Tetrahedron Letters 49 (2008) 5056–5058

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

First total synthesis of the antifungal antibiotic thiobutacin

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ARTICLE INFO

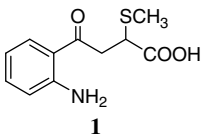
Article history:
Received 24 April 2008
Revised 3 June 2008
Accepted 9 June 2008
Available online 12 June 2008

Keywords:
Antifungal
Thiobutacin
Synthesis
Natural product

ABSTRACT

The first total synthesis of thiobutacin, a butanoic acid with antifungal activity recently isolated from the culture broth of a soil actinomycete, *Lechevalieria aerocolonigenes* strain VK-A9, is described. The five-step procedure involves readily available and cheap starting materials and can easily be transposed to the large scale. Fungal growth inhibition of thiobutacin is mediated by the pH of the growth medium. Maximum inhibitory activity was obtained between pH 6 and 7.

Thiobutacin (**1**, 4-(2-aminophenyl-4-oxo-2-methylthiobutanoic acid)) was recently isolated by Hwang and co-workers from the culture broth of a soil actinomycete, *Lechevalieria aerocolonigenes* strain VK-A9.[1] Although thiobutacin presents a stereogenic centre at carbon C-2, no optical data were reported for the natural compound, most probably due to the easy racemization. It is well known that mercapto-bearing carbons adjacent to an electron-withdrawing group like an acid, ester, amide or nitrile are extremely sensitive to racemization, either complete or partial.[2] Thiobutacin showed actinomycete activity against *Phytophthora capsici* in microtiter broth dilution assay (MIC 10 μg/mL) and antifungal activity against *Botrytis cinerea* (MIC 50 μg/mL) and the yeast *Saccharomyces cerevisiae* (MIC 30 μg/mL).[1] In a following paper, the same authors confirmed the in vitro actinomycete activity of thiobutacin against *P. capsici* and its control efficacy against *Phytophthora* blight in vivo.[3]



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As part of our studies on natural compounds with antifungal activity, we became interested in developing a general method for synthesizing **1**, which might also be amenable to the synthesis of analogues.

In this Letter, we describe a preparatively simple five-step route to thiobutacin that is depicted in Scheme 1.

Crotonic condensation of 2-nitroacetophenone with glyoxylic acid monohydrate afforded compound **3** in 77% yield. The reaction was accomplished by heating at 96 °C under reduced pressure in the presence of water and a catalytic amount of concd H_2SO_4, using a modified version of the procedures described by Bianchi[4a] and Kameo.[4b] Michael addition of thioacetic acid to **3** provided 2-acetylsulfanyl-4-(2-nitrophenyl)-4-oxobutyric acid **4**[5] that was easily converted to **5** (92% yield) by refluxing with concd H_2SO_4 and AcOH. Subsequently, compound **5** was methylated with methyl iodide to furnish 2-methylsulfanyl-4-(2-nitrophenyl)-4-oxobutyric acid **6** in good yield.

To verify the regioselectivity of the Michael addition, compound **6** was converted to the methyl ester and reduced with $NaBH_4$ in ethanol/water giving the corresponding diol **8** as a mixture of diastereomers. Analysis of the ^{1}H NMR spectra of the two (±)-diastereomers **8a** and **8b** confirmed that the thioacetate was bound at the α position with respect to the carboxylic acid group. In fact the hydrogen on C-4, which bears the hydroxy group, gives in both compounds a double doublet at 5.45 ppm in **8a** (J = 2.6, 9.6 Hz), and at 5.55 ppm in **8b** (J = 2.6, 9.9 Hz), thus indicating the presence of 2 hydrogens on C-3. Moreover, the multiplicity of H-3A (in **8a**: ddd, J = 2.6, 6.3, 14.7 in **8b**: ddd, J = 2.6, 11.4, 14.7, Hz), and H-3B (in **8a**: ddd, J = 6.6, 9.6, 14.7 in **8b**: ddd, J = 5.5, 9.9, 14.7 Hz) clearly demonstrates the proposed structure.

