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The Antioxidant Activity of Dihydropyridine Derivatives

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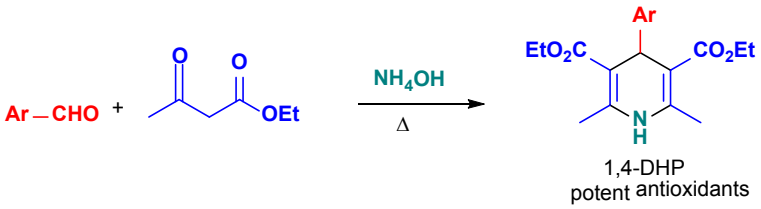
Abstract

1,4-Dihydropyridines **6a-i** were synthesised in the laboratory by the Hantzsch reaction and purified by flash column chromatography. These molecules bear a close resemblance to the biological reducing agent Nicotinamide Adenine Dinucleotide (NAD) and were evaluated for their antioxidant activities by two complementary assays, DPPH and β-carotene/linoleic acid. The relative antioxidant activity (RAA) results obtained by β-carotene/linoleic acid were more reliable and showed compounds with electron donating groups on the aromatic rings gave higher RAA values compared with L-ascorbic acid (AA). Compounds **6a**, **6c**, **6d** and **6g** possessed the most potent antioxidant activity of 71%, 80%, 78% and 45% respectively compared with AA with RAA 49%. It was found that the free radical scavenging ability of compounds **6b** and **6f** showed a small concentration dependence profile in DPPH assay but both had low antioxidant activity in both DPPH and b-carotene/linoleic assays.

Graphical Abstract

The antioxidant activities of 1,4-dihydropyridines synthesised by the Hantzsch reaction



Keywords: Antioxidant activity; 1,4-Dihydropyridines; Hantzsch synthesis; NADH

Introduction

Aromatic heterocyclic molecules comprise the largest class of therapeutic compounds in clinical use and continue to feature as promising candidates in future drug discovery programmes. In this context, 1,4-dihydropyridines (1,4-DHP) constitute a large group of structurally diverse group of drugs that possess a wide variety of biological activities such as calcium-channel modulating, anti-hypertensive, antioxidant, antimicrobial, vasodilator, bronchodilator, anti-atherosclerotic, anti-aggregation, anti-ischemic, anti-diabetic, and antitumor agents [1,2] agents. For example, nifedipine **1**, nitrendipine **2** and amlodipine **3** (Figure1) are 1,4-DHP clinically important drugs used in pharmacology for the treatment of cardiovascular diseases, including hypertension [3].

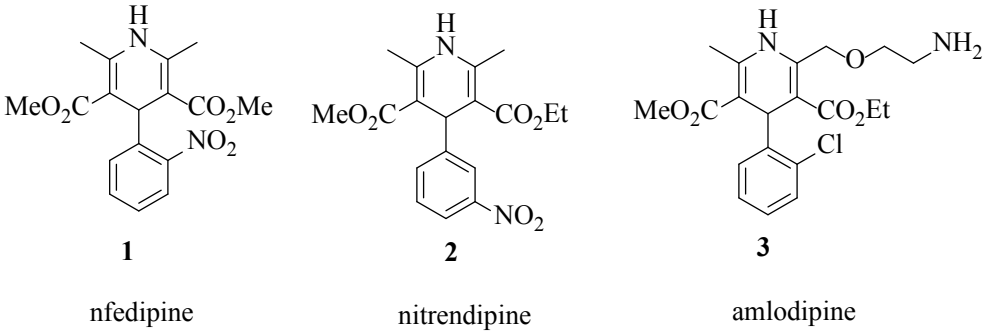


Figure 1: Structures of three commonly used drugs as calcium channel blockers.

As a result of the widely recognised well established pharmacological activities of 1,4-DHPs research interest in this area has continued to grow and generate new molecules. Calcium antagonist DHPs have been evaluated for their antioxidant activity via a competitive kinetic procedure. It was found that calcium antagonist DHPs such as nifedipine **1** and nitrendipine **2**, possessed antioxidant activity related to their electron density on the DPH ring. The process of anti-oxidation took place via a primary one-electron accompanied by a fast proton release, resulting in the creation of a neutral radical undergoing an easier one-electron step. This results in the generation of a final product being a protonated form of the parent pyridine. Therefore, as the radical is more prone to be oxidised than reduced, this prevents the propagation of the oxidative chain reaction. In another study the synthesis of a new group of 1,4-DHPs has been reported for their antioxidant properties [2,4,5]. One interesting feature of 1,4-DHPs is their close resemblance to the biological reducing agent Nicotinamide Adenine Dinucleotide (NADH) and their interaction with cellular enzymes. Based on this, two new synthetic series of 1,4-DHP derivatives containing substituted pyrazole moiety have been reported for antioxidant activity. These compounds were found to possess both antioxidant and antimicrobial properties [4,6] and displayed good safety profiles when administered in *in vivo* experiments in large doses (2000mg).

