Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 14 (2004) 3379–3384
Bioorganic & Medicinal Chemistry Letters

3,4-Dihydroquinazoline derivatives as novel selective T-type Ca^{2+} channel blockers

Yong Sup Lee,[a] Bum Hoon Lee,[a,b] Seong Jun Park,[a,b] Soon Bang Kang,[a] Hyewhon Rhim,[a] Jin-Yong Park,[c] Jung-Ha Lee,[c] Seong-Woo Jeong[d] and Jae Yeol Lee[e,*]

[a]*Life Sciences Division, Korea Institute of Science & Technology, PO Box 131, Cheongryang, Seoul 130-650, South Korea*
[b]*Department of Chemistry, Korea University, 1-Anamdong, Seoul 136-701, South Korea*
[c]*Department of Life Science, Sogang University, Shinsu-1-Dong, Seoul 121-742, South Korea*
[d]*Department of Physiology and Institute of Basic Medical Science, Yonsei University, Wonju College of Medicine, Ilsan-Dong 162, Wonju, Kangwon-Do 220-701, South Korea*
[e]*Research Institute for Basic Sciences and Department of Chemistry, College of Sciences, Kyung Hee University, 1 Hoegi- Dong, Seoul 130-701, South Korea*

Received 9 April 2004; revised 27 April 2004; accepted 28 April 2004

Abstract—For LVA T-type Ca^{2+} channel blockers, 3,4-dihydroquinazoline derivatives as new scaffolds were prepared and evaluated for the inhibitory activity against two members of the recombinant T-type Ca^{2+} channel family. Among them, **8a** (KYS05001, $IC_{50} = 0.9\,\mu M$) was nearly equipotent with mibefradil ($IC_{50} = 0.84\,\mu M$) and inhibited LVA T-type Ca^{2+} channel with greater efficacy than HVA Ca^{2+} channel.

Low voltage-activated (LVA) Ca^{2+} channels are strongly associated with the generation of rhythmical firing patterns in the mammalian CNS.[1–3] Furthermore, many reports have suggested that T-type channels are implicated in pathogenesis of epilepsy and neuropathic pain.[4–7] This view is supported by an antiepiletic drug that is known to alleviate the generation of neuronal hyperexcitability in the brain: Ethosuximide (Zarontin®), a succinimide derivative, is an anticonvulsant effective in the treatment of absence epilepsy (Fig. 1).[8,9]

Until recently, three genes encoding T-type Ca^{2+} channel pore-forming subunits were identified and designated $Ca_v3.1$ (α_{1G}), $Ca_v3.2$ (α_{1H}), and $Ca_v3.1$ (α_{1I}).[10–13] However, only limited progress has been made to date in the quest to identify both potent and selective compounds for T-type channel blockade. Only Kurtoxin ($IC_{50} = 15\,nM$) isolated from the scorpion and mibefradil ($IC_{50} = 1\,\mu M$) developed by Roche have been known as most potent T-type channel antagonists (Fig. 1).[14–16] However, Kurtoxin also inhibits the HVA Ca^{2+}

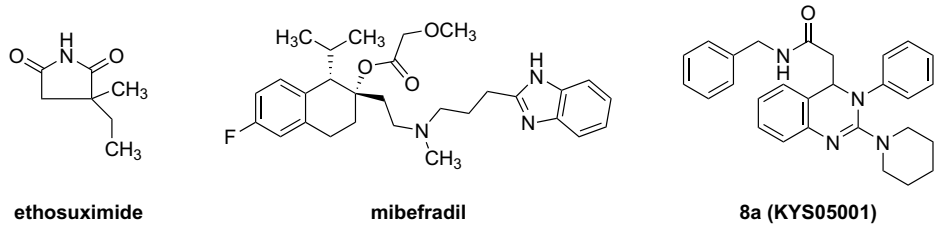


Figure 1. Structures of T-type Ca^{2+} channel blockers.

