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Optimization of isoxazoline amide benzoxaboroles for identification of a development candidate as an oral long acting animal ectoparasiticide

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ABSTRACT

Novel isoxazoline amide benzoxaboroles were designed and synthesized to optimize the ectoparasiticide activity of this chemistry series against ticks and fleas. The study identified an orally bioavailable molecule, (*S*)-*N*-((1-hydroxy-3,3-dimethyl-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)methyl)-2-methyl-4-(5-(3,4,5-trichlorophenyl)-5-(trifluoromethyl)-4,5-dihydroisoxazol-3-yl)benzamide (**23**), with a favorable pharmacodynamics profile in dogs (C_{max} = 7.42 ng/mL; T_{max} = 26.0 h; terminal half-life $t_{1/2}$ = 127 h). Compound **23**, a development candidate, demonstrated 100% therapeutic effectiveness within 24 h of treatment, with residual efficacy of 97% against American dog ticks (*Dermacentor variabilis*) on day 30 and 98% against cat fleas (*Ctenocephalides felis*) on day 32 after a single oral dose at 25 mg/kg in dogs.

Isoxazolines are a relatively new class of ectoparasiticide agents inhibiting γ-aminobutyric acid (GABA)-gated and L-glutamate-gated chloride channels, with several examples approved for use in the companion animal industry.[1] Three efficacious isoxazoline compounds[1a–t] (Fig. 1) have been approved for the treatment of tick and flea infestations in dogs.[1u–w]

We previously reported a novel series of isoxazoline benzoxaborole small molecules exhibiting excellent oral-systemic activity against ticks and fleas; the lead compound (**AN8030**, Fig. 2) was shown to provide 95% efficacy against American dog ticks (*Dermacentor variabilis*) on day 30, following a single oral dose at 50 mg/kg in dogs.[2]

As a continuation of the research program with a goal of lowering the dosage while maintaining acceptable clinical effectiveness (i.e., ⩾95%) for at least one month, we designed and synthesized a series of novel isoxazoline amide benzoxaboroles (**1**–**23**, Figs. 3 and 4) for a structure-activity relationship (SAR) study to optimize the ectoparasitic activity. Specifically, these molecules were designed to examine the effects of oxaborole 3-substituent variation (**1** vs **2**, **9** and **11**; **7** vs **10**, **13** and **14**), amide *N*-substituent change (**2** vs **3**), halogen addition on the C(5)-aryl (**4** vs **5**, and **2** vs **6** and **7**), linking position variation to the benzoxaborole (**2** vs **4**, **5** vs **6**, and **7** vs **8**), linkage length (**2** vs **15**, **6** vs **16**, and **7** vs **17**), substituent changes on the benzylic carbon between the amide and benzoxaborole (**7** vs **18** and **19**), and the chiral configuration (**20** vs **21**, and **22** vs **23**). Herein, we report the synthesis, pharmacokinetic profile and ectoparasiticide activity against ticks and fleas of this 'extended' series.

The synthetic routes used for the preparation of compounds **1**–**19** are outlined in Schemes 1–3.[3a] In Scheme 1, aldehyde **24**[3b] reacted with hydroxylamine to give the resulting oxime **25**, which was reduced to the aminomethyl intermediate **26**. Amidation reaction between amine **26** and acid **27** gave the corresponding amides (**1**, **2**, **4**–**14**). Reductive amination of **24** with methylamine provided the amine **28**, which was followed by reaction with acid **27** to generate **3**. Aldehyde **24** reacted with nitromethane providing **29**, which was reduced to aminoethyl molecule **30** followed by an amidation reaction with **27**, giving final compounds **15**–**17**. In Scheme 2, Grignard reaction of **31** with MeMgBr generated the ketone alcohol **32**, which was protected to give **33** followed by catalytic boronylation to afford

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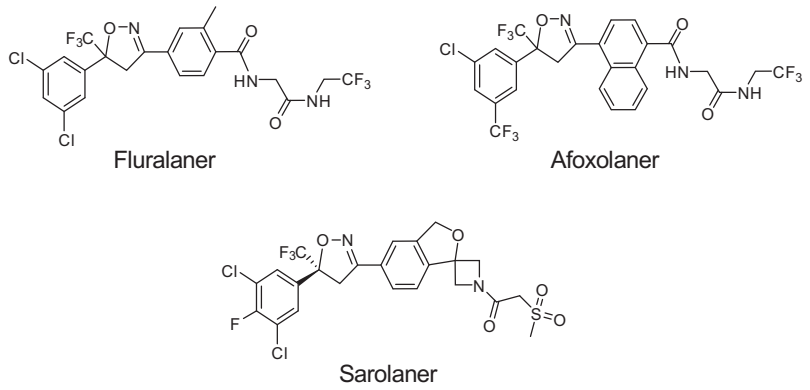


Figure 1. Chemical structures of three typical isoxazoline compounds.

