DESIGN, SYNTHESIS AND EVALUATION OF ACYL DERIVATIVES OF HYDROXYLATED EUDESMANOLIDES AS ANTI-COLORECTAL CANCER AGENTS

Rawinnipha WETTAYANUGUL[1] and Khanitha PUDHOM[1*]
1 Faculty of Science, Chulalongkorn University, Thailand; rawinnipha_1997@hotmail.com (R. W.); Khanitha.P@chula.ac.th (K. P.) (Corresponding Author)

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ABSTRACT
Colorectal cancer (CRC) is the third most common cancer worldwide. Due to side effects of current cancer therapies, the development of therapeutic colorectal cancer agents with safer and more effective are urgently required. Acyls of hydroxylated eudesmanolides, RNS **(2)** and STM **(3)** were reported to possess diverse bioactivities, especially anti-cancer activity. This study aimed to synthesize acyl derivatives, RNS **(2)** and STM **(3)** and to evaluate their cytotoxicity against human colorectal HCT-116 cancer cells. The hydroxylated eudesmanolide, a $\Delta^{3,4}$ double bond containing STM **(3)**, showed slightly more potent than RNS **(2)**, a $\Delta^{4,15}$ double bond derivative, with IC_{50} values 15.41 and 18.17 μM, respectively. Among acyl derivatives, compound **3d** showed the most potent activity against HCT-116 cells with IC_{50} value of 13.51 μM, as well as slightly stronger than parent compound. Additionally, the results obtained beneficial SAR information for further study. Benzoyl derivatives, **2d** and **3d**, displayed stronger cytotoxicity than cinnamoyl derivative series (**a-c**), indicating the C2 extension between benzene ring and carbonyl moiety caused the decrease in activity. It was also seen that the existence of *p*-nitro group on benzene ring and the double bond hydrogenation of cinnamoyl moiety decreased activity compared to original cinnamoyl derivatives (**2a** and **3a**)
Keywords: Acyl Derivatives, Hydroxylated Eudesmanolides, Anti-Colorectal Cancer, Cytotoxicity

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INTRODUCTION

Cancer is one of the leading causes of death worldwide characterized by uncontrolled growth and spread of abnormal cells. Among cancers, colorectal cancer (CRC) is the third most common cancer worldwide. In 2020, more than 1.9 million new CRC cases with the 935,000 deaths have been reported, as well as the mortality rate is estimated to increase up to 60% by the year 2030 (Yenuganti, 2021). Conventional methods to treat CRC include chemotherapy, radiotherapy and surgery,however, chemotherapy is a major approach for the treatment. Nevertheless, most current chemotherapeutic drugs not only kill cancer cells but also damage normal cells, with the cause of severe side effects to the patients (Huang, 2018). Therefore, anti-colorectal cancer agents with safer and more effective still need to be developed.

Costunolide, a naturally well-known sesquiterpene lactone, presents in many plant species, particularly in the root of *Saussurea lappa* (Okugawa, 1996). It was reported to possess broad biological activities such as anti-ulcer, anti-microbial, anti-inflammatory, anti-melanogenic and anti-cancer activities (Choi, 2008; Koch, 2001; Luna-Herrea, 2007; Rasul, 2012; Yamahara, 1985). Based on these pharmacological effects, costunolide was received considerable attention from medicinal chemists. However, the problem is that costunolide is easily degraded and difficult to handle. Transformation of costunolide to its derivatives like hydroxylated eudesmanolides, reynosin (RNS) and santamarine (STM), could be considered as one of the approaches to solve this problem. Although some publications reported that bioactivities of these derivatives, acyl derivatives of eudesmonolides played a potential role to boost their activity, particularly anti-cancer activity due to its high diffusion coefficient, their inhibition against CRC has not been investigated (Mejías, 2021; Wang, 2021). Therefore, the current research focuses on design and synthesis of acyl derivatives of hydroxylated eudesmanolides, both RNS **(2)** and STM **(3)**. All synthesized compounds were evaluated their anti-cancer activity against human colorectal HCT-116 cancer cells compared with parent compounds.

LITERATURE REVIEWS

1) Colorectal cancer

Colorectal (CRC) is the third most leading cancer in both males and females worldwide which occurs in the colon or rectum (Gad, 2022). According to World Health Organization (WHO), 1.93 million cases and nearly 1 million deaths due to colon and rectal cancer were reported in the year 2020 (Biradar, 2023). The new cases and CRC-associated deaths were predicted to increase to 2.2 million and 1.1 million by 2030, respectively (Arnold, 2017). Various factors, including alcohol over-consumption, smoking, obesity, diabetes, lack of physical exercise and intestinal microbiota disorder, were reported to play an important role in increasing CRC risk (Dai, 2023). There are some available methods to treat CRC including chemotherapy, radiotherapy and surgery, but these methods have severe effects and toxicity. Among all treatments, chemotherapy is the main method for treatment of CRC, although it damages both cancer and normal cells (Huang, 2018). Therefore, developing low side-effect and more effective anti-cancer agents with natural products is one of interesting options.

