Kynurenic Acid Derivatives Inhibit the Binding of Nerve Growth Factor (NGF) to the Low-Affinity p75 NGF Receptor

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The ability of a series of substituted kynurenic acids, thienopyridinonecarboxylic acids, and related compounds to inhibit the binding of nerve growth factor (NGF) to the p75 NGF receptor (NGFR) was evaluated in a radioligand binding assay that utilized a biotinylated derivative of the extracellular domain of p75 NGFR ($p75_{ext}$) fixed to streptavidin-coated plastic wells. Two compounds, 6-aminokynurenic acid (**5h**) and the 3-methyl ester of 4,7-dihydro-2-methyl-7-oxothieno[3,2-*b*]pyridine-3,5-dicarboxylic acid (**16**), were found to inhibit the binding of [^{125}I]NGF to $p75_{ext}$ with IC_{50} values in the low micromolar range. Other amino-substituted kynurenic acids also possessed activity at slightly higher concentrations. Several structural features seem to be essential, including the carboxylic acid, a polar group on the benzene ring (or thiophene ring, in the case of analogues of **16**), and the C-4 carbonyl group in the pyridinone ring. These compounds were also found to inhibit the binding of [^{125}I]NGF to its receptors in membranes from PC12 cells (which express p75 as well as trk_a receptors for NGF) and DG44-CHO cells (transfected with full length p75 NGFR). The available data for **5h** and **16** do not allow the determination of whether the effects of these compounds are mediated by their interaction with NGF or the NGF receptors.

Introduction

Neurotrophic (neuron-nurturing) factors are target-derived peptides that play an essential role in the development and survival of responsive neuronal populations. Developing neurons compete for a limited supply of these factors. The death of those neurons least successful at securing an adequate supply of trophic factor represents a form of neuronal sculpturing, which ultimately produces the mature nervous system.[1,2] Additionally, many mature neurons require continued trophic support for survival. Neurotrophic factor removal can lead to the apoptotic death of the dependent neurons.[3]

Nerve growth factor (NGF) is one of the most extensively studied neurotrophic factors. This 118 amino acid polypeptide (depending on the species) is essential for the development and survival of peripheral sympathetic and neural crest derived sensory neurons.[4-7] It also provides trophic support to basal forebrain cholinergic neurons, in vitro[8,9] as well as in vivo.[10-12]

NGF is a member of the neurotrophin family, which includes brain-derived neurotrophic factor (BDNF),[13,14] neurotrophin 3 (NT-3),[15-17] and neurotrophin 4/5 (NT-4/5).[18,19] A high level of sequence homology (50–60%) exists among the various neurotrophins. These factors display neurotrophic activity on a variety of peripheral and central neurons, and the supported neuronal populations overlap in many cases. In recent years, the ability of neurotrophins to promote the survival of peripheral and central neurons during development and after neuronal damage has stimulated wide interest in their use as potential treatments for neurodegenerative diseases and nervous system injuries.[20-22] For example, Alzheimer's disease, which is characterized by a loss of basal forebrain cholinergic neurons,[23] represents a reasonable therapeutic target of NGF treatment.[24,25] In support of this expectation, chronic NGF treatment to animals has been shown to enhance the survival of lesioned septohippocampal cholinergic neurons[26,27] and to ameliorate the age-related atrophy of central cholinergic neurons.[28]

Two types of neurotrophin cell surface receptors have been identified to date. The neuronal specificity of the different factors is believed to arise from their selective interaction with members of the trk family of tyrosine kinase receptors. NGF binds selectively to trk (also known as trkA),[29-31] while BDNF and NT-4/5 bind to the related receptor trkB[32] and NT-3 binds preferentially to trkC, the third member of this receptor family.[33] The second type of receptor, $p75^{NGFR}$ (also known as p75), is recognized with similar affinity (in the low nanomolar range) by NGF and the other neurotrophins.[34-37]

Although p75 was initially thought to be a component of a functional high-affinity NGF receptor[38] and involved in its biological activity,[39] recent evidence disputes the direct role of p75 in neurotrophin-mediated signal transduction.[40-42] This receptor is a member of a family of membrane-bound receptors that possess several extracellular cysteine-rich domains. Other members of this family include the B cell antigen CD40 and the tumor necrosis factor receptor.[43] Recent reports have suggested a role for the p75 receptor in NGF internalization[44] and in neuronal apoptotic death.[45]

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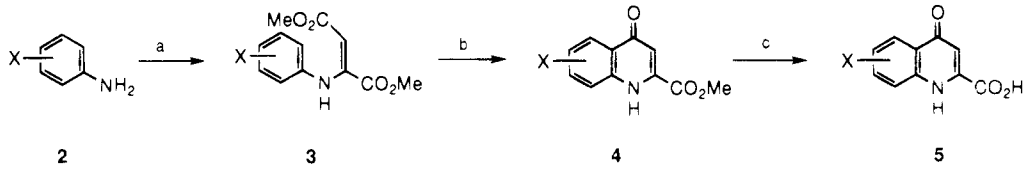
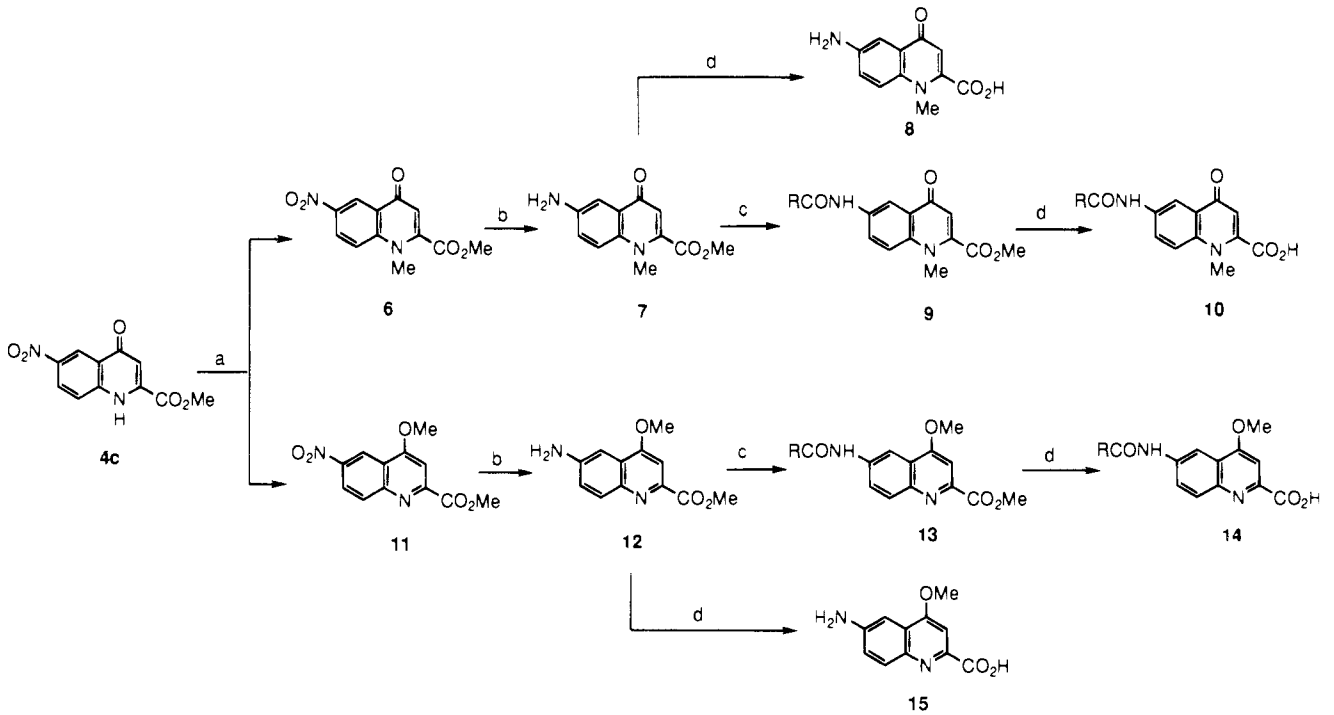
Scheme 1[a]



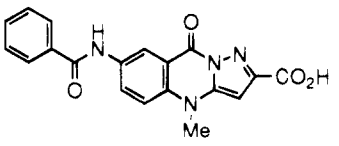
[a] Reagents: (a) $MeO_2CC{\equiv}CCO_2Me$, MeOH, reflux; (b) Dowtherm A, 235 °C; (c) NaOH, MeOH, 20 °C.