Reactive oxygen species (ROS) are formed during normal cell aerobic respiration [7] and are the main cause of cell damage involved in chronic diseases like diabetes cancer, cardiovascular and others [8]. Antioxidants play an important role in neutralising (ROS) and protecting the cells from oxidative damage. In an on-going work on heterocyclic compounds, in our laboratories, we have synthesised some 1,4-DHPs and have developed assays to assess their antioxidant activities. In this endeavour, here, we report the antioxidant activities of nine 1,4-DHPs (**6a-i**) with considerable structural diversity (Figure 2).

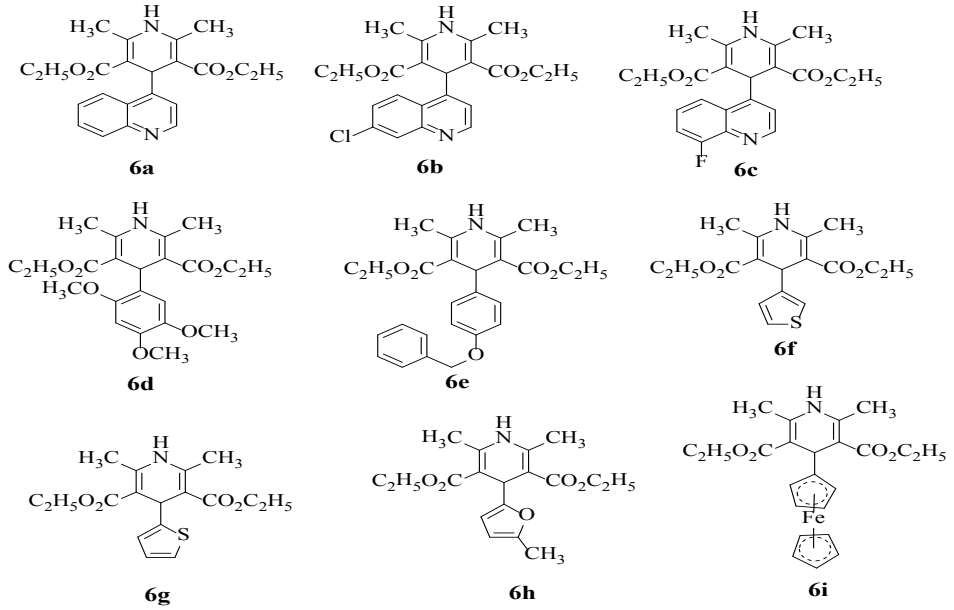


Figure 2: Structures of dihydropyridines synthesised by the Hantzsch synthesis.

Experimental section

Chemistry: materials and method

Melting points were recorded on Stuart SMP3 digital apparatus ; IR spectra were recorded on Perkin-Elmer Spectrum 100 FTIR spectrophotometer with a universal ATR sampling accessory; ^{1}H NMR spectra were recorded on a Bruker AC 250 MHz and ^{13}C NMR spectra were recorded on a Bruker Avance III 400 MHz spectrometers. Mass spectra (MS) were obtained on VG 770E spectrometer operated in EI mode at 70 eV. TLC analyses were done using Merck aluminium coated silica gel sheets and flash chromatography was performed using BDH flash silica gel and the eluents are indicated in parenthesis for each compound.

General method for synthesis of dihydropyridine (6a-i) by the Hantzsch reaction.

A typical procedure is illustrated by the synthesis of diethyl 2,6-dimethyl-4-(thiophen-3-yl)-1,4-dihydropyridine-3,5-dicarboxalate (6f). To a round bottom flask equipped with a magnetic stirrer thiophene-3-carboxaldehyde (2.24g,0.02moles) was added ethyl acetoacetate (5.20g, 0.04moles), isopropanol (3ml) and conc. ammonia (0.5ml). The mixture was refluxed in an oil bath at 120°C for 3h and then rotary evaporated at 60°C to remove the solvent to give the crude product as a solid (4.70g) which was purified by silica gel flash chromatography [1:2, ethyl acetate: heptane] to give pure product (6f) (4.10g, 61.2%) as a brown coloured solid; R_f = 0.34 (1:2, ethyl acetate: heptane); m.p.168.8-169.5°C; FTIR (solid) n (cm^{-1}) 3342.80 (NH), 1694.64 (ester >C=O), 1647.84 (alkene, C=C). ^{1}H NMR: (δ, ppm, $CDCl_3$) 1.20 (6H, triplet, J=7.12 Hz, 2 x -OCH_2-$\underline{CH_3}$), 2.30 (6H, s, 2 x -CH_3), 4.12 (4H, q, J= 7.12 Hz, 2 x -O-CH_2-), 5.17 (1H, s, >CH-), 5.80 (1H, singlet, NH), 6.93 (1H, fine d, J = 1.3 Hz, H-2), 7.00 (1H, d, J = 3.3 Hz, H-4), 7.13 (1H, dd, J= 1.3 Hz and 3.3 Hz, H-5);^{13}C NMR (δ, ppm, $CDCl_3$) 14.34 -H_2C-$\underline{CH_3}$), 18.40 and 19.33 (=C-CH_3), 34.61(>C-H-),59.61 and 59.81 (-O-$\underline{CH_2}$-), 102.86 and 103.28 (=C-CO_2Et), 120.29 (C-5), 124.60 (C-4), 127.60 (C-2), 144.68 (C-3), 147.94 (>$\underline{C}$-NH-), 167.76 (>C=O); High resolution EIMS (M^+) found (calculated): 335.1370 (335.1191).

