Bioorganic & Medicinal Chemistry 16 (2008) 6423–6435

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Design, synthesis and biological evaluation of bis(hydroxyphenyl) azoles as potent and selective non-steroidal inhibitors of 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1) for the treatment of estrogen-dependent diseases

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ARTICLE INFO

Article history:
Received 14 March 2008
Revised 24 April 2008
Accepted 30 April 2008
Available online 3 May 2008

Keywords:
17β-HSD1
17β-HSD2
Non-steroidal inhibitor
ER
Estrogen-dependent diseases
Bis(hydroxyphenyl) azoles

ABSTRACT

The 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1) catalyses the reduction of the weakly active estrone (E1) into the most potent estrogen, 17β-estradiol (E2). E2 stimulates the growth of hormone-dependent diseases via activation of the estrogen receptors (ERs). 17β-HSD1 is often over-expressed in breast cancer cells. Thus, it is an attractive target for the treatment of mammary tumours. The combination of a ligand- and a structure-based drug design approach led to the identification of bis(hydroxyphenyl) azoles as potential inhibitors of 17β-HSD1. Different azoles and hydroxy substitution patterns were investigated. The compounds were evaluated for activity and selectivity with regard to 17β-HSD2, ERα and ERβ. The most potent compound is 3-[5-(4-hydroxyphenyl)-1,3-oxazol-2-yl]phenol (**18**, IC_{50} = 0.31 μM), showing very good selectivity, high cell permeability and medium CaCo-2 permeability.

1. Introduction

The naturally occurring steroidal hormones, estrone (E1) and 17β-estradiol (E2), are responsible for the development and differentiation of estrogen-sensitive tissues. It is well known, however, that E2, the most active estrogen, also plays a pivotal role in the growth of estrogen-dependent breast cancer,[1] and is involved in the pathophysiology of endometriosis.[2]

Until now three endocrine therapies have been established for the treatment of breast cancer.[3,4] Selective estrogen receptor modulators (SERMs) and pure antiestrogens[5] like fulvestrant block the estrogen action at the receptor level whilst aromatase inhibitors and GnRH-analogues restrain the formation of estrogens. This strong reduction of systemic estrogen action is a rather radical approach. A softer therapy could be the inhibition of the enzyme catalysing the last step of the E2 biosynthesis: 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1).

17β-HSD1 is a member of the 17β-hydroxysteroid dehydrogenase family which is responsible for the intracellular regulation of steroidal sex hormones activities.[6,7] Until now, fourteen members of this enzyme class are known.[8] In humans, eleven of them regulate the concentration of active androgens and estrogens in a tissue-specific manner.[8,9]

17β-HSD1 (EC1.1.1.62) is a cytosolic enzyme, which converts the weakly active estrone (E1) into the highly potent E2 using NAD(P)H as cofactor (Chart 1). The enzyme is expressed in different organs like ovaries, placenta, breast, endometrium[10] and often over-expressed in many breast cancer tissues.[11–14] Inhibition of 17β-HSD1 should be a good strategy to selectively reduce the E2 level in diseased tissues, and might therefore be a new therapeutic approach with probably less side effects for the treatment of estrogen-dependent diseases.

As a biological counterpart, the membrane-bound 17β-hydroxysteroid dehydrogenase type 2 (17β-HSD2) catalyses the

Abbreviations: 17β-HSD1, 17β-hydroxysteroid dehydrogenase type 1; 17β-HSD2, 17β-hydroxysteroid dehydrogenase type 2; E1, estrone; E2, 17β-estradiol; ER, estrogen receptor; PDB-ID, protein data bank identification code; P_{app}, apparent permeability coefficient; RBA, relative-binding affinity; SAR, structure–activity relationship; CC, column chromatography; MES, 2-(morpholino)ethanesulfonic acid; EDTA, ethylene diaminetetraacetate; TE, Tris–EDTA; FCS, foetal calf serum; DMEM, Dulbecco's modified Eagle's medium; AUC, area under the curve

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doi:10.1016/j.bmc.2008.04.073

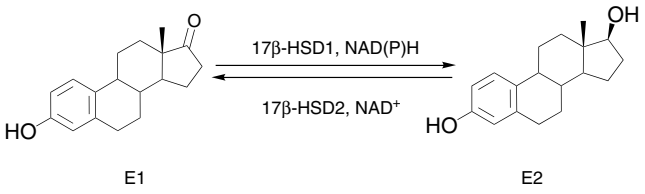


Chart 1. Interconversion of estrone (E1) to 17β-estradiol (E2).

NAD^+-dependent oxidation of E2 into E1 (Chart 1). This enzyme should not be affected by potential inhibitors of 17β-HSD1, as it might protect the cell from excessively high concentrations of active estrogen.[15] Nor should 17β-HSD1 inhibitors show affinity to the estrogen receptors α and β (ERα and ERβ) to avoid intrinsic estrogenic effects.

Over the last decade, several groups have reported on 17β-HSD1 inhibitors, most of them showing steroidal structures.[6,16–30] Until now, only three classes of non-steroidal compounds have been described.[31–34]

In the following, we will report on the design, synthesis and biological evaluation of potent and selective non-steroidal inhibitors of 17β-HSD1 that are appropriate for drug development.

2. Design of the inhibitors

2.1. Characterisation of the binding site

Sixteen crystal structures[35–43] with different steroidal ligands are available from the Protein Data Bank.[44] The three dimensional architecture of the enzyme was investigated using the ternary complex of 17β-HSD1 with E2 and $NADP^+$ (PDB-ID: 1FDT).[35] A substrate-binding site, a cofactor-binding pocket and an entry channel can be defined. The former is a narrow hydrophobic tunnel showing a high degree of complementarity to the steroid. Two polar regions can be identified at each extremity of the substrate-binding site corresponding to the binding positions of the 3- and 17-hydroxy group of E2. Each of them establish two hydrogen bonds with His221/Glu282 and Ser142/Tyr155, respectively. This area also contains a mainly apolar region, corresponding to the B/C ring of the steroid (Leu149, Val225, Phe226, Phe259). Interestingly, two polar amino acids (Tyr218, Ser222) are located in the same domain (Fig. 1).

The catalytic centre of the enzyme is formed by a tetrad of amino acids[45] (Asn114, Ser142, Tyr155 and Lys159) which stabilises the steroid and the nicotinamide moiety during hydride transfer. The active site is limited by a flexible loop (amino acids 188–201) which is not well resolved in all of the X-ray structures.

2.2. Design of steroidomimetics

In the design process, the substrate-binding site was defined as a binding region for the potential inhibitors as a lack of selectivity is expected for the compounds targeting only the cofactor-binding pocket: this area consists of Rossmann fold motifs which are highly conserved in the majority of dehydrogenases. We focused on non-steroidal structures as it is known that steroidal compounds often show side effects which are caused by agonistic or antagonistic effects at steroid receptors. As the compounds have to mimic the steroidal substrate, they should contain two hydroxyphenyl (or two methoxyphenyl) moieties to imitate the A-ring (interaction with His221/Glu282) and the D-ring (interaction with Ser142/Tyr155) of the steroid. Additionally, the O–O distance should be in the same range as observed for the steroid (d = 11 Å). Different substitution patterns concerning the position of the phenolic OH groups were therefore investigated to find out the one which fits best into the active site (Chart 2). To mimic the flat shape of the substrate, the two substituted benzenes should be linked by an additional aromatic ring. The five-membered heterocycles imidazole, triazole, pyrazole, isoxazole and oxazole seemed to be especially suitable as their heteroatoms might be able to establish additional interactions with the amino acids Tyr218 and/or Ser222, which are located close to the C6 position of the steroidal B-ring. Position, number and nature of the heteroatoms in the five-membered ring were varied in order to identify the most appropriate heterocycle. 1,2- and 1,3-bis(hydroxyphenyl) and tris(hydroxyphenyl) azoles have been described by Fink et al.[46] as novel ER ligands. The fact

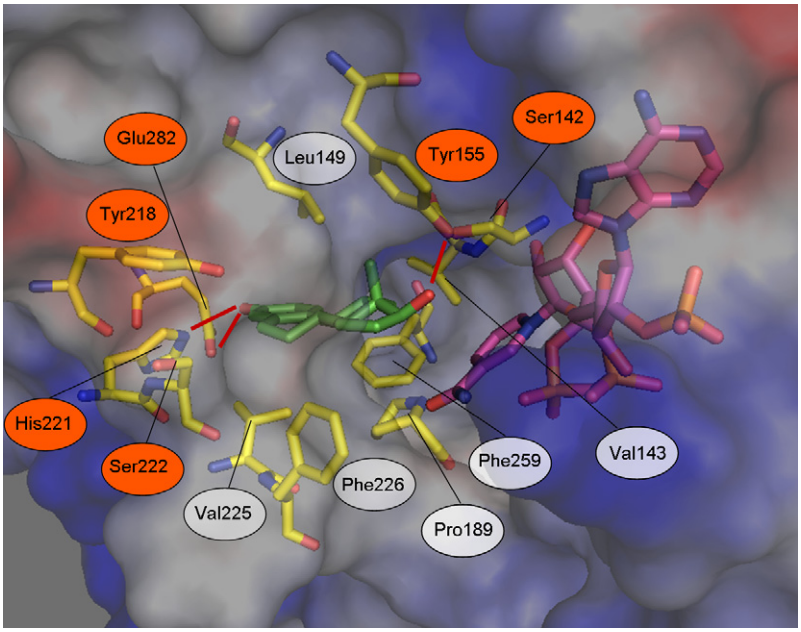
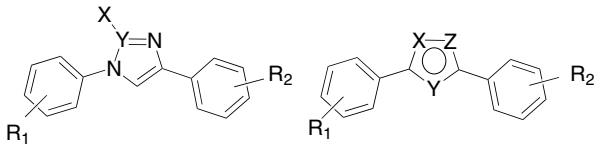


Figure 1. Schematic presentation of the active site of 17β-HSD1 containing E2 (PDB-ID: 1FDT). Orange labels denote polar amino acids and white labels stand for lipophilic amino acids. Hydrogen bonds are marked in red and cofactor in purple.



