Research Article

Synthesis and Biological Evaluation of Novel γ -Alkylidene Butenolides

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Three new series of 4-substituted-5-alkylidene-2,5-dihydrofuran-2-ones were synthesized. The in vitro activity test results showed that some of them exhibited good antibacterial and cytotoxic activities. Among them compound **5c** showed the most potent antibacterial activity against *Escherichia coli* with the MIC value of 20.00 $\mu\text{g/mL}$. Compound **9c** showed good cytotoxic activity against Ec9706 cells with IC_{50} value of 19.39 μM , better than that of the reference compound fluorouracil ($\text{IC}_{50} = 37.74 \mu\text{M}$).

1. Introduction

The biological importance of unsaturated lactones is well known [1–8]. In particular, γ -alkylidene butenolide is an important class of organic compounds that is present in many synthetic bioactive molecules and natural products, such as rubrolides (**a**) [9, 10], nostoclidides (**b**) [11–13], and uncinine (**1**) [14, 15] (Figure 1). Most of them exhibit various interesting biological activities, such as antibacterial, anticancer, antibiotic, and phospholipase A2 inhibition activity [16–26]. Over the past few decades, many researchers have been engaged in the synthesis of γ -alkylidene butenolides and their analogues due to their promising biological activities.

In the previous literatures [27, 28], pulvinones (**2**) were synthesized and evaluated as inhibitors of early stage cell wall biosynthesis enzymes MurA-MurD. Several pulvinones inhibited Mur enzymes with IC_{50} s in the 1–10 μM range and demonstrated antibacterial activity against Gram-positive bacteria including methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus faecalis*, and penicillin-resistant *Streptococcus pneumoniae*. A series of α,β -unsaturated- γ -lactone-free nitrogen-containing heterocyclic analogues of solamin, a natural mono-THF acetogenin, have been synthesized, and their cytotoxicity was investigated against 39 tumor cell lines. One of them, 1-methyl-pyrazol-5-yl derivative, showed selective increase of cytotoxicity against

NCI-H23 with 80 times higher potency than solamin [29–37]. The synthesis and bioactivity of the α,β -unsaturated- γ -alkylidene butenolides containing nitrogen atom or nitrogen heterocyclic unit were reported few times [14, 15].

2. Results and Discussion

Based on the previous researches and our previous work on the synthesis and bioactivity of γ -alkylidene butenolides [38–41], series of new α,β -unsaturated- γ -lactones bearing a nitrogen-containing unit such as piperazine and piperidine are designed and synthesized in the present work. The antibacterial and cytotoxic activities of the synthetic compounds were also evaluated.

The synthesis of compound **3** was reported in early studies [42–44]. The butenolide intermediates **4**, **6**, and **8** were prepared via introducing nitrogen-containing moiety such as piperazine or piperidine to the C-4 position of the lactone **3** in good yields (Scheme 1). In this reaction, secondary amines were logical choice as nucleophiles because of the base-sensitive nature of the butenolide **3**. In addition, lower alkalinity catalysts such as Na_2CO_3 were used in the nucleophilic substitution reaction. The corresponding amines piperidine, methyl benzylamine, and benzoyl piperazine are all secondary amines, so the catalyst must be relatively weak alkaline in order to avoid the occurrence of adverse

FIGURE 1: Representative examples of γ -alkylidene butenolides. SCHEME 1: Reagents and conditions: (i) 1.2 eq. piperidine, CH_3CN , Na_2CO_3 , r.t. 3 h, 85%; (ii) 1.2 eq. aldehyde, Na_2CO_3 , CH_3OH , r.t. 3–12 h; (iii) 1.2 eq. methyl benzylamine, CH_3CN , Na_2CO_3 , 60°C , 3 h, 90%; (iv) 1.1 eq. benzoyl piperazine, CH_3CN , Na_2CO_3 , r.t. 2 h, 95%.

reaction. Compounds **4**, **6**, and **8** were reacted with various aromatic aldehydes to produce a series of novel γ -alkylidene butenolides **5**, **7**, and **9** except for compound **5d** (compound **4** was reacted with acetone to produce **5d**). The structures of compounds **5**, **7**, and **9**, including the stereochemistry of the exocyclic carbon-carbon double bond, were established by 1D and 2D NMR spectroscopy. In the NMR spectrum of **5c** and **9c** (Figure 2), single peaks at δH 6.41 and δH 6.40, respectively, could be observed, suggesting the products were single isomers. In order to determine the geometry at the double bonds ($\Delta^{5,11}$) of **5c** and **9c** (Figure 2), NOESY experiment was investigated. The correlation between H-11 at δH 6.41 of **5c** and H-6 proved that the geometry of the double bond ($\Delta^{5,11}$) in the structure of **5c** is Z configuration. The stereochemistry of **9c** was similar to that of **5c**. Similarly, most of the other synthetic compounds were confirmed to be single isomers, in which the geometry of the double bond ($\Delta^{5,11}$) is Z configuration.

