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Chemical and biological explorations of the electrophilic reactivity of the bioactive marine natural product halenaquinone with biomimetic nucleophiles

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ABSTRACT

The electrophilic reactivity of the bioactive marine sponge natural product halenaquinone has been investigated by reaction with the biomimetic nucleophiles *N*-acetyl-L-cysteine and N_α-acetyl-L-lysine. While cysteine reacted at the vacant quinone positions C-14 and C-15, lysine was found to react preferentially at the keto-furan position C-1. A small library of analogues was prepared by reaction of halenaquinone with primary amines, and evaluated against a range of biological targets including phospholipase A_2, farnesyltransferases (FTases) and *Plasmodium falciparum*. Geranylamine analogue **11** exhibited the most potent activity towards FTases (IC_{50} 0.017–0.031 μM) and malaria (IC_{50} 0.53–0.62 μM).

Halenaquinone (**1**)[1] and xestoquinone (**2**)[2] were the first examples of a now large family of polycyclic aromatic natural products isolated from marine sponges of the genera *Xestospongia*, *Neopetrosia* and *Adocia*.[3] While halenaquinone was originally reported to exhibit antibacterial properties, subsequent studies revealed it also to be a potent irreversible inhibitor of phosphatidylinositol 3-kinase (PI3-kinase)[4] and protein tyrosine kinase.[3d,5] A number of research groups have noted differing biological activities, and potency of activity, between halenaquinone (**1**) and xestoquinone (**2**), despite the only structural difference between the compounds being oxidation at C-3. For example, halenaquinone is a more potent PI3-kinase[4] and protein tyrosine kinase[3d,5] inhibitor and more potent growth inhibitor of fungi,[6] while xestoquinone is a more potent cytotoxin[6] and a stronger growth inhibitor of *Plasmodium falciparum*.[7,3n] Both **1** and **2** contain a *para*-quinone moiety, the electrophilic nature of which has been confirmed in the case of **2** by formation of a di(2-mercaptoethanol) adduct.[8] The 3-ketofuran fragment of halenaquinone (**1**) is also expected to be electrophilic (at C-1),[3d] being reminiscent of a similar substructural fragment in the irreversibly PI3-kinase inhibiting natural product wortmannin (**3**).[9] As part of our ongoing investigation of bioactive natural products isolated from South Pacific marine sponges, we recently identified orhalquinone (**4**) as an inhibitor of bee venom phospholipase A_2 and human and yeast farnesyltransferase enzymes and a growth inhibitor of *P. falciparum*.[3n] Comparative biological evaluation with halenaquinone identified that loss of the C-1 electrophilic center was detrimental to PLA_2 inhibition, but was requisite for *in vitro* antimalarial activity. Our isolation of orhalquinone (**4**) from *Xestospongia* sp. also yielded considerable quantity of halenaquinone (**1**),[10] which provided an opportunity to investigate the reactivity of the natural product with biomimetic-type thiol and amine nucleophiles, which in turn led to the preparation and biological evaluation of a library of novel amine analogues. In this letter, we present the results of these studies.

Reaction of halenaquinone with *N*-acetyl-L-cysteine (1 equiv) in DMF and triethylamine (1 equiv) for 3 h yielded a mixture of unreacted halenaquinone and a new product **5** (2:1 ratio) as judged by ^{1}H NMR spectroscopy.[11] While many of the resonances of the two compounds were overlapped, quinonoid proton H-15 (δ_H 6.93, s) and furan proton H-1 (δ_H 8.78, s) of the new derivative were distinct, allowing characterization of **5**. Inspection of ^{1}H, ^{13}C and HSQC NMR data observed for **5** indicated loss of one of the two chemically equivalent quinonoid proton resonances of halenaquinone and the presence of an intact furan ring (δ_H 8.78, δ_C 151.8), suggest-

