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# Synthesis, and the antioxidant, neuroprotective and P-glycoprotein induction activity of 4-arylquinoline-2-carboxylates†‡

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An efficient formic acid catalyzed one-pot synthesis of 4-arylquinoline 2-carboxylates in water *via* three-component coupling of arylamines, glyoxylates and phenylacetylenes has been described. 4-Arylquinoline 2-carboxylates **1o** and **1q** displayed significant antioxidant activity as indicated by their Fe-reducing power in the ferric reducing ability of plasma (FRAP) assay. The compounds were found to react directly with hydrogen peroxide, which might be one of the mechanisms of their antioxidant effect. Compounds **1o** and **1q** effectively quenched $H_2O_2$ and amyloid-β-generated reactive oxygen species (ROS) and also displayed significant protection against $H_2O_2$-induced neurotoxicity in human neuroblastoma SH-SY5Y cells. Additionally, all compounds exhibited promising P-glycoprotein induction activity in human adenocarcinoma LS-180 cells, indicating their potential to enhance amyloid-β clearance from Alzheimer's brains. Furthermore, all compounds were relatively non-toxic to SH-SY5Y and LS-180 cells ($IC_{50}$ > 50 μM). The promising antioxidant, ROS quenching, neuroprotective and Pgp-induction activity of these compounds strongly indicate their potential as anti-Alzheimer's agents.



## Introduction

The brains of Alzheimer's patients are characterized by accumulation of amyloid-β plaques, neurofibrillary tangles of hyperphosphorylated tau protein and loss of neurons. The neurons loaded with amyloid-β plaques and neurofibrillary tangles are stressed by unusually high levels of reactive oxygen species (ROS).[1] Amyloid-β induced oxidative damage plays an important role in the pathogenesis of Alzheimer's disease. Numerous reports suggest that deposition of amyloid-β protein can lead to accumulation of hydrogen peroxide and thus result in oxidative damage to the neurons.[2] There is considerable evidence for involvement of ROS in amyloid-β induced oxidative damage.[3] Evidence also exists for the role of ROS in the enhanced accumulation of amyloid-β protein.[4] Thus, ROS does damage to the nerve cells by this feed forward loop mechanism. The use of antioxidants has been shown to reduce the accumulation of amyloid-β protein in the mouse Alzheimer's disease (AD) model and slowed functional decline in clinical studies in mild to moderate AD.[5] Therefore, discovery of new antioxidants for the effective treatment of Alzheimer's disease is one of the current areas in the development of Alzheimer's disease therapeutics. Furthermore, the amyloid hypothesis proposes that Alzheimer's disease is caused by an imbalance between amyloid-β production and clearance, resulting in increased amounts of amyloid-β in various forms such as monomers, oligomers, insoluble fibrils and plaques in the CNS.[6] The Aβ efflux is regulated through a P-glycoprotein (Pgp) efflux pump, and thus the Pgp deficiency at the blood–brain barrier has been reported to increase amyloid-β deposition.[7] *In vivo* studies indicated that Pgp inducers are able to increase amyloid-β clearance.[8] With the aim of discovering effective anti-Alzheimer's agents, herein we screened a series of 4-arylquinoline carboxylate esters for their antioxidant and Pgp-induction activity.

The quinoline ring system occurs widely among alkaloids[9] and is a key structural component of several pharmaceuticals, agrochemicals, dyestuffs, and materials. The quinoline scaffold has been reported to possess diverse range of pharmacological activities.[9,10] The well known antimalarial natural products quinine and quinidine alkaloids isolated

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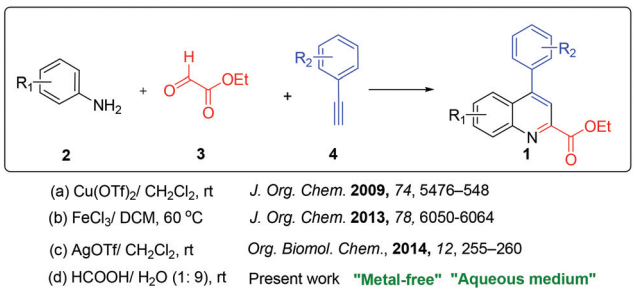



**Fig. 1** Methods for one-pot synthesis of 4-arylquinoline-2-carboxylates from arylamines, glyoxylates and phenylacetylenes.

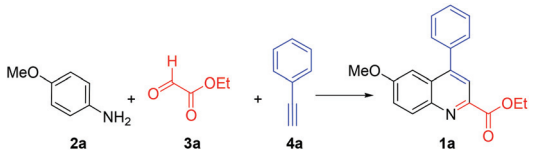
from Cinchona bark comprise the quinoline scaffold.[11] Because of the promising biological activities of the scaffold, methods to construct structurally diverse quinoline derivatives have been extensively studied.[12] Most of the well known classical methods such as the Skraup synthesis (involves sulphuric acid),[12c] the Doebner–Miller reaction ($SnCl_4$),[12d,e] and the Friedlander synthesis (Lewis acids)[12f,g,i] involve harsh reaction conditions. All of these are two-component reactions, and thus have limitations for diversity-oriented synthesis. Diversity-oriented one-pot protocols such as the Povarov reaction (3CC of aromatic amine, aldehyde and alkyne),[13] and 3CC of two molecules of *ortho*-haloacetophenones with urea or primary amines[14] have also been reported. Amongst these multicomponent protocols, the Povarov reaction[15] is one of the most elegant and direct one-pot approach for synthesis of functionalized quinolines. In the literature, the reagents used for the Povarov reaction include $HClO_4$-montmorillonite,[13] Cu(OTf)$_2$,[16] $I_2$/$MeNO_2$,[17] CuI/La(OTf)$_2$,[18] $AuCl_3$/CuBr,[19] $FeCl_3$,[20] and AgOTf.[21] Most of the protocols involve the use of a metal catalyst, organic solvents and also require heating or longer reaction times. Thus, development of a metal-free eco-friendly green protocol for synthesis of this class of compounds will be of great use. Herein, we report metal-free formic acid-catalyzed multicomponent synthesis of functionalized quinolines **1** through 3CC of arylamines **2**, glyoxylates **3**, and phenylacetylenes **4** in water (Fig. 1). 4-Arylquinolines displayed promising antioxidant, neuroprotective and Pgp-induction activity, indicating their potential as anti-Alzheimer's agents.

## Results and discussion

### Chemistry

Initially, experimental exploration of reaction parameters, including catalyst, solvent, temperature and reaction time, was conducted using the model multicomponent reaction (MCR) between aniline **2a**, ethyl glyoxylate **3a** and phenyl acetylene **4a** (Table 1). Catalyst-free MCR was first attempted by varying solvent, reaction temperature and time; however, no product was formed. To start with, first we investigated the catalytic effect of heterogeneous metal catalysts Cu–Mn spinel oxide,[22] and Fe–PILC;[23] however, the reaction does not proceed with

**Table 1** Solvent and catalyst optimization studies[a]




| Entry | Reaction medium (catalyst and solvent) | Temp. | Time | Yield[b] (%) |
|---|---|---|---|---|
| 1 | 50 mol% Cu–Mn B, $CH_2Cl_2$ | rt | 12 h | 0 |
| 2 | 50 mol% Cu–Mn B, water | rt | 12 h | 0 |
| 3 | 50 mol% Cu–Mn B, $CH_2Cl_2$ | 70 °C | 12 h | 0 |
| 4 | 20 mol% Fe–PILC, $CH_2Cl_2$ | rt | 12 h | 0 |
| 5 | 20 mol% Fe–PILC, water | rt | 12 h | 0 |
| 6 | 20 mol% Cu-turnings, $CH_2Cl_2$ | rt | 12 h | 0 |
| 7 | 20 mol% $Fe_2O_3$, $CH_2Cl_2$ | rt | 12 h | 0 |
| 8 | 10 mol% Pd(OAC)$_2$, $CH_2Cl_2$ | rt | 12 h | 0 |
| 9 | 50 mol%, $PdCl_2$, $CH_2Cl_2$ | rt | 12 h | 0 |
| 10 | 10 mol%, Pd (TFA)$_2$, $CH_2Cl_2$ | rt | 12 h | 0 |
| 11 | 10 mol% In(OTf)$_2$, $CH_2Cl_2$ | rt | 45 min | 75 |
| 12 | 10 mol% TFA, water | rt | 45 min | 50 |
| 13 | 10 mol% formic acid, water | rt | 45 min | 70 |
| 14 | 100 mol% formic acid, water | rt | 30 min | 95 |
| 15 | 50% formic acid in water | rt | 30 min | 92 |
| **16**[c] | **10% formic acid in water** | **rt** | **45 min** | **88** |

rt, room temperature. [a] Reagents and conditions: **2a** (1 mmol), **3a** (1 mmol), **4a** (1.2 mmol) and reaction medium (catalyst and solvent). [b] Isolated yields. [c] Optimized reaction conditions.