2) Costunolide and its derivatives with anti-cancer activity

Costunolide, a natural germacranolide type sesquiterpene lactone, presents in many plant species (Rasul, 2012), particularly in the root of *S. lappa* of Asteraceae family. (Okugawa, 1996). Its chemical structure consists of α-methylene-γ-lactone functional group **(Figure 1)**, a significant characteristic of most sesquiterpene lactones, which is required for bioactivities (Li, 2020). This compound was reported to possess broad biological activities including anti-cancer, anti-ulcer, anti-microbial, anti-inflammatory and anti-melanogenic activities (Choi, 2008; Koch, 2001; Luna-Herrea, 2007; Rasul, 2012; Yamahara, 1985). However, costunolide is easily degraded and difficult to handle, when compared to its hydroxylated eudesmanolide

derivatives, especially RNS and STM **(Figure 1)**. The hydroxylated compounds could be obtained by one-pot reaction from costunolide by a cyclization reaction in the presence of the epoxidation agent *m*-CPBA followed by the addition of a catalytic amount of *p*-TsOH or $BF_3 \cdot OEt_2$ to open the epoxy ring (Choodej, 2018; Mejías, 2021).

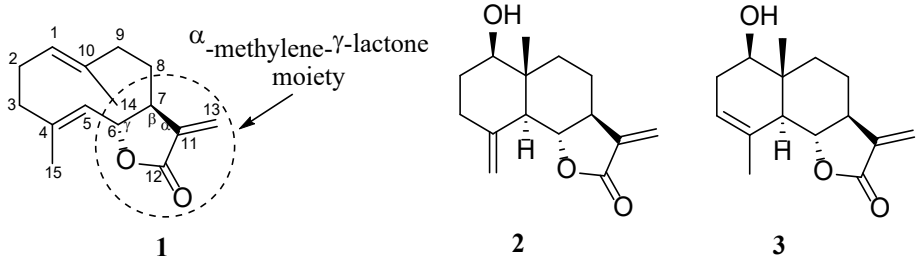

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Figure 1 Chemical structures of costunolide **(1)** and hydroxylated eudesmanolides, RNS **(2)** and STM **(3)**

Very recently (2020) Mejías, F.J. and co-workers revealed that acyl derivatives of eudesmonolides showed potential to boost their anti-cancer activity, particularly aryl derivatives. They were more active than a current anti-cancer drug, etoposide, in terms of both selectivity and activity. Furthermore, computational studies gave a support that the lipophilicity of the compounds played an important role for activity by making them pass easily through the cell membrane. The most potent compound was 4-fluorobenzylreynosin **(4) (Figure 2)** with IC_{50} value of 2.85 μM toward cervical carcinoma (HeLa) cells (Mejías, 2021).

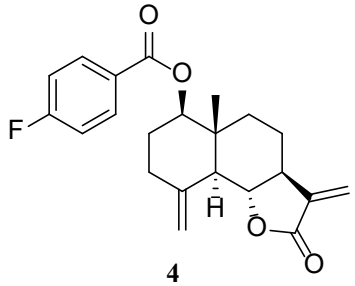

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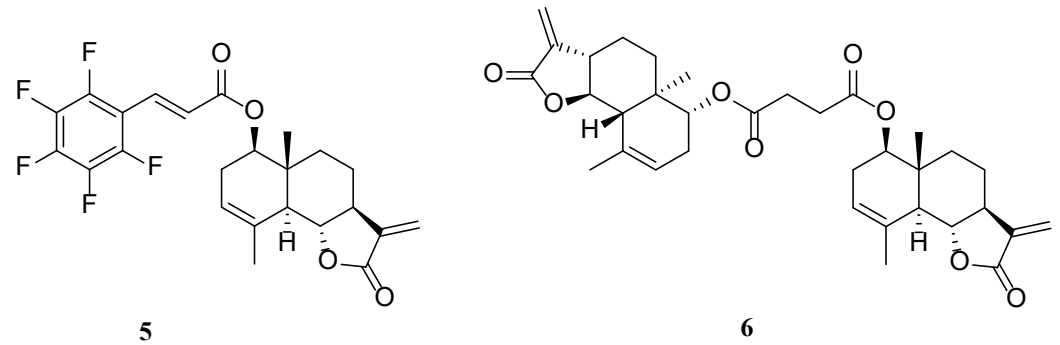
Figure 2 Chemical structure of 4-fluorobenzylreynosin **(4)**

In addition, Wang and colleagues (2021) synthesized a series of ester derivatives of STM and investigated their anti-fibrotic activity. Refer to this work, 1-*O*-(2', 3', 4', 5', 6'-pentafluorocinnamyl)santamarine **(5)** and disantamarine-yl-succinate **(6) (Figure 3)** showed potent cytotoxicity against human hepatic stellate cell lines (HSC-LX2) with IC_{50} values of 4.6 and 3.5 μM, respectively (Wang, 2021).



5 **6**

Figure 3 Chemical structures of 1-*O*-(2', 3', 4', 5', 6'-pentafluorocinnamyl) santamarine **(5)** and disantamarine-yl-succinate **(6)**

