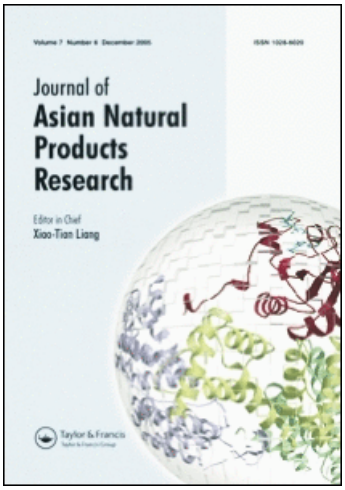


Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

Isolation and characterization of cytotoxic compounds from *Euphorbia cornigera* Boiss.

Imam Bakhsh Baloch[a]; Musa Kaleem Baloch[a]
[a] Department of Chemistry, Gomal University, Dera Ismail Khan, Pakistan

Online publication date: 08 November 2010

To cite this Article Baloch, Imam Bakhsh and Baloch, Musa Kaleem(2010) 'Isolation and characterization of cytotoxic compounds from *Euphorbia cornigera* Boiss.', Journal of Asian Natural Products Research, 12: 11, 985 — 991
To link to this Article: DOI: 10.1080/10286020.2010.512007
URL: http://dx.doi.org/10.1080/10286020.2010.512007

Journal of Asian Natural Products Research
Vol. 12, No. 11, November 2010, 985–991

Taylor & Francis
Taylor & Francis Group

Isolation and characterization of cytotoxic compounds from *Euphorbia cornigera* Boiss.

Imam Bakhsh Baloch and Musa Kaleem Baloch*

Department of Chemistry, Gomal University, Dera Ismail Khan 29050, Pakistan

(*Received 3 May 2010; final version received 27 July 2010*)

Methanolic extract of *Euphorbia cornigera* shoots was separated using HPLC, affording compounds **1**–**4**. Their structures and relative stereochemistry were established after obtaining their spectroscopic (IR, ^{1}H, ^{13}C NMR COSY-45°, HOHAHA, HSQC, HMBC, NOESY, and mass measurement) data. On the basis of these data, the compounds were characterized as 3-*O*-(2,3-dimethylbutanoyl)-13-*O*-dodecanoyl-20-*O*-tetradecanoylingenol (**1**), 3-*O*-decanoyl-20-*O*-hexanoylingenol (**2**), 3-*O*-(2,3-dimethylbutanoyl)-13-*O*-dodecanoyl-20-*O*-hexadecanoylingenol (**3**), and 13-*O*-dodecanoyl-20-*O*-hexanoylingenol (**4**); among these compounds, two (**1** and **2**) were new metabolites while the rest (**3** and **4**) were known. The MTT cytotoxicity assay was carried out using amrubicin hydrochloride as a positive control. Compound **1** displayed IC_{50} as 5.0 and 2.9 μM against RAW and HT-29 cell lines, respectively, which is 5- and 1.5-folds stronger than the control with IC_{50} values of 25 and 4.36 μM, respectively.

Keywords: *Euphorbia cornigera* Boiss.; Euphorbiaceae; cytotoxicity; ingenol

1. Introduction

Importance of plants is well recognized as the valuable source of novel anti-cancer and other drugs throughout medical history [1–5]. Most of the anti-cancer drugs such as vinblastine, vincristine, taxol, camptothecin, topotecan, irinotecan, podophyllotoxin, etoposide, and teniposide are derived from plants [1]. However, many tumors are showing resistance against available drugs [6,7] and hence these drugs have only limited anti-solid tumor activities [6,8,9]. Therefore, there is a need to discover new plant-derived anti-cancer drugs, which may have more potential than the present drugs or may show a different mechanism to cure tumors.

Fortunately, Euphorbiaceae family, which is one of the largest families of plants, comprising 300 genera and 7500 species, is commonly available in plain, mountainous, and desert areas of Pakistan [10,11]. These plants are popular among the local people for the treatment of various ailments including cancer [11–13]. The Euphorbiaceae family is also famous for its bio-diversity and enrichment in highly toxic, carcinogenic, co-carcinogenic, tumor-promoter to nontoxic, anti-tumor larvicidal, and molluscacidal compounds [12–14]. These aspects prompted us to investigate indigenous medicinal plants of Pakistan. In this paper, two new (**1** and **2**) and two known (**3** and **4**) cytotoxic compounds from *Euphorbia cornigera* are presented.

