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9-Dihydroerythromycins as non-antibiotic motilin receptor agonists

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ABSTRACT

A series of 9-dihydroerythromycin A and B analogues with modification of the desosamine nitrogen have been synthesized and screened for motilin agonist activity, antibiotic activity, tachyphylaxis and hERG channel current inhibition. Small alkyl groups resulted in the potency while compounds with a primary or secondary amine resulted in the low motilin agonist potency. Several compounds were identified as non-antibiotic motilin receptor agonists with minimal tachyphylaxis and low hERG interaction.

The motilin receptor is a G-protein-coupled receptor (GPCR) found in the gastrointestinal smooth muscle and enteric neuronal tissues. Motilin, a 22-amino acid peptide hormone, binds to the receptor and stimulates coordinated gastric motility.[1–3] The motilin receptor is a target for the treatment of gastrointestinal (GI) disorders such as gastroesophageal reflux disease (GERD) and gastroparesis. It has been shown that IV infusion of motilin or oral administration of the macrolide antibiotic erythromycin A (EryA) stimulates GI motility in man.[4,5] However, while EryA may be used to treat gastroparesis, its antibiotic activity makes it unsuitable for chronic administration.

Attempts to optimize the motilin agonist activity of EryA while engineering out the antibiotic activity have resulted in compounds derived from the erythromycin enol ether, ABT-229 and GM-611 (Fig. 1).[6,7] ABT-229 was withdrawn from phase II clinical trials, due to a lack of efficacy upon repeated administration, a phenomenon known as tachyphylaxis.[8]

We also aimed to identify non-antibiotic motilin receptor agonists that were effective upon repeated administration. We were conscious of the acid instability of EryA, which results in cyclization of 6-hydroxyl onto the 9-ketone to form a reactive 6,9-enol ether that is 50–100 fold more potent as a motilin agonist. Further,

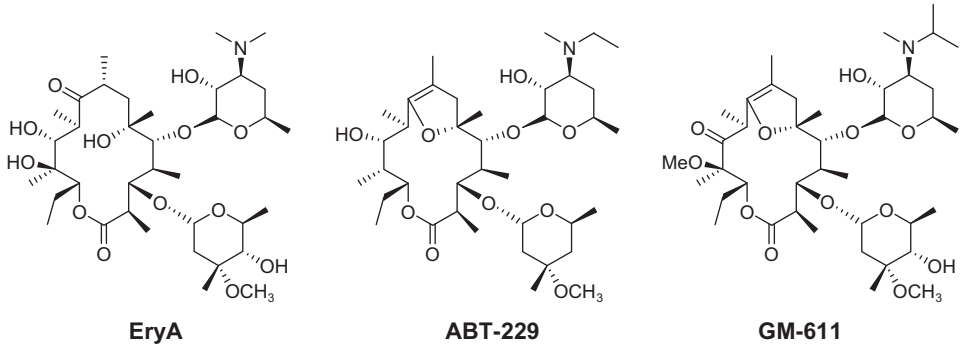

Figure 1. Chemical structures of erythromycin A (EryA), ABT-229 and GM-611.

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upon continued exposure to acid, the enol ether decomposes by reaction with the 12-hydroxyl to form an inactive spiroketal.[9] By contrast, reduction of the 9-ketone results in the acid-stable 9-dihydroerythromycin **1**. Compound **1** is a weaker antibiotic but a potent motilin agonist comparable to EryA.[10] We decided to investigate 9-dihydroerythromycin A as a motilin agonist scaffold. In contrast to the enol ether, which shows little antibiotic activity, **1** retains moderate antibiotic activity. Thus, we focused our efforts on developing SAR around the dimethylamine of the desosamine, which is crucial for antibacterial potency.[11]

9-Dihydro-*N*-desmethylerythromycin A was synthesized by sodium borohydride reduction of EryA to the 9(*S*)-isomer **1**,[12] followed by reaction with iodine in the presence of sodium acetate to yield the *N*-desmethyl compound **2**[13] (Fig. 2). *N*-Alkyl derivatives were available through alkylation of **2** with the variety of alkyl halides in the presence of Hünig's base (compounds **3**–**13** and **18**–**20**)[14] or with the corresponding epoxide in methanol at 40 °C (**14**–**17**). In two cases the remaining *N*-methyl group of **2** was removed again by treatment with iodine to obtain secondary amines **21** and **22**.

