Tetrahedron 67 (2011) 7593–7597

Contents lists available at ScienceDirect

Tetrahedron

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

Antimalarial and antituberculosis substances from *Streptomyces* sp. BCC26924

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ARTICLE INFO

Article history:
Received 26 April 2011
Received in revised form 28 June 2011
Accepted 18 July 2011
Available online 22 July 2011

Keywords:
Actinomycetes
Streptomyces
Antimalaria
Antituberculosis
Carbazomycin derivatives
Carbazomycin

ABSTRACT

Two new carbazomycin dimers (**6** and **7**) and 3-hydroxy-1,2-dimethyl-2,3-dihydro-1*H*-carbazol-4-one (**9**) together with six known compounds, carbazomycins A–D, cyclomarin C, and pimprinine have been isolated from *Streptomyces* sp. BCC26924. Carbazomycins B, C, and cyclomarin C exhibited antimalarial activity (against *Plasmodium falciparum*, K1 multi-drug resistant strain) with IC_{50} in a range of 0.24–2.37 μg/mL. Cyclomarin C exhibited anti-TB activity with a minimum inhibitory concentration value of 0.10 μg/mL, while carbazomycin D, compound **7**, and pimprinine displayed MIC values in a range of 12.5–25.0 μg/mL. In addition, compounds **2**, **5**, **6**, and **7** showed weak cytotoxicity against cancerous (MCF-7, KB, NCI-H187) and non-cancerous (Vero) cells.

1. Introduction

Streptomyces, a common genus in Actinomycetes, has long been of interest due to its diversity of chemical constituents and biological activities. Several well-known antibiotics have been isolated from this genus, for example, chloramphenicol, streptomycin, lincomycin, tetracycline, to name just a few. Recently, researchers at BIOTEC have collected and identified many genera of Actinomycetes from various habitats in Thailand. These specimens have been deposited at BIOTEC Culture Collection (BCC). As part of our continuing search for bioactive compounds from microorganisms, we noted that the genus *Streptomyces* has shown a broad spectrum of both biological activities and chemical diversity. One of the crude extracts from *Streptomyces*, registered as BCC26924, showed a productive HPLC profile and also exhibited antimalaria and antifungal activity (*Candida albicans*) with IC_{50} values of 0.77 and 13.8 μg/mL, respectively. It also showed antituberculosis activity with MIC value of <1.56 μg/mL, Therefore, we decided to investigate the chemical ingredients of the specimen BCC26924.

2. Results and discussion

Compounds **1**–**5** and **8** were identical to those reported as cyclic heptapeptide cyclomarin C (**1**),[1] carbazomycins A–D (**2**–**5**),[2,3] and pimprinine (**8**).[4]

Compound **6**, obtained as a brown solid, had the molecular formula $C_{30}H_{28}O_4N_2Na$ with the mass peak at 503.1949 in HRESIMS. There are thirty carbons consisting of five methyl, one methylene, seven methine, and seventeen quaternary carbons. The ^1H NMR spectrum showed the disappearance of four protons at δ_H 8.90, 9.24, 10.65, and 11.07 after addition of D_2O. Seven aromatic protons resonated between 7 and 8 ppm, whose HMQC correlations with ^{13}C are shown in Table 1. Four of these protons at positions 5, 6, 7, and 8 showed HMBC correlations as follows; the doublet proton at δ_H 7.39 (8-H) showed correlations to the carbons at δ_C 123.4 (4b-C) and 118.5 (6-CH); the triplet methine at δ_H 7.26 (7-H) to the carbons at δ_C 122.3 (5-CH) and 140.1 (8a-C); the triplet at δ_H 7.08 (6-H) to the carbons at δ_C 110.8 (8-CH) and 123.4 (4b-C); the doublet proton at δ_H 8.13 (5-H) to the methine at δ_C 124.4 (7-CH) and two quaternary carbons at δ_C 110.3 (4a-C) and 140.1 (8a-C). The hydroxyl proton at δ_H 9.24 (4-OH) showed HMBC correlations to δ_C 110.3 (4a-C), 138.9 (3-C), and 143.7 (4-C), respectively. The methoxyl signal at δ_H 3.66 displayed HMBC correlations to the quaternary carbon at δ_C 138.9 (3-C) and the methyl at δ_H 2.25 also correlated to three quaternary carbons at δ_C 113.4 (2-C), 128.3 (1-C), and 138.9 (3-C). The ^1H NMR spectrum of the described moiety is similar to that of carbazomycin B. In addition, the double doublet proton at δ_H 7.08 (7′-H), bearing a carbon at δ_C 124.9 in HMQC, correlated in HMBC spectrum to the methylene at δ_C 33.7 (10-CH_2), to the methine at δ_C 121.4 (5′-CH), and to the quaternary carbon at δ_C 138.5 (8′a-C). The doublet methine at δ_H 7.26 (8′-H) showed HMBC correlations to two quaternary carbons at 123.5 (4′b-C) and 130.4 (6′-C). The singlet methine at δ_H 7.83 (5′-H) correlated to the methylene carbon at δ_C 33.7 (10-CH_2), to methine carbon at δ_C 124.9

