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Syntheses of tricyclic pyrones and pyridinones and protection of Aβ-peptide induced MC65 neuronal cell death

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ABSTRACT

The SβC gene is conditionally expressed a 99-residue carboxy terminal fragment, C99, of amyloid precursor protein in MC65 cells and causes cell death. Consequently, MC65 cell line was used to identify inhibitors of toxicity related to intracellular amyloid β (Aβ) oligomers. Compounds that reduce the level of Aβ peptides, prevent Aβ aggregation, or eliminate existing Aβ aggregates may be used in the treatment of Alzheimer's disease (AD). Previously, we found that a tricyclic pyrone (TP) molecule, compound **1**, prevents MC65 cell death and inhibits Aβ aggregation. Hence various TPs containing heterocycle at C7 side chain and a nitrogen at position 2 or 5 were synthesized and their MC65 cell protective activities evaluated. TPs containing N3′-adenine moiety such as compounds **1** and **11** are most active with EC_{50} values of 0.31 and 0.35 μM, respectively. EC_{50} values of tricyclic N5-analog, pyranoisoquinolinone **13**, and N2-analog, pyranopyridinone **20**, are 2.49 and 1.25 μM, respectively, despite the lack of adenine moiety. Further investigation of tricyclic N2- and N5-analogs is warranted.

Alzheimer's disease (AD) is a progressive and irreversible brain disorder with no known cure. An Alzheimer's patient's brain has two classical lesions: extracellular senile (neuritic) plaques and intracellular neurofibrillary tangles.[1–3] A small protein, amyloid β peptide (Aβ) containing 39–42 amino acids, is widely considered a culprit for the disease. The main alloforms of Aβ deposits in AD brain are 40 and 42 amino acids long (designated as Aβ40 and Aβ42). Recent evidence indicates that soluble oligomers of Aβ may represent the primary toxic species of amyloid in AD.[4–7] It is accepted that newly produced Aβ is monomeric, soluble and non-toxic, adopting random coil/α-helix mixture structures under normal physiological conditions.[8–10] In AD, Aβ undergoes conformational changes or misfolding from random coil/α-helix to β-sheet structure, resulting in oligomerization and fibril formation.[11] At the earlier stage of this oligomerization, the soluble oligomeric Aβ are small oligomers (5–10 nm width and 6 nm height), which undergo aggregation to form larger oligomers (50–100 nm in width) and protofibrils (~180 nm in length).[11] Further oligomerization of Aβ protofibrils leads to fibril formation, which is insoluble and deposited in the neurophile. A compound that blocks this conformational change and/or disaggregate oligomeric Aβ and protofibrils will possibly serve as a drug for the treatment of AD.[12] To search for potential drugs, we use MC65 cell line to screen bioactive compounds.[13] MC65 is a line of human neuroblastoma that conditionally expresses C99, a 99-residue carboxy terminal fragment derived from the cleavage of amyloid precursor protein (APP) by β-secretase.[14] C99 is cleaved by γ-secretase to generate Aβ peptides. Following transgene induction, significant cell death occurs after 68 h. The death is likely derived from intracellularly induced toxic Aβ oligomers.[13,14]

In search of such compounds, we discovered that a class of tricyclic pyrones (TP), especially tricyclic pyrone **1** (Fig. 1), prevents the death of human neuroblastoma MC65 cells related to intracellular accumulation of Aβ oligomers.[15] This protection effect is intimately related to compound **1**'s ability to inhibit the formation of Aβ aggregates.[15] Based on our previous studies,[16] compound **1** containing an adenine moiety (at N-3′) attached at C7-alkyl side chain of the tricyclic pyrone ring structure, shows a great MC65 cell protective activity. The N-9′ analog **2** is about 10 times less active comparing with **1**.[16] To examine whether TP analogs containing other purine- and pyrimidine-tethered alkyl group at C7, a double bond at C12–C14, or nitrogen atom at position 2 or 5, possess greater bioactivities than **1**, we synthesized various heterocycle and nitrogen containing TP analogs, **3**–**13** (Fig. 1) and studied their MC65 cell protective activities.

