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Brief Article

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Optimization of Hydroxybenzothiazoles as Novel Potent and Selective Inhibitors of 17β-HSD1

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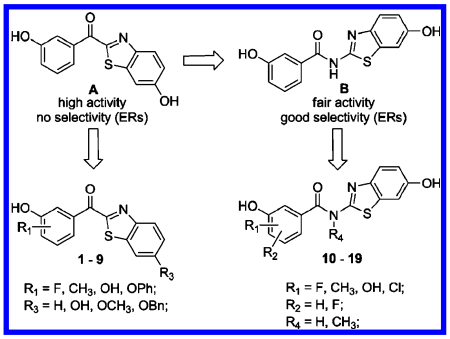
Supporting Information

ABSTRACT: 17β-HSD1 is a novel target for the treatment of estrogen-dependent diseases, as it catalyzes intracellular estradiol formation. Starting from two recently described compounds, highly active and selective inhibitors were developed. Benzoyl **6** and benzamide **17** are the most selective compounds toward 17β-HSD2 described so far. They also showed a promising profile regarding activity in T47-D cells, selectivity toward ERα and ERβ, inhibition of hepatic CYP enzymes, metabolic stability, and inhibition of marmoset 17β-HSD1 and 17β-HSD2.

INTRODUCTION

For the treatment of steroid hormone-dependent diseases biosynthesis inhibitors blocking hormone formation in the corresponding endocrine gland provide an excellent strategy as demonstrated by the use of CYP19 inhibitors for the treatment of breast cancer (BC).[1] Three further cytochrome P450 (CYP) enzymes are presently in the focus of drug development: CYP17,[2] CYP11B2,[3] and CYP11B1[4] for the treatment of prostate cancer, congestive heart failure and myocardial fibrosis, and Cushing's syndrome, respectively. As these therapeutic options result in systemic reductions of steroid hormone levels that often are associated with side effects, a softer option could be the inhibition of hormone activation in the diseased cell. For the treatment of benign prostatic hyperplasia, this strategy has already been proven to be successful with inhibitors of 5α-reductase.[5] More recently 17β-hydroxysteroid dehydrogenase 1 (17β-HSD1) came into the focus of interest as a novel therapeutic target for the treatment of estrogen dependent diseases like BC and endometriosis.[6] 17β-HSD1 catalyzes the conversion of the weakly active estrone (E1) to the highly active estradiol (E2) and is overexpressed in some BC tissues.[6] Efficacy of 17β-HSD1 inhibitors against human breast cancer cell lines has already been reported in vitro and in nude mice.[7] Besides some approaches to develop steroidal inhibitors,[8] there have also been attempts by us and other groups to develop nonsteroidal inhibitors that should be advantageous with respect to side effects.[9] Recently we reported on two new 17β-HSD1 inhibitors with different activity/selectivity profiles, the 1,3-benzothiazol-2-ylphenylmethanone **A** and the *N*-(1,3-benzothiazol-2-yl)benzamide **B** (Chart 1).[10] Here we report on the structure optimization of these two lead compounds with regard to 17β-HSD1 inhibitory activity and selectivity toward 17β-HSD2 (the enzyme catalyzing the reverse reaction) and the estrogen receptors (ERs) α and β. For selected compounds, 17β-HSD1 inhibition in human T47-D breast cancer cells, inhibition of 17β-HSD1 and 17β-HSD2 from *Callithrix jacchus* (marmoset, animal model for endometriosis[11]), of human hepatic CYP enzymes, and metabolic stability were determined.

