RESEARCH ARTICLE

 View Article Online
 View Journal | View Issue

 Cite this: *Med. Chem. Commun.*,
 2017, 8, 445

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

 Thi Dao Phi,^{ab} Huong Doan Thi Mai,^a Van Hieu Tran,^a Bich Ngan Truong,^a
 Tuan Anh Tran,^{ac} Van Loi Vu,^a Van Minh Chau^a and Van Cuong Pham^{*a}

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** (2'R) and **8a** (2'S). Additionally, comparison of cytotoxicity of the protected acetonide compounds with that of their corresponding deprotected bengamide analogues suggested that the flexibility of the ketide side chain should be required for their cytotoxic activity.

 Received 24th October 2016,
 Accepted 18th December 2016

DOI: 10.1039/c6md00587j

www.rsc.org/medchemcomm

Introduction

Natural products from terrestrial sources 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} On the other hand, the marine environment represents an enormous additional reservoir for novel secondary metabolites.^{4,5} Marine sponges of the family Jaspidae have been proven to be an important source of bioactive secondary metabolites. The sponge-derived bengamides were firstly reported in 1986, and have unique molecular structures (Fig. 1).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 interesting pharmacological applications, 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, their synthetic analogues have been prepared by modification of the different stereocenters located in their polyketide chain,^{10–14} variation of the substituent located at the terminal olefinic position,^{15–17} or modifica-

tion of the caprolactam unit.^{17–20} These modifications led to the acquisition of more potent bengamide derivatives. Modification of the side chain by replacement of isopropyl by a *tert*-butyl group has been proven successful. This modification simplified the synthesis of their analogues. Also, the introduction of the *tert*-butyl group at the terminal side chain led to the acquisition of more stable structures by avoiding olefin isomerization. A bengamide analogue, LAF389 with *tert*-butyl at the 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 prompted the search for new bengamide analogues with better therapeutic indices. To our knowledge, except for *ent*-bengamide,²³ no synthesis of bengamide analogues with modification of chirality at C-2' carbons has 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 evaluation of their cytotoxicity against six cancer cell lines.

Bengamide A: R₁ = H, R₂ = O₂C(CH₂)₁₂CH₃
 B: R₁ = Me, R₂ = O₂C(CH₂)₁₂CH₃
 E: R₁ = R₂ = H
 F: R₁ = Me, R₂ = H
 Y: R₁ = H, R₂ = OH
 Z: R₁ = Me, R₂ = OH

Fig. 1 Representative bengamide structures.

^a Advanced Center for Bioorganic Chemistry, Institute of Marine Biochemistry, VAST, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam.

E-mail: phamvc@imb.vast.vn; Fax: +84437917054; Tel: +84437917049

^b Graduate University of Science and Technology, VAST, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam

^c University of Science and Technology of Hanoi, VAST, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam

† The authors declare no competing interests.

‡ Electronic supplementary information (ESI) available: HRESIMS, 1D and 2D NMR spectra of compounds **3a** and **b**, *rac*-**4a** and **b**, **6a** and **b**, **7a**-**d**, **8a** and **b**, and **9a**-**d**. See DOI: 10.1039/c6md00587j

Results and discussion

The synthetic approach to bengamide analogues is described in Schemes 1–4. The general strategy for the preparation of bengamide analogues is based on the lactone **5** and aminocaprolactam intermediates, *via* an amide coupling reaction.

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 the use of expensive reagents or the need for operating under high pressure, at high temperature and for long reaction time (methods a, b and c, Scheme 1). In this work, the preparation of α -aminolactams was examined under microwave irradiation. With the aid of microwave irradiation, the cyclization reactions proceed faster under mild conditions (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** ($2S^*,5S^*$) and **4b** ($2R^*,5S^*$) were also prepared from the corresponding racemic **2a** and **2b** (Scheme 3).

The coupling reaction of the lactone **5** (which was prepared according to the previously reported method)^{20,21} with each enantiomeric aminolactam, **3a** and **3b**, and two racemic mixtures, *rac*-**4a** and *rac*-**4b**, was optimized. An initial attempt for the coupling of **5** with these aminolactams was performed

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%).

