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The discovery of fluazaindolizine: A new product for the control of plant parasitic nematodes

George P. Lahm,[a]* Johan Desaeger,[a] Ben K. Smith,[a] Thomas F. Pahutski,[a] Michel A. Rivera,[a] Tony Meloro,[a] Roman Kucharczyk,[a] Renee M. Lett,[a] Anne Daly,[a] Brenton T. Smith,[a] Daniel Cordova,[a] Tim Thoden,[b] John A. Wiles[c]

[a]*DuPont Crop Protection, Stine-Haskell Research Center, 1090 Elkton Rd., Newark, DE 19714, USA*
[b]*DuPont de Nemours (France) S.A.S., European Research Center, 68740 Nambsheim, France*
[c]*DuPont (U.K.) Limited, 4th Floor, Kings Court, London Road, Stevenage, SG1 2NG, United Kingdom*

Abstract - Fluazaindolizine is a new highly effective and selective product for the control of plant parasitic nematodes. Specificity for nematodes coupled with absence of activity against the target sites of commercial nematicides suggests that fluazaindolizine has a novel mode of action. The discovery, structure-activity development and biological properties for this new class of nematicides are presented.

The discovery and development of new nematicides that are highly effective against the target pest, work by new modes of action, and meet societal demands of safety to humans and the environment are essential in the defense of crops. Soil dwelling nematodes are responsible for significant crop damage and yield loss in agricultural production. Furthermore, many current nematicidal products are under regulatory pressure due to a range of toxicological and environmental issues. Herein we describe the discovery of fluazaindolizine (**1**), a new product for the control of plant parasitic nematodes.[1]

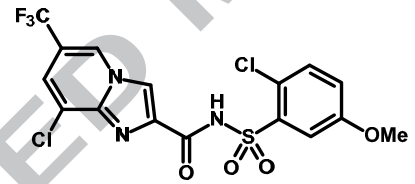

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Figure 1. Chemical structure of fluazaindolizine.

Plant-parasitic nematodes are ubiquitous microscopic soil pests that feed on plant roots resulting in severe crop losses. The damage often goes unnoticed due to the hidden nature of nematodes and the non-specific damage symptoms, which can be confused with soil fertility, drought or other soil pest or pathogen problems. The most recent nematode damage survey valued global crop losses at $100 billion annually.[2] The increasing need for food production and the growing pressure on agricultural land will likely cause crop losses to increase.

More than 4,000 species of plant-parasitic nematodes have been described but only a fraction of these cause economic damage to crops.[3] The most important nematode pest worldwide is the root-knot nematode (*Meloidogyne* spp.), which is estimated to account for greater than 50% of all nematicide use and 5% of crop loss globally.[4] Other agriculturally important plant-parasitic nematodes include cyst nematodes (*Globodera* and *Heterodera* spp.), lesion nematodes (*Pratylenchus* spp.), reniform nematodes (*Rotylenchulus* spp.) and sting nematodes (*Belonolaimus* spp.).

Soil fumigants account for about 50% of all commercial nematicides, with organophosphates and carbamates comprising nearly all of the remaining nematicides.[5] Many of these products are under regulatory pressure, and certain fumigants and organophosphates have been banned. Despite increasing regulatory pressure and limited choice, the global nematicide market continues to grow. Valued at $1 billion in 2011, the market is estimated to

increase to $1.4 billion by 2020.[5] Recently there has been a resurgence in nematicide discovery across the crop protection industry, and several new nematicides including fluensulfone and fluopyram have been introduced.[6-8] The renewed interest in nematicide discovery is long overdue and offers hope for growers that are currently struggling to manage nematode problems. The challenge is to align the needs of the grower with the demands of regulators and society. An ideal nematicide would 1) offer highly effective plant parasitic nematode control, without negatively impacting beneficial soil organisms, beneficial arthropods or pollinators, 2) have good soil root zone movement, but low leaching potential, 3) provide effective residual plant parasitic nematode control without persisting in soil for unacceptably long durations and 4) provide an appropriate level of plant root protection, by either contact or systemic activity, without resulting in residues in crop produce.

