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SYNTHESIS AND BIOLOGICAL ACTIVITY OF ANTHELMINTIC THIADIAZOLES USING AN AF-2 RECEPTOR BINDING ASSAY

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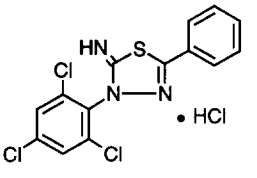
Abstract: Following our discovery of the strong binding of thiadiazole **1** to the AF-2 neuropeptide receptor of gastrointestinal nematodes (e.g., *Ascaris suum*), we prepared two series of analogs. Only the series containing the thiadiazole ring had potencies comparable to that of compound **1**. Analog **50** exhibited an apparent potency in the AF-2 binding assay 300 times that of compound **1**. © 1999 Elsevier Science Ltd. All rights reserved.

Helminths, especially parasitic nematodes, cause substantial health problems in humans and domestic animals. Currently, three distinct chemical classes are used for broad spectrum control of gastrointestinal nematodes: benzimidazoles, imidazothiazoles, and macrocyclic lactones.[1] No single drug from these chemical classes is ideally suited for all therapeutic situations, and each class has been challenged by the development of drug-resistant nematode strains.[2] Expansion of the anthelmintic arsenal is thus an urgent goal.

Recently, several natural products such as parherquamides,[3] marcfortines,[4] and PF-1022A[5] have shown promising biological activity, but undesired side effects, inadequate bioavailability or difficulty of production prohibit development of these compounds.

Our objective is to discover novel compounds that act specifically at FMRFamide-related peptide (FaRP) receptors in parasitic helminths. Since FaRPs are not useful candidates for drug development due to their poor pharmacodynamic properties, we have utilized a high-throughput screen[6] for identifying organic



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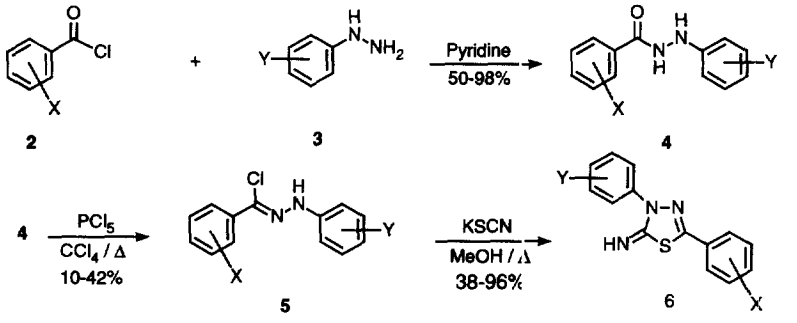
compounds that compete with the excitatory FaRP, AF-2 (Lys-His-Glu-Tyr-Leu-Arg-Phe-NH_2), for binding sites in nematode tissue. AF-2 was originally identified in extracts of the gastrointestinal nematode *Ascaris suum*, where it induces excitation and spastic paralysis *in vitro* when applied to neuromuscular strip preparations.[7] AF-2 was subsequently shown to be present in other nematodes, including *Panagrellus redivivus*,[8] *Caenorhabditis elegans*,[9] and *Haemonchus contortus*.[10] Following our discovery of the binding activity of compound **1**, we undertook an analog program to enhance this activity with the view in mind that it would ultimately lead to anthelmintic activity.

Chemistry

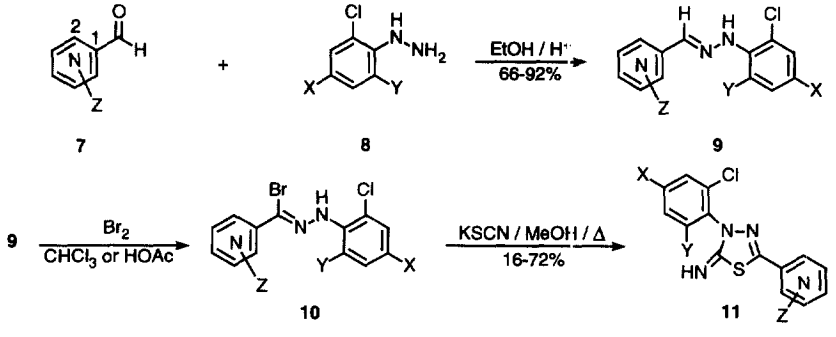
Several thiadiazole analogs were prepared from acid chloride **2** by treating it with phenylhydrazine **3** to produce hydrazide **4**. This material was chlorinated using PCl_5 followed by chromatography to give



X = H and various substituents, Y = various substituents

hydrazonoyl chloride **5**. Treatment of **5** with potassium thiocyanate in refluxing MeOH resulted in cyclization[11] which gave the thiadiazole product **6**.