The final step of the synthesis was the reduction of the nitro group. This was first attempted using $Na_2S_2O_4$ in a mixture of dioxane/H_2O (1.2:1)[6] but the troublesome workup and the unsatisfactory yield prompted us to search for an alternative route. After

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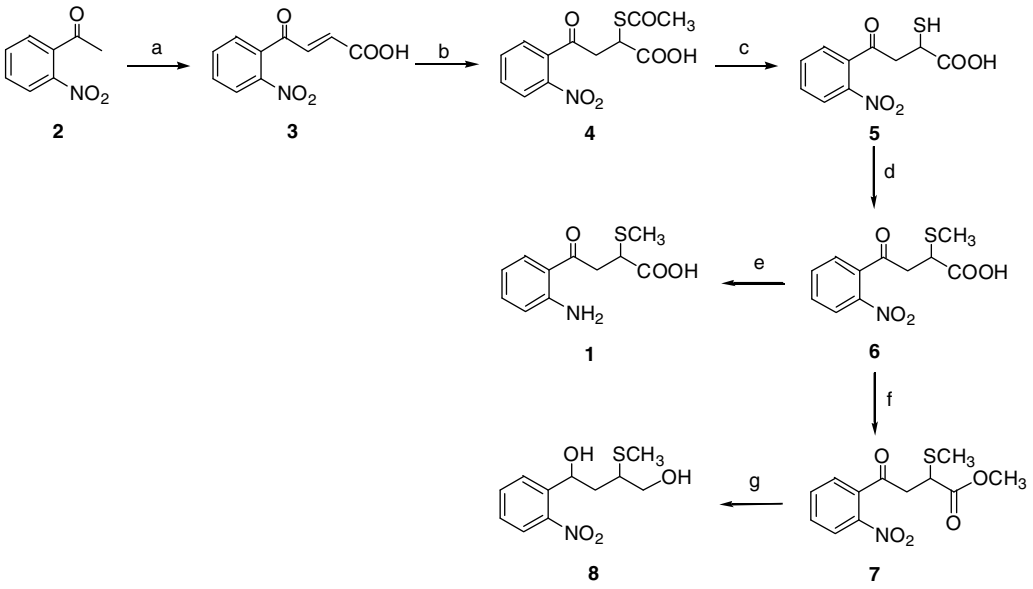
doi:10.1016/j.tetlet.2008.06.036



Scheme 1. Reagents and conditions: (a) glyoxylic acid monohydrate, 96 °C, reduced pressure, concd H_2SO_4, H_2O, 1.5 h, 77%; (b) thioacetic acid, CH_2Cl_2, rt, 3.5 h, 94%; (c) AcOH, concd H_2SO_4, H_2O, reflux, 2 h, 92%; (d) MeI, TFA, CH_2Cl_2, rt, 3 h, 69%; (e) HI, 90 °C, 3 h, 70%; (f) MeOH, H_2SO_4, rt, 14 h, 83%; (g) $NaBH_4$, ethanol/water, rt, 1.5 h, 72%.

several other attempts, it was found that the use of 57% HI[7] at non-refluxing conditions (90 °C) for 3 h yielded the corresponding amine thiobutacin (**1**) with good chemoselectivity, that is, without affecting the carbonyl group.[8]

The spectroscopic data, including ^{1}H NMR, ^{13}C NMR, HMBC, COSY and MS spectra of the synthetic thiobutacin matched with those reported in the literature[1] for the natural compound, thus confirming its structure.

Thiobutacin was then tested for its biological activity. As we were unable to secure an authentic sample for direct comparison, the experiments were performed only on the synthetic sample.