Scheme 2[a]



[a] Reagents: (a) NaH, MeI, 80 °C; (b) H_2, Raney Ni, MeOH; (c) 4-MePhCOCl, $(^iPr)_2NEt$, CH_2Cl_2, DMF; (d) NaOH, MeOH, 20 °C.

Selective inhibitors of the actions of NGF at the p75 receptor would be useful tools in further elucidating the biological role of the p75 receptor. No selective antagonists of the p75 receptor have been described in the literature. Recently, we described a novel compound, PD 90780 (1), as a potent and selective inhibitor of NGF binding to p75.[46,47] This report describes the inhibition of NGF-p75 interaction and structure–activity relationship of a series of kynurenic acid derivatives that bear some structural resemblance to PD 90780.



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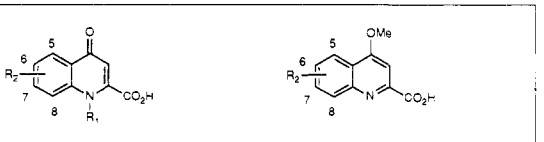
Chemistry

We decided to prepare a series of substituted kynurenic acids (**5**, **8**, and **10**) and 4-methoxyquinoline-2-carboxylic acids (**14** and **15**) based on our understanding of the structure–activity relationship (SAR) of the PD 90780 series[46,47] and our discovery, through random screening of a compound library, of thienopyridones **16** and **17** as inhibitors of NGF–p75 binding. In general, well-established methodology for the synthesis of kynurenic acids was used to prepare the target compounds of general structure **5** (Scheme 1).[48–50] Reaction of appropriately substituted anilines with dimethyl acetylenedicarboxylcate produced the corresponding Michael adducts **3**, which were thermally cyclized to produce kynurenic esters **4**. Amino-substituted kynurenic esters (**4g–i**) were prepared by catalytic reduction of the corresponding nitro compounds. Alkaline hydrolysis of esters **4** readily produced the corresponding substituted kynurenic acids **5**. Reaction of the sodium anion of **4c** with methyl iodide produced a mixture of N-alkylated and O-alkylated products (**6** and **11**, in 22 and 78% yield, respectively) (Scheme 2). Standard methodology, involving catalytic reduction of the nitro group, in some cases derivatization of the amino group with 4-methylbenzoyl chloride, and finally alkaline ester hydrolysis, produced the target compounds **8**, **10**, **14**, and **15**.

Pharmacology

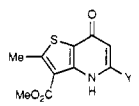
Multiple NGF-sensitive cell lines exist that express the p75 receptor and can be used for competitive [^{125}I]-NGF binding experiments. However, high-volume screening of a proprietary compound library required a large total amount of receptor protein, and a modified receptor system was developed for binding experiments. The ability of test compounds to inhibit the binding of [^{125}I]NGF to the biotinylated recombinant extracellular domain of human p75 ($p75_{ext}$), plated onto streptavidin-coated polyethylene wells, was used as a high-capacity initial screen.[51] In this assay, NGF displays an IC_{50}

Table 1. Inhibition of NGF Binding to $p75_{ext}$ by Test Compounds



5, 8, 10 14, 15

Compound	R_1	R_2	Inhibn of NGF Binding[a]
1	--	--	200 ± 50
5a	H	H	IA[b]
5b[c]	H	5,7-diCl	IA
5c[d]	H	6-NO_2	IA
5d	H	5-CO_2H	IA
5e[d]	H	6-CO_2H	IA
5f	H	7-CO_2H	IA
5g[e]	H	5-NH_2	[50%]
5h[d]	H	6-NH_2	3100 ± 900
5i	H	7-NH_2	[37%]
5j	H	6-$CONH_2$	IA
5k	H	5-NHCO-(4-Me)C_6H_4	[38%]
5l	H	6-NHCO-(4-Me)C_6H_4	IA
5m	H	7-NHCO-(4-Me)C_6H_4	IA
8	Me	6-NH_2	IA
10	Me	6-NHCO-(4-Me)C_6H_4	IA
14	--	6-NHCO-(4-Me)C_6H_4	[29%]
15	--	6-NH_2	[28%]



16	--	Y = CO_2H	2300 ± 600
17	--	Y = CONH-Tetrazole	1300 ± 400

[a]Screening results; inhibition of [^{125}I]NGF binding to recombinant extracellular domain of the p75 receptor (see experimental). Percent inhibition at screening concentration of 10 μM or IC_{50} (nM) ± SEM. [b]IA = inactive; less than 25% inhibition at 10 μM. [c]Reference 52. [d]Reference 53. [e]Reference 54.

value of about 2−5 nM, similar to the binding of NGF to the native p75 receptor.[34,35] The activity of the most potent compounds in this initial screen was confirmed using the binding of [^{125}I]NGF to full-length p75 receptors expressed in DG44-CHO and PC12 cells, as described in the Experimental Section.

Results and Discussion

The inhibition by test compounds of [^{125}I]NGF binding to $p75_{ext}$ is summarized in Table 1. Thienopyridones **16** and **17**, which were identified by random screening of a proprietary compound collection, displayed IC_{50} values of 2300 and 1300 nM, respectively. Consistent with the SAR of PD 90780 (**1**) and analogues, the acidic functionalities of **16** (carboxylic acid) and **17** (tetrazolylamide) were found to be essential for activity; other ester and amide derivatives were found to be inactive.[47] Either acidic functionality produced compounds of comparable activity. Benzene analogues of **16**, kynurenic acids of general formula **5**, were targeted for synthesis because they are more readily accessible and provide more opportunities for structural variations. Particular attention was paid to polar substituents, such as carboxylic, amino, and amide groups on the benzene ring of analogues **5**, based on the SAR of compounds related to **1**.[46,47]

As shown in Table 1, kynurenic acid (**5a**) and its 5,7-dichloro derivative (**5b**), a potent glycine/NMDA antagonist,[52,53] were both found to be inactive as inhibitors of NGF binding to p75, as were the 6-nitro (**5c**) and the various carboxy-substituted analogues (**5d**−**f**). Unlike compounds in the PD 90780 series,[47] arylamide derivatives of kynurenic acid (**5k**−**m**) were mostly inactive, although **5k** possessed some activity (38% inhibition) at the screening concentration of 10 μM. The amino-substituted analogues (**5g**−**i**) were the most potent kynurenic acid derivatives tested. Thus, **5g** displayed an IC_{50} value of about 10 μM, while the 6-amino analogue **5h** possessed an IC_{50} of 3.1 μM. Interestingly, **8**, the N1 methyl derivative of **5h**, was inactive, suggesting that the high degree of phenolic character present in the C4 carbonyl group of an N1 unsubstituted kynurenic acid[52] is important for inhibition of NGF binding to the p75 receptor within this series of compounds. Compared to **5h**, the reduced activity of **15** (28% inh. at 10 μM), a compound with a 4-methoxy substituent, suggests that the C4 functionality of **5h** may act as a hydrogen bond donor instead of a hydrogen bond acceptor.

