Cinchona Alkaloids from *Cinchona succirubra* and *Cinchona ledgeriana*

Authors
Gui-Guang Cheng[1,2], Xiang-Hai Cai[1], Bao-Hong Zhang[3], Yan Li[1], Ji Gu[1,2], Mei-Fen Bao[1,2], Ya-Ping Liu[1], Xiao-Dong Luo[1]

Affiliations
[1] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, P. R. China
[2] Graduate School of Chinese Academy of Sciences, Beijing, P. R. China
[3] Yunnan University of Traditional Chinese Medicine, Kunming, P. R. China

Key words
- *Cinchona surrirubra*
- *Cinchona ledgeriana*
- Rubiaceae
- cinchonanines A–G
- cinchona alkaloids
- cytotoxicity

Abstract

Seven new cinchona alkaloids, cinchonanines A–G (**1**–**7**), and 29 known alkaloids were isolated from the barks of *Cinchona surrirubra* and *C. ledgeriana* collected from Yunnan Province in China. The new structures were elucidated by extensive spectroscopic analysis. All compounds were evaluated for their cytotoxicity against five human cancer cell lines. Compounds **2, 13, 14**, and **15** showed moderate cytotoxicity.

Supporting information available online at http://www.thieme-connect.de/ejournals/toc/plantamedica

received August 28, 2013
revised October 22, 2013
accepted Dec. 16, 2013

Bibliography
DOI http://dx.doi.org/10.1055/s-0033-1360279
Published online January 22, 2014
Planta Med 2014; 80: 223–230

ISSN 0032-0943

Correspondence
Prof. Dr. Xiao-Dong Luo
State Key Laboratory of Phytochemistry and Plant Resources in West China
Kunming Institute of Botany, Chinese Academy of Sciences
Lanhei Road 132
Kunming 650201
P. R. China
Phone: + 86 8 71 65 22 31 77
Fax: + 86 8 71 65 15 02 27
xdluo@mail.kib.ac.cn

Correspondence
Dr. Ya-Ping Liu
State Key Laboratory of Phytochemistry and Plant Resources in West China
Kunming Institute of Botany, Chinese Academy of Sciences
Lanhei Road 132
Kunming 650201
P. R. China
liuyaping@mail.kib.ac.cn

Introduction

Cinchona alkaloids, which originate from the condensation of tryptophan with secologanin and then develop to give an impressive array of structural variants [1], are an important class of medicinal natural products. Some of the remarkable cinchona alkaloids, particularly quinine, have played a pivotal medicinal role in human society for over 300 years in the treatment of malaria, a disease caused by protozoans, of which the most troublesome is *Plasmodium falciparum* [2]. Structurally, these alkaloids can be divided into three groups, indole alkaloids, quinoline alkaloids, and quasi-dimeric cinchophyllines, regarding their oxidative cleavage, ring rearrangement, cyclization, etc. [1,3–7]. Pharmacological investigations on these alkaloids and their derivatives demonstrated cytotoxic [8], antimalarial [9,10], antiarrhythmic [11], antibacterial [12,13], antifebrile, and MAO-inhibitory activities [14]. Over the last thirty years, cinchona alkaloids have become increasingly popular in organic chemistry, being used as chiral catalysts, ligands, and NMR discriminating agents, among others [15–18].

The barks of several species of *Cinchona* and *Remijia* (Rubiaceae) trees have been proven to be good sources of cinchona alkaloids [1,3,4,14,19–22]. Up to now, over 30 cinchona alkaloids have been characterized by structural and stereochemical investigations. Most of them are quinoline alkaloids, which consist of two relatively rigid entities, an aromatic quinoline ring and an aliphatic quinuclidine moiety, connected by two carbon-carbon single bonds differing only in their configuration at the C-2 and C-3 chiral centers [18,23]. Among these quinoline alkaloids, cinchonine-HCl and acetylcupreine, isolated from *R. peruviana*, showed cytotoxic activities toward murine colon adenocarcinoma (CT26), human colon adenocarcinoma (SW480), human cervical adenocarcinoma (Hela), human melanoma (SkMel25), and human malignant melanoma (SkMel28) cancer cell lines, while cinchonine was more cytotoxic than cinchonine-HCl on Chinese hamster ovary (CHO) cancer cell line [19]. Although quinidine and its derivatives hydrocinchonine and cinchonine had weak cytotoxicity against human uterus sarcoma cells (MES-SA/DX5) and human sarcoma cells (MES-SA), the compounds enhanced paclitaxel (TAX)-induced cytotoxicity and P-glycoprotein (gp) substrate rhodamine accumulation in P-gp positive expressing MES-SA/DX5 cells and facilitated paclitaxel-induced apoptosis in MES-SA/DX5 cells [24]. Similarly, cinchonine has been reported to modulate doxorubicin-induced apoptosis by enhancing Fas expression in multidrug resistance cells and reverse the drug resistance of tumoral cells more efficiently than quinine through P-gp binding [8,25].

The genus *Cinchona*, comprising about 40 species, is native to the eastern slopes of the Andes and cultivated in tropical regions of the world. Previous phytochemical studies on two species of

this genus, *C. succirubra* and *C. ledgeriana*, collected in India, South America, and Europe, showed diverse secondary metabolites, including quinoline alkaloids [26–30], indole alkaloids [4], and polyphenols [21,31,32] (henolic acids, anthocyanins, and flavonoids). Four of the quinoline alkaloids: quinine, quinidine, cinchonidine, and cinchonine, account for over 50% of the alkaloid content. *C. succirubra* and *C. ledgeriana* have been introduced from Indonesia and cultivated in Yunnan Province of China since the 1930s [33]. The secondary metabolites would plausibly be influenced by the ecological environment, which encourage us to search for structurally unique and biological active terpenoid alkaloids from them. As a result, seven new cinchona alkaloids, including three quinoline alkaloids (**1**–**3**) and four indole alkaloids (**4**–**7**), together with 29 known compounds were isolated. The new alkaloids were elucidated by means of spectroscopic methods, while the known alkaloids were identified as cinchoninone (**8**) [26,34], cinchotoxine (**9**) [14], remijinine (**10**) [20], cinchonamine (**11**) [3], quinamine (**12**) [3], liriodenine (**13**) [35], lyscamine (**14**) [36], cinchophylline (**15**) [4], quinidinone (**16**) [37], quininone (**17**) [37], cinchonidinone (**18**) [38], quinine (**19**) [39], quinidine (**20**) [38], cinchonine (**21**) [5,19], cinchonidine (**22**) [39], 9-epiquinine (**23**) [39], 9-epiquinidine (**24**) [39], dihydroquinine (**25**) [39], dihydroquinidine (**26**) [39], quinine-*N*(4)-Oxide (**27**) [40], quinidine-*N*(4)-oxide (**28**) [40], 10-methoxycinchonamine (**29**) [21], cinchonaminone (**30**) [14], cinchonicinol (**31**) [14], epi-3-quinamine (**32**) [4], isocinchophyllamine (**33**) [4], alkaloid LA 5 (**34**) [41], 10-hydroxyscandine (**35**) [42], and alkaloid 376 (**36**) [43], by comparison with data in the literature. All compounds were evaluated for their cytotoxicity against five human cancer cell lines. The isolation, structural elucidation, and cytotoxicity evaluation of these alkaloids are reported in this paper.

