DESIGN, SYNTHESIS, AND CYTOTOXICITY EVALUATION OF NOVEL OPEN-CHAIN ANALOGUES OF ANTIMYCIN A<sub>3</sub> AS POTENTIAL ANTI-COLORECTAL CANCER AGENTS

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ABSTRACT

Objective: Colorectal cancer is the third most common diagnosed cancer in the world. The aim of this work was to design, to synthesize, and to evaluate the novel open-chain analogues of antimycin A<sub>3</sub> as highly potent anti-colorectal cancer agents.

Methods: Our analogue synthesis was designed by modifying the nine-membered dilactone moiety of antimycin A<sub>3</sub> with a simple open-chain moiety, as well as introducing the stereocenter, and the hydroxyl groups on the side chain of the ester group. The synthesis was conducted through a sequence of reactions from Boc-L-threonine by esterification, amidation, and sharpless asymmetric dihydroxylation. After completion the synthesis, cytotoxicities of the analogues were evaluated as inhibitors of colorectal HCT-116 cells by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide cell proliferation assay.

Results: Novel open-chain analogues of antimycin A<sub>3</sub> were successfully synthesized in a good yield. The analogues exhibited a greater anticancer activity against colorectal HCT-116 cells than the original antimycin A<sub>3</sub> with 50% inhibitory concentrations ranging of 35.0-47.0 µM. The results indicated that the presence of stereocenter and a hydroxylated open-chain moiety in the analogues were successfully improved its anti-colorectal cancer activity.

Conclusion: Our results clearly demonstrate that the open-chain analogues of antimycin A<sub>3</sub> as promising candidates of new anti-colorectal cancer agents.

Keywords: Design, Synthesis, Open-chain, Analogue, Antimycin A<sub>3</sub>, Anti-colorectal cancer.

INTRODUCTION

Colorectal cancer is the third most common diagnosed cancer in the world with nearly 1.4 million new cases in 2012. It is estimated that worldwide the number of colorectal cancer cases will raise to 2.44 million by 2035 with approximately 394,000 people would die annually. Chemotherapy is a standard method which can alter tumor growth and improves survival rates in a patient with colorectal cancer. However, cancer chemotherapy is often failure due to the development of resistance by cancer cells against current anti-colorectal cancer agents [1,2]. Consequently, there is a significant need for new agents which more effective, safe, and potentially extend the survival of colorectal cancer patients.

Antimycin A<sub>3</sub>[1] is an active agent isolated from Streptomyces sp. in 1949 that inhibit the electron transfer activity of ubiquinol cytochrome c oxidoreductase, induce apoptosis of cancer cells, as well as shows a strong growth inhibitory activity against human colorectal cancer COLO205 cells [3]. The unique biological activity of antimycin A<sub>3</sub> inspired us to carry out the synthesis of its novel analogues, as well as evaluated its cytotoxicity against HCT-116 cells of colorectal cancer. Antimycin A<sub>3</sub> consists of a nine-membered dilactone core which is similar to antifungal antibiotic UK-3A that was also isolated from Streptomyces sp. in 1997 [3,4]. Previously, in 2010, we have reported the synthesis of novel 2-hydroxynicotinoyl-serine-butyl esters related to antimycin A<sub>3</sub>, which showed a strong antimicrobial activity against Bacillus subtilis and Staphylococcus aureus [5]. Subsequently, in 2012, we succeeded in synthesizing polyhydroxylated 18-membered analogue of antimycin A<sub>3</sub>, which strongly inhibited the growth of HeLa cells, breast MDA-MB-231 cells and prostate PC-3 cells [6]. More recently, in 2014, we have simulated some antimycin A<sub>3</sub> analogues as inhibitors of anti-apoptotic Bcl-2 of breast cancer by computational molecular docking [7]. In this research, as the continuing research to develop antimycin A<sub>3</sub> analogues as anticancer agents, we conduct the synthesis of open-chain analogues of antimycin A<sub>3</sub>.