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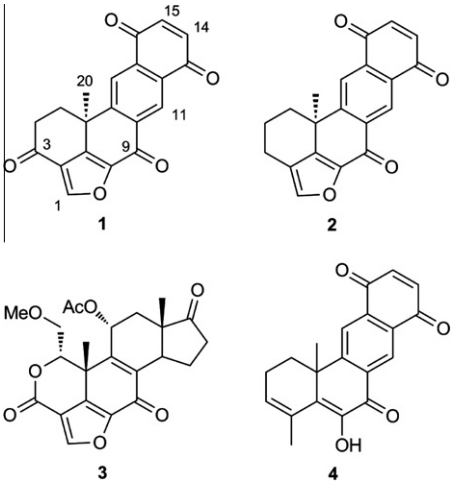
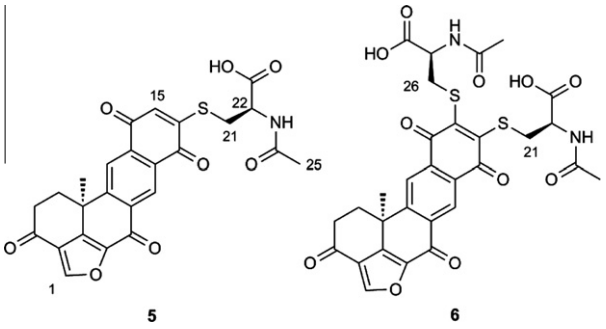
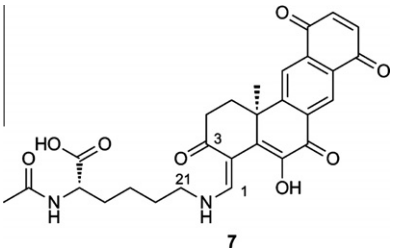


ing thiol attack at C-14 or C-15 of **1**. HMBC NMR correlations observed between H-11 (δ_H 8.69) and C-17 (δ_C 135.0) and between the remaining quinonoid proton resonance (δ_H 6.93, s) and C-17 established the identity of **5** as 14-(*N*-acetyl-L-cysteinyl)-halenaquinone. Extended reaction of halenaquinone with excess *N*-acetyl-L-cysteine ($\geqslant$5 equiv) yielded an unstable product that was characterized as the 14,15-dicysteinyl compound **6**.[12] Thus the reactivity of halenaquinone towards thiol nucleophiles mirrors that observed for xestoquinone,[8] in that the reactive centers are C-14 and C-15 of the quinone ring.

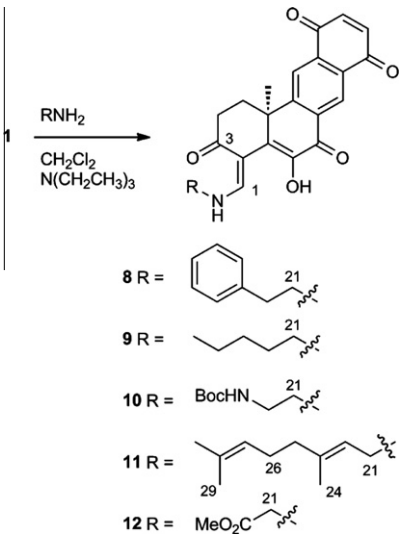


Reaction of halenaquinone with N_α-acetyl-L-lysine (5 equiv) in a mixed solvent system (DMSO/MeOH/H_2O, 1:1:0.1) for 1 hr yielded an unstable single product **7**.[13] Mass spectrometry indicated the product to be a mono-*N*-acetyllysine adduct of halenaquinone ((+)-ESIMS *m/z* 521.1921 MH^+, calcd for $C_{28}H_{29}N_2O_8$, 521.1918). Analysis of ^1H, ^{13}C and 2-D NMR data established the presence of H-14 and H-15 resonances (δ_H 7.13, 2H, s) and the absence of resonances associated with the furan moiety of halenaquinone. The sharp H-1 (δ_H 8.90, s) resonance of halenaquinone was noticeably absent from the ^1H NMR spectrum of **7**, being replaced by an olefinic methine at δ_H 8.47 (1H, br d, J = 12.9 Hz, H-1), which in an HMBC spectrum correlated weakly to an sp^2 resonance at δ_C 194.0 (C-3). From the COSY NMR spectrum, connectivity between the acetyllysine $N_\varepsilon H$ resonance (δ_H 11.48, br m) and δ_H 8.47 (H-1) was observed, establishing the covalent linkage between the two reactants. Instability of the product prevented conclusive determination of the geometry of Δ^1, but subsequent studies (see below) suggest it to be *Z* as shown. Further reaction of halenaquinone with 10-fold excess of N_α-acetyl-L-lysine afforded a complex mixture of products, (−)-ESI mass spectrometric analysis of which detected an ion at *m/z* 705.2761 $[M-H]^-$, consistent with the presence of a di(N_α-acetyl-L-lysine)halenaquinone adduct (calcd for $C_{36}H_{41}N_4O_{11}$, 705.2777). Thus in contrast to the reactivity towards thiol nucleophiles, amines preferentially attack halenaquinone at C-1.



