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Der Pharmacia Sinica, 2011, 2 (6):172-184

Der Pharmacia Sinica
ISSN: 0976-8688
CODEN (USA): PSHIBD

Synthesis, Characterization and evaluated for Antibacterial Activity of Novel Erythromycin Derivatives

Dhermesh Vekariya, Sourabh Jain, Jitender K Malik*

Department of Pharmaceutical Chemistry, NRI Institutes of Pharmaceutical Sciences-Bhopal(MP) India

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ABSTRACT

*A novel series of Substituted Erythromycin derivatives (**5-7**) were synthesized by methylation of substituted S-MOP (**4**) The structures of the synthesized compounds were established by IR, ^{1}H NMR, ^{13}C NMR and Mass spectroscopical data. All the synthesized compounds were screened for their in-vitro antibacterial activity against Gram-positive, Gram-negative bacteria. The investigation of antibacterial screening data revealed that most of the compounds tested have demonstrated congruent activity against Staphylococcus aureus, Bacillus subtilis, Escherichia coli, and Klebsiella as compared with the standard Erythromycin. Among the series, compounds 7 exhibited excellent an antibacterial activity profile as compared with the standard. In summary, preliminary results indicate that some of the newly synthesized title compounds exhibited promising antibacterial activities and they warrant more consideration as prospective antimicrobials.*

Key Words: Erythromycin; in-vitro antibacterial activity.

INTRODUCTION

The macrolide antibiotics are derived from Streptomyces bacteria, and got their name because they all have a macrocyclic lactone chemical structure. The macrolides are bacteriostatic, binding with bacterial ribosomes to inhibit protein synthesis. Erythromycin, the prototype of this class, has a spectrum and use similar to penicillin. Macrolide antibiotics are used to treat respiratory tract infections (such as pharyngitis, sinusitis, and bronchitis), genital, gastrointestinal tract, and skin infections.The most commonly prescribed macrolide antibiotics are: erythromycin, clarithromycin, azithromycin, roxithromycin, troleandomycin.[1]

Erythromycin, a typical macrolide antibiotic, was the first employed clinically and it has provided new opportunities for the discovery of potential therapeutic agents.[2] Over the past

decades, numerous clinical studies have confirmed that erythromycin and its derivatives have additional anti- inflammatory and prokinetic activity[3-4]. Erythromycin A is a 14-membered ring macrolide of an important family of oral antibiotics. It is used in the treatment of infectious diseases caused by aerobic Gram-positive bacteria and some anaerobic Gram-negative bacteria, mycoplasma and chlamydia.[5]

Erythromycin is a widely used broad spectrum antibiotic. It is a secondary metabolite produced by soil inhabating actinomycete family of bacteria Saccharopolyspora erythraea (formerly known as Streptomyces erythraea). First isolated in 1952 by J. M. McGuire and coworkers at Eli Lilly from soil collected in Philippines,[6] this substance was launched, also in 1952 as commercial antibiotic by Eli Lilly under the brand name llosone Streptomyces, the largest antibiotic producing genus of bacteria,[7] is also the source of some other well known antibiotics such as streptomycin, vancomycin, and tetracycline among others. Since its discovery, erythromycin was known to exert antibiotic activity by blocking bacterial protein synthesis. But only recently the molecular details have been uncovered. An X-ray crystal structure of a ribosome-erythromycin complex revealed that erythromycin binds to the 50S ribosomonal subunit and consequently blocks protein translation.[8]

The presence of the two deoxysugar units appears to be key to the antibacterial activity. Other congeners of erythromycin show weaker antibiotic activity. The biological study of erythromycin is still under investigation, especially the aspect of bacterial resistance towards erythromycin and in general macrolide antibiotics.[9]

The rapid development of antibiotic resistance among the major respiratory pathogens has created a serious problem for the effective management of respiratory tract infections.[10-15] There is a great need for new antibiotics that address the problem of antibiotic resistance. Under these circumstances, a substantial amount study has been carried out on novel macrolides.[16-18]