Structure-activity relationship (SAR) studies of antimycin A<sub>3</sub> by Miyoshi et al. in 1995 had shown that the anticancer activity of antimycin A<sub>3</sub> highly depend on the presence of hydroxyl group, amide bond and 3-formamido group. Whereas, the nine-membered dilactone core in antimycin A<sub>3</sub> was less necessary for anticancer activity compared to 3-formamidosalicylyl moiety [8]. These reports suggesting that the nine-membered dilactone core in antimycin A<sub>3</sub> can be modified by another active core in order to increase its anticancer activity. To date, there are several reports on the analogue synthesis of antimycin A<sub>3</sub>. However, studies on the synthesis of open-chain analogues of antimycin A<sub>3</sub> are still limited. Therefore, in this work, we conducted the synthesis of antimycin A<sub>3</sub> analogues by modifying the nine-membered dilactone core of antimycin A<sub>3</sub> with an open-chain moiety in our desired analogue 1 and analogue 2 (Fig. 1).

It has been reported that introduction of hydroxyl groups into biologically active compound resulted in increasing of its biological activity due to the enhancement of its solubility in water, which is the one of important factors influencing the efficacy of drugs [9]. Thus, the introduction hydroxyl group in the open-chain moiety of the analogues is expected to greatly improve its anticancer activity. Furthermore, to study whether the stereochemistry influences the anticancer activity of the analogues; we designed hydroxyl group with bottom facial stereochemistry on the open-chain moiety of analogue 1, in contrast to those analogue, with the top facial stereochemistry in analogue 2.
METHODS

General experimental method
Unless otherwise noted, all reactions were performed in oven-dried glassware, sealed with a rubber septum under nitrogen atmosphere. Anhydrous tetrahydrofuran (THF) and CHCl₃ were purchased from Kanto Chemical Co., dimethyl formamide (DMF) and t-butyl alcohol was distilled prior to use. Methanol, ethyl acetate (EtOAc), n-hexane, chloroform, Et₂O and dimethyl sulfoxide (DMSO) were purchased from Wako Pure Chemical Industries. Boc-L-threonine, allyl bromide, NaHCO₃, K₂CO₃, Na⁺-4-dimethylamidopyridine, N-methylmorpholine (NMM), 1-hydroxybenzotriazole (HOBT), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI), iodomethane (MeI), benzylbromide, formamide and osmium (VIII) oxide (OsO₄) were obtained from Wako Pure Chemical Industries. 3-Aminosalicylic acid was obtained from Sigma-Aldrich Chemical Company. Flash column chromatography was carried out using Merck silica gel 60 (spherical/40-63 μm). Reactions were monitored by thin layer chromatography (TLC) using Merck silica gel 60 F₂₅₄ plates (Merck). Compounds were visualized using an ultraviolet lamp (254 nm) and/or by staining with ninhydrin (in EtOH), p-anisaldehyde (in EtOH) and ammonium molybdate (in 10% H₂SO₄). Proton nuclear magnetic resonance (NMR) and ¹³C NMR spectra were recorded on JEDL JNM-EC500 (500 MHz) spectrometers with tetramethylsilane (δ 0) as internal standard. Mass spectra were recorded on Shimadzu GCMS QP-5000 or JEOL JNM-ECP500 (500 MHz) spectrometers with tetramethylsilane (δ 0) as standard. Specific rotation, [α]D, were measured using a PerkinElmer 341 polarimeter. Carbon, hydrogen, and nitrogen contents were determined on a Carlo Erba 1106 elemental analyzer. All reactions were performed in oven-dried and pre-flushed glassware, sealed with a rubber septum under nitrogen atmosphere.

