



Synergistic Induction of Avian Immune Gene Expression by Sirtuin Modulators & Histone Methyltransferase Inhibitors



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Objective

Investigate the potential for sirtuin modulating compounds to synergize with histone methyltransferase inhibitors to upregulate the avian innate immune gene response with the goal of developing them as host-directed therapeutics for disease control and prevention

Background

- 85% of emerging antibiotics in clinical trials are insufficient to combat antibiotic resistant bacteria, directing the need for novel antimicrobial alternatives¹
- Host defense peptides (HDPs) are critical components of innate immunity and are quickly mobilize to fight off infection²
- Epigenetic compounds such as histone deacetylase inhibitors (HDACi) and methyltransferase inhibitors (HMTi) are known to enhance HDP gene expression.³
- The compounds that modulate the activity of sirtuins (SIRT), a distinct class of HDAC⁴, and their possible synergy with HMTi in HDP gene induction is yet unknown.

Methods

I Cell Culture & Stimulation

- Chicken macrophage cells (HTC)
- Time-course (0, 12, 24, 48 h)
- Dose-dependent response

II Isolation of Total RNA & Reverse Transcription

- RNAzol RT
- iScript cDNA Synthesis Kit

III Analysis of Gene Expression

- Reverse transcription
- Genes:
 - HDPs (AvBD1-10 & 14)
 - Inflammation (IL-1 β)
 - Barrier protection (MUC2 & CLDN1)

IV Data Analysis

- One-way ANOVA & *post hoc* Tukey's test ($P \leq 0.05$)

