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View Article Online
DOI: 10.1039/C6MD00587J

MedChemComm

RESEARCH ARTICLE

Design, synthesis and cytotoxicity of bengamide analogues and their epimers†‡

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

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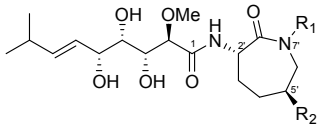
Starting from D-glycero-D-gulo-heptonic acid γ-lactone and amino acids, a number of diastereoisomeric bengamide analogues were synthesized. Optimization of the reaction conditions revealed that microwave irradiation assistance is a powerful method for the preparation of aminolactams, as well as for the coupling reactions of the lactone **5** with aminolactams. Cytotoxic activity evaluation against six cancer cell lines, KB, HepG2, LU1, MCF7, HL60, and Hela demonstrated that the configuration of C-2' seems to be critical for the cytotoxic activity of compounds **8b** (3'*R*) and **8b** (3'*S*). Additionlly, comparison of cytotoxicity of the protected acetonide compounds with their corresponding deprotedted bengamide analogues suggested that the flexibility of the ketide side chain should be require for their cytotoxic activity.

Introduction

Natural products from terrestrial source play an important role in biomedical research, as the majority of antibacterial and cytotoxic anticancer drugs in clinical use are either such secondary metabolites or their derivatives.[1-3] Although the marine environment represents an enormous additional reservoir for novel secondary metabolites.[4-5] Marine sponges of the family Jaspidae have proven to be an important source of bioactive secondary metabolites. The sponge-derived bengamides, have been firstly reported in 1986, and have a unique molecular structure.[6] These compounds were found to have a broad spectrum of biological activities such as antitumor, antibiotic, and anthelmintic properties.[7-9] Due to their pharmacological interest, these molecules have attracted the attention of many organic chemists. The structural modification of bengamides has focused mainly on improving their water solubility, stability or biological activity. Accordingly, the synthetic analogues have been prepared by modifications of the different stereocenters located at the polyketide chain;[10-14] changes of the substituent located at the terminal olefinic position;[15-17] or modification of the caprolactam unit.[17-20] These modifications led to the obtainment of more potent bengamide derivatives. Modification of side chain by replacement of isopropyl by *tert*-butyl group has proven successful. This modification simplified the synthesis of their analogues. Also, the introduction of *tert*-butyl group at the terminal side chain led to the obtainment of more stable structures by avoiding olefin isomerization. A bengamide analogue, LAF389 with *tert*-butyl at terminal side chain,[21] has been used in a clinical trial. However, the poor pharmacokinetic properties and unclear side effects of LAF389, which appeared early in the trial, have prevented its further development.[22] This prompt the search for new bengamide analogues with better therapeutic index. To our knowledge, except for the ent-bengamide,[23] no synthesis of bengamide analogues with modification of chirality at C-2' carbons have been reported. In this communication, we report the synthesis of bengamide analogues with replacement of isopropyl by *tert*-butyl, and modification of C-2' or C-5' of the lactam ring, and their cytotoxicity evaluation against six cancer cell lines.



Bengamide A: R_1 = H, R_2 = $O_2C(CH_2)_{12}CH_3$
B: R_1 = Me, R_2 = $O_2C(CH_2)_{12}CH_3$
E: R_1 = R_2 = H
F: R_1 = Me, R_2 = H
Y: R_1 = H, R_2 = OH
Z: R_1 = Me, R_2 = OH

Fig. 1 Representative bengamide structures

Results and discussion

Synthetic approach of bengamide analogues was described in Schemes 1 - 4. The general strategy for the preparation of bengamide analogues is based on the lactone **5** and

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† The authors declare no competing interests.
‡ Electronic Supplementary Information (ESI) available: HRESIMS, 1D and 2D NMR spectra of compounds **3a-b**, *rac*-**4a-b**, **6a-b**, **7a-d**, **8a-b** and **9a-d**. See DOI:

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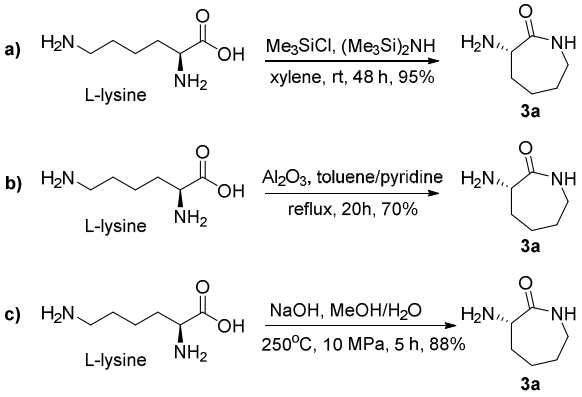
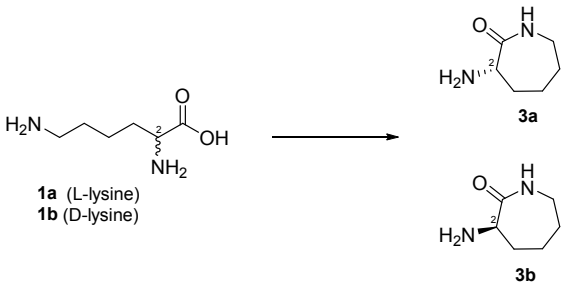
DOI: 10.1039/C6MD00587J

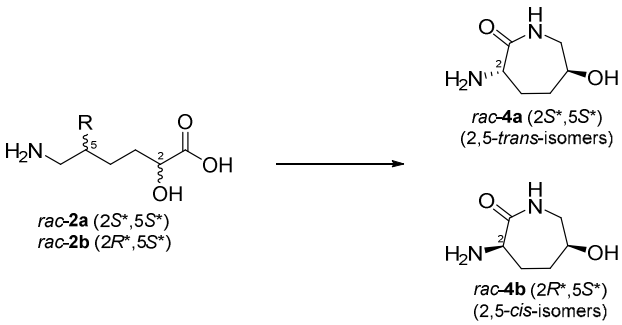
aminocaprolactam intermediates, via an amide coupling reaction.



