



Synthesis of New Zerumbone Derivatives and Their *In vitro* Anti-cancer Activity

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Received: 5 April 2019

Revised: 25 July 2019

Accepted: 3 August 2019

ABSTRACT

Twelve novel derivatives (16-18, 21-23, 26, 27 and 31-34) of azazerumbone and azazerumbone oxide with dihydroartemisinin (DHA), zidovudine (AZT), [4-(4-fluorophenyl)-6-isopropyl-2-(N-methyl-N-methylsulfonylamino)pyrimidin-5-yl] methanol (PM), benzotriazole and piperazine derivatives were synthesized by N-alkylation, acylation and click triazole cyclisation. Their structures were confirmed by 1D-, 2D-NMR and HRMS spectra. Screening for cytotoxicity indicated that the conjugates 21-23 of azazerumbone and azazerumbone oxide with DHA showed potent cytotoxic activity to human cancer cell lines including HepG-2, LU-1, RD and FL. In addition, the anti-mutagenic activity role of azazerumbone (4, 5) in the improvement of cytotoxicity was also discussed.

Keywords: azazerumbone, azazerumbone oxide, click reaction, N-alkylation and cytotoxicity

1. INTRODUCTION

Zerumbone or (*E,E,E*)-2,6,9,9-2,6,10-trien-tetramethylcycloundeca-1-one is a promising cytotoxic sesquiterpene ketone abundantly occurring in the rhizomes of *Zingiber zerumbet*, a wild plant in Vietnam [1]. This sesquiterpene contains an α,β -unsaturated ketone moiety that is reported to be the center of bioactivity and as considered an active Michael acceptor with preferential activity towards thiol group in certain protein due to conjugate addition [2]. In 2009, Giang and colleagues [3] studied the inhibition

of NF- κ B factor by zerumbone and also pointed out that the α,β -unsaturated ketone group is the key to the activity of this compound. Zerumbone was also reported to inhibit several human cancer cell lines including HepG-2 ($IC_{50}=3.15\mu\text{g}/\text{ml}$) [4], HeLa ($IC_{50}=2.5\mu\text{g}/\text{ml}$) [5], HL60 [6] and P-338D1 [7]. Recently, chemical modification of zerumbone has attracted significant attention of chemists to find out the suitable structures for application in preventing and treatment of cancer. However, recent chemical interventions

have mainly concentrated on α,β -unsaturated ketone moiety leading to a significant reduction of anticancer activity. In 2013, Kumar and colleagues [8] recognized that azazerumbone, a derivative containing lactam moiety of zerumbone showed anti-mutagenic activity, and it could be suitable to conjugate with other bioactive molecules. In our previous study [9], two series of conjugates between azazerumbone with 2'-hydroxychalcones and 2', 4'-dihydroxychalcones were successfully synthesized and evaluated the anti-proliferative activity. The conjugate of azazerumbone with 3,4,5-trimethoxy-4-hydroxychalcone *via* trimethylene linker was found to be strongest activity against HepG-2, LU-1, MCF-7, P338 and SW480 cell lines with IC₅₀ values of 1.01, 0.99, 0.58, 0.77 and 0.71 $\mu\text{g}/\text{mL}$, respectively. The use of azazerumbones as anti-mutagenic substrates for the conjugate with other active molecules can produce valuable derivatives in activity against cancer cell lines. Therefore, we present herein the synthesis of novel derivatives from azazerumbone and their *in vitro* anticancer activity evaluation.

2. MATERIAL AND METHODS

All chemicals and reaction solvents were purchased from Merck and Aldrich. Melting points were determined in open capillaries on a Shimadzu Electrothermal IA 9200 apparatus and uncorrected. IR spectra were recorded on a FT-IR IMPACT-410 using KBr discs. 1D- and 2D-NMR Spectra were recorded on a Bruker AVANCE 500 MHz spectrometer in CDCl_3 and $\text{DMSO}-d_6$. Optical rotations were measured with an A. KROSS OPTRONIC polarimeter. Chemical shifts (δ) are in ppm relative to TMS, multiplicities are shown as follows: s (singlet), d (doublet), t (triplet), m (multiplet) and coupling constants (J) are expressed in hertz (Hz). HRMS was recorded by using a FTICR MS and an ESI-TOF-MS Agilent 6230 TOF-MS spectrophotometer (Varian). Progress of the reaction was monitored by thin-layer chromatography (TLC) using precoated TLC sheets with ultraviolet (UV) fluorescent silica gel

(Merck 60F254) and spots were visualized by UV lamp at 254 nm. Column chromatography was carried out using silica gel (40-230 mesh).

The cytotoxic activity evaluation was performed according to the described protocol [10, 11]. The IC₅₀ value of the assay was evaluated using four human cancer cell lines: HepG-2, RD, LU-1, FL and VERO-B4. The derivatives of azazerumbones and azazerumbone oxide were judged to have no cytotoxicity when their IC₅₀ values $> 20 \mu\text{g}/\text{mL}$.

2.1 Synthesis of Azazerumbone 4 and 5

Azazerumbones **4**, **5** were synthesized from zerumbone according to a known procedure [8].

2.2 Synthesis of Zerumbone oxide 6

Zerumbone oxide was synthesized by epoxidation of zerumbone with *m*-CPBA using procedure in reference [10].

¹H-NMR (500 MHz, $\text{DMSO}-d_6$, ppm) δ 6.11 (m, 1H, H-3), 6.08 (s, 2H, H-10, H-11), 2.81 (dd, $J_1=1.5$ Hz, $J_2=11.5$ Hz, 1H, H-7), 2.38 (m, 1H, H4a), 2.30 (m, 1H, H5a), 2.12 (m, 1H, H-4b), 1.79 (d, $J = 14.0$ Hz, 1H, H-8a), 1.75 (s, 3H, H-12), 1.40 (dd, $J_1=2.5$ Hz, $J_2=14.0$ Hz, 1H, H-8b), 1.30 (m, 1H, H-5b), 1.27 (s, 3H, H-14), 1.15 (s, 3H, H-13), 1.03 (s, 3H, H-15). ¹³C-NMR (125 MHz, $\text{DMSO}-d_6$, ppm) δ 202.1 (C-1), 159.2 (C-10), 147.8 (C-3), 138.6 (C-2), 128.1 (C-11), 61.4 (C-7), 61.0 (C-6), 42.1 (C-8), 37.4 (C-5), 35.7 (C-9), 29.4 (C-14), 24.3 (C-4), 23.6 (C-15), 15.4 (C-13), 11.9 (C-12)

2.3 Synthesis of Zerumbone oxide oxime 7, 8

To a solution of zerumbone oxide (**6**) (1.38 mmol) in absolute ethanol (10 mL), hydroxylamine hydrochloride (0.9 g, 13.76 mmol) and K_2CO_3 (1.9 g, 13.76 mmol) were added at room temperature. The mixture was stirred and the progress of the reaction was monitored by TLC using *n*-hexane : EtOAc = 4 : 1. After completion of reaction, the mixture was filtered and washed with absolute ethanol. The solvent was removed under reduced pressure to afford an oil mass, which was then dissolved in dichloromethane (20 mL). The organic

solution was washed with water (10 mL \times 3) and dried over anhydrous sodium sulfate. Solvent was evaporated under reduced pressure to obtain a couple of diastereomers (zerumbone oxide oxime **7** and **8**) that were purified by chromatography on silica gel eluting with *n*-hexane:EtOAc = 4:1.

Zerumbone oxide oxime 7, 8: Yield 92.0 %; White solid; mp: decomp. $^1\text{H-NMR}$ (500 MHz, DMSO- d_6 , ppm) δ 1.01 (3H, s, H-15), 1.11 (3H, s, H-13), 1.20 (1H, m, H-5a), 1.25 (3H, s, H-14), 1.37 (1H, dd, $J_1 = 13.5$ Hz, $J_2 = 11.0$ Hz, H-8a), 1.73 (1H, d, $J = 13.5$ Hz, H-8b), 1.83 (3H, s, H-12), 2.06 (1H, m, H-5b), 2.19 (1H, m, H-4a), 2.28 (1H, m, H-4b), 2.73 (1H, d, $J = 10.5$ Hz, H-7), 5.60 (1H, d, $J = 9.5$ Hz, H-3), 5.62 (1H, d, $J = 16.5$ Hz, H-10), 6.33 (1H, d, $J = 16.5$ Hz, H-11). $^{13}\text{C-NMR}$ (125 MHz, DMSO- d_6 , ppm) δ 15.1 (C-12), 15.4 (C-13), 23.7 (C-15), 24.0 (C-4), 30.2 (C-14), 35.3 (C-9), 37.9 (C-5), 42.3 (C-8), 61.0 (C-6), 61.6 (C-7), 121.7 (C-11), 134.6 (C-2), 137.7 (C-3), 150.8 (C-10), 159.4 (C-1). ESI-HRMS calculated for $\text{C}_{15}\text{H}_{23}\text{NO}_2$: $[\text{M}+\text{H}]^+$ 250.18070, Found: 250.18053.

2.4 Synthesis of Azazerumbone oxides **9**, **10**

To a solution of zerumbone oxide oximes **7**, **8** (1.5 g, 6.02 mmol) in acetonitrile (20 mL), anhydrous ZnCl_2 (0.17 g, 1.28 mmol) was added and the mixture was refluxed on water bath until TLC (*n*-hexane : EtOAc = 4 : 1) indicated that zerumbone oxime **7**, **8** were absolutely disappeared (6 h). Solvent was removed under reduced pressure and residue was dissolved in dichloromethane (30 mL). The organic solution was washed with water (3 \times 20 mL) and dried over anhydrous sodium sulfate. Solvent was evaporated in vacuum to afford crude product that was purified by chromatography on silica gel eluting with *n*-hexane:EtOAc = 4:1. Azazerumbone oxides **9** and **10** obtained as white solids with the yields 9.1 and 39.1 %, respectively.

