Synthesis and Anti-bacterial Properties of Mono-carbonyl Analogues of Curcumin

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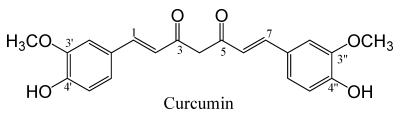
The synthesis of three series of curcumin analogues with mono-carbonyl is described. Their *in vitro* anti-bacterial activities against seven Gram-positive and Gram-negative bacteria were tested and the effect of substituents on the aryl ring and the space structure of the linking strain were discussed. It was observed that part of the derivatives displayed significant activity when compared with curcumin and most of them exhibited activity against the ampicillin-resisted *Enterobacter cloacae*. Compounds A12, B09, B13, B14 and C09 show remarkable antibacterial activity *in vitro*. The result showed that heterocycle or long-chain substituents may enhance the activity of curcumin analogues.

Key words curcumin; analogue; anti-bacterial activity; substituent effect; *Enterobacter cloacae*

Curcumin [1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, Fig. 1], a compound isolated from *Curcuma longa* L., has been used for centuries as dietary pigment and spice. Curcumin has been found to possess a variety of traditional pharmaceutical applications on diseases, including external/internal wounds, liver diseases (particularly jaundice), blood purification, microbial effects and inflamed joints.[1—4] Over a period of research, curcumin was reported to inhibit carcinogen-induced mutations and the formation of tumour in several experimental systems,[5—7] and exhibit anti-proliferation capability as a potent tool in cancer therapy.[8,9] Curcumin has also been reported to inhibit bacterial lipopolysaccharide-induced TNF-α overexpression and the transcription factor nuclear factor kappa B (NF-κB) activation which are involved in several pathogen-infected diseases.[2,10]

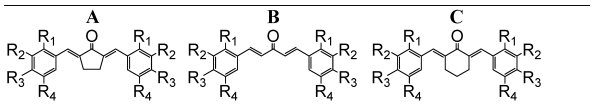
Preclinical and clinic studies showed that, however, curcumin possesses several disadvantages in pharmacokinetics such as poor bioavailability, fast metabolism and requiring repetitive oral doses,[11,12] which limited its applications. However, curcumin is still an excellent lead compound for drug design and development on the basis of the explicit bioactivities, non-toxicity and easy synthesis.[13—16] Curcumin is stable at a pH below 6.5. The instability of curcumin at a pH above 6.5 is caused by the methylene group.[17] Omitting the methylene group and one carbonyl group, B. M. Markaverich,[18] M. Artico,[19] and H. I. El-Subbagh[20] synthesized series of mono-carbonyl curcumin analogues, 1,5-diaryl-1,4-pentadiene-3-ones, and evaluated their bioactivity. The result that the mono-carbonyl analogues exhibit more powerful inhibition in a variety of cancer cells than curcumin indicated that the central methylene group which had been considered the main active group of curcuminoids in anti-tumor property may be of decreasing importance.

Therefore, in the present paper three series of mono-carbonyl curcumin analogues without the central methylene functional groups, 1,5-diaryl-1,4-pentadiene-3-ones (B), together with cyclopentanone (A) and cyclohexanone (C) analogues (Fig. 2), were prepared and their anti-bacterial properties *in vitro* were evaluated and compared using seven multidrug-resistant bacteria specially causing secondary infections in human being.[21] These compounds were also designed to examine the role of different substitutes in the benzene ring and the influence of the space structure of the linking C-strain. It is hoped that continued research will lead to development of new lead compounds from curcumin as anti-bacterial agents and extrapolated agents for bacteria-infected diseases.



Curcumin

Fig. 1. Chemical Structure of Curcumin



	R_1	R_2	R_3	R_4	
1	H	H	OCH_2CH_3	H	
2	H	H	F	H	
3	H	OCH_3	OCH_3	OCH_3	**A09**
4	H	H	$N(CH_3)_2$	H	
6	Br	H	H	H	
7	H	H		H	**B09**
8	F	CF_3	H	H	
10	H	Br	H	H	**C09**
11	H	OCH_3		H	
12	H	H		H	
13	H	H	OH	H	**A15**
14	H	OCH_3	OH	H	
16	H	H	$OCH_2CH{=}CH_2$	H	
17	H	OCH_3	$OCH_2CH{=}CH_2$	H	**B15**

Fig. 2. Structure of Mono-carbonyl Analogues of Curcumin

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Results and Discussion

Chemistry The structure and general synthesis of analogues designed are shown in Fig. 2 and Chart 1. Most of three classes of compounds (**A**, **B**, **C**) were synthesized by coupling the appropriate aromatic aldehyde with cyclohexanone, acetone or cyclopentanone in an alkaline medium, respectively.[22] Reaction routes, yields, melting points and spectroscopic analysis such as NMR and mass spectroscopy are shown in Experiment Part. All reaction were carried out with a rate 2 : 1 of substituted arylaldehydes and ketones. The ^{1}H-NMR evidences that there did not appear the peaks of methyl adjacent to the carbonyl group in acetone analogues (or methylene peaks in cyclohexanone and cyclopentanone analogues) confirmed the absence of compounds substituted by single aryl ring.

For the synthesis of **13** and **14** analogues in alkaline condition, the use of 4-hydroxybenzaldehyde and 3-methoxy-4-hydroxybenzaldehyde required preventive protection of the hydroxyl groups, preferably with easily removable groups such as tetrahydropyran-2-yl.[19] The protected 4-(tetrahydropyran-2-yloxy)benzaldehyde, therefore, was obtained by reacting 4-hydroxybenzaldehyde with 3,4-dihydro-α-pyran in the presence of pyridinium *p*-toluenesulfonate (illustrated in Chart 2 as a representative example of the general procedure). To couple the protected derivatives with acetone, cyclopentanone and cyclohexanone, respectively gave compounds **07** and **11**. Hydroxylated analogues **13** and **14** were then obtained by hydrolization with a catalytic amount of *p*-toluenesulfonic acid.

Compound **09** was prepared by coupling 5-bromofuran-2-aldehyde with three ketones respectively in alkaline medium, so did compound **15**.

