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Synthesis of lactones from fatty acids by ring-closing metathesis and their biological evaluation

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ABSTRACT

The present study involves the synthesis of macrocyclic lactones by the esterification of unsaturated fatty acids (oleic acid, undecenoic acid, and erucic acid) with unsaturated alcohols (allyl alcohol, prop-2-ene-1-ol, oleyl alcohol, and undecenol) followed by a ring closing metathesis reaction employing Grubbs' second generation catalyst (1.0-1.5 mmol). The structure of the compounds was confirmed by ^{1}H NMR, ^{13}C NMR, FT-IR, and ESI-Mass spectral studies. The antibacterial activity of the synthesised lactones was evaluated. The larger ring-sized lactone, namely, erucic acid lactone, exhibited excellent antibacterial activity against three bacterial cell lines, namely, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Bacillus subtilis*. Undecenoic acid-based lactones exhibited excellent antibacterial activity selectively against only *Staphylococcus epidermidis*. The assay of macrolactones for their *in vitro* anticancer activity was carried out by MTT for different cancer cell lines, namely, human prostate epithelial cancer cells (ATCC HTB-81), HepG2 derived from hepatic cancer cells (ATCC HB-8065), SKOV3 derived from human ovarian cancer cells (ATCC HTB-77), MDAMB-231 derived from human breast cancer cells (ATCC HTB-26) and Chinese hamster ovarian (CHO-K1) cell lines. The molecules selectively exhibited anticancer activity against Chinese hamster ovarian (CHO-K1) cell lines. Among macrolactones, (*E*)-oxacyclotridec-11-en-2-one (MALUN) was more active and its activity was much higher compared to others and on par with the reference standard Mitomycin C. This was followed by (*E*)-oxacyclotricos-14-en-2-one (MOLER) and (*E*)-oxacyclononadec-10-en-2-one (MOLOH). The fatty acid-based cyclic lactones with selective antibacterial and anticancer activities can be further explored for a variety of pharmaceutical formulations.

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1. Introduction

Natural feed stocks such as vegetable oils and their derivatives (*e.g.*, fatty acids and fatty acid esters) can be converted into industrially useful chemicals *through the* metathesis of olefins [1]. Olefin functionalisation approaches a new transformation, with high potential applications in natural products and the synthesis of bioactive compounds. Olefin metathesis is a powerful and versatile method for forming carbon-carbon bonds [2]. The metabolization of fatty acids helps in the synthesis of the required molecules, employing only a few steps, avoiding multiple steps. Transformations include ring-opening metathesis polymerisation (ROMP), ring-closing metathesis (RCM), acyclic diene metathesis polymerisation (ADMET), ring-opening metathesis (ROM), cross metathesis (CM), and self-metathesis (SM) [3]. The most widely used method is the RCM of olefins used in organic synthesis for the synthesis of various unsaturated rings, which in turn forms the cycloalkene as the *E* or *Z* isomers widely used in the preparation of medium-large rings into complex drug molecules [4], supramolecular assemblies [5] or small molecule libraries [6,7]. The formation of biologically important lactones is one of the very good examples of RCM. Lactones are important components of naturally occurring compounds widely present as structural subunits in a large number of natural compounds, are of great importance in pharmaceuticals, agrichemicals, flavour components, materials, and polymers and have significant biological activities such as antibiotic, antifungal, antitumor, cytostatic, estrogenic, and anabolic activities [8-11].

The ring-closing metathesis (RCM) reaction represents a powerful tool to obtain macrolactones from diolefinic esters [12]. Diolefinic esters are suitable substrates for these reactions, and the various metathesis catalysts employed are complexes based on molybdenum, tungsten, or ruthenium [13]. However, catalyst efficiency and product conversion can vary dramatically depending on the purity of the feedstock being metathesised [14]. In general, several steps are involved in the synthesis of lactones, for example: macrocyclic acetylenic lactones by ring expansion of oxabicyclo alkenones [15], Pd-catalysed C-H lactonization for the synthesis of biaryl lactones [16], C-C bond formation by intramolecular addition of enolate ion, intramolecular diacetylene ester coupling, intramolecular Wittig or Horner-Emmons reactions and olefin metathesis using WCl_6-Me_4Sn or WCl_6-Cp_2TiMe_2 or $WOCl_4$-Cp_2-$TiMe_2$ as catalysts [17-20]. However, one-pot synthesis is a powerful tool, because it offers significant advantages, such as the

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construction of macromolecules from readily available building blocks with high yields and has significance over multisteps in terms of cost, waste, time, and atom economy [21]. In this context, Grubbs catalysts have made it possible to cyclize substrates containing various functionalities.