Azazerumbone oxide **9:** Yield 9.1 %; White solid; mp: 174-176 °C. $^1\text{H-NMR}$ (500 MHz, DMSO- d_6 , ppm) δ 0.96 (3H, s, H-16), 1.12 (3H, s, H-15), 1.16 (3H, s, H-14), 1.26 (1H, m, H-6a), 1.36 (1H, d, J

= 15.5 Hz, H-9a), 1.76 (3H, s, H-13), 1.80 (1H, dd, $J_1 = 7.0$ Hz, $J_2 = 15.5$ Hz, H-9b), 2.12 (2H, m, H-5a, H-6b), 2.38 (1H, m, H-5b), 2.62 (1H, d, $J = 7.0$ Hz, H-8), 4.87 (1H, d, $J = 14.5$ Hz, H-11), 5.48 (1H, d, $J = 10.5$ Hz; H-4), 6.08 (1H, dd, $J_1 = 9.5$ Hz, $J_2 = 14.5$ Hz, H-12), 9.37 (1H, d, $J = 9.5$ Hz, N-H). $^{13}\text{C-NMR}$ (125 MHz, DMSO- d_6 , ppm) δ 13.3 (C-13), 15.9 (C-14), 24.0 (C-5), 26.9 (C-15), 32.5 (C-16), 34.6 (C-10), 37.4 (C-6), 37.6 (C-9), 60.0 (C-7), 61.2 (C-8), 117.9 (C-11), 126.2 (C-12), 129.5 (C-3), 135.8 (C-4), 171.7 (C-2). ESI-HRMS calculated for $\text{C}_{15}\text{H}_{23}\text{NO}_2$: $[\text{M}+\text{H}]^+$ 250.18070, Found: 250.17959.

Azazerumbone oxide (**10**): Yield 39.1 %; White solid; mp: 161-163 °C. Optical rotation: (c.0.99 and c. 0.24, 0.00). $^1\text{H-NMR}$ (500 MHz, DMSO- d_6 , ppm) δ 1.00 (3H, s, H-15), 1.18 (3H, s, H-14), 1.19 (3H, s, H-16), 1.27 (1H, m, H-9a), 1.46 (1H, d, $J = 15.0$ Hz, H-6a), 1.73 (3H, s, H-13), 1.83 (1H, dd; $J_1 = 8.5$ Hz, $J_2 = 15.5$ Hz, H-6b), 2.06 (1H, m, H-9b), 2.14 (1H, m, H-10a), 2.22 (1H, m, H-10b), 2.58 (1H; d, br, $J = 7.0$ Hz, H-7), 4.79 (1H, d, $J = 11.0$ Hz, H-11), 5.89 (1H, d, $J = 15.75$ Hz, H-3), 6.35 (1H, d, $J = 15.75$ Hz, H-4), 8.55 (1H, s, -N-H). $^{13}\text{C-NMR}$ (125 MHz, DMSO- d_6 , ppm) δ 15.8 (C-14), 16.9 (C-13), 23.8 (C-10), 25.8 (C-16), 30.6 (C-15), 36.2 (C-5), 37.5 (C-6), 37.8 (C-9), 60.3 (C-8), 61.4 (C-7), 120.8 (C-3), 123.9 (C-11), 131.7 (C-12), 148.5 (C-4), 166.2 (C-2). ESI-HRMS calculated for $\text{C}_{15}\text{H}_{23}\text{NO}_2$: $[\text{M}+\text{H}]^+$ 250.18070, Found: 250.17964.

General procedure for the synthesis of propargylazazerumbones **12**, **13** and propargylazazerumbone oxide **15**.

A stirred mixture of each azazerumbone **4**, **5** or **10** (1 mmol) in THF (20 mL) at 0 °C was added sodium hydride (0.0288 g, 1.2 mmol), followed by propargyl bromide **11** (0.1368 g, 1.15 mmol). The reaction was stirred at room temperature for overnight then cooled to 5 °C. The mixture was diluted with ice water (15 mL) and extracted with dichloromethane (3 \times 15 mL). The combined organic layer was washed with

water (15 mL) and brine (15 mL), and dried over anhydrous sodium sulfate. Solvent was removed under reduced pressure to give crude of each **12**, **13** or **14** respectively, that were purified by flash chromatography (5% MeOH in CHCl₃).

N-propargylazaserumbone **12**: Yield 76.1%; Colorless oil. ¹H-NMR (500 MHz, DMSO-*d*₆, ppm) δ 1.06 (6H, s, H-15, H-16), 1.53 (3H, s, H-14), 1.75 (3H, s, H-13), 2.09 (2H, d, *J* = 6.5 Hz, H-9), 2.23 (2H, t, *J* = 6.0 Hz, H-6), 2.32 (2H, m, H-5), 3.08 (1H, t, *J* = 2.5 Hz, H-19), 4.30 (2H, d, *J* = 2.5 Hz, H-17), 4.93 (1H, d, *J* = 15.0 Hz, H-11), 5.13 (1H, t, *J* = 6.0 Hz, H-8), 5.33 (1H, t, *J* = 7.25 Hz, H-4), 6.41 (1H, d, *J* = 15.0 Hz, H-12). ¹³C-NMR (125 MHz, DMSO-*d*₆, ppm) δ 13.5 (C-13), 14.8 (C-14), 24.5 (C-5), 30.1 (C-15), 30.1 (C-16), 32.0 (C-17), 34.8 (C-10), 38.0 (C-9), 38.4 (C-6), 73.4 (C-19), 79.4 (C-18), 119.1 (C-11), 124.7 (C-8), 128.6 (C-12), 128.8 (C-3), 133.7 (C-7), 136.4 (C-4), 170.8 (C-2). ESI-HRMS calculated for C₁₈H₂₅NO: [M+H]⁺ 272.20143, Found: 272.20053.

N-propargylazaserumbone (**13**): Yield 87.0%; Colorless oil. ¹H-NMR (500 MHz, DMSO-*d*₆, ppm) δ 1.06 (6H, s, H-15, H-16), 1.56 (3H, s, H-14), 1.82 (3H, s, H-13), 2.11 (2H, m, H-6), 2.19 (2H, m, H-9), 2.28 (2H, m, H-10), 3.02 (1H, t, *J* = 2.5 Hz, H-19), 4.16 (2H, s, H-17), 4.99 (1H, m, H-11), 5.01 (1H, m, H-7), 5.81 (1H, d, *J* = 16.0 Hz, H-3), 6.21 (1H, d, *J* = 16.0 Hz, H-4). ¹³C-NMR (125 MHz, DMSO-*d*₆, ppm) δ 14.8 (C-14), 14.9 (C-13), 24.9 (C-10), 33.0 (C-17), 36.0 (C-6), 38.1 (C-5), 39.3 (C-9), 73.3 (C-19), 80.1 (C-18), 122.6 (C-3), 124.5 (C-7), 132.1 (C-11), 133.5 (C-12), 133.6 (C-8), 148.1 (C-4), 165.0 (C-2). ESI-HRMS calculated for C₁₈H₂₅NO: [M+H]⁺ 272.20143, Found: 272.20053.

N-propargylazaserumbone oxide (**14**): Yield 77.2%; Colorless oil. ¹H-NMR (500 MHz, DMSO-*d*₆, ppm) δ 1.01 (3H, s, H-15), 1.19 (3H, s, H-16), 1.20 (1H, m, H-9a), 1.22 (3H, s, H-14), 1.42 (1H, d, *J* = 15.5 Hz, H-6a); 1.89 (3H, s, H-13), 1.91 (1H, m, H-6b), 2.07 (1H, m, H-9b), 2.14 (1H, m, H-10a), 2.35 (1H, m, H-10b), 2.53 (1H, d, *J* = 6.5 Hz, H-7), 3.06 (1H, t, *J* = 2.5 Hz, H-19), 4.00 (1H, dd, *J*₁ = 2.5 Hz, *J*₂ = 17.5 Hz, H-17a), 4.41 (1H, dd, *J*₁ =

2.5 Hz, *J*₂ = 17.5 Hz, H-17b), 5.07 (dd, *J* = 2 Hz, *J* = 11 Hz, H-11), 5.90 (1H; d, *J* = 15.5 Hz; H-3), 6.46 (1H, d, *J* = 15.5 Hz, H-4). ¹³C-NMR (125 MHz, DMSO-*d*₆, ppm) δ 15.0 (C-14), 16.3 (C-13), 24.2 (C-10), 26.1 (C-16), 30.4 (C-15), 32.9 (C-17), 36.2 (C-5), 37.5 (C-6), 37.6 (C-9), 60.0 (C-8), 61.2 (C-7), 73.5 (C-19), 80.0 (C-18), 120.7 (C-3), 131.2 (C-11), 133.9 (C-12), 149.5 (C-4), 164.6 (C-2). ESI-HRMS calculated for C₁₈H₂₅NO₂: [M+H]⁺ 288.19635, Found: 288.19532.

General procedure for the synthesis of conjugates **16**, **17** and **18** of *N*-propargylazaserumbones and *N*-propargylazaserumbone oxide with AZT.

A catalytic amount of CuI was added to a solution of zidovudine AZT (**15**) (1 mmol) and appropriate **12**, **13** or **14** (1mmol) in DMSO (5 mL). The reaction mixture was stirred for overnight at room temperature and then poured in ice water (80 mL). Solid was filtered and washed thoroughly with water and then dried in vacuum. Crude **16-18** were purified by flash chromatography (50% CHCl₃ in *n*-hexane).