Diethyl 2,6-dimethyl-4-(quinolin-4-yl)-1,4-dihydropyridine-3,5-dicarboxylate (6a) was obtained as grey solid (59% yield); m.p.193-201°C; R_f = 0.09 (1:2, ethyl acetate: heptane); FTIR (solid) n (cm^{-1}) 3266.05 (NH), 1698.05 (ester >C=O); ^{1}H NMR: (δ, ppm, $CDCl_3$) 0.95 (6H, triplet, J=7.12 Hz, 2 x -OCH_2-CH_3), 2.40 (6H, s, 2 x -CH_3), 3.95 (4H, q, J= 7.12 Hz, 2 x -O-CH_2-), 5.85 (1H, s, >CH-), 6.04 (1H, s, >NH), 7.43 (1H, d, 4.60 Hz, H-3), 7.57 (1H, t, J = 7.80 Hz, H-5), 7.67 (1H, t, J = 7.80 Hz, H-6), 8.02 (1H, d, 7.80 Hz, H-5), 8.60 (1H, d, J = 7.80 Hz, H-8), 8.80 (1H, d, J = 4.60 Hz, H-2); ^{13}CNMR ((δ, ppm,$CDCl_3$): 13.97(CH_3), 21.57(CH_3), 41.03(>CH-), 61.22 (OCH_2), 118.55(C-3), 122.68 (C-5), 125.11(C-6), 129.90(C-10),129.97(C-7), 136.32(C-8), 146.19(C-4), 148.20(C-9), 150.10(C-2), 1167.60 (>C=O); High resolution EIMS (M^+) found (calculated): 380.1670 (380.1736).

Diethyl 4-(7-chloroquinolin-4-yl)-2,6-dimethyl--1,4-dihydropyridine-3,5-dicarboxylate (6b) was obtained as a white solid (72% yield); 198-2050C; R_f = 0.06 (1:2, ethyl acetate: heptane); FTIR (solid) n (cm^{-1}) 3265.51 (NH), 1697.77 (ester >C=O); ^{1}H NMR: (δ, ppm, $CDCl_3$) 1.00 (6H, triplet, J=7.12 Hz, 2 x -OCH_2-CH_3), 2.40 (6H, s, 2 x -CH_3), 3.92 (4H, q, J= 7.12 Hz, 2 x -O-CH_2-), 5.80 (1H, s, >CH-), 5.90 (1H, s, >NH), 7.40 (1H, d, 4.60 Hz, H-3), 7.56 (1H, t, J = 7.80 Hz, H-5), 7.57 (1H, t, J = 7.80 Hz, H-6), 8.05 (1H, d, 7.80 Hz, H-5), 8.60 (1H, d, J = 7.80 Hz, H-8), 8.80 (1H, d, J = 4.60 Hz, H-2); ^{13}CNMR (δ, ppm, $CDCl_3$): 13.97(CH_3), 21.57(CH_3), 41.03(>CH-), 61.22(OCH_2), 119.38(C-3), 124.55(C-5), 125.19(C-10), 127.69(C-6), 129.00(C-8), 135.08(C-7), 146.53(C-4), 148.84(C-9), 151.27(C-2), 167.10 (>C=O); High resolution EIMS (M^+) found (calculated): 414.1276 (380.1346).

Diethyl 4-(8-fluoroquinolin-4-yl)-2,6-dimethyl--1,4-dihydropyridine-3,5-dicarboxylate (6c) was obtained as a white solid (82% yield); 198-2050C; R_f = 0.10 (1:2, ethyl acetate: heptane); FTIR (solid) n (cm^{-1}) 3262.41 (NH), 1695.87 (ester >C=O); ^{1}H NMR: (δ, ppm, $CDCl_3$) 1.00 (6H, triplet, J=7.12 Hz, 2 x -OCH_2-CH_3), 2.40 (6H, s, 2 x -CH_3), 3.90 (4H, q, J= 7.12 Hz, 2 x -O-CH_2-), 5.75 (1H, s, >NH), 5.82 (1H, s, >CH-), 7.35 (1H, d, 7.90 Hz, H-7), 7.40 (1H, d, J = 4.50 Hz, H-3), 7.57 (1H, t, J = 7.90 Hz, H-6), 8.40 (1H, d, J = 7.90 Hz, H-5), 8.80 (1H, d, J = 4.50 Hz, H-2); ^{13}CNMR (δ, ppm,$CDCl_3$): 13.97(CH_3), 21.57(CH_3), 41.03(>CH-), 61.22(OCH_2), 113.25(C-7), 118.83(C-5), 120.14(C-3), 126.48(C-6), 128.44(C-8), 138.85(C-7), 146.38(C-4), 150.34(C-9), 158.53(C-2), 167.10 (>C=O); High resolution EIMS (M^+) found (calculated): 398.1856 (398.1642).

