Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 2223–2225

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Antitumor Agents. Part 218: Cappamensin A, a New In Vitro Anticancer Principle, from *Capparis sikkimensis*†

Jiu-Hong Wu,[a] Fang-Rong Chang,[a] Ken-ichiro Hayashi,[a] Hiroaki Shiraki,[a] Chih-Chuang Liaw,[a] Yuka Nakanishi,[a] Kenneth F. Bastow,[a] Donglei Yu,[a] Ih-Sheng Chen[b] and Kuo-Hsiung Lee[a,*]

[a]*Natural Products Laboratory, School of Pharmacy, University of North Carolina, Chapel Hill, NC 27599, USA*
[b]*School of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan*

Received 5 September 2002; accepted 19 March 2003

Abstract—A new inhibitor of in vitro tumor cell replication, cappamensin A (**1**) (2*H*-1,4-benzoxazin-3(4*H*)-one, 6-methoxy-2-methyl-4-carbaldehyde), was isolated from the roots of *Capparis sikkimensis* subsp. *formosana* using bioactivity-guided fractionation. The structure of **1** was established by spectroscopic methods, including 2D NMR analyses. Compound **1** displayed significant in vitro anticancer activity against ovarian (1A9), lung (A549), ileocecal (HCT-8), breast (MCF-7), nasopharyngeal (KB), and vincristine resistant (KB-VIN) human tumor cell lines with ED_{50} values ≤ 4 μg/mL (mean GI_{50} value of 15.1 μM).

Capparis sikkimensis subsp. *Formosana* (Capparaceae) is a native Taiwanese shrub with overhanging, climbing branches. The roots and seeds of the genus *Capparis* have been used as antirheumatic, tonic, expectorant, antispasmodic, and analgesic agents in Chinese folk medicine.[2] Their healing properties have also been known since antiquity among numerous tribes in different Mediterranean countries.[3,4]

In a screening program dedicated to isolating antitumor compounds from plant sources, the chloroform extract of *Capparis sikkimensis*[5] showed significant in vitro cytotoxicity against various human tumor cell lines. Bioactivity-directed fractionation[6] of the active extract against A594 lung cancer and 1A9 ovarian cancer cells in tissue culture led to the isolation of compound **1** as the major novel active principle. The structure of **1**, which is provisionally named cappamensin A, was determined by chemical modification and 2D NMR spectra, including ^1H-^1H COSY, HMQC, HSQC, and HMBC techniques.[7] We report herein the isolation and structural characterization of cappamensin A.

Cappamensin A (**1**) has the molecular formula $C_{11}H_{11}O_4N$ as determined by ESI-MS (positive: *m*/*z* 244, $[M+Na]^+$, negative: *m*/*z* 220, $[M-H]^+$) and elemental analysis. Its ^1H NMR spectrum indicated allyl methyl (δ 2.51, s, 3H), methoxy (δ 3.71, s, 3H), 5, 6, 8 trisubstituted benzene (δ 8.58, d, $J=8.7$ Hz, 1H; δ 7.12, dd, $J=8.7$ Hz, $J=2.4$ Hz, 1H; δ 7.04, d, $J=2.4$ Hz, 1H), amide aldehyde (δ 10.60, s, 1H), and chelated hydroxy (δ 13.32, s, 1H) protons. The ^{13}C NMR spectrum showed 11 signals including an amido aldehyde, two olefinic, and six benzene ring signals. The presence of an amido aldehyde and a hydroxy group was also supported by IR absorptions at 1733 and 3450 cm^{-1}, respectively. The position of the methoxy group at C-7 in the benzene ring was confirmed by HMBC correlation of δ_H 7.12 (H-6, dd, 8.7, 2.4 Hz) with δ_C 120.8 (C-4a). Cross peaks in the HMBC spectrum between the amido aldehyde proton and C-4a indicated that the nitrogen was attached to C-4a. The chemical shifts of the OH proton at δ 13.32 and of C-3 at δ 143.98 suggested that the hydroxy group was located at C-3 and interacted with the aldehyde by hydrogen bonding. ^1H–^{13}C long-range coupling between the hydroxy group and C-3 supported this conclusion. The position of the methyl group at C-2 was determined by a HMBC cross peak between the methyl protons and C-3 and by the NMR chemical shifts (δ_H 2.51 ppm and δ_C 18.5 ppm).

