Supporting Information

Identification of 4,6-diaryl-1,4-dihidropyridines as a new class of neuroprotective agents

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1. Experimental procedures

1.1. Chemistry

Experimental section

General experimental information
All reagents (Aldrich, Fluka, SDS, Probus) and solvents (SDS), were of commercial quality and were used as received. Reactions were monitored by thin layer chromatography, on aluminum plates coated with silica gel with fluorescent indicator (SDS CCM221254). Separations by flash chromatography were performed on silica gel (SDS 60 ACC 40-63 μm) or neutral alumina (Merck S22). Melting points were measured on a Reichert 723 hot stage microscope, and are uncorrected. Infrared spectra were recorded on a Perkin Elmer Paragon 1000 FT-IR spectrophotometer, with all compounds examined as KBr pellets or as thin films on NaCl disks. NMR spectra were obtained on a Bruker Avance 250 spectrometer operating at 250 MHz for $^1$H and 63 MHz for $^{13}$C (CAI de Resonancia Magnética Nuclear, Universidad Complutense). Elemental analyses were determined by CAI de Microanálisis Elemental, Universidad Complutense, using a Leco 932 CHNS combustion microanalyzer.

General procedure for the synthesis of 1,4-dihydropyridine derivatives (3a-k) and 4,6,7,8-tetrahydroquinolin-5(1H)-one (4)
To a stirred solution of 1,3-diphenyl-2-propen-1-one derivatives (1 equiv, 2 mmol), 1,3-dicarbonyl compounds (1.1 equiv, 2.2 mmol) and ammonium acetate (3 equiv, 6 mmol) in ethanol (2 mL) was added ceric ammonium nitrate (CAN, 10% mol) and the resulting mixture was refluxed for 4 hours. After this time, another portion of ammonium acetate (1.5 equiv, 3 mmol) was added and stirring was continued at the same conditions for an additional period of 4 hours. After completion of the reaction (checked by TLC), the mixture was allowed to cool to room temperature, diluted with CH$_2$Cl$_2$ (20 mL) and washed with water to remove CAN and the excess of ammonium acetate. The organic layer was then washed with brine and dried over anhydrous Na$_2$SO$_4$ and the solvent was evaporated under reduced pressure.

The crude residue was crystallized from EtOH or purified by silica gel column chromatography using petroleum ether-ethyl acetate mixture (12:1 v/v) as eluent to give pure compounds (3a-k or 4). Characterization data for all final compounds follow.
**Ethyl 2-methyl-4,6-diphenyl-1,4-dihydropyridine-3-carboxylate (3a)**

Yellow solid, mp 244-246 °C; $^1$H-NMR (CDCl$_3$, 250 MHz) $\delta$ 1.28 (t, $J = 7.1$ Hz, 3H, OCH$_2$CH$_3$), 2.56 (s, 3H, C-2CH$_3$), 4.17 (q, $J = 7.1$ Hz, 2H, OCH$_2$CH$_3$), 4.84 (d, $J = 5.5$ Hz, 1H, C-4H), 5.34 (dd, $J = 1.8$, 5.5 Hz, 1H, C-5H), 5.73 (bs, 1H, NH), 7.30-7.33 (m, 1H, ArH), 7.38-7.52 (m, 9H, ArH); $^{13}$C-NMR (CDCl$_3$, 63 MHz) $\delta$ 14.7 (OCH$_2$CH$_3$), 21.1 (C-2CH$_3$), 41.4 (C-4), 59.7 (OCH$_2$CH$_3$), 99.5 (C-3), 105.5 (C-5), 125.5 (2xCHAr), 126.5 (CHAr), 128.2 (2xCHAr), 128.6 (2xCHAr), 128.9 (CHAr), 129.2 (2xCHAr), 134.7, 136.3 (C-6Car, C-6), 147.3, 149.3 (C-4Car, C-2), 168.8 (COOR); IR (NaCl) ν 2984, 1717, 1666, 1481, 1382 cm$^{-1}$; elemental analysis calcd (%) for C$_{21}$H$_{22}$NO$_2$: C 78.97, H 6.63, N 4.39; found: C 78.87, H 6.71, N 4.42.