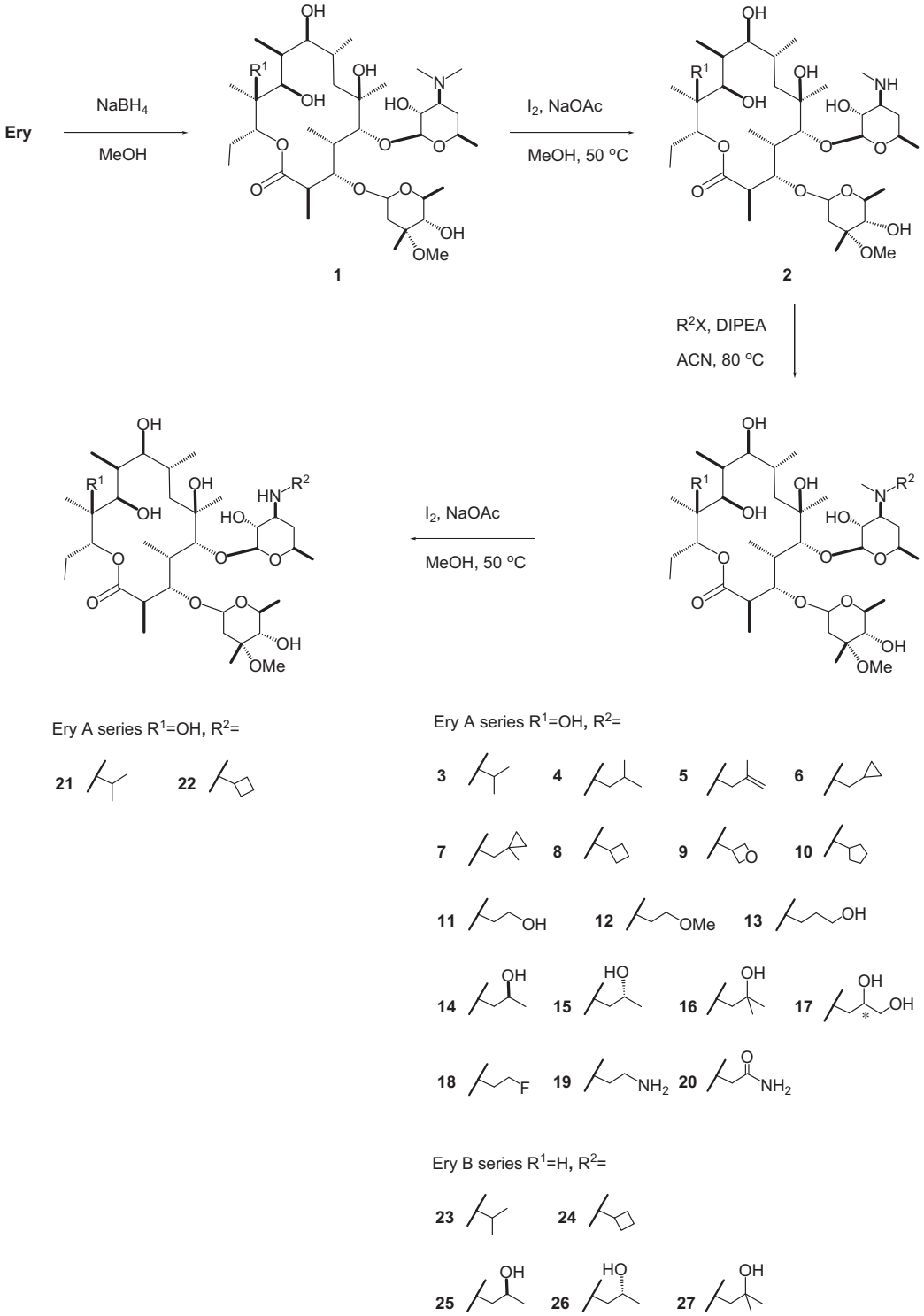

Figure 2. Desosamine modification of 9-dihydroerythromycins.

Table 1
Assay results for Ery A and 9-dihydroerythromycin A analogues

Compounds	Agonist EC_{50} (nM)	MIC (μg/mL)	Tachyphylaxis (% @ 4th dose)
EryA	1200	0.03	97 ± 1
ABT-229	7	64	22 ± 10
3	260	32	76 ± 6
4	363	nd	81 ± 3
5	2400	nd	nd
6	310	32	nd
7	2000	64	nd
8	350	128	90 ± 5
9	5700	32	nd
10	>5000	128	nd
11	210	8	86 ± 6
12	7700	nd	nd
13	700	nd	nd
14	360	128	95
15	460	>128	93
16	100	>128	89 ± 5
17	2500	nd	nd
18	3800	nd	nd
19	7300	nd	nd
20	>5000	nd	nd
21	>5000	>128	nd
22	>5000	16	nd

Motilin agonist potency and tachyphylaxis were measured in a rabbit smooth muscle contractility assays as previously reported.[15,18] 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. Antibacterial activity was assessed by determining the minimal growth inhibitory concentration[19] (MIC) with the highly erythromycin-sensitive strain *Streptococcus pneumoniae* ATCC6301. nd = Not determined.

Table 2
Assay results for 9-dihydroerythromycin B analogues

Compounds	Agonist EC_{50} (nM)	MIC (μg/mL)	Tachyphylaxis (% @ 4th dose)
23	106	128	79 ± 7
24	300	>128	71 ± 12
25	180	>128	70
26	280	>128	89 ± 5
27	100	>128	56

Using a similar synthetic route a subset of these derivatives was generated in the erythromycin B (EryB) scaffold, which lacks the 12-hydroxyl group. In this way it might be possible to take advantage of the lower antibacterial activity of this scaffold (Fig. 2).

