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Utilization of a copper-catalyzed diaryl ether synthesis for the preparation of verbenachalcone

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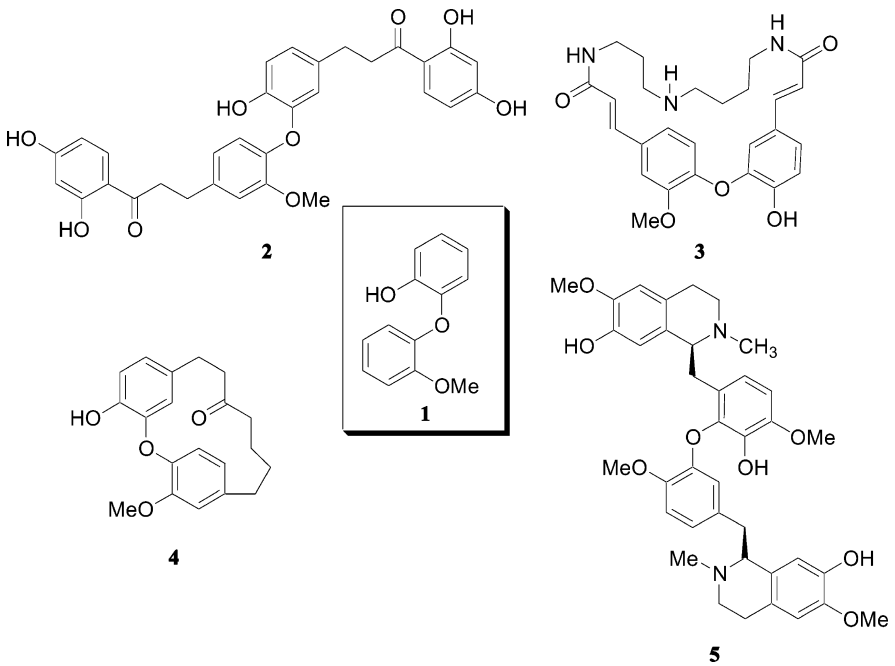
Abstract—2-Hydroxy-2′-methoxydiphenyl ethers were efficiently assembled utilizing a catalytic copper mediated coupling of 2-benzyloxybromobenzene derivatives and 2-methoxyphenol derivatives in the presence of cesium carbonate in pyridine followed by debenzylation. Utilization of this method allowed for a concise synthesis of verbenachalcone, a compound reported to enhance nerve growth factor's ability to stimulate neurite outgrowths in PC12D cells. Initial SAR data is also presented.

1. Introduction

Compounds containing diaryl ethers are abundant in nature. In many instances, the diaryl ether is part of a complex acyclic or macrocyclic structure. A significant proportion of these compounds contain 2-hydroxy-2′-methoxydiphenyl ethers **1**. For example, the recently isolated dimeric dihydrochalcone verbenachalcone (**2**),[1] the cyclic alkaloid isocodonocarpine (**3**),[2] the cyclic ketone galleon (**4**),[3] and the isoquinoline alkaloid (+)-vateamine (**5**)[4] all contain this substructure.

Many compounds comprising substructure **1** have interesting biological properties. For example, **2** has been reported to enhance nerve growth factor's (NGF's) ability to stimulate neurite outgrowths from PC12D cells.[1] Compounds that possess this property may be useful in the treatment of both acute (e.g. trauma or stroke) and chronic



Keywords: copper; catalysis; diaryl ether; verbenachalcone; nerve growth factor.

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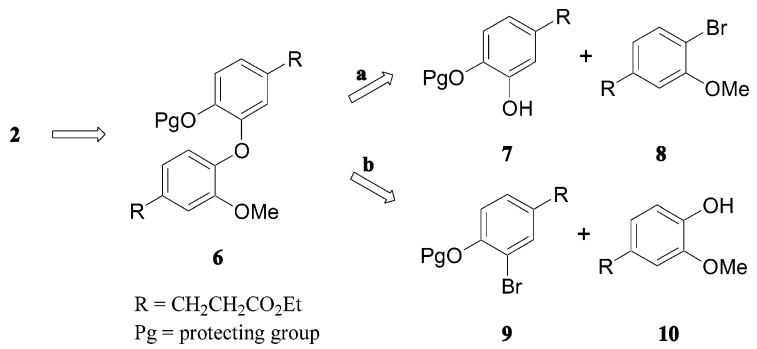


Scheme 1.

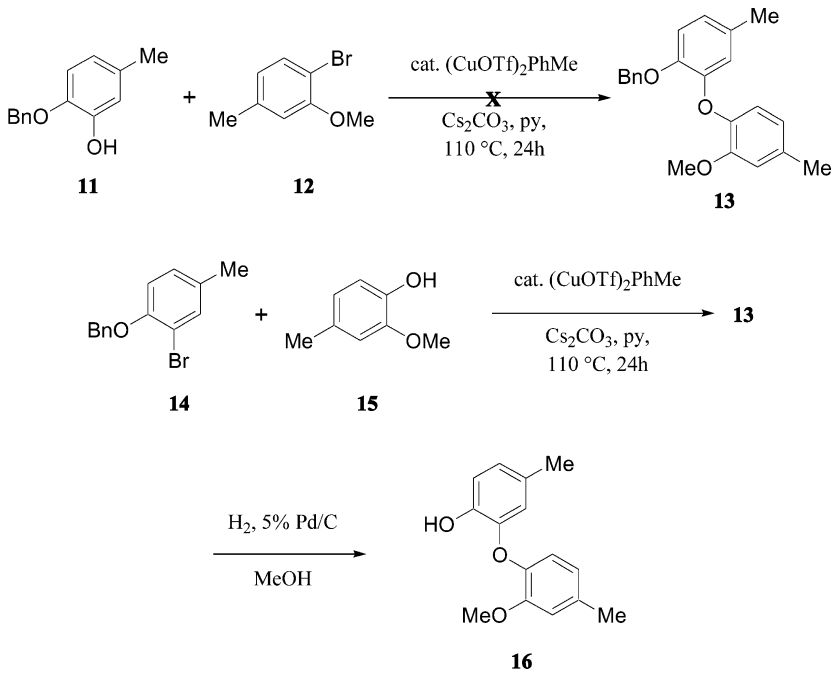
neurological disorders, such as Parkinson's disease (PD), Alzheimer's disease (AD), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS), and human immunodeficiency virus associated dementia (HAD).

