Cryptocaryol A and B: Total Syntheses, Stereochemical Revision, Structure Elucidation and Structure-Activity Relationship

Yanping Wang and **George A. O'Doherty**[*]
Department of Chemistry and Chemical Biology, Northeastern University, Boston, MA 02115

Abstract

The first total syntheses and structural elucidation of cryptocaryol A and cryptocaryol B were achieved in 23 and 25 linear steps, respectively. The synthesis relied on the use of a key pseudo-C_s symmetric pentaol intermediate, which in a stereo-chemically divergent manner was converted into either enantiomer as well as diastereomers. This synthetic effort enabled the first structure-activity relationships of this class of PDCD4 stabilizing natural products.

The early success and subsequent limitation found with the development of PKC as a target for cancer and other diseases, have led to the search for alternative downstream kinase targets for development (e.g., mTOR, Akt).[1] It is believed that the regulation of these new targets will selectively produce all the desired outcomes (e.g., tumor suppression) without side effects (e.g., non-cancer cell toxicity).[2] Programmed cell death 4 (PDCD4), a downstream target of Akt, is a novel tumor suppressor protein. PDCD4 interaction with eukaryotic initiation factor 4A (eIF4A) inhibits protein synthesis.[3] In addition, PDCD4 suppresses the activation of activator protein-1 (AP-1) through c-Jun.[4] Not surprisingly, the stabilization of PDCD4 is linked to the induction of apoptosis.[5] Conversely, its low expression levels are linked with the progression of several cancers (e.g., lung, liver, ovary and brain).[6]

In an effort to find natural products that stabilize levels of PDCD4, Gustafson *et al.* developed a high-throughput *in vivo* cell-based assay that identified cryptocaryols A–H (**1**–**8**) (Figure 1).[7] This class of natural products isolated from *cryptocarya sp.* shares a 5,6-dihydro- -pyranone and a 1,3-polyol segment. In addition, the eight cryptocaryols stablized PDCD4 in 12-*O*-tetradecanoylphorbol-13-acetate (TPA) challenged cells with EC_{50} ranging from 1.3 to 4.9 mM. The structures of these compounds were elucidated by a combination of NMR, HRMS and CD analyses. The all *syn*-tetraol relative configuration was assigned using Kishi's ^{13}C NMR database,[8] and the absolute configuration of pyranone at C-6 was assigned as *R* from its Cotton effect.[9] Unfortunately, knowledge gained from the structure-activity relationship (SAR) study was limited by the ambiguities associated with the absolute and relative stereochemistry of these structures.[10]

Thus, we devised a plan for the synthesis of cryptocaryol A and B with the aims of establishing the 3D structure and providing material for SAR studies (Scheme 1). In

Corresponding Author g.odoherty@neu.edu.

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Supporting Information
Detailed experimental procedures, full characterization data, and copies of spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

particular, we envisioned an approach that would take advantage of the pseudo-C_s symmetry of a tetraol fragment in **13**,[11] which would be amenable for the synthesis of the purported structures of these natural products (**1** and **2**), along with their enantiomer (**12**) and C6/16-diastereomers (e.g., **9**, **10** and **11**). Recently, we developed an iterative hydration of polyene strategy to build 1,3-polyols,[12] which has proved to be extremely successful for the syntheses of related 1,3-polyol-natural products[13] as well as more complicated variants.[14]

Towards this end, we began with the synthesis of orthogonally protected pentaol **13** from commercially available 5-hexyn-1-ol (**16**) (Scheme 2). The primary alcohol was protected as a PMB ether and the terminal alkyne was homologated (*n*-BuLi/methyl chloroformate, **16** to **17**) and then subsequently isomerized (PPh$_3$/PhOH)[15] to give dienoate **18** in excellent overall yield for 3 steps (88%). The distal double bond of dienoate **18** was asymmetrically oxidized under the Sharpless conditions ((DHQ)$_2$PHAL) to give a 2-enoate-4,5-diol,[16] which upon treatment with triphosgene and pyridine gave carbonate **19**. A Pd-catalyzed regioselective reduction of **19** with (Et$_3$N/HCO$_2$H, catalytic Pd/PPh$_3$) produced -hydroxy enoate **20**. Acetal formation using the Evans' conditions (benzaldehyde/KO*t*-Bu) diastereoselectively transformed **20** into benzylidene protected *syn*-1,3-diol **21**.[17] Thus in 4 steps, the initial protected diol fragment of **13** was installed in **21** from **18**.

