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Do *Epichloë* Endophytes and Their Grass Symbiosis Only Produce Toxic Alkaloids to Insects and Livestock?

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ABSTRACT: *Epichloë* endophytes in forage grasses have attracted widespread attention and interest of chemistry researchers as a result of the various unique chemical structures and interesting biological activities of their secondary metabolites. This review describes the diversity of unique chemical structures of taxa from *Epichloë* endophytes and grass infected with *Epichloë* endophytes and demonstrates their reported biological activities. Until now, nearly 160 secondary metabolites (alkaloids, peptides, indole derivatives, pyrimidines, sesquiterpenoids, flavonoids, phenol and phenolic acid derivatives, aliphatic metabolites, sterols, amines and amides, and others) have been reported from *Epichloë* endophytes and grass infected with *Epichloë* endophytes. Among these, non-alkaloids account for half of the population of total metabolites, indicating that they also play an important role in *Epichloë* endophytes and grass infected with *Epichloë* endophytes. Also, a diverse array of secondary metabolites isolated from *Epichloë* endophytes and symbionts is a rich source for developing new pesticides and drugs. Bioassays disclose that, in addition to toxic alkaloids, the other metabolites isolated from *Epichloë* endophytes and symbionts have notable biological activities, such as antifungal, anti-insect, and phytotoxic activities. Accordingly, the biological functions of non-alkaloids should not be neglected in the future investigation of *Epichloë* endophytes and symbionts.

KEYWORDS: *Epichloë, symbiont, metabolites, chemistry, bioactivity*

1. INTRODUCTION

A grass−endophyte fungus is a fungal microorganism, which spends all or part of its life cycle colonizing healthy tissues of grasses at the inter- and/or intracellular levels, typically causing no apparent symptoms of disease.[1,2] The endophytic population of a given grass species varies from a few to several dozens of fungal strains.[3,4] To date, endophytic fungi have been found in more than 80 genera and 290 species of Gramineae in the world.[5] In the year 1996, asexual *Neotyphodium* species included previously reported species within the asexual *Acremonium* species by Glenn et al., which usually inhabit cool-season grasses, such as *Festuca arundinacea* and *Lolium perenne*.[6] In the year 2014, asexual *Neotyphodium* species were known to be derived from sexual *Epichloë* species and have been reclassified in the genus of *Epichloë* according to the morphological similarity of conidia and the characteristics of hybridization sources of anamorphic endophytic fungi.[7] To date, 46 kinds of *Epichloë* have been reported in plants, including those previously found in the genus *Neotyphodium*.[7−9]

Under biotic and abiotic stresses, Gramineae plants infected with *Epichloë* have advantages of rapid growth and resistance to stress, diseases, and animal predation, improving their survival competitiveness and nutrient utilization efficiency compared to non-infected plants.[10−17] This is attributed to the production of abundant and diverse secondary metabolites, as observed in a host and pure culture, including four kinds of alkaloids: peramine, loline, indole diterpene, and ergot alkaloids.[11,18−22] Some alkaloids isolated from endophytic fungi are neurotoxic to animals and insects, especially ergot alkaloids and indole diterpene alkaloids, which can cause livestock poisoning or death with high intake.[22−29] Thus, the study of alkaloids has attracted much attention. Less research has been conducted on other types of secondary metabolites in *Epichloë*, particularly with regard to their role in infected grasses. In this review, we summarize the chemical structures and biological activities of secondary metabolites of *Epichloë* and their symbionts reported before 2019.

2. SECONDARY METABOLITES

All secondary metabolites were found in endophytes of *Epichloë* and grasses infected with *Epichloë* endophytes. The types of metabolites (**1−159**) (Table 1) include alkaloids (ergot alkaloids, indole diterpene alkaloids, loline alkaloids, and peramines), other peptides, indole derivatives, pyrimidines, sesquiterpenoids, flavonoids, phenol and phenolic acid derivatives, aliphatic metabolites, sterols, amines and amides, and others (Figure 1). To date, these secondary metabolites were obtained from 6 kinds of endophytic fungi (*Epichloë typhina*, *Epichloë lolii*, *Epichloë festucae*, *Epichloë bromicola*, *Epichloë bromicola* N1, and *Epichloë* sp.) and more than 10 kinds of symbionts (*Epichloë gansuense*−*Achnatherum inebrians*, *Epichloë festucae* var. *lolii*−*Lolium perenne*, *Epichloë* endophyte−*Stipa*

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Table 1. List of Secondary Metabolites from *Epichloë* Endophytes and Their Symbionts

number	name	MF	occurrence: fungi	occurrence: symbiont	reference
1	ergosine	$C_{30}H_{37}N_5O_5$	*Epichloë typhina*		30
2	ergosinine	$C_{30}H_{37}N_5O_5$			
3	chanoclavine I	$C_{19}H_{22}N_2O_1$			
4	agroclavine	$C_{16}H_{18}N_2$			31
5	elymoclavine	$C_{18}H_{22}N_2O_1$			
6	penniclavine	$C_{16}H_{18}N_2O_2$			
7	festuclavine	$C_{16}H_{20}N_2$			
8	6,7-secoagroclavine	$C_{16}H_{20}N_2$			
9	ergovaline	$C_{29}H_{35}N_5O_5$			
10	ergovalinine	$C_{29}H_{35}N_5O_5$			
11	ergonovine	$C_{19}H_{23}N_3O_2$		*Epichloë gansuense*−*Achnatherum inebrians*	33
12	ergonovinine	$C_{19}H_{23}N_3O_2$			
13	ergine	$C_{16}H_{17}N_3O$			
14	setoclavine	$C_{16}H_{18}N_2O_1$		*Epichloë* sp. Lp1−*Lolium perenne*	26
15	chanoclavine	$C_{16}H_{18}N_2O_1$		*Epichloë* sp. Lp1−*Lolium perenne*, *Epichloë festucae* var. *lolii*−*Lolium perenne*, and *Epichloë* endophyte−*Stipa robusta*	26, 35, 36, 43, and 45
16	lysergol	$C_{16}H_{18}N_2O_1$		*Epichloë festucae* var. *lolii*−*Lolium perenne*	36
17	lysergic acid	$C_{16}H_{16}N_2O_2$			
18	6,7-secolysergine	$C_{17}H_{22}N_2$		*Epichloë* sp. Lp1−*Lolium perenne*	26
19	isolysergamide	$C_{16}H_{17}N_3O_1$		*Epichloë* spp.−*Achnatherum robustum*, *Epichloë* species−*Achnatherum inebrians*, and *Epichloë* endophyte−*Stipa robusta*	35, 38, 43, and 45
20	lysergamide	$C_{16}H_{17}N_3O_1$		*Epichloë festucae* var. *lolii*−*Lolium perenne*, *Epichloë* spp.−*Achnatherum robustum*, *Epichloë* species−*Achnatherum inebrians*, and *Epichloë* endophyte−*Stipa robusta*	35, 36, 38, 43, and 45
21	8-hydroxylysergamide	$C_{16}H_{17}N_3O_2$		*Epichloë* endophyte−*Stipa robusta*	43 and 45
22	erginine	$C_{16}H_{17}N_3O_1$		*Epichloë coenophialum*−*Festuca arundinacea*	45, 46, 51, and 53
23	ergotamine	$C_{33}H_{35}N_5O_5$		*Epichloë typhina*−*Festuca arundinacea*	45, 46, 48, 51, and 53
24	ergotaminine	$C_{33}H_{35}N_5O_5$		*Epichloë coenophialum*−*Festuca arundinacea* and *Epichloë lolii*−*Lolium perenne*	45, 46, and 48
25	*β*-ergosine	$C_{30}H_{37}N_5O_5$		*Epichloë coenophialum*−*Festuca arundinacea* and *Epichloë typhina*−*Festuca arundinacea*	45, 46, 51, and 53
26	*β*-ergosinine	$C_{30}H_{37}N_5O_5$		*Epichloë coenophialum*−*Festuca arundinacea*	45 and 46
27	ergonine	$C_{30}H_{37}N_5O_5$		*Epichloë coenophialum*−*Festuca arundinacea* and *Epichloë typhina*−*Festuca arundinacea*	45, 46, 51, and 53
28	ergoninine	$C_{30}H_{37}N_5O_5$		*Epichloë coenophialum*−*Festuca arundinacea*	45 and 46
29	ergostine	$C_{34}H_{37}N_5O_5$			
30	ergostinine	$C_{34}H_{37}N_5O_5$			
31	ergoptine	$C_{31}H_{39}N_5O_5$			
32	ergoptinine	$C_{31}H_{39}N_5O_5$			
33	*β*-ergoptine	$C_{31}H_{39}N_5O_5$			
34	*β*-ergoptinine	$C_{31}H_{39}N_5O_5$			
35	ergosine	$C_{32}H_{41}N_5O_5$		*Epichloë coenophialum*−*Festuca arundinacea* and *Epichloë typhina*−*Festuca arundinacea*	45, 46, 51, and 53

