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# Discovery and structure-guided drug design of inhibitors of 11β-hydroxysteroid-dehydrogenase type I based on a spiro-carboxamide scaffold

Franck Lepifre, Serge Christmann-Franck *, Didier Roche, Caroline Leriche †, Denis Carniato ‡, Christine Charon, Sophie Bozec, Liliane Doare, Fabien Schmidlin §, Marc Lecomte, Eric Valeur ¶

*Merck-Serono, 4 Avenue du Président Mitterrand, 91380 Chilly-Mazarin, France*

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ABSTRACT

Spiro-carboxamides were identified as inhibitors of 11β-hydroxysteroid-dehydrogenase type 1 by high-throughput screening. Structure-based drug design was used to optimise the initial hit yielding a sub-nanomolar $IC_{50}$ inhibitor (0.5 nM) on human 11β-HSD1 with a high binding efficiency index (BEI of 32.7) which was selective against human 11β-HSD2 (selectivity ratio > 200000).



Hydroxysteroid dehydrogenases regulate ligand-binding to diverse steroid hormone receptors at the pre-receptor level, like a switch, converting steroid hormones to the inactive or precursor forms and vice-versa.[1] Among these, 11β-hydroxysteroid dehydrogenases 1 and 2 enzymes tune glucocorticoid chemistry, oxidizing the 11β-hydroxyl position or reducing the 11-oxo group, which determine whether or not steroids are able to bind to glucocorticoid (and mineralocorticoid) receptors.[2]

Binding to glucocorticoid receptor is restricted to active steroids bearing 11β-hydroxyl moiety, i.e. mainly cortisol in humans and corticosterone in rodents. 11β-hydroxy-steroid dehydrogenase type 2 (11β-HSD2) is expressed in tissues containing the mineralocorticoid receptor, predominantly kidney, but also gut and placenta.[3] 11β-HSD2 inactivates active glucocorticoids (such as cortisol and corticosterone) to cortisone or 11-dehydrocorticosterone.[2] At the opposite, the other isoform 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) catalyses intracellular regeneration of active glucocorticoids from inert precursors (cortisone, 11-dehydrocorticosterone) by 11-oxoreductase activity in liver, adipose tissue, brain, skeletal muscle, vascular smooth muscle cells and other organs.[4] Several transgenic and in vivo models performed in rodents have documented the physiological importance of this glucocorticoid converting enzyme in insulin action. In particular, 11β-HSD1 knockout mice were shown to resist hyperglycemia and to have increased hepatic insulin sensitivity.[5] In mirror of this, 11β-HSD1 selective over-expression in mouse adipose tissue resulted in development of visceral adiposity, hyperglycemia and insulin resistance,[6] landmarks of metabolic syndrome. In addition, 11β-HSD2 over-expression in mouse adipose tissue, leading to the inactivation of glucocorticoids, improved glucose tolerance and insulin sensitivity.[7]

These promising studies, as well as others,[8] strongly suggest that 11β-HSD1 inhibition is a potential therapeutic target for a broad range of disorders that could be improved by decreased intracellular glucocorticoid levels (i.e., cortisol), including the obesity-induced metabolic syndrome. Selectivity against the 11β-HSD2 isoform is expected to be crucial as deficiency or inhibition (i.e., licorice) of 11β-HSD2 are conditions allowing mineralocorticoid receptor to be occupied by cortisol, leading to the 'essential' form of hypertension.[9] In view of this, many efforts towards the discovery and development of selective 11β-HSD1 inhibitors have been carried out.[10]

We wish to report herein the structural bases which led us to rapidly identify spirocycloalkyl-pyrrolidine amides[11] as a new privileged structure.

