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Rynaxypyr™: A new insecticidal anthranilic diamide that acts as a potent and selective ryanodine receptor activator

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Abstract—Rynaxypyr™ is a highly potent and selective activator of insect ryanodine receptors with exceptional activity on a broad range of Lepidoptera. A strong correlation between insecticidal activity and ryanodine receptor activation is observed along with selective activity against insect over mammalian receptors. The synthesis and biological results are presented.

The ryanodine receptor (RyR) is a non-voltage-gated calcium channel located in the sarcoplasmic reticulum of muscle cells and endoplasmic reticulum of non-muscle cells. RyRs regulate the release of intracellular calcium stores critical for muscle contraction. Its name is derived from the natural insecticide ryanodine, a plant metabolite from *Ryania speciosa*, that has been found to affect calcium release by locking channels in a partially opened state.[1] We recently reported the discovery of a new class of insecticides, the anthranilic diamides, which exhibit their action through activation of the ryanodine receptor.[2,3] In this paper we describe the discovery of Rynaxypyr™ (chlorantraniliprole, DPX-E2Y45), a potent ryanodine receptor activator, as the first new insecticide from this class (Fig. 1).[4,5] Rynaxypyr™ is characterized by its high levels of insecticidal activity and low toxicity to mammals attributed to a high selectivity for insect over mammalian ryanodine receptors.[6,7]

The ability of insects to rapidly develop resistance to conventional pesticides poses a significant problem for effective pest management. The discovery of control agents that work by new biochemical mechanisms is therefore of critical importance in crop protection. Calcium channel regulation and, in particular, the ryanodine receptor (RyR) represents a new biochemical target for insect control and thus offers excellent promise in integrated pest management strategies. Rynaxypyr™ binds to a receptor site distinct from that of ryanodine and appears to be impacted by the channel's state. Activation causes unregulated release of internal calcium stores leading to Ca^{2+} depletion, feeding cessation, lethargy, muscle paralysis, and ultimately insect death. Through cloning and expression of multiple insect RyRs we have been able to provide genetic validation for the mode of action.[3,6]

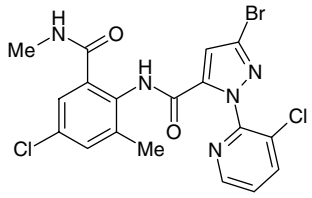

Figure 1. Rynaxypyr™ (chlorantraniliprole, DPX-E2Y45).

We previously described the 3-chloropyridyl group of the anthranilic diamides as an optimum substituent on the pyrazole-5-carboxamide.[2] Additionally, we found the 6-methyl group of the anthranilamide to be one of the more preferred substituents. Here we describe the synthesis, insecticidal activity, and RyR activity of 1-(3-chlororpyridyl)-pyrazole-6-methylanthranilic diamides

