Bioorganic & Medicinal Chemistry Letters 19 (2009) 6740–6744

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Structure-based design, synthesis and in vitro characterization of potent 17β-hydroxysteroid dehydrogenase type 1 inhibitors based on 2-substitutions of estrone and D-homo-estrone

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ARTICLE INFO

Article history:
Received 22 July 2009
Revised 27 September 2009
Accepted 29 September 2009
Available online 3 October 2009

Keywords:
Steroid metabolism
Cancer
Inhibitors
Molecular docking
Structure-based drug design

ABSTRACT

In search for specific drugs against steroid-dependent cancers we have developed a novel set of potent inhibitors of steroidogenic human 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD 1). The X-ray structure of 17β-HSD 1 in complex with estradiol served as basis for the design of the inhibitors. 2-Substituted estrone and D-homo-estrone derivatives were synthesized and tested for 17β-HSD 1 inhibition. The best 17β-HSD 1 inhibitor, 2-phenethyl-D-homo-estrone, revealed an IC_{50} of 15 nM in vitro. The inhibitory potency of compounds is comparable or better to that of previously described inhibitors. An interaction within the cofactor binding site is not necessary to obtain this high binding affinity for substances developed.

Human diseases can be treated by manipulation of selective targets contributing to pathogenesis. Several such targets are defined as enzymes including 17β-hydroxysteroid dehydrogenases (17β-HSDs).[1,2] The latter control the biological potency of steroid hormones by redox reactions at position 17 of the steroid scaffold.[3–5] The 17β-HSDs belong to the short-chain dehydrogenase/reductase superfamily (SDR)[6] except for 17β-HSD 5 which is an aldo-ketoreductase.[7]

Observations on the prognostic value of 17β-HSDs in breast cancer,[8–10] prostate cancer,[11] and in endometriosis[12–14] have boosted research on these enzymes. Approaches against breast and prostate cancers involve the development of new safe and 17β-HSD-specific drugs.[15–18] Several strategies include 17β-HSD 1 as a target and drug design has been facilitated by the known crystal structure of the enzyme.[19] An effective inhibitor of conversion of estrone to estradiol by 17β-HSD 1 should deplete active hormone from the signal transduction pathway.

Among naturally available substances the phytoestrogens are the most potent inhibitors of 17β-HSDs[20,21] but unfortunately they are often non-specific.[22] Therefore, distinct highly specific 17β-HSD 1 inhibitors have been developed.[14,23] Strategies for that included modifications of the steroid scaffold at positions 6, 16, and/or 17[16,24–28] and the substitution of hydroxyl moieties with sulfamates[29,30] or fluorine.[31] Even hybrid inhibitors based on estradiol derivatized with adenosine were reported.[17,32,33] Non-steroidal inhibitors of 17β-HSD 1 have as well been identified.[34] However, none of the published inhibitors has progressed to clinical trials and further research is necessary.

In this work we present the development of an inhibitor for 17β-HSD 1 for treatment of estradiol-dependent diseases as described in our patents.[35,36] We show the design, synthesis, and in vitro characterization of several 2-substituted derivatives of estrone and D-homo-estrone. The main difference from previous reports is the substitution of estrone and D-homo-estrone in position 2 at the aromatic A-ring. The X-ray structure of 17β-HSD 1 shows an unoccupied lipophilic subpocket there and modifications at C2 were only marginally pursued so far.[16,37] In addition, these 2-substituted inhibitors should show reduced intrinsic estrogenici-

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doi:10.1016/j.bmcl.2009.09.113

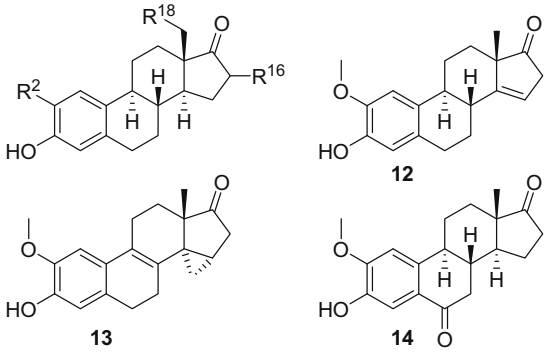
ty,[37] since these do not fit well into the ERα binding site,[27] and should posses altered properties with respect to phase II metabolism, due to shielding of the phenolic 3-hydroxy group.[27]