Conjugate **16** of azaserumbone **4** with AZT: Yield 58.0%; White solid; mp: 114-116 °C. ¹H-NMR (500 MHz, DMSO-*d*₆, ppm) δ 0.99 (6H, s, H-15, H-16), 1.53 (3H, s, H-14), 1.78 (3H, s, H-13), 1.80 (3H, s, H-7'''), 2.06 (2H, d, *J* = 6.0 Hz, H-9), 2.23 (2H, m, H-5a, H-6a), 2.31 (2H, m, H-5b, H-6b), 2.62 (1H, m, H-2''a), 2.69 (1H, m, H-2''b), 3.59 (1H, m, H-5''a), 3.68 (1H, m, H-5''b), 4.17 (1H, m, H-4''), 4.77 (2H, s, H-17), 4.94 (1H, d, *J* = 15.0 Hz, H-11), 5.10 (1H, t, *J* = 5.75 Hz, H-8), 5.26 (1H, t, *J* = 5.0 Hz, 5''-OH), 5.34 (2H, m, H-3'', H-4), 6.42 (2H, m, H-1'', H-12), 7.80 (1H, s, H-6'''), 8.04 (1H, s, H-5'), 11.3 (1H, s, 3'''-NH). ¹³C-NMR (125 MHz, DMSO-*d*₆, ppm) δ 12.2 (C-7'''), 13.6 (C-13), 14.9 (C-14), 24.5 (C-5), 30.0 (C-15), 30.0 (C-16), 34.8 (C-10), 37.1 (C-2''), 37.9 (C-9), 38.3 (C-17), 38.4 (C-6), 59.0 (C-3''), 60.7 (C-5''), 83.9 (C-1''), 84.4 (C-4''), 109.6 (C-5'''), 119.2 (C-11), 122.8 (C-5'), 124.7 (C-8), 129.0 (C-3), 129.1 (C-12), 133.6 (C-7), 136.1 (C-4), 136.2 (C-6'''), 143.6 (C-4'), 150.4 (C-2''), 163.7 (C-4'''), 171.3 (C-2).

ESI-HRMS calculated for $C_{28}H_{38}N_6O_5$: $[M+H]^+$ 539.29819, Found: 539.29783.

Conjugate **17** of azazerumbone **5** with AZT: Yield 63.3 %; White solid; mp: 119-121 °C. 1H -NMR (500 MHz, DMSO- d_6 , ppm) δ 1.05 (6H, s, H-15, H-16), 1.55 (3H, s, H-14), 1.80 (3H, s, H-5'''-CH₃), 1.83 (3H, s, H-13), 2.15 (2H, s, brd, H-9), 2.15 (6H, s, brd, H-6, H-9, H-10), 2.60 (1H, m, H-2''a), 2.70 (1H, m, H-2''b), 3.58 (1H, m, H-5''a), 3.67 (1H, m, H-5''b), 4.16 (1H, m, H-4''), 4.60 (2H, s, H-17), 4.86 (1H, t, $J = 7.25$ Hz, H-11), 4.97 (1H, t, $J = 6.0$ Hz, H-7), 5.25 (1H, t, $J = 5.75$ Hz, 5''-OH), 5.32 (1H, m, H-3''), 5.85 (1H, d, $J = 15.5$ Hz, H-3), 6.21 (1H, d, $J = 15.5$ Hz, H-4), 6.40 (1H, t, $J = 6.5$ Hz, H-1''), 7.80 (1H, s, H-6'''), 8.06 (1H, s, H-5'), 11.3 (1H, s, 3'''-NH). ^{13}C -NMR (125 MHz, DMSO- d_6 , ppm) δ 12.2 (C-7'''), 14.9 (C-13), 15.0 (C-14), 25.0 (C-10), 36.1 (C-5), 37.1 (C-2''), 38.1 (C-6), 38.9 (C-17), 39.0 (C-9), 59.0 (C-3''), 60.7 (C-5''), 83.9 (C-1''), 84.4 (C-4''), 109.6 (C-5'''), 122.9 (C-5'), 123.1 (C-3), 124.5 (C-7), 131.9 (C-11), 133.7 (C-12), 134.0 (C-8), 136.2 (C-6'''), 143.9 (C-4'), 147.8 (C-4), 150.4 (C-2''), 163.7 (C-4'''), 165.4 (C-2). ESI-HRMS calculated for $C_{28}H_{38}N_6O_5$: $[M+H]^+$ 539.29819, Found: 539.29760.

Conjugate **18** of azazerumbone oxide **10** with AZT: Yield 54.8 %; White solid; mp: 145-147 °C. 1H -NMR (500 MHz, DMSO- d_6 , ppm) δ 1.01 (3H, s, H-15), 1.14 (1H, m, H-9a), 1.19 (3H, s, H-16), 1.21 (3H, s, H-14), 1.42 (1H, d, $J = 15.5$ Hz, H-6a), 1.80 (3H, s, H-7'''), 1.89 (4H, m, H-6b, H-13), 2.06 (2H, m, H-9b, H-10a), 2.29 (1H, m, H-10b), 2.50 (1H, m, H-7), 2.60 (1H, m, H-2''a), 2.70 (1H, m, H-2''b), 3.58 (1H, m, H-5''a), 3.67 (1H, m, H-5''b), 4.17 (1H, m, H-4''+), 4.17 (1H, m, H-17a), 4.82 (1H, m, H-17b), 4.95 (1H, d, brd, $J = 11.0$ Hz, H-11), 5.26 (1H, t, $J = 5.5$ Hz, 5''-OH), 5.32 (1H, m, H-3''), 5.93 (1H, d, $J = 16.0$ Hz, H-3), 6.40 (1H, m, H-1''), 6.46 (1H, d, $J = 16.0$ Hz, H-4), 7.80 (1H, s, H-6'''), 8.09 (1H, s, H-5'), 11.33 (1H, s, 3'''-NH). ^{13}C -NMR (125 MHz, DMSO- d_6 , ppm) δ 12.2 (C-7'''), 15.2 (C-14), 16.3 (C-13), 24.1 (C-10), 26.2 (C-16), 30.5

(C-15), 36.2 (C-5), 37.1 (C-2''), 37.5 (C-6), 37.7 (C-9), 40.4 (C-17), 59.0 (C-3''), 60.1 (C-8), 60.7 (C-5''), 61.3 (C-7), 83.8 (C-1''), 84.4 (C-4''), 109.6 (C-5'''), 121.0 (C-3), 123.2 (C-5'), 130.9 (C-11), 134.4 (C-12), 136.2 (C-6'''), 143.8 (C-4'), 149.1 (C-4), 150.4 (C-2'''), 163.7 (C-4'''), 164.8 (C-2). ESI-HRMS $C_{28}H_{38}N_6O_6$: $[M+H]^+$ 555.29310, Found: 539.29243.

Synthesis of conjugates **21-23** of azazerumbones and azazerumbone oxide with dihydroxyartemisinin (DHA)

A mixture of appropriate azazerumbones **4**, **5** and azazerumbone oxide **10** (1 mmol, 585 mg), (1.5 mmol) 2-(10 β -dihydroarteminoxy)ethyl bromide **20** (207 mg, 1.5 mmol), K₂CO₃ (16 mg, 10% mol) KI and (1-butyl)triethylammonium bromide (23.8 mg, 10% mol) in DMF (6 mL) was thoroughly stirred and subjected to microwave irradiation in a microwave oven operating at 70 °C with maximum power output of 275 W for 25 minutes. The mixture was concentrated under reduced pressure and dissolved with EtOAc (30 mL), then extracted with distilled water (30 mL \times 3). The organic phase was dried over anhydrous sodium sulfate, then concentrated under reduced pressure to afford crude **21-23**, that were purified by flash chromatography eluting with *n*-hexane : EtOAc 3:1.

Conjugate **21** of azazerumbone **4** with DHA: Yield 49.4 %; White solid; mp: 75-77 °C. 1H -NMR (500 MHz, DMSO- d_6 , ppm) δ 0.81 (3H, d, $J = 7.5$ Hz, H-15'), 0.87 (1H, m, H-17'), 0.91 (3H, d, $J = 6.5$ Hz, H-14'), 1.04 (3H, s, H-15), 1.06 (3H, s, H-16), 1.15 (1H, m, H-5'a), 1.28 (3H, s, H-13'), 1.34 (3H, m, H-5', H-6', H-8'a), 1.53 (2H, m, H-7', H-8''), 1.57 (3H, s, H-14), 1.72 (1H, m, H-8''), 1.80 (3H, s, H-13), 1.83 (1H, m, H-5'), 1.99 (2H, m, H-4'), 2.18 (7H, m, H-6, H-9, H-10, H-17), 2.36 (1H, m, H-17), 2.36 (1H, m, H-9'), 3.34 (1H, m, H-18), 3.69 (1H, s, brd, H-18), 4.64 (1H, d, $J = 3.5$ Hz, H-10'), 4.96 (1H, t, $J = 5.5$ Hz, H-7), 5.02 (1H, t, $J = 7.0$ Hz, H-11), 5.28 (1H, s, H-12'), 5.83 (1H, d, $J = 15.5$ Hz, H-3), 6.15 (1H, d, $J = 15.5$ Hz, H-4). ^{13}C -NMR (125 MHz, DMSO- d_6 ,

ppm) δ 12.7 (C-13'), 14.6 (C-13), 15.0 (C-14), 20.2 (C-14'), 23.7 (C-8'), 24.3 (C-5'), 25.1 (C-10), 25.6 (C-15'), 30.4 (C-9'), 34.2 (C-7'), 36.0 (C-5, C-17, C-4'), 36.6 (C-6'), 38.1 (C-6), 39.0 (C-9), 43.8 (C-8a'), 52.0 (C-5a'), 65.0 (C-18), 80.4 (C-12a'), 87.0 (C-12'), 100.8 (C-10'), 103.2 (C-3'), 123.3 (C-3), 124.5 (C-7), 131.9 (C-11), 133.4 (C-12), 133.7 (C-8), 147.1 (C-4), 165.5 (C-2). ESI-HRMS calculated for $C_{32}H_{49}NO_6$: $[M+H]^+$ 544.36381, Found: 544.36709.