High throughput screening of our internal compound libraries against root-knot nematode (RKN) identified several active N-phenylsulfonylimidazopyridine-2-carboxamides, such as **2** and **3** (Figure 2). Testing at lower rates showed **3** to be highly effective on the root-knot nematode, however, some associated plant phytotoxicity was observed. The resulting optimization program focused on the dual objective of maximizing nematicidal activity with a corresponding reduction or elimination of the plant effects.

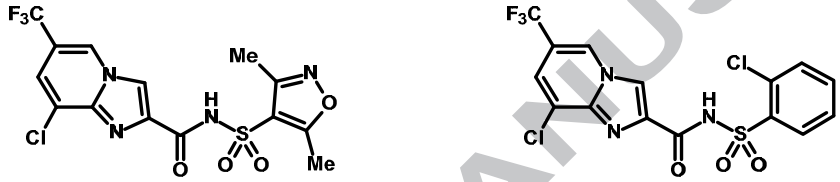
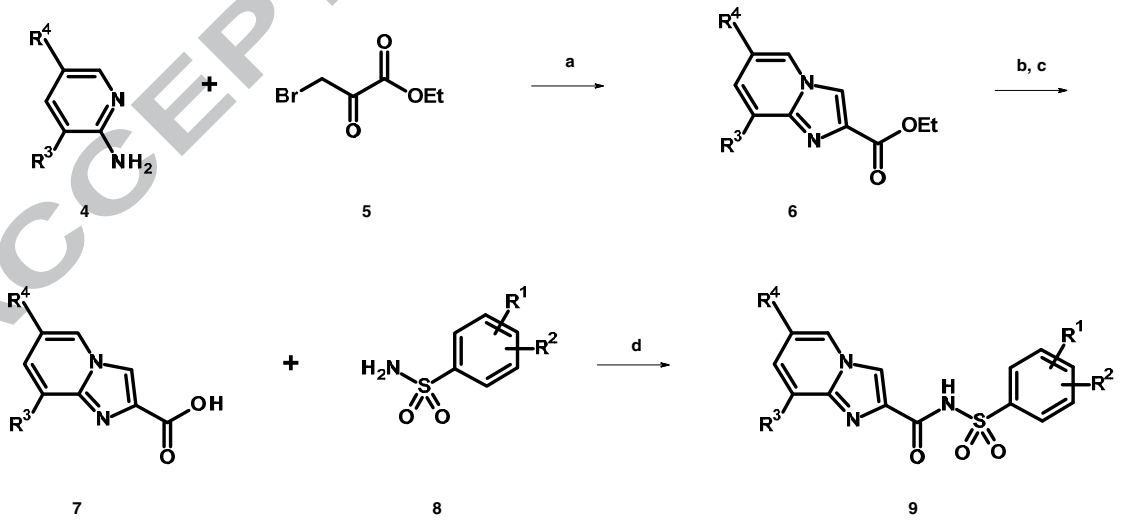


Figure 2. N-phenylsulfonylimidazopyridine-2-carboxamide lead compounds.

The compounds of Tables 1-3 were prepared as outlined in Scheme 1. The imidazopyridine-2-carboxylic acid **7** was prepared by the reaction of 2-amino-3,5-disubstituted pyridines with ethyl bromopyruvate to afford the ethyl esters of formula **6**, which were subsequently hydrolyzed to the acids **7**. The acids were then coupled with a series of phenylsulfonamides **8**, typically using EDC as the coupling reagent, to afford the N-phenylsulfonylimidazopyridine-2-carboxamides **9** in yields generally ranging from 40-60%.[9]



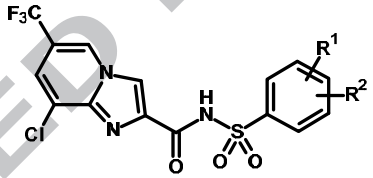
Scheme 1. (a) DME, 0°C to RT or heat (b) aq. NaOH, EtOH (c) aq. HCl (d) EDC, DMAP, DCM:tBuOH (1:1).