Biological Activity All compounds were evaluated and compared with curcumin for anti-bacterial activities against multidrug-resistant bacteria specially causing secondary infections in human being, Gram-positive pathogens *viz.* *Staphylococcus aureus*, *Micrococcus luteus*, *Staphylococcus saprophyticus* and *Staphylococcus epidermidis*, and Gram-negative pathogens *viz.* *Enterobacter cloacae*, *Enterococus* sp. and *Escherichia coli* were selected. Standard anti-bacterial agent, ampicillin, was evaluated under identical conditions for comparison. Preliminary susceptibility test *in vitro* was done in serially diluted concentration 10, 5, 2.5 mM by the method of testing zone of inhibition according to the literature.[21,23] Each test was performed in triplicate and the final results have been tabulated (Table 1). It has been observed that part of the analogues exhibited interesting biological activity in different degree.

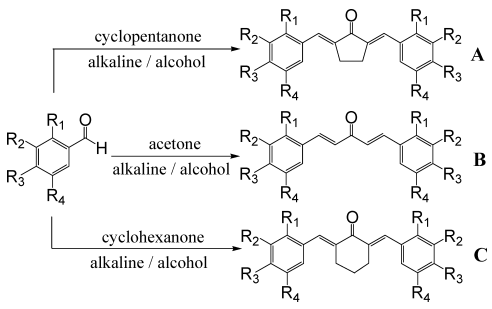

Chart 1. General Synthesis of 1,5-Diphenyl-1,4-pentadiene-3-ones and Cyclic Analogues

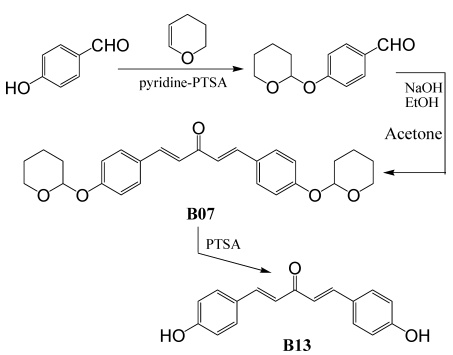

Chart 2. Synthesis of 1,5-Bis(4-hydroxyphenyl)penta-1,4-dien-3-one

As can be seen in Table 1, although not as active as the positive ampicillin, some compounds were generally found to be more active than the leading compound curcumin. Among the heterocycle substituted compounds, **B09** and **C09** showed good activities against all seven organisms, especially **C09** against *S. saprophyticus* that appeared the largest zone of inhibition (21 mm) in our current experiments and was equal to ampicillin. **B09** against *S. aureus* and *S. saprophyticus* as well as **C09** against *S. aureus* and *Micrococcus luteus* also showed high activities. These indicated that the heterocycle (furan) substitution may enhance the bioactivities of curcumin analogues and moreover the acetone (**B09**) and cyclohexanone analogues (**C09**) are more active than cyclopentanone analogues (**A09**).

The compound **A12**, a novel compound with a long chain substituent of 3-(dimethylamino)propoxy, possessed excellent *in vitro* activity against all of 7 target pathogens. However, the dimethylamino substituted compounds (**A04**, **B04**, **C04**) showed weak activity, indicating the long and flexible chain out of the conjugation structure in **A12** may play a important role in exhibiting bioactivity.

Among the six compounds with the phenolic groups (**A13**, **A14**, **B13**, **B14**, **C13**, **C14**), 1,4-pentadiene-3-one analogues **B13** and **B14** exhibited high activities against four Gram-positive bacterial strains and moderate activity against three Gram-negatives. The cyclopentanone (**A14**) and cyclohexanone (**C14**) analogues showed moderate activity and **A13** and **C13**, however, had low activities, which illustrated that the space structure of the linking C-strain can make some influence on the anti-bacterial ability.

Active compounds here were more effective against Gram-positive organisms than against Gram-negatives. But the most encouraging result was that most of compounds exhibit activities against the Gram-negative *E. cloacae* that is completely resistant to the clinical drug ampicillin, which indicated that there may be a different mechanism of their antimicrobial action. So, it is possible to develop a novel agent against nosopoietic *E. cloacae* from mono-carbonyl curcumin analogues.

Although these compounds that possessed different substituents showed varying degrees of inhibition, among the three serial analogues designed, **B** compounds were more effective than the **A** and **C** compounds. Thus the nature and position of the substituent and the space of the linking strain have strong influence on the spectrum and extent of anti-bacterial activities.

Table 1. Anti-bacterial Activity of Curcumin Analogues against Bacterial Strains in Different Dilutions (10, 5, 2.5 mM)

