Bioorganic & Medicinal Chemistry Letters 25 (2015) 811–814

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Evaluation of synthetic naphthalene derivatives as novel chemical chaperones that mimic 4-phenylbutyric acid

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ARTICLE INFO

Article history:
Received 18 October 2014
Revised 22 December 2014
Accepted 24 December 2014
Available online 5 January 2015

Keywords:
Chemical chaperone
4-Phenylbutyric acid (4-PBA)
Naphthalene
Parkinson's disease (PD)
Endoplasmic reticulum (ER) stress

ABSTRACT

The chemical chaperone 4-phenylbutyric acid (4-PBA) has potential as an agent for the treatment of neurodegenerative diseases. However, the requirement of high concentrations warrants chemical optimization for clinical use. In this study, novel naphthalene derivatives with a greater chemical chaperone activity than 4-PBA were synthesized with analogy to the benzene ring. All novel compounds showed chemical chaperone activity, and **2** and **5** possessed high activity. In subsequent experiments, the protective effects of the compounds were examined in Parkinson's disease model cells, and low toxicity of **9** and **11** was related to amphiphilic substitution with naphthalene.

Aggregation of insoluble proteins is common to neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD), involving phosphorylated tau and amyloid-β (Aβ) peptides, and α-synuclein, respectively. Dysfunctions associated with these aggregates have prompted the discovery of genetic predispositions and inhibitors for use as prophylactics or treatments. In particular, geldanamycin induces molecular chaperones and suppresses neurodegeneration in a drosophila model of PD.[1,2]

Molecular chaperones such as heat shock proteins (Hsps) are required for the functional folding of nascent proteins and the repair of higher-order protein structure. In particular, Hsp70 reduces the overexpression of Aβ, which is the major component of neurofibrillary tangles in AD, and protects neuronal cells from Aβ-dependent neurotoxicity in vivo and in vitro.[3,4] Moreover, inducible Hsp70 is protective against ischemic injury in vivo and in vitro.[5]

Although chemical chaperones are low molecular weight compounds, their actions are similar to those of molecular chaperones, and the well-known molecular chaperone 4-PBA prevents the aggregation of denatured proteins. In previous studies, 4-PBA has been shown to protect against cerebral ischemic injury and endoplasmic reticulum (ER) stress-induced neuronal cell death,[6,7] and

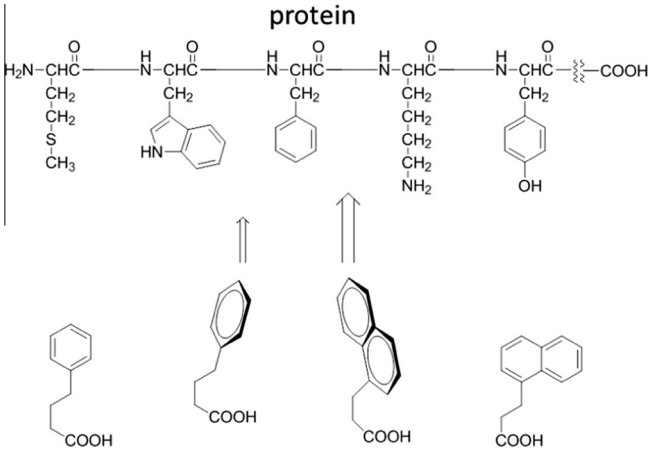

Figure 1. Strategy for synthesis of novel chemical chaperones.