The installation of the second protected diol fragment of **13** began with an ester to aldehyde reduction of **21** (DIBALH) followed by Leighton allylation to give homoallylic alcohol **22**.[18] The homoallylic alcohol stereochemistry of **22** was used to stereospecifically install the final benzylidene protected diol fragment. This was accomplished with a 2-step cross metathesis (ethyl acrylate/Grubbs II) and Evans' acetal formation sequence to furnish the pentaol **13**.[19]

With the key pentaol **13** in hand, our efforts were turned to the synthesis of the purported cryptocaryol A (**1**) and B (**2**). The PMB group in **13** was deprotected with DDQ to release the primary alcohol, which then was oxidized with DMP to afford aldehyde **23** (Scheme 3). Nucleophilic alkyne addition (1-pentadecyne/*n*-BuLi, −78 °C) to aldehyde **23** gave a propargyl alcohol, which upon oxidation (Dess-Martin) and reduction (Noyori) diastereoselectively gave propargyl alcohol **24**.[20] The alkyne in **24** was reduced to alkane **25** with excess diimide (NBSH/Et$_3$N). A 2-step DIBALH reduction and alcohol acylation procedure on ester **25** produced aldehyde **26**. The final stereocenter in **2** was installed with the use of a second Leighton allylation, which after acylation (acrylic acid/DCC) was then converted into diene **27**. A ring closing metathesis (Grubbs I) installed the desired pyranone, which after benzylidene deprotection (AcOH/H$_2$O) furnished the structure purported to be cryptocaryol B (**2**).[10,21]

Although great similarities existed between the ^{1}H and ^{13}C NMR spectra of **2** and the data reported for cryptocaryol B,[7] our analysis led us to conclude that they did not match.[10] This included discrepancies in the ^{1}H NMR (e.g., H-5a/H-5b, H-6, H-7a/H-7b, and H-8) and the ^{13}C NMR (C-6, C-7 and C-8), with the variances (0.6 to 0.9 ppm) in the ^{13}C NMR values being the hardest to reconcile. In order to gain a locus for further comparison, we attempted to convert **2** into the structure reported for cryptocaryol A (**1**). Unfortunately, we were unable to find conditions to selectively hydrolyze the C-16 acetate without concomitant hydrolysis of the pyranone ring. Next, we targeted the C-6 diastereomers of **1** and **2** (**30a** and **30b**, respectively), as the stereochemical relationship between the C-6 and C-8 positions was ambiguously assigned by Gustafson.[7] Moreover, we found the greatest variance in the C-5 to C-9 positions in our comparison of the ^{1}H and ^{13}C NMR.

These revised efforts returned to alcohol **25** and involved the use of the enantiomeric (*R,R*)-Leighton reagent (Scheme 4). In practice, we protected the secondary alcohol in **25** as a TBS

ether and reduced the ester to aldehyde **28**. Application of the diasteromeric Leighton allylation, acylation (acrylic acid/DCC) gave diene **29a**, which in 2 steps (Grubbs I; AcOH/ H_2O) was converted into **30a**. The ^1H NMR and ^{13}C NMR spectral data for synthetic **30a** were found to be identical to the data reported for cryptocaryol A. While the optical rotation data was consistent in magnitude, it was opposite in sign (reported: [ ]$_D$ = +12 (c = 0.1, MeOH); synthetic: [ ]$_D^{21}$ = –13.4 (c = 0.1, MeOH)). Replacing the TBS group in **29a** with an acetate group (TBAF; Ac_2O/EtN$_3$) gave **29b**, the precursor for *ent*-cryptocaryol B, which in 2 steps (Grubbs I; AcOH/H_2O) was converted into **30b**. Once again, the spectral data for synthetic **30b** were identical to the data reported for cryptocaryol B.[22] Thus the structures for cryptocaryol A and B should be reassigned to **9** and **10**, respectively.[21]

