An efficient one-pot synthesis and biological evaluation of novel (E)-2-aroyl-4-arylidene-5-oxotetrahydrofuran derivatives

Hong-Mei Wang¹,²,³, *, Xiu-Lian Zhu¹,²,³, *, Qin-Hua Chen², Ming-Wu Ding⁴ and Xiao-Hua Zeng³

Abstract
An efficient one-pot base-mediated approach to (E)-2-aroyl-4-arylidene-5-oxotetrahydrofurans is developed. Nine (E)-2-aroyl-4-arylidene-5-oxotetrahydrofurans are synthesized in good yields via tandem Passerini and cyclization reactions, starting from Baylis–Hillman acids, aryl glyoxals, and isocyanides at room temperature in the presence of Cs₂CO₃. In addition, the MTT assay is used to evaluate their cytotoxicities toward the cervical cancer cell lines C-33A, CaSki, and SiHa and the hepatocarcinoma cell line HepG2. The results show that some of the compounds inhibit the proliferation of cancer cells significantly.

Keywords
Cancer, (E)-2-aroyl-4-arylidene-5-oxotetrahydrofurans, one-pot, proliferation, synthesis

Date received: 10 July 2020; accepted: 25 August 2020

Introduction
Heterocyclic compounds, especially pyrroles, pyridines, furans, and β-lactams, are used extensively in medicinal chemistry, industry, and pesticides due to their useful properties. The structural modification, synthesis, and bioactivity of heterocyclic compounds are important research fields in organic and pharmaceutical chemistry. Derivatives of heterocycles containing a tetrahydrofuranone moiety are widely found in plants.¹,² They have significant anticancer³ and anti-Toxoplasma gondii activity⁴ and can also be used as seed germination stimulants,⁵ in dermatology, and in capillary drug preparation.⁶

Studies have shown that the alkaloids rhopaladins A-D, which are present in marine cysts, have remarkable cytotoxicity against human tumor cell lines,⁷ especially rhopaladin B (Scheme 1).⁸ We have previously synthesized 2-aroyl-4-arylidene-5-oxopyrrolidines via tandem Ugi and cyclization reactions from Baylis–Hillman acids, aryl glyoxals, and isocyanides⁹ and found that they are bioisosteres of rhopaladins because of the presence of five-membered nitrogen heterocycles with arylidene and aroyl groups. Furthermore, (E)-2-aroyl-4-arylidene-5-oxopyrrolidines have significant cytotoxicities toward cervical cancer cells.⁹ In order to find improved or novel heterocyclic

¹The Experiment Center of Medicine, Dongfeng Hospital, Hubei University of Medicine, Shiyan, P.R. China
²Shenzhen Bao’an Authentic TCM Therapy Hospital, Shenzhen, P.R. China
³Hubei Key Laboratory of Wudang Local Chinese Medicine Research, School of Pharmaceutical Sciences, Hubei University of Medicine, Shiyan, P.R. China
⁴Key Laboratory of Pesticide & Chemical Biology of Ministry of Education, Central China Normal University, Wuhan, P.R. China
⁵Hong-Mei Wang and Xiu-Lian Zhu are the first authors.
cores, we used the Baylis–Hillman and Passerini reactions to synthesize a series of tetrahydrofurane derivatives that are bioisosteres of 2-aryl-4-arylidene-5-oxopyrrolidines. The strategy for the preparation of (E)-2-aryl-4-arylidene-5-oxotetrahydrofurans is shown in Scheme 1. Meanwhile, the effects of these novel compounds on the proliferation of cervical cancer cells and hepatoma cells were studied by the MTT assay.10

Results and discussion
Initially, the Passerini reaction condensation intermediate (4a) was prepared from α-bromomethyl cinnamic acid (1a), 4-nitrophenylglyoxal (2a), and tert-butyl isocyanide (3). When the reactants were stirred at room temperature in methanol or ether for 15 days, the reaction was incomplete. In acetonitrile, the reaction did not proceed at room temperature. When the temperature was raised to 40 °C, the reaction was complete in 4h, but the quantity of side-products increased and the yield of intermediate (4a) was reduced. In CH₂Cl₂, the condensation reaction was complete in 48 h at room temperature (Scheme 2), and the presence of the condensation intermediate (4a) and compound (5a) was verified by 1H NMR spectroscopy. Finally, intermediate (4a) was completely converted into compound (5a) via a base-mediated approach.

