Synthesis and Activity Optimization of Herbicidal Substituted 4-Aryl-1,2,4-triazole-5(1*H*)-thiones

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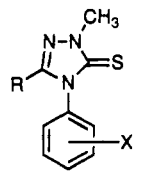
The process of sorting leads identified via screening can be made more efficient by the application of experimental design strategies. Such strategies were applied during the course of optimization of a series of 4-aryl-1,2,4-triazole-5(1*H*)-thiones which were initially identified as being herbicidal in greenhouse screens. Through application of a Free–Wilson strategy and subsequent sequential simplex optimization, potency improvements of 25 000-fold over the unsubstituted phenyl compound have been realized in a hydroponic cucumber assay. Synthesis of these analogues will be discussed as well as the development of the QSAR model which relates structural modifications to potency.

INTRODUCTION

Many agricultural and pharmaceutical companies supplement in-house discovery programs with compounds acquired from sources outside the organization. When compounds possessing lead level activity are uncovered through in-vivo screening, chemists are confronted with evaluating a lead's potential with little if any, knowledge of the mode of action or toxophore. During a 3-year period we were involved with identifying and optimizing the potency of leads identified through greenhouse screening. Our working hypothesis was the potential of a lead is best evaluated via the structure–activity response of a set of well-designed analogues rather than from the level of activity of the initially identified molecule. This requires all leads to be explored synthetically to some degree, and strategies were developed to make this exploration more efficient. This process will be illustrated by optimization of a series of substituted 4-aryl-1,2,4-triazole-5(1*H*)-thiones (I) in which the lead molecule exhibited weak activity in a herbicide greenhouse screen at 8 kg/ha.

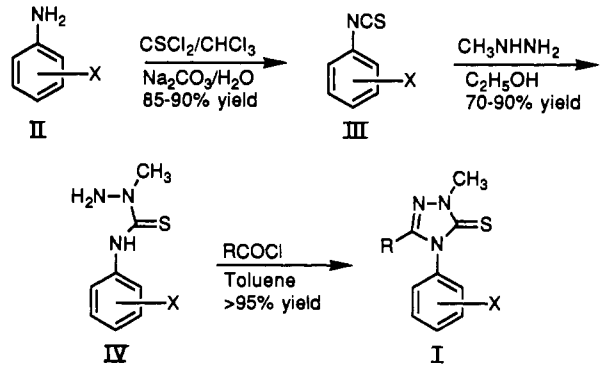


I

MATERIALS AND METHODS

Strategy. Once compounds were identified as being herbicidal in greenhouse whole-plant screens, subsequent activity optimization was driven by a laboratory hydroponic-based cucumber assay. Use of this type of assay avoids the potential complicating effects of soil binding, degradation, and root uptake. In this way, even initially weakly active analogues can be assessed as to their potential for potency improvements. Initial synthesis strategies involved identification of the allowed points of substitution in aromatic rings, followed by the application of efficient optimization strategies such as sequential simplex optimization (Burton and Nickless, 1987; Spendley et al., 1962; Darvas, 1974; Gilliom et al., 1977). The product of these strategies was identification of the likely substitution patterns required for activity and a set of analogues varying widely in physical properties in these patterns. Biological evaluation of this set of compounds afforded an assessment of the structure–activity response surface. If this response surface seemed steep, further synthesis would be warranted.

Scheme I. Synthesis of 1,2,4-Triazole-5(1*H*)-thiones[a]



[a] See Table VIII.

Biological Evaluations. *Hydroponic Cucumber Assay.* This assay was adapted from a published procedure (Ross, 1974). Cucumbers (*Cucumis sativus* cv. Wisconsin) were grown for 10 days in the required medium treated with 3-fold dilutions of the experimental compound starting at 100 μM. A pI_{50} for each compound was calculated from the relative growth inhibition measured as the weight gain after treatment relative to that of untreated controls. The fit of the experimental data to a line was evaluated using regression analysis plotting percent inhibition against the logarithm of the dose.

Greenhouse Herbicide Assay. Whole plant efficacy of the herbicides was determined in greenhouse herbicide screens on several crop and weed species. These tests were completed by filling pressed fiber pots (10 in. × 6 in. × 3 in.) with a sandy loam soil, pH 6.7, 1.9% organic matter, and planting the test species in rows, in two pots. The test species included cotton (*Gossypium hirsutum* cv. DPL61), soybean (*Glycine max* cv. Williams 82), wheat (*Triticum aestivum* cv. Wheaton), rice (*Oryza sativa* cv. Labelle), field corn (*Zea mays* cv. PN3733), ivyleaf morningglory [*Ipomoea hederacea* (L.) Jacq.], wild mustard (*Sinapsis arvensis* L.), velvetleaf (*Abutilon theophrasti* Medik.), barnyardgrass [*Echinochloa crusgalli* (L.) Beauv.], green foxtail [*Setaria viridis* (L.) Beauv.], and seeding johnsongrass [*Sorghum halepense* (L.) Pers.]. Candidate herbicides were dissolved in a 50/50 (v/v) acetone/water solution containing 0.5% (vol) Tween 20 surfactant. The herbicide solutions, serially diluted 2-fold, were applied to the pots using an overhead spray system with 8003 flat fan nozzles set to deliver 1000 L/ha at a spray pressure

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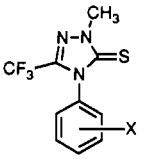
Table I. Free–Wilson Matrix



compd	X	2-Cl	3-Cl	4-Cl	5-Cl	6-Cl	cucumber pI_{50},[a] M obsd	cucumber pI_{50},[a] M predicted[b]
1	H	0	0	0	0	0	4.1	3.8
2	4-Cl	0	0	1	0	0	5.1	5.2
3	3-Cl	0	1	0	0	0	5.0	4.9
4	2-Cl	1	0	0	0	0	4.7	4.6
5	2,3-Cl_2	1	1	0	0	0	5.5	5.7
6	2,4-Cl_2	1	0	1	0	0	5.8	6.0
7	2,5-Cl_2	1	0	0	1	0	5.7	6.6
8	2,6-Cl_2	1	0	0	0	1	5.1	4.6
9	3,4-Cl_2	0	1	1	0	0	6.1	6.0
10	3,5-Cl_2	0	1	0	1	0	5.8	5.8
11	2,3,4-Cl_3	1	1	1	0	0	6.5	6.0
12	2,4,5-Cl_3	1	0	1	1	0	6.9	6.8
13	3,4,5-Cl_3	0	1	1	1	0	6.7	6.0
14	2,4,6-Cl_3	1	0	1	0	1	6.2	6.0

[a] Cucumber pI_{50} is defined under Materials and Methods. [b] Using eq 6.

of 15 psi. Following application of the herbicides the pots were placed in the greenhouse and grown under a 14-h day-length regime with temperatures of 30 °C during the day and 25 °C at night. Herbicidal activity was determined visually, 14 days after treatment, as an estimate (0–100%) of biomass reduction compared to untreated controls.

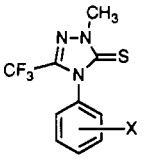
Chemical Synthesis. The synthesis of analogues of I proved to be reasonably straightforward (see Scheme I). Treatment of the substituted anilines with thiophosgene afforded the aryl isothiocyanates in excellent yields. Condensation of the isothiocyanates with alkylhydrazines in ethanol provided the 2-substituted-4-aryl thiosemicarbazides. No 1-substituted isomers could be detected. Cyclization of the thiosemicarbazides with the appropriate acid halides or anhydrides occurred smoothly in toluene at 0–20 °C. The structures of I in all cases were consistent with spectral data (^{1}H NMR, ^{13}C NMR, elemental analysis, and IR) and were confirmed for two examples by X-ray diffraction to be 1,2,4-triazole-5(1*H*)-thiones.

Aryl Isothiocyanates (III). The aniline (II) (60 mmol in 100 mL of $CHCl_3$) was added dropwise to a mixture of Na_2CO_3 (300 mmol), 60 mL of H_2O, and thiophosgene (120 mmol) cooled to 10 °C. After the addition was completed, the reaction was followed by TLC. When the aniline was consumed (typically ca. 3 h), 100 mL of $CHCl_3$ was added, the aqueous phase separated, and the organic phase dried over anhydrous Na_2CO_3. This solution was concentrated under vacuum and passed through a short column of silica gel. Solvent evaporation under vacuum left a reddish oil: IR (neat) 2050 cm^{-1}. Yields of isolated products were typically 85–90%.

4-Aryl-2-methylthiosemicarbazides (IV). Methylhydrazine (100 mmol) was added dropwise to a solution of the aryl isothiocyanate (III) (100 mmol) in 50 mL of ethanol at room temperature. After the addition was completed, the reaction mixture was stirred for about 1 h, chilled on ice, and filtered. The product was first washed with ice-cold ethanol (15 mL) and then with petroleum ether. Yields of isolated products were typically 70–90%.

