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Design, Synthesis, and Biological Evaluation of Ring-Opened Bengamide Analogues

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Bengamides are marine natural products, isolated from Jaspidae sponges,[1] that display a wide and interesting range of biological activities, including antitumor,[2] antibiotic,[3] and anthelmintic properties.[1] In particular, their antitumor properties have stimulated intense research.[4–6] Bengamide B, the most promising member of this family, and its 5′-ester analogues were investigated fully by Kinder et al.[4] One analogue, LAF389, was considered as a clinical candidate, however, poor pharmacokinetic properties and unclear side effects were observed during the clinical trial.[7] In our previous work, we reported the identification of a new potent analogue, **1o**′, which is N-substituted rather than 5′-substituted, as in the natural product, and displays more potent activity and greater water solubility than LAF389 (Figure 1).[6]

Herein, we report a novel series of ring-opened bengamide analogues obtained by replacing the caprolactam ring with linear alkyl chains. Among these analogues, several compounds showed potent antitumor activity, as well as good water solubility (Figure 1). These compounds also present a novel structure type different from natural bengamides and allow us to refine the antitumor potency.

A general synthetic procedure for **1**–**3** is depicted in Scheme 1. α-Amino acid derivatives **4**–**6** were prepared by employing simple and versatile methodologies including esterification and amidation. These derivatives were deprotected and coupled with lactone **10**[8] to obtain ketals **11**–**13**, followed by removal of the ketal group to give the target compounds **1**–**3**.

Key intermediates **4a**–**4f** (Scheme 2) were synthesized by coupling Boc-glycine analogues and compound **14**. The latter was easily prepared from aminoethanol through Boc protection, esterification and deprotection. Compounds **5a**–**5k** and **6a**–**6c** were prepared from the common intermediate **15a** (Scheme 2). Coupling with various amino alcohols provides intermediates **16** and **18a**–**18c**, and further esterification with various acids or acyl chlorides gave the target compounds.

This study had three key objectives: 1) to simplify the structure of bengamide analogues and improve their synthetic accessibility; 2) to identify more potent and water soluble analogues; 3) to further explore the structure–activity relationships

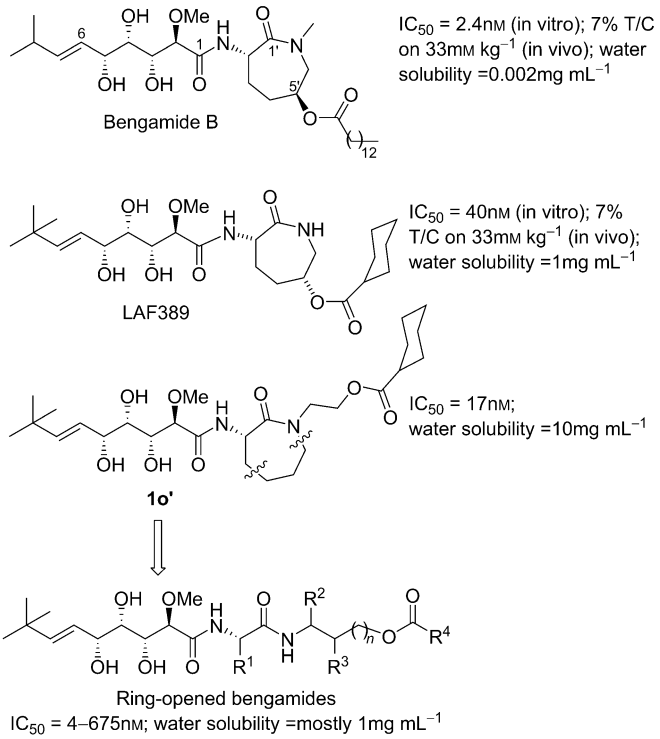

Figure 1. The structures of bengamide B, LAF389, and **1o**′ and their key biological activities,[4–6] and design strategy for the preparation of ring-opened bengamide analogues.

(SAR) of the bengamides. For simplifying the structure of bengamide analogues, the seven-membered ring in **1o**′ was opened, and a series of compounds (**1a**–**1f**) modified at the R^1 position were synthesized and evaluated against human breast carcinoma cells (Table 1). It was found that the simple methyl-substituted analogue **1a** showed high potency, with an IC_{50} value of 31 nM, while compounds with longer chain lengths and bulky substituents at this position (**1b**–**1f**) showed lower potency compared to **1a** (Table 1).

