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Synthesis and biological evaluation of calycanthaceous alkaloid analogs

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ABSTRACT

Starting from 9-methyl-1,2,3,4,9,9a-hexahydro-4aH-pyrido[2,3-*b*]indol-4a-ol, or indole-3-acetonitrile, 40 new calycanthaceous alkaloid analogs were synthesized in excellent yields. The prepared compounds were evaluated for biological activity against acetylcholinesterase and a broad range of plant pathogen fungi. The results of bioassays indicated that the majority of tested compounds displayed comparable or better *in vitro* bioactivity than the positive control. Notably, compounds **b8** and **b9** showed higher activity against *Verticillium dahlia* than chlorothalonil, with MIC values of 62.5 and 7.81 μg mL^{-1}, respectively. Compound **b3** had a higher activity against *Bacillus cereus*, with a MIC value of 15.63 μg mL^{-1}. Compounds **c2** and **c11** revealed potent activity against acetylcholinesterase, with MIC values of 0.01 and 0.1 ng mL^{-1}, respectively. Analysis of the molecular docking modes of **c2** and **c11** with *Torpedo californica* acetylcholinesterase indicated a medium strong hydrogen bond interaction between the hydroxyl groups of both the ligands and the phenolic hydroxyl of Try121 at a distance of approximately 2.4 Å. The results obtained in this study will be useful for the further design and structural optimization of calycanthaceous alkaloids as potential agrochemical lead compounds for plant disease control.

Recently, fragment-based pesticide discovery methods have become an increasing focus in agricultural chemistry.[1] Fragments with the features of high modifiability, diversified scaffolds, and small molecular weights, are an ideal resource for pesticide development. Compared with traditional pesticide design methods, agrochemists can evolve, connect, and integrate fragments into pesticide candidates via structure-based modification.[2,3]

In nature, hexahydropyrroloindole skeletons are very important moieties that are widespread in a large family of natural products that possess potential bioactivity. The Calycanthaceae plants (Fig. 1), which contain hexahydropyrroloindole skeletons, have been used as traditional Chinese medicines for the treatment of fungal infection,[4] hypertension, tumors, inflammatory conditions, and melanogenesis.[5–8] Because of the broad spectrum of biological properties, a number of studies investigating the synthesis and antimicrobial activity of calycanthaceous alkaloids have been reported.[7,9–18]

Our group has recently reported the preparation and potent antimicrobial activity of calycanthaceous alkaloid derivatives.[19–24] These findings inspired us to further modify the structure of calycanthaceous alkaloids with functional motifs so as to acquire potential agrochemical leads for plant disease control.

As a continuation of the development of new natural-product-based antifungal agents, a series of *N*-substituted calycanthaceous alkaloid analogs were designed and synthesized, and the structures were characterized by ^{1}H NMR, ^{13}C NMR, and MS.

To the best of our knowledge, the biological activities of the prepared analogs are reported here for the first time.

The synthetic route to the compounds is given in Scheme 1. The calycanthaceous alkaloid analogs were prepared according to a previously reported procedure by our group and the spectral data were consistent with reported values.[19–22] The derivatives of calycanthaceous alkaloids were prepared from indole-3-acetonitrile, or 9-methyl-1,2,3,4,9,9a-hexahydro-4aH-pyrido[2,3-*b*]indol-4a-ol (**4**), via acylation at the *N*-position. A total of 40 calycanthaceous analogs were prepared, and characterized by ^{1}H NMR, ^{13}C NMR spectroscopy, and ESI-MS.

The inhibitory effects of the calycanthaceous alkaloid derivatives against a broad range of plant pathogen fungi are outlined in Tables 1–4. The MIC values were evaluated with chlorothalonil, gentamicin, streptomycin, amphotericin B, carbendazim, fluconazole, or penicillin as positive controls, to assay the activities of the prepared calycanthaceous alkaloid derivatives against *Cytospora juglandis, Aspergillus flavus, Penicillium citrinum, Fusarium oxysperium* sp. *vasinfectum,*

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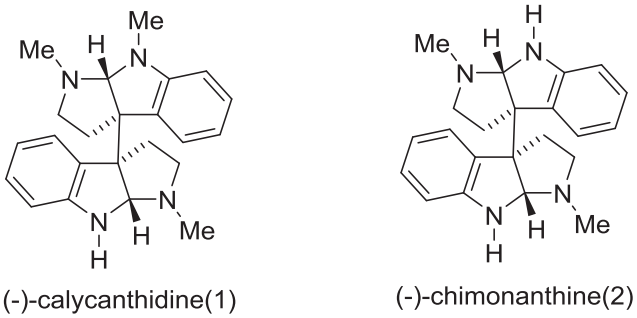


Fig. 1. Structures of calycanthaceous alkaloids.

