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Synthesis of the intermediate for fumimycin: a natural peptide deformylase inhibitor

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Abstract Synthetic efforts toward the potential bacterial peptide deformylase inhibitor fumimycin are reported. The synthetic approach features a tandem Friedel–Crafts alkylation/lactonization access as key reaction to the α, α-disubstituted amino acid unit, and results in the synthesis of an advanced racemic intermediate with an *Z* configuration propenyl group starting from vanillin with 18 % total yield in five steps.

Keywords Fumimycin · Peptide deformylase · Inhibitors · Friedel–Crafts alkylation · Synthesis

Introduction

Bacterium infection is a common and frequently-occurring disease in our daily lives, and various bacterium infections can be a fatal threat to mankind. Despite the past success of antibiotic drug discovery, at least in the industrially developed world, infectious diseases remain the second-leading cause of death. Bacterial infections cause 17 million deaths globally, particularly in children and the elderly [1]. Since penicillin was discovered at the beginning of the twentieth century, the antibiotic family has expanded day by day to become the most effective approach to cure bacterium infection

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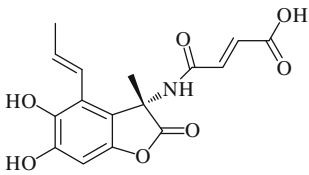
diseases. However, the emergence of bacterial resistance has gradually lessened the effectiveness during long-term clinical use. Especially during the past 10 years, the drug-resistant bacteria has brought lots of difficulties to clinical treatment such as the abuse of antibiotics and the rapid growth of various drug-resistant germs [2]. With the emergence of bacterial resistance to all known classes of antibiotics, the search for new antibiotics with novel modes of action is of utmost urgency.

One novel antibacterial target that has attracted more and more attention lately is the bacterial peptide deformylase (PDF) (EC 3.5.1.31) [3, 4]. PDF is an essential enzyme for bacterial growth that catalyzes the removal of the formyl group at the N-terminus of bacterial proteins, while it is not required in mammalian cells. PDF inhibitors are potentially non-toxic, broad-spectrum and selective mechanism-based antibacterial agents [5, 6]. Although PDF offers a new target for antibacterial agent discovery, only a few PDF inhibitors have been reported so far, and most of them are peptidic [7–11]. In the course of screening for new PDF inhibitors, a novel nonpeptidic metabolite named fumimycin (1, Fig. 1) was isolated from the fermentation broth of *Aspergillus fumisynnematus* F746 by Kim and coworkers [12]. It possesses an unusual skeleton incorporating an aromatic ring fused to a five-membered lactone with an alanine unit of which the amino group is linked to a fumaric acid residue. Fumimycin exhibits potent inhibition on *S. aureus* PDF with an IC_{50} of 4.1 μM and also displays antibacterial activity against methicillin-resistant *S. aureus* (MRSA) and quinolone-resistant *S. aureus* (QRSA). Therefore, fumimycin may represent a lead structure of PDF inhibitors for further structural optimization.

Due to our research interests in discovering new PDF inhibitors, the significant biological properties and the distinctive structural features of fumimycin prompted us to synthesize this compound. The challenge for the synthesis of fumimycin is to establish the stereogenic quaternary carbon center of the α,α-disubstituted amino acid unit. Bräse and coworkers [13, 14] carried out considerable work on the synthesis of fumimycin and its analogue, including racemic synthesis of fumimycin and methoxyfumimycin and enantioselective synthesis of (+)-fumimycin [15], as well as an organocatalytic amination approach to an advanced intermediate [16]; however, the key step in most of these reactions was the 1, 2-addition of a Grignard reagent to ketimine, which makes the reaction steps too long and the reaction conditions too tedious. Here, we report a racemic synthetic effort toward fumimycin and a concise approach to give rise to the benzofuran-2-one skeleton with a α,α-disubstituted amino unit, with commercial vanillin as the starting material, which undergoes allylation, Dakin oxidation, tandem Friedel–Crafts alkylation/lactonization [17, 18], Claisen rearrangement, and olefin isomerization to get the key racemic intermediate, 3-acetylamino-5-hydroxy-6-methoxy-3-methyl-4-[(*Z*)-propenyl]-3*H*-benzofuran-2-one **10**.



