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A facile synthesis, antibacterial activity of pulvinone and its derivatives

Hai-Wei Xu *, Chao Xu, Zi-qi Fan, Ling-Jie Zhao, Hong-Min Liu *

New Drug Research & Development Center, School of Pharmaceutical Sciences, Zhengzhou University, No. 100, KeXue DaDao, Zhengzhou 450001, China

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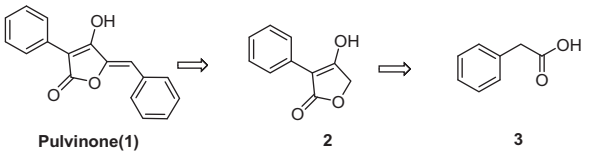
ABSTRACT

Pulvinone and several 3-fluoro-4-morpholino substituted pulvinone derivatives were synthesized in five steps from a common precursor, phenyl acetic acid. Most of synthetic morpholine substituted pulvinones showed inhibitory activity against *Escherichia coli*. For the first time, the inhibition of pulvinone and its derivatives against Gram-negative bacteria was reported.

Pulvinone (**1**) was 5-benzylidene-4-hydroxy-3-phenyl-5-furan-2(5*H*)-one isolated from several fungi, including boletus *Suillus grevillei*[1] and mold *Aspergillus terreus*.[2] Natural or synthetic compounds of this class display interesting biological activities, such as anticoagulant[3] or anti-inflammatory[4] properties. Inhibitors of bacterial cell wall biosynthesis have been recently reported.[5] It was first obtained as a by-product in the synthesis of pulvinic acid by Volhard. Several syntheses were then reported.[6–11]

As for us, we focused on an adaptation of our previously described facile synthesis of γ-AIBs.[12] According to this strategy, pulvinone would then be accessible from the 4-hydroxy-3-phenyl-5-furan-2(5*H*)-one (**2**) obtained from a phenyl acetic acid (**3**). The retrosynthetic analysis is summarized in the Scheme 1. In connection with previous studies concerning the synthesis and bioactivities of butenolide, we became interested in an efficient synthetic access to pulvinones and its antibacterial activities. Herein we wish to present the implementation of this scheme, which led to the synthesis of several new pulvinone derivatives having 3-fluoro-4-morpholino group which was the pharmacophores of antibacterial drug Linezolid (**9**), and investigated the antibacterial activities.



Scheme 1. Planned synthesis of pulvinones.

The intermediate, 4-hydroxy-3-phenyl-5-furan-2(5*H*)-one (**2**) was easily prepared by the reaction of ethyl chloroacetate and phenyl acetic acid (**3**). But pulvinone (**1**) could not be obtained by the vinylogous adol reaction of **2** and benzaldehyde, which may be due to the effects of the hydroxyl at C-4 of compound **2**. Accordingly, 4-methoxyl-3-phenyl-5-furan-2(5*H*)-one (**4**) was prepared by the reaction of **2** and the dimethyl sulfate. 5-(hydroxyl(phenyl)-methyl)-4-methoxy-3-phenylfuran-2(5*H*)-one (**5**) instead of **6** was obtained by the vinylogous adol reaction of **4** and benzaldehyde. This may result from a stable hydrogen bonds between the 4-methoxy and newly generated hydroxyl groups, which affected the elimination reaction of the hydroxyl. The 5-benzylidene-4-methoxy-3-phenylfuran-2(5*H*)-one (**6**) was realized by the dehydration reaction of 5-(hydroxyl(phenyl)methyl)-4-methoxy-3-phenylfuran-2(5*H*)-one (**5**) in the presence of toluene sulfonyl chloride. Pulvinone (**1**) could be obtained from compound **5** which was treated using the LiBr (Scheme 2).[13–17] Pulvinone (**1**) was isolated as *Z*-isomer with the yield of 85.6% by evaporation crystallization from ethyl acetate. The spectral characteristic was in agreement with that reported of the literature. The methenic proton of the *Z*-isomer appear at δ 6.67 ppm in $CO(CD_3)$.