Conjugate **22** of azazerumbone **5** with DHA: Yield 46.5 %; White solid; mp: 78-80 °C. 1H -NMR (500 MHz, DMSO- d_6 , ppm) δ 0.83 (3H, d, $J = 7$ Hz, H-15'), 0.90 (3H, d, $J = 6.5$ Hz, H-14'), 0.92 (1H, m; H1-7'), 1.05 (6H, s, H-15, H-16), 1.16 (1H, m, H-5'a), 1.27 (3H, s, H-13'), 1.35 (3H, m, H1-5', H-6', H-8'a), 1.52 (3H, s, H-14), 1.55 (1H, m, H1-8'), 1.63 (2H, m, H1-7', H2-8'), 1.74 (3H, s, H-13), 1.80 (1H, m, H2-5'), 2.00 (2H, m, H-4'), 2.22 (6H, m, H-5, H-6, H-9), 2.37 (2H, m, H-9', H1-17), 3.67 (1H, m, H2-17), 3.77 (1H, m, H1-18), 3.88 (1H, m, H2-18), 4.66 (1H, d, $J = 3.0$ Hz, H-10'), 4.89 (1H, d, $J = 14.5$ Hz, H-11), 5.03 (1H, t, $J = 6.0$ Hz, H-8), 5.21 (1H, s, H-12'), 5.31 (1H, t, $J = 7.0$ Hz, H-4), 6.33 (d, $J = 14.5$ Hz, H-12). ^{13}C -NMR (125 MHz, DMSO- d_6 , ppm) δ 12.7 (C-13'), 13.5 (C-13), 14.8 (C-14), 20.1 (C-14'), 24.1 (C-8'), 24.3 (C-5), 24.5 (C-5'), 25.6 (C-15'), 29.6 (C-9'), 30.4 (C-15), 30.6 (C-16), 34.2 (C-7'), 34.8 (C-10), 36.0 (C-4'), 36.9 (C-6'), 38.0 (C-9), 38.5 (C-6), 34.8 (C-17), 43.7 (C-8a'), 52.0 (C-5a'), 63.9 (C-18), 80.3 (C-12a'), 86.8 (C-12'), 100.9 (C-10'), 103.3 (C-3'), 119.3 (C-11), 124.7 (C-8), 129.6 (C-12), 130.0 (C-3), 133.5 (C-7), 135.7 (C-4), 171.4 (C-2). ESI-HRMS calculated for $C_{32}H_{49}NO_6$: $[M+H]^+$ 544.36399, Found: 544.36699.

Conjugate **23** of azazerumbone oxide **10** with DHA: Yield 48.6 %; White solid; mp: 80-82 °C. 1H -NMR (500 MHz, DMSO- d_6 , ppm) δ 0.81 (3H, d, $J = 7.5$ Hz, H-15'), 0.87 (1H, m, H1-7'), 0.91 (3H, d, $J = 6.5$ Hz, H-14'), 1.01 (3H, s, H-15), 1.19 (3H, s, H-16), 1.23 (3H, s, H-14), 1.28 (3H, s, H-13'), 1.31 (4H, m, H-5'a, H-6', H-8'a, H1-9), 1.43 (1H, d, $J = 15.0$ Hz, H1-6), 1.55 (1H, m, H2-

7', H1-8'), 1.77 (1H, m, H2-6, H2-5', H2-8'), 1.87 (3H, s, H-13), 1.94 (4H, m, H2-9, H1-10, H-4'), 2.17 (1H, m, H1-17), 2.17 (1H, m, H2-10), 2.37 (2H, m, H2-17, H-9'), 2.46 (1H, d, $J = 6.0$ Hz, H-7), 3.38 (1H, m, H1-18), 4.16 (1H, m, H2-18), 4.63 (1H, d, $J = 3.0$ Hz, H-10'), 5.12 (1H, d, $J = 10.5$ Hz, H-11), 5.21 (1H, s, H-12'), 5.93 (1H, d, $J = 15.5$ Hz, H-3), 6.40 (1H, d, $J = 16.0$ Hz, H-4). ^{13}C -NMR (125 MHz, DMSO- d_6 , ppm) δ 12.7 (C-13'), 14.7 (C-14), 16.3 (C-13), 20.2 (C-14'), 23.7 (C-8'), 24.2 (C-10), 24.3 (C-5'), 25.6 (C-15'), 26.4 (C-16), 30.3 (C-9'), 30.5 (C-15), 34.2 (C-7'), 36.0 (C-4', C-17), 36.1 (C-5), 36.7 (C-6'), 37.6 (C-6), 37.9 (C-9), 43.7 (C-8'a), 51.9 (C-5'a), 59.9 (C-8), 61.2 (C-7), 65.3 (C-18), 80.4 (C-12'a), 87.0 (C-12'), 100.8 (C-10'), 103.2 (C-3'), 121.4 (C-3), 131.1 (C-11), 133.8 (C-12), 148.4 (C-4), 164.8 (C-2). ESI-HRMS calculated for $C_{32}H_{49}NO_7$: $[M+H]^+$ 560.35872, Found: 560.35796.

General procedure for the synthesis of conjugates **26-27** of azazerumbone and azazerumbone oxide with PM

A mixture of appropriate azazerumbone **5** or azazerumbone oxide **10** (1mmol), K_2CO_3 (0.621 g, 4.5 mmol), PBr_3 (1.25 g, 3 mmol) and (1-butyl)triethylammonium bromide (23.8 mg, 10 % mmol) in dried DMF (15 mL) was stirred at 80 °C for 24h. Solvent was removed under reduced pressure and the residue was suspended in water (20 mL), then extracted with EtOAc (20 mL \times 3). The combined EtOAc extract was dried over anhydrous sodium sulfate, then concentrated under reduced pressure to afford crude **26** or **27**, that were purified by flash chromatography eluting with *n*-hexane : EtOAc.

Conjugate **26** of azazerumbone **5** with PM: Yield 43.5 %; White solid; mp: 142-144 °C. 1H -NMR (500 MHz, DMSO- d_6 , ppm) δ 1.00 (6H, s; H-15, H-16), 1.09 (3H, s, H-14), 1.18 (6H, d, $J = 7.0$ Hz, H-8', H-9'), 1.45 (3H, s, H-13), 2.03 (6H, s brd, H-6, H-9, H-10), 3.41 (3H, s, H-11'), 3.52 (3H, s, 10^1 -SO $_2$ CH $_3$), 3.58 (1H, m, H-7'), 4.45 (1H, t, $J = 6.75$ Hz, H-7), 4.83 (1H, t, $J =$

5.75 Hz, H-11), 5.70 (1H, d, $J = 16.0$ Hz, H-3), 6.09 (1H, d, $J = 16.0$ Hz, H-4), 7.32 (2H, t, $J = 8.5$ Hz, H-3", H-5"), 7.53 (2H, dd; $J_1 = 5.5$ Hz, $J_2 = 8.5$ Hz, H-2", H-6"). $^{13}\text{C-NMR}$ (125 MHz, DMSO- d_6 , ppm) δ 14.7 (C-13), 15.1 (C-14), 21.9 (C-15), 21.9 (C-16), 24.9 (C-10), 30.6 (C-7'), 33.1 (C-10'), 36.0 (C-5), 38.0 (C-6), 38.1 (C-17), 38.5 (C-9), 41.6 (C-11'), 115.1 (C-3", C-5"), 119.1 (C-5'), 122.4 (C-3), 124.4 (C-6), 131.6 (d; $J = 8.4$ Hz; C-2", C-6"), 133.9 (C-8), 133.1 (C-11), 133.4 (C-12), 134.6 (C-1"), 147.7 (C-4), 157.0 (C-2'), 162.4 (d; $J = 245.4$ Hz; C-4"), 164.9 (C-4'), 166.8 (C-2), 176.7 (C-6'). ESI-HRMS calculated for $\text{C}_{31}\text{H}_{41}\text{FN}_4\text{O}_3\text{S}$: $[\text{M}+\text{H}]^+$ 569.29618, Found: 569.29404.

