Inhibition of ACAT, overexpression of cholesterol transporter gene, and protection of amyloid β oligomers-induced neuronal cell death by tricyclic pyrone molecules

Laxman Pokhrel, Izumi Maezawa, Thi D. T. Nguyen, Kyeong-Ok Chang, Lee-Way Jin, and Duy H. Hua

Published Version Information

Citation: Pokhrel, L., Maezawa, I., Nguyen, T. D. T., Chang, K., Jin, L., & Hua, D. H. (2012). Inhibition of Acyl-CoA: Cholesterol acyltransferase (ACAT), overexpression of cholesterol transporter gene, and protection of amyloid β (Aβ) oligomers-induced neuronal cell death by tricyclic pyrone molecules. Journal of Medicinal Chemistry, 55(20), 8969–8973.

Digital Object Identifier (DOI): doi:10.1021/jm3012189

Publisher's Link: http://pubs.acs.org/doi/abs/10.1021/jm3012189

Inhibition of ACAT, Overexpression of Cholesterol Transporter Gene, and Protection of Amyloid β Oligomers-Induced Neuronal Cell Death by Tricyclic Pyrone Molecules

Laxman Pokhrel,[a] Izumi Maezawa,[b] Thi D. T. Nguyen,[a] Kyeong-Ok Chang,[c] Lee-Way Jin,[b] and Duy H. Hua[a,*]

[a]Department of Chemistry, 213 CBC Building, Kansas State University, Manhattan, KS 66506, United States

[b]M.I.N.D. Institute and Department of Pathology, 2825 50th Street, UC Davis Health System, Sacramento, CA 95817, United States

[c]Department of Diagnostic Medicine and Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506, United States

ABSTRACT: Alzheimer's disease (AD) is the most common cause of dementia in the elderly. A major effort in AD therapeutic development has targeted Aβ and downstream events. We have taken a rational design approach and synthesized a small library of tricyclic pyrone compounds based on CP2. Their protective action in MC65 cells and the inhibition of acyl-CoA:cholesterol acyltransferase along with the upregulation of cholesterol transporter gene were investigated. Five most active compounds exhibited potencies in the nanomolar to low micromolar ranges. The multiple effects of the compounds on Aβ and cellular cholesterol pathways could be potential mechanisms underlying the protective effects in *vivo*.

INTRODUCTION

Over 35 million people worldwide suffer from Alzheimer's disease (AD), and current available treatments for AD, donepezil, rivastigmine, galantamine and memantine, temporarily ameliorate some symptoms but do not modify the underlying disease. New drug discovery is urgently needed.[1] In search of small molecules that against intracellular Aβ oligomers (AβO) toxicity, we have used MC65 cells protection assay as our primary screen for bioactive compounds.[2–4] MC65 cells are neuroblastoma cells that degenerate after induction of intraneuronal accumulation of AβO. This line has been used in a high throughput assay that reliably selects compounds that penetrate the membranes, bind, neutralize, and reduce intraneuronal levels of AβO.[4,5] This assay generates few false positive results and gives a high likelihood of identifying leads that penetrate cells and ameliorate AβO-induced toxicity.[5] Previously, we identified a tricyclic pyrone (TP) molecule, CP2 (code name; **1**; Figure 1),[3,6] from MC65 cells assay that prevented cell death associated with intracellular AβO and inhibits Aβ aggregation *in vitro* and reduced amyloid plaques and soluble AβO *in vivo*.[3,6] In search of other TP molecules that possess greater cell protective action, we synthesized a library of TPs and evaluated their MC65 cells protective potencies and additional beneficial effects such as lipid modulation activities including the inhibition of acyl-CoA:cholesterol acyltransferase (ACAT) and upregulation of cholesterol transporter gene, ATP-binding cassette sub-family A, member 1 (ABCA1).