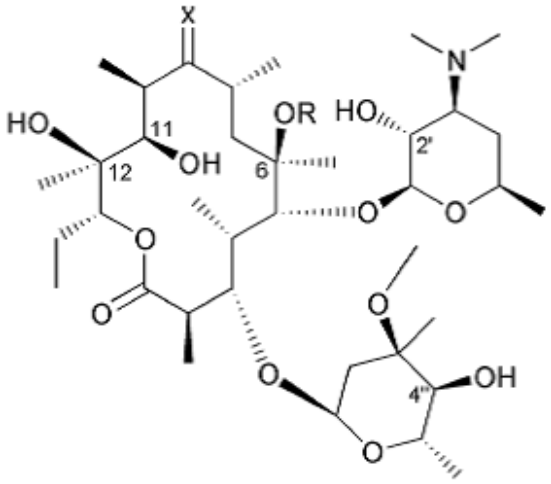
The C-3 cladinose sugar attached to the 14-membered ring macrolides is believed to be responsible for the induction of macrolide resistance. This moiety also seems to be responsible for efflux resistance.[19] Many new derivatives of macrolides for the effective management of erythromycin resistancehave been investigated.[20]

Chemistry

Erythromycin, a macrolide derived from Streptomyces erythreus, contains a 14-member macrocyclic lactone ring to which are attached two sugar moieties, desosamine and cladinose.Azithromycin and clarithromycin are semi-synthetic macrolides similar in structure to erythromycin.Clarithromycin differs from erythromycin by the methylation of a hydroxyl group at the 6-position of the lactone ring. Clarithromycin is a 14-member macrolide with a lactone ring attached to two sugar moieties, which confers acid stability and improved antimicrobial and pharmacokinetic properties. A primary metabolite of clarithromycin is the 14-hydroxyepimer that possesses antimicrobial activity, which is thought to have an additive or synergistic action with the parent compound against various microorganisms.Azithromycin is an azalide which differs from erythromycin by the addition of a methyl-substituted nitrogen atom into the lactone ring. These modifications in structure result in better gastrointestinal tolerability and tissue penetration. In addition, there is a decreased risk of interaction with other drugs metabolized by

the cytochrome P-450 enzyme system, and increased half-life.[21]

The selectivity of the methylation of the C-6 hydroxy group was studied by using a variety of erythromycin A derivatives. Before methylation of C-6, the 2' and 4" hydroxy groups were protected starting with erythromycin 9-oxime and protecting the OH groups on the cladinose and desosamine rings with chlorotrimethylsilane [$(CH_3)_3SiCl$]. This protected compound was allowed to react with methyl iodide (CH_3I) and potassium hydroxide (KOH) in a mixture of dimethyl sulfoxide (DMSO) and tetrahydrofuran (THF) (1 : 1). In the presence of a bulky derivative of an oxime group (9-[*O*-(dimethylthexylsilyl)]) † the C-11 and C-12 hydroxy groups were automatically protected against the attack of the methylating reagent (CH_3I)



1 R= H, X= O
2 R= CH_3, X= O
3 R= H, X= $NOSi(CH_3)_2C(CH_3)_2CH(CH_3)_2$

Structures of erythromycin A (1), 6-0-methyleryrithromycin (2) and erythromycin A 9-[*O*-(dimethylthexylsilyl)oxime] (3).

The structure of clarithromycin generated by MD calculations and optimized with AM1 was chosen a the initial structure for the QM calculations. The methyl group at C-6 was replaced by a hydrogen to generate erythromycin and a conformationalsearch was performed in order to determine the orientation of the substituent at position 9. Each of the five hydroxy groups of the erythromycin A 9-[O-(dimethylthexylsilyl)oxime][22] was protected one by one with chlorotrimethylsilane (CH3)3SiCl.

In order to assess the protection mechanism of alcohols by (CH3)3SiCl, several pathways were considered for which methanol was adopted as a model alcohol. An SN1 mechanism through which $(CH3)3Si^+$ attacks methanol did not yield a stationary transition structure. An SN2 mechanism in which (CH3)3SiCl attacks the O atom of methanol as Cl leaves, yielded a transition structure which was 51 kcal mol^{-1} higher in energy than the reactants. Finally, a four-membered transition struc- ture was located in which the Si–O and H–Cl bonds form while the Si–Cl and O–H bonds are broken. This last stationary structure was found to have a barrier of 31.5 kcal/ mol.

