Synthesis of NG-061 and Its Analogs, and Their Biological Evaluation as an Enhancer of Nerve Growth Factor

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A novel potentiator of nerve growth factor (NGF), NG-061, which had been isolated from the fermentation broth of *Penicillium minioluteum* F-4627, was synthesized from methoxybenzoquinone and phenylacetylhydrazine in a single step. A series of acyl hydrazone derivatives were also synthesized and their potentiator activity of neurotrophic effect of NGF on neurite outgrowth was evaluated by assay with a rat pheochromocytoma cell line PC12.

Key words NG-061; nerve growth factor; biological activity

Nerve growth factor (NGF) is a polypeptide which acts as a prototypical neurotrophic factor essential for growth and development of neurons in the central as well as peripheral nervous systems.[1,2] Survival and growth of cultured neuronal cells are also affected by such biological polypeptides as fibroblast growth factor. These neurotrophic factors have been shown to protect the neuron from dysfunction and death *in vivo* in animal models of injury and neurologic diseases. It was reported that NGF treatment could ameliorate age-related memory impairment and prevent lesion-induced loss of septal cholinergic neurons in rats.[3—5] Also known is that NGF can prevent neuronal loss of the hippocampus in the cerebral ischemia model in the Mongolian gerbil.[6] These findings suggest that NGF can be effective for the treatment of dementia and cerebral paralysis. However, it is very difficult to use NGF as a medicine since it must be administered to a patient intraventricularly because of its impermeability across the blood-brain barrier. To resolve these difficulties, it is anticipated that low molecular-weight compounds exhibiting and/or enhancing neurotrophic actions may have significant potential for development as therapeutic agenst to prevent neuronal cell death.

During the course of our screening program for low molecular-weight natural products with ability to potentiate and/or mimic the neurotrophic effect of NGF, we isolated a novel fungal metabolite, NG-061, from the fermentation broth of *Penicillium minioluteum* F-4627, and determined the structure by spectroscopic means and X-ray diffraction method as shown in Fig. 1.[7,8] In this paper, we wish to describe the synthesis of NG-061 and its analogs as well as their biological evaluation as a potentiator of NGF.

Results and Discussion

The synthesis of NG-061 was straightforward. Simple treatment of 2-methoxy-1,4-benzoquinone with phenylacetylhydrazide in $CHCl_3$ at room temperature for 2 d as shown in Fig. 2 gave, after recrystallization, **4a** in 12% yield. Spectroscopic data of synthesized **4a** was completely identical with those of natural NG-061. Further, a series of analogs of NG-061 were synthesized similarly as also shown in the figure. The compounds **4a**—**e**, **4g**, **4h**, **4j**, **4l**, **4m**, **4o**, **4q**—**u**, **4x**, and **4y** were obtained as a mixture of isomers (*ca.* 1 : 1—10 : 1). Because these compounds were derived from asymmetrical quinones, the presence of isomer appeared to be due to the geometrical isomer of hydrazone moiety. As reported previously,[8] natural NG-061 exists in equilibrium between the geometrical isomer in solution depending on the solvent: 1) *ca.* 4 : 1 in $CDCl_3$, 2) 8 : 1—10 : 1 in dimethyl sulfoxide (DMSO)-d_6, or 3) 1 : 1 in pyridine-d_5. Although not absolutely comfirmed, the above mentioned analogs should also be in equilibrium in solution.

The biological activity of the synthesized NG-061 and its analogs (**4a**—**y**) for the enhancement activity of neurite outgrowth effect of NGF was examined in PC12 cells, and the results are shown in Fig. 3. Unfortunately, since compounds **4c**—**e**, **4m**, **4r**—**u**, **4w**, and **4y** showed cytotoxicity against PC12 cells at the dose of 10 μg/ml, the activities of these compounds were not determined. The synthesized NG-061 **4a** showed the same level of activity as natural NG-061 in enhancing neurite outgrowth effect by NGF, therefore, the active principle from the fermentation broth of *Penicillium minioluteum* F-4627, has now been proven to be NG-061.

The structure–activity relationship of NG-061 was studied by structurally modifing it. Modification of the phenylacetyl moiety into an acetyl group compound **4b** significantly reduced the activity and slightly inhibited the effect of NGF. This result suggested that the presence of an aromatic ring in the acyl hydrazone moiety plays an important role in the biological acitivity. The replacement of the methoxy group of NG-061 into a hydrogen atom compound **4f** also reduced the activity. Introduction of alkyl groups into the quinone moiety generally diminished the activity of NG-061. Particularly, the bulky di-*tert*-butylated compounds **4k** and **4p** were found to

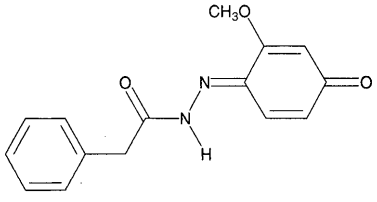

Fig. 1. Structure of NG-061

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Dedicated to the memory of Dr. Kyosuke Tsuda.

have an inhibitory effect against NGF. As far as tested, the synthetic analogs **4b**—**y** turned out to be less active or toxic, and the compound **4a** (NG-061) showed the highest activity.