NGF belongs to a class of proteins called neurotrophins.[5] These proteins are a subclass of growth factors whose effects are almost exclusively on the central and peripheral nervous system. Other members of this family include brain-derived neurotrophic factor (BDNF), NT-3, NT-4/5, NT-6 and NT-7. Several related classes of growth factors also specific to the nervous system have been identified, such as glial cell-derived neurotrophic factor (GDNF) and ciliary neurotrophic factor (CNTF). These proteins serve a vital role in the development and homeostasis of the nervous system. Recently, numerous studies have demonstrated the potential benefits of enhancing, augmenting or mimicking the actions of neurotrophins in both in vitro and in vivo models of neurodegenerative diseases.[6,7] However, direct in vivo application of neurotrophins is limited by several factors, including the inherent problems frequently associated with peptide therapeutics (e.g. lack of both blood–brain-barrier penetration and stability).

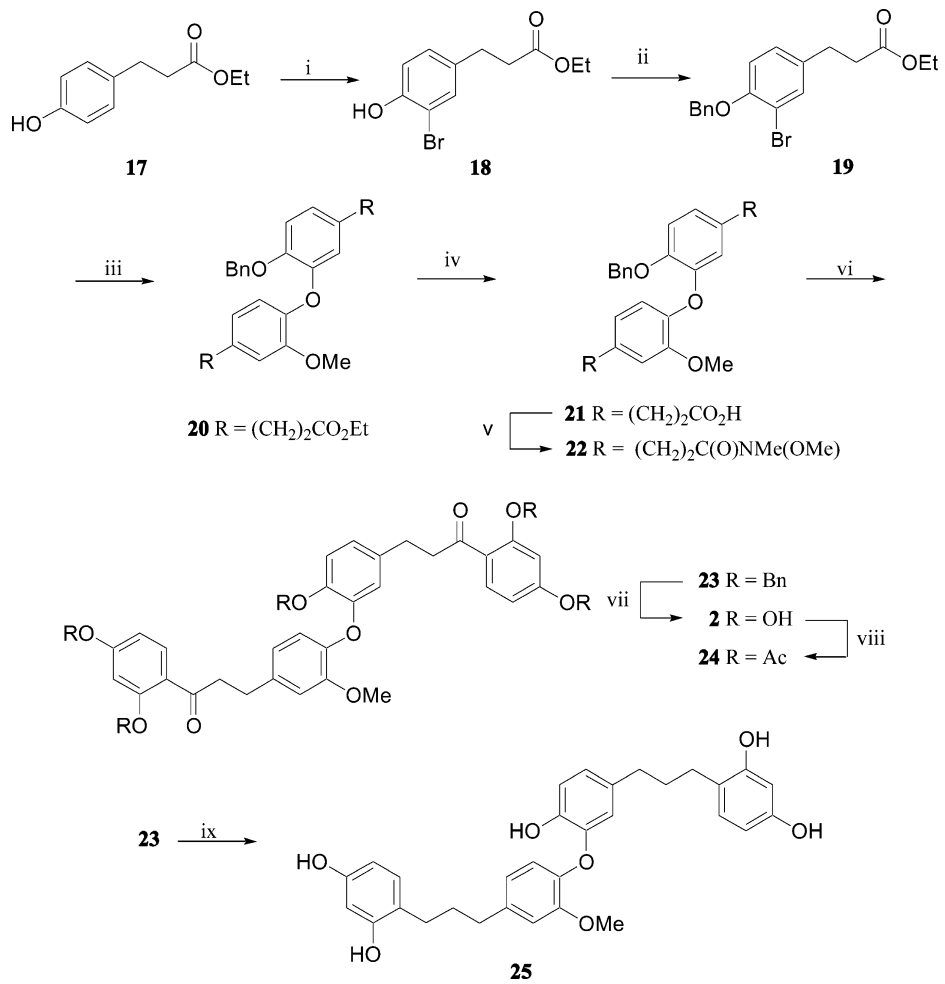
Identifying and preparing low molecular weight molecules, such as **2**, that enhance the activity of NGF can assist in elucidating the role of neurotrophins in the pathophysiology of neurological diseases and can provide lead compounds for therapeutic development. In the present study, we report methodology for the assembly of 2-hydroxy-2′-methoxydiphenyl ethers and its application to the synthesis of **2**. The methodology described herein will be applicable for the preparation of derivatives of **2** for structure–activity relationship (SAR) studies related to enhancing NGF activity on neurite outgrowths and for the synthesis of other natural and non-natural substances containing 2-hydroxy-2′-methoxydiphenyl ethers.

2. Results and discussion

The interesting biological activity reported for **2** prompted an investigation into its synthesis. A retrosynthetic analysis of **2** began with disconnecting the resorcinol groups to give **6**. Further disconnection of the protected 2-hydroxy-2′-methoxydiphenyl ether gave phenol derivative **7** and 2-bromoanisole derivative **8** (Scheme 1, pathway **a**).



Scheme 2.



