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Synthesis of 13-amino costunolide derivatives as anticancer agents

Sanjay K. Srivastava,[a,*] Aji Abraham,[b] Beena Bhat,[b] Manu Jaggi,[c,*] Anu T. Singh,[d] Vinod K. Sanna,[c] Gurvinder Singh,[d] Shiv K. Agarwal,[a,b] Rama Mukherjee[a,b,c,d] and Anand C. Burman[a,b,c,d]

[a]*Divisions of Medicinal Chemistry, Dabur Research Foundation, 22, Site IV, Sahibabad, Ghaziabad, 201 010 UP, India*
[b]*Divisions of Phytochemistry, Dabur Research Foundation, 22, Site IV, Sahibabad, Ghaziabad, 201 010 UP, India*
[c]*Divisions of Experimental Oncology, Dabur Research Foundation, 22, Site IV, Sahibabad, Ghaziabad, 201 010 UP, India*
[d]*Divisions of Molecular Oncology, Dabur Research Foundation, 22, Site IV, Sahibabad, Ghaziabad, 201 010 UP, India*

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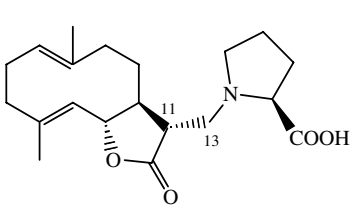
Abstract—A number of costunolide derivatives (**4a**–**p**) have been synthesized and evaluated for their in vitro cytotoxicity against eight tumor and a non-tumor cell lines. Compound **4d** showed around 2-fold better cytotoxicity against SW-620 (colon) cell line with improved safety index than costunolide (**1**). While compounds **4e**, **4g**, and **4p** have shown around 2- to 3-fold better cytotoxicity against MIAPaCa2 (pancreas), K-562 (leukemia) and PA-1 (ovary) cell lines as well as better safety index in comparison to costunolide (**1**). Compound **4p** also exhibited cytotoxicity against HBL100 (breast) cell line with 2-fold better safety index. Structure–activity relationship has been described.

Thousands of sesquiterepene lactones have been isolated from the natural plants and several of them have shown a variety of biological activities.[1] Sesquiterpenes are reported to exert antitumor activity by triggering apoptosis in human leukemia cells.[2] One of the sesquiterpene lactones, costunolide (**1**) is being isolated from *Saussurea radix*, the dried root of *Saussurea lappa* Clarks.[3] Costunolide (**1**) has shown potential anticancer activity and is considered as a potential candidate for various types of tumors.[1,4–6] However, structural modifications in costunolide (**1**), is required to improve the cytotoxicity as well as to establish the meaningful structure–activity relationship.

It has been reported that *exo*-methylene group on lactone part of sesquiterpene lactones is required for eliciting cytotoxicity.[1,6] However, we assumed that upon introduction of nitrogen containing functionality at position-13 in costunolide (**1**), additional hydrogen bonding potential and selectivity might be realized and may lead to derivatives with improved cytotoxic profile. We therefore designed, synthesized a number of 13-amino costunolide derivatives and evaluated them for their anticancer activity in vitro (**4a**–**p**). The details are being reported here.



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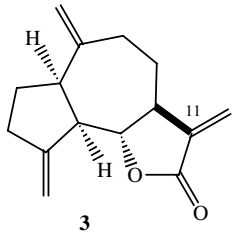

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Synthesis of costunolide derivatives (**4a**–**p**) from costunolide (**1**) has been described in Scheme 1. Michael-type addition of amine (R^1R^2NH) with α-methylene-γ-lactone part of the costunolide (**1**) afforded the corresponding 13-amino derivatives (**4a**–**p**).[7] Matsuda et al.[8] reported that Michael-type addition for α-methylene-γ-lactone of costunolide with L-proline resulted saussureamine (**2**), selectively. In addition, the Michael-type addition for α-methylene-γ-lactone of costunolide (**1**) or dehydrocostus lactone (**3**) with other amino acids afforded, stereoselectively, the less hindered

