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# Synthesis and biological evaluation of 2-(arylethynyl)quinoline derivatives as mGluR5 antagonists for the treatment of neuropathic pain

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ABSTRACT

We described here the synthesis and biological evaluation of mGluR5 antagonists containing a quinoline ring structure. Using intracellular calcium mobilization assay (FDSS assay), we identified compound **5n**, showing high inhibitory activity against mGluR5. In addition, it was found that compound **5n** has excellent stability profile. Finally, this compound exhibited favorable analgesic effects in spinal nerve ligation model of neuropathic pain, which is comparable to gabapentin.



Glutamate, the major excitatory neurotransmitter in the mammalian central nervous system, is involved in a wide range of physiological functions acting through various glutamatergic transmission.[1] At the synaptic level, its transmission of neuronal signals is mediated by two main families of glutamate receptors: ionotropic receptors (iGluRs) and metabotropic glutamate receptors (mGluRs). The iGluRs, known as ligand-gated ion channels, are classified into three different receptors on the basis of the interaction with their specific ligands, that is, *N*-methyl-D-aspartic acid (NMDA), kainate, and (*S*)-2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid (AMPA). In general, they are responsible for fast excitatory synaptic transmission and plasticity. On the other hand, the mGluRs are members of the G-protein-coupled receptors (GPCRs) and divided into three groups according to their sequence homology, receptor pharmacology, and signal transduction pathways.[2] Currently, eight distinct mGluR subtypes are discovered. Group I consists of mGluR1 and mGluR5, which are mainly distributed in postsynaptic regions and positively coupled to phospholipase C via a Gq protein, whereas group II (mGluR2 and mGluR3) and group III (mGluR4, mGluR6, mGluR7, and mGluR8) are located in presynaptic regions and inhibit activated adenylate cyclase activity by coupling to a Gi protein.[3] mGluR5 is highly expressed in brain regions and its modulation represents a potential therapeutic approach for treatment of a wide range of CNS-related disorders including pain,[4] anxiety,[5] Parkinson's disease[6] and drug dependency.[7]

There has been a huge study to indentify selective mGluR5 modulators suitable for clinical use (Fig. 1). For example, MPEP, 2-methyl-6-(phenylethynyl)-pyridine[8] was first reported as a potent, selective mGluR5 NAM (negative allosteric modulator) showing high efficacies in various preclinical disease models, such as pain, anxiety, and depression although it did not proceed to clinical trials due to low pharmacokinetic profile. In addition, different kinds of mGluR5 NAMs such as fenobam,[9] ADX10059,[10] and AFQ056[11] have been discovered and known to be effective for Fragile X syndrome, gastro-esophageal reflux disease (GERD), and migraine. Many of mGluR5 antagonists contain an alkyne subunit as a key structural component. Much effort has focused on synthesis of compounds bearing acetylene itself or its isostere.[12] Despite the substantial activity of the reported acetylenic analogues, however, the identification of selective and clinically efficacious mGluR5 antagonists still remains a challenge.

In this context, we report the synthesis and biological evaluation of novel acetylenic quinoline derivatives as selective mGluR5

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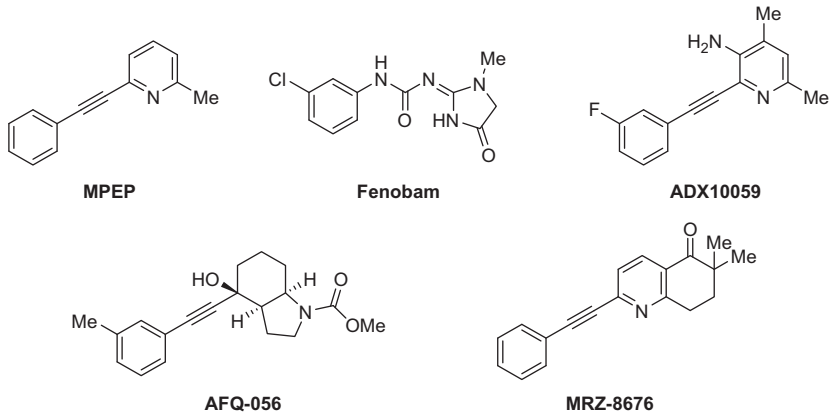


