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PAPER

Synthesis, 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 ferric reducing ability of plasma (FRAP) assay. The compounds were found to react directly with hydrogen peroxide, which might be one of the mechanism 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 brains. Further, 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 agents.

Introduction

The brains of Alzheimer 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 a 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 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 p-glycoprotein (Pgp) efflux pump, and thus the Pgp deficiency at 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 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

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activities.[9-10] The well known antimalarial natural products quinine and quinidine alkaloids isolated from Cinchona bark comprises 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] Doebner–Miller reaction ($SnCl_4$),[12d, 12e] Friedlander synthesis (Lewis acids)[12f, 12g, 12i] involves 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 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 involves use of a metal catalyst, organic solvents and also requires heating or longer reaction times. Thus, development of 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 (Figure 1). 4-Arylquinolines displayed promising antioxidant, neuroprotective and Pgp-induction activity, indicating their potential as anti-Alzheimer agents.

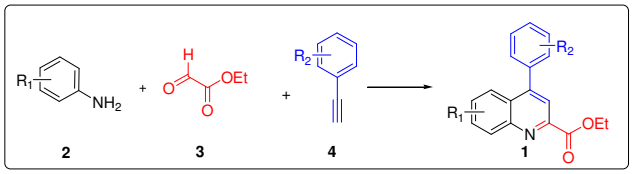
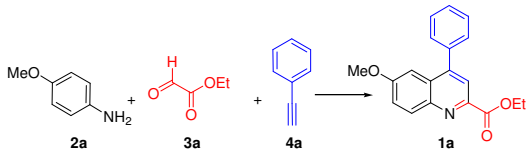


Figure 1 Methods for one-pot synthesis of 4-arylquinoline-2-carboxylates from arylamines, glyoxylates and phenylacetylenes

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 reaction does not proceed with 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 as an optimal condition to produce desired product in good yield (entry 16), which was selected for further studies.

Table 1 Solvent and catalyst optimization studies[a]

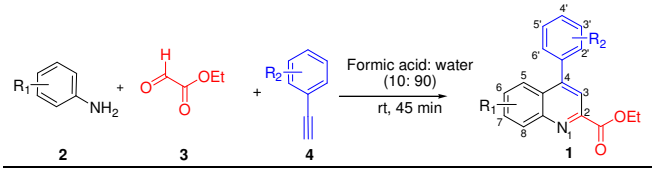


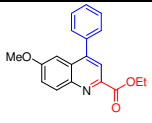
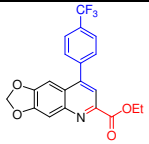
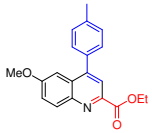
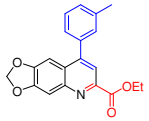
Entry	Reaction medium (catalyst and solvent)	Temp.	Time	Yield[b] (%)
1.	50 mol% Cu-Mn B, CH_2Cl_2	rt	12 h	0
2.	50 mol% Cu-Mn B, water	rt	12 h	0
3.	50 mol% Cu-Mn B, CH_2Cl_2	70 °C	12 h	0
4.	20 mol% Fe-PILC, CH_2Cl_2	rt	12 h	0
5.	20 mol% Fe-PILC, water	rt	12 h	0
6.	20 mol% Cu-turnings, CH_2Cl_2	rt	12 h	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**