Name of bacteria	Gram-positive				Gram-negative		
	S. aureus	*Micrococcus*	*S. saprophyticus*	*S. epidermidis*	*E. cloacae*	*Enterococus*	*E. coli*
A01	—	14, –, –	—	—	10, –, –	—	—
A02	—	—	—	—	10, 9, –	—	9, –, –
A03	—	10, –, –	—	—	10, –, –	9, –, –	—
A04	—	12, 12, –	—	—	9, –, –	—	—
A06	—	—	—	—	10, 9, –	—	—
A07	—	—	—	—	13, 9, –	—	—
A08	—	—	—	—	11, –, –	—	—
A09	12, 9, –	10, –, –	10, –, –	9, –, –	10, 9, –	—	—
A10	—	12, 10, –	—	—	10, –, –	—	—
A11	—	—	—	—	11, –, –	—	—
A12	20, 15, 13	17, 17, 14	17, 14, 12	18, 16, 14	13, 12, 10	17, 15, 14	14, 14, 13
A13	—	12, –, –	—	—	10, –, –	—	—
A14	12, –, –	12, –, –	10, –, –	10, –, –	11, 10, 9	—	—
A15	12, 9, –	14, –, –	—	10, 8, –	12, –, –	—	—
A16	—	—	—	—	10, –, –	—	—
A17	—	—	—	—	11, 10, –	—	—
B01	—	—	—	—	9, –, –	—	10, 9, –
B02	10, 8, –	11, –, –	10, –, –	11, 10, –	13, –, –	—	10, 9, –
B03	14, –, –	10, –, –	—	—	11, –, –	—	—
B04	—	—	—	—	10, –, –	—	—
B06	—	10, 9, –	—	15, –, –	12, 11, 10	—	11, 10, –
B07	—	11, –, –	—	—	10, –, –	—	—
B09	18, 14, 10	13, 12, 8	15, 12, 10	13, 11, –	10, 10, –	9, –, –	11, 11, 9
B13	17, 14, 12	14, 14, 13	13, 12, 12	16, 13, –	11, 10, –	12, –, –	9, –, –
B14	15, 13, 11	13, 10, –	16, 12, 10	15, 12, 9	12, 10, –	10, –, –	10, 9, –
B15	10, –, –	—	—	—	—	10, –, –	—
B16	—	—	—	—	9, –, –	—	9, –, –
C01	—	10, –, –	—	—	10, –, –	—	—
C02	—	8, –, –	—	—	9, –, –	—	—
C03	—	9, –, –	—	—	10, –, –	—	—
C04	—	—	—	12, –, –	—	—	—
C06	—	—	—	—	—	—	—
C07	—	—	—	—	10, –, –	—	—
C09	15, 10, 8	14, 14, –	21, 11, –	13, 11, –	11, 10, –	10, –, –	11, –, –
C10	—	—	—	—	—	—	—
C11	—	—	—	—	—	—	11, –, –
C13	—	—	—	—	—	—	9, –, –
C14	11, –, –	13, 9, –	10, 9, –	13, 10, –	10, –, –	—	—
C16	—	—	—	—	10, 9, –	—	—
C17	—	—	—	—	—	—	—
Curcumin	11, 10, –	9, –, –	10, 10, –	11, 9, –	11, 11, –	10, 10, –	10, 10, –
Ampicillin	30, 26, 20	23, 18, –	18, 16, –	18, 14, 10	—	23, 20, 16	31, 26, 24

Numerals show size of zone of inhibition in mm; the size (mm) of pores on agar is 4 mm.

Conclusion

Curcumin has been reported on the therapy of bacteria-infected diseases through inhibiting the bacterial endotoxin-induced cytokines secretion and pathways activation, and directly suppressing pathogen cell growth. So, the anti-bacterial screening presented here may be also useful to find more advanced compounds for bacteria-induced inflammation and tumorigenesis. The bacteria used in this research were isolated from the clinical patients from First Affiliated Hospital of Wenzhou Medical College, and the inhibition to them also possessed more significant meanings in clinically medicinal therapy. In this paper, three series of mono-canbonyl analogues of curcumin, totaled to 40 compounds, have been synthesized and evaluated for anti-bacterial activities against seven multidrug resistant bacteria. It was observed that heterocycle or long-chain substituents may enhance the activity of curcumin analogues and the ampicillin-resisted Gram-negative *E. cloacae* was sensitive to most mono-canbonyl curcumin analogues.

Experimental

Chemistry Melting points were determined on a Fisher–Johns melting apparatus. ^{1}H-NMR spectra were recorded on a Varian INOVA-400 spectrometer. The chemical shifts are presented in terms of ppm with TMS as the internal reference. Electron-spray ionization mass spectra in positive mode (ESI-MS) were recorded on a Bruker Esquire 3000$^+$ spectrometer. Column chromatography purifications were carried out on Silica Gel 60 (E. Merck, 70—230 mesh). The purity of all new compounds was checked by thin-layer chromatography (TLC) and ^{1}H-NMR. All reactions were monitored by TLC on pre-coated Silica Gel F254 plates (purchased from Qingdao Marine Chemical Factory, China) with detection by UV. All reagents used were of analytical grade. 4-Fluorobenzaldehyde, 2-bromobenzaldehyde, 3,4-dihydro-α-pyran, 2-fluoro-3-(trifluoromethyl)benzaldehyde, 5-bromofuran-2-aldehyde and 3-bromobenzaldehyde were purchased from Aldrich, U.S.A. Other reagents were purchased from Sinopharm Chemical Reagent Co., Ltd., China.

General Procedure of 01, 02, 03, 04, 06, 08, 09, 10 and 12 To a solution of 15 mmol arylaldehyde in MeOH (10 ml) was added 7.5 mmol ketone. The solution was stirred at room temperature for 20 min, followed by added

dropwise 20% (w/v) NaOH (1.5 ml, 7.5 mmol). The mixture was stirred at room temperature and monitored with TLC. When the reaction finished, the residue was poured into saturated NH_4Cl solution and filtered. The precipitate was washed with water, cold ethanol, and then cold acetone and dried in vacuum. The solid was purified by chromatography over silica gel using CH_2Cl_2/CH_3OH as the eluent to afford compounds **01**, **02**, **03**, **04**, **06**, **08**, **09**, **10** and **12**.

(2*E*,5*E*)-2,5-Bis(4-ethoxybenzylidene)cyclopentanone (**A01**): The product was recrystallized from ethanol/water (9 : 1). Orange powder, 76% yield, mp 191—192 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.44 (6H, t, J=7 Hz, $CH_3\times2$), 3.08 (4H, s, CH_2–CH_2), 4.09 (4H, q, J=7 Hz, O–$CH_2\times2$), 6.95 (4H, d, J=8.8 Hz, Ar–$H^{2,6}\times2$), 7.54 (2H, s, Ar–CH=C×2), 7.56 (4H, d, J=8.8 Hz, Ar–$H^{3,5}\times2$). ESI-MS m/z: 349.15 $(M+1)^+$, Calcd for $C_{23}H_{24}O_3$: 348.43.

(2*E*,5*E*)-2,5-Bis(4-fluorobenzylidene)cyclopentanone (**A02**): The product was recrystallized from ethanol/water (9 : 1). Yellow crystal, 88% yield, mp 239—240 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.10 (4H, s, CH_2–CH_2), 7.14 (4H, t, J=8.4 Hz, Ar–$H^{2,6}\times2$), 7.59 (4H, t, J=9 Hz, Ar–$H^{3,5}\times2$), 7.61 (2H, s, Ar–CH=C×2). ESI-MS m/z: 297.13 $(M+1)^+$, Calcd for $C_{19}H_{14}F_2O$: 296.31.