4-Aryl-1-methyl-3-(trifluoromethyl)-1,2,4-triazole-5(1H)-thiones (I). A slurry of 4-aryl-2-methylthiosemicarbazide (IV) (30 mmol) in 75 mL of dry toluene was cooled to 0–10 °C, and trifluoroacetic anhydride (30 mmol) was added dropwise, during which time the reaction mixture cleared. After the reaction mixture was stirred at 0–10 °C for several hours, the starting material was completely consumed (by TLC). Fifty milliliters of 1 N Na_2CO_3 was added and the organic phase separated, dried over anhydrous Na_2CO_3, and concentrated under reduced pressure. The product was purified by flash chromatography on silica gel (eluting with CH_2Cl_2/petroleum ether 1:5). Yields of isolated products were generally greater than 95% for this cyclization.

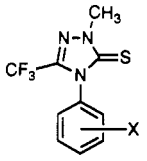
Table II. Analogues of I Synthesized during Simplex Optimizations



compd	X	cucumber pI_{50}, M obsd[a]	cucumber pI_{50}, M predicted[c]
1	H	4.1	3.8
2	4-Cl	5.1	5.2
15	4-COPh	<4	5.1
16	4-O-*n*-C_5H_{11}	5.3	5.4
17	4-$N(CH_3)_2$	5.5	5.8
18	4-$CH(CH_3)_2$	5.6	5.5
19	4-OC_2H_5	5.3[R]	5.4
20	4-$OCH(CH_3)_2$	5.5[R]	5.8
21	4-O-*n*-C_4H_9	5.3[R]	5.6
22	4-CH_3	5.4[R]	5.5
23	4-$N(C_2H_5)_2$	6.7[R]	6.2
24	4-NHPh	6.2[R]	5.8
25	4-$NCH_3(i\text{-}C_3H_7)$	6.1[R]	6.2
26	4-$N(n\text{-}C_3H_7)_2$	6.1[R]	5.8
27	4-NHC_2H_5	5.5[R]	5.5
3	3-Cl	5.0	4.9
28	3-$N(CH_3)_2$	5.1	4.9
29	3-COPh	–[b]	4.9
30	3-O-*n*-C_5H_{11}	4.7	4.9
31	3-$OCH(CH_3)_2$	5.1	4.9
32	3-CH_3	4.6[R]	4.9
33	3-CCH	5.2[R]	4.9
4	2-Cl	4.7	4.6
34	2-F	4.2	4.2
35	2-CH_3	4.6	4.3
36	2-OCH_3	3.8	4.2
37	2-CCH	4.0	4.4
38	2-CF_3	4.8[R]	4.8

[a] R designates a substituent derived from the sequential application of the simplex optimization strategy. [b] This data point is missing since 7 and 29 had been mislabeled when submitted for testing. Insufficient sample remained for retesting when the error was discovered. [c] Using eq 6.

Table III. Greenhouse Herbicide Activity of Selected Analogues

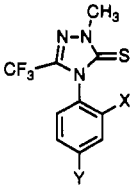


compd	X	ED_{85},[a] mol/ha
6	2,4-Cl_2	1.4
7	2,5-Cl_2	inactive[b]
9	3,4-Cl_2	3.3
10	3,5-Cl_2	3.6
12	2,4,5-Cl_3	1.2
13	3,4,5-Cl_3	1.3
23	4-$N(C_2H_5)_2$	3.2

[a] ED_{85} is the averaged value of the rate required for 85% control of barnyardgrass, green foxtail, and johnsongrass. [b] This data point is suspect as we later found that 7 and 29 had been cross-labeled during the submission process for biological testing.

N(N)-(Di)substituted-4-amino-2,6-dichloroanilines. A mixture of 2,6-dichloro-4-nitroaniline (75 mmol), 90 mL of 30% H_2O_2, 10 mL of concentrated H_2SO_4, and 300 mL of glacial acetic acid was heated at 100 °C for 2.5 h. Additional H_2O_2 (90 mL) was added and the mixture heated at 100 °C overnight. Diluting the reaction with 600 mL of H_2O, cooling to room temperature, and filtering the solid afforded 13.0 g (75% yield) of 2,6-dichloro-1,4-dinitrobenzene [mp 110–113 °C (lit. 114 °C; Pollock and

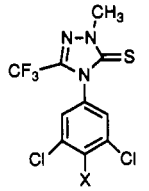
Table IV. 2,4-Disubstituted Analogues of I



compd	X	Y	cucumber pI_{50}, M obsd	cucumber pI_{50}, M predicted[a]
6	Cl	Cl	5.8	6.0
39	OCH_3	NO_2	4.6	4.3
40	$NHCOCH_3$	Cl	5.6	5.7
41	CF_3	NHCOPh	<4	6.4
42	CH_3	F	5.1	5.5
43	OC_2H_5	OC_2H_5	5.4	5.8
44	NO_2	CF_3	5.7	5.6
45	Cl	CCPh	5.9	5.2
46	CH_3	CCH	6.1	5.5
47	Cl	$N(C_2H_5)_2$	7.8	7.0
48	CF_3	$N(CH_3)_2$	6.8	6.7
49	F	F	4.6	5.3
50	Cl	$N(CH_3)_2$	6.5	6.6
51	CF_3	$OCH(CH_3)_2$	6.4	6.8
52	NO_2	$NHCOCH_3$	4.2	4.4

[a] Using eq 6.

Table V. 3,4,5-Substituted Analogues of I



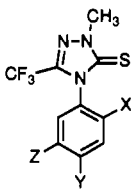
compd	X	cucumber pI_{50}, M obsd	cucumber pI_{50}, M predicted[a]
10	H	5.8	5.8
13	Cl	6.7	6.0
53	$OCH(CH_3)_2$	6.8	6.7
54	$N(C_2H_5)_2$	6.8	7.1
55	$N(CH_3)_2$	6.6	6.6
56	NH-*n*-C_4H_9	6.1	6.7
57	N(*n*-Pr)$_2$	5.6	6.6
23	4-$N(C_2H_5)_2$	6.7	6.2
47	2-Cl-4-$N(C_2H_5)_2$	7.8	7.0

[a] Using eq 6.

Stevens, 1965)]. Heating 20 mmol of this product in 40 mL of DMF with 60 mmol of the (di)alkylamine at 70 °C overnight cleanly afforded the *N*(*N*)-(di)substituted-4-nitro-2,6-dichloroaniline. Yields were typically greater than 90%. Alternatively, the reaction could be carried out using the amine component as the solvent. The 4-nitro group was readily hydrogenated in ethanol over PtO_2 to afford the desired *N*(*N*)-(di)substituted-4-amino-2,6-dichloroanilines.

2,5-Dichloro-4-alkoxyanilines. A solution of 2,5-dichlorophenol (100 mmol) in 45 mL of $CHCl_3$ was cooled to 0–10 °C. Seven milliliters of 70% HNO_3 (sp gr 1.42) was added dropwise with good stirring maintaining the reaction temperature below 10 °C. By the end of the addition the product began to crystallize. The solid was filtered and washed with ice-cold 1:1 $CHCl_3$/petroleum ether. Yield was 10.8 g (52%) of 2,5-dichloro-4-nitrophenol [mp 111–112 °C (lit. 117 °C; Pollack and Stevens, 1965)]. 2,5-Dichloro-4-nitrophenol (100 mmol), 150 mL of acetone, Na_2CO_3 (150 mmol), and the alkyl iodide (125 mmol) were combined and heated to reflux overnight. The reaction mixture was concentrated under vacuum, diluted with 200 mL of H_2O, and extracted with diethyl ether. The ether extracts were dried over anhydrous Na_2CO_3 and evaporated under vacuum to afford the 1-alkoxy-2,5-dichloro-4-nitrobenzenes. Yields were typically greater than 90%. The 4-nitro group was readily hydrogenated in ethanol over PtO_2 to provide the 2,5-dichloro-4-alkoxyanilines.