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* Corresponding authors. Tel.: +91 120 4378400; fax: +91 120 4376902; e-mail addresses: srivastavasa@dabur.com; jaggim@dabur.com

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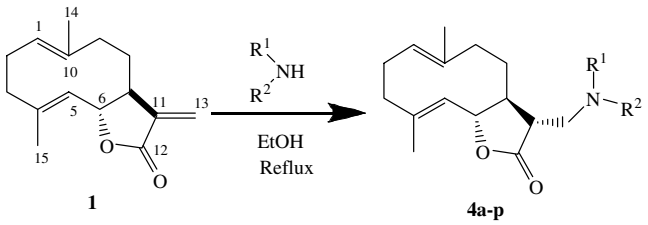


Scheme 1.

11α-configurated derivatives.[8] Based on these literature results, the stereochemistry at position-11 in the synthesized costunolide derivatives (**4a**–**p**) has been assumed. The synthesized 13-amino costunolide derivatives (**4a**–**p**) have been listed in Table 1.

Results and discussion. Costunolide (**1**) and its derivatives (**4a**–**p**) were tested for in vitro cytotoxicity on tumor as well as a non-tumorous cell lines and IC_{50} values were calculated in micro mole (μM).[9] The human tumor cell lines used in the study are breast (HBL100), lung (A-549), pancreas (MIAPaCa2), leukemia (K562), ovary (PA1), prostate (DU145), oral (KB), and colon (SW620) cancers. Costunolide (**1**) and its derivatives (**4a**–**p**) were also screened against normal mouse fibroblast (NIH3T3) to evaluate their cancer cell specificity (safety index).[9] The cytotoxicity data are summarized in Table 2. It is evident from Table 2 that the parent molecule, costunolide (**1**), exhibited broad spectrum of cytotoxicity ($IC_{50} > 10$ μM except against SW-620 cell line) and had shown poor safety index (<2). While several derivatives of 13-amino costunolide have shown, in general, better cytotoxicity and selectivity with improved safety index. Structure–activity relationship (SAR) of these derivatives has been discussed below.

The unsubstituted piperidine or pyrrolidine derivatives (compounds **4a** and **4b**) did not show major improvement in the cytotoxicity. While amongst substituted piperidine or pyrrolidine derivatives (**4c**–**f**), nature of the group and its position in the ring played an important role in eliciting cytotoxicity. For example, 4-methyl piperidine derivative (**4c**) did not show improvement in the cytotoxicity, whereas 3-methyl piperidine derivative (**4d**) exhibited 2-fold better cytotoxicity ($IC_{50} = 3.3$ μM) than costunolide (**1**, $IC_{50} = 7.8$ μM) against SW-620 cell line. In addition, compound **4d** has shown safety index >6 for colon cancer cell line. It indicated that the position of methyl group was critical for eliciting cytotoxicity. While those costunolide derivatives, which possess hydroxy group in place of methyl, exhibited a different cytotoxicity profile. For example, upon replacement of 4-methyl group in compound **4c** with 4-hydroxy (compound **4e**), the cytotoxicity ($IC_{50} = 4.3$–8.0 μM) improved up to 2- to 3-fold as compared to costunolide (**1**) and 3- to 5-fold when compared to compound **4c**, against MIAPaCa2, K-562, and PA-1 cell lines. Moreover, compound **4e** has also shown improvement in safety index (>2) in the same cell lines. On the other hand, 4-hydroxymethyl derivative (**4f**) did not provide better cytotoxicity. While, as described the cytotoxicity profile for 4-hydroxypiperidine derivative (**4e**), 3-hydroxypyrrolidine derivative (**4g**) also showed strong cytotoxicity

Table 1. List of 13-amino costunolide derivatives (**4a**–**p**)