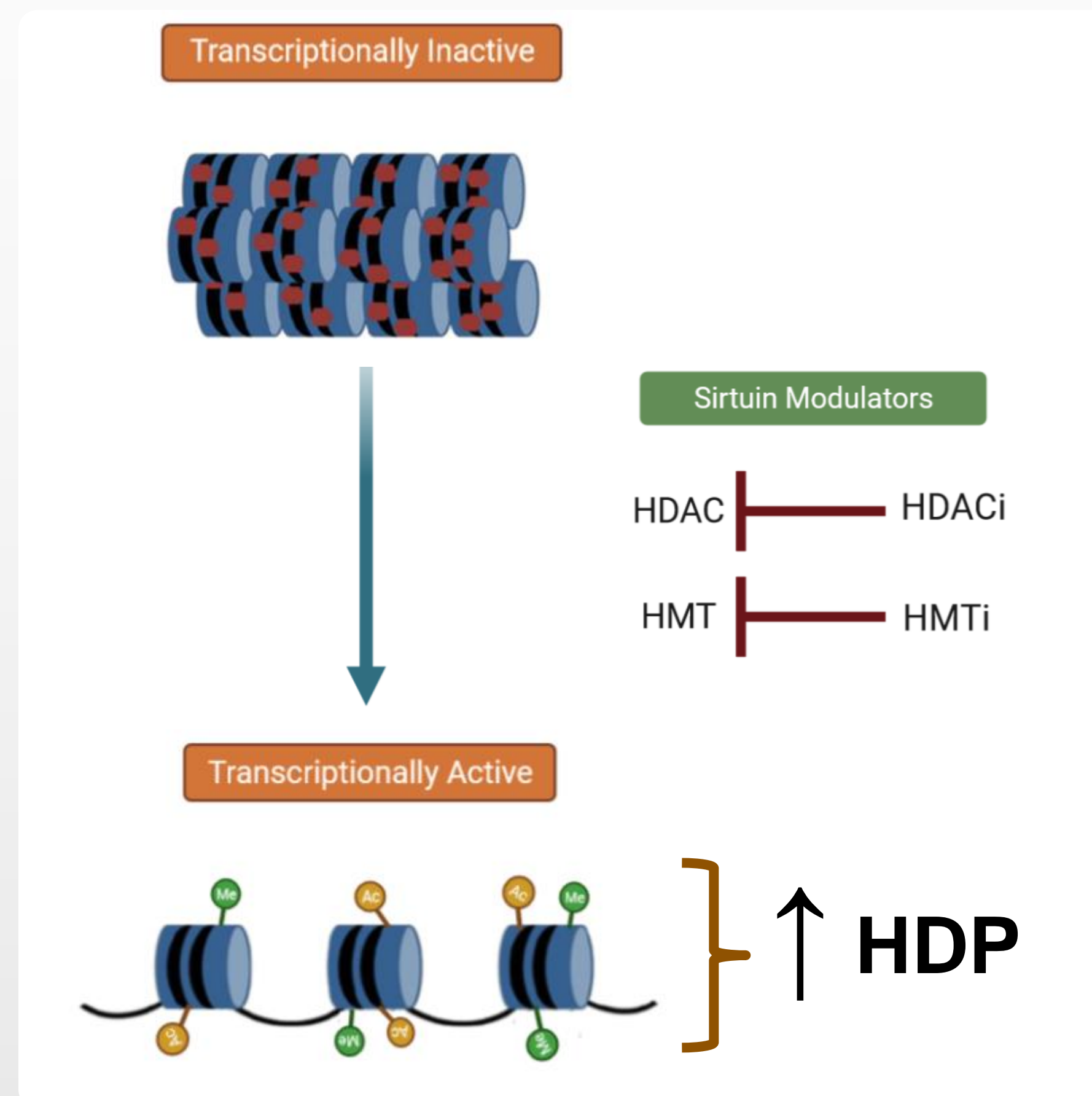


Figure 1. Proposed epigenetic effect of sirtuin modulators on chromatin relaxation and HDP gene expression.

Sirtuin Activator (Histone Deacetylase)	↓ acetylation	Resveratrol SRT2104 SRT1720 Kaempferol
Sirtuin Inhibitor (Histone Deacetylase Inhibitor)	↑ acetylation	Sirtinol EX527
Histone Methyltransferase Inhibitor (HMTi)	↓ methylation	BIX01294

Table 1. Sirtuin activators, sirtuin inhibitors, and histone methyltransferase inhibitor used in this study and their respective effect on histones.

Discussion & Conclusion

- Sirtuin activators and inhibitors synergize respectively with HMTi to induce HDP gene expression
- Sirtuin activators may modulate HDP gene expression through alternative pathways or selectively activate and inhibit sirtuin proteins⁵
- Ex vivo* results show promise for translational potential
- Epigenetic regulation of HDP gene expression provides a promising novel, therapeutic strategy for disease control and prevention without relying on antibiotics

Future Directions

- Further characterize the synergy between sirtuin modulators and HMTi
 - Test additional HDP genes
 - Test synergy with other HMTi
 - Test additional *in vivo* conditions
- Identify the mechanism of synergy on HDP expression
 - Analyze histone acetylation/methylation and chromatin accessibility of HDP gene promoters

References

- World Health Organization. (2017, September 20). *The world is running out of antibiotics, WHO report confirms*. <https://www.who.int/news/item/20-09-2017-the-world-is-running-out-of-antibiotics-who-report-confirms>
- Hancock, R. E. W., Haney, E. F., & Gill, E. E. (2016). The immunology of host defense peptides: Beyond antimicrobial activity. *Nature Reviews Immunology*, 16, 321-34. <https://doi.org/10.1038/nri.2016.29>
- Whitmore MA, Li H, Lyu W, Khanam S, and Zhang G. Epigenetic Regulation of Host Defense Peptide Synthesis: Synergy between Histone Deacetylase Inhibitors and DNA/Histone Methyltransferase Inhibitors. *Frontiers of Immunology* 2022; 13: 874706.
- Dai, H., Sinclair, D. A., Ellis, J. L., & Steegborn, C. (2018). Sirtuin activators and inhibitors: Promises, achievements, and challenges. *Pharmacology & Therapeutics*, 188, 140-154. <https://doi.org/10.1016/j.pharmthera.2018.03.004>
- Singh, C., Panackal, J., Siddiqui, S., Ahmad, N., & Nihal, M. (2020). Combined inhibition of specific sirtuins as a potential strategy to inhibit melanoma growth. *Frontiers of Oncology*, 10, 591972. doi: <https://doi.org/10.3389/fonc.2020.591972>

Acknowledgements

I appreciate Dr. Glenn Zhang, Melanie Whitmore, and the Zhang lab members for dedicating their time and mentorship towards my research training. I am grateful for the Wentz Research Scholar Program and Office of Scholar Development & Undergraduate Research at OSU for their generous funding and program support.