Keywords: Rynaxypyr™; Ryanodine; Receptor; Insecticide.
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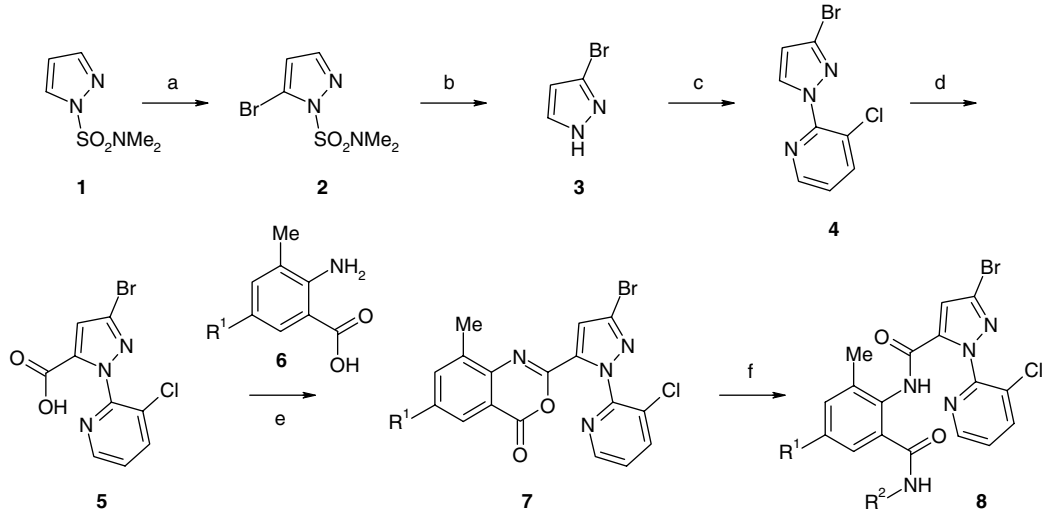
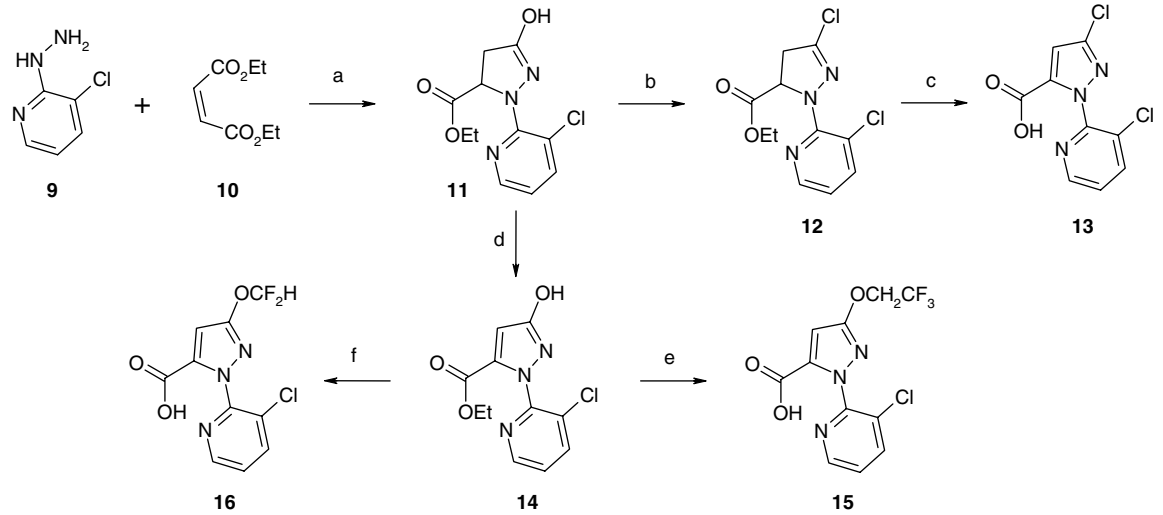
with varied 4-anthranilic and 3-pyrazole substituents, leading to the discovery of Rynaxypyr™.

Schemes 1–3 illustrate the routes by which the compounds of Table 1 were synthesized. Anthranilic diamides **8** containing a 3-bromopyrazole (R^3 = Br) could be prepared as outlined in Scheme 1. A new method for the preparation of the intermediate 3-bromopyrazole **3** was developed that takes advantage of the known selective lithiation of *N*,*N*-dimethyl-sulfamoylpyrazole **1** at the 5-position.[8] Thus, treatment of **1** with *n*-butyllithium followed by bromination with 1,2-dibromo-1,1,2,2-tetrachloroethane affords 5-bromo-1-*N*,*N*-dimethylsulfamoylpyrazole **2**. Removal of the *N*,*N*-dimethylsulfamoyl protecting group with TFA affords the 3-bromopyrazole **3**, which upon reaction with 2,3-dichloropyridine provides 1-pyridyl-3-bromopyrazole **4**. Metallation of **4** with lithium diisopropylamide followed by addition of carbon dioxide affords the key intermediate 3-bromopyrazole-5-carboxylic acid **5**.

