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Neural network-based QSAR and insecticide discovery: spinetoram

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Abstract Improvements in the efficacy and spectrum of the spinosyns, novel fermentation derived insecticide, has long been a goal within Dow AgroSciences. As large and complex fermentation products identifying specific modifications to the spinosyns likely to result in improved activity was a difficult process, since most modifications decreased the activity. A variety of approaches were investigated to identify new synthetic directions for the spinosyn chemistry including several explorations of the quantitative structure activity relationships (QSAR) of spinosyns, which initially were unsuccessful. However, application of artificial neural networks (ANN) to the spinosyn QSAR problem identified new directions for improved activity in the chemistry, which subsequent synthesis and testing confirmed. The ANN-based analogs coupled with other information on substitution effects resulting from spinosyn structure activity relationships lead to the discovery of spinetoram (XDE-175). Launched in late 2007, spinetoram provides both improved efficacy and an expanded spectrum while maintaining the exceptional environmental and toxicological profile already established for the spinosyn chemistry.

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Abbreviations

ANN	Artificial neural network
MLR	Multiple linear regression
SAR	Structure activity relationships
QSAR	Quantitative structure activity relationships
UV	Ultraviolet
ppm	Parts per million
LC_{50}	Lethal concentration resulting in mortality in 50% of the population

Introduction

The spinosyns are a novel class of natural products possessing a unique structure [1], a novel mode of action [2] and commercial levels of insecticidal activity [3–5]. Produced by the actinomycete, *Saccharopolyspora spinosa* [1, 6], the fermentation-derived spinosyns, and their insecticidal activity were discovered in the mid-1980s, eventually leading to the development and the 1997 registration of spinosad, a naturally occurring mixture of two of the most active spinosyns, A and D [5, 7].

As exemplified by spinosad, the spinosyns are primarily active against a wide range of lepidopterous and dipterous insect pests. The spinosyns also exhibit activity on a variety of other insect pests, however the activity is not always at a level that would provide useful for controlling these other insect pests in the field. Thus, it was deemed desirable to investigate ways to potentially enhance the biological efficacy of this novel chemistry with the goal of

providing broader utility in the insecticide marketplace. As such, diverse approaches were explored to identify or derive new spinosyns with improved activity, expanded spectrum, and improved physicochemical attributes. Included among the approaches investigated were searching for new, naturally occurring spinosyns [8, 9], genetic engineering of the biochemical pathways that produce the spinosyns [10], biotransformation [11] and synthetic modification of the naturally occurring spinosyns [9, 12–15]. While all of the aforementioned approaches produced analogs that were active to varying degrees, the semi-synthetic spinosyn analogs were explored in the greatest detail. As a part of the semi-synthetic effort, some of the most dramatic improvements in activity were the direct result of studies into the quantitative structure activity relationships (QSAR) of the spinosyns. What follows is a short overview of that effort and the subsequent discovery of spinetoram (XDE-175). This short review in not intended to be an in-depth review, or an explanation of the methodology used, but rather, to present the role of a novel QSAR approach in the discovery of a new, novel commercial insecticide.

Spinosyn SAR

Within the naturally occurring spinosyns, spinosyn A is the most biologically active, and is also the major component of spinosad [3, 4].

Among the other naturally occurring spinosyns, many of which are only produced in very minor amounts, removal of the methyl groups on the forosamine nitrogen (Fig. 1) (spinosyn B and C) only slightly reduced lepidopteran activity, as did addition of a methyl group at C6 (spinosyn D). Conversely, a reduction in the size/presence of alkyl groups on the tetracycle at C21 or C16 (Fig. 1) reduced lepidopteran activity by about 10-fold (Table 1; [4, 6]).