Recent results from genetic, cell-culture, mouse model, and epidemiologic data suggest that cellular cholesterol (lipid) metabolism is important in the control of the production and/or accumulation of Aβ.[7] For example, natural and synthetic liver X receptor (LXR) agonists including oxysterols, retinoic acids, T0901317 and GW3965 have been shown to induce cholesterol efflux,[8] which associates with the reduction of Aβ formation and secretion of Aβ *in vitro* and *in vivo*.[9,10] The induction of ABCA1 is important for cholesterol efflux and is also shown to mediate the secretion of Aβ from the cells.[9] Moreover, a well-established AD biomarker, ε4 allele[11] of apolipoprotein E (APOE) is involved in the cholesterol homeostasis. ACAT plays an important role in the cholesterol homeostasis by converting free cholesterol to neutral cholesteryl ester for storage, and ACAT inhibitors have been implicated in anti-atherosclerosis and reduction of amyloid pathology by regulating cholesterol homeostasis.[12–14] Also ACAT inhibitors were shown to induce cholesterol efflux.[15]

RESULTS AND DISCUSSION

In mimicking CP2,[16] various TP compounds (Figure 1) containing aryl substituted alkylamino functions attached at the C7 isopropyl side chain were synthesized from a facile reductive amination reaction starting from amine **7** (Scheme 1). Two TP amides, compounds **6a** and **6b**, were also prepared for comparison of their MC65 cells protective activities with that of TP amines **2** – **5**. Hence, treatment of TP amine **7**[17] with aldehydes **8** – **11** in methanol followed by sodium cyanoborohydride and acetic acid afforded compounds **2** – **5** in good yields. Various functionalities including hydroxyl, methyl ester, aromatic halides, cyanide, amine, and nitro remain intact under the reaction conditions.

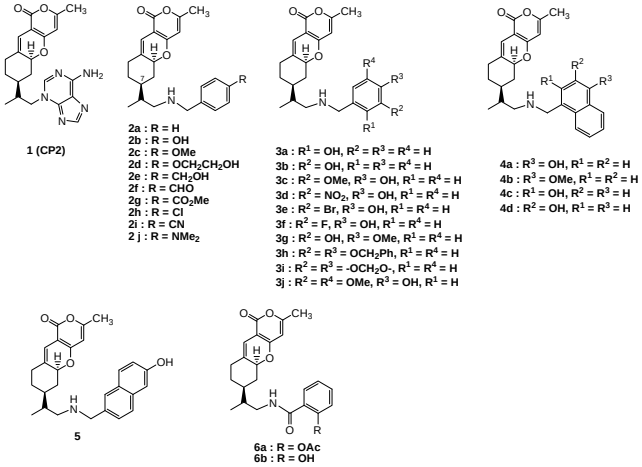
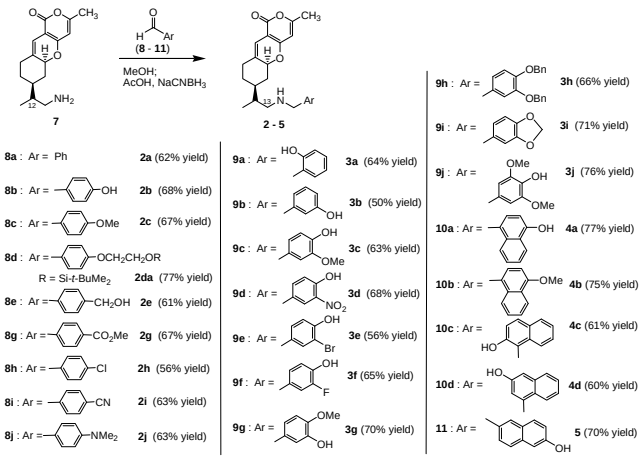


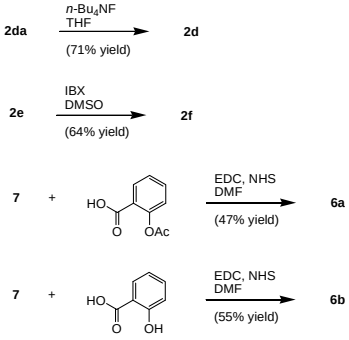
Figure 1. Synthesized and Bioevaluated Tricyclic Pyrone Compounds **1 - 6** using MC65 cells.



Scheme 1. Synthesis of TP Compounds **2 - 5**.