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Table 1. continued

number	name	MF	occurrence: fungi	occurrence: symbiont	reference
36	ergosinine	$C_{32}H_{41}N_5O_5$		*Epichloë coenophialum*–*Festuca arundinacea*	45 and 46
37	*α*-ergocryptine	$C_{32}H_{41}N_5O_5$			
38	*α*-ergocryptinine	$C_{32}H_{41}N_5O_5$			
39	*β*-ergocryptine	$C_{32}H_{41}N_5O_5$			
40	*β*-ergocryptinine	$C_{32}H_{41}N_5O_5$			
41	ergocornine	$C_{31}H_{39}N_5O_5$		*Epichloë coenophialum*–*Festuca arundinacea* and *Epichloë typhina*–*Festuca arundinacea*	45, 46, 51, and 53
42	ergocorninine	$C_{31}H_{39}N_5O_5$		*Epichloë coenophialum*–*Festuca arundinacea*	45 and 46
43	ergocristine	$C_{35}H_{39}N_5O_5$		*Epichloë coenophialum*–*Festuca arundinacea* and *Epichloë typhina*–*Festuca arundinacea*	45, 46, 51, and 53
44	ergocristinine	$C_{35}H_{39}N_5O_5$		*Epichloë coenophialum*–*Festuca arundinacea*	
45	lolitrem A	$C_{42}H_{55}N_1O_8$		*Epichloë lolii*–*Lolium perenne*	24 and 57
46	lolitrem B	$C_{42}H_{55}N_1O_7$			24, 27, 55–57, 60, and 62
47	lolitrem C	$C_{42}H_{57}N_1O_7$			56, 57, and 60
48	lolitrem E	$C_{42}H_{57}N_1O_7$			27, 56, and 57
49	lolitrem F	$C_{42}H_{55}N_1O_7$			58
50	lolilline	$C_{37}H_{47}N_1O_6$			59
51	lolicine A	$C_{38}H_{54}N_1O_4$			19
52	lolicine A 11-*O*-propionate	$C_{41}H_{57}N_1O_5$			
53	lolicine B	$C_{39}H_{53}N_1O_5$			
54	lolicine B 11-*O*-propionate	$C_{41}H_{55}N_1O_6$			
55	lolitrem N	$C_{37}H_{49}N_1O_7$			
56	lolitrem N 10-*O*-acetate	$C_{39}H_{51}N_1O_8$			
57	lolitriol	$C_{37}H_{49}N_1O_7$			
58	lolitriol 10-*O*-acetate	$C_{39}H_{51}N_1O_8$			
59	terpendole M	$C_{32}H_{41}N_1O_6$			61
60	paspaline	$C_{28}H_{39}N_1O_2$			27, 40, 60, and 61
61	13-desoxypaxilline	$C_{27}H_{33}N_1O_3$			40 and 61
62	epoxy-janthitrem I	$C_{39}H_{51}N_1O_7$		*Epichloë* species–*Lolium* species	40
63	paspaline B	$C_{28}H_{37}N_1O_3$			
64	paxilline	$C_{27}H_{33}N_1O_4$			
65	terpendole C	$C_{32}H_{41}N_1O_5$			40
66	terpendole B	$C_{27}H_{35}N_1O_3$			27 and 40
67	terpendole E	$C_{28}H_{39}N_1O_3$			40
68	1′-*O*-acetylpaxilline	$C_{29}H_{35}N_1O_5$			63
69	loline	$C_8H_{14}N_2O_1$		*Epichloë* endophyte–*Festuca* grasses	44, 64, 66, and 68
70	norloline	$C_7H_{12}N_2O_1$			44
71	*N*-acetylloline	$C_{10}H_{16}N_2O_2$			44 and 68
72	*N*-acetylnorloline	$C_8H_{12}N_2O_2$			44 and 66

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Table 1. continued

number	name	MF	occurrence: fungi	occurrence: symbiont	reference
73	*N*-formylloline	$C_9H_{14}N_2O_2$			44
74	*N*-formylnorloline	$C_8H_{12}N_2O_2$			44, 65, 66, and 68
75	rotamer of *N*-formylloline	$C_9H_{14}N_2O_2$			44 and 66
76	*N*-methylloline	$C_9H_{16}N_2O_1$			44, 66, and 68
77	*N*-ethylnorloline	$C_9H_{16}N_2O_1$		*Epichloë uncinata*−*Festuca pratensis* and *Epichloë siegelii*−*Lolium perenne*	44
78	5,6-dehydro-*N*-acetylloline	$C_{11}H_{16}N_2O_2$		*Epichloë* endophyte−*Festuca argentina*	68
79	peramine	$C_{12}H_{17}N_5O_1$	*Epichloë lolii*		60 and 69
80	diacetylperamine	$C_{16}H_{21}N_5O_3$			60
81	cyclo-(L-Pro-L-Leu)	$C_{11}H_{18}N_2O_2$	*Epichloë typhina*		70 and 71
82	cyclo-(L-Pro-L-Phe)	$C_{14}H_{16}N_2O_2$			70 and 72
83	epichlicin	$C_{48}H_{74}N_{12}O_{14}$			73
84	epichloenin A	$C_{46}H_{74}N_{12}O_{18}$	*Epichloë festucae*		74
85	epichloenin B	$C_{46}H_{75}N_7O_{17}$			
86	epichloeamide	$C_{46}H_{74}N_{12}O_{15}$			
87	cyclosporin T	$C_{61}H_{109}N_{11}O_{12}$	*Epichloë bromicola*		71
88	dahurelmusin A	$C_{18}H_{30}N_2O_5$		*Epichloë bromicola*−*Elymus dahuricus*	75
89	indole-3-carboxaldehyde	$C_{10}H_9N_1O_2$	*Epichloë festucae*		77
90	methylindole-3-carboxylate	$C_9H_7N_1O_1$			
91	indole-3-ethanol	$C_{10}H_{11}N_1O_1$			
92	indole-3-acetic acid	$C_{10}H_9N_1O_2$			71 and 77
93	indoleacetic acid	$C_{10}H_9N_1O_2$	*Epichloë* sp.		72
94	uracil	$C_4H_4N_2O_2$	*Epichloë bromicola*		71
95	5-methyluracil	$C_5H_6N_2O_2$	*Epichloë bromicola* and *Epichloë bromicola* N1		
96	thymidine	$C_{10}H_{14}N_2O_5$	*Epichloë bromicola* N1		79
97	chokol A	$C_{10}H_{22}O_2$	*Epichloë typhina*		80 and 81
98	chokol B	$C_{15}H_{26}O_2$			
99	chokol C	$C_{15}H_{26}O_2$			
100	chokol D	$C_{15}H_{26}O_2$			80
101	chokol E	$C_{15}H_{28}O_3$			
102	chokol F	$C_{14}H_{24}O_3$			
103	chokol G	$C_{11}H_{22}O_2$			
104	cyclonerodiol	$C_{15}H_{28}O_2$	*Epichloë festucae*		77
105	(−)-sydonic acid	$C_{15}H_{22}O_4$	*Epichloë bromicola*		71
106	isoorientin	$C_{21}H_{20}O_{11}$		*Epichloë typhnium*−*Poa ampla*	82
107	tricin	$C_{17}H_{14}O_7$			
108	7-*O*-(*β*-D-glucopyranosyl)tricin	$C_{23}H_{24}O_{11}$			
109	7-*O*-[*α*-L-rhamnopyranosyl(1−6)-*β*-D-glucopyranosyl]tricin	$C_{23}H_{28}O_{21}$			
110	*P*-hydroxybenzoic acid	$C_7H_6O_3$	*Epichloë typhina*		71 and 83
111	2-(4-hydroxyphenyl)-ethanol	$C_8H_{10}O_2$			
112	(4-hydroxyphenyl)-acetic acid	$C_8H_8O_3$			
113	(*E*)-3-(4-hydroxyphenyl)acrylic acid	$C_9H_8O_3$			83

