SYNTHESIS, PHYSICOCHEMICAL CHARACTERIZATION AND NEUROPROTECTIVE EVALUATION OF NOVEL 1-HYDROXYPRAZIN-2(1H)-ONE IRON CHELATORS IN AN IN VITRO CELL MODEL OF PARKINSON’S DISEASE

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1. Organic Synthesis

Discussion

The target 1-hydroxy pyrazin-2(1H)-ones 6 were synthesized in two steps from amino acid ethyl esters following the literature procedures as shown below in Scheme S1. Initially, reaction of glycine ethyl ester hydrochloride 4a with hydroxylamine hydrochloride in alkaline water afforded the known glycine hydroxamic acid 5a in 64 % yield. Condensation reaction of 5a with 2,3-butanedione afforded the known 1-hydroxy pyrazin-2(1H)-one 6a in 24 % yield (Scheme S1). Unfortunately, application of this two-step procedure to the synthesis of 6b from alanine ethyl ester 4b failed to give the desired product, due to the high solubility of the hydroxamic acid 5b in water. We subsequently modified this procedure by using methanol as the solvent and we were able to obtain 5a from 4a in 56 % yield (Scheme S1). However, application of this modified procedure to the synthesis of alanine hydroxamic acid 5b from alanine ethyl ester 4b gave a mixture of 5b and another compound (presumed to be the corresponding diketopiperazine) in low yield as judged by 1H NMR spectroscopy. Reaction of this mixture with 2,3-butanedione gave an intractable mixture of products from which the novel 1-hydroxy pyrazin-2(1H)-one 6b could not be isolated by chromatography. However, 1-hydroxy pyrazin-2(1H)-ones 6c and 6d were successfully obtained by this modified procedure from the known hydroxamic acids 5c and 5d, albeit in only 13 % and 14 % overall yields from 4c and 4d, respectively (Scheme S1). There are some reports of multifunctional hydroxypyridinone metal chelators containing phenolic antioxidant moieties that show promising efficacy against neurodegenerative diseases by acting as radical traps as well as metal chelators. Accordingly, we synthesized 1-hydroxy pyrazin-2(1H)-one 6d that contains a phenol moiety which could provide a beneficial antioxidant mode of action in addition to iron chelation. Unfortunately, all our attempts to isolate hydroxamic acids 5e–5g from amino esters 4e–4g met with no success.
Due to the low yields obtained above and the failure to synthesize certain 1-hydroxypyrazin-2(1H)-ones 6 by the procedure shown in Scheme S1, we sought a more general synthetic method which could be applied to the synthesis of a broader range of these compounds. The synthesis of 1-hydroxypyrazin-2(1H)-ones 6 in 4 steps from N-Boc amino acids via their protected hydroxamic acid benzyl esters was previously reported.\textsuperscript{3–5,10} Inspired by this approach, we explored a new synthesis of 1-hydroxypyrazin-2(1H)-ones 6 from activated N-Boc amino acid N-hydroxysuccinimide esters 7 as shown below in Scheme S2.

Reaction of N-Boc-protected N-hydroxysuccinimide esters 7a, 7b, 7c, 7e and 7f with O-benzylhydroxylamine generated the Boc-protected aminohydroxamic acid benzyl esters 8a, 8b, 8c, 8e and 8f in high yields. Subsequent N-Boc deprotection (TFA in DCM) gave the free aminohydroxamic acid benzyl esters 9b, 9c, 9e and 9f in excellent yields. However, despite the known formation of 6’a from 9a (as HCl salt) and 2,3-butanedione reported in the literature,\textsuperscript{5,11} attempted condensation reactions of compounds 9b, 9c, 9e and 9f with 2,3-butanedione in our hands failed to generate the desired 1-benzyloxypyrazin-2(1H)-ones 6’b, 6’c, 6’e and 6’f. This synthetic approach was subsequently abandoned in favour of the approach outlined above in Scheme S1.
We also explored the reactions of glycine hydroxamic acid 5a with both aromatic and aliphatic \( \alpha \)-ketoaldehydes (glyoxals) as shown below in Scheme S3. Reaction of 5a with phenylglyoxal in ethanol/water at reflux afforded the novel 1-hydroxyprazin-2(1\( H \))-one 10a in 30% yield as a single regioisomer. Similarly, reaction of 5a with 4-methoxyphenylglyoxal and 4-fluorophenylglyoxal gave 10b and 10c as single regioisomers in 27% and 24% yields, respectively. As with 1-hydroxyprazin-2(1\( H \))-one 6d, we sought to convert 10b into a 1-hydroxyprazin-2(1\( H \))-one bearing a phenol moiety with potential antioxidant activity. Accordingly, deprotection of the methoxy group of 10b with boron tribromide in DCM afforded the novel 1-hydroxyprazin-2(1\( H \))-one 10d in 21% yield. Reaction of 5a with pyruvaldehyde gave the novel 1-hydroxyprazin-2(1\( H \))-ones 11a and 12a as a 12:1 mixture of regioisomers, as judged by \( ^1 \)H NMR spectroscopy (Scheme S3). The major regioisomer was tentatively assigned as 11a on the basis that the free primary amino group of 5a would preferentially react with the aldehyde carbonyl group of the glyoxal, rather than the less electrophilic ketone carbonyl group. These regioisomers proved inseparable by recrystallisation or chromatography, and were studied without further purification.
Scheme S3. Synthesis of 1-hydroxypyrizin-2(1H)-ones 10a–10d, 11a and 12a.
2. Experimental Procedures