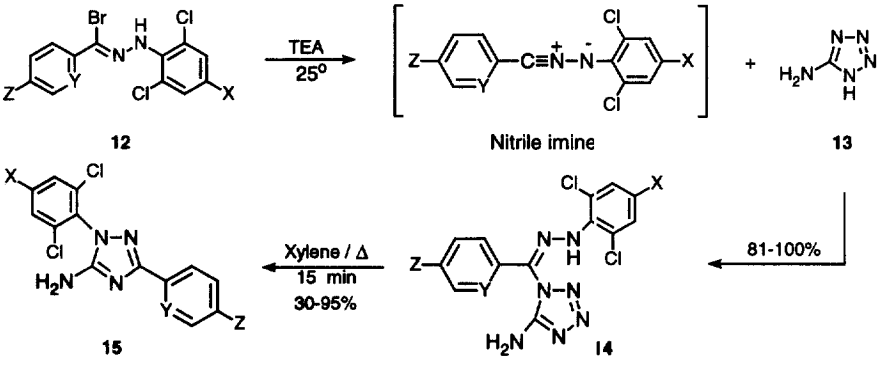
Often, it was either necessary or more convenient to start with an aldehyde rather than an acid chloride. In this alternative procedure, carboxyaldehyde **7** was treated with hydrazine **8** giving hydrazone **9**, which was then brominated to produce hydrazonoyl bromide **10**. This material was taken up in MeOH, potassium



X = Cl or CF_3, Y = Cl or CF_3, Z = various substituents, N = zero, one or two annular nitrogens

thiocyanate added and the mixture heated under reflux for 60-90 minutes to give thiadiazole **11**. In the case of some thiadiazole analogs containing a nitrogen heterocycle this method was necessary because attempts to chlorinate a hydrazide with PCl_5 failed to give the required hydrazonoyl chloride.

A second series of analogs were prepared in which the sulfur atom of the thiadiazole ring was first replaced with a nitrogen atom to give 1,2,4-triazole analogs, and later replaced with a carbon atom to give pyrazole analogs.

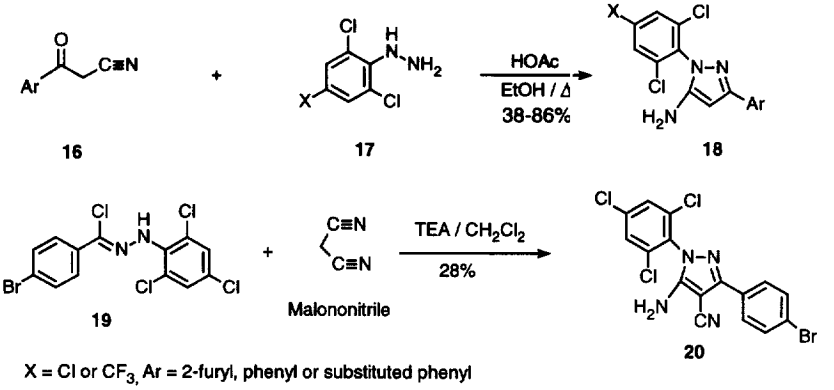


12 13 15 14

X = Cl or CF_3, Y = C or N, Z = various substituents

1,2,4-Triazoles were prepared in two steps from hydrozonoyl bromide **12**. Treatment of this material with 5-aminotetrazole **13** at room temperature in the presence of triethylamine (TEA) gave an intermediate nitrile imine followed by hydrazonoyl tetrazole **14**. Upon heating in xylene tetrazole **14** cyclized to give the triazole **15**.[12]

With the exception of one pyrazole, these analogs were prepared in a single step by reacting aroylacetonitrile **16** with phenylhydrazine **17** to give pyrazole **18**.[13] One pyrazole, compound **20**, was prepared



16 17 18 19 20

X = Cl or CF_3, Ar = 2-furyl, phenyl or substituted phenyl

by treating hydrazonoyl chloride **19** with malononitrile[14] in the presence of triethylamine.

