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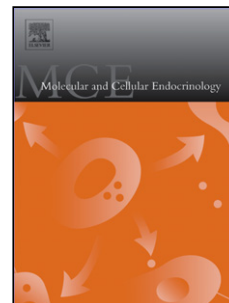
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The Role of the Heterocycle in Bis(hydroxyphenyl)triazoles for Inhibition of 17 β -Hydroxysteroid Dehydrogenase (17 β -HSD) Type 1 and Type 2

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Abbreviations: 17 β -HSD1, 17 β -hydroxysteroid dehydrogenase type 1; 17 β -HSD2, 17 β -hydroxysteroid dehydrogenase type 2; E1, estrone; E2, 17 β -estradiol; NADP(H), nicotinamide adenine dinucleotide phosphate; NAD(H), nicotinamide adenine dinucleotide; SAR, structure activity relationship.

Remark: For the sake of clarity, IUPAC nomenclature is not strictly followed except for the supplementary material section where the correct IUPAC names are given.

Keywords: 17 β -HSD; non-steroidal inhibitor; estrogen-dependent diseases; breast cancer, endometriosis, bis(hydroxyphenyl)azoles.

Abstract

17 β Hydroxysteroid dehydrogenase type 1 (17 β -HSD1) is responsible for the catalytic reduction of the weak estrogen estrone (E1) into the highly potent 17 β -estradiol (E2). As 17 β -HSD1 is often overexpressed in mammary tumors and endometriosis, the selective inhibition of this enzyme is discussed as a promising approach for the treatment of estrogen-dependent diseases. Recently, we reported on bis(hydroxyphenyl)azoles as a new class of potent inhibitors of 17 β -HSD1. In this paper, we focused on bis(hydroxyphenyl)triazoles. The influence of nitrogens on the potency as well as the space available around the heterocycle was investigated. Substituents were introduced on the triazole core in order to establish additional interactions with the enzyme active site. The compounds were evaluated for activity towards 17 β -HSD1 and selectivity with regard to 17 β -HSD2, the enzyme which is responsible for the deactivation of E2 into E1. 3-[4-(4-Hydroxyphenyl)-1*H*-1,2,3-triazol-1-yl]phenol (**3**) was the most active compound discovered in this study with an IC₅₀ value of 840 nM and a reasonable selectivity toward 17 β -HSD2.

1. Introduction

Estrogens are responsible for the development and differentiation of estrogen-sensitive tissues. Furthermore, it is well known that 17β -estradiol (E2), the most active estrogen, also plays a crucial role in the growth of estrogen-dependent mammary tumors (Travis et al., 2003) and is involved in the pathophysiology of endometriosis (Dizerega et al., 1980).

The potency of steroidal sex hormones like E2 is modulated by enzymes of the 17β -hydroxysteroid dehydrogenase (17β -HSD) family (Lukacik et al., 2006), especially 17β -HSD1 and 17β -HSD2, which catalyze the activation of E2 from estrone (E1) or the deactivation of E2 into E1, respectively (Chart 1). 17β -HSD1 is a cytosolic enzyme present in different organs like ovaries, placenta, breast, endometrium (Vihko et al., 2001) which is often overexpressed in breast cancer tissues (Suzuki et al., 2000; Gunnarsson et al., 2001; Miyoshi et al., 2001; Gunnarsson et al., 2005) and endometriosis (Šmuc et al., 2007). Consequently, selective inhibition of 17β -HSD1 is discussed to be a good strategy to reduce the intracellular E2 levels and regarded as a new therapeutic approach for the treatment of estrogen-dependent diseases. As a biological counterpart, 17β -HSD2 should not be affected by potential inhibitors of 17β -HSD1, as it protects the cell from excessively high concentrations of active estrogen (Vihko et al., 2004).