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Table 1. continued

number	name	MF	occurrence: fungi	occurrence: symbiont	reference
114	(*Z*)-3-(4-hydroxyphenyl)acrylic acid	$C_9H_8O_3$			
115	1,2-*O*-di-*trans*-*p*-coumaroylglycerol	$C_{21}H_{20}O_7$			
116	1,3-*O*-di-*trans*-*p*-coumaroylglycerol	$C_{21}H_{20}O_7$			
117	chokorm	$C_{42}H_{40}O_{14}$			
118	vanillic acid	$C_8H_8O_4$			71
119	4-hydroxybenzaldehyde	$C_7H_6O_2$			70
120	butyl 4-hydroxybenzoate	$C_{11}H_{14}O_3$	*Epichloë bromicola* N1		79
121	3-(2′-(4″-hydroxyphenyl)acetoxy)-2*S*-methylpropanoic acid	$C_{12}H_{14}O_5$	*Epichloë bromicola*		71
122	3,3′-dihydroxy-5,5′-dimethyldiphenyl ether	$C_{14}H_{14}O_3$	*Epichloë bromicola* and *Epichloë bromicola* N1		71 and 79
123	benzeneacetic acid	$C_8H_8O_2$	*Epichloë* sp.		72
124	1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester	$C_{14}H_{18}O_4$			
125	phenol, 2,2′-methylenebis[6-(1,1-dimethylethyl)-4-methyl]-	$C_{23}H_{32}O_2$			
126	10-hydroxy-8*E*, 12*Z*-octadecadienoid acid.	$C_{19}H_{34}O_3$	*Epichloë typhina*		84
127	12-hydroxy-9*Z*,13*E*-octadecadienoic acid.	$C_{19}H_{34}O_3$			
128	10-hydroxy-8*E*-octadecenoic acid	$C_{19}H_{36}O_3$			
129	9-hydroxy-10*E*-octadecenoic acid,	$C_{19}H_{36}O_3$			
130	ethyl *trans*-9,10-epoxy-11-oxoundecanoate	$C_{13}H_{22}O_4$			85
131	ethyl 9-oxononanoate	$C_{11}H_{20}O_3$			
132	ethyl azelate	$C_{11}H_{20}O_4$			
133	hydroxydihydrobovolide	$C_{12}H_{20}O_3$			
134	hexadecanoic acid	$C_{16}H_{32}O_2$	*Epichloë* sp.		72
135	*cis*-13-octadecenoic acid	$C_{17}H_{32}O_2$			
136	9,12-octadecadienoic acid (*Z*, *Z*)	$C_{19}H_{34}O_2$			
137	fumaric acid methyl ester	$C_5H_6O_4$	*Epichloë bromicola*		71
138	3-acetoxy-2*S*-methylpropanoic acid	$C_6H_{10}O_4$			
139	1(10→6)*abeo*-ergosta-5,7,9,22-tetraen-3*α*-ol	$C_{28}H_{42}O_1$	*Epichloë typhina*		86
140	5*α*,8*α*-epidioxyergosta-6,22-dien-3*β*-ol	$C_{28}H_{44}O_3$	*Epichloë lolii*		87
141	5*α*,8*α*-epidioxyergosta-6,9(11),22-trien-3*β*-ol	$C_{28}H_{42}O_3$			
142	ergosta-4,6,8(14),22-tetraen-3-one	$C_{28}H_{40}O_1$	*Epichloë bromicola* N1		79
143	ergosta-5,7,22-trien-3*β*-ol	$C_{28}H_{44}O_1$			
144	3-hydroxy-9-oxo-4-tetradecyl-5-oxa-l-azabicyclo[4.3.0]nonane-2-methanol	$C_{22}H_{39}NO_4$	*Epichloë typhina*		88
145	3-hydroxy-9-oxo-4-(4*E*-tetradecenyl)-5-oxa-1-azabicyclo[4.3.0]nonane-2-methanol	$C_{22}H_{41}NO_4$			
146	diacetamide	$C_4H_7NO_2$	*Epichloë festucae*		77
147	2-phenylacetamide	C_8H_9NO	*Epichloë bromicola* N1		79
148	2-(acetylamino)-2-deoxy-*β*-D-talopyranose	$C_8H_{15}NO_6$			
149	4-(phenylamino)phenol	$C_{12}H_{11}NO$			
150	gamahonolide A	$C_{12}H_{20}O_3$	*Epichloë typhina*		89
151	gamahonolide B	$C_{18}H_{28}O_6$			
152	gamahorin	$C_{12}H_{14}O_4$			
153	5-hydroxy-4-phenyl-2(5*H*)-furanone	$C_{10}H_8O_3$			
154	alternariol	$C_{14}H_{10}O_5$	*Epichloë bromicola* N1		79
155	alternariol monomethyl ether	$C_{15}H_{12}O_5$			

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Table 1. continued

number	name	MF	occurrence: fungi	occurrence: symbiont	reference
156	1*H*-indazole	$C_7H_6N_2$	*Epichloë bromicola*		71
157	2-benzothiazolinone	C_7H_5NOS	*Epichloë bromicola* N1		71 and 79
158	D-mannitol	$C_6H_{14}O_6$	*Epichloë bromicola* and *Epichloë bromicola* N1		
159	furan-2-carboxylic acid	$C_5H_4O_3$	*Epichloë bromicola*		71

robusta, *Epichloë coenophialum*—*F. arundinacea*, *E. typhina*—*F. arundinacea*, *E. lolii*—*L. perenne*, *Epichloë uncinata*—*Festuca pratensis*, *Epichloë siegelii*—*L. perenne*, *E. bromicola*—*Elymus dahuricus*, *Epichloë typhnium*—*Poa ampla*, and some endophyte fungi not being determined, such as *Epichloë endophyte*—*Festuca argentina*, *Epichloë* sp. Lp1—*L. perenne*, *Epichloë* species—*Lolium* species, *Epichloë* species—*A. inebrians*, *Epichloë* sp.—*Achnatherum robustum*, etc.). However, the four types of alkaloids are mainly isolated from the grass infected with *Epichloë* (*F. arundinacea*—*E. typhina*, *F. arundinacea*—*E. coenophialum*, *F. arundinacea*—*E. lolii*, *L. perenne*—*E. festucae*, *L. perenne*—*E. lolii*, *A. inebrians*—*E. gansuense*, and *Lolium* or *Festuca* species—*Epichloë* species), and alkaloids account for half of the total obtained secondary metabolites. There are still many other types of secondary metabolites of endophytic fungi and their symbionts to be further studied. The structures and names of these isolated secondary metabolites from *Epichloë* and their symbionts are shown below.

2.1. Ergot Alkaloids. Ergosine/ergosinine and chanoclavine I (**1–3**) have been isolated from *E. typhina*, the fungus obtained from toxic K-31 tall fescue (*F. arundinacea*) grass by Porter et al.[30] Porter et al. also isolated agroclavine, elymoclavine, penniclavine, festuclavine, 6,7-secoagroclavine, and ergovaline/ergovalinine (**4–10**) from this fungus.[31] In addition, the two ergot peptide alkaloids (ergosine/ergosinine) were determined as ergovaline/ergovalinine with isobutane chemical ionization mass spectroscopy. Their names were consistent with their structures to avoid confusion. Ergonovine/ergonovinine and ergine (**11–13**) have been obtained from the aerial parts of drunken horse grass (*A. inebrians*) infected with endophyte (*E. gansuense*) (Figure 2).[32] The above ergot alkaloids were obtained by various separation and purification techniques.

Although setoclavine (**14**), chanoclavine (**15**), lysergol (**16**), lysergic acid (**17**), 6,7-secolysergine (**18**), isolysergamide (**19**), lysergamide (**20**), 8-hydroxylysergamide (**21**), ergine/erginine (**13/22**), ergotamine/ergotaminine (**23/24**), *β*-ergosine/*β*-ergosinine (**25/26**), ergonine/ergoninine (**27/28**), ergostine/ergostinine (**29/30**), ergoptine/ergoptinine (**31/32**), *β*-ergoptine/*β*-ergoptinine (**33/34**), ergosine/ergosinine (**35/36**), *α*-ergocryptine/*α*-ergocryptinine (**37/38**), *β*-ergocryptine/*β*-ergocryptinine (**39/40**), ergocornine/ergocorninine (**41/42**), and ergocristine/ergocristinine (**43/44**) have been isolated from the genus *Clavicep*, the following alkaloids were also detected and confirmed in symbionts: *F. arundinacea* infected with *E. typhina*, *E. lolii*, and *E. coenophialum*, *A. inebrians* infected with *Epichloë*-like endophytic fungus, and *L. perenne* infected with *E. lolii*, *E. festucae*, and *Epichloë* species, using various analysis methods (Figure 3).[11,33–54] Because the structures of aciergovaline/aciergovalinine and didehydroergovaline/didehydroergovalinine are uncertain, their chemical structures are not included in this report.[46]

2.2. Indole Diterpene Alkaloids. Lolitrem A, lolitrem B, lolitrem C, lolitrem E, lolitrem F, and lolilline (**45–50**) have been isolated from extracts of the seed of *L. perenne* infected with the endophytic fungus *E. lolii* (Figure 4).[55–60] Lolitrem F, lolicine A, lolicine A 11-*O*-propionate, lolicine B, lolicine B 11-*O*-propionate, lolitrem N, lolitrem N 10-*O*-acetate, lolitriol, lolitriol 10-*O*-acetate, and terpendole M (**49** and **51–59**) were identified in extracts of *L. perenne* infected with the endophytic fungus *E. lolii*.[19,27] Terpendole M, paspaline, and 13-desoxypaxilline (**59–61**) were obtained from *L. perenne* infected with the endophytic fungus *E. lolii*.[61,62] Thus, the above indole diterpene alkaloids are mainly isolated from *L. perenne* infected

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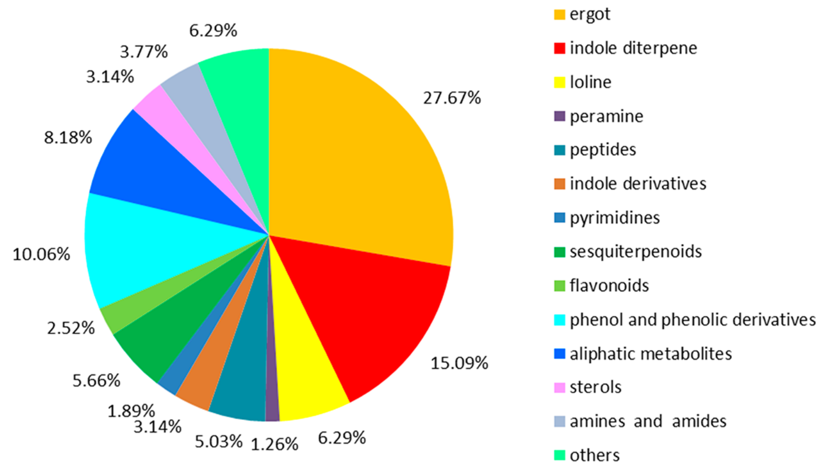

Figure 1. All secondary metabolites obtained from *Epichloë* and their symbionts.

