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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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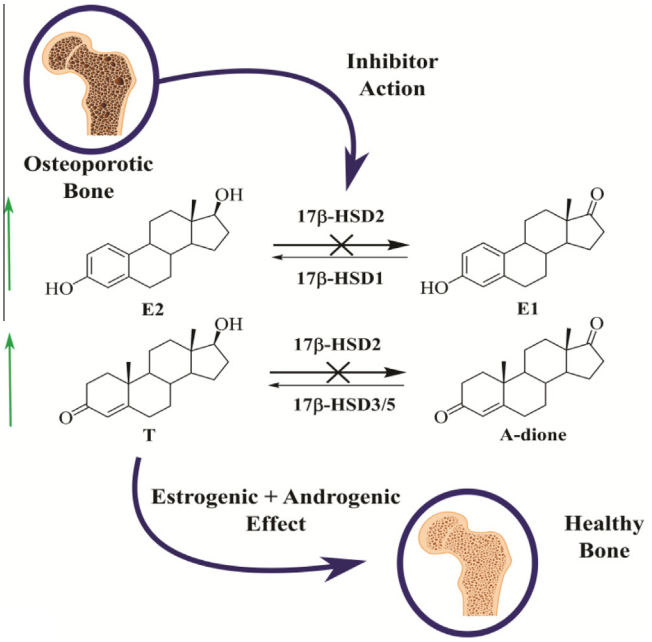

Figure 1. Estrogens (E2) and androgens (T) contribute to the maintenance of the overall bone quality. Blocking the oxidation of estradiol and testosterone by using an inhibitor of 17β-HSD2 should rebalance the steroid level in the bones.

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.

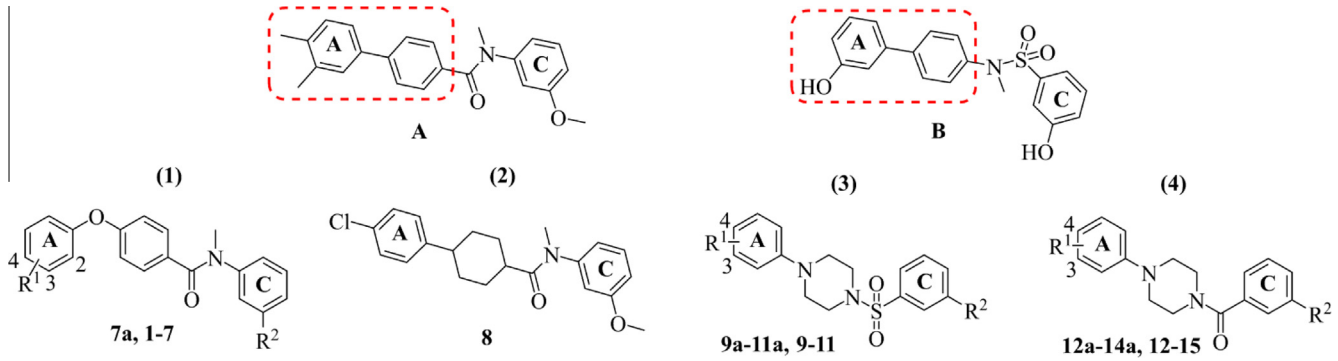

Figure 2. Four different classes of inhibitors derived from the lead compounds **A** and **B**.