New methodologies, such as ring-closing sequence of dienes, allowed the construction of a fused ring system with carbon-carbon bond formation. One of the major applications of RCM has been the synthesis of medium- to large-ring systems. The RCM has wide applications in drug discovery; the macrolactones prepared using this methodology have enhanced potency relative to those of the corresponding acyclic analogues. RCMs include medium-sized heterocyclic rings containing phosphorus [22], sulphur [22,23], oxygen [23], or nitrogen [24] compounds containing (E) alkene units synthesised in moderate to very good yields using Grubbs first- and second-generation catalysts [25]. The synthesis involved metathesis-based macro-lactones and the reactions were optimised with respect to solvent, temperature, and catalyst concentrations. RCM of acyclic diene esters with Grubbs' first-generation catalyst (0.01 mmol) in benzene resulted in different macrolactones such as 13-, 19-, 20-, and 21-membered macrolactones with 6, 65, 63, and 82% yields, the yield of the reaction decreases due to steric hindrance [26]. Furstner and Langemann reported the synthesis of exaltolide, which was a metathesised and hydrogenated product of a diene ester [27]. Kraft and Cadalbert reported the structure-odour correlation of musk, (12*R*)-12-methyl-13-tridecanolide, a macrocyclic musk, and 13-tridecanolide, while its nonmusky odour-based molecule, a demethyl analogue, was conformationally restricted by the introduction of methylene bridges between C-3 and C-8 or C-9 [28]. Lehmann and Tochtermann synthesised a series of 16-membered macrocyclics with double and triple bonds at various positions and regioselectively at different positions of the macrocyclic rings [29].

However, fatty acid-based lactones employing RCM of diesters have not been reported so far. Furthermore, fatty acids, being excellent raw materials for different industrial oleochemicals and biologically important compounds, have been exploited for the synthesis of macrocyclic lactones of varied sizes. The title molecules were synthesised by esterification of unsaturated fatty acids (Oleic acid, undecenoic acid, and erucic acid) with unsaturated alcohols (Allyl alcohol, prop-2-ene-1-ol, oleyl alcohol and undecenol) followed by a ring closing metathesis reaction using Grubbs second generation catalyst. On the other hand, because macrocyclic lactones are well known as antibiotic agents, the synthesised lactones were explored for their antibacterial and anticancer activities.

2. Experimental

2.1. Materials

Oleic acid (99%), erucic acid, undecenoic acid, allyl alcohol, hex-5-en-1-ol, oleyl alcohol, and tricyclohexylphosphine [1,3-*bis*(2,4,6-trimethylphenyl)-4,5-dihydroimidazol-2-ylidene benzylidene ruthenium(IV) dichloride (Grubbs' second generation catalyst) (II), hexane, ethyl acetate, *N*-ethyl-*N*′-(3-dimethyl aminopropyl) carbodiimide hydrochloride (EDC·HCl), 4-dimethylaminopyridine (DMAP), dry methanol and dry dichloro methane (99.9%) were purchased from Sigma Aldrich Chemical Co., Hyderabad, India. Silica gel (60-120 mesh) for column chromatography was purchased from Acme synthetic chemicals (Mumbai, India) and precoated TLC plates (silica gel $60F_{254}$) were purchased from Merck (Darmstadt, Germany).

2.2. Instrumentation

All synthesised compounds were purified by silica gel column chromatography and identified by thin-layer chromatography (TLC) performed on precoated silica gel $60F_{254}$ plates from Merck (Darmstadt, Germany). Infrared (IR) spectra were recorded as neat samples in dichloromethane on a PerkinElmer FT-IR instrument (Model: Spectrum BX; Perkin-Elmer, Connecticut, USA). The ^{1}H NMR and ^{13}C NMR spectral data was recorded on a Bruker Avance 500 and 75 MHz spectrometer, respectively, in deuterated chloroform ($CDCl_3$) and tetramethyl silane (TMS) was used as an internal standard. The chemical shift values were reported in units of δ (ppm) downfield from the TMS. The coupling constant (*J*) values were measured in hertz (Hz). Mass spectra were recorded using electron spray ionisation on ESI/MS using the Waters e2695 separator module (Waters, Milford, MA, USA) mass spectrometer. All high-resolution spectra were recorded on the QSTARXL hybrid MS/MS system (Applied Biosystems, USA) under electron spray ionisation.

2.3. Typical procedure for the synthesis of allyl oleate (diolefinic esters)

A mixture of undecenoic acid (185 mg; 1 mol), allyl alcohol (58 mg; 1 mol), EDC·HCl (286 mg; 1.5 mol) and DMAP (126 mg; 1 mol) was dissolved in dry 60 ml of DCM in a 100 ml round bottom flask. The reaction mixture was stirred at 0 °C for 9 h. The progress of the reaction was monitored by TLC eluted with hexane: ethyl acetate (80:20, *v*:*v*). After maximum conversion, the reaction mixture was taken into ethyl acetate and the solution was washed with distilled water. The solvent was passed through anhydrous Na_2SO_4 and concentrated under reduced pressure. The reaction mixture was purified by column chromatography on silica gel using hexane: ethyl acetate solvent system (80:20, *v*:*v*) to obtain the viscous oil corresponding to allylundec-10-enoateas, 175 mg, 75% yield.