Chart 1. Compounds A and B and Synthesized Compounds



RESULTS

Chemistry. The synthesis of **1**−**9** started from 1,3-benzothiazol-2-ylamine or 1,3-benzothiazole. **1**−**3** and **5**−**7** were prepared in a four-step synthesis previously described[10] (steps a−d in Scheme 1). **4** was synthesized in three steps starting with the nucleophilic addition of **4iii** to 4-fluoro-3-methoxybenzaldehyde. For **8**, **8iiii** and **8iii** were synthesized and coupled via nucleophilic addition. Oxidation of the resulting alcohol and ether cleavage of the silanyl ether using tetra-*n*-butylammonium fluoride (TBAF) gave the final compound. **9** was synthesized by coupling **1iii** and **9iiiii**, followed by oxidation, cleavage with TBAF, boronic acid−phenol cross-coupling reaction mediated by copper(II) acetate, and cleavage of the two methoxy groups with boron tribromide (BBr_3). **10**−**18** were prepared via two steps as described[10] (steps a and b in Scheme 2): (a) amide coupling between 6-methoxy-1,3-benzothiazol-2-ylamine and the corresponding benzoyl chloride and (b) ether cleavage. **19** was synthesized by N-methylation of **11i** with methyl iodide and sodium hydride in DMF and subsequent ether cleavage.

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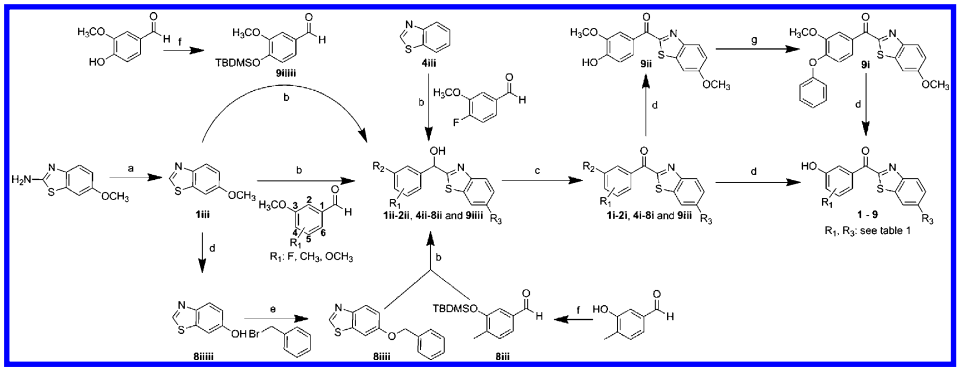
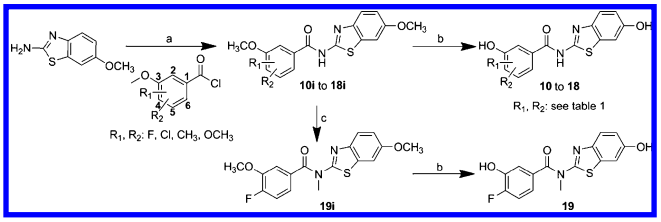
Scheme 1. Synthesis of Compounds 1−9[a]



[a]Reagents and conditions: (a) (1) $NaNO_2$, H_3PO_4 (85%), −10 °C, 20 min, (2) H_3PO_2, H_3PO_4 (85%), −10 °C to room temp, 20 h. (b) Method A: (1) *n*-BuLi, anhydrous THF, −70 to −20 °C, 1 h, (2) methoxybenzaldehyde, anhydrous THF, −15 °C, 90 min. (c) Method C: SIBX, anhydrous THF, 0−60 °C, 20 h. (d) For **1**−**6**, method D: pyridinium hydrochloride, 220 °C, 4 h. For **7**, **8iiiii** and **9**, method E: BBr_3, CH_2Cl_2, −78 °C to room temp, 20 h. (e) Benzyl bromide, K_2CO_3, acetone, 100 °C, 20 h. For **9ii** and **8**: TBAF, THF, room temp, 2 h. (f) TBDMSiCl, imidazole, DMF, room temp, 20 h; (g) benzeneboronic acid, $Cu(AcO)_2$, Et_3N, anhydrous CH_2Cl_2, room temp, 18 h.

