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Design, synthesis and in vivo activity of 9-(*S*)-dihydroerythromycin derivatives as potent anti-inflammatory agents

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Abstract—The synthesis of a new class of 9-(*S*)-dihydroerythromycin derivatives and their anti-inflammatory activity on in vivo PMA assay are described. Modifying the desosamine sugar on the C-3′ amino group, it was possible to differentiate between anti-biotic and anti-flammatory action. The compounds are completely devoid of anti-microbial effects but their anti-inflammatory properties are enhanced. These results strongly suggest the potential of macrolides as a new class of anti-inflammatory agents.

Macrolide anti-biotics have been used effectively and safely for the treatment of respiratory tract infections for more than 40 years. The first macrolide, erythromycin A (Scheme 1), was introduced in the 1950s and has enjoyed widespread clinical use especially in patients with allergic reactions to penicillin.[1]

There are growing evidences that macrolide anti-biotics may have beneficial effects in chronic inflammatory airway diseases such as asthma, diffuse panbronchiolitis (DPB) and chronic sinusitis that are independent of their anti-bacterial effects.[2–4] However, the anti-inflammatory activities seem to be limited to the 14-membered ring macrolides like erythromycin and clarithromycin, and 15-membered ring macrolides like azithromycin, but are not shared by 16-membered ring macrolide like josamycin.[5]

Great caution must be used for administering anti-microbial drugs as anti-inflammatory agents for chronic treatment in order to avoid selecting microbial resistance. A better approach would involve chemical modification of the basic structure in order to enhance effects on the inflammatory cascade and avoid anti-microbial activity.

In this report, we describe the preliminary results regarding a study aimed at the design and synthesis of a new class of erythromycin derivatives provides with potent in vivo anti-inflammatory properties. Several compounds were prepared and tested in an iterative approach in order to enhance the anti-inflammatory activity but at the same time eradicating the anti-bacterial effect.

During this study, we have found that erythromycin anti-bacterial activity can be reduced by structural modifications at different functional groups:

(1) C-3′ dimethylamino modification,
(2) Cladinose removal,[6]
(3) C-9 carbonyl modification.

The dimethyl amino group is directly involved in the anti-bacterial mechanism and its modification drastically reduces this effect.[7]

One of the major problems of erythromycin is its instability in the acidic environment of the stomach. The acid degradation products generated by intramolecular hemiketal formation between the hydroxyls at C-6 and C-12 with the C-9 carbonyl are responsible for its poor bioavailibility and gastrointestinal (GI) side effects.[8]

Keywords: Macrolide; Erythromycin; Erythronoid; Anti-inflammatory, amide; Amine; Desosamine; Cladinose; PMA induced ear edema; CD1 mice.
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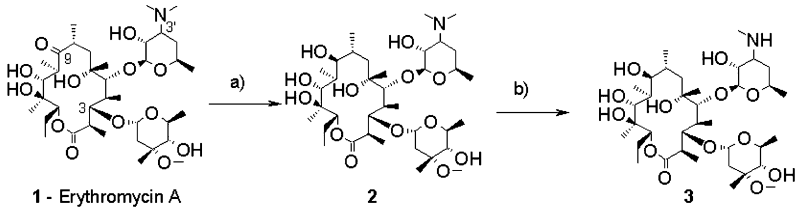


Scheme 1. Reagents and conditions: (a) $NaBH_4$ (1 equiv), 5% H_2O/THF, 0 °C, 1 h, 72%, 95:5 isomer ratio; (b) DEAD (1 equiv), CH_2Cl_2, 16 h, 98%.

Among all the possible C-9 carbonyl modifications in erythromycins, we selected the reduction to secondary alcohol.[9] C-9 carbonyl reduction increased metabolic stability and played an important role in reducing the anti-bacterial effect, which was completely eliminated by the concomitant modification of the dimethylamino group.[7]

