

## SYNTHESIS OF 1,3,4-OXADIAZOLES AS SELECTIVE T-TYPE CALCIUM CHANNEL INHIBITORS

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This manuscript is in celebration of Professor Kaoru Fuji's 80 birthday and for his  
dedication to research and education

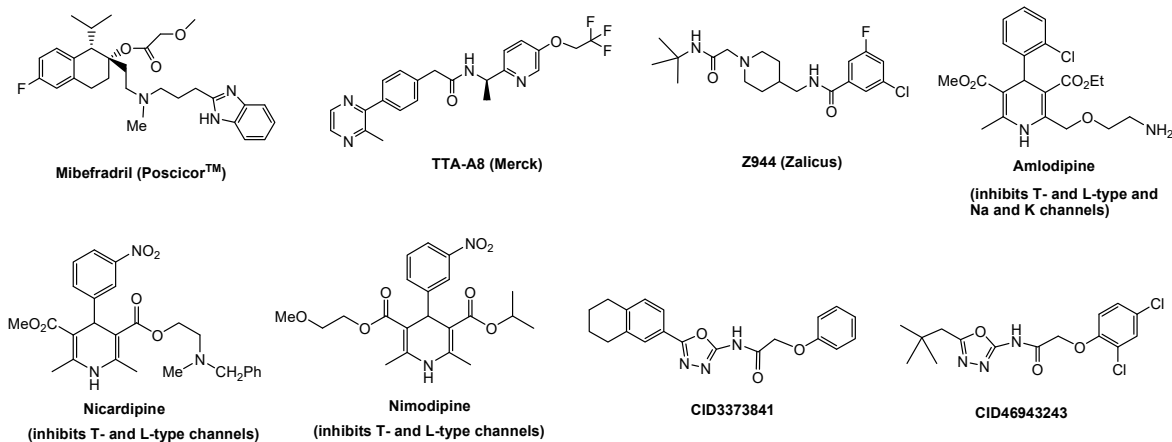
**Abstract** – Neuropathic pain, epilepsy, insomnia, and tremor disorder may arrive from an increase of intracellular Ca<sup>2+</sup> concentration through a dysfunction of T-type Ca<sup>2+</sup> channels. Thus, T-type calcium channels could be a target in drug discovery for the treatments of neuropathic pain and epilepsy. From rational drug design approach, a group of 2,5-disubstituted 1,3,4-oxadiazole molecules was synthesized and their selective T-type channel inhibitions were evaluated. The synthetic strategy consists of a short sequence of three reactions: (i) condensation of thiosemicarbazide with acid chlorides; (ii) ring closing by 1,3-dibromo-5,5-dimethylhydantoin; and (iii) coupling with various acid chlorides. 5-Chloro-*N*-(5-phenyl-1,3,4-oxadiazol-2-yl)thiophene-2-carboxamide (**11**) was found to selectively inhibit T-type Ca<sup>2+</sup> channel over Na<sup>+</sup> and K<sup>+</sup> channels in mouse dorsal root ganglion neurons and/or human embryonic kidney (HEK)-293 cells and to suppress seizure-induced death in mouse model. Consequently, compound **11** is a useful probe for investigation of physiologic and pathophysiologic roles of the T-channel, and provides a basis to develop a novel therapeutic to treat chronic neuropathic and inflammatory pains.

## INTRODUCTION

Voltage-gated calcium channels (VGCCs) are transmembrane, multi-subunit proteins that modulate influx of calcium ions ( $\text{Ca}^{2+}$ ) into the cell in response to membrane depolarization. According to different activation potentials, generally VGCCs can be classified as high voltage-activated (L-, N-, P-/Q- and R-types) and low voltage-activated (T-type) channels.<sup>1,2</sup> T-type  $\text{Ca}^{2+}$  channels (or T-channels) consist of three distinct channel proteins,  $\text{Ca}_v3.1$ ,  $\text{Ca}_v3.2$ , and  $\text{Ca}_v3.3$  (or  $\alpha 1\text{-G}$ ,  $\alpha 1\text{-H}$ , and  $\alpha 1\text{-I}$ ) that regulate neural excitability and are involved in pathophysiological disorders, such as neuropathic pain, epilepsy, insomnia, and tremor disorders.<sup>3-7</sup> These three sub-types are heterogeneously expressed in the brain and organs such as the heart, vascular smooth muscle, non-vascular smooth muscle, skeletal muscle and others.<sup>8-13</sup> The opening of calcium channels leads to an increase of intracellular  $\text{Ca}^{2+}$  concentration and subsequent membrane depolarization. This has an effect on several important processes including muscle contraction,<sup>13</sup> electrical conduction, neurotransmission, and neuropathic pain.<sup>3</sup> The mechanism in neuropathic pains involving over-activation of T-type channel remains unclear and current pharmaco-therapeutics do not adequately control neuropathic pain conditions. In addition, T-type calcium channels also involve in hormone secretion, mechanosensation, and epilepsy.<sup>14</sup> Epilepsy leads to disturbances in brain electrical activity and seizure, which is one of the most frequently occurring neurological diseases.<sup>15</sup> In rodent models, an increase of T-type current was found in thalamic neurons,<sup>16,17</sup> and there was no effect on the amplitude of L-type current, indicating that epilepsy resulted from a selective increase of T-type current. Thus, T-type calcium channel is a good target for the treatments of neuropathic pains and epilepsy.

A number of T-type calcium channel inhibitors have been reported<sup>18-25</sup> and representative bioactive molecules are summarized in Figure 1. Mibefradril was a FDA approved anti-hypertension drug and selectively inhibits T-type ( $\text{IC}_{50} = 0.1 \mu\text{M}$ ) over L-type ( $\text{IC}_{50} = 3 \mu\text{M}$ ) calcium channels.<sup>21</sup> However, due to severe drug-drug interactions, it was retracted from market.<sup>26</sup> Pyrazine TTA-A8 has been reported to display excellent potency and short-acting selective inhibition of T-type channels and evaluated in human clinical trials in 2010.<sup>27</sup> Piperidine Z944 has been reported to selectively inhibit T-type calcium channel ( $\text{Ca}_v3.2$ ),<sup>28</sup> but it showed only weak protective effect on seizures from pentylentetrazole-induced fatality rat model (33% at 30 mg/Kg).<sup>29</sup> Despite the presence of a number of calcium channel inhibitors, satisfactory drugs remain to be developed due to various issues including selectivity, efficacy, and safety profile. Only amlodipine, nifedipine, and nimodipine are FDA-approved drugs for clinical use, and these molecules not only block T-type calcium channels but also L-type and Na- and K-channels.<sup>30</sup> Several 1,3,4-oxadiazoles have been reported to act as L- and T-type calcium channel blockers.<sup>25,31,32</sup> For instance, through several high throughput screening studies, compound CID3373841 (Figure 1) was found to selectively inhibit T-type calcium channel towards L- and N-type channels.<sup>20</sup> Further structural optimization led to a more potent inhibitor CID46943243, which suggested that 1,3,4-oxadiazole scaffold could be a good pharmacophore

for the inhibition of T-type calcium channels. To discover potent and selective T-channel inhibitors for the treatment of neuropathic pain, we took a rational drug design approach and report herein the synthesis and selective T-type channel inhibitions of a group of 2,5-disubstituted 1,3,4-oxadiazole molecules.



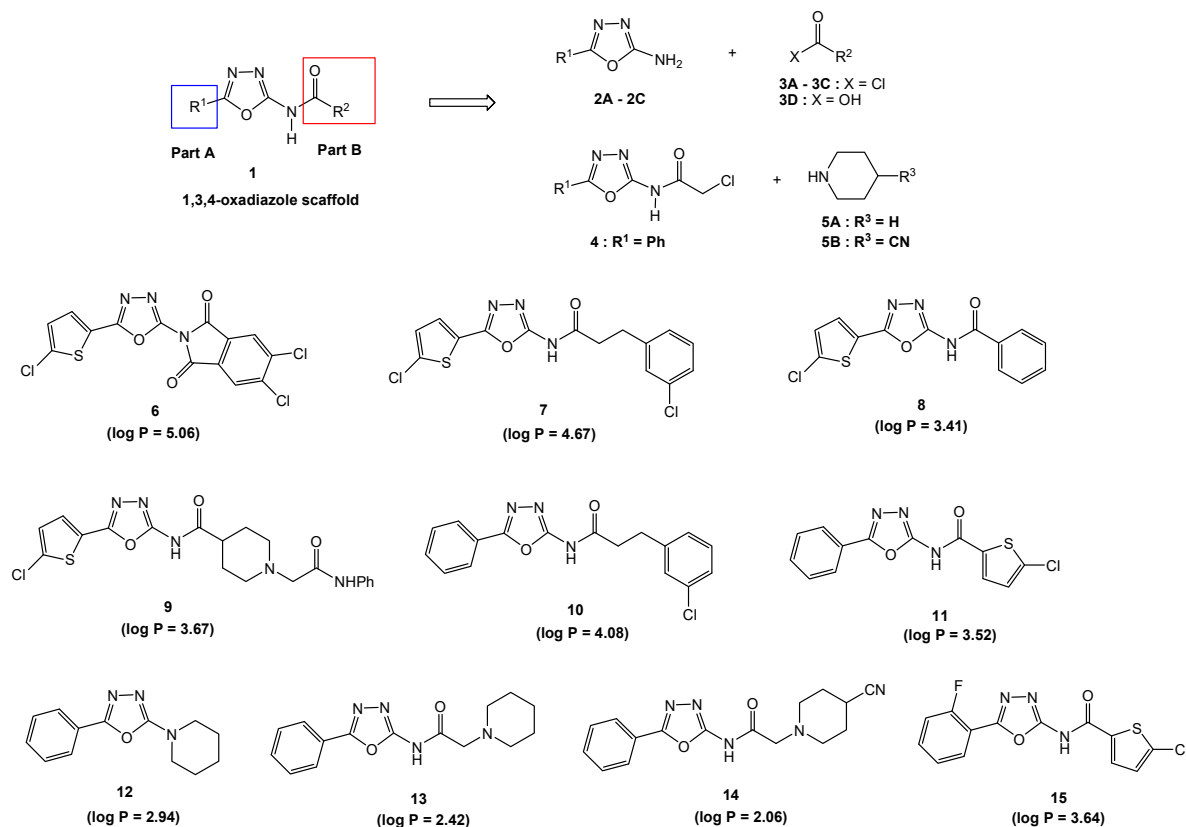
**Figure 1.** Representative T-type calcium channel blockers

