Bioorganic & Medicinal Chemistry Letters xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Discovery of an orally bioavailable isoxazoline benzoxaborole (AN8030) as a long acting animal ectoparasiticide

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ARTICLE INFO

Article history:
Received 26 September 2015
Revised 13 October 2015
Accepted 15 October 2015
Available online xxxx

Keywords:
Benzoxaborole
Isoxazoline
Structure–activity relationship
Ectoparasiticide
Tick and flea

ABSTRACT

A novel series of isoxazoline benzoxaborole small molecules was designed and synthesized for a structure–activity relationship (SAR) investigation to assess the ectoparasiticide activity against ticks and fleas. The study identified an orally bioavailable molecule, (*S*)-3,3-dimethyl-5-(5-(3,4,5-trichlorophenyl)-5-(trifluoromethyl)-4,5-dihydroisoxazol-3-yl)benzo[*c*][1,2]oxaborol-1(3*H*)-ol (**38**, **AN8030**), which was long lasting in dogs ($t_{1/2}$ = 22 days). Compound **38** demonstrated 97.6% therapeutic effectiveness within 24 h of treatment, with residual efficacy of 95.3% against American dog ticks (*Dermacentor variabilis*) on day 30 and 100% against cat fleas (*Ctenocephalides felis*) on day 32 after a single oral dose at 50 mg/kg in dogs.

Isoxazoline compounds, as a new class of ectoparasiticide agents inhibiting γ-aminobutyric acid (GABA)-gated and L-glutamate-gated chloride channels, have recently been discovered and drawn broad attention in the companion animal industry.[1] Two efficacious isoxazoline compounds[1a–s] (Fig. 1) have recently been approved for the treatment of tick and flea infestations in dogs.[1t,u]

Previously, benzoxaboroles have been reported with selective activity against parasites[2] including the parasite, *Plasmodium falciparum*, the trypanosome, *Trypanosoma brucei* and the filarial worm, *Brugia malayi*. As an extension of our research, we rationalized exploring the potential of benzoxaboroles as ectoparasiticides for the treatment of ticks and fleas in animals including cats and dogs.

It was disclosed that compound **1** (Fig. 2) has pesticide activities against several pests tested including fall armyworm larvae (*Spodoptera frugiperda*) and mustard beetle larvae (*Phaedon cochleariae*).[3] The five-membered lactone scaffold in **1** has similar pharmacophore feature to the oxaborole ring in **2**. Both the lactone carbonyl carbon in **1** and the boron in **2** are electron-deficient and can interact with nucleophilic components. Therefore, starting from molecule **2**, a series of its close analogs (**3**–**38** in Figs. 3 and 4) was designed and synthesized for a structure–activity relationship (SAR) study to examine the effects from oxaborole 3-substituent variation (**2**–**6**), fluoro position change on the benzoxaborole benzene ring (**7**–**9**), substituent variation on the isoxazoline ring (**10** vs **3**, and **17**–**19**), fluoro substitution on 3,3-dimethyls (**20** vs **17**), di-substitution (**11**–**16**), tri-substitution (**21**–**34**) and tetra-substitution on the left benzene ring (**35** vs **36**), and the chiral configuration (**37** vs **38**). Herein, we report the chemistry, pharmacokinetic results and ectoparasiticide activity against ticks and fleas.

The syntheses of compounds **2**–**36** were convenient and the general route is shown in Scheme 1.[4] Aldehyde **39**[4] reacted with hydroxylamine to give carbaldehyde oxime **40**, which was converted to carbimidoyl chloride **41** by reacting to *N*-chlorosuccinimide (NCS). The last step was the cycloaddition reaction of **41** with substituted styrene intermediates **42** to give the final compounds **2**–**36**. As an example, the experimental procedure for the synthesis of **17**, and its chiral separation method for obtaining **37** and **38** are described in the reference and note section.[7a,b] It has been reported that the active enantiomer of Fluralaner has the (*S*)-configuration of the carbon chiral center on the isoxazoline ring.[1a]

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http://dx.doi.org/10.1016/j.bmcl.2015.10.044
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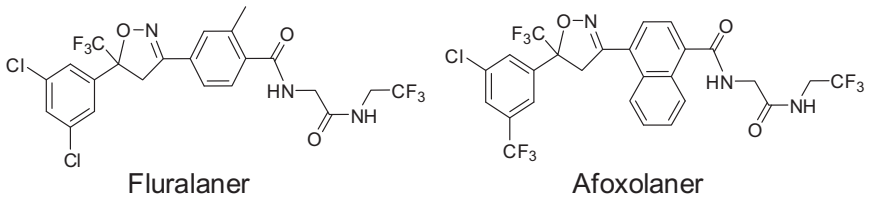


Figure 1. Chemical structures of two typical isoxazoline compounds.

