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Chemical Modification of Nodulisporic Acid A: Preliminary Structure–Activity Relationships

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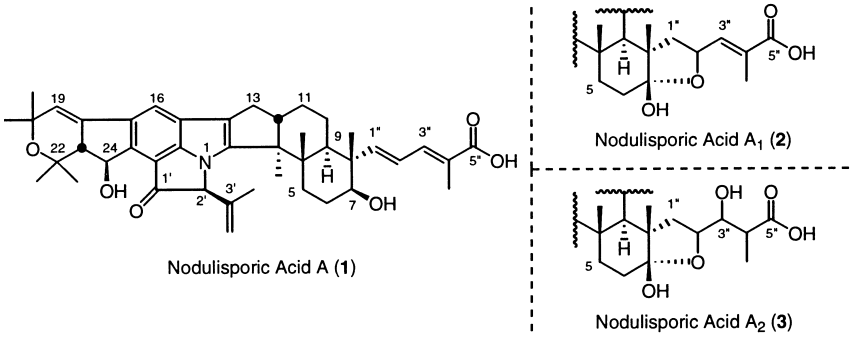
Abstract—Medicinal chemistry efforts were initiated to identify the key constituents of the nodulisporic acid A (**1**) pharmacophore that are integral to its potent insecticidal activity. New semisynthetic derivatives delineated **1** into 'permissive' and 'nonpermissive' regions and led to the discovery of new nodulisporamides with significantly improved flea efficacy.

At Merck, we have had a long-standing interest in the development of new, biologically active natural products, or derivatives thereof, with potent insecticidal and antiparasitic activity, as exemplified by the discoveries of avermectin B_{1a}/B_{1b}[1] (1984), ivermectin[2] (1986), eprinomectin[3–5] (1995), and emamectin[6,7] (1995). Our continued interest in antiparasitic agents led to the recent isolation and characterization of the potent, fermentation-derived insecticidal agent, nodulisporic acid (NsA) A[8,9] (**1**), as well as the two fermentation congeners NsA A_1[10] (**2**) and NsA A_2 (**3**). The nodulisporic acids are remarkable in that they exhibit *systemic* efficacy (as opposed to contact activity) against fleas which are killed following ingestion of drug-treated blood.[11] Significantly, this systemic antiparasitic activity was reproduced in dogs following oral dosing, successfully killing fleas subsequent to their ingestion of a blood meal without apparent toxicity to the host animal. NsA A achieves this lethality to insects by selectively modulating a subset of the invertebrate-specific glutamate-gated chloride ion channels targeted by ivermectin.[12]

Flea control on companion animals such as dogs and cats represents a major market and total expenditures worldwide to combat these pests were in excess of $1.2 billion in 1999. Consumer dissatisfaction with past flea control treatment protocols prompted drug discovery efforts which culminated in the recent introduction of



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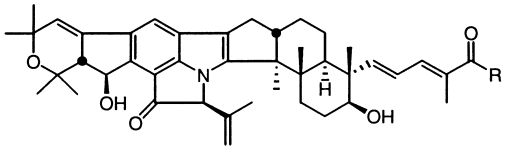
five new flea control products: lufenuron,[13] nitenpyram,[14] fipronil,[15] imidacloprid,[16] and selamectin.[17] Lufenuron is an *orally* efficacious insect growth regulator, which disrupts the flea's life cycle by preventing maturation of flea larvae but, lacking lethality to adult fleas, is frequently used in conjunction with nitenpyram, an orally active flea adulticide that serves as a 'knock-down' agent. Nitenpyram by itself, although very efficacious, has no residual activity beyond 1 day and reinfestations occur quickly. Fipronil, imidacloprid, and selamectin, on the other hand, are *topically* applied agents that are toxic to adult fleas, but because the drugs are exposed to the environment, their residual efficacies can be variable. Therefore, the identification of an oral, *systemically* active agent that selectively kills adult fleas on cats and dogs, possesses good residual control, yet lacks mammalian toxicity is highly desirable. Consequently, a medicinal chemistry program was initiated to modify these structurally intriguing indole diterpenes in an effort to discover new NsA derivatives with enhanced flea activity. We disclose herein the preliminary chemical alterations that were made at positions 7, 23, 24, 1′, 2′, 1″, 2″, 3″, 4″, and 5″ and the resultant biological consequences that facilitated identification of the key constituents of the NsA pharmacophore.

To discern if the 5″-carboxylic acid of NsA A was essential for biological activity, **1** was treated with $Me_3SiCH=N_2$ in MeOH, leading to methyl ester **4**. Additional esters, thioesters, and amides were prepared via modular protocols; representative examples are shown in Table 1. Esters **4**–**7** and thioester **8** were prepared by reacting **1** using Coste's conditions[18] (ROH, CH_2Cl_2, BOP, $-20\,^{\circ}C \rightarrow$ rt) while amides **9**–**25** were generated following Castro's protocol[19] (R_1R_2NH, CH_2Cl_2, BOP, DIEA, $0\,^{\circ}C \rightarrow$ rt).

