Synthesis and Biological Activity of Chiral Rho Kinase Inhibitors

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It was found that (S)-6H-1-(5-isoquinolinesulfonyl)-2-hydroxymethyl-1-pyrrolidine and (S)-6H-1-(5-isoquinolinesulfonyl)-2-chloromethyl-1-pyrrolidine displayed excellent Rho inhibitory activity in previous work. Therefore, with these two compounds as lead compounds, we designed and synthesized their enantiomers in order to reveal the effects of chirality on the combination of inhibitor and Rho kinase. Their biological activity evaluation results indicated that the chirality of inhibitors indeed presented important influences on their Rho kinase inhibitory activities. Furthermore, several analogues of the lead compounds were designed and synthesized. It was found that the isostere, (S)-6H-1-(5-isoquinolinesulfonyl)-2-fluorinated-methyl-pyrrolidine (compound 7) exhibited much better Rho kinase inhibitory activity than the lead compounds and strongly promoted synapse formation. Thus, it is believed to be a potential candidate as Rho kinase inhibitors.

Keywords: Rho kinase inhibitors, Fasudil, Biological activity, Synapse formation.

INTRODUCTION

Rho kinase is also termed as Rho-associated coiled-coil forming protein serine kinase (ROCK). Its activation normally leads to diverse diseases, including hypertension¹, erectile dysfunction², coronary and cerebral vasospasm³, glaucoma^{4,5}, atherosclerosis⁶, asthma⁷, multiple sclerosis (MS)⁸, cancer⁹ and stroke¹⁰. Presently, Rho kinases are regarded as attractive drug targets that may provide new therapeutic strategies for the treatment of several conditions. In 2009, Taniguchi *et al.*¹¹ and others¹²⁻¹⁵ demonstrated that the deactivation of Rho-kinase with inhibitors were related to neurite cell outgrowth, neurite cell length and cell regeneration. Specific ROCK inhibitors, such as Y-27632 and dimethylfasudil, have been shown to facilitate axon regeneration after on crush *in vivo*¹⁶. Rho-associated kinase inhibition enhanced neurite outgrowth in Ntera-2 or PC-12 cells *in vitro*¹⁷. It has been reported that the documented neurite outgrowth inhibitors utilize the Rho-ROCK pathway for their inhibitory functions¹⁸. These findings indicate that Rho-ROCK is an appropriate target to promote the synapse formation and improve learning and memory ability.

In previous work, with fasudil as the reference compound¹⁹⁻²¹, we designed and synthesized a group of isoquinoline Rho kinase inhibitors targeting region D of the kinase on the basis of the ligand-binding pocket theory²². Biological assays

revealed that (S)-6H-1-(5-isoquinolinesulfonyl)-2-hydroxymethyl-1-pyrrolidine (compound 1) and (S)-6H-1-(5-isoquinolinesulfonyl)-2-chloromethyl-1-pyrrolidine (compound 2) displayed excellent Rho kinase inhibitory activity and also promoted synapse formation (Fig. 1). Encouraged by this result, the enantiomers of compounds 1 and 2 were synthesized in our laboratory and the influence of chirality on their Rho kinase inhibitory activity was also evaluated. Furthermore, several analogues of compound 1 were synthesized by the replacement of hydroxymethyl with carboxyl group and the substitution of hydroxyl group with fluorine on the pyrrolidine moiety of compound 1. We evaluated their biological activities, including assays of Rho kinase inhibitory activity, synapse formation.

Fig. 1. Chemical structures of compounds 1 and 2

As shown in **Scheme-I**, starting from isoquinoline sulfonyl chloride hydrochloride, compound **3** was prepared by nucleophilic substitution with D-prolinol. Then, chlorination of compound **3** with SOCl_2 afforded compound **4** in 75% yield. Esterification of compound **1** with methylsulfonyl chloride, followed by treatment with tetrabutylammonium fluoride (TMFA), produced the fluorosubstituted compound **7** in 27% yield. Compounds **5**, **6** and **8** were synthesized by a similar procedure, in 44, 44 and 46% yields, respectively.

