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Optimization of Marine Triterpene Sipholenols as Inhibitors of Breast Cancer Migration and Invasion

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Sipholenol A, a sipholane triterpene isolated from the Red Sea sponge *Callyspongia siphonella*, has the ability to reverse multidrug resistance in cancer cells that overexpress P-glycoprotein (P-gp). Here, the antimigratory activity of sipholenol A and analogues are reported against the highly metastatic human breast cancer cell line MDA-MB-231 in a wound-healing assay. Sipholenol A and sipholenone A were semisynthetically optimized using ligand-based strategies to generate structurally diverse analogues in an attempt to maximize their antimigratory activity. A total of 22 semisynthetic ester, ether, oxime, and carbamate analogues were generated and identified by extensive one- and two-dimensional NMR spectroscopy and high-resolution mass spectrometry analyses. Sipholenol A 4β-4-chlorobenzoate and 19,20-anhydrosipholenol A 4β-4-chlorobenzoate esters were the most potent of all tested analogues in the wound-healing assay, with IC_{50} values of 5.3 and 5.9 μM, respectively. Generally, ester derivatives showed better antimigratory activities than the carbamate analogues. A KINOME*scan* of 19,20-anhydrosipholenol A 4β-benzoate ester against 451 human protein kinases identified protein tyrosine kinase 6 (PTK6) as a potential target. In breast tumor cells, PTK6 promotes growth factor signaling and migration, and as such the semisynthetic sipholanes were evaluated for their ability to inhibit PTK6 phosphorylation in vitro. The two analogues with the highest antimigratory activities, sipholenol A 4β-4-chlorobenzoate and 19,20-anhydrosipholenol A 4β-4-chlorobenzoate esters, also exhibited the most potent inhibition of PTK6 phosphorylation inhibition. None of the compounds exhibited cytotoxicity in a normal epithelial breast cell line. These derivatives were evaluated in an in vitro invasion assay, where sipholenol A succinate potently inhibited MDA-MB-231 cell invasion at 10 μM. These results highlight sipholane triterpenoids as novel antimigratory marine natural products with potential for further development as agents for the control of metastatic breast malignancies.

Introduction

Marine-derived natural products are among the important resources for drug discovery and development.[1–3] In the last three decades, thousands of new marine-derived compounds have been discovered and shown to have potential anticancer activities.[4,5] Marine-derived sipholane triterpenoids sipholenol A (**1**) and sipholenone A (**2**) were first isolated from the Red Sea sponge *Callyspongia (Siphonochalina) siphonella* by Kashman and co-workers.[6] Thirty sipholane triterpenoids have been isolated so far, possessing four different skeletons, namely, the sipholane, siphonellane, neviotane, and dahabinane.[6–11] The sipholanes contain a perhydrobenzoxepine (rings A and B) and a [5,3,0]bicyclodecane system (rings C and D), linked together through an ethylene bridge.[7,8] In recent studies, the potential of sipholane triterpenoids as P-glycoprotein (P-gp) modulators and their ability to reverse multidrug resistance (MDR) in human epidermoid cancer cells was investigated.[9,10] Accordingly, **1** was reported to potentiate the cytotoxicity of several P-gp-substrate anticancer drugs, including colchicine, vinblastine, and paclitaxel, but not non-P-gp substrate drugs, such as cisplatin; compound **1** was also shown to significantly reverse the MDR of P-gp-overexpressing human epidermal carcinoma cell lines KB-C2 and KB-V1 in a concentration-dependent manner.[10,11] Furthermore, **1** showed no cytotoxicity towards numerous cell lines, regardless of their membrane transporter status.[11–13] Sipholenol A (**1**) efficiently inhibited the function of P-gp through direct interaction, and sipholane triterpenoids were proposed as a novel class of potential P-gp inhibitors for the reversal of MDR in P-gp-overexpressing tumors.[12,13]

Breast cancer is the most common cancer and the second leading cause of cancer-related death among women in the USA.[14] Breast tumor kinase (Brk), also referred to as protein tyrosine kinase 6 (PTK6), is a member of the Src family of tyrosine kinases and has been cloned from metastatic breast tumor samples and cultured human melanocytes.[15] Brk is normally expressed in the differentiating epithelial cells of the intestine, skin, prostate, and oral cavity where it has been shown to promote cellular differentiation, apoptosis, and more recently, to mediate migration/wound healing.[16] Overexpression of Brk has been implicated as a mediator of cancer cell phenotypes,

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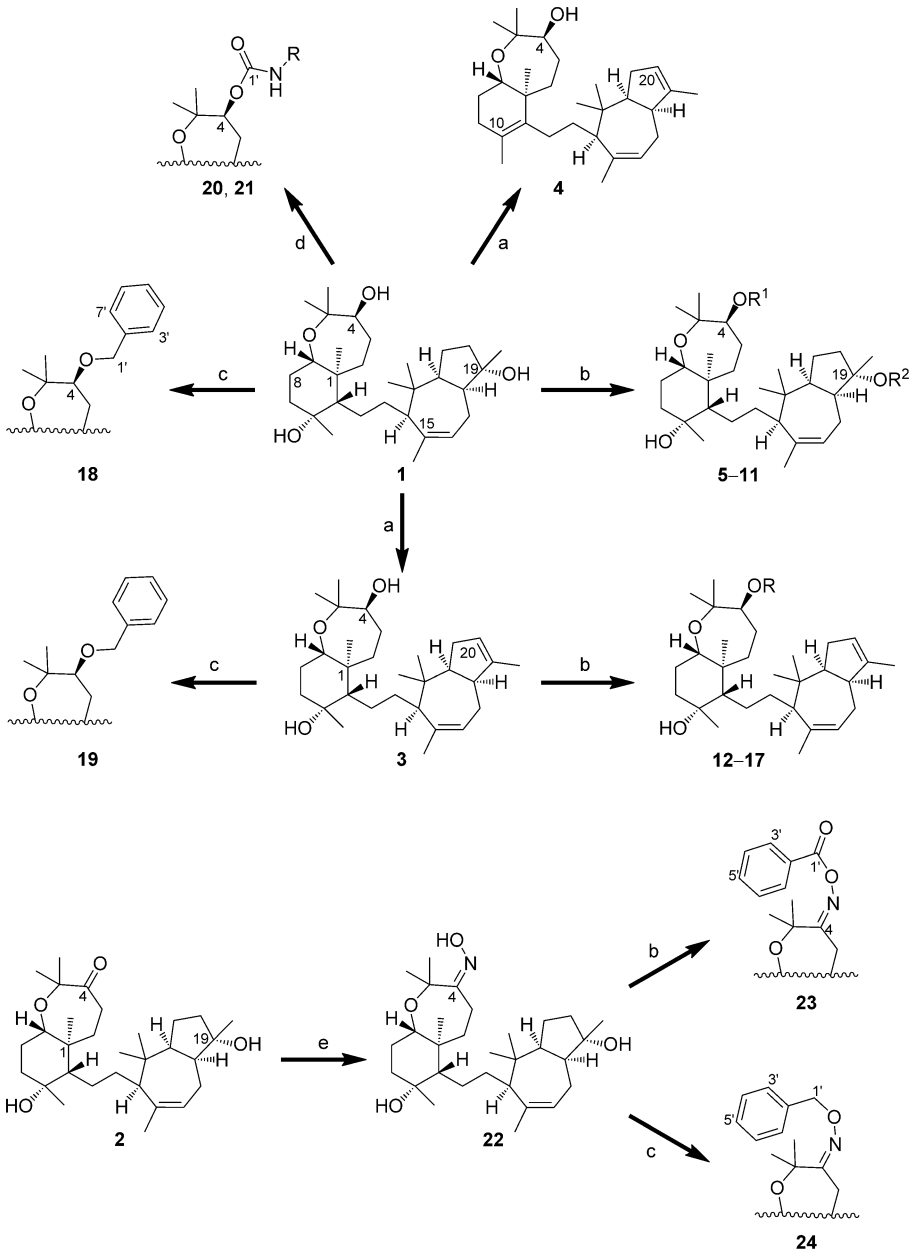
including increased proliferation, survival, and migration. Brk is overexpressed in up to 86% of invasive human breast tumors, prostate and colon carcinomas.[17,18] Its expression levels increase in association with the carcinoma content of breast tumors, tumor grade, and invasiveness.[15–20] Inhibition of Brk activity might provide a potentially novel approach to sensitize tumor cells to other chemotherapeutics and prevent or inhibit metastasis with enhanced therapeutic windows.

To the best of our knowledge, sipholanes **1** and **2** have never been tested for their ability to inhibit the migration or invasion of breast cancer cell lines. Structurally related polyepoxysqualene-derived, cyclic ether triterpenoids (e.g., sodwanones from the Indian Ocean sponge *Axinella weltneri*) were reported to possess cytotoxicity against human cancer cell lines.[21] Several sodwanones were reported to possess activity against various cancer cells. For example, sodwanones G–I were found to possess cytotoxicity at 20 μM or less concentrations against P-388 murine leukemia, A-549 lung carcinoma, HT-29 human colon carcinoma, and MEL-28 human melanoma cells.[21,22] Sodwanone A was found to be cytotoxic in nanomolar concentrations to IGROV-ET human ovarian cancer cells resistant to the cancer drug ET-743, PANC1 human pancreatic carcinoma cells, and LOVO-DOX multidrug-resistant human colon adenocarcinoma cells. Sodwanone A was also cytotoxic to human lung cancer NSCLC-N6 cells with an IC_{50} value of 12 μM.[23] Sodwanones G and S also displayed cytotoxicity to IGROV-ET, PANC1, and LOVO-DOX cells with IC_{50} values of less than 10 μM.[24] Recently, 3-*epi*-sodwanone K-3 acetate was found to be cytotoxic against T47D breast cancer cells (IC_{50} = 22 μM), while sodwanone V was cytotoxic to MDA-MB-231 breast cancer cells (IC_{50} = 23 μM). Sodwanone V also inhibited hypoxia-inducible factor 1 (HIF-1) activation in both T47D breast and PC3 prostate cancer cells, with an IC_{50} value of 15 μM.[25] Therefore, we hypothesized that sipholane triterpenoids could be potential anticancer hits.

Bioactivity screening of our library of natural products for the discovery of new potential antimigratory agents suggested sipholanes **1** and **2** as inhibitors of the migration of the highly metastatic MDA-MB-231 breast cancer cell line in a wound-healing assay at mid-micromolar concentrations. The aim of this study was to prepare different semisynthetic analogues of **1** and **2** with enhanced antimigratory and anti-invasive activity against the highly metastatic MDA-MB-231 breast cancer cell line, assess their PTK6 phosphorylation inhibitory activity as a possible molecular target, and identify key pharmacophores required for enhanced bioactivity.



Scheme 1. Semisynthetic transformations of sipholenol A (**1**) and sipholenone A (**2**). *Reagents and conditions*: a) *p*-toluenesulfonic acid, $CHCl_3$, reflux, 3 h; b) acid anhydride, DMAP, anhyd CH_2Cl_2, reflux, 12 h; c) BnBr, DMF, NaH, RT, 12 h; d) isocyanate, toluene, Et_3N, reflux, 3 h; e) NH_2OH, pyridine, EtOH, reflux, 4 h.

Results and Discussion

Chemistry

The sipholane triterpenoids **1** and **2** were subjected to semisynthetic modifications (Scheme 1) to generate structurally diverse analogues, to optimize their antimigratory activities, and to establish preliminary structure–activity relationships (SARs). The five different reactions types, including acid-catalyzed elimination, esterification, etherification, carbamoylation and oxime formation, were used to afford four known (**3**–**6**) and several new (**7**–**24**) semisynthetic analogues of **1** and **2** (Table 1).

Acid-catalyzed elimination

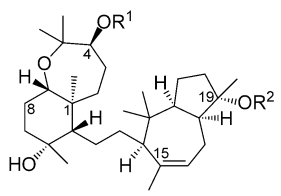
Sipholenol A (**1**) was converted into the known 19,20-anhydrosipholenol A (**3**) and 10,11,19,20-dianhydrosipholenol A (**4**), by treatment with *p*-toluenesulfonic acid monohydrate (*p*-TsOH·H_2O) in chloroform.[3,6,7] The reaction aimed to eliminate the tertiary alcohol groups at C-10 and C-19 and introduce additional double bonds (Δ) at specific positions in the structure of **1**, to assess their effects on the antimigratory activity. The major reaction product, 19,20-anhydrosipholenol A (**3**) was formed due to the elimination of one molecule of water, which resulted in the formation of a $\Delta^{19,20}$ bond. Similarly, 10,11,19,20-dianhydrosipholenol A (**4**) was a result of the loss of two molecules of water with the formation of $\Delta^{10,11}$ and $\Delta^{19,20}$ bonds. The identity of **3** and **4** was established by comparing their ^{1}H and ^{13}C NMR data with that reported in the literature.[6]

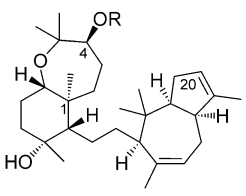
Esterification

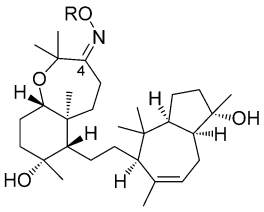
Several known and new esters of **1** were prepared using acetic, succinic, benzoic and 4-methylbenzoic anhydrides, isonicotinoyl, and 4-chlorobenzoyl chloride to assess the significance of the C-4 hydroxy group and nearby space on the antimigratory activity of this class of compounds. Acetylation of **1** in pyridine was carried out to afford the corresponding monoacetate (**5**) and diacetate (**6**) esters.[6] The new esters of **1** include the succinate (**7**), benzoate (**8**), isonicotinate (**9**), 4-methylbenzoate (**10**), and 4-chlorobenzoate (**11**) esters. In a similar fashion, the acetate (**12**), succinate (**13**), benzoate (**14**), isonicotinate (**15**), 4-methylbenzoate (**16**), 4-chlorobenzoate (**17**) esters of 19,20-anhydrosipholenol A (**3**) were also prepared. The identification of derivatives **7**–**17** was based on extensive spectral analyses.

The high-resolution mass spectrometry within (HRMS) of **7** showed an $[M+Na]^+$ peak at *m/z* 599.3929, suggesting the molecular formula $C_{34}H_{56}O_7Na$ and possible ester analogue of sipholenol A. The ^{1}H and ^{13}C NMR data also showed the presence of peaks corresponding to two ester carbonyl carbons at δ values of 171.5 ppm and 176.5 ppm assigned to C-1′ and C-4′, respectively. The esterification at the C-4 position was evident from the downfield shifting of the methine H-4 doublet to a δ value of 5.01 ppm ($J = 6.8$ Hz) compared with its parent **1** ($\Delta\delta = +1.27$ ppm). This was further supported by a ^3J-HMBC correlation of H-4 with C-1′. The four-proton peak at 2.72 ppm

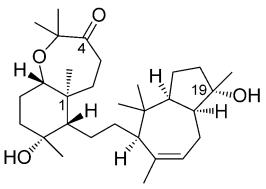
Table 1. Structures of sipholenol A (**1**) and sipholenone A (**2**) analogues.