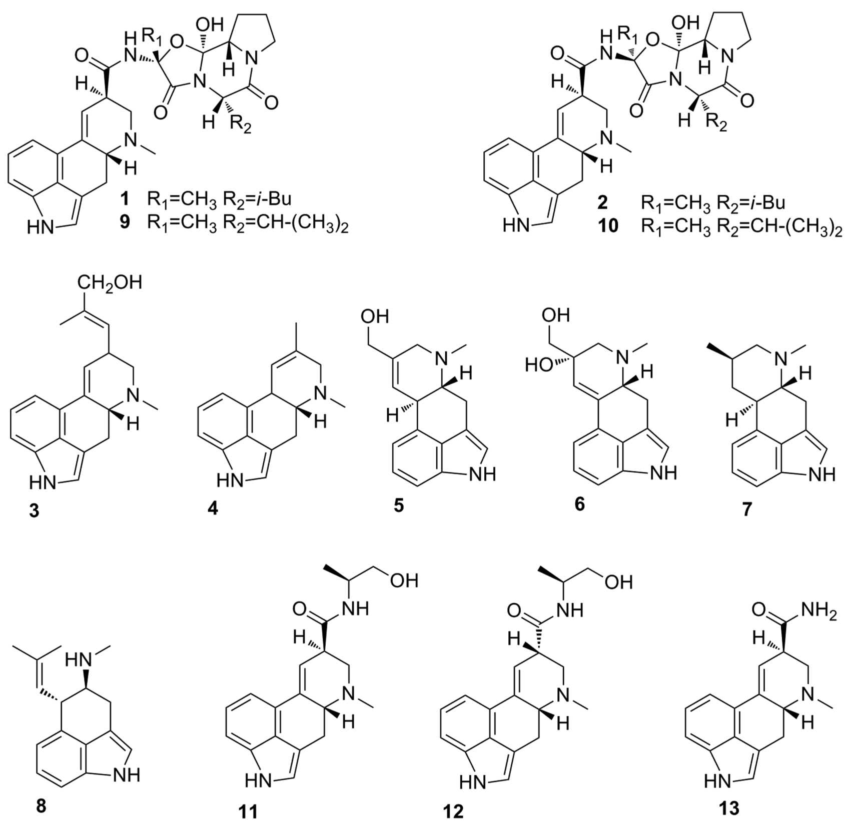

Figure 2. Structures of compounds **1**–**13**.

with *E. lolii*. Epoxy-janthitrem I, paspaline B, paxilline, terpendole C, terpendole B, terpendole E, and 1′-*O*-acetylpaxilline (**62**–**68**) were detected in *Lolium* species infected with *Epichloë* species by high-performance liquid chromatography (HPLC) and gas chromatography with flame ionization detection (GC–FID).[40,60,63] These *Lolium* species contain *L. arundinacea*, *L. multiflorum*, *L. perenne*, *L. persicum*, *L. rigidum*, and *L. temulentum*.

2.3. Loline Alkaloids. Loline, norloline, *N*-acetylloline, *N*-acetylnorloline, rotamer of *N*-formylloline, *N*-formylnorloline, *N*-methylloline, and *N*-ethylnorloline (**69**–**76**) have mainly been found in the genus of *Festuca* grasses infected with *Epichloë* endophytes (Figure 5).[44,64,65] Loline (**69**), *N*-acetylnorloline (**72**), and rotamer of *N*-formylloline (**75** and**76**) were isolated from *Epichloë uncinatum*.[66] *N*-Ethylnorloline (**77**) was detected in both *F. pratensis* infected with *E. uncinata* and *L. perenne* infected with *E. siegelii*.[13,67] In addition, loline (**69**), *N*-acetylloline (**71**), *N*-formylloline (**74**), *N*-methylloline (**76**), and 5,6-dehydro-*N*-acetylloline (**78**) were obtained from *F. argentina* infected with *Epichloë* endophyte.[68] Loline alkaloids can be isolated from the pure culture of *Epichloë* endophyte.

2.4. Peramine Alkaloids. Peramine and diacetylperamine (**79** and **80**) have been identified in the mycelium of *E. lolii* by mass spectroscopy, ultraviolet spectroscopy, and thin-layer chromatography (Figure 6).[60,69] However, only peramine was obtained from *L. perenne* infected with the fungal endophyte *E. lolii*.

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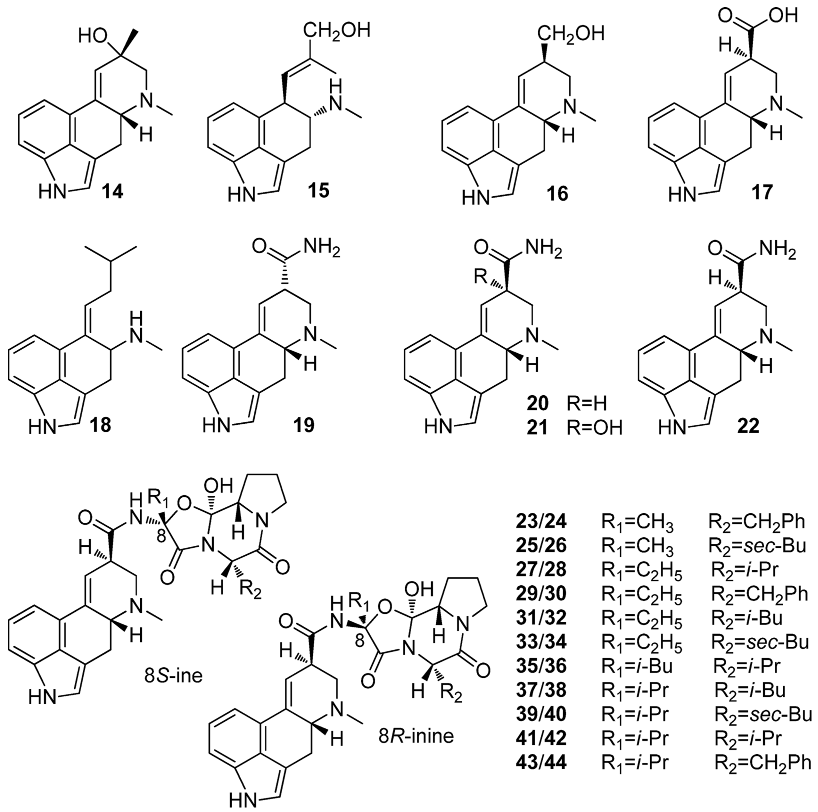

Figure 3. Structures of compounds 14−44.

2.5. Peptides. Two cyclic dipeptides, cyclo-(L-Pro-L-Leu) and cyclo-(L-Pro-L-Phe) (**81** and **82**), have been identified from the culture filtrate of *E. typhina* (Figure 7).[70,71] These two metabolites (**81** and **82**) were also obtained from *E. bromicola* and the asexual endophyte of *Epichloë* sp. from *Festuca sinensis*, respectively, indicating that cyclic dipeptide can be used as marker chemicals to distinguish the difference between *Epichloë* and the other types of endophytic fungi.[71,72] Epichlicin (**83**) was identified from *E. typhina*, an endophytic fungus of *Phleum pretense*.[73] Epichloenin A, epichloenin B, and epichloeamide (**84−86**) from the endophytic fungus *E. festucae* have been identified in culture and endophyte-infected *L. perenne*.[74] Cyclosporin T (**87**) was obtained from a fungus of *E. bromicola* (isolated from *Elymus tangutorum*).[71] A novel hybrid peptide−polyketide, dahurelmusin A (**88**), was first isolated from *E. dahuricus* infected by *E. bromicola* endophyte.[75] Dahurelmusin A possesses a 5-hydroxy-2,2,4,6-tetramethyl-3-oxooctanoic acid residue and a novel 5-hydroxy-2,2,4,6-tetramethyl-3-oxooctanoic acid unit. This type of hybrid peptide−polyketide is derived from microorganisms. Therefore, we speculate that *E. bromicola* infected with *E. dahuricus* yielded dahurelmusin A, which is attributed to *E. bromicola* living within *E. dahuricus*. At the same time, this type of metabolite was first obtained from the grass infected with *Epichloë* endophytes. In addition to four major alkaloids, dahurelmusin A is a new non-alkaloid with insecticidal activity, which is consistent with the reported literature.[76]

2.6. Indole Derivatives. Four indole metabolites, methylindole-3-carboxylate, indole-3-carboxaldehyde, indole-3-ethanol, and indole-3-acetic acid (**89−92**), were obtained from the fermentation of *E. festucae* through the bioassay-directed fraction, and *Cryphonectria parasitica* was used as the test target strain (Figure 8).[77] Indole-3-acetic acid and indoleacetic acid (**92** and **93**) were identified from the asexual endophyte of *Epichloë* sp. from *F. sinensis*.[72] Indole-3-acetic acid (**92**), as a plant auxin, can promote the formation of top buds of shoots.[78] Numerous studies have shown that *Epichloë* endophytes may improve the competitiveness of host plants. Thus, we conclude that indole-3-acetic acid (**92**) produced by the endophytic fungi plays a key role in promoting the growth of host plants.