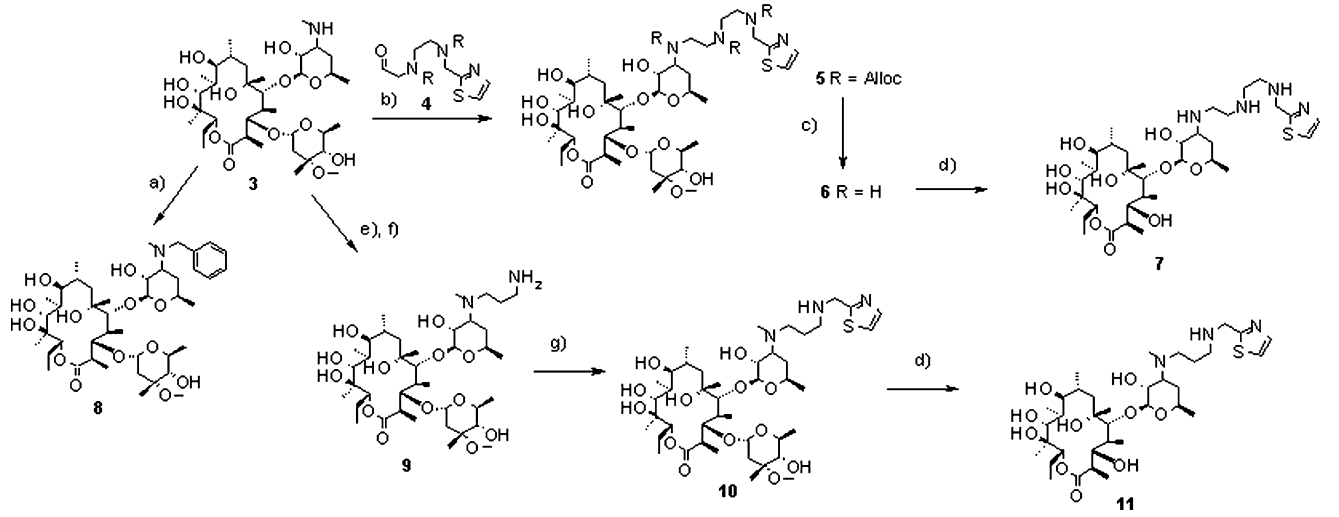
A study on the diastereoselective reduction of C-9 carboxyl group was performed. The diastereoselection was induced by the presence of 18 stereogenic centers in erythromycin and its 3D-conformation. The highest isomer ratio was obtained using polar, aprotic solvents such as THF, rather than protic solvents such as MeOH or H_2O. On the other hand, the global yield was quantitative in protic solvents but much lower in THF. The best condition consisted of using a 5% H_2O/THF solution at 0 °C to give the 9-(*S*)-dihydroerythromycin **2** in good yield and isomer ratio (Scheme 1).

Functionalization of the desosamine sugar by introduction of a C-3′ substituent was envisaged as a suitable modification. The amino group was demethylated following two protocols. The first one, an Iodine-promoted demethylation in MeOH, could be performed either refluxing[10] or irradiating with a 400 W lamp[11] bringing to 50–60% of isolated yield. The second one, a DEAD mediated reaction,[12] gave quantitative yield of the desmethyl-alcohol **3** and it was chosen for its synthesis (Scheme 1).