Compound TP **2d** was obtained from removal of the silyl ether protecting group of compound **2da**, derived from the above reductive amination of **7** and **8d**, in 71% yield, and TP aldehyde **2f** from oxidation of alcohol **2e** with 2-iodoxybenzoic acid (IBX) and DMSO[18] in 64% yield (Scheme 2). TP amides **6a** and **6b** were prepared from coupling reactions of amine **7** with acetylsalicylic acid and salicylic acid, respectively, using 3-(3-dimethylaminopropyl)-1-ethylcarbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) as the activating reagents.

Aryl aldehydes **8a** – **8c**, **8h** – **8**j, **9a** – **9d**, **9g**, **9i**, **9j**, **10a,** and **10c** were obtained from commercial sources. Aryl aldehydes **8d**, **8e**, **8g**, **9e**, **9f**, **9h**, **10b**, **11**, and **10d** were prepared from alkylation of *p*-hydroxybenzaldehyde (**8b**) with 2-bromo-1-(*t*-butyldimethylsilyloxy)ethane,[19] reduction of 1,4-benzenedicarboxaldehyde with sodium borohydride,[20] methylation of 4-formylbenzoic acid with potassium carbonate and methyl iodide in DMF,[21] bromination of **8b** with bromine in chloroform and 1,2-dimethoxyethane (4:1),[22] a modified reduction of 4-cyano-2-flourophenol with platinum oxide in formic acid,[23] dibenzylation of 3,4-dihydroxybenzaldehyde with potassium carbonate and benzyl bromide,[24] methylation of 4-hydroxynaphthalenecarboxaldehyde with potassium carbonate and methyl iodide,[25] and metallation/Vilsmeier-Haack reaction of 6-bromo-2-naphthol with sodium hydride, *n*-BuLi, and DMF,[26] respectively.



Scheme 2. Syntheses of TP compounds **2d**, **2f**, **6a**, and **6b**.

3-Hydroxynaphthalenecarboxaldehyde (**10d**) was similarly made from the metallation/Vilsmeier-Haack reaction of 4-bromo-2-naphthol,[27] which derived from a sequence of bromination, oxydiazotization, and reduction of 1-aminonaphthalene.[28]

As described previously we used MC65 cell line to screen bioactive compounds.[4] The cells are readily propagated and cell death occurs after three days and is measured quantitatively by a simple 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.[4] Tetracycline (TC) is used to suppress the induction of SβC gene which produces C99 fragment of amyloid precursor protein (APP). Hence, in the presence of TC, MC65 cells survive, and absence of TC leads to cell death. In the absence of TC, compounds that protect neuron cell death could be used for screening of new leads in anti-Aβ. In the presence of TC and the compound, toxicity of the compound to MC65 cells is revealed. The EC_{50} (median effective concentration), TD_{50} (median toxic concentration), and TI (therapeutic index; equals to TD_{50}/EC_{50}) values of various TP compounds are listed in Table 1. Compounds **2b**, **2e**, **3f**, and **4a** showed the greatest potencies in protecting MC65 cells death having EC_{50} values of 94, 91, 92, and 95 nM, respectively. These four compounds are more active than our initial lead compound, **1** (CP2), EC_{50} value of 120 nM. It appears that a hydroxyl (**2b** and **4a**) or hydroxymethyl (**2e**) substituent at the *para*-position of the C13 phenyl- and naphthyl-methylamino moieties (for numbering, see structures **2** – **5** of Scheme 1) enhances the bioactivity, and additional fluorine atom at the *meta*-position provides the highest activity. Other substituents such as hydrogen, methoxy, 2-hydroxyethyloxy, aldehyde, ester, chlorine, and dimethylamino at the *para*-position of the phenylmethylamino group decrease the activity. The *para*-cyano group of compound **2i** abolishes the activity. The *ortho*- or *meta*-hydroxyl group lowers the activity. It is encouraging that additional functionality to the phenyl ring of compound **2b** only lowers the activity moderately and fluorine increases the activity (compound **3f**), implying further modification of compound **2b** is possible. Other regioisomers such as **4c**, **4d**, and **5** of 4-hydroxynaphthyl analog **4a** also possess weaker activities.

