

Communication

Synthesis and Antifungal Activity of Carabrone Derivatives

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Abstract: Nine derivatives **6-14** of carabrone (**1**) were synthesized and tested *in vitro* against *Colletotrichum lagenarium* Ell et Halst using the spore germination method. Among all of the derivatives, compounds **6-8** and **12** showed more potent antifungal activity than **1**. Structure-activity relationships (SAR) demonstrated that the γ -lactone was necessary for the antifungal activity of **1**, and the substituents on the C-4 position of **1** could significantly affect the antifungal activity.

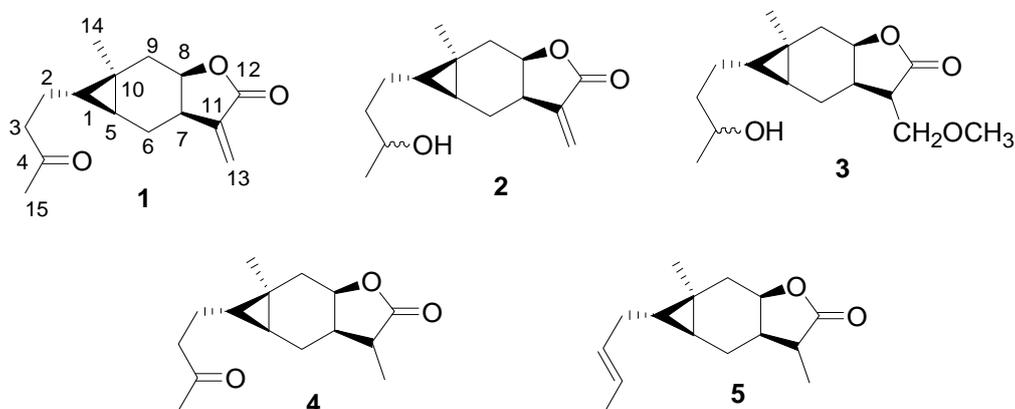
Keywords: carabrone; structural modification; synthesis; antifungal activity

1. Introduction

Carabrone (**1**, Figure 1), containing cyclopropane and sesquiterpene lactone moieties, was first isolated from the fruits of *Carpesium abrotanoides* [1], and is widely distributed in feverfew and other plant species [2-9]. It was demonstrated that compound **1** displays cytotoxic [10], antibacterial [11,12], and antitumor activity [13]. In our course of screening for novel naturally occurring phytofungicides from the plants in northwestern China, compound **1** was obtained from *Carpesium macrocephalum*, and exhibited antifungal activities *in vitro* and *in vivo* against *Botrytis cinerea*, *Colletotrichum lagenarium*, and *Erysiphe graminis* [14]. Subsequently, we prepared four derivatives (**2-5**, Figure 1) from **1**, and found that the 11,13-double bond and the carbonyl group on the C-4 position of **1** are two

active sites [15,16]. In order to further investigate the effect of lactone and substituents on the C-4 position of **1** on the antifungal activity, herein we synthesized nine new carbabrone derivatives **6-14** as potential antifungal agents.

