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**ORIGINAL PAPER**

# Antibacterial and Cytotoxic Activities of Rhodomyrtone Derivatives

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**Abstract**: Rhodomyrtone, a bioactive acylphloroglucinols compound, was obtained from the leaves of *Rhodomyrtus tomentosa* (Aiton.) Hassk and had been scientifically evidenced as multiple potential pharmacological activities. The purpose of research is to synthesize rhodomyrtone derivatives and e valuate for their antibacterial and anticancer activities. Rhodomyrtone derivatives **2**-**12** were synthesized and their structures were elucidated by spectroscopic techniques. The results indicated that rhodomyrtone showed antibacterial activity similar to that of a standard drug (vancomycin). It exhibited higher activity than its derivatives. Rhodomyrtone (**1**) exhibited highest anticancer activity against H460 and PC3 cells with $IC_{50}$ value of 6.12 and 13.30 μM. Whereas, derivatives **8** and **9** gave higher anticancer activity against HeLa cell than rhodomyrtone.

**Keywords**: Rhodomyrtone, Rhodomyrtone derivatives, *Rhodomyrtus tomentosa*, Anticancer, Antibacterial, and Acylphloroglucinols

## 1. Introduction

*Rhodomyrtus tomentosa* (Aiton.) Hassk is a plant of the family *Myrtaceae* that can be found in Southeast Asia such as Vietnam, China, Malaysia, Thailand, Cambodia, Taiwan, Philippines, and Indonesia. *R. tomentosa* exhibits a wide spectrum of pharmacological effects and has been used to treat colic diarrhea, wounds, heartburn, abscesses, gynecopathy, and as a pain killer. It has also been used in traditional Chinese medicine to treat urinary tract infection. *R. tomentosa* that has been documented several biological activities including antibacterial, antifungal, antimalarial, osteogenic, antioxidant, and anti-inflammatory activities. It has also been studied extensively for alternative antimicrobial agents.

Phloroglucinol, flavonoid, terpenoid, anthracene glycoside, tannin, and other compounds have been isolated and structurally elucidated from this plant (Bach *et al.* 2020). Rhodomyrtone (**1**) is a member of acylphloroglucinols isolated from *R. tomentosa* (Aiton.) Hassk which is the most representative compound with multiple potential pharmacology activities (Zhang *et al.* 2018). Rhodomyrtone (**1**) exhibited pronounced antibacterial activity against a wide range of Gram-positive bacteria (Limsuwan *et al.* 2009; Sianglum *et al.* 2011; Hamid *et al.* 2017). Proteomics study indicated that GAPDH expression in *S. pyrogenes*, a key enzyme in glycolysis pathway, was down-regulated when it was treated with rhodomyrtone

(**1**) (Limsuwan *et al.* 2011). It also exhibited anti-caries activity and biofilm formation caused by *S. mutans* (Bach *et al.* 2020). Furthermore, *R. tomentosa* extract was also found to be a potential inhibitor of quorum sensing system (QSS), which is an important factor in controlling biofilm formation by *S. pyrogenes* (Limsuwan and Voravuthikunchai 2008; Wunnoo *et al.* 2017). Treatment of *Propionibacterium acnes* with rhodomyrtone (**1**) resulted in a reduction in biofilm formation (Zhao *et al.* 2019). Moreover, rhodomyrtone (**1**) was found to be the most potent antitumor effect on HeLa cells (Zhao *et al.* 2019 and Zhang et al. 2018). It also showed time-dependently inhibited the proliferation of HaCaT keratinocyte cells *in vitro* and without skin irritation observed *in vivo*. In addition, inhibition of cancer metastasis by reducing cell migration, cell adhesive ability and cell invasion on epidermoid carcinoma A431 cells by rhodomyrtone (**1**) was also reported (Chorachoo *et al.* 2016; Tayeh *et al.* 2017).

Rhodomyrtone (**1**) has been subjected to structural modification and rhodomyrtone 6,8-diacetate and ketoxime analogues demonstrated similar activity as the parent compound against *B. subtilis* and *S. epidermidis* (Leejae *et al.* 2012). However, rhodomyrtone derivatives have not been designed, synthesized and tested for anticancer activity. Thus, in this study rhodomyrtone derivatives **2** – **12** were synthesized and tested for their antibacterial and anticancer activities.

## 2. Materials and Methods

### *2.1 Chemistry*

### *2.1.1 General method*

The organic solvents (commercial grade reagents) were purchased from commercial suppliers and dried according to standard procedures. Reagents were purchased from Sigma-Aldrich. The rhodomyrtone was isolated from *Rhodomyrtus tomentosa* (Aiton.) Hassk leaves. IR spectra were recorded on a Perkin-Elmer FT-IR 400 spectrometer (ATR). $^1$H-NMR and $^{13}$C-NMR spectra were recorded on an Ascend™ 400 spectrometer. High-resolution electrospray ionization mass spectrometry (HR-ESI-TOF-MS) was measured on a Bruker micrOTOF-II mass spectrometer. All reactions were carried out under an air atmosphere and monitored by using thin layer chromatography (TLC Silica gel 60 $F_{254}$, Merck, Germany).

### *2.2 Synthesis of rhodomyrtone derivatives*

#### *2.1.2 Synthesis of 6-O-(2-hydroxyethyl) rhodomyrtone (**2**)*

Rhodomyrtone (**1**) (20 mg, 0.045 mmol) and 1-bromoethanol (8.5 mg, 0.068 mmol) were dissolved in DMF (5 mL). $K_2CO_3$ (31 mg, 0.226 mmol) was then added and the reaction mixture was stirred at 80 °C for 8 h. Water (100 mL) was then added and the mixture extracted with EtOAc (3×50 mL). The organic layer was dried over sodium sulphate anhydrous ($Na_2SO_4$) and concentrated *in vacuo*. The crude residue was purified by column chromatography on silica gel. The elution was conducted gradient with n-hexane and n-hexane-EtOAc, respectively, to obtain the title compound as colorless oil (15.5 mg, 70 % yield). IR $\nu_{max}$ 2954, 2871, 1716, 1647, 1614, 1461, 1424, 1379, 1280, 1160, 1106, 1039, 996, 958, 856, 802, 767 cm$^{-1}$; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.81 (*d*, *J* = 6.0 Hz, 3H, H-3″), 0.84 (*d*, *J* = 6.0 Hz, 3H, H-4″), 0.96 (*d*, *J* = 6.7 Hz, 6H, H-4′, H-5′), 1.33 (*s*, 3H, H-10), 1.38 (*s*, 3H, H-11), 1.34 (*overlapping,* 3H, H-1″, H-2″), 1.42 (*s*, 3H, H-12),1.54 (*s*, 3H, H-13), 2.25 (*m*, 1H, H-3′), 2.95 (*m*, 2H, H-2′), 4.01 (*t*, *J* = 4.6 Hz, 2H, H-*b*), 4.17 (*m*, 2H, H-*a*), 4.27 (*t*, *J* = 5.5 Hz, 1H, H-9), 6.10 (*s*, 1H, H-5), 14.10 (*H-Bond of C8-OH*); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 22.7 ($CH_3$, C-5′), 22.8 ($CH_3$, C-4′), 23.2 ($CH_3$, C-3′) 23.5 ($CH_3$, C-4′), 24.1 (CH, C-2), 24.6 ($2CH_3$, C-10, C-11), 24.7 ($2CH_3$, C-12, C-13), 25.0 (CH, C-3′), 25.2 (CH, C-9), 45.8 ($CH_2$, C-1″), 47.1 (C-4), 53.6 ($CH_2$, C-2′), 56.1 (C-2), 61.1 ($CH_2$, C-*b*), 70.2 ($CH_2$, C-*a*), 90.9 (CH, C-5), 107.7 (C-8*a*), 108.5 (C-7), 114.3 (C-9*a*), 156.0 (C-5*a*), 159.8 (C-OH, C-6) 163.9 (C-OH, C-8) 166.6 (C-4*a*), 197.4 (C=O, C-1), 206.1 (C=O, C-

