DEVELOPMENT OF NOVEL ANTI-HBV AGENTS: CHARACTERIZATION OF THE *N*-HYDROXYPYRIDINEDIONES (HPDs) AS HBV RNASE H INHIBITORS

Maria Makri*, Dimitrios Moianos*, Georgia-Myrto Prifti*, Vasiliki Pardali*, Erofili Giannakopoulou*, Molly E. Woodson**, Tiffany C. Edwards**, Razia Tajwar**, John E. Tavis**, Grigoris Zoidis*,[1]

*School of Health Sciences, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, National and Kapodistrian University of Athens, Greece
**Saint Louis University School of Medicine, Department of Molecular Microbiology and Immunology, St. Louis MO USA
[1]zoidis@pharm.uoa.gr

Hepatitis B Virus (HBV) is a DNA virus in the *Hepadnaviridae* family. Long-term HBV infections constitute a major cause of end-stage liver disease, cirrhosis, liver failure and hepatocellular carcinoma. Current antiviral therapy (immunomodulators, nucleos(t)ide analogues) rarely eradicates the virus and cleared HBV infections can be reactivated. Moreover, HBV's high mutation rate can lead to drug resistance. To cure HBV infection, it is crucial to develop new strategies.

HBV ribonuclease H (RNaseH) is a metalloenzyme that belongs to the nucleotidyl transferase superfamily and its active site contains four carboxylates that bind to two Mg^{2+} ions required for RNA cleavage. However, the potential of RNaseH as a drug target for HBV treatment was not explored until recently. The importance of the RNaseH and its low amino acid homology with the cellular RNaseHs, prompted the development of novel scaffolds, bearing a metal-chelating motif, as potent inhibitors.[2]

Utilizing findings in the literature and our previous publications,[3] we rationally designed and synthesized a series of metal chelating agents (*N*-hydroxyimides) to optimize our lead compound. HPDs are compounds that inhibit metalloenzymes. They are effective against the HBV RNaseH, suppressing HBV replication at sub-micromolar concentrations. Previously,[2] the best compounds from these classes had selectivity indexes (SIs) of ~350 (HPD). We describe the synthesis and characterization of efficacy, cytotoxicity, pharmacological properties, and off-target effects of newly synthesized HPDs. All compounds tested had CC_{50}s >100 μM in PHHs. Additionally, compounds had a satisfying $t_{1/2}$ > 4 hr. Moreover, the HPDs were soluble and passively permeable at all pHs, and significantly effective at suppressing plus polarity strand synthesis – two had EC_{50} values of 90 and 60 nM, resulting in SIs of 1063 and 1120. These results indicate that the HPDs hold significant potential for antiviral development. Our future studies will be informed by our growing structure activity relationships.

References

1) Prifti, G.-M., Moianos, D., Giannakopoulou, E., Pardali, V., Tavis, J.E., Zoidis, G. *Pharmaceuticals*, 2021, 14(5), 417.
DOI: https://doi.org/10.3390/ph14050417
2) J. E. Tavis, G. Zoidis, M. J. Meyers, R. P. Murelli, *ACS Infect Dis.*, 2019, 5(5), pp. 655–658.
DOI: https://doi.org/10.1021/acsinfecdis.8b00045
3) T. C. Edwards, E. Lomonosova , J. A. Patel, Q. Li, J. A. Villa, A. K. Gupta, L. A. Morrison, F. Bailly, P. Cotelle, E. Giannakopoulou, G. Zoidis, J. E. Tavis, *Antiviral Res.*, 2017, 143, 205-217.
DOI: https://doi.org/10.1016/j.antiviral.2017.04.012