Bioorganic & Medicinal Chemistry Letters 20 (2010) 5282–5285

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Novel Notch-sparing γ-secretase inhibitors derived from a peroxisome proliferator-activated receptor agonist library

Motonori Kurosumi [a], Yoshino Nishio [a], Satoko Osawa [b], Hisayoshi Kobayashi [c], Takeshi Iwatsubo [b,d,e], Taisuke Tomita [b,d], Hiroyuki Miyachi [a,*]

[a] Division of Pharmaceutical Sciences, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 1-1-1, Tsushima-Naka, Kita-ku, Okayama 700-8530, Japan
[b] Department of Neuropathology and Neuroscience, Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo 113-0033, Japan
[c] Institute of Molecular and Cellular Biosciences, The University of Tokyo, 1-1-1, Yayoi, Bunkyo-ku, Tokyo 113-0032, Japan
[d] Core Research for Evolutional Science and Technology, Japan Science and Technology Corporation, Tokyo 113-0033, Japan
[e] Department of Neuropathology, Graduate School of Medicine, The University of Tokyo, Tokyo 113-0033, Japan

ARTICLE INFO

Article history:
Received 1 May 2010
Revised 24 June 2010
Accepted 28 June 2010
Available online 3 July 2010

Keywords:
γ-Secretase
γ-Secretase inhibitor
Notch-sparing γ-secretase inhibitor
PPAR

ABSTRACT

Screening of our library of peroxisome proliferator-activated receptor (PPAR) agonists yielded several phenylpropanoic acid-derived γ-secretase inhibitors (GSIs). Structure–activity relationship studies indicated that (*R*)-configuration of α-substituted phenylpropanoic acid structure and cinnamic acid structure is favorable to prepare Notch-sparing GSIs.

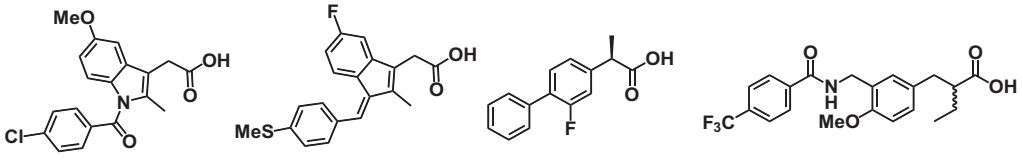
Alzheimer's disease (AD) is a chronic neurodegenerative disorder characterized by memory loss, personality changes and a decline in cognitive abilities, and is predicted to affect 20 million people worldwide within 20 years.[1] AD is pathologically characterized by the accumulation of senile plaques, neurofibrillary tangles, synaptic loss, and neuronal death. Several lines of evidence suggest that extracellular deposition of amyloid-β peptide (Aβ) as senile plaques is involved in the pathogenesis of AD. Aβ is proteolytically derived from amyloid precursor protein (APP) via sequential cleavages by β- and γ-secretases. β-Secretase-cleavage results in production of soluble APPβ and the membrane-bound C-terminal fragment C99. γ-Secretase, which is a membrane protein complex consisting of at least four proteins (i.e., presenilin 1 (PS1), nicastrin, anterior pharynx defective-1 and presenilin enhancer-2,[2]) cleaves C99 within its transmembrane domain to generate Aβ peptides composed of 38-, 40- and 42-amino acids (designated as $Aβ_{38}$, $Aβ_{40}$, $Aβ_{42}$). Among these, $Aβ_{42}$ is a highly amyloidogenic species and is predominantly deposited in AD brains.[3–5] Thus, increased levels of $Aβ_{42}$ in the brain are considered to trigger a neuropathological cascade which ultimately leads to neurodegeneration and dementia.

Development of γ-secretase inhibitors (GSIs) is a promising therapeutic approach for AD.[1] However, the γ-secretase cleaves not only APP, but also several other transmembrane proteins, including Notch, the latter being indispensable for the differentiation and development of a variety of organs and tissues. Thus, most of the reported GSIs cause severe adverse effects, because these compounds are not substrate-specific and inhibit Notch signalling.[6–8] Recently, compounds capable of modulating the γ-secretase activity to alter and/or block Aβ production with little or no effect on Notch cleavage have been identified.[9–12] Such compounds, called Notch-sparing GSIs (NS-GSIs) or γ-secretase modulators (GSMs), would be good candidates for AD therapeutics.