MATERIALS AND METHODS

1) Materials and instruments

Solvents and reagents were analytical, AR grade or purified according to standard methods. 3-Chloroperoxybenzoic acid (*m*-CPBA), Na_2SO_4, NaOH, $NaHCO_3$ and 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI·HCl) were purchased from Merck KGaA, Germany, NaCl, AR. Benzene and Conc. HCl were purchased from CARLO ERBA Reagents S. A. S.,Italy, A.R. Chroloform ($CHCl_3$) and dichloromethane (CH_2Cl_2) were obtained from RCI Labscan limited, Thailand. Boron trifuoride-ethtyl- ether ($BF_3 \cdot OEt_2$), *trans*-cinnamic acid, 3-phenylpropionic acid, 4-nitrocinnamic acid and benzoic acid were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Human colorectal HCT-116 cancer cell line was purchased from American Type Culture Collection (Manassas, Virginia, United States). Fetal bovine serum (FBS), and Dulbecco's Modified Eagle's Medium (DMEM) were obtained from Sigma-Aldrich (St. Louis, Missouri, United States). Nuclear magnetic resonance (NMR) spectra were measured in chloroform-d ($CDCl_3$) and using JEOL 500 and 125 MHz NMR (JEOL, Tokyo, Japan) SilicaFlash® P60 was purchased from SILICYCLE Inc. UltraPure SILICA GELS, Canada. Analytical thin layer chromatography (TLC) was performed on pre-coated silica gel 60 F254 aluminium plate (Merck, German).

2) Plant material

S. lappa roots were purchased from a dispensary pharmacy (Chao Krom Poe) at Chakkrawat, Samphanthawong, Bangkok province, Thailand, in June 2021.

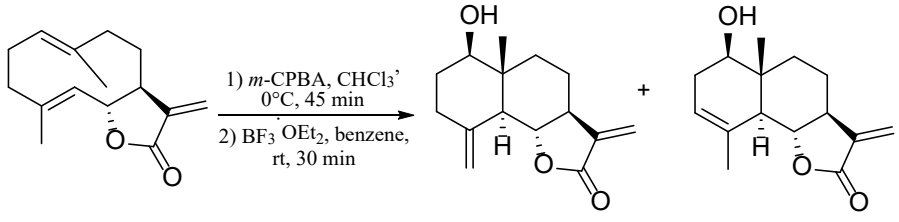
3) Isolation and purification of costunolide (1) from *S. lappa* roots

Air-dried roots of *S. lappa* were powdered (1kg) and extracted three times with *n*-hexane. The solvent was removed under reduced pressure to give the hexane extract. Costunolide **(1)** was isolated from the hexane crude extract by normal phase silica gel column chromatography with a 9:1 mixture of hexane and EtOAc. Its structure was identified by NMR spectroscopy and confirmed by comparing NMR data with the previously reported (Li, 2005).

Costunolide **(1)**; colorless crystals, 2.18 g, 4.35% yield. ^{1}H NMR data (600 MHz, $CDCl_3$) ; δ_H 6.23 (1H, d, J = 6.0 Hz, H-13), 5.50 (1H, d, J = 12.0 Hz, H-13), 4.82 (1H, dd, J = 12.0, 6.0 Hz, H-1), 4.71 (1H, d, J = 12.0 Hz, H-5), 4.54 (1H, t, J = 12.0 Hz, H-6), 2.55 (1H, m, H-7), 1.41 (3H, s, H-14), 1.66 (3H, s, H-15). ^{13}C NMR data (125 MHz, $CDCl_3$); δ_C 170.5 (C-12), 141.5 (C-4), 140.2 (C-11), 137.0 (C-10), 127.4 (C-5),127.1 (C-1), 119.7 (C-13), 82.0 (C-6), 50.0 (C-7), 17.4 (C-15), 16.2 (C-14) (Li, 2005).

4) Synthesis of hydroxylated eudesmanolides, reynosin (RNS) and santamarine (STM)

Costunolide (**1,** 500 mg, 2.15 mmol) and *m*-CPBA (557.40 mg, 3.23 mmol) was dissolved in dry $CHCl_3$ and stirred at 0°C under nitrogen for 45 min. The reaction mixture was poured into cold water and then extracted with EtOAc, washed with brine, and dried with Na_2SO_4. After removal of solvent, the residue was passed through a short silica gel column (*n*-hexane: EtoAc, 7:3) to give an intermediate. The intermediate was treated with $BF_3 \cdot OEt_2$ in benzene at room temperature. After 30 min, the reaction mixture was poured into cold water and extracted with EtOAc. The organic layer was washed with 5% aqueous $NaHCO_3$, brine and dried with Na_2SO_4. The crude extract was purified by silica gel column chromatography to give RNS **(2)** and STM **(3)** as white crystals in 28% and 50% yields, respectively **(Scheme 1)** (Choodej, 2018).



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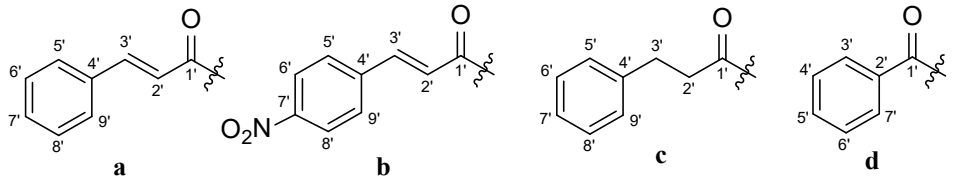
Scheme 1 Synthesis of hydroxylated eudesmanolides