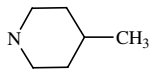
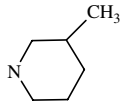
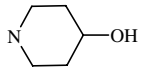
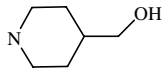
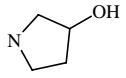
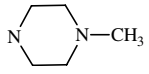
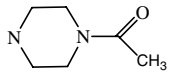
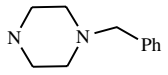
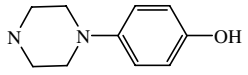
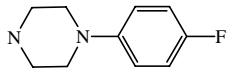
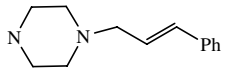
Compound	R^1 N R^2
4a	
4b	
4c	
4d	
4e	
4f	
4g	
4h	
4i	
4j	
4k	
4l	
4m	
4n	
4o	
4p	

Table 2. In vitro cytotoxicity data of costunolide (**1**) and its 13-amino derivatives (**4a**–**p**)

Compound	Cell lines (IC_{50} μM)																
	HBL 100	Cancer cell specificity	A549	Cancer cell specificity	MiaPaca2	Cancer cell specificity	K 562	Cancer cell specificity	PA1	Cancer cell specificity	DU145	Cancer cell specificity	KB	Cancer cell specificity	SW 620	Cancer cell specificity	NIH3T3
1	10.3	1.3	12.3	1.1	17.7	0.8	14.5	1.0	10.6	1.3	29.2	0.5	28.9	0.5	7.8	1.8	13.9
4a	28.5	0.9	31.1	0.8	12.9	2.0	15.7	1.6	11.4	2.3	26.5	1.0	28.3	0.9	56.1	0.5	25.8
4b	20.3	0.9	32.5	0.6	10.9	1.7	10.6	1.7	9.4	2.0	25.1	0.7	28.7	0.6	19.9	0.9	18.5
4c	55.1	0.3	22.7	0.8	23.4	0.8	21.4	0.8	27.5	0.7	30.6	0.6	17.3	1.0	12.4	1.4	17.9
4d	28.6	0.7	21.3	0.9	17	1.2	12.2	1.6	33.4	0.6	35.1	0.6	20.9	1.0	3.3	6.0	19.9
4e	14.6	1.3	27.7	0.7	8	2.5	4.3	4.6	5.3	3.7	28.7	0.7	37	0.5	20.9	0.9	19.7
4f	12.8	1.3	18.1	0.9	9.7	1.8	10.2	1.7	16.8	1.0	26.3	0.7	12.6	1.4	10.7	1.6	17.1
4g	11.2	2.0	26.3	0.9	5.3	4.3	4.8	4.8	4.3	5.3	23.8	1.0	34.9	0.7	8.9	2.6	22.8
4h	36.5	0.8	57.1	0.5	33.9	0.8	57	0.5	14.3	2.0	62.9	0.4	85.2	0.3	20.2	1.4	28.3
4i	63.3	0.5	73.1	0.5	22.9	1.5	84.4	0.4	47.8	0.7	67.4	0.5	43.1	0.8	23.1	1.5	34.7
4j	NA	—	NA	—	NA	—	NA	—	NA	—	NA	—	NA	—	NA	—	NA
4k	NA	—	NA	—	NA	—	NA	—	NA	—	NA	—	NA	—	NA	—	NA
4l	NA	—	NA	—	NA	—	NA	—	NA	—	NA	—	NA	—	NA	—	NA
4m	54.7	0.4	74.7	0.3	51.7	0.4	159.3	0.1	453.1	0.0	120.8	0.2	285.2	0.1	280.4	0.1	19.3
4n	NA	—	NA	—	NA	—	NA	—	NA	—	247.1	>1.0	195.1	>1.3	92.8	>2.7	NA
4o	31.5	1.3	62.1	0.7	98.9	0.4	42.2	1.0	36.4	1.1	117.6	0.3	102.6	0.4	54.1	0.8	40.8
4p	6.1	2.6	27	0.6	5.4	2.9	4.4	3.6	3.4	4.6	22.7	0.7	21.1	0.7	9.1	1.7	15.7

NA, not active with IC_{50} greater than the highest concentration tested; —, cancer cell specificity cannot be calculated.