EXPERIMENTAL

Unless specified otherwise, the raw chemical materials were purchased from Guangfu Technology Development Co., Ltd., Tianjin (China) and Chase Sun Pharmaceutical Co., Ltd., Tianjin (China). The reagents were purchased from commercial suppliers without further purification. Solvents were dried and

purified before use according to standard procedures. The reactions were monitored by thin-layer chromatography (TLC) methods (silica gel GF254s). Flash chromatography was also performed, on 200-300 mesh silica gel. ^1H NMR and ^{13}C NMR spectra were recorded on an INOVA 400/600 Hz spectrometer with TMS as internal standard. HR-MS was recorded on MicroOTOF-Q II.

(R)-6H-1-(5-isoquinolinesulfonyl)-2-hydroxymethylpyrrolidine (3): Isoquinoline sulfonyl chloride hydrochloride (5.00 g, 18.9 mmol) was slowly added to a sodium bicarbonate solution (2.52 g, 30 mmol). The pH of the reaction mixture was kept at 5-6 and stirred for 0.5 h, extracted with 20 mL CH_2Cl_2 . The organic layer was dried over Na_2SO_4 and concentrated. The residue dissolved in 20 mL CH_2Cl_2 was added dropwise to the flask containing a mixture of D-prolinol (2.29 g, 22.6 mmol) and triethylamine (1.91 g, 18.9 mmol). The

Scheme-I: Synthesis of compounds **3-8**. (a) CH_2Cl_2 , 0 °C, 2 h, 95% yield. (b) SOCl_2 , 40 °C, 8 h, 75% yield. (c) CH_2Cl_2 , $\text{CH}_3\text{SO}_2\text{Cl}$, 0 °C, 2 h, 90% yield. (d) THF, $(\text{CH}_3)_4\text{NF}$, 65 °C, 8 h, 27% yield. (e) H_2O , Na_2CO_3 , 0 °C, 2 h, 44% yield

mixture was stirred for 2 h at 0-5 °C, then concentrated. The residue was dissolved in 50 mL ethyl acetate, washed with 20 mL water. The organic phase was dried and concentrated. The crude product was purified by flash column chromatography (ethyl acetate) to give a yellow oil (5.29 g, 95%). ¹H NMR (400 MHz, CDCl₃) δ: 9.42 (s, 1H), 8.74 (s, 2H), 8.49 (d, *J* = 7.3 Hz, 1H), 8.29 (d, *J* = 8.1 Hz, 1H), 7.78 (t, *J* = 7.8 Hz, 1H), 3.97-3.82 (m, 1H), 3.71 (dd, *J* = 11.4, 4.9 Hz, 2H), 3.56-3.32 (m, 2H), 1.99-1.70 (m, 2H), 1.66-1.51 (m, 2H). ¹³C NMR (100 MHz, CDCl₃) δ: 25.27, 29.39, 50.44, 62.70, 65.04, 124.31, 130.13, 131.35, 134.52, 136.42, 136.71, 137.41, 139.97, 149.74.

(R)-6H-1-(5-Isoquinolinesulfonyl)-2-chloromethylpyrrolidine (4): Compound **3** (5 g, 0.02 mol) was added to thionyl chloride (20 mL, 0.27 mol) and the reaction mixture was stirred for 8 h at 40 °C. Thionyl chloride was then distilled. Saturated aqueous solution of sodium bicarbonate was added to make the pH of the solution between 7-8. The residue was dissolved in 60 mL CH₂Cl₂. The crude product was purified by flash column chromatography (ethyl acetate) to yield a yellow oil (3.91 g, 75%). ¹H NMR (400 MHz, CD₃OD) δ: 9.87 (s, 1H), 8.93 (d, *J* = 5.8 Hz, 1H), 8.72 (s, 1H), 8.61 (d, *J* = 7.3 Hz, 2H), 7.92 (t, *J* = 7.4 Hz, 1H), 4.14 (s, 1H), 3.78 (d, *J* = 11.0 Hz, 1H), 3.66-3.50 (m, 1H), 3.40 (d, *J* = 16.5 Hz, 2H), 2.18-1.56 (m, 5H). ¹³C NMR (100 MHz, CD₃OD) δ: 25.12, 30.46, 47.69, 49.47, 50.87, 62.00, 124.20, 130.17, 131.45, 134.51, 136.13, 136.72, 137.68, 140.17, 149.80.