1-8 **9-19**

compound	X	Y	Z	R_1	R_2
1	SH	C		4-OH	3-OH
2	SH	C		4-OH	4-OH
3	SH	C		3-OH	4-OH
4	H	C		4-OH	3-OH
5	H	C		4-OH	4-OH
6	H	C		3-OH	4-OH
7		N		4-OH	3-OH
8		N		3-OH	4-OH
9	N	NH	CH	4-OH	3-OH
10	N	NH	CH	4-OH	4-OH
11	N	NH	CH	3-OH	4-OH
12	NH	CH	N	4-OH	3-OH
13	NH	CH	N	4-OH	4-OH
14	O	CH	N	4-OH	3-OH
15	O	CH	N	4-OH	4-OH
16	O	CH	N	3-OH	4-OH
17	N	O	CH	4-OH	4-OH
18	N	O	CH	3-OH	4-OH
19	O	N	CH	3-OH	4-OH

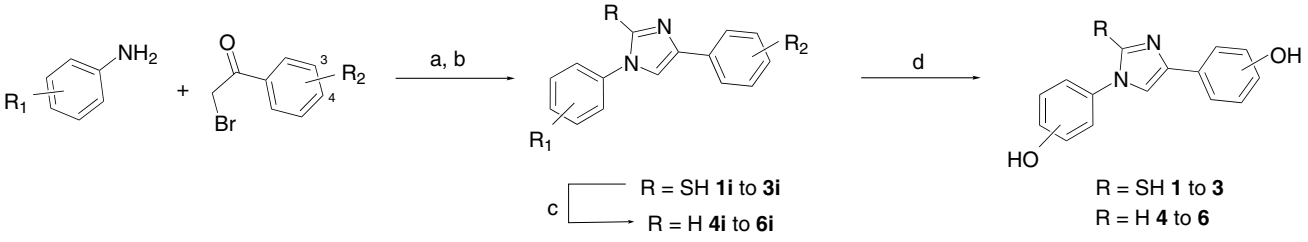
Chart 2. Title compounds.

that the compounds bearing two hydroxyphenyl moieties did not show any binding affinity to the ERs[46] is supportive of our design concept. In the following, we describe the synthesis of compounds **1**–**19** (Chart 2) and their biological evaluation using human 17β-HSD1, 17β-HSD2, ERα and ERβ as well as T-47D and CaCo-2 cell lines.

3. Chemistry

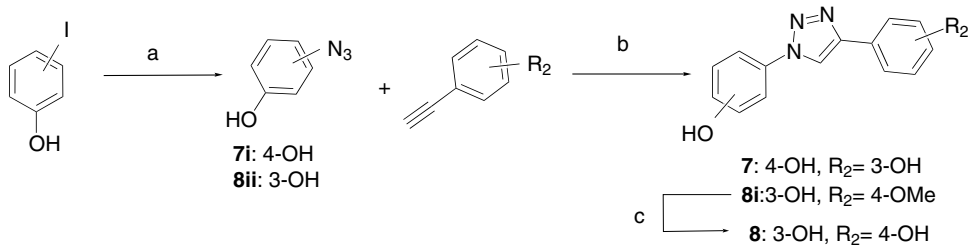
The synthesis of the title compounds is depicted in Schemes 1–4. 1,4-Bis(hydroxyphenyl) imidazoles were synthesised according to the route presented in Scheme 1. Intermediates **1i**–**3i** were prepared by nucleophilic substitution followed by cyclisation with potassium thiocyanate as described by Prakash et al.[47] The sulfur removal (**4i**–**6i**) was performed under strong acidic conditions according to Dodson and Ross.[48] Compounds **1i**–**6i** were submitted to ether cleavage with boron trifluoride dimethyl sulfide complex[46] as reagent.

Synthesis of the 1,2,3-triazoles **7** and **8** was performed according to the synthetic pathway shown in Scheme 2. Aromatic nucleophilic substitution of iodophenol derivatives by sodium azide led to intermediates **7i** and **8ii**. The resulting azides were converted to

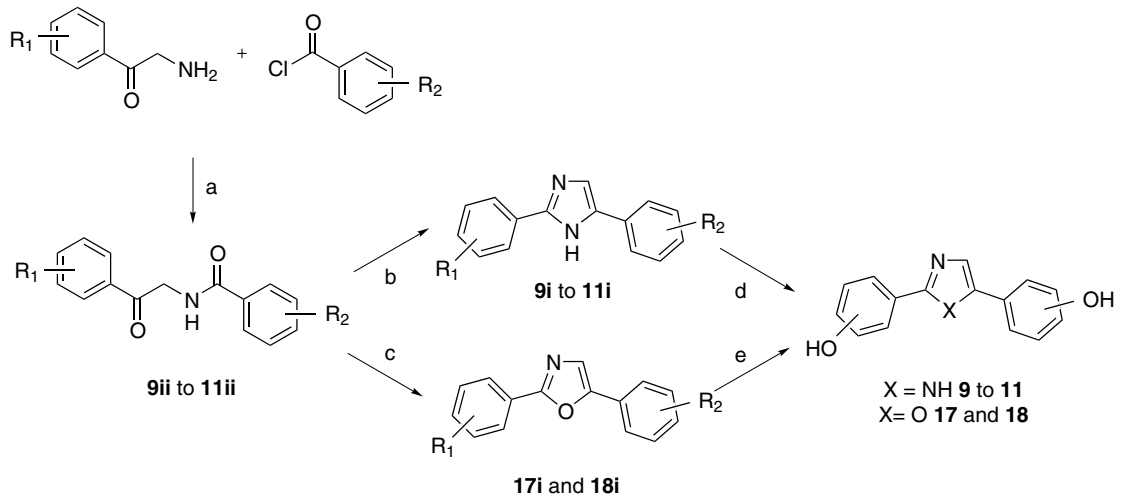


compound	R_1	R_2
1i	4-OMe	3-OMe
2i	4-OMe	4-OMe
3i	3-OMe	4-OMe
4i	4-OMe	3-OMe
5i	4-OMe	4-OMe
6i	3-OMe	4-OMe

Scheme 1. Synthesis of compounds **1**–**6**. Reagents and conditions: (a) NEt_3, DMF, rt, 7 h; (b) KSCN, cat. HCl, MeOH, reflux, 18 h; (c) HNO_3, $NaNO_2$, glacial acetic acid, 0 °C, 20 min; (d) $BF_3{\cdot}SMe_2$, CH_2Cl_2, rt, 20 h.



Scheme 2. Synthesis of compounds **7** and **8**. Reagents and conditions: (a) NaN_3, CuI, L-proline, NaOH, DMSO, 60 °C, 10 h; (b) Na-ascorbate, $CuSO_4$, H_2O/*t*-BuOH (1:1), 60 °C, 24 h; (c) $BF_3{\cdot}SMe_2$, CH_2Cl_2, rt, 20 h.



compound	R_1	R_2	compound	R_1	R_2
9ii	4-OMe	3-OMe	**9i**	4-OMe	3-OMe
10ii	4-OMe	4-OMe	**10i**	4-OMe	4-OMe
11ii	3-OMe	4-OMe	**11i**	3-OMe	4-OMe
			17i	4-OMe	4-OMe
			18i	3-OMe	4-OMe

Scheme 3. Synthesis of compounds **9**–**11**, **17** and **18**. Reagents and conditions: (a) NEt_3, rt, 30 min.; (b) NH_4OAc, AcOH, reflux, 2 h; (c) $POCl_3$, pyridine, reflux, 8 h; (d) $BF_3{\cdot}SMe_2$, CH_2Cl_2, rt, 20 h; (e) for **17i**: pyridinium hydrochloride, 220 °C, 18 h, for **18i**: BBr_3, CH_2Cl_2, −78 °C to rt, 20 h.

compounds **7** and **8i** in the presence of the corresponding phenyl acetylene derivative using the method of Sharpless.[49] The methoxy group of **8i** was cleaved with boron trifluoride dimethyl sulfide complex.

The synthesis of the 2,5-bis(hydroxyphenyl) imidazoles and 2,5-bis(hydroxyphenyl) oxazoles is presented in Scheme 3. The commercially available amine derivatives were N-acylated with two different acid chlorides to build the key intermediates **9ii**–**11ii**. The latter were cyclised to the 2,4-disubstituted imidazoles (**9i**–**11i**) according to Suzuki et al.[50] and to the 2,5-disubstituted oxazoles (**17i** and **18i**) following Nicolaou et al.[51] Ether cleavage was successful with boron trifluoride dimethyl sulfide complex for the imidazole compounds **9**–**11**, pyridinium hydrochloride for **17** and boron tribromide for **18**.

