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Synthesis of new hemiasterlin derivatives with *α,β*-unsaturated carbonyl-thiophene groups in fragment A

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Abstract

Hemiasterlin, an antimitotic tripeptide, exhibits cytotoxicity in the nanomolar range against a variety of cultured human and murine cell lines. For this reason, the synthesis of new hemiasterlin derivatives has attracted a lot of interest in the organic chemistry community recently. In this article, we synthesized new simplified derivatives of hemiasterlin in which the *α,α*-dimethylbenzylic group in fragment A is replaced by *α,β*-unsaturated carbonyl-thiophene group. These new compounds will be prepared by classical peptide coupling approach between the carboxylic acid fragments A **12** and dipeptide **13**. We expect that this derivative will possess interesting biological activities due to the high reactivity of the *α,β*-unsaturated carbonyl group as Michael receptor with biological nucleophiles, such as DNA, RN5A, and enzymes.

Keywords. Synthesis, hemiasterlin, *α,β*-unsaturated; tripeptides, nanomolar.

1. INTRODUCTION

Hemiasterlins belong to a family of naturally occurring tripeptides from marine sponges [1]. The important derivatives of hemiasterlin are hemiasterlin A, hemiasterlin B, and hemiasterlin C, which were isolated from marine sponge *Auletta* and *Cymbastella* (figure 1) [2, 3]. These naturally occurring substances exhibited potent cytotoxicity *in vitro* against murine leukemia P388 and human breast, ovarian, colon, and lung cancer cell lines [2,3,5]. Hemiasterlins suppress microtubule depolymerization presumably by binding to the vinca alkaloid site of tubulin and causing mitotic arrest and cell death [4]. This active mechanism makes them very attractive molecules for new anticancer drugs. However, synthesis of the stereospecific amine group and especially the *gem*-dimethyl moiety in segment A was proved to be highly problematic. To overcome this difficulty, several studies explored the modifications of segment A that eliminated the *gem*-dimethyl moiety [6]. Some derivatives from this approach (**7**, **8**) showed promising cytotoxic test results.

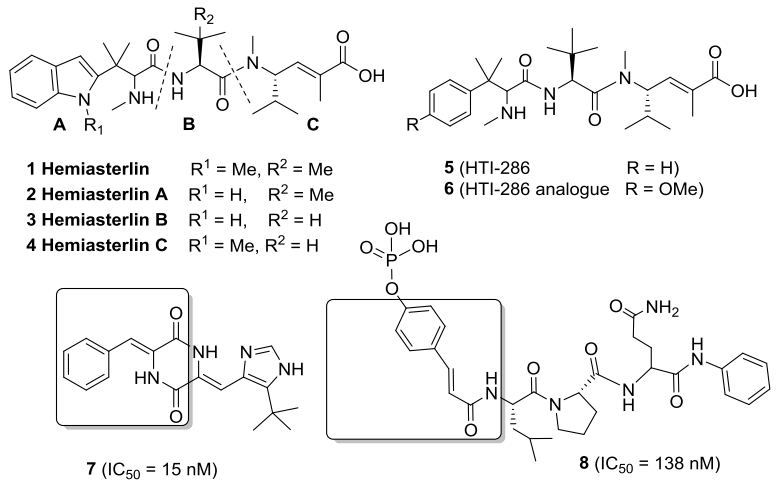


Figure 1: Examples of hemiasterlin derivatives and modification of segment A of hemiasterli

Recently, Hayashi et al. and McMurray et al. prepared new synthetic peptides (**7, 8**) with *α,β*-unsaturated carbonyl systems which were thought to play a role in the remarkably strong cytotoxicity of these compounds [7, 8]. The *α,β*-unsaturated carbonyl moieties are well-known Michael type acceptors. They are particularly reactive and interact strongly with electron-rich biological macromolecules such as DNA, protein, and enzyme, resulting in a wide range of biological effects including general toxicity, allergenic reactions, mutagenicity, and carcinogenicity. In this article, we report the synthesis of new derivatives of hemiasterlin with a novel direction in the construction of fragment A that contained *α,β*-unsaturated-thiophene group.