Diethyl 2,6-dimethyl-4-(2,4,5-trimethoxyphenyl)-1,4-dihydropyridine-3,5-dicarboxylate (6d) as a creamy coloured solid (85.9% yield); m.p.178-180°C; R_f = 0.12 (1:2, ethyl acetate: heptane); FTIR (solid) n (cm^{-1}) 3347.13 (NH), 1690.02 (ester >C=O), 1202.22 (C-O-C); ^{1}H NMR: (δ, ppm, $CDCl_3$) 1.30 (6H, triplet, J=7.12 Hz, 2 x -OCH_2-CH_3), 2.37 (6H, s, 2 x -CH_3), 3.85 (6H, s, 2 x OCH_3), 3.92 (3H, s, OCH_3), 4.15 (4H, q, J= 7.12 Hz, 2 x -O-CH_2-), 5.20 (1H, s, >CH-), 5.92 (1H, s, >NH), 6.54 (1H, s, H-3), 6.88 (1H, s, H-6); ^{13}CNMR (δ, ppm,$CDCl_3$): 14.34 (CH_3),19.29 (CH_3), 36.16 (>CH-), 55.95 (-OCH_3), 56.53 (OCH_3) 59.43 (OCH_2), 98.05(C-3), 102.58 (CO-C<), 115.36(C-6), 127.52 (C-1), 142.29(C-5), 143.78(C-4), 148.06(C-2), 152.31(NH-C<), 168.71 (>C=O); High resolution EIMS (M^+) found (calculated): 419.2085 (419.1944).

Diethyl 4-(4-(benzyloxy)phenyl)-1,4-dihydro-2,6-dimethyl pyridine-3,5-dicarboxylate (6f) white solid (53.6% yield); m.p.170°C; R_f = 0.31 (1:2, ethyl acetate: heptane); FTIR (solid) n (cm^{-1}) 3356.57 (NH), 1692.10 (ester >C=O), 1197.57 (C-O-C); ^{1}H NMR: (δ, ppm, $CDCl_3$) 1.35 (6H, triplet, J=7.12 Hz, 2 x -OCH_2-CH_3), 2.44 (6H, s, 2 x -CH_3), 4.23 (4H, q, J= 7.12 Hz, 2 x -O-CH_2-), 5.11 (1H, s, >CH-), 5.13 (2H, s, OCH_2Ph), 6.00 (1H, s, >NH), 6.94

(2H, d, AB system J = 7.8 Hz, H-3 and H-5), 7.14 (2H, d, AB system J = 7.8 Hz, H-2 and H-6) 7.27 (3H, m, Ph) 7.34 (2H, m, Ph); ^{13}CNMR (δ, ppm,$CDCl_3$): 14.31(CH_3),19.51(CH_3), 38.79 (>CH-), 59.73 (2 x -OCH_2), 69.99 (OCH_2Ph), 104.27(C-3), 102.58(CO-C<), 114.14(C-3), 127.55, 127.87, 128.54, 129.00, 137.30, 140.67, 143.77, 157.22 (Ar =C-O-), 167.79 (>C=O); High resolution EIMS (M^+) found (calculated): 435.2070 (435.2046).

Diethyl 2,6-dimethyl-4-(thiophen-2-yl)-1,4-dihydropyridine-3,5-dicarboxalate (6g) brown coloured solid (89.2% yield); m.p.162.4-162.6°C; R_f = 0.34 (1:2, ethyl acetate: heptane); FTIR (solid) n (cm^{-1}) 3343.40 (NH), 1692.27 (ester >C=O), 1649.13 (alkene, C=C). ^{1}H NMR: (δ, ppm, $CDCl_3$) δ1.27 (6H, triplet, J=7.12 Hz, 2 x -OCH_2-CH_3), 2.33 (6H, s, 2 x -CH_3), 4.17 (4H, q, J= 7.12 Hz, 2 x -O-CH_2-), 5.34 (1H, s, >CH-), 6.03 (1H, singlet, NH), 6.77 (1H, d, J = 3.3 Hz, H-2), 6.83 (1H, dd, J = 3.3 Hz, H-3), 7.03 (1H, d, J = 3.3 Hz, H-4); ^{13}C NMR (δ, ppm, $CDCl_3$) 14.33 -H_2C-CH_3), 18.42 and 19.38 (=C-CH_3), 34.41(>C-H-),58.40 and 59.92 (-O-CH_2-), 103.43 (=C-CO_2Et), 123.10, 123.14, 126.34 (C-2), 144.74, 151.63 (>C-NH-), 167.46 (>C=O); High resolution EIMS (M^+) found (calculated): 335.1407 (335.1191).

Diethyl 2,6-dimethyl-4-(5-methylfuran-2-yl)-1-4-dihydropyridine-3,5-dicarboxylate (6h) dark brown solid (55.8% yield); m.p.145-147°C; R_f = 0.35 (1:2, ethyl acetate: heptane); FTIR (solid) n (cm^{-1}) 3342.35 (NH), 1697.09 (ester >C=O), 1204.99.13 (-C-O-C-). ^{1}H NMR: (δ, ppm, $CDCl_3$) 1.32 (6H, triplet, J=7.12 Hz, 2 x -OCH_2-CH_3), 2.24 (3H, s, Ar-CH_3), 2.37 (6H, s, 2 x -CH_3), 4.23 (4H, q, J = 7.12 Hz, 2 x -O-CH_2-), 5.19 (1H, s, >CH-), 5.83 (2H, s, Ar-H), 6.02 (1H, s, > NH); ^{13}C NMR (δ, ppm, $CDCl_3$) 13.69 (CH_3-Ar), 14.34 (-H_2C-CH_3), 19.36 (=C-CH_3), 33.35(>C-H-),58.37 and 59.73 (-O-CH_2-), 100.77 (=C-CO_2Et), 105.01, 105.85, 145.07, 150.22, 157.05 (>C-NH-), 167.71 (>C=O); High resolution EIMS (M^+) found (calculated): 333.1732 (333.1576).

