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A facile synthesis and antimicrobial activity of erythromycin-2'ester

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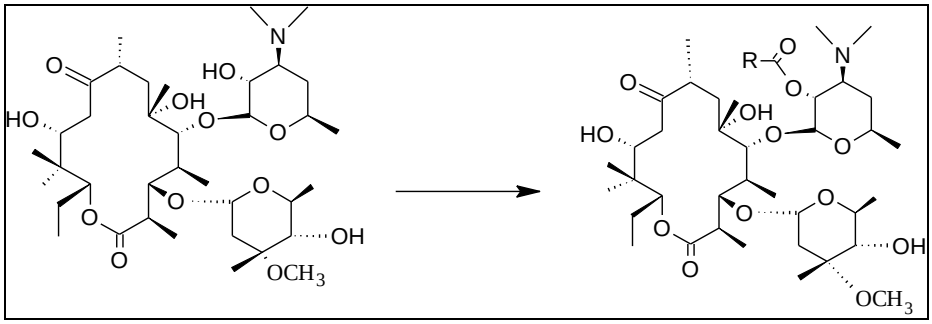
Abstract
Erythromycin-2'esters were synthesized regiospecifically using some Boc-, Fmoc- and Z protected amino acids. These ester analogs were tested for their antimicrobial activity *in vitro* against few bacterial strains *viz Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumoniae* and *Escherichia coli* taking erythromycin as reference. Some of the compounds synthesized have shown promising antibacterial activity as compared to the reference erythromycin.

Keywords: Erythromycin-2'esters, prodrug, protected aminoacids, antibacterial activity

Introduction
Structural modification of erythromycin has been one of the most important approaches for the development of new derivatives for the control and treatment of bacterial infection including resistant pathogens. This has started right after the discovery of erythromycin, a very powerful and safe antibiotic, since macrolide resistance has been observed after its prolonged administration. There are two major problems associated with erythromycin therapy *viz*. its acidic degradation [2, 3] and acquired resistance. In the past several decades efforts are being made for the development of derivatives, which are more stable to acidic degradation and are active against resistant strains of pathogens. Several strategies were adapted to generate molecules which are acid stable as well as active against resistant strain of bacteria. The most important approach is to develop molecules without cladinose sugar to eliminate the acid sensitive part from the macrolide skeleton. However it was reported that removal of cladinose sugar leads to drastic loss of antibacterial activity in spite of the fact that cladinose moiety is not involved at the binding site of 50S and 23S subunit of bacterial ribosome[1]. It has been proposed that cladinose moiety provides structural rigidity and optimal topology to the molecule at the binding site. This prompted us to investigate structural modification in the macrolide skeleton keeping the cladinose moiety intact. We have designed few prototypes, which may be better as compared to parent molecule. This include synthesis molecule as prodrugs as well as modifications that may influence the binding of the drug at the RNA binging site.

There have been several structural modifications exercised earlier on the macrolide skeleton, to overcome the above-mentioned problems. Some early structural modifications involve the synthesis of a few ester prodrugs of erythromycin namely 2'-Ethyl succinyl erythromycin 1, 2'-Acetyl erythromycin 2, 2'-Propionyl erythromycin 3, 2'-Benzoyl erythromycin 4 [figure-1] in 1960s and 1970s[4]. Erythromycin ethyl succinate has overcome the problem of vile taste and erythromycin is normally administered to children as a suspension of this compound. Although these ester derivatives have enhanced acid stability relative to erythromycin but they have suffered the problem of loss in antibacterial activity [5]. It is very well known that various amino acid ester prodrugs *viz*. valacyclovir (acyclovir), an antiviral agent and 5'-L-valyl and 5'-L-isolucyl esters of gemcitabine, an anticancer agent and some amino acid ester prodrugs of nalidixic acid (an antibacterial agent); have been proven to be more effective than their parent compound[5]. Although ester pro drug of macrolides is known in the literature but prodrugs derived from the amino acid remains unexplored. Inspired by the observation that amino acid prodrugs have been successfully developed as better drugs, we have decided to make some amino acid based prodrugs of erythromycin, and tested them for their antibacterial activity. The most reactive site of the erythromycin *viz*. 2' hydroxy group of desosamine sugar was derivatised to appropriate amino acid esters.