Similar four-membered transition structures have already been proposed in the literature for the formation of formamide and water from ammonia and formic acid.[23] Thus, in the course of the reaction, the attack of the (CH3)3SiCl on the hydroxy group was simulated by a four-membered transition state. In each case (C-6, C-11, C-12, C-2, C-4"), the geometries of the four-membered ring have been analyzed. The results show that the O–Si bond in the transition state is longer for the C-6 case rather than for the other hydroxy groups. The energy barrier (ΔE‡) between the reactants and the transition states for each hydroxy group was also evaluated. The energy barriers for the protection of C-2' and C-4" are somewhat lower than those for the protection of C-6, C-11 and C-12. Even though the difference in energy between the barriers for the protection of C-4" and C-11 is only 1 kcal mol^{-1}, the general trend is such that C-2' and C-4" tend to be protected faster than the others.[24]

Pharmacology

Antibacterial activity

All three newly synthesized compounds (5-7) were evaluated for their in-vitro antibacterial activity against two Gram-positive bacterial strains namely, Staphylococcus aureus and Bacillus subtilis and two Gram-negative bacterial strains namely, Escherichia coli and Klebsiella using the conventional agar-dilution method . Erythromycin was used as the reference standard. The results of the in-vitro antibacterial activity screening of the novel series of substituted erythromycin (5-7) are summarized in Table 1. Among the series tested, seventh compounds (7) exhibited excellent antibacterial activity against both Gram-positive and Gram-negative bacteria. However, all other compounds in the series were found to have moderate to good antibacterial activity against both Gram-positive and Gram-negative bacteria as compared to the standard. Minimum inhibitory concentration (MIC) was recorded as the lowest concentration of a compound that inhibits the growth of the tested microorganisms.

The results in the table-1 shows the antibacterial activity of 6-o-methylerythromycin, 6-11-o-dimethylerythromycin and 6,11,12-o-trimethylerithromycin. We obtained some new derivatives of 6,11-di-O-methylerythromycin A by modifying the 400 OH group with various carbamate groups such as alkylcarbamoyl, hydroxy-alkylcarbamoyl, alkoxy-alkylcarbamoyl, heterocyclic-carbamoyl, substituted benzylcarbamoyl and substituted phenethylcarbamoyl. To our surprise, most of the derivatives tested had potent activity against most resistant bacteria.[25]

Methods for studying antimicrobial activity

Biological evaluation involves testing of the microbial susceptibility to chemotherapeutic agents. Determination of antimicrobial effectiveness against pathogens is essential for proper therapy. Testing can show the efficacy of the antibiotic against pathogen and give an estimate of proper therapeutic dose. The idea of the effectiveness of a chemotherapeutic agent against a specific pathogen can be obtained from the Minimum Inhibitory Concentration (MIC). The MIC is the lowest concentration of a drug that can prevent growth of a specific pathogen.

Antimicrobial activity is determined based on the *in-vitro* activity in pure cultures. *In-vitro* susceptibility tests are done by the following methods.[26-27]

Agar diffusion method

In this technique petridishes of agar are prepared by pouring method. The agar is inoculated with microorganisms. In the agar dilution method different antibiotic concentrations are incorporated in to an agar medium for both aerobes and anaerobes. The plates are incubated at a temperature of 37 ºC for 24 hours. The antimicrobial substance diffuses through the agar and produces a clear zone of inhibition. The diameter of this zone can be measured and an estimation of the degree of activity of the antimicrobial substance can be obtained.

Drug diffusion methods[26-29]

Because the well method is still a bit awkward to be performed as a routine laboratory procedure, impregnated paper disc have received wide acceptance. A. Bondi in 1947 reported using filter paper discs containing specified concentration of antibiotics. Several factors can affect the size of the zone of antibacterial activity. These include: the depth of the medium used, the choice of the medium, the size of the inoculum, the diffusion rate of a particular antibiotic and the last factor in particular has resulted in unfortunate misinterpretation of results. Some laboratories used single or double disk methods. The single disk method use one disk of either a high or low antibiotic concentration.

Determining the relative sensitivity of the organism to the drug requires interpretations of zone sizes. With double disk method, the interpretation is simpler. Here both high and low strength disks are applied for each antibiotic to be tested. The organism is reported as being sensitive if a clear zone appears around both disks. If the zone appears around the high concentration alone, the organism is called moderately susceptible. If zones are lacking in both the disks, the organism is considered resistant to drug.

In our current study, the antimicrobial activity was carried out by the agar diffusion method. Here responses of microorganisms to the synthesized compounds were measured and compared with the response of the standard reference drug. The standard reference drug used in the present work was ERYTHROMYCIN.