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 the 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 produced the corresponding compounds **6a** and **6b** in 95 and 76% yields, respectively. Thus, with microwave irradiation assistance, the coupling reaction occurred significantly faster with the yields comparable to those obtained using the 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** ($2R^*,5S^*$) provided the two diastereoisomers **7c** and **7d**. Finally, acetonide deprotection of compounds **6a** and **6b** and **7a**–**7d** was achieved by treatment with TFA and H₂O in THF at 0 °C to afford the

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, H₂O, 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.

corresponding bengamide analogues **8a** and **b** and **9a–d** (Fig. 2).

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 those of the previously reported compound (in the 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 that for **9b** was -20.0 (*c* 0.1, MeOH). Bengamide Y (2',5',5'S), a related structure to **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** and **d** and **9c** and **d**, comparison of NMR data revealed that **7c** had values identical to those reported for the 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.

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 the six tested cancer cell lines and exhibited slightly selective inhibition toward MCF7 cells (Table 1). Comparison of 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 all of the tested cancer cell lines. Additionally, in order to under-

stand the flexibility effects of the ketide side chain on the cytotoxicity, the acetonide compounds were also tested for their activity against the six abovementioned 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 μ M. 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 rotational 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 C-3, C-4 and C-5 of the polyketide chain are involved in the activity of bengamides by forming a 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 a similar configuration at C-2' to natural bengamides. Furthermore, since the acetonide compounds were much less active than their corresponding acetonide deprotected analogues, the flexibility of the ketide side chain should be required for their cytotoxic activity. This is consistent with the previous study on the mode of action of bengamides in which the hydroxyl groups at C-2, C-4 and C-5 are required for forming a complex with MetAps.

Experimental

General information

Optical rotations were recorded on a Polax-2L polarimeter in CHCl₃ 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 ¹³C NMR spectroscopy, respectively. ¹H NMR chemical shifts were referenced to CHCl₃ at 7.27 ppm, and ¹³C NMR chemical shifts to the central peak of CDCl₃ at 77.0 ppm. 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 and 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

Table 1 Cytotoxicity of the synthetic bengamide analogues (IC₅₀ values are expressed in μ M)

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

microwaves at 284 W for 60 min. The corresponding aminolactam compounds were obtained by chromatographic purification on a silica gel (gradient acetone/MeOH). NMR data for **3a** and **3b**: $^1\text{H-NMR}$ (500 MHz, $\text{DMSO-}d_6$): 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, $J = 1.5$ and 11.0 Hz), 7.56 (1H, br. s, NH). $^{13}\text{C-NMR}$ (125 MHz, $\text{DMSO-}d_6$): 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**: $^1\text{H-NMR}$ (500 MHz, $\text{DMSO-}d_6$): 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). $^{13}\text{C-NMR}$ (125 MHz, $\text{DMSO-}d_6$): 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**: $^1\text{H-NMR}$ (500 MHz, $\text{DMSO-}d_6$): 1.44 (m, 1H), 1.58–1.78 (m, 3H), 2.98–3.66 (m, 4H), 7.18 (br. s, NH). $^{13}\text{C-NMR}$ (125 MHz, $\text{DMSO-}d_6$): 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 the coupling reaction of lactone **5** with aminolactams

To a solution of each amino lactam, **3a** and **b**, *rac*-**4a** and *rac*-**4b** (0.5 mmol, 5.0 eq.), in 0.2 mL anhydrous THF were added the ketide **5** (1.0 eq.) and sodium 2-ethylhexanoate (1.2 eq.) under a nitrogen atmosphere. The solution mixture was irradiated with microwaves at 100 W for 60–120 min. The solvent was removed under vacuum. The residue was chromatographed on a silica gel column, eluting with a gradient mixture of $\text{CH}_2\text{Cl}_2/\text{MeOH}$ to afford the corresponding protected bengamide analogues.