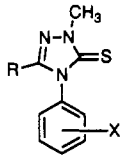
Table VI. 2,4,5-Substituted Analogues of I



compd	X	Y	Z	cucumber pI_{50}, M obsd	cucumber pI_{50}, M predicted[a]
7	Cl	H	Cl	5.7	6.6
12	Cl	Cl	Cl	6.9	6.8
58	Cl	$OCH(CH_3)_2$	Cl	7.2	7.4
59	Cl	$N(C_2H_5)_2$	Cl	8.0	7.8
60	Cl	$N(CH_3)_2$	Cl	8.5	7.4
61	OCF_2H	$OCH(CH_3)_2$	Cl	7.8	7.0
62	CH_3	CH_3	CH_3	6.0	6.6
63	Cl	$OCH(CH_3)_2$	CH_3	7.2	7.3
64	Cl	$OCH(CH_3)_2$	OCF_2H	7.5	7.3
65	Cl	$OCH(CH_3)_2$	OCH_3	6.2	6.6
66	F	$OCH(CH_3)_2$	Cl	7.4	7.0
67	Br	$OCH(CH_3)_2$	Br	7.8	7.8
68	CF_3	$OCH(CH_3)_2$	Cl	6.8	7.6
69	F	$N(C_2H_5)_2$	F	6.6	6.7
70	SCH_3	$OCH(CH_3)_2$	Cl	7.4	7.4
71	Cl	OCH_3	Cl	6.7	6.8
72	F	OCH_3	Cl	6.8	6.3
73	Cl	NHC_2H_5	Cl	7.8	7.1
74	Cl	$OCH(CH_2)_4$	Cl	6.8	7.4
75	Cl	$OCH(CH_2)_3$	Cl	8.1	–[b]
76	Cl	$OC(CH_3)_3$	Cl	7.2	–[b]

[a] Using eq 6. [b] We did not have σ_p substituent constant values for these analogues.

Table VII. 3′-Substituted Analogues of I



compd	R	X	pI_{50}, M	cucumber –log RP[a] obsd	cucumber –log RP[a] predicted[b]
23	CF_3	4-NEt_2	6.7	0.0	–0.05
77	$CF_2CF_2CF_3$	4-NEt_2	5.1	–1.6	–1.8
78	CO_2CH_3	4-NEt_2	<4	<–2.7	–1.7
79	COC_2H_5	4-NEt_2	<4	<–2.7	–4.4
80	$CON(CH_3)(OCH_3)$	4-NEt_2	<4	<–2.7	–2.6
58	CF_3	2,5-Cl_2 4-OiPr	7.2	0.0	–0.05
81	CF_2H	2,5-Cl_2 4-OiPr	7.1	–0.1	–0.9
82	CF_2Cl	2,5-Cl_2 4-OiPr	6.8	–0.4	–0.3
83	CF_2CF_3	2,5-Cl_2 4-OiPr	6.2	–1.0	–0.8
84	CF_2CH_3	2,5-Cl_2 4-OiPr	6.1	–1.1	–0.5
85	Cl	2,5-Cl_2 4-OiPr	5.0	–2.2	–0.8
86	CN	2,5-Cl_2 4-OiPr	5.1	–2.1	–2.1
87	$N(CH_3)_2$	2,5-Cl_2 4-OiPr	<5	<–2.2	–1.8
88	OCH_3	2,5-Cl_2 4-OiPr	<5	<–2.2	–2.7
89	H	2,5-Cl_2 4-OiPr	4.8	–2.4	–2.1

[a] log RP is the relative potency defined as [pI_{50}(R ≠ CF_3) – pI_{50}(R = CF_3)] for each substitution pattern in the aromatic ring. [b] Using eq 7.

N-Substituted-4-amino-2,5-dichloroanilines. 2,5-Dichloroaniline (100 mmol) and 4-methylbenzenesulfonyl chloride (100 mmol) were combined in 35 mL of dry pyridine and heated to reflux overnight. The reaction was cooled to room temperature and poured into 85 mL of 6 N HCl. The solid was filtered, washed with H_2O, and vacuum dried to afford the *N*-(2,5-dichlorophenyl)-