It has been reported that the hydrolysis of '2' esters (alkyl or arylkyl) is sluggish and incomplete. The amino acid esters are expected to release the drug more efficiently because of the abundance of amino acid ester hydrolases in the biophase. We have chosen some Boc-, Z- and Fmoc protected amino acids for preparing 2'ester of erythromycin.

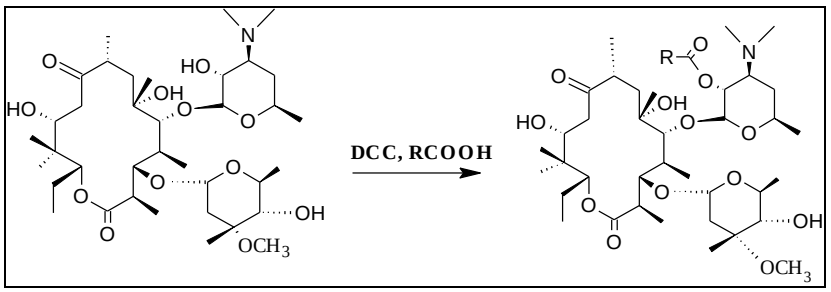


R = $-CH_2CH_2COOC_2H_5$ 1
$-CH_3$ 2
$-C_3H_7$ 3
$-C_6H_5$ 4

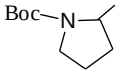
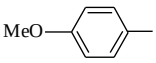
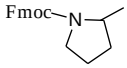
Fig 1: Erythromycin 2'ester

Materials and Methods

The ester derivatives of erythromycin were made regio-selectively on the 2'hydroxyl. Erythromycin molecule contains five hydroxyls, most reactive among them is the 2'hydroxyl of cladinose sugar. The most probable reason behind the increased reactivity of 2' hydroxyl over others is the existence of neighboring dimethylamino functionality.



R=

Z-NH-CH_2-	**5a**	$BocNHCH_2$-	**5i**
ZNHCH(CH_3)-	**5b**	Boc-NHCH(CH_3)-	**5j**
Z-NHCH(C_6H_5)-	**5c**	Boc-NHCH(C_6H_5)-	**5k**
	**5d**		**5l**
$FmocNHCH_2$-	**5e**		**5m**
Fmoc-NHCH(CH_3)-	**5f**		**5n**
Fmoc-NHCH(C_6H_5)-	**5g**		
	**5h**		

Scheme 1: Synthesis of erythromycin 2' esters

The amino acid ester prodrugs of erythromycin were made by the one pot dicyclohexyl carbodiimde (DCC) mediated coupling protocol (Scheme 1). Synthesis of 2'-alkyl and arylakyl esters reported earlier were made using acid chlorides under basic conditions. We have developed a new procedure for making 2' esters of erythromycin under extremely mild and neutral experimental conditions using DCC as condensation reagent. The 2'hydroxy group is very reactive and the quantitative esterification was observed without the use of any catalyst. For this purpose erythromycin was reacted regio-specifically with different appropriately protected amino acids. The esters prodrugs were generated selectively on the 2' position of the macrolide skeleton using different protected amino acids as well as carboxylic acids. The 4" hydroxyl group of cladinose sugar remains unaffected as no traces of 4" esters was observed. In the synthetic protocol, erythromycin was dissolved in DCM at 0°C and subsequently added the solution of corresponding amino acids, followed by the addition of DCC. Initially the reaction mixture was stirred at

0°C for half hr then next one hr at room temperature. The progress of reaction was monitored by tlc. The DCU formed during the reaction was separated through filtration, and the usual workup of reaction mixture furnished the desired 2'esters. It contains traces of DCU as an impurity which was removed by recrystallization with Ethyl acetate.

Due to highly acid sensitive nature of macrolides and restricted stability' of these esters, free amino functionality couldn't be generated [2, 4]. This rendered us to screen the mass as such for their antibacterial activity. The compounds 5a-o were tested in vitro for their antibacterial activity using standard broth dilution assay[6] against *S. aureus*, *B. subtlis*, *K. pneumoniae* and *E. Coli* strains taking erythromycin as reference.