1′), 212.1 (C=O, C-3); HR-ESI-TOF-MS (positive-ion mode) *m/z* 509.2509 [M+Na]$^+$ (calcd for $C_{28}H_{38}NaO_7$, 509.2515).

### *2.1.3 Synthesis of rhodomyrtone 5-sulfate (**3**)*

Rhodomyrtone (**1**) (50 mg, 0.113 mmol) and sulfur trioxide pyridine complex (90 mg, 0.585 mmol) were dissolved in DMF (3 mL). The reaction mixture was stirred at room temperature for 48 h. After usual work-up, the crude product was purified by column chromatography to obtain compound **3** (12.5 mg, 20 % yield) as a white solid. IR $\nu_{max}$ 3465, 2955, 2860, 1716, 1642, 1601, 1468, 1424, 1386, 1269, 1231, 1168, 1020, 974, 755 cm$^{-1}$; $^1$H NMR (400 MHz, $CD_3OD$) δ 0.72 (*d*, *J* = 6.1 Hz, 3H, H-3″), 0.77 (*d*, *J* = 6.0 Hz, 3H, H-4″), 0.88 (*d*, *J* = 6.7 Hz, 6H, H-4′, H-5′), 1.24 (*d*, *J* = 1.6 Hz, 6H, H-10, H-11), 1.31 (*overlapping*, 3H, H-1″, H-2″), 1.47 (*s*, 3H, H-13), 1.50 (*s*, 3H, H-12), 2.16 (*m*, 1H, H-3′), 2.92 (*m*, 2H, H-2′), 4.13 (*t*, *J* = 5.8 Hz, 1H, H-9), 4.50 (*Br*, C6-OH); $^{13}$C NMR (100 MHz, $CD_3OD$) δ 21.7 ($CH_3$, C-5′, C-4′), 22.3 ($CH_3$, C-3″), 22.4 (C-4″), 23.1 ($CH_3$, C-10), 23.4 ($CH_3$, C-11), 23.6 ($CH_3$, C-12), 24.2 ($CH_3$, C-13), 24.6 (CH, C-9), 24.8 (CH, C-2″), 25.0 (CH, C-3′), 45.8 ($CH_2$, C-1″), 47.3 (C-4), 53.1 ($CH_2$, C-2′), 55.6 (C-2), 105.4 (C-5), 106.9 (C-8*a*), 108.2 (C-7), 113.5 (C-9*a*), 153.5 (C-5*a*), 157.5 (C-OH, C-6), 163.8 (C-OH, C-8), 167.7 (C-4*a*), 198.3 (C=O, C-1), 207.3 (C=O, C-1′), 212.3 (C=O, C-3); HR-ESI-TOF-MS (positive-ion mode) *m/z* 567.1614 [M+2Na]$^+$ (calcd for $C_{26}H_{33}Na_2O_9S$, 567.1640).

### *2.1.4 Alkylation of rhodomyrtone (1) with 1,2-dibromoethane*

Rhodomyrtone (**1**) (200 mg, 0.452 mmol) and 1,2-dibromoethane (169.8 mg, 0.904 mmol) were dissolved in DMF (5 mL). $K_2CO_3$ (125 mg, 0.904 mmol) was then added to the solution. The reaction mixture was stirred at 80 °C for 8 h. Water (100 mL) was added and the mixture extracted with EtOAc (3x100 mL). The organic layer was dried over sodium sulphate anhydrous ($Na_2SO_4$) and concentrated *in vacuo*. The crude residue was purified by column chromatography to yield 6,8-*O*-di(2-bromoethyl) rhodomyrtone (**4**) (209 mg, 70 % yield) and 6-*O*-(2-bromoethyl) rhodomyrtone (**5**) (50 mg, 20%) were obtained.

**Compound 4.** Colorless oil; IR $\nu_{max}$ 3415, 2950, 2877, 2825, 1866, 1705, 1644, 1608, 1585, 1458, 1382, 1363, 1275, 1163, 1103, 1039, 950, 854, 822, 755 cm$^{-1}$; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.76 (*d*, *J* = 6.1 Hz, 3H, H-3″), 0.86 (*d*, *J* = 6.0 Hz , 3H, H-4″), 0.97 (*d*, *J* = 6.7 Hz, 6H, H-4′, H-5′), 1.35 (*s*, 3H, H-10), 1.37 (*s*, 3H, H-11), 1.39 (*overlapping*, 3H, H-1″, H-2″), 1.43 (*s*, 3H, H-12), 1.54 (*s*, 3H, H-13), 2.20 (*m*, 1H, H-3′), 2.74 (*m*, 2H, H-2′), 3.54 (*d*, *J* = 2.7 Hz, 2H, H-*b*), 3.60 (*t*, *J* = 6.01 Hz, 2H, H-*b*′), 4.15 (*overlapping*, 2H, H-*a*), 4.18 (*t*, *J* = 5.6 Hz, 1H, H-9), 4.28 (*overlapping*, 2H, H-*a*′), 6.42 (*s*, 1H, H-5); $^{13}$C NMR (100 MHz, $CDCl_3$): δ 22.6 ($CH_3$, C-4′), 22.8 ($CH_3$, C-3″), 23.3 (C-4″), 23.4 ($CH_3$, C-5′), 24.3 (CH, C-2″), 24.6 (2$CH_3$, C-10, C-11), 24.8 (2$CH_3$, C-12, C-13), 24.9 (2CH, C-3′, C-9), 28.4 ($CH_2$, C-*b*), 29.2 ($CH_2$, C-*b*′), 46.9 ($CH_2$, C-1″), 47.2 (C-4), 54.1 ($CH_2$, C-2′), 56.0 (C-2), 68.6 ($CH_2$, C-*a*), 75.3 ($CH_2$, C-*a*′), 96.9 (CH, C-5), 113.3 (C-8*a*), 113.7 (C-7), 123.4 (C-9*a*), 152.7 (C-5a), 153.4 ($\underline{C}$-O-$CH_2$, C-6), 154.1 ($\underline{C}$-O-$CH_2$, C-8), 167.3 (C-4*a*), 197.5 (C=O, C-1), 203.7 (C=O, C-1′), 212.1 (C=O, C-3); HR-ESI-TOF-MS (positive-ion mode) *m/z* 677.1109 [M+Na]$^+$ (calcd for $C_{30}H_{40}Br_2NaO_6$, 677.1089.