The antifungal activity was evaluated against *B. cinerea, Penicillium* sp., *Mucor mucedo, Alternaria alternata* and the yeast *S. cerevisiae*.[9] The higher inhibitory effect was observed on the growth of *S. cerevisiae*, with a 30% inhibition at a dose of 250 μg/mL. A 10% inhibition was observed on *B. cinerea* and *M. mucedo* at the same dose, whilst the compound was ineffective on *A. alternata* and *Penicillium* sp. The lower antifungal activity of synthetic thiobutacin as compared to the one reported by Lee et al.[1] led us to hypothesize that the medium pH, and consequently thiobutacin charge, could influence its biological activity. There are examples in the literature of such effects.[10] Therefore, we tested the effectiveness on *B. cinerea* growth at 5 different pH conditions, from 4 to 8 (Fig. 1). We found that growth inhibition was strongly affected by the pH of the culture medium, as reported in Figure 1, being higher at pH between 6 and 7 and steeply decreasing at higher or lower pH conditions. These results could explain the discrepancy of our data with those of Lee et al.

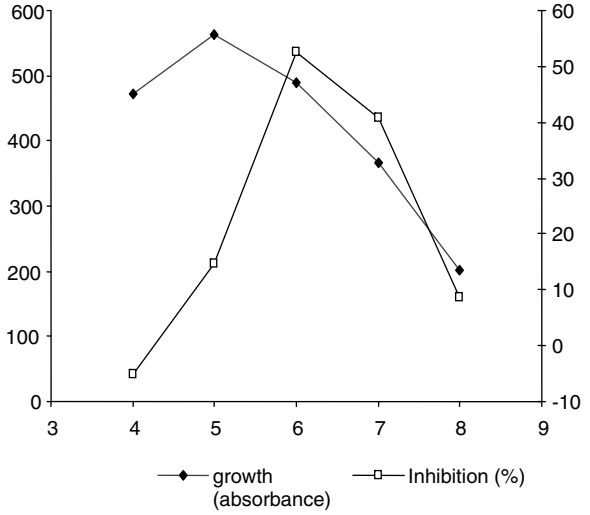


Figure 1. Effect of the pH of the culture medium on the growth of *B. cinerea* (measured from absorbance at 492 nm, left scale) and on its growth inhibition induced by 100 μg/mL thiobutacin (right scale).

In conclusion, we have accomplished the first synthesis of the natural antifungal thiobutacin; the proposed route is concise and modular, making it convenient for large scale preparation and rapid synthesis of analogues. Antifungal tests against *B. cinerea* highlighted that the pH of the medium strongly affects the growth inhibition activity, this latter being highest at pH between 6 and 7.

Acknowledgement

This work was supported by the University of Milano (FIRST funds).

Supplementry data

Supplementary data (synthetic procedures, characterization data and copies of ^{1}H, ^{13}C and HMBC NMR spectra of thiobutacin) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.06.036.