Modifications of our previously reported methodologies[16,17] were made for the synthesis of compounds **3**–**13**. Compound **3** was synthesized from tricyclic pyrone **14**[18] in four steps (Scheme 1). Selective allylic oxidation of C7-isopropenyl tether of **14** with palladium acetate (0.1 equiv), benzoquinone (3 equiv), and diethyl malonate (0.2 equiv) in acetic acid[19] under heat followed by basic methanolysis with potassium carbonate in methanol at 0 °C for 1 h afforded alcohol **15** in 49% overall yield. Direct allylic oxidation of

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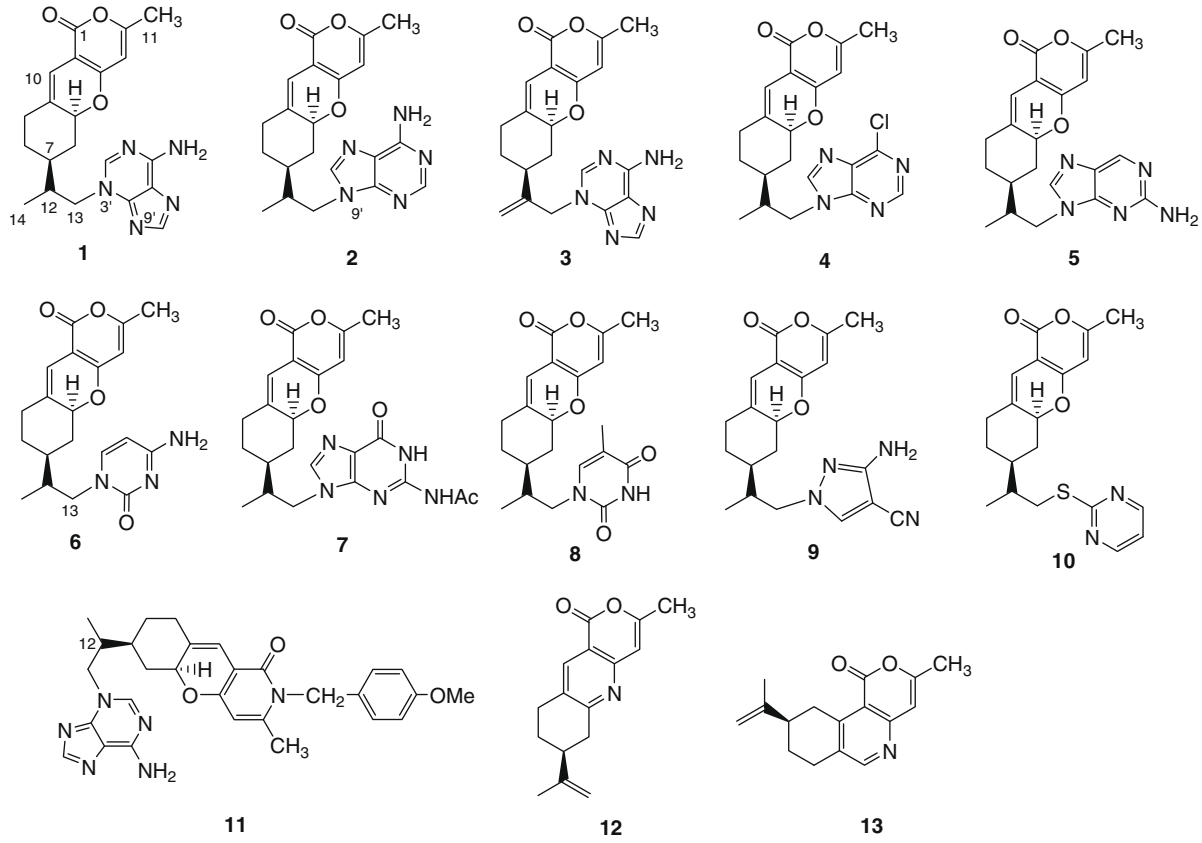
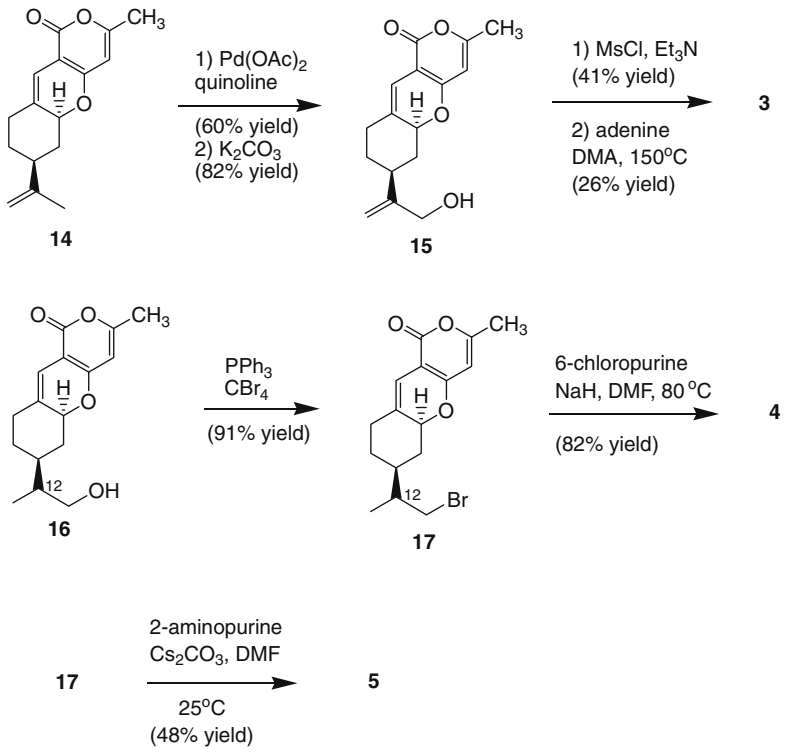