Keywords: 3,4-Dihydroquinazolines; T-type calcium channel; Blockers; Mibefradil.
* Corresponding author. Tel.: +82-2-9610726; fax: +82-2-9663701; e-mail: ljy@khu.ac.kr

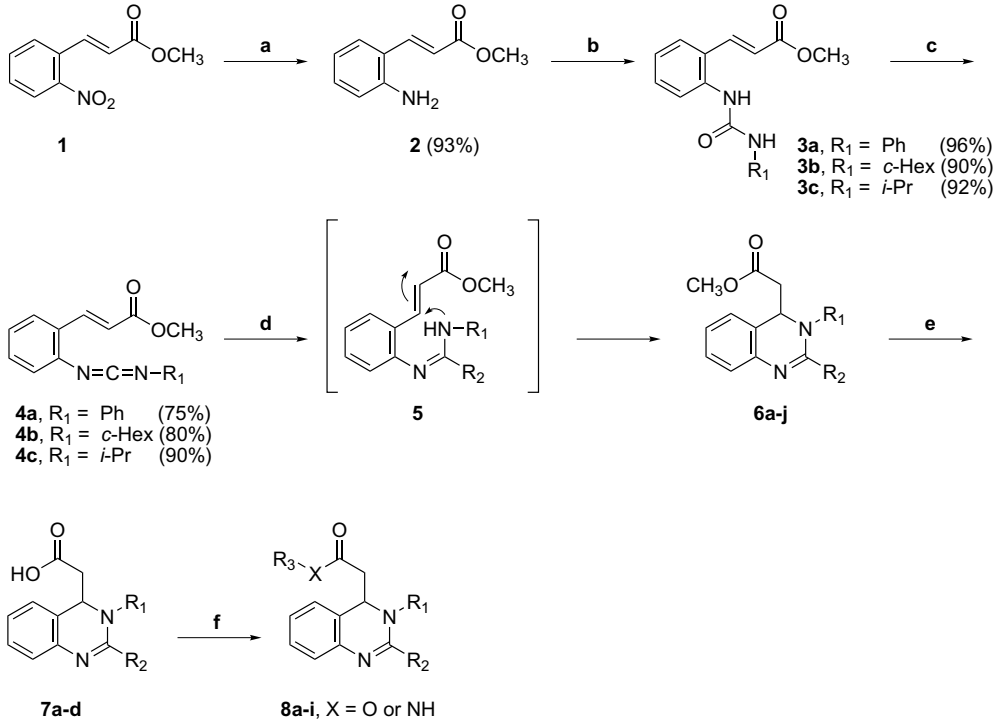
0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.04.090

channels and its peptide nature (63 amino acids) has a problem for the therapeutic agent.[17] In the meanwhile, mibefradil is nonselective as well and regarded as a broad spectrum ion channel blocker with activities on HVA Ca^{2+} channels, Na^+ channels, K^+ channels, and Cl^- channels.[18–21] As a result, mibefradil was withdrawn from U.S. market in May 1988 due to the toxicity from drug–drug interaction. Therefore, new T-type Ca^{2+} channel blockers having new scaffolds are needed in understanding the exact role of T-type Ca^{2+} channel in cellular functions. Herein we describe the synthesis and in vitro T-type Ca^{2+} channel blocking effects of 3,4-dihydroquinazoline derivatives for the development of both potent and selective T-type Ca^{2+} channel blockers (Fig. 1).

The 3,4-dihydroquinazoline derivatives (**6**) were prepared by using a known method, as illustrated in Scheme 1.[22] Thus, carbodiimide (**4**), an intermediate of this reaction, was prepared by the reaction of urea (**3**) with $Ph_3P{\cdot}Br_2$ and Et_3N.[23] The urea (**3**) was prepared in two steps starting from methyl 2-nitrocinnamate (**1**) according to a published procedure.[24] The regioselective addition of secondary amine into the carbodiimide (**4**) followed by intramolecular conjugate addition of the resulting amine species (**5**) to an α,β-unsaturated ester finally afforded the 3,4-dihydroquinazoline derivative (**6**). The methyl ester group of compound **6** was hydrolyzed with LiOH to provide the free carboxylic compound **7** in quantitative yield. The acid compound **7** was coupled with alcohol or amine by using EDC and HOBT to give the respective ester or amide as illustrated in Scheme 1.[25] The structure and yield of each step for all compounds were summarized in Table 1.

