Supporting information

Discovery of 3-arylcoumarin-tetracyclic tacrine hybrids as multifunctional agents against Parkinson's disease

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1. General Information:

All reagents were commercial and were used without further purification. Chromatography was carried on silica gel (60-120 and 100-200 mesh). All reactions were monitored by TLC; silica gel plates with fluorescence F254 were used. Melting points were uncorrected. The ^{1}H NMR, and ^{13}C NMR spectra were determined on 200, 300, 400 MHz and 50, 75, 100 MHz, respectively, using $CDCl_3$ and DMSO- d_6 as solvents and TMS as internal standard. All chemical shifts were given in ppm. IR spectra were recorded on in the range of 500~4000 cm^{-1} and multiplicity (s = singlet, brs = broad singlet, d = doublet, brd = broad doublet, dd = double doublet, t = triplet, q = quartet, m = multiplet). All final compounds (**16**-**24**) purity was checked by Waters high performance liquid chromatography (HPLC) system and found to be > 95% purity. Purity was determined using analytical reverse-phase Thermo Scientific C8 1 Å column (250 mm x 4.6 mm, particle size (μ)-5) and a binary solvent system; solvent A, 0.01% TFA/H_2O; solvent B, 0.01% TFA/CH_3CN. Isocratic elution was carried out having 25min, 15% of H_2O and 85% of CH_3CN; at a flow rate of 0.8 mL/ min monitored at 254 nm using a Waters 2998 photodiode array detector.

1.2. General synthetic procedure for preparation of 5-alkyl-4-hydroxy-benzene-1,3-dicarbaldehyde. (**3**, **4**)

2-alkyl phenol (1.0 equiv.) and hexamethylenetetramine (1.2 equiv.) were dissolved in TFA and the solution was heated at 120 ºC for 3 h. After cooling to room temperature 10 % aq.H_2SO_4 (25 mL) was added and again the temperature maintained (at 90-100 °C) for two more hours. The solution was basified with $NaHCO_3$ to pH 8 and extracted 3-fold with 50 mL of $CHCl_3$. The combined organic layers were dried on Na_2SO_4, filtered, and concentrated to dryness under reduced pressure. The crude product was purified on a silica gel column (100-200 mesh) using hexane-ethylacetate (12:88, v/v) as eluent to afford compounds **3**, **4** in good yields.

1.2.1. 5-Isopropyl-4-Hydroxyisophthalaldehyde (**3**):

White solid, yield: 65%; mp: 163-164 °C; IR (KBr): 3028, 1677, 1665, 1615, 1229, 768 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ: 11.94 (s, 1H), 9.97 (s, 1H), 9.91 (s, 1H), 7.99 (brs, 1H), 7.96(d, *J*=1.47 Hz,1H), 3.43-3.33 (m, 1H), 1.27 (d, *J*=5.19 HZ, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) : 196.6, 189.9, 164.3, 138.9, 134.8, 133.2, 129.0, 119.9, 26.5, 22.1;ESI-MS: m/z: 193 $(M+H)^+$.

*1.2.2. 5-Sec-butyl-4-hydroxy-benzene-1,3-dicarbaldehyde (***4***)*

Oily, Yield 55%, IR (neat): 3267, 2862, 1709, 1622, 1018 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ: 11.99 (s, 1H), 10.05 (s, 1H), 9.97 (s, 1H), 8.09 (brs, 1H), 8.01 (brs, 1H), 3.27-3.10 (m, 1H), 1.74-1.57 (m, 2H), 1.26 (d, 3H, *J* = 7.0 Hz), 0.86 (t, 3H, *J* = 7.3 Hz); ESI-MS: m/z: 207 $(M+H)^+$.

*1.3. General synthetic procedure for preparation of compounds (***5-9***)*:

To a mixture of cyanuric chloride (1.0 equiv) and *N*-methyl morpholine (1.5 equiv) in DMF appropriate substituted phenyl acetic acid (1.0 equiv) was added and stirred at room temperature for 10 min. After that, dicarbaldehyde (**3** or **4**, 1.0 equiv) was added, subsequently, the resulting reaction mixture was refluxed for 45 min (monitored by TLC). The reaction mixture was diluted with water and extracted with EtOAc. The combined organic layers were dried on Na_2SO_4, filtered, and concentrated to dryness under reduced pressure. The residue was purified over column chromatography (Al_2O_3, 70-230 mesh, neutral, hexane/DCM) provided the pure compounds (**5-9)**.

*1.3.1. 8-Sec-butyl-2-oxo-3-(3,4,5-trimethoxyphenyl)-2H-chromene-6-carbaldehyde (***9***)*:

Light yellow solid; yield: 95%; mp: 195-196 °C; IR (KBr): 3070, 1714, 1624, 1580, 1010 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) *δ*: 10.03 (s, 1H), 7.93 (s, 2H), 7.87 (s, 1H), 6.95 (s, 2H), 3.92

(s, 6H), 3.90 (s, 3H), 3.54–3.45 (m, 1H), 1.81–1.71 (m, 2H), 1.34 (d, 3H, *J* = 6.8Hz), 0.90 (t, 3H, *J* = 7.2 Hz);ESI-MS (*m/z*): 397 $(M+H)^+$.

*1.4. General synthetic procedure for preparation of compounds (***10-15***)*:

Into a mixture of 3-phenylcoumarin intermediates (1 mmol) and malononitrile (1 mmol) in 4 mL of ethanol was added the catalyst DMAP (0.2 mmol) and kept for stirring at room temperature. The solid precipitate was formed immediately after 20-30 min. Then C–H different 1, 3-cyclohexadione (1 mmol) was added into the reaction mixture and it was kept for stirring under reflux conditions (monitored by TLC). The reaction mixture was concentrated to dryness under reduced pressure.The crude product was purified on a silica gel column (100-200 mesh) using ethylacetate-hexane (50:50, v/v) as eluent to afford compounds **10-15** in good yields.

*1.4.1. (±)2-amino-8'-isopropyl-3'-(4-methoxyphenyl)-7,7-dimethyl-2',5-dioxo-5,6,7,8-tetrahydro-2'H,4H-[4,6'-bichromene]-3-carbonitrile (***10***)*.

Light yellow solid, yield: 70%; mp: 195-196 °C; IR (KBr): 3411, 3012, 2983, 2187, 1665,1590, 1040 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 8.19 (s, 1H), 7.70 (d, 2H, *J* = 8.3Hz), 7.38 (s, 1H), 7.25 (s, 1H), 7.07-7.00 (m, 4H), 4.27 (s, 1H), 3.80 (s, 3H), 3.48-3.38 (m, 1H), 2.62-2.49 (m, 2H), 2.31-2.07 (m, 2H), 1.26 (d, 6H, *J* = 7.2Hz), 1.05 (s, 3H), 0.97 (S, 3H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ:196.1, 163.1, 160.2, 160.0, 158.9, 149.2, 141.3, 140.0, 135.2, 130.2, 127.4, 127.3, 126.5. 124.8, 119.7, 114.1, 112.8, 58.5, 55.6, 50.4, 35.8, 32.2, 29.0, 27.0, 26.6, 22.8; ESI-MS (*m/z*): 511 $(M+H)^+$.

*1.4.2. (±)2-Amino-3'-(3,4-dimethoxyphenyl)-8'-isopropyl-7,7-dimethyl-2',5-dioxo-5,6,7,8-tetrahydro-2'H,4H-4,6'-bichromene-3-carbonitrile (***11***)*.

Light yellow solid, yield: 65%; mp: 175-176 °C; IR (KBr): 3414, 3018, 2983, 2200, 1668, 1584, 1092 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 8.24 (s, 1H), 7.39 (s, 1H), 7.36 (s, 2H), 7.26 (s, 1H), 7.06 (brs, 3H), 4.28 (s, 1H), 3.82 (s, 6H), 3.44 (brs, 1H), 2.62-2.50 (m, 2H), 2.31-2.08 (m, 2H), 1.27 (d, 6H, J = 6.5Hz), 1.05 (s, 3H), 0.97 (s, 3H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ:195.7, 162.7, 159.7, 158.4, 149.2, 148.7, 148.3, 140.9, 139.7, 134.7, 127.0, 126.1, 124.4, 121.3, 119.3, 112.4, 112.1, 111.3, 58.0, 55.6, 49.9, 35.4, 31.8, 28.5, 26.5, 26.2, 22.0; ESI-MS (*m/z*): 541 $(M+H)^+$.

*1.4.3. (±)2-Amino-8'-isopropyl-2',5-dioxo-3'-(3,4,5-trimethoxyphenyl)-5,6,7,8-tetrahydro-2'H,4H-4,6'-bichromene-3-carbonitrile (***12***).*

Light yellow solid, yield: 60%; mp: 183-184 °C; IR (KBr): 3397, 3011, 2953, 2194, 1652, 1590, 1096 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 8.28 (s, 1H), 7.36 (s, 2H), 7.08-7.05 (m, 4H), 4.30 (s, 1H), 3.82 (s, 6H), 3.71 (s, 3H), 3.52-3.41 (m, 1H), 2.63 (brs, 2H), 2.33-2.28 (m, 2H), 1.96-1.89 (m, 2H), 1.28 (d, 6H, J = 6.8Hz); ^{13}C NMR (DMSO-d_6, 100 MHz) δ:195.9, 164.6, 159.6, 158.4, 152.5, 148.8, 141.0, 140.7, 137.9, 134.5, 130.0, 127.8, 126.0, 124.2, 119.6, 119.2, 113.4, 106.2, 60.0, 57.9, 56.0, 36.3, 35.2, 26.5, 26.4, 22.3, 22.2, 19.8; ESI-MS (*m/z*): 543 $(M+H)^+$.

1.4.4. (±)2-amino-8'-sec-butyl-3'-(4-methoxyphenyl)-7,7-dimethyl-2',5-dioxo-5,6,7,8-tetrahydro-2'H,4H-4,6'-bichromene-3-carbonitrile (**13**).

Light yellow solid, yield: 70%; mp: 208-209 °C; IR (KBr): 3418, 3016, 2963, 2195, 1674, 1604, 1035 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 8.19 (s, 1H), 7.71 (d, 2H, J = 8.8Hz), 7.39 (s, 1H), 7.23 (s, 1H), 7.06-7.00 (m, 4H), 4.28 (s, 1H), 3.81 (s, 3H), 3.28-3.21 (m, 1H), 2.62-2.50 (m, 2H), 2.31-2.07 (m, 2H), 1.69-1.62 (m, 2H), 1.25-1.21 (m, 3H), 1.06 (s, 3H), 0.97 (d, 3H, J = 3.7Hz), 0.81 (t, 3H, J = 7.3Hz);ESI-MS (*m/z*): 525 $(M+H)^+$.

1.4.5. (±)2-Amino-8'-sec-butyl-2',5-dioxo-3'-(3,4,5-trimethoxyphenyl)-5,6,7,8-tetrahydro-2'H,4H-4,6'-bichromene-3-carbonitrile (**14**).

Light yellow solid, yield: 60%; mp: 194-195 °C; IR (KBr): 3402, 3020, 2965, 2198, 1677, 1589, 998 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 8.28 (s, 1H), 7.46-7.32 (m, 2H), 7.08-7.06 (m, 4H), 4.30 (s, 1H), 3.83 (s, 6H), 3.71 (s, 3H), 3.27-3.21 (m, 1H), 2.64 (s, 2H), 2.28 (brs, 2H), 1.99-1.97 (m, 2H), 1.71-1.66 (m, 2H), 1.27 (d, 3H, J = 6.8Hz), 0.83 (t, 3H, J = 7.1Hz);ESI-MS (*m/z*): 557 $(M+H)^+$.

1.4.6. (±)2-Amino-8'-sec-butyl-7,7-dimethyl-2',5-dioxo-3'-(3,4,5-trimethoxyphenyl)-5,6,7,8-tetrahydro-2'H,4H-4,6'-bichromene-3-carbonitrile (**15**).

Light yellow solid, yield: 62%; mp: 223-224 °C; IR (KBr): 3385, 3016, 2963, 2194, 1675, 1588, 1032 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 8.28 (s, 1H), 7.40 (s, 1H), 7.25 (s, 1H), 7.08 (brs, 4H), 4.29 (s, 1H), 3.83 (s, 6H), 3.71 (s, 3H), 3.26-3.21 (m, 1H), 2.62-2.50 (m, 2H), 2.31-2.07 (m, 2H), 1.67-1.65 (m, 2H), 1.24 (s, 3H), 1.05 (s, 3H), 0.81 (t, 3H, J = 6.7Hz); ^{13}C NMR (DMSO-d_6, 100 MHz) δ:195.6, 162.6, 162.5, 159.5, 158.5, 158.4, 152.5, 149.2, 140.7,137.9, 133.5, 133.4, 130.0, 126.2, 124.5, 119.5, 119.1, 112.4,112.3, 106.2, 60.0, 58.0, 57.9, 56.0, 49.9, 35.3, 31.7, 28.9, 28.5, 28.4, 26.5, 26.4, 20.3, 20.2, 11.8, 11.7; ESI-MS (*m/z*): 584 $(M+H)^+$.

*1.5. General synthetic procedure of Friedlander reaction for preparation of compounds (***16-24***)*:

Aluminium chloride (1.2 equiv) was added in dry 1, 2-dichloroethane (10 mL) at rt. The corresponding coumarin-4*H*-chromene intermediates (**10-15**) (1 equiv) and different cyclic ketones (cyclopentanone, cyclohexanone) (1.2 equiv) were added, the resulting reaction mixture was refluxed for 6h (monitored by TLC). When the reaction was completed, a

mixture of THF/H_2O (1:1) was added at rt and basified (pH 8) with aqueous solution of sodium hydroxide (10%). The mixture was extracted three times with dichloromethane. The organic layer was washed with brine solution, dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated. The crude product was purified on a silica gel column (100-200 mesh) using methanol-chloroform (05:95, v/v) as eluent to afford compounds **16-24** in good yields.

*1.5.1. (±)11-amino-10-(8-isopropyl-3-(4-methoxyphenyl)-2-oxo-2H-chromen-6-yl)-7,7-dimethyl-2,3,6,7,8,10-hexahydrochromeno[2,3-b]cyclopenta[e]pyridin-9(1H)-one (***16***)*

white solid, yield: 64%; mp: 201-202 °C; IR (neat): 1023, 1220, 1507, 1637, 1713, 3016, 3405 cm^{-1}; ^1H (DMSO-d_6, 400 MHz) δ: 8.05 (s, 1H), 7.68 (d, 3H, J = 6.1 Hz), 7.39 (s, 1H), 6.99 (s, 1H), 6.97 (s, 1H), 5.89 (s, 2H), 5.05 (s, 1H), 3.78 (s, 3H), 3.41-3.34 (m, 1H), 2.70-2.50 (m, 6H), 2.33-2.05 (m, 2H), 1.98-1.93 (m, 2H), 1.23 (d, 6H, J = 6.9 Hz), 1.05 (s, 3H), 0.90 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 196.8, 164.9, 162.5, 160.8, 160.1, 156.5, 149.6, 149.0, 139.5, 139.1, 136.6, 129.8, 128.5, 127.5, 127.2, 124.8, 119.7, 118.8, 114.0, 113.5, 99.9, 55.4, 50.8, 41.5, 34.6, 34.3, 32.3, 29.2, 27.2, 26.7, 22.8, 22.6, 22.3; HPLC t_R : 3.78 min, Purity > 97 % (% of area); ESI-MS: m/z: 577 $(M+H)^+$.

*1.5.2. (±)11-amino-10-(3-(3,4-dimethoxyphenyl)-8-isopropyl-2-oxo-2H-chromen-6-yl)-7,7-dimethyl-2,3,6,7,8,10-hexahydrochromeno[2,3-b]cyclopenta[e]pyridin-9(1H)-one (***17***)*

white solid, yield: 67%; mp: 282-283 °C; IR (neat): 1028, 1216, 1516, 1646, 1715, 3020, 3406 cm^{-1}; ^1H (DMSO-d_6, 400 MHz) δ: 8.11 (s, 1H), 7.72 (d, 1H, J = 2.0 Hz), 7.37-7.32 (m, 3H), 7.00 (d, 1H, J = 8.0 Hz), 5.90 (s, 2H), 5.07 (s, 1H), 3.80 (s, 6H), 3.44-3.37 (m, 1H), 2.71-2.50 (m, 6H), 2.33-2.06 (m, 2H), 1.96-1.91 (m, 2H), 1.25 (d, 6H, J = 6.8 Hz), 1.06 (s, 3H), 0.91 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 196.8, 164.9, 162.6, 160.7, 156.6, 149.7,

149.0, 139.3, 136.6, 128.5, 127.5, 124.8, 121.2, 119.6, 118.8, 113.5,111.7, 111.1, 99.9, 56.1, 56.0, 50.8, 41.5, 34.6, 34.3, 32.3, 29.2, 27.1, 26.8, 22.8, 22.5, 22.3; HPLC t_R : 3.82 min, Purity > 96 % (% of area); ESI-MS: m/z: 607 $(M+H)^+$.