With the elucidation of the structures for the cryptocaryol A and B, we set out to undertake their enantioselective synthesis and biological evaluation as anticancer agents. This effort began with pseudo-C_s symmetric protected pentaol **13**, and requires the reversal in the order of pyranone and side chain installation (Scheme 5). The revised route begins with the conversion of ester **13** into ynone **31** (DIBALH; 1-penadecyne; Dess-Martin). The C-16 stereochemistry was installed in alcohol **32** by a 2-step Noyori asymmetric and diimide reduction procedure. Adjustments of the protecting groups involved the protection of the C-16 alcohol of **32** as a TBS group (TBSCl) followed by PMB-deprotection (DDQ) to give **33a**. Oxidation of the primary alcohol in **33a** (Dess-Martin) followed by Leighton allylation and acrylate acylation (acrylic acid/DCC) provided diene **34a**, from which **34b** was prepared with the required C-16 acetate group.

Using the same ring closing metathesis/deprotection sequence, the dienes **34a** and **34b** were uneventfully converted into cryptocaryol A (**9**) and B (**10**). The ^1H and ^{13}C NMR data for the synthetic material were identical to the data reported for the isolated material.[21] In addition to providing ample material for structural elucidation, the route also provided enough material for the cancer cell cytotoxicity studies. As part of these SAR studies, additional analogues (hexaol **35a**, hexaol acetate **35b** and saturated pyranone compound **36**) were required for evaluation. These analogues were readily prepared from intermediates **33a/b** and cryptocaryol B (**10**) by deprotection of benzylidene and hydrogenation of alkene, respectively (Scheme 6).

While other PDCD4 stabilizers are known to be cytotoxic, there is very little data to correlate their activity to PDCD4 stabilization.[23] With access to cryptocaryol A and B, two known PDCD4 stabilizers (4.9 and 3.0 mM, see Gustafson's assay), this comparison can be made. We chose to study MCF-7 breast cancer cells (Table 1 and Figure 2),[24] because of their high expression level of PDCD4.[25] We found that both cryptocaryol A and B possessed growth inhibitory activity against MCF-7 in the mircomolar range and their relative activity was consistent with their PDCD4 stabilizing activity (i.e., **10** slightly more active than **9**). The two analogues without a pyranone ring **35a/b** (>10 fold) and the one without the double bond **36** (>100 fold) were the least active. The surprisingly greater loss in activity for **36**, could be a result of its propensity to ring open (e.g., unstable in CD_3OD). The diastereomer **2** (with only the C-6 pyranostereocenter retained) had a small loss in activity (~2 fold). The effect of C-16 acylation could be seen in the comparison between cryptocaryol A and B (**9/10**), as well as, **35a/35b**. Surprisingly, the stereochemistry of natural products did not have a significant effect on activity as *ent*-cryptocaryol A (**30a**) had only a ~3 fold loss of activity.

In conclusion, the first total synthesis, structural elucidation/correction and SAR of cryptocaryol A and B have been achieved. The enantioselective synthesis was accomplished in 23 and 25-step linear sequence, respectively, from commercially available 5-hexyn-1-ol. The stereochemically divergent synthesis concisely enabled the exact stereochemical

assignment, as well as, the SAR for cryptocaryol A and B in a cancer cell cytotoxicity assay. It is worth noting that the difficulties in distinguishing between the two diastereomers (e.g., **1** and **9**) demonstrate the need for stereochemically divergent approaches for structural determination, as well as, enabling SAR-studies that probe the effects of stereochemistry on activity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

References

1. Mochly-Rosen D, Das K, Grimes KV. Nat Rev Drug Discov. 2012; 11:937–957. [PubMed: 23197040]

2. (a) Watanabe R, Wei L, Huang J. J Nucl Med. 2011; 52:497–500. [PubMed: 21421716] (b) Cheng JQ, Lindsley CW, Cheng GZ, Yang H, Nicosia SV. Oncogene. 2005; 24:7482–7492. [PubMed: 16288295]