The antibacterial activity¹ *in vitro* of compounds **5**, **7**, and **9** was evaluated against *Escherichia coli* and *Staphylococcus*

aureus as described in Table 1. Among them is compound **5c** with potent antibacterial activity against *Escherichia coli* (MIC = 20 $\mu\text{g/mL}$), which was better than antibacterial drug chloramphenicol (MIC₉₀ = 110 $\mu\text{g/mL}$). The results indicated that the introduction of the nitrogen heterocyclic and γ -alkylidene to the lactone was favorable for the antibacterial activity in some cases.

The cytotoxic activity² *in vitro* of synthetic γ -alkylidene butenolides was also investigated against Ec9706 cells, Hela cells, SPCA1 cells, and HepG-2 cells. In cytotoxic activity test, first, each compound was tested at 100 μM concentration if the compounds show no inhibitory activity for each selected cell, their IC₅₀ values would not be tested. The results were described in Table 2. Some of them showed good cytotoxic activity. Among them compound **9c** showed excellent cytotoxic activity against Ec9706 cells with IC₅₀ value of 19.39 μM , which was better than that of the standard drug Fluorouracil (IC₅₀ = 37.74 μM). Meanwhile, it exhibited good cytotoxic activity against HepG-2 and SPCA1 with IC₅₀ values of 49.96 μM and 50.15 μM , respectively. The results

FIGURE 2: The NOE spectra of compounds **5c** and **9c**.TABLE 1: MIC^{a,b} of butenolide derivatives against *Escherichia coli* and *Staphylococcus aureus*.

Com	EC ^c	SA ^d	Com	EC ^c	SA ^d
3	120.13	101.46	7b	82.58	82.58
4	90.83	90.83	7c	Nd ^e	Nd
5a	79.30	93.59	7d	Nd	Nd
5b	>200	>200	8	93.59	93.59
5c	20.00	99.10	9a	132.58	132.58
5d	82.58	100.56	9b	93.59	93.59
5e	82.58	82.58	9c	90.83	110.62
5f	93.59	93.59	9d	90.83	90.83
6	Nd ^e	Nd	9e	82.58	82.58
7a	82.58	>200	Chloramphenicol	64	110 ^f

^aMIC ($\mu\text{g/mL}$): minimum inhibitory concentration.^bDefined as 100% inhibition of the growth of control. ^c*Escherichia coli*;^d*Staphylococcus aureus*. ^eNo detection. ^fMIC₉₀ ($\mu\text{g/mL}$).

indicated that the introduction of the nitrogen heterocyclic and γ -alkylidene to the lactone was efficient for the cytotoxic activity in some case.

3. Experimental

3.1. Chemistry. All reagents and solvents were obtained from commercial suppliers. All the reactions were monitored by TLC. Melting points were determined on a Beijing Keyi XT5 apparatus, and the temperature was not corrected. IR spectra were recorded as KBr pellets on a Thermo Nicolet (IR200) Spectrometer. ¹H and ¹³C NMR spectra were recorded on a Bruker DPX-400 spectrometer at 400 and 100 MHz with TMS as internal standard. Mass spectra were taken by Waters Q-ToF micromass spectrometer.

3.1.1. 4-(Piperidin-1-ylmethyl)furan-2(5H)-one (4). To an CH₃CN (50 mL) solvent of compound **3** (0.1 mol) and anhydrous sodium carbonate (0.06 mol) 1.2 eq. piperidine (0.12 mol) was added. The mixture was stirred at room temperature for 3 h, and the reaction was monitored by TLC until completion, the solid salt was filtered, and the filtrate was evaporated under reduced pressure to steam out of most of the acetonitrile, the reaction mixture was washed with water and ethyl acetate, dried and purified by column chromatography (elution with chloroform ether-ethylacetate,

3 : 1) which afforded 15.4 g compound **4**. Yield: 85%, yellow oil. IR (KBr, cm⁻¹): 1762, 1630, 1489, 1462, 1438, 1284, 1247, 1024, 994, and 758. ¹H NMR (400 MHz, CDCl₃, ppm) δ : 5.98 (s, 1H, =CH), 4.82 (s, 2H), 3.31 (s, 2H), 2.38 (s, 4H), and 1.61–1.26 (m, overlap, 6H); ¹³C NMR (100.6 MHz, CDCl₃, ppm) δ : 173.8 (C2), 168.4, 117.1, 72.7, 56.7, 55.0, 25.8, and 23.9. HR-MS (ESI), calcd. C₁₀H₁₅NO₂: [M+H]⁺ m/z : 182.1181; found: 182.1160 (see Supplementary Material available online at <http://dx.doi.org/10.1155/2013/926723>).