*1.5.3. (±)11-amino-12-(8-isopropyl-2-oxo-3-(3,4,5-trimethoxyphenyl)-2H-chromen-6-yl)-2,3,4,7,8,9,10,12-octahydro-1H-chromeno[2,3-b]quinolin-1-one (**18**)*

white solid, yield: 69%; mp: 272-273 °C; IR (neat): 1034, 1217, 1518, 1642, 1714, 3015, 3394 cm^{-1}; ^1H (DMSO-d_6, 400 MHz) δ: 8.18 (s, 1H), 7.90 (d, 1H, *J* = 2.0 Hz), 7.25 (d, 1H, *J* = 2.0 Hz), 7.05 (s, 2H), 5.72 (s, 2H), 5.09 (s, 1H), 3.81 (s, 6H), 3.70 (s, 3H), 3.45-3.38 (m, 1H), 2.69-2.66 (m, 2H), 2.56-2.50 (m, 2H), 2.34-2.13 (m, 4H), 1.99-1.84 (m, 2H), 1.68 (brs, 4H), 1.29 (d, 6H, *J* = 6.8 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) *δ*: 196.9, 166.6, 160.6, 154.5, 153.2, 150.8, 149.7, 140.1, 138.8, 136.4, 130.2, 129.1, 127.5, 125.1, 119.7, 114.9, 114.2, 105.9, 99.6, 61.0, 56.3, 37.0, 34.5, 32.6, 28.0, 27.0, 23.0, 22.6, 22.4, 20.3; HPLC t_R : 4.96 min, Purity > 99 % (% of area); ESI-MS: m/z: 623 $(M+H)^+$.

*1.5.4. (±)11-amino-12-(8-(sec-butyl)-3-(4-methoxyphenyl)-2-oxo-2H-chromen-6-yl)-3,3-dimethyl-2,3,4,7,8,9,10,12-octahydro-1H-chromeno[2,3-b]quinolin-1-one (**19**)*

white solid, yield: 61%; mp: 230-231 °C; IR (neat): 1034, 1216, 1513, 1649, 1702, 3019, 3406 cm^{-1}; ^1H (DMSO-d_6, 400 MHz) δ: 8.06 (s, 1H), 7.67 (d, 3H, *J* = 1.8 Hz), 7.35 (d, 1H, *J* = 1.8 Hz), 6.97 (d, 2H, *J* = 8.8 Hz), 5.71 (s, 1H), 5.06 (s, 1H), 3.78 (s, 3H), 3.21-3.16 (m, 1H), 2.64-2.50 (m, 4H), 2.34-2.27 (m, 2H), 2.16-2.04 (m, 2H), 1.67-1.60 (m, 6H), 1.22-1.21 (m, 3H), 1.04 (s, 3H), 0.89 (d, 3H, *J* = 1.8 Hz), 0.81-0.76 (m, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) *δ*: 196.7, 164.9, 160.8, 160.1, 154.4, 150.9, 149.9, 139.2, 135.4, 135.3, 129.8, 124.8, 119.8, 113.9, 99.6, 99.5, 68.2, 55.4, 50.8, 41.5, 32.5, 32.2, 29.2, 27.2, 23.0, 22.4, 20.7, 12.0, 11.9; HPLC t_R : 3.93 min, Purity > 95 % (% of area); ESI-MS: m/z: 605 $(M+H)^+$.

*1.5.5. (±)11-amino-10-(8-(sec-butyl)-2-oxo-3-(3,4,5-trimethoxyphenyl)-2H-chromen-6-yl)-2,3,6,7,8,10-hexahydrochromeno[2,3-b]cyclopenta[e]pyridin-9(1H)-one (**20**)*

white solid, yield: 58%; mp: 258-259 °C; IR (neat): 1109, 1215, 1510, 1646, 1703, 3019, 3411 cm^{-1}; ^1H (DMSO-d_6, 400 MHz) δ: 8.16 (s, 1H), 7.84 (s, 1H), 7.24 (d, 1H, J = 8.0 Hz), 7.04 (s, 2H), 5.88 (s, 2H), 5.07 (s, 1H), 3.80 (s, 6H), 3.69 (s, 3H), 3.24-3.17 (m, 1H), 2.67 (brs, 4H), 2.35-2.20 (m, 2H), 1.98-1.81 (m, 4H), 1.74-1.64 (m, 2H), 1.27-1.22 (m, 5H), 0.81 (t, 3H, J = 7.3 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 196.9, 166.6, 162.7, 160.6, 156.6, 153.2, 150.0, 148.9, 140.1, 138.8, 135.3, 130.2, 129.8, 127.5, 125.0, 118.8, 114.8, 105.9, 99.8, 61.0, 56.3, 37.0, 34.3, 33.8, 29.6, 29.4, 27.9, 27.1, 22.4, 20.3, 11.9; HPLC t_R : 3.92 min, Purity > 95 % (% of area); ESI-MS: m/z: 623 $(M+H)^+$.

*1.5.6. (±)11-amino-12-(8-(sec-butyl)-2-oxo-3-(3,4,5-trimethoxyphenyl)-2H-chromen-6-yl)-3,3-dimethyl-2,3,4,7,8,9,10,12-octahydro-1H-chromeno[2,3-b]quinolin-1-one (**21**)*

white solid, yield: 60%; mp: 240-241 °C; IR (neat): 1030, 1216, 1513, 1645, 1712, 3019, 3401 cm^{-1}; ^1H (DMSO-d_6, 400 MHz) δ: 8.14 (s, 1H), 7.72 (d, 1H, J = 2.3 Hz), 7.33 (d, 1H, J = 8.0 Hz), 7.03 (s, 2H), 5.70 (s, 2H), 5.07 (s, 1H), 3.80 (s, 6H), 3.69 (s, 3H), 3.22-3.15 (m, 1H), 2.64-2.50 (m, 4H), 2.34-2.27 (m, 2H), 2.15-2.04 (m, 2H), 1.67 (brs, 6H), 1.23 (t, 3H, J = 6.0 Hz), 1.04 (s, 3H), 0.88 (d, 3H, J = 6.0 Hz), 0.81-0.76 (m, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 196.8, 164.9, 160.6, 154.5, 153.2, 150.9, 150.0, 140.2, 138.8, 135.5, 130.3, 129.8, 127.6, 125.0, 119.6, 114.2, 113.5, 105.9, 99.5, 61.0, 56.3, 50.8, 41.5, 34.5, 33.8, 32.6, 32.3, 29.7, 29.2, 27.2, 23.0, 22.6, 20.4, 12.0, 11.9; HPLC t_R : 3.95 min, Purity > 95 % (% of area); ESI-MS: m/z: 665 $(M+H)^+$.

*1.5.7. (±)11-amino-12-(8-isopropyl-3-(4-methoxyphenyl)-2-oxo-2H-chromen-6-yl)-3,3-dimethyl-2,3,4,7,8,9,10,12-octahydro-1H-chromeno[2,3-b]quinolin-1-one (**22**)*

white solid, yield: 65%; mp: 194-195 °C; IR (neat): 1031, 1218, 1516, 1653, 1716, 3026, 3403 cm^{-1}; ^1H (DMSO-d_6, 400 MHz) δ: 8.05 (s, 1H), 7.67 (d, 3H, *J* = 8.8 Hz), 7.40 (d, 1H, *J* = 2.0 Hz), 6.97 (d, 2H, *J* = 8.8 Hz), 5.73 (s, 2H), 5.07 (s, 1H), 3.78 (s, 3H), 3.35 (brs, 1H), 2.65-2.50 (m, 4H), 2.31-2.27 (m, 2H), 2.17-2.04 (m, 2H), 1.66 (brs, 4H), 1.23 (d, 6H, *J* = 6.8 Hz), 1.04 (s, 3H), 0.90 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) *δ*: 196.7, 164.9, 160.8, 160.1, 154.4, 150.9, 149.6, 139.4, 139.1, 136.6, 130.9, 129.8, 128.5, 127.5, 124.8, 119.7, 114.0, 99.6, 68.2, 55.4, 50.8, 41.5, 38.8, 34.6, 32.5, 32.3, 29.7, 29.2, 27.2, 26.7, 22.8, 22.6, 14.1, 11.0; HPLC t_R : 3.81 min, Purity > 96 % (% of area); ESI-MS: m/z: 591 $(M+H)^+$.

*1.5.8. (±)11-amino-12-(3-(3,4-dimethoxyphenyl)-8-isopropyl-2-oxo-2H-chromen-6-yl)-3,3-dimethyl-2,3,4,7,8,9,10,12-octahydro-1H-chromeno[2,3-b]quinolin-1-one (***23***)*

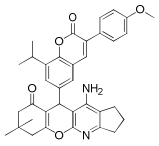
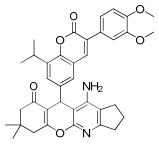
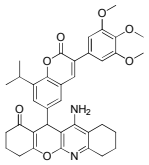
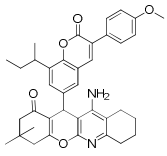
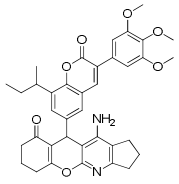
white solid, yield: 58%; mp: 205-206 °C; IR (neat): 1038, 1229, 1514, 1653, 1720, 3018, 3409 cm^{-1}; ^1H (DMSO-d_6, 400 MHz) δ: 8.10 (s, 1H), 7.72 (d, 1H, *J* = 2.0 Hz), 7.37-7.30 (m, 3H), 6.99 (d, 1H, *J* = 8.0 Hz), 5.73 (s, 2H), 5.06 (s, 1H), 3.79 (s, 6H), 3.43-3.36 (m, 1H), 2.65-2.50 (m, 4H), 2.32-2.28 (m, 2H), 2.17-2.05 (m, 2H), 1.67 (brs, 4H), 1.25 (d, 6H, *J* = 2.0 Hz), 1.05 (s, 3H), 0.90 (s,3H); ^{13}C NMR ($CDCl_3$, 100 MHz) *δ*: 196.7, 164.9, 160.7, 154.4, 154.3, 150.9, 149.7, 149.8, 139.5, 139.3, 136.6, 128.6, 127.5, 124.8, 121.2, 119.6, 114.2, 113.6, 111.7, 111.1, 99.6, 56.1, 56.0, 50.8, 41.5, 34.6,32.5, 32.3, 29.2, 27.2, 26.8, 23.0, 22.5; HPLC t_R : 3.63 min, Purity > 96 % (% of area); ESI-MS: m/z: 621 $(M+H)^+$.

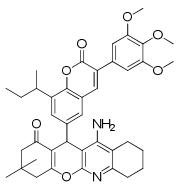
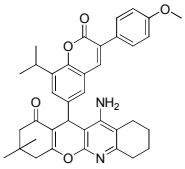
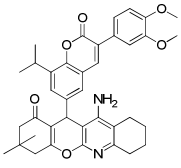
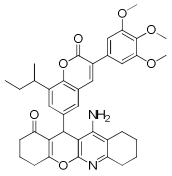
*1.5.9. (±)11-amino-12-(8-(sec-butyl)-2-oxo-3-(3,4,5-trimethoxyphenyl)-2H-chromen-6-yl)-2,3,4,7,8,9,10,12-octahydro-1H-chromeno[2,3-b]quinolin-1-one (***24***)*

white solid, yield: 64%; mp: 235-236 °C; IR (neat):1126,1217, 1507, 1642, 1717, 3015, 3394 cm^{-1}; ^1H (DMSO-d_6, 400 MHz) δ: 8.17 (s, 1H), 7.85 (d, 1H, *J* = 2.0 Hz), 7.24 (d, 1H, *J* = 7.4 Hz), 7.04 (s, 2H), 5.71 (s, 2H), 5.08 (s, 1H), 3.80 (s, 6H), 3.69 (s, 3H), 3.23-3.17 (m, 1H), 2.66 (brs, 2H), 2.54 (brs, 2H), 2.36-2.11 (m, 4H), 1.98-1.81 (m, 2H), 1.67 (s, 6H), 1.27-1.24

(m, 3H), 0.81 (t, 3H, J = 8.0 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 196.9, 166.6, 160.6, 154.5, 153.2, 150.8, 150.0, 140.1, 138.8, 135.2, 129.9, 127.5, 125.1, 119.7, 114.8, 114.2, 105.9, 99.5, 61.0, 56.3, 37.0, 34.6, 34.5, 33.8, 33.5, 32.6, 29.5, 29.4, 27.9, 23.0, 22.6, 22.4, 20.3, 12.0, 11.9; HPLC t_R : 3.85 min, Purity > 95 % (% of area); ESI-MS: m/z: 637 $(M+H)^+$.

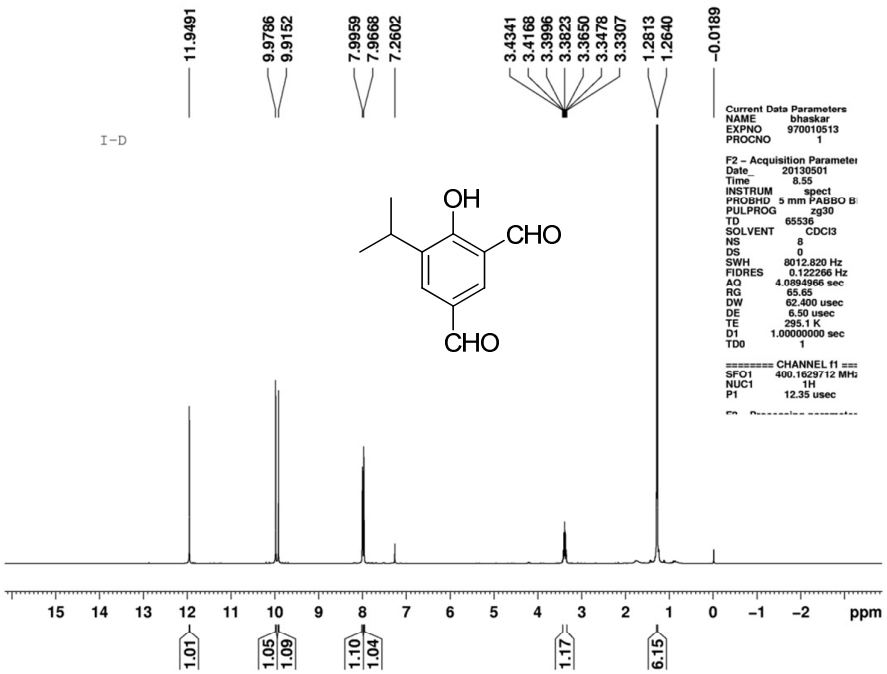
Compounds characterization table

Compound No.	Structure	Mol. Formula	M. P.	Yield
16		$C_{36}H_{36}N_2O_5$	201-202 °C	64%
17		$C_{37}H_{38}N_2O_6$	282-283 °C	67%
18		$C_{37}H_{38}N_2O_7$	272-273 °C	69%
19		$C_{38}H_{40}N_2O_5$	230-231 °C	61%
20		$C_{37}H_{38}N_2O_7$	258-259 °C	58%

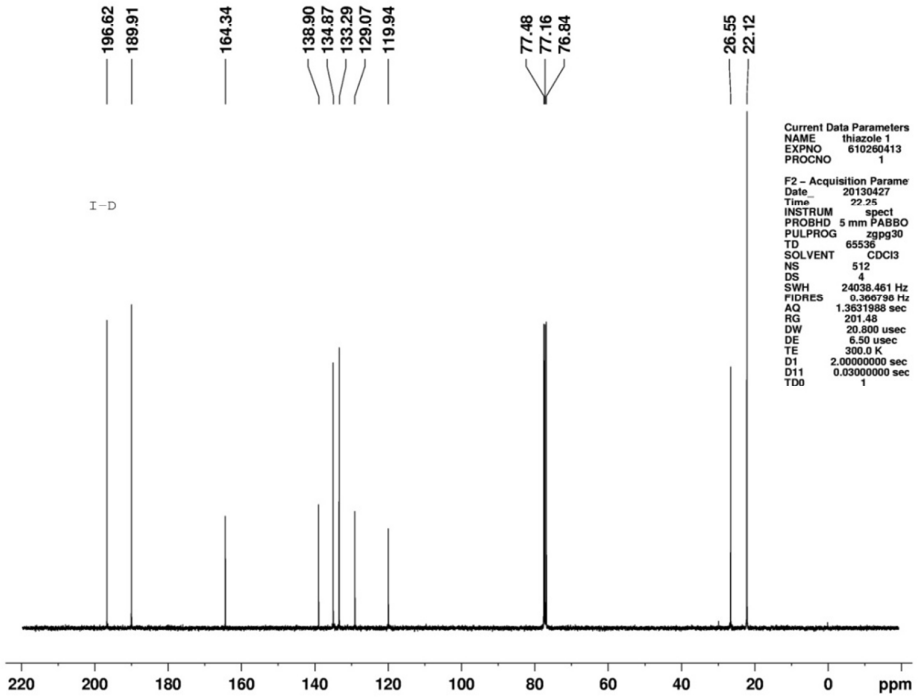
21		$C_{40}H_{44}N_2O_7$	240-241 °C	60%
22		$C_{37}H_{38}N_2O_5$	194-195 °C	65%
23		$C_{38}H_{40}N_2O_6$	205-206 °C	58%
24		$C_{38}H_{40}N_2O_7$	235-236 °C	64%