Conjugate **27** of azazerumbone **10** with PM: Yield 37.4 %; White solid; mp: 121-123 °C. $^1\text{H-NMR}$ (500 MHz, DMSO- d_6 , ppm) δ 0.96 (3H, m, H-15), 1.11 (3H, m, H-8'), 1.12 (3H, m, H-9'), 1.16 (3H, m, H-16), 1.18 (3H, s, H-14), 1.22 (3H, s, H-13), 1.30 (1H, m, H-9a), 1.34 (1H, d, $J_1 = 15.5$ Hz; H-6a), 1.82 (1H, m, H-10a), 1.97 (1H, m, H-9b), 1.97 (1H, m, H-10b), 2.07 (1H, s brd, H-6b), 2.35 (1H, t, $J = 6.0$ Hz, H-7), 3.40 (3H, s, H-11'), 3.52 (3H, s, 10'-SO $_2$ CH $_3$), 3.62 (1H, m, H-7'), 4.55 (2H, d, $J = 14.5$ Hz, H-17), 5.22 (1H, d, $J = 15.0$ Hz, H-11), 5.72 (1H, d, $J = 15.75$ Hz, H-3), 6.34 (1H, d, $J = 15.75$ Hz, H-4), 7.33 (2H, t, $J = 9.0$ Hz, H-3", H-5"), 7.56 (2H, dd, $J_1 = 8.5$ Hz, $J_2 = 5.5$ Hz, H-2", H-6"). $^{13}\text{C-NMR}$ (125 MHz, DMSO- d_6 , ppm) δ 14.8 (C-14), 16.4 (C-13), 21.6 (C-9'), 22.2 (C-8'), 24.0 (C-10), 26.0 (C-16), 30.3 (C-15), 30.7 (C-7'), 33.1 (C-10'), 36.2 (C-5), 37.5 (C-6), 37.6 (C-17), 38.3 (C-9), 41.6 (C-11'), 59.7 (C-8), 60.8 (C-7), 115.1 (C-3", C-5"), 118.9 (C-5'), 120.5 (C-3), 133.0 (C-11), 133.5 (C-12), 149.1 (C-4), 131.6 (C-2", C-6"), 134.7 (C-1"), 157.0 (C-2'), 162.4 (C-4"), 164.4 (C-4'), 170.3 (C-2), 176.7 (C-6'). ESI-HRMS calculated for $\text{C}_{31}\text{H}_{41}\text{FN}_4\text{O}_4\text{S}$: $[\text{M}+\text{H}]^+$ 585.29109, Found: 585.28975.

General procedure for the synthesis of azazerumbone derivatives **31-34**

Synthesis of intermediate **29**

A solution of azazerumbone **5** (108) (1 g,

4.2 mmol) in dried THF (20 mL) was added triethylamine (TEA, 1.1 mL), followed by a solution of 4-chloromethylbenzoyl chloride **28** (1.2 g, 6.4 mmol) in THF (7 mL). The reaction was stirred at room temperature for overnight and diluted with water (80 mL). The reaction mixture was extracted with EtOAc (3×30 mL) and organic phase was dried over anhydrous sodium sulfate. Solvent was removed under reduced pressure to get crude **29** that was purified by flash column chromatography using *n*-hexane:EtOAc 5:1.

N-(4-Chloromethylbenzoyl)azazerumbone (**29**): Yield 47.8 %; White solid; mp: 96-98 °C. $^1\text{H-NMR}$ (500 MHz, DMSO- d_6 , ppm); δ 1.11 (6H, s, H-15, H-16), 1.57 (3H, s, H-14), 1.87 (3H, s, H-13), 2.14 (2H, d, brd, $J = 5.5$ Hz, H-6), 2.22 (2H, t, $J = 6.25$ Hz, H-9), 2.34 (2H, s, brd, H-10), 4.79 (2H, s, 4'-CH $_2$ -), 4.97 (1H, t, $J = 6.5$ Hz, H-11), 5.12 (1H, t, $J = 8.0$ Hz, H-7), 5.91 (1H, d, $J = 16.0$ Hz, H-3), 5.93 (1H, d, $J = 16.0$ Hz, H-3), 6.39 (1H, d, $J = 16.0$ Hz, H-4), 7.49 (2H, d, $J = 7.5$ Hz, H-3', H-5'), 7.56 (2H, d, $J = 7.5$ Hz, H-2', H-6'). $^{13}\text{C-NMR}$ (125 MHz, DMSO- d_6 , ppm); δ 15.1 (C-14), 15.3 (C-13), 24.7 (C-10), 28.0 (C-15), 36.8 (C-6), 38.6 (C-5), 39.5 (C-9), 45.3 (4'-CH $_2$ -), 123.9 (C-7), 124.0 (C-3), 128.2 (C-2', C-6'), 128.7 (C-3', C-5'), 132.8 (C-11), 132.9 (C-1'), 134.0 (C-12), 135.8 (C-8), 140.9 (C-4'), 168.7 (C-2), 171.5 (C-7'). ESI-HRMS calculated for $\text{C}_{23}\text{H}_{28}\text{ClNO}_2$: $[\text{M}+\text{H}]^+$ 386.18868, Found: 386.18862.

General procedure for the synthesis of azazerumbone derivatives **31-34**

To a stirred solution of each amines **33a-d** (0.5 mmol), K $_2$ CO $_3$ (200 mg, 1.44 mmol) in DMF (5 mL), intermediate **29** (195 mg, 0.5 mmol) and (1-butyl) triethyl ammonium bromide 12 mg (0.05 mmol) was added. The mixture was stirred at room temperature for overnight then poured into water (50 mL) and extracted with CH $_2$ Cl $_2$ (3×40 mL). The combined dichloromethane extract was dried over anhydrous sodium sulfate and solvent was removed by rotary evaporation to obtain crude **31-34** that were purified by flash chromatography

eluting with chloroform: MeOH 9:1.

4-[4-Methylpiperazylmethyl]benzoyl-*N*-azazerumbone (**31**): Yield 42.2%; Pale yellow oil. ¹H-NMR (500 MHz, DMSO-*d*₆, ppm); δ 1.08 (6H, s, H-15, H-16), 1.57 (3H, s, H-14), 1.85 (3H, s, H-13), 2.15 (3H, s, 4"-CH₃), 2.33 (8H, s, H-2", H-6", H-3", H-5"), 3.48 (2H, s, 4'-CH₂-), 4.97 (1H, t, *J* = 5.75 Hz, H-11), 5.10 (1H, t, *J* = 7.25, H-7), 5.93 (1H, d, *J* = 16.0 Hz, H-3), 6.39 (1H, d, *J* = 16.0 Hz, H-4), 7.34 (2H, d, *J* = 8.0 Hz, H-3', H-5'), 7.51 (2H, d, *J* = 8.0 Hz, H-2', H-6'). ¹³C-NMR (125 MHz, DMSO-*d*₆, ppm); δ 15.1 (C-14), 15.3 (C-13), 24.7 (C-10), 28.0 (C-15, C-16), 36.8 (C-6), 38.7 (C-5), 39.5 (C-9), 45.6 (4"-CH₃), 52.5 (C-2", C-6"), 54.6 (C-3", C-5"), 61.6 (4'-CH₂), 124.0 (C-3, C-7), 128.0 (C-3', C-5'), 128.5 (C-2', C-6'), 132.7 (C-11), 132.9 (C-1'), 133.9 (C-12), 134.4 (C-8), 142.4 (C-4'), 153.3 (C-4), 168.6 (C-2), 171.8 (C-7). ESI-HRMS calculated for C₂₈H₃₉N₃O₂: [M+H]⁺ 450.31205, Found: 450.31253.

N-[4-(4-Ethylpiperazylmethyl)benzoyl]azazerumbone (**32**). Yield 45.6%; Pale yellow oil. ¹H-NMR (500 MHz, DMSO-*d*₆, ppm); δ 0.97 (3H, t, *J* = 7.25 Hz, 4"-CH₂-CH₃), 1.11 (6H, s, H-15, H-16), 1.57 (3H, s, H-14), 1.85 (3H, s, H-13), 2.13 (2H, s, H-6), 2.22 (2H, d, *J* = 5.0 Hz, H-9), 2.32 (10H, m, H-2", H-6", H-3", H-5", 4"-CH₂-CH₃), 3.48 (2H, s, 4'-CH₂-), 4.95 (1H, t, *J* = 6.25 Hz, H-11), 5.10 (1H, t, *J* = 7.5 Hz, H-7), 5.93 (1H, d, *J* = 16.0 Hz, H-3), 6.39 (1H, d, *J* = 16.0 Hz, H-4), 7.34 (2H, d, *J* = 8.0 Hz, H-3', H-5'), 7.50 (2H, d, *J* = 8.0 Hz, H-2', H-6'). ¹³C-NMR (125 MHz, DMSO-*d*₆, ppm); δ 11.9 (C-4", -CH₂-CH₃), 15.1 (C-14), 15.4 (C-13), 24.7 (C-10), 28.0 (C-15, C-16), 36.8 (C-6), 38.7 (C-5), 39.5 (C-9), 51.5 (4"-CH₂-), 52.3 (C-2", C-6"), 52.6 (C-3", C-5"), 61.6 (4'-CH₂-), 124.0 (C-3, C-7), 128.0 (C-3', C-5'), 128.5 (C-2', C-6'), 132.7 (C-11), 132.9 (C-1'), 133.9 (C-12), 134.4 (C-8), 142.4 (C-4'), 153.3 (C-4), 168.6 (C-2), 171.8 (C-7). ESI-HRMS calculated for C₂₉H₄₁N₃O₂: [M+H]⁺ 464.32770, Found: 464.32775.

4-[4-(2-Hydroxyethyl)piperazinylmethyl]benzoyl-*N*-azazerumbone (**33**). Yield 46.4%; Yellow oil. ¹H-NMR (500 MHz, DMSO-*d*₆, ppm);

1.11 (6H, s, H-15, H-16), 1.57 (3H, s, H-14), 1.85 (3H, s, H-13), 2.14 (2H, d, *J* = 6.0 Hz, H-6), 2.21 (2H, t, *J* = 6.0 Hz, H-9), 2.37 (10H, m, H-2", H-6", H-3", H-5", H-10), 3.47 (4H, s, 4'-CH₂-, 4"-CH₂-CH₂-OH), 4.35 (1H, t, *J* = 5.5 Hz, -OH), 4.96 (1H, t, *J* = 6.5 Hz, H-11), 5.10 (1H, t, *J* = 7.0 Hz, H-7), 5.93 (1H, d, *J* = 15.75 Hz, H-3), 6.38 (1H, d, *J* = 15.75 Hz, H-4), 7.34 (2H, d, *J* = 8.0 Hz, H-3', H-5'), 7.51 (d, *J* = 8.0 Hz, 2H, H-2', H-6'). ¹³C-NMR (125 MHz, DMSO-*d*₆, ppm); δ (ppm): 15.2 (C-14), 15.5 (C-13), 24.8 (C-10), 28.1 (C-15, C-16), 36.9 (C-6), 38.8 (C-5), 39.5 (C-9), 52.7 (C-2", C-6"), 53.2 (C-3", C-5"), 58.5 (4"-CH₂-CH₂-OH), 60.2 (4"-CH₂-CH₂-OH), 61.7(4'-CH₂-), 124.1 (C-3, C-7), 128.1 (C-3', C-5'), 128.7 (C-2', C-6'), 132.9 (C-11), 133.0 (C-1'), 134.1 (C-12), 134.5 (C-8), 142.4 (C-4'), 153.3 (C-4), 168.8 (C-2), 172.0 (C-7). ESI-HRMS calculated for C₂₉H₄₁N₃O₃: [M+H]⁺ 480.32261, Found: 480.32275.