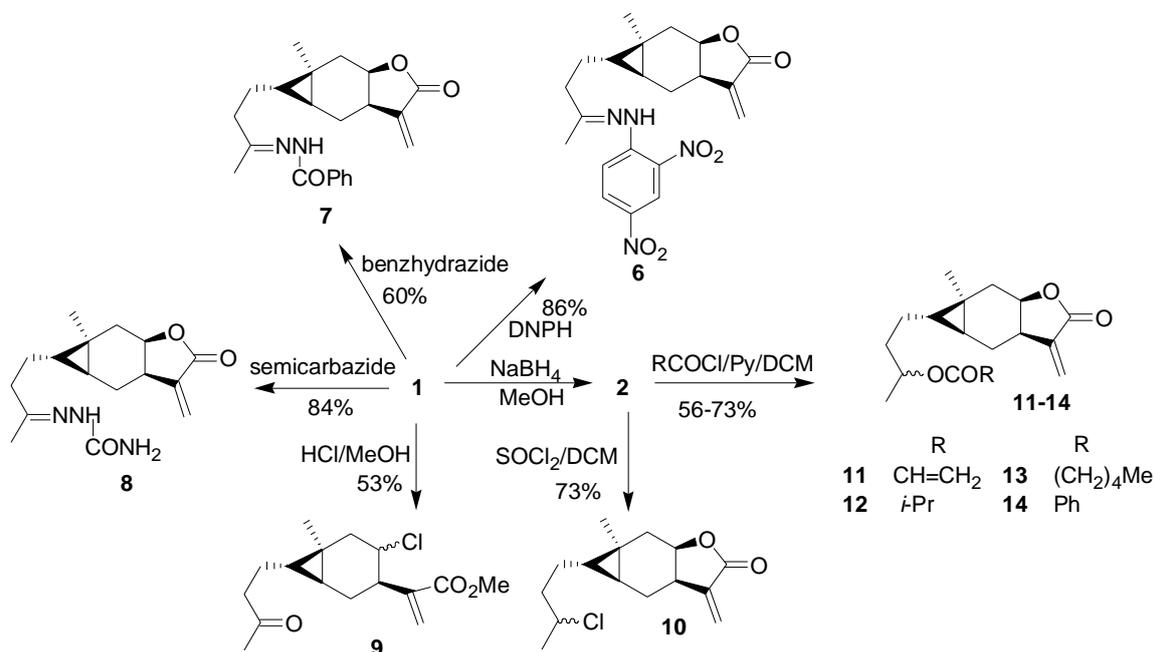
Figure 1. The chemical structures of carbabrone and its derivatives.



2. Results and Discussion

Nine carbabrone derivatives **6-14** were synthesized as shown in Scheme 1. Compound **6** was prepared by the reaction of 2,4-dinitrophenyl hydrazine (DNPH) and **1** in the presence of hydrogen chloride (HCl). Benzhydrazide or semicarbazide reacted with **1** to give compounds **7** and **8**, respectively. Compound **9** was prepared from **1** with dry HCl. Compound **10** was synthesized by the reduction of the carbonyl group of **1** in the presence of NaBH₄, followed by chlorination of the 4-OH group of **2** with thionyl chloride (SOCl₂). Compound **2** reacted with acyl chlorides in the presence of pyridine to afford compounds **11-14**. All compounds were characterized by ¹H-NMR, IR, and HR-MS spectra.

Scheme 1. The synthetic route to carbabrone derivatives **6-14**.



The antifungal activity was assayed *in vitro* against *Colletotrichum lagenarium* Ell *et* Halst by the spore germination method. Chlorothalonil was used as a positive control. As described in Table 1, compounds **6-8** exhibited the most potent antifungal activity with the EC₅₀ values of 2.24, 4.32 and 3.03 µg/mL, respectively, *i.e.*, the antifungal activity of **6**, **7** and **8** was 1.5-3 times more potent than that of **1**. However, the antifungal activity of other compounds was 1-8 times less than that of **1**. Obviously, substituents on the C-4 position of **1** could significantly affect the antifungal activity. For example, introducing the hydrazone substituents on the C-4 position of **1** lead to the most potent compounds (e.g., **6-8**), while when other substituents, such as the hydroxy group, chloro atom, and ester groups (except isobutyryloxy group), were introduced on the C-4 position of **1**, the corresponding compounds showed the less potent activity than **1** (e.g., **10**, **11**, **13** and **14**). Interestingly, when the isobutyryloxy group was introduced on the C-4 position of **1** to give **12**, the EC₅₀ value of **12** was 6.39 µg/mL, which was more potent than that of **1**. Meanwhile, compound **1** was nearly eightfold more potent than **9** (EC₅₀ 7.10 µg/mL for **1** vs. EC₅₀ 56.30 µg/mL for **9**). This demonstrated that the γ -lactone was necessary for the antifungal activity of **1**, and opening the lactone would lead to a less potent compound (**1** vs. **9**).

