Phytochemistry Letters xxx (2013) xxx–xxx

Contents lists available at ScienceDirect

Phytochemistry Letters

journal homepage: www.elsevier.com/locate/phytol

Nigrosphaerin A a new isochromene derivative from the endophytic fungus *Nigrospora sphaerica*

Ahmed M. Metwaly [a,b], Hazem A. Kadry [b], Atef A. El-Hela [b], Abd-Elsalam I. Mohammad [b], Guoyi Ma [a], Stephen J. Cutler [c], Samir A. Ross [a,d,*]

[a] National Center for Natural Products Research, School of Pharmacy, The University of Mississippi, University, MS 38677, USA
[b] Department of Pharmacognosy, Faculty of Pharmacy, University of Al-Azhar, Cairo, Egypt
[c] Department of Medicinal Chemistry, School of Pharmacy, The University of Mississippi, University, MS 38677, USA
[d] Department of Pharmacognosy, School of Pharmacy, The University of Mississippi, University, MS 38677, USA

ARTICLE INFO

Article history:
Received 27 June 2013
Received in revised form 23 August 2013
Accepted 3 September 2013
Available online xxx

Keywords:
Nigrospora sphaerica
Nigrosphaerin A
Isochromene
Antileukemic
Antileishmanial

ABSTRACT

Nigrosphaerin A, a new isochromene derivative (**1**), was isolated from the endophytic fungus *Nigrospora sphaerica* and chemically identified as 3-(3,4-dihydroxyphenyl)-4,6,8-trihydroxy-1H-isochromen-1-one-6-O-β-D-glucopyranoside. In addition nineteen known compounds (**2**–**20**) were isolated from the same fungus and chemically identified. Compounds (**1**–**3**, **5**, and **7**–**16**) were isolated for the first time from this fungus. *In vitro* antileukemic, antileishmanial, antifungal, antibacterial and antimalarial activities of (**1**–**20**) were examined. Compounds **5**, **7**, **9** and **10** showed good antileukemic activity against HL60 cells with IC_{50} values of 0.03, 0.39, 0.2 and 0.4 μg/mL, respectively and against K562 cells with IC_{50} values of 0.35, 0.35, 0.49 and 0.01 μg/mL, respectively. Compounds **3**, **4** and **6** showed moderate antileishmanial activity with IC_{50} values of 30.2, 26.4 and 36.4 μg/ml, respectively. Compound **7** showed moderate antifungal activity against *Cryptococcus neoformans* with IC_{50} value of 14.8 μg/mL.

1. Introduction

Endophytic fungi are prospective producers of an abundant source of bioactive chemically novel compounds with potential for exploitation in a wide variety of medical areas (Tenguria et al., 2011). Fungi belonging to the genus *Nigrospora* have been a rich source of bioactive secondary metabolites, such as nigrosporolides which found to have plant growth-inhibiting activity (Kim et al., 2001), phomalactones with good anti plant pathogenic fungi effect (Kim et al., 2001), phytotoxic antibacterial nigrosporins (Tanaka et al., 1997), phytotoxic lactones (Fukushima et al., 1998), epoxydons and pyrones (Trisuwan et al., 2008).

The fungus *Nigrospora sphaerica* has been reported as an endophyte in several plants and marine organisms (Zhang et al., 2009). *N. sphaerica* has been found to be a source of biologically active secondary metabolites, including diterpenes (Turner and Aldridge, 1983), diketopiperazines (Cutler et al., 1991), lactones (Kim et al., 2001) and nigrosporolides (Zhang et al., 2009).

Chemical and biological investigation for the endophytic fungus *N. sphaerica* (Fig. 1), led to the isolation of Nigrosphaerin A, a new isochromene derivative (**1**), along with nineteen known compounds (**2**–**20**). The antileukemic, antileishmanial, antifungal and antibacterial activities of the isolated compounds were studied.

2. Results and discussion

Compound **1** (Fig. 2A) was isolated as a greenish yellow amorphous powder. The molecular formula $C_{21}H_{20}O_{12}$ was determined by HR-ESI-MS (*+ve* mode) showing molecular ion peak $[M+H]^+$ at *m/z* 465.0990 (calcd. for $C_{21}H_{21}O_{12}$, 465.1033) indicating twelve degrees of unsaturation. The IR spectrum showed that **1** contained hydroxyl (ν_{max} 3405 cm^{-1}), and carbonyl (ν_{max} 1648 cm^{-1}) functional groups. The UV spectrum showed absorption bands at λ_{max} 255, 290 (sh) and 365.0 nm.