Scheme 3. (i) Br_2, AcOH, 85%; (ii) BnBr, EtOH, K_2CO_3, Δ, 85%; (iii) **10**, 5 mol% $(CuOTf)_2PhMe$, Cs_2CO_3, py, 110°C, 78%; (iv) 8N NaOH, THF/MeOH (2:1), room temperature, 95%; (v) MeNHOMe, HBTU, DIEA, DCM, 94%; (vi) 1-Li-2,4-$(OBn)_2Ph$, THF, −78°C then 1N HCl, 70%; (vii) 1 atm H_2, 5% Pd/C, AcOH, 2 h, 88%; (viii) Ac_2O, py, room temperature, 90%; (ix) 1 atm H_2, 5% Pd/C, MeOH/THF, 16 h, 74%.

Alternatively, disconnection of **6** gave protected 2-hydroxybromobenzene derivative **9** and 2-methoxyphenol derivative **10** (Scheme 1, pathway **b**). Initially, a model system was used to assess the feasibility of both approaches and to determine an appropriate hydroxyl protecting group.

Due to the prevalence of diaryl ethers in many natural products and medicinally interesting compounds, various strategies have been devised for their construction. Most prominent among these methods are the classic Ullmann ether synthesis and nucleophilic aromatic substitution reactions (S_NAr).[8] Typically, the S_NAr reaction is applicable for coupling electron-deficient aryl halides to phenols under basic conditions. Recently, catalytic metal mediated (copper[9a–e] and palladium[10]) couplings of phenols with aryl halides have been reported. Successful implementation of these reactions can be less dependent of the electronic nature of the aryl halide. Since the present work envisioned coupling electron-rich aryl halides, a modified copper-catalyzed reaction was employed.

The model systems used for accessing methods of constructing 2-hydroxy-2′-methoxydiphenyl ethers are illustrated in Scheme 2. In the presence of 5 mol% $(CuOTf)_2PhMe$ and cesium carbonate in pyridine at 110°C for 24 h, the 2-benzyloxyphenol derivative **11** failed to react with the 2-bromoanisole derivative **12**.[11] However, utilizing identical reaction conditions, the 2-benzyloxybromobenzene derivative **14** reacted with the 2-methoxyphenol derivative **15** (0.83 equiv.) to give the diaryl ether **13** in moderate yield (51%).[9f] The reaction produced one product that could be easily purified from remaining starting materials by flash chromatography on basic aluminum oxide. Afterward, the benzyl group was readily removed by catalytic hydrogenation (1 atm H_2, 5% Pd/C, MeOH, 1.5 h) to give **16** in excellent yield (94%). These results demonstrated that disconnection **b** in Scheme 1 would be best for the synthesis of **2** and that a benzyl protecting group would be tolerated in the coupling reaction and readily removed in a subsequent transformation.

Having established a convenient means of assembling 2-hydroxy-2′-methoxydiphenyl ethers, the methodology was applied to the synthesis of verbenachalcone (**2**). The synthesis began by first converting ethyl 3-(4-hydroxyphenyl)propionate (**17**) to the aryl bromide **18** by treatment with bromine in acetic acid (Scheme 3).[12] Benzylation of the phenol was accomplished with benzyl bromide in refluxing ethanol in the presence of potassium carbonate to give **19**.[13] Next, the aryl bromide **19** was coupled to ethyl

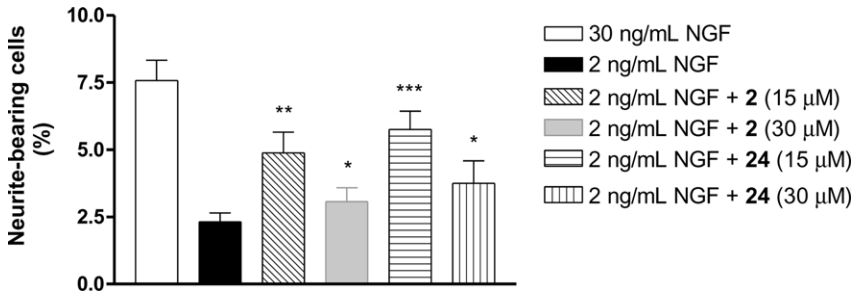


Figure 1. Enhancement of NGF's effects for stimulating neurite outgrowths in PC12 cells with **2** and **24**. Cells were incubated in the presence of NGF (2 or 30 ng/mL) alone and in the presence of **2** (15 or 30 μM) or **24** (15 or 30 μM) plus NGF (2 ng/mL) for 48 h before being fixed with 2% glutaraldehyde (37°C, 1 h). Neurite outgrowth was accessed under a phase-contrast microscope. Neurite processes with a length equal to or greater than the diameter of the neuron cell body were scored as a neurite-bearing cell. The ratio of neurite-bearing cells to total cells was determined and expressed as a percentage±standard deviation (N=12). The data was analyzed by Student unpaired t-test. * Indicates no significant difference verses 2 ng/mL NGF; ** indicates a significant difference ($p<0.007$) verses 2 ng/mL NGF; *** indicates a significant difference ($p<0.0002$) verses 2 ng/mL NGF.

3-(4-hydroxy-3-methoxyphenyl)propionate (**10**), which was readily prepared from commercially available 3-(4-hydroxy-3-methoxyphenyl)propionic acid, to give **20** in 78% yield (based on recovered **19**; 66% conversion). The diaryl ether coupling was accomplished utilizing the method previously developed (5 mol% $(CuOTf)_2PhMe$, Cs_2CO_3, pyridine, 110°C, 24 h) with the model system. The esters were hydrolyzed with sodium hydroxide (8N) in a mixture of THF and methanol (2:1) to give **21**.[14] This material was converted to its corresponding Weinreb amide **22** utilizing *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyl-uronium tetrafluoroborate (HBTU) in dichloromethane (DCM) in the presence of diisopropyl ethylamine (DIEA).[15] Treatment of the amide with 4 equiv. of 1-lithio-2,4-dibenzyloxybenzene (generated in situ from 1-bromo-2,4-dibenzyloxybenzene and *n*-BuLi in THF at −78°C for 1.5 h) followed by the addition of hydrochloric acid (1N) yielded **23**.[16,17] Finally, debenzylation was accomplished by hydrogenation (1 atm, 2 h) in the presence of 5% Pd/C in acetic acid to give the natural product **2**.[18] The ^1H and ^{13}C NMR spectra of the synthetic product were identical to those reported for the natural product.