Keeping R^1 as a methyl group and replacing the cyclohexyl carboxyl group with a series of alkyl groups, a series of ester compounds (**2a**–**2k**) were synthesized (Table 2). The ester substituents were selected on the basis of lipophilicity, size, and shape, and fall into three categories: 1) linear alkyl esters (**2a**–**2b**); 2) branched alkyl esters (**2c**–**2e**); and 3) cycloalkyl-substituted esters (**2f**–**2k**). These analogues were evaluated in the same bioassay as compounds **1a**–**1f**, the results of which are given in Table 2. With a few exceptions, most of these analogues were near equipotent with LAF389. Linear chains (**2a** and **2b**) gave higher activity than bulky branched esters (**2c**–**2e**). Cycloalkyl esters other than **2j** gave high potency with IC_{50} values below 31 nM, cyclopentyl-substituted compound **2i**

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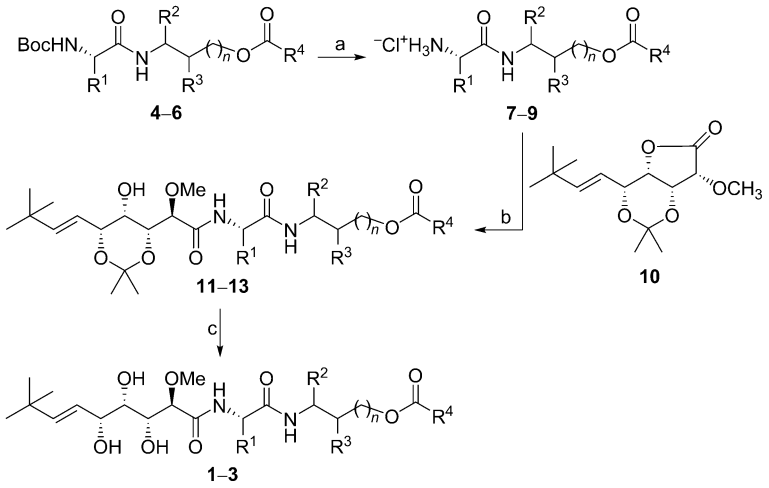


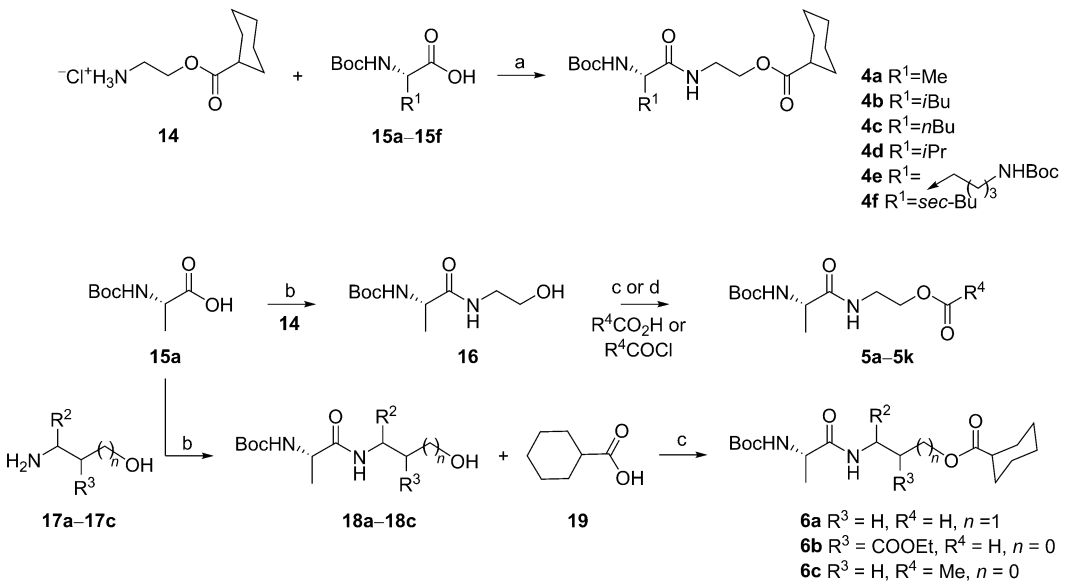
Scheme 1. Synthesis of ring-opened bengamide analogues **1**–**3**. a) 2 N HCl/EtOAc, RT, 1 h, 99%; b) sodium 2-ethylhexanoate, THF, 40 °C, overnight, 40–50%; c) 1 N HCl/H_2O, THF, RT, 2 h, 50–60%.

was the most active derivative of the series (IC_{50} = 9 nM), and the compound with a five-membered ring showed improved activity over the compounds containing three-, four- or six-membered rings. Derivatives containing other functional groups also showed significant antitumor activity (Table 2).

At the same time, we turned our attention to investigating the effects of steric hindrance and chain length of the linker when R^1 is a methyl group. Substitution of R^2 and R^3 led to compounds with retained high potency compared to compound **1a** (Table 3), while a one-carbon extension (**3a**) produced almost an eightfold improvement. These results indicated that the three-carbon linker is better than two-carbon linker.