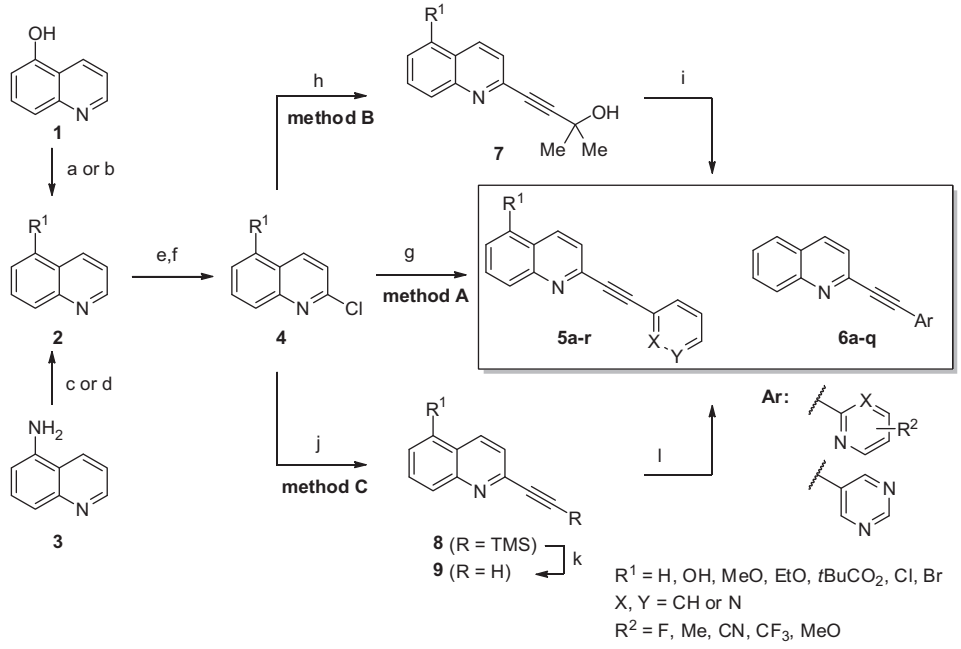

**Figure 1.** Representative mGluR5 antagonists.

antagonists including in vivo study of a lead compound in animal models of neuropathic pain.

Considering the previously reported mGluR5 NAMs, we first designed 2-(arylethynyl)quinoline as a potential starting scaffold. We reasoned that a quinoline group can act as a potential isostere of heteoaromatic groups existing in most acetylene-based mGluR5 NAMs such as MPEP, MTEP, and AFQ056. It has been reported that MRZ-8676[13] having a hydroquinolinone scaffold (Fig. 1) showed a significant negative modulation against mGluR5 with an $IC_{50}$ value of 20 nM, which also supports our idea that the quinoline structure may be a suitable scaffold for an alternative pharmacophore. Moreover, we thought that introduction of various substituents to the quinoline ring system provides a good strategy to lead optimization through potency improvement or physicochemical modulation.

The synthesis of 2-(arylethynyl)quinoline derivatives is depicted in Scheme 1. Starting from 5-substituted quinolines **2**, prepared from alkylation/acylation of 5-hydroxyquinoline **1** or Sandmeyer reaction of 5-aminoquinoline **3**,[14] 2-chloroquinoline derivatives **4** having different substituents at the 5-postion were synthesized via *N*-oxidation followed by chlorination. Installation of arylethynyl groups was conducted in three different ways. Sonogashira reaction of **4** with arylethyne[15] directly afforded the 5-substituted 2-(arylethynyl)quinolines **5** (method A).[16] Alternatively, the 2-arylethynyl components were introduced by a stepwise procedure. Thus, palladium-catalyzed coupling reaction of 2-chloroquinolines **4** with 2-methylbut-3-yn-2-ol or ethynyltrimethylsilane gave the C-terminal protected ethynyl derivatives **7** and **8**. Compound **7** was subjected to another Sonogashira reaction in the presence of potassium hydroxide to give its corresponding 2-ethynylquinolines **5** or **6** through in situ deprotection of 2-propanol group (method B).[17] Removal of trimethylsilyl group of **8** and subsequent Sonogashira reaction produced the desired 2-(arylethynyl)quinolines **6** containing pyridine or pyrimidine rings (method C).