The selectivity of reaction between amines and halenaquinone at the C-1 position was further explored. While reaction of halenaquinone with secondary amines yielded complex mixtures of products (data not shown), primary amines afforded single products that were stable to chromatographic purification (Scheme 1).[14] Reaction of halenaquinone with phenethylamine (1 equiv) in CH_2Cl_2 in the presence of triethylamine for 1 h followed by purification via silica gel flash chromatography yielded halenaquinone analogue **8** in 99% yield.[15] As observed previously for lysine analogue **7**, analysis of ^1H and ^{13}C NMR data acquired for **8** established the presence of resonances attributable to H-14/15 and C-14/15 and the absence of furan ring resonances. A COSY NMR spectrum established spin-system connectivity from the phenethylamine methylene protons (δ_H 3.69, 3.62, m, H_2-21; δ_H 2.97, m, H_2-22) to an exchangeable proton (δ_H 11.68, dt, J = 6.5, 13.2 Hz, NH) to a resonance at δ_H 8.42 (1H, d, J = 13.2 Hz, H-1). HMBC NMR correlations observed for the δ_H 8.42 resonance to C-21 (δ_C 51.9) and C-3 (δ_C 195.1) placed this proton at C-1. An additional HMBC correlation observed between the H_2-21 (δ_H 3.69 and 3.62) resonances and C-1 (δ_C 160.5) further confirmed the structure of **8**. A *J*-HMBC NMR experiment[16] determined the heteronuclear coupling constant between C-3 and H-1 to be 7.5 Hz, indicative of a *trans* H-1/C-3 geometry and a Δ^1 *Z* configuration.[17] Following this general protocol, reaction of halenaquinone with *n*-pentylamine, *tert*-butoxycarbonyl ethylenediamine, geranylamine and glycine methylester afforded analogues **9** (93% yield),[18] **10** (78%),[19] **11** (78%)[20] and **12** (77%).[21] Analysis of ^1H and ^{13}C one-dimensional and two-dimensional NMR data and mass spectrometric data was used to confirm the structures of **9**–**12**.

Compounds **8**–**12** were evaluated for activity against bee venom phospholipase A_2, yeast (*Saccharomyces cerevisiae*) and human protein farnesyltransferases, FcB1 and 3D7 strains of *P. falciparum* and VERO cells. The results from the in vitro assays are presented



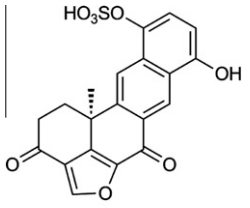
Scheme 1. Semisynthesis of **8**–**12** from halenaquinone (**1**).

Table 1
Biological activities of halenaquinone (**1**), orhalquinone (**4**) and derivatives **8–12**

Compound	PLA_2[a]	*y*FTase[b]	*h*FTase[c]	Pf-FcB1[d]	Pf-3D7[e]	VERO[f]
Halenaquinone **1**[g]	3.7 ± 0.3	1.6 ± 0.1	0.93 ± 0.18	>30	>30	>60
Orhalquinone **4**[g]	1570 ± 92.6	0.40 ± 0.01	0.41 ± 0.03	9.2 ± 0.4	10.9 ± 0.3	>62
8	17.7 ± 0.4	0.31 ± 0.04	0.44 ± 0.03	8.8 ± 0.3	2.1 ± 0.8	4.7 ± 2.0
9	23.9 ± 2.6	0.041 ± 0.004	0.057 ± 0.004	9.2 ± 1.2	7.4 ± 1.7	6.3 ± 1.5
10	12.8 ± 0.8	nt[h]	nt	52.8 ± 14.4	nt	7.4 ± 0.2
11	34.4 ± 3.7	0.017 ± 0.001	0.031 ± 0.003	0.62 ± 0.25	0.53 ± 0.12	8.6 ± 1.6
12	76.8 ± 11.0	nt	nt	55.8 ± 21.8	16.5 ± 6.2	13.4 ± 7.2

