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Addressing cytotoxicity of 1,4-biphenyl amide derivatives: Discovery of new potent and selective 17β-hydroxysteroid dehydrogenase type 2 inhibitors

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ABSTRACT

Four different classes of new 17β-hydroxysteroid dehydrogenase type 2 (17β-HSD2) inhibitors were synthesized, in order to lower the cytotoxicity exhibited by the lead compound **A**, via disrupting the linearity and the aromaticity of the biphenyl moiety. Compounds **3**, **4**, **7a** and **8** displayed comparable or better inhibitory activity and selectivity, as well as a lower cytotoxic effect, compared to the reference compound **A**. The best compound **4** (IC_{50} = 160 nM, selectivity factor = 168, $LD_{50} \approx 25$ μM) turned out as new lead compound for inhibition of 17β-HSD2.

Osteoporosis affects more than 75 million people in the United States, Europe and Japan, causing almost 9 million bone fractures annualy.[1] The current available therapies lack of sufficient safety and effectiveness,[2] and as consequence development of new treatments is needed.

17β-Hydroxysteroid dehydrogenase type 2 (17β-HSD2) is responsible for the local reduction of the highly biologically active estradiol (E2) and testosterone (T) into the much less active estrone (E1) and androstenedione (A-dione), whereas 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1), a target for the treatment of endometriosis,[3–5] type 3 (17β-HSD3) and type 5 (17β-HSD5) catalyze the opposite reaction (Fig. 1).[6]

The age-related decrease in the local levels of E2 and T is responsible for osteoporosis onset and progression.[7,8] Therefore the inhibition of 17β-HSD2, which is present in the bones,[9] should rebalance the steroid levels in this tissue and represents an appealing strategy for the treatment of this disease. Since 17β-HSD2 and 17β-HSD1 were shown to be both expressed in bone tissue,[9] 17β-HSD2 inhibitors should be selective over 17β-HSD1 (Fig. 1).

We previously reported on the discovery of compound **A** (Fig. 2), which showed a good 17β-HSD2 inhibitory activity (IC_{50} = 300 nM) and good selectivity over 17β-HSD1 (IC_{50} = 13.3 μM, selectivity factor = 44) as well as an improved metabolic stability in human S9 fraction ($t_{1/2}$ = 107 min) compared to the other 17β-HSD2 inhibitors described so far.[10] However, this lead compound **A** was found to exert some cytotoxicity. Only 34% of the cells were still alive were still alive after treatment with 6.25 μM of compound **A** in a MTT assay.[11]

The 1,1′-biphenyl moiety is known for its toxicity.[12] It might partly come from its planarity and the presence of two aromatic rings next to each other which might result in DNA intercalation by interaction with the nucleobases.[13]

Decrease in cytotoxicity should therefore be achieved by disrupting the planarity of the biphenyl moiety and/or avoiding the biphenyl moiety.

We already reported on compound **B** (Fig. 2) showing a 17β-HSD2 inhibitory activity (IC_{50} = 510 nM) slightly weaker compared to the one of **A**.[14] Whereas **B** and **A** share the biphenyl moiety, compound **B** bears, in place of a carboxamide, a sulfonamide linker. The O of the carbonamide **A** and of the sulfonamide **B** explores different regions of the protein and potentially achieves different H-bond interactions. Therefore the carboxamide of **A** and

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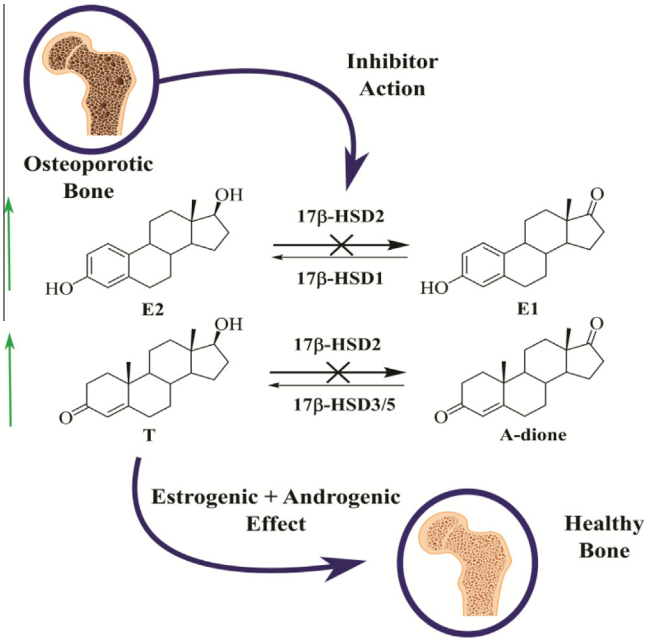


