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Perspective

Perspectives in Animal Health: Old Targets and New Opportunities

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Introduction

The past decade has witnessed a period of considerable turmoil in the field of animal health research. Not unlike the human health arena, these changes manifested themselves in part by consolidations, acquisitions, and divestitures among animal health research organizations. Traditionally, veterinary drug discovery programs have focused on the identification of therapeutic and prophylactic agents for food animals (swine, poultry, cattle, and sheep) as these represented major economic targets. However, in recent years, animal health research shifted its emphasis to the development of therapies for companion and performance animals, specifically dogs, cats, and horses.

Due to economic issues, animal health drug discovery efforts typically were ancillary to human health research. It was common for approved human health medicines to simply be reformulated and/or used off-label for animal health applications, often resulting in suboptimal therapies for animals. Interestingly, human health drug discovery is littered with molecules that, while fully efficacious in animals, failed to achieve the desired biological profiles in humans. The development of these drugs exclusively for animal health applications can potentially yield superior therapeutic agents. Many of the therapies described herein rose out of human health drug discovery programs, indicative of the significance of leveraging research efforts in one area to create opportunities in a completely different arena. Additionally, these opportunities are not one-sided. Human health and insecticidal agents have found application in animal health, while medicines developed originally for animals have been extended to encompass the treatment of human diseases or found utility in crop protection. Examples of each will be presented here.

Distinct advantages exist in the animal health arena that have no counterpart in human health. Veterinary chemotherapeutics can be evaluated directly in the intended target species during the earliest stages of the drug discovery process, providing prompt and timely in vivo confirmation of efficacy. It should be noted that these studies proceed at a significantly earlier stage than for human health, as FDA regulations preclude comparable evaluations in humans. An additional benefit derived from early compound evaluation is that deleterious safety issues associated with a given therapeutic agent may be discerned prior to the commitment of considerable resources. As a direct consequence, a high percentage of developmental candidates selected for animal health survive the winnowing process of safety assessment, while typically only 1 in 10 potential human health drugs successfully traverses these hurdles to reach the marketplace. Furthermore, decreased regulatory hurdles exist for non-food-animal therapeutic agents relative to those destined for food animals, leading to accelerated drug development periods.

Today, the veterinary profession is being transformed as growing health care expenditures for canine, feline, and equine health reach levels previously deemed untenable. Even though these animals represent 25% of the total market, in 1999 alone, veterinary pharmaceutical sales increased almost 25% over the previous year.[1] Factors responsible for these profound changes include an altered perception of the human/animal bond (e.g. pets are perceived as “family members”), an aging

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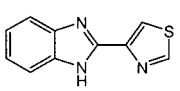
pet population, the emergence of new therapies for conditions that previously were poorly or under-treated as well as an increasingly affluent society. It is self-evident that increased animal longevity correlates with increasingly prevalent afflictions more commonly associated with the human geriatric population, particularly obesity and musculoskeletal disorders.

The current transformation in the veterinary field is not solely a recent phenomenon. In actuality, the seeds of change were quietly sown two decades previously with the discovery of ivermectin. The introduction of this first true broad-spectrum endectocide dramatically expanded the anthelmintic and insecticide markets for cattle and sheep and additionally created entirely new opportunities in companion animals for the control of systemic parasites such as heartworm; currently, the worldwide anthelmintic market exceeds $2.1 billion annually.[2] As a more recent example of the creation and expansion of new markets, in 1995 in the United States alone, spending reached $860 million for the control of fleas and ticks on companion animals using often highly variable and ineffective over-the-counter (OTC) remedies. Introduction of the superior prescription flea control agents (lufenuron, fipronil, and imidacloprid) increased this market to over $1.2 billion within 2 years while simultaneously capturing a significant percentage of the OTC spending.[2] Indeed, by 1996, lufenuron became the world's second best-selling animal health product, second only to ivermectin. Creative drug discovery efforts such as these which identify effective and innovative therapeutic agents can create and exploit untapped opportunities for new growth in veterinary pharmaceutical health care.

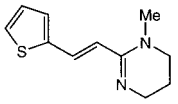
This Perspective is not intended to serve as an all-inclusive review of the field of animal health drug discovery but will highlight recent specific trends occurring in the industry and the nature of opportunities available for therapeutic intervention. Particular emphasis will center on canine, feline, and equine targets by focusing on several illustrative areas: treatments for endo- and ectoparasites, equine gastric ulceration, obesity, and antiinflammatory agents. Last, research directed toward the discovery of companion-animal drugs may not be readily separated from those efforts focused on food animals and may be discussed interchangeably.

Endectocides and Anthelmintics

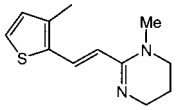
Helminths, including parasitic nematodes, cause significant health afflictions in both humans and animals, and treatment of diseases induced by these parasitic worms literally goes back thousands of years. Historically, therapeutic agents were primarily herbal concoctions of little or no demonstrable efficacy and frequently posed significant health risks to the host. Ideally, an anthelmintic agent should exhibit efficacy against multiple stages of the parasite's life cycle to minimize resistance potential, and for strategic reasons, anthelmintic agents tend to be used prophylactically. Evolution of contemporary drug discovery techniques for compound assessment ultimately has led to less toxic and more efficacious agents for controlling helminthic infections. For instance, thiabendazole (**1**), discovered in 1961, presaged the modern era of increasingly safe compounds with medium- to broad-spectrum antinematocidal activities.[3] Superseding thiabendazole was a host of increasingly potent anthelmintic agents including pyrantel (**2**), morantel (**3**), and levamisole (**4**), in addition to a large family of benzimidazole derivatives (exemplified by flubendazole (**5**) or albendazole (**6**)). While these



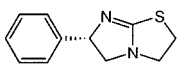
1: Thiabendazole



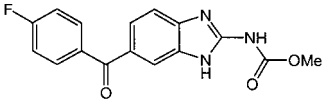
2: Pyrantel



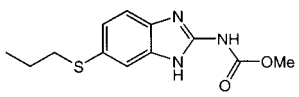
3: Morantel



4: Levamisole



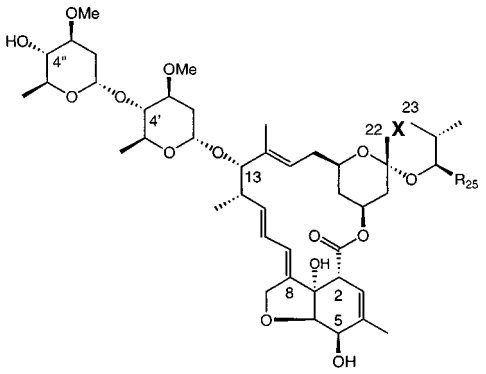
5: Flubendazole



6: Albendazole

anthelmintic agents were developed primarily for use in domestic animals, several found significant applications in human health as well and remain in use today. The benzimidazoles **1**, **5**, and **6** all share a common mechanism of action in which they selectively bind to β-tubulin, thereby inhibiting microtubule formation in the parasite's intestinal cells.[4] As a consequence, the nematodes are not able to absorb glucose and other nutrients efficiently and ultimately starve. Pyrantel, morentel, and levamisole, on the other hand, exert their parasite-specific toxic effects by modulating the ligand-gated nicotinic−acetylcholine ion channels.[5−7]

While the biological spectrum of these latter molecules (e.g. **5** and **6**) had expanded to encompass additional intestinal worms, profound deficiencies in the control of extraintestinal worms remained. Ongoing research to identify superior anthelmintic agents led to the discovery in 1976 by Merck researchers of a remarkable new class of macrocyclic lactones called the avermectins[8] (AVMs). Abamectin (**7**) was isolated from



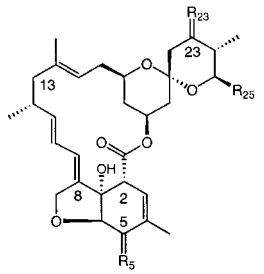
Entry	Name	R_{25}	C_{22} - C_{23}
7:	Avermectin B_{1a}/B_{1b}	*s*-Bu/*i*-Pr	double
8:	Ivermectin	*s*-Bu/*i*-Pr	single

Table 1

cattle	**sheep**	**humans**
Haemonchus	Haemonchus	*Onchocerca volvulus*
Trichostrongylus	Trichostrongylus	*Wucheraria bancrofti*
Oesophagastomum	Oesophagastomum	*Ascaris lumbricoides*
Ostertagia	Ostertagia	Strongyloides
Cooperia	Cooperia	Enterboius
Strongyloides	Strongyloides	Trichuris
Toxocara	Dictyocaulus	
Dictyocaulus	itch mites	**dogs**
cattle grubs	nasal bots	*Dirofilaria immitis*
lice		Toxocara
mites	**horses**	Ancylostoma
ticks	Parascaris	Strongyloides
screwworm larvae	Strongylus	Capillaria
	Dictyocaulus	Trichuris
swine	*Onchocerca cervicalis*	sarcoptic mange
Ascaris	bots	fleas
Trichuris	sarcoptic mange	
Strongyloides		**cats**
Metastrongyloids	**crop protection**	Toxocara
Stephanurus	>25 agricultural pests	Ancyclostoma

the fermentation extracts of the soil-dwelling *Streptomyces avermitilis* as a mixture of avermectin B_{1a}/B_{1b} (~85/15 *sec*-butyl/isopropyl). This natural product possessed dramatic anthelmintic activity and served as starting material for the preparation of ivermectin (IVM, **8**), a semisynthetic 22,23-dihydro analogue.[9] The introduction of IVM in 1981 as an antiparasitic drug ushered in a new era in the treatment of helminths. IVM's special advantage over conventional nematocidal agents such as **1**−**6** is its wide spectrum against not only gastrointestinal and systemic parasites but also ectoparasitic insects and mites at the unprecedented low dose of 200−300 μg/kg in cattle, sheep, swine, and horses (Table 1). Its unique potency against a broad spectrum of animal endo- *and* ectoparasites following a single parental administration led to the coining of the term 'endectocide' to describe these remarkable properties. IVM's worldwide acceptance in livestock production and companion animals has made it a major commercial success. Additionally, IVM has found application in preventing human onchocerciasis (river blindness), an insidious and intractable tropical disease. Up to 10 million people will receive IVM treatment to help prevent the affliction this year alone. While a single drug entity that has satisfactory activity against all nematodes has yet to be developed, the level of anthelmintic efficacy and low toxicity presented by these macrolides is remarkable.