Microorganisms

The four microorganisms used were *Klebsiella* (Gram –ve), *Escherichia coli* (Gram –ve), *Staphylococcus aureus* (Gram +ve) and *Bacillus subtillis* (Gram +ve).[30]

Composition of the Mueller Hinton agar[31,32]

Beef infusion	300 g
Casamino acids	17.5 g
Starch	1.5 g
Agar	17 g
Distilled water	1,000 ml
Final pH	7.3

Preparation of test solutions

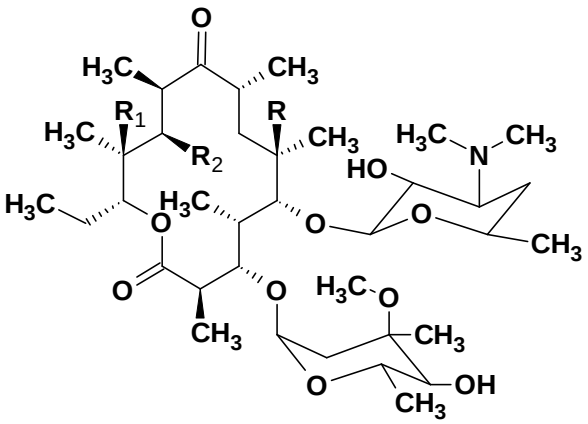
Each test compound was dissolved in DMSO to get a concentration of 500 µg/ml. This concentration was used for testing antibacterial activity.

Preparation of Mueller Hinton agar media[31,32]
The beef extract was taken in a 1000 ml beaker and made up the volume to 1000 ml with water. To this mixture known quantities of beef infusion, agar, starch and casamino acids were added and dissolved by heating the mixture. The pH was adjusted to 7.3. Finally the media was sterilized by autoclaving at 121 ºC for 15 minutes at 15-PSI pressure. Afterwards the mixture was cooled to 45 ºC and then inoculums were added to the above cooled media, mixed properly and poured into the sterile petridishes for solidifying. Bores were made on the medium using sterile borer. 0.1 ml of test solution and standard solution at a concentration of 50 µg/0.1 ml were taken. A standard (Streptomycin) was maintained with same concentration in each plate and a control having only DMSO in one plate. Then the petridishes were incubated at 37 ºC for 24 hours and zones of inhibition were observed and measured.

Preparation of inoculum
The suspensions of all the organisms were prepared as per standard procedure. (Mcfarland Nephelometer Standard). A 24 hour-old culture was used for the preparation of bacterial suspension. Suspensions of organisms were made in sterile isotonic solution of sodium chloride (0.9 % w/v).

Table 1: In-vitro antibacterial activity of substituted erythromycin (5-7) against strains (µg/mL)*.

Compound	R	R_1	R_2	Zone of inhibition (mm.)											
				Klebsiella Gram (-ve)			*E. coli* Gram (-ve)			*S. aureus* Gram (+ve)			*B. subtillis* Gram (+ve)		
				I	II	Mean ±S.D.	I	II	Mean ±S.D.	I	II	Mean ±S.D.	I	II	Mean ±S.D.
5	OCH_3	OH	OH	12	11	11.5 ±0.707	12	10	11 ±1.414	09	07	08 ±1.414	07	06	6.5 ±0.707
6	OCH_3	OH	OCH_3	13	11	12 ±1.414	10	11	10.5 ±0.707	10	07	8.5 ±2.12	08	08	08 ±0.0
7	OCH_3	OCH_3	OCH_3	14	12	13 ±1.414	09	08	8.5 ±0.707	08	08	08 ±0.0	07	08	7.5 ±0.707
Erithromycin	--			25	23	24 ±1.414	19	20	19.5 ±0.707	18	16	17 ±1.414	14	15	14.5 ±0.707