Table VIII. Physical Property Data for I

compd	mp, °C	molecular formula	anal. (C, H, N) calcd/found	^{1}H NMR ($CDCl_3$), δ, multiplicity[a] (area)
1	oil	$C_{10}H_8N_3F_3S$	46.33/46.12, 3.11/2.88, 16.21/16.41	3.90 s (3 H), 7.35 m (2 H), 7.59 m (3 H)
2	137–140	$C_{10}H_7ClN_3F_3S$	40.96/40.94, 2.41/2.45, 14.34/14.43	3.90 s (3 H), 7.25 m (2 H), 7.55 m (2 H)
3	81–83	$C_{10}H_7ClN_3F_3S$	40.96/40.97, 2.41/2.27, 14.34/14.36	3.88 s (3 H), 7.20–7.60 m (4 H)
4	119–121	$C_{10}H_7ClN_3F_3S$	40.96/40.66, 2.41/2.26, 14.34/14.28	3.90 s (3 H), 7.35–7.65 m (4 H)
5	118–120	$C_{10}H_6Cl_2N_3F_3S$	36.60/36.82, 1.85/2.02, 12.85/12.55	3.90 s (3 H), 7.30 dd (1 H), 7.45 t (1 H), 7.70 dd (1 H)
6	oil	$C_{10}H_6Cl_2N_3F_3S$	36.60/36.42, 1.84/1.70, 12.81/13.03	3.90 s (3 H), 7.30 d (1 H), 7.45 dd (1 H), 7.63 d (1 H)
7	149–151	$C_{10}H_6Cl_2N_3F_3S$	36.60/36.51, 1.85/1.95, 12.85/12.59	3.90 s (3 H), 7.40 d (1 H), 7.50–7.60 m (2 H)
8	117–119	$C_{10}H_6Cl_2N_3F_3S$	36.60/36.88, 1.85/1.99, 12.85/12.61	3.90 s (3 H), 7.4–7.55 m (3 H)
9	96–97	$C_{10}H_6Cl_2N_3F_3S$	36.60/36.47, 1.85/1.74, 12.85/12.83	3.90 s (3 H), 7.20 dd (1 H), 7.45 d (1 H), 7.65 d (1 H)
10	130–131	$C_{10}H_6Cl_2N_3F_3S$	36.60/36.41, 1.85/1.71, 12.85/12.70	3.85 s (3 H), 7.25 d (2 H), 7.58 d (1 H)
11	125–127	$C_{10}H_5Cl_3N_3F_3S$	33.13/32.99, 1.39/1.33, 11.59/11.52	3.90 s (3 H), 7.23 d (1 H), 7.60 d (1 H)
12	123–125	$C_{10}H_5Cl_3N_3F_3S$	MS $[M - Cl]^+$ 325.9533/325.9531	3.85 s (3 H), 7.45 s (1 H), 7.75 s (1 H)
13	118–121	$C_{10}H_5Cl_3N_3F_3S$	33.13/33.28, 1.39/1.28, 11.59/11.77	3.90 s (3 H), 7.43 s (2 H)
14	80–84	$C_{10}H_5Cl_3N_3F_3S$	33.13/33.25, 1.39/1.30, 11.59/11.09	3.90 s (3 H), 7.60 s (2 H)
15	141–142	$C_{17}H_{12}N_3F_3OS$	56.19/56.32, 3.33/3.30, 11.57/11.29	3.90 s (3 H), 7.5 m (4 H), 7.63 m (1 H), 7.85 d (2 H), 8.00 d (2 H)
16	52–53	$C_{15}H_{18}N_3F_3OS$	52.16/52.39, 5.25/5.20, 12.17/12.09	0.95 t (3 H), 1.43 m (4 H), 1.83 m (2 H), 3.90 s (3 H), 4.03 t (2 H), 7.05 d (2 H), 7.25 d (2 H)
17	151–152	$C_{12}H_{13}N_4F_3S$	47.67/47.41, 4.34/4.16, 18.54/18.38	3.00 s (6 H), 3.90 s (3 H), 6.75 d (2 H), 7.15 d (2 H)
18	oil	$C_{13}H_{14}N_3F_3S$	51.81/52.60, 4.69/4.27, 13.95/13.64	1.30 d (6 H), 3.00 m (1 H), 3.90 s (3 H), 7.25 d (2 H), 7.40 d (2 H)
19	93–95	$C_{12}H_{12}N_3F_3OS$	47.51/47.67, 3.99/4.15, 13.86/13.73	1.43 t (3 H), 3.85 s (3 H), 4.05 q (2 H), 7.00 d (2 H), 7.20 d (2 H)
20	89–91	$C_{13}H_{14}N_3F_3OS$	49.20/49.02, 4.45/4.28, 13.25/13.13	1.35 d (6 H), 3.85 s (3 H), 4.60 m (1 H), 7.00 d (2 H), 7.25 d (2 H)
21	69–70	$C_{14}H_{16}N_3F_3OS$	50.74/50.91, 4.87/4.85, 12.69/12.39	0.95 t (3 H), 1.45 m (2 H), 1.70 m (2 H), 3.85 s (3 H), 4.00 t (2 H), 7.05 d (2 H), 7.20 d (2 H)
22	102–104	$C_{11}H_{10}N_3F_3S$	48.34/48.24, 3.69/3.72, 15.38/15.58	2.43 s (3 H), 3.90 s (3 H), 7.20 d (2 H), 7.35 d (2 H)
23	110–113	$C_{14}H_{17}N_4F_3S$	50.90/50.88, 5.19/5.17, 16.96/17.19	1.20 t (6 H), 3.40 q (4 H), 3.90 s (3 H), 6.70 d (2 H), 7.10 d (2 H)
24	177–179	$C_{16}H_{13}N_4F_3S$	54.85/55.00, 3.74/3.86, 15.99/15.77	3.90 s (3 H), 6.05 br s (1 H), 7.00–7.20 m (7 H), 7.30–7.40 m (2 H)
25	85–86	$C_{14}H_{17}N_4F_3S$	50.90/50.79, 5.19/4.95, 16.96/16.78	1.15 d (6 H), 2.75 s (3 H), 3.85 s (3 H), 4.15 m (1 H), 6.80 d (2 H), 7.15 d (2 H)
26	gum	$C_{16}H_{21}N_4F_3S$	53.62/53.34, 5.90/5.74, 15.63/15.31	0.95 t (6 H), 1.62 m (4 H), 3.25 t (4 H), 3.90 s (3 H), 6.63 d (2 H), 7.05 d (2 H)
27	139–142	$C_{12}H_{13}N_4F_3S$	47.67/47.76, 4.34/4.43, 18.85/18.42	1.25 t (3 H), 3.20 m (2 H), 3.90 s (3 H), 3.95 br s (1 H), 6.65 d (2 H), 7.10 d (2 H)
28	oil	$C_{12}H_{13}N_4F_3S$	47.68/47.34, 4.33/4.32, 18.53/18.14	2.95 s (6 H), 3.85 s (3 H), 6.60 m (2 H), 6.85 dd (1 H), 7.35 t (1 H)
29	122–124	$C_{17}H_{12}N_3F_3OS$	56.20/56.32, 3.31/3.48, 11.57/11.30	3.90 s (3 H), 7.40–8.10 m (9 H)
30	oil	$C_{15}H_{18}N_3F_3OS$	52.16/51.96, 5.25/5.18, 12.17/12.42	0.95 t (3 H), 1.40 m (4 H), 1.80 m (2 H), 3.85 s (3 H), 3.95 t (2 H), 6.85 m (2 H), 7.05 dd (1 H), 7.40 t (1 H)
31	oil	$C_{13}H_{14}N_3F_3OS$	49.21/49.50, 4.42/4.71, 13.25/13.09	1.30 d (6 H), 3.85 s (3 H), 4.55 m (1 H), 6.90 m (2 H), 7.05 dd (1 H), 7.40 t (1 H)
32	oil	$C_{11}H_{10}N_3F_3S$	48.35/48.45, 3.69/3.55, 15.38/15.27	2.40 s (3 H), 3.85 s (3 H), 7.15 m (2 H), 7.40 m (2 H)
33		$C_{12}H_8N_3F_3S$	MS M^+ 283.0391/283.0390	3.18 s (1 H), 3.90 s (3 H), 7.30 dd (1 H), 7.45 d (1 H), 7.55 t (1 H), 7.65 dd (1 H)
34	59–61	$C_{10}H_7N_3F_4S$	43.32/43.60, 2.54/2.57, 15.16/14.98	3.86 s (3 H), 7.30 m (3 H), 7.55 m (1 H)
35	89–91	$C_{11}H_{10}N_3F_3S$	48.35/48.07, 3.69/3.63, 15.38/15.50	2.15 s (3 H), 3.90 s (3 H), 7.15 d (1 H), 7.30–7.50 m (3 H)
36	138–140	$C_{11}H_{10}N_3F_3OS$	45.67/45.62, 3.46/3.18, 14.53/14.37	3.78 s (3 H), 3.90 s (3 H), 7.10 d (2 H), 7.26 d (1 H), 7.52 dt (1 H)
37	90–91	$C_{12}H_8N_3F_3S$	50.88/51.28, 2.83/2.81, 14.84/14.12	3.20 s (1 H), 3.90 s (3 H), 7.30 m (1 H), 7.55 m (2 H), 7.70 m (1 H)
38	145–147	$C_{11}H_7N_3F_6S$	40.37/40.68, 2.16/2.17, 12.84/12.61	3.90 s (3 H), 7.30–7.90 m (4 H)
39	138–140	$C_{11}H_9N_4F_3O_3S$	39.52/39.63, 2.71/2.54, 16.76/16.83	3.85 s (3 H), 3.95 s (3 H), 7.50 d (1 H), 8.00 m (2 H)
40	207–210	$C_{12}H_{10}ClN_4F_3OS$	41.14/40.81, 2.88/2.62, 16.00/15.76	2.00 s (3 H), 3.80 s (3 H), 7.45 dd (1 H), 7.50 d (1 H), 8.30 d (1 H), 9.50 s (1 H)
41	220–222	$C_{18}H_{12}N_4F_6OS$	MS M^+ 446	3.90 s (3 H), 7.35 d (1 H), 7.50–7.65 m (3 H), 7.90 d (2 H), 8.20 m (3 H)
42	75–76	$C_{11}H_9N_3F_4S$	45.36/45.28, 3.11/3.06, 14.43/14.15	2.15 s (3 H), 3.90 s (3 H), 7.00–7.20 m (3 H)
43	oil	$C_{14}H_{16}N_3F_3OS$	MS M^+ 347.0915/347.0916	1.30 t (3 H), 1.43 t (3 H), 3.90 s (3 H), 4.05 m (4 H), 6.55 m (2 H), 7.15 d (1 H)
44		$C_{11}H_6N_4F_6O_2S$	MS M^+ 372.0116/372.0124	3.90 s (3 H), 7.65 d (1 H), 8.15 d (1 H), 8.60 s (1 H)
45	50–53	$C_{18}H_{11}ClN_3F_3S$	54.89/54.79, 2.80/2.69, 10.67/10.70	3.90 s (3 H), 7.30–7.40 m (4 H), 7.50–7.60 m (3 H), 7.75 d (1 H)
46	oil	$C_{13}H_{10}N_3F_3S$	52.53/52.29, 3.37/3.09, 14.14/13.87	2.15 s (3 H), 3.20 s (1 H), 3.85 s (3 H), 7.15 d (1 H), 7.45 dd (1 H), 7.55 bs (1 H)
47	108–110	$C_{14}H_{16}ClN_4F_3S$	MS M^+ 364	1.20 t (6 H), 3.37 q (4 H), 3.90 s (3 H), 6.60 dd (1 H), 6.75 d (1 H), 7.05 d (1 H)
48	114–116	$C_{13}H_{12}N_4F_6S$	42.16/42.40, 3.27/2.98, 15.13/15.02	3.05 s (6 H), 3.86 s (3 H), 6.85 dd (1 H), 6.95 d (1 H), 7.10 d (1 H)
49	80–85	$C_{10}H_6N_3F_5S$	MS $[M - F]^+$ 276.0219/276.0217	3.92 s (3 H), 7.25 m (1 H), 7.40 m (1 H), 7.58 m (1 H)
50	122–125	$C_{12}H_{12}ClN_4F_3S$	42.79/42.83, 3.57/3.42, 16.64/16.70	3.00 s (6 H), 3.90 s (3 H), 6.63 dd (1 H), 6.80 d (1 H), 7.13 d (1 H)
51	78–80	$C_{14}H_{13}N_3F_6OS$	MS M^+ 385.0683/385.0670	1.38 s (3 H), 1.42 s (3 H), 3.90 s (3 H), 4.63 m (1 H), 7.15–7.30 m (3 H)
52	gum	$C_{12}H_{10}N_5F_3O_3S$		2.25 s (3 H), 3.90 s (3 H), 7.40 d (1 H), 7.70 br s (1 H), 8.13 dd (1 H), 8.45 d (1 H)
53		$C_{13}H_{12}Cl_2N_3F_3OS$	40.43/40.78, 3.13/3.02, 10.88/10.74	1.40 d (6 H), 3.86 s (3 H), 4.75 m (1 H), 7.30 s (2 H)
54	128–130	$C_{14}H_{15}Cl_2N_4F_3S$	MS M^+ 398	1.05 t (6 H), 3.25 q (4 H), 3.90 s (3 H), 7.30 s (2 H)
55	gum	$C_{12}H_{11}Cl_2N_4F_3S$	38.81/38.55, 2.96/3.14, 15.09/14.84	2.95 s (6 H), 3.85 s (3 H), 7.25 s (2 H)
56	73–75	$C_{14}H_{15}Cl_2N_4F_3S$	42.11/42.15, 3.76/3.74, 14.04/14.11	0.95 t (3 H), 1.45 m (2 H), 1.60 m (2 H), 3.50 t (2 H), 3.85 s (3 H), 4.35 br s (1 H), 7.20 s (2 H)
57	118–121	$C_{16}H_{19}Cl_2N_4F_3S$	45.06/45.51, 4.48/4.51, 13.15/13.04	0.85 t (6 H), 1.50 m (4 H), 3.15 t (4 H), 3.85 s (3 H), 7.25 s (2 H)
58	97–99	$C_{13}H_{12}Cl_2N_3F_3OS$	40.43/40.70, 3.13/3.17, 10.88/10.71	1.40 d (3 H), 1.45 d (3 H), 3.90 s (3 H), 4.60 m (1 H), 7.07 s (1 H), 7.35 s (1 H)
59	oil	$C_{14}H_{14}Cl_2N_4F_3S$	42.12/42.39, 3.79/3.79, 14.03/13.89	1.13 t (6 H), 3.23 q (4 H), 3.87 s (3 H), 7.15 s (1 H), 7.30 s (1 H)
60	105–106	$C_{12}H_{11}Cl_2N_4F_3S$	38.83/38.53, 2.99/2.72, 15.09/15.04	2.90 s (6 H), 3.90 s (3 H), 7.12 s (1 H), 7.28 s (1 H)
61	104–105	$C_{14}H_{13}ClN_3F_5O_2S$	40.25/40.39, 3.14/3.43, 10.06/9.99	1.40 s (3 H), 1.44 s (3 H), 3.87 s (3 H), 4.60 m (1 H), 6.53 dd (1 H), 6.90 s (1 H), 7.35 s (1 H)
62	63–66	$C_{13}H_{14}N_3F_3S$	51.82/51.95, 4.68/4.76, 13.94/13.94	2.05 s (3 H), 2.20 s (3 H), 2.25 s (3 H), 3.85 s (3 H), 6.95 s (1 H), 7.15 s (1 H)
63	83–84	$C_{14}H_{15}ClN_3F_3OS$	MS $[M - Cl]^+$ 330.0888/330.0893	1.40 s (3 H), 1.43 s (3 H), 2.20 s (3 H), 3.90 s (3 H), 4.60 m (1 H), 6.95 s (1 H), 7.06 d (1 H)
64	oil	$C_{14}H_{13}ClN_3F_5O_2S$	MS $[M - Cl]^+$ 382.0649/382.0656	1.40 s (3 H), 1.43 s (3 H), 3.85 s (3 H), 4.63 m (1 H), 6.55 t (1 H), 7.10 s (1 H), 7.15 s (1 H)