Fusarium oxysporum, Colletotrichum orbiculare, Aspergillus niger, Curvularia lunata, Escherichia sp., *Verticillium dahliae, Pseudomonas aeruginosa, Ralstonia solanacearum, Bacillus cereus, Staphylococcus aureus* subsp. *aureus, Candida krolimus,* and *Cryptococcus neoformans.*

The synthesized series of analogs exhibited better antimicrobial activity compared with the positive controls. Compounds **a4**, **a5**, **a16**, **b7**, and **b8** showed moderate activity against *F. oxysperium* sp. *Vasinfectum*, *C. juglandis*, *A. flavus*, *V. dahliae, P. citrinum*, *F. oxysporum, C. orbiculare*, *A. niger,* and *C. lunata.* Thirty-six compounds exhibited activity against *V. dahliae.* In particular, compounds **a2**, **a5**, **b8**, **b9**, **b11**, and **b14** showed more effective activity against *V. dahliae* compared with chlorothalonil. Compounds **b8** and **b9** displayed the most effective activity among the tested analogs, with MIC values of 62.5 and 7.81 μg mL^{-1}, respectively. Compounds **b7**, **b9**, and **c7** displayed slightly better activity against *C. lunata* than carbendazim or chlorothalonil, with the same MIC value of 62.50 μg mL^{-1}. Compounds **b14** and **b16** had improved activity compared with the positive control chlorothalonil against *F. oxysperium*, both with the same MIC value of 62.50 μg mL^{-1}.

As shown in Table 2, **15** analogs displayed potent *in vitro* antimicrobial activity against *Escherichia* sp., *P. aeruginosa,* and *R. solanacearum.* Compounds **a5**, **b8**, **c5**, **c7**, **c12**, and **c14** exhibited improved activity against *R. solanacearum* compared with streptomycin, all with the same MIC value of 62.50 μg mL^{-1}. Compounds **c2**, **c3**, and **c8** displayed better activity against *R. solanacearum* compared with gentamicin and streptomycin.

As indicated in Table 3, compounds **a2**, **a5**, **a6**, **b1**, **b2**, **b5**, **b7**, **b8**, **c7**, and **c8** showed some activity against *Staphylococcus aureus* subsp. *aureus* and *B. cereus,* with compound **b3** being the most effective, with a MIC value of 15.63 μg mL^{-1}.

The results of the MIC values of compounds against human pathogenic fungi are summarized in Table 4. Compounds **a2**, **a5**, **b8**, and **b11** had better activity against *C. tropicalis* than amphotericin B or fluconazole, both with the same MIC value of 125.00 μg mL^{-1}. Compounds **a5**, **b8**, **c7**, and **c8** showed greater activity against *C. neoformans* than fluconazole, with MIC values of 62.50, 125.00, 125.00, and 125.00 μg mL^{-1}, respectively. Compounds **a5** and **b8** showed better activity against *C. krolimus* than amphotericin B or fluconazole, both with the same MIC value of 31.25 μg mL^{-1}. Compounds **a5**, **b8**, **c7**, and **c8**, with a long chain at the *N*-position, showed potent activity. Compounds **a6** and **b9** also showed potent activity because of the *n*-benzyl group at the *N*-position. These results laid the foundation for the study of the structure–activity relationship of the alkaloid analogs.

