Supplementary Material

Curcumin Pyrazole and its derivative (N-(3-Nitrophenylpyrazole) Curcumin inhibit aggregation, disrupt fibrils and modulate toxicity of Wild type and Mutant α-Synuclein

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Supplementary material 1

Chemistry

Reaction progress was monitored by TLC using Merck silica gel 60 F_{254} with detection by UV. Column chromatography was performed using Merck silica gel 230–400 mesh. Melting points were determined in Pyrex capillary tube using Büchi Melting Point B-540 apparatus. ^{1}H NMR spectra was recorded on 300 and 400 MHz Bruker NMR spectrometers using tetramethylsilane as internal standard and the chemical shifts are reported in (δ) units. Coupling constants are reported as a *J* value in Hertz (Hz).The sample concentration in each case was approximately 10 mg in chloroform-d/methanol-d_4/DMSO-d_6 (0.6 mL). Mass spectra were recorded on an Electrospray–MS (Bruker Daltonis) instrument.

Isoxazole analogue of curcumin **(2)** and pyrazole analogs of curcumin **(3)** were prepared according to our previous reported procedure. The purity of all the compounds were >95% as determined by HPLC.

General procedure for the preparation of *N*-(substituted) phenylcurcumin pyrazole Analogs:

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Prepared according to previously reported procedure by us and others with some modifications [20-23]. Curcumin (1 mM) was dissolved in glacial acetic acid (5 mL), and hydrazine hydrate/various phenyl substituted hydrazine hydrochlorides (1.5 mM) were added to the solution. The solution was refluxed for 8 -24 h, and then the solvent was removed in vacuo. Residue was dissolved in ethyl acetate and washed with water. Organic portion was collected, dried over sodium sulfate, and concentrated in vacuo. Crude product was purified by column chromatography. All the curcumin pyrazoles analogues were prepared by using this procedure.

***N*-Phenylpyrazole curcumin (4):** The crude product was purified by column chromatography (EtOAc/hexanes, 4:6) on silica gel to get the desired product. R_f = 0.62 (EtOAc/Hexane 5:5), yield: 65%, mp 89 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.89 (s, 3H, -OCH_3), 3.91 (s, 3H, -OCH_3), 5.60 (s, br, 2H, -*OH*), 6.79-6.91 (m, 3H, C_4-*H,* C_2-*H* and C_6-*H*), 7.04 (d, 2H, *J* = 15.8 Hz, C_1-*H* and C_7-*H*), 7.18–7.34 (m, 6H, *Ar-H*), 7.48–7.54 (m, 5H, *Ar-H*). ESI HRMS *m/z* calcd for $(C_{27}H_{24}N_2O_4 + H)^+$ 441.1808, found $[M + H]^+$ 441.1810.

N-(2-Flurophenylpyrazole) curcumin (5): The crude product was purified by column chromatography (EtOAc/hexanes, 40:60) on silica gel to get the desired product, R_f = 0.55 (EtOAc/Hexane 4:6), Yield 48%; mp 118-120°C, ^{1}H NMR (300 MHz, DMSO d_6): δ 3.73 (s, 3H, -*OCH*$_3$), 3.83 (s, 3H, -*OCH*$_3$), 6.45 (d, 1H, *J* = 4.6 Hz, C_4-H), 6.5-6.7 (m, 2H, C_2-H and, C_6-H), 6.8-6.98 (m, 8H, C_1-H and, C_7-H, *Ar-H*), 7.27-7.63 (m, 4H, *Ar-H*), 9.32 (s, 1H, -*OH*), 9.68 (s, 1H, -*OH*). ESI HRMS *m/z* calcd for ($C_{27}H_{23}FN_2O_4$ + H^+) 459.1714, found $[M+H]^+$ 459.1714.