Synthesis of \( N \)-Boc hydroxamic acid benzyl esters 8a–8f: General procedure

To a solution of the appropriate \( N \)-Boc amino acid \( N \)-hydroxysuccinimide (OSu) ester 7 (1.47 mmol) in DCM (20 mL) at room temperature was added \( O \)-benzylhydroxylamine (1.47 mmol, 1 eq). The solution was allowed to stir at room temperature for 24 hours. The solvent was evaporated to afford the crude \( N \)-Boc hydroxamic acid benzyl ester 8 as an oil that crystallised contaminated with \( N \)-hydroxysuccinimide. This mixture was used in the next step without further purification.

\( N \)-Boc glycine hydroxamic acid benzyl ester 8a
\( \delta_H(399.8 \text{ MHz}, \text{CDCl}_3, \text{Me}_4\text{Si}) 1.40 \text{ (9H, s, } (\text{CH}_3)_3\text{)}, \ 3.67 \text{ (2H, s, } CH_2\text{NHBoc)}\), 4.82 (1H, br s, NH), 4.88 (2H, s, OCH\textsubscript{2}Ph), 5.25 (1H, br s, NHBoc), 7.36 (5H, s, ArH).

\( N \)-Boc alanine hydroxamic acid benzyl ester 8b
\( \delta_H(399.8 \text{ MHz}, \text{CDCl}_3, \text{Me}_4\text{Si}) 1.28 \text{ (3H, d, } J 6.8, \text{CH}_3\text{CH)}, \ 1.38 \text{ (9H, s, } (\text{CH}_3)_3\text{)}, \ 4.05 \text{ (1H, app t, } J 6.8, \text{CH}_3CH)\), 4.86 (2H, s, OCH\textsubscript{2}Ph), 5.29 (1H, d, J 6.4, NHBoc), 7.27–7.36 (5H, m, ArH).

\( N \)-Boc phenylalanine hydroxamic acid benzyl ester 8c
\( \delta_H(399.8 \text{ MHz}, \text{CDCl}_3, \text{Me}_4\text{Si}) 1.36 \text{ (9H, s, } (\text{CH}_3)_3\text{)}, \ 2.96–3.06 \text{ (2H, m, CHC}H_2\text{Ph)}, \ 4.20 \text{ (1H, q, } J 7.6, \text{CH}_3CH)\), 4.86 (2H, s, OCH\textsubscript{2}Ph), 5.26 (1H, d, J 7.6, CHNHBoc), 7.17–7.36 (10H, m, ArH).

\( N \)-Boc valine hydroxamic acid benzyl ester 8e
\( \delta_H(399.8 \text{ MHz}, \text{CDCl}_3, \text{Me}_4\text{Si}) 0.88 \text{ (3H, d, } J 6.4, \text{ } (\text{CH}_3)_2\text{CH)}, \ 0.90 \text{ (3H, d, } J 6.4, \text{ } (\text{CH}_3)_2\text{CH)}, \ 1.39 \text{ (9H, s, } (\text{CH}_3)_3\text{)}, \ 1.96–2.02 \text{ (1H, m, } (\text{CH}_3)_2\text{CH})\), 3.70 (1H, t, J 8.4, CHNHBoc), 4.88 (2H, s, OCH\textsubscript{2}Ph), 5.28 (1H, d, J 8.4, CHNHBoc), 7.28–7.37 (5H, m, ArH), 9.45 (1H, br s, NHOCH\textsubscript{2}Ph).

\( N \)-Boc leucine hydroxamic acid benzyl ester 8f
\( \delta_H(399.8 \text{ MHz}, \text{CDCl}_3, \text{Me}_4\text{Si}) 0.85–0.87 \text{ (6H, m, } (\text{CH}_3)_2\text{CH)}, \ 1.39 \text{ (9H, s, } (\text{CH}_3)_3\text{)}, \ 1.41–1.47 \text{ (1H, m, } (\text{CH}_3)_2\text{CH})\), 1.54–1.60 (2H, m, \text{CH}_2\text{CH}(\text{CH}_3)_2), ...
3.97 (1H, q, J 8.0, CHNHBOc), 4.88 (2H, s, OCH<sub>2</sub>Ph), 5.17 (1H, d, J 8.0, CHNHBOc), 7.24–7.37 (5H, m, ArH), 9.45 (1H, br s, NHOC<sub>2</sub>Ph).