(S)-2-(Isoquinolin-5-ylsulfonyl)cyclopentane carboxylic acid (5): Isoquinoline sulfonyl chloride hydrochloride (2.00 g, 7.60 mmol) was dissolved in 25 mL methanol at 0-5 °C and added dropwise to a mixture of L-proline (1.00g, 8.69 mmol) and sodium carbonate (1.11g, 10.4 mmol). The reaction mixture was stirred at 0-5 °C for 2 h. The methanol was distilled off. The residue was dissolved in 30 mL CH₂Cl₂ and washed with water. The organic phase was dried over Na₂SO₄ and concentrated. The crude product was purified by flash column chromatography (ethyl acetate) to afford a dense yellow oil (0.23 g, 44%). ¹H NMR (600 MHz, CDCl₃) δ: 9.47 (s, 1H), 8.87-8.62 (d, *J* = 9.6 Hz, 2H), 8.67 (s, 1H), 8.55 (d, *J* = 10.8 Hz, 1H), 8.37 (s, 1H), 7.86 (t, *J* = 11.7 Hz, 1H), 4.27 (t, *J* = 11.4 Hz, 1H), 3.5 (m, 1H), 2.47-2.45 (m, 2H), 2.16-2.13 (m, 2H), 2.13-2.09 (m, 2H). ¹³C NMR (150 MHz, CDCl₃) δ: 25.86, 32.25, 53.44, 61.93, 123.94, 130.04, 131.25, 135.25, 136.27, 137.76, 139.81, 149.95, 174.07, 175.26.

(R)-2-(Isoquinolin-5-ylsulfonyl)cyclopentane carboxylic acid (6): Compound **6** was similarly prepared from D-proline. The crude product was purified by flash column chromatography (ethyl acetate) to afford a dense yellow oil (0.23 g, 44%). ¹H NMR (600 MHz, CDCl₃) δ: 9.47 (s, 1H), 8.87-8.82 (d, *J* = 9.6 Hz, 2H), 8.67 (s, 1H), 8.55 (d, *J* = 10.8 Hz, 1H), 8.37 (s, 1H), 7.86 (t, *J* = 11.7 Hz, 1H), 4.27 (t, *J* = 11.4 Hz, 1H), 3.5 (m, 1H), 2.47-2.45 (m, 2H), 2.16-2.13 (m, 2H), 2.13-2.09 (m, 2H). ¹³C NMR (150 MHz, CDCl₃) δ: 25.86, 32.38, 53.39, 61.84, 123.73, 130.04, 131.25, 135.25, 136.17, 137.62, 139.76, 149.92, 174.07, 175.26.

(S)-6H-1-(5-Isoquinolinesulfonyl)-2-fluorinated-methylpyrrolidine (7): Methanesulfonyl chloride (1.71 g, 15 mmol) was added dropwise to the mixture of (S)-6H-1-(5-

isoquinolinesulfonyl)-2-hydroxymethyl-pyrrolidine (3.80 g, 13 mmol) and triethylamine (1.85 g, 18.3 mmol) in 40 mL dichloromethane. The reaction mixture was stirred for 2 h at 0-5 °C. The reaction mixture was washed with 20 mL water and then the organic layer was dried over Na₂SO₄ and concentrated to afford a yellow oil (4.30 g, 90%).