**Compound 5.** Colorless oil; IR $\nu_{max}$ 3421, 2956, 2872, 1710, 1645, 1610, 1585, 1459, 1364, 1278, 1163, 1111, 1088, 1040, 997, 952, 826, 756 cm$^{-1}$; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.81 (*d*, *J* = 5.9 Hz, 3H, H-3″), 0.85 (*d*, *J* = 6.1 Hz, 3H, H-4″), 0.96 (*d*, *J* = 6.7 Hz, 6H, H-4′, H-5′), 1.34 (*s*, 3H, H-10), 1.38 (*s*, 3H, H-11), 1.40 (*overlaping*, 3H, H-1″, H-2″), 1.42 (*s*, 3H, H-12), 1.53 (*s*, 3H, H-13), 2.27 (*m*, 1H, H-3′), 3.04 (*m*, 2H, H-2′), 3.72 (*t*, *J* = 5.6 Hz, 2H, H-*b*), 4.27 (*t*, *J* = 5.6 Hz, 1H, H-9), 4.38 (*m*, 2H, H-*a*), 6.03 (*s*, 1H, H-5), 14.20 (*H-Bond of C8-OH*);

$^{13}$C NMR (100 MHz, $CDCl_3$) δ 22.8 ($CH_3$, C-4′), 23.2 ($CH_3$, C-5’), 23.3 ($CH_3$, C-3″), 23.4 (C-4″), 24.1 (CH, C-2″), 24.6 (2$CH_3$, C-10, C-11), 24.7 (2$CH_3$, C-12, C-13), 25.2 (2CH, C-3′, C-9), 28.3 ($CH_2$, C-*b*), 45.9 ($CH_2$, C-1″), 47.1 (C-4), 53.7 ($CH_2$, C-2′), 56.2 (C-2), 68.7 ($CH_2$, C-*a*), 90.5 (CH, C-5), 108.0 (C-8*a*), 108.4 (C-7), 114.3 (C-9*a*), 155.8 (C-5*a*), 159.1 (C-OH, C-6), 164.0 (C-OH, C-8), 166.6 (C-4*a*), 197.4 (C=O, C-1), 206.3 (C=O, C-1′), 212.2 (C=O, C-3); HR-ESI-TOF-MS (positive-ion mode) *m/z* 549.1811 $[M+H]^+$ (calcd for $C_{28}H_{38}BrO_6$, 549.1851).

### *2.1.5 Synthesis of 6,8-O-di-(2-pyrrolidinylethyl) rhodomyrtone (**6**)*

Rhodomyrtone derivative **4** (50 mg, 0.076 mmol) and pyrrolidine (21.7 mg, 0.152 mmol) were dissolved in DMF (5 mL) and stirred. $K_2CO_3$ (32 mg, 0.228 mmol) was then added and the reaction mixture was stirred at room temperature. The progress of reaction was monitored by TLC. Water (100 mL) was added and the mixture was extracted with EtOAc (3×100 mL). The organic layer was combined, dried over sodium sulphate anhydrous ($Na_2SO_4$) and concentrated *in vacuo*. The crude residue was purified by column chromatography to obtain rhodomyrtone derivative **6** as yellow oil (42 mg, 85% yield). IR $\nu_{max}$ 2955, 2873, 1759, 1712, 1648, 1611, 1587, 1463, 1382, 1274, 1186, 1161, 1123, 1094, 1031, 956, 855, 826, 714, 629, 582 cm$^{-1}$; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.79 (*d*, *J* = 5.9 Hz, 3H, H-3″), 0.89 (*d*, *J* = 6.1 Hz, 3H, H-4″), 0.99 (*d*, *J* = 1.5 Hz, 3H, H-4′), 1.01 (*d*, *J* = 1.5 Hz, 3H, H-5′), 1.35 (*s*, 3H, H-10), 1.38 (*s*, 3H, H-11 ), 1.43 (*overlapping*, 3H, H-1″, H-2″), 1.48 (*s*, 3H, H-12), 1.58 (*s*, 3H, H-13), 1.84 (*overlapping*, 8H, $N(CH_2\underline{CH_2})$), 2.14 (*m*, 1H, H-3′), 2.66 (*overlapping*, 9H, ($N(\underline{CH_2}CH_2)$, H-2′), 2.94 (*overlapping*, 4H, H-*b*, H-*b*′), 4.02 (*overlapping*, 2H, H-*a*), 4.18 (*overlapping*, 2H, H-*a*′), 4.24 (*t*, *J* = 5.5 Hz, 1H, H-9), 6.77 (*s*, 1H, H-5); $^{13}$C NMR (100 MHz, $CD_3OD$): δ 21.7 ($CH_3$, C-3″), 21.8 ($CH_3$, C-4″), 22.5 (2$CH_3$, C-4′, C-5′), 22.7 ($CH_3$, C-10), 22.8 (2 × $N(CH_2)_2(\underline{CH_2})_2$), 22.9 (2CH, C-3′), 23.5($CH_3$, C-12), 23.9 ($CH_3$, C-13), 24.1 (CH, C-2″), 24.7 ($CH_3$, C-11), 26.0 (CH, C-9), 46.6 ($CH_2$, C-1″), 46.9 (C-4), 54.0 (2$CH_2$, C-*b*, C-*b*′), 54.2 ($N(\underline{CH_2}CH_2)_2$), 54.4 ($N(\underline{CH_2}CH_2)_2$), 55.1 ($CH_2$, C-2′), 55.5 (C-2), 67.8 ($CH_2$, C-*a*), 74.3 ($CH_2$, C-*a*′), 96.7 (CH, C-5), 112.7 (C-8*a*), 113.1′ (C-7), 122.9 (C-9*a*), 153.0 (C-5*a*), 153.9 (C-OH, C-6), 155.1 (C-OH, C-8), 168.0 (C-4*a*), 197.8 (C=O, C-1), 204.3 (C=O, C-1′), 211.8 (C=O, C-3); HR-ESI-TOF-MS (positive-ion mode) *m/z* 637.4269 $[M+H]^+$, (calcd for $C_{38}H_{57}N_2O_8$, 669.4114).