During the synthetic studies of NG-061 and its analogs, we noticed that the low product yields were due to the formation of several by-products, and a major by-product was found to be the Michael adduct of acylhydrazide to the quinone-type structure. It was speculated that the Michael addition to NG-061 by a nucleophilic residue of a targeted biological polymer leading to a covalent bond formation might be a plausible mechanism of action of NG-061. In fact, the treatment of NG-061 with *N*-acetylcysteamine in the presence of triethylamine in a CH_2Cl_2 solution afforded, after auto-oxidation, the Michael addtion product **5** in moderate yield as shown in Fig. 4. Further detail of the mechanism of NG-061 action is intriguing and is to be investigated.

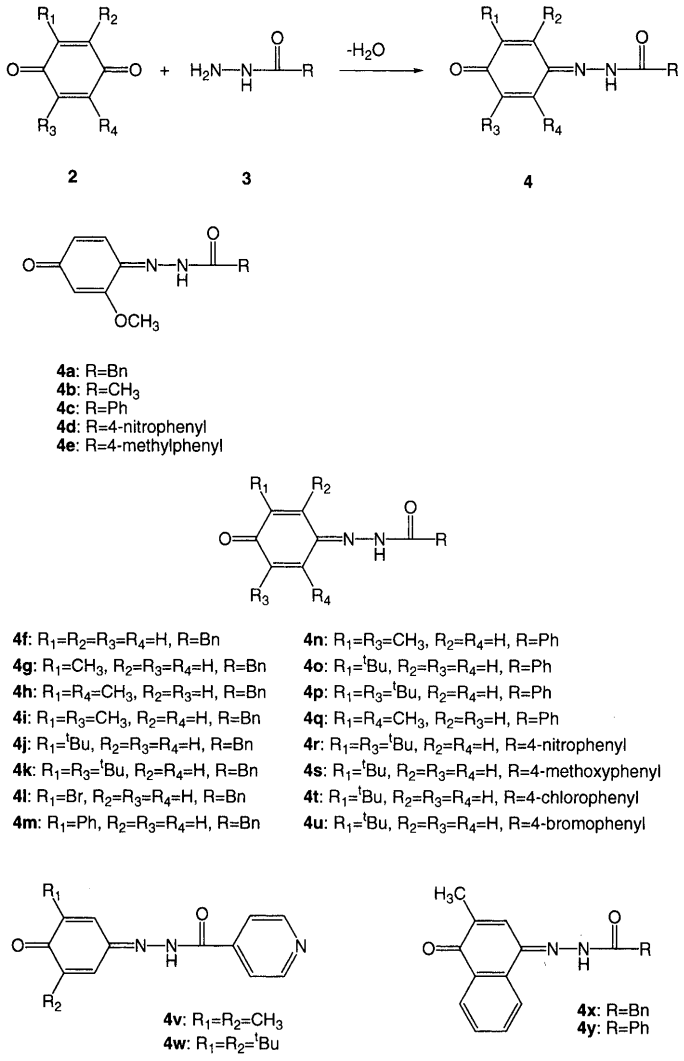

Fig. 2. Synthesis of NG-061 and Its Analogs

Experimental

General Melting points were determined with a Yanagimoto micro-melting point apparatus and are uncorrected. IR spectra were taken on a Horiba FT-710 Fourier transform IR spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL LA-300 spectrometer. Deuteriochloroform (99.8% atom ^{2}H, Merck) was used as the solvent for NMR spectra, unless otherwise stated. Chemical shifts were reported in δ values based on the internal tetramethylsilane (δ_H=0) or the solvent signal of $CDCl_3$ (δ_C=77.0) as the reference. Mass spectrum was obtained on a Finnigan LCQ LC/MS spectrometer in atmospheric pressure chemical ionization mode. Elemental analyses were performed with a Perkin Elmer 2400 apparatus.

Compound 4a (NG-061) A mixture of 2-methoxy-1,4-benzoquinone (1.32 g, 9.54 mmol) and phenylacetic hydrazide (1.42 g, 9.46 mmol) in $CHCl_3$ (150 ml) was stirred at room temperature for 2 d. After evaporation of the solvent, the residual solid was recrystallized from $CHCl_3$–ether to afford NG-061 (310 mg, 12%) as yellow powder; mp 189—190 °C (from $CHCl_3$–ether); IR (KBr) cm^{-1}: 1685, 1639, 1567, 1529; ^{1}H-NMR (major : minor=4 : 1) δ (major): 3.90 (s, 3H), 4.10 (s, 2H), 5.90 (s, 1H), 6.44 (d, 1H, J=9.7 Hz), 7.09 (d, 1H, J=9.7 Hz), 7.19—7.50 (m, 5H), 11.36 (br, 1H); ^{13}C-NMR δ: 29.65, 38.72, 43.27, 55.62, 56.24, 106.91, 127.13, 128.10 (br), 128.50, 128.62, 128.87 (br), 129.47, 130.10 (br), 132.81, 133.75, 138.70, 139.13, 159.87, 174.04, 186.60. *Anal.* Calcd for $C_{15}H_{14}N_2O_3$: C, 66.66; H, 5.22; N, 10.36. Found: C, 66.66; H, 5.35; N, 10.35.