Figure 1. Estrogens (E2) and androgens (T) contribute to the maintenance of the overall bone quality. Blocking the oxidation of estradiol and testosterone by using an inhibitor of 17β-HSD2 should rebalance the steroid level in the bones.

the sulfonamide of **B** were both taken as starting point for the design of the new inhibitors, in order to obtain a greater chemical diversity.

In order to develop new 17β-HSD2 inhibitors with a better toxicity profile and a good 17β-HSD2 inhibitory activity, we applied four strategies: (1) introduction of an ether bridge between the two phenyl rings, compounds **1–7**; (2) exchange of the phenyl central ring by a cyclohexane ring, compound **8**; (3) the exchange of the central ring by a piperazine ring linked to a sulfonyl group, compounds **9a–11a** and **9–11** and (4) the exchange of the sulfonyl function by an acyl function, compounds **12a–14a** and **12–15** (Fig. 2).

The reaction steps involved in the synthesis of the target compounds **1–8** are shown in Scheme 1. The 4-phenoxybenzoyl chlorides were obtained from the commercially available corresponding 4-phenoxybenzoic acids **1a–6a** and **7b** by reaction with $SOCl_2$ and subsequently reacted with different anilines, according to the already described procedure,[15] providing compounds **1–6** and **7a**.

Compound **7a** was submitted to ether cleavage, using boron trifluoride–dimethyl sulfide complex $BF_3{\cdot}SMe_2$ yielding the hydroxy compound **7**, as previously described.[15]

Compound **8** was synthesized using an identical method, starting from the commercially available 4-(4-chlorophenyl)cyclohexane-1-carboxylic acid **8a** (Scheme 1).

The 3-(4-phenylpiperazin-1yl) sulfonyls **9a–11a** were prepared through the sulfonamide bond formation (Scheme 2), achieved by reaction of commercially available 1-phenylpiperazines **9b–11b** with 3-methoxybenzenesulfonyl chloride **9c**, according to a described procedure.[14] The following ether cleavage of **9a–11a**, using $BF_3{\cdot}SMe_2$ in presence of triethylamine, as already described,[14] yielded the hydroxy compounds **9–11**.

The synthesis of the phenylpiperazin-1-yl methanones **12–14** and **15a** are depicted in Scheme 3. The amide bond was formed, by reacting the commercially available 1-phenylpiperazines **9b**, **10b**, **12b** and **13b** and 3-methoxybenzoyl chloride **10c**, using triethylamine and dichloromethane as solvent. Compounds **12a–14a** were submitted to ether cleavage using boron trifluoride–dimethyl sulfide complex yielding the hydroxy compounds **12–14**. Conditions of both reactions were previously described.[7]

All final compounds as well as their intermediates were fully characterized (^{1}H NMR, ^{13}C NMR and LRMS) to confirm their chemical structure. The data of the representative compounds **4**, **8**, **9a** and **12** are presented as examples.[16–19]

The inhibitory activities of compounds **7a**, **10a**, **11a**, **13a–15a** and **1–15** on 17β-HSD2 and 17β-HSD1 obtained from human placental source, were determined as previously described.[20]

The 4-phenoxybenzamides **7a** and **1–7**, as well as the phenylcyclohexanecarboxamide **8** (Table 1) displayed a good inhibition of 17β-HSD2. Compound **4**, with a bent shape and **8**, lacking the central aromatic ring, but conserving the overall linear shape, showed an inhibitory activity in the same order of magnitude as compound **A** and significantly improved selectivity against 17β-HSD1, thus demonstrating that neither the linearity of the biphenyl moiety nor the aromaticity of the central ring are essential for inhibitory activity.