3. (a) Yang HS, Jansen AP, Komar AA, Zheng XJ, Merrick WC, Costes S, Lockett SJ, Sonenberg N, Colburn NH. Mol Cell Biol. 2003; 23:26–37. [PubMed: 12482958] (b) Suzuki C, Garces RG, Edmonds KA, Hiller S, Hyberts SG, Marintchev A, Wagner G. Proc Natl Acad Sci U S A. 2008; 105:3274–3279. [PubMed: 18296639] (c) Chang JH, Cho YH, Sohn SY, Choi JM, Kim A, Kim YC, Jang SK, Cho Y. Proc Natl Acad Sci U S A. 2009; 106:3148–3153. [PubMed: 19204291] (d) Göke A, Göke R, Knolle A, Trusheim H, Schmidt H, Wilmen A, Carmody R, Göke B, Chen YH. Biochem Biophys Res Commun. 2002; 297:78–82. [PubMed: 12220511] (e) Yang HS, Cho MH, Zakowicz H, Hegamyer G, Sonenberg N, Colburn NH. Mol Cell Biol. 2004; 24:3894–3906. [PubMed: 15082783] (f) Frankel LB, Christoffersen NR, Jacobsen A, Lindow M, Krogh A, Lund AH. J Biol Chem. 2008; 283:1026–1033. [PubMed: 17991735] (g) Dorrello NV, Peschiaroli A, Guardavaccaro D, Colburn NH, Sherman NE, Pagano M. Science. 2006; 314:467–471. [PubMed: 17053147] (h) Palamarchuk A, Efanov A, Maximov V, Aqeilan RI, Croce CM, Pekarsky Y. Cancer Res. 2005; 65:11282–11286. [PubMed: 16357133]

4. Yang HS, Knies JL, Stark C, Colburn NH. Oncogene. 2003; 22:3712–3720. [PubMed: 12802278]

5. Afonja O, Juste D, Das S, Matsuhashi S, Samuels HH. Oncogene. 2004; 23:8135–8145. [PubMed: 15361828]

6. Wang Q, Sun Z, Yang HS. Oncogene. 2007; 27:1527–1535. [PubMed: 17828298]

7. Grkovic T, Blees JS, Colburn NH, Schmid T, Thomas CL, Henrich CJ, McMahon JB, Gustafson KR. J Nat Prod. 2011; 74:1015–1020. [PubMed: 21539301]

8. (a) Kobayashi Y, Tan CH, Kishi Y. Helv Chim Acta. 2000; 83:2562–2571.(b) Higashibayashi S, Czechtizky W, Kobayashi Y, Kishi Y. J Am Chem Soc. 2003; 125:14379–14393. [PubMed: 14624586]

9. Snatzke G. Angew Chem Int Ed. 1968; 7:14–25.

10. As this manuscript was in preparation, Mohapatra et al. described the synthesis of purported cryptocaryol A (1) and mistakenly reported it as Cryptocaryol A (9), see: Reddy DS, Mohapatra DK. Eur J Org Chem. 2013; 2013:1051–1057.

11. Hunter, TJ. PhD Thesis. the University of Minnesota; 2003.

12. (a) Wang Y, Xing Y, Zhang Q, O'Doherty GA. Chem Commun. 2011; 47:8493–8505.(b) Ahmed, MdM; Mortensen, MS.; O'Doherty, GA. J Org Chem. 2006; 71:7741–7746. [PubMed: 16995681] (c) Hunter TJ, O'Doherty GA. Org Lett. 2001; 3:1049–1052. [PubMed: 11277792]

13. Hunter TJ, O'Doherty GA. Org Lett. 2001; 3:2777–2780. [PubMed: 11506632] Smith CM, O'Doherty GA. Org Lett. 2003; 5:1959–1962. [PubMed: 12762696] Garaas SD, Hunter TJ,

O'Doherty GA. J Org Chem. 2002; 67:2682–2685. [PubMed: 11950317] For alternative approaches, see, ref. 10 and Melillo B, Smith AB. Org Lett. 2013; 15:2282–2285. [PubMed: 23614670]

14. (a) Guo H, Mortensen MS, O'Doherty GA. Org Lett. 2008; 10:3149–3152. [PubMed: 18549226] (b) Gao D, O'Doherty GA. Org Lett. 2010; 12:3752–3755. [PubMed: 20687585] (c) Li M, O'Doherty GA. Org Lett. 2006; 8:6087–6090. [PubMed: 17165936] (d) Li M, O'Doherty GA. Org Lett. 2006; 8:3987–3990. [PubMed: 16928055]

15. (a) Rychnovsky SD, Kim J. J Org Chem. 1994; 59:2659–2660.(b) Trost BM, Kazmaier U. J Am Chem Soc. 1992; 114:7933–7935.