Figure 1. Synthesized and bioevaluated tricyclic pyrones.



Scheme 1. Syntheses of tricyclic pyrones **3**–**5**.

14 with 0.5 equiv of selenium dioxide and 2 equiv of *tert*-butyl hydroperoxide in dichloromethane at 25 °C produced **15** in only 20% yield. Mesylation of **15** with methanesulfonyl chloride and triethylamine followed by adenine in *N*,*N*-dimethylacetamide (DMA) at 150 °C gave analog **3** (26% yield) along with decomposition by-products which were not investigated. 2D NOESY NMR spectrum

of **3** reveals NOE correlation between C2′-H at δ 8.07 ppm, and CH_2N at δ 5.14 and 4.92 ppm, and the chemical shift assignments of C2′ and C8′ protons are in agreement with the values of compound **1**.[16] In order to obtain N-3′ displacement product, bases such as NaH, $NaHCO_3$, and K_2CO_3 cannot be used. The presence of an inorganic base will lead to a nucleophilic attack at N-9 (vide infra).[16] N-9′ analog of **3** was not investigated because N-9′ analog, such as compound **2**, is less active compared to **1**.

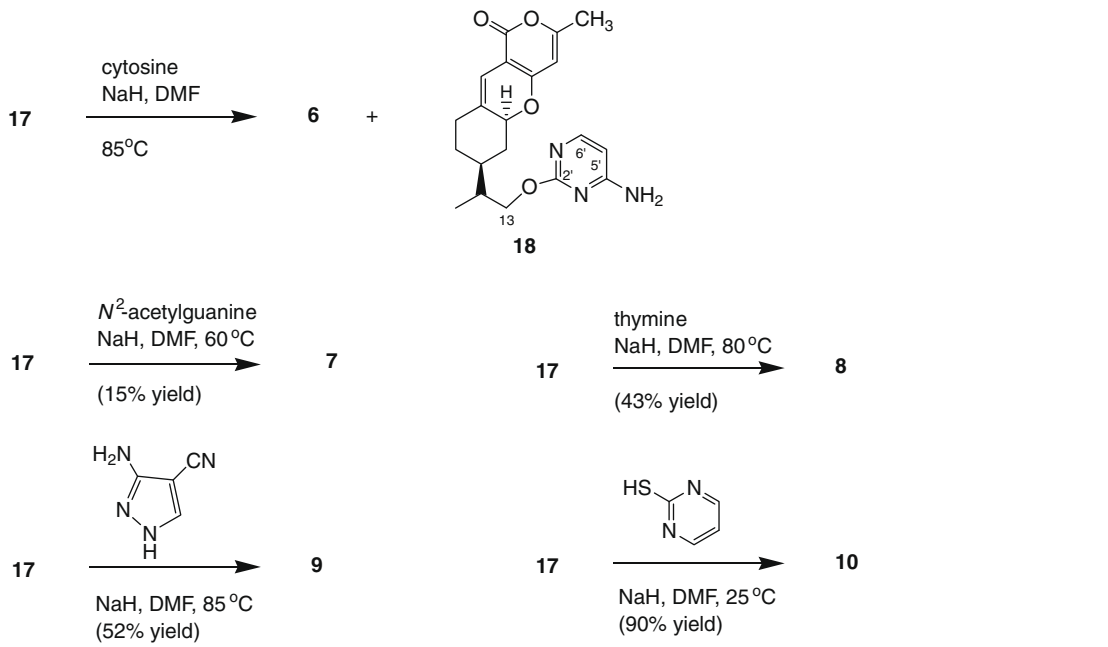
Electron donating effect of the C6-amino function of adenine apparently leads to the nucleophilic attack at N-3 (vide supra). Hence, 6-chloropurine, absence of amino function, does not undergo nucleophilic displacement reaction with bromide **17** (or the mesylate derivative of **15**) under similar reaction conditions. However, treatment of **17** with 6-chloropurine and sodium hydride (1 equiv each) in DMF at 80 °C yielded N-9′ analog **4** (82%). Since chloropurine and other purines used in the present studies have different structures to that of adenine, their N-9′ alkylated TPs were investigated. Pure C12-*R* and pure C12-*S* diastereomer of **1** have been separated by HPLC and shown to possess similar bioactivities,[15] hence neither separation of diastereomers **4** nor stereoselective synthesis of an enantiomer of **16** (at C12) was studied. Bromide **17** was obtained in 91% yield from the bromination of alcohol **16**[16] with triphenylphosphine and carbon tetrabromide in dichloromethane at 0 °C for 1 h. Similar treatment of **17** with 2-aminopurine and Cs_2CO_3 gave 48% yield of analog **5**. In the syntheses of TP **5** and **6**–**10** (vide infra) bromide **17** produced better yields of the displacement products than that of the corresponding mesylate derivative[16] of **16**, hence reactions of **17** are reported here. Compound **5** is a fluorescent molecule suitable for study of the staining of amyloid plaques with fluorescence microscopy. Again, in the absence of a base, 2-aminopurine alone does not react with bromide **17** in DMA under heat (>150 °C) to provide the desired displacement product. The regiochemistry of **5** was determined through a 2D NOESY NMR spectroscopic study. C8′-H, δ 7.71 ppm, shows NOE correlations with CH_2N proton at δ 4.10 and 3.89 ppm, and C6′-H, δ 8.66 ppm, shows no correlation. The chemical shift assignments of C6′ and C8′ protons are in agreement with the reported values.[20]