Scheme 1 Main reported methods for synthesis of **3a** from L-lysine

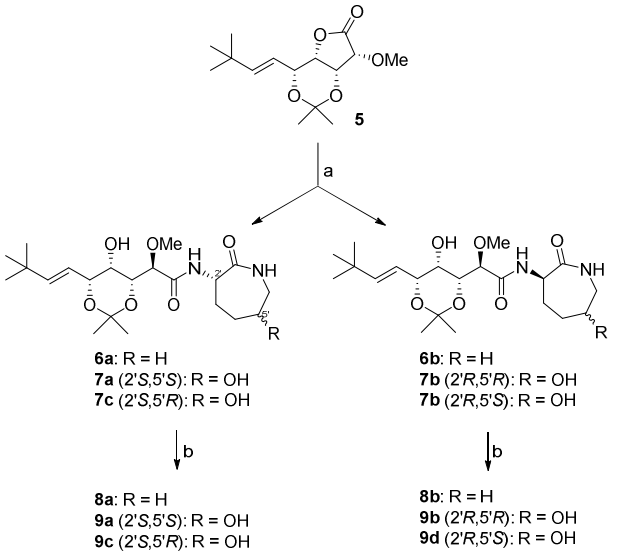


Scheme 2 Reagents and conditions: Ethylene glycol, MW at 284 W, 60 min; **3a** (79%), **3b** (82%).



Scheme 3 Reagents and conditions: Ethylene glycol, MW at 284 W, 60 min; *rac*-**4a** (51%), *rac*-**4b** (39%).

In the first step, the aminocaprolactams were synthesized from the corresponding amino acids. Several methods for synthesis of α-aminolactams from the corresponding amino acids have been reported.[24-26] However, these methods involved to use expensive reagents or need performing under high pressure, at high temperature and long reaction time (methods a, b and c, Scheme 1). In this work, the preparation of α-aminolactams were examined under microwave irradiation. With the aid of microwave irradiation, the cyclization reactions proceed faster under mild condition (Scheme 2). The α-aminolactams **3a** and **3b** were obtained from L-lysine and D-lysine in 79 and 82 % yields, respectively. Similarly, the racemic **4a** (2*S**,5*S**) and **4b** (2*R**,5*S**) were also prepared from the corresponding racemic **2a** and **2b** (Scheme 3).



Scheme 4 Reagents and conditions: (a) THF, MW at 100 W, 60 - 120 min, **6a** (95%), **6b** (76%), **7a** (48%), **7b** (44%) (93% overall yield for **7a**+**7b**), **7c** (28%) and **7d** (25%) (53% overall yield for **7c**+**7d**); (b) TFA, H_2O, THF, 0°C, 1 h, **8a** (69%), **8b** (58%), **9a** (45%), **9b** (63%), **9c** (42%) and **9d** (40%).

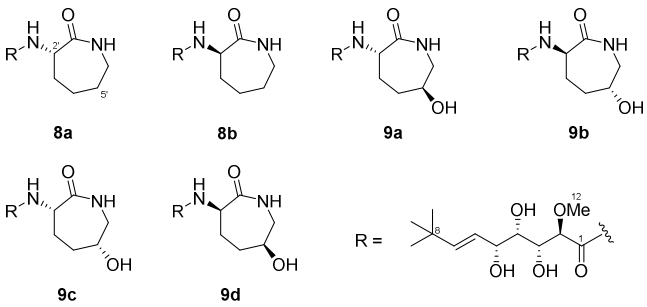


Fig. 2 Structures of the synthetic bengamide analogues.

Coupling reaction of the lactone **5** (which was prepared according to the previously reported method)[20,21] with each enantiomeric aminolactams **3a** and **3b**, and two racemic mixtures, *rac*-**4a** and *rac*-**4b** was optimized. Initial attempt for the coupling of **5** with these aminolactams was performed according to the previously described method by using sodium 2-ethylhexanoate as a base at room temperature for 15 – 20 h.[21] The lactone ring opening by aminolactams was then examined under microwave irradiation. Accordingly, treatment of **5** with the aminolactams **3a** and **3b** under microwave irradiation at 100 W for 1 - 2 h to afford the corresponding compounds **6a** and **6b** in 95 and 76% yield, respectively. Thus, with microwave irradiation assistance, the coupling reaction occurred significantly faster with the yields comparable with previously described method. By using the same procedure with microwave irradiation assistance, the lactone **5** reacted with the racemic **4a** (2S*,5S*), affording two diastereoisomers **7a** and **7b**, while the coupling reaction of **5** with the racemic **4b** (2*R**,5*S**) provided the two diastereoisomers **7c** and **7d**.