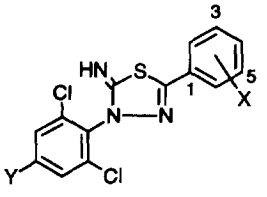
Results and Discussion

The essentiality of the 1,3,4-thiadiazole ring for AF-2 binding activity was made clear by the lack of any discernible activity in the compounds of Series 2, namely the pyrazole and triazole analogs. Only compounds in Series 1 competed with AF-2 for binding to the nematode membrane.

Among the analogs of Series 1, substituted 5-phenyl-1,3,4-thiadiazoles (Table 1), in which the Y-substituent is chlorine, changes to the X-substituent at position-4 generally reduced activity. While a fluorine in this position (**28**, 0.9 μM) marginally improved activity relative to the lead compound (**1**, ~3 μM), a trifluoromethyl group (**43**, 20 μM) in this position dramatically reduced binding. Other X-substituents at position-4 also had a marginal to deleterious effect on activity, for example **32** (4 μM) and **47** (80 μM).

Analogs in which the Y-substituent is chloro while there is an X-substituent at position-2 were also examined. A fluorine in this position (**25**, 0.5 μM) improved binding about six-fold while a methyl group (**31**,

Table 1. Series 1, Substituted 5-Phenyl-1,3,4-Thiadiazoles



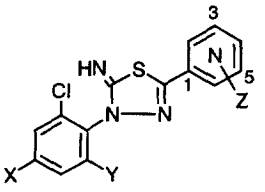
Structure (Generic)*	X	Y	IC_{50} (μM)	Structure (Generic)*	X	Y	IC_{50} (μM)
21 (11)	2-F-5-CF_3	CF_3	0.063	**35 (11)**	2-5-OMe	CF_3	4
22 (11)	2-F-5-CF_3	Cl	0.10	**36 (6)**	4-Cl	Cl	5
23 (11)	2-F	CF_3	0.15	**37 (6)**	4-NO_2	Cl	5
24 (11)	2,5-DiF	CF_3	0.37	**38 (6)**	4-OCF_3	Cl	5
25 (11)	2-F	Cl	0.50	**39 (6)**	4-CN	Cl	6
26 (11)	2-F-5-Br	CF_3	0.63	**40 (6)**	4-Me	Cl	7
27 (11)	2-F-5-NO_2	CF_3	0.63	**41 (6)**	Penta-F	Cl	8
28 (6)	4-F	Cl	0.90	**42 (6)**	4-CO_2Me	Cl	11
29 (11)	2-F-4-CF_3	Cl	1	**43 (6)**	4-CF_3	Cl	20
1 (6)	H	Cl	3	**44 (11)**	2-SCF_3	CF_3	56
30 (11)	2-F-3-CF_3	Cl	4	**45 (11)**	4-$NHCOCH_3$	Cl	70
31 (6)	2-Me	Cl	4	**46 (6)**	4-OMe	Cl	80
32 (6)	4-Br	Cl	4	**47 (11)**	2-OCF_3	CF_3	85
33 (6)	4-OCO_2Et	Cl	4	**48 (11)**	2-F-6-CF_3	CF_3	>100
34 (6)	4-OH	Cl	4	**49 (11)**	2-CO_2Me	CF_3	>100

* Generic structure identifying method of preparation.

4 μM) had little effect. When the X-substituent is 2-fluoro and the Y-substituent is changed from chloro (**25**, 0.5 μM) to trifluoromethyl (**23**, 0.15 μM) the binding activity increases three-fold, or about 20 times relative to compound **1**. A similar, albeit less dramatic, improvement in activity occurs when the X-substituent is 2-F-5-CF_3 and the Y-substituent is again changed from chloro (**22**, 0.1 μM) to trifluoromethyl (**21**, 0.063 μM), a 59%

increase in activity, making compound **21** about 48 times more potent than the original lead compound. These observations strongly suggest that potency can be increased by replacing the chlorine at position-Y with trifluoromethyl. Also, 2-F (**23**, 0.15 μM) seems to be a more effective X-substituent than 2-SCF_3 (**44**, 56 μM) and 2-OCF_3 (**47**, 85 μM). Interestingly, compound **48**, a very close analog of **21** in which the CF_3 group has