2. Results and discussion

The MeOH extract of *E. cornigera* shoots after dispersing in hot water was further

*Corresponding author. Email: musakaleem2001@yahoo.com

ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020.2010.512007
http://www.informaworld.com

extracted with Et_2O, which showed promising cytotoxicity. The extract was subjected to silica gel column chromatography to obtain semi-pure active material. After separation on HPLC, the active portion afforded compounds **1**–**4** (Figure 1).

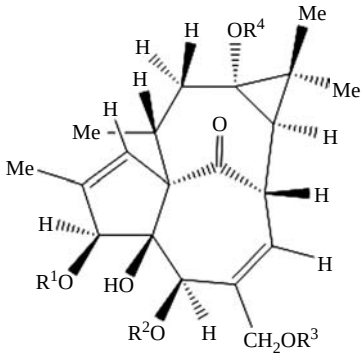
The active fraction of the ether extract (compounds **1**–**4**) was hydrolyzed with 0.5 M HCl and was separated through HPLC. The product was identified through GC-MS and (^{1}H, ^{13}C) NMR spectrometry (Table 1) and identified as dodecanoic, 2,3-dimethylbutanoic, decanoic, hexanoic, tetradecanoic, and hexadecanoic acid.

Compound **1** was obtained as a pale yellow oil displaying a molecular ion $[M]^+$ peak at *m/z* 854.6276 in HR-EI-MS, corresponding to the molecular formula $C_{52}H_{86}O_9$, suggesting 10 degrees of unsaturation in the molecule. In EI-MS, peaks at *m/z* 682 $[M - 172]^+$, 738 $[M - 116]^+$ and *m/z* 626 $[M - 228]^+$ suggested it as a tri-ester of dodecanoic, 2,3-dimethybutanoic, and tetradecanoic acid. The observed IR spectrum of **1** was assigned as 3534 (OH), 1767, 1754, 1745 (ester), 1704 (ketone), 1652 (C═C), and 1146 (C—O) cm^{-1}. The functional group moieties were further confirmed by ^{13}C NMR (DEPT) spectral data (Table 2). The singlets appearing at δ 205.2 and 172–174 were attributed to the carbonyls of ketone and of ester, respectively. Four peaks observed in the middle region of the NMR spectrum at δ 128.4, 131.7, 136.3, and 137.2 were due to two C═C (trisubstituted) bonds in the skeleton. In the same spectrum, peaks in the region at δ 63–82 were due to the *gem*-hydroxy–acyloxy groups. In addition, four Me groups appeared at δ 22.6, 16.7, 15.5, and 18.3. The chemical shift, multiplicity, and coupling constant values in the ^{1}H NMR spectrum revealed that two Me groups are tertiary (δ 1.07 s, 1.21 s), one secondary (δ 0.96 d, $J = 7.5$ Hz), and one vinylic (δ 1.86 d, $J = 1.2$ Hz) in nature. In the HSQC spectrum, two Me singlets (δ 1.07, 1.21 s) showing correlations with carbons at δ 22.6, 16.7 were assigned to Me-16, 17, respectively; the third Me protons (δ 1.86) showed correlation with the carbon at δ 15.5 (Me-19), and the fourth Me protons (δ 0.96) with Me-18 resonated at δ 18.2. In the HMBC spectrum, Me-16, 17 showed correlations with C-15 at δ 30.5, indicating a *gem*-dimethyl moiety at C-15. Me-19 protons (δ 1.86) showed correlation with C-2 (δ 136.3), and Me-18 (δ 0.96) showed correlation with secondary C-11 resonated at δ 37.7.

The COSY-45° spectrum revealed two spin systems (i) CH^7—CH^8—CH^{14} and (ii) CH_2^{12}—CH^{11}—CH_3^{18} in the molecule, indicating 13-hydroxyingenol as a parent system (Figure 2) [14–16].

NMR (^{1}H, ^{13}C) spectra of **1** showed peaks for H-3 (δ 5.45), H_2-20 (δ 4.42, 4.73), C-3 (δ 80.2), and C-20 (δ 66.4), indicating ester moieties at these sites.