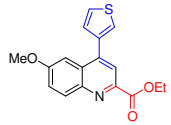
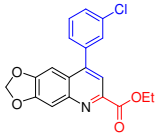
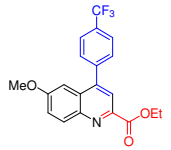
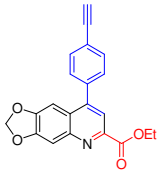
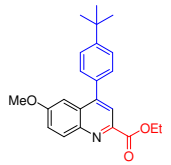
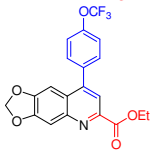
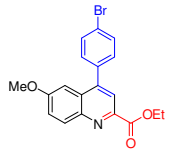
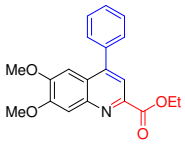
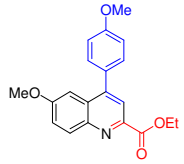
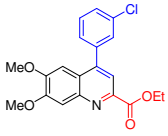
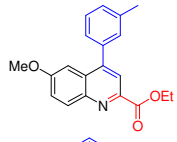
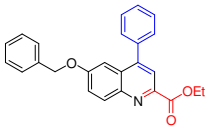
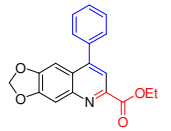
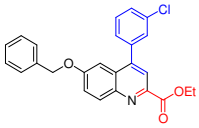
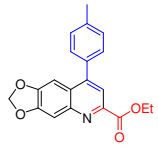
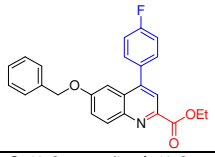
rt, room temperature; [a] Reagents and conditions: **2a** (1 mmol), **3a** (1 mmol), **4a** (1.2 mmol) and reaction medium (catalyst and solvent); [b] isolated yields; [c] optimized reaction condition.

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

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
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

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 Figure 2. The reaction mechanism involves 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**.

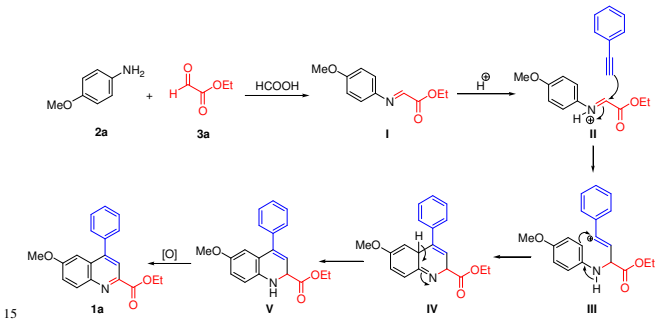


Figure 2 Proposed mechanism

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 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 IC_{50} value of >50 µM (Table 3).

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

Entry	Antioxidant activity		% viability of SH-SY5Y cells at 50 µM[d]
	FRAP assay[ac]	DPPH assay[bc]	
	% Fe reducing power	% free-radical scavenging activity	
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 to 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 to ascorbic acid. [c]Data in the FRAP and DPPH assay are mean ± SD of three independent experiments. [d]Data for cell viability assay are mean ± SD of three similar experiments.

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. The compounds **1o** and **1q** displayed instantaneous rate of reaction 3.0×10^{-3} and 2.0×10^{-3} moles/sec, respectively. Thus, the ability of these compounds to directly interact with hydrogen peroxide should be one of the mechanism of their observed antioxidant activity.

Next, the electrochemical behaviour of compound **1o** (1.0×10^{-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 conjugated imine group of quinoline which possess lone pair of electrons. The cyclic voltammetry measurement indicates that the peak at 0.4 V vs. Ag/Ag^+ is the oxidation peak of imino nitrogen atom.

Further, the ROS-quenching activity of compounds **1o** and **1q** was investigated using 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 decreased level of DCF fluorescence to 53% and 76% by these compounds, respectively (Figure 3).