Compound **1**, based on a spiro-chromanone scaffold, was identified as an initial hit by high-throughput screening (Fig. 1). It displayed a moderate potency on the human isolated 11β-HSD1

* Corresponding author.
*E-mail address:* serge.christmann-franck@merck.fr (S. Christmann-Franck).
† Present address: Fovea Pharmaceuticals, 12 rue Jean-Antoine de Baif, 75013 Paris, France.
‡ Present address: Cytomics Pharmaceuticals, 86 rue de Paris, Bâtiment Mélèze, 91400 Orsay, France.
§ Present address: Ipsen, 5 avenue du Canada, 91966 Les Ulis Cedex, France.
¶ Present address: Novartis Pharma AG, FAB 16-4.06.6 CH-4002 Basel, Switzerland.

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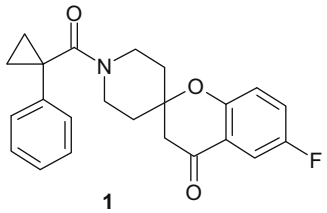



**1**

$IC_{50}$ = 2.5 μM

BEI = 14.8

**Figure 1.** Initial hit discovered by HTS.

(2.5 μM) and moderate Binding Efficiency Index (BEI)[12] of 14.8 (molecular weight of 379 Da). In a hit characterisation context, docking of compound **1** was performed to confirm the spiro-chromanone as a valuable starting point for optimisation and to guide chemistry programs. The program GOLD 4.0[13] was used to run the docking in the human 11β-HSD1–adamantane sulfone co-crystal structure (PDB code 2ILT).[14] The population size and number of operations per docking were set to auto with maximum search efficiency (200%). Water molecules 4288 and 4285 were set to toggle and spin, and a maximum of 20 poses per ligand was set, using the Chemscore scoring function.[15]

The carbonyl of the amide group established hydrogen bonds with hydroxyl groups of residues Ser170 and Tyr183 (Fig. 2). In-house and public previous observations had led us to the conclusion that a cyclopropane ring can direct hydrophobic groups away from the catalytic residues, in the direction of the solvent-connected channel, through a Thorpe–Ingold effect.[16] Surprisingly, the phenyl was located in the same subpocket as the adamantyl in the original crystal structure, close the NADP(H) cofactor, and the spiro-chromanone moiety pointed away from the catalytic residues, towards the solvent, in the vicinity of Tyr177. This reversed binding mode was suggested to be induced by steric clashes of the initial hit chromanone moiety within the adamantyl pocket.

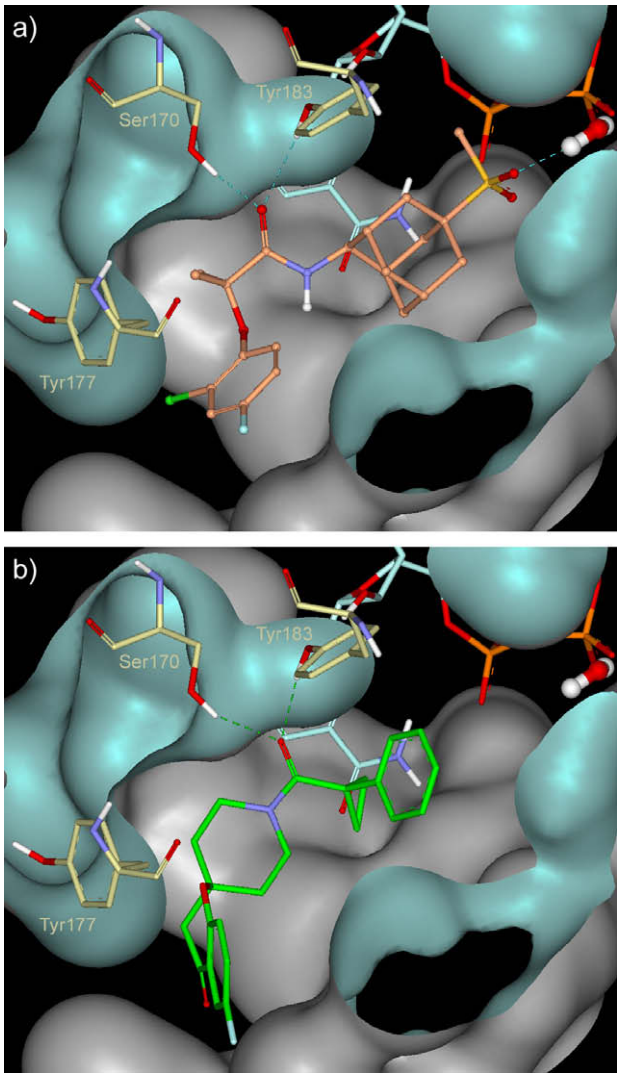
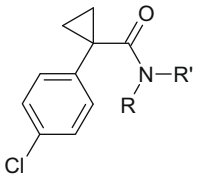
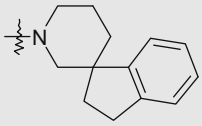
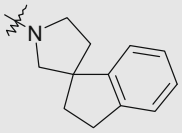
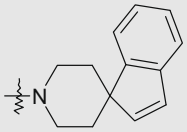
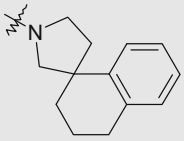
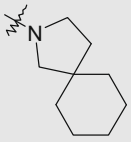