### *2.1.6 Synthesis of 6,8-O-di-(2-morpholinylethyl) rhodomyrtone (**7**)*

6,8-*O*-Di-(2-morpholinylethyl) rhodomyrtone (**7**) was synthesized from rhodomyrtone derivative **4** (50 mg, 0.076 mmol) and morpholine (20 mg, 0.228 mmol) using the same manner as described for the synthesis of **6**. Rhodomyrtone derivative **7** was obtained as yellow oil (45 mg, 88 %). IR $\nu_{max}$ 3352, 3089, 2949, 2870, 2776, 1661, 1609, 1537, 1463, 1382, 1365, 1263, 1163, 1115, 1088, 1039, 1002, 964, 852, 830, 748, 689, 625, 578 cm$^{-1}$; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.77 (*d*, *J* = 5.9 Hz, 3H, H-3″), 0.86 (*d*, *J* = 6.1 Hz, 3H, H-4″), 0.96 (*d*, *J* = 1.5 Hz, 3H, H-4′), 0.98 (*d*, *J* = 1.5 Hz, 3H, H-5′), 1.34 (*s*, 3H, H-10), 1.37 (*s*, 3H, H-11 ), 1.38 (*overlapping*, 3H, H-1″, H-2″), 1.42 (*s*, 3H, H-12), 1.54 (*s*, 3H, H-13), 2.16 (*m*, 1H, H-3′), 2.58 (*overlapping*, 8H, $O(CH_2\underline{CH_2})_2N$, 2.68 (*overlapping*, 2H, H-*b*′), 2.70 (*overlapping*, 2H, H-2′), 2.73 (*overlapping*, 2H, H-*b*), 3.69 (*t*, *J* = 4.6 Hz, 8H, $O(\underline{CH_2}CH_2)_2N$, 3.91 (*m*, 2H, H-*a*), 4.06 (*m*, 2H, H-*a*′), 4.18 (*t*, *J* = 5.5 Hz, 1H, H-9), 6.42 (*s*, 1H, H-5); $^{13}$C NMR (100 MHz, $CDCl_3$): δ 22.7 (2$CH_3$, C-3″, C-4″), 22.8 (2$CH_3$, C-4′, C-5′), 23.2, ($CH_3$, C-10), 23.3(2CH, C-3′) 23.9 ($CH_3$, C-12), 24.3 ($CH_3$, C-13), 24.6 (CH, C-2″), 24.8 ($CH_3$, C-11), 25.9 (CH, C-9), 29.7 ($CH_2$, $O(\underline{CH_2}CH_2)_2)N$, 47.1 ($CH_2$, C-1″), 47.2 (C-4), 53.9 ($CH_2$, C-*b*), 54.0 ($CH_2$, C-*b*′), 57.3 ($CH_2$, $(O(CH_2\underline{CH_2})_2)N$), 58.3 ($CH_2$, C-2′), 63.1 (C-2), 66.9 ($CH_2$, C-*a*), 73.2 ($CH_2$, C-*a*′), 96.4

(CH, C-5), 113.2 (C-8*a*), 113.5 (C-7), 123.1 (C-9*a*), 152.6 (C-5*a*), 154.2 (C-OH, C-6), 154.8 (C-OH, C-8), 167.4 (C-4*a*), 197.5 (C=O, C-1), 204.0 (C=O, C-1′), 212.2 (C=O, C-3); HR-ESI-TOF-MS m/z 669.4109 $[M+H]^+$ (calcd for $C_{38}H_{57}N_2O_8$, 669.4114).

### *2.1.7 Alkylation of rhodomyrtone (**1**) with ethyl chloroacetate*

Rhodomyrtone (**1**) (200 mg, 0.452 mmol) and ethyl-chloroacetate (110 mg, 0.904 mmol) were dissolved in DMF (5 mL). $K_2CO_3$ (125 mg, 0.904 mmol) was added to the solution. The reaction mixture was stirred at room temperature for 8 h. After usual work-up, the crude product was purified by column chromatography to obtain compounds **8** (56.5 mg, 20 %) and **9** (209.5 mg, 75%).

**Compound 8.** IR $\nu_{max}$ 3305, 2815, 2848, 1713, 1645, 1608, 1582, 1463, 1381, 1362, 1267, 1240, 1165, 1114, 1039, 948, 859, 719 cm$^{-1}$;$^1$H NMR (400 MHz, $CDCl_3$) δ 0.80 (*d*, *J* = 6.0 Hz, 3H, H-3″), 0.84 (*d*, *J* = 6.0 Hz, 3H, H-4″), 0.95 (*d*, *J* = 6.7 Hz, 6H, H-4′, H-5′), 1.31 (*t*, *J* = 7.1 Hz, 3H, H-*d*), 1.33 (*s*, 3H, H-10), 1.37 (*s*, 3H, H-11), 1.38 (*overlapping*, 3H, H-1″, H-2″), 1.41 (*s*, 3H, H-12), 1.53 (*s*, 3H, H-13), 2.19 (*m*, 1H, H-3′), 2.75 (*m*, 2H, H-2′), 4.27 (*t*, *J* = 5.5 Hz, 1H, H-9), 4.30 (*m*, 2H, H-*c*), 4.61 (*s*, 2H, H-a), 6.32 (*s*, 1H, H-5); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.2 ($CH_3$,C-*d*), 22.7 ($CH_3$, C-4′), 22.8 ($CH_3$, C-5′), 23.1 ($CH_3$, C-3″), 23.4 ($CH_3$, C-4″), 24.1 (CH, C-2″), 24.5 (2$CH_3$, C-10, C-11), 24.6 (2$CH_3$, C-12, C-13), 24.8 (2CH, C-3′), 25.2 (CH, C-9), 45.9 ($CH_2$, C-1″), 47.1 (C-4), 53.4 ($CH_2$, C-2′), 56.1 (C-2), 61.7 ($CH_2$, C-c), 65.7 ($CH_2$, C-a), 90.6 (CH, C-5), 108.4 (C-8*a*), 108.5 (C-7), 114.3 (C-9*a*), 155.7 (C-5*a*), 158.7 ($\underline{C}$-O-$CH_2$, C-6), 163.9 ($\underline{C}$-O-$CH_2$, C-8), 166.5 (C-4*a*), 167.5 (C=O, C-*b*), 197.4 (C=O, C-1), 206.6 (C=O, C-1′), 212.1(C=O, C-3); HR-ESI-TOF-MS *m/z* 551.2615 $[M+Na]^+$ (calcd for $C_{30}H_{40}NaO_8$, 551.2620)

**Compound 9.** IR $\nu_{max}$ 2955, 2877, 1763, 1711, 1648, 1611, 1590, 1463, 1383, 1279, 1163, 1126, 1093, 1036, 1000, 964, 922, 853, 766, 713, 632, 589 cm$^{-1}$; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.71 (*d*, *J* = 6.0 Hz, 3H, H-3″), 0.81 (*d*, *J* = 6.0 Hz , 3H, H-4″), 0.92 (*d*, *J* = 6.7 Hz, 6H, H-4′, H-5′), 1.24 (*m*, 6H, H-d, H-*d*′), 1.30 (*s*, 3H, H-10), 1.33 (*s*, 3H, H-11), 1.35 (*overlapping,* 3H, H-1″, H-2″), 1.38 (*s*, 3H, H-12), 1.49 (*s*, 3H, H-13), 2.15 (*m*, 1H, H-3′), 2.73 (*m*, 2H, H-2′), 4.14 (*t*, *J* = 5.5 Hz, 1H, H-9), 4.20 (*m*, 4H, H-*c*, H-*c*), 4.44 (*m*, 2H, H-*a'*), 4.57 (*s*, 2H, H-*a*), 6.32 (*s*, 1H, H-5), $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.1 (2$CH_3$, C-*d*, C-*d*′), 22.5 ($CH_3$, C-4′), 22.6 ($CH_3$, C-5′), 23.2 ($CH_3$, C-3″), 23.2 ($CH_3$, C-4″), 23.9 (CH, C-2″), 24.5 (2$CH_3$, C-10, C-11), 24.8 (2$CH_3$, C-12, C-13), 24.9 (CH, C-3′), 26.0 (2CH, C-9), 46.9 ($CH_2$, C-1″), 47.2 (C-4), 53.7 ($CH_2$, C-2′), 56.0 (C-2), 61.2 ($CH_2$, C-*c*′), 61.6 ($CH_2$, C-*c*), 65.7 ($CH_2$, C-*a*′), 72.5 ($CH_2$, C-*a*), 97.0 (CH, C-5), 113.3 (C-8*a*), 114.1 (C-7), 123.4 (C-9*a*), 152.6 (C-5*a*), 153.3 ($\underline{C}$-O-$CH_2$, C-6), 154.0 ($\underline{C}$-O-$CH_2$, C-8), 167.2 (C-4*a*), 167.9 (2(C=O), C-*b*, C-*b*′), 197.3 (C=O, C-1), 203.6 (C=O, C-1′), 212.0 (C=O, C-3); HR-ESI-TOF-MS *m/z* 637.2938 $[M+Na]^+$ (calcd for $C_{34}H_{46}NaO_{10}$ 637.2988.

