Novel steroid receptor modulators identified by high-throughput profiling with panel of reporter cell lines

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1. Introduction
Steroid hormone receptors (SR) are evolutionary and functionally related transcription factors regulating a broad variety of physiological functions. SRs consist of two receptors for estrogens (ERa and EBl), an androgen receptor (AR), a glucocorticoid receptor (GR), a mineralocorticoid receptor (MR) and a progesterone receptor (PR). The regulation of transcription by SRs is highly complex but it shows a common trait: the activity can be modulated by low molecular weight ligands, which makes them one of the most prominent drug targets. In addition to their role in the physiology of healthy organism, SRs have a role in pathophysiological processes including cancer, metabolic disorders, neurodegeneration or inflammation.

Current efforts in SR drug research focus on the identification of new active ligands, and on the improvement of their function, selectivity, mode of action and tissue selectivity. Here, we have used a recently developed complex chemical and informatics platform for SRs. The core of the platform is formed by a selection of selected luciferase reporter cell lines created on the unified cellular background in U2OS osteosarcoma cells. Our collection consists of two panels. The first panel is based on the expression of a chimeric receptor containing DNA binding domain from the yeast transcription factor Gα4. This system is suitable for the primary screens as it is less sensitive to the crosstalk from other cellular signaling pathways.

In the second panel, the activation of steroid response elements containing promoter is mediated by a full-length SR and this system provides better information about ligand-induced transactivation by steroid receptors for the general chemists.

We have systematically profiled a chemical library containing 3000 unique steroid derivatives (ICCB, Prague) using SR reporter cell lines. Each receptor was assayed in 3 concentrations (1 µM, 100 nM, 10 nM) both in the agonist and antagonist mode. Data were normalized and analyzed with several clustering algorithms using iChIB, an interactive cluster heat map library integrated within an in-house built laboratory information management system. Our expectations of a high hit rate in the steroid library were confirmed. ~1000 compounds (one third of the library) were active on at least one of the tested receptors. Structure-activity analysis on all receptors revealed surprising and complex activity profiles. For example, some compounds were active only on one of the 6 receptors in the whole concentration range suggesting high selectivity for the receptor. However, we have also identified compounds with remarkable activity profiles across multiple steroid receptors. One such example are activity profiles consisting of the agonism on ERβ and antagonism on ERα.

By completing the preliminary study, we see a strong potential in this data set containing rich information about structure-activity relationships not only on a specific target, but rather on a complete receptor family in diverse mode of actions. Ligands, exhibiting specific combinations of activities, are useful in the treatment of several oncologic disorders like prostate and breast cancer.

2. Panels of luciferase reporter cell lines

(A) A series of reporter U2OS-based cell-lines stably transfected with reporter vector pGL4.30( minimalGal4UAS/LBD+Luc) and the expression vector encoding a fusion of the yeast Gα4 (GR) with the LBD of the corresponding human steroid receptor (ERa, EBl, AR, MR and PR) was created (Gα4/GR reporter system). (B) Set of ligands with known activities was used to validate the panel of reporter cell lines. Comparison of the Gα4/GR reporter system with the reporter system using the full-length steroid receptors (C) Correlation of compound potencies obtained from the full-length steroid receptor reporter system while using different promoters: synthetic glucocorticoid response element (GRe) and viral promoter MMTV. Both promoters provide comparable response to tested ligands as shown by calculated Pearson correlation coefficients: AR (P<0.05, 1.2 ± 0.8), EBl (P<0.001, 1.07 ± 0.7).

3. Workflow for steroid library profiling

(A) Workflow for the high-throughput profiling of chemical library containing 3173 unique steroid derivatives (provided by ICCB, Prague) in both agonist and antagonist mode, in 3 concentrations ranging from 1µM to 10nM. 16 main screens were carried out along with 2 counter screens to filter out false positive and negative hits. (B) Hierarchical clustering of ~1300 active compounds in the agonist mode (2010 inactive compounds are not shown) using iChIB (http://openscreen.cz/software/iChIB).

4. Activity profiles of the active compounds across the whole steroid receptor family

(A) Ligands active in the sex steroid receptors (ERa, EBl, AR and PR) at 100 nM, in the agonist mode. (B) Activity profiles of estrogenic compounds were analyzed at 100nM in the agonist mode and at 1µM in the antagonist mode. In total, 328 ligands of ERa and 319 ligands for EBl were identified. (C) Identification of estrogen receptor selective ligands using stringent selection criteria: the potency of the selective ligand is at least 100% higher for one of the estrogen receptors. (D) 17α-aryl and 17α-perfluoroalkylated estradiols represent novel classes of ERa and EBl selective ligands.

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