Nematocidal Activity of Quassinoids against a Species of Diplogastridae

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The nematocidal activity of 38 quassinoids, C₁₉ or C₂₀ compounds isolated from Simaroubaceae, was measured using a species of Diplogastridae (Nematoda) to develop lead parasiticides. Of the various quassinoids tested, samaderines B and E displayed the most potent nematocidal activity with a minimum lethal concentration (MLC) of 2.0×10^{-5} M. The nematocidal activities of samaderines B and E were 15-fold greater than that of albendazole (3.0×10^{-4} M), 10-fold greater than that of thiabendazole (2.0×10^{-4} M) and 7.5-fold greater than that of avermectin (1.5×10^{-4} M). Thus, samaderines B and E may eventually be used as lead parasiticides. In light of the relationship between the structures of quassinoids and their nematocidal activities, those with potent nematocidal activity may require the elements mentioned. These results should help to further our understanding of nematocidal activity.

Key words quassinoid; Simaroubaceae; Nematoda; Diplogastridae; nematocidal activity; structure–activity relationship

The control of parasitic nematode diseases is of great importance due to their widespread presence and massive effects on the health of human beings and animals. Therefore, new anthelmintics are needed. We previously reported a simple but effective method for screening nematocidal compounds,¹⁾ and our experimental results on the structure–activity relationship of picrodendrins.²⁾ In this paper, we wish to report the nematocidal activities and structure–activity relationships of compounds from the family Simaroubaceae.

Quassinoids are all-chair cyclization products of squalene. Quassinoids have been isolated from these Simaroubaceae plants: *Brucea mollis* WALL. var. *tonkinensis* LECOMTE, an Indonesian medicinal plant,³⁾ *B. amarissima*, a popular medicinal plant in Java, *Quassia indica* (GARTN.) NOOTEBOOM, an Indonesian folk medicine,^{4,5)} and Indonesian species *Picrasma javanica* BL,^{6–16)} and *P. quassioides* BENNETT,¹⁷⁾ which are commonly used to treat dysentery, and are also used in Riau to treat the same disease. Quassinoids are known to have various biological activities, including anti-inflammatory, antimalarial, amoebicidal, antifeedant, insecticidal, anti-tumor, and herbicidal activities.¹⁸⁾ For these reasons, we searched for nematocidal compounds *in vitro* to develop a lead compound to treat this parasitic disease.

Using a previously established assay,¹⁾ we investigated the nematocidal activity of 38 quassinoids using a species of Diplogastridae (Nematoda) in third-stage larva. To develop lead nematocidal agent, nematocidal activity was assessed in terms of the structure–activity relationship.

MATERIALS AND METHOD

Subculture and Nematocidal Assay The subculture medium for free-living nematode (Diplogastridae) contained 0.01% bean curd lees extract as the main nutrient, 1.1% agar powder and 0.1% nutrient agar. Subculture provided stable life-cycles for the larvae, and enabled us to collect enough larvae for a subsequent nematocidal assay. Approximately 20 larva were placed in a well of 96-well plate and the well was filled with a sample solution. And the assay was done at least 4 times at one sample. The rapid microplate assay lasted for just 4 h and consumed only small amounts of sample. Mobility of larvae in the well was observed under a microscope.

The minimum lethal concentration (MLC) of the tested compounds was calculated from the results of the assay.¹⁾

