Testing the Specificity and Cytotoxicity of Biotin-Ferrocene Derivatives on Cancer Cells

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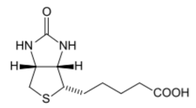
Abstract

Cancer is the second-leading cause of death in the US. A problem with current cancer treatment is that the chemotherapeutics often are not specific to cancer cells and can lead to negative side effects. Cancer cells are unique from normal cells in two ways. First, cancer cells overexpress biotin receptors on their surface relative to normal cells. Secondly, cancer cells have a dysregulated redox balance such that the cellular environment favors the production of harmful reactive oxygen species (ROS).

This research project focuses on testing the cytotoxic properties of various molecules that combine biotin and ferrocene, which has been shown to facilitate the generation of ROS, on cancer (HeLa, MCF7) and non-cancer (293HEK) cell lines. We hypothesize that the biotin-ferrocene compounds will enter the biotin receptor-overexpressing cancer cells more efficiently than non-cancer cells, allowing for the ferrocene moiety to facilitate ROS generation to high levels that lead to cell death. MTT cytotoxicity assays are used to quantify cell death.

Altered Metabolism and Biotin

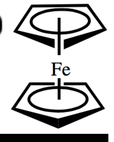
- Cancer cells overexpress glucose and vitamin transporters
- Allows cancer cells to uptake more nutrients to drive rapid cell division
- Biotin, or vitamin B_7, can bind to vitamin receptors and enter cells
- Thus, compounds or drugs that have biotin should preferably bind and enter cancer cells relative to non-cancer cells.



Aberrant Redox and Ferrocene

- Certain cancer cells have dysregulated redox balance
- Leads to a more reducing cellular environment, which favors the production of ROS that damage DNA and other cellular molecules
- Cancer cells have higher levels of ROS compared to normal cells
- High levels of ROS can cause cell death
- Ferrocene has been shown to facilitate the generation of ROS through the bottom redox reaction with its iron center

$$O_2 \rightarrow O_2^{\bullet -} \rightarrow H_2O_2 \rightarrow OH^{\bullet} \rightarrow H_2O$$

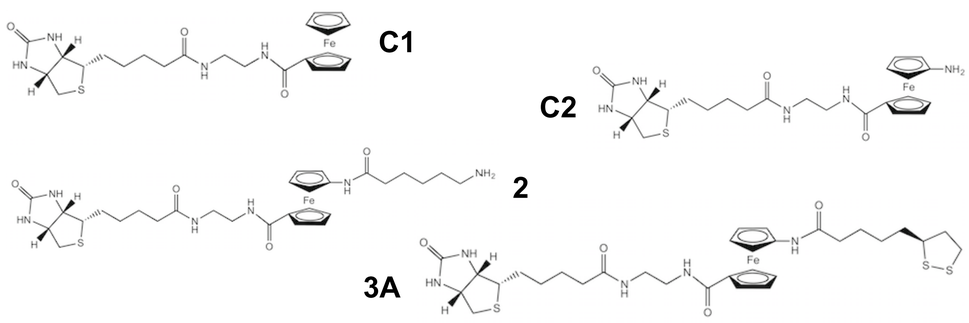


$$Fe^{II} + H_2O_2 \rightarrow Fe^{III} + OH^{\bullet} + OH^{-}$$

Hypothesis

The addition of biotin to ferrocene makes the resulting compound more selective and, therefore, more toxic to biotin receptor-expressing cancer cells compared to non-cancer cells.