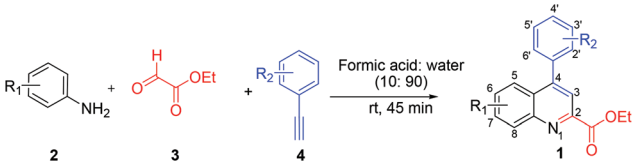
these catalysts (entries 1–5). Several other commercial catalysts such as Cu-turnings, $Fe_2O_3$, Pd(OAc)$_2$, $PdCl_2$, Pd(TFA)$_2$ were also unsuccessful (entries 6–10). The use of 10 mol% In(OTf)$_2$ produced product **1a** in good yield (entry 11). Trifluoroacetic acid and formic acid produced product **1a** in good yields (50–95%; entries 12–16). A brief examination of formic acid loading showed that 10% formic acid in water is an optimal condition to produce the desired product in good yield (entry 16), which was selected for further studies.

Next, the scope of the optimized protocol was investigated for a variety of arylamines and phenylacetylenes (Table 2). A variety of aromatic amines such as 4-methoxy-substituted, 3,4-methylene-dioxy, and 4-benzyloxy substituted anilines participated well in this reaction. The phenylacetylenes substituted with various electron-donating (*e.g.* methyl, *t*-butyl, OMe, acetylene, $OCF_3$) as well as electron-withdrawing (*e.g.* F, Cl, Br, $CF_3$) groups produced the corresponding 4-arylquinoline-2-carboxylates in excellent yields (Table 2).

A possible mechanism for formic acid-catalyzed synthesis of 4-arylquinoline 2-carboxylate **1a** from aniline **2a**, glyoxylate **3a** and phenylacetylene **4a** is depicted in Fig. 2. The reaction mechanism involves a cascade of reactions involving initial condensation of aniline **2a** and ethyl glyoxylate **3a** to form imine intermediate **I**. Next, there is a protonation of the nitrogen of the imine which facilitates the attack by phenylacetylene **4a**, leading to cyclization to produce dihydroquinoline **V**. The dihydroquinoline **V** on oxidation produces 4-arylquinoline 2-carboxylate **1a**.

Table 2 Scope of the reaction[a]



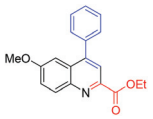
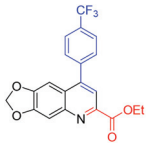
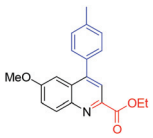
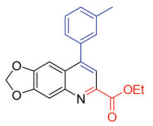
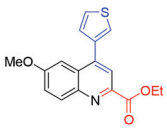
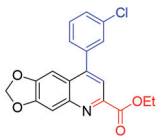
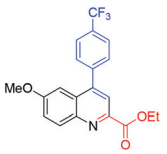
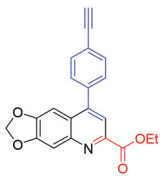
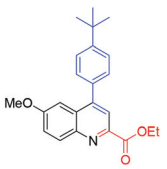
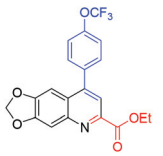
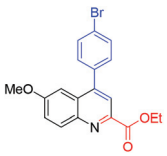
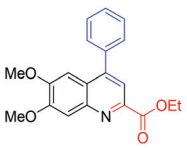
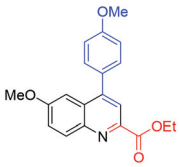
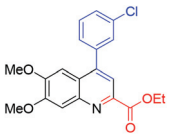
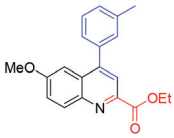
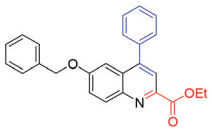
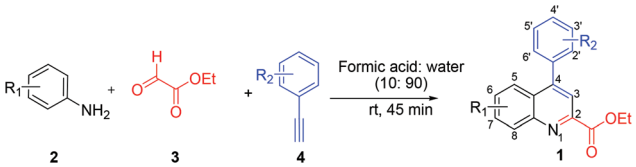
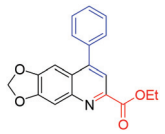
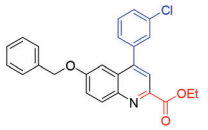
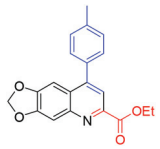
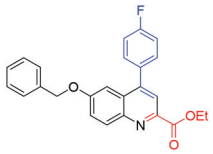

| Entry | Product | Yield[b] (%) | Entry | Product | Yield[b] (%) |
|---|---|---|---|---|---|
| **1a** |  | 88 | **1k** |  | 85 |
| **1b** |  | 88 | **1l** |  | 82 |
| **1c** |  | 82 | **1m** |  | 78 |
| **1d** |  | 92 | **1n** |  | 78 |
| **1e** |  | 92 | **1o** |  | 88 |
| **1f** |  | 85 | **1p** |  | 80 |
| **1g** |  | 80 | **1q** |  | 78 |
| **1h** |  | 82 | **1r** |  | 86 |

Table 2 *(Contd.)*




| Entry | Product | Yield[b] (%) | Entry | Product | Yield[b] (%) |
|---|---|---|---|---|---|
| **1i** |  | 92 | **1s** |  | 88 |
| **1j** |  | 92 | **1t** |  | 92 |

[a] Reagents and conditions: **2** (1.0 mmol), **3** (1.0 mmol), **4** (1.2 mmol) in 10% formic acid in water, stirred at rt for 45 min. [b] Isolated yields.

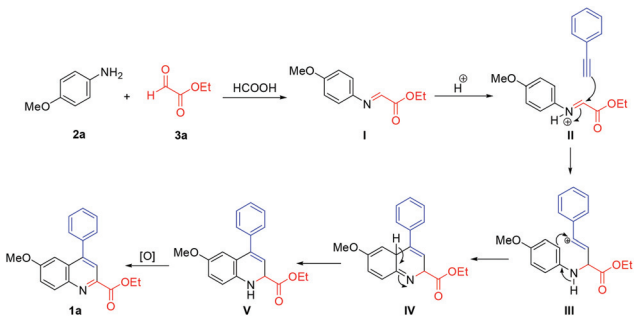



Fig. 2 Proposed mechanism.

## Antioxidant and neuroprotection activity

Quinoline class of compounds are known to possess antioxidant activity due to their metal chelation ability.[24] Therefore, herein all synthesized compounds were screened for antioxidant potential *via* checking their Fe-reducing power and free-radical scavenging activity. Several analogs showed significant antioxidant activity at 100 μM in the ferric reducing ability of plasma (FRAP) assay, where compounds **1o** and **1q** were found to be better than others. None of the compound displayed significant DPPH free radical scavenging activity. Further experiments showed that all analogs were relatively non-toxic in human neuroblastoma SH-SY5Y cells with an $IC_{50}$ value of >50 μM (Table 3).

With a view that compounds **1o** and **1q** may interact directly with hydrogen peroxide to reduce its damaging effect on cells, the direct reaction of hydrogen peroxide with these compounds was investigated. The rate of disappearance of hydrogen peroxide was calculated by measuring the reduction of optical density of hydrogen peroxide at 240 nm.[25] The reaction of hydrogen peroxide started immediately after the addition of compounds **1o** and **1q**. The OD was taken 10 seconds after the addition of compounds. Compounds **1o** and **1q** displayed instantaneous rate of reaction $3.0 \times 10^{-3}$ and $2.0 \times 10^{-3}$ moles $s^{-1}$, respectively. Thus, the ability of these compounds to directly interact with hydrogen peroxide should be one of the mechanisms of their observed antioxidant activity.

