Bioorganic & Medicinal Chemistry 19 (2011) 4928–4934

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Semisynthetic analogues of the marine cembranoid sarcophine as prostate and breast cancer migration inhibitors

Hossam M. Hassan [a,b], Asmaa A. Sallam [a], Rabab Mohammed [b], Mohamed S. Hifnawy [c], Diaa T. A. Youssef [d], Khalid A. El Sayed [a,*]

[a] Department of Basic Pharmaceutical Sciences, College of Pharmacy, University of Louisiana at Monroe, Monroe, LA 71201, USA
[b] Department of Pharmacognosy, College of Pharmacy, Beni-Suief University, Egypt
[c] Department of Pharmacognosy, College of Pharmacy, Nahda University, Egypt
[d] Department of Natural Products and Alternative Medicine, College of Pharmacy, King Abdulaziz University, Jeddah 21589, Saudi Arabia

ARTICLE INFO

Article history:
Received 26 April 2011
Revised 15 June 2011
Accepted 21 June 2011
Available online 28 June 2011

Keywords:
Antimigratory
Breast cancer
Carbamoylation
Cembranoids
Etherification
Prostate cancer
Semisynthesis
Wound-healing

ABSTRACT

Sarcophine (**1**) is a bioactive cembranoid diterpene isolated from the Red Sea soft coral *Sarcophyton glaucum*. Previous semisynthesis attempts resulted in decreased or complete loss of **1**'s anticancer activity. Sarcophine and analogues showed antimigratory activity against breast and prostate cancer cell lines. This encouraged further semisynthetic optimizations to improve its activity and establish a preliminary structure–activity relationship. Eight new and five known semisynthetic analogues were generated. These compounds were evaluated for their ability to inhibit growth, proliferation, and migration of the prostate and breast metastatic cancer cell lines PC-3 and MDA-MB-231, respectively. Most analogues exhibited enhanced antimigratory activity.

© 2011 Elsevier Ltd. All rights reserved.

1. Introduction

Cembranoids are natural diterpenes possessing 14-membered macrocyclic rings substituted by an isopropyl residue at C-1 and by three symmetrically disposed methyl groups at positions C-4, C-8, and C-12.[1] They exhibit a wide range of biological activities including neuroprotective, antimicrobial, and antitumor properties.[1,2] Cembranoids are common secondary metabolites within both the plant and animal kingdoms.[1,2] Examples of plant-derived cembranoids are those isolated from tobacco, pine oleoresin, olibanum (frankincense, *Boswellia carteri* and *Boswellia sacra*), and *Cleome* species.[1,2] The majority of natural cembranoids were isolated from marine invertebrates including soft corals, octacorals, and gorgonians.[1,2]

Sarcophine (**1**) is a bioactive cembranoid first isolated from the Red Sea soft coral *Sarcophyton glaucum* by Kashman group in 1974 in up to 3% wet weight yield.[3,4] In 1998, sarcophine and its metabolite 7α-hydroxy-$\Delta^{8,(19)}$-deepoxysarcophine effectively suppressed TPA-induced anchorage-independent JB6 cell transformation at dose range of 0.4–10 μM.[5] This report pointed the critical importance of functional groups at carbons C-7/C-8 for the anticancer activity. The antiproliferative effect of sarcophine was attributed to its ability to increase the expression of cyclin dependant kinase (CDK) inhibitor, p21, and to enhance the residual DNA repair without affecting the DNA adduct formation in MCF-7 cells.[6] Allylic oxidation of sarcophine and its $LiAlH_4$-reduced products showed enhanced anticancer activity, especially those containing 1,2-propanediol at C-15/C-16, suggesting that opening of the sarcophine lactone greatly enhances the activity.[7] This product, named sarcophine-diol, decreased cell growth and induced apoptosis through caspase-dependent extrinsic pathway in A431 cells, which justified its overall chemopreventive effects in mouse skin cancer models.[8,9]

Sarcophine, 16-deoxysarcophine, and 2-*epi*-16-deoxysarcophine (**1**–**3**) showed the most potent antimigratory activity out of several other tested cembranoids against the highly metastatic mouse melanoma B16B15b cell line.[10] Oxymercuration–demercuration of **1** produced rearranged and hydroxylated products that displayed no antiproliferative effects, confirming the importance of the macrocyclic double bonds for the activity.[11] Bromination of sarcophine improved the antiproliferative activity against the highly malignant +SA mammary epithelial cells.[11] The oxidation product of **1**, (+)-sarcophytoxin B, showed antiproliferative activity

* Corresponding author. Tel.: +1 318 342 1725; fax: +1 318 342 1737.
E-mail address: elsayed@ulm.edu (K.A. El Sayed).

0968-0896/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2011.06.060

equivalent to **1**, suggesting that C-7/C-8 epoxide is not required for activity.[11] Attempts to insert sulfur functionalities into sarcophine afforded the *cis*-oxathiolan-2-imine and the cyclic oxathiophospholane-2-sulfide analogues, which inhibited +SA cells' growth better than **1**.[12] These analogues induced cell cycle arrest in G0/G1 phase with concomitant decrease in the cell populations at S and G2+M phases in the estrogen receptor alpha positive MCF-7 cells but had no effect on the estrogen-receptor negative MDA-MB-231 breast cancer cells.[6] These studies clearly highlighted the importance of further optimization of C-7/C-8 epoxide functionality of **1**. Therefore, etherification and carbamoylation reactions at C-7/C-8 of **1** were conducted to study the effect of using diverse side chains at these positions on the antimigratory activity.