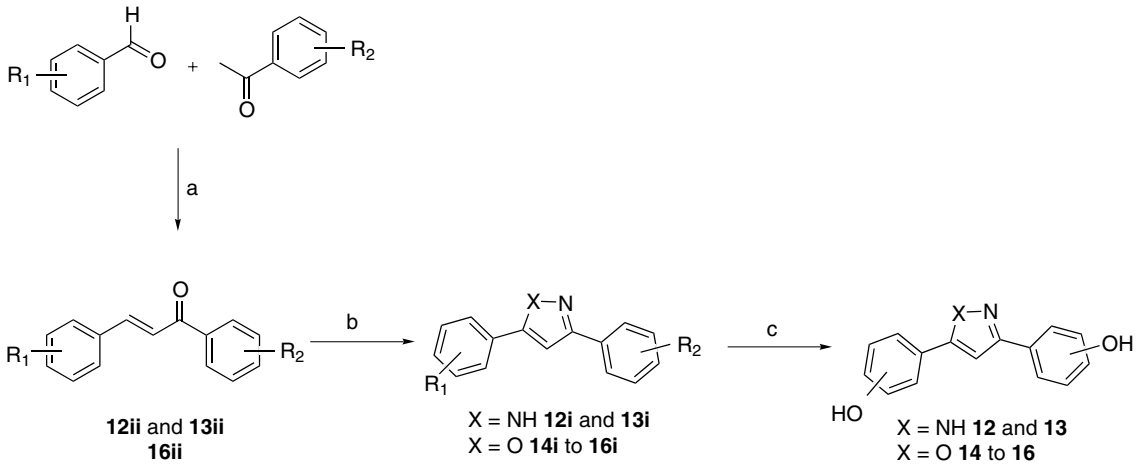
The pyrazoles **12** and **13** and the isoxazoles **14** and **16** were synthesised according to the route shown in Scheme 4. Key intermediates **12ii**, **13ii** and **16ii** were prepared via Claisen condensation of the commercially available methoxylated acetophenone derivatives with the appropriate benzaldehydes under strong basic conditions. The cyclisation step for the pyrazoles[52] **12i** and **13i** was carried out with hydrazine monohydrate (Method A). For compounds **14i**–**16i**, the α,β-unsaturated ketones **12ii**, **13ii** and **16ii** were first activated with bromine and cyclised to isoxazole with hydroxylamine hydrochloride (Method B). Ether cleavage was performed with boron tribromide to yield **12**–**16**.

The 2, 4 oxazole **19i** was prepared in a one-pot synthesis following the procedure of Lee et al.[53] Briefly, 4-methoxyacetophenone, [hydroxy(2,4-dinitrobenzensulfonyloxy)iodo]benzene and 3-methoxybenzamide were heated under reflux in acetonitrile for 10 h to yield the intermediate **19i**. The ether groups of **19i** were cleaved with boron tribromide.

4. Biological results

4.1. Inhibition of human 17β-HSD1

Placental enzyme was isolated following a described procedure.[54] Tritiated E1 was incubated with 17β-HSD1, cofactor and inhibitor. The amount of formed E2 was quantified by HPLC. All methoxy compounds and *para*–*para*di (hydroxylated) derivatives are inactive (data not shown). In contrast, some of the unsymmetrically substituted compounds were active (Table 1). Interestingly



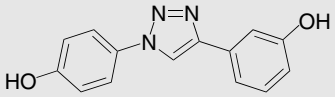
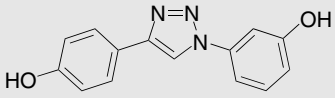
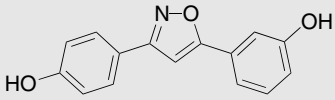
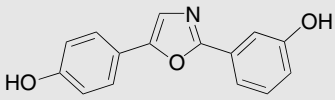
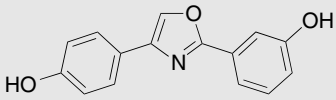
compound	R_1	R_2	method
12i	4-OMe	3-OMe	A
13i	4-OMe	4-OMe	A
14i	4-OMe	3-OMe	B
15i	4-OMe	4-OMe	B
16i	3-OMe	4-OMe	B

Scheme 4. Synthesis of compounds **12**–**16**. Reagents and conditions: (a) EtOH, Na, rt, 30 min; (b) Method A: hydrazine monohydrate, AcOH, EtOH, reflux, 24 h; Method B: bromine, diethylether, 0 °C, 1 h, hydroxylamine hydrochloride, abs. EtOH, reflux 24 h; (c) BBr_3, CH_2Cl_2, −78 °C to rt, 20 h.

the *para–meta* disubstituted isoxazole **14** is inactive whilst its *meta–para* analogue **16** (IC_{50} = 1.61 μM) shows inhibitory activity. This exemplifies the importance of the positions of the OH groups. The triazole **7**, isoxazole **16** and 2,4-disubstituted oxazole **19** are only weak inhibitors of 17β-HSD1, whilst derivatives **8** and **18** show IC_{50} values in the nanomolar range. The 2,5-disubstituted oxazole **18** was the most potent compound identified with an IC_{50} value of 0.31 μM.

It is striking that only compounds containing a heterocycle which can function as a hydrogen bond acceptor (nitrogen and/or oxygen) are active. The position of the heteroatoms in the heterocyclic skeleton is also a decisive criterion for inhibitory activity.

Table 1
Inhibition of human 17β-HSD1 and 17β-HSD2 by selected inhibitors

Compound	Structure	IC_{50}[a] (μM)		Selectivity factors[d]
		17β-HSD1[b]	17β-HSD2[c]	
7		1.32	8.12	6
8		0.84	7.28	9
16		1.61	0.27	0.2
18		0.31	17.5	56
19		1.85	0.25	0.1

[a] Mean value of three determinations, standard deviation less than 10%.
[b] Human placental, cytosolic fraction, substrate E1, 500 nM, cofactor NADH, 500 μM.
[c] Human placental, microsomal fraction, substrate E2, 500 nM, cofactor NAD^+, 1500 μM.
[d] IC_{50} HSD2/IC_{50} HSD1.

The 2,5-disubstituted oxazole (**18**, IC_{50} = 0.31 μM) showed a stronger inhibition of the enzyme as its 2,4-disubstituted oxazole (**19**, IC_{50} = 1.85 μM) and 3,5-disubstituted isoxazole (**16**, IC_{50} = 1.61 μM) analogues.

4.2. Selectivity

Since 17β-HSD2 catalyses the inactivation of E2 into E1, inhibitory activity towards this enzyme must be avoided. The 17β-HSD2 inhibition was determined using an assay similar to the 17β-HSD1 test. Placental microsomes were incubated with tritiated E2 in the presence of NAD^+ and inhibitor. Quantification of labelled product (E1) was performed by HPLC and following radio detection. IC_{50} values and selectivity factors (IC_{50} HSD2/ IC_{50} HSD1) are presented in Table 1.

Compound **18** is only a weak inhibitor of the type 2 enzyme (IC_{50} = 17.5 μM) with a selectivity factor of 56, whilst the isoxazole **16** (IC_{50} = 0.27 μM) and the oxazole **19** (IC_{50} = 0.25 μM) isomers show stronger inhibition for the type 2 versus type 1 enzyme (selectivity factors: 0.2 and 0.1, respectively).

Furthermore, as 17β-HSD1 inhibitors should have no or low affinity to ERα and ERβ, binding affinity was measured for selected compounds. Using recombinant human protein, a competition assay applying tritium labelled E2 (RBA = 100%) and hydroxyapatite was performed. The triazoles **7** and **8**, isoxazole **16** and oxazole **19** show very low binding affinity to both ERs, whereas for the most interesting compound, the 2,5-disubstituted oxazole **18**, a very low affinity to ERα (0.01 < RBA(%) < 0.1) and a marginally higher affinity to ERβ (RBA = 0.5%, Table 2) is observed.

4.3. Further biological evaluation of compound 18 using T-47D and CaCo-2 cell lines

The most promising inhibitor of this series, compound **18** was evaluated for estrogenic effects on the ER-positive, mammary tumour T-47D cell line. No agonistic, that is no stimulatory effect, was observed after application of compound **18** even at a concentration 1000-fold higher compared to E2.

Compound **18** was additionally evaluated using the same cell line which expresses both 17β-HSD1 and 17β-HSD2. The oxazole **18** inhibits the formation of E2 after incubation with labelled E1 exhibiting an IC_{50} value of 0.38 μM. As this value is very similar to the one observed in the cell free assay (0.31 μM, see Table 1) it can be concluded that the compound is capable of unfolding its activity in intact cells.

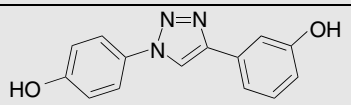
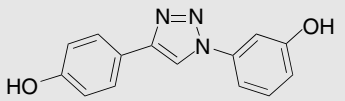
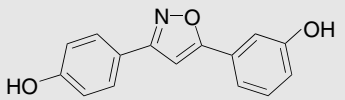
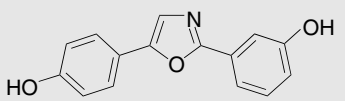
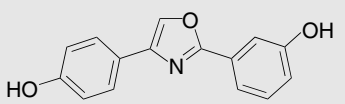
Further investigations were performed using CaCo-2 cells. These cells exhibit morphological and physiological properties of the human small intestine[55] and are generally accepted to be an appropriate model for the prediction of peroral absorption. Depending on the P_{app} data obtained, compounds are usually classified as low (P_{app} (10^{-6} cm/s) < 1), medium (1 < P_{app} < 10) or highly permeable (P_{app} > 10). Compound **18**, showing a P_{app} value of 7.9 10^{-6} cm/s, is a medium cell permeator like for example acetyl salicylic acid.

5. Molecular modelling

In order to get a better insight into the molecular interactions between the most potent steroidomimetic **18** and 17β-HSD1, the compound was docked into the active site of the protein (PDB-ID: 1FDT, E2 removed) using the docking software Gold 3.0 (rigid protein, flexible ligand).