[a] Bee venom PLA_2. IC_{50} values (μM ± SEM; $n = 2$). Manoalide (positive control) IC_{50} 0.5 ± 0.05 μM. Assay protocol is described in Ref. 3n.
[b] Farnesyltransferase from the yeast *Saccharomyces cerevisiae*. IC_{50} values (μM ± SEM; $n = 4$). FTase inhibitor I (positive control) IC_{50} 0.032 μM. Assay protocol is described in Ref. 3n.
[c] Farnesyltransferase from human. IC_{50} values (μM ± SEM; $n = 4$). FTI-276 (positive control) IC_{50} 0.015 μM. Assay protocol is described in Ref. 3n.
[d] *Plasmodium falciparum* strain FcB1 (chloroquine resistant). IC_{50} values (μM ± SEM; n = 2). Highest concentration tested 10 μg/mL. Chloroquine (positive control) IC_{50} 150 nM. Assay protocol is described in Ref. 3n.
[e] *Plasmodium falciparum* strain 3D7 (chloroquine sensitive). IC_{50} values (μM ± SEM; $n = 2$). Highest concentration tested 10 μg/mL. Chloroquine (positive control) IC_{50} 30 nM. Assay protocol is described in Ref. 3n.
[f] VERO mammalian non-malignant cell line. IC_{50} values (μM ± SEM; $n = 2$). Doxorubicin (positive control) IC_{50} 5.12 ± 0.17 μM. Highest concentration tested 20 μg/mL. Assay protocol is described in Ref. 3n
[g] Data taken from Ref. 3n.
[h] Not tested.

in Table 1. We have previously reported that while halenaquinone (**1**) is a micromolar inhibitor of PLA_2, the furan ring-opened natural product orhalquinone (**4**) and halenaquinol sulfate (**13**) are significantly less potent (IC_{50} >1 mM).[3n] All of **8**–**12** were found to be modest inhibitors of PLA_2 enzymatic activity, with IC_{50} values of 12.8–76.8 μM. Taking the poor biological activities of **4** and **13** towards PLA_2 into account, these current results indicate the importance of the presence of a quinone ring and oxygen functionality at C-3, but not the presence of an electrophilic center at C-1, for PLA_2 inhibition.



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Sub-micromolar inhibition of both yeast and mammalian FTases was observed for **8**, **9** and **11**, with the lipophilic geranyl amine analogue **11** being particularly potent (IC_{50} 0.017 and 0.031 μM). This leads to the conclusion that inhibition of FTase enzymes by this compound class does not require an electrophilic center at C-1, and that further modulation of activity may be possible by variation of lipophilicity. While the furan-ring containing natural product halenaquinone was inactive as a growth inhibitor of *P. falciparum*,[3n] orhalquinone (**4**) and analogues **8**, **9** and **11** were found to be active. In particular the geranyl amine analogue **11** displayed the most potent antiplasmodial activity (IC_{50} 0.62 and 0.53 μM). As previously found, the antiplasmodial activity did not depend on the chloroquine sensitivity of the strain tested and there did appear to be some correlation between FTase and *P. falciparum* activities.

Although good selectivity was observed previously for orhalquinone (VERO cytotoxicity IC_{50} >62 μM), analogues **8**–**12** all exhibited enhanced cytotoxicity towards the VERO non-malignant cell line. Of the new compounds studied, geranyl analogue **11** exhibited the best selectivity towards malaria or FTases.