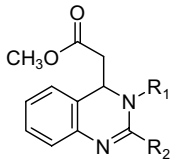
The in vitro calcium channel blocking activities of 3,4-dihydroquinazoline derivatives (**6a–j**, **7a–d**, and **8a–i**) were determined in T-type channels expressed *Xenopus* oocytes (α_{1H}) or HEK293 cells (α_{1G}). As preliminary assays, all synthetic compounds (100 μM) were evaluated for their inhibitory effects on α_{1H} T-type Ca^{2+} channels expressed in *Xenopus* oocytes by a two-electrode voltage clamp method.[26] The compounds shown more than 50% inhibition were re-evaluated for the blocking effects on α_{1G} T-type Ca^{2+} channels expressed in HEK293 cells at 10 μM concentration by whole-cells patch clamp methods.[27] The molar concentrations (IC_{50}) of test compounds required to produce 50% inhibition of α_{1G} T-type currents were determined from fitting raw data into dose–response curves. In vitro blocking effects of all 3,4-dihydroquinazoline derivatives were summarized in Table 1.

Irrespective of the kinds of substituents at R_1 and R_2 position, as a glance, compounds (**6a–j**, **7a–d**, and **8f–i**) possessing methyl or benzyl ester and free carboxylic group at 4-position of quinazoline ring showed poor inhibitory activities (0.0–32.7% inhibition range) against the α_{1H} T-type Ca^{2+} channel with exception of compound **6d** (51.5% inhibition), which has the phenyl rings at R_1 and R_2 position.[28] On the other hand, compounds (**8a–d**) having a benzyl amide substituent always showed good efficacies (63.8–91.9% inhibition range) except for **8e** (14.8%), which was obtained from compound **6d**. Among them, the compound **8d** (91.9%) bearing a piperidine ring at R_2 position and a 4-nitrobenzylamide functional group at 4-position of quinazoline ring was particularly more potent than the reference drug mibefradil (86%). This result implies that both *N*-heterocycles

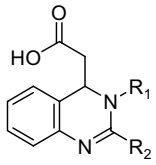


Scheme 1. Reagents and conditions: (a) $SnCl_2{\cdot}2H_2O$, EtOAc, 70 °C, 1 h; (b) R_1NCO, benzene, rt, 12 h; (c) $Ph_3P{\cdot}Br_2$, Et_3N, CH_2Cl_2, 0 °C, 1 h; (d) R_2H or R_2MgBr, THF, rt or reflux; (e) $LiOH{\cdot}H_2O$, THF–H_2O (1:1), 60 °C, 2 h; (f) R_3OH or R_3NH_2, HOBT, EDC, rt, 5 h.

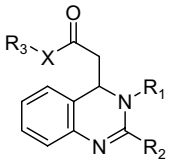
Table 1. In vitro calcium channel blocking effects of 3,4-dihydroquinazolines derivatives



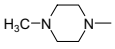
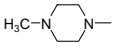
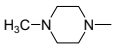
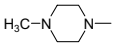
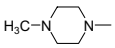
6



7

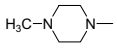


8

Compound[a]	Yield[b] (%)	R_1	R_2	R_3	X	*Xenopus* oocyte (α_{1H})	HEK293 cells (α_{1G})	
						% Inhibition (100 μM)[c]	% Inhibition (10 μM)[c]	IC_{50} (μM)[d]
6a	80					11.5	18.3 ± 6.4	27.70
6b	70					14.0		
6c	75					15.0		
6d[e]						51.5	50.0 ± 3.1	10.70
6e	50					27.0		
6f	75					12.2		
6g	50					3.0		
6h	40					8.0		
6i	70					~0		
6j	50					~0		
7a	~99					8.0		
7b	~99					9.0		
7c	~99					15.0		
7d	~99					7.2		
8a (KYS05001)	60				NH	77.0	90.1 ± 2.3	0.90
8b	78				NH	63.8	40.5 ± 5.5	21.60
8c	50				NH	70.3	29.0 ± 0.8	35.80
8d (KYS05034)	60				NH	91.9	92.3 ± 1.3	2.50
8e	89				NH	14.8		

(*continued on next page*)

Table 1 (*continued*)

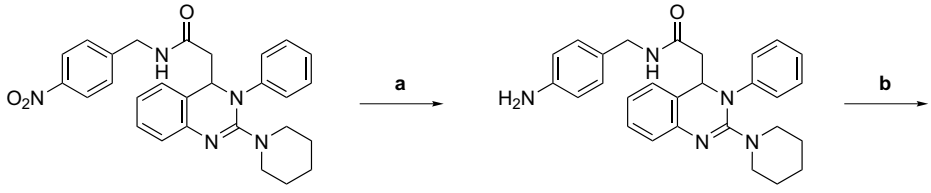
Compound[a]	Yield[b] (%)	R_1	R_2	R_3	X	*Xenopus* oocyte (α_{1H})	HEK293 cells (α_{1G})	
						% Inhibition (100 μM)[c]	% Inhibition (10 μM)[c]	IC_{50} (μM)[d]
8f	74				O	14.2		
8g	86				O	15.6		
8h	88				O	20.9		
8i	70				O	32.7		
Mibefradil						86.0	95.9 ± 1.7	0.84

