Antiproliferative Compounds from *Cleistanthus boivinianus* from the Madagascar Dry Forest

Yixi Liu,† Kelly Young,† L. Harinantenaina Rakotondraibe,‡ Peggy J. Brodie,† Jessica D. Wiley,‡ Maria B. Cassera,† Martin W. Callmander,† R. Rakotondrajaona,† Etienne Rakotobe,⊥ Vincent E. Rasamison,⊥ Karen TenDyke,∥ Yongchun Shen,⊥ and David G. I. Kingston*†

†Department of Chemistry and the Virginia Tech Center for Drug Discovery and Safety Evaluation, Virginia Tech, Blacksburg, Virginia 24061, United States
‡Missouri Botanical Garden, P.O. Box 299, St. Louis, Missouri 63166, United States
⊥Centre National d’Application des Recherches Pharmaceutiques, B.P. 702, Antananarivo 101, Madagascar
∥Eisai Inc, 4 Corporate Drive, Andover, Massachusetts 01810, United States

Supporting Information

**ABSTRACT:** The two new lignans 3α-O-[(β-D-glucopyranosyl)desoxyxypodophyllotoxin (1) and 4-O-[(β-D-glucopyranosyl)dehydropodophyllotoxin (2) were isolated from *Cleistanthus boivinianus*, together with the known lignans deoxypodophyllotoxin (3), (+)-β-apipicropodophyllin (4), (−)-desoxyxypodophyllotoxin (5), (−)-yatein (6), and β-peltatin-5-β-β-D-glucopyranoside (7). The structures of all compounds were characterized by spectroscopic techniques. Compounds 1, 4, and 5 showed potent antiproliferative activities against the A2780 ovarian cancer cell line, with IC50 values of 33.0 ± 3.6, 63.1 ± 6.7, and 230 ± 1 nM, respectively. Compounds 2 and 7 showed only modest A2780 activities, with IC50 values of 2.1 ± 0.3 and 4.9 ± 0.1 μM, respectively, while compounds 3 and 6 had IC50 values of 10 μM. Compound 1 also had potent antiproliferative activity against the HCT-116 human colon carcinoma cell line, with an IC50 value of 20.5 nM, and compound 4 exhibited modest antiproliferative activity against the A2058 human caucasian metastatic melanoma and MES-SA human uterine sarcoma cell lines, with IC50 values of 4.6 and 4.0 μM, respectively.

In the course of work as part of the Madagascar International Cooperative Biodiversity Group (ICBG) program, an ethanol extract of the stems of *Cleistanthus boivinianus* (Baill.) Mull. Arg. (Phyllanthaceae) was found to have moderate antiproliferative activity against the A2780 ovarian cancer cell line (IC50 4.5 μg/mL) and was thus selected for evaluation of the presence of novel antiproliferative agents. The genus *Cleistanthus* is native to Africa, India, and Australia and comprises approximately 140 species, but only five of these have been investigated chemically: *C. collinus*, *C. patulus*, *C. schlechteri* var. *schlechteri*, *C. gracilis*, and *C. indochinensis*. Arylnaphthalide lignans are major constituents of the genus, while aryltetralin, furofuranoid, and dibenzylbutane lignans and terpenoids have also been isolated from them and are known for their cytotoxic properties.

**RESULTS AND DISCUSSION**

Dereplication of an active EtOAc-soluble fraction obtained from liquid–liquid partition of the extract (100 mg) as previously described indicated that it contained at least one new bioactive compound, so a larger sample was investigated. Fractionation of this extract yielded an antiproliferative EtOAc fraction, which was further subjected to size-exclusion column chromatography on Sephadex LH-20 followed by reverse-phase semipreparative chromatography to yield compounds 3, 5, and 6 and three semipreparative active fractions. Further purification of these fractions by silica gel or diol HPLC furnished compounds 1, 2, 4, and 7.

Compound 1 was isolated as a white solid with the molecular formula C28H32O13 based its HRESIMS data. Its 1H NMR spectrum contained three singlet aromatic signals together with signals for a methylenedioxy group and three aromatic methoxy groups. Signals suggestive of a sugar unit were also present. Analysis of COSY, HSQC, and HMBC data assigned the sugar −glucopyranosyl unit. The remaining aliphatic signals were assigned by HSQC and COSY analysis to two isolated arylnaphthalide lignan groups. Signals suggestive of a sugar unit were also present. Further characterization of these fractions by silica gel or diol HPLC furnished compounds 1, 2, 4, and 7.