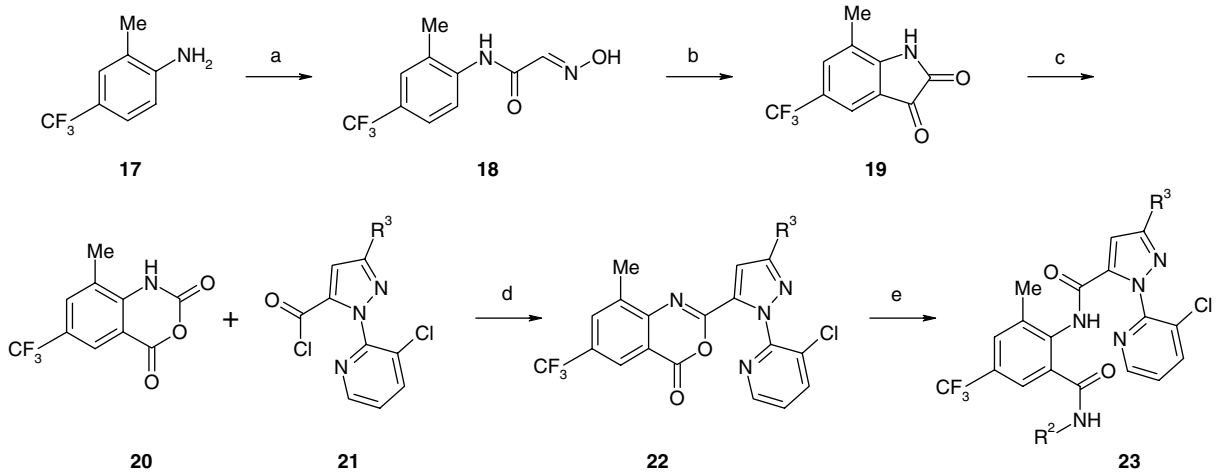
The benzoxazinone intermediates **7** could be prepared by the coupling of the pyrazole carboxylic acids **5** with anthranilic acids **6**. A preferred method for direct conversion of **5** to the benzoxazinone involves sequential treatment with one equivalent of triethylamine and methanesulfonyl chloride, followed by sequential addition of the anthranilic acid **6**, triethylamine, and methanesulfonyl chloride to provide benzoxazinone **7**. Treatment of **7** with amines, R^2NH_2, provided the anthranilic diamides **8** which generally precipitated from the reaction medium. Anthranilic diamides containing a 3-chloropyrazole (R^3 = Cl) could be prepared in an analogous fashion from 3-chloropyrazole prepared from



Scheme 1. Reagents and conditions: (a) i—*n*BuLi, THF, −60 °C; ii—$BrCCl_2CCl_2Br$, THF, −70 °C, 89%; (b) TFA, 25 °C, 78%; (c) 2,3-dichloropyridine, K_2CO_3, DMF, 125 °C, 64%; (d) i—LDA, THF, −78 °C; ii—CO_2 gas; iii—HCl, 87%; (e) i—**5**, $MeSO_2Cl$, Et_3N, MeCN; ii—**6**; iii—Et_3N, MeCN; iv—$MeSO_2Cl$, 50–60%; (f) R^2NH_2, THF, 70–95%.

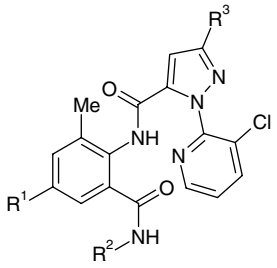


Scheme 2. Reagents and conditions: (a) NaOEt, EtOH, reflux, 55%; (b) $POCl_3$, MeCN, 80 °C, 91%; (c) i—MeCN, H_2SO_4, $K_2S_2O_8$, reflux, 82%; ii—aq NaOH, MeOH, 83%; (d) MeCN, H_2SO_4, $K_2S_2O_8$, reflux, 82%; (e) i—CF_3CH_2I, K_2CO_3, DMF, 100 °C, 44%; ii—aq NaOH, MeOH, 87%; (f) i—$CHClF_2$, K_2CO_3, DMF 42%; ii—aq NaOH, MeOH, 80%.