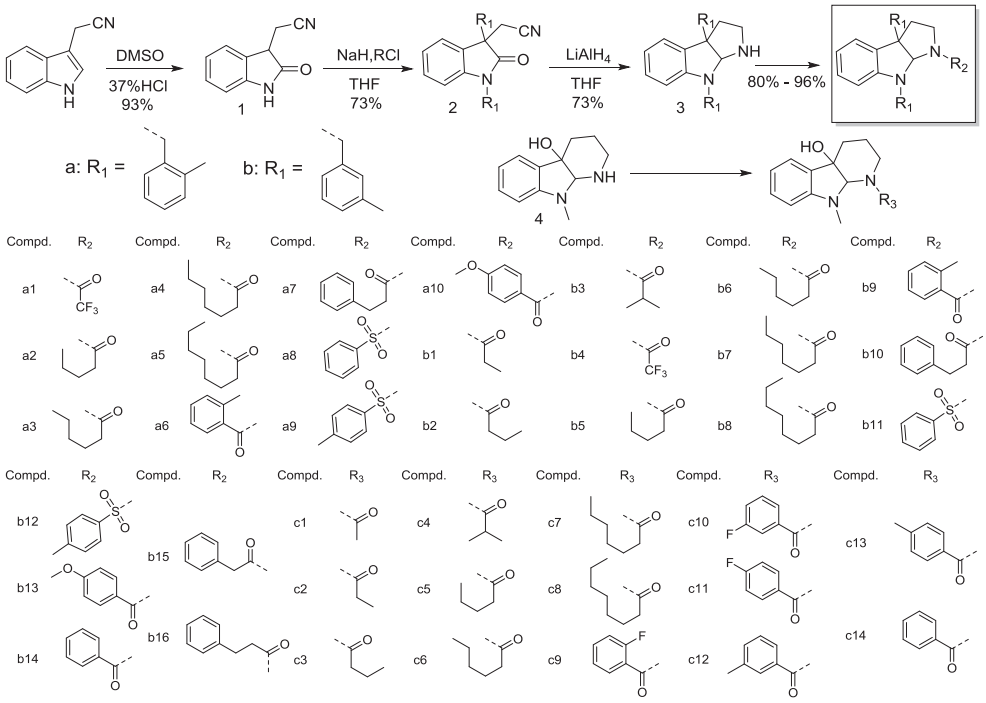
As shown in Fig. 2, compound **c7** exhibited a better inhibitory effect against the mycelium of *C. lunata* at 250.00 μg mL^{-1} than carbendazim or chlorothalonil, and the inhibition rate was positively correlated with the concentration. When the concentrations of compound **c7** were 62.50, 125.00, and 250.00 μg mL^{-1}, the inhibition rates of the mycelium were 15.50%, 31.32%, and 77.73%, respectively.

As shown in Fig. 3, compound **a5** exhibited better inhibitory activity against *V. dahlia* than the positive control chlorothalonil at 250.00 μg mL^{-1}. When the concentrations of compound **a5** were 31.25, 62.50, 125.00, and 250.00 μg mL^{-1}, the inhibition rates of mycelium were 46.24%, 46.95%, 63.44%, and 67.74%, respectively.

Fig. 4 shows that the inhibitory rate of compound **b8** on the spore germination of *C. krolimus* was 87.46% at 500.00 μg mL^{-1}.

Fig. 5 shows that compound **a8** exhibited potent inhibitory activity against *C. lunata* at 125.00 μg mL^{-1}.

Fig. 6 shows that the inhibitory rate of compound **c7** on the spore germination of *C. lunata* was 76.23% at 125.00 μg mL^{-1}, which was superior to chlorothalonil.



Scheme 1. Synthetic route to the compounds **a1–a10**, **b1–b16**, and **c1–c14**.

Table 1
MIC values of compounds against plant pathogenic fungi.

Comp.	*V.d.*	*F.v.*	*C.j.*	*A.f.*	*P.c.*	*F.o.*	*C.o.*	*A.n.*	*C.l.*
	MIC (μg/mL)								
a1	125.00	–	250.00	250.00	–	250.00	250.00	250.00	250.00
a2	31.25	–	250.00	250.00	250.00	125.00	–	250.00	–
a3	62.50	–	125.00	250.00	250.00	125.00	250.00	250.00	125.00
a4	62.50	250.00	62.50	250.00	125.00	125.00	250.00	250.00	125.00
a5	31.25	250.00	125.00	250.00	250.00	125.00	250.00	250.00	125.00
a6	250.00	250.00	250.00	250.00	250.00	250.00	–	125.00	62.50
a7	250.00	–	250.00	250.00	125.00	250.00	250.00	250.00	–
a8	125.00	250.00	250.00	250.00	–	250.00	250.00	250.00	–
a9	62.50	250.00	250.00	250.00	–	250.00	250.00	250.00	–
a10	250.00	–	250.00	250.00	–	250.00	–	250.00	–
b1	250.00	–	62.50	250.00	–	125.00	250.00	125.00	250.00
b2	62.50	–	62.50	250.00	–	125.00	250.00	125.00	–
b3	250.00	–	250.00	250.00	–	125.00	250.00	250.00	–
b4	–	–	250.00	250.00	–	250.00	–	–	–
b5	31.25	–	250.00	250.00	250.00	125.00	–	250.00	–
b6	15.63	–	125.00	250.00	250.00	125.00	250.00	250.00	–
b7	125.00	250.00	62.50	250.00	250.00	125.00	250.00	250.00	62.50
b8	62.50	250.00	125.00	250.00	250.00	125.00	250.00	250.00	125.00
b9	7.81	125.00	125.00	250.00	–	125.00	250.00	250.00	62.50
b10	250.00	250.00	125.00	250.00	250.00	250.00	250.00	250.00	–
b11	31.25	250.00	250.00	250.00	–	250.00	250.00	250.00	–
b12	62.50	125.00	250.00	250.00	–	250.00	250.00	250.00	–
b13	125.00	–	250.00	250.00	250.00	250.00	–	250.00	–
b14	31.25	62.50	62.50	250.00	250.00	250.00	250.00	250.00	–
b15	250.00	125.00	250.00	250.00	250.00	250.00	–	250.00	–
b16	–	62.50	250.00	250.00	–	250.00	–	–	–
c1	–	–	–	–	–	–	–	–	–
c2	250.00	–	–	–	–	–	–	–	–
c3	250.00	–	–	–	–	–	–	–	–
c4	250.00	–	–	–	–	–	–	–	–
c5	250.00	–	–	–	–	–	–	–	–
c6	125.00	250.00	125.00	–	–	250.00	250.00	250.00	250.00
c7	62.50	250.00	125.00	–	–	250.00	250.00	250.00	62.50
c8	62.50	250.00	125.00	125.00	–	–	250.00	250.00	125.00
c9	125.00	–	–	–	–	–	–	–	–
c10	250.00	–	–	–	–	–	–	–	–
c11	250.00	250.00	250.00	250.00	250.00	250.00	250.00	–	125.00
c12	250.00	–	–	–	–	–	–	–	–
c13	250.00	–	–	–	–	–	–	–	–
c14	–	–	–	–	–	–	–	–	–
Ca	7.81	62.50	31.25	7.81	1.96	125.00	125.00	–	250.00
Ch	31.25	250.00	62.50	7.81	15.63	62.50	250.00	15.63	125.00