**Synthesis of hydroxamic acid benzyl ester TFA salts 9b–9f: General procedure**

![Chemical structure](image)

The appropriate crude N-Boc hydroxamic acid benzyl ester 8 (1.47 mmol) was dissolved in DCM (10 mL) and trifluoroacetic acid (10 mL) was added. The solution was allowed to stir at room temperature for 24 hours. The solvents were evaporated to afford the crude TFA salt 9 as a clear oil. The oil was triturated with diethyl ether (10 mL) and the resulting white solid was filtered and washed with diethyl ether (10 mL) and allowed to dry in air to afford the pure TFA salt 9 as a white solid.

**Alanine hydroxamic acid benzyl ester TFA salt 9b**<sup>4,11</sup> Obtained from 7b in 87% overall yield.

δ<sup>H</sup>(399.8 MHz, DMSO-d<sub>6</sub>) 1.24 (3H, d, J 7.2, C<sub>H</sub>CH), 3.64 (1H, br s, CH<sub>3</sub>C<sub>H</sub>), 4.77 (1H, d, J 11.2, OC<sub>H</sub>Ph), 4.81 (1H, d, J 11.2, OCH<sub>2</sub>Ph), 7.34–7.38 (5H, m, ArH).

**Phenylalanine hydroxamic acid benzyl ester TFA salt 9c**<sup>12,13</sup> Obtained from 7c in 78% overall yield.

δ<sup>H</sup>(399.8 MHz, D<sub>2</sub>O) 2.91 (1H, dd, J 14.0 and 8.4, CHCH<sub>2</sub>Ph), 2.99 (1H, dd, J 14.0 and 6.8, CHCH<sub>2</sub>Ph), 3.82 (1H, dd, J 8.4 and 6.8, CHCH<sub>2</sub>Ph), 4.41 (1H, d, J 11.0, OCH<sub>2</sub>Ph), 4.59 (1H, d, J 11.0, OCH<sub>2</sub>Ph), 7.06–7.13 (4H, m, ArH), 7.20–7.26 (6H, m, ArH).

**Valine hydroxamic acid benzyl ester TFA salt 9e**<sup>12,13</sup> Obtained from 7e in 91% overall yield.

δ<sup>H</sup>(399.8 MHz, D<sub>2</sub>O) 0.69 (3H, d, J 6.4, (CH<sub>3</sub>)<sub>2</sub>CH), 0.73 (3H, d, J 6.4, (CH<sub>3</sub>)<sub>2</sub>CH), 1.88 (1H, sp, J 6.4, (CH<sub>3</sub>)<sub>2</sub>CH), 3.38 (1H, d, J 6.4, CHNH<sub>3</sub>), 4.75 (1H, d, J 11.2, OCH<sub>2</sub>Ph), 4.80 (1H, d, J 11.2, OCH<sub>2</sub>Ph), 7.27–7.32 (5H, m, ArH).

**Leucine hydroxamic acid benzyl ester TFA salt 9f**<sup>11</sup> Obtained from 7f in 84% overall yield. δ<sup>H</sup>(399.8 MHz, D<sub>2</sub>O) 0.65 (3H, d, J 6.0, (CH<sub>3</sub>)<sub>2</sub>CH), 0.67 (3H, d, J 6.0, (CH<sub>3</sub>)<sub>2</sub>CH), 1.01–1.10 (1H, m, (CH<sub>3</sub>)<sub>2</sub>CH), 1.37 (2H, t, J 7.2, CH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>), 3.56 (1H, t, J 7.2, CHNH<sub>3</sub>), 4.74 (1H, d, J 11.2, OCH<sub>2</sub>Ph), 4.82 (1H, d, J 11.2, OCH<sub>2</sub>Ph), 7.25–7.34 (5H, m, ArH).
3. NMR Spectra