Compound 1 was isolated as a white solid with the molecular formula C28H32O13 based its HRESIMS data. Its 1H NMR spectrum contained three singlet aromatic signals together with signals for a methylenedioxy group and three aromatic methoxy groups. Signals suggestive of a sugar unit were also present. Analysis of COSY, HSQC, and HMBC data assigned the sugar −glucopyranosyl unit. The remaining aliphatic signals were assigned by HSQC and COSY analysis to two isolated arylnaphthalide lignan groups. Signals suggestive of a sugar unit were also present. Further characterization of these fractions by silica gel or diol HPLC furnished compounds 1, 2, 4, and 7.

Received: December 17, 2014
Published: June 19, 2015
δ assigned based on cross-peaks from the two methoxy signals at group. Finally, the positions of the methoxy groups were C-6 and C-7 indicated the position of the methylenedioxy carbon C-2a. Correlations of the methylenedioxy protons with between the methylene protons H-2a and the ester carbonyl H-5 suggested that compound 1 was assigned as 3α-O-(β-D-glucopyranosyl)desoxypodophyllotoxin.

As indicated by the experiment described above, compound 1 is prone to conversion to its α,β-unsaturated derivative 4a, and a sample of 1 left in MeOH–CHCl₃ for a few days underwent decomposition, most likely caused by traces of HCl in the CHCl₃. Attempted enzymatic hydrolysis of 1 did not proceed under normal conditions, so the aglycone could not be isolated.

Compound 2 had the molecular formula of C₂₈H₂₉O₁₃, based on its HRESIMS data. Its ¹H NMR spectrum was similar to that obtained for 1, and its UV absorption maxima at 260, 315, and 351 nm indicated the presence of a naphthalene nucleus, suggesting that 2 is an arylnaphthalide lignan. The ¹H NMR spectrum of 2 exhibited signals for four aromatic protons at δH 8.04 and 6.95 (s, each 1H, H-5 and H-8) and 6.59 (s, 2H, H-2a and H-5') and for an oxymethylene group at δH 5.75 and 5.53 (J = 15.4 Hz, each 1H, H-3a), but lacked the signals for the two methine protons at C-1 and C-2 and the methylene protons at C-4 observed in 1. These facts confirmed the presence of a naphthalene unit in 2. In the HMBC spectrum, the correlation from H-5 (δH 8.04) and H₂-3a (δH 5.75 and 5.53) to δC 144.3 (C-4) confirmed the linkage of C-4 to both the B- and D-rings, while the correlations of δH 6.59 (1H, H-2a and/or H-6') and δH 6.95 (H-8) to δC 128.6 (C-1) connected the A- and B-rings to C-1. A comparison of the NMR spectra of 2 with those of dehydropodophyllotoxin (2a) suggested that compound 2 is a glycosylated derivative of 2a.

The HMBC correlation between the anomeric proton signal at δH 4.89 (H-1') and δC 144.3 (C-4) confirmed that the sugar is located at C-4 of the aglycone. Acid hydrolysis of 2 gave dehydropodophyllotoxin (2a) as the aglycon and a sugar that was identified as d-glucose by ¹H NMR, TLC, and optical rotation comparison with a standard sample. The complete assignments of all protons and carbons of 2 (Table 1) were accomplished by analysis of the HSQC, HMBC, and NOESY spectra. Compound 2 was thus assigned as 4-O-(β-D-glucopyranosyl)-dehydropodophyllotoxin.

The five known aryltetralin lignans deoxypicropodophyllotoxin (3), (±)-β-apicarpodophyllin (4), (±)-desoxydeoxypodophyllotoxin (5), and β-peltatins-5-O-β-D-glucopyranoside (7) were also isolated. Their structures were determined by comparison of their ¹H NMR spectroscopic, mass spectrometric, and optical rotation values

Figure 1. Key HMBC and NOESY correlations of I and key HMBC correlations of 2.
with the data reported in the literature, except for the case of β-apopiocarpodoplylhin (4), which was optically inactive. The isolation of racemic 4 is noteworthy, since all previous isolates have been of the dextrorotatory isomer, and indicates that it is not formed by elimination of glucose from 1.