2. Results and discussion

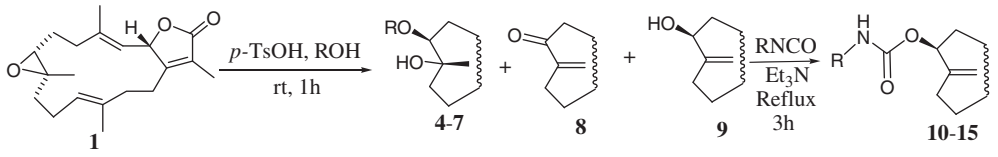
2.1. Semisynthesis of sarcophine analogues

Reaction of methanol, *n*-propanol, or *n*-butanol and *p*-toluenesulfonic acid with sarcophine (**1**) afforded the corresponding C-7-ethers **4**–**6**, along with the known 7β,8α-dihydroxy-deepoxysarcophine (**7**),[5,13] 7-keto-$\Delta^{8(19)}$-deepoxysarcophine (**8**),[11] and 7β-hydroxy-$\Delta^{8(19)}$-deepoxysarcophine (**9**) (Scheme 1).[5,13] Compound **9** was prepared in high-yield using the previously reported etherification method using *n*-butanol.[13] Compound **9** was the C-7 epimer of the sarcophine's bioconversion product, which was the most active in suppressing the TPA-induced anchorage-independent JB6 cell transformation.[5] The stereochemistry of **9** was based on the fact that the epoxide opening in acidic condition should retain the original configuration of the original epoxide.[5,13] Compound **9** occurred as oil and had identical physical, chemical, and NMR properties of the same compound reported by Czarkie, et al. 1985.[13] This compound was selected as a scaffold to prepare diverse aliphatic, olefinic, and aromatic C-7-carbamates **10**–**15** by reaction with their corresponding isocyanates (Scheme 1). The C-7 ether and carbamate functionalities offer diverse hydrogen bonding donor (HBD), acceptor (HBA) and π-aromatic groups at this critical position with diverse functionalized side chain to optimize their binding affinities toward their plausible molecular target.

The HRTOFMS data of **5** suggested the molecular formula $C_{23}H_{36}O_4$ and possible C-7 propyl ether formation. The ^1H and ^{13}C NMR data (Tables 1 and 3) further supported this conclusion. The NMR data also showed the characteristic signals for the propyl ether moiety. The β-orientation of C-7 hydroxy was based on the coupling constant, splitting and chemical shift similarity of H-7 with that of the previously related methyl ether.[13] Thus, **5** was determined to be 7β-propyloxy-8α-hydroxy-deepoxysarcophine. Similarly, compound **6** was determined to be 7β-butyloxy-8α-hydroxy-deepoxysarcophine.

The HRTOFMS data of **10** and **11** suggested possible butylcarbamoylation of C-7. The ^1H and ^{13}C NMR data (Tables 1 and 3) further supported this fact by showing signals for butylcarbamate and pentylcarbamate moieties, respectively, and the downfield shift of C-7/H-7 compared to their parent **9**. Thus, compounds **10** and **11** were determined to be 7β-butylcarbamoyl-$\Delta^{8(19)}$-deepoxysarcophine and 7β-pentylcarbamoyl-$\Delta^{8(19)}$-deepoxysarcophine, respectively.

The HRTOFMS of **12** suggested the molecular formula $C_{24}H_{34}NO_4Cl$ and showed the M and M+2, 3:1, isotopic cluster characteristic pattern for a monochlorinated compound. The ^1H, and ^{13}C NMR data of **12** (Tables 2 and 3) confirmed the attachment of a chloropropylcarbomyl moiety to C-7. Thus, compound **12** was determined to be 7β-chloropropylcarbamoyl-$\Delta^{8(19)}$-deepoxysarcophine. Similarly, compound **13** was determined to be 7β-cyclohexylcarbamoyl-$\Delta^{8(19)}$-deepoxysarcophine.



Scheme 1. Semisynthesis of sarcophine ether and carbamate analogues.

Table 1
^1H NMR data of compounds **5**, **6**, **10**, **11**[a]

Position	δ_H (*J* in Hz)			
	5	**6**	**10**	**11**
2	5.56, dq (10.2, 1.8)	5.56, dq (10.2, 1.8)	5.60, dq (10.2, 2.2)	5.60, dq (10.2, 2.2)
3	4.94, dq (10.3, 1.1)	4.94, dq (10.3, 1.1)	5.00, d (10.3)	5.00, d (10.3)
5	2.40, ddd (13.8, 13.1, 3.3); 2.16, m	2.40, ddd (13.8, 13.1, 3.3); 2.16, m	2.44, ddd (13.8, 12.8, 3.8); 2.16, m	2.44, ddd (13.8, 12.8, 3.8); 2.16, m
6	2.09, m; 1.58, m	2.09, m; 1.58, m	2.06, m; 1.41, m	2.06, m; 1.41, m
7	3.41, dd (9.5, 9.5)	3.41, dd (9.5, 9.5)	4.64, d, (10.2)	4.64, d, (10.2)
9	2.35, m; 1.95, m	2.35, m; 1.95, m	2.34, m; 2.02, m	2.34, m; 2.02, m
10	2.28, m; 2.25, m	2.28, m; 2.25, m	2.23, m; 2.18, m	2.23, m; 2.18, m
11	5.07, dd (6.6, 6.6)	5.07, dd (6.6, 6.6)	5.10, dd (7.0, 7.0)	5.10, dd (7.0, 7.0)
13	2.13, m; 2.08, m	2.13, m; 2.08, m	2.19, m; 2.12, m	2.19, m; 2.12, m
14	2.56, m; 2.20, m	2.56, m; 2.20, m	2.71, m; 1.91, m	2.71, m; 1.91, m
17	1.82, s	1.82, s	1.83, s	1.83, s
18	1.86, s	1.86, s	1.88, s	1.88, s
19	1.10, s	1.10, s	5.92, br s; 4.87, br s	5.92, br s; 4.87, br s
20	1.63, s	1.63, s	1.61, s	1.61, s
1′	3.26, t (6.2)	3.30, t (6.2)		
2′	1.47, m	1.48, m	3.23, m	3.12, m, 3.25, m
3′	0.90, t (7.7)	1.35, m	1.80, m	1.28, m
4′		0.90, t (7.4)	1.31, m	1.44, m
5′			0.88, t (7.3)	1.60, m
6′				0.87, t (6.9)

[a] In $CDCl_3$, 400 MHz. Coupling constants (*J*) are in Hz.