Chart 1

Until now, several groups have reported on 17β -HSD1 inhibitors, most of them showing steroidal structures (for reviews see: Poirier, 2003; Brožic et al., 2008). Concerning the non-steroidal cores, only four compound classes have been described so far: thienopyrimidinones (Messinger et al., 2006; Karkola et al., 2008), biphenyl ethanones (Allan et al., 2008), from our group 6-(hydroxyphenyl) naphthols (Frotscher et al., 2008; Marchais-Oberwinkler et al., 2008) and recently, we reported on bis(hydroxyphenyl)azoles (Bey et al., 2008) as potent and selective inhibitors of 17β -HSD1. Different core structures were investigated (Chart 2): imidazoles like compounds **A** and **B**, pyrazoles like compound **C** and oxazoles like compound **D**. The most active was compound **D** with an IC_{50} value of 310 nM and a selectivity factor of 56 against 17β -HSD2.

Chart 2

In the following, we will report on the design, synthesis and biological evaluation of a new family of azoles: bis(hydroxyphenyl)triazoles (Chart 3).

Chart 3

2. Design of the inhibitors

The analysis of the available ternary complex of 17β -HSD1 with E2 and $NADP^+$ (PDB-ID: 1FDT; Breton et al., 1996) provided useful informations about the architecture of the active site of the enzyme. A substrate binding site and a cofactor binding pocket can be defined. The former is a narrow hydrophobic tunnel containing two polar areas at each end. His221/Glu282 are located on the one side and Ser142/Tyr155 on the other side, corresponding to the binding oxygens in 3- and 17-hydroxy group of E2. Interestingly, close to the hydrophobic B/C region of the estrogen two polar amino acids, Tyr218 and Ser222, can be found which do not interact with the steroid.

In our design process, we focused on non-steroidal structures to avoid binding to other steroid receptors. The compounds should be able to mimic the substrate of the enzyme. We have previously reported on the importance of the two hydroxyphenyl moieties present in our steroidomimetics (Bey et al., 2008; Chart 2). The positions of the OH substituents on the phenyl groups is decisive for the inhibitory potency of the compounds: only inhibitors bearing a *meta-para* di-OH substitution pattern (O-O distance in the same range as observed for the steroid, $d = 11 \text{ \AA}$) were active probably due to the establishment of hydrogen bond interactions with His221/Glu282 and Ser142/Tyr155. *Meta-meta* disubstituted compounds which were not investigated so far might also show a favorable geometry to establish these interactions.

Furthermore, the nature of the heterocycle present between the two hydroxyphenyl moieties also influences the activity of the compounds. Heterocycles like oxazole **D** which can act as hydrogen bond acceptors were active while inhibitors bearing a H-bond donator function (NH) like 2,4-disubstituted imidazoles (e.g. compound **B**) and 3,5-disubstituted pyrazoles (e.g. compound **C**) were devoid of activity. It was hypothesized that the activity of compound **D** was linked to its ability to establish hydrogen bond interaction with Tyr218 and/or Ser222 (Bey et al., 2008).

It is striking that 1,4-disubstituted imidazoles like compound **A**, although pure hydrogen bond acceptors, were inactive. To obtain an insight into the impact of an additional nitrogen atom and on distribution of the nitrogen atom in the central ring on inhibitory activity, 1,2,3-triazoles **1-3** and 1,2,4-triazoles **4-9** were designed.

In addition, a non polar substituent was introduced on the heterocycle, anticipating supplementary hydrophobic interactions with amino acid residues of the substrate binding site. Compounds **4-9** bearing a methyl or a phenyl on the triazole core could give additional informations on the feasibility of these interactions (π -stacking interactions with Tyr218 for example in case of compounds **7-9**) as well as on the space available around the heterocycle.

3. Chemistry

1,2,3-Triazoles **1-3** were obtained according to the synthetic pathway previously described by Bey et al. (Bey et al., 2008).

The synthesis of 1,2,4-triazoles **4-9** is presented in Scheme 1. Starting from the *N*-acylimidates **4b-6b** prepared according to the method described by Kelarev et al. (Kelarev et al., 2003), the dimethoxylated 1,2,4-triazoles **4a-9a** were synthesized by nucleophilic addition of methylhydrazine for compounds **4a-6a** and phenylhydrazine for compounds **7a-9a**. The deprotection of the methoxy groups of compounds **4a-9a** was performed with borontrifluoride dimethyl sulfide complex (Bey et al., 2008).