2. Compounds Spectra

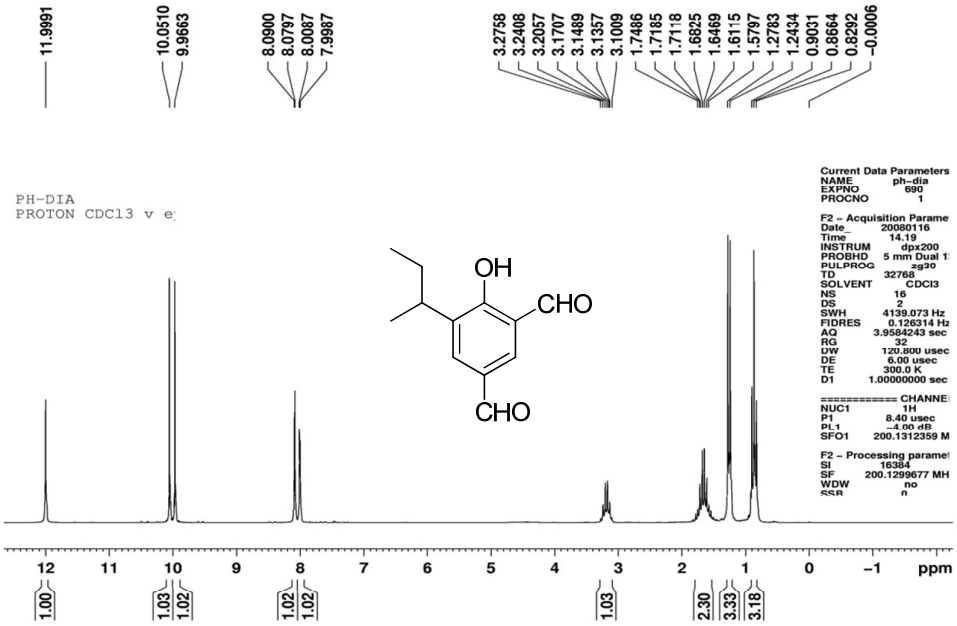
^{1}H NMR of compound **3** at 300 MHz ($CDCl_3$)



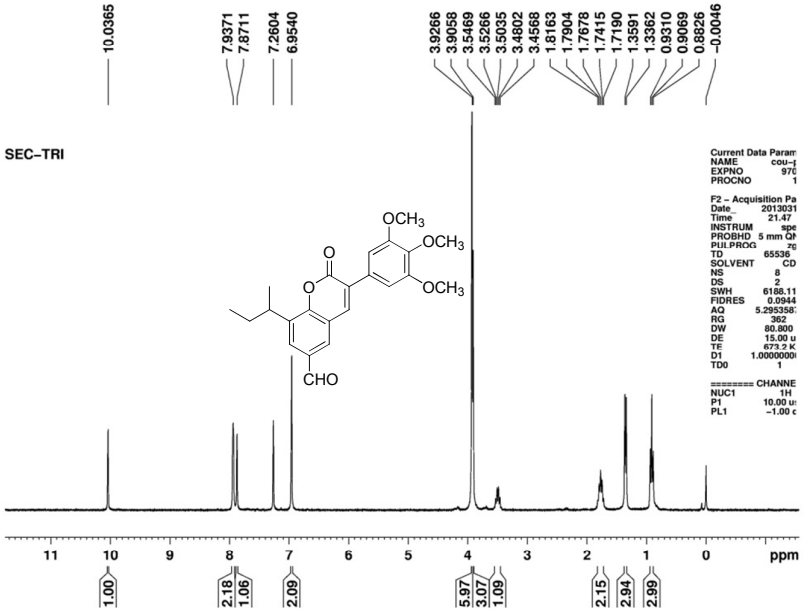
^{13}C NMR of compound **3** at 50 MHz ($CDCl_3$)



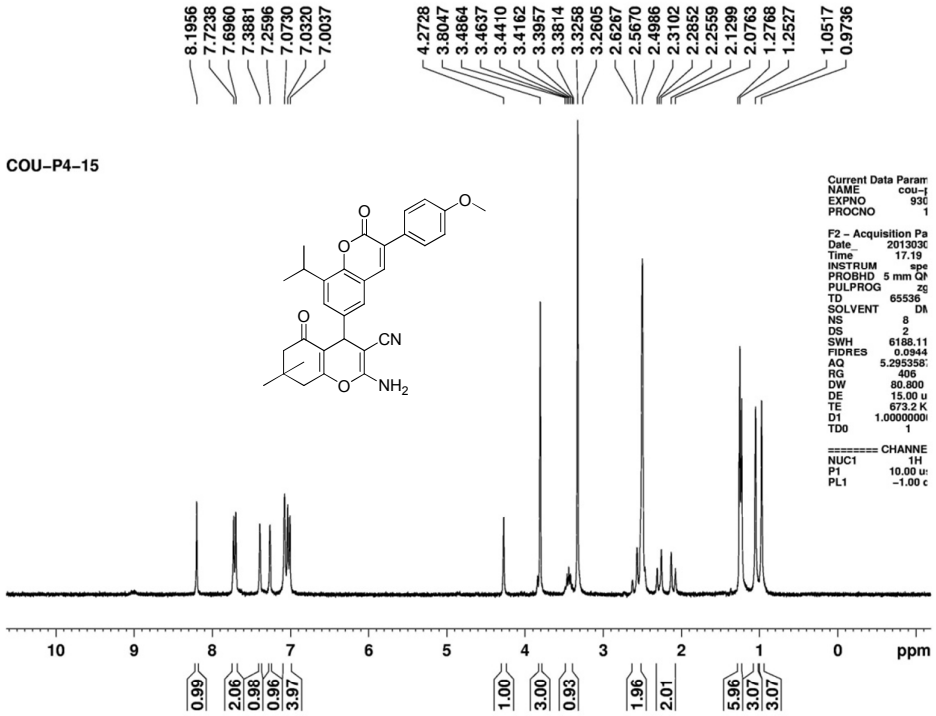
^{1}H NMR of compound **4** at 300 MHz ($CDCl_3$)



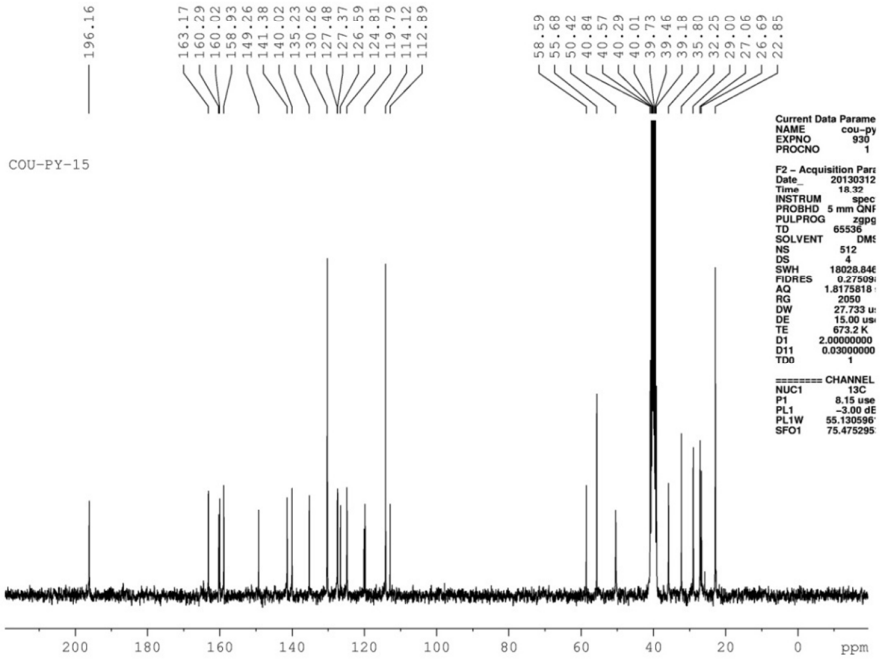
^{1}H NMR of compound **9** at 300 MHz ($CDCl_3$)



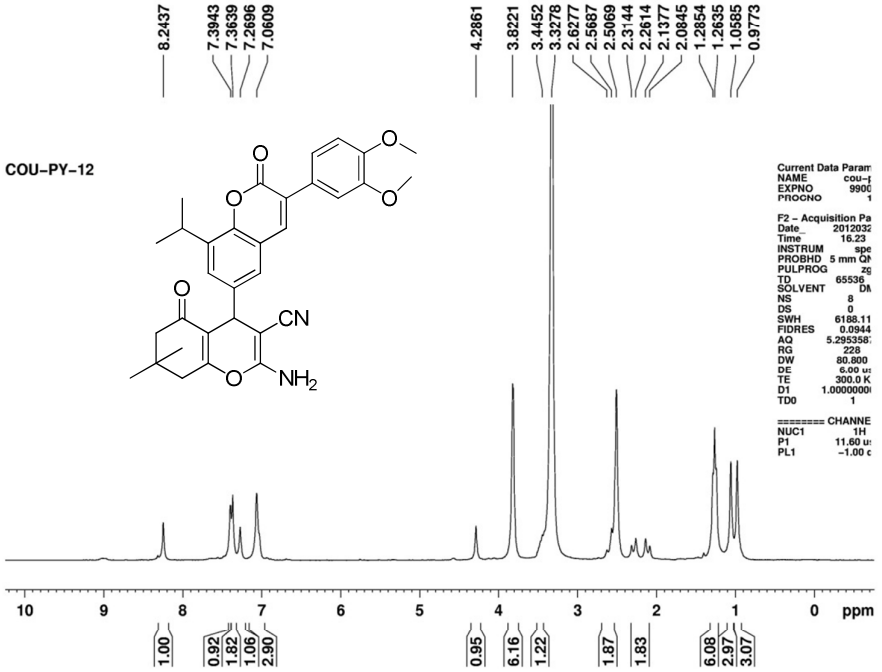
^{1}H NMR of compound **10** at 300 MHz (DMSO-d_6)



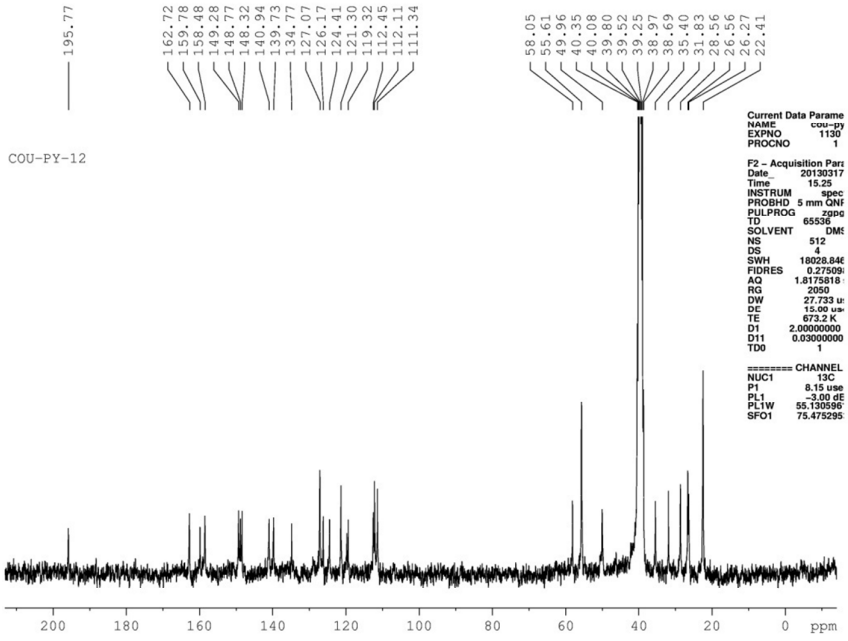
^{13}C NMR of compound **10** at 75 MHz (DMSO-d_6)



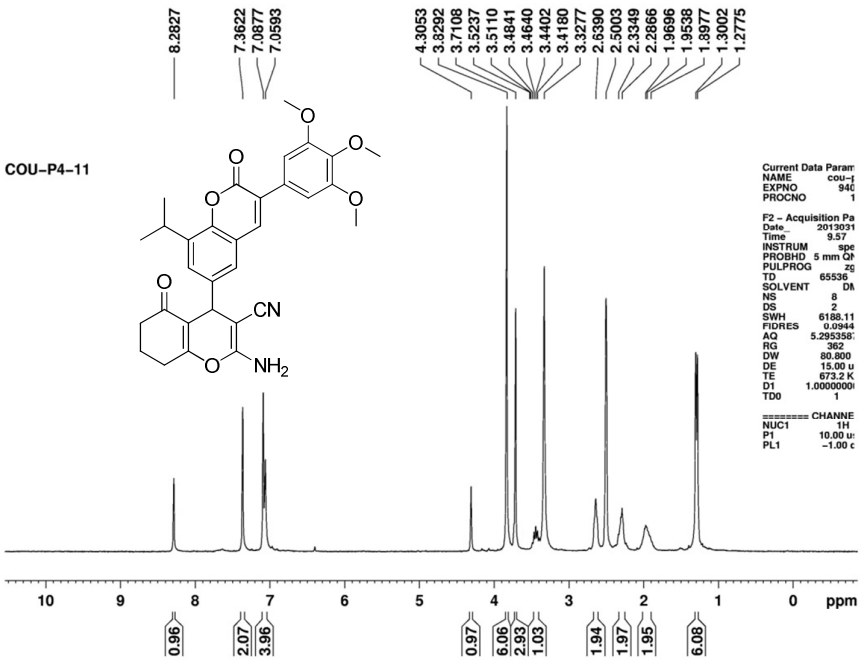
^{1}H NMR of compound **11** at 300 MHz (DMSO-d_6)



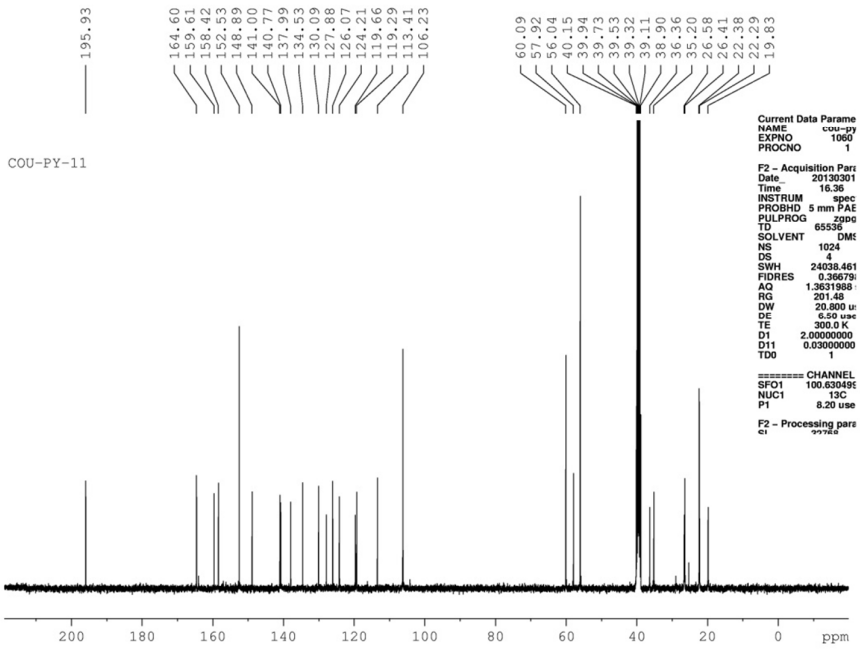
^{13}C NMR of compound **11**at 75 MHz (DMSO-d_6)