Compounds **1** (estrone, E1) and **6** (Table 1) are commercially available while the synthesis of compounds **2**–**4**[38], **5**[37], **7**–**8**[39] has been published in the literature. The synthesis of the other derivatives was performed as described by us in full in our patents.[35,36] In brief, incorporation of the 2-pentanoyl moiety into 3-methoxyestra-1,3,5(10)-trien-17-one was accomplished under Friedel–Crafts conditions with valeroyl chloride and aluminium trichloride at 5 °C to give the 2-acylated intermediate.[40] Cleavage of the 3-methyl ether with hydrobromide in acetic acid under reflux afforded product **9**. Derivative **10** was synthesized from 3-acetoxy-2-iodo-estra-1,3,5(10)-trien-17-one[38] via a Sonogashira coupling with phenylacetylene and subsequent deacetylation. Hydrogenation of the acetylated 2-phenethynyl intermediate with palladium on charcoal furnished the 2-phenethyl-substituted derivative **11** (Scheme 1).

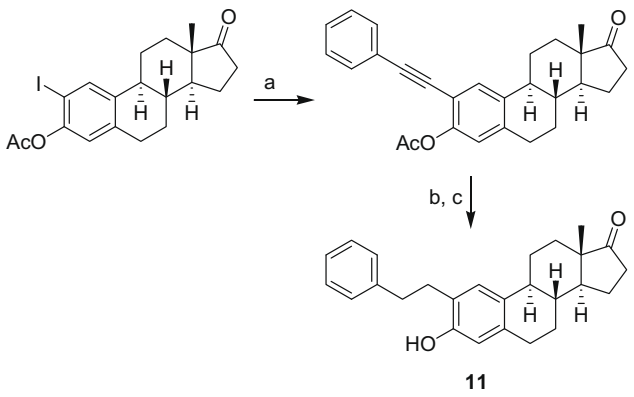
Modification of the steroidal core towards the 14,15-dehydro derivative **12** was accessible by application of Saegusa's general procedure to compound **6**.[41] The 14α,15α-cyclopropa[*a*]estra-1,3,5(10),8-tetraene core[42] was substituted in the 2-position by *ortho*-lithiation similar as previously described[43] to give product **13**. Incorporation of the 6-oxo moiety towards compound **14** was accomplished with chromium trioxide in acetic acid as described with similar derivatives previously.[44]

Halogenation of 18-homo-1,3,5(10)-trien-17-one was executed as described for derivative **2** to give compound **15**.[38] Fluorination was accomplished by silylation of ketone **2** and reaction of the resulting enol ether with *N*-fluoropyridinium triflate (NFPT) to give compound **16** (Scheme 2).[45] Sodium methoxide catalyzed epimerization[45] of derivative **16** in methanol afforded an equimolar mixture, wherein the 16β-fluoro derivative **17** was separated by HPLC in 33% yield.

Table 1
Inhibition of 17β-HSD 1 by 2-substituted estrone derivatives



Compd	R^2	R^{16}	R^{18}	% Inhibition @ 2 μM	IC_{50} (nM)
1 (E1)	H	H	H	90	109
2	Cl	H	H	95	140
3	Br	H	H	95	233
4	NC	H	H	96	148
5	Et	H	H	86	89
6	MeO	H	H	70	207
7	Allyl	H	H	94	109
8	*n*-Propyl	H	H	91	545
9	$CH_3(CH_2)_3CO$	H	H	85	218
10	Ph-C≡C	H	H	90	56
11	Phenethyl	H	H	97	47
12	—	—	—	84	633
13	—	—	—	97	193
14	—	—	—	80	392
15	Cl	H	Me	86	121
16	Cl	α-F	H	99	101
17	Cl	β-F	H	92	35



Scheme 1. Reagents and conditions: (a) PhC≡CH, $Pd(OAc)_2$, PPh_3, CuI, NEt_3/THF (3:2), rt, 89%; (b) H_2 (1 bar), Pd/C, EtOAc, rt, 3 h; (c) NaOMe, MeOH/THF, rt, 89% for both steps.

