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Discovery of Novel 11β-HSD1 Inhibitors by Pharmacophore-Based Virtual Screening

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The 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) enzyme is involved in modulation of glucocorticoid activity within target tissues. This enzyme may contribute to obesity and/or metabolic disease through its action in adipose or liver tissue. Inhibition of 11β-HSD1 has major therapeutic potential for glucocorticoid-associated diseases, including obesity, diabetes (wound healing), and muscle atrophy. To develop such therapeutics, we performed a pharmacophore-based virtual screening (VS) for identification of novel 11β-HSD1 inhibitors and found that the VS hit compounds show potent inhibition of 11β-HSD1 enzyme activity. Further, we present a binding model for active compounds. The proposed pharmacophore may serve as a useful guideline for future design of new chemical entities as 11β-HSD1-targeted antidiabetic agents.

Key Words : 11β-HSD1, Pharmacophore-based virtual screening, Antidiabetic, Glucocorticoid, Molecular docking

Introduction

In Cushing's syndrome, the notable excess of glucocorticoids causes metabolic abnormalities, such as visceral obesity, impaired glucose tolerance, atherosclerosis, dyslipidemia, and hyperglycemia.[1,2] These features of metabolic syndrome can be reversed through normalization of glucocorticoid levels.[3] The principal glucocorticoid hormone is cortisol, which is modulated by tissue-specific enzymes: 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) and type 2 (11β-HSD2). 11β-HSD1 catalyzes the enzymatic conversion of inactive cortisone in human to their active forms (cortisol, corticosterone), whereas 11β-HSD2 catalyzes the reverse reaction. It was reported that 11β-HSD1-knockout mice fed a high-fat diet showed reduced weight gain, improved glucose tolerance and insulin sensitivity, and a decreased hepatic gluconeogenic response to fasting.[4] In contrast, animals with elevated adipose 11β-HSD1 expression develop metabolic-syndrome-like phenotypes.[5] In addition, transgenic mice with increased 11β-HSD2 expression in adipose tissue and maintained on a high-fat diet. Transgenic mice resist weight gain that associate with increased energy expenditure and improved glucose tolerance, as well as insulin sensitivity.[6] These data suggest that 11β-HSD1 could be a potential target for treatment of patients with diabetes and metabolic syndrome.[7,8] Numerous efforts have been made to investigate 11β-HSD1 inhibitors. At present, 3 11β-HSD1 inhibitors, namely, INCB-13739, INCB-20817, and AMG-221, are used in clinical practice.

Pharmacophore modeling provides a productive tool in the discovery of compounds with improved potency and pharmacokinetic properties. This modeling includes ligand-based and structure-based methods. The former uses information provided by a set of known active compounds to build the pharmacophore model (PCM), whereas structure-based pharmacophore modeling using a 3 dimensional known receptor-ligand complex to build the PCM. The structure-based PCM has become increasingly prominent because of the rising number of available protein structures. It has been suggested that protein structure is provided as a good source of structure-based PCM that can be used for the primary screening of ligands before post processing of docking studies.[9,10]

Ligand-based PCMs were first generated to identify 11β-HSD1 inhibitors.[11,12] Usually, suitable 11β-HSD1 inhibitors have been released earlier than a corresponding favorable 11β-HSD1 complex structure. Recently, an X-ray crystal structure (PDB code 3FCO) satisfactory for structure-based molecular modeling has been released.[13] This X-ray structure consists of a complex of human 11β-HSD1 with a potent synthetic inhibitor and with the co-substrate nicotinamide adenine dinucleotide phosphate (NADP). The interactions of the inhibitor with 11β-HSD1 and NADP could be interpreted in a very specific way by building a PCM. Recently AMG-221, MK-0916, PF-915275, and drug candidates are currently not being developed any more, there is a need to develop potential drug candidates. For indentifying the hit compounds, pharmacophore modeling provide initial hits for starting medicinal chemistry program instead of high throughput screening (HTS). Herein, we present structure-based PCMs useful for VS. To identify virtual hits satisfying the combination of hydrogen bond acceptor (HBA) and lipophilic (LP) features of the models, we carried out VS. The virtual hits were purchased and evaluated by enzymatic assay. With this procedure, several selective 11β-HSD1 inhibitors with new scaffolds were discovered. The efficacy

of this strategy was confirmed by positive biological results.

