Synthesis and evaluation of compounds designed to prevent oxidative neurodegeneration

Victoria Goldberg, supervised by Dr Geoffrey Wells and Uzma Jaffry

UCL School of Pharmacy

Background

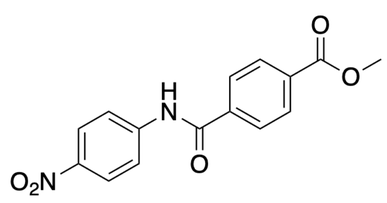
- Oxidative stress is caused by an imbalance of antioxidants and free radicals in cells
- If free radicals are not destroyed by cell defence mechanisms they can cause serious cellular and DNA damage
- This damage is thought to be linked to neurodegenerative diseases including Alzheimer's and Parkinson's disease, and development of some cancers
- Nrf2 is a protein that regulates the expression of cell protecting antioxidant proteins
- Under normal conditions, Nrf2 is retained in the cytoplasm by another protein, Keap1, where it is degraded quickly
- When there is oxidative stress, Nrf2 travels to the nucleus and binds to a DNA promoter to initiate transcription of antioxidant genes
- Inhibition of the binding between Keap1 and Nrf2 prevents Nrf2 from being degraded, allowing it to continue to initiate synthesis of antioxidant proteins to protect cells

Methodology

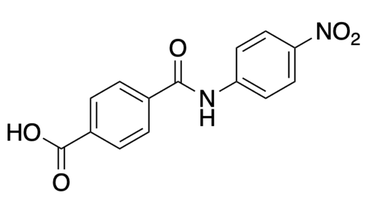
- Target structures were devised based on *Bertrand, H. C. et al (2015)* paper[1]
- Docking calculations were performed on each potential structure and UCSF Chimera was used to anaylse the potential binding of each structure
- For each amide coupling reaction, the amines were first dissolved in anhydrous DCM and then a 1:1 equivalence of the acid reagent was added
- 2 equiv. of EDCI and 2.5 equiv. of DMAP were added and the reactions were stirred for 24 hours[2]
- TLC was then performed for the reaction mixture to see if the reaction had been completed and then any precipitates were filtered off and collected
- If the compound needed to be hydrolysed, a base catalysed hydrolysis was performed using 25 equiv. NaOH, 1.5mL of methanol (65℃/reflux/1hr) and then the mixture was quenched with HCl until it reached ~pH 2
- NMR and LCMS data were collected for each sample and analysed

Results

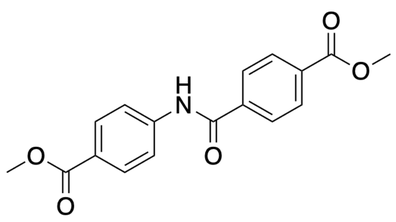
- Successfully synthesized compounds:
- **VG1A:** **^{1}H NMR** (400MHz, DMSO-d_6) δ ppm: 10.99 (s, 1H), 8.249 (J=8.0Hz, 2H), 8.09-7.96 (m, 3H), 3.91 (s, 3H) **LC-MS** : *m/z (ESI)* = 301 $[M+H]^+$, 299 $[M-H]^-$
- **VG1A(H):** **^{1}H NMR** (400MHz, DMSO-d_6) δ ppm: 13.12 (s, br, 1H), 10.96 (s, J=16.0 Hz, 1H), 8.37-7.93 (m, 2H), 6.72-6.59 (m, 6H) **LC-MS** : *m/z (ESI)* = 288 $[M+2H]^+$ 285 $[M-H]^-$
- **VG1C:** **^{1}H NMR** (400MHz, DMSO-d_6) δ ppm: 10.75 (s, 1H), 8.24-8.11 (m, 1H), 8.04-7.98 (m, 3H) , 3.85 (s, 1H), 3.81 (s, 3H) **LC-MS** : *m/z (ESI)* = 314 $[M+H]^+$, 312 $[M-H]^-$
- **VG1D:** **^{1}H NMR** (400MHz, DMSO-d_6) δ ppm: 10.43 (s, 1H), 8.09 (t, J=12.0Hz, 4H), 7.77 (d, J=12.0Hz, 2H), 7.35 (t, J=8.0Hz, 2H), 7.11 (t, J=8.0Hz, 1H), 3.91 (s, 3H) **LC-MS** : *m/z (ESI)* = 256 $[M+H]^+$, 254 $[M-H]^-$
- **VG1D(H):** **^{1}H NMR** (400MHz, DMSO-d_6) δ ppm: 10.40 (s, 1H), 8.06-8.03 (m, 4H), 7.97-7.89 (m, 2H), 7.35 (t, J=8.0Hz, 2H), 7.11 (t, J=8.0Hz, 1H) **LC-MS** : *m/z (ESI)* = 242 $[M+H]^+$, 2400 $[M-H]^-$



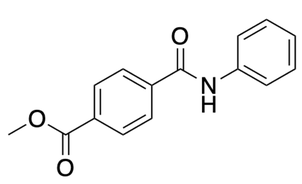
VG1A



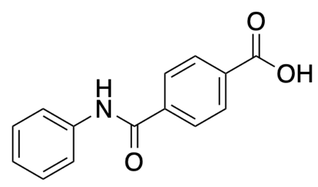
VG1A(H)