2.7. Pyrimidines. Uracil (**94**) and thymidine (**96**) were isolated from the fungus of *E. bromicola* and *E. bromicola* N1, respectively, and 5-methyluracil (**95**) was obtained from *E. bromicola* and *E. bromicola* N1 (Figure 9).[71,79] Thus far, these pyrimidines have only been found in this *Epichloë* fungus.

2.8. Sesquiterpenoids. Seven sesquiterpenoids, chokols A−G (**97−103**), were obtained from the stromata of *E. typhina* (Figure 10).[80,81] Cyclonerodiol (**104**) was obtained from the stromata of *E. festucae* and inhibited the growth of *C. parasitica*.[77] Another type of sesquiterpenoid, (−)-sydonic acid (**105**), was identified from a fungus of *E. bromicola*.[71] Although there are many types of sesquiterpenes, only nine sesquiterpenoids have been found in *Epichloë* endophytes.

2.9. Flavonoids. Bioassay-guided fractionation of the ethyl acetate fraction of the leaves of *P. ampla* infected with *E. typhnium* yielded isoorientin, tricin, 7-*O*-(*β*-D-glucopyranosyl)-tricin, and 7-*O*-[*α*-L-rhamnopyranosyl(1−6)-*β*-D-glucopy ranosyl]tricin (**106−109**) (Figure 11).[82] There are a large number of flavonoids obtained from Gramineae plants, but at present, only these four flavonoids are isolated from *E. typhnium*−*P. ampla* symbiosis. There is no study on the content and structure difference of flavonoids in Gramineae plants with and without endophytes.

2.10. Phenol and Phenolic Acid Derivatives. *p*-Hydroxybenzoic acid, 2-(4-hydroxyphenyl)-ethanol, (4-hydroxyphenyl)-acetic acid, (*E*)-3-(4-hydroxyphenyl)acrylic acid, and (*Z*)-3-(4-hydroxyphenyl)acrylic acid (**110−114**), together with

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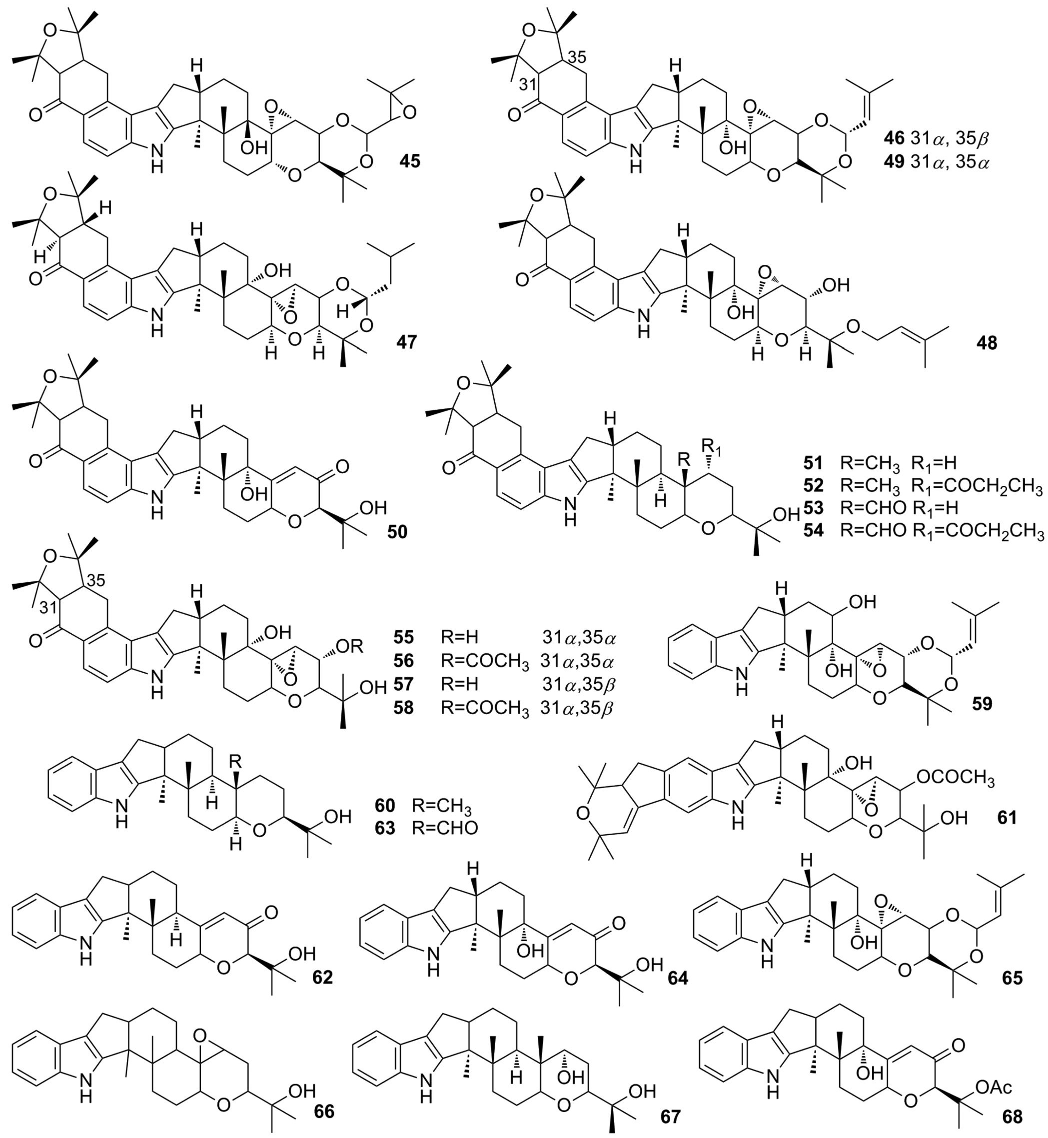

Figure 4. Structures of compounds **45**−**68**.

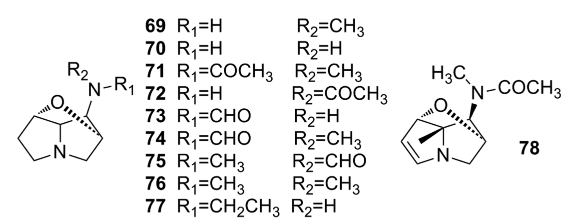

Figure 5. Structures of compounds **69**−**78**.

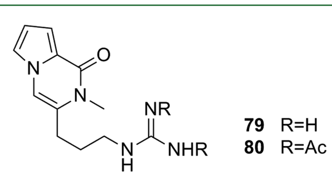

Figure 6. Structures of compounds **79** and **80**.

four phenolic acid derivatives (1,2-*O*-di-*trans*-*p*-coumaroylglycerol, 1,3-*O*-di-*trans*-*p*-coumaroylglycerol, chokorm, and 4-hydroxybenzaldehyde) (**115**−**117** and **119**) were obtained from the stromata of *E. typhina* (Figure 12).[70,83] Vanillic acid, 3-(2′-(4″-hydroxyphenyl)acetoxy)-2*S*-methylpropanoic acid, and 3,3′-dihydroxy-5,5′-dimethyldiphenyl ether (**118**, **121**, and **122**) were characterized from *E. bromicola*.[71] Butyl 4-hydroxybenzoate and 3,3′-dihydroxy-5,5′-dimethyldiphenyl ether (**120**, and **122**) were isolated from *E. bromicola* N1.[79] The phenol and phenolic acid derivatives, 2-(4-hydroxyphenyl)-ethanol, benzeneacetic acid, 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester, and phenol, 2,2′-methylenebis[6-(1,1-dimethylethyl)-4-methyl]- (**111**, and **123**−**125**), were identified from *Epichloë* sp.[72] A total of 16 phenol and phenolic acid derivatives (**110**−**125**) were isolated from *Epichloë* endophytes; however, it is unclear why these metabolites exist in *Epichloë* endophytes and what biological functions they provide for host plants.