Results and Discussion

Cinchonanine A (**1**) was isolated as colourless oil and gave a positive reaction with Dragendorff's reagent, characteristic of alkaloids. Its molecular formula $C_{20}H_{20}N_2O_2$ was determined by the molecular ion at *m/z* 320.1526 $[M]^+$ in the HREIMS, indicating twelve degrees of unsaturation. The UV spectrum of compound **1** demonstrated the presence of a quinoline moiety by presenting maximum absorptions at 206 and 334 nm [44]. The IR spectrum showed a strong absorption band at 1622 cm^{-1}, consistent with the presence of an *α,β*-unsaturated carbonyl functionality. According to the above data, together with one methyl at δ_H 3.83 (s, 3H), one characteristic terminal vinyl group, and five olefinic protons in its ^1H NMR spectrum, compound **1** was readily identified as a quinoline alkaloid, with a disubstituted quinoline ring moiety and a methoxyl signal at C-10 [39].

Analysis of the ^{13}C NMR and DEPT spectra (**Table 3**) of **1** revealed the presence of 20 carbon resonances, ascribed to one methoxyl group, four methylene, nine methine, and six quaternary carbons (one ketonic carbonyl group and four aromatic carbons). These data suggested that **1** was a cinchona alkaloid related to quinidinone with identical quinoline ring [40]. Besides one quinoline moiety, one ketonic carbonyl group, and one characteristic terminal vinyl group, the remaining three degrees of unsaturation should reside in the quinuclidine ring moiety. Because no sp^2 carbons were observed in the quinuclidine moiety in **1**, an additional ring structure should be assigned. Its ^1H and ^{13}C NMR data (**Tables 1** and **3**) indicated that it was similar to quinidinone. A significant difference was that a new carbon-carbon bond between C-3 and C-17 was formed in **1**. The conjecture was supported by the HMBC correlations of δ_H 1.87 (1H, br. d, J = 13.1 Hz, H-16ex) with δ_C 52.6 (s, C-3) and 41.2 (d, C-20), and of δ_H 2.21 (1H, m, H-14ex) with δ_C 32.8 (t, C-16), 47.1 (d, C-17), and 41.2 (d, C-20). The ROESY spectrum showed correlations of H-19/H-21c, H-21c/H-14ex, H-20/H-15, and H-20/H-16ex. Based on the consideration of the biosynthesis of cinchona alkaloids such as quinione, quinidine, cinchonine, and cinchonidine, which have a quinuclidine moiety, the absolute configurations at C-15 and C-20 were concluded to be *S* and *R*, respectively. In addition, the ROESY correlations of H-17ex/H-16ex, H-16ex/H-20, and H-17ex/H-21t suggested an *R* configuration for C-17. Thus, the structure of cinchonanine A (**1**) was established as shown in **Figs. 1** and **2**.

Cinchonanine B (**2**) had a molecular formula of $C_{19}H_{20}N_2O_2$ as established by HREIMS. The UV absorption bands at 294 and 206 nm suggested the presence of a quinoline chromophore, while the IR spectrum absorption bands at 3422 and 1725 cm^{-1} showed the existence of -OH and carbonyl groups. The ^1H and ^{13}C NMR (**Tables 1** and **3**) data of **2** were similar to those of cinchoninone [26,34], except for a hydroxyl substituent at C-17 in **2**. The assumption was supported by HMBC correlations of δ_H 4.24 (1H, dd, J = 9.0, 4.2 Hz, H-3), 3.08 (1H, dd, J = 10.7, 4.8 Hz, H-21t), 2.48 (1H, m, H-21c), 1.72 (1H, m, H-16en), and 1.57 (1H, d, J = 11.9 Hz, H-16ex), with δ_C 69.8 (d, C-17). The relative configuration of **2** was confirmed on the basis of the ROESY experiment, in which ROESY correlations of H-17ex with H-16ex, H-14en, H-21t, and H-3 suggested the configuration of C-17. The configurations of C-3, C-15, and C-20 in this cinchona alkaloid were confirmed as 3*R*, 15*S*, and 20*R*, respectively, on the basis of its biogenetic pathway. Detailed analysis of its 2D NMR data (HSQC, HMBC, and ROESY) established the structure of **2** to be 17-hydroxy cinchoninone, and named cinchonanine B.

Cinchonanine C (**3**) was isolated as colourless oil and had a molecular ion peak $[M]^+$ at *m/z* 310.1686 in its HREIMS, identified as $C_{19}H_{22}N_2O_2$, 16 mass units higher than that of cinchotoxine. The ^{13}C NMR spectrum of **3** showed a ketonic carbonyl group, two methylenes, a *cis*-4-alkyl-3-ethenylpiperidine, and a monosubstituted quinoline moiety. Thus, it was readily identified as cinchotoxine-*N*(4)-oxide from its ^1H and ^{13}C NMR data (**Tables 1** and **3**), in particular the characteristic downfield shifts of the carbon resonances of C-17 (δ_C 60.1) and C-21 (δ_C 65.5), compared with those of cinchotoxine [14]. Other parts of **3** were identical to those of cinchotoxine, supported by its HSQC, HMBC, and ROESY spectral data.

The molecular formula $C_{20}H_{26}N_2O_3$ of cinchonanine D (**4**) was established by HREIMS ($[M]^+$ at *m/z* 342.1940). Its UV spectra showed absorption maxima at 207, 260, and 302 nm, which is characteristic for oxindole chromophores [45], while the IR spectrum revealed the presence of a hydroxyl group at 3332 cm^{-1}, an amide carbonyl group at 1686 cm^{-1}, and an aromatic ring at 1610 and 1493 cm^{-1}. The ^1H and ^{13}C NMR spectra of compound **4** (**Tables 2** and **3**) suggested an indoylquinuclidine-type alkaloid with a *β*-hydroxyethyl side chain. The protons assignments of the quinuclidine moiety were established by HSQC, HMBC, and ROESY experiments, which were in agreement with a previous work [19]. The 1D (**Tables 2** and **3**) and 2D NMR data of compound **4** were similar to those of remijinine (**10**) [20]. A significant difference was a methoxyl group (δ_H 3.78, δ_C 56.3) substituted at C-10 of the benzene ring in **4**, which presented the ABX proton spin system signals, and then was further supported by the HMBC cor-

Table 1 ^{1}H NMR data of **1**–**3**^{a} (δ in ppm and J in Hz).

