

## Synthesis of 1,4- and 1,4,4-substituted piperidines for the inhibition of neuronal T-type $\text{Ca}^{2+}$ channels and mitigation of neuropathic pain in mice

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This manuscript is in celebration of Professor George A. Kraus for his dedication to research and education

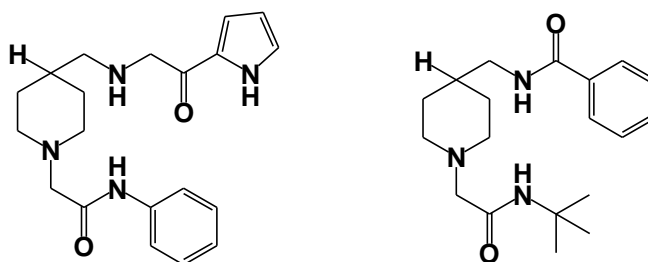
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### Abstract

A dysregulation in function of neuronal low-voltage-activated T-channel causes neuropathic pain. A group of ten 1,4-disubstituted and 1,4,4-trisubstituted piperidines were synthesized through short sequences of reactions from 4-cyanopiperidine, which may potentially produce a library of analogs. Inhibitions of T-channel in rat dorsal root ganglion neurons and/or  $\text{Ca}_{v3.2}$  channel in human embryonic kidney-293 cells were screened, and subsequent analgesic effects and inhibition of seizure in pentylenetetrazole-induced seizure mouse model were examined. The two most promising piperidine molecules showed analgesic effects in rat model using a spared nerve injury protocol. One displayed a long withdrawal latency in response to thermal stimulation, and an 80% increase in mechanical threshold assessment studies.



**Keywords:** Neuropathic pain, patch clamp recording, 1,4-disubstituted piperidines, 1,4,4-trisubstituted piperidines, T-type calcium channel inhibitors, alkylation of 4-cyanopiperidines

## Introduction

Intracellular calcium concentrations play a vital role in neuronal functions and signal transduction.<sup>1,2</sup> Control of calcium entry into cells through the cell membrane is partly regulated by a family of transmembrane proteins called voltage-gated calcium channels.<sup>3</sup> Voltage-gated calcium channels modulate the entry of extracellular calcium ions into cells in response to the changes in electrical potential differences across the cell membrane.<sup>4,5</sup> Depending on the membrane potential required for activation, voltage-gated calcium channels are divided into two types.<sup>5</sup> They are called low-voltage-activated channels (T-type) and high-voltage-activated channels (L-, N-, P/Q-, and R-types).<sup>5-7</sup>

Low-voltage-activated channels stimulate by negative membrane depolarization potentials (from about -40 mV to -70 mV) and have fast voltage-dependent inactivations.<sup>3,5,6,8</sup> Therefore, they produce transient currents.<sup>3-6</sup> High-voltage-activated channels are provoked by relatively positive membrane depolarization potentials (from about -35 mV to +15 mV).<sup>3</sup> Voltage-gated calcium channels are divided into three main channel families called  $Ca_v1$  (L-type),  $Ca_v2$  (P/Q, N, and R-types) and  $Ca_v3$  (T-type).<sup>8</sup> Based on the sequence of their membrane pore-forming  $\alpha1$  subunit, each channel family is further classified into subtypes such as  $Ca_{v1.1}$  -  $Ca_{v1.4}$ ,  $Ca_{v2.1}$  -  $Ca_{v2.3}$ , and  $Ca_{v3.1}$  -  $Ca_{v3.3}$ .<sup>8</sup> L-type calcium channels are mainly distributed in cardiac, skeletal, vascular smooth muscles and endocrine cells; P/Q-, N- and R- type channels are expressed in neuronal presynaptic terminals.<sup>8-11</sup> T-type calcium channels  $Ca_{v3.1}$  and  $Ca_{v3.2}$  are mainly expressed in the brain, peripheral nervous tissues (dorsal root ganglia and autonomic ganglia) and peripheral tissues such as heart, smooth muscle, skeletal muscle and endocrine cells,<sup>12</sup> while  $Ca_{v3.3}$  is mainly expressed in the brain and peripheral nerve tissues such as dorsal root ganglia and autonomic ganglia.<sup>12-14</sup>

T-type calcium channels are involved in regulating neuronal excitability, burst firing and transmitter release.<sup>15-19</sup> Therefore, dysregulation in function of neuronal T-type calcium currents has been proposed to be a link to various types of neurological disorders, including neuropathic pain, hypertension, and absence seizures.<sup>17,18,20,21</sup> Neuropathic pain is a dysfunction of the central and peripheral nervous systems, resulting from nerve injury or disease conditions such as inflammation, multiple sclerosis, autoimmune diseases, diabetes or cancer.<sup>22,23</sup> Neuropathic pain is often characterized by pain responses such as dysesthesia (abnormal sense of touch), hyperalgesia (increased sensitivity to painful stimuli) and allodynia (painful response to normally not painful stimuli).<sup>22</sup>

Although the molecular mechanisms underlying the development of neuropathic pain are poorly understood, it is suggested that injured nerve fibers induce neuropathic pain by altering ion channels which affect the sensory signaling.<sup>24</sup> It has been found that T-type calcium currents were up-regulated in sensory neurons in rat models of neuropathic pain<sup>25</sup>, and an increase of T-type  $Ca^{2+}$  (mainly  $Ca_{v3.2}$ ) currents promoted the nociceptor excitability and hyperalgesia in rat dorsal root ganglion (DRG) neurons, subsequently influencing pain processing.<sup>26</sup> Another study has shown that axonal accumulation of  $Ca_{v3.2}$  T-type calcium channels in uninjured rat sural nerves with spared nerve injury (SNI) enhanced sensitization, and subsequently contributed to neuropathic pain.<sup>27</sup>

Since T-type calcium channel is involved in the progression of the neuropathic pain, T-type calcium channel blockers have been investigated intensely for the discovery of neuropathic-pain therapeutics.<sup>17,28</sup> It has been shown that perineural administration of a T-type calcium channel blocker, mibefradil, increased mechanical thresholds of A $\beta$ -, A $\delta$ - and C-fibers in the sural nerve of neuropathic pain models.<sup>17</sup> A number of studies using the antisense of  $Ca_{v3.2}$  T-type channel have shown to reduce nociceptive response in mouse models of neuropathic pain.<sup>26,29,30</sup>

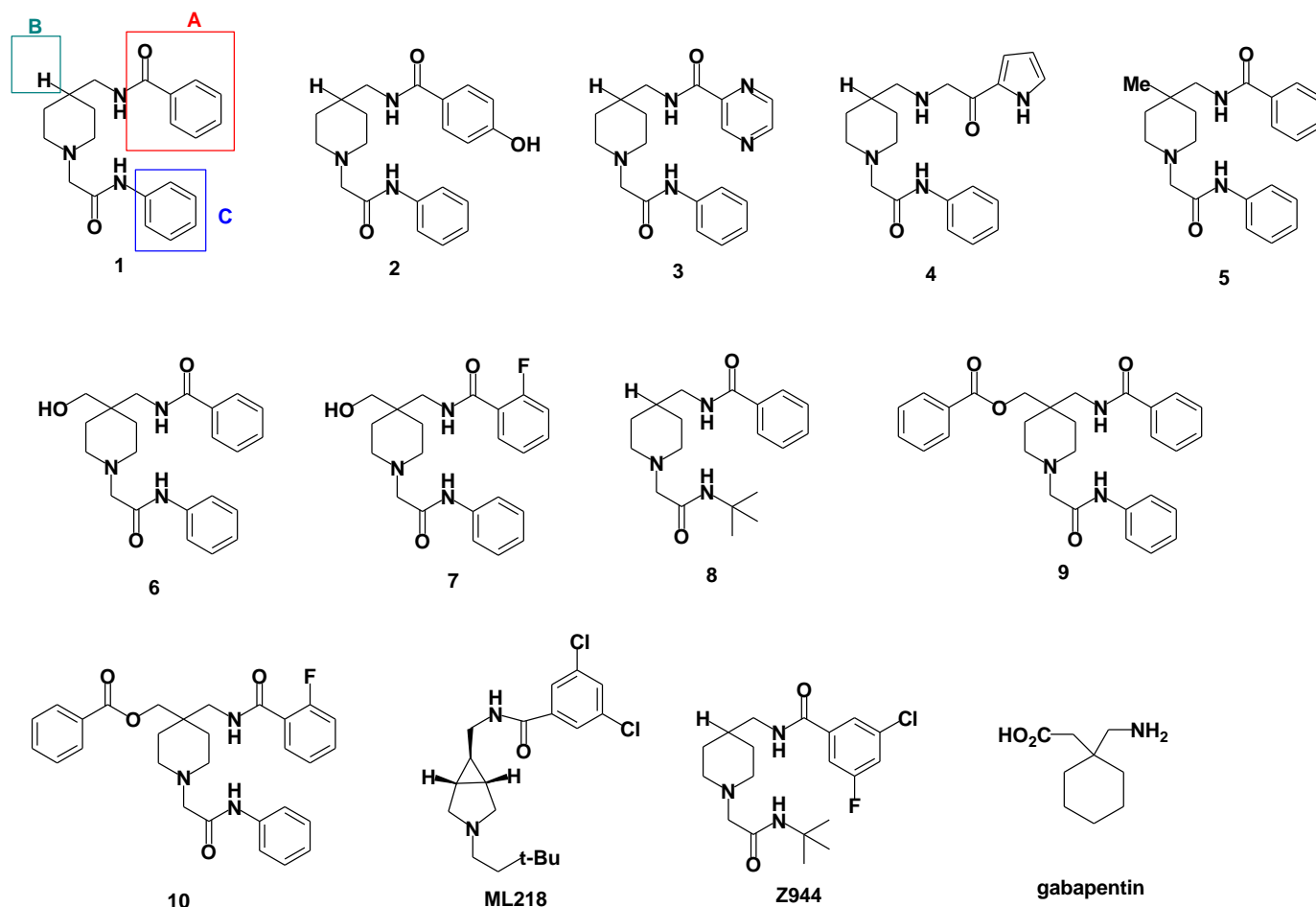
Literature data have shown that T-type channel blockers with a piperidine scaffold have good potencies, with minimal effects on L-type  $\text{Ca}^{2+}$ ,  $\text{K}^{+}$ , human ether-a-go-go related gene (hERG) channels and other off-targets. hERG is a gene that codes a protein known as  $\text{K}_{v11.1}$ , the  $\alpha$  subunit of a potassium ion channel in the heart that coordinates heart beating. Therefore, inhibition of the hERG channel is detrimental.<sup>31</sup>

Although several T-type calcium channel inhibitors have shown success in relieving neuropathic pain in rat models, there has been no FDA-approved T-type calcium channel blocker available for clinical use until now. Therefore, there is a high demand for the discovery of novel, potent T-type-channel-specific inhibitors. Herein, we report the synthesis of a series of ten 1,4-disubstituted and 1,4,4-trisubstituted piperidines, and their inhibitory efficacy of neuropathic pain.

## Results and Discussion

In search of potent and selective T-channel inhibitors for the treatment of neuropathic pain, we used a rational drug-design approach. A series of 1,4-di- and 1,4,4-trisubstituted piperidines were synthesized due to their broad scope of potential therapeutic applications, and their activities against T-type calcium channels were evaluated for the possible treatment of neuropathic pain.