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Finally, acetonide deprotection of compounds **6a-b** and **7a-d**, was achieved by treatment with TFA and H_2O in THF at 0 ^oC to afford the corresponding bengamide analogues **8a-b** and **9a-d**. The absolute configuration of C-2' and C-5' of compounds **7a**-**d** and **9a**-**d** was established by comparison of their NMR data and optical rotation activity with the previously reported compound (in case of **7c**) or related structures. Accordingly, for the 2',5'-*trans*-isomer series, **9a** had a positive rotation activity {$[\alpha]_D^{25}$ +7.0 (*c* 0.1, MeOH)}, while this value was -20.0 (*c* 0.1, MeOH) for **9b**. The bengamide Y (2'*S*,5'*S*), a related structure with **9a** and **9b** displayed a positive rotation activity {$[\alpha]_D^{25}$ +14.0 (*c* 0.11, MeOH)}.[9] This observation suggested the absolute configurations 2'*S* and 5'*S* for **9a**, as well as for **7a**. Thus, the configurations 2'*R* and 5'*R* were assigned for **9b** and **7b**. Similarly, for the 2',5'-*cis*-isomers **7c**-**d** and **9c**-**d**, comparison of NMR data revealed that **7c** was identical with the reported values for 2'*S*,5'*R*-isomer.[20] The configurations 2'*S* and 5'*R* were distributed for **7c** and **9c**. Therefore, **7d** and **9d** had the configurations 2'*R* and 5'*S*.

Table 1 Cytotoxicity of the synthetic bengamide analogues (IC_{50} values are expressed in μM)

Compd	KB	LU1	HepG2	MCF7	HL60	Hela
8a	21.0	4.3	21.1	1.3	83.3	60.7
8b	1.1	0.5	1.1	0.2	5.3	2.1
9a	19.8	10.0	103.1	144.5	>150.0	>150.0
9b	5.1	17.2	21.3	164.9	12.1	15.4
9c	62.6	23.4	>150.0	23.4	>150.0	>150.0
9d	23.7	36.3	137.6	80.4	>150.0	>150.0
6a	>150.0	>150.0	>150.0	>150.0	>150.0	>150.0
6b	>150.0	>150.0	>150.0	>150.0	>150.0	>150.0
Ellipticine	1.2	1.6	1.2	2.4	2.0	1.6

The synthetic bengamide analogues were evaluated for their cytotoxicity against six cancer cell lines, KB (mouth epidermal carcinoma cells), HepG2 (human liver hepatocellular carcinoma cells), LU (human lung adenocarcinoma cells), MCF7 (human breast cancer cells), HL60 (human promyelocytic leukemia cells), and Hela (human cervical carcinoma cells). Compound **8b** (2'*R*) was the most active against six tested cancer cell lines and exhibited slightly selective inhibition toward MCF7 cells (Table 1). Comparison of the compound **8b** (2'*R*) with its 2'-epimer (**8a**) which had the same stereochemistry at C-2' as natural bengamides revealed that **8b** (2'*R*) was more active than **8a** (2'*S*) against almost tested cancer cell lines. Additionally, in order to understand the flexibility effects of the ketide side chain to the cytotoxicity, the acetonide compounds were also tested for their activity against six above cancer cell lines. However, all of them were much less active than their corresponding deprotected compounds. No inhibition was observed for the restricted compounds even at the concentration of 150 μM. As an example indicated in Table 1, the two compounds **6a** and **6b** with restricted C-3/C-4/C-5 bonds are significantly less active than **8a** and **8b** which had free rotation bonds for C-3/C-4/C-5. This suggested that the flexibility of the polyketide side chain of bengamides seems to be critical for their cytotoxicity. This observation is in agreement with the previous report that the hydroxyl groups at C3, C-4 and C-5 of the polyketide chain are involved in the activity of bengamides by forming complex with MetAps,[27] enzymes playing important roles in cell proliferation and angiogenesis.[28-29]

Conclusions

Six diastereoisomeric bengamide analogues were synthesized. Microwave irradiation assistance was found to be useful for the preparation of aminolactams, and especially for the coupling reactions of the lactone **5** with aminolactams. Cytotoxicity evaluation of these diastereoisomeric bengamide analogues revealed that the analogue **8b** was remarkably more active than its 2'-epimer (**8a**) which represented similar configuration at C-2' as natural bengamides. Furthermore, since the acetonide compounds were much less active than their corresponding acetonide deprotection analogues, the flexibility of the ketide side chain should be require for their cytotoxic activity. This is consistent with the previous study on mode of action for bengamides in which the hydroxyl groups at C-2, C-4 and C-5 are required for forming complex with MetAps.

Experimental

General information

Optical rotations were recorded on a Polax-2L polarimeter in $CHCl_3$ or MeOH. HRESIMS data were recorded on a VARIAN 910 spectrometer. NMR spectra were recorded on a Bruker AM500 FT-NMR spectrometer operating at 500.13 MHz and 125.76 MHz for ^1H NMR and ^{13}C NMR spectra, respectively. ^1H NMR chemical shifts were referenced to $CHCl_3$ at 7.27 ppm, and ^{13}C NMR chemical shifts to the central peak of $CDCl_3$ at 77.0 ppm. The HMBC measurements were optimized to 7.0 Hz long-range couplings, and NOESY experiments were run with 150 ms mixing time. All chemicals were purchased from Sigma-Aldrich and used without further purification.