Results and Discussions (Antibacterial activity of 2'ester derivative)

The results of antibacterial screen were quite encouraging. All the ester derivatives we have synthesized have exhibited remarkable antibacterial activity. A few of them have shown antibacterial profile better than erythromycin itself, which is in contrast with the previous results obtained for the antibacterial activity of erythromycin-ester prodrugs[4]. The increase in antibacterial activity was recorded primarily in the case of aromatic group containing macrolides. These findings somewhere coincide with the macrolide binding model proposed by Schlunzen *et al*. according to which 2'-OH plays a crucial role in macrolide binding[7]. Aromatic side chain appended with this functionality can enhance its binding through the target. It has been proved by various findings that attachment of aromatic functionality enhances the antibacterial activity of macrolide [8, 9]. The other reasons responsible for this behavior can be the enhanced penetration of the amino acid ester prodrugs through the bacterial cell wall due to presence of lipophilic functionality [10]. All the Z protected amino acid esters excluding 5d have exhibited enhanced activity against *S. aureus* strain in comparison with erythromycin. Among the synthesized compound 5b proved to be the most active one with two dilution factor enhanced activity against ATCC9144 (MIC 0.09 μg/mL).

Table 1: *In vitro* Antibacterial activity of Erythromycin-2'ester analogs

Compound	Minimum Inhibitory Concentration (µg/mL)				
	S. aureus		*B. subtlis*	*K. pneumonie*	*E. coli*
	ATCC9144	ATCC12598	ATCC6633	ATCC13833	ATCC1053
Erythromycin	0.39	0.39	0.19	0.04	25
5a	0.19	0.19	0.19	0.04	25
5b	0.09	0.78	0.39	0.19	25
5c	0.19	0.39	0.19	-	1.56
5d	0.39	50	0.19	50	100
5e	0.19	0.39	0.39	0.09	3.12
5f	0.39	0.19	0.39	0.39	1.56
5g	0.78	6.25	-	-	-
5h	0.78	50	0.39	50	100
5i	1.56	1.56	-	-	-
5j	1.56	0.78	-	-	-
5k	0.78	0.78	-	-	-
5l	3.12	1.56	-	-	-
5m	0.78	0.78	-	-	-
5n	0.39	0.78	0.78	0.39	6.25

In the case of Fmoc protected amino acid esters, enhanced activity was recorded in the case of 5e and 5f, whereas loss in antibacterial activity was observed in case of 5g and 5h. As far as the antibacterial activity of Boc protected amino acid esters is concerned, all these compounds have resulted in loss in antibacterial activity, probably bulky t-butyl group hinders the binding of macrolide towards its target. However 5k being the most active in this class with MIC value 0.78μg/mL against both *S. aureus* strains. Compounds having improved activity profile against *S. aureus* were tested against *B. subtlis*, *K. pneumoniae* and *E. coli* strains also to obtain their expanded spectra of antibacterial activity. There was no remarkable improvement in the antibacterial activity of these compounds against *B. subtilis* and *K. pneumoniae* or in other words the entire compounds tested have exhibited activity lower than erythromycin itself. Interestingly, all the compounds (except 5a and 5b) have exhibited enhanced activity against gram-negative *E. coli* strain (6.251.56μg/mL) compared to erythromycin (25μg/mL). Among the carboxylic acid esters only 5o have shown antibacterial activity at par with erythromycin.

Experimental: All the reagents and solvents were purchased commercially and used without purification unless otherwise noted. ^{1}H nuclear magnetic resonance (NMR) spectra were recorded in $CDCl_3$ on a DRX-400 Bruker FT-NMR or DRX-200 Bruker FT-NMR spectrophotometers. Chemical shifts are reported in part per million (ppm) with tetramethylsilane (TMS) as an internal standard. Mass spectra (MS) were obtained with a JEOL-SX-102 instrument using fast atom bombardment (FAB positive) technique.