The AVMs are structurally related to the milbemycins, which differ predominantly by the absence of the α-L-oleandrosyl-α-L-oleandrosyl disaccharide at position 13 and aliphatic substitutions at position 25. While milbemycins had been discovered prior to the AVMs in 1972 and their potent acaricidal and miticidal efficacy was known,[10] their anthelmintic properties were realized only after AVM's. As in the case of the milbemycins, AVM's biological activities are not restricted solely to animal health applications. Abamectin exhibits comparable efficacy against many agriculturally important mite and insect pests and was introduced as an agricultural pesticide in 1985. Ultimately, four milbemycin analogues were developed as anthelmintic agents, and these are shown below. Milbemycins **9**−**12** exhibit the characteristic biological activity profile of this class, although some improvements in spectrum were achieved.



Entry	Name	R_5	R_{23}	R_{25}
9:	Nemadectin	β-OH	H, α-OH	(Z)-C(Me)=CHCHMe$_2$
10:	Milbemycin oxime	=NHOH	H, H	Me/Et (7/3)
11:	Moxidectin	β-OH	=NHOMe	(Z)-C(Me)=CHCHMe$_2$
12:	Milbemycin D	β-OH	H, H	iPr

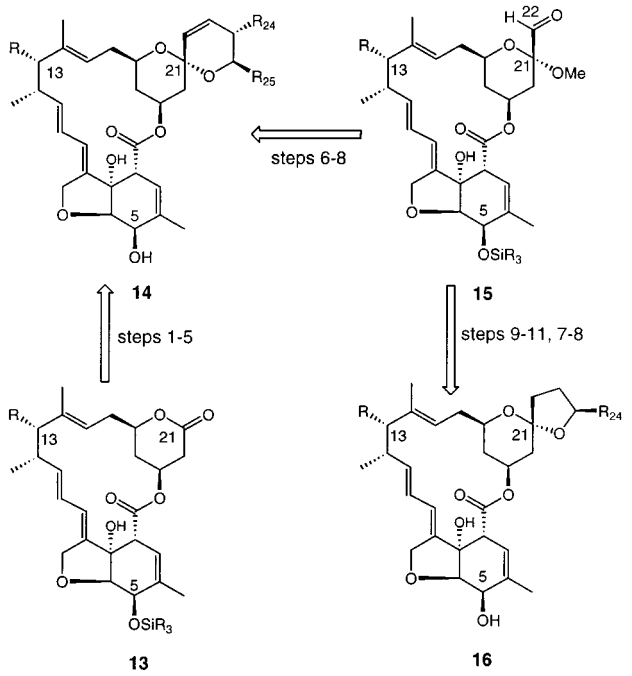
Heartworm is a serious and potentially fatal condition caused by the roundworm *Dirofilaria immitis* residing in the heart and major blood vessels of dogs and cats. Adult worms in the host produce microfilarial offspring that circulate in the blood and are transmitted to the next host via mosquitos. IVM and milbemycins **9**−**12** are unusually potent against heartworm microfilariae (larval stage) and are used for the monthly prevention of heartworm infection. Prevention of heartworm is important as limited options exist[11] for the treatment of adult heartworm due to potential for complications related to cardiac tissue damage.

Broad-spectrum activity as noted in production animals has not been observed in dogs using these macrolides, however. To date, no endectocidal activities in dogs have been noted at marketed levels for these anthelmintics, and insect, mite, and nematocidal claims remain relatively narrow. The primary reason for these decreased intestinal nematocidal activities derives from the observation that certain canine breeds (predominantly collies) were exquisitely sensitive to both AVMs and milbemycins. This toxicity is derived from deficiencies in the *p*-glycoprotein efflux mechanism leading to increased CNS concentration by a given AVM derivative. To ensure lack of toxicity in sensitive breeds, minimal dosages (6 μg/kg in dogs vs 200 μg/kg in cattle) were employed, thereby limiting efficacy and claims against additional parasites other than *D. immitis*. Consequently, neither IVM nor the milbemycins are approved for the treatment of gastrointestinal worms, fleas, ticks, or flies in dogs.

The complex molecular architecture and remarkable spectrum of bioactivities of these macrolactones stimulated intense scientific interest in diverse fields, and particular highlights will be noted. Perhaps one measure of the significance of the AVM class is the appearance of over 50 total (or formal) syntheses of various AVM or milbemycin derivatives in the scientific literature. Considerable resources were directed to the preparation of diverse, structurally modified analogues with enhanced biological activity profiles,[12] permitting synthetic access to virtually every site in the molecule.

As noted previously, different aliphatic substituents were well-tolerated at position 25 in natural milbemycins and AVMs. To extend this diversity further, novel C25-substituents were introduced synthetically by careful chemical degradation and reconstitution of the 6,6-spiroketal region (e.g. **13**[13] → **14** or **15**[14] → **14** in Scheme 1). In addition, the related, conformationally constrained

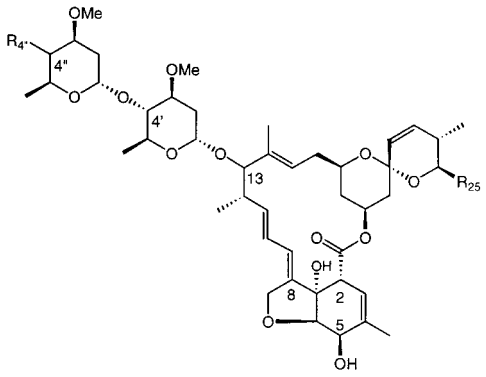
Scheme 1[a]



[a] Steps: (1) LiC≡CCHR$_{24}$CHR$_{25}$OSiR$_3$; (2) MeOH, TsOH; (3) Lindlar reduction; (4) PPTS, MeOH; (5) desilylate; (6) Ph$_3$P=CH-CHR$_{24}$CHR$_{25}$OSiMe$_3$; (7) PPTS, MeOH; (8) HF·pyridine; (9) (MeO)$_2$P(O)CH$_2$C(O)R$_{24}$; (10) Na$_2$S$_2$O$_3$, NaHCO$_3$, Adogen 464, 80 °C; (11) chiral reduction.

and potent ring-contracted 6,5-spiroketal derivatives **16** were generated.[15] Perhaps the most powerful and versatile approach, however, to unnatural spiroketal-modified AVM derivatives utilized directed fermentation.[16] Normally, AVM's C25-*sec*-butyl group was derived from leucine which was oxidatively degraded to (*S*)-2-methylbutyric acid and subsequently incorporated into the spiroketal.[17] A mutant *S. avermitilis* auxotroph lacking branched chain keto−acid dehydrogenase activity was generated by Pfizer scientists which, when fed exogenously added carboxylic acids, incorporated them at C25.[16] Unnatural AVM derivatives were produced containing substituents at C25 which were amenable to further chemical manipulation.[18] These efforts led to the discovery of doramectin (**17**) which differs from AVM by the presence of a cyclohexyl moiety at C25 in lieu of *sec*-butyl or isopropyl. The more lipophilic doramectin has a spectrum of biological activity profile comparable to that of IVM and moxidectin.[19]

Surprisingly small changes in the chemical structure of the AVMs often led to pronounced differences in physical and biological properties. For instance, conversion of AVM's terminal 4″-hydroxyl (**7**) into an *epi*-acetylamino function led to the identification of eprinomectin (**18**).[20−22] Eprinomectin exhibits superior anthelmintic activity relative to IVM (approximately 4-fold as judged by the dosage limiting parasite, *Cooperia oncophora*),[21,22] and unlike all the other AVM and milbemycins, it may be used in lactating dairy cattle with no withdrawal period as it does not partition into milk. Eprinomectin's improved safety profile is reflected in the zero time withholding periods for beef and milk in livestock, effectively eliminating both endoparasites and ectoparasites while lacking a slaughter withdrawal interval. Emamectin (**19**), on the other hand, which bears a 4″-*epi*-methylamino group,[20,23,24] is among the most potent agents yet identified for control of lepidoptera larvae[24,25] and has been developed for agricultural applications.



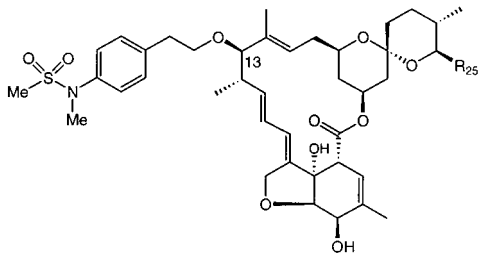
Entry	Name	$R_{4''}$	R_{25}	C_{13} - O
17:	Doramectin	α-OH	*c*-C$_6$H$_{11}$	α
18:	Eprinomectin	β-NHAc	*s*-Bu/*i*-Pr	α
19:	Emamectin	β-NHMe	*s*-Bu/*i*-Pr	α
20:	13-*epi*-Avermectin B$_{1a}$/B$_{1b}$	α-OH	*s*-Bu/*i*-Pr	β

Additional improvements to the AVM safety profile could be achieved without concomitant cost to anthelmintic efficacy. Conversion of **7** using a seven-step synthetic sequence (disaccharide removal, inversion of the pendent 13-hydroxyl followed by sugar reinstallation) yielded 13-*epi*-avermectin B$_{1a}$/B$_{1b}$ (**20**).[26] Remarkably, in vivo evaluation of **20** in sheep against *Haemonchus contortus*, *Ostertagia circumcincta*, *Trichostrongylus axei*, *Cooperia* spp., and *Oesophagostomum columbianum* indicated no loss of anthelmintic efficacy relative to the parent AVM **7**, while mouse safety studies established that a dramatic decrease in toxicity occurred (Table 2). The enhanced safety profile of **20** may permit its use in a broader range of applications.

Table 2. Acute Toxicity of AVMs in Mice[26]

entry	compound	LD_{50} (mg/kg)
7	avermectin B$_{1a}$/B$_{1b}$	19
20	13-*epi*-avermectin B$_{1a}$/B$_{1b}$	160

Sankyo workers reported[27] the preparation of a series of potent, 13-*epi*-modified milbemycin analogues which culminated in the identification of fuladectin **21**. They

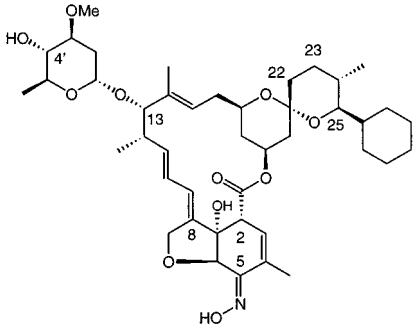


21: Fuladectin (R_{25} = Et/Me, 8/2)

noted that while 13-alkoxymilbemycin analogues may mimic the disaccharide moiety of AVM, synthetic challenges had limited the diversity of derivatives prepared. The C13-ethers were generated by *syn*-displacement of the corresponding C13-iodo analogue, and many of these new ethers were potent anthelmintic agents. Fuladectin,

in particular, when evaluated in vivo using an oral rat *Nipponstrongylus brasiliensis* model, was fully active at 125 μg/kg and equipotent to IVM.