Amide **6b** showed poor activity and its acetyl ester analog, **6a**, is inactive, hence, other amide derivatives were not investigated. Notably, data presented in our previous report suggests that TP compounds achieve MC65 protection neither by inhibiting γ-secretase-catalyzed Aβ production, nor by a general anti-oxidation effect.[6]

Table 1. EC_{50}, TD_{50} and TI values of Compounds **1** – **6** from MC65 cell protection assays.[29]

Compound	EC_{50} (μM)	TD_{50} (μM)	TI
1	0.120 ± 0.015	39.0 ± 1.12	325
2a	3.24 ± 0.160	37.3 ± 1.77	11.5
2b	0.094 ± 0.035	49.3 ± 0.127	524
2c	2.77 ± 0.191	>50	>18.1
2d	24.5 ± 1.31	>50	>2.04
2e	0.091 ± 0.027	>50	>549
2f	6.41 ± 0.961	39.4 ± 1.87	6.15
2g	0.769 ± 0.033	>50	>65.0
2h	2.79 ± 0.107	48.0 ± 0.080	17.2
2i	>50	>50	–
2j	4.36 ± 0.055	38.2 ± 1.02	8.76
3a	2.44 ± 0.111	30.7 ± 0.429	12.6
3b	4.66 ± 0.339	>50	>10.7
3c	0.242 ± 0.014	26.3 ± 1.34	109
3d	3.85 ± 0.416	>50	>13.0
3e	0.639 ± 0.001	>50	>78.2
3f	0.0920 ± 0.012	>50	>543
3g	1.56 ± 0.025	>50	>32.1
3h	0.662 ± 0.070	9.38 ± 0.217	14.2
3i	1.18 ± 0.010	>50	>42.4
3j	1.26 ± 0.182	>50	>39.7
4a	0.095 ± 0.297	13.7 ± 0.704	144
4b	0.621 ± 0.051	15.3 ± 0.183	24.6
4c	0.198 ± 0.013	26.4 ± 0.264	133
4d	0.586 ± 0.045	18.2 ± 1.84	31.1
5	0.459 ± 0.026	24.1 ± 1.48	52.5
6a	>50	8.35 ± 0.319	–
6b	6.25 ± 0.171	6.69 ± 0.105	1.07

In light of the finding of alleviation of cholesterol accumulation in Huntington's disease neurons by CP2[30] and to explore other proteins that TPs may affect, inhibitions of ACAT in MC65 cells by five most active compounds selected from MC65 assay, **1**, **2b**, **2e**, **3f**, and **4a**, were carried out. Cells were incubated with mock-medium, TP compounds and CI-976 (an ACAT inhibitor) for 24 h. ACAT activities were examined by staining with NBD-cholesterol, which is a fluorescent probe for cholesteryl ester (CE)-rich lipid droplets.[31] The intensity of fluorescence were measured on a fluorescent plate reader equipped with 485 nm excitation and 535 nm emission filters. The ACAT activity in the presence of each compound was assessed by the comparison of fluorescence intensity with mock-treated cells. Like CI-976, the incubation with TP compounds significantly reduced the fluorescence intensity in MC65 cells, and results are summarized in Table 2. Compound **2b** with IC_{50} value of 0.3 μM possesses similar inhibitory activity as that of CI-976. Compounds **1**, **2e**, **3f**, and **4a** are slightly less active with IC_{50} values in the range of 0.8 – 1.8 μM. It appears that TPs' ACAT inhibitory activities are correlated with MC65 cells protective activities. Anti-ACAT effects of each compound were also confirmed in human hepatoma cells (Huh-7 cells) and similar results were obtained (data not shown).

The inhibition of ACAT may increase the level of free cholesterol, subsequently induce oxidation of cholesterol (oxycholesterol) and activate LXR pathway. We examined whether these compounds induced the expression of cholesterol efflux-related protein gene, ABCA1, in MC65 cells. Cells were incubated with mock-medium, TP compounds, and CI-976 for 24 h, and expression of the gene was assessed with the Gene Expression Assay.[32] The treatment with compounds **1**, **2b**, **4a** and CI-976 in both MC65 (Table 2) and Huh-7 cells, significantly increased the expression of ABCA1 with EC_{50} values in the range of 0.6 – 1.1 μM, compared to mock-treated cells (Table 2). Compounds **2e** and **3f** are less active having EC_{50} values of 2.5 and 2.2 μM, respectively. Because TP compounds and CI-976 inhibit ACAT activity in the cells and the role of ACAT in cholesterol homeostasis, we speculate that the induction of expression is due to the inhibition of ACAT activity.