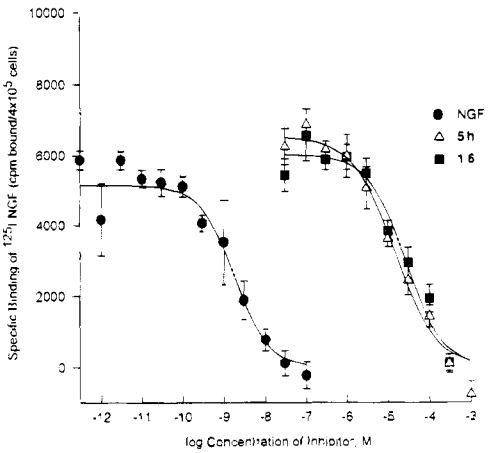


Figure 1. Inhibition of [^{125}I]NGF binding to DG44 CHO cells.

The activity of compounds **5h** and **16** in the $p75_{ext}$ assay was confirmed by evaluating their inhibitor of [^{125}I]NGF binding to full-length p75 receptors in intact DG44-CHO cells (Figure 1). Both compounds displaced [^{125}I]NGF binding to p75 receptors in a concentration-dependent manner, with IC_{50} values of about 10 μM for both compounds, only slightly higher than the values from the solid-supported $p75_{ext}$ protein.

Interestingly, the activity of **5h** and **16** in the PC12 cell assay provides some insight into another relevant question, the selectivity of these compounds in inhibiting NGF binding to p75 versus the trk receptor. As illustrated in Figure 2, binding of [^{125}I]NGF to intact PC12 cells is inhibited in a concentration-dependent manner by both compounds, a profile very similar to that seen in p75-expressing DG44-CHO cells. Unlike the CHO cells, however, PC12 cells express both p75 and trk receptors. Thus, these results suggest that **5h** and **16** block the binding of NGF to both types of receptors. Although we have no direct evidence in the case of **5h** and **16**, PD 90780 and analogues have been shown to prevent binding of NGF to p75 by specifically binding to the NGF molecule, not to p75. Given that the PD 90780 and kynurenic acid series share several structural requirements for NGF/p75 inhibition (such as the carboxylic acid group or an equivalent, the carbonyl group corresponding to C4 in the kynurenic

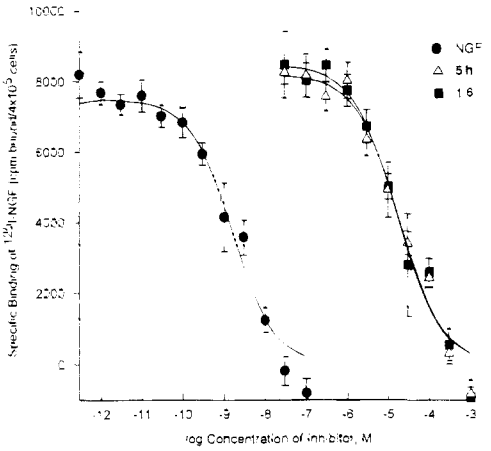


Figure 2. Inhibition of [^{125}I]NGF binding to PC12 cells.

acid structure, and to a limited extent the SAR of the substitution pattern on the benzene portion of the molecule), we hypothesize that compounds such as **5h** and **16** may also bind to NGF instead of the NGF receptors. The observed blockade of NGF binding to both p75 and trk receptors suggests that these compounds bind to an NGF epitope that is important for its interaction with both receptor types.

As indicated above, glial cells have been shown to possess a high expression level of p75 receptors. On the other hand, they lack trk receptors. Glial cells are capable of internalizing and degrading significant amounts of NGF, suggesting that this may be a regulatory mechanism involved in controlling the amount of NGF available to neurons.[44] In this case, inhibition of the NGF-p75 interaction by compounds such as **5h** might increase the amount of NGF available to neurons and might be a viable approach to developing small molecules that enhance endogenous neurotrophic activity. Another potential therapeutic use of this type of compound would be as an antineoplastic agent. Excessive synthesis of trophic factors or their receptors has been implicated in the neoplastic transformation of some cells.[56] Additionally, several types of brain tumors have been shown to secrete a variety of growth factors, including NGF.[57] Thus, NGF antagonists may possess utility in the treatment of some tumors of neuronal origin. Finally, some authors have argued that aberrant neuronal growth takes place in the earliest stages of AD and that this growth is being promoted by growth factors. In this line of thinking, an effective strategy might be to block the effects of such factors using neurotrophic antagonists.[58] Clearly, a better understanding of the role of NGF in these and other disease processes is required before compounds such as **5h** and **16** can be considered as potential therapeutic agents. Several reports on the activity of PD 90780 and related compounds, which seem to share much of the profile of **5h** and **16** but with increased potency, are currently in preparation.

Experimental Section

Air- or moisture-sensitive reactions were carried out in flame-dried glassware under an atmosphere of nitrogen or argon. Kynurenic acid (**5a**) was purchased from Aldrich Chemical Co. Tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl, dioxane from sodium, and dimethylformamide (DMF) from calcium hydride. Organic solutions were dried over anhydrous $MgSO_4$ and concentrated under reduced pressure on a rotary evaporator. Thin-layer chromatography (TLC) was carried out on E. Merck silica gel 60-F254 precoated glass plates (0.25 mm thickness). Flash column chromatography was performed with E. Merck silica gel 60, 230–400 mesh ASTM. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infrared spectra were recorded on a Nicolet MX-1 FTIR spectrometer. ^{1}H-NMR spectra were recorded on a Varian XL200 (200 MHz) or a Bruker AM250 (250 MHz) spectrometer. Chemical shifts are reported in ppm downfield from tetramethylsilane (internal standard). Mass spectra were recorded on a Finnigan 4500 mass spectrometer or a VG analytical 7070E/HF mass spectrometer; the spectra are described by the molecular peak (M) and its relative intensity as well as the base peak (100). Elemental analyses were performed on a CEC 240XA elemental analyzer. Where analyses are indicated by the symbols of the elements, the results are within 0.4% of the theoretical values. The purity of the final products was determined by high-performance liquid chromatography on a system composed of a LKB 2150 pump, a LKB 2152 controller, and an Applied Biosystems 783A programmable absorbance detector.

General Procedure for the Synthesis of Substituted Kynurenic Acids. Method A: Dimethyl 2-[(4-Nitrophenyl)amino]-2-butenedioate (3c). Dimethyl acetylenedicarboxylate (15.63 g, 110 mmol) was added dropwise to a stirred solution of *p*-nitroaniline (13.81 g, 100 mmol) in 150 mL of methanol.[55,59] The resulting suspension was heated at gentle reflux for 2 h and then allowed to cool to room temperature. The precipitated solids were filtered, washed with cold methanol and ether, and dried in vacuo at 40 °C to give the title compound (20.60 g, 74%) as a yellow solid. Mp: 118.5–119.5 °C (lit.[60] mp 124.5–125 °C). IR (KBr): 1629, 1729 cm^{-1}. ^{1}H-NMR ($CDCl_3$): 3.78 (3H, s), 3.80 (3H, s), 5.70 (1H, s), 6.89 (2H, d, J = 9.0 Hz), 8.17 (2H, d, J = 9.0 Hz), 9.80 (1H, broad s). MS: 281 (M$^+$ + 1, 13), 189 (100). Anal. ($C_{12}H_{12}N_2O_6$) C, H, N.

Method B: Methyl 1,4-Dihydro-6-nitro-4-oxo-2-quinolinecarboxylate (4c). Compound **3c** (10.0 g, 35.7 mmol) was added in small portions to 100 mL of Dowtherm A at 235 °C.[55,59] After 5 min at 235–240 °C, the solution was allowed to cool to room temperature and was further stirred at this temperature overnight. The precipitated solid was filtered, washed several times with ether, and dried in vacuo at 40 °C to give **4c** (5.21 g). The filtrate and washings were concentrated, and the residue was heated again at 240 °C for 30 min. Upon cooling, a second crop of product was obtained (2.72 g, 89% overall). Mp: >260 °C (lit.[59] mp 295–297 °C). IR (KBr): 1636, 1737 cm^{-1}. ^{1}H-NMR ($CDCl_3$): 3.99 (3H, s), 6.73 (1H, s), 8.10 (1H, d, J = 9.3 Hz), 8.48 (1H, dd, J = 2.6, 9.3 Hz), 8.79 (1H, d, J = 2.4 Hz), 12.58 (1H, broad s). MS: 248 (M$^+$, 57), 84 (100). Anal. ($C_{11}H_8N_2O_5$) C, H, N.