Table VIII. (Continued) Physical Property Data for I

compd	mp, °C	molecular formula	anal. (C, H, N) calcd/found	^{1}H NMR ($CDCl_3$), δ, multiplicity[a] (area)
65	gum	$C_{14}H_{15}ClN_3F_3O_2S$	44.04/43.84, 3.96/4.20, 11.00/10.95	1.40 s (3 H), 1.43 s (3 H), 3.83 s (3 H), 3.90 s (3 H), 4.62 m (1 H), 6.80 s (1 H), 7.05 s (1 H)
66	oil	$C_{13}H_{12}ClN_3F_4OS$	42.23/42.03, 3.27/3.09, 11.36/11.42	1.38 d (3 H), 1.43 d (3 H), 3.90 s (3 H), 4.60 m (1 H), 6.85 d (1 H), 7.35 d (1 H)
67	48–52	$C_{13}H_{12}Br_2N_3F_3O$	32.84/33.09, 2.53/2.59, 8.84/8.91	1.40 d (3 H), 1.45 d (3 H), 3.90 s (3 H), 4.60 m (1 H), 7.20 s (1 H), 7.5 s (1 H)
68	70–72	$C_{14}H_{12}ClN_3F_6OS$	40.06/40.09, 2.88/2.82, 10.01/9.67	1.43 d (3 H), 1.45 d (3 H), 3.90 s (3 H), 4.70 m (1 H), 7.25 s (1 H), 7.35 s (1 H)
69	88–89	$C_{14}H_{15}N_4F_5S$	45.90/45.64, 4.13/3.95, 15.29/15.16	1.20 t (6 H), 3.35 q (4 H), 3.90 s (3 H), 6.63 dd (1 H), 6.95 dd (1 H)
70	120–121	$C_{14}H_{15}ClN_3F_3OS_2$	42.27/42.54, 3.80/3.92, 10.56/10.60	1.38 d (3 H), 1.43 d (3 H), 2.43 s (3 H), 3.85 s (3 H), 4.63 m (1 H), 6.95 s (1 H), 7.25 s (1 H)
71	141–143	$C_{11}H_8Cl_2N_3F_3OS$	36.89/37.02, 2.25/2.19, 11.73/11.44	3.85 s (3 H), 3.96 s (3 H), 7.15 s (1 H), 7.40 s (1 H)
72	123–124	$C_{11}H_8ClN_3F_4OS$	38.66/38.42, 2.36/2.38, 12.30/12.30	3.85 s (3 H), 3.95 s (3 H), 6.85 d (1 H), 7.35 d (1 H)
73	129–131	$C_{12}H_{11}Cl_2N_4F_3S$	38.83/38.59, 2.99/2.90, 15.09/14.95	1.35 t (3 H), 3.25 br q (2 H), 3.90 s (3 H), 4.60 br m (1 H), 6.85 s (1 H), 7.20 s (1 H)
74	133–136	$C_{15}H_{14}Cl_2N_3F_3OS$	43.69/43.82, 3.40/3.53, 10.19/10.05	1.65 m (2 H), 1.90 m (2 H), 1.96 m (4 H), 3.90 s (3 H), 4.83 m (1 H), 7.10 s (1 H), 7.35 s (1 H)
75	104–108	$C_{14}H_{12}Cl_2N_3F_3OS$	42.21/42.38, 3.02/2.84, 10.55/10.85	1.75 m (1 H), 1.95 m (1 H), 2.30 m (2 H), 2.55 m (2 H), 3.90 s (3 H), 4.70 m (1 H), 6.95 s (1 H), 7.37 s (1 H)
76	87–88	$C_{14}H_{14}Cl_2N_3F_3OS$	MS M^+ 399	1.52 s (9 H), 3.90 s (3 H), 7.35 s (1 H), 7.40 s (1 H)
77	109–110	$C_{16}H_{17}N_4F_7S$	44.65/44.69, 3.98/3.73, 13.02/13.06	1.20 t (6 H), 3.40 q (4 H), 3.93 s (3 H), 6.70 d (2 H), 7.05 d (2 H)
78	168–169	$C_{15}H_{20}N_4O_2S$	56.23/56.10, 6.29/6.13	1.15 t (6 H), 3.40 q (4 H), 3.75 s (3 H), 3.80 s (3 H), 6.70 d (2 H), 7.10 d (2 H)
79	148–149	$C_{16}H_{22}N_4OS$	60.35/60.52, 6.96/6.77, 17.59/17.43	1.10 t (3 H), 1.18 t (6 H), 2.95 q (2 H), 3.35 q (4 H), 3.90 s (3 H), 6.70 d (2 H), 7.05 d (2 H)
80	191–193	$C_{16}H_{23}N_5O_2S$	54.99/55.21, 6.63/6.69, 20.04/19.71	1.15 t (6 H), 3.20 s (3 H), 3.35 q (4 H), 2.65 s (3 H), 3.90 s (3 H), 6.65 d (2 H), 7.15 d (2 H)
81	110–113	$C_{13}H_{13}Cl_2N_3F_2OS$	42.39/42.32, 3.53/3.39, 11.41/11.28	1.40 s (3 H), 1.43 s (3 H), 3.85 s (3 H), 4.60 m (1 H), 6.55 t (1 H), 7.15 s (1 H), 7.40 s (1 H)
82	oil	$C_{13}H_{12}Cl_3N_3F_2OS$	MS M^+ 401	1.43 d (6 H), 3.90 s (3 H), 4.65 m (1 H), 7.15 s (1 H), 7.22 s (1 H)
83	87–90	$C_{14}H_{12}Cl_2N_3F_5OS$	38.53/38.73, 2.75/2.71, 9.63/9.56	1.40 d (6 H), 3.90 s (3 H), 4.55 m (1 H), 7.05 s (1 H), 7.35 s (1 H)
84	99–102	$C_{14}H_{15}Cl_2N_3F_2OS$	43.98/43.93, 3.93/3.71, 10.99/10.80	1.40 s (3 H), 1.43 s (3 H), 2.03 t (3 H), 3.85 s (3 H), 4.60 m (1 H), 7.05 s (1 H), 7.35 s (1 H)
85	69–72	$C_{12}H_{12}Cl_3N_3OS$	40.85/41.12, 3.40/3.66, 11.91/11.99	1.40 s (3 H), 1.43 s (3 H), 3.65 s (3 H), 4.45 m (1 H), 6.98 s (1 H), 7.01 s (1 H)
86	103–105	$C_{13}H_{12}Cl_2N_4OS$	MS M^+ 342.0109/342.0111	1.38 s (3 H), 1.41 s (3 H), 3.80 s (3 H), 4.50 m (1 H), 7.00 s (1 H), 7.02 s (1 H)
87	131–134	$C_{14}H_{18}Cl_2N_4OS$	MS M^+ 360	1.40–1.43 br s (6 H), 2.68 s (6 H), 3.78 s (3 H), 4.60 m (1 H), 7.10 s (1 H), 7.38 s (1 H)
88	oil	$C_{13}H_{18}Cl_2N_3O_2S$	MS M^+ 347.0262/347.0262	1.38 s (3 H), 1.40 s (3 H), 3.58 s (3 H), 3.95 s (3 H), 4.48 m (1 H), 7.00 s (1 H), 7.03 s (1 H)
89	101–103	$C_{12}H_{13}Cl_2N_3OS$	MS M^+ 317.0156/317.0109	1.42 d (6 H), 3.85 s (3 H), 4.62 m (1 H), 7.10 s (1 H), 7.50 s (1 H), 7.85 s (1 H)