A similar procedure was followed for the synthesis of allyloleate with 254 mg, 79% yield from allyl alcohol (58 mg; 1 mol) and oleic acid (282 mg; 1 mol); allyldicosa-13-enoate with 323 mg, 85% yield from allyl alcohol (58 mg; 1 mol) and erucic acid (340 mg; 1 mol); octadec-9-ene-1-yloleate with 462 mg, 87% yield from oleyl alcohol (268 mg; 1 mol) and oleic acid (282 mg; 1 mol); (*Z*)-octadec-9-en-1-yl (*Z*)-docos-13-enoate with 483 mg, 82% yield from oleyl alcohol (268 mg; 1 mol) and erucic acid (340 mg; 1 mol); (*Z*)-octadec-9-en-1-yl undec-10-enoate with 382 mg, 88% yield from oleyl alcohol (268 mg;1 mol) and undecenoic acid (185 mg; 1 mol); hex-5-en-1-yl oleate with 294 mg, 81% yield from hex-5-en-1-ol (100 mg; 1 mol) and oleic acid (282 mg; 1 mol);hex-5-en-1-yl undec-10-enoate with 200 mg, 75% yield from undecenoic acid (185 mg; 1 mol) andhex-5-en-1-ol (100 mg; 1 mol);hex-5-en-1-yl (*Z*)-docos-13-enoate with 337 mg, 80% yield from erucic acid (340 mg; 1 mol) and hex-5-en-1-ol (100 mg; 1 mol). The structure of the compounds was characterised by IR, ^{1}H NMR, and ^{13}C NMR techniques.

2.4. Typical procedure for the synthesis of (E)-oxacyclo tridec-11-en-2-one-MALUN (Macro lactones) by ring-closing metathesis reaction

A mixture of allylundec-10-enoate (diolefinic ester, 223 mg, 1 mol) Grubbs second generation catalyst (8.4 mg, 0.01 mmol) was taken in a round bottom flask with two necks (100 mL). One neck was fixed with a septum and another with a condenser. Two N_2 balloons were arranged, one in the septum and another in the condenser. 20 mL of dry DCM was added through the septum with the help of a syringe keeping the temperature at 45 ° C and the reaction was carried out for 8-10 h. The completion of the reaction was monitored by TLC, eluted with hexane: ethyl acetate (80:20, *v*:*v*). The reaction mixture was extracted with ethyl acetate and washed with water. The solvent was evaporated using a rotary evaporator and dried under vacuum. The reaction mixture was purified by column

chromatography with silica gel using hexane: ethyl acetate (60:40, *v*:*v*) solvent system to obtain the corresponding (*E*)-oxacyclotridec-11-en-2-one (MALUN) as viscous oil with 141 mg and 72% yield.

A similar procedure was followed for the synthesis of (*E*)-oxacyclododec-10-en-2-one (MALOL) with 138 mg, 75% yield of allyloleate; (*E*)-oxacyclohexadec-14-en-2-one (MALER) with 195 mg, 82% yield of allyldicosa-13-enoate; (*E*)-oxacyclononadec-10-en-2-one (MOLOH) with 235 mg, 84% yield of octadec-9-ene-1-yloleate; (*E*)-oxacyclotricos-14-en-2-one (MOLER) with 285 mg, 85% yield from (*Z*)-octadec-9-en-1-yl (*Z*)-docos-13-enoate; (*E*)-oxacycloicos-11-en-2-one (MOLUN) with 238 mg, 81% yield from (*Z*)-octadec-9-en-1-yl undec-10-enoate; (*E*)-oxacyclopentadec-10-en-2-one (MOL5H) with 168mg, 75% yield from hex-5-en-1-yl oleate; (*E*)-oxacyclohexadec-11-en-2-one (MUN5H) with 183 mg, 71% yield from hex-5-en-1-yl undec-10-enoate; (*E*)-oxacyclononadec-14-en-2-one (MER5H) with 198 mg, 77% yield from hex-5-en-1-yl (*Z*)-docos-13-enoate. The structure of the compounds was characterised by IR, ESI-MS, HRMS, ^{1}H NMR, and ^{13}C NMR. The spectral data of the compounds are given below (Scheme 1).

(*E*)-Oxacyclotridec-11-en-2-one (MALUN): FT-IR (NaCl, $CHCl_3$, ν, cm^{-1}): 3518, 2925, 1721, 1540, 1384. ^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm): 5.97-5.81 (m, 2H, -*H*C=C*H*-), 4.58-4.56 (t, *J* = 7.4 Hz, 2H, -CH_2-O-C=O-CH$_2$-), 2.31-2.28 (t, *J* = 6.1 Hz, 2H, -O-C=O-CH_2-), 1.63 (m, 2H, -CH_2-HC=CH-CH$_2$-O-), 1.28-1.24 (m, *J* = 5.9 Hz, 12H, -[CH_2]$_6$-). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 173.7, 138.9, 129.7, 113.8, 76.9, 64.1, 35.8, 34.1, 31.6, 33.5, 28.4, 26.9, 24.7, 18.5, 13.8, 11.1. ESI-MS (*m/z*): 219.24 [M+Na]$^+$. HRMS (ESI, *m/z*): 219.31.