Herein, we report structurally new GSIs with substrate selectivity. We have been engaged in structural development studies of PPAR ligands for the treatment of metabolic syndrome, cancer and so on, based on phenylpropanoic acid structure as a basic framework (Fig. 1).[13] PPAR ligands are constructed from three components, that is, the acidic head group, the hydrophobic tail group, and the linking group connected the acidic head group and the hydrophobic tail group. Previous studies have indicated that a subset of nonsteroidal anti-inflammatory drugs (NSAIDs), such as indomethacin, sulindac sulfide and (*R*)-flurbiprofen, specifically reduce the $Aβ_{42}$ production, acting as NS-GSIs independently of their inhibitory activity for cyclooxygenases.[9] Considering the

* Corresponding author.
E-mail address: miyachi@pharm.okayama-u.ac.jp (H. Miyachi).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.06.131



1 Indomethacin **2** Sulindac Sulfide **3** (*R*)-Flurbiprofen **4**

Figure 1. Structures of the representative γ-secretase modulators **1**–**3**, and our representative PPAR ligand **4**.

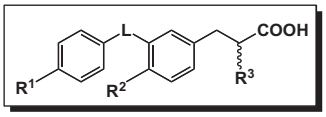
similar structural profile of NSAID-type NS-GSIs, which contain a highly lipophilic component in combination with an acidic functional group, we searched for compounds that inhibit or modulate γ-secretase activity by screening our PPAR agonist library. Cell-based γ-secretase assay was performed using an HEK293 cell line (NLNTK) stably co-overexpressing enhanced green fluorescent protein (EGFP), Swedish mutant APP, truncated Notch (NotchΔE) and Notch-responsive CSL-luciferase reporter.[14] Using this cell line, we are able to analyze simultaneously the effect of compounds on Aβ secretion and Notch signaling. The activities were expressed as relative percentage inhibitory activity compared to that of 10 μM DAPT (*N*-[*N*-(3,5-difluorophenylacetyl)-L-alanyl]-*S*-phenylglycine *t*-butyl ester), an authentic potent GSI.[15] In each set of experiments, the assay was performed in duplicate or triplicate.

Our initial screening results are summarized in Table 1. The $CONHCH_2$ linker compound **4**,[16] which exhibits potent dual PPARα/δ agonistic activity with EC_{50} values in the low nanomolar range, did not exhibit any apparent effect on APP, while it exhibited NOTCH cleavage-inhibitory activity at the concentration of 1 μM. At 100 μM, Aβ secretion was increased by twofold. Notably, a concomitant increase of Notch signaling was observed, suggesting that this compound differentially modulated the γ-secretase activity in a substrate-specific manner. This differential modulation was affected by the shape of the substituent at the α-position of the carboxyl group (**5**, **6**) and the steric bulkiness of the 4-position substituent on the distal benzene ring of the molecule (**7**, **8**). In contrast, compound **9**,[17] a weaker PPAR agonist than **4** with a reversed CH_2NHCO linker, exhibited inhibition of Aβ secretion as well as Notch signaling at both assay concentrations. The potency of **9** seems comparable to that of sulindac sulfide (IC_{50} values of sulindac sulfide for $Aβ_{40}$, $Aβ_{42}$ and Notch are 215 μM, 82.5 μM and 53.0 μM respectively). These results indicated the importance of the shape and/or nature of the linking group for inhibition of the Aβ-generating γ-secretase activity.

The central methoxy group could be replaced with a fluorine atom. Compound **10** exhibited similar activity to **9**, but with a reduced Notch-inhibitory activity. The steric hindrance of the distal benzene substituent R^1 might be important for substrate specificity. Increasing steric bulkiness decreased the inhibitory activity for Aβ secretion (**10** > **11** > **12**), but increased that for Notch cleavage (**12** > **11** > **10**).