Table 2
^{1}H NMR data of compounds **12**–**15**[a]

Position	δ_H (J in Hz)			
	12	**13**	**14**	**15**
2	5.60, dq (10.2, 2.2)	5.60, dq (10.2, 2.2)	5.60, dq (10.2, 2.2)	5.60, dq (10.2, 2.2)
3	5.00, d (10.3)	5.00, d (10.3)	5.00, d (10.3)	5.00, d (10.3)
5	2.44, ddd (13.8, 12.8, 3.8); 2.16, m	2.44, ddd (13.8, 12.8, 3.8); 2.16, m	2.44, ddd (13.8, 12.8, 3.8); 2.16, m	2.44, ddd (13.8, 12.8, 3.8); 2.16, m
6	2.06, m; 1.41, m	2.06, m; 1.41, m	2.06, m; 1.41, m	2.06, m; 1.41, m
7	4.64, d (10.2)	4.54, d (10.2)	4.54, d (10.2)	4.54, d (10.2)
9	2.34, m; 2.02, m	2.34, m; 2.02, m	2.34, m; 2.02, m	2.34, m; 2.02, m
10	2.23, m; 2.18, m	2.23, m; 2.18, m	2.23, m; 2.18, m	2.23, m; 2.18, m
11	5.10, dd (7.0, 7.0)	5.10, dd (7.0, 7.0)	5.10, dd (7.0, 7.0)	5.10, dd (7.0, 7.0)
13	2.19, m; 2.12, m	2.19, m; 2.12, m	2.19, m; 2.12, m	2.19, m; 2.12, m
14	2.71, m; 1.91, m	2.71, m; 1.91, m	2.71, m; 1.91, m	2.71, m; 1.91, m
17	1.83, s	1.83, s	1.83, s	1.83, s
18	1.88, s	1.88, s	1.88, s	1.88, s
19	5.92, br s; 4.87, br s	5.92, br s; 4.87, br s	5.92, br s; 4.87, br s	5.92, br s; 4.87, br s
20	1.61, s	1.61, s	1.61, s	1.61, s
2′	3.56, t (3.7)	3.40, m	4.41, 2H, ddd (5.2, 1.5, 1.5)	
3′	1.97, m	1.64, m; 1.39 m	5.94, m	7.04, dd (7.3, 1.8)
4′	3.3, t (6.2)	1.21, m; 1.11 m	5.21, m; 5.29, dd (1.8, 1.8)	7.13, m
5′		1.33, m		7.12, m
6′		1.21, m; 1.11, m	3.87, ddd (5.5, 1.5, 1.5)	7.13, m
7′		1.64, m; 1.39, m	5.87, m	7.04, dd (7.3, 1.8)
8′			5.12, m, 5.20, m	
9′				
10′			3.88, ddd (5.5, 1.5, 1.5)	
11′			5.84, m	
12′			5.11, m; 5.20, m	

[a] In $CDCl_3$, 400 MHz. Coupling constants (J) are in Hz.

Table 3
^{13}C NMR Data of Compounds **5**, **6**, and **10**–**15**[a]

Position	δ_C							
	5	**6**	**10**	**11**	**12**	**13**	**14**	**15**
1	163.0, qC	163.0, qC	162.8, qC	162.8, qC	162.8, qC	162.8, qC	162.8, qC	162.8, qC
2	79.2, CH	79.2, CH	79.0, CH	79.0, CH	79.0, CH	79.0, CH	79.0, CH	79.0, CH
3	121.0, CH	121.0, CH	121.5, CH	121.5, CH	121.5, CH	121.5, CH	121.5, CH	121.5, CH
4	144.3, qC	144.3, qC	1437, qC	1437, qC	1437, qC	1437, qC	1437, qC	1437, qC
5	32.7, CH_2	32.7, CH_2	36.1, CH_2	36.1, CH_2	36.1, CH_2	36.1, CH_2	36.1, CH_2	36.1, CH_2
6	26.9, CH_2	26.9, CH_2	32.2, CH_2	32.2, CH_2	32.2, CH_2	32.2, CH_2	32.2, CH_2	32.2, CH_2
7	72.8, CH	72.9, CH	72.9, CH	72.9, CH	72.9, CH	72.9, CH	72.9, CH	72.9, CH
8	78.9, qC	78.9, qC	154.6, qC	154.6, qC	154.6, qC	154.6, qC	154.6, qC	154.6, qC
9	36.5, CH_2	36.5, CH_2	33.9, CH_2	33.9, CH_2	33.9, CH_2	33.9, CH_2	33.9, CH_2	33.9, CH_2
10	19.6, CH_2	19.6, CH_2	30.0, CH_2	30.0, CH_2	30.0, CH_2	30.0, CH_2	30.0, CH_2	30.0, CH_2
11	125.6, CH	125.6, CH	125.9, CH	125.9, CH_2	125.9, CH	125.9, CH	125.9, CH	125.9, CH
12	134.2, qC	134.2, qC	135.1, qC	135.1, qC	135.1, qC	135.1, qC	135.1, qC	135.1, qC
13	35.6, CH_2	35.6, CH_2	36.9, CH_2	36.9, CH_2	36.9, CH_2	36.9, CH_2	36.9, CH_2	36.9, CH_2
14	26.6, CH_2	26.6, CH_2	26.4, CH_2	26.4, CH_2	26.4, CH_2	26.4, CH_2	26.4, CH_2	26.4, CH_2
15	122.8, qC	122.8, qC	123.0, qC	123.0, qC	123.0, qC	123.0, qC	123.0, qC	123.0, qC
16	174.9, qC	174.9, qC	174.8, qC	174.8, qC	174.8, qC	174.8, qC	174.8, qC	174.8, qC
17	9.0, CH_3	9.1, CH_3	9.0, CH_3	9.0, CH_3	9.0, CH_3	9.0, CH_3	9.0, CH_3	9.0, CH_3
18	16.1, CH_3	16.1, CH_3	16.7, CH_3	16.7, CH_3	16.7, CH_3	16.7, CH_3	16.7, CH_3	16.7, CH_3
19	19.1, CH_3	19.1, CH_3	110.6, CH_2	110.6, CH_2	110.6, CH_2	110.6, CH_2	110.6, CH_2	110.6, CH_2
20	15.8, CH_3	15.8, CH_3	16.1, CH_3	16.1, CH_3	16.1, CH_3	16.1, CH_3	16.1, CH_3	16.1, CH_3
1′	62.5, CH_2	60.6 CH_2	155.1, qC	154.0, qC	155.4, qC	155.0, qC	154.2, qC	154.1, qC
2′	23.8, CH_2	31.7, CH_2	43.1, CH_2	43.1, CH_2	42.4, CH_2	49.6, CH	46.1, CH_2	138.0, qC
3′	11.0, CH_3	23.5, CH_2	34.4, CH_2	34.4, CH_2	32.5, CH_2	29.6, CH_2	134.8, CH	118.6, CH
4′		14.1, CH_3	20.0, CH_2	25.5, CH_2	38.3, CH_2	25.6, CH_2	118.0, CH_2	129.2, CH
5′			13.8, CH_3	22.5, CH_2		30.4, CH_2	154.3, qC	122.0, CH
6′				14.0, CH_3		25.5, CH_2	43.1, CH_2	129.2, CH
7′						29.8, CH_2	134.6, CH	118.6, CH
8′							117.1, CH_2	
9′							154.3, qC	
10′							43.1, CH_2	
11′							134.3, CH	
12′							116.2, CH_2	