Synthetic procedures

Synthesis of the aminolactams 3a-b, *rac*-4a and *rac*-4b

To a solution of each amino acid, L-lysine, D-lysine, *rac*-**2a** and *rac*-**2b** (0.3 mmol) in ethylene glycol (0.1 mL) was added pyridine (0.1 mL). The mixture was irradiated with microwaves at 284 W for 60 min. The corresponding aminolactam compounds were obtained by chromatographic purification on silica gel (gradient acetone/MeOH). NMR data for **3b** and **3b**: ^1H-NMR (500 MHz, DMSO-d_6): 1.17 (1H, m), 1.32 (1H, m), 1.56 (1H, m), 1.68 (2H, m), 1.83 (1H, m), 3.05 (2H, m), 3.41 (1H, dd, *J* = 1.5, 11.0 Hz), 7.56 (1H, br. s, NH). ^{13}C-NMR (125 MHz, DMSO-d_6): 27.9 (CH_2), 29.1 (CH_2), 34.1 (CH_2), 40.5 (CH_2), 52.8 (CH), 177.9 (C=O); NMR data for *rac*-**4a**: ^1H-NMR (500 MHz, DMSO-d_6): 1.36-1.67 (m,3H), 1.95 (m, 1H), 2.90-3.50 (m, 4H), 4.85 (br. s, 1H), 7.55 (br. s, 1H). ^{13}C-NMR (125 MHz, DMSO-d_6): 32.5 (CH_2), 36.9 (CH_2), 47.4 (CH_2), 52.6 (CH), 69.3 (CH), 177.8 (C=O); NMR data for *rac*-**4b**: ^1H-NMR (500 MHz, DMSO-d_6): 1.44 (m,1H), 1.58-1.78 (m, 3H), 2.98-3.66 (m, 4H), 7.18 (br. s,

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NH). ^{13}C-NMR (125 MHz, DMSO-d6): 28.3 (CH_2), 34.4 (CH_2), 45.2 (CH_2), 52.9 (CH), 64.3 (CH), 177.6 (C=O).

General procedure for coupling reaction of lactone 5 with aminolactams

To a solution of each amino lactam, **3a**-**b**, *rac*-**4a** and *rac*-**4b** (0.5 mmol, 5.0 eq) in 0.2 mL anhydrous THF was added the ketide **5** (1.0 eq) and sodium-2-ethylhexanoate (1.2 eq) under nitrogen atmosphere. The solution mixture was irradiated with microwaves at 100 W for 60 - 120 min. The solvent was removed in vacuum. The residue was chromatographed on a silica gel column, eluting with gradient mixture of CH_2Cl_2/MeOH to afford the corresponding protected bengamide analogues.

6a: $C_{21}H_{36}N_2O_6$; White amorphous solids, $[\alpha]_D^{25}$ -7.0 (*c* 0.8 $CHCl_3$); ESIMS: *m/z* 413 $[M+H]^+$; ^{1}H-NMR (500 MHz, $CDCl_3$): 7.78 (1H, d, *J* = 6.0 Hz, NH-13), 5.91 (1H, br. s, NH-7'), 5.77 (1H, d, *J* = 16.0 Hz, H-7), 5.56 (1H, dd, *J* = 7.0 and 16.0 Hz, H-6), 4.58 (1H, ddd, *J* = 1.5, 6.0 and 10.5 Hz, H-2'), 4.26 (1H, br. d, *J* = 7.0 Hz, H-5), 4.07 (1H, br. d, *J* = 6.0 Hz, H-3), 3.88 (1H, d, *J* = 6.0 Hz, H-2), 3.54 (1H, br. d, *J* = 7.5 Hz, H-4), 3.51 (3H, OMe-12), 3.39 (1H, d, *J* = 7.5 Hz, OH), 3.28 (2H, m, CH_2-6'), 2.06 (1H, m, H_b-4'), 2.01 (1H, m, H_b-3'), 1.85 (2H, m, H_a-4', H_b-5'), 1.53 (1H, m, H_a-3'), 1.48 (3H, s, Me-15), 1.45 (3H, s, Me-16), 1.42 (1H, m, H_b-5'), 1.03 (9H, s, Me-9, 10 and 11). ^{13}C-NMR (125 MHz, $CDCl_3$): 175.0 (C-1'), 169.5 (C-1), 145.3 (C-7), 121.7 (C-6), 99.7 (C-14), 81.6 (C-2), 74.7 (C-5), 73.3 (C-3), 66.3 (C-4), 59.6 (OMe-12), 51.9 (C-2'), 42.2 (C-6'), 33.1 (C-8), 31.7 (C-3'), 29.5 (Me-15), 29.4 (C-9, 10 and 11), 28.9 (C-5'), 27.9 (C-4'), 19.0 (C-16).