Next, the electrochemical behaviour of compound **1o** ($1.0 \times 10^{-3}$ M) was investigated by cyclic voltammetry. One anodic peak at potential 0.4 V *vs.* $Ag/Ag^+$ was observed, which may be due to the presence of a conjugated imine group of quinoline which possesses lone pair of electrons. The cyclic voltammetry measurement indicates that the peak at 0.4 V *vs.* $Ag/Ag^+$ is the oxidation peak of the imino nitrogen atom.

Further, the ROS-quenching activity of compounds **1o** and **1q** was investigated using the *in vitro* assay in human neuroblastoma SH-SY5Y cells. Cells were differentiated for seven days by exposure to 10 μM of retinoic acid. The ROS was generated by treatment of differentiated SH-SY5Y cells with 500 μM of hydrogen peroxide for 30 min. The DCFH-DA fluorescence for $H_2O_2$ treated cells was considered to be 100%. Compounds **1o** and **1q** at 100 μM effectively quenched the acute ROS as indicated by the decreased level of DCF fluorescence to 53% and 76% by these compounds, respectively (Fig. 3).

Next, the effect of compounds on amyloid-β generated ROS was investigated. The SH-SY5Y cells treated with aggregated amyloid-β 1–42 peptide (10 μM) displayed 15% rise in the ROS level in comparison with control samples (100%). The SH-SY5Y cells co-treated with compounds **1o** and **1q** showed significantly reduced level of ROS to 96% and 99% respectively (Fig. 4).

Table 3 Antioxidant activity and effect of compounds on cell viability of SH-SY5Y cells

| | Antioxidant activity | | |
|---|---|---|---|
| | FRAP assay[a,c] | DPPH assay[b,c] | |
| Entry | % Fe reducing power | % free-radical scavenging activity | % viability of SH-SY5Y cells at 50 μM[d] |
| Control | — | — | 100 |
| Ascorbic acid | 100 ± 0.0 | 100 ± 0.0 | nd |
| **1a** | 5.8 ± 0.9 | 5.0 ± 0.32 | 87.7 ± 7.3 |
| **1b** | 8.4 ± 1.2 | 1.1 ± 0.35 | 99.8 ± 4.7 |
| **1c** | 32.8 ± 2.5 | 2.2 ± 0.41 | 115.7 ± 11.7 |
| **1d** | 12.3 ± 1.4 | 4.3 ± 0.19 | 107.8 ± 7.9 |
| **1e** | 15.7 ± 0.8 | 7.6 ± 1.5 | 99.1 ± 2.2 |
| **1f** | 7.0 ± 1.5 | 7.8 ± 0.64 | 69.3 ± 1.1 |
| **1g** | 20.5 ± 1.9 | 5.4 ± 1.4 | 68.7 ± 2.4 |
| **1h** | 16.9 ± 0.7 | 6.8 ± 1.02 | 96.6 ± 3.2 |
| **1i** | 13.9 ± 1.6 | 5.0 ± 0.06 | 98.41 ± 4.3 |
| **1j** | 7.4 ± 1.1 | 7.2 ± 0.82 | 71.1 ± 2.5 |
| **1k** | 10.0 ± 1.7 | 6.4 ± 0.36 | 90.7 ± 1.9 |
| **1l** | 5.4 ± 1.1 | 5.7 ± 0.79 | 92.0 ± 2.2 |
| **1m** | 3.4 ± 0.17 | 7.2 ± 1.52 | 99.6 ± 10.8 |
| **1n** | 10.8 ± 1.6 | 6.1 ± 0.27 | 56.0 ± 10.4 |
| **1o** | **41.5 ± 3.2** | 5.3 ± 1.28 | 96.4 ± 9.4 |
| **1p** | 5.4 ± 0.9 | 6.4 ± 0.57 | 88.1 ± 6.6 |
| **1q** | **48.3 ± 0.1** | 8.5 ± 0.39 | 82.3 ± 11.4 |
| **1r** | 8.7 ± 0.5 | 5.8 ± 0.88 | 111.9 ± 9.2 |
| **1s** | 6.7 ± 0.06 | 5.1 ± 0.80 | 92.5 ± 6.4 |
| **1t** | 2.9 ± 0.3 | 7.9 ± 0.72 | 89.6 ± 10.3 |

nd, not determined. [a] Ferric reducing ability of ascorbic acid (100 μM) was considered to be 100%, whereas FRAP value for other compounds was calculated in comparison with ascorbic acid. [b] DPPH free radical scavenging activity of ascorbic acid was considered to be 100%. The free radical scavenging activity of test compounds was calculated in comparison with ascorbic acid. [c] Data in the FRAP and DPPH assays are mean ± SD of three independent experiments. [d] Data for the cell viability assay are mean ± SD of three similar experiments.

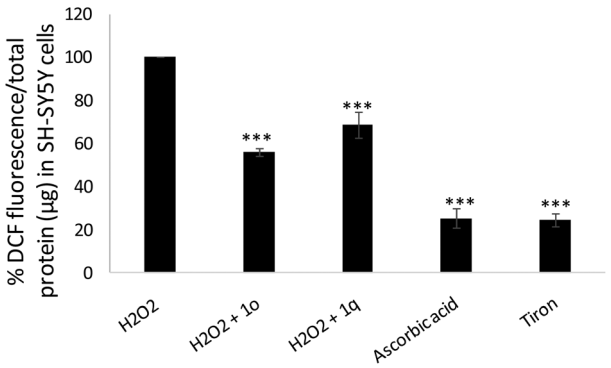



Fig. 3 ROS quenching by compounds **1o** and **1q** in SH-SY5Y cells. Cells were treated with 100 μM of **1o** and **1q**, 30 minutes before the treatment of $H_2O_2$ (500 μM). Data are mean of three independent experiments. Statistical comparisons were made using the Bonferroni test. The *p* value <0.05 was considered to be significant. *p* value *<0.05, **<0.01, ***<0.001.

Encouraged by these results, we hypothesized that these compounds may effectively provide neuroprotection to differentiated SH-SY5Y cells against oxidative stress. Interestingly,

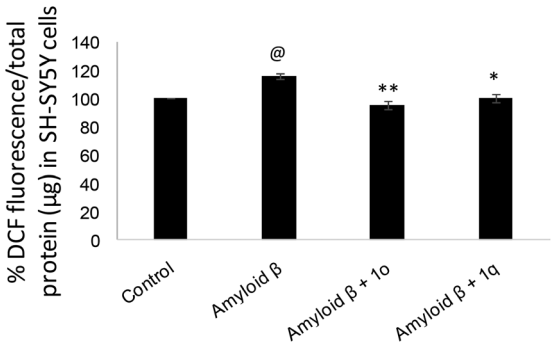



Fig. 4 Quenching of amyloid-β induced ROS by compounds **1o** and **1q** in SH-SY5Y cells. Cells were treated with **1o** and **1q** (at 50 μM), 30 min before the treatment of aggregated amyloid-β 1–42 peptide (10 μM). Data are mean of three independent experiments. Statistical comparisons were made using the Bonferroni test. The *p* value <0.05 was considered to be significant. *p* value *<0.05, **<0.01, ***<0.001. The symbol @ represents comparison between control and samples treated with amyloid-β, whereas the symbol * represents comparisons between samples treated with amyloid-β and those of treated with compounds **1o** and **1q** along with amyloid-β.