16. Xu D, Crispino GA, Sharpless KB. J Am Chem Soc. 1992; 114:7570–7571.

17. Evans DA, Gauchet-Prunet JA. J Org Chem. 1993; 58:2446–2453.

18. Kubota K, Leighton JL. Angew Chem Int Ed. 2003; 115:976–978.

19. Scholl M, Trnka TM, Morgan JP, Grubbs RH. Tetrahedron Lett. 1999; 40:2247–2250.

20. Hashiguchi S, Fujii A, Takehara J, Ikariya T, Noyori R. J Am Chem Soc. 1995; 117:7562–7563.

21. The absolute and relative configuration for all stereocenters was confirmed by a combination of Mosher and ^{1}H NMR analyses, see SI.

22. Unfortunately, no optical rotation data for cryptocaryol B was reported, see: ref. 7.

23. (a) Yin CH, Bach EA, Baeg GH. J Biomol Screen. 2011; 16:443–449. [PubMed: 21393628] (b) Wee JL, Sundermann K, Licari P, Galazzo J. J Nat Prod. 2006; 69:1456–1459. [PubMed: 17067161]

24. (a) Mosmann T. J Immunol Methods. 1983; 65:55–63. [PubMed: 6606682] (b) Wang HYL, Wu B, Zhang Q, Kang SW, Rojanasakul Y, O'Doherty GA. ACS Med Chem Lett. 2011; 2:259–263. [PubMed: 21572583]

25. Jansen AP, Camalier CE, Stark C, Colburn NH. Mol Cancer Ther. 2004; 3:103–110. [PubMed: 14985450]

Figure 1.
Purported structures of cryptocaryols A–H and revised structures of cryptocaryol A (**9**) and B (**10**). EC_{50} = mM conc. for recovery of 50% PDCD4 concentration from TPA-induced degradation.[7]
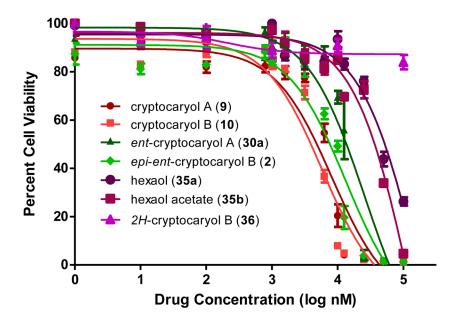
Figure 2.
Graph of MCF-7 cell viability for cryptocaryols and their analogues. The dose-response curve of cell viability from a 72 h drug treatment (1 nM to 100 μM).

Scheme 1.
Retrosynthetic analysis of cryptocaryol A and B

Scheme 2. Synthesis of pseudo-C_s symmetric intermediate
Reagents and conditions: a) PMBCl, NaH, DMF, TBAB, 0 °C, 99%; b) ClCO$_2$Me, n-BuLi, THF, −78 to 0 °C, 99%; c) PPh$_3$, PhOH, benzene, 50 °C, 90%; d) **AD-mix-** * = K$_3$Fe(CN)$_6$ (3 equiv), K$_2$CO$_3$ (3 equiv), MeSO$_2$NH$_2$ (1 equiv), (DHQ)$_2$PHAL (2 mol %), OsO$_4$ (1 mol %), t-BuOH/H$_2$O, 0 °C, 85%; e) triphosgene, pyridine, DMAP, CH$_2$Cl$_2$, −78 °C, 89%; f) **PdPPh$_3$** = Pd$_2$(dba)$_3$•CHCl$_3$/PPh$_3$ (0.3 mol %), Et$_3$N, HCO$_2$H, THF, reflux, 95%; g) PhCHO, KOt-Bu, THF, 0 °C, 67%; h) DIBALH, CH$_2$Cl$_2$, −78 °C, 95%; i) (S,S)-**Leighton**, Sc(OTf)$_3$ (2.5 mol %), CH$_2$Cl$_2$, −10 °C, 72%, dr = 8.7:1.0; j) ethyl acrylate, **Grubbs II** (1.5 mol %), CH$_2$Cl$_2$, 99%; k) PhCHO, KOt-Bu, THF, 0 °C, 77%.