A promising new entrant to the companion-animal market is the doramectin monosaccharide derivative, selamectin[28] (**22**). Selamectin is not only the first

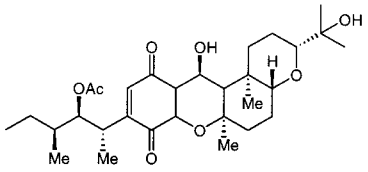


22: Selamectin

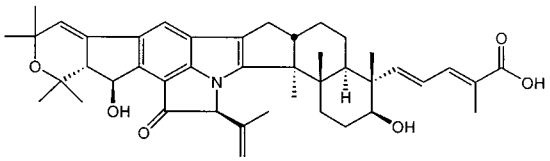
topically applied therapeutic agent approved for the prevention of heartworm disease but also the first AVM derivative to be granted flea and tick claims on dogs.[29] A single topical application of **22** to the base of an animal's neck conferred 1-month protection against adult fleas (>99% control at 30 days) and reduced the quantity of eggs and larvae produced. Selamectin's efficacy against fleas on dogs was determined to be comparable to that of fipronil (topical) and imidacloprid (topical) and somewhat superior to that of lufenuron/milbemycin (oral); similar results were obtained in cats (vide infra).[30] The efficacy of **22** against *Rhipicephalus sanguineus* and *Dermacentor variabilis* ticks is somewhat more modest, even when using a 5-day tick challenge protocol,[31,32] and ultimately, only claims for aid in the control of *D. variabilis* were granted by the FDA. Selamectin has additional claims for the control of ear mites (*Otodectes cynotis*) for both dogs and cats, treatment of sacrcoptic mange (*Sarcoptes scabiei*) in dogs, and treatment of roundworm (*Toxocara cati*) infections in cats.

Mode of Action and Receptor Isolation Studies

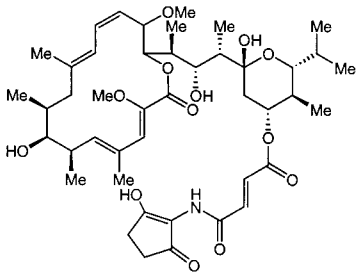
AVMs and milbemycins share the same mode of action, deriving their biological activity by modulating an invertebrate-specific chloride ion channel located on the plasma membranes of target neuronal cells[33−35] as well as in invertebrate muscle.[36] While the resultant chloride ion flux is presumed to induce paralysis and death to arthropods and nematodes, its exact mechanism of action initially was unclear.[37] In invertebrates, electrophysiological experiments showed that AVM enhances γ-aminobutyric acid (GABA)-mediated increases in membrane permeability to chloride ions.[38,39] AVMs also increase chloride ion permeability in systems that do not possess GABA receptors[36] and subsequently were demonstrated to modulate invertebrate-specific glutamate-gated anion channels.[37,40,41] This significant accomplishment was achieved by directly correlating activation of glutamate-sensitive chloride current, membrane binding, and biological activity of a series of AVM derivatives.[41] Interestingly, AVM's mode of action is not unique, as other structurally distinct ligands also have been discovered that are competitive inhibitors of AVM at the glutamate- and GABA-binding sites. These include cochlioquinone A (**23**, nematocidal glutamate-gated chloride channel modulator),[43] nodulisporic acid A (**24**, glutamate-gated chloride channel modulator that targets a subset (~50%) of the receptors modulated by IVM[44] with potent insecticidal activity),[45] and bafilomycin A_1 (**25**, GABA-releasing potency comparable to that of the AVMs).[46,47]



23: Cochlioquinone A

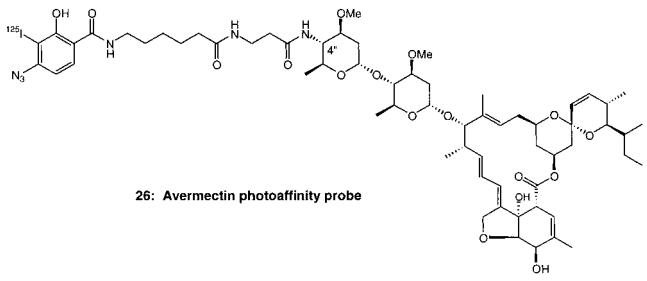


24: Nodulisporic Acid A



25: Bafilomycin A_1

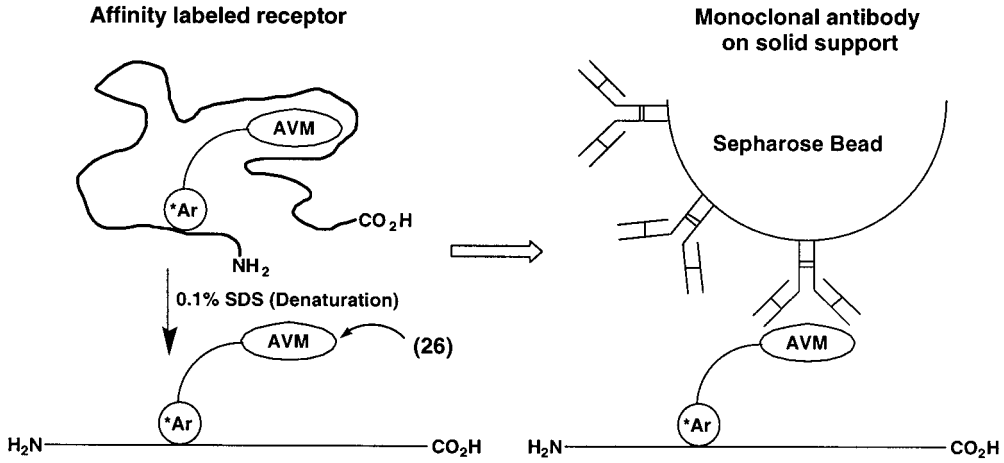
To further clarify the mechanism of AVM-modulated chloride ion transport, a series of AVM affinity probes were designed and synthesized to facilitate receptor identification, isolation, and structure determination.[48] Due to the paucity of AVM-binding proteins in the target tissue, early attempts at utilizing AVM affinity chromatography reagents failed to yield AVM-binding proteins present in detergent-solubilized *C. elegans* tissue[49] in a pure, biologically active form. This failure occurred despite their quantitative removal from solution, as determined by subsequent IVM-binding experiments with the eluant. Substitution of an ^{125}I-labeled AVM photoaffinity probe (**26**) in these experiments led



26: Avermectin photoaffinity probe

to the successful, specific labeling of three polypeptides with apparent molecular weights of 8, 47, and 53 kDa.[50]

Scheme 2



Again, extensive efforts to purify the cross-linked proteins to homogeneity proved unsuccessful due to the inherent technical difficulties associated with isolating large, lipophilic proteins, the minute amounts of tagged receptor available, and the significant quantities of contaminating proteins present.

This failure led to the development of a versatile, alternative protocol to obtain homogeneous, affinity-labeled AVM-binding proteins.[50,51] This receptor purification strategy is schematically illustrated in Scheme 2. Anti-AVM monoclonal antibodies were generated and conjugated to a solid support via their heavy chains. The photoaffinity cross-linking of **26** with *C. elegans*-derived proteins was repeated, and these labeled AVM-binding proteins were partially purified by size-exclusion chromatography. The tagged receptors were then incubated with the anti-AVM monoclonal beads, the solution was filtered to remove contaminating proteins, and then the receptor subsequently was eluted from the antibody. The utility of this approach in the purification of AVM-binding proteins is illustrated in Figure 1.

The recent advances in the control of parasitic worms is best-illustrated graphically. In Figure 2, typical use levels in sheep[3] for a given new chemotherapeutic at the time of its introduction are shown, providing further appreciation for the dramatic advance represented by the AVM/milbemycin structure class. For instance, for comparison purposes, the commercialized AVM analogues (IVM, abamectin, doramectin, and moxidectin) require identical use levels as subcutaneous injectables of 0.2 mg/kg for optimal efficacy. Additionally, it should be noted that this figure does not address the concurrent increase in the spectrum of parasite control that occurred. Currently, none of the distinct chemical classes previously described are used to control gastrointestinal nematodes in veterinary medicine nor are they ideally suited for all therapeutic situations. Each class has been challenged by the development of drug-resistant nematode strains.[52] Resistance to the AVM/milbemycin class of anthelmintic agents has appeared.[53−56] Given concerns associated with the inevitable evolutionary development of resistance, expansion of the anthelmintic arsenal therefore remains an urgent goal.

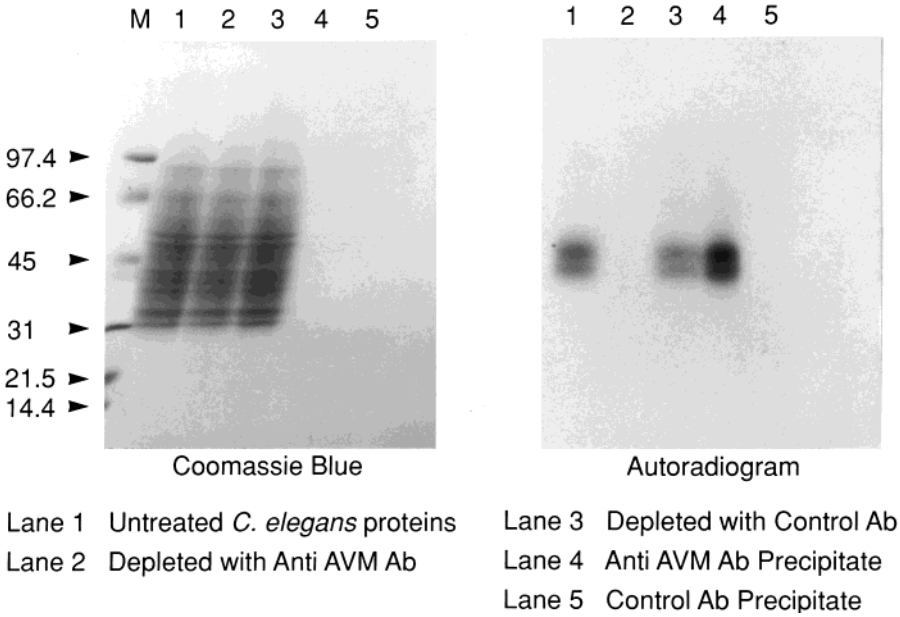


Figure 1. Immunoprecipitation of the *C. elegans* AVM-binding proteins with a monoclonal antibody to AVM.