Methods

Pharmacophore Model Generation. In the present study, a X-ray crystal structure of human 11β-HSD1 in complex with a synthetic inhibitor (PDB code 3FCO) was used as the starting structure for PCM generation. Structure-based PCM were generated using a structure-based focusing (SBF) module (Accelrys, Inc., San Diego, CA, USA). The program generates a LUDI interaction map describing all the possible interactions - hydrogen bonding (donors and acceptors) and hydrophobic - that a ligand can establish with a given active site. A map was generated for all atoms within a radius of 10 Å from the geometric center of the inhibitor. The pharmacophore features correspond to inhibitor interactions with active-site residues of 11β-HSD1. Exclusion volume spheres were placed on all heavy atoms of the protein, using a 0.9 Å radius. A three-dimensional (3D) compound database was built with a commercially available chemical library from ChemBridge, Ltd. (http://www.chembridge.com). Compounds in ChemBridge library were generated using 3D multiple conformers with the catDB command in the software Catalyst.[14] The Catalyst-formatted database was screened with the generated pharmacophores, as 3D PCMs, using Catalyst's catSearch. After assessing the query PCMs, virtual screening was carried out using Catalyst. The Fast Flexible Search mode[15] was adopted to screen the ChemBridge library, which contains structural information for 190,000 chemicals. Among the pharmacophore-based virtual screening hit compounds, those exhibiting unfavorable interactions with the binding site or unrealistic conformations were filtered out by visual inspection. Finally, we selected 28 compounds for further testing *in vitro*.

Biological Testing. Inhibition of human and mouse 11β-HSD1 and 11β-HSD2 enzymatic activity was determined through scintillation proximity assay (SPA), using microsomes containing 11β-HSD1 or 11β-HSD2.[12-16] Enoxolone was used as a positive control. The human and murine 11β-HSD1 and 11β-HSD2 enzymes were expressed in HEK-293 cells. Briefly, the sequences of human and murine 11β-HSD1 and 11β-HSD2 were obtained from clones provided by the NIH Mammalian Gene Collection. pcDNA3-derived expression plasmids were constructed by inserting the sequences into the multiple clone site of pcDNA3 purchased from Invitrogen. HEK-293 cells were transfected with the pcDNA3-derived expression plasmid and were selected by cultivation in the presence of 700 μg/mL of G-418. A microsomal fraction overexpressing 11β-HSD1 or 11β-HSD2 was prepared from HEK-293 cells stably transfected with either 11β-HSD1 or 11β-HSD2 and was used as the enzyme source for SPA. 11β-HSD1-containing microsomes were incubated with NADPH and [^{3}H]cortisone. Subsequently, the product [^{3}H]cortisol was specifically captured with a monoclonal antibody coupled to protein-A-coated SPA beads. The 11β-HSD2 screening was performed by incubating 11β-HSD2 microsomes with [^{3}H]cortisol and NAD+ and monitoring substrate disappearance.

Results and Discussion

As shown with a known inhibitor in Figure 1, the PCM automatically generated by the Structure-based Focusing (SBF) program includes 6 features: 1 HBA and 5 LP features. In addition, the program automatically generated several excluded volumes in the model. The HBA features point from the carbonyl group of the ligand to Tyr183 or to Ser170. The 5 LP features are located on the 2 cyclopropyl rings, on the cyclohexyl, on the ethyl and on the aromatic group of the ligand. Two modifications were made on this model to obtain an appropriate model for VS. The first modification involved the cyclohexyl of the ligand. While a cyclohexyl group is clearly hydrophobic, SBF could not automatically interpret the cyclohexyl ring as a hydrophobic group. Therefore, a hydrophobic group was added manually to describe this feature, resulting in Model 1. The other modification involved 2 HBA features located on 1 oxygen atom for describing 2 hydrogen bond interactions. Because Catalyst only supports 1 feature on 1 heavy atom, Model 1 was converted into 2 additional Catalyst query PCMs, Models 2 and 3. The HBA describing the hydrogen bond between Tyr183 and the ligand was retained in Model 2, whereas the other HBA of interaction with Ser170 was conserved in Model 3. Based on the above modifications, 2 Catalyst query PCMs with 6 features were prepared for subsequent VS. The prepared 3D database from ChemBridge library was searched with PCMs 2 and 3, employing the Fast Flexible Search algorithm. The resulting hits were submitted for best fit value calculations. The 28 compounds selected by VS with PCMs were examined using the same docking protocol, resulting in 14 compounds from Model 1. 14 compounds were additionally present among the 9 and 5