Design and synthesis

Retrosynthetic analysis
Synthesis of target analogues was designed by retrosynthetic approach through several steps chemical reaction. Starting from the target analogue 1 and 2, break it down by series of disconnection, interconversion of functional group, and modification into the starting material (8). Retrosynthetic analysis of the analogues is outlined in Scheme 1. As shown, analogue 1 can be built by hydrogenolysis of the benzyl group of amide 3, whereas analogue 2 can be derived from hydrogenolysis of the benzyl group of amide 4. Amide 3 and amide 4 can be prepared from sharpless asymmetric dihydroxylation (AD) [10,11] of amide 5 in the presence of (DHQ)₂PHAL ligand and (DHQD)₂PHAL ligand, respectively. Amide 5 can be constructed from amidation of ester 6 with 3-formamido-2-benzoyloxy-benzoic acid (7) which can be prepared from 3-formamidobenzoic acid according to known procedure as reported previously by Pettit et al. [12]. Ester 6 can be synthesized from esterification of commercially available Boc-L-threonine (8) followed by Boc deprotection.

Synthesis of Boc-L-threonine-Allyl ester (9)
To a stirred solution of Boc-L-threonine (2.0 g, 9.12 mmol) in DMF (50 mL) was added Na₂CO₃ (1.93 g, 4.56 mmol), followed by allyl bromide (0.98 mL, 10.94 mmol) and water (1.6 mL). The resulting mixture was stirred for 4 h at room temperature. The solvent was removed from the reaction mixture in vacuo and water (50 mL) was added. The aqueous layer was extracted with EtOAc (3 × 50 mL) and the combined organic layers were dried over anhydrous Na₂SO₄, filtered and concentrated in vacuo. The crude material was purified by silica gel column chromatography using 1:1 hexane:EtOAc as the elution solvent. Ester 6 (3437 mg, 91%) as pale yellow solid. Rf = 0.63 (1:1 hexane:EtOAc).

Synthesis of Boc-L-threonine-Allyl ester ammonium chloride (6)
A round-bottomed flask was charged with ester 9 (5 g, 19.2 mmol) dissolved in EtOAc (200 mL). 35% (w/v) of HCl (20 mL) was added to this solution and the mixture was stirred at room temperature for 10 hrs. After the reaction was complete, the solvent was evaporated in vacuo, and the crude was purified by flash column chromatography on silica gel (gradient elution 20:1 to 1:1, CHCl₃:CH₃OH) gave an ammonium chloride salt of 6 (3437 mg, 91%) as pale yellow solid. Rf = 0.23 (5:1 CHCl₃:CH₃OH).

Synthesis of amide 5
EDCI (0.3 mL, 1.83 mmol) and NMM (2.5 mL, 22.95 mmol) were added to a mixture solution of Boc-L-threonine-allyl ester ammonium chloride 6 (0.93 mL, 10.94 mmol) and water (1.6 mL). The resulting mixture was stirred for 4 h at room temperature. The solvent was removed from the reaction mixture in vacuo and water (50 mL) was added. The aqueous layer was dried over anhydrous Na₂SO₄, filtered and concentrated in vacuo, followed by purification by column chromatography on silica gel (gradient elution 20:1 to 18:2, hexane:EtOAc to give ester 8 (2.09 g, 96%) as colorless liquid. Rf = 0.63 (1:1 hexane:EtOAc). IR (neat) 3440, 2979, 2935, 2362, 1718, 1507, 1367, 1165, 1067, 989/cm; [α]D = 5.0 Hz, 1H), 4.16 (s, 1H), 3.98 (s, 1H), 1.22 (d, J = 10.2 Hz, 1H), 4.69 (d, J = 5.0 Hz, 1H), 4.16 (s, 1H), 3.98 (s, 1H), 1.22 (d, J = 6.0 Hz, 3H), 1H); C NMR (125 MHz, CDCl₃): δ 171.3, 156.3, 131.6, 118.7, 80.0, 68.0, 66.0, 59.0, 28.3, 19.9. HRMS FAB` calculated for C₂₂H₂₁NO₂ [M-H²⁺]: 326.1498, found: 326.1506.