(2*E*,5*E*)-2,5-Bis(3,4,5-trimethoxybenzylidene)cyclopentanone (**A03**): Yellow powder, 90% yield, mp 202—204 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.15 (4H, s, CH_2–CH_2), 3.91 (18H, s, O–$CH_3\times6$), 6.85 (4H, s, Ar–$H^{2,6}\times2$), 7.53 (2H, s, Ar–CH=C×2). ESI-MS m/z: 441.16 $(M+1)^+$, Calcd for $C_{25}H_{28}O_7$: 440.49.

(2*E*,5*E*)-2,5-Bis(4-(dimethylamino)benzylidene)cyclopentanone (**A04**): Red powder, 71.1% yield, mp >260 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.54 (4H, s, CH_2–CH_2), 2.98 (12H, s, N–$CH_3\times4$), 6.74 (4H, d, J=8.8 Hz, Ar–$H^{2,6}\times2$), 7.41 (4H, d, J=8.8 Hz, Ar–$H^{3,5}\times2$), 7.77 (2H, s, Ar–CH=C×2). ESI-MS m/z: 347.21 $(M+1)^+$, Calcd for $C_{23}H_{26}N_2O$: 346.47.

(2*E*,5*E*)-2,5-Bis(2-bromobenzylidene)cyclopentanone (**A06**): Yellow powder, 87% yield, mp 163 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.98 (4H, s, CH_2–CH_2), 7.22 (2H, q, J=3.8 Hz, Ar–$H^4\times2$), 7.36 (2H, q, J=7.4 Hz, Ar–$H^6\times2$), 7.53 (2H, q, J=3.8 Hz, Ar–$H^5\times2$), 7.66 (2H, q, J=4 Hz, Ar–$H^3\times2$), 7.86 (2H, s, Ar–CH=C×2). ESI-MS m/z: 418.94 $(M+1)^+$, Calcd for $C_{19}H_{14}Br_2O$: 418.12.

(2*E*,5*E*)-2,5-Bis(2-fluoro-3-(trifluoromethyl)benzylidene)cyclopentanone (**A08**): Yellow powder, 91% yield, mp 223 °C; ^{1}H-NMR ($CDCl_3$) δ: 3.05 (4H, s, CH_2–CH_2), 7.32 (2H, t, J=8 Hz, Ar–$H_6\times2$), 7.64 (2H, t, J=7 Hz, Ar–$H_5\times2$), 7.66 (2H, t, J=7 Hz, Ar–$H_4\times2$), 7.79 (2H, s, Ar–CH=C×2). ESI-MS m/z: 430.92 $(M-1)^-$, Calcd for $C_{21}H_{12}F_8O$: 432.31.

(2*E*,5*E*)-2,5-Bis((5-bromofuran-2-yl)methylene)cyclopentanone (**A09**): Yellow powder, 77% yield, mp 176 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.06 (4H, s, CH_2–CH_2), 6.47 (2H, d, J=3.6 Hz, Ar–$H^4\times2$), 6.65 (2H, d, J=3.6 Hz, Ar–$H^3\times2$), 7.25 (2H, s, Ar–CH=C×2). ESI-MS m/z: 398.81 $(M+1)^+$, Calcd for $C_{15}H_{10}Br_2O_3$: 398.05.

(2*E*,5*E*)-2,5-Bis(3-bromobenzylidene)cyclopentanone (**A10**): Yellow powder, 91% yield, mp 186 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.12 (4H, s, CH_2–CH_2), 7.30 (2H, t, J=15.6 Hz, Ar–$H^5\times2$), 7.50—7.52 (6H, m), 7.73 (2H, s, Ar–CH=C×2). ESI-MS m/z: 418.98 $(M+1)^+$, Calcd for $C_{19}H_{14}Br_2O$: 418.12.

(2*E*,5*E*)-2,5-Bis(4-(3-(dimethylamino)propoxy)benzylidene)cyclopentanone (**A12**): Yellow powder, 72% yield, mp 154 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.98 (4H, m, $CH_2\times2$), 2.26 (12H, s, N–$CH_3\times4$), 2.46 (4H, t, J=7.4 Hz, CH_2–N×2), 3.08 (4H, s, CH_2–CH_2), 4.07 (4H, t, J=6.2 Hz, O–$CH_2\times2$), 6.96 (4H, d, J=8.8 Hz, Ar–$H^{2,6}\times2$), 7.73 (2H, s, Ar–CH=C×2), 7.56 (4H, d, J=8.8 Hz, Ar–$H^{3,5}\times2$). ESI-MS m/z: 463.33 $(M+1)^+$, Calcd for $C_{29}H_{38}N_2O_3$: 462.62.

(2*E*,5*E*)-2,5-Bis((*E*)-3-phenylallylidene)cyclopentanone (**A15**): Yellow powder, 81.1% yield, mp 229 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.93 (4H, s, CH_2–CH_2), 6.98 (4H, t, J=6.8 Hz, Ar–$H^{3,5}\times2$), 7.26—7.39 (8H, m), 7.53(4H, t). ESI-MS m/z: 313.11 $(M+1)^+$, Calcd for $C_{23}H_{20}O$: 312.40.

(1*E*,4*E*)-1,5-Bis(4-ethoxyphenyl)penta-1,4-dien-3-one (**B01**): Yellow powder, 77% yield, mp 123 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.39 (6H, t, J=8.2 Hz, $CH_3\times2$), 4.10 (4H, q, J=8.2 Hz, O–$CH_2\times2$), 6.99 (4H, m, J=7.2, Ar–H), 7.14 (2H, d, J=16 Hz, CH–C=O×2), 7.68—7.74 (6H, m). ESI-MS m/z: 323.16 $(M+1)^+$, Calcd for $C_{21}H_{22}O_3$: 322.40.

(1*E*,4*E*)-1,5-Bis(4-fluorophenyl)penta-1,4-dien-3-one (**B02**): The product was recrystallized from ethanol/water (9 : 1). Yellow crystal, 67% yield, mp 152 °C [lit.[22] 152—154 °C].