Table 1. Inhibition rates of carabrone derivates (**6-14**) against spore germination of *Colletotrichum lagenarium*.^a

Compd.	Regression equation (Y = a + bX)	r	EC ₅₀ ^b (µg/mL)	EC ₅₀ 95% CL/(µg/mL)
1	Y = 3.6090 + 1.6337X	0.9974	7.10	6.19~8.02
6	Y = 4.5130 + 1.3891X	0.9923	2.24	1.97~2.55
7	Y = 3.4038 + 2.5118X	0.9817	4.32	3.81~4.85
8	Y = 4.3577 + 1.3351X	0.9979	3.03	2.58~3.55
9	Y = 3.1867 + 1.0358X	0.9942	56.30	42.95~73.80
10	Y = 3.2442 + 1.1736X	0.9969	31.34	26.08~37.66
11	Y = 4.2780 + 0.6720X	0.9882	10.78	9.16~12.68
12	Y = 4.3568 + 0.7209X	0.9920	6.39	5.33~7.65
13	Y = 3.7775 + 0.9365X	0.9970	20.20	16.85~24.22
14	Y = 3.7676 + 1.0011X	0.9962	17.02	14.41~20.11
chlorothalonil ^c	Y = 5.1247 + 1.0081X	0.9935	0.75	0.63~0.90

^a Values are means of three separate experiments; ^b EC₅₀ (50% effective concentration), concentration of compound that reduces spore germination by 50%; ^c Chlorothalonil was used as a positive control.

3. Experimental

3.1. General

All the solvents were of analytical grade and the reagents were commercially available. Thin-layer chromatography (TLC) and silica gel-column chromatography were performed with silica gel plates using silica gel 60 GF₂₅₄, and 200-300 mesh (Qingdao Haiyang Chemical Co., Ltd., China). Melting points were determined on a digital melting-point apparatus and uncorrected. All compounds were characterized by proton nuclear magnetic resonance (¹H-NMR), high-resolution mass spectra (HR-MS), mass spectra (MS-ESI), and infrared spectra (IR), respectively.

3.2. Synthesis

(3*aR*, 4*aS*, 5*S*, 5*aR*, 6*aR*)-5-(3-keto 2, 4-dinitrophenyl hydrazone-butyl)-5*a*-methyl-3-methylene-3*a*, 4, 4*a*, 5, 6, 6*a*-hexahydrocyclopropa[*ff*]benzofuran-2-one (**6**). A mixture of compound **1** (124 mg, 0.5 mmol), 2,4-dinitrophenyl hydrazine (DNPH, 39.6 mg, 2 mmol) and hydrochloric acid (0.2 mL, 6 mol/L) in anhydrous methanol (MeOH, 10 mL) was reacted at 60 °C until a precipitate formed. The reaction mixture was then filtered, and the filtrate was evaporated under reduced pressure. The residue was recrystallized in dimethyl sulfoxide (DMSO) to produce **6** as a yellow solid. Yield: 86%, m.p. 181–182 °C [17]; IR (KBr) cm^{-1} : 3321, 2956, 1756, 1593, 1530, 1345; $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ : 10.79 (s, 1H, =NNH), 8.84 (m, 1H, H-3'), 8.35 (d, $J = 6.8$ Hz, 1H, H-5'), 7.82 (d, $J = 10.0$ Hz, 1H, H-6'), 5.99 (d, $J = 2.4$ Hz, 1H, H-13), 5.62 (d, $J = 2.4$ Hz, 1H, H-13), 4.80 (m, 1H, H-8), 3.31 (m, 2H), 3.18 (m, 1H, H-7), 2.43~2.55 (m, 1H), 2.26~2.35 (m, 1H), 2.05 (s, 3H, H-15), 1.54~1.64 (m, 2H), 1.05 (s, 3H, H-14), 0.85~0.91 (m, 2H), 0.53 (m, 1H, H-5), 0.35 (m, 1H, H-1); HR-MS (ESI): m/z calcd for $\text{C}_{21}\text{H}_{25}\text{N}_4\text{O}_6$ ($[\text{M}+\text{H}]^+$), 429.1769; found, 429.1763.

