Synthesis of Analogs of Amathamide A and Their Preliminary Antimicrobial Activity

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Abstract: Syntheses of three non-brominated analogs of amathamide A (1), a natural alkaloid isolated from the Tasmanian marine bryozoan Amathia wilsoni, are described. Antimicrobial activity against Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Candida albicans was tested. Test results for amathamide A (1) showed a weak activity against C. albicans and E. coli. The three non-natural analogs 2-4 proved to be inactive compounds.

Keywords: Organic synthesis, Amathamide A, Antimicrobial activity.

Introduction

Amathamide A (1) is a brominated alkaloid isolated from the Tasmanian bryozoan Amathia wilsoni Kirkpatrick [1,2]. Biological activity of this type of enamide alkaloids has not been well studied, and only nematocidal, antifungal, and antibacterial activity has been described for amathamides A (1), B, G, H, and I, isolated from A. wilsoni and A. convolute species [3]. Our previous work relating to the synthesis of two natural amathamides (A and B) [4] prompted us to investigate the
synthesis and biological evaluation of analogs of this type of alkaloid. Therefore, we selected amathamide A (1) as a lead compound and we carried out the synthesis of three non-brominated analogs 2-4, maintaining the N-methyl or substituting it by a single hydrogen or t-butyloxycarbamate (Figure 1). Antimicrobial activity was then determined against two Gram-positive bacteria (B. subtilis and S. aureus), two Gram-negative bacteria (E. coli and P. aeruginosa) and a yeast (C. albicans).

Results and Discussion

Amathamide A (1) was synthesized as previously reported [4]. For the three non-natural analogs, the synthetic route was modified as shown in Scheme 1. m-Anisaldehyde was condensed with nitromethane in 81 % yield in the presence of ammonium acetate and then thiophenol was incorporated via Michael addition with a catalytic amount of N-isopropylcyclohexylamine to obtain the product 6 in 90 % yield. Nitro group reduction was achieved in 45% yield in the conventional manner [5] using Zn in AcOH/HCl. Acylation of the amine with (S)-N-t-butoxycarbonylproline in presence of DCC/HOBt gave 8 in 20 % yield. Removal of the Boc (t-butoxycarbonyl) protective group from compound 8 affords 9 in very good yield and acylation of the latter with formaldehyde and reduction with NaBH₃CN afford 10 (80 % yield). Elimination of thiophenol of 10 via oxidation with NaIO₄ to the sulfoxide and refluxing in toluene/K₂CO₃, gave the non-brominated analogue 2. On the other hand, elimination of thiophenol from 8 led to analogue 3, and removal of Boc afforded 4. In this manner, we obtained the three non-natural analogs of amathamide A (1).

The preliminary antimicrobial activity of amathamide A (1) and the three non-natural analogs 2-4 was determined against the Gram-positive bacterias S. aureus and B. subtilis, the Gram-negative bacteria E. coli and P. aeruginosa and the yeast C. albicans by using the agar dilution-streak assay (Mitscher method) [6]. Only compound 1 was active in the bioassay at the final concentration of 200 µg/mL. Minimal inhibitory concentrations (MIC) for 1 were determined [7,8]. The MICs of 1 for C. albicans and E. coli were weak, and less active (MICs greater than 128 µg/mL) for P. aeruginosa, S. aureus, and B. subtilis (Table 1).

Scheme 1.
Table 1. Minimal Inhibitory Concentration (MIC) of 1, Nystatin and Streptomycin toward Pathogenic Bacteria and yeast.

| Pathogen         | 1\(^a\) | Nystatin\(^a\) | Streptomycin\(^a\) |
|------------------|---------|----------------|-------------------|
| *C. albicans*    | 142.8   | 10.0           | -                 |
| *E. coli*        | 71.4    | -              | 10.0              |
| *P. aeruginosa*  | 71.4    | -              | 0.5               |
| *S. aureus*      | 142.8   | -              | 1.6               |
| *B. subtilis*    | 142.8   | -              | 3.1               |

\(^a\)Results are means of two determinations and are expressed as µg/mL.
Conclusions

The non-brominated analogues of amathamides obtained following a simpler synthetic route proved to be inactive compounds. Thus, we conclude that the presence of bromine on the aryl ring contributes to the antimicrobial activity of amathamide A \( (1) \). According to Narkowicz et al. [3], the \( E \) orientation of the double bond of amathamide A \( (1) \) conferred the highest biological activity compared with the less constrained saturated version, as in amathamide I, or the \( Z \) orientation of the double bond as in amathamide B. This information leaves only the possibility of altering the acyl group and/or substitution on the amide nitrogen in an effort to obtain analogues with enhanced antimicrobial activity for further biological testing. The effect of other aryl substituents could also be explored.

