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GABA receptor antagonists and insecticides: common structural features of 4-alkyl-1-phenylpyrazoles and 4-alkyl-1-phenyltrioxabicyclooctanes

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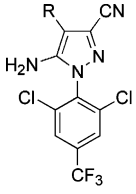
Abstract—Fipronil [5-amino-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-trifluoromethylsulfinylpyrazole] is one of the most important insecticides. Structure–activity studies described here reveal that fipronil retains its very high binding potency at the human β3 and house fly γ-aminobutyric acid (GABA) receptors and toxicity to house flies on replacing the pyrazole trifluoromethylsulfinyl moiety with *tert*-butyl or isopropyl and the phenyl trifluoromethyl substituent with ethynyl, trifluoromethoxy, bromo or chloro. Among the compounds studied, those with other alkyl groups at the 4-position of the pyrazole, as well as phenyl substitution without one or both of the 2,6-dichloro groups, are less effective. 5-Amino-4-*tert*-butyl-3-cyano-1-(2,6-dichloro-4-ethynylphenyl)pyrazole is highly effective and almost isosteric with 4-*tert*-butyl-3-cyano-1-(4-ethynylphenyl)-2,6,7-trioxabicyclo[2.2.2]octane (the most potent 4-alkyl-1-phenyltrioxabicyclooctane) as a noncompetitive GABA antagonist and insecticide. These findings are interpreted as three binding subsites in the GABA receptor: a hydrophobic site undergoing steric interaction with the *tert*-butyl or equivalent group; a hydrogen bonding site to pyrazole N-2; a pi bonding site to the face of the phenyl moiety; with supplemental enhancement by the 3-cyano and 4-ethynyl substituents.

1. Introduction

Fipronil (**1**) (Fig. 1) is one of the most important insect control chemicals.[1–3] Similar insecticidal activity is observed for its sulfide (**2**) photoproduct, sulfone (**3**) photoproduct and metabolite, and desulfinyl photoproduct (**4**).[4,5] Ethiprole (**5**), with 4-EtSO replacing the 4-CF_3SO of **1**, is a newer analog in advanced development.[6] Desulfinylethiprole (**6**) (the 4-Et analog), a 4-alkyl-1-phenylpyrazole, was prepared as a candidate photoproduct and found not to be formed photochemically but surprisingly to have high insecticidal activity.[7] The biochemical target of **1**–**6** in both insects and mammals is the γ-aminobutyric acid (GABA) receptor and more specifically its noncompetitive blocker site.[8–12] To better understand the interactions between 4-alkyl-1-phenylpyrazoles and the GABA receptor, a series of compounds was synthesized by varying the 4-position of the



1 R = $SOCF_3$
2 R = SCF_3
3 R = SO_2CF_3
4 R = CF_3
5 R = SOEt
6 R = Et
7 R = *t*-Bu

Figure 1. Fipronil (**1**), ethiprole (**5**) and analogs (**2**–**4**, **6**, and **7**).

Abbreviations: Bu, butyl; *c*, cyclo; DCM, dichloromethane; DIPA, diisopropylamine; [^{3}H]EBOB, 4-*n*-[^{3}H]propyl-1-(4-ethynylphenyl)-2,6,7-trioxabicyclo[2.2.2]octane, also known as 4-*n*-[^{3}H]propyl-4′-ethynylbicycloorthobenzoate; EI, electron impact; Et, ethyl; GABA, γ-aminobutyric acid; Hex, hexyl; HRMS, high-resolution MS; *i*, iso; IC_{50}, concentration for 50% inhibition; Me, methyl; mp, melting point; MS, mass spectrometry or mass spectra; *n*, normal; PB, piperonyl butoxide; Pen, pentyl; Pr, propyl; *s*, secondary; SAR, structure–activity relationship; SE, standard error; *t*, tertiary; TBO, 2,6,7-trioxabicyclo[2.2.2]octane.

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pyrazole moiety. A second library was prepared to study the phenyl substituents. The findings show unexpected common structural features of 4-alkyl-1-phenylpyrazoles and 4-alkyl-1-phenyl-2,6,7-trioxabicyclo[2.2.2]octanes (4-alkyl-1-phenylTBOs) in their action as GABA receptor antagonists and insecticides.

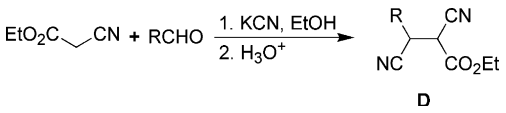
Potency evaluations are conveniently made with a binding assay using 4-*n*-[^{3}H]propyl-1-(4-ethynylphenyl)TBO ([^{3}H]EBOB).[13] This determination with house fly head membranes is predictive of insecticidal activity as topical LD_{50} in house flies pretreated with the CYP450 inhibitor piperonyl butoxide (PB) to minimize oxidative detoxification.[14] Mammalian brain membranes also bind [^{3}H]EBOB in a manner sensitive to many insecticides and convulsants.[15] The β subunit contains the binding site[16] and the human recombinant homooligomeric β3 receptor is very similar to the housefly receptor in sensitivity and specificity and therefore provides a very convenient, well defined and validated binding assay.[17] Accordingly, the present study uses both the house fly and human β3 receptors to define the structure–activity relationships (SAR) and the target site conformation. House fly topical toxicity assays alone and with PB are used to evaluate organismal sensitivity and synergistic effects.

2. Chemistry

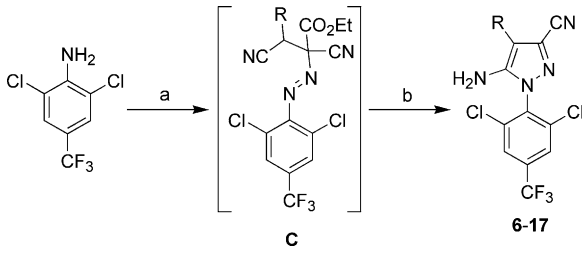
2.1. 4-Alkyl-5-amino-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl)pyrazoles

Retrosynthetically, these 4-alkyl-1-phenylpyrazoles (**A**, Scheme 1) can be obtained from base-induced cyclization of phenylhydrazone **B**. Intermediate **B** arises from hydrolysis and decarboxylation of diazo compound **C**, which can be prepared by addition of **D** to the diazonium salt of 2,6-dichloro-4-trifluoromethylaniline. Structure **D** would originate from ethyl cyanoacetate, potassium cyanide and the corresponding aliphatic aldehyde via Knoevenagel condensation/Michael addition protocol.[18,19] This route has been employed for the unsubstituted case (compound **8**, R = H) but is novel for substituted examples.

The synthesis commenced with the preparation of the ethyl 2,3-dicyanoalkanoates (**D**) from ethyl cyanoacetate, potassium cyanide, and aldehyde in ethanol (Scheme 2). In all cases, except when R = H, mixtures of



Scheme 2. Preparation of ethyl 2,3-dicyanoalkanoate intermediates (**D**).

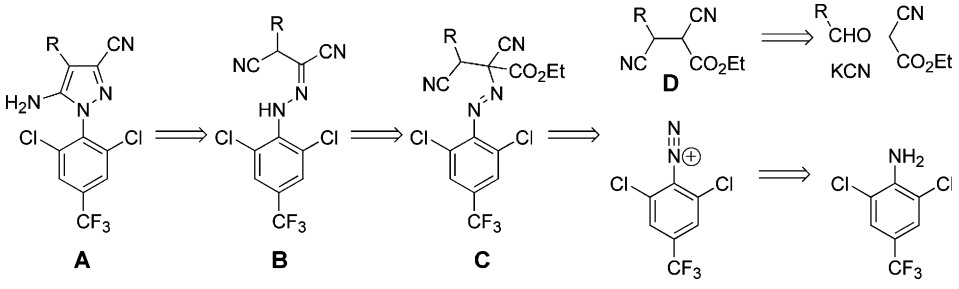


Scheme 3. Synthesis of 4-alkyl analogs of fipronil. (a) i. H_2SO_4, $NaNO_2$, CH_3CO_2H; ii. D, CH_3CO_2H, H_2O; (b) NH_3, H_2O, DCM.