(3*aR*, 4*aS*, 5*S*, 5*aR*, 6*aR*)-5-(3-keto benzoyl hydrazone-butyl)-5*a*-methyl-3-methylene-3*a*, 4, 4*a*, 5, 6, 6*a*-hexahydrocyclopropa[*ff*]benzofuran-2-one (**7**). A mixture of **1** (125 mg, 0.5 mmol), benzhydrazide (82 mg, 0.6 mmol), and 1-2 drops of HOAc in absolute ethanol (10 mL) was stirred at 80 °C. After 3 h, the solvent was removed under reduced pressure to give a residue, which was dissolved in CH_2Cl_2 . Then the organic phase was washed with H_2O , dried by anhydrous Na_2SO_4 , and evaporated under reduced pressure. Finally, the residue was purified by silica gel-column chromatography using CH_2Cl_2 -EtOAc as the eluent to give **7** as a pale yellow solid. Yield: 60%, m.p. 45–46 °C; $^1\text{H-NMR}$ (500 MHz, CDCl_3) δ : 8.77 (s, 1H, CONH), 7.79 (s, 2H, Ar-H), 7.46 (s, 1H, Ar-H), 7.42 (s, 2H, Ar-H), 6.19-6.20 (m, 1H, H-13), 5.22 (d, $J = 2.2$ Hz, 1H, H-13), 4.72-4.4.77 (m, 1H, H-8), 3.11-3.13 (m, 1H, H-7), 2.48-2.51 (m, 2H), 2.26-2.33 (m, 3H), 2.21 (s, 1H), 1.94 (s, 2H), 1.51-1.73 (m, 2H), 1.23 (s, 1H), 1.06 (s, 3H, H-14), 0.83-0.97 (m, 2H, H-2). MS (ESI): m/z 389 ($[\text{M}+\text{Na}]^+$), 100.

(3*aR*, 4*aS*, 5*S*, 5*aR*, 6*aR*)-5-(3-keto carbamoyl hydrazone-butyl)-5*a*-methyl-3-methylene-3*a*, 4, 4*a*, 5, 6, 6*a*-hexahydrocyclopropa[*ff*]benzofuran-2-one (**8**). A mixture of **1** (223.2 mg, 0.9 mmol), semicarbazide hydrochloride (111.5 mg, 1 mmol) and sodium acetate (176.8 mg, 1.3 mmol) in distilled H_2O (5 mL) was stirred at 60 °C until a white precipitate was produced, then the reaction mixture was filtered, and the filtrate was evaporated under reduced pressure. The residue was recrystallized from distilled H_2O -MeOH to give **8** as a white solid. Yield: 84%, m.p. 171–173 °C; IR (KBr) cm^{-1} : 3317, 2963, 1753, 1690, 1620; $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ : 6.14 (d, $J = 2.6$ Hz, 1H, H-13), 5.64 (d, $J = 2.4$ Hz, 1H, H-13), 4.88 (m, 1H, H-8), 4.78 (s, 1H, =NNH), 4.60 (s, 2H, O=CNH₂), 3.36 (m, 2H), 3.20 (m, 1H, H-7), 2.24~2.27 (m, 1H), 1.97~2.13 (m, 1H), 1.85 (s, 3H, H-15), 1.81~1.84 (m, 1H), 1.43~1.51 (m, 1H), 1.04 (s, 3H, H-14), 0.85~0.92 (m, 2H), 0.53 (m, 1H, H-5), 0.38 (m, 1H, H-1); HR-MS (ESI): m/z calcd for $\text{C}_{16}\text{H}_{24}\text{N}_3\text{O}_3$ ($[\text{M}+\text{H}]^+$), 306.1812; found, 306.1812.