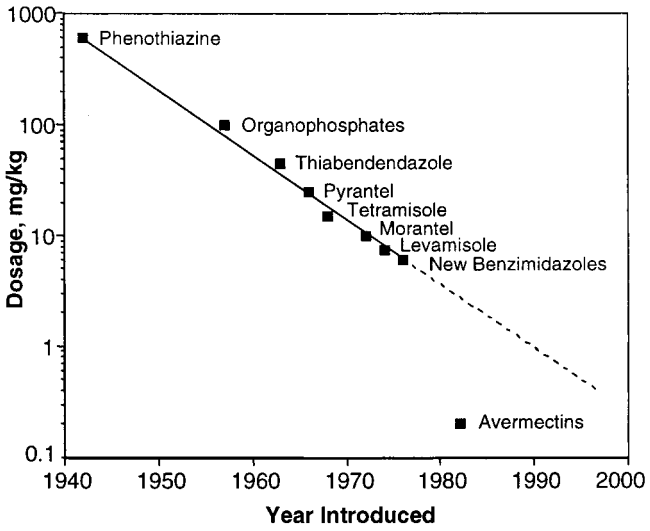
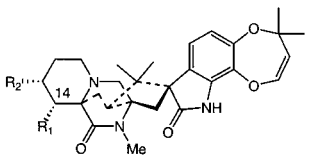


Figure 2. Anthelmintic agents through the years.

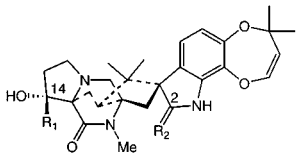
New Anthelmintic Agents

The broad-spectrum anthelmintic agent paraherquamide A (**30**, PHQ) is a toxic metabolite produced by *Penicillium paraherquei*.[57] PHQ is closely related to the previously identified oxindole marcfortine A (**27**)[58] and structurally distinct from the AVM class. Marcfortine and PHQ structural differences reside exclusively in the left-hand G ring (5- vs 6-membered, presence of hydroxyl group). PHQ exhibits potent antiparasitic activity in sheep,[59] controlling the adult stages of *H. contortus*, *O. circumcincta*, *T. colubriformis*, *T. axe*, and *C. curticei* following a single oral dose. PHQ, when evaluated in a cattle anthelmintic model against nine common gastrointestinal and lung nematodes, exhibited efficacy comparable to that obtained in sheep using single oral dosing. Among currently marketed products, only IVM is more potent, and no adverse reactions were noted for either cattle or sheep. Although PHQ is less potent than the AVMs, it still exhibits striking activity, approximately 5-fold more potent than levamisole (**4**) in vivo. Unfortunately, despite its high anthelmintic efficacy in ruminants, PHQ exhibits unexpected and significant toxicosis in canine models at dosages considerably lower than those that were safe in ruminants,[11,60] and the origin of these adverse effects remains poorly understood today. Given the species-specific toxicities noted for PHQ, additional structural refinement will be required to further exploit this class of molecules.

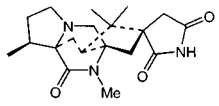
Pfizer workers recently reported the isolation and structure elucidation of aspergillimide (**33**, also known as asperparaline A) and VM54159 (**34**), which are new anthelmintic agents structurally related to PHQ.[61] While **34** was only modestly active, aspergillimide's in vivo anthelmintic potency and efficacy were superior to that of PHQ in rodent models. Sclerotiamide (**35**) also is closely related to PHQ and marcfortine and is uniquely hydroxylated on the central 5-membered ring.[62] While no anthelmintic efficacy has been reported for **35**, it possesses striking insecticidal and antifeedent properties.[62] Among the simplest members of this structural class yet discovered is brevianamide A (**36**). Both **27** and **30** contain an unusual dioxygenated 7-membered ring, while in brevianamide this ring is lacking and in aspergillimide the phenyl ring has also been excised.



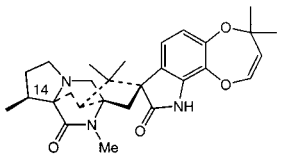
27: Marcfortine A (R_1 = R_2 = H)
28: R_1 = OH, R_2 = H
29: R_1 = OH, R_2 = Me



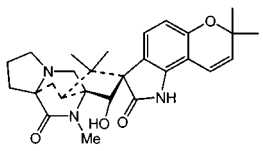
30: Paraherquamide A (R_1 = Me, R_2 = O)
31: R_1 = Et, R_2 = O
32: R_1 = Me, R_2 = H,H



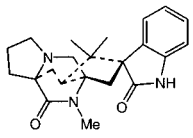
33: Aspergillimide



34: VM54159
(14-desoxy-paraherquamide)



35: Sclerotiamide



36: Brevianamide A

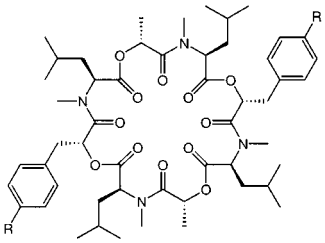
As with the previously described AVMs, the anthelmintic properties of these oxindole alkaloids stimulated much interest among medicinal chemists in their chemical modification. Unfortunately, however, synthetic alteration of these classes of molecules generally resulted in a loss of anthelmintic and ectoparasite potency, with few analogues possessing efficacy comparable to the parent PHQ and none significantly more potent. The effects of G-ring hydroxylation were probed by introducing an α-hydroxyl at C14 of marcfortine yielding **28**, which was equipotent to PHQ.[63] Similarly, incorporation of a methyl at C13 and a hydroxyl at C14 (e.g. **29**) yielded a biologically active derivative.[64] The *N*-oxide of PHQ also was reported to be equipotent to PHQ in vivo.[65] One early exception entailed the replacement of PHQ's C14 methyl group with an ethyl (**31**), leading to a 3-fold increase in in vitro efficacy using a *C. elegans* model.[66]

More recently, Lee et al.[67] employing a high-yielding four-step protocol (i, FmocCl; ii, $NaBH_4$; iii, piperidine; iv, $NaBH_4$) prepared 2-desoxoparaherquamide A (**32**) from **30**. This selective removal of the 2-oxo group not only increased intrinsic anthelmintic potency (2−4-fold) but more significantly, for the first time, divorced toxicity from efficacy. This comparatively minor structural modification led to remarkably decreased toxicity as no untoward effects were observed at 200 mg/kg in mouse models for **32** (vs LD_{50} < 15 mg/kg for **30**). Similarly, in dogs only mild and transitory mydriasis was noted at 25 mg/kg, in stark contrast to the lethality seen at 0.5 mg/kg for **30**.

It should be noted that while the *H. contortus* used in the PHQ sheep study (vide supra) was resistant to IVM and the *T. colubriformis* was resistant to both IVM and benzimidazoles, both remained sensitive to PHQ. Indeed, *H. contortus* isolates that are AVM-resistant were discovered to be up to 10-fold more sensitive to PHQ.[53] In addition, nematodes resistant to both thiabendazole and IVM remain sensitive to PHQ treatment in vitro.[59] Evidence of cross-resistance between levami-

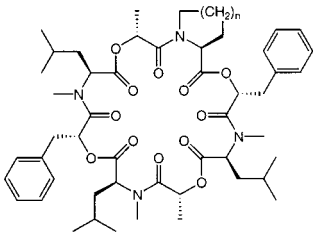
sole, the benzimidazoles, or IVM has not been reported, supplying strong evidence that they exert their biological effects by independent modes of action. PHQ's striking efficacy against resistant nematode strains suggests that it has a mode of action distinct from other major anthelmintic classes. While the mechanism of action of these new anthelmintic agents is poorly understood, they appear to share a common binding site with phenothiazines.[68] Interestingly, PHQ which also binds to the housefly nAChR with comparable affinity to imidacloprid, is a poor insecticidal agent and was shown electrophysiologically to function as a receptor antagonist.[69]

Recently, reports describing a new cyclooctadepsipeptide of fungal origin, PF1022A (**37**), with interesting

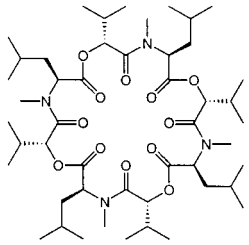


37: PF1022A (R = H)

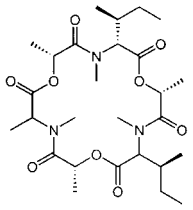
38: R =



39: n = 1 or 2



40: Bassianolide



41: Enniatin A

anthelmintic efficacy both in vitro and in vivo have begun to appear, largely in the patent literature. This 24-membered macrocycle was examined for intrinsic anthelmintic potency and breadth of spectrum. For example, **37** controlled *T. canis* and *T. cati* in dogs following oral dosing (200 μg/kg) and additionally controls *H. contortus* and *O. ostertagi* in cattle at doses comparable to commercial anthelmintic agents.[70] These depsipeptides are almost completely devoid of significant efficacy against arthropods,[71] and no adverse reactions to PF1022A have been noted in animals. Radiolabeling studies have established that PF1022A functions as a neurotoxin in nematodes, exerting its biological effects by modulating GABA receptors[72] in a manner distinct from the AVM class and unrelated to its ionophoric potential.[71] The lipophilicity of PF1022A restricts its utility as a parasiticide, as its parenteral coverage of all nematodes is incomplete; also it does not penetrate the blood−brain barrier.

Solid-phase synthesis techniques using Kaiser oxime resins ammenable for rapid or combinatorial chemistry were employed to rapidly synthesize PF1022A derivatives.[73] A variety of PF1022A analogues, including, among others,[74] the conformationally constrained prolyl and pipicolic derivatives **39** were prepared.[75] While active in sheep models, these analogues were generally less efficacious than the parent depsipeptide.[75] Replacing each of the four *N*-methyl groups of **37** with ethyl or propyl groups significantly increased the molecule's lipophilicity with a concomitant decrease in in vivo anthelmintic efficacy in sheep.[76] Similarly, systematic replacement of the four isoleucines of **37** with four valines or leucines led to a significant loss of anthelmintic efficacy. Incorporation of basic groups such as morpholino (**38**), however, at the *para*-position of PF1022A's phenylalanines did lead to significant improvements in observed in vivo anthelmintic activity. Orally dosed **38** was 15 times more active in a rat *N. brasiliensis* model than was PF1022A (ED_{95} = 0.63 mg/kg vs 10 mg/kg).[77,78] Also, PF1022A is structurally related to a series of other depsipeptides exhibiting modest anthelmintic activity including bassianolide (**40**), enniatin A[71] (**41**), and the cyclododecapeptides omphalotins (not shown).[79]

Peptidergic targets of helminth nervous systems are potentially promising targets for drug discovery for which resistance development is least likely to occur. These nematocidal regulatory neuropeptides have been discovered with increasing frequency in recent years[80] as the neurobiological field expands. Moreover, they may be distinguished pharmacologically from their mammalian counterparts, reducing potential toxicity issues. The discovery of small molecules that block or mimic their effects would confer a new pathway to control parasitic worms. Implementation of new, mechanism-based screens conceivably can circumvent certain limitations inherent in whole organism screening and has immense potential for the identification of novel anthelmintics.