Two derivatives were also prepared for a preliminary SAR study. Treatment of **2** with acetic anhydride in pyridine gave verbenachalcone pentaacetate (**24**). This compound was designed to probe the relevance of the hydroxyl substituents for activity. Similarly, a derivative was made to explore the importance of the ketone functional groups. Hydrogenation (1 atm H_2, 5% Pd/C, MeOH/THF) of **23** for an extended period of time (16 h) resulted in reduction of the ketones to give **25** in 74% yield.

The ability of **2**, **24**, and **25** to enhance NGF's effects for stimulating neurite outgrowths was accessed utilizing methodology previous reported.[7d] In control experiments, the percentage of neurite-bearing cells was 2.5% following incubation with 2 ng/mL NGF and 7.5% with 30 ng/mL NGF after 48 h exposure in agreement with a previous report (Fig. 1).[19] A significant enhancement of NGF's effects was demonstrated with **2** (15 μM) in agreement with Li et al.[1] However, this effect was not observed at 30 μM. The failure to see enhancement at the higher dose was most likely due to cell toxicity, which was apparent during microscopic examination of the cells. Likewise, **24** at 15 μM significantly enhanced NGF's effects. These results were similar to those observed with the 30 ng/mL NGF exposure. Again, this effect was not observed at 30 μM due to toxicity. The increase in activity of **24** compared to **2** maybe due to an increase in intrinsic activity at the molecular target or an increase in cell permeability. Further experiments will be necessary to elucidate the reason. Neither compound was active in the absence of NGF. Compound **25** did not enhance NGF's effects for stimulating neurite outgrowth at either 15 or 30 μM. This result illustrated the importance of the ketones for activity.

3. Conclusions

2-Hydroxy-2′-methoxydiphenyl ethers were efficiently assembled utilizing a catalytic copper mediated coupling of 2-benzyloxybromobenzene derivatives and 2-methoxy-phenol derivatives in the presence of cesium carbonate in pyridine followed by debenzylation. This method allowed for a concise synthesis of verbenachalcone (**2**), a compound reported to significantly enhance NGF's effects for stimulating neurite outgrowths in PC12D cells.[1] A preliminary SAR study demonstrated that verbenachalcone pentaacetate (**24**) was more active compared to the natural product. However, a derivative of verbenachalcone that lacked the ketone functional groups was devoid of activity. The methodology employed for the synthesis of verbenachalcone should be amenable to the preparation of additional derivatives for SAR studies to enhance NGF's effects on neurite outgrowths and for the synthesis of other natural and non-natural substances containing 2-hydroxy-2′-methoxydiphenyl ethers.

4. Experimental

4.1. General experimental procedures

Unless otherwise noted, all reagents and solvents were purchased from commercial sources and used without further purification. The NMR spectra were obtained using a Bruker 400 MHz, Varian 400 MHz, or Varian 500 MHz spectrometer. All ^1H NMR spectra are reported in δ units ppm and referenced to tetramethylsilane (TMS). All ^{13}C NMR spectra are reported in δ units ppm and referenced to the central line of the triplet at 77.23 ppm. Column

chromatography was performed on silica gel (Merck, grade 60, 230–400 mesh) or utilizing a CombiFlash Sg 100c separation system (ISCO) with RediSep disposable silica gel columns (ISCO), or activated basic aluminum oxide (Brockmann I, standard grade, ~150 mesh, 58Å). High-resolution mass spectra were obtained by using an SX-102A mass spectrometer (JEOL USA, Inc., Peabody, MA) or an LCT mass spectrometer (Micromass Inc., Beverly, MA).

4.1.1. 2-Benzyloxy-2′-methoxy-4′,5-dimethyldiphenylether (13). A Schlenk flask was charged with 2-benzyloxy-5-methylbromobenzene (**14**, 166 mg, 0.6 mmol), pyridine (2 mL), 2-methoxy-4-methylphenol (**15**, 63.3 μL, 0.5 mmol), cesium carbonate (179 mg, 0.55 mmol), and copper(I) trifluoromethanesulfonate toluene complex (6.5 mg, 0.0125 mmol). The flask was purged with argon, sealed, and heated at 110°C for 24 h. The reaction was poured into 2N hydrochloric acid (25 mL). The mixture was extracted with ethyl acetate (50 mL). The organic extract was stirred with 10% sodium hydroxide (40 mL) for 15 min and then washed with brine (25 mL), dried over anhydrous magnesium sulfate, filtered, and concentrated to give a brown oil. The oil was purified by column chromatography on silica gel using hexane/ethyl acetate (95:5) as eluant to give 95 mg of **13** as a colorless oil (57%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.25 (s, 3H), 2.38 (s, 3H), 3.86 (s, 3H), 5.15 (s, 2H), 6.70–6.84 (m, 5H), 6.90 (d, 1H, J=8.4 Hz), 7.30–7.34 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.78, 21.32, 56.03, 71.26, 113.54, 115.53, 118.44, 120.04, 120.97, 123.78, 127.04 (2C), 127.36, 128.10 (2C), 131.22, 133.17, 137.27, 144.08, 146.57, 147.06, 149.94; HREIMS $[M+H]^+$: 335.1642 (calcd for $[C_{22}H_{22}O_3+H]^+$, 335.1647).

