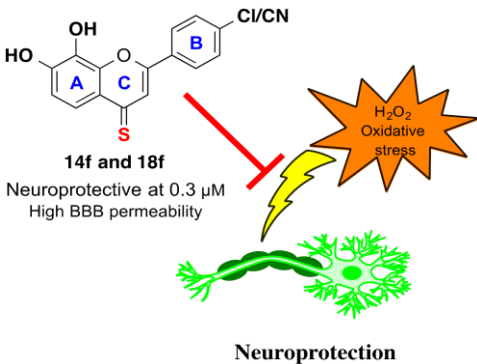


## Graphical Abstract

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<h3>Thioflavones as novel neuroprotective agents</h3>	Leave this area blank for abstract info.
<p>Divyashree Ravishankar<sup>a</sup>, Giulia Corona<sup>b,##</sup>, Stephanie M Hogan<sup>a</sup>, Jeremy P E Spencer<sup>b</sup>, Francesca Greco<sup>a,*</sup>, Helen M I Osborn<sup>a,*</sup></p>	
<div style="text-align: center;"><p><b>14f and 18f</b> Neuroprotective at 0.3 <math>\mu</math>M High BBB permeability</p><p><b>Neuroprotection</b></p></div>	



## Thioflavones as novel neuroprotective agents

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### ABSTRACT

Oxidative stress is associated with the pathology of neurodegenerative diseases. Identification of small molecules capable of protecting against oxidative stress is therefore of significant importance. In this context, a library of 76 hydroxy flavones, methoxy flavones and their 4-thio analogues has been evaluated for neuroprotection against H<sub>2</sub>O<sub>2</sub>-induced oxidative stress. This revealed the synthetic 7,8-dihydroxy 4-thioflavones as neuroprotective compounds, with **14d** and **18d** showing highest neuroprotective effects at lower concentrations (0.3 μM). Neuroprotection was found to be mediated via activation of the anti-apoptotic cell survival proteins of the ERK1/2 and PI3K/Akt pathways. Structure-activity relationship analysis revealed the B-ring phenyl group as essential for greater neuroprotection. Replacing the 4-C=O moiety with a 4-C=S moiety also generally enhanced neuroprotection.

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## 1 Introduction

Increased levels of oxidative stress are closely linked with many neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, and Amyotrophic Lateral Sclerosis<sup>1-3</sup>. Whilst antioxidants have received attention as potential agents for managing such conditions, some clinical trials using recognised antioxidants such as vitamin E, vitamin C, pure scavenger molecules such as boldine, and NMDA receptor blockers, have resulted in conflicting results and conclusions<sup>4,5</sup>. It is now believed that antioxidants not only reduce oxidative stress, but can also halt beneficial cellular processes. Therefore, it has been hypothesised that restoring the redox equilibrium by activation of intracellular signals involved in cell survival is more important<sup>6</sup> than solely restoring the redox equilibrium by direct scavenging of reactive oxygen species in the course of cellular oxidative stress. Hence, therapeutic strategies that aim to identify small molecules that confer neuroprotection against oxidative stress either by activating pro-survival regulatory pathways, or by increasing endogenous cellular antioxidant defences, may offer effective treatment for these neurodegenerative diseases<sup>7,8</sup>. Identification of novel neuroprotective agents with favourable pharmacokinetic profiles and CNS distribution is also of pivotal importance for a successful clinical translation.

Flavones, a subclass of flavonoids, are polyphenolic phytochemicals that have been well recognised for their diverse pharmacological activities including neuroprotective<sup>9,10</sup> activities. In particular, several epidemiological *in vitro* and *in vivo* studies have highlighted the potential of flavonoids as neuroprotective agents. For example, natural flavonoids such as fisetin<sup>8</sup>, luteolin,

quercetin, myricetin and hesperetin<sup>4,11,12</sup> have been reported to protect neurons against oxidative damage. Further, several synthetic flavones<sup>13,14</sup> and thioflavones<sup>15</sup> have been reported to limit neurodegeneration associated with a variety of neurological disorders, namely Alzheimer's disease<sup>16</sup> (AD) and Parkinson's disease<sup>17</sup> (PD). Interestingly, flavonoids have been reported to increase cell survival in an oxidative stress model where scavenging antioxidants (vitamin E, boldine) failed to protect cells from the oxidative insult<sup>4</sup>. Also, growing bodies of evidence have attributed the neuroprotective abilities of flavonoids to their signalling regulation abilities<sup>18-21</sup>. Further, studies focusing on the blood-brain barrier (BBB) permeability of flavonoids have highlighted that the lipophilic flavonoids possess greater BBB permeability than the polar flavonoids<sup>22,23</sup>; the permeability potency of the compounds also correlated with their lipophilicity (log P)<sup>24-26</sup>. With our interest in developing novel flavone derivatives as therapeutic agents, we had previously synthesised and characterised a library of 76 hydroxy flavones, methoxy flavones and their 4-thio derivatives<sup>27</sup> (Figure-1). Intrigued by the neuroprotective potentials of flavones and by the higher lipophilicity of 4-thioflavones and methoxy flavones than their corresponding hydroxy flavones, in this study, an assessment of the neuroprotective abilities of the library of 76 flavones presented herein was carried out. Also, due to the absence of a systematic investigation of the effect of substitution of the 4-carbonyl (4-C=O) group by a 4-thiocarbonyl (4-C=S) group on the neuroprotective activities of flavones, we aimed to explore the structure-activity relationships (SARs) of the library of 76 flavones presented herein

