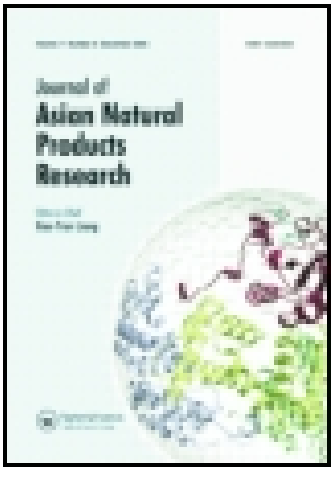

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Synthesis, antifungal activity, and QSAR study of novel trichodermin derivatives

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In an attempt to discover more potential antifungal agents, in this study, 21 novel trichodermin derivatives containing conjugated oxime ester (**5a**–**5u**) were designed and synthesized and were screened for *in vitro* antifungal activity. The bioassay tests showed that some of them exhibited good inhibitory activity against the tested pathogenic fungi. Compound **5a** exhibited better activity against *Pyricularia oryzae* and *Sclerotonia sclerotiorum* than trichodermin, and compound **5j** showed particular activity against *P. oryzae* and *Botrytis cinerea*. The quantitative structure–activity relationship (QSAR) indicated that log *P* and hardness were two critical parameters for the biological activities. The result suggested that these would be potential lead compounds for the development of fungicides with further structure modification.

Keywords: trichodermin derivatives; conjugated oxime ester; antifungal activity; QSAR

1. Introduction

The extensive application of traditional fungicides for crop disease control has led to the rapid development of fungicide resistance and severe environmental problems [1]. This situation has prompted an increased demand for more eco-friendly agents to replace conventional chemical fungicides. For decades, natural products have attracted widespread attentions due to their broad-spectrum biological activities, such as insecticidal [2], anti-inflammatory [3], antifungal [4], antitumor [5], and antibacterial activities [6]. The development and commercial success of some natural products, such as validamycin and streptomycin, make them become ideal lead compounds for novel fungicide discovery. Therefore, it is worth searching for new antifungal agents based on natural products as the bioactive prototypes for further synthesis and structure optimization.

Trichodermin (**1**, Figure 1), a sesquiterpene antibiotic originally isolated from metabolites of fungi, has been found to exhibit excellent antifungal activity against a broad spectrum of pathogenic fungi [7]. It has already been discovered that the antifungal activity of trichodermin is mainly owing to its novel mechanism of action by inhibiting protein synthesis in eukaryotes [8]. Thus, compounds derived from trichodermin are considered worthy of exploring.

In our previous research, we successfully isolated a new fungus *Trichoderma taxi* sp. nov. (ZJUF0986) which could produce trichodermin in a fairly high yield. Moreover, several series of structure modifications on trichodermin were carried out in order to study the structure–

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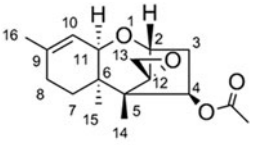


Trichodermin (1)

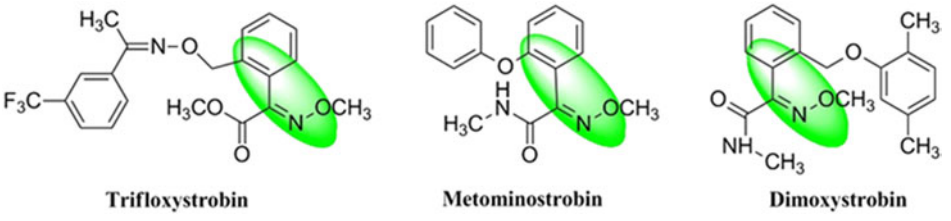
Figure 1. Chemical structure of trichodermin (**1**).

activity relationship (SAR) [9–11]. As indicated by the SAR analysis, appropriate substituents introduced to the C-4 and C-8 positions could help improving the inhibitory activities of the compounds, and the conjugated structure could promote intensive efforts to enhance their bioactivity [12]. On the other hand, since the first commercial oxime ether fungicide, cymoxanil, was first marketed in the mid-1970s, there have been numerous researches on oxime ether compounds [13], and some agents by incorporating conjugated oxime ether into mother nucleus have been commercialized, such as trifloxystrobin, metominostrobin, and dimoxystrobin (Figure 2). Thus, conjugated oxime ether can be considered as potential pharmacophore.