The basicity plays an important role in the base-mediated approach. In order to avoid side reactions caused by too high a pH, a base was added to adjust the pH of the solution to 8 during the reaction (Table 1). The best result was obtained when solid cesium carbonate was used as the base (Table 1, entry 4). With optimized condition, we synthesized (E)-2-aryl-4-arylidene-5-oxotetrahydrofurane derivatives 5 from various Baylis–Hillman acids 1, aryl glyoxals 2, and isocyanide 3 (Scheme 3). All the reactions occurred smoothly to give compounds 5 in yields of 66%–80% (Table 2). It was found that the yields of compounds 5 were mainly related to the nature of the Ar² substituent on aryl glyoxals 2. The stronger the electron-withdrawing effect of Ar² substituted, the higher the yield of compounds 5. The Ar² substituent on Baylis–Hillman acids 1 had little impact on the reaction yields (Table 3).

The structures of compounds 5a–i were confirmed by their spectroscopic data. The 1H NMR spectra of compounds 5 showed chemical shifts for the aromatic hydrogens generally between 8.39 and 6.90 ppm, the alkene hydrogen is generally around 7.60 ppm, and the NH is around 6.60 ppm. When the electron-withdrawing effect of Ar² is enhanced, the chemical shift moves to a lower field. The signal for the CH₃ of the furan ring occurs as two doublets at around 4.44 and 3.34 ppm, respectively. The 13C NMR spectral data of compounds 5 show the characteristic chemical shift of a carbonyl carbon around 189 ppm, and the amide and ester carboxyls around 169 and 167 ppm, respectively. The characteristic quaternary carbon on the furan ring occurs around 86 ppm. The products are racemates, but the configuration of the double bond of the Baylis–Hillman acid is known to be retained after the condensation and cyclization reactions.8,9,11

The cytotoxicities of compounds 5 were tested by the MTT assay; meanwhile, cisplatin was used as the positive control. The results indicated that there were three compounds, 5d, 5g, and 5h, which could inhibit the proliferation of most of the cells. Other compounds were hardly cytotoxic to cancer cells. The IC₅₀ of 5d to C-33A cells was 32.5 μmol, which can significantly inhibit cancer cell proliferation.

Conclusion
By combining the Baylis–Hillman reaction with the Passerini reaction, nine (E)-2-aryl-4-arylidene-5-oxotetrahydrofuran derivatives have been synthesized via tandem Passerini and cyclization reactions starting from Baylis–Hillman acids. None of the compounds have previously been reported in the literature. The products are easy to obtain under mild conditions, via a simple procedure and in high yields. In the experimental process, the Passerini reaction intermediates do not need to be separated and purified, but are directly used in the subsequent step. The results of cytotoxicity studies indicated that compound 5d had a significant inhibitory effect on cancer cells.

Experimental section
General information
Melting points were measured with an X-4 melting point instrument (uncorrected thermometer) produced by Beijing Ruili Analytical Instrument Co., Ltd. Mass spectrometry was performed with a Finnigan trace MS analyzer (direct injection method). Elemental analysis was performed using a Vario EL III analyzer. 1H NMR and 13C NMR spectra were measured at 600 or 400 MHz using spectrometers. The solvent was CDCl₃ or DMSO-d₆ with tetramethylsilane (TMS) as the internal standard.