Glycine hydroxamic acid 5a
Alanine hydroxamic acid 5b
Phenylalanine hydroxamic acid 5c
Tyrosine hydroxamic acid 5d
1-Hydroxy-5,6-dimethylpyrazin-2(1H)-one 6a
1-Hydroxy-3-benzyl-5,6-dimethylpyrazin-2(1H)-one 6c
1-Hydroxy-3-(4-hydroxybenzyl)-5,6-dimethylpyrazin-2(1H)-one 6d
$N$-Boc glycine hydroxamic acid benzyl ester 8a
N-Boc alanine hydroxamic acid benzyl ester 8b
N-Boc phenylalanine hydroxamic acid benzyl ester 8c
N-Boc valine hydroxamic acid benzyl ester 8e
N-Boc leucine hydroxamic acid benzyl ester 8f
Alanine hydroxamic acid benzyl ester TFA salt 9b
Phenylalanine hydroxamic acid benzyl ester TFA salt 9c
Valine hydroxamic acid benzyl ester TFA salt 9e
Leucine hydroxamic acid benzyl ester TFA salt 9f
1-Hydroxy-6-phenylpyrazin-2(1H)-one 10a
1-Hydroxy-6-(4-methoxyphenyl)-pyrazin-2(1H)-one 10b
1-Hydroxy-6-(4-fluorophenyl)-pyrazin-2(1H)-one 10c
1-Hydroxy-6-(4-hydroxyphenyl)-pyrazin-2(1H)-one 10d
1-Hydroxy-6-methylpyrazin-2(1H)-one 11a and 1-Hydroxy-5-methylpyrazin-2(1H)-one 12a
+ regioisomer
4. Mass Spectra

1-Hydroxy-3-(4-hydroxybenzyl)-5,6-dimethylpyrazin-2(1H)-one 6d
1-Hydroxy-6-phenylpyrazin-2(1H)-one 10a
1-Hydroxy-6-(4-methoxyphenyl)-pyrazin-2(1H)-one 10b
1-Hydroxy-6-(4-fluorophenyl)-pyrazin-2(1H)-one 10c
1-Hydroxy-6-(4-hydroxyphenyl)-pyrazin-2(1H)-one 10d
1-Hydroxy-6-methylpyrazin-2(1H)-one 11a and 1-Hydroxy-5-methylpyrazin-2(1H)-one 12a
5. Determination of pKa Values of the Ligands and Stability Constants of the Complexes

Protonation studies with ligand 11a

Figure S1. Spectrophotometric titrations vs pH of ligand 11a between (A) −0.5 < pH < 2.08 (batch titration, [11a] = 2.57 × 10⁻⁴ M) and (B) 2.61 < pH < 10.17 (direct titration, [11a] = 2.55 × 10⁻⁴ M). (C) Electronic spectra and (D) distribution curves ([11a] = 2.55 × 10⁻⁴ M) of the protonated species of ligand 11a. Solvent: H₂O, I = 0.1 M (NaClO₄), T = 25.0 °C.
Protonation studies with ligand 10a in water

**Figure S2.** Spectrophotometric titrations vs pH of ligand 10a between (A) $-0.75 < \text{pH} < 2$ (batch titration, $[10a] = 1.95 \times 10^{-4}$ M) and (B) $2.12 < \text{pH} < 11.79$ (direct titration, $[10a] = 1.02 \times 10^{-4}$ M). (C) Electronic spectra and (D) distribution curves ($[10a] = 1.95 \times 10^{-4}$ M) of the protonated species of ligand 10a. Solvent: H$_2$O, $I = 0.1$ M (NaClO$_4$), $T= 25.0 \text{ °C}$. 
Protonation studies with ligand 10a in MeOH/H₂O (80/20 w/w)

Figure S3. Spectrophotometric titrations vs pH of ligand 10a between (A) −0.37 < pH < 0.63 (batch titration, [10a] = 1.02 × 10⁻⁴ M) and (B) 2.43 < pH < 11.88 (direct titration, [10a] = 1.01 × 10⁻³ M). (C) Electronic spectra and (D) distribution curves ([10a] = 1.02 × 10⁻⁴ M) of the protonated species of ligand 10a. Solvent: MeOH/H₂O (80/20 w/w), I = 0.1 M (NaClO₄), T= 25.0 °C.
Protonation studies with ligand 6d

Figure S4. Spectrophotometric titrations vs pH of ligand 6d between (A) −0.36 < pH < 2.36 (batch titration, [6d] = 3.0 × 10^{-4} M) and (B) 1.78 < pH < 11.47 (direct titration, [6d] = 9.98 × 10^{-5} M). (C) Electronic spectra and (D) distribution curves ([6d] = 3.0 × 10^{-4} M) of the protonated species of ligand 6d. Solvent: MeOH/H2O (80/20 w/w), I = 0.1 M (NaClO4), T= 25.0 °C.
Protonation studies with ligand 6c

Figure S5. Spectrophotometric titrations vs pH of ligand 6c between (A) −0.04 < pH < 2.11 (batch titration, [6c] = 3.0 × 10⁻⁴ M) and (B) 2.49 < pH < 11.74 (direct titration, [6c] = 9.98 × 10⁻⁵ M). (C) Electronic spectra and (D) distribution curves ([6c] = 1.54 × 10⁻⁴ M) of the protonated species of ligand 6c. Solvent: MeOH/H₂O (80/20 w/w), I = 0.1 M (NaClO₄), T= 25.0 °C.
Fe$^{3+}$ complexation studies with ligand 6a

