Med Chem Res (2013) 22:3536–3540
DOI 10.1007/s00044-013-0615-3

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, characterization, and in vitro antiproliferative activity of novel β-elemene monosubstituted derivatives

Guifeng Liu · Zhenwu Kong · Yumei Shen

Received: 22 December 2012 / Accepted: 3 May 2013 / Published online: 16 May 2013

Abstract A series of β-elemene monosubstituted ester, carbamate, acylamide, and carbamidine derivatives were synthesized via intermediates, β-elemene alcohol and β-elemene amine, which were synthesized from the traditional Chinese medicine, β-elemene. The structures of all the new compounds were characterized by NMR, IR, and HRMS. Their in vitro antiproliferative activities on HeLa cell line were tested through the WST-1 assay. The results show that the in vitro antiproliferative activities of the novel compounds are improved compared to that of the parent β-elemene.

Keywords β-Elemene · Carbamate · Acylamide · Carbamidine · Antiproliferative activity

Introduction

β-Elemene, (5S,7R,10S)-(−)-(1-methyl-1-vinyl-2,4-diisopropenyl-cyclohexane), a natural sesquiterpene extracted from the traditional Chinese drugs curcuma *wenyujin* (Guo *et al.*, 1983), is the main effective monomer of elemene emulsion, and the chemical structure are shown in Fig. 1.

β-Elemene has many advantages when it is used as anticancer drug: it has a broad-spectrum antitumor effect in many types of cancer (lung, breast, prostate, cervical, gastric, ovarian, bladder cancer, and osteosarcoma) without severe side effects (Li *et al.*, 2005, 2010, 2013; Wang *et al.*, 2005; Liu *et al.*, 2011; Liang *et al.*, 2012; Zhang *et al.*, 2012). No bone marrow suppression and drug resistance have been observed in the clinical studies, as well as patient immunity was improved during the therapy with β-elemene (Peng *et al.*, 2006). Furthermore, it has low toxicity and is therefore well-tolerated and accepted by patients.

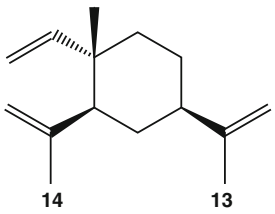
However, β-elemene suffers from limited bioavailability due to poor water solubility, short half life, and rapid clearance from the body (Cheng *et al.*, 2008). As we know, the water solubility of compounds could be improved by adding polar groups such as an ether, ester, amide, and so on into the molecular structure. Although many β-elemene derivatives with better properties have already been synthesized (Jia *et al.*, 1991; Peng *et al.*, 2006; Xu *et al.*, 2006; Ren *et al.*, 2009; Sun *et al.*, 2009), β-elemene derivatives with carbamate, acylamide, and carbamidine groups have not been reported. In this study, a series of novel β-elemene-monosubstituted ester, carbamate, acylamide, and carbamidine derivatives were synthesized from intermediates β-elemene amine and β-elemene alcohol at ambient temperature. And by controlling the reaction conditions, we prepared β-elemene 13-monosubstituted derivatives as the major product. The in vitro antiproliferative activities of pure β-elemene and its derivatives over HeLa cell lines were evaluated through the WST-1 assay. To our pleasure that some of the derivatives has much higher antiproliferative activities compared to that of parent β-elemene.

G. Liu · Z. Kong
Institute of Chemical Industry of Forest Products, CAF, Nanjing 210042, China

G. Liu · Z. Kong
Institute of New Technology of Forestry, CAF, Beijing 100091, China

Y. Shen (✉)
Shanghai Center for Systems Biomedicine, Key Laboratory of Systems Biomedicine (Ministry of Education), Shanghai Jiao Tong University, Shanghai 200240, China
e-mail: yumeishen@sjtu.edu.cn

Fig. 1 Chemical structure of β-elemene



Experimental

β-Elemene was obtained from WenTe Research Institute of Oleum Curcumae Wenchowensis in Yue Qing city, Zhejiang province (purity 98 %). Other materials were purchased from Fluka Co. and Sinopharm Chemical Reagent Co. Ltds. The NMR data were obtained using a Bruker DRX 500 MHz FT spectrometer. The chemical shifts as d are reported in ppm relative to TMS. Infrared (IR) spectra were recorded on a Perkin-Elmer FT-IR spectrometer. Mass spectral data were collected using positive mode on a Finnigan LCQ classic mass spectrometer. Elemental analysis was performed using a Perkin-Elmer Series III analyzer.