(1*E*,4*E*)-1,5-Bis(3,4,5-trimethoxyphenyl)penta-1,4-dien-3-one (**B03**): Yellow powder, 81% yield, mp 136 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.89 (18H, s, O–$CH_3\times6$), 6.84 (4H, s, Ar–H), 6.98 (2H, q, J=16 Hz, CH–C=O×2), 7.65 (2H, q, J=16 Hz, Ar–CH=C×2). ESI-MS m/z: 415.14 $(M+1)^+$, Calcd for $C_{23}H_{26}O_7$: 414.45.

(1*E*,4*E*)-1,5-Bis(4-(dimethylamino)phenyl)penta-1,4-dien-3-one (**B04**): Red powder, 91% yield, mp 185 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.04 (12H, s, N–$CH_3\times4$), 6.69 (4H, d, J=8.4 Hz, Ar–H), 6.90 (2H, d, J=15.6 Hz, C=CH–CO×2), 7.52 (4H, d, J=8.4 Hz, Ar–H), 7.68 (2H, d, J=15.6 Hz, Ar–CH=C×2). ESI-MS m/z: 321.18 $(M+1)^+$, Calcd for $C_{21}H_{24}N_2O$: 320.43.

(1*E*,4*E*)-1,5-Bis(2-bromophenyl)penta-1,4-dien-3-one (**B06**): Orange powder, 59% yield, mp 97 °C. ^{1}H-NMR ($CDCl_3$) δ: 7.03 (2H, d, J=16 Hz, C=CH–CO×2), 7.22—7.63 (8H, m, Ar–H), 8.09 (2H, d, J=16 Hz, Ar–CH=C×2). ESI-MS m/z: 393.10 $(M+1)^+$, Calcd for $C_{17}H_{12}Br_2O$: 392.08.

(1*E*,4*E*)-1,5-Bis(5-bromofuran-2-yl)penta-1,4-dien-3-one (**B09**): Yellow powder, 79% yield, mp 138 °C. ^{1}H-NMR ($CDCl_3$) δ: 6.44 (2H, d, J=3.6 Hz, Ar–$H^4\times2$), 6.63 (2H, d, J=3.6 Hz, Ar–$H^3\times2$), 6.89 (2H, d, J=15.6 Hz, C=CH–CO×2), 7.36 (2H, d, J=15.6 Hz, Ar–CH=C×2). ESI-MS m/z: 372.88 $(M+1)^+$, Calcd for $C_{13}H_8Br_2O_3$: 372.01.

(1*E*,4*E*)-1,5-Bis(3-bromophenyl)penta-1,4-dien-3-one (**B10**): Yellow powder, 63% yield, mp 117 °C. ^{1}H-NMR ($CDCl_3$) δ: 7.06 (2H, d, J=16 Hz, C=CH–CO×2), 7.22—7.52 (8H, m, Ar–H), 7.65 (2H, d, J=16 Hz, Ar–CH=C×2). ESI-MS m/z: 392.95 $(M+1)^+$, Calcd for $C_{17}H_{12}Br_2O$: 392.08.

(1*E*,3*E*,6*E*,8*E*)-1,9-Diphenylnona-1,3,6,8-tetraen-5-one (**B15**): Yellow powder, 90% yield, mp 142 °C. ^{1}H-NMR ($CDCl_3$) δ: 6.58 (2H, d, J=15.2 Hz, C=CH–CO×2), 6.97—7.00 (4H, m, C=CH–CH=C×2), 7.27—7.52 (12H, m, Ar–H). ESI-MS m/z: 287.07 $(M+1)^+$, Calcd for $C_{21}H_{18}O$: 286.37.

(2*E*,6*E*)-2,6-Bis(4-ethoxybenzylidene)cyclohexanone (**C01**): Yellow powder, 77% yield, mp 147-149 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.56 (6H, t, J=7.4 Hz, CH_3), 1.81 (2H, q, J=11.8 Hz, CH_2), 3.08 (4H, t, J=6 Hz, C–$CH_2\times2$), 3.89 (4H, q, J=7.4 Hz, O–$CH_2\times2$), 6.79—7.21 (8H, m, Ar–H), 7.73 (2H, s, Ar–CH=C×2). ESI-MS m/z: 363.18 $(M-1)^+$, Calcd for $C_{24}H_{26}O_3$: 362.46.

(2*E*,6*E*)-2,6-Bis(4-fluorobenzylidene)cyclohexanone (**C02**): Yellow powder, 69% yield, mp 156 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.81 (2H, q, J=12 Hz, CH_2), 2.90 (4H, t, J=5.4 Hz, $CH_2\times2$), 7.12 (4H, m, Ar–$H^{2,6}\times2$), 7.45 (4H, m, Ar–$H^{3,5}\times2$), 7.76 (2H, s, Ar–CH=C×2). ESI-MS m/z: 311.11 $(M+1)^+$, Calcd for $C_{20}H_{16}F_2O$: 310.34.

(2*E*,6*E*)-2,6-Bis(3,4,5-trimethoxybenzylidene)cyclohexanone (**C03**): Yellow powder, 71% yield, mp 196 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.83 (2H, t, J=5.6 Hz, CH_2), 2.97 (4H, t, J=5.2 Hz, C–$CH_2\times2$), 3.90 (18H, s, O–$CH_3\times6$), 6.71 (4H, s, Ar–H), 7.73 (2H, s, Ar–CH=C×2). ESI-MS m/z: 455.15 $(M+1)^+$, Calcd for $C_{26}H_{30}O_7$: 454.51.

(2*E*,6*E*)-2,6-Bis(4-(dimethylamino)benzylidene)cyclohexanone (**C04**): Yellow powder, 55% yield, mp 160 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.82 (2H, t, J=6 Hz, CH_2), 2.93 (4H, t, J=5.6 Hz, C–$CH_2\times2$), 3.01 (12H, s, N–$CH_3\times4$), 6.72 (4H, d, J=8.8 Hz, Ar–$H_{2,6}\times4$), 7.45 (4H, d, J=8.8 Hz, Ar–$H_{3,5}\times4$), 7.76 (2H, s, Ar–CH=C×2). ESI-MS m/z: 361.24 $(M+1)^+$, Calcd for $C_{24}H_{28}N_2O$: 360.49.