Synthesis and characterization of some compounds

2'O-Z-glycyl erythromycin-A (5a)

Yield 85%; M.P. 124 - 127°C; MS (FAB) 925 $(M + H)^+$; ' H NMR (200MHz, CDCl3) : δ2.84 (dq, 1H)C_2 - H, 1.17 (m, 3H) 2 - Me, 1.97 (m, 1H) C_4 - H, 1.09 (d, 3H) 4 - Me, 3.56 (d, 1H) C_5 - H, 1.42 (s, 3H) 6 - Me, 1.56 (dd, 1H) &1.90 - 1.96 (m, 1H) C_7 - H, 2.93 (m, 1H) C_8 - H, 1.12 (d, 3H) 8 - Me, 2.79 (m, 1H) C_{10} - H, 1.33 (d, 3H) 10-Me, 3.83 (br s, 1H) C11 - H, 1.15 (s, 3H) 12-Me, 5.18 (dd, 1H) C_{13} -H, 1.49 (m, 1H) &1.90 - 1.96 (m, 1H) C14 - H, 0.77 (t, 3H) $C\underline{H}_3CH_2$, 4.38 (d, 1H) C_1' -H, 3.24 (dd, 1H) C_2'-H, 2.50-4 (m, 1H)C_3'-H, 2.28 (s, 6H) C_3'-NMe_2, 1.20-1.28 (m,1H) C_4'-H, 3.52 (m, 1H) C_5-H, 1.26 (d, 3H) $C_{5'}$-Me, 4.98 (d, 1H)

1''H, 1.59 (dd, 1H) 2"Hax, 2.38 (dd, 1H) 2",eq, 3.04 (dd, 1H) 4" H, 4.04 (dq, 1H) 5' H, 3.34 (s, 3H) 3"-OCH_3, 7.20-7.27 (m, 5H) C_6H_5, 5.05 (s, 2H) CH_2Phe, 3.94 - 3.96 (d, 2H) CH_2.

2'O-Z-alanyl erythromycin-A (5b)

Yield 51%; M.P. 115 - 116^{0}C; MS (FAB) 939 $(M + H)^+$; ^{1}H NMR (200MHz, CDCl3) : δ2.84 (dq, 1H) C2 - H, 1.17 (m, 3H) 2 -Me, 1.97 (m, 1H) C4-H, 1.09 (d, 3H) 4-Me, 3.56 (d, 1H) C5-H, 1.42 (s, 3H) 6- Me, 1.56(dd, 1H) &1.90-1.96 (m, 1H) C7 - H, 2.93 (m, 1H) C8 - H, 1.12 (d, 3H) 8-Me, 2.79 (m,1H) C10-H, 1.33 (d, 3H) 10-Me, 3.83 (br s, 1H) C11-H, 1.15 (s, 3H) 12-Me, 5.18 (dd, 1H) C13 H, 1.49 (m, 1H) &1.90-1.96 (m, 1H) C14-H, 0.77 (t, 3H) CH3CH2, 4.38 (d, 1H) C1'- H, 3.24 (dd, 1H) C2′ - H, 2.50 - 4 (m, 1H) C3′ - H, 2.28 (s, 6H) C3′ - NMe2, 1.20 -1.28 (m, 1H) C4′ - H, 3.52 (m, 1H) C5'- H, 1.26 (d, 3H) C5'-Me, 4.98 (d, 1H) 1"H, 1.59 (dd,'1H) 2"Hax, 2.38 (dd, 1H) 2'eq, 3.04 (dd, 1H) 4 " H, 4.04 (dq, 1H) 5 " H, 3.34 (s, 3H) 3 "- OCH3, 7.26 (m, 5H) C6H5, 5.11 (s, 2H) CH2, 4.64 (s, 1H) CH_{aa}, 1.43 (s, 3H) CH3.

2'O-Z-Phenylalanyl erythromycin-A (5c)