Method C: Methyl 6-Amino-1,4-dihydro-4-oxo-2-quinolinecarboxylate (4h). A suspension of **4c** (4.12 g, 16.6 mmol) and Raney nickel (1.0 g) in 250 mL of methanol was hydrogenated in a Parr apparatus at ca. 50 psi for 1.5 h. The catalyst was filtered, and the filtrate was concentrated to give **4h** (3.30 g, 91%) as a yellow solid. Mp: 225–226 °C (lit.[59] mp 235 °C dec). IR (KBr): 1717 cm^{-1}. ^{1}H-NMR (DMSO-d_6): 3.93 (3H, s), 5.59 (2H, broad s, NH_2), 6.50–6.70 (1H, m), 7.07–7.15 (2H, m), 7.71 (1H, d, J = 8.8 Hz), 11.62–11.90 (1H, m). MS: 218 (M$^+$, 94), 158 (100). Anal. ($C_{11}H_{10}N_2O_3$) C, H, N.

Method D: 6-Amino-1,4-dihydro-4-oxo-2-quinolinecarboxylic Acid (5h). A solution of **4h** (0.75 g, 3.4 mmol) and 5 mL of 1 N NaOH in 30 mL of methanol was stirred at room temperature for 48 h. The mixture was filtered to remove some insoluble material, and the filtrate was evaporated to dryness. The residue was dissolved in water (20 mL), and the solution was titrated to pH 5 with 6 N HCl and cooled to 0 °C. The precipitated solid was filtered, washed with cold methanol and ether, and dried in vacuo to give **5h** (0.48 g, 69%). Mp: >240 °C (lit.[59] mp 308 °C). IR (KBr): 1639 cm^{-1}. ^{1}H-NMR (DMSO-d_6): 6.71 (1H, s), 7.11 (1H, dd, J = 2.4, 8.9 Hz), 7.17 (1H, d, J = 2.3 Hz), 7.78 (1H, d, J = 8.9), MS: 205 (M$^+$ + 1, 42), 161 (100). Anal. ($C_{10}H_8N_2O_3 \cdot 1.2H_2O$) C, H, N.

In another series of experiments, ethyl 4-aminobenzoate was transformed (methods A, B, and D) into a mixture of **5d** and **5f**, which were separated by MPLC (silica: EtOAc:hexane, 1:1) at the diester stage, prior to the final hydrolysis.

1,4-Dihydro-4-oxo-2,5-quinolinedicarboxylic Acid (5d). Mp: >280 °C. IR (KBr): 1695 cm^{-1}. ^{1}H-NMR (TFA-*d*): 7.93 (1H, s), 8.18 (1H, dd, J = 8.9 and 7.2 Hz), 8.62 (1H, d, J = 8.9 Hz), 8.90 (1H, d, J = 7.2 Hz). MS: 234 (M + 1, 44), 216 (100). Anal. ($C_{11}H_7NO_5 \cdot 1.5H_2O$) C, H, N.

1,4-Dihydro-4-oxo-2,7-quinolinedicarboxylic Acid (5f). Mp: >300 °C. IR (KBr): 1701 cm^{-1}. ^{1}H-NMR (TFA-*d*): 8.21 (1H, s), 8.66 (1H, d, J = 8.4 Hz), 8.82 (1H, d, J = 8.6 Hz), 9.22 (1H, s). MS: 233 (M, 4), 44 (100). Anal. ($C_{11}H_7NO_5 \cdot 1.4HCl$) C, H, N.

Starting with 3-nitroaniline, approximately equal amounts of the 5- and 7-nitro derivatives of methyl kynurinate were produced (methods A and B; 70% combined yield). Reduction of this mixture (method C) followed by column chromatography (silica: EtOAC:hexane, 1:1) produced a 40% yield of methyl 5-amino-1,4-dihydro-4-oxo-2-quinolinecarboxylate (**4g**) and a 45% yield of methyl 7-amino-1,4-dihydro-4-oxo-2-quinolinecarboxylate (**4i**). These crude esters were hydrolyzed (method D) to the corresponding acids.

5-Amino-1,4-dihydro-4-oxo-2-quinolinecarboxylic Acid (5g).[54] Monohydrochloride salt. Mp: >260 °C. IR (KBr): 3444, 1733 cm^{-1}. ^{1}H-NMR (DMSO-d_6): 6.24 (1H, d, J = 7.8 Hz), 6.31 (1H, s), 6.88 (1H, d, J = 8.0 Hz), 7.14 (1H, t, J = 8.0 Hz). MS: 205 (M + 1, 15), 161 (100). Anal. ($C_{10}H_8N_2O_3 \cdot HCl$) C, H; N: calcd, 11.64; found, 10.95.

7-Amino-1,4-dihydro-4-oxo-2-quinolinecarboxylic Acid (5i). Monohydrochloride salt. Mp: >260 °C. IR (KBr): 3327, 1715 cm^{-1}. ^{1}H-NMR (DMSO-d_6): 7.00 (1H, dd, J = 9.1 and 1.8 Hz), 7.07 (1H, d, J = 1.7 Hz), 7.21 (1H, s), 7.93 (1H, d, J = 9.1 Hz). MS: 205 (M + 1, 8), 161 (100). Anal. ($C_{10}H_8N_2O_3 \cdot HCl \cdot 0.25H_2O$) C, H, N.

6-Formamido-1,4-dihydro-4-oxo-2-quinolinecarboxylic Acid (5j). This compound was prepared in 55% overall yield from 4-carboxamidoaniline by methods A, B, D. Mp: >270 °C. IR (KBr): 1665, 1670 cm^{-1}. ^{1}H-NMR (TFA-*d*): 8.21 (1H, s), 8.56 (1H, d, J = 8.9 Hz), 8.75 (1H, d, J = 8.2 Hz), 9.27 (1H, s). MS: 232 (M, 3), 44 (100). Anal. ($C_{11}H_8N_2O_4 \cdot 0.7HCl$): C, H, N.

Method E: Methyl 1,4-Dihydro-6-[(4-methylbenzoyl)amino]-4-oxo-2-quinolinecarboxylate (4l). To a solution of **4h** (0.73 g, 3.3 mmol) and diisopropylethylamine (2.58 g, 20 mmol) in 50 mL of dichloromethane and 2 mL of DMF was added 4-methylbenzoyl chloride (1.25 g, 8 mmol) dropwise, at 0 °C. The mixture was stired at room temperature for 65 h and then concentrated in vacuo. The resulting solid was taken up in about 10 mL of methanol:ether (1:10), filtered, washed with ether, and dried. The solid was dissolved in 40 mL of methanol and treated with 5 mL of 1 N sodium hydroxide for 30 min. The solution was neutralized with 1 N HCl, and the resulting solid was filtered, washed with methanol and ether, and dried in vacuo to give crude **4l** (0.20 g, 20%), mp >250 °C. This material was used in the next step without further purification.

Method F: 1,4-Dihydro-6-[(4-methylbenzoyl)amino]-4-oxo-2-quinolinecarboxylic Acid (5l). A solution of **4l** (0.15 g, 0.45 mmol) and 3 mL of 1 N sodium hydroxide in 30 mL of water was stirred at room temperature for 8 h. The resulting mixture was filtered, and the filtrate was titrated to pH 3.8 with 6 N HCl. The precipitated solid was collected, washed consecutively with cold water, methanol, and ether, and dried in vacuo at 40 °C to give **5l** (80 mg, 55%). Mp: >250 °C. IR (KBr): 1674, 1734 cm^{-1}. ^{1}H-NMR (TFA-*d*): 2.51 (3H, s), 7.44–7.46 (2H, m), 7.86–7.88 (2H, m), 8.12 (1H, s), 8.47–8.56 (2H, m), 9.10 (1H, s). MS: 322 (M, 2), 119 (100). Anal. ($C_{18}H_{14}N_2O_4 \cdot 0.3H_2O$) C, H, N.