We designed this series of molecules by changing substituents independently in three regions (A, B and C) of the 1,4-disubstituted piperidine core **1** (Figure 1). The selection of core **1** was based on reported piperidine inhibitors such as ML218 and Z944.<sup>28,32,33</sup> We hypothesize that the piperidine ring adopts a chair conformation and, with the *N*-1 and C4 substituents oriented at the equatorial positions, leads to an extended structure for hydrophilic (from amide and amine functions) and hydrophobic (aryl or alkyl group) interactions with the targeted calcium channels. Modification in part A focused on the substitution of different aromatic-ring equivalents, while modifications in part B focused on the replacement of C4-hydrogen with methyl, hydroxymethyl or arylcarbonyloxymethyl groups. Modification in part C focused on the replacement of benzoyl amide with a *tertiary*-butyl amide. These structural modifications were expected to change the hydrophilic, hydrophobic, rigidity, planarity, steric hindrance, and number of hydrogen bondings of each molecule, which may allow future studies for improving the potency and selectivity.



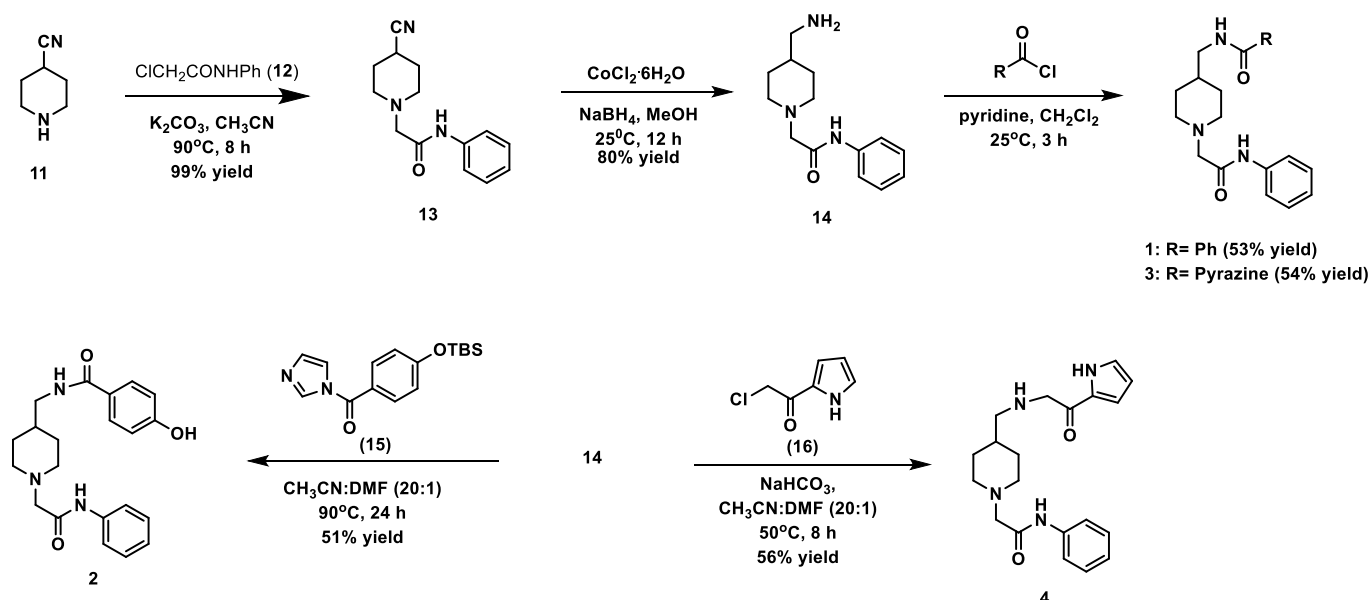
**Figure 1.** Designed 1,4- and 1,4,4-substituted piperidine compounds as T-type calcium channel inhibitors and reported inhibitors ML218, Z944, and gabapentin

### Synthesis of T-type calcium channel inhibitors

The synthetic routes of compounds **1-10** are shown in Scheme 1 - Scheme 4 starting from commercially available 4-cyanopiperidine (**11**). The synthetic methods for the syntheses of these molecules are relatively short. Hence, in the syntheses of **1** and **3**, an initial *N*-alkylation was carried out by the treatment of 4-cyanopiperidine with *N*-phenyl-2-chloroacetamide under basic conditions that yielded compound **13** (Scheme 1). Reduction of the nitrile function of **13** by cobalt(II) chloride•hexahydrate and sodium borohydride yielded amine **14**. Coupling of amine **14** with benzoyl chloride and pyrazinoyl chloride, separately, in the presence of pyridine, gave compounds **1** and **3**, respectively.

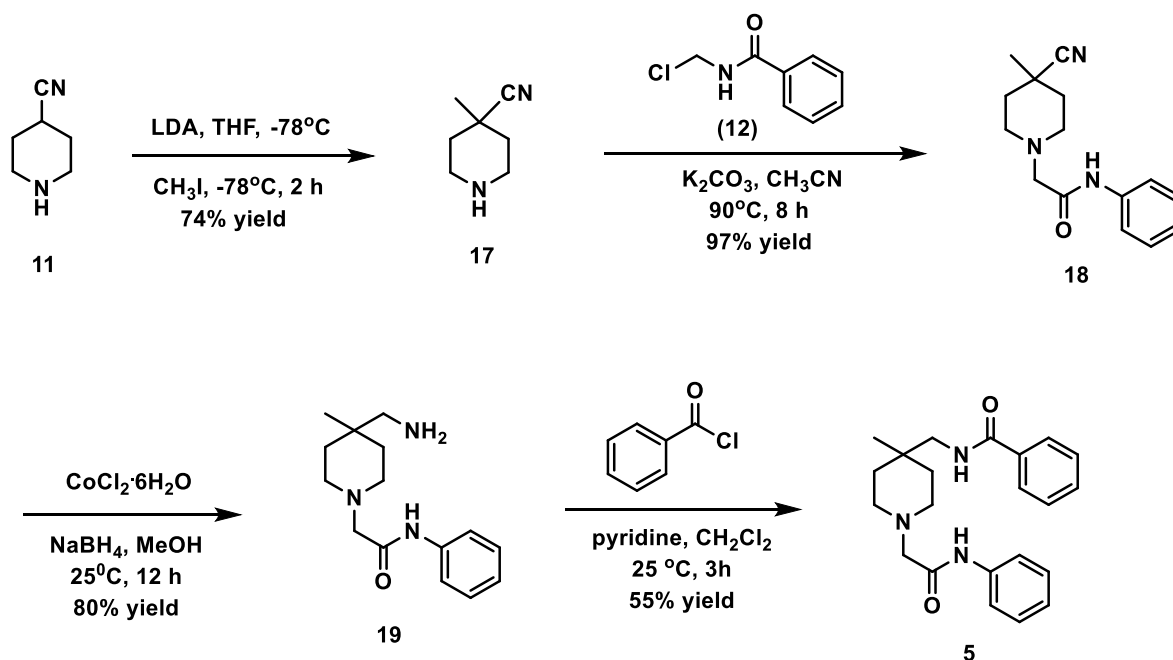
Similar amide coupling of compound **14** with *p*-hydroxybenzoic acid via activation of the acid with 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide hydrochloride (EDC) in the presence of a catalytic amount of *N,N'*-dimethylaminopyridine (DMAP), gave a poor yield of amide **2**. An improved synthesis was found by using acyl imidazole **15**, derived from 4-(*tert*-butyldimethylsilyloxy)benzoic acid and 1,1'-carbonyldiimidazole (CDI). Hence, coupling of **14** and **15** in acetonitrile:DMF (20:1) at 90 °C gave a 51% yield of **2**.

Amine **14** was treated with 2-chloroacetylpyrrole (**16**) in the presence of sodium bicarbonate in acetonitrile:DMF (20:1) at 50 °C to yield compound **4** (56% yield). It is important to keep a higher ratio of amine to alkyl chloride to prevent the formation of *N,N*-dialkylated product. The desired product **4** and unreacted amine **14** were separated by silica gel column chromatography.



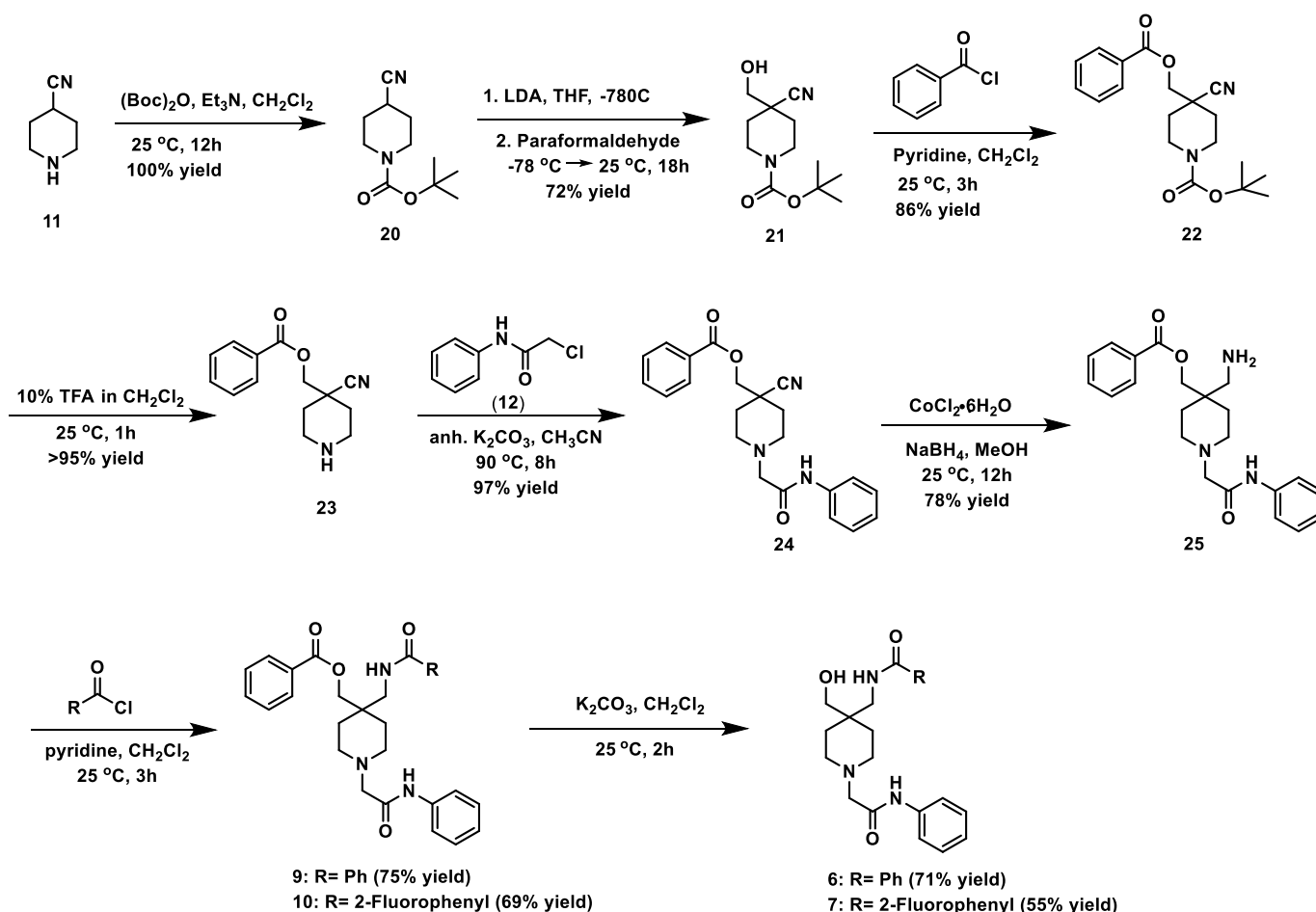
**Scheme 1.** Syntheses of compounds **1** to **4**

The introduction of a hydrophilic or hydrophobic group at C-4 of piperidines **1** – **4** may increase binding to the targeted  $\text{Ca}^{2+}$  channel. Hence compounds **5-7**, **9**, and **10** were synthesized. Deprotonation of 4-cyanopiperidine (**11**) using 1 equivalent of lithium diisopropylamide (LDA) followed by methylation with methyl iodide (1 equivalent) furnished 4-cyano-4-methylpiperidine (**17**) (Scheme 2).<sup>34</sup> The C4-H (adjacent to CN group) is more acidic ( $\text{pK}_a \sim 27$ ) than N1-H ( $\text{pK}_a \sim 40$ ); hence, methylation took place preferably at the C4-carbanion instead of N1. However, we cannot rule out that methylation at N-1 (as a minor by-product) did not occur to yield a minor byproduct since the isolated yield of **17** was not quantitative (74%). Installation of the usual phenylamidomethyl group to the piperidine nitrogen of **17** with *N*-phenyl-2-chloroacetamide under basic conditions gave compound **18**. Reduction of the cyano function, followed by amide formation of **18** under a similar reaction sequence as described above, afforded piperidine derivative **5** containing a hydrophobic methyl group at C4 of the piperidine core (Scheme 2). Proton and carbon-13 NMR spectra of molecule **5** showed a single isomer in which the larger C4-benzoylaminomethyl group, compared with the methyl substituent, likely orients in the equatorial position.