4.1.2. 2-Hydroxy-2′-methoxy-4′,5-dimethyldiphenylether (16). A solution of **13** (42 mg, 0.171 mmol) and 5% Pd/C (5 mg) in methanol (5 mL) was stirred at room temperature for 1.5 h under 1 atm H_2. The reaction mixture was filtered and the filtrate was concentrated to give a colorless oil. The oil was purified by column chromatography on silica gel using hexane/ethyl acetate (90:10) as eluant to give 25 mg of **16** as a colorless oil (94%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.16 (s, 3H), 2.32 (s, 3H), 3.81 (s, 3H), 6.00 (bs, 1H), 6.62 (d, 1H, J=1.6 Hz), 6.68–6.77 (m, 3H), 6.85 (d, 1H, J=8.8 Hz), 6.92 (d, 1H, J=8.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.84, 21.44, 55.98, 113.44, 115.42, 118.29, 120.66, 121.37, 124.37, 129.60, 134.93, 143.00, 144.72 (2C), 150.44; HREIMS $[M+H]^+$: 245.1167 (calcd for $[C_{15}H_{16}O_3+H]^+$, 245.1178).

4.1.3. Ethyl 3-(3-bromo-4-hydroxyphenyl)propionate (18). To a stirred solution of ethyl 3-(4-hydroxyphenyl)-propionate (**17**, 971 mg, 5 mmol) in acetic acid (10 mL) was slowly added a solution of bromine (0.13 mL, 2.5 mmol) in acetic acid (10 mL) at room temperature. After the addition, stirring was continued for 30 min. The reaction mixture was then diluted with 80 mL of ethyl acetate and washed with brine (2×30 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated. The crude product was purified by column chromatography on silica gel using hexane/ethyl acetate (90:10) as eluant to afford 1.16 g of **18** as a white solid (85%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.24 (t, 3H, J=7.0 Hz), 2.57 (t, 2H, J=8.0 Hz), 2.86 (t, 2H, J=8.0 Hz), 4.13 (q, 2H, J=7.0 Hz), 5.47 (s, 1H), 6.93 (d, 1H, J=8.5 Hz), 7.05 (dd, 1H, J=2.0, 8.5 Hz), 7.30 (d, 1H, J=2.0 Hz); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 14.56 (CH_3), 30.08 (CH_2), 36.26 (CH_2), 60.77 (CH_2), 110.13 (C), 116.11 (CH), 129.19 (CH), 131.70 (CH), 134.29 (C), 150.72 (C), 172.66 (C=O).

4.1.4. Ethyl 3-(4-benzyloxy-3-bromophenyl)propionate (19). A mixture of **18** (546 mg, 2 mmol), benzyl bromide (0.36 mL, 3 mmol), K_2CO_3 (833 mg, 6 mmol) and ethanol (18 mL) was refluxed for 2 h, then allowed to cool to room temperature and concentrated. The residue was partitioned between 30 mL of water and 80 mL of ethyl acetate. The organic solution was washed with sat. $NaHCO_3$ (20 mL) and brine (15 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated. The residue was purified by chromatography on silica gel using hexane/ethyl acetate (95:5) as eluant to give **19** as a colorless oil (620 mg, 85%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.23 (t, 3H, J=7.0 Hz), 2.57 (t, 2H, J=8.0 Hz), 2.86 (t, 2H, J=8.0 Hz), 4.12 (q, 2H, J=7.0 Hz), 5.12 (s, 2H), 6.85 (d, 1H, J=8.5 Hz), 7.06 (dd, 1H, J=2.0, 8.5 Hz), 7.31 (t, 1H, J=7.5 Hz), 7.38 (t, 2H, J=7.5 Hz), 7.41 (d, 1H, J=2.0 Hz), 7.46 (d, 2H, J=7.5 Hz); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 14.57 (CH_3), 30.08 (CH_2), 36.22 (CH_2), 60.73 (CH_2), 71.14 (CH_2), 112.51 (C), 114.05 (CH), 127.09 (2×CH), 127.98 (CH), 128.30 (CH), 128.64 (2×CH), 133.27 (CH), 134.69 (C), 136.70 (C), 153.52 (C), 172.61 (C=O); FT-IR (film, ν_{max}, cm^{-1}): 3032w, 2980m, 2934m, 2868w, 1732s, 1604w, 1496s, 1282s, 1254s, 1183s, 1051s, 809w, 737m, 696w.

4.1.5. Ethyl 3-{4-[2-benzyloxy-5-(2-ethoxycarbonylethyl)phenoxy]-3-methoxyphenyl}propionate (20). A mixture of **19** (175 mg, 0.5 mmol), ethyl 3-(4-hydroxy-3-methoxyphenyl)propionate (**10**, 168 mg, 0.75 mmol), $(CF_3SO_3Cu)_2{\cdot}C_6H_5CH_3$ (14 mg, 0.026 mmol), Cs_2CO_3 (179 mg, 0.55 mmol) and pyridine (2 mL) was stirred at 110°C under argon for 24 h. The reaction mixture was allowed to cool before being diluted with 30 mL of ethyl acetate and washed with 1N HCl (3×10 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography on basic aluminum oxide using hexane/ethyl acetate (85:15) as eluant to give 60 mg of recovered **19** and 130 mg of the coupling product **20** (66% conversion) as a colorless oil (78%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.21 (t, 3H, J=7.5 Hz), 1.24 (t, 3H, J=7.5 Hz), 2.52 (t, 2H, J=8.0 Hz), 2.63 (t, 2H, J=8.0 Hz), 2.82 (t, 2H, J=8.0 Hz), 2.93 (t, 2H, J=8.0 Hz), 3.82 (s, 3H), 4.09 (q, 2H, J=7.5 Hz), 4.14 (q, 2H, J=7.5 Hz), 5.08 (s, 2H), 6.68 (dd, 1H, J=2.0, 8.5 Hz), 6.70 (d, 1H, J=8.5 Hz), 6.76 (d, 1H, J=2.0 Hz), 6.82 (d, 1H, J=2.0 Hz), 6.83 (dd, 1H, J=2.0, 8.5 Hz), 6.89 (d, 1H, J=8.5 Hz), 7.20–7.31 (br m, 5H); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 14.56 (CH_3), 14.61 (CH_3), 30.55 (CH_2), 31.08 (CH_2), 36.33 (CH_2), 36.47 (CH_2), 56.30 (CH_3), 60.63 (CH_2), 60.70 (CH_2), 71.31 (CH_2), 112.98 (CH), 115.60 (CH), 118.22 (CH), 120.05 (CH), 120.43 (CH), 123.70 (CH), 127.22 (2×CH), 127.67 (CH), 128.36 (2×CH), 134.17 (C), 135.97 (C), 137.29 (C), 145.12 (C), 146.44 (C), 148.12 (C), 150.11 (C), 172.78 (C=O), 172.90 (C=O); FT-IR (film, ν_{max}, cm^{-1}): 3062w, 3033w, 2980m, 2835m, 2871w, 1732s, 1594m, 1510s, 1421m, 1263s,