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Experimental

General

Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. (IR) spectra were taken on a Perkin Elmer FT-IR 1600 spectrometer. \(^1\)H- (200 MHz) and \(^13\)C-NMR (75 MHz) spectra were recorded on a Bruker Avance DPX-300 MHz spectrometer. Spectra were run in CDCl\(_3\) with tetramethylsilane (TMS) used as internal standard. The EIMS data was obtained on a Finnigan MAT-90 instrument and all experiments were performed in the electron-impact mode (EI) at 70 eV using a direct insertion probe.

\((E)-3\text{-Methoxy-}\beta\text{-nitrostyrene} \( (5) \): To a solution of \( m \)-anisaldehyde (73.4 mmol, 10.0 g) in glacial AcOH (120 mL) was added AcONH\(_4\) (2.71 g) and nitromethane (13.1 mL). The solution was heated under reflux for 1 h. The mixture was cooled and the precipitate was washed with water and recrystallized from AcOH/H\(_2\)O to give a yellowish solid (10.6 g, 81%); mp 88-89º C; IR (KBr): 3108, 1636, 1577, 1511 cm\(^{-1}\); \(^1\)H-NMR: \( \delta 7.97 \) (d, \( J = 14.0 \) Hz), 7.56 (d, \( J = 14.0 \) Hz), 7.41-7.02 (m, 4H), 3.85 (s, 3H, OCH\(_3\)).

\(2\text{-}(3\text{-Methoxyphenyl})\text{-}2\text{-}(thiophenyl)-1\text{-nitroethane} \( (6) \): Compound \( 5 \) (22.3 mmol, 4.0 g) was dissolved in CH\(_2\)Cl\(_2\) (100 mL) and was added thiophenol (22.9 mmol, 2.6 mL) and 0.5 mL of \( N \)-isopropylcyclohexylamine. The resulting solution was stirred for 1 h. at rt. The solution was concentrated and subjected to flash chromatography using hexane/CH\(_2\)Cl\(_2\) (80:20). Removal of the solvent gave a brownish oil (5.8 g, 90%). IR (film): 3057, 2957, 1599, 1554, 1262 cm\(^{-1}\); \(^1\)H-NMR: \( \delta 7.45\text{-}7.22 \) (m, 6H), 6.85-6.79 (m, 3H), 4.83-4.60 (m, 3H), 3.75 (s, 3H, OCH\(_3\)).

\(2\text{-}(3\text{-Methoxyphenyl})\text{-}2\text{-}(thiophenyl)-1\text{-aminoethane} \( (7) \): Compound \( 6 \) (17.3 mmol, 5.0 g) was dissolved in AcOH (46 mL) and Zn powder (172.4 mmol, 11.3 g) was added. Then conc. HCl (37 mL)
was added dropwise and the solution was stirred overnight at rt. The solution was neutralized with 2 N NH₄OH. The residue was extracted into ethyl acetate (2 × 100 mL) and the solvent was removed under reduced pressure to give a yellowish green oil (2.0 g, 45%); IR (film): 3372, 3057, 2936, 1587, 1483, 1261, 1044, 694 cm⁻¹; ¹H-NMR: δ 7.4-7.18 (m, 6H), 6.96-6.70 (m, 3H), 4.12 (t, J = 8 Hz), 3.77 (s, 3H, OCH₃), 3.20-3.00 (m, 2H), 2.00-1.40 (bs, 2H, NH₂).

2(S)-N-[2(3-Methoxyphenyl)-2-(thiophenyl)ethanyl]-1-t-butoxycarbonylproline (8): To a solution of (S)-N-t-butoxycarbonylproline (19.5 mmol, 4.1 g) in dry THF (100 mL) was added dicyclohexyl-carbodiimide (19.5 mmol, 4.0 g), 1-hydroxybenzotriazole (19.5 mmol, 2.6 g) and 4-N,N-dimethyl-aminopyridine in catalytic amounts. A solution of 7 (19.5 mmol, 5.05 g) in dry THF (20 mL) was added and the suspension was stirred for 24 h, filtered and the solvent was removed in vacuo. The residue was purified by flash chromatography using CH₂Cl₂/MeOH (95:5). Removal of the solvent gave a brownish oil (1.8 g, 20%); IR (film): 3313, 3056, 2974, 1697 cm⁻¹; ¹H-NMR: δ 7.50-7.20 (m, 6H), 6.90-6.76 (m, 3H), 4.50-4.30 (m, 1H), 4.30-4.10 (m, 1H), 3.75 (s, 3H, OCH₃), 3.90-3.50 (m, 2H), 3.50-3.20 (m, 2H), 2.40-1.60 (m, 4H), 1.39 (s, 9H, boc); ¹³C-NMR: δ 159.6, 140.7, 133.7, 132.2, 129.5, 128.8, 127.3, 127.2, 120.0, 113.3, 113.2, 113.1, 80.3, 55.1, 52.3, 46.9, 43.8, 43.6, 28.3: IEMS, m/z: M⁺ 456 (nd), [M-PhSH]⁺ 346 (23), 198 (3), 149 (62), 132 (2), 70 (100), 57 (33).

