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Supplemental Data

1α,25-dihydroxyvitamin D_3 Interacts with Curcuminoids to Stimulate Amyloid-β Clearance by Macrophages of Alzheimer's Disease Patients

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SUPPLEMENTARY MATERIAL

General

All reactions were run under an argon atmosphere with magnetic stirring using oven-dried glassware unless otherwise indicated. Air- and moisture-sensitive liquids were transferred via syringe through rubber septa. Silica gel (60 Å, 40–63 μm mesh) from Silicycle was used for column chromatography. Ethylacetate was dried by filtration through neutral alumina and was stored over activated 4 Å molecular sieves under Argon prior to use. All other solvents and reagents were used as received. ^{1}H NMR was recorded at 300.0 MHz with a Varian Mercury 300 instrument. Chemical shifts were reported in ppm (δ) relative to $CDCl_3$ at 7.26 ppm. NMR spectra were recorded in $CDCl_3$ unless stated otherwise. ESI-MS data were obtained using a Hitachi model M-8000 mass spectrometer.

Synthesis of *5-Hydroxy-1,7-bis-(4-hydroxy-phenyl)-hepta-1,4,6-trien-3-one* (*Bisdemethoxycurcumin*), *2*. A mixture of acetylacetone (2 mL, 19.5 mmol) and boric anhydride (1 g, 12.8 mmol) was stirred at room temperature for 1 h under an atmosphere of argon. 4-Hydroxybenzaldehyde (4.9 g, 40 mmol) and tributyl borate (21.6 mL, 80 mmol) were dissolved in dry ethyl acetate (50 mL) in a round bottom flask under an atmosphere of argon. The boron complex was added to this mixture and the reaction mixture was stirred at room temperature for 5–10 min. n-Butylamine (0.4 mL) was added in 0.1 mL portions every 10 min. The resulting mixture was stirred for another 4 h and then allowed to stand overnight. The reaction was heated to 60 °C and hydrochloric acid (0.4 N, 30 ml) was added. After stir-

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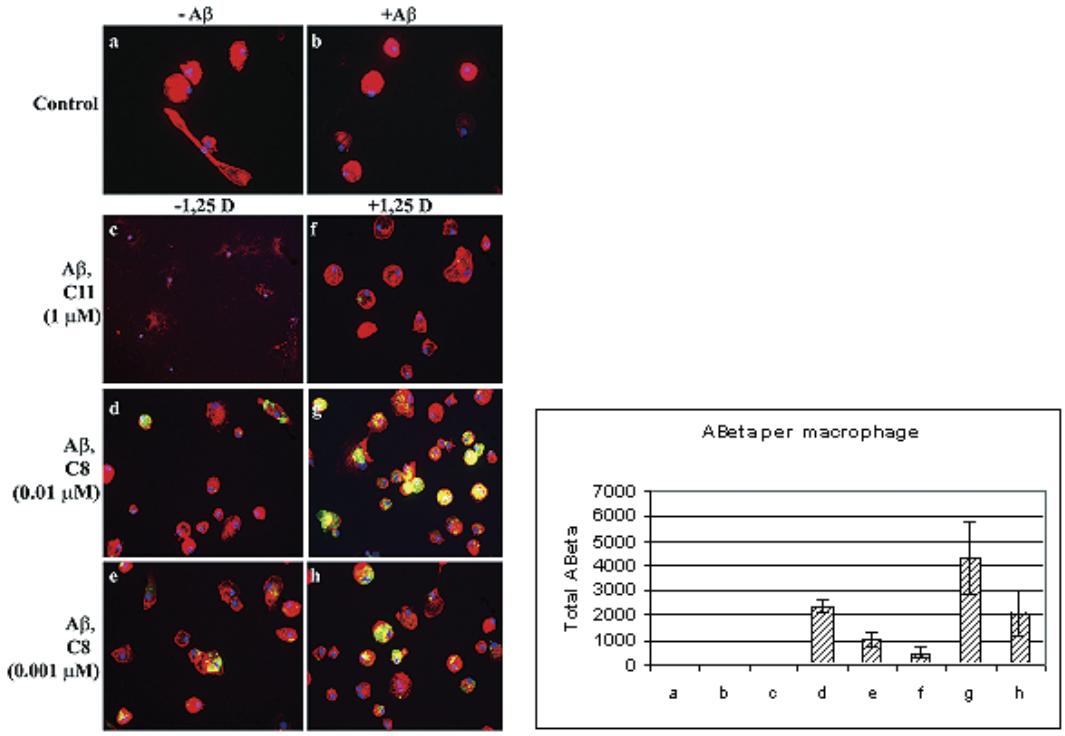


Supplemental Fig. 1. 1,25D3 strongly stimulates Aβ uptake in Type I macrophages of an MCI patient; SG-IV-176 (C11) or SG-IV-180 (C8) have additive effects. Replicate cultures of macrophages of a Type I MCI patient, which were either untreated or treated with a combination of SG-IV-180 (**C8**) or SG-IV-176 (**C11**) with 1,25D3 were exposed to FAM-Aβ (2 μM) and stained with phalloidin (red).