[a] s, singlet; d, doublet; t, triplet; q, quartet; br, broadened; dd, doublet of doublets; dt, doublet of triplets; m, multiplet.

4′-methylbenzenesulfonamide in a yield of 98% (mp 130–134 °C). The sulfonamide (100 mmol) was added to a mixture of 145 mL of H_2O and 4.2 mL of fuming HNO_3. Then 145 mL of glacial acetic acid and 0.8 g of $NaNO_2$ were added, and the mixture was heated overnight at reflux. The next day, after the mixture was cooled to 25 °C, 0.8 mL of fuming HNO_3 and 0.3 g of $NaNO_2$ were added and the mixture was heated to reflux for 3 h. The reaction was allowed to cool and diluted with 165 mL of H_2O, and the solid was filtered. Recrystallization from 95% ethanol afforded 25 g (69%) of *N*-(2,5-dichloro-4-nitrophenyl)-4′-methylbenzenesulfonamide (mp 180–183 °C). The sulfonamide (100 mmol) was heated to 90 °C for 6 h with 90% H_2SO_4 (48 mL). The reaction was cooled to room temperature and poured onto 1000 g of ice. The solid was filtered, washed with water, and vacuum dried to afford 2,5-dichloro-4-nitroaniline [97%, mp 152–154 °C (lit. 157 °C; Pollack and Stevens, 1965)]. The 2,5-dichloro-4-nitroaniline was elaborated into the *N*-substituted-2,5-dichloro-4-nitroanilines through two approaches. The first involved acylation of the aniline in CH_2Cl_2 with a carboxylic anhydride and a catalytic amount of 4-(dimethylamino)pyridine. The amides could be readily reduced to the *N*-alkylanilines using borane/methyl sulfide in THF. This cycle could be repeated if *N*,*N*-dialkylanilines were desired. In those cases where the *N*,*N*-dialkylanilines bore the same alkyl groups, the Eschweiler–Clark alkylation of the aniline was performed using the aldehyde and formic acid (Moore, 1977). The 4-nitro group was readily hydrogenated in ethanol over PtO_2 to afford the *N*-substituted-4-amino-2,5-dichloroanilines in excellent yields.

Structure–Activity Studies. Values for the physiochemical parameters π, σ, *F*, *R*, *L*, *B*1, and *B*4 were taken from the literature (Hansch and Leo, 1978; Hansch et al., 1991). π is the Hansch hydrophobicity index (Hansch and Fujita, 1964); σ is the Hammett sigma constant (Hammett, 1940; Hansch and Fujita, 1964); *F* and *R* are the Swain and Lupton field and resonance parameters (Swain and Lupton, 1968; Hansch et al., 1973); and *L*, *B*1 and *B*4 are the sterimol length, minimum radius, and maximum radius parameters (Verloop et al., 1976). Regression analyses were performed using standard statistical packages (Dixon, 1988) operating on a VAX 6350 computer.

RESULTS AND DISCUSSION

On the basis of biological evaluation of a small set of analogues, synthesis was directed at identifying an optimal pattern of substitution in the aromatic ring while keeping R trifluoromethyl in I. Various aromatic substitution patterns were explored using a Free–Wilson approach by synthesizing and testing chlorine-substituted analogues (Free and Wilson, 1964). The Free–Wilson matrix for this set is presented in Table I. Statistical analysis of this matrix using standard regression techniques afforded the following contributions to potency for chlorine in each position of the aromatic ring (Kubinyi, 1988):

2-Cl	0.7 (±0.06)	5-Cl	1.0 (±0.06)
3-Cl	0.7 (±0.06)	6-Cl	0.4 (±0.08)
4-Cl	1.0 (±0.05)		

These data suggested that substitution by chlorine in the 2-, 3-, 4-, or 5-position contributes significantly to potency. The group contribution for the 6-position was probably not significant since its magnitude was comparable to the test-to-test variance of the hydroponic cucumber assay. If one's goal was to explore only monosubstituted analogues, then optimization should occur in the 4-position. If one wanted to explore disubstituted analogues, then the

optimization should be focused on the 4,5-positions except that in this case, due to the free rotation of the phenyl group, a unique 5-position requires a substituent in the 2- or 3-position. Other disubstituted patterns to explore, based upon the size of the group potency contributions, would be the 2,4-, 2,5-, 3,4-, or 3,5-positions. Exploring trisubstituted analogues would lead one to work in the 2,4,5- or 3,4,5-positions. Using the Free–Wilson analysis in this way avoids exploring all positions in a molecule at the early stages and keeps one focused on those positions that are most interesting.

Concurrent with establishing the substitution pattern in the aromatic ring, sets of analogues were synthesized in each of the ortho, meta, and para positions. These sets were selected to represent the starting points of a simplex design in the physiochemical parameter space of π, F, R, L, and $B1$. These parameters were chosen upon the expectation that lipophilicity, electronics, and steric effects of substituents often influence biological activity. Each simplex set was processed through several reflections to explore the structure–activity response surface of I. The initial simplex sets and those targets predicted via application of the simplex algorithm are presented in Table II. The simplex algorithm used in these studies was adapted from published procedures (Plummer, 1991).

The structure–activity surface for this lead was responsive and, as such, justified further synthesis directed at understanding this response surface. Some of the most potent analogues identified by the Free–Wilson and simplex optimizations were evaluated side-by-side in a replicated pre-emergent greenhouse test. The direction of further work was prioritized based upon the results in Table III.

Compounds **6**, **12**, and **13** were the most efficacious in greenhouse testing. This information, coupled with the 630-fold improvement in potency relative to the parent compound (Table I, **12** vs **1**), suggested exploration of each of these substitution patterns (i.e., 2,4, 2,4,5, and 3,4,5). Exploration commenced with the 2,4-pattern, since 2,4-disubstituted anilines were more readily available and less costly than the other substituted anilines.

Cluster analysis (Hansch and Unger, 1973) was used to select a set of 2,4-disubstituted analogues varying widely in the physiochemical parameters π, F, R, and MR (Hansch and Leo, 1978). As before, parameters were chosen to represent lipophilicity, electronics, and sterics. To represent two positions simultaneously in the clustering process, pairs of substituents were created and their physiochemical properties (π, F, R, and MR in this instance) summed across positions. The clustering algorithm was applied to the property sums. Although clustering on properties summed across positions does not assure good variation in the properties at each position, this was the best that could be achieved at that time (Simmons, 1990). The observed laboratory potency of this analogue set is presented in Table IV. Across this small set of compounds a 6300-fold potency range was observed.