RNS **(2)**; white crystals, 140 mg, 28% yield. ^{1}H NMR data (500 MHz, $CDCl_3$); δ_H 6.08 (1H, d, *J* = 3.2 Hz, H-13), 5.41 (1H, d, *J* = 3.1 Hz, H-13), 4.98 (1H, d, *J* = 0.9 Hz, H-15), 4.85 (1H, d, *J* = 1.5 Hz, H-15), 4.03 (1H, t, *J* = 10.9 Hz, H-6), 3.52 (1H, dd, *J* = 11.6, 4.6 Hz, H-1), 2.57-2.50 (1H, m, H-7), 2.33 (1H, ddd, *J* = 13.8, 5.1, 2.2 Hz, H-3), 2.19-2.16 (1H, m, H-5), 2.16-2.10 (1H, m, H-9), 2.10-2.00 (2H, m, H-3, H-8), 1.84 (1H, m, H-2), 1.64-1.51 (2H, m, H-2, H-8), 1.40-1.30 (1H, m, H-9), 0.81 (3H, s, H-14). ^{13}C NMR data (125 MHz, $CDCl_3$); δ_C 170.8 (C-12), 142.6 (C-4), 139.3 (C-11), 117.3 (C-13), 110.8 (C-15), 79.7 (C-6), 78.3 (C-1), 53.0 (C-5), 49.7 (C-7), 43.1 (C-10), 35.7 (C-3), 33.6 (C-9), 31.5 (C-2), 21.5 (C-8), 11.7 (C-14).
STM **(3)**; white crystals, 250 mg, 50% yield. ^{1}H NMR data (500 MHz, $CDCl_3$); δ_H 6.06 (1H, d, *J* = 3.2 Hz, H-13), 5.40 (1H, d, *J* = 3.2 Hz, H-13), 5.34 (1H, m, H-3), 3.94 (1H , t, *J* = 11.0 Hz, H-6), 3.67 (1H, dd, *J* = 10.0, 6.7 Hz, H-1), 2.53-2.46 (1H, m, H-7), 2.42-2.36 (1H, m, H-2), 2.36-2.30 (1H, m, H-5), 2.10-2.02 (2H, m, H-8, H-9), 2.00-1.92 (1H, m, H-2), 1.83 (3H, s, H-15), 1.69-1.60 (1H, m, H-8), 1.34-1.26 (1H, m, H-9), 0.87 (3H, s, H-14). ^{13}C NMR data (125 MHz, $CDCl_3$); δ_C 171.1 (C-12), 139.0 (C-11), 133.5 (C-4), 121.5 (C-3), 117.1 (C-13), 81.7 (C-6), 75.2 (C-1), 51.2 (C-5), 51.1 (C-7), 41.0 (C-10), 34.3 (C-9), 32.8 (C-2), 23.5 (C-15), 21.3 (C-8), 11.2 (C-14).

5) Synthesis of acyl derivatives of hydroxylated eudesmanolides

A solution of carboxylic acid (0.20 mmol) in dry CH_2Cl_2 (1.25 mL) was added with EDCI·HCl (38.58 mg, 0.20 mmol) and a catalytic amount of 4-dimethylaminopyridine (DMAP) under an inert atmosphere. After stirring for 10 min, hydroxylated eudesmanolides, RNS **(2)** or STM **(3)** (25 mg, 0.10 mmol) was added**.** The reaction mixture was stirred overnight, after that it was quenched with 5% HCl aqueous solution and extracted with CH_2Cl_2. The organic layer was washed with brine and dried with Na_2SO_4. After removal solvent, the acyl derivatives of hydroxylated eudesmanolides were purified by silica gel column chromatography (**Scheme 2)** (Wang, 2021).

R=



Scheme 2 Synthesis of acyl derivatives of hydroxylated eudesmanolides

1-*O*-cinnamoylreynosin **(2a)**; white solid, 15.6 mg, 41% yield. ^{1}H NMR data (500 MHz, $CDCl_3$); δ_H 7.68 (1H, d, *J* = 16.0 Hz, H-3'), 7.56-7.51 (2H, m, H-5', H-9'), 7.41-7.37 (3H, m, H-6', H-7', H-8'), 6.43 (1H, d, *J* = 15.9 Hz, H-2'), 6.10 (1H, d, *J* = 3.2 Hz, H-13), 5.42 (1H, d, *J* = 3.1 Hz, H-13), 5.04 (1H, *J* = 0.9 Hz, H-15), 4.95 (1H, dd, *J* = 11.7, 4.7 Hz, H-1), 4.91 (1H, *J* = 0.9 Hz, H-15), 4.03 (1H, t, *J* = 10.9 Hz, H-6), 2.60-2.52 (1H, m, H-7), 2.42-2.32 (2H, m, H-3, H-5), 2.23 (1H, ddd, *J* = 13.9, 13.9, 5.1 Hz, H-3), 2.07-2.00 (1H, m, H-8), 1.99-1.92 (1H, m, H-2), 1.90-1.85 (1H, m, H-9), 1.75-1.65 (1H, m, H-2), 1.64-1.53 (1H, m, H-8), 1.46-1.38 (1H, dd, *J* = 13.1, 4.1 Hz, H-9), 0.98 (3H, s, H-14). ^{13}C NMR data (125 MHz, $CDCl_3$); δ_C 170.6 (C-12), 166.5 (C-1'), 145.2 (C-3'), 142.1 (C-4), 139.2 (C-11), 134.4 (C-4'), 130.6 (C-6', C-8'), 129.1 (C-5', C-9'), 128.2 (C-7'), 118.3 (C-13), 117.4 (C-2'), 111.3 (C-15), 79.9 (C-1), 53.2 (C-5), 49.6 (C-7), 42.3 (C-10), 35.5 (C-9), 33.4 (C-3), 28.0 (C-2), 21.4 (C-8), 13.1 (C-14).
1-*O*-(4-nitrocinnamoyl)reynosin **(2b)**; yellow solid, 5.9 mg, 13.8% yield. ^{1}H NMR data (500 MHz, $CDCl_3$); δ_H 8.26 (2H, d, *J* = 8.8 Hz, H-6', H-8'), 7.73-7.67 (3H, m, H-3', H-5', H-9'), 6.55 (1H, d, *J* = 16.1 Hz, H-2'), 6.10 (1H, d, *J* = 3.2 Hz, H-13), 5.42 (1H, d, *J* = 3.1 Hz, H-13), 5.05 (1H, d, *J* = 1.6 Hz, H-15), 4.96 (1H, dd, *J* = 11.7, 4.7 Hz, H-1), 4.92 (1H, d, *J* = 1.4 Hz, H-15),