In vitro activities of our synthesized compounds against mGluR5 were determined by the ability of the compound to inhibit



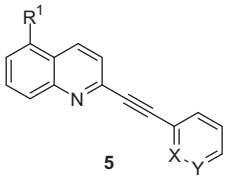

**Scheme 1.** Reagents and conditions: (a) $TMSCHN_2$, MeOH, rt, 42% or $K_2CO_3$, EtI, DMF, 89%; (b) PvCl, pyridine, $CH_2Cl_2$, rt, 84%; (c) CuCl, aq HCl, $NaNO_2$, 0 °C to rt, 68%; (d) CuBr, $NaNO_2$, 48% HBr, 0 °C to rt, 35%; (e) *m*CPBA, DCM, 0 °C to rt; (f) $POCl_3$, $CH_2Cl_2$, reflux, 32–47% (2 steps); (g) arylacetylene, $PdCl_2(PPh_3)_2$, CuI, $Et_3N$, 80 °C, 9–100%; (h) 2-methylbut-3-yn-2-ol, $PdCl_2(PPh_3)_2$, CuI, $Et_3N$, 80 °C, 52–100%; (i) aryl bromide, KOH, $PdCl_2(PPh_3)_2$, CuI, $iPr_2NH$, 95 °C, 8–35%; (j) TMS–acetylene, $PdCl_2(PPh_3)_2$, CuI, $Et_3N$, 60 °C, 93%; (k) $K_2CO_3$, $CH_2Cl_2$/MeOH, rt, 85%; (l) 2-bromopyrimidine or 5-bromopyrimidine, $PdCl_2(PPh_3)_2$, CuI, $Et_3N$, 80 °C, 11–32%

calcium mobilization caused by a high concentration of glutamate in human mGluR5/HEK293 cells using FDSS6000 system.[18] First, we investigated the effect of substituents at the 5-position of quinolines (Table 1). 2-Phenylethynyl or 2-(pyridine-3-ylethynyl)quinolines having hydrogen and alkoxy groups at the 5-position (**5a**, **b**, **g–i**, **n**) gave good inhibitory activities with higher than 60% and 30% inhibition values at the concentration of 10 and 1 μM, respectively. On the other hand, most 2-(pyridine-2-yl)quinoline derivatives maintain the high negative modulation of mGluR5 except R = OH. This result indicated that hydrogen bonding acceptors at the 5-postion of quinoline moiety as well as pyridine-2-yl group on the opposite side are crucial to interact with the mGluR5 receptor.

Based on the SAR of the first series, we then examined a second set of compounds, which derived from compound **5n**, to study the influence of substituted pyridine-2-yl groups on the potency (Table 2). In general, inhibitory activities of most compounds in this series against the mGluR5 were not superior or comparable to that of **5n** at 10 μM. Best results were obtained with small substituents such as nitrile, fluorine, and methyl group at the 4- and 5-positions (**6b**, **c**, **e**, **f**, **h**, **i**). Although we found that the compound **6e** was the most potent derivative of this set at 10 μM, its potency at 1 μM was two-fold lower than that of **5n**. In addition, replacement of the pyridin-2-yl group with pyrimidin-2-yl or 5-yl group (**6n–q**) exhibited low potency regardless of a substituent character, which also supports that the position of nitrogen atom in the pyridine fragment of this series is closely associated with the binding site of the mGluR5 receptor.

Next, we selected several compounds with over 60% (10 μM) and 50% (1 μM) inhibition in the FDSS assay and examined their $IC_{50}$ values, hERG inhibition, microsomal stability, and CYP inhibition. The results are summarized in Table 3. In fact, most of the compounds showed good $IC_{50}$ values against the mGluR5 and low activity against the hERG channel blockade whereas only the compound **5n** displayed excellent pharmacological properties in terms of microsomal stability and CYP inhibition.

