SYNTHESIS AND CYTOTOXIC ACTIVITY EVALUATION OF NOVEL DERIVATIVES OF MURRAYAFOLINE A

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ABSTRACT

Two new conjugates of murrayafoline A with zerumbone and artemisinin 3, 11 were prepared by N-alkylation, in which, compound 3 was synthesized from two consecutive N-alkylation reactions. Their cytotoxicity was evaluated on four human cancer cell lines Hep-G2, LU, RD and Fl. The result showed that both compounds exhibited no activity against the tested cell lines.

Keywords: murrayafoline A, N-alkylation, cytotoxicity, sesquiterpene, conjugate.

1. INTRODUCTION

A number of natural carbazole alkaloids were recognized to exhibit potential biological activities such as antimicrobial [1, 2], anti-HIV [3], anticancer [4, 5, 6] activities. Among them, several compounds were used as drugs for treatment of cancer [7]. Especially, murrayafoline A, a well-known carbazole alkaloid is promising cytotoxic carbazole in the roots of G. stenocarpa in Vietnam. This carbazole was reported to show significant growth suppression of the human leukemia cell line HL-60 due to apoptosis mediated by the activation of the caspase-9/caspase-3 pathway [7] and prevent heart diseases [8]. Recently, some of the N-substituted derivatives were synthesized and evaluated the anti-inflammatory activity [9]. However, no reports on the synthesis and biological evaluation of conjugates between murrayafoline A and other bioactive compounds are available. In this respect, we report herein the synthesis and cytotoxicity evaluation of two new conjugates of murrayafoline A and sesquiterpenes.

2. MATERIALS AND METHODS

All chemicals were purchased from Sigma-Aldrich (USA). Murrayafoline A was isolated from rhizome of Glycosmis stenocarpa in Vietnam. Melting points were measured in open capillary tubes on a Buchi 530 (Switzerland) melting point apparatus and were uncorrected. NMR spectra were recorded on a Bruker Advance 500M using tetramethylsilane (TMS) as
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internal standard. Mass spectra were recorded on a FTICR MS Varian and an Agilent 1200 Series LC-MSD 6310 Ion Trap LC/MS.

1.1. Synthesis of conjugate 3

2.1.1. Synthesis of 3-(1-methoxy-3-methyl-N-carbazolyl)propyl bromide 2

Compound 2 was synthesized from murrayafoline A according to the procedure as described in reference [9].

2.1.2. Synthesis of azazerumbone 7, 8

Synthesis of zerumbone oximes 5, 6

To a solution of zerumbone (0.3 g, 1.38 mmol) in absolute ethanol (10 mL), hydroxylamine hydrochloride (0.92 g, 13.8 mmol) and K$_2$CO$_3$ (0.96 g, 13.8 mmol) were added at room temperature. The mixture was stirred for overnight and the solid was filtered off and washed with ethanol. The filtrate was concentrated under reduced pressure to afford a white solid mass, which was dissolved in dichloromethane (10 ml). The organic layer was washed with water (3 × 10 mL) and dried over anhydrous sodium sulfate, the solvent was removed by rotary evaporation to give a mixture (0.25 g, 90% yield) of E- and Z- zerumbone as white solid which was used for next step without purification.

Synthesis of azazerrumbones 7, 8

To a solution of zerumbone oximes 5, 6 (1.5 g, 6.43 mmol) in dry acetonitrile (20 mL), anhydrous zinc chloride (0.17 g, 1.28 mmol) was added. The reaction mixture was reflux under nitrogen stream line and the progress of the reaction was monitored on TLC using n-H:EtOAc 3:1. After completion of the reaction, solvent was removed under reduced pressure and the residue was taken in dichloromethane (20 mL). The organic layer was washed with water (3 × 20 mL), followed by brine (20 mL) and then dried over anhydrous sodium sulfate. The solvent was evaporated to give crude product that was chromatographed on silica gel using n-hexane : ethylacetate 3:1 to afford 7 (200 mg) and 8 (600 mg).