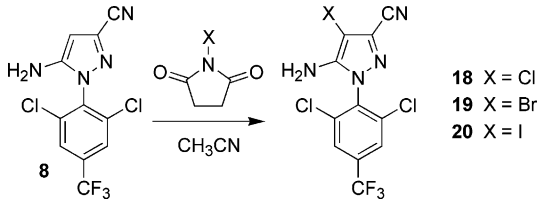
diastereomers were obtained after stirring for 4 h at room temperature. The yields and purities of each of these reactions were excellent and these mixtures of stereoisomers were utilized without separation or purification after aqueous extraction. Diazotization of 2,6-dichloro-4-trifluoromethylaniline with sulfuric acid and sodium nitrite in acetic acid was followed by reaction with **D** to give the yellow diazo addition product **C** (Scheme 3). This crude mixture of stereoisomers was extracted with dichloromethane, made basic, and the resultant orange solution stirred with 50% ammonium hydroxide overnight. The substituted derivatives, especially hindered ones like *t*-Bu, cyclized at a slower rate than the unsubstituted derivative (R = H) but under these conditions it did not affect the overall yield. Extraction with dichloromethane and concentration of the organic layer were followed by crystallization or chromatography to provide the desired 4-alkyl-1-phenylpyrazoles (**6**, **7**, and **9**–**17**).

2.2. 5-Amino-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-halopyrazoles

Compound **8** was synthesized as above for biological evaluation and was used as a precursor for the 4-halopyrazole derivatives. Halogenation at the 4-position of **8** with *N*-chlorosuccinimide at 80 °C, *N*-bromosuccin-



Scheme 1. Retrosynthetic approach to 4-alkyl analogs of fipronil.



Scheme 4. Synthesis of 4-halo analogs of fipronil.

imide at 50 °C, and *N*-iodosuccinimide at 20 °C in acetonitrile provided the 4-Cl (**18**), 4-Br (**19**), and 4-I (**20**) derivatives, respectively (Scheme 4).

2.3. 5-Amino-3-cyano-1-(2,4,6-substituted-phenyl)-4-(isopropyl or *tert*-butyl)pyrazoles

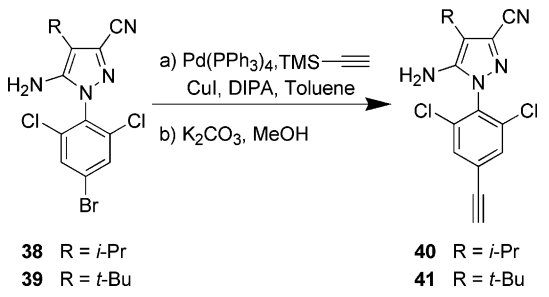
Analogs **21**–**39** were prepared as above for **7** and **11** but in cases where one or both of the 2,6-dichloro substituents were absent 2 N hydrochloric acid was used instead of concentrated sulfuric acid. Some substituents give slower cyclization (requiring up to 4 d) and the *t*-Bu derivatives also called for heating to reflux in several cases.

2.4. 5-Amino-3-cyano-1-(2,6-dichloro-4-ethynylphenyl)-4-(isopropyl or *tert*-butyl)pyrazoles

Ethynyl derivatives **40** and **41** were obtained from Sonagishira coupling of bromo analogs **38** and **39**, respectively. The coupling reaction was carried out with trimethylsilylacetylene, tetrakistriphenyl phosphine palladium and cuprous iodide in diisopropylamine and toluene (Scheme 5). Desilylation was achieved with potassium carbonate in methanol.

3. Biology

Fifteen 5-amino-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-(alkyl or halo)pyrazoles were first assayed as competitors for [^{3}H]EBOB binding to the house fly GABA receptor with potency expressed as IC_{50} values (Table 1). Then a discriminating concentration of 10 nM was used to determine possible sensitivity differences between house fly and human β3 receptors. In most cases, the inhibition increased slightly from the house fly to the human β3 receptor, the exceptions being *c*-Hex and possibly *neo*-Pen. House fly percentage mortality was then determined at 1.2 μg/g alone and



Scheme 5. Synthesis of two 4-alkyl-5-amino-3-cyano-1-(2,6-dichloro-4-ethynylphenyl)pyrazoles.

with PB to inhibit cytochrome P450-catalyzed oxidative detoxification.[5,14] These assays allowed ranking the 4-alkyl substituent effects (Table 1). Next, 22 isopropyl- or *tert*-butylpyrazoles with varied phenyl substituents were compared for human β3 GABA receptor IC_{50} and house fly mortality at the 1.2 μg/g discriminating dose alone and with PB (Table 2). This permitted selection of two of the most active compounds reported here (**7** and **11**) for direct potency comparisons to **1** and **5** (Table 3).

4. Results and discussion

4.1. Effect of 4-(alkyl or halo)pyrazole substituents on GABA receptor potency and insecticidal activity

The effect of 4-(alkyl or halo)pyrazole substituents was studied first in the 5-amino-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl)pyrazole series (Table 1). The potency order falls into the same five distinct groups based on house fly and human β3 receptors and toxicity to house flies alone and with PB (Table 1). The highest potency is for *t*-Bu followed by outstanding potency for the branched chain *3*-Pen, *i*-Bu and *i*-Pr substituents. High potency is retained for the *n*-Pr, *s*-Bu, *n*-Bu and Et substituents and medium potency for *neo*-Pen, *c*-Hex and the halo analogs (I, Br and Cl). Phenylpyrazoles halogenated at the 4-position of the pyrazole showed only a small variation in activity but generally the larger and more sterically hindered substituent is preferred and I is marginally better than Br or Cl for receptor binding and toxicity. There is a considerable drop to low potency for compounds with H and Me (the smallest substituents).

Further comparisons were made of the effect of three 4-alkylpyrazole substituents on potency as inhibitors of the human β3 receptors in 2,6-dichlorophenyl compounds with 4-CF_3 and 4-Cl substituents (Table 2). In each case, as above, the potency decreases in the order for pyrazole substituents of 4-*t*-Bu $\geqslant$ 4-*i*-Pr $\gg$ 4-Et.

4.2. Effect of phenyl substituents on GABA receptor potency and insecticidal activity

The position, type and extent of substitution on the phenyl ring have pronounced effects on human β3 receptor potency and house fly mortality (Table 2). The receptor potency order for derivatives with mono substitution is 4-CF_3>4-Cl>4-CN and for disubstituted compounds is 2-Cl, 4-CF_3>2,4-Cl_2 = 2,6-Cl_2. In the trisubstituted series, receptor potency and toxicity to house flies (judged both with and without PB) decreases in the order 2,4-Cl_2-4-CF_3 = 2,4-Cl_2-4-CF_3O>2,4-Cl_2-4-Br $\geqslant$ 2,4,6-Cl_3. Importantly, the 2,6-Cl_2-4-C≡CH substitution pattern (compounds **40** and **41**) confers very good receptor potency and, for **41**, also insecticidal activity with PB.

4.3. Comparisons of the most active 4-alkyl-1-phenylpyrazoles with commercial insecticide standards

It is of interest to compare the activity of the best new 4-alkyl-1-phenylpyrazoles with commercial insecticide

Table 1. Effect of alkyl or halo substituent (R in Fig. 1) on the potency of 5-amino-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-(alkyl or halo)pyrazoles as inhibitors of [^{3}H]EBOB binding to house fly and human β3 GABA receptors and toxicants to house flies alone and with piperonyl butoxide

