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Synthesis and biological activities of 2,3-dihydro-1,3,4-oxadiazole compounds and its derivatives as potential activator of ryanodine receptors

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ABSTRACT

A series of novel 2,3-dihydro-1,3,4-oxadiazoles containing *N*-pyridylpyrazole carboxamides moieties were obtained by applying a new synthetic route. Their insecticidal tests against oriental armyworm (*Mythimna separata*) and diamondback moth (*Plutella xylostella*) indicated that most of the compounds showed moderate to excellent activities at the testing concentrations. In particular, compound **6a** showed 40% larvicidal activities against oriental armyworm at 1 mg/L, while **7a** against diamondback was 100% at 0.01 mg/L. Calcium imaging results demonstrated that **6a**, **6d** and **7a** stimulated a transient elevation in $[Ca^{2+}]_i$ in the absence of external calcium after the central neurons dye loading with fluo-3 AM, implying that these novel compounds were potential activators of the ligand-gated calcium channel on the endoplasmic reticulum.

Ryanodine receptor (RyR) is a distinct class of ligand-gated calcium channels controlling the release of calcium from intracellular stores.[1] Anthranilic diamides were discovered by DuPont as a new kind of pesticides targeting at the insect RyRs.[2] The two diamides, Chlorantraniliprole and Cyantraniliprole (Fig. 1) with excellent insecticidal activity have been marketed,[3] which are highly potent activator of the insect RyRs.[4] Due to the intrinsic selectivity for the insect receptor and the low toxicity to mammals,[5] this category of insecticide has received considerable attention.

Since the discovery of Chlorantraniliprole, some structural modification have been reported.[6–10] We noticed that most optimizations focused on the phenyl and *N*-pyridylpyrazole moieties, but the modification of two amide moieties has not been fully reported. In hope of enhancing their insecticidal activities, it is an explorative study for us to modify the amido bridge in anthranilic diamides.[11,12]

The introduction of dihydroquinazolinone moiety[13] displayed good insecticidal activity and solubility (Fig. 1), and this results encouraged us to synthesize other structures with such bridge-modified structure. On the basis of the above consideration, a replacement of NCH_3 with NNH_2 group in dihydroquinazolinone moiety was designed through a bioisosterism approach. Unexpectedly, though the compound **A** was failed to obtain, a series of structures (**B**) containing new oxadiazoline ring was found. The compounds which containing the oxadiazole ring have shown diverse biological activities, such as, anticancer,[14] antibacterial,[15] antifungal,[16] etc., and our study found that these compounds have good insecticidal activities.

Thus, a series of novel dimethyl-2,3-dihydro-1,3,4-oxadiazolinylaniline derivatives containing *N*-pyridylpyrazole group were subsequently synthesized. Their insecticidal activities against oriental armyworms (*Mythimna separata*) and diamondback moth (*Plutella xylostella*) were tested, which showed that some exhibited excellent insecticidal activities. The structure–activity relationship (SAR) was discussed as well. On the other hand, calcium imaging technique was also adopted to investigate the effects on calcium channels in the central neurons of *Spodoptera exigua*.

Compounds **1a–f** were synthesized using 2-aminobenzoic acid derivatives as starting materials. At first, 2-aminobenzoic acid derivatives were converted to the acyl chloride by treatment with thionyl chloride, then coupled with hydrazine hydrate (80%), resulting in low yields. A different synthetic route via the intermediates of isatoic anhydride was applied and **1a–f** were successfully obtained with satisfactory yields and purity (Scheme 1).

According to the literature,[17,18] compounds **2** and **3** could be all obtained in the presence of acetone as shown in Scheme 1 via step iii. Compounds **3** were obtained under the same conditions, but attempts to synthesize compounds **2** with the literature procedure were failed. In the ^1H NMR spectra of compound **3a**, a broad signal peak disappeared at 3.92–4.19 ppm compared with its starting material **1a**, demonstrating the condensation of NH_2 in the –CON-