**Ethyl 2-ethyl-4,6-diphenyl-1,4-dihydropyridine-3-carboxylate (3b)**

White solid, mp 143-145 °C; $^1$H-NMR (CDCl$_3$, 250 MHz) $\delta$ 1.28 (t, $J = 7.2$ Hz, 3H, CH$_2$CH$_3$), 1.44 (t, $J = 7.5$ Hz, 3H, OCH$_2$CH$_3$), 2.97 (dq, $J = 4.3$, 7.2 Hz, 2H, CH$_2$CH$_3$), 4.17 (dq, $J = 0.6$, 7.5 Hz, 2H, OCH$_2$CH$_3$), 4.84 (d, $J = 5.6$ Hz, 1H, C-4H), 5.33 (dd, $J = 1.9$, 5.6 Hz, 1H, C-5H), 5.79 (bs, 1H, NH), 7.23-7.33 (m, 1H, ArH), 7.38-7.57 (m, 9H, ArH); $^{13}$C-NMR (CDCl$_3$, 63 MHz) $\delta$ 13.5 (CH$_2$CH$_3$), 14.6 (OCH$_2$CH$_3$), 27.4 (CH$_2$CH$_3$), 41.4 (C-4), 59.6 (OCH$_2$CH$_3$), 98.6 (C-3), 105.4 (C-5), 125.5 (2xCHAr), 126.4 (CHAr), 128.1 (2xCHAr), 128.6 (2xCHAr), 128.9 (CHAr), 129.2 (2xCHAr), 134.6, 136.4 (C-6Car, C-6), 149.4, 152.7 (C-4Car, C-2), 168.3 (COOR); IR (NaCl) ν 3325, 2974, 1666, 1487, 1366 cm$^{-1}$; elemental analysis calcd (%) for C$_{22}$H$_{23}$NO$_2$: C 79.25, H 6.95, N 4.20; found: C 78.94, H 6.88, N 4.40.

**Ethyl 6-(4-chlorophenyl)-2-methyl-4-phenyl-1,4-dihydropyridine-3-carboxylate (3c)**

Yellow solid, mp > 250 °C; $^1$H-NMR (CDCl$_3$, 250 MHz) $\delta$ 1.16 (t, $J = 7.1$ Hz, 3H, OCH$_2$CH$_3$), 2.44 (s, 3H, C-2CH$_3$), 4.05 (dq, $J = 1.6$, 7.1 Hz, 2H, OCH$_2$CH$_3$), 4.71 (d, $J = 5.5$ Hz, 1H, C-4H), 5.19 (dd, $J = 1.8$, 5.5 Hz, 1H, C-5H), 5.52 (bs, 1H, NH), 7.16-7.22 (m, 1H, ArH), 7.27-7.36 (m, 8H, ArH); $^{13}$C-NMR (CDCl$_3$, 63 MHz) $\delta$ 14.6 (OCH$_2$CH$_3$), 21.1 (C-2CH$_3$), 41.3 (C-4), 59.7 (OCH$_2$CH$_3$), 99.7 (C-3), 106.0 (C-5), 126.5 (CHAr), 126.8 (2xCHAr), 128.1 (2xCHAr), 128.7 (2xCHAr), 129.3 (2xCHAr), 133.7, 134.7, 134.8 (C-6Car, C-6, ArCCI), 147.0, 149.0 (C-4Car, C-2), 168.7 (COOR); IR (NaCl) ν 2981, 2363, 1722, 1586, 1491, 1382 cm$^{-1}$; elemental analysis calcd (%) for C$_{21}$H$_{20}$ClNO$_2$: C 71.28, H 5.70, N 3.96; found: C 70.91, H 5.68, N 4.00.

