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Original article

# Structure-based virtual screening for identification of novel 11β-HSD1 inhibitors

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**Abstract**

Structure-based pharmacophore models were built by using LigandScout and used for virtual screening of the SPECS database to identify new potential 11β-HSD1 inhibitors. As a refinement of the results obtained from virtual 3D pharmacophore screening, the best fitting virtual hits were subjected to docking study. The resulting compounds were tested in an enzyme assay and revealed several compounds with novel scaffolds that show sub-micromolar activity and high selectivity for 11β-HSD1 against 11β-HSD2.


*Keywords:* 11β-HSD1; 11β-HSD2; Inhibitor; Pharmacophore; Docking

## 1. Introduction

In Cushing's syndrome, the notably excess of glucocorticoids causes metabolic abnormalities, such as visceral obesity, impaired glucose tolerance, atherosclerosis, dyslipidaemia and hyperglycemia [1,2]. These features of metabolic syndrome can be reversed through normalization of GC levels [3]. The principal glucocorticoid 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 conversion of inactive cortisone into glucocorticoid receptor-active cortisol, while 11β-HSD2 catalyzes the reverse reaction. It was reported that 11β-HSD1 knockout mice showed reduced weight gain on a high-fat diet, 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 resist weight gain on high-fat diet, which is associated 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 diabetes and metabolic syndrome [7,8]. Numerous efforts have been made to discover 11β-HSD1 inhibitors. In the time being, three 11β-HSD1 inhibitors, namely INCB-13739, INCB-20817, and AMG-221, are 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 pharmacophore model (PCM), while the structure-based pharmacophore modeling adopts receptor–ligand complex to build PCM. The structure-based method becomes more and more important because more and more protein structures have been and are being identified. It has been suggested that protein structure

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is a good source of pharmacophore and can be used as first-screening before docking studies [9,10].

Ligand-based PCMs rather than structure-based PCMs were first generated to identify 11β-HSD1 inhibitors [11,12]. Usually suitable 11β-HSD1 inhibitors are released earlier than a corresponding favorable structure of 11β-HSD1 complex. Last year, an X-ray crystal structure (PDB code 2IRW) satisfactory for structure-based modeling was released [13]. This complex structure is composed of human 11β-HSD1, a synthetic inhibitor with high activity, and a co-substrate nicotinamide–adenine–dinucleotide phosphate (NADP). The interactions of the inhibitor to 11β-HSD1 and NADP could be interpreted in a very specific way by building a PCM. Herein, we present structure-based PCMs which are used for first-screening. In order to select hits satisfying the hydrogen bond acceptor (HBA) features of the models, molecular docking was carried out as second-screening. These hits were purchased and evaluated by the enzyme assay. With this procedure several selective 11β-HSD1 inhibitors with new scaffolds were discovered. The efficiency of this strategy is confirmed by the positive biological results.

## 2. Methods

### 2.1. Pharmacophore model generation

In the present study crystal structure of human 11β-HSD1 with a synthetic inhibitor (PDB code 2IRW) was used as starting structure for the generation of PCM. The software LigandScout [14] was applied to detection and interpretation of crucial interaction patterns between 11β-HSD1 and the ligand. LigandScout extracts and interprets ligands and their macromolecular environment from PDB files and automatically creates and visualizes advanced 3D PCMs. In the present study, the 3D PCM produced by LigandScout was exported and converted into two Catalyst [15] query PCMs: **2** and **3** (see Figs. 1 and 2).

### 2.2. In silico screening

After assessing the query PCMs, virtual screening was carried out by using the software Catalyst. The Fast Flexible

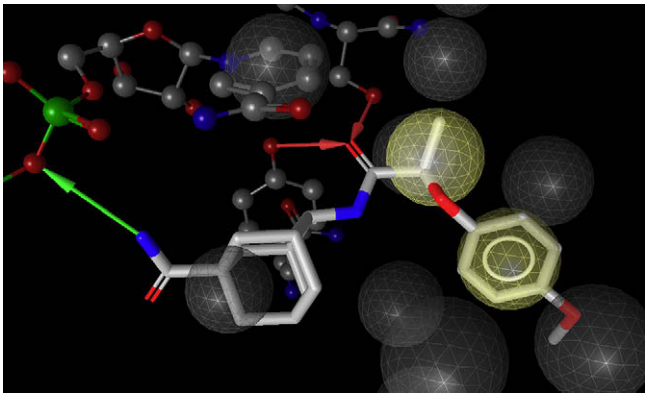

Fig. 1. LigandScout pharmacophore model generated from 11β-HSD1/ligand complex (red arrows, HBA; green arrow, HBD; yellow spheres, hydrophobic sites; gray spheres, excluded volumes).