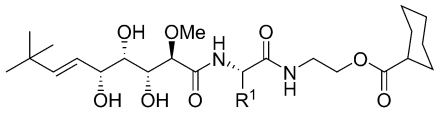
Water solubility was determined experimentally using the following procedure at 25 ± 1.5 °C.[9] An excess of the test compound (**1d**, **2d**, **2i** and **3a**) was added to ultrapure water (0.1 mL), and a suspension of the mixture was equilibrated during1 h of sonication and 24 h of shaking, followed by centrifugation (5 min, 12000 r min^{-1}). The water supernatant (50 μL) was further diluted in water (0.45 mL). A regression curve for each compound was obtained from seven standard stock solutions ($r > 0.99$) using HPLC analysis. The absolute amount of each compound was then calculated (Table 4). The selected compounds showed greater solubility in water than LAF389 (1 mg mL^{-1}), and **2h** was even better than the parent compound **1o′** (10 mg mL^{-1}), showing that the ring-opened bengamides possess improved water solubility.

Bengamides have been known as effective antitumor agents for decades, but the development of this type of natural compound as a drug candidate suffers from several key problems: 1) the amount that can be isolated from natural sources is insufficient to satisfy research needs, while known syntheses are long and unsuitable for large-scale preparation; 2) little SAR data is currently available in the literature, and there is no direct proof of their biological target; 3) the poor solubility of



Scheme 2. Synthesis of ester compounds **4a**–**4f**, **5a**–**5k**, and **6a**–**6c**. a) EDCI, DMAP, Et_3N, CH_2Cl_2, RT, overnight, 40–80%; b) EDCI, imidazole, CH_2Cl_2, RT, overnight, 50–80%; c) EDCI, DMAP, CH_2Cl_2, RT, overnight, 50–90%; d) imidazole, CH_2Cl_2, RT, overnight, 50–90%. Abbreviations: 4-dimethylaminopyridine (DMAP); 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI).

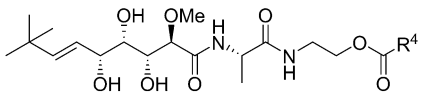
Table 1. Structures and in vitro activity (IC_{50}) of ring-opened bengamide analogues **1a**–**1f** and **2a**–**2k**.

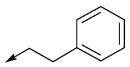
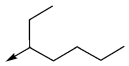
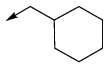


Compd	R^1	IC_{50}[a] [nM]
1a	Methyl	31 ± 14
1b	*i*Butyl	125 ± 9
1c	*n*Butyl	157 ± 8
1d	*i*Propyl	105 ± 10
1e	$BocNH(CH_2)_4^-$	141 ± 22
1f	*sec*-Butyl	208 ± 76
LAF389	–	40 ± 2
1o′	–	17 ± 8

[a] IC_{50} values are the half-maximal inhibitory concentrations as measured in MDA-MB-435 human breast carcinoma cells; data represent the mean value ± SD. See the Experimental Section for details.

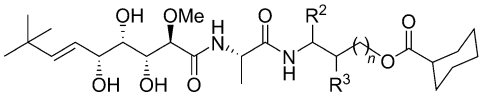
Table 2. Structures and in vitro activity (IC_{50}) of ring-opened bengamide analogues **2a**–**2k**.



Compd	R^4	IC_{50}[a] [nM]	Compd	R^4	IC_{50}[a] [nM]
2a		19 ± 4	**2g**		16 ± 37
2b		10 ± 1	**2h**		12 ± 2
2c		293 ± 80	**2i**		9 ± 7
2d		125 ± 35	**2j**		675 ± 15
2e		154 ± 37	**2k**		20 ± 13
2f		31 ± 14			

[a] For details of the assay conditions and data significance see Table 1.

Table 3. Structures and in vitro activity (IC_{50}) of ring-opened bengamide analogues **3a**–**3c**.



Compd	R^2	R^3	*n*	IC_{50}[a] [nM]
3a	H	H	1	4 ± 11
3b	COOEt	H	0	65 ± 6
3c	H	Me	0	17 ± 11

[a] For details of the assay conditions and data significance see Table 1.

Table 4. The solubility of ring-opened bengamide analogues **1d**, **2d**, **2i** and **3a** in water.

Compd	Solubility [mg mL^{-1}]
1d	3.45
2d	2.84
2i	25.84
3a	7.57

analogues containing caprolactam rings and long-chain ester groups makes development challenging. In this work, we simplified the structure by opening the caprolactam ring, making the syntheses more concise. The analogues with greatly simplified structures showed potent antitumor activity against MDA-MB-435 human breast cancer cells, In addition, most of the compounds reported in this work showed good solubility in an aqueous medium. Taken together, these advantages provide possibilities for further SAR studies that could afford novel candidates for further development.