6a. $\text{C}_{21}\text{H}_{36}\text{N}_2\text{O}_6$; white amorphous solids; $[\alpha]_{\text{D}}^{25} -7.0$ (c 0.8, CHCl_3); ESIMS: m/z 413 $[\text{M} + \text{H}]^+$; $^1\text{H-NMR}$ (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). $^{13}\text{C-NMR}$ (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. $\text{C}_{21}\text{H}_{36}\text{N}_2\text{O}_6$; white amorphous solids; $[\alpha]_{\text{D}}^{25} +8.8$ (c 1.05, CHCl_3); ESIMS: m/z 413 $[\text{M} + \text{H}]^+$; $^1\text{H-NMR}$ (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). $^{13}\text{C-NMR}$ (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. $\text{C}_{21}\text{H}_{36}\text{N}_2\text{O}_7$; white amorphous; ESIMS: m/z 451 $[\text{M} + \text{Na}]^+$; $^1\text{H-NMR}$ (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). $^{13}\text{C-NMR}$ (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. $\text{C}_{21}\text{H}_{36}\text{N}_2\text{O}_7$; white amorphous; $[\alpha]_{\text{D}}^{25} +23.4$ (c 0.3, CHCl_3); ESIMS: m/z 451 $[\text{M} + \text{Na}]^+$; $^1\text{H-NMR}$ (500 MHz, CDCl_3): 7.57 (1H, d, $J = 6.0$ Hz, NH-13), 6.19 (1H, t, $J = 6.0$ Hz, NH-7'), 5.78 (1H, d, $J = 16.0$ Hz, H-7), 5.52 (1H, dd, $J = 7.0$ and 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). $^{13}\text{C-NMR}$ (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. $\text{C}_{21}\text{H}_{36}\text{N}_2\text{O}_7$; white amorphous; $[\alpha]_{\text{D}}^{25} +26.9$ (c 0.45, CHCl_3); ESIMS: m/z 451 $[\text{M} + \text{Na}]^+$; $^1\text{H-NMR}$ (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). $^{13}\text{C-NMR}$ (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. $\text{C}_{21}\text{H}_{36}\text{N}_2\text{O}_7$; white amorphous; $[\alpha]_{\text{D}}^{25} -19.7$ (c 0.3, CHCl_3); ESIMS: m/z 451 $[\text{M} + \text{Na}]^+$; $^1\text{H-NMR}$ (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, $J = 7.0$ and 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). ¹³C-NMR (125 MHz, CDCl₃): 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 and b and 9a-d

A solution of each protected compound, 6a and b and 7a-d, (0.02 mmol) in THF (0.15 mL) was cooled down to 0 °C. To this solution, TFA (8 eq.) and H₂O (30 μL) were successively added. The solution was stirred at 0 °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.

8a. C₁₈H₃₂N₂O₆; white amorphous solid; $[\alpha]_D^{25} +5.0$ (c 0.4, CHCl₃); HRESIMS: m/z 395.2135 [M + Na]⁺ (calcd 395.2158 for C₁₈H₃₂N₂O₆Na); ¹H-NMR (500 MHz, CDCl₃): 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, CH₂-6'), 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). ¹³C-NMR (125 MHz, CDCl₃): 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. C₁₈H₃₂N₂O₆; white amorphous solid; $[\alpha]_D^{25} +37.7$ (c 0.26, CHCl₃); HRESIMS: m/z 395.2161 [M + Na]⁺ (calcd 395.2158 for C₁₈H₃₂N₂O₆Na); ¹H-NMR (500 MHz, CDCl₃): 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, CH₂-6'), 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). ¹³C-NMR (125 MHz, CDCl₃): 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 C₁₈H₃₂N₂O₇Na); ¹H-NMR (500 MHz, CD₃OD): 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). ¹³C-NMR (125 MHz, CD₃OD): 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 C₁₈H₃₂N₂O₇Na); ¹H-NMR (500 MHz, CD₃OD): 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, CH₂-6'), 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). ¹³C-NMR (125 MHz, CD₃OD): 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 C₁₈H₃₂N₂O₇Na); ¹H-NMR (500 MHz, CD₃OD): 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, CH₂-3'), 1.61 (2H, m, CH₂-4'), 1.06 (9H, s, Me-9, 10 and 11). ¹³C-NMR (125 MHz, CD₃OD): 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 C₁₈H₃₂N₂O₇Na); ¹H-NMR (500 MHz, CD₃OD): 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, CH₂-4'), 1.94 (1H, m, H_b-3'), 1.77 (1H, m, H_a-3'), 1.04 (9H, s, Me-9, 10 and 11). ¹³C-NMR (125 MHz, CD₃OD): 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