All isolated compounds were evaluated for their antiproliferative activity against the A2780 human ovarian cancer cell line, and some were evaluated in other cell lines and for antimalarial activity (Table 2). Compound 1 showed the highest antiproliferative activity (IC$_{50}$ 33.0 ± 3.6 nM) against the A2780 cell line, followed by 4 (63.1 ± 6.7 nM) and 5 (230 ± 1 nM). The potency of 1 is similar to that of the anticancer drug paclitaxel, which has an IC$_{50}$ value of 73 nM in this assay. Compounds 2 and 7 showed only modest antiproliferative activities, with IC$_{50}$ values of 2.1 ± 0.3 and 4.9 ± 0.1 μM, respectively. The glycosylated compound 1 had slightly improved activity compared with the racemic compound 4. The unsaturated D-ring of 4 increased the activity about 4-fold compared with the trans dihydro D-ring analogue 5, while the cis D-ring analogue 3 was much less potent than 4, consistent with previous studies indicating the significance of the trans-fused lactone for activity. A glucose moiety at C-5 or C-4 and the aromatization of the C-ring reduced activity, as shown by the fact that compounds 2 and 7 were about 10- and 20-fold less potent than 5, respectively. Furthermore, although good antiproliferative activity has been observed in other cell lines for (−)-yatein (6), it was only weakly active against the A2780 cell line. Compound 1 also displayed potent antiproliferative activity against the HCT-116 human colon carcinoma cell line, with an IC$_{50}$ value of 20.5 nM, and weak antimalarial activity against *Plasmodium falciparum* with an IC$_{50}$ value of 12.6 ± 3.2 μM. Compound 4 displayed moderate antiproliferative activity against A2058 human caucasian metastatic melanoma and MES-SA human uterine sarcoma cells, with IC$_{50}$ values of 4.6 and 4.0 μM, respectively.

In summary, compound 1 is a new lignan with potent antiproliferative activity against the A2780 cell line. It is also the first reported C-3 substituted podophyllotoxin analogue. It would be an attractive substrate for further studies to explore its mechanism of action were it not for its lability under acidic conditions, which suggests that it would not be stable enough for drug use.

### EXPERIMENTAL SECTION

**General Experimental Procedures.** IR and UV spectra were measured on MIDAC M-series FTIR and Shimadzu UV-1201 spectrophotometers, respectively. $^1$H and $^{13}$C NMR spectra were recorded on a Bruker Avance 500 spectrometer in CD$_3$OD (with CD$_3$OD as reference) and CDCl$_3$ (with CDCl$_3$ as reference). Massspectra were obtained on an Agilent 6220 mass spectrometer. Open column chromatography was performed using Sephadex LH-20, and solid-phase extraction was performed using C$_{18}$ cartridges. Semi-preparative HPLC was performed using Shimadzu LC-10AT pumps coupled with a semi-preparative Phenomenex C$_{18}$ column (5 μm, 250 × 10 mm), a Shimadzu SPD M10A diode array detector, and a SCL-10A system controller. All isolated compounds were purified to 95% purity or better, as judged by HPLC (both UV and ELSD detection) before determining bioactivity.

**Plant Material.** Leaves of *Cleistanthus boivinianus* (collection: Stéphan Rakotonandrasana et al. 1036) were obtained at an elevation of 31 m from a 3 m tall tree with yellow flowers. Collection was made 5 km northeast of the village of Marivoraha, PK 123,
Andohanantsihoby, in a mosaic of dry forest and savanna trees; coordinates 13°06′37″ S 049°09′39″ E. Collection was made by Stéphan Rakotonandravina with assistance from R. Randrianarivo, R. Rakotonandravina, C. Claude, V. Benjara, and M. Modeste. Duplicate voucher specimens are deposited at the Centre National d’Application des Recherches Pharmaceutiques (CNRAP), the Herbarium of the Parc Botanique et Zoologique de Tsimbazaza, Antananarivo, Madagascar (TAN), the Missouri Botanical Garden, St. Louis, Missouri (MO), and the Museum National d’Histoire Naturelle in Paris, France (P).

