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Synthesis and insecticidal activity of fluorinated 2-(2,6-dichloro-4-trifluoromethylphenyl)-2,4,5,6-tetrahydrocyclopentapyrazoles

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Abstract—A number of fluorinated 1-aryl-tetrahydrocyclopentapyrazoles were synthesized and their insecticidal activity was evaluated. Some of the fluorinated compounds had significant insecticidal properties.

Prior to 1995, a large proportion of market sales in the area of animal flea-killing agents came from generic, 'over-the-counter' drugs. In the late 1990s' three new anti-flea products drove market sales to veterinarians. These products are: Program™ (Novartis, 1995), Advantage™ (Bayer, 1996), and Frontline™ (Rhone-Merieux Merial, 1996).

The active pharmaceutical ingredient in each of these three products has a different mechanism of action. Lufenuron (**1**), the active ingredient of Program™, is a chitin synthesis/polymerization inhibitor. Bayer's anti-flea agent Advantage™ contains imidacloprid (**2**), which is a nicotinic acetylcholine receptor agonist. The active ingredient of Frontline™ is fipronil (**3**), a phenylpyrazole which acts as a GABA-gated chloride channel inhibitor which causes hyperactivity in neurons, leading to eventual death.

Recently, we described[1] the synthesis and structure–insecticidal activity relationships of a series of 3,4-fused-cycloalkyl-1-arylpyrazoles (generic structure **4**). These could be formally considered as rotationally constrained analogs of fipronil (**3**).

More specifically, we became interested in 2-(2,6-dichloro-4-trifluoromethylphenyl)-2,4,5,6-tetrahydrocyclopentapyrazoles (**5**) and 2-(2,6-dichloro-4-trifluoromethylphenyl)-4-methyl-2,4,5,6-tetrahydrocyclopentapyrazoles (**6**), which showed good binding affinity for the GABA receptor in housefly brain preparations (83 and 30 nM, respectively), with 19- and 126-fold selectivity against mammalian GABA receptors, respectively. Although **5** and **6** caused only 30% and 10% mortality in a housefly contact assay, respectively, both compounds produced high mortality in three flea strains tested (Table 1).

Unfortunately, compounds **5** and **6** were ineffective as anti-flea agents in in vivo efficacy studies in dogs after either topical (10 mg/kg) or oral (20 mg/kg) administration. Although dogs orally dosed with compound **5** had quantifiable levels of compound in plasma for as long as 3 h post-dosing, only trace plasma levels of **5** were detected after topical dosing over a comparable period of time. In vivo metabolite identification studies, carried out by LC/MS/MS analysis of plasma samples from dogs that had been orally dosed with compound **5**, revealed that the test article was extensively hydroxylated at benzylic positions 4 and 6. In agreement with this observation, in vitro metabolic stability studies on **5** showed it had a short half-life ($t_{1/2}$ = 2.5 min) in dog liver microsomal preparations (unpublished data).

The structure–insecticidal binding affinity studies (unpublished data) in this series of compounds demonstrated

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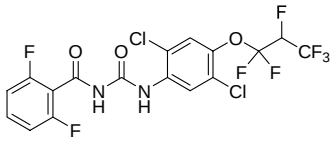

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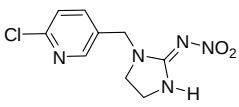

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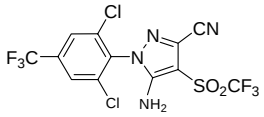

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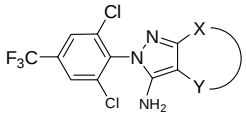

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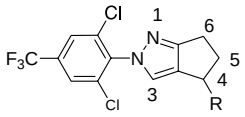
Table 1. Insecticidal activity of fused cyclopentylpyrazoles (**5**) and (**6**)



5: R = H, **6**: R = CH_3

Compound	Binding assay IC_{50} (nM)			Contact assay LC_{50} (mM) or % mortality at 100 mM[4]			
	Fly	Mouse	Selectivity	Fly (%)	S/S fleas	S/A fleas	A/A fleas (%)
5	83	1600	19	30	8	7	100
6	30	3800	126	10	10	3	100

no tolerance for bulky substituents at the C-6 position, while the C-4 position tolerated the presence of small substituents, which, in addition, increased the selectivity against mammalian GABA receptors. Thus, it appeared that the selective fluorination of the alicyclic portion of this series of compounds could be a viable option to address their metabolic liabilities while retaining insecticidal activity. Herein we report the results from our efforts on the synthesis of novel fluorinated 2-(2,6-dichloro-4-trifluoromethylphenyl)-2,4,5,6-tetrahydrocyclopentapyrazoles and their insecticidal properties.

