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Discovery of ectoparasiticidal hydrazonotrifluoromethanesulfonanilides

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ABSTRACT

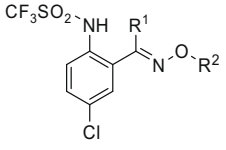
A series of hydrazonotrifluorosulfonanilide derivatives were synthesized and evaluated for in vitro activity against the ectoparasites *Ctenocephalides felis* and *Rhipicephalus sanguineus*. Some compounds with excellent activity against tick were identified.

Trifluoromethanesulfonanilide (TFMS) derivatives display insecticidal and acaricidal properties[1] which are exerted through uncoupling of oxidative phosphorylation in mitochondria.[2] As part of a program to discover new drugs to control commercially significant ectoparasites on companion animals,[3] we recently reported the discovery of alkoxy and aryloxy iminoalkyl (oxime ether) TFMS derivatives (e.g., **1–3**, Fig. 1) with significant in vitro insecticidal and acaricidal activity.[4] As an extension to that study, compound **4** was prepared as a prototype hydrazone analog of the oxime ethers and gave 100% mortality in a single-dose (1.26 μg/cm^2) rapid screen for cat flea (*Ctenocephalides felis*) activity and 98% mortality in a rapid screen (10 μg/tick) against brown dog tick (*Rhipicephalus sanguineus*).[5] By virtue of their additional site of substitution, hydrazones enable exploration of considerably greater chemical space than the corresponding oxime ethers. The 4-chloro-TFMS pharmacophore present in the most active oxime ether TFMS derivatives was maintained in the current study. The substituents on the hydrazone moiety were systematically varied in an effort to optimize parasiticidal activity. Compounds were initially screened against cat flea, then selected active compounds were subjected to a rapid dog tick screen and, where appropriate, LC_{50} (flea) and LD_{50} (tick) determination.[5]

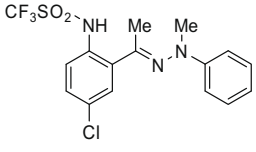
Hydrazone TFMS derivatives **4** and **7–45** were synthesized by condensation of ketones **5**[6,7] with hydrazines **6**[8] as indicated in Scheme 1 and Table 1.[9,10] The hydrazones were obtained either as single isomers or mixtures of geometrical isomers.[11] Cyclic analogs of **4** (compounds **46** and **47**) were prepared by condensation of phenylhydrazine with nitroaryl enone **48**,[12] using a modification of the method of Mannich and Lammering,[13] followed by nitro reduction[14] and amine triflation to give pyrazoline **46**. Dehydrogenation of **46** with DDQ gave pyrazole **47**.

Taking active compound **4** as the lead compound for an SAR study of hydrazone TFMS derivatives, we first varied R^1, while maintaining R^2 = Me and R^3 = Ph. Phenyl and cyclohexyl groups (compounds **7** and **8**, respectively) both gave inferior flea activity to methyl.

By fixing R^1 as methyl or ethyl and R^3 as phenyl, the effect of varying R^2 was next examined. While R^2 = H (compounds **9** and **10**) gave poor flea activity, various small alkyl groups (compounds **11–13**) gave good flea activity. Compounds **11** and **12** were also effective in the rapid tick assay, with the latter demonstrating an excellent LD_{50}. In contrast, allyl and propargyl substituents (compounds **14** and **15**, respectively) gave poor flea activity, while benzyl (compound **16**) gave good flea activity but poor tick activity.



1 R^1 = Me, R^2 = $(CH_2)_2OMe$
2 R^1 = Et, R^2 = 4-Chlorobenzyl
3 R^1 = Et, R^2 = 3,4-diCl-Ph



4

Figure 1. Oxime ether trifluoromethanesulfonanilides and prototype hydrazone analog.