Five heterocyclic analogs **6**–**10** were similarly synthesized from reactions of bromide **17** with cytosine, guanine, thymine, 3-aminopyrazole-4-carbonitrile, and 2-mercaptopyrimidine, respectively (Scheme 2). Treatment of cytosine with sodium hydride in DMF followed by 1 equiv of **17** gave N1′-alkylated product **6** (28% yield) along with O-alkylated isomer **18** (10% yield). The ^{13}C chemical shift value of carbon-13 of compound **6** is 53.9 ppm (carbon attached to nitrogen) while that of compound **18** is 69.7 ppm, indicative of a carbon attached to oxygen. Moreover, the 2D NOESY spectrum of **6** shows correlation between CH_2N, δ 3.93 and 3.37 ppm, and C6′-H, δ 7.21 ppm, of the cytosine moiety, while the 2D NOESY spectrum of **18** reveals no correlation between CH_2N, δ 4.18 ppm, and C6′-H, δ 8.02 ppm. The ^1H NMR chemical shift value of C6′-H of **6** and N1′-alkylation of cytosine are in agreement with that reported,[21–23] and *O*-alkylation of cytosine has been reported previously.[24] Sodiation of N^2-acetylguanine with sodium hydride in DMF followed by bromide **17** afforded N9′-alkylated product **7**. The chemical shift of N8′-H, δ 7.73 ppm, of **7** is similar to the reported value,[25] and the 2D NOESY spectrum reveals a NOE correlation between N8′-H, δ 7.73 ppm, with CH_2N, δ 4.46 and 4.03 ppm. In order to obtain N-9′ alkylation, acetylation of N^2-H of guanine is needed.[26] The reaction of guanine (without N^2-protection) with sodium hydride and bromide **17** or the mesylate analog (derived from the mesylation of alcohol **16**) produced no displacement product, only E2 reaction product, compound **14**, was obtained. Reactions of bromide **17** with thymine, 3-aminopyrazole-4-carbonitrile, and 2-mercaptopyrimidine, separately, and sodium hydride in DMF afforded displacement products **8**, **9**, and **10**, respectively. The regiochemistry of **8** and **9** were verified from their 2D NOESY spectra and in agreement with that reported.