(*E*)-Oxacyclododec-10-en-2-one (MALOL): FT-IR (NaCl, $CHCl_3$, ν, cm^{-1}): 3451, 2742, 1717, 1572, 1357. ^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm): 5.8-5.7 (m, 2H, -*H*C=C*H*-), 4.91 (t, *J* = 7.4 Hz, 2H, -CH_2-O-C=O-CH$_2$-), 2.03 (t, *J* = 6.1 Hz, 2H, -O-C=O-CH_2-), 1.55 (m, 2H, -O-C=O-CH$_2$-CH_2-), 1.25 (m, *J* = 5.9 Hz, 10H, -[CH_2]$_5$-). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 172.4, 138.9, 129.5, 113.8, 76.9, 64.1, 28.6, 25.6, 33.5, 13.8, 11.1. ESI-MS (*m/z*): 205.24 [M+Na]$^+$. HRMS (ESI, *m/z*): 205.31.

(*E*)-Oxacyclohexadec-14-en-2-one (MALER): FT-IR (NaCl, $CHCl_3$, ν, cm^{-1}): 3439, 2753, 1719, 1569, 1348. ^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm): 5.8-5.7 (m, 2H, -*H*C=C*H*-), 4.9 (t, *J* = 7.4 Hz, 2H, -CH_2-O-C=O-), 2.28-2.27 (t, *J* = 6.1 Hz, 2H, -O-C=O-CH_2-), 2.05-2.02 (m, *J* = 6.1 Hz, 2H, -CH_2-HC=CH-CH$_2$-O-), 1.63-1.56 (m, 2H, -O-C=O-CH$_2$-CH_2-), 1.36-1.25 (m, *J* = 5.9 Hz, 16H, -[CH_2]$_8$-). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 173.9, 138.8, 124.04, 113.8, 76.9, 51.12, 34.37, 31.70, 28.60, 22.7, 13.7. ESI-MS (*m/z*): 261.41 [M+Na]$^+$. HRMS (ESI, *m/z*): 261.23.

(*E*)-Oxacyclononadec-10-en-2-one (MOLOH): FT-IR (NaCl, $CHCl_3$, ν, cm^{-1}): 3449, 2751, 1717, 1569, 1348. ^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm): 5.38-5.33 (m, 2H, -*H*C=C*H*-), 4.13-4.09 (t, *J* = 7.4 Hz, 2H, -CH_2-O-C=O-), 2.04-1.94 (t, *J* = 6.1 Hz, 2H, -O-C=O-CH_2-), 1.56 (m, *J* = 6.1 Hz, 4H, -CH_2-HC=CH-CH_2-), 1.32-1.24 (m, *J* = 5.9 Hz, 22H, -[CH_2]$_{11}$-). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 173.24, 138.83, 132.2, 124.04, 113.8, 76.4, 73.3, 51.12, 34.3, 31.4, 28.6, 24.636, 22.27, 13.7. ESI-MS (*m/z*): 312.09 [M+Na]$^+$. HRMS (ESI, *m/z*): 312.03.

(*E*)-Oxacyclotricos-14-en-2-one (MOLER): FT-IR (NaCl, $CHCl_3$, ν, cm^{-1}): 3538, 2647, 1715, 1575, 1347. ^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm): 5.84-5.76 (m, 2H, -*H*C=C*H*-), 3.87 (t, *J* = 7.4 Hz, 2H, -CH_2-O-C=O-), 2.33-2.29 (t, *J* = 6.1 Hz, 2H, -O-C=O-CH_2-), 2.04-2.01 (m, *J* = 6.1 Hz, 4H, -CH_2-HC=CH-CH_2-), 1.61 (m, 2H, -O-C=O-CH$_2$-CH_2-), 1.37-1.28. (m, *J* = 5.9 Hz, 28H, -[CH_2]$_{14}$-). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 175.5, 130.0, 76.3, 32.31, 31.6, 29.4, 26.9, 22.3, 13.7. ESI-MS (*m/z*): 359.24 [M+Na]$^+$. HRMS (ESI, *m/z*): 359.17.

(*E*)-Oxacycloicos-11-en-2-one (MOLUN): FT-IR (NaCl, $CHCl_3$, ν, cm^{-1}): 3451, 2742, 1717, 1572, 1357. ^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm): 5.38-5.30 (m, 2H, -*H*C=C*H*-), 4.07-4.03 (t, *J* = 7.4 Hz, 2H, -CH_2-O-C=O-), 2.28 (t, *J* = 6.1 Hz, 2H, -O-C=O-CH_2-), 1.98 (m, 4H, -CH_2-HC=CH-CH_2-), 1.61 (m, *J* = 6.1 Hz, 2H, -O-C=O-CH$_2$-CH_2-), 1.25 (m, *J* = 5.9 Hz, 22H, -[CH_2]$_{11}$). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 174.2, 139.4, 130.02, 114.37, 77.50, 64.63, 34.65, 34.03, 29.31, 22.93, 14.36, 11.67. ESI-MS (*m/z*): 317.17 [M+Na]$^+$. HRMS (ESI, *m/z*): 317.27.