The phenylpiperazin-1-yl sulfonyls **9a–11a** and **9–11** (Table 2) displayed poor inhibition of the 17β-HSD2 enzyme, when compared to compounds **A** and **B**. Compound **10**, which can be directly compared to **B**, is a much weaker inhibitor of 17β-HSD2. Compound **11** is the best in the series. The improvement in activity between **10** and **11** comes from the introduction of the fluorine in *ortho* to the OH group. The F likely positively influences the hydrogen bond on the OH group next to it. In comparison to compound **B**, the sulfonyl derivatives bear a much more hydrophilic central ring, which might explain the loss of activity. They also bear a sulfonamide function condensed in the piperazine ring, which renders the molecules shorter than **B**. This feature might lead to a loss of important interactions with the enzyme, thus further explaining the lower inhibitory activity.

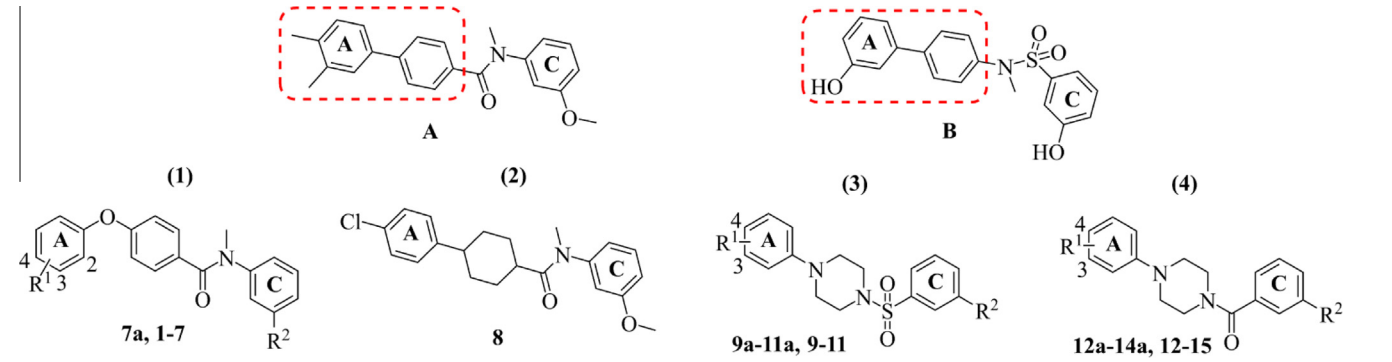
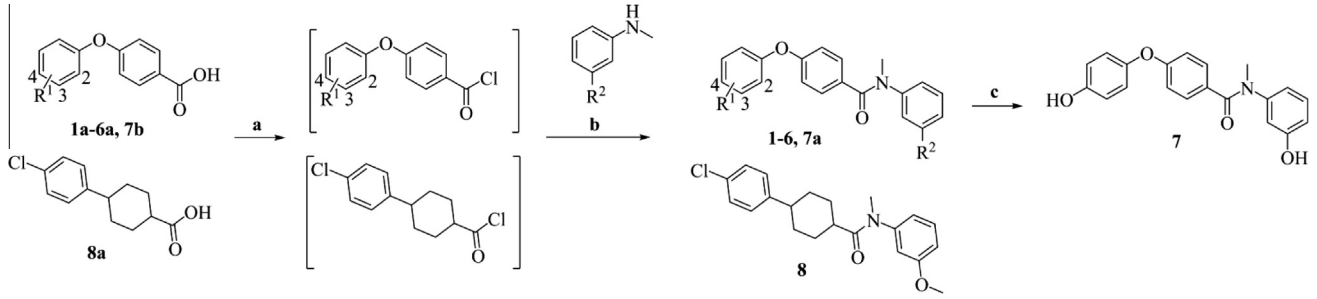
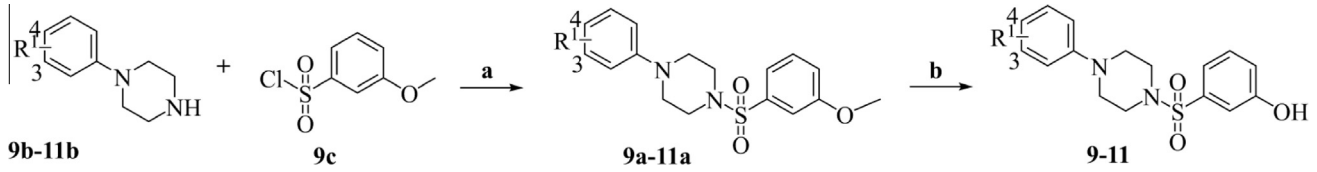