The newly prepared NsA derivatives were evaluated for systemic activity against adult cat fleas (*Ctenocephalides felis*) using an artificial membrane feeding apparatus.[20] In this assay, fleas (20–25 fleas/level) ingested fresh bovine blood containing 10, 1, 0.1, and 0.01 ppm concentrations of a given compound. An analogue was deemed fully active at a given dosage if greater than 80% fleas were killed, partially active (∼) if 51–79% of fleas died and inactive if fewer than 50% of the fleas succumbed. Generally, esters and thioesters were poorly active relative to the parent acid, although incorporation of a polar hydroxyl group served to reintroduce limited efficacy against fleas. Indeed, **7**, the most potent ester derivative prepared, incorporated both an ether and a hydroxyl in its side chain and showed a 10-fold improvement in flea activity relative to **1**.

The new nodulisporamides were subdivided into three broad classifications: those bearing polar substituents or those prepared from lipophilic mono- or disubstituted amines. Polar functionality (i.e., basic amines, carboxylic acids, carboxamides, etc.) was poorly tolerated in the amide side chains, with the exception, once again, of a hydroxyl group whose presence conferred modest potency enhancements (e.g., **9**). Small (**13**, **14**, and **20**) to intermediate-sized (**15**–**19** and **21**–**25**) mono- and disubstituted amides exhibit improved flea activity, while larger amides generally demonstrated decreased efficacy (data not shown). The promising biological activity observed with certain amide derivatives prompted the screening of additional nodulisporamides and led to the discovery of analogues with significantly enhanced flea efficacy. Of particular interest were **19** and **25**, which are almost two orders of magnitude more potent than NsA A in the systemic flea assay. It is also interesting to compare their activity with that of less active, but structurally similar nodulisporamides **18**, **23**, and **24**.

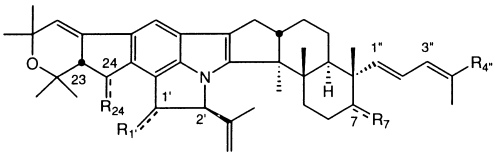
Table 1. Ester and amide derivatives of nodulisporic acid A[22]



Compound	R	Flea (ppm)
1	OH	1
Esters and thioesters		
4	OMe	∼10
5	OCH_2CH_2OH	∼1
6	$OCH_2CH_2CH_2OH$	10
7	$OCH_2CH_2OCH_2CH_2OH$	0.1
8	SEt	>1
Polar amides		
9	$NHCH_2CH_2OH$	0.5
10	$NHCH_2CH_2NMe_2$	10
11	$NHCH_2CH_2CO_2H$	>10
12	$NHCH_2C(O)NH_2$	10
Monosubstituted amides		
13	NH_2	∼0.1
14	NH-Me	∼0.1
15	NH-*n*-Hexyl	∼0.5
16	$NHCH_2Ph(4\text{-}Cl)$	0.1
17	NHPh(4-F)	∼1
18	$NHC(Me)_2CO_2Me$	1
19	$NHC(Me)_2C(O)NMe_2$	0.01
Disubstituted amides		
20	NMe_2	∼0.1
21	N(Et)*n*Pr	∼0.1
22	*N*-1-Piperidinyl	∼1
23	*N*-1-[(4-Me)Piperazinyl]	1
24	*N*-1-[(4-CO_2Et)Piperazinyl]	0.1
25	*N*-1-[(4-SO_2Me)Piperazinyl]	∼0.01

The biological consequences of additional synthetic modifications to the core of nodulisporic acid were probed primarily using *N*-methyl nodulisporamide **14** as starting material (Table 2). The 7- and 24-hydroxyls could be oxidized (Dess–Martin reagent, CH_2Cl_2, rt, 10 min) or acetylated (AcCl, pyridine, $0\,^{\circ}C$) to smoothly generate **28** and **34**, respectively. Alternatively, statistical silylation of **14** (TBDMSCl, DMF, imidazole) yielded, following chromatographic separation, both the 7- and 24-mono-protected ethers. The individual mono-protected nodulisporamides were subsequently oxidized or acetylated at either position 7 or 24 as noted above and deprotected (PPTS, EtOH, rt, 30 min). The stereochemistry of the 2′-propenyl group was quantitatively inverted by treatment of **14** in MeOH with Et_3N at rt to form the thermodynamically more stable 2′-epimeric