[a] All compounds were obtained as racemates.
[b] Isolated yield.
[c] % Inhibition value (±SEM) was obtained by repeated procedures ($n \geqslant 3$).
[d] IC_{50} value was determined from the dose–response curve.
[e] Compound obtained from our previous work.[28]

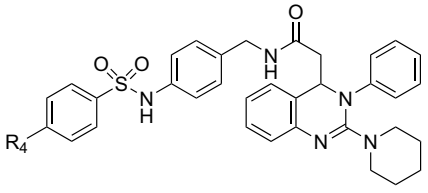
at R_2 position and benzylamide group at 4-position of quinazoline ring would be the essential pharmacophores for calcium entry blocking activity.

Next, the effective compounds (**6a**, **6d**, and **8a**–**d**) were further evaluated in HEK293 cells (α_{1G}) at lower concentration (10 μM). Our experimental results are summarized as follows. First, the inhibitory effects of compounds were quite similar in HEK293 cells (α_{1G}) as *Xenopus* oocyte (α_{1H}) except for the compound **8c**, which displayed surprisingly the decreased magnitude of inhibition (29.0 ± 0.80%) compared with the inhibitory activity on the α_{1H} expressed in *Xenopus* oocytes (70.3%). Secondly, the structure–activity relationships showed that the relative potency order was piperidine (**8a**, **8d**) > morpholine (**8b**) > 4-methylpiperazine (**8c**) with respect to R_2 substituent and the compound **8a** (KYS05001) and **8d** (KYS05034) were nearly equipotent (90.1 ± 2.3, 92.3 ± 1.3% inhibition) comparable with mibefradil (95.9 ± 1.7% inhibition). With respect to the IC_{50} values, however, the compound **8a** (KYS05001, $IC_{50} = 0.9$ μM) was shown to be 2.8-fold more potent than **8d** (KYS05034, $IC_{50} = 2.5$ μM) and nearly equipotent with mibefradil ($IC_{50} = 0.84$ μM).

Based on the SAR data, we embarked on the synthesis of **8d** (KYS05034) analogs in order to increase the potency and the pharmacological profile, as illustrated in Scheme 2. Hydrogenation of the nitro group of compound **8d** using 10% Pd(C) in MeOH afforded *N*-(4-aminobenzylamido)-3,4-dihydroquinazoline (**9**, KYS05040) in 97% yield, which was coupled with the respective phenylsulfonyl chloride in the presence of pyridine to provide the sulfonamido-3,4-dihydroquinazoline derivative **10a**



8d (KYS05034) **9 (KYS05040):** (97%)



10a (KYS05041), $R_4 = CH_3$ (94%)
10b (KYS05042), $R_4 = F$ (73%)

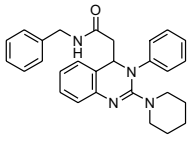
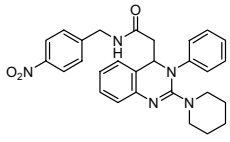
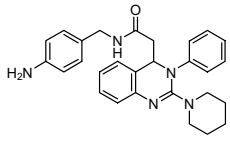
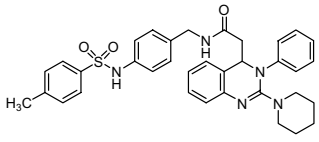
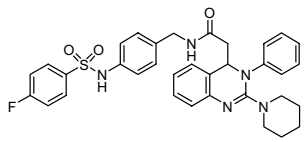
Scheme 2. Reagents and conditions: (a) 10% Pd(C), MeOH, rt, 2 h; (b) *p*-CH_3–$PhSO_2Cl$ (for **10a**), or *p*-F–$PhSO_2Cl$ (for **10b**), pyridine, 0 °C to rt, 24 h.

