

Article

Synthesis, Antifungal Activities and Qualitative Structure Activity Relationship of Carabrone Hydrazone Derivatives as Potential Antifungal Agents

Hao Wang, Shuang-Xi Ren, Ze-Yu He, De-Long Wang, Xiao-Nan Yan, Jun-Tao Feng * and Xing Zhang

Research & Development Center of Biorational Pesticide, Key Laboratory of Plant Protection Resources and Pest Management of Ministry of Education, Northwest A&F University, Xinong Road 22, Yangling 712100, Shaanxi, China; E-Mails: k2wanghao@gmail.com (H.W.); rensx123@gmail.com (S.-X.R.); skyhugh2012@gmail.com (Z.-Y.H.); moraldragonwang@gmail.com (D.-L.W.); orgshylock@gmail.com (X.-N.Y.); zhxing1952@126.com (X.Z.)

* Author to whom correspondence should be addressed; E-Mail: fengjt67@hotmail.com; Tel./Fax: +86-29-8709-2122.

Received: 23 January 2014; in revised form: 24 February 2014 / Accepted: 4 March 2014 / Published: 11 March 2014

Abstract: Aimed at developing novel fungicides for relieving the ever-increasing pressure of agricultural production caused by phytopathogenic fungi, 28 new hydrazone derivatives of carabrone, a natural bioactive sesquiterpene, in three types were designed, synthesized and their antifungal activities against *Botrytis cinerea* and *Colletotrichum lagenarium* were evaluated. The result revealed that all the derivatives synthesized exhibited considerable antifungal activities *in vitro* and *in vivo*, which led to the improved activities for carabrone and its analogues and further confirmed their potential as antifungal agents.

Keywords: carabrone; hydrazone derivatives; chemical modification; antifungal activity; structure–activity relationship

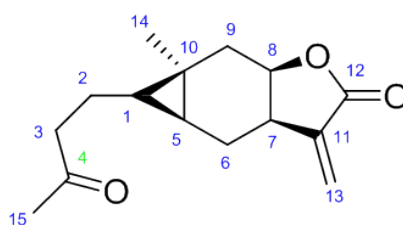
1. Introduction

Emerging infectious diseases caused by fungi are increasingly recognized as presenting a worldwide threat to food security, and pathogenic fungi have long been known to constitute a

widespread threat to plant species [1,2]. Plant disease epidemics caused by fungi or the fungal-like oomycetes can infect diverse economically important crops and result in severe yield losses and quality reduction of agricultural production [3–5]. Chemical fungicides are widely used to protect crops against these losses at present, but the application of chemical fungicides is challenged by the incidence of resistance and residues [4]. Thus, the discovery of natural antifungal compounds along with further development is important to address these challenges.

Carabrone (Figure 1), first isolated from the fruits of *Carpesium abrotanoides* [6], is a well-known sesquiterpene and exhibits significant antibacterial [7,8] and antitumor activities [9,10]. We have demonstrated the antifungal activities of carabrone and its derivatives against *Colletotrichum lagenarium* [11,12] with α -methylene- γ -butyrolactone [13,14] previously, and found the C-4 position of carabrone was a key to their activities [11,12]. Besides, as part of an effort to develop environment friendly antifungal agents from natural products, researchers from our laboratory have designed and synthesized a series of carabrone derivatives to explore the qualitative structure–activity relationship (QSAR) hidden in them and the result implied that the derivatives of carabrone with a C=N double bond on the C-4 position may display stronger antifungal activity against *C. lagenarium* than others.

Figure 1. The structure of carabrone.



As a continuation of our project aimed at developing novel fungicides with carabrone as the lead compound, three types of hydrazone derivatives of carabrone were designed in this study by adding a hydrazine or hydrazide group to the C-4 site. To our best knowledge, the type of carabrone derivatives formed in sulfonyl hydrazone has not been reported before. Besides, in order to determine whether the carabrone hydrazone derivatives (Figure 2, **6a–q**, **7r–s**, **8a–i**) have the potential for further development, their antifungal activities against *C. lagenarium* *in vitro* and against *Botrytis cinerea* *in vitro* and *in vivo* were evaluated [15]. In addition, the QSAR of these compounds were also investigated; it is the first time the QSAR of carabrone derivatives have been obtained based on the experiments both *in vitro* and *in vivo*.

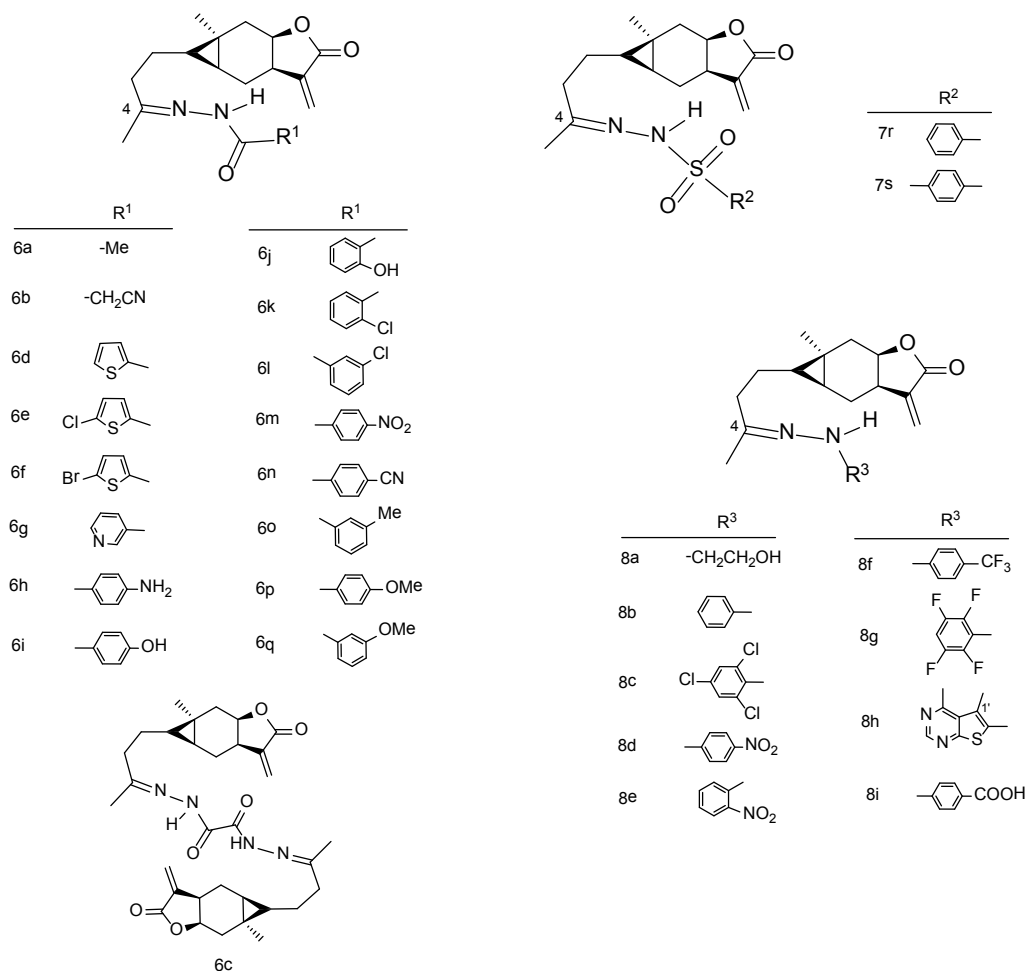
2. Results and Discussion

2.1. Synthesis

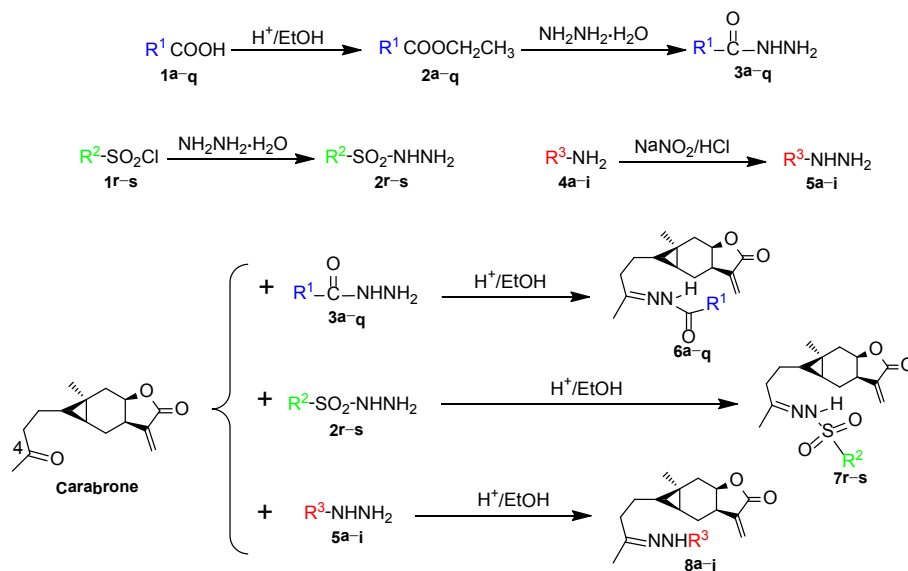
Structure modification on active natural compounds is an important way to develop new drugs with improved activities and reduced side effects. Based on our previous finding that antifungal activity can be improved significantly by the introduction of a C=N group to the C-4 position of carabrone, catalyzed by glacial acetic acid, 28 new title compounds forming in hydrazone, acyl hydrazone and sulfonyl hydrazone were obtained (Scheme 1) by reacting carabrone with the intermediates prepared

prior to use. To our best knowledge, it is the first time to synthesize carabrone derivatives in sulfonyl hydrazone (7r–s). The structures of all the derivatives were characterized by ^1H NMR, ^{13}C NMR and high-resolution electrospray ionization mass spectrometry (HR-ESI-MS).