2.11. Aliphatic Metabolites. Four fungitoxic C-18 hydroxy unsaturated fatty acids (**126**−**129**) were obtained from the stromata of *E. typhina* in 1987 by Koshino et al. (Figure 13).[84] In 1989, Koshino et al. also isolated ethyl *trans*-9,10-epoxy-11-oxoundecanoate (**130**), ethyl 9-oxononanoate (**131**), ethyl azelate (**132**), and cyclonerodiol (**133**) from this fungus.[85] Hexadecanoic acid, *cis*-13-octadecenoic acid, and 9,12-octadecadienoic acid (*Z*,*Z*) (**134**−**136**) were identified from *Epichloë*

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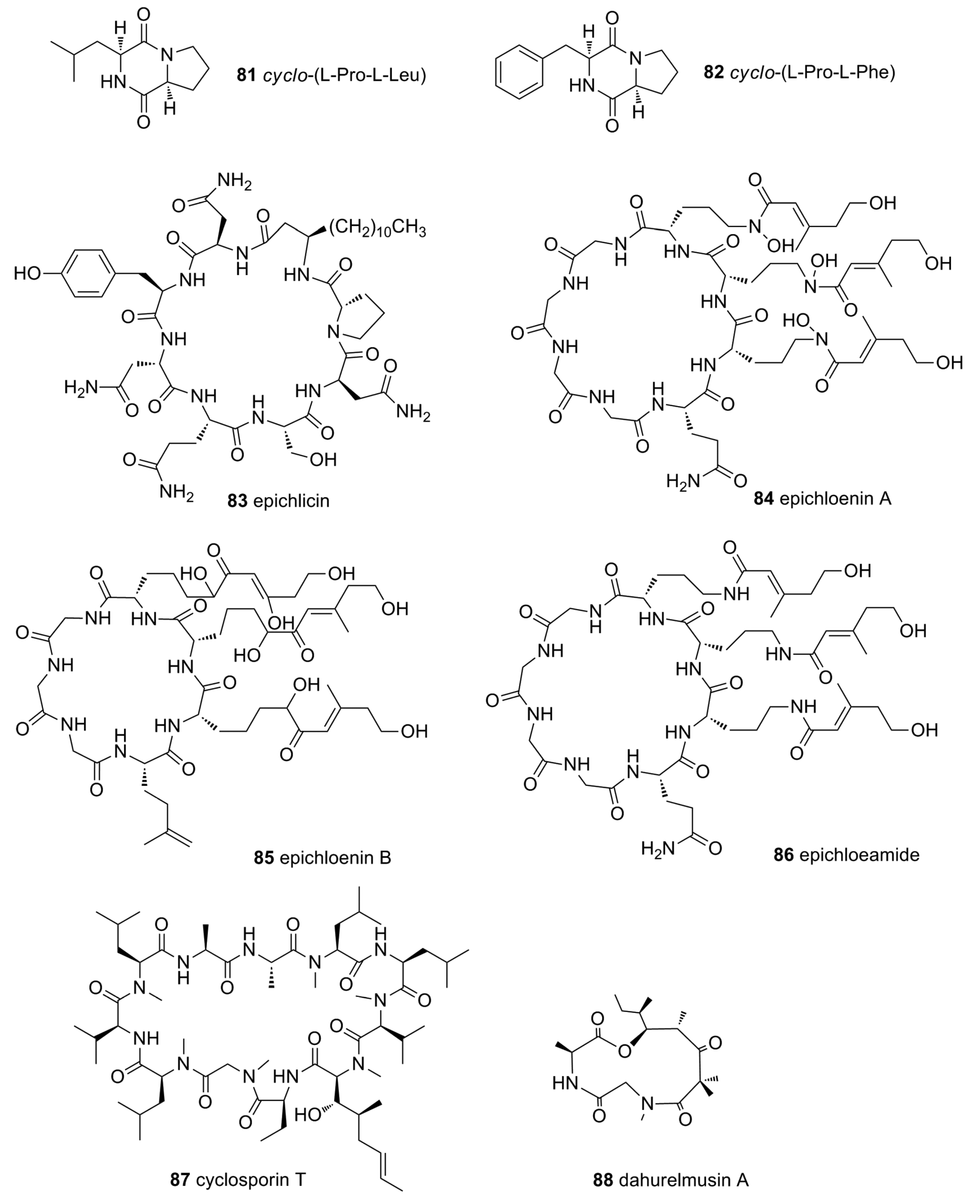

Figure 7. Structures of compounds **81**−**88**.

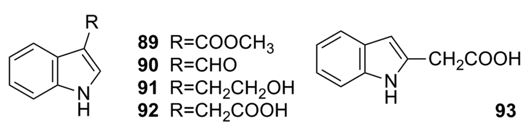

Figure 8. Structures of compounds **89**−**93**.

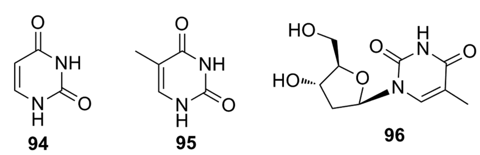

Figure 9. Structures of compounds **94**−**96**.

sp.[72] Fumaric acid methyl ester and 3-acetoxy-2*S*-methylpropanoic acid (**137** and **138**) were obtained from *E. bromicola*, which are simple ester metabolites.[71] A total of 13 fatty acid compounds (**126**−**138**) were isolated from *Epichloë* endophytes, but there are no reports about the biological activity of these aliphatic metabolites. Accordingly, more attention should be given to the roles of these metabolites of *Epichloë* endophytes in the future.

2.12. Sterols. A sterol with an aromatized B ring (**139**) was obtained from *E. typhina*.[86] Two 5α,8α-epidioxyergosta-3β-ols (**140** and **141**) were isolated from the endophyte, *E. lolii*.[87] The sterols ergosta-4,6,8(14),22-tetraen-3-one and ergosta-5,7,22-trien-3β-ol (**142** and **143**) were identified from *E. bromicola* N1 (Figure 14).[79] Among these, ergosta-5,7,22-trien-3β-ol (**143**) was found in all tested endophytic fungi. The content of ergosta-5,7,22-trien-3β-ol (**143**) in tested grass seeds had a high correlation with the endophyte content. Therefore, analysis of sterol **143** may be used to predict the endophyte content in grass infected with endophytic fungi.

2.13. Amines and Amides. Two sphingoid derivatives, 3-hydroxy-9-oxo-4-tetradecyl-5-oxa-l-azabicyclo[4.3.0]nonane-2-methanol (**144**) and 3-hydroxy-9-oxo-4-(4*E*-tetradecenyl)-5-oxa-1-azabicyclo[4.3.0]nonane-2-methanol (**145**), were obtained from the stromata of *E. typhina* (Figure 15).[88] Diacetamide (**146**) was isolated from the culture of *E. festucae*.[77] Three secondary metabolites, 2-phenylacetamide (**147**), 2-

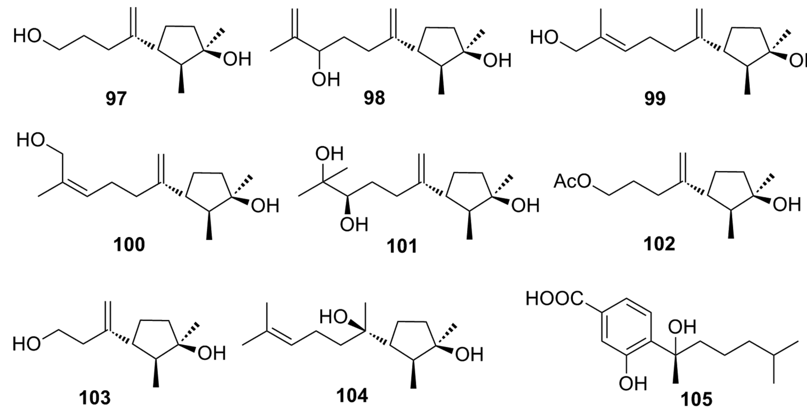

Figure 10. Structures of compounds **97**−**105**.

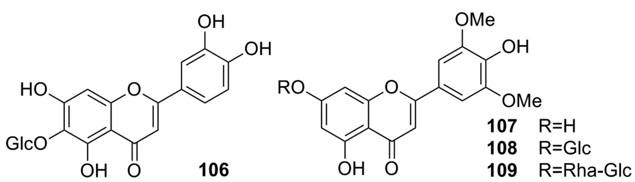

Figure 11. Structures of compounds **106**−**109**.

(acetylamino)-2-deoxy-*β*-D-talopyranose (**148**), and 4-(phenylamino)phenol (**149**), were identified from *E. bromicola* N1.[79] To date, the biological activities of these amines and amides in *Epichloë* have not been studied.