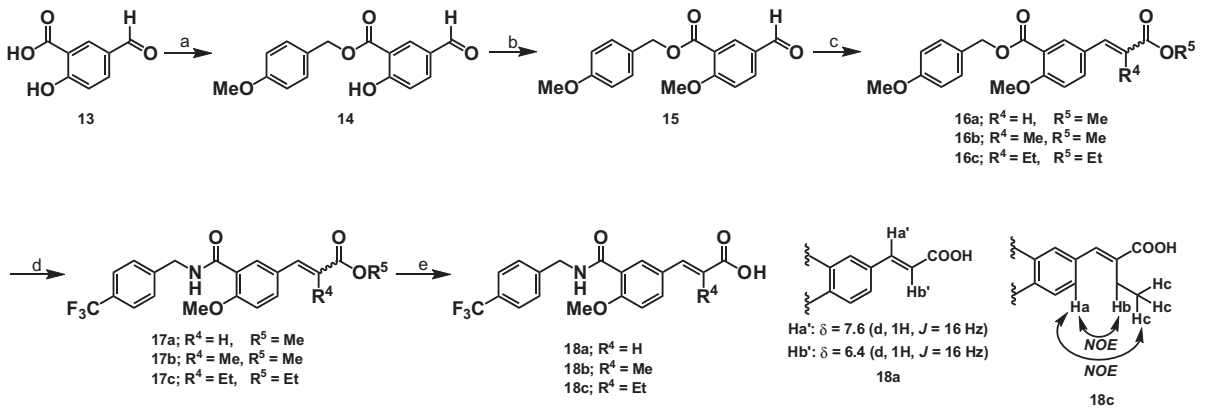
Based on the initial screen, we concluded that phenylpropanoic acid structure with a CH_2NHCO linker was effective, as in **9**. However, this compound had been assayed as a racemate. In order to ascertain the stereochemistry–activity relationship, we then assayed both enantiomers of **9**. Its cinnamic acid derivatives **18a–c** were also assayed, because in the case of PPAR, both frameworks are effective as PPAR agonists. Compounds **18a–c** were prepared from 5-formylsalicylic acid (**13**) in five steps. Compound **13** was benzylated with 4-methoxybenzyl bromide, followed by methylation of the phenolic hydroxyl group to afford the benzaldehyde derivative (**15**). Compound **15** was treated with trialkyl 2-phosphonoalkylate in the presence of *t*BuOK as a base, followed by hydrolysis with trifluoroacetic acid and amidation with 4-trifluoromethylbenzylamine to afford alkyl cinnamate derivatives (**17a–c**). Alkaline hydrolysis of **17a–c** and recrystallization afforded the desired **18a–c**. The *E*-stereochemistry was assigned based on the coupling constant (in the case of **18a**, Scheme 1) or the presence of the NOESY correlation peak between Ha and Hb (in the cases of **18b** and **18c**, Scheme 1). The correlation peak between Ha and

Table 1
Cleavage activities of compounds **4**–**12**



No.	L	R^1	R^2	R^3	$Aβ_{40}$		$Aβ_{42}$		Notch	
					1 μM	100 μM	1 μM	100 μM	1 μM	100 μM
4	$CONHCH_2$	CF_3	OCH_3	CH_2CH_3	89.5	186	101	220	31.2	0.00
5	$CONHCH_2$	CF_3	OCH_3	CH_3	114	132	109	139	190	190
6	$CONHCH_2$	CF_3	OCH_3	H	104	112	102	104	178	162
7	$CONHCH_2$	OCH_2CF_3	OCH_3	CH_2CH_3	118	136	119	133	170	84.0
8	$CONHCH_2$	OPh	OCH_3	CH_2CH_3	103	119	103	145	200	162
9	CH_2NHCO	CF_3	OCH_3	CH_2CH_3	82.0	41.0	88.5	15.2	102	8.90
10	CH_2NHCO	CF_3	F	CH_2CH_3	77.6	13.0	63.3	0.00	53.8	38.5
11	CH_2NHCO	OCF_3	F	CH_2CH_3	90.6	42.6	87.4	55.7	67.2	0.00
12	CH_2NHCO	OPh	F	CH_2CH_3	89.8	63.2	99.7	106	6.30	6.80
DMSO					100		100		100	
DAPT					0.00[a]		0.00[a]		0.00[a]	
Sulindac sulfide					98.4[b]	88.6[c]	97.1[b]	60.3[c]	90.7[b]	43.9[c]

[a] The relative γ-secretase activity elicited with 10 μM DAPT was designated as 0%.
[b] 3 μM sulindac sulfide was used.
[c] 30 μM sulindac sulfide was used.