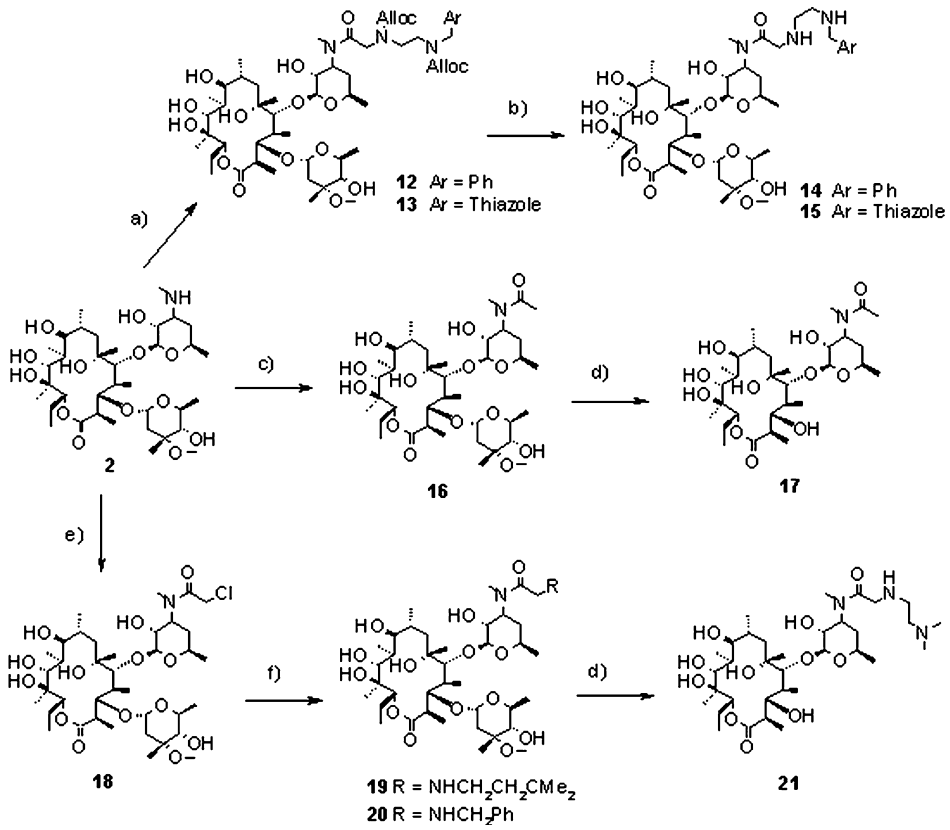
Reductive amination of desmethyldihydroerythromycin **3** with benzaldehyde gave product **8** (Scheme 2). Similarly intermediate **5** was obtained reacting **2** with an alloc-protected thiazole-diamino-aldehyde **4**. Alloc removal under standard conditions[13] gave product **6**, which was further treated in acid media to remove cladinose sugar, yielding the cladinose-free analogue **7** (Scheme 2). Acid promoted cladinose removal gave a class of erythronoids that were found to be inactive in many anti-bacterial tests even without further structural modifications.[6]

Macrolide **10** and its cladinose-free analogue **11** have been prepared from **3** by a three-step procedure (Scheme 2). Michael addition of **3** to refluxing acrylonitrile, followed by catalytic hydrogenation gave amine **9**. This latter was in turn subjected to reductive amination with thiazolecarboxaldehyde to give product **10** in satisfactory yield (Scheme 3).

We finally devised another class of compounds featuring a substituent connected to the desosamine sugar through an amide bond in C-3′ position. This modification completely suppressed the unwanted anti-bacterial effect.



Scheme 2. Reagents and conditions: (a) benzaldehyde (1.1 equiv), $Me_4NBH(OAc)_3$ (2 equiv), AcOH (1.5 equiv), dichloroethane, 67%; (b) 4 (1 equiv), $Me_4NBH(OAc)_3$ (2 equiv), AcOH (1.5 equiv), dichloroethane, 55%; (c) pyrrolidine (2.5 equiv), tetrakis(triphenylphospin)palladium (0.05 equiv), $CHCl_3$, 2 h, 56%; (d) HCl 2 N in MeOH, 50–80%; (e) acrylonitrile, reflux, 6 h; 60%; (f) H_2, 5 atm, 5% Pd/C, MeOH, 95%; (g) thiazolecarboxaldehyde (1 equiv), $Me_4NBH(OAc)_3$ (2 equiv), AcOH (1.5 equiv), dichloroethane, 87%.



Scheme 3. Reagents and conditions: (a) long-chain acid (1.5 equiv), DCC-resin (2 equiv), dichloroethane, 40 °C, 16 h, 50–60%; (b) pyrrolidine (2 equiv), tetrakis(triphenylphospine)palladium (0.05 equiv), CH_2Cl_2, 60–80%; (c) acetyl chloride (1 equiv), NEt_3 (1.2 equiv), CH_2Cl_2, 1 h, 90%; (d) HCl 1 N in MeOH, 90%; (e) 2-Cl-acetyl (1.1 equiv), DCC-resin (2 equiv), CH_2Cl_2, 40 h, 66%; (f) dimethylaminoethylenamine or benzylamine (1.1 equiv) THF, 40 °C, 1 h, 75–80%.