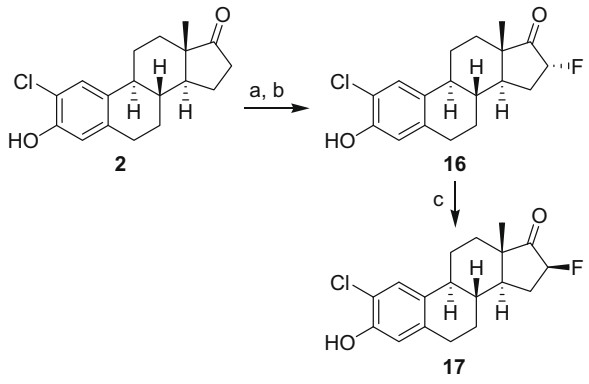
The D-homo moiety (Table 2) was incorporated via a modified Tiffenau–Demjanow ring expansion, starting from the 17-keto derivatives.[46,47]

For example, reaction of **1** with trimethylsulfonium iodide and potassium *tert*-butylate afforded the oxirane, which was opened with sodium azide and then cyclized with trimethylsilyliodide to afford the D-homo derivative **18** in high overall yield (Scheme 3).

Starting with compound **6**, product **22** was obtained on the same route. After acetylation of compound **18**, incorporation of the halogen moiety in 2-position was accomplished via an *ortho*-thallation as described previously,[38] albeit in lower yield (Scheme 4). Subsequent deprotection furnished the derivatives **19**, **20**, and **21**, respectively. The 2-allylated product **23** was obtained via the route described for the corresponding estrone derivative (Scheme 3).[39] Starting material **18** was reacted with allyl bromide and cesium carbonate as base in *N*,*N*-dimethylformamide to give the 3-*O*-allyl intermediate, which was rearranged in refluxing diethylaniline to afford a mixture of the 2-allyl and 4-allyl isomer (ratio ≈1:2). Separation by HPLC furnished the desired 2-allyl substituted product **23**. Derivative **24** was synthesized similar as described above for derivative **11** (Scheme 4). α-Fluorination of ketone **19** with Accufluor® NFTh was conducted as previously described[48] and furnished a diastereomeric mixture, which was separated by HPLC to give pure compounds **25** and **26**.

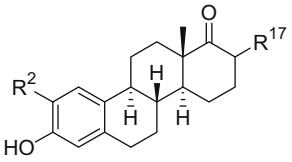
Inhibition of catalytic activity of human 17β-HSD 1 towards E1 was assessed as originally described by us and a detailed description for that is given in the Supplementary data section.[31,49]

The X-ray structure of 17β-HSD 1 in complex with estradiol (E2) and $NADP^+$ (PDB entry code 1FDT)[19] was used for the docking

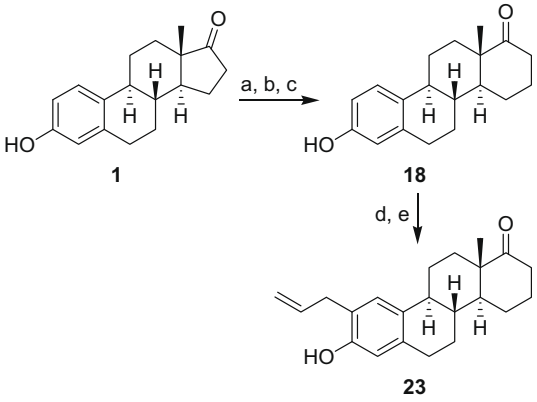


Scheme 2. Reagents and conditions: (a) TMSOTf, NEt_3, toluene, reflux; (b) NFPT, CH_2Cl_2, rt, 1 N HCl; (c) NaOMe, MeOH, rt, 11 d, 33%.

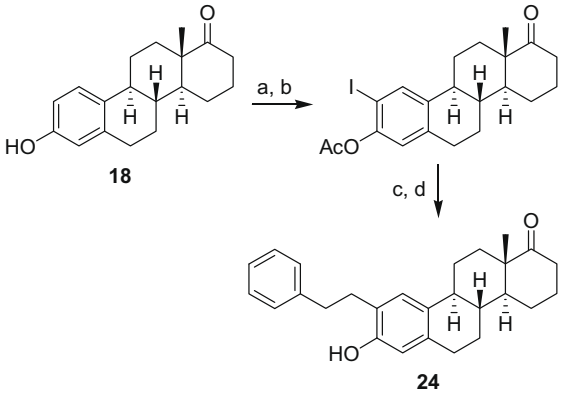
Table 2
Inhibition of 17β-HSD 1 by 2-substituted D-homo-derivatives of estrone