Scheme 1

4. Inhibition of human 17 β -HSD1 and 17 β -HSD2

The 17 β -HSD1 and 17 β -HSD2 inhibition assays were carried out following a previously described procedure (Bey et al., 2008). Briefly, the cytosolic (17 β -HSD1) or microsomal (17 β -HSD2) fraction of human placental was incubated with tritiated substrate, cofactor and inhibitor at 37 °C. After HPLC separation of substrate and product, the amount of radiolabeled E2 (17 β -HSD1) or E1 (17 β -HSD2) formed was quantified. The inhibition values of compounds **1-9** are shown in Table 1.

The *meta-meta* disubstituted 1,2,3-triazole **1** is a weak inhibitor of 17 β -HSD1 (17 % inhibition at 1 μ M). Shifting one group from the *meta* to the *para* position (compounds **2** and

3) leads to an increase in activity, compound **3** being the most active one (IC_{50} = 0.84 μ M and 1.32 μ M for **3** and **2**, respectively). Concerning selectivity, the 1,2,3-triazoles **2** and **3** are very weak inhibitors of the type 2 enzyme (IC_{50} = 8.12 μ M and 7.28 μ M, respectively).

None of the 1,2,4-triazoles (compounds **4-9**) shows inhibition of 17 β -HSD1. Surprisingly, the *N*-1 phenyl substituted 1,2,4-triazoles (compounds **7** and **8**) are selective inhibitors of 17 β -HSD2 (44 % and 41 % inhibition at 1 μ M, respectively).

The introduction of an additional nitrogen into the inactive imidazole core structure (compound **A**) enhances inhibition of 17 β -HSD1 strongly (compound **2**, IC_{50} = 1.32 μ M) whereas a supplementary substituent (methyl for compounds **4-6** and phenyl for compounds **7-9**) is obviously not tolerated by the enzyme.

Table 1

5. Discussion and conclusion

As already mentioned in a previous report (Bey et al., 2008), the OH substitution pattern on the phenyl ring is a decisive criterion for inhibitory activity. The distance between the *meta-meta* hydroxy group (compound **1**) which is shorter compared to the *para-meta* (compound **2**) and the *meta-para* (compound **3**) might be one explanation for the lower activity of compound **1**.

Varying the heterocycle, two active 1,2,3-triazoles **2** and **3** have been identified. They differ from the inactive 1,4-disubstituted imidazole (compound **A**) by the presence of an additional nitrogen in position 2. It is interesting that the introduction of this supplementary heteroatom leads to a strong increase in activity (**A**: inactive, IC_{50} = 1.32 μ M for **2** and 0.84 μ M for **3**, respectively). It can be concluded that this nitrogen in position 2 is stabilizing the molecule in the active site via additional interactions. A hydrogen bond interaction between the nitrogen of the heterocycle and the OH of Tyr218 might be responsible for this gain in activity.

We also wanted to investigate whether an additional substituent on the heterocycle might be able to increase the potency of the inhibitor by establishing supplementary interactions. 1,2,4-Triazole was chosen as core structure as none of the nitrogen of the 1,2,3-triazoles can be substituted in an appropriate position where space is available.

The 1,2,4-triazoles are structurally related to the 2,4-disubstituted imidazoles like compound **B** and to the 3,5-disubstituted pyrazoles like compound **C** which are both inactive. It was previously hypothesized that this lack of activity was due to the presence of a hydrogen bond donator (NH). Introduction of a methyl or phenyl group in this position will remove the hydrogen bond donating function of the heteroatom and was hypothesized to increase the ability of the inhibitors to induce hydrophobic interactions with the enzyme active site. The fact that all 1,2,4-triazoles (compounds **4-9**) are inactive indicates that either the nitrogen in the vertex position on the heterocycle and/or the supplementary substituent are not tolerated by the enzyme. The introduction of a phenyl moiety into the 1,2,4-triazole core (compounds **7-9**) induces conformational constraints: to adopt a stable conformation the hydroxyphenyl moiety close to the phenyl substituent and/or the phenyl itself will rotate out of the heterocyclic plane. This conformational change disturbs the flatness of the inhibitor considerably. The hydroxyphenyl moieties might therefore not be able to adopt an appropriate conformation to establish hydrogen bond interactions with His221/Glu282 and Ser142/Tyr155, thus leading to compounds devoid of 17 β -HSD1 inhibitory activity. The loss of planarity, however, is correlated with an increase of 17 β -HSD2 inhibition. This finding is in agreement with the structures of previously described inhibitors of this enzyme (Bydal P. et al, 2004) which also lack planarity.