Scheme 1. Synthesis of the γ-secretase modulator **18**s. Reagents and conditions: (a) 4-methoxybenzylamine, $KHCO_3$, DMF, rt, 24 h, quant.; (b) MeI, K_2CO_3, DMF, 24 h, 93%; (c) (1) *t*BuOK, trialkylphosphonoalkylate, dehydrated THF, rt, 6 h, 61–82%, (2) trifluoroacetic acid, rt, 24 h, 77–84%; (d) 4-(trifluoromethyl)benzylamine, DEPC, TEA, DMF, rt, 24 h, 77–89%; (e) (1) 2 mol/L NaOH, EtOH, 60 °C, 24 h, (2) recrystallization from *n*-hexane/AcOEt, 28–54% (two steps).

Hc was also observed in the case of **18c** (Scheme 1). The preparation of the optically active α-ethylphenylpropanoic acid derivatives, (*S*)-**9** and (*R*)-**9**, was reported previously.[17]

The inhibitory activities of these compounds are summarized in Figure 2. Compound (*S*)-**9** did not inhibit Aβ-generating γ-secretase activity at the high concentration of 100 μM, for either $Aβ_{40}$ or $Aβ_{42}$. On the other hand, the antipodal (*R*)-**9** inhibited γ-secretase activity leading to both $Aβ_{40}$ and $Aβ_{42}$ at 100 μM. These results clearly indicated that inhibitory activity of **9** towards Aβ generation resides mainly in the (*R*)-enantiomer. Notably, the PPAR-agonistic activity of **9** resides mainly in the (*S*)-enantiomer[17] suggesting that the inhibition of Aβ-generating activity occurs independently of the modulation of the transcriptional activity of PPAR. In accordance with this notion, (*R*)-**9** inhibited the γ-secretase activity in in vitro assay using recombinant substrate (IC_{50} values for de novo generation of $Aβ_{40}$ and $Aβ_{42}$ are 15.2 μM and 10.1 μM, respectively),[18] indicating that these compounds act directly on the γ-secretase.

It is very important to note the contrasting results that effective Notch-inhibitory activity was seen at the low concentration of 1 μM (*S*)-**9**, whereas (*R*)-**9** did not exhibit apparent Notch-inhibitory activity at the higher concentration of 100 μM. This suggests that inhibitory activities for Aβ generation and Notch signaling might be separated by controlling the stereochemistry of the substituent at the α-position of phenylpropanoic acid derivatives. The IC_{50}s of (*S*)-**9** for $Aβ_{40,\ Aβ42}$ and Notch are >100 μM, >100 μM and 24.0 μM, respectively, while those of (*R*)-**9** are 37.8 μM, 34.2 μM and >100 μM, respectively.

The cinnamic acid derivatives (**18a–c**) exhibited similar pharmacological character to (*R*)-**9**. That is, **18a–c** exhibited dose-