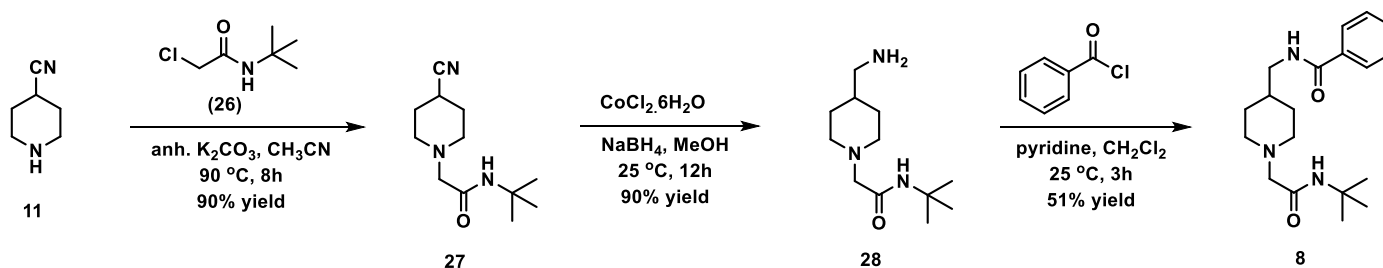
**Scheme 2.** Synthesis of compound 5

Piperidine derivatives **6**, **7**, **9** and **10**, containing a hydrophilic group, were synthesized via a modification of Scheme 2 due to the competing reactivity of piperidinyl amine and the newly installed hydroxyl group (Scheme 3). Hence, 4-cyanopiperidine was first protected using di-*tert*-butyl dicarbonate ( $\text{Boc}_2\text{O}$ ) and triethylamine to give *N*-Boc protected compound **20**. Deprotonation of compound **20**, using an equivalent of LDA followed by paraformaldehyde, and quenching with ammonium hydroxide/ammonium chloride, yielded the C4-hydroxymethylated molecule **21**. Benzoylation of **21** with benzoyl chloride under basic conditions resulted in benzoate **22**. The Boc protecting group of **22** was removed using 10% trifluoroacetic acid (TFA) in methylene chloride to give the amine intermediate **23**. Subsequent alkylation of **23** using *N*-phenyl-2-chloroacetamide under basic conditions provided compound **24**. Reduction of the cyano moiety of compound **24** was carried out by the treatment with cobalt(II) chloride hexahydrate and sodium borohydride to obtain the corresponding amine compound **25**. Amidation of **25** with benzoyl chloride and 2-fluorobenzoyl chloride, separately, under basic conditions, resulted in compounds **9** and **10**, respectively. Basic hydrolysis of compounds **9** and **10** with potassium carbonate gave the debenzoylated products **6** and **7**, respectively. Molecules **6**, **7**, **9**, and **10** (*vide infra*) are mixtures of *cis*- and *trans*-isomers based on their NMR spectra.



Scheme 3. Synthesis of compounds 6, 7, 9, and 10

We considered, and subsequently investigated, whether a replacement of the phenyl ring of the amido group at C1 of **1** with a *t*-butyl group, such as molecule **8**, would enhance the bioactivity. The synthesis of the 1,4-diamide analog **8** was similar to that of compound **1** except in the initial *N*-alkylation step of the piperidine ring (Scheme 4). Treatment of piperidine **11** with *N*-*tert*-butyl-2-chloroacetamide under basic conditions afforded compound **27**. The cyano function of **27** was reduced with cobalt(II) chloride•hexahydrate and sodium borohydride to obtain the corresponding amine molecule **28**, which, upon benzoylation with benzoyl chloride, furnished analog **8**.



Scheme 4. Synthesis of compound 8

Biological evaluation of the synthesized molecules was carried out using patch clamp recordings of either dissociated mouse dorsal root ganglion (DRG) neurons or  $\text{Ca}_{\text{v}3.2}$  channel expressed in human embryonic kidney (HEK)-293 cells from a tetracycline-inducible expression system [see Table S1 and supporting information published in the Supplementary Material (SM) for details]. The tested compounds **4** and **8** have increased thermal and mechanical thresholds compared to vehicle. The tested compounds produced analgesic effects on both thermal and mechanical pain thresholds, while the vehicle group continued to produce thermal hyperalgesia and mechanical allodynia conditions (see SM). Therefore, compounds **4** and **8** have the ability to mitigate induced-neuropathic pain in rats, and **4** appears to have a greater effect than **8** at the 3-hour time point (an 80% increase in mechanical threshold for **4** and ~60% for **8**) (see Figures S1 and S2 in SM). We have also examined the prolonged latency to seizures and decreased fatality rate in pentylenetetrazole (PTZ)-induced mouse model to assess the inhibition of T-channel activity. In agreement with the aforementioned studies, molecules **4** and **8** exhibited 86 and 89%, respectively, seizure-inhibitory activities.

## Conclusions

In summary, a series of ten 1,4-disubstituted and 1,4,4-trisubstituted piperidines were synthesized from 4-cyanopiperidine through short sequences of reactions. Molecule **4**, containing a pyrrolylcarbonylmethylaminomethyl at C4 and *N*-phenylacetamide at *N*-1, showed strong inhibition of the  $\text{Ca}_{\text{v}3.2}$  channel and equal potency of analgesic effect as two known potent T-channel inhibitors, ML218 and Z944. Importantly, **4** revealed a much stronger inhibition of seizure in PTZ-induced fatality than the two known inhibitors, suggesting that **4** can be a lead compound for discovery of novel analgesics in the future.

## Experimental Section

**General.** Chemicals were purchased from Fisher Scientific, VWR international LLC and Chem-Impex International, Inc. All solvents were dried over appropriate drying agents such as  $\text{CaH}_2$  (for DMF, dichloromethane and acetonitrile) or Na/benzophenone (for THF and diethyl ether) followed by distillation. Column chromatography was carried out on silica gel (200-400 mesh).  $^1\text{H}$  NMR spectra (400 MHz) and  $^{13}\text{C}$  NMR spectra (100 MHz) were recorded on a Varian Unity plus 400 MHz Spectrometer in  $\text{CDCl}_3$  or  $\text{DMSO}-d_6$  relative to TMS ( $\delta = 0$  ppm) or  $\text{CHCl}_3$  ( $\delta = 7.26$  ppm) ( $^1\text{H}$  NMR) or  $\text{CDCl}_3$  ( $\delta = 77.0$  ppm) or  $\text{DMSO}$  ( $\delta = 39.5$  ppm) ( $^{13}\text{C}$  NMR). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, dd = doublet of doublets, td = triplet of doublets, ddd = doublet of doublet of doublets, m = multiplet, br = broad), coupling constant (Hz). Mass spectra were obtained from an API 2000-triple quadrupole ESI-MS/MS mass spectrometer (Applied Biosystems).

### Synthesis of T-type calcium channel inhibitors

***N*-({1-[2-Oxo-2-(phenylamino)ethyl]piperidin-4-yl)methyl}benzamide (1).** A mixture of 4-cyanopiperidine (**11**) (1.0 g, 9.1 mmol), *N*-phenyl-2-chloroacetamide (**12**) (1.54 g, 9.1 mmol), and anhydrous potassium carbonate (2.5 g, 18.2 mmol) in acetonitrile (30 mL) was heated to reflux at 90 °C under argon for 8 hours. The resulting mixture was cooled and filtered to remove inorganic salts. The filtrate was concentrated, and column chromatographed on silica gel using a mixture of dichloromethane and methanol (30:1) as an eluent



to give 2-(4-cyanopiperidin-1-yl)-*N*-phenylacetamide (**13**) (2.2 g, 99.7% yield) as a white solid.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta_{\text{H}}$  8.94 - 8.90 (1H, br. s., NH), 7.52 (2H, d,  $J$  7.8 Hz, CH x 2 *o*-aromatic), 7.29 (2H, t,  $J$  8.0 Hz, CH x 2 *m*-Aromatic), 7.08 (1H, t,  $J$  7.4 Hz, CH *p*-aromatic), 3.08 (2H, s,  $\text{NCH}_2\text{CO}$ ), 2.72 - 2.79 (2H, m,  $\text{CH}_2\text{N}$ ), 2.63 (1H, td,  $J$  7.7, 3.8 Hz,  $\text{CHCN}$ ), 2.44 (2H, t,  $J$  8.4 Hz,  $\text{CH}_2\text{N}$ ), 1.84 - 2.00 (4H, m, 2  $\text{CH}_2$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta_{\text{C}}$  167.8 (C=O), 137.4 (C aromatic), 128.9 (CH x 2 aromatic), 124.2 (CH aromatic), 121.2 (CN), 119.4 (CH x 2 aromatic), 62.1 ( $\text{CH}_2\text{N}$ ), 51.7 (2  $\text{CH}_2\text{N}$ ), 28.8 (2  $\text{CH}_2$ ), 25.4 ( $\text{CHCN}$ ); MS (ESI):  $m/z$  244.5 ( $\text{M}+\text{H}$ ) $^+$  (75%), 123.1 (100%).