Compound 4b In the same manner as described in the synthesis of **4a**, the compound **4b** was obtained from the corresponding quinone and hydrazide in 22% yield; yellow powder; mp 220—221 °C (from $CHCl_3$–MeOH); IR (KBr) cm^{-1}: 1722, 1689, 1639, 1621, 1565, 1542; ^{1}H-NMR (major : minor=4 : 1) δ (major): 2.38 (s, 3H), 3.92 (s, 3H), 5.91 (d, 1H, J=1.7 Hz), 6.42 (dd, 1H, J=1.7, 9.8 Hz), 7.04 (d, 1H, J=9.8 Hz), 11.32 (br, 1H); ^{13}C-NMR δ: 19.64, 56.23, 105.01, 106.88, 128.38, 138.84, 159.90, 174.01, 186.64. *Anal.* Calcd for $C_9H_{10}N_2O_3$: C, 55.67; H, 5.19; N, 14.43. Found: C, 55.44; H, 5.32; N, 14.30.

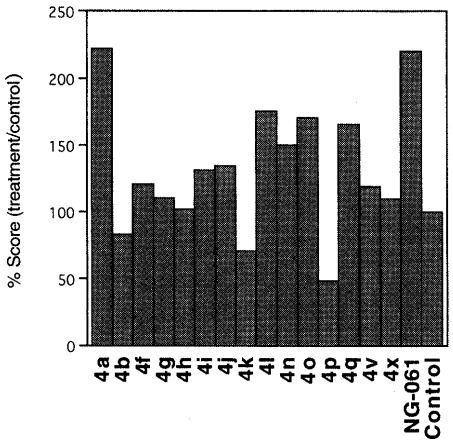

Fig. 3. The Activity of Synthesized NG-061 and Its Analogs (**4a**—**y**) in Enhancing the Neurite Outgrowth Effect of NGF in PC12 Cells

Compounds **4c**—**e**, **4r**—**u**, and **4y** showed toxicity against PC12 cells at doses of 10 μg/ml, therefore, the activities of these compounds were not determined.

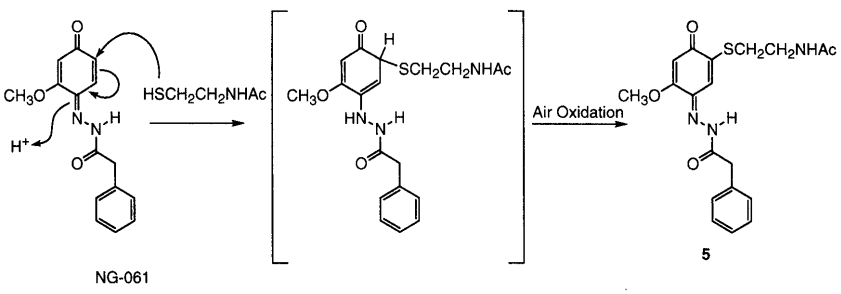

Fig. 4. Reaction of NG-061 with *N*-Acetylcysteamine

Compound 4c In the same manner as described in the synthesis of **4a**, the compound **4c** was obtained from the corresponding quinone and hydrazide in 27% yield; yellow powder; mp 187—188 °C (from $CHCl_3$–hexane); IR (KBr) cm^{-1}: 1700, 1644, 1612, 1573, 1529; ^{1}H-NMR (major : minor=8 : 1) δ (major): 4.01 (s, 3H), 5.95 (d, 1H, J=1.7 Hz), 6.44 (dd, 1H, J=1.7, 10.0 Hz), 7.25 (d, 1H, J=10.0 Hz), 7.53 (m, 2H), 7.63 (m, 1H), 7.87 (m, 2H); ^{13}C-NMR δ: 56.56, 107.21, 127.19, 127.89, 128.65, 128.92, 132.00, 132.95, 132.95, 135.77 (br), 139.17, 159.70, 186.40. *Anal.* Calcd for $C_{14}H_{12}N_2O_3$: C, 65.62; H, 4.72; N, 10.93. Found: C, 65.45; H, 4.90; N, 10.93.