Compd	R^2	R^{17}	% Inhibition @ 2 μM	IC_{50} (nM)
18	H	H	91	30
19	Cl	H	100	77
20	Br	H	100	73
21	I	H	100	123
22	MeO	H	92	85
23	Allyl	H	97	32
24	Phenethyl	H	95	15
25	Cl	α-F	86	87
26	Cl	β-F	100	126



Scheme 3. Reagents and conditions: (a) MeSI (2 equiv), KO*t*Bu (2.2 equiv), DMSO, rt, 80%; (b) NaN_3 (1.3 equiv), DMF, 60 °C, 57%; (c) NaI, Me_3SiCl, CH_3CN, rt, 75%; (d) AllBr, Cs_2CO_3, DMF, 60 °C, 3 h, 89%; (e) $PhNEt_2$, reflux, 8 h, 34% (and 4-allyl isomer).



Scheme 4. Reagents and conditions: (a) Ac_2O, pyridine, cat. DMAP, rt, quant.; (b) $Tl(OCOCF_3)_3$, TFA, 10 °C, then KI, 40%; (c) PhC≡CH, $Pd(OAc)_2$, PPh_3, CuI, NEt_3/THF (3:1), rt, 80%; (d) H_2 (1 bar), Pd/C, EtOAc/THF, rt; NaOMe, MeOH/CH_2Cl_2, rt, 65%.

experiments to support structure–activity relationships (SAR) studies and design of inhibitors. The 3D-structures of the steroidal compounds were generated within SYBYL 6.7 (Tripos Inc. SYBYL, 2000) and energy minimized using the MMFF94 force field.[50] The FLEXX program version 1.8[51] interfaced with SYBYL was used to perform the docking experiments to 17β-HSD 1. Glu282 was treated as charged residue, whereas His221 was kept uncharged. All default FLEXX parameters, as implemented in the 6.7 release of SYBYL, were used. Substrate binding was analyzed using the program MOLCAD in SYBYL.

The crystal structure of 17β-HSD 1 in complex with E2 was previously applied in molecular dynamics simulations and ligand–protein docking studies of non-steroidal[52–54] as well as steroidal derivatives.[16,28,30,55] In the latter studies mainly the region in the 16 and 17 position of the steroid was investigated, which points towards the $NADP^+$ cofactor. In our study, we focused on position 2 of estrone and D-homo-estrone. From the surface of the substrate-binding pocket in the 17β-HSD 1 X-ray structures it is clearly visible that the position 2 of E2 is located in a lipophilic environment consisting of the side-chains of Val143, Met147, Phe259, Leu262 and Met279. In this region the substrate binding pocket forms a lipophilic tunnel to the exterior of the protein. The existence of such a lipophilic tunnel inspired us to synthesize steroidal compounds substituted with lipophilic residues (such as halogen or alkyl) in position 2. In addition, fluorine substituents were introduced in position 16α and β to stabilize the 17 carbonyl group of estrone from metabolic attack. The five-membered D-ring was also enlarged to a six-membered (D-homo) ring.

Several 2-substituted estrone derivatives were tested for their ability to inhibit recombinant 17β-HSD 1 activity (Table 1). Structure–activity relationships focused mainly on the role (size and polarity) of substitutions at position 2 of the steroidal A-ring and some modifications of the B- and D-ring (Fig. 1).

Based on the observed IC_{50}-values all compounds in this series are submicromolar to nanomolar inhibitors of 17β-HSD 1. To facilitate normalized comparison we analyzed the natural substrate estrone (E1) which shows under the same conditions an IC_{50} of 109 nM. Compared to halogen/pseudohalogen substituents such as chlorine, bromine and carbonitrile (compounds **2**–**4**) in 2-position do not lead to increased inhibition. The same is true for the 18-homo estrone derivative **15** and the 2-methoxy-derivatives (compounds **6**, **12**–**14**). We had made similar observations for 17-fluorine-substituted estrogens in a previous study.[31] It is proposed that an intramolecular hydrogen bond between the 3-hydroxyl group and the oxygen atom of the 2-methoxy substituent of the steroid reduces binding to His221 and Glu282 of 17β-HSD 1 and positions the methoxy group in the plane of the steroidal A-ring. This might lead to repulsive interactions with Phe259. This intramolecular hydrogen bond and the respective conformation of the methoxy group is observed in a number of small molecule X-ray structures bearing the *O*-methoxy-phenyl ring system.[56]