Tetrabutylammonium fluoride (1.88 g, 7.2 mmol) was added to the above obtained yellow oil (4.30 g, 11.6 mmol) dissolved in 30 mL tetrahydrofuran. The reaction mixture was stirred at 65 °C for 8 h, concentrated. The crude product was purified by flash column chromatography (EA/PE, 2:1, v/v) to yield a dense yellow oil (0.92 g, 27%). ¹H NMR (600 MHz, CDCl₃) δ: 10.23 (d, *J* = 10.7 Hz, 1H), 9.25 (d, *J* = 6.8 Hz, 1H), 8.97-8.65 (m, 2H), 8.11 (t, *J* = 7.8 Hz, 1H), 7.28 (s, 1H), 4.34 (m, 1H), 4.20 (m, 1H), 3.75-3.67 (m, 2H), 2.13-2.10 (m, 2H), 1.52-1.03 (t, *J* = 5.4 Hz, 1H). ¹³C NMR (150 MHz, CDCl₃) δ: 25.13, 30.44, 47.73, 50.90, 61.96, 123.95, 130.19, 131.29, 135.09, 135.89, 136.53, 137.63, 139.94, 150.05.

(1S,4R)-4-Hydroxyl-2-(isoquinolin-5-ylsulfonyl)cyclopentane carboxylic acid (8): Compound **8** was similarly prepared from *trans*-4-hydroxy-L-proline in 46 % yield. ¹H NMR (600 MHz, D₂O) δ: 9.36 (s, 1H), 8.78 (s, 1H), 8.76 (s, 1H), 8.58-8.62 (d, *J* = 9.6 Hz, 2H), 7.82 (d, *J* = 11.4 Hz, 1H), 4.27 (t, *J* = 11.4 Hz, 1H), 3.63 (s, 1H), 3.40-3.32 (m, 2H), 2.13-2.11 (m, 2H). ¹³C NMR (150 MHz, D₂O) δ: 26.34, 30.65, 51.97, 71.51, 128.73, 130.04, 133.25, 135.25, 136.17, 137.62, 142.76, 150.92, 176.07, 179.26.

BV-2 Microglia cells culture: Mice BV-2 microglia lines were purchased from American Type Culture Collection (ATCC) and cultured in Dulbecco's modified eagle's medium (DMEM; Gibco). DMEM nutrient solution was prepared with 10 % fetal bovine serum (FBS; Gibco), 100 U/mL penicillin and 100 µg/mL streptomycin (Gibco). BV-2 microglial cells were cultured in a humidified cell incubator at 37 °C with 5 % CO₂. BV-2 microglial cells were treated with fasudil and compounds **1-8** (15 µg/mL) and cultured for 24 h.

Primary neurons culture: Mouse cortex from 16-18 embryonic day old mice was dissected under a microscope and then dissociated in neurobasal medium (Gibco) containing 2 % B27 (Gibco). 100 µg/mL streptomycin and 100 U/mL penicillin (Gibco) were then added to tissue samples. Blood vessels and other fibres were removed by filtration using a 40 µm sterile nylon filter. The cell suspension (1 × 10⁶ cells/cm²) was then plated onto flasks coated with poly-D-lysine (0.1 mg/mL, Sigma). Primary neurons were cultured at 37 °C in a humidified cell incubator under 5% CO₂ for approximately 7-10 days. Primary neurons were cultured at 37 °C in a humidified cell incubator under 5 % CO₂ for approximately 7-10 days. Primary neurons were then cultured for 24 h in the presence of fasudil and compounds **1-8** (15 µg/mL).