## RESULTS AND DISCUSSION

There are some common structural features shared by the reported oxadiazole blockers (Figure 1) and they include (i) all of them have at least one aromatic ring and one or more basic nitrogen atom(s); (ii) some have amide bond(s) and flexible short aliphatic alkane chains; and (iii) a molecular planarity is not required. These are good guidances in the molecular design. Based on these common features, computational modeling of the pharmacophores, and predictive Log P values (partition coefficient), a small library of ten 1,3,4-oxadiazole containing molecules, **6** – **15**, were designed, synthesized and bio-evaluated (Figure 2). A retrosynthesis of 1,3,4-oxadiazole scaffold **1** is depicted in Figure 2 involving the preparations of four representative 2-amino-5-aryl-1,3,4-oxadiazole molecules, **2A** – **2C** and **4**, followed by either simple coupling reactions with various acid halides **3A** – **3D** or displacement reactions with **5A** or **5B**. The amine  $\text{NH}_2$  function of **2A** – **2C** serves as a nucleophile while the chloroamide function of **4** serves as an electrophile. The 5-substituted 2-amino-1,3,4-oxadiazoles can be made by the amide bond formation of thiosemicarbazide (**16**) and acid chlorides followed by ring closing through halogenation. Different substituents were installed on C2 and C5 of the 1,3,4-oxadiazole ring to achieve bioactivities. Modification in part A,  $\text{R}^1$  substituent, focused on different aromatic rings to adjust the molecular lipophilicity. Diverse functional groups were introduced in part B,  $\text{R}^2\text{-C=O}$ , to explore the structural features such as rigidity, flexibility, planarity,  $\pi\text{-}\pi$  interaction, and steric hindrance, which may have an effect on potency. 5-Chlorothiophenyl moiety was chosen in  $\text{R}^1$  or  $\text{R}^2$  due in part based on the reported bioactivities of the thiophene scaffold including that in the central nervous system.<sup>33</sup>

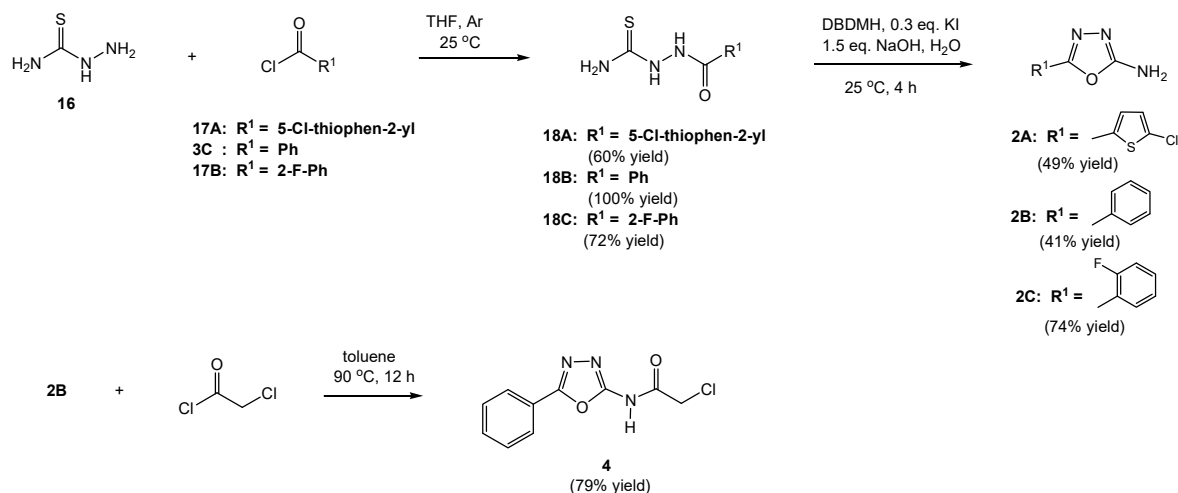
Predicted log P values of compounds **6** - **15** were calculated using interactive log P calculator

(Molinspiration Cheminformatics 2017; <http://www.molinspiration.com/services/logp.html>) and the calculated values are described in Figure 2. Except compound **6** with log P value slightly over 5, all other designed 1,3,4-oxadiazole derivatives have log P values between 2 to 5, indicating a suitable hydrophobicity as drug-like molecules.



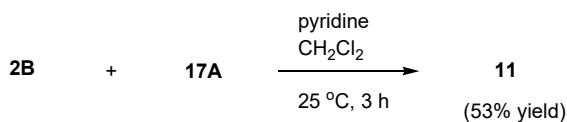
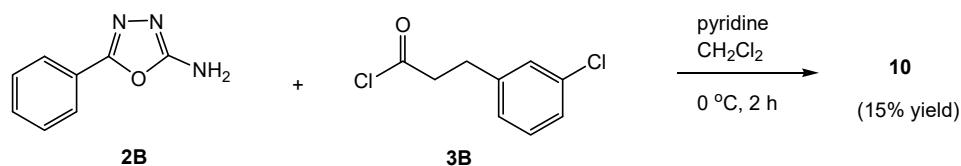
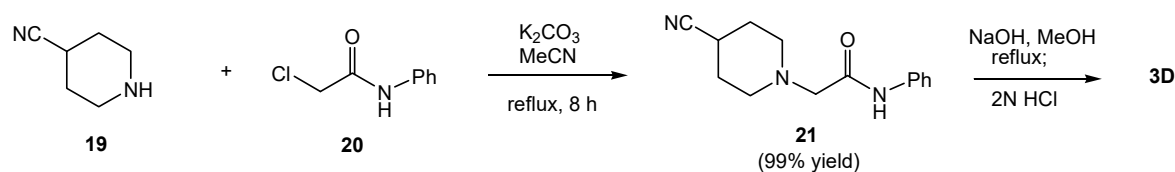
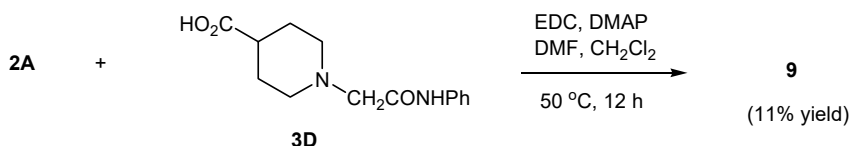
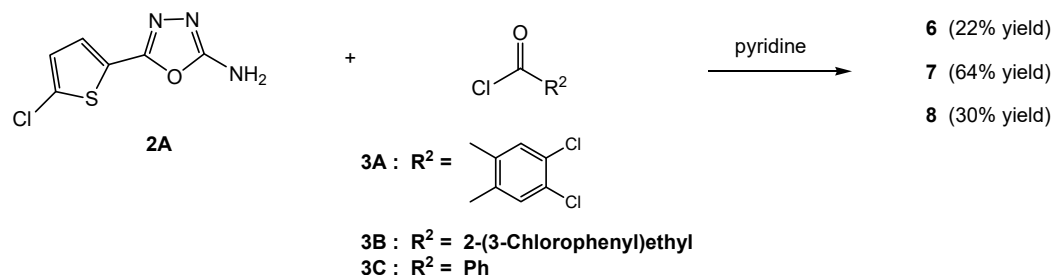
**Figure 2.** Designed substituted 1,3,4-oxadiazole compounds as T-type calcium channel inhibitors

The syntheses of 2-amino-5-aryl-1,3,4-oxadiazoles<sup>34</sup> appear to be general and are depicted in Scheme 1. Hence, coupling of thiosemicarbazide (**16**) with different acyl chlorides **17A**, **3C**, and **17B** in THF at 25 °C gave 1-aryl-3-thiosemicarbazides **18A** – **18C** in 60 – 100% yields (Scheme 1). The cyclization reactions of **18A** – **18C** were affected by the treatment with 1,3-dibromo-5,5-dimethylhydantoin (DBDMH) and potassium iodide under basic conditions to give 1,3,4-oxadiazoles **2A** – **2C**, respectively, in moderate to good yields. A possible mechanism for the cyclization has been reported,<sup>35</sup> involving iodination (I<sub>2</sub> is generated from DBDMH and KI) of the thioamide moiety followed by elimination of NaSH, H<sub>2</sub>O and NaI, and annulation from an intramolecular addition reaction of the resulting diimide function by the adjacent amido oxygen.  $\alpha$ -Chloroamide **4** was readily prepared in a 79% yield by the coupling of oxadiazole **2B** with chloroacetyl chloride.