**Table 1**
In vitro inhibitory activity of the synthesized compounds against mGluR5

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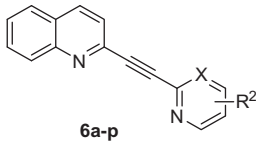
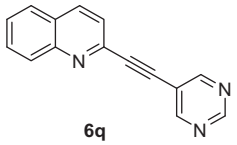
| Compounds | $R^1$ | X | Y | % Inhibition (mGluR5)[a] | | Method[b] |
|---|---|---|---|---|---|---|
| | | | | 10 μM | 1 μM | |
| **5a** | H | CH | CH | 67.71 | 35.88 | A |
| **5b** | MeO | CH | CH | 68.03 | 63.58 | A |
| **5c** | $t$BuCO$_2$ | CH | CH | 23.77 | 24.03 | A |
| **5d** | OH | CH | CH | 24.32 | 18.01 | A |
| **5e** | Cl | CH | CH | 8.02 | 11.72 | A |
| **5f** | Br | CH | CH | 20.67 | 21.09 | A |
| **5g** | H | CH | N | 65.78 | 43.93 | A |
| **5h** | MeO | CH | N | 67.72 | 67.68 | A |
| **5i** | EtO | CH | N | 66.63 | 47.16 | A |
| **5j** | $t$BuCO$_2$ | CH | N | 28.5 | 10.56 | A |
| **5k** | OH | CH | N | 17.58 | 25.22 | A |
| **5l** | Cl | CH | N | 28.35 | 11.16 | A |
| **5m** | Br | CH | N | 40.07 | 22.02 | A |
| **5n** | H | N | CH | 76.66 | 58.81 | A |
| **5o** | $t$BuCO$_2$ | N | CH | 62.44 | 17.12 | A |
| **5p** | OH | N | CH | 22.15 | 17.36 | A |
| **5q** | Cl | N | CH | 64.31 | 31.02 | B |
| **5r** | Br | N | CH | 77.52 | 56.73 | B |

[a] $Ca^{2+}$ flux assay using glutamate as agonist.
[b] See Scheme 1 for synthetic methods.

**Table 2**
In vitro inhibitory activity of the synthesized compounds against mGluR5




6a-p




6q

| Compounds | $R^2$ | X | % Inhibition (mGluR5)[a] | | Method[b] |
|---|---|---|---|---|---|
| | | | 10 μM | 1 μM | |
| **6a** | 3-$CF_3$ | CH | 16.43 | 9.49 | C |
| **6b** | 3-F | CH | 55.24 | 31.31 | B |
| **6c** | 3-Me | CH | 40.09 | 18.85 | B |
| **6d** | 4-$CF_3$ | CH | 58.04 | 23.26 | C |
| **6e** | 4-CN | CH | 76.09 | 35.22 | C |
| **6f** | 4-Me | CH | 66.48 | 21.89 | B |
| **6g** | 5-$CF_3$ | CH | 50.66 | 12.99 | C |
| **6h** | 5-F | CH | 63.29 | 33.87 | C |
| **6i** | 5-Me | CH | 66.27 | 29.00 | B |
| **6j** | 6-$CF_3$ | CH | 28.66 | 15.77 | C |
| **6k** | 6-CN | CH | 64.82 | 32.96 | C |
| **6l** | 6-F | CH | 46.90 | 29.59 | B |
| **6m** | 6-MeO | CH | 48.62 | 16.60 | C |
| **6n** | H | N | 28.63 | 17.08 | C |
| **6o** | 4-$CF_3$ | N | 15.87 | 17.34 | C |
| **6p** | 5-F | N | 15.59 | 15.96 | C |
| **6q** | | | 22.45 | 13.60 | C |

[a] $Ca^{2+}$ flux assay using glutamate as agonist.
[b] See Scheme 1 for synthetic methods.

The pharmacokinetic parameters for compound **5n** following intravenous and oral administration in rats are presented in Table 4. Despite of high stability in human liver microsomes and CYP enzymes, compound **5n** showed high clearance and low bioavailability. The considerably high value of the mean volume of distribution suggests that it tends to bind to tissue components or plasma proteins. The brain to plasma ratios in intravenous and oral administration were low and moderate, which might be due to nonspecific binding in brain or high clearance.