Figure S6. Spectrophotometric titration vs pH of Fe$^{3+}$ complexes of ligand 6a between (A) $-0.5 \leq \text{pH} \leq 1.25$ (batch titration, $[6a] = 3.78 \times 10^{-4}$ M, $[\text{Fe}^{3+}] = 1.26 \times 10^{-4}$ M) and (B) $1.97 \leq \text{pH} \leq 12.04$ (direct titration, $[6a] = 2.38 \times 10^{-4}$ M, $[\text{Fe}^{3+}] = 7.14 \times 10^{-5}$ M). (C) Electronic spectra and (D) distribution curves ($[6a] = 2.38 \times 10^{-4}$ M, $[\text{Fe}^{3+}] = 7.14 \times 10^{-5}$ M) of the Fe$^{3+}$ complexes of 6a.

Solvent: $\text{H}_2\text{O}$, $I = 0.1$ M (NaClO$_4$), $T= 25.0 \, ^\circ\text{C}$. 
**Fe$^{3+}$ complexation studies with ligand 10a**

Figure S7. Spectrophotometric titration vs pH of Fe$^{3+}$ complexes of ligand 10a between (A) $-0.36 \leq pH \leq 1.80$ (batch titration, $[10a] = 1.02 \times 10^{-4}$ M, [Fe$^{3+}$] = $3.20 \times 10^{-5}$ M) and (B) $2.34 \leq pH \leq 8.03$ (direct titration, $[10a] = 1.02 \times 10^{-3}$ M, [Fe$^{3+}$] = $3.12 \times 10^{-4}$ M). (C) Electronic spectra and (D) distribution curves ($[10a] = 1.02 \times 10^{-4}$ M, [Fe$^{3+}$] = $3.20 \times 10^{-5}$ M) of the Fe$^{3+}$ complexes of 10a.

Solvent: MeOH/H$_2$O (80/20 w/w), $I = 0.1$ M (NaClO$_4$), $T = 25.0$ °C.
Fe$^{3+}$ complexation studies with ligand 6d

**Figure S8.** Spectrophotometric titration vs pH of Fe$^{3+}$ complexes of ligand 6d between (A) $-0.36 \leq$ pH $\leq 2.36$ (batch titration, [6d] = 3.0 $\times$ 10$^{-4}$ M, [Fe$^{3+}$] = 8.0 $\times$ 10$^{-5}$ M) and (B) 1.97 $\leq$ pH $\leq$ 12.04 (direct titration, [6d] = 1.04 $\times$ 10$^{-4}$ M, [Fe$^{3+}$] = 3.23 $\times$ 10$^{-5}$ M). (C) Electronic spectra and (D) distribution curves ([6d] = 1.04 $\times$ 10$^{-4}$ M, [Fe$^{3+}$] = 3.23 $\times$ 10$^{-5}$ M) of the Fe$^{3+}$ complexes of 6d.

Solvent: MeOH/H$_2$O (80/20 w/w), I = 0.1 M (NaClO$_4$), T= 25.0 °C.
Fe$^{3+}$ complexation studies with ligand 6c

Figure S9. Spectrophotometric titration vs pH of Fe$^{3+}$ complexes of ligand 6c between (A) −0.36 ≤ pH ≤ 1.83 (batch titration, [6c] = 3.0 × 10$^{-4}$ M, [Fe$^{3+}$] = 9.38 × 10$^{-5}$ M) and (B) 1.97 ≤ pH ≤ 12.04 (direct titration, [6c] = 1.02 × 10$^{-4}$ M, [Fe$^{3+}$] = 3.12 × 10$^{-5}$ M). (C) Electronic spectra and (D) distribution curves ([6c] = 1.02 × 10$^{-4}$ M, [Fe$^{3+}$] = 3.12 × 10$^{-5}$ M) of the Fe$^{3+}$ complexes of 6c.

Solvent: MeOH/H$_2$O (80/20 w/w), I = 0.1 M (NaClO$_4$), T= 25.0 °C.
### 6. BBB Penetration Scores

#### Table S1. Predicted BBB score of compound 6a.

| Property                                         | Value | To  |
|--------------------------------------------------|-------|-----|
| Number of Aromatic Rings (Aro_R)                 | 1     | 0.82|
| Number of Heavy Atoms (HA)                       | 10    | 0.65|
| Molecular Weight (MW)                            | 140.14|     |
| Number of Hydrogen Bond Acceptor (HBA)           | 3     |     |
| Number of Hydrogen Bond Donor (HBD)              | 1     |     |
| MWHBN \( [\text{MWHBN} = (\text{MW}^{(-0.5)} \times \text{HBN}), \text{where } \text{HBN} = \text{HBA} + \text{HBD}] \) | 0.34  | 0.71|
| Topological Polar Surface Area(TPSA)             | 55.12 | 0.62|
| pKa                                              | 4.58  | 0.23|
| **BBB SCORE**                                    | 3.88  |     |
**Table S2.** Predicted BBB score of compound 6c.