**Scheme 1.** Syntheses of 1,3,4-oxadiazoles **2A** – **2C** and **4**

With these four 1,3,4-oxadiazoles, **2A** – **2C** and **4**, on hands, the intended 2,5-disubstituted oxadiazoles **6** – **15** were synthesized and the synthetic reactions are depicted in Schemes 2 and 3. First, amine **2A** was condensed with aryl halides **3A** – **3C**, separately, in the presence of pyridine to give oxadiazoles **6** – **8** in 22, 64, and 30% yield, respectively, along with unidentifiable byproducts and small amounts of starting material, 2-aminooxadiazole **2A**. An attempt to improve the yields by changing the reaction solvent to *N,N*-dimethylformamide failed, and no desired products were obtained. The poor solubility of the products such as **6** and **8** in common organic solvents (diethyl ether, ethyl acetate, and dichloromethane) likely led to the decrease of isolated chemical yields. Oxadiazole **9** was synthesized by the condensation of **2A** and carboxylic acid **3D** in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and 4-(dimethylamino)pyridine (DMAP). After purification, an 11% yield of **9** and 18% recovery of **2A** were isolated. Treatment of **3D** with oxalyl chloride failed to produce the corresponding acid chloride. Carboxylic acid **3D** was made from the coupling of 4-cyanopiperidine (**19**) and 2-chloro-*N*-phenylacetamide (**20**) in the presence of potassium carbonate in acetonitrile followed by basic hydrolysis of the cyanide group. It was used in the synthesis of **9** as described above without purification. Oxadiazoles **10** and **11**, containing phenyl ring at  $\text{R}^1$ , were synthesized in 15% and 53% yield, respectively, from the coupling reactions of 2-amino-5-phenyl-1,3,4-oxadiazole (**2B**) with 3-(3-chlorophenyl)propanoyl chloride (**3B**) and 5-chlorothiophene-2-carbonyl chloride (**17A**) separately in dichloromethane (Scheme 2). Acid chlorides **3B** and **17A** were prepared from the corresponding carboxylic acids and oxalyl chloride in ethyl acetate in the presence of a catalytic amount of DMF.

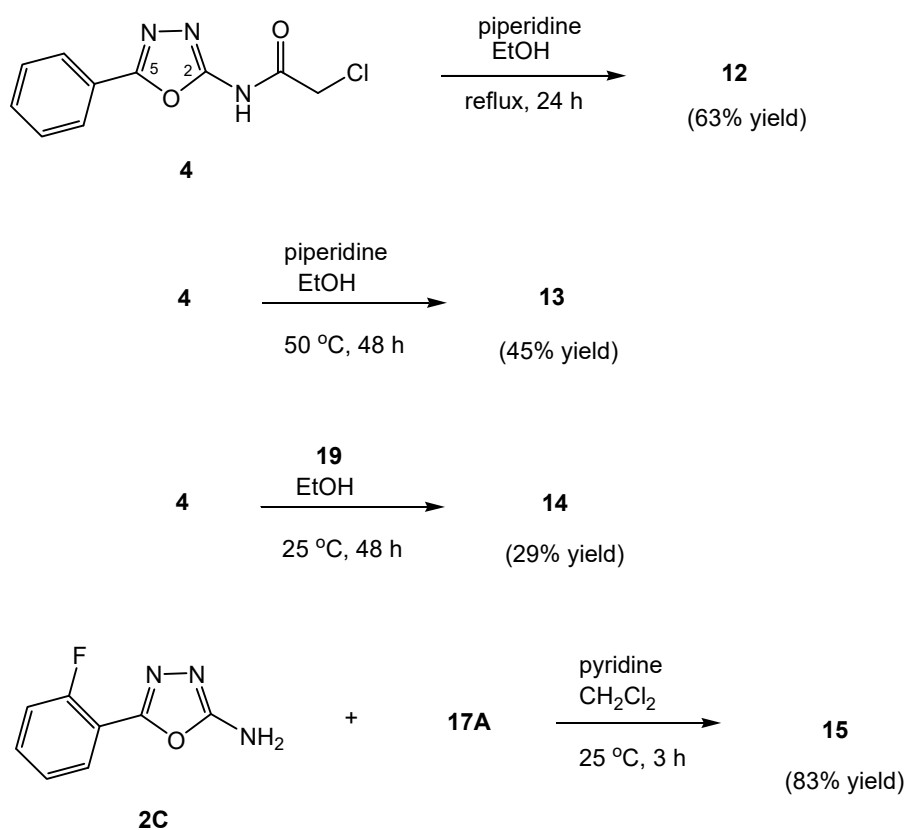


**Scheme 2.** Syntheses of 1,3,4-oxadiazoles **6 - 11**

Chloroacetamide **4** serves as an electrophile for various substitution reactions through a displacement of the chlorine atom. To our surprise, treatment of **4** with piperidine in refluxing ethanol gave a 63% yield of an unexpected product, **12**, in which the chloroacetamide unit was replaced by the piperidine molecule (Scheme 3). The reaction temperature, 82 °C, was considered to be the culprit, leading to this unusual displacement reaction. Piperidine likely adds to C2 of the oxadiazole ring providing the conjugate iminoamine anion, which in turn undergoes elimination of the chloroacetamide anion ( $\text{ClCH}_2\text{CONH}^-$ ) producing 2-phenyl-5-piperidyl-1,3,4-oxadiazole (**12**). To verify such consideration, **4** was treated with piperidine in ethanol at 50 °C for 48 h and the expected displacement product **13** was isolated in 45% yield along with unidentifiable byproducts after silica gel column chromatographic separation. There was no reaction at 25 °C. To generate a similar analog, compound **4** was allowed to react with 4-cyanopiperidine (**19**) in ethanol. Interestingly, the reaction took place at 25 °C and afforded the expected displacement

product, **14**, in a 29% yield along with other byproducts. Likely, product **14** undergoes further alkylation reaction with **4** producing the byproducts. The C4-cyano group lowers the pK<sub>a</sub> value of the NH of **19**, which may lead to a faster rate of the S<sub>N</sub>2 displacement reaction.

Based on the favorable bioactivities found from oxadiazole **11** (*vide infra*), 2-fluorophenyl analog **15** was synthesized in an 83% yield via a similar coupling reaction of amine **2C** and thiophenecarbonyl chloride **17A** (Scheme 3). A higher yield in this reaction, comparing with those found in the syntheses of **6 – 8** and **11**, suggests that the fluorine atom in the *ortho* position of the C5-phenyl ring preventing undesired acylation on the oxadiazole ring.



**Scheme 3.** Syntheses of 1,3,4-oxadiazoles **12 - 15**

Oxadiazoles **6 – 15** and a reported T-type calcium channel blocker, Z944 (Figure 1), were first studied their inhibitions of T-Ca<sub>v</sub>3.2-, Na-, and K-channels, separately, in mouse dorsal root ganglion (DRG) neurons using whole-cell patch clamp recording protocol,<sup>29</sup> and results are summarized in Table 1. A few compounds were tested in T-Ca<sub>v</sub>3.2-expressed in human embryonic kidney (HEK)-293 cells from a tetracycline inducible expression system (Invitrogen; see Experimental Section shown below). It has been reported that an over activation of T-channel resulted in the generation of seizure activity,<sup>36</sup> hence prolonged latencies to seizures and decreased the fatality rate in seizure mouse model would affirm the

inhibition of T-channel activity. The active compounds were consequently evaluated in mouse model for resistance of neuropathic pain by pentylenetetrazole (PTZ)-induced seizures method.<sup>36</sup> The inhibition ratio (5<sup>th</sup> column in Table 1) is the number of mice died among the tested mice, there is no mean or standard error of the mean. For example, if 2 of 6 mice have died, the death ratio would be 33.3% and the inhibition ratio of fatality would be 100 - 33.3 = 66.6%. The latency to fatality during the first 20 minutes of testing is also shown in Table 1 (6<sup>th</sup> column). There are mean and standard error of the mean because each mouse has a value of latency.