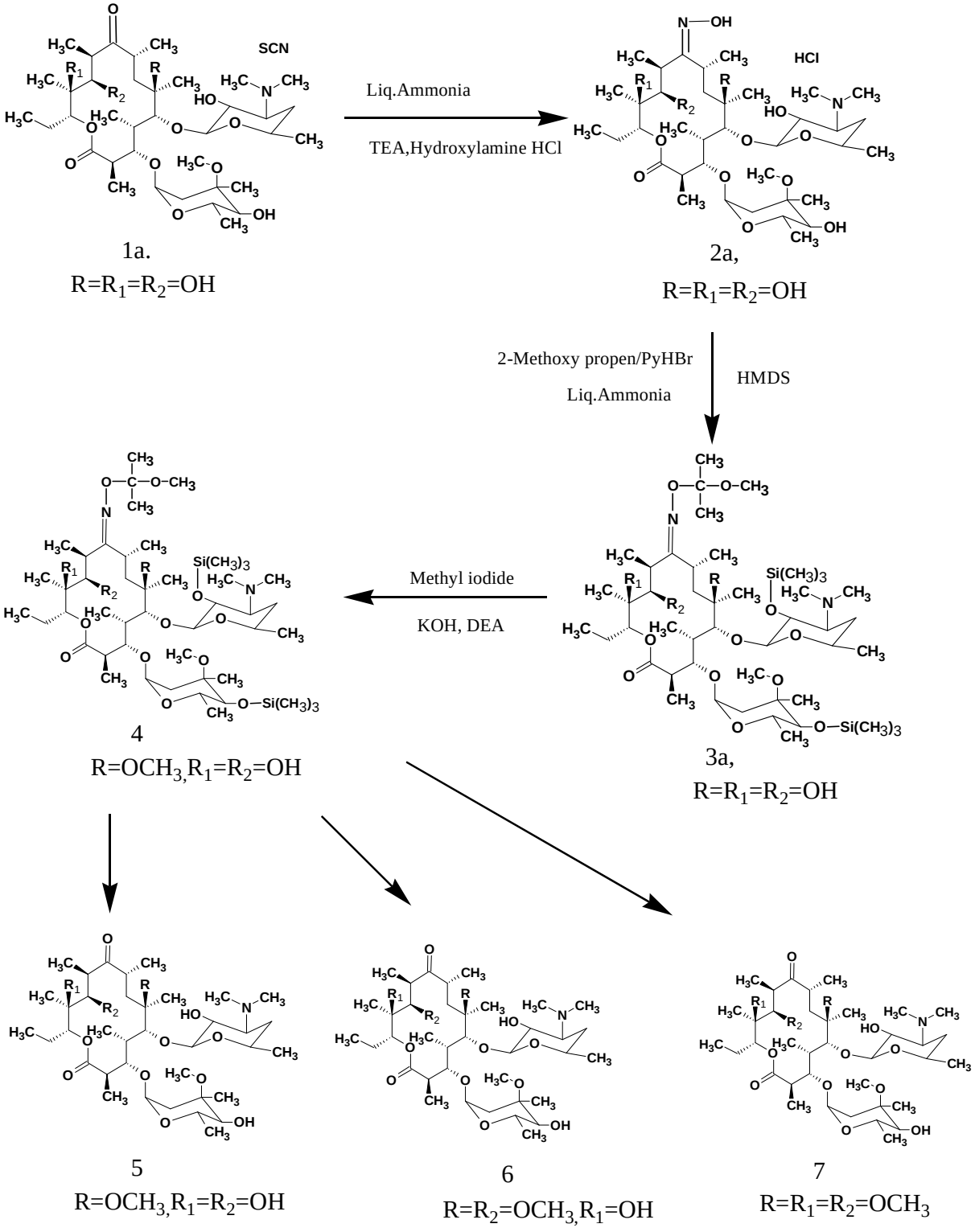


MATERIALS AND METHODS

^{1}H NMR spectra were measured at 300 MHz with a JEOL GSX 270FT NMR spectrometer. Chemical shifts were measured relative to internal standard TMS . ^{13}C NMR spectra were recorded at 67.8 MHz on the same instrument with internal TMS ($CDCl_3$). IR spectra were recorded on a UNICAM series FT-instrument. Mass spectra were recorded on AEI MS 902 or VG ZAB-E- instruments. Microanalyses were performed by MEDAC Ltd, Surrey. Melting points were determined on Gallen Kamp capillary melting point apparatus and are uncorrected. Optical rotations were measured in chloroform solution using a Bellingham and Stanley ADP 220 polarimeter. Flash chromatography was performed using Fluka silica gel 60 (230-400 mesh). Thin layer chromatography was carried out using pre-coated aluminum plates (Merck Kieselghur 60 F_{254}) which were visualized under UV light and then with either phosphomolybdic acid or basic aqueous potassium permanganate as appropriate.