Following the above results, we embarked in the preparations of several pulvinone derivatives (**7** and **8**) containing the moiety of 3-*F*-4-morpholino which was the pharmacophore of antibacterial drugs Linezolid (**9**) (Fig. 1, Table 1). Conditions similar to those described for the preparation of **1** were then applied to the synthesis of several pulvinone derivatives (**7**, **8**).[18,19] Compounds **7** and **8** were purified by precipitation or by silica gel chromatography, and isolated as mixture isomers in 56–85% yield. In the mixture isomers of **8**, the *Z*-isomer was the predominant. The results were summarized in Table 1.

Antibacterial activities of synthetic compounds were evaluated versus Gram-positive bacteria, *Staphyloccocus aureus* and

* Corresponding authors. Tel.: +86 371 65959833 (H.-W. X.); tel.: +86 371 67781739 (H.-M.L.).
E-mail addresses: xhwei01@126.com (H.-W. Xu), liuhm@zzu.edu.cn (H.-M. Liu).

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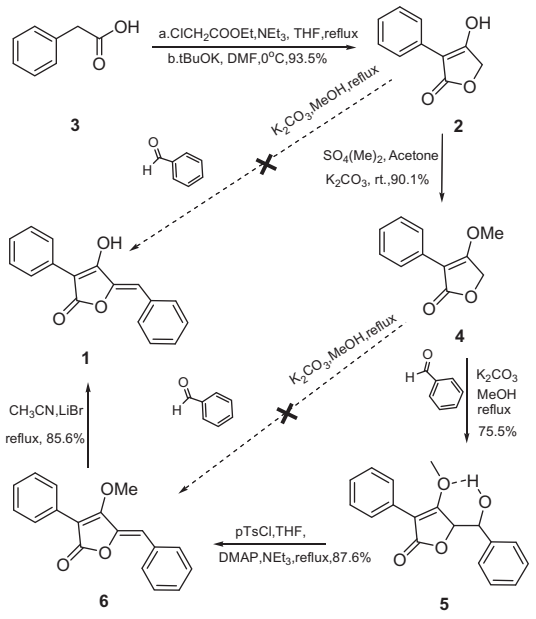

Scheme 2. Synthesis of pulvinone.

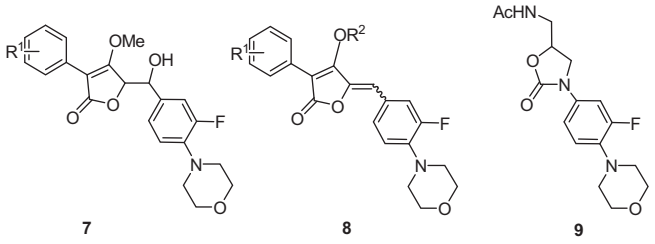

Figure 1. Structures of pulvinone derivatives (**7**, **8**) and Linezolide (**9**).

Table 1
Structures of synthetic compounds

Compd	R^1	R^2	Z/E ration[a]	Yield (%)
7a	4-F	—	—	65.3
7b	4-Cl	—	—	59.4
7c	4-Br	—	—	76.8
7d	4-Me	—	—	61.8
7e	3-F	—	—	56.7
7f	H	—	—	60.5
8a	4-F	CH_3	0.66/0.33	85.3
8b	4-Cl	CH_3	0.61/0.39	82.4
8c	H	CH_3	0.85/0.15	74.6
8d	4-F	H	0.71/0.23	75.9

[a] The ration was identified according to ^{1}H NMR.