3.1.2. General Procedure for the Preparation of (5a–f). Compound **4** (0.01 mol) and anhydrous sodium carbonate (0.01 mol) were dissolved in MeOH (50 mL) solvent, then the respective aromatic aldehyde (acetone for compound **5d**) (0.012 mol) was added. The mixture was stirred at room temperature for 3–12 h, and the reaction was monitored by TLC until completion. The reaction solution was evaporated under reduced pressure to steam out of most of the methanol solvent. the reaction mixture was washed with water and ethyl acetate, dried, and purified by column chromatography.

(Z)-5-(Furan-2-ylmethylene)-4-(piperidin-1-ylmethyl)furan-2(5H)-one (5a). Yield: 60%, brown oil. IR (KBr, cm⁻¹): 2923, 2848, 1627, 1446, and 1382. ¹H NMR (400 MHz, CDCl₃, ppm) δ : 7.50 (d, J = 1.38 Hz, 1H, Ar), 7.05 (d, J = 3.39 Hz, 1H, Ar), 6.54 (m, 1H, Ar), 6.53 (s, 1H, =CH), 6.09 (s, 1H, =CH), 3.42 (s, 2H), 2.43 (s, 4H), 1.63 (m, 4H), and 1.47 (m, 2H); ¹³C NMR (100.6 MHz, CDCl₃, ppm) δ : 168.9 (C2), 156.4, 149.0, 146.1, 143.9, 116.0, 115.3, 113.0 (C11), 100.2, 54.9, 25.9, and 24.0. HR-MS (ESI), calcd. C₁₅H₁₇NO₃: [M+H]⁺ m/z : 260.1287; found: 260.1243.

(Z)-5-(4-Hydroxy-3-methoxybenzylidene)-4-(piperidin-1-ylmethyl)furan-2(5H)-one (5b). Yield: 65%, yellow oil. IR (KBr, cm⁻¹): 2924, 1623, 1516, 1382, and 1124. ¹H NMR (400 MHz, CDCl₃, ppm) δ : 7.53 (d, J = 1.76 Hz, 1H, Ph), 7.18 (dd, J = 1.80 Hz, 8.24 Hz, 1H, Ph), 6.93 (d, J = 8.24 Hz, 1H, Ph), 6.34 (s, 1H, =CH), 6.08 (s, 1H, =CH), 3.96 (s, 3H, OCH₃), 3.46 (s, 2H), 2.46 (s, 4H), 1.63 (m, 4H), and 1.46 (m, 2H); ¹³C NMR (100.6 MHz, CDCl₃, ppm) δ : 169.6 (C2), 157.2, 147.1, 146.8, 146.5, 127.5, 125.7, 115.0, 114.6, 111.5, 111.7 (C11), 56.1, 54.9, 54.8 (OCH₃), 25.9, and 24.0. HR-MS (ESI), calcd. C₁₈H₂₁NO₄: [M+H]⁺ m/z : 316.1549; found: 316.1537.

(Z)-5-(4-Hydroxybenzylidene)-4-(piperidin-1-ylmethyl)furan-2(5H)-one (5c). Yield: 85%, sallow crystal mp: 178.3–179.5°C.

TABLE 2: $^1\text{IC}_{50}$ values of butenolides against Ec9706 cells, Hela cells, SPCA1 cells and HepG-2 cells.

Com	Ec9706	HepG-2	SPCA1	Hela	Com	Ec9706	HepG-2	SPCA1	Hela
3	89.45	97.38	100.02	92.87	7b	81.34	NI	NI	113
4	78.23	80.16	108	70.36	7c	NI ^b	116	NI	84.28
5a	50.69	56.87	NI	NI	7d	104	178	89.19	Nd ^c
5b	100	88.64	200	90.16	8	NI ^b	102	116	128
5c	76.34	66.58	80.25	56.37	9a	77.59	67.51	102	86.31
5d	NI	NI	NI	Nd	9b	131	59.13	106	156
5e	96.12	132	101	87.69	9c	19.39	49.96	50.15	80.04
5f	55.87	86.92	NI	77.42	9d	Nd	200	Nd	Nd
6	98.39	123	147	NI	9e	151	125	143	74.13
7a	69.36	86.29	73.56	99.13	5-Fu^d	37.74	18.26	32.50	25.39

^a IC_{50} (μM): the concentration of substance that provides 50% inhibition.

^bNo inhibition at 100 μM . ^cNo detection. ^dFluorouracil.