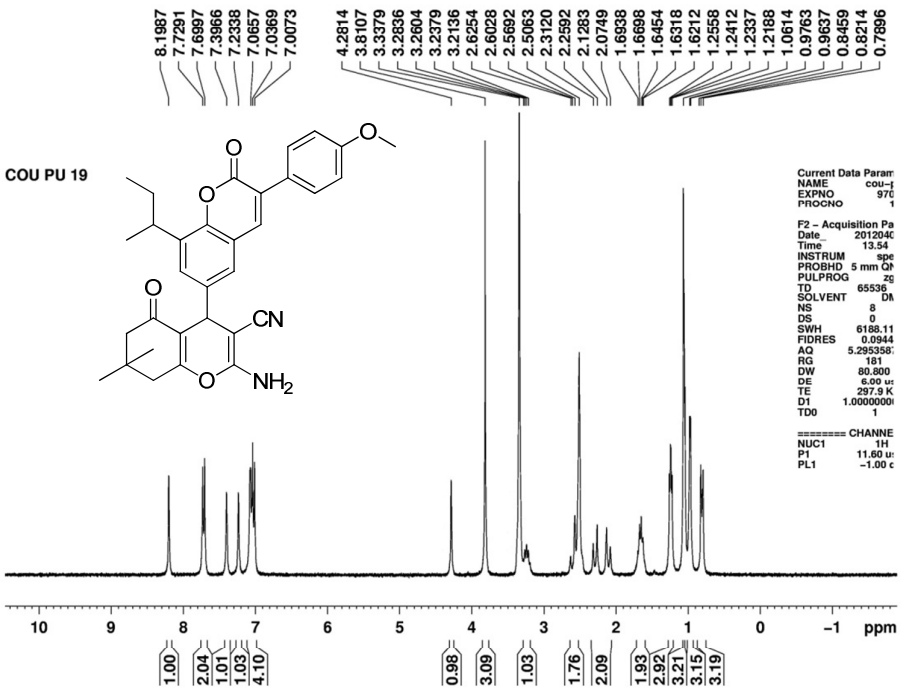
^{1}H NMR of compound **12** at 300 MHz (DMSO-d_6)



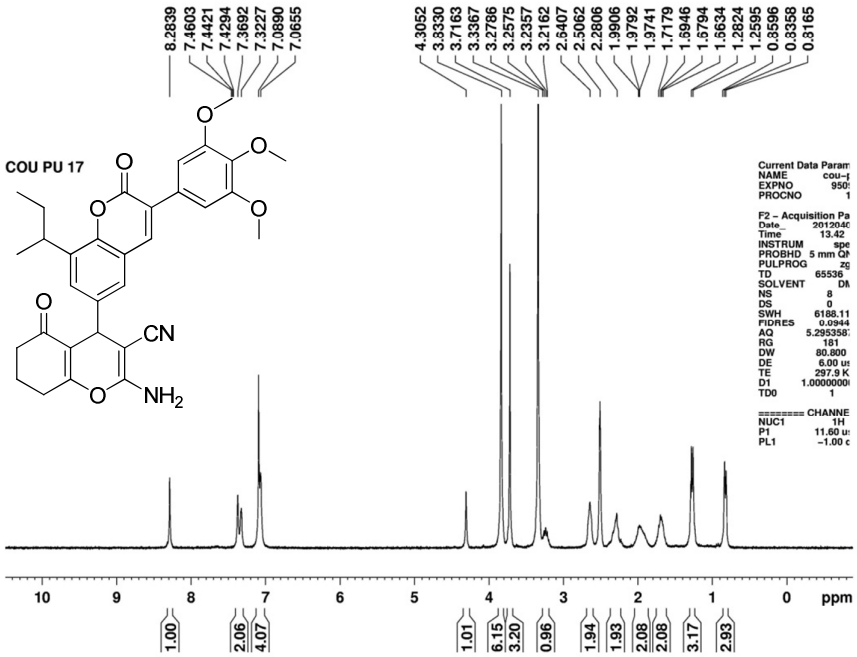
^{13}C NMR of compound **12** at 75 MHz (DMSO-d_6)



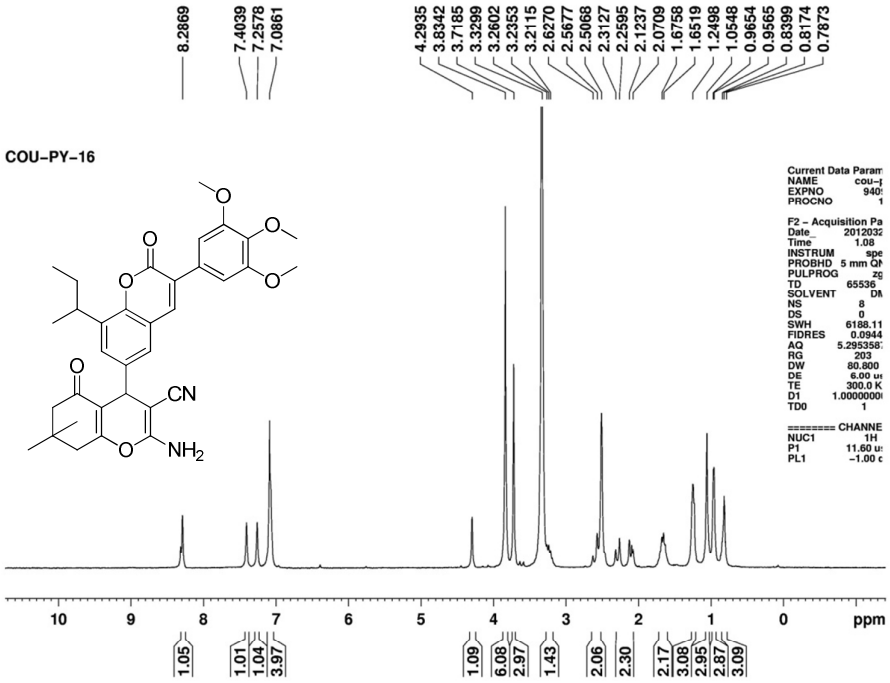
^{1}H NMR of compound 1**3** at 300 MHz (DMSO-d_6)



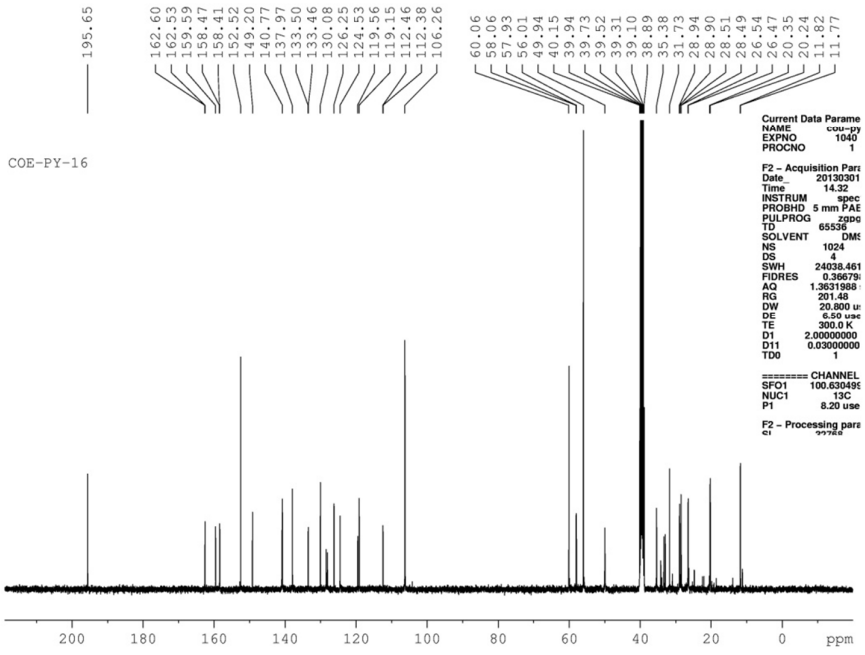
^{1}H NMR of compound **14** at 300 MHz (DMSO-d_6)



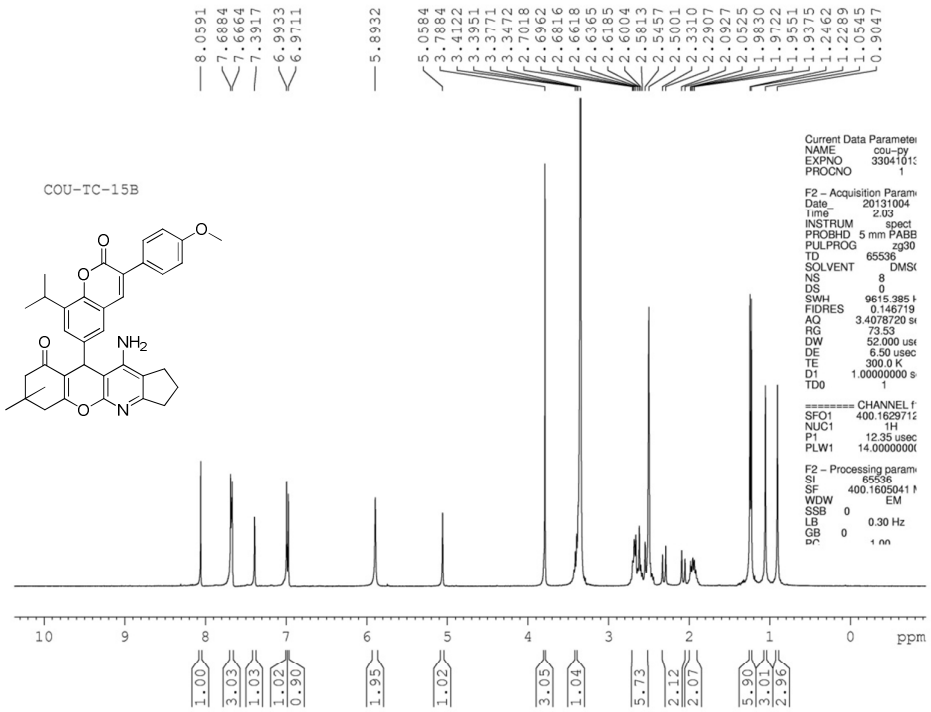
^{1}H NMR of compound **15** at 300 MHz (DMSO-d_6)



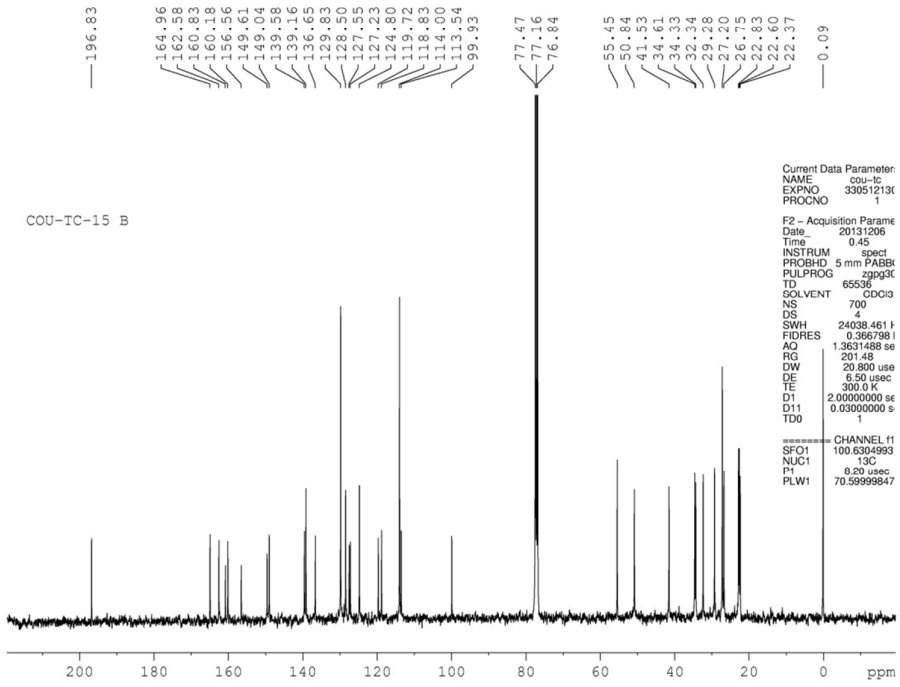
^{13}C NMR of compound **15** at 75 MHz (DMSO-d_6)



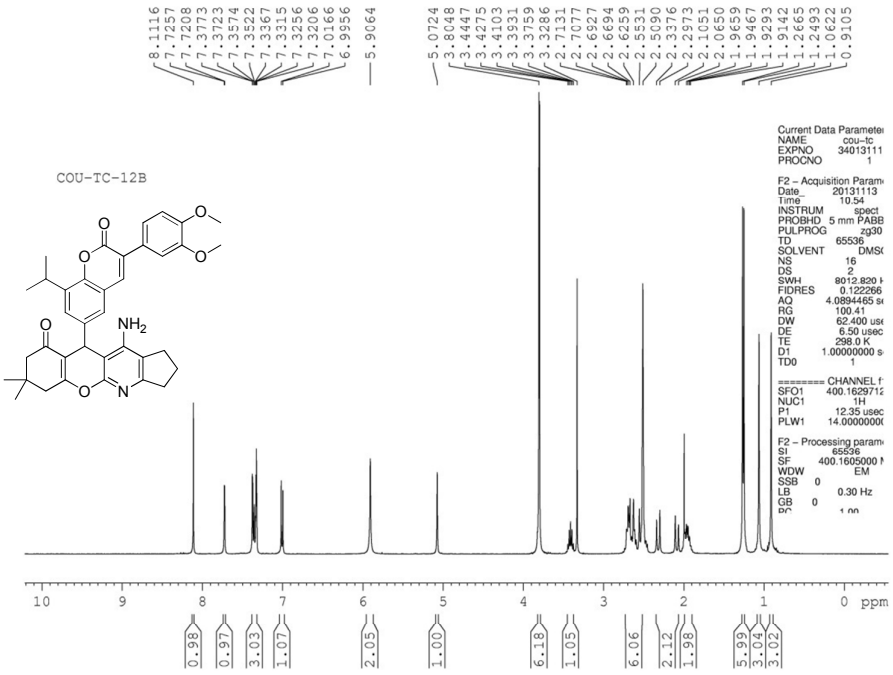
^{1}H NMR of compound **16** at 400 MHz (DMSO-d_6)



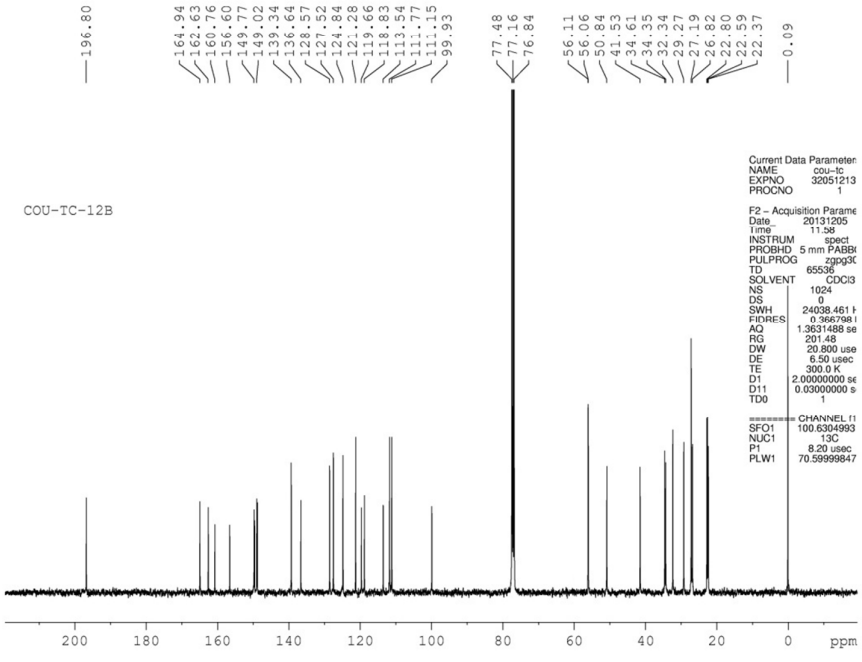
^{13}C NMR of compound **16** at 100 MHz ($CDCl_3$)