R in Figure 1	Compd	GABA receptor			House fly mortality at 1.2 μg/g (% ± SE)	
		House fly IC_{50} (nM ± SE)	Inhibition at 10 nM (% ± SE)			
			House fly	Human β3	Alone	PB
Highest potency						
t-Bu	**7**	2.4 ± 1.1	77 ± 8	97 ± 8	100	100
Outstanding potency						
3-Pen	**15**	3.3 ± 1.4	75 ± 11	81 ± 4	7 ± 11	100
i-Bu	**13**	4.8 ± 0.2	57 ± 7	85 ± 7	23 ± 21	100
i-Pr	**11**	5.3 ± 1.7	67 ± 7	89 ± 9	78 ± 22	100
High potency						
n-Pr	**10**	9.6 ± 4.4	55 ± 14	88 ± 12	23 ± 21	100
s-Bu	**14**	10 ± 1	51 ± 4	73 ± 12	37 ± 23	100
n-Bu	**12**	12 ± 6	52 ± 6	76 ± 22	3 ± 6	93 ± 12
Et	**6**	13 ± 3	53 ± 19	65 ± 8	20 ± 22	100
Medium potency						
neo-Pen	**16**	16 ± 8	47 ± 5	37 ± 19	7 ± 11	83 ± 29
I	**20**	24 ± 16	40 ± 6	78 ± 15	0	90 ± 17
c-Hex	**17**	26 ± 1	20 ± 4	4 ± 1	0	40 ± 35
Br	**19**	30 ± 15	31 ± 7	68 ± 8	0	63 ± 25
Cl	**18**	31 ± 19	25 ± 11	66 ± 13	0	53 ± 21
Low potency						
H	**8**	123 ± 35	24 ± 9	41 ± 16	0	2 ± 5
Me	**9**	424 ± 58	1 ± 1	19 ± 17	10 ± 14	15 ± 19

Table 2. Effect of phenyl substituents on the potency of 5-amino-3-cyano-1-(substituted-phenyl)-4-(isopropyl or *tert*-butyl)pyrazoles as inhibitors of [^{3}H]EBOB binding to human β3 $GABA_A$ receptors and toxicants to house flies alone and with piperonyl butoxide

Phenyl substituents			Compd		Human β3 GABA receptor IC_{50} (nM ± SE)		House fly mortality at 1.2 μg/g (% ± SE)			
							Alone		PB	
2	4	6	*i*-Pr	*t*-Bu	*i*-Pr	*t*-Bu	*i*-Pr	*t*-Bu	*i*-Pr	*t*-Bu
H	Cl	H	**21**	**22**	10,000 ± 3500	2470 ± 500	0	0	13 ± 6	7 ± 6
H	CF_3	H	**23**	**24**	714 ± 22	177 ± 47	0	0	3 ± 5	10 ± 0
H	CN	H	**25**	**26**	>10,000[a]	>10,000[a]	0	0	10 ± 12	8 ± 5
Cl	Cl	H	**27**	**28**	337 ± 124	74 ± 24	0	0	0	10 ± 11
Cl	CF_3	H	**29**	**30**	15 ± 3	5.6 ± 2.1	0	0	13 ± 15	97 ± 6
Cl	H	Cl	**31**	**32**	101,145	253, 319	0	5 ± 6	25 ± 17	40 ± 20
Cl	CF_3O	Cl	**33**	**34**	3.4 ± 0.4	2.2 ± 0.3	83 ± 21	100	100	100
Cl	Cl	Cl	**36**	**37**	6.2 ± 0.3[b]	6.2 ± 4.0	6 ± 6	0	20 ± 14	96 ± 6
Cl	Br	Cl	**38**	**39**	6.3, 6.7	3.8, 4.3	3 ± 5	18 ± 5	48 ± 13	100
Cl	CF_3	Cl	**11**	**7**	1.9 ± 1.1[b]	1.8 ± 0.7	34 ± 23	97 ± 5	100	100
Cl	C≡CH	Cl	**40**	**41**	7.1 ± 0.7	5.4, 3.6	0	8 ± 13	8 ± 8	94 ± 13

Mortality values at 1.2 μg/g (% ± SE) for **6** and **35** are 5 ± 6 and 0 ± 0, respectively, without PB and 78 ± 17 and 5 ± 6, respectively, with PB.
[a] Inhibition of 23–29% at 10,000 nM.
[b] IC_{50} values (nM ± SE) with 4-Et substituent are: 2,6-Cl_2-4-CF_3 (compound **6**) 5.1 ± 2.4 nM; 2,4,6-Cl_3 (compound **35**) 29 ± 4 nM.

standards. The most effective new compounds (both binding potency and insecticidal activity) are 5-amino-4-alkyl-3-cyano-1-(2,4-dichloro-6-substituted-phenyl)-pyrazoles with *i*-Pr or *t*-Bu substituents (compounds **7**, **11**, and **33**–**41**) and particularly **7** and **34**. The *t*-Bu (**7**) and *i*-Pr (**11**) analogs were selected for direct comparison with fipronil (**1**) and ethiprole (**5**) (Table 3). Ethiprole is less potent than the others on the receptors. Compounds **1** and **7** are not significantly different in activity and are generally the most potent of the series. The synergist factor was particularly high for compound **11**.

4.4. Common structural requirements for alkyl substituents of 4-alkyl-1-phenylpyrazoles, 4-alkyl-1-phenylTBOs, 4-alkyl-1-phosphaTBO 1-oxides and related compounds

A similar, and potentially the same, structural specificity is observed for the alkyl substituents in several series of

Table 3. Comparison of the potency of fipronil (**1**), ethiprole (**5**) and their *t*-Bu (**7**) and *i*-Pr (**11**) analogs as inhibitors of [^{3}H]EBOB binding to house fly and human β3 GABA receptors and toxicants to house flies alone and with piperonyl butoxide

		GABA receptor IC_{50} (nM ± SE)		Fly LD_{50} (μg/g ± SE)		
R in Figure 1	Compd	House fly	Human β3	Alone	PB	PB Synergism factor
$SOCF_3$	**1**	2.3 ± 0.7[a]	3.1 ± 0.6[a]	0.16 ± 0.01[a]	0.023 ± 0.004[a]	7
SOEt	**5**	15 ± 3[a]	12 ± 2[a]	0.50 ± 0.03[a]	0.30 ± 0.02[a]	2
t-Bu	**7**	2.4 ± 1.1	1.8 ± 0.7	0.15 ± 0.07	0.019 ± 0.010	8
i-Pr	**11**	5.3 ± 1.7	1.9 ± 1.1	0.74 ± 0.50	0.067 ± 0.052	11

[a] Ref. 7.

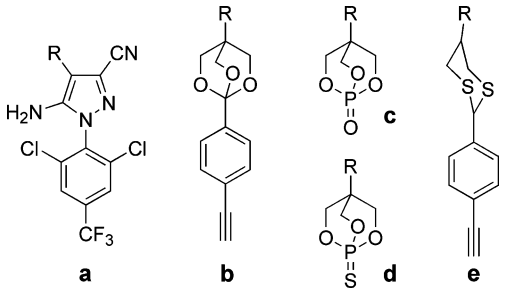


Figure 2. Common structural requirements for the optimal alkyl substituents of 4-alkyl-5-amino-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl)pyrazoles (**a**) compared with 4-alkyl-1-(4-ethynylphenyl)TBOs (**b**), 4-alkyl-1-phosphaTBO 1-oxides (**c**), 4-alkyl-1-phosphaTBO 1-sulfides (**d**), and 5-alkyl-2-(4-ethynylphenyl)-1,3-dithianes (**e**).

compounds (Fig. 2). This is evident on comparing 4-alkyl-1-phenylpyrazoles (**a**) at the house fly GABA receptor [^{3}H]EBOB binding site (this study) with 4-alkyl-1-(4-ethynylphenyl)TBOs (**b**) as toxicants to mice[20] ($r = 0.87$, $n = 8$) (Fig. 3A) and 4-alkyl-1-phosphaTBO 1-oxides (**c**) at the human brain membrane GABA receptor ([^{35}S]TBPS binding)[21] ($r = 0.83$, $n = 6$) (Fig. 3B). Similar correlations to that shown in Figure 3B are obtained with mouse ip LD_{50} values of 4-alkyl-1-phosphaTBO 1-oxides (**c**) ($r = 0.83$, $n = 6$)[22,23] and 4-alkyl-1-phosphaTBO 1-sulfides (**d**) ($r = 0.99$, $n = 5$).[23] When these last three are combined into a single plot (not shown), an excellent overall correlation is observed ($r = 0.92$, $n = 19$). The same potency order *t*-Bu> *i*-Pr>*n*-Pr>Et is also observed with 5-alkyl-2-(4-ethynylphenyl)-1,3-dithanes (**e**).[24,25] Thus, the shorter and more hindered alkyl moieties are better in the 4-alkyl-1-phenylpyrazoles as in the 4-alkyl-1-phenylTBOs, 1-phosphaTBO 1-oxides, 1-phosphaTBO 1-sulfides and 5-alkyl-2-(4-ethynylphenyl)-1,3-dithanes. The *t*-Bu substituent is also present in other active phenyl heterocyclic analogs.[26]

4.5. Structural requirements for phenyl substituents of 4-alkyl-1-phenylpyrazoles and 4-alkyl-1-phenylTBOs

4-Isopropyl- or 4-*tert*-butyl-1-phenylpyrazoles were examined with one, two or three phenyl substituents relative to optimization for potency (Table 2). With mono substitution, the receptor potency decreases in the order 4-CF_3>4-Cl>4-CN. The 4-CN moiety confers high potency in 4-alkyl-1-(4-cyanophenyl)TBOs[21] but not in the 4-alkyl-1-(4-cyanophenyl)pyrazoles, that is the substituent effect varies with the compound series.