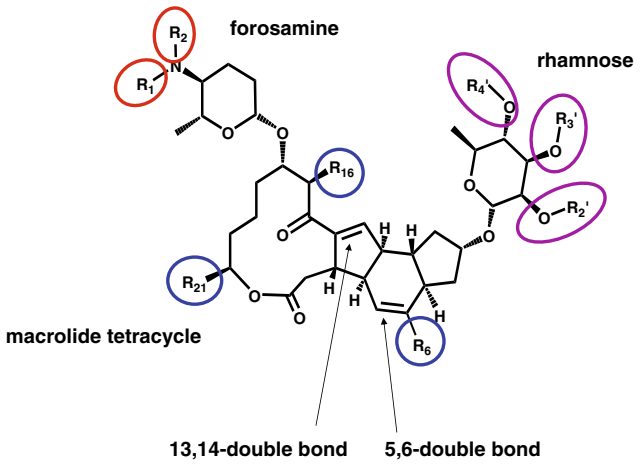


Fig. 1 Structure of the spinosyns

Likewise *O*-demethylation on the rhamnose sugar in either the 2′ or 4′-position reduced lepidopteran activity, while *O*-demethylation at the 3′-position nearly eliminated activity (Table 1; [4, 6]). Loss of one or both sugars also essentially eliminates activity [6, 17]. The structure activity relationship (SAR) just described was similar for other insect pests with one exception; for non-lepidopterans such as aphids and mites, loss of a methyl group from the forosamine or the 4′-position of the rhamnose typically resulted in a modest improvement in activity compared to spinosyn A (Table 1; [6, 9]).

Early synthetic efforts focused on simple modifications to the most available starting material, spinosyn A. The activity of the semi-synthetics quickly confirmed the observations made for the naturally occurring spinosyns; specifically, that with few exceptions [18] lepidopteran activity was easily diminished, if not eliminated, by virtually all modifications to the spinosyn structure, including modifications to, or replacement of, forosamine or rhamnose. Likewise, modifications to the tetracycle in a variety of forms also tended to negatively impact lepidopteran activity [9, 13].

Among the many modifications of the spinosyn structure that were explored, hydrogenation of the 5,6-double bond (Fig. 1) was found to have a small detrimental effect on lepidopteran activity, as measured by newly hatched (neonate) tobacco budworm larval activity (Table 1). However, compared to spinosyn A, the 5,6-dihydro analog did show an improvement in activity towards other insect pests such as the cotton aphid, two-spotted spider mite, and whitefly (Table 1; [9, 14, 19]). Thus, while not improving upon the activity of spinosyn A for lepidopterous pests, modification of the 5,6-double bond did provide some improvement in the spectrum.

Spinosyn QSAR and neural networks

Following the synthesis of several hundred analogs without significant improvement in lepidopteran activity, attempts were made to apply quantitative structure activity relationships (QSAR) to seek new synthetic directions for improving activity. The early approaches involved the analysis of large sets (>100) of the spinosyns and semi-synthetic analogs. Among the approaches examined were classical Hansch-style multiple linear regression (MLR), using spinosyn whole molecule properties, and 3D techniques such as comparative molecular field analysis (CoMFA). At the time, none of these approaches produced directions for further synthesis, or useful models to better understand the basis of the activity [20].

The lack of success with conventional approaches to QSAR resulted in the exploration of other tactics. Early

Table 1 Structure of selected examples of spinosyns and semi-synthetic spinosyn analogs

