Management of the Soybean Cyst Nematode by Using a Biorational Strategy

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Abstract
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Disciplines
Chemistry | Environmental Chemistry | Inorganic Chemistry | Organic Chemistry | Other Chemistry

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Chapter 11

Management of the Soybean Cyst Nematode by Using a Biorational Strategy

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Analogs of glycinoclepin A have been shown to affect hatching of soybean cyst nematode cysts in laboratory conditions. Most analogs are hatch inhibitors. More complex bicyclic compounds may be hatch accelerators.

Introduction

Soybean cyst nematode (SCN), also known as Heterodera glycines, is one of the most widely distributed and economically devastating soybean pests. SCN was first reported in Japan by Hori in 1915 and first appeared in the United States in North Carolina in 1954 (1). SCN has since been confirmed in 28 states. SCN was first detected in Iowa in 1978 in Winnebago County and currently the majority of Iowa counties are known to be infested with SCN. It can be assumed that undetected infestations are probably present in many other counties as well. Documenting the economic impact of SCN is difficult, because detection of SCN is difficult in the early stages and the producers may attribute the loss in yields to factors other than SCN. If nationwide loss is conservatively estimated at 1%, SCN costs soybean producers $121 million in 1992 alone. Typically, the estimated percentage losses are not 1%, but range from 1.1- 5.8%. An Iowa farm field ravaged by SCN is illustrated below in Figure 1. The light green, yellow, and brown areas are areas of significant SCN damage.
SCN survives in the soil as eggs contained within protective cysts (2). Many of the eggs contain fully developed second-stage juveniles that will hatch under the proper conditions. The cyst typically contains 200 or more eggs. Because of the relatively short life cycle of the SCN (24 to 30 days), under the proper conditions, three to four generations of SCN can be produced in a single growing season. This ability of the SCN to rapidly proliferate, combined with its hardiness and longevity make the SCN difficult to control. The SCN is readily dispersed by the movement of infested soil through adherence to machinery, and may also be dispersed by wind, runoff water, livestock, wildlife, and migrating birds.

Currently, management of the SCN is achieved by incorporation of a crop rotation strategy, use of resistant soybean varieties, and the use of nematicides (3). Because of environmental and economic concerns, the use of nematicides has dwindled over the years. In place of nematicides, crop rotation strategies have been utilized where two years of non-host crops are used followed by one year of SCN resistant soybeans. This strategy has been effective in controlling SCN population densities, but market considerations make this strategy unattractive, if not unfeasible, to producers. Also, the overuse of SCN-resistant soybean varieties may lead to the development of SCN races which can readily reproduce on resistant soybeans.

In recent years there has been more interest in the possible development of herbicides that affect the hatch of the SCN (4,5). Herbicides which stimulate or inhibit the hatch could be used to manage the SCN populations if they could cause the SCN to hatch prematurely in the absence of a host plant or completely.
suppress the hatch when soybeans are being grown. In the absence of a host plant, the SCN would not be able to reproduce and would ultimately die from starvation, parasitism, or predation. If the SCN was found to be present during the growing season, a herbicide that suppressed hatch could be used to keep the population densities from proliferating.

Glycinoclepin A is a hatching stimulus capable of initiating hatch of SCN eggs at concentrations as low as $10^{-12}$ g/mL (6). It is a naturally occurring compound that should be readily biodegradable. Glycinoclepin A has been isolated by extraction from kidney bean roots, but only milligram quantities were obtained from thousands of kilograms of roots. The structure of glycinoclepin A is shown in Figure 2.

![Figure 2. Structure of glycinoclepin A](image)

Murai and coworkers set out to determine the minimum functionality needed in order to stimulate SCN hatch (7). They synthesized numerous compounds and measured the minimum concentration accelerating the hatching of the juveniles from half the number of eggs. It was determined from this study that the minimum functionality to induce hatch are the axial hydroxyl group and the two carboxylic acids. Of significant interest from a synthetic chemistry perspective, the side chain functionality in the oxabicyclic ring system and also the position of the cross-conjugated diene has little effect on the activity of the analogs. Kraus and Tylka also reported their studies on the functionality necessary for biological activity (8). A summary of the Mauai and Kraus findings is collated in Figure 3.

![Figure 3. Key functional groups for bioactivity](image)
The structure activity relationship information proved to be invaluable in
determining the course of our research project. Corey synthesized
glycinoclepin A by a multistep route (9). Murai (10, 11) and Mori (12, 13)
also synthesized glycinoeclepin A by multistep routes. Corey later published a
more direct synthesis using a novel rearrangement reaction (14). Miwa and
coworkers have reported a clever approach to a key fragment of glycinoeclepin
A (15).

We wished to develop a series of small molecule activators that would
allow us to imitate the activity of glycinoclepin A. Our overall goal was to find
a route to a small molecule analog that would be practical and would be
amenable to scale up for industrial production. These small-molecule activators
should be synthetically accessible in the fewest number of steps possible. It was
also crucial that the starting materials for the syntheses were inexpensive and
readily available. With these goals in mind, we set out to discover new and
potent compounds to control the proliferation of the soybean cyst nematode.