(2*E*,6*E*)-2,6-Bis(2-bromobenzylidene)cyclohexanone (**C06**): Yellow powder, 83% yield, mp 120 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.75 (2H, q, J=9.3 Hz, CH_2), 2.75 (4H, t, J=6.1 Hz, C–$CH_2\times2$), 7.18—7.35 (6H, m, Ar–H), 7.64 (2H, m, Ar–H), 7.86 (2H, s, Ar–CH=C×2). ESI-MS m/z: 432.98 $(M+1)^+$, Calcd for $C_{20}H_{16}Br_2O$: 432.15.

(2*E*,6*E*)-2,6-Bis((5-bromofuran-2-yl)methylene)cyclohexanone (**C09**): Yellow powder, 61% yield, mp 127 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.90 (2H, q, J=9.4 Hz, CH_2), 2.92 (4H, m, J=6.2 Hz, C–$CH_2\times2$), 6.44 (2H, d, J=3.6 Hz, Ar–$H^4\times2$), 6.66 (2H, d, J=3.6 Hz, Ar–$H^3\times2$), 7.43 (2H, s, Ar–CH=C×2). ESI-MS m/z: 412.87 $(M+1)^+$, Calcd for $C_{16}H_{12}Br_2O_3$: 412.07.

(2*E*,6*E*)-2,6-Bis(3-bromobenzylidene)cyclohexanone (**C10**): Yellow powder, 52% yield, mp 112 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.81 (2H, s, CH_2), 2.90 (4H, s, C–$CH_2\times2$), 7.28 (2H, t, J=7.8 Hz, Ar–$H^5\times2$), 7.37 (2H, d, J=7.6 Hz, Ar–$H^6\times2$), 7.47 (2H, d, J=7.6 Hz, Ar–$H^4\times2$), 7.59 (2H, s, Ar–$H^2\times2$), 7.70 (2H, s, Ar–CH=C×2). ESI-MS m/z: 433.32 $(M+1)^+$, Calcd for $C_{20}H_{16}Br_2O$: 432.15.

General Procedure of 07 and 11 A solution of 3,4-dihydro-α-pyran (22 mmol) in dichloromethane (20 ml) was added dropwise onto a well-stirred suspension of hydroxyl benzaldehyde (14.4 mmol) and pyridinium *p*-toluenesulfonate (0.16 mmol) in the same solvent (40 ml). The mixture was stirred at room temperature and monitored by TLC, then was washed with saturated $NaHCO_3$ solution (50 ml×3) and brine (50 ml×3) and dried over Na_2SO_4. Evaporation of the solvent gave crude product which was purified by column chromatography on silica gel (hexane/ethyl acetate, 7 : 3, as eluent) to obtain the mediate 4-(tetrahydropyran-2-yloxy) benzaldehyde as a lightly yellow oil (69—78% yield).

A mixture of protected derivative (6.94 mmol) and ketone (3.47 mmol) in C_2H_5OH (10 ml) was stirred at room temperature for 20 min; then 20% (w/v) NaOH (0.7 ml, 3.47 mmol) was added dropwise. After stirring for 60 min, the mixture was treated with 100 ml cold water and filtered. The residue was washed with ethanol and acetone and dried in vacuum. The product was purified by column chromatography on silica gel (hexane/ethyl acetate 9 : 1) to obtain **07** and **11**.

(2*E*,5*E*)-2,5-Bis(4-(tetrahydro-2*H*-pyran-2-yloxy)benzylidene)cyclopen-

tanone (**A07**): Yellow powder, 72% yield, mp 209 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.60—1.90 (12H, m), 3.09 (4H, s, CH_2×2), 3.61 (2H, q, J=5.2 Hz, O–CH_2^e×2), 3.90 (2H, q, J=10.7 Hz, O–CH_2^a×2), 5.50 (2H, t, O–CH–O×2), 7.11 (4H, d, J=8.8 Hz, Ar–$H^{2,6}$×2), 7.56 (6H, m). ESI-MS m/z: 460.92 (M^+), Calcd for $C_{29}H_{32}O_5$: 460.56.

(2*E*,5*E*)-2,5-Bis(3-methoxy-4-(tetrahydro-2*H*-pyran-2-yloxy)benzylidene)cyclopentanone (**A11**): Yellow powder, 67% yield, mp 138 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.70—1.95 (12H, m), 3.11 (4H, s, CH_2–CH_2), 3.63 (2H, q, J=7.2 Hz, O–CH_2^e×2), 3.90 (2H, q, J=11.6 Hz, O–CH_2^a×2), 3.91 (6H, s, O–CH_3×2), 5.50 (2H, s, O–CH–O×2), 7.09—7.23 (6H, m), 7.54 (2H, s, Ar–CH=C×2). ESI-MS m/z: 520.86 (M^+), Calcd for $C_{31}H_{36}O_7$: 520.61.

(1*E*,4*E*)-1,5-Bis(4-(tetrahydro-2*H*-pyran-2-yloxy)phenyl)penta-1,4-dien-3-one (**B07**): Yellow powder, 71% yield, mp 146—150 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.65 (4H, m), 1.76 (4H, m), 1.94 (4H, m), 3.61 (2H, q, J=11 Hz, O–CH_2^e×2), 3.88 (2H, q, J=14.8 Hz, O–CH_2^a×2), 5.49 (2H, s, O–CH–O×2), 6.95 (2H, d, J=16 Hz, C=CH–CO×2), 7.08 (4H, m), 7.56 (4H, m), 7.70 (2H, d, J=16 Hz, Ar–CH=C×2). ESI-MS m/z: 434.95 (M^+), Calcd for $C_{27}H_{30}O_5$: 434.52.

