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Short Note

(8*R*,10a*S*)-Methyl 2,4-diamino-8-(*tert*-butyl)-6-oxo-6,8,10,10a-tetrahydrooxazolo[3″,4″:1′,5′]-pyrrolo[3′,4′:5,6]pyrido[2,3-*d*]pyrimidine-10a-carboxylate

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Abstract: A fused polyheterocyclic derivative is available by annulation of a tetramate scaffold, and has been shown to have some Gram-negative, but not Gram-positive, antibacterial activity.

Keywords: antibacterial; tetramate; polyheterocycle; heteroaromatic; annulation

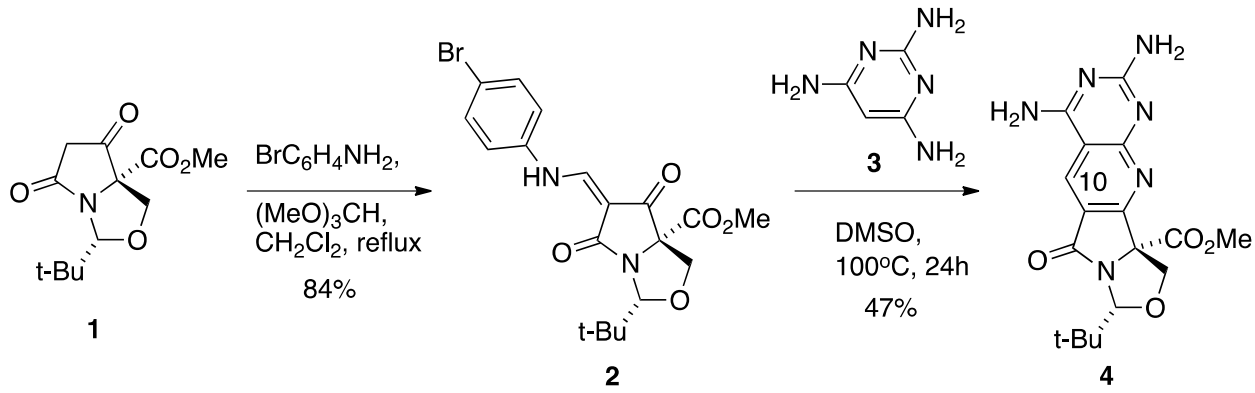
1. Introduction

It is now widely appreciated that the antibacterial drug pipeline is poorly populated [1], and that the emergence of antibacterial resistance creates a constant need for new drugs [2–4]. Historically, natural products have provided a crucial start point in antibacterial drug discovery [5] and still remain worthy of investigation. The tetramic acid core is a template widely found in natural products with antibacterial activity [6,7] and although the core tetramic motif tends to have no antibacterial activity [8], we have shown that an appropriately modified tetramic core can exhibit high levels of antibacterial activity [9–13]. Some fused ring quinoline-containing compounds which are structurally similar to tetramates, such as pyrrolo[3,4-*c*]quinoline-1,3-dione derivatives, exhibit inhibition of Gram-positive and Gram-negative bacteria [14] and, of interest to us, was the construction of a similarly fused heterocyclic-tetramate and an examination of its bioactivity.

2. Results and Discussion

2.1. Chemical Synthesis

The desired compound used bicyclic template **1**, prepared as reported [15], which could be readily converted to enamine **2** by treatment with trimethyl orthoformate and an aromatic amine in refluxing dichloromethane, and similar to a recently reported protocol [16] (Scheme 1 and Figure 1). The pure product **2** could be obtained by column chromatography, as a mixture of *E*- and *Z*-isomers, with the major being that as shown. This material was readily reacted with 2,4,6-triaminopyrimidine **3** which, under prolonged heating in DMSO, gave heterocyclic product **4** in 47% yield, assigned on the basis-detailed NMR (HSQC, HMBC) analysis. The bulk structure was confirmed by clear correlations of 0.88 (-C(C*H*$_3$)$_3$)) with a carbon at 23.96, of 4.81 (C-2*H*) with 97.37 (*C*-2), of 8.61 (1H, s, C-10*H*) with 131.3 (*C*-10), and of the coupled pair at 3.25 and 4.89 (C-4H*H*) with 68.93 (*C*-4). This HMBC data gives the expected correlations, but does not secure the regiochemistry of the original addition to enamine **2**. The structure of this compound could not be confirmed by NOE analysis either, but is assigned on the basis of comparison with the outcome of related annulation processes [17] and by chemical shift calculations (MestReNova™) of 8.42 ppm (observed, 8.61) for C(10)H and of 131.2 ppm (observed, 131.0) for C(10).