In conclusion, we have identified the sites of thiol reactivity of halenaquinone to be the vacant quinonoid positions C-14 and C-15, while primary amines react preferentially at the keto-furan C-1 position. The preparation and biological evaluation of a library of amine analogues of halenaquinone highlighted the importance of a quinonoid ring and oxygen functionality at C-3 for bioactivity and also demonstrated that the electrophilic center at C-1 of the natural product is not a necessary requirement for activity. The ability to functionalize the halenaquinone scaffold selectively at either C-14/C-15 or C-1 by the judicious choice of linker chemistry will facilitate biotinylation[22] or click chemistry[23] activity-based protein profiling experiments to identify cellular targets[24] of this intriguing marine natural product.

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10. Halenaquinone (**1**) was isolated from *Xestospongia* sp. as previously described.[3n] $[\alpha]_D$ +38 (*c*. 0.212, CH_2Cl_2) (lit.[1] +62.1 (*c* 0.066, CH_2Cl_2)); ^1H NMR (DMSO-d_6, 300 MHz) δ 8.90 (1H, s, H-1), 8.72 (1H, s, H-11), 8.35 (1H, s, H-18), 7.20 (2H, s, H-14 and H-15), 3.12 (1H, ddd, *J* = 5.0, 13.0, 18.5 Hz, H-4a), 2.94 (1H, ddd, *J* = 1.5, 5.0, 13.0 Hz, H-5a), 2.69 (1H, *J* = 1.5, 4.6, 18.5 Hz, H-4b), 2.22 (1H, ddd, *J* = 4.6, 13.0, 13.0 Hz, H-5b), 1.67 (3H, s, H-20); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 191.4 (C-3), 184.1 (C-16), 183.7 (C-13), 169.8 (C-9), 154.4 (C-19), 150.9 (C-1), 148.4 (C-7), 143.9 (C-8), 139.0 (C-14 and C-15), 136.3 (C-10), 133.5 (C-17), 130.2 (C-12), 125.0 (C-11), 123.7 (C-18), 122.3 (C-2), 36.6 (C-6), 36.2 (C-4), 32.2 (C-5), 29.7 (C-20).
11. 14-(*N*-Acetyl-L-cysteinyl)-halenaquinone (**5**): ^1H NMR (DMSO-d_6, 400 MHz) δ 8.78 (1H, s, H-1), 8.69 (1H, s, H-11), 8.48 (1H, d, *J* = 8.2 Hz, NH), 8.31 (1H, s, H-18), 6.93 (1H, s, H-15), 4.57 (1H, m, H-22), 3.38 (1H, m, H-21a), 3.24 (1H, m, H-21b), 3.08 (1H, ddd, *J* = 5.0, 13.3, 18.5 Hz, H-4a), 2.92 (1H, m, H-5a), 2.70 (1H, m, H-4b), 2.21 (1H, ddd, *J* = 4.7, 13.2, 17.9 Hz, H-5b), 1.88 (3H, s, H-25), 1.65 (3H, s, H-20); (+)-ESIMS *m/z* 494 $[M+H]^+$; (+)-HRESIMS $[M+H]^+$ 494.0901, calcd for $C_{25}H_{20}NO_8S$ 494.0904.