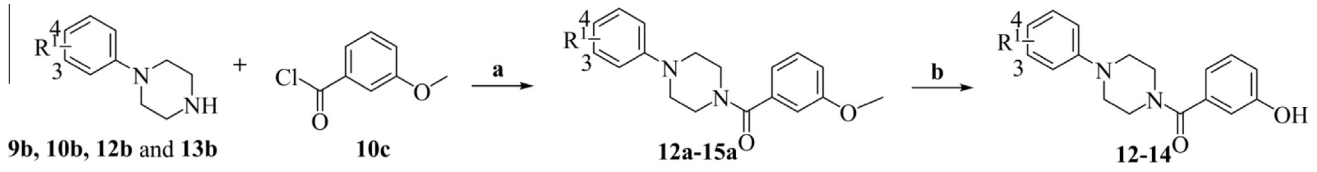
Figure 2. Four different classes of inhibitors derived from the lead compounds **A** and **B**.



Scheme 1. Synthesis of 4-phenoxybenzamide derivatives **1–7** and **7a** and 4-phenylcyclohexane carboxamide **8**. Reagents and conditions: (a) $SOCl_2$, DMF cat, toluene, reflux, 4 h; (b) Et_3N, CH_2Cl_2, room temperature, overnight; (c) $BF_3{\cdot}SMe_2$, CH_2Cl_2, 0 °C to room temperature, overnight.



Scheme 2. Synthesis of 3-(4-phenylpiperazin-1yl)sulfonyls **9–11**. Reagents and conditions: (a) $(Bu_4)N{\cdot}HSO_4$, NaOH 50%, CH_2Cl_2, room temperature, 3 h; (b) $BF_3{\cdot}SMe_2$, CH_2Cl_2, Et_3N, 0 °C to room temperature, overnight.



Scheme 3. Synthesis of phenylpiperazine-1-yl methanones **12–14** and **15a**. Reagents and conditions: (a) Et_3N, CH_2Cl_2, room temperature, overnight; (b) $BF_3{\cdot}SMe_2$, CH_2Cl_2, Et_3N, 0 °C to room temperature, overnight.

Table 1
Inhibitory activities toward 17β-HSD2 and 17β-HSD1 of compounds **7a** and **1–8**

Compd	R^1	R^2	IC_{50} (nM)[a] or % Inh. at 1 μM[a]		s.f.[e,f]
			17β-HSD2[b]	17β-HSD1[c,d]	
A	–	–	75% (300)	13,300	44
1	-H	-Me	59%	n.i.	n.d.
2	2-Me	-Me	55%	n.i.	n.d.
3	3-Me	-Me	300	16,100	54
4	4-Me	-Me	160	26,300	168
5	4-Cl	-OMe	51%	13%	n.d.
6	4-NO_2	-OMe	43%	n.i.	n.d.
7a	4-OMe	-OMe	310	9600	31
7	4-OH	-OH	43%	n.i.	n.d.
8	–	–	290	60,100	209

[a] Mean value of at least two determinations, standard deviation less than 20%.
[b] Human placental, microsomal fraction, substrate E2[500 nM], cofactor NAD^+[1500 μM].
[c] Human placental, cytosolic fraction, substrate E1[500 nM], cofactor NADH[500 μM].
[d] n.i.: no inhibition.
[e] s.f.: selectivity factor.
[f] n.d.: not determined.