6b: $C_{21}H_{36}N_2O_6$; White amorphous solids; $[\alpha]_D^{25}$ +8.8 (*c* 1.05 $CHCl_3$); ESIMS: *m/z* 413 $[M+H]^+$; ^{1}H-NMR (500 MHz, $CDCl_3$): 7.55 (1H, d, *J* = 6.0 Hz, NH-13), 6.05 (1H, t, *J* = 5.5 Hz, NH-7'), 5.77 (1H, d, *J* = 16.0 Hz, H-7), 5.52 (1H, dd, *J* = 6.5 and 16.0 Hz, H-6), 4.59 (1H, br. dd, *J* = 6.0 and 10.5 Hz, H-2'), 4.27 (1H, br. d, *J* = 6.5 Hz, H-5), 4.06 (1H, br. d, *J* = 7.5 Hz, H-3), 3.87 (1H, d, *J* = 7.5 Hz, H-2), 3.54 (1H, br. s, H-4), 3.48 (3H, s, OMe-12), 3.27 (2H, m, CH_2-6'), 2.09 (1H, m, H_b-4'), 2.01 (1H, m, H_b-3'), 1.85 (2H, m, H_a-4', H_b-5'), 1.65 (1H, m, H_a-3'), 1.46 (3H, s, Me-15), 1.45 (3H, s, Me-16), 1.44 (1H, m, H_b-5'), 1.02 (9H, s, Me-9, 10 and 11). ^{13}C-NMR (125 MHz, $CDCl_3$): 175.2 (C-1'), 169.4 (C-1), 145.3 (C-7), 121.6 (C-6), 99.7 (C-14), 80.7 (C-2), 74.5 (C-5), 73.2 (C-3), 65.9 (C-4), 59.2 (OMe-12), 52.0 (C-2'), 42.1 (C-6'), 33.1 (C-8), 31.3 (C-3'), 29.6 (Me-15), 29.4 (C-9, 10 and 11), 28.9 (C-5'), 27.9 (C-6'), 19.1 (C-16).

7a: $C_{21}H_{36}N_2O_7$; White amorphous; ESIMS: *m/z* 451 $[M+Na]^+$; ^{1}H-NMR (500 MHz, $CDCl_3$): 7.77 (1H, d, *J* = 6.0 Hz, NH-13), 6.09 (1H, t, *J* = 6.0 Hz, NH-7'), 5.77 (1H, d, *J* = 16.0 Hz, H-7), 5.54 (1H, dd, *J* = 6.5 and 16.0 Hz, H-6), 4.61 (1H, m, H-2'), 4.26 (1H, br. d, *J* = 6.5 Hz, H-5), 4.05 (1H, br. d, *J* = 6.5 Hz, H-3), 3.88 (1H, d, *J* = 6.5 Hz, H-2), 3.62 (1H, m, H-5'), 3.53 (1H, br. s, H-4), 3.50 (3H, s, OMe-12), 3.33 (1H, m, H_b-6'), 3.29 (1H, m, H_a-6'), 2.21 (1H, m, H_b-4'), 2.10 (1H, m, H_b-3'), 1.89 (1H, m, H_a-4'), 1.65 (1H, m, H_a-3'), 1.47 (3H, s, Me-15), 1.45 (3H, s, Me-16), 1.02 (9H, s, Me-9, 10 and 11). ^{13}C-NMR (125 MHz, $CDCl_3$): 173.6 (C-1'), 168.8 (C-1), 144.4 (C-7), 120.6 (C-6), 98.7 (C-14), 80.4 (C-2), 73.6 (C-5), 72.3 (C-3), 68.9 (C-5'), 65.2 (C-4), 58.6 (OMe-12), 50.6 (C-2'), 47.0 (C-6'), 35.9 (C-4'), 32.1 (C-8), 28.5 (C-3'), 28.3 (C-9, 10 and 11), 28.6 (C-15), 17.9 (C-16).

7b: $C_{21}H_{36}N_2O_7$; White amorphous; $[\alpha]_D^{25}$ +23.4 (c 0.3 $CHCl_3$), ESIMS: m/z 451 $[M+Na]^+$; ^{1}H-NMR (500 MHz, $CDCl_3$): 7.57 (1H, d, *J* = 6.0 Hz, NH-13), 6.19 (1H, t, *J* = 6.0, NH-7'), 5.78 (1H, d, *J* = 16.0 Hz, H-7), 5.52 (1H, dd, *J* = 7.0, 16.0 Hz, H-6), 4.62 (1H, br. dd, *J* = 6.0 and 9.5 Hz, H-2'), 4.28 (1H, br. d, *J* = 7.0 Hz, H-5), 4.06 (1H, br. d, *J* = 7.5 Hz, H-3), 3.88 (1H, d, *J* = 7.5 Hz, H-2), 3.61 (1H, m, H-5'), 3.53 (1H, br. s, *J* = 5.5 Hz, H-4), 3.48 (3H, s, OMe-12), 3.35 (1H, m, H_b-6'), 3.28 (1H, m, H_a-6'), 2.22 (1H, m, H_b-4'), 2.13 (1H, m, H_b-3'), 1.88 (1H, m, H_a-4'), 1.63 (1H, m, H_a-3'), 1.46 (3H, s, Me-15), 1.45 (3H, s, Me-16), 1.03 (9H, s, Me-9, 10 and 11). ^{13}C-NMR (125 MHz, $CDCl_3$): 174.7 (C-1'), 169.7 (C-1), 145.4 (C-7), 121.5 (C-6), 99.7 (C-14), 80.7 (C-2), 74.4 (C-5), 73.2 (C-3), 69.9 (C-5'), 65.8 (C-4), 59.3 (OMe-12), 51.7 (C-2'), 48.1 (C-6'), 36.8 (C-4'), 33.1 (C-8), 29.6 (C-15), 29.4 (C-9, 10 and 11), 29.3 (C-3'), 19.1 (C-16).