**Figure 2.** Steroid substrate binding site of human 11β-HSD1 (PDB code 2ILT) with (a) the adamantane sulfone compound from the experimental structure and (b) docked compound **1**; for the sake of clarity, only Tyr177 and catalytic residues Ser170 and Tyr183, and only polar hydrogen atoms have been displayed; colour coding by atoms is as follows: oxygen in red, nitrogen in blue, hydrogen in white, fluorine in light blue, chlorine in light green, phosphorus in orange; carbon atoms are coloured in light orange for the experimental ligand (a), in green for compound **1** (b), in light yellow for the protein and in light blue for the NADP(H) cofactor (protonation state not determined in the X-ray structure).

**Table 1**
Variations of the spiro moiety




| Compounds | NRR′ | h-HSD1 $IC_{50}$ [a], nM | BEI [b] |
|---|---|---|---|
| **2** |  | 94 | 19.2 |
| **3** |  | 25 | 21.6 |
| **4** |  | 2100 | 15.6 |
| **5** |  | 22 | 20.9 |
| **6** |  | 6 | 25.9 |

[a] 11β-HSD1 enzyme activity was assessed in 30 mM Hepes buffer, pH 7.4, containing 1 mM EDTA, 1 mM G-6P, and a substrate mixture cortisone/NADPH (200 nM / 200 μM). Reaction was initiated by addition of 3 μg of human recombinant 11β-HSD1 expressed in *E. Coli* and terminated with addition of 18β-glycyrrhetinic acid stop solution after 150 min incubation at 37 °C. Determinations of cortisol levels were monitored by HTRF assay (Cisbio International).

[b] Binding efficiency index = $pK_i$ or $pIC_{50}$/molecular weight (kDa), $pIC_{50}$ was used in this study.

This prompted us not to focus on spiro-chromanones in our first round of optimisation. Therefore, a small library of diversified spiro-compounds based on commercially available building blocks was prepared. Based on our experience, p-chlorophenyl cyclopropane carboxylic acid was used as a preferred capping reagent. New analogues were synthesized through standard amide bond formation and led to a 2 log potency improvement as exemplified with compounds **2**, **3** and **5** (average BEI of 20, Table 1).

Dockings confirmed our first hypothesis with a binding mode of the newly synthesized compounds similar to the experimental structure (Figs. 2a and 3). Interestingly, the incorporation of a pyrrolidine or a 3-piperidine within the spiro moiety was necessary to improve significantly the potency, while a 4-piperidine (compound **4**) proved to be detrimental.

Although the subpocket close to the NADP(H) cofactor can accommodate bulky, hydrophobic groups such as adamantyl,[13] the presence of the cofactor phosphate groups and of a network of conserved water molecules also offers possibilities for polar contacts. Since the spiro-indane aromatic ring was inserted close to these hydrophilic groups (Fig. 3), we postulated that removal of this aromatic ring to afford a smaller, non-aromatic spiro moiety may improve potency.