IR (KBr, cm^{-1}): 3347, 3103, 2935, 2800, 2758, 1749, 1605, 1588, 1515, 1445, 1278, 1227, 1172, 944, 840. ^1H NMR (400 MHz, CDCl_3 and CD_3COCD_3 , ppm) δ : 7.73 (d, J = 8.72 Hz, 2H, Ph), 6.96 (d, J = 8.56 Hz, 2H, Ph), 6.41 (s, 1H, =CH), 6.10 (s, 1H, =CH), 3.46 (s, 2H), 2.46 (s, 4H), 1.65 (m, 4H), and 1.26 (m, 2H); ^{13}C NMR (100.6 MHz, CDCl_3 , ppm) δ : 157.7 (C2), 146.4, 132.8, 125.5, 116.1, 114.8, 112.0, 55.0, 25.9, and 24.0. HR-MS (ESI), calcd. $\text{C}_{17}\text{H}_{19}\text{NO}_3$: $[\text{M}+\text{H}]^+$ m/z : 286.1443; found: 286.1459.

4-(Piperidin-1-ylmethyl)-5-(propan-2-ylidene)furan-2(5H)-one (**5d**). Yield: 50%, brown oil. IR (KBr, cm^{-1}): 2938, 2856, 1744, 1624, 1588, 1447, 1129, 1032, 947, 909, and 876. ^1H NMR (400 MHz, CDCl_3 , ppm) δ : 6.07 (s, 1H, =CH), 3.37 (d, J = 1.13 Hz, 2H), 2.42 (s, 4H), 2.04, 2.03 (s, 6H, $=\text{C}(\text{CH}_3)_2$), 1.61 (m, 4H), and 1.46 (m, 2H); ^{13}C NMR (100.6 MHz, CDCl_3 , ppm) δ : 169.2 (C1), 155.9 (C3), 145.2 (C4), 124.2 (C11), 118.1 (C2), 57.3, 54.9, 26.0, 24.1, 21.0, and 19.1. HR-MS (ESI), calcd. $\text{C}_{13}\text{H}_{19}\text{NO}_2$: $[\text{M}+\text{H}]^+$ m/z : 222.1494; found: 222.1468.

(Z)-5-(2-Methoxybenzylidene)-4-(piperidin-1-ylmethyl)furan-2(5H)-one (**5e**). Yield: 46%, pink oil. IR (KBr, cm^{-1}): 2932, 1740, 1638, 1596, 1491, 1461, 1239, 1023, 982, and 754. ^1H NMR (400 MHz, CDCl_3 , ppm) δ : 7.29–6.80 (overlap, m, 4H, Ph), 6.23 (s, 1H, $=\text{C}_{(11)}\text{H}$), 5.89 (s, 1H, $=\text{C}_{(2)}\text{H}$), 3.86 (s, 3H, OCH_3), 3.73 (m, 2H), 2.53 (br, 4H), 1.72 (m, 4H), and 1.51 (m, 2H). ^{13}C NMR (100.6 MHz, CDCl_3 , ppm) δ : 171.7, 166.2, 157.2, 129.2, 128.5, 127.4, 121.8, 120.8, 111.0, 87.6, 68.7, 56.6, 55.6, 54.6, 25.4, and 23.6. HR-MS (ESI), calcd. $\text{C}_{18}\text{H}_{21}\text{NO}_3$: $[\text{M}+\text{H}]^+$ m/z : 300.1600; found: 300.1598.

(Z)-5-(Benzo[d][1,3]dioxol-5-ylmethylene)-4-(piperidin-1-ylmethyl)furan-2(5H)-one (**5f**). Yield: 55%, pink oil. IR (KBr, cm^{-1}): 2929, 2854, 1761, 1623, 1489, 1447, 1005, and 470. ^1H NMR (400 MHz, CDCl_3 , ppm) δ : 7.48 (s, 1H, Ph), 7.18 (d, J = 7.68 Hz, 1H, Ph), 6.83 (d, J = 8.13 Hz, 1H, Ph), 6.34 (s, 1H, $=\text{C}_{(11)}\text{H}$), 6.08 (s, 1H, $=\text{C}_{(2)}\text{H}$), 6.01 (s, 2H, OCH_2O), 3.49 (d, J = 19.46 Hz, 2H), 2.44 (s, 4H), 1.62 (m, 4H), and 1.26 (m, 2H); ^{13}C NMR (100.6 MHz, CDCl_3 , ppm) δ : 169.5 (C1), 157.4 (C3), 148.4 (C4), 148.2 (Ph), 146.6 (Ph), 127.2 (Ph), 126.1 (Ph), 115.0 (Ph), 111.3 (Ph), 110.1 (OCH_2O), 108.4 (C2),

101.4 (C11), 54.9, 25.9, 24.0. HR-MS (ESI), calcd. $\text{C}_{18}\text{H}_{19}\text{NO}_4$: $[\text{M}+\text{H}]^+$ m/z : 314.1392; found: 314.1362.