1215s, 1157s, 1037s, 886m, 809m, 739m, 697m; HRESMS $[M+H]^+$: 507.2399 (calcd for $[C_{30}H_{34}O_7+H]^+$, 507.2383).

4.1.6. 3-{4-[2-Benzyloxy-5-(2-carboxyethyl)phenoxy]-3-methoxyphenyl}propionic acid (21). A mixture of **20** (133 mg, 0.26 mmol), NaOH (1.28 g, 32 mmol), and 7 mL of H_2O/THF/MeOH (4:2:1) was stirred at room temperature for 2 h and then acidified (pH 1) with concentrated HCl. The mixture was extracted with ethyl acetate (80 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated to give 110 mg of **21** as a pale yellow solid (94%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.53 (t, 2H, J=6.5 Hz), 2.74 (t, 2H, J=7.5 Hz), 2.82 (t, 2H, J=6.5 Hz), 2.99 (t, 2H, J=7.5 Hz), 3.79 (s, 3H), 5.18 (s, 2H), 6.52 (d, 1H, J=2.0 Hz), 6.72 (dd, 1H, J=2.0, 8.5 Hz), 6.76 (dd, 1H, J=2.0, 8.5 Hz), 6.83 (d, 1H, J=2.0 Hz), 6.86 (d, 1H, J=8.5 Hz), 6.87 (d, 1H, J=8.5 Hz), 7.25–7.29 (br m, 5H); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 30.07 (CH_2), 30.91 (CH_2), 35.94 (2×CH_2), 56.21 (CH_3), 71.50 (CH_2), 113.56 (CH), 115.71 (CH), 118.12 (CH), 120.14 (CH), 120.93 (CH), 123.10 (CH), 127.30 (2×CH), 127.74 (CH), 128.45 (2×CH), 134.03 (C), 135.89 (C), 137.41 (C), 144.44 (C), 147.67 (C), 147.68 (C), 150.67 (C), 178.95 (C═O), 178.96 (C═O); HRESMS $[M+NH_4]^+$: 468.2031 (calcd for $(C_{26}H_{26}O_7+NH_4]^+$, 468.2022).

4.1.7. 3-(4-{2-Benzyloxy-5-[2-(methoxymethylcarbamoyl)ethyl]phenoxy}-3-methoxyphenyl)-*N*-methoxy-*N*-methylpropionamide (22). A mixture of **21** (45 mg, 0.1 mmol), HBTU (76 mg, 0.2 mmol), DIEA (0.12 mL, 0.7 mmol), and *N*,*O*-dimethylhydroxylamine hydrochloride (29 mg, 0.3 mmol) in 2 mL of CH_2Cl_2 was stirred at room temperature for 1 h before removal of the solvent. The residue was partitioned between ethyl acetate (40 mL) and 1N HCl (20 mL). The organic layer was washed sequentially with 1N HCl (10 mL), saturated $NaHCO_3$ (10 mL) and brine (10 mL). The organic solution was then dried over anhydrous Na_2SO_4, filtered and concentrated. The product was purified by column chromatography on silica gel using hexane/ethyl acetate (50:50 to 30:70) as eluant to give 51 mg of **22** as a colorless oil (95%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.65 (t, 2H, J=8.0 Hz), 2.75 (t, 2H, J=8.0 Hz), 2.83 (t, 2H, J=8.5 Hz), 2.95 (t, 2H, J=8.5 Hz), 3.15 (s, 3H), 3.18 (s, 3H), 3.58 (s, 3H), 3.61 (s, 3H), 3.84 (s, 3H), 5.08 (s, 2H), 6.70 (d, 2H, J=1.0 Hz), 6.80 (d, 1H, J=2.0 Hz), 6.85–6.90 (br m, 3H), 7.22–7.30 (br m, 5H); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 30.25 (CH_2), 30.82 (CH_2), 32.49 (2×CH_3), 34.05 (CH_2), 34.25 (CH_2), 56.32 (CH_3), 61.46 (2×CH_3), 71.30 (CH_2), 113.17 (CH), 115.60 (CH), 118.11 (CH), 120.21 (CH), 120.50 (CH), 123.85 (CH), 127.21 (2×CH), 127.65 (CH), 128.24 (2×CH), 134.92 (C), 136.79 (C), 137.32 (C), 145.05 (C), 146.39 (C), 148.02 (C), 150.04 (C), 173.61 (2×C═O); FT-IR (film, ν_{max}, cm^{-1}): 3032w, 2936m, 2872w, 1661s, 1594w, 1509s, 1420s, 1266s, 1215s, 1131m, 1034m, 990m, 854w, 808w, 740w, 698w; HRESMS $[M+H]^+$: 537.2596 (calcd for $[C_{30}H_{36}N_2O_7+H]^+$, 537.2601).