4.03 (1H, t, *J* = 10.9 Hz, H-6), 2.60-2.54 (1H, m, H-7), 2.40 (1H, ddd, *J* = 14.0, 5.1, 2.2 Hz, H-3), 2.35 (1H, d, *J* = 10.9 Hz, H-5), 2.28-2.20 (1H, m, H-3), 2.08-2.02 (1H, m, H-8), 1.99-1.92 (1H, m, H-2), 1.87 (1H, m, H-9), 1.75-1.69 (1H, m, H-2), 1.63-1.60 (1H, m, H-8), 1.43 (1H, ddd, *J* = 13.2, 6.7, 1.1 Hz, H-9), 0.98 (3H, s, H-14). ^{13}C NMR data (125 MHz, $CDCl_3$); δ_C 170.6 (C-12), 165.5 (C-1'), 148.7 (C-7'), 142.2 (C-3'), 141.8 (C-4), 140.4 (C-4'), 138.9 (C-11), 128.8 (C-5', C-9'), 124.3 (C-6', C-8'), 122.2 (C-13), 117.5 (C-2'), 111.5 (C-15), 80.0 (C-1), 79.2 (C-6), 53.1 (C-5), 49.5 (C-7), 42.2 (C-10), 35.5 (C-9), 33.3 (C-3), 27.9 (C-2), 21.3 (C-8), 13.0 (C-14).

1-*O*-(3-phenylpropanoyl)reynosin **(2c)**, white solid, 30.2 mg, 78% yield. ^{1}H NMR data (500 MHz, $CDCl_3$); δ_H 7.30-7.26 (2H, m, H-6', H-8'), 7.22-7.18 (3H, m, H-5', H-7', H-9'), 6.08 (1H, d, *J* = 3.2 Hz, H-13), 5.41 (1H, d, *J* = 3.0 Hz, H-13), 5.00 (1H, d, *J* = 1.6 Hz, H-15), 4.87 (1H, d, *J* = 1.6 Hz, H-15), 4.76 (1H, dd, *J* = 11.6, 4.7 Hz, H-1), 3.97 (1H, t, *J* = 10.9 Hz, H-6), 2.95 (2H, t, *J* = 7.1 Hz, H-3'), 2.67-2.63 (2H, m, H-2'), 2.53-2.46 (1H, m, H-7), 2.33 (1H, ddd, *J* = 13.9, 5.2, 2.2 Hz, H-3), 2.25 (1H, m, H-5), 2.18-2.11 (1H, m, H-8), 1.97-1.91 (1H, m, H-2), 1.83-1.81 (1H, m, H-9), 1.61-1.43 (2H, m, H-2, H-8), 1.21 (1H, ddd, *J* = 13.2, 6.7, 1.1 Hz, H-9), 0.84 (3H, s H-14). ^{13}C NMR data (125 MHz, $CDCl_3$); δ_C 172.4 (C-1), 170.6 (C-12), 141.9 (C-4), 140.3 (C-4'), 139.1 (C-11), 128.6 (C-6', C-8'), 128.4 (C-5', C-9'), 126.4 (C-7'), 117.4 (C-13), 111.2 (C-15), 79.4 (C-6), 79.3 (C-1), 53.1 (C-5), 49.5 (C-7), 42.0 (C-10), 36.1 (C-2'), 35.2 (C-9), 33.3 (C-3'), 31.1 (C-2), 27.8 (C-3), 21.3 (C-8), 12.9 (C-14).

Benzoylreynosin **(2d)**; white solid, 14.9 mg, 40 % yield. ^{1}H NMR data (500 MHz, $CDCl_3$); δ_H 8.03 (2H, d, *J* = 7.1 Hz, H3', H7'), 7.60-7.56 (1H, *J* = 7.45 Hz, m, H-5'), 7.48-7.46 (2H, m, t, *J* = 7.55 Hz, H-4', H-6'), 6.10 (1H, d, *J* = 3.05 Hz, H-13), 5.42 (1H, d, *J* = 3.05 Hz, C-13), 5.05 (1H, dd, *J* = 11.6, 4.7 Hz, H-1), 5.05 (1H, *J* = 1.6 Hz, H-15), 4.93 (1H, d, *J* = 1.4 Hz, H-15), 4.05 (1H, t, *J* = 10.85 Hz, H-6), 2.61-2.53 (1H, m, H-7), 2.45-2.39 (1H, m, H-3), 2.39-2.36 (1H, m, H-5), 2.26 (1H, ddd, *J* = 13.9, 13.9, 5.1 H, H-3), 2.06-2.00 (2H, m, H-2, H-8), 1.91 (1H, ddd, *J* = 13.9, 13.9, 5.1 Hz, H-9), 1.81-1.71 (1H, m, H-2), 1.64-1.53 (1H, m, H-8), 1.48-1.42 (1H, m, ddd, *J* = 13.5, 13.5, 4.1 Hz, H-9), 1.04 (3H, s, H-14). ^{13}C NMR data (125 MHz, $CDCl_3$); δ_C 170.6 (C-12), 166.00 (C-1'), 142.0 (C-4), 139.1 (C-11), 133.3 (C-5'), 130.4 (C-2'), 129.7 (C-3', C-7'), 128.6 (C-4', C-6'), 117.5 (C-13), 111.4 (C-15), 79.9 (C-1), 79.8 (C-6), 53.2 (C-5), 49.6 (C-7), 42.4 (C-10), 35.6 (C-9), 33.3 (C-3), 28.0 (C-2), 21.4 (C-8), 13.2 (C-14).