4-oxo-7 β -(2-methylformate) propene-8-chloro-carabrane (**9**) [18]. Compound **1** (248 mg, 1 mmol) was dissolved in anhydrous MeOH (40 mL) under reflux, and a flow of dry HCl was passed for 6 h. When the starting material was nearly complete as checked by TLC, the reaction mixture was cooled to room temperature. Sodium bicarbonate (NaHCO_3 , 20 mg) and distilled H_2O (30 mL) were added to

the above mixture, which was extracted with CH₂Cl₂ (50 mL × 3). The organic phases were combined, and washed with 2% aq. NaHCO₃, and distilled H₂O, dried over anhydrous Na₂SO₄, and evaporated under reduced pressure. Finally, the residue was purified by silica gel-column chromatography using petroleum ether-EtOAc as the eluent to give **9** as a colorless oily liquid. Yield: 53%, [α]_D¹⁸ +49.7 (c 0.35, CHCl₃); IR (KBr) cm⁻¹ 2942, 1756, 1695, 1632; ¹H-NMR (400 MHz, CDCl₃) δ: 6.13 (d, J = 2.6 Hz, 1H, H-13), 5.58 (d, J = 2.4 Hz, 1H, H-13), 3.85 (m, 1H, H-8), 3.76 (s, 3H, OCH₃), 3.31 (m, 2H), 2.82 (m, 1H, H-7), 2.26~2.13 (m, 2H), 1.94 (s, 3H, H-15), 1.58~1.63 (m, 2H), 1.16 (s, 3H, H-14), 0.86~0.93 (m, 2H), 0.54 (m, 1H, H-5), 0.32 (m, 1H, H-1); HR-MS (ESI): m/z calcd for C₁₆H₂₇NO₃Cl ([M+NH₄]⁺), 316.1697; found, 316.1695.

(3aR, 4aS, 5S, 5aR, 6aR)-5-(3-chloro-butyl)-5a-methyl-3-methylene-3a, 4, 4a, 5, 6, 6a-hexahydrocyclopropa[*ff*]benzofuran-2-one (**10**) [19]. A mixture of **2** (100 mg, 0.4 mmol) and pyridine (0.1 mL) in anhydrous CH₂Cl₂ (15 mL) was stirred at 0 °C. Thionyl chloride (SOCl₂, 0.1 mL) was added dropwise. After the addition was complete, the mixture was stirred under reflux. When the starting material was nearly completely consumed, as checked by TLC, NaHCO₃ (20 mg) and distilled H₂O (10 mL) were added to the above mixture, which was extracted with CH₂Cl₂ (20 mL × 3). The organic phases were combined, washed with 0.3% HCl, saturated aq. Na₂CO₃ and brine, dried over anhydrous Na₂SO₄, and evaporated under reduced pressure. Finally, the residue was purified by silica gel-column chromatography using petroleum ether-EtOAc as the eluent to produce **10** as colorless acicular crystals. Yield: 73%, m.p. 97–99 °C; IR (KBr) cm⁻¹: 2943, 1757, 1625, 1148, 628; ¹H NMR (400 MHz, CDCl₃) δ: 6.24 (d, J = 2.6 Hz, 1H, H-13), 5.56 (d, J = 2.4 Hz, 1H, H-13), 4.81 (m, 1H, H-8), 4.04 (m, 1H, H-4), 3.14 (m, 1H, H-7), 2.17~2.37 (m, 2H, H-3), 1.79~1.83 (m, 2H), 1.56 (d, J = 6.0 Hz, 3H, H-15), 1.43~1.46 (m, 2H), 1.09 (s, 3H, H-14), 0.88~0.95 (m, 2H), 0.47 (m, 1H, H-5), 0.38 (m, 1H, H-1); HR-MS (ESI): m/z calcd for C₁₅H₂₅NO₂Cl ([M+NH₄]⁺), 286.1568; found, 286.1565.

General procedure for the synthesis of compounds 11-14 [20]

A mixture of **2** (100 mg, 0.4 mmol) and pyridine (0.1 mL) in anhydrous CH₂Cl₂ (15 mL) was stirred at 0 °C. Acyl chloride (0.1 mL) in anhydrous CH₂Cl₂ (2 mL) was added dropwise. After the addition, the mixture was stirred under reflux. When the reaction was nearly complete, as checked by TLC, NaHCO₃ (20 mg) and distilled H₂O (10 mL) were added to the above mixture, which was extracted with CH₂Cl₂ (30 mL × 3). The organic phases were combined, washed by 0.3% HCl, saturated aq. Na₂CO₃ and brine, and evaporated under the reduced pressure. Finally, the residue was purified by silica gel column chromatography using petroleum ether-acetone as the eluent to give compounds **11-14**.