#	Spinosyn	R1[a]	R2	R6	R16	R21	R2′	R3′	R4′	DB[b]	TBW[c] LC_{50}	CA[d] LC_{50}	TSSM[e] LC_{50}
1	A	Me	Me	H	Me	Et	OMe	OMe	OMe	56DB	0.31	42–88	5.3
Forosamine modifications													
2	B	H	–[f]	–	–	–	–	–	–	–	0.4	11	0.9
3	C	H	H	–	–	–	–	–	–	–	0.8	33	8–29
Tetracycle modifications													
4	D	–	–	Me	–	–	–	–	–	–	0.8	50	3–19
5	E	–	–	–	–	Me	–	–	–	–	4.6	>50	100
6	F	–	–	–	H	–	–	–	–	–	4.5	>50	16
Rhamnose modifications													
7	H	–	–	–	–	–	OH	–	–	–	5.1	>50	>50
8	J	–	–	–	–	–	–	OH	–	–	>80	>50	~63
9	K	–	–	–	–	–	–	–	OH	–	3.5	12	1.4
Rhamnose + forosamine or tetracycle modifications													
10	L	–	–	Me	–	–	–	OH	–	–	26	>50	48–67
11	M	H	–	–	–	–	–	OH	–	–	22.6	–	–
12	N	H	–	Me	–	–	–	OH	–	–	40	–	>50
13	O	–	–	Me	–	–	–	–	OH	–	1.4	11	0.8
14	P	–	–	–	–	–	–	OH	OH	–	>64	–	–
15	Q	–	–	Me	–	–	OH	–	–	–	0.5	>50	14
16	R	H	–	–	–	–	OH	–	–	–	14.5	–	–
17	S	–	–	–	–	Me	OH	–	–	–	53	–	114
18	T	–	–	–	–	–	OH	OH	–	–	>64	–	–
19	U	–	–	–	–	–	OH	–	OH	–	22	–	>50
20	V	–	–	Me	–	–	OH	–	OH	–	17	–	–
21	W	–	–	Me	–	–	–	OH	OH	–	>64	–	–
22	Y	–	–	–	–	Me	–	–	OH	–	20	–	–
Semi-synthetic − forosamine modifications													
23	*N*-demethyl D	H	–	Me	–	–	–	–	–	–	5.6	>50	0.1
24	*N*-demethyl K	H	–	–	–	–	–	–	OH	–	9.9	–	0.5
25	*N,N*-dideMe K	H	H	–	–	–	–	–	OH	–	7.4	–	3.4
26	*N*-demethyl P	H	–	–	–	–	–	–	OH	OH	>64	–	–
27	*N*-ethyl	Et	–	–	–	–	–	–	–	–	0.24	15.2	0.3
28	*N,N*-diethyl	Et	Et	–	–	–	–	–	–	–	>64	4.8	0.3
29	*N*-acetyl	acetyl	–	–	–	–	–	–	–	–	5.7	>50	~50
30	*N*-allyl	allyl	–	–	–	–	–	–	–	–	1.3	>50	>50
31	*N*-benzyl	benzyl	–	–	–	–	–	–	–	–	10.8	>50	~100
32	4″-OH	na[g]	na	–	–	–	–	–	–	–	3.7	–	–
Semi-synthetic − tetracycle modifications													
33	5,6-DH	–	–	–	–	–	–	–	–	56SB	0.46	6.4	0.44
34	5,6-α-epoxy	–	–	–	–	–	–	–	–	56 α-epoxy	8.0	–	–
35	5,6-*β*-epoxy	–	–	–	–	–	–	–	–	56 *β*-epoxy	0.63	–	0.50
36	13,14-α-DH	–	–	–	–	–	–	–	–	1314-αSB	4.7	–	18
37	13,14-*β*-DH	–	–	–	–	–	–	–	–	1314-*β*SB	>80	–	>50
Semi-synthetic − rhamnose modifications													
38	2′,3′,4′-tri-Oet	–	–	–	–	–	OEt	OEt	OEt	–	0.02	6.8	1.3
39	2′-OEt	–	–	–	–	–	OEt	–	–	–	0.3	–	3.5
40	2′-O*n*Prop	–	–	–	–	–	O*n*Pr	–	–	–	0.9	–	–
41	2′-O*n*Pent	–	–	–	–	–	O*n*Pn	–	–	–	2.0	–	–

Table 1 continued

#	Spinosyn	R1[a]	R2	R6	R16	R21	R2′	R3′	R4′	DB[b]	TBW[c] LC_{50}	CA[d] LC_{50}	TSSM[e] LC_{50}
42	3′-OEt	–	–	–	–	–	–	OEt	–	–	0.03	12.7	2.0
43	3′-O*n*Prop	–	–	–	–	–	–	O*n*Pr	–	–	0.05	9.3	2.6
44	3′-O*n*But	–	–	–	–	–	–	O*n*Bu	–	–	0.38	2.5	1.5
45	4′-OEt	–	–	–	–	–	–	–	OEt	–	0.24	>50	4.1
46	4′-O*n*Prop	–	–	–	–	–	–	–	O*n*Pr	–	0.97	–	–
Semi-synthetic − rhamnose + tetracycle modifications													
47	2′-OEt 56DH	–	–	–	–	–	OEt	–	–	56SB	0.53	9.8	3.5
48	3′-OEt 56DH	–	–	–	–	–	–	OEt	–	56SB	0.05	15	0.7
49	3′-O*n*Prop 56DH	–	–	–	–	–	–	O*n*Pr	–	56SB	0.04	1.7	0.4
50	3′-OEt, 6Me	–	–	Me	–	–	–	OEt	–	–	0.12	–	0.4
Forosamine replacement													
51	H (no forosamine)	na	na	–	–	–	–	–	–	–	>64	–	100
52	4-NMe_2butyrate	na	na	–	–	–	–	–	–	–	16.6	–	–
53	3-NMe_2proprionate	na	na	–	–	–	–	–	–	–	16.0	–	–
54	2-NMe_2acetate	na	na	–	–	–	–	–	–	–	>64	–	21

Data adapted, in part, from [6, 9, 14, 16]

[a] Position on spinosyn structure (see Fig. 1)

[b] DB = double bond; location and presence (DB) or absence (SB = single bond)

[c] TBW = tobacco budworm drench assay using newly emerged (neonate) larvae, LC_{50} ppm (median lethal concentration resulting in 50% mortality)