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⁻¹), streptomycin (100 µg mL⁻¹) and gentamicin (10 µg mL⁻¹). Stock solutions of the compounds were prepared in DMSO/H₂O (1/9), and the cytotoxicity assays were carried out in 96-well microtiter plates against cancer or normal cells (3 × 10³ cells per mL) using a modification of the published method.³⁰ After 72 h of incubation at 37 °C in air/CO₂ (95 : 5) with or without the test compounds, cell growth was estimated by colorimetric measurement of stained living cells using neutral red. Optical density was determined at 540 nm with a Titertek Multiscan photometer. The IC₅₀ value was defined as the concentration of sample necessary to inhibit 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-DHUD.2012-G/05).

References

- 1 D. J. Newman and G. M. Cragg, Natural products as sources of new drugs over the 30 years from 1981 to 2010, *J. Nat. Prod.*, 2012, 75, 311–335.
- 2 A. Bauer and M. Bronstrup, Industrial natural product chemistry for drug discovery and development, *Nat. Prod. Rep.*, 2014, 31, 35–60.
- 3 M. S. Butler, A. A. Robertson and M. A. Cooper, Natural product and natural product derived drugs in clinical trials, *Nat. Prod. Rep.*, 2014, 31, 1612–1661.
- 4 J. W. Blunt, B. R. Copp, R. A. Keyzers, M. H. Munro and M. R. Prinsep, Marine natural products, *Nat. Prod. Rep.*, 2015, 32, 116–211.
- 5 T. F. Molinski, D. S. Dalisay, S. L. Lievens and J. P. Saludes, Drug development from marine natural products, *Nat. Rev. Drug Discovery*, 2009, 8, 69–85.
- 6 E. Quinoa, M. Adamczeski, P. Crews and G. J. Bakus, Bengamides, heterocyclic anthelmintics from a Jaspidae marine sponge, *J. Org. Chem.*, 1986, 51, 4494–4497.
- 7 Z. Thale, F. R. Kinder, K. W. Bair, J. Bontempo, A. M. Czuchta, R. W. Versace, P. E. Phillips, M. L. Sanders, S. Wattanasin and P. Crews, Bengamides revisited: New structures and antitumor studies, *J. Org. Chem.*, 2001, 66, 1733–1741.
- 8 S. Weltchek, A. Wood, S. D. Zabudoff and P. E. Phillips, Bengamides are novel marine natural products with broad spectrum antitumor activity, *Proc. Am. Assoc. Cancer Res.*, 2000, 41, 600.
- 9 A. Groweiss, J. J. Newcomer, B. R. O'Keefe, A. Blackman and M. R. Boyd, Cytotoxic metabolites from an Australian collection of the sponge *Jaspis* species, *J. Nat. Prod.*, 1999, 62, 1691–1693.
- 10 W. Zhang, Q. Liang, H. Li, X. Meng and Z. Li, Concise synthesis and antitumor activity of bengamide E and its analogs, *Tetrahedron*, 2013, 69, 664–672.
- 11 Q. J. Liu, H. Li, S. P. Chen and G. C. Zhou, Synthesis of (3*S*,4*R*)-bengamide E, *Chin. Chem. Lett.*, 2011, 22, 505–507.
- 12 F. Sarabia, F. Martín-Gálvez, C. García-Ruiz, A. Sánchez-Ruiz and C. Vivar-García, Epi-, epoxy-, and C2-modified bengamides: Synthesis and biological evaluation, *J. Org. Chem.*, 2013, 78, 5239–5253.
- 13 F. Sarabia and A. Sánchez-Ruiz, Total Synthesis of Bengamide E and analogues by modification at C-2 and at terminal olefinic positions, *J. Org. Chem.*, 2005, 70, 9514–9520.
- 14 F. Sarabia and A. Sánchez-Ruiz, A diversity-oriented synthetic approach to bengamides, *Tetrahedron Lett.*, 2005, 46, 1131–1135.