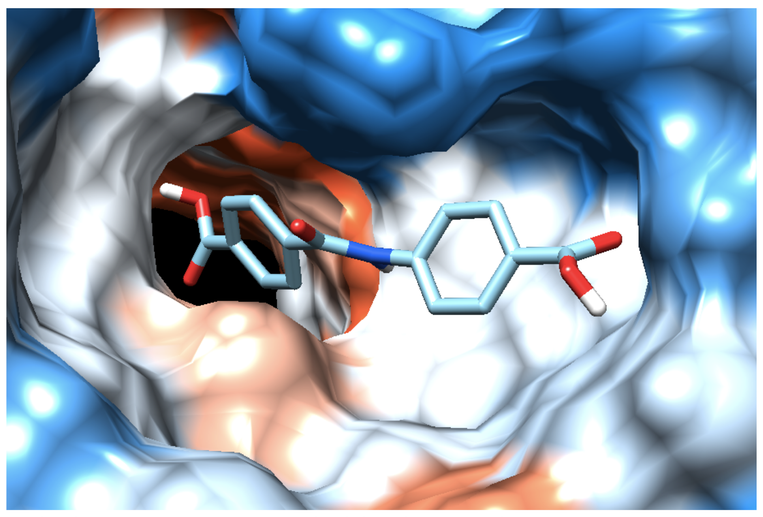
VG1C



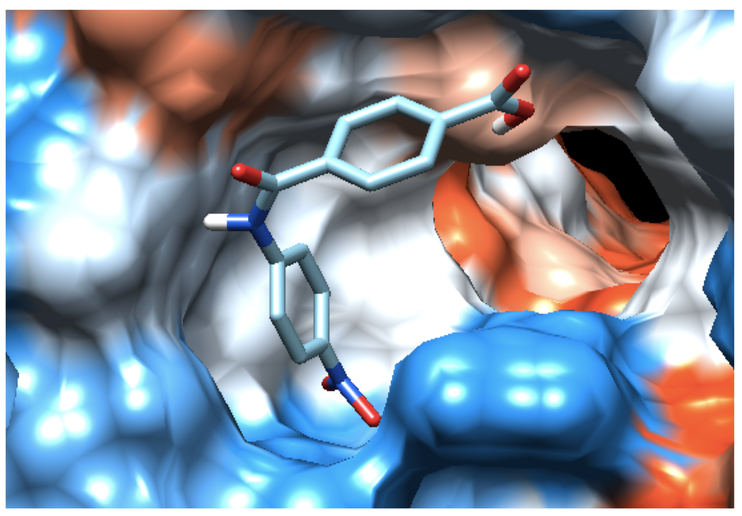
VG1D



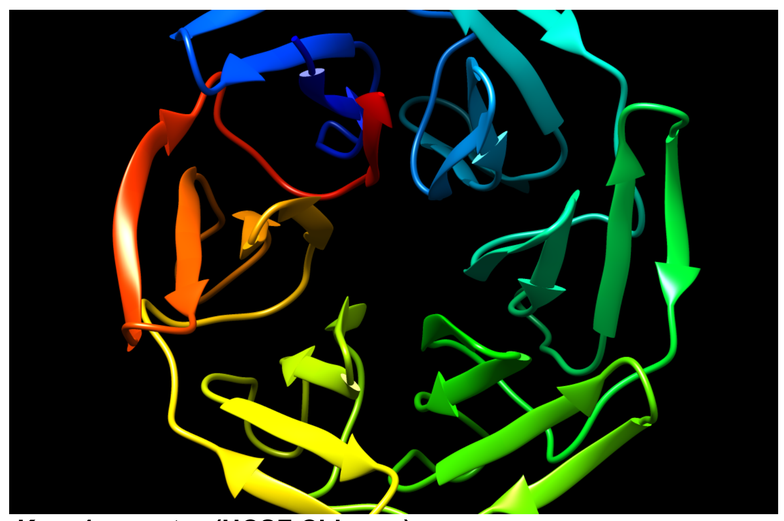
VG1D(H)



VG1C binding to Keap1 (UCSF Chimera)



VG1A(H) binding to Keap1 (UCSF Chimera)



Keap1 receptor (UCSF Chimera)

Discussion

These novel structures present interesting opportunities for further research and development, in particular working on the optimisation of the substituted groups on either side of the amide bond to result in the strongest binding affinity to Keap1. From this set of compounds, from the docking calculations performed, the strongest binding compounds were **VG1C(H)**, **VG1C**, **VG1D(H)** and **VG1A(H)** with Vina binding energies of -8.2, -8.1, -7.8 and -7.7kcal/mol respectively. This shows that there is an overall trend of the compounds with carboxylic acid substituted groups having the highest binding affinity so it would be worth experimenting with more acid compounds. The next step is to complete biological Keap1 binding assays for each compound, testing whether these results correlate with the values generated by the docking calculations, and then creating more acid compounds and repeating the same again.

References

[1]Bertrand, H. C. et al; 2015.
Design, Synthesis, and Evaluation of Triazole Derivatives That Induce Nrf2 Dependent Gene Products and Inhibit the Keap1−Nrf2 Protein− Protein Interaction. J. Med. Chem, 58, p. 7189. https://doi.org/10.1021/acs.jmedchem.5b00602

[2]Wells, G. et al.; 2006. *Design, Synthesis, and Biophysical and Biological Evaluation of a Series of Pyrrolobenzodiazepine-Poly(N-methylpyrrole) Conjugates.* J. Med. Chem, 49, p. 5456. https://doi.org/10.1021/jm051199z

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