Note: Carbendazim and chlorothalonil were used as the positive controls; "−" means no inhibition effect. MIC: minimal inhibitory concentration; *V.d.: V. dahliae; F.v.: F. oxysperium* sp. *Vasinfectum; C.j.: C. juglandis; A.f.: A. flavus; P.c.: P. citrinum; F.o.: F. oxysporum; C.o.: C. orbiculare: A.n.: A. niger; C.l.: C. lunata; Ca: carbendazim; Ch: chlorothalonil.*

Table 2
MIC values against Gram-negative bacteria.

Comp.	*E.s.*	*P.a.*	*R.s.*	Comp.	*E.s.*	*P.a.*	*R.s.*	Comp.	*E.s.*	*P.a.*	*R.s.*
	MIC (μg/mL)				MIC (μg/mL)				MIC (μg/mL)		
a1	–	–	–	b5	250.00	125.00	250.00	c3	–	250.00	31.25
a2	250.00	250.00	250.00	b6	–	125.00	250.00	c4	–	250.00	–
a3	–	125.00	250.00	b7	250.00	125.00	125.00	c5	250.00	250.00	62.50
a4	250.00	250.00	125.00	b8	250.00	125.00	62.50	c6	250.00	250.00	125.00
a5	125.00	125.00	62.50	b9	–	–	125.00	c7	125.00	250.00	62.50
a6	–	–	125.00	b10	–	–	–	c8	62.50	250.00	31.25
a7	–	–	–	b11	250.00	125.00	–	c9	250.00	250.00	125.00
a8	–	–	–	b12	–	–	–	c10	250.00	250.00	250.00
a9	250.00	–	250.00	b13	–	–	–	c11	250.00	250.00	125.00
a10	–	–	–	b14	–	–	–	c12	–	250.00	62.50
b1	250.00	–	–	b15	–	–	–	c13	–	250.00	–
b2	250.00	250.00	250.00	b16	–	–	–	c14	250.00	250.00	62.50
b3	250.00	–	–	c1	–	250.00	250.00	g	1.96	1.96	62.50
b4	–	–	–	c2	–	250.00	31.25	s	31.25	–	250.00

Note: Gentamicin and streptomycin were used as the positive controls; "−" means no inhibition effect. MIC: minimal inhibitory concentration; *E.s.: Escherichia* sp.*; P.a.: P. aeruginosa; R.s.: R. solanacearum; g: gentamicin; s: streptomycin.*

Table 3
MIC values against Gram-positive bacteria.

