83	1.87	1.86	1.91	1.91
H-14b	1.36	1.40	1.45	1.52	1.53
H-15	0.80	0.86	0.90	0.93	0.94
H-1′	4.56	4.61	4.63	4.61	4.63
H-2′	3.14	3.21	3.23	3.28	3.31
H-3′	2.53	2.59	2.63	2.58	2.58
H-4′a	1.74	1.78	1.62	1.67	1.70
H-4′b	1.54	1.59	1.38	1.45	1.46
H-5′	3.51	3.57	3.58	3.62	3.63
NCH_3	2.12	2.17	2.21	2.25	2.27
NCH	2.81	2.85	2.63	2.94	2.96
H-1″	4.96	5.04	5.07	5.09	5.13
H-2″a	2.31	2.37	2.38	2.44	2.47
H-2″b	1.48	1.53	1.54	1.61	1.63
H-4″	3.12	3.18	2.90	3.04	3.36
H-5″	3.88	3.95	3.91	4.03	4.08
OCH_3	3.26	3.32	3.33	3.37	3.39
H-9′a	4.75	4.78	4.94	4.93	4.85
H-9′b	4.48	4.46	4.42	4.91	4.82

4.9.3. Compound 14c

Using the general procedure with 3-(bromomethyl)-5-methylisoxazole as alkylating agent yielded **14c** (0.082 g, 61%) as a white solid; ^1H NMR (400 MHz) δ (Table 5) 6.00 (1H, d, *J* 1.0 Hz, ArH), 2.35 (3H, d, *J* 0.5 Hz, $ArCH_3$); ^{13}C NMR (100 MHz) 177.4, 170.1, 161.5, 102.3, 101.2, 95.1, 92.6, 85.1, 78.2, 77.7, 77.4, 75.7, 74.2, 73.3, 70.5, 70.4, 69.8, 66.0, 64.4, 62.4, 53.0, 49.7, 46.5, 44.5, 44.4, 38.6, 35.0, 33.3, 32.6, 31.5, 30.7, 22.8, 22.5, 22.0, 21.6 (2C), 20.7, 19.4, 18.2, 17.0, 15.1, 13.3, 12.6, 12.0, 11.8, 9.6; *m*/*z*: 860 $[M+H]^+$ (found $[M+H]^+$, 859.5494. $C_{44}H_{78}N_2O_{14}$ requires $[M+H]^+$ 859.5526).

4.9.4. Compound 14d

Using the general procedure with 4-(bromomethyl)pyridine as alkylating agent yielded **14d** as a white solid; ^1H NMR (400 MHz) (Table 5) 8.55 (2H, d, *J* 4.5 Hz, 2 × *m*-ArH), 7.21 (2H, d, *J* 4.0 Hz, 2 × *o*-ArH); ^{13}C NMR (100 MHz) δ 177.5, 150.2 (2C), 147.5, 130.9, 122.0 (2C), 102.3, 94.9, 92.8, 85.3, 78.1, 77.7, 77.4, 75.9, 74.4, 73.3, 71.1, 70.6, 70.4, 69.8, 65.9, 62.4, 53.2, 49.7, 44.7, 44.4, 38.8, 35.0, 33.3, 33.0, 31.6, 30.5, 23.0, 22.2, 21.6, 21.5, 20.7, 19.5, 18.0, 17.1, 15.1, 13.2, 12.1, 9.7; *m*/*z*: 856 $[M+H]^+$ (found $[M+H]^+$, 855.5613. $C_{45}H_{78}N_2O_{13}$ requires $[M+H]^+$ 855.5577).

4.9.5. Compound 14e

Using the general procedure with 3-chloromethyl-2-trityl-1,2,4-triazole as alkylating agent yielded the trityl protected material (0.170 g). To a solution of the trityl protected compound (0.170 g, 0.157 mmol) in methanol (6 mL), was added pyridine hydrochloride (0.007 g, 0.061 mmol, 0.4 equiv) and pyridine *para*-toluenesulfonate (0.010 g, 0.04 mmol, 0.25 equiv). The solution was maintained at 50 °C for 16 h. The reaction was quenched with saturated $NaHCO_3$ solution (20 mL) and extracted with

chloroform–methanol (5:1) (3 × 20 mL). The organic extracts were dried (Na_2SO_4). Column chromatography (silica, 9% MeOH–CH_2Cl_2, 0.5% NH_4OH) yielded **14e** (0.035 g, 27% over two steps) as white solid; ^{1}H NMR (400 MHz) (Table 5) 7.95 (1H, br s, NH), 4.99 (1H, br s, ArH); *m*/*z*: 846 $[M+H]^+$.

4.9.6. Compound 14f

Using the general procedure with 2-chloromethylimidazole hydrochloride as alkylating agent yielded **14f** (0.061 g, 46%) as a white solid; ^{1}H NMR (400 MHz) (Table 5) 6.97 (2H, br s, 2 × ArH); *m*/*z*: 845 $[M+H]^+$.

4.9.7. In vitro assays

Motilin agonist potency and tachyphylaxis were measured in a rabbit smooth muscle contractility assays as previously reported.[29,30] EC_{50} values are the concentration that caused 50% of the maximal possible contraction. Tachyphylaxis is reported as the % contractility response obtained from an EC_{90} drug concentration following three cycles of administration and washout. hERG inhibition was measured at 37 °C using a stably transfected HEK cell line at expressing the hERG mRNA.[33] For routine screening, compounds were tested in replicate at 30 and 300 μM. For measurement of the IC_{50} of **1**, **3**, and **7a**, duplicate measurements were made at 1, 3, 10, 50, 100, and 300 μM.

Antibacterial activity was assessed by determining the minimal growth inhibitory concentration (MIC) of each compound.[39] For routine screening, the highly erythromycin-sensitive strain *S. pneumoniae* ATCC6301 was used. For **7a**, a panel of ~200 strains were used and were representative of those commonly found in the gut flora, and as well as those which are commonly know to develop resistance to antibiotics. The strains were isolated from a variety of clinical specimens and are part of a collection maintained at the Clinical Microbiology Laboratories at the University of Rochester Medical Center and included approximately 10 isolates each of the following strains: *Enterococcus faecalis*, *Enterococcus faecium*, *Micrococcus luteus*, *Staphylococcus aureus*, *Staphylococcus Coagulase*, *Streptococcus bovis*, *S. pneumoniae*, *Streptococcus pyogenes*, *Corynebacterium jeikeium*, *Corynebacterium species* (not jeikeium), *Lactobacillus species*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Bacteroides fragilis*, *Clostridium difficle*, *Clostridium perfringenes*, and *Propionbacterium acnes*.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.08.035. These data include MOL files and InChiKeys of the most important compounds described in this article.

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