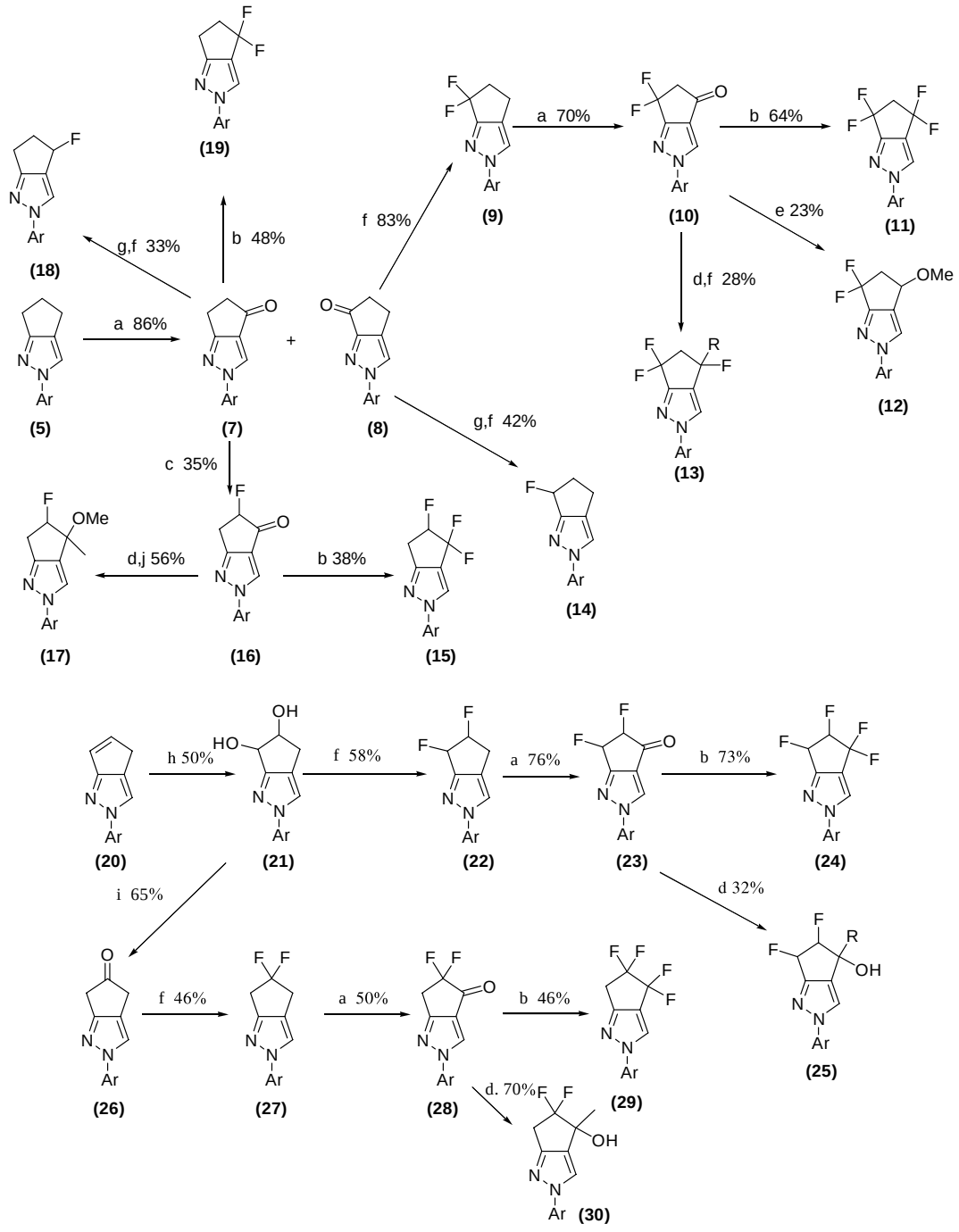
The reaction sequence leading to the synthesis of fluorinated analogs of compound **5** is shown in Scheme 1. Tetrahydrocyclopentylpyrazole **5**, synthesized in two steps from commercially available cylopentanone and 2,6-dichloro-4-trifluoromethylpyrazole, served as key starting material for the preparation of all analogs studied. After some experimentation, compound **5** was oxidized to a mixture of benzylic ketones **7** and **8** in 86% combined yield (10:1 ratio). These could be easily separated by flash chromatography on silica gel. Introduction of fluorine atoms on the fused cyclopentane rings of **7** and **8** was carried out following four different synthetic protocols.[2]

1. Reduction of the ketone to alcohol or reaction of the ketone with a Grignard reagent to obtain the corresponding tertiary alcohol, followed by conversion of the alcohol to fluoride by the action of diethylaminosulfurtrifluoride (DAST).
2. Conversion of the ketone to the corresponding silylenol ethers, followed by α-fluorination with SELECTFLUOR.
3. Direct *gem* difluorination of the ketone with DAST or conversion of the ketone to the corresponding dithioketal, followed by *gem* difluorination with PPHF/NIS.
4. Conversion of the ketone to alkene, vicinal bis hydroxylation, and vicinal bis fluorination with DAST.