In this study, the 1,2,3-triazoles (compounds **2** and **3**) have been identified as a new class of potent and selective inhibitors of 17 β -HSD1. The hydroxy substitution pattern (*meta-para* and

para-meta) of the most active compounds confirms the reduced flexibility of the enzyme active site already observed. The introduction of an additional nitrogen in the inactive 2,4-disubstituted imidazole core structure leads to a highly active inhibitor of 17 β -HSD1. In contrast introduction of a substituent on the heterocycle is not tolerated by this enzyme but in case of a phenyl moiety results in fairly potent 17 β -HSD2 inhibitors. They might be considered as leads for further structural optimization in the development of selective 17 β -HSD2 inhibitors.

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Supplementary material

Synthetic procedures, spectroscopic data of compounds **4-9**. This material is available via internet at <http://www.sciencedirect.com>.

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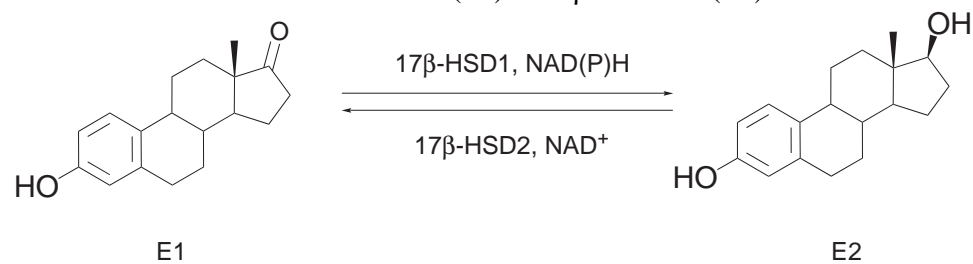
Chart 1: Interconversion of estrone (E1) to 17 β -estradiol (E2)

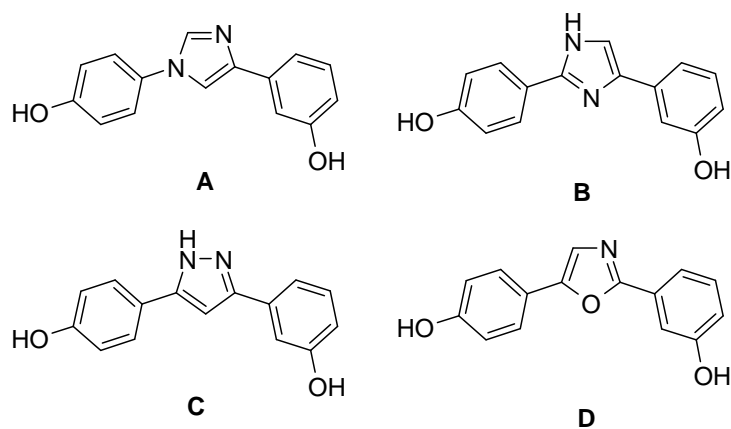
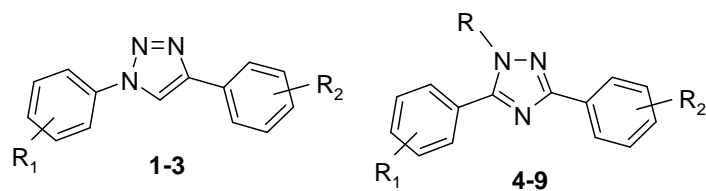
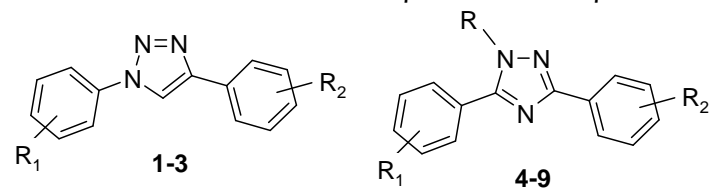
Chart 2: Described bis(hydroxyphenyl) azoles

