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Synthesis and GABA receptor potency of 3-thiomethyl-4-(hetero)aryl-5-amino-1-phenylpyrazoles

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Abstract—A convenient synthetic route to novel 4-arylpyrazoles is described. The potential for insecticidal activity through GABA channel blockage by this series of compounds, as well as their selectivity for insect versus mammalian receptors, are explored through in vitro and in vivo assays.

The 1-phenylpyrazole core has been shown to bestow pharmacological activity in a number of areas in the pharmaceutical and agrochemical industries. In the latter field, select examples of biological activities include insecticidal, miticidal, and herbicidal. More specifically, 1-phenylpyrazoles with alkyl, acyl, thioalkyl or cyano substituents at the 4-position exhibit potent insecticidal activity.[1] In particular, 5-amino-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-trifluoromethanesulfinyl-1*H*-pyrazole-3-carbonitrile (Fipronil®, **1**) is one of the most commercially successful insecticides. In fleas, ticks, and other arthropods it acts as a GABA-gated chloride channel inhibitor causing neuronal cell hyperexcitability and eventual death.

As a part of our own program searching for novel, orally bioavailable small molecule insecticidal agents, we learned that we could maintain high binding affinity for insect GABA receptors by swapping the thioalkyl and nitrile functionalities on C-3 and C-4 of **1**, leading to 3-thiomethyl-4-cyano-5-amino-1-aylpyrazoles such as **2a**. This observation led us to the discovery of a potent insect GABA radiolabeled ligand (CTOM, **2b**), with 500-fold selectivity against the mammalian (mouse brain) GABA receptor.[2] Presence of the C-3 thiomethyl on **2** was essential to maintain the high affinity of this class of compounds. Indeed, early reports had shown that removal of both substituents on C-3 and C-5, leading to 1-(2,6-dichloro-4-trifluoromethylphenyl)-4-trifluoromethanesulfinyl-1*H*-pyrazole (**3**, $IC_{50} = 4.2$ nM), had no detrimental effect on the binding affinity for the housefly GABA receptor.[3] We reasoned that these experimental facts reflected the tolerance of this binding site of the GABA receptor for lipophilic substituents at the C-4 position of 1-arylpyrazoles of this type. Thus we contemplated the possibility of increasing affinity for this receptor by exploring diverse lipophilic functionalities at C-4, while maintaining the thioalkyl functionality at the C-3 position (generic structure **4**)[4] (Fig. 1).

The chemistry employed in our synthesis is depicted in Scheme 1. The key step in this synthesis is palladium-catalyzed cross coupling of iodopyrazole **11** and an appropriate arylboronic acid or arylstannane.[5] This strategy, carried out through a parallel synthesis format, enabled us to efficiently prepare a number of 4-arylpyrazoles with various substitution patterns in a short time. The reaction of ethylcyanoacetate **5** with CS_2 in the presence of 2 equiv of NaH, followed by addition of 3 equiv of MeI afforded ketedithioacetal **6** in 58% isolated yield.[6] Compound **6** was then cyclocondensed with 2,6-dichloro-4-trifluoromethylphenylhydrazine **7** in refluxing ethanol to obtain the 5-aminopyrazole ester **8** in 73% yield. The ester group of **8** was saponified by treatment with LiOH in methanol–water to obtain the corresponding carboxylic acid **9**, which contained 15–20% of decarboxylation product **10**. The conversion of

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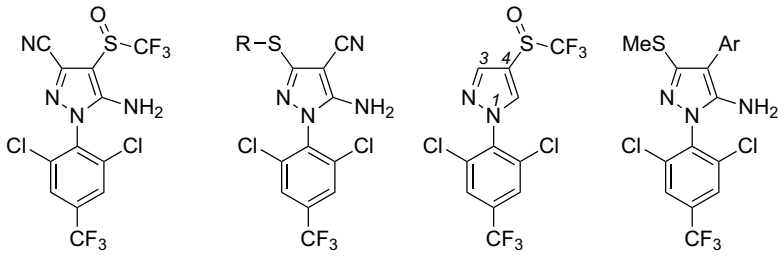
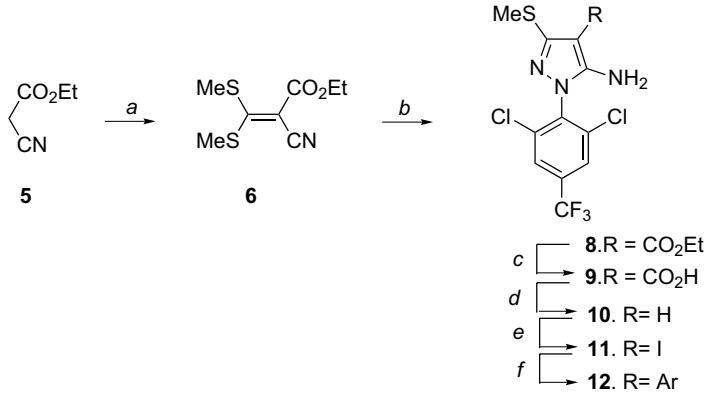


Figure 1.