4.1.8. 3-(4-{2-Benzyloxy-5-[3-(2,4-bis-benzyloxyphenyl)-3-oxopropyl]phenoxy}-3-methoxyphenyl)-1-(2,4-bis-benzyloxyphenyl)propan-1-one (23). A solution of *n*-BuLi (2.5 M in hexane, 0.26 mL, 0.64 mmol) was added to a stirred solution of 1-bromo-2,4-dibenzyloxybenzene (236 mg, 0.64 mmol) in THF (2 mL) at −78°C under argon. The mixture was stirred at −78°C for 1.5 h before adding a solution of **22** (86 mg, 0.16 mmol) in THF (2 mL). After the addition, stirring was continued at −78°C for another 2 h, then the reaction was quenched with 1N HCl (10 mL) before allowing the reaction mixture to warm to room temperature. The mixture was extracted with ethyl acetate (40 mL). The extracts were washed with 1N HCl (10 mL) and brine (10 mL). The organic solution was dried over anhydrous $MgSO_4$, filtered, and concentrated. The product was isolated by column chromatography on silica gel using hexane/ethyl acetate (85:15) as eluant to afford **23** as a pale yellow oil (111 mg, 70%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.82 (t, 2H, J=8.0 Hz), 2.92 (t, 2H, J=8.0 Hz), 3.18 (t, 2H, J=8.0 Hz), 3.28 (t, 2H, J=8.0 Hz), 3.72 (s, 3H), 5.04 (s, 2H), 5.07 (s, 4H), 5.08 (s, 2H), 5.08 (s, 2H), 6.47 (dd, 1H, J=2.0, 8.5 Hz), 6.57–6.65 (br m, 6H), 6.68 (d, 1H, J=2.0 Hz), 6.71 (d, 1H, J=2.0 Hz), 6.80 (d, 1H, J=8.5 Hz), 7.24–7.41 (br m, 25H), 7.77 (d, 1H, J=8.5 Hz), 7.82 (d, 1H, J=9.0 Hz); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 30.02 (CH_2), 30.49 (CH_2), 45.65 (CH_2), 45.90 (CH_2), 56.19 (CH_3), 70.47 (2×CH_2), 70.96 (CH_2), 70.99 (CH_2), 71.40 (CH_2), 100.45 (CH), 100.50 (CH), 106.51 (2×CH), 113.09 (CH), 115.66 (CH), 118.10 (CH), 120.11 (CH), 120.29 (CH), 121.63 (C), 121.72 (C), 123.44 (CH), 127.21 (2×CH), 127.60 (6×CH), 127.68 (2×CH), 127.71 (2×CH), 128.35 (6×CH), 128.76 (7×CH), 132.80 (2×CH), 135.37 (C), 135.85 (2×C), 136.22 (2×C), 137.12 (C), 137.59 (C), 144.74 (C), 146.41 (C), 147.73 (C), 149.91 (C), 159.74 (C), 159.78 (C), 163.32 (C), 163.35 (C), 199.27 (C═O), 199.39 (C═O); FT-IR (film, ν_{max}, cm^{-1}): 3032w, 2926m, 2869w, 1663s, 1598s, 1508s, 1454m, 1257s, 1177s, 1124s, 1026s, 832m, 736m, 697m; HRESMS $[M+H]^+$: 995.4174 (calcd for $[C_{66}H_{58}O_9+H]^+$, 995.4159).

4.1.9. Verbenachalcone (2). A mixture of **23** (25 mg, 0.025 mmol), 5% Pd/C (25 mg) and acetic acid (2 mL) was stirred at room temperature under hydrogen (1 atm) for 2 h, before being diluted with 5 mL of methanol. After removal of the catalyst by filtration through anhydrous Na_2SO_4, the solution was concentrated and the product purified by column chromatography on silica gel using $CHCl_3$/MeOH (100:2) as eluant to afford **2** as a pale yellow oil (12 mg, 88%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.88 (t, 2H, J=7.5 Hz), 3.05 (t, 2H, J=7.5 Hz), 3.12 (t, 2H, J=7.5 Hz), 3.22 (t, 2H, J=7.5 Hz), 3.80 (s, 3H), 5.76 (br s, 1H), 5.98 (s, 1H), 6.13 (br s, 1H), 6.34 (dd, 1H, J=2.5, 8.5 Hz), 6.34 (dd, 1H, J=2.5, 8.5 Hz), 6.58 (d, 1H, J=2.0 Hz), 6.75 (dd, 1H, J=2.0, 8.5 Hz), 6.81 (d, 1H, J=2.0 Hz), 6.84 (dd, 1H, J=2.0, 8.5 Hz), 6.90 (d, 1H, J=8.0 Hz), 6.92 (d, 1H, J=8.0 Hz), 7.56 (d, 1H, J=8.5 Hz), 7.58 (d, 1H, J=8.5 Hz), 12.62 (s, 1H), 12.76 (s, 1H); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 30.37 (CH_2), 31.16 (CH_2), 39.68 (CH_2), 40.10 (CH_2), 56.26 (CH_3), 103.75 (CH), 103.78 (CH), 107.87 (CH), 107.99 (CH), 113.25, 113.92 (C), 114.19 (C), 116.01 (CH), 117.54 (CH), 121.01 (2×CH), 123.77 (CH), 132.49 (CH), 132.51 (CH), 132.71 (C), 138.08 (C), 143.46 (C), 144.97 (C), 145.47 (C), 150.82 (C), 162.69 (C), 162.79 (C), 165.28 (C), 165.46 (C), 203.85 (C═O), 204.07 (C═O); HRESMS $[M+H]^+$: 545.1815 (calcd for $[C_{31}H_{28}O_9+H]^+$, 545.1811).

