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Communication

Oxygenated Analogues of Santacruzamate A

Plamen Angelov [1,*], Stanimir Manolov [1], Pavel Yanev [1] and Mladen Naydenov [2]

[1] Department of Organic Chemistry, University of Plovdiv Paisii Hilendarski, 24 Tsar Asen Str., 4000 Plovdiv, Bulgaria; manolov@uni-plovdiv.net (S.M.); qnev@uni-plovdiv.net (P.Y.)
[2] Department of Human Anatomy and Physiology, University of Plovdiv Paisii Hilendarski, 24 Tsar Asen Str., 4000 Plovdiv, Bulgaria; naydenov@plantgene.eu
* Correspondence: angelov@uni-plovdiv.bg; Tel.: +359-32-261349

Abstract: A new approach for the synthesis of Santacruzamate A analogues is demonstrated. The method allows functionalization at position 3 of the gamma-aminobutyric fragment and carbon chain variation.

Keywords: Santacruzamate A; β-ketoamide; β-hydroxyamide

1. Introduction

Santacruzamate A (Figure 1a) is a structurally simple natural product isolated from marine cyanobacterium *Symploca* sp. [1]. This natural product bears some structural similarity to the clinically used histone deacetylase (HDAC) inhibitor vorinostat (SAHA) [2]. The initial publication about its discovery also reported picomolar-level selective inhibitory activity against HDAC2 (IC_{50} = 119 pM), with a relatively weak inhibition of HDAC4 and HDAC6 [1]. Although these data were not entirely corroborated by later publications from the same group [3] and others [4–6], the interest drawn by this natural product has led to the preparation of many analogues, some with promising bioactivity [4–8]. Considering the current interest in this topic and the ongoing investigations of the structure-activity relationships, we saw an opportunity to contribute to the availability of structurally diverse Santacruzamate A analogues with our method for β-keto amide synthesis [9]. In a previous publication we demonstrated that this enamine-based domino approach provides access to β-keto amides **IV** functionalized with protected amino group in the side chain (Scheme 1, R^1 = H, Ph) [10]. If *N*-ethoxycarbonyl glycine is used as the amino acid component **III** in this methodology and R^1 is set to phenethyl, then the products **IV** would be structurally similar to Santacruzamate A, with introduced carbonyl functionality in the gamma-aminobutyric part and possible variation of the chain length by the choice of an appropriate R^1 substituent in the acetoacetamide **I** (Figure 1b).

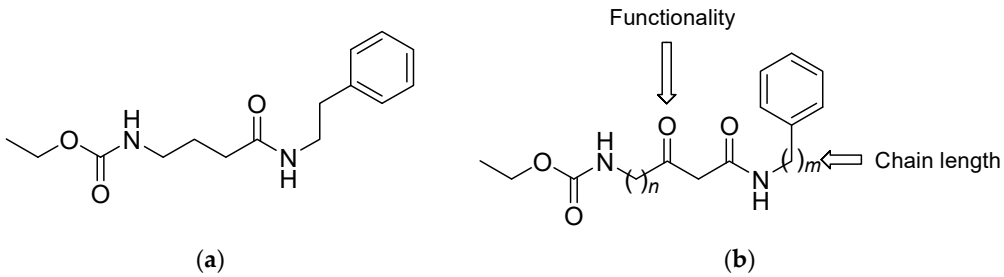

Figure 1. Santacruzamate A (**a**) and oxygenated analogues (**b**).

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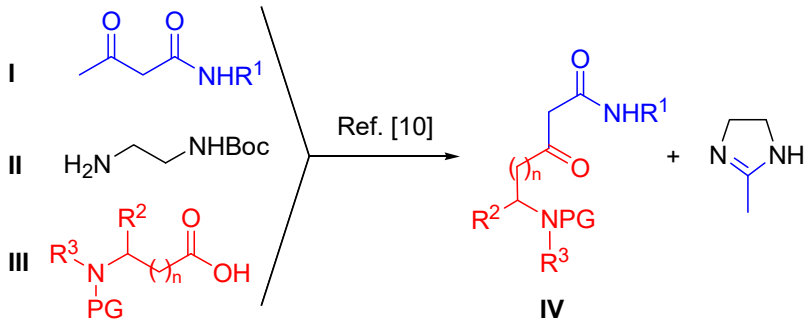
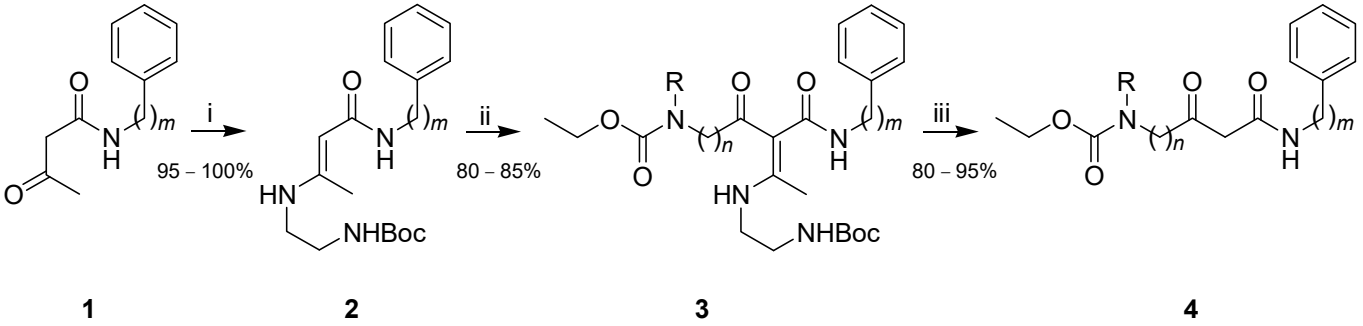