Figure 2. Structures of carabrone hydrazone derivatives.



Scheme 1. Synthetic route of compounds 6a–q, 7r–s and 8a–i.



2.2. Antifungal Activity

All the derivatives exhibited significant antifungal activities in spore germination assay with IC_{50} (50% inhibitory concentration, analyzed by SPSS for windows) values ranging from 1.27–27.33 $\mu\text{g/mL}$ against *B. cinerea* and 0.77–15.23 $\mu\text{g/mL}$ against *C. lagenarium* (Table 1). In addition, all derivatives also showed strong antifungal activities against *B. cinerea in vivo* (IC_{50} ranged from 2.10–30.80 $\mu\text{g/mL}$), when testing their inhibition on colonial growth of *B. cinerea* using tomato fruits. Among them, compounds **8g** with the IC_{50} value of 1.27 $\mu\text{g/mL}$ against *B. cinerea* and **6q** with 0.77 $\mu\text{g/mL}$ against *C. lagenarium* successively showed the strongest activities to corresponding pathogens *in vitro*, and no significant differences were showed to a commercial fungicide, chlorothalonil. In addition, compound **8c**, with the IC_{50} of 2.10 $\mu\text{g/mL}$ against *B. cinerea*, exhibited the strongest activity *in vivo*.

Comparing among carabrone, carabrol and their derivatives [12], it was confirmed that antifungal activity against *C. lagenarium* increased significantly with the introduction of a C=N double bond to the C-4 position. Only two compounds as sulfonyl hydrazones of carabrone were designed in our project; they both showed medium antifungal activities. The exact QSAR should be obtained from more derivatives of this type in the future. In addition, it should be especially noted that in our ongoing research, carabrone hydrazone derivatives also showed higher antifungal activities than their oxime ester analogues with the same C=N double bond on the C-4 position, both *in vitro* and *in vivo*. This may indicate that the changes in spatial conformation except for the functional group had significant influence on its antifungal activity. In a word, focusing on the lead compound, this study led to an improved activity of carabrone and its analogues and further confirmed their potential as antifungal agents.

2.3. Qualitative Structure–Activity Relationship

QSAR analysis based on the activities of hydrazone derivatives of carabrone *in vitro* and *in vivo* would provide some insight into potential rational optimization of active natural compounds to develop new fungicides.

Generally, the introduction of chlorine atom or bromine atom on the substituent group may improve the antifungal activity of the hydrazone derivatives of carabrone, both *in vitro* and *in vivo*. For example, when a chlorine atom was introduced into compound **6d**, affording **6e**, the antifungal activity increased, with the *in vitro* and *in vivo* IC_{50} against *B. cinerea* decreasing from 7.81 and 12.84 $\mu\text{g/mL}$ to 5.57 and 9.57 $\mu\text{g/mL}$, and with the *in vitro* IC_{50} against *C. lagenarium* decreasing from 10.83–9.98 $\mu\text{g/mL}$. When a bromine atom was introduced into **6d**, generating **6f**, the *in vitro* antifungal activities against *B. cinerea* and *C. lagenarium* were enhanced, with an IC_{50} of 4.83 and 10.02 $\mu\text{g/mL}$, respectively, along with an improved antifungal activity against *B. cinerea in vivo* at 7.03 $\mu\text{g/mL}$.

The hydrazone derivatives of carabrone containing meta-substituted R groups showed higher antifungal activities than those derivatives with para-substituted R groups. On the other hand, the antifungal activities of the derivatives with ortho-substituted R groups were lower than those of derivatives with para-substituted R groups. For example, compound **6q** (*in vitro* IC_{50} : 6.37 $\mu\text{g/mL}$ against *B. cinerea* and 0.77 $\mu\text{g/mL}$ against *C. lagenarium*; *in vivo* IC_{50} : 12.52 $\mu\text{g/mL}$ against *B. cinerea*) showed stronger antifungal activities than that of **6p** (*in vitro* IC_{50} : 10.30 $\mu\text{g/mL}$ against *B. cinerea* and

2.56 $\mu\text{g/mL}$ against *C. lagenarium*; *in vivo* IC_{50} : 16.42 $\mu\text{g/mL}$ against *B. cinerea*). Compound **8e** (*in vitro* IC_{50} : 10.31 $\mu\text{g/mL}$ against *B. cinerea* and 4.27 $\mu\text{g/mL}$ against *C. lagenarium*; *in vivo* IC_{50} : 8.93 $\mu\text{g/mL}$ against *B. cinerea*) exhibited a weaker activity than that of compound **8d** (*in vitro* IC_{50} : 3.79 $\mu\text{g/mL}$ against *B. cinerea* and 3.95 $\mu\text{g/mL}$ against *C. lagenarium*; *in vivo* IC_{50} : 4.62 $\mu\text{g/mL}$ against *B. cinerea*). In addition, compound **6j** exhibited a weaker activity than that of compound **6i**, following the same rules.

Table 1. Chemical yields and antifungal activities of the 28 hydrazone derivatives of carabrone.

Compound No.	Yield ^a (%)	<i>In vitro</i> (IC_{50} ^b , $\mu\text{g/mL}$)		<i>In vivo</i> (IC_{50} ^b , $\mu\text{g/mL}$)
		<i>B. cinerea</i>	<i>C. lagenarium</i>	<i>B. cinerea</i>
6a	78	27.33 \pm 1.29	10.18 \pm 1.02	29.62 \pm 3.12
6b	82	9.77 \pm 0.68	8.14 \pm 0.34	7.55 \pm 0.68
6c	93	22.07 \pm 0.70	10.22 \pm 0.62	30.80 \pm 1.51
6d	78	7.81 \pm 0.37	10.83 \pm 1.79	12.84 \pm 0.31
6e	87	5.57 \pm 0.89	9.98 \pm 0.30	9.57 \pm 0.99
6f	72	4.83 \pm 0.41	10.02 \pm 0.72	7.03 \pm 0.58
6g	70	8.50 \pm 0.69	2.06 \pm 0.86	4.02 \pm 0.26
6h	78	2.67 \pm 0.10	2.10 \pm 0.19	4.85 \pm 0.52
6i	59	3.46 \pm 0.39	1.24 \pm 0.47	4.73 \pm 1.13
6j	48	13.16 \pm 0.23	2.01 \pm 0.34	13.44 \pm 1.02
6k	77	3.35 \pm 0.65	3.52 \pm 0.21	9.67 \pm 0.95
6l	78	2.57 \pm 0.12	1.97 \pm 0.78	8.76 \pm 1.08
6m	61	3.39 \pm 0.56	5.49 \pm 0.73	6.67 \pm 0.49
6n	88	2.47 \pm 0.72	1.69 \pm 0.54	4.29 \pm 0.51
6o	65	9.00 \pm 1.09	0.98 \pm 0.19	18.08 \pm 0.91
6p	53	10.30 \pm 0.86	2.56 \pm 0.32	16.42 \pm 1.24
6q	47	6.37 \pm 0.71	0.77 \pm 0.27	12.52 \pm 0.96
7r	66	13.32 \pm 0.87	6.43 \pm 0.95	17.46 \pm 0.88
7s	73	12.99 \pm 0.62	5.33 \pm 0.89	17.26 \pm 1.05
8a	58	17.37 \pm 0.91	15.23 \pm 1.14	16.69 \pm 2.37
8b	47	16.32 \pm 0.58	7.62 \pm 0.19	18.85 \pm 1.25
8c	75	1.51 \pm 0.73	1.53 \pm 0.46	2.10 \pm 0.47
8d	65	3.79 \pm 0.82	3.95 \pm 0.28	4.62 \pm 0.29
8e	77	10.31 \pm 1.31	4.27 \pm 0.35	8.93 \pm 0.96
8f	72	1.99 \pm 0.34	4.05 \pm 0.57	5.52 \pm 0.37
8g	63	1.27 \pm 0.16	2.65 \pm 0.91	2.59 \pm 0.63
8h	81	13.32 \pm 0.87	1.63 \pm 0.45	11.46 \pm 1.09
8i	45	14.77 \pm 0.59	7.06 \pm 0.77	14.59 \pm 0.95
Carabrone		14.14 \pm 0.94	8.29 \pm 0.51	16.74 \pm 1.32
Chlorothalonil ^c		0.49 \pm 0.28	0.52 \pm 0.17	1.29 \pm 0.33

^a chemical yield at the final step of the synthesis; ^b IC_{50} represents 50% inhibitory concentration that are presented as the means \pm SD ($n = 3$), $\mu\text{g/mL}$; ^c positive control.