4.1.10. Verbenachalcone pentaacetate (24). A mixture of **2** (8 mg, 0.015 mmol), acetic anhydride (0.5 mL) and

anhydrous pyridine (2 mL) was stirred at room temperature under argon for 3 h, then diluted with 30 mL of ethyl acetate. The organic solution was washed with 1N HCl (3×10 mL), dried over anhydrous $MgSO_4$, filtered and concentrated. The product was purified by column chromatography on silica gel using hexane/ethyl acetate (50:50) as eluant to give **24** as a pale yellow oil (10 mg, 90%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.23 (s, 3H), 2.28 (s, 3H), 2.31 (s, 3H), 2.32 (s, 3H), 2.32 (s, 3H), 2.89 (t, 2H, J=7.5 Hz), 3.00 (t, 2H, J=7.5 Hz), 3.11 (t, 2H, J=7.5 Hz), 3.22 (t, 2H, J=7.5 Hz), 3.81 (s, 3H), 6.65 (d, 1H, J=2.0 Hz), 6.73 (dd, 1H, J=2.0, 8.0 Hz), 6.84 (d, 1H, J=2.0 Hz), 6.87 (d, 1H, J=8.0 Hz), 6.89 (dd, 1H, J=2.0, 8.5 Hz), 6.95 (d, 1H, J=2.0 Hz), 6.97 (d, 1H, J=2.0 Hz), 7.03 (d, 1H, J=8.0 Hz), 7.08 (dd, 1H, J=8.5 Hz), 7.10 (dd, 1H, J=8.5 Hz), 7.74 (d, 1H, J=8.5 Hz), 7.81 (d, 1H, J=8.5 Hz). HRESMS $[M+NH_4]^+$: 772.2607 (calcd for $[C_{31}H_{28}O_9+NH_4]^+$, 772.2605).

4.1.11. 1-(2,4-Dihydroxyphenyl)-3-(4-{5-[3-(2,4-dihydroxyphenyl)propyl]-2-hydroxyphenoxy}-3-methoxyphenyl)propane (25). A mixture of **23** (25 mg, 0.025 mmol), 5% Pd/C (20 mg) and THF/MeOH (2:3, 2.5 mL) was stirred at room temperature under hydrogen (1 atm) for 16 h, before being diluted with 5 mL of methanol. After removal of the catalyst by filtration through anhydrous Na_2SO_4, the solution was concentrated and the residue purified by column chromatography on silica gel using $CHCl_3$/MeOH (100:7) as eluant to afford **25** as a pale yellow solid (10 mg, 74%). ^{1}H NMR (500 MHz, $CDCl_3$/CD_3OD (3:1)): δ 1.78 (quintet, 2H, J=7.5 Hz), 1.91 (quintet, 2H, J=7.5 Hz), 2.48 (t, 2H, J=7.5 Hz), 2.50 (t, 2H, J=7.5 Hz), 2.57 (t, 2H, J=7.5 Hz), 2.64 (t, 2H, J=7.5 Hz), 3.85 (s, 3H), 6.24 (d, 1H, J=2.5 Hz), 6.26 (dd, 1H, J=2.5, 7.5 Hz), 6.28 (d, 1H, J=2.5 Hz), 6.30 (dd, 1H, J=2.5, 8.0 Hz), 6.67 (d, 1H, J=2.0 Hz), 6.74 (dd, 1H, J=2.0, 8.5 Hz), 6.80 (dd, 1H, J=2.0, 8.5 Hz), 6.83 (d, 1H, J=2.0 Hz), 6.83 (d, 1H, J=8.0 Hz), 6.89 (d, 1H, J=8.5 Hz), 6.90 (d, 1H, J=8.5 Hz), 6.91 (d, 1H, J=8.5 Hz); ^{13}C NMR (100.5 MHz, $CDCl_3$/CD_3OD (3:1)): δ 29.04 (CH_2), 29.27 (CH_2), 31.85 (2×CH_2), 34.99 (CH_2), 35.57 (CH_2), 56.22 (CH_3), 102.53 (CH), 102.59 (CH), 106.78 (CH), 106.83 (CH), 113.06 (CH), 115.92 (CH), 118.04 (CH), 119.89 (CH), 120.23 (C), 120.26 (C), 121.07 (CH), 124.00 (CH), 130.57 (CH), 130.80 (CH), 134.70 (C), 139.51 (C), 143.37 (C), 144.49 (C), 145.00 (C), 150.20 (C), 155.00 (C), 155.06 (C), 155.21 (C), 155.36 (C); HRESMS $[M+H]^+$: 517.2222 (calcd for $[C_{31}H_{32}O_7+H]^+$, 517.2226).

4.2. Neurite outgrowth assay

The ability of compounds to enhance NGF's effects for stimulating neurite outgrowths was accessed utilizing the methodology previous reported.[7d] PC12 cells were dissociated by incubation with 1 mM ethylene glycol-bis-(2-aminoethylether)-*N*,*N*,*N*′*N*′-tetraacetic acid (EGTA) in phosphate-buffered saline (PBS) for 1 h and then were seeded in 24-well culture plates (2×10^4 cells/well) coated with collagen. After 24 h, the medium was changed to test medium containing various concentrations of NGF (30 ng/mL for control and 2 ng/mL for test samples), 1% fetal calf serum, 2% horse serum, and various concentrations of test compounds (15 and 30 μM). All test compound stock solutions were prepared at 10 mM in DMSO. The DMSO concentration in the assays was 0.3%. After 48 h the cells were fixed with 2% glutaraldehyde at 37°C for 1 h. The neurite outgrowth was accessed under a phase-contrast microscope. Neurite processes with a length equal to or greater than the diameter of the neuron cell body were scored as a neurite-bearing cell. The ratio of neurite-bearing cells to total cells (with at least 100 cells examined/viewing area; 3 viewing areas/well; 4 wells/sample) was determined and expressed as a percentage. The data was analyzed by Student unpaired *t*-test.

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