N-(3-Fluorophenylpyrazole) Curcumin (6): The crude product was purified by column chromatography (EtOAc/hexanes, 40:60) on silica gel to get the desired product, R_f = 0.60 (EtOAc/Hexane 40:60), Yield 46%; mp 122-123°C, ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.78 (s, 3H, -*OCH*$_3$) 3.83 (s, 3H, -*OCH*$_3$), 6.76-6.84 (m, 3H, C_4-*H*, C_2-*H* and, C_6-*H*), 6.98-7.43 (m, 10H, C_1-*H*, C_7-*H*, *Ar-H*), 7.56-7.74 (m, 2H, *Ar-H*). ESI HRMS *m/z* calcd for ($C_{27}H_{23}FN_2O_4$ + H^+) 459.1714, found $[M+H]^+$ 459.1714.

***N*-(4-Fluorophenylpyrazole) curcumin (7):** The crude product was purified by column chromatography (EtOAc/hexanes, 30:70). R_f = 0.56 (EtOAc/Hexane 5:5), yield: 52%. mp 65 °C. ^{1}H NMR (300 MHz, $CDCl_3$): *δ* 3.88 (s, 3H, - *OCH*$_3$), *δ* 3.90 (s, 3H, - *OCH*$_3$), 6.79 (s, 1H, C_4-*H*), 6.98 (d, 2H, *J* = 8.6 Hz, C_2-*H* and, C_6-*H*), 7.02 (d, 2H, *J* = 8.6 Hz, C_1-*H* and C_7-*H*), 7.10–7.22 (m, 6H, *Ar-H*), 7.45–7.69 (m, 4H, *Ar-H*). ESI HRMS *m/z* cald for ($C_{27}H_{23}FN_2O_4$ + H)$^+$ 459.1714, found $[M + H]^+$ 459.1720.

N-(2-Chlorophenylpyrazole) curcumin (8): The crude product was purified by column chromatography (EtOAc/hexanes, 40:60) on silica gel to get the desired curcumin pyrazole product, R_f = 0.60 (EtOAc/Hexane 4:6), mp 128 °C yield: 39%, 1H NMR (300 MHz, DMSO d_6): δ 3.85 (s, 3H, -*OCH*$_3$) 3.91 (s, 3H, -*OCH*$_3$), 6.80-6.64 (m, 2H; H-9, H-18), 6.86-7.05 (m, 6H), 7.24 (d, 2H, *J* = 15.8 Hz), 7.3-7.52 (m, 4H, *Ar-H*), 7.6-7.78 (m, 1H, *Ar-H*). ESI HRMS m/z cald for ($C_{27}H_{23}ClN_2O_4$ + H)$^+$ 475.1419, found $[M + H]^+$ 475.1430.

N-(3-Chlorophenylpyrazole) Curcumin (9): The The crude product was purified by column chromatography (EtOAc/hexanes, 30:70) on silica gel to get the desired curcumin pyrazole product, R_f = 0.56 (EtOAc/Hexane 4:6), mp 154°C, yield: 42%, 1H NMR (300 MHz, DMSO-d_6): δ 3.76 (s, 3H, -*OCH*$_3$) 3.82 (s, 3H, -*OCH*$_3$), 6.63 (d, 1H, *J* = 13.9 Hz, C_4-H), 6.43-6.66 (m, 2H, C_2-H and, C_6-H), 6.72-7.20 (m, 9H, C_1-H, C_7-H, *Ar-H*), 7.66 (s, 2H, *Ar-H*), 7.92 (s, 1H, *Ar-H*), 9.21 (s, 1H, -*OH*), 9.31 (s, 1H, -*OH*). ESI HRMS m/z cald for ($C_{27}H_{23}ClN_2O_4$ + H)$^+$ 475.1419, found $[M + H]^+$ 475.1432.

***N*-(2,4-Dichlorophenylpyrazole) curcumin (10):** The crude product was purified by column chromatography (EtOAc/hexanes, 40:60) on silica gel to get the desired curcumin pyrazole product, yield: 54%, mp 142 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.83 (s, 3H, -OCH_3), 3.88 (s, 3H, -OCH_3), 6.65 (d, 1H, *J* = 8.6 Hz, C_7-*H*), 6.89 (d, 2H, *J* = 15.6 Hz, C_2-*H* and, C_6-*H*), 7.08 (m, 4H, C_1-*H* and C_7-*H, Ar-H*), 7.12–7.22 (m, 5H, *Ar-H*), 7.35-7.58 (m, 2H, *Ar-H*). ESI HRMS m/z cald for $(C_{27}H_{22}Cl_2N_2O_4+ H)^+$ 509.1029, found $[M + H]^+$ 509.1038.