[a] In $CDCl_3$, 100 MHz. Carbon multiplicities were determined by APT experiments, C = quaternary, CH = methine, CH_2 = methylene, CH_3 = methyl carbons.

The HRTOFMS, ^{1}H, and ^{13}C NMR data of **14** (Tables 2 and 3) suggested the molecular formula $C_{32}H_{43}N_3O_6$, and the possibility of reaction of three molecules of allylisocyanate with the C-7 secondary alcohol in **9** to form trimeric carbamates. The previous conclusion was supported by the presence of three non-symmetric allyl groups along with three chemically non-equivalent carbamoyl

carbonyl carbons (C-1′, C-5′, and C-9′, δ_C 154.2, 154.3, and 154.4, respectively). This was further supported via the ^3J-HMBC correlations of H_2-2′ (δ_H 4.41) with C-1′, C-4′, and C-5′, H_2-6′ (δ_H 3.87) with C-5′, C-8′, and C-9′, and H_2-10′ (δ_H 3.88) with C-9′ and C-12′. Thus, **14** was determined to be 7β-allyl-(allyl-(allylcarbamoyl)-carbamoyl)-carbamate-$\Delta^{8(19)}$-deepoxysarcophine.

The HRTOFMS of **15** suggested the molecular formula $C_{27}H_{33}NO_4$ and possible C-7 phenylcarbamoylation. The ^{1}H and ^{13}C NMR data (Tables 2 and 3) further supported this conclusion. Thus, **15** was proved to be 7β-phenylcarbamoyl-$\Delta^{8(19)}$-deepoxysarcophine.

2.2. Biological activity and preliminary structure–activity relationship

Metastasis is the process by which cancer cells leave the primary tumor to form secondary tumors at distant sites, causing the morbidity and mortality of most cancer patients.[14] Metastasis results from a complex interconnected molecular cascade via several adhesive interactions and invasive processes and responses to chemotactic stimuli.[14] The main elements of metastasis include 1-angiogenesis; 2-disaggregation of tumor cells from the primary tumor mass, mediated by cadherins and catenins; 3-invasion of, and migration through, the basement membrane and extracellular matrix surrounding tumor epithelium, mediated through integrins and proteases including urokinase form of plasminogen activator, matrix metalloproteinases and cathepsins; 4-intravasation of the tumor cells into the blood vessels; 5-adhesion of the circulating tumor cells to the endothelial cell lining mediated by selectins, integrins and members of the immunoglobulin superfamily; 6-invasion of the tumor cells through the endothelial cell layer and surrounding basement membrane and target organ tissue; and 7-development of secondary tumor foci at the target organ site.[14] The wound-healing assay (WHA) is a simple method for the study of directional cell migration in vitro.[14] The scratched monolayer heals in a characteristic manner; therefore, this assay is widely used to study cell migration rates, cell polarization, and matrix remodeling studies.[15–17] WHA proved to be useful as a proxy for metastasis, angiogenesis and other pathophysiological and physiological processes.[18–21] Therefore, WHA was used in this study to assess and rank the antimigratory potential of semisynthetic sarcophine derivatives.

All cembranoids were evaluated for their ability to inhibit the proliferation and migration of the human metastatic prostate cancer PC-3 and breast cancer MDA-MB-231 cell lines using MTT and

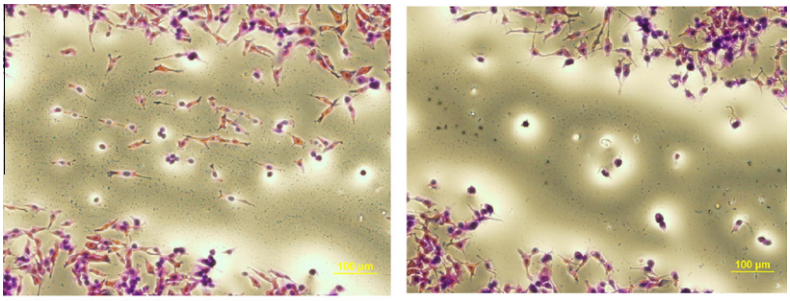


Figure 1. Relative number of migrated MDA-MB-231 cells after 24 h treatment with vehicle control and 50 μM dose of **6** in wound-healing assay. Magnification = 100×.