(*E*)-Oxacyclopentadec-10-en-2-one (MOL5H): FT-IR (NaCl, $CHCl_3$, ν, cm^{-1}): 3441, 2442, 1710, 1565, 1349. ^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm): 5.33-5.21 (m, 2H, -*H*C=C*H*-), 4.56 (t, *J* = 7.4 Hz, 2H, -CH_2-O-C=O-), 2.34-2.31 (t, *J* = 6.1 Hz, 2H, -O-C=O-CH_2-), 2.06-2.00 (m, *J* = 6.1 Hz, 4H, -CH_2-HC=CH-CH_2-), 1.65-1.58 (m, 2H, -O-C=O-CH$_2$-CH_2-), 1.42-1.29 (m, *J* = 5.9 Hz, 12H, -[CH_2]$_6$). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 171.5, 130.7, 77.84, 60.78, 33.01, 32.32, 27.62, 23.09, 21.42, 14.60. ESI-MS (*m/z*): 247.24 [M+Na]$^+$. HRMS (ESI, *m/z*): 247.02.

(*E*)-Oxacyclohexadec-11-en-2-one (MUN5H): FT-IR (NaCl, $CHCl_3$, ν, cm^{-1}): 3467, 2738, 1705, 1546, 1387. ^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm):5.36-5.33 (m, 2H, -*H*C=C*H*-), 4.06-4.04 (t, *J* = 7.4 Hz, 2H, -CH_2-O-C=O-), 2.30-2.27 (t, *J* = 6.1 Hz, 2H, -O-C=O-CH_2-), 2.05-1.96 (m, *J* = 6.1 Hz, 4H, -CH_2-HC=CH-CH_2-) 1.65-1.52 (m, 2H, -O-C=O-CH$_2$-CH_2-), 1.38-1.25 (m, *J* = 5.9 Hz, 14H, -[CH_2]$_7$). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm):172.6, 138.7, 137.39, 134.86, 130.9, 115.14, 113.6, 76.15, 70.08, 34.79, 33.3, 31.14, 28.45, 25.55, 22.2, 24.55, 19.4, 13.6, 10.96. ESI-MS (*m/z*): 261.17 [M+Na]$^+$. HRMS (ESI, *m/z*):261.78.

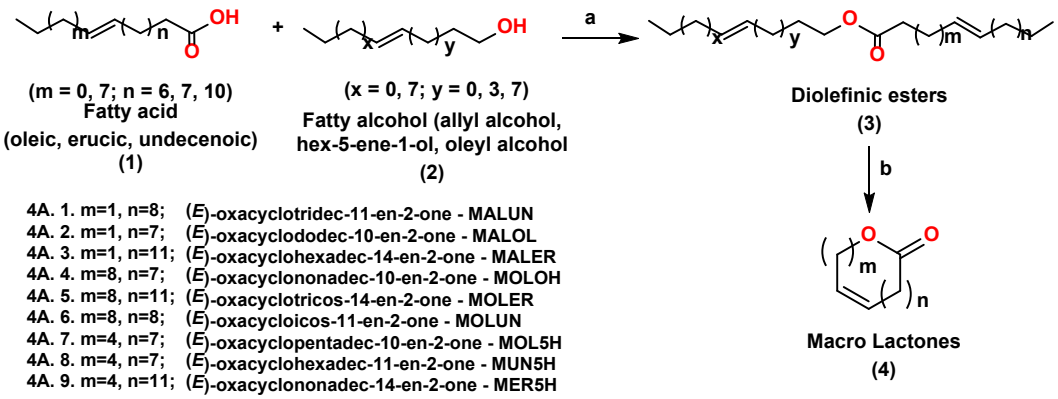
(*E*)-Oxacyclononadec-14-en-2-one (MER5H): FT-IR (NaCl, $CHCl_3$, ν, cm^{-1}): 3541, 2724, 1771, 1564, 1348. ^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm):5.34-5.39 (m, 2H, -*H*C=C*H*-), 4.05-4.03 (t, *J* = 7.4 Hz, 2H, -CH_2-O-C=O-), 2.30-2.26 (t, *J* = 6.1 Hz, 2H, -O-C=O-CH_2-), 2.04-1.94 (m, *J* = 6.1 Hz, 4H, -CH_2-HC=CH-CH_2-), 1.63-1.56 (m, 4H, -CH_2-CH$_2$-O-C=O-CH$_2$-CH_2-), 1.30-1.25 (m, *J* = 5.9 Hz, 18H, -[CH_2]$_9$). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm):174.2, 130.06, 76.99, 64.67, 34.7, 32.2, 29.6, 27.5, 26.23, 22.98, 14.41. ESI-MS (*m/z*): 205. 42 [M+Na]$^+$. HRMS (ESI, *m/z*):205.07.