Given the good in vitro potency and stability along with moderate pharmacokinetic profile in rats, the in vivo efficacy of compound **5n** was evaluated in the SNL (spinal nerve ligation) neuropathic pain model (Fig. 2).[19] In this model, a neuropathic pain state was induced by tight ligation of the L5 spinal nerve at a site distal to the DRG. Two behavioral tests (mechanical allodynia and cold allodynia) were performed after 14 days of surgical manipulation.[20] The rats were treated orally with 100 mg/kg of compound **5n** or gabapentin (a positive control). While **5n** exhibited high suppression effect on mechanical allodynia at 5 h, high paw withdrawal response was observed in cold allodynia at 3 h. This result showed that the efficacy of compound **5n** is comparable to that of Gabapentin in this behavior test. Therefore, it should be further investigated as a viable mGluR5 antagonist for treatment of neuropathic pain.

In conclusion, we have synthesized and evaluated 2-(arylethynyl)quinolines **5** and **6** as potential mGluR5 antagonists. The SAR study of acetylenic quinoline derivatives on the mGluR5 receptor led to the identification of **5n**, a compound showing high inhibitory activity against mGluR5 and excellent stability profile. Despite relatively low oral bioavailability, **5n** was evaluated in animal model of neuropathic pain and it significantly reduced both mechanical allodynia and cold allodynia by oral administration, which is comparable to gabapentin. Based on the in vitro and in vivo data

**Table 3**
The results of $IC_{50}$ (mGluR5 and hERG channel), microsomal stability, and CYP inhibition

| Compounds | mGluR5 $IC_{50}$[a] (μM) | hERG $IC_{50}$[a] (μM) | HLM% remaining @ 1 μM after 30 min | CYP (% remaining @ 10 μM) | | |
|---|---|---|---|---|---|---|
| | | | | CYP2D6 | CYP2C9 | CYP3A4 |
| **5b** | 0.41 ± 0.07 | 61.70 ± 11.20 | <1 | >99 | 55 | 50 |
| **5h** | 0.43 ± 0.02 | 65.30 ± 17.70 | <1 | 86 | >99 | 29 |
| **5n** | 0.94 ± 0.25 | 20.30 ± 6.11 | 88 | 98 | >99 | 77 |
| **5r** | 0.75 ± 0.17 | 22.70 ± 7.04 | 98 | 78 | 52 | 56 |

[a] $IC_{50}$ value(±SD) was obtained from a dose–response curve.

**Table 4**
Mean pharmacokinetic parameters in rat plasma following intravenous ($n$ = 4) and oral ($n$ = 3) administration (10 mg/kg) of **5n**

| | Intravenous | Oral |
|---|---|---|
| $C_{max}$ (μg/mL) | — | 0.43(±0.18) |
| $T_{max}$ (min) | — | 10 (5–30)[a] |
| $T_{1/2}$ (min) | 58.24 (±62.41) | 48.53 (±8.06) |
| CL (ml/min/kg) | 84.93 ± 8.98 | |
| $V_{dss}$ (mL/kg) | 1269.02 (±158.55) | — |
| B/P ratio at 2 h | 0.08 | 0.35 |
| $F$ (%) | — | 15.9% |

Values are presented as mean (standard deviation in parentheses). $C_{max}$, peak plasma concentration; $T_{max}$, time to reach $C_{max}$; $V_{dss}$, apparent volume of distribution at steady state; $F$, bioavailability.
[a] Median (range) for $T_{max}$.

generated to date, mGluR5 antagonists such as **5n** will be further optimized as a potential lead compound for the treatment of neuropathic pain.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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.12.056.