N-(4-Bromophenylpyrazole) curcumin (11): The crude product was purified by column chromatography (EtOAc/hexanes, 40:60) on silica gel to get the desired curcumin pyrazole product, R_f = 0.65 (EtOAc/Hexane 4:6), Yield 54%, mp 149-151°C, ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.78 (s, 3H, -OCH_3), 3.83 (s, 3H, -OCH_3), 6.76 (m, 3H, C_4-H, C_2-H, C_6-H), 6.97-7.21 (m, 8H, *J* = 15.8 Hz, C_1-H and, C_7-H, *Ar-H*), 7.49 (d, 2H, *J* = 8.6 Hz, *Ar-H*), 7.75 (d, 2H, *J* = 8.6 Hz, *Ar-H*), 9.21 (s, 1H, -*OH*), 9.32 (s, 1H, -*OH*). ESI HRMS *m/z* cald for $(C_{27}H_{23}BrN_2O_4 + H)^+$ 519.0913, found $[M + H]^+$ 519.0921.

N-(2-Methylphenylpyrazole) curcumin (12): The crude product was purified by column chromatography (EtOAc/hexanes, 30:70) on silica gel to get the desired curcumin pyrazole product, Yield 46 %; mp. 97-98 °C 1H NMR (300 MHz, DMSO-d_6): δ 2.31 (s, 3H, -CH_3), 3.77 (s, 3H, -OCH_3), 3.84 (s, 3H, -OCH_3), 6.38 (d, 2H, *J* = 8.4 Hz, C_1-H & C_7-H), 6.68 (m, 3H, C_2-H, C_6-H & C_4-H), 7.05 (m, 3H, *Ar-H*), 7.12 (m, 2H, *Ar-H*), 7.36 (m, 1H, *Ar-H*), 7.48 (m, 4H, *Ar-H*), 9.20 (s, 1H, -*OH*), 9.35 (s, 1H, -*OH*). 454.14. ESI HRMS m/z cald for $(C_{28}H_{26}N_2O_4 + H)^+$ 455.1965, found $[M + H]^+$ 455. 1941.

N-(3-Methylphenylpyrazole) curcumin (13): The crude product was purified by column chromatography (EtOAc/hexanes, 30:70) on silica gel to get the desired curcumin pyrazole product, Yield 57 %; mp. 120-122 °C 1H NMR (300 MHz, DMSO-d_6): δ 2.34 (s, 3H, -CH_3), 3.85 (s, 3H, -OCH_3), 3.90 (s, 3H, -OCH_3), 6.56 (m, 2H, C_1-H & C_7-H), 6.86 (m, 3H, C_2-H, C_6-H & C_4-H), 7.05 (m, 3H, *Ar-H*), 7.20 (m, 2H, *Ar-H*), 7.36 (m, 1H, *Ar-H*), 7.43 (m, 4H, *Ar-H*), 9.20 (s, 1H, -*OH*), 9.35 (s, 1H, -*OH*). ESI HRMS m/z cald for $(C_{28}H_{26}N_2O_4 + H)^+$ 455.1965, found $[M + H]^+$ 455. 1953.

N-(2-Nitrophenylpyrazole) curcumin (14): The crude product was purified by column chromatography (EtOAc/hexanes, 30:70) on silica gel to get the desired curcumin pyrazole product,. R_f = 0.62 (EtOAc/Hexane 4:6), yield: 38%, mp 198-199 °C. 1H NMR (300 MHz, DMSO- d_6): δ 3.78 (s, 3H, -OCH_3) 3.85 (s, 3H, -OCH_3), 6.65 (s, 1H, C_4-H), 6.96 (d, 2H, *J* = 12.8 Hz, C_2- H and, C_6-H), 6.98-7.12 (m, 4H, C_1-H and C_7-H, *Ar-H*), 7.2-7.38 (m, 2H, *Ar-H*), 7.46 (d, 2H, *J* = 12.8 Hz, *Ar-H*), 7.62-7.94

(m, 3H, *Ar-H*), 8.20 (d, 1H, *J* = 7.8 Hz), 9.19 (s, 1H, -*OH*), 9.38 (s, 1H, -*OH*). ESI HRMS m/z cald for $(C_{27}H_{23}N_3O_6 + H)^+$ 486.1459, found $[M + H]^+$ 486.1455.