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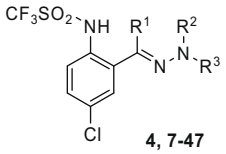
Table 1
Activity of hydrazonotrifluoromethanesulfonanilides **4**, **7–47** against *C. felis* (*C.f.*) and *R. sanguineus* (*R.s.*)



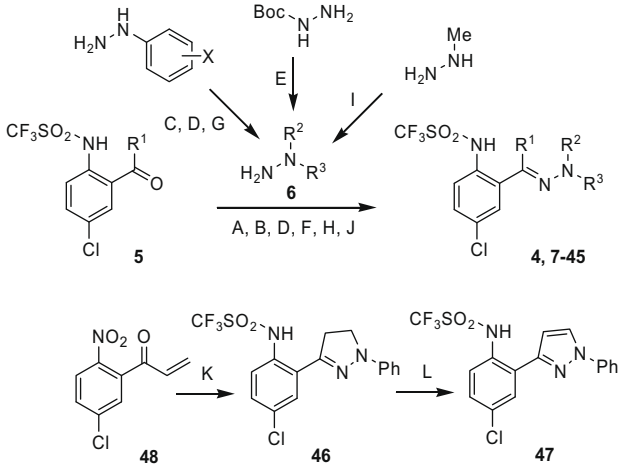
4, 7-47

Compound	Method of preparation[a]	R^1	R^2	R^3	*C.f.* % mortality 24 h	*C.f.* LC_{50}[b] (ng/cm^2)	*R.s.* % mortality 24 h[b]	*R.s.* LD_{50}[b] (μg/tick)
4	A	Me	Me	Ph	100		98	
7	A	Ph	Me	Ph	59			
8	A	Cyclohexyl	Me	Ph	14			
9	A	Me	H	Ph	20[c]			
10	B	Et	H	2,4-DiCl–Ph	5[c]			
11	C, A	Me	Et	Ph	100		100	
12	C, A	Me	*n*-Pr	Ph	100		75	1.2
13	A, D	Me	*i*-Pr	Ph	100			
14	C, A	Me	Allyl	Ph	59			
15	C, A	Me	Propargyl	Ph	38			
16	C, A	Me	Bn	Ph	100		33	
17	A	Me	Me	Me	66			
18	E, F	Me	Me	Cyclopentyl	85		98	1.01
19	E, F	Me	Me	Cyclohexyl	100		73	1.29
20	A, D	Me	Me	4-Cl–Ph	100	52.3	95	1.4
21	A, D	Me	Et	4-Cl–Ph	100		97	
22	A, D	Me	Et	4-F–Ph	100		100	
23	A, D	Me	Me	4-Br–Ph	100	42.7	100	
24	A, D	Me	Et	4-Br–Ph	100	27.3	95	5.6
25	A, D	Me	Me	4-CF_3–Ph	100		100	
26	A, D	Me	Me	4-Me–Ph	100	24.8	100	
27	A, D	Me	Me	3-Cl–Ph	100	14.6	100	2.6
28	A, D	Me	Me	3-F–Ph	100	13.4	100	2.0
29	A, D	Me	Et	3-F–Ph	100	7.46	98	2.1
30	G, H	Me	Me	3-CF_3–Ph	98		95	0.68
31	C, A	Me	Me	2-Cl–Ph	100		90	
32	A, D	Me	Et	2-Cl–Ph	89		83	
33	A, D	Me	Me	2-F–Ph	100	8.66	100	1.6
34	A, D	Me	Me	3,4-DiCl–Ph	99	35.3	78	3.0
35	A, D	Me	Me	3,5-DiCl–Ph	100	33.0	95	3.1
36	A, D	Me	Me	2,3-DiCl–Ph	100	30.8	70	4.0
37	A, D	Me	Et	2,4-DiCl–Ph	100		90	8.5
38	A, D	Me	*n*-Pr	2,6-DiCl–Ph	100		40	
39	A, D	Me	Me	2,5-DiCl–Ph	75			
40	I, A	Me	Me	2-Pyridyl	30			
41	A	Me	$CH_2CH_2OCH_2CH_2$		100		95	0.75
42	A	Me	$-(CH_2)_5-$		100		78	0.64
43	A	Me	$-(CH_2)_6-$		23			
44	J	Me	$-(CH_2)_4-$		100		93	0.74
45	A	Me	$CH_2CH_2N(Me)CH_2CH_2$		48			
46	K	CH_2CH_2		Ph	37			
47	L	CH=CH		Ph	43			
Fipronil						0.6–1.0		
Permethrin								0.13–0.35

[a] See Scheme 1 for reaction conditions.
[b] No entry indicates that the compound was not assayed.
[c] Measured at 8 h.