Compound 4d In the same manner as described in the synthesis of **4a**, the compound **4d** was obtained from the corresponding quinone and hydrazide in 16% yield; pale yellow powder; mp 232—233 °C (from $CHCl_3$–MeOH); IR (KBr) cm^{-1}: 1710, 1675, 1644, 1571, 1525; ^{1}H-NMR (major : minor=9 : 1) δ (major): 4.00 (s, 3H), 5.97 (d, 1H, J=1.5 Hz), 6.46 (dd, 1H, J=1.5, 9.8 Hz), 7.14 (d, 1H, J=9.8 Hz), 8.03 (m, 2H), 8.36 (m, 2H); ^{13}C-NMR (DMSO-d_6) δ: 56.08, 104.72 (br), 106.63, 123.26, 123.93, 124.33, 128.72, 129.28 (br), 129.65, 129.96, 130.64 (br), 137.75, 138.54, 138.73, 149.38, 149.62, 159.71, 162.95, 185.94. *Anal.* Calcd for $C_{14}H_{11}N_3O_5$: C, 55.82; H, 3.68; N, 13.95. Found: C, 55.56; H, 3.41; N, 13.80.

Compound 4e In the same manner as described in the synthesis of **4a**, the compound **4e** was obtained from the corresponding quinone and hydrazide in 16% yield; yellow powder; mp 214—215 °C (from $CHCl_3$–MeOH); IR (KBr) cm^{-1}: 1698, 1644, 1608, 1571, 1525; ^{1}H-NMR (major : minor=8 : 1) δ (major): 2.46 (s, 3H), 4.00 (s, 3H), 5.95 (d, 1H, J=2.0 Hz), 6.45 (dd, 1H, J=1.7, 10.0 Hz), 7.28 (d, 1H, J=10.0 Hz), 7.33 (m, 2H), 7.77 (m, 2H); ^{13}C-NMR δ: 21.61, 56.55, 107.17, 127.16, 127.95, 128.55, 129.15, 129.39, 129.63, 135.54, 139.26, 143.89, 159.76, 186.46. *Anal.* Calcd for $C_{15}H_{14}N_2O_3$: C, 66.66; H, 5.22; N, 10.36. Found C, 66.37; H, 5.12; N, 10.07.

Compound 4f In the same manner as described in the synthesis of **4a**, the compound **4f** was obtained from the corresponding quinone and hydrazide in 13% yield; yellow powder; mp 134—135 °C (from $CHCl_3$–hexane); IR (KBr) cm^{-1}: 1677, 1641, 1533; ^{1}H-NMR δ: 4.13 (s, 2H), 6.46 (dd, 1H, J=2.2, 10.2 Hz), 6.55 (dd, 1H, J=2.2, 10.0 Hz), 7.24—7.36 (m, 6H), 7.68 (dd, 1H, J=2.7, 10.5 Hz); ^{13}C-NMR δ: 39.17, 123.53, 127.28, 128.56, 129.62, 130.33, 132.72, 133.60, 140.31, 140.97, 176.34, 187.03. *Anal.* Calcd for $C_{14}H_{12}N_2O_2$: C, 69.99; H, 5.03; N, 11.66. Found: C, 70.17; H, 5.19; N, 11.78.

Compound 4g In the same manner as described in the synthesis of **4a**, the compound **4g** was obtained from the corresponding quinone and hydrazide in 14% yield; brown powder; mp 185—186 °C (from $CHCl_3$–hexane); IR (KBr) cm^{-1}: 1691, 1675, 1639, 1616, 1533; ^{1}H-NMR (major : minor=3 : 1) δ (major): 1.91 (d, 3H, J=1.2 Hz), 4.14 (s, 2H), 6.53 (d, 1H, J=10.0 Hz), 7.19 (dd, 1H, J=2.9, 10.0 Hz), 7.25—7.35 (m, 5H), 7.57 (br, 1H), 11.35 (br, 1H); ^{13}C-NMR δ: 15.74, 16.65, 39.23, 120.56, 123.04, 127.16, 128.57, 129.66, 130.11, 132.57, 133.83, 136.63, 138.40, 139.92, 141.52, 141.57, 176.04, 176.14, 187.25, 187.36. *Anal.* Calcd for $C_{15}H_{14}N_2O_2$: C, 70.85; H, 5.55; N, 11.02. Found: C, 70.62; H, 5.83, N, 10.93.

Compound 4h In the same manner as described in the synthesis of **4a**, the compound **4h** was obtained from the corresponding quinone and hydrazide in 10% yield; yellow powder; mp 245—247 °C (from $CHCl_3$–MeOH); IR (KBr) cm^{-1}: 1680, 1623, 1537; ^{1}H-NMR (DMSO-d_6) (major : minor=2 : 1) δ (major): 1.89 (s, 3H), 2.18 (s, 3H), 4.05 (s, 2H), 6.36 (s, 1H), 7.2—7.35 (m, 5H), 7.85 (s, 1H), 10.11 (s, 1H); ^{13}C-NMR (DMSO-d_6) δ: 15.92, 17.34, 122.22, 126.49, 126.62, 127.97, 128.21, 128.35, 129.01, 129.37, 135.01, 135.72, 138.45, 168.91, 186.34. *Anal.* Calcd for $C_{16}H_{16}N_2O_2$: C, 71.62; H, 6.01; N, 10.44. Found: C, 71.46; H, 6.19; N, 10.23.