Compd	R^1	R^2	Compd	R	Compd	R
1	H	H	**3**	H	**22**	H
5	a	H	**12**	a	**23**	c
6	a	a	**13**	b	**24**	g
7	b	H	**14**	c		
8	c	H	**15**	d		
9	d	H	**16**	e		
10	e	H	**17**	f		
11	f	H	**19**	g		
18	g	H				
20	h	H				
21	i	H				



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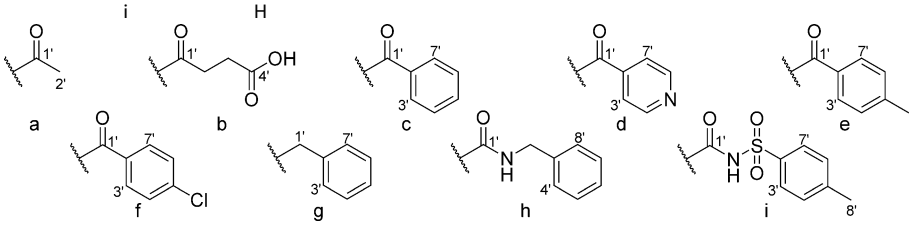

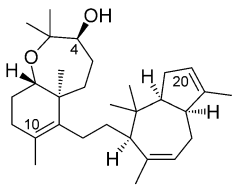

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was assigned to methylene protons of succinate (H_2-2′ and H_2-3′). Therefore, compound **7** was proved to be sipholenol A 4β-succinate.

The HRMS spectrum of **8** showed an $[M+Na]^+$ peak at *m/z* 603.4019, suggesting the molecular formula $C_{37}H_{56}O_5Na$ and possible ester analogue of sipholenol A. The ^1H and ^{13}C NMR data suggested benzoylation of **1** at C-4. The peak corresponding to the methine proton H-4 was shifted downfield to a δ value of 5.19 ppm (d, $J=6.8$ Hz) in a similar fashion to **7**, and showed a ^3J-HMBC correlation with C-1′ ($\delta_C=165.8$ ppm). The peak corresponding to two aromatic methine protons at a δ value of 8.07 ppm (dd, $J=7.7$, 1.4 Hz) was assigned to H-3′ and H-7′ based on the ^3J-HMBC correlations with C-1′ ($\delta_C=165.8$ ppm) and C-5′ ($\delta_C=133.1$ ppm). Therefore, compound **8** was found to be the 4β-benzoate ester of **1**. Similarly, analysis of the ^1H and ^{13}C NMR data of compounds **10** and **11** confirmed their identity as 4β-methylbenzoate and 4β-chlorobenzoate esters of **1**, respectively.

The HRMS spectrum of **9** showed an $[M+H]^+$ peak at *m/z* 582.4157, suggesting the molecular formula $C_{36}H_{56}NO_5$ and possible ester analogue of sipholenol A. In a similar fashion, the ^1H and ^{13}C NMR data of **9** confirmed the formation of isonicotinate ester at C-4. The downfield peak at a δ of 8.83 ppm corresponding to aromatic methine protons was assigned to H-4′ and H-6′ because they bear a heterocyclic nitrogen atom N-5′, which caused a downfield shift. The remaining aromatic methine proton peak at a δ value of 7.86 ppm (2H, d, $J=$ 4.4 Hz) was assigned to H-3′ and H-7′. Therefore, compound **9** was proved to be the 4β-isonicotinate ester of **1**.

The HRMS spectrum of **12** showed an $[M+H]^+$ peak at *m/z* 501.3912, suggesting the molecular formula $C_{32}H_{53}O_4$ and possible ester analogue of sipholenol A. The ^1H and ^{13}C NMR data of **12** showed acetylation of **3** at C-4 position. The methyl singlet H_3-2′ ($\delta=2.14$ ppm) and methine doublet H-4 ($\delta=$ 4.97 ppm, $J=6.6$ Hz) showed HMBC correlations with the carbonyl carbon C-1′ ($\delta_C=170.4$ ppm). Therefore, compound **12** was proved to be 19,20-anhydrosipholenol A 4β-acetate. Similarly, ^1H and ^{13}C NMR data of **13**–**17** confirmed esterification of **3** at C-4. The assignments of proton and carbon chemical shifts of compounds **13**–**17** were carried out in a similar fashion to those of compounds **7**–**11**, respectively. Compounds **13**–**17** were found to be the 4β-succinate, 4β-benzoate, 4β-isonicotinate, 4β-methylbenzoate, and 4β-chlorobenzoate esters of 19,20-anhydrosipholenol A (**3**).

Etherification

Like semisynthetic sipholane esters (**5**–**17**), 4β-*O*-benzyl ethers of **1** and **3** were prepared to test the significance of a free secondary alcohol group at C-4 on antimigratory activity. The benzyl ethers were also prepared to explore whether an ether (**18** and **19**) in this position imparts improved antimigratory activity as compared with the ester analogues (**8** and **14**). Reaction of **1** with benzyl bromide (BnBr) afforded the expected 4β-*O*-benzyl sipholenol A (**18**). The HRMS spectrum of **18** showed an $[M+H]^+$ peak at *m/z* 567.4405, suggesting the molecular formula $C_{37}H_{59}O_4$. The same reaction with **3** afforded the 4β-*O*-benzyl-19,20-anhydrosipholenol A (**19**), based on its HRMS spectrum, which showed an $[M-H]^+$ peak at *m/z* 547.4120, suggesting the molecular formula $C_{37}H_{55}O_3$. The structures of **18** and **19** were confirmed by analyses of their ^1H and ^{13}C NMR data. The upfield shift of the H-4 methine doublet ($\delta=3.40$ ppm, $J=6.6$ Hz) and the downfield shift of the C-4 carbon atom peak ($\delta_C=84.6$ ppm) indicated the selective etherification of the C-4 secondary alcohol in **18** and **19**. This was further supported by the ^3J-HMBC correlation of H-4 with the benzylic methylene carbon C-1′ ($\delta_C=71.6$ and 71.5 ppm, in **18** and **19**, respectively).

Carbamoylation

The HRMS spectrum of **20** showed an $[M+Na]^+$ peak at *m/z* 632.4283, suggesting the molecular formula $C_{38}H_{59}NO_5Na$ and possible carbamoyl analogue of sipholenol A. The ^1H and ^{13}C NMR data of **20** suggested the C-4 benzyl carbamoylation of **1**. The benzylic methylene protons H_2-2′ ($\delta=4.46$ ppm) showed ^3J-HMBC correlations with C-4′/C-8′ ($\delta_C=127.6$ ppm) and the carbamate carbonyl C-1′ ($\delta_C=155.6$ ppm). Thus, compound **20** was determined to be the 4β-benzylcarbamate of **1**.

The HRMS spectrum of **21** showed an $[M+H]^+$ peak at *m/z* 674.4079, suggesting the molecular formula $C_{38}H_{60}NO_7S$ and possible ester analogue of sipholenol A. The ^1H and ^{13}C NMR data of **21** indicated 4β-methyl tosylcarbamoylation has occurred. The benzylic methyl group at H_3-8′ ($\delta=2.44$ ppm) showed a ^2J-HMBC correlation with the quaternary aromatic carbon C-5′ ($\delta_C=135.0$ ppm) and ^3J-HMBC correlations with the methine carbons C-4′ and C-6′ ($\delta_C=129.7$ and 128.6 ppm, respectively). Thus, compound **21** was determined to be the 4β-tosylcarbamate of **1**.

Oxime formation

The HRMS spectrum of **22** showed an $[M+H]^+$ peak at *m/z* 490.3859, suggesting the molecular formula $C_{30}H_{52}NO_4$ and possible oxime analogue of **2**. The ^1H and ^{13}C NMR data also showed the upfield shift of the C-4 peak ($\delta_C=167.9$ ppm), indicating oxime formation. This was further supported by a ^3J-HMBC correlation between the methylene proton multiplets H_2-2 ($\delta=1.21$ and 1.90 ppm) with the quaternary carbon C-4 peak ($\delta_C=167.9$ ppm).

The HRMS spectrum of **23** showed an $[M+Na]^+$ peak at *m/z* 616.3967, suggesting the molecular formula $C_{37}H_{55}NO_5Na$ and possible oxime ester analogue of sipholenone A. The ^1H and ^{13}C NMR data showed the downfield shift of the C-4 peak ($\delta_C=$ 176.0 ppm), indicating benzoylation of the C-4 oxime hydroxy group of **22**. This was further supported by the ^3J-HMBC correlation of the methylene proton H_2-2 peaks ($\delta=1.21$ and 1.90 ppm) with the quaternary carbon C-4 peak ($\delta_C=$ 176.0 ppm). The peak corresponding to the two aromatic methine protons at a δ value of 8.01 ppm (dd, $J=7.7$, 1.4 Hz) were assigned to H-3′ and H-7′ based on their ^3J-HMBC correlations with the C-1′ ($\delta_C=163.9$ ppm) and C-5′ ($\delta_C=133.3$ ppm) peaks.

The HRMS spectrum of **24** showed an $[M+H]^+$ peak at *m/z* 580.4370, suggesting the molecular formula $C_{37}H_{58}NO_4$ and possible oxime ether analogue of **22**. The structure of **24** was confirmed by analysis of its ^1H and ^{13}C NMR data, which showed ^3J-HMBC correlations between the methylene proton H_2-2 multiplets ($\delta = 1.21$ and 1.90 ppm) with the quaternary carbon C-4 peak ($\delta_C = 167.0$ ppm). The assignment of the benzyl moiety was achieved in a similar fashion as described for **23**.

The geometry of the oxime double bond in compounds **22**–**24** was confirmed to be the *Z*-orientation based on NOESY data. The peak corresponding to the aromatic H-3′ proton in **24** showed strong correlation with the H_3-26 methyl singlet proton peak in a NOESY experiment, indicating a close distance range through space (< 5 Å). The distance between the aromatic H-3′ proton and the H_3-26 protons in compound **24** was calculated by using SYBYL version 8.0 to be 2.403 Å, which is in the range of NOE coupling (Figure S1 in the Supporting Information). Thus, the same double bond geometry was also assumed in compounds **22** and **23**.

Biological activity

The antimigratory, anti-invasive, and cytotoxic activities of compounds **1**–**24** were evaluated using a wound-healing assay, a Cultrex invasion assay and an MTT assay, respectively. The highly metastatic human breast cancer cell line MDA-MB-231 was used to assess the antimigratory and anti-invasive activities. These cells as well as the normal breast cell line MCF10A were used to evaluate the cytotoxicity in an MTT assay. Furthermore, Kinome profiling of analogue **14** was applied against a panel of 451 kinases. Additionally, a Z′-LYTE kinase assay was used to evaluate the ability of compounds **1**–**24** to inhibit PTK6 phosphorylation.

Antimigratory (wound-healing) assay

The wound-healing assay[26,27] is a simple method for the study of directional cell migration in vitro. The known antimigratory lead 4-*S*-ethylphenylmethylene hydantoin (*S*-Ethyl) was used as a positive control at a 50 μM dose.[20] Figure 1 shows the effect of active analogues **11** and **17** on cell migration across the wound inflicted in the MDA-MB231 cell monolayer compared with the vehicle and positive controls. Generally, all sipholenol A analogues, except **4** and **22**, showed better antimigratory activities than parent compound **1**, which indicates the removal of the hydrogen-bond donor capability of the free C-4 secondary alcohol group is beneficial for antimigratory activity (Table 2). Of the diverse ester analogues tested, aromatic were found to be more active than aliphatic esters. In contrast, carbamate analogues **20** and **21** were less active than the aromatic esters. Furthermore, benzyl ethers **18** and **19** were less active than benzoate esters **8** and **14**, which illustrates the important role of the carbonyl group in **8** and **14** for enhanced antimigratory activities. The activity of esters versus ether and carbamate analogues suggests that esters fulfill the required optimal distance between the side chain aromatic ring system and C-4 oxygen for maximal antimigratory activity. Shorter or longer distances as represented by ethers or carbamates significantly decrease the antimigratory activity.

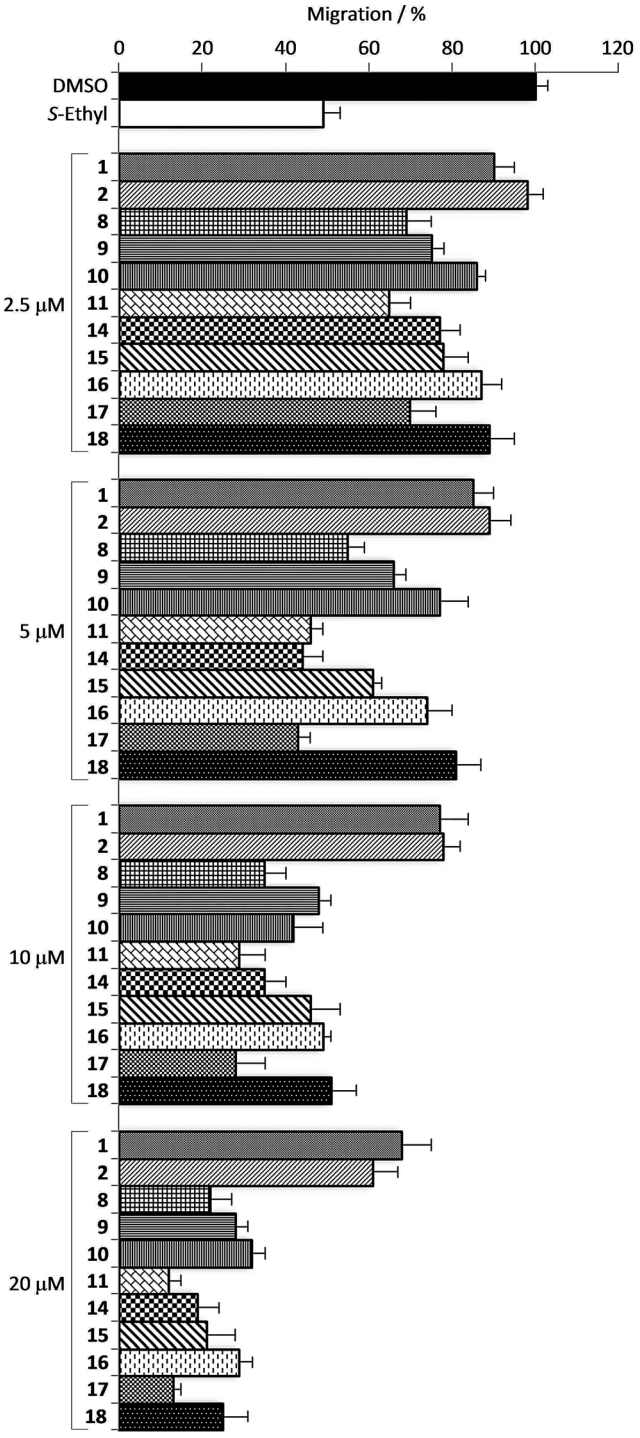

Figure 1. Antimigratory activity of **1**, **2**, **8**–**11** and **14**–**18** against the human breast cancer cells MDA-MB-231. Error bars indicate the SD of $n = 3$/dose. DMSO was used as a vehicle control, while 4-*S*-ethylphenylmethylene hydantoin (*S*-Ethyl)[27] was used as a positive control at 50 μM.

To assess the importance of *para*-substitution (C-5′) on the activity of compounds **8** and **14**, electron-donating (methyl) and -withdrawing (chloro) groups were inserted in this position

Table 2. IC_{50} values of analogues **1**–**24** determined using the wound-healing assay (WHA) against MDA-MB-231 cells.