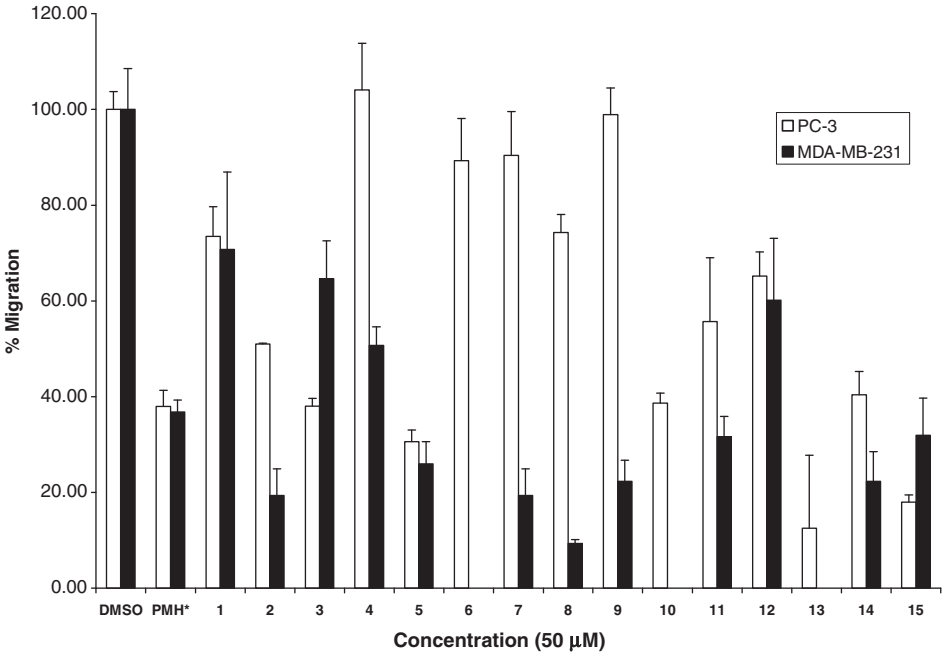


Figure 2. Effect of 50 μM dose of each of **1**–**15** on the percent of migration on the highly metastatic PC-3 and MDA-MB-231 cells in wound healing assay. A 200 μM dose of 4-hydroxyphenylmethylene hydantoin (PMH) was used a positive drug control.[12,13]

wound-healing assays (Figs. 1 and 2).[22–24] Most compounds showed no effect on the proliferation of PC-3 or MDA-MB-231 cells up to a 50 μM dose, indicating the lack of cytotoxicity toward these cell lines. Only compounds **6**, **10**, and **13** showed antiproliferative activity against MDA-MB-231 cells, with IC_{50} values 20, 7 and 45 μM, respectively. The antimigratory activity of all compounds was assessed in the wound-healing assay at a 50 μM dose, except compounds **6**, **10**, and **13** which were tested at lower concentrations (Fig. 3) against MDA-MB-231 and PC-3 cells, to avoid false positive activity due to their possible cytotoxicity. The activity was compared to a 200 μM dose of the antimetastatic marine natural product lead 4-hydroxyphenylmethylene hydantoin (PMH).[25,26] Compound **13** showed potent antimigratory activity against the prostate cancer PC-3 cell line, followed by **14** and **15**, which showed moderate activity (Figs. 1 and 2 and Table 4). Cembranoids **3**, **5**, **10**, and **13**–**15** were more active against PC-3 at 50 μM than a 200 μM of the known marine natural product PMH. The IC_{50} values of **13**–**15** were 15.53, 24.28, and 16.29 μM, respectively (Table 4). On the other hand, a 50 μM dose of **8** showed a potent antimigratory activity against the estrogen negative breast cancer MDA-MB-231 cell line, followed by **7**, **2**, **9**, **14**, **5**, **11**, and **15**, respectively, with better activities than the 200 μM dose of the PMH positive control,[25,26] while **3**, **4**, and **12** showed moderate activity but better than the parent compound **1**, which showed a very limited activity (Fig. 2). This was consistent with literature which limited the activity of **1** only to the estrogen positive breast cancer cell lines.[6] The IC_{50} of the most active antimigratory analogues **6**, **10**, and **13** were 30.02, 4.83, and 17.23 μM, respectively, against MDA-MB-231 cells (Table 4). To avoid the correlation of the antimigratory activity with possible cytotoxic activity, compounds **6**, **10**, and **13** were retested at different doses lower than their antiproliferative IC_{50} values (Fig. 3). A dose of 5 and 30 μM of **10** and **13**, respectively, induced antimigratory effects comparable to that produced by a 200 μM dose of PMH without affecting the cell shape or viability, while compound **6** showed no significant activity up to 30 μM.

showed better activity compared to olefinic or aromatic carbamates. Butyloxy, butyl or hexylcarbamate functionalities were optimal for enhanced antimigratory activity. Inclusion of halogens in the side chain as represented by the carbamate **12** remarkably reduced the activity. Cembranoids can be potential scaffolds for the design of potent antimetastatic leads.

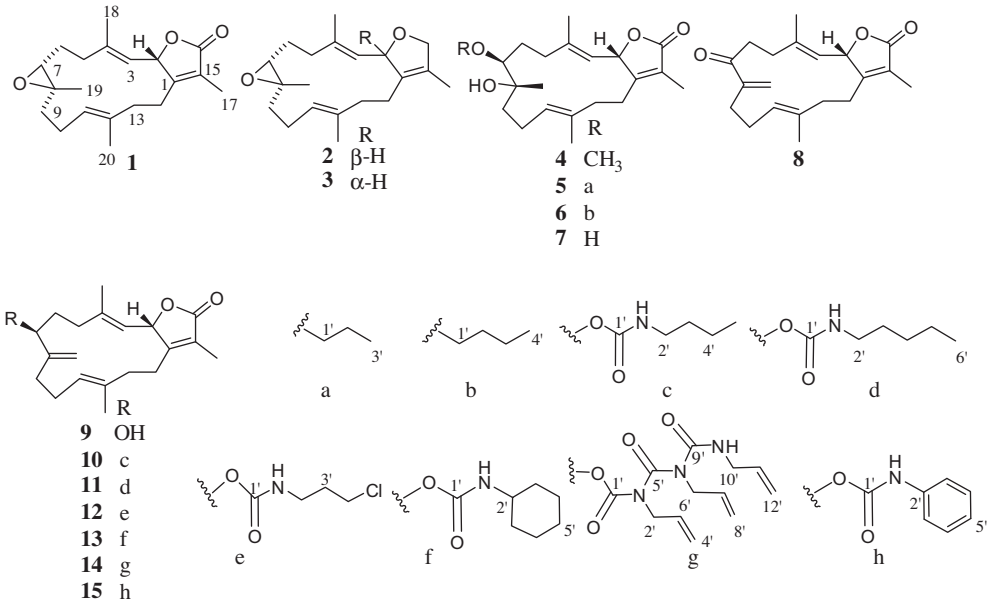
3. Experimental

3.1. General experimental procedures

Optical rotations were measured on a Rudolph Research Analytical Autopol III polarimeter. IR spectra were recorded on a Varian 800 FT-IR spectrophotometer. The ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$, using TMS as internal standard, on a JEOL Eclipse NMR spectrometer operating at 400 MHz for ^1H and 100 MHz for ^{13}C. The HREIMS experiments were conducted at Louisiana State University on Agilent 6200-TOF LCMS. TLC analysis was carried on precoated Si gel 60 F_{254} 500 μm TLC plates (EMD Chemicals), using isocratic *n*-hexane-EtOAc (1:1) as a developing system. For column chromatography, Si gel 60 (Natland, 63–200 μm) and *n*-hexane-EtOAc (9.5:0.5) with increasing polarity to *n*-hexane-EtOAc (7:3) systems were used. A purity of >95% was established for each of **1**–**15** as assessed by TLC, HPLC, and/or ^1H NMR spectroscopy.