1-*O*-cinnamoylsantamarine **(3a)**; white solid, 17.2 mg, 45% yield. ^{1}H NMR data (500 MHz, $CDCl_3$); δ_H 7.68 (1H, d, *J* =16.0 Hz, H-3'), 7.56-7.52 (2H, m, H5', H9'), 7.41-7.38 (2H, m, H6', H8'), 6.44 (1H, d, *J* = 16.0 Hz, H-2'), 6.10 (1H, d, *J* = 3.2 Hz, H-13), 5.41 (1H, d, *J* = 3.0 Hz, H-13), 5.39 (1H, m, H-3), 5.05 (1H, dd, *J* = 9.9, 6.7 Hz, H-1), 3.95 (1H, t, *J* = 10.9 Hz, H-6), 2.58-2.47 (3H, m, H-2, H-5, H-7), 2.15-2.06 (1H, m, H-2), 2.06-2.00 (1H, m, H-8), 1.90-1.85 (4H, m, H-9, H-15), 1.68-1.59 (1H, m, H-8), 1.41-1.33 (1H, ddd, *J* = 13.9, 13.9, 5.1 Hz, H-9), 1.05 (3H, s, H-14). ^{13}C NMR data (125 MHz, $CDCl_3$); δ_C 170.8 (C-12), 166.6 (C-1'), 145.1 (C-3'), 138.9 (C-11), 134.4 (C-4'), 133.5 (C-4), 130.5 (C-6', C-8'), 129.0 (C-5', C-9'), 128.2 (C-7'), 121.1 (C-3), 118.2 (C-13), 117.1 (C-2'), 81.3 (C-6), 76.9 (C-1), 51.0 (C-5), 51.0 (C-7), 40.1 (C-10), 34.2 (C-9), 29.6 (C-2), 23.4 (C-15), 21.1 (C-8), 12.6 (C-14).

1-*O*-(4-nitrocinnamoyl)santamarine **(3b)**; yellow solid, 7.3 mg, 17.2% yield. ^{1}H NMR data (500 MHz, $CDCl_3$); δ_H 8.26 (2H, d, *J* = 8.8 Hz, H-6', H-8'), 7.70 (3H, m, H-3', H-5', H-9'), 6.56 (1H, d, *J* = 16.1 Hz, H-2'), 6.09 (1H, d, *J* = 3.15 Hz, H-13), 5.41 (1H, d, *J* = 3.10 Hz, H-13), 5.39 (1H, m, H-3), 5.07 (1H, dd, *J* = 9.9, 6.7 Hz, H-1), 3.95 (1H, t, *J* = 10.9 Hz, H-6), 2.56-2.45 (3H, m, H-2, H-5, H-7), 2.15-2.07 (1H, m, H-2), 2.07-2.00 (1H, m, H-8), 1.90-1.85 (4H, m, H-9, H-15), 1.68-1.59 (1H, m, H-8), 1.41-1.33 (1H, ddd, *J* = 13.9, 13.9, 5.1 Hz, H-9), 1.05 (3H, s, H-14). ^{13}C NMR data (125 MHz, $CDCl_3$); δ_C 170.7 (C-12), 165.7 (C-1'), 148.7 (C-7'), 142.2 (C-3'), 140.5 (C-4'), 138.8 (C-11), 133.6 (C-4), 128.2 (C-5', C-9'), 124.2 (C-6', C-8'), 122.5 (C-3), 121.1 (C-3), 117.1 (C-2'), 81.1 (C-6), 77.6 (C-1), 51.1 (C-5), 51.0 (C-7), 40.0 (C-10), 29.6 (C-2), 24.0 (C-9), 23.4 (C-15), 21.1(C-8), 12.6 (C-14).