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http://dx.doi.org/10.1016/j.bmcl.2014.12.080
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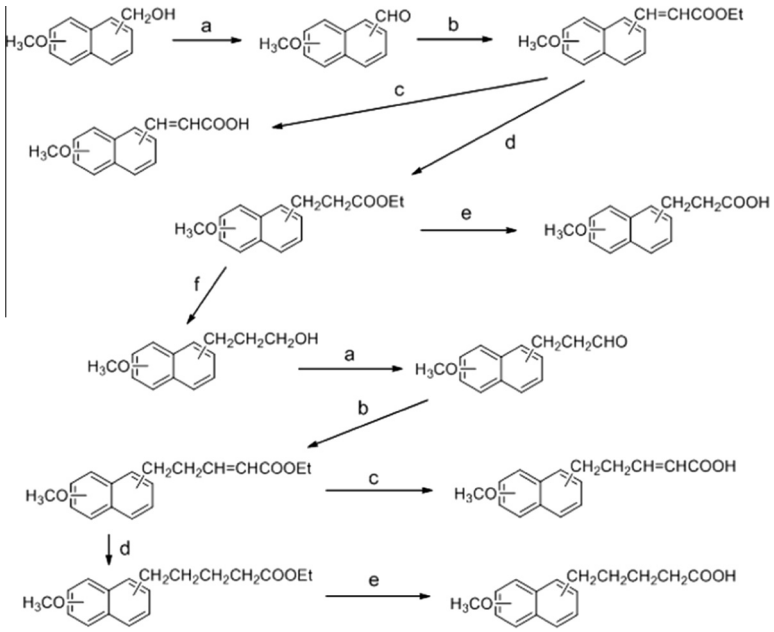

Scheme 1. Reagents and conditions: (a) pyridinium chlorochromate (PCC), methylene chloride, RT, 2–4 h, 64–72%; (b) ethyl diethylphosphonoacetate, NaH, THF, RT, 66–99%; (c) alkaline hydrolysis, EtOH, 10% NaOH, RT, 83–97%; (d) H_2, 5% palladium carbon, AcOEt, RT, 56–90%; (e) alkaline hydrolysis, EtOH, 10% NaOH, RT, 80–98%; (f) $LiAH_4$, THF, RT, 1.5–3 h, 91–97%.

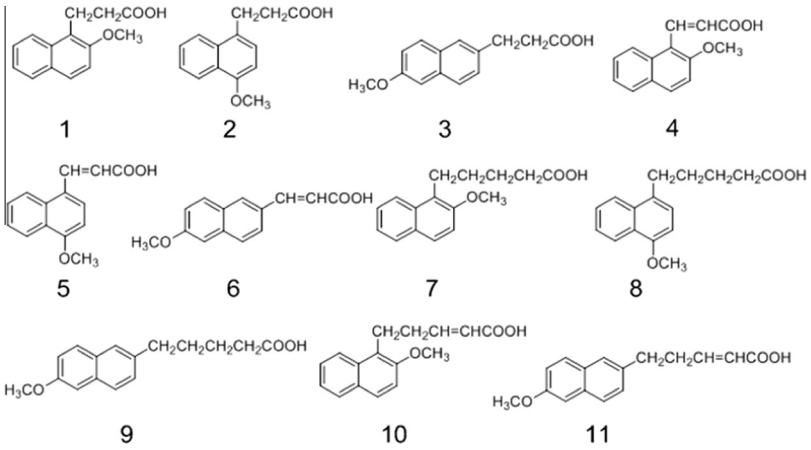

Figure 2. Structures of synthetic naphthalene analogs.

significant neuroprotective effects are shown in mouse models of AD and PD.[8–10] Moreover, 4-PBA has been approved for clinical use as an ammonia scavenger in children with urea cycle disorders.[11] Recently, 4-PBA has also shown remarkable potential as a novel therapeutic agent for type 2 diabetes[12] and familial hypercholesterolemia.[13]

Although these effects are promising, the required doses of 4-PBA are prohibitively high. Thus, we designed synthetic 4-PBA analogs to optimize chemical chaperone activity against denatured proteins.

In a recent study, we showed that chemical chaperone activities and protective effects against ER stress-induced neuronal cell death are dependent on the numbers of fatty acid carbon atoms on 4-PBA derivatives.[14] In further studies, we evaluated the chemical chaperone activities of synthetic methoxy-substituted 4-PBA derivatives.[15] Subsequently, we showed that although 4-PBA inhibits histone deacetylase (HDAC), it acts as a chemical chaperone and protects against ER stress-induced neuronal cell death.[16]

Previous data indicate that the benzene ring of 4-PBA is essential for chemical chaperone activity, suggesting that hydrophobic amino acids are involved in protein aggregation (Fig. 1). Thus, we assumed that the electron clouds of benzene rings interact with the aggregates of denatured proteins, and intended to expand the electron cloud area by synthesizing 4-PBA analogs based on naphthalene.