ROCK activity assay: ROCK activity was measured using the CycLex Research Product ROCK assay kit (cat. # CY-1160). It has been used to determine ROCK activity in cell extracts and tissue cytosol (CycLex Co., Ltd, Nagano, Japan). BV-2 microglial cells were homogenized on ice in four volumes of an appropriate extraction buffer (pH 8), which included 0.1 % Triton X-100, 50 mM Tris-HCl, 1 mM EGTA, 1 mM EDTA, 10 mM NaF, 0.5 mM PMSF and 10 mM

β -mercapto-ethanol. BV-2 microglial cells were then centrifuged for 0.5 h to collect the supernatant. Protein concentration was determined by a Bicinchoninic acid protein assay kit (Pierce, USA). ROCK activity in the supernatant fraction (100 μ L fraction containing 50 ng protein) was determined following the manufacturer's instructions. Optical density was measured at 490 nm. Experiments were also performed in the presence of fasudil and the synthesized compounds (10 μ g/mL) for 24 h.

Observation of cell synapse: BV-2 microglial cells were cultured as previously described. Cells were treated with fasudil (15 μ g/mL) and the synthesized compounds (15 μ g/mL) for 24 h. Cells were fixed with 4% paraformaldehyde in 0.1 M phosphate-buffered saline at room temperature for 20 min. BV-2 microglial cells were directly observed under a phase-contrast microscope. Primary neurons were stained with anti-mouse monoclonal MAP-2 antibody overnight at 4 °C. The next day, cells were washed with PBS and incubated with fluorescent labelled secondary antibody at room temperature for 2 h. Cells were then sealed with 50 % glycerol and observed under fluorescence microscope.

RESULTS AND DISCUSSION

In our previous work, it was found that (*S*)-6*H*-1-(5-isoquinolinesulfonyl)-2-hydroxymethyl-1- pyrrolidine (**1**) and (*S*)-6*H*-1-(5-isoquinolinesulfonyl)-2-chloromethyl-1- pyrrolidine (**2**) exhibited excellent Rho kinase inhibitory activity, accelerated synapse formation. Based on these findings, with compounds **1** and **2** as lead compounds, compounds **3-8** were designed and synthesized by the method mentioned above (Scheme 1). The synthesized Rho kinase inhibitors were fully characterized by ¹H NMR and ¹³C NMR spectroscopy, infrared spectroscopy and high-resolution mass-spectroscopy. Their biological activities, including Rho kinase inhibitory activity, synapse formation were systematically evaluated by MTT assay and LDH assay.

Rho kinase inhibitory activity: The results of Rho kinase inhibition with fasudil and compounds **1-8** are shown in Fig. 2, compounds **2-4** and **7** presented excellent Rho kinase inhibitory activity. Compound **3** exhibited much better Rho kinase inhibitory activity than its enantiomer (compound **1**) while compound **2** displayed slightly better activity than compound **4**. As seen from the results of chiral Rho kinase inhibitors (**1-4**), chirality of inhibitors may have an obvious influence on their Rho kinase inhibitory activity. Importantly, compound **7**, synthesized by the replacement of chlorine atom in compound **2** with fluorine atom, also demonstrated excellent inhibitory activity. However, compounds **5**, **6** and **8** unfortunately yielded poor inhibitory activities. The hydroxyl groups, chlorines and fluorine available in compounds **1-4** and **7** possibly offer as the binding sites to region D of the Rho kinase, appear to be related with the enhancement of Rho kinase inhibitory activity. Therefore, these results suggested that the hydroxyl group, the chlorine and fluorine atom existed in inhibitors (**1-4**, **7**) might play an important role in Rho kinase inhibitory activity.

Synapse formation in primary neuronal cells and BV-2 microglia cells: As shown in Fig. 3(a), compound **3** more

Fig. 2. ROCK kinase inhibitory activity of BV-2 microglial cells treated with fasudil and compounds **1-8** (10 μ g/mL)

effectively enhanced the synapse formation of primary primary neurons than compound **1** while compound **2** was better than its enantiomer (**4**). Therefore, chiral inhibitors indeed exhibited different activity on the synapse formation of primary neurons. Compound **7** strongly enhanced synapse formation of primary neurons while compounds **5**, **6** and **8** presented poor impact on it. It is interesting to note that compound **7** exhibited greater efficacy than fasudil on the synapse formation.