Scheme 1. Synthetic methodology for functionalized β-keto amides [10].

2. Results

To synthesize the oxo-analogue of Santacruzamate A, we first prepared the enaminoamide **2a** (Scheme 2, *m* = 2) by condensation of *N*-phenethyl acetoacetamide with Boc-monoprotected ethylenediamine. This compound was then reacted with mixed carbonic anhydride of *N*-ethoxycarbonyl glycine to provide the intermediate **3a** in 85% yield. Upon the subsequent Boc-deprotection and buffering with NaOAc solution, **3a** gave the expected analogue **4a** in 80% yield. By analogy, this procedure was applied for the preparation of *N*-methylated analogue **4b** and chain-shifted analogue **4c** (Scheme 2, Table 1). The entire synthetic sequence was carried out without any chromatographic purification of the intermediates. The final step gave practically pure keto amides **4** with only small proportion of the enol tautomer visible in the ^{1}H NMR spectra (Supplementary materials).



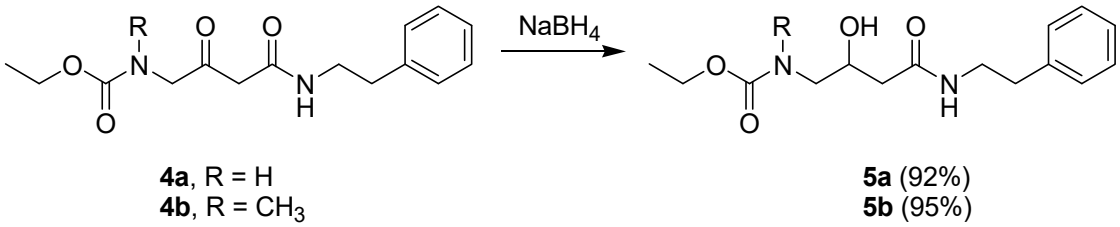
Scheme 2. Reagents and conditions: (i) $BocNHCH_2CH_2NH_2$, CH_2Cl_2, Na_2SO_4, 24 h r.t.; (ii) *N*-Ethoxycarbonyl amino acid, NMM, EtOCOCl, CH_2Cl_2, 0 °C, 5 min; Then **2** and DMAP (0.2 equiv.) in CH_2Cl_2, 0 °C to r.t., 1 h.; (iii) TFA, 5 min, r.t., then aq. CH_3COONa, 2 h, r.t.

Table 1. Yields of keto amides **4**, prepared according to Scheme 2.

4	*n*	*m*	R	Yield (%) [1]
a	1	2	H	68
b	1	2	CH_3	76
c	2	1	H	71

[1] Overall yield after three steps, based on the starting acetoacetamide **1**.

The introduction of carbonyl group in the gamma-aminobutyric fragment of Santacruzamate A provides a useful handle for various further manipulations. For example, the reduction of **4a**,**b** with $NaBH_4$ was straightforward and gave the corresponding alcohols **5a**,**b** in quantitative yields (Scheme 3).



4a, R = H
4b, R = CH_3

5a (92%)
5b (95%)

Scheme 3. Reduction of oxo-analogues to hydroxy-analogues.

3. Materials and Methods

All reagents and solvents were purchased from commercial suppliers (Sigma-Aldrich or Merck, Darmstadt, Germany) and were used without further purification. *Boc*-monoprotected ethylenediamine [11], acetoacetamides **1** [12,13], and enaminoamides **2** [9] were prepared according to the published procedures. NMR spectra were run on Bruker Avance AV600 (600/150 MHz $^{1}H/^{13}C$) spectrometer (Bruker, Billerica, MA, USA) at BAS-IOCCP—Sofia and chemical shifts (δ, ppm) are downfield from TMS. High resolution mass spectral measurements were performed on a Thermo Scientific Q Exactive hybrid quadrupole-orbitrap mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). TLC was done on aluminium-backed Silica gel 60 sheets (Merck) with $KMnO_4$ staining; Melting points were measured on Boetius hot stage apparatus (Boetius, Germany) and are not corrected.