Scheme 2. Synthesis of Compounds 10−19[a]



[a]Reagents and conditions. (a) Method B: pyridine, 100 °C, 4 h. (b) Method E: BBr_3, CH_2Cl_2, −78 °C to room temp, 20 h. (c) **11i**, NaH, CH_3I, DMF, 0 °C to room temp, 3 h.

Inhibition of Human 17β-HSD1 and 17β-HSD2. For the determination of 17β-HSD1 and 17β-HSD2 inhibition, tritiated substrates E1 or E2 and the placental enzymes 17β-HSD1 (cytosolic fraction) or 17β-HSD2 (microsomal fraction) were used. The labeled products formed were quantified by HPLC using radiodetection. The inhibition values (IC_{50}) and the selectivity factors (SFs) are shown in Table 1. **A** and **B** were used as references. In the series of the ketones, **1**−**3** and **5**−**7** strongly inhibited 17β-HSD1. Also, in the series of the amide derivatives stronger 17β-HSD1 inhibition than the one shown by **B** was observed, with **10** and **17** exhibiting IC_{50} in the low nanomolar range. Furthermore, **6** and **17** displayed strong selectivity for 17β-HSD1 over 17β-HSD2, exhibiting the highest selectivity factors (>130) reported in the literature.

Affinities to ERα and ERβ. The relative binding affinities (RBA) of the most selective compounds of this study were determined using recombinant human ERα and ERβ in a competition assay[12] with [^{3}H]E2 (E2 was used as reference, and its RBA value was set to 100%). Only **2** showed a slight affinity to the ERs, while all other compounds were more than 1000-fold less potent compared to E2.

Further Biological Evaluation. Additionally, the inhibition of intracellular E2 formation for selected compounds was determined in T47-D cells (Table 2). The high cellular activities of **2**, **10**, and **17** indicate that they easily permeate the cell membrane and are not metabolized quickly. Furthermore, **2**, **6**, **10**, and **17** were tested for inhibition of *Callithrix jacchus* 17β-HSD1 and 17β-HSD2 (Table 2). They showed comparable (**6**) or even stronger (**2**, **10**, and **17**) inhibition of 17β-HSD1 but apparently lower selectivity compared to the human enzymes. Compounds **2**, **6**, and **17** were further investigated for inhibition of the human hepatic CYP enzymes: 3A4, 2D6, 2C9, 2C19, 1A2, and 2B6. They showed very little inhibition (54 μM > IC_{50} > 3 μM) except for 1A2 which was inhibited by **2** and **6** moderately (IC_{50} = 1.8 and 0.57 μM). Furthermore, metabolic stability of **6**, **7**, **10**, and **17** was determined using human liver microsomes. The two ketones **6** and **7** showed high intrinsic clearance (Cl_{int}) of 94 and 108 (μL/min)/mg protein, respectively. On the other hand, the amides **10** and **17** were metabolically more stable, as they displayed Cl_{int} of 38 and 28, respectively.

■ DISCUSSION AND CONCLUSION

Recently we discovered two new 17β-HSD1 inhibitors, **A** and **B** (Chart 1).[10] In the present study they should be optimized regarding activity and selectivity to obtain compounds suitable for in vivo use. Introduction of various substituents with different electronic, lipophilic, steric, and H-bonding properties into the benzoyl moiety of **A** and **B** led to the discovery of two new lead compounds, **6** and **17**. As suggested in our previous study,[10] where **A** and **B** were hypothesized to interact differently with the binding site, the introduction of a variety of substituents into the benzoyl moiety of **A** and **B** had different influence on inhibitory activity: comparing **1** and **10**, **2** and **11**, **5** and **12**, **6** and **13**, and **7** and **15** with their parent compounds shows that only the fluorine increased inhibitory activity in both classes. The introduction of methyl in the 4-position and OH in the 6-position turned out to be beneficial in the case of the ketones (**6** and **7**) but was deleterious for the amides (**13** and **15**). The introduction of a bulky substituent decreased inhibition (**8**, **9**). Interestingly, methylation of the OH group of **2** (**3**) or replacement by hydrogen (**4**) on the benzothiazole resulted only in a slight decrease of 17β-HSD1 inhibition but in a complete loss of selectivity toward 17β-HSD2. Changing the F position and adding a second fluorine in the amide series increased 17β-HSD1 inhibitory activity: **17** showed the same IC_{50} of 13 nM as the best ketone **2**. This may be due to favorable electronic effects exerted by the fluorines on the H-bonding property of the OH, to the increased lipophilicity, or to the change in π-interacting properties of the aromatic ring as suggested by previous SAR studies.[10] Regarding selectivity in the ketone series, introduction of the methyl group in the 4-position (**6**) not only increased activity but also decreased