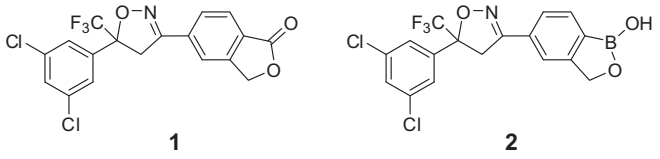
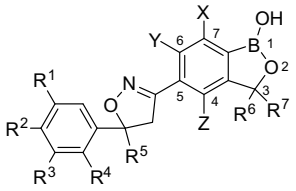


Figure 2. Structural comparison of an initial isoxazoline benzoxaborole (**2**) with a reported isoxazoline benzolactone pesticide (**1**).

Activity of compounds **2**–**38** against larval-stage Lone star ticks (*Amblyomma americanum*) was tested in an in vitro larval immersion microassay (LIM assay).[7c] In this assay, tick larvae were submerged in solutions containing compounds for 30 min, taken out to air dry and then incubated for mortality-counting in 24 h. If the compounds showed good activity in the LIM assay, they were further tested for efficacy against nymphal-stage American dog ticks (*Dermacentor variabilis*) on rats. In this in vivo rodent model, tick nymphs were allowed to attach and begin feeding on rats for



3: $R^1 = R^3 = Cl$, $R^2 = R^4 = H$, $R^5 = CF_3$, $X = Y = Z = H$, $R^6 = R^7 = Me$;
4: $R^1 = R^3 = Cl$, $R^2 = R^4 = H$, $R^5 = CF_3$, $X = Y = Z = H$, $R^6 = R^7 = Et$;
5: $R^1 = R^3 = Cl$, $R^2 = R^4 = H$, $R^5 = CF_3$, $X = Y = Z = H$, R^6 & $R^7 = (CH_2)_4$;
6: $R^1 = R^3 = Cl$, $R^2 = R^4 = H$, $R^5 = CF_3$, $X = Y = Z = H$, R^6 & $R^7 = (CH_2)_5$;
7: $R^1 = R^3 = Cl$, $R^2 = R^4 = H$, $R^5 = CF_3$, $X = Y = H$, $Z = F$, $R^6 = R^7 = Me$;
8: $R^1 = R^3 = Cl$, $R^2 = R^4 = H$, $R^5 = CF_3$, $X = Z = H$, $Y = F$, $R^6 = R^7 = Me$;
9: $R^1 = R^3 = Cl$, $R^2 = R^4 = H$, $R^5 = CF_3$, $X = F$, $Y = Z = H$, $R^6 = R^7 = Me$;
10: $R^1 = R^3 = Cl$, $R^2 = R^4 = H$, $R^5 = CF_2CF_3$, $X = Y = Z = H$, $R^6 = R^7 = Me$;
11: $R^1 = R^3 = Br$, $R^2 = R^4 = H$, $R^5 = CF_3$, $X = Y = Z = H$, $R^6 = R^7 = Me$;
12: $R^1 = CF_3$, $R^3 = Cl$, $R^2 = R^4 = H$, $R^5 = CF_3$, $X = Y = Z = H$, $R^6 = R^7 = Me$;
13: $R^1 = R^3 = CF_3$, $R^2 = R^4 = H$, $R^5 = CF_3$, $X = Y = Z = H$, $R^6 = R^7 = Me$;
14: $R^1 = Cl$, $R^2 = F$, $R^3 = R^4 = H$, $R^5 = CF_3$, $X = Y = Z = H$, $R^6 = R^7 = Me$;
15: $R^1 = R^2 = Cl$, $R^3 = R^4 = H$, $R^5 = CF_3$, $X = Y = Z = H$, $R^6 = R^7 = Me$;