Nematicidal activity is summarized in Tables 1-3. Control of RKN through contact and/or systemic means was evaluated using standard laboratory procedures. Efficacy was determined by the amount of root gall formation observed in a 7-day evaluation when compared to an untreated control.[10] Lack of gall formation was indicative of 100% nematode control, whereas gall formation equivalent to that found in the untreated control was indicative of 0% control. Both EC_{50} and EC_{90} values are reported in Tables 1-3. Compounds showing a wider range between the EC_{50} and EC_{90} tended to have a flatter dose response with generally higher use rates required for economic control.

Compounds **D01** through **D07** of Table 1 compare a series of mono-substituted aryl sulfonamides containing a common left side, i.e. the 8-chloro-6-trifluoromethyl-imidazopyridine-2-carboxamide group. Lead compound **D01** demonstrated excellent efficacy in our RKN assay and was in fact one of the more potent analogs we evaluated (EC_{50} = 3.9 ppm). However, significant plant effects were observed which precluded further advancement. The corresponding 2-Me analog, **D02**, also demonstrated strong nematicidal activity but with similar phytotoxicity. While the meta-substituted derivatives **D03**-**D05** were less active as nematicides (EC_{50} = 16.2-57.3 ppm), several analogs demonstrated significant safety to plants including the 3-OMe (**D04**) and 3-CF_3 (**D05**) derivatives. Compounds **D06** and **D07**, containing substituents at the para position, were inactive against RKN.

Compounds **D08**-**D11** of Table 1 compare a series of substitution patterns for dichloro-arylsulfonamides. The 2,5- and 2,6-analogs, **D09** and **D10** respectively, were similar in nematicidal efficacy (EC_{50} = 4.7-8.3 ppm). However, phytoxicity was observed with the 2,6-analog but not with the 2,5-analog. This improvement in plant safety was observed with other 2,5-disubstituted analogs. Nematicidal activity was not observed for the 3,5-dichloro analog **D11**.

Table 1. Root-knot nematode (*Meloidogyne incognita*, RKN) activity of 8-chloro-N-phenylsulfonyl-6-(trifluoromethyl) imidazo[1,2a]pyridine-2-carboxamides.



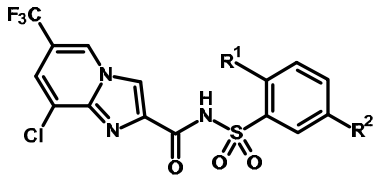
Entry	R^1	R^2	**RKN**[a] EC_{50} ppm	**RKN**[a] EC_{90} ppm
D01	2-Cl	H	3.9	19.3
D02	2-Me	H	4.3	71.6
D03	3-Me	H	16.2	63.4
D04	3-OMe	H	38.0	149.1
D05	3-CF_3	H	57.3	>250
D06	4-Me	H	>250	>250
D07	4-Cl	H	>250	>250
D08	2-Cl	3-Cl	25.5	185.7
D09	2-Cl	5-Cl	4.7	40.4
D10	2-Cl	6-Cl	8.3	55.7
D11	3-Cl	5-Cl	>250	>250

a. Mortality values were obtained for multiple test rates, each tested in replicate. EC_{50} and EC_{90} calculations were determined by Probit analysis. Confidence intervals are contained within the supplementary information.