In the HMBC spectrum of **1**, H-3 showed correlation with the carbonyl carbon (δ 177.4) of 2,3-dimethylbutanoyl (Bu) (Figure 2) and the NOE experiment showed the interaction between H-3 and H-



Compound	R^1	R^2	R^3	R^4
1	Bu	H	Tet	Dod
2	Dec	H	Hex	H
3	Bu	H	Hexd	Dod
4	H	H	Hex	Dod

Figure 1. Structures of compounds **1**–**4**. Bu, 2,3-di-Me-butanoyl; Dec, decanoyl; Dod, dodecanoyl; Hex, hexanoyl; Hexd, hexadecanolyl; Tet, tetradecanoyl.

Table 1. ^{1}H (300 MHz) and ^{13}C NMR spectral data of acid moieties for compounds **1** and **2** in $CDCl_3$ (δ, ppm, J in Hz).

	Tetradecanoate		Dodecanoate		Decanoate		Hexanoate		2,3-Dimethylbutanoate	
Acid no.	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		174.3 s		174.3 s		173.3 s		172.3 s		176.3 s
2	2.30 t (6.2)	34.4 t	2.31 t (6.2)	34.4 t	2.32 t (6.2)	34.4 t	2.21 t (6.2)	36.3 t	2.19 m	46.2 d
3	1.25 br s	25.0 t	1.25 br s	25.0 t	1.25 br s	25.0 t	1.63 br s	16.3 t	1.83 m	30.0 d
4	1.24 br s	29.7 t	1.25 br s	29.7 t	1.24 br s	29.7 t	1.24 br s	16.2 t	0.85 d (7.2)	20.7 q
5	1.23 br s	29.7 t	1.25 br s	29.7 t	1.23 br s	29.7 t	1.23 br s	16.1 t	1.04 d (7.2)	13.9 q
6	1.23 br s	29.7 t	1.25 br s	29.7 t	1.23 br s	29.7 t	0.96 t (7.2)	13.7 t	0.84 d (7.3)	18.7 q
7	1.22 br s	29.7 t	1.25 br s	29.7 t	1.22 br s	29.7 t				
8	1.24 br s	29.7 t	1.25 br s	29.7 t	1.24 br s	29.7 t				
9	1.24 br s	29.7 t	1.25 br s	29.7 t	1.23 br s	22.7 t				
10	1.25 br s	29.7 t	1.25 br s	29.7 t	0.88 t (7.5)	14.2 q				
11	1.25 br s	29.7 t	1.23 br s	22.7 t						
12	1.25 br s	29.4 t	0.88 t (7.3)	14.2 q						
13	1.23 br s	22.7 t								
14	0.88 t (7.2)	14.2 q								

Table 2. ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectral data of compounds **1** and **2** in $CDCl_3$ (δ, ppm, J in Hz).

	1		**2**	
No.	δ_H	δ_C	δ_H	δ_C
1	6.01 q (1.2)	131.7 d	6.02 q (1.2)	131.8 d
2	–	136.3 s	–	136.5 s
3	5.43 s	82.8 d	3.87 s	80.7 d
4	–	74.7 s	–	74.6 s
5	3.83 br s	75.0 d	3.86 br s	74.9 d
6	–	137.2 s	–	136.9 s
7	5.63 d (4.6)	128.4 d	5.61 d (4.6)	128.2 d
8	4.14 dd (4.6, 12.5)	42.8 d	4.12 dd (4.6, 12.5)	42.6 d
9		205.2 s		205.5 s
10		72.0 s		72.1 s
11	2.45 ddq (4.5, 3.5, 7.5)	37.7 d	2.44 ddq (4.5, 3.5, 7.5)	37.8 d
12 α	2.83 ddd (15.5, 4.5, 3.5)	35.2 t	2.81 ddd (15.5, 4.2, 3.5)	35.1 t
12 β	2.37 dd (15.5, 4.5)		2.35 dd (15.5, 4.5)	
13	–	69.2 s	–	69.1 s
14	1.64 d (12.4)	28.4 d	1.65 d (12.4)	28.5 d
15	–	30.4 s	–	30.3 s
16	1.21 s	22.6 q	1.23 s	22.5 q
17	1.07 s	16.7 q	1.07 s	16.6 q
18	0.96 d (7.2)	18.3 q	0.97 d (7.2)	18.1 q
19	1.86 d (1.2)	15.5 q	1.87 d (1.2)	15.4 q
20 α	4.421 d (12.5)	66.4 t	4.43 d (12.5)	66.2 t
20 β	4.72 d (12.5)		4.75 d (12.5)	

2 (acyl part) concluding the attachment of 2,3-dimethylbutanoate at C-3. In the same spectrum, CH_2-20 showed correlation with the carbonyl carbon of the dodecanoyl (Dod) moiety and the same was proved by NOE. The third ester moiety, which did not display any interaction, was located at C-13, since the corresponding carbon (C-13) resonated in the low field at δ 69.2 in the ^{13}C NMR spectrum.