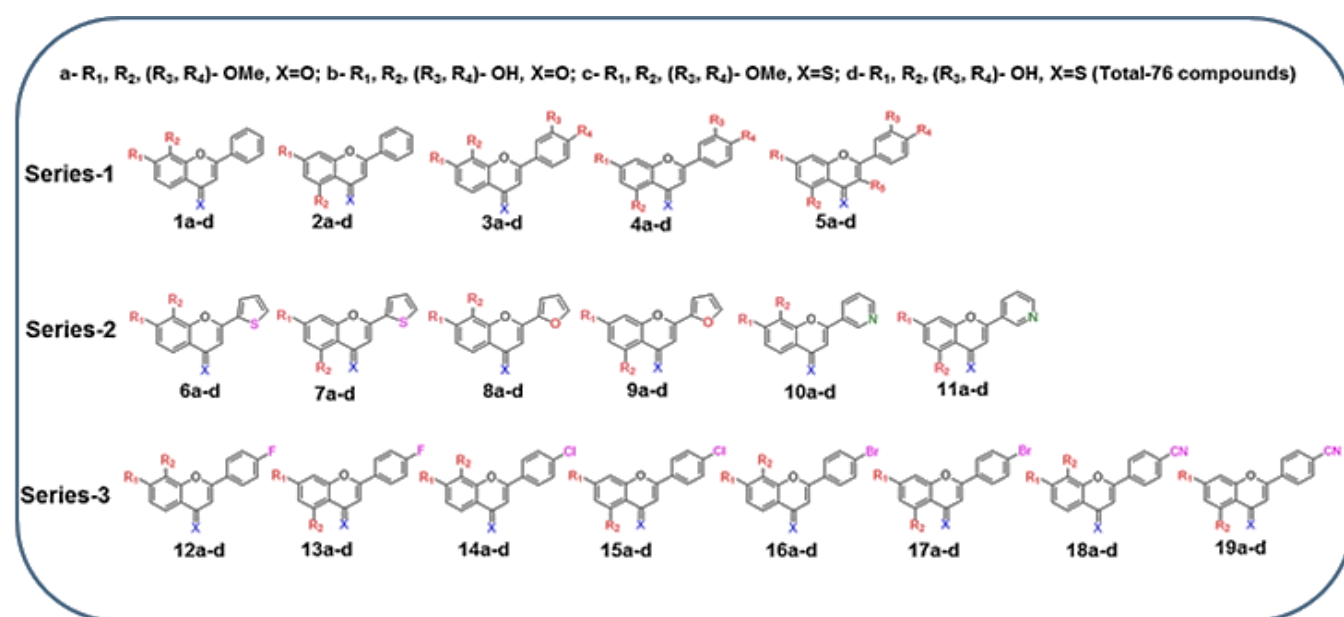


Figure 1. Structures of flavones investigated in this study.

## 2 Materials and methods

Please see supplementary information.

## 3 Results and discussion

### 3.1 Library design

As illustrated in Figure-1, the library of flavones is composed of three series. Each series contained methoxy flavones (designated **a**), hydroxy flavones (designated **b**), methoxy 4-thioflavones (designated **c**) and hydroxy 4-thioflavones (designated **d**). The compounds in series-1 (**1a-d** to **5a-d**) were derived from well-known flavones with different numbers and positions of hydroxyls. Series-2 was based on bioisosteric analogues of the B-ring of the active flavones from series-1 (**6a-d** to **11a-d**). In series-3 further functionalization was incorporated via the inclusion of electron-withdrawing groups (EWGs) onto the B-ring (**12a-d** to **19a-d**) (Figure-1). The purities of all compounds were established prior to evaluation, by reverse phase HPLC, and were found to be >95%.