Compound 4i In the same manner as described in the synthesis of **4a**, the compound **4i** was obtained from the corresponding quinone and hydrazide in 63% yield; yellow powder; mp 191—192 °C (from $CHCl_3$–ether); IR (KBr) cm^{-1}: 1677, 1623, 1535; ^{1}H-NMR δ: 1.91 (d, 3H, J=1.5 Hz), 2.05 (d, 3H, J=1.2 Hz), 4.13 (s, 2H), 7.02 (m, 1H), 7.24—7.33 (m, 5H), 7.52 (m, 1H), 11.24 (br, 1H); ^{13}C-NMR δ: 15.95, 16.85, 39.22, 120.22, 127.07, 128.55, 129.69, 134.06, 136.28, 138.02, 141.25, 141.68, 176.00, 187.54. *Anal.* Calcd for $C_{16}H_{16}N_2O_2$: C, 71.62; H, 6.01; N, 10.44. Found: C, 71.34; H, 6.13; N, 10.40.

Compound 4j In the same manner as described in the synthesis of **4a**, the compound **4j** was obtained from the corresponding quinone and hydrazide in 16% yield; pale yellow powder; mp 186—188 °C (from $CHCl_3$–ether); IR (KBr) cm^{-1}: 1670, 1643, 1590, 1540; ^{1}H-NMR (major : minor=5 : 4) δ (major): 1.27 (s, 9H), 4.13 (s, 2H), 6.47 (d, 1H, J=10.0 Hz), 7.14 (dd, 1H, J=2.7, 10.0 Hz), 7.22—7.34 (m, 5H), 7.40 (d, 1H, J=2.7 Hz), 10.93 (br, 1H); ^{13}C-NMR δ: 29.07, 29.23, 34.86, 36.02, 39.16, 39.41, 117.69, 121.31, 127.17, 128.61, 129.57, 132.03, 133.92, 134.69, 138.33, 141.41, 141.79, 145.60, 149.11, 152.25, 175.54, 175.62, 186.91. *Anal.* Calcd for $C_{18}H_{20}N_2O_2$: C, 72.95; H, 6.80; N, 9.45. Found: C, 72.66; H, 6.92; N, 9.41.

Compound 4k In the same manner as described in the synthesis of **4a**, the compound **4k** was obtained from the corresponding quinone and hydrazide in 26% yield; yellow powder; mp 193—194 °C (from $CHCl_3$); IR (KBr) cm^{-1}: 1683, 1621, 1583, 1533; ^{1}H-NMR δ: 1.26 (s, 9H), 1.31 (s, 9H), 4.13 (s, 2H), 6.96 (d, 1H, J=2.7 Hz), 7.25—7.33 (m, 5H), 7.38 (d, 1H, J=2.7 Hz), 11.20 (br, 1H); ^{13}C-NMR δ: 29.38, 29.54, 35.05, 36.25, 39.35, 116.74, 127.00, 128.57, 129.53, 133.07, 134.33, 142.13, 150.10, 153.45, 175.67, 186.83. *Anal.* Calcd for $C_{22}H_{28}N_2O_2$: C, 74.53; H, 7.74; N, 8.28. Found: C: 74.48, H: 7.65, N: 8.06.

Compound 4l In the same manner as described in the synthesis of **4a**, the compound **4l** was obtained from the corresponding quinone and hydrazide in 12% yield; reddish powder; mp 160—161 °C (from $CHCl_3$); IR (KBr) cm^{-1}: 1689, 1637, 1529; ^{1}H-NMR (major : minor=5 : 4) δ (major): 4.14 (s, 2H), 6.70 (d, 1H, J=9.8 Hz), 7.23—7.35 (m, 5H), 7.29 (dd, 1H, J=2.2, 9.8 Hz), 8.29 (d, 1H, J=2.2 Hz), 11.30 (br, 1H); ^{13}C-NMR δ: 39.20, 125.60, 127.39, 128.73, 129.16, 129.60, 131.59, 133.00, 133,25, 139.79, 140,24, 141.38, 176.04, 179.24, 182.73. *Anal.* Calcd for $C_{14}H_{11}BrN_2O_2$: C, 52.69; H, 3.47; N, 8.78. Found: C, 52.90; H, 3.65; N, 8.85.

Compound 4m In the same manner as described in the synthesis of **4a**, the compound **4m** was obtained from the corresponding quinone and hydrazide in 36% yield; brown powder; mp 154—155 °C (from $CHCl_3$–MeOH); IR (KBr) cm^{-1}: 1677, 1635, 1525; ^{1}H-NMR (major : minor=4 : 3) δ (major): 4.12 (s, 2H), 6.67 (d, 1H, J=9.8 Hz), 7.27 (m, 5H), 7.30 (dd, 1H, J=2.7, 9.8 Hz), 7.40 (m, 3H), 7.60 (m, 2H), 7.88 (d, 1H, J=2.7 Hz), 11.43 (br, 1H); ^{13}C-NMR δ: 39.29, 39.34, 120.71, 122.84, 127.21, 128.22, 128.28, 128.61, 128.85, 129.21, 129.41, 129.47, 130.94, 133.33, 133.71, 134.70, 134.91, 137.38, 139,45, 140.22, 141.48, 141.54, 142.08, 176.03, 176.05, 185.66, 185.88. *Anal.* Calcd for $C_{20}H_{16}N_2O_2$: C, 75.93; H, 5.10; N, 8.86. Found: C, 75.94; H, 5.33; N, 8.57.