No.	1	2	3
3		4.24 dd (9.0, 4.2)	3.16 m
5	8.78 d (3.7)	9.05 d (4.4)	9.04 d (4.3)
6	7.32 overlap	8.15 d (4.4)	7.85 d (4.3)
9	7.10 d (2.7)	8.28 d (8.4)	8.29 d (8.4)
10		7.67 t (8.4)	7.67 t (8.4)
11	7.39 overlap	7.81 t (8.4)	7.81 t (8.4)
12	8.01 d (9.2)	8.12 d (8.4)	8.12 d (8.4)
14en	2.24 m	1.76 m	1.62 m
14ex	2.21 m	2.26 overlap	1.62 m
15	2.19 m	2.26 overlap	1.54 m
16en	2.02 m	1.72 m	2.08 m
16ex	1.87 br. d (13.1)	1.57 d (11.9)	1.28 m
17en			2.39 t (10.4)
17ex	2.87 br. s	3.62 d (4.9)	2.39 t (10.4)
18 t	5.04 d (18.4)	5.06 d (17.1)	5.16 d (17.2)
18c	5.01 d (10.8)	5.03 d (10.4)	5.05 d (10.2)
19	5.78 ddd (17.2, 10.8, 6.8)	5.77 ddd (17.1, 10.6, 6.3)	6.11 dt (17.4, 10.0)
20	2.28 m	2.38 m	2.53 m
21 t	3.48 m	3.08 dd (10.7, 4.8)	3.12 dd (17.4, 7.5)
21c	3.08 dd (14.8, 7.4)	2.48 m	2.58 dd (14.3, 11.7)
10-OMe	3.90 s		

^{a} Compound **1** was measured in $CDCl_3$; **2** and **3** in acetone-d_6

Table 2 ^{1}H NMR data of **4**–**7**^{a} (δ in ppm and J in Hz).

No.	4	5	6	7
3	3.30 overlap			3.71 t (9.6)
5a	3.23 td (10.1, 5.9)	3.90 t (6.0)	3.77 m	4.06 t (8.3)
5b	3.08 td (10.0, 5.2)	3.90 t (6.0)	3.77 m	3.50 m
6a	2.18 ddd (13.0, 9.6, 6.0)	3.19 t (6.0)	3.15 m	2.86 br. s
6b	2.05 ddd (13.1, 9.7, 5.2)	3.19 t (6.0)	3.08 m	2.29 dd (11.5, 4.1)
9	6.91 d (2.2)	7.55 d (7.9)	7.57 d (7.8)	7.37 d (7.4)
10		7.10 t (7.9)	6.98 t (7.8)	6.83 t (7.4)
11	6.79 dd (8.2, 2.2)	7.17 t (7.9)	7.07 t (7.8)	7.10 t (7.4)
12	6.82 d (8.2)	7.31 d (7.9)	7.44 d (7.8)	6.54 d (7.4)
14en	2.11 m	6.76 d (6.5)	2.55 br. d (13.7)	1.61 m
14ex	1.95 m		2.10 overlap	2.47 t (11.6)
15	1.80 s	2.75 overlap	2.06 m	2.08 overlap
16en	1.56 m	1.80 m	1.81 m	2.11 overlap
16ex	1.42 m	1.61 m	1.55 m	2.02 overlap
17en	3.00 m	3.01 m	3.09 overlap	4.40 m
17ex	2.47 m	2.70 overlap	2.68 m	3.17 t (10.4)
18 t	5.08 d (17.4)	4.98 d (17.1)	4.87 d (17.4)	5.17 m
18c	5.06 d (10.0)	4.91 d (10.1)	4.84 d (9.6)	5.17 m
19	6.04 ddd (17.3, 10.3, 7.8)	5.61 m	5.69 m	5.85 m
20	2.29 d (8.4)	2.61 dd (14.3, 7.8)	2.32 m	2.98 dd (20.1, 12,1)
21 t	2.93 dd (13.6, 10.2)	3.27 dd (13.0, 8.8)	3.16 dd (14.2, 9.6)	3.58 m
21c	2.63 m	2.51 d (12.7)	2.61 dd (13.1, 9.1)	3.34 d (12.9)
10-OMe	3.78 s			
3-OMe			2.83 s	

^{a} Compound **4** was measured in methanol-d_4; **5** and **7** in $CDCl_3$; **6** in acetone-d_6

relations of δ_H 6.91 (1H, d, J = 2.2 Hz, H-9) with δ_C 55.5 (s, C-7), 157.7 (d, C-10), and 136.5 (s, C-13), and of δ_H 6.82 (1H, d, J = 8.2 Hz, H-12) with δ_C 157.7 (d, C-10) and 134.6 (s, C-8). The ROESY spectrum showed correlations of H-3/H-21c, H-21t/H-20, H-19/H-14ex, H-15/H-20, and H-20/H-16ex. These were in good agreement with those of **10**, which showed the same configurations at C-3, C-15, and C-20. The specific rotation of compound **4** [− 11.3 (*c* 0.08, MeOH)] had the same sign and similar value to that of remijinine [− 21.9 (*c* 0.56, MeOH)], whose absolute configuration was determined by X-ray diffraction, but opposite to that of epiremijinine [+ 41.6 (*c* 0.13, MeOH)] [33]. Thus, the structure of cinchonanine D (**4**) was elucidated as 10-methoxy remijinine.

The HREIMS of cinchonanine E (**5**) displayed its molecular ion peak $[M]^+$ at *m/z* 294.1728 ($C_{19}H_{22}N_2O$). The UV spectrum showed absorption maxima characteristic of an indole chromophore (307, 233, and 206 nm) [46]. Its ^{1}H NMR spectrum displayed an ortho-disubstituted phenyl ring, two triplet signals at δ_H 3.19 and 3.90, assigned to two connected methylene groups

Table 3 ^{13}C NMR data of **1**–**7**[a] (δ in ppm).