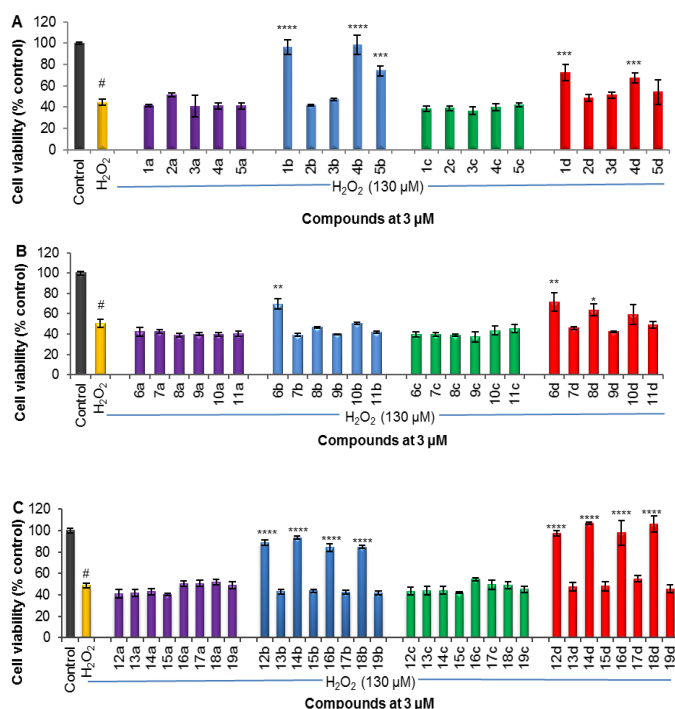
### 3.2 Neuroprotective evaluation

All compounds were evaluated for their ability to protect neurons from H<sub>2</sub>O<sub>2</sub>-induced oxidative stress in an *in vitro* model using the SH-SY5Y human neuroblastoma cell line<sup>28,29</sup>. An appropriate concentration of H<sub>2</sub>O<sub>2</sub> for inducing oxidative stress in SH-SY5Y cells was determined by exposing the SH-SY5Y cells to various concentrations of H<sub>2</sub>O<sub>2</sub> (5-500 μM) for 18 h. From the cell viability assessment, using the MTT assay, it was found that H<sub>2</sub>O<sub>2</sub> at 130 μM (IC<sub>50</sub> value) induced a 50% reduction in the SH-SY5Y cell viability (Supplementary info, Figure-S1). Hence, this concentration (130 μM) was used for further experiments to induce oxidative stress in SH-SY5Y cells. Next, the SH-SY5Y cells were treated with each of the compounds at the physiologically relevant concentration of 3 μM concentration<sup>30</sup> for 24 h prior to exposure to H<sub>2</sub>O<sub>2</sub> (18 h). The cell viability was then determined by the MTT assay (Figure-2).

By comparing the neuroprotective activities of series-1, series-2 and series-3 flavones (**1a-d** to **19c-d**) it was generally found that: -

- i) Flavones bearing catecholic hydroxyl (C-7,8 hydroxyl, *o*-hydroxy) substitutions either on ring-A or -B were significantly more neuroprotective than those with *m*-hydroxyl substitutions. For example **2b** and **2d** with C-5,7 hydroxyl (*m*-hydroxy) groups) were found to be inactive (cell viability < 50%) whereas **1b**, **4b** and **5b** with catecholic hydroxyl groups confer neuroprotection by restoring the cell viability > 80%. This suggests that the catecholic hydroxyl substitution is indispensable for neuroprotective activity.