To a cold (0 °C) solution of **13** (0.80 g, 3.29 mmol) in MeOH (25 mL), cobalt(II) chloride•hexahydrate (0.39 g, 1.65 mmol) was added followed by the addition of sodium borohydride (0.62 g, 16.5 mmol). After stirring for 15 hours at 25 °C, the reaction solution was diluted with 25 mL of 5% aqueous ammonium hydroxide and extracted three times with dichloromethane. The combined organic layer was washed with brine, dried over anhydrous  $\text{Na}_2\text{SO}_4$ , concentrated, and column chromatographed on silica gel using a mixture of dichloromethane and methanol (2:1) as an eluent to give 2-[4-(aminomethyl)piperidin-1-yl]-*N*-phenylacetamide (**14**) (0.81 g, 80% yield) as a colorless oil.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta_{\text{H}}$  9.18 (1H, br. s., NH), 7.56 (2H, d,  $J$  8.2 Hz, CH x 2 *o*-aromatic), 7.32 (2H, t,  $J$  7.8 Hz, CH x 2 *m*-aromatic), 7.09 (1H, t,  $J$  7.4 Hz, CH *p*-aromatic), 3.09 (2H, s,  $\text{CH}_2\text{N}$ ), 2.92 (2H, d,  $J$  11.3 Hz,  $\text{CH}_2\text{N}$ ), 2.63 (1H, d,  $J$  5.5 Hz, CH), 2.18 - 2.30 (2H, m,  $\text{CH}_2\text{N}$ ), 1.78 (2H, d,  $J$  10.5 Hz,  $\text{CH}_2\text{N}$ ), 1.21 - 1.37 (4H, m, 2  $\text{CH}_2$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta_{\text{C}}$  168.9 (C=O), 137.8 (C aromatic), 129.1 (CH x 2 aromatic), 124.2 (*p*-CH aromatic), 119.5 (CH x 2 aromatic), 62.5 ( $\text{CH}_2\text{N}$ ), 54.2 (3  $\text{CH}_2\text{N}$ ), 48.0 (CH), 30.4 (2  $\text{CH}_2$ ); MS (ESI):  $m/z$  270.2 ( $\text{M}+\text{Na}$ ) $^+$ , 248.2 ( $\text{M}+\text{H}$ ) $^+$  (100%), 231.3.

To a cold (0 °C) solution of 0.13 g (0.61 mmol) of **14** in 3 mL of freshly distilled dichloromethane under argon, pyridine 78  $\mu\text{L}$  (0.915 mmol) was added followed by the addition of 70  $\mu\text{L}$  (0.61 mmol) of benzoyl chloride. The reaction was stirred at 0 °C for 30 min. and 25 °C for 3 hours. The reaction solution was diluted with 20 mL of aqueous sodium bicarbonate and extracted three times with dichloromethane. The combined organic layer was washed with brine, dried (anhydrous  $\text{Na}_2\text{SO}_4$ ), concentrated, and column chromatographed on silica gel using a mixture of dichloromethane and methanol (10:1) as an eluent to give *N*-({1-[2-oxo-2-(phenylamino)ethyl]piperidin-4-yl}methyl)benzamide (**1**) (0.114 g, 53% yield) as a white solid.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta_{\text{H}}$  9.85 (1H, br. s., NH), 9.59 (1H, br. s., NH), 8.09 (2H, d,  $J$  7.4 Hz, CH 2 x aromatic), 7.79 (1H, d,  $J$  7.4 Hz, CH aromatic), 7.54 - 7.58 (2H, m, CH x 2 aromatic), 7.40 - 7.43 (2H, m, CH x 2 aromatic), 7.30 (1H, t,  $J$  7.8 Hz, CH aromatic), 7.09 (1H, t,  $J$  7.4 Hz, CH aromatic), 6.65 (1H, t,  $J$  5.9 Hz, CH aromatic), 3.40 (2H, t,  $J$  6.4 Hz,  $\text{CH}_2\text{N}$ ), 3.31 (2H, s,  $\text{O}=\text{CCH}_2\text{N}$ ), 3.08 (2H, d,  $J$  11.7 Hz,  $\text{CH}_2\text{N}$ ), 2.34 - 2.45 (2H, m,  $\text{CH}_2\text{N}$ ), 1.82 (2H, d,  $J$  12.5 Hz,  $\text{CH}_2$ ), 1.66-1.77 (1H, m, CH), 1.44-1.57 (2H, m,  $\text{CH}_2$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta_{\text{C}}$  168.3 (C=O), 167.6 (C=O), 137.8 (C aromatic), 134.6 (C aromatic), 131.8 (CH aromatic), 129.2 (CH x 2 aromatic), 128.6 (CH x 2 aromatic), 127.2 (CH x 2 aromatic), 124.6 (CH aromatic), 119.9 (CH x 2 aromatic), 62.0 ( $\text{CH}_2\text{N}$ ), 53.7 (2  $\text{CH}_2\text{N}$ ), 45.3 ( $\text{CH}_2\text{N}$ ), 35.5 (CH), 29.7 (2  $\text{CH}_2$ ); MS (ESI):  $m/z$  374.2 ( $\text{M}+\text{Na}$ ) $^+$ , 352.1 ( $\text{M}+\text{H}$ ) $^+$  (100%).

**4-Hydroxy-*N*-((1-(2-oxo-2-(phenylamino)ethyl)piperidin-4-yl)methyl)benzamide (2).** To a solution of 0.10 g (0.28 mmol) of **14** in a freshly distilled 20:1 mixture of acetonitrile:DMF under argon, 0.86 g (0.28 mmol) of (4-(*tert*-butyldimethylsilyloxy)phenyl)(1*H*-imidazol-1-yl)methanone (**15**) was added. The reaction mixture was heated to reflux at 90 °C for 24 hours, cooled to room temperature, diluted with 20 mL of aqueous sodium bicarbonate, and extracted three times with dichloromethane. The combined organic layer was washed with brine, dried (anhydrous  $\text{Na}_2\text{SO}_4$ ), concentrated, and column chromatographed on silica gel using a mixture of dichloromethane and methanol (15:1) as an eluent to give **2** (52 mg, 51% yield) as a white solid.  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ ):  $\delta_{\text{H}}$  9.16 (1H, br. s., OH), 8.25 (1H, s, NH), 7.68 (2H, d,  $J$  8.6 Hz, CH 2 x aromatic), 7.52 - 7.61 (2H, m, CH x 2 aromatic), 7.35 (2H, d,  $J$  7.4 Hz, CH x 2 aromatic), 7.07 - 7.18 (1H, m, CH aromatic), 6.87 (2H, d,  $J$  8.6 Hz, CH x 2 aromatic), 3.12 (2H, t,  $J$  6.4 Hz,  $\text{CH}_2\text{N}$ ), 3.04 - 3.15 (2H, s,  $\text{CH}_2\text{N}$ ), 2.93 (2H, d,  $J$  11.7 Hz,  $\text{CH}_2\text{N}$ ), 2.25 (2H, t,

$J$  10.7 Hz, CH<sub>2</sub>N), 1.81 (2H, d,  $J$  10.5 Hz, CH<sub>2</sub>), 1.60-1.58 (1H, m, CH), 1.37 - 1.46 (2H, m, CH<sub>2</sub>); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>):  $\delta_c$  168.6 (C=O), 166.0 (C=O), 160.0 (C aromatic), 138.6 (C aromatic), 129.1 (CH x 2 aromatic), 128.7 (CH x 2 aromatic), 125.4 (C aromatic), 123.4 (C aromatic), 119.5 (CH x 2 aromatic), 114.8 (CH x 2 aromatic), 62.3 (CH<sub>2</sub>N), 53.3 (2 CH<sub>2</sub>N), 44.7 (CH<sub>2</sub>N), 35.4 (CH), 29.8 (2 CH<sub>2</sub>); MS (ESI):  $m/z$  389.9 (M+Na)<sup>+</sup>, 368.4 (M+H)<sup>+</sup> (100%).

***N*-({1-[2-Oxo-2-(phenylamino)ethyl]piperidin-4-yl)methyl}pyrazine-2-carboxamide (3).** To a cold (0 °C) solution of **14** (80 mg, 0.32 mmol) in 3 mL of freshly distilled dichloromethane under argon, pyridine 40  $\mu$ L (0.48 mmol) was added followed by the addition of 52 mg (0.36 mmol) of pyrazinoyl chloride. The reaction was stirred at 0 °C for 30 min. and 25 °C for 3 hours. The reaction solution was diluted with 20 mL of aqueous sodium bicarbonate and extracted three times with dichloromethane. The combined organic layer was washed with brine, dried (anhydrous Na<sub>2</sub>SO<sub>4</sub>), concentrated, and column chromatographed on silica gel using a mixture of dichloromethane and methanol (10:1) as an eluent to give **3** (62 mg, 54% yield) as a white solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta_H$  9.42 (1H, s, CH pyrazine), 9.14 (1H, br. s., NH), 8.77 (1H, d,  $J$  2.3 Hz, CH pyrazine), 8.53 (1H, s, CH pyrazine), 7.86 - 7.99 (1H, m, NH), 7.56 (2H, d,  $J$  8.2 Hz, CH x 2 *o*-aromatic), 7.33 (2H, t,  $J$  7.8 Hz, CH x 2 *m*-aromatic), 7.11 (1H, t,  $J$  7.4 Hz, CH *p*-aromatic), 3.44 (1H, t,  $J$  6.6 Hz, CH or CH<sub>2</sub>N), 3.11 (2H, s, CH<sub>2</sub>N), 2.95 (2H, d,  $J$  11.7 Hz, CH<sub>2</sub>N), 2.26 (2H, t,  $J$  10.7 Hz, CH<sub>2</sub>N), 1.84 (2H, d,  $J$  12.1 Hz, CH<sub>2</sub>), 1.69 (1H, ddd,  $J$  3.9, 7.3, 11.0 Hz, CH), 1.39 - 1.49 (2H, m, CH<sub>2</sub>); <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta_c$  168.6 (C=O), 163.3 (C=O), 147.5 (CH pyrazine), 144.6 (CH pyrazine), 144.5 (C pyrazine), 142.6 (CH pyrazine), 137.7 (C aromatic), 129.2 (CH x 2 aromatic), 124.3 (CH aromatic), 119.6 (CH x 2 aromatic), 62.5 (CH<sub>2</sub>N), 55.0 (2 CH<sub>2</sub>N), 44.9 (CH<sub>2</sub>N), 35.9 (CH), 30.5 (2 CH<sub>2</sub>); MS (ESI):  $m/z$  376.1 (M+Na)<sup>+</sup>, 354.3 (M+H)<sup>+</sup> (100%), 233.4 (75%).

**2-[4-{{2-Oxo-2-(1H-pyrrol-2-yl)ethylamino}methyl}piperidin-1-yl]-*N*-phenylacetamide (4).** A mixture of **14** (0.10 g, 0.40 mmol), 2-chloroacetyl pyrrole (**16**) (0.04 g, 0.28 mmol), and sodium bicarbonate (0.11 g, 0.40 mmol) in a mixture of 20:1 distilled acetonitrile:DMF (5 mL) was heated at 50°C under argon for 8 hours. The resulting mixture was cooled to room temperature and filtered to remove insoluble inorganic salt. The filtrate was concentrated and column chromatographed on silica gel using a mixture of dichloromethane and methanol (30:1) as an eluent to give **4** (56 mg, 56% yield) as a red solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta_H$  9.96 (1H, br. s., NH), 9.19 (1H, br. s., NH), 7.53 (2H, d,  $J$  1.0 Hz, CH x 2 *o*-aromatic), 7.33 (2H, t,  $J$  1.0 Hz, CH x 2 *m*-aromatic), 7.10 (1H, t,  $J$  7.4 Hz, CH *p*-aromatic), 7.06 (1H, br. s., CH imidazole), 6.93 (1H, d,  $J$  3.5 Hz, CH imidazole), 6.27 (1H, dd,  $J$  1.0 Hz, CH imidazole), 3.89 (2H, s, CH<sub>2</sub>N), 3.18 (2H, s, CH<sub>2</sub>N), 2.90 (2H, d,  $J$  11.3 Hz, CH<sub>2</sub>N), 2.64 (2H, d,  $J$  6.6 Hz, CH<sub>2</sub>N), 2.38 (1H, s, NH), 2.23 (2H, t,  $J$  10.7 Hz, CH<sub>2</sub>N), 1.84 (2H, d,  $J$  11.7 Hz, CH<sub>2</sub>), 1.62-1.70 (1H, m, CH), 1.13 - 1.47 (2H, m, CH<sub>2</sub>); <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta_c$  188.6 (C=O), 169.1 (C=O), 137.9 (C aromatic), 130.6 (C imidazole), 129.2 (CH x 2 aromatic), 124.9 (CH imidazole), 124.3 (CH aromatic), 119.6 (CH x 2 aromatic), 116.0 (CH imidazole), 111.0 (CH imidazole), 62.6 (CH<sub>2</sub>N), 55.9 (CH<sub>2</sub>N), 55.0 (CH<sub>2</sub>N), 54.3 (2 CH<sub>2</sub>N), 36.0 (CH), 31.0 (2 CH<sub>2</sub>); MS (ESI):  $m/z$  355.4 (M+H)<sup>+</sup> (30%), 377.5 (M+Na)<sup>+</sup>, 231.3 (100%).