Chart 3: Title compounds

compound	R	R_1	R_2
1		3-OH	3-OH
2		4-OH	3-OH
3		3-OH	4-OH
4	CH ₃	3-OH	3-OH
5	CH ₃	4-OH	3-OH
6	CH ₃	3-OH	4-OH
7	Ph	3-OH	3-OH
8	Ph	4-OH	3-OH
9	Ph	3-OH	4-OH

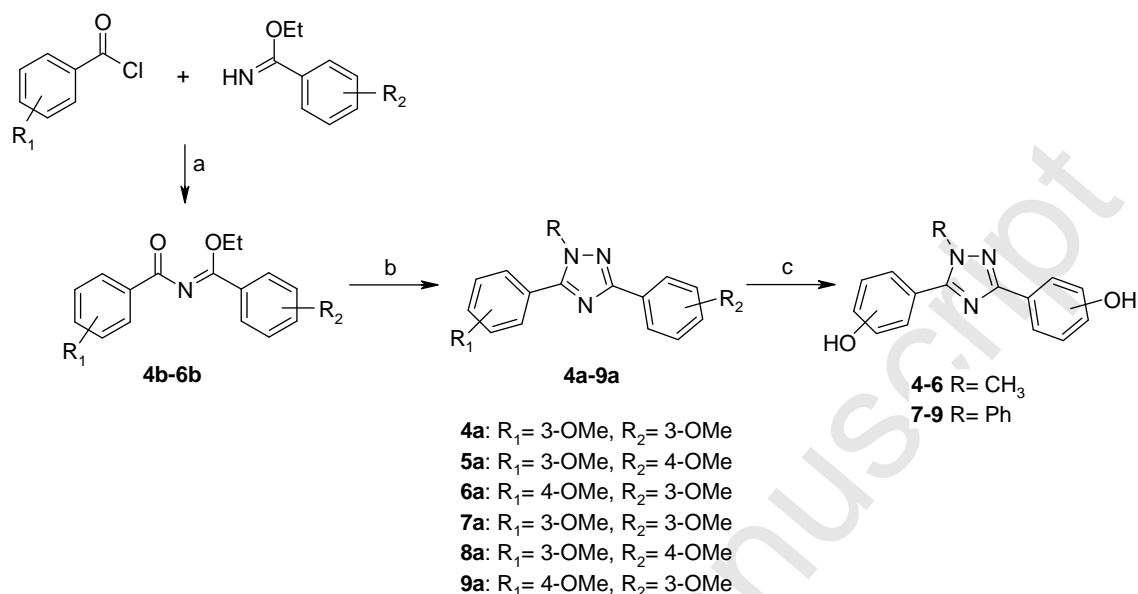
Table 1: Inhibition of human 17 β -HSD1 and 17 β -HSD2 by compounds **1-9**

compound	R	R ₁	R ₂	IC ₅₀ value (μM) ^a	
				17 β -HSD1 ^b	17 β -HSD2 ^c
1		3-OH	3-OH	17 ^d	ni
2		4-OH	3-OH	1.32	8.12
3		3-OH	4-OH	0.84	7.28
4	CH ₃	3-OH	3-OH	ni	ni
5	CH ₃	4-OH	3-OH	ni	20 ^d
6	CH ₃	3-OH	4-OH	ni	21 ^d
7	Ph	3-OH	3-OH	ni	41 ^d
8	Ph	4-OH	3-OH	ni	44 ^d
9	Ph	3-OH	4-OH	ni	ni

^aMean values of three determinations, standard deviation less than 18 %; ^bHuman placental, cytosolic fraction, substrate [³H]-E1 + E1 [500 nM], cofactor NADH [500 μM]; ^cHuman placental, microsomal fraction, substrate [³H]-E2 + E2 [500 nM], cofactor NAD⁺ [1500 μM];

^d% inhibition at 1 μM; ni: no inhibition (<10 % inhibition at 1 μM).

Scheme 1^a: Synthesis of compounds **4-9**



^a**Reagents and conditions:** (a) CH₂Cl₂, NEt₃, 30-40 °C, 6 h; (b) for compounds **4a-6a** H₃CNHNH₂ and for compounds **7a-9a** PhNHNH₂, CH₂Cl₂, 30-40 °C, 4 h; (c) BF₃·SMe₂, CH₂Cl₂, rt, 20 h.