Yield 38%; M.P. 119-120^{0}C; MS (FAB) 1015 $(M + H)^+$; ^{1}H NMR (200MHz, $CDCl_3$) : δ2.84 (dq, 1H) C_2-H, 1.17 (m, 3H) 2-Me, 1.97 (m, 1H) C_4-H, 1.09 (d, 3H) 4-Me, 3.56 (d, 1H) C_5-H, 1.42 (s, 3H) 6-Me, 1.56 (dd, 1H) & 1.90-1.96 (m, 1H) C_7-H, 2.93 (m, 1H) C_8 -H, 1.12 (d, 3H) 8-Me, 2.79 (m, 1H) C_{10} -H, 1.33 (d, 3H) 10-Me,3.83 (br s, 1H) C_{11} - H, 1.15 (s, 3H) 12-Me, 5.18 (dd, 1H) C_{13} - H, 1.49 (m, 1H) & 1.90-1.96 (m, 1H) C_{14} - H, 0.77 (t, 3H) CH_3CH_2, 4.38 (d, 1H) C_1 '- H, 3.24 (dd, lH) C_2′ -H, 2.50.4 (m, 1H) C_3′-H, 2.28 (s, 6H) C_3′-NMe_2, 1.20-1.28 (m, 1H) C_4′-H, 3.52 (m,1H) C_5 - H, 1.26 (d, 3H) C_5'-Me, 4.98 (d, 1H) 1"H, 1.59 (dd, 1H) 2"Hax, 2.38 (dd, 1H) 2"eq, 3.04 (dd, 1H) 4''H, 4.04 (dq, 1H) 5''H, 3.34 (s, 3H) 3''- OCH_3, 7.10 (s, 5H) C_6H_5, 5.03 (s,CH_2), 4.94 (s, 1H) CHaa, 3.04 (d, 2H) CH_2, 6.90 - 7.25 (m, 5H) C_6H_5.

2'O-FmocGlycinyl erythromycin-A (5e)

Yield 51%; M.P. 125 - 126◦C; MS (FAB) 1013 $(M + H)^+$; ^{1} H NMR (200 MHz, $CDCl_3$): 82.84 (dq, 1H) C2 H, 1.17 (m, 3H) 2-Me, 1.97 (m, 1H) C4-H, 1.09 (d, 3H) 4-Me, 3.56 (d, 1H) C_5 - H, 1.42 (s, 3H) 6-Me, 1.56 (dd, 1H) &1.90-1.96 (m, 1H) C -H, 2.93 (m, 1H) C_8 - H, 1.12 (d, 3H) 8-Me, 2.79 (m, 1H) C_{10} - H, 1.33 (d, 3H) 10-Me, 3.83 (br s, 1H) C_{11} - H, 1.15 (s, 3H) 12-Me, 5.18 (dd, 1H) C_{13}H, 1.49 (m, 1H) & 1.90-1.96 (m, 1H) C_{14} - H, 0.77 (t, 3H) CH_3CH_2, 4.38 (d, 1H) C_1 '- H, 3.24 (dd,1H) C_2′-H, 2.50-4 (m, 1H) C_3 ′-H, 2.28 (s, 6H) C_3′-NMe_2, 1.20-1.28 (m, 1H) C_4′-H, 3.52 (m, 1H) C_5 '-H, 1.26 (d, 3H) C_5'-Me, 4.98 (d, 1H) 1' 'H, 1.59 (dd, 1H) 2''Hax, 2.38 (dd, 1H) 2"eq, 3.04 (dd, 1H) 4' H, 4.04 (dq, 1H) 5" H, 3.34 (s, 3H)3 " -OCH_3, 1.35 (s, 9H) $(CH_3)_3$, 4.94 (s, 1H) CHaa, 3.94 - 3.96 (d, 2H) CH_2, 3.95 (d, 2H) CH_2, 4.3 - 4.5 (dd, 2H) OCH_2, 4.18 (dd, 1H) $(Ph)_2$CH-, 7.25 - 7.78 (m, 8H) Ph.

2'O-Fmoc-Alanyl erythromycin-A (5f)