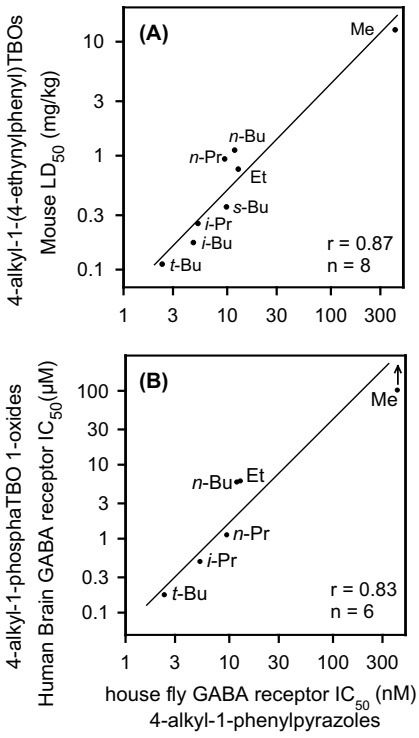


Figure 3. Structural specificity of alkyl substituents for three types of GABA receptor antagonists. IC_{50} values for house fly [^{3}H]EBOB binding site of 4-alkyl-5-amino-3-cyano-1-(2,6-dichloro-4-trifluoromethyl)pyrazoles (**a**) are compared with (panel **A**) mouse ip LD_{50} values of 4-alkyl-1-(4-ethynylphenyl)TBOs (**b**) and (panel **B**) IC_{50} values for human [^{35}S]TBPS binding site of 4-alkyl-1-phosphaTBO 1-oxides (**c**).

The disubstituted compounds are more potent at the receptor than the monosubstituted in the order 2-Cl, 4-CF_3>2,4-Cl_2 = 2,6-Cl_2. The most potent derivatives at the receptor and as insecticides are 2,6-dichloro-4-substituted compounds with 2,6-Cl_2-4-CF_3 = 2,6-Cl_2-4-OCF_3>2,4,6-Cl_3 = 2,6-Cl_2-4-Br. Importantly, 2,6-Cl_2-4-HC≡C-phenyl is an effective substitution pattern in the 4-alkyl-1-phenylpyrazoles (compounds **40** and **41**) just as HC≡C-phenyl is for the 4-alkyl-1-phenylTBOs.[27,28]

4.6. Common structural features of 4-alkyl-1-phenylpyrazoles and 4-alkyl-1-phenylTBOs

Four aspects of the SARs developed here for 4-alkyl-1-phenylpyrazoles are similar to those found earlier in optimization of 4-alkyl-1-phenylTBOs (Fig. 4). The first is the preference in both cases for *tert*-butyl among the 4-alkyl groups examined. Other bulky alkyl groups also

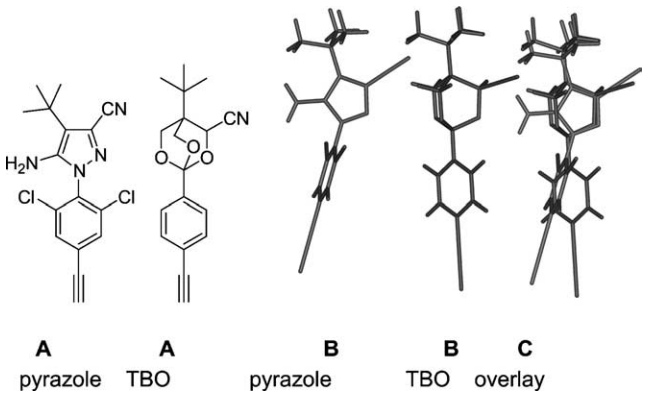


Figure 4. Comparison of 5-amino-4-*tert*-butyl-3-cyano-1-(2,6-dichloro-4-ethynylphenyl)pyrazole (pyrazole) and 4-*tert*-butyl-3-cyano-1-(4-ethynylphenyl)-2,6,7-trioxabicyclo[2.2.2]octane (TBO) as molecular structure (**A**) and wireframe individual (**B**) and overlay (**C**) images.

confer outstanding potency and it seems that a sterically hindered moiety at this position is significant with respect to binding. Second, there is a potentially corresponding electronegative atom in both heterocyclic rings. Third, the cyano substituent enhances activity but is not absolutely essential in either series.[8,26,29,30] Finally, 4-ethynyl confers high activity in the phenylpyrazoles and phenylTBOs.

A reasonably good overlay is achieved for the two series by focusing on the tertiary C of the *tert*-butyl moieties, the ring heteroatoms 2-N of pyrazole and O vicinal to CN of TBO, and the phenyl rings (Fig. 4). The two molecules overall occupy the same volume, that is 245.48 $Å^3$ for the phenylpyrazole and 238.57 $Å^3$ for the phenylTBO. The volumes in this case were calculated using the algorithm in the Search/Compare module of InsightII (Accelrys Inc, San Diego, CA), each representing a grid of the van der Waals surface of the molecule in question. The X-ray structure of **4**[4] has a volume of 213.44 $Å^3$, somewhat smaller than key phenylpyrazole **41** due to the CF_3 versus *t*-Bu moieties at the 4 position of the pyrazole rings, even though both have similar hydrophobic character.

The *tert*-butyl moieties of phenylpyrazole **41** and the corresponding TBO (**A** pyrazole and **A** TBO of Fig. 4) with the single attached C [$(CH_3)_3C$–C] occupy very much the same space of about 76 $Å^3$ using the volume algorithm as above. The difference in volumes in the overlaid mode is 15.6 $Å^3$. On comparing the volume/charge relationship of model systems, the volume of [$CF_3S(O)$–CH_3] is 73.22 $Å^3$ whereas that of [$(CH_3)_3$ C–CH_3] is 80.89 $Å^3$. If these are overlaid as C–S–C versus C–C–C then the difference in the volumes is 15.28 $Å^3$. Electronically, they both have a balance of charges. Electron density mapped onto the electrostatic potential shows the methyl hydrogens bearing a partial positive charge. There is a partial negative charge on the F atoms and the O in [$CF_3S(O)$–CH_3] and the same is true for the C atoms in [$(CH_3)_3C$–CH_3]. Despite these similarities, there are also obvious differences between the structural units, for example, the sulfoxide has the potential to participate in hydrogen bonds whereas the *tert*-butyl moiety does not.

An electronegative atom in the heterocyclic ring (pyrazole, TBO) is another feature common to both systems and superimposed in the overlays. These atoms may participate in hydrogen bonding relationships. The configuration of the CN in the TBO may have little or no significance since the pyrazole N is planar at that point and while it may pucker on binding, the nature of the pucker is not obvious. It seems that either enantiomer of the phenylTBO may be a suitable fit.

The phenyl rings have reasonably good overlap in the phenylpyrazole and phenylTBO systems and the availability of a pi face is probably important to binding. However there are some plane angle deviations. Considering a plane defined by the 1,2,6 carbons of the phenyl rings, the angle defined by the pyrazole 2-N, 1-N, and the phenyl plane is 55° whereas for the pyrazole 2-N, 1-N, and the 1-C of the phenyl is 125°. The corresponding values in the TBO system are 68° (O (vicinal to CN)-C-phenyl plane) and 111°. Rotation of the phenyl is locked orthogonal to the pyrazole by the dichloro substituents. This orientation is easily achieved by the phenylTBO. The commonality of these series is in the availability of the pi face, even though the exact angle of the pi face differs somewhat between the species. Pi–pi interactions can occur in a sandwich style stacking configuration, a parallel displaced configuration, or in a T-shaped configuration. These latter two forms are close to isoenergetic in simply substituted benzene rings with the sandwich form being higher in energy. The strength of the interaction is dependent on the ring substituents.[31,32] As a result, there are a variety of pi interactions, which can occur depending on the amino acids available in the receptor pocket.