No.	1	2	3	4	5	6	7
2	204.8 (s)	205.9 (s)	204.7 (s)	183.1 (s)	131.3 (s)	136.8 (s)	102.5 (s)
3	52.6 (s)	48.5 (d)	40.5 (t)	65.5 (d)	144.4 (s)	88.6 (s)	69.1 (d)
5	146.7 (d)	151.2 (d)	151.2 (d)	58.9 (t)	62.3 (t)	63.0 (t)	66.0 (t)
6	118.8 (d)	121.1 (d)	120.5 (d)	39.3 (t)	28.0 (t)	29.4 (t)	41.3 (t)
7	142.3 (s)	150.0 (s)	150.0 (s)	55.5 (s)	109.6 (s)	110.2 (s)	89.3 (s)
8	125.6 (s)	125.1 (s)	124.6 (s)	134.6 (s)	129.7 (s)	130.3 (s)	131.3 (s)
9	102.5 (d)	126.4 (d)	126.4 (d)	111.8 (d)	118.6 (d)	119.4 (d)	124.6 (d)
10	158.4 (s)	128.8 (d)	128.7 (d)	157.7 (s)	119.5 (d)	119.4 (d)	120.0 (d)
11	122.5 (d)	130.5 (d)	130.5 (d)	113.8 (d)	122.5 (d)	122.2 (d)	129.3 (d)
12	131.4 (d)	130.9 (d)	130.8 (d)	111.5 (d)	110.8 (d)	112.4 (d)	108.5 (d)
13	144.7 (s)	144.1 (s)	144.4 (s)	136.5 (s)	134.9 (s)	135.2 (s)	147.3 (s)
14	24.1 (t)	26.5 (t)	28.4 (t)	23.3 (t)	127.1 (d)	37.3 (t)	26.0 (t)
15	33.2 (d)	39.4 (d)	38.2 (d)	29.5 (d)	33.4 (d)	30.7 (d)	27.4 (d)
16	32.8 (t)	36.1 (t)	28.9 (t)	28.4 (t)	28.5 (t)	27.0 (t)	27.6 (t)
17	47.1 (d)	69.8 (d)	60.1 (t)	44.1 (t)	47.4 (t)	41.9 (t)	59.2 (t)
18	114.9 (t)	115.3 (t)	116.7 (t)	115.2 (t)	114.1 (t)	114.7 (t)	116.9 (t)
19	140.0 (d)	140.0 (d)	138.9 (d)	143.5 (d)	142.3 (d)	141.8 (d)	137.9 (d)
20	41.2 (d)	43.9 (d)	45.3 (d)	41.5 (d)	45.3 (d)	39.9 (d)	40.6 (d)
21	47.0 (t)	56.0 (t)	65.5 (t)	59.1 (t)	55.3 (t)	51.1(t)	72.3 (t)
10-OMe	55.6 (q)			56.3 (q)			
3-OMe						51.7 (q)	

[a] Compounds **1, 5**, and **7** were measured in $CDCl_3$; **2, 3**, and **6** in acetone-d_6; **4** in methanol-d_4

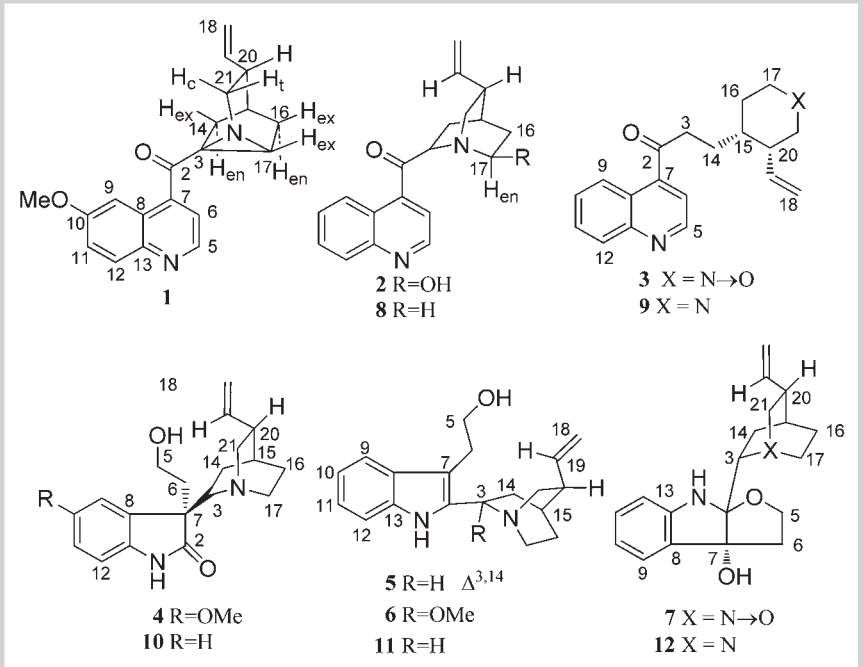

Fig. 1 Chemical structures of the new cinchonanines A–G (**1**–**7**) and the known **8**–**12**.

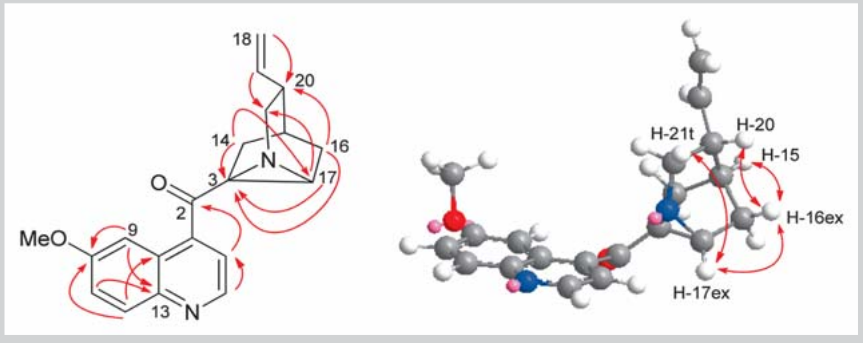

Fig. 2 Selected HMBC (→) and ROESY (↔) correlations of **1**. (Color figure available online only.)

Entry	HL-60	SMMC-7721	A-549	MCF-7	SW480
2	4.4	18.1	25.0	13.0	14.2
3	16.7	> 40	> 40	> 40	> 40
13	6.4	12.9	14.2	25.5	28.5
14	12.5	12.7	13.8	12.9	11.7
15	5.8	11.7	16.5	14.1	13.2
Cisplatin	1.1	14.5	12.7	17.1	16.8

Table 4 Cytotoxicity of compounds **2, 3**, and **13–15** (IC_{50}, μM).

(J = 6.0 Hz), a doublet at δ_H 6.76 (1H, d, J = 6.5 Hz) ascribed to an olefinic proton, and one characteristic terminal vinyl group. The ^1H and ^{13}C NMR data of **5** were similar to those of cinchonamine [3], except for two olefinic carbons [δ_C 127.1 (d, C-14), 144.4 (s, C-3)] appearing in **5** instead of two sp^3 carbons of C-3 and C-14 in cinchonamine. The assumption was supported by HMBC correlations of δ_H 6.76 (1H, d, J = 6.5 Hz, H-14) with δ_C 131.3 (s, C-2), 144.4 (s, C-3), 33.4 (d, C-15), and 45.3 (d, C-20). Furthermore, detailed analysis of 1D and 2D NMR data allowed the establishment of the structure of **5** as 3,14-dehydrocinchonamine.

Cinchonanine F (**6**) possessed a molecular formula of $C_{20}H_{26}N_2O_2$, as deduced from HREIMS ($[M]^+$, at m/z 326.1991, calcd. for 326.1994). Comparison of the ^1H and ^{13}C NMR spectra (**Tables 2** and **3**) of compound **6** with those of cinchonamine showed a close relationship between both alkaloids [3], with one more methoxyl group (δ_H 2.83, δ_C 51.7) at C-3 appearing in **6**. The suggestion was supported by HMBC correlations from δ_H 2.06 (1H, m, H-15), 3.16 (1H, dd, J = 14.2, 9.6 Hz, H-21t), 2.61 (1H, dd, J = 13.1, 9.1 Hz, H-21c), and 2.68 (1H, m H-17ex) to δ_C 88.6 (s, C-3). The relative configuration of C-3 was established by NOE correlations of H-16ex/H-15, H-17ex/H-16ex, and H-17en with the methoxyl in its ROESY spectrum. Complete analysis of 2D NMR data confirmed that the other parts of **6** were identical to those of cinchonamine. Hence, cinchonanine F (**6**) was elucidated to be 3-methoxy-cinchonamine.