2. EXPERIMENTAL

2.1. General information

All reactions were performed in the appropriate oven-dried glass apparatus and under nitrogen atmosphere. Unless otherwise stated, solvents and chemicals were obtained from commercial sources and used without further purification. Column chromatography was performed using silica gel (60 Å, particle size 40-60 µm). NMR spectra were recorded on a Bruker Avance (500 MHz). Chemical shifts (δ) are given in parts per million (ppm) and coupling constants (J) in hertz (Hz). Mass spectrometry analysis (MS) were recorded on a Q-exactive or a Q-TOF2 instrument. IR analysis was recorded on Perkin Elmer Spectrum Two.

2.2. Synthesis of compound 11

A solution of *N*-acetylglycine (**10**) (500 mg, 4.27 mmol), thiophene-2-carbaldehyde (360 mg, 3.20 mmol), acetic anhydride (15 mL), and fused sodium acetate (350 mg, 4.27 mmol) was heated at 90 °C with stirring for 12 h. Afterwards, the acetic anhydride was evaporated *in vacuum*, the residue was extracted with CH_2Cl_2 and the combined CH_2Cl_2 extract was washed with brine, dried ($MgSO_4$). The solvent was removed *in vacuum* to give crude azalactone **11** which was purified by column chromatography on silica gel (*n*-hexane/EtOAc 98/2) to afford pure azalactone **11** (328 mg, 53 %). M.p. 168-170 °C. IR (KBr): 3063; 1793; 1676; 1655; 1624; 1561; 1537; 1468; 1381; 1305; 1247; 1189; 1017; 1003; 941; 896; 873; 824; 766 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 8.41 (1H, s, C<u>H</u>=C-); 7.66 (1H, brs, H-5'); 7.32 (1H, brs, H-3'); 6.95 (1H, brs, H-4'); 2.45 (3H, s, C<u>H</u>$_3$).

2.3. Synthesis of compound 12

A solution of azalactone **11** (300 mg, 1.55 mmol) in NaOH 1 N (5 mL) was heated at 100 ^{o}C for 30 min, followed by addition of a solution of HCl 12 N (5 mL). After stirring for 4 h, the solvent was evaporated *in vacuum* to give a residue which was extracted with EtOAc and the combined EtOAc extract was washed with brine and dried ($MgSO_4$). The solvent was removed *in vacuum* to give amide **12** which was purified by column chromatography on silica gel (*n*-hexane/EtOAc: 95/5) to afford the pure desired product **12** (200 mg, 61 %). M.p. 214-215 °C. IR (KBr): 3422; 3288; 3121; 2915; 1687; 1640; 1624; 1558; 1527; 1471; 1519; 1302; 1244; 1216; 1190; 1024; 941; 886; 869; 767; 749; 695 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 7.48 (1H, brs, H-4'); 7.15 (1H, brs, H-3'); 6.57 (1H, brs, H-5'); 6.44 (1H, s, H-3); 2.11 (3H, s, C<u>H</u>$_3$). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ ppm: 170.6 (<u>C</u>O, Ac), 166.4 (<u>C</u>=O), 149.1 (C-2'), 144.5 (C-5'), 121.7 (NH-<u>C</u>=CH); 120.6 (NH-C=<u>C</u>H), 115.5 (<u>C</u>-3'), 111.9 (<u>C</u>-4'), 22.0 (<u>C</u>H$_3$).

2.4. Synthesis of compounds 14a,b

To a solution of compound **13a** (100 mg, 0.24 mmol), EDC (50 mg, 0.26 mmol), HOBt (35 mg, 0.26 mmol) and *i*-PrNHEt (124 mg, 0.48 mmol) in DMF (3 mL) was added compound **12** (55 mg, 0.26 mmol) and the solution was stirred at room temperature for 12 h. The reaction mixture was partitioned between water and EtOAc and extracted with EtOAc. The combined EtOAc extract was washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give the crude product. This material was purified by column chromatography on silica gel (hexane/EtOAc: 80/20) to obtain pure compound **14a** (59 mg, 50 %). The compound **14b** (62 %) was prepared in the same way using acid **12** as starting material.