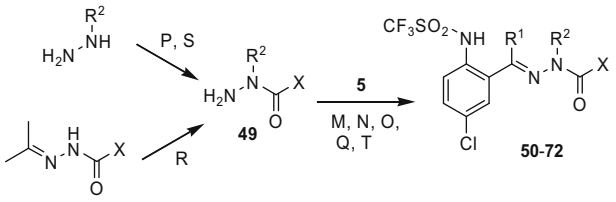
With R^1 fixed as methyl and R^2 as methyl or ethyl, the effect of varying R^3 was examined. Poor activity was observed when R^3 = methyl (compound **17**), while cycloalkyl groups in this position (compounds **18** and **19**) showed good flea and excellent tick activity. Several 2, 3 or 4-monosubstituted aryl rings gave good flea and tick activity (compounds **20–33**) with **29** and **33** being representative of compounds which exhibited good potency across both dose response assays, and **30** giving the best tick activity. Various dichloro substituted aryl groups (compounds **34–39**) were generally effective in both rapid screens, but tick potency (as measured by LD_{50}) was decreased relative to the monohaloaryl compounds. Replacement of the phenyl ring with the 2-pyridyl group (compound **40**) gave poor activity in the rapid flea screen.

Compounds **41**, **42** and **44,** in which R^2 and R^3 formed morpholine, piperidine and pyrrolidine rings, respectively, were effective in the rapid flea screen and (together with **30**) showed the best tick activity of all the alkylhydrazones in Table 1. In contrast to compound **4**, conformationally-restricted, cyclic analogs **46** and **47** gave poor activity in the rapid flea assay.



Scheme 1. Reagents and conditions:[15] (A) EtOH, rt; (B) arylhydrazine·HCl, KOAc, EtOH, rt; (C) NaOH (aq), *n*-Bu_4NCl, R^2I, rt;[16] (D) (i) NaH, DMF; (ii) R^2I, rt, 16 h; (E) (i) cycloalkyl ketone, MeOH, rt; (ii) $Na(CN)BH_3$ HOAc, H_2O;[17] (iii) R^2I, K_2CO_3, CH_3CN, microwave, 120 °C; (iv) 6 N HCl, rt; (F) **6**·HCl, $NaHCO_3$, toluene, reflux (Dean–Stark); (G) (i) arylhydrazine·HCl, PhCHO, K_2CO_3, EtOH; (ii) NaH, DMF, R^2X; (iii) 12% HCl, reflux (Dean–Stark); (iv) NaOMe, MeOH; (H) toluene, reflux (Dean–Stark); (I) 2-chloropyridine, K_2CO_3, *i*-PrOH, microwave, 180 °C, 18 h; (J) **6**·HCl, K_2CO_3, EtOH, rt; (K) (i) phenylhydrazine, EtOH, rt; (ii) $SnCl_2$, EtOH or H_2, Pd–C, HOAc, HCl (aq); (iii) Tf_2O, C_5H_5N; (L) DDQ, CH_2Cl_2.

In an effort to further optimize the insecticidal and/or acaricidal activity of hydrazone TFMS derivatives, the scope of the study was extended by condensing ketone TFMS **5** with a selection of acylhydrazines, semicarbazides, carbazates and *N*-amino heterocycles **49** to give compounds **50**–**72** (Scheme 2 and Table 2).

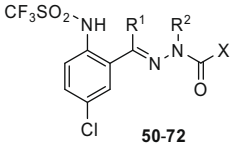


Scheme 2. Reagents and conditions:[15] (M) EtOH, 100 °C, sealed tube, 16 h; (N) EtOH, rt, 16 h; (O) EtOH, 45 °C, 16 h; (P) (i) HCO_2H/H_2O (1:1), rt; (ii) Ac_2O, 100 °C;[18] (iii) HCl, rt, 5 h;[19] (Q) toluene, reflux (Dean–Stark); (R) (i) DMF, R^2X, rt to 60 °C; (ii) $EtOH/H_2O$ (3:2), reflux 6 h; (iii) EtOH, rt, 16 h; (S) (i) (R_2 = H) acrylonitrile; (ii) acid chloride, THF, NEt_3, −70 °C to rt; (T) 1-aminohydantoin·HCl, K_2CO_3, EtOH, 80 °C, 10 h.