[d] CA = cotton aphid (*Aphis gossypii*), whole sprayed plant assay, LC_{50} ppm

[e] TSSM = two spotted spider mite (*Tetranychus urticae*), treated leaf disk assay, LC_{50} ppm

[f] Dash = no change in substitution from spinosyn A

[g] na = not applicable

papers on artificial neural networks (ANN) and QSAR (e.g. [21]), suggested that ANN could be used for QSAR, with the fit of the resulting ANN models often exceeding that provided by MLR. This exploration was facilitated by the availability of inexpensive, easy to use ANN software (Braincel 2.3, Promised Land Technologies, New Haven, CT). Application of ANN analysis to spinosyn QSAR was further aided by simplifying the problem and applying a more targeted approach to the evaluation of the spinosyns. Specifically, the initial analyses were limited to examining the effects of specific substitutions at targeted sites around the periphery of the molecule that were associated with large changes in the biological activity.

Typically, the ANN analysis examined eight specific points around the spinosyn structure (Figs. 1 and 2), and these points became the neurons or nodes of the input layer for the ANN (Table 2, Fig. 2). Typical models consisted of a three layer network comprised of input layer containing eight (sometimes ten) nodes (the eight points of substitution around the spinosyn structure), a hidden layer containing a number of nodes equal to that of the input layer, and an output layer comprised of a single node (Fig. 2). The output was the LC_{50} (lethal concentration resulting in mortality in 50% of the population) in ppm for neonate tobacco budworm (*Heliothis virescens*) larvae, or an activity index using spinosyn A as the reference compound [4, 20]. The initial analyses were limited to a small targeted set (21–26 compounds) of spinosyns and semi-synthetics (e.g. Table 2). Given the limited number of compounds, the training set comprised the bulk (90%) of the compounds, with only a small percentage (10%) held back as the test set (as suggested by the software manufacturer). For these early analyses, the input data were restricted to a proton or simple alkyl substitutions around the spinosyn structure. Although later studies used molecular radius, molecular weight, molecular volume, etc. as the input data, the initial studies were conducted using simple numeric values (0, 1, 2, 3) as inputs for the number of carbon atoms (Table 2, [20]). Each model was usually trained to an error level of between 5 and 10%. The effect of overtraining was minimized by averaging the results of several separately trained models. Averaging the results of several models also provides a measure of cross-validation [22, 23].

The ANNs were able to provide good models for the activity patterns of the spinosyns in the training and test sets (Fig. 3; [20]). Subsequently, a series of selected "virtual" spinosyns was examined using these ANN models (Table 2). Predicted activity for these virtual

Table 2 Example of a targeted spinosyn data set and virtual spinosyns

#	Spinosyn	R1[a]	R2	R6	R16	R21	R2′	R3′	R4′	TBW LC_{50}	TBW[a] Pred1	SpinA[b] ratio	TBW Pred2	SpinA ratio
1	A	1	1	0	1	2	1	1	1	0.31	1.00	1.00	3.25	1.00
2	B	0	1	0	1	2	1	1	1	0.36	0.31	0.31	2.25	0.69
3	C	0	0	0	1	2	1	1	1	0.19	1.34	1.34	2.85	0.88
4	D	1	1	1	1	2	1	1	1	0.8	0.35	0.35	0.99	0.31
5	E	1	1	0	1	1	1	1	1	4.6	7.16	7.16	7.44	2.29
6	F	1	1	0	0	2	1	1	1	4.5	1.78	1.78	3.48	1.07
7	H	1	1	0	1	2	0	1	1	5.7	2.55	2.55	6.57	2.02
8	J	1	1	0	1	2	1	0	1	>80	48.1	48.1	69.1	21.3
9	K	1	1	0	1	2	1	1	0	3.5	2.70	2.70	4.46	1.37
10	L	1	1	1	1	2	1	0	1	26	25.9	25.9	32.4	9.98
11	M	0	1	0	1	2	1	0	1	22.6	23.1	23.1	29.9	9.22
12	N	0	1	1	1	2	1	0	1	40	33.3	33.3	37.6	11.6
13	O	1	1	1	1	2	1	1	0	1.4	1.28	1.28	3.69	1.14
14	P	1	1	0	1	2	1	0	0	>64	56.8	56.8	68.4	21.1
15	Q	1	1	1	1	2	0	1	1	0.5	4.53	4.53	4.39	1.35
16	R	0	1	0	1	2	0	1	1	14.5	3.00	3.00	13.2	4.06
17	S	1	1	0	1	1	0	1	1	53	45.0	45.0	55.0	17.0
18	T	1	1	0	1	2	0	0	1	>64	68.6	68.6	68.1	21.0
19	U	1	1	0	1	2	0	1	0	22	7.55	7.55	21.5	6.64
21	W	1	1	1	1	2	1	0	0	>64	55.3	55.3	56.6	17.4
22	Y	1	1	0	1	1	1	1	0	20	19.5	19.5	21.5	6.62
23	*N*-demethyl D	0	1	1	1	2	1	1	1	5.8	2.68	2.68	1.99	0.61
24	*N*-demethyl K	0	1	0	1	2	1	1	0	9.8	6.66	6.66	8.05	2.48
25	*N*,*N*-didemethyl K	0	0	0	1	2	1	1	0	7.5	5.74	5.74	8.74	2.60
26	*N*-demethyl P	0	1	0	1	2	1	0	0	>64	60.4	60.4	71.3	22.0
Examples of virtual compounds in the first round of analysis														
38	2′,3′,4′-tri-OEt	1	1	0	1	2	2	2	2	0.02	0.09	0.09	0.30	0.09
–	All nProp	3	3	3	3	3	3	3	3	–	0.09	0.09	18.7	5.76
–	All H	0	0	0	0	0	0	0	0	–	99.8	99.8	77.8	24.0
28	*N*,*N*-diEt	2	2	0	1	2	1	1	1	>64	3.43	3.43	17.4	5.37
39	2′-OEt	1	1	0	1	2	2	1	1	0.30	0.92	0.92	3.42	1.05
–	21-nProp	1	1	0	1	3	1	1	1	0.16	0.41	0.41	3.31	1.02