**Extraction and Isolation.** A ground sample of *C. bovinus* leaves (250 g) was extracted with EtOH at room temperature to yield 33.4 g of crude EtOH extract designated MG4031. A total of 5.88 g of this extract was made available to Virginia Tech. An active EtOAc-soluble fraction obtained from liquid–liquid partition of the extract (100 mg) was subjected to dereplication studies using size-exclusion chromatography, reversed-phase HPLC coupled with bioassay, high-resolution ESI-MS, H NMR spectroscopy, and a database search using the online Dictionary of Natural Products. The results indicated the extract to contain at least one new bioactive component, and so a 3.0 g sample was investigated. The crude EtOH extract was dissolved in 90% MeOH (200 mL) and extracted with hexanes (3 × 200 mL). Evaporation of the hexane-soluble fraction afforded 271 mg of residue. The 90% MeOH (aq) layer was then evaporated, suspended in H2O (300 mL), and extracted with EtOAc (3 × 200 mL) to yield 640 mg of an EtOAc-soluble fraction with an IC50 value of 0.19 μg/mL. The EtOAc fraction was subjected to Sephadex LH-20 open-column chromatography (CH2Cl2–MeOH, 1:1) to give six fractions. The most active fraction, Fr 3 (1.2 mg, 0.11 μg/mL), was then divided into three subfractions by C18 solid-phase extraction using 40% MeOH (Fr 3-1), 70% MeOH (Fr 3-2), and 100% MeOH (Fr 3-3). Further purification of the most active subfraction, Fr 3-3 (2.8 mg, 0.11 μg/mL), by HPLC on a C8 column with a solvent gradient of CHCl3–MeOH, from 95:5 to 90:10 from 0 to 5 min, to 80:20 from 5 to 12 min, and ending with a 100% MeOH wash from 16 to 25 min, yielded compounds 3 (1.2 mg, 0.95 μg/mL), 5 (1.5 mg, tR 24.5 min), and 6 (2.1 mg, tR 26.5 min). Purification of the subfraction Fr 3-2 (1.5 mg, 0.1 μg/mL) by C18 HPLC furnished eight fractions (solvent gradient of H2O–CH3CN, from 60:40 to 50:50 from 0 to 17 min, to 40:60 from 17 to 22 min, to 40:60 from 22 to 27 min, and ending with 100% CH3CN from 27 to 35 min, yielded compounds 4 (2.1 mg, tR 23.5 min), 7 (1.5 mg, tR 24.5 min), and 6 (2.1 mg, tR 26.5 min). Further purification of the third fraction by HPLC on a silica gel column with a solvent gradient of CHCl3–MeOH, from 95:5 to 90:10 from 0 to 5 min, to 88:12 from 5 to 12 min, and ending with a 100% MeOH wash from 13 to 20 min, yielded compound 7 (2.8 mg, tR 13.5 min). Further purification of the sixth fraction by HPLC on a silica gel column with a solvent gradient of CHCl3–MeOH, from 95:5 to 90:10 from 0 to 5 min, to 80:20 from 5 to 10 min, to 70:30 from 10 to 15 min, and ending with a 100% MeOH wash from 16 to 25 min, yielded compounds 4 (5 mg, tR 6 min) and 1 (19 mg, tR 14 min). Further purification of the seventh fraction by HPLC on a diol column with a solvent gradient of CHCl3–MeOH, from 100:0 to 95:5 from 0 to 5 min, to 90:10 from 5 to 15 min, to 87.5:12.5 from 15 to 20 min, and ending with a 100% MeOH wash from 20 to 25 min, yielded compound 2 (3 mg, tR 22.5 min).

**Acid Hydrolysis of Compounds 1 and 2.** Compound 1 (2 mg) was dissolved in dilute citrate–phosphate buffer (pH 7.0, 4 mL), and β-glucosidase (4 mg) was added. The mixture was incubated for 2 weeks at 37 °C; no reaction was observed. The reaction mixture was then adjusted to pH 5.0 with dilute HCl and stirred at 37 °C overnight. The mixture was then briefly heated to boiling and extracted with EtOAc (3 × 10 mL), and both the organic and the water layers were evaporated to dryness under reduced pressure. The structure of the white powder (1.2 mg) derived from the organic layer was determined to be 4a by its H NMR spectrum and optical rotation ([α]25c +52.1°, c 1.2, CHCl3). The H and 13C NMR spectra were identical with literature data.20−22

(±)-β-Apicropodophyllin (4): amorphous powder; [α]25c +0° (c 0.5, CHCl3). The H and 13C NMR spectra were identical with literature data.20,23,24

**Notes**

1. H NMR spectra of compounds 1–7. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/np501020m.

**AUTHOR INFORMATION**

Corresponding Author

Tel: +1-540-231-6570. Fax: +1-540-231-3255. E-mail: dkingston@vt.edu (D. G. I. Kingston).

Present Address

College of Pharmacy, Division of Medicinal Chemistry and Pharmacognosy, The Ohio State University, Columbus, Ohio 43210, United States.