[27,28] NOESY spectrum of thymine adduct **8** shows correlation between C6′-H (of thymine nucleus) at δ 6.94 ppm and CH_2N at δ 3.82 and 3.40 ppm, while spectrum of **9** reveals correlation between C5′-H (of pyrazole) at δ 7.51 and 7.48 ppm (2 diastereomers at C12) and CH_2N at δ 3.95 and 3.69 ppm. The *S*-alkylations of 2-mercaptopyrimidine with alkyl halides have been reported previously.[29]

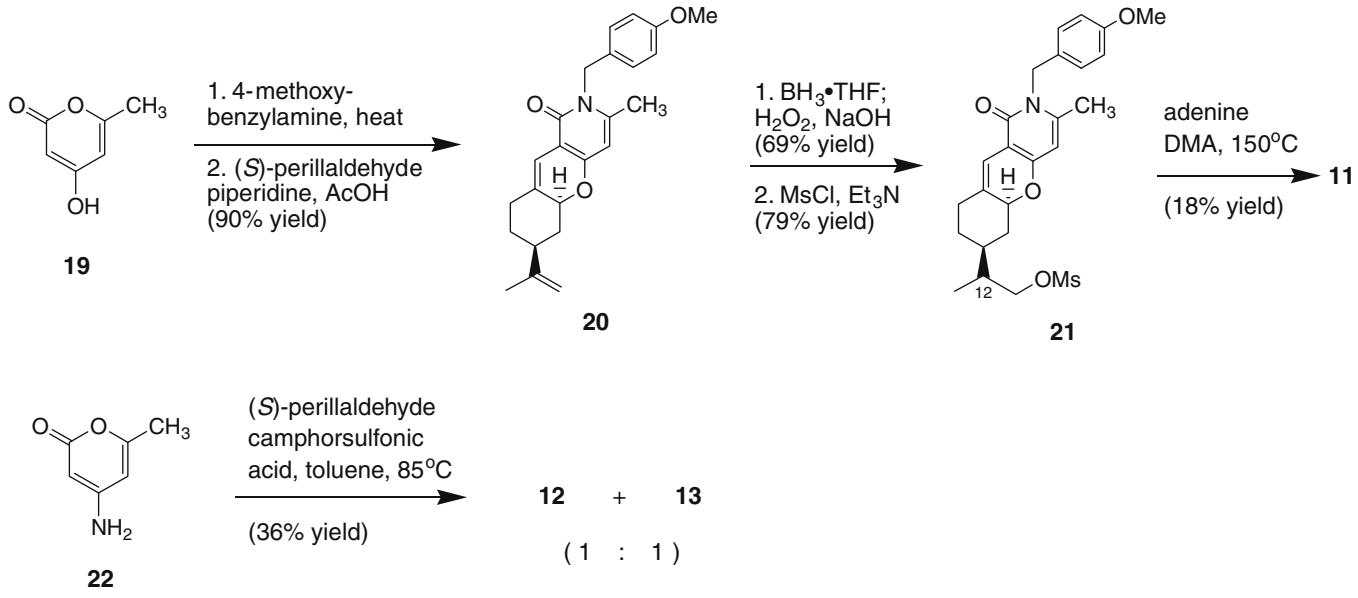
Since nitrogen containing compounds often increase bioactivities and brain-penetrating ability, we investigated N2- and N5-analogs of TP. Tricyclic pyridinone **20** was obtained in 90% yield from a condensation reaction of 4-hydroxy-*N*-(4-methoxybenzyl)-6-methyl-2-pyridinone and (*S*)-perillaldehyde in the presence of piperidine and acetic acid in chloroform under reflux (Scheme 3).[18,30,31] Following a similar procedure as that reported,[32] 4-hydroxy-*N*-(4-methoxybenzyl)-6-methyl-2-pyridinone was prepared by the treatment of 4-hydroxy-6-methyl-2-pyrone with 4-methoxybenzylamine under refluxing ethanol. Chemoselective hydroboration of **20** with borane in THF at −20 °C followed by 30% H_2O_2 and NaOH, and mesylation with triethylamine and methanesulfonyl chloride gave mesylate **21** as two diastereomers at C12 in 1:1 ratio (determined from its C13 NMR spectrum). The two diastereomers are inseparable by silica gel column chromatography or crystallization. No stereoselectivity resulted from the hydroboration reaction.[18] Displacement reaction of **21** with adenine in DMA at 150 °C afforded tricyclic pyranopyridinone **11** (18% yield). The N3′-regiochemistry is similar to that of compound **1** and supported by its 2D NOESY NMR spectrum in which the NOE correlation is shown between C2′H at δ 8.08 ppm and CH_2N at δ 4.51 and 4.08 ppm. The use of the bromo derivative of compound **21** did not improve the yield of **11**.