Synthesis of L-threonine-allyl ester ammonium chloride (5)
A round-bottomed flask was charged with ester 9 (5 g, 19.2 mmol) dissolved in EtOAc (200 mL). 35% (w/v) of HCl (20 mL) was added to this solution and the mixture was stirred at room temperature for 10 hrs. After the reaction was complete, the solvent was evaporated in vacuo, and the crude was purified by flash column chromatography on silica gel (gradient elution 20:1 to 1:1, CHCl₃:CH₃OH) gave an ammonium chloride salt of 5 (6347 mg, 91%) as pale yellow solid. Rf = 0.23 (5:1 CHCl₃:CH₃OH): [α]D = 8.46 (s, 3H), 5.97-5.91 (m, 1H), 5.69-5.60 (m, 1H), 5.41 (d, J = 17.1 Hz, 1H), 5.27 (d, J = 10.2 Hz, 1H), 4.69 (d, J = 5.0 Hz, 1H), 4.16 (s, 1H), 3.98 (s, 1H), 1.22 (d, J = 6.0 Hz, 3H), 1H); C NMR (125 MHz, CDCl₃): δ 171.3, 156.3, 131.6, 118.7, 80.0, 68.0, 66.0, 59.0, 28.3, 19.9. HRMS FAB` calculated for C₂₂H₂₁NO₂ [M+H]⁺: 326.1498, found: 326.1506.

Synthesis of amide 5
Ester 6 (0.3 mL, 1.83 mmol) and NMM (2.5 mL, 22.95 mmol) were added to a mixture solution of L-threonine-allyl ester ammonium chloride 6 (0.93 mL, 10.94 mmol) and water (1.6 mL). The resulting mixture was stirred for 4 h at room temperature. The solvent was removed from the reaction mixture in vacuo and water (50 mL) was added. The aqueous layer was dried over anhydrous Na₂SO₄, filtered and concentrated in vacuo, followed by purification by column chromatography on silica gel (gradient elution 20:1 to 18:2, hexane:EtOAc) to give 8 (2.09 g, 96%) as colorless liquid. Rf = 0.63 (1:1 hexane:EtOAc). IR (neat) 3440, 2979, 2935, 2362, 1718, 1507, 1367, 1165, 1067, 989/cm; [α]D = 5.0 Hz, 1H), 4.16 (s, 1H), 3.98 (s, 1H), 1.22 (d, J = 10.2 Hz, 1H), 4.69 (d, J = 5.0 Hz, 1H), 4.16 (s, 1H), 3.98 (s, 1H), 1.22 (d, J = 6.0 Hz, 3H), 1H); C NMR (125 MHz, CDCl₃): δ 171.3, 156.3, 131.6, 118.7, 80.0, 68.0, 66.0, 59.0, 28.3, 19.9. HRMS FAB` calculated for C₂₂H₂₁NO₂ [M-H²⁺]: 326.1498, found: 326.1506.
acid 7 (0.51 g, 1.83 mmol) and HOBt (0.31 g, 2.295 mmol) in DMF (22 mL). The mixture was stirred at room temperature for 17 hrs. The mixture was then diluted by addition of EtOAc (200 mL), and washed repeatedly by water (4 × 75 mL) and saturated NaCl (2 × 75 mL). EtOAc phase was dried over MgSO4 anhydrous and evaporated the residue was flash chromatographed on silica gel (gradient elution 6:1 to 3:1, hexane:EtoAc), gave amide 5 (535.1 mg, 85%) as pale yellow oil.

Rf=0.56 (1:1 hexane:EtOAc); H NMR (500 MHz, DMSO-d6): δ 7.94 (s, 1H), 8.47 (d, J=8.0 Hz, 1H), 8.31 (d, J=6.0 Hz, 1H), 8.17 (d, J=8.0 Hz, 1H), 7.50-7.74 (m, 2H), 7.39-7.31 (m, 4H), 7.20 (t, J=8.0 Hz, 1H), 5.88-5.85 (m, 1H), 5.34 (dd, J=18.9 and 5.4 Hz, 1H), 5.19 (dd, J=10.9 and 5.4 Hz, 1H), 5.04-4.94 (dd, J=17.2 and 5.7 Hz, 2H), 4.97-4.84 (m, 1H), 4.53-4.51 (m, 1H), 4.25-4.15 (m, 1H), 1.22 (d, J=6.3 Hz, 3H); 13C NMR (125 MHz, DMSO-d6): δ 166.5 (s), 165.9 (s), 164.0 (s), 154.9 (d), 154.2 (s), 153.5 (s), 152.3 (s), 151.6 (s), 129.0 (d), 128.9 (d), 128.2 (d), 128.0 (d), 124.8 (d), 124.3 (d), 124.1 (d), 117.1 (t), 76.15 (t), 66.27 (d), 64.96 (t), 58.62 (d), 20.3 (q); HRMS ESI+ calc'd for C45H26N2O9Na+ [M+Na]+: 649.1587, found: 649.1586; [α]21 = −9 (c=0.71, CH2OH, 26°C).