Table 2. ACAT inhibitory activity and increase of ABCA1 gene expression of the most active TP compounds and CI-976 in MC65 cells.

Compound	IC_{50} value of ACAT inhibition (μM)	EC_{50} value of ABCA1 gene expression (μM)
1 (CP2)	1.2 ± 0.2	0.9 ± 0.1
2b	0.3 ± 0.08	1.1 ± 0.1
2e	1.4 ± 0.2	2.5 ± 0.4
3f	1.8 ± 0.3	2.2 ± 0.2
4a	0.8 ± 0.06	1.3 ± 0.1
CI-976	0.2 ± 0.1	0.6 ± 0.07

CONCLUSION

Newly synthesized TP compounds containing 4-(hydroxymethyl)phenyl-, 4-hydroxyphenyl-, and 4-hydroxynaphthyl-methylamino moiety at C13 of the tricyclic pyrone skeleton possess strong cell protective properties against intracellularly induced Aβ toxicity, inhibitory activities against ACAT, and enhancing properties of ABCA1 cholesterol transporter gene in nanomolar to low micromolar ranges. Additional fluorine atom at C3 on the 4-hydroxyphenyl ring of compound **2b** retains cell protective activity. The therapeutic index values of the TP compounds in MC65 cells are high (>100) and they may serve as lead compounds for the discovery of AD drugs.

EXPERIMENTAL SECTION

Chemistry. A representative synthesis of compound **2b** is described below. The general bioassays, experimental information, and synthesis of all other compounds are supplied in the Supporting Information. Purity of all final compounds determined by HPLC analysis is >95%.

(5a*S*,7*S*)-3-Methyl-7-[(1*R*) and (1*S*)1–(4-hydroxybenzylamino)propan-2-yl]-1H,7H-5a,6,8,9-tetrahydro-1-oxopyrano[4,3-b][1]benzopyran (2b): A solution of 85 mg (0.31 mmol) of amine **7** and 38 mg (0.31 mmol) of aldehyde **8b** in 5 mL of dry MeOH was stirred under argon at 25°C for 12 h. To it, were added acetic acid (5 drops) and a solution of 68 mg (1.1 mmol) of $NaBH_3CN$ in MeOH. After stirring for 1 h, the reaction solution was diluted with 40 mL of 5% aqueous ammonium hydroxide and extracted three times with dichloromethane. The combined organic layer was washed with brine, dried ($MgSO_4$), concentrated, and column chromatographed on silica gel using a gradient mixture of CH_2Cl_2 and MeOH as eluant to give 80 mg (68% yield) of light yellow solid **2b**. ^{1}H NMR δ 7.16 (d, *J* = 8.0 Hz, 2 H), 6.76 (d, *J* = 8.4 Hz, 2 H), 6.06 (s, 1 H), 5.71 (s, 1 H), 5.09 – 4.99 (m, 1 H), 3.70 (s, 2 H), 2.66 – 2.57 (m, 1 H), 2.51 – 2.39 (m, 2 H), 2.19 (s, 3 H), 2.07 – 1.90 (m, 2 H), 1.73 – 1.47 (m, 4 H), 1.29 – 1.08 (m, 1 H), 0.90 (d, *J* = 6.4 Hz, 1.5 H, CH_3), 0.89 (d, *J* = 6.4 Hz, 1.5 H, CH_3); ^{13}C NMR δ 163.7, 163.6, 163.0, 161.8, 156.5, 133.0, 129.9, 129.7, 115.9, 109.1, 100.1, 97.5, 79.7, 79.65, 53.5, 52.8, 52.7, 39.2, 38.8, 38.7, 37.2, 37.1, 36.8, 32.4, 32.3, 31.0, 28.4, 20.2, 14.7, 14.6; MS (electrospray ionization) m/z 382.4 ($M+H^+$), 276.5; HRMS calcd for $C_{23}H_{28}NO_4^+$ ($M+H^+$) 382.2018, found 382.2013.