Azazerumbone 7: Yield 39.1 % (white crystals); mp: 145-147°C; ESI-MS (m/z): 234.1 [M + H]$^+$; $^1$H-NMR (500 MHz, DMSO-$_d_6$, ppm)$^\delta$ 8.35 (s, 1H, H-1'), 6.13 (d, $J$ = 15.5 Hz, 1H, H-4'), 5.05 (t, $J$ = 7 Hz, 1H, H-11'), 4.72 (t, $J$ = 7 Hz, 1H, H-7'), 2.24 (m, 2H, 2H-10'), 2.25 (d, 2H, 2H-9'), 2.12 (d, broad, $J$ = 6.5 Hz, 2H, H-6'), 1.68 (s, 3H, H-13'), 1.52 (s, 3H, H-14'), 1.05 (s, 3H, H-15'), 1.05 (s, 3H, H-16'). $^{13}$C-NMR (125 MHz, DMSO-$_d_6$, ppm) $^\delta$ 166.7 (C-2'), 147.5 (C-4'), 133.7 (C-8'), 130.8 (C-12'), 125.1 (C-11'), 124.4 (C-7'), 122.6 (C-3'), 39.0 (C-9'), 38.0 (C-6'), 36.2 (C-5'), 28.5 (C-15'), 28.5 (C-16'), 24.4 (C-10'), 16.7 (C-14'), 14.7 (C-13').

Azazerumbone 8: Yield 9.7 % (white crystals); mp: 165-167°C; ESI-MS (m/z): 234.3 [M + H]$^+$; $^1$H-NMR (500 MHz, DMSO-$_d_6$, ppm)$^\delta$ 9.20 (d, $J$ = 9.5 Hz, 1H, H-1'), 6.05 (d, d, $J_1$ = 10 Hz, $J_2$ = 14.5 Hz, 1H, H-12'), 5.37 (t, $J$ = 6.5 Hz, 1H, H-4'), 5.13 (t, $J$ = 6 Hz, 1H, H-8'), 4.68 (d, $J$ = 14.5 Hz, 1H, H-7'), 2.29 (m, 2H, 2H-5'), 2.23 (t, $J$ = 6.0 Hz, 2H, H-9'), 2.06 (d, $J$ = 6.5 Hz, 2H, H-9'), 1.74 (s, 3H, 3H-13'), 1.52 (s, 3H, H-14'), 1.00 (s, 3H, H-15'), 1.00 (s, 3H, H-16'). $^{13}$C-NMR (125 MHz, DMSO-$_d_6$, ppm) $^\delta$ 172.0 (C-2'), 136.0 (C-4'), 133.2 (C-7'), 128.7 (C-3'), 127.6 (C-12'), 125.0 (C-8'), 117.3 (C-11'), 39.0 (C-6'), 38.1 (C-9'), 34.7 (C-10'), 32.2 (C-15'), 30.2 (C-16'), 24.1 (C-5'), 14.6 (C-14'), 13.1 (C-13').
2.1.3. Synthesis of conjugate 3

A mixture of 7 (233 mg, 1 mmol), K$_2$CO$_3$ (0.21 g, 1.5 mmol), compound 2 (0.42 g, 1.3 mmol) and (1-butyl)triethylammonium bromide (23.8 mg, 0.1 mmol) in dry dimethylformamide (15 mL) was thoroughly stirred at room temperature for overnight and solvent was removed under reduced pressure. The resulting mixture was dissolved in water (20 mL) and extracted with EtOAc (3 × 20 mL). The combined extract was dried over anhydrous sodium sulfate and solvent was removed under reduced pressure. Crude 3 were purified by column chromatography on silica gel eluting with $n$-hexane : ethylacetate.