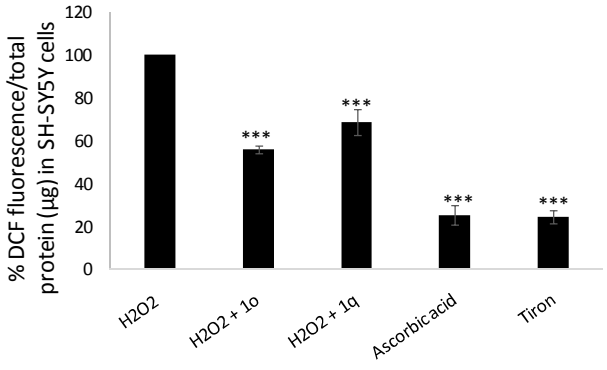


Figure 3. ROS quenching by compounds **1o** and **1q** in SH-SY5Y cells. Cells were treated with 100 µM of **1o** and **1q**, 30 minutes before the treatment of H_2O_2 (500 µM). Data are mean of three independent experiments. Statistical comparisons were made by using Bonferroni test. The p value <0.5 was considered to be significant. P value *<0.5, **<0.01, ***<0.001.

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 to 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 (Figure 4).

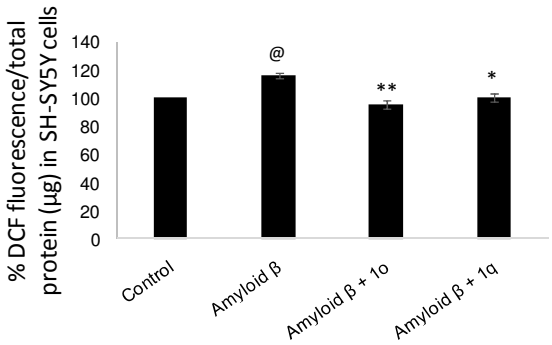


Figure 4. Quenching of amyloid-β induced ROS by compounds **1o** and **1q** in SH-SY5Y cells. Cells were treated with **1o** and **1q** (at 50 µM), 30 min before the treatment of aggregated amyloid-β1-42 peptide (10 µM). Data are mean of three independent experiments. Statistical comparisons were made using Bonferroni test. The p value <0.5 was considered to be significant. P value *<0.5, **<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.

Encouraged by these results, we hypothesized that these compounds may effectively provide neuroprotection to differentiated SH-SY5Y cells against oxidative stress. Interestingly, 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 (Figure 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, neuroprotective effect of compounds **1o** and **1q** against Aβ induced neurotoxicity could not be analysed in this study.

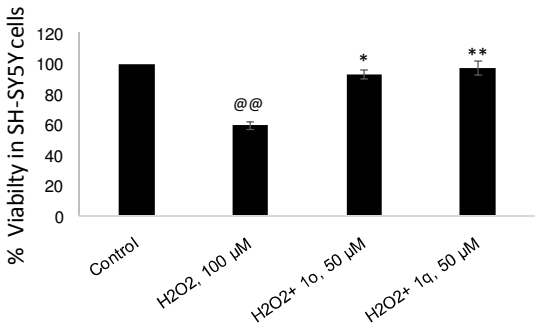


Figure 5. Neuroprotective effect of compounds **1o** and **1q** against H_2O_2-induced toxicity in SH-SY5Y cells. Cells were treated with **1o** and **1q** at indicated concentrations 30 minutes prior to the treatment with H_2O_2 (100 µM) for 24 h. Data are mean of three independent experiments. Statistical comparisons were made by using Bonferroni test. The p value <0.5 was considered to be significant. P value *<0.5, **<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.

Pgp-induction activity. The recent two independent clinical studies[6, 26] observed that AD patients have decreased clearance of CNS amyloid-ß compared to healthy volunteers. The Aß-clearance occurs primarily via Pgp efflux pump, thus drugs with 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 compounds 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 to untreated control samples. Furthermore, these compounds were found to be non-toxic to LS-180 cells (IC_{50}> 50 µM).

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[ab]	% 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 *< 0.5, **<0.01, ***<0.001.

[b] values are shown as average of three experiments ± SD.

Conclusion

In summary, we have developed 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 AD brain. These findings clearly indicates their potential as a new lead for anti-Alzheimer 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 Brucker-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, δ 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 Agilent 1100 LC-Q-TOF and HRMS-6540-UHD machines. IR spectra were recorded on Perkin-Elmer IR spectrophotometer. Melting points were recorded on digital melting point apparatus.