Scheme 3. Reagents and conditions: (a) $Cl_3CCH(OH)_2$, Na_2SO_4, $(NH_2OH)_2$–H_2SO_4, HCl, H_2O, 35%; (b) H_2SO_4, H_2O, 85%; (c) HOAc, H_2O_2, 75 °C, 60%; (d) Et_3N, CH_3CN, 70 °C, 23%; (e) R^2NH_2, THF, 40–50%.

Table 1. Insecticidal potency and *Hv* ryanodine receptor activity (*Hv* RyR EC_{50}) of anthranilic diamides[15]



Entry	R^1	R^2	R^3	*Sf*[a] LC_{50} (ppm)	*Px*[a] LC_{50} (ppm)	*Hv*[a] LC_{50} (ppm)	*Hv* RyR[a] EC_{50} nM (SEM)
D1	Cl	Me	CF_3	0.02	0.01[c]	0.05	73 (4)
D2	Cl	*i*-Pr	CF_3	0.03	0.01	0.02[b]	1834 (111)
D3	Cl	Me	Br	0.02[b]	0.02	0.04[b]	52 (3)
D4	Cl	*i*-Pr	Br	0.04[b]	0.04	0.02[c]	458 (23)
D5	Cl	Me	Cl	0.03	0.03	0.07	69 (3)
D6	Cl	*i*-Pr	Cl	0.05	0.09	0.05[c]	270 (17)
D7	Br	*i*-Pr	CF_3	0.03	0.01	0.03	1574 (97)
D8	Br	Me	Br	0.18	0.06	0.11	63 (4)
D9	I	Me	CF_3	0.26	0.10	0.21	205 (10)
D10	I	Me	Br	0.13	0.07[b]	0.16	118 (6)
D11	CF_3	Me	CF_3	0.53	0.09	0.66	NT
D12	CF_3	*i*-Pr	CF_3	0.39	0.07	0.11	3704 (80)
D13	Cl	Me	OCH_3	0.68	0.33	2.43	101 (9)
D14	Cl	*i*-Pr	OCH_3	0.30	0.18	1.14	387 (9)
D15	Cl	Me	OCF_2H	0.21	0.38	1.08	60 (3)
D16	Cl	*i*-Pr	OCF_2H	0.14	0.10	0.12	511 (34)
D17	Cl	Me	OCH_2CF_3	0.11	0.04	0.24	240 (4)
D18	Cl	*i*-Pr	OCH_2CF_3	0.03[b]	0.01	0.09	2734 (284)

Insect LC_{50}s (ppm) are on fall armyworm (*Sf*, *Spodoptera frugiperda*), diamondback moth (*Px*, *Plutella xylostella*), and tobacco budworm (*Hv*, *Heliothis virescens*).

[a] Mortality and RyR EC_{50} values were obtained for multiple test rates, each tested in replicate ($n \geqslant 16$ for LC_{50} values), ($n \geqslant 3$ for EC_{50} values). LC_{50} and EC_{50} calculations were determined by Probit analysis using a maximum quasi-likelihood curve fitting algorithm.[16] For each of the LC_{50} values listed, the range between the calculated LC_{50} and the corresponding lower or upper 90%-confidence interval value is less than 50% of the calculated value unless otherwise noted.

[b] The range for the upper and/or lower 90%-confidence interval is less than 100% of the calculated value.

[c] The range for the upper and/or lower 90%-confidence interval is less than 300% of the calculated value.