(1*E*,4*E*)-1,5-Bis(3-methoxy-4-(tetrahydro-2*H*-pyran-2-yloxy)phenyl)penta-1,4-dien-3-one (**B11**): Red oil, 66% yield. ^{1}H-NMR ($CDCl_3$) δ: 1.65—1.95 (12H, m), 3.63 (2H, d, J=12 Hz, O–CH_2^e×2), 3.86 (2H, q, J=15.2 Hz, O–CH_2^a×2), 3.94 (6H, s, O–CH_3×2), 5.49 (2H, s, O–CH–O×2), 6.90 (2H, d, J=13.2 Hz, C=CH–CO×2), 6.91—7.26 (6H, m), 7.68 (2H, d, J=16 Hz, Ar–CH=C×2). ESI-MS m/z: 494.88 (M^+), Calcd for $C_{29}H_{34}O_7$: 494.57.

(2*E*,6*E*)-2,6-Bis(4-(tetrahydro-2*H*-pyran-2-yloxy)benzylidene)cyclohexanone (**C07**): Yellow powder, 63% yield, mp 160 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.65—2.00 (12H, m), 2.04 (2H, m), 2.92 (4H, t, J=5.4 Hz, C–CH_2×2), 3.62 (2H, d, J=11.6 Hz, O–CH_2^e×2), 3.90 (2H, t, J=9.2 Hz, O–CH_2^a×2), 5.48 (2H, t, O–CH–O×2), 7.03 (4H, d, J=8.4 Hz, Ar–$H^{2,6}$×2), 7.43 (4H, d, J=8.4 Hz, Ar–$H^{3,5}$×2), 7.82 (2H, s, Ar–CH=C×2). ESI-MS m/z: 475.03 (M^+), Calcd for $C_{30}H_{34}O_5$: 474.59.

(1*E*,4*E*)-1,5-Bis(3-methoxy-4-(tetrahydro-2*H*-pyran-2-yloxy)phenyl)penta-1,4-dien-3-one (**C11**): Yellow powder, 59% yield, mp 138 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.64—2.01 (14H, m), 2.94 (4H, t, J=5.2 Hz, C–CH_2×2), 3.62 (2H, d, J=11.2 Hz, O–CH_2^e×2), 3.89 (6H, s, O–CH_3×2), 3.97 (2H, t, J=9.0 Hz, O–CH_2^a×2), 5.47 (2H, s, O–CH–O×2), 6.98—7.17 (6H, m), 7.74 (2H, s, Ar–CH=C×2). ESI-MS m/z: 534.88 (M^+), Calcd for $C_{32}H_{38}O_7$: 534.64.

General Procedure of 13 and 14 The protected product (**07** or **11**, 10 mmol) was suspended in methanol (40 ml) and treated with a catalytic amount of *p*-toluenesulfonic acid. After stirring at room temperature for 10 h the solvent was removed. The residue was treated with water and neutralized with a saturated solution of $NaHCO_3$. The suspension was extracted with ethyl acetate (50 ml×3) and the collected organic extract was washed with brine (100 ml×3), followed by dried with Na_2SO_4. Evaporation of the solvent gave crude product and then recrystallization from C_2H_5OH/H_2O to obtain pure **13** or **14**.

(2*E*,5*E*)-2,5-Bis(4-hydroxybenzylidene)cyclopentanone (**A13**): Yellow crystal, 89.3% yield, mp >300 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.01 (4H, s, CH_2–CH_2), 6.88 (4H, d, J=8.4 Hz, Ar–$H^{2,6}$×2),7.33 (2H, s, Ar–CH=C×2), 7.53 (4H, d, J=8.4 Hz, Ar–$H^{3,5}$×2), 10.01 (2H, br, Ar–OH×2). ESI-MS m/z: 291.13 $(M-1)^+$, Calcd for $C_{19}H_{16}O_3$: 292.33.

(2*E*,5*E*)-2,5-Bis(4-hydroxy-3-methoxybenzylidene)cyclopentanone (**A14**): Yellow powder, 76% yield, mp 214 °C [lit.[17] 212—214 °C].

(1*E*,4*E*)-1,5-Bis(4-hydroxyphenyl)penta-1,4-dien-3-one (**B13**): Orange crystal, 92% yield, mp 246—248 °C [lit.[17] 243—245 °C].

(1*E*,4*E*)-1,5-Bis(4-hydroxy-3-methoxyphenyl)penta-1,4-dien-3-one (**B14**): Yellow powder, 85% yield, mp 87—89 °C [lit.[22] 84—86 °C].

(2*E*,6*E*)-2,6-Bis(4-hydroxybenzylidene)cyclohexanone (**C13**): Yellow crystal, 59% yield, mp >300 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.71 (2H, m), 2.84 (4H, s, C–CH_2×2), 6.83 (4H, d, J=6.0 Hz, Ar–$H^{2,6}$×2), 7.40 (4H, d, J=6.0 Hz, Ar–$H^{3,5}$×2), 7.53 (2H, s, Ar–CH=C×2), 9.50 (2H, br, Ar–OH×2). ESI-MS m/z: 305.18 $(M-1)^+$, Calcd for $C_{20}H_{18}O_3$: 306.36.

(2*E*,6*E*)-2,6-Bis(4-hydroxy-3-methoxybenzylidene)cyclohexanone (**C14**): Yellow powder, 67% yield, mp 174—176 °C [lit.[17] 178—179 °C].

General Procedure of 16 and 17 A stirred suspension of 4-hydroxylbenzaldehyde (or 3-methoxyl-4-hydroxylbenzaldehyde, 10 mmol) and anhydrous K_2CO_3 (1.39 g, 10 mmol) in dry acetone (30 ml) was refluxed for 30 min under nitrogen atmosphere. Then, a solution of allylbromide (15 mmol) in acetone (10 ml) was added and refluxed for 3.5 h under nitrogen atmosphere and monitored by TLC. The resulting solution was evaporated to remove most of acetone and then poured with AcOEt (100 ml). The combined organic layers were washed with brine (100 ml×3), dried over Na_2SO_4 and evaporated *in vacuo* to afford the crude product 4-(allyloxy)benzaldehyde (or 4-(allyloxy)-3-methoxybenzaldehyde) as a colorless oil.