Reduction of ketone **8** with $NaBH_4$ followed by acid catalyzed dehydration of the resulting alcohol yielded cyclopentene **20** and its regioisomer (6:1 ratio). Since these two compounds could not be separated by chromatography, they were converted to the corresponding vicinal diols, which were easily separable.

In order to evaluate the insecticidal activity and selectivity of the test compounds prepared, the following assays were used. Binding affinity for the housefly neuronal membrane GABA receptor was measured by displacing radioligand [^{3}H]EBOB. Selectivity against the mammalian receptor was determined by binding affinities to mouse brain GABA receptors. The compounds were also assayed in 'contact assays' with houseflies and two different flea strains as 'in vivo' screens. The two flea strains were used to assess insecticidal activity differences among the genetic variants of the cyclodiene resistant allele-homozygous serine (resistant, CO fleas) and homozygous alanine (sensitive, ARC fleas).[3] (Table 2)

In general, most compounds induced some degree of mortality in sensitive ARC fleas. The notable exceptions are the ketones **10** and **28**, which showed poor activity in contact assays. As is evident from the data, high activity in housefly binding assays did not necessarily translate into high anti-flea activity. Although monofluorination at C-6 slightly decreased the LD_{50} in CO flea contact assay, it caused a 3-fold decrease in binding affinity in housefly brain preparations. In contrast, the presence of fluorine at C-4 position in compound **18** caused a very significant decrease in both binding affinity and activity in the contact



Scheme 1. Synthesis of fluorinated tetrahydrocyclopentapyrazoles. Reagents and conditions: (a) CrO_3/HOAc/50 °C; (b) 1. $HSCH_2CH_2SH/BF_3{\cdot}OEt/CH_2Cl_2$, 2. PPHF/NIS/$CH_2Cl_2$/−30 °C; (c) 1. TMSOTf, Et_3N, 2. SELECTFLUOR/CH_3CN/12 h; (d) RMgX/ether/0 °C; (e) 1. $NaBH_4$/ether; 2. K-O-*t*Bu/MeI; (f) DAST/CH_2Cl_2; (g) $NaBH_4$/ether; (h) cat. OsO_4/NMO/*t*-BuOH/H_2O, (i) *p*-TSA/benzene/reflux/15 min; (j) K-O-*t*Bu/MeI

assay. However, addition of an extra fluorine atom at C-4 (**19**) led to improved binding as well as activity in the contact assay. Compound **9** containing a *gem*-difluoro substituent at C-6 maintained the activity of monofluoro compound **14**. Compounds with apical *gem*-difluoro substitution (e.g., **27**) showed a 4-fold increase in anti-flea activity in the CO flea contact assay with no selectivity against mammalian GABA receptors. The 5,6-vicinal difluoro compound (**22**) also showed an increase in anti-CO flea activity compared to that of the parent cyclopentane analog **5**. Trifluoro compound **15**, 4,4,5,6-tetrafluoro compound **24**, and 4,4,5,5-tetrafluoro compound **29** exhibited excellent anti-flea activity in the CO flea contact assay. Although both tetrafluoro compounds showed LD_{50} values near 500 nM in the CO flea contact assay, they showed a relatively high affinity for mouse GABA receptors as well. The observation that the presence of small substituents at C-4 yields compounds with appropriate selectivity prompted us to study the

Table 2. Insecticidal activities of fluorinated tetrahydrocyclopentapyrazoles

Compound	Binding assay IC_{50} (nM)			Contact assay LD_{50} (mM) or % mortality at 100 mM[4]		
	Fly	Mouse	Selectivity	Fly	S/S fleas	A/A fleas (%)
7	470	20,000	42	81	0	16.5
9	ND	2,800	NA	NA	15	100
10	ND	ND	NA	6%	1%	40
11	74	300	4	93.9%	60.7%	100
12	ND	3,700	NA	22%	3%	100
13 (R = Me)	ND	>10,000	NA	16.7%	3%	100
14	290	2900	10	85%	11	95
15	<100	1,000	>10	100%	1	100
16	320	3,200	10	57.5%	10.8%	95.4
17	100	12,000	120	ND	1.8	100
18	1700	>10,000	>5	7%	36%	ND
19	>100	1,000	<10	88	1	100
22	200	1,000	5	79	1.5	100
24	ND	175	ND	0.4	<0.5	100
25 (R = Me)	350	>10,000	> 28	100%	5	100
27	200	175	1	87.9%	2	100
28	ND	ND	NA	6%	1%	40
29	≪100	50	≫0.5	ND	<0.5	100
30	100	2,300	23	72%	10	100