7c: $C_{21}H_{36}N_2O_7$; White amorphous, $[\alpha]_D^{25}$ +26.9 (*c* 0.45 $CHCl_3$); ESIMS: *m/z* 451 $[M+Na]^+$; ^{1}H-NMR (500 MHz, $CDCl_3$): 7.60 (1H, d, *J* = 7.0 Hz, NH-13), 6.27 (1H, t, *J* = 5.0 Hz, NH-7'), 5.78 (1H, d, *J* = 16.0 Hz, H-7), 5.53 (1H, dd, *J* = 7.0 and 16.0 Hz, H-6), 4.57 (1H, m, H-2'), 4.28 (1H, br. d, *J* = 7.0 Hz, H-5), 4.07 (1H, br. d, *J* = 7.0 Hz, H-3), 4.01 (1H, m, H-5'), 3.90 (1H, d, *J* = 7.0 Hz, H-2), 3.55 (1H, br. s, H-4), 3.50 (1H, m, H_b-6'), 3.48 (3H, s, OMe-12), 3.35 (1H, m, H_a-6'), 2.05 (1H, m, H_b-4'), 2.00 (1H, m, H_a-4'), 1.89 (1H, m, H_b-3'), 1.84 (1H, m, H_a-3'), 1.46 (3H, s, Me-16), 1.45 (3H, s, Me-15), 1.03 (9H, s, Me-9, 10 and 11). ^{13}C-NMR (125 MHz, $CDCl_3$): 175.1 (C-1'), 169.7 (C-1), 145.4 (C-7), 121.5 (C-6), 99.7 (C-14), 80.6 (C-2), 74.5 (C-5), 73.2 (C-3), 65.8 (C-4), 64.7 (C-5'), 59.2 (OMe-12), 51.8 (C-2'), 46.0 (C-6'), 34.6 (C-4'), 33.1 (C-8), 29.6 (C-15), 29.3 (C-9, 10 and 11), 25.1 (C-3'), 19.1 (C-16).

7d: $C_{21}H_{36}N_2O_7$; White amorphous, $[\alpha]_D^{25}$ -19.7 (*c* 0.3 $CHCl_3$); ESIMS: *m/z* 451 $[M+Na]^+$; ^{1}H-NMR (500 MHz, $CDCl_3$): 7.78 (1H, d, *J* = 6.0 Hz, NH-13), 6.10 (1H, br. s, H-7'), 5.78 (1H, d, *J* = 15.5 Hz, H-7), 5.55 (1H, dd, *J* = 7.0, 15.5 Hz, H-6), 4.55 (1H, m, H-2'), 4.26 (1H, br. d, *J* = 7.0 Hz, H-5), 4.06 (1H, br. d, *J* = 6.5 Hz, H-3), 4.01 (1H, m, H-5'), 3.89 (1H, d, *J* = 6.5 Hz, H-2), 3.53 (1H, br. s, H-4), 3.51 (1H, m, H_b-6'), 3.50 (3H, s, OMe-12), 3.34 (1H, m, H_a-6'), 2.04 (1H, m, H_b-4'), 2.01 (1H, m, H_a-4'), 1.88 (1H, m, H_b-3'), 1.84 (1H, m, H_a-3'), 1.47 (3H, s, Me-15), 1.45 (3H, s, H-16), 1.03 (9H, s, Me-9, 10 and 11). ^{13}C-NMR (125 MHz, $CDCl_3$): 175.0 (C-1'), 169.7 (C-1), 145.4 (C-7), 121.6 (C-6), 99.7 (C-14), 81.4 (C-2), 74.6 (C-5), 73.3 (C-3), 66.3 (C-4), 64.7 (C-5'), 59.6 (OMe-12), 51.7 (C-2'), 46.1 (C-6'), 34.6 (C-4'), 33.1 (C-8), 29.5 (C-15), 29.4 (C-9, 10 and 11), 25.4 (C-3'), 19.0 (C-16).

General procedure for synthesis of compounds 8a-b and 9a-d

A solution of each protected compound, **6a**-**b**, and **7a**-**d**, (0.02 mmol) in THF (0.15 mL) was cooled down to 0^{o}C. To this solution, TFA (8 eq) and H_2O (30 µL) were successively added. The solution was stirred at 0 ^{o}C for 1 h, and passed through a Sephadex LH-20 column (eluting with MeOH). The solvent was removed under diminished pressure and the residue was purified by preparative TLC to give the corresponding title compounds.

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8a: $C_{18}H_{32}N_2O_6$; White amorphous solid; $[\alpha]_D^{25}$ +5.0 (c 0.4 $CHCl_3$); HRESI-MS: *m/z* 395.2135 $[M+Na]^+$ (calcd 395.2158 for $C_{18}H_{32}N_2O_6Na$); ^{1}H-NMR (500 MHz, $CDCl_3$): 7.96 (1H, d, *J* = 6.5 Hz, NH-13), 6.13 (1H, br. s, NH-7'), 5.82 (1H, d, *J* = 15.5 Hz, H-7), 5.41 (1H, dd, *J* = 7.5 and 15.5 Hz, H-6), 4.55 (1H, br. dd, *J* = 6.5 and 10.5 Hz, H-2'), 4.22 (1H, dd, *J* = 5.0 and 7.5 Hz, H-5), 3.80 (1H, br. d, *J* = 7.0 Hz, H-3), 3.75 (1H, d, *J* = 7.0 Hz, H-2), 3.62 (1H, br. d, *J* = 5.0 Hz, H-4), 3.55 (3H, s, OMe-12), 3.28 (2H, CH_2-6'), 2.03 (1H, m, H_b-4'), 2.01 (1H, m, H_b-3'), 1.87 (1H, m, H_b-5'), 1.82 (1H, m, H_a-4'), 1.56 (1H, m, H_a-3'), 1.42 (1H, m, H_a-5'), 1.02 (9H, s, Me-9, 10 and 11). ^{13}C-NMR (125 MHz, $CDCl_3$): 174.8 (C-1'), 171.5 (C-1), 145.7 (C-7), 123.2 (C-6), 81.3 (C-2), 74.5 (C-5), 72.7 (C-3), 72.3 (C-4), 59.9 (OMe-12), 51.9 (C-2'), 42.1 (C-6'), 33.0 (C-8), 31.4 (C-3'), 29.4 (C-9, 10 and 11), 28.8 (C-5'), 27.9 (C-4').