In a similar manner, compounds **5k** and **5m** were prepared (methods E and F) from the amino esters **4g** and **4i**:

1,4-Dihydro-5-[(4-methylbenzoyl)amino]-4-oxo-2-quinolinecarboxylic Acid (5k). Mp: >260 °C. IR (KBr): 1624 cm^{-1}. ^{1}H-NMR (DMSO-d_6): 2.41 (3H, s), 6.72 (1H, s), 7.41 (2H, d, J = 8.0 Hz), 7.68–7.74 (2H, m), 7.95 (2H, d, J = 8.1 Hz), 8.64 (1H, t, J = 4.5 Hz). MS: 323 (M − 1, 23), 261 (100). Anal. ($C_{18}H_{14}N_2O_4 \cdot 2H_2O$) C, H, N.

1,4-Dihydro-7-[(4-methylbenzoyl)amino]-4-oxo-2-quinolinecarboxylic Acid (5m). Mp: >260 °C. IR (KBr): 1659 cm^{-1}. ^{1}H-NMR (DMSO-d_6): 2.41 (3H, s), 6.58 (1H, s), 7.37 (2H, d, J = 8.1 Hz), 7.60 (1H, d, J = 8.8 Hz), 7.92 (2H, d, J = 8.1 Hz), 8.04 (1H, d, J = 8.8 Hz), 8.62 (1H, s), 10.54 (1H, s, CO_2H). MS: 323 (M + 1, 100). Anal. ($C_{18}H_{14}N_2O_4 \cdot 0.75H_2O$) C, H, N.

Methyl 1,4-Dihydro-1-methyl-6-nitro-4-oxo-2-quinolinecarboxylate (6) and Methyl 4-Methoxy-6-nitro-2-quinolinecarboxylate (11). To a suspension of sodium hydride (0.77 g, 32 mmol) in 100 mL of DMF was added solid **4c** (4.00 g, 16 mmol) in small portions. The solution was heated at 80 °C for 35 min, iodomethane (3.55 g, 25 mmol) was added, and heating was continued at 80 °C for 3 h. Another portion of iodomethane (3.0 g, 21 mmol) was added, and the reaction mixture was heated for an additional 2 h. The cooled reaction mixture was concentrated under reduced pressure, and the residue was partitioned between ethyl acetate and water. The organic layer was dried over $MgSO_4$, concentrated, and purified by flash column chromatography (hexanes:EtOAc, 1:1) to give **6** (0.91 g, 22%) and 11 (3.32 g, 78%).

Compound 6. Mp: 169–172 °C. IR (KBr): 1644, 1735 cm^{-1}. ^{1}H-NMR (DMSO-d_6): 3.85 (3H, s), 3.99 (3H, s), 6.54 (1H, s), 8.04 (1H, d, J = 9.5 Hz), 8.53 (1H, dd, J = 2.7, 9.5 Hz), 8.85 (1H, d, J = 2.8 Hz). MS: 262 (M, 100). Anal. ($C_{12}H_{10}N_2O_5$) C, H, N.

Compound 11. Mp: 194.0–194.5 °C. IR (KBr): 1623, 1718 cm^{-1}. ^{1}H-NMR (DMSO-d_6): 3.99 (3H, s), 4.22 (3H, s), 7.68 (1H, s), 8.29 (1H, d, J = 9.2 Hz), 8.52 (1H, dd, J = 2.7, 9.2 Hz), 8.95 (1H, d, J = 2.6 Hz). MS: 263 (M + 1, 91) 204 (100). Anal. ($C_{12}H_{10}N_2O_5 \cdot 0.25H_2O$) C, H, N.

Methyl 6-Amino-1,4-dihydro-1-methyl-4-oxo-2-quinolinecarboxylate (7). Catalytic reduction of **6**, in a manner similar to the reduction of **4c** to give **4h**, produced **7** (79% yield). Mp: 189–190 °C. IR (KBr): 1595, 1740 cm^{-1}. ^{1}H-NMR ($CDCl_3$): 3.83 (3H, s), 3.99 (3H, s), 6.53 (1H, s), 7.20 (1H, dd, J = 2.7, 9.1 Hz), 7.45 (1H, d, J = 9.1 Hz), 7.57 (1H, d, J = 2.5 Hz). MS: 233 (M + 1, 100). Anal. ($C_{12}H_{12}N_2O_3 \cdot 0.25H_2O$) C, H, N.

6-Amino-1,4-dihydro-1-methyl-4-oxo-2-quinolinecarboxylic Acid (8). Alkaline hydrolysis of **7**, in a manner similar to the hydrolysis of **4h** to **5h**, produced **8** (41% yield). Mp: >265 °C. ^{1}H-NMR (DMSO-d_6): 3.72 (3H, s), 5.86 (1H, s), 7.05 (1H, dd, J = 2.6, 9.1 Hz), 7.29 (1H, d, J = 2.6 Hz), 7.46 (1H, d, J = 9.1 Hz). MS: 174 (M − 44, 100), 159 (17). Anal. ($C_{13}H_{12}N_2O_4 \cdot HCl \cdot 0.5H_2O$): C, H, N.

Methyl 1,4-Dihydro-1-methyl-6-[(4-methylbenzoyl)amino]-4-oxo-2-quinolinecarboxylate (9). Reaction of **7** with 4-methylbenzoyl chloride in a manner similar to the procedure described above for the synthesis of **4l** produced the title compound (82%). Mp: 269–272 °C. IR (KBr): 1666, 1736 cm^{-1}. ^{1}H-NMR (TFA-*d*): 2.51 (3H, s), 4.28 (3H, s), 4.56 (3H, s), 7.45 (2H, d, J = 7.8 Hz), 7.73 (1H, s), 7.89 (2H, d, J = 7.8 Hz), 8.38 (1H, d, J = 9.7 Hz), 8.72 (1H, d, J = 9.6 Hz), 9.05 (1H, s). MS: 350 (M, 52), 119 (100). This crude compound was used in the next step without further purification.

1,4-Dihydro-1-methyl-6-[(4-methylbenzoyl)amino]-4-oxo-2-quinolinecarboxylic Acid (10). Hydrolysis of **9** in a manner similar to the hydrolysis of **4l** produced the title compound (97% yield). Mp: >265 °C. IR (KBr): 1668, 1724 cm^{-1}. ^{1}H-NMR (TFA-*d*): 2.51 (3H, s), 4.63 (3H, s), 7.45 (2H, d, J = 7.7 Hz), 7.84 (1H, s), 7.89 (2H, d, J = 7.7 Hz), 8.40 (1H, d, J = 9.6 Hz), 8.72 (1H, d, J = 9.3 Hz), 9.07 (1H, s). MS: 337 (M + 1, 100). Anal. ($C_{19}H_{16}N_2O_4 \cdot 1.1H_2O$): C, N; H: calcd, 5.15; found, 4.61.

Methyl 6-Amino-4-methoxy-2-quinolinecarboxylate (12). Catalytic hydrogenation of 11 in a manner similar to the reduction of **4c** produced the title compound (100% yield). Mp: 232–235 °C. IR (KBr): 1624, 1720 cm^{-1}. ^{1}H-NMR (DMSO-d_6): 3.90 (3H, s), 4.04 (3H, s), 6.00 (2H, s, NH_2), 7.06 (1H, d, J = 2.3 Hz), 7.20 (1H, dd, J = 2.4, 9.1 Hz), 7.37 (1H, s), 7.77 (1H, d, J = 9.0 Hz). MS: 232 (M, 54), 174 (100). Anal. ($C_{12}H_{12}N_2O_3$) C, H, N.