12. 14,15-Di(*N*-acetyl-L-cysteinyl)-halenaquinone (**6**): ^1H NMR (DMSO-d_6, 400 MHz) δ 8.90 (1H, s, H-1), 8.70 (1H, s, H-11), 8.32 (1H, s, H-18), 8.29 (2H, m, NH), 4.49 (2H, m, H-22 and H-27), 3.67 (2H, m, H-21a and H-26a), 3.43 (2H, m, H-21b and H-26b), 3.08 (1H, m, H-4a), 2.93 (1H, m, H-5a), 2.65 (1H, m, H-4b), 2.18 (1H, m, H-5b), 1.74 (6H, s, H-25 and H-30), 1.66 (3H, s, H-20); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 194.1 (C-3), 177.6 (C-16), 171.6 (C-23 and C-28), 170.0 (C-9), 169.4 (C-24 and C-29), 154.3 (C-19), 151.0 (C-1, $^1J_{CH}$ 215 Hz), 148.5 (C-7), 148.0 (C-14 and C-15), 144.0 (C-8), 136.0 (C-10), 134.8 (C-17), 131.3 (C-12), 125.6 (C-11), 124.4 (C-18), 122.5 (C-2), 52.7 (C-22 and C-27), 38.8 (C-6), 36.6 (C-4), 36.3 (C-21 and C-26), 35.1 (C-5), 29.7 (C-20), 22.2 (C-25 and C-30); (+)-ESIMS *m/z* 655 $[M+H]^+$; (+)-HRESIMS $[M+H]^+$ 655.1056 (calcd for $C_{30}H_{27}N_2O_{11}S_2$, 655.1051).
13. (*N*α-Acetyl-L-lysinyl)-halenaquinone analogue **7**: ^1H NMR (DMSO-d_6, 300 MHz) δ 11.48 (1H, br m, NH), 8.64 (1H, s, H-11), 8.47 (1H, br d, *J* = 12.9 Hz, H-1), 8.33 (1H, s, H-18), 8.03 (1H, d, *J* = 7.9 Hz, N_2H), 7.13 (2H, s, H-14 and H-15), 4.21 (1H, m, H-25), 3.40 (2H, obsc, H-21), 2.75 (1H, m, H-5a), 2.55 (2H, m, H-5b and H-4a), 1.85 (3H, s, H-28), 1.74 (1H, m, H-4b), 1.67 (2H, m, H-24), 1.62 (2H, m, H-22), 1.51 (3H, s, H-20), 1.40 (2H, m, H-23); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 194.0 (C-3), 184.4 (C-16), 184.0 (C-13), 175.6 (C-9), 173.8 (C-26), 169.4 (C-27), 159.9 (C-1), 154.4 (C-19), 138.6 (C-14 and C-15), 138.3 (C-8), 137.2 (C-7), 133.0 (C-17), 132.9 (C-10), 130.1 (C-12), 124.4 (C-18), 123.4 (C-11), 101.4 (C-2), 51.3 (C-25), 48.8 (C-21), 39.4 (C-6), 32.5 (C-4), 32.4 (C-5), 30.7 (C-24), 29.7 (C-22), 27.4 (C-20); 23.2 (C-28), 22.0 (C-23); (+)-ESIMS *m/z* 521 $[M+H]^+$; (+)-HRESIMS $[M+H]^+$ 521.1921 (calcd for $C_{28}H_{29}N_2O_8$, 521.1918).
14. *Representative experimental.* An aliquot of phenethylamine (495 μL, 0.020 mmol of a 4.81 mg mL^{-1} solution in CH_2Cl_2) was added to a solution of halenaquinone (7.25 mg, 0.022 mmol) in CH_2Cl_2 (5 mL). The solution was stirred for 1 hr at room temperature and then dried in vacuo. The crude residue was purified by silica gel column chromatography (0.5% MeOH/CH_2Cl_2) to obtain **8** (9.8 mg) as a dark red oil in 99.0% yield.
15. Phenylethylamino analogue **8**: $[\alpha]_D$ +883 (*c*. 0.08, CH_2Cl_2); R_f (SiO_2, 1% MeOH/CH_2Cl_2) 0.27; IR ν_{max} (smear) 1672, 1278 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 11.68 (1H, br m, NH), 8.90 (1H, s, H-11), 8.42 (1H, d, *J* = 13.2 Hz, H-1), 8.29 (1H, s, H-18), 7.32 (2H, t, *J* = 7.6 Hz, H-25 and H-27), 7.24 (1H, m, H-26), 7.20 (2H, d, *J* = 7.6 Hz, H-24 and H-28), 7.04 and 7.03 (2H, ABq, *J* = 10.3 Hz, H-14 and H-15), 3.69 (1H, m, H-21a), 3.62 (1H, m, H-21b), 2.97 (2H, m, H-22), 2.78 (1H, ddd, J = 7.0, 13.0, 19.0 Hz, H-4a), 2.60 (1H, dd, *J* = 6.6, 19.0 Hz, H-4b), 2.54 (1H, dd, *J* = 7.0, 13.0 Hz, H-5a), 1.84 (1H, ddd, *J* = 6.6, 13.0, 13.0 Hz, H-5b), 1.53 (3H, s, H-20); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 195.1 (C-3), 184.6 (C-16), 183.8 (C-13), 176.1 (C-9), 160.5 (C-1), 154.8 (C-19), 139.4 (C-15*), 138.9 (C-14*), 138.2 (C-8), 137.4 (C-23), 136.4 (C-7), 133.3 (C-17), 132.6 (C-10), 130.4 (C-12), 128.9 (C-24/C-25/C-27/C-28), 128.8 (C-24/C-25/C-27/C-28), 127.0 (C-26), 125.7 (C-11), 125.0 (C-18), 102.2 (C-2), 51.9 (C-21), 39.7 (C-6), 37.1 (C-22), 33.0 (C-4), 32.7 (C-5), 28.3 (C-20); (+)-ESIMS *m/z* 454 $[M+H]^+$; (+)-HRESIMS $[M+H]^+$ 454.1651 (calcd for $C_{28}H_{24}NO_5$, 454.1649).
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18. *n*-Pentylamino analogue **9**: $[\alpha]_D$ +1351 (*c*. 0.02, CH_2Cl_2); R_f (SiO_2, 1% MeOH/CH_2Cl_2) 0.32; IR ν_{max} (smear) 1673, 1276 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 11.72 (1H, br m, NH), 8.91 (1H, s, H-11), 8.59 (1H, d, *J* = 13.6 Hz, H-1), 8.31 (1H, s, H-18), 7.04 (2H, s, H-14 and H-15), 3.41 (2H, q, *J* = 6.8 Hz, H-21), 2.80 (1H, m, H-4a), 2.62 (1H, m, H-4b), 2.56 (1H, m, H-5a), 1.86 (1H, m, H-5b), 1.69 (2H, m, H-22), 1.56 (3H, s, H-20), 1.38 (4H, obsc, H-23 and H-24), 0.92 (3H, t, *J* = 7.0 Hz, H-25); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 195.0 (C-3), 184.5 (C-16), 183.8 (C-13), 176.0 (C-9), 160.7 (C-1), 154.8 (C-19), 139.4 (C-14/C-15), 138.9 (C-15/C-14), 138.1 (C-8), 136.6 (C-7), 133.2 (C-17), 133.2 (C-10), 130.4 (C-12), 125.6 (C-11), 124.9 (C-18), 102.0 (C-2), 50.4 (C-21), 39.7 (C-6), 33.0 (C-4), 32.7 (C-5), 30.2 (C-22), 28.6 (C-23), 28.7 (C-20), 22.2 (C-24), 13.9 (C-25); (+)-ESIMS *m/z* 420 $[M+H]^+$; (+)-HRESIMS $[M+H]^+$ 420.1810 (calcd for $C_{25}H_{26}NO_5$, 420.1805).
19. *tert*-Butoxycarbonylethylenediamine analogue **10**: $[\alpha]_D$ +794 (*c* 0.06, CH_2Cl_2); R_f (SiO_2, 1% MeOH/CH_2Cl_2) 0.31; IR ν_{max} (smear) 1702, 1671, 1281 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 11.62 (1H, br s, NH), 8.91 (1H, s, H-11), 8.53 (1H, d, *J* = 13.2 Hz, H-1), 8.30 (1H, s, H-18), 7.05 (2H, s, H-14 and H-15), 4.86 (1H, br s, NH), 3.53 (2H, m, H-21), 3.36 (2H, m, H-22), 2.79 (1H, m, H-4a), 2.57 (2H, m, H-4b and H-5a), 1.85 (1H, m, H-5b), 1.56 (3H, s, H-20), 1.44 (9H, s, H-25); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 195.1 (C-3), 184.8 (C-16), 183.6 (C-13), 176.5 (C-9), 161.0 (C-1), 154.5 (C-19), 139.4 (C-14/C-15), 138.9 (C-15/C-14), 136.0 (C-7), 132.9 (C-17), 131.5 (C-12 and C-10), 125.8 (C-11), 125.0 (C-18), 102.6 (C-2), 79.9 (C-24), 50.1 (C-21), 41.3 (C-22), 39.5 (C-6), 33.0 (C-4), 32.8 (C-5), 28.3 (C-25), 28.2 (C-20), C-23 not observed; (+)-ESIMS *m/z* 493 $[M+H]^+$; (+)-HRESIMS $[M+H]^+$ 493.1983 (calcd for $C_{27}H_{29}N2O_7$, 493.1969).