As shown in Fig. 3(b), the result is consistent with the above results, the enhancement of the synapse formation of BV-2 microglial cells was attributed to the deactivation of Rho kinase. It is worth noted that compound **7** behaved much better than fasudil in synaptic formation of BV-2 microglial cells. The longest synapses were found in the presence of compound **7** while compounds **2** and **3** took second place in the enhancement of synapse formation as shown in Fig. 3(c). This result further demonstrated that the fluorine atom available in compound **7** served as an important binding site with region D of Rho kinase, resulting in the inhibition of Rho kinase, leading to enhance synapse formation. Furthermore, Longer synapses were observed in the presence of compound **3** compared to compound **1** while longer synapses were observed with compound **2** than with compound **4**. This consequence indicated that the chirality of inhibitors indeed play an important role in the inhibition of Rho kinase.

To our best of knowledge, Rho kinase was closely related to synapse formation. Because the activation of Rho kinase exhibited a key role for axonal growth inhibition, the blockade of the Rho-ROCK can promote axonal regeneration²³. Rho kinase inhibitors Y-27632 also enhanced retina ganglion cell axon growth on glial scar tissue²⁴. In a streptozotocin-treated rat model, learning and memory were impaired. However, the administration of fasudil can improved synaptic transmission in the CA1 region of the hippocampus, increased the expression of synaptophysin and improved learning and memory ability²⁵. These findings indicate Rho-ROCK is an appropriate target to promote the synapse formation and improve learning and memory ability. Compound **7** as the novel ROCK inhibitors, showed better synapse formation than fasudil, providing a therapeutic potential against injuries to the human CNS, such as spinal cord injuries, stroke and neurodegenerative disorders.

Fig. 3. Representative images of synaptic formation. (a) primary neurons cultured with fasudil and compounds **1-8** (15 $\mu\text{g/mL}$). (b) BV-2 microglia cells cultured with fasudil and compounds **1-8** (15 $\mu\text{g/mL}$). (c) The Length of synaptic formation in BV-2 microglia cultured with fasudil and compounds **1-8** (15 $\mu\text{g/mL}$). All data represent means $\pm$ standard deviations (error bars) for three separate experiments

Conclusion

Several Rho kinase inhibitors were designed and synthesized and their biological activities were systematically evaluated, including Rho kinase inhibition and synapse formation. The obtained results indicated that the chirality of

inhibitors presented important impact on their Rho kinase inhibitory activity, compound **2** exhibited better biological activity than its enantiomer (**4**) while compound **3** is much more effective than its enantiomer (**1**). Fortunately, compound **7** exhibited much better Rho kinase inhibitory activity than the lead compounds and strongly promoted synapse formation compounds **3** and **7** with a chlorine atom or fluorine atom as an isostere of hydroxyl, might effectively improve Rho kinase inhibitory activity.