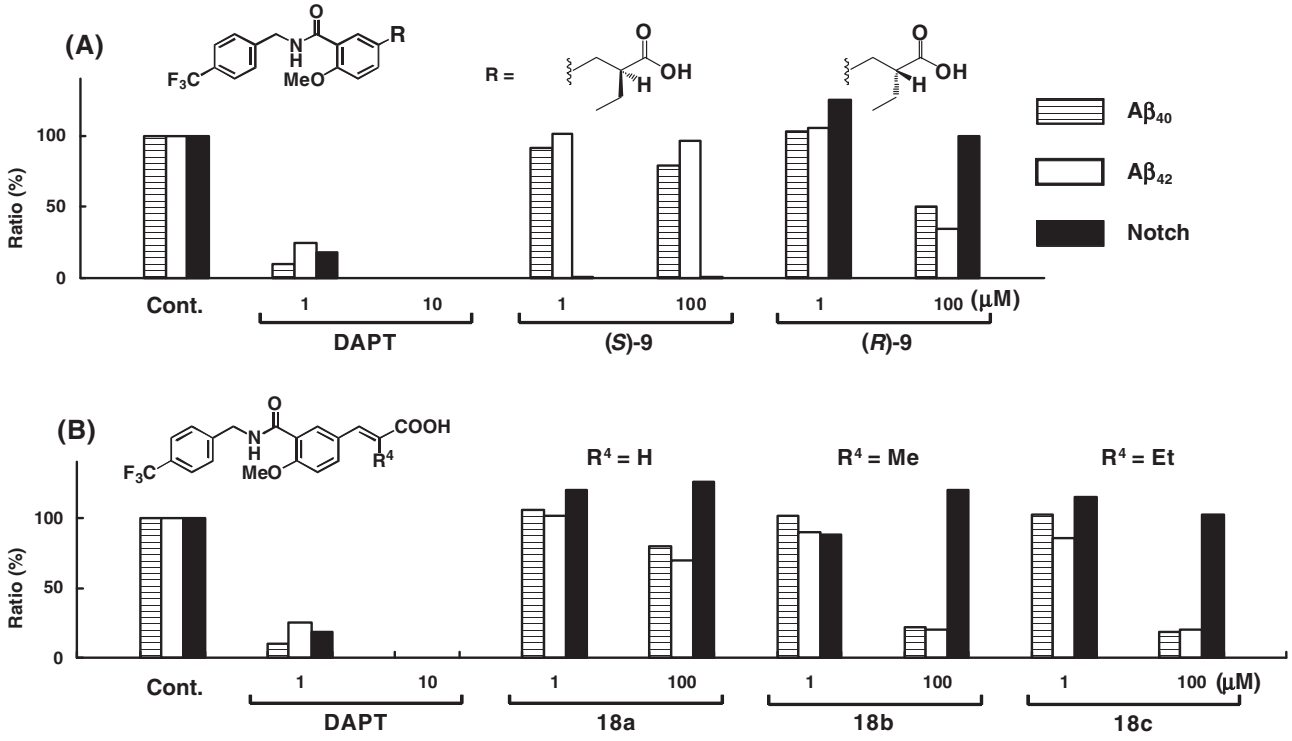


Figure 2. Cleavage activities of DAPT, (*S*)-**9**, (*R*)-**9**, and **18a**,**b**,**c**. Relative γ-secretase activities are shown as the ratio (%) to the control (left), taken as 100%, and 10 μM DAPT as 0%.

dependent inhibitory activity for both $A\beta_{40}$ and $A\beta_{42}$-generating activities without affecting Notch signaling. An α-alkyl substituent was required for potent γ-secretase-inhibitory activity, because **18b** and **18c** exhibited more potent γ-secretase inhibitory activity than **18a**. The IC_{50}s for $A\beta_{40,\ A\beta42}$ and Notch are calculated to 32.7 μM, 22.4 μM and >100 μM, respectively. Based on the calculated IC_{50}s, **18c** exhibited more potent GSI activity than sulindac sulfide, with a reduced effect on the Notch signaling pathway. Nevertheless, further mechanistic study on the mode of action of these compounds is still needed.

In conclusion, we found that the phenylpropanoic acid with a CH_2NHCO linker and cinnamic acid with a CH_2NHCO linker can be employed as scaffolds for the creation of structurally novel NS-GSIs. Moreover, we identified phenylpropanoic acid with a $CONHCH_2$ linker as a Notch-specific GSI. Notch inhibition is considered to be effective against cancer cell induction and/or proliferation in a subset of malignant neoplasms.[19] Therefore, such compounds could be useful as a scaffold for developing agents to treat Notch-dependent cancer. Further chemical modification studies are on-going.

Acknowledgements

This work is supported in part by Grants-in-Aid for Young Scientists (S) from Japan Society for the Promotion of Science (JSPS), Scientific Research on Priority Areas 'Research on Pathomechanisms of Brain Disorders' from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), by Targeted Proteins Research Program of the Japan Science and Technology Corporation (JST) and by Core Research for Evolutional Science and Technology of JST, Japan.