***N*-({4-Methyl-1-[2-oxo-2-(phenylamino)ethyl]piperidin-4-yl)methyl}benzamide (5).** To a cold (-78 °C) solution of **11** (0.5 g, 4.54 mmol) in 25 mL of dry THF under argon was added a solution of lithium diisopropylamide (LDA) (0.23 M in 20 mL of THF, 4.54 mmol) slowly over 30 min. A pale brown solution resulted. Stirring was continued for 2 hours at -78 °C and iodomethane (0.64 g, 4.54 mmol) was added dropwise into the reaction solution while maintaining the temperature at -78 °C. The reaction solution was stirred at -78 °C for 2 hours, diluted with 5 mL of saturated aqueous NH<sub>4</sub>Cl solution at -78 °C, and then warmed to room temperature. The organic layer was separated, and the aqueous layer was extracted three times (100 mL each) with dichloromethane. The combined organic layers were washed with brine (100 mL), 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 4-methylpiperidine-4-carbonitrile (**17**)<sup>34</sup> (0.42 g, 74% yield) as a white

solid.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta_{\text{H}}$  4.82 (1H, br. s., NH), 3.23 (2H, d,  $J$  11.4 Hz,  $\text{CH}_2\text{N}$ ), 2.98 (2H, t,  $J$  12.6 Hz,  $\text{CH}_2\text{N}$ ), 1.97 (2H, d,  $J$  13.5 Hz,  $\text{CH}_2$ ), 1.72-1.63 (2H, m,  $\text{CH}_2$ ), 1.41 (3H, s,  $\text{CH}_3$ ); MS (ESI):  $m/z$  125.1 ( $\text{M}+\text{H}$ ) $^+$  (100%).

To a solution of **17** (0.15 g, 1.21 mmol) in dry  $\text{CH}_3\text{CN}$  (8 mL) under argon, *N*-(chloromethyl)benzamide (0.21 g, 1.21 mmol) and anhydrous  $\text{K}_2\text{CO}_3$  (0.33 g, 2.42 mmol) were added, and the mixture was stirred at 85 °C for 8 hours. The reaction mixture was cooled to 25 °C, filtered, concentrated, and column chromatographed on silica gel using a mixture of ethyl acetate and hexane (5:1) as an eluent to give 2-(4-cyano-4-methylpiperidin-1-yl)-*N*-phenylacetamide (**18**) (0.30 g, 97% yield).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta_{\text{H}}$  8.94 (1H, br. s., NH), 7.57 - 7.52 (2H, m, CH x 2 *o*-aromatic), 7.35 - 7.29 (2H, m, CH x 2 *m*-aromatic), 7.18 - 7.06 (1H, m, CH *p*-aromatic), 3.15 (2H, s,  $\text{CH}_2\text{N}$ ), 2.91 - 2.84 (2H, m,  $\text{CH}_2\text{N}$ ), 2.58 (2H, dt,  $J$  2.3, 12.3 Hz,  $\text{CH}_2\text{N}$ ), 2.00 - 1.94 (2H, m,  $\text{CH}_2$ ), 1.69 - 1.58 (2H, m,  $\text{CH}_2$ ), 1.42 (3H, s,  $\text{CH}_3$ ); MS (ESI):  $m/z$  258.6 ( $\text{M}+\text{H}$ ) $^+$ , 280.1 ( $\text{M}+\text{Na}$ ) $^+$  (100%).

To a solution of **18** (200 mg, 0.78 mmol) in 8 mL of methanol,  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  (94 mg, 0.39 mmol) was added, and the solution was stirred for 5 min. The reaction solution was cooled to 0 °C and sodium borohydride (132 mg, 3.51 mmol) was added portion-wise over 30 min. The resulting solution was stirred for 18 hours at room temperature, diluted with 15 mL of cold saturated  $\text{NH}_4\text{Cl}$ , 15 mL of water and extracted three times with ethyl acetate. The combined organic layers were washed with brine (10 mL), dried (anhydrous  $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated to yield 165 mg (80% yield) of 2-[4-(aminomethyl)-4-methylpiperidin-1-yl]-*N*-phenylacetamide (**19**), as a light yellow oil. MS (ESI):  $m/z$  284.3 ( $\text{M}+\text{Na}$ ) $^+$ , 262.3 ( $\text{M}+\text{H}$ ) $^+$  (70%), 245.5 (100%). This compound was used in the following reaction without further purification.

To a solution of compound **19** (150 mg, 0.57 mmol) in distilled dichloromethane (7.5 mL) under argon, dry pyridine (74  $\mu\text{L}$ , 0.57 mmol) was added and stirred at 0 °C. To it, benzoyl chloride (80 mg, 0.57 mmol) was added and stirred for 3 hours at room temperature. The reaction mixture was partitioned between dichloromethane and water and basified to pH 8 using aqueous  $\text{NaHCO}_3$ . The organic layer was separated, and the aqueous layer was extracted three times with dichloromethane. The combined organic layers were washed with brine, dried (anhydrous  $\text{Na}_2\text{SO}_4$ ), filtered, concentrated, and purified by column chromatography on silica gel using a gradient mixture of  $\text{CH}_2\text{Cl}_2$  and MeOH as eluent to obtained 115 mg (55% yield) of *N*-(4-methyl-1-[2-oxo-2-(phenylamino)ethyl]piperidin-4-yl)methyl)benzamide (**5**) as a white solid.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta_{\text{H}}$  9.39 (1H, br. s., NH), 7.82 - 7.77 (2H, m, CH x 2 *o*-aromatic), 7.57 (2H, m, CH x 2 aromatic), 7.52 - 7.49 (1H, m, CH aromatic), 7.46 - 7.42 (2H, m, CH x 2 aromatic), 7.35 - 7.29 (2H, m, CH x 2 aromatic), 7.14 - 7.06 (1H, m, CH aromatic), 6.41 (1H, t,  $J$  5.9 Hz, NH), 3.45 - 3.37 (2H, m,  $\text{CH}_2\text{N}$ ), 3.23 (2H, s,  $\text{CH}_2\text{N}$ ), 2.85 - 2.75 (2H, m,  $\text{CH}_2\text{N}$ ), 2.66 - 2.56 (2H, m,  $\text{CH}_2\text{N}$ ), 1.70 (2H, ddd,  $J$  3.7, 9.5, 13.4 Hz,  $\text{CH}_2$ ), 1.56 - 1.46 (2H, m,  $\text{CH}_2$ ), 1.05 (3H, s,  $\text{CH}_3$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta_{\text{C}}$  168.0 (C=O), 167.7 (C=O), 137.5 (C aromatic), 134.5 (C aromatic), 131.4 (CH aromatic), 128.9 (CH x 2 aromatic), 128.5 (CH x 2 aromatic), 126.9 (CH x 2 aromatic), 124.2 (CH aromatic), 119.5 (CH x 2 aromatic), 61.8 ( $\text{CH}_2\text{N}$ ), 53.6 ( $\text{CH}_2\text{N}$ ), 49.6 (2  $\text{CH}_2\text{N}$ ), 35.4 (C), 34.4 (2  $\text{CH}_2$ ), 32.7 ( $\text{CH}_3$ ); MS (ESI):  $m/z$  = 366.1 ( $\text{M}+\text{H}$ ) $^+$  (100%), 245.5.

**{4-(Benzamidomethyl)-1-[2-oxo-2-(phenylamino)ethyl]piperidin-4-yl)methyl benzoate (9).** To a solution of 4-cyanopiperidine (**11**) (0.5 g, 4.54 mmol) in 25 mL of distilled dichloromethane under argon, dried triethylamine (1.3 mL, 9.08 mmol) was added. While stirring, the solution was cooled to 0 °C and di-*tert*-butyl dicarbonate (1.19 g, 5.4 mmol) was added in several portions over 15 min (bubbling observed). The resulting mixture was stirred at 25 °C for 12 hours, then the solvent was evaporated and the residue purified by column chromatography on silica gel using a mixture of dichloromethane and methanol (30:1) as an eluent to give *tert*-butyl 4-cyanopiperidine-1-carboxylate (**20**), 0.95 g (100% yield) as a colorless viscous oil, that solidifies on standing.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta_{\text{H}}$  3.64 (2H, ddd,  $J$  3.91, 7.03, 13.67 Hz,  $\text{CH}_2\text{N}$ ), 3.31 (2H, ddd,  $J$  3.7, 7.8, 13.9 Hz,

CH<sub>2</sub>N), 2.73 - 2.83 (1H, m, CHCN), 1.82 - 1.92 (2H, m, CH<sub>2</sub>), 1.72 - 1.82 (2H, m, CH<sub>2</sub>), 1.44 (9 H, s, 3 CH<sub>3</sub>); <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ<sub>c</sub> 153.6 (C=O), 120.5 (CN), 79.0 (C-O), 40.8 (2 CH<sub>2</sub>N), 27.8 (CH), 27.7 (3 CH<sub>3</sub>), 25.5 (2 CH<sub>2</sub>).

A solution of *tert*-butyl 4-cyanopiperidine-1-carboxylate (**20**) (0.95 g, 4.54 mmol) was dissolved in 25 mL of dry THF under argon and cooled to -78 °C. Lithium diisopropylamide (0.23M in 20 mL, 4.54 mmol) was slowly added to the reaction solution over 30 min at -78 °C. A pale brown solution was obtained. Stirring was continued for 2 hours at -78 °C, and a solution of paraformaldehyde (0.16 g, 5.4 mmol) in 25 mL of freshly distilled THF was added slowly into the reaction solution while keeping the temperature at -78 °C. The reaction mixture was allowed to reach room temperature while being stirred for 15 hours under argon. It was diluted with 100 mL of water and 50 mL of saturated NaCl, and the resulting solution was extracted with dichloromethane three times (250 mL each). The combined organic layers were washed with brine (100 mL), dried (anhydrous Na<sub>2</sub>SO<sub>4</sub>), concentrated, and column chromatographed on silica gel using a gradient mixture of hexane and ethyl acetate as eluent to give *tert*-butyl 4-cyano-4-(hydroxymethyl)piperidine-1-carboxylate (**21**), 0.78 g (72% yield) as a white solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ<sub>H</sub> 4.10 - 4.24 (2H, m, CH<sub>2</sub>O), 3.67 (2H, d, *J* 6.3 Hz, CH<sub>2</sub>N), 2.03 - 2.11 (2H, m, CH<sub>2</sub>N), 1.91 - 2.00 (2H, m, CH<sub>2</sub>), 1.95 - 1.85 (2H, m, CH<sub>2</sub>), 1.46 (9H, s, 3 CH<sub>3</sub>); <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ<sub>c</sub> 154.4 (C=O), 121.4 (CN), 80.1 (C-O), 67.4 (CH<sub>2</sub>O), 40.6 (2 CH<sub>2</sub>N), 40.0 (2 CH<sub>2</sub>), 30.8 (C), 28.1 (3 CH<sub>3</sub>); MS (ESI): *m/z* 263.5 (M+Na)<sup>+</sup> (100%).