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8. *Spectral data:* Compound **3**: IR (film) 3060, 3010, 1720, 1540, 1430, 1370, 1280, 1230, 750, 720 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 13.25 (br s, 1H, –COOH), 8.21 (d, 1H, H-3′, J = 8.2 Hz), 7.90 (dd, 1H, H-5′, J = 8.2, 8.2 Hz), 7.82 (dd, 1H, H-4′, J = 8.2 Hz), 7.65 (d, 1H, H-6′, J = 8.2 Hz), 7.20 (d, 1H, J = 16.0 Hz), 6.38 (d, 1H, J = 16.0 Hz). ^{13}C NMR (acetone-d_6) δ: 191.92; 165.32; 146.70; 139.28; 134.86; 134.71; 133.15; 131.74; 128.96; 124.63. HRMS (ESI$^-$) calcd for $C_{10}H_6NO_5$ [M−H]$^-$ 220.02515, found 220.02488; calcd for $C_{20}H_{13}N_2O_{10}$ [2M−H]$^-$ 441.05757, found 441.05630; calcd for $C_{20}H_{12}N_2O_{10}Na$ [2M−2H+Na]− 463.03951, found 463.03822.
Compound **4**: IR (film) 3060, 3000, 1720, 1540, 1430, 1355, 1280, 750, 720 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 8.13 (1H, d, H-3′, J = 8.2 Hz), 7.75 (1H, dd, H-5′, J = 7.4, 7.4 Hz), 7.62 (1H, dd, H-4′, J = 7.4, 8.2 Hz), 7.49 (1H, d, H-6′, J = 7.4 Hz), 4.76 (1H, dd, H-2, J = 7.4, 4.8 Hz), 3.56 (1H, H-3A, J = 7.4, 18.2 Hz), 3.40 (1H, dd, H-3B, J = 4.8, 18.2 Hz), 2.42 (3H, s, $COCH_3$). ^{13}C NMR (acetone-d_6) δ: 192.68; 170.93; 146.34; 135.79; 134.10; 131.57; 128.16; 124.33; 43.88; 39.79; 28.74. HRMS (ESI$^-$) calcd for $C_{12}H_{10}NO_6S$ [M−H]$^-$ 296.02343, found 296.02304; calcd for $C_{24}H_{21}N_2O_{12}S_2$ [2M−H]$^-$ 593.05414, found 593.05140; calcd for $C_{24}H_{20}N_2O_{12}S_2Na$ [2M−2H+Na]$^-$ 615.03608, found 615.03414.
Compound **5**: IR (film) 3060, 3000, 1720, 1560, 1430, 1280, 750, 720 cm^{-1}; ^1H NMR (acetone-d_6) δ: 8.11 (1H, d, H-3′, J = 8.2 Hz), 7.89 (1H, dd, H-5′, J = 7.4, 7.4 Hz), 7.79 (1H, dd, H-4′, J = 7.4, 8.2 Hz), 7.75 (1H, d, H-6′, J = 7.4 Hz), 3.93 (1H, ddd, H-2, J = 4.0, 9.3, 9.3 Hz), 3.63 (1H, H-3A, J = 18.2, 9.3 Hz), 3.38 (1H, dd, H-3B, J = 18.2, 4.8 Hz), 2.76 (1H, d, –SH, J = 9.3 Hz). ^{13}C NMR (acetone-d_6) δ: 198.73; 172.88; 146.37; 135.89; 134.10; 131.53; 128.10; 124.33; 47.25; 34.67. HRMS (ESI$^-$) calcd for $C_{10}H_8NO_5S$ [M−H]$^-$ 254.01287, found 254.01356; calcd for $C_{20}H_{17}N_2O_{10}S_2$ [2M−H]$^-$ 509.03301, found 509.03234; calcd for $C_{20}H_{16}N_2O_{10}S_2Na$ [2M−2H+Na]$^-$ 531.01495, found 531.01523.
Compound **6**: IR (film) 3060, 3000, 1720, 1540, 1430, 1350, 1270, 910, 750, 720 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 8.13 (1H, d, H-3′, J = 8.2 Hz), 7.75 (1H, dd, H-5′, J = 7.4, 7.4 Hz), 7.62 (1H, dd, H-4′, J = 7.4, 8.2 Hz), 7.48 (1H, d, H-6′, J = 7.4 Hz), 3.87 (1H, dd, H-2, J = 4.5, 10.1 Hz), 3.48 (1H, H-3A, J = 10.1, 18.2 Hz), 3.24 (1H, dd, H-3B, J = 18.2, 4.5 Hz), 2.27 (3H, s, –SCH_3). ^{13}C NMR ($CDCl_3$) δ: 199.24, 177.35, 145.44, 136.90, 134.45, 130.88, 127.52, 124.49, 44.05, 41.66, 14.78. HRMS (ESI$^-$) calcd for $C_{11}H_{10}NO_5S$ [M−H]$^-$ 268.02852, found 268.02861; calcd for $C_{22}H_{21}N_2O_{10}S_2$ [2M−H]$^-$ 537.06431, found 537.06278; calcd for $C_{22}H_{20}N_2O_{10}S_2Na$ [2M−2H+Na]$^-$ 559.04625, found 559.04489.