Inspired by the above result, we first introduced conjugated oxime ester groups with different lengths of carbon chain into position C-8. In addition, with appropriate lipophilicity of derivatives taken into account, we also incorporated different ester moieties into position C-4 (Figure 3). Therefore, in this paper, 21 novel trichodermin derivatives containing conjugated oxime ester have been designed and synthesized as an important part of our program to discovery novel compounds with superior performance [10–12]. Furthermore, to further explore the structural requirements of the derivatives for the activity, the quantitative structure–activity relationship (QSAR) were also studied.

2. Results and discussion

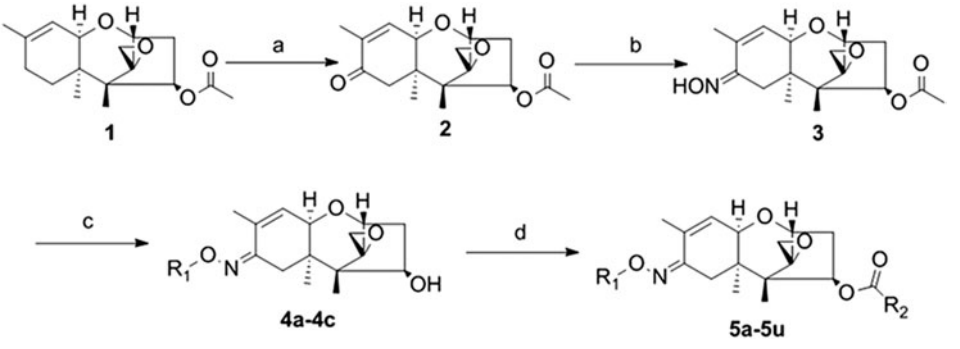
A series of novel trichodermin derivatives containing conjugated oxime ester (**5a**–**5u**) were synthesized by a four-step procedure as shown in Scheme 1. The key intermediate **2**, obtained according to our previously described procedure [11], reacted with hydroxylamine hydrochloride in the presence of K_2CO_3 to form compound **3** in good yield. With **3** in hand, conversion of **3** to **4** was then carried out with iodoalkane in the anhydrous *N*,*N*-dimethylformamide using sodium hydride as a base. Finally, different carboxylic acids were treated with **4** in the presence of *N*,*N*-dimethylpyridin-4-amine (DMAP) and *N*,*N*-dicyclohexylcarbodiimide (DCC) to afford the desired compounds **5a**–**5u**. All of the target compounds were obtained in moderate to good yields (47.76–85.32%). However, it was worth noting that the volume of the groups at C-4 position affected the yields to an extent. That is, a bulk group generally made the reaction run much slower with a relatively lower yield. Of course, all spectral and analytical data were in good agreement



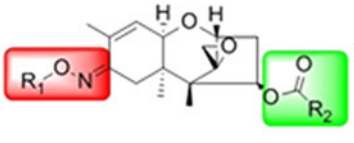
Trifloxystrobin Metominostrobin Dimoxystrobin

Figure 2. Commercial fungicides with conjugated oxime ester groups.

Figure 3. Design strategy of the target molecules.



Scheme 1. Synthesis route of target compounds. Reagents and conditions: (a) $CrO_3{\cdot}Py_2$, CH_2Cl_2, reflux, 12 h; (b) $NH_2OH{\cdot}HCl$, K_2CO_3, CH_3OH, reflux, 5 h; (c) NaH, R_1I, DMF, Rt, 4 h; (d) R_2COOH, DMAP, DCC, CH_2Cl_2, Rt, 12 h.