Data adapted, in part, from [9, 14, 16, 20]

[a] TBW pred = predicted LC_{50} (ppm) for neonate tobacco budworm larvae

[b] SpinA ratio = predicted LC_{50} compound/predicted LC_{50} spinosyn A

spinosyns ranged from rather inactive to potentially more active than spinosyn A. The initial models suggested that expanding the size of the alkyl groups at all points around the spinosyn structure could improve activity, while reducing the size of all of the alkyl groups to just a proton would reduce activity (Table 2). However, the models also indicated that larger alkyl groups on the forosamine (*N*,*N*-diethyl) would be less active, while also suggesting that expanding the size of the alkyl groups on just the rhamnose might be effective in improving activity. Specifically, the models predicted that the 2′,3′,4′-tri-*O*-ethyl analog of spinosyn A (compound 38) could be significantly more active than spinosyn A (Table 2). Subsequent synthesis and testing confirmed this prediction (Tables 1 and 2; [20]), and the prediction that the 2′-*O*-ethyl analog would be similar in activity to spinosyn A. Like the modification to the 2′-position on the rhamnose, the models also suggested that larger alkyl groups at the C21 position of the spinosyn tetracycle (e.g. *n*-propyl) might be as active, or perhaps slightly better, than spinosyn A against neonate tobacco budworm larvae (Table 2). Interestingly this prediction was confirmed when the C21-*n*-propyl analog became available [9, 10].

Since modifications to the rhamnose appeared to provide a large benefit in activity, further ANN modeling focused on determining which of the three positions on the

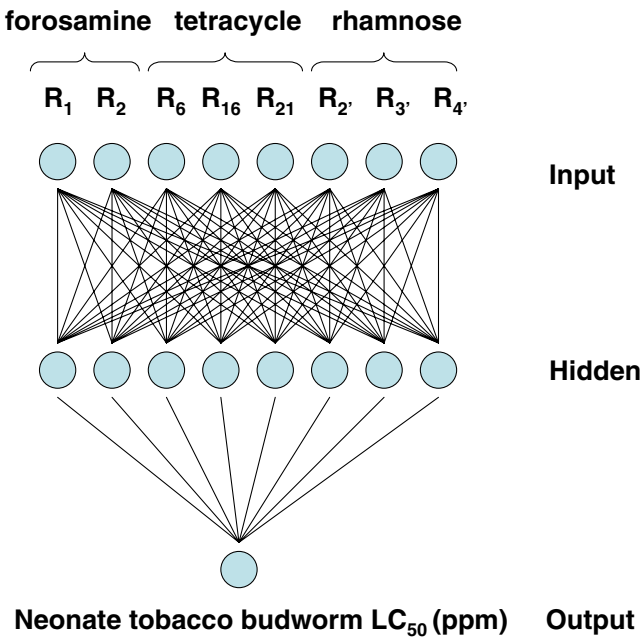


Fig. 2 Network architecture for analyzing larval (neonate) tobacco budworm activity of the spinosyns. Adapted from [20], with permission from Elsevier