Gram-negative bacteria, *Esherichia coli*, *Pseudonmonas aeruginosa*, *Stenotrophomonas maltophilia*, *Acinetobacter baumannii* by microdilution broth method.[20] The results suggested that most of them except for pulvinone with inhibition of 59.2% in 50 μg/mL against *Staphyloccocus aureus* showed less inhibitory activities against Gram-positive bacteria, *Staphyloccocus aureus* and Gram-negative bacteria, *Pseudonmonas aeruginosa, Stenotrophomonas maltophilia, Acinetobacter baumannii*. But they showed good inhibitory activity against Gram-negative bacteria, *Esherichia coli*, with the MIC_{50} of 1.01–36.47 μg/ml (Table 2). The results seemed to be agreement with the literature data that showed pulvinoes good Gram-negative enzyme, MurA-D from *Esherichia coli*, inhibitiory activity.[5a]

Table 2
Antibacterial activities of synthetic compounds

Compd	Bacteria				
	E.c[a]	P.a[b]	S.m[c]	A.b[d]	S.a[e]
1	(1.38)[f]	(>50)	(12.79)	(>50)	59.2
5	61.7 [g] (5.86)	53.2	18.0	25.9	—[h]
6	60.2 (3.91)	45.4	19.7	18.2	—
7a	42.0 (>50)	35.2	31.9	22.8	—
7b	61.8 (30.26)	31.7	/[i]	22.1	—
7c	45.5 (1.01)	35.6	(11.85)	32.9	—
7d	58.3 (3.67)	31.8	1.1	4.9	—
7e	72.0 (4.09)	38.2	41.5	30.9	—
7f	52.4 (36.47)	34.8	17.1	29.2	—
8a	37.6 (>50)	23.4	59.4 (20.64)	20.1	—
8b	70.7 (7.40)	45.3	/	/	—
8c	45.4 (>50)	17.6	/	10.0	—
8d	56.8 (15.6)	23.2	27.8	15.6	—
OFL[j]	(<0.25)	(<0.25)	(<0.25)	(<0.25)	(<0.25)

[a] E.c: *Esherichia coli*.
[b] P.a: *Pseudomonas aeruginosa*.
[c] S.m: *Stenotrophomonas maltophilia*.
[d] A.b: *Acinetobacter baumannii*.
[e] S.a: *Staphyloccocus aureus*.
[f] Values in parentheses are MIC_{50} (μg/ml).
[g] Percentage inhibition (%) in 50 μg/ml.
[h] No inhibition in 50 μg/ml.
[i] No detection.
[j] OFL: levofloxacin, positive control drug.

Among the synthetic derivatives, compound **8d** containing 4-OH and 5-idene substituted butenolide showed better inhibitory activity against *Esherichia coli* than that of compounds **7a** and **8a** with MIC_{50} of >50 μg/ml. Compounds **1**, **5**, **6** and compounds **7b**, **8b** had the similar SAR. The results showed that the 4-hydroxyl and 5-idene were beneficial for the bioactivity. Compound **7c** substituted by bomide (MIC_{50}, 1.01 μg/ml) showed comparable inhibition against *Esherichia coli* with pulvione (MIC_{50}, 1.38 μg/ml), and which showed better bioactivity than compounds **7a**, **7b**, **7e** containing other halogens. Compound **7d** containing 4-methyl phenyl substituted butenolide showed good inhibitory activity against *Esherichia coli* with MIC_{50} of 3.67 μg/ml. Besides, pulvinone(**1**), compound **7c** and **8a** showed inhibitory activity against *Stenotrophomonas maltophilia* with MIC_{50} of 12.79, 11.85 and 20.64 μg/ml respectively.

To summary, a facile synthesis of pulvinone was reported, which was used to the synthesis of morpholine substituted pulvinone derivatives. Most of synthetic morpholine substituted pulvinones showed inhibitory activity against *Esherichia coli*. But the inhibitory activity was not enough good.