The ^1H NMR spectroscopic data of **1** displayed signals at δ_H 7.59 (1H, d, J = 2.0 Hz, H-2′), δ_H 7.45 (1H, dd, J = 8.4, 2.0 Hz, H-6′) and δ 6.86$_H$ (1H, d, J = 8.4, H-5′) indicating a 1,3,4-trisubstituted benzene ring. These protons were found to be correlated to δ_C 114.8, δ_C 119.7 and δ_C 115.8 in the HMQC spectrum, respectively. Two meta coupled aromatic protons resonating at δ_H 6.64(1H, d, J = 1.6 Hz, H-5) and δ_H 6.75(1H, d, J = 1.6 Hz, H-7) were correlated to δ_C 97.5 and to δ_C 103.4 in the HMQC spectrum, respectively. The hydroxyl proton (C-8) found to be resonating at δ_H 13.48 due to intermolecular hydrogen bonding with the carbonyl group (C-1). The ^{13}C NMR, DEPT and HMQC spectroscopic data of **1** displayed 21 signals, including one methylene, ten methine and ten quaternary

* Corresponding author at: National Center for Natural Products Research, School of Pharmacy, The University of Mississippi, University, MS 38677, USA. Tel.: +1 662 915 1031; fax: +1 662 915 7989.
E-mail address: sross@olemiss.edu (S.A. Ross).

1874-3900/$ – see front matter © 2013 Published by Elsevier B.V. on behalf of Phytochemical Society of Europe.
http://dx.doi.org/10.1016/j.phytol.2013.09.001

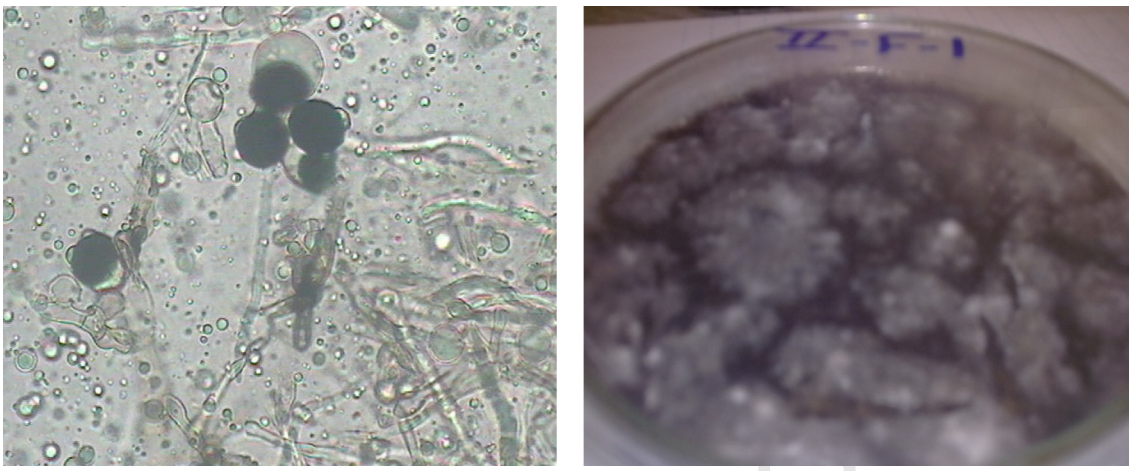
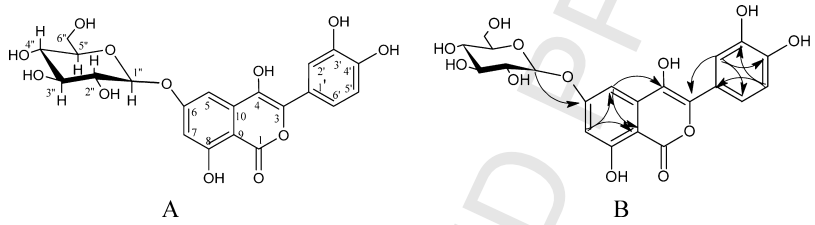


Fig. 1. *Nigrospora sphaerica.*



A B

Fig. 2. Compound **1** (A) and key HMBC (H → C) of **1** (B).