***N*-(3-Nitrophenylpyrazole) Curcumin (15):** The crude product was purified by column chromatography (hexane/ethyl acetate, 60:40) on silica gel to get the desired product, R_f = 0.65 (DCM/MeOH 9:1), yield: 46%, mp 93 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.78 (s, 3H, -*OCH*$_3$), 3.84 (s, 3H, -*OCH*$_3$), 6.70-6.86 (m, 3H, *C*$_4$-*H, C*$_2$-*H* and, *C*$_6$-*H*), 7.10 (d, 2H, *J* = 14.8 Hz, *C*$_1$-*H* and *C*$_7$-*H*), 6.56–7.91 (m, 7H, *Ar-H*), 7.81 (t, 1H, J= 8.13 Hz, *Ar-H*), 8.10 (dd, 1 H, J_1 = 8.13Hz, J_2= 7.6Hz, *Ar-H*), 8.30 (s, 1H, *J* = 8.2 Hz, *Ar-H*), 9.71 (s, 1H, -*OH*), 9.80 (s, 1H, -*OH*). ESI HRMS *m/z* calculated. for $(C_{27}H_{23}N_3O_6 + H^+)$ 486.1459, found $[M+H]^+$ 486.1452.

***N*-(4-Methoxyphenylpyrazole) curcumin (16):** The crude product was purified by column chromatography (EtOAc/hexanes, 30:70) on silica gel to get the desired curcumin pyrazole product, R_f = 0.65 (EtOAc/Hexane 4:6), yield: 35%, mp 106 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.85 (s, 3H, -*OCH*$_3$), 3.89(s, 3H, -*OCH*$_3$), 3.91(s, 3H, -*OCH*$_3$), 6.79 -6.95 (m, 3H, *C*$_4$-*H, C*$_2$-*H* and, *C*$_6$-*H*), 7.04 (d, 2H, *J* = 16.2 Hz, *C*$_1$-*H* and *C*$_7$-*H*), 7.09–7.20(m, 6H, *Ar-H*), 7.28–7.48 (m, 4H, *Ar-H*). ESI HRMS m/z cald for $(C_{28}H_{26}N_2O_5 + H)^+$ 471.1914 found $[M + H]^+$ 471.1906.

N-(2-Carboxypyrazole) curcumin (17): The crude product was purified by column chromatography (EtOAc/hexanes, 40:60) on silica gel to get the desired curcumin pyrazole product, Yellow powder (38%), mp 201-204°C. ^{1}H NMR (300 MHz, DMSO-*d*$_6$): δ 3.75 (s, 3H, -*OCH*$_3$), 3.86 (s, 3H, -*OCH*$_3$), 6.43 (1H, d, *J* = 6.8 Hz, C_4-H), 6.58-7.16 (m, 10H, C_1-H, C_7-H, C_2-H, C_6-H, *Ar-H*), 7.54 (d, 1H, *J* = 7.6 Hz, *Ar-H*), 7.62 (d, 1H, *J* = 7.2 Hz, *Ar-H*), 7.82 (d, 1H, *J* = 5.6 Hz, *Ar-H*), 8.06 (d, 1H, *J* = 8.2 Hz, *Ar-H*), 9.18 (s, 1H, -*OH*), 9.26 (s, 1H, -*OH*). ESI HRMS *m/z* cald for $(C_{28}H_{24}N_2O_6 + H)^+$ 485.1707, found $[M+H]^+$ 485.1738.