Compound **7**: IR (film) 3060, 2990, 1740, 1540, 1440, 1430, 1360, 1280, 750, 720 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 8.12 (1H, d, H-3′, J = 8.1 Hz) 7.74 (1H, dd, H-5′, J = 7.7, 7.7 Hz), 7.61 (1H, dd, H-4′, J = 7.7, 8.1 Hz), 7.49 (1H, d, H-6′, J = 7.7 Hz), 3.86 (1H, dd, H-2, J = 4.4, 10.3 Hz), 3.84 (3H, s, OCH_3), 3.51 (1H, dd, H-3A, J = 10.3, 18.0 Hz), 3.21 (1H, dd, H-3B, J = 4.4, 18.0 Hz), 2.20 (3H, s, SCH_3). ^{13}C NMR ($CDCl_3$) δ: 199.41, 171.97, 145.46, 136.98, 134.34, 128.65, 124.60, 52.77, 45.14, 41.52, 14.33.
Compound **8a**: IR (film) 3350, 3060, 3000, 1530, 1440, 1330, 1275, 1100, 900, 750, 720 cm^{-1}; ^1H NMR($CDCl_3$) δ: 7.91 (1H, d, H-3′, J = 8.5 Hz) 7.86 (1H, d, H-6′, J = 8.1 Hz), 7.64 (1H, dd, H-5′, J = 8.1, 8.1 Hz), 7.41 (1H, dd, H-4′, J = 8.5, 8.1 Hz), 5.45 (1H, dd, H-4, J = 2.6, 9.6 Hz), 3.77 (1H, dd, H-1A, J = 6.3, 11.4 Hz), 3.67 (1H, dd, H-1B, J = 6.3, 11.4 Hz), 3.00 (1H, m, H-2), 2.15 (1H, ddd, H-3A, J = 2.6, 6.3, 14.7 Hz), 2.10 (3H, s, SCH_3), 2.01 (1H, ddd, H-3B, J = 6.6, 9.6, 14.7 Hz); ^{13}C NMR ($CDCl_3$) δ: 147.36, 139.68, 132.65, 128.12 (x2), 124.43, 68.33, 63.49, 48.50, 39.99, 12.72; HRMS (ESI$^+$) calcd for $C_{11}H_{15}NO_2S$ [M+Na]$^+$ 280.06195, found 280.06219.
Compound **8b**: IR (film) 3350, 3060, 3000, 1530, 1440, 1330, 1275, 1100, 900, 750, 720 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 7.91 (1H, d, H-3′, J = 8.5 Hz) 7.85 (1H, d, H-6′, J = 8.1 Hz), 7.64 (1H, dd, H-5′, J = 8.1, 8.1 Hz), 7.49 (1H, dd, H-4′, J = 8.5, 8.1 Hz), 5.55 (1H, dd, H-4, J = 2.6, 9.9 Hz), 3.68 (2H, d, H-1, J = 6.3), 3.09 (1H, m, H-2), 2.10 (3H, s, SCH_3), 2.04 (1H, ddd, H-3A, J = 2.6, 11.4, 14.7 Hz), 1.91 (1H, ddd, H-3B, J = 5.5, 9.9, 14.7 Hz). ^{13}C NMR ($CDCl_3$) δ:147.41, 139.65, 133.69, 128.23, 128.12, 124.38, 67.43, 63.22, 46.57, 39.58, 11.84. HRMS (ESI$^+$) calcd for $C_{11}H_{15}NO_2S$ [M+Na]$^+$ 280.06195, found 280.06176.
9. Bioassays procedure: Antimicrobial activity of the newly synthesized thiobutacin was at first assessed on *B. cinerea, Penicillium* sp., *Alternaria alternata, Mucor mucedo* and *Saccharomyces cerevisiae* in 96-well microtiter dishes with the modified method described by Lee et al.[1] Briefly 50 μL of fungal spore (2×10^3 spores/mL) and yeast cell (2×102 CFU/mL) suspensions in potato dextrose broth PDB (Difco) were added to each well containing 50 μL of PDB amended with thiobutacin at different concentrations from 0 to 250 μg/mL. To assess the influence of pH on thiobutacin activity, an experiment was performed with *B. cinerea* grown at different pH conditions, from 4 to 8, adding an adequate amount of HCl or KOH to PDB. The absorbance was measured with a microplate reader at 492 nm wavelength twice, the first just after filling the plates and the second at the end of the experiment after incubation for 2 days at 25 °C. Final absorbance values were calculated subtracting the values of the first reading from those of the second reading. Percent growth inhibition at the different thiobutacin doses was calculated with respect to the control wells.
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