Scheme 1. Annulation of tetramate **1** to product **4**.

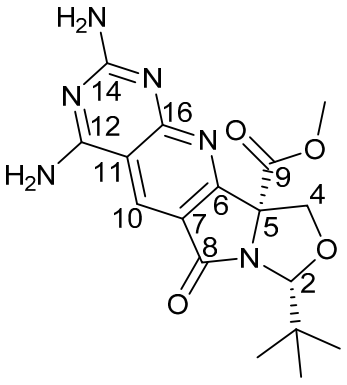


Figure 1. Structure of compound **4**.

2.2. Antibacterial Activity

Biological activity of this derivative was assessed against Gram-positive *S. aureus* and Gram-negative *E. coli*, in triplicate by hole-plate bioassay, with cephalosporin C as a positive control, after overnight incubation in a 1:1 mixture of DMSO and water with a concentration of 4 mg mL^{-1}. Compound **4** showed activity against *E. coli*, but not against *S. aureus*, in contrast to most tetramates, which generally display selective Gram positive activity (Table S1 and Figure S9 in the supplementary materials) [9–12].

3. Experimental Section

(8R,10aS)-Methyl 2,4-diamino-8-(tert-butyl)-6-oxo-6,8,10,10a-tetrahydrooxazolo[3'',4'':1',5']-pyrrolo[3',4':5,6]pyrido[2,3-d]pyrimidine-10a-carboxylate **4**

Under a nitrogen atmosphere, tetramic acid **1** (225 mg, 1.0 mmol) was dissolved in anhydrous DCM (10 mL), and *para*-bromoaniline (189 mg, 1.1 mmol) and trimethyl orthoformate (0.12 mL, 1.0 mmol) were added. The solution was refluxed at 45 °C for five hours, following the reaction using TLC. The reaction mixture was then cooled to room temperature and the DCM was removed in vacuo to give the crude product. Flash column chromatography (DCM–EtOAc 4:1) was used to purify the crude product to give **2** as a yellow solid in the ratio of 1.8:1 diastereomers (325 mg, 84%) [18].

Compound **2** (70 mg, 0.16 mmol) was dissolved in DMSO (5 mL), and 2,4,6-triaminopyrimidine (26 mg, 0.21 mmol) was added. The solution was heated to 100 °C for 24 h, following the reaction using TLC. The reaction mixture was then cooled to room temperature and diluted with EtOAc (20 mL). Brine (15 mL) was added, and the organic layer was extracted, and then washed with brine (15 mL) four more times. The EtOAc was removed *in vacuo* to give the crude product. This was then columned on aluminium oxide (EtOAc–MeOH 5:1) to give the product **4** as a pink solid (28 mg, 47%); **δH** (500 MHz, MeOD) 0.88 (9H, s, -C(C*H*$_3$)$_3$), 3.25 (1H, d, *J* = 8.6, C-4*H*H′), 3.62 (3H, s, -OC*H*$_3$), 4.81 (1H, s, C-2*H*), 4.89 (1H, d, *J* = 8.6, C-4H*H*′), 8.61 (1H, s, C-10*H*); **δC** (500 MHz, MeOD) 23.96 (-C(*C*H$_3$)$_3$), 34.98 (-*C*(CH$_3$)$_3$), 52.35 (-O*C*H$_3$), 68.93 (*C*-4), 75.60 (*C*-5), 97.37 (*C*-2), 105.7, 117.9 (*C*-7, *C*-11), 131.3 (*C*-10), 163.8, 164.4, 164.6, 168.1 (*C*-6, *C*-12, *C*-14, *C*-16), 168.8 (*C*-9), 172.8 (*C*-8); ***m/z*** (ESI$^+$) 373.2 ([M + H]$^+$ 100%); **HRMS** (ESI$^+$) found 373.16187 ([M + H]$^+$) requires 373.16188; ν_{max} 3344 (N-H), 3184 (N-H), 2957 (C-H), 1715 (C=O), 1607 (C=O), 1547, 1488, 1260, 1207, 818; **m.p.** 190–192 °C.