The compounds of Table 2 represent a series of the 2,5-disubstituted arylsulfonamides containing both 2-Me (**D12**-**D21**) and 2-Cl (**D22**-**D32**) substituents. Examination of the EC_{50} and EC_{90} values show a wide range of efficacy with the most active analogs containing 5-Me, 5-OMe and 5-OEt substituents, i.e. compounds **D17**, **D22**, **D25** and **D26** (EC_{50} = 2.0-4.2 ppm). Analogs containing other 5-substituents such as SMe, SO_2Me, OCF_2H, OCF_3, cyano, nitro,

carbomethoxy, and dimethylamino generally showed a modest to significant reduction in RKN activity. Among the most active compounds **D17** and **D22** showed some plant phytotoxicity, whereas the 2-chloro-5-alkoxy analogs **D25** (5-OMe) and **D26** (5-OEt) provided the highest margins of plant safety combined with exceptional nematicidal activity.

Table 2. Root-knot nematode (*Meloidogyne incognita*, RKN) activity of 8-chloro-N-phenylsulfonyl-6-(trifluoromethyl) imidazo[1,2a]pyridine-2-carboxamides.

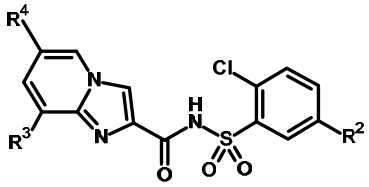


Entry	R^1	R^2	**RKN**[a] EC_{50} ppm	**RKN**[a] EC_{90} ppm
D12	Me	Me	7.0	34.8
D13	Me	F	20.9	56.9
D14	Me	Cl	23.1	91.0
D15	Me	Br	14.8	237.7
D16	Me	CF_3	5.7	53.4
D17	Me	OMe	2.6	10.1
D18	Me	OCF_2H	12.3	93.1
D19	Me	C(O)Me	6.1	58.9
D20	Me	SO_2Me	>250	>250
D21	Me	NO_2	21.2	>250
D22	Cl	Me	2.0	6.0
D23	Cl	Br	17.3	64.0
D24	Cl	CF_3	4.5	42.5
D25	Cl	OMe	3.1	14.9
D26	Cl	OEt	4.2	15.8
D27	Cl	O-iPr	19.2	40.5
D28	Cl	OCF_3	32.6	>250
D29	Cl	NMe_2	18.2	114.3
D30	Cl	SMe	>250	>250
D31	Cl	CO_2Me	91.1	>250
D32	Cl	CN	17.2	>250

a. Mortality values were obtained for multiple test rates, each tested in replicate. EC_{50} and EC_{90} calculations were determined by Probit analysis. Confidence intervals are contained within the supplementary information.

The compounds of Table 3 compare modifications to the 8-chloro-6-trifluoromethylimidazopyridine-2-carboxamide group. Removal of the 8-chloro-6-trifluoromethyl substituents, i.e. the unsubstituted analog **D33**, resulted in a loss of activity. Replacement of the 6-trifluoromethyl group with 6-Cl, **D34**, also resulted in a loss of activity. The dibromo analogs, **D35** and **D36**, similarly resulted in a significant loss of nematicidal activity with **D35** showing only modest RKN control. In contrast, compounds **D37** through **D42**, which all retain the 6-trifluoromethyl substituent and replace the 8-chloro group with H, F, Br or Me show moderate to good levels of RKN control indicating the importance of the 6-trifluoromethyl substituent. However, none of these compounds were more potent than the analogous 8-chloro analogs **D24** or **D25**. Beyond testing compounds in the RKN assay for comparison of intrinsic potency the most preferred analogs were evaluated in field trials against a broad spectrum of nematodes and in a variety of studies to evaluate toxicological and environmental attributes. From this group **D25** (fluazaindolizine) was selected for development.

Table 3. Root-knot nematode (*Meloidogyne incognita*, RKN) activity of imidazopyridine-2-carboxylic-N-(2-chlorophenyl)sulfonamides.