carbons. The carbon resonating at δ_C 171.8 ppm is characteristic for carbonyl group (C-1). The ^{13}C, HMQC and HMBC NMR data further established four phenolic hydroxyl groups at C-4 (δ_C 157.4), C-8 (δ_C 163.3), C-3′ (δ_C 145.3) and C-4′ (δ_C 147.5). Six carbons for sugar were found to be resonating at δ_C 103.9 (C-1″), δ_C 73.8 (C-2″), δ_C 77.6 (C-3″), δ_C 69.8 (C-4″), δ_C 75.8 (C-5″) and δ_C 60.8 (C-6″). Anomeric proton at δ_H 4.77 found to be correlated to C-1″ at δ_C 103.9 in the HMQC spectrum and to δ_C 158.5 in the HMBC spectrum, indicating that the sugar is attached to C-6. The large coupling constant of the anomeric proton J = 7.2 Hz indicated the β-configuration of the sugar (Avilov et al., 2003; Gao et al., 2008). The following correlations have been found in the HMBC spectrum (Fig. 2B): H-7 (δ_H 6.75) to C-5 (δ_C 97.5) and C-9 (δ_C 106.1), from H-5 (δ_H 6.64) to C-4 (δ_C 157.4) and C-9 (δ_C 106.1), from H-2′ (δ_H 7.59) to C-3 (δ_C 143.6), C-4′ (δ_C 147.5) and C-6′ (δ_C 119.7), from H-5′ (δ_H 6.86) to C-1′ (δ_C 122.2) and C-3′ (δ_C 145.3) and from H-6′ (δ_H 7.45) to C-2′ (δ_C 114.8) and C-4′ (δ_C 147.5). The hydrolysis of **1** with 0.5 N HCl yielded 3-(3,4-dihydroxyphenyl)-4,6,8-trihydroxy-1H-isochromen-1-one and glucose which was identified by co-chromatography with standard sugars using TLC. Based on the above evidences, the structure of **1** was established as 3-(3,4-dihydroxyphenyl)-4,6,8-trihydroxy-1H-isochromen-1-one-6-O-β-D-glucopyranoside.

Fifteen compounds (**2**–**16**, Fig. 3) have been isolated and were chemically identified using 1D NMR (^{1}H, ^{13}C, DEPT135) and 2D NMR (COSY, HMQC, HMBC) as well as HR-ESI-MS and found to be ergosta-6,22-diene-3β,5α,8α-triol (**2**) (Cateni et al., 2007), ergosta-7,22-diene-3β-ol (**3**) (Gong et al., 2010), ergosta-4,6,8(14),22-tetraene-3β-ol (**4**) (Pang and Sterner, 1993), ergosta-4,6,8(14),22-tetraene-3one (**5**) (Lee et al., 2005), ergosta-5(6),7,22-triene-3β-ol (**6**) (Li et al., 2007), ergosta-7,9(14),22-triene-3β-ol (**7**) (Li et al., 2008a), ergosta-7,22-epidioxy-3β-ol (**8**) (Cateni et al., 2007), 4-(hydroxymethyl)-3,5-dimethyl dihydrofuran-2(3H)-one (**9**) (Rukachaisirikul et al., 2009), 3-(1-hydroxyethyl)-4-methyl dihydrofuran-2(3H)-one (**10**) (Choi et al., 2008), uridine (**11**) (Mantsch and Smith, 1973), adenosine (**12**) (Liu et al., 2011), 3-benzylhexahydropyrrolo[1,2-a]pyrazine-1,4-dione (**13**) (Fdhila et al., 2003), 3-methylhexahydro pyrrolo[1,2-a]pyrazine-1,4-dione (**14**) (Hendea et al., 2006) and methyl 4-hydroxybenzoate (**15**) (Li et al., 2008b), 4-(2-hydroxyethyl)phenol (**16**) (Li et al., 2012). Four fatty acids (**17**–**20**) have been also isolated and chemically identified as stearic acid (**17**), oleic acid (**18**), palmitic acid (**19**), and myristic acid (**20**) using ^{1}H NMR and GC/MS after methylation.

Compounds **5**, **7**, **9** and **10** showed good antileukemic activity (Table 1 and Fig. 4) against acute HL60 cells with IC_{50} values of 0.03, 0.39, 0.2 and 0.4 μM/mL, respectively and against chronic K562 cells with IC_{50} values of 0.35, 0.35, 0.49 and 0.01 μM/mL, respectively. Standard taxol showed IC_{50} values of 0.0005 μM/mL and 0.0023 μM/mL, respectively. Compounds **3**, **4** and **6** showed moderate antileishmanial activity with IC_{50} values of 30.2, 26.4 and 36.4 μg/mL, respectively. (Value of IC_{50} for standard pentamidine was found to be 1.01 μg/mL). Compound **7** showed moderate antifungal activity against *Cryptococcus neoformans* with IC_{50} value of 14.81 μg/mL. It was found to be 0.28 μg/mL for standard amphotericin B.

