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Design and synthesis of sarolaner, a novel, once-a-month, oral isoxazoline for the control of fleas and ticks on dogs

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ABSTRACT

Over the last decade, the isoxazoline motif has become the intense focus of crop protection and animal health companies in their search for novel pesticides and ectoparasiticides. Herein we report the discovery of sarolaner, a proprietary, optimized-for-animal health use isoxazoline, for once-a-month oral treatment of flea and tick infestation on dogs.

The companion animal parasiticide market represents a major and rapidly growing segment in the animal health-industry, with an estimated value of approximately US $4.3 billion in 2013 (Source: Vetnosis). Ectoparasiticides, which control flea, tick, lice and mite infestations, are important to pet owners since they not only protect their pets against the direct impact of these parasites, but also may help to prevent the transmission of diseases by these parasites (*e.g.*, lyme disease).[1] The introduction of fipronil in 1994 generated a significant paradigm shift among ectoparasiticides, providing for the first time a highly effective and convenient *topical* monthly control method.[2] Significant market success resulted with annual global sales of Frontline® (Merial) and other animal health fipronil products reaching over US $800 million in 2012.[3] Consequently, over the last two decades, many animal health companies have dedicated significant efforts toward the development of novel and differentiated products, in specific, once-a-month, *orally* administered flea and tick agents for companion animals.[4] Much of this work has been based on leveraging the discovery efforts for new pesticides conducted by agrochemical companies. At the time that we embarked on our own active research in this arena, there were multiple reports in the patent literature of isoxazolines exhibiting insecticidal activity from such sources and representative examples of this class have recently entered the market for animal health use (Fig. 1).[5] Herein we report the discovery of sarolaner, a once-a-month, oral isoxazoline specifically optimized for the treatment of flea and tick infestation on dogs.

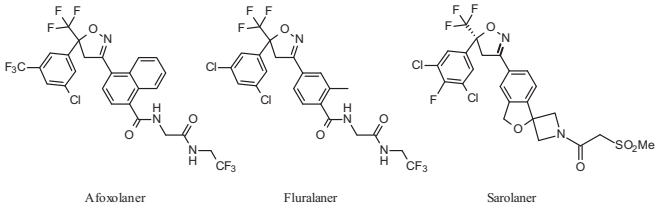

Figure 1. Ectoparasiticidal isoxazolies.

Our initial work in the area of isoxazolines culminated in the discovery of the novel isoxazoline azetidines with oral flea and tick activity in dogs.[6] The in vitro ectoparasiticidal data and oral dog pharmacokinetic (PK) profiles of two representative examples, **1** and **2**, synthesized in the course of that effort, are presented in Table 1.[7,8] With the understanding that plasma is the efficacious compartment, or at least an excellent surrogate for the efficacious compartment, an inspection of these data reveals that while **1**

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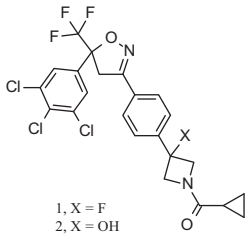
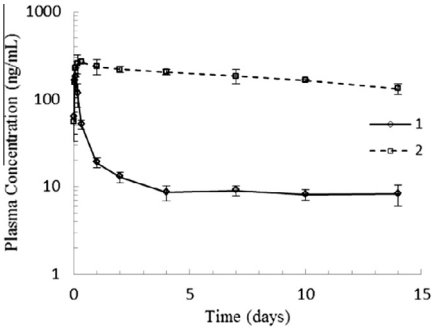
0960-894X/

Table 1
In vitro ectoparasiticidal activities and oral dog PK profiles for representative isoxazoline azetidines **1** and **2**

Compound	Flea feed[a]	Soft Tick feed[a]		Computed properties[b]		
	LD_{80} (μg/mL)	ED_{50} (μg/mL)	LD_{100} (μg/mL)	M.W.	*A*log*P*	PSA
1	1	0.03	0.03	534	6.3	42
2	10	N.D.[c]	N.D.[c]	532	5.3	62





[a] Ref. 7.
[b] Pipeline pilot.
[c] Compounds inactive or weakly active in the flea feed assay were not progressed into the soft tick feed assay.