Chemistry: Naphthalene derivatives were synthesized according to a previous study[15] as shown in Scheme 1. Additional details of the synthetic process are presented in Supporting information[15] and all synthesized compounds are shown in Figure 2. All derivatives were characterized using spectrometric methods,[17] and ^1H NMR experiments were performed using a Unity 400 MHz spectrometer. Silica gel column chromatography was conducted using a Merck Silica gel 60 (0.040–0.063; 230–400 mesh ASTM).

Biology: Initially, we examined the effects of chemical chaperones on the aggregation of r-LA with BSA in vitro, as previously described.[14,16] All compounds inhibited the aggregation of

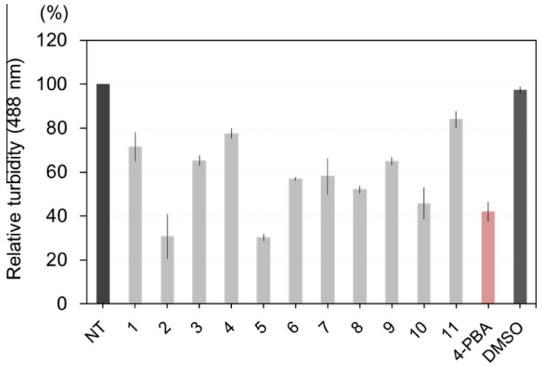

Figure 3. Inhibitory effects of naphthalene analogs on the aggregation of reduced α-lactalbumin. Experiments were conducted as previously described.[14–16] Briefly, r-LA was prepared by incubating α-LA with 5 mM dithiothreitol and 2.5 mM ethylenediamine tetraacetic acid at 25 °C for 30 min. Aggregation of r-LA was induced by the addition of denatured bovine serum albumin at 37 °C. Protein aggregation was monitored by measuring optical density at 488 nm (turbidity) after the addition of a vehicle (DMSO), 0.3 mM test compound, or 3 mM 4-PBA; NT indicates no treatment. Turbidities in the presence of naphthalene derivatives are expressed relative to corresponding controls and are presented as means ± SE of four independent experiments.

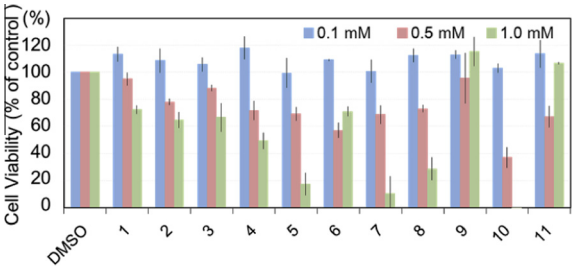

Figure 4. Protective effects of synthetic naphthalene derivatives on Pael-R-induced cell death in human neuroblastoma SH-SY5Y cells. Experiments were conducted as we previously described.[14] Briefly, SH-SY5Y cells stably expressing Pael-R–FLAG were incubated in the presence or absence of indicated naphthalene derivatives at 0.1, 0.5, or 1.0 mM for 48 h. About 10% of control Pael-R overexpressing cells died. Cell viability was determined using crystal violet assays; Data are presented as means ± SE of three independent experiments.

denatured BSA and r-LA (Fig. 3), and although 4-PBA was active at 3 mM, synthetic compounds were active at 0.3 mM. However, further studies are required to elucidate the associated structure–activity relationships.

Pael-R is a substrate of the protein Parkin,[18] which is a PD-associated ubiquitin ligase E3.[19] Thus, in subsequent experiments, we investigated the protective effects of the naphthalene derivatives on Pael-R-induced cell death in SH-SY5Y cells using crystal violet assays, as previously described.[14] At 0.1 mM, the present synthetic analogs were not significantly more effective than 4-PBA (Fig. 4). Moreover, naphthalene has been used as an insecticide and may have exerted some toxicity.

In conclusion, we have developed naphthalene based analogs of 4-PBA that have wider electron clouds and exhibit chemical chaperone activity. Among the tested naphthalene derivatives, **9** had the weakest toxicity and may protect against Pael-R-induced cell death. However, no significant protective effects were observed in PD model cells. Thus, in further studies, we will examine atomic substitutes for carbon in chemical chaperones.