In addition, the hydrazone derivatives of carabrone with a N-containing heterocyclic (**6g,8h**) or S-containing heterocyclic (**6d–f**) all showed stronger antifungal activities against *B. cinerea* *in vitro* and *in vivo* than that of the lead compound.

3. Experimental Section

3.1. General

The melting points of the compounds were determined using an X-4 apparatus (uncorrected, Beijing Tech. Instrument Co., Beijing, China). ^1H NMR, ^{13}C NMR and 2D-NMR spectra were obtained using a Bruker Avance 500 MHz spectrometer (Bruker, Bremerhaven, Germany) in CDCl_3 or CD_3COCD_3 solution with TMS as the internal standard. HR-ESI-MS spectra were carried out using Bruker/Germany apex-ultra 7.0 T spectrometer (Bruker, Bremerhaven, Germany). Carabrone was isolated from *Carpesium macrocephalu* collected in Gansu province, China. Carboxylic acid reagents were purchased from J&K China Chemical Ltd. (Beijing, China). All organic solvents were commercial products and were purified by standard techniques prior to use. Silica gel for TLC and CC was obtained from Qingdao Haiyang Chemical Co. Ltd. (Qingdao, China).

3.2. Synthesis of Title Compounds

As shown in Scheme 1, 10 mmol carboxylic acid (**1a–q**) was first reacted with excess ethanol in a three-necked, round-bottomed flask containing concentrated sulfuric acid as catalyst, reflux for 5 h, to get the corresponding esters (**2a–q**) [16]. The esters were then reacted with excess hydrazine hydrate directly without further purification. The second reaction was performed under reflux for 5–8 h until the reaction was complete as determined by TLC. The intermediates (**3a–q**) were isolated by re-crystallization and then reacted with carabrone (100 mg, 0.4 mmol) to obtain the target compounds (**6a–q**, Figure 2), catalyzed by glacial acetic acid [17] (See Supplementary for more information).

3.2.1. *N'*-(4-((4a*S*,5*S*,5a*R*)-5a-Methyl-3-methylene-2-oxooctahydro-2*H*-cyclopropa[*f*]benzofuran-5-yl)butan-2-ylidene)acetohydrazide (**6a**)

^1H NMR (500 MHz, CDCl_3) δ : 9.16 (1H, s), 6.22 (1H, d, $J = 2.6$ Hz), 5.56 (1H, d, $J = 2.6$ Hz), 4.77–4.82 (1H, m), 3.17–3.18 (1H, m), 2.37 (2H, t, $J = 7.6$ Hz), 2.30–2.34 (2H, m), 2.23 (3H, s), 1.88 (3H, s), 1.49–1.64 (2H, m), 1.09 (3H, s), 0.89–1.00 (2H, m), 0.47–0.49 (1H, m), 0.36–0.40 (m, 1H, *H*-5); ^{13}C NMR (125 MHz, CDCl_3) δ : 173.7, 170.5, 152.0, 139.0, 122.5, 75.7, 38.8, 37.7, 37.3, 34.4, 30.8, 25.7, 22.9, 20.5, 18.3, 17.1, 15.5; HR-MS (ESI): m/z calcd for $\text{C}_{17}\text{H}_{25}\text{N}_2\text{O}_3$ ($[\text{M} + \text{H}]^+$), 305.1860; found, 305.1860.

3.2.2. 2-Cyano-*N'*-(4-((4a*S*,5*S*,5a*R*)-5a-methyl-3-methylene-2-oxooctahydro-2*H*-cyclopropa[*f*]benzofuran-5-yl)butan-2-ylidene)acetohydrazide (**6b**)

^1H NMR (500 MHz, CDCl_3) δ : 10.02 (1H, s), 6.24 (1H, d, $J = 2.6$ Hz), 5.56 (1H, d, $J = 2.6$ Hz), 4.75–4.81 (1H, m), 3.81 (2H, s), 3.12–3.23 (1H, m), 2.38 (2H, t, $J = 7.6$ Hz), 2.25–2.31 (2H, m), 1.95 (3H, s), 1.48–1.63 (2H, m), 1.09 (3H, s), 0.90–1.01 (2H, m), 0.40–0.46 (1H, m), 0.37–0.41 (1H, m); ^{13}C NMR (125 MHz, CDCl_3) δ : 170.6, 165.1, 156.0, 139.1, 129.0, 122.5, 75.7, 38.8, 37.5, 37.1, 33.9, 30.5, 25.6, 24.4, 22.8, 18.1, 17.0, 16.2; HR-MS (ESI): m/z calcd for $\text{C}_{18}\text{H}_{24}\text{N}_3\text{O}_3$ ($[\text{M} + \text{H}]^+$), 330.1812; found, 330.1812.

3.2.3. *N*'1,*N*'2-Bis(4-((4*aS*,5*S*,5*aR*)-5*a*-methyl-3-methylene-2-oxooctahydro-2*H*-cyclo-propa[f]benzofuran-5-yl)butan-2-ylidene)oxalohydrazide (**6c**)

¹H NMR (500 MHz, CDCl₃) δ: 9.93 (2H, s), 6.23 (2H, d, *J* = 2.6 Hz), 5.56 (2H, d, *J* = 2.6 Hz), 4.85–4.98 (2H, m), 3.11–3.19 (2H, m), 2.49 (4H, t, *J* = 7.6 Hz), 2.30–2.37 (4H, m), 2.01 (6H, s), 1.48–1.75 (4H, m), 1.09 (6H, s), 0.89–1.01 (4H, m), 0.45–0.51 (2H, m), 0.36–0.42 (2H, m); ¹³C NMR (125 MHz, CDCl₃) δ: 170.5, 162.4, 155.2, 139.0, 122.6, 75.6, 39.1, 37.7, 37.2, 34.3, 30.7, 26.1, 23.0, 18.4, 17.3, 15.9; HR-MS (ESI): *m/z* calcd for C₃₂H₄₃N₄O₆ ([M + H]⁺), 579.3177; found, 579.3183.

3.2.4. *N*'-(4-((4*aS*,5*S*,5*aR*)-5*a*-Methyl-3-methylene-2-oxooctahydro-2*H*-cyclopropa[f]-benzofuran-5-yl)butan-2-ylidene)thiophene-2-carbohydrazide (**6d**)

¹H NMR (500 MHz, CDCl₃) δ: 10.39 (1H, s), 8.14 (1H, d, *J* = 3.7 Hz), 7.62 (1H, d, *J* = 4.9 Hz), 7.10 (1H, dd, *J* = 4.9, 3.7 Hz), 6.16 (1H, d, *J* = 2.6 Hz), 5.48 (1H, d, *J* = 2.6 Hz), 4.72–4.77 (1H, m), 3.05–3.14 (1H, m), 2.45–2.48 (2H, t, *J* = 7.1 Hz, *H*-3), 2.25–2.32 (2H, m), 2.05 (3H, s), 1.62–1.74 (2H, m), 1.09 (3H, s), 0.82–0.96 (2H, m), 0.48–0.54 (1H, m), 0.35–0.39 (1H, m); ¹³C NMR (125 MHz, CDCl₃) δ: 170.5, 163.1, 154.0, 139.1, 135.0, 134.5, 133.3, 126.2, 122.4, 75.7, 38.8, 37.6, 37.3, 34.4, 30.7, 26.2, 22.9, 18.3, 17.1, 16.0; HR-MS (ESI): *m/z* calcd for C₂₀H₂₅N₂O₃S ([M + H]⁺), 373.1580; found, 373.1581.

3.2.5. 5-Chloro-*N*'-(4-((4*aS*,5*S*,5*aR*)-5*a*-methyl-3-methylene-2-oxooctahydro-2*H*-cyclopropa[f]benzofuran-5-yl)butan-2-ylidene)thiophene-2-carbohydrazide (**6e**)

¹H NMR (500 MHz, CDCl₃) δ: 9.78 (1H, s), 7.90 (1H, d, *J* = 4.2 Hz), 6.96 (1H, d, *J* = 4.2 Hz), 6.23 (1H, d, *J* = 2.6 Hz), 5.53 (1H, d, *J* = 2.6 Hz), 4.76–4.81 (1H, m), 3.12–3.17 (1H, m), 2.50 (2H, t, *J* = 7.6 Hz), 2.28–2.39 (2H, m), 2.00 (3H, s), 1.49–1.81 (2H, m), 1.14 (3H, s), 0.89–1.00 (2H, m), 0.51–0.56 (1H, m), 0.38–0.43 (1H, m); ¹³C NMR (125 MHz, CDCl₃) δ: 170.5, 162.0, 153.6, 139.9, 139.0, 134.5, 130.2, 125.5, 122.6, 75.7, 38.7, 37.7, 37.3, 34.3, 30.8, 26.3, 23.1, 18.4, 17.2, 15.8; HR-MS (ESI): *m/z* calcd for C₂₀H₂₄ClN₂O₃S ([M + H]⁺), 407.1191; found, 407.1191.