Comp.	*B.c.*	*S.a.*	Comp.	*B.c.*	*S.a.*	Comp.	*B.c.*	*S.a.*	Comp.	*B.c.*	*S.a.*
	MIC(μg/mL)			MIC(μg/mL)			MIC(μg/mL)			MIC(μg/mL)	
a1	–	–	b1	31.25	62.50	b11	125.00	–	c5	–	125.00
a2	250.00	250.00	b2	250.00	250.00	b12	125.00	–	c6	–	125.00
a3	–	–	b3	15.63	–	b13	–	–	c7	250.00	62.50
a4	–	–	b4	–	–	b14	–	–	c8	250.00	62.50
a5	62.50	125.00	b5	250.00	250.00	b15	–	–	c9	–	–
a6	250.00	250.00	b6	250.00	–	b16	–	–	c10	–	–
a7	250.00	–	b7	125.00	250.00	c1	–	250.00	c11	–	250.00
a8	125.00	–	b8	62.50	125.00	c2	–	–	c12	–	250.00
a9	–	250.00	b9	125.00	–	c3	–	–	c13	–	–
a10	–	–	b10	250.00	–	c4	–	–	c14	–	250.00
p	7.81	15.63									

Note: Gentamicin and streptomycin were used as the positive controls; "–" means no inhibition effect. MIC: minimal inhibitory concentration; *p: penicillin; B.c.: B. cereus; S.a.: S. aureus.*

Table 4
MIC values of compounds against human pathogenic fungi.

Comp.	*C.k*	*C.N.*	*C.t*	Comp.	*C.k*	*C.N.*	*C.t*	Comp.	*C.k*	*C.N.*	*C.t*
	MIC (μg/mL)				MIC (μg/mL)				MIC (μg/mL)		
a1	250.00	–	125.00	b5	250.00	–	250.00	c3	–	–	–
a2	250.00	–	125.00	b6	250.00	–	250.00	c4	–	–	–
a3	125.00	–	–	b7	250.00	–	250.00	c5	–	–	–
a4	250.00	–	250.00	b8	31.25	125.00	125.00	c6	–	250.00	–
a5	31.25	62.50	125.00	b9	250.00	–	250.00	c7	–	125.00	250.00
a6	250.00	–	250.00	b10	250.00	–	125.00	c8	–	125.00	–
a7	250.00	–	250.00	b11	250.00	–	125.00	c9	–	–	–
a8	250.00	–	–	b12	250.00	–	–	c10	–	–	–
a9	250.00	–	250.00	b13	–	–	–	c11	–	–	250.00
a10	–	–	–	b14	–	–	–	c12	–	–	250.00
b1	250.00	–	250.00	b15	–	–	–	c13	–	–	–
b2	250.00	–	125.00	b16	–	–	–	c14	–	–	250.00
b3	250.00	–	250.00	c1	–	–	–	A	250.00	1.96	
b4	–	–	–	c2	–	–	–	F	62.50		250.00

Note: Gentamicin and streptomycin were used as the positive controls; "–" means no inhibition effect. MIC: minimal inhibitory concentration; *C.k: C. krolimus; C.N.: C. neoformans; C.t.: C. tropicalis; A: amphotericin B; F: fluconazole.*