3. Experimental

3.1. General

NMR spectra were recorded on a Bruker Avance DRX-500 instrument at 500 (^{1}H) and 125 MHz (^{13}C), and a Varian Mercury 400 MHz spectrometer at 400 (^{1}H) and 100 MHz (^{13}C). The HR-ESI-MS spectra were measured using a Bruker Bioapex-FTMS with electrospray ionization (ESI). The GC–MS was interfaced to a HP 5973 quadrupole mass selective detector. The injector temperature was 250 °C, and 1 μL injections were performed in the split (1:10) mode using helium as carrier gas. Column chromatographic separation was performed on silica gel 60 (0.04–0.063 mm) and

Please cite this article in press as: Metwaly, A.M., et al., Nigrosphaerin A a new isochromene derivative from the endophytic fungus *Nigrospora sphaerica*. Phytochem. Lett. (2013), http://dx.doi.org/10.1016/j.phytol.2013.09.001

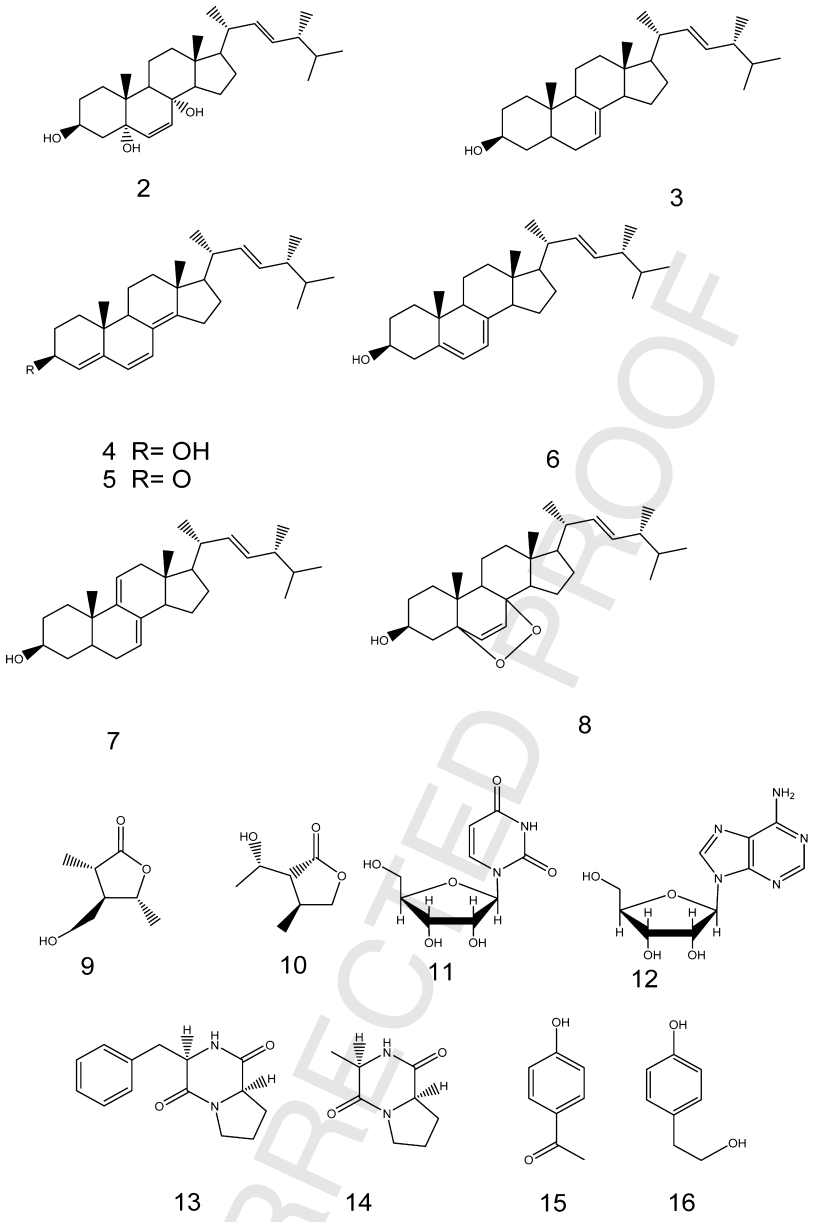


Fig. 3. Compounds **2**–**16**.