Methyl 4-Methoxy-6-[(4-methylbenzoyl)amino]-2-quinolinecarboxylate (13). Reaction of **12** with 4-methylbenzoyl

chloride in a manner similar to the procedure described above for the synthesis of **4l** produced the title compound (77%). Mp: 262–265 °C. IR (KBr): 1673, 1732 cm^{-1}. ^{1}H-NMR (TFA-*d*): 2.51 (3H, s), 4.35 (3H, s), 4.56 (3H, s), 7.44 (2H, d, J = 8.0 Hz), 7.88 (2H, d, J = 8.0 Hz), 8.02 (1H, s), 8.38–8.62 (2H, m), 9.11 (1H, s). MS: 350 (M, 20), 119 (100). Anal. ($C_{20}H_{18}N_2O_4 \cdot 0.75H_2O$) C, H, N.

4-Methoxy-6-[(4-methylbenzoyl)amino]-2-quinolinecarboxylic Acid (14). Hydrolysis of **13** in a manner similar to the hydrolysis of **4h** produced the title compound (79%). Mp: >270 °C. IR (KBr): 1590, 1631, 1649 cm^{-1}. ^{1}H-NMR (TFA-*d*): 2.51 (3H, s), 4.58 (3H, s), 7.44 (2H, d, J = 8.1 Hz), 7.89 (2H, d, J = 8.1 Hz), 8.09 (1H, s), 8.42–8.58 (2H, m), 9.13 (1H, s). MS: 336 (M, 9), 119 (100). Anal. ($C_{18}H_{16}N_2O_3 \cdot 2H_2O$) C, H, N.

6-Amino-4-methoxy-2-quinolinecarboxylic Acid (15). Hydrolysis of **12** in a manner similar to the hydrolysis of **4h** produced the title compound (59%). Mp: 229–230 °C. IR (KBr): 1649 cm^{-1}. ^{1}H-NMR (DMSO-d_6): 4.32 (3H, s), 7.20 (1H, d, J = 1.9 Hz), 7.54 (1H, d, J = 9.1 Hz), 7.65 (1H, s), 8.25 (1H, d, J = 9.3 Hz). MS: 218 (M, 30), 174 (100). Anal. ($C_{11}H_{10}N_2O_3 \cdot H_2O$) C, H, N.

4,7-Dihydro-2-methyl-7-oxo-thieno[3,2-*b*]pyridine-3,5-dicarboxylic Acid, 3-Methyl Ester (16). Reaction of methyl 4-amino-2-methylthiophene-3-carboxylate[61] with dimethyl acetylenedicarboxylate in a manner similar to the procedure described for the synthesis of **3c** produced 2-[[4-(methoxycarbonyl)-5-methyl-3-thienyl]amino]-2-butenedioic acid, dimethyl ester (59%), recrystallized from EtOH:water (1:1). Mp: 67–69 °C. ^{1}H-NMR ($CDCl_3$): 2.64 (3H, s), 3.76 (6H, s), 3.91 (3H, s), 5.27 (1H, s), 5.99 (1H, s), 10.67 (1H, bs).

Cyclization of the above compound in a manner similar to the cyclization of **4c** gave 4,7-dihydro-2-methyl-7-oxo-thieno-[3,2-*b*]pyridine-3,5-dicarboxylic acid, dimethyl ester (58%), recrystallized from EtOAc. Mp: 130–133 °C. ^{1}H NMR (DMSO-d_6): 2.74 (3H, s), 3.91 (3H, s), 3.96 (3H, s), 6.60 (1H, s), 10.77 (1H, bs).

A suspension of the above diester (3.5 g, 12.4 mmol) in 2 N HCl (125 mL) was refluxed for 3 h. The mixture was cooled and filtered, and the solid was washed with water followed by methanol. The purple solid was recrystallized from DMF (160 mL) to give **16** (2.0 g, 60%). Mp: 275 °C dec. ^{1}H-NMR (TFA-*d*): 3.18 (3H, s), 4.27 (3H, s), 8.11 (1H, s). Anal. ($C_{11}H_9NO_5S$) C, H, N.

4,7-Dihydro-2-methyl-7-oxo-5-[(1*H*-tetrazol-5-ylamino)-carbonyl]thieno[3,2-*b*]pyridine-3-carboxylic Acid, Methyl Ester (17). A mixture of **16** (1.5 g, 6 mmol) and 1,1'-carbonyldiimidazole (1.9 g, 12 mmol) in 50 mL of DMF was heated under N_2 at 95 °C for 75 min. 5-Aminotetrazole monohydrate (0.58 g, 6 mmol) was added, and heating was continued for 2 h. The mixture was cooled and filtered. The solid was washed with water, resuspended in 0.5 N $NaHCO_3$ solution, stirred at room temperature for 1 h, and filtered. The filtrate was cooled and acidified with 1 N HCl. At pH ~ 3, a solid formed. The mixture was filtered, and the solid was washed with water and acetone. The solid was recrystallized from a mixture of EtOH:MeOH:DMF (200:50:10 mL) to give the title compound (0.41 g, 22%). Mp: 253–254 °C dec. ^{1}H-NMR (DMSO-d_6): 2.77 (3H, s), 3.92 (3H, s), 7.38 (1H, s). Anal. ($C_{12}H_{10}N_6O_4S \cdot 0.1H_2O$) C, H, N.

Inhibition of [^{125}I]NGF Binding to p75$_{ext}$. Routine binding assays were performed using a truncated form of the p75 receptor (residues 1–222, molecular mass = 56 kDa), originally obtained from Chiron Corp. (Emeryville, CA) but also commercially available from Austral Biologicals, Inc. (San Diego, CA), on plastic 96-well microtiter plates (Immulon 11 Dynatech). The wells were coated with 50 μL/well of 20 μg/mL streptavidin in a diphosphate buffer solution (DPBS). The plates were incubated at room temperature for 6 h. The excess streptavidin solution was poured out, and the wells were washed with a 0.1% solution of bovine serum albumin (BSA) in DPBS. To each well was then added 50 μg/mL of a DPBS solution containing the truncated p75 receptor, which had been previously reacted with biotin by standard procedures. The plates were incubated at room temperature for 2 h and washed with 0.2% BSA/DPBS. To each well was added 98 μL of 2 nM [^{125}I]NGF, followed by 2 μL/well of a concentration of test compound 50 times greater than the desired final test concentration. The plates were incubated for 2 h at room temperature and washed with 0.1% BSA/DPBS. A gamma counter was used to determine the amount of [^{125}I]NGF bound to the plates. Nonspecific binding was defined as the binding of [^{125}I]NGF in the presence of 2 μM cold NGF. The activity of test compounds is expressed as percent inhibition of [^{125}I]-NGF binding at the test concentration of 10 μM, or as the IC_{50} value (the concentration of test compound that blocks 50% of the binding of [^{125}I]NGF to the truncated p75 receptor).

[^{125}I]NGF Binding Studies. PC12 cells from American Type Culture Collection were cultured on rat tail collagen-coated tissue culture plastic at a density of 5×10^4 cells/cm^2 in a culture medium consisting of 85% RPMI 1640, 10% horse serum, 5% dialyzed fetal calf serum, 125 ng/mL fungizone (amphotericin B), and 50 μg/mL gentamycin (GIBCO). Q1 CHO cells (engineered from the parent DG44 line to express full length p75 NGF receptors) were a generous gift from Dr. B. Chapman (Chiron Corp.) and were cultured on tissue culture plastic in a medium consisting of 90% Dulbecco's modified Eagle's medium (DMEM, high glucose, GIBCO), 10% dialyzed fetal calf serum, 125 ng/mL fungizone, 50 μg/mL gentamycin, and 150 μg/ml L-proline.

The binding of [^{125}I]NGF to intact Q1 CHO cells and PC12 cells was performed in suspension. The cells were suspended at a concentration of 4 and 5×10^5 cells/mL, respectively, in binding buffer (Dulbecco's phosphate buffered saline containing 1 mg/mL glucose and 1 mg/mL bovine serum albumin). The cells were incubated with 50 or 10 pM [^{125}I]NGF (Q1 CHO and PC12 cells, respectively) in the absence and presence of unlabeled inhibitors for 1 h at 37 °C. The cells were then centrifuged in a microcentrifuge, the supernatant was aspirated off, and the pellets were counted in a gamma counter. Nonspecific binding was defined as the binding of [^{125}I]NGF in the presence of 50 nM cold NGF.