Compd	IC_{50} [μM]	Compd	IC_{50} [μM]	Compd	IC_{50} [μM]
1	37.5	**9**	10.6	**17**	5.9
2	28.1	**10**	11.8	**18**	12.2
3	35.3	**11**	5.3	**19**	13.4
4	46.7	**12**	16.0	**20**	13.9
5	16.8	**13**	21.0	**21**	18.0
6	20.3	**14**	7.2	**22**	52.7
7	18.0	**15**	9.5	**23**	28.5
8	7.9	**16**	12.3	**24**	31.4

to give compounds **10**, **11**, **16**, and **17**. 4-Chlorobenzoate esters **11** and **17** were the most potent among all sipholanes tested, with IC_{50} values of 5.3 and 5.9 μM, respectively, which correlates well with their PTK6 phosphorylation inhibitory activity. The presence of an electron-withdrawing substituent at the *para*-position of the aromatic moiety (C-5′), as in compounds **11** and **17**, enhanced the antimigratory activity by twofold when compared with an electron-donating substituent, as in compounds **10** and **16**. Compounds **8**, **10**, **14**, and **16** exhibited antimigratory activities with IC_{50} values of 7.9, 11.8, 7.2 and 12.3 μM, respectively, but with weaker inhibitory activity against PTK6 phosphorylation, which could indicate that this is not their only molecular target.

Invasion assay

The Cultrex basement membrane extract (BME) cell invasion assay[28] is an accelerated in vitro screening process for compounds that influence cell migration through extracellular matrices, which is a fundamental function of cellular processes such as angiogenesis, embryonic development, immune response, and metastasis of cancer cells.[29] The anti-invasive activities of compounds **1**–**24** were evaluated in the BME cell invasion assay using highly metastatic MDA-MB-231 human breast cancer cells (Figure 2). In a preliminary screening at two different doses (10 and 20 μM), sipholenol A succinate (**7**) showed the most potent activity, allowing only 52% and 34% invasion at the two concentrations, respectively. In addition, compounds **5**, **6**, **12**, and **13** showed more than 50% inhibition of invasion at 20 μM, which suggests the importance of a carbonyl group especially in the aliphatic ester analogues of **1**. Moreover, derivative **7** highlighted the significance of increased side chain length and an additional carboxy carbonyl group for enhancement of the anti-invasion activity.

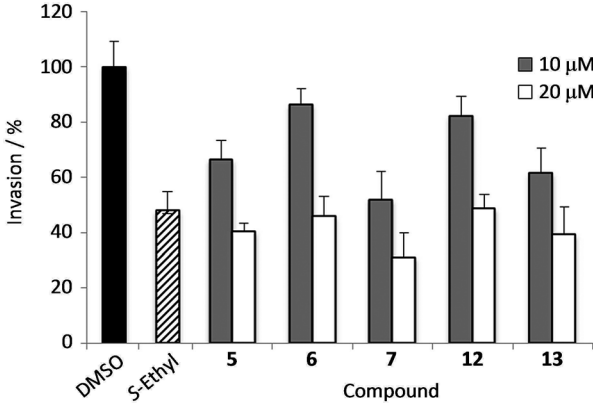

Figure 2. Anti-invasive activities of **5**–**7**, **12** and **13** against the human breast cancer cell line MDA-MB-231 using a Cultrex BME cell invasion assay kit. Each concentration was run in triplicate, and the data are expressed as the mean ± SEM. 4-*S*-Ethylphenylmethylene hydantoin (S-Ethyl)[27] was used as a positive control at 50 μM.

MTT cytotoxicity assay

The MTT assay[30,31] allows for the measurement of cell viability and populations in a quantitative colorimetric fashion by utilizing cellular ability to reduce the MTT reagent to insoluble purple formazan dye. The cytotoxic effects of compounds **1**–**24** were evaluated in MDA-MB-231 (malignant) and MCF10A (normal) cells. Cells were treated with test compound under the same conditions to those used for the wound-healing assay before the addition of MTT (for full details, see the Experimental Section), thus allowing the valid comparison of cytotoxicity and migration inhibition. Against MDA-MB-231 cells, all compounds were found to be noncytotoxic at 20 μM. The cytotoxicity of the most active analogues in the wound-healing assay was also assessed in normal mammary epithelial cells (Figure 3). All active analogues were nontoxic at concentrations equal their IC_{50} values in the wound-healing assay, suggesting

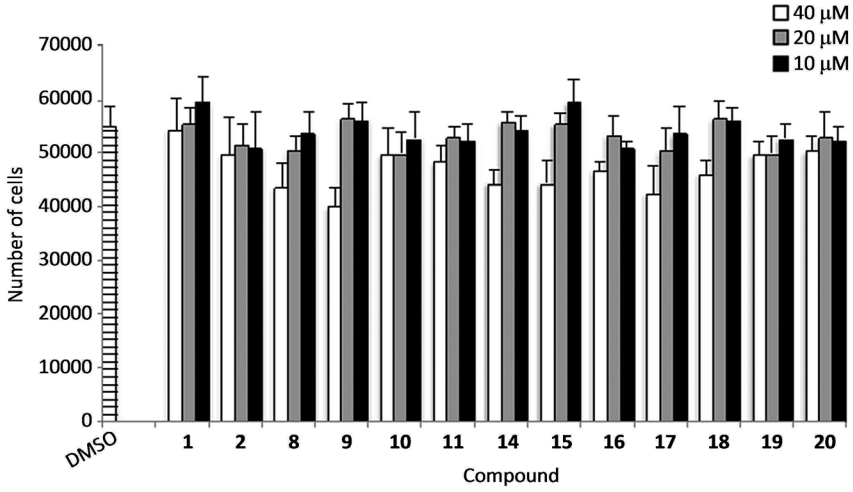

Figure 3. Cytotoxic activity of **1**, **2**, **8**–**11** and **14**–**20** against the normal human mammary epithelium cells MCF10A. Error bars indicate the SD of $n = 3$/dose.

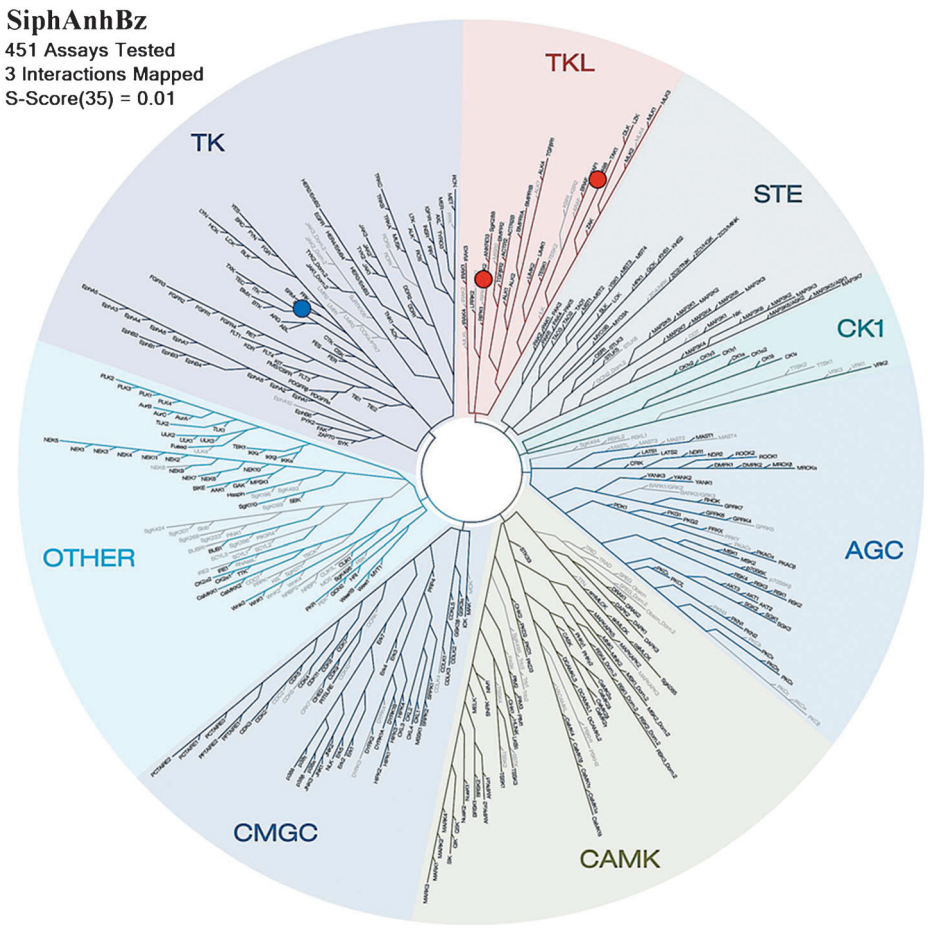

Figure 4. Kinome dendrogram of the selectivity profiling of compound **14** at 10 μM. PTK6 is colored blue, and other target kinases are colored red. Dendrogram was generated using TREE*spot* Software tool with 35% cutoff. Dendrogram reprinted with permission from KINOME*scan*.

selectivity towards malignant cells. Ester and ether analogues **5**–**18** did not show any effect on P-gp accumulation or expression in P-gp-expressing cell lines (data not shown).

KINOME*scan* kinase assay

The KINOME*scan* screening platform employs a novel and proprietary active site-directed competition binding assay to quantitatively measure interactions between test compound and a diverse kinase panel. Analogue **14** was screened against 451 human kinases using an ATP site competition binding assay at a concentration of 10 μM. In the KINOME*scan* panel, only three kinases showed greater than 65% displacement from an immobilized ligand by **14**, which indicate the selectivity of this compound as a kinase inhibitor with selectivity score (S_{35}) less than 0.01 (Figure 4). The S_{35} value is calculated by dividing the number of kinases with less than 35% binding compared with a control by the total number of tested kinases.

Z′-LYTE PTK6 kinase assay

A Z′-LYTE kinase assay[32] was used to assess the ability of the natural products **1** and **2**, and semisynthetic analogues **3**–**24** to inhibit PTK6 phosphorylation in vitro. Table 3 shows the percentage of inhibition at a single dose concentration (25 μM) for compounds **1**–**24**; the results of those compounds with the most positive inhibitory effects are also shown in Figure 5a. Based on this initial assay, analogues **18** and **19** were identified as the most potent inhibitors of PTK6 phosphorylation, with IC_{50} values of 15.1 and 12.3 μM, respectively (Figure 5b). In contrast, parent compounds **1** and **2** showed weak inhibition even at high concentrations, which is consistent with their moderate antimigratory activities.

Table 3. PTK6 inhibitory activity of sipholane analogues **1**–**24** in Z-Lyte kinase assay.[a]

Compd	*I* [%]	Compd	*I* [%]	Compd	*I* [%]
1	−12.0	**9**	−22.4	**17**	67.2
2	−4.2	**10**	25.8	**18**	95.5
3	−17.5	**11**	58.8	**19**	89.6
4	−6.4	**12**	−15.3	**20**	−6.5
5	−9.0	**13**	−12.9	**21**	−10.2
6	−6.8	**14**	38.6	**22**	−3.0
7	−9.0	**15**	−1.5	**23**	−6.0
8	30.2	**16**	29.3	**24**	−4.8

[a] % Inhibition (*I*) of phosphorylation was determined at a compound concentration of 25 μM.

Conclusions

Aromatic sipholenol A esters showed improved antimigratory activity over analogous ether and carbamate derivatives. *p*-Chloro substitution in the aromatic C-4 esters significantly improved the activity of these compounds. In most cases, the antimigratory activity is paralleled with their ability to inhibit PTK6 phosphorylation, and in all cases, no cytotoxicity to normal cells was observed. Aliphatic polar esters, such as compound **7**, showed better activity in the invasion assay compared with aromatic esters. These results demonstrate the potential of the marine natural products sipholanes as a new scaffold for the design of novel PTK6 inhibitors and their potential for development for use to control metastatic breast cancer.

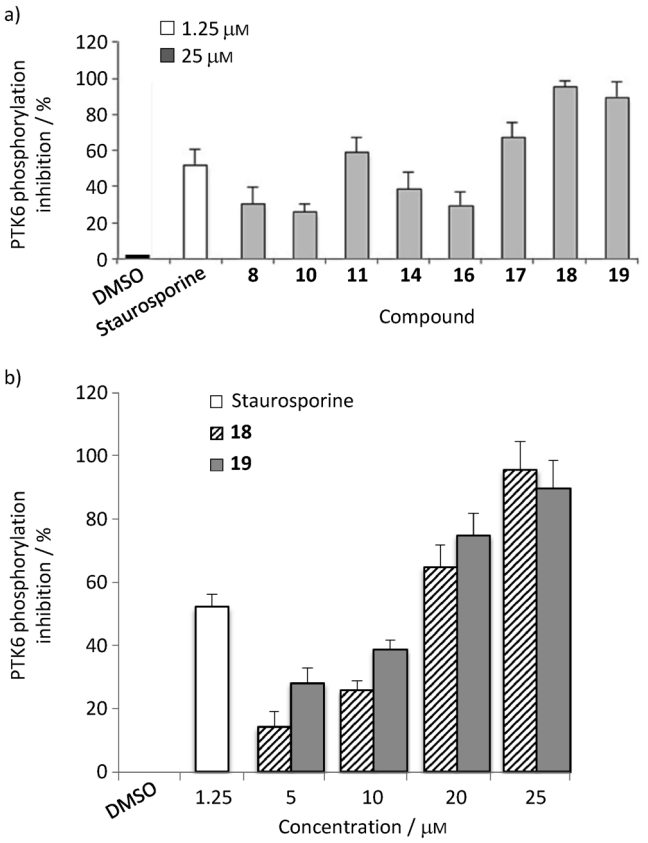

Figure 5. PTK6 phosphorylation inhibition of a) compounds **8**, **10**, **11**, **14** and **16**–**19** at a concentration of 25 μM, and b) compounds **18** and **19** at various concentrations. Results were obtained using a Z′-LYTE assay kit; error bars indicate the SD of $n = 3$/dose; staurosporine was used as a positive control.

Experimental Section

Chemistry

General: Optical rotations ($[\alpha]_D^{25}$) were measured on an analytical Autopol III polarimeter (Rudolph Research, Hackettstown, NJ, USA). IR spectra were recorded on a Varian 800 FT-IR spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$, using tetramethylsilane (TMS) as an internal standard, on a JEOL Eclipse-400 NMR spectrometer operating at 400 MHz (^{1}H) and 100 MHz (^{13}C). High-resolution mass spectrometry (HRMS) experiments were conducted at Louisiana State University on a 6200-TOF LC-MS (Agilent) equipped with multimode source: electrospray ionization (ESI) or atmospheric-pressure chemical ionization (APCI). Analytical HPLC analyses were performed on a DIONEX Summit III system using a Phenomenex Luna 250×4.6 mm, RP-C18 column, and isocratic elution (100% MeOH) with UV detection set at 210–235 nm to verify the purity of each sipholenol and (*Z*)-5-(4-[ethylthio]benzylidene)-imidazolidine-2,4-dione (S-Ethyl). A minimum of 98% HPLC purity for each compound was verified. TLC analysis was carried out on precoated silica gel 60 F_{254} 500 μm TLC plates (EMD Chemicals), using variable proportions of *n*-hexane/EtOAc as a mobile phase. 1% Vanillin in concd H_2SO_4 was used as a visualizing reagent. For column chromatography, silica gel 60 (63–200 μm; Natland Intl, Research Triangle Park, NC, USA) was used.

General procedure A:[6] A solution of sipholenol A (1 equiv) in $CHCl_3$ (5 mL/equiv) was treated with *p*-TsOH·H_2O (0.1 equiv), and the reaction mixture was refluxed for 3 h. Water (5 mL) was added with stirring, and the $CHCl_3$ layer was separated, dried with anhydrous Na_2SO_4, filtered and concentrated to obtain a residue, which was purified by column chromatography (silica gel 60) with *n*-hexane/EtOAc gradient elution to afford compounds **3** and **4**.