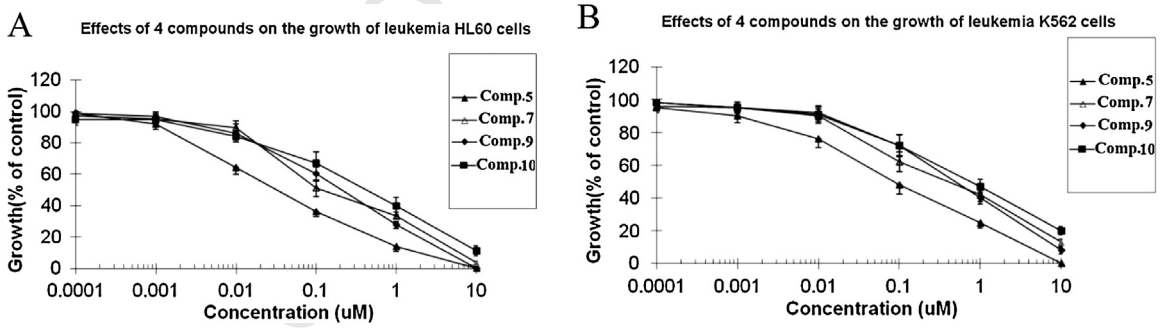


Fig. 4. The concentration-dependent effects of compounds **5**, **7**, **9** and **10** on the growths of acute leukemia HL60 (A) and chronic leukemia K562 (B) cells. Cells were treated with compounds for 48 h and then cell numbers were determined by the trypan blue exclusion test. Results shown are mean ± SD (bars) of triplicate experiments.

sephadex LH-20 (0.25–0.1 mm, Merck). TLC was performed on precoated TLC plates with silica gel 60 F254 (0.2 mm, Merck). Semi preparative HPLC (Waters Delta Prep 4000) was performed using Luna® RP-18 (250,10 mm × 5 μm; flow rate 5 mL/min).

3.2. Fungal material

The fungus *N. sphaerica* was isolated from surface sterilized fresh leaves of an apparently healthy *vinca rosea* (Apocynaceae)

Please cite this article in press as: Metwaly, A.M., et al., Nigrosphaerin A a new isochromene derivative from the endophytic fungus *Nigrospora sphaerica*. Phytochem. Lett. (2013), http://dx.doi.org/10.1016/j.phytol.2013.09.001

Table 1
Inhibitory effects of compounds **5**, **7**, **9** and **10** on the growth of human leukemia cells *in vitro* (48 h drug exposure for HL60 and K562 cells). Experiments were carried out in triplicate.

Compounds	Estimated IC_{50} (μM)	
	HL60 cells	K562 cells
Compound **5**	0.03 ± 0.05	0.35 ± 0.002
Compound **7**	0.39 ± 0.03	0.35 ± 0.002
Compound **9**	0.2 ± 0.001	0.49 ± 0.0012
Compound **10**	0.4 ± 0.001	0.01 ± 0.003
Taxol	0.0005 ± 0.00008	0.0023 ± 0.0005

collected in March 2010 in Cairo, Egypt. The leaves were rinsed with water and followed by surface sterilization in 70% EtOH for 1 min, rinsed with sterilized water, then cut into small pieces (2 cm in length and width) and deposited in on a petri dish containing PDA medium (200 g potato, 20 g glucose, and 15 g agar in 1 L distilled water, supplemented with 100 mg/L chloramphenicol) and cultivated at 28 °C for 3 days. The hypha tips were observed and transferred to new PDA plates and subcultured until pure culture was obtained. The fungus was identified by the regional center for mycology and biotechnology, Cairo, Egypt. Identification was based on The Data Base Identification Program of the Regional Center for Mycology and Biotechnology (RCMB) for fungi, using an Image Analysis System and on current universal keys (Fisher and Cook, 1998; Hoog et al., 2000). After purification the fungus was grown on PDA at 28 °C for 5 days. Ten pieces (0.5×0.5 cm^2) of mycelial agar plugs were inoculated into ten 1000 mL Erlenmeyer flasks containing sterilized (100 g Asian rice and 100 ml distilled water) at room temperature for 40 days.

3.3. Extraction and Isolation

The fungus was extracted by adding 2 L EtOAc to each flask and homogenized. The homogenized suspensions were collected, filtrated, concentrated under vacuum and partitioned with distilled water. Ethyl acetate portion was evaporated to dryness and fractionated using hexane and 90% MeOH to afford hexane fraction (7.6 g) and MeOH fraction (8.0 g). Water portion was fractionated against *n*-butanol to afford water fraction (36 g) and butanol fraction (7 g). *N. sphaerica* MeOH fraction (8 g) was subjected to Si gel VLC eluted with hexane, EtOAc and finally MeOH. Six fractions were collected (500 mL each). Fractions 2–3 (410.9 mg) were chromatographed on sephadex LH-20 using eluent MeOH:$CHCl_3$ (5:5) to yield nine subfractions. Subfraction 2 was chromatographed with Si-SPE column eluted with $CHCl_3$, MeOH in a matter of increasing polarity to afford compound **2** (3 mg), compound **4** (5 mg) and compound **5** (4 mg). Subfraction 3 was chromatographed on Si-SPE column eluted with $CHCl_3$, MeOH to give compounds **6** (12.6 mg) and **8** (1.3 mg). Subfraction 4 was chromatographed on sephadex LH-20 using eluent MeOH:$CHCl_3$ (5:5) to afford compound **7** (2.4 mg) and compound **3** (3.9 mg). Subfractions 5–6 was chromatographed on Si gel eluted with $CHCl_3$, MeOH in a matter of increasing polarity to afford compounds **9** (5.8 mg) and compound **10** (2.7 mg).