Acknowledgment

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13. *Preparation of 4-hydroxy-3-phenyl-5-furan-2(5H)-one (**2**).* To a stirred solution of 4-fluorophenylacetic acid (4.08 g, 0.03 mol) in THF (20 ml) was added triethylamine (4.55 g, 0.045 mol) and ethyl chloroacetate (5.51 g, 0.045 mol). The mixture was refluxed for 8 h. After the reaction was complete (monitored by TLC), the reaction mixture was cooled to room temperature and filtered through a sintered funnel. Then the filter cake was washed with THF (3 × 10 ml). The solvent was removed under reduced pressure and yellow oil was obtained. To a cooled (0 °C) solution of this yellow oil obtained above in DMF (20 ml) was added potassium *t*-butoxide (6.73 g, 0.06 mol) slowly and the solution was stirred for 30 min. Then the reaction mixture was warmed to room temperature and stirred at the same temperature for 5 h. After the reaction was complete (monitored by TLC), it was diluted by addition of water (20 ml) and its pH was adjusted to 3 by addition of diluted hydrochloric acid. Finally, a great amount of white solid precipitated from the solution after addition of water (150 ml). The mixture was filtered and the filter cake was washed with H_2O (3 × 10 ml). **2** (4.93 g, 93.5%) was obtained as a white solid.
14. *Preparation of 4-methoxyl-3-phenyl-5-furan-2(5H)-one (**4**).* To a stirred solution of **2** (3.52 g, 0.02 mol) in acetone (25 ml) was added potassium carbonate (3.86 g, 0.028 mol). Dimethyl sulphate (3.02 g, 0.024 mol) in acetone (5 ml) was added dropwise over a period of 30 min under N_2 atmosphere. The mixture was stirred at room temperature for 8–10 h. After the reaction was complete (monitored by TLC), H_2O (30 ml) was added and its pH was adjusted to 3 by addition of diluted hydrochloric acid. The solvent was removed under reduced pressure. The mixture was filtered and **4** (3.29 g, 90.1%) was obtained as a slightly yellow solid.
15. *Preparation of 5-(hydroxyl(phenyl)methyl)-4-methoxy-3-phenylfuran-2(5H)-one (**5**).* To a stirred solution of **4** (3.80 g, 0.02 mol) in methanol (25 ml) was added potassium carbonate (3.86 g, 0.028 mol) and benzaldehyde (2.54 g, 0.024 mol). The mixture was stirred at room temperature. After the reaction was complete (monitored by TLC), the solvent was removed under reduced pressure. H_2O (15 ml) was added and its pH was adjusted to 3 by addition of diluted hydrochloric acid. The reaction mixture was extracted with EtOAc (3 × 10 ml) and the combined organic extracts dried (Na_2SO_4), filtered and the solvent removed in vacuo. The residue obtained was purified by silica gel column chromatography with petroleum ether: EtOAc (2:1) as an eluent to give **5** (4.47 g, 75.5%) as a white solid
16. *Preparation of 5-benzylidene-4-methoxy-3-phenylfuran-2(5H)-one (**6**).* To a cooled (0 °C) solution of **5** (1.48, 0.005 mol) in dry THF (15 ml) was added paratoluensulfonyl chloride (1.91 g, 0.01 mol) and DMAP (0.183 g, 0.0015 mol). Triethylamine (0.506 g, 0.005 mol) was introduced into the reaction mixture at the same temperature. Then the mixture was allowed to stir for 20 min. The reaction mixture was warmed to room temperature and stirred for 2 h. Then the mixture was heated at 90 °C for 10 h. After the reaction was complete (monitored by TLC), the solvent was removed under reduced pressure and H_2O (15 ml) was added. The reaction mixture was extracted with EtOAc (3 × 10 ml) and the organic extracts were washed with water (3 × 10 mL) and brine (3 × 10 mL) respectively. The combined organic extracts dried (Na_2SO_4), filtered and the solvent removed in vacuo. **6** (1.22 g, 87.6%) was obtained as a yellow solid.