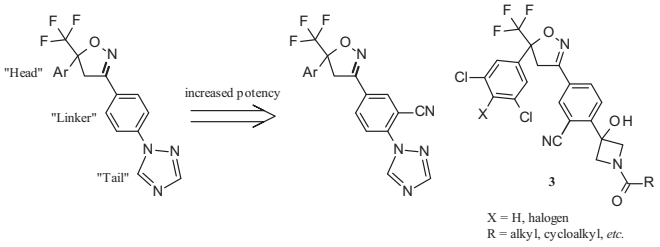


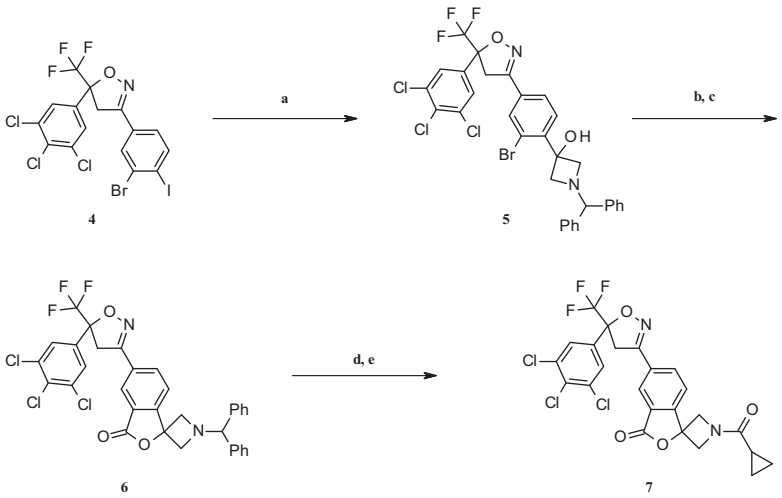
Figure 2. Hypothesized potency optimization of **2** through placement of a CN group.

exhibits superior in vitro flea and tick potency, **2** affords a superior dog PK profile due to its higher systemic plasma concentrations.[9] The challenge in front of us was to incorporate both of these features in a single molecule; thus, we intended to modulate the structure of **2** to improve in vitro potency (flea feed LD_{80} <1 μg/mL; soft tick feed ED_{50} <0.1 μg/mL) while maintaining its dog PK profile.

Previous reports in the patent literature demonstrated the superior ability of an *ortho* substitution in the linker aryl ring of an isoxazoline to improve insecticidal potency. Thus, the placement of a cyano substituent *ortho* to the triazole ring (Fig. 2) was shown to drastically improve insecticidal potency.[10] From this, we postulated that we could enhance the in vitro potency of **2**, while maintaining its favorable PK profile, by placing a cyano substituent *ortho* to the azetidine attachment on the linker phenyl as shown in the generic structure **3**.

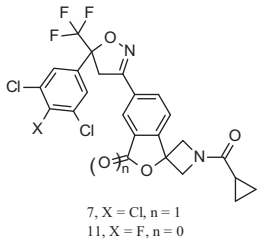
Attempted synthesis of a representative analog of the generic structure **3** started with the bromoiodo arene intermediate **4** (Scheme 1).[11] In situ Grignard generation, followed by the addition of the azetidinone building block led to hydroxyazetidine **5**. Somewhat unexpectedly, cyanation of **5**, in place of affording the intended cyano substituted hydroxyazetidine **3**, led to the cyclized lactone **6**, presumably arising from facile intramolecular attack of the hydroxyl on the cyano functional group and hydrolysis of the intermediate oxoamidine.