20. Geranylamino analogue **11**: $[\alpha]_D$ +975 (*c*. 0.12, CH_2Cl_2); R_f (SiO_2, 1% MeOH/CH_2Cl_2) 0.51; IR ν_{max} (smear) 1671, 1277 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 11.65 (1H, br m, NH), 8.91 (1H, s, H-11), 8.62 (1H, d, *J* = 13.5 Hz, H-1), 8.30 (1H, s, H-18), 7.04 (2H, s, H-14 and H-15), 5.30 (1H, m, H-22), 5.07 (1H, m, H-27), 4.02 (2H, t, *J* = 6.1 Hz, H-21), 2.80 (1H, ddd, *J* = 6.9, 13.0, 19.3 Hz, H-4a), 2.58 (2H, m, H-4b and H-5a), 2.08 (4H, obsc, H-25 and H-26), 1.85 (1H, ddd, *J* = 6.9, 13.0, 13.0 Hz, H-5b), 1.72 (3H, d, *J* = 1.0 Hz, H-24), 1.66 (3H, d, *J* = 1.0 Hz, H-30), 1.60 (3H, s, H-29), 1.56 (3H, s, H-20); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 194.9 (C-3), 184.6 (C-16), 183.8 (C-13), 176.0 (C-9), 160.2 (C-1), 154.9 (C-19), 142.5 (C-23), 139.4 (C-14/C-15), 138.9 (C-15/C-14), 138.1 (C-8), 136.8 (C-7), 133.3 (C-17), 132.7 (C-10), 132.1 (C-28), 130.4 (C-12), 125.7 (C-11), 125.0 (C-18), 123.6 (C-27), 118.1 (C-22), 102.3 (C-2), 47.3 (C-21), 39.8 (C-25 or C-26), 39.5 (C-6), 33.0 (C-4), 32.7 (C-5), 28.4 (C-20), 26.3 (C-26 or C-25), 25.6 (C-30), 17.7 (C-29), 16.5 (C-24); (+)-ESIMS *m/z* 486 $[M+H]^+$; (+)-HRESIMS $[M+H]^+$ 486.2286 (calcd for $C_{30}H_{32}NO_5$, 486.2275).
21. Glycylmethylester analogue **12**: $[\alpha]_D$ +889 (*c*. 0.09, CH_2Cl_2); R_f (SiO_2, 100% CH_2Cl_2) 0.14; IR ν_{max} (smear) 1746, 1671, 1199 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 11.72 (1H, br m, NH), 8.91 (1H, s, H-11), 8.50 (1H, d, *J* = 13.0 Hz, H-1), 8.32 (1H, s, H-18), 7.05 (2H, s, H-14 and H-15), 4.19 (2H, dd, *J* = 3.7, 5.8 Hz, H-21), 3.82 (3H, s, H-23), 2.82 (1H, ddd, *J* = 7.2, 12.7, 19.6 Hz, H-4a), 2.63 (2H, m, H-4b and H-5a), 1.88 (1H, ddd, *J* = 6.7, 13.0, 13.0 Hz, H-5b), 1.57 (3H, s, H-20); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 196.0 (C-3), 184.5 (C-16), 183.8 (C-13), 176.5 (C-9), 168.6 (C-22), 160.6 (C-1), 154.9 (C-19), 139.4 (C-14 or C-15), 139.0 (C-15 or C-14), 138.5 (C-8), 135.5 (C-7), 133.4 (C-17), 132.4 (C-10), 130.4 (C-12), 125.8 (C-11), 125.1 (C-18), 102.4 (C-2), 52.8 (C-23), 50.6 (C-21), 39.7 (C-6), 33.1 (C-4), 32.7 (C-5), 28.2 (C-20); (+)-ESIMS *m/z* 422 $[M+H]^+$; (+)-HRESIMS $[M+H]^+$ 422.1240 (calcd for $C_{23}H_{20}NO_7$, 422.1234).
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