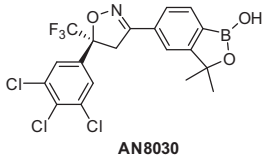
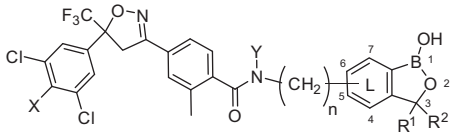


Figure 2. Structure of an isoxazoline benzoxaborole lead providing at least 1 month of ectoparasitic activity against experimental tick and flea infestations on dogs.[2]



1: X = H, Y = H, L (link) to 6, n = 1, $R^1 = R^2$ = H;
2: X = H, Y = H, L to 6, n = 1, $R^1 = R^2$ = Me;
3: X = H, Y = Me, L to 6, n = 1, $R^1 = R^2$ = Me;
4: X = H, Y = H, L to 5, n = 1, $R^1 = R^2$ = Me;
5: X = F, Y = H, L to 5, n = 1, $R^1 = R^2$ = Me;
6: X = F, Y = H, L to 6, n = 1, $R^1 = R^2$ = Me;
7: X = Cl, Y = H, L to 6, n = 1, $R^1 = R^2$ = Me;
8: X = Cl, Y = H, L to 5, n = 1, $R^1 = R^2$ = Me;
9: X = H, Y = H, L to 6, n = 1, $R^1 = R^2 = CH_2F$;
10: X = Cl, Y = H, L to 6, n = 1, $R^1 = R^2 = CH_2F$;
11: X = H, Y = H, L to 6, n = 1, $R^1 = R^2$ = Et;
12: X = F, Y = H, L to 6, n = 1, $R^1 = R^2$ = Et;
13: X = Cl, Y = H, L to 6, n = 1, $R^1 = R^2$ = Et;
14: X = Cl, Y = H, L to 6, n = 1, R^1 & $R^2 = (CH_2)_4$;
15: X = H, Y = H, L to 6, n = 2, $R^1 = R^2$ = Me;
16: X = F, Y = H, L to 6, n = 2, $R^1 = R^2$ = Me;
17: X = Cl, Y = H, L to 6, n = 2, $R^1 = R^2$ = Me;
18: X = Cl, Y = H, L to 6, n = 1 of CHMe instead of CH_2, $R^1 = R^2$ = Me;
19: X = Cl, Y = H, L to 6, n = 1 of CMe_2 instead of CH_2, $R^1 = R^2$ = Me;

Figure 3. Chemical structures of the isoxazoline amide benzoxaboroles investigated in a SAR study to identify novel ectoparasiticides.

34. Deprotection of **34** and subsequent simultaneous cyclization provided **35** which was converted to oxime **36**. Reduction of the oxime group in **36** yielded amine **37**, which reacted with acid **27** to produce the final amide **18**. In Scheme 3, the diester **38** reacted with MeMgBr to give the dialcohol **39** followed by protection to provide **40**, which was catalytically boronylated to afford **41**. Hydrolysis of **41** generated the alcohol **42** that was converted to the azide **43**. The azide group in **43** was reduced to the amine of **44**, which reacted with the acid **27** to produce the final amide **19**. The racemic compounds **6** and **7** were separated by chiral SFC to obtain each enantiomer (Fig. 4). As an example, the experimental procedure for the synthesis of **7**, and its chiral separation method for obtaining **22** and **23** are described in the reference and note section.[4a,b]

Activity of compounds **1**–**23** against larval-stage Lone Star ticks (*Amblyomma americanum*) was tested in an in vitro larval immersion microassay (LIM assay).[2] In this assay, tick larvae were submerged in solutions containing compounds for 30 min, taken out to air dry, and incubated for 24 h, at which time mortality was assessed. If the compounds showed good activity in the LIM assay, they were advanced for in vivo testing against nymphal-stage American dog tick (*D. variabilis*) infestations on rats. In this in vivo rodent model, tick nymphs were allowed to attach and begin feeding on rats for 24 h. Rats in treated groups received compounds administered orally, and 48 h after treatment, live and dead ticks were removed from animals and counted.[2]

Starting with compound **1** that had good activity in the LIM assay (100% at 300 μM and EC_{50} = 61.3 μM; see Table 1) and encouraging in vivo efficacy (93.0% at 25 mg/kg) in the rodent model, compound **2** was designed to improve metabolic stability and its pharmacokinetic profile by installing 3,3-dimethyl groups on the oxaborole ring. This structural modification resulted in better in vivo efficacy (100% at 25 or 10 mg/kg). *N*-methylation on the amide decreased the activity (**3**, 13.9% at 10 mg/kg). Switching the linking position on the benzoxaborole from 6- to 5-position also decreased the activity (**4**, 16.3% at 10 mg/kg). Replacement of the hydrogen on the left phenyl with a halogen, such as F or Cl, generally improved the activity in the examples of **5** (100% at 10 mg/kg) vs **4**, **6** (10% at 5 mg/kg) vs **2** (0% at 5 mg/kg), and **7** (100% at 5 mg/kg) vs **2**. Decreased activity of **8** (11.2% at 5 mg/kg, vs **7**) reconfirmed the previous observation that linking 6-position to the benzoxaborole provided better activity, which led us to focus on this chemistry. Replacement of 3,3-dimethyl groups with 3,3-di(fluoromethyl) substituents maintained the activity (**9** and **10** vs **2** and **7**, respectively). Increasing the 3,3-substituent size using diethyl and spirocyclopentyl groups for more lipophilicity decreased the activity (**11**–**14**). Increasing the linkage length between the amide and the benzoxaborole from a carbon to two carbons also resulted in the decreased activity (**15**–**17**). With regard to the substitution on the methylene moiety between the amide and the benzoxaborole, a methyl group maintained the activity (**18**) while dimethyl substituents significantly lowered the activity (**19**).

The SAR study identified three top tier molecules (**7**, **10** and **18**) that exhibited 90–100% efficacy at 5 mg/kg in the in vivo rat model, and two second tier compounds (**5** and **6**) that exhibited 100% efficacy at a slightly higher dose of 10 mg/kg. Two compounds (**6** and **7**) from each of the tiers were selected for chiral chromatography