Cinchonanine G (**7**) gave the molecular formula of $C_{19}H_{24}N_2O_3$ on the basis of HREIMS ($[M]^+$, at m/z 328.1782), with an index of hydrogen deficiency of nine. Its ^1H NMR spectrum suggested an indolylquinuclidine type alkaloid [3,6]. The ^{13}C NMR and DEPT data of **7** were similar to those of quinamine [3], except for three downfield carbon signals at δ_C 69.1 (d, C-3), 72.3 (t, C-21), and 59.2 (t, C-17) caused by the *N*(4)-oxide, which was consistent with its molecular formula. The ROESY correlations indicated that the relative configuration of **7** was the same to that of quinamine. Thus, compound **7** was elucidated to be quinamine-*N*(4)-oxide.

Alkaloids **2, 3, 9**, and **14** were isolated from *C. succirubra*, while alkaloids **1, 5, 6, 10, 15, 24–26, 29–31**, and **33–36** were obtained from *C. ledgeriana*, and the other alkaloids **4, 7, 8, 11–13, 16–23, 27, 28**, and **32** were ubiquitous in the two species. Comparison of reported cinchona alkaloids showed that two aporphine alkaloids, liriodenine (**13**) and lyscamine (**14**), one dimeric pyridine alkaloid, alkaloid LA 5 (**34**), one quinoline alkaloid, 10-hydroxyscandine (**35**), and one indole alkaloid, alkaloid 376 (**36**), without quinoline or quinuclidine ring, were first isolated from plants of the genus *Cinchona*.

All alkaloids (purities > 90%) were evaluated for their cytotoxicity against five human cancer cell lines, HL-60, SMMC-7721, A-549, MCF-7, and SW-480, using MTT method as reported previously [47]. Cisplatin (Sigma, > 98%) was used as the positive control. The results showed that compounds **2, 13**, and **15** exhibited significant cytotoxicity against HL-60 cell line, with IC_{50} values of 4.4, 6.4, and 5.8 μM, respectively. Furthermore, they showed moderate inhibitory effects against other four human cancer cell lines, with IC_{50} values comparable to those of cisplatin (IC_{50} values from 11.7 to 28.5 μM, **Table 4**). Compound **14** showed also moderate cytotoxicity against five human cancer cell lines (IC_{50}: 11.7–13.8 μM), while compound **3** displayed selective cytotoxicity against HL-60 (IC_{50} 16.7 μM). The other alkaloids were inactive (IC_{50} values of > 40 μM).

Materials and Methods

General experimental procedures

Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrometer. IR spectra were obtained by a Bruker FT-IR Tensor 27 spectrometer using KBr pellets. 1D and 2D spectra were run on an Avance III–600 MHz or a Bruker DRX-500 MHz spectrometer or an AV-400 MHz spectrometer with TMS as an internal standard. Chemical shifts (δ) were expressed in ppm with reference to solvent signals. HREIMS was recorded on a Waters Auto Premier P776 spectrometer. Column chromatography (CC) was performed on silica gel (200–300 mesh, Qingdao Marine Chemical Ltd.), RP-18 gel (20–45 μm, Fuji Silysia Chemical Ltd.), and Sephadex LH-20 (Pharmacia Fine Chemical Co., Ltd.). Fractions were monitored by TLC (GF 254, Qingdao Haiyang Chemical Co., Ltd.), and spots were visualized by Dragendorff's reagent. HPLC was performed using Waters 600 pumps coupled with analytical and semipreparative Sunfire C18 columns (150 × 4.6 and 150 × 10 mm, respectively). The HPLC system employed a Waters 2996 photodiode array detector and a Waters fraction collector II.

Plant material

C. succirubra and *C. ledgeriana* were collected from Yunnan Province, P.R. China, and authenticated by Mr. Jing-Yun Cui, Xishuangbanna Tropical Plant Garden. Two voucher specimens (No. Cui20090428 and No. Cui20090429) have been deposited in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and isolation

The air-dried and powdered barks of *Cinchona succirubra* (16 kg) and *C. ledgeriana* (11 kg) were extracted with 90% MeOH (40 L × 3, 2 days each) at room temperature, respectively. The extracts were partitioned between EtOAc and 0.5% HCl solution. The acidic water-soluble material, adjusted to pH 9–10 with 10% ammonia solution, was repeatedly extracted with EtOAc for three times, to give two crude alkaloidal extracts (118 g and 79 g).

The alkaloidal extract of *C. succirubra* (118 g) was subjected to a silica gel column (200–300 mesh, 8 × 150 cm, 1.2 kg) eluted with $CHCl_3$/MeOH (20 : 1, 10 : 1, 5 : 1, 1 : 1, 0 : 1, each 10 L) to afford frac-

tions I–V. Fraction I (1.7 g) was separated by silica gel CC (200–300 mesh, 5 × 40 cm, 60 g, eluted with petroleum ether–Me_2CO from 10 : 1 to 4 : 1) to afford **13** (8 mg). Fraction II (1.0 g) was gradually purified by RP-18 (2.5 × 25 cm, 50 g, MeOH–H_2O, 2 : 8 → 8 : 2), then followed by silica gel CC (200–300 mesh, 2.5 × 50 cm, 30 g, eluted with petroleum ether–EtOAc from 6 : 1 to 2 : 1) to yield an epimer, **16** and **17** (13 mg). Fraction III (3.4 g) was subjected to RP-18 (3 × 40 cm, 100 g, MeOH-H_2O, from 1 : 9 to 8 : 2) and afforded two subfractions, III-a and III-b. Subfraction III-a (1.6 g) was further purified by silica gel CC (200–300 mesh, 5 × 40 cm, 60 g, petroleum ether–Me_2CO, v/v, 4 : 1 → 1 : 1) to yield **8** (10 mg), **11** (24 mg), **12** (31 mg), and **18** (18 mg). Subfraction III-b (710 mg) was chromatographed on a silica gel column (200–300 mesh, 1.5 × 30 cm, 25 g, $CHCl_3$–MeOH, 20 : 1) to afford **7** (13 mg), **14** (3 mg), and **32** (21 mg). Fraction IV (55 g) was separated by silica gel CC (200–300 mesh, 7 × 80 cm, 700 g, $CHCl_3$ → MeOH, v/v, 15 : 1 to 5 : 1), then by RP-18 CC (3 × 40 cm, 100 g), eluted with MeOH–H_2O (3 : 7 → 7 : 3) to afford **19** (16.2 g), **20** (4.6 g), **21** (6.4 g), **22** (3.1 g), and a mixture. The mixture was further purified by Sephadex LH-20 CC (1.5 × 100 cm, 50 g, $CHCl_3$–MeOH, v/v, 1 : 1), then by silica gel CC ($CHCl_3$–MeOH, 15 : 1) to give **4** (7 mg) and **9** (9 mg). Fraction V (20 g) was separated by RP-18 column (4.9 × 46 cm, 450 g), eluted with MeOH–H_2O (3 : 7 → 7 : 3) and then by silica gel CC (200–300 mesh, 4 × 50 cm, 70 g, $CHCl_3$–MeOH, 10 : 1) to yield **23** (660 mg), **27** (6630 mg), **28** (1660 mg), and a mixture. The mixture was further separated on a semipreparative C_{18} HPLC column (4.6 × 150 mm) with a gradient MeOH–H_2O (3 : 7–4 : 6) to **2** (2 mg) and **3** (2 mg).