Other substitution patterns on the phenyl in the phenylpyrazole series also confer good activity (Table 2). The 5-amino moiety on the pyrazole coupled with any 2 or 6 phenyl substituent acts to make the two rings twist out of plane, thus making the orthogonal orientation energetically available.

4.7. Common binding subsites for 4-alkyl-1-phenylpyrazoles and 4-alkyl-1-phenylTBOs

Three binding subsites can be envisioned that recognize and interact to varying degrees with the common structural features of the antagonists. Quantitative SAR-type analyses based on a wide variety of compounds and large amounts of data (Ozoe and Akamatsu[12], Brooks[33] and references cited therein) have lead to models for the binding site somewhat different than those proposed here. From the present findings, the first subsite interacts with the *t*-Bu or *i*-Pr substituent but in the pyrazole series also accepts CF_3, CF_3S, CF_3SO and CF_3SO_2 almost equally well and as such appears to be involved more in a hydrophobic and steric than an electronic interaction. The second subsite is proposed to undergo hydrogen bonding with pyrazole N-2 and TBO

O-2. Support for pyrazole N-2 comes from the good activity of 5-*tert*-butyl-2-(4-ethynylphenyl)pyrimidine with an equivalent pyrimidine N-2.[34] The pi face of the phenyl moiety is probably important for binding. Finally, the 3-cyano and 4-ethynyl substituents may be supplemental in binding since they enhance potency but are not absolutely required in either the pyrazole or TBO series.

5. Experimental

5.1. Synthetic procedures

5.1.1. General. Melting points (mp) are uncorrected. Reagents from Aldrich (Milwaukee, WI) and solvents from Fisher Scientific (Tustin, CA) were used without further purification. ^{1}H and ^{13}C NMR spectra were obtained on a 300 MHz spectrophotometer using deuteriochloroform as solvent and standard where $\delta = 7.27$ and 77.0, respectively. Mass spectra (MS) were obtained by GC/MS on a Hewlett–Packard Model 5989A mass spectrometer using electron impact (EI) and are reported as m/z. Combustion analysis were performed in the elemental analysis laboratory at the University of California, Berkeley. High-resolution mass spectra (HRMS) were obtained from the University of Notre Dame.

5.1.2. General procedure for synthesis of ethyl 2,3-dicyanoalkanoates (D in Schemes 1 and 2). To a stirred solution of ethyl cyanoacetate (10 mmol) and aldehyde (10–12 mmol) in ethanol at room temperature was added potassium cyanide (10 mmol). The reaction was initially exothermic and went to completion within 4 h but could stand overnight without problems. Water and dichloromethane (DCM) were added and then it was acidified with 2 M hydrochloric acid (6 mL). *Caution*: traces of hydrogen cyanide can be produced and this should always be carried out in a well-ventilated fume hood. The layers were separated and the aqueous phase was extracted twice more with DCM. Drying with sodium sulfate, filtering, and concentrating provided **D** in excellent yields (89–100%), which was used without further purification.

5.1.3. General procedure for synthesis of 4-alkyl-1-phenylpyrazoles (Schemes 1 and 3). Concentrated sulfuric acid (0.30 mL) or 2 M hydrochloric acid (2 mL) was added to sodium nitrite (69 mg, 1 mmol) at room temperature followed after 10 min by glacial acetic acid (0.25 mL). This nitrosyl bisulfate or nitrosyl chloride was added to 2,6-dichloro-4-trifluoromethylaniline (230 mg, 1 mmol) or other anilines in glacial acetic acid (0.25 mL). After 30 min this mixture was introduced to **D** (1 mmol) in 40% aqueous acetic acid (1.67 mL) and after 1 h DCM and water were added and the aqueous layer extracted a second time with DCM. The combined organic layers were made basic with careful addition of 10% ammonium hydroxide. The aqueous layer was removed and the organic phase stirred rapidly 12 h–4 days with 50% aqueous ammonium hydroxide at room temperature. The layers were separated and the aqueous phase extracted with DCM once. The combined organic layers were dried, filtered, and concentrated to give the crude alkylphenylpyrazoles, which were purified by crystallization (DCM/hexane), preparative TLC or flash chromatography (20% ethyl acetate in hexane). The yields reported are for isolated product based on starting aniline.

5.1.4. 5-Amino-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-ethylpyrazole (6).[7] ^{1}H NMR δ 7.78 (s, 2H), 3.49 (br s, 2H), 2.56 (q, $J = 7.5$ Hz, 2H), 1.29 (t, $J = 7.5$ Hz, 2H).

5.1.5. 5-Amino-4-*tert*-butyl-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl)pyrazole (7). (72%): mp 159–161 °C; ^{1}H NMR δ 7.78 (s, 2H), 3.57 (br s, 2H), 1.51 (s, 9H); ^{13}C NMR δ 142.5, 136.5, 135.8, 134.2 (q, $J = 34$ Hz), 126.1, 125.6, 121.9 (q, $J = 274$ Hz), 115.9, 115.0, 30.6, 30.0. MS m/z (% relative abundance) 361 (100), 376 $[M]^+$ (12). Anal. Calcd for $C_{15}H_{13}Cl_2F_3N_4$: C, 47.76; H, 3.47; N, 14.85. Found: C, 47.66; H, 3.52; N, 14.62.

5.1.6. 5-Amino-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl)pyrazole (8).[18] ^{1}H NMR δ 7.79 (s, 2H), 6.06 (s, 1H), 3.80 (br s, 2H).

5.1.7. 5-Amino-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-methylpyrazole (9). (37%): mp 162–166 °C; ^{1}H NMR δ 7.74 (s, 2H), 3.75 (br s, 2H), 2.08 (s, 3H); ^{13}C NMR δ 147.5, 137.7, 137.5, 134.4 (q, $J = 34$ Hz), 128.9, 128.4, 123.5 (q, $J = 271$ Hz), 114.7, 101.8, 7.4. MS m/z (% relative abundance) 334 $[M]^+$ (100), 213 (13). Anal. Calcd for $C_{12}H_7Cl_2F_3N_4$: C, 43.01; H, 2.11; N, 16.72. Found: C, 43.39; H, 2.41; N, 16.35.

5.1.8. 5-Amino-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-propylpyrazole (10). (55%): mp 132–134 °C; ^{1}H NMR δ 7.78 (s, 2H), 3.49 (br s, 2H), 2.51 (t, $J = 7.4$ Hz, 2H), 1.69 (sext, $J = 7.4$ Hz, 2H), 0.99 (t, $J = 7.4$ Hz, 3H); ^{13}C NMR δ 144.2, 136.3, 135.8, 134.0 (q, $J = 34$ Hz), 127.2, 125.9, 121.9 (q, $J = 274$ Hz), 113.5, 108.7, 24.4, 22.6, 13.2. MS m/z (% relative abundance) 333 (100), 362 $[M]^+$ (16). Anal. Calcd for $C_{14}H_{11}Cl_2F_3N_4$: C, 46.30; H, 3.05; N, 15.43. Found: C, 46.35; H, 3.09; N, 15.59.

5.1.9. 5-Amino-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-isopropylpyrazole (11). (48%): mp 158–160 °C; ^{1}H NMR δ 7.76 (s, 2H), 3.56 (br s, 2H), 2.89 (sept, $J = 7.2$ Hz, 1H), 1.37 (d, $J = 7.2$ Hz, 6H); ^{13}C NMR δ 142.7, 136.4, 135.9, 134.2 (q, $J = 35$ Hz), 126.1, 125.7, 121.9 (q, $J = 274$ Hz), 114.9, 114.1, 24.2, 22.1. MS m/z (% relative abundance) 347 (100), 362 $[M]^+$ (18). Anal. Calcd for $C_{14}H_{11}Cl_2F_3N_4$: C, 46.30; H, 3.05; N, 15.43. Found: C, 46.35; H, 3.20; N, 15.28.