Test Compounds As reported in previous studies, bruceins B (1), D (2), bruceoside B (5), brusatol (6), soulameanone (36) and yadanzolid A (38), were isolated from the stems and bark of *B. mollis* WALL. var. *tonkinensis* LECOMTE,³⁾ bruceins E (3) and F (4) were isolated from stems and bark of *B. amarissima* DESV., cedronin (7), dihydrosamaderine B (8), indaquassins C (9), D (10), E (11), F (12), samaderines B (32), C (33), D (34), E (35), soulameolide (37) were isolated from the stems and bark of *Q. indica* (GARTN.) NOOTEBOOM,^{4,5)} javanicins A (13), F (14), H (15), J (16), K (17), O (18), Z (19), javanicinosides A (20), B (21), C (22), D (23), H (24), nigakilactone B (27), picrasin A (nigakilactone G) (28) and quassin (nigakilactone D) (31) were isolated from the stems and bark of *P. javanica* BL,^{6–16)} and nigakihemiacetal F (25), nigakilactone A (26), picrasin B (29) and picrasinol B (30) were isolated from the stems and bark of *P. quassioides* BENNETT.¹⁷⁾

RESULTS AND DISCUSSION

Nematocidal Activities The nematocidal activities and structures of 38 quassinoids, including 32 and 35, are shown in Table 1 and Figs. 1–3. Of the quassinoids studied, 32 and 35 had the greatest nematocidal activities (minimum lethal concentrations (MLC): 2.0×10^{-5} M), followed by 2 (1.0×10^{-4} M) and 7 (2.0×10^{-4} M). The nematocidal activities of 32 and 35 were comparable with those of well-known parasitic remedies, and the results are shown below. The nematocidal activities of 32 and 35 were 2000-fold higher than those of kainic acid (anti-nematode agent, 4.0×10^{-2} M) and santonin (anti-roundworm agent, 4.0×10^{-2} M), and 1500-fold higher than that of diethylcarbamazine citrate (anti-filaria agent, 3.0×10^{-2} M). Furthermore, these activities were 30 times greater than that of piperazine (anti-roundworm and -pinworm agent, 6.0×10^{-4} M), 15 times greater than that of albendazole (anti-nematode agent, 3.0×10^{-4} M), 10 times greater than that of thiabendazole (anti-nematode agent, 2.0×10^{-4} M) and 7.5 times greater than that of avermectin (anti-filaria agent, 1.5×10^{-4} M).

On the other hand, quassinoids have been reported on the

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Fig. 1. Structure of Quassinoids (I)

cytotoxicity to KB cell. The cytotoxicities of **32** and **35** to KB cell were strong and had shown the strongest nematocidal activities too. And it is reported to show a strong cytotoxicity compared with the nematocidal activity as for **33**.¹⁹⁾ In another report of brucein, as for **2**, stronger nematocidal activity than **1** and **6** was shown, but the cytotoxicity was weaker than these compounds. Moreover, the activity of **31** was low in both nematode and KB cell.^{20,21)} The compound like **2** would be effective as the anthelmintics *in vivo*.

Structure-Activity Relationships When roughly seeing, compounds with an epoxide group bridging C-8 and C-13, such as **32** and **35**, exhibited more potent activity. And C₁₉ quassinoids with a γ -lactone that bridges C-12 and C-14 were high activities.

As shown in Fig. 1 and Table 1, the nematocidal activities of **20** and **23**, which have an α -glucosyl group, were less than those of **13** and **15**, which have a carbonyl group at C-16. **26** was less potent than **25**, which has a β -methoxy group at C-16. In compounds (**16**, **17**, **21**, and **24**) with an esterified benzene at C-11, an α -glucosyl group at C-16, as in **21**, was associated with a weaker activity than a carbonyl group, as in **16**.

As shown in Fig. 2 and Table 1, among quassinoids (**14**, **22**, **29** and **31**) with a double bond at C-12 and C-13, the nematocidal activity of **31**, which has an α -methyl group at C-

