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Ligand-Protein Binding of Cantharidin and Norcantharidin on HSF1: A Docking Study

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Ligand-Protein Binding of Cantharidin and Norcantharidin on HSF1: A Docking Study

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Overview

- Cantharidin has been shown to display anti-tumor properties through inhibitory activity on heat shock factor 1 protein (HSF1)[1].
- In the same study, norcantharidin displayed no inhibitory activity on the same protein.

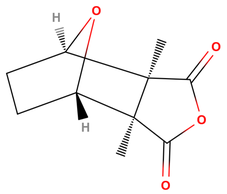

Figure 1: Cantharidin.

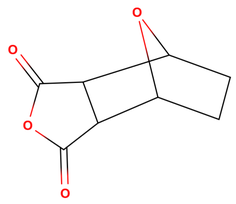

Figure 2: Norcantharidin.

- No literature on a binding site or binding strength is available at the time of this study.
- Our goal is to present potential binding sites and strengths for cantharidin and norcantharidin on HSF1 (PDB ID: 5HDG).
- Another goal is to supply binding energies to explain the inhibitory/non-inhibitory activity of cantharidin/norcantharidin on 5HDG.

Methods

- Docking simulations through AutoDock 4.2[2].
- Ligand structure files generated using *Avogadro*/ORCA.
- Water molecules stripped from 5HDG, Gasteiger charges added
- AutoGrid with 0.425 Angstrom spacing, failed dockings increased from 10 to 25.
- Small GridBox in pocket, high AutoDock simulations
- Ligand-protein renderings were done using the Chimera[3].

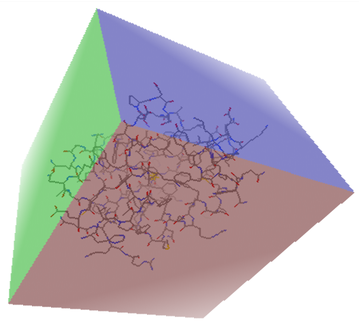

Figure 3: Example of the full protein AutoGrid.

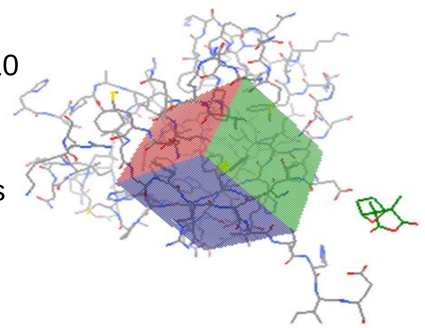

Figure 4: Example of the P1 AutoGrid.

Binding Sites

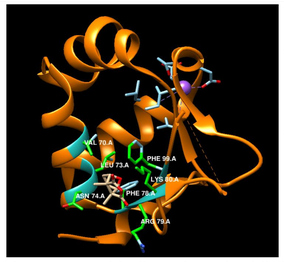

Figure 5: P1 binding of cantharidin/ 5HDG with residue labels.

- Two probable identical binding sites for both cantharidin and norcantharidin.
- "Pocket 1" or P1 consisted of residues *Val70, Leu73, Asn74, Phe99, Lys80, Phe78,* and *Arg79.*
- "Pocket 2" or P2 consisted of *Ile35, Asp32, Cys36, Val26, Ser38, Trp37, Arg106, Pro29, Asp28,* and *Ser27.*
- Simulations done assuming a rigid protein structure.
- It is unknown if P1/P2 is the active site of HSF1.

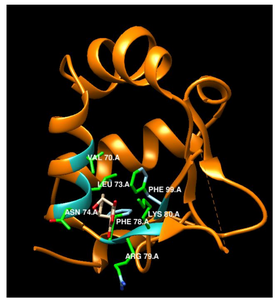

Figure 6: P1 binding of norcantharidin/ 5HDG with residue labels.

Interactions

Table 1: Summary of ligand-protein docking simulations in AutoDock.

Protein	Ligand	Pocket	Binding Energy kcal · mol⁻¹
5HDG	Cantharidin	P1	-4.75
5HDG	Cantharidin	P2	-4.65
5HDG	Norcantharidin	P1	-4.46
5HDG	Norcantharidin	P2	-4.09

- Cantharidin hydrogen bond in area of LYS80 and ARG79
- Norcantharidin hydrogen bond in area of ASN74

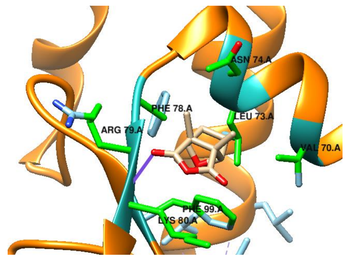

Figure 7: Cantharidin hydrogen bonding (purple) in P1.

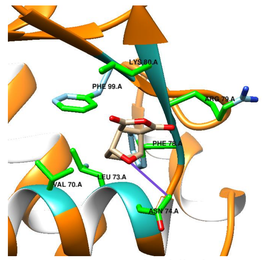

Figure 8: Norcantharidin hydrogen bonding (purple) in P1.

- Cantharidin bond to backbone (carboxyls/amines), norcantharidin to side chain.
- Lack of methyl groups could allow norcantharidin to settle in a different confirmation within P1 during docking.
- The binding energies may help explain why cantharidin inhibits HSF1 and norcantharidin does not.

Additional Ligands

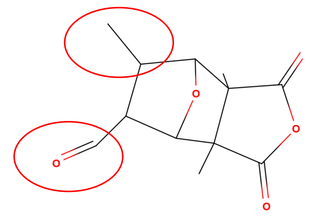

Figure 9: Ligand 5 had a binding energies of -5.15 and -4.87 kcal mol $^{-1}$ to 5HDG.

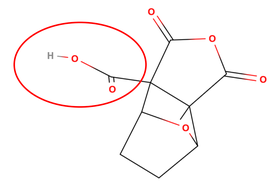

Figure 10: Ligand 3 had binding energies of -4.47 and -4.08 kcal mol $^{-1}$ to 5HDG.

- Cantharidin-based compounds could prove to be even more effective HSF1 inhibitors than cantharidin.
- Based on binding energies ligand 5 (**9**) would be a compound to advance to further screening, ligand 3 (**10**) would not.

Conclusions

- Regardless of binding site, cantharidin consistently binds stronger to HSF1 than norcantharidin.
- Computational investigation reinforced the experimental evidence of cantharidin inhibitory activity on HSF1.
- Two potential binding sites (P1 and P2) were put forth for cantharidin-5HDG.

Future Work

- Further studies forthcoming using GROMACS to account for protein structure changes and thus more accurate binding free energy.
- Increase number of ligands screened to determine most effective inhibitor of HSF1 while considering possible toxicities.
- Synthesis of such ligands.
- More explicitly account for solvent interactions.

References

[1] Kim, J.A.; Kim, Y.; Kwon, B; Han, D.C; The Natural Compound Cantharidin Induces Cancer Cell Death through Inhibtion of Heat Shock Factor Protein 70 (HSP70) and Bcl-2-associated Athanogene Domain 3 (BAG3) Expression by Blocking Heat Shock Factor 1 (HSF1) Binding to Promoters , *J. Biol. Chem.*, **2013**, *288*, 28713-28726.

[2] Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S. and Olson, A. J. (2009) Autodock4 and AutoDockTools4: automated docking with selective receptor flexiblity. *J. Computational Chemistry* 2009, **16**: 2785-91

[3] Molecular graphics and analyses performed with UCSF Chimera, developed by the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco, with support from NIH P41-GM103311