Synthetic Procedures

*Oxo-analogues of Santacruzamate A (***4a–c***), general procedure:* To a magnetically stirred solution of the corresponding *N*-ethoxycarbonyl amino acid (1 mmol) in CH_2Cl_2 (5 mL), *N*-methylmorpholine (1 mmol, 0.11 mL) was added. The solution was then put in an ice bath and ethyl chloroformate (1 mmol, 0.1 mL) was added. The mixture was left to stir for 5 min and after that a solution of enamino amide **2** (1 mmol) and DMAP (0.2 mmol) in CH_2Cl_2 (10 mL) was added in one go. The ice bath was then removed, and the reaction mixture was left to stir for one more hour at r.t. The reaction mixture was then transferred to a separatory funnel with additional 30 mL of CH_2Cl_2 and washed with aqueous (10:1) HCl. The aqueous layer was extracted with 30 more mL of CH_2Cl_2, the combined organic layers were dried with anhydrous sodium sulfate, the drying agent was removed by filtration, and the solvent was evaporated under reduced pressure. The intermediates **3** solidified upon trituration with small volume of diethyl ether. The ethereal washings were filtered off and the crude compounds **3** were dissolved in TFA (1 mL TFA per 100 mg of **3**). The TFA solutions were stirred for 5 min at r.t. and then 3 mol/L aqueous solution of NaOAc (10 mL for each mL of TFA) was added, followed by CH_2Cl_2 (30–50 mL). The mixture was left to stir intensely for 2 h. The layers were then separated, and the aqueous layer was extracted two more times with CH_2Cl_2. The organic layers were combined, washed with saturated aqueous $NaHCO_3$ (20 mL), and then dried over Na_2SO_4. The solvent was removed on a rotary evaporator. Compounds **4a** and **4c** crystallized and were rinsed with small volumes of diethyl ether or ether-petroleum. Compound **4b** was isolated as clear oil. Chromatography of the ethereal washings through a short plug of silica gel can afford small additional amounts of **4a**,**c**.

(2-Oxo-3-phenethylcarbamoyl-propyl)-carbamic acid ethyl ester (**4a**): white solid, mp 137 –139 °C; R_f = 0.55 (Et_2O:CH_3OH 20:1); ^{1}H-NMR (600 MHz, $CDCl_3$, δ ppm, *J* Hz): 7.34–7.20 (m, 5H), 6.71 (br s, 1H), 5.40 (br s, 1H), 4.15 (q, *J* = 7.0, 2H) overlapped with 4.12 (d, *J* = 5.3, 2H), 3.55 (dt, *J* = 6.4, *J* = 7.0, 2H), 3.39 (s, 2H), 2.84 (t, *J* = 7.0, 2H), 1.27 (t, *J* = 7.0, 3H); ^{13}C-NMR (150 MHz, $CDCl_3$, δ ppm): 201.6, 164.8, 156.5, 138.6, 128.8, 128.7, 126.6, 61.4, 51.1, 47.0, 40.9, 35.5, 14.6; ESI-MS (*m*/*z*): 315.1320 $[M + Na]^+$ (calcd for $C_{15}H_{20}N_2NaO_4^+$ 315.1315); 291.1353 $[M - H]^-$ (calcd for $C_{15}H_{19}N_2O_4^-$ 291.1350).

Methyl-(2-oxo-3-phenethylcarbamoyl-propyl)-carbamic acid ethyl ester (**4b**): clear oil; R_f = 0.35 (Et_2O:CH_3OH 20:1); ^{1}H-NMR (600 MHz, $CDCl_3$, δ ppm, *J* Hz), only signals for the major rotamer are listed: 7.34–7.21 (m, 5H), 6.87 (br s, 1H), 4.17–4.09 (m, 4H) 3.55 (m, 2H), 3.38

(s, 2H), 2.94 (s, 3H), 2.85 (t, *J* = 7.0, 2H), 1.30 (t, *J* = 7.0, 3H); ^{13}C-NMR (150 MHz, $CDCl_3$, δ ppm): 202.0, 165.1, 157.0, 138.7, 128.8, 128.6, 126.6, 62.0, 58.9, 46.7, 41.0, 35.6, 35.5, 14.6; ESI-MS (*m*/*z*): 329.1475 $[M + Na]^+$ (calcd for $C_{16}H_{22}N_2NaO_4^+$ 329.1472).