3.2. Biological material

The soft coral *S. glaucum* was collected by SCUBA June 2003 from Hurghada, at the Egyptian Red Sea coast. A voucher specimen (03RS24) was deposited in the Department of Basic Pharmaceutical Sciences, College of Pharmacy, University of Louisiana at Monroe. The frozen soft coral (680 g) was extracted four times with isopropanol. The extract (86 g) was then concentrated under vacuum and chromatographed on silica gel using *n*-hexane/EtOAc to yield 1.5 g



Acid catalyzed conversion of the epoxide moiety of **1** to secondary alcohol or ketone at C-7 with a $\Delta^{8(19)}$ exomethylene system significantly improved the antimigratory activity. Modification of C-7, including etherification and carbamoylation, enhanced the activity and demonstrated its significant contribution to the binding at the plausible molecular target(s). Saturated ethers and carbamates

of sarcophine (**1**), which was further recrystallized from EtOAc. Further chromatographic separation of the extract afforded the less polar 16-deoxysarcophine (**2**, 1.5 g, R_f 0.52 *n*-hexane/EtOAc 7:3) and 2-*epi*-16-deoxysarcophine (**3**, 4.1 g, R_f 0.50 *n*-hexane/EtOAc 7:3). Identity was confirmed via spectral analysis and comparison with literature.[3,4,6,10]

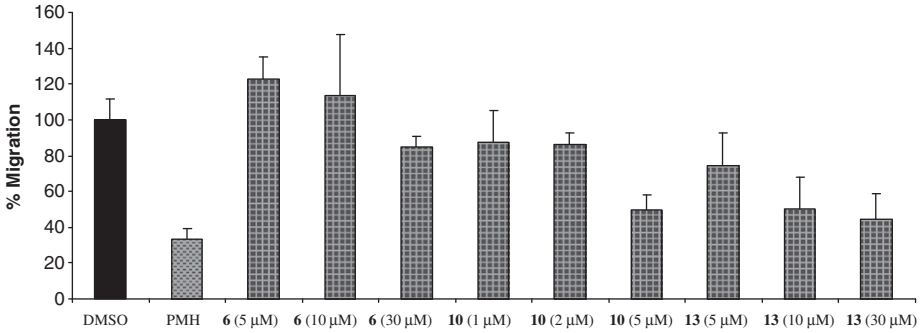


Figure 3. Effect of different doses of **6**, **10** and **13** on the percent of migration on the highly metastatic MDA-MB-231 in wound healing assay. A 200 μM dose of 4-hydroxyphenylmethylene hydantoin (PMH) was used a positive drug control.[12,13]

Table 4
IC_{50} values of selected sarcophine analogues against the highly metastatic MDA-MB-231 (breast) and PC-3 (prostate) cancer cell lines[a]

MDA-MB-231		PC-3	
Compound	IC_{50} (μM)	Compound	IC_{50} (μM)
6	30.02	**13**	15.53
10	4.83	**14**	24.28
13	17.23	**15**	16.29

[a] The values are the mean ± SD. Each experiment was conducted in triplicate.

3.3. Chemical reactions

3.3.1. Etherification of 1[3,10]

About 100 mg of sarcophine dissolved in 2.5 mL of each of MeOH, *n*-propanol, and *n*-butanol and each stirred for 1 h at rt with *p*-toluenesulfonic acid (5 mg) (Scheme 1). After neutralization with $NaHCO_3$, organic solvent was evaporated under vacuum and $CHCl_3$ (5 mL) was added to each reaction mixture. Each solution was then washed with water (5 mL), dried over anhydrous Na_2SO_4 and evaporated. Each residue was subjected to repeated column chromatography to afford compounds **4**–**9**[3,10].

3.3.2. Carbamoylation of 9[27–29]

Compound **9** (10 mg) in toluene (2 mL) was reacted with various isocyanates and catalytic amount of triethylamine (Et_3N, 10 μL) (Scheme 1).[27–29] Each solution was separately refluxed for 3 h. Water (10 mL) was added and each reaction mixture was extracted with EtOAc (3 × 10 mL). Each EtOAc extract was dried over anhydrous Na_2SO_4, concentrated under vacuum and subjected to repeated column chromatography to afford **10**–**15**.

3.3.3. 7β-Propyloxy-8α-hydroxy-deepoxysarcophine (5)

Compound **5** was prepared in 15% yield using the reaction conditions described above. White powder; $[\alpha]_D^{25}$ +15.5 (*c* 0.53, $CHCl_3$); IR ν_{max} ($CHCl_3$) 3689, 3565, 3055, 2927, 2875, 1747, 1680, 1455, 1387, 1096, 999 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 3; HRTOFMS *m*/*z* 399.2506 $[M+Na]^+$ (calcd for $C_{23}H_{36}O_4Na$, 399.2511).

3.3.4. 7β-Butyoxy-8α-hydroxy-deepoxysarcophine (6)

Compound **6** was prepared in 20% yield. White powder; $[\alpha]_D^{25}$ +13.7 (*c* 0.57, $CHCl_3$); IR ν_{max} ($CHCl_3$) 3689, 3055, 2959, 2932, 2872, 1747, 1681, 1422, 1096, 989, 896 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 3; HRTOFMS *m*/*z* 413.2662 $[M+Na]^+$ (calcd for $C_{24}H_{38}O_4Na$, 413.2667).

3.3.5. 7β-Butylcarbamoyl-$\Delta^{8(19)}$-deepoxysarcophine (10)

Compound **10** was prepared in 98% yield using 3.1 μL of butylisocyanate. Colorless oil; $[\alpha]_D^{25}$ +33.4 (*c* 0.23, $CHCl_3$); IR ν_{max} ($CHCl_3$) 3689, 3565, 3055, 2927, 2875, 1747, 1680, 1455, 1387, 1096, 999 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 3; HRTOFMS *m*/*z* 438.2615 $[M+Na]^+$ (calcd for $C_{25}H_{37}NO_4Na$, 438.2620).