Yield 62.5%; M.P. 87-88◦C; MS (FAB) 1028(M+H)+; ^{1}H NMR (200 MHz, $CDCl_3$) : δ2.84 (dq, 1H) C_2-H, 1.17(m, 3H) 2-Me, 1.97 (m, 1H) C_4-H, 1.09 (d, 3H) 4- Me, 3.56 (d, 1H) C_5 - H, 1.42 (s, 3H) 6 - Me, 1.56 (dd, 1H) &1.90 - 1.96 (m, 1H) C_7 -H, 2.93 (m, 1H) C_8 - H, 1.12 (d, 3H) 8 - Me, 2.79 (m, 1H) C_{10} - H, 1.33 (d, 3H) 10 -Me, 3.83 (brs, 1H) C_{11} - H, 1.15 (s, 3H) 12 -Me, 5.18 (dd, 1H) C_{13} - H, 1.49 (m, 1H) &1.90-1.96 (m, 1H) C_{14}-H, 0.77 (t, 3H) CH3CH2, 4.38 (d, 1H) C_1 ' H, 3.24 (dd, 1H) C_2′-H, 2.50 - 4 (m, 1H) C_3′ - H, 2.28 (s, 6H) C_3′ - NMe_2, 1.20 - 1.28 (m, 1H) C_4′ - H, 3.52 (m, 1H) C_5′ - H, 1.26 (d, 3H) C_5 '-Me, 4.98 (d, 1H) 1 "H, 1.59 (dd,1H) 2"Hax, 2.38 (dd,1H) 2"eq, 3.04 (dd,1H) 4''H, 4.04 (dq, 1H) 5" H, 3.34 (s,3H) 3"- -OCH_3, 1.58 (d, 2H) CH_3, 4.70 (s, 1H) CH 4.3-4.5 (dd, 2H) OCH_2-, 4.2 (dd, 1H) $(Ph)_2$CH-, 7.26 -7.78 (m, 8H) Ph.

Conclusion

In summary, synthesis of several 2'amino acid esters of erythromycin has been carried out. A new DCC mediated procedure for the synthesis of 2'esters of erythromycin has been developed. Using this procedure synthesis of exclusively 2' amino acid esters of erythromycin was carried out under very mild experimental conditions without any protection of other functional groups. This procedure, although used for the synthesis of amino acid ester for the present work but can be used for a variety of organic acids. The antibacterial activity of some of the new prodrugs was better than erythromycin itself. Some of these derivatives have exhibited promising antibacterial activity towards gram-positive *S. aureus* strain, 5b found to be the most active compound of this series with MIC value 0.09 *μg*/mL against *S. aureus* strain ATCC9144. Compounds 5c and 5f have also shown enhanced antibacterial activity against gram-negative *E. coli* strain compared to erythromycin. Thus the amino acid esters prodrugs have a potential as antibacterial drug.

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References

1. Noel BA, Cuot TP, Courvalin P. Mechanism of action of spiramycin and other macrolides. J Antimicrob Chemother. 1988;22(2):13-23.
2. Kurath P, Jones PH, Egan RS, Perun TJ. Acid degradation of erythromycin A and erythromycin B. Experientia. 1971 Apr 15;27(4):362. DOI: 10.1007/BF02137246. PMID: 5581079.
3. Itoh Z, Nakaya M, Suzuki T, Arai H, Wakabayashi K. Erythromycin mimics exogenous motilin in gastrointestinal contractile activity in the dog. Am J Physiol. 1984;247-G694. DOI: 10.1152/ajpgi.1984.247.6.G688.
4. Tardrew PL, Mao JC, Kenney D. Antibacterial activity of 2'-esters of erythromycin. Appl Microbiol. 1969 Aug;18(2):159-165.
5. National Committee for Clinical Laboratory Standards. Methods for determining bacteriocidal activity of antimicrobial agents: approved standard M26-A. NCCLS; c1999.
6. Schlünzen F, Zarivach R, Harms J, *et al*. Structural basis for the interaction of antibiotics with the peptidyl transferase centre in eubacteria. Nature. 2001;413:814-821. DOI: 10.1038/35101544.
7. Ma Z, Clark RF, Brazzale A, *et al*. Novel erythromycin derivatives with aryl groups tethered to the C-6 position

are potent protein synthesis inhibitors and active against multidrug-resistant respiratory pathogens. J Med Chem. 2001 Nov 22;44(24):4137-56.
DOI: 10.1021/jm0102349. PMID: 11708916.

8. Or YS, Clark RF, Wang S, *et al*. Design, synthesis, and antimicrobial activity of 6-O-substituted ketolides active against resistant respiratory tract pathogens. J Med Chem. 2000;43(6):1045-1049. DOI: 10.1021/jm990618n.
9. Von Daehne W, Godtfredsen WO, Roholt K, Tybring L. Pivampicillin, a new orally active ampicillin ester. Antimicrob Agents Chemother. 1970;10:431-437. PMID: 5521364.