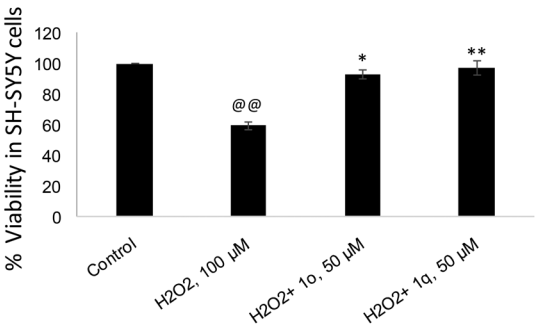



Fig. 5 Neuroprotective effect of compounds **1o** and **1q** against $H_2O_2$-induced toxicity in SH-SY5Y cells. Cells were treated with **1o** and **1q** at indicated concentrations 30 minutes prior to the treatment with $H_2O_2$ (100 μM) for 24 h. Data are mean of three independent experiments. Statistical comparisons were made using the Bonferroni test. The *p* value <0.05 was considered to be significant. *p* value *<0.05, **<0.01, ***<0.001. The symbol @ represents comparison between control and samples treated with $H_2O_2$, whereas the symbol * represents comparisons between samples treated with $H_2O_2$ and those of treated with **1o** and **1q** along with $H_2O_2$.

compounds **1o** and **1q** strongly reversed the $H_2O_2$-induced neurotoxicity. The viability of hydrogen peroxide treated cells (59%) was restored to 93% and 97% by pre-treatments with **1o** (50 μM) and **1q** (50 μM) respectively (Fig. 5). However, the treatment with Aβ 1–42 (10 μM) did not induce any significant damage to SH-SY5Y cells in 24 h (data not shown), therefore, the neuroprotective effect of compounds **1o** and **1q** against Aβ induced neurotoxicity could not be analysed in this study.

## Pgp-induction activity

The recent two independent clinical studies[6,26] observed that AD patients have decreased clearance of CNS amyloid-β compared to healthy volunteers. The Aβ-clearance occurs primarily

Table 4 Pgp-induction activity and effect of 4-arylquinoline-2-carboxylates on cell viability in LS-180 cells

| Entry | % Rh123 accumulation in LS-180 cells after 48 h[a,b] | % viability of LS-180 cells at 50 μM[b] |
|---|---|---|
| Control | 100 | 100 |
| Rifampicin | 70.6 ± 7.9** | nd |
| **1a** | 69.8 ± 3.2** | 106.1 ± 9.3 |
| **1b** | 78.3 ± 3.8** | 100 ± 6.5 |
| **1c** | 60.9 ± 8.4*** | 115.6 ± 6.2 |
| **1d** | 69.6 ± 9.1** | 114.8 ± 6.0 |
| **1e** | 76.7 ± 5.0** | 134.8 ± 1.1 |
| **1f** | 93.0 ± 6.9 | 98.1 ± 6.6 |
| **1g** | 72.0 ± 7.1** | 127.1 ± 5.2 |
| **1h** | 71.9 ± 9.9** | 135.5 ± 4.8 |
| **1i** | 81.5 ± 2.5* | 61.9 ± 2.3 |
| **1j** | 79.0 ± 3.8** | 65.5 ± 4.9 |
| **1k** | 71.0 ± 6.4** | 98.6 ± 0.9 |
| **1l** | 65.1 ± 11.4** | 99.2 ± 6.4 |
| **1m** | 61.0 ± 2.3*** | 111.9 ± 7.4 |
| **1n** | 70.9 ± 3.3** | 45.6 ± 5.0 |
| **1o** | 59.6 ± 2.1*** | 86.2 ± 4.2 |
| **1p** | 69.9 ± 7.9** | 80.9 ± 4.8 |
| **1q** | 60.2 ± 4.7*** | 64.2 ± 3.7 |
| **1r** | 57.8 ± 8.2*** | 95.6 ± 9.2 |
| **1s** | 71.4 ± 5.9** | 117.0 ± 12.2 |
| **1t** | 66.5 ± 6.9*** | 117.0 ± 6.4 |

nd, not determined. [a] Pgp induction activity of compounds was checked at 5 μM and was measured in terms of the % intracellular accumulation of rhodamine 123/total protein (μg) inside LS-180 cells. The decrease in % intracellular accumulation (compared to control) of Rh123 indicates induction of Pgp. Rifampicin (10 μM) was used as a reference Pgp inducer. The statistical comparisons were made between control *vs.* compounds. The $p$ value <0.5 was considered to be significant. $p$ value *<0.05, **<0.01, ***<0.001. [b] Values are shown as average of three experiments ± SD.

*via* a Pgp efflux pump, thus drugs with the ability to induce Pgp have a great potential to emerge as novel AD therapeutics. The compounds prepared herein are structurally similar to nifedipine, a 4-(phenyl)-1,4-dihydropyridine-3,5-dicarboxylate methyl ester, which is known to induce Pgp-expression.[27] Therefore, we decided to screen all the compounds for Pgp induction activity. The Pgp induction activity was determined in colorectal adenocarcinoma LS-180 cells, which are known to have constitutively high expression of Pgp. Cells treated with 5 μM of each compound for 48 h displayed significant induction of Pgp activity, as displayed by the increased efflux of rhodamine-123 (Table 4). The compounds **1o** and **1q** showed % intracellular rhodamine-123 level of 57% and 60% respectively in comparison with untreated control samples. Furthermore, these compounds were found to be non-toxic to LS-180 cells ($IC_{50}$ > 50 μM).

# Conclusion

In summary, we have developed a simple and efficient, economically viable formic acid-catalyzed one-pot multicomponent protocol for preparation of structurally diverse 4-arylquinoline 2-carboxylates. Compounds displayed significant antioxidant and Pgp-induction activity and relatively low toxicity. The antioxidant activity of these compounds appears to be due to their ability to directly interact with ROS. Furthermore, the significant Pgp-induction activity of 4-arylquinolines indicates their potential application in promoting efflux of toxins from the body and enhancing amyloid-β clearance from the AD brain. These findings clearly indicate their potential as a new lead for anti-Alzheimer's therapeutics.

# Experimental section

### General

All chemicals were obtained from Sigma-Aldrich Company and were used as received. $^1$H, $^{13}$C and DEPT NMR spectra were recorded on Bruker-Avance DPX FT-NMR 500 and 400 MHz instruments. Chemical data for protons are reported in parts per million (ppm) downfield from tetramethylsilane and are referenced to the residual proton in the NMR solvent ($CDCl_3$, 7.26 ppm). Carbon nuclear magnetic resonance spectra ($^{13}$C NMR) were recorded at 125 MHz or 100 MHz: chemical data for carbons are reported in parts per million (ppm, $\delta$ scale) downfield from tetramethylsilane and referenced to the carbon resonance of the solvent ($CDCl_3$, 77 ppm). ESI-MS and HRMS spectra were recorded on an Agilent 1100 LC-Q-TOF and HRMS-6540-UHD machines. IR spectra were recorded on a Perkin-Elmer IR spectrophotometer. Melting points were recorded on digital melting point apparatus.

### General procedure for preparation of 4-arylquinoline-2-carboxylates

A mixture of arylamine (1.0 mmol), ethyl glyoxylate (1.0 mmol), and phenylacetylene (1 mmol) in 10% formic acid–water was stirred at room temperature for 45 min. The reaction mixture was then extracted with ethyl acetate and combined organic layers were evaporated on a vacuum rotavapor. The crude product was purified by silica-gel column chromatography (mesh 100–200) to obtain desired quinoline-2-carboxylates **1a–t** in 78–92% yield.

**Ethyl 6-methoxy-4-phenylquinoline-2-carboxylate (1a).**[28] Yellow solid; m.p. 155–156 °C; $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ 8.29 (d, $J$ = 12.0 Hz, 1H), 8.10 (s, 1H), 7.56–7.50 (m, 5H), 7.45 (d, $J$ = 8.0 Hz, 1H), 7.22 (s, 1H), 4.58–4.53 (q, $J$ = 8.0, 12.0 Hz, 2H), 3.81 (s, 3H), 1.49 (t, $J$ = 4.0 Hz, 3H); $^{13}$C NMR ($CDCl_3$, 100 MHz): $\delta$ 165.6, 159.5, 148.0, 145.4, 144.3, 137.9, 132.7, 129.3, 129.2, 128.8, 128.7, 122.8, 121.8, 103.2, 62.1, 55.5, 14.4; IR ($CHCl_3$): $\nu_{max}$ 3400, 3055, 2925, 1729, 1715, 1620, 1492, 1413, 1366, 1224, 1107 cm$^{-1}$; ESI-MS: $m/z$ 308.00 $[M + H]^+$; HRMS: $m/z$ 308.1279 (ESI) calcd for $C_{19}H_{17}NO_3 + H^+$ (308.1281).