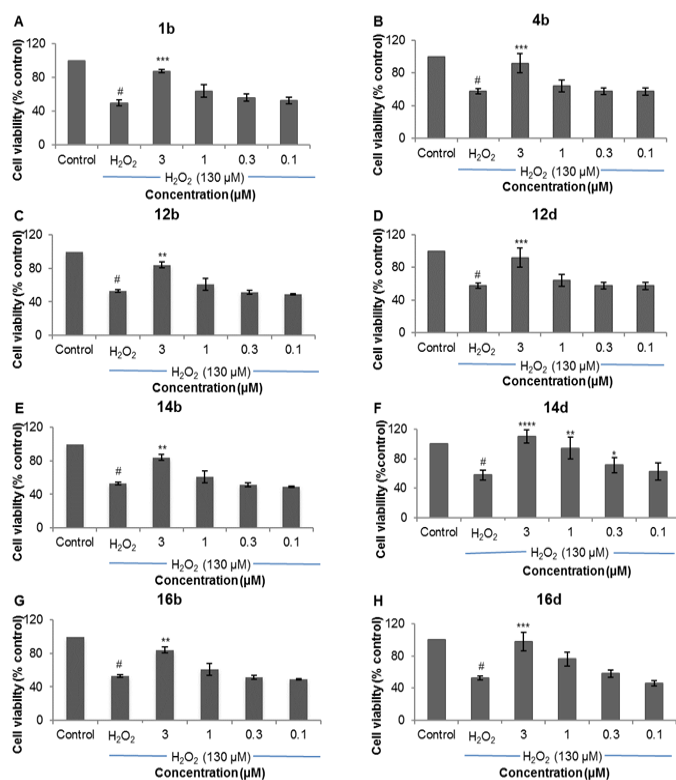
- ii) The B-ring phenyl group was vital for neuroprotective activity of catecholic flavones; replacing the B-ring phenyl group with its bioisosteres [series-2 (**6b**, **8b**, **10b**, **6d**, **8d** and **10d**); cell viability < 60%] was detrimental for neuroprotective activity but incorporation of electron withdrawing groups on the B-ring phenyl group [series-3 (**12b**, **14b**, **16b**, **12d**, **14d** and **18d**); cell viability > 85%] was beneficial.
- iii) Methoxy flavones were less neuroprotective than their analogous hydroxyl flavones.
- iv) For the synthetic flavones in series 2 and 3, the 4-thioflavones (C=S) [**12f** (cell viability = 97.4 ± 4.3%), **14f** (cell viability = 106.7 ± 1.2%), **16f** (cell viability = 97.8 ± 2.6%) and **18f** (cell viability = 105.8 ± 3.7%)] were significantly more neuroprotective than the flavones (C=O) [**12d** (cell viability = 88.8 ± 2.5%), **14d** (cell viability = 93.4 ± 1.4%), **16d** (cell viability = 84.0 ± 3.6%), and **18d** (cell viability = 84.6 ± 1.6%)]. However, the opposite trend was evident for the natural flavones in series 1 [**1d** (cell viability = 96.2 ± 1.3%) vs **1f** (cell viability = 72.4 ± 1.2%); **4d** (cell viability = 98.3 ± 2.1%) vs **4f** (cell viability = 67.4 ± 1.3%)].



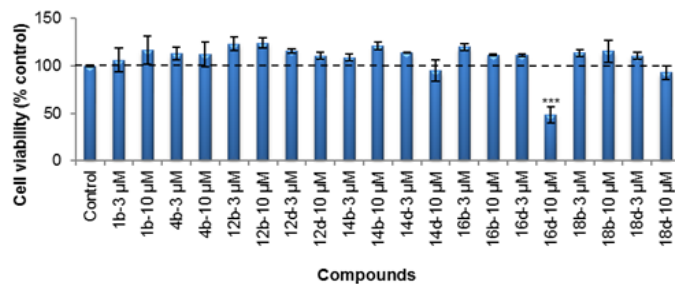
**Figure 2.** Neuroprotective effects of (A) Series-1 (**1a-d** to **5a-d**) (B) Series-2 (**6a-d** to **11a-d**) and (C) Series-3 (**12a-d** to **19a-d**) at 3 μM concentration against H<sub>2</sub>O<sub>2</sub>-induced oxidative stress in SH-SY5Y cells. Cells without treatment serve as control. Cell viability was measured by MTT assay. Statistical significance was estimated by one-way ANOVA followed by Bonferroni's *post hoc* test, (#)-significance with respect to the control ( $p < 0.0001$ ) and (\*)-significance with respect to H<sub>2</sub>O<sub>2</sub> (\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  and \*\*\*\* $p < 0.0001$ ). Colour coding: purple-methoxy flavone (-OMe, 4-C=O), blue-hydroxy flavone (-OH, 4-C=O), green-

methoxy 4-thioflavone (-OMe, 4-C=S) and red-hydroxy 4-thioflavone (-OH, 4-C=S).

Compounds **1b**, **4d**, **12b**, **12d**, **14b**, **14d**, **16b**, **16d**, **18b** and **18d** that exhibited the highest neuroprotective effects at the single dose of 3  $\mu\text{M}$  were further evaluated at lower concentrations to determine their ability to exert neuronal protection against  $\text{H}_2\text{O}_2$ -induced oxidative stress. Whilst no significant protective effects were observed at lower concentrations for **1b**, **4b**, **12b**, **12d**, **14b**, **16b**, compounds **14d** and **18d** were found to be effective even at 0.3  $\mu\text{M}$  concentration (cell viability >70%) (Figure-3). Importantly, compounds **1b**, **4d**, **12b**, **12d**, **14d**, **16b** and **18d** were found to be nontoxic to SH-SY5Y cells at 3  $\mu\text{M}$  and 10  $\mu\text{M}$  doses for 24 h (as evidenced using the MTT assay). However, compound **16d** caused 52% reduction in the cell viability at 10  $\mu\text{M}$ ,  $p < 0.001$  (Figure-4).