References and notes

1. Tomita, T. *Expert Rev. Neurother.* **2009**, *9*, 661.
2. Takasugi, N.; Tomita, T.; Hayashi, I.; Tsuruoka, M.; Niimura, M.; Takahashi, Y.; Thinakaran, G.; Iwatsubo, T. *Nature* **2003**, *422*, 438.
3. Jarrett, J. T.; Berger, E. P.; Lansbury, P. T., Jr. *Biochemistry* **1993**, *32*, 4693.
4. Roher, A. E.; Lowenson, J. D.; Clarke, S.; Woods, A. S.; Cotter, R. J.; Gowing, E.; Ball, M. J. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 10836.
5. Iwatsubo, T.; Odaka, A.; Suzuki, N.; Mizusawa, H.; Nukina, N.; Ihara, Y. *Neuron* **1994**, *13*, 45.
6. Searfoss, G. H.; Jordan, W. H.; Calligaro, D. O.; Galbreath, E. J.; Schirtzinger, L. M.; Berridge, B. R.; Gao, H.; Higgins, M. A.; May, P. C.; Ryan, T. P. *J. Biol. Chem.* **2003**, *278*, 46107.
7. Wong, G. T.; Manfra, D.; Poulet, F. M.; Zhang, Q.; Josien, H.; Bara, T.; Engstrom, L.; Pinzon-Ortiz, M.; Fine, J. S.; Lee, H. J.; Zhang, L.; Higgins, G. A.; Parker, E. M. *J. Biol. Chem.* **2004**, *279*, 12876.
8. Milano, J.; McKay, J.; Dagenais, C.; Foster-Brown, L.; Pognan, F.; Gadient, R.; Jacobs, R. T.; Zacco, A.; Greenberg, B.; Ciaccio, P. J. *Toxicol. Sci.* **2004**, *82*, 341.
9. Petit, A.; Bihel, F.; Alves da Costa, C.; Pourquie, O.; Checler, F.; Kraus, J. L. *Nat. Cell Biol.* **2001**, *3*, 507.
10. (a) Weggen, S.; Eriksen, J. L.; Das, P.; Sagi, S. A.; Wang, R.; Pietrzik, C. U.; Findlay, K. A.; Smith, T. E.; Murphy, M. P.; Bulter, T.; Kang, D. E.; Marquez-Sterling, N.; Golde, T. E.; Koo, E. H. *Nature* **2001**, *414*, 212; (b) Kukar, T.; Murphy, M. P.; Eriksen, J. L.; Sagi, S. A.; Weggen, S.; Smith, T. E.; Ladd, T.; Khan, M. A.; Kache, R.; Beard, J.; Dodson, M.; Merit, S.; Ozols, V. V.; Anastasiadis, P. Z.; Das, P.; Fauq, A.; Koo, E. H.; Golde, T. E. *Nat. Med.* **2005**, *11*, 545; (c) Kukar, T. L.; Ladd, T. B.; Bann, M. A.; Fraering, P. C.; Narlawar, R.; Maharvi, G. M.; Healy, B.; Chapman, R.; Welzel, A. T.; Price, R. W.; Moore, B.; Rangachari, V.; Cusack, B.; Eriksen, J.; Jansen-West, K.; Verbeeck, C.; Yager, D.; Eckman, C.; Ye, W.; Sagi, S.; Cottrell, B. A.; Torpey, J.; Rosenberry, T. L.; Fauq, A.; Wolfe, M. S.; Schmidt, B.; Walsh, D. M.; Koo, E. H.; Golde, T. E. *Nature* **2008**, *453*, 925.
11. (a) Barten, D. M.; Guss, V. L.; Corsa, J. A.; Loo, A.; Hansel, S. B.; Zheng, M.; Munoz, B.; Srinivasan, K.; Wang, B.; Robertson, B. J.; Polson, C. T.; Wang, J.; Roberts, S. B.; Hendrick, J. P.; Anderson, J. J.; Loy, J. K.; Denton, R.; Verdoorn, T. A.; Smith, D. W.; Felsenstein, K. M. *J. Pharmacol. Exp. Ther.* **2005**, *312*, 635; (b) Kreft, A.; Harrison, B.; Aschmies, S.; Atchison, K.; Casebier, D.; Cole, D. C.; Diamantidis, G.; Ellingboe, J.; Hauze, D.; Hu, Y.; Huryn, D.; Jin, M.; Kubrak, D.; Lu, P.; Lundquist, J.; Mann, C.; Martone, R.; Moore, W.; Oganesian, A.; Porte, A.; Riddell, D. R.; Sonnenberg-Reines, J.; Stock, J. R.; Sun, S. C.; Wagner, E.; Woller, K.; Xu, Z.; Zhou, H.; Steven Jacobsen, J. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4232.