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doi:10.1016/j.tet.2011.07.053

Table 1
^{1}H and ^{13}C NMR assignments of compounds **6** and **7** in DMSO-d_6

Position	**6**		**7**	
	^{1}H NMR, ppm	^{13}C NMR, ppm	^{1}H NMR, ppm	^{13}C NMR, ppm
1	—	128.3	—	128.2
2	—	113.4	—	113.4
3	—	138.9	—	138.6
4	—	143.7	—	143.5
4a	—	110.3	—	110.4
4b	—	123.4	—	123.7
5	8.13 (1H, d, *J* 7.75 Hz)	122.3	7.66 (1H, d, *J* 2.49 Hz)	105.4
6	7.08 (1H, t, *J* 7.69 Hz)[a]	118.5	—	153.0
7	7.26 (1H, t, *J* 7.70 Hz)[a]	124.4	6.92 (1H, dd, *J* 8.67, 2.49 Hz)	113.4
8	7.39 (1H, d, *J* 8.04 Hz)	110.8	7.29 (1H, d, *J* 8.67 Hz)	111.3
8a	—	140.1	—	135.0
9	11.07 (1H, s, NH)	—	10.85 (1H, s, NH)	—
9a	—	137.9	—	138.4
10	4.36 (2H, s)	33.7	4.33 (2H, s)	33.7
1′	—	127.2	—	127.2
2′	—	108.8	—	108.9
3′	—	138.5	—	138.5
4′	—	142.9	—	142.9
4′a	—	109.9	—	109.9
4′b	—	123.5	—	123.5
5′	7.83 (1H, s)	121.4	7.83 (1H, s)	121.4
6′	—	130.4	—	130.3
7′	7.08 (1H, dd, *J* 8.10, 1.43 Hz)[a]	124.9	7.08 (1H, dd, *J* 8.27, 1.39 Hz)	124.9
8′	7.26 (1H, d, *J* 8.10 Hz)[a]	110.3	7.26 (1H, d, *J* 8.27 Hz)	110.3
8′a	—	138.5	—	138.5
9′	10.65 (1H, s, NH)	—	10.65 (1H, s)	—
9′a	—	137.6	—	137.6
2-CH_3	2.25 (3H, s)	13.0	2.24 (3H, s)	13.0
3-OCH_3	3.66 (3H, s)	61.1	3.67 (3H, s)	61.1
4-OH	9.24 (1H, s, OH)	—	9.23 (1H, s, OH)	—
6-OCH_3	—	—	3.82 (3H, s)	56.0
1′-CH_3	2.30 (3H, s)	13.8	2.29 (3H, s)	13.8
2′-CH_3	2.23 (3H,s)	13.0	2.23 (3H, s)	13.0
3′-OCH_3	3.57 (3H,s)	61.0	3.57 (3H, s)	61.0
4′-OH	8.90 (1H, s, OH)	—	8.91 (1H, s, OH)	—

[a] Coupling constants are observed in acetone-d_6 due to signals overlapping in DMSO-d_6.