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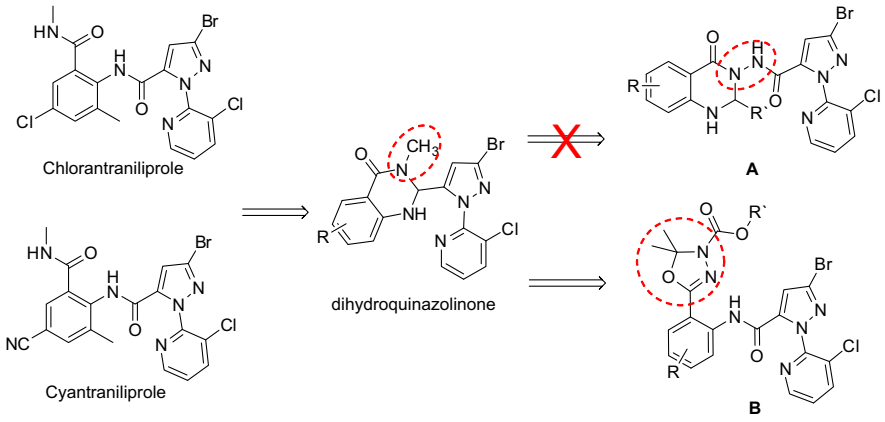
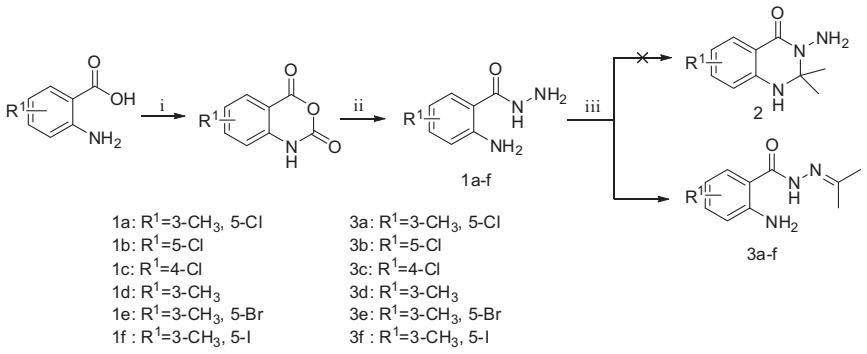


Figure 1. Design of target compounds.



Scheme 1. Reagents and conditions: (i) triphosgene, THF, room temperature; (ii) $N_2H_4{\cdot}H_2O$ (80%), EtOH, room temperature; (iii) acetone, room temperature.

HNH_2 with acetone. The structure of **3a** was further proved by the single crystal structure of its corresponding derivative **6a**. It is rationalized that the structure stated in former literature[14] might not be correct.

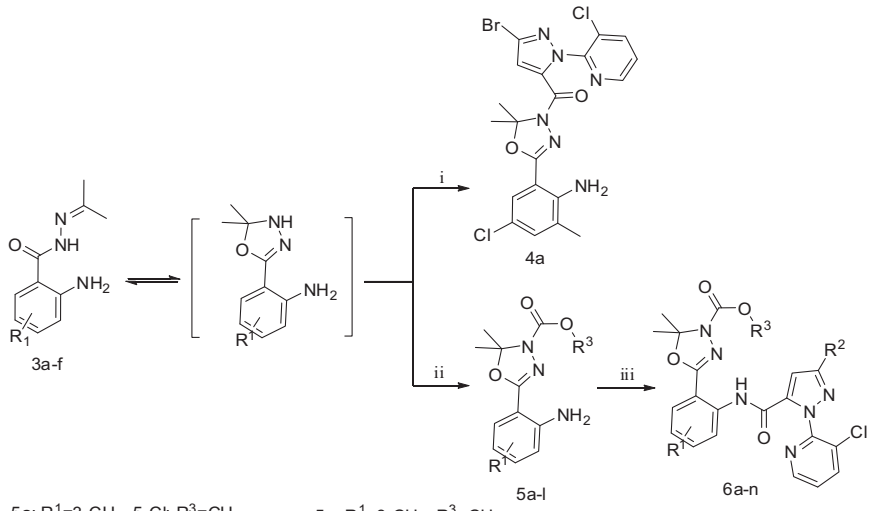
Surprisingly, when **3a** reacted with methyl chloroformate, an unprecedented structure **5a** was obtained as the sole product in high yield (Scheme 2). Referring to literature,[19] a similar mechanism was proposed for the formation of **5a** (Scheme 3). In the presence of Et_3N, the hydrazide underwent a deprotonation process, the amide bond tautomerized, and the nucleophilic oxygen attacked the carbon of imine to form the oxadiazolidine intermediate. Therefore **5a–l** were successfully obtained by using corresponding acylhydrazone and chlorocarbonate. Furthermore, the oxadiazolidine intermediate could be acylated by pyridylpyrazole carbonyl chloride to form product **4a**.