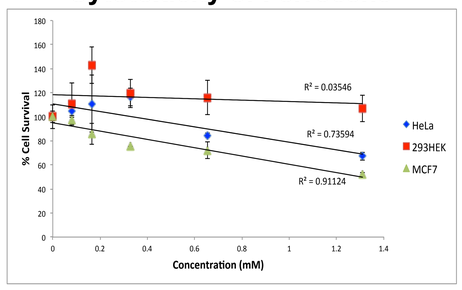
Biotin-Ferrocene Derivatives



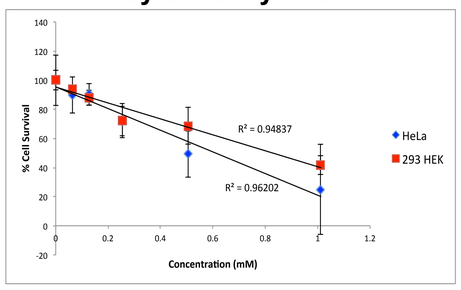
Synthesized by M. Burnett and K. Green; Dept. of Chemistry

Results

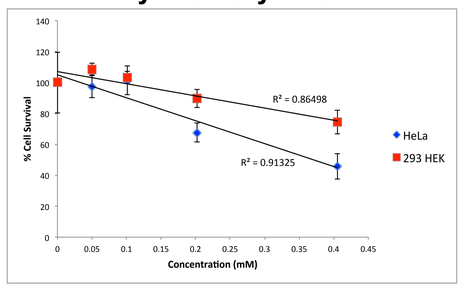
Cytotoxicity of Ferrocene



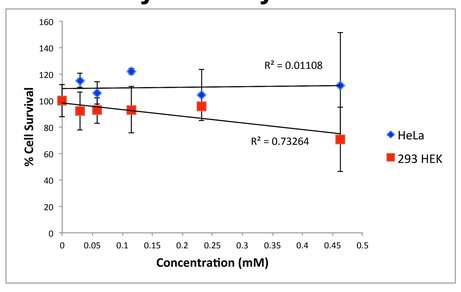
Cytotoxicity of C1



Cytotoxicity of C2



Cytotoxicity of 3A



Cytotoxicity of 2

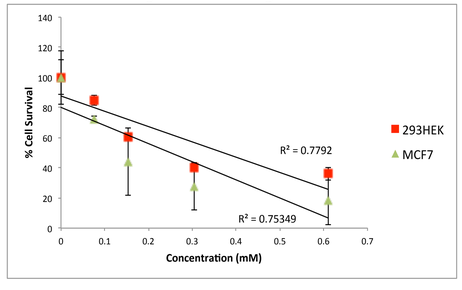


Table of EC_{50} Values (mM)

Cell Line	Fc	3A	C2	C1	2
293HEK	12.09	0.96	0.37	0.83	0.73
HeLa	1.91	-	N.D.	0.61	0.37
MCF7	1.30	N.D.	0.25	N.D.	N.D.

"N.D." indicates not determined. "-" indicates no EC_{50} could be calculated.

Discussion

- **Ferrocene** is toxic to cancer cell types (HeLa and MCF7) specifically
- **Ferrocene** is not toxic to non-cancer cells (293HEK)
- It is predicted that this observation is related to the more reducing environment within cancer cells
- Suggests cancer cells are more susceptible to ferrocene-mediated generation of high levels of ROS within cells that cause cell death

- **C1**, **C2**, and **2** are toxic to both cancer and non-cancer cells
- For each, cytotoxicity is greater in cancer cells relative to non-cancer cells as indicated by EC_{50} values
- Support the hypothesis that biotin-ferrocene compounds bind and enter cancer cells with greater specificity relative to non-cancer cells
- Again, it is predicted that ferrocene moiety can generate high levels of ROS within cells that cause cell death

- **3A** appears to be toxic to non-cancer cells (293HEK) specifically
- **3A** appears to not be toxic to cancer cells (HeLa)
- Perhaps, the finding is related to the lipoic acid side chain group
- Fails to support the hypothesis
- Underscores our incomplete understanding of how these compounds interact with both cell types

- Further research is required to understand how these compounds interact with cells at the molecular level and how the variable side chain group can modify cytotoxicity.

Conclusions

- **Ferrocene** shows dose-dependent cytotoxicity specific to cancer cells
- **C1**, **C2**, and **2** are more toxic to cancer cells than non-cancer cells
- **3A** shows dose-dependent cytotoxicity specific to non-cancer cells

Acknowledgements

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