3.3.6. 7β-Pentylcarbamoyl-$\Delta^{8(19)}$-deepoxysarcophine (11)

Compound **11** was prepared in 98% yield using 3.5 μL of pentylisocyanate. Colorless oil; $[\alpha]_D^{25}$ +31.1 (*c* 0.27, $CHCl_3$); IR ν_{max} ($CHCl_3$) 3690, 3481, 3446, 3054, 2986, 2874, 1747, 1695, 1645, 1513, 1422, 1258, 1166, 896 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 3; HRTOFMS *m*/*z* 452.2773 $[M+Na]^+$ (calcd for $C_{26}H_{39}NO_4Na$, 452.2776).

3.3.7. 7β-Chloropropylcarbamoyl-$\Delta^{8(19)}$-deepoxysarcophine (12)

Compound **12** was prepared in 58% yield using 3.7 μL of chloropropylisocyanate. Colorless oil; $[\alpha]_D^{25}$ +34 (*c* 0.1, $CHCl_3$); IR ν_{max} ($CHCl_3$) 3689, 3447, 3054, 2927, 2855, 1748, 1720, 1510, 1422, 1216, 989 cm^{-1}; ^1H and ^{13}C NMR, see Tables 2 and 3; HRTOFMS *m*/*z* 470.1862 $[M+Cl]^+$ (calcd for $C_{24}H_{34}NO_4Cl_2$, 470.1864)

3.3.8. 7β-Cyclohexylcarbamoyl-$\Delta^{8(19)}$-deepoxysarcophine (13)

Compound **13** was prepared in 98% yield using 3.6 μL of cyclohexylisocyanate afforded **13** in a 98% yield. Colorless oil; $[\alpha]_D^{25}$ +42.0 (*c* 0.2, $CHCl_3$); IR ν_{max} ($CHCl_3$) 3689, 3434, 3055, 2930, 2856, 1746, 1716, 1506, 1452, 1216,1096, 989 cm^{-1}; ^1H and ^{13}C NMR, see Tables 2 and 3; HRTOFMS *m*/*z* 464.2771 $[M+Na]^+$ (calcd for $C_{27}H_{39}NO_4Na$, 464.2776).

3.3.9. 7β-Allyl(allyl(allylcarbamoyl)carbamoyl)-$\Delta^{8(19)}$-deepoxysarcophine (14)

Compound **14** was prepared in 98% yield using 5.2 μL of allylisocyanate. Colorless oil; $[\alpha]_D^{25}$ +40.5 (c 0.13, CHCl3); IR ν_{max} ($CHCl_3$) 3688, 3445, 3056, 3020, 2928, 2856, 1747, 1700, 1509, 1216, 1096, 993 cm^{-1}; ^1H and ^{13}C NMR, see Tables 2 and 3; HRTOFMS m/z 588.3045 $[M+Na]^+$ (calcd for $C_{32}H_{43}N_3O_6Na$, 588.3049).

3.3.10. 7β-Phenylcarbamoyl-$\Delta^{8(19)}$-deepoxysarcophine (15)

Compound **15** was prepared in 68% yield using 3.7 μL of phenylisocyanate. Colorless oil; $[\alpha]_D^{25}$ +51.0 (*c* 0.13, $CHCl_3$); IR ν_{max} ($CHCl_3$) 3687, 3600, 3425, 3054, 2986, 2928, 2855, 1746, 1703, 1597, 1525, 1443, 1209, 1095, 896 cm^{-1}; ^1H and ^{13}C NMR, see Tables 2 and 3; HRTOFMS *m*/*z* 458.2302 $[M+Na]^+$ (calcd for $C_{27}H_{33}NO_4Na$ 458.2307).

4. Biological assays

4.1. Cell culture

Prostate and breast cancer cell lines PC-3 and MDA-MB-231 were purchased from ATCC. Cells were grown in 10% fetal bovine serum (FBS) and RPMI 1640 (GIBCO-Invitrogen) supplemented

with 2 mmol/L glutamine, 100 μg/mL penicillin G, and 100 μg/mL streptomycin at 37 °C under 5% CO_2.

4.1.1. Proliferation assay

The antiproliferative effects of the isolated compounds were tested in culture on malignant PC-3 and MDA-MB-231 epithelial cell lines using MTT kit (TACS, Trevigen, Inc.). After passing the cells for 3–4 times, growing cells were incubated in a 96-well plate at a density of 8×10^3 cells per well, and allowed to attach for 24 h. Complete growth medium was then replaced with 100 μL of RPMI serum free medium (GIBCO-Invitrogen) containing various doses (50, 20, 10, and 5 μM) of each cembranoid as previously described.[28,29] The number of cells per well was calculated against a standard curve prepared by plating various concentration of cells. Growth curves were determined to ensure that cells used in experiments were within the exponential growth phase. Cell proliferation was assessed by monitoring the conversion of 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide (MTT) to formazan. The reduction of MTT is catalyzed by mitochondrial dehydrogenase enzymes and is therefore a measure for cell viability.[28,29]

4.1.2. Wound-healing assay (WHA)

PC-3 and MDA-MB-231 cells were cultured and plated onto sterile 24-well and allowed to form a confluent cell monolayer in each well (>90% confluence) as previously reported.[29] Wounds were then inflicted to each cell monolayer using a sterile 200 μL pipette tip. Test compounds were prepared in DMSO at different concentrations and added to the plates, each in triplicate using PMH as positive control and DMSO as a vehicle control.[25,26] The incubation was carried out for 24 h under serum-starved conditions, after which media was removed and cells were fixed and stained using Diff Quick staining (Dade Behring Diagnostics). The number of cells migrated on the scratched wound were counted under the microscope in three or more randomly selected fields (magnification: 400×). Final results are expressed as mean ± SEM per 400× field. All treatments, including the controls, were documented photographically.

Acknowledgments

The Louisiana Board of Regents is acknowledged for supporting the new NMR facility (LEQSF(2010-11)-ENH-TR-41). The Egyptian government is acknowledged for the fellowship support of H.H.