N-[4-(Benzotriazolyl-2)methylbenzoyl]azazerumbone (**34**): Yield 43.2%; white solid; mp: 128-130 °C. ¹H-NMR (500 MHz, DMSO-*d*₆, ppm); 1.09 (6H, s, H-15, H-16), 1.55 (3H, s, H-14), 1.84 (3H, s, H-13), 2.12 (2H, s, brd, H-6), 2.20 (2H, t, *J* = 5.75 Hz, H-9), 2.32 (2H, s, H-6), 4.95 (1H, t, *J* = 6.25 Hz, H-11), 5.10 (1H, t, *J* = 7.5 Hz, H-7), 5.91 (1H, d, *J* = 15.75 Hz, H-3), 6.02 (2H, s, 4'-CH₂-), 6.37 (1H, d, *J* = 15.75 Hz, H-4), 7.43 (m, 4H, H-4", H-5", H-3', H-5'), 7.54 (2H, d, *J* = 8.0 Hz, H-2', H-6'), 7.92 (m, 2H, H-3", H-6"). ¹³C-NMR (125 MHz, DMSO-*d*₆, ppm); 15.1 (C-14), 15.3 (C-13), 24.7 (C-10), 27.9 (C-15, C-16), 36.8 (C-6), 38.6 (C-5), 39.3 (C-9), 59.0 (4'-CH₂-), 117.8 (C-4", C-7"), 123.9 (C-7), 124.0 (C-3), 126.6 (C-5", C-6"), 128.1 (C-3', C-5'), 128.3 (C-2', C-6'), 132.8 (C-11, C-12), 133.9 (C-1'), 135.8 (C-8), 138.5 (C-4), 143.9 (C-8", C-9"), 153.5 (C-4), 168.6 (C-2), 171.5 (C-7). ESI-HRMS calculated for C₂₉H₃₂N₄O₂: [M+H]⁺ 479.26015, Found: 479.26039.

3. RESULTS AND DISCUSSION

3.1 Chemistry

The preparation of key intermediates **4**, **5** and

9, 10 was outlined in Scheme 1. Azazerumbones **4** and **5** were synthesized from zerumbone (**1**) using a known procedure [8]. For the synthesis of azazerumbone oxide **9, 10**, zerumbone was first reacted with *m*-chloroperbenzoic acid in CH₂Cl₂ at -5 °C to give zerumbone oxide (**6**) in 73% yield according to a known procedure [12] and : (c.0.99, 0.00) [13]. In the next step, **6** was reacted with hydroxylamine hydrochloride in ethanol in the presence of K₂CO₃ to afford a mixture of zerumbone oxide oxime diastereomers **7** and **8**. In the reaction of zerumbone and zerumbone oxide with hydroxylamine, 1,2-addition reaction of hydroxylamine with dienone moiety in zerumbone and zerumbone oxide occurred under the catalyst of a weak base K₂CO₃ to form oximes **2,3** and **7,8** instead of 1,4-addition of Michael reaction (Scheme 1) [8,14,15], followed by Beckman rearrangement in acetonitrile [8] to obtain designed azazerumbone oxides **9** and **10** in 9.9 and 39.1 % yields, respectively. In addition to designed products, some unexpected by-products may be formed in Beckman rearrangement under the Lewis catalyst, ZnCl₂ as described in reference [16]. However the yield of products was too low to isolate. Structures of azazerumbone oxides **9** and **10** were determined by 1-D, 2D-NMR and HRMS spectra.

Reagents and conditions: (i) HO-NH₂·HCl, K₂CO₃, EtOH, rt, 90.0 %; (ii) ZnCl₂, CH₃CN, reflux, 6 h, 13.1 and 39.0 %; (iii) HO-NH₂·HCl, K₂CO₃, EtOH, rt, 92.0 %; (iv) ZnCl₂, CH₃CN, reflux, 6 h, 9.1 and 39.1 %.

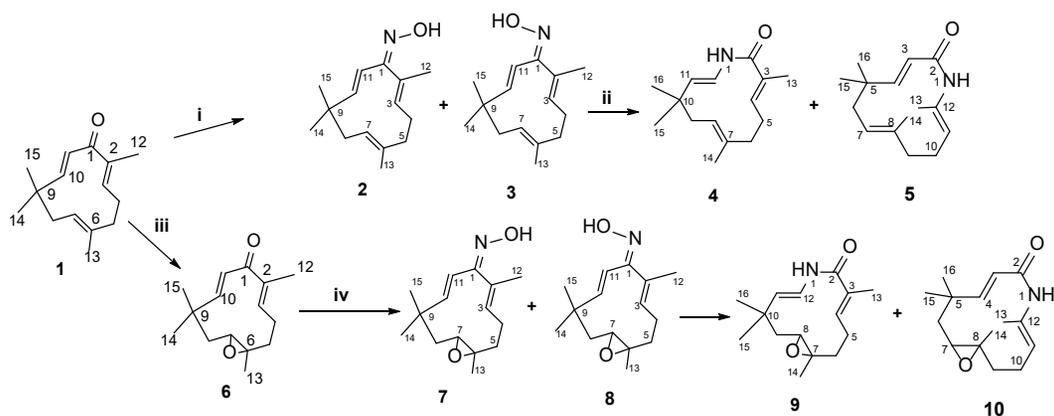
The synthesis of derivatives **16-18** was started from azazerumbone **4, 5** and azazerumbone oxide **10** in two steps. In the first step, propargyl group was introduced into **4, 5** and **10** by *N*-alkylation with propargyl bromide **11** in THF catalyzed by a strong base NaH to give **12, 13** and **14** in 76.1, 87.0 and 77.2 % yields, respectively (Scheme 2). The presence of propargyl group in the intermediates **12, 13** and **14** was characterized by the signals at 3.02-3.08 ppm of protons H-19 and signals at 4.16-4.41 ppm of protons H-17 in ¹H-NMR

spectra and signals at 32-33ppm for carbons: C-17, 80.1-80.0 ppm for carbons C-18 and 73.30-73.5 for carbons C-19 in ¹³C-NMR spectra. In the next step, the triazole click cyclization of alkynes **12, 13** and **14** with AZT **15** were performed in DMSO catalyzed by CuI to afford designed derivatives **16-18** in 54.8-63.3 % yields.

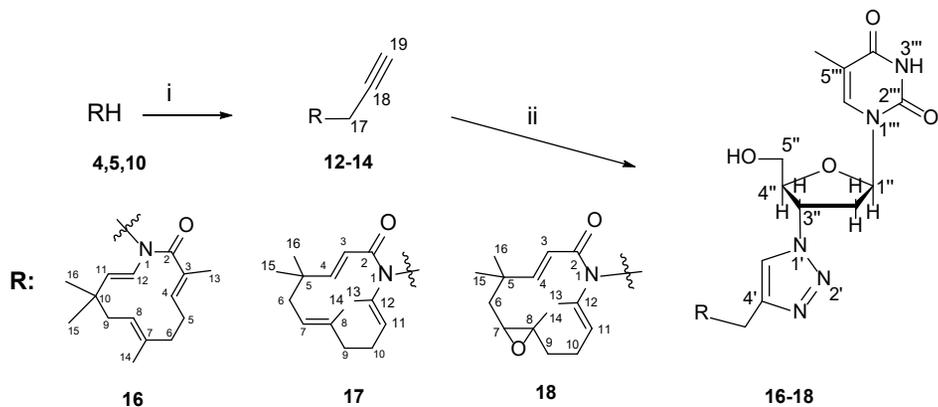
Reagents and conditions: (i) propargyl bromide, NaH, THF, 0 °C-rt, 76.1-87.0 %; (ii) AZT, CuI, DMSO, rt, 54.8-63.3 %.

The structure of **16-18** was confirmed by 1D, 2D and HRMS spectra. The similarity of the multiplicity and signal position of AZT and triazole moieties was easily observed in ¹H- and ¹³C-NMR spectra of compounds **16-18**. The spectral signal distinction among derivatives was found due to the resonance of protons and carbons in azazerumbones and azazerumbone oxide moieties. The presence of 4-methylene-1,2,3-triazole linker in **16, 17** and **18** was determined by singlet signals of proton 4'-CH₂- in the range of 4,60- 4.82 ppm and other singlet signals of proton H-5' from 8.05-8.09 ppm. The signals of the corresponding carbons were easily determined for example: 4'-CH₂- (38.3-40.4 ppm), C-4' (143.6-149.1 ppm) and C-5' (122.8-123.2 ppm). Compound **16** was selected to assign data of structure by 1D- and 2D-NMR spectra. The signals of protons H-17 and H-5' appeared as singlets at 4.77 and 8.04 ppm, respectively. The correlations in HSQC spectrum indicated all carbons 4'-CH₂- (38.3 ppm) and C-5' (122.8 ppm) attached to these protons. Finally, The key cross-picks of 4'-CH₂- (4.77 ppm) with C-2 (171.3 ppm), C-4' (143.6 ppm), C-5' (122.8 ppm) and C-12 (129.1 ppm) in HMBC spectrum proved the connection of azazerumbone **4** with AZT.