8b: $C_{18}H_{32}N_2O_6$; White amorphous solid; $[\alpha]_D^{25}$ +37.7 (*c* 0.26 $CHCl_3$); HRESIMS: *m/z* 395.2161 $[M+Na]^+$ (calcd 395.2158 for $C_{18}H_{32}N_2O_6Na$); ^{1}H-NMR (500 MHz, $CDCl_3$): 7.88 (1H, br. s, NH-13), 5.84 (1H, d, *J* = 15.5 Hz, H-7), 5.39 (1H, dd, *J* = 7.5 and 15.5 Hz, H-6), 4.53 (1H, br. dd, *J* = 6.5 and 9.5 Hz, H-2'), 4.16 (1H, dd, *J* = 5.0 and 7.5 Hz, H-5), 3.93 (1H, m, H-3), 3.72 (1H, m, H-2), 3.67 (1H, m, H-4), 3.47 (3H, s, OMe-12), 3.30 (2H, CH_2-6'), 2.03 (1H, m, H_b-4'), 2.02 (1H, m, H_b-3'), 1.85 (1H, m, H_b-5'), 1.78 (1H, m, H_a-4'), 1.67 (1H, m, H_a-3'), 1.44 (1H, m, H_a-5'), 1.01 (9H, s, Me-9, 10 and 11). ^{13}C-NMR (125 MHz, $CDCl_3$): 175.1 (C-1'), 171.8 (C-1), 146.0 (C-7), 123.3 (C-6), 81.5 (C-2), 74.1 (C-5), 72.7 (C-4), 72.1 (C-3), 59.1 (OMe-12), 52.2 (C-2'), 41.9 (C-6'), 33.0 (C-8), 30.3 (C-3'), 29.4 (C-9, 10 and 11), 28.6 (C-5'), 27.9 (C-4').

9a: White amorphous solid; $[\alpha]_D^{25}$ +7.0 (*c* 0.1, MeOH); HRESIMS: *m/z* 411.2111 $[M+Na]^+$ (calcd 411.2107 for $C_{18}H_{32}N_2O_7Na$); ^{1}H-NMR (500 MHz, CD_3OD): 5.82 (1H, d, *J* = 16.0 Hz, H-7), 5.43 (1H, dd, *J* = 8.0 and 16.0 Hz, H-6), 4.64 (1H, dd, *J* = 1.5 and 11.5 Hz, H-2'), 4.16 (1H, dd, *J* = 8.0 and 8.0 Hz, H-5), 3.83 (1H, d, *J* = 7.0 Hz, H-2), 3.74 (1H, dd, *J* = 1.5 and 7.0 Hz, H-3), 3.60 (1H, dd, *J* = 1.5 and 8.0 Hz, H-4), 3.51 (1H, m, H-5'), 3.43 (3H, s, OMe-12), 3.32 (1H, m, H_b-6'), 3.26 (1H, m, H_a-6'), 2.20 (1H, m, H_b-4'), 2.03 (1H, m, H_b-3'), 1.82 (1H, m, H_a-4'), 1.73 (1H, m, H_a-3'), 1.05 (9H, s, Me-9, 10 and 11). ^{13}C-NMR (125 MHz, CD_3OD): 176.7 (C-1'), 173.1 (C-1), 146.0 (C-7), 125.4 (C-6), 83.6 (C-2), 75.4 (C-5), 74.3 (C-4), 72.7 (C-3), 70.5 (C-5'), 58.8 (OMe-12), 53.0 (C-2'), 48.6 (C-6'), 37.5 (C-4'), 33.7 (C-8), 30.2 (C-3'), 29.8 (C-9, 10 and 11).

9b: White amorphous solid; $[\alpha]_D^{25}$ -20.0 (*c* 0.1, MeOH); HRESIMS: *m/z* 411.2103 $[M+Na]^+$ (calcd 411.2107 for $C_{18}H_{32}N_2O_7Na$); ^{1}H-NMR (500 MHz, CD_3OD): 5.83 (1H, d, *J* = 16.0 Hz, H-7), 5.42 (1H, dd, *J* = 7.0 and 16.0 Hz, H-6), 4.58 (1H, dd, *J* = 6.5 and 11.0 Hz, H-2'), 4.23 (1H, dd, *J* = 7.0 and 7.0 Hz, H-5), 3.84 (1H, br. d, *J* = 6.5 Hz, H-3), 3.79 (1H, d, *J* = 6.5 Hz, H-2), 3.63 (1H, m, H-5'), 3.62 (1H, m, H-4), 3.53 (3H, s, OMe-12), 3.23 (2H, m, CH_2-6'), 2.22 (1H, m, H_b-4'), 2.12 (1H, m, H_b-3'), 1.87 (1H, m, H_a-4'), 1.67 (1H, m, H_a-3'), 1.02 (9H, s, Me-9, 10 and 11). ^{13}C-NMR (125 MHz, CD_3OD): 174.3 (C-1'), 172.2 (C-1), 145.8 (C-7), 123.2 (C-6), 81.4 (C-2), 74.5 (C-5), 72.7 (C-3), 72.5 (C-4), 69.8 (C-5'), 59.8 (OMe-12), 51.7 (C-2'), 48.0 (C-6'), 36.8 (C-4'), 33.0 (C-8), 29.4 (C-9, 10 and 11), 28.9 (C-3').