General procedure for preparation of 4-arylquinoline-2-carboxylates. To 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 vacuo rotavapor. The crude product was purified by silica-gel column chromatography (mesh 100-200) to get 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): δ 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): δ 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 ($CHCl_3$): ν_{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): δ 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): δ 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 ($CHCl_3$): ν_{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): δ 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): δ 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 ($CHCl_3$): ν_{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): δ8.31 (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): δ 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, $CDCl_3$): δ -62.81 (s, 3F); IR ($CHCl_3$): ν_{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): δ 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): δ 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 ($CHCl_3$): ν_{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): δ 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): δ 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 ($CHCl_3$): ν_{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): δ 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 ($CHCl_3$): ν_{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): δ 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): δ 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 ($CHCl_3$): ν_{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): δ 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): δ 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 ($CHCl_3$): ν_{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): δ 7.98 (s, 1 H), 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): δ 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 ($CHCl_3$): ν_{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): δ 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): δ 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, $CDCl_3$) δ -62.95 (s, 3F); IR ($CHCl_3$): ν_{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): δ 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): δ 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 ($CHCl_3$): ν_{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; ^{1}H NMR ($CDCl_3$, 400 MHz): δ7.97 (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): δ 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 ($CHCl_3$): ν_{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): δ 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): δ 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 ($CHCl_3$): ν_{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): δ 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): δ 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, $CDCl_3$) δ -57.72 (s, 3F); IR ($CHCl_3$): ν_{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): δ 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): δ 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 ($CHCl_3$): ν_{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): δ 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): δ 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 ($CHCl_3$): ν_{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): δ 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): δ 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 ($CHCl_3$): ν_{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): δ 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): δ 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 ($CHCl_3$): ν_{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): δ 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): δ 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, $CDCl_3$): δ -112.96 (s, 1F); IR ($CHCl_3$): ν_{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. FRAP assay was performed in 96-well microplate using 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 $FeCl_3.6H_2O$. The freshly prepared FRAP reagent (195 µl) was added to all wells of 96 well plate. Test compounds dissolved in methanol (5 µl) were added to the final concentration of 100 µM. Ascorbic acid (40 µM) was used as a positive control. The absorbance was read at 593 nm after 30 min incubation in the dark.

DPPH Assay. DPPH assay was carried out in 96-well microplate. Briefly, 190 µl of each test compound dissolved in methanol was added into each well of a 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 following formula:

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

where, A_0 is the absorbance of control and A_1 is the absorbance of 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 was supplemented with 1% MEM non-essential amino acids along with 10% FCS, 100 U penicillin G and 100 µg/ml 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 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 media every 48 h.[30]

Cell viability assay. The cell proliferation assay was done in human colorectal adenocarcinoma LS-180 and human neuroblastoma SH-SY5Y cells (differentiated with 10 µM of retinoic acid for seven days). Cells ($1x10^4$) were seeded into each well of 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 taking 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 done using the protocol of Beers and Sizer (1952).[25] A standard curve for hydrogen peroxide was generated using its different concentrations (0.0125 to 0.1 M). The rate of disappearance of hydrgen peroxide in presence of compounds **1o** and **1q** was calculated by measuring optical denstity at different time points. The rate of reaction was calculated using following formula:

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

where, Δ[A] is the change in concentration and Δt is corresponding change in time.

Measurement of redox potential. The electrochemical behaviour of compound **1o** (1.0×10^{-3} M) was investigated by cyclic voltammetry at platinum electrode in 0.01 M TBAP (tetrabutylammonium perchlorate)/acetonitrile solution with a scan rate of 50 mV/s. The cyclic voltammogram of compound **1o** was scanned between 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 at 1 mM. The *Aβ* peptides 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. 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 in PBS, trypsinized and resuspended in PBS for the measurement of fluorescence on fluorimeter (BioTek SYNERGYMx) at 504/529 nm. Total level of ROS was calculated by dividing fluorescence with that of total protein present in each sample.