3.2.6. 5-Bromo-*N*'-(4-((4*aS*,5*S*,5*aR*)-5*a*-methyl-3-methylene-2-oxooctahydro-2*H*-cyclo-propa[f]benzofuran-5-yl)butan-2-ylidene)thiophene-2-carbohydrazide (**6f**)

¹H NMR (500 MHz, CDCl₃) δ: 9.86 (1H, s), 7.86 (1H, d, *J* = 4.2 Hz), 7.09 (1H, d, *J* = 4.2 Hz), 6.22 (1H, d, *J* = 2.5 Hz), 5.53 (1H, d, *J* = 2.5 Hz), 4.76–4.81 (1H, m), 3.12–3.18 (1H, m), 2.49 (2H, t, *J* = 7.1 Hz, *H*-3), 2.28–2.39 (2H, m), 2.01 (3H, s), 1.57–1.80 (2H, m), 1.15 (3H, s), 0.89–1.00 (2H, m), 0.51–0.56 (1H, m), 0.38–0.44 (1H, m); ¹³C NMR (125 MHz, CDCl₃) δ: 170.5, 162.0, 153.8, 139.0, 135.1, 133.2, 129.1, 123.6, 122.6, 75.7, 38.7, 37.8, 37.3, 34.3, 30.8, 26.3, 23.1, 18.4, 17.2, 15.9; HR-MS (ESI): *m/z* calcd for C₂₀H₂₄BrN₂O₃S ([M + H]⁺), 451.0686; found, 451.0684.

3.2.7. *N*'-(4-((4*aS*,5*S*,5*aR*)-5*a*-Methyl-3-methylene-2-oxooctahydro-2*H*-cyclopropa[f]-benzofuran-5-yl)butan-2-ylidene)nicotinohydrazide (**6g**)

¹H NMR (500 MHz, CDCl₃) δ: 9.79 (1H, s), 9.02 (1H, s), 8.65 (1H, d, *J* = 6.7 Hz), 8.16 (1H, d, *J* = 7.5 Hz), 7.34 (1H, dd, *J* = 7.5, 6.7 Hz), 6.16 (1H, d, *J* = 2.6 Hz), 5.54 (1H, d, *J* = 2.6 Hz), 4.74–4.79

(1H, m), 3.10–3.17 (1H, m), 2.42 (2H, t, $J = 7.6$ Hz), 2.24–2.29 (2H, m), 2.04 (3H, s), 1.41–1.68 (2H, m), 1.08 (3H, s), 0.85–0.98 (2H, m), 0.31–0.49 (2H, m); ^{13}C NMR (125 MHz, CDCl_3) δ : 170.6, 161.6, 152.0, 148.4, 148.3, 139.0, 135.5, 129.7, 123.3, 122.5, 75.7, 39.0, 37.4, 37.0, 34.1, 30.5, 26.2, 22.8, 18.1, 17.0, 14.1; HR-MS (ESI): m/z calcd for $\text{C}_{21}\text{H}_{26}\text{N}_3\text{O}_3$ ($[\text{M} + \text{H}]^+$), 368.1969; found, 368.1969.

3.2.8. 4-Amino-*N'*-(4-((4*aS*,5*S*,5*aR*)-5*a*-methyl-3-methylene-2-oxooctahydro-2*H*-cyclopropa[*f*]benzofuran-5-yl)butan-2-ylidene)benzohydrazide (**6h**)

^1H NMR (500 MHz, CDCl_3) δ : 9.04 (1H, s), 7.62 (2H, d, $J = 7.9$ Hz), 6.60 (2H, d, $J = 7.9$ Hz), 6.14 (1H, d, $J = 2.6$ Hz), 5.54 (1H, d, $J = 2.6$ Hz), 4.66–4.77 (1H, m), 4.29 (2H, s), 3.07–3.11 (1H, m), 2.42 (2H, t, $J = 7.6$ Hz), 2.25–2.29 (2H, m), 1.95 (3H, s), 1.41–1.66 (2H, m), 1.03 (3H, s), 0.79–0.92 (2H, m), 0.40–0.45 (1H, m), 0.29–0.35 (1H, m); ^{13}C NMR (125 MHz, CDCl_3) δ : 170.7, 164.2, 158.0, 150.8, 139.0, 129.2, 122.6, 121.7, 113.8, 75.8, 38.9, 37.4, 37.0, 34.1, 30.5, 26.2, 22.8, 18.2, 17.0, 15.5; HR-MS (ESI): m/z calcd for $\text{C}_{22}\text{H}_{28}\text{N}_3\text{O}_3$ ($[\text{M} + \text{H}]^+$), 382.2125; found, 382.2124.

3.2.9. 4-Hydroxy-*N'*-(4-((4*aS*,5*S*,5*aR*)-5*a*-methyl-3-methylene-2-oxooctahydro-2*H*-cyclopropa[*f*]benzofuran-5-yl)butan-2-ylidene)benzohydrazide (**6i**)

^1H NMR (500 MHz, CDCl_3) δ : 9.24 (1H, s), 7.60 (2H, d, $J = 7.3$ Hz), 6.88 (2H, d, $J = 7.3$ Hz), 6.23 (1H, d, $J = 2.6$ Hz), 5.57 (1H, d, $J = 2.6$ Hz), 4.66–4.74 (1H, m), 3.03–3.11 (1H, m), 2.46 (2H, t, $J = 7.6$ Hz), 2.25–2.30 (2H, m), 2.16 (3H, s), 1.59–1.62 (2H, m), 0.99 (3H, s), 0.79–0.94 (2H, m), 0.29–0.45 (2H, m); ^{13}C NMR (125 MHz, CDCl_3) δ : 171.0, 165.0, 161.0, 155.1, 139.0, 129.3, 123.8, 122.7, 115.8, 75.8, 38.8, 37.7, 37.3, 34.2, 30.7, 26.0, 22.9, 18.2, 17.0, 15.9; HR-MS (ESI): m/z calcd for $\text{C}_{22}\text{H}_{27}\text{N}_2\text{O}_4$ ($[\text{M} + \text{H}]^+$), 383.1965; found, 383.1963.

3.2.10. 2-Hydroxy-*N'*-(4-((4*aS*,5*S*,5*aR*)-5*a*-methyl-3-methylene-2-oxooctahydro-2*H*-cyclopropa[*f*]benzofuran-5-yl)butan-2-ylidene)benzohydrazide (**6j**)

^1H NMR (500 MHz, CDCl_3) δ : 6.86–7.62 (4H, m), 6.21 (1H, d, $J = 2.6$ Hz), 5.55 (1H, d, $J = 2.6$ Hz), 4.74–4.78 (1H, m), 3.13–3.15 (1H, m), 2.46 (2H, t, $J = 7.6$ Hz), 2.23–2.38 (2H, m), 2.01 (3H, s), 1.52–1.74 (2H, m), 1.07 (3H, s), 0.84–0.99 (2H, m), 0.35–0.45 (2H, m); ^{13}C NMR (125 MHz, CDCl_3) δ : 170.7, 166.1, 160.3, 139.2, 134.4, 126.3, 122.7, 119.0, 118.5, 114.2, 75.8, 38.9, 37.7, 37.3, 34.3, 30.5, 26.1, 22.9, 18.4, 17.1, 16.0; HR-MS (ESI): m/z calcd for $\text{C}_{22}\text{H}_{27}\text{N}_2\text{O}_4$ ($[\text{M} + \text{H}]^+$), 383.1965; found, 383.1964.

3.2.11. 2-Chloro-*N'*-(4-((4*aS*,5*S*,5*aR*)-5*a*-methyl-3-methylene-2-oxooctahydro-2*H*-cyclopropa[*f*]benzofuran-5-yl)butan-2-ylidene)benzohydrazide (**6k**)

^1H NMR (500 MHz, CDCl_3) δ : 9.33 (1H, s), 7.25–7.63 (4H, m), 6.14 (1H, d, $J = 2.6$ Hz), 5.53 (1H, d, $J = 2.6$ Hz), 4.68–4.78 (1H, m), 3.09–3.15 (1H, m), 2.46 (2H, t, $J = 7.6$ Hz), 2.25–2.31 (2H, m), 1.96 (3H, s), 1.21–1.74 (2H, m), 1.09 (3H, s), 0.74–0.98 (2H, m), 0.20–0.48 (2H, m); ^{13}C NMR (125 MHz, CDCl_3) δ : 170.4, 162.6, 159.9, 152.4, 139.0, 131.4, 130.8, 130.4, 130.0, 127.0, 122.4, 75.7, 39.0, 37.5, 37.1, 34.2, 30.6, 26.1, 22.9, 18.3, 17.1, 16.1; HR-MS (ESI): m/z calcd for $\text{C}_{22}\text{H}_{26}\text{ClN}_2\text{O}_3$ ($[\text{M} + \text{H}]^+$), 401.1627; found, 401.1626.