**1-(2-Methyl-4,6-diphenyl-1,4-dihydropyridin-3-yl)ethanone (3d)**

Yellow solid, mp 153-155 °C; $^1$H-NMR (CDCl$_3$, 250 MHz) $\delta$ 2.07 (s, 3H, COCH$_3$), 2.48 (s, 3H, C-2CH$_3$), 4.74 (d, $J = 5.6$ Hz, 1H, C-4H), 5.30 (dd, $J = 1.9$, 5.6 Hz, 1H, C-5H), 5.70 (bs, 1H, NH), 7.19-7.25 (m, 1H, ArH), 7.30-7.40 (m, 9H, ArH); $^{13}$C-NMR (CDCl$_3$, 63 MHz) $\delta$ 22.2 (C-2CH$_3$), 29.9 (COCH$_3$), 42.5 (C-4), 106.3 (C-5), 107.8 (C-3), 125.4 (2xCHAr), 126.8 (CHAr), 127.7 (2xCHAr), 129.0 (CHAr), 129.17

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(2xCHAr), 129.21 (2xCHAr), 134.1, 136.0 (C-6Car, C-6), 147.3, 148.3 (C-4Car, C-2), 199.2 (COCH₃); IR (NaCl) ν 3022, 2962, 1637, 1574, 1474, 1289 cm⁻¹; elemental analysis calcd (%) for C₂₅H₃₉NO: C 83.01, H 6.62, N 4.84; found: C 82.83, H 6.45, N 4.98.

**S-tert-Butyl 2-methyl-4,6-diphenyl-1,4-dihydropyridine-3-carbothioate (3e)**

Yellow solid, mp 158-160 °C; ¹H-NMR (CDCl₃, 250 MHz) δ 1.44 (s, 9H, SC(CH₃)₃), 2.45 (s, 3H, C-2CH₃), 3.92 (s, 3H, OCH₃), 4.92 (d, J = 6.0 Hz, 1H, C-4H), 5.40 (dd, J = 1.6, 6.0 Hz, 1H, C-5H), 5.78 (bs, 1H, NH), 6.97 (d, J = 8.6 Hz, 2H, C-4Ar-H3 and C-4Ar-H5), 7.38 (d, J = 8.6 Hz, 2H, C-4Ar-H2 and C-4Ar-H6), 7.49-7.51 (m, 5H, ArH); ¹³C-NMR (CDCl₃, 63 MHz) δ 21.7 (C-2CH₃), 30.6 (SC(CH₃)₃), 40.5 (C-4), 47.6 (SC(CH₃)₃), 55.6(OCH₃), 108.4 (C-5), 114.1 (2xCHAr), 125.5 (2xCHAr), 128.8 (2xCHAr), 128.91 (CHAr), 134.3, 136.1 (C-6Car, C-6), 140.5, 144.4 (C-4Car, C-2), 158.4 (ArC-OCH₃), 193.2 (COSR); IR (NaCl) ν 2960, 1534, 1506, 1472, 1382 cm⁻¹; elemental analysis calcd (%) for C₂₄H₂₇NO₂S: C 73.25, H 6.92, N 3.56; found: C 73.11, H 6.75, N 3.73; S 7.91.