Q5 The butanol fraction (7.0 mg) was subjected to Si gel VLC gradient eluted with $CHCl_3$/MeOH and finally MeOH. Eight fractions were collected (500 mL each). Fraction 2 (17.8 mg) was chromatographed on sephadex LH-20 eluting with MeOH/H_2O, followed by purification with semi-preparative HPLC eluted with 75% MeOH/H_2O to give compound **1** (6.3 mg). Fractions 3–4 (702.9 mg) were chromatographed on sephadex LH-20 eluting with MeOH to yield 5 subfractions. Subfraction 2 was chromatographed on RP-Si-SPE (C18) column using eluent H_2O:MeOH (6:4) to give compounds **11** (6.7 mg) and **15** (7.3 mg). Subfraction 3 was chromatographed on sephadex LH-20 eluting with MeOH to give compound **12** (15.9 mg). Subfraction 4 was chromatographed with semi-preparative HPLC with linear gradient elution 20–85% aqueous methanol to obtain compounds **13** (7.8 mg), **14** (4.3 mg) and **16** (1.4 mg).

3.4. 3-(3,4-Dihydroxyphenyl)-4,6,8-trihydroxy-1H-isochromen-1-one-6-O-β-D-glucopyranoside (**1**)

Greenish yellow amorphous powder; UV (MeOH): λ_{max} (log ε) nm: 255 (3.93), 290(sh) (3.71) and 365.0 (3.92); IR ν_{max}: 3405, 1648 and 1024 cm^{-1}; HR-ESI-MS *m/z* 465.0990 $[M+H]^+$ (calcd. for $C_{21}H_{21}O_{12}$, 465.1033). ^1H NMR (DMSO, δ, 400 MHz, ppm): 6.64 (1H, d, *J* = 1.6 Hz, H-5), 6.75 (1H, d, *J* = 1.6 Hz, H-7), 7.59 (1H, d, *J* = 2.0 Hz, H-2′), 7.45 (1H, dd, *J* = 8.4, 2.0 Hz, H-6′), 6.86 (1H, d, *J* = 8.4, H-5′), 4.77 (1H, d, *J* = 7.2 Hz, H-1″), 3.33 (1H, H-2″), 3.30 (1H, H-3″), 3.18 (1H, H-4″), 3.27 (1H, H-5″), 3.65 and 3.70 (2H, H-6″); ^{13}C NMR (DMSO, δ, 125 MHz, ppm): 171.9 (C-1), δ143.6 (C-3), 157.4 (C-4), 97.5 (C-5), 158.5 (C-6), 103.4 (C-7), 163.3 (C-8), 106.1 (C-9), 137.4 (C-10), 122.2 (C-1′), 114.8 (C-2′), 145.3 (C-3′), 147.5 (C-4′), 115.8 (C-5′), 119.7 (C-6′), 103.9 (C-1″), 73.8 (C-2″), 77.6 (C-3″), 69.8 (C-4″), 75.8 (C-5″), 60.8 (C-6″).

3.5. Acid hydrolysis of compound **1**

2 mg of compound 1 was refluxed with HCl (0.5 N, 2 mL) for 2 h. The hydrolyzed product was extracted with CH_2Cl_2/H_2O. The sugar was extracted from the aqueous layer using pyridine (1 mL). The sugar was identified as D-glucose by co-chromatography with authentic samples of different sugars using silica gel TLC using solvent system EtOAc/MeOH/HOAc/H_2O (11:2:2:2) followed by spraying with anisaldehyde/H_2SO_4 and heating at 100 °C.

3.6. Antileukemic assay

Human acute leukemia HL60 cells and human chronic leukemia 562 cells were purchased from American Type Culture Collection, Rockville MD, USA. Both cell lines were grown in suspension culture at 37 °C in RPMI-1640 medium supplemented with 10% non-dialysed fetal bovine serum (FBS), 2 mM L-glutamine, 100 units/mL of penicillin and 10 μg/mL of streptomycin. For the cell growth inhibition assay, HL60 and K562 cells were set up at 1×10^5 cells/well in Costar 24-well plates. Cells were allowed to grow undisturbed for 24 h before addition of "compounds". After 48 h incubation with drugs at 37 °C, viable cell counts were made by using the trypan blue exclusion method to assess cell viability (Roper and Drewinko, 1976).