Compounds **16** (or **17**) was prepared according the procedure of **01** starting from 4-(allyloxy)benzaldehyde (or 4-(allyloxy)-3-methoxybenzaldehyde) and ketones, respectively.

(2*E*,5*E*)-2,5-Bis(4-(allyloxy)benzylidene)cyclopentanone (**A16**): Yellow powder, 67% yield, mp 192 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.09 (4H, s, C–CH_2×2), 4.60 (4H, d, J=5.2 Hz, CH_2–O×2), 5.33 (2H, q, J=10.8 Hz, CH_2^{α}=C×2), 5.44 (2H, m, J=17.2 Hz, CH_2^{β}=C×2), 6.07 (2H, m), 6.98 (4H, d, J=8.8 Hz, Ar–$H^{2,6}$×2), 7.51 (2H, s, Ar–CH=C×2), 7.57 (4H, d, J=8.8 Hz, Ar–$H^{3,5}$×2). ESI-MS m/z: 372.89 (M^+), Calcd for $C_{25}H_{24}O_3$: 372.5.

(2*E*,5*E*)-2,5-Bis(4-(allyloxy)-3-methoxybenzylidene)cyclopentanone (**A17**): Yellow powder, 67% yield, mp 116—117 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.02 (4H, s, CH_2–CH_2), 4.10 (6H, s, O–CH_3×2), 4.59 (4H, d, J=6.4 Hz, O–CH_2×2), 5.27 (2H, t, J=9.8 Hz, CH_2^{α}=C×2), 5.44 (2H, m, J=15.4 Hz, CH_2^{β}=C×2), 6.10 (2H, m), 6.71—7.32 (6H, m), 7.78 (2H, s, CH=C×2). ESI-MS m/z: 431.41 $(M-1)^+$, Calcd for $C_{27}H_{28}O_5$: 432.5.

(1*E*,4*E*)-1,5-Bis(4-(allyloxy)phenyl)penta-1,4-dien-3-one (**B16**): Yellow powder, 67% yield, mp 123—124 °C. ^{1}H-NMR ($CDCl_3$) δ: 4.55 (4H, t, J=4.2, CH_2–O×2), 5.33 (2H, d, J=10.4 Hz, CH_2^{α}=C×2), 5.44 (2H, m, J=17.6 Hz, CH_2^{β}=C×2), 6.08 (2H, m), 6.92 (2H, d, J=15.6 Hz, CH–C=O×2), 6.97 (4H, d, J=10 Hz, Ar–$H^{2,6}$×2), 7.57 (4H, d, J=10 Hz, Ar–$H^{3,5}$×2), 7.70 (2H, d, J=15.6 Hz, Ar–CH=C×2). ESI-MS m/z: 245.66 $(M-1)^+$, Calcd for $C_{23}H_{22}O_3$: 346.4.

(2*E*,6*E*)-2,6-Bis(4-(allyloxy)benzylidene)cyclohexanone (**C16**): Yellow powder, 67% yield, mp 120—122 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.81 (2H, t, J=5.6 Hz, CH_2), 2.92 (4H, t, J=5.6 Hz, C–CH_2×2), 4.59 (4H, d, J=5.2 Hz, O–CH_2×2), 5.32 (2H, d, J=10.4 Hz, CH_2^{α}=C×2), 5.44 (2H, m, J=16 Hz, CH_2^{β}=C×2), 6.09 (2H, m), 6.95 (4H, d, J=8.8 Hz, Ar–$H^{2,6}$×2), 7.45 (4H, d, J=8.8 Hz, Ar–$H^{3,5}$×2), 7.76 (2H, s, CH=C×2). ESI-MS m/z: 387.81 $(M+1)^+$, Calcd for $C_{26}H_{26}O_3$: 386.5.

(2*E*,6*E*)-2,6-Bis(4-(allyloxy)-3-methoxybenzylidene)cyclohexanone (**C17**): Yellow powder, 67% yield, mp 141 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.88 (2H, s, CH_2), 2.89 (4H, t, J=5.6 Hz, C–CH_2×2), 3.91 (6H, s, O–CH_3×2), 4.66 (4H, m), 5.31 (2H, t, J=10.2 Hz, CH_2^{α}=C×2), 5.42 (2H, m, J=16.4 Hz, CH_2^{β}=C×2), 6.09 (2H, m), 6.59—7.26 (6H, m), 7.40—7.75 (2H, s, CH=C×2). ESI-MS m/z: 446.67 (M^+), Calcd for $C_{28}H_{30}O_5$: 446.5.

Biology The anti-bacterial susceptibility test was done by determining zone of inhibition. The curcumin analogues were weighed and dissolved in DMSO to make a solution of concentration 10 mM. From this stock solution serial dilution has been done to 5, 2.5, 1.25 and 0.625 mM with DMSO in sterile test tubes. Seven different bacteria were selected *viz. Staphylococcus aureus* 26112 (ATCC 25923), *Micrococcus luteus* 28001, *Staphylococcus saprophyticus* 3-87, *Staphylococcus epidermidis* 26069, *Enterococus* sp. 050901, *Enterobacter cloacae* 45301 and *Escherichia coli* 2765, which were obtained from the diagnosed patients from the First Affiliated Hospital of Wenzhou Medical College without antibiotic for testing isolates. Then they were subcultured on sabourad dextrose agar slant and incubated at 37 °C for 10—12 d. LB solid medium (prepared by: yeast extract 5 g, tryptone 10 g, NaCl 5 g and powdered agar 15 g was solved in deionized water, metered volume to 1000 ml, autoclaved and then kept under 4 °C) were autoclaved and then cooled to about 40 °C, then an overnight culture of bacteria were added and intensively mixed. The bacteria-bearing mixture was infunded to the flat plate and allowed to solidify. In each plate, 3 pores (4 mm) were dug in aequalis distribution by sterile perforex and 50 μl curcumin analogues in different concentrations were put into a pore. Antibiotic ampicillin, curcumin and DMSO were used for comparison in the same method. The flat plates were incubated at 37 °C for 24 h under 5% CO_2. The diameter of the zone of inhibition was measured using scale.

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