REFERENCES

1. J.K. Liao, M. Seto and K. Noma, *J. Cardiovasc. Pharmacol.*, **50**, 17 (2007).
2. I.A. Abdel-Hamid, *Drug Discov. Today*, **10**, 1459 (2005).
3. A. Masumoto, M. Masahiro, H. Shimokawa, L. Urakami, M. Usui and A. Takeshita, *Circulation*, **105**, 1545 (2002).
4. V.P. Rao and D.L. Epstein, *BioDrugs*, **21**, 167 (2007).
5. H. Tokushige, M. Inatani, S. Nemoto, H. Sakaki, K. Katayama, M. Uehata and H. Tanihara, *Invest. Ophthalmol. Vis. Sci.*, **48**, 3216 (2007).
6. I. Cicha, M. Goppelt-Strube, S. Muehlich, A. Yilmaz, D. Raaz, W.G. Daniel and C.D. Garlich, *Atherosclerosis*, **196**, 136 (2008).
7. M. Kobayashi, H. Kume, T. Oguma, Y. Makino, Y. Ito and K. Shimokata, *Clin. Exp. Allergy*, **38**, 135 (2008).
8. B.K. Mueller, H. Mack and N. Teusch, *Nat. Rev. Drug Discov.*, **4**, 387 (2005).
9. L. Yin, K. Morishige, T. Takahashi, K. Hashimoto, S. Ogata, S. Tsutsumi, K. Takata, T. Ohta, J. Kawagoe, K. Takahashi and H. Kurachi, *Mol. Cancer Ther.*, **6**, 1517 (2007).
10. K. Noma, N. Oyama and J.K. Liao, *Am. J. Physiol. Cell Physiol.*, **290**, 661 (2005).
11. J. Taniguchi, S. Sawai, M. Mori, T. Kubo, K. Kanai, S. Misawa, S. Iose, T. Yamashita and S. Kuwabara, *Ann. Neurol.*, **66**, 694 (2009).
12. B.S.P. Takekazu Kubo, B.S.P. Katsuhiko Hata, B.S.P. Atsushi Yamaguchi and B.S.P. Toshihide Yamashita, *Curr. Pharm. Des.*, **13**, 2493 (2007).
13. S. Tang, Y.J. Shen, M.E. DeBellard and G. Mukhopadhyay, *J. Cell. Biol.*, **138**, 1355 (1997).
14. S. Tang, R.W. Woodhall, Y.J. Shen, M.E. deBellard, J.L. Saffell, P. Doherty, F. Walsh and M.T. Filbin, *Mol. Cell. Neurosci.*, **9**, 333 (1997).
15. T. Yamashita, M. Fujitani, S. Yamagishi, K. Hata and F. Mimura, *Mol. Neurobiol.*, **32**, 105 (2005).
16. P. Lingor, N. Teusch, K. Schwarz, R. Mueller, H. Mack, M. Bahr and B.K. Mueller, *J. Neurochem.*, **103**, 181 (2007).
17. Z. Zhang, A.K. Ottens, S.F. Larner, F.H. Kobeissy, M.L. Williams, R.L. Hayes and K.K. Wang, *Cell. Mol. Biol. Lett.*, **11**, 12 (2006).
18. P.P. Monnier, A. Sierra, J.M. Schwab, S. Henke-Fahle and B.K. Mueller, *Mol. Cell. Neurosci.*, **22**, 319 (2003).
19. S. Satoh, T. Utsunomiya, K. Tsurui, T. Kobayashi, I. Ikegaki, Y. Sasaki and T. Asano, *Life Sci.*, **69**, 1441 (2001).
20. M. Iwakubo, A. Takami, Y. Okada, T. Kawata, Y. Tagami, H. Ohashi, M. Sato, T. Sugiyama, K. Fukushima and H. Iijima, *Bioorg. Med. Chem.*, **15**, 350 (2007).
21. T. Asano, I. Ikegaki, S. Satoh, Y. Suzuki, M. Shibuya, M. Takayasu and H. Hidaka, *J. Pharmacol. Exp. Ther.*, **241**, 1033 (1987).
22. M. Iwakubo, A. Takami, Y. Okada, T. Kawata, Y. Tagami, M. Sato, T. Sugiyama, K. Fukushima, S. Taya, M. Amano, K. Kaibuchi and H. Iijima, *Bioorg. Med. Chem.*, **15**, 1022 (2007).
23. B.S.P. Takekazu Kubo, B.S.P. Katsuhiko Hata, B.S.P. Atsushi Yamaguchi and B.S.P. Toshihide Yamashita, *Curr. Pharm. Des.*, **13**, 2493 (2007).
24. P.P. Monnier, A. Sierra, J.M. Schwab, S. Henke-Fahle and B.K. Mueller, *Mol. Cell. Neurosci.*, **22**, 319 (2003).
25. Y. Hou, L. Zhou, Q.D. Yang, X.P. Du, M. Li, M. Yuan and Z.W. Zhou, *Neuroscience*, **200**, 120 (2012).