**Table 1.** *In vitro* and *in vivo* bioactivities of 1,3,4-oxadiazoles **6** – **15** and Z944. n = number of experiment; IC<sub>50</sub> is half maximal inhibitory concentration; *I<sub>A</sub>* stands for rapid inactivating potassium current; *I<sub>Kd</sub>* stands for delayed rectifier potassium current; NT: not tested.

1,3,4-Oxadiazoles	Inhibition (%) of T-Cav <sub>3.2</sub> -channel in DRG neurons at 1 μM (unless specified)	Inhibition (%) of Na-channel in DRG neurons at 1 μM (unless specified)	Inhibition (%) of K-channel in DRG neurons at 1 μM (unless specified)	Inhibition ratio (%) of fatality in PTZ-model of mice, at 30 mg/Kg	Latency to fatality (minutes) in PTZ model of mice, at 30 mg/Kg
<b>6</b>	9.8 (3.3 μM) (n = 1)	100 (3.3 μM) (n = 1)	-11.6 ( <i>I<sub>A</sub></i> ) -17.8 ( <i>I<sub>Kd</sub></i> ) (3.3 μM) (n = 1)	0 (n = 8)	1.9±0.3 (n = 8)
<b>7</b>	20.4±1.9 (n = 2)	82.2 (3.3 μM) (n = 1)	-26.0 ( <i>I<sub>A</sub></i> ) -15.6 ( <i>I<sub>Kd</sub></i> ) (3.3 μM) (n = 1)	28.6 (n = 7)	13.6±1.8 (n = 7)
<b>8</b>	24.4±9.6 (n = 3)	-71.8 (n = 1)	-18.7 ( <i>I<sub>A</sub></i> ) -28.6 ( <i>I<sub>Kd</sub></i> ) (n = 1)	NT	
<b>11</b>	50 ±9.7 (IC <sub>50</sub> =0.93 μM) (n = 8)	16.5±8.9 (n = 7)	18.9±11.8 ( <i>I<sub>A</sub></i> ) 24.1±14.6 ( <i>I<sub>Kd</sub></i> ) (n = 4)	72.7 (n = 11)	15.3±2.4 (n = 11)
<b>12</b>	44.2±5.6 64.6±14.1 (in HEK293 cells) (n = 2)	25.7±6.3 (IC <sub>50</sub> =2.1 μM) (n = 5)	21.9±8.6 ( <i>I<sub>A</sub></i> ) (IC <sub>50</sub> =19.6 μM) (n = 4) 31.6±5.5 ( <i>I<sub>Kd</sub></i> ) (IC <sub>50</sub> =2.6 μM) (n = 4)	NT	
<b>13</b>	55.4±28.7 (n = 2)	-2.7 (n = 1)	19.9 ( <i>I<sub>A</sub></i> ) 36.5 ( <i>I<sub>Kd</sub></i> ) (n = 1)	0 (n = 7)	3.7±1.8 (n = 7)
<b>14</b>	69.6±6.2 (n = 2)	-4.7 (n = 1)	12.9 ( <i>I<sub>A</sub></i> ) 30.0 ( <i>I<sub>Kd</sub></i> ) (n = 1)	14 (n = 7)	11.8±2.5 (n = 7)



Z944	74.5±7.8 (0.25 μM, in HEK293 cells) (n = 6)	49.7±6 (IC <sub>50</sub> =0.51 μM) (n = 3)	34.5±15.0 ( <i>I<sub>A</sub></i> ) 44.9±17.7 ( <i>I<sub>Kd</sub></i> ) (n = 2)	33.3 (n = 6)	15.2±3.1 (n = 6)
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As described in Table 1 all ten oxadiazoles possess weak to strong inhibitions of T-type calcium channel and particularly molecules **11** - **14** exhibited strong activities at 1 μM concentration. Molecules **9** and **15** although are active in the inhibition of Ca-channel, 79.5 and 60.9%, respectively, were not investigated further due to its low solubility issue. Compound **10** showed weak inhibition of 5% and was not studied. The three compounds, **9**, **10**, and **15**, are not listed in Table 1. Oxadiazole **6** - **8** and **11** - **14** were also studied for their inhibition selectivity towards sodium and potassium channels. Molecules **6** and **7** weakly inhibited Ca-channels with 9.8 and 20.4% inhibition, respectively, and strongly inhibited Na-channel with respective 100 and 82.2%, hence they were not investigated further. Molecule **8** showed weak inhibition, 24.4%, in Ca-channel and negative inhibition in Na- and K-channel. Molecules **12** - **14** although revealed promising Ca-channel inhibition (44.2, 55.4 and 69.6%, respectively), but they also inhibited K-channel and were not pursued further. Compound **11** showed good inhibition (50%) in Ca-channel and lesser potencies in Na- and K-channels (~16 – 19%). The reported inhibitor, Z944, on the other hand showed a much stronger inhibitory activity against Ca-channel, 74.5% at 0.25 μM. However, it also blocked Na- and K-channels in 49.7 and 34.5%, respectively. Notably, an increase of potassium (K<sup>+</sup>) current has been shown to decrease the excitability of hippocampal neurons such as CA1 pyramidal neurons, after ischemia.<sup>37</sup> Moreover, increases of the amplitude of rapid inactivating potassium currents, *I<sub>A</sub>*, and delayed rectifier potassium currents, *I<sub>Kd</sub>*, in neurons are implicated in the damage of neuronal death after ischemia. Hence, detailed studies of the inhibition of K<sup>+</sup> channels by measuring *I<sub>A</sub>* and *I<sub>Kd</sub>* values were carried out and results are listed in Table 1. Interestingly, oxadiazoles **11** – **14** showed moderate inhibitory activities with *I<sub>A</sub>* and *I<sub>Kd</sub>* values range from 12.9 to 31.6%. The positive control molecule, Z944, also possesses moderate activities with *I<sub>A</sub>* and *I<sub>Kd</sub>* values of 34.5 and 44.9%, respectively. Based on these initial channel inhibitory studies, compounds **6**, **7**, **11**, and Z944 were further bio-evaluated towards their inhibition of seizure-induced mouse model. Compounds **6** and **7** were used as negative controls to verify that the inability in inhibition of Ca-channel resulted in fatality. In this study, fatality rates were calculated as the percentage of mice within each treatment group that died within the 20-min observation period, which revealed 1,3,4-oxadiazole molecules' ability to inhibit neuropathic pains. As predicted, oxadiazoles **6** and **7** showed none to low suppression (0 and 30%) of seizure-induced death. Compound **11** was the best inhibitor that produced a 72.1% of suppression of seizure-induced death. Under similar conditions, Z944 affected only 33% of inhibition of seizures. Results of the seizure studies indicated that inhibition of T-type calcium channels would lead to inhibition of seizures. Thus, T-type calcium channels could potentially be the

biological target for the treatment of neuropathic pain and epilepsy. It appears that aryl and rigid rings in parts A and B (see Figure 1) provide Ca-channel inhibition and reduce neuropathic pain, while aliphatic chain in part B reduces the activities.

## CONCLUSION

A series of 1,3,4-oxadiazole derivatives was designed and synthesized as T-type calcium channel blockers. The synthetic sequence is relatively short and should provide a general route for the construction of a library of 1,3,4-oxadiazole molecules for structure-activity relationship study. An unusual displacement reaction of a C2-chloroacetamide unit of oxadiazole **4** by piperidine was found. The synthesized molecules were screened for their ability and selectivity towards inhibition of T-type calcium channel. Two hit compounds, **11** and **15**, were found to possess good inhibitory activities on T-type Ca<sup>2+</sup> currents and lower activities on voltage-gated Na<sup>+</sup> or K<sup>+</sup> currents. Enhancement of T-type calcium channel inhibition may be achieved through further structural modification of C5-aryl ring of 1,3,4-oxadiazole scaffold. Studies on seizure-induced mouse model showed that the inhibition of T-type calcium channel could lead to inhibition of seizures or epilepsy. Among various 1,3,4-oxadiazole derivatives, compound **11** was found to be the lead compound for future structural optimization in the treatment of neuropathic pain and epilepsy.