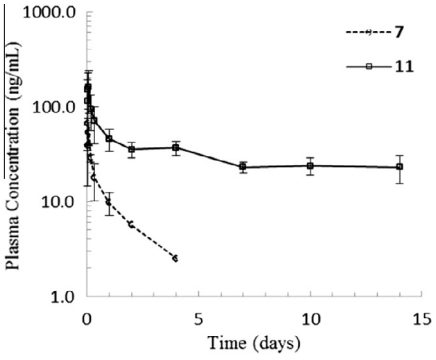
Opportunistic synthesis and in vitro testing of amide **7** revealed it to possess promising insecticidal potency (cf. **2**); even more



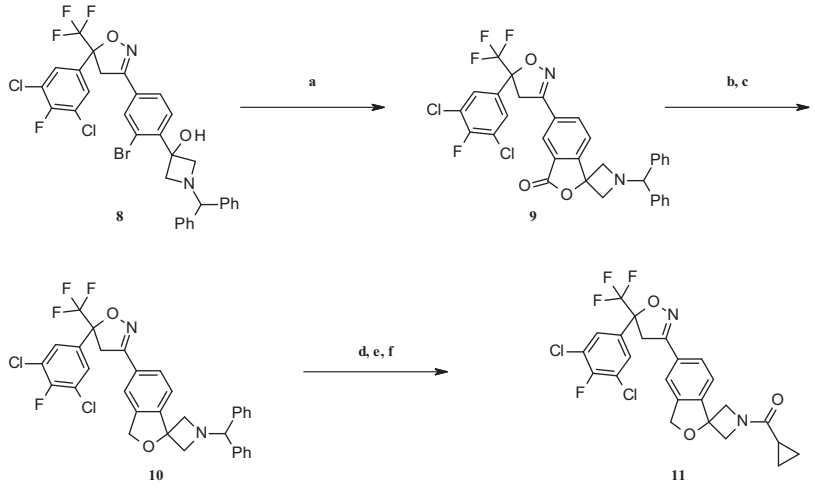
Scheme 1. Reagents and conditions: (a) *i*PrMgCl, THF, azetidinone, −40 °C to −10 °C, 52%; (b) $Zn(CN)_2$, $Pd(PPh_3)_4$, DMF, microwave, 150 °C; (c) H_2O, 70 °C, 58% (steps b–c); (d) chloroethyl chloroformate, DCM-acetonitrile, MeOH, reflux, 71%; (e) *c*-PrCOCl, TEA, DCM, 46%.

Table 2
In vitro ectoparasiticidal activities and oral dog PK profiles for spirolactone **7** and spiroether **11**

	Compound	Flea feed[a]	Soft tick feed[a]		Computed properties[b]		
		LD_{80} (μg/mL)	ED_{50} (μg/mL)	LD_{100} (μg/mL)	M.W.	*A*log*P*	PSA
 7, X = Cl, n = 1 11, X = F, n = 0	**7**	1	0.03	0.1	558	5.5	68
	11	0.3	0.01	0.03	528	5.0	51



[a] Ref. 7.
[b] Pipeline pilot.



Scheme 2. Reagents and conditions: (a) $Pd(OAc)_2$, Xantphos, MeOH, CO (1 atm), 70%; (b) $LiBH_4$, THF, 95%; (c) MsCl, DCM, 0 °C to 35 °C, 80%; (d) chloroethyl chloroformate, DCM, acetonitrile, reflux; (e) MeOH, reflux, 85% (steps d–e); (f) *c*-PrCOCl, TEA, DCM, 95%.

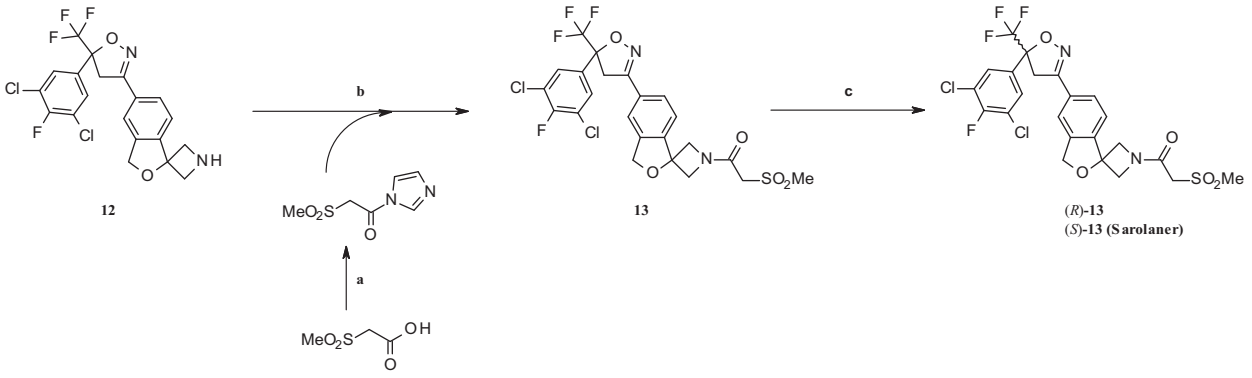
interestingly, evaluation of **7** in a dog PK study showed it to exhibit a reasonable enough PK profile (factoring in the potential liability of the lactone functionality) following oral administration (Table 2) that rendered it worthy of further exploration.