5a $R_1 = CH_3$; $R_2 = CH_3$
5b $R_1 = CH_3$; $R_2 = C_2H_5$
5c $R_1 = CH_3$; $R_2 = C_6H_5$
5d $R_1 = CH_3$; $R_2 = C_6H_5CH_2$
5e $R_1 = CH_3$; $R_2 = 4\text{-Me-}C_6H_4CH_2$
5f $R_1 = CH_3$; $R_2 = C_6H_5CH{=}CH$
5g $R_1 = CH_3$; $R_2 = 4\text{-}CH_3OC_6H_4CH{=}CH$
5h $R_1 = CH_3$; $R_2 = 4\text{-F-}C_6H_4CH{=}CH$
5i $R_1 = C_2H_5$; $R_2 = CH_3$
5j $R_1 = C_2H_5$; $R_2 = C_2H_5$
5k $R_1 = C_2H_5$; $R_2 = C_6H_5$
5l $R_1 = C_2H_5$; $R_2 = C_6H_5CH_2$
5m $R_1 = C_2H_5$; $R_2 = 4\text{-Me-}C_6H_4CH_2$
5n $R_1 = C_2H_5$; $R_2 = C_6H_5CH{=}CH$
5o $R_1 = C_2H_5$; $R_2 = 4\text{-}CH_3OC_6H_4CH{=}CH$
5p $R_1 = C_2H_5$; $R_2 = 4\text{-F-}C_6H_4CH{=}CH$
5q $R_1 = n\text{-}C_5H_{11}$; $R_2 = CH_3$
5r $R_1 = n\text{-}C_5H_{11}$; $R_2 = C_2H_5$
5s $R_1 = n\text{-}C_5H_{11}$; $R_2 = C_6H_5CH{=}CH$
5t $R_1 = n\text{-}C_5H_{11}$; $R_2 = 4\text{-}CH_3OC_6H_4CH{=}CH$
5u $R_1 = n\text{-}C_5H_{11}$; $R_2 = 4\text{-F-}C_6H_4CH{=}CH$

Figure 4. Structures of the target compounds.

with the proposed structures of the compounds.

With the above synthesized compounds in hand (Figure 4), the *in vitro* antifungal activities of these trichodermin derivatives (**5a**–**5u**) were evaluated on three representative plant pathogenic fungi including *Pyricularia oryzae*, *Botrytis cinerea*, and *Sclerotonia sclerotiorum* by using the mycelium growth rate method

[14]. Trichodermin, prochloraz, pyrimethanil, and chlorothalonil were used as comparative controls. Each treatment was performed three times, and the effective concentration 50 (EC_{50}) defined by the concentration corresponding to 50% growth inhibition *in vitro* was used to describe the inhibitory activity of the compounds.

The data presented in Table 1 demonstrated that the biological activities of these molecules were obviously dependent on not only the length of carbon chain at the C-8 position but also the nature of the substituents at the C-4 position. All of these assayed trichodermin derivatives possessed moderate to good inhibitory activity against *P. oryzae*, which is one of the most serious threats against rice productivity worldwide, with the EC_{50} values of 2.40–30.51 mg L^{-1}. Among these derivatives, compounds **5b**, **5d**, and **5j** showed excellent antifungal activity, with EC_{50} values of 4.83, 5.71, and 5.31 mg L^{-1}, respectively, comparable with the lead compound trichodermin (EC_{50}: 4.25 mg L^{-1}). Compounds **5a** (EC_{50}: 2.40 mg L^{-1}) and **5i** (EC_{50}: 3.61 mg L^{-1}) displayed better inhibitory activity against *P. oryzae* than that of trichodermin. Moreover, compound **5a** (EC_{50}: 1.59 mg L^{-1}) exhibited the highest antifungal activity against *Sclerotonia. Sclerotiorum*, which is higher than that of trichodermin (EC_{50}: 3.20 mg L^{-1}) and commercial fungicide chlorothalonil (EC_{50}: 7.21 mg L^{-1}). In addition, compounds **5i** (EC_{50}: 7.49 mg L^{-1}) and **5j** (EC_{50}: 6.34 mg L^{-1}) were found to exhibit the nearest approximation of inhibitory activity against *B. cinerea* to pyrimethanil (EC_{50}: 6.98 mg L^{-1}).

Table 1. Antifungal activities of compounds **5a**–**5u** expressed as EC_{50} (mg L^{-1}).

Compd	*P. ory.*	*B. cin.*	*S. scl.*
5a	2.40	8.83	1.59
5b	4.83	12.90	7.52
5c	8.32	16.92	14.31
5d	5.71	12.78	10.34
5e	5.95	9.36	7.91
5f	11.28	26.31	21.28
5g	16.59	>30	>30
5h	7.85	20.12	>30
5i	3.61	7.49	3.17
5j	5.31	6.34	4.34
5k	9.77	18.26	16.51
5l	6.79	10.37	11.27
5m	9.46	17.38	14.05
5n	14.33	14.29	20.41
5o	20.45	>30	>30
5p	11.29	10.25	26.28
5q	10.96	21.74	>30
5r	14.02	28.93	>30
5s	26.69	>30	>30
5t	30.51	>30	>30
5u	21.32	>30	>30
1	4.25	0.45	3.20
Prochloraz	0.96	ND	ND
Pyrimethanil	ND	6.98	ND
Chlorothalonil	ND	ND	7.21

Note: *Pyricularia oryzae* (*P. ory.*), *Botrytis cinerea* (*B. cin.*), and *Sclerotonia sclerotiorum* (*S. scl.*).
ND: Not determined.