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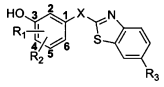
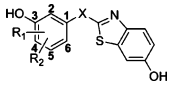
Table 1. 17β-HSD1 Inhibitory Activities and Selectivities of Compounds 1−19



compd	X	R_1	R_2	R_3	IC_{50} (nM)[c,f] HSD1[a]	IC_{50} (nM)[c,f] HSD2[b]	SF[d,f]
A	CO	H	H	OH	44	1035	24
1		2-F	H	OH	5	83	17
2		4-F	H	OH	13	121	9
3		4-F	H	OCH_3	38	59	2
4		4-F	H	H	136	104	1
5		6-F	H	OH	13	1416	109
6		4-CH_3	H	OH	27	4003	148
7		6-OH	H	OH	78	1538	20
8		4-CH_3	H	OBn	ni	ni	nd
9		4-OPh	H	OH	863	1457	2
B	CONH	H	H	OH	243	9264	38
10		2-F	H	OH	29	1224	42
11		4-F	H	OH	171	1480	9
12		6-F	H	OH	112	3804	34
13		4-CH_3	H	OH	ni	nd	nd
14		5-OH	H	OH	3000[e]	nd	nd
15		6-OH	H	OH	ni	nd	nd
16		2-Cl	6-F	OH	221	469	2
17		2-F	6-F	OH	13	1774	136
18		2-F	4-F	OH	99	541	5
19	$CONCH_3$	4-F	H	OH	667[e]	2704[e]	4

[a]Human placenta, cytosolic fraction, substrate [^{3}H]E1 + E1 [500 nM], cofactor NADH [500 μM]. [b]Human placenta, microsomal fraction, substrate [^{3}H]E2 + E2 [500 nM], cofactor NAD^+ [1500 μM]. [c]Mean values of three determinations, standard deviation less than 10%. [d]Selectivity factor: IC_{50}(17β-HSD2)/IC_{50}(17β-HSD1). [e]Calculated with LOGIT transformation. [f]nd: not determined. ni: no inhibition (less than 10% inhibition at 1 μM).

Table 2. Binding Affinities for the ERα and ERβ, 17β-HSD1 Inhibitory Activity in T47-D Cells, and Inhibition of *Callithrix jacchus* 17β-HSD1 and 17β-HSD2 by Selected Compounds



compd	X	R_1	R_2	RBA (%)[b] ERα[a]	RBA (%)[b] ERβ[a]	IC_{50} (nM),[c] T47-D	% inhibition calHSD1[d] 5 nM	% inhibition calHSD1[d] 50 nM	% inhibition calHSD2,[e] 50 nM
A	CO	H	H	<10	<1				
1		2-F	H	0.1	<0.1				
2		4-F	H	<1	<1	11	80		34
5		6-F	H	<0.1	0.1				
6		4-CH_3	H	<0.1	<0.1	258		83	40
7		6-OH	H	<0.1	<0.1	365[f]			
B	CONH	H	H	<0.1	<0.1	245			
10		2-F	H	<0.1	<0.1	73[g]	67		48
12		6-F	H	<0.1	<0.1	152[g]			
17		2-F	6-F	<0.1	<0.1	37[g]	87		51