Experimental procedures
A solution of dried Baylis–Hillman acid 1 (1 mmol) and dichloromethane (10 mL) was stirred in a 25-mL round-bottom flask. The aryl glyoxal 2 (1 mmol) and isocyanide 3 (1 mmol) were added consecutively to the solution, and the mixture was stirred at room temperature for 48 h. The pH value of the reaction system was adjusted to about 8 by adding solid cesium carbonate intermittently during the reaction process. Dichloromethane was removed under reduced pressure, and the target compounds 5 were recrystallized from ether. (E)-4-Benzylidene-N-tert-butyl-2-(4-nitrobenzoyl)-5-oxotetrahydrofuran-2-carboxamide (5a). White crystals (0.31 g, 73%), m.p. 210–211 °C; 1H NMR (CDCl₃, 600 MHz): δ 8.31 (d, J=8.4 Hz, 2H, Ar-H), 8.23 (d, J=8.4 Hz, 2H, Ar-H), 7.64–7.48 (m, 6H, Ar-H and =CH), 6.58 (s, 1H, NH), 4.44 (d, J=18.6 Hz, 1H, CH₂), 3.38 (d, J=18.0 Hz, 1H, CH₂), 1.36 (s, 9H, 3CH₃); 13C NMR (CDCl₃, 150 MHz): δ 189.4, 169.3, 167.1, 150.5, 140.3, 138.4, 133.6, 130.6, 129.2, 123.6, 123.5, 119.9, 85.8, 52.5, 29.0, 28.4. MS (EI): m/z (%)=422 (M⁺), (4), 322 (69), 272 (100), 216 (86), 150 (25), 113 (69), 57...
(E)-N-tert-butyl-4-(4-chlorobenzylidene)-2-(4-nitrobenzoyl)-5-oxotetrahydrofuran-2-carboxamide (5b). White crystals (0.35 g, 78%), m.p. 143–144 °C; 1H NMR (CDCl₃, 600 MHz): δ 8.31 (d, J = 8.4 Hz, 2H, Ar-H), 8.22 (d, J = 7.8 Hz, 2H, Ar-H), 7.58–7.46 (m, 5H, Ar-H and =CH), 6.55 (s, 1H, NH), 4.40 (d, J = 18.6 Hz, 1H, CH₂a), 3.40 (d, J = 18.0 Hz, 1H, CH₂b), 1.36 (s, 9H, 3CH₃); 13C NMR (CDCl₃, 150 MHz): δ 189.2, 169.0, 167.0, 150.5, 138.7, 138.2, 137.1, 132.0, 131.7, 130.6, 129.4, 123.6, 120.5, 85.7, 52.6, 29.0, 28.4. MS (EI): m/z (%) = 456 (M⁺), (4), 356 (66), 306 (95), 250 (100), 150 (66), 57 (74). Anal. calcd for C₂₃H₂₂ClN₂O₆: C, 60.46; H, 4.63; N, 3.07. Found: C, 60.45; H, 4.92; N, 3.02.

Table 1. Optimization of the synthesis of compound 5a.

| Entry | Base     | Reaction conditions | Yield (%) |
|-------|----------|--------------------|-----------|
| 1     | K₂CO₃    | rt/12 h            | 52        |
| 2     | NaHCO₃   | rt/12 h            | 38        |
| 3     | NEt₃     | rt/12 h            | –         |
| 4     | Cs₂CO₃   | rt/12 h            | 73        |

(397) Anal. calcd for C₂₃H₂₂N₂O₆: C, 65.39; H, 5.25; N, 6.63. Found: C, 65.33; H, 5.33; N, 6.78.

Table 2. Synthesis of compounds 5.

| Product | Ar¹ | Ar²          | R    | Yield (%) |
|---------|-----|--------------|------|-----------|
| 5a      | Ph  | 4-O₂NC₆H₄   | t-Bu | 73        |
| 5b      | 4-ClC₆H₄ | 4-O₂NC₆H₄ | t-Bu | 78        |
| 5c      | Ph  | 4-BrC₆H₄   | n-Bu | 66        |
| 5d      | Ph  | 2,4-Cl₂C₆H₄| t-Bu | 75        |
| 5e      | 4-ClC₆H₄ | 4-BrC₆H₄ | t-Bu | 80        |
| 5f      | 4-F₃CC₆H₄ | 4-BrC₆H₄ | t-Bu | 72        |
| 5g      | 4-ClC₆H₄ | 4-BrC₆H₄ | t-Bu | 79        |
| 5h      | 4-CH₃OC₆H₄ | 4-ClC₆H₄ | t-Bu | 80        |
| 5i      | Ph  | 4-BrC₆H₄   | t-Bu | 73        |

(43) Anal. calcd for C₂₃H₂₁ClN₂O₆: C, 65.39; H, 5.25; N, 6.63. Found: C, 65.33; H, 5.33; N, 6.78.

(5g) Anal. calcd for C₂₃H₂₂Br₂N₂O₆: C, 51.81; H, 3.94; N, 7.69. Found: C, 51.84; H, 3.91; N, 7.72.