One recent report describes the discovery of a series of small-molecule inhibitors of the *Ascaris suum* AF-2 neuropeptide receptor.[81] This neuropeptide is broadly distributed among nematode species and is one of the most abundant neuropeptides identified in *C. elegans*.[82] As polypeptides generally represent poor drug development candidates, initial screening efforts used radiolabeled AF-2 hexapeptide **42**. This work identified thiadiazole **43** as a weak, competitive inhibitor of AF-2. While the researchers were not able to replace the thiadiazole ring and retain potency, medicinal chemistry efforts did lead to the synthesis of **44** (10 nM), which was almost 300 times more potent than **43** (~3 μM). Unfortunately, however, **44** exhibited poor physiological activity on intact nematodes.

Lys-His-Glu-Tyr-Arg-Phe-NH_2

42

43: R_1 = H; R_2 = Cl
44: R_1 = CN; R_2 = CF_3

Ectoparasite Control for Companion Animals

Fleas and ticks are among the most common nuisance ectoparasites plaguing dogs and cats, particularly in climates with high relative humidity and high temperatures. In addition to causing blood loss anemia and

allergic dermatitis, these blood-sucking pests are a major vector for disease transmission in domestic animals, exceeded only by mosquitos. Ticks, for instance, transmit a diverse array of pathogenic organisms, including cat scratch disease (*Bartonella henselae*), Lyme disease, anaplasmosis, babesiosis, ehrlichiosis, and Rocky Mountain spotted fever.

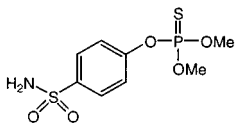
As with the anthelmintic agents previously described, a long and rich tradition existed for the treatment of companion-animal ectoparasites. The compounds traditionally used were applied topically to the skin of the host animal or were sprayed around the household environment and were modestly successful.[83] Historical treatments commonly included nonspecific toxins such as arsenicals, mercury-containing agents, tobacco extracts containing nicotine, or even a diverse array of liquid hydrocarbons. Virtually all the drugs described herein employed to control fleas and ticks on dogs and cats constitute a subset of agricultural chemicals. New classes of compounds were introduced for topical use, including various chlorinated hydrocarbons such as DDT, a substantial family of organophosphates and carbamates, synthetic pyrethroids, and insect growth regulators (IGRs). Traditional flea control products marketed included powders, sprays, shampoos, dips, collars, and premise sprays, but these commercial products were largely ineffective or exhibited highly variable efficacy. In addition, resistance was observed in flea populations to many of the older, topically applied pesticidal agents.[84] Ideally, an ectoparasiticide should satisfy the pet owner's expectations for a rapid and visible effect on flea infestation. In response to these market needs, new and more reliable treatments, including those that exhibit systemic activity following oral or parenteral dosing, recently have been developed. Unfortunately, no single entity that is orally active which controls fleas, ticks, and parasitic worms with a single monthly dose has yet been discovered. Recently, four new medicines (lufenuron, imidacloprid, fipronil, and selamectin) were introduced that are clearly superior to previous agents and provide monthly flea and/or tick control. These new substances are generally quite safe to dogs and cats.

Worldwide, there are approximately 1350 different species of fleas in the class Insecta, but the most common flea affecting companion animals is the cat flea, *Ctenocephalides felis*. Under ideal conditions after ingesting a blood meal, fleas either deposit eggs (up to 20 000 eggs in its lifetime) on the animal or in its surrounding environment.[85] From egg to larvae to adult takes 3−6 weeks, and these larvae literally can remain dormant for years under diverse conditions. To completely eradicate fleas it is essential that this cycle somehow be disrupted. Traditional *C. felis* bioassays functioned by direct *contact* wherein fleas were exposed to various compounds impregnated on some surface or on an animal. Recently, an artificial membrane-feeding system for fleas was developed.[86] This assay has facilitated the discovery of new compounds with *systemic* flea activity, which exploits the fact that the cat flea requires blood to complete all stages of its life cycle. This assay has also increased compound through-put, greatly reducing on-animal screening needs. In this assay, blood (typically bovine) containing varying concentrations of a given drug is placed in a small, thermally jacketed vessel. Next, a Parafilm membrane is stretched over this vessel which then is inverted and housed in a temperature- and humidity-controlled environment. Fleas pierce this membrane with their mouthparts and ingest treated blood. An additional advantage of this assay is that all fleas (both dead and alive) and their eggs may be readily recovered or counted. This provided not only mortality data but also information on all stages of the fleas' life cycle.

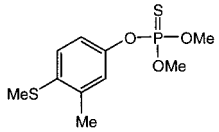
Unlike fleas, the approximately 850 known species of ticks are members of the class Arachnida (not Insecta), and not surprisingly, they often exhibit different sensitivities to pesticidal agents. Ticks may be further divided into two families: the Ixodidae or hard ticks and the Argasidae or soft ticks. Argasids have a soft, leathery cuticle, lack of scutum, and subterminal mouthparts that are localized on the underside of the tick. These ticks feed rapidly (in a matter of hours) and drop off the host when engorged. On the other hand, hard ticks are distinguished by a hard plate on their dorsal surface and terminal mouthparts. Prior to feeding, they secrete a cement that hardens and keeps the tick attached to the host. This attachment process typically takes a day at which juncture feeding subsequently commences. The total feeding time may be up to 6 additional days before the tick drops off its host. Common tick species include *Ixodes scapularis* (deer tick), *I. pacificus* (Western black-legged tick), *Rhipicephalus sanguineus* (Brown dog tick), *Amblyomma americanum* (Lone Star tick), and *Dermacentor variabilis* (American dog tick). Both hard and soft ticks may ingest up to 100 times their body weight in a blood meal, and they may need to be fully engorged before a systemic ectoparasiticide shows activity.

Current Flea and Tick Control Therapies

A number of organophosphates were developed for oral (e.g. cythioate, **45**) or topical (e.g. fenthion, **46**) flea control on dogs (not cats). Both **45** and **46** are systemically active acetylcholinesterase inhibitors (fenthion is absorbed into the animal's bloodstream), and lethality is achieved only following flea ingestion of canine blood. However, due to duration of efficacy, animal toxicity, and resistance issues, organophosphate use has largely been supplanted in recent years by pyrethroids.

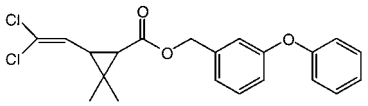


45: Cythioate

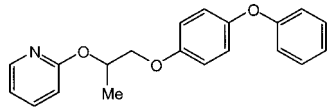


46: Fenthion

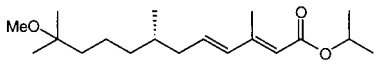
Pyrethroids, such as permethrin (**47**), are topically applied synthetic insecticides structurally related to pyrethrins and frequently see use in conjunction with IGRs. The IGRs pyriproxyfen (**48**) and (*S*)-methoprene (**49**) are synthetic mimics of an insect's natural endogenous juvenile hormone. They disrupt the transition of insect larvae development to pupae by artificially maintaining high hormonal levels[87,88] and may be used in conjunction with a flea adulticide such as the pyrethroid permethrin. In a highly concentrated form, topically applied permethrin, the most commonly used



47: Permethrin



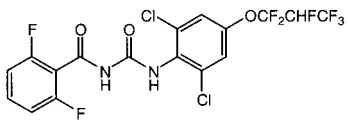
48: Pyriproxyfen



49: (S)-Methoprene

pyrethroid, may be used to control fleas and ticks on dogs for up to 2−3 weeks following a single application. These concentrations, typically 20−30 times those suitable for cats, were designed exclusively for the canine market and often carry warning labels indicating that secondary exposure to cats should be prevented.

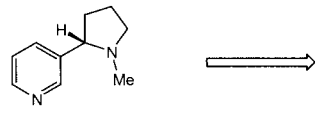
Lufenuron (**50**) is the first and only monthly oral agent available for the control of fleas, and its introduction in 1995 initiated a new method for companion-animal ectoparasite control. Lufenuron also is an IGR but with a mechanism of action distinct from that of **48** or **49**. Analogous to other structurally related benozylureas,[89,90] lufenuron inhibits chitin development, an integral protein required for maturation and function of the flea exoskeleton.[91] After oral dosing, **50** partitions into the animal's adipose tissue and then slowly diffuses into the bloodstream where fleas feeding on the treated host ingest the drug. Egg and larval developmental inhibition subsequently occurs because excreted flea feces are the major dietary component of their larvae and lufenuron is present in these feces.[90] With continued exposure to **50**, the population of fleas gradually diminishes over several months.[92] Lufenuron is typical of the IGR class and lacks direct toxic effects to the adult flea and also is devoid of tick efficacy. Consequently, the additional use of a "knock-down" spray, powder, or dip is required to reduce an existing adult flea infestation. In addition, effective use of **50** requires that all pets in a household be treated and fares poorly outside of a closed environment.



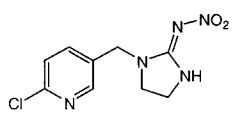
50: Lufenuron

Combination products and products with broad-spectrum potential are the next wave of flea control products for companion animals. Unlike topical agents, lufenuron has been combined with milbemycin oxime (**10**) for use as a once-a-month oral medication. In addition to the previously described flea control activity, the milbemycin in this combination confers protection against heartworm and roundworm (*Toxocara canis* and *T. leonina*), hookworm (*Ancyclostoma caninum*), and whipworm (*Trichuris vulpis*) infections. More recently, **50** was reformulated as a long-acting injectable, conferring 6-month protection against flea larval development following a single subcutaneous injection.