In the Supplementary Information, Figures S1–S8 give NMR spectra, Table S1 gives the results of the antibacterial assay, and Figure S9 gives the standard calibration curve.

4. Conclusions

Annulation of a bicyclic tetramate to generate a multiply fused heterocylic system can be achieved rapidly and in good yield, and this system possesses some antibacterial bioactivity.

Author Contributions

GC designed, conducted and analysed the data from experiments, and MGM analysed the data from experiments and wrote the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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18. Data for compound **2**: **δH** (500 MHz, $CDCl_3$) Major: 0.88 (9H, s, -C(C*H*$_3$)$_3$), 3.46 (1H, d, *J* = 8.8, C-4*H*H'), 3.72 (3H, s, -OC*H*$_3$), 4.78 (1H, d, *J* = 8.8, C-4H*H*'), 4.81 (1H, s, C-2*H*), 7.00–7.05 (2H, m, ArH), 7.46–7.51 (2H, m, ArH), 8.10 (1H, d, *J* = 13.2, C-10*H*), 10.93 (1H, d, *J* = 12.9, -N*H*Ar), Minor: 0.87 (9H, s, -C(C*H*$_3$)$_3$), 3.45 (1H, d, *J* = 8.8, C-4*H*H'), 3.73 (3H, s, -OC*H*$_3$), 4.77 (1H, d, *J* = 8.8, C-4H*H*'), 4.84 (1H, s, C-2*H*), 7.00–7.05 (2H, m, ArH), 7.46–7.51 (2H, m, ArH), 8.02 (1H, s, C-10*H*), 10.82 (1H, br s, -N*H*Ar); **δC** (500 MHz, $CDCl_3$): Major: 24.73 (-C(*C*H$_3$)$_3$), 35.29 (-*C*(CH$_3$)$_3$), 53.23 (-O*C*H$_3$), 68.42 (*C*-4), 78.04 (*C*-5), 98.18 (*C*-2), 99.06 (*C*-7), 119.1 (*C*-13), 120.1 (*C*-15), 133.3 (*C*-14), 136.7 (*C*-12), 146.3 (*C*-10), 168.3 (*C*-9), 177.6 (*C*-8), 189.0 (*C*-6), Minor: 24.77 (-C(*C*H$_3$)$_3$), 35.35 (-*C*(CH$_3$)$_3$), 53.24 (-O*C*H$_3$), 68.32 (*C*-4), 77.29 (*C*-5), 98.28 (*C*-2), 99.05 (*C*-7), 119.3 (*C*-13), 120.3 (*C*-15), 133.3 (*C*-14), 136.7 (*C*-12), 147.0 (*C*-10), 168.3 (*C*-9), 174.2 (*C*-8), 192.5 (*C*-6); ***m/z*** (ESI$^+$) 437.1 ([M + H]$^+$ 100%); **HRMS** (ESI$^+$) found 436.0777, 438.0661 ([M + H]$^+$) requires 436.0634, 438.0616; $\boldsymbol{\nu}_{\mathbf{max}}$ 3214 (N-H), 2959 (C-H), 2871 (C-H), 1747 (C=O), 1708, (C=O) 1619 (C=O), **1474**, *1300*, 1205, 731; **m.p.** 116–117 °C.