3.2.12. 3-Chloro-*N'*-(4-((4*aS*,5*S*,5*aR*)-5*a*-methyl-3-methylene-2-oxooctahydro-2*H*-cyclopropa[*f*]benzofuran-5-yl)butan-2-ylidene)benzohydrazide (**6l**)

¹H NMR (500 MHz, CDCl₃) δ: 9.09 (1H, s), 7.80 (1H, d, *J* = 7.4 Hz), 7.69 (1H, s), 7.46 (1H, d, *J* = 7.7 Hz), 7.34 (1H, dd, *J* = 7.7, 7.4 Hz), 6.18 (1H, d, *J* = 2.5 Hz), 5.54 (1H, d, *J* = 2.5 Hz), 4.73–4.78 (1H, m), 3.12–3.16 (1H, m), 2.47 (2H, t, *J* = 7.6 Hz), 2.27–2.33 (2H, m), 2.11 (3H, s), 1.47–1.68 (2H, m), 1.07 (3H, s), 0.89–0.99 (2H, m), 0.38–0.45 (2H, m); ¹³C NMR (125 MHz, CDCl₃) δ: 170.5, 162.9, 160.5, 139.1, 135.6, 134.7, 131.6, 129.9, 127.6, 125.5, 122.5, 75.7, 39.0, 37.7, 37.1, 34.2, 30.6, 26.2, 22.9, 18.2, 17.1, 16.0; HR-MS (ESI): *m/z* calcd for C₂₂H₂₆ClN₂O₃ ([M + H]⁺), 401.1627; found, 401.1626.

3.2.13. *N'*-(4-((4*aS*,5*S*,5*aR*)-5*a*-Methyl-3-methylene-2-oxooctahydro-2*H*-cyclopropa[*f*]-benzofuran-5-yl)butan-2-ylidene)-4-nitrobenzohydrazide (**6m**)

¹H NMR (500 MHz, CDCl₃) δ: 9.21 (1H, s), 7.80 (1H, d, *J* = 7.7 Hz), 7.69 (1H, d, *J* = 7.7 Hz), 7.43 (1H, d, *J* = 7.4 Hz), 7.35 (1H, d, *J* = 7.4 Hz), 6.17 (1H, d, *J* = 2.6 Hz), 5.54 (1H, d, *J* = 2.6 Hz), 4.72–4.76 (1H, m), 3.13–3.14 (1H, m), 2.46 (2H, t, *J* = 7.6 Hz), 2.24–2.31 (2H, m), 2.01 (3H, s), 1.48–1.67 (2H, m), 1.07 (3H, s), 0.84–0.99 (2H, m), 0.42–0.45 (1H, m), 0.29–0.40 (1H, m); ¹³C NMR (125 MHz, CDCl₃) δ: 170.5, 163.0, 160.7, 145.6, 139.1, 134.5, 130.5, 129.8, 125.7, 125.5, 122.5, 75.7, 39.0, 37.6, 37.1, 34.2, 30.5, 26.2, 22.9, 18.2, 17.1, 16.1; HR-MS (ESI): *m/z* calcd for C₂₂H₂₆N₃O₅ ([M + H]⁺), 412.1867; found, 412.1866.

3.2.14. 4-Cyano-*N'*-(4-((4*aS*,5*S*,5*aR*)-5*a*-methyl-3-methylene-2-oxooctahydro-2*H*-cyclopropa[*f*]benzofuran-5-yl)butan-2-ylidene)benzohydrazide (**6n**)

¹H NMR (500 MHz, CDCl₃) δ: 9.38 (1H, s), 7.96 (2H, d, *J* = 7.7 Hz), 7.76 (2H, d, *J* = 7.7 Hz), 6.18 (1H, d, *J* = 2.6 Hz), 5.55 (1H, d, *J* = 2.6 Hz), 4.74–4.79 (1H, m), 3.15–3.16 (1H, m), 2.46 (2H, t, *J* = 7.6 Hz), 2.26–2.33 (2H, m), 1.52–1.55 (2H, m), 1.08 (3H, s), 0.84–0.99 (2H, m), 0.41–0.46 (2H, m); ¹³C NMR (125 MHz, CDCl₃) δ: 170.6, 161.9, 154.4, 139.0, 137.8, 132.3, 128.2, 122.6, 118.0, 115.0, 75.7, 39.0, 37.7, 37.2, 34.2, 30.7, 26.2, 22.9, 18.2, 17.1, 16.2; HR-MS (ESI): *m/z* calcd for C₂₃H₂₆N₃O₃ ([M + H]⁺), 392.1969; found, 392.1965.

3.2.15. 3-Methyl-*N'*-(4-((4*aS*,5*S*,5*aR*)-5*a*-methyl-3-methylene-2-oxooctahydro-2*H*-cyclopropa[*f*]benzofuran-5-yl)butan-2-ylidene)benzohydrazide (**6o**)

¹H NMR (500 MHz, CDCl₃) δ: 9.16 (1H, s), 7.63 (1H, d, *J* = 7.9 Hz), 7.61 (1H, dd, *J* = 8.1, 7.9 Hz), 7.38 (1H, s), 7.28 (1H, d, *J* = 8.1 Hz), 6.16 (1H, d, *J* = 2.6 Hz), 5.53 (1H, d, *J* = 2.6 Hz), 4.71–4.76 (1H, m), 3.82 (3H, s), 3.10–3.14 (1H, m), 2.44 (2H, t, *J* = 7.6 Hz), 2.37 (3H, s), 2.26–2.31 (2H, m), 2.01 (3H, s), 1.46–1.61 (2H, m), 1.07 (3H, s), 0.89–0.95 (2H, m), 0.37–0.45 (2H, m); ¹³C NMR (125 MHz, CDCl₃) δ: 170.5, 164.2, 159.3, 139.1, 138.3, 133.7, 132.3, 128.3, 128.0, 124.2, 122.4, 75.7, 39.0, 37.5, 37.1, 34.1, 30.5, 26.2, 22.9, 21.2, 18.3, 17.0, 15.8; HR-MS (ESI): *m/z* calcd for C₂₃H₂₉N₂O₃ ([M + H]⁺), 381.2173; found, 381.2172.

3.2.16. 4-Methoxy-*N'*-(4-((4a*S*,5*S*,5a*R*)-5a-methyl-3-methylene-2-oxooctahydro-2*H*-cyclopropa[*f*]benzofuran-5-yl)butan-2-ylidene)benzohydrazide (**6p**)

¹H NMR (500 MHz, CDCl₃) δ: 8.78 (1H, s), 7.81 (2H, d, *J* = 7.7 Hz), 6.93 (2H, d, *J* = 7.7 Hz), 6.22 (1H, d, *J* = 2.6 Hz), 5.54 (1H, d, *J* = 2.6 Hz), 4.74–4.79 (1H, m), 3.85 (3H, s), 3.12–3.18 (1H, m), 2.48 (2H, t, *J* = 7.6 Hz), 2.28–2.35 (2H, m), 2.16 (3H, s), 1.52–1.61 (2H, m), 1.09 (3H, s), 0.92–0.97 (2H, m), 0.37–0.47 (2H, m); ¹³C NMR (125 MHz, CDCl₃) δ: 170.4, 162.4, 160.2, 156.4, 139.0, 129.2, 125.8, 122.5, 113.9, 75.6, 55.4, 39.0, 37.7, 37.3, 34.2, 30.7, 26.2, 22.9, 18.2, 17.2, 15.4; HR-MS (ESI): *m/z* calcd for C₂₃H₂₉N₂O₄ ([*M* + *H*]⁺), 397.2122; found, 397.2121.

3.2.17. 3-Methoxy-*N'*-(4-((4a*S*,5*S*,5a*R*)-5a-methyl-3-methylene-2-oxooctahydro-2*H*-cyclopropa[*f*]benzofuran-5-yl)butan-2-ylidene)benzohydrazide (**6q**)

¹H NMR (500 MHz, CDCl₃) δ: 9.03 (1H, s), 7.29–7.37 (3H, m), 7.03 (1H, s), 6.18 (1H, d, *J* = 2.6 Hz), 5.54 (1H, d, *J* = 2.6 Hz), 4.73–4.78 (1H, m), 3.82 (3H, s), 3.13–3.15 (1H, m), 2.48 (2H, t, *J* = 7.6 Hz), 2.26–2.31 (2H, m), 2.14 (3H, s), 1.51–1.61 (2H, m), 1.07 (3H, s), 0.89–0.96 (2H, m), 0.38–0.46 (2H, m); ¹³C NMR (125 MHz, CDCl₃) δ: 170.5, 163.8, 159.7, 153.6, 139.0, 135.1, 129.6, 122.5, 119.0, 117.7, 112.8, 75.7, 55.4, 39.0, 37.6, 37.1, 34.2, 30.6, 26.2, 22.9, 18.2, 17.1, 15.8; HR-MS (ESI): *m/z* calcd for C₂₃H₂₉N₂O₄ ([*M* + *H*]⁺), 397.2122; found, 397.2121.