General procedure B:[33] *1. Using acid chlorides*: A solution of sipholenol A (1 equiv) in anhydrous CH_2Cl_2 (10 mL/equiv) was treated with Et_3N (5 equiv) and acid chloride (2 equiv), and the reaction mixture was heated at reflux overnight. The reaction was then partitioned with water (10 mL), the organic phase was collected, washed with 1 N HCl (3×2 mL), dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo to obtain a residue, which was purified by column chromatography (silica gel 60) with *n*-hexane/EtOAc gradient elution to afford esters **9**, **11**, **15**, and **17**.

2. Using acid anhydrides: A solution of sipholenol A (1 equiv) in anhydrous CH_2Cl_2 or pyridine (10 mL/equiv) was treated with 4-dimethylaminopyridine (DMAP, 1 equiv) and acid anhydride (2 equiv), and the reaction mixture was heated at reflux overnight. Water (5 mL) was added to stop the reaction, and the resulting mixture was partitioned to collect the organic phase, which was dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The residue obtained was purified by column chromatography (silica gel 60) with $CHCl_3$/MeOH (95:5) isocratic elution to afford esters **5**–**8**, **10**, **12**–**14**, and **16**.

General procedure C (etherification): A solution of sipholenol A (0.1 mmol) in dry DMF (1 mL/equiv) was treated with excess NaH (10 equiv, 60% dispersion in mineral oil) and BnBr (1 equiv). Excess NaH was used to increase the reaction yield. The reaction mixture was stirred overnight at RT, and later for 15 min at 0 °C at the end of reaction. Gradually, 0.2 N HCl (5 mL) was added, and the vapors were allowed to subside. The resulting aqueous phase was extracted with $CHCl_3$ (3×6 mL), and the combined organic phase was dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The residue obtained was purified by column chromatography (silica gel 60) with *n*-hexane/EtOAc gradient elution to afford benzyl ethers **18** and **19**.

General procedure D (carbamoylation):[34] A solution of sipholenol A (1 equiv) in toluene (2 mL/equiv) was treated with the appropriate isocyanate (3 equiv) and Et_3N (10 μL, 0.05 mmol, 0.5 equiv). The reaction was heated at refluxed with stirring for 3 h. Water (10 mL) was then added, and the mixture was extracted with EtOAc (3×10 mL). The combined organic phase was dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by column chromatography (silica gel 60) with $CHCl_3$/MeOH (95:5) isocratic elution to afford carbamates **20** and **21**.

General procedure E: A solution of sipholenone A (1 equiv) in pyridine/EtOH (1:1, 2 mL/equiv) was treated with NH_2OH·HCl (1 equiv), and the solution was heated at reflux for 4 h. Water (10 mL) was added, and the mixture was extracted with EtOAc (3×10 mL). The combined organic phase was dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by column chromatography (silica gel 60) with $CHCl_3$/MeOH (95:5) isocratic elution to afford oxime **22**.

19,20-Anhydrosipholenol A (3) and 10,11,19,20-dianhydrosipholenol A (4): Prepared according to the general procedure A from **1** (150 mg, 0.31 mmol), $CHCl_3$ (5 mL), *p*-TsOH·H_2O (6 mg, 0.031 mmol). Purification by flash chromatography (*n*-hexane/

EtOAc, 80:20) afforded **3** (120 mg, 83.2%) and **4** (5 mg, 3.6%). Characterization data were as reported previously.[6]

4β-*O*-Acetylsipholenol A (5) and **4,19β-*O*-diacetylsipholenol A (6)**: Prepared according to procedure B from **1** (50 mg, 0.1 mmol), anhydrous pyridine (0.5 mL), and Ac_2O (20 μL, 0.21 mmol). Purification by flash chromatography (*n*-hexane/EtOAc, 80:20) afforded **5** (40 mg, 73.5%) and **6** (13 mg, 22.1%). Characterization data were as reported previously.[6]

4β-*O*-Succinylsipholenol A (7): Prepared according to procedure B from **1** (288 mg, 0.6 mmol), anhydrous CH_2Cl_2 (10 mL), DMAP (72 mg, 0.5 mmol) and succinic anhydride (60 mg, 1.2 mmol). Purification by HPLC was performed on a Phenomenex Gemini-NX C18 column (250×4.6 mm, 5 μm) at λ_{210} nm, using a flow rate of 1 mL min^{-1} and H_2O/CH_3CN (4:6) as a mobile phase (isocratic elution) to afford **7** (t_R = 7.5 min) as a colorless oil (167 mg, 47.9%): $[\alpha]_D^{25} = -41.5$ (c = 0.57, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.75 (1H, m, H-11), 0.98 (3H, s, H-24), 1.02 (3H, s, H-31), 1.08 (3H, s, H-30), 1.10 (3H, s, H-27), 1.13 (3H, s, H-26), 1.18 (3H, s, H-25), 1.18 (1H, m, H-12a), 1.24 (1H, m, H-2a), 1.25 (3H, s, H-29), 1.37 (1H, m, H-8a), 1.43 (1H, m, H-2b), 1.48 (1H, m, H-9a), 1.52 (1H, m, H-12b), 1.57 (1H, m, H-20a), 1.62 (1H, m, H-14), 1.63 (1H, m, H-9b), 1.70 (2H, m, H-13a/H-20b), 1.74 (3H, s, H-28), 1.75 (1H, m, H-8b), 1.78 (2H, m, H-3a/H-17a), 1.80 (2H, m, H-18/H-21a), 1.95 (1H, m, H-21b), 1.98 (1H, m, H-3b), 2.01 (1H, m, H-13b), 2.02 (1H, m, H-17b), 2.46 (1H, m, H-22), 2.72 (4H, m, H-2′/H-3′), 3.34 (1H, dd, J = 11.9, 4.0 Hz, H-7), 5.01 (1H, d, J = 6.8 Hz, H-4), 5.45 ppm (1H, m, H-16); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 12.9 (CH_3, C-24), 21.6 (CH_3, C-26), 23.1 (CH_2, C-3), 24.8 (CH_2, C-17), 25.0 (CH_2, C-21), 25.6 (CH_3, C-29), 26.6 (CH_2, C-8), 26.9 (CH_2, C-12), 28.9 (CH_2, C-2′), 29.1 (CH_3, C-25), 29.3 (CH_2, C-3′), 29.6 (CH_3, C-31), 30.0 (CH_3, C-27), 30.3 (CH_3, C-28), 31.8 (CH_3, C-30), 33.7 (CH_2, C-13), 35.0 (CH_2, C-2), 35.5 (qC, C-23), 37.1 (CH_2, C-20), 39.2 (CH_2, C-9), 42.6 (qC, C-1), 48.9 (CH, C-18), 52.8 (CH, C-22), 56.0 (CH, C-11), 57.5 (CH, C-14), 72.5 (qC, C-10), 77.0 (CH, C-7), 77.4 (qC, C-5), 79.7 (CH, C-4), 82.5 (qC, C-19), 121.4 (CH, C-16), 143.2 (qC, C-15), 171.5 (qC, C-1′), 176.5 ppm (qC, C-4′); IR ($CHCl_3$): ν_{max} = 3169, 2987, 2949, 2864, 1730, 1717, 1364, 1162, 1080, 909 cm^{-1}; HRMS-ESI: m/z $[M+Na]^+$ calcd for $C_{34}H_{56}O_7Na$: 599.3924, found: 599.3929.

4β-*O*-Benzoylsipholenol A (8): Prepared according to procedure B from **1** (25 mg, 0.052 mmol), anhydrous CH_2Cl_2 (10 mL), DMAP (6 mg, 0.05 mmol) and benzoyl anhydride (23.8 mg, 0.1 mmol). Purification by flash chromatography ($CHCl_3$/MeOH, 95:5) afforded **8** as an amorphous solid (30 mg, 98.5%): $[\alpha]_D^{25} = -59.6$ (c = 0.96, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.76 (1H, m, H-11), 0.90 (3H, s, H-31), 1.04 (6H, s, H-24/H-30), 1.12 (3H, s, H-27), 1.16 (1H, m, H-12a), 1.24 (6H, s, H-26/H-29), 1.26 (3H, s, H-25), 1.38 (1H, m, H-2a), 1.45 (1H, m, H-8a), 1.52 (1H, m, H-12b), 1.56 (1H, m, H-2b), 1.57 (1H, m, H-14), 1.60 (1H, m, H-20), 1.64 (2H, m, H-9), 1.66 (1H, m, H-13a), 1.68 (1H, m, H-21a), 1.73 (3H, s, H-28), 1.77 (1H, m, H-18), 1.78 (1H, m, H-17a), 1.82 (1H, m, H-8b), 1.87 (1H, m, H-21b), 1.94 (1H, m, H-3a), 1.95 (1H, m, H-13b), 1.98 (1H, m, H-17b), 2.12 (1H, m, H-3b), 2.41 (1H, m, H-22), 3.55 (1H, dd, J = 11.3, 3.6 Hz, H-7), 5.19 (1H, d, J = 6.6 Hz, H-4), 5.44 (1H, dd, J = 9.2, 5.1 Hz, H-16), 7.48 (2H, dd, J = 7.7, 7.7 Hz, H-4′/H-6′), 7.60 (1H, m, H-5′), 8.07 ppm (2H, dd, J = 7.7, 1.4 Hz, H-3′/H-7′); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 13.2 (CH_3, C-24), 21.8 (CH_3, C-26), 23.2 (CH_2, C-3), 24.8 (CH_2, C-17), 24.9 (CH_2, C-21), 25.7 (CH_3, C-29), 26.7 (CH_2, C-8), 26.8 (CH_2, C-12), 29.1 (CH_3, C-25), 29.5 (CH_3, C-31), 30.1 (2C, CH_3, C-27/C-28), 31.7 (CH_3, C-30), 33.8 (CH_2, C-13), 35.1 (CH_2, C-2), 35.4 (qC, C-23), 37.2 (CH_2, C-20), 39.5 (CH_2, C-9), 42.7 (qC, C-1), 48.9 (CH, C-18), 52.8 (CH, C-22), 56.5 (CH, C-11), 57.4 (CH, C-14), 72.4 (qC, C-10), 77.3 (CH, C-7), 77.8 (qC, C-5), 80.2 (CH, C-4), 82.2 (qC, C-19), 121.4 (CH, C-16), 128.6 (2C, CH, C-4′/C-6′), 129.6 (2C, CH, C-3′/C-7′), 130.6 (qC, C-2′), 133.1 (CH, C-5′), 143.2 (qC, C-15), 165.8 ppm (qC, C-1′); IR ($CHCl_3$): ν_{max} = 3454, 2987, 2950, 2930, 2858, 1713, 1282, 1114, 910 cm^{-1}; HRMS-ESI: m/z $[M+Na]^+$ calcd for $C_{37}H_{56}O_5Na$: 603.4020, found: 603.4019.

4β-*O*-Isonicotinoylsipholenol A (9): Prepared according to procedure B from **1** (30 mg, 0.063 mmol), anhydrous CH_2Cl_2 (10 mL), Et_3N (43.5 μL, 0.06 mmol) and isonicotinoyl chloride hydrochloride (22.4 mg, 0.12 mmol). Purification by flash chromatography (*n*-hexane/EtOAc, 70:30) afforded **9** as an amorphous solid (24 mg, 65.5%): $[\alpha]_D^{25} = -59.6$ (c = 0.72, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.76 (1H, m, H-11), 0.90 (3H, s, H-31), 1.04 (6H, s, H-24/H-30), 1.12 (3H, s, H-27), 1.16 (1H, m, H-12a), 1.24 (6H, s, H-26/H-29), 1.26 (3H, s, H-25), 1.31 (1H, m, H-2a), 1.45 (1H, m, H-8a), 1.52 (1H, m, H-12b), 1.54 (1H, m, H-2b), 1.57 (1H, m, H-14), 1.60 (1H, m, H-20), 1.62 (2H, m, H-9), 1.66 (1H, m, H-13a), 1.68 (1H, m, H-21a), 1.73 (3H, s, H-28), 1.77 (1H, m, H-18), 1.78 (1H, m, H-17a), 1.82 (1H, m, H-8b), 1.87 (1H, m, H-21b), 1.95 (1H, m, H-13b), 1.97 (1H, m, H-3a), 1.98 (1H, m, H-17b), 2.13 (1H, m, H-3b), 2.41 (1H, m, H-22), 3.47 (1H, dd, J = 11.7, 4.0 Hz, H-7), 5.21 (1H, d, J = 6.6 Hz, H-4), 5.43 (1H, m, H-16), 7.86 (2H, d, J = 4.4 Hz, H-3′/H-7′), 8.83 ppm (2H, br s, H-4′/H-6′); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 13.2 (CH_3, C-24), 21.8 (CH_3, C-26), 23.2 (CH_2, C-3), 24.8 (CH_2, C-17), 24.9 (CH_2, C-21), 25.7 (CH_3, C-29), 26.7 (CH_2, C-8), 26.8 (CH_2, C-12), 29.1 (CH_3, C-25), 29.5 (CH_3, C-31), 30.1 (2C, CH_3, C-27/C-28), 31.7 (CH_3, C-30), 33.8 (CH_2, C-13), 35.1 (CH_2, C-2), 35.4 (qC, C-23), 37.2 (CH_2, C-20), 39.5 (CH_2, C-9), 42.7 (qC, C-1), 48.9 (CH, C-18), 52.8 (CH, C-22), 56.5 (CH, C-11), 57.4 (CH, C-14), 72.4 (qC, C-10), 77.3 (CH, C-7), 77.8 (qC, C-5), 80.2 (CH, C-4), 82.2 (qC, C-19), 121.4 (CH, C-16), 123.9 (2C, CH, C-3′/C-7′), 137.9 (qC, C-2′), 143.0 (qC, C-15), 150.8 (2C, CH, C-4′/C-6′), 164.3 ppm (qC, C-1′); IR ($CHCl_3$): ν_{max} = 3432, 2987, 2952, 2931, 2859, 1724, 1287, 1122, 910 cm^{-1}; HRMS-ESI: m/z $[M+H]^+$ calcd for $C_{36}H_{56}NO_5$: 582.4153, found: 582.4157.