| Property                                      | Value | To  |
|-----------------------------------------------|-------|-----|
| Number of Aromatic Rings (Aro_R)              | 2     | 1.00|
| Number of Heavy Atoms (HA)                    | 17    | 0.98|
| Molecular Weight (MW)                         | 230.26|     |
| Number of Hydrogen Bond Acceptor (HBA)        | 3     |     |
| Number of Hydrogen Bond Donor (HBD)           | 1     |     |
| $\text{MWHBN} = (\text{MW}^{(-0.5)}*\text{HBN})$, where $\text{HBN} = \text{HBA} + \text{HBD}$ | 0.26  | 0.93|
| Topological Polar Surface Area (TPSA)          | 65.42 | 0.54|
| $pK_a$                                         | 5.53  | 0.47|
| **BBB SCORE**                                  |       | 4.70|
**Table S3.** Predicted BBB score of compound 6d.

| Property                                           | Value     | $T_0$ |
|----------------------------------------------------|-----------|-------|
| Number of Aromatic Rings (Aro_R)                   | 2         | 1.00  |
| Number of Heavy Atoms (HA)                         | 18        | 0.99  |
| Molecular Weight (MW)                              | 246.26    |       |
| Number of Hydrogen Bond Acceptor (HBA)             | 4         |       |
| Number of Hydrogen Bond Donor (HBD)                | 2         |       |
| MWHBN [$MWHBN = (MW^{(-0.5)}*HBN)$, where $HBN=HBA+HBD$] | 0.38      | 0.55  |
| Topological Polar Surface Area (TPSA)              | 75.35     | 0.47  |
| pKa                                                | 5.96      | 0.58  |
| **BBB SCORE**                                      |           | 4.05  |
### Table S4. Predicted BBB score of compound 10a.

| Property                                             | Value | To |
|-------------------------------------------------------|-------|----|
| Number of Aromatic Rings (Aro_R)                      | 2     | 1.00 |
| Number of Heavy Atoms (HA)                           | 14    | 0.89 |
| Molecular Weight (MW)                                | 188.18|     |
| Number of Hydrogen Bond Acceptor (HBA)               | 3     |     |
| Number of Hydrogen Bond Donor (HBD)                  | 1     |     |
| MWHBN [MWHBN = (MW^(-0.5)) * HBN], where HBN=HBA+HBD| 0.29  | 0.87 |
| Topological Polar Surface Area (TPSA)                | 51.48 | 0.64 |
| pKa                                                   | 3.18  | 0.00 |
| **BBB SCORE**                                         |       | 4.47 |
**Table S5.** Predicted BBB score of compound 10d.

| Property                                           | Value | $T_0$ |
|----------------------------------------------------|-------|-------|
| Number of Aromatic Rings (Aro_R)                   | 2     | 1.00  |
| Number of Heavy Atoms (HA)                         | 15    | 0.93  |
| Molecular Weight (MW)                              | 204.18|       |
| Number of Hydrogen Bond Acceptor (HBA)             | 4     |       |
| Number of Hydrogen Bond Donor (HBD)                | 2     |       |
| $\text{MWHBN} = \left(\text{MW}^{(-0.5)}\times\text{HBN}\right)$, where $\text{HBN}=\text{HBA}+\text{HBD}$ | 0.42  | 0.40  |
| Topological Polar Surface Area(TPSA)               | 53.5  | 0.63  |
| pKa                                                | 4     | 0.11  |
| **BBB SCORE**                                      | 3.83  |       |
Table S6. Predicted BBB score of compound 11a.

| Property                                               | Value | To |
|--------------------------------------------------------|-------|----|
| Number of Aromatic Rings (Aro_R)                        | 1     | 0.82 |
| Number of Heavy Atoms (HA)                              | 9     | 0.56 |
| Molecular Weight (MW)                                  | 126.11|     |
| Number of Hydrogen Bond Acceptor (HBA)                 | 3     |     |
| Number of Hydrogen Bond Donor (HBD)                    | 1     |     |
| MWHBN [MWHBN = (MW^(-0.5))*HBN], where HBN=HBA+HBD    | 0.36  | 0.65 |
| Topological Polar Surface Area (TPSA)                   | 31.01 | 0.78 |
| pKa                                                    | 3.98  | 0.11 |
| **BBB SCORE**                                          | **3.97**|    |
Table S7. Predicted BBB score of compound 2.