**Figure 3.** Neuroprotective effects of (A) Compound-**1b**, (B) Compound-**4b**, (C) Compound-**12b**, (D) Compound-**12d**, (E) Compound-**14b**, (F) Compound-**14d**, (G) Compound-**16b**, (H) Compound-**16d**, (I) Compound-**18b**, and (J) Compound-**18d** in a dose dependent manner in the concentration range 0.1-3  $\mu\text{M}$  against  $\text{H}_2\text{O}_2$ -induced oxidative stress in SH-SY5Y cells. Cells without treatment serve as control. Data are expressed as the mean  $\pm$  standard error of the mean (SEM) ( $n = 3$ ). Statistical significance was estimated by one-way ANOVA followed by Bonferroni's *post hoc* test, (#)-significance with respect to the control ( $p < 0.0001$ ) and (\*)-significance with respect to  $\text{H}_2\text{O}_2$  (\*\* $p < 0.01$  and \*\*\*  $p < 0.001$ ).



**Figure 4.** Toxic effects of compounds **1b**, **4d**, **12b**, **12d**, **14b**, **14d**, **16b**, **16d**, **18b** and **18d** evaluated at 3 and 10  $\mu\text{M}$  concentrations against SH-SY5Y cells. Cells without treatment serve as control. Data are expressed as the mean  $\pm$  standard error of the mean (SEM) ( $n = 3$ ). Statistical significance was estimated with respect to the control by one-way ANOVA, followed by Bonferroni's *post hoc* test (\*\* $p < 0.001$ ).

### 3.3 Molecular mechanism of neuroprotection

Flavones can act either as a free radical scavenger (antioxidant) or can trigger intracellular pathways for cell survival. Therefore, to gain insight into the mechanism of neuroprotection, their antioxidant potentials and impact on certain intracellular signalling targets were explored.

#### 3.3.1 Antioxidant activity

Flavones are very well known for their antioxidant activities<sup>31,32</sup>, therefore the antioxidant properties of flavones **1b** (7,8-dihydroxy flavone), **4b** (luteolin, a well-known natural flavone) and compound-**14b** with a 4-C=O moiety, as well as the most neuroprotective 4-thioflavones (with 4-C=S) **14d**, **16d** and **18d** were studied. For this, the flavones were evaluated at their neuroprotective concentration of 3  $\mu\text{M}$  both for their ability to directly interact with free radicals (primary antioxidant activity) using a DPPH free radical scavenging assay and for their ability to bind to ferrous ( $\text{Fe}^{2+}$ ) ion that catalyses oxidation (secondary antioxidant activity), using a metal chelating assay (Table-1). As shown in Table 1, all the aforementioned flavones showed very low scavenging activity (only up to 1.9% inhibition of DPPH radical) at 3  $\mu\text{M}$ . Also, based on previous reports on DPPH scavenging data for the well-known flavones (**1b** and **4d**, Series-1), no correlation was found between the order of their neuroprotective abilities [**1b** (Cell viability-97%) > **4d** (Cell viability-73%,  $p < 0.05$ )] and the order of their scavenging activity [DPPH radical scavenging activity-  $\text{IC}_{50}$ -**4d** ( $11.04 \pm 0.38 \mu\text{M}$ ) > **1b** ( $15.50 \pm 0.12 \mu\text{M}$ )]<sup>33</sup>. Since, in general, compounds with catechol groups are defined as effective metal chelators<sup>34-36</sup>, the iron-chelating ability of flavones **1b**, **4d**, **14b**, **14d**, **16d** and **18d** at 3  $\mu\text{M}$  was further studied. Interestingly, a low degree of  $\text{Fe}^{2+}$  chelation (only up to 5%) was exhibited by these flavones at 3  $\mu\text{M}$  concentration (Table 1).

Taken together, there was no observed correlation between the neuroprotective profiles and the antioxidant activities of flavones at their tested concentration. Therefore, it is possible that the flavones directly interact with cellular events leading to cell death after oxidative stress. Hence, the intracellular



signalling pathways triggered by flavones in oxidative-stress induced SH-SY5Y cells were probed.