**S-tert-Butyl 4-(4-methoxyphenyl)-2-methyl-6-phenyl-1,4-dihydropyridine-3-carbothioate (3f)**

Yellow solid, mp 138-140 °C; ¹H-NMR (CDCl₃, 250 MHz) δ 1.56 (s, 9H, SC(CH₃)₃), 2.55 (s, 3H, C-2CH₃), 2.49 (s, 3H, CH₃), 2.55 (s, 3H, CH₃), 4.93 (d, J = 5.9 Hz, 1H, C-4H), 5.32 (dd, J = 1.6, 5.9 Hz, 1H, C-5H), 5.79 (bs, 1H, NH), 7.28-7.40 (m, 6H, ArH), 7.54 (d, J = 8.3 Hz, 2H, ArH); ¹³C-NMR (CDCl₃, 63 MHz) δ 21.3 (CH₃), 21.4 (CH₃), 30.2 (SC(CH₃)₃), 40.6 (C-4), 47.4 (SC(CH₃)₃), 104.0 (C-5), 107.3 (C-3), 120.0 (ArBr), 125.1 (2xCHAr), 129.3 (2xCHAr), 129.6 (2xCHAr), 131.5 (2xCHAr), 132.7, 134.4, 138.8 (C-6Car, C-6, ArCCH₃), 144.7, 146.8 (C-4Car, C-2), 192.5 (COSR); IR (NaCl) ν 2929, 2892, 1612, 1553, 1470, 1387, 1159 cm⁻¹; elemental analysis calcd (%) for C₂₄H₂₅BrNO: C 63.15, H 5.74, N 3.07; S 7.91; found: C 62.98, H 5.78, N 3.36, S 6.99.

**S-tert-Butyl 2-methyl-6-phenyl-4-(4-tolyl)-1,4-dihydropyridine-3-carbothioate (3g)**

Light yellow solid, mp 164-166 °C; ¹H-NMR (CDCl₃, 250 MHz) δ 1.56 (s, 9H, SC(CH₃)₃), 2.49 (s, 3H, CH₃), 2.55 (s, 3H, CH₃), 4.93 (d, J = 5.9 Hz, 1H, C-4H), 5.32 (dd, J = 1.6, 5.9 Hz, 1H, C-5H), 5.79 (bs, 1H, NH), 7.28-7.40 (m, 6H, ArH), 7.54 (d, J = 8.3 Hz, 2H, ArH); ¹³C-NMR (CDCl₃, 63 MHz) δ 21.3 (CH₃), 21.4 (CH₃), 30.2 (SC(CH₃)₃), 40.6 (C-4), 47.4 (SC(CH₃)₃), 104.0 (C-5), 107.3 (C-3), 120.0 (ArBr), 125.1 (2xCHAr), 129.3 (2xCHAr), 129.6 (2xCHAr), 131.5 (2xCHAr), 132.7, 134.4, 138.8 (C-6Car, C-6, ArCCH₃), 144.7, 146.8 (C-4Car, C-2), 192.5 (COSR); IR (NaCl) ν 3066, 2960, 1638, 1571, 1470, 1385, 1158 cm⁻¹; elemental analysis calcd (%) for C₂₄H₂₅BrNO: C 63.15, H 5.74, N 3.07, S 7.03; found: C 62.98, H 5.78, N 3.36, S 6.99.
**S-tert-Butyl 2-methyl-4,6-di(4-tolyl)-1,4-dihydropyridine-3-carbothioate (3i)**

Yellow solid, mp 169-171 °C; $^1$H-NMR (CDCl$_3$, 250 MHz) $\delta$ 1.46 (s, 9H, SC(CH$_3$)$_3$), 2.35 (s, 3H, CH$_3$), 2.38 (s, 3H, CH$_3$), 2.44 (s, 3H, CH$_3$), 4.82 (d, $J$ = 6.1 Hz, 1H, C-4H), 5.27 (dd, $J$ = 1.7, 6.1 Hz, 1H, C-5H), 5.67 (bs, 1H, NH), 7.04-7.38 (m, 8H, ArH); $^{13}$C-NMR (CDCl$_3$, 63 MHz) $\delta$ 21.5 (CH$_3$), 21.6 (CH$_3$), 21.7 (CH$_3$), 30.6 (SC(CH$_3$)$_3$), 40.8 (C-4), 47.5 (SC(CH$_3$)$_3$), 105.1 (C-5), 108.1 (C-3), 124.5 (2xCHAr), 127.7 (2xCHAr), 129.5 (2xCHAr), 129.8 (2xCHAr), 133.2, 134.3, 136.0, 138.8 (C-6Ar, C-6, 2xArCH$_3$), 144.8, 145.2 (C-4Ar, C-2), 193.1 (COSR); IR (NaCl) v 3279, 2968, 1612, 1474, 1379 cm$^{-1}$; elemental analysis calcd (%) for C$_{25}$H$_{27}$NOS: C 76.35, H 7.21, N 3.71, S 8.49; found: C 75.92, H 7.10, N 4.01, S 8.11.