Interestingly, product **6k** (R^3=CH_3) could easily convert to **7a** quantitatively (Scheme 4), when the amount of Et_3N was less than 1 equiv or during the regular chromatographic processing. Application of 1.2 equiv of Et_3N to the reaction mixture and addition of 1% Et_3N to the chromatography eluent were able to avoid this problem and smoothly afforded **6k** in high yield and purity. In addition, **6k** had much better solubility than **7a**.

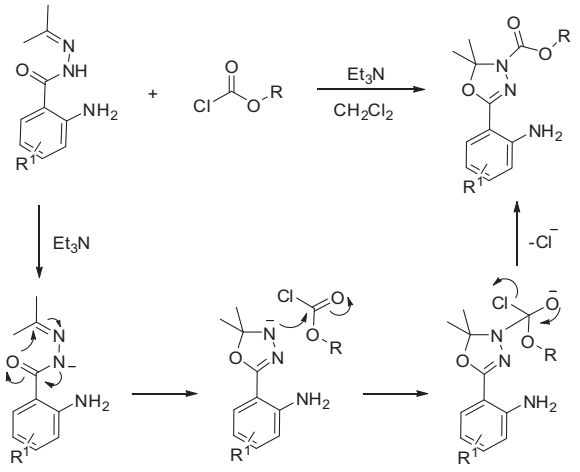
Compound **6a** (deposition #CCDC 992835) was recrystallized from ethyl acetate to give colorless crystal suitable for single crystal X-ray diffraction with the following crystallographic parameters: $a = 12.644(3)$ Å, $b = 14.171(3)$ Å, $c = 14.501(3)$ Å, $\alpha = 90°$, $\beta = 109.50(3)°$, $\gamma = 90°$, $\mu = 1.938$ mm^{-1}, $V = 2449.3(8)$ Å^3, $Z = 4$. $D_c = 1.579$ g cm^{-3}, $F\ (000) = 1176$, $T = 293.15$ K, $3.06° \leqslant \theta \leqslant 25.02°$, $R = 0.0440$, $wR = 0.1110$. The crystal structure of **6a** is monoclinic and contains following four plane subunits: the benzene ring, the pyridine ring, the pyrazole ring, and the oxadiazoline ring (Fig. 2). The average bond lengths and bond angles of the ring systems (pyridine, pyrazole, benzene and oxadiazoline ring) are within normal ranges.[20–27] The pyridine ring is oblique with pyrazole ring and benzene ring with dihedral angles (θ) of 62.54° and 66.18°, respectively. The benzene ring is nearly planar with oxadiazoline ring with a small dihedral angle (θ) of 6.65°. In the molecular packing of **6a** (Fig. 3), intermolecular C–H···O and C–H···Cl hydrogen bonds link the molecules, stacking them along the *c* axis.

Table 1 shows the insecticidal activities data of the title compounds **4a**, **6a–n**, **7a** and Chlorantraniliprole against oriental armyworm. The results indicated that some of the title compounds exhibited good activities against oriental armyworm, the mortality of **6a** and **6c** at 1 mg/L were 40%, 20%, respectively. And **7a** showed 80% activity at 0.25 mg/L.

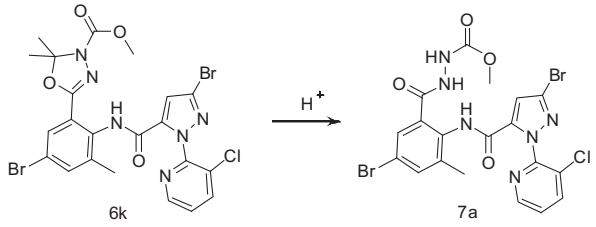
Activities varied significantly relating to the structures. For instance, **6a–n** with an amido bond showed an insecticidal activity at low concentration, while **4a** containing 1,3,4-oxadiazoline moiety had no activity. The structural–activity relationship of substituted phenyl ring was summarized as follows: When R^2 was fixed as Br, R^3 was fixed as CH_3, the insecticidal activities of compounds with different R^1 indicated the sequence of 3-CH_3, 5-Cl (**6a**) > 3-CH_3 (**6i**) ≈ 3-CH_3, 5-Br (**6k**) > 5-Cl (**6e**) ≈ 4-Cl (**6g**) ≈ 3-CH_3, 5-I (**6m**). The compounds without CH_3 in benzene ring led to a significant decrease in activity, compounds **6e–h** showed no activity at 100 mg/L, while no halogen on the benzene ring had relatively small impact on the larvicidal activities, such as **6i** and **6j** had about 50% activity at 10 mg/L. In addition, different halogen substituted in benzene ring showed a clear trend in insecticidal activity -Cl > -Br > -I. For example, compound **6m** had a significantly decrease in insecticidal activity at 200 mg/L. These