Table 3. Metabolic stability data

Compound	Microsomal $t_{1/2}$ (min)		Clearance (pmol/min/1 mg/protein)	
	Mouse	Dog	Mouse	Dog
15	64.7	Stable[a]	109.2	Stable[a]
17	145	Stable[a]	34.1	Stable[a]
22	22.4	Stable[a]	255.1	Stable[a]
30	Stable[a]	506.7	Stable[a]	296.9

[a] 80% of test substance was detected after 30 min of incubation.

particular structure–activity relationship at this position. Among this type of compounds, compound **17** showed the highest anti-flea activity with CO flea, with an LD_{50} of 1.8 mM and 120-fold selectivity against the insect GABA receptors. The other two compounds in this subgroup, **25** and **30**, were about 25-fold selective for insect GABA receptors with 5 and 10 mM LD_{50}s in CO flea contact assays.[3]

Limited in vitro metabolic stability studies of promising analogs were undertaken for determining half-lives in mouse and dog liver microsomal preparations (Table 3). These studies revealed that, in general, compounds containing a C-5 apical fluorine substitution were more stable than unsubstituted analogs. Unfortunately, although the introduced substituents OH and/or CH_3 at C-4 improved the selectivity of the test compounds against insect GABA receptors, they also became more vulnerable to microsomal metabolism.

In conclusion, we have successfully employed selective fluorination as a tool to address observed metabolic instabilities of parent compound (**5**). Some of the fluorinated compounds exhibited a several-fold increase in activity compared to that of the parent compound (**5**).

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References and notes

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2. Olah, G. A.; Chambers, R. D.; Prakash, G. K. *Synthetic Fluorine Chemistry*; John Wiley & Sons Inc: USA, 1992.
3. Dhanoa, D. S.; Meegalla, S.; Soll, R. M.; Doller, D.; Sha; DeYou; Liu; RuiPing; Silver, G.; Lee, Y.; U.S. Patent 6,545,033.
4. *In vitro binding assay.* Test compounds were dissolved in DMSO at concentrations ranging from about 2 nM to 100 mM. One microliter of dissolved test compound was dispensed into the well of a 96-well polystyrene plate. 100 μL of ice-cold assay buffer (10 mM phosphate, 300 mM NaCl, pH 7.5) containing 5.2 nM 4″-ethynyl-4-*n*-[2,3-$^3H_2$]propyl-bicycloorthobenzoate ([^{3}H]EBOB, 38 Ci/mmol) was added followed by 100 μL of ice-cold assay buffer containing 0.5–1.0 mg/mL neuronal membranes. Control wells were prepared the same way, except the neuronal membranes were omitted. The samples were incubated for 45 min at 24 °C and filtered on a 0.1% (wt/v) polyethylenimine-soaked glass fiber Filtermat A rinse of cold assay buffer using Harvester 96-cell harvester. The Filtermat was air-dried and radioactivity bound to the Filtermat was detected. Compounds that displaced [^{3}H]EBOB specifically bound to the housefly were then tested at 24–48 different final concentrations, varying from about 0.1 nm to about 125 mM, in order to determine the concentration at which 50% of the maximum inhibition due to the addition of that compound was observed (IC_{50}).
In vivo housefly assay. Newly emerged houseflies were sedated with CO_2 gas, collected in 50 mL polypropylene conical tubes containing filter paper saturated with 10% (wt/wt) sucrose in water, and allowed to feed at room temper-

ature for about 2–4 h. Test compounds were dissolved in DMSO at concentrations ranging from 0.05 to 100 mM. One microliter of dissolved test compound and 100 mL of isopropanol were dispensed into the bottom of a 9 mL screw-top glass test tube. Positive control was prepared in the same manner, except that no test compounds were dissolved in DMSO. Each test tube was rolled to coat the sides with the chemical solution and allowed to air-dry for 24–48 h. Twenty houseflies were sedated by refrigeration at 0–4 °C and transferred to each test tube. Each tube was sealed with organdy cloth secured by an open top screw cap and laid horizontally in dark. After 24 h the healthy, moribund, and dead houseflies in each tube were counted. The percentage of dead houseflies in each tube was then calculated.

In vivo flea assay. Test compounds were dissolved in DMSO at concentrations ranging from 0.05 to 100 mM. One microliter of dissolved test compound was dispensed onto a 6 mm GF/C filter disk in the bottom of a 4 mL screw-top glass vial and allowed to air-dry for 24–48 h. Positive control was prepared in the same manner, except that no test compounds were dissolved in DMSO. Twenty newly emerged cat fleas were sedated by refrigeration at 0–4 °C and transferred to each vial. Each vial was sealed with thin, perforated Teflon septum secured by an open top screw cap and held vertically in dark. After 24 h, the healthy, moribund, and dead houseflies in each tube were counted. The percentage of dead fleas in each vial was then calculated.