ASSOCIATED CONTENT

Supporting Information. Experimental procedures and spectroscopic data for compounds **2** – **6**, detailed protocols for MC65 cells assay, inhibition of ACAT, and upregulation of ABCA1. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*For D.H.H.: phone, 785–532–6699; fax, 785–532–6666; E-mail, duy@ksu.edu; address, Department of Chemistry, 213 CBC Building, Kansas State University, Manhattan, KS 66506, United States.

Author Contributions

Professors Hua, Jin, and Chang directed and designed the chemistry and biological chemistry. Mr. Pokhrel and Ms. Nguyen carried out the chemical synthesis, Dr. Maezawa conducted the MC65 cell assays, and Dr. Chang performed the inhibition of ACAT and expression of ABCA1 transporter gene.

Funding Sources

This work was generously supported by the NIH grant RO1 AG025500 to DHH and LWJ, U01 AI081891 and K-INBRE 5P20RR016475–11 to KOC and DHH.

ACKNOWLEDGMENT

We thank Mr. Mahendra Thapa for technical assistance with the synthesis of compounds **6a** and **6b**.

ABBREVIATIONS

AβO, amyloid beta oligomers; ACAT, acyl-CoA:cholesterol acyltransferase; ABCA1, ATP-binding cassette sub-family A member 1; AD, Alzheimer's disease; APP, amyloid precursor protein; APOE, apolipoprotein E; CE, cholesteryl ester; DMF, *N,N*-dimethylformamide; DMSO, dimethyl sulfoxide; EDC, 3–(3-dimethylaminopropyl)-1-ethylcarbodiimide hydrochloride; IBX, 2-iodoxybenzoic acid; IC_{50}, inhibition concentration at 50%; LXR, liver X receptor; MTT, 3–(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NBD, 25-[*N*-[(7-nitro-2–1,3-benzoxadiazol-4-yl)methyl]amino]-27-norcholesterol; NHS, *N*-hydroxysuccinimide; TC, tetracycline; TP, tricyclic pyrone.

REFERENCES

1. Reitz, C. Alzheimer's disease and the amyloid cascade hypothesis: a critical review. *Int. J. Alz. Dis*. **2012**, article ID 369808, 11 pages.
2. Jin, J. –W.; Hua, D. H.; Shie, F. –S.; Maezawa, I.; Sopher, B.; Martin, G. M. Novel tricyclic pyrone compounds prevent intracellular APP C99-induced cell death. *J. Mol. Neurosci*. **2002**, *19*, 57–61.
3. 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. A novel tricyclic pyrone compound ameliorates cell death associated with intracellular amyloid-beta oligomeric complexes. *J. Neurochem*. **2006**, *98*, 57–67.
4. Hong, H. –S.; Maezawa, I.; Yao, N.; Diaz-Avalos, R.; Rana, S.; Hua, D. H.; Cheng, R. H.; Lam, K. S.; Jin, L. -W. Combining the rapid MTT formazan exocytosis assay and the MC65 protection assay led to the discovery of carbazole analogs as small molecule inhibitors of Aβ oligomer-induced cytotoxicity. *Brain Research*, **2007**, *1130*, 223–234.
5. Jin, L. -W.; Shie, F. S,; Maezawa, I.; Vincent, I.; Bird, T. Intracellular accumulation of amyloidogenic fragments of amyloid-beta precursor protein in neurons with Niemann-Pick type C defects is associated with endosomal abnormalities. *Am. J. Pathol*. **2004**, *164*, 975–985.
6. Hong, H. S.; Rana, S.; Barrigan, L.; Shi, A.; Zhang, Y.; Zhou, F.; Jin, L. –W.; Hua, D. H. A novel tricyclic pyrone compound ameliorates cell death associated with intracellular amyloid-β oligomeric complexes. *J. Neurochem*. **2009**, *108*, 1097–1108.