Two different binding modes can be expected because of the pseudo-symmetry of our steroidomimetic: each hydroxyphenyl group could mimic the A-ring of the steroid. The energetically most favourable pose is depicted in Figure 2. The *para*-hydroxyphenyl substituent and the heterocycle are in the same plane, whilst the *meta*-hydroxyphenyl moiety is rotated 32° out of this plane. This conformation allows the inhibitor to establish hydrogen bond interactions with His221/Glu282 (*para*-hydroxyphenyl moiety) and Ser142/Tyr155 (*meta*-hydroxyphenyl substituent). Interestingly, the $N_{oxazole}$–O_{Tyr218} distance is 2.89 Å making an additional hydrogen bond interaction very likely. On the other hand, an inter-

Table 2
Binding affinities for the estrogen receptors α and β by selected compounds

Compound	Structure	RBA[a] (%)	
		ERα	ERβ
7		<0.01	<0.01
8		0.01 < RBA < 0.1	0.01 < RBA < 0.1
16		0.01 < RBA < 0.1	<0.01
18		0.01 < RBA < 0.1	0.5
19		0.01 < RBA < 0.1	0.01 < RBA < 0.1

[a] RBA: relative-binding affinity, E2: 100%, mean value of three determinations, standard deviation less than 10%.

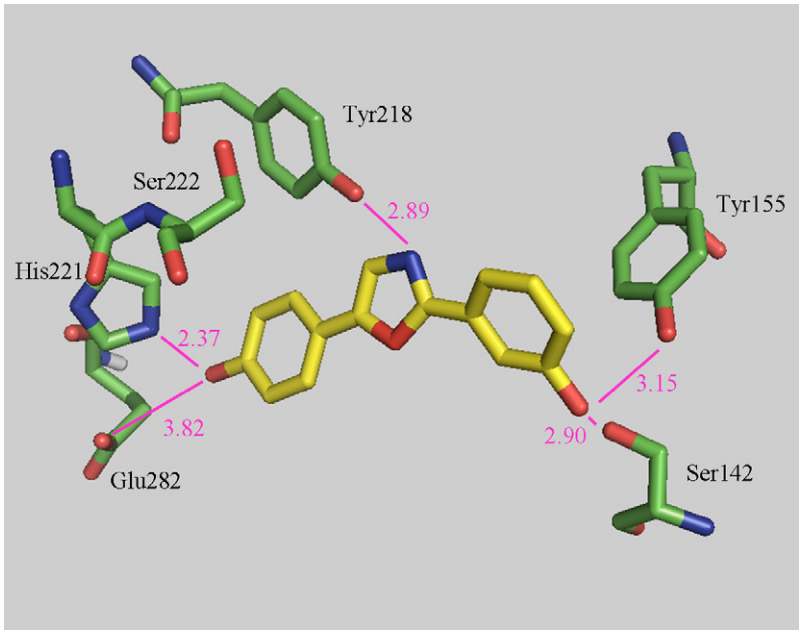


Figure 2. 17β-HSD1-binding pocket (green amino acids) with docked compound **18** (yellow). Hydrogen bonding interactions are marked by violet lines. All distances are expressed in Å. For clarity, only selected amino acids are represented.

action with the O_{Ser222} cannot be observed as in the rigid protein structure the CH_2OH moiety is turned away from the heterocycle. However, it cannot be excluded that there is a conformational change in that functional group after binding of this ligand. Additionally, it is likely that hydrophobic interactions (Van der Waals and π–π stacking) are also involved in the binding of **18** into the active site.

6. Discussion and conclusion

Looking at the SARs of the synthesised compounds, it becomes apparent that the positions of the OH groups at the hydroxyphenyl moieties are crucial for the activities of the corresponding derivatives: all *para–para* substituted compounds are inactive, whilst several of the *meta–para*/*para–meta* isomers are active. This suggests that 17β-HSD1 is not as flexible to adjust its geometry to the two OH groups as it is reported for the ERs,[56] and is in agreement with the non-flexibility already observed for 17β-HSD1 inhibitors in the class of 6-(hydroxyphenyl)naphthalenes.[34] The fact that not all *meta–para*/*para–meta* bis (hydroxyphenyl) derivatives (with O–O distances comparable to E2) are active, indicates that the nature of the heterocycle is also playing an important role for the inhibitory potencies of the compounds.

Obviously hydrogen bond donors are unfavourable for activity (e.g., imidazoles **9**–**11**, pyrazoles **12** and **13**), whilst several compounds with only hydrogen bond acceptor groups show reasonable activities (e.g., triazoles **7** and **8**, isoxazole **16**, oxazoles **18** and **19**).

It is interesting to have a closer look at the inactive isoxazole **14** (*para–meta*) and its highly active isomer, the oxazole **18** (*para–meta*). Provided that both compounds interact with the active site in the same manner, for example, the *para*-hydroxyphenyl moiety establishes hydrogen bond contacts with His221/Glu282, the structure of the inhibitors only differ by the position of the oxygen on the heterocycle. It has been described[57] that only the nitrogen of isoxazole and oxazole (and not the oxygen) is able to establish hydrogen bond interaction. The position of the oxygen on the azole structure seems to be determinant for the ability of the compound for forming hydrogen bonds involving the nitrogen: in case of the isoxazole, the electronic density on the nitrogen is reduced compared to the oxazole. This is obviously due to the electronegative effect of the oxygen. It can therefore be assumed that only in case of compound **18**, hydrogen bond interactions with Tyr218 and Ser222 (Fig. 2) are possible making the latter compound a potent inhibitor. These explanations are supported by the fact that the oxazole **19** shows a weak inhibitory activity.

The insertion of a third nitrogen (triazoles **7** and **8**) in the heterocycle increases the inhibitory potency of the correspondingly substituted imidazoles **4**, **6** and imidazoles-2-thiol **1**, **3** which are inactive. Obviously, this nitrogen plays the same role for the interaction with the amino acid residues in the binding site as observed for compound **18**.

The most interesting compound of the present study is **18** showing a high 17β-HSD1 inhibition and a good selectivity towards 17β-HSD2. In addition, compound **18** exhibits very low affinity to ERα and ERβ. It is striking that the high-binding affinity of similar ER ligands like the tris(hydroxyphenyl) pyrazoles described by Katzenellenbogen's group[46,58–60] depends on the presence of three hydroxyphenyl moieties. No polar amino acids in the B/C ring region of E2 can be identified in the X-ray structures of the ERs as groups to establish interactions with the heterocycle. Obviously, the pyrazole is playing a passive role in this class of ER ligands. In case of our bis(hydroxyphenyl) substituted azoles the combination of at least two parameters is implicated in the 17β-HSD1 inhibitory potency: an optimal OH substitution pattern and an appropriate heterocycle.

Activation of the ERs would be detrimental for the treatment of estrogen-dependent diseases. Therefore, agonism must be avoided. Antagonistic activity would be less critical. The diseased cells certainly could benefit from compounds with dual activity. However, antiestrogens would also exert systemic effects in other healthy steroidogenic tissues leading to unwanted effects. Consequently, we focused on the discovery of compounds without affinity to the ERs.

The fact that the 2,5-disubstituted oxazole **18** inhibits the cellular formation of E2 with an IC_{50} value in the nanomolar range shows that this compound is able to enter the cell and to be active at the target enzyme. The fact that the inhibitor shows a good permeation is supported by the CaCo-2 data. These results are indicative of a sufficient intestinal absorption.

Compound **18** might therefore be a good lead compound for the development of a clinically applicable therapeutic for the treatment of estrogen-dependent diseases.

7. Experimental Section

7.1. Chemical methods

Chemical names follow IUPAC nomenclature. Starting materials were purchased from Aldrich, Acros, Lancaster, Roth, Merck or Fluka and were used without purification.

Column chromatography (CC) was performed on silica gel (70–200 μm) coated with silica, preparative thin layer chromatography (TLC) on 1 mm SIL G-100 UV_{254} glass plates (Macherey-Nagel) and reaction progress was monitored by TLC on Alugram SIL G UV_{254} (Macherey-Nagel).

Melting points were measured on a Mettler FP1 melting point apparatus and are uncorrected. IR spectra were recorded on a Bruker Vector 33 spectrometer (neat sample).

^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker AM500 spectrometer (500 MHz) at 300 K. Chemical shifts are reported in δ (parts per million: ppm), by reference to the hydrogenated residues of deuteriated solvent as internal standard $CDCl_3$: δ = 7.24 ppm (^{1}H NMR) and δ = 77 ppm (^{13}C NMR), CD_3OD: δ = 3.35 ppm (^{1}H NMR) and δ = 49.3 ppm (^{13}C NMR), CD_3COCD_3: δ = 2.05 ppm (^{1}H NMR) and δ = 29.9 ppm (^{13}C NMR), CD_3SOCD_3: δ = 2.50 ppm (^{1}H NMR) and δ = 39.5 ppm (^{13}C NMR). Signals are described as s, d, t, dd, m, dt, q for singlet, doublet, triplet, doublet of doublets, multiplet, doublet of triplets and quadruplet, respectively. All coupling constants (*J*) are given in hertz (Hz).

Mass spectra (ESI and APCI) were recorded on a TSQ Quantum (Thermo Finnigan) instrument. Elemental analyses were performed at the Department of Instrumental Analysis and Bioanalysis, Saarland University.