## EXPERIMENTAL

Reagent oxalyl chloride was purchased from Fisher Scientific Inc., 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and *N,N*-dimethylaminopyridine (DMAP) from VWR international LLC, and 5-chlorothiophene-2-carboxylic acid, 1,3-dibromo-5,5-dimethylhydantoin, 3-(3-chlorophenyl)propionic acid, 4-cyanopiperidine, and thiosemicarbazide from Chem-Impex International, Inc. Dry THF and diethyl ether were freshly distilled over sodium-benzophenone under argon. Dichloromethane, toluene, and *N,N*-dimethyl formamide (DMF) were distilled (under 5 mm Hg of vacuum for DMF) over calcium hydride under argon. <sup>1</sup>H NMR (400 MHz) and <sup>13</sup>C NMR spectra (100 MHz) spectra were obtained from a 400-MHz Varian NMR spectrometer, and were measured from a solution in CDCl<sub>3</sub> unless otherwise mentioned. The chemical shift data for each signal on <sup>1</sup>H NMR are given in units of δ relative to TMS (δ = 0) or CHCl<sub>3</sub> (δ = 7.26 ppm). For <sup>13</sup>C NMR spectra, the chemical shifts are recorded relative to CDCl<sub>3</sub> (δ = 77.0 ppm). Mass spectra were taken from an API 2000-triple quadrupole Electrospray ionization-MS/MS mass spectrometer (Applied Biosystems).

***N*-(Carbamothioylamino)-5-chlorothiophene-2-carboxamide (18A).** To a solution of 1.0 g (6.1 mmol) of 5-chlorothiophene-2-carboxylic acid in 13 mL of distilled EtOAc at 0 °C under argon, 0.94 g (7.4 mmol) of oxalyl chloride was added followed by the addition of 2 drops of distilled DMF. The resulting solution stirred at 0 °C for 2 h and concentrated to dryness under vacuum to yield 5-chlorothiophene-2-carbonyl chloride (**17A**), which was used in the subsequent step without purification. To a cold (0 °C) suspension of 1.22 g (13 mmol) of thiosemicarbazide (**16**) in 10 mL of distilled THF under argon, was added slowly a solution of 1.10 g (6.1 mmol) of **17A** in 10 mL of THF. The resulting mixture warmed to room temperature and stirred for 12 h. To it, 100 mL of water was added and the precipitated white solids were collected by filtration, washed twice with water, and dried under vacuum to yield 0.87 g (60% yield) of compound **18A** as white solids, mp 198.5 – 199.5 °C. - <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 10.50 (s, 1H), 9.37 (s, 1H), 7.22 (d, *J* = 4.3 Hz, 1H), 7.92 (s, 1H), 7.61 - 7.76 (m, 2H) ppm. - <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): δ 183.1, 160.8, 137.5, 134.7, 130.5, 129.1 ppm. - MS (positive mode): *m/z* calcd for C<sub>6</sub>H<sub>6</sub>ClN<sub>3</sub>OS<sub>2</sub>Na (M+Na)<sup>+</sup>: 258.0, found 258.3.

**1-Benzoyl-3-thiosemicarbazide (18B).** To a cold (0 °C) suspension of 4.0 g (43.8 mmol) of thiosemicarbazide (**16**) in 100 mL of THF under argon was added a solution of 2.8 g (19.9 mmol) of benzoyl chloride (**3C**) in 20 mL of THF. The resulting reaction mixture was stirred at 25 °C for 12 h, concentrated on a rotary evaporator to remove THF, and diluted with 100 mL of water and 10 mL of aqueous NaHCO<sub>3</sub> solution. The precipitated white solids collected by filtration, washed with hexane, and dried under vacuum to yield 3.8 g (100% yield) of compound **18B** as white solids: mp 115 – 116 °C. - <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 9.36 (s, 1H), 7.79 - 7.93 (m, 3H), 7.62 (s, 1H), 7.51 - 7.59 (m, 2H), 7.47 (t, *J* = 7.1 Hz, 2H) ppm. - <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): δ 183.5, 168.6, 134.5, 132.9, 130.4, 129.6 ppm. - MS (positive mode): *m/z* calcd for C<sub>8</sub>H<sub>9</sub>N<sub>3</sub>OSNa (M+Na)<sup>+</sup>: 218.05, found 218.1.

**1-(2-Fluorobenzoyl)-3-thiosemicarbazide (18C).** To a cold (0 °C) suspension of 1.43 g (15.7 mmol) of **16** in 30 mL of THF under argon was added a solution of 1.13 g (7.1 mmol) of 2-fluorobenzoyl chloride (**17B**) in 15 mL each of THF and CH<sub>2</sub>Cl<sub>2</sub> via cannula. The mixture was stirred at 25 °C for 12 h, concentrated on a rotary evaporator to remove most of the solvents, and the precipitated solids were filtered, washed with CH<sub>2</sub>Cl<sub>2</sub> and water, and dried under vacuum to give 1.11 g (72% yield) of **18C** as white solids, mp 203 – 203.5 °C. - <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 10.19 (s, 1H), 9.44 (s, 1H), 7.96 – 7.90 (m, 1H), 7.86 – 7.80 (m, 1H), 7.56 – 7.50 (m, 2H), 7.30 – 7.26 (m, 2H) ppm. - <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): δ 182.8, 164.3, 160.4 (d *J* = 250 Hz, CF), 134.1, 131.6, 125.2, 122.9, 117.2 ppm. - MS (positive mode): *m/z* calcd for C<sub>8</sub>H<sub>8</sub>FN<sub>3</sub>OSNa<sup>+</sup> (M+Na)<sup>+</sup>: 236.2, found 236.1.

**5-(5-Chlorothiophen-2-yl)-1,3,4-oxadiazol-2-amine (2A).** To a cold (0 °C) solution of 0.61 g (3.69 mmol) of KI in 7 mL of water were added 2.9 g (12.3 mmol) of **18A**, 20 mL of water, and 4.6 mL of 4 N NaOH solution (18.5 mmol) and the resulting solution was stirred for 5 min. To it, was added 3.87 g (13.5 mmol) of 1,3-dibromo-5,5-dimethylhydantoin (DBDMH) and the solution was stirred at 25 °C for 7 h, diluted with 3.6 mL of saturated NaHSO<sub>3</sub> aqueous solution, and extracted four times with EtOAc (150 mL each). The combined organic layers were washed with brine, dried (anhydrous Na<sub>2</sub>SO<sub>4</sub>), concentrated, and crystallized from EtOAc (80 mL) to yield 1.20 g (49% yield) of compound **2A** as white solids, mp 250 – 253 °C. - <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 7.33 - 7.38 (m, 3H), 7.24 (d, *J* = 4.3 Hz, 1H) ppm. - <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): δ 163.5, 152.6, 130.7, 128.3, 126.9, 124.6 ppm. - MS (positive mode): *m/z* calcd for C<sub>6</sub>H<sub>5</sub>ClN<sub>3</sub>OS (M+H)<sup>+</sup> 202.0, found 202.0.

**5-Phenyl-1,3,4-oxadiazol-2-amine (2B).** The aforementioned procedure was followed and starting from 3.8 g (19.5 mmol) of compound **18B**, 1.3 g (41% yield) of compound **2B** was obtained as yellow solids, mp 156 – 158 °C. - <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 7.68 - 7.94 (m, 2H), 7.40 - 7.62 (m, 3H), 4.90 – 4.80 (bs, 2H) ppm. - <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): δ 164.9, 156.9, 131.3, 130.2, 126.0, 125.4 ppm. - MS (positive mode): *m/z* calcd for C<sub>8</sub>H<sub>8</sub>N<sub>3</sub>O (M+H)<sup>+</sup> 162.06, found 162.3.

**5-(2-Fluorophenyl)-1,3,4-oxadiazol-2-amine (2C).** The aforementioned procedure for the synthesis of **2A** was followed. Starting from 1.08 g (5.07 mmol) of **18C**, 0.25 g (1.52 mmol), 0.3 g (7.52 mmol) of NaOH, and 1.59 g (5.57 mmol) of DBDMH, after purification using silica gel column chromatography and a mixture of CH<sub>2</sub>Cl<sub>2</sub> and MeOH (10:1) as an eluent, 0.68 g (74% yield) of compound **2C** was obtained as light yellow solids, mp 218.5 – 219.5 °C. - <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 7.83 (t, *J* = 8 Hz, 1H), 7.58 - 7.53 (m, 1H), 7.42 – 7.33 (m, 4H) ppm. - <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): δ 165.0, 159.5 (d, *J* = 251 Hz, CF), 156.9, 133.4, 129.4, 126.0, 118.0, 113.6 ppm. - MS (positive mode): *m/z* calcd for C<sub>8</sub>H<sub>7</sub>FN<sub>3</sub>O<sup>+</sup> (M+H)<sup>+</sup> 180.2, found 180.1.

**2-Chloro-N-(5-phenyl-1,3,4-oxadiazol-2-yl)acetamide (4).** To a cold (0 °C) solution of 0.331 g (2.05 mmol) of compound **2B** in 10 mL of toluene under argon, was added 0.2 mL (2.47 mmol) of chloroacetyl chloride and the solution was stirred under reflux for 12 h. The reaction solution was cooled to 25 °C, concentrated on a rotary evaporator, and column chromatographed on silica gel using a gradient mixture of CH<sub>2</sub>Cl<sub>2</sub> and MeOH as eluent to give 0.385 g (79% yield) of compound **4**. - <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 12.35 - 12.18 (bs, 1 H), 8.02 - 7.82 (m, 2 H), 7.72 - 7.47 (m, 3 H), 4.46 (s, 2 H) ppm. - <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): δ 164.5, 160.7, 157.0, 131.9, 129.6, 126.1, 123.3, 43.2 ppm. - MS (positive mode): *m/z* calcd for C<sub>10</sub>H<sub>8</sub>ClN<sub>3</sub>O<sub>2</sub>Na (M+Na)<sup>+</sup>: 260.6, found 260.4; and C<sub>10</sub>H<sub>9</sub>ClN<sub>3</sub>O<sub>2</sub><sup>+</sup> (M+H)<sup>+</sup>: 238.65, found 238.2 (100%).