For the synthesis of conjugates **21-23**, 2-(10β-dihydroarteminoxy)ethyl bromide (**20**) was first formed in 46% yield by the etherification of dihydroxyartemisinin (**19**) (DHA) with 2-bromoethanol in CH₂Cl₂ in the presence of BF₃·Et₂O according to a known procedure [17]. Next, 2-(10β-dihydroarteminoxy)ethyl bromide (**20**) was used for *N*-alkylation reaction of azazerumbones



Scheme 1. Synthesis of intermediates 4, 5 and 9, 10.



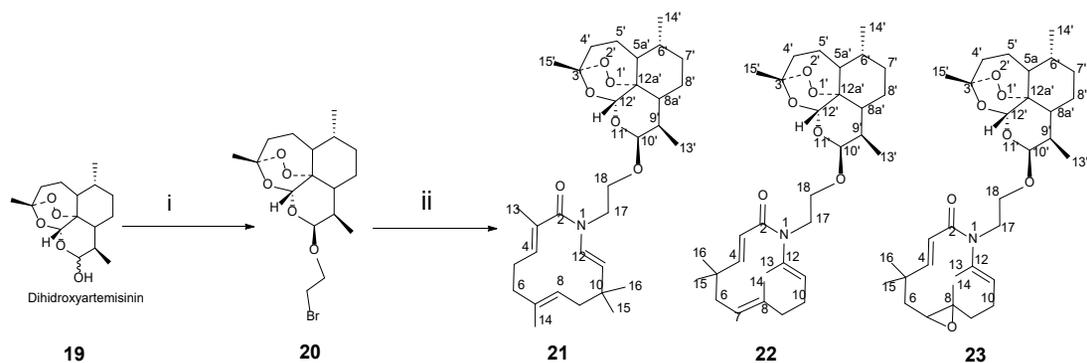
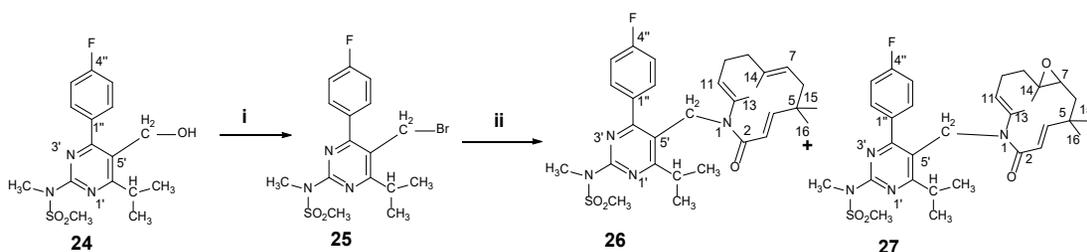
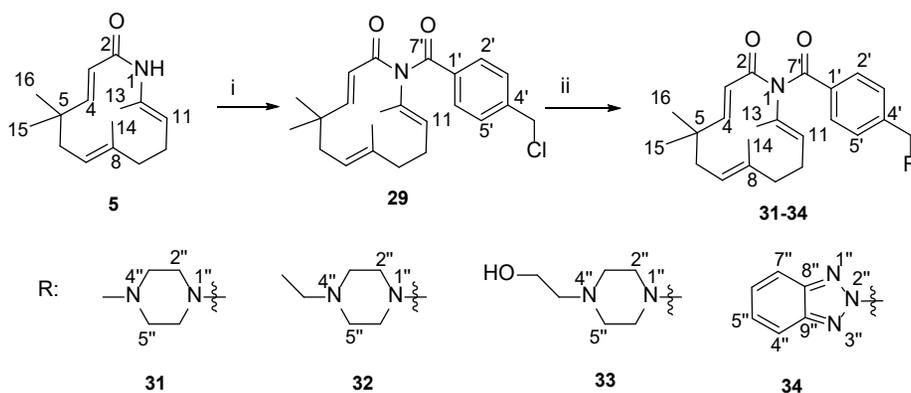
Scheme 2. Synthesis of 16-18.

4, 5 and azazerumbone oxide 10 in DMF and catalyzed by a mixture of K_2CO_3 and (1-butyl) triethylammonium bromide in microwave oven at $70^\circ C$ with maximum power output of 275 W for 25 minutes to give 21-23 in 46.5, 49.4 and 48.6% yields, respectively (Scheme 3). Here, strong bases as NaOH or NaH were not used to avoid the removal of endoperoxide bridge in DHA [18,19].

Reagents and conditions: (i) 2-bromoethanol, $BF_3 \cdot Et_2O$, CH_2Cl_2 , $0^\circ C$ -rt, 46.0%; (ii) azazerumbones 4, 5 and azazerumbone oxide 10, K_2CO_3 , KI (10% mol), (1-butyl)triethylammonium bromide (10% mol), DMF, MW 250 W, $70^\circ C$, 25 min. 46.5-49.4%.

Structure of conjugates 21-23 was confirmed by NMR and HRMS spectra. Among the synthesized

conjugates, compound 22 was selected to assign the proton and carbon signals by support of HSQC and HMBIC spectra. In 1H -NMR spectrum of 21, the assignment of signals in DHA and azazerumbone moieties based on 1H - and ^{13}C -NMR data of azazerumbone 5 and 2-(10 β -dihydroarteminoxy)ethyl bromide (20), dimethylene bridge was interpreted by multiplet signals of H-17 at 2.18 and 2.36 ppm, other multiplet signals at 3.34 and 3.69 ppm arose H-18. Correlations in HSQC spectrum indicated the corresponding carbons: C-17 (36.0 ppm) and C-18 (65.0 ppm). The connection of azazerumbone 5 and 2-(10 β -dihydroarteminoxy)ethyl bromide (20) by dimethylene bridge was confirmed by the presence of signals from protons and carbons of

Scheme 3. Synthesis of conjugates **21**, **22** and **23**.Scheme 4. Synthesis of conjugates **26**, **27**.Scheme 5. Synthesis of **30-33**

moieties **5** and **20** in $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ and HRMS of conjugate **22**.

The synthesis of conjugates **26** and **27** was outlined in Scheme 4 and started from PM **24**, a starting material for the synthesis of rosuvastatin that was used to treat several types of high cholesterol and prevent heart disease. Firstly, PM **24** was reacted with PBr_3 in acetonitrile to yield *N*-(5-(bromomethyl)-

4-(4-fluorophenyl)-6-isopropylpyrimidin-2-yl)-*N*-methylmethanesulfonamide (PBr) **25** by a known procedure [20]. Next, *N*-alkylation of azaserumbone **5** and azaserumbone oxide **10** with PBr was carried out at 80°C in DMF and catalyzed by a mixture of K_2CO_3 and (1-butyl) triethylammonium bromide to give conjugates **26**

and **27** in 43.5 and 37.4 %, respectively. Obtained NMR data agreed well with their structures.

Reagents and conditions: (i) PBr_3 , MeCN, 10-15 °C, %; (ii) azazerumbone **5**, **10**, K_2CO_3 , (1-butyl)triethylammonium bromide (10% mol), DMF, 80 °C, 24h, 43.5 and 37.4 %.

In last series **31-34**, two steps for the synthesis of **31-34** were outlined in Scheme 5. Firstly, *N*-acylation of azazerumbone **5** by 4-chloromethylbenzoyl chloride **28** was carried out in THF to afford intermediate **29** in 47.8 % yield. The acyl chlorides of rotudic and asiatic acids were also used for the acylation of azazerumbone **5**. However, the low conversion and by-products prevented the isolation of designed products. Next, several *N*-alkylations of **29** with various heterocyclic amines: *N*-methylpiperzine

30a, *N*-ethylpiperazine **30b**, *N*-(2-hydroxyethyl)piperazine **30c** and benzotriazole **30d** were performed in DMF at room temperature to give **31-34** in 42.2-46.4 % yields.

Reagents and conditions: (i) *p*-chloromethylbenzoyl chloride, THF, TEA, rt, 24 h, 47.8 %; (ii) amines **30a-d**, DMF, rt, 42.2-46.4 %.

^1H - and ^{13}C -NMR spectra of series **31-34** indicated the similarity of the multiplicity and signal position of protons and carbons in structural part of the intermediate **29**. Spectral signal distinction in ^1H - and ^{13}C -NMR spectra among derivatives **31-34** arose from protons and carbons in the attached amines. The assignment of protons and carbons in amine moieties of **31-34** based on reference [21].

Table 1. *In vitro* cytotoxic activity of target compounds.

No	Compounds	IC ₅₀ (µg/mL)				
		HepG2	LU-1	RD	FL	VERO-B4
1	16	-	-	23.70	-	-
2	17	-	-	21.16	-	-
3	18	-	-	-	-	-
4	21	0.86	2.82	0.67	0.90	9.56
5	22	0.82	3.81	0.69	0.20	10.02
6	23	1.96	12.46	1.90	4.07	-
7	26	3.94	-	2.70	5.61	-
8	27	11.78	-	10.23	-	-
9	29	15.89	16.25	18.69	-	-
10	31	18.34	19.94	-	-	-
11	32	18.50	17.68	17.36	-	-
12	33	19.92	-	-	-	-
13	34	19.44	-	16.72	11.07	-
14	Azazerumbone 4	-	-	-	-	-
15	Azazerumbone 5	-	17.82	-	-	-
16	Azazerumbone oxide 10	-	-	-	-	-
	Ellipticine	0.46	0.42	0.46	0.44	2.23

Note: The reference substance, ellipticine, exhibited cytotoxic activity against HepG-2 (ATCC-HB-8065), LU-1 (ATCC-HTB-57), RD (ATCC-CCL-136), FL (ATCC-CCL2) and African green monkey kidney cell VERO-B4 (ATCC-CCL-81) cells with IC₅₀ values of 0.46, 0.42, 0.46, 0.44 and 0.39 µg/mL, respectively. The values shown for these compounds are the average of three determinations.