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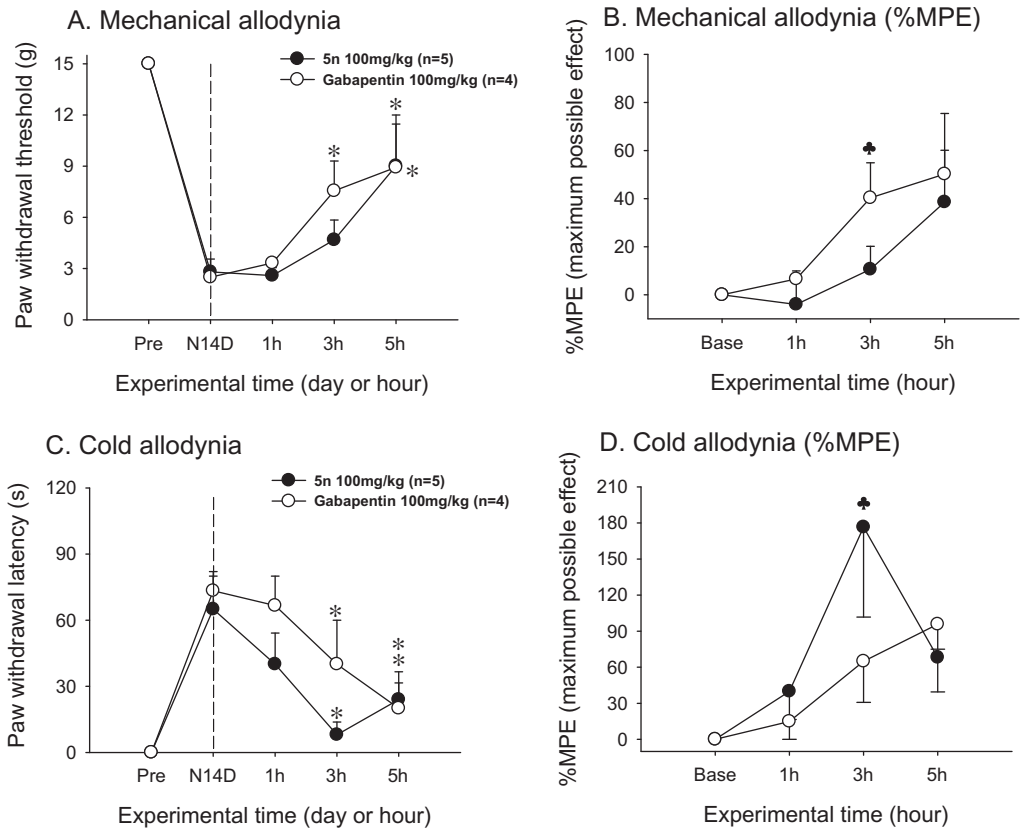


**Figure 2.** Effect on mechanical allodynia (A and B) and cold allodynia (C and D) after oral administration of gabapentin (○, 100 mg/kg, $n$ = 4) and **5n** (●, 100 mg/kg, $n$ = 5) to neuropathic pain-induced rats. Experimental time expressed as D for days after neuropathic injury (N) and h for hours after gabapentin or **5n** administration, $^{*}P$ <0.05 (gabapentin), $^{\#}P$ <0.05 (**5n**) versus pre-administration value (paired $t$-test), $^{\clubsuit}P$ <0.05 gabapentin versus **5n** (unpaired $t$-test).