Modification of compound **6** with an additional carbonyl group in position 6 (compound **14**) or double bond in position 14–15 (compound **12**) leads to diminished inhibition while introduction of a 8–9 double bond together with a 14α,15α-cyclopropyl substituent does not alter the IC_{50} in comparison with compound **6**. The docking studies suggest that the 6-oxo group in compound **14** could lead to repulsive interactions with Ser222 and Tyr218. Slightly increased inhibition was observed with the 2-ethyl substituent (compound **5** with IC_{50} = 89 nM) while 2-allyl, propyl, and pentanoyl resulted in equally potent or weaker inhibitors. Especially the terminal CH_3-group of the 2-ethyl substituent is proposed to fill a subpocket formed by Val143, Met147, Leu149, Phe259, and Leu262 of 17β-HSD 1 while the other substituents are longer and interact with Leu262, which is more exposed to the solvent. A significant increase in inhibitory activity compared to E1 was observed for the 2-phenethynyl and 2-phenethyl substituents (compounds **10**, IC_{50} = 56 nM and **11**, IC_{50} = 47 nM). These longer substituents are proposed to form lipophilic contacts with Leu262 and Phe259, which might explain the increased inhibition.

The most potent inhibitor in this series was obtained by an additional substitution of compound **2** (2-chloro-estrone) in posi-