Entry	R^2	R^3	R^4	**RKN**[a] EC_{50} ppm	**RKN**[a] EC_{90} ppm
D33	OMe	H	H	>250	>250
D34	CF_3	Cl	Cl	>250	>250
D35	OMe	Br	Br	58.2	>250
D36	CF_3	Br	Br	>250	>250
D37	OMe	H	CF_3	15.6	127.4
D38	OMe	Br	CF_3	3.5	11.5
D39	CF_3	Br	CF_3	8.2	48.9
D40	OMe	F	CF_3	5.2	47.0
D41	CF_3	F	CF_3	9.9	132.6
D42	CF_3	Me	CF_3	12.9	>250

a. Mortality values were obtained for multiple test rates, each tested in replicate. EC_{50} and EC_{90} calculations were determined by Probit analysis. Confidence intervals are contained within the supplementary information.

In aqueous testing, juvenile RKN (J2) treated with fluazaindolizine (5-50 ppm) exhibit increasing immobility and ultimately death between 24-96 hours without significant symptoms being observed in the first hours of exposure. However, even at lower doses (1-5 ppm) and with short exposure periods the RKN fitness was reduced to an extent that they were unable to detect and infect roots. Adult *C. elegans* (a model non-plant parasitic nematode) treated with fluazaindolizine (300 ppm) showed no mortality or significant effect on motility over a 120 hour period. Further, the life cycle (egg to adult) of the fruit fly, *Drosophila melanogaster*, was unaffected when grown in diet containing fluazaindolizine (200 ppm). The nematicide was tested against *in vitro* assays for acetylcholinesterase (*Diabrotica undecimpunctata*), mitochondrial electron transport (*C. elegans*) nicotinic acetylcholine receptors (*C. elegans*), and glutamate-gated chloride channels (*P. americana*) with no significant activity observed at concentrations up to 30 uM. Specificity for plant parasitic nematodes coupled with absence of activity against targets of commercial nematicides suggests that fluazaindolizine has a novel mode of action.

Fluazaindolizine has been extensively tested in laboratory, greenhouse, micro-plot and field trials in North America, Latin America, Europe and in the Asia-Pacific region. In these trials fluazaindolizine has proven extremely effective against a wide range of root-knot nematode species (*Meloidogyne spp.*), and other important plant parasitic nematodes, such as reniform (*Rotylenchulus reniformis*), dagger (*Xiphinema spp*.), spiral (*Helicotylenchus spp.*) and some lesion nematode species (*Pratylenchus spp*.), as well as other important plant parasitic nematode species in certain circumstances.

Global development of fluazaindolizine is primarily as a suspension concentrate (500SC) liquid formulation, with granular formulations also under development for certain markets. The physico-chemical properties of fluazaindolizine lend it to be well balanced in terms of soil mobility (average Kfoc of 128) and residual properties (DT_{50} ~ 35 days) in the soil root zone. As such it is compatible with a variety of grower application methods; such as drip irrigation, bed sprays, micro-jets, pre-plant hole drench, in furrow applications and soil incorporation. Thus, agronomically, it has a fit in a range of crops including fruiting and cucurbit vegetables, root vegetables (carrot, sweet potato, potato), soybean, sugarcane, coffee, corn, citrus, tree nuts, stone fruit and grapes. Application rates vary by crop and application method and range typically from 0.25 to 2 kg ai/ha. The formulated products will be

commercialized under the brand DuPont™ Salibro™ nematode control, and the active ingredient (fluazaindolizine) will be branded as Vellozine™ nematode control. Use of the formulated product in the field, under the proposed directions for use, has demonstrated consistent root protection and the potential for increased yields in the crops tested.

Intrinsic sensitivities differ to fluazaindolizine, with plant parasitic nematodes being of higher sensitivity than other groups of the soil nematode community. Fluazaindolizine works by contact with nematodes in the soil pore water, and is not considered systemic in plants by soil application. It has a highly favourable profile for operators and also for non-target organisms making it an ideal product for use in integrated pest management (IPM) systems. In particular, fluazaindolizine has been shown to be highly compatible with a broad range of naturally occurring or introduced biological control agents, such as beneficial fungi and nematodes, bacteria and other important non target organisms that inhabit the soil rhizosphere and help sustain crop and soil health.