1 by chlorination with hexachloroethane. The intermediate 4-halo-6-methyl-anthranilic acids **6** (R^1 = Cl, Br, I) were prepared by halogenation of 6-methylanthranilic acid with the corresponding *N*-halosuccinimide.[5]

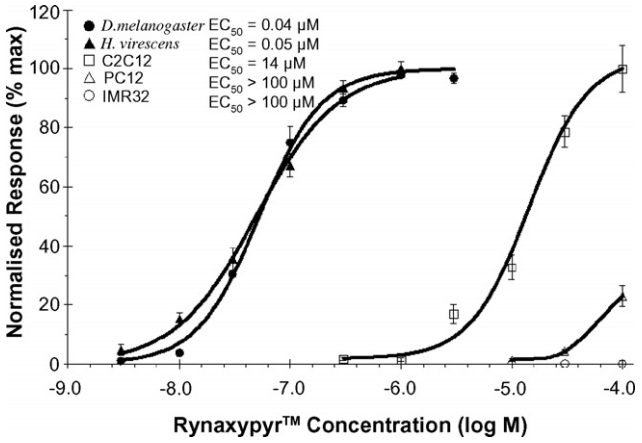


Chart 1. Differential receptor selectivity of Rynaxypyr™ in insect and mammalian cell lines expressing ryanodine receptors.

Preparation of the pyrazole-5-carboxylic acids created several problems associated with practical aspects of the lithiation on scale up. An alternate route for the preparation of halopyrazole carboxylic acids (R^3 is Cl or Br) was developed and is outlined in Scheme 2 for 3-chloropyrazole-5-carboxylic acid **13**.[9] Reaction of diethylmaleate **10** with 3-chloro-2-hydrazinopyridine **9** in the presence of sodium ethoxide afforded the pyrazolone **11** in 55% yield. Subsequent treatment of **11** with phosphoryl chloride in acetonitrile at 80 °C afforded the chloropyrazoline **12** in excellent yield. The bromopyrazoline analog of **12** could also be prepared with similar results using phosphoryl bromide as the bromination reagent. A variety of reagents and conditions were explored for oxidation of **12** to the pyrazole **13**, and potassium persulfate was found to be one of the preferred oxidants.[9]

The fluoroalkoxy derivatives **15** and **16** were prepared in two steps from pyrazolone **11**. Persulfate oxidation of **11** provided pyrazolone **14** albeit in lower yields than the chloro analog. Trifluoroethoxy pyrazole **15** was prepared by alkylation with trifluoroethyliodide. The difluoromethoxy analog **16** was prepared from **14** via difluorocarbene addition generated from Freon 22 and base. The pyrazole acids **13**, **15**, and **16** were used to prepare anthranilic diamides by procedures analogous to those of Scheme 1.

Anthranilic diamides containing a CF_3 substituent at R^1 (i.e. **23)** were prepared by the procedure shown in Scheme 3. Isatin **19** was prepared in two steps from 2-methyl-4-trifluoromethyl aniline by first reaction with chloral hydrate and hydroxylamine to yield **18** followed by cyclization with sulfuric acid.[10,11] Oxidation of **19** to the isatoic anhydride **20** was accomplished with hydrogen peroxide in acetic acid.[12] The isatoic anhydride **20** could be coupled directly with pyrazole acid chlorides such as **21** to yield the benzoxazinones **22** in modest yield.[13] Reaction of **22** with amines afforded the anthranilic diamides **23**.