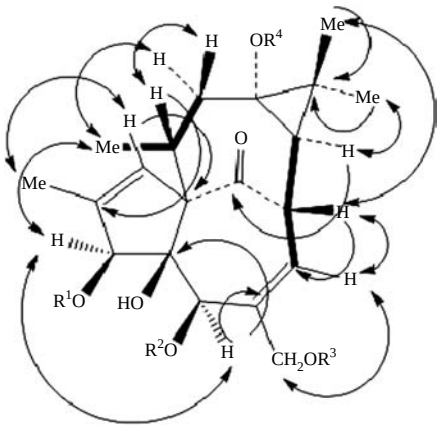


Figure 2. Key COSY and HOHAHA 45° (bold bonds), HMBC (↷) and NOESY (↶) correlations of compounds **1**–**4**.

The ^{13}C NMR (BBD) spectrum of **1** showed 52 carbon atoms and DEPT spectra afforded 9 Me, 24 CH_2 and 9 CH, and 10 C. In the light of the spectral evidences, the structure of **1** was elucidated as 3-*O*-(2,3-dimethylbutanoyl)-13-*O*-tetradecanoyl-20-*O*-dodecanoylingenol.

The relative stereochemistry of **1** was established with the help of the NOESY spectrum displayed in Figure 2.

Compound **2** was obtained as a colorless oil. IR and UV spectral data were identical to those of **1**. Compound **2** displayed a molecular ion peak [M^+] at *m/z* 616.3977 in HR-EI-MS corresponding to the molecular formula $C_{36}H_{56}O_8$. From the EI-MS fragmentation, peaks at *m/z* 516 [M − 116]$^+$,

498, and 444 $[M - 172]^+$ indicated **2** as a diester of decanoic and hexanoic acid. In the NMR spectrum of **2**, H_2-20 resonated as an AB doublet at δ 4.43, 4.75 ($J = 12.5$ Hz), indicating the presence of one of the ester moiety at C-20. The HMBC spectrum showed the correlation of the carbonyl carbon of the hexanoyl moiety with H_2-20, suggested the attachment of the hexanoyl moiety at C-20, and the decanoyl carbonyl carbon displayed no interactions with any proton and hence placed at C-13. On the basis of the obtained results, **2** was named as 13-*O*-decanoyl-20-*O*-hexanoylingenol. The relative stereochemistry of all the stereogenic centers was established with the analysis of the NOE and NOESY spectra and found to be similar to that of **1** (Figure 2).

After comparing the available physical and spectral data of **3** and **4** with those in the literature [14,15], their structures were established as 3-*O*-(2,3-dimethylbutanoyl)-13-*O*-dodecanoyl-20-*O*-hexadecanoylingenol (**3**) and 13-*O*-dodecanoyl-20-*O*-hexanoylingenol (**4**). The relative stereochemistry of compounds **3** and **4** was also confirmed by recording the NOESY spectra and the measurement of NOE interaction. It was concluded that compounds **3** and **4** have the same stereochemistry as ingenols except for the presence of the ester moiety at C-13, having α-orientation.

In vitro cytotoxicity of isolates **1**–**4** was evaluated against RAW (mouse macrophage cells) and HT-29 (colon cancer cell lines) using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay method. Compound **1** exhibited significant cytotoxicity with IC_{50} 5.0 (RAW) and 2.90 (HT-29) μM, concluding that **1** was 5- and 1.5-folds more potent against RAW and HT-29 cancer cell lines than standard amrubicin hydrochloride. Compound **2** showed moderate cytotoxic activity against both RAW and HT-29 cell lines (Table 3). The cytotoxicity of compound **4** against RAW cell lines was more potent than that of compound **2**. However, compound **3** showed no prominent activity against any cancer lines tested here.

Table 3. *In vitro* cytotoxicity (IC_{50}, μM) of compounds **1**–**4** against RAW and HT-29 cell lines.