In summary fluazaindolizine has demonstrated excellent control of plant parasitic nematodes and the damage they cause to plant roots, resulting in higher quality crops and increased potential in crop yields. Specificity for nematodes, coupled with the absence of activity against target sites of commercial nematicides, suggests that it has a novel mode of action. Fluazaindolizine promises to offer farmers around the world a valuable new tool for crop protection and plant parasitic nematode management.

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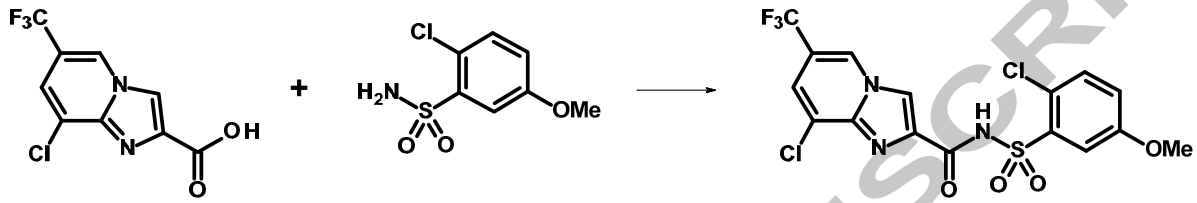
9) Synthesis and characterization of compound **D25**: To 8-chloro-6-(trifluoromethyl)-imidazo[1,2-a]pyridine-2-carboxylic acid (243 mg, 0.92 mmol) was added a solution of 4-(dimethylamino)pyridine (340 mg, 2.76 mmol) and 1-(3-dimethyl-aminopropyl)-3-ethylcarbodiimide hydrochloride (232 mg, 2.3 mmol) in t-butanol (5 mL) and dichloromethane (5 mL). The reaction mixture was stirred for 15 min and then 2-chloro-5-methoxybenzenesulfonamide (190 mg, 0.86 mmol) was added, and the mixture was left to stir at room temperature overnight. Dichloromethane (200 mL) was then added and the mixture was extracted with 1 N hydrochloric acid (3 x 100 mL). The organic phase was dried over magnesium sulfate and concentrated under reduced pressure to afford a solid. The solid was rinsed with diethyl ether to afford 240 mg of **D25** as a white solid, m.p. 211-212 °C. ^{1}HNMR ($CDCl_3$) δ10.10 (br s, 1H), 8.46 (s, 1H), 8.27 (s, 1H), 7.86 (d, 1H), 7.54 (s, 1H), 7.38 (d, 1H), 7.09 (dd, 1H), 3.91 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ56.57, 116.45 (q, J_{C-F} 34.5 Hz),

117.99, 120.89, 121.49, 121.92, 122.01, 123.41 (q, J_{C-F} 271.6 Hz), 124.10, 127.98 (q, J_{C-F} 5.9 Hz), 133.23, 135.67, 137.87, 138.28, 142.07, 158.28, 160.48; HRMS (APESI, M+) $C_{21}H_{19}ClF_3N_5O_2$: m/z calcd 466.9721, m/z found 466.9724.

10) Control of the southern root-knot nematode (Meloidogyne incognita) through contact and/or systemic means was evaluated in test units consisting of small open containers filled with approximately 10 ml of a sandy soil mixture and cucumber seedlings. Test compounds were formulated using a solution containing 50% acetone and 50% water. Test compounds (330 µl) were applied directly to the soil of the test units at concentrations of 250, 50, 10 and 2 ppm active ingredient. Each test was replicated 3 times. After treatment, the test units were allowed to dry for 1 hour, after which time about 250 second-stage juvenile (J2) larvae were pipetted into the soil. The test units were held at 27° C. and watered as needed for 7 days. Nematicidal efficacy was determined by the amount of root gall formation observed when compared to an untreated control. No gall formation was indicative of 100% nematode control. Gall formation equivalent to that found in the untreated control was indicative of 0% control. No nematode control rating was given to compounds showing significant phytotoxicity.

Graphical abstract



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