2.14. Others. Gamahonolides A and B (**150** and **151**), gamahorin (**152**), and 5-hydroxy-4-phenyl-2(5*H*)-furanone (**153**) were identified from the stromata of *E. typhina* (Figure 16).[89] Alternariol, alternariol monomethyl ether, 1*H*-indazole, 2-benzothiazolinone, D-mannitol, furan-2-carboxylic acid, and 5-hydroxy-4-phenyl-2(5*H*)-furanone (**154**−**159**) were identified from *E. bromicola* and *E. bromicola* N1, and the fungi were isolated from plants of the genus *Elymus*.[71,79]

3. BIOLOGICAL ACTIVITIES

3.1. Toxicity to Livestock. Ergot alkaloids are thought to be responsible for the toxicity of *F. arundinacea* and *L. perenne* to livestock and insects.[18,21,90−92] Endophyte−grass symbiosis

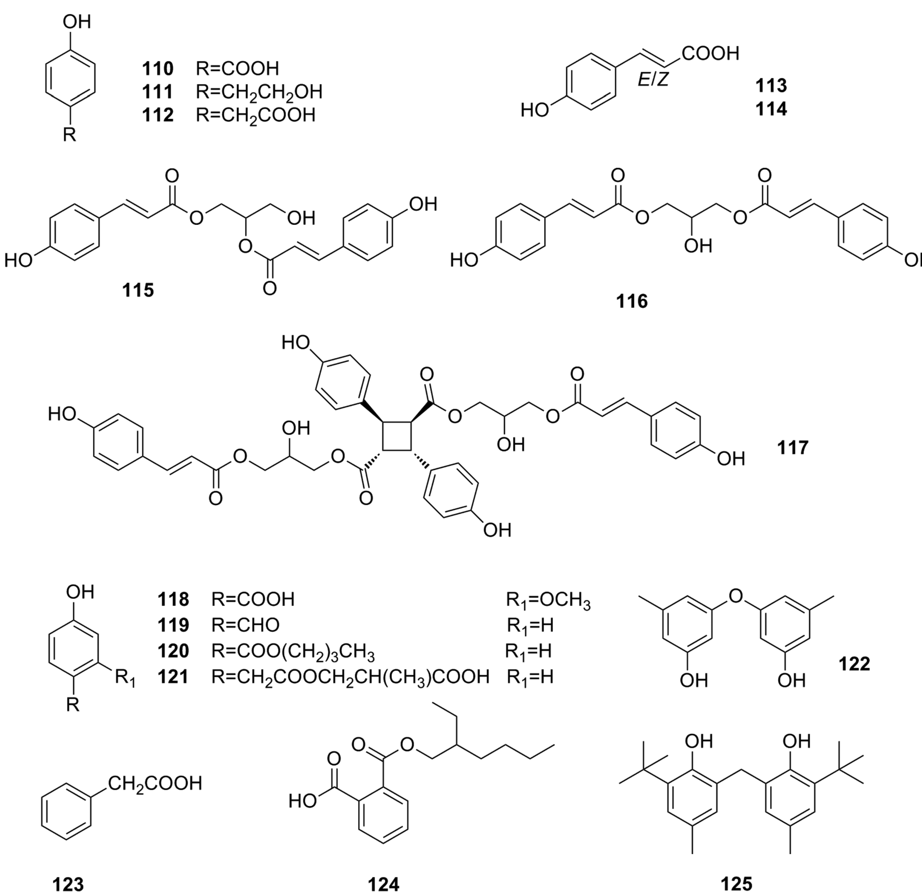

Figure 12. Structures of compounds **110**−**125**.

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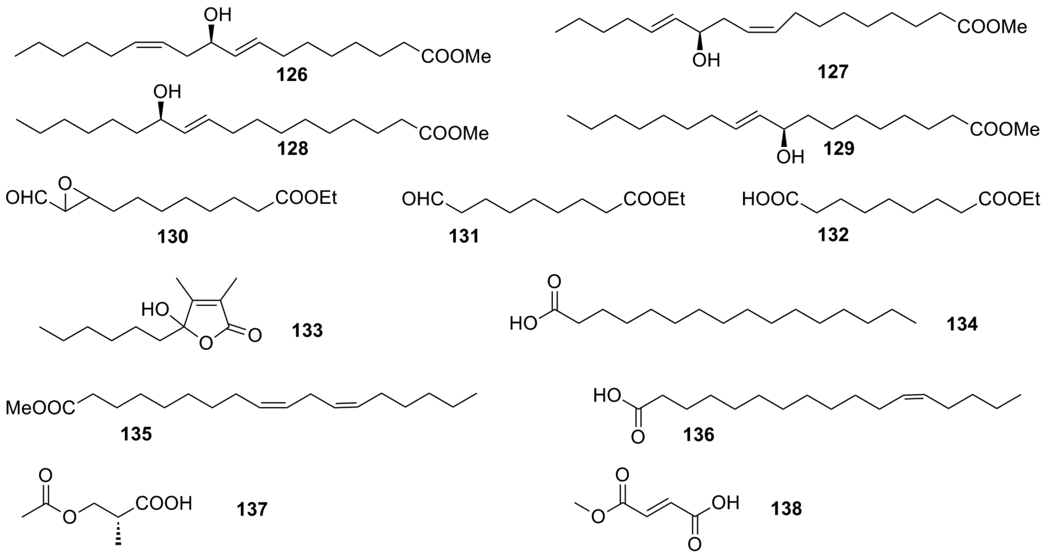

Figure 13. Structures of compounds **126**−**138**.

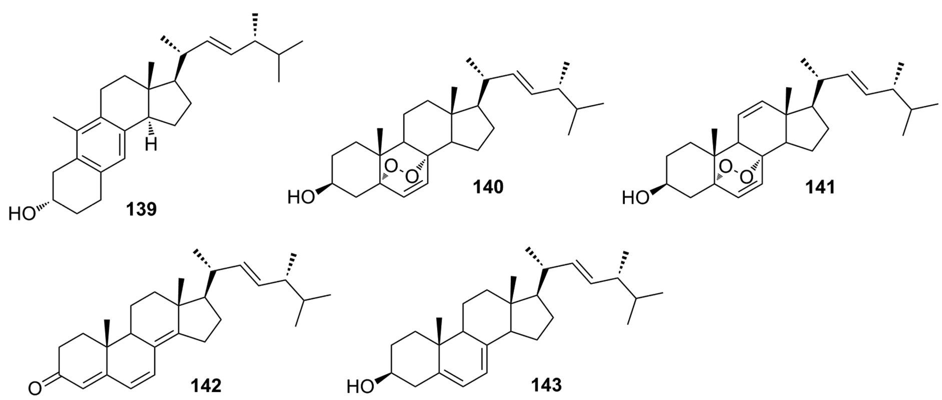

Figure 14. Structures of compounds **139**−**143**.

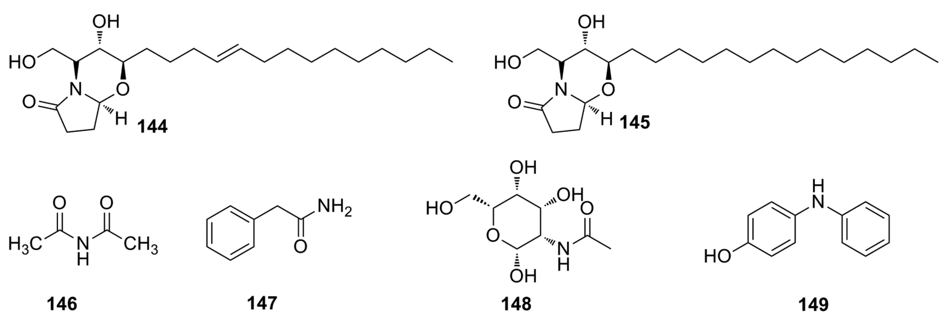

Figure 15. Structures of compounds **144**−**149**.

often yields various ergot alkaloids.[93] Different environmental factors lead to different alkaloid contents.[94] Ergovaline is the most abundant ergopeptide alkaloid in grass infected with endophytes and has been associated with "fescue toxicosis".[46,95−99] Ergonovine and ergine are thought to be major alkaloids of *A. inebrians* infected by *E. gansuensis*, which are known for their ability to cause stupor in livestock.[38,100−103] Chanoclavine I is the main alkaloid of *A. robustum* infected by *Epichloë funkii* and does not have toxic effects for livestock.[104,105] When the toxicity of ergot alkaloids in endophyte−grass symbiosis needs to be known, the contents of ergovaline, ergonovine, and ergine should be detected.

Lolitrem B is found to be the most abundant in *L. perenne* and thought to be primarily responsible for ryegrass staggers in sheep, horses, and cattle.[55,106] Besides lolitrem B, substantially higher concentrations of paxilline are also found in toxic pastures in New Zealand. Lolitrem B and paxilline are potent blockers of calcium-activated large conductance potassium channels.[63,107,108] Thus, ergot alkaloids and lolitrem B may play a vital role in the occurrence of tremorgenic mycotoxins and should be detected in endophyte-infected grasses in the future.

The reason why *F. arundinacea* and *L. perenne* are called poisonous grasses is that they are infected by *Epichloë* endophytes to produce ergovaline and lolitrem B. When *Epichloë* endophytes are used to cultivate new forage varieties,

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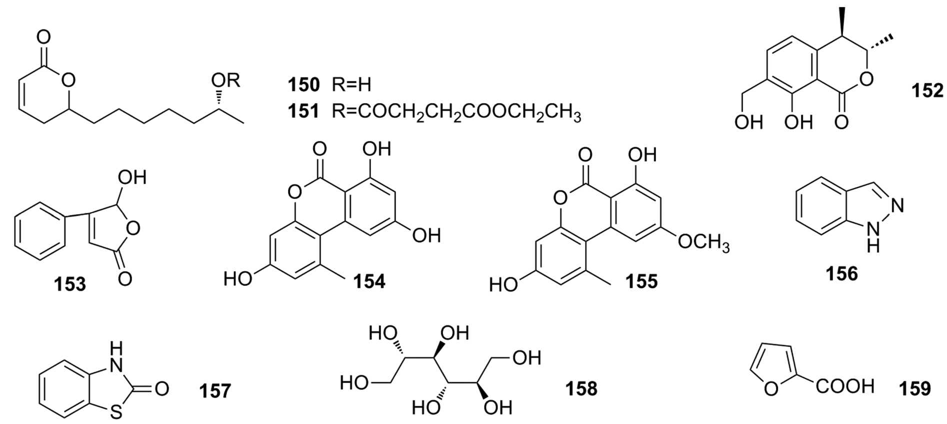

Figure 16. Structures of compounds **150−159**.

we must consider whether this *Epichloë* endophyte and its grass symbiosis will produce ergovaline and lolitrem B alkaloids.