Notes

The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

This project was supported by the Fogarty International Center, the National Cancer Institute, the National Institute of Allergy and Infectious Diseases, the National Institute of Mental Health, the National Institute on Drug Abuse, the National Heart Lung and Blood Institute, the National Center for Complementary and Alternative Medicine, the Office of Dietary Supplements, the National Institute of General Medical Sciences, the Biological Sciences Directorate of the National Science Foundation, and the Office of Biological and Environmental Research of the U.S. Department of Energy under Cooperative Agreement U01 TW00313 with the International Cooperative Biodiversity Groups. This project
was also supported by the National Research Initiative of the Cooperative State Research, Education and Extension Service, USDA, Grant 2008-35621-04732. J.D.W. was supported by NSF S-STEM award DUE-0850198. These supports are gratefully acknowledged. Work at Virginia Tech was supported by a multilateral agreement between the ICBG and the National Science Foundation under Grant CHE-0722638 for the purchase of the Agilent 6220 mass spectrometer. We thank Mr. B. Bebout for obtaining the mass spectra. Fieldwork essential for this project was conducted under a collaborative agreement between the Missouri Botanical Garden and the Parc Botanique et Zoologique de Tsimbazaza and a multilateral agreement between the ICBG partners, including the Centre National d’Application des Recherches Pharmaceutiques. We thank R. Randrianarivo, R. Rakotonandrasana, C. Claude, V. Benjara, and M. Modeste for assistance with plant collection, and we gratefully acknowledge courtesies extended by the Government of Madagascar (Ministère des Eaux et Forêts).

**REFERENCES**

(1) Biodiversity Conservation and Drug Discovery in Madagascar, Part 63. For Part 62, see: Liu, Y.; Cheng, E.; Rakotondraibe, L. H.; Brodie, P. J.; Wiley, J. D.; Cassera, M. B.; Applequist, W.; Birkinshaw, C.; Rakotondrafa, A.; Ratsimbason, M.; Rasmenson, V. E.; Kingston, D. G. I. *Tetrahedron Lett.* 2015, 56, 3630–3632.

(2) Rakotondraibe, H. L.; Graupner, P. R.; Xiong, Q.; Olson, M.; Wiley, J. D.; Krai, P.; Brodie, P. J.; Callmader, M. W.; Rakotobe, E.; Ratovoson, F.; Rasamison, V. E.; Cassera, M. B.; Hahn, D. R.; Kingston, D. G. I.; Fotsot; S. J. Nat. Prod. 2015, 78, 431–440.

(3) Kingston, D. G. I. *J. Org. Chem.* 2008, 73, 3975–3984.

(4) Pinho, P. M.; Kijjoa, A. *Phytochem. Rev.* 2007, 6, 175–182.

(5) Thanh, V. T. T.; Pham, V. C.; Mai, H. D. T.; Litaudon, M.; Gueritte, F.; Retailleau, P.; Nguyen, V. H.; Chau, V. M. *J. Nat. Prod.* 2012, 75, 1577–1583.

(6) Thanh, V. T. T.; Pham, V. C.; Mai, H. D. T.; Litaudon, M.; Gueritte, F.; Nguyen, V. H.; Chau, V. M. *Planta Med.* 2014, 80, 695–702.

(7) Thanh, V. T. T.; Pham, V. C.; Nguyen, H. H.; Mai, H. D. T.; Minh, H. N. T.; Nguyen, V. H.; Litaudon, M.; Gueritte, F.; Chau, V. M. *Eur. J. Org. Chem.* 2011, 2011, S4108/1–S4108/22 and S4108–4111.

(8) Parasuraman, S.; Raveendran, R. *Pharmacogn. Mag.* 2011, 7, 243–247.

(9) Pinho, P. M.; Naengchomnong, W.; Kijjoa, A.; Nazareth, N.; Silva, A. M. S.; Eaton, G.; Herz, W. *Phytochemistry* 2006, 67, 1789–1792.

(10) Ramesh, C.; Ravindranath, N.; Ram, T. S.; Das, B. *Chem. Pharm. Bull.* 2003, 51, 1299–1300.

(11) Sastry, K. V.; Rao, E. V.; Buchanan, J. G.; Sturgeon, R. J. *Phytochemistry* 1987, 26, 1153–1154.

(12) Pradheepkumar, C. P.; Shamugam, G. *Oncol. Res.* 1999, 11, 225–232.