3.7. Antimicrobial assay

Crude extracts and isolated compounds were tested for antimicrobial activity against *Candida albicans* ATCC 90028, *Candida glabrata* ATCC90030, *Candida krusei* ATCC 6258, *Aspergillus fumigates* ATCC 90906, Methicillin-resistant *Staphylococcus aureus* ATCC 33591, *Cryptococcus neoformans* ATCC 90113, *Staphylococcus aureus* ATCC 2921, *Escherichia coli* ATCC 35218, *Pseudomonus aeruginosa* ATCC 27853, *Mycobacterium intracellulare* ATCC 23068, Ciprofloxacin and Amphotericin B were used as positive standards (Bharate et al., 2007; Radwan et al., 2009).

3.8. Antimalarial assay

Crude extracts were tested on chloroquine sensitive (D6, Sierraleon) and resistant (W2, Indo-china) strains of *Plasmodium falciparum* using previously reported method; Artemisinin and Chloroquine were used as positive standards (Bharate et al., 2007).

3.9. Antileishmanial assay

The antileishmanial activity of the isolated metabolites was tested *in vitro* against a culture of *L. donovani* promastigotes; Pentamidine and Amphoterecin B were used as positive standards (Abdel-Mageed et al., 2012).

Acknowledgments

We are grateful to the Egyptian Government and National Center for Natural Products Research, School of Pharmacy, University of Mississippi, for financial support. We are also thankful for Dr. Baharthi Avula for HRMS, Drs. Melissa Jacob, Babu Tekwani and Shabana Khan for antimicrobial, antileishmanial and antimalarial assays. This investigation was conducted in part in a facility constructed with support from the research facilities improvement program C06 RR-14503-01 from the NIH NCRR. This work is supported in part by United States Department of Agriculture ARS Specific Cooperative Agreement No. 58-6408-2-0009.

Appendix A. Supplementary data

(^{1}H NMR, ^{13}C NMR, DEPT 135, HMQC, HMBC, HR-ESI-MS, UV and IR) spectral data of compound **1**.

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.phytol.2013.09.001.

References

Abdel-Mageed, W.M., Backheet, E.Y., Khalifa, A.A., Ibraheim, Z.Z., Ross, S.A., 2012. Antiparasitic antioxidant phenylpropanoids and iridoid glycosides from *Tecoma mollis*. Fitoterapia 83, 500–507.

Avilov, S.A., Antonov, A.S., Silchenko, A.S., Kalinin, V.I., Kalinovsky, A.I., Dmitrenok, P.S., Stonik, V.A., Riguera, R., Jimenez, C., 2003. Triterpene glycosides from the Far Eastern sea cucumber *Cucumaria conicospermium*. J. Nat. Prod. 66, 910–916.

Bharate, S.B., Khan, S.I., Yunus, N.A.M., Chauthe, S.K., Jacob, M.R., Tekwani, B.L., Khan, I.A., Singh, I.P., 2007. Antiprotozoal and antimicrobial activities of O-alkylated and formylated acylphloroglucinols. Bioorg. Med. Chem. 15, 87–96.

Cateni, F., Doljak, B., Zacchigna, M., Anderluh, M., Piltaver, A., Scialino, G., Banfi, E., 2007. New biologically active epidioxysterols from *Stereum hirsutum*. Bioorg. Med. Chem. Lett. 17, 6330–6334.

Choi, J.-H., Horikawa, M., Okumura, H., Kodani, S., Nagai, K., Hashizume, D., Koshino, H., Kawagishi, H., 2008. Endoplasmic reticulum (ER) stress protecting compounds from the mushroom *Mycoleptodonoides aitchisonii*. Tetrahedron 65, 221–224.

Cutler, H.G., Hoogsteen, K., Littrell, R.H., Arison, B.H., 1991. Epoxyexserohilone, a novel metabolite from *Nigrospora sphaerica*. Agric. Biol. Chem. 55, 2037–2042.

Fdhila, F., Vazquez, V., Sanchez, J.L., Riguera, R., 2003. dd-Diketopiperazines: antibiotics active against *Vibrio anguillarum* isolated from marine bacteria associated with cultures of *Pecten maximus*. J. Nat. Prod. 66, 1299–1301.

Fisher, F.W., Cook, N.B., 1998. Fundamentals of Diagnostic Mycology. WB Saunders, Philadelphia, PA.

Fukushima, T., Tanaka, M., Gohbara, M., Fujimori, T., 1998. Phytotoxicity of three lactones from *Nigrospora sacchari*. Phytochemistry 48, 625–630.

Gao, Z., Ali, Z., Khan, I.A., 2008. Glycerogalactolipids from the fruit of *Lycium barbarum*. Phytochemistry 69, 2856–2861.

Gong, Q., Zhang, Y., Tan, N., Chen, Z., 2010. Chemical constituents of three poisonous mushrooms. Tianran Chanwu Yanjiu Yu Kaifa 22, 185–188.