A general procedure for the formation of 13-chloro-β-elemene **2**

β-Elemene in acetic acid was cooled to 0 °C, and sodium hypochlorite was added over a 4 h period, then the mixture was stirred for 2 h at room temperature and extracted with EtOAc. The combined organic extracts were washed with water, dried over anhydrous $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give the mixture as yellow oil used in the next step directly without further purification.

A general procedure for the formation of β-elemene alcohol **3**

A chlorinated β-elemene mixture (1.2 g) containing chlorinated β-elemene (2.5 mmol) was dissolved in dry CH_2Cl_2, then anhydrous CH_3COONa (0.82 g, 10 mmol), HMPT (1.0 mL), and KI (0.76 g, 4.5 mmol) were added to the solution. The reaction mixture was stirred at 95 °C for 12 h, then extracted with hexane, dried over anhydrous Na_2SO_4, filtered, and evaporated. Purification was accomplished by chromatograph to give β-elemene acetate, which was hydrolyzed to give β-elemene alcohol (0.33 g, 60 %).

^{1}H NMR ($CDCl_3$, TMS, 500 MHz): δ 1.06 (s, 3H, CH_3), 1.34–1.66 (m, 6H), 1.73 (s, 3H, CH_3), 2.01–2.11 (m, 2H), 4.14 (s, 2H), 4.59 (s, 1H), 4.82 (s, 1H), 4.91 (s, 1H), 4.92(d, $J = 5.55$, 1H), 4.94 (s, 1H), 5.05 (s, 1H), 5.82 (dd, $J = 17.12$, 11.18, 1H). IR (cm^{-1}): 3334 (O–H), 3081 (=CH), 2968, 2858 (CH), 1638 (C=C), 1440, 1375 (CH), 910 (CH). HRMS (*m*/*z*): 220.20.

A general procedure for the formation of β-elemene amine **4**

A β-elemene chlorination mixture (0.78 g) containing chlorinated β-elemene (1.6 mmol) was dissolved in 5-mL CH_3CN, and then KI (78 mg) was added to the solution. Liquid ammonia was introduced into the autoclave until the pressure is up to 4 atm. The reaction mixture was stirred at 60 °C for 24 h. Purification was accomplished by chromatograph to give β-elemene amine **4** (70 mg, 19.4 %).

^{1}H NMR ($CDCl_3$, TMS, 500 MHz): δ 1.00 (s, 3H, CH_3), 1.32–1.77(m, 6H), 1.71 (s, 3H, CH_3), 2.04–2.18 (m, 2H), 3.62 (s, 2H), 4.58 (s, 1H), 4.83 (s, 1H), 4.89 (s, 1H), 4.92 (d, $J = 4.66$, 1H), 5.15 (s, 1H), 5.23 (s, 1H), 5.81 (dd, $J = 17.70$, 10.65, 1H). IR (cm^{-1}): 3390 (N–H), 3081 (=CH), 2976, 2867 (CH), 1642 (C=C), 1442, 1375 (CH), 909 (CH). HRMS (*m*/*z*): 219.17.

A general procedure for the formation of β-elemene carbamate and ester **5**

A mixture of β-elemene alcohol **3** (50 mg, 0.23 mmol) and isocyanate (0.5 mmol) was dissolved in 10-mL toluene and stirred at 67 °C for 10 h. Concentrated in vacuo to give the mixture as yellow oil, purification was accomplished by chromatograph to give compounds **5.**

The formation of compound **5a**. A colorless oil, yield 58 %;

^{1}H NMR (CD_3OD, TMS, 400 MHz): δ 1.02 (s, 3H), 1.51–1.77 (m, 6H), 1.71 (s, 3H), 2.01–2.16 (m, 2H), 4.60 (s, 1H), 4.84 (s, 2H), 4.84 (s, 1H), 4.89 (s, 1H), 4.92 (d, $J = 6.63$, 1H), 5.07 (s, 1H), 5.16 (s, 1H), 5.82 (dd, $J = 17.69$, 11.07, 1H), 7.45 (t, $J = 7.64$, 2H), 7.57 (t, $J = 6.55$, 1H). IR (cm^{-1}): 3321 (N–H), 3081 (=CH), 2929, 2865 (CH), 1701 (C=O), 1643 (C=C), 1463, 1374 (CH), 907(CH). HRMS (*m*/*z*): 324.21.