We next investigated the effect of modification of the lactone ring to improve both activity and pharmacokinetic parameters. Gratifyingly, reduction of the lactone to the cyclic ether, concomitant with optimization of the head aryl ring (Cl to F substitution),[12] afforded **11** (Scheme 2), exhibiting improved flea and tick activities in vitro as well as a more desirable oral dog PK profile (Table 2).

Lead compound **11** presented an exciting advance on the path to meeting our initial laboratory objectives vis-à-vis **1** and **2**. In an attempt to further optimize the PK properties of **11**, while maintaining or even improving in vitro potency, a focused set of amide tail replacements with a range of physicochemical properties (lipophilic to polar) was investigated.[13] From this investigation, **13**, bearing a polar SO_2Me substitution on the amide tail (Scheme 3) was identified as exhibiting the optimal balance of in vitro potency, dog PK and physicochemical properties to potentially target a once-a-month oral ectoparasiticidal profile (Table 3). Since previous reports on antiparasitic isoxazolines had demonstrated superior activity for the (*S*)-enantiomer, racemic **13** was further separated and tested as its (*R*)- and (*S*)-enantiomers; the in vitro activity and dog oral PK profile for the active (*S*)-enantiomer ((*S*)-**13**; sarolaner) are included in Table 3.[14,15]

(*S*)-**13** (sarolaner) was subsequently progressed into a dog efficacy study wherein, at 2.5 mg/kg oral dose, it exhibited ⩾99.9% efficacy for fleas (*Ctenocephalides felis*) and 100% for ticks (*Rhipicephalus sanguineus*) for the entire duration of the 28 day study period (Table 4).[16] Based on its promising PK profile (Table 5), efficacy profile and additional in vitro and in vivo biological profiling, (*S*)-**13** (sarolaner) was progressed into development; details of these studies will be reported separately.

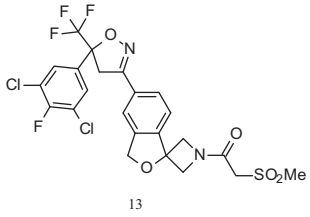
In summary, this communication outlines the discovery of sarolaner, a proprietary, optimized-for-animal health use isoxazoline for once-a-month, oral treatment of flea and tick infestation on dogs, starting from an azetidine based isoxazoline lead series.

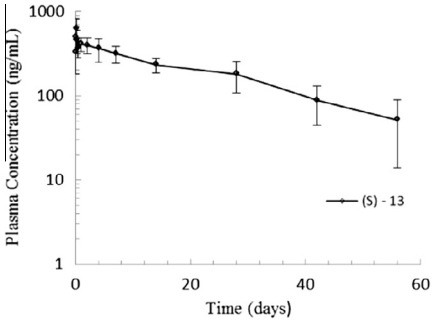


Scheme 3. Reagents and conditions: (a) CDI, DMF; (b) MTBE, 90%; (c) chiral separation.

Table 3
In vitro ectoparasiticidal activities and oral dog PK profile for sarolaner (*S*)-**13**

Compound	Flea feed[a]	Soft tick feed[a]		Computed properties[b]		
	LD_{80} (μg/mL)	ED_{50} (μg/mL)	LD_{100} (μg/mL)	M.W.	*A*log*P*	PSA
rac-**13**	1	0.03	0.03	580	3.7	94
(*R*)-**13**	3	>0.1	>0.1			
(*S*)-**13** (Sarolaner)	0.3	0.003	0.003			





[a] Ref. 7.
[b] Pipeline pilot.