The phenylpiperazin-1-yl methanones **12a–15a** and **12–14** (Table 3) displayed no or very low 17β-HSD2 inhibitory activity, indicating that the acyl group does not bring any advantage in comparison with the sulfonyl group.

Cell viability in HEK293 cells was determined for the best compounds **3**, **4**, **7a** and **8**, using a MTT assay according to a described procedure.[11] The results are displayed in Figure 3.

In comparison to compound **A** (LD_{50} less than 6.25 μM), all four compounds showed a better safety profile, with a LD_{50} around 25 μM for **3**, **4** and **7a** and above 12.5 μM for compound **8**. Compound **4** displays improved 17β-HSD2 inhibitory activity and much better selectivity against 17β-HSD1 as well as significantly decrease in cytotoxicity, when compared to compound **A**. These results confirm compound **4** as new lead compound for inhibition of 17β-HSD2.

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Table 2
Inhibitory activities toward 17β-HSD2 and 17β-HSD1 of compounds **9a–11a** and **9–11**

Compd	R^1	R^2	% Inh. at 1 μM[a]	
			17β-HSD2[b,c]	17β-HSD1[d,c]
B	—		66%	22%
9a	3-Me	–OMe	16%	n.i.
9	3-Me	–OH	27%	n.i.
10a	3-OMe	–OMe	n.i	n.i.
10	3-OH	–OH	25%	n.i.
11a	4-F, 3-OMe	–OMe	15%	n.i.
11	4-F, 3-OH	–OH	48%	n.i.

[a] Mean value of at least two determinations, standard deviation less than 20%.
[b] Human placental, microsomal fraction, substrate E2[500 nM], cofactor NAD^+[1500 μM].
[c] n.i.: no inhibition.
[d] Human placental, cytosolic fraction, substrate E1[500 nM], cofactor NADH [500 μM].

Table 3
Inhibitory activities toward 17β-HSD2 and 17β-HSD1 of compounds **12a–15a** and **12–14**

Compd	R^1	R^2	% Inh. at 1 μM[a]	
			17β-HSD2[b,c]	17β-HSD1[d,c]
12a	–H	–OMe	14%	n.i.
12	–H	–OH	27%	n.i.
13a	3-Me	–OMe	n.i	n.i.
13	3-Me	–OH	16%	n.i.
14a	4-Me	–OMe	16%	n.i.
14	4-Me	–OH	n.i.	n.i.
15a	3-OMe	–OMe	n.i.	n.i.

[a] Mean value of at least two determinations, standard deviation less than 20%.
[b] Human placental, microsomal fraction, substrate E2[500 nM], cofactor NAD^+[1500 μM].
[c] n.i.: no inhibition.
[d] Human placental, cytosolic fraction, substrate E1[500 nM], cofactor NADH [500 μM].

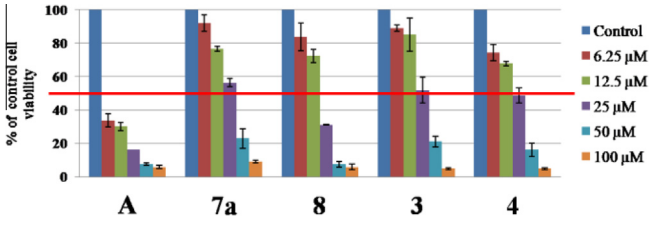

Figure 3. Cytotoxicity of selected compounds is displayed in the order of increasing 17β-HSD2 inhibitory activity. Incubation was carried out at the indicated inhibitor concentrations for 66 hours at 37 °C. 100%-values were determined without inhibitor.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.11.047. These data include MOL files and InChiKeys of the most important compounds described in this article.