Yield 53.6 % (White powder); mp: 130-132°C; $^1$H-NMR (500 MHz, DMSO-d$_6$, ppm) δ 8.03 (d, $J = 7.5$ Hz, 1H, H-8), 7.50 (s, broad, 2H, H-4, H-5), 7.39 (t, $J = 7.5$ Hz, 1H, H-7), 7.13 (t, $J = 7.5$ Hz, 1H, H-6), 6.85 (s, 1H, H-2), 6.14 (d, $J = 15.5$ Hz, 1H, H-4 ), 5.79 (d, $J = 15.5$ Hz, 1H, H-3 ), 4.94 (m, 2H, H-7 , H-11 ), 4.55 (m, 2H, 9-CH$_2$), 2.14 (s, broad, 4H, 9-CH$_2$CH$_2$-CH$_2$-), 1.83-1.98 (s, broad, 6 H, H-10 , H-6 , H-9 ), 1.68 (s, 3H, H-13 ), 1.53 (s, 6H, H-15 , H-16 ). $^{13}$C-NMR (125 MHz, DMSO-d$_6$, ppm) δ 165.3 (C-2), 147.3 (C-4), 146.1 (C-1), 140.3 (C-13), 133.8 (C-8), 133.7 (C-12), 131.8 (C-11), 128.6 (C-3), 127.3 (C-10), 125.4 (C-7), 124.5 (C-7), 124.1 (C-12), 123.2 (C-3), 122.2 (C-11), 121.0 (C-6), 118.6 (C-8), 112.5 (C-4), 109.2 (C-2, C-5), 55.7 (1-OCH$_3$), 42.7 (9-CH$_2$), 40.6 (1'-CH$_3$), 38.1 (C-9'), 36.0 (C-5'), 28.6 (9-CH$_2$CH$_2$-), 25.0 (C-10'), 21.3 (3-CH$_3$), 15.0 (C-14'), 14.7 (C-13'). HR-MS (m/z): Found 485.31675 [M+H]$^+$, calculated for C$_{32}$H$_{41}$N$_2$O$_2$: 485.31625

2.2. Synthesis of conjugate 11

2.2.1. Synthesis of 2-(10β-dihydroartemisinoxy)ethyl bromide 10

The intermediate 10 was synthesized from dihydroartemisinin and 2-bromoethanol according to the procedure as described in reference [10].

2.2.2. Synthesis of conjugate 11

A mixture of murrayafoline A (1) (0.211 g, 1 mmol), K$_2$CO$_3$ (0.621 g, 4.5 mmol), compound 10 (1.25 g, 3 mmol) and (1-butyl)triethylammonium bromide (35.7 mg, 0.15 mmol) in dry dimethylformamide (15 mL) was stirred at 70°C for 48 h and solvent was removed under reduced pressure. The resulting mixture was dissolved in water (20 mL) and extracted with EtOAc (3 × 20 mL). The combined extract was dried over anhydrous sodium sulfate and solvent was removed under reduced pressure to afford crude 11 that was purified by column chromatography on silica gel eluting with $n$-hexane : ethylacetate 2:1.

Yield 45.8 % (white crystals); mp: 176 – 178 °C; $^1$H-NMR (500 MHz, DMSO-d$_6$, ppm) δ 7.99 (d, $J = 7.5$ Hz, 1H, H-8), 7.53 (d, $J = 8$ Hz, 1H, H-5), 7.49 (s, 1H, H-4), 7.37 (t, $J = 8$ Hz, 1H, H-7), 7.12 (t, $J = 7.5$ Hz, 1H, H-6), 6.85 (s, 1H, H-2), 4.80 (m, 1H, 9-CH$_2$-), 4.69 (m, 1H, 9-CH$_2$-), 4.59 (s, 1H, H-12), 4.54 (d, $J = 3.5$ Hz, 1H, H-10'), 4.17 (m, 1H, 10' -O-CH$_3$-), 3.96 (s, 3H, 1-OCH$_3$), 3.64 (m, 1H, 10' -O-CH$_2$-), 2.47 (s, 3H, 3-CH$_3$), 2.22 (m, 1H, H-9'), 2.08 (m, 1H, 4'-CH$_2$-), 1.88 (m, 1H, 4' -CH$_2$-), 1.66 (m, 1H, 5'-CH$_2$-), 1.09 (m, 1H, 5'-CH$_2$-), 1.20 (s, 3H, 3' -CH$_3$), 1.15 (m, 1H, H-8'), 1.09 (m, 1H, 7'-CH$_3$-), 0.59 (m, 1H, 7'-CH$_2$-), 1.03 (m, 1H, 8'-CH$_3$-), 0.89 (m, 1H, 8' -CH$_3$-), 0.89 (m, 1H, H-5 a), 0.79 (d, $J = 6$Hz, 3H, 6'-CH$_3$), 0.59 (m, 1H, H-6'). $^{13}$C-NMR (125 MHz, DMSO-d$_6$, ppm) δ 147.0 (C-1), 141.7 (C-8), 129.2 (C-3), 128.4 (C-10), 125.7 (C-7), 125.1 (C-12), 123.0 (C-11), 120.3 (C-6),
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119.0 (C-8), 113.0 (C-4), 110.4 (C-2), 110.0 (C-5), 103.6 (C-3 ), 101.3 (C-10 ), 87.3 (C-12 ), 80.8 (C-12 a), 66.9 (10 -O-CH2-), 56.4 (15-OCH3), 52.6 (C-5 a), 44.9 (C-8 a), 44.3 (9-CH2-), 36.9 (C-6 ), 36.7 (C-4 ), 34.7 (C-7 ), 30.9 (C-9 ), 26.2 (C-15 ), 24.7 (C-5 ), 23.9 (C-8 ), 21.8 (3-CH3), 20.4 (C-14 ), 12.8 (C-13 ). HR-MS (m/z): 522.28550 [M+H]^+, calculated for C31H40NO6: 522.28502