12. Shelton, C. C.; Zhu, L.; Chau, D.; Yang, L.; Wang, R.; Djaballah, H.; Zheng, H.; Li, Y. M. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 20228.
13. (a) Kasuga, J.; Yamasaki, D.; Ogura, K.; Shimizu, M.; Sato, M.; Makishima, M.; Doi, T.; Hashimoto, Y.; Miyachi, H. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1110; (b) Kasuga, J.; Nakagome, I.; Aoyama, A.; Sako, K.; Ishizawa, M.; Ogura, M.; Makishima, M.; Hirono, S.; Hashimoto, Y.; Miyachi, H. *Bioorg. Med. Chem.* **2007**, *15*, 5177; (c) Kasuga, J.; Hashimoto, Y.; Miyachi, H. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 771; (d) Kasuga, J.; Yamasaki, D.; Araya, Y.; Nakagawa, A.; Makishima, M.; Doi, T.; Hashimoto, Y.; Miyachi, H. *Bioorg. Med. Chem.* **2006**, *14*, 8405.
14. (a) Isoo, N.; Sato, C.; Miyashita, H.; Shinohara, M.; Takasugi, N.; Morohashi, Y.; Tsuji, S.; Tomita, T.; Iwatsubo, T. *J. Biol. Chem.* **2007**, *282*, 12388; (b) Kopan, R.; Schroeter, E. H.; Weintraub, H.; Nye, J. S. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 1683; (c) Strobl, L. J.; Hofelmayr, H.; Stein, C.; Marschall, G.; Brielmeier, M.; Laux, G.; Bornkamm, G. W.; Zimber-Strobl, U. *Immunobiology* **1997**, *198*, 299; (d) Imamura, Y.; Watanabe, N.; Umezawa, N.; Iwatsubo, T.; Kato, N.; Tomita, T.; Higuchi, T. *J. Am. Chem. Soc.* **2009**, *131*, 7353.
15. Dovey, H. F.; John, V.; Anderson, J. P.; Chen, L. Z.; de Saint, A. P.; Fang, L. Y.; Freedman, S. B.; Folmer, B.; Goldbach, E.; Holsztynska, E. J.; Hu, K. L.; Johnson-Wood, K. L.; Kennedy, S. L.; Kholodenko, D.; Knops, J. E.; Latimer, L. H.; Lee, M. Z.; Lieberburg, I. M.; Motter, R. N.; Mutter, L. C.; Nietz, J.; Quinn, K. P.; Sacchi, K. L.; Seubert, P. A.; Shopp, G. M.; Thorsett, E. D.; Tung, J. S.; Wu, J.; Yang, S.; Yin, C. T.; Schenk, D. B.; May, P. C.; Altstiel, L. D.; Bender, M. H.; Boggs, L. N.; Britton, T. C.; Clemens, J. C.; Czilli, D. L.; Dieckman-McGinty, D. K.; Droste, J. J.; Fuson, K. S.; Gitter, B. D.; Hyslop, P. A.; Johnstone, E. M.; Li, W. Y.; Little, S. P.; Mabry, T. E.; Miller, F. D.; Audia, J. E. *J. Neurochem.* **2001**, *76*, 173.
16. Kasuga, J.; Makishima, M.; Hashimoto, Y.; Miyachi, H. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 554.
17. Nomura, M.; Tanase, T.; Ide, T.; Tsunoda, M.; Suzuki, M.; Uchiki, H.; Murakami, K.; Miyachi, H. *J. Med. Chem.* **2003**, *46*, 3581.
18. Takahashi, Y.; Hayashi, I.; Tominari, Y.; Rikimaru, K.; Morohashi, Y.; Kan, T.; Natsugari, H.; Fukuyama, T.; Tomita, T.; Iwatsubo, T. *J. Biol. Chem.* **2003**, *278*, 18664.
19. (a) Sikandar, S. S.; Pate, K. T.; Anderson, S.; Dizon, D.; Edwards, R. A.; Waterman, M. L.; Lipkin, S. M. *Cancer Res.* **2010**, *70*, 1469; (b) Tammam, J.; Ware, C.; Efferson, C.; O'Neil, J.; Rao, S.; Qu, X.; Gorenstein, J.; Angagaw, M.; Kim, H.; Kenific, C.; Kunii, K.; Leach, K. J.; Nikov, G.; Zhao, J.; Dai, X.; Hardwick, J.; Scott, M.; Winter, C.; Bristow, L.; Elbi, C.; Reilly, J. F.; Look, T.; Draetta, G.; Van der Ploeg, L.; Kohl, N. E.; Strack, P. R.; Majumder, P. K. *Br. J. Pharmacol.* **2009**, *58*, 1183.