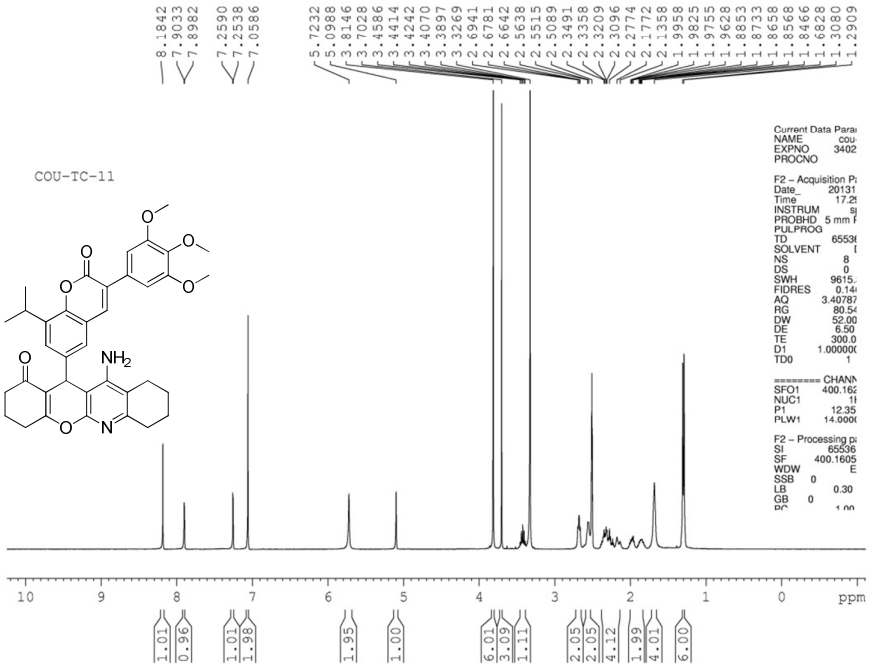
^{1}H NMR of compound **17** at 400 MHz (DMSO-d_6)



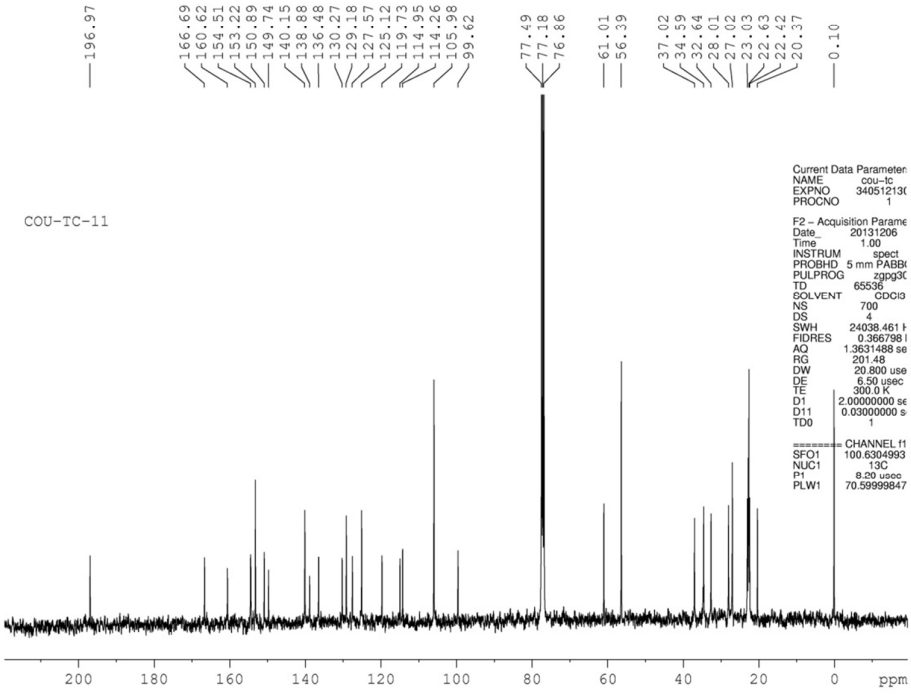
^{13}C NMR of compound **17** at 100 MHz ($CDCl_3$)



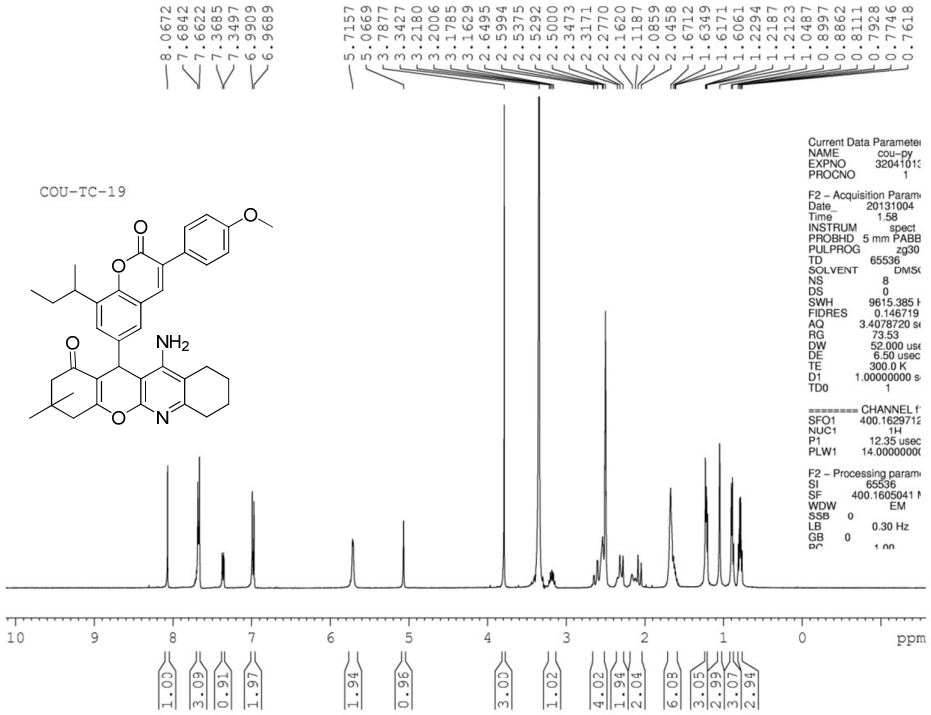
^{1}H NMR of compound **18** at 400 MHz (DMSO-d_6)



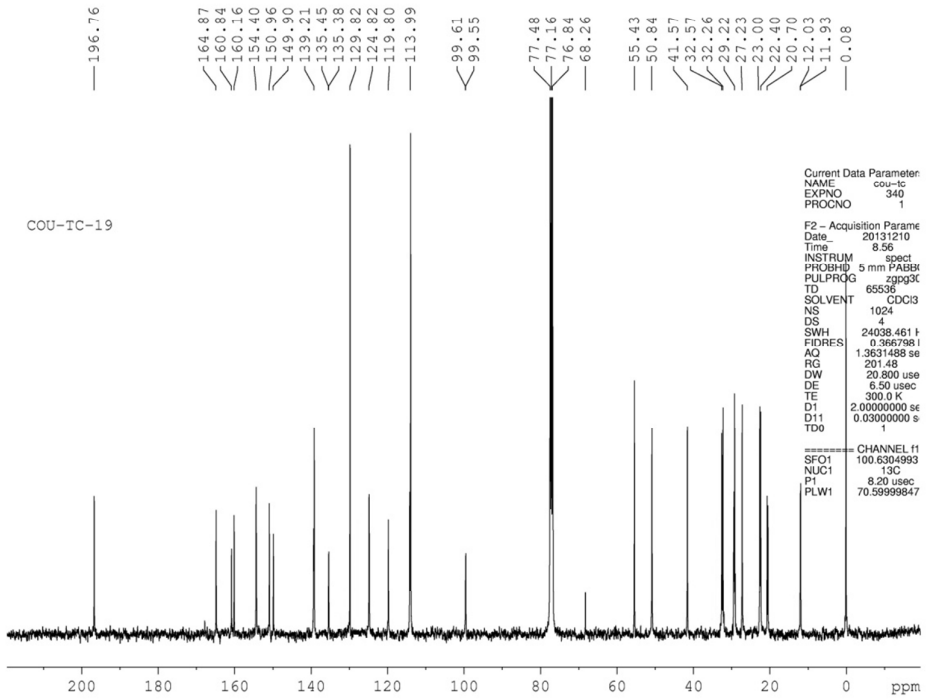
^{13}C NMR of compound **18** at 100 MHz ($CDCl_3$)



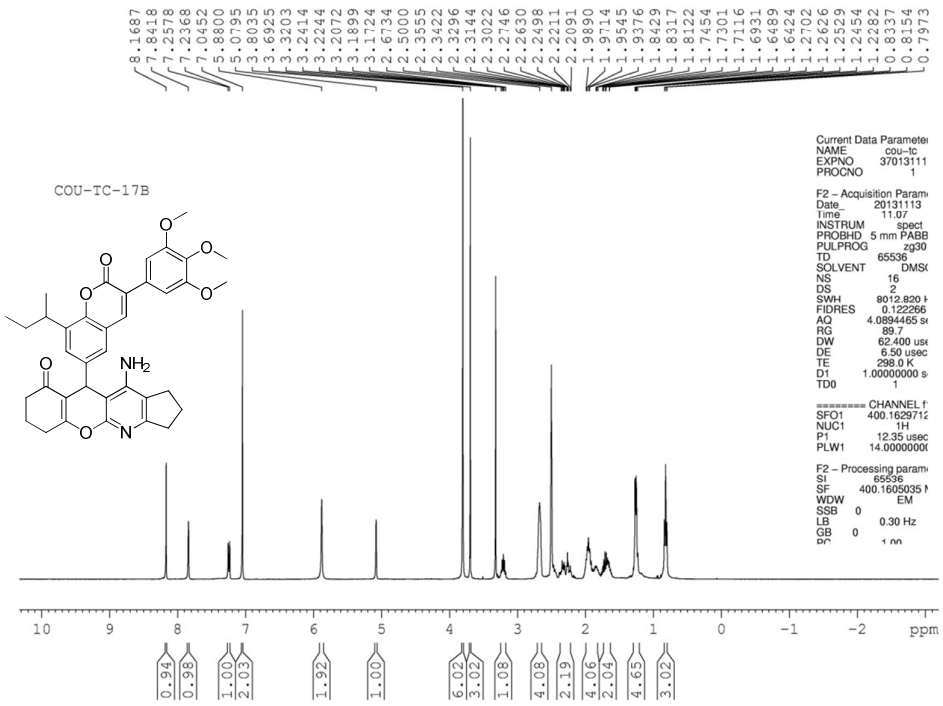
^{1}H NMR of compound **19** at 400 MHz (DMSO-d_6)



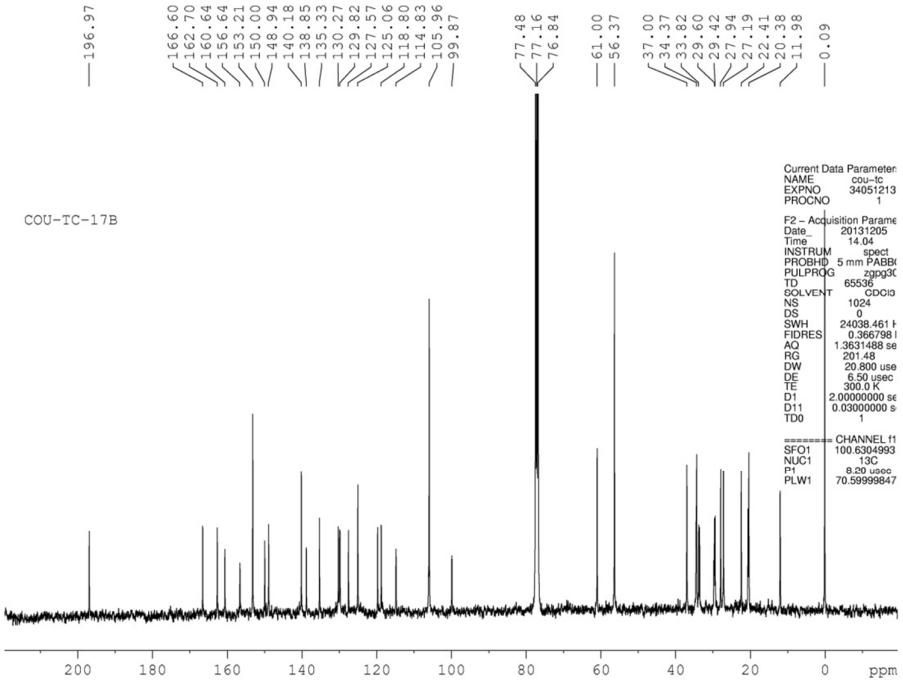
^{13}C NMR of compound **19** at 100 MHz ($CDCl_3$)



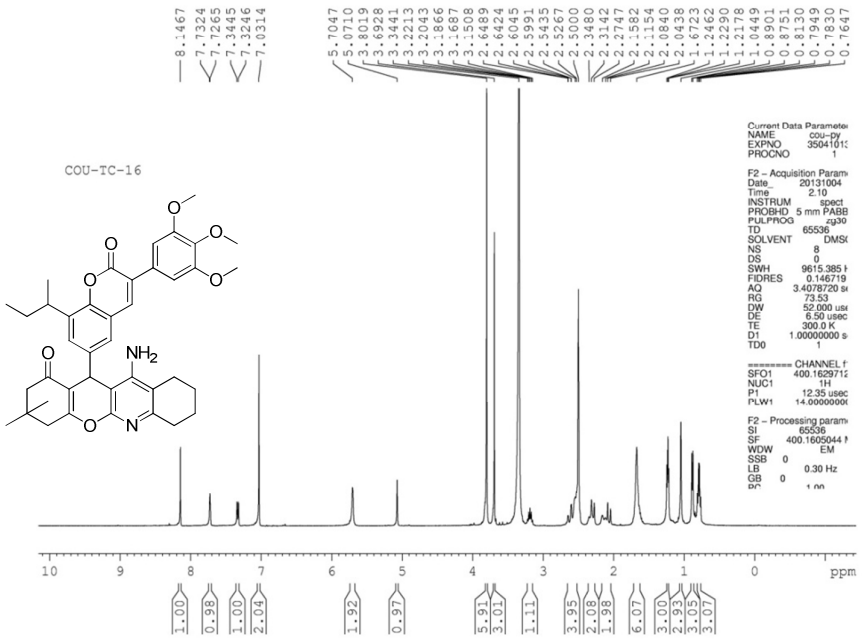
^{1}H NMR of compound **20** at 400 MHz (DMSO-d_6)



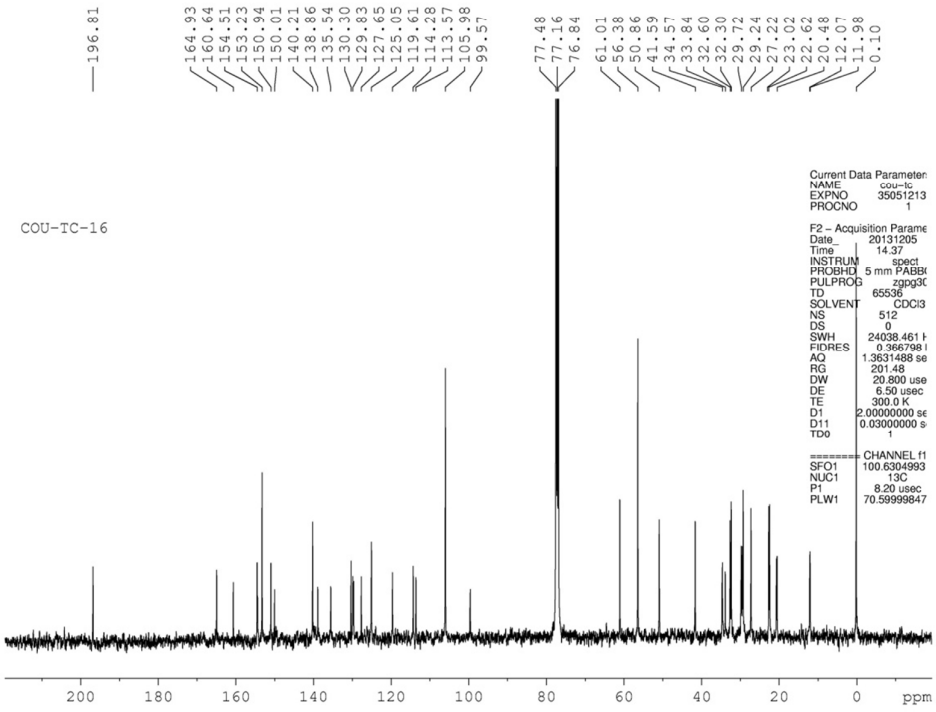
^{13}C NMR of compound **20** at 100 MHz ($CDCl_3$)