3.1.3. 4-((Benzyl(methyl)amino)methyl)furan-2(5H)-one (**6**). To a CH_3CN (50 mL) solvent of compound **3** (0.1 mol) 1.2 eq methyl benzylamine (0.12 mol) and catalytic amount of anhydrous sodium carbonate were added. The mixture was stirred at room temperature for 3 h and the reaction was monitored by TLC until completion. The reaction solution was evaporated under reduced pressure to steam out most of the CH_3CN solvent, the reaction mixture was washed with water and ethyl acetate, dried and purified by column chromatography (elution with chloroform ether-ethylacetate, 4:1) which afforded 19.5 g compound **6**. Yellow oil. Yield: 90%. ^1H NMR (400 MHz, CDCl_3 , ppm) δ : 7.31 (m, 5H, Ph), 6.01 (s, 1H, $=\text{C}_{(2)}\text{H}$), 4.81 (s, 2H, $\text{C}_{(4)}\text{H}_2$), 3.54 (s, 2H, $\text{C}_{(7)}\text{H}_2$), 3.34 (s, 2H, $\text{C}_{(7)}\text{H}_2$), 2.25 (s, 3H, NCH_3); ^{13}C NMR (100.6 MHz, CDCl_3 , ppm) δ : 173.7 (C1), 168.5 (C3), 138.0 (Ph), 128.6 (Ph), 127.4 (Ph), 117.0 (C2), 72.4 (C4), 62.2 (C5), 54.5 (C6), 42.8 (NCH_3). HR-MS (ESI), calcd. $\text{C}_{13}\text{H}_{15}\text{NO}_2$: $[\text{M}+\text{H}]^+$ m/z : 218.1181; found: 218.1149.

3.1.4. General Procedure for the Preparation of (**7a–d**). Compound **5** (0.01 mol) and anhydrous sodium carbonate (0.01 mol) were dissolved in methanol solvent, and then the respective aromatic aldehyde (0.012 mol) was added. The mixture was stirred at room temperature for 3–12 h and the reaction was monitored by TLC until completion. The reaction solution was evaporated under reduced pressure to steam out of most of the methanol solvent. The reaction mixture was washed with water and ethyl acetate, dried and purified by column chromatography.

(Z)-4-((Benzyl(methyl)amino)methyl)-5-(furan-2-ylmethylene)furan-2(5H)-one (**7a**). Yield: 44%, thick brown oil. IR (KBr, cm^{-1}): 1755, 1645, 1607, 1470, 1145, 994, 745. ^1H NMR (400 MHz, CDCl_3 , ppm) δ : 7.51 (d, J = 1.96 Hz, 1H, Ar), 7.32–7.26 (m, 5H, Ph), 7.07 (d, J = 3.60 Hz, 1H, Ar), 6.54 (m, 1H, Ar), 6.27 (s, 1H, $=\text{C}_{(8)}\text{H}$), 5.74 (s, 1H, $=\text{C}_{(2)}\text{H}$), 3.77 (d, J = 14.08 Hz, 1H), 3.37 (d, J = 14.08 Hz, 1H), 3.66–3.45 (m, 2H),

and 2.21 (s, 3H, CH₃); ¹³C NMR (100.6 MHz, CDCl₃, ppm) δ: 167.6 (C2), 154.2 (C4), 149.0 (C5), 145.5 (Ph), 135.6, 129.7, 128.7, 128.0 (Ph), 115.8, 113.3, 110.4, 99.8, 62.7, 51.1, and 41.8 (NCH₃). HR-MS (ESI), calcd. C₁₈H₁₇NO₃: [M+H]⁺ *m/z*: 296.1287; found: 296.1277.

(Z)-4-((Benzyl(methyl)amino)methyl)-5-(4-(dimethylamino)benzylidene)furan-2(5H)-one (**7b**). Yield: 70%, thick brown. oil. IR (KBr, cm⁻¹): 2917, 2848, 2801, 1744, 1604, 1526, 1444, 1367, 1168, 930, and 818. ¹H NMR (400 MHz, CDCl₃, ppm) δ: 7.69 (d, *J* = 8.96 Hz, 2H, Ph), 7.36–7.26 (m, 5H, Ph), 6.69 (d, *J* = 8.96 Hz, 2H, Ph), 6.22 (s, 1H, =C₍₈₎H), 6.05 (s, 1H, =C₍₂₎H), 3.58 (s, 2H), 3.08 (s, 2H), 3.03 (s, 6H, N(CH₃)₂), and 2.12 (s, 3H, NCH₃); ¹³C NMR (100.6 MHz, CDCl₃, ppm) δ: 170.2 (C1), 157.2 (C3), 150.8 (C4), 145.1, 138.4, 132.6, 129.0, 128.5, 127.4, 120.9, 113.6, 112.7, 112.0, 111.0, 62.4 (C5), 52.8 (C6), 42.9 (NCH₃), and 40.1 (N(CH₃)₂). HR-MS (ESI), calcd. C₂₂H₂₄N₂O₂: [M+H]⁺ *m/z*: 349.1916; found: 349.1934.