Scheme 1. The strategy for the choice of (E)-2-aryloyl-4-arylidene-5-oxotetrahydrofuran as targets.

Scheme 2. Synthesis of intermediate 4a.

Scheme 3. Synthesis of (E)-2-aryloyl-4-arylidene-5-oxotetrahydrofuran derivatives 5.
Table 3. Cytotoxicity of compounds 5 in vitro (IC_{50}, μM).

| Compound | C-33A | CaSki | SiHa | HepG2 | L02 |
|----------|-------|-------|------|-------|-----|
| 5a       | >100  | >100  | >100 | >100  | >100 |
| 5b       | >100  | >100  | >100 | >100  | >100 |
| 5c       | >100  | >100  | >100 | >100  | >100 |
| 5d       | 35.2  | 46.7  | 57.8 | 89.4  | >100 |
| 5e       | >100  | >100  | >100 | >100  | >100 |
| 5f       | 49    | >100  | >100 | >100  | >100 |
| 5g       | 75    | 89    | >100 | >100  | >100 |
| 5h       | >100  | >100  | >100 | >100  | >100 |
| Cisplatin|       |       |      |       |      |

**Declaration of conflicting interests**

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**Funding**

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: We

**MTT assay**

Cell proliferation was evaluated using the MTT assay. Cervical cancer cell lines C-33A, SiHa, and CaSk were cultured in DMEM/MEM/1640 medium; HepG2 and L02 were cultured in 1640 medium, which contained 10% fetal bovine serum (FBS). The cells were grown in an incubator with a humidified atmosphere of 5% CO₂ at 37°C. The cells were incubated for 24h in 96-well plates at a density of 5 × 10³ cells/well. The cells were treated with varying concentrations of compounds 5 and cisplatin for 48h, and cultured in the same medium containing 5 mg/mL MTT for 4h. The crystal was then dissolved in DMSO (150 μL). The absorbance at 490nm (A490nm) was measured using an enzyme-labeled meter (BioTEK Inc., Biotek MQX200). The effect of treatment with compounds 5 and cisplatin on cell viability was evaluated relative to the readings of the control cells. A490nm represents the number of viable cells.

**Declaration of conflicting interests**

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**Funding**

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: We
gratefully acknowledge financial support of this work by the National Natural Science Foundation of China (81872509), the Hubei Provincial Technology Innovation Project (2017ACA176), the Shiyan Municipal Science and Technology Bureau Science and Technology Project (Grant No. 18K79), the Scientific Research Project of Educational Commission of Hubei Province of China (Grant No. B2018111), and the Postgraduate Innovation Project of Hubei University of Medicine (Grant No. YC2020042).

**ORCID iD**
Xiao-Hua Zeng https://orcid.org/0000-0002-6140-3626

**Supplemental material**
Supplemental material for this article is available online.

**References**
1. Wang GC, Fan YY, Sajan LS, et al. *Org Lett* 2017; 19: 2182–2185.
2. Guo LN, Pei YH, Chen G, et al. *J Asian Nat Prod Res* 2012; 14: 210–215
3. Chen C, Han X, Zou X, et al. *J Biol Chem* 2014; 289: 17184–17194.
4. Zhang HB, Shen QK, Wang H, et al. *Eur J Med Chem* 2018; 15: 414–427.
5. Yokota T, Nomura T, Yoneyama K, et al. *J Pestic Sci* 2017; 42: 58–61.
6. Santos LM, Legendre AO, Villis PCM Jr, et al. *Acta Crystallographica* 2012; 68: o294–297.
7. Poli G, Giambastiani G, Malacria M, et al. *Tetrahedron Lett* 2001; 42: 6287–6289.
8. Sá MM, Fernandes L, Ferreira M, et al. *Tetrahedron Lett* 2008; 49: 1228–1232.
9. Wang HM, Zhu XL, Deng SH, et al. *J Chem Res* 2020; 0: 1–4.
10. Imtara H, Kmail A, Touzani S, et al. *Evid-Based Compl Alt* 2019; 2019: 8768210.
11. Zeng XH, Wang HM, Wu L, et al. *Tetrahedron* 2013: 3823–3828.