Compound 4n In the same manner as described in the synthesis of **4a**, the compound **4n** was obtained from the corresponding quinone and hydrazide in 51% yield; yellow powder; mp 210—211 °C (from $CHCl_3$–hexane); IR (KBr) cm^{-1}: 1646, 1627, 1538; ^{1}H-NMR δ: 2.03 (d, 3H, J=1.2 Hz), 2.11 (d, 3H, J=1.2 Hz), 7.11 (br s, 2H), 7.45—7.65 (m, 3H), 7.92 (m, 2H); ^{13}C-NMR δ: 15.95, 17.18, 118.64 (br), 128.52 (br), 128.77, 132.16 (br), 132.62, 136.51, 138.30, 141.58 (br), 187.47. *Anal.* Calcd for $C_{15}H_{14}N_2O_2$, C, 70.85; H: 5.55, N: 11.02. Found: C, 71.01; H, 5.71; N, 11.27.

Compound 4o In the same manner as described in the synthesis of **4a**, the compound **4o** was obtained from the corresponding quinone and hydrazide in 14% yield; orange powder; mp 160 °C (from ether); IR (KBr) cm^{-1}: 1681, 1664, 1533; ^{1}H-NMR (major : minor=2 : 1) δ (major): 1.34 (s, 9H), 6.46 (d, 1H, J=9.7 Hz), 7.1—7.4 (br, 2H), 7.52 (m, 2H), 7.62 (m, 1H), 7.90 (m, 2H); ^{13}C-NMR δ: 29.03, 29.21, 34.97, 35.96, 117.21, 120.71, 127.30, 128.51 (br), 128.74, 132.00, 132.53, 132.67, 134.61, 135.07, 138.56, 149.35, 152.19, 186.92. *Anal.* Calcd for $C_{17}H_{18}N_2O_2$: C, 72.32; H, 6.43; N, 9.92. Found: C, 72.28; H, 6.71; N, 9.84.

Compound 4p In the same manner as described in the synthesis of **4a**, the compound **4p** was obtained from the corresponding quinone and hydrazide in 25% yield; yellow powder; mp 210—211 °C (from $CHCl_3$); IR (KBr) cm^{-1}: 1658, 1637, 1581, 1535; ^{1}H-NMR δ: 1.28 (s, 9H), 1.33 (s, 9H), 7.05 (br, 1H), 7.29 (br, 1H), 7.51 (m, 2H), 7.60 (m, 1H), 7.89 (m, 2H); ^{13}C-NMR δ: 29.37, 29.53, 35.20, 36.22, 115.42 (br), 128.37 (br), 132.37, 133.27, 150.43, 153.72, 186.81. *Anal.* Calcd for $C_{21}H_{26}N_2O_2$: C, 74.70; H, 7.90; N, 8.28. Found, C, 74.53; H, 7.74; N, 8.38.

Compound 4q In the same manner as described in the synthesis of **4a**, the compound **4q** was obtained from the corresponding quinone and hydrazide in 25% yield; pale yellow powder; mp 206—207 °C (from $CHCl_3$–ether); IR (KBr) cm^{-1}: 1643, 1619, 1575, 1535; ^{1}H-NMR (DMSO-d_6) (major : minor=2 : 1) δ (major): 1.95 (s, 3H), 2.16 (s, 3H), 6.40 (s, 1H), 7.53 (m, 2H), 7.60 (m, 1H), 7.91 (m, 2H), 8.31 (s, 1H), 10.52 (br, 1H); ^{13}C-NMR (DMSO-d_6) δ: 15.78, 17.25, 122.36, 127.46, 128.26, 128.53, 129.04, 131.88, 132.19, 132.56, 133.13, 148.09, 165.83. *Anal.* Calcd for $C_{15}H_{14}N_2O_2$: C, 70.85; H, 5.55; N, 11.02. Found: C, 70.70; H, 5.68; N, 10.95.

Compound 4r In the same manner as described in the synthesis of **4a**, the compound **4r** was obtained from the corresponding quinone

and hydrazide in 23% yield; yellow powder; mp 229—230 °C (from $CHCl_3$–MeOH); IR (KBr) cm^{-1}: 1675, 1658, 1625, 1536; ^{1}H-NMR δ: 1.27 (s, 9H), 1.32 (s, 9H), 6.81 (br, 1H), 7.43 (br, 1H), 8.04 (d, 2H, J=8.8 Hz), 8.36 (d, 2H, J=8.8 Hz), 11.11 (br, 1H); ^{13}C-NMR δ: 29.37, 29.58, 35.57, 36.39, 115.46 (br), 123.20 (br), 131.19 (br), 132.53, 138.13, 149.65, 151.13, 154.50, 186.63. *Anal.* Calcd for $C_{21}H_{25}N_3O_4$: C, 65.78; H, 6.57; N, 10.96. Found: C, 65.59; H, 6.68; N, 10.81.