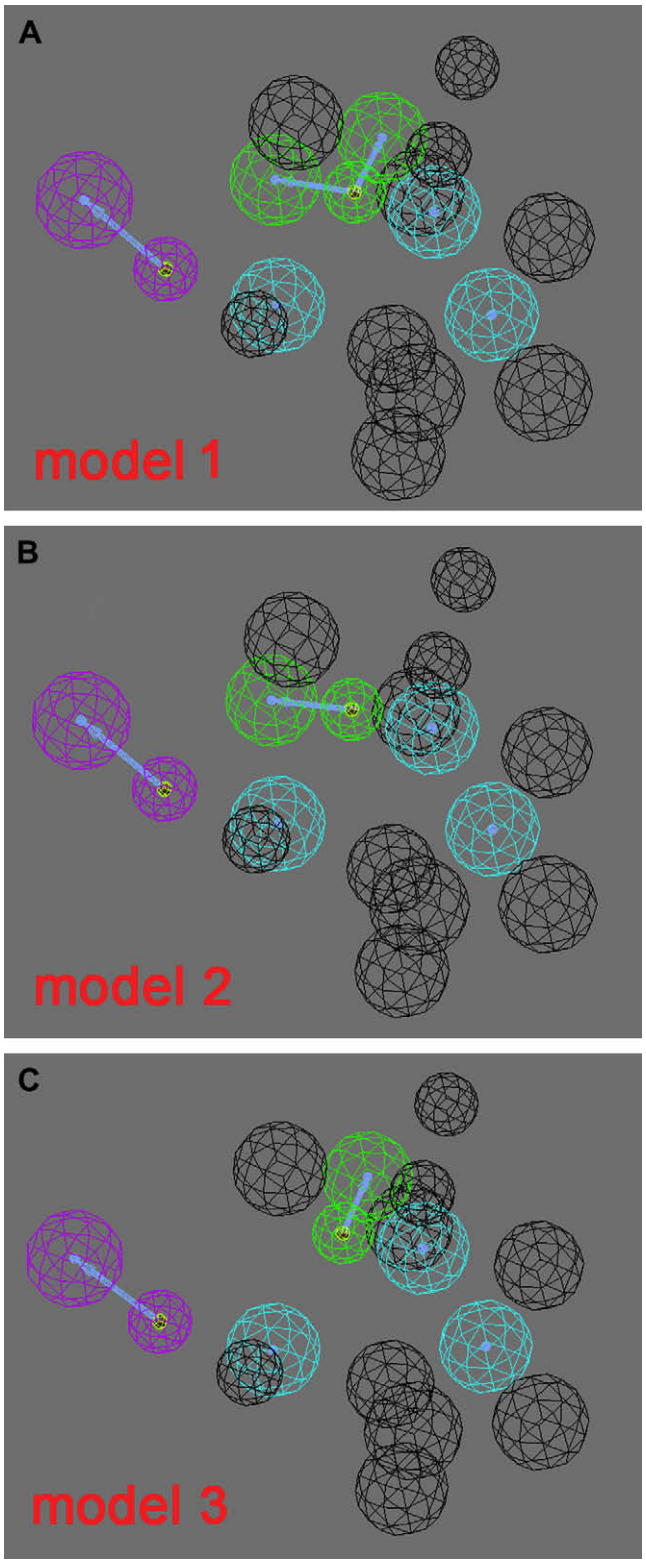


Fig. 2. Pharmacophore models **1** (A), **2** (B) and **3** (C) used within Catalyst (green, HBA; magenta, HBD; blue, hydrophobic sites; gray, excluded volumes).

Search mode was adopted to screen the SPECS database which contains the structural information of 190,000 chemicals (http://www.specs.net). The resulting hit molecules were ranked according to their Best Fit values. The compounds

with highest Best Fit values were extracted and subjected to docking study to select hits which satisfy the HBA feature of the models. Rigid docking studies were performed by using the Glide program [16]. The starting structure was PDB entry 2IRW. Receptor was prepared by using the Protein Preparation and Grid Preparation tools in the Schrödinger Maestro interface. The default settings were adopted for the cutoff, neutralization, scaling, dimensions of the binding pocket used for grid preparation, and treatment of the co-substrate NADP. The centroid of the ligand in the crystal structure was used as the center of the enclosing box. The cutoffs of each side of the box are 10 Å. Compounds were evaluated by using Gscores and one pose per ligand was written out.