(KYS05041) and **10b** (KYS05042), respectively, in 94% and 73% yields. Calcium blocking effects of these analogs (**9**, **10a**, and **10b**) were determined using two calcium channel isoforms (α_{1H} and α_{1G}) and also their IC_{50} values for inhibition of HEK293 cells (α_{1G}) were determined from the dose–response curve. In addition, these compounds were screened against Na^+ channels and high voltage-activated (HVA) Ca^{2+} channels of major pelvic ganglion (MPG) neurons for the evaluation of ion channel selectivity including compounds **8a** and **8d**.[29] All inhibitory activity data of these compounds were summarized in Table 2.[30]

Compared with the parent compound **8d**, the current inhibition by the reduced compound **9** was decreased in both *Xenopus* oocytes (66.8% inhibition) and HEK293 cells (43.5 ± 4.5% inhibition). The IC_{50} value (14.4 μM) for the compound **9** was also higher than that of compound **8d** (2.5 μM). In the meanwhile, compound **10a** (KYS05041) and compound **10b** (KYS05042) showed increased potencies, and were 3.4- and 4.2-fold more potent ($IC_{50} = 0.25$ and 0.20 μM, respectively) than mibefradil ($IC_{50} = 0.84$ μM).

As shown in Table 2, all compounds generally showed less inhibitory potencies in major pelvic ganglion (MPG) neurons than *Xenopus* oocytes and HEK293 cells and these results would be caused by the dilution effect connected with their nonspecific binding affinities for the present background of MPG neurons. With respect to calcium channel selectivity, all compounds except **8a** showed similar blocking effects against both LVA T-type and HVA Ca^{2+} channel expressed in MPG neurons at 10 μM concentration, as shown in Table 2. On the other hand, compound **8a** (KYS05001) bearing simple benzylamide exhibited the higher selectivity for LVA T-type over HVA Ca^{2+} channel, although its IC_{50} value (0.9 μM) was similar to that of mibefradil (0.84 μM). More importantly, all compounds (**8a**, **8d**, **9**, **10a**, and **10b**) did not exhibit Na^+ channel blocking effect in MPG neurons (the data were not shown) and also had no cytotoxicities on HEK293 cells at 10 μM concentration as confirmed using MTT assay method (the data were not shown). Therefore, it is likely that compound **8a** (KYS05001) would be regarded as a new potent and selective T-type Ca^{2+} channel blocker based on these biological data.

Table 2. The inhibitory activities and selectivity data for some 3,4-dihydroquinazolines

Compound	Structure	*Xenopus* oocyte (α_{1H})	HEK293 cells (α_{1G})		MPG neuron assay (% inhibition; 10 μM)	
		% Inhibition (100 μM)	% Inhibition (10 μM)[a]	IC_{50} (μM)[b]	LVA (T-type)	HVA (N-type)
8a (KYS05001)		77.0	90.1 ± 2.3	0.90	76.0[c]	12.0[c]
8d (KYS05034)		91.9	92.3 ± 1.3	2.50	37.7	32.2
9 (KYS05040)		66.8	43.5 ± 4.5	14.40	4.7	18.4
10a (KYS05041)		84.7	89.9 ± 1.3	0.25	29.0	38.7
10b (KYS05042)		80.5	89.0 ± 2.3	0.20	49.6	50.8

[a] % Inhibition value (±SEM) was obtained by repeated procedures ($n \geqslant 3$).
[b] IC_{50} value was determined from the dose–response curve.
[c] The values were obtained at 50 μg/mL concentration.

In summary, 3,4-dihydroquinazoline derivatives as novel scaffolds for nonpeptic T-type Ca^{2+} channel blocker were prepared and evaluated for the blocking effects against two isoforms of T-type Ca^{2+} channel subfamily. Among them, compound **8a** (KYS05001) has a great potential as chemical platform for the future development of useful pharmacophores without the side effects resulting from nonselective blocking on HVA Ca^{2+} channels and other types of ion channels.

Acknowledgements

This study was supported by Vision 21 Program from Korea Institute of Science and Technology (2E18000-04-024).