For the preparation of carabrone sulfonyl hydrazone (**7r–s**), a solution (20 mL) of benzenesulfonyl chloride (**1r**, 1 mmol) or *p*-toluene sulfonyl chloride (**1s**, 1 mmol) in acetone and an appropriate amount of hydrazine hydrate were treated with 5% NaOH solution (0.5 mL) firstly. The mixture was then shaken vigorously for 10 min, cooled and poured into 1:1 (*v/v*) HCl. The precipitate formed was filtered, washed with water and recrystallized from alcohol to obtain the intermediates (**2r–s**) [18]. Finally, the intermediates (0.5 mmol) was added to a three-necked, round-bottomed flask containing carabrone (100 mg, 0.4 mmol) in absolute ethyl alcohol, reflux until the reaction was complete, catalyzed by glacial acetic acid [17] as shown in Scheme 1.

3.2.18. *N'*-((*E*)-4-((4a*S*,5*S*,5a*R*)-5a-Methyl-3-methylene-2-oxooctahydro-2*H*-cyclopropa[*f*]benzofuran-5-yl)butan-2-ylidene)benzenesulfonylhydrazide (**7r**)

¹H NMR (500 MHz, CDCl₃) δ: 7.96 (2H, d, *J* = 8.7 Hz), 7.45–7.58 (3H, m), 6.22 (1H, d, *J* = 2.5 Hz), 5.56 (1H, d, *J* = 2.5 Hz), 4.70–4.75 (1H, m), 3.07–3.13 (1H, m), 2.48 (2H, t, *J* = 7.6 Hz), 2.26–2.31 (2H, m), 1.22–1.37 (2H, m), 0.99 (3H, s), 0.78–0.91 (2H, m), 0.21–0.39 (*H*-1, m); ¹³C NMR (125 MHz, CDCl₃) δ: 170.8, 158.8, 139.0, 138.4, 133.1, 128.8, 128.0, 122.7, 75.7, 38.5, 37.6, 37.1, 33.8, 30.5, 25.4, 22.9, 18.2, 16.8, 16.1; HR-MS (ESI): *m/z* calcd for C₂₁H₂₇N₂O₄S ([*M* + *H*]⁺), 403.1686; found, 403.1689.

3.2.19. 4-Methyl-*N'*-((*E*)-4-((4a*S*,5*S*,5a*R*)-5a-methyl-3-methylene-2-oxooctahydro-2*H*-cyclopropa[*f*]benzofuran-5-yl)butan-2-ylidene)benzenesulfonylhydrazide (**7s**)

¹H NMR (500 MHz, CDCl₃) δ: 7.74 (2H, d, *J* = 7.9 Hz), 7.29 (2H, d, *J* = 7.9 Hz), 6.24 (1H, d, *J* = 2.6 Hz), 5.56 (1H, d, *J* = 2.6 Hz), 4.71–4.75 (1H, m), 3.09–3.18 (1H, m), 2.40 (2H, t, *J* = 7.6 Hz), 2.24–2.30 (2H, m), 1.79 (3H, s), 1.51–1.65 (2H, m), 1.26 (3H, s), 1.02 (3H, s), 0.81–0.97 (2H, m),

0.28–0.47 (2H, m); ^{13}C NMR (125 MHz, CDCl_3) δ : 170.5, 158.1, 144.0, 139.1, 135.5, 129.4, 128.1, 122.5, 75.6, 38.5, 37.7, 37.2, 34.0, 30.6, 25.5, 23.0, 21.5, 18.2, 17.0, 16.0; HR-MS (ESI): m/z calcd for $\text{C}_{22}\text{H}_{29}\text{N}_2\text{O}_4\text{S}$ ($[\text{M} + \text{H}]^+$), 417.1843; found, 417.1845.

As shown in Scheme 1, for the preparation of the intermediates (**5a–i**), 2-hydroxyl ethanamine (**4a**, 1 mmol) or phenylamine derivatives (**4b–i**, 1 mmol) was first reacted with sodium nitrite (2 mmol) and hydrochloric acid (5 mL) at 0 °C, reduced by sodium pyrosulfite, washed with hydrochloric acid (5 mL) and dissolved in water (10 mL). Concentrated hydrochloric acid was then added to this solution after decolorization with activated carbon to produce intermediates (**5a–i**) [19]. Finally, the target compounds (**8a–i**, Figure 2) were successively obtained with the yields of 45%–93% after carbonyl (100 mg, 0.4 mmol) reacting with the intermediates (0.5 mmol) and catalyzed by glacial acetic acid [17].

3.2.20. (4a*S*,5*S*,5a*R*)-5-(3-(2-(2-Hydroxyethyl)hydrazono)butyl)-5a-methyl-3-methyl-eneoctahydro-2*H*-cyclopropa[*f*]benzofuran-2-one (**8a**)

^1H NMR (500 MHz, CDCl_3) δ : 6.24 (1H, d, $J = 2.6$ Hz), 5.5 (1H, d, $J = 2.6$ Hz), 4.74–4.81 (1H, m), 3.82 (2H, t, $J = 4.6$ Hz), 3.72 (1H, s), 3.27 (2H, t, $J = 4.6$ Hz), 3.13–3.19 (1H, m), 2.40 (2H, t, $J = 7.6$ Hz), 2.24–2.30 (2H, m), 2.17 (3H, s), 1.48–1.63 (2H, m), 1.09 (3H, s), 0.86–0.98 (2H, m), 0.28–0.46 (2H, m); ^{13}C NMR (125 MHz, CDCl_3) δ : 170.4, 150.0, 139.1, 122.5, 75.6, 63.7, 51.9, 38.8, 37.7, 37.3, 34.3, 30.7, 23.4, 23.0, 18.2, 17.2, 14.5; HR-MS (ESI): m/z calcd for $\text{C}_{17}\text{H}_{27}\text{N}_2\text{O}_3$ ($[\text{M} + \text{H}]^+$), 307.1940; found, 307.1938.

3.2.21. (4a*S*,5*S*,5a*R*)-5a-Methyl-3-methylene-5-(3-(2-phenylhydrazono)butyl)-octa-hydro-2*H*-cyclopropa[*f*]benzofuran-2-one (**8b**)

^1H NMR (500 MHz, CDCl_3) δ : 7.35–7.47 (6H, m), 6.22 (1H, d, $J = 2.5$ Hz), 5.54 (1H, d, $J = 2.5$ Hz), 4.75–4.77 (1H, m), 3.09–3.17 (1H, m), 2.44 (2H, t, $J = 7.6$ Hz), 2.25–2.31 (2H, m), 1.55 (s, 3H, H -15), 1.22–1.45 (2H, m), 1.06 (3H, s), 0.88–0.96 (2H, m), 0.33–0.47 (2H, m); ^{13}C NMR (125 MHz, CDCl_3) δ : 170.7, 151.0, 139.1, 131.4, 129.2, 122.6, 122.5, 104.9, 75.8, 38.5, 37.7, 37.3, 34.2, 30.8, 26.3, 22.9, 18.2, 17.2, 15.7; HR-MS (ESI): m/z calcd for $\text{C}_{21}\text{H}_{27}\text{N}_2\text{O}_2$ ($[\text{M} + \text{H}]^+$), 339.2067; found, 339.2068.

3.2.22. (4a*S*,5*S*,5a*R*)-5a-Methyl-3-methylene-5-(3-(2-(2,4,6-trichlorophenyl)-hydrazono)butyl)-octahydro-2*H*-cyclopropa[*f*]benzofuran-2-one (**8c**)

^1H NMR (500 MHz; CDCl_3) δ : 9.21 (1H; s); 7.42 (2H; s); 6.23 (1H,d; $J = 2.6$ Hz); 5.55 (1H; d; $J = 2.6$ Hz); 4.76–4.81 (1H; m); 3.13–3.19 (1H; m); 2.46 (2H; t; $J = 7.6$ Hz); 2.25–2.31 (2H; m); 1.62 (3H; s); 1.41–1.68 (2H; m); 1.10 (3H; s); 0.87–1.00 (2H; m); 0.36–0.52 (2H; m); ^{13}C NMR (125 MHz; CDCl_3) δ : 170.6; 153.3; 145.5; 139.1; 128.7; 127.0; 122.5; 75.7; 38.6; 37.8; 37.4; 34.5; 30.8; 26.1; 23.0; 18.3; 17.2; 15.1; HR-MS (ESI): m/z calcd for $\text{C}_{21}\text{H}_{24}\text{Cl}_3\text{N}_2\text{O}_2$ ($[\text{M} + \text{H}]^+$); 441.0898; found; 441.0896.