(Z)-4-((Benzyl(methyl)amino)methyl)-5-(2-methoxybenzylidene)furan-2(5H)-one (**7c**). Yield: 65%, light yellow oil. IR (KBr, cm⁻¹): 2926, 1767, 1642, 1599, 1491, 1460, 1247, 1025, 929, 764. ¹H NMR (400 MHz, CDCl₃, ppm) δ: 7.41–7.26 (m, 9H, Ph), 7.01 (s, 1H, =C₍₈₎H), 6.19 (s, 1H, =C₍₂₎H), 3.91 (s, 3H, OCH₃), 3.54 (s, 2H), 3.37 (s, 2H), 2.28 (s, 3H, NCH₃); ¹³C NMR (100.6 MHz, CDCl₃, ppm) δ: 169.7 (C1), 158.1 (C3), 157.6, 147.7, 138.4, 131.9, 130.6, 128.8, 128.7, 128.5, 128.4, 127.4, 121.8, 121.1, 115.6, 110.5 (C2), 105.3 (C8), 62.6 (C5), 55.66 (C6), 53.2 (OCH₃), 42.7 (NCH₃). HR-MS (ESI), calcd. C₂₁H₂₁NO₃: [M+H]⁺ *m/z*: 336.1600; found: 336.1598.

(Z)-4-((Benzyl(methyl)amino)methyl)-5-(3,4,5-trimethoxybenzylidene)furan-2(5H)-one (**7d**). Yield: 58%, light yellow oil. IR (KBr, cm⁻¹): 2939, 2841, 1753, 1585, 1504, 1457, 1422, 1331, 1235, 1126, 1001. ¹H NMR (400 MHz, CDCl₃, ppm) δ: 7.37 (m, 4H, Ph), 7.14 (s, 1H, Ph), 7.01 (s, 2H, Ph), 6.18 (s, 1H, =C₍₈₎H), 6.17 (s, 1H, =C₍₂₎H), 3.89 (s, 6H, OCH₃), 3.88 (s, 3H, OCH₃), 3.61 (s, 2H), 3.49 (s, 2H), 2.33 (s, 3H, NCH₃); ¹³C NMR (100.6 MHz, CDCl₃, ppm) δ: 169.7 (C1), 158.2, 157.6, 147.7, 138.5, 131.9, 131.6, 130.6, 130.5, 128.8, 128.4, 127.4, 121.8, 121.2, 115.6, 115.0, 110.4, 105.3, 103.8, 62.6, 55.6, 53.2, 42.7, and 29.7. HR-MS (ESI), calcd. C₂₃H₂₅NO₅: [M+H]⁺ *m/z*: 396.1811; found: 396.1807.

3.1.5. 4-((4-Benzoylpiperazin-1-yl)methyl)furan-2(5H)-one (**8**). To a CH₃CN (50 mL) solvent of compound **3** (0.1 mol) 1.1 eq. benzoyl piperazine¹² (0.11 mol) and catalytic amount of anhydrous sodium carbonate were added. The mixture was stirred at room temperature for 2 h, and the reaction was monitored by TLC until completion. The reaction solution was evaporated under reduced pressure to steam out of most of the CH₃CN solvent, the reaction mixture was washed with water and ethyl acetate, dried and purified by ethyl acetate crystallize to afforded 27.2 g compound **8**. White crystal. Yield: 95%, mp: 112.5–113.0°C. IR (KBr, cm⁻¹): 2820, 1787, 1742, 1624, 1434, 1275, 1143, 1025, 894, 868, 709. ¹H NMR (400 MHz, CDCl₃, ppm) δ: 7.43 (m, 5H, Ph), 6.02 (d, *J* = 1.42 Hz, 1H, =C₍₂₎H), 4.82 (d, *J* = 1.54 Hz, 2H), 3.80–3.46 (br,

4H), 3.40 (s, 2H), 2.56–2.42 (br, 4H); ¹³C NMR (100.6 MHz, CDCl₃, ppm) δ: 173.4 (C1), 170.4, 166.6 (C3), 135.4, 129.9, 128.7, 127.0 (Ph), 117.9 (C2), 72.4 (C4), 55.7 (C5), 53.7, 53.7, 47.5, 41.9. HR-MS (ESI), calcd. C₁₆H₁₈N₂O₃: [M+H]⁺ *m/z*: 287.1396; found: 287.1392.

3.1.6. General Procedure for the Preparation of (**9a–f**). Compound **8** (0.01 mol) and anhydrous sodium carbonate (0.01 mol) was dissolved in methanol solvent, then the respective aromatic aldehyde (0.012 mol) was added. The mixture was stirred at room temperature for 3–12 h and the reaction was monitored by TLC to complete. The reaction solution was vaporated under reduced pressure to steam out of most of the methanol solvent, the reaction mixture was washed with water and ethyl acetate, dried and purified by column chromatography.