Substituted phenacylhydrazones **51**–**54** showed good activity against flea and excellent activity against ticks. Replacement of the R^2 alkyl group with hydrogen was not as detrimental to the flea activity of the phenacylhydrazones **55** and **56** as it was to the alkylhydrazones in Table 1. When R^2 was a phenyl group (compound **57**) poor flea activity was obtained, while R^2 = cyanoethyl gave good tick control over a range of substituents X (compounds **58**–**62**). Semicarbazones **63** and **64** (R^2 = H) showed poor activity in the rapid flea assay. Alkoxycarbonyl hydrazones **65**–**69** retained high flea activity whilst delivering outstanding tick activity, with compounds **65** (LD_{50} = 0.39 μg/tick) and **66** (LD_{50} = 0.28 μg/tick) showing in vitro efficacy comparable to that of Permethrin. Oxazolidinones **70** and **71** and hydantoin **72** were active against both parasites.

The excellent cat flea and dog tick dose response results shown by several compounds in this study provide encouragement that a single compound may be developed to possess potent insecticidal and acaricidal activity. In particular, the potent acaricides **65** and **66** are candidates for subsequent in vivo evaluation as ectoparasi-

Table 2
Activity of acyl hydrazonotrifluoromethanesulfonanilides **50**–**72** against *C. felis* (*C.f.*) and *R. sanguineus* (*R.s.*)



Compound	Method of preparation[a]	R^1	R^2	X	*C.f.* % mortality 24 h	*C.f.* LC_{50}[b] (ng/cm^2)	*R.s.* % mortality 24h[b]	*R.s.* LD_{50}[b] (μg/tick)
50	M	Me	Me	Ph	66			
51	N	Me	Me	4-Cl-Ph	100	51.3	100	1.8
52	O	Et	Me	4-Cl-Ph	100	39.3	100	1.49
53	N	Me	Me	2,4-DiCl–Ph	100	71.8	100	1.6
54	O	Et	Me	2,4-DiCl–Ph	88	42.3	100	2.0
55	N	Et	H	4-Cl-Ph	100	54.8	75	7.1
56	O	Et	H	2-Cl-Ph	86			
57	P, Q	Me	Ph	Me	22			
58	S, N	Me	CH_2CH_2CN	Me	100		90	0.53
59	S, N	Me	CH_2CH_2CN	Et	73			
60	S, N	Me	CH_2CH_2CN	*i*-Pr	100		100	
61	S, N	Me	CH_2CH_2CN	*n*-Pentyl	100		100	0.71
62	S, N	Me	CH_2CH_2CN	Ph	89		88	0.56
63	N	Me	H	NHPh	17			
64	N	Me	H	NH(4-CF_3–Ph)	18			
65	R	Me	Et	OMe	100		100	0.39
66	R	Et	Et	OMe	100		100	0.28
67	R	Me	Et	OEt	100		95	0.64
68	N	Me	Me	OEt	97		100	0.69
69	R, N	Et	Et	OEt	100		100	0.54
70	N	Me		–CH_2CH_2O–	100		95	0.99
71	Q	Et		–CH_2CH_2O–	100		100	
72	T	Me		–CH_2CONH–	100	43.8	100	1.7
Fipronil						0.6–1.0		
Permethrin								0.13–0.35

[a] See Scheme 2 for reaction conditions.
[b] No entry indicates that the compound was not assayed.

ticides. In view of the mechanism of action of the compounds reported herein, it is difficult to discern clear trends in the SAR data. It is likely that subtle structural differences which affect the physicochemical and pharmacokinetic properties of the hydrazone TFMS derivatives give rise to the observed differences in activity.[20]

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