(7′-CH) and to the carbons at δ_C 109.9 (4′a-C) and 138.5 (8′a-C), respectively. The hydroxyl proton at δ_H 8.90 (4′-OH) correlated to three quaternary carbons at δ_C 109.9 (4′a-C), 138.5 (3′-C), and 142.9 (4′-C). The methoxyl at δ_H 3.57 (3′-OCH_3) showed HMBC correlation to the quaternary carbon at 138.5 (3′-C). Two methyls at δ_H 2.23 and 2.30 correlated to three quaternary carbons at 1′-C, 2′-C, 3′-C and 1′-C, 2′-C, 9′a-C, respectively. Two protons at δ_H 10.65 and 11.07, thought to be amine protons due to connections to the quaternary carbons between 137 and 140 ppm, displayed HMBC correlations to the carbons at 4′a, 4′b, 8′a, 9′a and 4a, 4b, 8a, 9a, respectively. The methylene proton at δ_H 4.36 (10-CH_2) showed HMBC correlations to the carbons at 128.3 (1-C), 137.9 (9a-C), 113.4 (2-C), 121.4 (5′-CH), 130.4 (6′-C), and 124.9 (7′-CH). The latter correlations led to the linking of the methylene group with two units of carbazomycins at positions 1 and 6′. The above evidence suggested that compound **6** should be depicted as shown in Fig. 1.

Compound **7** was obtained as a brown solid. Its molecular formula of $C_{31}H_{30}O_5N_2Na$ was determined by HRESIMS analysis with a mass peak at 533.2042. The ^{1}H NMR spectrum of compound **7** is similar to that of compound **6**. The main differences are the additional methoxyl proton at δ_H 3.82, the methine proton at δ_H 7.66 (5-H) with a *meta* coupling (*J*=2.49 Hz), and a double doublet methine at δ_H 6.92 (7-H, *J*=8.67, 2.49 Hz). The changing of their coupling patterns at 5-H and 7-H and the higher field shifts of both carbons (5-C and 7-C), compared with compound **6** in Table 1, suggested an additional methoxyl substituent at position 6. The HMBC spectrum showed correlations from the 6-methoxy group to the carbon at δ_C 153.0 (6-C); the doublet methine proton at δ_H 7.29 (8-H) to carbons at 6-C, 4b-C; the doublet methine at δ_H 7.66 (5-H) to 7-C and 8a-C; the double doublet methine at δ_H 6.92 (7-H) to 5-C and 8a-C. Therefore, the compound **7** can be depicted as shown in Fig. 1.

3-Hydroxy-1,2-dimethyl-2,3-dihydro-1*H*-carbazol-4-one (**9**) was obtained as brown gum. HRESIMS revealed the molecular formula of $C_{14}H_{15}NO_2Na$ with a mass peak of 252.0999. The ^{13}C NMR spectrum revealed the presence of two methyl, seven methine, and five quaternary carbons. The COSY spectrum showed connectivities from 1-H to 3-H and from 5-H to 8-H. The HMBC spectrum displayed correlations from the doublet methyl at δ_H 1.00 (2-CH_3) to the carbons at 1-CH, 2-CH, and 3-CH; the doublet methyl at δ_H 1.43 (1-CH_3) to the carbons at 1-CH, 2-CH, and 9a-C; the doublet methine at δ_H 7.91 (5-H) to the carbons at 7-CH and 4b-C; from the amine proton at δ_H 11.88 (9-H) to the carbons at 4a-C and 4b-C. The NOESY spectrum, showed cross-peaks correlations from the methyl at δ_H 1.00 (2-CH_3) to the protons at 1-H, 1-CH_3, 3-H, and 3-OH; from the methyl at δ_H 1.43 (1-CH_3) to the protons at 2-H, 2-CH_3, and 3-H. The observation in the NOESY spectrum of two methyl groups suggesting these two methyl groups are in *pseudo*-equatorial positions. The disappearance of correlation between protons at 1-H and 3-H indicated the opposite orientation of these two protons. The spectroscopic information, therefore, led to the chemical structure of compound **9** with the relative configuration as depicted in Fig. 1.