Neuroprotection assay against hydrogen peroxide. Neuroprotection assay was done in differentiated human neuroblastoma SH-SY5Y cells. Briefly, cells (1×10^4) were seeded into each well 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 MTT assay as described under the heading cell viability assay.

Pgp-induction assay. All synthesized compounds were screened for their ability to induce Pgp using rhodamine123 (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 2×10^4 per well of 96 well plate and were allowed to grow for next 24 h. Cells were further incubated with the test compounds, and were diluted 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%. 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 is expressed as mean ± SD of three independent experiments unless otherwise indicated. The comparisons were made between control and treated groups or the entire intra group using Bonferroni test through Instat-2 software. p -values *<0.5 were considered significant.

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Notes and References

1. (a) M. A. Smith, C. A. Rottkamp, A. Nunomura, A. K. Raina and G. Perry, *Biochim. Biophys. Acta*, 2000, **1502**, 139-144; (b) K. Ono, T. Hamaguchi, H. Naiki and M. Yamada, *Biochim. Biophys. Acta*, 2006, **1762**, 575-586.
2. (a) A. J. Hanson, J. E. Prasad, P. Nahreini, C. Andreatta, B. Kumar, X. D. Yan and K. N. Prasad, *J. Neurosci. Res.*, 2003, **74**, 148–159; (b) B. J. Tabner, O. M. El-Agnaf, S. Turnbull, M. J. German, K. E. Paleologou, Y. Hayashi, L. J. Cooper, N. J. Fullwood and D. Allsop, *J. Biol. Chem.*, 2005, **280**, 35789-35792.
3. (a) D. A. Butterfield, *Free Radic. Res.*, 2002, **36**, 1307-1313; (b) D. A. Butterfield, A. M. Swomley and R. Sultana, *Antiox. Redox Signal.*, 2013, **19**, 823-835.
4. (a) P. H. Frederikse, D. Garland, J. S. Zigler and J. Piatigorsky, *J. Biol. Chem.*, 1996, **271**, 10169-10174; (b) H. Misonou, M. Morishima-Kawashima and Y. Ihara, *Biochemistry*, 2000, **39**, 6951-6959; (c) C. Shen, Y. Chen, H. Liu, K. Zhang, T. Zhang, A. Lin and N. Jing, *J. Biol. Chem.*, 2008, **283**, 17721-17730.
5. (a) S. Sung, Y. Yao, K. Uryu, H. Yang, V. M. Lee, J. Q. Trojanowski and D. Pratico, *FASEB J.*, 2004, **18**, 323–325; (b) M. W. Dysken, M. Sano, S. Asthana, J. E. Vertrees, M. Pallaki, M. Llorente, S. Love, G. D. Schellenberg, J. R. McCarten, J. Malphurs, S. Prieto, P. Chen, D. J. Loreck, G. Trapp, R. S. Bakshi, J. E. Mintzer, J. L. Heidebrink, A. Vidal-Cardona, L. M. Arroyo, A. R. Cruz, S. Zachariah, N. W. Kowall, M. P. Chopra, S. Craft, S. Thielke, C. L. Turvey, C. Woodman, K. A. Monnell, K. Gordon, J. Tomaska, Y. Segal, P. N. Peduzzi and P. D. Guarino, *JAMA*, 2014, **311**, 33-44.