Gratifyingly, this hypothesis was confirmed experimentally with compound **6** displaying a single digit nanomolar $IC_{50}$. Combining optimised interactions within the binding site and reduced molecular weight led to an improved BEI of 25.9.

We then investigated the tuning of potency through the variation of the aromatic cyclopropane carboxamide moiety (Table 2). As expected, orientating effect of the cyclopropane group (Thorpe–Ingold effect)[16] was necessary for achieving high potency as compounds **8** and **9** displayed a dramatic loss in potency. The replacement of the phenyl group by a pyridine also proved to be detrimental (compounds **10** and **11**). The most active compounds reached a BEI above 31, twice as high as the initial hit **1** (Table 2), and above the average BEI calculated based on mean molecular weight of marketed oral drugs and arbitrary potency of 1 nM (BEI = 27.0).[12] Sub-nanomolar $IC_{50}$ was obtained through removal of the halogen on the phenyl group (compound **13**). Again, docking confirmed the expected binding mode for this compound (Fig. 4). This achievement was not associated with an increased molecular weight, since the most active compound **13** gained 3.5 log in potency over hit **1** while its molecular weight was reduced by 100 Da.

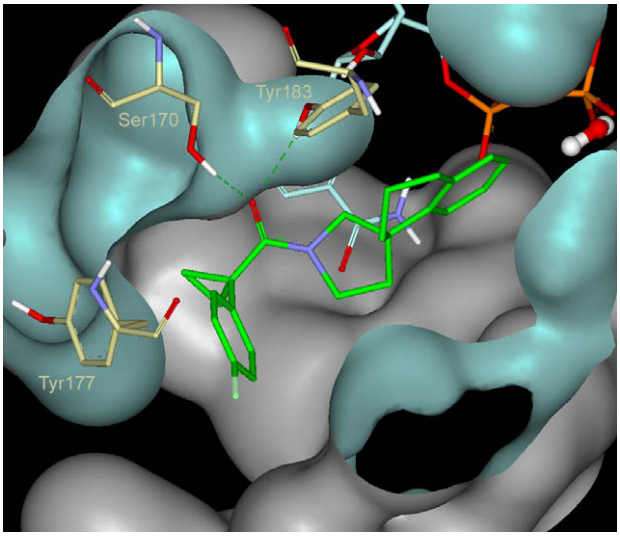
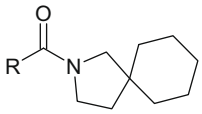
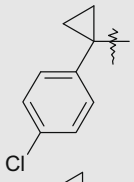
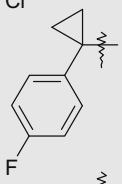
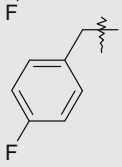
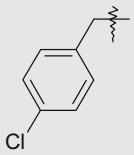
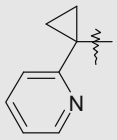
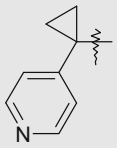
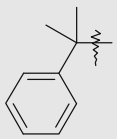
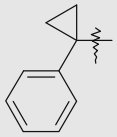



**Figure 3.** Steroid substrate binding site of human 11β-HSD1 (PDB code 2ILT) with docked compound **3**; displayed details and colour coding by atoms are identical to Figure 2b.

**Table 2**
Variation of the aromatic group




| Compounds | NRR' | h-HSD1 $IC_{50}$[a] (nM) | BEI[b] |
|---|---|---|---|
| **6** |  | 6 | 25.9 |
| **7** |  | 3 | 28.2 |
| **8** |  | 1410 | 21.2 |
| **9** |  | 445 | 21.8 |
| **10** |  | 92 | 24.7 |
| **11** |  | 252 | 23.2 |
| **12** |  | 1.5 | 31 |
| **13** |  | 0.5 | 32.7 |

[a] h 11β-HSD1 experimental protocol as described in Table 1.
[b] BEI as described in Table 1.