The formation of compound **5b**. A colorless oil, yield 65 %;

^{1}H NMR ($CDCl_3$, TMS, 500 MHz): δ 0.93 (t, $J = 7.34$, 3H), 1.01 (s, 3H), 1.31–1.38 (m, 2H), 1.42–1.53 (m, 5H), 1.55–1.62 (m, 2H), 1.65–1.75 (m, 2H), 1.71 (s, 3H), 1.99–2.05 (m, 1H), 3.19 (t, $J = 6.32$, 2H), 4.57 (s, 2H), 4.58 (s, 1H), 4.82 (s, 1H), 4.89 (s, 1H), 4.92 (d, $J = 5.29$, 1H), 4.98 (s, 1H), 5.04 (s, 1H), 5.82 (dd, $J = 17.96$, 10.48, 1H); IR (cm^{-1}): 3338 (N–H), 3081 (=CH), 2931, 2863 (CH), 1702 (C=O), 1643 (C=C), 1463, 1375 (CH), 907 (CH). HRMS (*m*/*z*): 319.25.

The formation of compound **5c**. A colorless oil, yield 70 %;

^{1}H NMR ($CDCl_3$, TMS, 500 MHz): δ 1.01 (s, 3H), 1.16 (d, $J = 6.51$, 6H), 1.44–1.74 (m, 6H), 1.68 (s, 3H), 1.99–2.02 (m, 2H), 3.80–4.02 (m, 1H), 4.56 (s, 1H), 4.58 (s, 2H), 4.82–5.04 (m, 5H), 5.81 (dd, $J = 18.00$, 11.25,

1H). IR (cm^{-1}): 3329 (N–H), 3081 (=CH), 2931, 2863 (CH), 1701 (C=O), 1643 (C=C), 1457, 1368 (CH), 907 (CH). HRMS (*m/z*): 305.23.

The formation of compound **5d.** A colorless oil, yield 67 %;

^{1}H NMR ($CDCl_3$, TMS, 500 MHz): δ 1.01 (s, 3H), 1.44–1.52 (m, 3H), 1.58–1.66 (m, 3H), 1.71 (s, 3H), 1.69–1.75 (m, 1H), 2.00–2.09 (m, 1H), 4.59 (s, 1H), 4.68 (s, 2H), 4.83 (s, 1H), 4.89 (s, 1H), 4.92 (d, $J = 6.14$, 1H), 5.03 (s, 1H), 5.10 (s, 1H), 5.82 (dd, $J = 17.86, 10.41$, 1H), 6.70 (s, 1H, NH), 7.05–7.08 (m, 1H), 7.29–7.32 (m, 2H), 7.37–7.39 (m, 2H). IR (cm^{-1}): 3319 (N–H), 3081 (=CH), 2929, 2859 (CH), 1708 (C=O), 1640 (C=C), 1444, 1375 (CH), 907 (CH). HRMS (*m/z*): 339.21.

The formation of compound **5e**. A colorless oil, yield 74 %;

^{1}H NMR ($CDCl_3$, TMS, 500 MHz): δ 1.01 (s, 3H), 1.09–1.20 (m, 3H), 1.30–1.40 (m, 2H), 1.42–1.51 (m, 3H), 1.56–1.62 (m, 3H), 1.66–1.76 (m, 3H), 1.71 (s, 3H), 1.90–1.98 (m, 2H), 1.99–2.07 (m, 2H), 3.45–3.55 (m, 1H), 4.55 (s, 1H), 4.58 (s, 2H), 4.82 (s, 1H), 4.89 (s, 1H), 4.92 (d, $J = 5.35$, 1H), 4.98 (s, 1H), 5.04 (s, 1H), 5.82 (dd, $J = 17.94, 10.44$, 1H). IR (cm^{-1}): 3332 (N–H), 3081 (=CH), 2931, 2855 (CH), 1705 (C=O), 1643 (C=C), 1451, 1374 (CH), 907 (CH). HRMS (*m/z*): 345.26.