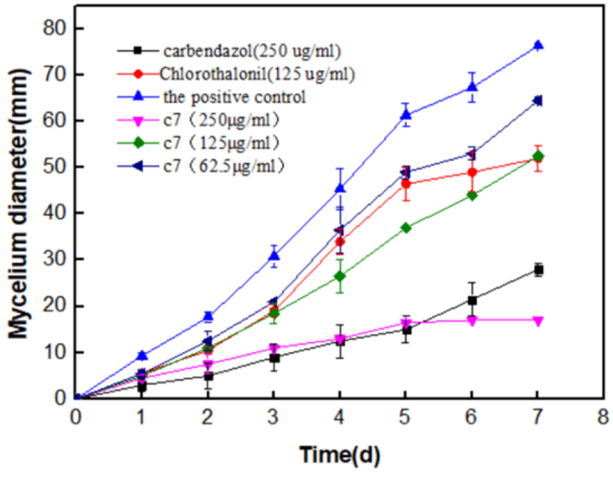


Fig. 2. Inhibitory effect of compound **c7** on the mycelium of *C. lunata.*

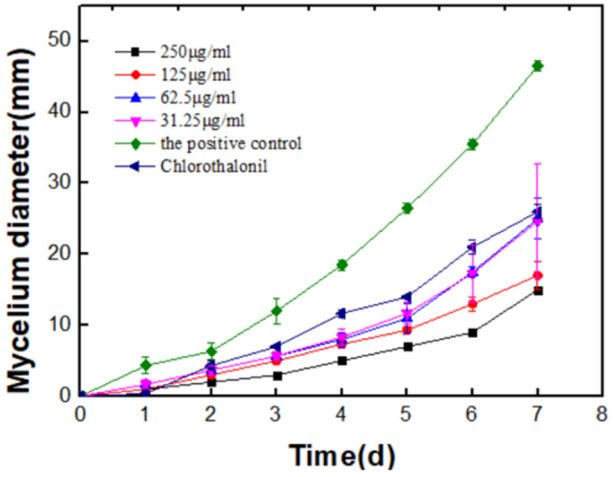


Fig. 3. Inhibitory effect of compound **a5** on the mycelium of *V. dahlia.*

The inhibitory rate of compound **c7** on the spore germination of *C. lunata* was 43.68% at 250.00 μg mL^{-1}, which was better than carbendazim or chlorothalonil (Fig 7).

Fig. 8 shows that bacteria in the control group proliferated quickly within 20 h. However, the bacteria proliferated relatively slowly after 20 h indicating that the bacteria grew slowly into a stable state with typical bacterial growth characteristics. Bacterial reproduction showed a slow upward trend from 1 to 6 h. However, the bacterial growth proliferated quickly from 12 to 20 h, and then stabilized, indicating that the growth of *R. solanacearum* was inhibited, and this was positively correlated with the sample concentration. The growth of bacteria was almost completely inhibited at 500 μg mL^{-1}.

The acetylcholinesterase activity of the compounds was determined by Ellman colorimetry. The inhibition rates of seven different

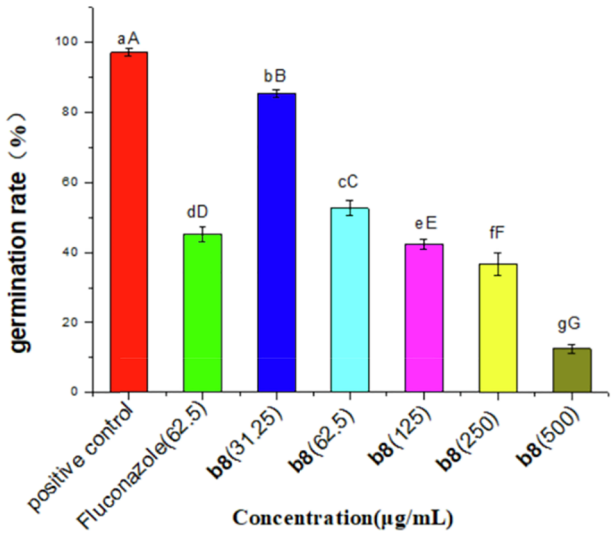


Fig. 4. Effect of compound **b8** on the spore germination of *C. krolimus*.

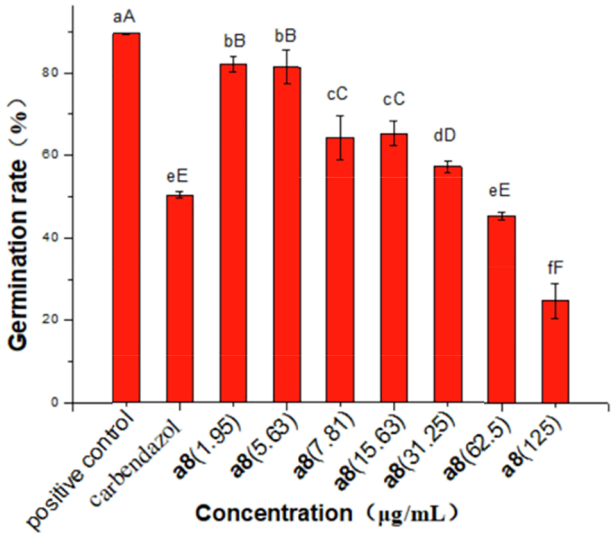


Fig. 5. Effect of compound **a8** on the spore germination of *C. juglandis*.

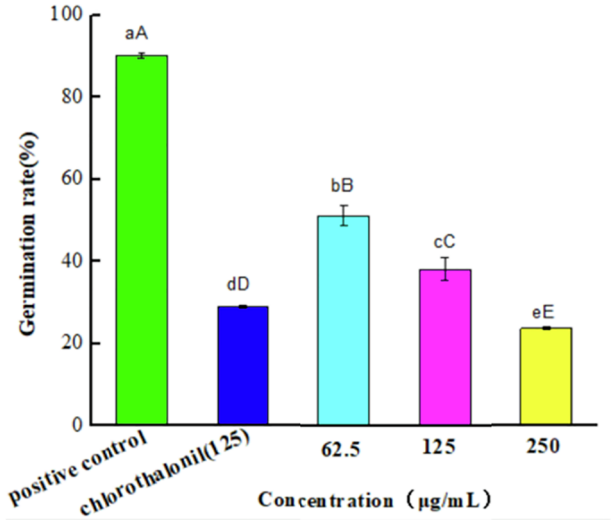


Fig. 6. Effect of compound **c7** on the spore germination of *C. lunata*.