9c: White amorphous solid; $[\alpha]_D^{25}$ +16.0 (*c* 0.1, MeOH); HRESIMS: *m/z* 411.2108 $[M+Na]^+$ (calcd 411.2107 for $C_{18}H_{32}N_2O_7Na$); ^{1}H-NMR (500 MHz, CD_3OD): 5.82 (1H, d, *J* = 15.5 Hz, H-7), 5.43 (1H, dd, *J* = 7.0 and 15.5 Hz, H-6), 4.59 (1H, br. d, *J* = 9.0 Hz, H-2'), 4.14 (1H, dd, *J* = 7.0 and 7.0 Hz, H-5), 3.83 (1H, overlap, H-2), 3.79 (1H, m, H-5'), 3.76 (1H, m, H-3), 3.59 (1H, m, H-4), 3.44 (3H, s, OMe-12), 3.03 (1H, m, H_b-6'), 2.80 (1H, m, H_a-6'), 1.96 (2H, m, CH_2-3'), 1.61 (2H, m, CH_2-4'), 1.06 (9H, s, Me-9, 10 and 11). ^{13}C-NMR (125 MHz, CD_3OD): 174.7 (C-1'), 172.2 (C-1), 146.0 (C-7), 125.4 (C-6), 83.4 (C-2), 75.2 (C-5), 74.3 (C-4), 72.6 (C-3), 68.4 (C-5'), 58.7 (OMe-12), 53.1 (C-2'), 45.9 (C-6'), 35.4 (C-3'), 33.8 (C-8), 32.2 (C-4'), 29.9 (C-9, 10 and 11).

9d: White amorphous solid; $[\alpha]_D^{25}$ -35.0 (*c* 0.1, MeOH); HRESIMS: *m/z* 411.2107 $[M+Na]^+$ (calcd 411.2107 for $C_{18}H_{32}N_2O_7Na$); ^{1}H-NMR (500 MHz, CD_3OD): 5.80 (1H, d, *J* = 15.5 Hz, H-7), 5.42 (1H, dd, *J* = 7.5 and 15.5 Hz, H-6), 4.59 (1H, m, H-2'), 4.15 (1H, dd, *J* = 7.5 and 7.5 Hz, H-5), 3.92 (1H, m, H-5'), 3.83 (1H, d, *J* = 7.5 Hz, H-2), 3.74 (1H, dd, *J* = 1.7 and 7.5 Hz, H-3), 3.60 (1H, dd, *J* = 1.7 and 7.5 Hz, H-4), 3.51 (1H, br. d, *J* = 15.0 Hz, H_b-6'), 3.42 (3H, s, OMe-12), 3.26 (1H, dd, *J* = 6.5 and 15.0 Hz, H_a-6'), 1.99 (2H, m, CH_2-4'), 1.94 (1H, m, H_b-3'), 1.77 (1H, m, H_a-3'), 1.04 (9H, s, Me-9, 10 and 11). ^{13}C-NMR (125 MHz, CD_3OD): 176.6 (C-1'), 173.1 (C-1), 146.1 (C-7), 125.4 (C-6), 83.5 (C-2), 75.5 (C-5), 74.2 (C-4), 72.8 (C-3), 65.7 (C-5'), 58.8 (OMe-12), 53.2 (C-2'), 46.5 (C-6'), 35.4 (C-4'), 33.8 (C-8), 29.8 (C-9, 10 and 11), 25.9 (C-3').

Cytotoxic activity assay

The cytotoxicity assays were carried out in triplicate in 96-well microtiter plates against KB, HepG2, LU, MCF7, HL60, and Hela. Cells were maintained in Dulbecco's D-MEM medium, supplemented with 10% fetal calf serum, L-glutamine (2 mM), penicillin G (100 UI/mL), streptomycin (100 μg/mL) and gentamicin (10 μg/mL). Stock solutions of compounds were prepared in DMSO/H_2O (1/9), and the cytotoxicity assays were carried out in 96-well microtiter plates against cancer or normal cells (3 x 10^3 cells/mL) using a modification of the published method.[30] After 72 h incubation at 37 °C in air/CO_2 (95:5) with or without test compounds, cell growth was estimated by colorimetric measurement of stained living cells by neutral red. Optical density was determined at 540 nm with a Titertek Multiscan photometer. The IC_{50} value was defined as the concentration of sample necessary to inhibit the cell growth to 50% of the control. Ellipticine was used as a reference compound.

Acknowledgements

The authors thank the National Foundation for Science and Technology (NAFOSTED), and the Ministry of Science and Technology of Vietnam (MOST) for financial support (NCCB-ĐHUD.2012-G/05).

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DOI: 10.1039/C6MD00587J

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View Article Online
DOI: 10.1039/C6MD00587J

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The C-2' of the lactam rings and the flexibility of polyketide chain should be critical for the cytotoxicity of bengamides.