**Ethyl 2-methyl-4-(thiophen-2-yl)-6-(4-tolyl)-1,4-dihydropyridine-3-carboxylate (3j)**

Orange syrup; $^1$H-NMR (CDCl$_3$, 250 MHz) $\delta$ 1.30 (t, $J$ = 7.1 Hz, 3H, OCH$_2$CH$_3$), 2.40 (s, 3H, CH$_3$), 2.41 (s, 3H, CH$_3$), 4.18 (q, $J$ = 7.1 Hz, 2H, OCH$_2$CH$_3$), 5.04 (d, $J$ = 5.8 Hz, 1H, C-4H), 5.28 (dd, $J$ = 1.8, 5.8 Hz, 1H, C-5H), 5.76 (bs, 1H, NH), 6.92-6.95 (m, 2H, thienyl-H), 7.14 (dd, $J$ = 1.6, 4.7 Hz, 1H, thienyl-H), 7.22 (d, $J$ = 8.0 Hz, 2H, C-4ArH), 7.36 (d, $J$ = 8.0 Hz, 2H, C-4ArH); $^{13}$C-NMR (CDCl$_3$, 63 MHz) $\delta$ 14.8 (OCH$_2$CH$_3$), 21.2 (CH$_3$), 21.6 (CH$_3$), 35.5 (C-4), 59.9 (OCH$_2$CH$_3$), 99.6 (C-3), 103.3 (C-5), 123.3 (CH-thienyl), 123.9 (CH-thienyl), 125.6 (2xCHAr), 127.0 (CH-thienyl), 129.9 (2xCHAr), 133.3, 135.7, 139.1 (C-6Ar, C-6, ArCH$_3$), 147.1, 153.9 (C-thienyl, C-2), 168.5 (COOR); IR (NaCl) v 2978, 1718, 1670, 1584, 1475, 1383 cm$^{-1}$; elemental analysis calcd (%) for C$_{20}$H$_{21}$NO$_2$S: C 70.77, H 6.24, N 4.13, S 9.45; found: C 70.76, H 6.06, N 4.25, S 9.46.

**S-tert-Butyl 2-methyl-6-phenyl-4-(1-phenylprop-1-en-2-yl)-1,4-dihydropyridine-3-carbothioate (3k)**

Yellow solid, mp 113-115 °C; $^1$H-NMR (CDCl$_3$, 250 MHz) $\delta$ 1.5 (s, 9H, SC(CH$_3$)$_3$), 1.95 (d, $J$ = 1.3 Hz, 3H, CH=CHCH$_3$), 2.39 (s, 3H, C=CHCH$_3$), 4.41 (d, $J$ = 5.8 Hz, 1H, C-4H), 5.19 (dd, $J$ = 1.9, 5.8 Hz, 1H, C-5H), 5.55 (bs, 1H, NH), 6.40 (s, 1H, CH=CHCH$_3$), 7.20-7.24 (m, 1H, ArH), 7.27-7.45 (m, 9H, ArH); $^{13}$C-NMR (CDCl$_3$, 63 MHz) $\delta$ 15.7 (CH=CHCH$_3$), 21.3 (C-2CH$_3$), 30.6 (SC(CH$_3$)$_3$), 45.7 (C-4), 47.4 (SC(CH$_3$)$_3$), 103.8 (C-5), 106.9 (C-3), 125.5 (2xCHAr), 125.7 (CH=CHCH$_3$), 126.2 (CHAr), 128.3 (2xCHAr), 129.0 (CHAr), 129.2 (2xCHAr), 129.4 (2xCHAr), 135.8, 136.3, 139.2, 142.6, 144.7 (C-6Ar, C-6, CH=CHCH$_3$, C=CHAr, C-2), 193.7 (COSR); IR (NaCl) v 3405, 3357, 2343, 1633, 1470, 1373, 1174, 1154 cm$^{-1}$; elemental
analysis calcd (%) for C_{26}H_{29}NOS: C 77.38, H 7.24, N 3.47, S 7.95; found: C 77.21, H 7.04, N 3.61, S 7.85.