Compound 4s In the same manner as described in the synthesis of **4a**, the compound **4s** was obtained from the corresponding quinone and hydrazide in 30% yield; yellow powder; mp 199—200 °C (from $CHCl_3$–MeOH); IR (KBr) cm^{-1}: 1650, 1633, 1606, 1540; ^{1}H-NMR (major : minor=1.1 : 1) δ (major): 1.30 (s, 9H), 3.89 (s, 3H), 6.52 (d, 1H, J=10.0 Hz), 6.99 (m, 2H), 7.22 (br, 1H), 7.46 (br, 1H), 7.94 (m, 2H), 10.14 (br, 1H); ^{13}C-NMR δ: 29.09, 29.26, 34.98, 35.96, 55.50, 113.70, 113.87, 116.68, 116.90, 120.33, 124.06, 131.10 (br), 131.61 (br), 131.90, 134.62, 135.08, 138.59, 142.32 (br), 143.48, 149.24, 152.18, 163.16, 163.24, 186.92. *Anal.* Calcd for $C_{18}H_{20}N_2O_3$: C, 69.21; H, 6.45; N, 8.97. Found: C, 68.92; H, 6.36; N, 8.87.

Compound 4t In the same manner as described in the synthesis of **4a**, the compound **4t** was obtained from the corresponding quinone and hydrazide in 5% yield; reddish powder; mp 140—141 °C (from ether–hexane); IR (KBr) cm^{-1}: 1654, 1635, 1533; ^{1}H-NMR (major : minor=2 : 1) δ (major): 1.29 (s, 9H), 6.46 (d, 1H, J=9.7 Hz), 7.14 (br, 1H), 7.35 (br, 1H), 7.48 (m, 2H), 7.87 (m, 2H), 10.19 (br, 1H); ^{13}C-NMR δ: 29.17, 35.56 (br), 120.64 (br), 128.65, 130.39, 130.93 (br), 134.57 (br), 138.96, 143.38 (br), 186.56. *Anal.* Calcd for $C_{17}H_{17}ClN_2O_2$: C, 64.46; H, 5.41; N, 8.84. Found: C, 64.69; H, 5.37; N, 8.88.

Compound 4u In the same manner as described in the synthesis of **4a**, the compound **4u** was obtained from the corresponding quinone and hydrazide in 6% yield; yellow powder; mp 160—161 °C (from $CHCl_3$–MeOH); IR (KBr) cm^{-1}: 1654, 1635, 1589, 1533; ^{1}H-NMR (major : minor=1.1 : 1) δ (major): 1.29 (s, 9H), 6.52 (d, 1H, J=10.0 Hz), 7.13 (br, 1H), 7.40 (br, 1H), 7.65 (m, 2H), 7.79 (m, 2H), 10.54 (br, 1H); ^{13}C-NMR δ: 29.07, 29.29, 35.01, 36.06, 117.05, 120.52, 127.35, 127.44, 127.58, 130.82, 131.53, 131.75, 132.21, 134.76, 134.90, 138.32, 149.66, 152.58, 186.82. *Anal.* Calcd for $C_{17}H_{17}BrN_2O_2$: C, 56.52; H, 4.74; N, 7.75. Found: C, 56.39; H, 5.04; N, 7.49.

Compound 4v In the same manner as described in the synthesis of **4a**, the compound **4v** was obtained from the corresponding quinone and hydrazide in 17% yield; yellow powder; mp 205—206 °C (from $CHCl_3$–MeOH); IR (KBr) cm^{-1}: 1683, 1625, 1546; ^{1}H-NMR δ: 2.04 (s, 3H), 2.11 (s, 3H), 6.96 (br, 1H), 7.64 (br, 1H), 7.74 (d, 2H, J=5.7 Hz), 8.80 (d, 2H, J=5.7 Hz), 11.17 (br, 1H); ^{13}C-NMR δ: 15.90, 17.19, 118.93 (br), 123.63 (br), 135.97, 138.88, 139.61, 142.03, 150.09 (br), 187.30. *Anal.* Calcd for $C_{14}H_{13}N_3O_2$: C, 65.87; H, 5.13; N, 16.46. Found: C, 65.84; H, 5.34; N, 16.34.