[a]Human recombinant protein, incubation with 10 nM [^{3}H]E2 and inhibitor for 1 h. [b]RBA: relative binding affinity in percent (E2 = 100%), mean value of three determinations, standard deviation less than 10%. [c]Mean value of three determinations, standard deviation less than 10%; [d]Marmoset placenta, cytosolic fraction, substrate [^{3}H]E1 + E1 [500 nM], cofactor NADH [500 μM]. [e]Marmoset placenta, microsomal fraction, substrate [^{3}H]E2 + E2 [500 nM], cofactor NAD^+ [1500 μM]. [f]Standard deviation less than 15%. [g]Calculated with LOGIT transformation at 50 nM inhibitor.

inhibition of 17β-HSD2 and affinity to the ERs. Thus, **6** shows the highest selectivity toward 17β-HSD2 described so far (SF = 148). In the amide series introduction of fluorine in the 4-position (**11**) led to a decrease in selectivity toward 17β-HSD2, as observed for **2** in the ketone series, while introducing a fluorine in the 2-position (**10**) and the 6-position (**12**) did not alter selectivity. The difluoro-substituted **17** showed the best selectivity in this series toward 17β-HSD2 and the ERs with values similar to those of **6**. To summarize, we have discovered two highly potent 17β-HSD1 inhibitors that are the most selective compounds described so far. Compounds **6** and **17** also show very good intracellular activity and high potency

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toward the marmoset target enzyme (IC_{50} < 50 nM in the case of **6** and IC_{50} < 5 nM in the case of **17**). Recently published sequence alignment studies[13] showed that human and marmoset 17β-HSD1 share high homology (80% identity and 85% similarity). Interestingly, among the seven human 17β-HSD1 amino acid residues (S142, N152, Y155, Y218, H221, R258, and E282) described[10] as potential interaction partners of **A** and **B**, only one amino acid variation was observed in the *Callithrix jacchus* enzyme: E282N. Similar binding modes in the marmoset enzyme as described for the two classes in human 17β-HSD1 can be postulated. In summary **6** and **17** should be suitable candidates that after optimization could be evaluated in a marmoset model of endometriosis.

EXPERIMENTAL SECTION

Chemistry. General. Chemical names follow IUPAC nomenclature. Starting materials were purchased and used without purification. ^1H and ^{13}C NMR spectra were measured on a Bruker AM500 spectrometer (500 MHz), and mass spectra (ESI) were recorded on a MSQ electro mass spectrometer (ThermoFisher). The purity for final compounds was ≥95% and was determined by HPLC-MS (see SI).

The following compounds were prepared according to previously described procedures: 6-methoxy-1,3-benzothiazole (**1iii**),[14] 6-hydroxy-1,3-benzothiazole (**8iiiii**),[14] [3-(*tert*-butyldimethylsilanyloxy)-4-methyl]benzaldehyde (**8iii**),[15] 3-methoxy-4-(*tert*-butyldimethylsilanyloxy)benzaldehyde (**9iiiii**).[16] Synthetic details and characterization of compounds can be found in SI. Method A involves formation of methanols, method B amide formation, method C oxidation with SIBX, method D ether cleavage with PyHCl, and method E ether cleavage with BBr_3. **6ii**, **17i**, **6i**, **6**, and **17** are presented as examples.

(6-Methoxy-1,3-benzothiazol-2-yl)(4-methyl-3-methoxyphenyl)methanol (6ii). **6ii** was prepared by reaction of 6-methoxy-1,3-benzothiazole (**1iii**) (0.25 g, 1.5 mmol), *n*-BuLi (0.61 mL, 1.5 mmol), and 4-methyl-3-methoxybenzaldehyde (0.18 g, 1.2 mmol) according to method A. The product was purified by CC (hexane/ethyl acetate, 60:40): yield, 89% (0.42 g).