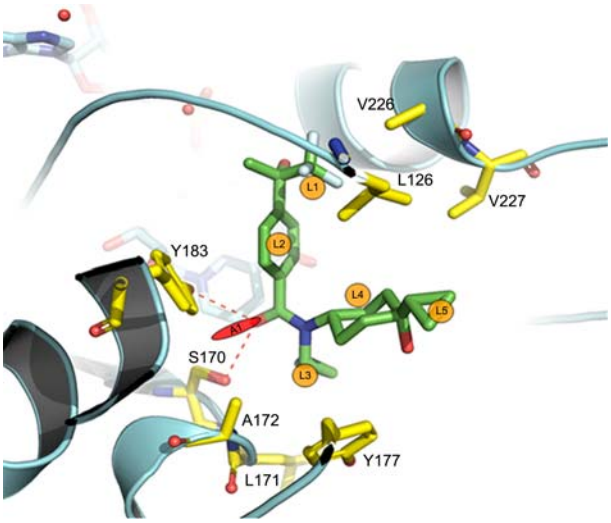
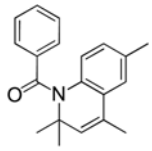
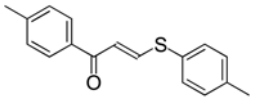
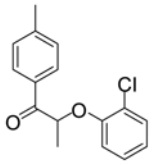
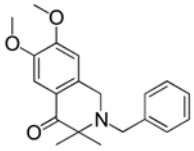
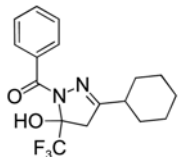


Figure 1. Co-crystal structure of an inhibitor with the 11β-HSD1 enzyme and detailed map of the proposed pharmacophore. The red circle indicates the hydrogen bond acceptor of the ligand. The yellow circles denote the hydrophobic interaction sites of the ligand.

Table 1. Inhibition percentages for 5 compounds at 100 μM against human 11β-HSD1 and 11β-HSD2

Compound	Structure	11β-HSD1 % inhibition	11β-HSD2 % inhibition	11β-HSD1 IC_{50} (μM)
1		95.3	11.7	6.1
2		90.8	9.0	7.7
3		83.1	2.4	13.1
4		66.9	3.2	30.1
5		59.8	5.5	49.1

compounds selected by Model 2 and Model 3, respectively. Thereafter, 28 compounds were finally extracted and evaluated by enzymatic assay.

These 28 compounds were purchased and were evaluated by enzyme assay for their inhibition of human 11β-HSD1. Compounds that showed more than 50% inhibition of human 11β-HSD1 at the concentration of 100 μM were put into dose-dependent studies. Among them, 5 compounds exhibited dose-dependent inhibition of human 11β-HSD1, with IC_{50} values ranging from 6.1 to 49.1 μM. Chemical structures and enzymatic activity of virtual screening hit compounds were tabulated in Table 1. The common scaffold for the final 5 compounds is an acetophenone moiety, which is consistently conserved with a known inhibitor in Figure 1.