2.3. In vitro anticancer assay

The in vitro evaluation of cytotoxicity was undertaken at Institute of Natural Products Chemistry (VAST) according to the protocol of Likhitwitayawid [10]. The determination of IC50 was carried out using four human cancer cell lines: HepG2, RD, LU-1 and FL with ellipticine was used as a positive control. The IC50 values were determined from dose-dependent curve plotted from five different concentration regimens (0 - 20 µM).

3. RESULT AND DISCUSSION

The aim of our synthesis was to introduce a murrayafoline A moiety connected to sesquiterpenes, artemisinin and zerumbone by 1,3-propane or 1,2-ethane bridges to form the conjugates 3, 11 and evaluate their cytotoxicity. The synthesis of new conjugates 3 and 11 was outlined in schemes 1, 2. For the synthesis of conjugates 3, on the one hand, 1-bromo-3-propyl substituent was first introduced into the position N-9 of murrayafoline A by N-alkylation reaction of 1 with 1,3-dibromopropane in THF in the presence of strong base NaH as catalyst to give the key intermediate 2 in 83.0 % yield and by-product 3 in 10 % [10] (scheme 1). On the other hand, azazerumbone 7 was also prepared from zerumbone by a procedure in two steps. Firstly, zerumbone was condensed with hydroxylamine hydrochloride in ethanol with potassium carbonate as catalyst to create zerumbone oximes 5 and 6 with E- and Z-configuration in 90 % yield. In the next step, Beckmann rearrangement of 5 and 6 was conducted in the presence of anhydrous zinc chloride in acetonitrile to afford azazerumbone 7 and 8 in 39.1 and 9.7 % yields, respectively. The confirmed structures of 7 and 8 agreed with 1H-, 13C-NMR and MS data. Finally, conjugate 3 was successfully synthesized in 53.6 % yield by N-alkylation of 7 with 2 using 10 % equivalent of transfer catalyst (1-butyl)triethylammonium bromide.

Scheme 1. Preparation of conjugate 3.

Reagents and conditions: (i) HO-NH2·HCl,EtOH, rt, 12 h, 90 %; (ii) ZnCl2, CH3CN, Reflux, 6 h, 39.1 % and 9.7 %; (iii) 1,3-Dibromopropane, NaH, THF, 72 h, 83 % (2) and 11 % (2a); (iv) 2, K2CO3, transfercatalyst 10 %, DMF, overnight, 53.6 %.
For the synthesis of conjugate 11, derivative 10 was prepared from dihydroartemisinin by etherification with 2-bromoethanol using BF3.Et2O in CH2Cl2 to give intermediate 10 in 46 % yield. This agent was used for N-alkylation of murrayafoline A in DMF in the presence of transfer catalyst (1-butyl)triethylammonium bromide at 70 °C for 48 h to obtain the target compound 11 in 45.8 % yield (Scheme 2).

\[ \text{Scheme 2. Preparation of conjugate 11.} \]