### *2.1.8 Base-catalysed hydrolysis of derivative **9***

Rhodomyrtone derivative **9** (200 mg, 0.325mmol) was dissolved in MeOH (5 mL) and stirred. A solution of litium hydroxide (LiOH) in THF: MeOH: $H_2O$ (3:2:1) was added. The reaction mixture was stirred at room temperature for 3 h. Water (100 mL) was added and extracted with EtOAc (3x100 mL). The organic layer was combined, dried over sodium sulphate anhydrous ($Na_2SO_4$) and concentrated *in vacuo*. The crude residue was purified by column chromatography to obtain acid **10** as colorless oil (82 mg, 90 %). IR $\nu_{max}$ 3367, 3260, 2952, 2828, 1789, 1593, 1456, 1386, 1264, 1174, 1097, 1037, 837, 749 cm$^{-1}$; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.75 (*d, J = 6.0,* 3H, H-3″), 0.84 (*d*, *J = 6.0,* 3H, H-4″), 0.95 (*d*, *J* = 6.7 Hz, 6H, H-4′, H-5′), 1.28 (*s*, 3H, H-10), 1.33 (*s*, 3H, H-11), 1.37 *(overlapping*, 3H, H-1″, H-2″), 1.42 (*s*, 3H, H-12), 1.53 (*s*, 3H, H-13), 2.19 (*m*, 1H, H-3′), 2.75 (*m*, 2H, H-2′), 4.18 (*t*, *J* = 5.5 Hz, 1H, H-9), 4.49 (*q*, 2H, H-*a*′), 4.61 (*s*, 2H, H-*a*), 6.34(*s*, 1H, H-5); $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 22.5 ($CH_3$, C-4′), 22.6 ($CH_3$, C-5′), 23.1 ($CH_3$, C-3″), 23.2 ($CH_3$, C-4″), 23.9 (CH, C-2″), 24.3 ($CH_3$, C-10), 24.5($CH_3$, C-11), 24.8 (2$CH_3$, C-12, C-13), 24.9 (CH, C-3′), 26.0 (2CH, C-9), 47.1 ($CH_2$, C-1″), 47.2 (C-4), 53.8 ($CH_2$, C-2′), 56.0 (C-2), 65.7 ($CH_2$, C-*a*′), 72.4 ($CH_2$, C-*a*), 97.1 (CH, C-5), 113.4 (C-8*a*), 114.4 (C-7), 123.5 (C-9*a*), 152.6 (C-5*a*), 153.4 (C-O-$CH_2$, C-6), 154.0 (C-O-$CH_2$, C-8), 167.2 (C-4*a*), 168.3 (2(C=O), C-*b*, C-*b*′), 197.4 (C=O, C-1), 203.5 (C=O, C-1′), 212.0 (C=O, C-3); HRMS (ESI-TOF) m/z 581.2357 $[M+Na]^+$ (calcd for $C_{30}H_{38}NaO_{10}$, 581.2362)

### *2.1.9 Synthesis of rhodomyrtone derivative **11***

Rhodomyrtone derivative **10** (50 mg. 0.089 mmol) was dissolved in DMF (3 mL) and stirred. A solution of *N*-(2-aminoethyl)ethanolamine (18.6 mg, 0.179 mmol) and HBTU (33.9 mg, 0.089 mmol) in DMF (2 mL) was added. TEA (18.1 mg, 0.179 mmol) was then added and continued stirring at room temperature for 4 h. Water (100 mL) was added and extracted with EtOAc (3×100 mL). The organic layer was combined, dried over sodium sulphate anhydrous ($Na_2SO_4$) and concentrated *in vacuo*. The crude residue was purified by column chromatography to yield rhodomyrtone derivative **11** as colorless oil (55 mg, 84%). IR $\nu_{max}$ 3307, 3079, 2951, 2871, 1790, 1642, 1542, 1463, 1442, 1383, 1365, 1276, 1164, 1115, 1087, 1044, 959, 843, 746 $cm^{-1}$; $^1H$ NMR (400 MHz, $CD_3OD$) δ 0.79 (*d*, *J* = 6.0 Hz, 3H, H-3″), 0.90 (*d*, *J* = 6.0 Hz, 3H, H-4″), 0.99 (*d*, *J* = 6.7 Hz, 6H, H-4′, H-5′), 1.34 (*s*, 3H, H-10), 1.37 (*s*, 3H, H-11 ), 1.44 (*overlapping*, 3H, H-1″, H-2″), 1.47 (*s*, 3H, H-12), 1.57 (*s*, 3H, H-13), 2.18 (*m*, 1H, H-3′), 2.87 (*m*, 4H, $CONHCH_2\underline{CH_2}NH\underline{CH_2}CH_2OH$), 2.93 (*m*, 4H, $CONHCH_2\underline{CH_2}NH\underline{CH_2}CH_2OH$), 3.00 (*overlapping*, 2H, H-2′), 3.48 (*m*, 2H, $CONH\underline{CH_2}CH_2NH$),3.53 (*m*, 2H, $CONH\underline{CH_2}CH_2NH$), 3.69 (*m*, 2H, $NHCH_2\underline{CH_2}OH$), 3.72 (*m*, 2H, $NHCH_2\underline{CH_2}OH$), 4.21 (*t*, *J* = 5.5 Hz, 1H, H-9), 4.40 (*s*, 2H, H-*a*′), 4.67 (*s*, 2H, H-*a*), 6.81 (*s*, 1H, H-5), 7.26 (*m*, 1H, $CONHCH_2CH_2\underline{NH}$), 7.69 (*t*, *J* = 7.4 Hz, 1H, $CO\underline{NH}CH_2CH_2NH$), $^{13}C$ NMR (100 MHz, $CD_3OD$): δ 25.5 (2$CH_3$, C-4′, C-5′), 26.1($CH_3$, C-4″), 26.4 (*$CH_3$*, C-3″), 26.8 ($CH_3$, C-10), 27.4 ($CH_3$, C-12), 27.8 (CH, C-2″), 28.1 ($CH_3$, C-13), 28.2 (*CH*, C-3′), 28.7 ($CH_3$, C-11), 29.8 (CH, C-9), 41.2 ($CH_2$, $CH_2CONH\underline{CH_2}CH_2NH$), 41.4 ($CH_2$, $CH_2CONH\underline{CH_2}CH_2NH$), 50.6 (C-4, $CH_2$, C-1″), 51.6 (*$CH_2$*, $CONHCH_2\underline{CH_2}NH$), 52.72 (*$CH_2$*, $CH_2CONHCH_2\underline{CH_2}NH$), 54.2 (*$CH_2$*, $NH\underline{CH_2}CH_2OH$), 54.2 (*$CH_2$*, $NH\underline{CH_2}CH_2OH$), 57.3 ($CH_2$, C-2′), 59.6 (C-2), 62.8 ($CH_2$, $NHCH_2\underline{CH_2}OH$), 63.0 ($CH_2$, $NHCH_2\underline{CH_2}OH$), 71.8 ($CH_2$, C-*a*′), 77.8 ($CH_2$, C-*a*), 102.3 (CH, C-5), 116.9 (C-8*a*), 118.0 (C-7), 126.7 (C-9*a*), 156.8 (C-5*a*), 157.3 ($\underline{C}$-O-$CH_2$, C-6), 158.7 ($\underline{C}$-O-$CH_2$, C-8), 171.9 (C-4*a*), 173.2 (2(C=O), C-*b*′, C-*b*), 201.8 (C=O, C-1), 208.2 (C=O, C-1′), 215.5 (C=O, C-3); HRMS (ESI-TOF) m/z 753.4045 $[M+Na]^+$ (calcd for $C_{38}H_{58}N_4NaO_{10}$, 753.4050).