To a solution of *tert*-butyl 4-cyano-4-(hydroxymethyl)piperidine-1-carboxylate (**21**) (0.49 g, 2.0 mmol) and pyridine (0.24 mL, 3.03 mmol) in dichloromethane (8 mL) under argon at 0 °C, was added benzoyl chloride (0.25 mL, 2.0 mmol), and the reaction mixture was stirred for 8 hours at room temperature. The reaction mixture was partitioned between dichloromethane and water and adjusted the pH to 8 using aqueous NaHCO<sub>3</sub>. The organic layer was separated, and the aqueous layer was extracted three times with dichloromethane. The combined organic layers were washed with brine (10 mL), dried (anhydrous Na<sub>2</sub>SO<sub>4</sub>), filtered, concentrated, and purified by column chromatography on silica gel using a mixture of dichloromethane and methanol (30:1) to obtain 0.21 g (86% yield) of *tert*-butyl 4-[(benzoyloxy)methyl]-4-cyanopiperidine-1-carboxylate (**22**), as a yellow solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ<sub>H</sub> 7.88 - 8.04 (2H, m, CH x 2 *o*-aromatic), 6.81 - 6.95 (2H, m, CH x 2 *m*-aromatic), 6.41 (1H, s, CH *p*-aromatic), 4.33 (2H, s, CH<sub>2</sub>O), 4.3 - 4.17 (2H, m, CH<sub>2</sub>N), 3.10 - 3.0 (2H, m, CH<sub>2</sub>N), 2.05 - 2.01 (2H, m, CH<sub>2</sub>), 1.53 - 1.61 (2H, m, CH<sub>2</sub>), 1.47 (9H, s, 3 CH<sub>3</sub>); <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ<sub>c</sub> 165.6 (C=O), 161.0 (C=O), 154.7 (C aromatic), 132.3 (CH x 2 aromatic), 121.3 (CN), 120.5 (CH aromatic), 115.6 (CH x 2 aromatic), 80.7 (C-O), 68.1 (CH<sub>2</sub>O), 42.0-39.0 (m, 2 CH<sub>2</sub>N), 38.8 (CH<sub>2</sub>), 31.8 (CH<sub>2</sub>), 28.5 (3 CH<sub>3</sub>); MS (ESI): *m/z* 367.0 (M+Na)<sup>+</sup> (100%), 245.4.

A solution of 4-[(benzoyloxy)methyl]-4-cyanopiperidine-1-carboxylate (**22**) (1.12 mmol) in 2 mL dichloromethane containing 10% trifluoroacetic acid (TFA) was stirred at room temperature for 1 hour. The solvent was removed and the remaining solid was dried under high vacuum to yield 0.27 g (95% yield) of (4-cyanopiperidin-4-yl)methyl benzoate (**23**) as a yellow solid. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ<sub>H</sub> 8.8-8.6 (1H, br. s., NH), 8.02 (2H, d, *J* 7.42 Hz, CH x 2 *o*-aromatic), 7.71 (1H, t, *J* 7.42 Hz, CH *p*-aromatic), 7.57 (2H, t, *J* 7.62 Hz, CH x 2 *m*-aromatic), 4.49 (2H, s, CH<sub>2</sub>O), 3.46 - 3.48 (2H, m, CH<sub>2</sub>N), 3.02 (2H, t, *J* 12.1 Hz, CH<sub>2</sub>N), 2.28 (2H, d, *J* 14.5 Hz, CH<sub>2</sub>), 1.92 - 2.02 (2H, m, CH<sub>2</sub>); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): δ<sub>c</sub> 165.1 (C=O), 134.0 (C aromatic), 129.5 (CH x 2 aromatic), 129.0 (CH x 2 aromatic), 128.9 (CH aromatic), 120.3 (CN), 67.2 (CH<sub>2</sub>O), 40.3 (2 CH<sub>2</sub>N), 39.0 (C, overlap with DMSO-*d*<sub>6</sub>), 36.7 (CH<sub>2</sub>), 27.8 (CH<sub>2</sub>). This molecule was used in the following reaction without purification.

To a solution of (4-cyanopiperidin-4-yl)methyl benzoate (**23**) (0.10 g, 0.41 mmol) in dry CH<sub>3</sub>CN under argon, *N*-(chloromethyl)benzamide (**12**) (57 mg, 0.41 mmol) and anhydrous K<sub>2</sub>CO<sub>3</sub> (0.17 g, 1.23 mmol) were added and the mixture was refluxed at 85 °C for 12 hours. The reaction mixture was cooled, filtered through Celite, concentrated, and purified by column chromatography on silica gel using a mixture of dichloromethane and

methanol (30:1) as an eluent to give {4-cyano-1-[2-oxo-2-(phenylamino)ethyl]piperidin-4-yl}methyl benzoate (**24**), 0.15 g (97% yield).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta_{\text{H}}$  8.9 - 8.8 (1H, br. s., NH), 8.04 - 8.12 (2H, m, CH x 2 aromatic), 7.58 - 7.64 (1H, m, CH aromatic), 7.52 - 7.57 (2H, m, 2H, CH x 2 aromatic), 7.44 - 7.51 (2H, m, CH x 2 aromatic), 7.28 - 7.36 (2H, m, 2H, CH x 2 aromatic), 7.08 - 7.14 (1H, m, CH aromatic), 4.40 (2H, s,  $\text{CH}_2\text{O}$ ), 3.21 (2H, s,  $\text{CH}_2\text{N}$ ), 2.98 (2H, d,  $J$  12.5 Hz,  $\text{CH}_2\text{N}$ ), 2.69 (2H, dt,  $J$  2.3, 12.3 Hz,  $\text{CH}_2\text{N}$ ), 2.13 (2H, d,  $J$  13.3 Hz,  $\text{CH}_2$ ), 1.77 - 1.86 (2H, m,  $\text{CH}_2$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta_{\text{C}}$  167.8 (C=O), 165.9 (C=O), 137.4 (C aromatic), 133.8 (CH aromatic), 129.9 (CH x 2 aromatic), 129.1 (CH x 2 aromatic), 128.7 (CH x 2 aromatic), 124.5 (CH aromatic), 124.4 (C aromatic), 120.5 ( $\text{C}\equiv\text{N}$ ), 119.6 (CH x 2 aromatic), 68.3 ( $\text{CH}_2\text{O}$ ), 62.0 ( $\text{CH}_2\text{N}$ ), 50.3 (2  $\text{CH}_2\text{N}$ ), 37.7 (C), 32.2 ( $\text{CH}_2$ ), 27.1 ( $\text{CH}_2$ ); MS (ESI):  $m/z$  378.5 ( $\text{M}+\text{H}$ ) $^+$  (100%), 257.3.

To a solution of {4-cyano-1-[2-oxo-2-(phenylamino)ethyl]piperidin-4-yl}methyl benzoate (**24**) (0.16 g, 0.42 mmol) in 5 mL of methanol under argon,  $\text{CoCl}_2\cdot 6\text{H}_2\text{O}$  (51 mg 0.21 mmol) was added and stirred for 5 min. The reaction mixture was cooled to 0 °C and sodium borohydride (86 mg, 2.27 mmol) was added in several portions over 30 min. The resulting solution was stirred for 18 hours at 25 °C, diluted with 15 mL of cold saturated aqueous  $\text{NH}_4\text{Cl}$  solution and 15 mL of water, and extracted three times with ethyl acetate. The combined organic layers were washed with brine (10 mL), dried (anhydrous  $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated to yield 0.13 g (78% yield) of {4-(aminomethyl)-1-[2-oxo-2-(phenylamino)ethyl]piperidin-4-yl}methyl benzoate (**25**), as a white solid. MS (ESI)  $m/z$  = 382.2 ( $\text{M}+\text{H}$ ) $^+$  (100%). This material was used in the following reaction without purification.

To a solution of {4-(aminomethyl)-1-[2-oxo-2-(phenylamino)ethyl]piperidin-4-yl}methyl benzoate (**25**) (50 mg, 0.13 mmol) in dichloromethane (5 mL) under argon, dry pyridine (19  $\mu\text{L}$ , 0.24 mmol) was added and the mixture was stirred at 0 °C. To it, benzoyl chloride (19  $\mu\text{L}$ , 0.16 mmol) was added and the mixture was stirred for 3 hours at room temperature, partitioned between dichloromethane and water, and basified to pH 8 using aqueous  $\text{NaHCO}_3$ . The organic layer was separated, and the aqueous layer was extracted three times with dichloromethane. The combined organic layers were washed with brine (10 mL), dried (anhydrous  $\text{Na}_2\text{SO}_4$ ), filtered, concentrated, and purified by column chromatography on silica gel using a mixture of dichloromethane and methanol (30:1) as an eluent to obtain 48 mg (75% yield) of {4-(benzamidomethyl)-1-[2-oxo-2-(phenylamino)ethyl]piperidin-4-yl}methyl benzoate (**9**), as a white solid (as a mixture of *cis*- and *trans*-isomers).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta_{\text{H}}$  9.35 - 9.27 (1H, br. s., NH), 8.70 - 8.65 (1H, br. s., NH), 8.11 (2H, d,  $J$  8.2 Hz, CH x 2 aromatic), 8.07 (2H, d,  $J$  8.2 Hz, CH x 2 aromatic), 7.87 (2H, d,  $J$  8.2 Hz, CH x 2 aromatic), 7.57 - 7.60 (2H, m, CH x 2 aromatic), 7.44 - 7.49 (4H, m, CH x 4 aromatic), 7.33 (2H, t,  $J$  7.62 Hz, CH x 2 aromatic), 7.08 - 7.14 (1H, m, CH aromatic), 4.40 (2H, s,  $\text{CH}_2\text{O}$ ), 3.53 (2H, d,  $J$  5.86 Hz,  $\text{CH}_2\text{N}$ ), 3.26 (2H, s,  $\text{CH}_2\text{N}$ ), 2.84 (2H, dd,  $J$  4.3, 5.9 Hz,  $\text{CH}_2\text{N}$ ), 2.71 - 2.79 (2H, m,  $\text{CH}_2\text{N}$ ), 1.67 - 1.85 (4H, m, 2  $\text{CH}_2$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta_{\text{C}}$  168.1 (C=O), 167.6 (C=O), 167.4 (C=O), 137.5 (C aromatic), 133.7 (CH aromatic), 133.2 (C aromatic), 131.6 (C aromatic), 130.0 (CH aromatic), 129.8 (CH x 2 aromatic), 129.0 (CH x 2 aromatic), 128.7 (CH x 2 aromatic), 128.4 (CH x 2 aromatic), 127.0 (CH x 2 aromatic), 124.2 (CH aromatic), 119.6 (CH x 2 aromatic), 68.4 ( $\text{CH}_2\text{O}$ ), 62.1 ( $\text{CH}_2\text{N}$ ), 49.5 ( $\text{CH}_2\text{N}$ ), 42.1 (C), 37.0 (2  $\text{CH}_2\text{N}$ ), 30.6 (2  $\text{CH}_2$ ); MS (ESI)  $m/z$  = 486.2 ( $\text{M}+\text{H}$ ) $^+$  (100%), 365.4.