5.1.10. 5-Amino-4-butyl-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl)pyrazole (12). (44%): mp 83–85 °C; ^{1}H NMR δ 7.77 (s, 2H), 3.54 (br s, 2H), 2.52 (t, $J = 6$ Hz,

2H), 1.62, (quint, $J = 7.4$ Hz, 2H), 1.38, (sext, $J = 7.4$ Hz, 2H), 0.96, (t, $J = 7.4$ Hz, 3H); ^{13}C NMR δ 143.6, 136.4, 134.9, 133.3 (q, $J = 34$ Hz), 127.2, 126.1, 121.9 (q, $J = 274$ Hz), 113.5, 109.6, 31.6, 22.5, 22.2, 13.8. MS m/z (% relative abundance) 333 (100), 376 $[M]^+$ (17), 246 (11). Anal. Calcd for $C_{15}H_{13}Cl_2F_3N_4$: C, 47.76; H, 3.47; N, 14.85. Found: C, 48.09; H, 3.68; N, 14.47.

5.1.11. 5-Amino-4-isobutyl-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl)pyrazole (13). (62%): mp 120–121 °C; ^{1}H NMR δ 7.78 (s, 2H), 3.48 (br s, 2H), 2.40 (d, $J = 7.2$ Hz, 2H), 2.05–1.88 (m, 1H), 0.98 (d, $J = 6.7$ Hz, 6H); ^{13}C NMR δ 144.2, 136.4, 135.9, 134.2 (q, $J = 34$ Hz), 127.9, 126.0, 121.9 (q, $J = 274$ Hz), 113.6, 108.3, 31.7, 29.3, 22.0. MS m/z (% relative abundance) 333 (100), 376 $[M]^+$ (12). Anal. Calcd for $C_{15}H_{13}Cl_2F_3N_4$: C, 47.76; H, 3.47; N, 14.85. Found: C, 47.91; H, 3.68; N, 14.60.

5.1.12. 5-Amino-4-*sec*-butyl-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenylpyrazole (14). (75%): mp 144–146 °C; ^{1}H NMR δ 7.78 (s, 2H), 3.44 (br s, 2H), 2.62 (sext, $J = 7.3$ Hz, 1H), 1.75 (quint, $J = 7.4$ Hz, 2H), 1.42 (d, $J = 7.2$ Hz, 3H), 0.92 (t, $J = 7.2$ Hz, 3H); ^{13}C NMR δ 143.5, 136.4, 135.9, 134.1 (q, $J = 34$ Hz), 126.1, 126.0, 121.9 (q, $J = 274$ Hz), 114.0, 113.3, 31.3, 29.5, 19.9, 12.2. MS m/z (% relative abundance) 347 (100), 376 $[M]^+$ (11). Anal. Calcd for $C_{15}H_{13}Cl_2F_3N_4$: C, 47.76; H, 3.47; N, 14.85. Found: C, 47.37; H, 3.54; N, 14.46.

5.1.13. 5-Amino-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-(3-pentyl)pyrazole (15). (71%): mp 145–147 °C; ^{1}H NMR δ 7.78 (s, 2H), 3.46 (br s, 2H), 2.41–2.31 (m, 1H), 1.88–1.66 (m, 4H), 0.90 (t, $J = 7.4$ Hz, 6H); ^{13}C NMR δ 144.1, 136.4, 135.9, 134.2 (q, $J = 34$ Hz), 126.5, 126.1, 121.9 (q, $J = 274$ Hz), 114.0, 111.5, 39.2, 27.5, 12.2. MS m/z (% relative abundance) 361 (100), 333 (15), 390 $[M]^+$ (7). Anal. Calcd for $C_{16}H_{15}Cl_2F_3N_4$: C, 49.12; H, 3.86; N, 14.32. Found: C, 49.30; H, 3.99; N, 14.13.

5.1.14. 5-Amino-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-*neo*-pentylpyrazole (16). (64%): mp 189–191 °C; ^{1}H NMR δ 7.78 (s, 2H), 3.52 (br s, 2H), 2.40 (s, 2H), 1.02 (s, 9H); ^{13}C NMR δ 144.7, 136.4, 136.0, 134.2 (q, $J = 34$ Hz), 128.9, 126.2, 121.9 (q, $J = 274$ Hz), 114.0, 107.1, 37.0, 34.0, 29.2. MS m/z (% relative abundance) 333 (100), 57 (28), 299 (21), 247 (18), 390 $[M]^+$ (16), 375 (15). Anal. Calcd for $C_{16}H_{15}Cl_2F_3N_4$: C, 49.12; H, 3.86; N, 14.32. Found: C, 49.16; H, 3.98; N, 14.16.

5.1.15. 5-Amino-3-cyano-4-cyclohexyl-1-(2,6-dichloro-4-trifluoromethylphenyl)pyrazole (17). (66%): mp 162–164 °C; ^{1}H NMR δ 7.77 (s, 2H), 3.48 (br s, 2H), 2.53–2.43 (m, 1H), 1.91–1.88 (m, 4H), 1.79–1.71 (m, 3H), 1.41–1.33 (m, 3H); ^{13}C NMR δ 143.0, 136.3, 135.9, 134.2 (q, $J = 34$ Hz), 126.0, 125.7, 121.9 (q, $J = 274$ Hz), 114.2, 114.0, 34.2, 32.2, 26.6, 25.5. MS m/z (% relative abundance) 359 (100), 402 $[M]^+$ (44), 333 (36). Anal. Calcd for $C_{17}H_{15}Cl_2F_3N_4$: C, 50.64; H, 3.75; N, 13.89. Found: C, 50.73; H, 3.68; N, 14.04.

5.1.16. General procedure for halogenation of 8 (Scheme 4). Phenylpyrazole **8** (64 mg, 0.20 mmol) was dissolved in acetonitrile (1 mL) and N-halosuccinimide (0.21 mmol) was added at room temperature. After reacting for 1 h at reflux (X = I), 50 °C (X = Br), or room temperature (X = C1) ethyl acetate and water were added. The separated organic layer was washed with water and brine, dried and concentrated to provide the desired products after purification by preparative TLC.

5.1.17. 5-Amino-4-chloro-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl)pyrazole (18). (94%): mp 187–188 °C; ^{1}H NMR δ 7.80 (s, 2H), 3.92 (br s, 2H); ^{13}C NMR δ 143.3, 136.4, 135.2, 134.8 (q, $J = 34$ Hz), 127.3, 126.3, 121.9 (q, $J = 274$ Hz), 111.4, 97.1. MS m/z (% relative abundance) 354 $[M]^+$ (100), 213 (17), 240 (14), 77 (14). Anal. Calcd for $C_{11}H_{14}Cl_3F_3N_4$: C, 37.16; H, 1.13; N, 15.76. Found: C, 37.27; H, 1.27; N, 15.81.

5.1.18. 5-Amino-4-bromo-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl)pyrazole (19). (87%): mp 208–210 °C; ^{1}H NMR δ 7.80 (s, 2H), 4.01 (br s, 2H); ^{13}C NMR δ 144.9, 136.4, 135.3, 134.8 (q, $J = 34$ Hz), 128.1, 126.3, 121.9 (q, $J = 274$ Hz), 111.9, 81.1. MS m/z (% relative abundance) 400 $[M]^+$ (100), 213 (19), 77 (12). Anal. Calcd for $C_{11}H_{14}BrCl_2F_3N_4$: C, 33.03; H, 1.01; N, 14.01. Found: C, 33.22; H, 1.00; N, 14.11.

5.1.19. 5-Amino-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-iodopyrazole (20).[7] ^{1}H NMR 7.80 (s, 2H), 3.98 (br s, 2H).

5.1.20. 5-Amino-1-(4-chlorophenyl)-3-cyano-4-isopropylpyrazole (21). (42%): ^{1}H NMR 7.52–7.45 (m, 4H), 3.79 (br s, 2H), 2.84 (sept, $J = 7$ Hz, 1H), 1.36 (d, $J = 7$ Hz, 6H); ^{13}C NMR 141.5, 136.0, 134.6, 129.8, 125.5, 123.8, 114.7, 114.5, 24.2, 22.1. HRMS Calcd for $C_{13}H_{14}ClN_4$: 261.0907, found: 261.0888.