References and notes

1. Llinas, R.; Yarom, Y. *J. Physiol.* **1981**, *315*, 549.
2. Gutnick, M. J.; Yarom, Y. *J. Neurosci. Meth.* **1989**, *28*, 93.
3. Bal, T.; McCormick, D. A. *J. Physiol.* **1993**, *468*, 669.
4. Huguenard, J. R.; Prince, D. A. *J. Neurosci.* **1994**, *14*, 5485.
5. Huguenard, J. R. *Annu. Rev. Physiol.* **1996**, *58*, 329.
6. Tsakiridou, E.; Bertollini, L.; De Curtis, M.; Avanzini, G.; Pape, H. *Neuroscience* **1995**, *15*, 3110.
7. Hogan, Q. H.; McCallum, J. B.; Sarantopoulos, C.; Aason, M.; Mynlieff, M.; Kwok, W. M.; Bosnjak, Z. J. *Pain* **2000**, *86*, 43.
8. Macdonald, R. L.; McLean, M. J. *Adv. Neurol.* **1986**, *44*, 713.
9. Coulter, D. A.; Huguenard, J. R.; Prince, D. A. *Neurosci. Lett.* **1989**, *98*, 74.
10. Cribbs, L. L.; Lee, J.-H.; Yang, J.; Satin, J.; Zhang, Y.; Daud, A.; Barclay, J.; Williamson, M. P.; Fox, M.; Rees, M.; Perez-Reyes, E. *Circ. Res.* **1998**, *83*, 103.
11. Lee, J.-H.; Daud, A. N.; Cribbs, L. L.; Lacerda, A. E.; Pereverzev, A.; Klöckner, U.; Schneider, T.; Perez-Reyes, E. *J. Neurosci.* **1999**, *19*, 1912.
12. Perez-Reyes, E.; Cribbs, L. L.; Daud, A.; Lacerda, A. E.; Barclay, J.; Williamson, M. P.; Fox, M.; Ress, M.; Lee, J.-H. *Nature* **1998**, *391*, 896.
13. Randall, A.; Benham, C. D. *Mol. Cell. Neurosci.* **1999**, *14*, 255.
14. Chuang, R. S.; Jaffe, H.; Cribbs, L.; Perez-Reyes, E.; Swartz, K. J. *Nat. Neurosci.* **1998**, *1*, 668.
15. Clozel, J. P.; Ertel, E. A.; Ertel, S. I. *J. Hypertens.* (Suppl. 15) **1997**, S17.
16. Mishra, S. K.; Hermsmeyer, K. *Circ. Res.* **1994**, *75*, 144.
17. Sidach, S. S.; Mintz, I. M. *J. Neurosci.* **2002**, *22*, 2023.
18. Randall, A. D.; Tsien, R. W. *Neuropharmacology* **1997**, *36*, 879–893.
19. Randall, A. D. *J. Physiol.* **1995**, *485*, 49P.
20. Gomora, J. C.; Enyeart, J. A.; Enyeart, J. *J. Mol. Pharmacol.* **1999**, *56*, 1192.
21. Nilius, B.; Prenen, J.; Kamouchi, M.; Viana, F.; Voets, T.; Droogmans, G. *Br. J. Pharmacol.* **1997**, *121*, 547.
22. (a) Molina, P.; Aller, E.; Lorenzo, A. *Synthesis* **1998**, 283; (b) Saito, T.; Tsuda, K.; Saito, Y. *Tetrahedron Lett.* **1996**, *37*, 209.
23. (a) Gololobov, Y. G.; Kasukhin, L. F. *Tetrahedron* **1992**, *48*, 1353; (b) Larksarp, C.; Alper, H. *J. Org. Chem.* **1998**, *63*, 6229; (c) Palomo, C.; Mestres, R. *Synthesis* **1981**, 373.
24. Wang, F.; Hauske, J. R. *Tetrahedron Lett.* **1997**, *38*, 8651.
25. (a) Gaucher, A.; Zuliani, Y.; Cabaret, D.; Wakselman, M.; Mazaleyrat, J.-P. *Tetrahedron: Asymmetry* **2001**, *12*, 2571; (b) Dhaon, M. K.; Olesen, R. K.; Ramasamy, K. *J. Org. Chem.* **1982**, *47*, 1962.
26. Lee, J.-H.; Gomora, J. C.; Cribbs, L. L.; Perez-Reyes, E. *J. Biophys.* **1999**, *77*, 3034.
27. Monteil, A.; Chemin, J.; Bourinet, E.; Mennessier, G.; Lory, P.; Nargeot, J. *J. Biol. Chem.* **2000**, *275*, 6090.