2.5. Antibacterial activity

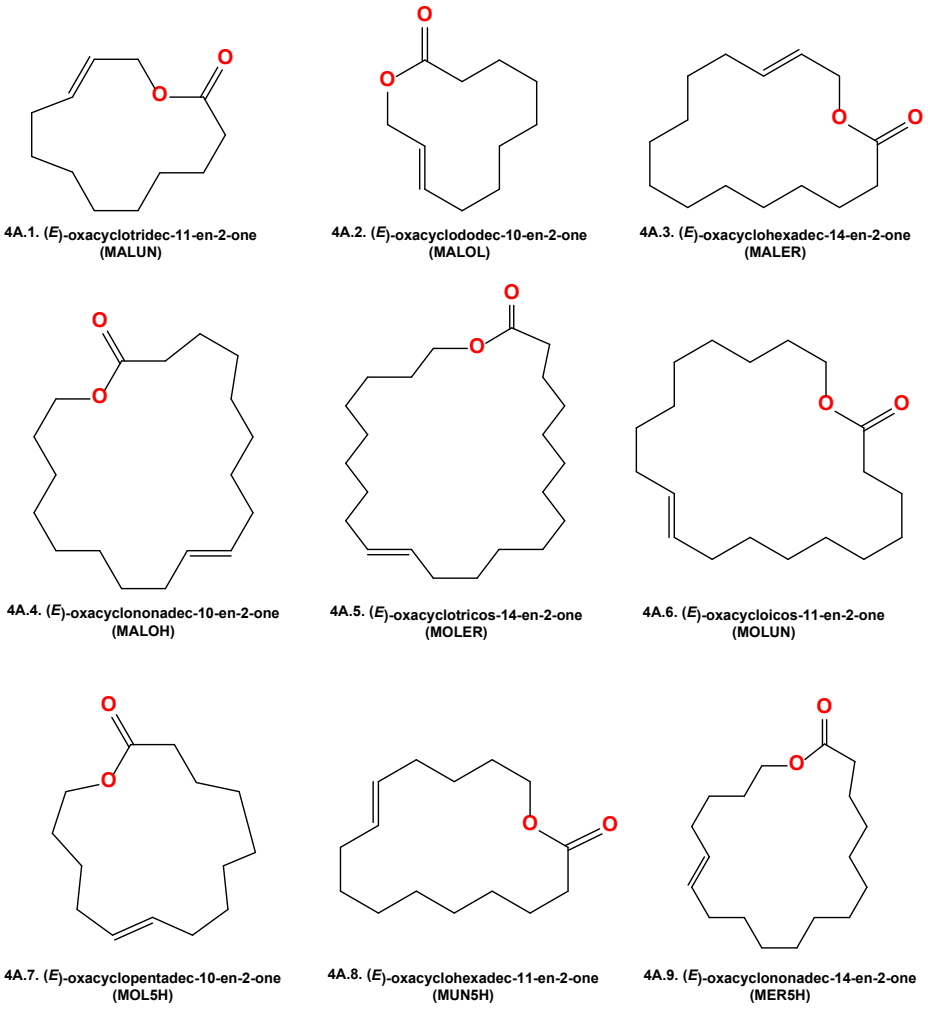
The bactericidal assay [30] (NCCLS, 2000) was performed in sterile 2.0 mL microfuge tubes against a panel of pathogenic bacterial strains including Gram positive bacteria; *Staphylococcus epidermidis* (MTCC 435), *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 96), and Gram-negative bacteria; *Pseudomonas aeruginosa* (MTCC 741), *E. coli* (MTCC 443) and *Klebsiella pneumoniae* (MTCC 618), which were cultured overnight in Mueller Hinton broth. Serial dilutions of the tested compounds were prepared in Mueller Hinton broth with different concentrations ranging from 0 to 125 µg/mL. To the test compounds,100 µL of overnight cultured bacterial suspensions were added to reach a final concentration of 1.5×10^8 cfu/mL (equal to 0.5 McFarland) and incubated at 37 °C for 24 h. After 24 h of incubation, the minimum bactericidal concentration (MBC) was determined by sampling 10 µL of suspension from the tubes in Mueller Hintonagar plates and incubated for 24 h at 37 °C to observe the growth of the test organisms. MBCs are the lowest concentration of test compounds required to kill a particular bacterium strain. All experiments were carried out in duplicates.

2.6. Anticancer activity

The anticancer activity of the synthesised macrolactones was measured using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) against different cancer cell lines obtained from the American Type Culture Collection (ATCC), Manassas, VA, USA, such as DU145 derived from human prostate epithelial cancer cells (ATCC HTB-81), HepG2 derived from liver cancer cells (ATCC HB-8065), SKOV3 derived from human ovarian cancer cells (ATCC HTB-77), and MDAMB-231 derived from human breast cancer cells (ATCC HTB-26) according to the method of Mosmann [31].



Scheme 1. Synthesis of macro lactones from fatty acids through olefin metathesis. Reagents and conditions: (a) EDC·HCl, DMAP dry DCM, 9 h, room temperature and (b) Grubbs' catalyst-I/II, dry toluene, 8-10 h, 90 °C.



Scheme 2. Macrolactones of varying ring sizes.