N5-Analogs, pyranoquinolinone **12** and pyranoisoquinolinone **13**, were produced in a ratio of 1:1 from the condensation reaction[17] of 4-amino-6-methyl-2-pyrone (**22**) and (*S*)-perillaldehyde in the presence of camphor-10-sulfonic acid under refluxing toluene (36% yield). Compounds **12** and **13** were readily separated by silica gel column chromatography and their structures were assigned by comparing their NMR spectra to those reported previously.[17]

MC65 cell protective activities: The MC65 cell line was used to screen bioactive compounds.[13,15,33] The cells are readily propagated and cell death occurs after 3 days and is measured quantitatively by a simple 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.[13] Compounds that protect neuron cell death induced by Aβ could be used for drug development in Alzheimer's disease.[15] The EC_{50} (effective concentration at 50%) values of compounds **1**–**13**, **18**, and **20** are summarized in Table 1. Different batches of MC65 cells produce small variations in activities. Three batches of MC65 cells were used in the studies of compounds **2**–**13**, **18**, and **20**, and compound **1** was used as a standard in all three batches of cells and an average EC_{50} value of 0.31 ± 0.01 μM was obtained. This value is comparable to that reported previously.[16] Surprisingly, compound **3** which has a similar structure as that of compound **1** containing a C12 double bond is ~6 times less active than **1**. Other heterocycles attached tricyclic pyrones, compounds **4**–**10** are less active compared with **1**. Chloropurine analog **4** for instance is 3 times less active compared with **1**, and 2-aminopurine **5** and thymine **8** are ~3 and 22 times, respectively, less active compared with **1**. Guanine derivative **7** and cytosine derivative **6** surprisingly are inactive. However, cytosine *O*-alkylated analog **18** has an EC_{50} value of 2.94 μM. Although pyrazole derivative **9** is 26 times less active compared with **1**, pyrimidine analog **10** is only 4 times less active. It is gratifying that the simple tricyclic pyridinone **20**, containing a nitrogen atom at 2 position, possesses a EC_{50} value of 1.25 μM and its adenine



Scheme 2. Syntheses of tricyclic pyrones **6**–**10**.



Scheme 3. Syntheses of tricyclic pyridinone **11** and pyrones **12** and **13**.

attached derivative **11** has similar cell protective activity as that of **1**. Compared with pyridinone **20**, linearly fused pyranoquinolinone **12** is inactive, however, the L-shape fused pyranoisoquinolinone **13** is only ~8 times less active compared to **1**, despite the lack of adenine attachment. Thus, the bioactivities of pyridinones **11** and **20** and pyranoisoquinolinone **13** warrant further investigation on N-2 and N-5 containing tricyclic pyrones.