28. Compound **6d** was obtained from our previous work. See: Lee, B. H.; Lee, J. Y.; Chung, B. Y.; Lee, Y. S. *Heterocycles* **2004**, *63*, 95.
29. Lee, J.-H.; Kim, E.-G.; Park, B. G.; Kim, K. H.; Cha, S. K.; Kong, I. D.; Lee, J. W.; Jeong, S. W. *J. Neurophysiol.* **2002**, *87*, 2844.
30. Spectra data of selected compounds, **8a** (KYS05001): mp 168 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.71 (br, 1H, –CO–N*H*–CH_2–Ph), 7.35–7.31 (m, 2H, aromatic), 7.29–7.19 (m, 5H, aromatic), 7.16–7.03 (m, 5H, aromatic), 6.96–6.92 (m, 2H, aromatic), 5.18 (dd, J = 5.0 and 10.1 Hz, 1H, –CH_2–C*H*–N–), 4.53 (dd, J = 6.0 and 14.4 Hz, 1H, –NH–CH_2–Ph), 4.42 (dd, J = 6.3 and 14.4 Hz, 1H, –NH–CH_2–Ph), 3.17 (br, 4H, piperidinyl), 2.68 (dd, J = 10.1 and 14.0 Hz, 1H, –CO–CH_2–), 2.23 (dd, J = 5.0 and 14.1 Hz, 1H, –CO–CH_2–), 1.37–1.33 (m, 2H, piperidinyl), 1.18 (br, 4H, piperidinyl); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.4, 153.9, 146.1, 143.2, 138.6, 129.3, 128.8, 128.5, 128.4, 127.7, 127.0, 125.2, 124.9, 124.4, 123.2, 122.8, 122.3, 61.1, 47.4, 43.9, 41.9, 25.3, 24.7; HRMS (FAB, M+H) Calcd for $C_{28}H_{31}N_4O$ 439.2498, found 439.2534; **10a** (KYS05041); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.66 (d, J = 8.4 Hz, 1H, aromatic), 7.58–7.73 (m, 3H, aromatic), 7.28–7.21 (m, 3H, aromatic), 7.18–6.95 (m, 12H, aromatic), 5.19 (dd, J = 5.2 and 10.1 Hz, 1H, –CH_2–C*H*–N–), 4.35 (dd, J = 6.1 and 14.2 Hz, 1H, –NH–CH_2–), 4.24 (dd, J = 5.5 and 14.8 Hz, 1H, –NH–CH_2–), 3.28 (br, 4H, piperidinyl), 2.82 (dd, J = 10.5 and 14.4 Hz, 1H, –CO–CH_2), 2.36 (dd, J = 5.2 and 14.0 Hz, 1H, –CO–CH_2–), 2.29 (s, 3H, –SO_2–C_4H_4–C*H*$_3$), 1.33 (br, 2H, piperidinyl), 1.20 (br, 4H, piperidinyl); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.4, 154.0, 144.9, 143.6, 138.9, 136.9, 136.7, 134.7, 129.8, 129.2, 129.0, 128.9, 127.3, 126.2, 125.9, 125,4, 124.4, 124.0, 121.1, 121.0, 61.7, 48.6, 43.2, 41.9, 24.8, 24.2, 21.7; HRMS (FAB, M+H) Calcd for $C_{35}H_{38}N_5O_3S$ 608.2695, found 608.2680; **10b** (KYS05042); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.66–7.62 (m, 2H, aromatic), 7.28–7.23 (m, 2H, aromatic), 7.20–7.06 (m, 9H, aromatic), 7.04–6.90 (m, 4H, aromatic), 6.72 (br, 1H, –CO–N*H*–CH_2–), 5.19 (dd, J = 6.0 and 9.9 Hz, 1H, –CH_2–C*H*–N–), 4.41 (dd, J = 6.1 and 14.8 Hz, –NH–CH_2–), 4.24 (dd, J = 5.5 and 14.8 Hz, –NH–CH_2–), 3.28 (br, 4H, piperidinyl), 2.74 (dd, J = 9.6 Hz and 14.1 Hz, 1H, –CO–CH_2), 2.44 (dd, J = 6.0 and 14.1 Hz, 1H, –CO–CH_2–), 1.39 (br, 2H, piperidinyl), 1.25 (br, 4H, piperidinyl); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.1, 166.7, 153.9, 145.3, 141.7, 135.9, 135.0, 129.9, 129.7, 129.3, 128.9, 128.4, 126.7, 125.2, 124.8, 123.2, 121.5, 116.3, 116.0, 61.4, 47.9, 43.3, 42.0, 25.2, 24.6; HRMS (FAB, M+H) Calcd for $C_{34}H_{35}FN_5O_3S$ 612.2445, found 612.2436.