2(S)-N-[2(3-Methoxyphenyl)-2-(thiophenyl)ethanyl]-2-pyrrolinecarboxamide (9): Compound 8 (4.38 mmol, 2.0 g) was dissolved in acetonitrile (30 mL) and HF (50%, 0.7 mL) was added and the mixture heated overnight under reflux with magnetic stirring. The mixture was cooled and the solvent was removed in vacuo. The residue was treated with K₂CO₃ (50%, 20 mL) and extracted with dichloromethane (2 × 20 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated to give 9 (1.51 g, 97%). Compound 9 was used in the next step without further purification. IR (film): 3322, 3054, 2950, 1665 cm⁻¹.

2(S)-N-[2(3-Methoxyphenyl)-2-(thiophenyl)ethanyl]-1-methyl-2-pyrrolinecarboxamide (10): 9 (4.24 mmol, 1.5 g) was dissolved in acetonitrile (14 mL) and formaldehyde (37%, 2.3 mL) and sodium cyanoborohydride (9.19 mmol, 0.6 g) were added and the solution was stirred for 2 h. at rt. The solution was neutralized with AcOH and stirring was continued for 2 h. The solvent was removed under vacuum and the residue was washed with water and extracted with CH₂Cl₂ (2 × 15 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated to give a brownish oil of the title compound as a mixture of diastereomers (1.2 g, 80%). IR (film): 3328, 3056, 2942, 1663, 1261 cm⁻¹; ¹H-NMR: δ 7.56 (bs, 2H, N-H), 7.38-7.16 (m, 12H), 6.88-6.75 (m, 6H), 4.42-4.32 (m, 2H), 3.87-3.55 (m, 4H), 3.76 (s, 6H, OCH₃), 3.00-2.93 (m, 2H), 2.80 (dd, J =10.0, 4.0 Hz, 2H), 2.32-2.00 (m, 4H), 2.22 (s, 3H, NCH₃), 2.15 (s, 3H, NCH₃), 1.50-1.80 (m, 6H); ¹³C-NMR: δ 174.9, 174.8, 159.8, 141.0, 141.0, 134.1, 132.1, 132.1, 129.9, 129.6, 129.1, 128.9, 127.5, 127.4, 127.3, 120.4, 120.3, 113.4, 113.4, 113.4, 68.8, 56.6, 56.5, 55.2, 52.2, 43.6, 43.5, 41.6, 41.5, 31.0, 30.9, 24.22.

2(S)-N-[E]-2(3-Methoxyphenyl)ethanyl]-1-methyl-2-pyrrolinecarboxamide (2): To a solution of 10 (1.7 mmol, 0.6 g) in methanol (10 mL) was added a solution of sodium periodate (1.9 mmol, 0.4 g) in water (10 mL) and the resulting mixture heated under reflux for 1.5 h. The methanol was removed in vacuo.
vacuo and the aqueous residue was extracted with dichloromethane (2 × 20 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated to give sulfoxide as a brownish oil (0.57 g, 70%). The crude product was then used in the next step without purification. The sulfoxide (1.20 mmol, 0.465 g) was dissolved in toluene (30 mL), sodium carbonate was added (166 mg) and the mixture heated under reflux for 2 h. The solvent was removed under vacuum and the residue was purified by circular chromatography using CH$_2$Cl$_2$/MeOH (95:5). Removal of the solvent gave a brownish oil (0.16 g, 35%). IR (film): 3286, 2942, 1681, 1650, 1504, 1262 cm$^{-1}$; $^1$H-NMR: $\delta$ 9.21 (d, $J$ = 10.8 Hz, 1H, N-H), 7.40 (dd, $J$ = 14.0 Hz, 1H), 7.20 (t, $J$ = 8.0 Hz, 1H), 6.94-6.85 (m, 2H), 6.73 (dd, $J$ = 8.2, 2.6, 0.8 Hz, 1H), 6.17 (d, $J$ = 14.0 Hz, 1H), 3.81 (s, 3H, OCH$_3$), 3.24-3.17 (m, 1H), 3.07 (dd, $J$ = 9.8, 4.8 Hz, 1H), 2.42 (s, 3H, NCH$_3$), 2.50-2.20 (m, 2H), 2.00-1.70 (m, 3H); $^{13}$C-NMR: $\delta$ 172.2, 159.9, 137.7, 129.6, 122.5, 118.4, 113.3, 112.5, 110.6, 68.7, 56.7, 55.2, 41.9, 31.0, 24.5; IEMS, $m/z$: M$^+$ 260 (26), 132 (15), 112 (18), 70 (100).

