Patients with loss–of–-function mutations in the 11β-HSD2 gene suffer from sodium retention. The Ddirect inhibition of this enzyme may therefore be an excellentrepresent a potential therapy for chronic hyponatremia, a condition which causes problemsprevalent 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 with may have favourablefavorable physicochemical and structural characteristics that would enable them to specifically bind to and to inhibit 11β-HSD2. We examined the top hits candidate compounds produced identified by through the virtual screening and tested their interaction with recombinant human 11β-HSD2. enzyme and found oneOne compound, DE2300c5, that strongly bound to 11β-HSD2 without affecting either 11β-HSD1 and or 17β-HSD2. Binding was confirmed in a HEK293 cell culture, and administration of DE2300c5 increased intracellular sodium levels in renal cortical cells, DE2300c5 increased intracellular sodium levels. miR-401 expression was unchanged by DE2300c5 administration. It is clear from ourThe findings of the present study suggest that DE2300c5 represents an effective treatment for hyponatremia, which is a significant health burden, and other electrolyte imbalances.

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INTRODUCTION

11β-hHydroxysteroid Dehydrogenase dehydrogenase type 2 (11β-HSD2) catalyses catalyzes the reaction conversion of the biologically active steroid hormone cortisol to cortisone (Fig. 1). This reaction is co-factor dependent, as it dependsrelies on NAD^{+2}. Cortisol binds to the MR, and interestingly, it binds as tightly as its other natural ligand, which is aldosterone. In vivo, 11β-HSD211bHSD2 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 Eepithelial Na(+) channel subunit alpha, as well as and other ion transport machinery.

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Dose-–Response Curves

For ~~our~~ dose-–response curve analysis~~s method~~, the 11β-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 reaction volume ~~of the reaction~~ in each well of the plate was 100 µl and~~,~~ contained~~ing~~ the following: 30 µl of each inhibitor (see ~~the~~ Supplementary Information), 30 µl of 10 mM NADH, 30 µl of 4 mM cortisol and 60 µl of 160 µg/ml 11β-HSD2~~11β-Hydroxysteroid Dehydrogenase 2~~. ~~Then, we~~We subsequently ~~inclubated~~ 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 the fluorometer to measure the~~ fluorescence was measured using a fluorometer. 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 ~~M~~master mix was used to initiate the reaction, and ~~we used~~ blanks for all inhibitors were included as controls. The pH of each reaction ~~well~~ was ~~checked~~determined.

Crystallographic A~~a~~nalysis

~~Crystallisation~~ Crystallization ~~trails~~ trials were ~~carried out~~performed with bound ~~ligand~~ DE2300c5 to investigate the binding mechanism. Human recombinant 11β-HSD2~~11β-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 ~~a~~the Bradford p~~P~~rotein a~~A~~ssay to determine the concentration of the purified extract. The enzyme was then concentrated in 500 µM NADH buffer ~~-~~at pH 8 ~~which also contained:~~ containing 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°~~e~~C to remove dust, precipitated protein, ~~etc~~and other particulates. Human recombinant 11β-HSD2 ~~11β-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).

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For illustrative purposes only.

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

Figure 4. Cpm vs. inhibitor concentration for 11β-HSD2 ~~-~~transfected HEK293 cells. A dose~~-~~response plot is shown for each compound screened for use as an inhibitor~~,~~ ~~t~~. 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 ~~form~~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 µM	1.2 µM	N/A
5	1.8 ± 4.04 µM	2.5 µM	N/A
DE2300c5	1.82 ± 0.03 nM	15 nM	1.7 nM ± 0.3
9	10.11 ± 8.1 µM	2.8 µM	N/A
10	9.61 ± 12.4 µM	5.7 µM	N/A
21	6.73 ± 2.06 µM	2.2 µM	N/A

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

[Table and other text deleted]

For illustrative purposes only.