

Patients with loss-of-function ~~mutations~~ in the 11 β -HSD2 gene suffer from sodium retention. ~~The~~ ~~Direct~~ inhibition of this enzyme may therefore be an excellent potential therapy for chronic hyponatremia, ~~a condition which causes problems that is prevalent in among elderly patients.~~ To develop drugs to inhibit this enzyme, ~~a process of virtual screening was employed on~~ several compound libraries ~~were~~ ~~virtually screened~~ using a newly developed algorithm for detecting which compounds may have ~~favourable~~ favorable physicochemical and structural characteristics that would enable them to specifically bind to and ~~to~~ inhibit 11 β -HSD2. We examined the ~~top hits-candidate compounds produced-identified by-through the~~ virtual screening and tested ~~their~~ interaction with recombinant human 11 β -HSD2 enzyme and found one compound, DE2300c5, ~~-~~that strongly bound to 11 β -HSD2 without affecting ~~either~~ 11 β -HSD1 ~~and-or~~ 17 β -HSD2. ~~-~~ Binding was confirmed in a HEK293 cell culture, and ~~in-renal-cortical-cells,~~ ~~administration of~~ DE2300c5 increased intracellular sodium levels ~~in renal cortical cells,~~ miR-401 ~~expression~~ was unchanged by DE2300c5 administration. It is clear from our findings that DE2300c5 represents an effective treatment for hyponatremia, which is a significant health burden, and other electrolyte imbalances.

Commented [QCE1]: Please ensure that the intended meaning has been maintained in this edit.

Commented [QCE2]: Please ensure that the intended meaning has been maintained in this edit.

INTRODUCTION

11 β -~~h~~Hydroxysteroid ~~Dehydrogenase-dehydrogenase~~ type 2 ~~catalyses-catalyzes~~ the ~~reaction conversion of the~~ biologically active steroid hormone cortisol to cortisone (Fig. 1). This reaction is co-factor dependent, as it depends on NAD⁺². Cortisol binds to the MR, ~~and interestingly, it binds~~ as tightly as its other natural ligand, ~~which-is~~ aldosterone. In vivo, ~~11 β -HSD2+11 β -HSD2~~ is co-localized in tissues where MR expression is high, and ~~has-the-its~~ main role ~~involves~~ ~~-of~~ regulating the local concentrations of cortisol to prevent it ~~from~~ excessively binding to the MR. Activation of the MR results in re-absorption of sodium, excretion of potassium and an associated increase in blood pressure. The MR is a member of the nuclear receptor family and transcriptionally regulates the ~~ion-channel-alpha subunit of the E~~ epithelial Na(+) channel ~~-subunit alpha,~~ as well as other ion transport machinery.

Commented [QCE3]: Abbreviations are typically defined the first time the term is used within the abstract and again in the main text and then used exclusively throughout the remainder of the document. Please consider adhering to this convention. The target journal may have a list of abbreviations that are considered common enough that they do not need to be defined.

[Other text deleted]

Dose-Response Curves

For ~~our~~ dose-response curve ~~analysis~~ ~~method~~, the ~~11 β -HSD2~~ ~~performed~~ ~~inhibition~~ ~~or~~ assays ~~are~~ ~~were~~ ~~performed~~ at room temperature (25°C) in pH 8.0 35 mM Tris buffer, ~~also~~ containing 20 mM NaCl. The total volume of the reaction in each well of the plate was 100 μ l, containing 30 μ l of each inhibitor (see ~~the~~ Supplementary Information), 30 μ l of 10 mM NADH, 30 μ l of 4 mM cortisol and 60 μ l of 160 μ g/ml ~~11 β -Hydroxysteroid~~ ~~dehydrogenase~~ ~~dehydrogenase~~ 2. Then, we ~~incubated~~ ~~incubated~~ the reaction mixture at the same temperature as described above, and then added ~~the~~ substrate, ~~and then transferred~~ ~~the~~ ~~The~~ product ~~was transferred~~ into the wells before ~~putting in~~ ~~using~~ the fluorometer to measure the fluorescence. ~~Each reaction was performed in triplicate using well contained a different inhibitors at a different concentrations except that we did each reaction in triplicates.~~ A Master mix was used to initiate the reaction, and we used blanks for all inhibitors as controls. The pH of each reaction ~~well~~ was ~~checked~~ ~~determined~~.

Commented [QCE4]: Please ensure that the intended meaning has been maintained in this edit.

Crystallographic analysis

~~Crystallisation~~ ~~Crystallization~~ ~~trials~~ ~~trials~~ were carried out with bound ~~ligand~~ DE2300c5 to investigate the binding mechanism. Human recombinant 11 β -hydroxysteroid dehydrogenase-2 was over-expressed in ~~the BL21 strain of E. coli~~ ~~BL21~~ ~~E. coli~~ ~~bacterial cells~~ and the purified extract was determined to be 98-% pure using SDS-PAGE ~~gels~~ and a densitometric analysis. We also carried out ~~at~~ ~~the~~ Bradford ~~p~~Protein ~~a~~Assay to determine ~~the concentration of the purified extract~~. The enzyme was then concentrated in 500 μ M NADH buffer, which ~~also~~ contained ~~1~~ 1% glycerol, 1 mM EDTA, 50 mM Tris, ~~pH 8~~, and 100 mM NaCl. The enzyme was ~~then~~ assayed for activity. Finally, the sample was centrifuged at 2200 rpm; ~~for between~~ ~~5 to and~~ 10 minutes at 5°C to remove dust, precipitated protein, etc. Human recombinant 11 β -hydroxysteroid dehydrogenase-2 ~~was crystallised~~ ~~crystallized in a preparation of at a final concentration of~~ 32 mg/ml in pH 7.8 crystal buffer (1-% glycerol, 30 mM Tris, 45 mM NaCl, ~~and~~ 5 mM EDTA).

Commented [QCE5]: Please ensure that the intended meaning has been maintained in this edit.

Commented [QCE6]: Please ensure that the intended meaning has been maintained in this edit.

[Other text deleted]

Figure 3. Bar charts showing the average % inhibition of 11 β -HSD2 from each potential inhibitor in transfected cells. DE2300c5 is compound 8. The experiments were carried out ~~in~~using HEK293 cells.

Figure 4. Cpm vs. inhibitor concentration for 11 β -HSD2-transfected HEK293 cells. A dose-response plot is shown for each compound screened for use as an inhibitor. ~~The~~ chemical structure and the IC50 ~~are also~~ is shown. The maximum line corresponds to substrate binding in the absence of a competitor. DE2300c5 is compound 8. Counts per minute describes the signal detected by scintillation counting. Error bars = S.E., N = 2.

Table 1. A summary of ~~the~~ results ~~from~~from the SPA for 6 selected compounds. Please see ~~the~~Supplementary Information for further ~~information~~details.

Compound number	IC ₅₀ cell SPA	Calculated K _i of SPA (cell)	Calculated K _i of SPA (recombinant)
3	10.4 ± 1.1 μ M	1.2 μ M	N/A
5	1.8 ± 4.04 μ M	2.5 μ M	N/A
DE2300c5	1.82 ± 0.03 nM	15 nM	1.7 nM ± 0.3
9	10.11 ± 8.1 μ M	2.8 μ M	N/A
10	9.61 ± 12.4 μ M	5.7 μ M	N/A
21	6.73 ± 2.06 μ M	2.2 μ M	N/A

Table 2. Summary information relating to the top compounds inhibiting 11 β -HSD2, and the query molecules.

[Table and other text deleted]