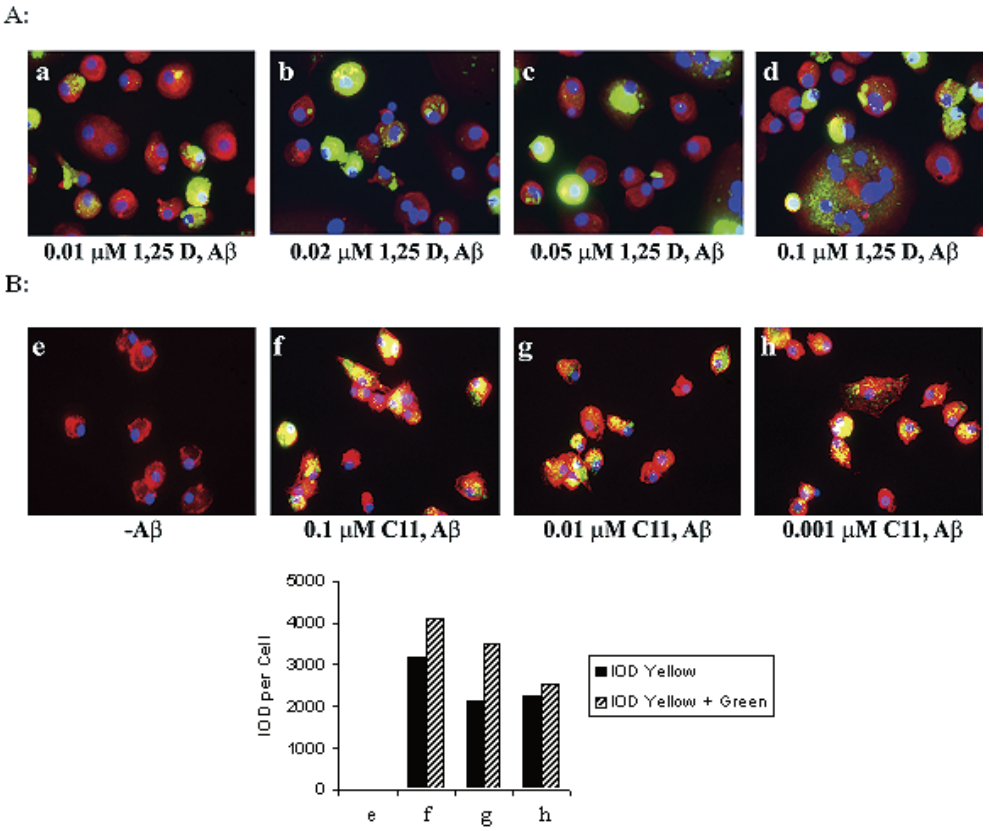
ring at 60°C for 1 h, the layers were separated and the organic layer was successively washed with water and brine. The solution was dried over Na_2SO_4, filtered and concentrated to dryness. The crude product was dissolved in ethyl acetate (75 mL) and methanol (50 mL) and the solution was kept in the fridge overnight. The precipitate was washed with cold methanol to afford 3.62 g, 60% yield of the product as an orange powder. R_f = 0.15 (hexane/EtOAc, 2:1, v:v), mp= 199.9 °C. ESI-MS: m/z 309 (MH^+), 331 (MNa^+), 307 $(MH)^-$; ^{1}H NMR ($CDCl_3$) δ 7.50 (d, 2H, Ph-CH-), 7.29 (m, 4H, Ph), 6.62–6.78 (m, 4H, Ph), 6.37 (d, 2H, -CH-), 5.70 (s, 1H, -CO-CH-CO-).