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16. Data for *N*-methyl-*N*-(*m*-tolyl)-4-(*p*-tolyloxy)benzamide (compound **4**): yellow oil; $C_{22}H_{21}NO_2$; ^{1}H NMR-300 MHz (acetone-d_6, δ, ppm) 2.25 (s, 3H), 2.30 (s, 3H), 3.39 (s, 3H), 6.71–6.76 (m, 2H), 6.84–6.92 (m, 3H), 6.98–7.01 (m, 2H), 7.12–7.20 (m, 3H), 7.27–7.32 (m, 2H); ^{13}C NMR-75 MHz (acetone-d_6, δ, ppm) 20.8, 21.3, 38.6, 117.5, 120.4, 125.1, 127.9, 128.5, 129.8, 131.4, 131.7, 132.0, 134.5, 139.9, 146.5, 154.9, 159.8, 169.9; LRMS (*m*/*z*) calcd for $C_{22}H_{22}NO_2$ $[M+H]^+$ 332.16, found 332.18.
17. Data for 4-(4-chlorophenyl)-*N*-(3-methoxyphenyl)-*N*-methylcyclohexane-1-carboxamide (compound **8**): yellow solid; m.p. = 108–109 °C; $C_{21}H_{24}ClNO_2$. ^{1}H NMR-500 MHz (acetone-d_6, δ, ppm) 1.18–1.25 (m, 2H), 1.62–1.71 (m, 2H), 1.77–1.82 (m, 4H), 2.38 (br s, 1H), 2.48–2.54 (m, 1H), 3.18 (s, 3H), 3.84 (s, 3H), 6.89–6.97 (m, 2H), 6.96–6.98 (m, 1H), 7.17 (d, *J* = 9 Hz, 2H), 7.23–7.25 (m, 2H), 7.39 (t, *J* = 8 Hz, 1H); ^{13}C NMR-125 MHz (acetone-d_6, δ, ppm) 30.5, 34.0, 37.4, 41.4, 43.9, 55.9, 114.0, 114.2, 120.4, 129.1, 129.4, 131.3, 131.9, 146.8, 147.1, 161.7, 175.5; LRMS (*m*/*z*) calcd for $C_{21}H_{25}ClNO_2$ $[M+H]^+$ 358.16, found 358.21.
18. Data for 1-((3-methoxyphenyl)sulfonyl)-4-(m-tolyl)piperazine (compound **9a**): white solid; m.p. = 129–130 °C; $C_{18}H_{22}N_2O_3S$; ^{1}H NMR-300 MHz (acetone-d_6, δ, ppm) 2.24 (s, 3H), 3.11–3.14 (m, 4H), 3.22–3.25 (m, 4H), 3.91 (s, 3H), 6.63–6.66 (m, 1H), 6.70–6.76 (m, 2H), 7.08 (t, *J* = 8 Hz, 1H), 7.25–7.30 (m, 2H), 7.36–7.40 (m, 1H), 7.58 (t, *J* = 8 Hz, 1H); ^{13}C NMR-75 MHz 21.7, 47.1, 49.7, 56.1, 113.7, 114.6, 118.2, 119.7, 120.7, 121.8, 129.7, 131.2, 137.9, 139.3, 151.9, 161.0; LRMS (*m*/*z*) calcd for $C_{18}H_{23}N_2O_3S$ $[M+H]^+$ 346.14, found 346.89.
19. Data for (3-hydroxyphenyl)(4-phenylpiperazin-1-yl)methanone (compound **12**): white solid; m.p. = 174–175 °C; $C_{17}H_{18}N_2O_2$; ^{1}H NMR-300 MHz (acetone-d_6, δ, ppm) 4.00–4.14 (m, 8H), 6.94–6.97 (m, 1H), 6.95–7.02 (m, 3H), 7.30 (t, *J* = 8 Hz, 1H), 7.56–7.68 (m, 3H), 7.83–7.86 (m, 1H), 8.64 (br s, 1H); ^{13}C NMR-75 MHz (acetone-d_6, δ, ppm) 56.5, 115.1, 118.0, 119.1, 122.0, 130.6, 131.0, 131.4, 137.1, 142.9, 158.4, 170.4; LRMS (*m*/*z*) calcd for $C_{17}H_{19}N_2O_2$ $[M+H]^+$ 283.14, found 283.20.
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