A general procedure for the formation of β-elemene acylamide and carbamide **6**

A mixture of β-elemene amine **4** (1.3 g) and excess isocyanate was dissolved in 10-mL toluene and stirred at room temperature for 3 h. Concentrated in vacuo to give the mixture as yellow oil, purification was accomplished by chromatograph to give **6**.

The formation of compound **6a**. A colorless oil, yield 58 %;

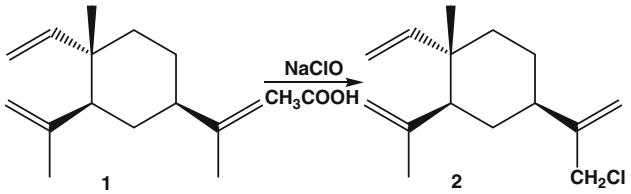
^{1}H NMR ($CDCl_3$, TMS, 500 MHz): δ 1.00 (s, 3H), 1.40–1.66 (m, 6H), 1.71 (s, 3H), 2.04–2.27 (m, 2H), 3.62 (s, 2H), 4.58 (s, 1H), 4.83 (s, 1H), 4.89 (s, 1H), 4.92 (d, $J = 4.66$, 1H), 5.16 (s, 1H), 5.23 (s, 1H), 5.81 (dd, $J = 17.7, 10.65$, 1H), 7.31–7.45 (m, 5H). IR (cm^{-1}): 3339 (N–H), 3071 (=CH), 2924, 2852 (CH), 1685 (C=O), 1641 (C=C), 1452, 1383 (CH), 907 (CH). HRMS (*m/z*): 323.20.

The formation of compound **6b**. A colorless oil, yield 58 %;

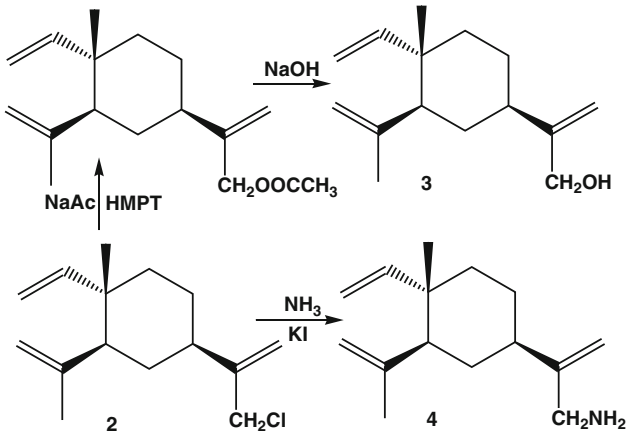
^{1}H NMR ($CDCl_3$, TMS, 500 MHz): δ 1.01 (s, 3H), 1.16 (d, $J = 6.39$, 6H), 1.45–1.52 (m, 4H), 1.57–1.75 (m, 3H), 1.70 (s, 3H), 1.93–2.03 (m, 1H), 3.89–4.00 (m, 1H), 4.40 (d, $J = 3.34$, 2H), 4.58 (s, 1H), 4.83 (s, 1H), 4.89 (s, 1H), 4.91–4.95 (m, 2H), 5.05 (s, 1H), 5.81 (dd, $J = 17.74, 10.56$, 1H). IR (cm^{-1}): 3418 (N–H), 3081 (=CH), 2930, 2868 (CH), 1646 (C=C), 1456, 1367 (CH), 906 (CH). HRMS (*m/z*): 305.24.

The antiproliferative evaluation by WST-1 method

The antiproliferative effect of the monosubstituted ether derivatives of β-elemene in human HeLa cervix carcinoma cell (ATCC CCL-2) was evaluated by WST-1 method (Beyotime). HeLa cells were maintained in RPMI 1640 (GIBCO) with 10 % inactivated fetal bovine serum (GIBCO). The cell lines were grown in logarithmic growth at 37 °C in a humidified atmosphere consisting of 5 % CO_2 and 95 % air. The HeLa cells were harvested using 0.25 %