4β-*O*-4′-Methylbenzoylsipholenol A (10): Prepared according to procedure B from **1** (25 mg, 0.052 mmol), anhydrous CH_2Cl_2 (10 mL), DMAP (6 mg, 0.05 mmol) and 4-methylbenzoic anhydride (23.8 mg, 0.1 mmol). Purification by flash chromatography ($CHCl_3$/MeOH, 95:5) afforded **10** as an amorphous solid (30 mg, 98.5%): $[\alpha]_D^{25} = -57.6$ (c = 0.36, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.76 (1H, m, H-11), 0.90 (3H, s, H-31), 1.04 (6H, s, H-24/H-30), 1.12 (3H, s, H-27), 1.16 (1H, m, H-12a), 1.24 (6H, s, H-26/H-29), 1.26 (3H, s, H-25), 1.38 (1H, m, H-2a), 1.45 (1H, m, H-8a), 1.52 (1H, m, H-12b), 1.56 (1H, m, H-2b), 1.57 (1H, m, H-14), 1.60 (1H, m, H-20), 1.64 (2H, m, H-9), 1.66 (1H, m, H-13a), 1.68 (1H, m, H-21a), 1.73 (3H, s, H-28), 1.77 (1H, m, H-18), 1.78 (1H, m, H-17a), 1.82 (1H, m, H-8b), 1.87 (1H, m, H-21b), 1.94 (1H, m, H-3a), 1.95 (1H, m, H-13b), 1.98 (1H, m, H-17b), 2.12 (1H, m, H-3b), 2.41 (1H, m, H-22), 2.44 (3H, m, H-8′), 3.55 (1H, dd, J = 11.3, 3.6 Hz, H-7), 5.19 (1H, d, J = 6.6 Hz, H-4), 5.44 (1H, dd, J = 9.2, 5.1 Hz, H-16), 7.26 (2H, d, J = 8.2 Hz, H-4′/H-6′), 7.98 ppm (2H, d, J = 8.2 Hz, H-3′/H-7′); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 13.2 (CH_3, C-24), 21.3 (CH_3, C-8′), 21.8 (CH_3, C-26), 23.2 (CH_2, C-3), 24.8 (CH_2, C-17), 24.9 (CH_2, C-21), 25.7 (CH_3, C-29), 26.7 (CH_2, C-8), 26.8 (CH_2, C-12), 29.1 (CH_3, C-25), 29.5 (CH_3, C-31), 30.1 (2C, CH_3, C-27/C-28), 31.7 (CH_3, C-30), 33.8 (CH_2, C-13), 35.1 (CH_2, C-2), 35.4 (qC, C-23), 37.2 (CH_2, C-20), 39.5 (CH_2, C-9), 42.7 (qC, C-1), 48.9 (CH, C-18), 52.8 (CH, C-22), 56.5 (CH, C-11), 57.4 (CH, C-14), 72.4 (qC, C-10), 77.3 (CH, C-7), 77.8 (qC, C-5), 80.2 (CH, C-4), 82.2 (qC, C-19), 121.4 (CH, C-16), 127.8 (qC, C-5′), 129.6 (2C, CH, C-4′/C-6′), 130.2 (2C, CH, C-3′/C-7′), 143.2 (qC, C-15), 143.8 (qC, C-2′), 165.8 ppm (qC, C-1′); IR ($CHCl_3$): ν_{max} = 3454, 2987, 2950, 2930, 2858, 1713, 1282, 1114, 910 cm^{-1}; HRMS-ESI: m/z $[M+H]^+$ calcd for $C_{38}H_{59}O_5$: 595.4357, found: 595.4324.

4β-*O*-4′-Chlorobenzoylsipholenol A (11): Prepared according to procedure B from **1** (25 mg, 0.052 mmol), anhydrous CH_2Cl_2 (10 mL), DMAP (6 mg, 0.05 mmol) and 4-chlorobenzoyl anhydride (23.8 mg, 0.12 mmol). Purification by flash chromatography ($CHCl_3$/MeOH, 95:5) gave **11** as an amorphous solid (30 mg, 98.5%): $[\alpha]_D^{25} = -61.3$ ($c = 0.52$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.76$ (1H, m, H-11), 0.90 (3H, s, H-31), 1.04 (6H, s, H-24/H-30), 1.12 (3H, s, H-27), 1.16 (1H, m, H-12a), 1.24 (6H, s, H-26/H-29), 1.26 (3H, s, H-25), 1.38 (1H, m, H-2a), 1.45 (1H, m, H-8a), 1.52 (1H, m, H-12b), 1.56 (1H, m, H-2b), 1.57 (1H, m, H-14), 1.60 (1H, m, H-20), 1.64 (2H, m, H-9), 1.66 (1H, m, H-13a), 1.68 (1H, m, H-21a), 1.73 (3H, s, H-28), 1.77 (1H, m, H-18), 1.78 (1H, m, H-17a), 1.82 (1H, m, H-8b), 1.87 (1H, m, H-21b), 1.94 (1H, m, H-3a), 1.95 (1H, m, H-13b), 1.98 (1H, m, H-17b), 2.12 (1H, m, H-3b), 2.41 (1H, m, H-22), 3.55 (1H, dd, J = 11.3, 3.6 Hz, H-7), 5.19 (1H, d, J = 6.6 Hz, H-4), 5.44 (1H, dd, J = 9.2, 5.1 Hz, H-16), 7.44 (2H, d, J = 8.6 Hz, H-4′/H-6′), 7.97 ppm (2H, d, J = 8.6 Hz, H-3′/H-7′); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 13.2$ (CH_3, C-24), 21.8 (CH_3, C-26), 23.2 (CH_2, C-3), 24.8 (CH_2, C-17), 24.9 (CH_2, C-21), 25.7 (CH_3, C-29), 26.7 (CH_2, C-8), 26.8 (CH_2, C-12), 29.1 (CH_3, C-25), 29.5 (CH_3, C-31), 30.1 (2C, CH_3, C-27/C-28), 31.7 (CH_3, C-30), 33.8 (CH_2, C-13), 35.1 (CH_2, C-2), 35.4 (qC, C-23), 37.2 (CH_2, C-20), 39.5 (CH_2, C-9), 42.7 (qC, C-1), 48.9 (CH, C-18), 52.8 (CH, C-22), 56.5 (CH, C-11), 57.4 (CH, C-14), 72.4 (qC, C-10), 77.3 (CH, C-7), 77.8 (qC, C-5), 80.2 (CH, C-4), 82.2 (qC, C-19), 121.4 (CH, C-16), 128.9 (qC, C-5′), 129.0 (2C, CH, C-4′/C-6′), 130.9 (2C, CH, C-3′/C-7′), 139.6 (qC, C-2′), 143.2 (qC, C-15), 164.9 ppm (qC, C-1′); IR ($CHCl_3$): $\nu_{max} = 3454$, 2987, 2950, 2930, 2858, 1713, 1282, 1114, 910 cm^{-1}; HRMS-ESI: m/z $[M+Na]^+$ calcd for $C_{37}H_{55}ClO_5Na$: 637.3636, found: 637.3685.

4β-*O*-Acetyl-19,20-anhydrosipholenol A (12): Prepared according to procedure B from **3** (30 mg, 0.065 mmol), anhydrous CH_2Cl_2 (3 mL), DMAP (8 mg, 0.06 mmol) and Ac_2O (12.3 μL, 0.18 mmol). Purification by flash chromatography (*n*-hexane/EtOAc, 80:20) gave **12** as a colorless oil (22 mg, 67.2%): $[\alpha]_D^{25} = -4.6$ ($c = 0.69$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.74$ (1H, m, H-11), 0.98 (3H, s, H-24), 1.01 (3H, s, H-31), 1.08 (6H, s, H-27/H-30), 1.13 (3H, s, H-26), 1.18 (3H, s, H-25), 1.19 (1H, m, H-12a), 1.26 (1H, m, H-2a), 1.37 (1H, m, H-8a), 1.46 (1H, m, H-2b), 1.53 (1H, m, H-12b), 1.60 (1H, m, H-9), 1.66 (3H, s, H-29), 1.68 (2H, m, H-14/H-21a), 1.74 (1H, m, H-8b), 1.75 (3H, s, H-28), 1.76 (1H, m, H-3a), 1.84 (1H, m, H-17a), 1.94 (1H, m, H-3b), 2.05 (1H, m, H-21b), 2.06 (1H, m, H-17b), 2.07 (1H, m, H-22), 2.14 (3H, s, H-2′), 2.14 (1H, m, H-13a), 2.31 (1H, m, H-18), 2.41 (1H, m, H-13b), 3.34 (1H, dd, J = 11.9, 4.2 Hz, H-7), 4.97 (1H, d, J = 6.6 Hz, H-4), 5.27 (1H, br s, H-20), 5.48 ppm (1H, m, H-16); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 12.9$ (CH_3, C-24), 15.4 (CH_3, C-29), 21.4 (CH_3, C-2′), 21.6 (CH_3, C-26), 23.0 (CH_2, C-3), 26.5 (CH_2, C-12), 26.6 (CH_2, C-8), 26.9 (CH_2, C-17), 29.1 (CH_3, C-25), 29.4 (CH_3, C-31), 30.1 (CH_3, C-27), 30.3 (CH_3, C-28), 31.3 (CH_3, C-30), 33.3 (CH_2, C-21), 34.0 (CH_2, C-13), 35.0 (CH_2, C-2), 35.3 (qC, C-23), 39.2 (CH_2, C-9), 42.6 (qC, C-1), 45.2 (CH, C-18), 56.0 (CH, C-11), 56.4 (CH, C-22), 57.1 (CH, C-14), 72.3 (qC, C-10), 76.9 (CH, C-7), 77.3 (qC, C-5), 79.2 (CH, C-4), 121.6 (CH, C-16), 123.4 (CH, C-20), 143.0 (qC, C-15), 146.7 (qC, C-19), 170.4 ppm (qC, C-1′); IR ($CHCl_3$): $\nu_{max} = 2986$, 2930, 2858, 1728, 1461, 1365, 1262, 1080, 910 cm^{-1}; HRMS-ESI: m/z $[M+H]^+$ calcd for $C_{32}H_{53}O_4$: 501.3938, found: 501.3912.

4β-*O*-Succinyl-19,20-anhydrosipholenol A (13): Prepared according to procedure B from **3** (25 mg, 0.055 mmol), anhydrous CH_2Cl_2 (10 mL), DMAP (6.7 mg, 0.06 mmol) and succinic anhydride (22 mg, 0.16 mmol). Purification by flash chromatography ($CHCl_3$/MeOH, 95:5) gave **13** as a colorless oil (19.6 mg, 64.4%): $[\alpha]_D^{25} = -4.3$ ($c = 0.49$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.74$ (1H, m, H-11), 0.99 (3H, s, H-24), 1.01 (3H, s, H-31), 1.09 (6H, s, H-27/H-30), 1.13 (3H, s, H-26), 1.19 (1H, m, H-12a), 1.19 (3H, s, H-25), 1.27 (1H, m, H-2a), 1.38 (1H, m, H-8a), 1.47 (1H, m, H-9a), 1.48 (1H, m, H-2b), 1.52 (1H, m, H-12b), 1.62 (1H, m, H-9b), 1.67 (1H, m, H-21a), 1.67 (3H, s, H-29), 1.68 (1H, m, H-14), 1.75 (3H, s, H-28), 1.76 (1H, m, H-8b), 1.78 (1H, m, H-3a), 1.86 (1H, m, H-17a), 1.98 (1H, m, H-3b), 2.06 (1H, m, H-21b), 2.07 (1H, m, H-17b), 2.08 (1H, m, H-22), 2.16 (1H, m, H-13a), 2.32 (1H, m, H-18), 2.42 (1H, m, H-13b), 2.73 (2H, m, H-2′), 2.73 (2H, m, H-3′), 3.34 (1H, dd, J = 11.7, 4.0 Hz, H-7), 5.01 (1H, d, J = 6.6 Hz, H-4), 5.27 (1H, br s, H-20), 5.48 ppm (1H, m, H-16); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 13.0$ (CH_3, C-24), 15.5 (CH_3, C-29), 21.5 (CH_3, C-26), 23.1 (CH_2, C-3), 26.5 (CH_2, C-12), 26.6 (CH_2, C-8), 26.9 (CH_2, C-17), 29.0 (CH_2, C-3′), 29.1 (CH_3, C-25), 29.2 (CH_2, C-2′), 29.6 (CH_3, C-31), 30.0 (CH_3, C-27), 30.2 (CH_3, C-28), 31.3 (CH_3, C-30), 33.3 (CH_2, C-21), 34.0 (CH_2, C-13), 35.0 (CH_2, C-2), 35.3 (qC, C-23), 39.2 (CH_2, C-9), 42.6 (qC, C-1), 45.2 (CH, C-18), 56.0 (CH, C-11), 56.4 (CH, C-22), 57.1 (CH, C-14), 72.5 (qC, C-10), 77.0 (CH, C-7), 77.8 (qC, C-5), 79.8 (CH, C-4), 121.7 (CH, C-16), 123.5 (CH, C-20), 143.0 (qC, C-15), 146.7 (qC, C-19), 171.4 (qC, C-1′), 177.2 ppm (qC, C-4′); IR ($CHCl_3$): $\nu_{max} = 3165$, 2987, 2929, 2857, 1729, 1717, 1364, 1164, 1080, 909 cm^{-1}; HRMS-ESI: m/z $[M+H]^+$ calcd for $C_{34}H_{55}O_6$: 559.3993, found: 559.3969.

4β-*O*-Benzoyl-19,20-anhydrosipholenol A (14): Prepared according to procedure B using **3** (20 mg, 0.044 mmol), anhydrous CH_2Cl_2 (10 mL), DMAP (5.3 mg, 0.05 mmol) and benzoic anhydride (30 mg, 0.12 mmol). Purification by flash chromatography (*n*-hexane/EtOAc, 70:30) gave **14** as a colorless oil (10.4 mg, 42.4%): $[\alpha]_D^{25} = -25.9$ ($c = 0.21$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.76$ (1H, m, H-11), 0.89 (3H, s, H-31), 1.04 (6H, s, H-24/H-30), 1.11 (3H, s, H-27), 1.18 (1H, m, H-12a), 1.24 (3H, s, H-26), 1.27 (3H, s, H-25), 1.40 (1H, m, H-2a), 1.45 (1H, m, H-8a), 1.50 (1H, m, H-9a), 1.52 (1H, m, H-12b), 1.56 (1H, m, H-2b), 1.66 (2H, m, H-14/H-21a), 1.66 (3H, s, H-29), 1.68 (1H, m, H-9b), 1.74 (3H, s, H-28), 1.82 (1H, m, H-8b), 1.84 (1H, m, H-17a), 1.96 (1H, m, H-3a), 1.98 (1H, m, H-21b), 2.01 (1H, m, H-22), 2.02 (1H, m, H-13a), 2.03 (1H, m, H-17b), 2.12 (1H, m, H-3b), 2.26 (2H, m, H-13b/H-18), 3.55 (1H, dd, J = 11.9, 4.2 Hz, H-7), 5.20 (1H, d, J = 6.6 Hz, H-4), 5.23 (1H, br s, H-20), 5.47 (1H, m, H-16), 7.50 (2H, dd, J = 7.7, 7.7 Hz, H-4′/H-6′), 7.61 (1H, dd, J = 7.3, 7.3 Hz, H-5′), 8.07 ppm (2H, dd, J = 7.9, 1.1 Hz, H-3′/H-7′); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 13.2$ (CH_3, C-24), 15.4 (CH_3, C-29), 21.8 (CH_3, C-26), 23.2 (CH_2, C-3), 26.3 (CH_2, C-12), 26.7 (CH_2, C-8), 26.9 (CH_2, C-17), 29.1 (CH_3, C-25), 29.2 (CH_3, C-31), 30.2 (CH_3, C-28), 30.8 (CH_3, C-27), 31.2 (CH_3, C-30), 33.3 (CH_2, C-21), 34.0 (CH_2, C-13), 35.1 (CH_2, C-2), 35.2 (qC, C-23), 39.5 (CH_2, C-9), 42.7 (qC, C-1), 45.2 (CH, C-18), 56.3 (CH, C-22), 56.5 (CH, C-11), 57.1 (CH, C-14), 72.4 (qC, C-10), 77.3 (CH, C-7), 77.8 (qC, C-5), 80.1 (CH, C-4), 121.8 (CH, C-16), 123.4 (CH, C-20), 128.6 (2C, CH, C-4′/C-6′), 129.5 (2C, CH, C-3′/C-7′), 130.5 (qC, C-2′), 133.2 (CH, C-5′), 143.0 (qC, C-15), 146.5 (qC, C-19), 165.8 ppm (qC, C-1′); IR ($CHCl_3$): $\nu_{max} = 2985$, 2929, 2857, 1713, 1451, 1364, 1280, 1114, 909 cm^{-1}; HRMS-ESI: m/z $[M+H]^+$ calcd for $C_{37}H_{55}O_4$: 63.4095, found: 563.4095.