Acknowledgments

This work was partly supported by a Sasakawa Scientific Research Grant from The Japan Science Society. In addition, this work was supported by Grants-in-Aid for Scientific Research (24790085, 245901119, and 24300135) from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

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17. *Spectral data for representative compounds:* 3-(2-Methoxynaphthalen-1-yl)propanoic acid (**1**): ^{1}H NMR (400 MHz; $CDCl_3$): δ 4.13 (2H, t, J = 16 Hz, C_2-H), 6.54 (2H, t, J = 16 Hz, C_3-H), 6.90 (3H, s, $-OCH_3$), 7.62–7.86 (1H, m, naph-H), 8.24 (2H, m, naph-H), 8.31 (1H, d, J = 8.3 Hz, naph-C_5-H), 8.64 (1H, d, J = 16 Hz, naph-C_8-H) HRMS (EI method): Calcd for $C_{14}H_{14}O_3$ (M^+): 230.0943. Found: 230.0943. Mp: 132–133 °C. 3-(4-Methoxynaphthalen-1-yl)propanoic acid (**2**): ^{1}H NMR (400 MHz; $CDCl_3$): δ 4.05 (2H, m, C_2-H), 6.53 (2H, t, J = 16 Hz, C_3-H), 6.89 (3H, s, J = 8.1 Hz, $-OCH_3$) 7.55–7.84 (1H, m, naph-H), 8.24 (2H, m, naph-H), 8.25 (1H, d, J = 8.3 Hz, naph-C_5-H), 8.60 (1H, d, J = 16 Hz, naph-C_8-H) HRMS (EI method): Calcd for $C_{14}H_{14}O_3$ (M^+): 230.0943. Found: 230.0943. Mp: 170–172 °C. 3-(6-Methoxynaphthalen-2-yl)acrylic acid (**3**): ^{1}H NMR (400 MHz; $CDCl_3$): δ 2.76 (2H, t, J = 8.0, C_2-H), 3.09 (2H, t, J = 8.0 Hz, C_3-H), 3.91 (3H, s, $-OCH_3$), 7.10–7.69 (6H, m, naph-H) HRMS (EI method): Calcd for $C_{14}H_{14}O_3$ (M^+): 230.0943. Found: 230.0943. Mp: 158–161 °C. 3-(2-Methoxynaphthalen-1-yl)acrylic acid (**4**): ^{1}H NMR (400 MHz; $CDCl_3$): δ 4.03 (3H, s, $-OCH_3$), 6.84 (1H, d, J = 16 Hz, C_3-H), 7.29–7.54 (3H, m, naph-H), 7.80 (1H, d, J = 8.0 Hz, naph-H), 7.88 (1H, d, J = 9.0 Hz, naph-H), 8.21 (1H, d, J = 8.6 Hz, naph-H), 8.48 (1H, d, J = 16 Hz, C_2-H) HRMS (EI method): Calcd for $C_{14}H_{12}O_3$ (M^+): 228.0787. Found: 228.0788. Mp: 162–164 °C. 3-(4-Methoxynaphthalen-1-yl)acrylic acid (**5**): ^{1}H NMR (400 MHz; $CDCl_3$): δ 4.06 (3H, s, $-OCH_3$), 6.46 (1H, d, J = 16 Hz, naph-C_3-H), 6.88 (1H, d, J = 8.1 Hz), 7.53–7.82 (2H, m, naph-H), 8.20 (1H, d, J = 8.4 Hz, naph-H), 8.32 (1H, d, J = 8.3 Hz, naph-H), 8.57 (1H, d, J = 16 Hz, naph-C_8-H) HRMS (EI method): Calcd for $C_{14}H_{12}O_3$ (M^+): 228.0787. Found: 228.0789. Mp: 216–220 °C. 3-(6-Methoxynaphthalen-2-yl)propanoic acid (**6**): ^{1}H NMR (400 