Table 1
EC_{50} values of compounds **1**–**13**, **18**, and **20** in the protection of MC65 cells induced by APP C99

Compound	EC_{50} (μM)	Compound	EC_{50} (μM)
1	0.31 ± 0.03	**9**	8.30 ± 0.83
2	3.00 ± 0.30	**10**	1.38 ± 0.14
3	1.95 ± 0.20	**11**	0.35 ± 0.03
4	0.93 ± 0.09	**12**	Inactive
5	0.86 ± 0.09	**13**	2.49 ± 0.25
6	Inactive	**18**	2.94 ± 0.30
7	Inactive	**20**	1.25 ± 0.13
8	6.92 ± 0.69		

In summary, various heterocycle and nitrogen containing tricyclic pyrone derivatives were synthesized. The attachment of N3′-adenine at C7 side chain of the tricyclic pyrone provides the strongest MC65 protective activity. Possibly, the 6′-amino function of the adenine nucleus enhances the bioactivity. Replacement of the oxygen at position 2 with a nitrogen provides similar protective activity as that of the most active compound, **1**. Tricyclic pyranoisoquinolinone possesses protective activity despite lacking an adenine moiety. Further studies on N2- and N5-analogs and mechanisms of action are in progress.

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Supplementary data

Synthetic procedure, analysis data, cell line and cell culture, and protocols for cell-viability assay. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.12.060.