$2(S)$-N-[(E)-2(3-Methoxyphenyl)ethanyl]-1-t-butoxycarbonyl-2-pyrrolinecarboxamide (3): Compound 3 was synthesized from 8 (2.2 mmol, 1.0 g) using the same procedure described for amide 2. Compound 3 was obtained as a white solid (0.30 g, 30%); IR (KBr): 3283, 2974, 1695, 1653, 1400, 1257, 1161, 774 cm$^{-1}$; $^1$H-NMR: $\delta$ 9.28 (bs, 1H, N-H), 7.46 (dd, $J$ = 14.6, 10.8 Hz, 1H), 7.19 (t, $J$ = 7.8 Hz, 1H), 6.90 (d, $J$ = 7.8 Hz, 1H), 6.85 (d, $J$ = 2.4 Hz, 1H), 6.73 (dd, $J$ = 8.2, 2.4 Hz, 1H), 6.08 (d, $J$ = 14.6 Hz, 1H), 4.42-4.22 (m, 1H), 3.77 (s, 3H, OCH$_3$), 3.56-3.2 (m, 2H), 2.60-1.80 (m, 4H), 1.49 (s, 9H); IEMS, $m/z$: M$^+$ 346 (23), 245 (1), 198 (2), 149 (42), 132 (6), 70 (100), 57 (33).

$2(S)$-N-[(E)-2(3-Methoxyphenyl)ethanyl]-2-pyrrolinecarboxamide (4): Compound 4 was obtained from 3 (0.6 mmol, 0.2 g) as a brownish oil (0.13 g, 90%) using the same procedure described for amide 9. IR (film) 3272, 3062, 2956, 1679, 1649, 1528, 1260, 1043, 692 cm$^{-1}$; $^1$H-NMR: $\delta$ 9.58 (d, $J$ = 10.6 Hz, 1H, N-H), 7.44 (dd, $J$ = 15.0, 11.3 Hz, 1H), 7.19 (t, $J$ = 8.0 Hz, 1H), 6.91 (d, $J$ = 7.8, 1H), 6.87 (d, $J$ = 2.0 Hz, 1H), 6.72 (dd, $J$ = 8.0, 2.4, 0.8 Hz, 1H), 6.16 (d, $J$ = 15 Hz, 1H), 3.91-3.75 (m, 1H), 3.80 (s, 3H, OCH$_3$), 3.10-2.90 (m, 2H), 2.65-2.3 (bs, 1H, NH), 2.26-2.15 (m, 1H), 2.06-1.90 (m, 1H), 1.81-1.71 (m, 2H); $^{13}$C-NMR: $\delta$ 172.9, 160.0, 138.0, 129.8, 122.6, 118.5, 113.5, 112.6, 110.7, 60.5, 55.4, 47.5, 30.9, 26.4; IEMS $m/z$: M$^+$ 246 (25), 149 (16), 132 (3), 70 (100).

Bioassay Evaluation Procedures.

Antimicrobial activity against Gram-positive bacteria Staphylococcus aureus ATCC 6538 and Bacillus subtilis ATCC 6633, Gram-negative bacteria Escherichia coli ATCC 8739 and Pseudomonas aeruginosa ATCC 9027, and yeast Candida albicans ATCC 10231 was determined by the agar Mitscher method as previously described [5]. Test samples were dissolved (2 mg/mL) in 10 mL nutrient agar medium No. 1 (Merck) for bacterial cultures and Sabouraud agar (Merck) supplemented with glucose (4 %) for yeast, and added aseptically to each Petri dish and swirled carefully until the agar began to set. After 24 h. of incubation (sterility test) the bacteria or yeast in a concentration of approximately 0.5 McFarland were streaked in radial patterns on the agar plates containing the samples, incubated at 37°C for 24 h, except for C. albicans which was incubated at 25°C. Complete inhibition of bacterial growth was expected for a sample to be declared active. Inhibition was present
at 200 µg/mL only for amathamide A (1). Minimal inhibitory concentrations (MICs) values were determined by the dilution method previously described [6,7]. Two-fold serial dilutions were tested in nutrient broth (Merck) for B. subtilis, S. aureus, E. coli and P. aeruginosa, and Sabouraud-glucose (2 %) nutrient broth (Merck) for C. albicans. An initial concentration of 1 mg/mL of sample was prepared by dissolving in dimethyl sulphoxide. Serial dilutions (100-0.1 µg/mL) of sample were prepared, and the liquid medium was inoculated by cultures in stationary phase at concentration of 10^5 CFU/mL. After overnight incubation, the MIC was determined as the lowest concentration of compound preventing any visible growth. Streptomycin sulfate (Sigma) (1 µg/mL) and nystatin (Sigma) (3 µg/mL) were used as positive controls.

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