### *2.1.10 Synthesis of rhodomyrtone derivative **12***

The synthesis of derivative **12** was carried out in the same manner as the synthesis of **11** except 1,1-dimethylethylenediamine was used instead of *N*-(2-aminoethyl)ethanolamine. After chromatographic separation, derivative **12** was obtained as colorless oil (56.5 mg, 87%); IR $\nu_{max}$ 3318, 2919, 2850, 1720, 1621, 1463, 1430, 1389, 1342, 1305, 1258, 1166, 1111, 1042, 1010, 886, 848, 757 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) δ 0.73 (*d*, *J* = 6.0 Hz, 3H, H-3″), 0.86 (*d*, *J* = 6.0 Hz, 3H, H-4″), 0.95 (*d*, *J* = 6.7 Hz, 6H, H-4′, H-5′), 1.33 (*s*, 3H, H-10), 1.37 (*s*, 3H, H-11), 1.39 (*overlapping*, 3H, H-1″, H-2″), 1.43 (*s*, 3H, H-12), 1.55 (*s*, 3H, H-13), 1.68 (*m*, 2H, $NHCH_2\underline{CH_2}CH_2N(CH_3)_2$), 1.78 (*m*, 2H, $NHCH_2\underline{CH_2}CH_2N(CH_3)_2$), 2.14 (*s*, 6H, $NHCH_2CH_2CH_2N(\underline{CH_3})_2$), 2.19 (*overlapping*, 1H, H-3′), 2.30 (*s*, 6H, $NHCH_2CH_2CH_2N(\underline{CH_3})_2$), 2.37 (*m*, *overlapping*, 2H, $NHCH_2CH_2\underline{CH_2}N(CH_3)_2$), 2.48 (*overlapping*, 2H, $NHCH_2CH_2\underline{CH_2}N(CH_3)_2$), 2.70 (*m*, 2H, H2′), 3.39 (*overlapping*, 2H, $NH\underline{CH_2}CH_2CH_2N(CH_3)_2$), 3.44 (*overlapping*, 2H,

$\mathrm{NH\underline{CH}_2CH_2CH_2N(CH_3)_2}$), 4.12 (*t*, *J* = 5.5 Hz, 1H, H-9), 4.33 (*q*, 2H, H-*a'*), 4.51 (*d*, *J* = 2.7 Hz, 2H, H-*a*), 6.47 (*s*, 1H, H-5), 7.41 (*t*, *J* = 5.6 Hz, 1H, $\mathrm{\underline{NH}CH_2CH_2CH_2N(CH_3)_2}$), 7.72 (*t*, *J* = 5.2 Hz, 1H, $\mathrm{\underline{NH}CH_2CH_2CH_2N(CH_3)_2}$); $^{13}$C NMR (100 MHz, $\mathrm{CDCl_3}$) δ 22.6 ($\mathrm{2CH_3}$, C-4′, C-5′), 23.1 ($\mathrm{2CH_3}$, C-3″, C-4″), 23.5 (CH, C-2″), 24.2 ($\mathrm{CH_3}$, C-10), 24.5 ($\mathrm{CH_3}$, C-11), 24.6 ($\mathrm{CH_3}$, C-12), 24.8 ($\mathrm{CH_3}$, C-13), 24.9 (CH, C-3′), 26.0 (CH, C-9), 26.1 ($\mathrm{CH_2}$, $\mathrm{CH_2CH_2\underline{C}H_2N(CH_3)_2}$), 26.4 ($\mathrm{CH_2}$, $\mathrm{CH_2CH_2\underline{C}H_2N(CH_3)_2}$), 38.1 ($\mathrm{CH_2}$, $\mathrm{\underline{C}H_2CH_2CH_2N(CH_3)_2}$), 38.6 ($\mathrm{CH_2}$, $\mathrm{\underline{C}H_2CH_2CH_2N(CH_3)_2}$), 45.0 ($\mathrm{CH_3}$, $\mathrm{CH_2CH_2CH_2N(\underline{C}H_3)_2}$), 45.2 ($\mathrm{CH_3}$, $\mathrm{CH_2CH_2CH_2N(\underline{C}H_3)_2}$), 47.3 (C-4, $\mathrm{CH_2}$, C-1″), 53.9 ($\mathrm{CH_2}$, C-2′), 56.0 (C-2), 57.6 ($\mathrm{CH_2}$, $\mathrm{CH_2\underline{C}H_2CH_2N(CH_3)_2}$), 57.9 ($\mathrm{CH_2}$, $\mathrm{CH_2\underline{C}H_2CH_2N(CH_3)_2}$), 68.4 ($\mathrm{CH_2}$, C-*a*′), 74.2 ($\mathrm{CH_2}$, C-*a*), 98.2 (CH, C-5), 113.0 (C-8*a*), 114.2 (C-7), 123.5 (C-9*a*), 152.8 (C-5*a*), 153.3 ($\mathrm{\underline{C}\text{-}O\text{-}CH_2}$, C-6), 154.2 ($\mathrm{\underline{C}\text{-}O\text{-}CH_2}$, C-8), 167.0 (C-4*a*), 167.3 (C=O, C-*b*), 167.6 (C=O, C-*b*′), 197.3 (C=O, C-1), 203.0 (C=O, C-1′), 211.7 (C=O, C-3); HRMS (ESI-TOF) m/z 727.4601 $[\mathrm{M+H}]^+$ (calcd for $\mathrm{C_{40}H_{63}N_4O_8}$ 727.4646).

### *2.2 Bacterial strain and culture condition*

*Staphylococcus aureus* ATCC 25923 was used in this study. The bacterial strain was cultured on tryptic soy agar (TSA) and incubated at 37°C for 24 h. The bacterial culture was stored in Mueller-Hilton broth (MHB) containing 40% glycerol at -80°C until use.