Insecticidal activity of the anthranilic diamides is summarized in Table 1. Compounds were tested against a series of Lepidoptera under standard laboratory procedures.[14] Potency on fall armyworm (*Spodoptera frugiperda, Sf*), diamondback moth (*Plutella xylostella, Px*), and tobacco budworm (*Heliothis virescens, Hv*) was evaluated. Insecticidal activity is reported as an LC_{50} in ppm, the lethal concentration required for 50% mortality. Also reported in Table 1 is the *Hv* RyR EC_{50}, a measure of receptor activity from a recombinant Spodopteran cell line that stably expresses the *H. virescens* RyR. Sf9 cells are devoid of endogenous RyRs, however expression of recombinant *H. virescens* RyR confers sensitivity to anthranilic diamides and the known RyR agent, caffeine.[5b] These recombinant cells were plated into a 96-well plate and the compound-induced calcium mobilization was monitored using a FlexStation™ plate reader as previously described.[2] As these cells are devoid of endogenous RyRs, the activity reflects compound potency against the recombinant *H. virescens* RyR. As a general rule all new compounds showed high potency against the three species of Lepidoptera, with the greatest sensitivity to *Px* and the least sensitivity to *Hv*. Compound **D13**, the least active compound of Table 1, still proved substantially more active than many insecticide standards. Structure–activity relationships for compounds **D1**–**D6**, where R^1 was fixed as Cl, and R^3 was varied as chloro, bromo, and trifluoromethyl, suggested the trend Br ~ CF_3 > Cl, although these differences were very small. In addition, the isopropyl amides of compounds **D1**–**D6** tended to show slightly greater levels of insecticidal activity on Lepidoptera than their corresponding methyl amides particularly on the least susceptible *Hv* species. However, we observed the opposite trend at the Heliothis ryanodine receptor. In particular we noted that *Hv* RyR activity for each of the methyl amides **D1**, **D3**, and **D5** to be consistently more potent than the corresponding isopropyl amides **D2**, **D4**, and **D6**. While we cannot rule out decreased cell permeability of the isopropyl amides as a possible cause for the reduced receptor activity, one would expect a similar reduction in insect toxicity and this is not observed. We therefore believe the higher whole-organism toxicity for the isopropyl analogs may be the result of improved bioavailability.

Replacement of the chlorine substituent at R^1 with the groups Br, I, and CF_3 (compounds **D7**–**D12**) indicated sensitivity to substituents at R^1. While the Br analog **D7** was of equal potency to the Cl analog **D2**, the methyl amide **D8** and both sets of I and CF_3 analogs **D9**–**D12** were somewhat less active.

Alkoxy and haloalkoxy derivatives at R^3 **(D13**–**D18)** followed the structure–activity trend on *Hv* of OCH_2CF_3 > $OCHF_2$ > OCH_3. In particular, we observed lower activity for the methoxy derivatives **D13**–**D14** in spite of their strong activity at the Heliothis ryanodine receptor for both the methyl and isopropyl amides. This trend was observed for other methoxy derivatives and may be the result of metabolic detoxification via demethylation. In support of this, we observed a >30- to 50-fold decrease in insect toxicity for the hydroxy (desmethyl) derivatives. Additionally, the reduced Lepidopteran activity of the difluoromethoxy

analog **D15** was a bit of a surprise owing to the strong activity of the analogous *i*-Pr amide **D16**, and the exceptional activity at the Heliothis ryanodine receptor. Also, consistent with the trend for **D1**–**D6** we observed greater activity for the methyl amides of **D13**–**D18** at the *Hv* RyR.

Rynaxypyr™ possesses low acute mammalian toxicity with an acute oral LD_{50} of >5000 mg/kg in rats, and little to no toxicity in 90-day studies, at dosing as high as 1500 mg/kg/day. In addition, no developmental toxicity is observed in rats or rabbits with doses as high as 1000 mg/kg/day. Comparative studies of ryanodine receptor activation between insect and mammalian cell lines were conducted to determine if differential receptor selectivity is a contributing factor to the low mammalian toxicity. Unlike insects, mammals express three RyR isoforms. RyR1 and RyR2 are predominately found in skeletal and cardiac muscle, respectively, whereas RyR3 is heterogeneously distributed throughout various tissues. As the data in Chart 1 shows, Rynaxypyr™ is ~300-fold less potent against RyRs in the mouse myoblast cell line, C2C12, than in insect RyRs from *Drosophila melanogaster* and *H. virescens*. This mouse cell line has been shown to predominately express RyR1.[17] Increased selectivity is observed with the rat cell line, PC12, which predominately expresses RyR2.[17] In this cell line Rynaxypyr™ has an EC_{50} value >100 μM (~25% activation at 100 μM, >2000-fold selectivity). Of the mammalian cell lines tested, the human cell line, IMR32, was the least sensitive to Rynaxypyr™, with no receptor activation observed at 100 μM. This cell line has been shown to express functional RyRs however it is unclear which isoforms are being expressed.[18] Overall, Rynaxypyr™ exhibits strong differential selectivity for insect over mammalian RyRs. Therefore, it is likely that such selectivity contributes significantly to the observed low mammalian toxicity.