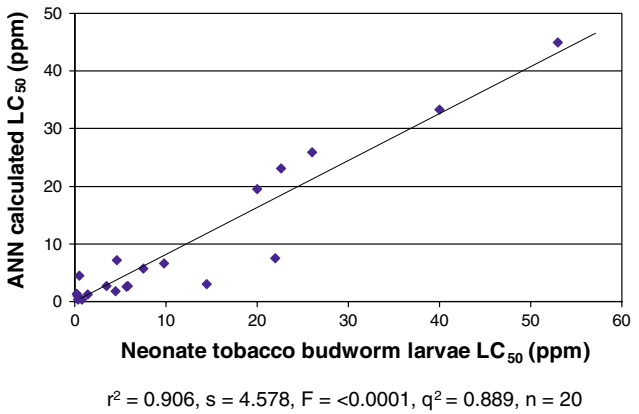


Fig. 3 Response of observed versus ANN calculated larval (neonate) tobacco budworm LC_{50}s Data from Table 2

rhamnose was responsible for the bulk of the improved activity. In these studies, the ANN predicted that increasing the size of the alkyl group at the 3′-position should provide the largest boost in activity, while increasing the size of the alkyl groups at the other two positions should provide little improvement in activity compared to spinosyn A. Again, subsequent synthesis and testing of the *O*-ethyl and *O*-*n*-propyl analogs confirmed the new predictions (Fig. 4; [20]). Further testing indicated that the 3′-*O*-ethyl analog (compound 42) was essentially as active as the 2′,3′,4′-tri-*O*-ethyl analog and equivalent to, or slightly better, than the corresponding 3′-*O*-*n*-propyl analog (Table 2; Fig. 5; [14, 16, 20]). Thus, the use of ANN-based QSAR provided a new direction for the spinosyn chemistry that led to significant improvements in activity. Of particular importance was the identification of the 3′-*O*-ethyl analog of spinosyn A and its associated improved lepidopteran activity compared to spinosyn A. In addition the 3′-*O*-ethyl analog also showed improvements in insecticidal activity towards other insect pests including cotton aphids (*Aphis gossypii*) and two-spotted spider mites (*Tetranyuchus urticae*) (Table 3; [9, 14]).

Subsequent to the ANN studies, Hansch-style MLR analysis was re-examined using targeted data sets similar to those used for the ANN studies. These subsequent studies were successful in demonstrating that the neonate tobacco budworm activity could be well explained by simple whole molecule properties such as calculated logP and dipole

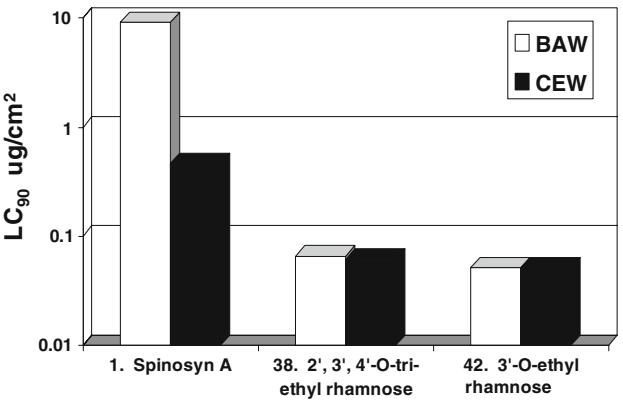


Fig. 5 Modified spinosyns: more active than spinosyn A on beet armyworm (BAW, *Spodoptera exigua*), and corn earworm (CEW, *Helicoverpa zea*); diet assays, 4th instar larvae

Fig. 4 ANN predicted and observed activity for *O*-ethyl and *O*-*n*-propyl spinosyn rhamnose analogs. Data adapted from [20]