Diethyl 2,6-dimethyl-4-(ferrocenyl)-1-4-dihydropyridine-3,5-dicarboxylate (6i) golden yellow solid, (57% yield); m.p. 226-228°C; R_f = 0.38(1:2, ethyl acetate: heptane); FTIR (solid) n (cm^{-1}) 3339.7 (NH), 1695.8 (ester >C=O), 1204.99.13 (-C-O-C-). ^{1}H NMR: (δ, ppm, $CDCl_3$) 1.36 (6H, triplet, J=7.12 Hz, 2 x -OCH_2-CH_3), 2.37 (6H, s, 2 x -CH_3), 3.96 (4H, d, J= 5.1 Hz, ferrocene H), 4.06 (5H, s, ferrocene H), 4.27 (4H, q, J=7.12, 2 x -O-CH_2-), 4.85 (1H, s, >CH), 5.67 (1H, broad s, >NH); ^{13}C NMR (δ, ppm, $CDCl_3$) 14.53 (-H_2C-CH_3), 19.65 (=C-CH_3), 32.07(>C-H-), 59.90 (-O-CH_2-), 69.95 (ferrocene), 76.71 (ferrocene), 103.79 (=C-CO_2Et), 143.77 (>C-NH-), 167.95 (>C=O); High resolution EIMS (M^+) found (calculated): 437.1410 (437.1290).

DPPH assay

This assay spectrophotometrically measures the colour decay of the stable free radical diphenylpicrylhydrazyl (DPPH) by interaction with an antioxidant [9]. Fifty mL of various concentrations of methanolic solution of the sample was added to 5 mL of a 101 µmol methanolic solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was read against a blank at λ517 nm. Inhibition of free radical DPPH in percent (I%) was calculated in following way:

$$I\,\% = (A_{blank} - A_{sample} / A_{blank}) \times 100$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. Concentration providing 50% inhibition (IC_{50}) was calculated from the graph by plotting inhibition percentage against sample concentration. Assays were carried out in triplicate. Synthetic antioxidant Butylated Hydroxy Toluene (BHT) was used as positive control.

ß Carotene-linoleic acid assay

In this assay, antioxidant capacity of the compound is determined by measuring the conjugated dienes produced from linoleic acid oxidation. A stock solution of b-carotene-linoleic acid mixture was prepared as following: 0.5 mg b-carotene was dissolved in 1 mL of chloroform (HPLC grade), 25 mL linoleic acid and 200 mg Tween 40 was added. The chloroform was completely evaporated using a vacuum evaporator. Distilled water (100 mL) saturated with oxygen (30 min, 100 mL/min.) was added with vigorous shaking. 2.5 mL of this mixture was added to three test tubes and ethanolic solution (350 mL) of the test compound (concentration 2 mg/mL) was added and the emulsion thus produced was incubated for up for 24 hours at room temperature. The same procedure was repeated with positive control BHT and a blank. After completion of the incubation period absorbance of the mixture was taken at λ 490 nm. Antioxidant capacities of the synthetic curcuminoids were compared with BHT and blank run under identical conditions.

Results and Discussion

Limited methods exist for the synthesis of unsymmetric 1,4-DHPs [2]. However, for the synthesis of symmetrical 1,4-DHPs the Hantzsch synthesis is the method of choice and we employed this method to produce our compounds **6a-i.** The aldehydes **5 a-i** were heated with the β-dicarbonyl compound ethyl acetoacetate **4** and ammonia (Figure 3). The 1,4-DHPs **6a-i** were isolated, purified by flash column chromatography and characterised spectroscopically by FTIR, ^{1}H NMR, ^{13}C NMR and MS. Characteristic features of the 1,4-DHPs were the absorbance of the >NH functional group in **6a-i** as a single peak in the FTIR spectra at around 3300 cm^{-1} and its resonance as a singlet at about 6ppm in the ^{1}HNMR spectra. The other characteristic feature in the ^{1}HNMR spectra was the resonance of the tertiary SP^3 proton at position-4 of 1,4-DHP as a singlet at around 4.9 ppm. The molecules **6a-i** all gave the correct molecular ions in the mass spectra.