Synthesis of amide 3

To a solution of amide 5 (0.3 g, 0.73 mmol), [DHQD]2PHAL (57 mg, 10 mol%) and N-methylmorpholine-N-oxide (NMO) (256 mg, 2.18 mmol) in t-BuOH/THF:H2O (6:6:1.2) was added OsO4 (19 mg 10 mol%).

The resulting mixture was stirred at room temperature and monitoring by thin-layer chromatography (TLC) until disappearance of starting material (5 hrs). The reaction was quenched with addition of Na2SO4 (0.3g) and water (7 mL). The resulting mixture was extracted by CHCl3 (3× 15 mL). The combined CHCl3 layers were dried over Na2SO4 filtered and concentrated under reduced pressure. The crude residue was purified by column chromatography on silica (gradient elution 20:1 to 8:1, CH2Cl2:CH3OH) to give a colorless oil of 10:1 dr of dihydroxylated amide products (0.26 g, 64%), with an inseparable diastereomeric mixture was then used for the next step.

The reaction mixture was then diluted by addition of EtOAc (200 mL) and washed with water (22 ml). The mixture was stirred at room temperature for 17 hrs. The reaction was complete, the solution phase was filtered through celite and the solid phase was washed with 1:1 EtOAc-MeOH (40 mL). The combined solvent filtrate and washings was evaporated and the residue was flash chromatographed on silica (gradient elution 20:1 to 9:1, CHCl3:CH3OH) gave a mixture of 10:1 dr of the corresponding Bn-deprotected products. This mixture was separated by medium pressure liquid chromatography afforded major diastereomer 1 (37.3 mg, 74%) as a pale brown oil. Rf=0.29 (4:1 CHCl3:CH3OH); H NMR (500 MHz, acetone-d6): δ 8.16 (s, 1H), 8.51 (s, 1H), 8.47 (d, J=7.5 Hz, 1H), 8.13 (d, J=9.0 Hz, 1H), 7.71 (d, J=8.5 Hz, 1H), 6.90 (t, J=8.0, 1H), 4.80-4.76 (m, 1H), 4.53-4.45 (m, 1H), 4.32-4.14 (m, 2H), 3.91-3.89 (m, 1H), 3.75-3.72 (m, 1H), 3.59-3.55 (m, 2H), 3.29 (s, 2H), 1.24 (d, J=6.0 Hz, 3H); 13C NMR (125 MHz, acetone-d6): δ 171.2 (s), 170.8 (s), 160.9 (d), 151.6 (s), 128.3 (s), 124.9 (d), 122.1 (d), 119.1 (d), 114.4 (s), 70.5 (d), 67.9 (d), 67.1 (t), 63.6 (t), 59.0 (d), 20.3 (q); HRMS ESI+ calc'd for C37H23N2O6Na+ [M+Na]+: 579.1117, found: 579.1118; [α]21 = +1 (c=0.59, CH2OH, 22°C).

Synthesis of amide 4

To a solution of amide 5 (0.3 g, 0.73 mmol), [DHQD]2PHAL (57 mg, 10 mol%) and NMO (256 mg, 2.18 mmol) in t-BuOH/THF:H2O (6:6:1.2) was added OsO4 (19 mg, 10 mol%). The resulting mixture was stirred at room temperature and monitoring by TLC until disappearance of starting material (5 hrs). The reaction was quenched with addition of Na2SO4 (0.3g) and water (7 mL). The resulting mixture was extracted by CHCl3 (3× 15 mL). The combined CHCl3 layers were dried over Na2SO4 filtered and concentrated under reduced pressure. The crude residue was purified by column chromatography on silica (gradient elution 20:1 to 8:1, CH2Cl2:CH3OH) to give a colorless oil of 10:1 dr of dihydroxylated amide products (0.26 g, 64%), with 3 as major diastereomer. This inseparable diastereomeric mixture was then used for the next step.