General procedure for the synthesis of substituted silylated derivative of oxime (3a) *[33]*
Erythromycin thiocynate (0.06moles) was suspended in283.5 ml dichloromethane. 132-140 ml of 10-12% Liquid ammonia solution was added at a temperature of 27-33° C and adjusts the pH 10-10.5. The mixture was stirred, so that the solid would completely dissolve. The lower dichloromethane layer was separated from the aqueous layer. The aqueous layer was then extracted with 70 ml dichloromethane. Both dichloromethane layers were combined and washed with 150 ml process water. Dichloromethane was distilled out from the solution at 35-45°C. 33.5 ml Methanol was added. The methanol was distilled out to remove traces of dichloromethane at 65-70°C.Apply the vacuum to remove the traces of MDC at 65-70°C. The viscous liquid was cooled up to 30° C. 125 ml Methanol, triethylamine (0.155moles), hydroxyl amine hydrochloride (0.11mole) were charged. The mass was heated to reflux temperature and refluxed for 24 hrs. After completion of the reaction, distilled out solvent 18-34 ml the reaction mass was cooled to 0-5° C. The product was filtered & washed with 10.5 ml chilled methanol to obtain a wet cake (0.065-0.08 moles).

0.065-0.08 moles wet cake was suspended in 600 ml dichloromethane. Slowly 137-156 ml 10-12% Liq ammonia was added at 28-33° C and adjusts the pH 10.2-10.5 and stirred for 30 minutes. The dichloromethane layer was separated out and then washed with 100X100 ml process water. Dichloromethane was distilled out azetropic from the mass for removing moisture at 35-45°C. The resulting product was cooled down to 4-6°C. 2-methoxy propane (0.20moles) and pyridine hydro bromide(0.11moles) were added. The mass was stirred to 12-17° C. for 180 minutes, and then 17 gm hexamethyldisilazene was charged at 10-12°C and stirred for 60 min at 15-17°C. Sodium bicarbonate solution (106ml water & $NaHCO_3$ (0.10mole)) was charged into the reaction mixture and stirred for 30 min at 27-33° C. The dichloromethane layer was separated out, and then the dichloromethane layer was washed with brine solution. The dichloromethane layer was separated out, and then the dichloromethane layer was washed with 106 ml water. The dichloromethane was distilled out in such a way that the distillation of dichloromethane and addition of 500 ml water remained same. The mass was slowly heated up to 40-75°C. And vacuum was applied to remove traces of dichloromethane. The slurry was cooled to 15-20° C. and stirred for 1 hr. The product was filtered and the wet product was dried at a temperature of 50-60° **(3a).**



1a.
R=R_1=R_2=OH

2a,
R=R_1=R_2=OH

3a,
R=R_1=R_2=OH

4
R=OCH_3,R_1=R_2=OH

5
R=OCH_3,R_1=R_2=OH

6
R=R_2=OCH_3,R_1=OH

7
R=R_1=R_2=OCH_3

(Scheme 1)

General procedure for the synthesis of substituted S-MOP (4) *[33]*
Methyl tertiary butyl ether (600 ml) was charged, 0.38 moles of the silyl ester derivative was added at ambient temperature,500 ml Dimethyl sulphoxide was added and the mass was cooled to 12-14° C. methyl iodide (0.06) and powder potassium hydroxide(0.08) were added. The mixture was stirred at a temperature of 12-14° C. for 10-40 min. After completion of the reaction, 30 ml Dimethyl amine solution and 50 ml process water were added. The mixture was stirred for 30 minutes and the bottom aqueous layer was removed. The aqueous layer was extracted with 500 ml methyl tert-butyl ether, the methyl tert-butyl ether was combined and washed with brine solution and water. The methyl tert-butyl ether was distilled out and 400 ml hot water was added to remove traces of the methyl tert-butyl ether. The slurry was cooled down to 20-25° C., filtered and washed with 50 ml water. The wet product was dried at 50-55° C. until the moisture content was less than 2%. (**4**).

General procedure for the synthesis 6,-O-methylerythromycin (5) *[33,34]*
0.05 mole of SMOP oxime was suspended in 150 ml ethyl alcohol. 165 ml Water was added at below 35° C. Then sodium metabisulphite(0.57mole) was added and formic acid(0.19 mole) was added. The temperature of the mass was raised to 60-68° C. The mass was stirred at 60-68° C. for 5 hours, and then cooled to a temperature of 50-55° C. Sodium metabisulphite(0.08 mole) was added. The mass was heated to 60-68° C. The mass was stirred and the temperature of the reaction was maintained reflux at 60-68° C for 5 hours. The mass was cooled to 30-35°C. and a caustic lye solution was slowly added to adjust the pH to 10.5-11.5. The mass was stirred at 30-35°C. For 30 minute slurry was filtered and washed with 104 ml ethanol water (50:54). The wet solid was stirred in hot water 425 ml at 40-45°C. Filter the solid and spin dried, unloaded. The product was dried at 85-95°C for 8 to 10hrs.