Results

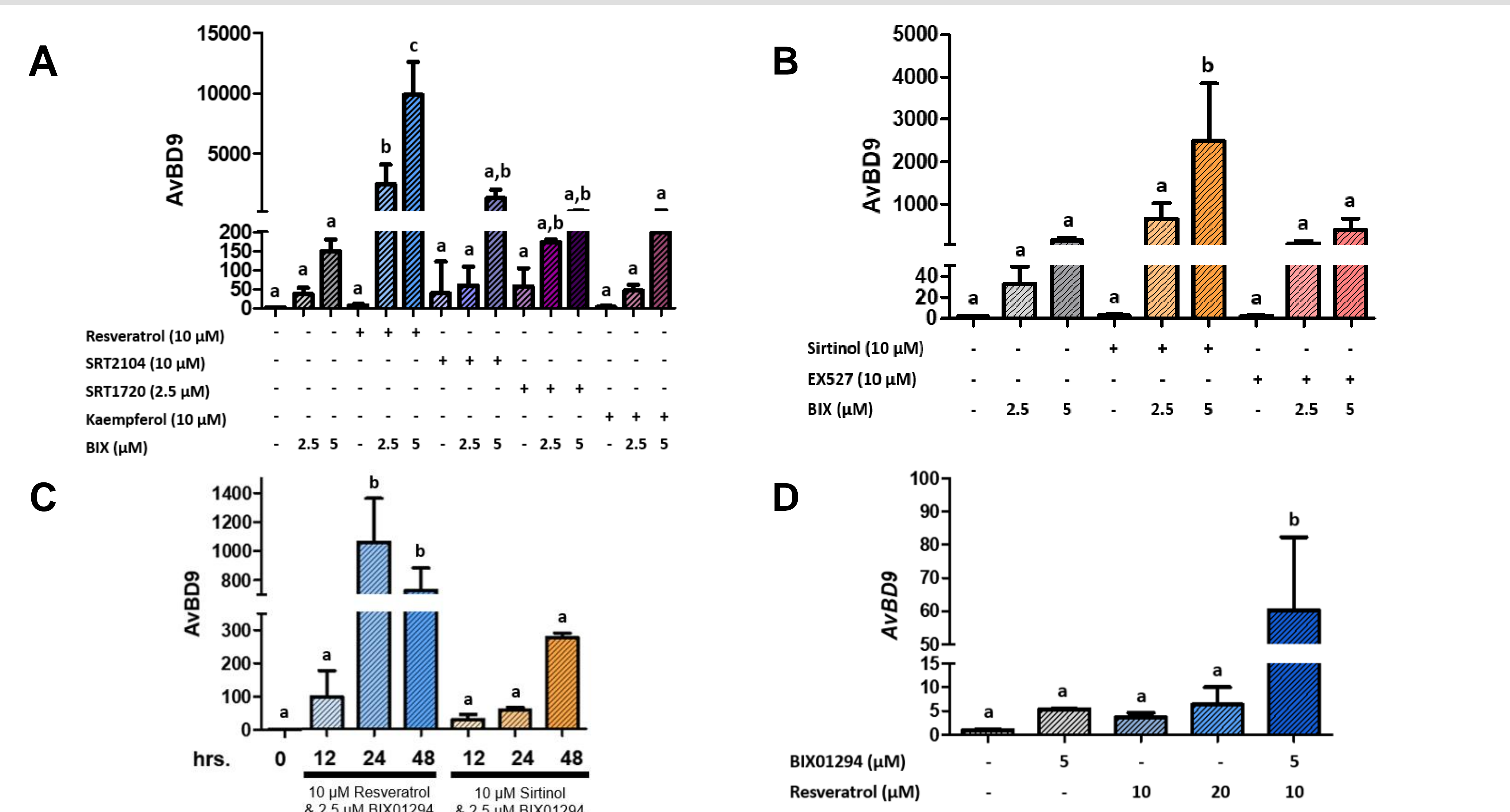


Figure 2. Synergistic induction of avian HDP gene expression by sirtuin modulators and a histone methyltransferase inhibitor (BIX01294). Chicken HTC cells were treated with sirtuin activators (A) or sirtuin inhibitors (B) individually or in combination with BIX01294 for 24 h, followed by AvBD9 expression analysis. (C) Time-dependent induction of AvBD9 expression in HTC cells. (D) Induction of AvBD9 expression in chicken peripheral blood mononuclear cells. Treatments without common superscripts are considered statistically different ($P \leq 0.05$).