A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.06.060. These data include MOL files and InChiKeys of the most important compounds described in this article.

References and notes

1. El Sayed, K. A.; Sylvester, P. W. *Expt. Opin. Invest. Drugs* **2007**, *16*, 877.
2. Li, Y.; Peng, L.; Zhang, T. Progress of studies on the natural cembranoids from soft coral species of *Sarcophyton* genus. In *Medicinal Chemistry of Bioactive Natural Products*; Liang, X. T., Fang, W. S., Eds.; Wiley-Interscience: New Jersey, 2006; pp 257–300.
3. Bernstein, J.; Shmeuli, U.; Zadock, E.; Kashman, Y.; Neeman, I. *Tetrahedron* **1974**, *30*, 2817.
4. Ne'eman, I.; Fishelson, L.; Kashman, Y. *Toxicon* **1974**, *12*, 593.
5. El Sayed, K. A.; Hamann, M. T.; Waddling, C. A.; Jensen, C.; Lee, S. K.; Dunstan, C. A.; Pezzuto, J. M. *J. Org. Chem.* **1998**, *63*, 7449.
6. Arif, J. M.; Sawant, S. S.; El Sayed, K. A.; Kunhi, M.; Subramanian, M. P.; Siddiqui, Y. M.; Youssef, D. T. A.; Al-Hussain, K.; Mohammed, N.; Al-Ahdal, M. N.; Al-Khodairy, F. *J. Nat. Med.* **2007**, *61*, 154.
7. Katsuyama, I.; Fahmy, H.; Zjawiony, J. K.; Khalifa, S. I.; Kilada, R. W.; Konoshima, T.; Takasaki, M.; Tokuda, H. *J. Nat. Prod.* **2002**, *65*, 1809.
8. Zhang, X.; Bommareddy, A.; Chen, W.; Khalifa, S.; Kaushik, R. S.; Fahmy, H.; Dwivedi, C. *Transl. Oncol.* **2009**, *2*, 21.
9. Zhang, X.; Bommareddy, A.; Chen, W.; Hildreth, M. B.; Kaushik, R. S.; Zeman, D.; Khalifa, S.; Fahmy, H.; Dwivedi, C. *Mar. Drugs* **2009**, *7*, 153.
10. Sawant, S. S.; Youssef, D. T. A.; Reiland, J.; Ferniz, M.; Marchetti, D.; El Sayed, K. A. *J. Nat. Prod.* **2006**, *69*, 1010.
11. Sawant, S. S.; Sylvester, P. W.; Avery, M. A.; Desai, P.; Youssef, T. A.; El Sayed, K. A. *J. Nat. Prod.* **2004**, *67*, 2017.
12. Sawant, S. S.; Youssef, D. T. A.; Mayer, A. M. S.; Sylvester, P. W.; Wali, V.; Arant, M. E.; El Sayed, K. A. *Chem. Pharm. Bull.* **2006**, *54*, 1019.
13. Czarkie, D.; Carmely, S.; Groweiss, A.; Kashaman, Y. *Tetrahedron* **1985**, *41*, 1049.
14. Brooks, S. A.; Lomax-Browne, H. J.; Carter, T. M.; Kinch, C. E.; Hall, D. M. *Acta Histochem.* **2010**, *112*, 3.
15. Finder, I. J. *Nat. Rev. Cancer* **2003**, *3*, 453.
16. Lampugnani, M. G. *Methods Mol. Biol.* **1999**, *96*, 177.
17. Lu, K. V.; Jong, K. A.; Rajasekaran, A. K.; Cloughesy, T. F.; Mischel, P. S. *Lab Invest.* **2004**, *84*, 8.
18. Herren, B.; Garton, K. J.; Coats, S.; Bowen-Pope, D. F.; Ross, R.; Raines, E. W. *Exp. Cell Res.* **2001**, *271*, 152.
19. Huang, C.; Rajfur, Z.; Borchers, C.; Schaller, M. D.; Jacobson, K. *Nature* **2003**, *424*, 219.
20. Mc Henry, K. T.; Ankala, S. V.; Ghosh, A. K.; Fenteany, G. *ChemBioChem* **2002**, *3*, 1105.
21. Vogt, A.; Pestell, K. E.; Day, B. W.; Lazo, J. S.; Wipf, P. *Mol. Cancer Ther.* **2002**, *1*, 885.
22. Moon, E. J.; Lee, Y. M.; Lee, O. H.; Lee, M. J.; Lee, S. K.; Chung, M. H.; Park, Y. I.; Sung, C. K.; Choi, J. S.; Kim, K. W. *Angiogenesis* **1999**, *3*, 117.
23. Ma, P. C.; Kijima, T.; Maulik, G.; Fox, E. A.; Sattler, M.; Griffin, J. D.; Johnson, B. E.; Salgia, R. *Cancer Res.* **2003**, *63*, 6272.
24. Shah, S. J.; Sylvester, P. W. *Exp. Biol. Med.* **2005**, *230*, 235.
25. Mudit, M.; Khanfar, M.; Muralidharan, A.; Thomas, S.; Shah, G. V.; van Soest, R. W.; El Sayed, K. A. *Bioorg. Med. Chem.* **2009**, *17*, 1731.
26. Shah, G. V.; Muralidharan, A.; Thomas, S.; Gokulgandhi, M.; Mudit, M.; Khanfar, M.; El Sayed, K. *Mol. Cancer Ther.* **2009**, *8*, 509.
27. El Sayed, K. A.; Khanfar, M.; Shallal, H.; Muralidharan, A.; Awate, B.; Youssef, D. T.; Shah, G. V. *J. Nat. Prod.* **2008**, *71*, 396.
28. Hassan, H. M.; Khanfar, M. A.; Elnagar, A. Y.; Mohammed, R.; Shaala, L. A.; Youssef, D. T. A.; Hifnawy, M. S.; El Sayed, K. A. *J. Nat. Prod.* **2010**, *73*, 848.
29. Hassan, H. M.; Khanfar, M. A.; Elnagar, A. Y.; Mohammed, R.; Shaala, L. A.; Youssef, D. T. A.; Hifnawy, M. S.; El Sayed, K. A. *Eur. J. Med. Chem.* **2011**, *46*, 1122.