675 ml Ethanol and 0.06 mole of crude Clarithromycin was charged in a reactor. The mass was heated to reflux. 0.50 gm hyflow were added. The mass was filtered and washed with 100 ml hot ethanol. The filtrate was collected, distilled out 100 ml and cooled up to 55-60° C with in 3-4 hrs. Further cooled to 35-40°c with in 2-3 hrs, cooled to 18-20°C with in 2-3 hrs. The product was filtered.

General procedure for the 6, 11-O-dimethylerythromycin (6) *[33,34]*
Charge 1.0 lit Toluene and add 0.10 moe of SMOP oxime under stirring at RT. Add DMSO 1.0 lit at RT under stirring.Cool the reaction mass to 8°C to 10°C.Add Methyl iodide 48 gm and KOH powder (0.64)at 8°C to 10°C. Stir for 5 hrs at 8°C to 10°C.Add DMA 50 ml and 700 ml process water.Stir for 30 minutes, stop stirring and settling for 15 minutes.Separate lower aqueous layer and collect above organic layer.Distilled toluene under vacuum below 50°C.Add DNS 50 ml and distilled completely, add 100 ml DNS.Heat the reaction mass to reflux 70-78°C maintain for 30 minutes.Cool to 25-30°C, further cool to 15-20°C.Filter the reaction mass, suck dry well.Collect wet cake=0.06 mole.Charge above wet cake in RBF, add 600 ml DNS. Heat the reaction mass to reflux 70-78°C maintain for 30 minutes.Cool to 25-30°C, further cool to 5-10°C.Stir for 60 minutes at 5-10°C.Collect wet cake=65 gm. Dry at 50-60 under vacuum for 5 hrs.Dry weight=0.08 mole.

General procedure for the synthesis 6,11,12-O-trimethylerythromycin (7) [33-35]
Charge 1.0 lit Toluene and add 0.10 moe of SMOP oxime under stirring at RT. Add DMSO 1.0 lit at RT under stirring.Cool the reaction mass to 8°C to 10°C.Add Methyl iodide 48 gm and KOH powder (0.64)at 8°C to 10°C. Stir for 10 hrs at 8°C to 10°C.Add DMA 50 ml and 700 ml process water.Stir for 30 minutes, stop stirring and settling for 15 minutes.Separate lower aqueous layer and collect above organic layer.Distilled toluene under vacuum below 50°C.Add DNS 50 ml and distilled completely, add 100 ml DNS.Heat the reaction mass to reflux 70-78°C maintain for 30 minutes.Cool to 25-30°C, further cool to 15-20°C.Filter the reaction mass, suck dry well.Collect wet cake=0.06 mole.Charge above wet cake in RBF, add 600 ml DNS. Heat the reaction mass to reflux 120-130°C maintain for 30 minutes.Cool to 25-30°C, further cool to 5-10°C.Stir for 60 minutes at 5-10°C.Collect wet cake=65 gm. Dry at 50-60 under vacuum for 5 hrs.Dry weight=0.08 mole.

6-(4-dimethylamino-3-hydroxy-6-methyl-tetrahydropyran-2-yl)oxy-14-ethyl-12,13-dihydroxy-4-(5-hydroxy-4-methoxy-4,6-dimethyl-tetrahydropyran-2-yl)oxy-7-methoxy-3,5,7,9,11,13 hexamethyl-1-oxacyclotetradecane-2,10-dione. ***(5)***
This compound was prepared as per the above mentioned procedure purified and isolated as white crystalline : yield 78.7%; mp 217°C; IR (KBr) v_{max} 3475, 2980, 2836, 1730, 1632, 1457, 1374, 1320, 1285, 1250, 1172, 1106, 1050, 1010, 938, 623, 586, 502, 405. cm^{-1}; ^1H NMR ($CDCl_3$) δ ppm δ 12.09 (OCH_3), 7.12-8.99 (m,14 H, Ar-H), 5.13 (OH); ^{13}C NMR ($CDCl_3$) δ ppm; 169.4,155.5, 142.3, 138.4,135.7,135.0, 133.7, 129.6, 128.1, 24.3. HRMS (EI) m/z calcd for $C_{38}H_{69}NO_{13}$: 747.953; found: 748.5.