***N*-{[4-(Hydroxymethyl)-1-[2-oxo-2-(phenylamino)ethyl]piperidin-4-yl]methyl}benzamide (6).** To a solution of {4-(benzamidomethyl)-1-[2-oxo-2-(phenylamino)ethyl]piperidin-4-yl}methyl benzoate (**9**) (45 mg, 0.09 mmol) in methanol, anhydrous  $\text{K}_2\text{CO}_3$  (130 mg, 0.93 mmol) was added and the mixture was stirred for 2 hours at 25 °C. The reaction mixture was filtered and purified by column chromatography on silica gel using dichloromethane:methanol (30:1) as an eluent to give *N*-{[4-(hydroxymethyl)-1-[2-oxo-2-(phenylamino)ethyl]piperidin-4-yl]methyl}benzamide (**6**), 25 mg (71% yield) as a white solid (as a mixture of *trans*- and *cis*-isomers).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta_{\text{H}}$  9.0 - 8.96 (1H, br. s., NH, major isomer), 8.4 - 8.35 (1H, br. s., NH, minor isomer),

8.15 – 8.10 (2H, m, CH x 2 *o*-aromatic, minor isomer), 7.76 – 7.85 (3H, m, CH x 3 aromatic, minor isomer), 7.51 – 7.62 (3H, m, CH x 3 aromatic, major isomer), 7.42 – 7.50 (2H, m, CH x 2 aromatic, major isomer), 7.34 (3H, t, *J* 8.01 Hz, CH x 3 aromatic, major isomer), 7.13 (2H, t, *J* 7.42 Hz, CH x 2 aromatic major isomer), 3.72 (2H, s, CH<sub>2</sub>O), 3.67 (2H, s, O=CCH<sub>2</sub>N), 3.22 (2H, s, CH<sub>2</sub>NC=O), 3.0 – 2.8 (1H, m, CHN) 2.63 (2H, dt, *J* 2.0, 12.3 Hz, CH<sub>2</sub>N), 2.34 (1H, t, *J* 7.4 Hz, CHN), 2.06 (2H, d, *J* 12.9 Hz, CHN + OH), 1.63 – 1.73 (4H, m, 2 CH<sub>2</sub>); <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ<sub>c</sub> 168.1 (C=O), 168.0 (C=O), 133.2 (C aromatic), 129.8 (CH x 2 aromatic), 129.3 (CH x 2 aromatic), 129.0 (C aromatic) 128.6 (CH aromatic), 127.3 (CH aromatic), 124.7 (CH x 2 aromatic), 119.8 (CH x 2 aromatic), 68.3 (CH<sub>2</sub>O), 62.1 (CH<sub>2</sub>N), 53.0 (CH<sub>2</sub>N), 50.8 (CH<sub>2</sub>N), 40.0 (CH<sub>2</sub>N), 33.8 (C), 31.7 (CH<sub>2</sub>), 28.6 (CH<sub>2</sub>); MS (ESI): *m/z* 382.2 (M+H)<sup>+</sup> (100%).

**{4-[(2-Fluorobenzamido)methyl]-1-[2-oxo-2-(phenylamino)ethyl]piperidin-4-yl)methyl benzoate (10).** To a solution of {4-(aminomethyl)-1-[2-oxo-2-(phenylamino)ethyl]piperidin-4-yl)methyl benzoate (**25**) (50 mg, 0.13 mmol) in dried dichloromethane (5 mL) under argon was added pyridine (19 μL, 0.17 mmol) and stirred at 0 °C for 10 min. To the reaction solution, 2-fluorobenzoyl chloride (27 mg, 0.17 mmol) was added and stirred for 3 hours at room temperature. The reaction mixture was partitioned between dichloromethane and water and basified to pH 8 using aqueous NaHCO<sub>3</sub>. The organic layer was separated, and the aqueous layer was extracted three times with dichloromethane. The combined organic layers were washed with brine (10 mL), dried (anhydrous Na<sub>2</sub>SO<sub>4</sub>), filtered, concentrated, and purified by column chromatography on silica gel using a mixture of dichloromethane and methanol (30:1) as an eluent to obtain 48 mg (69% yield) of 4-(benzamidomethyl)-1-[2-oxo-2-(phenylamino)ethyl]piperidin-4-yl)methyl benzoate (**10**), as a white solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ<sub>H</sub> 9.68-9.55 (1H, br. s., NH), 7.97 – 8.04 (2H, m, CH x 2 aromatic), 7.83 (2H, d, *J* 7.42 Hz, CH x 2 aromatic), 7.60 (2H, d, *J* 7.8 Hz, CH x 2 aromatic), 7.52 (2H, d, *J* 6.6 Hz, CH x 2 aromatic), 7.41 – 7.47 (2H, m, CH x 2 aromatic), 7.29 – 7.36 (2H, m, CH x 2 aromatic), 7.12 – 7.17 (2H, m, CH x 2 aromatic), 6.10 (1H, br. s., NH), 4.41 (2H, s, CH<sub>2</sub>O), 3.62 (2H, d, *J* 5.9 Hz, CH<sub>2</sub>N), 3.48 (2H, s, CH<sub>2</sub>N), 2.92 – 3.08 (4H, m, 2 CH<sub>2</sub>N), 1.85 (4H, m, 2 CH<sub>2</sub>); <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ<sub>c</sub> 167.9 (C=O), 167.8 (C=O), 166.6 (C=O), 164.4 (d, *J*<sub>C-F</sub> 160 Hz, CF aromatic), 137.6 (C aromatic), 135.2 (C aromatic), 134.6 (C aromatic), 132.6 (CH aromatic), 132.3 (CH aromatic), 131.7 (CH aromatic), 128.9 (CH x 2 aromatic), 128.6 (CH x 2 aromatic), 127.0 (CH x 2 aromatic), 124.4 (CH aromatic), 124.0 (CH aromatic), 119.7 (CH x 2 aromatic), 117.0 (CH aromatic), 69.2 (CH<sub>2</sub>O), 61.5 (CH<sub>2</sub>N), 49.2 (CH<sub>2</sub>N), 42.3 (C), 36.5 (2 CH<sub>2</sub>N), 29.7 (2 CH<sub>2</sub>); MS (ESI): *m/z* 504.2 (M+H)<sup>+</sup> (100%).

**2-Fluoro-*N*-{[4-(hydroxymethyl)-1-[2-oxo-2-(phenylamino)ethyl]piperidin-4-yl)methyl}benzamide (7).** To a solution of {4-(benzamidomethyl)-1-[2-oxo-2-(phenylamino)ethyl]piperidin-4-yl)methyl benzoate (**10**) (30 mg, 0.06 mmol) in methanol under argon, anhydrous K<sub>2</sub>CO<sub>3</sub> (83 mg, 0.6 mmol) was added and the mixture was stirred for 2 hours at 25 °C. The reaction mixture was filtered and purified by column chromatography on silica gel using a mixture of dichloromethane and methanol (30:1) as an eluent to give *N*-{[4-(hydroxymethyl)-1-[2-oxo-2-(phenylamino)ethyl]piperidin-4-yl)methyl}benzamide (**7**), 13 mg (55% yield) as a white solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ<sub>H</sub> 9.20 – 9.07 (1H, br. s., NH), 7.78 (1H, d, *J* 7.81 Hz, CH aromatic), 7.56 (2H, d, *J* 7.81 Hz, CH x 2 aromatic), 7.41 – 7.48 (2H, m, CH x 2 aromatic), 7.33 (2H, t, *J* 7.81 Hz, CH x 2 aromatic), 7.08 – 7.22 (1H, m, CH aromatic), 6.78 (1H, d, *J* 5.47 Hz, CH aromatic), 4.1 – 3.7 (1H, br. s., OH), 3.49 (2H, d, *J* 6.3 Hz, CH<sub>2</sub>O), 3.46 (2H, s, CH<sub>2</sub>N), 3.14 (2H, s, CH<sub>2</sub>N), 2.61 (4H, dd, *J* 7.0, 11.3 Hz, 2 CH<sub>2</sub>N), 1.60-1.68 (4H, m, 2 CH<sub>2</sub>); <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ<sub>c</sub> 169.1 (C=O), 168.5 (C=O), 135.6 (d, *J*<sub>C-F</sub> 380 Hz, CF aromatic), 131.9 (C aromatic), 131.8 (CH aromatic), 129.0 (CH x 2 aromatic), 128.7 (CH x 2 aromatic), 127.0 (CH x 2 aromatic), 124.3 (C aromatic), 119.5 (CH x 2 aromatic), 65.8 (CH<sub>2</sub>O), 62.3 (CH<sub>2</sub>N), 49.7 (2 CH<sub>2</sub>N), 37.0 (CH<sub>2</sub>N), 30.9 (CH<sub>2</sub>); MS (ESI) *m/z* = 400.0 (M+H)<sup>+</sup> (100%).

***N*-({1-[2-(*tert*-Butylamino)-2-oxoethyl]piperidin-4-yl}methyl)benzamide (8).** A mixture of 4-cyanopiperidine (**11**) (1.0 g, 9.1 mmol), *N*-*tert*-butyl-2-chloroacetamide (**26**) (1.35 g, 9.1 mmol), and anhydrous potassium carbonate (2.5 g, 18.2 mmol) in acetonitrile (30 mL) was heated to reflux under argon for 9 hours. The resulting mixture was cooled to room temperature and filtered to remove inorganic salts. The filtrate was concentrated and crystallized from diethyl ether to give *N*-*tert*-butyl-2-(4-cyanopiperidin-1-yl)acetamide (**27**) (1.82 g, 90% yield) as a white solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ<sub>H</sub> 2.89 (2H, s, CH<sub>2</sub>N), 2.68 - 2.78 (2H, m, CH<sub>2</sub>N), 2.67 - 2.55 (1H, m, CH), 2.31 - 2.44 (2H, m, CH<sub>2</sub>N), 1.93-2.03 (2H, m, CH<sub>2</sub>), 1.81-1.93 (2H, m, CH<sub>2</sub>), 1.35 (9H, s, 3 CH<sub>3</sub>); <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ<sub>C</sub> 168.9 (C=O), 121.5 (CN), 62.5 (CH<sub>2</sub>N), 51.8 (C-N), 50.6 (2 CH<sub>2</sub>N), 29.1 (2 CH<sub>2</sub>), 28.9 (3 CH<sub>3</sub>), 25.7 (CH); MS (ESI): *m/z* 262.4 (M+K)<sup>+</sup> (100%), 246.3 (M+Na)<sup>+</sup> (85%), 224.2 (M+H)<sup>+</sup>.

To a cold (0 °C) solution of *N*-*tert*-butyl-2-(4-cyanopiperidin-1-yl)acetamide (**27**) (0.7 g, 3.1 mmol) in methanol (20 mL) under argon, cobalt (II) chloride hexahydrate (0.37 g, 1.6 mmol) was added followed by the addition of sodium borohydride (0.53 g, 14.1 mmol). After stirring for 15 hours at 25 °C, the reaction solution was diluted with 25 mL of 5% aqueous ammonium hydroxide and extracted three times with dichloromethane. The combined organic layer was washed with brine, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and concentrated to give 2-[(4-(aminomethyl)piperidin-1-yl]-*N*-*tert*-butylacetamide (**28**) (0.65 g, 90% yield) as a crude product, which was used in the next step without further purification. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ<sub>H</sub> 7.12 - 7.05 (1H, br. s., NHC=O), 2.86 (2H, s, O=CCH<sub>2</sub>N), 2.81 (2H, br. s., CH<sub>2</sub>N), 2.61 (2H, d, *J* 6.3 Hz, CH<sub>2</sub>N), 2.12 (2H, t, *J* 11.1 Hz, CH<sub>2</sub>N), 1.74 (2H, d, *J* 11.7 Hz, CH<sub>2</sub>), 1.35 (9H, s, 3 CH<sub>3</sub>), 1.17 - 1.25 (3H, m, CH + CH<sub>2</sub>) ; MS (ESI): *m/z* 228.3 (M+H)<sup>+</sup> (100%).