(4-Benzylcarbamoyl-3-oxo-butyl)-carbamic acid ethyl ester (**4c**): white solid, mp 100–102 °C; R_f = 0.50 (Et_2O:CH_3OH 20:1); ^{1}H-NMR (600 MHz, DMSO-d_6, δ ppm, *J* Hz): 8.33 (br t, *J* = 5.9, 1H), 7.15–7.04 (m, 5H), 6.86 (br t, *J* = 5.9, 1H), 4.10 (d, *J* = 5.9, 2H) 3.77 (q, *J* = 7.0, 2H) 3.21 (s, 2H), 2.98(dt, *J* = 5.9, *J* = 7.0, 2H), 2.50 (t, *J* = 7.0, 2H), 0.95 (t, *J* = 7.0, 3H); ^{13}C-NMR (150 MHz, DMSO-d_6, δ ppm): 204.3, 166.5, 156.6, 139.6, 128.8, 127.7, 127.3, 60.0, 50.9, 42.9, 42.7, 35.7, 15.1; ESI-MS (*m*/*z*): 315.1317 $[M + Na]^+$ (calcd for $C_{15}H_{20}N_2NaO_4^+$ 315.1315);

*Hydroxy-analogues of Santacruzamate A (***5***):* To the corresponding keto amide **4** (100 mg) in methanol (10 mL) was added $NaBH_4$ in small portions (5–7 mg every 10 min) until TLC indicated the absence of the starting material. The mixture was then diluted with water (50 mL) and extracted with CH_2Cl_2 (3 × 20 mL). The organic layers were combined, dried over Na_2SO_4, and the solvent was removed on a rotary evaporator to afford practically clean hydroxy amides **5**.

(2-Hydroxy-3-phenethylcarbamoyl-propyl)-carbamic acid ethyl ester (**5a**): white solid, mp 104–105 °C; R_f = 0.50 (Et_2O:CH_3OH 20:1); ^{1}H-NMR (600 MHz, $CDCl_3$, δ ppm, *J* Hz): 7.25–7.11 (m, 5H), 6.20 (br s, 1H), 5.24 (br s, 1H), 4.02 (q, *J* = 7.0, 2H), 3.96 (m, 1H), 3.45 (dt, *J* = 5.9, *J* = 7.0, 2H), 3.22 (dt, ^2J = 14.1, ^3J = 4.7, 1H), 3.09 (dt, ^2J = 14.1, ^3J = 5.9, 1H), 2.75 (t, *J* = 7.0, 2H), 2.24 (m, 2H), 1.16 (t, *J* = 7.0, 3H); ^{13}C-NMR (150 MHz, $CDCl_3$, δ ppm): 172.04, 157.6, 138.6, 128.74, 128.70, 126.6, 68.2, 61.1, 45.9, 40.6, 39.6, 35.5, 14.6; ESI-MS (*m*/*z*): 317.1475 $[M + Na]^+$ (calcd for $C_{15}H_{22}N_2NaO_4^+$ 317.1472).

(2-Hydroxy-3-phenethylcarbamoyl-propyl)-methyl-carbamic acid ethyl ester (**5b**): white solid, mp 79–81 °C; R_f = 0.33 (Et_2O:CH_3OH 20:1); ^{1}H-NMR (600 MHz, $CDCl_3$, δ ppm, *J* Hz), only signals for the major rotamer are listed: 7.33–7.21 (m, 5H), 6.47 (br s, 1H), 4.15 (br s, 1H) overlapped with 4.13 (q, *J* = 7.0, 2H), 3.55 (m, 2H), 3.33 (m, 2H), 2.99 (s, 3H), 2.84 (t, *J* = 7.0, 2H), 2.33 (m, 2H), 1.28 (t, *J* = 7.0, 3H); ^{13}C-NMR (150 MHz, $CDCl_3$, δ ppm): 171.9, 158.0, 138.7, 128.8, 128.6, 126.6, 68.2, 61.8, 54.6, 40.6, 40.2, 36.2, 35.6, 14.7; ESI-MS (*m*/*z*): 331.1630 $[M + Na]^+$ (calcd for $C_{16}H_{24}N_2NaO_4^+$ 331.1628).

4. Conclusions

We successfully prepared new oxygenated analogues of Santacruzamate A. This extends the scope of the enamine-based domino approach to functionalized β-keto amides and demonstrates a viable route to many more analogues of the natural product.

Supplementary Materials: The following are available online, S1.PDF—processed ^{1}H and ^{13}C NMR spectra. S2.zip—Raw NMR data. S3.zip—mol files.

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Data Availability Statement: The data presented in this study are available in this article and supporting supplementary material.

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Conflicts of Interest: The authors declare no conflict of interest.

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