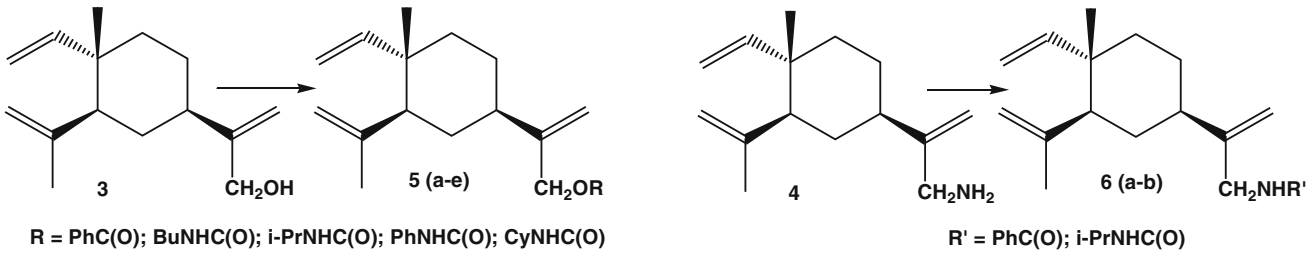
Scheme 1 Synthesis of β-elemene chloride **2**



Scheme 2 Synthesis of the intermediate β-elemene alcohol **3** and β-elemene amine **4**

Table 1 The antiproliferative activity of β-elemene monosubstituted derivatives over HeLa cell lines

Compounds	IC_{50} (μmol L^{-1})
β-elemene	336.2 ± 7.2
3	105.9 ± 14.4
4	5.90 ± 3.4
5a	529.9 ± 7.0
5b	529.7 ± 7.7
5c	280.8 ± 6.5
5d	264.1 ± 6.7
5e	318.0 ± 15.0
6a	219.0 ± 12.4
6b	364.6 ± 16.5



Scheme 3 Synthesis of β-elemene ester, carbamate, acylamide, and carbamidine derivatives

trypsin–EDTA (Beyotime) and seeded 5×10^3 cells in each well of a 96-well plate and incubated for 12 h. The β-elemene derivatives were added to wells of the plate at different concentrations (500, 250, 125, 62.5, 31.2 μg/mL) and continued to culture in CO_2 incubator for 48 h. WST-1 solution (10 μL) was added to wells absorbance values were got using a 96-well Opsys Microplate Reader (BIO-RAD) at 450 nm. IC_{50} were calculated with SPSS 11.5 statistical software according to the absorbance values. The differences between mean values were analyzed with students' tests. Differences were considered significant when $p < 0.05$. Data are presented as mean ± SD.

Results and discussion

The novel derivatives were synthesized from chlorination of β-elemene according to the procedure outlined in Scheme 1. In order to make the 13-monosubstituted chlorinated β-elemene as the absolutely major product, we optimized the reaction conditions and found that the reaction temperature should be kept below 0 °C, NaClO should be fresh and its mole ratio to β-elemene should be kept between 1.1 and 1.2, the mixture of products includes little 14-monosubstituted and disubstituted chlorinated β-elemene derivatives. Finally, the chlorinated β-elemene mixture was used directly without further purification.

The β-elemene monosubstituted alcohol **3** was synthesized according to literature (Jia *et al.*, 1991) with a little modification. The chlorinated β-elemene reacts with Ac_2O promoted by HMPT to give the ester at room temperature, followed by hydroxylation under basic conditions to yield the important intermediate **3**. For the intermediate **4**, it is the first time to be synthesized successfully. At first, we attempted to prepare it by the reaction of compound **2** with saturated ammonia hydroxide under reflux conditions, unfortunately, no product was identified. To improve the reaction's activity, liquid ammonia was employed instead of ammonia hydroxide in autoclave under basic conditions. The expected reaction took place efficiently. Purification was accomplished by chromatograph to give compound **4** in good yield (Scheme 2).

The reactions of intermediate **3** with benzoyl chloride and several isocyanates at room temperature forming the ester and carbamate derivatives. The products are purified by chromatograph. Intermediate **4** is more reactive than intermediate **3**, to produce less byproduct, the reactions of **4** with benzoylchloride and i-PrNCO at 0 °C. We tested the pure β-elemene and its derivatives antiproliferative activity in vitro by the WST-1 method. Results show that the β-elemene derivatives were synthesized from 13-monosubstituted chlorinated β-elemene with higher proliferative activity over HeLa cell lines than that of the mother β-elemene (Table 1).