†For Part 217 of this series, see ref 1.

*Corresponding author. Tel.: +1-919-962-0066; fax: +1-919-966-3893; e-mail: khlee@unc.edu

0960-894X/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0960-894X(03)00379-2

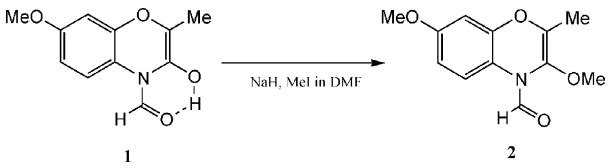

Figure 1. Methylation of cappamensin A.

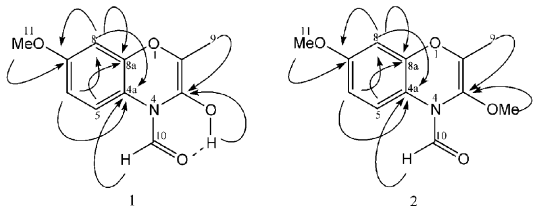

Figure 2. HMBC correlations for **1** and **2**.

Table 1. NMR spectral data for **1** and **2**

Position	**1** (Pyridine)		**2** ($CDCl_3$)	
	^{1}H ppm (*J*, Hz)	^{13}C	^{1}H ppm (*J*, Hz)	^{13}C
2		118.46		119.10
3		143.98		142.90
5	8.58 (d, 8.7)	121.98	8.34 (d, 9.0)	122.73
6	7.12 (dd, 8.7, 2.4)	112.23	7.08 (dd, 9.0, 2.4)	112.58
7		158.03		158.12
8	7.04 (d, 2.4)	95.72	6.92 (d, 2.4)	93.57
8a		139.08		138.98
4a		120.85		119.89
9-Me	2.51 s	18.52	2.54 s	21.08
10	10.60 s	184.68	10.41 s	186.77
11-OMe	3.71 s	55.51	3.99 s	55.68
3-OH	13.32 s			
3-OMe			4.01 s	30.13

Table 2. In vitro anticancer activity data for **1** and **2**

Compd	HCT-8[a]	U-87-MG	SK-MEL-2	KB	KB-VIN	1A9	PC-3	A549	MCF-7
1	3.6[b]	> 5 (36)	> 5 (30)	4.0	3.5	2.4	4.7	3.6	4.0
2	> 20 (39)	> 20 (19)	NA	> 20 (22)	> 20 (20)	20	> 20 (37)	> 20 (36)	> 20 (49)

[a]Cell lines include HCT-8: ileocecal cancer; KB, epidermoid nasopharyngeal carcinoma; KB-VIN, vincristine-resistant KB; 1A9, ovarian cancer; PC-3, prostate cancer; A549, lung cancer; MCF-7: breast cancer; U-87-MG, glioblastoma; SK-MEL-2, melanoma.
[b]ED_{50} values (the concentration that inhibits replication by 50% after 3 days of continuous treatment) are in μg/mL. Mean values are provided and variation between replicate treatments varied no more than 5%. If inhibition was less than 50%, the value in parentheses is percent inhibition observed. NA, not active at 20 μg/mL.

In addition to the five degrees of unsaturation resulting from the benzene, allyl, and aldehyde groups, compound **1** had one degree of unsaturation remaining. Therefore, the last oxygen connects C-2 to C-8a, forming the ether linkage of the 2*H*-1,4-benzoxazine-3(4*H*)-one skeleton. To further confirm the structure, **1** was treated with NaH and MeI (Fig. 1) to give a methylated derivative, methyl cappamensin A (**2**).[8] In the NMR spectrum of **2**, a second methoxy group (δ_H 4.01 and δ_C 55.7) appeared and the hydroxy group disappeared. In addition, the HMBC spectrum of **2** showed correlation between the additional methoxy group and C-3. Thus, the structure of **1** was determined to be 2*H*-1,4-benzoxazin-3(4*H*)-one, 6-methoxy-2-methyl-4-carbaldehyde, as indicated in Figure 1. Table 1 and Figure 2 show the assignment of NMR signals and HMBC correlations, respectively.