For the biological activities, cyclomarin C (**1**), carbazomycins B (**3**), and C (**4**) exhibited antimalarial activity with IC_{50} values of 0.24, 2.37, and 2.10 μg/mL, respectively. Cyclomarin C also showed anti-TB at MIC 0.10 μg/mL but was inactive against cancerous cells (KB, MCF-7, and

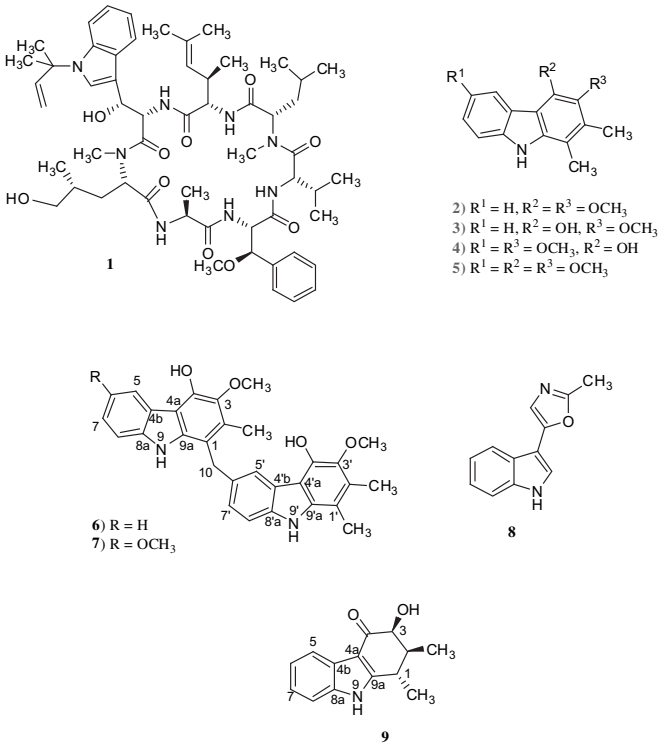

Fig. 1. Chemical structures of compounds **1**–**9**.

NCI-H187) and Vero cells at concentration of 50 μg/mL. This constitutes the first report on antimalarial and antituberculosis activities for cyclomarin C. Carbazomycin D (**5**), compound **7**, and pimprinine (**8**) also displayed anti-TB with MIC in a range of 12.5–25.0 μg/mL. Only carbazomycin B exhibited antifungal, *C. albicans*, with IC_{50} value of 19.6 μg/mL. Compounds **2**–**7** exhibited cytotoxicity against KB (IC_{50} in a range of 8.6–40.1 μg/mL), NCI-H187 (IC_{50} in a range of 4.2–25.3 μg/mL), and MCF-7 (IC_{50} in a range of 8.4–36.3 μg/mL) (also see Table 2).

Cyclomarin C (**1**) was first isolated from the culture broth of the marine bacterium *Streptomyces* sp. CNB982 as a minor component. Its biological activity has not yet been reported due to the limited amount. However, its derivative, cyclomarin A, displayed a potent anti-inflammatory activity.[5] Carbazomycins A–D (**2**–**5**) were isolated from *Streptoverticillium ehimense* (strain H1051 MY10).[6] They exhibited a broad range of antimicrobial activity (MIC 12.5–>200 μg/mL) against various organisms including yeast and bacteria, such as *Candida* spp., *Staphylococcus* spp., *Bacillus* spp., etc.[2,7] They also inhibited growth of phytopathogenic fungi (MIC 3.1–>200 μg/mL), such as *Alternaria kikuchiana*, *Aspergillus niger*, etc.[7] In addition, carbazomycins B and C showed 5-lipoxygenase (5-LPO) inhibitory at IC_{50} 1.5 and 1.9 μM, respectively.[8] Pimprinine showed weak antimicrobial activity against *Bacillus subtilis* (MIC 100 μg/mL), *Sarcina lutea* (MIC 2.5 μg/mL), *Saccharomyces cerevisiae* (MIC 100 μg/mL), and *Paecilomyces varioti* (MIC 1 μg/mL).[9]