Conclusion

In summary, a series of novel β-elemene monosubstituted ester, carbamate, acylamide, and carbamidine derivatives were synthesized via intermediates β-elemene alcohol and β-elemene amine at ambient temperature, especially, intermediate β-elemene amine is the first time to be synthesised successfully (Scheme 3). The monosubstituted derivatives in vitro antiproliferative activities over HeLa cell lines were tested by WST-1 method and some of their activities were improved compared to pure β-elemene.

Acknowledgments The authors gratefully acknowledge the financial supports provided by the National Natural Science Foundation of China (81071250), Major Projects in National Science and Technology, "Creation of major new drugs" (2011ZX09501-001-01), Institute of New Technology of Chinese Academy of Forestry (Special Fund for Fundamental Research) (CAFINT2012C04).

References

Cheng KM, Sun YH, Feng CL, Shen YM (2008) Synthesis and in vitro antiproliferative activity of β-elemene polyethylene glycol derivatives. Chin J Org Chem 28:65–68

Guo YT, Wu XY, Chen YR (1983) Separation and identification of element in essential oil from curcuma. Bull Chin Mater Med 8:31–33

Jia WM, Yang LM, Li ZH, Hu JH (1991) Synthesis of the antitumor β-elemene derivatives. Chin J Org Chem 11:608–610

Li X, Wang G, Zhao J, Ding H, Cunningham C, Chen F, Flynn DC, Reed E, Li QQ (2005) Antiproliferative effect of beta-elemene in

chemoresistant ovarian carcinoma cells is mediated through arrest of the cell cycle at the G2-M phase. Cell Mol Life Sci 62:894–904

Li QQ, Wang G, Huang F, Banda M, Reed E (2010) Antineoplastic effect of beta-elemene on prostate cancer cells and other types of solid tumour cells. J Pharm Pharmacol 62:1018–1027

Li QQ, Wang G, Liang HS, Zhong YH, Reed E (2013) β-Elemene promotes cisplatin-induced cell death in human bladder cancer and other carcinomas. Anticancer Res 33:1421–1428

Liang D, Yang M, Guo B, Yang L, Cao J, Zhang X (2012) HIF-1α induced by β-elemene protects human osteosarcoma cells from undergoing apoptosis. J Cancer Res Clin Oncol 138:1865–1887

Liu J, Zhang Y, Qu J, Xu L, Hou K, Zhang J, Qu X, Liu Y (2011) β-Elemene-induced autophagy protects human gastric cancer cells from undergoing apoptosis. BMC Cancer 11:183

Peng XX, Zhao YL, Liang XY, Wu LJ, Cui SQ, Guo AM, Wang W (2006) Assessing the quality of RCTs on the effect of β-elemene, one ingredient of a Chinese herb, against malignant tumors. Contemp Clin Trials 27:70–82

Ren YF, Sun YH, Liu GF, Shen YM (2009) Synthesis and radiolabelling of $Re(CO)_3$-β-elemene derivatives as potential therapeutic radiopharmaceuticals. J Labelled Compd Rad 52:139–145

Sun YH, Liu GF, Zhang YQ, Zhu H, Ren YF, Shen YM (2009) Synthesis and in vitro anti-proliferative activity of β-elemene monosubstituted derivatives in HeLa cells mediated through arrest of cell cycle at the G1 phase. Bioorg Med Chem 17:1118–1124

Wang G, Li X, Huang F, Zhao J, Ding H, Cunningham C, Coad JE, Flynn DC, Reed E, Li QQ (2005) Antitumor effect of beta-elemene in non-small-cell lung cancer cells is mediated via induction of cell cycle arrest and apoptotic cell death. Cell Mol Life Sci 62:881–893

Xu LY, Tao SJ, Wang XM, Yu ZY, Wang MW, Chen D, Jing YK, Dong JH (2006) The synthesis and anti-proliferative effects of β-elemene derivatives with mTOR inhibition activity. Bioorg Med Chem 14:5351–5356

Zhang B, Zhang X, Tang B, Zheng P, Zhang Y (2012) Investigation of elemene-induced reversal of tamoxifen resistance in MCF-7 cells through oestrogen receptor α(ERα) re-expression. Breast Cancer Res Treat 136:399–406