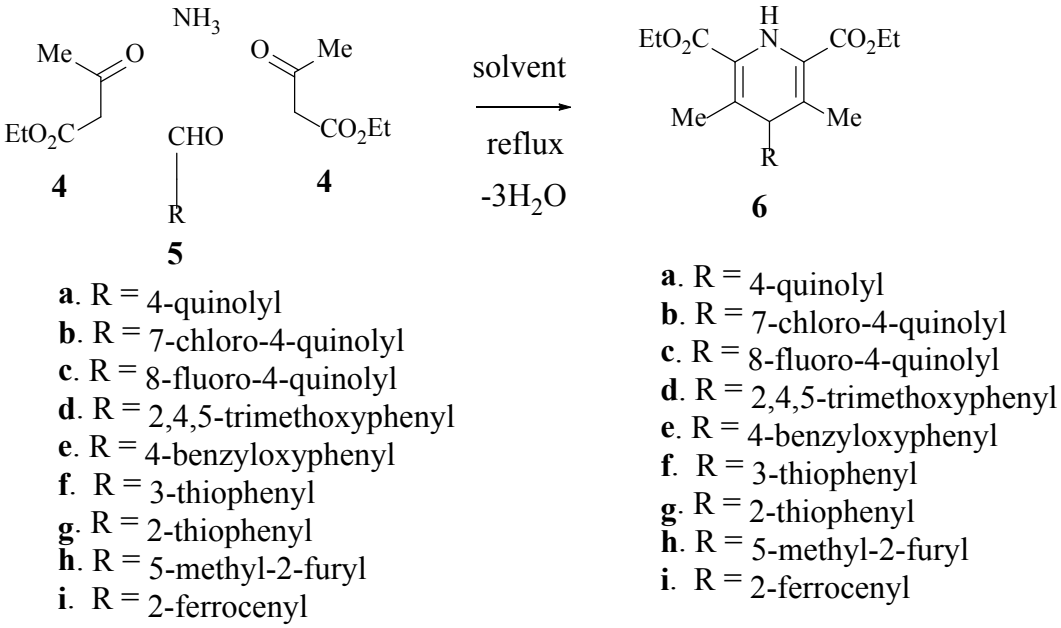


Figure 3: The Hantzsch synthesis for making 1,4-dihydropyridine derivatives **6a-i.**

Two complementary assays were employed for screening the antioxidative properties of the synthetic compounds **6a-i**. One of the assays measured the free radical scavenging activity-using 2,2-Diphenylhydroxyl Stable Free Radical (DPPH) and a second assay involved the inhibition of the lipid oxidation to determine antioxidant capacity of the samples. The inhibition of linoleic acid oxidation was determined by employing a modified b-carotene/linoleic acid assay. The principle of the β-carotene bleaching assay for evaluating antioxidant activity is based on the discoloration of yellowish colo*u*r of a β-carotene solution due to the breaking of π-conjugation by addition reaction of lipid or lipid peroxyl radical (L· or LOO·) to a C=C double bond of β-carotene. The radical species is generated from the autoxidation of linoleic acid by heating under air atmosphere. In the presence of antioxidants, oxidation of b-carotene is scavenged, preventing bleaching the colour of b-carotene.

The antioxidant activities of the 1,4-DHPs **6a-i** using the two assays, DPPH and b-carotene/linoleic acid are summarised in Table 1 and Figure 4. Both compounds **6b** and **6f** showed a small concentration dependence profile in DPPH assay but had low antioxidant activity in both DPPH and b-carotene/linoleic assays. The remaining 1,4-DHPs did not demonstrate any substantial antioxidant properties with DPPH assay and no concentration dependency as shown in Figures 4 And 5.

Absorbance Average (l- nm)									
Concentration (µg/ml)	**6a**	**6b**	**6c**	**6d**	**6e**	**6f**	**6g**	**6h**	**6i**
2	1.1	1.137	1.093	1.127	1.066	1.261	0.748	0.936	1.162
4	1.041	1.19	1.04	1.093	1.065	1.213	0.79	0.929	1.169
8	1.139	1.104	1.027	1.088	1.062	1.129	0.815	0.926	1.173
12	1.087	1.105	1.069	1.089	1.062	1.209	0.839	0.93	1.183
16	1.123	1.095	1.052	1.097	1.077	1.14	0.822	0.928	1.17
20	1.073	1.081	1.048	1.095	1.076	1.142	0.817	0.928	1.169
22	1.057	1.071	1.04	1.114	1.078	1.138	0.813	0.928	1.163
30	1.054	1.064	1.034	1.105	1.098	1.135	0.836	0.941	1.159
35	1.057	1.04	1.052	1.128	1.14	1.089	0.852	1.134	1.158
40	1.062	1.026	1.05	1.139	1.35	1.052	0.87	0.986	1.158
45	1.062	1.015	1.083	1.139	1.474	1.051	0.862	0.946	1.159
50	1.069	0.99	1.061	1.135	1.161	1.05	0.88	0.946	1.164

Table 1: The results from the DPPH assay test of the dihydropyridines **6a-i**. Results were compared against a blank at 1230 nm.