References and notes

1. Crowther, R. A. *Biochim. Biophys. Acta* **1991**, *1096*, 1.
2. Selkoe, D. J. *Physiol. Rev*. **2001**, *81*, 741.
3. De Felice, F. G.; Wu, D.; Lambert, M. P.; Fernandez, S. J.; Velasco, P. T.; Lacor, P. N.; Bigio, E. H.; Jerecic, J.; Acton, P. J.; Shughrue, P. J.; Chen-Dodson, E.; Kinney, G. G.; Klein, W. L. *Neurobiol. Aging* **2008**, *29*, 1334.
4. Lambert, M. P.; Barlow, A. K.; Chromy, B. A.; Edwards, C.; Freed, R.; Liosatos, M.; Morgan, T. E.; Rozovsky, I.; Trommer, B.; Viola, K. L.; Wals, P.; Zhang, C.; Finch, C. E.; Krafft, G. A.; Klein, W. L. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 6448.
5. Klein, W. L.; Krafft, G. A.; Finch, C. E. *Trends Neurosci.* **2001**, *24*, 219.
6. Walsh, D. M.; Klyubin, I.; Fadeeva, J. V.; Cullen, W. K.; Anwyl, R.; Wolfe, M. S.; Rowan, M. J.; Selkoe, D. J. *Nature* **2002**, *416*, 535.
7. De Felice, F. G.; Valasco, P. T.; Lambert, M. P.; Viola, K.; Fernandez, S. J.; Ferreira, S. T.; Klein, W. L. *J. Biol. Chem.* **2007**, *282*, 11590.
8. Hou, L.; Shao, H.; Zhang, Y.; Li, H.; Menon, N. K.; Neuhaus, E. B.; Brewer, J. M.; Byeon, I. L.; Ray, D. G.; Vitek, M. P.; Iwashita, T.; Majula, R. A.; Przybyla, A. B.; Zagorski, M. G. *J. Am. Chem. Soc.* **2004**, *126*, 1992.
9. Anand, P.; Nandel, F. S.; Hansmann, U. H. E. *J. Chem. Phys.* **2008**, *128*, 165102.
10. Hoyer, W.; Gronwall, C.; Jonsson, A.; Stahl, S.; Hard, T. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 5099.
11. Bitan, G.; Kirkitadze, M. D.; Lomakin, A.; Vollers, S. S.; Benedek, G. B.; Teplow, D. B. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 330.
12. Hardy, J.; Selkoe, D. J. *Science* **2002**, *297*, 353.
13. Jin, L.-W.; Hua, D. H.; Shie, F.-S.; Maezawa, I.; Sopher, B.; Martin, G. M. *J. Mol. Neurosci.* **2002**, *19*, 57.
14. Sopher, R. L.; Fukuchi, K.; Smith, A. C.; Leppig, K. A.; Furlong, C. E.; Martin, G. M. *Brain Res. Mol. Brain Res.* **1994**, *26*, 207.
15. Maezawa, I.; Hong, H.-S.; Wu, H.-C.; Battina, S. K.; Rana, S.; Iwamoto, T.; Radke, G. A.; Pettersson, E.; Martin, G. M.; Hua, D. H.; Jin, L. W. *J. Neurochem.* **2006**, *98*, 57.
16. Hua, D. H.; Huang, X.; Tamura, M.; Chen, Y.; Woltkamp, M.; Jin, L.-W.; Perchellet, E. M.; Perchellet, J.-P.; Chiang, P. K.; Namatame, I.; Tomoda, H. *Tetrahedron* **2003**, *59*, 4795.
17. Hua, D. H.; Chen, Y.; Sin, H.-S.; Robinson, P. D.; Meyers, C. Y.; Perchellet, E. M.; Perchellet, J.-P.; Chiang, P. K.; Biellmann, J.-F. *Acta Crystallogr.* **1999**, *C55*, 1698.
18. Hua, D. H.; Chen, Y.; Sin, H. -S.; Maroto, M. J.; Robinson, P. D.; Newell, S. W.; Perchellet, E. M.; Ladesich, J. B.; Freeman, J. A.; Perchellet, J.-P.; Chiang, P. K. *J. Org. Chem.* **1997**, *62*, 6888.
19. McMurry, J. E.; Kocovsky, P. *Tetrahedron Lett.* **1984**, *25*, 4187.
20. Busca, P.; McCort, I.; Prange, T.; Merrer, Y. L. *Eur. J. Org. Chem.* **2006**, *10*, 2403.
21. Overberger, C. G.; Chang, J. Y. *Tetrahedron Lett.* **1989**, *30*, 51.
22. Phadtare, S.; Zemlicka, J. *J. Am. Chem. Soc.* **1989**, *111*, 5925.
23. Lim, M.-I.; Moyer, J. D.; Cysyk, R. L.; Marquez, V. E. *J. Med. Chem.* **1984**, *27*, 1536.
24. Wang, F.; Yang, Z.-J.; Jin, H.-W.; Zhang, L.-R.; Zhang, L.-H. *Tetrahedron: Asymmetry* **2007**, *18*, 2139.
25. Papoulis, A.; Al-Abed, Y.; Bucala, R. *Biochemistry* **1995**, *34*, 648.
26. Li, N.-S.; Piccirilli, J. A. *J. Org. Chem.* **2006**, *71*, 4018.
27. Wang, B.; Wang, M.; Zhang, H.; Sobal, N. S.; Tong, W.; Gao, C.; Wang, Y.; Giersig, M.; Wang, D.; Mohwald, H. *Phys. Chem. Chem. Phys.* **2007**, *9*, 6313.
28. Da Settimo, F.; Primofiore, G.; La Motta, C.; Taliani, S.; Simorini, F.; Marini, A. M.; Mugnaini, L.; Lovecchia, A.; Novellino, E.; Tuscano, D.; Martini, C. *J. Med. Chem.* **2005**, *48*, 5162.
29. Brown, D. J.; Grigg, G. W.; Iwai, Y.; McAndrew, K. N.; Nagamatsu, T.; Van Heeswyck, R. *Aust. J. Chem.* **1979**, *32*, 2713.
30. Wang, X.; Lee, Y. R. *Synthesis* **2007**, *19*, 3044.
31. Wang, J.; Swidorski, J. J.; Sydorenko, N.; Hsung, R. P.; Coverdale, H. A.; Kuyava, J. M.; Liu, J. *Heterocycles* **2006**, *70*, 423.
32. Castillo, S.; Ouadahi, H.; Herault, V. *Bull. Soc. Chim. France* **1982**, *7–8*, 257.
33. Woltjer, R. L.; Nghiem, W.; Maezawa, I.; Milatovic, D.; Vaisar, T.; Montine, K. S.; Montine, T. J. *J. Neurochem.* **2005**, *93*, 1047.