**Table 1. Antioxidant activities of flavones**

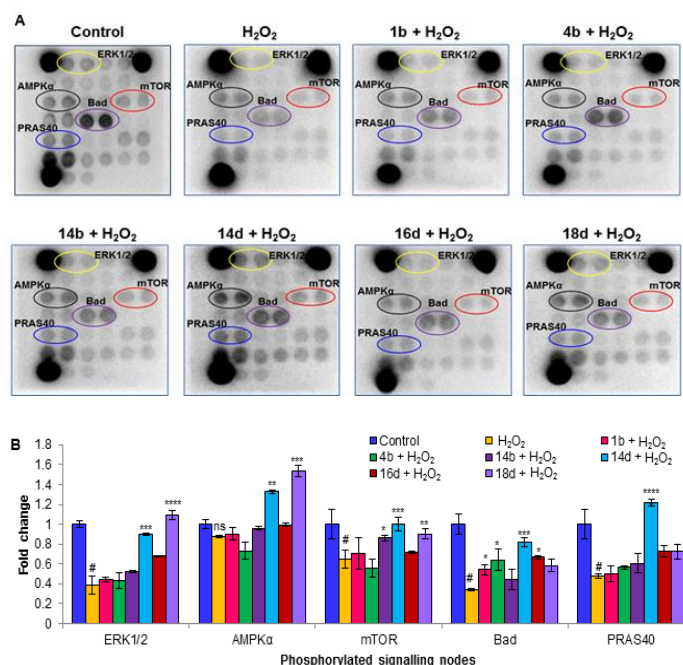
Flavone	Antioxidant activity at 3 $\mu\text{M}$ concentration <sup>a</sup>	
	DPPH scavenging assay (Scavenging activity, %)	Metal chelating assay (Fe <sup>2+</sup> chelating activity, %)
1b	1.4 $\pm$ 0.2	3.7 $\pm$ 1.0
4b	1.9 $\pm$ 0.2	3.4 $\pm$ 0.4
14b	1.4 $\pm$ 0.5	3.7 $\pm$ 1.2
14d	1.7 $\pm$ 0.2	5.0 $\pm$ 1.6
16d	1.6 $\pm$ 0.6	4.4 $\pm$ 1.7
18d	1.3 $\pm$ 0.2	3.9 $\pm$ 0.4
Ascorbic acid <sup>b</sup>	1.3 $\pm$ 0.5	-
EDTA <sup>c</sup>	-	6.9 $\pm$ 0.4

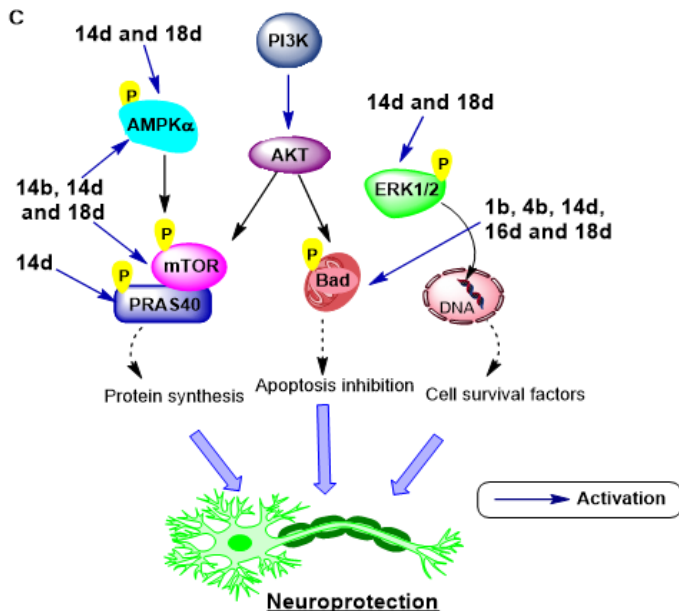
<sup>a</sup> Data expressed as Mean  $\pm$  SEM, n = 3; <sup>b</sup>Ascorbic acid was used as a reference standard for the DPPH scavenging assay, IC<sub>50</sub> value of ascorbic acid for the inhibition of DPPH radical formation was established to be 330  $\pm$  0.5  $\mu\text{M}$  (Supplementary info, Figure-S2); <sup>c</sup>EDTA was used as a reference standard for the metal chelating assay, IC<sub>50</sub> value of EDTA in metal chelating assay was determined to be 42  $\pm$  0.8  $\mu\text{M}$  (Supplementary info, Figure-S3).

