High Throughput Screening (HTS) for identifying novel DIO3 Inhibitors

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OBJECTIVES:

Iodothyronine Deiodinases (DIO) are essential enzymes catalyzing activation and inactivation of thyroid hormones thereby regulating their action on the cellular level. DIO3 is of specific interest, as this isoenzyme was shown to be (re-)expressed in certain neoplastic pathologies, consumptive hypothyroidism and regenerative processes.

The suggested role in cancer growth makes DIO3 an interesting novel target in oncology, as no specific and potent inhibitors are known so far.

We have recently developed a non-radioactive method for DIO measurements [1]. In combination with recombinant sources for the three DIO isoenzymes, it is possible to setup a comprehensive screening platform to identify specific modulators. Coming from the established 96-well microplate format, a HTS-assay on DIO3 interference was further developed and adapted to the 384-well format, which enabled screening a library of about 30000 drug like compounds including a structural diversity set of commercially available chemicals.

METHODS:

All DIO enzyme assays during the screening and specificity testing used the non-radioactive method based on the Sandell-Kolthoff-reaction utilizing HEK293 cell homogenates overexpressing high DIO activities (1). To verify the ability of the identified DIO3 inhibitors to enter the cell, the most potent and specific candidates were tested on intact DIO3-expressing HEK293 cells in order to identify the most promising lead compounds. Incubations on living cells included preincubation with the respective test compounds (3µM) and subsequent coincubation with the respective DIO3 substrate T3 (100nM) in FCS-free media. Cells and conditioned media were extracted and analyzed via LC-MS/MS. Iopanoic acid, an unspecific competitive pan-DIO-inhibitor, was used as positive control.

RESULTS:

From the primary library screening ~60 potent DIO3 inhibitors were preselected for further DIO specificity testing (IC50 <25µM, >75% decrease of enzymatic activity). Six compounds were then selected by their potency in the low µM range and DIO3 specificity and further analyzed in on-cell experiments for DIO3-inhibiton in the HEK293-hDIO3 cell line. All of these components have hereby been identified and verified as novel DIO3-inhibitors.

CONCLUSION:

The developed test platform is compatible with an HTS-setting in 386well format to identify DIO-inhibitors. From a library of about 30.000 compounds we identified a selection of potent hits with different specificity with regards to the DIO-isoenzymes. This strategy offers a hypothesis-free unbiased way to the current, DIO3 X-ray structure-driven approaches to develop DIO3-specific inhibitors for e.g. cancer treatment. To further verify and develop our current subset of lead structures we will, apply them to relevant tumor model systems to test their therapeutic effects in pathologies associated with DIO3 overexpression.

References: [1] Renko et al. 2012, 2015