Table 4
Oral ectoparasiticidal efficacy of sarolaner in dogs

Count Day	Arithmetic mean flea count (placebo control)	Arithmetic mean tick count (placebo control)	Flea efficacy (*C. felis*) 2.5 mg/kg sarolaner solution	Tick efficacy (*R. sanguineus*) 2.5 mg/kg sarolaner solution
2	62.5	20.0	0.0[a] (100)	0.0[a] (100)
28	76.5	18.0	0.1[a] (99.8)	0.0[a] (100)

[a] Mean counts are significantly different to control ($P \leqslant 0.05$). Percent efficacy is given in brackets.

Table 5
PK parameters of (*S*)-**13** (sarolaner) in dogs[a]

Treatment	PK parameter	LS mean
IV	CL (mL/min/kg)	0.12[b]
IV	Vd_{ss} (mL/kg)	2810[b]
IV	$t_{1/2}$ (h)	289
PO (fed)		297
PO (fed)	C_{max} (ng/mL)	919
PO (fed)	Bioavailability (%*F*)	107

IV, intravenous; PO, per os (oral).
[a] IV dose 2 mg/kg. PO dose 2.28–2.51 mg/kg. PO PK parameters, with the exception of $t_{1/2}$, have been dose-normalized to 2 mg/kg. Dose normalized PO $AUC_{0-\infty}$ was used to calculate %*F*.
[b] Arithmetic means reported in place of least-squares means.