(-)Fumimycin (1)

Fig. 1 (−)Fumimycin, a potential lead to antibacterial agents

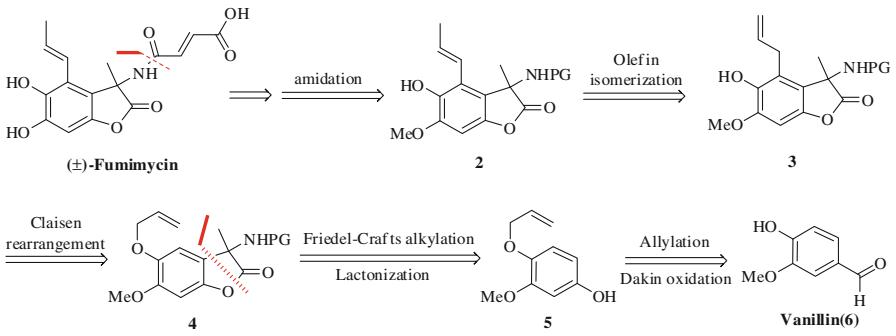
Results and discussion

The retrosynthetic analysis of (±)-fumimycin is shown in Scheme 1. Our retrosynthetic strategy employs a tandem Friedel–Crafts alkylation/lactonization of **5** as the key reaction [17, 18]. Foremost, we hypothesized that (±)-fumimycin could derive from amine **2** through amidation and deprotection; and **2** could be obtained by olefin isomerization from **3**. The Claisen rearrangement precursor for amine **3**, the benzofuran-2-one **4**, can be prepared from phenol **5** through Friedel–Crafts alkylation and subsequent lactonization, respectively, while **5** can be synthesized through Dakin oxidation and allylation by employing the readily available vanillin **6** as starting material.

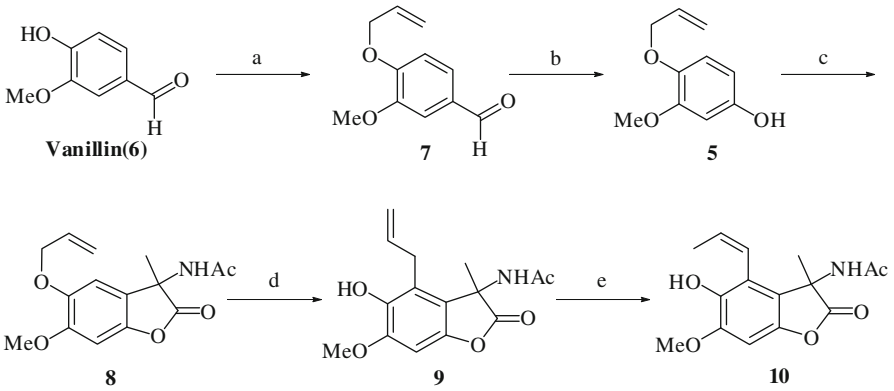
As illustrated in Scheme 2, the route from vanillin **6** to phenol **5** followed the protocol of Bräse [13, 14] with slight modifications. Firstly, vanillin was converted to its allyl ether **7** which underwent a Dakin oxidation to give the phenol **5**. A cascade lactonization followed by the Friedel–Crafts alkylation adduct of **5** with commercially available methyl 2-acetamidoacrylate gave rise to the benzofuranone **8** with 87 % yield. Claisen rearrangement of allyl ether **8** smoothly provided **9**. Isomerization of the terminal double bond was carried out as described by Bräse [13]. Disappointingly, when **9** was heated in the presence of $RhCl_3{\cdot}3H_2O$ in ethanol under 45 °C, only decomposition could be observed. When we increased the reaction temperature to 60 °C, a trace isomer product can be observed. Under refluxing after 4 h, the olefin isomerization surprisingly gave the *Z* configuration product **10**, exclusively evident from the $^3J_{HH}$ coupling constant of the double bond ($^3J_{HH} = 11.2$ Hz).

Conclusions

In summary, a tandem Friedel–Crafts alkylation/lactonization was used as the key reaction step to establish the α-arylalanine skeleton of fumimycin, which gave a yield as high as 87 %. The racemic advanced intermediate, 3-acetylamino-5-hydroxy-6-methoxy-3-methyl-4-[(*Z*)-propenyl]-3*H*-benzofuran-2-one, was synthesized with 18 % total yield in five steps with vanillin as the starting material.