(IC_{50} = 4.3–5.3 μM) against MIAPaCa2, K-562, and PA-1 cell lines and found 2- to 3-fold better than costunolide (**1**) against the same cell lines. However, unlike compound **4e**, compound **4g** showed relatively better activity against SW-620 cell line. The safety index for compound **4g** was observed in the range of 2.6–5.3. Thus, it seemed that hydroxy group in piperidine or in pyrrolidine ring was found crucial for eliciting cytotoxicity.

Upon replacing piperidine substituent with morpholine (**4h**) or thiomorpholine (**4i**), a low order of cytotoxicity was observed. Furthermore, the cytotoxicity was completely lost when 4-methylpiperazine (**4j**) or 4-acetylpiperazine (**4k**) or 4-benzylpiperazine (**4l**) was introduced, separately, at position-13 in costunolide (**1**). Further, upon incorporating bulkier group at position-4 in piperazine like 4-hydroxyphenyl (**4m**) or 4-fluorophenyl (**4n**) or longer chain like *trans*-cinnamyl group (**4o**), the cytotoxicity did not get better. It appeared that the presence of a heteroatom at position-4 in piperidine ring was not a good option to improve the cytotoxicity.

It was interesting to note that the substitution at position-13 with an open chain like *N*,*N*-dimethyl (compound **4p**) afforded a potent cytotoxic derivative. The cytotoxicity of compound **4p** was improved in all cancer cell lines except for A-549 and SW-620. However, as discussed for hydroxy derivatives **4e** and **4g**, compound **4p** also showed strong cytotoxicity (IC_{50} = 3.4–5.4 μM) against MIAPaCa2, K-562, and PA-1 cell lines and the safety index was found in the range of 2.9–4.6. In addition, compound **4p** also showed around $1\frac{1}{2}$-fold better cytotoxicity and 2-fold better safety index against HBL100 cell line when compared to costunolide (**1**). The cytotoxicity and safety index of compound **4p** and costunolide (**1**) were roughly identical against SW-620 cell line.

It clearly indicated that the piperidine or pyrrolidine substituents at position-13 in costunolide (**1**) played an important role in eliciting cytotoxicity. The hydroxy and methyl groups located either in piperidine or in pyrrolidine ring, separately, played crucial role in determining their selectivity toward cancer cell lines. However, it was noteworthy that *N*,*N*-dimethyl substituent at position-13 in costunolide played a significant role in order to improve the cytotoxicity.

In general, 13-amino costunolide derivatives were found selective towards pancreas, leukemia, and ovarian cancer cell lines except for 3-methylpiperidine derivative (**4d**), which was found selective for colon cancer. The 4-hydroxypiperidine (**4e**), 3-hydroxypyrrolidine (**4g**), and *N*,*N*-dimethyl (**4p**) derivatives have shown strong cytotoxicity against pancreas, leukemia, and ovarian cancer cell lines. All these derivatives have showed not only several fold better cytotoxicity but also exhibited better safety profile than costunolide (**1**). These molecules are under further biological studies.