### 2.3. Biological testing

Inhibition of human and mouse 11β-HSD1 and 11β-HSD2 enzymatic activities were determined by scintillation proximity assay (SPA) using microsomes containing 11β-HSD1 or 11β-HSD2 [12,18]. Enoxolone was tested as the positive control. The human and murine 11β-HSD1 and 11β-HSD2 enzymes were expressed in HEK-293 cells. Briefly, the sequence of human and murine 11β-HSD1 and 11β-HSD2 was obtained from the clones provided by NIH Mammalian Gene Collection. The pcDNA3-derived expression plasmids were constructed by inserting the sequence into the multiple clone site of pcDNA3 purchased from Invitrogen. HEK-293 cells were transfected with the pcDNA3-derived expression plasmid and selected by cultivation in the presence of 700 μg/ml of G-418. The microsomal fraction overexpressing 11β-HSD1 or 11β-HSD2 was prepared from the HEK-293 cells stable transfected with either 11β-HSD1 or 11β-HSD2 and used as the enzyme source for SPA. 11β-HSD1 containing microsomes was incubated with NADPH and [$^3$H]cortisone, then the product, [$^3$H]cortisol was specifically captured by 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.

## 3. Results and discussion

As shown in Fig. 1, the PCM automatically generated by the LigandScout program includes five features: one hydrogen bond donor (HBD), two hydrogen bond acceptors (HBA) and two hydrophobic groups. Besides, the program automatically generated several excluded volumes in the model. The HBD feature points from the $NH_2$ group of the ligand to the NADP. Both HBA features characterize the carbonyl group of the ligand which forms two hydrogen bonds with Tyr183 and Ser170. The two hydrophobic groups are located on the aromatic ring and the propyl group of the ligand, respectively.

Two modifications were made on this model to obtain appropriate model for VS. The first modification is about the adamantly of the ligand. It is clear that it is a hydrophobic group, but the LigandScout could not interpret the adamantly as a hydrophobic group automatically. Therefore, a hydrophobic group was added manually to describe this feature, resulting in model **1**. Another modification is about the two HBA features located on one oxygen atom. Because the Catalyst only supports one feature on one heavy atom, the model **1** was converted into two Catalyst query PCMs, **2** and **3**. The HBA describing the hydrogen bond between Tyr183 and the ligand was kept in model **2**, while the other HBA was conserved in model **3** (see Fig. 2). As a result, based on above modifications, two Catalyst query PCMs with five features (Fig. 2) were prepared for subsequent VS.

SPECS database was searched with PCMs **2** and **3** employing the Fast Flexible Search algorithm. The resulting hits were submitted into Best Fit value calculations. Then, for each model, 1000 compounds with highest Best Fit values were extracted and put into docking study.

Hydrogen bonds provide strong interactions between the ligand and the protein as well as its co-substrate. As shown in Fig. 1, the carbonyl group of the ligand forms two hydrogen bonds with Tyr183 and Ser170. Several reported 11β-HSD1 inhibitors, including the three compounds in clinical stage, have carbonyl group or similar sulphonyl group which might also form hydrogen bond with the two residues. Therefore more attentions were paid to the HBA features of the models **2** and **3**. Docking is a direct and simple method for finding hits which could form hydrogen bond with Tyr183 or Ser170. The docking pose could provide direct proof of that whether a compound forms such hydrogen bond.

The 1000 compounds selected by VS with PCM **2** were put into docking study by using the program Glide. Then the first 200 docking poses with highest Gscores were visually inspected. Compounds that formed hydrogen bond with Tyr183 or Ser170 were selected and put into druglikeness filtering [17]. The 1000 compounds selected by VS with PCM **3** were put into the same studies, resulting in 24 compounds selected. Seven compounds existed both in the 39 compounds selected by model **2** and the 24 compounds selected by model **3**. Therefore, totally 56 hits were selected after docking study.