### *2.3 Determination of minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC)*

Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of the derivatives and parent compound were determined by a broth micro-dilution method according to the Clinical and Laboratory Standards Institute (CLSI). Briefly, the two-fold dilutions of

MTT (2 mg/mL) in 90 µL of media was added to each well. After incubation at 37 °C for 4 h, the MTT solution was removed and replaced with 100 µL of DMSO. The absorbance of the solution was measured at 550 nm on a microplate reader (Thongbamrer *et al.* 2021).

the derivatives and parent compound were prepared. A suspension of *S. aureus* in MHB was prepared from the overnight broth culture and adjusted to McFarland No. 0.5 (approximately 1.5 × $10^8$ CFU/mL). The bacterial suspension was then diluted to the final bacterial cell concentration of 1 × $10^6$ CFU/mL. The bacterial suspension (100 µL) was mixed with the diluted test compound (100 µL) in a 96-well flat-bottom microtiter plate. The final concentration of the derivatives was ranging from 0.5 to 64 µg/mL except parent compound was ranging from 0.125 to 16 µg/mL. Untreated bacterial suspension and vancomycin were used as negative and positive control, respectively. Then microtiter plates were incubated at 37 °C for 16-18 h. MIC was recorded as the lowest concentration that produced a complete suppression of visible growth. To determine the MBC, aliquots (10 µL) of the broth with no visible growth were plated onto TSA and incubated in the same conditions. MBC was defined as the lowest concentration of fractions that completely prevent bacterial growth. The experiments were performed in triplicate.

### *2.4 Cytotoxicity assay*

The cytotoxicity of the derivatives and parent compound against HeLa, PC3, H460, and HEK293T cell lines was evaluated by MTT assay. The cells were seeded in a 96-well plate at a density of $1.0\times10^4$ cells/well and incubated at 37 °C under 5% $\mathrm{CO_2}$ for 24 h until reaching 70–80% confluent. The media were replaced with fresh DMEM (for HEK293T and HeLa) or RPMI1640 (for PC3 and H460) media. The derivatives and parent compound were added to the cells and incubated at 37 °C under 5% $\mathrm{CO_2}$ for 4 h. The media was replaced with fresh medium containing 10% FBS for 48 h. The media was removed and 10 µL of

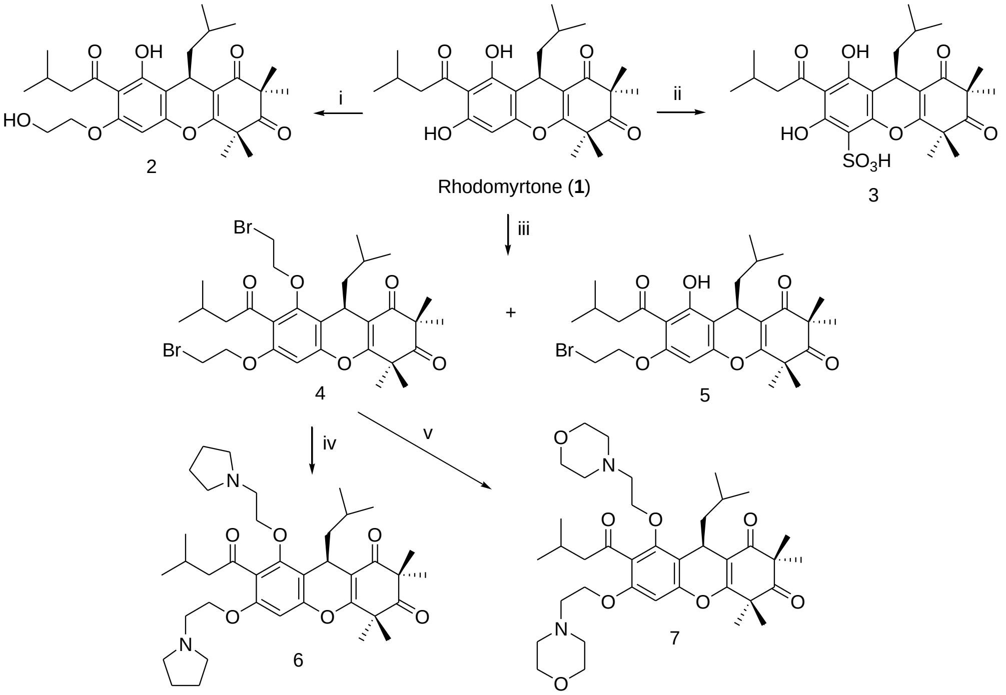
## 3. Results and Discussion

### 3.1 Chemistry

Rhodomyrtone (**1**) which was isolated from *R. tomentosa* exhibited interesting biological activities including anti-bacterial, anti-caries, antibiofilm formation, anti-inflammatory, antidepressant and anti-tumor activities. Rhodomyrtone (**1**) was subjected for structural modification and some of the derivatives exhibited comparable antibacterial activity to the parent compound. However, rhodomyrtone derivatives have not been evaluated for their anticancer activity. In order to explore the structure-activity relationship, new derivatives **2**–**12** were synthesized and tested for their antibacterial and anticancer activities.

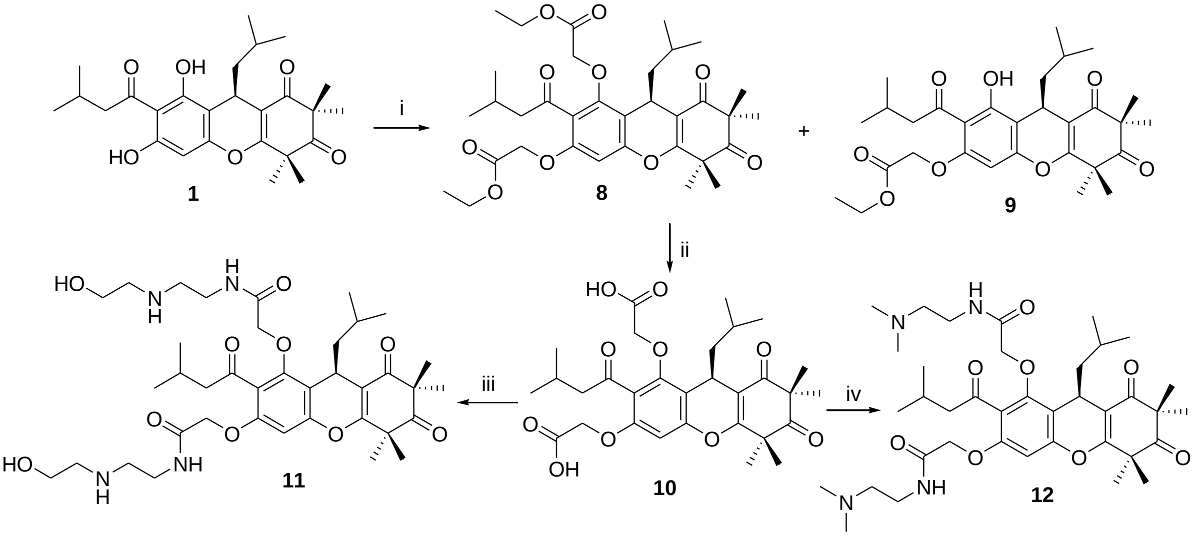
The derivatives **2** and **3** were synthesized as shown in Scheme 1. Rhodomyrtone (**1**) was reacted with bromoethanol in DMF in the presence of $K_2CO_3$ to afford derivative **2** in moderate yield. The spectroscopic ($^1$H NMR and HRMS) data obtained were consistent with the structure **2**. Thus the presence of methylene proton signals at $\delta$ 4.01 and 4.17 ppm was observed. Derivative **3** was successfully prepared by treating **1** with sulfur trioxide pyridine complex in DMF. However, the product was obtained in low yield. The addition of $SO_3H$ at C-5 position was evident from the absence of H-5 signal at $\delta$6.05 ppm. The HR-TOFMS (ES$^+$) showed a pseudomolecular ion [M+2Na]$^+$ at *m/z* 567.1614, compatible with the molecular $C_{26}H_{33}Na_2O_9S$.