3.2. Bioactivity

The synthesized derivatives were evaluated cytotoxicity with human cancer cell lines HepG-2, LU-1, RD, FL and normal cell VERO-B4 together with azazerumbones **4**, **5** and azazerumbone oxide **10** using ellipticin as a positive control.

The results in Table 1 showed that azazerumbone (**4**, **5**) and azazerumbone oxide **10** expressed weak or no activity against the tested cancer cell lines except weak activity of azazerumbone **5** against LU-1 cell line with IC₅₀ value of 17.82 µg/mL. Triazoles **16-18** of azazerumbones, azazerumbone oxide with AZT were also determined to have no activity. The derivatives of azazerumbone connected to heterocyclic amines **31-34** including 4-methyl-, 4-ethyl-, 4-(2-hydroxyethyl)piperazine and benzotriazole by *p*-methylbenzoyl linker had no improvement for their cytotoxicity. In particular, the conjugates **21-23**, **26-27** of azazerumbones and azazerumbone oxide with DHA and PM exhibited prominent cytotoxicity. Among those, conjugates **21-23** of azazerumbones and azazerumbone oxide with DHA via dimethylene linker had strong activity against HepG-2, LU-1, RD and FL cancer cell lines with the IC₅₀ values ranging from 0.2-3.81 µg/mL and much stronger than that of zerumbone. Dihydroartemisinin was also reported to have activity against HepG2 and LU with the IC₅₀ values of 21µM and 21.94 µg/mL [22, 23]. Despite exhibiting weak cytotoxicity, azazerumbones could be suitable anti-mutagenic substrates to carry active compounds and expression of their activities. In addition, comparing IC₅₀ of conjugates **22** and **23** with all four cancer cell lines tested also showed negligible contribution to activity of isolated double bond in azazerumbones. Next, all the derivatives were evaluated cytotoxic activity with normal cell VERO-B4. Among synthesized compounds, two conjugates of azazerumbone and azazerumbone oxide with DHA **22**, **23** expressed the activity with the IC₅₀ values of 9.56 and 10.52 µg/mL, the remaining derivatives had no activity against this cell line. Finally, two derivatives of azazerumbone with PM also exhibited cytotoxic

activity and selectivity to HepG-2, RD and FL in which compound **26** showed stronger cytotoxicity with the IC₅₀ values of 3.94, 2.7 and 5.61 µg/mL, respectively.

4. CONCLUSIONS

Structural diversity synthesis based on azazerumbones and azazerumbone oxide was carried out to obtain 12 novel derivatives for cytotoxicity screening on four human cancer cell lines: HepG-2, LU-1, RD and FL. Effective and selective characteristic for cytotoxicity of novel derivatives were found from the conjugates of azazerumbones and azazerumbone oxide with DHA and PM. The results of cytotoxic evaluation of derivatives also indicated that the isolated double bond in azazerumbones skeleton had a little influence in their cytotoxicity. Bioactive data could be useful suggestion for the use of azazerumbones as effective substrates to carry drugs without side effects.

ACKNOWLEDGEMENTS

This research is funded by Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number 106-NN.99-2015.26. Also the authors thank Assoc. Prof. Tran Van Loc-Institute of Chemistry, Vietnam Academy of Science and Technology (VAST) for providing acyl chlorides of rotundic and asiatic acids

REFERENCES

- [1] Dung N.X., Chinh T.D., Rang D.D. and Leclereq P.A., *J. Essent. Oil Res.*, 1993; **5**: 553-555. <http://doi.org/10.1080/10412905.1993.9698277>.
- [2] Xia Y., Yang Z.Y., Xia P., Bastow K.F., Nakanishi Y. and Lee K.H., *Bioorg. Med. Chem. Lett.*, 2000; **10**: 669-701. [http://doi.org/10.1016/S0960-894X\(00\)00072-X](http://doi.org/10.1016/S0960-894X(00)00072-X).
- [3] Giang P.M., Son P.T., Jin H.S., Lee J.H. and Lee J.J., *Sci. Pharm.*, 2009; **77**: 589-596. <http://doi.org/10.3797/scipharm.0907-16>.
- [4] Abdul A.B., Abdelwahad S.I., Al-zubairi A.S., Elhassan M.M. and Murali S.M., *Int. J.*

- Pharmacol.*, 2008; **4**: 301-304. DOI: 10.3923/ijp.2008.301.304.
- [5] Adbul A.B.H., Al-Zubairi A.S., Tailan N.D., Wahab S.I.A, Zain Z.N.M., Ruslay S. and Syam M.M., *Int. J. Pharmacol.*, 2008; **4**: 160-168. DOI: 10.3923/ijp.2008.160.168
- [6] Murakami A., Takahashi D., Kinoshita T., Koshimizu K., Kim H.W., Yoshihiro A., Nakamura Y., Jiwajinda S., Terao J. and Ohigashi H., *Carcinogenesis*, 2002; **23**: 795-802. <http://doi.org/10.1093/carcin/23.5.795>.
- [7] Huang G.C., Chien T.Y., Chen L.G. and Wang C.C., *Planta Med.*, 2005; **71**: 219-224. DOI: 10.1055/s-2005-837820.
- [8] Kumar S.C.S., Srinivas P., Negi P.S. and Betadaias B.K., *Food Chem.*, 2013; **141**: 1097-1103. <http://doi.org/10.1016/j.foodchem.2013.04.021>.
- [9] Truong V.V., Nam T.D., Hung T.N., Nga N.T., Quan P.M., Chinh L.V. and Jung S.H., *Bioorg. Med. Chem. Lett.*, 2015; **25**: 5182-5185. <http://doi.org/10.1016/j.bmcl.2015.09.069>.
- [10] Monks A., Scudiero D., Skehan P., Shoemaker R., Paull K., Vistica D., Hose C., Langley J., Cronise P., Campbell H., Mayo J. and Boyd M., *J. Natl. Cancer. Inst.*, 1991; **83**: 757-766. <http://doi.org/10.1093/jnci/83.11.757>.
- [11] Scudiero D. A., Shoe-maker R.H., Paull K.D., Monks A., Tierney S., Nofziger T.H., Currens M.J., Seniff D. and Boyd M.R., *Cancer Res.*, 1988; **48**: 4827-4833. DOI: Published September 1988.
- [12] Kitayama T., Yamamoto K., Utsumi R., Takatani M., Okamoto T., Hill R.K., Kawai Y. and Sawada S., *Biosci. Biotechnol. Biochem.*, 2001; **65**(10): pp. 2193-2199. DOI: 10.1271/bbb.65.2193.
- [13] Sadhu S.K., Khatun A., Ohtsuki T. and Ishibashi M., *Nat. Prod. Res.*, 2007; **21**(10): 1242-1247. <http://doi.org/10.1080/14786410701371421>.
- [14] Du Y., Mao D., Zhang-Negrerie D. and Zhao K., *PATAI's Chemistry of Functional Groups*, John Wiley & Sons, Ltd., 2009-2010. DOI: 10.1002/9780470682531.pat0501
- [15] Kitayama T., Yokoi T., Kawai Y., Hill R.K., Morita M., Okamoto T., Yamamoto Y., Fokin V.V., Sharpless K.B. and Sawada S., *Tetrahedron*, 2003; **59**: 4857-4866. [http://doi.org/10.1016/S0040-4020\(03\)00667-7](http://doi.org/10.1016/S0040-4020(03)00667-7)
- [16] Minassi A., Pollastro F., Chianese G., Caprioglio D., Tagliatela-Scafati O. and Appendino G., *Angew Chem. Int. Ed.*, 2017; **56**: 7935-7938. DOI: 10.1002/anie.2017034550.10.
- [17] Yajing L., Zijian L., Jiyue S., Huimin C., Bin M., Peng L. and Ping G., *Molecules*, 2013; **18**: 2864-2877. doi.org/10.3390/molecules18032864.
- [18] Liu J.M., Ni M.Y. and Fan J.F., *Acta Chim. Sin.*, 1979; **37**: 129-143.
- [19] Zeng M.Y., Li L.N., Chen S.F., Li G.Y., Liang X.T., Chen M. and Clardy J., *Tetrahedron*, 1983; **39**: 2941-2946. [doi.org/10.1016/S0040-4020\(01\)92155-6](http://doi.org/10.1016/S0040-4020(01)92155-6).
- [20] Nam P.D., Truong V.V. and Dan N.T., *Vietnam. J. Chem.*, 2012; **50**: 81-83.
- [21] Chinh L.V., Hung T.N., Nga N.T., Hang T.T.N., Mai T.T.N. and Tarasevich V. A., *Russ. J. Org. Chem.*, 2014; **50**: 1767-1774. DOI: 10.1134/S1070428014120094.
- [22] Gaur R., Pathania A.S., Malik F.A., Bhakuni R.S. and Verma R.K., *Eur. J. Med. Chem.*, 2016; **122**: 232-246. <http://dx.doi.org/10.1016/j.ejmech.2016.06.035>
- [23] Zhang B., Zhang Z., Wang J., Yang B., Zhao Y., Rao Z. and Gao J., *Oncol. Lett.*, 2018; **15**: 7531-7536. DOI: 10.3892/ol.2018.8276