Scheme 2. Reagents and conditions: (i) Et_3N, 3-bromo-1-(3-chloropyridin-2-yl)-1*H*-pyrazole-5-carbonyl chloride, CH_2Cl_2, room temperature; (ii) Et_3N, chlorocarbonate, CH_2Cl_2, room temperature; (iii) Et_3N, pyridylpyrazole carbonyl chloride, THF, room temperature.



Scheme 3. The possible mechanism to afford 2,3-dihydro-1,3,4-oxadiazole.



Scheme 4. The generation of compound **7a** from **6k**.

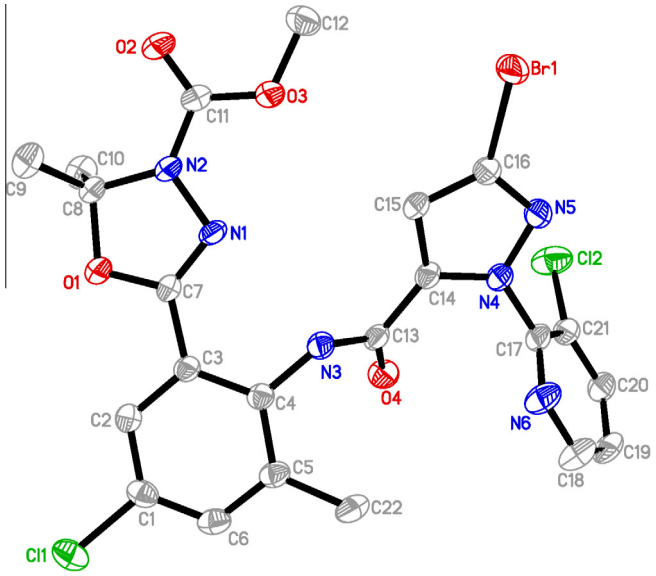


Figure 2. A molecular structure of compound **6a**.

results indicated that 3-CH_3 and 5-Cl groups were favorable on the benzene ring to enhance the insecticidal activity compared to other substituents.

However, compounds with different R^2 showed minimal impact on activities, with the sequence of -Br (**6a**) > -OCH_2CF_3 (**6c**) > -CF_3 (**6b**). Compound **6c** with the 2,2,2-trifluoroethoxy groups on the 3-position of pyrazole did not exhibit higher activities than bromine as we expected. The compounds with different R^3 also exhibited little influence on larvicidal activities.

The larvicidal activities of **4a**, **6a–n**, **7a** and Chlorantraniliprole against diamondback moth were also evaluated and the activity data were shown in Table 2. The results indicated that most of the compounds showed excellent insecticidal activities at 1 mg/L. In particular, at 0.01 mg/L the death rate of compound **6b**, **6l** and **7a** against diamondback moth were 43%, 43% and 100%, respectively. To our surprise, **6n** showed 29% killing effect to diamondback at the concentration of 0.01 mg/L.