1-*O*-(3-phenylpropanoyl)santamarine **(3c)**; white solid, 23 mg, 60% yield. ^{1}H NMR data (500 MHz, $CDCl_3$); δ_H 7.30-7.26 (2H, m, H-6', H-8'), 7.22-7.18 (3H, m, H-5', H-7', H-9'), 6.07 (1H, d, *J* = 3.15 Hz, H-13), 5.39 (1H, d, *J* = 3.15 Hz, H-13), 5.33 (1H, m, H-3), 4.88 (1H, dd, *J* = 9.9, 6.7 Hz, H-1), 3.89 (1H, t, *J* = 10.9 Hz, C-6), 2.95 (2H, t, *J* = 7.1 Hz, H-3'), 2.67-2.63 (2H, m, H-2'), 2.49-2.37 (3H, m, H-2, H-5, H-7), 1.99-1.90 (2H, ddd, *J* = 12.0, 6.3, 3.0 Hz, H-2, H-8), 1.83 (3H, s, H-15), 1.60-1.52 (2H, m, H-8, H-9), 1.23-1.14 (ddd, *J* = 13.9, 13.9, 5.1 Hz, H-9), 0.91 (3H, s, H-14). ^{13}C NMR data (125 MHz, $CDCl_3$); δ_C 172.6 (C-1'), 170.8 (C-12), 140.4 (C-4'), 138.9 (C-11), 133.4 (C-4), 128.6 (C-6', C-8'), 128.4 (C-5', C-9'), 126.4 (C-7'), 121.0 (C-3), 117.2 (C-13), 81.2 (C-6), 77.0 (C-1), 50.9 (C-70), 50.9 (C-5), 39.8 (C-10), 36.1 (C-2'), 33.9 (C-9), 31.1 (C-3'), 29.5 (C-2), 23.4 (C-15), 21.1 (C-8), 12.4 (C-14).

Benzoylsantamarine **(3d)**; white solid, 12.4 mg, 35% yield. ^{1}H NMR data (500 MHz, $CDCl_3$); δ_H 8.04 (2H, d, *J* = 7.1 Hz, H-3', H-7'), 7.57 (1H, t, *J* = 7.45 Hz, H-5'), 7.46 (2H, t, *J* = 7.55 Hz, H-4', H-6'), 6.09 (1H, d, *J* = 3.05 Hz, H-13), 5.41 (2H, d, *J* = 3.05 Hz, H-3, H-13), 5.16 (1H, dd, *J* = 9.9, 6.7 Hz, H-1), 3.97 (1H, t, *J* = 10.9 Hz, H-6), 2.65-2.50 (3H, m, H-2, H-5, H-7), 2.21-2.13 (1H, m, H-2), 2.31-2.22 (1H, m, H-3), 2.05-2.00 (1H, m, H-8), 1.93-1.88 (1H, m, H-9), 1.88 (3H, s, H-15), 1.68-1.60 (1H, m, H-8), 1.44-1.37 (1H, ddd, *J* = 13.9, 13.9, 5.1 Hz, H-9), 1.12 (3H, s, H-14). ^{13}C NMR data (125 MHz, $CDCl_3$); δ_C 170.7 (C-12), 166.1 (C-1'), 138.9 (C-11), 133.5 (C-5'), 133.2 (C-4), 130.4 (C-2'), 129.7 (C-3', C-7'), 128.6 (C-4', C-6'), 121.0 (C-3), 117.2 (C-13), 81.2 (C-6), 77.6 (C-1), 51.1 (C-7), 51.0 (C-5), 40.2 (C-10), 34.4 (C-9), 29.6 (C-2), 23.4 (C-15), 21.1 (C-8), 12.7 (C-14).

5) Evaluation of the cytotoxicity

The cytotoxicity effect of all compounds against human colorectal HCT-116 cancer cells was evaluated using the MTT method. HCT-116 cells were placed in a 96-wells plate (5 x 10^4 cells/well) in DMEM at 37°C under the atmosphere of 5% CO_2. After 24 h, the various concentrations of compounds were added then incubated for 24 h under the above conditions. At the end of the incubation, MTT reagent was added into each well, followed by further incubation for 3-4 hours, and then it was measured at absorbance 570 nm. The IC_{50} value was calculated by GraphPad prism^{TM} ver.8 program.

RESEARCH RESULTS

1) Synthesis of hydroxylated eudesmanolides, RNS (2) and STM (3)

Two hydroxylated eudesmanolides, RNS **(2)** and STM **(3)**, were synthesized from costunolide by a cyclization reaction in the presence of the epoxidation agent *m*-CPBA followed by the addition of a catalytic amount of $BF_3{\cdot}OEt_2$ to open the epoxy ring as shown in **Scheme 1**. Compounds **2** and **3** were obtained as white crystals with 28% and 50% yields, respectively. The presence of the C-1 hydroxyl group in both compounds was confirmed by analysis of ^{1}H and ^{13}C NMR spectroscopic data and compared to those previously reported [compound **2**; δ_H 3.52 (dd, *J* = 11.6, 4.6 Hz, H-1), δ_C 78.3; compound **3**; δ_H 3.67 (dd, *J* = 10.0, 6.7 Hz), δ_C 78.3]. The double bond moieties of **(2)** and **(3)** were confirmed by ^{1}H and ^{13}C NMR spectroscopic data [compound **2** ($\Delta^{4,15}$); δ_H 4.98 (1H, d, *J* = 0.9 Hz, H-15), 4.85 (1H, d, *J* = 1.5 Hz, H-15), δ_C 142.6 (C-4), δ_C 110.8 (C-15); compound **3** ($\Delta^{3,4}$); δ_H 5.34 (1H, m, H-3), δ_C 121.5 (C-3), δ_C 133.5 (C-4)].