**Ethyl 6-methoxy-4-*p*-tolylquinoline-2-carboxylate (1b).** White solid; m.p. 141–142 °C; $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ 8.28 (d, $J$ = 12.0 Hz, 1H), 8.08 (s, 1H), 7.46–7.42 (m, 4H), 7.37 (d, $J$ = 8.0 Hz, 2H), 4.58–4.52 (q, $J$ = 8.0, 16.0 Hz, 2H), 3.82 (s, 3H), 2.48 (s, 3H), 1.48 (t, $J$ = 8.0 Hz, 3H); $^{13}$C NMR ($CDCl_3$, 100 MHz): $\delta$ 165.7, 159.5, 148.1, 145.3, 144.3, 138.6, 134.8, 132.7, 129.5, 129.3, 122.8, 121.8, 103.3, 62.1, 55.6, 21.4, 14.3;

IR ($CHCl_3$): $\nu_{max}$ 3400, 2919, 1716, 1620, 1585, 1497, 1472, 1253, 1105 $cm^{-1}$; ESI-MS: *m*/*z* 322.00 $[M + H]^+$; HRMS: *m*/*z* 322.1445 (ESI) calcd for $C_{20}H_{19}NO_3 + H^+$ (322.1438).

**Ethyl 6-methoxy-4-(thiophen-3-yl)quinoline-2-carboxylate (1c).** Brown solid; m.p. 153–154 °C; $^1H$ NMR ($CDCl_3$, 400 MHz): $\delta$ 8.26 (d, *J* = 8.0 Hz, 1H), 8.14 (s, 1H), 7.60 (s, 1H), 7.55–7.53 (m, 1H), 7.44 (d, *J* = 8.0 Hz, 1H), 7.40–7.37 (m, 2H), 4.58–4.53 (m, 2H), 3.86 (s, 3H), 1.49 (t, *J* = 4.0 Hz, 3H); $^{13}C$ NMR ($CDCl_3$, 100 MHz): $\delta$ 165.6, 159.6, 145.4, 144.4, 142.8, 138.4, 132.8, 129.2, 128.5, 126.7, 125.1, 122.8, 121.6, 103.2, 62.1, 55.6, 14.4; IR ($CHCl_3$): $\nu_{max}$ 3415, 2960, 1732, 1714, 1620, 1555, 1475, 1268, 1226 $cm^{-1}$; ESI-MS: *m*/*z* 314.00; $[M + H]^+$; HRMS: *m*/*z* 314.0844 (ESI) calcd for $C_{17}H_{15}NO_3S + H^+$ (314.0845).

**Ethyl 4-(4-(trifluoromethyl)phenyl)-6-methoxyquinoline-2-carboxylate (1d).** Brown solid; m.p. 161–162 °C; $^1H$ NMR ($CDCl_3$, 400 MHz): $\delta$ 8.31 (d, *J* = 8.0 Hz, 1H), 8.08 (s, 1H), 7.84 (d, *J* = 8.0 Hz, 2H), 7.69 (d, *J* = 8.0 Hz, 2H), 7.48 (d, *J* = 8.0 Hz, 1H), 7.10 (s, 1H), 4.59–4.54 (q, *J* = 8.0, 16.0 Hz, 2H), 3.83 (s, 3H), 1.49 (t, *J* = 4.0 Hz, 3H); $^{13}C$ NMR ($CDCl_3$, 100 MHz): $\delta$ 165.4, 159.8, 146.4, 145.4, 144.3, 141.6, 133.0, 129.8, 128.8, 125.8, (t, $^1J_{CF}$ = 3.77 Hz), 123.1, 121.8, 102.8, 62.3, 55.7, 14.4; $^{19}F$ NMR (376.50 MHz, $CDCl_3$): $\delta$ −62.81 (s, 3F); IR ($CHCl_3$): $\nu_{max}$ 3434, 2927, 2851, 1620, 1512, 1476, 1324, 1228, 1066 $cm^{-1}$; ESI-MS: *m*/*z* 376.00 $[M + H]^+$; HRMS: *m*/*z* 376.1162 (ESI) calcd for $C_{20}H_{16}F_3NO_3 + H^+$ (376.1155).

**Ethyl 4-(4-*tert*-butylphenyl)-6-methoxyquinoline-2-carboxylate (1e).**[28] White solid; m.p. 174–175 °C; $^1H$ NMR ($CDCl_3$, 400 MHz): $\delta$ 8.28 (d, *J* = 8.0 Hz, 1H), 8.10 (s, 1H), 7.58 (d, *J* = 8.0 Hz, 2H), 7.52 (d, *J* = 12.0 Hz, 2H), 7.45–7.43 (dd, *J* = 4.0, 4.0 Hz, 1H), 7.31 (s, 1H), 4.57–4.52 (m, 2H), 3.84 (s, 3H), 1.48 (t, *J* = 8.0 Hz, 3H), 1.42 (s, 9H); $^{13}C$ NMR ($CDCl_3$, 100 MHz): $\delta$ 165.7, 159.4, 151.8, 148.1, 145.4, 144.4, 134.9, 132.7, 129.2, 129.1, 125.8, 122.6, 121.9, 103.6, 62.1, 55.6, 34.8, 31.4, 14.4; IR ($CHCl_3$): $\nu_{max}$ 3401, 2961, 2866, 1739, 1715, 1620, 1498, 1474, 1251, 1224 $cm^{-1}$; ESI-MS: *m*/*z* 364.10 $[M + H]^+$; HRMS: *m*/*z* 364.1903 (ESI) calcd for $C_{23}H_{25}NO_3 + H^+$ (364.1907).

**Ethyl 4-(4-bromophenyl)-6-methoxyquinoline-2-carboxylate (1f).** Pale yellow solid; m.p. 171–172 °C; $^1H$ NMR ($CDCl_3$, 400 MHz): $\delta$ 8.28 (d, *J* = 8.0 Hz, 1H), 8.06 (s, 1H), 7.70 (d, *J* = 8.0 Hz, 2H), 7.44 (d, *J* = 8.0 Hz, 3H), 7.13 (s, 1H), 4.58–4.53 (m, 2H), 3.82 (s, 3H), 1.49 (t, *J* = 8.0 Hz, 3H); $^{13}C$ NMR ($CDCl_3$, 100 MHz): $\delta$ 165.5, 159.7, 146.7, 145.4, 144.3, 136.8, 132.9, 132.1, 131.0, 130.9, 128.9, 123.1, 123.0, 121.7, 102.9, 102.8, 62.2, 55.6, 14.4; IR ($CHCl_3$): $\nu_{max}$ 3400, 2923, 2850, 1730, 1715, 1619, 1488, 1473, 1434, 1367, 1274, 1226 $cm^{-1}$; ESI-MS: *m*/*z* 387.90 $[M + H]^+$; HRMS: *m*/*z* 388.0374 (ESI) calcd for $C_{19}H_{16}BrNO_3 + H^+$ (386.0386).

**Ethyl 6-methoxy-4-(4-methoxyphenyl)quinoline-2-carboxylate (1g).**[28] Pale yellow solid; m.p. 119–120 °C; $^1H$ NMR ($CDCl_3$, 400 MHz): 8.27 (d, *J* = 8.0 Hz, 1H), 8.07 (s, 1H), 7.51 (d, *J* = 8.0 Hz, 2H), 7.44 (d, *J* = 8.0 Hz, 1H), 7.27 (s, 1H), 7.10 (s, 2H), 4.58–4.53 (m, 2H), 3.92 (s, 3H), 3.83 (s, 3H), 1.49 (t, *J* = 8.0 Hz, 3H); $^{13}C$ NMR ($CDCl_3$, 100 MHz): $\delta$ 165.8, 160.0, 159.4, 147.8, 145.4, 144.4, 132.7, 130.6, 130.2, 129.4, 122.8, 121.8, 114.2, 103.3, 62.1, 55.6, 55.4, 14.4; IR ($CHCl_3$): $\nu_{max}$ 3400, 2019, 2850, 1714, 1619, 1553, 1463, 1249, 1028 $cm^{-1}$; ESI-MS: *m*/*z* 338.10 $[M + H]^+$; HRMS: *m*/*z* 338.1391 (ESI) calcd for $C_{20}H_{19}NO_4 + H^+$ (338.1387).