The alkaloidal extract of *C. ledgeriana* (79 g) was chromatographed on a silica gel column (200–300 mesh, 7 × 120 cm, 1.0 kg), eluted with $CHCl_3$/MeOH (1 : 0 → 0 : 1), to yield fractions I–VII. Fraction I (1.4 g) was gradually purified by RP-18 (2.5 × 25 cm, 50 g, MeOH–H_2O, 4 : 6 → 7 : 3) to afford subfractions I-a and I-b. Subfraction I-a (1.7 g) was separated by silica gel CC (200–300 mesh, 5 × 40 cm, 60 g, petroleum ether–Me_2CO, 8 : 1 → 4 : 1) to yield **6** (1 mg), **12** (20 mg), and the epimer **16** and **17** (11 mg). Subfraction I-b was separated by silica gel CC (200–300 mesh, 1.5 × 25 cm, 20 g, petroleum ether–EtOAc, 4 : 1 → 1 : 1) to afford **1** (2 mg), **7** (38 mg), **11** (3 mg), **13** (6 mg), **29** (7 mg), and **34** (5 mg). Fraction II (590 mg) was gradually purified by RP-18 (2.2 × 25 cm, 30 g, MeOH–H_2O, 3 : 7 → 5 : 5), then followed by silica gel CC (1.0 × 25 cm, 10 g, petroleum ether–Me_2CO, 10 : 1 → 5 : 1) to yield **5** (14 mg). Fraction III (830 mg) was separated by silica gel CC (200–300 mesh, 3 × 40 cm, 30 g, petroleum ether–Me_2CO, 8 : 1 → 2 : 1), then by RP-18 (2.5 × 25 cm, 50 g), eluted with MeOH–H_2O (4 : 6 → 7 : 3) to afford **25** (11 mg) and the epimer **8** and **18** (6 mg). Fraction IV (16.7 g) was gradually purified by RP-18 (4.9 × 46 cm, 450 g, MeOH–H_2O, 2 : 8 → 6 : 4) to give subfractions IV-a (2.3 g) and IV-b (12.2 g). Subfraction IV-a was further purified by RP-18 (3 × 40 cm, 100 g), eluted with MeOH–H_2O (25 : 75 → 40 : 60) to afford **4** (25 mg), **10** (35 mg), and **26** (20 mg). Subfraction IV-b was further separated by silica gel CC (4 × 50 cm, 100 g, $CHCl_3$–MeOH, 15 : 1 → 10 : 1) to give **19** (4635 mg), **20** (920 mg), **21** (1423 mg), **22** (352 mg), **31** (27 mg), and a mixture. The mixture was further purified by Sephadex LH-20 CC (2 × 150 cm, 100 g, $CHCl_3$–MeOH, 1 : 1), then by silica gel CC (200–300 mesh, 1.5 × 25 cm, 20 g, petroleum ether–Me_2CO, 3 : 1) to give **15** (8 mg), **32** (132 mg), and **33** (4 mg). The separation of fraction VI (8.08 g) was gradually purified by RP-18 (3 × 40 cm, 100 g) eluted with MeOH–H_2O (3 : 7 → 8 : 2), and then by silica gel CC (200–300 mesh, 2.5 × 50 cm, 30 g, $CHCl_3$–MeOH, 10 : 1) to afford **23** (90 mg), **24** (152 mg), **27** (1643 mg), **28** (265 mg), **30** (3 mg), **35** (44 mg), and **36** (11 mg).

Cytotoxicity assay

Five human cancer cell lines, human myeloid leukemia HL-60, hepatocellular carcinoma SMMC-7721, lung cancer A-549, breast cancer MCF-7, and colon cancer SW480 cells, were used in the cytotoxic assay. All the cells were cultured in RPMI-1640 or DMEM medium (Hyclone), supplemented with 10% fetal bovine serum (Hyclone) in 5% CO_2 at 37 °C. The cytotoxicity assay was performed according to the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) method in 96-well microplates [47]. Briefly, 100 µL adherent cells were seeded into each well of 96-well cell culture plates and allowed to adhere for 12 h before drug addition, while suspended cells were seeded just before addition of the drug with initial density of 1×10^5 cells/ml. Each tumor cell line was exposed to the test compound at concentrations of 0.064, 0.32, 1.6, 8, and 40 µM in triplicate for 48 h, with cisplatin (Sigma) as a positive control. After compound treatment, cell viability was detected, and cell growth curve was graphed. IC_{50} value was calculated by Reed and Muench's method [48].

Cinchonanine A (**1**): colorless oil; $[\alpha]_D^{24}$ + 25.9 (*c* 0.18, MeOH); UV (MeOH) λ_{max} (log ε) 334 (2.71), 206 (3.61) nm; IR (KBr) ν_{max} 2955, 2925, 2854, 1622, 1506, 1471, 1465, 1430, 1418, 1384, 1269, 1229, 1082, 1028 cm^{-1}; ^1H (600 MHz) and ^{13}C NMR (150 MHz) data ($CDCl_3$), see ▶ **Tables 1** and **3**, respectively; HREIMS *m/z* 320.1526 (calcd. for $C_{20}H_{20}N_2O_2$ $[M]^+$, 320.1525).

Cinchonanine B (**2**): a white amorphous powder; $[\alpha]_D^{26}$ − 80.6 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 294 (3.82), 206 (3.10) nm; IR (KBr) ν_{max} 3422, 3072, 2926, 2855, 1725, 1688, 1642, 1616, 1566, 1461, 1355, 1266, 1214, 1109, 1027, 964, 771, 630, 529 cm^{-1}; ^1H (600 MHz) and ^{13}C NMR (150 MHz) data (Me_2CO-d_6), see ▶ **Tables 1** and **3**, respectively; HREIMS *m/z* 308.1514 (calcd. for $C_{19}H_{20}N_2O_2$ $[M]^+$, 308.1525).

Cinchonanine C (**3**): colorless oil; $[\alpha]_D^{26}$ − 8.6 (*c* 0.13, MeOH); UV (MeOH) λ_{max} (log ε) 294 (2.93), 206 (3.77) nm; IR (KBr) ν_{max} 3427, 2960, 2926, 1669, 1613, 1468, 1439, 1382, 1273, 1189, 1111, 809, 582 cm^{-1}; ^1H (600 MHz) and ^{13}C NMR (150 MHz) data (Me_2CO-d_6), see ▶ **Tables 1** and **3**, respectively; HREIMS *m/z* 310.1686 (calcd. for $C_{19}H_{22}N_2O_2$ $[M]^+$, 310.1681).

