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

Byung H. Lee, *^a Fred E. Dutton, ^a Michael F. Clothier, ^a Jerry W. Bowman, ^a John P. Davis, ^a Sandra S. Johnson, ^a Eileen M. Thomas, ^a Marjorie R. Zantello, ^a Erich W. Zinser, ^a James C. McGuire, ^b David P. Thompson, ^a and Timothy G. Geary^a

^a Animal Health Discovery Research, Pharmacia & Upjohn, Inc., Kalamazoo, MI 49001, U.S.A. ^b Discovery Technologies, Pharmacia & Upjohn, Inc., Kalamazoo, MI 49001, U.S.A.

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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. \odot 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-1022A⁵ 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 <u>FMRFamide-related peptide</u> (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 screen⁶ for identifying organic



compounds that compete with the excitatory FaRP, AF-2 (Lys-His-Glu-Tyr-Leu-Arg-Phe-NH₂), 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 cyclization¹¹ 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 = CI or CF₃, Y = CI or CF₃, 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 PCl₅ 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.



 $X = CI \text{ or } CF_3$, 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



X = CI or CF_{3.} Ar = 2-furyl, phenyl or substituted phenyl

by treating hydrazonoyl chloride 19 with malononitrile¹⁴ 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 μ M) marginally improved activity relative to the lead compound (1, ~3 μ M), a trifluoromethyl group (43, 20 μ M) 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 μ M) and 47 (80 μ M).

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 μ M) improved binding about six-fold while a methyl group (31,

$ \begin{array}{c} $										
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 ₂ Me	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	CF3	85			
33 (6)	4-OCO ₂ Et	Cl	4	48 (11)	2-F-6-CF ₃	CF ₃	>100			
34 (6)	4-OH	Cl	4	49 (11)	2-CO ₂ Me	CF ₃	>100			

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

* Generic structure identifying method of preparation.

4 μ M) had little effect. When the X-substituent is 2-fluoro and the Y-substituent is changed from chloro (25, 0.5 μ M) to trifluoromethyl (23, 0.15 μ M) 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-CF₃ and the Y-substituent is again changed from chloro (22, 0.1 μ M) to trifluoromethyl (21, 0.063 μ M), 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 μ M) seems to be a more effective X-substituent than 2-SCF₃ (44, 56 μ M) and 2-OCF₃ (47, 85 μ M). Interestingly, compound 48, a very close analog of 21 in which the CF₃ group has

$X \xrightarrow{C_{1}}^{N} N \xrightarrow{S_{1}}^{N} Z \xrightarrow{S_{2}}^{S}$										
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	Н	0.090					
55 (11)	2	CF ₃	Cl	5-CO ₂ Me	0.13					
56 (11)	2	Cl	CF ₃	Н	0.14					
57 (11)	2	CF ₃	Cl	5-Cl	0.16					
58 (11)	2,5	CF ₃	Cl	Н	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	Н	35					
65 (6)	3	Cl	Cl	Н	40					
66 (🗁)	2	CF ₃	Cl	5-CH ₂ NH ₂	47					
67 (11)	2	CF ₃	Cl	2-SMe-3-CN	70					

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

*Generic structure identifying method of preparation.

The formation 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 μ M) with **58** (0.2 μ M), **61** (2.6 μ M), and **64** (35 μ M). 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 μ M)

having a potency 300 times that of compound 1 while an aminomethyl group in that position produced an analog (66, 47 μ M) 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 = CF₃, 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). ¹H NMR (CDCl₃) 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 CHCl₃ (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 Et₃N (0.28 mL, 2.0 mmol). The mixture was heated under reflux for 2 h. After evaporating the solvent, the residue was partitioned between CH₂Cl₂ and H₂O. The organic layer was separated, dried (MgSO₄) 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). ¹H NMR (CDCl₃) δ 7.3 (br s, 1H), 7.55 (m, 1H), 7.79 (s, 2H), 8.78 (m, 1H).

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