3. Conclusion

The crude extract of *Streptomyces* sp. BCC26924 exhibited antimalarial, anti *C. albicans*, antituberculosis activities with IC_{50} values of 0.77, 13.8, and MIC value of <1.56 μg/mL, respectively. The investigation led to the isolation of nine compounds, three of which, compounds **6**, **7**, and **9**, are new. Cyclomarin C (**1**), carbazomycins B (**3**), and C (**4**) showed antimalarial activity with IC_{50} values of 0.24–2.37 μg/mL. Cyclomarin C (**1**), carbazomycin D (**5**), carbazomycin dimer **7**, and pimprinine (**8**) exhibited antituberculosis activity with MIC values of 0.1–25.0 μg/mL. Carbazomycin B displayed antifungal activity against *C. albicans* with IC_{50} value of 19.6 μg/mL. Carbazomycin A was active against cancer (KB, MCF-7, NCI-H187) and non-cancer (Vero) cells.

4. Experimental section

4.1. General experimental procedures

Optical rotations were measured on a JASCO P-1030 polarimeter in MeOH. UV spectra were recorded on a Cary 1E UV–Vis spectrophotometer in MeOH. IR spectra were taken on either a Bruker VECTOR 22 or Bruker ALPHA FT-IR spectrometer. NMR spectra, including COSY, NOESY, DEPT, HMQC, and HMBC experiments, were recorded on either a Bruker DRX400 (^1H at 400 MHz and ^{13}C at 100 MHz) or AV500D (^1H at 500 MHz and ^{13}C at 125 MHz) NMR spectrometer. ESIMS and HRESIMS data were determined on a Bruker MicrOTOF mass spectrometer. Semipreparative HPLC was performed using DIONEX, model UltiMate 3000, equipped with

Table 2
Biological activities of compounds **1**–**8**

Compound	Antimalaria	Anti-TB	Cytotoxicity (IC_{50}, μg/mL)				Anti-*Candida albicans*
	IC_{50}, μg/mL	MIC, μg/mL	MCF-7	KB	NCI-H187	Vero	IC_{50}, μg/mL
1	0.24	0.10	>50	>50	>50	>50	>50
2	>10	>50	26.2	30.1	18.4	32.6	>50
3	2.37	>50	8.4	8.6	4.2	48.9	19.6
4	2.10	>50	9.8	21.4	8.2	>50	>50
5	>10	25.0	21.3	33.2	12.9	34.3	>50
6	>10	>50	36.3	36.8	25.3	26.8	>50
7	>10	12.5	30.3	40.1	19.2	26.3	>50
8	>10	25.0	>50	>50	>50	> 50	>50
Ellipticine	—	—	—	0.44	0.44		—
Doxorubicin	—	—	9.2	0.61	0.09		—
Tomoxifen	—	—	4.9	—	—		—
AmphotericinB	—	—	—	—	—		0.08
Dihydroartemisinin	4.29×10^{-4}	—	—	—	—		—
Mefloquine	0.011	—	—	—	—		—
Rifampicin	—	0.00312–0.0250	—	—	—		—
Streptomycin	—	0.156–0.313	—	—	—		—
Isoniazid	—	0.0234–0.0468	—	—	—		—
Ofloxacin	—	0.391–0.781	—	—	—		—
Ethambutol	—	0.234–0.469	—	—	—		—

binary semipreparative system and Sunfire C18 column from Waters.

4.2. Biological material

Streptomyces sp. was collected from soil at Chat Trakan Waterfall National Park, Phitsanulok province, Thailand, and deposited at BIOTEC Culture Collection (BCC) with registration number BCC26924.