7. Wolozin, B. Cholesterol and the biology of Alzheimer's disease. *Neuron* **2004**, *41*, 7–10.

8. Patel, N.V.; Forman, B. M. Linking lipids, Alzheimer's and LXRs? *Nucl. Recept. Signal* **2004**, *2*, p. e001.

9. Fukumoto, H.; Deng, A.; Irizarry, M. C.; Fitzgerald, M. L.; Rebeck, G. W. Induction of the cholesterol transporter ABCA1 in central nervous system cells by liver X receptor agonists increases secreted Abeta levels. *J. Biol. Chem*. **2002**, *277*, 48508–48513.

10. Kim, W. S.; Chan, S. L.; Hill, A. F.; Guillemin, G. J.; Garner, B. Impact of 27-hydroxycholesterol on amyloid-beta peptide production and ATP-binding cassette transporter expression in primary human neurons. *J. Alzheimers Dis*. **2009**, *16*, 121–131.

11. Mahley, R. W.; Weisgraber, K. H.; Huang, Y. Apolipoprotein E4: a causative factor and therapeutic target in neuropathology, including Alzheimer's disease. *Proc. Natl. Acad. Sci. U. S. A*. **2006**, *103*, 5644–5651.

12. Puglielli, L.; Tanzi, R. E.; Kovacs, D. M. Alzheimer's disease: the cholesterol connection. *Nature Neurosci*. **2003**, *6*, 345–351.

13. Huttunen, H. J.; Kovacs, D. M. ACAT as a drug target for Alzheimer's disease. *Neurodegener. Dis.* **2008**, *5*, 212–214.

14. Hutter-Paier, B.; Huttunen, H. J.; Puglielli, L.; Eckman, C. B.; Kim, D. Y.; Hofmeister, A.; Moir, R. D.; Domnitz, S. B.; Frosch, M. P.; Windisch, M.; Kovacs, D. M. The ACAT inhibitor CP-113,818 markedly reduces amyloid pathology in a mouse model of Alzheimer's disease. *Neuron* **2004**, *44*, 227–238.

15. Schmitz, G.; Robenek, H.; Beuck, M.; Krause, R.; Schurek, A.; Niemann, R. Ca^{++} antagonists and ACAT inhibitors promote cholesterol efflux from macrophages by different mechanisms. I. Characterization of cellular lipid metabolism. *Arterioscler. Thromb. Vasc. Biol.* **1988**, *8*, 46–56.

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. Syntheses and Bioactivities of Tricyclic Pyrones. *Tetrahedron* **2003**, *59*, 4795–4803.

17. Pokhrel, L.; Kim, Y.; Nguyen, T. D. T.; Prior, A. M.; Lu, J.; Chang, K. –O.; Hua, D. H. Synthesis and anti-norovirus activity of pyranobenzopyrone compounds. *Bioorg. & Med. Chem. Lett*. **2012**, *22*, 3480–3484.

18. Nicolaou, K. C.; Montagnon, T.; Baran, P. S.; Zhong, Y. –L. Iodine(V) reagents in organic synthesis. Part 4. o-Iodoxybenzoic acid as a chemospecific tool for single electron transfer-based oxidation processes. *J. Am. Chem. Soc*. **2002**, *124*, 2245–2258.

19. Kemp, S. J.; Storey, L. J.; Storey, J. M. D.; Richard, J.; Harrington, C. R.; Wischik, C. M.; Clunas, S.; Heinrich, T. K. Preparation of benzothiazole, imidazothiazole, imidazopyrimidine and imidazopyridine compounds as ligands for aggregated tau molecules useful in diagnosis, prognosis and treatment of diseases. PCT Int. Appl. **2010**, WO 2010034982 A1 20100401.

20. Mak, C. C.; Bampos, N.; Darling, S. L.; Montalti, M.; Prodi, L.; Sanders, J. K. M. A strategy for the assembly of multiple porphyrin arrays based on the coordination chemistry of Ru-centered porphyrin pentamers. *J. Org. Chem*. **2001**, *66*, 4476–4486.