From a deep analysis of the data in Table 1, some interesting phenomena could be found. For example, with the comparisons of compounds **5a**–**5h**, **5i**–**5p**, and **5q**–**5u**, it can be seen that, when R_1 was replaced by substituents with different lengths of carbon chain, the antifungal activities of the corresponding compounds were changed obviously. Generally, compounds with a short carbon chain at C-8 position seemed to be more active than long ones. The substituent at C-4 position also has great effect on its antifungal activity, and aliphatic acyloxy group is the most preferred. For instance, compounds **5a** and **5b** with an aliphatic acyloxy group at C-4 position were generally more active than compounds **5c**–**5h** with an aromatic acyloxy group. Therefore, the volume of the groups at C-4 position may be an important factor for activity. Introducing a phenylacetyl group at C-4 position gives higher bioactivities than that of the benzoyl group. For example, compound **5d** (R_1 = methyl; R_2 = 4-benzyl) showed higher fungicidal activities than compound **5c**

(R_1 = methyl; R_2 = 4-phenyl). There may be a flexible methylene bridge in the phenylacetyl group, which favors bioactivity [15].

Because of the good antifungal effects against *P. oryzae* of the target compounds, our further studies in this work focused to determine the relationships between chemical structures and biological activity data for *P. oryzae*. Herein, a quantitative structure–activity relationships (QSAR) study was undertaken.

EC_{50} values are first transformed to log ($1/EC_{50}$) and used as dependent variables to get the linear relationship in the QSAR model [16]. These were correlated with different descriptors such as the highest occupied molecular orbital (HOMO) energy, the lowest unoccupied molecular orbital (LUMO) energy, hardness (HOMO–LUMO energy gap), *n*-octanol/water partition coefficient (log *P*), dipole moment (DM), and molar refractivity (MR) [17]. To obtain these parameters, the three-dimensional structures of the studied compounds were first sketched in the ChemBio3D Ultra 12.0 software [18], and then pre-optimized using molecular mechanics (MM) force field procedure, and the resulting geometric conclusions conformations were further refined by a more precise optimization using the semi-empirical AM1 method [19]. Finally, the geometry with the lowest energy for each derivative was then exported to Gaussian 03W (version 6.0) [20] to generate a set of molecular. The descriptor used in the present study was selected for its individual correlation with corresponding biological activity of compounds ($r > 0.5$), and the key descriptors correlating with the final equation are summarized in Table 2. Cross-correlation between these selected parameters is given in Table 3. Figure 5 displays the correlation relationship between experimental and predicted activity for the 21 compounds.

Correlation of the four descriptors demonstrated that LUMO was highly correlated with hardness (0.890) and log *P* was also strongly related to MR (0.955). Therefore, we must choose one between LUMO and hardness, as well as between log *P* and MR [21].

Table 2. Values of selected descriptors with correlation ($r > 0.5$).

Compd	LUMO	Hardness	Log *P*	MR
5a	−0.047	−0.186	0.84	87.31
5b	−0.044	−0.186	1.49	92.06
5c	−0.053	−0.178	2.73	107.44
5d	−0.042	−0.188	2.68	111.76
5e	−0.043	−0.186	3.17	117.65
5f	−0.069	−0.159	3.08	118.21
5g	−0.063	−0.154	2.95	125.46
5h	−0.074	−0.155	3.23	118.61
5i	−0.030	−0.184	1.18	92.11
5j	−0.028	−0.182	1.83	96.86
5k	−0.040	−0.173	3.07	112.24
5l	−0.029	−0.184	3.02	116.56
5m	−0.029	−0.182	3.50	122.45
5n	−0.075	−0.137	3.41	123.01
5o	−0.069	−0.141	3.29	130.26
5p	−0.080	−0.143	3.57	123.41
5q	−0.042	−0.186	2.50	105.91
5r	−0.041	−0.185	3.15	110.66
5s	−0.068	−0.158	4.73	136.81
5t	−0.063	−0.154	4.61	144.06
5u	−0.074	−0.154	4.89	137.21

The quality of each of the regression models was determined by the correlation coefficient (r), squared correlation coefficient (r^2), standard deviation (SE), and Fisher ratio (F) [22]. Of the combination of the parameters described before as independent variables, the best model for *P. oryzae* is Equation (1) as shown in Table 4.