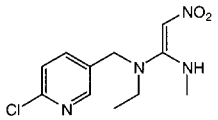
As noted previously, tobacco extracts containing nicotine (**51**) historically served as a pesticide when sprayed on crops. This highly potent and toxic alkaloid exerts its biological effects on insects by agonizing insect nicotinic acetylcholine receptors but lacks selectivity and has comparable activity on mammalian acetylcholine receptors. Structure−activity studies (SARs) led to the development of a new chemical class: neonicotinoids, of which imidacloprid (**52**) and nitenpyram (**53**) are members. Both **52** and **53** are insect-specific nicotinic−acetylcholine receptor agonists.[93]



51: Nicotine



52: Imidachloprid



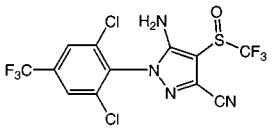
53: Nitenpyram

Nitenpyram has demonstrated fast-acting, adulticidal efficacy against fleas and currently is under investigation in combination with lufenuron as a "knock-down" agent. Preliminary studies indicated that dogs given a single oral dose of **53** were almost completely pest-free (99.6% of control) 4 h post-flea challenge.[94] The short half-life of **53**, however, precludes significant duration of action following oral administration. This was manifested when, in conjunction with a monthly dosing regimen of lufenuron, **53** was used "as needed" (but no more than once daily) to provide essentially complete control of fleas, not only those on the cat.[95] Comparable results were obtained in beagles using a monthly dose of **50** in conjunction with weekly doses of nitenpyram.[96]

Imidacloprid (**52**) was introduced in 1996 and confers full control of fleas (but lacks efficacy against ticks) on dogs and cats for 1 month after a single topical application.[97] Imidacloprid kills by contact, not by ingestion, so fleas do not require a bloodmeal to achieve lethal effects. Imidacloprid is rapid-acting and was shown to kill fleas within 20 min post-exposure.[98] The rapidity with which **52** kills fleas beneficially impacts the prevention of flea allergy dermatitis and alopecia.[99] Improvements in the control of fleas means that it is no longer necessary to treat the environment with pesticides to control fleas.

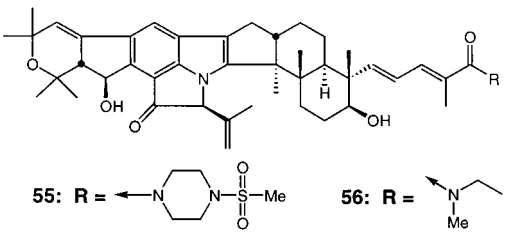
Fipronil (**54**), introduced in the United States in early 1996, is a long-acting topical flea/tick adulticide for dogs and cats that has rapidly attained the position as a world leader in sales for this market. An unusually potent member of the phenylpyrazole class of insecticides,[100] fipronil was until recently the only monthly product approved for companion animals that both is lethal to ticks and exhibits flea adulticidal activity. It remains the most potent product available for the treatment and control of ticks. In insects, ligand-gated

chloride channel blockers such as fipronil are very toxic proconvulsants. Mechanism of action studies demonstrated that **54** is a noncompetitive GABA antagonist in insects. Fipronil either acts by interacting with an allosteric binding site or by irreversible binding[100] and has a wide margin of safety because it exhibits little activity at the corresponding mammalian channel.[101]



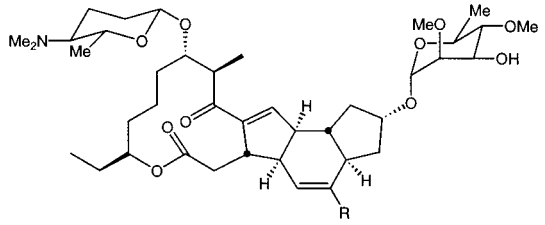
54: Fipronil

Evaluation of the indole diterpene nodulisporic acid A (**24**) using an artificial flea membrane-feeding apparatus[86] demonstrated that **24** killed fleas with a LC_{50} of 0.68 μg/mL and was approximately 10-fold more potent than the systemic insecticide IVM.[102] In a subsequent in vivo study,[102] dogs received a single oral dose of 15 mg/kg of **24** and were challenged repeatedly with 100 fleas at days 0, 4, 6, and 8. Nodulisporic acid exhibited potent systemic efficacy, reducing flea infestations by 99%, 97%, 51%, and 0% (relative to control) for the respective four flea challenges. It should be noted that no activity was noted against the nematode *A. caninum* in this study. Mechanistically, nodulisporic acid, as described previously, targets a subset of the receptors modulated by IVM. Unlike IVM, however, whose usage is restricted to minimal doses (6 μg/kg in dogs) to preclude toxicity, no mammalian toxicity for nodulisporic acid was observed in this investigation, despite the significantly higher dosage, consistent with its reported mechanism of action. Preliminary chemical modification of nodulisporic acid led to delineation of its pharmacophore as well as the identification of several derivatives, such as the nodulisporamide **55**, that exhibited enhanced flea efficacy (0.01 μg/mL).[103] Several additional nodulisporamide derivatives, typified by **56** (flea efficacy = 0.1 μg/mL), were evaluated in the dog flea model described above.[104] In dogs, **56** clearly was superior to **24**, exhibiting extended duration of efficacy (100%, 97%, 81%, and 47% flea lethality at days 12, 14, 16, and 18, respectively) following administration of a single oral dose of 15 mg/kg, again with no adverse effects noted.[104]



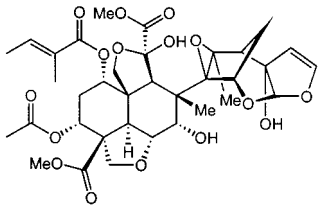
55: R = **56: R =**

The spinosyns are a recently discovered class of highly potent natural insecticidal agents produced by the soil microorganism *Saccharopolyspora spinosa* and first reported by Lilly scientists in 1989.[105] Spinosad (an 85/15 mixture of spinosyns A (**57**) and D (**58**)) demonstrates rapid contact and ingestion activity in insects which is unusual for a biological product.[106] The mode of action of spinosad is characterized by excitation of the insect nervous system, leading to involuntary muscle contractions and paralysis. These effects are consistent with the activation of nicotinic acetylcholine receptors[107] by a unique mechanism,[108] although the possibility of intracellular calcium release induced by spinosad may not be excluded. Known insect control products such as imidacloprid and other neonicotinoid receptor-based insecticides act at a different site,[69,107] and cross-resistance concerns appear minimal.[108] This macrolide also has effects on GABA receptor function that may contribute further to its insecticidal activity. To date, no activity of spinosad against fleas or ticks has been reported.



57: Spinosyn A (R = H)
58: Spinosyn D (R = Me)

For centuries, extracts from the seeds of the neem tree *Azadirachta indica* had multiple applications in India. Only in the 1960s was the most potent insecticidal component of these extracts fractionated and characterized.[109] The primary fermentation congener, a structurally complex tetranortriterpenoid natural product named azadirachtin (**59**), resisted structural elucidation until 1985.[110] While azadirachtin's mode of action is complex, it exhibits minimal nontarget toxicities. Its primary biological effects are derived from potent antifeedent properties[108] and IGR inhibitory activities in diverse insect species.[112] Despite numerous claims of ectoparasite efficacy in vitro, reports describing in vivo evaluations of azadirachtin against *C. felis* on either dogs or cats are sparse. However, a single topical application of a methanolic solution containing 1000 ppm azadirachtin eliminated fleas on cats completely for 1 week and up to 53−93% reduction relative to control after 19 days.[113] The activity of **59** against ticks is relatively modest,[114] as was its in vitro activity against *D. immitis*.[115] Simplification of the structure of **59** generally has resulted in pronounced decreases in insecticidal efficacy.



59: Azadirachtin

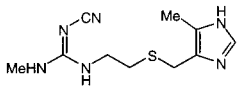
Equine Gastric Ulcers

Recognition that a substantial percentage of performance horses are prone to severe gastric ulceration is a relatively new finding. While nonperforating gastric ulcers were known to occur in horses of all ages, until recently these clinical syndromes were deemed insignificant when detected upon necropsy. Horses suffering from gastric ulcerations commonly appear healthy. If clinical signs of the disease are manifested, they are

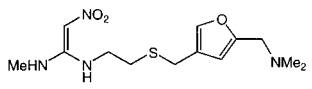
similar to those seen in medical colic. Indeed, not until 1986 had the extent of equine gastric ulceration begun to be fully appreciated. In a seminal study of 195 thoroughbred racehorses in Hong Kong,[116] routine postmortem examinations over a 2-year interval demonstrated that 66% had ulceration of the stomach. It was found that active training exacerbated the frequency of ulcer formation. Among horses euthanized after active training, the incidence of lesions was 80%, whereas retired horses had a lower frequency (52%). Furthermore, training exacerbated not only the frequency but also the severity of these lesions. Stomach lesions found in half these horses had severe gastric lesions versus only 5% in recently retired horses. Age differences also were found to exist. For instance, among retired horses the incidence of ulceration remained low between old and young horses, while for horses under active training, older horses showed the greatest increases in ulceration severity.[116] Confirmatory studies of equine gastric ulceration prevalence were greatly facilitated using long endoscopes (2 m in length is required), permitting in vivo examination of equine stomachs. In one study of 100 putatively healthy animals examined, 52 had gastric ulcers.[114] In another study of 111 horses with a history of depressed appetite, poor overall condition, and general abdominal discomfort, 81% had ulceration.[118]

Performance horses are those animals in vigorous competitive training, particularly racing. Horses having gastric ulcers train less effectively, and exercise regimens frequently are disrupted to permit this chronic inflammatory condition of the stomach and duodenum to heal.[111−113,116,118] While the etiology of equine gastric ulceration is multifactorial,[119] undoubtedly dietary factors as well as physical and psychological stresses (which are well-established contributory factors in the pathology of ulcers in humans) are similarly implicated in horses.[116] Diet is a particular culprit as these animals are fed grains and pelleted food concentrates which can stimulate gastrin secretion and ultimately sharply increase acid production in the stomach. Interestingly, unlike in humans, the pathogen *Helicobactor pylori* has not been observed in equine stomachs to date and currently is not associated with gastric ulceration in performance horses.[120]

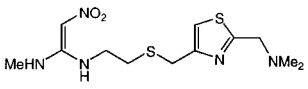
In horses, as in humans, secretion of hydrochloric acid is controlled via a H^+/K^+ ATPase pump in parietal cells,[120] and control of this acid secretion may be accomplished in several different ways. As treatments for equine ulceration were drawn extensively from research efforts directed toward human health, many protocols developed for ulcer treatments in humans were replicated in horses. These therapeutic strategies included the use of histamine H_2 receptor antagonists[121−124] (i.e. **60**−**64**) to decrease acid secretion, diverse antacids[124] (including $Al(OH)_3$, $Ca(OH)_2$, and $Mg(OH)_2$) to neutralize stomach acid, agents to protect the mucosal lining of the stomach (e.g. PGE analogues), and intravenous treatment using the proton pump inhibitor omeprazole[124,125] (**65**) to halt acid production. Significant limitations existed for each of these therapeutic protocols, not the least being that they required multiple dosing regimens due to their short duration of action, typically between two and six times daily. Often