4.3. Fermentation, extraction, and isolation

Streptomyces sp. BCC26924 was developed at 28 °C for 5 days in 2×1 L Erlenmeyer flasks, each contained 250 mL of the seed medium consisting of 2% (w/v) glucose, 0.5% (w/v) peptone, 0.3% (w/v) yeast, 0.5% (w/v) meat extract, 0.5% (w/v) NaCl, 0.3% (w/v) $CaCO_3$, 0.1% (w/v) vitamin B complex (Blackmore, Australia). It was then transferred into the fermentor, which contained 7 L of the liquid medium, and cultured for 8 days at 28 °C. The production medium comprised 2% (w/v) mannitol, 2% (w/v) soy meal, 0.025% (v/v) mixed trace elements [0.4% (w/v) $CaCl_2{\cdot}2H_2O$, 0.2% (w/v) $ZnSO_4{\cdot}7H_2O$, 0.01% (w/v) $Na_2B_4O_7{\cdot}10H_2O$, 0.5% (w/v) $FeSO_4{\cdot}7H_2O$, 0.005% (w/v) KI, 0.05% (w/v) $CoCl_2{\cdot}6H_2O$, 0.02% (w/v) $CuSO_4{\cdot}5H_2O$, 0.2% (w/v) $MnCl_2{\cdot}4H_2O$, 0.005% (w/v) $Na_2MoO_4{\cdot}2H_2O$, 0.1% (v/v) H_2SO_4 (95–97% p.a.)].

Broth and cell were together extracted three times with equal volume EtOAc. The EtOAc solution was dried over Na_2SO_4 and then evaporated to dryness to obtain a brown gum (4.02 g). The crude gum was passed through a Sephadex LH20 column, eluted with 80% MeOH in CH_2Cl_2, to obtain 5 fractions. The first fraction (0.15 g) was purified by semipreparative HPLC (column diam. 19×250 mm, 10 μm) using a linear gradient of 65–80% aqueous CH_3CN over 20 min at a flow rate of 20 mL/min to afford compounds **3** (11.3 mg) and **1** (77.6 mg), respectively. The second fraction (0.56 g) was purified by Sephadex LH20 column (eluted with 80% MeOH in CH_2Cl_2) to give two subfractions A (0.33 g) and B (0.14 g). Subfraction A was again purified by Sephadex LH20 column (eluted with 100% MeOH) to wash off diketopiparazines, followed by semipreparative HPLC (column diam. 19×150 mm, 5 μm) at a flow rate of 10 mL/min with a linear gradient of 5–45% aqueous CH_3CN over 20 min to yield compound **9** (2 mg). Subfraction B was further purified by semipreparative HPLC (column diam. 19×150 mm, 5 μm) using a linear gradient system of 10–75% aqueous CH_3CN over 20 min at a flow rate of 10 mL/min to afford compounds **8** (17.5 mg), **5** (11.0 mg), and **2** (23.5 mg), respectively. The third fraction (0.24 g) was purified by semipreparative HPLC (column diam. 19×150 mm, 5 μm) using a linear gradient system of 15–70% aqueous CH_3CN over 20 min at a flow rate 10 mL/min to give compounds **8** (9.6 mg), **4** (24.2 mg), **3** (0.11 g), **5** (8.1 mg), and **2** (23.6 mg), respectively. The fourth fraction was purified by semipreparative HPLC (column diam. 19×150 mm, 5 μm) using a linear gradient system of 25–65% aqueous CH_3CN over 20 min at a flow rate 10 mL/min to yield compounds **4** (30 mg), **3** (0.45 g), **7** (1.1 mg), and **6** (1.98 mg), respectively. The fifth fraction was purified by semipreparative HPLC (column diam. 19×150 mm, 5 μm) using a linear gradient system of 40–70% aqueous CH_3CN over 20 min at a flow rate 10 mL/min to afford compounds **7** (3.4 mg) and **6** (8.7 mg), respectively.

4.4. Characteristics of the new compounds

*4.4.1. Compound **6**.* Brown solid; UV (MeOH) λ_{max} (log ε) nm: 226 (4.40), 244 (4.44), 288 (4.12), 340 (3.73); IR ($CHCl_3$) ν_{max} cm^{-1}: 3267 (br), 2934, 1633, 1614, 1503, 1456, 1412, 1320, 1302, 1250, 1146, 1047, 1022, 1002, 750; ^1H and ^{13}C NMR (DMSO-d_6, 500/125 MHz), see Table 1; HRESIMS *m/z* 503.1949 $[M+Na]^+$ (calcd for $C_{30}H_{28}N_2O_4Na$: 503.1941).