The best docking poses of these compounds are shown in Figure 2. As mentioned previously, the ligand in the crystal structure forms 2 hydrogen bonds, 1 with Ser170 and 1 with Tyr183 (Figure 1). Interestingly, 5 active compounds form the same interactions with the enzyme in the best docking poses (Figure 2). Hydrogen bonds provide strong interactions between the ligand and the protein as well as its co-substrate. Figure 2 shows a key interaction, in which the carbonyl group of all the ligands consistently forms 2 hydrogen bonds with the side chains of Tyr183 and Ser170. Docking pose of active inhibitors were prepared using PyMOL.[17] The di-methyl group in compound **1** binds to the side chain of Ala172 with the hydrophobic interactions, which occurs the increasing activity. However, the di-methyl moiety in compound **4** shows same interactions, but di-methoxy group decreases the inhibiting activity. The hydroxyl and CF_3 groups in compound **5** interact the side chain of Ala172, which shows weaker inhibiting activity,. Several reported 11β-HSD1 inhibitors, including 3 compounds in the clinical stage, have a carbonyl group or the similar sulfonyl group, which might also form hydrogen bonds with the side chains of Tyr183 and Ser170.[18] Therefore, particular attention has been given to the HBA features of Models 2 and 3. Docking is a direct and simple method for finding hits that could form a hydrogen bond with Tyr183 or Ser170. A docking pose of an inhibitor can directly provide evidence that an inhibitor interacts with the side chains of Tyr 193 and Ser170 residues of 11β-HSD1.

To obtain highly selective inhibitors, these compounds were further tested for inhibition of human 11β-HSD2. All of the active compounds showed low inhibition against 11β-HSD2 at a concentration of 100 μM. Therefore, hit compounds are selective 11β-HSD1 inhibitors (Table 1).

In summary, structure-based PCMs were built and used in VS. The selected hits were further filtered by docking analysis. Finally, 28 compounds were selected and put into biological testing. Five compounds with IC_{50} values less than 50 μM were disclosed, providing 3 new chemical

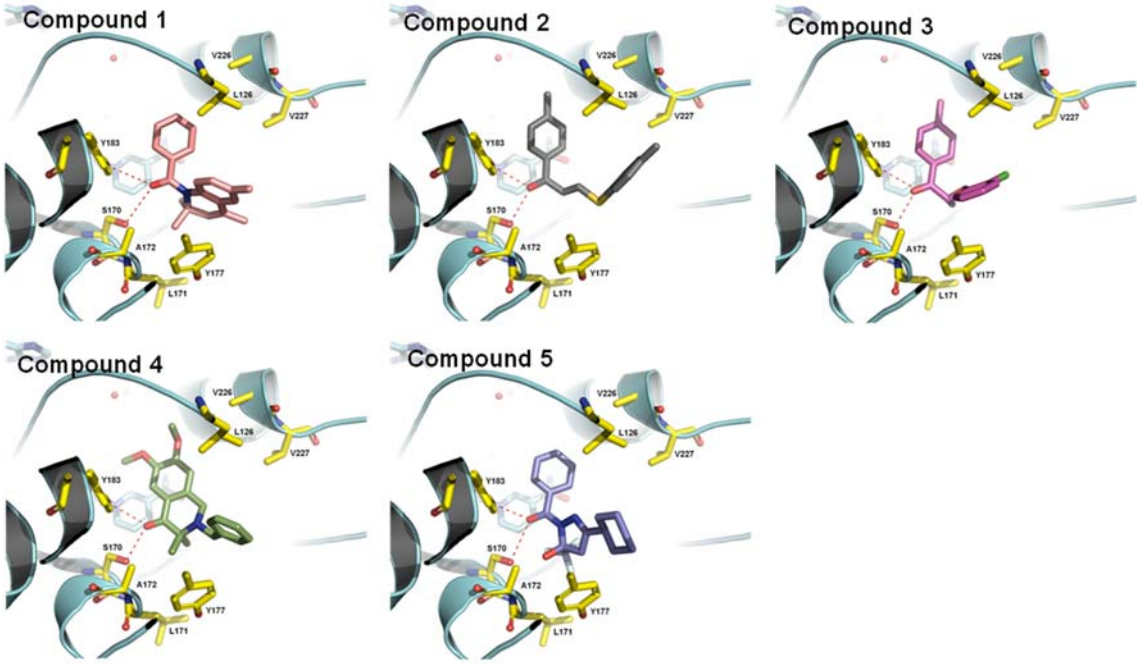


Figure 2. Molecular docking model of hit compounds **1-5** predicted. The carbonyl group of all the ligands forms 2 hydrogen bonds with the side chains of Tyr183 and Ser170.

scaffolds as 11β-HSD1-selective inhibitors. These new scaffolds provide useful information for further drug discovery of 11β-HSD1.

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