N-(Benzylpyrazole) curcumin (18): The crude product was purified by column chromatography (EtOAc/hexanes, 40:60) on silica gel to get the desired curcumin pyrazole product. Yield 48%; m.p. 135-137 °C; ^{1}H NMR (300 MHz, DMSO-*d*$_6$): 3.85 (s, 3H, -*OCH*$_3$), 3.86 (s, 3H, -*OCH*$_3$), 5.38 (s, 2H, –*CH*$_2$), 6.80 (dd, 2H, J_1 = 8 Hz, J_2 = 5.6 Hz), 6.89 (m, 1H), 6.92-7.08 (m, 4H), 7.12 (d, 1H, *J* = 12.4 Hz), 7.18 (d, 1H, *J* = 5.6 Hz), 7.22-7.28 (m, 5H), 7.35-7.39 (m, 2H), 9.14 (s, 1H, -*OH*), 9.27 (s, 1H, -*OH*) ESI HRMS *m/z* cald for $(C_{28}H_{26}N_2O_4 + H)^+$ 455.1965, found $[M + H]^+$ 455.1958.

N-(2-hydroxyethylpyrazole) curcumin (19): The crude product was purified by column chromatography (EtOAc/hexanes, 30:70) on silica gel to get the desired curcumin pyrazole product,

Light yellow powder (45%), mp 221-223°C. ^{1}H NMR (300 MHz, DMSO d_6): δ = 3.65 (t, 2H, *J* = 7.2 Hz, -CH_2), 3.88 (s, 3H, -OCH_3), 3.91 (s, 3H, -OCH_3), 4.30 (m, 2H, -CH_2), 4.84 (bs, 1H, -OH), 6.54-6.92 (m, 4H, *Ar-H*), 7.09-7.12 (m, 5H, *Ar-H*), 7.2-7.43 (m, 2H), 9.16 (s, 1H, -*OH*), 9.20 (s, IH, -*OH*). ESI HRMS *m/z* cald for $(C_{23}H_{24}N_2O_5 + H)^+$ 409.1757, found $[M + H]^+$ 409.1686, $[M-H]^+$ 407. 1650.