4β-*O*-Isonicotinoyl-19,20-anhydrosipholenol A (15): Prepared according to procedure B using **3** (18 mg, 0.065 mmol), anhydrous CH_2Cl_2 (10 mL), Et_3N (27.2 μL, 0.032 mmol) and isonicotinoyl chloride hydrochloride (14 mg, 0.2 mmol). Purification by flash chromatography (*n*-hexane/EtOAc, 70:30) gave **15** as a colorless oil (6 mg, 27.1%): $[\alpha]_D^{25} = -24.3$ ($c = 0.25$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.76$ (1H, m, H-11), 0.89 (3H, s, H-31), 1.04 (6H, s, H-24/H-30), 1.11 (3H, s, H-27), 1.18 (1H, m, H-12a), 1.24 (3H, s, H-26), 1.27 (3H, s, H-25), 1.35 (1H, m, H-2a), 1.45 (1H, m, H-8a), 1.50 (1H, m, H-9a), 1.52 (1H, m, H-12b), 1.54 (1H, m, H-2b), 1.66 (3H, s, H-29), 1.66 (2H, m, H-14/H-21a), 1.68 (1H, m, H-9b), 1.74 (3H, s, H-28), 1.82 (1H, m, H-8b), 1.84 (1H, m, H-17a), 1.95 (1H, m, H-3a), 1.98 (1H, m, H-21b), 2.01 (1H, m, H-22), 2.02 (1H, m, H-13a), 2.03 (1H, m, H-

17b), 2.12 (1H, m, H-3b), 2.26 (2H, m, H-13b/H-18), 3.47 (1H, dd, $J=11.7$, 4.0 Hz, H-7), 5.23 (1H, d, $J=7.0$ Hz, H-4), 5.25 (1H, br s, H-20), 5.47 (1H, m, H-16), 7.86 (2H, d, $J=4.0$ Hz, H-3′/H-7′), 8.85 ppm (2H, br s, H-4′/H-6′); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=13.2$ (CH_3, C-24), 15.4 (CH_3, C-29), 21.8 (CH_3, C-26), 23.2 (CH_2, C-3), 26.3 (CH_2, C-12), 26.7 (CH_2, C-8), 26.9 (CH_2, C-17), 29.1 (CH_3, C-25), 29.2 (CH_3, C-31), 30.8 (CH_3, C-27), 30.2 (CH_3, C-28), 31.2 (CH_3, C-30), 33.3 (CH_2, C-21), 34.0 (CH_2, C-13), 35.1 (CH_2, C-2), 35.2 (qC, C-23), 39.5 (CH_2, C-9), 42.7 (qC, C-1), 45.2 (CH, C-18), 56.3 (CH, C-22), 56.5 (CH, C-11), 57.1 (CH, C-14), 72.4 (qC, C-10), 77.3 (CH, C-7), 77.8 (qC, C-5), 80.1 (CH, C-4), 121.8 (CH, C-16), 122.9 (2C, CH, C-3′/C-7′), 123.4 (CH, C-20), 137.8 (qC, C-2′), 142.9 (qC, C-15), 146.6 (qC, C-19), 150.8 (2C, CH, C-4′/C-6′), 164.4 ppm (qC, C-1′); IR ($CHCl_3$): $\nu_{max}=2985$, 2953, 2929, 2856, 1724, 1286, 1123 cm^{-1}; HRMS-ESI: m/z $[M+H]^+$ calcd for $C_{36}H_{54}NO_4$, 564.4047, found: 564.4052.

4β-*O*-4′-Methylbenzoyl-19,20-anhydrosipholenol A (16): Prepared according to procedure B using **3** (20 mg, 0.044 mmol), anhydrous CH_2Cl_2 (10 mL), DMAP (5.3 mg, 0.05 mmol) and 4-methylbenzoic anhydride (30 mg, 0.18 mmol). Purification by flash chromatography (*n*-hexane/EtOAc, 95:5→80:20) gave **14** as a colorless oil (10.4 mg, 42.4%): $[\alpha]_D^{25}=-23.9$ ($c=0.36$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=0.76$ (1H, m, H-11), 0.89 (3H, s, H-31), 1.04 (6H, s, H-24/H-30), 1.11 (3H, s, H-27), 1.18 (1H, m, H-12a), 1.24 (3H, s, H-26), 1.27 (3H, s, H-25), 1.40 (1H, m, H-2a), 1.45 (1H, m, H-8a), 1.50 (1H, m, H-9a), 1.52 (1H, m, H-12b), 1.56 (1H, m, H-2b), 1.57 (1H, m, H-14), 1.66 (2H, m, H-13a/H-21a), 1.66 (3H, s, H-29), 1.68 (1H, m, H-9b), 1.74 (3H, s, H-28), 1.78 (1H, m, H-17a), 1.82 (1H, m, H-8b), 1.95 (1H, m, H-13b), 1.96 (1H, m, H-3a), 1.98 (2H, m, H-17b/H-21b), 2.01 (1H, m, H-22), 2.03 (1H, m, H-18), 2.12 (1H, m, H-3b), 2.44 (3H, m, H-8′), 3.55 (1H, dd, $J=11.9$, 4.2 Hz, H-7), 5.20 (1H, d, $J=6.6$ Hz, H-4), 5.23 (1H, br s, H-20), 5.47 (1H, m, H-16), 7.26 (2H, d, $J=7.8$ Hz, H-4′/H-6′), 7.98 ppm (2H, d, $J=7.8$ Hz, H-3′/H-7′); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=13.2$ (CH_3, C-24), 15.4 (CH_3, C-29), 21.3 (CH_3, C-8′), 21.8 (CH_3, C-26), 23.2 (CH_2, C-3), 26.3 (CH_2, C-12), 26.7 (CH_2, C-8), 26.9 (CH_2, C-17), 29.1 (CH_3, C-25), 29.2 (CH_3, C-31), 30.2 (CH_3, C-28), 30.8 (CH_3, C-27), 31.2 (CH_3, C-30), 33.3 (CH_2, C-21), 34.0 (CH_2, C-13), 35.1 (CH_2, C-2), 35.2 (qC, C-23), 39.5 (CH_2, C-9), 42.7 (qC, C-1), 45.2 (CH, C-18), 56.3 (CH, C-22), 56.5 (CH, C-11), 57.1 (CH, C-14), 72.4 (qC, C-10), 77.3 (CH, C-7), 77.8 (qC, C-5), 80.1 (CH, C-4), 121.8 (CH, C-16), 123.4 (CH, C-20), 127.8 (qC, C-5′), 129.6 (2C, CH, C-4′/C-6′), 130.2 (2C, CH, C-3′/C-7′), 143.0 (qC, C-15), 143.8 (qC, C-2′), 146.5 (qC, C-19), 165.8 ppm (qC, C-1′); IR ($CHCl_3$): $\nu_{max}=2985$, 2929, 2857, 1713, 1451, 1364, 1280, 1114, 909 cm^{-1}; HRMS-ESI: m/z $[M+H]^+$ calcd for $C_{38}H_{57}O_4$: 577.4251, found: 577.4272.

4β-*O*-4′-Chlorobenzoyl-19,20-anhydrosipholenol A (17): Prepared according to procedure B starting with **3** (25 mg, 0.052 mmol), anhydrous CH_2Cl_2 (10 mL), DMAP (6 mg, 0.05 mmol) and 4-chlorobenzoic anhydride (23.8 mg, 0.16 mmol). Purification by flash chromatography (*n*-hexane/EtOAc, 95:5) gave **11** as an amorphous solid (30 mg, 98.5%): $[\alpha]_D^{25}=-26.8$ ($c=0.27$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=0.76$ (1H, m, H-11), 0.89 (3H, s, H-31), 1.04 (6H, s, H-24/H-30), 1.11 (3H, s, H-27), 1.18 (1H, m, H-12a), 1.24 (3H, s, H-26), 1.27 (3H, s, H-25), 1.40 (1H, m, H-2a), 1.45 (1H, m, H-8a), 1.50 (1H, m, H-9a), 1.52 (1H, m, H-12b), 1.56 (1H, m, H-2b), 1.66 (3H, s, H-29), 1.66 (2H, m, H-14/H-21a), 1.68 (1H, m, H-9b), 1.74 (3H, s, H-28), 1.82 (1H, m, H-8b), 1.84 (1H, m, H-17a), 1.96 (1H, m, H-3a), 1.98 (1H, m, H-21b), 2.01 (1H, m, H-22), 2.02 (1H, m, H-13a), 2.03 (1H, m, H-17b), 2.12 (1H, m, H-3b), 2.26 (2H, m, H-13b/H-18), 3.55 (1H, dd, $J=11.9$, 4.2 Hz, H-7), 5.20 (1H, d, $J=6.6$ Hz, H-4), 5.23 (1H, br s, H-20), 5.47 (1H, m, H-16), 7.44 (2H, d, $J=8.6$ Hz, H-4′/H-6′), 7.97 ppm (2H, d, $J=8.6$ Hz, H-3′/H-7′); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=13.2$ (CH_3, C-24), 15.4 (CH_3, C-29), 21.8 (CH_3, C-26), 23.2 (CH_2, C-3), 26.3 (CH_2, C-12), 26.7 (CH_2, C-8), 26.9 (CH_2, C-17), 29.1 (CH_3, C-25), 29.2 (CH_3, C-31), 30.2 (CH_3, C-28), 30.8 (CH_3, C-27), 31.2 (CH_3, C-30), 33.3 (CH_2, C-21), 34.0 (CH_2, C-13), 35.1 (CH_2, C-2), 35.2 (qC, C-23), 39.5 (CH_2, C-9), 42.7 (qC, C-1), 45.2 (CH, C-18), 56.3 (CH, C-22), 56.5 (CH, C-11), 57.1 (CH, C-14), 72.4 (qC, C-10), 77.3 (CH, C-7), 77.8 (qC, C-5), 80.1 (CH, C-4), 121.8 (CH, C-16), 123.4 (CH, C-20), 128.9 (qC, C-5′), 129.0 (2C, CH, C-4′/C-6′), 130.9 (2C, CH, C-3′/C-7′), 139.6 (qC, C-2′), 143.0 (qC, C-15), 146.5 (qC, C-19), 164.9 ppm (qC, C-1′); IR ($CHCl_3$): $\nu_{max}=2985$, 2929, 2857, 1713, 1451, 1364, 1280, 1114, 909 cm^{-1}; HRMS-ESI: m/z $[M+H]^+$ calcd for $C_{37}H_{54}ClO_4$: 597.3711, found: 597.3711.

4β-*O*-Benzylsipholenol A (18): Prepared according to procedure C using **1** (48 mg, 0.1 mmol), dry DMF (1 mL), NaH (10 equiv) and BnBr (12 μL, 1 equiv). Purification by flash chromatography (*n*-hexane/EtOAc, 90:10) gave **18** as an amorphous solid (36 mg, 63.1%): $[\alpha]_D^{25}=-48.8$ ($c=0.53$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=0.78$ (1H, m, H-11), 0.98 (3H, s, H-24), 1.04 (3H, s, H-31), 1.06 (3H, s, H-27), 1.09 (3H, s, H-30), 1.11 (3H, s, H-25), 1.18 (1H, m, H-12a), 1.25 (3H, s, H-29), 1.27 (3H, s, H-26), 1.36 (1H, m, H-8a), 1.43 (2H, m, H-2), 1.50 (1H, m, H-9a), 1.51 (1H, m, H-12b), 1.57 (1H, m, H-9b), 1.61 (1H, m, H-14), 1.62 (1H, m, H-20a), 1.71 (1H, m, H-8b), 1.74 (1H, m, H-13a), 1.75 (3H, s, H-28), 1.78 (2H, m, H-17a/H-20b), 1.80 (2H, m, H-3a/H-18), 1.82 (1H, m, H-21a), 1.92 (1H, m, H-3b), 1.94 (1H, m, H-21b), 1.97 (1H, m, H-13b), 2.01 (1H, m, H-17b), 2.48 (1H, m, H-22), 3.40 (1H, d, $J=6.6$ Hz, H-4), 3.50 (1H, dd, $J=11.7$, 4.0 Hz, H-7), 4.31 (1H, d, $J=11.9$ Hz, H-1′b), 4.55 (1H, d, $J=11.9$ Hz, H-1′a), 5.45 (1H, dd, $J=9.1$, 5.1 Hz, H-16), 7.29 (1H, m, H-5′), 7.35 (2H, m, H-4′/H-6′), 7.36 ppm (2H, m, H-3′/H-7′); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=13.1$ (CH_3, C-24), 21.7 (CH_2, C-3), 22.2 (CH_3, C-26), 24.8 (CH_2, C-17), 25.1 (CH_2, C-21), 25.7 (CH_3, C-29), 26.6 (CH_2, C-8), 26.9 (CH_2, C-12), 29.5 (CH_3, C-31), 29.6 (CH_3, C-25), 29.9 (CH_3, C-27), 30.2 (CH_3, C-28), 31.7 (CH_3, C-30), 34.0 (CH_2, C-13), 34.1 (CH_2, C-2), 35.5 (qC, C-23), 37.4 (CH_2, C-20), 39.2 (CH_2, C-9), 42.5 (qC, C-1), 48.9 (CH, C-18), 52.8 (CH, C-22), 55.9 (CH, C-11), 57.6 (CH, C-14), 71.6 (CH_2, C-1′), 72.5 (qC, C-10), 76.5 (CH, C-7), 78.6 (qC, C-5), 82.2 (qC, C-19), 84.6 (CH, C-4), 121.3 (CH, C-16), 127.6 (3C, CH, C-3′/C-5′/C-7′), 128.4 (2C, CH, C-4′/C-6′), 139.2 (qC, C-2′), 143.2 ppm (qC, C-15); IR ($CHCl_3$): $\nu_{max}=3454$, 2987, 2949, 2863, 1463, 1455, 1376, 1363, 1082, 909 cm^{-1}; HRMS-ESI: m/z $[M+H]^+$ calcd for $C_{37}H_{59}O_4$: 567.4413, found: 567.4405.