References and notes

1. Sun, C.-H.; Syu, W.-J.; Don, M.-J.; Lu, J.-J.; Lee, G.-H. *J. Nat. Prod.* **2003**, *66*, 1175.
2. Hibasami, H.; Yamada, Y.; Moteki, H.; Katsuzaki, H.; Imai, K.; Yoshioka, K.; Komiya, T. *Int. J. Mol. Med.* **2003**, *12*, 147.
3. Okugawa, H.; Ueda, R.; Matsumoto, K.; Kawanishi, K.; Kato, A. *Phytomedicine* **1996**, 147.
4. Bocca, C.; Gabriel, L.; Bozzo, F.; Miglietta, A. *Chem. Biol. Interact.* **2004**, *147*, 79.
5. Lee, M.-G.; Lee, K.-T.; Chi, S.-G.; Park, J.-H. *Biol. Pharm. Bull.* **2001**, *24*, 303.
6. Zhang, S.; Won, Y.-K.; Ong, C.-N.; Shen, H.-M. *Curr. Med. Chem. Anti-Canc. Agents* **2005**, *5*, 239.
7. Isolation of costunolide (**1**): *Saussurea lappa* Clarks commonly known as Kuth in India is being widely used in traditional medicine all over the world for cure of various ailments. Dried roots were pulverized and extracted continuously in a soxhlet apparatus with hexane till complete extraction. The hexane extract was dried on a rotary evaporator to obtain a viscous mass. A part of hexane extract was subjected to column chromatography on silica gel (230–400 mesh) to obtain two enriched fractions A and B. Fraction B was dried in vacuuo to furnish crude product, which was washed with methanol. The residue, thus obtained, was crystallized from petroleum ether (60–80°) to yield costunolide (**1**) as colorless needles: mp 105–106 °C, ^{1}H and ^{13}C NMR data were in agreement with the previously reported data for costunolide.[10] Synthesis of 13-amino costunolide derivatives (**4a**–**p**): A mixture of costunolide (**1**, 0.15 g, 0.64 mmol) and 4-methyl piperidine (0.064 g, 0.64 mmol) in ethanol was refluxed for 12 h, and evaporated to dryness to afford the crude product. The crude product on silica gel (100–200 mesh) column chromatography using DCM/MeOH as eluent furnished compound **4c** as a yellow color semi-solid. Yield 80 mg (38%); R_f 0.5 (5% MeOH/DCM); IR 3017, 2928, 1760, 1207, 787 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.83–4.80 (m, 1H), 4.67–4.51 (m, 2H), 2.80–2.77 (m, 2H), 2.64–2.58 (m, 1H), 2.40–1.95 (m, 11H), 1.68 (s, 3H), 1.66–1.52 (3H), 1.41 (s, 3H), 1.25–1.61 (m, 4H), 0.91 (d, 3H, J = 6.3 Hz); MS (ES+) *m*/*z* (relative intensity) 332 (M+H) (100), 354 (M+H+Na) (30). Similarly, other 13-amino costunolide derivatives were synthesized and characterized by spectroscopic tools.
8. Matsuda, H.; Kageura, T.; Inoue, Y.; Morikawa, T.; Yoshikawa, M. *Tetrahedron* **2000**, *56*, 7763.
9. Various concentrations of costunolide (**1**) and its derivatives (**4a**–**p**) were tested for cytotoxic activity on earlier defined eight human tumor and one non-tumorous cell lines. Briefly, a three-day MTT in vitro cytotoxicity assay was performed, which is based on the principle of uptake of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide), a tetrazolium salt, by the metabolically active cells where it is metabolized by active mitochondria into a blue colored formazan product that is read spectrophotometrically.[11] MTT was dissolved in phosphate-buffered saline with a pH of 7.4 to obtain an MTT concentration of 5 mg/mL; the resulting mixture was filtered through a 0.22-micron filter to sterilize and remove a small amount of insoluble residue. For each type of tumor and normal cell, 5000–10,000 cells were seeded in a 96-well culture plate and incubated with various concentrations of costunolide (**1**) and its derivatives (**4a**–**p**) in a CO_2 incubator for 72 h. Control cells not treated with costunolide (**1**) and its derivatives (**4a**–**p**) were similarly incubated. The assay was terminated after 72 h by adding 125 μg (25 μL) MTT to each well, then incubating for 3 h,

and finally adding 50 μL of 10% SDS–0.01 N HCl to each well to lyse the cells and dissolve formazan. After incubating for 1 h, the plate was read spectrophotometrically at 540 nm and the cytotoxicity percentage calculated using the following formula: Cytotoxicity percentage = $100 \times [1 - (X/R_1)]$, where X = (absorbance of treated sample at 540 nm) − (absorbance of blank at 540 nm), R_1 = absorbance of control sample at 540 nm.

10. Park, H. J.; Jung, W. T.; Basnet, P.; Kadota, S.; Namba, T. *J. Nat. Prod.* **1996**, *59*, 1128.
11. Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55.