In conclusion, we synthesized a series of caprolactam-ring-opened bengamide analogues. Among the synthesized compounds, 20 novel analogues with greatly simplified structures showed potent antitumor activity against MDA-MB-435 human breast cancer cells and improved water solubility. In particular, compounds **3a** (IC_{50} = 4 nM) and **2i** (IC_{50} = 9 nM) showed more potent activity than LAF389 (IC_{50} = 40 nM) and the original caprolactam analogue **1o′** (IC_{50} = 17 nM). The ring-opened analogues also showed good water solubility (> 1 mg mL^{-1}), especially derivative **2i** (25.84 mg mL^{-1}) which showed improved solubility over **1o′** (10 mg mL^{-1}). These simplified bengamide analogues and the SARs documented in this study will, therefore, open new avenues of understanding for the further development of bengamides as antitumor agents.

Experimental Section

Following reported procedures,[4,8] bengamide analogues **1a**–**1f**, **2a**–**2k** and **3a**–**3c** were synthesized. Representative characterization data are give below for compounds **2i** and **3a**; data for all other bengamides analogues reported and general procedures for the synthesis of compounds **4a** are given in the Supporting Information.

2-((*S*)-2-((2*R*,3*R*,4*S*,5*R*,*E*)-3,4,5-trihydroxy-2-methoxy-8,8-dimethylnon-6-enamido)propanamido)ethyl cyclopentanecarboxylate (2i): yield, 52% as a light-yellow oil. ^{1}H NMR($CDCl_3$, 300 MHz): δ = 1.03 (s, 9H), 1.45 (d, J = 6.9 Hz, 3H), 1.75 (m, 4H), 1.90 (m, 4H), 2.75 (m,1H), 3.15 (d, J = 4.2 Hz, 1H), 3.50 (s, 3H), 3.52 (m, 2H), 3.65 (s, 1H), 3.80 (d, J = 4.5 Hz, 2H), 3.95 (m, 1H), 4.15 (m, 2H), 4.22 (m, 1H), 4.50 (m, 1H), 5.45 (dd, J = 15.6, 7.2 Hz, 1H), 5.92 (d, J = 15.6 Hz, 1H), 6.80 (m, 1H), 7.19 ppm (d, J = 8.1 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 18.1, 25.9, 29.5, 30.1, 33.1, 38.9, 43.8, 48.8, 59.2, 62.7, 73.0, 73.1, 74.7, 82.6, 123.5, 145.5, 172.0, 172.0, 177.3 ppm; LC–MS: *m/z* (%): 495.3 (100) $[M+Na]^+$; HRMS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{23}H_{40}N_2O_8$: 495.2682, found: 495.2702.

3-((*S*)-2-((2*R*,3*R*,4*S*,5*R*,*E*)-3,4,5-trihydroxy-2-methoxy-8,8-dimethylnon-6-enamido)propanamido)propyl cyclohexanecarboxylate (3a): yield, 55% as a light-yellow oil. ^{1}H NMR ($CDCl_3$,

300 MHz): $\delta = 1.03$ (s, 9H), 1.29 (m, 4H), 1.42 (d, $J = 7.2$ Hz, 3H), 1.80 (m, 8H), 2.30 (m, 1H), 3.20 (m, 2H), 3.35 (m, 1H), 3.46 (s, 3H), 3.68 (s, 1H), 3.93 (d, $J = 4.2$ Hz, 1H), 3.98 (m, 2H), 4.10 (t, $J = 6.6$ Hz, 2H), 4.20 (m, 2H), 4.45 (m, 1H), 5.45 (dd, $J = 15.6$, 6.9 Hz, 1H), 5.82 (d, $J = 15.6$ Hz, 1H), 6.83 (m, 1H), 7.18 ppm (d, $J = 7.8$ Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): $\delta = 18.1$, 25.5, 25.8, 28.6, 29.2, 29.6, 33.2, 36.4, 43.4, 48.9, 59.2, 61.8, 73.1, 73.2, 74.7, 82.7, 123.5, 145.6, 171.8, 172.0, 177.0 ppm; LC–MS: *m/z* (%): 523.3 (100) $[M+Na]^+$; HRMS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{25}H_{44}N_2O_8$: 523.2995, found: 523.2971.

MDA-MB-435S cell proliferation assay: MDA-MB-435S (3000 cells per well) were plated in 96-well plates; 24 h later they were treated with various concentrations of compounds (eight-dose in triplicate with fourfold serial dilution) or solvent control. Following incubation for 72 h, 40 μL MTT (5 mg mL^{-1}) was added into the wells and incubated for another 3 h. The media was then removed, and 100 μL DMSO was added. The absorbance at λ 550 nm was measured by a SpectraMAX 340 microplate reader (Molecular Devices, Sunnyvale, CA, USA) with a reference wavelength at 690 nm. Adriamycin was used as a positive control in the assay.

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Keywords: antitumor agents · bengamides · ring-opened analogues · water solubility

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