Compound 14a. White solid in 50 % yield, m.p. 96-98 ^{o}C. IR (KBr): 3292; 2962; 2878; 1700; 1650; 1529; 1370; 1270; 1240; 1109; 752 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 7.52 (1H, s, H-5'); 6.73 (1H, brs, H-3'); 6.62 (1H, brs, H-4'); 6.57 (1H, s, H-15); 6.48 (1H, s, H-3); 4.54 (1H, s, H-10); 4.39 (1H, d, J = 9.0 Hz, H-4); 4.13-4.20 (2H, m, C<u>H</u>$_2$ Et); 2.10 (3H, s, C<u>H</u>$_3$ Ac); 1.92 (3H, s, C<u>H</u>$_3$-C2); 1.77-1.82 (1H, m, H-5); 1.27 (3H, t, J = 7.0 Hz, C<u>H</u>$_3$ Et); 0.97 (9H, s, H-12); 0.86 (6H, d, J = 7.5 Hz; H-6, H-7). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ ppm: 169.8 (C-9); 168.2 (<u>C</u>=O Ac); 164.6 (<u>C</u>OOEt); 162.4 (C-13); 149.7 (C-2'); 143.9 (C-5'); 139.6 (C-14); 129.8 (C-3); 127.1 (C-2); 114.3 (C-15); 113.7 (C-3'); 112.0

(C-4'); 61.1 (C-10); 60.7 (C-4); 52.7 (CH$_2$ Et); 36.4 (C-11); 34.9 (C-5); 32.7 (CH$_3$ Ac); 26.6 (C-12); 18.6 (C-6, C-7); 14.1 (CH$_3$ Et); 13.1 (CH$_3$-C-2). HRMS (ESI) $[M+Na]^+$: calc. for $C_{25}H_{37}N_3NaO_5S$: 514.2346; Found: 514.2349.

Compound 14b. White solid in 62 % yield, m.p. 93-94^{o}C. IR (KBr): 3278; 2960; 2932; 2871; 1710; 1691; 1665; 1620; 1510; 1475; 1410; 1369; 1281; 1248; 1100; 1026; 981; 753 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 7.51 (1H, brs, H-5'); 6.89 (1H, brs, H-3'); 6.78 (1H, brs, H-4'); 6.62 (1H, dd, *J* = 1.5, 9.0 Hz,H-3); 6.46 (1H, s, H-15); 5.01 (1H, s, H-10); 4.92 (1H, d, *J* = 9.5 Hz, H-4); 4.16-4.22 (2H, m, CH$_2$ Et); 3.0 (3H, s, H-8); 2.19 (3H, s, CH$_3$ Ac); 1.91 (3H, s, CH$_3$-C-2); 1.88-1.89 (1H, m, H-5); 1.27 (3H, t, *J* = 7.0 Hz, CH$_3$ Et); 0.99 (9H, s, H-12); 0.84 (6H, d, *J* = 7.5; Hz, H-6, H-7). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ ppm: 170.2 (C-9); 167.7 (C=O Ac); 164.1 (COOEt); 162.0 (C-13); 149.8 (C-2'); 143.7 (C-5'); 138.2 (C-14); 132.6 (C-3); 127.0 (C-2); 121.2 (C-15); 113.8 (C-3'); 112.4 (C-4'); 60.9 (C-10); 56.2 (C-4); 55.7 (CH$_2$ Et); 35.2 (C-11); 34.5 (C-5); 31.1 (C-8); 29.9 (CH$_3$ Ac); 26.4 (C-12); 18.7 (C-6, C-7); 14.3 (CH$_3$ Et); 13.8 (CH$_3$-C-2). HRMS (ESI) $[M+Na]^+$: calc. for $C_{26}H_{39}N_3NaO_5S$: 528.2503; Found: 528.2511.

2.5. Synthesis of compound 15a,b

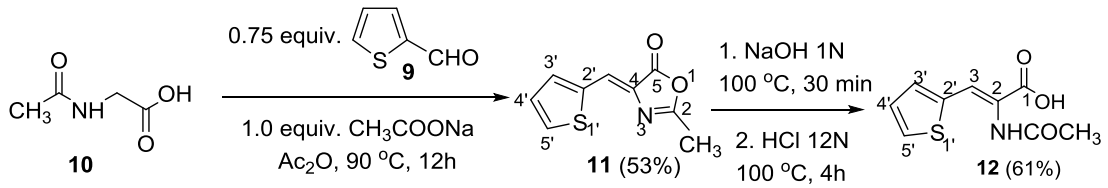
To a cooled (0 ^{o}C) solution of **14a** (50 mg, 0.102 mmol) in 2 mL MeOH/H_2O (2:1) was added LiOH (25 mg, 1.02 mmol). The cooling bath was removed, and the resulting mixture was stirred at room temperature for 10 h. The solvent was removed *in vacuum* and the residue was dissolved in a small amount of H_2O. The aqueous solution was cooled to 0 ^{o}C, and acidified to pH 5.5-6.0 with aqueous hydrochloride acid solution (pH = 2). Afterward, the reaction mixture was extracted with EtOAc. The combined EtOAc extract was washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give pure compound **15a** (45 mg, 95 %). The compound **15b** (97 %) was prepared in the same way from **14b**.