6. K. G. Mawuenyega, W. Sigurdson, V. Ovod, L. Munsell, T. Kasten, J. C. Morris, K. E. Yrasheski and R. J. Bateman, *Science* 2010, **330**, 1774.
7. J. R. Cirrito, R. Deane, A. M. Fagan, M. L. Spinner, M. Parsadanian, B. V. Zlokovic, D. Piwnica-Worms and D. M. Holtzman, *J. Clin. Invest.*, 2005, **115**, 3285-3290.
8. A. H. Abuznait, H. Qosa, B. A. Busnena, K. A. El Sayed and A. Kaddoumi, *ACS Chem. Neurosci.*, 2013, **4**, 973-982.
9. R. N. Kharwar, A. Mishra, S. K. Gond, A. Stierle, D. Stierle and S. Bawa, *Nat. Prod. Rep.*, 2011, **28**, 1208-1228.
10. (a) M. Orhan Püsküllü, B. Tekiner and S. Suzen, *Mini-Rev. Med. Chem.*, 2013, **13**, 365-372; (b) V. R. Solomon and H. Lee, *Curr. Med. Chem.*, 2011, **18**, 1488-1508; (c) J. Lavrado, R. Moreira and A. Paulo, *Curr. Med. Chem.*, 2010, **17**, 2348-2370; (d) S. Kumar, S. Bawa and H. Gupta, *Mini-Rev. Med. Chem.*, 2009, **9**, 1648-1654; (e) J. P. Michael, *Nat. Prod. Rep.*, 2002, **19**, 742–760; (f) J. P. Michael, *Nat. Prod. Rep.*, 1997, **14**, 605-618; (g) I. P. Singh and H. S. Bodiwala, *Nat. Prod. Rep.*, 2010, **27**, 1781-1800; (h) P. Williams, A. Sorribas and M.-J. R. Howes, *Nat. Prod. Rep.*, 2011, **28**, 48-77; (i) P. M. S. Chauhan and S. K. Srivastava, *Curr. Med. Chem.*, 2001, **8**, 1535; (j) Y.-L. Chen, K.-G. Fang, J.-Y. Sheu, S.-L. Hsu and C.-C. Tzeng, *J. Med. Chem.*, 2001, **44**, 2374; (k) G. Roma, M. D. Braccio, G. Grossi, F. Mattioli and M. Ghia, *Eur. J. Med. Chem.*, 2000, **35**, 1021-1035.
11. (a) H. BF, *Chem. News*, 1931, **142**, 129-133; (b) J. Achan, A. O. Talisuna, A. Erhart, A. Yeka, J. K. Tibenderana, F. N. Baliraine, P. J. Rosenthal and U. D'Alessandro, *Malaria J.*, 2011, **10**, 144.
12. (a) P. Liu, Z. Wang, J. Lin and X. Hu, *Eur. J. Org. Chem.*, 2012, 1583–1589; (b) Y. D. Wang, D. H. Boschelli, S. Johnson and E. Honores, *Tetrahedron*, 2004, **60**, 2937–2942; (c) Skraup and Z. H.Ber., *Dtsch. Chem. Ges.*, 1880, **13**, 2086; (d) A. R. Mackenzie, C. J. Moody and C. W. Rees, *Tetrahedron*, 1986, **42**, 3259-3268; (e) Y.-C. Wu, L. Liu, H.-J. Li, D. Wang and Y.-J. Chen, *J. Org. Chem.*, 2006, **71**, 6592-6595; (f) Friedlander and P. Berichte, 1882, **15**, 2572; (g) R. Varala, R. Enugala and S. R. Adapa, *Synthesis*, 2006, 3825–3830; (h) H. V. Mierde, P. V. D. Voort and F. Verpoort, *Tetrahedron Lett.*, 2009, **50**, 201–203; (i) S. Ghassamipour and A. R. Sardarian, *Tetrahedron Lett.*, 2009, **50**, 514-519.
13. S. K. Guchhait, K. Jadeja and C. Madaan, *Tetrahedron Lett.*, 2009, **50**, 6861–6865.