Spodoptera exigua and oriental armyworms are major Lepidoptera pests in China. According to previous method,[28] the calcium imaging technique was used to determine whether these novel

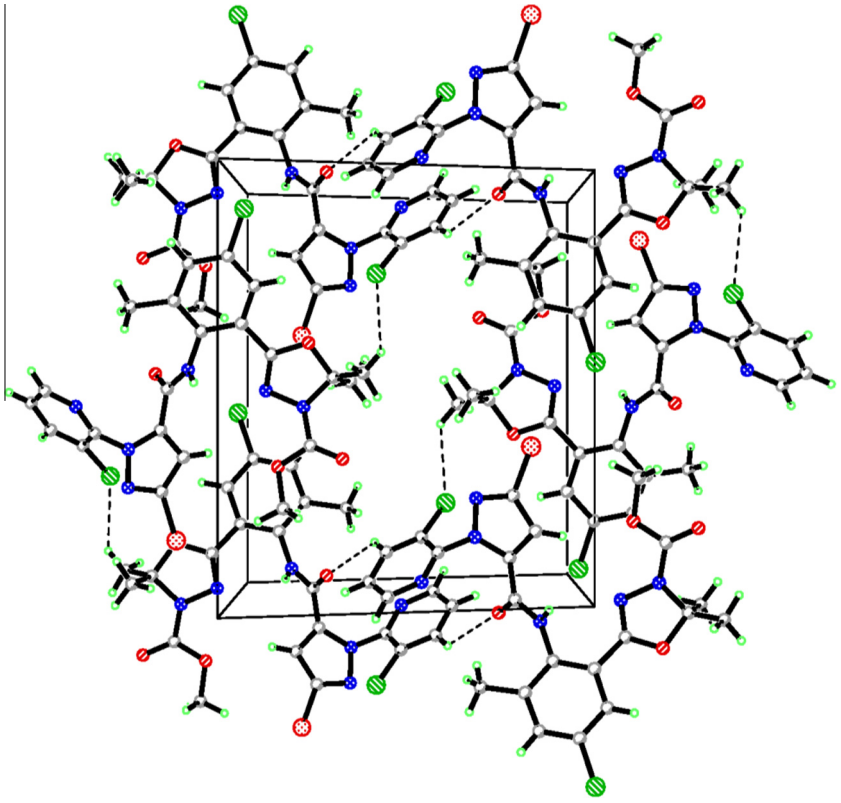


Figure 3. Packing diagram of compound **6a**.

Table 1
Insecticidal activities of compounds **4a**, **6a–n**, **7a** and Chlorantraniliprole against oriental armyworms

Compounds				Larvicidal activity (%) at concn (mg/L)								
No.	R^1	R^2	R^3	200	100	50	25	10	5	2.5	1	0.25
4a	3-CH_3, 5-Cl	Br	—	6.7								
6a	3-CH_3, 5-Cl	Br	CH_3	100	100	100	100	100	100	100	40	
6b	3-CH_3, 5-Cl	CF_3	CH_3	100	100	100	100	100	40			
6c	3-CH_3, 5-Cl	OCH_2CF_3	CH_3	100	100	100	100	100	100	60	20	
6d	3-CH_3, 5-Cl	Br	CH_2CH_3	100	100	100	100	100	60			
6e	5-Cl	Br	CH_3	50								
6f	5-Cl	Br	CH_2CH_3	25								
6g	4-Cl	Br	CH_3	26.7								
6h	4-Cl	Br	CH_2CH_3	10								
6i	3-CH_3	Br	CH_3	100	100	100	100	60				
6j	3-CH_3	Br	CH_2CH_3	100	100	100	100	40				
6k	3-CH_3, 5-Br	Br	CH_3	100	100	100	100	40				
6l	3-CH_3, 5-Br	Br	CH_2CH_3	100	100	100	100	60				
6m	3-CH_3, 5-I	Br	CH_3	65								
6n	3-CH_3, 5-I	Br	CH_2CH_3	45								
7a	3-CH_3, 5-Br	Br	CH_3	100	100	100	100	100	100	100	100	80
Chlorantraniliprole				100	100	100	100	100	100	100	100	100

compounds are involved in the calcium concentration and the correlation of $[Ca^{2+}]_i$ (F/F_0) with insecticidal activity. The effects on the central neurons of *S. exigua* on the calcium homeostasis were studied after the neurons were loaded with fluo-3 AM.

Figure 4 shows the change of $[Ca^{2+}]_i$ versus recording time when the neurons were treated with **6a**, **6d** and **7a**. The peaks of calcium concentration were elevated to be 108.74 ± 3.5% ($n = 9$), 106.74 ± 3.27% ($n = 9$), 110.56 ± 3.38% ($n = 9$) of the initial value when the cells were treated with 1000 mg/L of **6a**, **6d** and **7a**, respectively. Compared with the insecticidal activities data against oriental armyworms, the recorded $[Ca^{2+}]_i$ (F/F_0) had a good positive correlation with bioactivities. The results indicate that these novel compounds deliver calcium from endoplasmic reticulum to cytoplasm.