These compounds were purchased and were evaluated by the enzyme assay for their inhibition of the human and mouse 11β-HSD1. Compounds which showed more than 50% inhibition of human or mouse 11β-HSD1 at the concentration of

Table 1
Inhibition of 11β-HSD1

| Compound | $IC_{50}$ (μM) | |
|---|---|---|
| | Human | Mouse |
| **1** | 7.98 | 3.02 |
| **2** | 3.76 | 3.76 |
| **3** | 0.85 | 0.44 |
| **4** | 5.3 | >10 |
| **5** | 0.98 | 5.87 |
| **6** | 2.25 | >10 |
| **7** | 4.0 | >10 |
| **8** | 1.09 | >10 |
| **9** | 1.73 | >10 |
| **10** | >10 | 6.55 |
| **11** | >10 | 8.48 |
| Enoxolone | 0.013 | 0.005 |

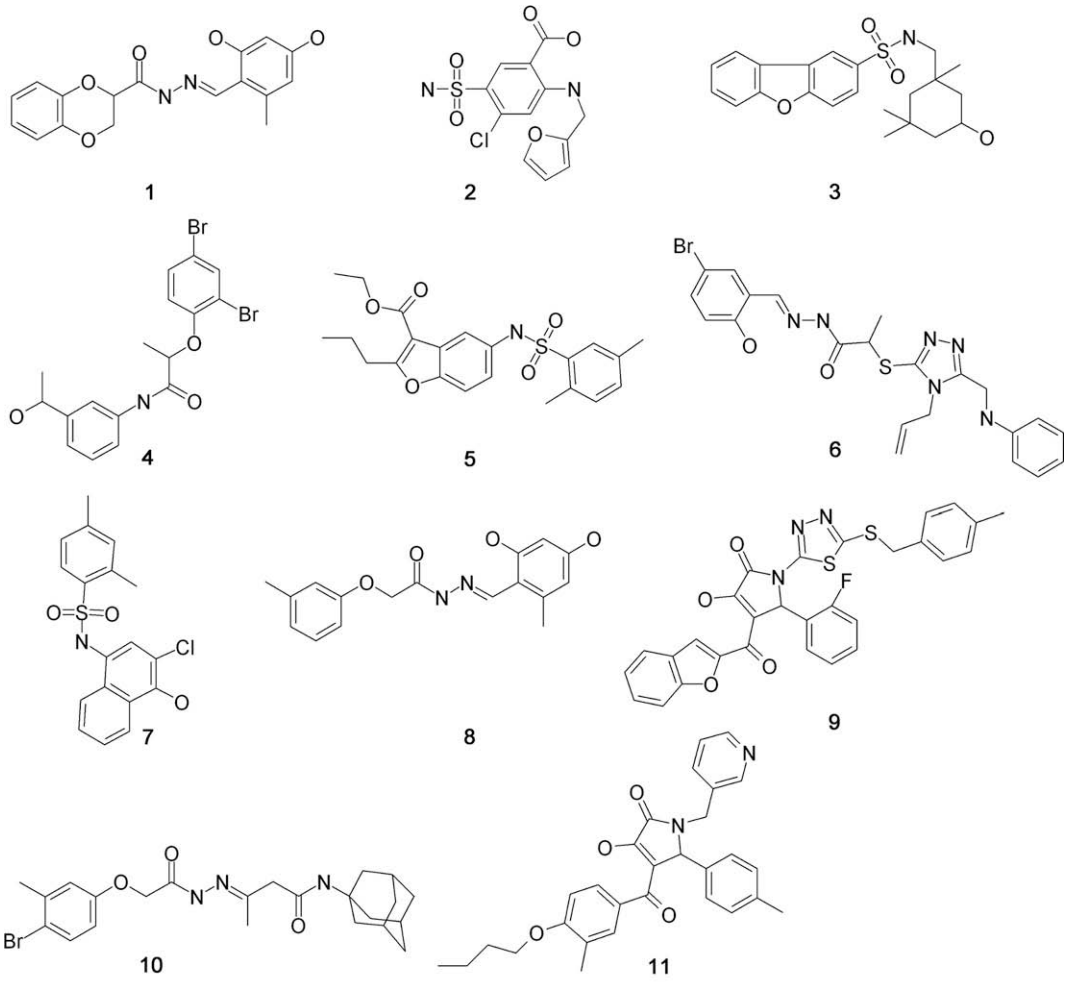


Fig. 3. Chemical structures of active compounds.

10 μM were put into concentration-response studies. Among them, nine compounds (**1**–**9**) exhibited dose-dependent inhibition of human 11β-HSD1 with $IC_{50}$ values ranging from 0.85 to 7.98 μM and six compounds (**1**, **2**, **3**, **5**, **10** and **11**) showed dose-dependent inhibition of mouse 11β-HSD1 with $IC_{50}$ values ranging from 0.44 to 8.48 μM (see Table 1 and Fig. 3).

In order to gain the highly selective inhibitor, these compounds were further tested for the inhibition against the human and mouse 11β-HSD2. All the compounds showed low inhibition against 11β-HSD2 at concentrations of 100 μM. Therefore these molecules are selective 11β-HSD1 inhibitors.