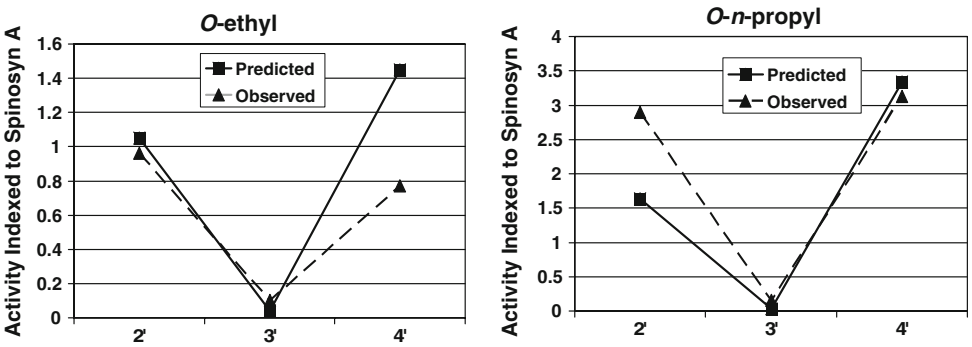


Table 3 Insect activity of spinosyn A and selected semi-synthetic spinosyns

#	Spinosyn	TBW	Ratio	BAW	Ratio	CL	Ratio	CA	Ratio	TSSM	Ratio	WF	Ratio
1	Spinosyn A	0.31	1.00	0.63	1.00	1.56	1.00	50	1.0	5.3	1.00	20	1.00
33	5,6-DH A	0.46	0.67	1.00	0.63	3.07	0.51	6.4	7.8	0.44	12.5	1.6	12.5
42	3′-O-ethyl A	0.03	10.3	0.039	6.2	0.67	2.32	12.7	3.9	2.0	2.65	5.5	3.64
48	3′-O-ethyl 5,6-DH	0.05	6.2	0.04	15.8	0.15	10.3	15.0	3.3	0.7	7.57	0.03	606.1
	Cypermethrin	0.18	–	–	–	–	–	–	–	–	–	–	–
	Imidacloprid	–	–	–	–	–	–	0.03	–	–	–	0.6	–
	Fenazaquin	–	–	–	–	–	–	–	–	1.0	–	–	

TBW = Tobacco budworm drench assay using newly emerged (neonate) larvae; LC_{50} ppm

BAW = Beet armyworm (*Spodoptera exigua*) topical assay using 4th instar larvae; median lethal dose for 50% of the population (LD_{50}), μg/larva

CL = Cabbage looper (*Trichoplusia ni*) leaf disk (feeding) assay using 2nd instar larvae, LC_{50} ppm

CA = Cotton aphid (*Aphis gossypii*), whole sprayed plant assay, LC_{50} ppm

TSSM = Two spotted spider mite (*Tetranychus urticae*), treated leaf disk assay, LC_{50} ppm

WF = Sweetpotato whitefly (*Bemisia tabaci*) whole plant treated assay, LC_{50} ppm

Ratio = LC_{50} spinosyn A/LC_{50} compound

moment [9, 16]. Further, the MLR results provided an understanding of the basis for the improved activity associated with the rhamnose modifications [16].

Spinetoram

In light of the broad improvement in insecticidal activity observed with the 3′-*O*-ethyl analog, this molecule then became the subject of further modification. Based on the improvement in non-lepidopteran activity noted for the 5,6-dihydro spinosyn A (compound 33), it was hypothesized that a similar improvement in spectrum might result from coupling of the 5,6-dihydro to the 3′-*O*-ethyl analog of spinosyn A. Interestingly, when this analog was synthesized (compound 48) and tested, it as was found to have retained both improved lepidopteran activity and the enhanced activity towards other insect pests (Table 3). The extension of the 3′-*O*-alkyl moiety from methyl (spinosyn A) to its 3′-*O*-ethyl derivative increased the lipophilic nature of the compound, resulting in a lower melting point and a greatly reduced ultraviolet (UV) stability [14]. Hydrogenation of the 5,6-double bond raised the melting point and generally restored the UV stability relative to spinosyn A [14]. Thus, the combination of the 3′-*O*-ethyl moiety and reduced 5,6-double bond led to improved insecticidal activity, enhanced duration of control, and an expanded pest spectrum (Fig. 6).

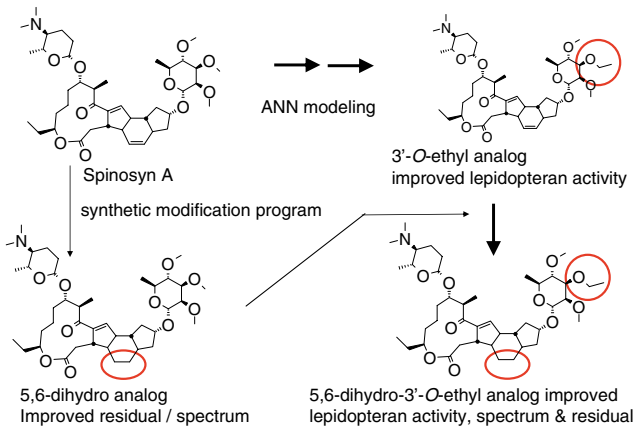
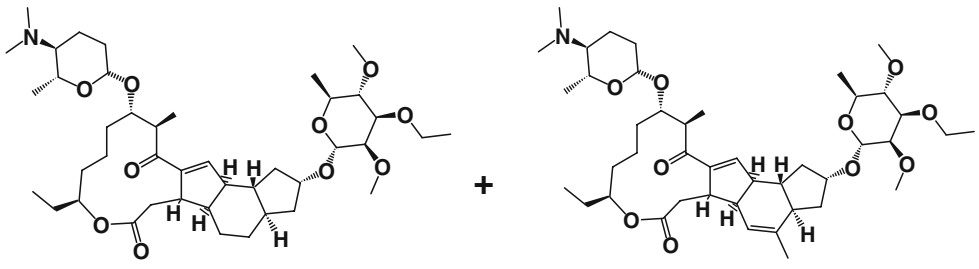