(13) Liu, Y.; Rakotondraibe, L. H.; Brodie, P. J.; Wiley, J. D.; Cassera, M. B.; Goetz, M.; Kingston, D. G. I. *Nat. Prod. Commun.* 2014, 9, 1403–1406.

(14) Yamaguchi, H.; Arimoto, M.; Tanoguchi, M.; Ishida, T.; Inoue, M. *Chem. Pharm. Bull.* 1982, 30, 3212–3218.

(15) Sun, Y.-J.; Li, Z.-L.; Chen, H.; Liu, X.-Q.; Zhou, W.; Hua, H.-M. *Biorg. Med. Chem. Lett.* 2011, 21, 3794–3797.

(16) Zhao, C.; Nagatsu, A.; Hatano, K.; Shirai, N.; Kato, S.; Ogihara, Y. *Chem. Pharm. Bull.* 2003, 51, 255–261.

(17) Gu, J.-Q.; Park, E. J.; Totoura, S.; Runwan, S.; Fong, H. H. S.; Pezzuto, J. M.; Kinghorn, A. D. *J. Nat. Prod.* 2002, 65, 1065–1068.

(18) Klyne, W.; Stevenson, R.; Swan, R. J. *J. Chem. Soc. C* 1966, 893–896.

(19) Swan, R. J.; Klyne, W.; MacLean, H. *Can. J. Chem.* 1967, 45, 319–324.

(20) Novelo, M.; Cruz, J. G.; Hernandez, L.; Pereda-Miranda, R.; Chai, H.; Mar, W.; Pezzuto, J. M. *J. Nat. Prod.* 1993, 56, 1728–1736.

(21) Atta-ur-Rahman; Ashraf, M.; Choudhary, M. I.; Habib-ur-Rehman; Kazmi, M. H. *Phytochemistry* 1995, 40, 427–431.

(22) Broomhead, A. J.; Dewick, P. M. *Phytochemistry* 1990, 29, 3839–3844.

(23) Pellet, A.; Ward, R. S.; Li, Q.; Pis, J. *Tetrahedron: Asymmetry* 1994, 5, 909–910.

(24) Andrews, R. C.; Teague, S. J.; Meyers, A. I. *J. Am. Chem. Soc.* 1988, 110, 7854–7858.

(25) San Feliciano, A.; Medarde, M.; Lopez, J. L.; Puebla, P.; del Corral, J. M. M.; Barrero, A. F. *Phytochemistry* 1989, 28, 2863–2866.

(26) Carpenter, C. D.; O’Neill, T.; Picot, N.; Johnson, J. A.; Robichaud, G. A.; Webster, D.; Gray, C. A. *J. Ethnopharmacol.* 2012, 143, 695–700.

(27) Tanoguchi, M.; Arimoto, M.; Saika, H.; Yamaguchi, H. *Chem. Pharm. Bull.* 1987, 35, 4162–4165.

(28) Rai, K. M. L.; Basavaraju, Y. B.; Sadashivamurthy, B. *Indian J. Pharm. Sci.* 2007, 69, 116–118.

(29) Gensler, W. J.; Gatsonis, C. D. *J. Org. Chem.* 1966, 31, 3224–3227.

(30) Cao, S.; Brodie, P. J.; Miller, J. S.; Randrianarivo, R.; Ratovoson, F.; Birkinshaw, C.; Andriantsiferana, R.; Rasamison, V. E.; Kingston, D. G. I. *Nat. Prod. Nat. Med. 2007, 70, 679–681.

(31) Pan, E.; Harinantenaina, L.; Brodie, P. J.; Miller, J. S.; Callmader, M. W.; Rakotondrasana, S.; Rakotobe, E.; Rasamison, V. E.; Kingston, D. G. I. *J. Nat. Prod.* 2010, 73, 1792–1795.

(32) Louie, K. G.; Behrens, B. C.; Kinsella, T. J.; Hamilton, T. C.; Grotzinger, K. R.; McKoy, W. M.; Winker, M. A.; Ozols, R. F. *Cancer Res.* 1985, 45, 2110–2115.

(33) Bennett, T. N.; Paguio, M.; Gligorijevic, B.; Seudieu, C.; Kosar, A. D.; Davidson, E.; Roepe, P. D. *Antimicrob. Agents Chemother.* 2004, 48, 1807–1810.

(34) Smilkstein, M.; Sriwilaijaroen, N.; Kelly, J. X.; Wilairat, P.; Rascoe, M. *Antimicrob. Agents Chemother.* 2004, 48, 1803–1806.