Rf=0.41 (4:1 CH2Cl2:CH3OH) for mixture of two diastereomers; H NMR (500 MHz, acetone-d6): δ 8.28 (dd, J=6.9 and 3.2 Hz, 1H), 0.94 (s, 6H), 7.85-7.28 (m, 2H), 7.10 (s), 6.80 (H2), 7.35-7.24 (m, 5H), 7.19-7.11 (m, 3H), 5.14 (d, J=10.3 Hz, 1H), 4.88-4.81 (m, 1H), 4.74 (d, J=9.2 Hz, 1H), 4.57-4.46 (m, 3H), 3.84 (br s, 1H), 3.73-3.62 (m, 1H), 3.59-3.45 (m, 2H), 2.10 (d, J=6.5 Hz, 3H), 3C NMR (125 MHz, acetone-d6): δ 181.7 (s), 166.5 (s), 160.5 (d), 147.0 (s), 136.7 (s), 132.9 (s), 132.8 (d), 132.0 (d), 129.9 (d), 129.0 (d), 128.7 (d), 126.1 (d), 124.9 (d), 78.1 (t), 70.5 (d), 67.8 (d), 67.1 (t), 63.7 (t), 59.4 (d), 20.7 (q); HRMS ESI+ calc'd for C46H32N2O11Na+ [M+Na]+:469.1587, found: 469.1582; [α]21 = +2 (c=0.42, CH2OH, 26°C).

In vitro cytotoxicity assay

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [13,14] is performed to measure the anti-proliferation effects of synthesized amide 5, amide 3, amide 4, analogue 2, analogue 1 and the original antimycin A, on the colon cancer HCT-116 cells. Synthesized amide 5, amide 3, amide 4, analogue 2, analogue 1 and the original antimycin A, are diluted and added to target cells in triplicates with final concentrations at 51.2, 25.6, 12.8, 6.4, 3.2, 1.6, 0.8, 0.4 μg/ml. The cells are incubated for 48 hrs and 20 μl of 5 mg/ml solution of MTT in phosphate-buffered saline is added to triplicate samples and the plates are incubated for additional 4 hrs. The plates are then centrifuged and the medium is removed. 200 μL of DMSO is added to each well to dissolve the purple blue sediment, the absorbance is determined at 590 nm on a microplate reader (Model 550, Bio-Rad, USA). The 50% inhibitory concentration (IC50) of the 48 hrs are calculated with Bliss assay. The inhibition rate is calculated as follows:

Inhibitionrate(%)=1−Absorbanceoftreatment groupAbsorbanceofcontrol group×100
RESULTS AND DISCUSSION