Regression models relating potency to structural changes were sequentially developed by position for all of the analogues explored up to now. A quantitative structure–activity relationship (QSAR) was first developed for the para-substituted analogues (Table II, **1**, **2**, **15–27**). Using the technique of all combinations regression on the 14 active analogues afforded the QSAR model in eq 1, where n is the number of analogues, s is the standard error for the model, r^2 is the explained variance, and F is the value for the F statistic. The r^2 value is adjusted for the number of variables in the equation. The inclusion of each term

$$pI_{50}\,(M) = 1.67\,(\pm0.38)\pi_p - 0.67\,(\pm0.17)\pi^2_p - 1.18\,(\pm0.22)\sigma_p + 4.34 \quad (1)$$

$$n = 14 \qquad r^2 = 0.79 \qquad s = 0.28 \qquad F = 17.6$$
$$\pi_{p(optimum)} = 1.2$$

was judged significant by comparing the value for r^2, s, and F to equations of fewer terms (Draper and Smith, 1966). The values in parentheses are the standard error for the coefficients. All terms were significant at the 99% confidence level based upon the Student t-test. Using eq 1 as a starting model for the para position, the 2,4-disubstituted analogues (Table IV, **6**, **39–52**) were included and resulted in eq 2. By all statistical measures (r^2, s,

$$pI_{50}\,(M) = 0.82\,(\pm0.28)\pi_p - 0.27\,(\pm0.13)\pi^2_p - 0.80\,(\pm0.24)\sigma_p + 5.16 \quad (2)$$

$$n = 28 \qquad r^2 = 0.46 \qquad s = 0.58 \qquad F = 8.8$$
$$\pi_{p(optimum)} = 1.5$$

and F) eq 2 is of lower significance than eq 1, having suffered from the inclusion of the 2,4-disubstituted analogues (Draper and Smith, 1966). Considering physiochemical descriptors for the ortho substituent by including one at a time from those on which the original design was based (π, F, R, and MR) indicated that potency was dependent upon the molar refractivity (MR) of the ortho substituent. We consider the molar refractivity substituent constant to approximate a steric effect (Charton, 1983). Exploring the Verloop steric substituent constants (L, $B1$, and $B4$) more clearly defined this steric effect. Specifically, including the minimum radius ($B1_0$) of the ortho substituent afforded the best model (eq 3). By these

$$pI_{50}\,(M) = 1.04\,(\pm0.18)\pi_p - 0.35\,(\pm0.08)\pi^2_p - 0.91\,(\pm0.15)\sigma_p + 1.23\,(\pm0.20)B1_0 + 3.42 \quad (3)$$

$$n = 28 \qquad r^2 = 0.79 \qquad s = 0.37 \qquad F = 26.6$$
$$\pi_{p(optimum)} = 1.5$$

same measures (r^2, s, and F) eq 3 is significantly better than eq 2 and is comparable to eq 1. The affect of $B1_0$ on potency suggests the two aromatic rings of I need to be nonplanar for maximal activity.

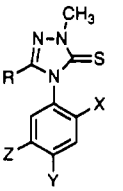
Inclusion of the meta-substituted analogues (Table II, **3**, **28–33**) in the model revealed that they were well predicted within the error of the model. However, to adequately predict the analogues that were only substituted ortho (Table II, **4**, **34–38**) required use of an indicator variable I_0 (Kubinyi, 1976; Fujita and Ban, 1971). This variable assumed the value of 1 when the aromatic ring was solely ortho substituted (i.e., 2- or 2,6-substituted or unsubstituted). Its high significance (t-test = −7.1, p-tail = 0.00, eq 4) and large negative coefficient suggested that

$$pI_{50}\,(M) = 0.92\,(\pm0.16)\pi_p - 0.33\,(\pm0.07)\pi^2_p - 0.86\,(\pm0.14)\sigma_p + 1.02\,(\pm0.15)B1_0 - 1.05\,(\pm0.15)I_0 + 3.81 \quad (4)$$

$$n = 40 \qquad r^2 = 0.85 \qquad s = 0.33 \qquad F = 45.0$$
$$\pi_{p(optimum)} = 1.4$$

I could undergo metabolism in the aromatic ring if not

Table IX. Physiochemical Data Used in QSAR Model Development



compd	π_y	π^2_y	σ_y	$B1_x$	I_0	π_z	$B1_R$	L_R	compd	π_y	π^2_y	σ_y	$B1_x$	I_0	π_z	$B1_R$	L_R
1	0.00	0.00	0.00	1.00	1	0.00	1.98	3.30	**46**	0.40	0.16	0.23	1.52	0	0.00	1.98	3.30
2	0.71	0.50	0.23	1.00	0	0.00	1.98	3.30	**47**	1.18	1.39	−0.90	1.80	0	0.00	1.98	3.30
3	0.00	0.00	0.00	1.00	0	0.00	1.98	3.30	**48**	0.18	0.03	−0.83	1.98	0	0.00	1.98	3.30
4	0.00	0.00	0.00	1.80	1	0.00	1.98	3.30	**49**	0.14	0.02	0.06	1.35	0	0.00	1.98	3.30
5	0.00	0.00	0.00	1.80	0	0.00	1.98	3.30	**50**	0.18	0.03	−0.83	1.80	0	0.00	1.98	3.30
6	0.71	0.50	0.23	1.80	0	0.00	1.98	3.30	**51**	1.05	1.10	−0.45	1.98	0	0.00	1.98	3.30
7	0.00	0.00	0.00	1.80	0	0.71	1.98	3.30	**52**	−0.97	0.94	0.00	1.70	0	0.00	1.98	3.30
8	0.00	0.00	0.00	1.80	1	0.00	1.98	3.30	**53**	1.05	1.10	−0.45	1.00	0	0.71	1.98	3.30
9	0.71	0.50	0.23	1.00	0	0.71	1.98	3.30	**54**	1.18	1.39	−0.90	1.00	0	0.71	1.98	3.30
10	0.00	0.00	0.00	1.00	0	0.71	1.98	3.30	**55**	0.18	0.03	−0.83	1.00	0	0.71	1.98	3.30
11	0.71	0.50	0.23	1.80	0	0.00	1.98	3.30	**56**	1.45	2.10	−0.51	1.00	0	0.71	1.98	3.30
12	0.71	0.50	0.23	1.80	0	0.71	1.98	3.30	**57**	2.18	4.75	−0.93	1.00	0	0.71	1.98	3.30
13	0.71	0.50	0.23	1.00	0	0.71	1.98	3.30	**58**	1.05	1.10	−0.45	1.80	0	0.71	1.98	3.30
14	0.71	0.50	0.23	1.80	0	0.00	1.98	3.30	**59**	1.18	1.39	−0.90	1.80	0	0.71	1.98	3.30
15	1.05	1.10	0.43	1.00	0	0.00	1.98	3.30	**60**	0.18	0.03	−0.83	1.80	0	0.71	1.98	3.30
16	2.04	4.16	−0.34	1.00	0	0.00	1.98	3.30	**61**	1.05	1.10	−0.45	1.35	0	0.71	1.98	3.30
17	0.18	0.03	−0.83	1.00	0	0.00	1.98	3.30	**62**	0.56	0.31	−0.17	1.52	0	0.56	1.98	3.30
18	1.53	2.34	−0.15	1.00	0	0.00	1.98	3.30	**63**	1.05	1.10	−0.45	1.80	0	0.56	1.98	3.30
19	0.38	0.14	−0.24	1.00	0	0.00	1.98	3.30	**64**	1.05	1.10	−0.45	1.80	0	0.58	1.98	3.30
20	1.05	1.10	−0.45	1.00	0	0.00	1.98	3.30	**65**	1.05	1.10	−0.45	1.80	0	−0.02	1.98	3.30
21	1.80	3.24	−0.32	1.00	0	0.00	1.98	3.30	**66**	1.05	1.10	−0.45	1.35	0	0.71	1.98	3.30
22	0.56	0.31	−0.17	1.00	0	0.00	1.98	3.30	**67**	1.05	1.10	−0.45	1.95	0	0.86	1.98	3.30
23	1.18	1.39	−0.90	1.00	0	0.00	1.98	3.30	**68**	1.50	1.10	−0.45	1.98	0	0.71	1.98	3.30
24	1.37	1.88	−0.40	1.00	0	0.00	1.98	3.30	**69**	1.18	1.39	−0.90	1.35	0	0.14	1.98	3.30
25	0.89	0.79	−0.93	1.00	0	0.00	1.98	3.30	**70**	1.05	1.10	−0.45	1.70	0	0.71	1.98	3.30
26	2.18	4.75	−0.93	1.00	0	0.00	1.98	3.30	**71**	−0.02	0.00	−0.27	1.80	0	0.71	1.98	3.30
27	0.08	0.01	−0.61	1.00	0	0.00	1.98	3.30	**72**	−0.02	0.00	−0.27	1.35	0	0.71	1.98	3.30
28	0.00	0.00	0.00	1.00	0	0.00	1.98	3.30	**73**	0.08	0.01	−0.61	1.80	0	0.71	1.98	3.30
29	0.00	0.00	0.00	1.00	0	0.00	1.98	3.30	**74**	1.39	1.93	−0.42	1.80	0	0.71	1.98	3.30
30	0.00	0.00	0.00	1.00	0	0.00	1.98	3.30	**75**	0.84	0.71	—[a]	1.80	0	0.71	1.98	3.30
31	0.00	0.00	0.00	1.00	0	0.00	1.98	3.30	**76**	1.16	1.34	—[a]	1.80	0	0.71	1.98	3.30
32	0.00	0.00	0.00	1.00	0	0.00	1.98	3.30	**77**	1.18	1.39	−0.90	1.00	0	0.00	1.99	5.35
33	0.00	0.00	0.00	1.00	0	0.00	1.98	3.30	**78**	1.18	1.39	−0.90	1.00	0	0.00	1.90	4.85
34	0.00	0.00	0.00	1.35	1	0.00	1.98	3.30	**79**	1.18	1.39	−0.90	1.00	0	0.00	1.67	7.20
35	0.00	0.00	0.00	1.52	1	0.00	1.98	3.30	**80**	1.18	1.39	−0.90	1.00	0	0.00	1.67	5.11
36	0.00	0.00	0.00	1.35	1	0.00	1.98	3.30	**81**	1.05	1.10	−0.45	1.80	0	0.71	1.71	3.30
37	0.00	0.00	0.00	1.60	1	0.00	1.98	3.30	**82**	1.05	1.10	−0.45	1.80	0	0.71	2.11	4.10
38	0.00	0.00	0.00	1.98	1	0.00	1.98	3.30	**83**	1.05	1.10	−0.45	1.80	0	0.71	1.98	4.11
39	−0.28	0.08	0.78	1.35	0	0.00	1.98	3.30	**84**	1.05	1.10	−0.45	1.80	0	0.71	2.10	4.30
40	0.71	0.50	0.23	1.50	0	0.00	1.98	3.30	**85**	1.05	1.10	−0.45	1.80	0	0.71	1.80	3.52
41	0.49	0.24	−0.19	1.98	0	0.00	1.98	3.30	**86**	1.05	1.10	−0.45	1.80	0	0.71	1.60	4.23
42	0.14	0.02	0.06	1.52	0	0.00	1.98	3.30	**87**	1.05	1.10	−0.45	1.80	0	0.71	1.50	3.53
43	0.38	0.14	−0.24	1.35	0	0.00	1.98	3.30	**88**	1.05	1.10	−0.45	1.80	0	0.71	1.35	3.98
44	0.88	0.77	0.54	1.70	0	0.00	1.98	3.30	**89**	1.05	1.10	−0.45	1.80	0	0.71	1.00	2.06
45	2.65	7.02	0.16	1.80	0	0.00	1.98	3.30									