(Z)-4-((4-Benzoylpiperazin-1-yl)methyl)-5-(furan-2-ylmethylene)furan-2(5H)-one (**9a**). Yield: 82%, brown-red oil. IR (KBr, cm⁻¹): 1757, 1629, 1466, 1435, 1282, 997, and 742. ¹H NMR (400 MHz, CDCl₃, ppm) δ: 7.51–7.07 (m, overlap, 6H, Ar), 6.34 (m, 2H, Ar), 6.31 (s, 1H, =C₍₁₁₎H), 5.73 (s, 1H, =C₍₂₎H), 3.88–3.51 (br, 4H), 3.50 (d, *J* = 14.28 Hz, 2H), 2.75–2.56 (br, 4H); ¹³C NMR (100.6 MHz, CDCl₃, ppm) δ: 170.4 (C1), 167.4, 153.9 (C3), 148.8 (C4), 147.2, 135.1, 130.0, 128.6, 127.0, 116.0, 113.3, 110.6, 107.4 (C11), 100.0 (C2), 62.9 (C5), 51.7, 47.6, and 42.1. HR-MS (ESI), calcd. C₂₁H₂₀N₂O₄: [M+H]⁺ *m/z*: 365.1501; found: 365.1497.

(Z)-4-((4-Benzoylpiperazin-1-yl)methyl)-5-(4-hydroxy-3-methoxybenzylidene)furan-2(5H)-one (**9b**). Yield: 88%, brown solid mp: 79.0–80.6°C. IR (KBr, cm⁻¹): 2921, 2852, 1752, 1630, 1580, 1515, 1431, 1284, 1163, 1131, and 1026. ¹H NMR (400 MHz, CDCl₃, ppm) δ: 7.40 (s, 1H, Ph), 7.36 (m, 5H, Ph), 7.15 (m, 1H), 6.86 (d, *J* = 8.24 Hz, 1H, Ph), 6.23 (s, 1H, =C₍₁₁₎H), 6.05 (s, 1H, =C₍₂₎H), 3.85 (s, 3H, OCH₃), 3.80, 3.44 (br, 4H), 3.48 (s, 2H), 2.58, 2.42 (br, 4H); ¹³C NMR (100.6 MHz, CDCl₃, ppm) δ: 170.5 (C1), 169.5 (C10), 156.1 (C3), 147.5, 147.1, 145.9, 135.3, 129.9, 128.5, 127.0, 125.6, 125.1, 115.0, 112.7 (C2), 112.1 (C11), 56.0 (C5), 53.7 (OCH₃), 53.5, 53.1, 47.6, and 42.1. HR-MS (ESI), calcd. C₂₄H₂₄N₂O₅: [M+H]⁺ *m/z*: 421.1763; found: 421.1735.

(Z)-4-((4-Benzoylpiperazin-1-yl)methyl)-5-(4-hydroxybenzylidene)furan-2(5H)-one (**9c**). Yield: 89%, yellow crystals, mp: 239.1–240.0°C. IR (KBr, cm⁻¹): 3447, 1762, 1604, 1513, 1446, 1279, 1021, and 998. ¹H NMR (400 MHz, CDCl₃ and CD₃COCD₃, ppm) δ: 7.72 (d, *J* = 8.72 Hz, 2H, Ph), 6.92 (d, *J* = 6.92 Hz, 2H, Ph), 7.42 (m, 5H, Ph), 6.40 (s, 1H, =C₍₁₁₎H), 6.11 (s, 1H, =C₍₃₎H), 3.85–3.46 (br, 4H), 3.85 (s, 2H), and 2.66–2.49 (br, 4H); ¹³C NMR (100.6 MHz, CDCl₃, ppm) δ: 170.4, 169.4, 156.1, 145.9, 129.9, 128.6, 127.1, 124.8, 116.1, 115.0, 56.0, 53.5, 47.6. HR-MS (ESI), calcd. C₂₃H₂₂N₂O₄: [M+H]⁺ *m/z*: 391.1658; found: 391.1627.

(Z)-4-((4-Benzoylpiperazin-1-yl)methyl)-5-benzylidenefuran-2(5H)-one (**9d**). Yield: 78%, light yellow solid, mp: 65.2–66.4°C. IR (KBr, cm⁻¹): 1758, 1627, 1434, 1280, 994, 697.

^1H NMR (400 MHz, CDCl_3 , ppm) δ : 7.80 (d, $J = 7.38$ Hz, 2H, Ph), 7.44–7.21 (m, 8H, Ph), 6.21 (s, 1H, $=\text{C}_{(11)}\text{H}$), 5.86 (s, 1H, $=\text{C}_{(2)}\text{H}$), 3.76–3.43 (br, 4H), 3.39 (d, $J = 10.70$ Hz, 2H), and 2.72–2.53 (br, 4H); ^{13}C NMR (100.6 MHz, CDCl_3 , ppm) δ : 170.4 (C1), 168.6, 147.6, 147.4, 141.9, 135.0, 132.5, 132.5, 130.8, 130.1, 129.6, 128.9, 128.6, 128.6, 128.4, 128.4, 127.7, 127.0, 125.8, 125.4, 111.1 (C11), 67.6 (C5), 53.2, 52.9, 47.0, 41.5. HR-MS (ESI), calcd. $\text{C}_{23}\text{H}_{22}\text{N}_2\text{O}_3$: $[\text{M}+\text{H}]^+$ m/z : 375.1709; found: 375.1716.