The following compounds were prepared according to previously described procedures: 1,4-bis(4-methoxyphenyl)-1*H*-imidazol-2-thiol (**2i**),[47] 4-(3-methoxyphenyl)-1-(4-methoxyphenyl)-1*H*-imidazole (**4i**),[61] 1,4-bis(4-methoxyphenyl)-1*H*-imidazole (**5i**),[62] 4-azidophenol (**7i**),[63] 3-[1-(4-hydroxyphenyl)-1*H*-1,2,3-triazol-4-yl]-phenol (**7**),[63] 3-azidophenol (**8ii**),[63] 3-[4-(4-hydroxyphenyl)-1*H*-1,2,3-triazol-1-yl]phenol (**8**),[63] 3-methoxy-*N*-[2-(4-methoxyphenyl)-2-oxo-ethyl]benzamide (**9ii**),[64] 4-methoxy-*N*-[2-(4-methoxyphenyl)-2-oxo-ethyl]benzamide (**10ii**),[65] 2,5-bis(4-methoxyphenyl)-1*H*-imidazole (**10i**),[66] 3-methoxy-*N*-[2-(4-methoxyphenyl)-2-oxo-ethyl]-benzamide (**11ii**),[67] (2*E*)-1-(3-methoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (**12ii**),[68] (2*E*)-1,3-bis(4-methoxyphenyl)prop-2-en-1-one (**13ii**),[68] 3,5-bis(4-methoxyphenyl)-1*H*-pyrazole (**13i**),[69] 4,4′-(1*H*-pyrazol-3,5-diyl)diphenol (**13**),[46] 3-(3-methoxyphenyl)-5-(4-methoxyphenyl)isoxazole (**14i**),[70] 3-[5-(4-hydroxyphenyl)isoxazol-3-yl]phenol (**14**),[71] 3,5-bis(4-methoxyphenyl)-isoxazole (**15i**),[71] 4,4′-(isoxazol-3,5-diyl)diphenol (**15**),[71] (2*E*)-3-(3-methoxyphenyl)-1-(4-methoxyphenyl)prop-2-en-1-one (**16ii**),[72] 3-[3-(4-hydroxyphenyl)isoxazol-5-yl]phenol (**16**),[71] 2,5-bis(4-methoxyphenyl)-1,3-oxazole (**17i**).[64]

7.1.1. General synthesis procedure for compounds 1i–3i

A mixture of methoxyaniline (1 equiv), methoxyphenacyl bromide (1 equiv) and triethylamine (1 equiv) was stirred at rt in 2 mL DMF for 7 h. The crude material was poured into ice water. The resulting precipitate was filtered and dried overnight in a desiccator. To a stirred solution of the precipitate (1 equiv) in 20 mL methanol, potassium thiocyanate (1 equiv) and 60 μL concentrated chlorhydric acid were added. The resulting mixture was refluxed for 18 h. After cooling to rt, the precipitate was filtered off and dried overnight in a desiccator. The crude product was purified by CC.

7.1.1.1. 4-(3-Methoxyphenyl)-1-(4-methoxyphenyl)-1*H*-imidazol-2-thiol (1i). The title compound was prepared by reaction of *para*-methoxyaniline (751 mg, 6.11 mmol), 3-methoxyphenacyl bromide (1.38 g, 6.11 mmol), triethylamine (0.89 mL, 6.11 mmol) and potassium thiocyanate (593 mg, 6.11 mmol) according to the procedure reported above. The product was purified by CC (hexane/ethyl acetate 9:1); yield: 28% (500 mg); yellow powder; mp: 248 °C; ^{1}H NMR ($CDCl_3$): 7.36 (d, *J* = 9.40 Hz, 2H), 7.30–7.26 (m, 2H), 7.10 (d, *J* = 7.80 Hz, 2H), 6.84 (m, 1H), 6.81 (d, *J* = 8.80 Hz, 2H), 3.82 (s, 3H, OMe), 3.72 (s, 3H, OMe); ^{13}C NMR ($CDCl_3$): 175.45, 160.05, 159.65, 129.70, 127.55, 117.45, 114.15 (2C), 113.95, 110.00, 55.45, 55.40. IR: 1626, 1514, 1222, 1037, 824 cm^{-1}; MS (APCI): 313 $(M+H)^+$.

7.1.1.2. 1-(3-Methoxyphenyl)-4-(4-methoxyphenyl)-1*H*-imidazol-2-thiol (3i). The title compound was prepared by reaction of *meta*-methoxyaniline (356 mg, 2.90 mmol), 4-methoxyphenacyl bromide (658 mg, 2.90 mmol) and triethylamine (0.37 mL, 2.90 mmol), potassium thiocyanate (282 mg, 2.90 mmol) according to the procedure reported above. The product was purified by CC (hexane/ethyl acetate 9:1); yield: 16% (140 mg); white powder; mp: 250 °C; ^{1}H NMR ($CDCl_3$): 7.51 (d, *J* = 8.50 Hz, 2H), 7.38 (t, *J* = 7.80 Hz, 1H), 7.27 (s, 1H), 7.18 (d, *J* = 7.80 Hz, 1H), 7.02 (m, 1H), 6.97 (dd, *J* = 2.50 and 8.50 Hz, 1H), 6.89 (d, *J* = 8.50 Hz, 2H), 3.84 (s, 3H, OMe), 3.79 (s, 3H, OMe); ^{13}C NMR ($CDCl_3$): 188.00, 160.00, 129.95, 126.50, 118.00, 114.65 (2C), 111.80, 55.60, 55.35; IR: 3055, 1601, 1455, 1181, 825 cm^{-1}; MS (ESI): 313 $(M+H)^+$.

7.1.2. 4-(4-Methoxyphenyl)-1-(3-methoxyphenyl)-1*H*-imidazole (6i)

The title compound was prepared by reaction of sodium nitrite (11 mg, 0.16 mmol, 1 equiv), nitric acid (152 μL, 87 μmol, 0.5 equiv) and 1-(3-methoxyphenyl)-4-(4-methoxyphenyl)-1*H*-imidazol-2-thiol (**3i**) (150 mg, 0.48 mmol, 3 equiv) mixed in glacial acetic acid (15 mL) at 0 °C and stirred for 20 min. The reaction was quenched with ammonium hydroxide (20 mL) and the resulting precipitate was filtered, dried overnight in a desiccator. The crude product was purified by CC (ethyl acetate/methanol 98:2); yield: 48% (22 mg); yellow powder; mp: 246 °C; ^{1}H NMR ($CDCl_3$): 8.90 (s, 1H) 7.60 (s, 1H), 7.53 (s, 1H), 7.48 (m, 3H), 7.32 (t, *J* = 7.80 Hz, 1H), 7.02 (d, *J* = 8.50 Hz, 2H), 6.99 (dd, *J* = 1.80 and 8.20 Hz, 1H), 3.95 (s, 3H, OMe), 3.85 (s, 3H, OMe); ^{13}C NMR ($CDCl_3$): 161.15, 160.55, 136.15, 133.10, 130.20, 127.20, 123.85, 117.90, 117.15, 115.85, 110.50, 56.25, 55.60; IR: 3200, 2966, 1520, 1255, 855 cm^{-1}; MS (ESI): 281 $(M+H)^+$.

7.1.3. 3-[4-(4-Methoxyphenyl)-1*H*-1,2,3-triazol-1-yl]phenol (8i)

3-Azidophenol (**8ii**) (500 mg, 3.70 mmol, 1 equiv) and 1-ethynyl-4-methoxybenzene (489 mg, 3.70 mmol, 1 equiv) were stirred in a mixture of water/*tert*-butanol (1:1, 20 mL) for 2 min. Copper(II)sulfate (9.24 mg, 0.04 mmol, 0.01 equiv) was suspended in a freshly prepared solution of sodium ascorbate (1 M, 73.7 mg, 0.37 mmol, 0.1 equiv) and added dropwise to the latter mixture. The reaction mixture was stirred at 60 °C for 24 h. After cooling to rt, the mixture was washed with water and the aqueous layer washed with ethyl acetate. The combined organic layers were dried over sodium sulfate and solvent was removed under reduced pressure. The crude product was purified by CC (ethyl acetate/methanol 9:1); yield: 37% (350 mg); yellow powder; ^{1}H NMR (CD_3COCD_3): 8.80 (s, 1H) 7.91 (d, *J* = 8.80 Hz, 2H), 7.46 (s, 1H), 7.41 (d, *J* = 7.90 Hz, 2H), 7.04 (d, *J* = 8.80 Hz, 2H), 6.96 (m, 1H), 3.85 (s, 3H, OMe); ^{13}C NMR (CD_3COCD_3): 170.90, 159.45, 131.60, 127.80 (2C), 118.50, 116.30, 115.10 (2C), 111.80, 108.10, 55.65; IR: 3148, 2928, 1614, 1499, 1257, 833 cm^{-1}.

7.1.4. General synthesis procedure for compounds 9i–11i

Benzamides **9ii**–**11ii** (1 equiv) were refluxed in acetic acid (10 mL) with ammonium acetate (8 equiv) for 2 h. Solvent was evaporated under reduced pressure and the crude product was suspended in a mixture of ethanol/water/dichloromethane (1:1:1). The dichloromethane layer was separated, washed with brine, dried over magnesium sulfate, evaporated under reduced pressure and purified by CC.