To a cold (0 °C) solution of 0.20 g (0.88 mmol) of 2-(4-(aminomethyl)piperidin-1-yl)-*N*-(*tert*-butyl)acetamide (**28**) in freshly distilled dichloromethane (2 mL) under argon, pyridine (1.3 mmol) was added followed by the addition of 0.12 g (0.88 mmol) of benzoyl chloride. The reaction was stirred at 0 °C for 30 min. and 25 °C for 2 hours. It was diluted with 20 mL of aqueous sodium bicarbonate and extracted three times with dichloromethane. The combined organic layer was washed with brine, dried (anhydrous Na<sub>2</sub>SO<sub>4</sub>), concentrated, and column chromatographed on silica gel using a mixture of dichloromethane and methanol (10:1) as an eluent to give *N*-({1-[2-(*tert*-butylamino)-2-oxoethyl]piperidin-4-yl}methyl)benzamide (**8**) (0.15 g, 51% yield) as a white solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ<sub>H</sub> 7.77 (2H, d, *J* 7.4 Hz, CH x 2 *o*-aromatic), 7.49 (1H, t, *J* 7 Hz, CH *p*-aromatic), 7.45 (2H, t, *J* 7 Hz, CH x 2 *m*-aromatic), 7.1 (1H, s, NH), 6.38 (1H, br. s., NH), 3.37 (2H, t, *J* 6.4 Hz, CH<sub>2</sub>N), 2.79 - 2.90 (6H, m, 3 CH<sub>2</sub>N), 2.12 (2H, t, *J* 11.3 Hz, CH<sub>2</sub>), 1.77 (2H, d, *J* 12.5 Hz, CH<sub>2</sub>), 1.55 - 1.67 (1H, m, CH), 1.36 (9H, s, 3 CH<sub>3</sub>); <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ<sub>C</sub> 169.5 (C=O), 167.6 (C=O), 134.6 (C aromatic), 131.4 (CH aromatic), 128.5 (CH x 2 aromatic), 126.8 (CH x 2 aromatic), 62.5 (CH<sub>2</sub>N), 53.7 (CH<sub>2</sub>N), 50.4 (C-N), 45.3 (2 CH<sub>2</sub>N), 35.9 (CH), 30.3 (2 CH<sub>2</sub>), 28.7 (3 CH<sub>3</sub>); MS (ESI): *m/z* 354 (M+Na)<sup>+</sup> (100%), 331.9 (M+H)<sup>+</sup> (70%).

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## Supplementary Material

Biological evaluation of the synthesized molecules is reported, and proton and carbon-13 NMR and mass spectra of compounds **1** – **10** and their synthetic intermediates are presented as supporting information in Supplementary Material. Readers will be able to access this supporting information using the link "Supplementary Material" in the journal issue contents page.

## References

1. Brini, M.; Carafoli, E. *Cell. Mol. Life Sci. CMLS* **2000**, *57*, 354.  
<https://doi:10.1007/PL00000698>
2. Kramer, I. M. Chapter 6, Intracellular Calcium, in *Signal Transduction*, 3rd Edn.; Kramer, I. M., Ed. Academic Press: Boston, 2016; pp 381-439.
3. Barrow, J. C.; Duffy, J. L. in *Annual reports in medicinal chemistry*, Elsevier: 2010; Vol. 45, pp 2-18.  
[https://doi.org/10.1016/S0065-7743\(10\)45001-0](https://doi.org/10.1016/S0065-7743(10)45001-0)
4. Giordanetto, F.; Knerr, L.; Wällberg, A. *Expert Opin. Ther. Patents* **2011**, *21*, 85.  
<https://doi:10.1517/13543776.2011.536532>
5. Huguenard, J. R. *Trends in Neurosci.* **1998**, *21*, 451.  
[https://doi.org/10.1016/S0166-2236\(98\)01331-9](https://doi.org/10.1016/S0166-2236(98)01331-9)
6. Catterall, W. A. *Annu. Rev. Cell Dev. Biol.* **2000**, *16*, 521.  
<https://doi.org/10.1146/annurev.cellbio.16.1.521>
7. Cheong, E.; Shin, H.-S. *Physiol. Rev.* **2013**, *93*, 961.  
<https://doi:10.1152/physrev.00010.2012>
8. Tyson, J. R.; Snutch, T. P. *Wiley Interdisciplinary Rev.: Membr. Transp. Signaling* **2013**, *2*, 181.  
<https://doi.org/10.1002/wmts.91>
9. Striessnig, J.; Pinggera, A.; Kaur, G.; Bock, G.; Tuluc, P. *Wiley Interdisciplinary Rev.: Membr. Transp. Signaling* **2014**, *3*, 15.  
<https://doi:10.1002/wmts.102>
10. Bean, B. P. *Am. J. Hypertension* **1991**, *4*, 406S.  
<https://doi.org/10.1093/ajh/4.7.406S>
11. Cao, Y.-Q.; Tsien, R. W. *J. Neurosci.* **2010**, *30*, 4536.  
<https://doi:10.1523/JNEUROSCI.5161-09.2010>
12. Iftinca, M. *J. Med. Life* **2011**, *4*, 126.  
<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3124264>
13. Talley, E. M.; Cribbs, L. L.; Lee, J. -H.; Daud, A.; Perez-Reyes, E.; Bayliss, D. A. *J. Neurosci* **1999**, *19*, 1895.  
<https://doi.org/10.1523/JNEUROSCI.19-06-01895.1999>
14. Watanabe, M.; Ueda, T.; Shibata, Y.; Kumamoto, N.; Shimada, S.; Ugawa, S. *PloS One* **2015**, *10*, e0127572.  
<https://doi:10.1371/journal.pone.0127572>
15. Iftinca, M. C.; Zamponi, G. W. *Trends Pharmacol. Sci.* **2009**, *30*, 32.  
<https://doi:10.1016/j.tips.2008.10.004>
16. Perez-Reyes, E. *Physiol. Rev.* **2003**, *83*, 117.  
<https://doi:10.1152/physrev.00018.2002>



17. Dogrul, A.; Gardell, L. R.; Ossipov, M. H.; Tulunay, F. C.; Lai, J.; Porreca, F. *Pain* **2003**, *105*, 159.  
[https://doi.org/10.1016/S0304-3959\(03\)00177-5](https://doi.org/10.1016/S0304-3959(03)00177-5)
18. Todorovic, S. M.; Jevtovic-Todorovic, V. *Br. J. Pharmacol.* **2011**, *163*, 484.  
<https://doi.org/10.1111/j.1476-5381.2011.01256.x>
19. Cain, S. M.; Snutch, T. P. *Channels* **2010**, *4*, 475.  
<https://doi.org/10.4161/chan.4.6.14106>
20. Todorovic, S. M.; Jevtovic-Todorovic, V. *Channels* **2007**, *1*, 238.  
<https://doi.org/10.4161/chan.4953>
21. Powell, K. L.; Cain, S. M.; Snutch, T. P.; O'Brien, T. J. *Br. J. Clin. Pharmacol.* **2014**, *77*, 729.  
<https://doi.org/10.1111/bcp.12205>
22. Marchettini, P.; Lacerenza, M.; Mauri, E.; Marangoni, C. *Curr. Neuropharmacol.* **2006**, *4*, 175.  
<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2430688>
23. Marchettini, P.; Formaglio, F.; Lacerenza, M. *Neurol. Sci.* **2006**, *27*, s294.  
<https://doi.org/10.1007/s10072-006-0643-6>
24. Colloca, L.; Ludman, T.; Bouhassira, D.; Baron, R.; Dickenson, A. H.; Yarnitsky, D.; Freeman, R.; Truini, A.; Attal, N.; Finnerup, N. B. *Nat. Rev. Dis. Primers* **2017**, *3*, 17002.  
<https://doi.org/10.1038/nrdp.2017.2>
25. Jagodic, M. M.; Pathirathna, S.; Joksovic, P. M.; Lee, W.; Nelson, M. T.; Naik, A. K.; Su, P.; Jevtovic-Todorovic, V.; Todorovic, S. M. *J. Neurophysiol.* **2008**, *99*, 3151.  
<https://doi.org/10.1152/jn.01031.2007>
26. Obradovic, A. L.; Hwang, S. M.; Scarpa, J.; Hong, S. J.; Todorovic, S. M.; Jevtovic-Todorovic, V. *PloS One* **2014**, *9*, e91467.  
<https://doi.org/10.1371/journal.pone.0091467>
27. Chen, W.; Chi, Y.-N.; Kang, X.-J.; Liu, Q.-Y.; Zhang, H.-L.; Li, Z.-H.; Zhao, Z.-F.; Yang, Y.; Su, L.; Cai, J. *Front. Mol. Neurosci.* **2018**, *11*, 24.  
<https://doi.org/10.3389/fnmol.2018.00024>
28. Choe, W.; Messinger, R. B.; Leach, E.; Eckle, V.-S.; Obradovic, A.; Salajegheh, R.; Jevtovic-Todorovic, V.; Todorovic, S. M. *Mol. Pharmacol.* **2011**, *80*, 900.  
<https://doi.org/10.1124/mol.111.073205>
29. Bourinet, E.; Alloui, A.; Monteil, A.; Barrere, C.; Couette, B.; Poirot, O.; Pages, A.; McRory, J.; Snutch, T. P.; Eschalier, A. *EMBO J.* **2005**, *24*, 315.  
<https://doi.org/10.1038/sj.emboj.7600515>
30. Choi, S.; Na, H.; Kim, J.; Lee, J.; Lee, S.; Kim, D.; Park, J.; Chen, C. C.; Campbell, K.; Shin, H. S. *Genes Brain Behav.* **2007**, *6*, 425.  
<https://doi.org/10.1111/j.1601-183X.2006.00268.x>
31. Lamothe, S. M.; Guo, J.; Li, W.; Yang, T.; Zhang, S. *J. Biol. Chem.* **2016**, *291*, 20387.  
<https://doi.org/10.1074/jbc.M116.743138>
32. Casillas-Espinosa, P. M.; Hicks, A.; Jeffreys, A.; Snutch, T. P.; O'Brien, T. J.; Powell, K. L. *PloS One* **2015**, *10*, e0130012.  
<https://doi.org/10.1371/journal.pone.0130012>
33. Yang, Z.-Q.; Barrow, J. C.; Shipe, W. D.; Schlegel, K.-A. S.; Shu, Y.; Yang, F. V.; Lindsley, C. W.; Rittle, K. E.; Bock, M. G.; Hartman, G. D. *J. Med. Chem.* **2008**, *51*, 6471.  
<https://doi.org/10.1021/jm800830n>

34. Yang, S. –M.; Martinez, N. J.; Yasgar, A.; Danchik, C.; Johansson, C.; Wang, Y.; Baljinnyam, B.; Wang, A. Q.; Xu, X.; Shah, P.; Cheff, D.; Wang, X. S.; Roth, J.; Lal-Nag. M.; Dunford, J. E.; Oppermann, U.; Vasiliou, V.; Simeonov, A.; Jadhav, A.; Maloney, D. J. *J. Med. Chem.* **2018**, *61*, 4883.  
<https://doi:10.1021/acs.jmedchem.8b00270>