MHz; $CDCl_3$): δ 3.49 (3H, s, $-OCH_3$), 3.95 (1H, d, J = 8.0 Hz, C_2-H), 6.53 (1H, d, J = 8.0 Hz, C_3-H), 7.14–8.26 (6H, m, naph-H) HRMS (EI method): Calcd for $C_{14}H_{12}O_3$ (M^+): 228.0787. Found: 228.0788. Mp: 215–217 °C. 5-(2-Methoxynaphthalen-1-yl)pentanoic acid (**7**): ^{1}H NMR (400 MHz; $CDCl_3$): δ 1.69 (4H, m), 2.42 (2H, t, J = 7.4 Hz, C_2-H), 3.10 (2H, t, J = 7.6 Hz, C_5-H), 3.93 (3H, s, $-OCH_3$), 7.25 (1H, d, naph-C_3-H), 7.27–7.47 (2H, m, naph-H), 7.73 (1H, m, naph-C_4-H), 7.79 (1H, m, naph-C_5-H), 7.93 (1H, d, J = 8.6 Hz, naph-C_8-H) HRMS (EI method): Calcd for $C_{16}H_{18}O_3$ (M^+): 258.1256. Found: 258.1256. Mp: 87–89 °C. 5-(4-Methoxynaphthalen-1-yl)pentanoic acid (**8**): ^{1}H NMR (400 MHz; $CDCl_3$): δ 1.75–1.79 (2H, m), 2.40 (2H, m), 3.01 (2H, m, C_5-H), 3.98 (3H, s, $-OCH_3$), 6.73 (1H, d, J = 7.8 Hz, naph-C_3-H), 7.21 (1H, d, J = 7.8 Hz, naph-C_2-H), 7.46 (2H, m), 7.94 (1H, d, J = 8.0 Hz, naph-C_5-H), 8.28 (1H, d, J = 1.3 Hz, naph-C_8-H) HRMS (EI method): Calcd for $C_{16}H_{18}O_3$ (M^+): 258.1256. Found: 258.1257. Mp: 141–143 °C. 5-(6-Methoxynaphthalen-2-yl)pentanoic acid (**9**): ^{1}H NMR (400 MHz; $CDCl_3$): δ 1.25–1.72 (4H, m), 2.39 (2H, t, J = 5.0 Hz, C_2-H), 2.76 (2H, t, J = 6.3 Hz, C_5-H), 3.91 (3H, s, $-OCH_3$), 7.10 (2H, m, naph-H), 7.29 (1H, m, naph-H), 7.53 (1H, s, naph-H), 7.66 (2H, m, naph-H) HRMS (EI method): Calcd for $C_{16}H_{18}O_3$ (M^+): 258.1256. Found: 258.1257. Mp: 130–133 °C. 5-(4-Methoxynaphthalen-1-yl)-2-pentenoic acid (**10**): ^{1}H NMR (400 MHz; $CDCl_3$): δ 2.66 (2H, m, C_4-H), 3.16 (2H, t, J = 7.8 Hz, C_5-H), 3.98 (3H, s, $-OCH_3$), 5.89 (1H, d, J = 16 Hz, C_2-H), 7.16–7.22 (2H, m, naph-H), 7.48–7.55 (2H, m, naph-H), 7.90 (1H, d, J = 8.0 Hz, naph-C_5-H), 8.30 (1H, d, J = 1.2 Hz, naph-C_8-H)

HRMS (EI method): Calcd for $C_{16}H_{16}O_3$ (M^+): 256.1100. Found: 256.1100. Mp: 155–158 °C. 5-(6-Methoxynaphthalen-2-yl)-2-pentenic acid (**11**): ^{1}H NMR (400 MHz; $CDCl_3$): δ 2.62 (2H, q, J = 7.3 Hz, C_4-H), 2.90 (2H, t, J = 7.5 Hz, C_5-H), 3.91 (3H, s, $-OCH_3$), 5.87 (1H, d, J = 16 Hz, C_2-H), 7.14 (3H, m, naph-H), 7.26 (2H, m, naph-H), 7.55 (1H, s, naph-C_1-H), 7.68 (2H, m, naph-H) HRMS (EI method): Calcd for $C_{16}H_{16}O_3$ (M^+): 256.1100. Found: 256.1101. Mp: 149–151 °C.

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