Reagents and conditions: (i) 2-bromoethanol, BF3.Et2O, CH2Cl2, 0 °C–rt, 46 %;
(ii) Murrayafoline A, K2CO3, transfer catalyst 10 %, DMF, 70 °C, 48 h, 45.8 %.

\[ ^1\text{H-NMR spectra of 11 indicated the presence of two components murrayafoline A and artemisinin. The aromatic protons of murrayafoline A appeared in the range with } \delta \text{ values from 6.90 – 8.11 ppm and the protons of artemisinin was found ranging from 0.53-4.92 ppm. Especially, the signals of carbon at 83 and 102 ppm confirmed that endoperoxide bridge of dihydroartemisinin was still preserved. The exact assignment for all protons and carbons in 11 was confirmed based on the HSQC and HMBC spectra. In HSQC spectrum, the correlation of carbons 9-CH2- and 9-CH2-CH2- with their protons indicated the signals of protons in 9-CH2- at 1.11 and 2.22 ppm and in 9-CH2-CH2- at 333 and 444 ppm, respectively. The key cross peaks between the protons of 9-CH2- with C-10 and C-13 of murrayafoline A were also found in HMBC. The structure of conjugate 11 agreed well with NMR and MS spectra.} \]

The evaluation of anti-proliferative activity of 3 and 11 was performed with the highest concentration of compounds 3 and 11 was used for the experiments was 20μg/mL. The IC50 values are listed in Table 1.

\[ \text{Table 1. The cytotoxic activity of 3 and 11.} \]

<table>
<thead>
<tr>
<th>No</th>
<th>Compounds</th>
<th>IC50 (μg/mL)</th>
<th>Hep-G2</th>
<th>RD</th>
<th>LU-1</th>
<th>FL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>&gt;</td>
<td>&gt;</td>
<td>&gt;</td>
<td>&gt;</td>
<td>&gt;</td>
</tr>
<tr>
<td>2</td>
<td>11</td>
<td>&gt;</td>
<td>&gt;</td>
<td>&gt;</td>
<td>&gt;</td>
<td>&gt;</td>
</tr>
<tr>
<td>3</td>
<td>Murrayafoline A</td>
<td>6.18</td>
<td>9.04</td>
<td>-</td>
<td>-</td>
<td>8.91</td>
</tr>
<tr>
<td>4</td>
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<td>0.32</td>
<td>0.18</td>
<td>0.21</td>
<td></td>
</tr>
</tbody>
</table>

\[ ^a\text{IC}_{50} \text{ shown for these compounds are the average of three determinations.} \]

\[ ^b\text{Cell lines: Hep-G2 (liver hepatocellular carcinoma, ATCC-HB-8065), LU (lung adenocarcinoma, ACTT-HBT-57), RD (rhabdomyosarcoma, ATCC-CCL-136) and FL (HeLa derivative, human cervix carcinoma).} \]
The results in Table 1 showed that the conjugates 3 and 11 had no cytotoxicity, while murrayafoline A exhibited activity against three human cancer cell lines including Hep-G2, RD and FL with IC_{50} values of 6.18, 9.04 and 8.91 µg/mL, respectively. The evaluation of cytotoxicity of murrayafoline A and its derivatives also revealed that the amine group (NH) at 9-position could play important role in the cytotoxicity of murrayafoline A. Finally, although the conjugates 3 and 11 did not show the activity as expected, but this is a good orientation in the design and synthesis of other bioactive derivatives of this carbazole.

3. CONCLUSION

Two new derivatives of murrayafoline A with zerumbone and artemisinin were successfully synthesized in good yields and their structures were elucidated by spectroscopic methods such as NMR and MS. The cytotoxic activity against the Hep-G2, LU, RD and FL of the conjugates was assayed and the structure-antiproliferative activity relationship was also discussed.

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REFERENCES


TÓM TÁT

TÓM HỘP VÀ ĐÁNH GIÁ HOẠT TÍNH GÂY ĐỘC TÊ BÀO CÁC DẦN XUẤT MỚI CỦA MURRAYAFOLINE A

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Từ khóa: murrayafoline A, N-alkyl hóa, hoạt tính gây độc tế bào, sesquiterpen, tổ hợp.