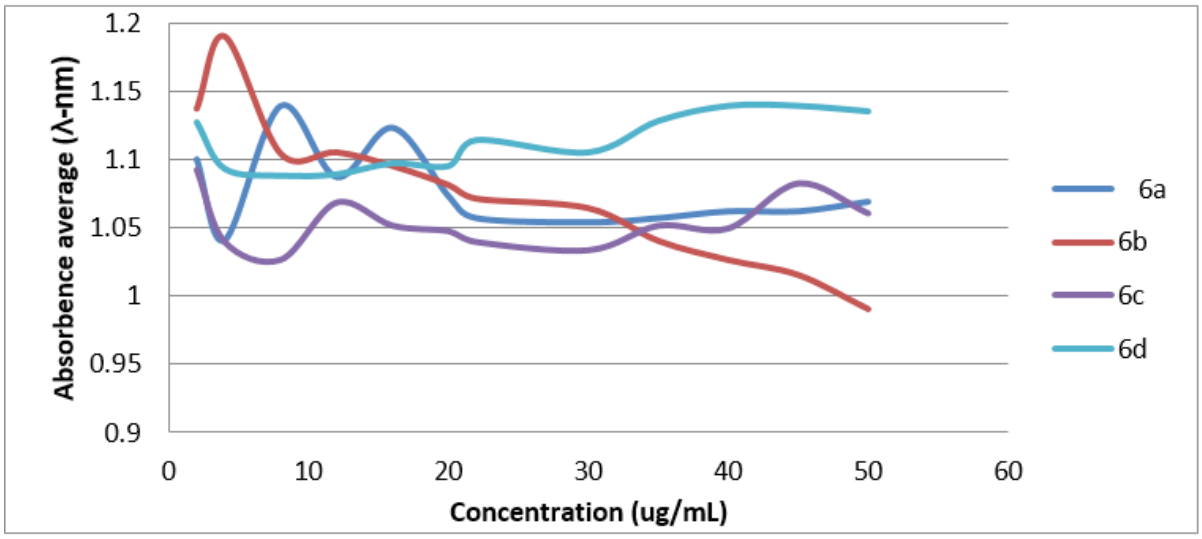


Figure 4: Antioxidative results by DPPH assay test for 1,4-DHP **6a-d.**

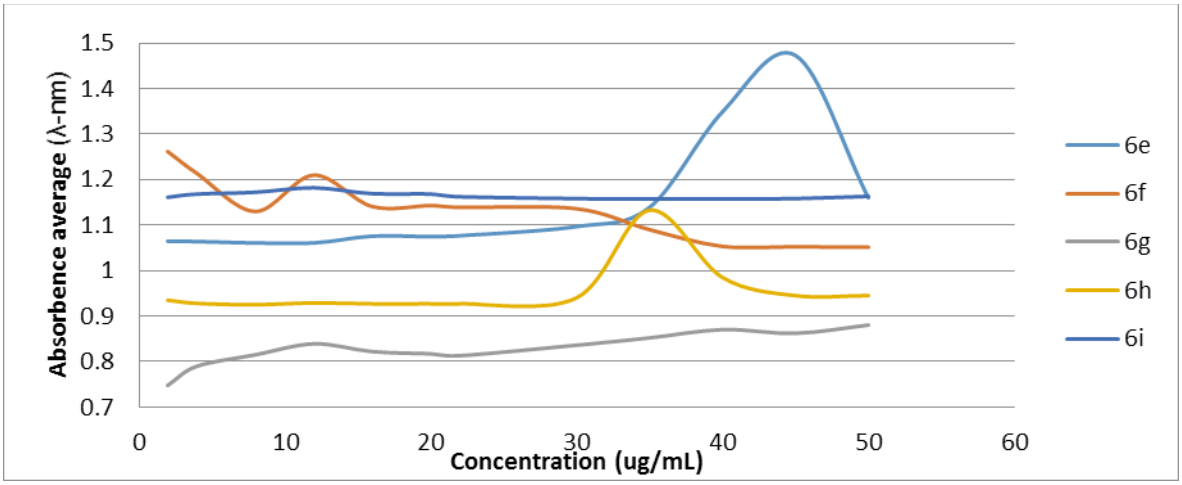


Figure 5: Antioxidative results DPPH assay test for 1,4-DHP **6e-i.**

However, in b-carotene/linoleic assay compounds **6a**, **6d** and **6e** showed remarkably good Relative Antioxidant Activity (RAA) of 71%, 80% and 78% respectively (Figure 6). Compound **6h** showed 45% RAA comparable with that for ascorbic acid of 49% RAA in b-carotene/linoleic assay. The three 1,4-DHPs with highest RAA values are **6a**, **6c** and **6d**. All these compounds have electron donating groups on the aromatic ring component. However, whilst **6d** with 78% RAA has strongly donating three methoxy groups on the aromatic ring the other two **6a** with 71% RAA and **6c** with 80% RAA have a chlorine and fluorine atoms on quinoline rings respectively. Halogen atoms on the aromatic ring are classed as weakly electron donating groups but the pyridine component of the quinoline ring is strongly electron withdrawing. On the other hand, compound **6g** with 45% RAA has a 2-thiophenyl ring attached which although aromatic is regarded as weakly electron donating as a result of the sulphur atom in the ring. That said the isomeric compound **6f** being a 3-thiophenyl derivative actually gives a much lower 20% RAA compared with the previously mentioned 2-thiophenyl derivative **6g** with 45% RAA. More structural activity research is needed to evaluate the effect of fluoro and methoxy groups in other positions of the aromatic ring for RAA activity. Generally, it appears that electron releasing groups on aromatic rings facilitate the RAA properties of the compounds.