2) Synthesis of acyl derivatives of hydroxylated eudesmanolides, RNS and STM

Cinnamoyl and benzoyl derivatives of hydroxylated eudesmanolide, RNS **(2)** and STM **(3)** were reported to exhibit human hepatic stellate (HSC-LX2) and cervical carcinoma (HeLa) cells. Nevertheless, these acyl derivatives have not been investigated for their activity against CRC cells. Therefore, acyl derivatives of RNS **(2)** and STM **(3)** were synthesized to evaluate their anti-cancer activity against human colorectal HCT-116 cancer cells. Four acyl derivatives of RNS **(2)** and STM **(3)** were prepared from a coupling reaction carried out with EDCI·HCl as a coupling reagent and DMAP as a catalytic agent at room temperature (**Scheme 2**). The

derivatives include cinnamoyl (**2a** and **3a**), 4-nitrocinnamoyl (**2b** and **3b**), 3-phenylpropanoyl (**2c** and **3c**) and benzoyl (**2d** and **3d**), obtained with the yields ranging from 13.8-78%.
Compounds **2a** and **3a** contained cinnamoyl moiety with NMR spectroscopic data; δ_H 7.68 (1H, d, *J* =16.0 Hz, H-3'), 7.56-7.51 (2H, m, H-5', H-9'), 7.41-7.37 (3H, m, H-6', H-7', H-8'), 6.43 (1H, d, *J* = 15.9 Hz, H-2'); δ_C 145.2, 134.4, 130.6, 129.1, 128.2, 117.4.
Compounds **2b** and **3b** contained 4-nitrocinnamoyl moiety with NMR spectroscopic data; δ_H 8.26 (2H, d, *J* = 8.8 Hz, H-6', H-8'), 7.73-7.67 (m, H-3', H-5', H-9'), 6.55 (1H, d, *J* = 16.1 Hz, H-2'); δ_C 145.1, 134.4, 130.5, 129.0, 128.2, 117.1.
Compounds **2c** and **3c** contained 3-phenylpropanoyl moiety with NMR spectroscopic data; δ_H 7.30-7.26 (2H, m, H-6', H-8'), 7.22-7.18 (3H, m, H-5', H-7', H-9'), 2.95 (2H, t, *J* = 7.1 Hz, H-3'), 2.67- 2.63 (2H, m, H-2'); δ_C 140.4, 128.6, 128.4, 126.4, 36.1, 31.1.
Compounds **2d** and **3d** contained benzoyl moiety with NMR spectroscopic data; δ_H 8.03 (2H, d, *J* =7.1 Hz, H-3', H-7'),7.60-7.56 (1H, *J* = 7.45 Hz, m, H5') and 7.48-7.46 (2H, t, *J*= 7.55 Hz, C-4', C-6'); δ_C 133.5, 130.4, 129.7, 128.6.

3) Evaluation of anti-colorectal activity

All acyl derivatives and the original hydroxylated RNS (**2)** and STM **(3)** were evaluated for their cytotoxic effect against human colorectal HCT-116 cancer cells by MTT method. Doxorubicin was used as a positive control. The results were shown in the **Table 1.**

Table 1 Anti-cancer activity of acyl derivatives of RNS **(2)** and STM **(3)** against the HCT-116 cells

Compound	**IC_{50} (μM)**
2	18.17
3	15.41
2a	34.23
2b	>50
2c	42.07
2d	16.37
3a	15.41
3b	23.42
3c	23.94
3d	13.51
Doxorubicin (positive control)	4.48

DISCUSSIONS AND CONCLUSION

The hydroxylated eudesmanolides, RNS (2) and STM (3), and their acyl derivatives had been reported to display potential anti-cancer activity (Mejías, 2021, Wang, 2021). Therefore, acyl derivatives of RNS (2) and STM (3) were synthesized and determined for their cytotoxic effect against human colorectal HCT-116 cancer cells. The results **(Table 1)** revealed that STM **(3)** showed slightly more potent activity than RNS **(2)** with IC_{50} values of 15.41 and 18.17 μM, respectively. This indicated that the position of the double bond of eudesmanolide did not give much effect on their cytotoxicity. Among acyl derivatives, benzoyl derivatives **(2d** and **3d)** were the most active compounds with IC_{50} values of 16.37 and 13.51 μM, respectively, in addition to their parent compounds. It was found that extension of two carbons (C-2'–C-3') between a benzene ring and a carbonyl moiety, as in cinnamoyl derivative series caused a decrease in activity. Moreover, an existence of nitro group at para position **(2b** and **2c)** and a C-2'–C-3' double bond hydrogenation (**2c** and **3c)** of cinnamoyl moiety decreased much effect on cancer cells, providing less cytotoxicity than the parent compounds. Although a more potent acyl derivative has not been obtained, but the beneficial SAR information could be seen and

used for the further compound design to obtain stronger compound against HCT-116 cancer cells.

In conclusion, hydroxylated eudesmanolides, RNS **(2)** and STM **(3)**, and their acyl derivatives were synthesized and evaluated for their cytotoxic effect against human colorectal HCT-116 cancer cells. Hydroxylated eudesmanolide, STM **(3)** showed slightly stronger activity than RNS **(2)** with IC_{50} values of 15.41 and 18.17 μM, respectively. Benzoyl derivatives (**2d** and **3d**) showed better inhibition than cinnamoyl derivatives (**2a** and **3a**) with IC_{50} values of 16.37 and 13.51 μM, respectively. Meanwhile, the presence of nitro group at para position and a C-2'–C-3' double bond hydrogenation of cinnamoyl moiety caused more decrease in activity. Among hydroxylated eudesmanolides and their acyl derivatives, compound **3d**, a benzoyl derivative, showed the most potent cytotoxicity. Therefore, further design and modification of benzoyl derivatives of STM **(2)** will be performed to develop a new anti-colorectal cancer agent.

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