4β-*O*-Benzyl-19,20-anhydrosipholenol A (19): Prepared according to procedure C from **3** (46 mg, 0.1 mmol), dry DMF (1 mL), NaH (10 equiv) and BnBr (12 μL, 1 equiv). Purification by flash chromatography (*n*-hexane/EtOAc, 70:30) gave **19** as a colorless oil (14 mg, 25.4%): $[\alpha]_D^{25}=+2.4$ ($c=0.38$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=0.78$ (1H, m, H-11), 0.98 (3H, s, H-24), 1.02 (3H, s, H-31), 1.05 (3H, s, H-27), 1.09 (3H, s, H-30), 1.11 (3H, s, H-25), 1.21 (1H, m, H-12a), 1.26 (3H, s, H-26), 1.36 (1H, m, H-8a), 1.46 (2H, m, H-2), 1.48 (1H, m, H-9a), 1.51 (1H, m, H-12b), 1.56 (1H, m, H-9b), 1.67 (3H, s, H-29), 1.70 (2H, m, H-14/H-21a), 1.72 (1H, m, H-8b), 1.75 (3H, s, H-28), 1.80 (1H, m, H-3a), 1.85 (1H, m, H-17a), 1.94 (1H, m, H-3b), 2.02 (1H, m, H-21b), 2.05 (1H, m, H-17b), 2.06 (1H, m, H-22), 2.14 (1H, m, H-13a), 2.32 (1H, m, H-18), 2.42 (1H, m, H-13b), 3.40 (1H, d, $J=6.6$ Hz, H-4), 3.50 (1H, dd, $J=11.9$, 4.2 Hz, H-7), 4.31 (1H, d, $J=11.7$ Hz, H-1′a), 4.56 (1H, d, $J=11.7$ Hz, H-1′b), 5.29 (1H, br s, H-20), 5.48 (1H, m, H-16), 7.30 (1H, m, H-5′), 7.35 (2H, m, H-4′/H-6′), 7.37 ppm (2H, m, H-3′/H-7′); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=13.2$ (CH_3, C-24), 15.4 (CH_3, C-29), 21.7 (CH_2, C-3), 22.2 (CH_3, C-26), 26.4 (CH_2, C-12), 26.6 (CH_2, C-8), 26.9 (CH_2, C-17), 29.5 (CH_3, C-31), 29.6 (CH_3, C-25), 30.0 (CH_3, C-27), 30.2 (CH_3, C-28), 31.3 (CH_3, C-30), 33.4 (CH_2, C-21), 34.0 (CH_2, C-13), 34.1 (CH_2, C-2), 35.5 (qC, C-23), 39.2 (CH_2, C-9), 42.4 (qC, C-1), 45.3 (CH, C-18), 56.0 (CH, C-11), 56.4 (CH,

C-22), 57.1 (CH, C-14), 71.5 (CH_2, C-1′), 72.5 (qC, C-10), 76.5 (CH, C-7), 78.5 (qC, C-5), 84.6 (CH, C-4), 121.6 (CH, C-16), 123.5 (CH, C-20), 127.5 (CH, C-5′), 127.6 (2C, CH, C-3′/C-7′), 128.4 (2C, CH, C-4′/C-6′), 139.2 (qC, C-2′), 143.0 (qC, C-15), 146.5 ppm (qC, C-19); IR ($CHCl_3$): ν_{max} = 2986, 2929, 2857, 1463, 1455, 1378, 1363, 1084, 908 cm^{-1}; HRMS-ESI: *m/z* $[M\text{-H}]^+$ calcd for $C_{37}H_{55}O_3$: 547.4145, found: 547.4120.

4β-*O*-Benzylcarbamoylsipholenol A (20): Prepared according to procedure D from **1** (30 mg, 0.052 mmol), toluene (2 mL), Et_3N (10 μL, 0.05 mmol) and benzyl isocyanate (25 mg, 0.16 mmol). Purification by flash chromatography ($CHCl_3$/MeOH, 95:5) gave **20** as a colorless oil (12 mg, 40%): $[\alpha]_D^{25} = -29.4$ (c = 0.48, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.76 (1H, m, H-11), 0.90 (3H, s, H-31), 1.04 (6H, s, H-24/H-30), 1.12 (3H, s, H-27), 1.16 (1H, m, H-12a), 1.24 (6H, s, H-26/H-29), 1.26 (3H, s, H-25), 1.38 (1H, m, H-2a), 1.45 (1H, m, H-8a), 1.52 (1H, m, H-12b), 1.56 (1H, m, H-2b), 1.57 (1H, m, H-14), 1.60 (2H, m, H-20), 1.64 (2H, m, H-9), 1.66 (1H, m, H-13a), 1.68 (1H, m, H-21a), 1.73 (3H, s, H-28), 1.77 (1H, m, H-18), 1.78 (1H, m, H-17a), 1.82 (1H, m, H-8b), 1.87 (1H, m, H-21b), 1.94 (1H, m, H-3a), 1.95 (1H, m, H-13b), 1.98 (1H, m, H-17b), 2.12 (1H, m, H-3b), 2.41 (1H, m, H-22), 3.55 (1H, dd, J = 11.3, 3.6 Hz, H-7), 4.46 (2H, d, J = 6.2 Hz, H-2′), 5.19 (1H, d, J = 6.6 Hz, H-4), 5.44 (1H, dd, J = 9.2, 5.1 Hz, H-16), 7.30 (2H, d, J = 6.9 Hz, H-4′/H-8′), 7.32 (1H, dd, J = 5.8, 5.8 Hz, H-6′), 7.34 ppm (2H, dd, J = 5.8, 5.8 Hz, H-5′/H-7′); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 13.1 (CH_3, C-24), 21.8 (CH_3, C-26), 23.2 (CH_2, C-3), 24.8 (CH_2, C-17), 24.9 (CH_2, C-21), 25.7 (CH_3, C-29), 26.7 (CH_2, C-8), 26.9 (CH_2, C-12), 29.1 (CH_3, C-25), 29.5 (CH_3, C-31), 29.9 (CH_3, C-27), 30.2 (CH_3, C-28), 31.7 (CH_3, C-30), 33.8 (CH_2, C-13), 35.1 (CH_2, C-2), 35.5 (qC, C-23), 37.4 (CH_2, C-20), 39.5 (CH_2, C-9), 42.7 (qC, C-1), 45.4 (CH_2, C-2′), 48.9 (CH, C-18), 52.8 (CH, C-22), 56.5 (CH, C-11), 57.4 (CH, C-14), 72.4 (qC, C-10), 77.3 (CH, C-7), 77.8 (qC, C-5), 80.2 (CH, C-4), 82.2 (qC, C-19), 121.4 (CH, C-16), 127.6 (2C, CH, C-4′/C-8′), 129.4 (2C, CH, C-5′/C-7′), 136.7 (CH, C-6′), 139.1 (qC, C-3′), 143.2 (qC, C-15), 155.6 ppm (qC, C-1′); IR ($CHCl_3$): ν_{max} = 3454, 2987, 2950, 2927, 1745, 1603, 1443, 1097 cm^{-1}; HRMS-ESI: *m/z* $[M+\text{Na}]^+$ calcd for $C_{38}H_{59}NO_5Na$: 632.4285, found: 632.4283.

4β-*O*-Tosylcarbamoylsipholenol A (21): Prepared according to procedure D from **1** (30 mg, 0.052 mmol), toluene (2 mL), Et_3N (10 μL, 0.05 mmol) and *p*-toluenesulfonyl isocyanate (34 mg, 0.16 mmol). Purification by flash chromatography ($CHCl_3$/MeOH, 95:5) gave **21** as a colorless oil (18 mg, 60%): $[\alpha]_D^{25} = -27.7$ (c = 0.41, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.72 (1H, m, H-11), 0.92 (3H, s, H-31), 1.04 (6H, s, H-24/H-30), 1.13 (3H, s, H-27), 1.16 (1H, m, H-12a), 1.23 (6H, s, H-26/H-29), 1.26 (3H, s, H-25), 1.31 (1H, m, H-2a), 1.45 (1H, m, H-8a), 1.54 (2H, m, H-2b/H-12b), 1.57 (1H, m, H-14), 1.60 (2H, m, H-20), 1.62 (2H, m, H-9), 1.68 (2H, m, H-13a/H-21a), 1.73 (3H, s, H-28), 1.77 (1H, m, H-18), 1.78 (1H, m, H-17a), 1.82 (1H, m, H-8b), 1.94 (1H, m, H-21b), 1.97 (2H, m, H-3a/H-17b), 1.98 (1H, m, H-13b), 2.13 (1H, m, H-3b), 2.43 (1H, m, H-22), 2.44 (3H, s, H-8′), 3.47 (1H, dd, J = 11.7, 4.0 Hz, H-7), 5.21 (1H, d, J = 6.6 Hz, H-4), 5.43 (1H, m, H-16), 7.33 (2H, d, J = 8.4 Hz, H-4′/H-6′), 7.90 ppm (2H, d, J = 8.4 Hz, H-3′/H-7′); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 13.1 (CH_3, C-24), 21.7 (CH_3, C-8′), 21.8 (CH_3, C-26), 23.2 (CH_2, C-3), 24.8 (CH_2, C-17), 24.9 (CH_2, C-21), 25.7 (CH_3, C-29), 26.7 (CH_2, C-8), 26.9 (CH_2, C-12), 29.1 (CH_3, C-25), 29.5 (CH_3, C-31), 29.9 (CH_3, C-27), 30.2 (CH_3, C-28), 31.7 (CH_3, C-30), 33.8 (CH_2, C-13), 35.2 (CH_2, C-2), 35.5 (qC, C-23), 37.3 (CH_2, C-20), 39.5 (CH_2, C-9), 42.7 (qC, C-1), 48.8 (CH, C-18), 52.7 (CH, C-22), 56.5 (CH, C-11), 57.4 (CH, C-14), 72.4 (qC, C-10), 77.4 (qC, C-5), 77.6 (CH, C-7), 81.4 (CH, C-4), 82.1 (qC, C-19), 121.5 (CH, C-16), 128.5 (CH, C-3′), 128.6 (CH, C-6′), 129.7 (2C, CH, C-4′/C-7′), 135.0 (qC, C-5′), 143.0 (qC, C-15), 145.1 (qC, C-2′), 150.8 ppm (qC, C-1′); IR ($CHCl_3$): ν_{max} = 3454, 2987, 2950, 2858, 1757, 1355, 1152, 1088, 890 cm^{-1}; HRMS-ESI: *m/z* $[M+\text{H}]^+$ calcd for $C_{38}H_{60}NO_7S$: 674.4090, found: 674.4079.

Sipholenone A-4*Z*-oxime (22): Prepared according to procedure E from **2** (25 mg, 0.05 mmol), pyridine/EtOH (1:1, 2 mL) and $NH_2OH{\cdot}HCl$ (3.5 mg, 0.10 mmol). Purification by flash chromatography ($CHCl_3$/MeOH, 95:5) gave **21** as a colorless oil (16 mg, 65%): $[\alpha]_D^{25} = -14.8$ (c = 0.33, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.71 (1H, m, H-11), 0.85 (3H, s, H-31), 1.04 (3H, s, H-30), 1.06 (3H, s, H-24), 1.13 (3H, s, H-27), 1.15 (1H, m, H-12a), 1.21 (1H, m, H-2a), 1.24 (6H, s, H-26/H-29), 1.25 (3H, s, H-25), 1.47 (1H, m, H-8a), 1.57 (1H, m, H-20), 1.61 (1H, m, H-14), 1.62 (1H, m, H-12b), 1.66 (2H, m, H-9), 1.73 (1H, m, H-13a), 1.73 (3H, s, H-28), 1.77 (1H, m, H-18), 1.79 (2H, m, H-17a/H-21a), 1.82 (1H, m, H-17b), 1.90 (1H, m, H-2b), 1.94 (2H, m, H-3a/H-8b), 1.98 (1H, m, H-21b), 1.99 (1H, m, H-13b), 2.17 (1H, m, H-3b), 2.45 (1H, m, H-22), 2.96 (1H, dd, J = 12.3, 3.6 Hz, H-7), 5.44 ppm (1H, dd, J = 9.2, 5.1 Hz, H-16); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 12.6 (CH_3, C-24), 14.2 (CH_3, C-25), 22.7 (CH_3, C-26), 24.8 (CH_2, C-17), 24.9 (CH_2, C-21), 25.6 (CH_3, C-29), 26.7 (CH_2, C-8), 26.8 (CH_2, C-12), 29.6 (CH_3, C-31), 30.0 (CH_3, C-27), 30.2 (CH_3, C-28), 31.7 (CH_3, C-30), 33.5 (CH_2, C-3), 33.8 (CH_2, C-13), 35.5 (qC, C-23), 37.2 (CH_2, C-20), 39.1 (CH_2, C-9), 39.3 (CH_2, C-2), 41.8 (qC, C-1), 48.9 (CH, C-18), 52.8 (CH, C-22), 56.5 (CH, C-11), 57.5 (CH, C-14), 72.4 (qC, C-10), 77.8 (qC, C-5), 78.7 (CH, C-7), 82.1 (qC, C-19), 121.4 (CH, C-16), 143.1 (qC, C-15), 167.9 ppm (qC, C-4); IR ($CHCl_3$): ν_{max} = 3456, 3328, 2986, 2935, 2861, 1665, 1363, 1082, 953 cm^{-1}; HRMS-ESI: *m/z* $[M+\text{H}]^+$ calcd for $C_{30}H_{52}NO_4$: 490.3890, found: 490.3859.

Sipholenone A-4*Z*-*N*-benzoyloxime (23): Prepared according to procedure B from **22** (25 mg, 0.052 mmol), anhydrous CH_2Cl_2 (10 mL), DMAP (6 mg, < 0.05 mmol) and benzoic anhydride (23.8 mg, < 0.11 mmol). Purification by flash chromatography (*n*-hexane/EtOAc, 70:30) afforded **23** as a colorless oil (58.5%): $[\alpha]_D^{25} = -19.6$ (c = 0.26, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.71 (1H, m, H-11), 0.85 (3H, s, H-31), 1.04 (3H, s, H-30), 1.06 (3H, s, H-24), 1.13 (3H, s, H-27), 1.15 (1H, m, H-12a), 1.21 (1H, m, H-2a), 1.24 (6H, s, H-26/H-29), 1.25 (3H, s, H-25), 1.47 (1H, m, H-8a), 1.57 (1H, m, H-20), 1.61 (1H, m, H-14), 1.62 (1H, m, H-12b), 1.66 (2H, m, H-9), 1.73 (1H, m, H-13a), 1.73 (3H, s, H-28), 1.77 (1H, m, H-18), 1.79 (1H, m, H-17a), 1.79 (1H, m, H-21a), 1.82 (1H, m, H-17b), 1.90 (1H, m, H-2b), 1.94 (2H, m, H-3a/H-8b), 1.98 (1H, m, H-21b), 1.99 (1H, m, H-13b), 2.17 (1H, m, H-3b), 2.45 (1H, m, H-22), 2.96 (1H, dd, J = 12.3, 3.6 Hz, H-7), 5.44 (1H, dd, J = 9.2, 5.1 Hz, H-16), 7.42 (2H, dd, J = 7.7, 7.7 Hz, H-4′/H-6′), 7.56 (1H, m, H-5′), 8.01 ppm (2H, dd, J = 7.7, 1.4 Hz, H-3′/H-7′); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 12.6 (CH_3, C-24), 14.2 (CH_3, C-25), 22.7 (CH_3, C-26), 24.8 (CH_2, C-17), 24.9 (CH_2, C-21), 25.6 (CH_3, C-29), 26.7 (CH_2, C-8), 26.8 (CH_2, C-12), 29.6 (CH_3, C-31), 30.0 (CH_3, C-27), 30.2 (CH_3, C-28), 31.7 (CH_3, C-30), 33.5 (CH_2, C-3), 33.8 (CH_2, C-13), 35.5 (qC, C-23), 37.2 (CH_2, C-20), 39.1 (CH_2, C-9), 39.3 (CH_2, C-2), 41.8 (qC, C-1), 48.9 (CH, C-18), 52.8 (CH, C-22), 55.6 (CH, C-11), 57.5 (CH, C-14), 72.4 (qC, C-10), 77.8 (qC, C-5), 78.7 (CH, C-7), 82.1 (qC, C-19), 121.4 (CH, C-16), 128.6 (2C, CH, C-4′/C-6′), 129.3 (qC, C-2′), 129.4 (2C, CH, C-3′/C-7′), 133.3 (CH, C-5′), 142.9 (qC, C-15), 163.9 (qC, C-1′), 176.0 ppm (qC, C-4); IR ($CHCl_3$): ν_{max} = 3454, 2987, 2950, 2930, 2858, 1713, 1659, 1282, 1114, 945 cm^{-1}; HRMS-ESI: *m/z* $[M+\text{Na}]^+$ calcd for $C_{37}H_{55}NO_5Na$: 616.3972, found: 616.3967.