As illustrated in Table 4, the inhibitory potential of each compound is highly correlated with log *P* and hardness. Log *P* is a measure of the lipophilicity of a chemical substance. A low log *P* indicates that the compound is lipophilic, while a high log *P* suggests a hydrophilic nature [23]. Lipophilicity is a very important molecular descriptor that often correlates well with the bioactivity of chemicals. In this study, the model shows that the

Table 3. Matrix of correlation between the selected variables used in equations.

	LUMO	Hardness	Log *P*	MR	Activity
LUMO	1.000				
Hardness	−0.890	1.000			
Log *P*	−0.561	−0.593	1.000		
MR	−0.617	−0.702	0.955	1.000	
Activity	0.599	−0.688	−0.879	−0.896	1.000

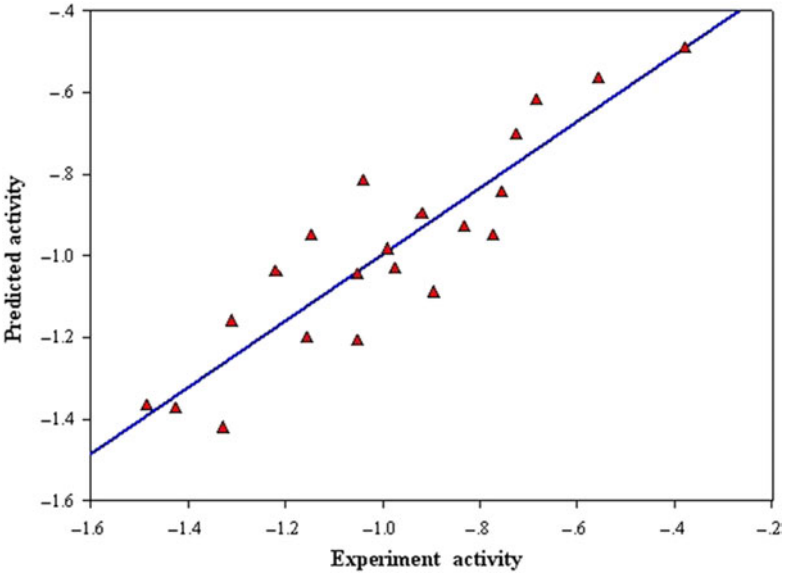


Figure 5. Plots of experiment versus predicted activity of compounds **5a**–**5u** screened against *Pyricularia oryzae* (Equation 1).

value of log *P* negatively correlates to antifungal activity. That is, a lower log *P* value will result in higher inhibitory activity, and high lipophilicity may be beneficial for derivatives to penetrate into the body of fungus.

It is worth noting that HOMO and LUMO are two most important factors which represent the ability to donate and accept electron, respectively [15]. Hardness is defined as the energy difference between HOMO and LUMO, which is widely used as a measure of chemical reactivity with bigger gaps implying larger excitation energies and higher stability [24]. It is indicated from Equation (1) that hardness places negative contribution towards the bioactivity.

Table 4. QSAR models for activity against *Pyricularia oryzae*.

QSAR models	*n*	*r*	r^2	*F*	SE
Log $(1/EC_{50}) = -1.102 - 0.197 \log P - 4.168$ Hardness (1)	21	0.903	0.815	39.710	0.129
Log $(1/EC_{50}) = 0.446 - 0.015$ MR $- 1.903$ Hardness (2)	21	0.900	0.809	38.161	0.131
Log $(1/EC_{50}) = -0.209 - 0.215 \log P + 2.535$ LUMO (3)	21	0.888	0.789	33.592	0.138
Log $(1/EC_{50}) = 0.911 - 0.016$ MR $+ 1.215$ LUMO (4)	21	0.898	0.806	37.287	0.133

In conclusion, a series of novel trichodermin derivatives with antifungal activity were designed and synthesized by incorporating the pharmacophore of conjugated oxime ether within the scaffold of trichodermin. Generally, most of the new compounds displayed excellent fungicidal activity, in which compounds **5a** and **5i** could be used as new lead compounds for the development of antifungal agent with further structure modification. In addition, the QSAR model indicated that log *P* and hardness were very important descriptors for the biological activities of the compounds.