Compound 4w In the same manner as described in the synthesis of **4a**, the compound **4w** was obtained from the corresponding quinone and hydrazide in 45% yield; yellow powder; mp 193—194 °C (from $CHCl_3$–MeOH); IR (KBr) cm^{-1}: 1662, 1627, 1533; ^{1}H-NMR δ: 1.28 (s, 9H), 1.30 (s, 9H), 6.82 (br, 1H), 7.54 (br, 1H), 7.69 (d, 2H, J=5.5 Hz), 8.78 (d, 2H, J=5.5 Hz), 11.63 (br, 1H); ^{13}C-NMR δ: 29.35, 29.57, 35.22, 36.35, 115.54 (br), 116.14 (br), 123.55 (br), 132.58, 140.01, 143.84 (br), 149.85 (br), 151.02 (br), 154.28, 170.48 (br), 186.69. *Anal.* Calcd for $C_{20}H_{25}N_3O_2$: C, 70.77; H, 7.42; N, 12.38. Found: C, 70.98; H, 7.62; N, 12.49.

Compound 4x In the same manner as described in the synthesis of **4a**, the compound **4x** was obtained from the corresponding quinone and hydrazide in 26% yield; pale yellow powder; mp 234—235 °C (from $CHCl_3$–MeOH); IR (KBr) cm^{-1}: 1681, 1639, 1600, 1546; ^{1}H-NMR (major : minor=10 : 1) δ (major): 2.07 (s, 3H), 4.19 (s, 2H), 7.20—7.33 (m, 5H), 7.60 (t, 1H, J=7.8 Hz), 7.73 (t, 1H, J=7.8 Hz), 8.02 (d, 1H, J=7.8 Hz), 8.14 (s, 1H), 8.31 (d, 1H, J=7.8 Hz), 10.10 (s, 1H); ^{13}C-NMR (DMSO-d_6) δ: 16.60, 79.14, 123.25, 124.10, 125.59, 126.45, 126.54, 128.17, 128.30, 128.98, 129.39, 129.97, 132.66, 135.26, 135.69, 168.87, 184.23. *Anal.* Calcd for $C_{19}H_{16}N_2O_2$: C, 74.98; H, 5.30; N, 9.20. Found: C, 74.70; H, 5.56; N, 9.40.

Compound 4y In the same manner as described in the synthesis of **4a**, the compound **4y** was obtained from the corresponding quinone and hydrazide in 42% yield; yellow powder; mp 220—222 °C (from $CHCl_3$–MeOH); IR (KBr) cm^{-1}: 1666, 1644, 1598, 1577, 1544; ^{1}H-NMR (major : minor=10 : 1) δ (major): 2.26 (s, 3H), 7.46—7.65 (m, 6H), 7.90 (d, 1H, J=8.0 Hz), 7.98 (m, 2H), 8.16 (d, 1H, J=7.8 Hz), 10.59 (br, 1H); ^{13}C-NMR (DMSO-d_6) δ: 16.46, 123.36, 124.70, 125.61, 127.44, 128.25, 128.49, 128.72 (br), 129.53, 130.04, 131.93 (br), 132.62, 133.40, 134.70, 138.80, 165.83, 184.37. *Anal.* Calcd for $C_{18}H_{14}N_2O_2$: C, 74.47; H, 4.86; N, 9.65. Found: C, 74.17; H, 5.02; N, 9.70.

Reaction of NG-061 with *N*-Acetylcysteamine A mixture of compound **4a** (5.5 mg, 0.020 mmol), *N*-acetylcysteamine (2.5 μl, 1.1 eq), and triethylamine (11 μl, 4 eq) in $CHCl_3$ (0.5 ml) was stirred at room temperature for 2 d. After evaporation of the solvent, the residue was purified by preparative TLC to give a product (3.3 mg, 42%) as yellow amorphous; ^{1}H-NMR δ: 2.05 (s, 3H), 2.95 (m, 2H), 3.45 (m, 2H), 3.80 (s, 3H), 4.15 (br s, 2H), 5.85 (s, 1H), 7.14—7.42 (m, 5H), 7.82 (s, 1H), 11.40 (br, 2H); MS: *m/z* [rel. intensity]; 388 [100%, $(M+H)^+$], 345 [7%, $(M+H)^+-CH_3CO$], 270 [72%, $(M+H)^+-SCH_2CH_2NHAc$].

Assay for Neurotrophic Activity in PC12 Cells PC12 cells were obtained from the RIKEN Cell Bank and maintained as monolayer culture in DULBECCO's modified Eagle's medium (DMEM, GIBCO) with 10% heat-inactivated fetal bovine serum (FBS), 5% horse serum (HS) 50U penicillin G and 50 μg/ml streptomycin. The cells were kept in a humidified incubator at 37 °C and 5% CO_2, then plated on collagen-coated 24-well plates (Corning) at a density of 1×10^4 per well. After 24 h of culture, NG-061 and its analogs at the concentration of 10 μg/ml were added to the medium in the presence of 0.5 ng/ml of NGF. The cells were further incubated for 48 h. To evaluate activity, 100 cells were observed under a phase-contrast microscope with scoring (round cells: 0, morphologically changed cells without neurite: 1, cells with neurites shorter than the diameter of the cell body: 2, cells with neurites longer than the diameter of the cell body: 3). One hundred cells were scored from a randomly chosen field and this was repeated 3 times (300 cells scored in total).

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