(3aR, 4aS, 5S, 5aR, 6aR)-5-(3-vinylCarbonate-butyl)-5a-methyl-3-methylene-3a, 4, 4a, 5, 6, 6a-hexahydrocyclopropa[*ff*]benzofuran-2-one (**11**). Yield: 73%, a colorless oily liquid; [α]_D¹⁸ +58.7 (c 0.47, CHCl₃); IR (KBr) cm⁻¹: 2977, 1757, 1715, 1642, 1203; ¹H-NMR (400 MHz, CDCl₃) δ: 6.40 (d, J = 16.8 Hz, 1H, CH=CH₂), 6.23 (d, J = 2.8 Hz, 1H, H-13), 6.10 (m, 1H, CH=CH₂), 5.82 (d, J = 10.8 Hz, 1H, CH=CH₂), 5.56 (d, J = 2.4 Hz, 1H, H-13), 4.99 (m, 1H, H-8), 4.78 (m, 1H, H-4), 3.16 (m, 1H, H-7), 2.31~2.39 (m, 2H), 1.59~1.62 (m, 2H), 1.36~1.45 (m, 2H), 1.27 (d, J = 6.0 Hz, 3H, H-

15), 1.06 (s, 3H, H-14), 0.85~0.99 (m, 2H), 0.43 (m, 1H, H-5), 0.35 (m, 1H, H-1); HR-MS (ESI): m/z calcd for $C_{18}H_{28}NO_4$ ($[M+NH_4]^+$), 322.2013; found, 322.2008.

(3*a*R, 4*a*S, 5*S*, 5*a*R, 6*a*R)-5-(3-isopropylCarbonate-butyl)-5*a*-methyl-3-methylene-3*a*, 4, 4*a*, 5, 6, 6*a*-hexahydrocyclopropa[*ff*]benzofuran-2-one (**12**). Yield: 72%; a colorless oily liquid; $[\alpha]_D^{18} +36.4$ (C 0.41, $CHCl_3$); IR (KBr) cm^{-1} : 2974, 1757, 1722, 1660, 1148; 1H -NMR (400 MHz, $CDCl_3$) δ : 6.23 (d, $J = 2.6$ Hz, 1H, H-13), 5.56 (d, $J = 2.4$ Hz, 1H, H-13), 4.95 (m, 1H, H-8), 4.80 (m, 1H, H-4), 3.16 (m, 1H, H-7), 2.51 (m, 1H, $CH(CH_3)_2$), 2.30~2.38 (m, 2H), 1.59~1.61 (m, 2H), 1.35~1.44 (m, 2H), 1.23 (d, $J = 6.0$ Hz, 3H, H-15), 1.18 (d, $J = 10.8$ Hz, 3H, $CHCH_3$), 1.14 (d, $J = 11.2$ Hz, 3H, $CHCH_3$), 1.07 (s, 3H, H-14), 0.87~0.98 (m, 2H), 0.43 (m, 1H, H-5), 0.34 (m, 1H, H-1); HR-MS (ESI): m/z calcd for $C_{19}H_{28}O_4Na$ ($[M+Na]^+$), 343.1880; found, 343.1875.

(3*a*R, 4*a*S, 5*S*, 5*a*R, 6*a*R)-5-(3-pentylCarbonate-butyl)-5*a*-methyl-3-methylene-3*a*, 4, 4*a*, 5, 6, 6*a*-hexahydrocyclopropa[*ff*]benzofuran-2-one (**13**). Yield: 70%; a colorless oily liquid; $[\alpha]_D^{18} +49.4$ (C 0.52, $CHCl_3$); IR (KBr) cm^{-1} : 2935, 1722, 1660, 1146; 1H -NMR (400 MHz, $CDCl_3$) δ : 6.23 (d, $J = 2.6$ Hz, 1H, H-13), 5.56 (d, $J = 2.4$ Hz, 1H, H-13), 4.95 (m, 1H, H-8), 4.78 (m, 1H, H-4), 3.16 (m, 1H, H-7), 3.01~3.04 (m, 1H, $O=CCH_2$), 2.51~2.54 (m, 1H, $O=CCH_2$), 2.30~2.38 (m, 2H), 1.59~1.61 (m, 2H), 1.27~1.51 (m, 8H), 1.23 (d, $J = 6.0$ Hz, 3H, H-15), 1.06 (s, 3H, H-14), 0.90~0.99 (m, 2H), 0.86~0.89 (m, 3H, CH_2CH_3), 0.43 (m, 1H, H-5), 0.35 (m, 1H, H-1); HR-MS (ESI): m/z calcd for $C_{21}H_{36}NO_4$ ($[M+NH_4]^+$), 366.2639; found, 366.2632.