Compound 15a. White solid in 95 % yield, m.p. 120-122 ^{o}C. IR (KBr): 3410; 3279; 2963; 2874; 1649; 1519; 1385; 1370; 1268; 1242; 1150; 1021; 751 cm^{-1}. ^{1}H-NMR (CD_3OD, 500 MHz) δ ppm: 7.69 (1H, d, *J* = 1.5 Hz, H-5'); 7.12 (1H, s, H-15); 6.73 (1H, d, *J* = 3.5 Hz, H-3'); 6.60 (1H, dd, *J* = 1.5, 10.0 Hz, H-3); 6.58 (1H, dd, *J* = 1.5, 3.5 Hz, H-4'); 4.50 (1H, s, H-10); 4.39 (1H, dd, *J* = 1.5, 10.0 Hz, H-4); 2.22 (3H, s, CH$_3$ Ac); 1.92 (3H, s, CH$_3$-C-2); 1.81-1.84 (1H, m, H-5); 1.00 (9H, s, H-12); 0.96 (3H, d, *J* = 6.5 Hz, CH$_3$ H-6); 0.91 (3H, d, *J* = 6.5 Hz, H-7). ^{13}C-NMR (CD_3OD, 125 MHz) δ ppm: 173.4 (C-1); 171.8 (C-9); 166.5 (CO Ac); 149.1 (C-2'); 146.7 (C-5'); 140.2 (C-3); 131.4 (C-14); 126.7 (C-3); 119.5 (C-15); 116.2 (C-4'); 113.2 (C-3'); 62.2 (C-10); 54.5 (C-4); 36.2 (C-11); 33.6 (C-5); 27.1 (C-12); 22.7 (CH$_3$ Ac); 19.3 (C-6); 19.1 (C-7); 13.3 (CH$_3$-C-2). HRMS (ESI) $[M+Na]^+$: calc. for $C_{23}H_{33}N_3NaO_5S$: 486.2033; Found: 486.2042.

Compound 15b. White solid in 97 % yield, m.p. 128-129 ^{o}C. IR (KBr): 3420; 3280; 2961; 2930; 2875; 1697; 1620; 1514; 1478; 1410; 1369; 1270; 1244; 1077; 1022; 980; 754 cm^{-1}. ^{1}H-NMR (CD_3OD, 500 MHz) δ ppm: 7.60 (1H, d, *J* = 1.5 Hz, H-5'); 7.12 (1H, s, H-15); 6.76 (2H, dd, *J* = 1.0, 3.5 Hz, H-3', H-4'); 6.58 (1H, dd, *J* = 1.5, 9.0 Hz, H-3); 5.08 (1H, s, H-10); 4.97 (1H, d, *J* = 9.5 Hz, H-4); 3.06 (3H, s, H-8); 2.21 (3H, s, CH$_3$ Ac); 1.99-2.03 (1H, m, H-5); 1.91 (3H, s, CH$_3$-C-2); 1.02 (9H, s, 3CH$_3$ H-12); 0.91 (3H, d, *J* = 6.5 Hz, H-6); 0.86 (3H, d, *J* = 6.5 Hz, H-7). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ ppm: 173.5 (C-1); 173.3 (C-9); 173.1 (C=O Ac); 166.7 (CO-NH); 151.0 (C-2'); 146.3 (C-5'); 139.5 (C-3); 133.8 (C-14); 126.6 (C-2); 119.6 (C-15); 116.2 (C-4'); 113.1 (C-3'); 58.9 (C-10); 56.8 (C-4); 37.0 (C-11); 31.7 (C-8); 30.9 (C-5); 26.8 (C-12); 22.7 (CH$_3$ Ac); 19.7 (C-6); 19.2 (C-7); 14.0 (CH$_3$-C-2).