Hendea, D., Laschat, S., Baro, A., Frey, W., 2006. Diastereoselective alkylation of a proline-derived bicyclic lactim ether. Helv. Chim. Acta 89, 1894–1909.

Hoog, G.D., Guarro, J., Gené, J., Figueras, M., 2000. Atlas of Clinical Fungi. Centraalbureau voor Schimmelcultures (CBS), Baarn.

Kim, J.-C., Choi, G.J., Park, J.-H., Kim, H.T., Cho, K.Y., 2001. Activity against plant pathogenic fungi of phomalactone isolated from *Nigrospora sphaerica*. Pest Manag. Sci. 57, 554–559.

Lee, W.Y., Park, Y., Ahn, J.-K., Park, S.-Y., Lee, H.-J., 2005. Cytotoxic activity of ergosta-4,6,8(14),22-tetraen-3-one from the sclerotia of *Polyporus umbellatus*. Bull. Korean Chem. Soc. 26, 1464–1466.

Li, D., Li, X., Cui, C., Wang, B., 2008a. Chemical constituents of endophytic fungus *Hypocreales* sp. derived from the red alga *Symphyocladia latiuscula*. Haiyang Kexue 32, 51–55.

Li, P., Zuo, T., Wang, X., Zhu, L., Wu, S., Zhang, G., 2008b. Chemical constituents of *Scutellaria barbata* D. Don (II). Zhongguo Yaowu Huaxue Zazhi 18, 374–376.

Li, X., Han, L., Cao, Y.-R., Liu, J., Jiang, Y., Huang, X.-S., 2012. Separation and identification of chemical constituents from a *Streptomyces* sp. isolated from sika deer feces. Huaxue Yu Shengwu Gongcheng 29 (31/32) 49.

Li, X., Sun, G.-Z., Zheng, Y.-N., Lin, W.-H., Sattler, I., 2007. Separation and structures of two steroids from mangrove endophyte *Penicillium*. Tianran Chanwu Yanjiu Yu Kaifa 19, 420–422.

Liu, D., Wang, S., Zhang, L., Li, L., 2011. Chemical composition of n-BuOH extract of *Potentilla anserina* L. and its protective effect of EAhy926 endothelial cells under hypoxia. Lat. Am. J. Pharm. 30, 1889–1894.

Mantsch, H.H., Smith, I.C.P., 1973. Solvent effects on the carbon-13 nuclear magnetic resonance spectra of cholesterol pyridine, and uridine. Can. J. Chem. 51, 1384–1391.

Pang, Z., Sterner, O., 1993. The isolation of ergosta-4,6,8(14),22-tetraen-3β-ol from injured fruit bodies of *Marasmius oreades*. Nat. Prod. Lett. 3, 193–196.

Radwan, M.M., Rodriguez-Guzman, R., Manly, S.P., Jacob, M., Ross, S.A., 2009. Sepicanin A—a new geranyl flavanone from *Artocarpus sepicanus* with activity against methicillin-resistant *Staphylococcus aureus* (MRSA). Phytochem. Lett. 2, 141–143.

Roper, P.R., Drewinko, B., 1976. Comparison of in vitro methods to determine drug-induced cell lethality. Cancer Res. 36, 2182–2188.

Rukachaisirikul, V., Arunpanichlert, J., Sukpondma, Y., Phongpaichit, S., Sakayaroj, J., 2009. Metabolites from the endophytic fungi *Botryosphaeria rhodina* PSU-M35 and PSU-M114. Tetrahedron 65, 10590–10595.

Tanaka, M., Fukushima, T., Tsujino, Y., Fujimori, T., 1997. Nigrosporins A and B, new phytotoxic and antibacterial metabolites produced by a fungus *Nigrospora oryzae*. Biosci. Biotechnol. Biochem. 61, 1848–1852.

Tenguria, R.K., Khan, F.N., Quereshi, S., 2011. Endophytes—mines of pharmacological therapeutics. World J. Sci. Technol. 1, 127–149.

Trisuwan, K., Rukachaisirikul, V., Sukpondma, Y., Preedanon, S., Phongpaichit, S., Rungjindamai, N., Sakayaroj, J., 2008. Epoxydons and a pyrone from the marine-derived fungus *Nigrospora* sp. PSU-F5. J. Nat. Prod 71, 1323–1326.

Turner, W.B., Aldridge, D.C., 1983. Fungal Metabolites, vol. II. Academic Press, New York.

Zhang, Q.-H., Tian, L., Zhou, L.-D., Zhang, Y., Li, Z.-F., Hua, H.-M., Pei, Y.-H., 2009. Two new compounds from the marine *Nigrospora sphaerica*. J. Asian Nat. Prod. Res. 11, 962–966.