Results and Discussion

The synthesis of the analogs began with a plan to create a molecule that
has as many of the necessary active groups as possible. Using literature
methods (16, 17), we synthesized compounds 2-8. Their structures are listed in
Figure 4. These compounds were prepared from readily available starting
materials such as ethyl acrylate, cyclohexanone, cyclopentanone and diethyl
oxalate. Compound 2 was synthesized by the method of Stork (16) from the
enamine of cyclohexanone and ethyl acrylate, followed by hydrolysis.

The compounds were tested using the chamber shown in Figure 5. He
used distilled water as a control and zinc sulfate, a known hatch stimulator, as a
standard for comparison. The results of the screening, shown in Figure 6,
surprised us. Unlike glycinoclepin A and zinc sulfate, compounds 7 and 8 were
not hatch stimulators but rather hatch suppressors. In addition, compounds 7
and 8 were also extremely potent, with 7 inhibiting the hatch at the 1 ppm level.
It is also worth noting that if the compounds were washed away after 30 days
and the eggs placed in water or zinc sulfate, the hatch profile resumed its normal
curve. Therefore, clearly compounds 7 and 8 were not killing the SCN, but
were repressing hatch by some unknown mechanism (19).

Realizing that our best hatch inhibitors contained an enolic diketone and
wanting to test the effect of the six member ring upon activity, an acyclic and six
Figure 4. Initial array of compounds tested.
Figure 5. SCN hatch chambers

Figure 6. Results of testing of compounds 7 and 8.
membered ring analog were synthesized and tested. Compounds 9 and 10 (Figure 7) showed significant hatch suppression activity, but were not as effective as 7 and 8, as a higher concentration of 9 and 10 were needed in order to achieve the same level of activity. Analyzing our results, we determined that of the compounds that are active in suppressing SCN hatch, they all had one of two common functional groups; the enolic dicarbonyl or the diacid.

![Figure 7. Compounds 9 and 10.](image)

Compounds 11, 12, 13, and 14 (Figure 8) were all submitted for testing and the results were encouraging. There was significant hatch suppression by 11 and 12, but the difference between the two was minimal. This demonstrated to us that only the enolic dicarbonyl was necessary. Similar results were found with 13 and 14. We can synthesize compounds in one step (from readily available and inexpensive starting materials) that effectively contained SCN hatch.

![Figure 8. Compounds 11 to 14.](image)
We felt it was worth our time to determine if ring size had anything to do with the activity. We synthesized the six and seven membered ring analogs shown in Figure 9 by literature methods and tested them for activity. The results were similar to the five member ring analogs, with the ester being just as active as the acid, but overall the larger ring size seems to diminish activity slightly.

![Figure 9. Six- and seven-membered ring analogs](image)

The final set of experiments to determine the minimum functionality required for hatch inhibition were centered around determining if the ester group of the enolic dicarbonyl was necessary for activity or if a simple enolic carbonyl was just as effective. To accomplish this, we synthesized the enolic derivative 19 shown in Figure 10 (20). This compound was as active as any other analog in hatch inhibition. This implies that the active portion of the molecule is centered around the interaction of the enol with the SCN (cyclopentanone had no activity). The addition of the side chain ester or acid offered no enhancement of activity.

![Figure 10. Hydroxymethylenecyclopentanone](image)
As the synthetic effort to make efficient inhibitors continued, we also studied new synthetic transformations to afford more complex molecules that would act as hatch initiators. As part of this research plan, we developed two new synthetic methods that would accelerate our ability to make new bicyclic compounds (21, 22). These are shown in Figure 11.

1. LiOH
2. TBSCI
3. $\text{si}a_2\text{BH}$
4. Jones ox
5. HF

Figure 11. New annulation method for synthesis of glycinoeclepin A analogs

The first version of our annulation involved an aldol reaction with a phosphonate aldehyde followed by acetylation and an intramolecular Emmons
cyclization. This three-step procedure afforded the bicyclic ring system shown above in approximately 20% overall yield from 2-methylcyclohexenone. We later identified reaction conditions that enabled us to achieve the same transformation in one pot in over 30% yield. The resulting bicyclic ester was converted into the acid by ester hydrolysis with LiOH. Protection of the alcohol as the tert-butyldimethylsilyl ether, elaboration of the allyl group into the propionic acid side chain and removal of the protecting group provided the desired diacid. This compound will be tested for inhibitory activity against SCN. The new annulation will permit us to easily synthesize a variety of more complex analogs.

Conclusions

Although our idea when we began this study was to develop small molecules that cause premature hatch of the SCN, we have synthesized nine compounds that effectively inhibit the hatch of the soybean cyst nematode. During the course of the study we have determined that the minimum functionality for activity is the enolic dicarbonyl. The five membered ring analogs are more effective than the six and seven membered ring analogs at inhibiting hatch. We have also developed an annulation reaction which will allow us to easily prepare more complex bicyclic analogs.

References

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