**Ethyl 6-methoxy-4-*m*-tolylquinoline-2-carboxylate (1h).**[28] Pale yellow solid; m.p. 139–140 °C; $^1H$ NMR ($CDCl_3$, 400 MHz): $\delta$ 8.28 (d, *J* = 8.0 Hz, 1H), 8.09 (s, 1H), 7.45–7.42 (m, 2H), 7.36–7.31 (m, 3H), 7.24 (s, 1H), 4.58–4.53 (m, 2H), 3.81 (s, 3H), 2.47 (s, 3H), 1.49 (t, *J* = 8.0 Hz, 3H); $^{13}C$ NMR ($CDCl_3$, 100 MHz): $\delta$ 165.7, 159.5, 148.2, 145.4, 144.3, 138.6, 137.9, 132.7, 130.1, 129.3, 129.2, 128.6, 126.4, 122.7, 121.7, 103.4, 62.1, 55.5, 21.5, 14.4; IR ($CHCl_3$): $\nu_{max}$ 2978, 2932, 1738, 1713, 1620, 1474, 1406, 1367, 1257, 1224, 1106 $cm^{-1}$; ESI-MS: *m*/*z* 322.10 $[M + H]^+$; HRMS: *m*/*z* 322.1410 (ESI) calcd for $C_{20}H_{19}NO_3 + H^+$ (322.1438).

**Ethyl 8-phenyl-[1,3]dioxolo[4,5-*g*]quinoline-6-carboxylate (1i).** Brown solid; m.p. 237–238 °C; $^1H$ NMR ($CDCl_3$, 400 MHz): $\delta$ 8.00 (s, 1H), 7.64 (s, 1H), 7.56–7.47 (m, 5H), 7.19 (s, 1H), 6.13 (s, 2H), 4.56–4.51 (m, 2H), 1.47 (t, *J* = 4.0 Hz, 3H); $^{13}C$ NMR ($CDCl_3$, 100 MHz): $\delta$ 165.6, 151.1, 149.9, 148.2, 146.8, 145.6, 138.1, 129.4, 128.8, 128.6, 125.6, 120.4, 107.0, 102.2, 100.9, 62.1, 14.4; IR ($CHCl_3$): $\nu_{max}$ 3435, 2918, 1696, 1462, 1276, 1239, 1111, 1036 $cm^{-1}$; ESI-MS: *m*/*z* 322.00 $[M + H]^+$; HRMS: *m*/*z* 322.1077 (ESI) calcd for $C_{19}H_{15}NO_4 + H^+$ (322.1074).

**Ethyl 8-*p*-tolyl-[1,3]dioxolo[4,5-*g*]quinoline-6-carboxylate (1j).** Yellow solid; m.p. 220–221 °C; $^1H$ NMR ($CDCl_3$, 400 MHz): $\delta$ 7.98 (s, 1H), 7.63 (s, 1H), 7.39–7.33 (m, 4H), 7.22 (s, 1H), 6.12 (s, 2H), 4.57–4.51 (m, 2H), 2.47 (s, 3H), 1.47 (t, *J* = 8.0 Hz, 3H); $^{13}C$ NMR ($CDCl_3$, 100 MHz): $\delta$ 165.7, 151.1, 149.8, 148.4, 146.8, 145.6, 138.6, 135.1, 129.4, 129.3, 125.7, 120.4, 106.1, 102.1, 101.0, 62.1, 21.3, 14.4; IR ($CHCl_3$): $\nu_{max}$ 3401, 2921, 1703, 1617, 1504, 1463, 1376, 1237, 1110 $cm^{-1}$; ESI-MS: *m*/*z* 336.00 $[M + H]^+$; HRMS: *m*/*z* 336.1236 (ESI) calcd for $C_{20}H_{17}NO_4 + H^+$ (336.1230).

**Ethyl 8-(4-(trifluoromethyl)phenyl)-[1,3]dioxolo[4,5-*g*]quinoline-6-carboxylate (1k).** Brown solid; m.p. 161–162 °C; $^1H$ NMR ($CDCl_3$, 400 MHz): $\delta$ 8.02 (s, 1H), 7.84 (d, *J* = 8.0 Hz, 2H), 7.74 (s, 1H), 7.63 (d, *J* = 8.0 Hz, 2H), 7.09 (s, 1H), 6.18 (s, 2H), 4.59–4.54 (m, 2H), 1.49 (t, *J* = 4.0 Hz, 3H); $^{13}C$ NMR ($CDCl_3$, 100 MHz): $\delta$ 165.0, 152.2, 150.7, 147.9, 145.7, 144.4, 141.2, 129.8, 125.9 (t, $^1J_{CF}$ = 3.77 Hz), 125.6, 131.3, 131.0, 120.3, 106.1, 102.7, 100.5, 62.7, 14.4; $^{19}F$ NMR (376.50 MHz, $CDCl_3$) $\delta$ −62.95 (s, 3F); IR ($CHCl_3$): $\nu_{max}$ 3400, 2919, 2850, 1617, 1419, 1090 $cm^{-1}$; ESI-MS: *m*/*z* 390.00 $[M + H]^+$; HRMS: *m*/*z* 390.0953 (ESI) calcd for $C_{20}H_{14}F_3NO_4 + H^+$ (390.0948).

**Ethyl 8-*m*-tolyl-[1,3]dioxolo[4,5-*g*]quinoline-6-carboxylate (1l).** Pale yellow solid; m.p. 126–127 °C; $^1H$ NMR ($CDCl_3$, 400 MHz): $\delta$ 7.98 (s, 1H), 7.63 (s, 1H), 7.42 (t, *J* = 8.0 Hz, 1H), 7.32–7.28 (m, 3H), 7.20 (s, 1H), 6.13 (s, 2H), 4.56–4.51 (m, 2H), 2.46 (s, 3H), 1.47 (t, *J* = 8.0 Hz, 3H); $^{13}C$ NMR ($CDCl_3$, 100 MHz): $\delta$ 165.7, 151.1, 149.8, 148.5, 146.8, 145.6, 138.5, 138.1, 130.0, 129.3, 128.4, 126.4, 125.8, 120.4, 107.0, 102.1, 101.0, 62.1, 21.5, 14.4; IR ($CHCl_3$): $\nu_{max}$ 3400, 2922, 1617, 1462, 1384, 1237, 1037 $cm^{-1}$; ESI-MS: *m*/*z* 336.3602 $[M + H]^+$; HRMS: *m*/*z* 336.3600 (ESI) calcd for $C_{20}H_{17}NO_4 + H^+$ (336.3607).

**Ethyl 8-(3-chlorophenyl)-[1,3]dioxolo[4,5-*g*]quinoline-6-carboxylate (1m).** Yellow solid; m.p. 133–134 °C; $^1H$ NMR ($CDCl_3$,

400 MHz): $\delta$ 7.97 (s, 1H), 7.64 (s, 1H), 7.48 (d, $J$ = 4.0 Hz, 3H), 7.37–7.35 (m, 1H), 7.12 (s, 1H), 6.14 (s, 2H), 4.57–4.52 (m, 2H), 1.48 (t, $J$ = 8.0 Hz, 3H); $^{13}$C NMR ($CDCl_3$, 100 MHz): $\delta$ 165.5, 151.3, 150.1, 146.8, 145.6, 139.8, 134.8, 130.1, 129.4, 128.7, 127.5, 125.3, 120.3, 107.1, 102.3, 100.5, 62.1, 29.6, 14.1; IR ($CHCl_3$): $\nu_{max}$ 3434, 2923, 1742, 1620, 1464, 1241, 1082 cm$^{-1}$; ESI-MS: $m/z$ 356.00 [M + H]$^+$; HRMS: $m/z$ 356.0656 (ESI) calcd for $C_{19}H_{14}ClNO_4$ + H$^+$ (356.0684).