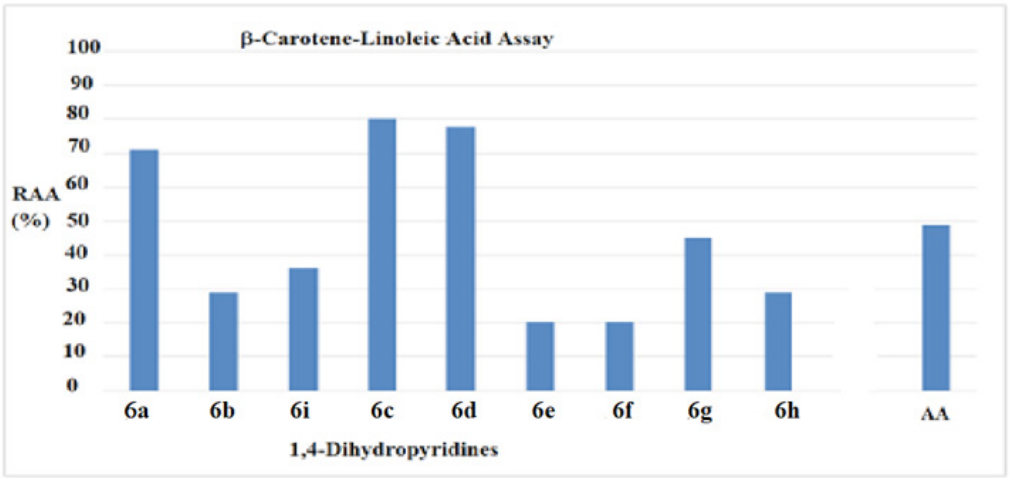


Figure 6: Antioxidant activity of 1,4-DHPs **6a-i** and ascorbic acid (AA) *(*control) with the b-carotene-linoleic acid antioxidant activity test compared to a blank after a 24 h period incubation.

The mechanism for the quenching of DPPH can occur by both electron and hydrogen atom transfer in different sequences is greatly influenced by solvent polarity and pH. Findings suggest that hydrogen-bonding solvents repress hydrogen atom transfer and favour electron transfer. This implies that compounds which are strongly active in hydrogen atom transfer appear to be slower reacting in protic solvents such as methanol and ethanol that are commonly used for the assay [10,11]. In the DPPH assay, an odd electron

displays a strong absorption band at a wavelength of 519 nm, which loses absorption once the odd electron is paired off by a hydrogen atom of electron-donating antioxidant (Figure 7). The limited viable data obtained for our 1,4-DHPs by the DPPH assay method may be associated with the comparable nature of the two nitrogen radicals involved in the reaction mechanism, the protonic solvents used and molecular steric factors (Figure 7).

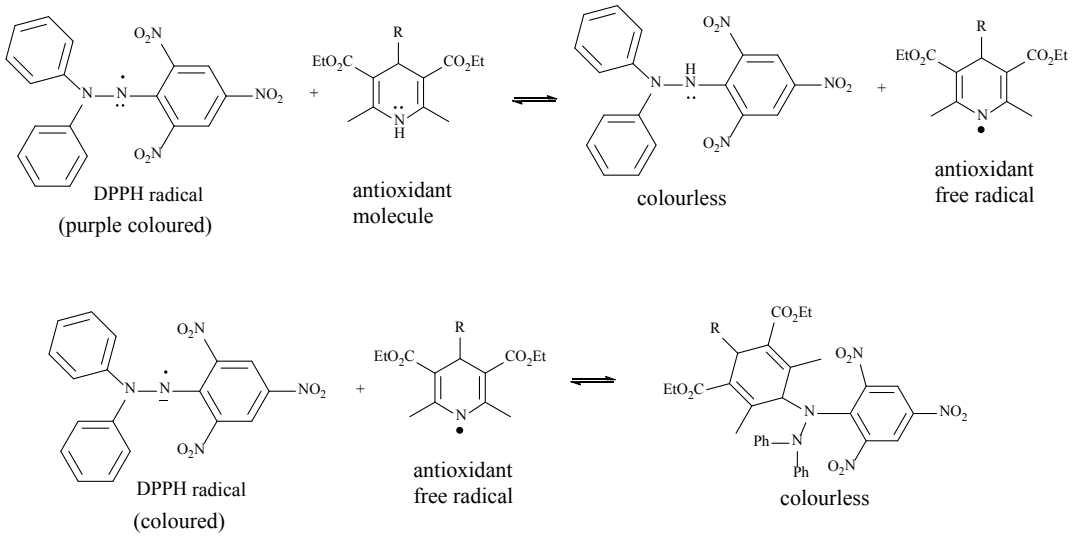


Figure 7: Reaction of DPPH radical with antioxidant molecules (1,4-DHPs).

Conclusion

Using the DPPH assay did not yield viable results except for two compounds **6b** and **6f** albeit in low RAA showing a small concentration dependant profile. Aromatic rings with methoxy, fluoro and chloro groups gave high % RAA values in the b-carotene/linoleic acid assay. Compounds **6a**, **6c**, **6d** and **6g** possessed the highest antioxidant activity of 71%, 80%, 78% and 45% respectively compared with AA of RAA 49%. Therefore, in general 1,4-DHPs with electron donating groups on the aromatic ring gave much higher RAA values compared with that for L-ascorbic acid as a reference. Further structural activity relationship work is needed to determine and substantiate the relative RAA values for isomeric compounds containing Cl, F and OCH_3 substituents on the aromatic rings.

Acknowledgments

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