5.1.21. 5-Amino-4-*tert*-butyl-1-(4-chlorophenyl)-3-cyanopyrazole (22). (13%): ^{1}H NMR 7.48 (s, 4H), 3.86 (br s, 2H), 1.46 (s, 9H); ^{13}C NMR 141.3, 135.7, 134.9, 129.9, 126.2, 124.0, 115.7, 115.5, 30.7, 30.4. HRMS Calcd for $C_{14}H_{16}ClN_4$: 275.1063, found 275.1056.

5.1.22. 5-Amino-3-cyano-1-(4-trifluoromethylphenyl)-4-isopropylpyrazole (23). (25%): ^{1}H NMR 7.77 (d, $J = 9$ Hz), 7.73 (d, $J = 9$ Hz), 3.88 (br s, 2H), 2.85 (sept, $J = 7$ Hz, 1H), 1.36 (d, $J = 7$ Hz, 6H); ^{13}C NMR 141.8, 140.5, 130.4 (q, $J = 33$ Hz), 126.8 (q, $J = 5$ Hz), 124.3, 124.1, 123.5 (q, $J = 270$ Hz), 115.2, 114.4, 24.1, 22.0.

Anal. Calcd for $C_{14}H_{13}F_3N_4$: C, 57.14; H, 4.45; N, 19.04. Found: C, 56.79; H, 4.48; N, 18.79.

5.1.23. 5-Amino-4-*tert*-butyl-3-cyano-1-(4-trifluoromethylphenyl)pyrazole (24). (16%): ^{1}H NMR 7.79 (d, $J = 9$ Hz), 7.72 (d, $J = 9$ Hz), 3.92 (br s, 2H), 1.48 (s, 9H); ^{13}C NMR 141.5, 140.3, 130.7 (q, $J = 33$ Hz), 128.9, 125.3, 124.8, 124.5, 116.1, 115.4, 30.7, 30.3. Anal. Calcd for $C_{15}H_{15}F_3N_4$: C, 58.44; H, 4.90; N, 18.17. Found: C, 58.10; H, 5.00; N, 17.85.

5.1.24. 5-Amino-3-cyano-1-(4-cyanophenyl)-4-isopropylpyrazole (25). (12%): ^{1}H NMR 7.82 (s, 4H), 3.80 (br s, 2H), 2.86 (sept, $J = 7$ Hz, 1H), 1.39 (d, $J = 7$ Hz, 6H); ^{13}C NMR 141.5, 141.3, 133.6, 125.1, 124.0, 117.8, 115.9, 114.1, 112.0, 24.2, 22.1. HRMS Calcd for $C_{14}H_{14}N_5$: 252.1249, found 252.1278.

5.1.25. 5-Amino-4-*tert*-butyl-3-cyano-1-(4-cyanophenyl-pyrazole (26). (15%): ^{1}H NMR 7.83–7.75 (m, 4H), 3.96 (br s, 2H), 1.47 (s, 9H); ^{13}C NMR 141.5, 141.0, 133.6, 125.0, 124.8, 117.7, 116.5, 115.2, 112.2, 30.7, 30.3. Anal. Calcd for $C_{15}H_{15}N_5$: C, 67.90; H, 5.70; N, 26.40. Found : C, 67.77; H, 5.79; N, 26.29.

5.1.26. 5-Amino-3-cyano-1-(2,4-dichlorophenyl)-4-isopropylpyrazole (27). (45%): ^{1}H NMR 7.60 (d, $J = 1$ Hz, 1H), 7.46–7.39 (m, 2H), 3.56 (br s, 2H), 2.88 (sept, $J = 7$ Hz, 1H), 1.38 (d, $J = 7$ Hz, 6H); ^{13}C NMR 142.7, 136.9, 133.3, 132.9, 130.6, 130.5, 128.4, 124.5, 114.3, 114.2, 24.1, 22.1. Anal. Calcd for $C_{13}H_{12}Cl_2N_4$: C, 52.90; H, 4.10; N, 18.98. Found: C, 52.94; H, 4.08; N, 18.76.

5.1.27. 5-Amino-4-*tert*-butyl-3-cyano-1-(2,4-dichlorophenyl)pyrazole (28). (28%): ^{1}H NMR 7.60 (d, $J = 1$ Hz, 1H), 7.46–7.39 (m, 2H), 3.65 (br s, 2H), 1.48 (s, 9H); ^{13}C NMR 142.6, 136.9, 133.2, 133.1, 130.7, 130.5, 128.4, 124.4, 115.3, 115.2, 30.5, 30.2. Anal. Calcd for $C_{14}H_{14}Cl_2N_4$: C, 54.38; H, 4.56; N, 18.12. Found: C, 54.41; H, 4.61; N, 18.06.

5.1.28. 5-Amino-1-(2-chloro-4-trifluoromethylphenyl)-3-cyano-4-isopropylpyrazole (29). (67%): ^{1}H NMR 7.86 (s, 1H), 7.73 (d, $J = 8$ Hz, 1H), 7.63 (d, $J = 8$ Hz, 1H), 3.58 (br s, 2H), 2.90 (sept, $J = 7$ Hz, 1H), 1.40 (d, $J = 7$ Hz, 6H); ^{13}C NMR 143.0, 137.9, 133.4 (q, $J = 34$ Hz), 132.7, 130.5, 127.9, 125.0, 124.8, 122.6 (q, $J = 270$ Hz), 114.6, 114.2, 24.1, 22.0. HRMS Calcd for $C_{14}H_{13}ClF_3N_4$: 329.0781, found 329.0777.

5.1.29. 5-Amino-4-*tert*-butyl-1-(2-chloro-4-trifluoromethylphenyl)-3-cyanopyrazole (30). (19%): ^{1}H NMR 7.85 (s, 1H), 7.72 (d, $J = 8$ Hz, 1H), 7.61 (d, $J = 8$ Hz, 1H), 3.64 (br s, 2H), 1.48 (s, 9H); ^{13}C NMR 142.7, 137.7, 133.4 (q, $J = 33$ Hz), 133.1, 130.6, 127.9, 125.0, 124.7, 123.6 (q, $J = 270$ Hz), 115.5, 115.2, 30.5, 30.2. Anal. Calcd for $C_{15}H_{14}ClF_3N_4$: C, 52.56; H, 4.12; N, 16.35. Found: C, 52.36; H, 4.36; N, 16.01.

5.1.30. 5-Amino-3-cyano-1-(2,6-dichlorophenyl)-4-isopropylpyrazole (31). (9%): ^{1}H NMR 7.51–7.41 (m, 3H), 3.48 (br s, 2H), 2.91 (sept, $J = 7$ Hz, 1H), 1.40 (d, $J = 7$ Hz, 6H); ^{13}C NMR 142.5, 135.4, 132.7, 131.9, 129.0, 125.2, 114.5, 114.3, 24.3, 22.2. HRMS Calcd for $C_{13}H_{13}Cl_2N_4$: 295.0517, found 295.0495.

5.1.31. 5-Amino-4-*tert*-butyl-3-cyano-1-(2,6-dichlorophenyl)pyrazole (32). (7%): ^{1}H NMR 7.53–7.44 (m, 3H), 3.59 (br s, 2H), 1.49 (s, 9H); ^{13}C NMR 142.4, 135.4, 132.5, 132.0, 129.0, 125.1, 115.5, 115.4, 30.7, 30.4. HRMS Calcd for $C_{14}H_{15}Cl_2N_4$: 309.0674, found 309.0669.

5.1.32. 5-Amino-3-cyano-1-(2,6-dichloro-4-trifluoromethoxyphenyl)-4-isopropylpyrazole (33). (45%): ^{1}H NMR 7.39 (s, 2H), 3.53 (br s, 2H), 2.90 (sept, $J = 7$ Hz, 1H), 1.39 (d, $J = 7$ Hz, 6H); ^{13}C NMR 150.1, 142.7, 136.5, 131.4, 125.6, 121.2, 120.0 (q, $J = 260$ Hz), 114.7, 114.1, 24.3, 22.2. Anal. Calcd for $C_{14}H_{11}Cl_2F_3N_4O_2$: C, 44.35; H, 2.92; N, 14.78. Found: C, 44.45; H, 2.90; N, 14.62.