3.2.23. (4a*S*,5*S*,5a*R*)-5a-Methyl-3-methylene-5-(3-(2-(4-nitrophenyl)hydrazono)-butyl)-octahydro-2*H*-cyclopropa[*f*]benzofuran-2-one (**8d**)

^1H NMR (500 MHz, CDCl_3) δ : 8.12 (2H, d, $J = 8.4$ Hz), 7.74 (1H, s), 7.06 (2H, d, $J = 8.4$ Hz), 6.21 (1H, d, $J = 2.6$ Hz), 5.54 (1H, d, $J = 2.6$ Hz), 4.78–4.80 (1H, m), 3.16–3.18 (1H, m), 2.42 (2H, t,

$J = 7.6$ Hz), 2.26–2.35 (2H, m), 1.93 (3H, s), 1.56–1.69 (2H, m), 1.11 (3H, s), 0.92–1.03 (2H, m), 0.48–0.52 (1H, m), 0.39–0.43 (1H, m); ^{13}C NMR (125 MHz, CDCl_3) δ : 170.7, 150.7, 150.5, 139.6, 139.1, 126.1, 122.7, 111.6, 75.8, 38.9, 37.6, 37.3, 34.3, 30.7, 26.0, 23.0, 18.4, 17.2, 15.0; HR-MS (ESI): m/z calcd for $\text{C}_{21}\text{H}_{26}\text{N}_3\text{O}_4$ ($[\text{M} + \text{H}]^+$), 384.1918; found, 384.1917.

3.2.24. (4a*S*,5*S*,5a*R*)-5a-Methyl-3-methylene-5-(3-(2-(2-nitrophenyl)hydrazono)butyl)-octahydro-2*H*-cyclopropa[*f*]benzofuran-2-one (**8e**)

^1H NMR (500 MHz, CDCl_3) δ : 10.67 (1H, s), 8.15 (1H, d, $J = 8.7$ Hz), 7.51–7.57 (2H, m), 6.77 (1H, d, $J = 8.0$ Hz), 6.22 (1H, d, $J = 2.6$ Hz), 5.52 (1H, d, $J = 2.6$ Hz), 4.76–4.79 (1H, m), 3.15–3.17 (1H, m), 2.48 (2H, t, $J = 7.7$ Hz), 2.28–2.38 (2H, m), 2.01 (3H, s), 1.52–1.78 (2H, m), 1.12 (3H, s), 0.92–1.01 (2H, m), 0.50–0.55 (1H, m), 0.37–0.42 (1H, m); ^{13}C NMR (125 MHz, CDCl_3) δ : 170.5, 152.8, 142.6, 139.1, 136.2, 130.7, 125.9, 122.5, 117.5, 115.8, 75.6, 39.0, 37.7, 37.4, 34.6, 30.8, 26.1, 23.0, 18.4, 17.2, 15.8; HR-MS (ESI): m/z calcd for $\text{C}_{21}\text{H}_{26}\text{N}_3\text{O}_4$ ($[\text{M} + \text{H}]^+$), 384.1918; found, 384.1917.

3.2.25. (4a*S*,5*S*,5a*R*)-5a-Methyl-3-methylene-5-(3-(2-(4-(trifluoromethyl)phenyl)-hydrazono)butyl)-octahydro-2*H*-cyclopropa[*f*]benzofuran-2-one (**8f**)

^1H NMR (500 MHz, CDCl_3) δ : 7.42 (2H, d, $J = 8.3$ Hz), 7.09 (2H, d, $J = 8.3$ Hz), 6.18 (1H, d, $J = 2.6$ Hz), 5.49 (1H, d, $J = 2.6$ Hz), 4.74–4.76 (1H, m), 3.11–3.13 (1H, m), 2.39 (2H, t, $J = 7.6$ Hz), 2.23–2.31 (2H, m), 1.86 (3H, s), 1.51–1.67 (2H, m), 1.08 (3H, s), 0.86–0.98 (2H, m), 0.45–0.50 (1H, m), 0.34–0.39 (1H, m); ^{13}C NMR (125 MHz, CDCl_3) δ : 170.7, 153.0, 148.6, 148.1, 139.2, 126.4, 122.8, 122.5, 112.2, 75.9, 38.8, 37.6, 37.3, 34.5, 30.8, 26.1, 22.9, 18.2, 17.1, 14.8; HR-MS (ESI): m/z calcd for $\text{C}_{22}\text{H}_{26}\text{F}_3\text{N}_2\text{O}_2$ ($[\text{M} + \text{H}]^+$), 407.1941; found, 407.1932.

3.2.26. (4a*S*,5*S*,5a*R*)-5a-Methyl-3-methylene-5-(3-(2-(2,3,5,6-tetrafluorophenyl)-hydrazono)butyl)-octahydro-2*H*-cyclopropa[*f*]benzofuran-2-one (**8g**)

^1H NMR (500 MHz, CDCl_3) δ : 6.78 (1H, s), 6.20 (1H, d, $J = 2.5$ Hz), 5.55 (1H, d, $J = 2.5$ Hz), 4.76–4.81 (1H, m), 3.16–3.20 (1H, m), 2.46 (2H, t, $J = 7.6$ Hz), 2.26–2.33 (2H, m), 1.94 (s, 3H, *H*-15), 1.49–1.69 (2H, m), 1.10 (3H, s), 0.88–1.00 (2H, m), 0.45–0.54 (1H, m), 0.36–0.42 (1H, m); ^{13}C NMR (125 MHz, CDCl_3) δ : 170.6, 153.9, 147.4, 145.4, 139.2, 126.7, 122.3, 96.3, 75.7, 38.5, 37.6, 37.2, 34.4, 30.7, 25.9, 22.9, 18.0, 17.1, 14.7; HR-MS (ESI): m/z calcd for $\text{C}_{21}\text{H}_{23}\text{F}_4\text{N}_2\text{O}_2$ ($[\text{M} + \text{H}]^+$), 411.1690; found, 411.1689.

3.2.27. (4a*S*,5*S*,5a*R*)-5-(3-(2-(5,6-Dimethylthieno[2,3-*d*]pyrimidin-4-yl)hydrazono)-butyl)-5a-methyl-3-methyleneoctahydro-2*H*-cyclopropa[*f*]benzofuran-2-one (**8h**)

^1H NMR (500 MHz, CDCl_3) δ : 8.42 (1H, s), 6.21 (1H, d, $J = 2.5$ Hz), 5.53 (1H, d, $J = 2.5$ Hz), 4.76–4.81 (1H, m), 3.12–3.18 (1H, m), 2.51 (3H, s), 2.44 (2H, t, $J = 7.6$ Hz), 2.40 (3H, s), 2.28–2.32 (2H, m), 2.07 (3H, s), 1.52–1.72 (2H, m), 1.09 (3H, s), 0.93–1.03 (2H, m), 0.46–0.51 (1H, m), 0.36–0.41 (1H, m); ^{13}C NMR (125 MHz, CDCl_3) δ : 170.4, 165.0, 157.4, 152.3, 139.1, 130.9, 130.8, 123.4, 122.5, 116.8, 75.6, 39.0, 37.6, 37.2, 34.4, 30.7, 26.6, 23.0, 18.4, 17.2, 15.5, 14.2, 13.2; HR-MS (ESI): m/z calcd for $\text{C}_{23}\text{H}_{29}\text{N}_4\text{O}_2\text{S}$ ($[\text{M} + \text{H}]^+$), 425.2006; found, 425.2004.

3.2.28. 4-(2-(4-((4a*S*,5*S*,5a*R*)-5a-Methyl-3-methylene-2-oxooctahydro-2*H*-cyclopropa[*f*]benzofuran-5-yl)butan-2-ylidene)hydrazinyl)benzoic acid (**8i**)

¹H NMR (500 MHz, (CD₃)₂CO) δ: 10.86 (1H, s), 8.20 (2H, d, *J* = 8.1 Hz), 7.85 (2H, d, *J* = 8.1 Hz), 6.07 (1H, d, *J* = 2.6 Hz), 5.61 (1H, d, *J* = 2.6 Hz), 4.80–4.86 (1H, m), 3.20–3.27 (1H, m), 2.40 (2H, t, *J* = 7.6 Hz), 2.24–2.33 (2H, m), 1.51–1.59 (2H, m), 1.46 (3H, s), 1.10 (3H, s), 0.89–1.03 (2H, m), 0.52–0.58 (1H, m), 0.40–0.45 (1H, m); ¹³C NMR (125 MHz, (CD₃)₂CO) δ: 170.7, 150.7, 150.5, 139.6, 139.1, 126.1, 122.7, 111.6, 75.8, 38.9, 37.6, 37.3, 34.3, 30.7, 26.0, 23.0, 18.4, 17.2, 15.0; HR-MS (ESI): *m/z* calcd for C₂₂H₂₇N₂O₄ ([M + H]⁺), 383.1965; found, 383.1966.

3.3. Microorganism and Preparation of Spore Suspension

The fungal pathogens *C. lagenarium* (Accession No. 36199) and *B. cinerea* were provided by Agricultural Culture Collection of China and the Institute of Plant Disease (Beijing, China), Northwest A&F University (Yangling, China), respectively. *C. lagenarium* was cultured for 2 weeks at 25 °C on potato dextrose agar (PDA, Difco) while *B. cinerea* was cultured at 20 °C on the same medium after being retrieved from the storage tube. Plates were then flooded with sterile distilled water, and the conidia were scraped with a glass rod. Mycelial debris was removed by filtrating through double-layer cheesecloth. The resulting spores were harvested and suspended in sterile distilled water containing 0.1% (*v/v*) Tween 20. Concentration of the spore suspension was adjusted to 1.0 × 10⁶ spore/mL by diluting with sterilized distilled water using a SUPERIOR hemocytometer (Marienfeld, Berlin, Germany) [12,20].