**5,6-Dichloro-2-(5-(5-chlorothiophen-2-yl)-1,3,4-oxadiazol-2-yl)isoindoline-1,3-dione (6).** To a solution of 94 mg (0.35 mmol) of 4,5-dichlorophthalic acid in 4 mL of CH<sub>2</sub>Cl<sub>2</sub> under argon at 0 °C, 0.107 g (0.84 mmol) of oxalyl chloride was added dropwise followed by 1 drop of DMF. The resulting solution was stirred under reflux for 3.5 h, cooled to 25 °C, and concentrated to dryness to give 95 mg (100% yield) of 4,5-dichlorobenzene-1,2-dioyl dichloride (**3A**) as white solids. This material was used in the subsequent step without purification. To a suspension of 70 mg (0.35 mmol) of compound **2A** in 3 mL of CH<sub>2</sub>Cl<sub>2</sub> under argon at 0 °C, were added 82 mg (1.04 mmol) of pyridine and a solution of 95 mg (0.35 mmol) of **3A** in 0.5 mL of dichloromethane. The resulting solution was stirred at 25 °C for 5 h, and diluted with 200 mL of CH<sub>2</sub>Cl<sub>2</sub> and 30 mL of 10% aqueous NaHCO<sub>3</sub> solution. The organic layer was separated and washed with brine, dried (anhydrous Na<sub>2</sub>SO<sub>4</sub>), concentrated, and column chromatographed on silica gel using a gradient mixture of hexane and EtOAc as eluent to give 30 mg (22% yield) of compound **6**. - <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 8.48 - 8.40 (m, 2H), 7.75 (d, *J* = 4 Hz, 1H), 7.40 (d, *J* = 4 Hz, 1H) ppm. - <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): δ 161.7, 159.3, 151.2, 138.7, 134.4, 131.1, 131.0, 129.1, 126.6, 122.2 ppm. - MS (positive mode): *m/z* calcd for C<sub>14</sub>H<sub>4</sub>Cl<sub>3</sub>N<sub>3</sub>O<sub>3</sub>SNa (M+Na)<sup>+</sup>: 421.9, found 422.0.

**3-(3-Chlorophenyl)propanoyl chloride (3B).** To a cold (0 °C) solution of 46 mg (0.25 mmol) of 3-(3-chlorophenyl)propionic acid in 3 mL of EtOAc under argon were added 38 mg (0.30 mmol) of oxalyl chloride and 1 drop of DMF. The resulting solution was stirred at 0 °C for 2 h, concentrated to dryness under vacuum to give 3-(3-chlorophenyl)propanoyl chloride (**3B**), which was used in the subsequent reaction without purification.

**3-(3-Chlorophenyl)-N-(5-(5-chlorothiophen-2-yl)-1,3,4-oxadiazol-2-yl)propanamide (7).** To a suspension of 50 mg (0.25 mmol) of compound **2A** in 3 mL of dichloromethane under argon, were added 30 mg (0.38 mmol) of pyridine and a solution of 50 mg (0.25 mmol) of **3B** in 0.3 mL of CH<sub>2</sub>Cl<sub>2</sub>. The resulting solution was stirred at 25 °C for 5 h and diluted with 300 mL of EtOAc and 40 mL of 10% aqueous NaHCO<sub>3</sub> solution. The organic layer was separated and washed with brine, dried (anhydrous Na<sub>2</sub>SO<sub>4</sub>), concentrated, and column chromatographed on silica gel using a gradient mixture of hexane and EtOAc as eluents to give 59 mg (64% yield) of compound **7**. - <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 7.55 (d, *J* = 3.9 Hz, 1H), 7.41 - 7.16 (m, 5H), 2.91 (t, *J* = 7.4 Hz, 2H), 2.78 (t, *J* = 7.4 Hz, 2H) ppm. - <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): δ 169.8, 157.0, 155.6, 143.3, 132.9, 132.6, 130.2, 129.1, 128.6, 128.2, 127.1, 126.1, 123.3, 36.8, 29.5 ppm. - MS (positive mode): *m/z* calcd for C<sub>15</sub>H<sub>11</sub>Cl<sub>2</sub>N<sub>3</sub>O<sub>2</sub>SNa (M+Na)<sup>+</sup> 390.0, found 389.9.

***N*-(5-(5-Chlorothiophen-2-yl)-1,3,4-oxadiazol-2-yl)benzamide (8).** To a hot (40 °C) solution of 50 mg (0.25 mmol) of compound **2A** and 31  $\mu$ L of pyridine (0.37 mmol) in 5 mL of THF under argon was added 35 mg (0.25 mmol) of benzoyl chloride. The resulting solution was stirred at 40 °C for 2 h, diluted with EtOAc, and washed with saturated aqueous NaHCO<sub>3</sub> solution and brine, dried (anhydrous Na<sub>2</sub>SO<sub>4</sub>), concentrated, and column chromatographed on silica gel using a gradient mixture of EtOAc and MeOH as eluent to give 23 mg (30% yield) of compound **8**. - <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>):  $\delta$  8.10 - 7.98 (m, 2H), 7.73 - 7.59 (m, 3H), 7.59 - 7.47 (m, 2H), 7.39 - 7.27 (m, 1H) ppm. - <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>):  $\delta$  161.3, 133.2, 132.9, 132.7, 132.0, 130.4, 129.5, 128.9 (2C), 128.0, 127.6 (2C), 127.2 ppm. - MS (positive mode): *m/z* calcd for C<sub>13</sub>H<sub>8</sub>CN<sub>3</sub>O<sub>2</sub>SNa (M+Na)<sup>+</sup> 328.0, found 328.0.

**2-(4-Cyanopiperidin-1-yl)-*N*-phenylacetamide (21).** To a mixture of 3.0 g (17.7 mmol) of 2-chloro-*N*-phenylacetamide (**20**) and 4.88 g (35.3 mmol) of anhydrous K<sub>2</sub>CO<sub>3</sub> in 25 mL of distilled MeCN under argon, was added 1.95 g (17.7 mmol) of 4-cyanopiperidine (**19**). The resulting mixture was stirred under reflux for 8 h, cooled to 25 °C, filtered through a layer of Celite, concentrated, and column chromatographed on silica gel using a gradient mixture of CH<sub>2</sub>Cl<sub>2</sub> and MeOH as eluent to give 4.28 g (99% yield) of compound **21**. - <sup>1</sup>H NMR:  $\delta$  8.90 – 8.80 (bs, 1H), 7.63 - 7.48 (m, 2H), 7.31 (t, *J* = 7.8 Hz, 2H), 7.09 (t, *J* = 7.8 Hz, 1H), 3.10 (s, 2H), 2.93 - 2.35 (m, 5H), 2.23 - 1.81 (m, 4H) ppm. - <sup>13</sup>C NMR:  $\delta$  168.0, 137.5, 129.2 (2C), 124.4, 121.3, 119.6 (2C), 62.3 (2C), 51.9, 29.1 (2C), 25.6 ppm. - MS (positive mode): *m/z* calcd for C<sub>14</sub>H<sub>18</sub>N<sub>3</sub>O (M+H)<sup>+</sup>: 244.0, found 244.5.

**1-(2-Oxo-2-(phenylamino)ethyl)piperidine-4-carboxylic acid (3D).** To a solution of 0.138 g (0.57 mmol) of compound **21** in 5 mL of MeOH, was added 2 mL of 3 *N* NaOH and the solution was stirred under reflux for 12 h. It was cooled to 25 °C, concentrated on a rotary evaporator to remove MeOH, neutralized with 2 *N* HCl solution, and lyophilized to dryness under vacuum to yield compound **3D** as a HCl salt together with NaCl as white solids. The crude mixture was used in the subsequent reaction without purification.