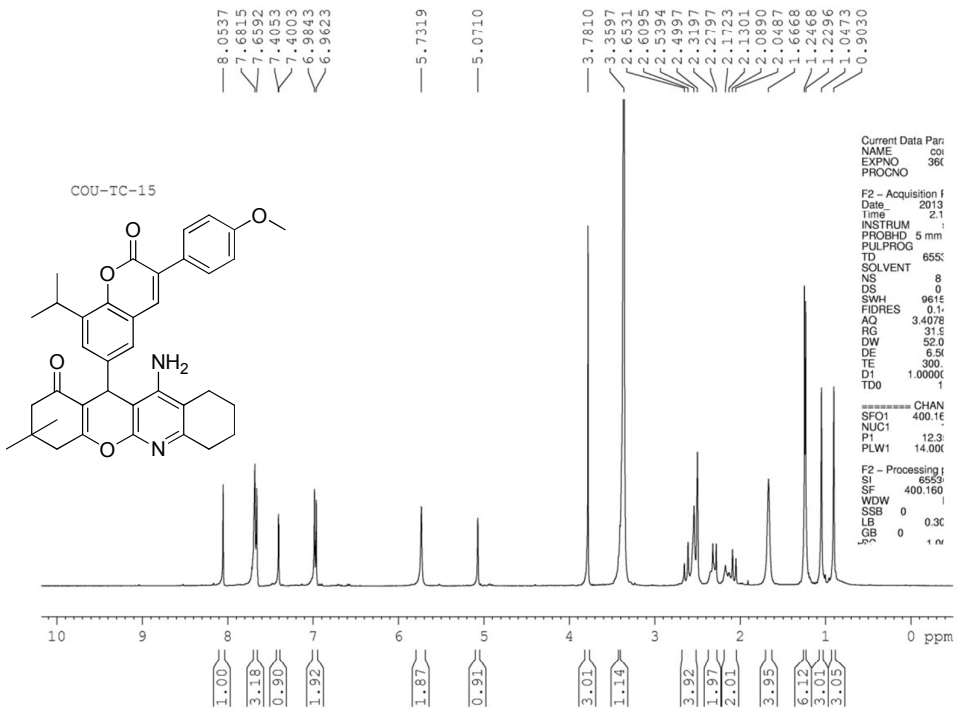
^{1}H NMR of compound **21** at 400 MHz (DMSO-d_6)



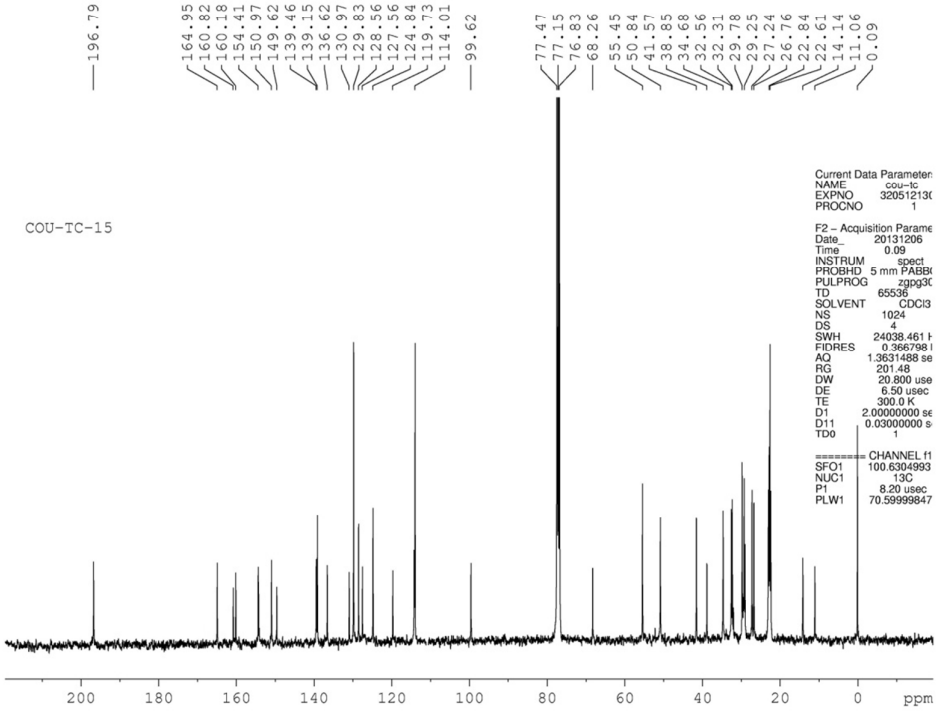
^{13}C NMR of compound **21** at 100 MHz ($CDCl_3$)



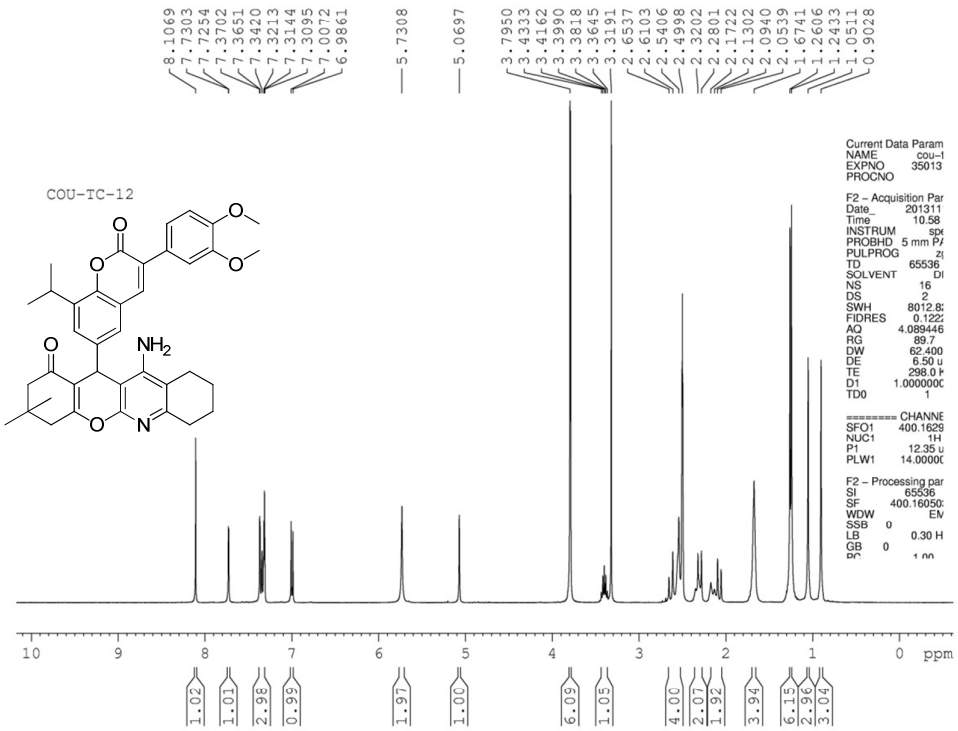
^{1}H NMR of compound **22** at 400 MHz (DMSO-d_6)



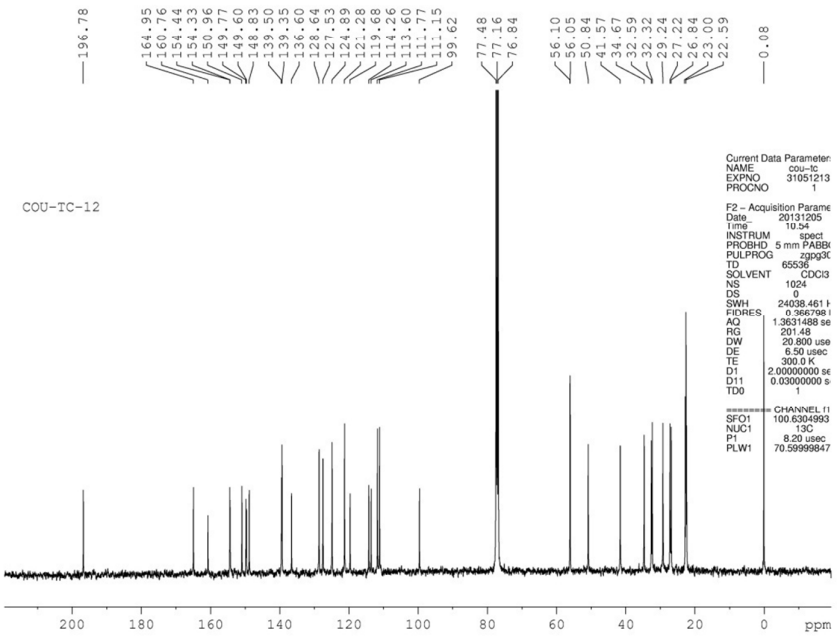
^{13}C NMR of compound **22** at 100 MHz ($CDCl_3$)



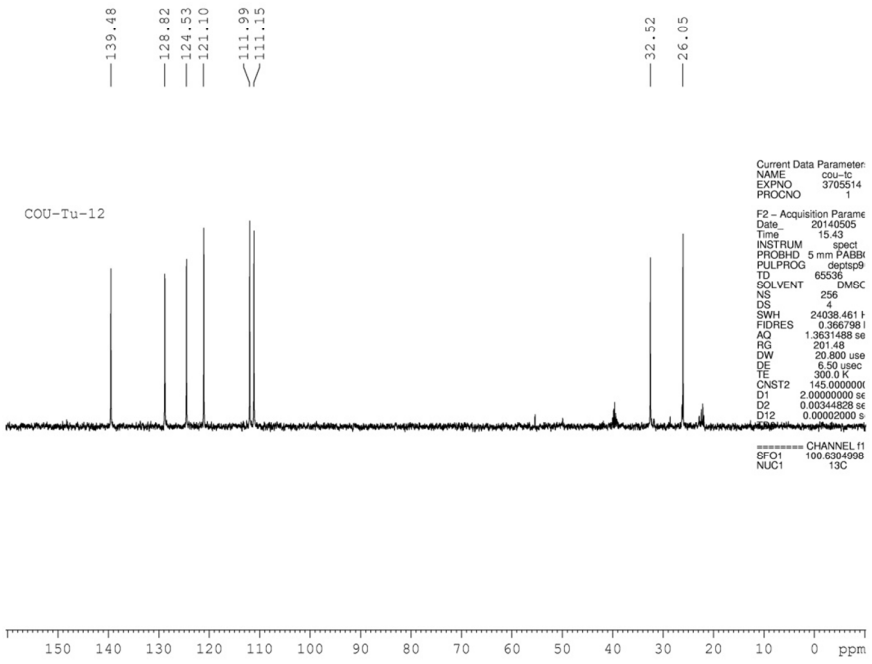
^{1}H NMR of compound **23** at 400 MHz (DMSO-d_6)



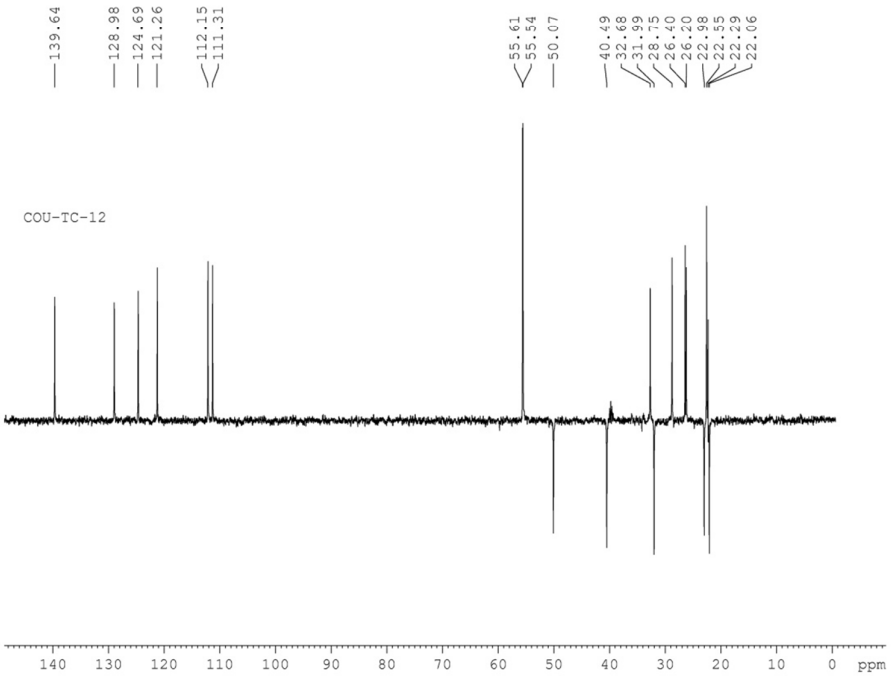
^{13}C NMR of compound **23** at 100 MHz ($CDCl_3$)



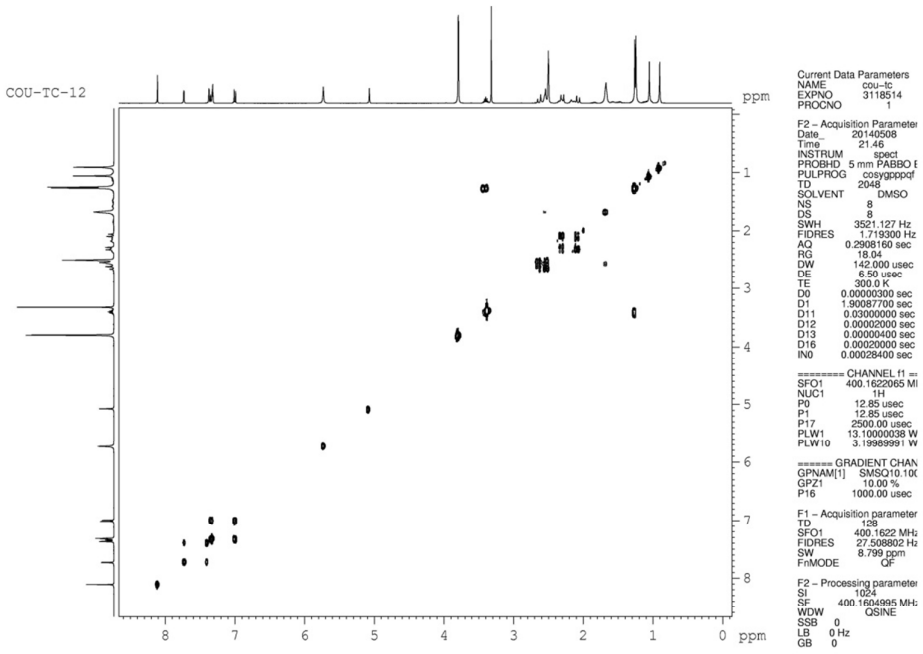
DEPT 90 of compound **23** at 400 MHz (DMSO-d_6)



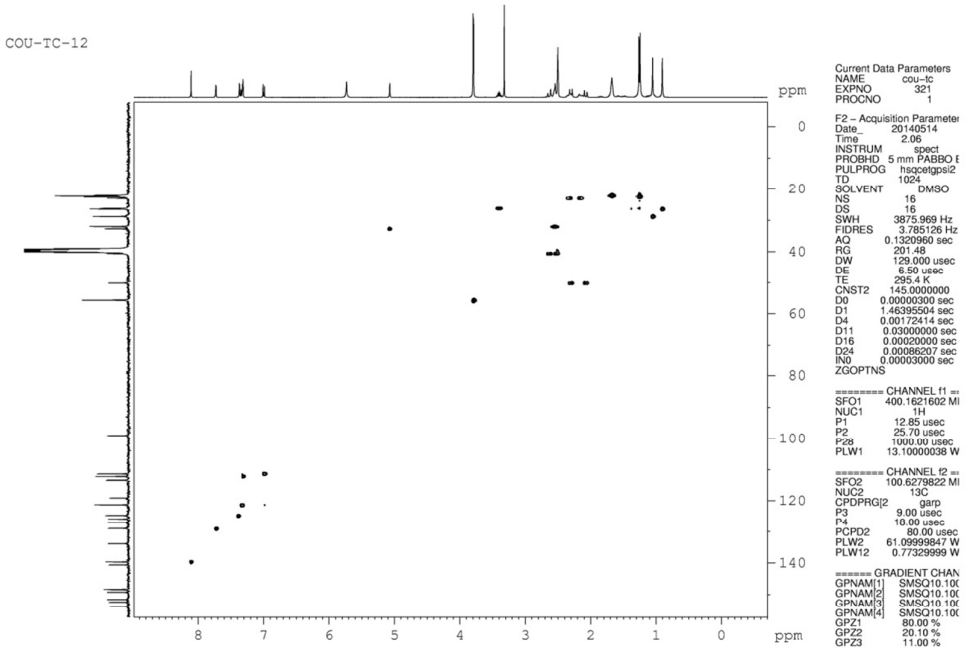
DEPT 135 of compound **23** at 400 MHz (DMSO-d_6)