Sipholenone A-4*Z*-*N*-benzyloxime (24): Prepared according to procedure C from **22** (25 mg, 0.052 mmol), dry DMF (1 mL), NaH (10 equiv) and BnBr (12 μL, 0.006 mmol). Purification by flash chromatography (*n*-hexane/EtOAc, 90:10) gave **24** as a colorless oil (43.1%): $[\alpha]_D^{25} = -17.9$ (c = 0.21, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.71 (1H, m, H-11), 0.85 (3H, s, H-31), 1.04 (3H, s, H-30), 1.06 (3H, s, H-24), 1.13 (3H, s, H-27), 1.15 (1H, m, H-12a), 1.21 (1H, m,

H-2a), 1.24 (6H, s, H-26/H-29), 1.25 (3H, s, H-25), 1.47 (1H, m, H-8a), 1.57 (1H, m, H-20), 1.61 (1H, m, H-14), 1.62 (1H, m, H-12b), 1.66 (2H, m, H-9), 1.73 (1H, m, H-13a), 1.73 (3H, s, H-28), 1.77 (1H, m, H-18), 1.79 (2H, m, H-17a/H-21a), 1.82 (1H, m, H-17b), 1.90 (1H, m, H-2b), 1.94 (2H, m, H-3a/H-8b), 1.98 (1H, m, H-21b), 1.99 (1H, m, H-13b), 2.17 (1H, m, H-3b), 2.45 (1H, m, H-22), 2.96 (1H, dd, J= 12.3, 3.6 Hz, H-7), 5.02 (1H, d, J=11.9 Hz, H-1′b), 5.11 (1H, d, J= 11.9 Hz, H-1′a), 5.44 (1H, dd, J=9.2, 5.1 Hz, H-16), 7.29 (1H, m, H-5′), 7.35 (2H, m, H-4′/H-6′), 7.36 ppm (2H, m, H-3′/H-7′); ^{13}C NMR (100 MHz, $CDCl_3$): δ=12.6 (CH_3, C-24), 14.2 (CH_3, C-25), 22.7 (CH_3, C-26), 24.8 (CH_2, C-17), 24.9 (CH_2, C-21), 25.6 (CH_3, C-29), 26.7 (CH_2, C-8), 26.8 (CH_2, C-12), 29.6 (CH_3, C-31), 30.0 (CH_3, C-27), 30.2 (CH_3, C-28), 31.7 (CH_3, C-30), 33.5 (CH_2, C-3), 33.8 (CH_2, C-13), 35.5 (qC, C-23), 37.2 (CH_2, C-20), 39.1 (CH_2, C-9), 39.3 (CH_2, C-2), 41.8 (qC, C-1), 48.9 (CH, C-18), 52.8 (CH, C-22), 55.6 (CH, C-11), 57.5 (CH, C-14), 72.4 (qC, C-10), 75.6 (CH_2, C-1′), 77.8 (qC, C-5), 78.7 (CH, C-7), 82.1 (qC, C-19), 121.3 (CH, C-16), 127.6 (2C, CH, C-3′/C-7′), 128.2 (CH, C-5′), 128.5 (2C, CH, C-4′/C-6′), 138.3 (qC, C-2′), 143.1 (qC, C-15), 167.0 ppm (qC, C-4); IR ($CHCl_3$): ν_{max}=3454, 2987, 2949, 2863, 1665, 1463, 1455, 1376, 1363, 1082, 947 cm^{-1}; HRMS-ESI: m/z $[M+H]^+$ calcd for $C_{37}H_{58}NO_4$: 580.4366, found: 580.4370.

Biology

Cell culture: Metastatic human mammary epithelial breast cancer (MDA-MB-231) and normal (MCF10A) cell lines were purchased from ATCC (Manassas, VA, USA). Cells were grown in RPMI 1640 medium (GIBCO-Invitrogen, Grand Island, NY, USA) with 10% fetal bovine serum (FBS) and supplemented with L-glutamine (2 mmol L^{-1}), penicillin G (100 μg mL^{-1}), and streptomycin (100 μg mL^{-1}) at 37 °C under 5% CO_2.

Preparation of solutions for cell-based assays: A stock solution of each analogue was prepared by dissolving the compound in DMSO at a concentration of 50 mM for all assays. An aliquot (~2 μL) of each stock solution was transferred to serum-free medium (998 μL) to obtain a solution with a concentration of 100 μM and 0.2% DMSO. Serial dilutions were then conducted to obtain the desired concentrations for each assay. The vehicle control was prepared as follows: *for the MTT assay*: DMSO (2 μL) was added to serum-free media (998 μL); *for the wound-healing assay*: DMSO (3 μL) was added to serum-free media (1497 μL) containing 0.5% FBS.

Antimigratory (wound-healing) assay:[26] MDA-MB-231 cells were cultured in the RPMI 1640 medium containing 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), L-glutamine (4 mM), 10% FBS, penicillin (100 IU mL^{-1}), and streptomycin (50 μg mL^{-1}), and incubated in 5% CO_2 at 37 °C. Cells were plated onto sterile 24-well plates and allowed to form a confluent monolayer in each well (>95% confluence). Wounds were then inflicted to each cell monolayer using a sterile 200 μL pipette tip. Media were removed, cells were washed twice with phosphate-buffered saline (PBS), and then test compound was added in fresh RPMI 1640 media (same as culture media but with only 0.5% FBS). Four non-cytotoxic concentrations of test compound were prepared in serum-free media containing 0.5% FBS. Each concentration was tested in triplicate. Incubation was carried out for 24 h in 5% CO_2 at 37 °C, after which time, the cell medium was removed, and cells were fixed and stained using a DiffQuick staining kit (Dade Behring Diagnostics, Aguada, Puerto Rico). The number of cells migrated across the wound was counted under the microscope in three or more randomly selected fields (magnification: 40×). Final results are expressed as the % migration ([number of cells in the wound of the treatment group/number of cells in the wound for vehicle control]×100) per 40× field. IC_{50} values (μM) are the doses that produced 50% migration and were calculated from nonlinear fitting of the dose–response curve using GraphPad Prism 5.04 (GraphPad Software, La Jolla, CA, USA).

Cultrex basement membrane extract (BME) cell invasion assay:[28] BME (~50 μL) was added to each well of the top chamber. After overnight incubation at 37 °C in 5% CO_2, MDA-MB-231 cells (50 000/50 μL) in 0.5% FBS RPMI medium were added to each well of the top chamber. RPMI 1640 medium (150 μL) was then added to the lower chamber; this media contained 10% FBS and penicillin/streptomycin, as well as fibronectin (1 μL mL^{-1}) and *N*-formyl-Met-Leu-Phe (10 nM) as chemoattractants. The test compound solutions were prepared at six-times the desired concentration, and an aliquot (10 μL) was added to each well of the top chamber. Cells were incubated at 37 °C in 5% CO_2, which allowed for cell invasion from the top to the lower chamber. After 24 h, the top and bottom chambers were aspirated and washed with washing buffer supplied with the assay kit. Cell dissociation solution/calcein-AM solution (~100 μL) was added to the bottom chamber, and cells were incubated at 37 °C under 5% CO_2 for 1 h. The cells internalize calcein-AM, and the intracellular esterases cleave the acetomethylester (AM) moiety to generate free calcein. Fluorescence of the samples was determined at $\lambda_{excitation}$ 485 nm and $\lambda_{emission}$ 528 nm using an ELISA plate reader (BioTek, Winooski, VT, USA). The number of cells that invaded through the BME coat was calculated using a standard curve.

MTT cytotoxicity assay: The cytotoxic effects of test compounds were evaluated in MDA-MB-231 and MCF10A cells using an MTT assay kit (TACS, Trevigen Inc., Gaithersburg, MD, USA). Cells in exponential growth were plated in a 96-well plate at a density of 20×10^3 cells per well and allowed to attach for 24 h at 37 °C in 5% CO_2. Complete growth medium was then replaced with RPMI 1640 or DMEM/F-12 serum-free medium (100 μL) containing various doses (5, 10, 20, and 40 μM) of test compound and incubation resumed at 37 °C in 5% CO_2 for 24 h. Cells were then treated with MTT solution (20 μL/well) and re-incubated for 4 h. The color reaction was stopped by addition of solubilization/stop solution (DMSO; 100 μL/well), and incubation at 37 °C was continued to ensure complete dissolution of the formazan product. Absorbance of the samples was determined at λ=570 nm using an ELISA plate reader (BioTek).

Phosphorylation inhibition assay: A Z′-LYTE kinase assay-Tyr 1 peptide kit (Invitrogen) was used to assess the ability of sipholenol A and its analogues to inhibit protein tyrosine kinase 6 (PTK6) phosphorylation. Briefly, 10 μL/well reactions were set up in 384-well plates containing kinase buffer, 150 μM ATP, 2 μM Z′-LYTE-Tyr 1 peptide substrate, 5000 ng mL^{-1} PTK6, and test compound (inhibitor). After 1 h of incubation at RT, 5 μL development solution containing site-specific protease was added to each well. Incubation was continued for 1 h. The reaction was then stopped, and the fluorescent signal ratio of 445 nm (coumarin)/520 nm (fluorescein) was determined on an FLx800 plate reader (BioTek), which reflects the peptide substrate cleavage status and/or the kinase inhibitory activity in the reaction.

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Keywords: antimigratory agents · breast cancer · PTK6 · sipholenol A · structure–activity relationships

[1] T. L. Simmons, E. Andrianasolo, K. McPhail, P. Flatt, W. H. Gerwick, *Mol. Cancer Ther.* **2005**, *4*, 333 – 342.
[2] R. Singh, M. Sharma, P. Joshi, D. S. Rawat, *Anti-Cancer Agents Med. Chem.* **2008**, *8*, 603 – 617.
[3] I. Ojima, *J. Med. Chem.* **2008**, *51*, 2587 – 2588.
[4] D. J. Newman, G. M. Cragg, *J. Nat. Prod.* **2012**, *75*, 311 – 335.
[5] D. J. Newman, G. M. Cragg, *J. Nat. Prod.* **2004**, *67*, 1216 – 1238.
[6] S. Carmely, Y. Kashman, *J. Org. Chem.* **1983**, *48*, 3517 – 3525.
[7] S. Carmely, Y. Kashman, *J. Org. Chem.* **1986**, *51*, 784 – 788.
[8] Y. Kashman, T. Yosief, S. Carmely, *J. Nat. Prod.* **2001**, *64*, 175 – 180.
[9] S. Jain, I. Abraham, P. Carvalho, Y.-H. Kuang, D. T. A. Youssef, M. A. Avery, Z. S. Chen, K. A. El Sayed, *J. Nat. Prod.* **2009**, *72*, 1291 – 1298.
[10] I. Abraham, S. Jain, C. P. Wu, Y. Kuanga, Z. Shia, X. Chen, M. Khanfar, L. Fu, S. V. Ambudkar, K. A. El Sayed, Z. S. Chen, *Biochem. Pharmacol.* **2010**, *80*, 1497 – 1506.
[11] a) S. Jain, S. Laphookhieo, Z. Shi, L. Fu, S. Akiyama, Z. S. Chen, D. T. A. Youssef, R. W. M. van Soest, K. A. El Sayed, *J. Nat. Prod.* **2007**, *70*, 928 – 931; b) S. Jain, A. Shirode, S. Yacoub, A. Barbo, P. W. Sylvester, E. Huntimer, F. Halaweish, K. A. El Sayed, *Planta Med.* **2007**, *73*, 591 – 596.
[12] G. Szakács, J. K. Paterson, J. A. Ludwig, C. Booth-Genthe, M. M. Gottesman, *Nat. Rev. Drug Discovery* **2006**, *5*, 219 – 234.
[13] V. Höll, M. Kouba, M. Dietel, G. Vogt, *Biochem. Pharmacol.* **1992**, *43*, 2601 – 2608.
[14] http://www.cancer.org/research/cancerfactsfigures/index (accessed October 20, 2012); American Cancer Society Statistics for 2011 – 2012.
[15] K. T. Barker, L. E. Jackson, *Oncogene* **1997**, *15*, 799 – 805.
[16] P. J. Mitchell, K. T. Barker, J. E. Martindale, T. Kamalati, P. N. Lowe, M. J. Page, B. A. Gusterson, M. R. Crompton, *Oncogene* **1994**, *9*, 2383 – 2390.
[17] M. Aubele, G. Auer, A. K. Walch, A. Munro, M. J. Atkinson, H. Braselmann, *Br. J. Cancer* **2007**, *96*, 801 – 807.
[18] P. J. Mitchell, K. T. Barker, J. Shipley, M. R. Crompton, *Oncogene* **1997**, *15*, 1497 – 1502.
[19] A. J. Harvey, C. J. Pennington, S. Porter, R. S. Burmi, D. R. Edwards, W. Court, *Am. J. Pathol.* **2009**, *175*, 1226 – 1234.
[20] P. M. Brauer, A. L. Tyner, *Biochim. Biophys. Acta Rev. Cancer* **2010**, *1806*, 66 – 73.
[21] A. Rudi, M. Aknin, E. M. Gaydou, Y. Kashman, *J. Nat. Prod.* **1997**, *60*, 700 – 703.
[22] A. Rudi, I. Goldberg, Z. Stein, Y. Kashman, Y. Benayahu, M. Schleyer, *J. Nat. Prod.* **1995**, *58*, 1702 – 1712.
[23] I. Carletti, C. Long, C. Funel, P. Amade, *J. Nat. Prod.* **2003**, *66*, 25 – 29.
[24] C. Funel-Le Bon, F. Berrué, O. P. Thomas, F. Reyes, P. Amade, *J. Nat. Prod.* **2005**, *68*, 1284 – 1287.
[25] J. Dai, J. A. Fishback, Y. D. Zhou, D. G. Nagle, *J. Nat. Prod.* **2006**, *69*, 1715 – 1720.
[26] M. Mudit, M. Khanfar, A. Muralidharan, S. Thomas, G. V. Shah, R. W. van Soest, K. A. El Sayed, *Bioorg. Med. Chem.* **2009**, *17*, 1731 – 1738.
[27] L. G. Rodriguez, X. Wu, J. L. Guan, *Methods Mol. Biol.* **2005**, *294*, 23 – 29.
[28] Cultrex BME cell invasion assay protocol, Trevigen Inc., Gaithersburg, MD, USA; http://www.trevigen.com (accessed on September 15, 2012).
[29] M. A. Khanfar, D. T. A. Youssef, K. A. El Sayed, *ChemMedChem* **2010**, *5*, 274 – 285.
[30] TACS MTT Cell proliferation assays protocol, Trevigen Inc., Gaithersburg, MD, USA; http://www.trevigen.com (accessed on May 22, 2011).
[31] T. Mosmann, *J. Immunol. Methods* **1983**, *65*, 55 – 63.
[32] S. M. Rodems, B. D. Hamman, C. Lin, J. Zhao, S. Shah, D. Heidary, L. Makings, J. H. Stack, B. A. Pollok, *Assay Drug Dev. Technol.* **2002**, *1*, 9 – 19.
[33] F. A. Behery, A. Y. Elnagar, M. R. Akl, V. B. Wali, B. Abuasal, A. Kaddoumi, P. W. Sylvester, K. A. El Sayed, *Bioorg. Med. Chem.* **2010**, *18*, 8066 – 8075.
[34] H. M. Hassan, A. Y. Elnagar, M. A. Khanfar, A. A. Sallam, R. Mohammed, L. A. Shaala, T. D. Diaa, S. M. Hifnawy, K. A. El Sayed, *Eur. J. Med. Chem.* **2011**, *46*, 1122 – 1130.

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