21. Meyer, E.; Joussef, A. C.; Gallardo, H.; de Souza, L. de B. P. Synthesis of new 4,5-dihydro-3(2H)-pyridazinone derivatives. *Syn. Commun*. **2004**, *34*, 783–793.

22. Silva, P. L.; Pires, P. A. R.; Trassi, M. A. S.; El Seoud, O. A. Solvation in pure liquids: what can be learned from the use of pairs of indicators? *J. Phys. Chem. B.* **2008**, *112*, 14976–14984.

23. Xi, F.; Kamal, F.; Schenerman, M. A. A novel and convenient transformation of nitriles to aldehydes. *Tetrahedron Lett*. **2002**, *43*, 1395–1396.

24. Hua, D. H.; Huang, X.; Chen, Y.; Battina, S. K.; Tamura, M.; No, S. –K.; Koo, S. I.; Namatame, I.; Tomoda, H.; Perchellet, E. M.; Perchellet, J. P. Total Syntheses of (+)-Chloropuupehenone and (+)-Chloropuupehenol, and their Analogs, and Evaluation of their Bioactivities. *J. Org. Chem*. **2004**, *69*, 6065–6078.

25. Goel, M.; Jayakannan, M. Supramolecular liquid crystalline π-conjugates: the role of aromatic π-stacking and van der Waals forces on the molecular self-assembly of oligophenylenevinylenes. *J. Phys. Chem. B.* **2010**, *114*, 12508–12519.

26. Chamontin, K.; Lokshin, V.; Rossollin, V.; Samat, A. Synthesis and reactivity of formyl-substituted photochromic 3,3-diphenyl-[3H]-naphtho[2,1-b]pyrans. *Tetrahedron* **1999**, *55*, 5821–5830.

27. Newman, M. S.; Sankaran, V.; Olson, D. R. Phenolic and ketonic tautomers in polycyclic aromatic hydrocarbons. *J. Am. Chem. Soc*. **1976**, *98*, 3237–3242.

28. Hodgson, H. H.; Birtwell, S. The diazotization of 1-amino-2-hydroxy-4-cyanonaphthalene and the preparation of 4-cyano-2-naphthol. *J. Chem. Soc*. **1944**, 539–540.

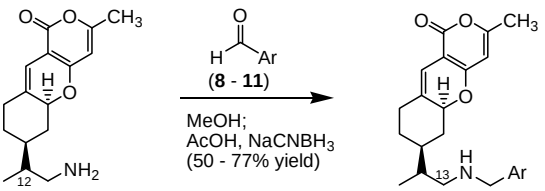
29. Each experiment was performed in triplicate and standard errors were calculated.

30. Trushina, E.; Rana, S.; McMurray, C. T.; Hua, D. H. Treatment with tricyclic pyrone compounds reverses cellular phenotype caused by expression of mutant huntingtin protein in striatal neurons. *BMC Neurosci*. **2009**, *10*:73 (Open Access; online publication; 14 pages).

31. Lada, A.T.; Davis, M.; Kent, C.; Chapman, J.; Tomoda, H.; Omura, S.; Rudel, L. L. Identification of ACAT1- and ACAT2-specific inhibitors using a novel, cell-based fluorescence assay: individual ACAT uniqueness. *J. Lipid Res*. **2004**, *45*, 378–386.

32. Chawla, A.; Boisvert, W. A.; Lee, C. H.; Laffitte, B. A.; Barak, Y.; Joseph, S. B.; Liao, D.; Nagy, L.; Edwards, P. A.; Curtiss, L. K.; Evans, R. M.; Tontonoz, P. A PPARg-LXR-ABCA1 pathway in macrophages is involved in cholesterol efflux and atherogenesis. *Mol. Cell* **2001**, *7*, 161–171.

Inhibition of ACAT, Overexpression of Cholesterol Transporter Gene, and Protection of Amyloid β Oligomers-Induced Neuronal Cell Death by Tricyclic Pyrone Molecules


O
O
CH3
H
O
12
NH2
O
H
Ar
(8 - 11)
MeOH;
AcOH, NaCNBH3
(50 - 77% yield)
O
O
CH3
H
O
13
H
N
Ar