Scheme 1 The retrosynthetic analysis of (±)fumimycin



Scheme 2 Reagents and conditions: **a** K_2CO3, allylbromide, acetone, reflux, 6 h, 98 %; **b** H_2O_2, H_2SO_4, $B(OH)_3$, THF, r.t., 5 h, 92 %; **c** methyl 2-acetamidoacrylate, $BF_3{\cdot}Et_2O$, THF, r.t., 2 d, 85 %; **d** DMF, 170 °C, 10 h, 84 %; **e** $RhCl_3{\cdot}3H_2O$, EtOH, reflux, 4 h, 28 %

Experimental section

General reagents and all solvents were analytically pure grade and were used without further purification. Column chromatography (CC) was performed on silica gel (200–300 mesh). Analytical thin layer chromatography (TLC) was performed on pre-coated silica gel GF_{254} plates (Qingdao Haiyang Chemical, Qingdao, PR of China). Visualization on TLC was achieved by the use of UV light (254 nm) and treatment with I_2. ^1H (600 MHz) and ^{13}C (150 MHz) NMR spectra were recorded on a Bruker AVANCE III 600 MHz spectrometer with $CDCl_3$ as solvent and TMS as internal standard. Chemical shifts are reported in ppm relative to the internal reference. ESIMS were obtained on a Waters ZQ4000/2695 HPLC–MS. HRMS were measured on a Finnigan MAT95 mass spectrometer or a VG ZAB-HS spectrometer.

4-Allyloxy-3-methoxybenzaldehyde (**7**) [13, 14]

To a suspension of vanillin **6** (2.87 g, 18.86 mmol) and K_2CO_3 (3.64 g, 26.4 mmol) in acetone (28 mL), allylbromide (2.1 mL, 24.51 mmol) was added. The mixture was heated to reflux for 6 h. After filtration, the filtrate was concentrated under reduced pressure. The crude product was purified by chromatography on silica gel (PE:AcOEt = 10:1) to afford the allylether **7** as brown oil (3.55 g, 98 %).

4-Allyloxy-3-methoxyphenol (**5**) [13, 14]

Boric acid (5.72 g, 92.3 mmol) was suspended in THF (50 mL), H_2O_2 (30 % in H_2O, 6 mL), and H_2SO_4 (2.66 mL, 98 %). After stirring for 30 min, **7** (3.55 g, 18.5 mmol) was added as solution in THF (20 mL) within 15 min. After additional stirring for 5 h, the mixture was filtered. The filtrate was neutralized by addition of sat. $NaHCO_3$ solution; the aqueous layer was extracted with EtOAc (3 × 90 mL).

The combined organic extracts were washed with brine (50 mL), dried over Na_2SO_4, and concentrated under reduced pressure. Flash chromatography (PE:AcOEt = 4:1) afforded the phenol **5** as brown oil (2.998 g, 92 %). ^{1}H NMR δ ($CDCl_3$, ppm): 3.68 (s, 3H), 4.49 (d, J = 6 Hz, 2H), 5.21 (dd, J = 10.2, 1.2 Hz, 1H), 5.32 (dd, J = 17.4, 1.2 Hz, 1H), 5.99–6.05 (m, 1H), 6.34 (dd, J = 8.4, 2.4 Hz, 1H), 6.46 (d, J = 2.4 Hz, 1H), 6.72 (d, J = 8.4 Hz, 1H), 7.20 (brs, 1H).

5-Allyloxy-3-acetylamino-6-methoxy-3-methyl-3*H*-benzofuran-2-one (**8**)

An amount of 1.58 mL $BF_3{\cdot}Et_2O$ was added at room temperature to a solution of methyl 2-acetamidoacrylate (429.4 mg, 3 mmol) in 10 mL dried THF. After 30 min of stirring, **5** (434 mg, 2.4 mmol) was added and the reaction mixture was stirred for 2 days. Work-up began by quenching with $Na_2CO_3{\cdot}10H_2O$ followed by filtration, evaporation of the solvent and column chromatography (PE:EtOAc = 1:1) to obtain the lactone **8** as a white solid (595 mg, 85 %). ^{1}H NMR δ ($CDCl_3$, ppm): 1.60 (s, 3H), 1.95 (s, 3H), 3.87 (s, 3H), 4.54–4.56 (m, 2H), 5.28 (dd, J = 10.2, 1.2 Hz, 1H), 5.39 (dd, J = 17.4, 1.2 Hz, 1H), 6.04–6.09 (m, 1H), 6.43 (s, 1H), 6.75 (s, 1H), 6.78 (s, 1H). ^{13}C NMR δ ($CDCl_3$, ppm): 176.4, 169.2, 151.3, 147.7, 145.3, 133.3, 119.5, 118.1, 108.9, 96.6, 71.2, 57.3, 56.3, 24.3, 22.3. (+)ESI–MS (MeOH): *m/z* 313.7 $[M + Na]^+$, 323.8 $[M + MeOH + H]^+$, 345.8 $[M + MeOH + Na]^+$; (−)ESI–MS (MeOH): *m/z* 289.7 $[M–H]^-$, 321.8 $[M + MeOH–H]^-$. HRMS (ESI): *m/z* $[M + Na]^+$ Calcd for C15H17NO5: 314.0999; found: 314.1011.