14. C. Qi, Q. Zheng and R. Hua, *Tetrahedron* 2009, **65**, 1316-1320.
15. V. V. Kouznetsov, *Tetrahedron* 2009, **65**, 2721-2750.
16. (a) Y. Wang, C. Chen, J. Peng and M. Li, *Angew. Chem. Int. Ed.*, 2013, **52**, 5323–5327; (b) H. Huang, H. Jiang, K. Chen and H. Liu, *J. Org. Chem.*, 2009, **74**, 5476–5480.
17. X. Li, Z. Mao, Y. Wang, W. Chen and X. Lin, *Tetrahedron* 2011, **67**, 3858-3862.
18. V. Gaddam, S. Ramesh and Nagarajan, *Tetrahedron*, 2010, **66**, 4218-4222.
19. F. Xiao, Y. Chen, Y. Liu and J. B. Wang, *Tetrahedron*, 2008, **64**, 2755-2761.
20. R. Rohlmann, S. T., H. Richter and O. G. Mancheño, *J. Org. Chem.*, 2013, **78**, 6050–6064.
21. J. McNulty, R. Vemula, C. Bordón, R. Yolken and L. Jones-Brando, *Org. Biomol. Chem.*, 2014, **12**, 255-260.
22. J. B. Bharate, S. K. Guru, S. K. Jain, S. Meena, P. P. Singh, S. Bhushan, B. Singh, S. B. Bharate and R. A. Vishwakarma, *RSC Adv.*, 2013, **3**, 20869–20876.
23. (a) J. B. Bharate, R. Sharma, B. Singh, S. Aravinda, V. K. Gupta, S. B. Bharate and R. A. Vishwakarma, *RSC Adv.*, 2013, **3**, 21736–21742; (b) S. B. Bharate, J. B. Bharate, S. I. Khan, B. L. Tekwani, M. R. Jacob, R. Mudududdla, R. R. Yadav, B. Singh, P. R. Sharma, S. Maity, B. Singh, I. A. Khan and R. A. Vishwakarma, *Eur. J. Med. Chem.*, 2013, **63**, 435-443.
24. (a) C. Praveen, P. DheenKumar, D. Muralidharan and P. T. Perumal, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 7292–7296; (b) H. R. P. Naik, H. S. B. Naik, T. R. R. Naik, H. R. Naika, K. Gouthamchandra, R. Mahmood and B. M. K. Ahamed, *Eur. J. Med. Chem.*, 2009, **44**, 981–989.
25. R. F. Beers and I. W. Sizer, *J. Biol. Chem.*, 1952, **195**, 133-140.
26. D. M. E. van Assema, M. Lubberink, M. Bauer, W. M. van der Flier, R. C. Schuit, A. D. Windhorst, E. F. I. Comans, N. J. Hoetjes, N. Tolboom, O. Langer, M. Müller, P. Scheltens, A. A. Lammertsma and B. N. M. van Berckel, *Brain*, 2012, **125**, 181-189.
27. E. G. Schuetz, W. T. Beck and J. D. Schuetz, *Mol. Pharmacol.*, 1996, **49**, 311-318.
28. H. Huang, H. Jiang, K. Chen and H. Liu, *J. Org. Chem.*, 2009, **74**, 5476–5480.
29. I. F. F. Benzie and J. J. Strain, *Anal. Biochem.*, 1996, **239**, 70-76.
30. L. Schneider, S. Giordano, B. R. Zelickson, M. Johnson, G. Benavides, X. Ouyang, N. Fineberg, V. M. Darley-Usmar and J. Zhang, *Free Radic. Biol. Med.*, 2011, **51**, 2007–2017.
31. A. Simić, D. Manojlović, D. Šegan and M. Todorović, *Molecules*, 2007, **12**, 2327-2340.
32. M. Kageyama, K. Fukushima, T. Togawa, K. Fujimoto, M. Taki, A. Nishimura, Y. Ito, N. Sugioka, N. Shibata and K. Takada, *Biol. Pharm. Bull.*, 2006, **29**, 779-784.