(Z)-4-((4-Benzoylpiperazin-1-yl)methyl)-5-(2-methoxybenzylidene)furan-2(5H)-one (**9e**). Yield: 85%, light yellow solid, mp: 199.3–200.5°C. ^1H NMR (400 MHz, CDCl_3 , ppm) δ : 7.42–6.83 (m, 9H, Ph), 6.68 (s, 1H, $=\text{C}_{(11)}\text{H}$), 5.93 (s, 1H, $=\text{C}_{(2)}\text{H}$), 3.90, 3.55 (br, 4H, $\text{C}_{(7,8)}\text{H}_2$), 3.74 (s, 3H, OCH_3), 3.29 (m, 2H, $\text{C}_{(5)}\text{H}_2$), and 2.92–2.53 (br, 4H, $\text{C}_{(6,9)}\text{H}_2$). ^{13}C NMR (100.6 MHz, CDCl_3 , ppm) δ : 170.4, 168.3, 157.4, 156.2, 147.4, 132.6, 131.6, 130.7, 128.9, 128.6, 128.5, 127.1, 127.0, 125.7, 121.1, 120.0, 110.6, 103.8, 63.6, 55.6, 55.3, and 50.7. HR-MS (ESI), calcd. $\text{C}_{24}\text{H}_{24}\text{N}_2\text{O}_4$: $[\text{M}+\text{H}]^+$ m/z : 405.1814; found: 405.1846.

4. Conclusions

In summary, a series of novel γ -alkylidene butenolides bearing nitrogen unit have been synthesized by a simple and general method in good yields. Their antibacterial activity and cytotoxic activity were evaluated. The results revealed that the introduction of nitrogen heterocyclic and γ -alkylidene to the lactone was efficient for the bioactivities.

Conflict of Interests

There is no conflict of interests to declare.

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Endnotes

1. *In vitro* antibacterial activity was studied with tube double dilution method. In order to study the bacteriostasis to *staphylococcus aureus* and *Escherichia coli*. The minimum inhibitory concentration (MIC) was measured. The sample was dissolved to 1.0 mg/mL high concentration solution, geometric diluted to less than 200 $\mu\text{g}/\text{mL}$ for several different concentration. The bacteria have been incubated 16–18 h and diluted 200 times, in the above-mentioned several different concentrations of liquid by adding 0.1 mL solution of bacteria. With a sample solution without bacteria as a test group, to observe whether the liquid was contaminated, a solution of diluted bacteria without sample was used as a control group to observe whether the bacteria can grow and another tube with only 1 mL medium was used as a blank control group to observe whether the medium was contaminated. Samples for each of the groups were

activated in a shaker with 225 r/min and 37°C for 16–18 h to check the results. With naked eye to observe the clear level of each group's medium and sequential observation of drug control tubes, control tubes, and control tubes of bacteria, control tubes needed to be clarified; otherwise it would mean that samples or medium has been contaminated. The control tube of bacteria that has become muddy proved to have normal bacterial growth. Comparison of the test tube, among the test tubes with clarified liquid medium, with the minimum sample concentration poses the drug concentration as the minimum inhibitory concentration of drugs. All the experiment was carried out in triplicate.

2. *In vitro* cytotoxicity study: the cells were purchased from the Type Culture Collection of the Chinese Academy of Sciences, Shanghai, China. The cytotoxicity of compounds at various concentrations or fluorouracil (10 $\mu\text{g}/\text{mL}$) was measured by MTT(3-[4,5]-dimethylthiazol-2-yl-2,5-di-phenyltetrazolium bromide) method. HepG-2.2.15 cells (3.0×10^4 cells/mL) were seeded in 96-wellplates for 24 h. After attachment to plates, the supernatants in each well were replaced very carefully with 200 μL of fresh RPMI1640 containing different concentrations of the compounds or fluorouracil. Untreated cells were used as the control. Remove the medium after HepG-2.2.15 cells were treated for 3 days as explained previously. Cells were cultured at 37°C for 4 h after 150 μL of DMEM medium containing MTT (5 mg/mL) was added into each well. Remove the medium and add 200 μL of DMSO into each well. The absorption (A) at 490 nm was measured after a ten-minute shaking. The IC_{50} value was the concentration that achieved 50% cytotoxicity to HepG-2.2.15. All the experiment was carried out in triplicate. Data were analyzed statistically by the SPSS11.5 program software package, and a P value of 0.05 was regarded as significant.

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