Supplementary material 2

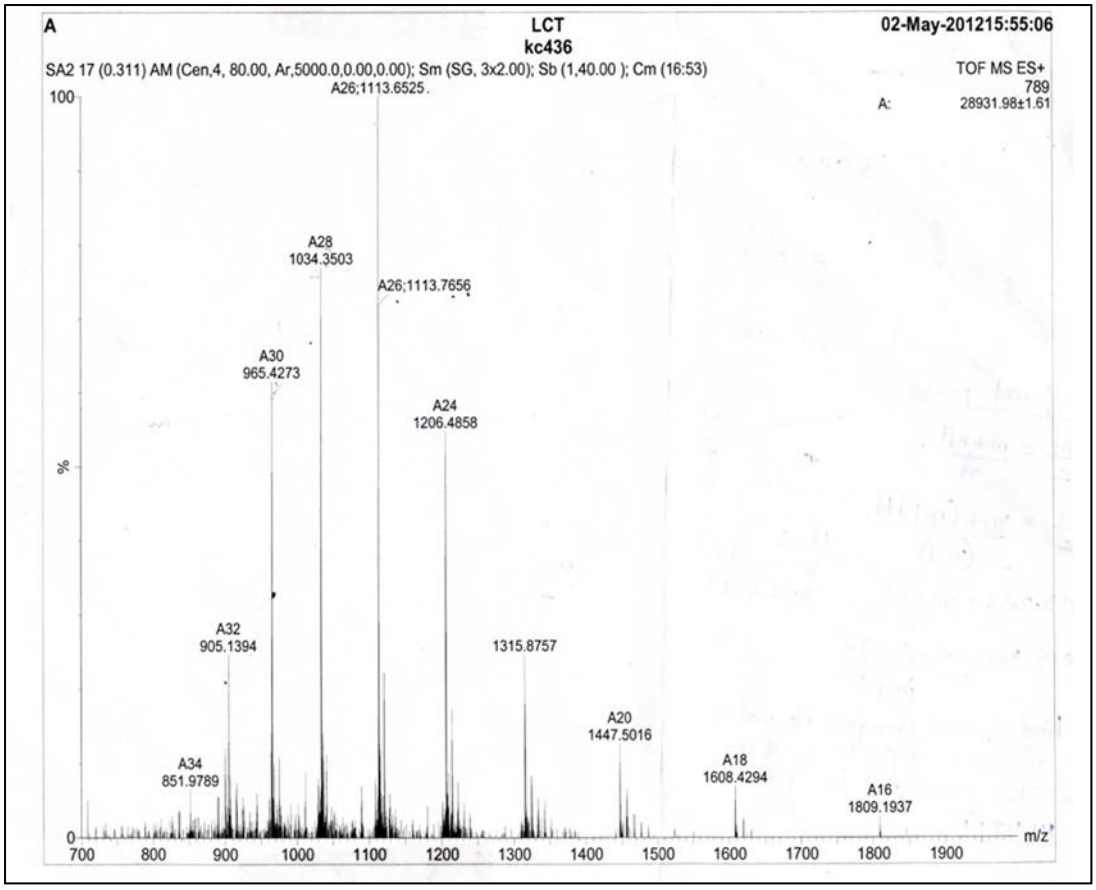

Fig.ESM_1 MALDI TOF MS of Wild type alpha-synuclein protein. Freshly prepared alpha-synuclein was dissolved in MilliQ and mass spectra acquired.

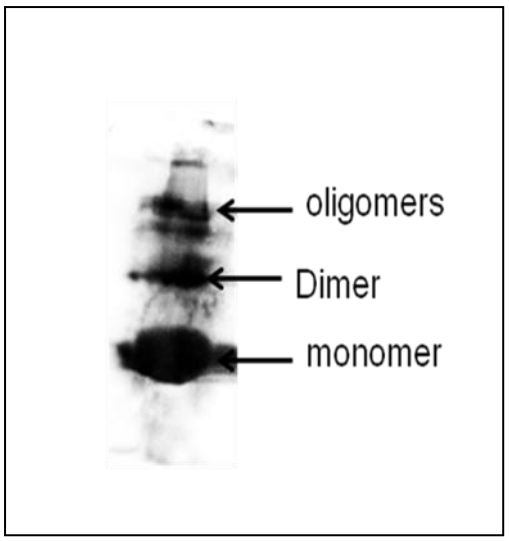

Fig.ESM_2 Representative Western blot of the purified protein using anti-α-synuclein antibody confirming the identity of the protein.

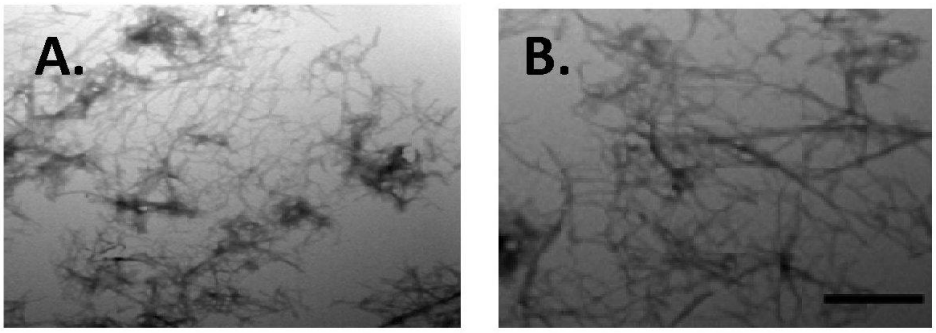

Fig.ESM_3 Representative TEM micrographs of 30 day aggregated (A) WtAS and (B) A53T mutant α-synuclein. 3 µl aggregated α-synuclein protein samples were adsorbed on carbon coated formavar grids and negatively stained with 2% uranyl acetate for TEM imaging. Scale bar is 200 nm.

Table S1: Size analysis of the species present in AFM images of Fig. 6

		Control	Compound 1	Compound 3	Compound 6	Compound 15
Diameter (nm)	Min	35.96	433.98	37.61	119.54	135
	Max	346.17	2943.38	276.4	576.41	627.57
	Mean	94.34	877.48	71.27	233.45	236.22
	SD	63.37	708.29	46.85	108.36	120.98
Z height (nm)	Min	21.20	8.98	4.92	6.9	21.63
	Max	21.58	46.92	5.762	8.84	24.58