Figure 5 exhibits change of $[Ca^{2+}]_i$ versus recording time when the neurons were treated with 500 mg/L of **6a** and **7a**. The peak of $[Ca^{2+}]_i$ decreased to 109.75 ± 3.11% ($n = 9$) and 102.6 ± 2.09% ($n = 9$) of the initial value after the neurons treated with 500 mg/L **7a** and **6a** shorter than 20 s, respectively. The elevations of calcium concentrations by **6a** and **7a** are in a concentration-dependent manner.

In summary, a series of new oxadiazole benzamide analogs containing 1,3,4-oxadiazoline group were synthesized. The structures were characterized by ^1H NMR and ^{13}C NMR spectroscopy, single

Table 2
Insecticidal activities of compounds **4a**, **6a–n**, **7a** and Chlorantraniliprole against diamondback moth

Compounds	Larvicidal activity (%) at concn (mg/L)			
	10	1	0.1	0.01
4a	0			
6a	100	100	30	
6b	100	100	90	43
6c	100	100	43	
6d	100	100	57	14
6e	0			
6f	0			
6g	100	43		
6h	86	43		
6i	100	100	86	14
6j	100	71		
6k	100	86	43	
6l	100	100	90	43
6m	100	43		
6n	100	100	71	29
7a	100	100	100	100
Chlorantraniliprole	100	100	100	100

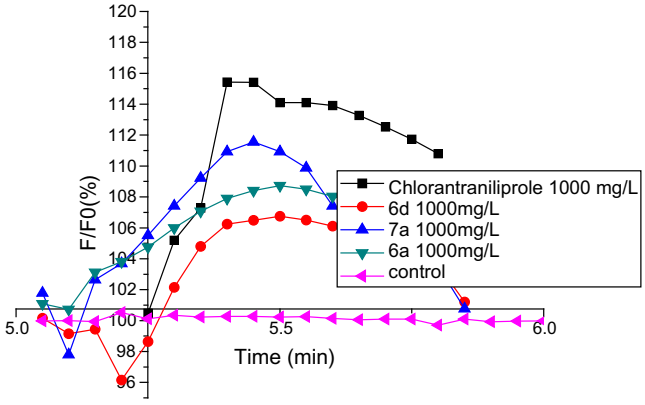


Figure 4. The change of $[Ca^{2+}]_i$ versus recording time when the neurons were treated with **6a**, **6d** and **7a**. The central neurons of *S. exigua* third larvae were dyed by loading with fluo-3 AM.

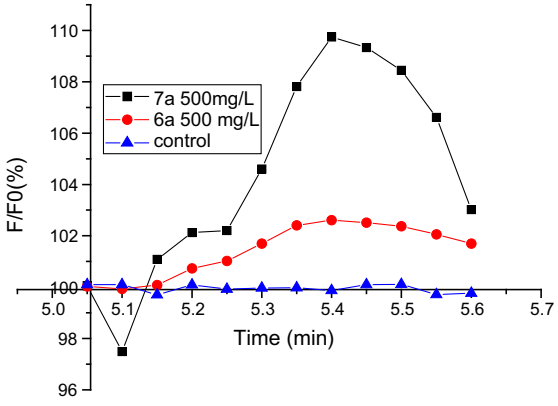


Figure 5. Effects of 500 mg/L of **6a** and **7a** on $[Ca^{2+}]_i$ in the central neurons of *S. exigua* when extracellular Ca^{2+} was in absence (EGTA replace Ca^{2+}). The central neurons of *S. exigua* third larvae were dyed by loading with fluo-3 AM.

crystal X-ray diffraction analysis and HRMS. The bioassays indicated that some of the compounds showed good insecticidal activities based on the oriental armyworm and diamondback moth tests. The preliminary structure–activity relationship of the title compounds indicated that the 3-CH_3 and 5-Cl groups on the benzene ring and 3-Br on pyrazole were preferred. The calcium imaging experiment demonstrated that these novel title compounds are potential activators of the ligand-gated calcium channel on the endoplasmic reticulum.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.03.077.

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