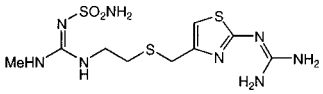
60: Cimetidine



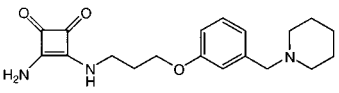
61: Ranitidine



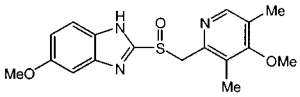
62: Nizatidine



63: Famotidine



64: BMY-25368



65: Omeprazole

these treatments only ameliorated the ulceration and were not curative due to incomplete control of acid secretion over time. As an example, treatment using either **60** or **61** three times daily for 3 weeks effectively controlled ulceration and promoted healing.[122] However, reduction of this dosing regimen to twice daily failed to maintain sufficiently low acid secretion levels. Consequently, this treatment strategy was not viable as only modest alterations in the lesions were apparent upon visual endoscopic examination.[122]

On the other hand, intravenous administration of a single dose of omeprazole significantly suppressed free gastric acid (>90%) for up to 7 h.[124] Omeprazole is known to exert its beneficial biological effects by irreversibly binding to the H^+/K^+ ATPase pump in gastric parietal cells.[126] Increases in the gastric acid levels occur only after the production of additional quantities of H^+/K^+ ATPase by the parietal cells, leading to superior control of acid secretion and longer duration of action. Intravenous administration of omeprazole was used in horses in recognition of the acid lability of **65** in the stomach following oral dosing. For human health applications, this problem was surmounted by encapsulating omeprazole with an acid-stable coating for protection during its passage through the stomach. This coating is unstable to base and efficiently degrades upon reaching the small intestine, releasing **65** intact. Consequently, the antisecretory effects using an acid-stable suspension of encapsulated **65** (1.5 mg/kg) in horses using once-a-day dosing following nasogastric administration were probed. This study[127] unambiguously demonstrated the curative properties of omeprazole, as the *entire* stomachs of treated horses were completely healed between 10 and 21 days. These results are superior to those obtained when applying other human health gastric ulcer treatment protocols to horses. To obviate the requirement for nasogastric drug administration, further formulation improvements were undertaken. These efforts led to the development of an oral paste suspension of omeprazole.[128] This suspension conferred sufficient protection of **65** during its transit through the acidic environment of the equine stomach such that the sufficient active ingredient remained to control gastric acid secretion and promote ulcer healing. No significant efficacy differences were noted between nasogastric and oral dosing protocols. For the first time,

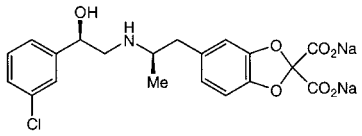
a convenient, easily administered, once-a-day oral dosing regimen using a proton pump inhibitor to heal gastroduodenal ulcerations in horses had been developed.

Canine and Feline Obesity

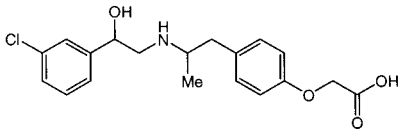
Veterinarians estimate that obesity affects between 25% and 50% of the canine and feline population (obesity is defined as >20% overweight).[129−131] In animals as in humans, obesity is an imbalance of caloric intake versus energy expenditure. While genetic and hypothalamic disorders are known to contribute to obese companion animals, diet-induced obesity is the most common reason for obesity and is exacerbated by the sedentary lifestyles and feeding habits of owners.[132] Often, this obesity derives from ad libitum feeding of high-fat diets, and controlling the weight loss has proved as challenging in companion animals as in humans. These risk factors are also negatively influenced by increased age and neutering. Also, obesity has additional negative ramifications and contributes to the pathogenesis of other significant disease states. Observed afflictions include hypertension, hyperinsulinemia, insulin resistance, increased incidence of hepatic disorders, cardiovascular diseases, musculoskeletal problems including osteoarthritis, diminished resistance to disease due to compromised immune function, and poorer overall health.[130,131,133] While obesity can be treated by a low-fat diet, reduced caloric intake by the animal, and increased exercise, owner compliance with these treatments and behavior modification of the animal frequently are poor and recidivism is high. As a consequence, opportunities for pharmaceutical intervention (obesity therapy) to manage feline and canine diet-induced obesity exist as even modest decreases in animal weight can have a substantial beneficial impact on its overall health.

A revolution in the understanding and treatment of obesity in humans is currently underway in laboratories around the world as new biochemical targets are being discovered with increasing frequency.[134] Potential anti-obesity targets include those which modulate satiety factors, agents which either increase lipolysis or decrease adipose tissue formation, and inhibitors of food absorption. Compounds that act on these new targets in vivo in various different species are beginning to appear in the scientific literature.

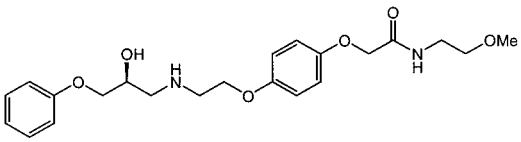
As one particularly promising example for therapeutic intervention, biochemical investigations have established that the β3-adrenergic receptor exists predominantly on adipocytes and stimulation of this receptor is anticipated to increase lipidolysis.[135,136] Also, adrenergic receptors on dog fat cells were shown to be structurally similar to those of humans.[137] In separate studies, beagles infused intravenously with selective β3-agonists **66**−**68** showed increased levels of free fatty acids for several hours.[138,139] Chronic oral dosing of BRL26830A (**69**) induced weight loss in obese dogs without a commensurate decrease in food intake.[140] The beneficial changes in girth and weight observed were due exclusively to lipid reduction as lean tissue was unaffected. These intriguing results indicate that agonists of this receptor have promise for in vivo lipolysis and diverse analogues have been reported.



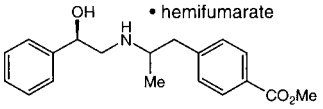
66: CL316,243



67: BRL 37344



68: ICI D7114



69: BRL 26830A

Nonsteroidal Antiinflammatory Agents

As companion animals age, diseases such as osteoarthritis have become more prevalent. Indeed, it is estimated that as many as 20% of the close to 53 million dogs in the United States suffer to some degree from arthritis, and as noted previously, these conditions are exacerbated by obesity. Equine osteoarthritis is equally widespread as athletic horses are particularly at risk as a natural consequence of both aging and rigorous training. Additional factors leading to inflamed joints and arthritic symptoms in animals include congenital defects (e.g. hip dysplasia in dogs) or joint damage from accidents. Degenerative joint disorders are a major source of chronic pain and/or disability for these animals and are increasingly recognized by veterinarians. While a goal of osteoarthritis therapy is to provide palliative control of pain, reduce inflammation, increase animal mobility, and prevent continued damage to the joint, pain control therapy remains suboptimal. This is particularly true in neuropathic pain deriving from joint disease where veterinary therapeutic use has been limited due to medicines with undesirable side effect profiles, leading to an unmet medical need and significant opportunities.

While aspirin has been used for over 100 years as a nonsteroidal antiinflammatory drug (NSAID), interest in NSAIDs has dramatically increased in the past two decades.[141] This interest has led to recent significant advances regarding the underlying pathogenesis of osteoarthritis and its treatment. Potential drug targets for osteoarthritis may be divided into two broad classes: those which modulate disease progression (i.e. corticosteroids) and those which function symptomatically (i.e. NSAIDs). All NSAIDs share a common mechanism of action and are effective for the treatment of inflammatory conditions in animals and will be the focus here. Also, each of the medicines described here for

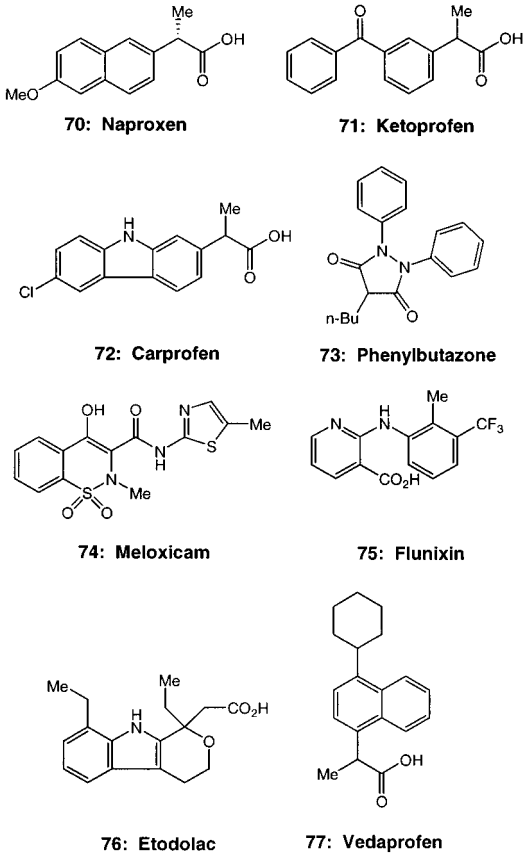
veterinary care originally saw application in human health for the treatment of arthritis pain. In veterinary medicine, the most common indication for these nonnarcotic analgesics is the treatment of degenerative joint disease causing mild to severe pain. Unfortunately, with increased usage of NSAIDs in veterinary care, a concomitant increase in NSAID-related toxicities has been noted.

While over 60 NSAIDs have been approved for use in humans, extrapolation of their use to animals is dangerous, and thus a much more constrained number have been approved for veterinary usage. Many NSAIDs share similar toxicities,[142,143] particularly ulceration of gastric or intestinal tissues and nephrotoxicity. Also, significant species to species differences with regard to NSAID efficacy and toxicity profiles exist as feline, canine, and equine susceptibility to the deleterious consequences of NSAID usage appears greater than that for humans.[144] For instance, while aspirin is the only NSAID currently recommended for treatment of OA in cats,[145] severe ulceration and renal toxicity in some cats still occur. Similarly, stomach ulceration and/or gastrointestinal bleeding was observed in a fair percentage of dogs, even when treated with buffered aspirin. Both cats and dogs have a well-recognized sensitivity to ibuprofen; this toxicity in felines derives from deficiencies in glucuronyl transferase enzyme levels. Indomethacin is contraindicated for canine veterinary care as very low dosages may be toxic. Needless to say, particular emphasis currently is being placed on the discovery of increasingly selective and safe NSAIDs for animal veterinary care.

It is now well-established that NSAIDs function primarily by inhibiting the enzyme cyclooxygenase (COX). NSAID treatment blocks conversion of arachidonic acid to eicosanoids, thereby suppressing prostaglandin formation and ultimately disrupting the inflammatory cascade. It is through this activity that NSAIDs derive their antiinflammatory, antipyretic, and analgesic activity. It took the recent discovery, however, of a second COX isoform[146] to regalvanize interest in this area.