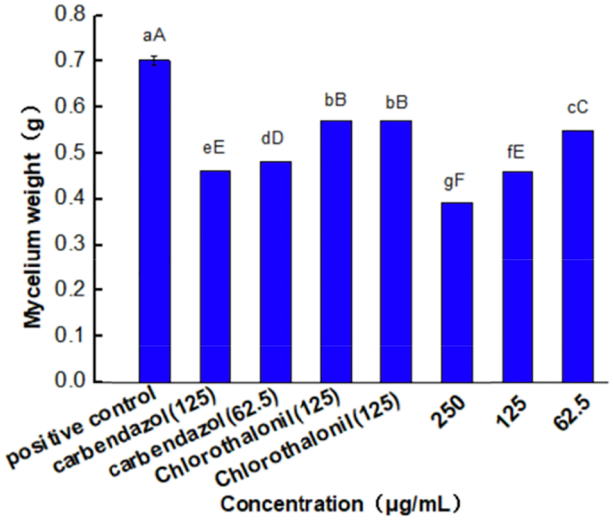


Fig. 7. Effect of compound **c7** on the spore germination of *C. lunata*.

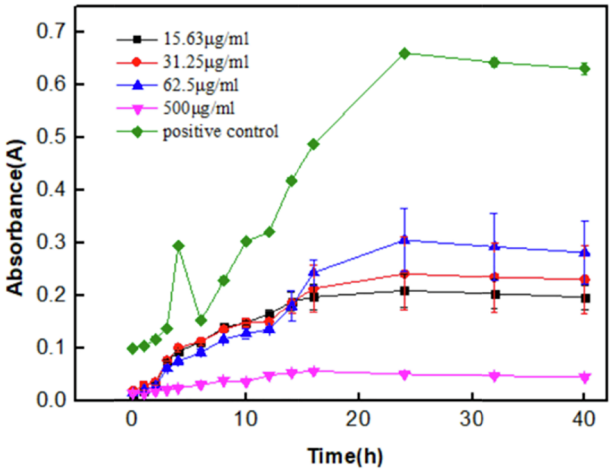


Fig. 8. Effect of compound **c8** on the growth curve of *R. solanacearum*.

Table 5
Inhibition of acetylcholinesterase.

Compd.	IC_{50} (μg/mL)	Compd.	IC_{50} (μg/mL)	Compd.	IC_{50} (μg/mL)	Compd.	IC_{50} (μg/mL)
a1	3.29	b1	0.39	b11	1.43	c5	7.90
a2	148.50	b2	2.86	b12	6.70	c6	2.29
a3	101.10	b3	5.96	b13	1.41	c7	1.33
a4	43.64	b4	1.57	b14	21.31	c8	0.12
a5	8.14	b5	0.06	b15	220.50	c9	0.32
a6	111.40	b6	66.12	b16	0.002	c10	0.11
a7	395.80	b7	8.74	c1	0.30	c11	0.0001
a8	0.14	b8	43.50	c2	0.00001	c12	1.78
a9	0.11	b9	54.29	c3	33.35	c13	0.92
a10	63.21	b10	3.14	c4	0.14	c14	0.08

concentrations and the IC_{50} values of the test compounds indicated that the inhibition rate of acetylcholinesterase increased with an increase in sample concentrations.

Table 5 shows the activity of compounds **a8**, **a9**, **b11**, **c2**, **c4**, **c6**, **c11**, **c13**, and **c14** against acetylcholinesterase. The results indicated that these compounds had potent activity at 1 mg mL^{-1} against acetylcholinesterase. The rate of inhibition of acetylcholinesterase was more than 80%. Compounds **c2** and **c11** were the most effective, with MIC values of 0.01 and 0.1 ng mL^{-1}, respectively.