**1-((Phenylcarbamoyl)methyl)-*N*-(5-(5-chlorothiophen-2-yl)-1,3,4-oxadiazol-2-yl)piperidine-4-carboxamide (9).** A mixture of 0.15 g (0.57 mmol) of aforementioned compound **3D**, 0.114 g (0.57 mmol) of compound **2A**, 0.33 g (1.71 mmol) of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and 0.21 g (1.71 mmol) of 4-(*N,N*-dimethylamino)pyridine (DMAP) in 2 mL of DMF and 2 mL of CH<sub>2</sub>Cl<sub>2</sub> was stirred under argon at 50 °C for 12 h. The resulting mixture was cooled to 25 °C, diluted with 40 mL of distilled water, adjusted the pH to 3 using 2 *N* HCl solution, and extracted with CH<sub>2</sub>Cl<sub>2</sub> twice. The combined organic layers were washed with brine, dried (anhydrous Na<sub>2</sub>SO<sub>4</sub>), concentrated, and column chromatographed on silica gel using a gradient mixture of CH<sub>2</sub>Cl<sub>2</sub> and MeOH as eluent to give 28 mg (11% yield) of compound **9** and 20 mg (18% recovery) of starting **2A**. Compound **9**: - <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>):  $\delta$

11.90 – 11.78 (bs, 1H, NH), 9.69 (s, 1H, NH), 7.64 (d,  $J = 8.2$  Hz, 2H), 7.57 (d,  $J = 3.9$  Hz, 1H), 7.36 - 7.26 (m, 3H), 7.05 (t,  $J = 7.2$  Hz, 1H), 3.15 - 3.13 (s, 2H), 2.97 - 2.87 (m, 3H), 2.2 (t,  $J = 11.1$  Hz, 2H), 1.90 - 1.67 (m, 4H) ppm. -  $^{13}\text{C}$  NMR (DMSO- $d_6$ ):  $\delta$  172.8, 168.4, 157.1, 155.7, 144.7, 138.6, 132.6, 129.7, 129.2, 128.7 (2C), 123.7, 119.8 (2C), 62.0, 52.5 (2C), 41.9, 27.9 (2C) ppm. - MS (positive mode),  $m/z$  calcd for  $\text{C}_{20}\text{H}_{21}\text{ClN}_5\text{O}_3\text{S}$  (M+H) $^+$ : 446.1, found 446.3.

**3-(3-Chlorophenyl)-*N*-(5-phenyl-1,3,4-oxadiazol-2-yl)propanamide (10).** To a cold (0 °C) solution of 0.10 g (0.62 mmol) of **2B** in 10 mL of  $\text{CH}_2\text{Cl}_2$  under argon, were added 75  $\mu\text{L}$  of pyridine and 0.126 g (0.62 mmol) of **3B**. The solution was stirred for 2 h, warmed to 25 °C, diluted with 20 mL of aqueous  $\text{NaHCO}_3$ , and extracted three times with  $\text{CH}_2\text{Cl}_2$ . The combined extract was washed with brine, dried (anhydrous  $\text{Na}_2\text{SO}_4$ ), concentrated, and column chromatographed on silica gel using a gradient mixture of hexane and EtOAc as eluent to give 30 mg (15% yield) of compound **10** as light yellow crystals, mp 201 – 203 °C. -  $^1\text{H}$  NMR:  $\delta$  8.07 (d,  $J = 8$  Hz, 2H), 7.60 – 7.50 (m, 3H), 7.36 (s, 1H, NH), 7.30 – 7.20 (m, 4H), 3.15 – 3.02 (m, 2H), 3.00 – 2.70 (m, 2H) ppm. -  $^{13}\text{C}$  NMR (DMSO- $d_6$ ):  $\delta$  166.0, 164.1, 155.2, 132.8, 133.4, 132.6, 131.1, 130.4, 129.5, 129.2, 128.7, 128.1, 126.9, 35.8, 30.8 ppm. - MS (positive mode):  $m/z$  calcd for  $\text{C}_{17}\text{H}_{15}\text{ClN}_3\text{O}_2$  (M+H) $^+$ : 329.8, found 330.1 ( $\text{Cl}^{37}$  isotope) and 328.0 ( $\text{Cl}^{35}$  isotope).

**5-Chloro-*N*-(5-phenyl-1,3,4-oxadiazol-2-yl)thiophene-2-carboxamide (11).** To a cold (0 °C) solution of 0.30 g (1.86 mmol) of compound **2B** and 0.22 g (2.79 mmol) of pyridine in 10 mL of  $\text{CH}_2\text{Cl}_2$  under argon, was added a solution of 0.34 g (1.86 mmol) of 5-chlorothiophene-2-carbonyl chloride (**17A**) in 5 mL of  $\text{CH}_2\text{Cl}_2$ . The resulting mixture was stirred at 25 °C for 3.5 h, diluted with 40 mL of 10% aqueous  $\text{NaHCO}_3$  solution, and extracted three times with  $\text{CH}_2\text{Cl}_2$ . The combined extract was washed with brine, dried (anh.  $\text{Na}_2\text{SO}_4$ ), concentrated, and column chromatographed on silica gel using a gradient mixture of hexane and EtOAc as eluent to give 0.30 g (53% yield) of compound **11**. -  $^1\text{H}$  NMR:  $\delta$  8.22 (bs, 1H, NH), 8.12 (d,  $J = 4.3$  Hz, 1H), 7.98 (d,  $J = 7.4$  Hz, 2H), 7.67 - 7.49 (m, 3H), 7.06 (d,  $J = 4.3$  Hz, 1H) ppm. -  $^{13}\text{C}$  NMR:  $\delta$  158.3, 154.3, 151.3, 142.1, 137.3 133.3, 129.9, 129.4, 127.2, 127.1, 122.4 ppm. - MS (positive mode):  $m/z$  calcd for  $\text{C}_{13}\text{H}_8\text{ClN}_3\text{O}_2\text{SNa}$  (M+Na) $^+$ : 328.0, found 327.8.

**1-(5-Phenyl-1,3,4-oxadiazol-2-yl)piperidine (12).** To a solution of 0.128 g (0.54 mmol) of **4** in 4 mL of EtOH under argon was added 60  $\mu\text{L}$  (0.65 mmol) of piperidine and the solution was stirred under reflux for 24 h. The solution was cooled to 25 °C, added three drops of 2 N NaOH solution, diluted with 50 mL of water, and extracted twice with  $\text{CH}_2\text{Cl}_2$ . The combined extracts were washed with brine, dried (anhydrous  $\text{Na}_2\text{SO}_4$ ), concentrated, and column chromatographed on silica gel using a gradient mixture of  $\text{CH}_2\text{Cl}_2$  and MeOH as eluent to give 78 mg (63% yield) of compound **12**. -  $^1\text{H}$  NMR:  $\delta$  7.98 (d,  $J = 7.8$  Hz, 2H), 7.46 - 7.35 (m, 3H), 3.45 (t,  $J = 5.3$  Hz, 4H), 1.69 (d,  $J = 5.1$  Hz, 4H), 1.66 - 1.62 (m, 2H) ppm. -  $^{13}\text{C}$  NMR:  $\delta$  161.3, 158.9, 130.5, 129.0, 128.4, 126.2, 47.3, 24.9, 23.8 ppm. - MS (positive mode):  $m/z$  calcd for

$C_{13}H_{16}N_3O^+$  (M+H)<sup>+</sup>: 230.3, found 230.1.

***N*-(5-Phenyl-1,3,4-oxadiazol-2-yl)-2-(piperidin-1-yl)acetamide (13).** To a solution of 0.13 g (0.55 mmol) of **4** in 3 mL of EtOH under argon was added 60  $\mu$ L (0.65 mmol) of piperidine and the solution was stirred at 50 °C for 48 h. It was cooled to 25 °C, added three drops of 2 N NaOH solution, diluted with 50 mL of water, and extracted twice with CH<sub>2</sub>Cl<sub>2</sub>. The combined extracts were washed with brine, dried (anhydrous Na<sub>2</sub>SO<sub>4</sub>), concentrated, and column chromatographed on silica gel using a gradient mixture of hexane and EtOAc as eluent to give 70 mg (45% yield) of compound **13**. - <sup>1</sup>H NMR:  $\delta$  8.00 (d,  $J$  = 6.6 Hz, 2H), 7.53 - 7.40 (m, 3H), 3.45 (s, 2H), 3.18 - 3.13 (m, 1H), 2.82 (m, 4H), 1.90 (td,  $J$  = 11.2, 5.9 Hz, 2H), 1.84 - 1.73 (m, 4H) ppm. - <sup>13</sup>C NMR:  $\delta$  167.6, 161.1, 158.7, 131.3, 128.9, 126.4, 123.8, 61.3, 54.7, 24.8, 22.4 ppm. - MS (positive mode):  $m/z$  calcd for C<sub>15</sub>H<sub>19</sub>N<sub>4</sub>O<sub>2</sub> (M+H)<sup>+</sup>: 287.3, found 287.0 (100%).