Scheme 3. Synthesis of 6-epi-ent-cryptocaryol B (2)
Reagents and conditions: a) DDQ, CH_2Cl_2, H_2O, 0 °C, 92%; b) Dess-Martin periodinane, CH_2Cl_2, 0 °C, 81%; c) 1-pentadecyne, n-BuLi, THF, −78 °C; d) Dess-Martin periodinane, CH_2Cl_2, 0 °C, 67 % (two steps); e) (S,S)-**Noyori** (5 mol %), Et_3N, HCO_2H, 94%; f) NBSH, Et_3N, CH_2Cl_2, 90%; g) DIBALH, CH_2Cl_2, −78 °C, 87%; h) Ac_2O, Et_3N, DMAP, CH_2Cl_2, 0 °C, 72%; i) (S,S)-**Leighton**, $Sc(OTf)_3$ (5 mol %), CH_2Cl_2, −10 °C, 75%; j) acrylic acid, DCC, DMAP, CH_2Cl_2, 61%; k) **Grubbs I** (5 mol %), CH_2Cl_2, reflux, 75%; l) $AcOH/H_2O$ (4:1), 80 °C, 75%.

Scheme 4. Synthesis of ent-cryptocaryol A and B (30a/b)
Reagents and conditions: a) TBSCl, imidazole, DMF, 96%; b) DIBALH, CH$_2$Cl$_2$, −78 °C, 90%; c) (R,R)-**Leighton**, Sc(OTf)$_3$ (5 mol %), CH$_2$Cl$_2$, −10 °C, 95%; d) acrylic acid, DCC, DMAP, CH$_2$Cl$_2$, 64%; e) **Grubbs I** (5–10 mol %), CH$_2$Cl$_2$, reflux, 87% or 78%; f) AcOH/ H$_2$O (4:1), 80 °C, 77% or 75%; g) TBAF, THF, 91%; h) Ac$_2$O, Et$_3$N, DMAP, CH$_2$Cl$_2$, 0 °C, 96%.

Scheme 5. Synthesis of cryptocaryol A and B (9 and 10)

Reagents and conditions: a) DIBALH, CH_2Cl_2, −78 °C, 90%; b) 1-pentadecyne, n-BuLi, THF, −78 °C; c) Dess–Martin periodinane, CH_2Cl_2, 0 °C, 68 % (2 steps); d) (R,R)-**Noyori** (5 mol %), Et_3N, HCO_2H, 98%; e) NBSH, Et_3N, CH_2Cl_2, 98%; f) TBSCl, imidazole, DMF, 94%; g) DDQ, CH_2Cl_2, H_2O, 0 °C, 92%; h) Dess-Martin periodinane, CH_2Cl_2, 0 °C, 62%; i) (S,S)-**Leighton**, $Sc(OTf)_3$ (5 mol %), CH_2Cl_2, −10 °C, 95%; j) acrylic acid, DCC, DMAP, CH_2Cl_2, 80%; k) **Grubbs I** (5–10 mol %), CH_2Cl_2, reflux, 76% or 70%; l) $AcOH/H_2O$ (4:1), 80 °C, 70%; m) TBAF, THF, 92%; n) Ac_2O, Et_3N, DMAP, CH_2Cl_2, 0 °C, 72%.

Scheme 6. Synthesis of cryptocaryol analogues for SAR

Reagents and conditions: a) Ac$_2$O, pyridine, DMAP, CH$_2$Cl$_2$, 0 °C, 98%; b) DDQ, CH$_2$Cl$_2$, H$_2$O, 0 °C, 72%; c) AcOH/H$_2$O (4:1), 80 °C, 65% or 69%; d) Pd/C (10% wt/wt, 10 mol %), H$_2$ (1 atm), 66%.

Table 1

Cytotoxicity of cryptocaryol analogues (MCF-7)

Compounds	IC$_{50}$ (µM)^a
cryptocaryol A (**9**)	8.5 ± 2.6
cryptocaryol B (**10**)	6.0 ± 1.6
6-*epi*-*ent*-cryptocaryol B (**2**)	14.0 ± 4.5
ent-cryptocaryol A (**30a**)	28.0 ± 10.7
hexaol (**35a**)	242 ± 180
hexaol acetate (**35b**)	170 ± 104
2H-cryptocaryol B (**36**)	>500
etoposide	1.2 ± 0.6

^{a}The IC$_{50}$ values were measured from 72 h treatment of MCF-7 cells in a MTT assay. All values represent the standard error of the mean value of three independent experiments with two duplicate determinations.