Cinchonanine D (**4**): a white amorphous powder; $[\alpha]_D^{26}$ − 11.3 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε) 302 (2.61), 260 (3.22), 207 (3.60), 192 (3.00) nm; IR (KBr) ν_{max} 3333, 2939, 2979, 2661, 2425, 1686, 1634, 1610, 1494, 1457, 1386, 1297, 1203, 1031, 910, 822, 744, 666, 608, 575 cm^{-1}; ^1H (600 MHz) and ^{13}C NMR (150 MHz) data (CD_3OD), see ▶ **Tables 2** and **3**, respectively; HREIMS *m/z* 342.1940 (calcd. for $C_{20}H_{26}N_2O_3$ $[M]^+$, 342.1943).

Cinchonanine E (**5**): a white amorphous powder; $[\alpha]_D^{25}$ − 10.4 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 307 (3.54), 233 (3.56), 206 (3.58) nm; IR (KBr) ν_{max} 3424, 2929, 2867, 2377, 2309, 1722, 1636, 1511, 1457, 1340, 1307, 1071, 1044, 911, 835, 742, 550 cm^{-1}; ^1H (400 MHz) and ^{13}C NMR (100 MHz) data ($CDCl_3$), see ▶ **Tables 2** and **3**, respectively; HREIMS *m/z* 294.1728 (calcd. for $C_{19}H_{22}N_2O$ $[M]^+$, 294.1732).

Cinchonanine F (**6**): colorless oil; $[\alpha]_D^{24}$ − 11.3 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 283 (3.11), 222 (3.73) nm; IR (KBr) ν_{max} 3441, 3426, 2933, 2869, 1634, 1456, 1436, 1326, 1312, 1203, 1155, 1092, 1041, 1003, 743 cm^{-1}; ^1H (600 MHz) and ^{13}C NMR (150 MHz) data (Me_2CO-d_6), see ▶ **Tables 2** and **3**, respectively; HREIMS *m/z* 326.1991 (calcd. for $C_{20}H_{26}N_2O_2$ $[M]^+$, 326.1994).

Cinchonanine G (**7**): a white amorphous powder; $[\alpha]_D^{26}$ + 51.0 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε) 299 (2.78), 239 (3.17), 206

(3.49) nm; IR (KBr) ν_{max} 3407, 2925, 2854, 1727, 1613, 1472, 1378, 1283, 1199, 1120, 1073, 1020, 927, 855, 747, 619, 504 cm^{-1}; ^1H (400 MHz) and ^{13}C NMR (100 MHz) data ($CDCl_3$), see **Tables 2** and **3**, respectively; HREIMS *m/z* 328.1782 (calcd. for $C_{19}H_{24}N_2O_3$ $[M]^+$, 328.1787).

Supporting information

1D, 2D NMR (HSQC, HMBC, ROESY), and MS spectra of cinchonanines A–G (**1**–**7**) are available as Supporting Information.

Acknowledgments

The authors are grateful to the National Natural Science Foundation of China (81225024, 31170334, 21072198) and the National Science and Technology Support Program of China (2013BAI11B02) for partial financial support.

Conflict of Interest

The authors declare no conflict of interest.