The derivatives **6** and **7** were synthesized from the intermediate **4** as shown in Scheme 1. The compound **4** together with **5** were obtained by reacting rhodomyrtone (**1**) with 1,2-dibromoethane using $K_2CO_3$ as a base in DMF. Compound **4** was reacted with pyrrolidine in the presence of $K_2CO_3$ as a base to generate derivative **6**. The HR-TOFMS (ES$^+$) of **6** showed a pseudomolecular ion [M+H]$^+$ at *m/z* 637.4269 which was consistent with the molecular formula of $C_{38}H_{57}N_2O_8$. Derivative **7** was also synthesized in the same manner as **6** except morpholine was used instead of pyrrolidine. The molecular formula of **7** was established as $C_{38}H_{57}N_2O_8$ by HR-TOFMS (positive ion mode, m/z 669.4109 [M+H]$^+$). The $^1$H NMR pattern of **6** was similar to those of **7**, except for the methylene proton signals of pyrrolidine and morpholine.



**Scheme 1.** Reagents and conditions: i) 1-bromoethanol, $K_2CO_3$, 80℃, DMF, 8 h.; ii) sulfur trioxide pyridine complex, DMF, rt, 48 h.; iii) 1,2-dibromoethane, $K_2CO_3$, DMF, 80℃, 8 h.; iv) pyrrolidine, $K_2CO_3$, DMF, rt, 4h.; v) morpholine, $K_2CO_3$, DMF, rt, 4h.

Rhodomyrtone derivatives **11** and **12** having nitrogen containing functionality were also synthesized as shown in Scheme 2. Rhodomyrtone (**1**) was reacted with ethyl chloroacetate using $K_2CO_3$ as a base in DMF afforded compounds **8** and **9**. The HR-TOFMS ($ES^+$) of **8** showed pseudomolecular ion $[M+Na]^+$ at *m/z* 637.2938, compatible with the molecular formula of $C_{34}H_{46}NaO_{10}$. The IR absorption band at 1763 $cm^{-1}$ indicated the presence of ester group. Assignments of the $^1H$ and $^{13}C$ NMR data were achieved by 2D techniques. The presence of the ethoxy function was evident from the multiplet signals at $\delta$ 4.20 ($OC\underline{H}_2CH_3$) and $\delta$ 1.24 ($OCH_2C\underline{H}_3$) in the $^1H$ NMR spectrum, which corresponded to the $^{13}C$ NMR at $\delta$ 61.2 and 61.6 ($O\underline{C}H_2CH_3$) and $\delta$ 14.1 ($OCH_2\underline{C}H_3$), respectively. Compound **8** was then subjected to base-catalyzed hydrolysis to furnish acid **10** in excellent yield. The structure of the obtained product was confirmed by spectroscopic means (IR, NMR and HR-TOFMS). This compound was subjected to couple with *N*-(2-aminoethyl)ethylamine using HBTU as coupling agent to give rhodomyrtone derivative **11** in 84% yield. Compound **12** was also synthesized in the same manner as compound **11** except 1,1-dimethylethylenediamine was used instead of N-(2-aminoethyl)ethylamine. The $^1H$ NMR spectral data was in agreement with the structures of rhodomyrtone derivatives **11** and **12**. The HR-TOFMS ($ES^+$) of **11** showed pseudomolecular ion $[M+Na]^+$ at *m/z* 753.4045, compatible with the molecular formula of $C_{38}H_{58}N_4NaO_{10}$, whereas, the pseudomolecular ion $[M+H]^+$ at m/z 727.4601 of **12** was consistent with the molecular formula of $C_{40}H_{63}N_4O_8$.



**Scheme 2**. Reagents and conditions: i) 1-ethyl chloroacetate, $K_2CO_3$, DMF, rt, 8 h.; ii) LiOH, THF: MeOH: $H_2O$ (3:2:1), rt, 3h.; iii) *N*-(2-aminoethyl)ethanolamine, TEA, HBTU, DMF, rt, 4h.; iv) 1,1-dimethylethylenediamine, TEA, HBTU, DMF, rt, 4h.

## 3.2 Biological activity

Rhodomyrtone (**1**) and derivatives **2-12** were assayed for their antibacterial potency using a broth micro-dilution method and cytotoxicity using MTT assay. Rhodomyrtone (**1**) demonstrated good activity against Gram-positive bacteria (*S. aureus ATCC 29213*) with minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of 0.55 µM and 1.1 µM, respectively. All of the derivatives showed lower activity than that of the parent compound. Among the derivatives, compound **11** exhibited highest antibacterial activity with MIC/MBC values of 47.56/47.56 μM. However, it was much lower activity than rhodomyrtone (**1**).

Rhodomyrtone (**1**) and its derivatives were evaluated for their anticancer activity against HeLa, H460, PC3 and HEK293T cells (Table 1). Rhodomyrtone (**1**) showed anticancer activity against HeLa, H460, PC3 with $IC_{50}$ values of 21.16, 6.12 and 13.30 μM, respectively. However, it was toxic to the HEK293T cells. Derivatives **8** and **9** exhibited greater cytotoxic activity than the parent compound.

**Table 1.** Anticancer activity of rhodomyrtone and derivatives

| **Compound** | **$IC_{50}$ (□M)** | | | |
|---|---|---|---|---|
| | **HeLa** | **H460** | **PC3** | **HEK293T** |
| **1** | 21.16 | 6.12 | 13.30 | 3.11 |
| **2** | 54.99 | 43.42 | 53.78 | 28.78 |
| **3** | 25.41 | 20.22 | 50.37 | 17.82 |
| **4** | ND | ND | ND | ND |
| **5** | ND | ND | ND | ND |
| **6** | ND | ND | ND | ND |
| **7** | ND | ND | ND | ND |
| **8** | 17.12 | 28.30 | 34.66 | 17.87 |
| **9** | 13.79 | 18.14 | 43.37 | 20.51 |
| **10** | ND | ND | ND | ND |
| **11** | 50.41 | 39.34 | 41.69 | 34.91 |
| **12** | 27.89 | 25.00 | 37.13 | 28.72 |
| Doxorubicin* | 0.28 | 3.12 | 1.59 | 0.64 |

*** Standard drug for anticancer activity test, ND=Not Detect**

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