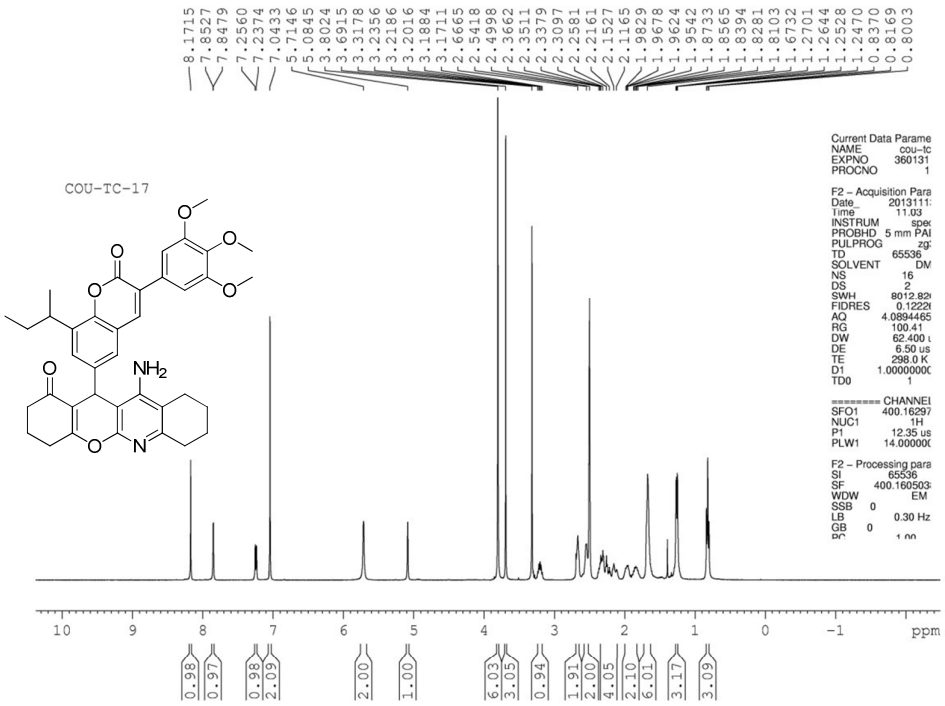
COSY of compound **23** at 400 MHz (DMSO-d_6)



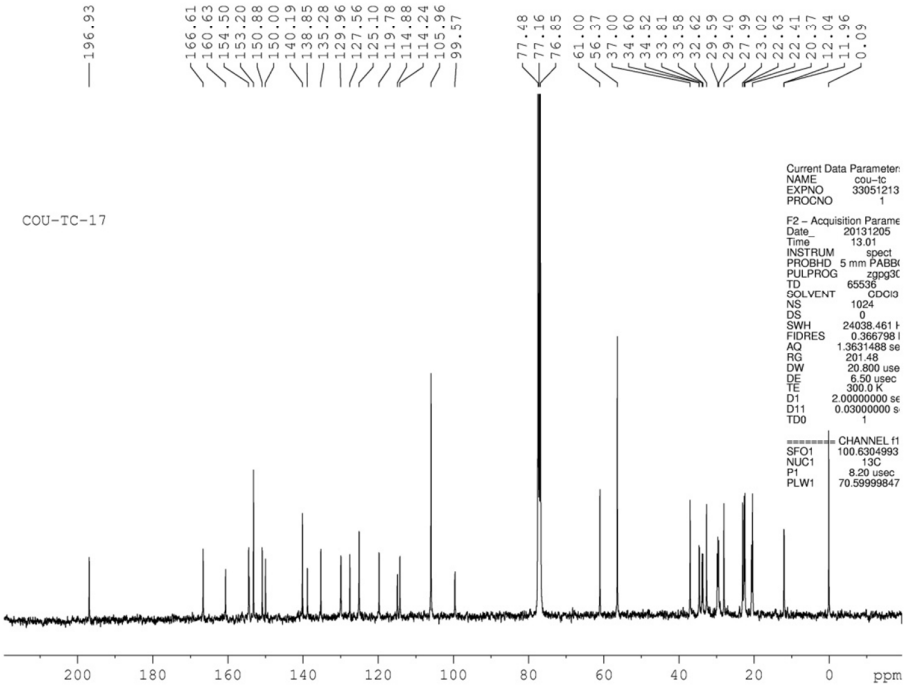
HSQC of compound **23** at 400 MHz (DMSO-d_6)



^{1}H NMR of compound **24** at 400 MHz (DMSO-d_6)

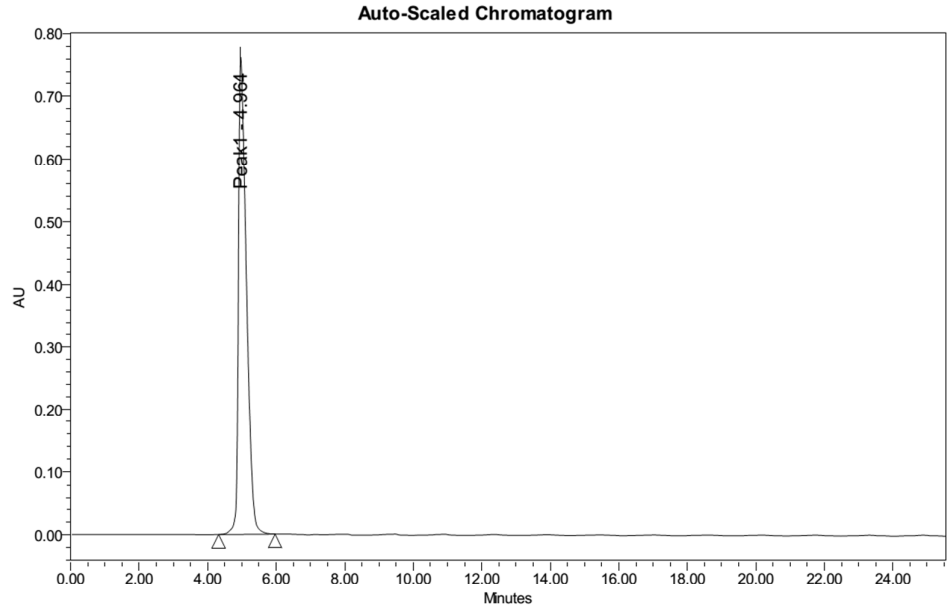


^{13}C NMR of compound **24** at 100 MHz ($CDCl_3$)



3. HPLC Report of most active compounds **18** and **24**

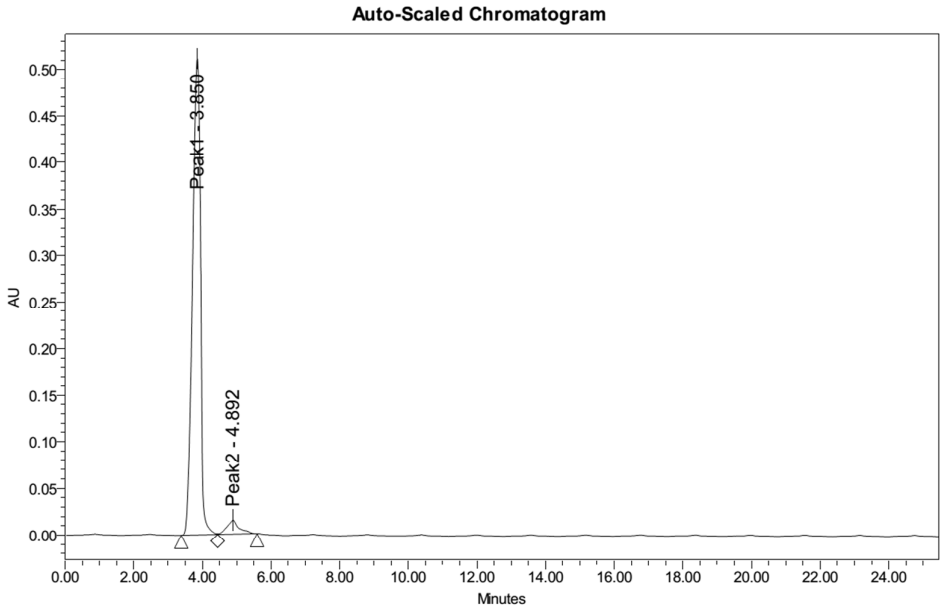
HPLC Report of compound **18**



Peak Results

	Name	RT	Area	Height	% Area
1	Peak1	4.964	12324478	762465	100.00

HPLC Report of compound **24**



Peak Results

	Name	RT	Area	Height	% Area
1	Peak1	3.850	8279891	511450	95.80
2	Peak2	4.892	363313	14915	4.20

4. Biological Experiments, Materials and Methods

4.1. C. elegans culture and maintenance

Worms's culture and maintenance were the same as previously described.[1] In brief, worms were cultivated on nematode growth medium (NGM) agar plates, which were seeded with *E. coli* -OP50 bacteria by the use of standard techniques at 22 °C. NGM media was prepared by adding 50 mM sodium chloride (Merck), 2.5 gL^{-1} peptone (Sigma), 17 gL^{-1} Agar (Hi-media) in 975 mL double distilled water and autoclaved for 30 to 40 minutes at 15 $lb/inch^2$. After the cooling of media to 50-60 °C, 5 μgmL^{-1} cholesterol solution (Sigma) prepared in ethanol, 1 mM calcium chloride (Sigma), 1 mM Magnesium Sulphate (Sigma) and 25 mM potassium dihydrogen phosphate (SRL) additives were added. In this study N2-Bristol strain was used, which is wild-type strain. The following transgenic strains were used: NL5901 (Punc-54:: α-synuclein:: YFP+unc-119; exp ressing human alpha α-synuclein protein with YFP expression in the muscles) and TJ356 (integrated DAF-16:: GFP roller, expressing GFP with DAF-16 were used. These strains were obtained from the *Caenorhabditis* Genetics Center (University of Minnesota, USA).

4.2. Obtaining synchronous nematode population by embryo isolation

For the isolation of embryos from various strains of *C. elegans*, worms were synchronized by alkaline sodium hypochlorite bleaching method. In this method, worms after being washed with M-9 buffer were treated with axenizing solution (2 mL of sodium hypochlorite and 5 mL of 1 M sodium hydroxide solution) and then subjected to intermittent vortexing. Thereafter, the worms were washed with M-9 buffer, twice, so as to obtain worms in their synchronized embryo stage.[2]

4.3. Treatment of Worms with Test Compounds

In this study, test compounds were initially dissolved in ethanol to prepare a stock of 20 mM. Working concentration of 2 mM was then prepared from the stock in OP50. For Bacopa

monnieri treatment concentrated mother tincture (SBL Private limited, Uttaranchal, India) was used. The mother tincture was diluted 10 folds in OP50 before seeding onto NGM plates. This OP50-test compound mixture was seeded onto 6-well NGM plates. The plates were incubated overnight at 37 °C for optimum growth of bacteria OP50. Age synchronized worms were grown on the plates, for further studies.

4.4. Assay for analysis of α-synuclein aggregation

Analysis of *α*-synuclein was carried out as described previously.[3] In brief, worms were grown on control and treated with different natural compounds for 48 hrs, washed with M9 buffer to remove adhering bacteria and immobilized with 100 mM sodium azide (Sigma, cat no. 71289) onto 2 % agar padded slides and sealed with a cover slip. Imaging of live (immobilised) worms using confocal microscopy (Carl Zeiss) was carried out to monitor the aggregation of *α*-synuclein protein, which was further quantified by measuring flourescence intensity using image J Software (Image J, National Institutes of Health, Bethesda, MD). At least 20-30 animals were analyzed on at least 3 separate days, and results were consistent between experiments.

4.5. Estimation of Reactive Oxygen Species (ROS)

To investigate the anti-oxidant properties of compounds, we conducted 2, 7-dichlorodihydrofluorescein-diacetate (H2-DCF-DA) assay (Cat. No – D399, Invitrogen) using standard protocol.[4] In this assay, control and compound treated worms of N2 strain were washed twice using PBS after washing with M-9 buffer. 100 µL volume of worm suspension having an approximate number of 100 worms was transferred into the wells of a 96-well plate in three replicates. After this, 100 µL of 100 µM H2DCFDA dye was added to each well and basal fluorescence was quantified immediately using an excitation wavelength of 485 nm and an emission wavelength of 520 nm. Plate was incubated for 1 hr and then second measurement was quantified. We further calculated fluorescence per worm by

dividing the delta by number of worms after subtracting initial fluorescence from the final reading. Experiments were conducted two times with three samples each time.

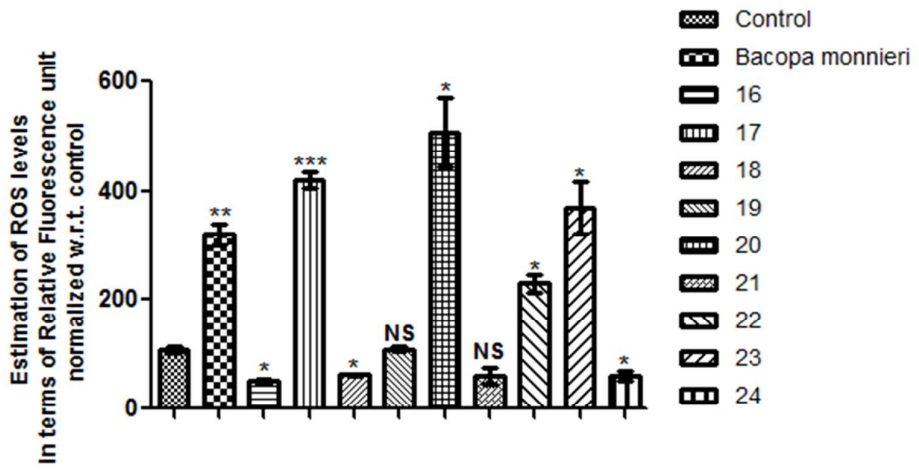


Figure S1 Effect of series of compounds on Relative formation of reactive oxygen species (ROS) measured by H2DCFDA assay in N2 strain of C. elegans.*p < 0.05, **p<0.01, ***p < 0.001, NS - Not significant.

Worms treated with compounds **16**, **18**, and **24** showed significantly 2.16, 1.72 and 1.84 folds ($p<0.05$) reduction in oxidative stress to that of untreated worms. On the contrary, worms fed on compounds BM, 17, 20, 22, and 23 showed 2.94 ($p<0.01$), 3.85 ($p<0.001$), 4.65 ($p<0.05$), 2.10 ($p<0.05$) and 3.38 ($p<0.05$) enhanced levels of ROS with respect to untreated worms. The results demonstrated the non-significant levels of ROS with respect to control, both in case of compounds **19** and **21** treated worms in wild type N2 strain of C. elegans. The results clearly indicated that the role of compounds **16**, **18**, and **24** in exhibiting anti-oxidant properties.

4.6. Acetylcholinesterase inhibition experiment

For AChE inhibition experiment for hybrids, we have employed an indirect method by the quantification of Acetylcholine (ACh) level using amplex red kit. Worms treated with compounds **16**, **17**, **18**, **19**, **20**, **22**, **23** and **24** except **21** showed a significant increase in the

ACh level indicating that the test compounds were inhibiting AChE which is being reflected in the ACh levels. The ACh levels serves as indirect reflection of AChE inhibition as reduction in the ACh breakdown rate, will lead to increase in the concentration of ACh in the worms.[7] Test compounds **16**, **17**, **18**, **19**, **20**, **22**, **23** and **24** exhibited a 1.50 ($p<0.01$), 1.19 ($p<0.001$), 1.20 ($p<0.01$), 1.51 ($p<0.001$), 1.14 ($p<0.05$), 1.88 ($p<0.001$), 1.16 ($p<0.01$) and 1.15 ($p<0.01$) folds increase in ACh levels to that of control worms (Fig. S2). The results demonstrated the inhibitory effect of the compounds on AChE and further advocate the displayed potent neuroprotective activity profile of coumarin and tetracyclic tacrine hybrids.