17. *Preparation of pulvinone (**1**).* To a stirred solution of **6** (1.39 g, 0.005 mol) in acetonitrile (10 ml) was added lithium bromide (646 mg, 0.005 mol). The mixture was heated at 120 °C. After the reaction was complete (monitored by TLC) the solvent was removed under reduced pressure and H_2O (10 ml) was added. The reaction mixture was extracted with dichloromethane (3 × 10 ml) and the combined organic extracts dried (Na_2SO_4), filtered and the solvent removed in vacuo. The residue obtained was purified by silica gel column chromatography with petroleum ether: EtOAc (3:1) as an eluent to give **1** (1.13 g, 85.6%) as a yellow solid. ^{1}H NMR (400 MHz, Acetone-d_6) δ: 8.32 (d, *J* = 7.6 Hz, 2H, ArH), 7.78 (d, *J* = 7.6 Hz, 2H, ArH), 7.39 (q, *J* = 7.2 Hz, 4H, ArH), 7.33–7.27 (m, 1H, ArH), 7.20 (t, *J* = 7.3 Hz, 1H, ArH), 6.87 (s, 1H, CH). ^{13}C NMR (100 MHz, Acetone-d_6) δ: 171.39, 169.90, 145.51, 134.26, 132.69, 130.06, 128.56, 127.82, 127.77, 127.61, 126.87, 126.46, 125.39, 105.29, 96.82.
18. *NMR data of **7a**.* ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.45–7.33 (m, 2H, ArH), 7.26–7.15 (m, 2H, ArH), 7.15–7.03 (m, 2H, ArH), 6.95 (t, *J* = 8.7 Hz, 1H, ArH), 5.05 (dd, *J* = 7.0, 2.4 Hz, 1H, CH), 5.01 (d, *J* = 2.5 Hz, 1H, CH), 3.93–3.86 (m, 4H, CH_2), 3.81 (s, 3H, OCH_3), 3.17–3.05 (m, 4H, CH_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 172.41, 171.73, 163.85, 161.38, 156.65, 154.19, 140.20, 140.12, 133.53, 133.47, 131.96, 131.88, 125.27, 125.24, 122.85, 118.56, 118.53, 115.41, 114.86, 114.64, 104.97, 79.78, 71.85, 66.92, 60.64, 50.77, 50.74.
19. *NMR data of **8a**.* ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.67–7.58 (m, 4H, ArH), 7.53 (dd, *J* = 8.7, 5.4 Hz, 2H, ArH), 7.15 (t, *J* = 8.6 Hz, 1H, ArH), 6.25 (s, 1H, CH), 3.93–3.88 (m, 4H, CH_2), 3.87 (s, 3H, OCH_3), 3.19 (m, 4H, CH_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ:166.25, 165.52, 156.15, 144.87, 143.99, 131.32, 131.24, 130.88, 130.80, 127.94, 127.19, 118.45, 118.26, 118.22, 118.03, 117.85, 115.94, 115.73, 110.61, 66.85, 66.80, 50.55, 50.52, 50.34, 50.30.
20. *General procedure for antimicrobial activity*. Antibacterial activity was determined using the broth microdilution method according to the National Committee for Clinical Laboratory Standards (NCCLS). Bacterial species were grown in Luria broth medium (RPMI 1640 medium for fungi) until exponential growth was achieved. Tests were performed in a 96-well microtiter plate. All the compounds were dissolved in dimethyl sulfoxide (DMSO) at an initial concentration of 5120 μg/ml and the solutions were diluted with the test medium. A series of concentrations ranging from 1 to 128 μg/ml to a final volume of 200 μl in plate was obtained by two-fold dilutions. Each well except for the blank well was inoculated with the test bacteria and incubated at 37 °C for 24 h. The plates were read using ELIASA at OD_{492}. The MIC_{50} is the lowest concentration of compound that inhibits growth to half the OD of the non-treated control.