3. Experimental

3.1 General experimental procedures

All reagents were commercially available and all anhydrous solvents were dried by standard techniques before use. All fungal materials were obtained from the National Engineering Research Center. Analytical thin-layer chromatography (TLC) was performed with silica gel plates using silica gel 60 GF_{254} (Qingdao Haiyang Chemical Co., Qingdao, China). Melting points were determined on a X-4 digital melting-point apparatus (Beijing Tech Instruments Co., Ltd., Beijing, China) and were uncorrected. ^{1}H and ^{13}C NMR (400 and 100 MHz, respectively) spectra were recorded on a Bruker AVANCE III instrument (Bruker CO., Bremen, Germany) in $CDCl_3$ using tetramethylsilane (TMS) as the internal standard. Mass spectra were recorded with a Finnigan Trace Mass 2000 spectra (Thermo Finnigan, California, USA) using the electrospray ionization (ESI) method. HRMS was determined with an APEX III 7.0 FT-ICR mass spectrometer (Bruker Daltonic Inc., New York, USA).

3.2 Bioassay

The antifungal activities of the target compounds were evaluated by the mycelium growth rate method [14]. Each of the test compounds was first dissolved in DMF, and a proper dilution was aseptically added to the medium at 45°C to generate a solution of corresponding concentration. *P. oryzae*, *B. cinerea*, and *S. sclerotiorum* were cultivated in potato dextrose agar (PDA) at 25 ± 1°C for 4 days to make a new mycelium for the identification of bioactivity. Then, mycelia dishes of approximately 4 mm diameter were cut from the culture. A mycelium was obtained using a germ-free inoculation needle and inoculated in the middle of the PDA plate aseptically. The inoculated plates were incubated at 25 ± 1°C for 4 days. The growth rate was conducted by measuring daily colony diameter for 4 days after the transport of the fungus onto dishes containing the substance to be tested. Each treatment had three repetitions. The relative inhibitory rates (I %) of the test compounds were calculated as follows:

$$I(\%) = \frac{\mathrm{CK} - \mathrm{PT}}{\mathrm{CK} - 0.4} \times 100,$$

where CK represents the diameter of fungal growth on untreated PDA, PT represents the diameter of fungi on treated PDA, and *I* represents the inhibition rate. Finally, the EC_{50} values were calculated to describe the inhibitory activity of the compounds.

3.3 Synthesis of title compounds

3.3.1 Synthesis of (4β)-4-acetoxy-8-oximido-12,13-epoxytrichothec-9-ene (3)

To a solution of compound **2** (0.92 g, 3 mmol) and hydroxylamine hydrochloride (0.63 g, 9 mmol) in anhydrous CH_3OH (10 ml) was added dropwise a solution of K_2CO_3 (1.24 g, 9 mmol) in water (10 ml) at 65°C. The reaction was stirred for 5 h at this temperature and monitored by TLC. After completion, the mixture was evaporated and extracted with ethyl acetate (3 × 5 ml). The combined extracts were washed with saturated brine, dried (anhy-

drous Na_2SO_4), and filtered. The filtrate was evaporated, and the crude product was purified via silica gel column chromatography, using a 1:3 (v/v) mixture of ethyl acetate and petroleum ether (boiling point range: 60–90°C) as the eluting solution to afford compound **3** as a colorless crystal; yield 89.83%; m.p.: 209–211°C; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): δ 8.57 (s, 1H, NO*H*), 5.86–5.84 (m, 1H, H-10), 4.31 (s, 1H, H-4), 3.89 (d, $J = 4.4$ Hz, 1H, H-11), 3.71 (d, $J = 4.8$ Hz, 1H, H-2), 3.15 (d, $J = 3.2$ Hz, 1H, H-13), 3.12 (d, $J = 12.4$ Hz, 1H, H-7), 2.94 (d, $J = 3.2$ Hz, 1H, H-13), 2.66 (dd, $J_1 = 6.0$ Hz, $J_2 = 12.8$ Hz, 1H, H-3), 2.38 (d, $J = 12.4$ Hz, 1H, H-7), 1.98–1.94 (m, 1H, H-3), 1.90 (s, 3H, O = CC*H*$_3$), 1.65 (s, 3H, H-16), 0.90 (s, 3H, H-14), 0.89 (s, 3H, H-15); ^{13}C NMR (100 MHz, $CDCl_3$): δ 4.88, 16.44, 17.46, 20.08, 24.60, 35.82, 40.24, 46.85, 47.97, 64.56, 69.11, 73.42, 78.35, 125.92, 133.71, 153.77, 170.01; ESI-MS (*m/z*): 322 $[M + H]^+$. HR-ESI-MS: *m/z* 322.1649 $[M + H]^+$ (calcd for $C_{17}H_{24}NO_5$, 322.1655).