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7. The in vitro ectoparasiticidal activities for compounds **1**, **2**, **7**, **11** and **13** were determined as follows:
   Flea (*Ctenocephalides felis)* Membrane Feed Assay: compounds were dissolved in DMSO and aliquots were added to citrated bovine blood in membrane covered wells warmed to 37 °C. Adult fleas were newly emerged (3–7 days) and unfed. Feeding chambers containing approximately 30 adult fleas were placed onto the treated blood wells, and the fleas were allowed to feed on the treated blood for 2 h. Fleas were observed for knockdown and/or death at 2 and 24 h. Each compound was tested at half-log intervals, and endpoint data was recorded as LD_{80} in μg/mL. LD_{80} is a subjective visual assessment of organism viability, and is the lowest dose to cause ⩾80% mortality.
   Soft Tick (*Ornithodorus turicata*) Membrane Feed Assay: compounds were dissolved in DMSO and aliquots were added to citrated bovine blood in membrane covered wells warmed to 37 °C. Five nymphs (N3–N4) were placed on the membrane, allowed to feed to repletion, and were placed in observation chambers. Nymphs were observed for knockdown and/or death at 24 and 72 h. Endpoint results for subjective visual assessment of organism viability were recorded as ED_{50} (the lowest dose to cause ⩾50% morbidity/mortality) and LD_{100} (the lowest dose to cause 100% mortality) in μg/mL.
8. All in vivo procedures were conducted according to state, national or international regulations, and after appropriate ethical reviews. Subjects for the pharmacokinetic studies were prepubertal to adult, purpose-bred beagles (⩾6 months of age and 6–14 kg).
   For the initial in vivo assessment of compounds **1**, **2**, **7**, **11** and **13**, three animals were randomly allocated to each treatment group. For compounds **1**, **2**, **7** and **11**, subjects were treated with the test materials (0.5 mg/kg) in a solution formulation (1 mg/mL) of DMSO: 2-pyrrolidinone: Tetraglycol: Solutol HS15: Tween80 (5:15:50:28:2% v/v) via oral gavage, followed by a water flush (10 mL). Blood sampling commenced at 0.5 h post dose and continued for two weeks post dose. For compound **13**, subjects were treated with the test material (2.5 mg/kg) in a solution formulation (20 mg/mL) of DMSO: 2-pyrrolidinone: Tetraglycol: Solutol HS15: Tween80 (5:15:50:28:2% v/v) via oral gavage, followed by a water flush (10 mL). Blood sampling commenced at 0.5 h post dose and continued for two months post dose. For the definitive PK assessment of compound **13**, as presented in Table 5, 12 animals (6 male, 6 female) were randomly allocated to two treatment groups. Subjects in Treatment group 1 were treated with **13** via an intravenous bolus injection (2 mg/kg) in a solution formulation (20 mg/mL) of DMSO: Glycerol Formal—Solutol HS15 (2:1, w/w): Sterile water for injection (5:50:45% v/v). Subjects in Treatment group 2 were each orally administered one 20 mg chewable tablet of **13**. Administration of the oral dose was followed by a water flush (approximately 15 mL). Blood sampling commenced at 2 min post dose, and continued for two months post dose. Blood was held on wet ice prior to centrifugation and removal of plasma. Plasma was stored at ⩽10 °C until analysis by protein precipitation and ultra performance liquid chromatography with tandem mass spectrometry.
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12. Aryl groups with varied combinations of halogen substitutions at the *ortho*, *meta* and *para* positions were explored since such substitutions were observed to impact in vitro potency. In the spirocyclic series, a Cl, F, Cl combination was identified to be one of the optimal aryl group substitutions for in vitro potency.
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14. (a) Ozoe, Y.; Asahi, M.; Ozoe, F.; Nakahira, K.; Mita, T. *Biochem. Biophys. Res. Commun.* **2010**, *391*, 744
    (b) Chiral separation was performed on a Berger Supercritical Fluid Chromatograph unit using the following set up: Column: OJ 30 mm x 250 mm, 5 μM; Mobile phase: A = CO_2, B = 1:1 MeOH: DCM with 0.1% TEA; Gradient: isocratic, 23% B; Temperature: 40 °C; Flow rate: 100 mL/min; Outlet pressure: 120 bar.
15. The synthesis of sarolaner is described in: Curtis, M.; Menon, S.; Vaillancourt, V. A.; Chubb, N. A. L.; Billen, D.; Greenwood, S. D. W.; Stuk, T. L. U. S. Patent 8 466 115, 2013. The spectral data for sarolaner is as follows:
    ^{1}H NMR (600 MHz, DMSO-d^6) δ: 7.82 (d, *J* = 6.24 Hz, 2H), 7.77 (d, *J* = 8.25 Hz, 1H), 7.70 (m, 2H), 5.13 (s, 2H), 4.58 (s, 2H), 4.37 (m, 2H), 4.26 (s, 2H), 4.23 (s, 2H), 3.16 (s, 3H); m/z (ESI) 579.0 $[M-H]^-$.
16. Subjects for the efficacy study were adult, purpose-bred beagles (⩾ 8 months of age and ⩽ 20 kg). Parasites used in the study were the cat flea (*C. felis*) and the brown dog tick (*R. sanguineus*). Day 0 was the day dogs were treated with test materials.
    Eight animals (four male and four female each) were randomly allocated to each treatment group (placebo and sarolaner) based on Day -5 tick counts. All subjects were treated via oral gavage (0.5 mL/kg) with either placebo (T01) or sarolaner (T02; 2.5 mg/kg) in a solution formulation of DMSO: 2-pyrrolidinone: Tetraglycol: Solutol HS15: Tween80 (5:15:50:28:2% v/v) on study Day 0. All dogs were infested with fleas and ticks prior to treatment and approximately weekly thereafter for 5 weeks. Infestations were conducted by applying 50 adult ticks (1:1 sex ratio) and 100 adult fleas gently to a site proximal or adjacent to the shoulder blades and allowed to crawl into the haircoat. Parasite counts, both flea and tick, were routinely conducted 24 hours after flea infestation (48 hours after tick infestation) except on Day 2 where both counts were conducted 48 hours post treatment with the test materials. Dogs were examined for ticks beginning from the head and moving posteriorly along the dorsal, lateral and ventral sides. Ticks were removed as they were found and subsequently identified and assessed for viability. Dogs were systematically combed for fleas using repeated strokes initially while standing, starting from the head, then posteriorly along the dorsal, lateral and ventral sides. Combing was continued until no fleas were recovered for about 5 minutes. Each animal was examined for a minimum of 10 minutes.