7.1.4.1. 2-(4-Methoxyphenyl)-5-(3-methoxyphenyl)-1*H*-imidazole (9i). The title compound was prepared by reaction of 3-methoxy-*N*-[2-(4-methoxyphenyl)-2-oxo-ethyl]benzamide (**9ii**) (917 mg, 3.20 mmol) and ammonium acetate (1.90 g, 25.6 mmol) according to the procedure reported above. The product was purified by CC (hexane/ethyl acetate 5:5); yield: 25% (224 mg); white powder; mp: 198 °C; ^{1}H NMR (CD_3SOCD_3): 8.07 (d, J = 2.50 Hz, 1H), 7.98 (d, J = 8.50 Hz, 2H), 7.78 (d, J = 8.50 Hz, 1H), 7.57 (s, 1H), 7.37 (s, 1H), 7.12–7.08 (m, 2H), 6.75 (s, 1H), 3.83 (s, 3H, OMe), 3.82 (s, 3H, OMe); ^{13}C NMR (CD_3SOCD_3): 162.10, 160.85, 151.40, 137.50, 128.65 (2C), 127.25, 125.40, 123.35, 120.75, 115.75 (2C), 113.70, 56.80, 56.50; IR: 3070, 2950, 1578, 1242, 742 cm^{-1}; MS (ESI): 281 (M+H)$^+$.

7.1.4.2. 2-(3-Methoxyphenyl)-5-(4-methoxyphenyl)-1*H*-imidazole (11i). The title compound was prepared by reaction of 3-methoxy-*N*-[2-(4-methoxyphenyl)-2-oxo-ethyl]benzamide (**11ii**) (917 mg, 3.20 mmol) and ammonium acetate (1.90 g, 25.6 mmol) according to the procedure reported above. The product was purified by CC (hexane/ethyl acetate 5:5); yield: 6% (54 mg); white powder; mp: 202 °C; ^{1}H NMR ($CDCl_3$): 8.04 (s, 1H), 7.85 (d, J = 8.20 Hz, 2H), 7.28–7.24 (m, 3H), 6.88 (d, J = 8.20 Hz, 2H), 6.78 (dq, J = 1.50 and 7.60 Hz, 1H), 3.79 (s, 3H, OMe), 3.77 (s, 3H, OMe); ^{13}C NMR ($CDCl_3$): 164.00, 132.35 (2C), 130.15, 120.60, 119.85, 113.75, 112.65, 55.60, 55.50; IR: 3077, 2965, 1678, 1468, 1240, 1031, 742 cm^{-1}; MS (ESI): 281 (M+H)$^+$.

7.1.5. 5-(4-Methoxyphenyl)-2-(3-methoxyphenyl)-1,3-oxazole (18i)

The title compound was prepared by reaction of 3-methoxy-*N*-[2-(4-methoxyphenyl)-2-oxo-ethyl]benzamide (**9ii**) (347 mg, 1.16 mmol, 1 equiv) and phosphorous oxychloride (12 mL, 0.89 mmol, 0.8 equiv) in pyridine (20 mL) and refluxed for 8 h. After cooling to rt, ethyl acetate (40 mL) was added and the crude material was poured into a saturated sodium hydrogencarbonate solution. The aqueous layer was extracted two times with ethyl acetate. The combined organic layers were washed with brine, dried over magnesium sulfate, evaporated under reduced pressure and purified by CC (hexane/ethyl acetate 5:5); yield: 36% (117 mg); yellow oil; ^{1}H NMR (CD_3COCD_3): 7.79 (d, J = 8.80 Hz, 2H), 7.70 (dt, J = 1.00 and 8.80 Hz, 1H), 7.64 (q, J = 1.00 Hz, 1H), 7.53 (s, 1H, H-oxazole), 7.44 (t, J = 7.90 Hz, 1H), 7.08 (m, 3H), 3.90 (s, 3H, OMe), 3.86 (s, 3H, OMe); ^{13}C NMR (CD_3COCD_3): 161.05, 160.95, 152.40, 130.95, 129.85, 126.65, 123.15, 121.65, 119.15, 116.95, 115.40, 111.85, 55.75, 55.70; IR: 2937, 1612, 1253, 1010, 872 cm^{-1}.

7.1.6. General synthesis procedure for compounds 12i and 13i (Method A)

A solution of hydrazine monohydrate (4 equiv) in glacial acetic acid (4 equiv) was added dropwise to the propenone intermediate **12ii** and **13ii** (1 equiv). The reaction mixture was heated at reflux for 24 h. After cooling to rt, the precipitate was filtered off. A mixture of water/ethyl acetate (1:1) was added to the filtrate. The combined organic layers were washed with brine, dried over magnesium sulfate, evaporated under reduced pressure and purified by CC.

7.1.6.1. 3-(4-Methoxyphenyl)-5-(3-methoxyphenyl)-1*H*-pyrazole (12i). The title compound was prepared by reaction of (2*E*)-1-(3-methoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (**12ii**) (250 mg, 0.93 mmol) and hydrazine monohydrate (212 μL, 3.72 mmol) according to described Method A. The product was purified by CC (hexane/ethyl acetate 5:5) followed by preparative TLC (dichloromethane/methanol 99:1); yield: 25% (60 mg); yellow oil; ^{1}H NMR ($CDCl_3$): 7.55 (d, J = 8.80 Hz, 2H), 7.21–7.19 (m, 2H), 7.18 (t, J = 7.80 Hz, 1H), 6.78–6.75 (m, 3H), 6.62 (s, 1H), 3.74 (s, 3H, OMe), 3.62 (s, 3H, OMe); ^{13}C NMR ($CDCl_3$): 159.85, 159.55, 129.70, 126.85, 126.85, 118.10, 114.15 (2C), 114.10, 110.50, 99.35, 55.20, 55.05; IR: 2933, 2837, 1601, 1439, 1250, 1033, 834 cm^{-1}.

7.1.7. General synthesis procedure for compounds 14i–16i (Method B)

Propenone intermediate (**12ii**, **13ii** and **16ii**, 1 equiv) was stirred at 0 °C in 5 mL dry diethylether and bromine (1 equiv) was added dropwise. After 1 h at 0 °C, the reaction was warmed up to rt, precipitate was filtered and washed with diethylether. The resulting di-brominated propenone was obtained in quantitative yield and used without further purification. The latter (1 equiv) was refluxed in 10 mL absolute ethanol with 1 equiv hydroxylamine hydrochloride and 1 equiv potassium hydroxide for 24 h. After cooling to rt, the mixture was poured into a cold water solution. The resulting precipitate was filtered, washed with cold water, dried overnight in a desiccator and purified by CC.

7.1.7.1. 5-(3-Methoxyphenyl)-3-(4-methoxyphenyl)isoxazole (16i). The title compound was prepared by reaction of (2*E*)-3-(3-methoxyphenyl)-1-(4-methoxyphenyl)prop-2-en-1-one (**16ii**) (275 mg, 1.03 mmol), bromine (52 μL, 1.03 mmol), hydroxylamine hydrochloride (71.6 mg, 1.03.mmol) and potassium hydroxide (57.8 mg, 1.03 mmol) according to Method B. The product was purified by CC (hexane/ethyl acetate 9:1) followed by preparative TLC (dichloromethane/methanol 1%); yield: 45% (130 mg); yellow powder; mp: 161 °C; ^{1}H NMR ($CDCl_3$): 7.20 (m, 2H), 6.75 (dd, J = 2.00 and 7.50 Hz, 1H), 6.80 (d, J = 7.50 Hz, 1H), 6.76 (s, 1H), 6.74 (s, 1H), 6.70 (m, 1H), 6.45 (d, J = 7.50 Hz, 2H), 3.72 (s, 3H, OMe), 3.56 (s, 3H, OMe); ^{13}C NMR ($CDCl_3$): 171.65, 164.15, 162.40, 161.25, 131.80, 131.20, 128.70 (2C), 121.60, 120.60, 117.35, 115.70, 113.00, 97.50; IR: 2925, 2853, 1602, 1248, 746 cm^{-1}.

7.1.8. 4-(4-Methoxyphenyl)-2-(3-methoxyphenyl)-1,3-oxazole (19i)

4-Methoxyacetophenone (500 mg, 3.33 mmol, 1 equiv) was refluxed for 2 h together with [hydroxy(2,4-dinitrobenzensulfonyloxy)-iodo]benzene (1.88 g, 3.99 mmol, 1.2 equiv) in acetonitrile (20 mL). After cooling to rt, 3-methoxybenzamide (1.52 g, 9.99 mmol, 3 equiv) was added and the reaction mixture was refluxed for 10 h. Acetonitrile was evaporated under reduced pressure. The crude product was suspended in dichloromethane. The resulting organic layer was washed with a saturated sodium bicarbonate solution and dried over magnesium sulfate. Solvent was removed under reduced pressure and the product was purified by CC (hexane/ethyl acetate 7:3); yield 50% (465 mg); white powder; mp: 165 °C; ^{1}H NMR (CD_3COCD_3): 8.23 (s, 1H, H-oxazole), 7.90 (d, J = 9.20 Hz, 2H), 7.32 (m, 2H), 7.20 (t, J = 7.50 Hz, 1H), 6.93 (d, J = 9.20 Hz, 2H), 6.76 (m, 1H), 3.73 (s, 3H, OMe), 3.70 (s, 3H, OMe); IR: 3015, 2925, 1625, 789 cm^{-1}.

7.1.9. Ether cleavage—General procedure for compounds 1–6 and 9–11

To a solution of bis (methoxyphenyl) derivative (1 equiv) in dry dichloromethane was added dropwise boron trifluoride dimethyl

sulfide complex (75 equiv). The reaction mixture was stirred at rt for 20 h. To quench the reaction, water was added and the aqueous layer was washed with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, evaporated under reduced pressure and purified by preparative TLC.