6-(4-dimethyl amino-3-hydroxy-6-methyl-tetra hydro pyran-2-yl) oxy-14-ethyl-13-dihydroxy-4-(5-hydroxy-4-methoxy-4,6-dimethyl-tetra hydro pyran-2-yl)oxy-7,12-dimethoxy-3,5,7,9,11,13-hexa methyl-1-oxacyclotetradecane-2,10-dione. ***(6)***
This compound was prepared as per the above mentioned procedure purified and isolated as white crystalline : yield 61.0%; mp 241°C; IR (KBr) v_{max} , 3454, 2974, 2938, 1725, 1632, 1457, 1374, 1320, 1285, 1250, 1172, 1106, 1050, 1010, 938, 623, 586, 502, 405. cm^{-1}; ^1H NMR ($CDCl_3$) δ ppm δ 12.09 (OCH_3), 7.12-8.99 (m,14 H, Ar-H), 5.90 (OH); ^{13}C NMR ($CDCl_3$) δ ppm; 169.4,155.5, 142.3, 138.4,135.7,135.0, 133.7, 129.6, 128.1, 24.3. HRMS (EI) m/z calcd for $C_{39}H_{71}NO_{13}$: 762.00; found: 761.5.

6-(4-dimethyl amino-3-hydroxy-6-methyl-tetra hydro pyran-2-yl) oxy-14-ethyl-4-(5-hydroxy-4-methoxy-4,6-dimethyl-tetra hydro pyran-2-yl)oxy-7,12,13-trimethoxy 3,5,7,9,11,13-hexa methyl-1-oxacyclotetradecane-2,10-dione. ***(7)***
This compound was prepared as per the above mentioned procedure purified and isolated as white amorphos : yield 45.7%; mp 271°C; IR (KBr) v_{max} 3461, 2975, 1727, 1626, 1458, 1371, 1285, 1252, 1173, 1107, 1051, 1009, 893, 626. cm^{-1}; ^1H NMR ($CDCl_3$) δ ppm δ 11.85 (OCH_3), 7.12-8.99 (m,14 H, Ar-H), 3.00 (CH_3); ^{13}C NMR ($CDCl_3$) δ ppm; 169.4,155.5, 142.3, 138.4,135.7,135.0, 133.7, 129.6, 128.1, 24.3. HRMS (EI) m/z calcd for $C_{40}H_{73}NO_{13}$: 776.00; found: 775.45.

RESULTS AND DISCUSSION

Synthesis of Erythromycin
Step 1: To synthesis of 6-o-methyl erythromycin

Erythromycin thiocynate was suspended with the liq.ammonia, Hydroxylamine HCl, and Triethyl amine to produce the Erythromycin-oxime. Than protect the hydroxyl group with HMDS, 2-Methoxy propene and to form silylated derivative of oxime. From the silylated derivative of oxime prepare the s-mop oxime by methylation process. Than after s-mop oxime converted to clarithromycin oxime by ethanol and formic acid and deprotect hydroxyl group. clarithromycin oxime converted the clarithromycin by sodium metabisulphide and MTBE.

Step 2: To synthesis of 6, 11-O-dimethylerythromycin
Starting from s-mop oxime, protect the hydroxyl group present in the s-mop oxime, by DMSO, etc…. and than the methylation process of hydroxyl group by Methyl iodide and KOH.

Step 3:.To synthesis of 6, 11, 12-O-trimethylerithromycin
Starting from s-mop oxime first prepare 6, 11,-o-dimethylerythromycin than from this prepare 6-11-12-o-trimethylerythromycin by methylation process.

A novel series of Substituted Erythromycin derivatives were synthesized as above mentioned procedure (Scheme No.1). The structures of the synthesized compounds were established by IR, ^{1}H NMR, ^{13}C NMR and Mass spectroscopic data. The synthesized compounds were screened for their in-vitro antibacterial activity (Table no.1).

CONCLUSION

A novel series of Substituted Erythromycin derivatives were synthesized. The structures of the synthesized compounds were established by IR, ^{1}H NMR, ^{13}C NMR and Mass spectroscopic data. All the synthesized compounds were screened for their in-vitro antibacterial activity against Gram-positive and gram-negative bacteria. as compared with the standard. In summary, preliminary results indicate that some of the newly synthesized title compounds exhibited promising antibacterial activities.

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