	Compounds				
Cell	**1**	**2**	**3**	**4**	Standard
RAW	5.0	10.0	19.7	9.7	25.2
HT-29	2.9	10.0	15.4	15.4	4.4

3. Experimental

3.1 General experimental procedures

Optical rotation was measured on a digital polarimeter supplied by OSK OGAWA Seiki Co. Ltd, Tokyo, Japan. IR spectra were recorded in nujol mull ν_{max} in cm^{-1} on a TENSOR 27 FT-IR spectrophotometer supplied by Bruker, Switzerland. ^1H and ^{13}C NMR (300 and 75 MHz) spectra were obtained in $CDCl_3$ at room temperature, with TMS as an internal standard using a Bruker Biospin-AMX 300-MHz FT NMR spectrometer (Bruker); in δ, ppm, coupling constant J in Hz. Mass measurements were made on a double-focusing Finnegan MAT 112 spectrometer (Bremen, Germany) and recorded in *m/z* (relative intensity, %). HR-EI-MS measurements were obtained on a JEOL HX 110 spectrometer (JEOL, Tokyo, Japan).

3.2 Plant material

The *E. cornigera* Boiss. shoots were collected from Murree Hills, Pakistan, during the flowering season. The sample was identified by Prof. Dr Qazi Najm-u-Saqib, Ex-Dean Faculty of Pharmacy, GU, DIKhan and authenticated by comparing with samples present in the Herbarium of Botany Department, Peshawar University, Pakistan. A voucher specimen (DG/EP/06/113) has been deposited in the herbarium.

3.3 Extraction and isolation

The air-dried powdered shoots (3400 g) were extracted thrice with MeOH (7 liters) at room temperature, to give a brown residue (54.3 g). The material was suspended in MeOH:H_2O (6:1), and extracted successively with *n*-C_6H_{14} (3 × 500 ml), $CHCl_3$ (3 × 500 ml), Et_2O (3 × 500 ml), EtOAc (3 × 500 ml), and *n*-BuOH (3 × 50 ml) to give corresponding 11.4, 5.3, 3.4, 2.3, and 1.2 g soluble materials, respectively. The Et_2O soluble material (3.3 g) was subjected to column chromatography over silica gel and eluted with the mixture of CH_2Cl_2:EtOAc:MeOH (9:1:0 → 0:1:9), to obtain nine crude fractions (**A**–**I**). All the fractions were tested against the cancer cell lines (RAW and HT-29), and only fractions **D** (137.2 mg) and **E** (134.3 mg) showed promising activities, and TLC results in CH_2Cl_2:EtOAc:MeOH (6:3:1) which displayed more than two components.

The active fractions were purified on preparative HPLC (Perkin-Elmer, Washington D.C., USA), HPLC RP-18 column (250 × 2.5 cm), after elution with a mixture of MeCN/H_2O, gradient scheme (**A** = H_2O; **B** = MeCN/H_2O 88/12; 5 min **A**, linear gradient to **B** in 20 min, 5 min **B**, linear gradient back to **A** in 2 min at 2 ml/min flow rate), as a mobile phase) to afford pure compounds **1** (23.3 mg), **2** (14 mg), **3** (14.4 mg), and **4** (79.1 mg) with retention times 7.3, 8.2, 9.2, and 10.7 min, respectively.

3.3.1 3-O-(2,3-Dimethylbutanoyl)-13-O-dodecanoyl-20-O-tetradecanoylingenol (*1*)

Pale yellow oil; $[\alpha]_D^{25}$ −26.8 ($c = 0.17$, $CHCl_3$). IR ν_{max} (dry): 3534, 1767, 1754, 1745, 1724, 1652, 1146 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$) spectral data are listed in Table 1. EI-MS *m/z*: 854 $[M, 3]^+$, 826 $[M - 28, 13]^+$, 836 $[M - 18, 13]^+$, 808 $[M - 28 - 18, 23]^+$, 738 $[M - 116, 12]^+$, 654 $[M - 200, 28]^+$, 626 $[M - 228, 22]^+$, 538 $[M - 116 - 200, 23]^+$, 510 $[M - 116 - 228, 32]^+$, 310 $[M - 116 - 200 - 228, 72]^+$, 292 (40), 187 (57), 160 (75), 151 (82), 133 (53), 123 (54), 83 (100). HR-EI-MS *m/z:* 854.6276 $[M]^+$ (calcd for $C_{52}H_{86}O_9$, 854.6272).