7.1.9.1. 3-[1-(4-Hydroxyphenyl)-2-sulfanyl-1*H*-imidazol-4-yl]-phenol (1). The title compound was prepared by reaction of 4-(3-methoxyphenyl)-1-(4-methoxyphenyl)-1*H*-imidazol-2-thiol (**1i**) (100 mg, 0.32 mmol) according to the procedure reported above. The product was purified by preparative TLC (ethyl acetate); yield: 61% (55 mg); orange powder; mp: 265 °C; ^{1}H NMR (CD_3SOCD_3): 12.76 (s, 1H, SH), 7.64 (s, 1H), 7.39 (d, J = 8.50 Hz, 2H), 7.19–7.15 (m, 2H), 7.09 (s, 1H), 6.84 (d, J = 8.50 Hz, 2H), 6.71–6.69 (m, 1H); ^{13}C NMR (CD_3SOCD_3): 162.30, 157.60, 156.85, 129.90, 129.20, 128.95, 127.15, 116.15, 115.10 (2C), 114.85, 111.10; IR: 3214, 1604, 1514, 1395, 1101, 833, 750 cm^{-1}; MS (APCI): 284 (M)$^+$; Anal. Calcd $C_{15}H_{12}N_2O_2S$: C, 63.36; H, 4.25; N, 9.85. Found: C, 63.20; H, 3.99; N, 9.80.

7.1.9.2. 4,4′-(2-Sulfanyl-1*H*-imidazol-1,4-diyl)diphenol (2). The title compound was prepared by reaction of 1,4-bis(4-methoxyphenyl)-1*H*-imidazol-2-thiol (**2i**) (100 mg, 0.32 mmol) according to the procedure reported above. The product was purified by preparative TLC (ethyl acetate); yield: 36% (35 mg); white powder; mp: 268 °C; ^{1}H NMR (CD_3OD): 7.49 (d, J = 8.80 Hz, 2H) 7.42 (d, J = 8.80 Hz, 2H), 7.34 (s, 1H), 6.92 (d, J = 8.80 Hz, 2H), 6.87 (d, J = 8.80 Hz, 2H); ^{13}C NMR (CD_3OD): 162.00, 159.05, 158.80, 131.20, 131.10, 131.00, 128.55, 127.30, 120.55, 116.90, 116.50, 115.95; IR: 3135, 2469, 2072, 1511, 1116, 973, 836 cm^{-1}; MS (APCI): 284 (M)$^+$, 285 (M+H)$^+$; Anal. Calcd $C_{15}H_{12}N_2O_2S$: C, 63.36; H, 4.25; N, 9.85. Found: C, 63.40; H, 4.24; N, 9.95.

7.1.9.3. 3-[4-(4-Hydroxyphenyl)-2-sulfanyl-1*H*-imidazol-1-yl]-phenol (3). The title compound was prepared by reaction of 1-(3-methoxyphenyl)-4-(4-methoxyphenyl)-1*H*-imidazol-2-thiol (**3i**) (100 mg, 0.32 mmol) according to the procedure reported above. The product was purified by preparative TLC (ethyl acetate); yield: 37% (40 mg); yellow powder; ^{1}H NMR (CD_3SOCD_3): 12.75 (s, 1H, SH), 7.62 (s, 1H), 7.40 (d, J = 8.50 Hz, 2H), 7.18–7.13 (m, 2H), 7.07 (s, 1H), 6.83 (d, J = 8.50 Hz, 2H), 6.69–6.66 (m, 1H); ^{13}C NMR (CD_3SOCD_3): 162.35, 157.65, 156.95, 129.85, 129.00, 128.90, 127.20, 116.20 (2C), 115.05, 114.90, 111.25; IR: 3213, 1600, 1514, 1392, 1100, 845, 750 cm^{-1}; MS (APCI): 284 (M)$^+$; Anal. Calcd $C_{15}H_{12}N_2O_2S$: C, 63.36; H, 4.25; N, 9.85. Found: C, 63.12; H, 4.22; N, 9.84.

7.1.9.4. 3-[1-(4-Hydroxyphenyl)-1*H*-imidazol-4-yl]phenol (4). The title compound was prepared by reaction of 4-(3-methoxyphenyl)-1-(4-methoxyphenyl)-1*H*-imidazole (**4i**) (100 mg, 0.36 mmol) according to the procedure reported above. The product was purified by preparative TLC (ethyl acetate); yield: 28% (25 mg); yellow oil; ^{1}H NMR (CD_3COCD_3): 9.32 (d, J = 1.20 Hz, 1H), 8.33 (d, J = 1.20 Hz, 1H), 7.70 (dd, J = 2.20 and 8.80 Hz, 2H), 7.36–7.33 (m, 2H), 7.29 (t, J = 1.90 Hz, 1H), 7.06 (dd, J = 2.20 and 8.80 Hz, 2H), 6.99 (m, 1H); ^{13}C NMR (CD_3COCD_3): 159.70, 158.95, 134.95, 131.60, 129.10, 128.10, 124.95, 118.25, 117.95, 117.80, 117.35, 113.35; IR: 3563, 1684, 1629, 1048, 836 cm^{-1}; MS (ESI): 253 (M+H)$^+$; Anal. Calcd $C_{15}H_{12}N_2O_2$: C, 71.42; H, 4.79; N, 11.10. Found: C, 71.11; H, 4.62; N, 11.01.

7.1.9.5. 4,4′-bis(1*H*-Imidazol-1,4-diyl)-diphenol (5). The title compound was prepared by reaction of 1,4-bis(4-methoxyphenyl)-1*H*-imidazole (**5i**) (100 mg, 0.36 mmol) according to the procedure reported above. The product was purified by preparative TLC (ethyl acetate), yield: 26% (24 mg); yellow powder; ^{1}H NMR (CD_3COCD_3): 9.43 (d, J = 1.50 Hz, 1H), 8.32 (d, J = 1.50 Hz, 1H), 7.74 (dd, J = 2.20 and 8.80 Hz, 2H), 7.71 (dd, J = 2.20 and 8.80 Hz, 2H), 7.10 (dd, J = 2.20 and 8.80 Hz, 2H), 7.08 (dd, J = 2.20 and 8.80 Hz, 2H); ^{13}C NMR (CD_3COCD_3): 160.40, 128.70 (2C), 125.30, 117.70 (2C), 117.45 (2C), 117.25; IR: 3563, 3155, 1684, 1048, 931, 836 cm^{-1}; MS (ESI): 253 (M+H)$^+$; Anal. Calcd $C_{15}H_{12}N_2O_2$: C, 71.42; H, 4.79; N, 11.10. Found: C, 71.43; H, 4.85; N, 11.11.

7.1.9.6. 3-[4-(4-Hydroxyphenyl)-1*H*-imidazol-4-yl]phenol (6). The title compound was prepared by reaction of 4-(4-methoxyphenyl)-1-(3-methoxyphenyl)-1*H*-imidazole (**6i**) (100 mg, 0.36 mmol) according to the procedure reported above. The product was purified by preparative TLC (ethyl acetate); yield: 26% (24 mg); yellow oil; ^{1}H NMR (CD_3COCD_3): 9.50 (d, J = 1.50 Hz, 1H), 8.40 (d, J = 1.50 Hz, 1H), 7.77 (m, 2H), 7.50 (t, J = 8.20 Hz, 1H), 7.36–7.34 (m, 2H), 7.16 (dd, J = 2.20 and 8.80 Hz, 1H), 7.04 (dt, J = 2.20 and 8.20 Hz, 2H); ^{13}C NMR (CD_3COCD_3): 137.45, 132.50, 128.80 (2C), 118.35, 117.50, 114.35, 110.70; IR: 3542, 3160, 2955, 1699, 1630, 1062, 841 cm^{-1}; MS (ESI): 253 (M+H)$^+$; Anal. Calcd $C_{15}H_{12}N_2O_2$: C, 71.42; H, 4.79; N, 11.10. Found: C, 71.59; H, 4.92; N, 10.97.

7.1.9.7. 3-[2-(4-Hydroxyphenyl)-1*H*-imidazol-5-yl]phenol (9). The title compound was prepared by reaction of 2-(4-methoxyphenyl)-5-(3-methoxyphenyl)-1*H*-imidazole (**9i**) (40 mg, 0.14 mmol) according to the procedure reported above. The product was purified by preparative TLC (hexane/ethyl acetate 5:5); yield: 45% (15 mg); yellow powder; ^{1}H NMR (CD_3COCD_3): 8.58 (s, 1H), 7.42 (t, J = 7.80 Hz, 2H), 7.40 (m, 1H), 7.33 (m, 1H), 7.27 (t, J = 7.80 Hz, 2H), 7.05 (dd, J = 0.90 and 1.50 Hz, 1H), 6.90 (dd, J = 0.90 and 1.50 Hz, 1H), 6.47 (s, 1H, NH); ^{13}C NMR (CD_3COCD_3): 168.95, 168.90, 158.25, 137.90, 136.95, 130.10 (2C), 119.40, 119.10, 118.70, 115.25; IR: 3450, 2950, 1604, 1580, 785 cm^{-1}. MS (ESI): 253 (M+H)$^+$; Anal. Calcd $C_{15}H_{12}N_2O_2$: C, 71.42; H, 4.79; N, 11.10. Found: C, 71.23; H, 4.64; N, 10.98.