Cappamensin A (**1**) was evaluated in vitro against a panel of nine human tumor cell lines.[9] It showed broad and significant activity against six human tumor cell lines, with a mean ED_{50} value of 3.7±0.7 μg/mL. The individual ED_{50} values against each cell line are given in Table 2. Compound **1** was not a substrate of P-glycoprotein, based on the relative susceptibilities of KB and KB-VIN cell lines. U87-MG and SK-MEL-2 cell replication were the least susceptible. Interestingly, methyl cappamensin A (**2**) showed no activity at 10 μg/mL and was only weakly active at 20 μg/mL, suggesting that the C-3 hydroxy group is crucial for the activity of **1** against tumor cell replication.

Cappamensin A thus represents a promising new lead structure for future development of new analogues as potential antitumor agents.

Acknowledgements

We thank Professor H. Nozaki (Department of Biochemistry, Okayama University of Science) for NMR measurement and Dr. Junko Ito for her helpful suggestions. This investigation was supported by grant CA-17625 from the National Cancer Institute awarded to K. H. Lee.

References and Notes

1. For Part 217, see: Ohtsu, H.; Xiao, Z.; Ishida, J.; Nagi, M.; Wang, H. K.; Itokawa, H.; Su, C. Y.; Shih, C.; Chiang, T.; Chang, E.; Lee, Y.; Tsai, M. Y.; Chang, C.; Lee, K. H. *J. Med. Chem*. In press.
2. Jiang Su New Medical College. *Chung Yao Da Tzu Dien* (*Dictionary of Chinese Materia Medicia*); Shanghai Science & Technology: Hong Kong, 1977; Vol. 2, pp. 297, 843, 1709.
3. Inocencio, C.; Rivera, D.; Alcaraz, F.; Tomás-Barberán, F. *Eur. Food Res. Technol*. **2000**, *212*, 70.
4. Calis, I.; Kuruuzum-Uz, A.; Lorenzetto, P. A.; Ruedi, P. *Phytochemistry* **2002**, *59*, 451.
5. The roots of *Capparis sikkimensis* subsp. *formosana* (Hemsl.) Jacobs were collected at Lai-Yi Village, Pintung County, in Taiwan. The voucher specimen is deposited at Kaohsiung Medical University, Taiwan.
6. **Extraction and isolation.** The air-dried roots of *C. sikkimensis* subsp. *formosana* (6.9 kg) were extracted with MeOH (20 L) for 72 h three times at room temperature. The MeOH extract was concentrated in vacuo to give a crude extract (690 g). The combined percolates were concentrated under reduced pressure to yield a residue (330 g), which was partitioned with hexane, $CHCl_3$, *n*-BuOH, and water. The $CHCl_3$ extract (6 g) was chromatographed on a silica gel (10–40 μ) column eluted with a $CHCl_3$–MeOH gradient (40:1–20:1). Active fractions

were combined and purified with repeated silica column gel chromatography guided by the cytotoxicity assay to give cappamensin A (**1**, 80 mg).

7. **Cappamensin A**, colorless crystals, mp: 204–206 °C; IR (neat) cm^{-1}: 3450, 2959, 1733, 1471, 1456, 1374; ^{1}H NMR and ^{13}C NMR data see Table 1; ESI-MS positive: *m*/*z* (%): 244, $[M+Na]^+$, negative: *m*/*z* 220, $[M-H]^+$. Elemental analysis: (Theory: H 5.01%, C 59.73%, N 6.33%. Found: H 4.99%, C 58.89%, N 6.10%).

8. Compound **2**: colorless amorphous solid; IR (neat) cm^{-1}: 2958, 1733, 1652, 1505, 1456, 1372; ^{1}H NMR, ^{13}C NMR and HMBC data see Table 1; ESI-MS [positive: *m*/*z* (%): 258, $[M+Na]^+$].

9. **Sulforhodamine B microtitre plate assay** [according to standard procedure referenced in *J.N.C.I.* **1990**, *82*, 1107)]. Treatment was for 3 days of continuous exposure. Cell culture: growth medium, RPMI-1640, was supplemented with 25 mM HEPES, 2% (w/v) sodium bicarbonate, 10% (v/v) fetal bovine serum and 100 μg/mL kanamycin. Cultures were maintained in 5% CO_2, humidified atmosphere at 37 °C. Cell lines are described in the legend to Table 2. Where possible, the ED_{50} value was interpolated from dose–response graphs.