Briefly, cells (2×10^4) were seeded in each well containing 0.1 mL of medium in 96 well plates. These well plates were incubated overnight at 37 °C in 5% CO_2. Cells were treated with 100 µL of different test concentrations, such as 0.1, 1, 5, and 10 µM of test compounds under identical conditions with three replicates each. The final test concentrations were equivalent to 10 to 100 µM. Cell viability was evaluated after 24 h, adding 10 µL of MTT (5 mg/mL) per well. The plates were incubated at 37 °C for an additional 3 h. The medium was discarded and the formazan blue formed in the cells was dissolved in 100 µL of DMSO. The absorbance was measured at 540 nm using the TRIAD multimode reader (Dynex Technologies, Inc., Chantilly, VA). The IC_{50} values (50% inhibitory concentration) were calculated from the absorbance data plotted on the dose-response curves. The assay was performed using Mitomycin C as a standard or positive control and 1% DMSO as vehicle control. To account for the toxicity of DMSO, the values obtained for the DMSO control were subtracted from those of the test compounds. The IC_{50} values (in µM) are expressed as the mean±SD of three independent experiments.

Table 1. Antibacterial activity of macro lactones *.

Compound	*Staphylococcus Aureus*	*Staphylococcus Epidermidis*	*Bacillus Subtilis*	*Escherichia Coli*	*Pseudomonas Aeruginosa*	*Klebsiella Pneumoniae*
MALUN	-	-	-	-	-	-
MALOL	-	-	-	-	-	-
MALER	-	-	-	-	-	-
MOLOH	-	-	-	-	-	-
MOLER	16	16	17	-	-	-
MOLUN	-	10	-	-	-	-
MOL5H	-	-	-	-	-	-
MUN5H	-	-	-	-	-	-
MER5H	-	-	-	-	-	-
Penicillin	1.562	3.125	1.562	12.5	12.5	6.25
Streptomycin	6.25	3.125	6.25	6.25	1.562	3.125

* MALUN: (*E*)-oxacyclotridec-11-en-2-one); MALOL: (*E*)-oxacyclododec-10-en-2one; MALER: (*E*)-oxacyclohexadec-14-en-2-one; MOLOH: (*E*)-oxacyclononadec-10-en-2-one; MOLER: (*E*)-oxacyclotricos-14-en-2-one; MOLUN: (*E*)-oxacycloicos-11-en-2-one; MOL5H: (*E*)-oxacyclopentadec-10-en-2-one; MER5H: (*E*)-oxacyclononadec-14-en-2-one; MUN5H: (*E*)-oxacyclohexadec-11-en-2-one.

Table 2. Cytotoxic activity of macro-lactones.

Compounds *	IC_{50} values (μM)
	Chinese hamster ovarian (CHO-K1)
MALUN	70.4±0.85
MALOL	29.5±0.62
MALER	31.1±0.76
MOLOH	44.7±0.67
MOLER	20.9±0.62
MOLUN	19.5±0.39
MOL5H	35.3±0.48
MUN5H	30.2±0.74
MER5H	27.8±0.53
Mitomycin C (Standard)	13.3±0.68

* MALUN: (*E*)-oxacyclotridec-11-en-2-one); MALOL: (*E*)-oxacyclododec-10-en-2-one; MALER: (*E*)-oxacyclohexadec-14-en-2-one; MOLOH: (*E*)-oxacyclononadec-10-en-2-one; MOLER: (*E*)-oxacyclotricos-14-en-2-one; MOLUN: (*E*)-oxacycloicos-11-en-2-one; MOL5H: (*E*)-oxacyclopentadec-10-en-2-one; MER5H: (*E*)-oxacyclononadec-14-en-2-one; MUN5H: (*E*)-oxacyclohexadec-11-en-2-one.

3. Results and discussion

3.1. Synthesis

The synthesis of macrolactones *via* ring closing metathesis was carried out employing Grubbs' second-generation catalyst using a two-step procedure with ring sizes ranging from 10-21 membered.

Step 1: Mono unsaturated fatty acids (oleic acid, erucic acid, and undecenoic acid undergo esterification with different monounsaturated alcohols (allyl alcohol, hex-5-en-1-ol and oleyl alcohol) using EDCI (*N*-(3-dimethylaminopropyl)-*N*-ethyl carbodiimide hydrochloride) and DMAP (4-dimethylamino pyridine) producing the desired diolefinic esters (allylundec-10-enoate, allyloleate, allyldicosa-13-enoate, octadec-9-ene-1-yloleate, (*Z*)-octadec-9-en-1-yl-(*Z*)-docos-13-enoate, (*Z*)-octadec-9-en-1-yl-undec-10-enoate, hex-5-en-1-yl-oleate, hex-5-en-1-yl-undec-10-enoate, hex-5-en-1-yl-(Z)-docos-13-enoate) in 75-88% yields (Scheme 1). Diolefinic esters are also good precursors for the production of polyesters [21,22].