2,6-Difluoro-3-methoxy-*N*-(6-methoxy-1,3-benzothiazol-2-yl)benzamide (17i). **17i** was prepared by reaction of 6-methoxy-1,3-benzothiazol-2-ylamine (0.3 g, 1.79 mmol) and 2,6-difluoro-3-methoxybenzoyl chloride (0.4 g, 1.79 mmol) according to method B. The product was purified by CC (dichloromethane/methanol, 95:05): yield, 29% (0.18 g).

(4-Methyl-3-methoxyphenyl)(6-methoxy-1,3-benzothiazol-2-yl)methanone (6i). **6i** was prepared by reaction of **6ii** (0.33 g, 1.03 mmol) and 2-iodoxybenzoic acid (0.58 g, 2.06 mmol) according to method C. The product was purified by CC (hexane/ethyl acetate, 85:15): yield, quantitative (0.33 g).

(4-Methyl-3-hydroxyphenyl)(6-hydroxy-1,3-benzothiazol-2-yl)methanone (6). **6** was prepared by reaction of **6i** (0.33 g, 1.05 mmol) and pyridinium hydrochloride (12.13 g, 105 mmol) according to method D. The product was purified by preparative HPLC: yield, 42% (0.12 g). ^1H NMR (CD_3COCD_3): 2.32 (s, 3H), 7.21 (dd, J = 8.8 Hz, J = 2.4 Hz, 1H), 7.32 (d, J = 7.6 Hz, 1H), 7.55 (d, J = 2.1 Hz, 1H), 8.02 (d, J = 1.5 Hz, 1H), 8.06 (d, J = 8.8 Hz, 1H), 8.10 (dd, J = 7.6 Hz, J = 1.5 Hz, 1H), 8.70 (s, 1H), 9.22 (s, 1H). ^{13}C NMR (CD_3COCD_3): 17.1, 107.9, 113.8, 117.7, 117.7, 119.0, 124.3, 127.8, 132.1, 135.5, 140.3, 142.7, 156.7, 159.3, 185.2. MS (ESI): 287.0 $(M + H)^+$.

2,6-Difluoro-3-hydroxy-*N*-(6-hydroxy-1,3-benzothiazol-2-yl)benzamide (17). **17** was prepared by reaction of **17i** (0.18 g, 0.51 mmol) and boron tribromide (5.1 mmol) according to method E. The product was purified by CC (dichloromethane/methanol, 90:10): yield, 30% (0.05 g). ^1H NMR (CD_3SOCD_3): 6.97−7.01 (m, 2H), 7.17−7.22 (m, 1H), 7.41 (d, J = 2.5 Hz, 1H), 7.59 (d, J = 8.2 Hz, 1H). ^{13}C NMR (CD_3SOCD_3): 107.2, 112.2, 112.3, 116.4, 120.6, 120.7, 122.7, 134.4, 142.6, 142.7, 142.7, 142.8, 143.3, 147.9, 147.9, 151.9, 151.9, 155.3, 155.6, 159.8, 167.1. MS (ESI): 323.1 $(M + H)^+$.

Biological Methods. See SI.

ASSOCIATED CONTENT

Supporting Information

Synthetic procedures, characterization, and HPLC purities of intermediates and final compounds and all biological assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

The manuscript was written with contributions of all authors. All authors have given approval to the final version.

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

BC, breast cancer; 17β-HSD1, 17β-hydroxysteroid dehydrogenase type 1; 17β-HSD2, 17β-hydroxysteroid dehydrogenase type 2; E1, estrone; E2, 17β-estradiol; EDD, estrogen-dependent disease; ER, estrogen receptor; SF, selectivity factor; RBA, relative binding affinity; CC, column chromatography

ADDITIONAL NOTE

For the sake of clarity, IUPAC nomenclature is not strictly followed except for the experimental part where the correct IUPAC names are given.

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