References

(1) Barde, Y.-A. Trophic factors and neuronal survival. *Neuron* **1989**, *2*, 1525–1534.
(2) Oppenheim, R. W. Neuronal cell death and some related regressive phenomena during neurogenesis: a selective historical review and progress report. In *Studies in Developmental Neurobiology*; Cowan, W. M., Ed.; Oxford University Press: Oxford, 1981; pp 74–133.
(3) Raff, M. C.; Barres, B. A.; Burne, J. F.; Coles, H. S.; Ishizaki, Y.; Jacobson, M. D. Programmed cell death and the control of cell survival: lessons from the nervous system. *Science* **1993**, *262*, 695–700.
(4) Levi-Montalcini, R.; Angeletti, P. Nerve growth factor. *Physiol. Rev.* **1968**, *48*, 534–569.
(5) Thoenen, H.; Barde, Y. A. Physiology of nerve growth factor. *Physiol. Rev.* **1980**, *60*, 1284–1335.
(6) Thoenen, H.; Bandtlow, C.; Heumann, R. The physiological function of nerve growth factor in the central nervous system: comparison with the periphery. *Rev. Physiol. Biochem. Pharmacol.* **1987**, *109*, 145–178.
(7) Levi-Montalcini, R. The nerve growth factor: Thirty-five years later. *EMBO J.* **1987**, *6*, 1145–1154.
(8) Hsiang, J.; Heller, A.; Hoffmann, P. C.; Mobley, W. C.; Wainer, B. H. The effects of nerve growth factor on the development of septal cholinergic neurons in reaggregate cell cultures. *Neuroscience* **1989**, *29*, 209–223.
(9) Cheng, B.; Mattson, M. P. NGF and bFGF protect rat hippocampal and human cortical neurons against hypoglycemic damage by stabilizing calcium homeostasis. *Neuron* **1991**, *7*, 1031–1041.
(10) Hefti, F. Nerve Growth Factor promotes survival of septal cholinergic neurons after fimbrial transections. *J. Neurosci.* **1986**, *6*, 2155–2162.
(11) Gage, F. H.; Armstrong, D. M.; Williams, L. R.; Varon, S. Morphological response of axotomized septal neurons to nerve growth factor. *J. Comp. Neurol.* **1988**, *269*, 147–155.
(12) Mobley, W. C.; Rutkowski, J. L.; Tennekoon, G. I.; Gemski, J.; Buchanan, K.; Johnston, M. V. Nerve growth factor increases choline acetyltransferase activity in developing basal forebrain neurons. *Brain Res.* **1986**, *387*, 53–62.
(13) Barde, Y.-A.; Edgar, D.; Thoenen, H. Purification of a new neurotrophic factor from mammalian brain. *EMBO J.* **1982**, *1*, 549–553.