3.3.2 *Synthesis of (4β)-8-methoxime-12, 13-epoxytrichothec-9-ene-4-ol (**4a**)*

To a stirred solution of **3** (0.96 g, 3 mmol) and 60% sodium hydride (0.24 g, 6 mmol) in *N*,*N*-dimethylformamide (10 ml) was slowly added iodomethane (0.57 g, 4 mmol). The reaction mixture was stirred at room temperature for 4 h. The mixture was poured into water (20 ml) and extracted with ethyl acetate (3 × 10 ml). The combined organic phase was washed with saturated brine (30 ml), dried over anhydrous Na_2SO_4, concentrated *in vacuo*, and purified via silica gel column chromatography to obtain compound **4a** as a colorless oil; yield 56.21%; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): δ 5.80–5.78 (m, 1H, H-10), 4.29 (s, 1H, H-4), 3.92 (s, 3H, NOC*H*$_3$), 3.87 (d, $J = 4.3$ Hz, 1H, H-11), 3.68 (d, $J = 4.7$ Hz, 1H, H-2), 3.12 (d, $J = 3.0$ Hz, 1H, H-13), 3.01 (d, $J = 12.6$ Hz, 1H, H-7), 2.92 (d, $J = 3.2$ Hz, 2H, H-13), 2.65 (dd, $J_1 = 6.0$ Hz, $J_2 = 12.6$ Hz, 1H, H-3), 2.30 (d, $J = 12.6$ Hz, 1H, H-7), 1.93–1.98 (m, 1H, H-3), 1.89 (s, 3H, H-16), 0.87 (s, 3H, H-14), 0.86 (s, 3H, H-15); ^{13}C NMR (100 MHz, $CDCl_3$): δ 5.88, 17.33, 18.31, 26.09, 30.90, 40.22, 47.55, 49.15, 61.97, 65.71, 69.80, 73.53, 78.92, 126.17, 135.17, 153.73; ESI-MS (*m/z*): 294 $[M + H]^+$.

Similar procedures as for the preparation of compound **4a** were used for the synthesis of compounds **4b** and **4c**.

3.3.3 *Synthesis of (4β)-8-methoxime-12, 13-epoxytrichothec-9-en-4-yl acetate (**5a**)*

Compound **4a** (0.59 g, 2 mmol), ethanoic acid (0.18 g, 3 mmol), DMAP (0.24 g, 2 mmol), and DCC (0.82 g, 4 mmol) were dissolved in dried dichloromethane (10 ml). The reaction was stirred at room temperature for 12 h. The process of the reaction was detected by TLC. After completion, the mixture was poured into water and extracted with dichloromethane (3 × 5 ml). The organic layer was washed with saturated brine (15 ml), dried over anhydrous Na_2SO_4, and filtered. The solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography to give compound **5a** as a colorless oil; yield 85.32%; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): δ 5.80–5.82 (m, 1H, H-10), 5.51 (dd, $J_1 = 2.8$, $J_2 = 6.4$ Hz, 1H, H-4), 3.93 (s, 3H, NOC*H*$_3$), 3.87 (d, $J = 4.3$ Hz, 1H, H-11), 3.78 (d, $J = 4.8$ Hz, 1H, H-2), 3.15 (d, $J = 3.2$ Hz, 1H, H-13), 3.00 (d, $J = 12.4$ Hz, 1H, H-7), 2.94 (d, $J = 3.2$ Hz, 1H, H-13), 2.58 (dd, $J_1 = 6.2$ Hz, $J_2 = 12.5$ Hz, 1H, H-3), 2.33 (d, $J = 12.4$ Hz, 1H, H-7), 2.10 (s, 3H, O = CC*H*$_3$), 2.04–2.00 (m, 1H, H-3), 1.90 (s, 3H, H-16), 0.94 (s, 3H, H-14), 0.79 (s, 3H, H-15); ESI-MS (*m/z*): 336 $[M + H]^+$.

The target compounds **5b**–**5u** were prepared by following the same procedures as for **5a**.

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