**Ethyl 8-(4-ethynylphenyl)-[1,3]dioxolo[4,5-*g*]quinoline-6-carboxylate (1n).** Pale yellow solid; m.p. 142–143 °C; $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ 7.97 (s, 1H), 7.67 (d, $J$ = 8.0 Hz, 3H), 7.46 (d, $J$ = 8.0 Hz, 2H), 7.13 (s, 1H), 6.14 (s, 2H), 4.57–4.51 (m, 2H), 3.19 (s, 1H), 1.48 (t, $J$ = 8.0 Hz, 3H); $^{13}$C NMR ($CDCl_3$, 100 MHz): $\delta$ 165.5, 151.2, 150.1, 147.4, 146.8, 145.6, 138.5, 132.5, 129.4, 125.3, 122.6, 120.3, 107.1, 102.2, 100.6, 83.0, 78.5, 62.1, 14.1; IR ($CHCl_3$): $\nu_{max}$ 3400, 2924, 1714, 1620, 1497, 1483, 1369, 1212, 1239, 1114 cm$^{-1}$; ESI-MS: $m/z$ 346.00 [M + H]$^+$; HRMS: $m/z$ 346.1082 (ESI) calcd for $C_{21}H_{15}NO_4$ + H$^+$ (346.1074).

**Ethyl 8-(4-(trifluoromethoxy)phenyl)-[1,3]dioxolo[4,5-*g*]quinoline-6-carboxylate (1o).** Brown solid; m.p. 117–118 °C; $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ 7.97 (s, 1H), 7.64 (s, 1H), 7.53 (d, $J$ = 8.0 Hz, 2H), 7.40 (d, $J$ = 8.0 Hz, 2H), 7.11 (s, 1H), 6.14 (s, 2H), 4.57–4.51 (m, 2H), 1.48 (t, $J$ = 8.0 Hz, 3H); $^{13}$C NMR ($CDCl_3$, 100 MHz): $\delta$ 165.5, 151.2, 150.1, 149.5, 146.8, 146.7, 145.7, 136.7, 130.9, 125.3, 121.8, 120.8 (d, $^1J_{CF}$ = 100.60 Hz), 119.2, 107.2, 102.3, 100.5, 62.1, 14.4; $^{19}$F NMR (376.50 MHz, $CDCl_3$) $\delta$ −57.72 (s, 3F); IR ($CHCl_3$): $\nu_{max}$ 3400, 2921, 2851, 1742, 1590, 1503, 1463, 1384, 1161, 1035 cm$^{-1}$; ESI-MS: $m/z$ 406.00 [M + H]$^+$; HRMS: $m/z$ 406.0936 (ESI) calcd for $C_{20}H_{14}F_3NO_5$ + H$^+$ (406.0897).

**Ethyl 6,7-dimethoxy-4-phenylquinoline-2-carboxylate (1p).** Yellow solid; m.p. 140–141 °C; $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ 8.02 (s, 1H), 7.69 (s, 1H), 7.56–7.53 (m, 5H), 7.20 (s, 1H), 4.58–4.53 (m, 2H), 4.06 (s, 3H), 3.87 (s, 3H), 1.48 (t, $J$ = 8.0 Hz, 3H); $^{13}$C NMR ($CDCl_3$, 100 MHz): $\delta$ 165.7, 152.8, 151.5, 147.6, 145.6, 145.5, 138.1, 129.3, 128.8, 128.6, 123.8, 120.3, 109.4, 102.9, 62.1, 56.4, 56.1, 14.5; IR ($CHCl_3$): $\nu_{max}$ 3400, 2924, 1735, 1714, 1497, 1483, 1239, 1212, 1113, 1027 cm$^{-1}$; ESI-MS: $m/z$ 338.10 [M + H]$^+$; HRMS: $m/z$ 338.1387 (ESI) calcd for $C_{20}H_{19}NO_4$ + H$^+$ (338.1387).

**Ethyl 4-(3-chlorophenyl)-6,7-dimethoxyquinoline-2-carboxylate (1q).** Brown solid; m.p. 141–142 °C; $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ 8.00 (s, 1H), 7.71 (s, 1H), 7.60 (d, $J$ = 8.0 Hz, 2H), 7.42 (d, $J$ = 8.0 Hz, 2H), 7.11 (s, 1H), 4.57–4.55 (m, 2H), 4.07 (s, 3H), 3.89 (s, 3H), 1.48 (t, $J$ = 8.0 Hz, 3H); $^{13}$C NMR ($CDCl_3$, 100 MHz): $\delta$ 165.6, 153.0, 151.8, 149.9, 146.0, 145.6, 145.6, 134.7, 130.1, 130.8, 128.8, 123.7, 121.2, 120.3, 109.5, 102.5, 62.1, 56.4, 56.1, 14.4; IR ($CHCl_3$): $\nu_{max}$ 3391, 2924, 1738, 1619, 1492, 1370, 1240, 771 cm$^{-1}$; ESI-MS: $m/z$ 372.00 [M + H]$^+$; HRMS: $m/z$ 372.1000 (ESI) calcd for $C_{20}H_{18}ClNO_4$ + H$^+$ (372.0997).

**Ethyl 6-(benzyloxy)-4-phenylquinoline-2-carboxylate (1r).** Pale yellow solid; m.p. 109–110 °C; $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ 8.30 (d, $J$ = 8.0 Hz, 1H), 8.08 (s, 1H), 7.53–7.50 (m, 5H), 7.45–7.43 (m, 2H), 7.38–7.36 (m, 5H), 5.07 (s, 2H), 4.58–4.52 (m, 2H), 1.48 (t, $J$ = 8.0 Hz, 3H); $^{13}$C NMR ($CDCl_3$, 100 MHz): $\delta$ 165.6, 158.4, 148.0, 145.5, 144.3, 137.8, 136.1, 132.8, 129.4, 129.0, 128.8, 128.7, 128.6, 128.2, 127.6, 123.2, 121.8, 104.9, 70.2, 62.1, 14.4; IR ($CHCl_3$): $\nu_{max}$ 3400, 2923, 1732, 1601, 1462, 1384, 1237, 1035 cm$^{-1}$; ESI-MS: $m/z$ 384.10 [M + H]$^+$; HRMS: $m/z$ 384.1595 (ESI) calcd for $C_{25}H_{21}NO_3$ + H$^+$ (384.1594).

**Ethyl 6-(benzyloxy)-4-(3-chlorophenyl)quinoline-2-carboxylate (1s).** Yellow solid; m.p. 153–154 °C; $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ 8.30 (d, $J$ = 12.0 Hz, 1H), 8.05 (s, 1H), 7.53 (m, 1H), 7.49 (s, 2H), 7.46–7.42 (m, 1H), 7.38–7.32 (m, 5H), 7.30 (s, 1H), 7.18 (d, $J$ = 4.0 Hz, 1H), 5.08 (s, 2H), 4.47–4.52 (m, 2H), 1.48 (t, $J$ = 8.0 Hz, 3H); $^{13}$C NMR ($CDCl_3$, 100 MHz): $\delta$ 165.4, 158.7, 146.5, 145.4, 144.3, 139.6, 136.1, 134.8, 132.6, 130.1, 128.7, 128.3, 129.3, 128.8, 127.6, 127.6, 123.5, 121.7, 104.7, 70.3, 62.2, 14.4; IR ($CHCl_3$): $\nu_{max}$ 3399, 2921, 1556, 1348, 1042, 772 cm$^{-1}$; ESI-MS: $m/z$ 418 [M + H]$^+$; HRMS: $m/z$ 418.1205 (ESI) calcd for $C_{25}H_{20}ClNO_3$ + H$^+$ (418.1204).