References

1 *Aerts RJ, Waal A, Pennings EJM, Verpoorte R.* The distribution of strictosidine-synthase activity and alkaloids in *Cinchona* plants. Planta 1991; 183: 536–541
2 *Hoffmann H, Martin R, Frackenpohl J.* Recent advances in *Cinchona* alkaloid chemistry. Eur J Org Chem 2004; 2004: 4293–4312
3 *Mulder-Krieger TH, Verpoorte R, Svendsen AB.* The ^{13}C-NMR spectrometry of cinchonamine and quinamine. Pharm World Sci 1982; 4: 91–92
4 *Zeches M, Richard B, Thepenier P, Le men-Olivier L, Le ment J.* Alcaloïdes des feuilles du *Cinchona ledgeriana*. Phytochemistry 1980; 19: 2451–2454
5 *Lyle GG, Keefer LK.* The configurations at C-9 of the cinchona alkaloids: NMR spectral study of the derived oxiranes. Tetrahedron 1967; 23: 3253–3263
6 *Bruix M, Rumbero A, Vazquez P.* Apodihydrocinchonamine, an indole alkaloid from *Isertia haenkeana*. Phytochemistry 1993; 33: 1257–1261
7 *Okunade AL, Lewis WH, Elvin-Lewis MP, Casper SJ, Goldberg DE.* Cinchonicine-derived alkaloids from the bark of the Peruvian *Ladenbergia oblongifolia*. Fitoterapia 2001; 72: 717–719
8 *Furusawa S, Nakano S, Wu J, Sakaguchi S, Takayanagi M, Sasaki K-I, Satoh S.* Apoptosis induced by doxorubicin and cinchonine in p388 multidrug-resistant cells. J Pharm Pharmacol 2001; 53: 1029–1039
9 *Karle JM, Karle IL, Gerena L, Milhous WK.* Stereochemical evaluation of the relative activities of the cinchona alkaloids against *Plasmodium falciparum*. Antimicrob Agents Chemother 1992; 36: 1538–1544
10 *Karle JM, Bhattacharjee AK.* Stereoelectronic features of the cinchona alkaloids determine their differential antimalarial activity. Bioorg Med Chem 1999; 7: 1769–1774
11 *Nwangwu PU, Holcslaw TL, Rosenberg H, Small LD, Stohs SJ.* The antiarrhythmic activities of 6′-hydroxycinchonine, 6′-benzyloxycinchonine and 6′-allyloxycinchonine compared with quinidine in mice. J Pharm Pharmacol 1979; 31: 488–489
12 *Yardley JP, Bright RE, Rane L, Rees RW, Russell PB, Smith H.* Antimalarial and other biological activities of some 2′-alkyl and 2′-aryl derivatives of cinchona alkaloids. J Med Chem 1971; 14: 62–65
13 *Skogman ME, Kujala J, Busygin I, Leino R, Vuorela PM, Fallarero A.* Evaluation of antibacterial and anti-biofilm activities of cinchona alkaloid derivatives against *Staphylococcus aureus*. Nat Prod Commun 2012; 7: 1173–1176
14 *Mitsui N, Noro T, Kuroyanagi M, Miyase T, Umehara K, Ueno A.* Monoamine oxidase inhibitors from Cinchonae Cortex. Chem Pharm Bull 1989; 37: 363–366
15 *Raheem IT, Goodman SN, Jacobsen EN.* Catalytic asymmetric total syntheses of quinine and quinidine. J Am Chem Soc 2003; 126: 706–707
16 *Zhao MX, Chen MX, Tang WH, Wei DK, Dai TL, Shi M.* Cinchona alkaloid catalyzed regio- and enantioselective allylic amination of Morita-Baylis-Hillman carbonates with isatins. Eur J Org Chem 2012; 2012: 3598–3606
17 *Quigley C, Rodriguez-Docampo Z, Connon SJ.* Highly tunable arylated cinchona alkaloids as bifunctional catalysts. Chem Commun 2012; 48: 1443–1445
18 *Dijkstra GDH, Kellogg RM, Wynberg H, Svendsen JS, Marko I, Sharpless KB.* Conformational study of cinchona alkaloids. A combined NMR, molecular mechanics and x-ray approach. J Am Chem Soc 1989; 111: 8069–8076
19 *Lastenia RM, Wilfredo RM, Matias R, Rafael MD, Concepcion DI, Guadano A, Azucena GC.* Bioactive cinchona alkaloids from *Remijia peruviana*. J Agric Food Chem 2005; 53: 1921–1926
20 *Diaz JG, Sazatornil JG, Rodriguez ML, Mesia LR, Arana GV.* Five new alkaloids from the leaves of *Remijia peruviana*. J Nat Prod 2004; 67: 1667–1671
21 *Mulder-Krieger T, Verpoorte R, de Water A, van Gessel M, van Oeveren BC, Svendsen AB.* Identification of the alkaloids and anthraquinones in *Cinchona ledgeriana* callus cultures. Planta Med 1982; 46: 19–24
22 *McCalley DV.* Analysis of the cinchona alkaloids by high-performance liquid chromatography and other separation techniques. J Chromatogr A 2002; 967: 1–19
23 *Dijkstra GDH, Kellogg RM, Wynberg H.* Conformational study of cinchona alkaloids. A combined NMR and molecular orbital approach. J Org Chem 1990; 55: 6121–6131
24 *Lee SY, Rhee YH, Jeong SJ, Lee HJ, Lee HJ, Jung MH, Kim SH, Lee EO, Ahn KS, Ahn KS, Kim SH.* Hydrocinchonine, cinchonine, and quinidine potentiate paclitaxel-induced cytotoxicity and apoptosis via multidrug resistance reversal in MES-SA/DX5 uterine sarcoma cells. Environ Toxicol 2011; 26: 424–431
25 *Solary E, Velay I, Chauffert B, Bidan JM, Caillot D, Dumas M, Guy H.* Sufficient levels of quinine in the serum circumvent the multidrug resistance of the human leukemic cell line K562/ADM. Cancer 1991; 68: 1714–1719
26 *Robins RJ, Rhodes MJC.* An evaluation of the tautomerism of cinchonine and quinidinone made using a combination of ^1H NMR and ^{13}C NMR spectroscopy. Phytochemistry 1987; 26: 551–556
27 *Staba EJ, Chung AC.* Quinine and quinidine production by cinchona leaf, root and unorganized cultures. Phytochemistry 1981; 20: 2495–2498
28 *Quevauviller A, Foussard-Blanpin O, Sarrazin G, Bourrinet P, Nakaji Y.* Pharmacodynamics of the alkaloids from *Cinchona ledgeriana* leaves. Ann Pharm Fr 1969; 27: 397–402
29 *Quevauviller A, Sarrazin G, Nakaji Y.* Cinchophyllamine, an alkaloid from *Cinchona ledgeriana* leaves. C R Acad Sci (Paris, Ser D) 1969; 268: 441–442
30 *Anderson LA, Keene AT, Phillipson JD.* Alkaloid production by leaf organ, root organ and cell suspension cultures of *Cinchona ledgeriana*. Planta Med 1982; 46: 25–27
31 *Dhar DN, Munjal RC.* Flavonoid constituents of the leaves of *Cinchona ledgeriana*. Curr Sci 1974; 43: 479
32 *Paris RR, Jacquemin H.* Leaves of two Cinchonas from Madagascar (*Cinchona ledgeriana* and *C. succirubra*) and their particular polyphenols (phenolic acids, anthocyanins, and flavonoids). Ann Pharm Fr 1975; 33: 73–76
33 *Luo XR.* Flora Republicae Popularis Sinicae (Zhongguo Zhiwu Zhi), Volume 71. Beijing: Science Press; 1999: 223–225
34 *Renfrew AG, Cretcher LH.* Cinchona alkaloids in pneumonia. II. Ketone formation with sodium amide. J Am Chem Soc 1935; 57: 738–739
35 *Zhang Z, ElSohly HN, Jacob MR, Pasco DS, Walker LA, Clark AM.* New sesquiterpenoids from the root of *Guatteria multivenia*. J Nat Prod 2002; 65: 856–859
36 *Hsieh TJ, Chang FR, Chia YC, Chen CY, Chiu HF, Wu YC.* Cytotoxic constituents of the fruits of *Cananga odorata*. J Nat Prod 2001; 64: 616–619
37 *Gutzwiller J, Uskokovic M.* Reinvestigation of the classical synthesis of *Cinchona* alkaloids. II. Synthesis of quinine and its naturally occurring diastereomers from quinotoxine. Helv Chim Acta 1973; 56: 1494–1503
38 *Lyle GG, Gaffield W.* Rotatory dispersion studies. V. The cinchona alkaloids. Tetrahedron 1967; 23: 51–63
39 *Moreland CG, Philip A, Carroll FI.* Carbon-13 nuclear magnetic resonance spectra of cinchona alkaloids. J Org Chem 1974; 39: 2413–2416
40 *Shibuya H, Kitamura C, Maehara S, Nagahata M, Winarno H, Simanjuntak P, Kim HS, Wataya Y, Ohashi K.* Transformation of cinchona alkaloids into 1-*N*-Oxide derivatives by endophytic *Xylaria* sp. isolated from *Cinchona pubescens*. Chem Pharm Bull 2003; 51: 71–74

41 *Willems M.* Dimeric pyridine alkaloids: artifacts, originated from secoiridoid glucosides from *Ligustrum vulgare* L. Arch Pharm 1988; 321: 229–230
42 *Zhou YL, Ye JH, Li ZM, Huang ZH.* Study on the alkaloids of *Melodinus tenuicaudatus*. Planta Med 1988; 54: 315–317
43 *Goh SH, Ali ARM, Wong WH.* Alkaloids of *Leuconotis griffithii* and *L. eugenifolia* (Apocynaceae). Tetrahedron 1989; 45: 7899–7920
44 *Novack L, Brodie BB.* Quinoline and its transformation products found in urine. J Biol Chem 1950; 187: 787–792
45 *Hinman R, Bauman C.* Reactions of 3-bromooxindoles. The synthesis of 3-methyleneoxindole1. J Org Chem 1964; 29: 2431–2437
46 *Sheludko Y, Gerasimenko I, Kolshorn H, Stoeckigt J.* New alkaloids of the sarpagine group from *Rauvolfia serpentina* hairy root culture. J Nat Prod 2002; 65: 1006–1010
47 *Mosmann T.* Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 1983; 65: 55–63
48 *Reed LJ, Muench H.* A simple method of estimating fifty percent endpoints. Am J Hygiene 1938; 27: 493–497