Chemistry

Scheme 2 outlines the synthesis of analogue 1 and 2. Starting from esterification of Boc-L-threonine (8) with allyl bromide under basic condition, which was conducted according to the procedure provided by Wu et al. [15], afforded Boc-L-threonine-allyl ester (9) in 96% yield. Subsequently, removal of Boc protecting the group from ester 9 was completed in the presence of 20 equivalents of HCl for 10 hrs, afforded 91% yield of ester 6 as ammonium chloride salts. With ester 6 in hands, our subsequent plan was to conduct the amidation of 6 with 3-formamido-benzoic acid (7) to form amide intermediate 5. The formation of amide intermediate 5 is the key step in this work, and it has accomplished by performing the reaction using the base NMM and the combination of EDG/1HOBt with DMF as a solvent, gave key intermediate amide 5 in 85% yield. In the next step, there are two synthetic pathways available which applying sharpless AD. In our initial attempt, introducing the stereocenter as well as addition of two hydroxyl groups at the terminal olefin of amide 5 was carried out by NMO-based catalytic dihydroxylation system in the presence of OsO₄, but in the absence of (DHQ)₂PHAL or (DHQD)₂PHAL ligand, to give hydroxylated amide product with very poor diastereoselectivity. Subsequently, the addition of 10 mol% of (DHQ)₂PHAL or (DHQD)₂PHAL ligand into this system was affected to significantly improved the diastereomeric diastereoselectivity. These results revealed that (DHQ)₂PHAL or (DHQD)₂PHAL ligand was very important to control stereoselectivity of sharpless dihydroxylation of amide 5, in order to obtain the product with satisfactory selectivity. Thus, in the first pathway, sharpless dihydroxylation of amide 5 in the presence of 6 equivalents of NMO with 10 mol % of both OsO₄ and (DHQ)₂PHAL ligand, proceeded smoothly to give inseparable diastereomeric mixture of hydroxylated amide products in 64% yield with satisfactory diastereoselectivity (dr=diastereomeric ratio = 10:1), while 3 as a major diastereomer. As the final step, hydrogenolysis of this diastereomeric mixture with 10% Pd/C resulted in cleavage of Bn group, and afforded a 10:1 diastereomeric mixture of the corresponding Bn deprotected hydroxylated amide products, which was successfully separated in this step by medium pressure liquid chromatography to give a pure major analogue 1 in 76% yield.

Cytotoxicity

After completion of the synthesis, cytotoxicities of the analogue 1, analogue 2, and three intermediate products, amide 5, amide 4 and amide 3 were evaluated as inhibitors of cancer cell growth versus colorectal cancer HCT-116 cells. The result is summarized in Table 1. As shown in Table 1, amide 5 with IC₅₀ over 200 µM showed no cytotoxicity against HCT-116 cells. In contrast to amide 5, amide 3 and amide 4 which two additional of hydroxyl groups showed the improvement in cytotoxicity with concentration 106.5 µM and 156.8 µM against HCT-116, respectively. The cytotoxicity of amide 3 and amide 4 are greatly improved by the presence of the hydroxyl groups compared to that of amide 5. This fact suggested that the hydroxyl groups are very important for the anti-colorectal cancer activity. Compared to amide 4, amide 3 which possess hydroxyl group with bottom facial stereochemistry, showed stronger cytotoxicity, indicating that introduction hydroxyl group with bottom facial stereochemistry was potentially responsible for the increase in its anticancer activity.

Compared to amide 3 and amide 4, analogue 1 and analogue 2 which have hydroxyl group instead of benzyloxy group on 3-formamidosalicylyl moiety, showed greater cytotoxicity, suggesting that the presence of hydroxyl group on 3-formamidosalicylyl moiety in both of analogue 1 and analogue 2 was very necessary to enhance its anticancer activity against HCT-116 cells. Moreover, analogue 1 and analogue 2 which contains open-chain moiety exhibited a greater anticancer activity compared to amide 5 and amide 4.
than that of the original antimycin A, on HCT-116 cells of colorectal cancer, with IC_{50}: 35 µM and 47 µM, respectively. These results indicated that modifying the nine-membered dilactone core of antimycin A with a hydroxylated open-chain moiety in analogue 1 and analogue 2 was successfully improved its anticancer activity. Furthermore, analogue 1 contains bottom facial stereocenter showed greater anti-colorectal cancer activity compared to analogue 2 which has top facial stereocenter. These results revealed that bottom facial stereocenter was more effective for anti-colorectal cancer activity than top facial stereocenter. Thus, analogue 1 which strongly inhibited the growth of colorectal HCT-116 cells should be considered as a promising candidate for the treatment of human colorectal cancer.

CONCLUSION

We synthesized novel open-chain analogues of antimycin A from Boc-L-threonine through esterification, amidation and Sharpless AD. Analogue 1 and analogue 2 showed greater anticancer activity against colorectal HCT-116 cells compared to the original antimycin A. The results and findings in this work are expected to be helpful to the medicinal chemist to develop synthesis strategy of antimycin A analogues, as well as to give a more thorough understanding of the SAR between the analogues and the original antimycin A.

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REFERENCES