Step 2: Ring closing metathesis of diolefinic esters employed Grubbs second generation catalyst was used to synthesise the desired macrolactones such as MALUN, MALOL, MALER, MOLOH, MOLER, MOLUN, MOL5H, MUN5H and MER5H isolated in 71-85% yields. In addition to macrolactones, the formation of mono- and di-esters at lower yields was also observed (Schemes 1 and 2). It was observed that the reaction time was a key parameter in determining the ultimate yield of cyclic products from oligomeric intermediates. As the size of the ring decreased, the yields of the reactions were found to decrease. The yields were found to improve when the DCM solvent was used as the medium. Litinas and Salteris observed a similar observation, where the synthesis of 13-, 19-, 20-, and 21-membered macrolactones was carried out using Grubbs' first-generation catalyst (0.01 m mol) was carried out in benzene at 60 ° C for 24 h [26]. 21-membered ring were obtained in 82% yield, 13-membered ring with 6% yield due to stearic effect [26], while yields were found to increase drastically with the use of DCM. Therefore, the closing metathesis of the ring uses the second-generation Grubbs catalyst, leading to the effective formation of macro-lactones in good yields (71-85%), and the formation of mono- and di-esters with lower yields compared to the cross-metathesis reaction [20].

3.2. Antibacterial activity

Antibacterial activity was carried out against a panel of pathogenic bacterial strains including Gram positive bacteria; *Staphylococcus epidermidis* (MTCC 435), *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 96), and Gram-negative bacteria; *Pseudomonas aeruginosa* (MTCC 741), *E. coli* (MTCC 443) and *Klebsiella pneumoniae* (MTCC 618) where penicillin and streptomycin were taken as standard. The macrolactones, which are also supposed to be good biologically important molecules, were also screened for the above activity exhibited by the erucic and undecenoic based lactones.

The MOLER compound obtained by esterification of oleyl alcohol and erucic acid followed by ring-closing metathesis resulted in a 21-ring-sized macrolactone that exhibited excellent antibacterial activity against three bacterial cell lines, namely *Staphylococcus aureus, Staphylococcus epidermidis*, and *Bacillus subtilis.* However, MOLUN, an 18-membered macrolactone obtained from esterification of undecanoic acid and oleyl alcohol followed by ring-closing metathesis, exhibited excellent antibacterial activity selectively against only *Staphylococcus epidermidis*. While the rest of the macrolactones did not exhibit any antibacterial activity. This further strengthens the lactones, especially those based on erucic and undecenoic acids that are good antibacterial agents because of the bigger ring size. The antibacterial activity of macrolactones is tabulated in Table 1.

3.3. Anticancer activity

The synthesised macrolactones were also evaluated for their *in vitro* anticancer activity by an MTT assay against different cancer cell lines, human prostate epithelial cancer cells (ATCC HTB-81), HepG2 derived from liver cancer cells

(ATCC HB-8065), SKOV3 derived from human ovarian cancer cells (ATCC HTB-77), MDAMB-231 derived from human breast cancer cells (ATCC HTB-26) and Chinese hamster ovarian (CHO-K1) cell lines. It was observed that macrolactones exhibited selective anticancer activity against Chinese hamster ovarian (CHO-K1) cell lines; however, the activities exhibited by MOLER and MOLUN were excellent but slightly inferior compared to standard Mitomycin C. The anticancer activities of macrolactones are tabulated in Table 2.

4. Conclusions

Ring-closing olefin metathesis reaction employing the second-generation Grubbs catalyst is one of the most efficient methods for the synthesis of macrocyclic lactones based on fatty acids from diolefinic esters, where multistep procedures can be avoided. The yields were found to decrease with decreasing ring size, which could be due to the stearic hindrance caused during the ring closing; bigger sized lactones exhibited good yields in DCM as a solvent. In conclusion, RCM of diolefinic esters is an excellent method for macrolactone formulation, especially for terminal alkenes. The synthesised macrolactones were evaluated for their antibacterial and anticancer activities. Oxacyclotricos-14-en-2-one, 21-membered macrolactone exhibited excellent antibacterial activity against three bacterial cell lines, namely, *Staphylococcus aureus, Staphylococcus epidermidis, and Bacillus subtilis,* similarly, oxacycloicos-11-en-2-one, 18-membered macrolactone exhibited excellent antibacterial activity selectively toward only *Staphylococcus epidermidis.* All macrolactones exhibited selective anticancer activity towards Chinese hamster ovarian (CHO-K1) cell lines. The compounds oxacyclotridec-11-en-2-one (MOLER) and oxacyclononadec-10-en-2-one (MOLUN) exhibited much superior activity compared to other macrolactones and slightly inferior to standard Mitomycin C.

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CRediT authorship contribution statement

Conceptualization: Mallampalli Sri Lakshmi Karuna, Methodology: Vyshnavi Yelchuri; Formal Analysis: Thirupathi Azmeera; Resources: Mallampalli Sri Lakshmi Karuna; Writing - Original Draft: Vyshnavi Yelchuri; Writing - Review and Editing: Mallampalli Sri Lakshmi Karuna; Thirupathi Azmeera; Supervision: Mallampalli Sri Lakshmi Karuna.

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