Scheme 1. Synthesis of 3-thiomethyl-4-aryl-5-amino-1-arylpyrazoles: (a) (1) NaH, (2) CS_2, (3) MeI, 63%; (b) EtOH, 2,6-Cl_2-4-CF_3-$_6H_2NHNH_2$ (**7**), reflux, 76%; (c) (1) LiOH; (d) 180 °C, 53%; (e) NIS, CH_2Cl_2 64%; (f) $ArB(OH)_2$, $Ph(PPh_3)_4$, toluene–$NaHCO_3$ (aq) 12–90%.

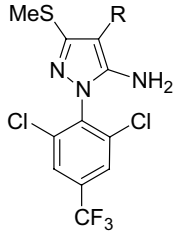
9 to **10** was completed by heating the vacuum-dried crude carboxylic acid **9** at 180–190 °C, yielding product **10** in 85% yield from ester **8**. The iodo functionality was then introduced on C-4 by treating pyrazole **10** with *N*-iodosuccinimide.[7] Similarly, reaction with *N*-chloro- or *N*-bromosuccinimide provided the 4-Cl or 4-Br analogs, respectively.

The cross-coupling reactions of **11** with appropriate arylboronic acids were carried out using catalytic $Pd(PPh_3)_4$ in a biphasic toluene/aq $NaHCO_3$ system.[5] Yields of isolated product under unoptimized reaction conditions ranged from 12% to 90%. The 5-oxadiazole analog **43** could only be obtained, albeit in low yield, through the arylstannane coupling reaction using the same Pd(0) catalyst in DMF at 75 °C; the corresponding oxadiazoleboronic acid failed to produce any detectable amounts of product.

The potential for insecticidal activity of the synthesized compounds was evaluated by determining their ability to bind to housefly neuronal membrane receptors displacing radioligand [3H]-EBOB.[8] Their in vitro selectivity was determined by measuring their binding affinities for mouse brain GABA receptors. The in vivo flea-killing efficacy of these compounds was also evaluated through a contact assay with house flies and three different flea strains.[8] Three different flea strains were used to assess differences in insecticidal activity among the three genetic variants of the cyclodiene resistant allele-homozygous serine (resistant, CO fleas), homozygous alanine (sensitive, ARC fleas), and a mixed homozygous and heterozygous population (NC fleas).

Table 1 shows affinity data for substituents smaller than an aryl group on C-4. The high affinity for the nitrile

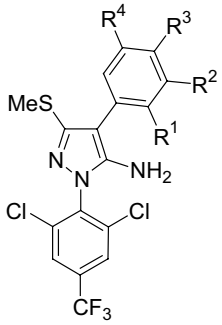
Table 1. C-4 small substituents



Compound	R	Housefly IC_{50}[a]	Selectivity[b]
10	H	400	250
12	Cl	740	210
13	Br	380	2500
11	I	160	43
2a	CN	8	500
14	C≡CH	105	>10,000
8a	CO_2Me	43	NA
8b	CO_2Et	25	400
9	CO_2H	700	45

[a] IC_{50} in nM.
[b] Ratio of mammalian receptor affinity to insect affinity.

Table 2. C-4 aryl substituents



Entry	R^1	R^2	R^3	R^4	Housefly IC_{50}[a]	Selectivity[b]
15	H	H	H	H	300	4
16			F		350	20
17			Cl		IA	—
18			Br		IA	—
19			CN		1500	666
20			CF_3		4000	2600
21			CH_3		120	66
22			OCH_3		170	180
23			SCH_3		52	230
24			CF_3		400	3
25			*i*-Pr		IA	—
26		CH_3	H		400	NA
27		Cl			800	31
28		OCH_3			26	346
29		*i*-Pr			IA	
30		CF_3			1000	5
31		*t*-Bu			IA	—
32	CH_3	H			50	400
33	SCH_3				IA	
34	OCH_3				500	NA
35	F				IA	—
36	CF_3				90	6000
37	H	CF_3		CF_3	700	12
38		Cl	F	H	1000	4
39		OCH_3	OCH_3		500	2000
40	OCH_3	H	OCH_3		325	49
41	H	OCH_2O			124	64
42		OCH_3	OCH_3	OCH_3	IA	—

[a] IC_{50} in nM.
[b] Ratio of mammalian receptor affinity to insect affinity. IA = inactive. NA = not available.

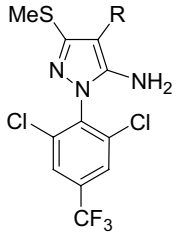
functionality is notable. Related functionalities, such as esters **8a** and **8b**, show fair levels of affinity, but halogens or the isosteric acetylene group bind less effectively.