4-Allyl-3-acetylamino-5-hydroxy-6-methoxy-3-methyl-3*H*-benzofuran-2-one (**9**)

A solution of **8** (1.018 g, 3.5 mmol) in DMF (80 mL) was heated at 170 °C to reflux for 10 h. The solvent was removed under reduced pressure, and 20 ml water was added to the residue. The mixture was extracted with EtOAc (3× 50 mL). The organic phase was washed with saturated brine, dried over Na_2SO_4 and concentrated under reduced pressure. Flash chromatography (PE:EtOAc = 1:2) afforded **9** as a white solid (852 mg, 84 %). ^{1}H NMR δ ($CDCl_3$, ppm): 1.67 (s, 3H), 1.93 (s, 3H), 3.38 (dd, J = 15.6, 5.4 Hz, 1H), 3.50 (dd, J = 15.6, 5.4 Hz, 1H), 3.90 (s, 3H), 4.90 (dd, J = 17.4, 1.8 Hz, 1H), 5.02 (dd, J = 10.2, 1.8 Hz, 1H), 5.48 (brs, 1H), 5.94–5.99 (m, 1H), 6.14 (s, 1H), 6.66 (s, 1H). ^{13}C NMR δ ($CDCl_3$, ppm): 176.4, 169.2, 146.9, 146.3, 140.7, 135.4, 121.3, 118.4, 115.3, 94.0, 57.8, 56.4, 29.6, 23.9, 22.2. (+)ESI–MS (MeOH): *m/z* 313.8 $[M + Na]^+$, 329.9 $[M + K]^+$, 345.8 $[M + MeOH + Na]^+$, 605.0 $[2M + Na]^+$; (−)ESI–MS (MeOH): *m/z* 289.7 $[M–H]^-$. HRMS (ESI): *m/z* $[2M + Na]^+$ Calcd for $C_{30}H_{34}N_2O_{10}Na$: 605.2111; found: 605.2110.

3-Acetylamino-5-hydroxy-6-methoxy-3-methyl-4-[(*Z*)-propenyl]-3*H*-benzofuran-2-one (**10**)

Under N_2 a mixture of **9** (130 mg, 0.45 mmol) and $RhCl_3{\cdot}3H_2O$ (11 mg, 40 μmol) in EtOH (10 mL) was heated to reflux. After 4 h, the reaction was allowed to cool to r.t. and filtered through a 3-cm pad of Celite. The fitrate was concentrated under

reduced pressure. Flash chromatography (PE:EtOAc = 1:2) afforded the white solid **10** (36.4 mg, 28 %). ^{1}H NMR δ ($CDCl_3$, ppm): 1.59 (d, J = 11.2 Hz, 3H), 1.62 (s, 3H), 1.96 (s, 3H), 3.91 (s, 3H), 5.51 (s, 1H), 6.01–6.09 (m, 2H), 6.22 (d, J = 11.2 Hz, 1H), 6.85 (s, 1H). ^{13}C NMR δ ($CDCl_3$, ppm): 176.2, 168.9, 147.3, 146.2, 139.4, 132.6, 119.9, 119.6, 117.7, 94.5, 70.6, 57.6, 56.4, 26.5, 22.6, 22.1, 15.1. (+)ESI–MS (MeOH): *m/z* 313.8 $[M + Na]^+$, 345.8 $[M + MeOH + Na]^+$; (−)ESI–MS (MeOH): *m/z* 289.7 $[M–H]^-$. HRMS (ESI): *m/z* $[M–H]^-$ Calcd for C15H17NO5: 290.1034; found: 290.1032.

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