2,4-Diphenyl-4,6,7,8-tetrahydroquinolin-5(1H)-one (4)
White solid, mp 208-210 °C; $^1$H-NMR (DMSO-d$_6$, 250 MHz) δ 1.75-1.99 (m, 2H, C-7H), 2.19-2.26 (m, 2H, C-8H), 2.55-2.71 (m, 2H, C-6H), 4.61 (d, J = 5.4 Hz, 1H, C-4H), 5.24 (dd, J = 1.6, 5.4 Hz, 1H, C-3H), 7.08-7.51 (m, 10H, ArH), 8.71 (bs, 1H, NH); $^{13}$C-NMR (DMSO-d$_6$, 63 MHz) δ 21.3 (C-7), 27.2 (C-8), 37.24 (C-4), 37.28 (C-6), 106.2 (C-3), 107.4 (C-4a), 125.91 (2xCHAr), 125.96 (CHAr), 127.7 (2xCHAr), 128.5 (2xCHAr), 128.7 (CHAr), 128.8 (2xCHAr), 134.8, 135.5 (C-2CAr, C-2), 148.6, 154.6 (C-4CAr, C-8a), 194.7 (C=O); IR (NaCl) ν 3213, 3167, 1584, 1487, 1389, 1329 cm$^{-1}$; elemental analysis calcd (%) for C$_{21}$H$_{19}$NO: C 83.69 H 6.35, N 4.65; found: C 83.45, H 6.01, N 4.51.
1.2. Pharmacology

Culture of SH-SY5Y Cells

SH-SY5Y cells were maintained in a 1:1 mixture of F-12 Nutrient Mixture (Ham12) (Sigma-Aldrich, Madrid, Spain) and Eagle’s minimum essential medium (EMEM) supplemented with 15 non-essential amino acids, 1 mM sodium pyruvate, 10% heat-inactivated foetal bovine serum (FBS), 100 units/mL penicillin, and 100 μg/mL streptomycin (reagents from Invitrogen, Madrid, Spain). Cultures were seeded into flasks containing supplemented medium and maintained at 37 °C in a humidified atmosphere of 5% CO₂ and 95% air. For assays, SH-SY5Y cells were sub-cultured in 48-well plates at a seeding density of 1 x 10⁵ cells per well. Cells were treated with the drugs before confluence in F-12/EMEM with 1% FBS. All the cells used in this study were used at a low passage number (<13).

Measurement of cytosolic Ca²⁺ transients in SH-SY5Y neuroblastoma cells

SHSY5Y cells were plated in black, bottom transparent 96-well plates and grown at confluence. Cells were loaded with 4 μM Fluo-4/AM for 45 min at 37 °C in EMEM. Then, cells were washed twice with Krebs-HEPES solution containing the following composition (in mM): 140 NaCl, 5.6 KCl, 1.2 MgCl₂, 2 CaCl₂, 10 HEPES, 11 D-glucose, at pH 7.4 and kept at room temperature for 15 min before the beginning of the experiment. Compounds were incubated 15 min before injecting potassium (70 mM) to enhance VDCC opening and [Ca²⁺]c increase. Fluorescence measurements were carried out for 14 seconds after the injection of the agonists in a microplate reader (FLUOstar Optima, BMG, Germany). Wavelengths of excitation and emission were 485 and 520 nm, respectively. At the end of the experiment, 50 μL of triton 5% was added to each well to calculate maximum fluorescence (F_max) and then 50 μL of MnCl₂ 1 M to obtain the minimum fluorescence (F_min). Drug-evoked responses were expressed as percentage of the fluorescence values at each time point (F) minus minimum fluorescence values (F₀) divided by F_max-F_min as follows:

\[ F_{520} = \frac{(F - F_0)}{(F_{max} - F_{min})}% \]