Profiling of these spiroalkyl-pyrrolidine compounds in the frame of hit characterisation revealed strong species selectivity in the enzymatic bioassay (Table 3): compounds were consistently several logs less potent against murine 11β-HSD1. This was identified as an issue since interspecies equipotency is required to afford predictive in vivo models. Compound **13** demonstrated that potency on mouse enzyme was achievable (murine 11β-HSD1 $IC_{50}$

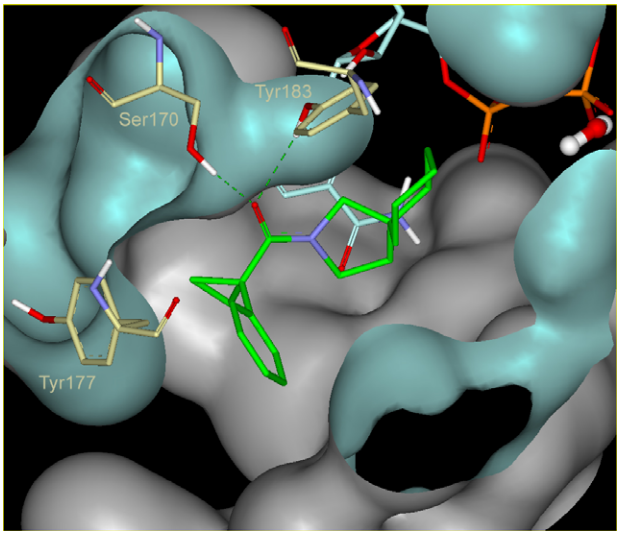



**Figure 4.** Steroid substrate binding site of human 11β-HSD1 (PDB code 2ILT) with docked compound **13**; displayed details and colour coding by atoms are identical to Figure 2b.

**Table 3**
Profile of compounds **3**, **5**, **6** and **13**

| Compounds | 3 | 5 | 6 | 13 |
|---|---|---|---|---|
| h 11β-HSD1 $IC_{50}$[a] (nM) | 25 | 22 | 6 | 0.5 |
| m 11β-HSD1 $IC_{50}$[a] (nM) | 2600 | 3400 | 3400 | 377 |
| h 11β-HSD2 $IC_{50}$[b] (μM) | >100 | >100 | >100 | >100 |
| h 11β-HSD2/ HSD1 $IC_{50}$ selectivity ratio | >4000 | >4500 | >16,000 | >200,000 |

[a] h 11β-HSD1 experimental protocol as described in Table 1; for murine (m) 11β-HSD1, 2.5 μg of microsome fraction from liver (Tebu) was used in the assay.

[b] 11β-HSD2 enzyme activity was assessed in 50 mM Tris buffer, pH 7.8, containing 1 mM $MgCl_2$, 0.5 mM NAD, and 1 μg of human recombinant 11β-HSD2 expressed in *E. Coli*. Reaction was initiated by addition of cortisol 20 nM and terminated with addition of carbenoxolone 10 μM stop solution after 60 min incubation at room temperature. Determinations of cortisol levels were monitored by HTRF assay (Cisbio International).

of 377 nM) but still exhibited the 3 log species selectivity ratio already observed with compounds **3**, **5** and **6**. Reaching equipotency through structure-based rationalisation of interspecies differences has been addressed, and is currently under evaluation.

Furthermore, all the synthesised compounds were also tested for selectivity against human 11β-HSD2 (Table 3). Very high $IC_{50}$ selectivity ratios (>4000 up to >200,000) were reached for these compounds by improving human 11β-HSD1 potency while keeping low potency on human 11β-HSD2 throughout optimisation of the initial hit.

In conclusion, we have identified spirocycloalkyl-pyrrolidine amides as potent and selective inhibitors of 11β-hydroxysteroid dehydrogenase type 1. Based on structural information, a weak hit was rapidly converted to a high affinity and BEI ligand allowing wide scope for optimisation. Work is currently under progress to further improve this profile towards the selection of a drug candidate.

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