Inducible cyclooxygenase (COX-2) is expressed during inflammation by inflammatory cells, while COX-1 is constitutively expressed in the stomach, platelets, kidney, and endothelial cells. COX-1 functions include modulation of renal blood flow and synthesis of the gastric mucosa; as a consequence, differential inhibition of COX-1 is associated with the commonly observed gastric and renal toxicities, with greater COX-2 selectivity leading to decreased risk of gastrointestinal insult. As currently utilized NSAIDs differ in their inhibitory potencies on COX-1 and COX-2, their antiinflammatory, antipyretic, and analgesic activities as well as their toxicities will differ. Inhibition of the arachidonic acid inflammatory cascade with highly selective COX-2 inhibitors could lead to more efficacious and safe veterinary therapeutics.

Several potent and effective NSAIDs (**70**−**77**) have been introduced for the treatment of canine and equine osteoarthritis in recent years. While several of these have been approved in Europe and Canada for feline applications, none have yet received approval in the United States. Of these new veterinary therapeutics, carprofen (**72**) is among the most selective COX-2 inhibitor (129-fold) against cell-free canine COX (COX-2 = 0.102 μM vs COX-1 = 13.2 μM).[147] In contrast, while potent, flunixin, etodolac, and ketoprofen are nonselective inhibitors of canine COX-2 and meloxicam exhibits modest selectivity (2.9-fold).[145,147,148] Phenylbutazone (**73**), while a less potent COX-2 inhibitor (3.79 μM), is at least 3-fold selective. It should be noted that COX isoform selectivity ratios vary greatly depending on the tissue source and assay type (pure enzymes, cell homogenates, or intact cells) used.[149,150] Carprofen's selectivity, for instance, drops from 129- to 15-fold using canine whole blood[151] and to one-to-one using murine or bovine cells.[150]



Orally administered **72** (F = ~90%) shows efficacy comparable to indomethacin in animal studies,[152] while renal failure and gastric ulceration, which are among the primary risks of NSAID use, have not been prominent problems. In horses, **73**−**75** are among the most commonly utilized NSAIDs. Vedaprofen (**77**) was recently introduced to the equine market, and while comparable in efficacy to phenylbutazone or flunixin, it may be safely used for extended periods (14 vs 5 days). In addition, certain NSAIDs may have additional beneficial properties, including inhibitory activity against either lipoxygenase (ketoprofen)[153] or phospholipase A_2 (carprofen).

Conclusions and Future Directions

Animal health drug discovery remains vibrant despite the upheaval currently underway. Historically, a substantial proportion of veterinary medicines originated as peripheral benefits of ongoing human health research

programs carried out in the laboratories of pharmaceutical companies, leading to registration of successful human health drugs for veterinary applications by exploiting physiological similarities between humans and animals. While antibiotics have long been used to treat bacterial infections in animals, overall comparatively few other human health drugs were available specifically for animals even though they suffer from similar afflictions, including, for example, hypertension, gastric ulceration, arthritis, diabetes, cancer, obesity, and pain. This apparent disconnect existed largely because animal health research was traditionally directed toward therapeutics for food animals, with particular emphasis on improving yields. The focus of the more recently created companion-animal health care market, however, is unambiguously distinct from the agricultural animal market.

While the animal health drug discovery research paradigm remains largely unchanged due in no small part to economic constraints, in recent years the pace of information transfer from the human health research arena has accelerated. This transformation occurred concurrently with commensurate dedication of substantial resources to specifically search for therapeutic agents for the corresponding companion-animal health applications. The expansion of animal health drug discovery research efforts directly resulted from enhanced appreciation for the value of new opportunities in previously underserved sectors of the veterinary market. Scientific advances in basic and/or applied research have been leveraged successfully to identify species-specific animal health drugs with improved therapeutic profiles and decreased adverse effects. The application of these innovative technologies and the development of new biological targets where previously only marginal or ineffective treatments existed led to the creation of entirely new markets for animal health drugs. Equally significant, evaluation of potential veterinary therapeutic agents in the target species occurs at the earliest stages in the drug discovery process, increasing the probability of success.

Additional factors integral to the renewed appreciation of animal health opportunities derived from the evolving perception of the human/animal bond, which has had profound impact on veterinary practices in our increasingly affluent society. Specifically, whereas medication of food animals is principally an economic investment, treatment of companion animals takes place for emotional reasons, suggesting that additional, largely untapped markets were waiting to be satisfied.

To more fully appreciate the current state of modern animal health science, a thorough appreciation of how the introduction of novel veterinary therapeutic agents created completely new markets is critical; and as previously noted, the genesis of these discoveries historically derived from early research efforts focusing on food animals. As an example, coccidiosis (a protozoan disease of chickens not discussed in this review) would not permit poultry to be raised in close confinement prior to the introduction of sulfaquinoxaline 50 years ago. The advent of superior anticoccidial agents subsequently altered the broiler industry to the extent that chicken is no longer a luxury but an inexpensive commodity today, and current animal husbandry practice requires that most chickens be medicated prophylactically. Similarly, worm infections of food animals (such as sheep, cattle, and pigs) were essentially uncontrolled until the modern anthelmintic age began with the introduction of thiabendazole in the 1960s. This discovery revolutionized the meat industry much as sulfaquinoxaline's discovery changed the poultry industry, and its success stimulated efforts to identify additional anthelmintic agents, culminating in the discovery of the AVMs.

The discovery of the AVMs in 1976 and the introduction of IVM in 1982 presaged a new era in the treatment of animal endo- and ectoparasites. These new endectocides vastly expanded the anthelmintic and ectoparasitic markets, and indeed, to this day, IVM remains the most successful animal health drug yet discovered. Its development also was coupled to significant basic science advances, including the recognition of IVM's activity against filarial worm infections in dogs and in humans. IVM now has been used successfully to prevent river blindness in many millions of people in equatorial Africa. The observation that several members of the AVM/milbemycin structure class could prevent canine heartworm infections and their successful commercialization contributed to the current shift in emphasis in animal health drugs from food animals to companion animals.

As evidence mounts that resistance to the AVM/milbemycin class has begun to appear, the identification of next-generation anthelmintic agents that possess their exquisite potency yet work by a different mechanism of action and lack cross-resistance will become paramount. Examples of compounds which exhibit considerable promise include the potent, broad-spectrum agent PHQ, one member of a large class of natural products. Interestingly, while toxic to dogs at very low doses, PHQ exhibits antiparasitic efficacy in sheep and cattle yet lacks apparent toxicity. Equally significant, PHQ retains full activity against IVM- and benzimidazole-resistant helminth strains. More recently, the discovery of 2-desoxoparaherquamide A with improved in vivo efficacy suggests that in this class toxicity may be effectively separated from anthelmintic activity. Other anthelmintic agents that exhibit promise include the cyclodepsipeptides. These compounds are comparable in potency to IVM, also lack overt mammalian toxicity, and may serve as effective starting points for the identification of small-molecule mimics. Additional effort to more rigorously characterize the mechanisms of action of PHQ and the depsipeptides will be critical to medicinal chemistry efforts to exploit these structure classes. Newer approaches for helminth control also will likely encompass the development of ligands which selectively target a variety of neuropeptide receptors, for which resistance is deemed unlikely. While preliminary reports indeed have described early successes in this area, research efforts in this arena remain in their infancy.

The introduction of the orally active IGR, lufenuron, as once-a-month treatment for flea control in dogs exposed the enormous and previously untapped potential for the control of ectoparasites in companion animals. Lufenuron increased the already substantial market for flea control by over one-third in a mere 2

years. This innovative product preceded the development of three topically applied monthly products with expanded activity profiles and diverse mechanisms of action that further capitalized on these newly created opportunities. Fipronil, for instance, also controls tick infestations, while selamectin's efficacy extends to the control of internal parasites as well. While the advent of these newer agents with ectoparasite adulticidal activity have largely supplanted lufenuron, the early commercial success of lufenuron illustrated the market potential of an orally active compound and provided the motivation for the development of oral combination products with cidal activity (lufenuron/nitenpyram) or anthelmintic efficacy (lufenuron/milbemycin oxime). In addition, the more recent discovery of nodulisporamide derivatives possessing ectoparasite adulticidal activity demonstrated that the identification of systemically active agents exhibiting extended duration of action while devoid of mammalian toxicity is viable.

Analgesics that effectively control chronic pain from mechanical injury or joint disease in cats, dogs, and horses represent a very large potential market. As the toxicity of aspirin and nonselective NSAIDs in companion animals has long been recognized, the possibility existed that selective COX-2 inhibitors represented a considerable opportunity. Animal health researchers indeed successfully exploited basic science research advances in the field of selective COX-2 inhibitors, leading to the introduction of numerous new therapeutic agents. While these newly identified NSAIDs with a range of selectivities offer the promise of improved treatments of pain for animals, continued efforts in this area will undoubtedly yield superior medications. It should be noted that suitable treatment for neuropathic pain in animals remains a condition awaiting a solution, and it is conceivable that current research in the mechanisms of pain signal transduction will result in new animal health therapies.

Obesity is an affliction that is as common in dogs and cats as it is in their owners, and diet restriction alone fares as poorly as recidivism is high. Obesity research is a very active area in human health, and some of the recently identified compounds in this field show particular promise for canine weight control, an area currently devoid of effective therapies. Need exists in this field for compounds to reduce appetite as well as for medicines that increase thermogenesis. A combination of both treatments will most likely be necessary to regulate obesity, since as diet is restricted, metabolic rate tends to decrease. Reversal of obesity has additional beneficial ramifications as it frequently alleviates commonly associated disorders (typified by diabetes) that are commonly seen in older, obese animals.

The future of animal health drug discovery is now heavily focused on drugs for the well being of companion animals, principally dogs, cats, and horses, but also includes much off-label use in other species, even extending to exotic and zoo animals. As in human health, many areas of animal health remain underserved due to inadequate therapies, and only by the introduction of new and innovative products which capitalize on advances in basic and applied science will animal health companies survive and the well being of their clients be improved. Little doubt remains that recent technological advances (i.e. genomics/proteomics/vaccine development) will exert a profound impact on the development of new treatments for human disease. The requirement that all new human health therapeutic agents must first be evaluated in animals for safety and efficacy ensures that there will be commensurate effects on the field of animal health, providing additional opportunities for the identification of new veterinary medicines. Finally, it remains to be noted that while opportunities for the treatment of disease of companion animals remain abundant, the older targets of parasitic infestations in both food and companion animals should not be neglected, as resistance will unavoidably develop to all of the current antiparasitic drugs.

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