**2-(4-Cyanopiperidin-1-yl)-*N*-(5-phenyl-1,3,4-oxadiazol-2-yl)acetamide (14).** To a solution of 0.107 g (0.45 mmol) of **4** in 3 mL of EtOH under argon, was added 60  $\mu$ L (0.54 mmol) of 4-cyanopiperidine (**19**) and the resulting solution was stirred at 80 °C for 24 h. It was cooled to 25 °C, added three drops of 2 N NaOH solution, diluted with 50 mL of water, and extracted twice with CH<sub>2</sub>Cl<sub>2</sub>. The combined extracts were washed with brine, dried (anhydrous Na<sub>2</sub>SO<sub>4</sub>), concentrated, and column chromatographed on silica gel using a gradient mixture of hexane and EtOAc as eluent to give 40 mg (29% yield) of compound **14**. - <sup>1</sup>H NMR:  $\delta$  10.00 (bs, 1H), 7.87 (d,  $J$  = 7.0 Hz, 2H), 7.43 - 7.41 (m, 3H), 3.29 (s, 2H), 2.94 - 2.72 (m, 4H), 2.64 (m, 1H), 2.10 - 1.95 (m, 4H) ppm. - <sup>13</sup>C NMR:  $\delta$  167.5, 160.8, 159.5, 130.9, 129.5, 127.0, 124.9, 122.5, 60.5, 56.1, 50.3, 27.4 ppm. - MS (positive mode):  $m/z$  calcd for C<sub>16</sub>H<sub>18</sub>N<sub>5</sub>O<sub>2</sub><sup>+</sup> (M+H)<sup>+</sup>: 312.3, found 312.2 (100%).

**5-Chloro-*N*-(5-(2-fluorophenyl)-1,3,4-oxadiazol-2-yl)thiophene-2-carboxamide (15).** To a cold (0 °C) solution of 60 mg (0.335 mmol) of compound **2C** and 40 mg (0.50 mmol) of pyridine in 3 mL of CH<sub>2</sub>Cl<sub>2</sub> under argon, was added a solution of 0.61 mg (0.335 mmol) of 5-chlorothiophene-2-carbonyl chloride (**17A**) in 1 mL of CH<sub>2</sub>Cl<sub>2</sub>. The resulting mixture was stirred at 25°C for 3.5 h, diluted with 15 mL of CH<sub>2</sub>Cl<sub>2</sub> and 8 mL of 10% aqueous NaHCO<sub>3</sub> solution, and extracted three times with CH<sub>2</sub>Cl<sub>2</sub>. The combined extract was washed with brine, dried (anhydrous Na<sub>2</sub>SO<sub>4</sub>), concentrated, and column chromatographed on silica gel using a gradient mixture of CH<sub>2</sub>Cl<sub>2</sub> and MeOH as eluent to give 90 mg (83% yield) of compound **15** as white solids, mp 150 – 151 °C. - <sup>1</sup>H NMR:  $\delta$  8.25 – 8.17 (bs, 1H, NH), 8.18 (d,  $J$  = 4 Hz, 1H), 7.93 (td,  $J$  = 8, 2 Hz, 1H), 7.65 – 7.58 (m, 1H), 7.34 (td,  $J$  = 8, 2 Hz, 1H), 7.28 (d,  $J$  = 8 Hz, 1H), 7.07 (d,  $J$  = 4 Hz, 1H). - <sup>13</sup>C NMR:  $\delta$  161.0 (d,  $J$  = 261 Hz, CF), 158.4, 150.8, 142.5, 137.5 135.0, 134.9, 130.4, 129.6, 127.5, 125.1, 117.5, 111.1 ppm. - MS (positive mode),  $m/z$  calcd for C<sub>13</sub>H<sub>8</sub>ClFN<sub>3</sub>O<sub>2</sub>S (M+H)<sup>+</sup>: 324.7, found 324.0.

**Biological studies:**



**Inhibition of T-channel in DRG neurons.** Mouse dorsal root ganglion (DRG) neurons were prepared from 1 – 6 month-old C57/BC6 mice. All the procedures related to animal were conducted at AfaSci Research Laboratories and were in strict accordance with NIH guidelines and IACUC approved protocols. After sacrificing the mice by decapitation, the spine was removed and split into two halves from the middle line. Lumbar DRG neurons were collected into a 1.5 mL Eppendorf tube with modified Krebs solution (130 mM NaCl, 10 mM HEPES-Na, 5 mM KCl, 1 mM CaCl<sub>2</sub>, 10 mM glucose, and 2 mM MgCl<sub>2</sub>, pH adjusted to 7.35 with 1 N HCl). Subsequently, the DRG neurons were removed for digestion into 0.5 mL of Hank's balanced salt solution (HBSS) with 1 mg/mL collagenase and 0.5 mg/mL trypsin added. The DRGs were minced with fine scissors and incubated at 35°C for 45-50 min. After removing the HBSS solution, the DRG neurons were dispersed into modified Krebs solution and triturated gently with 5 fire-polished glass pipettes in gradually shrunk opening until no clumps were visible. The cells were then dispersed onto poly-L-ornithine-coated cover slips and maintained in a modified Krebs solution at 21°C with antibiotics added (0.2 mM streptomycin sulfate, 0.3 mM Penicillin G Sodium, and 0.1 mM Gentamycin).

**Preparation of HEK293 cells expressing Ca<sub>v3.2</sub> channels.** Ca<sub>v3.2</sub> channels were expressed in human embryonic kidney (HEK)-293 cells using a tetracycline inducible expression system (Invitrogen). Cells were placed in 25-cm<sup>2</sup> tissue culture flasks at 37 °C, 5% CO<sub>2</sub>, and 100% relative humidity in D-MEM/F12 (Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12; Gibco) supplemented with fetal bovine serum (FBS, 10% v/v), sodium pyruvate (0.5 mM, Gibco), penicillin-streptomycin (100 U/mL, 100 µg/mL) and Geneticin<sup>R</sup> Selective antibiotic (G418; 0.5 mg/mL). Cells were detached from the flask base using a non-enzymatic cell dissociation solution (Cellstripper, Corning), removed and reseeded onto poly-D-lysine coated glass coverslips in 35-mm petri dishes. Cells in dishes were further supplemented with G418 at a final concentration of 1 mg/mL. After 24 hours, expression of Ca<sub>v3.2</sub> was induced with 1 µg/mL tetracycline. The medium for the HEK-293 cells was composed of D-MEM/F-12 and 15 mM HEPES buffer, with L-glutamine and pyridoxine hydrochloride. It was supplemented with FBS, P/S (penicillin/streptomycin) (Gibco, #15140-122), and 100 mM sodium pyruvate. Similarly, Na- and K-channel expressions in HEK293 cells were prepared.

**Patch clamp recordings.** Whole-cell voltage clamp recordings were performed on DRG neurons or cultured HEK293 cells expressing T-type channels (encoded by Ca<sub>v3.2</sub> channels). All experiments were performed at room temperature. Whole-cell currents were recorded using a MultiClamp 700B amplifier and analyzed using Clampfit of Pclamp software (version 10.4, Molecular Devices, LLC, Sunnyvale, CA, USA). To record calcium currents in HEK293 cells, the external solution was composed (in mM) of 115 choline-Cl, 30 TEA-Cl, 2 CaCl<sub>2</sub>, 10 glucose and 10 HEPES (pH 7.3 – 7.4 adjusted with TEA-OH; osmolality about 295 mOsm/kg). The internal solution was composed (in mM) of 125 CsCl, 10 HEPES

(acid), 10 EGTA (ethylene glycol tetraacetic acid), 1 CaCl<sub>2</sub>, 1 MgCl<sub>2</sub>, 4 MgATP, and 0.3 MgGTP (pH 7.3 – 7.4 adjusted with CsOH; osmolality about 295 mOsm/kg). Since sodium ions were absent in the external solution, tetrodotoxin was not added. Calcium currents were recorded at a holding potential of -100 mV and then depolarized in 10mV steps of 100 ms duration to activate Ca<sub>v</sub>3.2 expressed in HEK cells. An interpulse interval of 10 seconds allowed the channel recovery from inactivation with stable current recordings. All reagents were purchased from Sigma unless specified otherwise. Test compounds were applied through a rapid solution exchange system with 8 plastic tubings glued into a 27G needle and with opening located closely to the recorded cells. The current responses were normalized to the control. Inhibition percentage was calculated and sigmoidal dose-response curves were generated using KaleidaGraph, XLFit (IDBS, Surrey, UK) or Prism (GraphPad Software, La Jolla, CA, US) for calculation.

**Inhibition of seizure in pentylenetetrazol (PTZ)-induced fatality in mice.** It has been reported that over activation of T-channel involves in the generation of seizure activity. Following the reported protocol,<sup>36</sup> the pentylenetetrazol (PTZ; 40 mg/kg, by intraperitoneal injection)-induced seizure model in mice (n = 6 per group) was used to evaluate the inhibition of seizure. A prolonged latency to seizure and decrease of fatality rate were studied comparing to vehicle [2% DMSO in 0.5% hydroxypropyl cellulose (HPC)]. Fatality latency and rate – the percentage (%) of mice in each treatment group that died within a 20-minutes cutoff of the observational period, were recorded and calculated. Average fatality latency and rate of each treatment group were used to evaluate the molecule's ability to either prevent or delay the onset of PTZ-induced seizures and death. In all cases, experiments were conducted in a blind manner with respect to the experimenters.

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