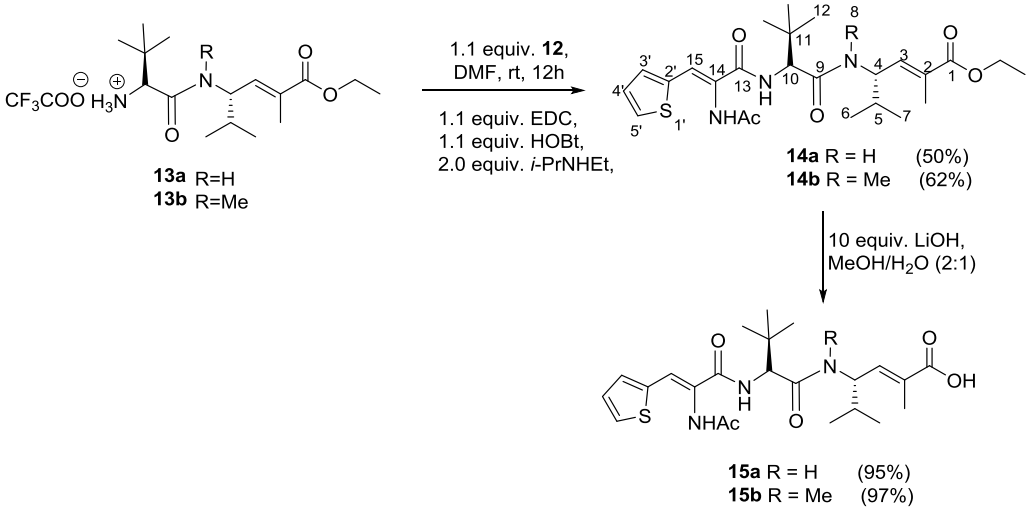
3. RESULTS AND DISCUSSION

Recently, we synthesized new hemiasterlin analogues in which the *α,α*-dimethylbenzylic group and amino NHMe moiety were replaced respectively by a *α,β*-unsaturated aryl and an amide NHAc group leading to the suppression of one chiral center [10]. However, derivatives of hemiasterlin containing 2-thiophenyl group moiety have not been investigated. As a part of our ongoing work, we continue to focus on the new hemiasterlin analogues. For this purpose, we investigated the synthesis of new hemiasterlin derivatives in which the *α,α*-dimethylbenzylic group in fragment A is replaced by *α,β*-unsaturated thiophen-2-yl group. These new compounds will be prepared by classical peptide coupling approach between the carboxylic acid fragments A **12** and dipeptide **13** [10]. A general procedure for the synthesis of compound **12** is outlined in Scheme 1 [10]. Compound **12** was synthesized from *N*-acetyl glycine (**10**) through two steps. The first step was condensation of *N*-acetyl glycine (**10**) with furan-2-carbaldehyde using sodium acetate in the presence of acetic anhydride at 90 °C for 12 h affording azalactone **11** in 80 % yield [9, 10]. Finally, azlactone **11** was hydrolyzed in aqueous sodium hydroxide, followed by treatment with hydrochloric acid (12 N) at 100 °C for 4 h to give compound **12** in 61 % yield.



Scheme 1

The hemiasterlins **15a**,**b** were prepared in two steps after peptide coupling reactions of **13a**,**b** with amides **12** followed by the saponification of ester using a 1N lithium hydroxide solution (scheme 2). The HRMS (ESI) of compound **14a** showed a pseudo-molecular ion peak at m/z 514.2349, corresponding to the molecular formula of $C_{25}H_{37}N_3NaO_5S$. The ^{1}H-NMR showed signals of a thiophene ring at 7.51 (1H, brs, H-5'); 6.89 (1H, brs, H-3') and 6.78 (1H, brs, H-4'). The ^{13}C-NMR revealed its function groups, as follows: an thiophene heterocycles 149.7, 113.7 and 112.0; four carbonyls 169.8, 168.2, 164.6 and 162.4; two double bonds 139.6, 114.3, 129.8 and 126.6; seven metyls including one metyl of acetyl, one vinyl-metyl, a *gem*-dimethyl group, one isopropyl and one *tert*-butyl group. All spectral data thus confirmed the structure of hemiasterlin **14a**. The structure of hemiasterlins **14b** and **15a**,**b** were elucidated the same way by IR, NMR and MS spectroscopic methods.



Scheme 2

In conclusion, a successful synthesis of new modified hemiasterlin derivatives was achieved in which the *α,α*-dimethylbenzylic group and amino NHMe moiety were replaced by *α,β*-unsaturated carbonyl-thiophene and amide NH-Ac group, respectively.

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