- 15 F. Martín-Gálvez, C. García-Ruiz, A. Sánchez-Ruiz, F. A. Valeriote and F. Sarabia, An array of bengamide E analogues modified at the terminal olefinic position: Synthesis and antitumor properties, *ChemMedChem*, 2013, 8, 819–831.
- 16 S. Kim, K. LaMontagne, M. Sabio, S. Sharma, R. W. Versace, N. Yusuff and P. E. Phillips, Depletion of methionine aminopeptidase 2 does not alter cell response to fumagillin or bengamides, *Cancer Res.*, 2004, 64, 2984–2987.
- 17 G. Liu, Y. M. Ma, W. Y. Tai, C. M. Xie, Y. L. Li, J. Li and F. J. Nan, Design, synthesis, and biological evaluation of caprolactam-modified bengamide analogues, *ChemMedChem*, 2008, 3, 74–78.
- 18 W. Y. Tai, R. T. Zhang, Y. M. Ma, M. Gu, G. Liu, J. Li and F. J. Nan, Design, synthesis, and biological evaluation of ring-opened bengamide analogues, *ChemMedChem*, 2011, 6, 1555–1558.
- 19 J. P. Lu, X. H. Yuan, H. Yuan, W. L. Wang, B. Wan, S. G. Franzblau and Q. Z. Ye, Inhibition of Mycobacterium tuberculosis methionine aminopeptidases by bengamide derivatives, *ChemMedChem*, 2011, 6, 1041–1048.
- 20 F. R. Kinder Jr., R. W. Versace, K. W. Bair, J. M. Bontempo, D. Cesarz, S. Chen, P. Crews, A. M. Czuchta, C. T. Jagoe, Y. Mou, R. Nemzek, P. E. Phillips, L. D. Tran, R. M. Wang, S. Weltchek and S. Zabudoff, Synthesis and antitumor activity of ester-modified analogues of bengamide B, *J. Med. Chem.*, 2001, 44, 3692–3699.
- 21 D. D. Xu, L. Waykole, J. V. Calienni, L. Ciszewski, G. T. Lee, W. Liu, J. Szweczyk, K. Vargars, K. Prasad, O. Repic and T. J. Blacklock, An expedient synthesis of LAF389, a bengamide B analogue, *Org. Process Res. Dev.*, 2003, 7, 856–865.
- 22 H. Dumez, H. Gall, R. Capdeville, C. Dutreix, A. T. van Oosterom and G. Giaccone, A phase I and pharmacokinetic study of LAF389 administered to patients with advanced cancer, *Anti-Cancer Drugs*, 2007, 18, 219–225.
- 23 M. G. Banwell and K. J. McRae, A Chemoenzymatic Total synthesis of ent-bengamide E, *J. Org. Chem.*, 2001, 66, 6768–6774.
- 24 R. Pellegata, G. Pinza and G. Pifferi, An improved synthesis of γ-, δ-, and ε-lactams, *Synthesis*, 1978, 614–616.
- 25 B. Front, Facile synthesis of γ-, δ-, and ε-lactams by cyclodehydration of ω-amino acids on alumina or silica gel, *Tetrahedron Lett.*, 1980, 21, 2443–2446.

- 26 H. Matsumoto and K. Kaiso, Preparation of α -amino- ϵ -caprolactam by cyclodehydration of lysine or its salts, *Jpn. Kokai Tokkyo Koho*, 2012, JP 2012162463.
- 27 W. Xu, J. P. Lu and Q. Z. Ye, Structural analysis of bengamide derivatives as inhibitors of methionine aminopeptidases, *J. Med. Chem.*, 2012, 55, 8021–8027.
- 28 E. C. Griffith, Z. Su, B. E. Turk, S. Chen, Y. H. Chang, Z. Wu, K. Biemann and J. O. Liu, Methionine aminopeptidase (type 2) is the common target for angiogenesis inhibitors AGM-1470 and ovalicin, *Chem. Biol.*, 1997, 4, 461–471.
- 29 X. Hu, A. Addlagatta, J. Lu, B. W. Matthews and J. O. Liu, Elucidation of the function of type 1 human methionine aminopeptidase during cell cycle progression, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, 103, 18148–18153.
- 30 T. Mosmann, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, *J. Immunol. Methods*, 1983, 65, 55–63.