5.1.33. 5-Amino-4-*tert*-butyl-3-cyano-1-(2,4-dichloro-4-trifluoromethoxyphenyl)pyrazole (34). (17%): ^{1}H NMR 7.39 (s, 2H), 3.65 (br s, 2H), 1.48 (s, 9H); ^{13}C NMR 150.1, 142.4, 136.6, 131.3, 125.6, 121.2, 120.0 (q, $J = 260$ Hz), 115.7, 115.1, 30.7, 30.3. HRMS Calcd for $C_{15}H_{14}Cl_2F_3N_4O$: 393.0497, found 393.0476.

5.1.34. 5-Amino-3-cyano-4-ethyl-1-(2,4,6-trichlorophenyl)pyrazole (35). (39%): ^{1}H NMR 7.52 (s, 2H), 3.51 (br s, 2H), 2.54 (q, $J = 8$ Hz, 2H), 1.27 (t, $J = 8$ Hz, 3H); ^{13}C NMR 143.5, 137.4, 135.9, 131.4, 129.0, 126.6, 113.7, 110.2, 16.1, 14.0. MS m/z (% relative abundance) 299 (100), 314 $[M]^+$ (33), 212 (12), 264 (11). Anal. Calcd for $C_{12}H_9Cl_3N_4$: C, 45.67; H, 2.87; N, 17.75. Found: C, 45.43; H, 2.95; N, 17.59.

5.1.35. 5-Amino-3-cyano-4-isopropyl-1-(2,4,6-trichlorophenyl)pyrazole (36). (42%): ^{1}H NMR 7.51 (s, 2H), 3.56 (br s, 2H), 2.88 (sept, $J = 7$ Hz, 1H), 1.37 (d, $J = 7$ Hz, 6H); ^{13}C NMR 142.7, 137.4, 135.9, 131.5, 129.0, 125.3, 114.4, 114.2, 24.2, 22.2. MS m/z (% relative abundance) 313 (100), 328 $[M]^+$ (17). Anal. Calcd for $C_{13}H_{11}Cl_3N_4$: C, 47.37; H, 3.36; N, 17.00. Found: C, 47.14; H, 3.51; N, 16.88.

5.1.36. 5-Amino-4-*tert*-butyl-3-cyano-1-(2,4,6-trichlorophenyl)pyrazole (37). (37%): ^{1}H NMR 7.52 (s, 2H), 3.59 (br s, 2H), 1.49 (s, 9H); ^{13}C NMR 142.3, 137.5, 136.1, 131.4, 129.1, 125.6, 115.8, 115.2, 30.7, 30.4. MS m/z (% relative abundance) 327 (100), 342 $[M]^+$ (10). Anal. Calcd for $C_{14}H_{13}Cl_3N_4$: C, 48.93; H, 3.81; N, 16.30. Found: C, 48.79; H, 3.86; N, 16.16.

5.1.37. 5-Amino-1-(4-bromo-2,6-dichlorophenyl)-3-cyano-4-isopropylpyrazole (38). (21%): ^{1}H NMR 7.67 (s, 2H), 3.50 (br s, 2H), 2.89 (sept, $J = 7$ Hz, 1H), 1.38 (d, $J = 7$ Hz, 6H); ^{13}C NMR 142.6, 136.1, 131.93, 131.87, 125.5, 124.9, 114.6, 114.1, 24.3, 22.2. Anal. Calcd for $C_{13}H_{11}BrCl_2N_4$: C, 41.74; H, 2.96; N, 14.98. Found: C, 41.67; H, 2.89; N, 14.62.

5.1.38. 5-Amino-4-*tert*-butyl-3-cyano-1-(4-bromo-2,6-dichlorophenyl)pyrazole (39). (19%): ^{1}H NMR 7.65 (s, 2H), 3.68 (br s, 2H), 1.45 (s, 9H); ^{13}C NMR 142.5, 136.1, 131.8, 131.7, 125.3, 124.9, 115.5, 115.2, 30.6, 30.3. HRMS Calcd for $C_{14}H_{14}BrCl_2N_4$: 386.9779, found 386.9798.

5.1.39. General procedure for synthesis of 1-(4-ethynylphenyl)pyrazoles (Scheme 5). The aryl bromide (100 μmol), tetrakis(triphenylphosphine)palladium (0) (2.3 mg, 2 mol), and copper (1) iodide were placed in a round-bottom flask under nitrogen. Toluene (2.3 mL) and diisopropylamine (DIPA) (700 μL) were added and the solution stirred for 20 min at room temperature before addition of (trimethylsilyl)acetylene (24.6 mg, 250 μmol). After heating to 60 °C for 14 h, the reaction was cooled and the dark mixture was filtered and evaporated to dryness, redissolved in toluene and filtered through a short silica gel column. The resulting product was mixed with potassium carbonate (100 mg) and methanol (6 mL) for 1 h. Water and dichloromethane were added and the organic layer was concentrated and purified via preparative TLC.

5.1.40. 5-Amino-3-cyano-1-(2,6-dichloro-4-ethynylphenyl)-4-isopropylpyrazole (40). (89%): ^{1}H NMR 7.60 (s, 2H), 3.47 (br s, 2H), 3.32 (s, 1H), 2.90 (sept, $J = 7$ Hz, 1H), 1.39 (d, $J = 7$ Hz, 6H); ^{13}C NMR 142.5, 135.3, 133.0, 132.2, 126.6, 125.5, 114.7, 114.2, 82.0, 79.8, 24.3, 22.2. HRMS Calcd for $C_{15}H_{13}Cl_2N_4$: 319.0517, found 319.0528.

5.1.41. 5-Amino-4-*tert*-butyl-3-cyano-1-(2,6-dichloro-4-ethynylphenyl)pyrazole (41). (84%): ^{1}H NMR 7.58 (s, 2H), 3.63 (br s, 2H), 3.32 (s, 1H), 1.47 (s, 9H); ^{13}C NMR 142.4, 135.3, 132.8, 132.1, 126.6, 125.3, 115.6, 115.2, 82.1, 79.7, 30.6, 30.3. HRMS Calcd for $C_{16}H_{15}Cl_2N_4$: 333.0674, found 333.0667.

5.2. [^{3}H]EBOB binding assays for house fly and human β3 GABA receptors

House fly head membranes were prepared in and washed with 0.25 M sucrose, 10 mM Tris–HCl buffer (pH 7.5) and finally reconstituted in 10 mM sodium phosphate buffer (pH 7.5) containing 300 mM sodium chloride.[7] Membranes with human homooligomeric β3 receptors expressed in Sf9 cells were prepared directly in 10 mM sodium phosphate pH 7.5–300 mM sodium chloride.[16] Protein was determined by the Lowry procedure[35] with bovine serum albumin as the standard. The assay mixture in a final volume of 500 μL contained 100–200 g membrane protein, 0.8 nM [^{3}H]EBOB, and the candidate inhibitor added in dimethyl sulfoxide (0.5% final concentration). Following incubation for 70 min at 25 °C with shaking, the samples were filtered through Whatman GF/B paper [presoaked in 0.1% polyethylenimine (v/v) for 1 h] and rinsed three times with 5 mL of ice cold rinsing buffer (0.9% sodium chloride w/v) using a Brandel 24-well harvester. Radioactivity bound to membranes on the filters was measured with a liquid scintillation counter. Nonspecific binding was determined in the presence of 0.5 μM-endosulfan. Sigma Plot version 4.01 was used for least-squares regression analysis to determine the concentration for 50% inhibition (IC_{50}) with three or four determinations for the standard error (SE) values.

5.3. Toxicity to house flies

Topical LD_{50} values or mortality at a discriminating dose were determined for adult female house flies 24 h after topical application of the test compound in 0.5 μL acetone to the ventrum of the abdomen.[14] For synergism studies, PB was applied topically to the thorax at 250 μg/g 2 h before the test compound to inhibit most cytochrome P450-catalyzed oxidative detoxification.[5,14]

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