(3*a*R, 4*a*S, 5*S*, 5*a*R, 6*a*R)-5-(3-phenylCarbonate-butyl)-5*a*-methyl-3-methylene-3*a*, 4, 4*a*, 5, 6, 6*a*-hexahydrocyclopropa[*ff*]benzofuran-2-one (**14**). Yield: 56%; a colorless oily liquid; $[\alpha]_D^{18} +73.9$ (C 0.31, $CHCl_3$); IR (KBr) cm^{-1} : 2942, 1757, 1711, 1485, 715; 1H -NMR (400 MHz, $CDCl_3$) δ : 8.03 (m, 2H, H-3', 5'), 7.57 (m, 1H, H-4'), 7.46 (m, 2H, H-2', 6'), 6.24 (d, $J = 2.6$ Hz, 1H, H-13), 5.55 (d, $J = 2.4$ Hz, 1H, H-13), 5.18 (m, 1H, H-8), 4.77 (m, 1H, H-4), 3.15 (m, 1H, H-7), 2.28~2.36 (m, 2H), 1.63~1.79 (m, 2H), 1.42~1.56 (m, 2H), 1.23 (d, $J = 6.0$ Hz, 3H, H-15), 1.08 (s, 3H, H-14), 0.88~0.92 (m, 2H), 0.47 (m, 1H, H-5), 0.35 (m, 1H, H-1); HR-MS (ESI): m/z calcd for $C_{22}H_{30}NO_4$ ($[M+NH_4]^+$), 372.2169; found, 372.2168.

3.3. Spore Germination Assay

Microorganisms and maintenance: the strain of *Colletotrichum lagenarium* (36199) was provided by Agricultural Culture Collection of China and maintained on potato dextrose agar (PDA). Compounds **1** and **6-14** were dissolved in acetone or DMSO and added to 2% water agar medium after sterilization to produce concentrations of 100, 75, 50, 25, 10, and 5 $\mu g/mL$ or 10, 5, 2, 1, 0.5 and 0.25 $\mu g/mL$ of medium. Conidial suspensions (0.2 mL) containing 1×10^5 conidia/mL, derived from cultures grown for 12 d on PDA plates, were spread on 2% water agar. Conidia were allowed to germinate 25 ± 1 °C for 8 h. Germination was quantified at three sites by counting 100 conidia per site. A conidium was scored as germinated if the germ tube had reached at least half the length of the conidium. Three plates for each concentration were used and the experiment was performed thrice, along with 98% chlorothalonil (Syngenta Crop Protection Co., Ltd., China) as a positive control. The

EC₅₀ for inhibition of spore germination was calculated for each isolate. Analysis of parameters was made with the statistical analysis system (SAS institute, Inc., Cary, NC, USA) [21].

4. Conclusions

In summary, nine new carabrone derivatives were synthesized and evaluated *in vitro* against *Colletotrichum lagenarium* Ell et Halst. Compounds **6-8**, and **12** displayed the more potent antifungal activity than **1**. Meanwhile, the structure-activity relationship (SAR) demonstrated that a γ -lactone moiety was necessary for the antifungal activity of **1**, and the substituents on the C-4 position of **1** could significantly affect their antifungal activity, e.g., introduction of the hydrazone substituents on the C-4 position of **1** lead to more potent compounds.

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Sample Availability: Samples of the compounds are available from the authors.

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