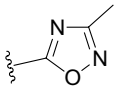
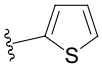
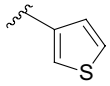
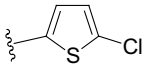
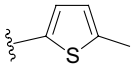
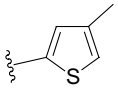
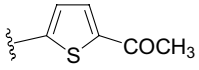
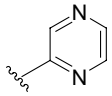
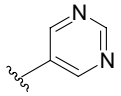
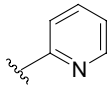
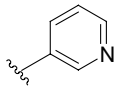
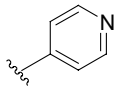
Analogs obtained by introduction of a substituted phenyl group on C-4 are shown in Table 2. While no analog shows improved affinity over nitrile **2a**, comparable levels of activity are seen with the 3-anisyl analog **28**, which shows selectivity greater than 300-fold. Increasingly lower affinity levels were obtained for 2-substituted analogs **32** and **36** (methyl and trifluoromethyl, respectively) and 4-substituted analogs **23**, **21**, and **22** (thiomethyl, methyl, and methoxy, respectively). Their selectivities (66–4000-fold) were comparable or better than that for **28**.

Table 3 shows binding results for analogs containing an heterocyclic C-4 substituent. Although 2-thiophenyl analog **44** exhibited 90 nM binding affinity for house fly brain preparations, chloro substitution at 5-position of thiophene resulted in ~4-fold increase in binding affinity in the same assay. However, other related thiophene analogs **45**, **47**, and **48** had no activity at all. Among nitrogen heterocycles, isomeric pyridines **52**–**54** are all inactive, but pyrazine substitution as in compound **50** and oxadiazole **43** only yielded fair binding affinity levels.

The next step was the evaluation of compounds with different levels of in vitro binding activity in the in vivo contact assay. The results of these experiments on selected analogs are shown in Table 4. Even at the relatively high concentration used in the assay (100 mM), only moderate activity was seen with compounds that had sub-micromolar affinity for the receptor. While binding to the receptor is necessary for an insecticide

Table 3. C-4 heterocyclic substituents



Compound	R	Housefly IC_{50}[a]	Selectivity[b]
43		110	9
44		90	44
45		IA	—
46		23	434
47		IA	—
48		IA	—
49		150	25
50		87	45
51		Negative inhibition	—
52		IA	—
53		IA	—
54		IA	—

[a] IC_{50} in nM.
[b] Ratio of mammalian receptor affinity to insect affinity. IA = inactive. NA = not available.

to work through this mechanism, the lack of in vivo activity in contact assays could be due to poor skin permeability, inappropriate disposition, and metabolism of the substrate which would preclude it from reaching the target site. Nevertheless, different profiles in terms of selectivity of in vivo insecticidal action emerged from our studies: dual acting **12**, fly selective **10**, **14**, **43**, **41**, or flea selective **32**, **36**, and **50** compounds. In addition

Table 4. In vivo efficacy of selected compounds evaluated by the contact assay

	Compound	Housefly IC_{50}[a]	CO fleas[b]	NC fleas[b]	Housefly[b]
Dual acting compound	**12**	740	32.3	3.3	92.8
Fly selective	**10**	160	4.4	0	42.4
	14	105	2.2	0	80.1
	43	110	0	0	95.4
	41	124	1.4	0	27.6
Flea selective	**50**	87	77.4	89.9	0
	36	90	58.7	0	3.9
	32	50	60.8	0	9.6

[a] Affinity for the housefly receptor, IC_{50} in nM.
[b] % Mortality at 100 mM.

compounds **32** and **36**, both with *ortho* substituents on the C-4 aryl group, showed selectivity between the flea colonies used. Based on these results it seems that the housefly receptor is not always a good predictor of activity in contact assays, both against fleas and flies.

In conclusion, a number of novel 4-substituted 1-aryl-3-thiomethyl-5-aminopyrazoles were made[9] and their insecticidal activities evaluated. Compounds with a variety of insecticidal profiles were obtained, including the desired broad anti-flea activity, as determined through contact assay models with various strains of cat fleas.