| Property                                      | Value | T0 |
|-----------------------------------------------|-------|----|
| Number of Aromatic Rings (Aro_R)              | 1     | 0.82 |
| Number of Heavy Atoms (HA)                    | 10    | 0.65 |
| Molecular Weight (MW)                         | 141.12|     |
| Number of Hydrogen Bond Acceptor (HBA)        | 3     |     |
| Number of Hydrogen Bond Donor (HBD)           | 2     |     |
| MWHBN [MWHBN = (MW^(-0.5))*HBN], where HBN=HBA+HBD | 0.42  | 0.39 |
| Topological Polar Surface Area (TPSA)         | 34.37 | 0.76 |
| pKa                                           | 6.02  | 0.60 |
| **BBB SCORE**                                 | 3.87  |     |
Table S8. Predicted BBB score of compound 3.

| Property                                         | Value | T₀  |
|--------------------------------------------------|-------|-----|
| Number of Aromatic Rings (Aro_R)                 | 1     | 0.82|
| Number of Heavy Atoms (HA)                       | 11    | 0.72|
| Molecular Weight (MW)                            | 155.11|     |
| Number of Hydrogen Bond Acceptor (HBA)           | 4     |     |
| Number of Hydrogen Bond Donor (HBD)              | 2     |     |
| MWHBN [MWHBN = (MW^(-0.5)) * HBN], where HBN=HBA+HBD | 0.48  | 0.00|
| Topological Polar Surface Area (TPSA)            | 79.53 | 0.44|
| pKa                                              | 3.7   | 0.06|
| **BBB SCORE**                                    |       | 2.46|
Table S9. Predicted BBB score of DFP 1.

| Property                                              | Value | To |
|-------------------------------------------------------|-------|----|
| Number of Aromatic Rings (Aro_R)                      | 1     | 0.82 |
| Number of Heavy Atoms (HA)                            | 10    | 0.65 |
| Molecular Weight (MW)                                 | 139.15|     |
| Number of Hydrogen Bond Acceptor (HBA)                | 2     |     |
| Number of Hydrogen Bond Donor (HBD)                   | 1     |     |
| MWHBN  [MWHBN = (MW^(-0.5))*HBN), where HBN=HBA+HBD] | 0.25  | 0.95 |
| Topological Polar Surface Area (TPSA)                 | 38.95 | 0.73 |
| pKa                                                   | 3.68  | 0.06 |
| **BBB SCORE**                                         |       | 4.37 |
Table S10. Comparison of predicted BBB scores with percentage neuronal rescue from 6-OHDA neurotoxicity at 100 µM dose of the compound.

| Compound | BBB Score | % 6-OHDA Rescue (at 100 µM) |
|----------|-----------|-----------------------------|
| 6a       | 3.88      | 100                         |
| 6c       | 4.70      | 89                          |
| 6d       | 4.05      | 64                          |
| 10a      | 4.47      | 63                          |
| 10d      | 3.83      | 76                          |
| 11a      | 3.97      | 60                          |
| DFP 1    | 4.37      | 117                         |
| 2        | 3.87      | 93                          |
| 3        | 2.46      | 84                          |
Figure S10. Plot of predicted BBB scores versus percentage neuronal rescue from 6-OHDA neurotoxicity at 100 µM dose of the compound, showing no clear correlation between the two properties (● = 6a–6d, 10a, 10d and 11a, ■ = 2 and 3, ▲ = DFP 1).
7. DPPH Antioxidant Assay

**Figure S11.** Percentage inhibition of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical by ligand 6a after 24 hours (24h) and 48 hours (48h).

**Figure S12.** Percentage inhibition of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical by ligand 11a after 1 hour (1h), 24 hours (24h) and 48 hours (48h).
Figure S13. Percentage inhibition of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical by ligand 10a after 1 hour (1h), 24 hours (24h) and 48 hours (48h).

Figure S14. Percentage inhibition of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical by ligand 6d after 24 hours (24h) and 48 hours (48h).
**Figure S15.** Percentage inhibition of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical by ligand 6c after 1 hour (1h), 24 hours (24h), 48 hours (48h) and 72 hours (72h).
8. Trolox Equivalent Antioxidant Capacity (TEAC) Assay

Figure S16. Percentage of ABTS inhibition (TEAC) by ligand 6a (ABTS = 2,2’-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)).

Figure S17. Percentage of ABTS inhibition (TEAC) by ligand 11a (ABTS = 2,2’-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)).
**Figure S18.** Percentage of ABTS inhibition (TEAC) by ligand 10a (ABTS = 2,2’-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)).

**Figure S19.** Percentage of ABTS inhibition (TEAC) by ligand 6d (ABTS = 2,2’-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)).
Figure S20. Percentage of ABTS inhibition (TEAC) by ligand 6c (ABTS = 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)).