3.2. Insecticidal Activity. Ergot alkaloids and lolitrem B are also neurotoxic to insects. In addition, loline alkaloids are known to be feeding deterrents against sucking insects, such as aphids (*Rhopalosphum pali* and *Skizophis graminum*).[109,110] Among these isolated loline alkaloids, *N*-acetylloline (**71**) and *N*-formylloline (**74**) exhibit insecticidal activity against *S. graminum*, which are as effective as the positive control nicotine sulfate. Injection of these loline alkaloids into mice does not cause poisoning, indicating that loline alkaloids exhibit different toxicities against animals and insects.[111] Accordingly, loline alkaloids are considered to be a good target for expression in cereal crops and can be used as natural grass protectants. Peramine can also deter herbivorous insects.[18] Therefore, an objective of many plant breeding programs is to cultivate grasses that are resistant to pests and drought and also safe for animal husbandry.

Dahurelmusin A (**88**) also displayed an obvious insecticidal activity against *Brevicoryne brassicae* and *R. padi*, with LC_{50} values of 0.251 and 0.092 mM, respectively.[79] Accordingly, dahurelmusin A could be considered as a lead compound for the development of a new kind of industrial insecticide to suppress aphids. Compounds **106−109** were tested for their mortality against mosquito larvae. Among these test metabolites, 7-*O*-(*β*-D-glucopyranosyl)tricin (**107**) was the most active against mosquito larvae, while 7-*O*-[*α*-L-rhamnopyranosyl(1−6)-*β*-D-glucopy ranosyl]tricin (**108**) possessed weak toxicity.[82]

Loline and peramine alkaloids are thought to play an important role in deterring insects in grasses infected with *Epichloë* endophytes. *Epichloë* endophyte−grass containing loline and peramine alkaloids with a high content is planted around the airport to prevent birds from eating, to reduce aircraft accidents caused by birds. However, non-alkaloids, hybrid peptide−polyketide (**88**) and flavonoid (**107**), also exhibited insecticidal activities against aphid and mosquito larvae, respectively. Accordingly, the role of these non-alkaloids in the enhancement of host resistance against insect should not be neglected.

3.3. Antifungal Activity. Epichlicin (**83**) showed inhibitory activity toward spore germination of *C. phlei*, with an IC_{50} value of 22 nM.[73] Cyclosporin T (**87**) showed significant antifungal activity against pathogenic fungi of grasses, including *Curvularia lunata*, *Bipolaris sorokiniana*, *Fusarium avenaceum*,, and *Alternaria alternata*, with EC_{50} values from 0.7 to 5.3 μM.[71] This compound demonstrated a stronger inhibitory activity than chlorothalonil as a positive control. The above two metabolites are peptides, which is consistent with the literature that peptides have significant antifungal activity.[112,113]

Indole derivatives also display antifungal activity. Metabolites **89**, **90**, and **92** showed similar inhibitory activity against *C. parasitica* at high concentrations, whereas indole-3-acetic acid (**92**) exhibited strong inhibitory activity at low concentrations.[77] In addition, indole-3-ethanol (**91**) and indole-3-acetic acid (**92**) were tested for their antifungal activity against *Lactisaria fusiformis*, *Magnaporthe poae*, and *Rhizoctonia solani*, and they demonstrated inhibitory activity toward these grass pathogens. All of the test metabolites were sensitive to *L. fusiformis* and displayed stronger inhibition activity at higher concentrations.

Chokols A−G (**97−103**), gamahonolides A and B (**150** and **151**), gamahorin (**152**), 5-hydroxy-4-phenyl-2(5*H*)-furanone (**153**), and aliphatic metabolites were also assayed for their antifungal activity against *Cladosporium herbarum* and *C. phlei* using a thin-layer chromatography (TLC) plate assay.[80,89] For *C. herbarum*, chokols B−D (**98−100**) were the most active, with the minimum amount of 5 μg/spot; gamahonolides A and B (**152 and 153**) exhibited significant inhibitory activity, with an amount of 10 μg/spot on a silica gel TLC plate; chokol A (**97**) and gamahonolide A (**150**) displayed moderate fungi toxic activity toward *C. herbarum*, with the minimum amount of 25 μg/spot; and in comparison to other compounds, choklols E (**101**) and G (**103**) showed a weaker inhibitory activity, with the minimum amount of 50 μg/spot. Cyclonerodiol (**133**) displayed a moderate activity against *C. phlei*, with a relatively high concentration of 10 μg/spot.

The above results exhibit that non-alkaloids (peptides, indole derivatives, sesquiterpenoids, aliphatic metabolites, and gamahonolides) display different degrees of antifungal activity; especially, epichlicin (**83**) and cyclosporin T (**87**) show better activity than the positive control. Therefore, these non-alkaloids produced by *Epichloë* endophytes may enhance the resistance of its host grass against various fungal diseases. However, there are few studies on the antifungal activity of alkaloids, which may be due to the focus of research on their toxicity. Therefore, these non-alkaloids produced by *Epichloë* endophytes may enhance its host grass resistance against various fungal diseases.

3.4. Phytotoxic Activity. A total of 17 metabolites (**87**, **92**, **94**, **95**, **105**, **110−112**, **118**, **121**, **122**, **137**, **138**, and **156−159**) produced by the endophyte of *E. bromicola* were tested for their phytotoxic activities against *L. perenne* seedlings at 200 ppm.[71] Among them, 3,3′-dihydroxy-5,5′-dimethyldiphenyl ether (**122**) can significantly inhibit the roots and shoots of *L. perenne*

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seedlings, with inhibitory rates of 83.9 and 59.8%, respectively. Indole-3-acetic acid (**92**) (74.8%) showed a similar activity to glyphosate (72.0%) in inhibiting the root growth; it displayed a weaker inhibition on the shoots (48.5%) than glyphosate as the positive control (82.3%). Metabolite **112**, (4-hydroxyphenyl)-acetic acid, exhibited 55.8 and 28.4% inhibitions against the roots and shoots, respectively. Other metabolites showed weak activity toward the shoots and roots, with their inhibition rates being from 0 to 35.6%. In addition, compound **122** possesses a marked inhibitory activity against the roots and shoots of *Poa crymophila*. Therefore, compound **122** produced by *E. bromicola* may also play a crucial role in promoting the growth and competitiveness of host plants.

3.5. Cytotoxicity. Ergonovine and ergonovinine alkaloids show cytotoxicity against animal smooth muscle cells. The IC_{50} values for ergonovine and ergonovinine were 71.95 and 72.75 μg/mL, respectively.[32] This result indicates that the two alkaloids produced by *E. gansuense* infecting *A. inebrians* were the cause of livestock poisoning caused by *A. inebrians*.

The endophyte of *E. bromicola* produced 17 metabolites, which were tested for their cytotoxicity against MDBK cells.[71] None of these metabolites exerted cytotoxicity toward MDBK cells. The IC_{50} values were above 100 μM. Moreover, the inhibition rates of the metabolites were between −1.6 and −17.0% at a concentration of 12.5 μM, indicating that they may promote mammalian cell growth at low concentrations. Thus, the metabolites did not exhibit cytotoxicity to MDBK cells. The results were quite different from those observed with the alkaloids isolated from *Epichloë* spp.; those alkaloids produced detrimental effects on their hosts.[114] This result may be attributed to the gene for the synthesis of these alkaloids being silent when *E. bromicola* is cultured alone. The present results indicate that it may be safe for animals to eat *E. tangutorum* grass infected with *E. bromicola*, ergot alkaloids exhibit cytotoxicity, and non-alkaloids have no toxicity.

4. CONCLUSION

In this paper, we systematically summarized the chemical structures and biological activities of secondary metabolites from the endophytic fungus genus of *Epichloë* and their symbionts. A total of 159 metabolites were discovered in this genus before the year 2019, and the number of non-alkaloids accounts for half. Some non-alkaloids with unprecedented carbon skeletons were obtained from *Epichloë*. Dahurelmusin A (**89**) from *E. dahuricus* infected by *E. bromicola* endophyte is an outstanding example, which will trigger further studies on the non-alkaloids of *Epichloë* and their symbionts in the coming year. In addition to insecticidal and animal toxicities, bioassays exhibited that antifungal activity is the most significant biological activity of these secondary metabolites identified from this genus. Some secondary metabolites are found to possess meaningful biological activity, as exemplified by epichlicin (**84**) and cyclosporin T (**88**), displaying obvious antifungal activity against plant pathogens. These findings will stimulate investigation of the bioactive metabolites of natural product chemistry in the future, with much attention given to non-alkaloids from *Epichloë* and their symbionts.

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Notes

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