**Ethyl 6-(benzyloxy)-4-(4-fluorophenyl)quinoline-2-carboxylate (1t).** Pale yellow solid; m.p. 134–135 °C; $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ 8.30 (d, $J$ = 12.0 Hz, 1H), 8.05 (s, 1H), 8.54–8.51 (dd, $J$ = 4.0, 12.0 Hz, 1H), 7.41–7.34 (m, 6H), 7.23–7.17 (m, 4H), 5.09 (s, 2H), 4.58–4.52 (m, 2H), 1.48 (t, $J$ = 8.0 Hz, 3H); $^{13}$C NMR ($CDCl_3$, 125 MHz): $\delta$ 165.6, 163.9 (d, $^1J_{CF}$ = 251.5 Hz), 158.5, 146.9, 145.4, 144.3, 136.1, 132.9, 131.14, 131.08, 129.0, 128.7, 128.2, 127.5, 123.3, 128.8, 116.0 (d, $^2J_{CF}$ = 21.8 Hz), 104.7, 70.2, 62.2, 14.4; $^{19}$F NMR (376.50 MHz, $CDCl_3$): $\delta$ −112.96 (s, 1F); IR ($CHCl_3$): $\nu_{max}$ 3400, 2925, 1715, 1618, 1605, 1497, 1378, 1224 cm$^{-1}$; ESI-MS: $m/z$ 402.00 [M + H]$^+$; HRMS: $m/z$ 402.1499 (ESI) calcd for $C_{25}H_{20}FNO_3$ + H$^+$ (402.1500).

### FRAP assay

The FRAP assay was performed in a 96-well microplate using a slightly modified protocol of Benzie and Strain (1996).[29] Briefly, the FRAP reagent was prepared by mixing 10 ml of 300 mM acetate buffer with 1 ml of 10 mM 2,4,6-tripyridyl-*S*-triazine (TPTZ) in 40 mM of hydrochloric acid and 1 ml of 20 mM $FeCl_3 \cdot 6H_2O$. The freshly prepared FRAP reagent (195 µl) was added to all wells of the 96-well plate. Test compounds dissolved in methanol (5 µl) were added to the final concentration of 100 µM. Ascorbic acid (100 µM) was used as a positive control. The absorbance was read at 593 nm after 30 min incubation in the dark.

### DPPH assay

The DPPH assay was carried out in a 96-well microplate. Briefly, 190 µl of each test compound dissolved in methanol was added into each well of the 96-well plate with a final concentration of 100 µM. Ascorbic acid (100 µM) was used as a positive control. The reaction was started by adding 10 µl methanolic solution of DPPH (100 µM) to all the samples. After 30 min incubation while shaking in the dark, absorbance was recorded at 517 nm. The DPPH free-radical scavenging activity was calculated as percentage inhibition using the

following formula:

$$\text{Inhibition } (\%) = (A_0 - A_1/A_0) \times 100$$

where $A_0$ is the absorbance of the control and $A_1$ is the absorbance of the test sample.

### Cell culture and treatments

Human colorectal adenocarcinoma LS-180 and human neuroblastoma SH-SY5Y cells were purchased from ECACC, England. LS-180 cells were grown in MEM growth medium and SH-SY5Y cells were grown in MEM : F12 nutrient medium mixed in the ratio of 1 : 1. The media for both the cell lines were supplemented with 1% MEM non-essential amino acids along with 10% FCS, 100 U penicillin G and 100 μg $ml^{-1}$ of streptomycin. Cells were grown in 5% $CO_2$ at 37 °C with 95% humidity. All the test compounds were dissolved in DMSO for treatment of either SH-SY5Y or LS-180 cells, while the untreated control cultures received only the vehicle (DMSO < 0.2%).

### Differentiation of SH-SY5Y cells

The SH-SY5Y cells were differentiated into neurons by treatment with 10 μM of retinoic acid for seven days before treatment with test compounds. During differentiation the retinoic acid concentration was maintained at 10 μM by replacing the media every 48 h.[30]

### Cell viability assay

The cell proliferation assay was performed in human colorectal adenocarcinoma LS-180 and human neuroblastoma SH-SY5Y cells (differentiated with 10 μM of retinoic acid for seven days). Cells ($1 \times 10^4$) were seeded into each well of the 96-well microplate for 24 h. Cells were treated with 50 μM of each compound for 24 h. The MTT dye was then added to each well 4 h prior to the termination of experiment. Formazan crystals were dissolved in DMSO before recording absorbance at 570 nm. Cell viability of the untreated control sample was considered to be 100%, while viability of test samples was calculated using the following formula:

$$\%\ \text{cell viability} = \frac{\text{OD (test)}}{\text{OD (control)}} \times 100$$

### Direct interaction of compounds with hydrogen peroxide

This assay was performed using the protocol of Beers and Sizer (1952).[25] A standard curve for hydrogen peroxide was generated using different concentrations (0.0125 to 0.1 M). The rate of disappearance of hydrogen peroxide in the presence of compounds **1o** and **1q** was calculated by measuring optical density at different time points. The rate of reaction was calculated using the following formula:

$$\text{Rate of disappearance of hydrogen peroxide} = -\frac{\Delta[\text{A}]}{\Delta t}$$

where $\Delta$[A] is the change in concentration and $\Delta t$ is the corresponding change in time.

### Measurement of redox potential

The electrochemical behaviour of compound **1o** ($1.0 \times 10^{-3}$ M) was investigated by cyclic voltammetry at the platinum electrode in 0.01 M TBAP (tetrabutylammonium perchlorate)/acetonitrile solution with a scan rate of 50 mV $s^{-1}$. The cyclic voltammogram of compound **1o** was scanned between the potential range of 1.2 V to −1.2 V *versus* $Ag/Ag^+$.[31]

### Preparation of Aβ 1–42 peptide

Aβ 1–42 was dissolved in water containing 0.1% $NH_3$ to prepare 1 mM stock. The Aβ peptide solution was further diluted with the same volume of PBS and the aggregation was induced by incubating the peptide at 37 °C for 3 days before use.

### ROS generation analysis

The ROS scavenging activity of compounds against acute oxidative stress produced by hydrogen peroxide was checked in differentiated SH-SY5Y cells. Cells were treated with the test compounds **1o** and **1q** at 50 or 100 μM 30 minutes prior to the treatment with 10 μM of Aβ 1–42 for 24 h or 500 μM of $H_2O_2$ for 30 min. The cell permeable ROS probe DCFH-DA (10 μM) was added to each well at the time of treatment with compounds **1o** and **1q**. The cells were washed once with PBS, trypsinized and resuspended in PBS for the measurement of fluorescence on a fluorimeter (BioTek SYNERGYMx) at 504/529 nm. The total level of ROS was calculated by dividing fluorescence with the total protein present in each sample.

### Neuroprotection assay against hydrogen peroxide

The neuroprotection assay was performed in differentiated human neuroblastoma SH-SY5Y cells. Briefly, cells ($1 \times 10^4$) were seeded into each well of 96-well plate for 24 h. Compounds **1o** and **1q** were added 30 min prior to the treatment with 100 μM of $H_2O_2$ for 24 h. At the end of treatments, cell viability was analysed by the MTT assay as described under the section heading Cell viability assay.

### Pgp-induction assay

All synthesized compounds were screened for their ability to induce Pgp using the rhodamine 123 (Rh123) cell exclusion method. In this method, the Pgp function was evaluated in terms of rhodamine 123 (Rh123) accumulation and efflux.[32] Briefly, the protocol used is as follows: colorectal LS-180 cells were seeded at a density of $2 \times 10^4$ per well of a 96-well plate and were allowed to grow for the next 24 h. Cells were further incubated with the test compounds to a final concentration of 5 μM and rifampicin (positive control) to a final concentration of 10 μM in complete media for 48 h. The final concentration of DMSO was kept at 0.1%. The drugs were removed and cells were incubated with HANKS buffer for 40 minutes before further incubation with HANKS buffer (containing 10 μM of Rh123 as a Pgp substrate) for 90 minutes. At the end of Rh123 treatment cells were washed four times with cold PBS followed by cell lysis for 1 h using 200 μl of lysis buffer (0.1% Triton X-100 and 0.2 N NaOH). A total of 100 μl of

lysate was used for reading fluorescence of Rh123 at 485/529 nm. Samples were normalized by dividing fluorescence of each sample with total protein present in the lysate.

### Statistical analysis

Data are expressed as mean ± SD of three independent experiments unless otherwise indicated. The comparisons were made between control and treated groups or the entire intragroup using the Bonferroni test through the Instat-2 software. $p$ values *<0.05 were considered significant. $p$ value *<0.05, **<0.01, ***<0.001.

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