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16. *Experimental procedure for synthesis of compound* ***5n***. To a solution of 2-chloroquinoline (871 mg, 5.26 mmol) in THF (1.5 mL) was added $PdCl_2(PPh_3)_2$ (183 mg, 0.26 mmol), CuI (110 mg, 0.58 mmol). The reaction mixture was stirred for 5 min and triethylamine (14.4 mL) and 2-ethynylpyridine (1.05 mL, 10.4 mmol) were added. After the resulting mixture was stirred at 100 °C for 48 h, it was allowed to cool to room temperature and filtered through a pad of Celite by the aid of EtOAc. The filtrate was treated with water and extracted with EtOAc (3 × 100 mL). The organic layer was washed with water and brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The crude oil was purified by column chromatography on silica gel (EtOAc/hexane = 1:1) to give 2-(pyridine-2-ylethynyl)quinoline **5n** (448 mg, 37%) as a green solid; mp 91–95 °C; $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 7.31–7.34 (m, 1H), 7.59 (dd, $J$ = 7.8, 7.2 Hz, 1H), 7.70–7.79 (m, 4H), 7.84 (d, $J$ = 8.4 Hz, 1H), 8.17 (t, $J$ = 8.7 Hz, 2H), 8.69 (d, $J$ = 4.8 Hz, 1H); $^{13}$C NMR ($CDCl_3$, 75 MHz) $\delta$ 88.3, 88.4, 123.5, 124.6, 127.4, 127.5, 127.6, 128.0, 129.4, 130.2, 136.3, 136.3, 142.5, 142.8, 148.2, 150.3; GC/MS (EI): $m/z$ 230M$^+$; HRMS ($m/z$): [M+H$^+$] calcd for $C_{16}H_{11}N_2$ 231.0922, Found 231.0929.
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18. *Experimental procedure for in vitro assay*. Human embryonic kidney cells which stably express mGluR5 were obtained from Yonsei University. Cells were grown in DMEM medium supplemented with 10% (v/v) fetal bovine serum, penicillin (100 U/mL), streptomycin (100 μg/mL), and puromycin (10 μg/mL) at 37 °C in a humid atmosphere of 5% $CO_2$ and 95% air. For calcium assay, cells were harvested and dispensed into 96-well black wall clear bottom plates at a density of 40,000 cells per a well. After 18 h of incubation, cells were treated with Calcium-5 assay reagent, which is prepared by manufacture's instruction (Molecular Devices Corporation, California). During fluorescence-based FDSS6000 assay, mGluR5 was activated using a high concentration of L-glutamate (10 μM) in HBSS, and various concentrations of synthesized compounds were treated to cells 75 s before mGluR activation. All data were collected and analyzed using FDSS6000 and related software (Hamamatsu, Japan).
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20. *Experimental procedure for in vivo assay*. Two behavioral tests (mechanical allodynia and cold allodynia) were conducted for rats at 1 day prior to surgery and 14 days after surgery. After the postoperative behavioral test, the animals were treated orally with 50 mg/kg compound **5n** or gabapentin. The tests were re-evaluated at 1 h, 3 h, and 5 h after administration. *Mechanical allodynia:* Testing was performed according to methods described in the literature (Chaplan, S. R.; Bach, F. W.; Pogrel, J. W.; Chung, J. M.; Yaksh, T. L. *J. Neurosci. Methods* **1994**, *53*, 55). Rats were acclimated in a transparent plastic boxes permitting freedom of movement with a wire mesh floor to allow access to the planter surface of the hind paws. A von Frey filament (Stoelting, Wood Dale, IL) was applied 5 times (once every 3–4 s) to hind paw. Von Frey filaments were used to assess the 50% mechanical threshold for paw withdrawal. The 50% withdrawal threshold was determined by using the up-down method and formula given by Dixon (Dixon, W. J. *Ann. Rev. Pharmacol. Toxicol.* **1980**, *20*, 441): 50% threshold = 10 ($X + kd$)/10$^4$, where $X$ is the value of the final von Frey hair used (in log units), $k$ is the tabular value for the pattern of positive/negative responses modified from the same literature, and $d$ is the mean difference between stimuli in log units (0.17). *Cold allodynia:* To quantify cold sensitivity of the paw, brisk paw withdrawal in response to acetone application was measured as reported previously (Choi, Y.; Yoon, Y. W.; Na, H. S.; Kim, S. H.; Chung, J. M. *Pain* **1994**, *59*, 369). The rat was placed under a transparent plastic box on a metal mesh floor and acetone was applied to the plantar surface of the hind paw. To do this, an acetone bubble was formed at the end of a small piece of polyethylene tubing which was connected to a syringe. The bubble was then gently touched to the heel. The acetone quickly spread over the proximal half of the plantar surface of the hind paw. The acetone was applied 5 times to hind paw at 2 min interval. The frequency of paw withdrawal was expressed as a percentage [(no. of trials accompanied by brisk foot withdrawal/total no. of trials) × l00]. *Data analysis:* The results of behavioral tests are expressed as a %MPE. For example, paw withdrawal thresholds were converted to %MPE by the following formula, by using a cutoff of 15 g (the threshold for normal rats) to define maximum possible effect: (post drug threshold − baseline threshold)/(cutoff − baseline threshold) × 100.%MPE values near 100 indicate normal mechanical thresholds (i.e., at or near 15 g), whereas values near 0 indicate allodynia. The result of cold allodynia was also expressed as %MPE.