Table 1. Nematocidal Activity of Quassinoids

No.	Compounds	MLC (M)
1	Brucein B	6.0×10^{-4}
2	Brucein D	1.0×10^{-4}
3	Brucein E	2.5×10^{-3}
4	Brucein F	2.0×10^{-2}
5	Bruceoside B	8.0×10^{-3}
6	Brusatol	6.0×10^{-4}
7	Cedronin	2.0×10^{-4}
8	Dihydrosamaderine B	1.0×10^{-3}
9	Indaquassin C	1.4×10^{-3}
10	Indaquassin D	2.5×10^{-3}
11	Indaquassin E	2.0×10^{-3}
12	Indaquassin F	7.2×10^{-3}
13	Javanicin A	4.0×10^{-2}
14	Javanicin F	4.0×10^{-2}
15	Javanicin H	4.0×10^{-2}
16	Javanicin J	4.0×10^{-2}
17	Javanicin K	$>4.0 \times 10^{-2}$
18	Javanicin O	4.0×10^{-2}
19	Javanicin Z	4.0×10^{-2}
20	Javanicinoside A	$>4.0 \times 10^{-2}$
21	Javanicinoside B	$>4.0 \times 10^{-2}$
22	Javanicinoside C	$>4.0 \times 10^{-2}$
23	Javanicinoside D	$>4.0 \times 10^{-2}$
24	Javanicinoside H	$>4.0 \times 10^{-2}$
25	Nigakihemiacetal F	1.0×10^{-2}
26	Nigakilactone A	4.0×10^{-2}
27	Nigakilactone B	4.0×10^{-2}
28	Picrasin A	4.0×10^{-2}
29	Picrasin B	4.0×10^{-2}
30	Picrasinol B	4.0×10^{-2}
31	Quassin	2.0×10^{-2}
32	Samaderine B	2.0×10^{-5}
33	Samaderine C	5.0×10^{-3}
34	Samaderine D	5.0×10^{-3}
35	Samaderine E	2.0×10^{-5}
36	Soulameanone	1.3×10^{-2}
37	Soulameolide	2.2×10^{-3}
38	Yadanzolide A	1.6×10^{-3}
	Kainic acid	4.0×10^{-2}
	Santonin	4.0×10^{-2}
	Diethylcarbamazine	3.0×10^{-2}
	Piperazine	6.3×10^{-4}
	Albendazole	3.1×10^{-4}
	Thiabendazole	2.0×10^{-4}
	Avermectin	1.5×10^{-4}

MLC: minimum lethal concentration.

4, was stronger than that of **14**. Moreover, as discussed above for **13**, **15**, **20** and **23** in Fig. 1, a carbonyl group at C-16, as in **14**, was associated with greater nematocidal activity than a α -glucosyl group, as in **22**. In compounds (**12** and **37**) which had a γ -lactone at C-13 β , a β -hydroxyl at C-1, as in **37**, was associated with greater nematocidal activity than an α -hydroxyl, as in **12**. In compounds (**2—4**, **9**, **35** and **38**) with an epoxide group between the C-8 methyl and C-13, a carbonyl group at C-2, as in **2** and yadanzolide A (**38**), was associated with greater nematocidal activity than an α -hydroxyl, as in **3** and **4**. The nematocidal activity of **35**, which lacks a hydroxyl at C-6, was 70 times greater than that of **9**, which has an α -hydroxyl. The nematocidal activity of **3**, which has an α -methyl group at C-13, was greater than that of **4**, which has an α -hydroxymethyl group. The activity of **35** was approximately 5 times greater than that of **2**, which had a β -hydroxy group substituted at C-15.

No.		R ₂	R ₄	R ₁₆
14	Javanicin F	OMe	H	O
22	Javanicinoside C	OMe	H	α-OGlc, β-H
29	Picrasin B	α-OH, β-H	Me	O ; 2,3-dihydro
31	Quassin	OMe	Me	O

No.		R ₁	R ₁₂	R ₁₃	R ₁₅
12	Indaquassin F	α-OH, β-H	H	R	H ; 14,15-dehydro
36	Soulameanone	α-H, β-OH	OH	OH	OH
37	Soulameolide	α-H, β-OH	H	R	H ; 14,15-dehydro