Acknowledgements

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References and notes

1. For examples of insecticidal activity, see: (a) Banks, B. J. Eur. Pat. Appl. EP 846686, 1998; (b) Banks, B. J. U.S. Patent 6,069,157, 2000; (c) Lankau, H.-J.; Menzer, M.; Rostock, A.; Arnold, T.; Rundfelt, C.; Unverfeth, K. *Pharmazie* **1999**, *54*, 705.
2. Meegalla, S. K.; Doller, D.; Silver, G. M.; Wisnewski, N.; Soll, R. M.; Dhanoa, D. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4035.
3. Cole, L. M.; Nicholson, R. A.; Casida, J. E. *Pestic. Biochem. Physiol.* **1993**, *46*, 47–54.
4. For other examples of C-4 substituted phenylpyrazole derivatives, see: Sammelson, R. E.; Caboni, P. C.; Durkin, K. A.; Casida, J. E. *Bioorg. Med. Chem.* **2004**, *12*, 3345.
5. Tsuji, J. *Palladium Reagents and Catalysis*; John Wiley & Sons Ltd, 1995.
6. Villemin, D.; Ben Alloum, A. *Synthesis* **1991**, 301.
7. Preliminary experiments showed that the presence of 5-amino group is necessary for successful iodination. The amino group could then be removed quantitatively by treatment with isoamyl nitrite in DMF at 60 °C.

8. Dhanoa, D. S.; Meegalla, S. K.; Doller, D.; Soll, R. M. U.S. Patent 6,409,988.

9. General synthetic procedures are as follows:
Synthesis of compound **8**: A solution of 2,6-dichloro-4-trifluoromethylphenylhydrazine **7** (245 mg, 1 mmol) and 3,3-(bismethylthio)-2-cyanoacrylic acid ethyl ester **6** (217 mg, 1 mmol) in isopropanol (15 mL) was heated at reflux for 16 h. The solvent was removed under reduced pressure. The residue was purified on silica EtOAc–hexanes (1:9) to afford compound **8** (75%). ^{1}H NMR (δ, $CDCl_3$): 1.4 (3H, t, J = 7.2 Hz), 2.48 (3H, s), 4.13 (2H, q, J = 7.2 Hz), 7.76 (2H, s).
Synthesis of compound **9**: Compound **8** (212 mg, 0.5 mmol) was dissolved in LiOH (96 mg, 4 mmol) in methanol/water (7 mL, 9:1 mixture). The resulting solution was stirred at reflux for 16 h and then cooled to room temperature. Methanol was removed and the reaction mixture was acidified to pH 4 by adding AcOH and extracted with CH_2Cl_2 (3×20 mL). The CH_2Cl_2 layers were combined and dried (Na_2SO_4) to yield compound **9** (90%) contaminated with compound **10** which was directly used in next step without further purification. For compound **9**: ^{1}H NMR (δ, $CDCl_3$): 2.5 (3H, s), 3.58 (2H, q, J = 7.2 Hz), 7.77 (2H, s).
Synthesis of compound **10**: Compound **9** (1.16 g) was heated at 180–190 °C for 20 min under N_2. The reaction mixture was allowed to cool to room temperature and purified on silica (EtOAc–hexanes 15:85) to yield compound **10** (65%). ^{1}H NMR (δ, $CDCl_3$): 2.5 (3H, s), 5.67 (1H, s), 7.75 (2H, s).
Synthesis of compound **11**: A solution of compound **10** (352 mg, 1 mmol) in acetonitrile (5 mL) was treated with NIS (1.2 mmol, 270 mg) at 0 °C. The resulting mixture was stirred at room temperature for 30 min and concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 (20 mL) and washed with water (10 mL), aq 10% $Na_2S_2O_3$ (10 mL), and saturated Na_2CO_3 (10 mL). The organic layer was separated, dried (Na_2SO_4), and concentrated. The residue was purified on silica (EtOAc–hexanes 15:85) to obtain the desired compound (63%). ^{1}H NMR (δ, $CDCl_3$): 2.54 (3H, s), 7.65 (2H, s).
General procedure for synthesis of 3-thiomethyl-4-(hetero)-aryl-5-amino-1-phenylpyrazoles:
(a) A solution of compound **11** (47.8 mg, 0.1 mmol) and boronic acid or ester (2 equiv, 0.2 mmol) in toluene (5 mL) was placed in a N_2-flushed vial. Aqueous $NaHCO_3$ (2 M, 2 mL) ethanol (2 mL) and tetrakis(triphenylphosphine) palladium(0) (12 mg, 0.01 mmol) was added. The reaction mixture was then heated at 100 °C for 6 h. The reaction mixture was allowed to cool to room temperature and the organic layer was separated. Solvents were removed and the residue was purified on silica with an appropriate solvent system.
(b) A solution of compound **11** (47.8 mg, 0.1 mmol) and the corresponding arylstannane (2 equiv, 0.2 mmol) in DMF (5 mL) was placed in a N_2-flushed vial. Tetrakis(triphenylphosphine) palladium(0) (12 mg, 0.01 mmol) was then added and the resulting mixture was heated at 75 °C for 12 h. The reaction mixture was allowed to cool to room temperature and poured into water. The water layer was extracted with CH_2Cl_2 (3×10 mL). The organic layers were separated, combined, dried, and concentrated. The residue obtained was purified on silica with the appropriate solvent system.