All the compounds were initially screened in a muscle strip contractility assay to test for motilin agonist activity. Compounds of interest were further examined for antibiotic activity against the EryA sensitive strain *Streptococcus pneumoniae* ATCC 6301 and in our tachyphylaxis assay[15] (Table 1).

The results show that there are several compounds that met our desired profile of motilin agonist potency of less than 350 nM. There appears to be a steric requirement about the desosamine nitrogen for compounds with small alkyl groups—the isopropyl **3**, isobutyl **4**, cyclopropylmethyl **6** and cyclobutyl **8**—show good potency while the larger 2-methylcycloproplymethyl **7** and cyclopentyl **10** are less potent. The potency is balanced by antibacterial activity where the smaller groups are more potent. However the *N*-cyclobutyl compound **8** does meet our profile showing good potency with acceptable antibacterial activity (⩾128 μg/mL) and minimal tachyphylaxis (⩾90% at 4th dose).

It is interesting to note that moving from *N*-cyclobutyl to *N*-oxetanyl as in **9** results in a significant drop in potency. It appears that in general increasing polarity of the desosamine group is deleterious to potency. The ethylamino compound **19** and the acetamide **20** show little motilin agonist activity, while the secondary amino compounds **21** and **22** are inactive. In the case of the hydroxy compounds the effects are more subtle, however, tertiary hydroxyl compound **16** shows the optimal profile of potency and off target effects. Indeed this compound meets the criteria we had set.

In the case of the EryB derivatives as expected all the compounds met our criteria in terms of lack of antibacterial activity (Table 2). The five compounds synthesized **23**–**27** are analogous to the EryA series compounds **3**, **8**, **14**–**16**, respectively and show similar or improved potency against the motilin receptor, however, which many of these compounds a significant amount of tachyphylaxis was observed with only one compound **26** meeting the desired profile.

Table 3
hERG inhibition for EryA, ABT-229 and selected compounds as a percentage of inhibition at 30 μM

Compounds	hERG
EryA	50
ABT-229	98
3	80
8	38
14	16
16	25
26	38

hERG inhibition was measured at 37 °C using a stably transfected HEK cell line at expressing the hERG mRNA.[20]

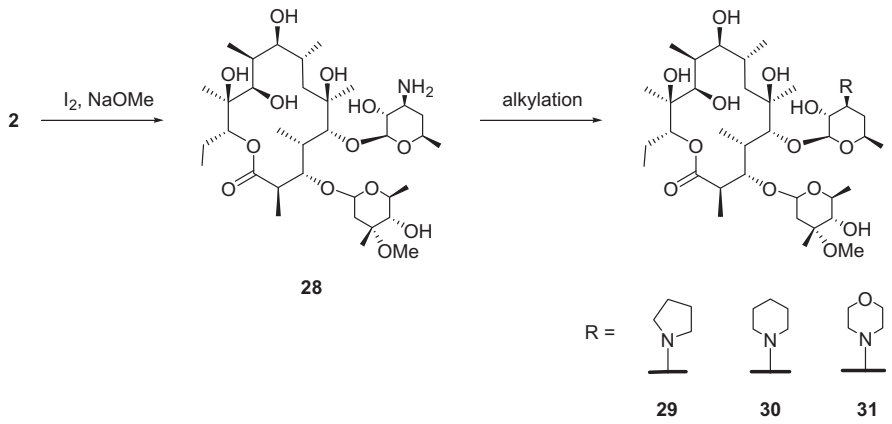

Figure 3.

With these results in hand, it was decided to investigate cyclic amines at the desosamine nitrogen. These were synthesized using the appropriate dibromide and the primary amine **28** which was obtained by demethylation of **2** with iodine and sodium methoxide,[13] (Fig. 3). In this way, the pyrrolidino, piperidinyl and morpholinyl compounds **29**–**31** were obtained. Unfortunately, these compounds showed little or no motilin agonist activity.

From the compounds synthesized there were several that met the profile which we had set, that is to say, a potent motilin agonist without showing both tachyphylaxis and significant antibiotic activity. However, we were conscious of the ability of macrolides to inhibit the hERG, a potassium ion channel in the heart that can result in potentially fatal cardiac arrythmia.[16] Therefore, the most promising analogues were examined for their ability to inhibit hERG (Table 3). Of the compounds investigated all were superior to ABT-229. In general, as the side-chain on the desosamine nitrogen becomes more polar the hERG inhibition decreases. Four compounds **8**, **14**, **16** and **26** showed hERG inhibition, which was lower than EryA.

In summary, we have shown that modification of desosamine nitrogen of 9-dihydroerythromycins can separate motilin agonist activity from its antibiotic activity. In general the EryB series shows less antibiotic activity but a more significant amount of tachyphylaxis. We have generated several erythromycin analogues **8**, **14**, **16** and **26** that meet our profile, motilin agonist potency without antibiotic activity. These compounds do not show significant tachyphylaxis and have an improved safety profile relative to EryA in terms of hERG inhibition. It was the ability of these compounds to inhibit hERG that led us to further investigate the 9-substituted-9-dihydroerythromycin series.[17]

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