Table 2. Series 1, 5-Pyridinyl and 5-Diazinyl-1,3,4-Thiadiazoles



Structure (Generic)*	N - Position	X	Y	Z	IC_{50} (μM)
50 (11)	2	CF_3	Cl	5-CN	0.010
51 (11)	4	CF_3	Cl	5-NO_2	0.013
52 (11)	3	CF_3	Cl	5- CF_3	0.021
53 (11)	4	CF_3	Cl	5-*tert*-Bu	0.037
54 (11)	2	CF_3	Cl	H	0.090
55 (11)	2	CF_3	Cl	5-CO_2Me	0.13
56 (11)	2	Cl	CF_3	H	0.14
57 (11)	2	CF_3	Cl	5-Cl	0.16
58 (11)	2,5	CF_3	Cl	H	0.20
59 (6)	2	Cl	Cl	H	0.40
60 (11)	2	CF_3	Cl	4- CF_3-6-Cl	2.4
61 (11)	2,4	CF_3	Cl	H	2.6
62 (11)	2	CF_3	Cl	6-Me	11
63 (11)	2	CF_3	Cl	3-Br	20
64 (11)	2,3	CF_3	Cl	H	35
65 (6)	3	Cl	Cl	H	40
66 (🗁)	2	CF_3	Cl	5-CH_2NH_2	47
67 (11)	2	CF_3	Cl	2-SMe-3-CN	70

*Generic structure identifying method of preparation.
🗁 From reduction of **50** (LAH in THF).

been switched from position-5 to position-6, had no activity at all.

The analogs of Series 1, 5-pyridinyl and 5-diazinyl-1,3,4-thiadiazoles (Table 2) show the gain in potency resulting from addition of one or two annular nitrogens to the aromatic ring attached at position-5 of the thiadiazole ring. Compounds **50-59** had activity better than the lead compound while four of the compounds (**50-53**) had better activity than any of the compounds of Table 1. The pyridinyl analogs with nitrogen atoms in positions-2, -3, or -4 of the aromatic ring had better activity than the diazinyl analogs: compare **54** (0.09 μM) with **58** (0.2 μM), **61** (2.6 μM), and **64** (35 μM). Z-substituents in position-5 had a profound effect on activity: a cyano group in that position produced our most active analog (**50**, 0.01 μM)

having a potency 300 times that of compound **1** while an aminomethyl group in that position produced an analog (**66**, 47 μM) which was 15 times less active than compound **1** and about 4700 times less active than compound **50**.[15]

Each compound in Tables 1 and 2 was also tested for physiological activity on intact nematodes, using the free-living nematode *C. elegans*. AF-2 is present in *C. elegans*, where it is the most abundant FaRP detected so far.[9] In this assay, which measures drug effects on motility and development of worms in liquid culture over 7 days,[16] compound **1** and several analogs were active at concentrations ≥5 uM. However, there was generally a poor correlation between binding and physiological activity. These results suggest that other factors, such as drug penetration or species-dependent differences in receptor pharmacology, may be important determinants in the biological actions of these compounds.

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15. Aldehyde **7** [N = 2, Z = 5-CN] (260 mg, 2 mmol) and hydrazine **8** [X = CF_3, Y = Cl] (490 mg, 2 mmol) were suspended in ethanol (20 mL) and heated under reflux for 2 h. The solvent was removed and the resulting solid triturated with ether. The solid was collected and dried to give **9** (600 mg, 83 %). MS (ES-) *m/e* 357, 359 (M - H). ^{1}H NMR ($CDCl_3$) δ 7.43 (dd, *J* = 1.4, 5.1 Hz, 1H), 7.63 (s, 2H), 7.91 (s, 1H), 8.19 (s, 1H), 8.29 (s, 1H), 8.72 (d, *J* = 5.1 Hz, 1H). A solution of compound **9** (360 mg, 1 mmol) in $CHCl_3$ (20 mL) was treated bromine (0.12 mL, 2 mmol) and the mixture stirred for 16 h at room temperature. The solvent was removed and crude compound **10**, which tended to be unstable, was redissolved in MeOH (30 mL) and treated with KSCN (100 mg, 1.03 mmol) and Et_3N (0.28 mL, 2.0 mmol). The mixture was heated under reflux for 2 h. After evaporating the solvent, the residue was partitioned between CH_2Cl_2 and H_2O. The organic layer was separated, dried ($MgSO_4$) and concentrated. The residue was subjected to silica gel chromatography (1/1, EtOAc/hexane) to give compound **50** as a white solid (210 mg, 50% yield). MS (ES+) *m/e* 416, 418 (M + H). ^{1}H NMR ($CDCl_3$) δ 7.3 (br s, 1H), 7.55 (m, 1H), 7.79 (s, 2H), 8.78 (m, 1H).
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