The maximum value of F₅₂₀ obtained for each experiment was considered as the Peak F₅₂₀ value.
Measurement of protection against high K⁺-induced toxicity

Concentrated solutions of drugs were prepared in DMSO. For the DMSO group (control), 0.1% DMSO was incubated, having the same DMSO concentration of the tested drugs group. Compounds, at the concentration of 5 μM, were co-incubated for 24 h with the drug in the presence of the toxic stimulus in serum-free medium.

In vitro OGD model of ischemia

Briefly, after 24 h of cell culture, the culture medium was changed to the glucose-free F-12/EMEM containing either melatonin, nifedipine (positive control) or target compounds at 5 μM before placing into an anaerobic chamber that was flushed with 5% CO₂ and 95% N₂ (v/v). The same anaerobic gas mixture was bubbled through the glucose-free PBS with 2-deoxyglucose. The cell cultures within the anaerobic chamber were kept in a humidified incubator at 37 °C for 4 h. To terminate the OGD, the PBS medium was changed to normal medium containing the same concentrations of tested compound alone before returning to the normoxic incubating conditions. In the sham-OGD groups, the cell cultures were subjected to the same experimental procedures plus vehicle,-without exposure to the glucose-free PBS plus anoxia.

Quantification of Viability by MTT in SH-SY5Y cells

Cell viability, virtually the mitochondrial activity of living cells, was measured by quantitative colorimetric assay with MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide, Sigma Aldrich, Madrid, Spain), as described previously. To evaluate viability after the treatment with the compounds, MTT was added to all wells (5 mg/mL) and allowed to incubate, in the dark at 37 °C for 2 h. The tetrazolium ring of MTT is cleaved by active reductases producing a precipitated formazan derivative. The formazan produced was dissolved by adding 300 μL DMSO, resulting in a coloured compound whose optical density was measured in an ELISA reader at 540 nm in 100 μL of the resulting solution for each well transferred to a 96-well plate. All MTT assays were performed in triplicate.
2. Copies of spectra

Ethyl 2-methyl-4,6-diphenyl-1,4-dihydropyridine-3-carboxylate (3a)
Ethyl 2-ethyl-4,6-diphenyl-1,4-dihydropyridine-3-carboxylate (3b)
Ethyl 6-(4-chlorophenyl)-2-methyl-4-phenyl-1,4-dihydropyridine-3-carboxylate (3c)
1-(2-Methyl-4,6-diphenyl-1,4-dihydropyridin-3-yl)ethanone (3d)
S-\textit{tert}-Butyl 2-methyl-4,6-diphenyl-1,4-dihydropyridine-3-carbothioate (3e)
S-tert-Butyl 4-(4-methoxyphenyl)-2-methyl-6-phenyl-1,4-dihydropyridine-3-carbothioate (3f)
S-<i>tert</i>-Butyl 4-(4-bromophenyl)-2-methyl-6-(4-tolyl)-1,4-dihydropyridine-3-carbothioate (3g)
S-\textit{tert}-Butyl 2-methyl-6-phenyl-4-(4-tolyl)-1,4-dihydropyridine-3-carbothioate (3h)
S-tert-Butyl 2-methyl-4,6-di-(4-tolyl)-1,4-dihydropyridine-3-carbothioate (3i)
Ethyl 2-methyl-4-(thiophen-2-yl)-6-(4-tolyl)-1,4-dihydropyridine-3-carboxylate (3j)
S-tert-Butyl 2-methyl-6-phenyl-4-(1-phenylprop-1-en-2-yl)-1,4-dihydropyridine-3-carbothioate (3k)
2,4-Diphenyl-4,6,7,8-tetrahydroquinolin-5(1H)-one (4)