(14) Leibrock, J.; Lottspeich, F.; Hohn, A.; Hofer, M.; Hengerer, B.; Masiakowski, P.; Thoenen, H.; Barde, Y.-A. Molecular cloning and expression of brain-derived neurotrophic factor. *Nature* **1989**, *341*, 149–152.
(15) Ernfors, P.; Ibañez, C. F.; Ebendal, T.; Olson, L.; Persson, H. Molecular cloning and neurotrophic activities of a protein with structural similarities to nerve growth factor: developmental and topographical expression in the brain. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 5454–5458.
(16) Hohn, A.; Leibrock, J.; Bailey, K.; Barde, Y.-A. Identification and characterization of a novel member of the nerve growth factor/brain-derived neurotrophic factor family. *Nature* **1990**, *344*, 339–341.
(17) Maisonpierre, P. C.; Belluscio, L.; Squinto, S.; Ip, N. Y.; Furth, M. E.; Lindsay, R. M.; Yancopoulos, G. D. Neurotrophin-3: a neurotrophic factor related to NGF and BDNF. *Science* **1990**, *247*, 1446–1451.
(18) Hallböök, F.; Ibañez, C. F.; Persson, H. Evolutionary studies of the nerve growth factor family reveal a novel member abundantly expressed in *Xenopus* ovary. *Neuron* **1991**, *6*, 845–858.
(19) Berkemeier, L. R.; Winslow, J. W.; Kaplan, D. R.; Nikolics, K.; Goeddel, D. V.; Rosenthal, A. Neurotrophin-5: a novel neurotrophic factor that activates trk and trkB. *Neuron* **1991**, *7*, 857–866.
(20) Sendtner, M.; Holtmann, B.; Kolbeck, R.; Thoenen, H.; Barde, Y.-A. Brain-derived neurotrophic factor prevents the death of motoneurons in newborn rats after nerve section. *Nature* **1992**, *360*, 757–759.
(21) Yan, Q.; Elliott, J.; Snider, W. D. Brain-derived neurotrophic factor rescues spinal motor neurons from axotomy-induced cell death. *Nature* **1992**, *360*, 753–759.
(22) Hyman, C.; Hofer, M.; Barde, Y.-A.; Juhasz, M.; Yancopoulos, G. D.; Squinto, S. P.; Lindsay, R. M. BDNF is a neurotrophic factor for dopaminergic neurons of the substantia nigra. *Nature* **1991**, *350*, 230–232.
(23) Whitehouse, P. J.; Price, D. L.; Clark, A. W.; Coyle, J. T.; DeLong, M. R. Alzheimer's disease: evidence for selective loss of cholinergic neurons in the nucleus basalis. *Ann. Neurol.* **1981**, *10*, 122–126.
(24) Phelps, C. H.; Gage, F. H.; Growdon, J. H.; Hefti, F.; Harbaugh, R.; Johnston, M. V.; Khachaturian, Z. S.; Mobley, W. C.; Price, D. L.; Raskind, M.; et al. Potential use of nerve growth factor to treat Alzheimer's disease. *Neurobiol. Aging* **1989**, *10*, 205–207.
(25) Hefti, F.; Schneider, L. S. Rationale for the planned clinical trials with nerve growth factor in Alzheimer's disease. *Psychiatry Dev.* **1989**, *7*, 297–315.
(26) Hefti, F. Nerve growth factor promotes survival of septal cholinergic neurons after fimbrial transections. *J. Neurosci.* **1986**, *6*, 2155–2162.
(27) Montero, C. N.; Hefti, F. Rescue of lesioned septal cholinergic neurons by nerve growth factor: specificity and requirement for chronic treatment. *J. Neurosci.* **1988**, *8*, 2986–2999.
(28) Fischer, W.; Wictorin, K.; Bjorklund, A.; Williams, L. R.; Varon, S.; Gage, F. H. Amelioration of cholinergic neuron atrophy and spatial memory impairment in aged rats by nerve growth factor. *Nature* **1987**, *329*, 65–68.
(29) Kaplan, D. R.; Martin-Zanca, D.; Parada, L. F. Tyrosine phosphorylation and tyrosine kinase activity of the trk proto-oncogene product induced by NGF. *Nature* **1991**, *350*, 158–160.
(30) Kaplan, D. R.; Hempstead, B. L.; Martin-Zanca, D.; Chao, M. V.; Parada, L. F. The trk proto-oncogene product: a signal transducing receptor for nerve growth factor *Science* **1991**, *252*, 554–558.
(31) Klein, R.; Jing, S. Q.; Nanduri, V.; O'Rourke, E.; Barbacid, M. The tkr proto-oncogene encodes a receptor for nerve growth factor. *Cell* **1991**, *65*, 189–197.
(32) Klein, R.; Nanduri, V.; Jing, S. A.; Lamballe, F.; Tapley, P.; Bryant, S.; Cordon-Cardo, C.; Jones, K. R.; Reichardt, L. F.; Barbacid, M. The trkB tyrosine protein kinase is a receptor for brain-derived neurotrophic factor and neurotrophin-3. *Cell* **1991**, *66*, 395–403.
(33) Lamballe, F.; Klein, R.; Barbacid, M. trkC, a new member of the trk family of tyrosine protein kinases, is a receptor for neurotrophin-3. *Cell* **1991**, *66*, 967–979.
(34) Chao, M. V.; Bothwell, M. A.; Ross, A. H.; Koprowski, H.; Lanahan, A. A.; Buck, C. R.; Sehgal, A. Gene-transfer and molecular cloning of the human NGF receptor. *Science* **1986**, *232*, 518–521.
(35) Radeke, M. J.; Misko, T. P.; Hsu, C.; Herzenberg, L. A.; Shooter, E. M. Gene transfer and molecular cloning of the rat nerve growth factor receptor. *Nature* **1987**, *325*, 593–597.
(36) Rodriguez-Tebar, A.; Dechant, G.; Barde, Y. A. Binding of brain-derived neurotrophic factor to the nerve growth factor receptor. *Neuron* **1990**, *4*, 487–492.
(37) Squinto, S. P.; Stitt, T. N.; Aldrich, T. H.; Davis, S.; Bianco, S. M.; Radziejewski, C.; Glass, D. J.; Masiakowski, P.; Furth, M. E.; Valenzuela, D. M.; et al. trkB encodes a functional receptor for brain-derived neurotrophic factor and neurotrophin-3 but not nerve growth factor. *Cell* **1991**, *65*, 885–893.
(38) Hempstead, B. L.; Martin-Zanca, D.; Kaplan, D. R.; Parada, L. F.; Chao, M. V. High affinity NGF binding requires coexpression of the trk proto-oncogene and the low-affinity NGF receptor. *Nature* **1991**, *350*, 678–683.
(39) Hempstead, B. L.; Schleifer, L. S.; Chao, M. V. High-affinity NGF binding requires coexpression of the trk proto-oncogene and the low-affinity NGF receptor. *Science* **1989**, *243*, 373–375.
(40) Weskamp, G.; Reichardt, L. F. Evidence that biological activity of NGF is mediated through a novel subclass of high affinity receptors. *Neuron* **1991**, *6*, 649–663.
(41) Ibañez, C. F.; Ebendal, T.; Barbany, G.; Murray-Rust, J.; Blundell, T. L.; Persson, H. Disruption of the low affinity receptor-binding site in NGF allows neuronal survival and differentiation by binding to the trk gene product. *Cell* **1992**, *69*, 329–341.
(42) Jing, S.; Tapley, P.; Barbacid, M. Nerve growth factor mediates signal transduction through trk homodimer receptors. *Neuron* **1992**, *9*, 1067–1079.
(43) Loetscher, H.; Pan, Y. C.; Lahm, H. W.; Gentz, R.; Brockhaus, M.; Tabuchi, H.; Lesslauer, W. Molecular cloning and expression of the human 55 kd tumor necrosis factor receptor. *Cell* **1990**, *61*, 351–359.
(44) Kahle, P.; Hertel, C. Nerve growth factor (NGF) receptor on rat glial cell lines. Evidence for NGF internalization via p75NGFR. *J. Biol. Chem.* **1992**, *267*, 13917–13923.
(45) Rabizadeh, S.; Oh, J.; Zhong, L.; Yang, J.; Bitler, C. M.; Butcher, L. L.; Bredesen, D. E. Induction of apoptosis by the low-affinity NGF receptor. *Science* **1993**, *261*, 345–348.
(46) Davis, R. E.; Dickerson, M.; Fergus, J.; Hepburn, T.; Hopkins, J.; Levine, H.; Marks, J.; Spiegel, K.; Jaen, J.; Moos, W. PD 90780: A selective non-peptide ligand for the p75 NGF receptor. *Soc. Neurosci. Abs.* **1992**, *18*, 544.4.
(47) Jaen, J. C. Neurotrophic Factors and Alzheimer's Disease: Discovery of Selective Non-Peptide Inhibitors of NGF-p75 Receptor Interaction. 205th ACS National Meeting, Denver: March 28–April 2, 1993. Abstract MEDI 168.
(48) Heindel, N. D.; Bechara, I. S.; Kennewell, P. D.; Molnar, J.; Ohnmacht, C. J.; Lemke, S. M.; Lemke, T. F. Cyclization of aniline-acetylenedicarboxylate adducts. A modified Conrad-Limpach method for the synthesis of potential antimalarials. *J. Med. Chem.* **1968**, *11*, 1218–1221.
(49) Baker, B. R.; Bramhall, R. R. Irreversible enzyme inhibitors. 189. Inhibition of some dehydrogenases by derivatives of 4-hydroxyquinoline-2- and -3-carboxylic acids. *J. Med. Chem.* **1972**, *15*, 230–233.
(50) Coltman, S. C. W.; Eyley, S. C.; Raphael, R. A. A new efficient route to 4-oxo-1,4-dihydroquinoline-2-carboxylic esters. *Synthesis* **1984**, 150–152.
(51) Kaufman, S. E.; Brown, S.; Stauber, G. B. Characterization of ligand binding to immobilized biotinylated extracellular domains of three growth factor receptors. *Anal. Biochem.* **1993**, *211*, 261–266.
(52) Leeson, P. D.; Baker, R.; Carling, R. W.; Curtis, N. R.; Moore, K. W.; Williams, B. J.; Foster, A. C.; Donald, A. E.; Kemp, J. A.; Marshall, G. R. Kynurenic acid derivatives. Structure-activity relationships for excitatory amino acid antagonism and identification of potent and selective antagonists at the glycine site on the N-methyl-D-aspartate receptor. *J. Med. Chem.* **1991**, *34*, 1243–1252.
(53) Hall, C. M.; Johnson, H. G.; Wright, J. B. Quinoline derivatives as antiallergy agents. *J. Med. Chem.* **1974**, *17*, 685–690.
(54) Kermack, W. O.; Tebrich, W. Synthesis of 4-hydroxy-5:6:2':3'-pyridoquinoline (4-hydroxy-m-phenanthroline). *J. Chem. Soc.* **1945**, 375–378.
(55) Kemp, J. A.; Foster, A. C.; Leeson, P. D.; Priestley, T.; Tridgett, R.; Iversen, L. L.; Woodruff, G. N. 7-Chlorokynurenic acid is a selective antagonist at the glycine modulatory site of the N-methyl-D-aspartate receptor complex. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 6547–6550.
(56) Levi-Montalcini, R. The nerve growth factor 35 years later. *Science* **1987**, *237*, 1154–1162.
(57) Westermann, R.; Hardung, M.; Meyer, D. K.; Ehrhard, P.; Otten, U.; Unsicker, K. Neuronotrophic factors released by C6 glioma cells. *J. Neurochem.* **1988**, *50*, 1747–1758.
(58) Butcher, L. L.; Woolf, N. J. Neurotrophic agents may exacerbate the pathologic cascade of Alzheimer's disease. *Neurobiol. Aging* **1989**, *10*, 557–570.
(59) Peet, N. P.; Baugh, L. E.; Sunder, S.; Lewis, J. E. Synthesis and antiallergic activity of some quinolinones and imidazoquinolines. *J. Med. Chem.* **1985**, *28*, 298–302.
(60) Heindel, N. D.; Bechara, I. S.; Lemke, T. F.; Fish, V. B. Cyclization of aniline-acetylenedicarboxylate adducts. Improved synthesis of 8-nitro-2-carbomethoxy-4(1H)-quinolines. *J. Org. Chem.* **1967**, *32*, 4155–4157.
(61) Binder, D.; Noe, C. R.; Zahora, M. Thiophene as a structural element of physiologically active substances. Part 8. 1H,5H-Imidazo[1,2-a]thieno[3,4-d]pyrimidin-2(3H)-ones. *Arch. Pharm.* **1981**, *314*, 557–564.

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