[a] For these compounds we did not have values for σ_p.

adequately substituted. Although no metabolism studies were conducted, hydroxylation of an aromatic ring in a position para to a nitrogen or oxygen substituent is well documented (Testa and Jenner, 1976).

Our attention was not directed at assessing the potential of the 3,4,5-substituted analogues. This was accomplished by synthesis of a set of analogues in which the 3- and 5-positions bear a chlorine and the 4-position bears substituents, which were expected to lead to maximum potency as expressed in eq 4, namely electron donating and a lipophilicity near the optimum of 1.4. Overall, the 3,4,5-substituted analogues (Table V, **10**, **13**, **53–57**) were no more potent than the current best para-substituted analogue (Table V, **23**) and markedly less potent than the current best 2,4-disubstituted analogue (Table V, **47**).

Exploration of the 2,4,5-substitution pattern became the next goal. As with the 3,4,5 pattern, initially the 2- and 5-positions were substituted with chlorine and the 4-position was substituted with a set of substituents whose physiochemical properties mirrored the QSAR model for that position. As evidenced in Table VI the 2,4,5-substituted analogues proved to be significantly more potent than the corresponding 3,4,5-substituted analogues (Table V, **53–55**, vs Table VI, **58–60**). Therefore, 2,4,5-substituted analogues were more completely explored (Table VI, **7**, **12**, **58–76**). Our selection of analogues in this series was directed by a growing understanding of the physical properties of these compounds which impacted their ability to express laboratory potency in a greenhouse and field environment (Simmons et al., 1992). With this set of compounds a potency improvement of 25 000-fold was realized relative to the parent compound (Table I, **1**).

Potency prediction of the 2,4,5- and 3,4,5-substituted analogues using eq 4 revealed that these analogues generally were not well predicted within the error of the model (±0.33). Inclusion of these analogues into the model

resulted in eq 5, which suffered from their inclusion. Sub-

$$pI_{50}\,(M) = 1.12\,(\pm0.23)\pi_p - 0.50\,(\pm0.11)\pi^2_p - 1.01\,(\pm0.19)\sigma_p + 1.19\,(\pm0.19)B1_0 - 1.38\,(\pm0.24)I_0 + 3.90 \quad (5)$$

$$n = 69 \qquad r^2 = 0.72 \qquad s = 0.57 \qquad F = 36.2$$
$$\pi_{p(optimum)} = 1.1$$

stituent constants (π, σ_m, and MR) for the substituents in the 5-position of the aromatic ring were explored one at a time to better predict the potency of these analogues and improve the overall model. The best equation (eq 6) is statistically more significant than eq 5 on the basis of

$$pI_{50}\,(M) = 0.90\,(\pm0.17)\pi_p - 0.39\,(\pm0.09)\pi^2_p - 0.82\,(\pm0.14)\sigma_p + 0.98\,(\pm0.14)B1_0 - 1.12\,(\pm0.19)I_0 + 1.18\,(\pm0.17)\pi_5 + 3.95 \quad (6)$$

$$n = 69 \qquad r^2 = 0.84 \qquad s = 0.43 \qquad F = 60.4$$
$$\pi_{p(optimum)} = 1.2$$

the values for r^2, s, and F. At this point we felt that the QSAR rules for the aromatic substitution were well understood. Earlier in the program, when the simplex optimization of the para position was completed, we had started a simplex optimization of the 3′-position keeping the para substituent as $N(C_2H_5)_2$. This first attempt was hampered when three of the five compounds synthesized as part of the simplex set were inactive (Table VII, **23**, **77–80**). Since the simplex method requires that the least active of the set be identified, we suspended our efforts. Since significantly more active compounds were in hand through substitution in the aromatic ring, the effort at understanding the 3′-position was resumed. The compound set prepared to explore this position is presented (Table VII, **58**, **81–89**). A relative potency for each compound was defined as the difference between its potency and the potency of the corresponding analogue with 3′-CF_3. In this way, sets could be combined which differed in the substitution in the aromatic ring. For these compounds a wide range of potencies was observed. Using the technique of all combinations regression analysis considering the parameters on which the simplex was designed (π, σ_p, L, and $B1$) provided eq 7 as a model for

$$-\log RP = 3.19\,(\pm0.60)B1 - 0.87\,(\pm0.23)L - 3.50 \quad (7)$$

$$n = 9 \qquad r^2 = 0.76 \qquad s = 0.44 \qquad F = 14.1$$

the 3′-position. In the development of this equation, compound **85** was excluded since it was a significant outlier when included (its residual >3 standard errors for the equation) and it is possible that the 3′-Cl could undergo metabolism, possibly displacement with glutathione. In fact, compounds **86–88** were prepared by nucleophilic displacement of the chlorine in compound **85**. After this analysis was completed, the potency of three analogues (**15**, **41**, and **78**) was still poorly predicted on the basis of the models developed (residuals >1.0). No apparent pattern was evident among them until we began to explore the aqueous solubility of these compounds in conjunction with a study to understand translation of laboratory potency to field activity. Based upon the measured water solubility (log S, in mol/L) for six analogues, a relationship (eq 8) was developed between each compound's water

$$\log S = -0.81\,(\pm0.085)\log P - 1.24(\pm0.44)[(mp - 25\,^\circ C)/100] - 0.83 \quad (8)$$

$$n = 6 \qquad r^2 = 0.95 \qquad s = 0.23 \qquad F = 45.3$$

solubility, log P, and melting point (Wakita et al., 1986). For the three analogues poorly predicted by the QSAR models, the concentrations (predicted by eq 6 or 7) required to inhibit cucumber growth 50% (IC_{50}) exceeded their predicted water solubilities by 2–6-fold. This indicates that potency of any analogue could be masked by potentially unanticipated factors.

CONCLUSION

During the course of optimization of the herbicidal activity of 4-aryl-1,2,4-triazole-5(1*H*)-thiones, an application of experimental design strategies resulted in a very significant improvement in herbicidal efficacy. Optimization afforded a QSAR-based predictive understanding of the effects of substitution on six positions in the lead molecule based upon 89 synthesized analogues. This is a significant efficiency improvement over the number of possible analogues that could have been made. On the basis of a hydroponic cucumber assay, potency improved 25 000-fold over the unsubstituted parent compound. While improvements of this magnitude cannot be realistically expected for all leads, we feel the use of efficient design strategies, such as sequential simplex optimization and Free–Wilson, can improve the odds of recognizing those leads that have the potential to do so. The increased effectiveness results from expending one's resources following these leads and more quickly recognizing the need to abandon others.

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