16: $R^1 = CF_3$, $R^2 = F$, $R^3 = R^4 = H$, $R^5 = CF_3$, $X = Y = Z = H$, $R^6 = R^7 = Me$;
17: $R^1 = R^2 = R^3 = Cl$, $R^4 = H$, $R^5 = CF_3$, $X = Y = Z = H$, $R^6 = R^7 = Me$;
18: $R^1 = R^2 = R^3 = Cl$, $R^4 = H$, $R^5 = CH_3$, $X = Y = Z = H$, $R^6 = R^7 = Me$;
19: $R^1 = R^2 = R^3 = Cl$, $R^4 = H$, $R^5 = CH_2F$, $X = Y = Z = H$, $R^6 = R^7 = Me$;
20: $R^1 = R^2 = R^3 = Cl$, $R^4 = H$, $R^5 = CF_3$, $X = Y = Z = H$, $R^6 = R^7 = CH_2F$;
21: $R^1 = R^3 = Cl$, $R^2 = F$, $R^4 = H$, $R^5 = CF_3$, $X = Y = Z = H$, $R^6 = R^7 = Me$;
22: $R^1 = R^3 = Cl$, $R^2 = Br$, $R^4 = H$, $R^5 = CF_3$, $X = Y = Z = H$, $R^6 = R^7 = Me$;
23: $R^1 = R^2 = R^3 = F$, $R^4 = H$, $R^5 = CF_3$, $X = Y = Z = H$, $R^6 = R^7 = Me$;
24: $R^1 = R^3 = Cl$, $R^2 = CF_3$, $R^4 = H$, $R^5 = CF_3$, $X = Y = Z = H$, $R^6 = R^7 = Me$;
25: $R^1 = R^3 = Cl$, $R^2 = CF_2H$, $R^4 = H$, $R^5 = CF_3$, $X = Y = Z = H$, $R^6 = R^7 = Me$;
26: $R^1 = R^3 = Cl$, $R^2 = Me$, $R^4 = H$, $R^5 = CF_3$, $X = Y = Z = H$, $R^6 = R^7 = Me$;
27: $R^1 = R^2 = Cl$, $R^3 = F$, $R^4 = H$, $R^5 = CF_3$, $X = Y = Z = H$, $R^6 = R^7 = Me$;
28: $R^1 = Cl$, $R^2 = R^3 = F$, $R^4 = H$, $R^5 = CF_3$, $X = Y = Z = H$, $R^6 = R^7 = Me$;
29: $R^1 = R^3 = Br$, $R^2 = Cl$, $R^4 = H$, $R^5 = CF_3$, $X = Y = Z = H$, $R^6 = R^7 = Me$;
30: $R^1 = R^3 = F$, $R^2 = Cl$, $R^4 = H$, $R^5 = CF_3$, $X = Y = Z = H$, $R^6 = R^7 = Me$;
31: $R^1 = R^3 = Br$, $R^2 = F$, $R^4 = H$, $R^5 = CF_3$, $X = Y = Z = H$, $R^6 = R^7 = Me$;
32: $R^1 = R^3 = Cl$, $R^2 = OMe$, $R^4 = H$, $R^5 = CF_3$, $X = Y = Z = H$, $R^6 = R^7 = Me$;
33: $R^1 = R^3 = Cl$, $R^2 = OCF_3$, $R^4 = H$, $R^5 = CF_3$, $X = Y = Z = H$, $R^6 = R^7 = Me$;
34: $R^1 = R^3 = Cl$, $R^2 = OCH_2CF_3$, $R^4 = H$, $R^5 = CF_3$, $X = Y = Z = H$, $R^6 = R^7 = Me$;
35: $R^1 = R^2 = R^3 = R^4 = Cl$, $R^5 = CF_3$, $X = Y = Z = H$, $R^6 = R^7 = Me$;
36: $R^1 = R^3 = Cl$, $R^2 = R^4 = F$, $R^5 = CF_3$, $X = Y = Z = H$, $R^6 = R^7 = Me$;

Figure 3. Chemical structures of the isoxazoline benzoxaboroles of compound **2** analogs investigated in a SAR study to identify novel ectoparasiticides.