*4.4.2. Compound **7**.* Brown solid; UV (MeOH) λ_{max} (log ε) nm: 237 (4.34), 292 (4.07), 341 (3.70); IR ($CHCl_3$) ν_{max} cm^{-1}: 3384 (br), 1639, 1637, 1460, 1409, 1321, 1294, 1269, 1216, 1137, 1106, 1024, 1003, 808, 764; ^1H and ^{13}C NMR (DMSO-d_6, 500/125 MHz), see Table 1; HRESIMS : *m/z* 533.2042 $[M+Na]^+$ (calcd for $C_{31}H_{30}N_2O_5Na$: 533.2047).

*4.4.3. 3-Hydroxy-1,2-dimethyl-2,3-dihydro-1H-carbazol-4-one (**9**).* Brown gum, $[\alpha]_D^{23}$ −3.44 (*c* 0.1000, MeOH); UV (MeOH) λ_{max} (log ε) nm: 217 (3.95), 241 (3.94), 265 (3.84), 299 (3.75); IR (ATR) ν_{max} cm^{-1}: 3255 (br), 1630, 1585, 1474, 1454, 1401, 1385, 1090, 1035, 979, 894, 751; ^1H NMR (DMSO-d_6, 500 MHz): δ 1.00 (d, *J*=6.76 Hz, CH_3-2, 3H), 1.43 (d, *J*=7.17 Hz, CH_3-1, 3H), 2.15–2.18 (m, H-2, 1H), 3.03 (dq, *J*=5.13, 7.17 Hz, H-1, 1H), 4.18 (t, *J*=3.83 Hz, H-3, 1H), 5.20 (d, *J*=4.20 Hz, OH-3, 1H), 7.13 (td, *J*=7.38, 1.42 Hz, H-6, 1H), 7.15 (td, *J*=7.29 Hz, 1.50, H-7, 1H), 7.39 (dd, *J*=7.10, 1.42 Hz, H-8, 1H), 7.91 (dd, *J*=6.78, 1.50 Hz, H-5, 1H), 11.88 (s, H-9, 1H); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 14.7 (CH_3-2), 18.2 (CH_3-1), 33.7 (C-1), 43.0 (C-2), 73.8 (C-3), 109.1 (C-4a), 112.2 (C-8), 120.6 (C-5), 122.1 (C-7), 123.0 (C-6), 125.1 (C-4b), 137.3 (C-8a), 155.1 (C-9a), 193.1 (C-4); HRESIMS: *m/z* 252.0999 $[M+Na]^+$ (calcd for $C_{14}H_{15}NO_2Na$: 252.0995).

4.5. Biological tests

Antimalarial assay against *Plasmodium falciparum* (K1, multidrug resistant strain) was performed as described by Desjardins et. al.[10] Dihydroaretemisinin and mefloquine were used as standard references. Anti-TB against *Mycobacterium tuberculosis* strain H37Ra, and cytotoxicity against Vero cells (African green monkey kidney fibroblasts; ATCC CCL-81) assays were evaluated by the green fluorescent protein microplate assay (GFPMA).[11] Isoniazid, ofloxacin, rifampicin, streptomycin, and ethambutol were used as references for antituberculosis assay and ellipticine was used as a reference for the cytotoxicity test, respectively. Antifungal assay against *C. albicans* and cytotoxicity against KB (human oral epidermoid carcinoma, ATCC CCL-17), MCF-7 (human breast cancer, ATCC HTB-22), and NCI-H187 cells (human small-cell lung cancer, ATCC CRL-5804) were performed by using the resazurin microplate assay (REMA).[12] Ellipticine was used as a reference for cytotoxicity tests against KB and NCI-H187 and doxorubicin was used as a reference for cytotoxicity tests against KB, MCF-7, and NCI-H187. In addition, tamoxifen and amphotericin B were used references for cytotoxicity against MCF-7 and for antifungal tests, respectively.

Acknowledgements

Financial support from National Center for Genetic Engineering and Biotechnology (BIOTEC) is gratefully acknowledged.

Supplementary data

Supplementary data includes NMR data of compounds **1**–**9** obtained. Supplementary data related to this article can be found online at doi:10.1016/j.tet.2011.07.053.

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