Amide **16** was prepared from **3** by simple acetylation.[14] DCC-resin supported coupling of **3** with the corresponding aromatic long-chain acids gave the alloc-protected amines that, after Pd° catalyzed deprotections,[13] yielded compounds **14** and **15**. Starting from the chloro acetamide intermediate **18**, compounds **19** and **20** were synthesized by nucleophilic substitution with the corresponding amines. Cladinose-free macrolides **17** and **21** were prepared reacting compounds **16** and **19** in methanolic HCl solution.

The prepared macrolides were preliminary screened through a first level of tests regarding cytotoxicity[15] and in vitro ROS inhibition.[16] The compounds whose synthesis are described above passed this first selection and their anti-inflammatory activity was evaluated in vivo by topical administration of 0.5 mg/ear on PMA-induced ear edema in mice using erythromycin as standard (Table 1).[17]

All the compounds were soluble, nontoxic and, except for compounds **8** and **10**, no effect was observed on several bacterial strains.[18]

Most of these compounds are as effective as erythromycin (i.e., aminomethylthiazoles **6**, **7**, and **11** or benzylamine **8**). The best anti-inflammatory activity on amino subclass was obtained by aminomethylthiazole **10**.

Table 1. Inhibition of ear edema in CD1 mice after topically application (500 μg/ear) of erythromycin derivatives[17]

Macrolide derivatives code no.	Subclass	Cladinose	Inhibition[a] (%)	SE (±)[b]
Erythromycin	Amine	+	42	
6	Amine	+	43	11
7	Amine	–	45	5
8	Amine	+	58	5
10	Amine	+	74	2
11	Amine	–	53	9
14	Amide	+	87	6
15	Amide	+	77	5
16	Amide	+	79	5
17	Amide	–	66	5
19	Amide	+	18	13
20	Amide	+	71	6
21	Amide	–	31	5

[a] Values are means of five experiments.
[b] Standard error.

The anti-bacterial action was greatly reduced or eliminated as the size of the C-3′ substituent increased.

The best results were achieved with the amide subclass, which never showed toxicity or anti-microbial effect. Their anti-inflammatory properties were improved ranging from 66% inhibition of cladinose-free acetamide **17**

to 86% of the benzylamine **14**. Only dimethyl amines **19** and **21** showed a low activity.

In general cladinose removal did not increase the anti-inflammatory effect, but these compounds showed a better physical–chemical profile and no anti-microbial action.

In summary, we have developed a new class of 9-(*S*)-dihydroerythromycin derivatives in order to obtain a new class of derivatives endowed with anti-inflammatory activity but devoid of anti-bacterial effects.

These results led us to further evaluate this class of compounds. In consideration of bioavailability, permeation, and in vivo toxicity studies, acetamido macrolide **17** was selected as drug candidate for preclinical and clinical development studies.

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14. Amide 16 was also prepared by Polonovsky protocol treating the corresponding dimethylamino-*N*-oxide with acetic anhydride in ethyl acetate at rt for 4 h followed by hydrolysis of 2′-*O*-acetyl functionality with K_2CO_3 in aqueous methanol media at 60 °C. Ku, Y.-Y.; Riley, D.; Patel, H.; Yang, C.; Liu, J.-H. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1203.
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16. The ability of macrolide compounds to modulate the oxidase activity of phagocytic cells (reactive oxygen species production) was tested in vitro in human whole blood.
17. Zunic, M.; Bahr, G. M.; Mudde, G. C.; Meingassner, J. G.; Lam, C. *J. Invest. Dermatol.* **1998**, *111*, 77, *PMA Method:* The PMA-induced edema in ear tissue was evaluated in five CD1 mice per compound. The compounds were solubilized in trans-phase delivery system (TPDS), a vehicle containing benzyl alcohol 10%, acetone 40%, and isopropanol 50%. The test item was topically applied at 0.5 mg/ear to the inner surface of one ear and 30 min later 15 μl of 0.01% PMA dissolved in acetone was applied to the same area of the test item application. Six hours later, mice were sacrificed by CO_2 inhalation. The edema was evaluated by weighting a constant portion of the treated ear pinna, in comparison with the untreated controlateral one.
18. The 'in vitro' antibacterial effect of macrolides was evaluated on different bacterial strains: Streptococcus pneumoniae (ATCC 49619), Staphylococcus aureus (ATCC 29213 or ATCC 6538), Enterococcus faecalis (ATCC 29212), and Streptococcus pyogenes (ATCC 19615).