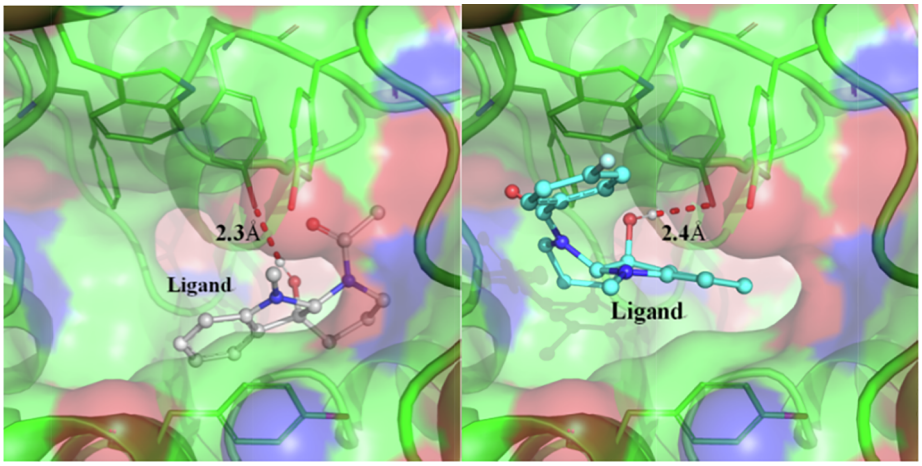


Fig. 9. The stereoisomer for each compound with the best docking score is circled. The top-scoring pose of **c2** and **c11** obtained by docking into the active site of acetylcholinesterase is shown. Hydrogen bonds are shown by red dotted lines.

Analysis of the molecular docking modes of compounds **c2** and **c11** with *Torpedo californica* acetylcholinesterase indicated a medium to strong hydrogen bond (HB) interaction between the hydroxyl groups of both ligands and the phenolic hydroxyl of Try121 at a distance of approximately 2.4 Å (Fig. 9). The quantitative HB interaction energy of A1-TcAChE and B2-Tc acetylcholinesterase, calculated based on the AutoDock scoring function, showed slight differences of −1.39 and −1.37 kcal/mol, respectively. Van der Waals (Vdw) interactions between the ligands and TcAChE was the key difference, these interactions benefited from replacing the methyl group with a *p*-fluorophenyl group, which allows a new π-π interaction between the *p*-fluorophenyl ring and Trp279 to be formed, which had −8.23 kcal/mol Vdw interaction energy in the B2-TcAChE complex, while the corresponding energy in the A1-TcAChE complex was −7.11 kcal/mol. The total binding energy difference was −1.27 kcal/mol (−8.07 kcal/mol in B2-TcAChE and −6.80 kcal/mol in A1-TcAChE), which is very close to the theoretical free-energy change of −1.36 kcal/mol, while the bioactivity was improved 10-fold.

In summary, 40 novel tetrahydropyrroloindole-based calycanthaceous alkaloids analogs were prepared using 9-methyl-1,2,3,4,9,9a-hexahydro-4aH-pyrido[2,3-*b*]indol-4a-ol(4), or indole-3-acetonitrile as the starting material via acylation at the *N*-position, and the activity against acetylcholinesterase, as well as a broad range of plant pathogen fungi was screened. The results of bioassays revealed that most of the compounds had moderate to potent activity against acetylcholinesterase as well as a broad variety of plant pathogen fungi, and were more effective than the positive controls. Compounds in the a and b series exhibited potent inhibition activity against plant pathogen fungi. The reason for this activity was the substitutions at the 1- and 3-positions and the presence of a long chain without fluorine at the *N*-position. Compounds in the c series exhibited potent inhibition activity against plant pathogen fungi and Gram-negative bacteria. The reason for this activity was that the structures contained hexahydropyrroloindole skeletons. Compounds **a5**, **b8**, **c7**, and **c8**, with a long chain at the *N*-position, also showed potent activity. Compounds **a6** and **b9** also showed potent activity because of the *n*-benzyl group at the *N*-position. Compounds **a10**, **b13**, and **c13**, with *m*-methoxybenzoyl and *m*-methylbenzoyl groups at the *N*-position showed no activity against bacteria or plant pathogen fungi.

Compounds **c2**, **c3**, and **c8** showed improved activity against *C. lunata* compared with gentamicin and streptomycin, both with the same MIC value of 31.25 μg mL^{-1}. Compounds **b8** and **b9** showed better activity against *V. dahliae* compared with chlorothalonil, with MIC values of 62.5 and 7.81 μg mL^{-1}, respectively. The activity of compound **b3** was more potent against *B. cereus*, with a MIC value of 15.63 μg mL^{-1}. Notably, compounds **c2** and **c11** had potent activity against acetylcholinesterase, with MIC values of 0.01 and 0.1 ng mL^{-1}, respectively. These results will pave the way for the further design, structural optimization, and development of calycanthaceous alkaloids as potential agrochemical leads for plant disease control.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmc.2019.115088.

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