3.3.2 13-O-Decanoyl-20-O-hexanoylingenol (*2*)

Colorless oil, $[\alpha]_D^{25}$ −33.7 ($c = 0.17$, $CHCl_3$). IR (dry) ν_{max}: 3503, 1754, 1735, 1625, 1552 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$) spectral data are listed in Table 1. EI-MS *m/z* (rel. int.): 616 $[M, 3]^+$, 598 $[M - 18, 13]^+$, 588 $[M - 28, 13]^+$, 570 $[M - 18 - 28, 17]^+$, 500 $[M - 116, 27]^+$, 482 $[M - 116 - 18, 23]^+$, 444 $[M - 172, 29]^+$, 426 $[M - 172 - 18, 31]^+$, 312 (73), 294 (69), 284 (77), 251 (53), 221 (57), 188 (59), 162 (45), 153 (77), 151 (73), 135 (83), 122 (84), 121 (52), 83 (100). HR-EI-MS *m/z:* 616.3977 $[M]^+$ (calcd for $C_{36}H_{56}O_8$, 616.3975).

3.4 Cytotoxicity assay

The cytotoxicity assay of samples **1**–**4** was evaluated on RAW and HT-29 cell lines, using the MTT assay as described previously [9,10]. The amrubicin hydrochloride salt was used as a control and all the measurements were taken in triplicate. Results in this connection are displayed in Table 3.

References

[1] M. Shoeb, *Bangladesh J. Pharmacol.* **1**, 35 (Minireview), and references cited therein (2006).
[2] I.B. Baloch, M.K. Baloch, and Q.N. Saqib, *Planta Med.* **72**, 830 (2006).
[3] I.B. Baloch, M.K. Baloch, and Q.N. Saqib, *Eur. J. Med. Chem.* **43**, 274 (2008).
[4] Y.B. Wang, R. Huang, H.B. Wang, H.Z. Jin, L.G. Lou, and G.W. Qin, *J. Nat. Prod.* **69**, 967 (2006).

[5] L. Su, S.G. Feng, L. Qiao, Y.Z. Zhou, R.P. Yang, and Y.H. Pei, *J. Asian Nat. Prod. Res.* **11**, 38 (2009).
[6] J.W. Yarbro, The scientific basis of cancer chemotherapy, in *The Chemotherapy Source Book*, edited by M.C. Perry (Williams & Wilkins, Baltimore, MD, 1992), p. 2.
[7] B.A. Chabner, Anti-cancer drugs, in *Cancer: Principles and Practice*, edited by V.T. DeVita Jr., S. Hellman, A.S. Rosenberg, 4th ed. (Lippincott, Philadelphia, MD, 1991), Chap. 18, p. 325.
[8] J.M. Jessup, L.S. McGinnis, D.P. Winchester, H. Eyre, A. Fremgen, G.P. Murphy, and H.R. Menck, *CA Cancer J. Clin.* **46**, 185 (1996).
[9] I.B. Baloch, M.K. Baloch, and Q.N. Saqib, *Helv. Chim. Acta* **88**, 3145 (2005).
[10] I.B. Baloch, M.K. Baloch, Q.N. Saqib, and M. Ahmed, *Chem. Pharm. Med. J. Int.* **2**, 107 (2004).
[11] I.B. Baloch, and M.K. Baloch, *J. Asian Nat. Prod. Res.* **12**, 600 (2010).
[12] I.B. Baloch, M.K. Baloch, and A.K. Baloch, *Planta Med.* **76**, 809 (2010).
[13] I.B. Baloch, M.K. Baloch, and A.K. Baloch, *Eur. J. Med. Chem.* **44**, 3188 (2009).
[14] D. Uemura and Y. Hirata, *Tetrahedron Lett.* **29**, 2529 (1974).
[15] T.T. Wu, Y.M. Lin, M. Haruna, D.J. Pan, and T. Shingu, *J. Nat. Prod.* **54**, 823 (1991).
[16] Z.Q. Lu, M. Yang, J.Q. Zhang, G.T. Chen, H.L. Huang, S.H. Guan, C. Ma, X. Liu, and D.A. Guo, *Phytochemistry* **69**, 812 (2008).