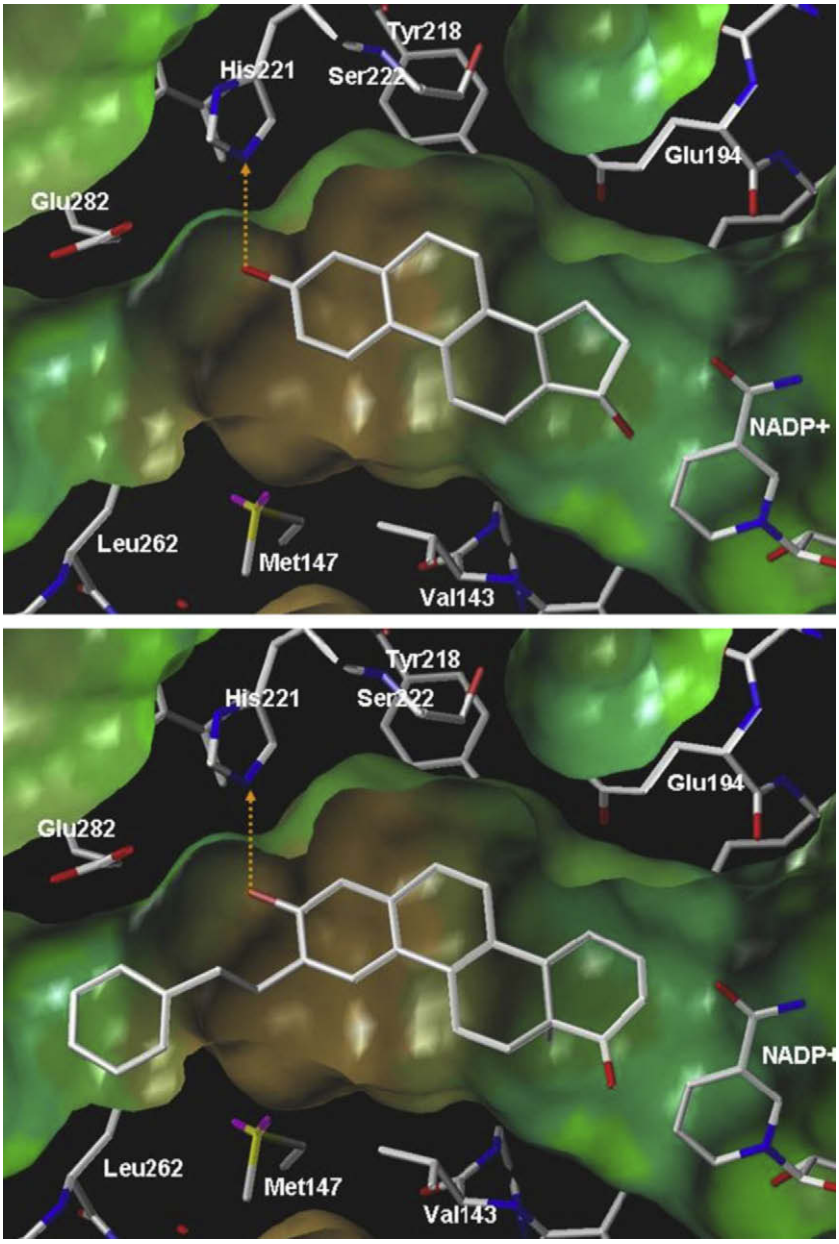


Figure 1. Molecular modelling. Lipophilic surface areas are colored brownish, green corresponds to neutral and blue to hydrophilic parts of the protein surface. Leu149 and Phe259 are not depicted since they are positioned far below and above the steroid plane. (Top) Substrate binding pocket of 17β-HSD 1 in complex with estradiol and $NADP^+$ according to PDB data 1FDT. (Bottom) Modelled complex of 2-phenethyl-D-homo-estrone **24** with 17β-HSD 1 and $NADP^+$.

tion 16 with fluorine. 2-chloro-16β-fluorine-estrone (compound **17**) is significantly more potent (IC_{50} = 35 nM) than the 16α-isomer (IC_{50} = 101 nM). An electrostatic repulsive interaction between the partially negatively charged 16α-fluorine substituent and the amide carbonyl oxygen of $NADP^+$ (distance ~3 Å) might explain this difference of the two stereoisomers.

The structure–activity study was extended to compounds with another steroid-like scaffold, the D-homo-estrone (Table 2), in which the five-membered D-ring of estrone is expanded to an aliphatic 6-ring. This modification was purely driven by the favourable synthetic accessibility of these compounds. It has been shown that androstanedione, androstenedione, and testosterone bind to 17β-HSD 1 in a reverse binding mode in comparison to E2.[57,58] When bound substrate conformations are compared, the six-membered A ring of the androgens overlays approximately with the five-membered D-ring of E2. This observation allows for the assumption that D-homo-estrogens probably behave like androgens on binding to 17β-HSD 1. The lead compound D-homo-estrone **18**, having a six-membered A and D-ring (Table 2), revealed an IC_{50} of 30 nM which is even better than that obtained with the most potent compound **17** from the series based on 2-substituted E1. This is surprising, because the SAR around the 2-position between the two lead series (estrone and D-homo-estrone) is completely parallel (Tables 1 and 2). Chlorine, bromine, and methoxy modifications lead to weaker inhibitors. Allyl is neutral in comparison to compound **18**, whereas 2-phenethyl increases inhibition and represents the most potent compound in this study (compound **24**, IC_{50} = 15 nM). This suggests a very similar binding mode of the two series which is also supported by our docking experiments. In contrast to the estrone series (compounds **16** and **17**), fluorine substitution in the position vicinal to the carbonyl moiety (position 17) leads to weaker inhibitors than plain D-homo-estrone **18**. This effect cannot be explained in detail with the applied docking procedures.

Table 3
Estrogenicity of selected estrone derivatives

Compound	IC_{50} of hERα binding (nM)
E2	12.5
1 (E1)	96
6	11,000
19	770
22	6200

Binding affinity of human hERα (hERα) was tested for selected 2-substituted estrogen derivatives and compared to that of estrone and estradiol (Table 3).[59] The binding affinity and accordingly estrogenicity is reduced by 100- to 1000-fold making the substances applicable inhibitor candidates as expected.[27,37]

In conclusion, novel and potent inhibitors of 17β-HSD 1 were identified applying structure based design, chemical synthesis and biochemical testing of the recombinant enzyme in an iterative manner. The inhibitors were derived from estrone, the natural substrate of 17β-HSD 1. The X-ray structure of 17β-HSD 1 guided the design of the compounds. The most potent compound is characterized by an IC_{50} in the low nanomolar range, sevenfold better than that of estrone. With respect to inhibitory potency, the compounds are comparable to previously described inhibitors such as non-steroidal compounds or compounds that are linked to cofactors in positions 16 and 17.[16,26,34e] However, the increase in potency is obtained by only adding relatively little molecular weight. Generally, the strategy presented in this work might be better suited to obtain more drug-like molecules for oral administration.

Acknowledgment

This project was supported by BMBF-Project 'Bioinstrumente Jena 0312255'.

Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bmcl.2009.09.113.

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