No.		R ₂	R ₆	R ₁₃	R ₁₅
2	Brucein D	O	H	Me	OH
3	Brucein E	α-OH, β-H	H	Me	OH
4	Brucein F	α-OH, β-H	H	CH ₂ OH	OH
9	Indaquassin C	O	OH	Me	H
35	Samaderine E	O	H	Me	H
38	Yadanzolide A	O	H	CH ₂ OH	OH

No.		R ₃	R ₁₅
1	Brucein B	OH	OCOMe
5	Bruceoside B	OGlc	OCOCH=CM ₂
6	Brusatol	OH	OCOCH=CM ₂

No.		R ₁₅
10	Indaquassin D	H
11	Indaquassin E	OH

No.		R ₂	R ₇
7	Cedronin	O	α-OH, β-H
8	Dihydrosamaderine B	O	O ; 3,4-dihydro
32	Samaderine B	O	O
33	Samaderine C	α-OH, β-H	O
34	Samaderine D	α-OH, β-H	O ; 5,6-dehydro

Fig. 2. Structure of Quassinoids (II)

Fig. 3. Structure of Quassinoids (III)

Among the compounds (**1**, **5** and **6**) with an epoxide group between the C-8 methyl group and C-13, and with a methyl ester at C-13 in Fig. 3 and Table 1, the nematocidal activity of **5**, which has a glucosyl group at C-3, was weaker than that of **6**, which has a hydroxy group. With respect to C₁₉ quassinoids (**7**, **8** and **32–34**), **32**, which has a carbonyl group at C-2, showed nematocidal activity that was 250-fold greater than that of **33**, which has an α-hydroxy group, as in **3** and **4**. Compound **32** had the greatest nematocidal activity in this assay, along with **35**. The α-hydroxy group at C-7 in **7** reduced the activity of **32** 10-fold. Dihydrosamaderine B (**8**), which has a dihydro group at C-3 and C-4, was 50 times weaker than **32**, which has a dehydro group.

Action Mechanisms The relationship between the action mechanism and the morphology of larvae has been assessed in previous studies.¹⁾ The morphology of larvae observed following the administration of quassinoids was the same as that observed for picrotoxinin (coil or S shape), which is a noncompetitive GABA_A receptor Cl[−] channel complex antagonist.^{22–24)} Chronological changes associated with these compounds' nematocidal activities were also the same. On the other hand, the partial structure of **32**, with regard to the C ring and the γ-lactone bridging C-12 and C-14, resembled that of picrotoxinin, with regard to the cyclohexane ring and γ-lactone bridging C-3 and C-5. These find-

ings suggest that the one of mechanism of actions of quassinoids may be related to GABA receptor.

Moreover, quassinoids has the report of inhibitting the protein synthesis of the animal cell and malaria. So it is necessary to take the protein synthesis inhibition into consideration as one of mechanism of actions to nematode.^{25,26)}

CONCLUSION

With regard to the various quassinoids evaluated in the present study, the nematocidal activities of **32** and **35** exceeded those of thiabendazole and avermectin (anti-nematode agents). In addition, **2** that the cytotoxicity was comparatively low will become a lead compound though the activity was not the strongest, too. Furthermore, potent nematocidal activity depended upon the structural components described above. Compounds that satisfy these conditions may eventually become lead nematocides or parasiticides. These structure-activity relationship would become clear by examining a lot of similar compounds further.

Moreover, the extract from the leaf of *Q. indica* which was the original plant of the compound group (**32**, **35**) with the strongest activity was assayed. The nematocidal activity of the MeOH extract was 5.0 mg/ml, which was comparatively weak. Next, the MeOH extract was distributed with CHCl₃ and H₂O. The activity value of the H₂O layer was 5.0 mg/ml

though that of the CHCl_3 layer rose with 1.25 mg/ml. As for the compound, the results are the above-mentioned. There is a possibility to overlook the compound like this time when judged only from the result of the extract. These results are expected to become one index of the actions of quassinoids.

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