Synthesis of the water soluble analogs

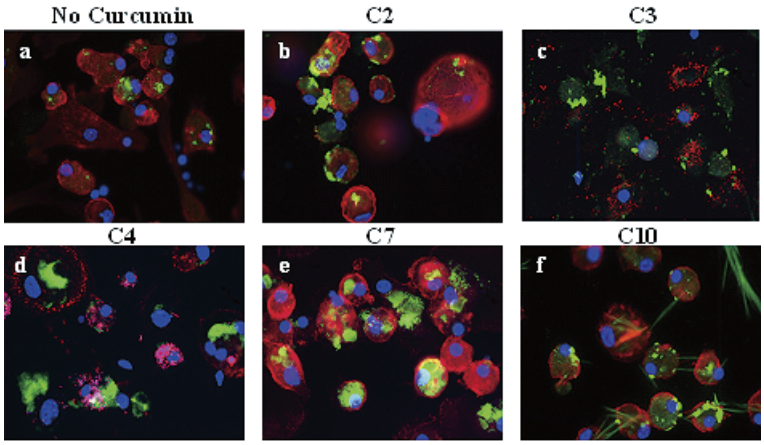
4,4'-(3,5-Dioxohepta-1,6-diene-1,7-diyl)bis(2-methoxy-4,1-phenylene)bis(2-amino-3-methylbutanoate) HCl salt(**10**), 4,4'-(3,5-dioxohepta-1,6-diene-1,7-diyl) bis(2-methoxy-4,1-phenylene) bis(2,6-diaminohexanoate) HCl salt(**11**), 4-(4-(2-Amino-3-methylbutanoyloxy)benzylidene)-2-oxocyclohexyl)-3-oxoprop-1-enyl)phenyl-2-amino-3-methylbutanoate HCl salt(**13**), and4-(3-(4-(2,6-Diaminohexanoyloxy)benzylidene)-2-oxocyclohexyl)-3-oxoprop-1-enyl)phenyl-2,6-diaminohexanoate HCl salt (**14**) were synthesized according to the procedure described below for compound **12**.

Synthesis of 4,4'-(3,5-Dioxohepta-1,6-diene-1,7-diyl) bis(4,1-phenylene) bis(2-amino-3-methylbutanoate) HCl salt, 12

A mixture of bisdemethoxycurcumin, **2** (50 mg, 0.16 mmol), dicyclohexylcarbodiimide (84 mg, 0.4 mmol), dimethylamino pyridine (4 mg, 0.03 mmol) and BOC-Val-OH (141 mg, 0.65 mmol) were dissolved in dichloromethane (13 mL) and DMF (0.5) in a pre dried scintillation vial and stirred at room temperature under Ar(g) for 2 h. The white precipitate was filtered off and solvent was removed. The oily residue was redissolved in dichloromethane and water was added. The solution was extracted with DCM, dried over Na_2SO_4, filtered and concentrated. The resulting oil was purified using PTLC (Hexane/EtOAc, 3:1, v/v) to give 60 mg, 53% yield of the Valine-(BOC)-ester-BDC product. ESI/MS: m/z = 729.13 (MNa^+), R_f = 0.3. ^{1}HNMR ($CDCl_3$) δ 7.64 (d, J = 16.5 Hz, 2H), 7.58 (d, J = 9.3 Hz, 4H), 7.14 (d, J = 9.3, 4 H),



Supplemental Fig. 2. Dose response in type I macrophages to (A) 1,25D3, and (B) SG-IV-176 (**C11**). **C11** was effective in the range 0.1 to 0.001 μM, and 1,25D3 was effective in the range 0.1 to 0.01 μM.



Supplemental Fig. 3. Comparative effects of novel synthetic curcuminoids on Aβ phagocytosis. SC-IV-85 (**C7**) was the most effective, **C2**, **C4**, and **C10** were less effective, and **C3** was toxic.

6.59 (d, J = 16.5 Hz, 2H), 5.84 (s, 1H), 5.06 (m, 2H), 4.46 (m, 2H), 2.34 (m, 2H), 1.47 (s, 18H), 1.09 (d, J = 6.9 Hz, 6H), 1.03 (d, J = 6.9 Hz, 6H). The BOC-protected amino acid ester (56 mg, 0.08 mmol) was dissolved in EtOAc (4 mL) and DCM (0.4 mL). The yellow solution was cooled to 0°C (ice bath). HCl(g) was bubbled through the solution for 2 min. The solution turned orange. The vial was capped and solution was

stirred for 3 h. Solvent was removed under a stream of argon and the residue was washed with ether to give the hydrochloride salt in 91% yield (42 mg). Solubility in water: 8 mg/mL. ESI/MS: $m/z = 506.87$ (MH^+). ^{1}HNMR (CD_3OD) δ 7.76 (d, $J = 8.4$ Hz, 4H), 7.69 (d, $J = 15.9$ Hz, 2H), 7.26 (d, $J = 8.4$ Hz, 4H), 6.85 (d, $J = 15.9$ Hz, 2H), 6.10 (s, 1H), 4.25 (d, $J = 4.8$ Hz, 2H), 2.5 (m, 2H), 1.22–1.19 (m, 12H).