4.7. Estimation of Acetylcholine (ACh) levels using Amplex Red

The Amplex®Red (Invitrogen, cat. No. A12217) is an Acetylcholine estimation Kit which provides an ultrasensitive method for estimation of acetylcholine level using fluorescence based technique [8]. For the measurement of ACh levels amplex red kit was utilised. Age-synchronized wild type *C. elegans* were transferred onto treatment plates. After 48 hrs, worms were washed thrice with sodium phosphate buffer (PBS) by centrifugation at 1000

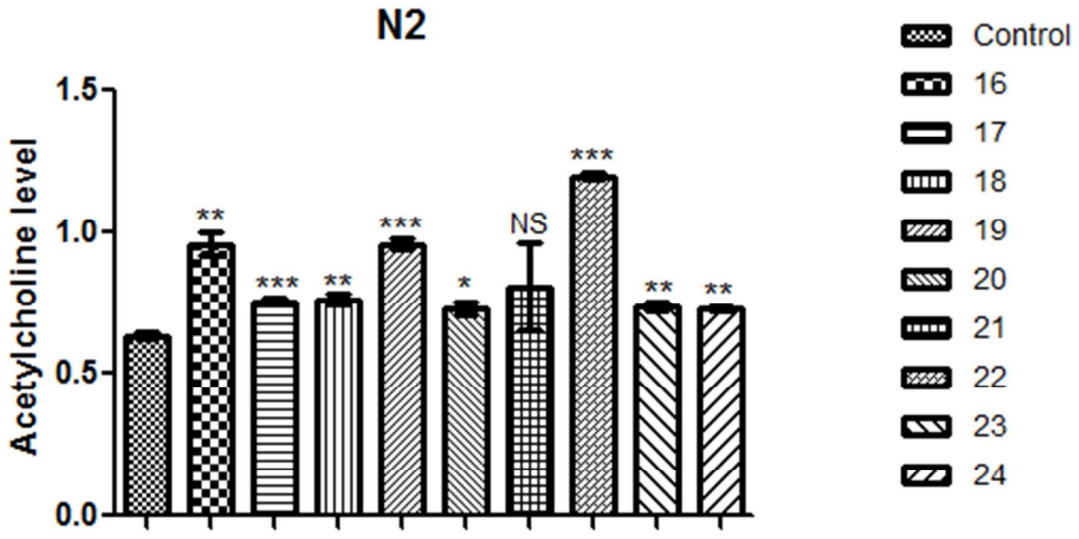


Figure S2 Effect of series of compounds on Acetylcholine levels measured by Amplex red assay in N2 strain of *C. elegans*.*p < 0.05, **p < 0.01 ***p < 0.001, NS - Not significant.

rpm for 2 mins to clear off any adhering bacteria. Then the worms were subjected to sonication on ice at 15 sec intervals using 25% amplitude for a period of 3 mins. The resulting extract was then centrifuged at 14,000 rpm for 30 min. and supernatant was assayed for ACh levels according to the manufacturer's protocol. Absorbance was recorded in a fluorimeter with an excitation wavelength of 544 nm and an emission wavelength of 590 nm. Assays were performed in triplicates and final fluorescence was normalized with respect to protein content (estimated by Bradford reagent).

4.8. Assay for analysis of dopamine signaling using nonanol repulsion assay

Dopamine signaling plays an important role in the regulation of motor function. Therefore, impaired dopamine signaling reduces responses to any volatile attractants or repellents. To evaluate the effect of compounds on the DA-related functions, we employed the odour based repellent assay using 1-nonanol following a method described previously.[5] In this assay, we studied the response of control and compound treated worms against 1-nonanol. In case of worms with altered DA function have a delayed repulsion response against the nonanol whereas healthy worms have a quick repulsion response against this repellent. After treatment of 48 hr, worms were washed off the culture plates and cleaned thrice with M9 buffer. A drop of 1-nonanol was placed near the head of a worm and the response time of worms against the repellent was recorded for ten worms per treatment condition. The experiment was repeated twice and the mean response time ± SE for each group was calculated and the statistical significance was evaluated by Student's t-test.

4.9. Analysis of DAF-16 nuclear localization

For analysis of *DAF-16* expression in worms treated with different compounds, fluorescent microscopy was performed using transgenic strain TJ356, which expresses GFP *with DAF-16.* In this assay, the embryos of TJ356 strain were obtained by alkaline sodium hypochlorite method of embryo isolation. These embryos were then placed on control and treatment plates.

After 48hr incubation, these worms were washed off with M-9 buffer and then mounted on to the slides after immobilizing them with sodium azide. The slides were then analyzed using a 40x objective of the fluorescence microscope (carl zeiss) equipped with a digital camera.[6]

4.10. Cytotoxicity Studies

Activity of compounds in HepG2 at 500µM and 2mM concentration

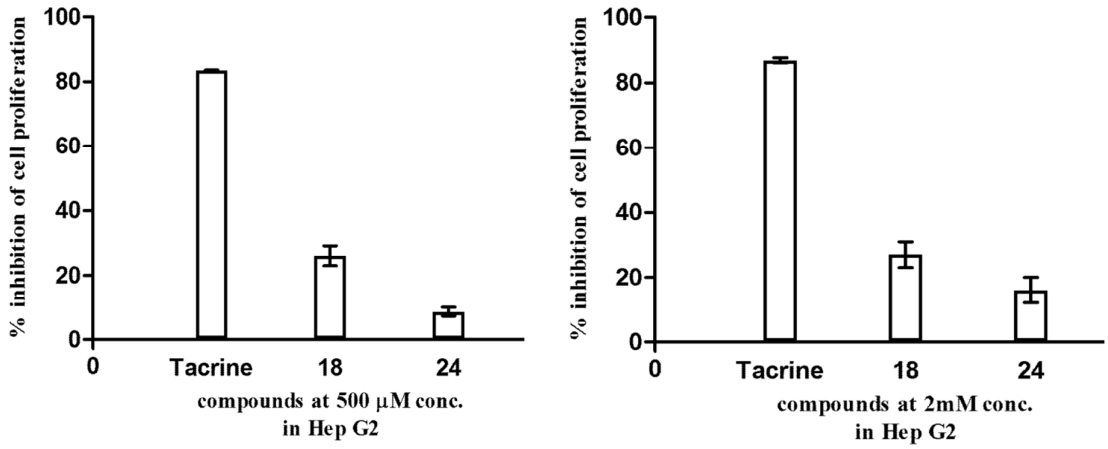


Figure S3 Activity of compound **18** and **24** in HepG2 at 500µM and 2mM concentration.

Inhibitors at 500µM conc. in Hep G2	% of inhibition of cell proliferation
Tacrine	83.42 ± 0.19
18	26.13 ± 3.11
24	8.66 ± 1.34

Inhibitors at 2mM conc. in Hep G2	% of inhibition of cell proliferation
Tacrine	86.91 ± 0.76

18	27.83 ± 3.17
24	16.53 ± 3.44

The compounds **18** and **24** show no apparent toxicity to Hep G2 (hepatocellular carcinoma) cells at 2mM concentration as compared to the parent Tacrine molecule that showed >80% growth inhibition at 2mM concentration.

Activity of compounds in HEK-293 at 500µM and 2mM concentration

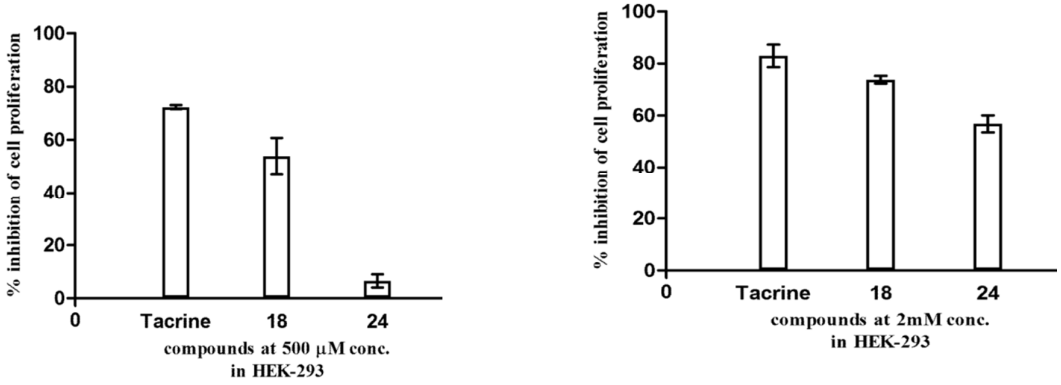


Figure S4 Activity of compounds **18** and **24** in HEK-293 at 500µM and 2mM concentration.

Inhibitors at 500 µM conc. in HEK-293	% of inhibition of cell proliferation
Tacrine	72.35 ± 0.79
18	53.97 ± 6.84
24	6.5 ± 2.5

Inhibitors at 2mM conc. in HEK-293	% of inhibition of cell proliferation

Tacrine	82.95 ± 4.35
18	73.83 ± 1.49
24	56.85 ± 3.28

HEK-293 cells are derived from normal human embryonic kidney cells. The test compounds **18** and **24** show reduced toxicity as compared to the parent molecules in this cell line. The compound **24** is the least toxic with negligible toxicity upto 500μM concentration. From the MTT assay it is clearly shown that, the tacrine have more cytotoxic effect in Hep G2 and HEK-293 cells as compared to compound **18** and **24** (Fig. S3 and S4). Tacrine is showing more cytotoxicity in Hep G2 as compared to HEK-293 cells whereas **18** and **24** are showing less cytotoxicity in Hep G2 as compared to HEK-293, but lesser than tacrine.

4.10.1. Cell proliferation by MTT assay

Cell proliferation was assessed by MTT assay, which is based on the reduction of MTT by mitochondrial dehydrogenases of viable cells to form a purple formazan product. The cell lines (Hep G2 and HEK-293) were grown in recommended media (DMEM and EMEM respectively) supplemented with 10% FBS and PenStrep in a 5% CO_2 humidified atmosphere at 37 ^{o}C. Briefly, cells (5000/well) were plated in 96 well plates. After incubating overnight, the cells were treated with 2 different concentrations (500μM and 2mM) in triplicates with compounds tacrine, **18** and **24** for 48 h. Subsequently, 10 μL of MTT (5 mg/mL) was added to each well and incubated for 3 h. The MTT formazan formed by viable cells was dissolved in 100 μL of Isopropanol and shaken for 10 min. The absorbance was measured on an ELISA plate reader at 590 nm wavelength.

4.11. Data Analysis

All graphs show the mean ± standard error of the mean of at least two independent experiments. Statistical analyses were carried out employing Student's t test using Graph Pad prism 5 software packages. Statistical significance was accepted only when $P < 0.05$.

5. Computational Methods

Computational docking studies were carried out with compound **18**, **24** and *α*-synuclein, using coordinates for *α*-synuclein protein (PDB code: 1XQ8). Interestingly it has been found that hydrophobic non-amyloidbeta component (NAC) region of *α*-synuclein is important in forming amyloid like fibrils, particularly tyrosine triad (Tyr125, Tyr133, and Tyr136), which predicted to play crucial role in fibril formation (Fig S5). Thus, binding of putative inhibitor to this

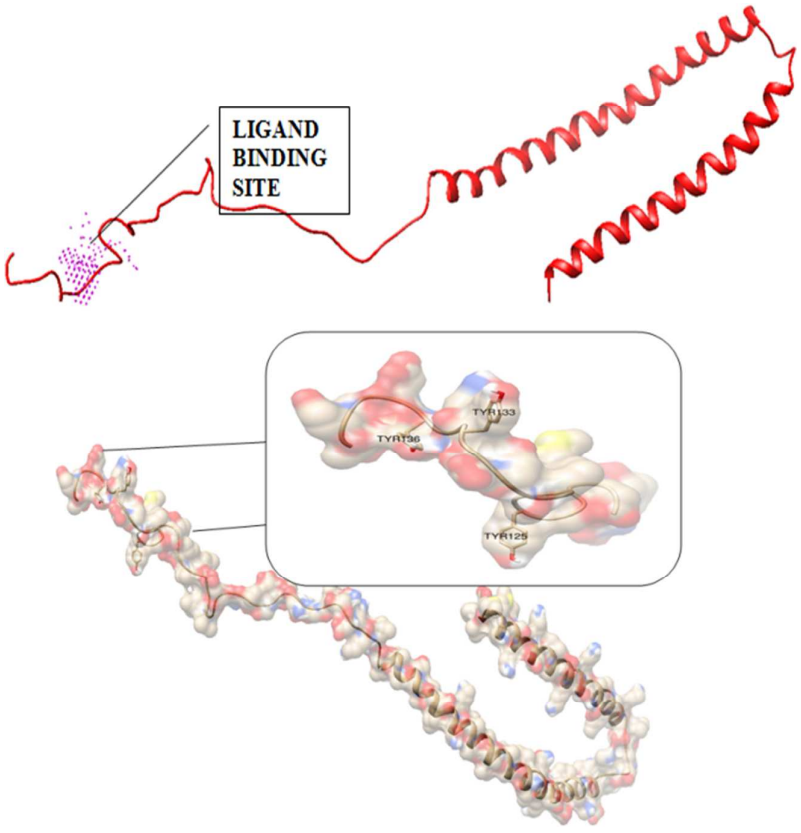


Figure S5 Ligand binding site identified by MetaPocket 2.0 and position of tyrosine triad in *α*-synuclein.

site should affect *α*- synuclein aggregation and thereby preventing intracellular accumulation of misfolded protein, through making misfolded region of protein on hydrophilic tail (Asp98-Ala140), which represents a good drug target. Ligand binding sites on protein surface were identified by using metapocket 2.0,[9] and were subjected to docking studies using autodock 4.2 [10] to generate 100 genetic algorithm runs, using long number of evaluations, and default docking Parameters thereby predicting molecular level binding of ligands to its binding

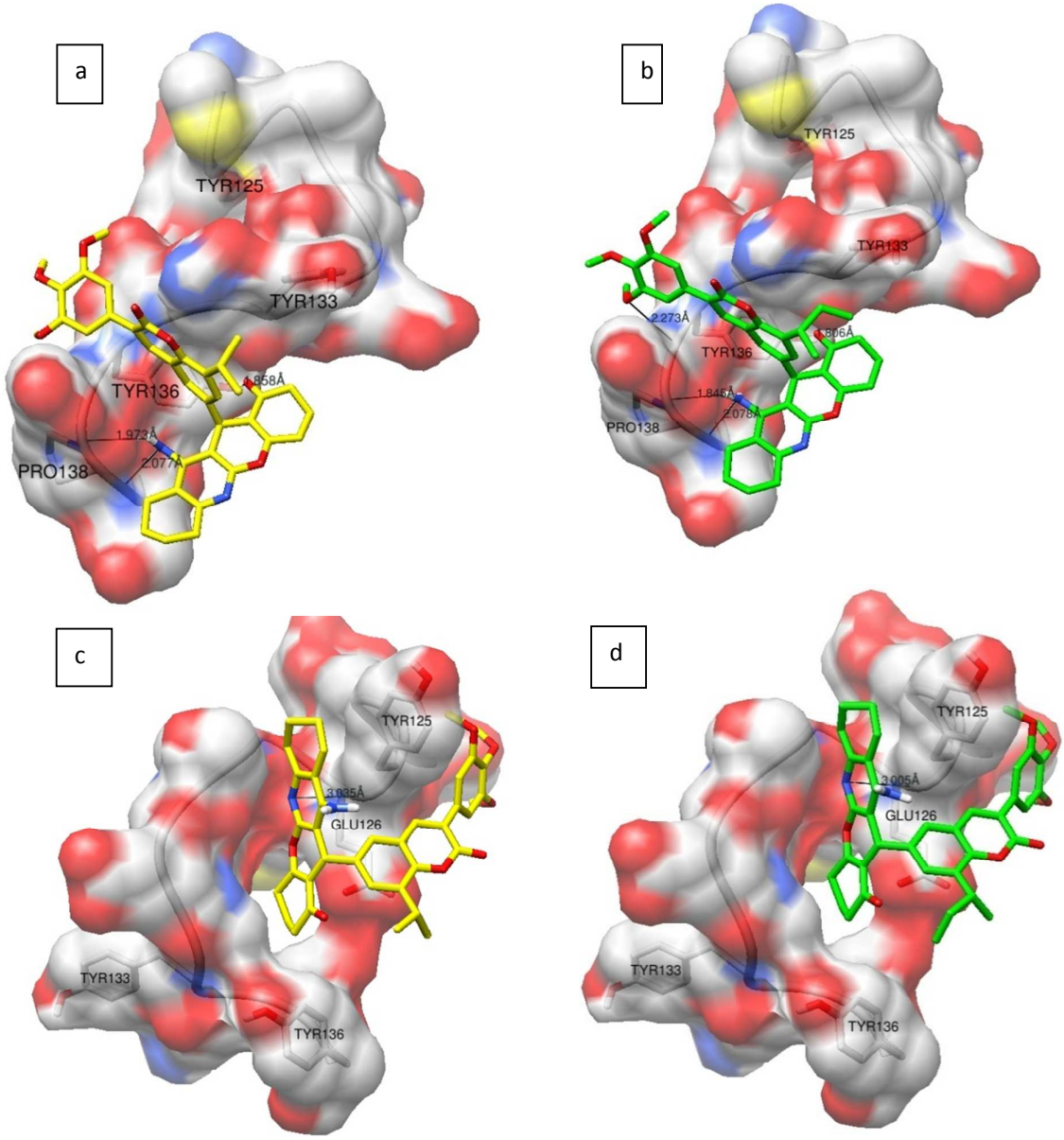


Figure S6 (a) Molecular interaction of compounds **18**-S (yellow), (b) compound **24**-S (green), (c) compound **18**-R (yellow) and (d) compound 18-R (green) with *α*-synuclein

pocket. Structure of compound **18** and **24**, used for docking studies were built and minimized using marvinsketch version 6.1.2 from chemaxon[11] and then clean in 3D using fine build method. The pocket so identified was docked with both compounds (**18** and **24**) Docking poses were visualized and images were processed by using Chimera 1.8.1.[12]

6. References

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