Fig. 6 Evolution of spinetoram: Combination of 3′-*O*-ethyl and 5,6-dihydro provides better activity, spectrum and residual

When the above synthetic modifications were applied to the naturally occurring mixture of spinosyns J and L, the result was spinetoram (XDE-175), a semi-synthetic insecticide that is a mixture of the above mentioned 3′-O-ethyl-5,6-dihydro-spinosyn J (major component, compound 48) and 3′-*O*-ethyl spinosyn L (minor component, compound 50) (Fig. 7; [15]). Like its primary component (3′-*O*-ethyl-5,6-dihydro-spinosyn J, compound 48), spinetoram is more active and has a longer duration of control than spinosad against many key insect pests, such as codling moth (*Cydia pomonella*), a major pest of pome fruits, and tobacco budworm, a major pest of cotton and vegetable crops. The results of two bioassays demonstrating this difference in activity are presented in Fig. 8, where spinetoram was 4.3-fold more active than spinosad against codling moth larvae, and more than 6-fold more active than spinosad against tobacco budworm larvae.

Although spinetoram is more active than spinosad against many important insect pests, the mammalian toxicity, the

Fig. 7 Structure of spinetoram



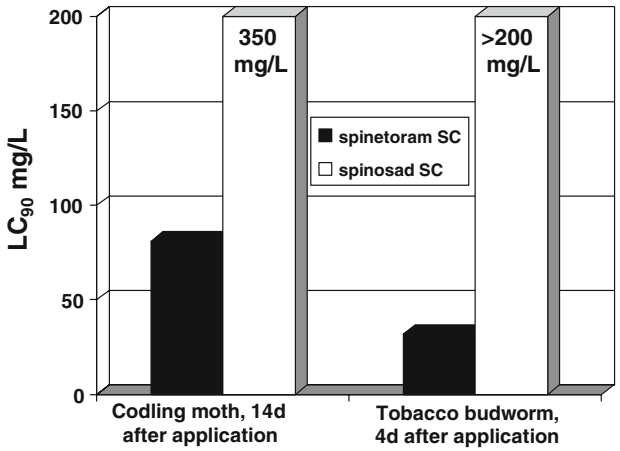


Fig. 8 Residual activity of spinetoram versus spinosad against codling moth larvae (14 days after treatment of the apples used as diet) and tobacco budworm larvae (4 days after treatment of the cotton plants). For codling moth, apple fruits were sprayed with suspension concentrate (SC) formulations of spinetoram or spinosad at a range of concentrations, held 14 days under lamps simulating sunlight, and then infested with codling moth larvae. For tobacco budworm, potted cotton plants were sprayed with SC formulations of spinetoram or spinosad at a range of concentrations, held 4 days under lamps simulating sunlight, and then infested with tobacco budworm larvae. In each assay, the number of live and dead larvae was determined after a few days of exposure to the treated plant material and the concentration expected to kill 90% of the population (LC_{90}) was calculated using probit analysis [24]

ecotoxicity, and the environmental fate characteristics of spinetoram are very similar to spinosad [25]. Spinetoram was evaluated by the US EPA under its reduced risk pesticide program and was granted registration in September 2007. Spinetoram is being developed and registered in many countries around the world to control insect pests in a wide range of crops [25].

QSAR has long been an important tool in understanding the physicochemical basis for insecticidal activity [26–28], and in at least one case QSAR played a central role in the discovery of a commercialized insecticide [29]. As outlined in this brief review, ANN-based QSAR was successful in identifying new directions for the synthesis of spinosyn analogs where other methods, at the time, had failed. The new analogs suggested by the ANN-based QSAR led to a breakthrough in improving the insecticidal activity of this unique class of chemistry. Thus, the use of ANN-based QSAR was pivotal to the discovery of spinetoram, the next generation spinosyn insecticide.

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