From a number of strong candidates identified in Table 1, Rynaxypyr™ was selected for development based on the combination of insecticidal potency and low mammalian toxicity. In numerous field trials we observed exceptional activity across a wide range of pests and with great consistency. We believe Ryanxypyr™ will be a valuable addition to the chemistry of crop protection based on the combination of an extremely favorable toxicology profile, the new mode of action and outstanding insecticidal properties.

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14. A representative testing protocol for *Spodoptera frugiperda* is described. Experimental compounds were formulated at 500 ppm using a solution containing 10% acetone, 90% water and 300 ppm X-77® Spreader (Loveland Industries, Inc.). Serial dilutions were made to obtain concentrations of 0.01, 0.03, 1, 3, 10, 30, 100 and 500 ppm. The formulated compounds were sprayed to run-off on 4-week old soybean plants. Once the plants had dried, a leaf (or trifoliate) was excised from the treated plant. The leaves were cut into 24 pieces and placed singly into a 5.5 cm by 3.5 cm cell of a 16-well plastic tray (Mullinix Packages, Inc.). Each cell also contained a 2.5 square of moistened chromatography paper (Whatman No. 3MM) to prevent desiccation. One third instar larvae of *Spodoptera frugiperda* was placed into each cell. A total of 16 insects were tested per rate. Larval mortality was assessed at 96 hours post-infestation. Percent control was evaluated, [(#dead/total number insects) × 100], and LC_{50}'s calculated.
15. All new compounds gave satisfactory spectral data consistent with their structures. Synthesis of Rynaxypyr™ **(D3)**: To a solution of 2-[3-bromo-1-(3-chloro-2-pyridinyl)-1*H*-pyrazol-5-yl]-6-chloro-8-methyl-4H-3,1-benzoxazin-4-one (5.5 g, 12.2 mmol) in tetrahydrofuran (100 mL) was added methylamine (2 M in THF, 15.0 mL, 30.0 mmol) dropwise at room temperature. The reaction mixture was stirred for 10 min and concentrated. The

residual solid was purified by chromatography on silica gel by elution with methylene chloride followed by ethyl acetate and concentrated to a white solid. Trituration of the solid with ether/hexane afforded 4.46 g of a white solid. Yield 76%; mp 239–240 °C; IR (Nujol) ν_{max} 3388, 3262, 1661, 1638, 1578, 1529, 1503, 1463, 1416, 1377 cm^{-1}; ^1H NMR (400 MHz $CDCl_3$) δ 1.25 (s, 3 H), 2.17 (s, 3H), 2.94 (d, 2H, J = 4.8 Hz), 6.20 (q, 1H, J = 4.8 Hz), 7.12 (s, 1H), 7.20 (d, 1H, J = 2.4 Hz), 7.23 (d, 1H, J = 2.4 Hz), 7.37 (dd, 1H, J = 4.7, 8.0 Hz), 7.84 (dd, 1H, 1.7, 8.0 Hz), 8.46 (dd, 1H, J = 1.7, 4.7 Hz), 10.08 (s, 1H); ^{13}C NMR (100 MHz, DMSO) δ 18.35, 26.77, 111.34, 126.03, 127.26, 127.48, 128.52, 131.61, 131.83, 132.22, 136.69, 139.47, 139.91, 140.05, 147.77, 149.09, 156.22, 166.81; HRMS (APESI, M+) $C_{18}H_{14}Cl_2BrN_5O_2$: *m/z* calcd 500.0868, *m/z* found 500.0851 (M^+).

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18. IMR32 cells challenged with caffeine exhibit a dose-dependent release of internal Ca^{2+} stores.