### 3.3.2 Intracellular signalling

Accumulative evidence has shown that flavonoids display signalling properties during neuroprotection<sup>12,21,37-40</sup>. Hence, the potential intracellular signalling involved in the neuroprotective function of flavones were probed. For this, the signal mediated in SH-SY5Y cells by the well-known compounds **1b**, **4b** (luteolin) and compound-**14b** with a 4-C=O moiety, as well as the neuroprotective 4-thioflavones (with 4-C=S) **14d**, **16d** and **18d** were studied. Pooled samples from three independent treatments of SH-SY5Y cells with and without H<sub>2</sub>O<sub>2</sub> (130  $\mu\text{M}$ ), and with these compounds at 3  $\mu\text{M}$  concentration for 24 h followed by the exposure to H<sub>2</sub>O<sub>2</sub> (1 h)<sup>38</sup>, were analysed using the PathScan® Intracellular Signalling Array Kit (Figure-5). These compounds were found to modulate the signalling molecules that are associated with cellular survival and apoptosis such as ERK1/2, mTOR, AMPK $\alpha$  and Akt targets such as Bad and PRAS40. Treatment of SH-SY5Y cells with H<sub>2</sub>O<sub>2</sub> (130  $\mu\text{M}$ ) resulted in a marked reduction in phosphorylation of ERK1/2, mTOR, Bad and PRAS40. Pre-treatment with 7,8-dihydroxy flavones (containing 4-C=O) showed that compounds **1b** and **4b** were able to confer neuroprotection by the inhibition of apoptosis through restoration of Bad (a pro-apoptotic protein) phosphorylation (by inactivating its apoptotic activity), whereas, compound **14b** was shown to elicit its activity through restoration of mTOR phosphorylation, which restores protein synthesis. In the case of 4-thioflavones, compound **14d** significantly restored phosphorylation of ERK1/2, mTOR, Bad and PRAS40 up to the same levels or higher than that observed in the control, along with activation of AMPK $\alpha$ . Also, compound **18d** was found to restore phosphorylation of

ERK1/2 and mTOR, along with activation of AMPK $\alpha$ , however, compound **16d** showed restoration of Bad phosphorylation only. These results support the *in vitro* observations and suggest that the neuroprotective effects of flavones are mediated via ERK1/2 and PI3K/Akt/mTOR pathways and that these flavones differentially activate the pro-survival protein kinases based on their chemical structure. Also, a comparison of intracellular signalling of the 4-thioflavone **14d** with its corresponding flavone **14b** highlights the beneficial influence of 4-C=S substitution on the neuroprotective activity. Thus, the enhanced neuroprotective effects of compounds **14d** and **18d** can be attributed to their potential to modulate multiple signalling targets. As several studies have highlighted compound-**1b** as a promising small molecular BDNF mimetic, with selective TrkB (Tropomyosin-related kinase B) receptor agonist activity<sup>41-47</sup>, it may be pertinent to explore the roles of compounds **14d** and **18d** in the modulation of the TrkB receptor in the future.

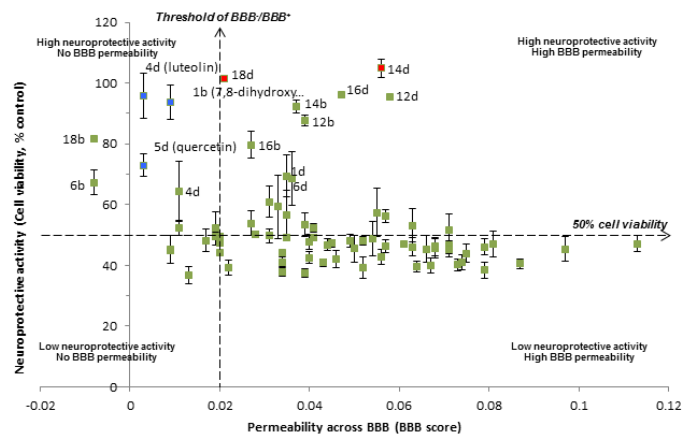




**Figure 5.** (A) Chemiluminescent array images of the PathScan Intracellular Signalling array kit revealing various phosphorylated in SH-SY5Y cells pre-treated with and without compounds **1b**, **4b**, **14b**, **14d**, **16d** and **18d** at 3  $\mu$ M for 24 h before exposure to  $H_2O_2$  (130  $\mu$ M). Cells without treatment serve as control. (B) Bar chart representing the fold change in the integrated density of phosphorylated signalling nodes in the array image of SH-SY5Y cells pre-treated with and without compounds **1b**, **4b**, **14b**, **14d**, **16d** and **18d** at 3  $\mu$ M for 24 h before exposure to  $H_2O_2$  (130  $\mu$ M). Data are expressed as the mean  $\pm$  standard error of the mean (SEM) (n = 4). Statistical significance was estimated with respect to the control (#) and with respect to  $H_2O_2$  (\*) by one-way ANOVA, followed by Bonferroni's *post hoc* test (ns-not significant, \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  and \*\*\*\* $p < 0.0001$ ). (C) Molecular mechanism of action of **1b**, **4b**, **14b**, **14d**, **16d** and **18d** in SH-SY5Y cell line.