The best docking poses of these compounds are shown in Fig. 4. As mentioned previously, the ligand in the crystal structure forms two hydrogen bonds with Ser170 and Tyr183, respectively (see Fig. 1). Interestingly, seven (**1**, **3**, **5**, **7**–**10**) of the 11 active compounds form the same interactions with the enzyme in the best docking poses (see Fig. 4). The remaining four compounds form hydrogen bond only with Ser170 in the best docking poses.

The scaffolds of the 11 compounds were analyzed. As shown in Fig. 3, the scaffold of arylsulfonamido is present in compounds **2**, **3**, **5** and **7**. In fact, this scaffold is already reported in some earlier important 11β-HSD1 inhibitors [7,20].

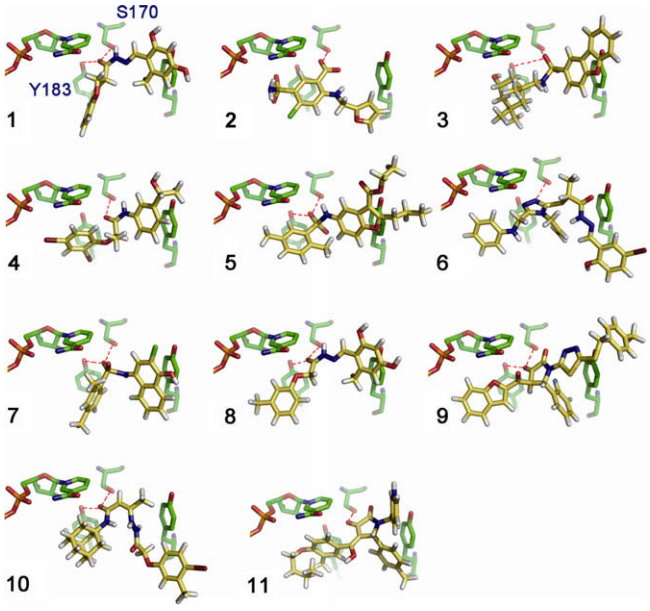


Fig. 4. Best binding poses of compounds **1**–**11** resulting from docking. The cofactor NADPH and three residues are shown in green. Compounds are displayed in yellow. The hydrogen bonds between the ligands and Ser170 or Y183 are shown as red dashed lines. This figure was prepared by using PyMOL [19].

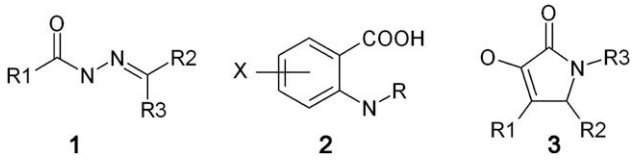


Fig. 5. New chemical scaffolds as 11β-HSD1 inhibitors.

For example, the scaffold exists in compound BVT-3498 (Bioovitrum Corp) which entered phase II trials but did not undergo further development because of phase II failure. Among the three 11β-HSD1 inhibitors which are presently in clinical practice, INCB-13739's structure was disclosed. Arylsulfonamido is also present in compound INCB-13739. Though the ligand in the starting structure (PDB:2IRW) for pharmacophore modeling and docking does not have the scaffold of arylsulfonamido, novel 11β-HSD1 inhibitors with the scaffold of arylsulfonamido were discovered by using the modeling approach. This result confirms the validity of the modeling method in finding new active compounds different from the starting ligand. Besides arylsulfonamido, the active compounds in Fig. 3 produce other three kinds of new scaffolds (**1**–**3**) as 11β-HSD1 inhibitors (see Fig. 5). Scaffold **1** appears in compounds **1**, **6**, **8** and **10**. Compound **2** generates the second scaffold. As shown in Fig. 4, the carboxyl in scaffold **2** forms hydrogen bond with Ser170. The last scaffold (**3**) is produced by two compounds (**9** and **11**). The hydroxyl in scaffold **3** forms two hydrogen bonds with Ser170 and Tyr183, respectively (see Fig. 4). These new scaffolds provide useful information for designing better chemical agents inhibiting 11β-HSD1.

In summary, structure-based Catalyst query PCMs **2** and **3** were built and used as first-screening. The hits with highest Best Fit values were further filtered by docking and druglikeness analysis. Finally, 56 compounds were selected and put into biological testing. Eleven compounds with $IC_{50}$ values less than 10 μM are disclosed, providing three new chemical scaffolds as 11β-HSD1 selective inhibitors.

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