3.4. Spore Germination Assay

The tested samples (10 mg) dissolved in acetone (0.1 mL) were diluted with sterile distilled water to get the test solutions, with the final concentration of acetone lower than 1% (*v/v*) [20]. A series of concentrations of tested samples and negative control (1% acetone with sterile distilled water) were tested on spore germination of *C. lagenarium* or *B. cinerea*. The samples were inoculated with spore suspension of *C. lagenarium* or *B. cinerea* containing 1.0 × 10⁶ spores/mL. Aliquots of 10 μL prepared spore suspension were placed on separate glass slides in triplicate. Slides containing the spores were incubated in a moisture chamber at 25 °C for 6–8 h. Each slide was then checked under the microscope for spore germination. Spores were considered to be germinated when the length of the germ tube reached to at least half of the spore length. The numbers of generated spores were counted, and the percentage of germinated spores was calculated [12]. Chlorothalonil, purchased from Xiangtan Huayuan Fine-Chem Co. Ltd. (Xiangtan, China), was used as the positive control.

3.5. Preparation of Tested Tomato Fruits

Tomato fruits, avoiding pesticides for more than one month, were harvested from greenhouse at the mature green stage (mature but fruit surface was green). Harvest fruits were then sorted based on their size and colour. Selected fruits were washed with water, air dried and the surface was then sterilized with sodium hypochlorite solution (1.0 g/L) for 5 min, rinsed twice by sterile distilled water and air dried [21].

3.6. Culture of *B. cinerea* and Inoculation

The *B. cinerea* strain was cultured on PDA at 25 °C for a week. Two symmetrical spots (5 mm in diameter and 3 mm deep) were punctured on the opposite side of tomato fruits with a sterile nail, tested samples were then sprayed on the surface and air dried. Twenty-four hours later, agar discs with mycelium (4 mm in diameter), taking from the edge of 7-day old colonies of *B. cinerea* on PDA, were placed on the punctured site of the tomato fruits with mycelia facing the surface of fruits. Every test was performed in triplicate. Chlorothalonil was used as the positive control. Treated fruits were put in plastic boxes containing sterile water to keep humidity and stored at 25 °C. After 6 days, lesion diameters were measured in two perpendicular directions and the average inhibition rate was calculated [21].

3.7. Statistical Analysis

All experimental data were calculated and analyzed using SPSS 16.0 for Windows (SPSS China, Shanghai, China).

4. Conclusions

Twenty-eight new hydrazone derivatives of carabrone were synthesized in this work, and most of them exhibited higher antifungal activities against *C. lagenarium* than the lead compound and their ester analogues [12]. The additional experiment carried out against *B. cinerea* *in vitro* and *in vivo* confirmed their promising potential for development of activities. It is worth noting that **8c** and **8g** exhibited the strongest antifungal activities among these compounds and have been identified as potential candidate compounds for the development of new fungicides for the sustainable agriculture. Given this interesting level of activity, further study on these compounds is necessary.

Acknowledgments

We greatly appreciate the funding support for this research provided by the National Natural Science Foundation of China (NSFC No. 30971934 and No. 31272074) and the National Department Public Benefit Research Foundation of China (Grant No. 200903052). We also thank Luo Du-qiang for the spectral data, and Sun Ping-Ping at the University of Minnesota for the corrections on this manuscript.

Author Contributions

Wang Hao designed and conducted the experiment and wrote the paper; Ren Shuang-Xi and Wang De-Long assisted in the completion of synthesis of the title compounds; He Ze-Yu and Yan Xiao-Nan took part in the experiments on activity evaluation; Feng Jun-Tao and Zhang Xing supervised the whole experiment and provided technical guidance. All the authors contributed to the analysis of the data.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Fisher, M.C.; Henk, D.A.; Briggs, C.J.; Brownstein, J.S.; Madoff, L.C.; McCraw, S.L.; Gurr, S.J. Emerging fungal threats to animal, plant and ecosystem health. *Nature* **2012**, *484*, 186–194.
2. Savary, S.; Ficke, A.; Aubertot, J.N.; Hollier, C. Crop losses due to diseases and their implications for global food production losses and food security. *Food Secur.* **2012**, *4*, 519–537.
3. Shimizu, M.; Yazawa, S.; Ushijima, Y. A promising strain of endophytic *Streptomyces* sp. for biological control of cucumber anthracnose. *J. Gen. Plant. Pathol.* **2009**, *75*, 27–36.
4. Oercke, E.C. Crop losses to pests. *J. Agric. Sci.* **2006**, *144*, 31–43.
5. Oercke, E.C.; Dehne, H.W. Safeguarding production—Losses in major crops and the role of crop protection. *Crop Prot.* **2004**, *23*, 275–285.
6. Minato, H.; Nosaka, S.; Horibe, I. Studies on sesquiterpenoids. Part VIII. The structure of carabrone, a new component of *Carpesium abrotanoides* Linn. *J. Chem. Soc.* **1964**, 5503–5510.
7. Maruyama, M.; Omura, S. Carpesiolin from *Carpesium abrotanoides*. *Phytochemistry* **1977**, *16*, 782–783.
8. Yang, C.; Shi, Y.P.; Jia, Z.J. Sesquiterpene lactone glycosides, eudesmanolides, and other constituents from *Carpesium macrocephalum*. *Planta Med.* **2002**, *68*, 626–630.
9. Jiang, J.W. *Manuscript of Active Ingredients of Vegetable Drug*; People's Health Press: Beijing, China, 1986; pp. 832–833.
10. Lee, J.; Min, B.; Lee, S.; Na, M.; Kwon, B.; Lee, C.; Bae, K. Cytotoxic sesquiterpene lactones from *Carpesium abrotanoides*. *Planta. Med.* **2002**, *68*, 745–747.
11. Feng, J.T.; Ma, Z.Q.; Li, J.H.; He, J.; Xu, H.; Zhang, X. Synthesis and antifungal activity of carabrone derivatives. *Molecules* **2010**, *15*, 6485–6492.
12. Feng, J.T.; Wang, H.; Ren, S.X.; He, J.; Liu, Y.; Zhang, X. Synthesis and antifungal activities of carabrol ester derivatives. *J. Agric. Food Chem.* **2012**, *60*, 3817–3823.
13. Fardella, G.; Barbetti, P.; Grandolini, G.; Chiappini, I.; Ambrogi, V.; Scarcia, V.; Candiani, A.F. Phenylthio-derivatives of α -methylene- γ -lactones as pro-drugs of cytotoxic agents. *Eur. J. Med. Chem.* **1999**, *34*, 515–523.
14. Lee, K.H.; Wu, Y.S.; Hall, I.H. Antitumor agents. 25. Synthesis and antitumor activity of uracil and thymine α -methylene- γ -lactones and related derivatives. *J. Med. Chem.* **1977**, *20*, 911–914.
15. Makovitzki, A.; Viterbo, A.; Brotman, Y.; Chet, I.; Shai, Y. Inhibition of fungal and bacterial plant pathogens *in vitro* and *in planta* with ultrashort cationic lipopeptides. *Appl. Environ. Microbiol.* **2007**, *73*, 6629–6636.
16. Ji, Y.L.; Liu, L.; Jia, D.Z. Synthesis and crystal structure of *N*-(1-phenyl-3-methyl-4-benzylidene-5-pyrazolone)*p*-nitrobenzoylhydrazide. *Chin. J. Struct. Chem.* **2002**, *21*, 553–556.
17. Rollas, S.; Kucukguzel, S.G. Biological activities of hydrazone derivatives. *Molecules* **2007**, *12*, 1910–1939.
18. Kumar, K.R.; Prasad, A.R.G.; Srilalitha, V.; Swamy, G.N.; Ravindranath, L.K. Synthesis and electrochemical investigations on certain pyrazolin-5-ones. *Sci. Iran. Trans. C* **2012**, *19*, 605–618.
19. Shen, Y.H.; Yu, J.L. A Synthetic Method of 3-Bromophenyl hydrazine. Chin. Patent CN 101157636, 9 April 2008.

20. Li, S.K.; Ji, Z.Q.; Zhang, J.W.; Guo, Z.Y.; Wu, W.J. Synthesis of 1-acyl-3-isopropenylbenzimidazolone derivatives and their activity against *Botrytis cinerea*. *J. Agric. Food Chem.* **2010**, *58*, 2668–2672.
21. Fang, X.L.; Li, Z.Z.; Wang, Y.H.; Zhang, X. *In vitro* and *in vivo* antimicrobial activity of *Xenorhabdus bovienii* YL002 against *Phytophthora capsici* and *Botrytis cinerea*. *J. Appl. Microbiol.* **2011**, *111*, 145–154.

© 2014 by the authors; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution license (<http://creativecommons.org/licenses/by/3.0/>).