Figure S21. Percentage of ABTS inhibition (TEAC) by Trolox (ABTS = 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)).
9. Neuroprotection against 6-OHDA Neurotoxicity

**Figure S22.** Comparison of the percentage neuroprotection against 6-hydroxydopamine (6-OHDA) neurotoxicity in SH-SY5Y neuroblastoma cells by compounds 6a, 6c, 2, 3 and DFP 1 (at 100 µM compound dose).
10. References

1. (a) S. R. Safir and J. H. Williams, *J. Org. Chem.*, 1952, **17**, 1298–1301; (b) K. Tanaka, K. Matsuo, A. Nakanishi, Y. Kataoka, K. Takase and S. Otsuki, *Chem. Pharm. Bull.*, 1988, **36**, 2323–2330.

2. (a) L. W. Jones and M. C. Sneed, *J. Am. Chem. Soc.*, 1917, **39**, 668–674; (b) K. G. Cunningham, G. T. Newbold, F. S. Spring and J. Stark, *J. Chem. Soc.*, 1949, 2091–2094; (c) A. Cordi, J.-M. Lacoste, V. Audinot and M. Millan, *Bioorg. Med. Chem. Lett.*, 1999, **9**, 1409–1414.

3. A. Katoh, J. Ohkanda, Y. Itoh and K. Mitsuhashi, *Chem. Lett.*, 1992, 2009–2012.

4. F. Gutierrez, C. Tedeschi, L. Maron, J.-P. Daudey, R. Pouteau, J. Azema, P. Tisnes and C. Picard, *Dalton Trans.*, 2004, 1334–1347.

5. J. Ohkanda, T. Tokumitsu, K. Mitsuhashi and A. Katoh, *Bull. Chem. Soc. Jpn.*, 1993, **66**, 841–847.

6. (a) M. Frankel, G. Zvilichovsky and Y. Knobler, *J. Chem. Soc.*, 1964, 3931–3940; (b) L. Marchio, N. Marchetti, C. Atzeri, V. Borghesani, M. Remelli and M. Tegoni, *Dalton Trans.*, 2015, **44**, 3237–3250.

7. (a) M. Tegoni, M. Furlotti, M. Tropiano, C.-S. Lim and V. L. Pecoraro, *Inorg. Chem.*, 2010, **49**, 5190–5201; (b) C. M. Zaleski, C.-S. Lim, A. D. Cutland-Van Noord, J. W. Kampf and V. L. Pecoraro, *Inorg. Chem.*, 2011, **50**, 7707–7717; (c) J. Jankolovits, C.-S. Lim, G. Mezei, J. W. Kampf and V. L. Pecoraro, *Inorg. Chem.*, 2012, **51**, 4527–4538.

8. E. E. Smissman and V. D. Warner, *J. Med. Chem.*, 1972, **15**, 681–682.

9. (a) D. Bebbington, N. J. T. Monck, S. Gaur, A. M. Palmer, K. Benwell, V. Harvey, C. S. Malcolm and R. H. P. Porter, *J. Med. Chem.*, 2000, **43**, 2779–2782; (b) D. Bebbington, C. E. Dawson, S. Gaur and J. Spencer, *Bioorg. Med. Chem. Lett.*, 2002, **12**, 3297–3300; (c) H. Schugar, D. E. Green, M. L. Bowen, L. E. Scott, T. Storr, K. Böhmerle, F. Thomas, D. D. Allen, P. R. Lockman, M. Merkel, K. H. Thompson and C. Orvig, *Angew. Chem. Int. Ed.*, 2007, **46**, 1716–1718; (d) D. E. Green, M. L. Bowen, L. E. Scott, T. Storr, M. Merkel, K. Böhmerle, K. H. Thompson, B. O. Patrick, H. J. Schugar and C. Orvig, *Dalton Trans.*, 2010, **39**, 1604–1615.

10. (a) J. Ohkanda and A. Katoh, *J. Org. Chem.*, 1995, **60**, 1583–1589; (b) J. Ohkanda and A. Katoh, *Tetrahedron*, 1995, **51**, 12995–13002; (c) J. Ohkanda and A. Katoh, *Chem. Lett.*, 1996, 423–424.

11. A.-H. Mai, S. Pawar and W. M. De Borggraeve, *Tetrahedron Lett.*, 2014, **55**, 4664–4666.

12. A. Volonterio, P. Bravo and M. Zanda, *Tetrahedron Lett.*, 2001, **42**, 3141–3144.

13. A. Volonterio, S. Bellosta, P. Bravo, M. Canavesi, E. Corradi, S. V. Meille, M. Monetti, N. Moussier and M. Zanda, *Eur. J. Org. Chem.*, 2002, 428–438.