### 3.3.3 In silico prediction of BBB permeability

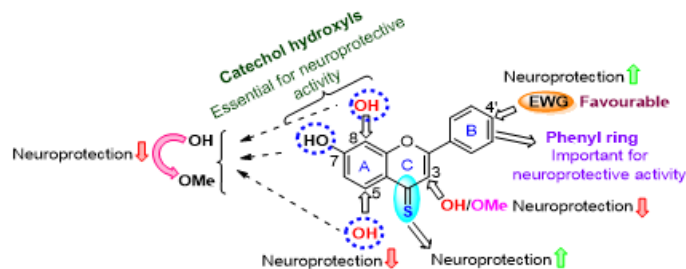
For the flavones to be able to exert their neuroprotective effect clinically, permeation across the BBB is required. Therefore, BBB permeability scores were calculated for each flavone. These BBB permeability scores were then plotted against the neuroprotective ability to identify the most promising candidates (i.e. compounds with both high predicted permeation across the BBB and high neuroprotective activity, Figure-6). This showed that synthetic thioflavone compounds **14d** and **18d** possess both high neuroprotective ability (cell viability > 95%, neuroprotective even at 0.3  $\mu$ M concentration) and high BBB permeability (BBB score > 0.02). In contrast, the natural/well-known flavones such as 7,8-dihydroxy flavone (**1b**), luteolin (**4b**) and quercetin (**5b**) were found to be neuroprotective but their poor BBB permeability scores might undermine the likelihood of achieving sufficiently high concentrations in the central nervous system. Therefore, the synthetic thioflavones **14d** and **18d** can be considered as lead candidates for further design and development of neuroprotective agents.



**Figure 6.** Graph represents BBB permeability of compounds (predicted BBB score) versus neuroprotective activity (cell viability) determined at 3  $\mu$ M using the SH-SY5Y neuroblastoma cell line. The dashed line at  $x = 0.02$  represents the threshold BBB-/BBB+ score and the dashed line at  $y = 50$  represents 50% cell viability in SH-SY5Y cells exposed to  $H_2O_2$ . Neuroprotective natural flavone/well-known flavones are highlighted in blue and the thioflavones that have been identified to be neuroprotective are highlighted in red. Lower left quadrant represents low neuroprotective activity and no BBB permeability, lower right quadrant represents low neuroprotective activity and high BBB permeability, upper left quadrant represents high neuroprotective activity and no BBB permeability and upper right quadrant represents high neuroprotective activity and high BBB permeability.

## 4 Conclusion

In summary, evaluation of the neuroprotective properties of structurally related methoxy flavones, hydroxy flavones and their 4-thio analogues has provided significant insights into the SARs of flavones, as summarised in Figure 7.



**Figure 7.** Summary of the SARs on the neuroprotective activities of flavones.

Overall, 7,8-dihydroxy 4-thioflavones such as compounds **14d** and **18d** were found to exhibit potent neuroprotective effects against  $H_2O_2$ -induced oxidative stress with their activity being restored even at 0.3  $\mu$ M concentration. For **14d** and **18d**, high BBB permeability is also predicted. Investigations of the molecular mechanism of action of **14d** and **18d** indicated that these compounds preferably mediate their neuroprotective effects through suppression of apoptosis by activating the anti-apoptotic proteins, and inactivating the pro-apoptotic proteins of the ERK1/2 and PI3K/Akt/mTOR pathways that are deregulated in AD<sup>48</sup> and PD<sup>49</sup>. As such,

small molecules have been identified that confer neuroprotection against oxidative stress by activating pro-survival regulatory pathways. Therefore, the synthetic flavones **14d** and **18d** can be considered as promising candidates for further optimisation and development as neuroprotective agents. In this regard, future studies that will decipher the pharmacokinetic and pharmacodynamic properties of these synthetic compounds will further guide the optimisation of these candidates for neuroprotective applications.

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## Abbreviations

MTT-3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, DPPH-2,2-diphenyl-1-picrylhydrazyl, EDTA-Ethylenediaminetetraacetic acid, ERK1/2-extracellular signal-regulated kinase, mTOR-mammalian target of rapamycin, AMPK $\alpha$ -5' adenosine monophosphate-activated protein kinase, GSK-3 $\beta$ - Glycogen synthase kinase-3 $\beta$ , Bad-Bcl-2-associated death promoter, PRAS40-proline-rich Akt substrate of 40 kDa, PI3K-Phosphatidylinositol-4,5-bisphosphate-3-kinase, BDNF-brain-derived neurotrophic factor.

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## Notes

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## Supplementary Material

Supporting Information Available: This includes materials and methods, the dose-dependent curves of H<sub>2</sub>O<sub>2</sub>, ascorbic acid and EDTA. Theoretical BBB scores calculated using the online prediction tools are also given.