Table 2. Additional structural modifications of nodulisporic acid A

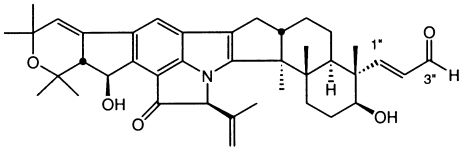


Entry	R_{24} group	$R_{1'}$ group	R_7 group	$R_{4''}$ group	Miscellany	Flea (ppm)
1	β-OH	oxo	β-OH	CO_2H		1
14	β-OH	oxo	β-OH	C(O)NHMe		~0.1
26	β-OH	oxo	oxo	C(O)NHMe		~10
27	oxo	oxo	β-OH	C(O)NHMe		>10
28	oxo	oxo	oxo	C(O)NHMe		>10
30	β-OH	β-OH	β-OH	C(O)NHMe		10
31	β-OH	oxo	β-OH	C(O)NHMe	2′-Epi	~10
32	β-OH	oxo	β-OAc	C(O)NHMe		~10
33	β-OAc	oxo	β-OH	C(O)NHMe		>10
34	β-OAc	oxo	β-OAc	C(O)NHMe		>10
35	β-OH	oxo	β-OH	CHO		>1
36	β-OH	oxo	β-OH	CH_2OH		>10
37	—	oxo	β-OH	C(O)NHMe	23,24-Dehydro	10
38	β-OH	oxo	β-OH	C(O)NHMe	1″,2″-Dihydro	~10
39	β-OH	oxo	β-OH	C(O)NHMe	3″,4″-Dihydro	~1
40	β-OH	oxo	β-OH	C(O)NHMe	1″,2″,3″,4″-Tetrahydro	>10
41	See structure below[23]					~10
42	See structure below					~10

adduct **31**. Reduction of the 1′-carbonyl was accomplished using $NaBH_4$ in MeOH at 0 °C to generate a separable mixture of the alcohol epimers **29** (not shown) and **30** in a ~1:9 ratio. The 4″-aldehyde **35** was prepared by Rosenmund reduction of the corresponding thioester **8**.[21] This aldehyde could be further reduced using 9-BBN to yield alcohol **36**. *N*-Methyl nodulisporamide's 24-hydroxyl was eliminated by treatment of **14** with TsOH in MeOH at rt to form the corresponding 23,24-dehydro **37**. The side-chain olefins were reduced nonselectively (5% Pd/C, EtOAc, H_2, 40 min), producing the requisite nodulisporamides **38**–**40** as a separable mixture of reduction products. The 24-hydroxyl is stabilized by a six-center internal hydrogen bonding array (the hydroxyl proton is commonly observed as a sharp singlet in the ^{1}H NMR at δ 3.2 ppm). Disrupting this array, for example, by reducing the 1′-carbonyl or acetylating the 24-hydroxyl, generated compounds that were prone to dehydration, forming the corresponding 23,24-dehydro analogue (i.e., **37**). While the 7-hydroxyl generally was more reactive towards acetylation or silylation than the 24-hydroxyl, oxidation of the benzylic 24-hydroxyl proceeded more readily than that of the 7-hydroxyl. Also, in its ^{1}H NMR spectrum, the 24-keto derivative is seen as a 1:2 mixture of keto:enol tautomers.

Biological evaluation of these new NsA derivatives demonstrated that flea efficacy is extremely sensitive to synthetic modications to the core of the molecule. Hydroxyl acetylation (**32**–**34**) or oxidation (**26**–**28**) led to complete loss of activity in the flea assay. Elimination of the 24-hydroxyl (**37**) or reduction of the 1′-carbonyl (**30**) resulted in similar consequences to flea efficacy. Epimerization at 2′ (**31**) decreased flea efficacy somewhat, as did reduction of the 3″,4″-olefin (**39**). Dihydro derivative **38** and tetrahydro analogue **40** exhibited even less potency than **39** in the flea assay. Conversion of NsA A's carboxylic acid into an aldehyde (**35**) or a hydroxymethyl group (**36**) led to a major loss of bioactivity. Finally, the side chain truncated 3″-aldehyde **41** and the 1″-aldehyde **42** also lost significant efficacy, indicative of the importance of a longer side chain for good potency against fleas.

In summary, synthetic alterations of nodulisporic acid A have successfully been accomplished at the multiple sites indicated in Figure 1. Preliminary structure–activity relationships discerned from these newly synthesized NsA A derivatives indicated that the molecule may be partitioned into so-called 'permissive' and 'non-permissive' regions. Changes in the 'non-permissive' polycyclic core have deleterious effects on observed biological activity as determined using a systemic assay for efficacy against fleas. Structural modifications in the 'permissive' side-chain region, with particular emphasis on the formation of amide analogues, led to the discovery of new nodulisporamides with enhanced systemic efficacy against fleas.



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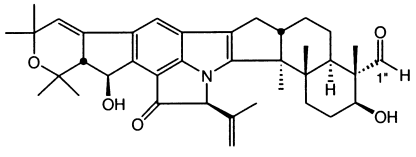

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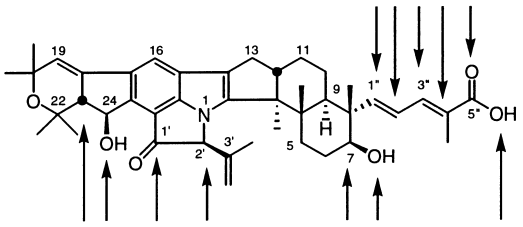

Figure 1. Synthetically modified sites NsA A.

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