7.1.9.8. 4,4′-(1*H*-Imidazol-2,5-diyl)diphenol (10). The title compound was prepared by reaction of 2,5-bis(4-methoxyphenyl)-1*H*-imidazole (**10i**) (82 mg, 0.29 mmol) according to the procedure reported above. The product was purified by preparative TLC (dichloromethane/methanol 99:1); yield: 17% (12 mg); yellow powder; ^{1}H NMR (CD_3OD): 7.83 (d, J = 8.70 Hz, 2H), 7.62 (d, J = 8.70 Hz, 2H), 7.60 (s, 1H), 7.03 (d, J = 8.70 Hz, 2H), 6.92 (d, J = 8.70 Hz, 2H); ^{13}C NMR (CD_3OD): 131.30, 129.15, 120.15 (2C), 119.80 (2C), 115.55 (2C), 114.75 (2C), 114.30; IR: 2590, 1645, 1488, 1114, 841 cm^{-1}; MS (ESI): 253 (M+H)$^+$; Anal. Calcd $C_{15}H_{12}N_2O_2$: C, 71.42; H, 4.79; N, 11.10. Found: C, 71.30; H, 4.52; N, 11.00.

7.1.9.9. 3-[5-(4-Hydroxyphenyl)-1*H*-imidazol-2-yl]phenol (11). The title compound was prepared by reaction of 2-(3-methoxyphenyl)-5-(4-methoxyphenyl)-1*H*-imidazole (**11i**) (40 mg, 0.14 mmol) according to the procedure reported above. The product was purified by preparative TLC (hexane/ethyl acetate 5:5); yield: 45% (15 mg); yellow powder; ^{1}H NMR (CD_3COCD_3): 8.56 (s, 1H), 7.40 (t, J = 7.80 Hz, 2H), 7.39 (m, 1H), 7.37 (m, 1H), 7.25 (t, J = 7.80 Hz, 2H), 6.99 (dd, J = 0.90 and 1.50 Hz, 1H), 6.97 (dd, J = 0.90 and 1.50 Hz, 1H), 6.47 (s, 1H, NH); ^{13}C NMR (CD_3COCD_3): 168.95, 168.90, 158.25, 137.95, 136.90, 130.15 (2C), 119.30 (2C), 118.95, 115.40; IR: 3350, 3045, 2922, 1664, 1582, 760 cm^{-1}; MS (ESI): 253 (M+H)$^+$; Anal. Calcd $C_{15}H_{12}N_2O_2$: C, 71.42; H, 4.79; N, 11.10. Found: C, 71.42; H, 4.89; N, 11.08.

7.1.10. Ether cleavage—General synthesis for compounds 12, 18 and 19

To a solution of bis(methoxyphenyl) derivative (1 equiv) in dry dichloromethane at −78 °C (dry ice/acetone bath), boron tribromide (1 M in dichloromethane, 6 equiv) was added dropwise and

the reaction mixture was stirred for 20 h. To quench the reaction, water was added and the aqueous layer was washed with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, evaporated under reduced pressure and purified by preparative TLC.

7.1.10.1. 3-[3-(4-Hydroxyphenyl)-1*H*-pyrazol-5-yl]phenol (12). The title compound was prepared by reaction of 3-(4-methoxyphenyl)-5-(3-methoxyphenyl)-1*H*-pyrazole (**12i**) (82 mg, 0.29 mmol) according to the procedure reported above. The product was purified by preparative TLC (dichloromethane/methanol 92:8); yield: 55% (40 mg); orange powder; mp: 262 °C; ^{1}H NMR (CD_3OD): 7.65 (d, J = 8.50 Hz, 2H), 7.22 (m, 1H), 6.83 (d, J = 8.50 Hz, 2H), 6.81 (s, 1H), 6.72–6.74 (m, 3H); ^{13}C NMR (CD_3OD): 160.50, 131.85, 122.10 (2C), 117.05, 116.80, 116.00, 113.30 (2C), 102.15; IR: 3500, 2935, 1620, 790 cm^{-1}; MS (ESI): 253 $(M+H)^+$; Anal. Calcd $C_{15}H_{12}N_2O_2$. C, 71.42; H, 4.79; N, 11.10. Found: C, 71.38; H, 4.71; N, 11.25.

7.1.10.2. 3-[5-(4-Hydroxyphenyl)-1,3-oxazol-2-yl]phenol (18). The title compound was prepared by reaction of 5-(4-methoxyphenyl)-2-(3-methoxyphenyl)-1,3-oxazole (**18i**) (100 mg, 0.35 mmol) according to the procedure reported above. The product was purified by preparative TLC (hexane/ethyl acetate 5:5); yield: 65% (59 mg); yellow powder; ^{1}H NMR (CD_3COCD_3): 8.80 (s, 1H, OH), 8.75 (s, 1H, OH), 7.69 (d, J = 8.20 Hz, 2H), 7.60 (m, 2H), 7.46 (s, 1H, H-oxazole), 7.35 (t, J = 8.20 Hz, 1H), 6.98–6.95 (m, 3H); ^{13}C NMR (CD_3COCD_3): 160.90, 158.95, 158.75, 152.55, 130.95, 129.80, 126.80, 122.50, 120.65, 118.25 (2C), 118.15, 116.85, 115.40, 113.55; IR: 3480, 1602, 1510, 852 cm^{-1}; MS (ESI): 254 $(M+H)^+$; Anal. Calcd $C_{15}H_{11}NO_3$: C, 71.14; H, 4.38; N, 5.53. Found: C, 70.92; H, 4.35; N, 5.60.

7.1.10.3. 3-[4-(4-Hydroxyphenyl)-1,3-oxazol-2-yl]phenol (19). The title compound was prepared by reaction of 4-(4-methoxyphenyl)-2-(3-methoxyphenyl)-1,3-oxazole (**19i**) (100 mg, 0.36 mmol) according to the procedure reported above. The product was purified by preparative TLC (hexane/ethyl acetate 5:5); yield: 82% (74 mg); yellow powder; ^{1}H NMR (CD_3COCD_3): 8.27 (s, 1H, H-oxazole), 7.93 (d, J = 8.50 Hz, 2H), 7.37 (s, 1H), 7.33 (d, J = 7.60 Hz, 1H), 7.20 (t, J = 7.60 Hz, 1H), 6.97 (d, J = 8.50 Hz, 2H), 6.79 (m, 1H); ^{13}C NMR (CD_3COCD_3): 161.80, 159.70, 157.75, 141.55, 133.70, 132.90, 129.70, 128.05, 119.25, 116.70, 115.75, 114.90, 112.35; IR: 3300, 1595, 1259, 804 cm^{-1}; MS (ESI): 252 $(M-H)^-$; Anal. Calcd $C_{15}H_{11}NO_3$: C, 71.14; H, 4.38; N, 5.53. Found: C, 71.00; H, 4.48; N, 5.58.

7.1.11. 4,4′-(1,3-Oxazol-2,5-diyl)diphenol (17)

The title compound was prepared by reaction of 2,5-bis(4-methoxyphenyl)-oxazole (**17i**) (260 mg, 0.90 mmol, 1 equiv) and pyridinium hydrochloride (2.90 g, 25.7 mmol, 37 equiv) heated to 220 °C for 18 h. After cooling to rt, water (10 mL) and ethyl acetate (20 mL) were added. The aqueous layer was washed with ethyl acetate. The combined organic layers were dried over sodium sulfate, filtered, evaporated under reduced pressure and purified by preparative TLC (hexane/ethyl acetate 5/5); yield: 82% (186 mg), yellow powder; mp: 163 °C; ^{1}H NMR (CD_3OD): 7.89 (d, J = 7.80 Hz, 2H), 7.60 (d, J = 8.80 Hz, 2H), 7.32 (s, 1H), 6.91–6.86 (m, 4H); ^{13}C NMR (CD_3OD): 162.35, 161.30, 159.35, 152.78, 132.80, 129.00 (2C), 126.80 (2C), 125.80 (2C), 116.90 (2C); IR: 3387, 1611, 1506, 1170, 834 cm^{-1}; MS (ESI): 254 $(M+H)^+$; Anal. Calcd $C_{15}H_{11}NO_3$: C, 71.14; H, 4.38; N, 5.53. Found: C, 71.02; H, 4.18; N, 5.63.

7.2. Biological methods

[2,4,6,7-^{3}H]-E2 and [2,4,6,7-^{3}H]-E1 were bought from Perkin Elmer, Boston. Quickszint Flow 302 scintillator fluid was bought from Zinsser Analytic, Frankfurt.

17β-HSD1 and 17β-HSD2 were obtained from human placenta according to previously described procedures.[40,73] Fresh human placenta was homogenised and the enzymes were separated by centrifugation. For the purification of 17β-HSD1, the cytosolic fraction was precipitated with ammonium sulfate. 17β-HSD2 was obtained from the microsomal fraction.

7.2.1. Inhibition of 17β-HSD1

Inhibitory activities were evaluated by an established method with minor modifications.[54,74,75] Briefly, the enzyme preparation was incubated with NADH [500 μM] in the presence of potential inhibitors at 37 °C in a phosphate buffer (50 mM) supplemented with 20% of glycerol and EDTA 1 mM. Inhibitor stock solutions were prepared in DMSO. The final concentration of DMSO was adjusted to 1% in all samples. The enzymatic reaction was started by addition of a mixture of unlabelled- and [2,4,6,7-^{3}H]-E1 (final concentration: 500 nM, 0.15 μCi). After 10 min, the incubation was stopped with $HgCl_2$ and the mixture was extracted with diethylether. After evaporation, the steroids were dissolved in acetonitrile. E1 and E2 were separated using acetonitrile/water (45:55) as mobile phase in a C18 reverse phase chromatography column (Nucleodur C18 Gravity, 3 μm, Macherey-Nagel, Düren) connected to a HPLC-system (Agilent 1100 Series, Agilent Technologies, Waldbronn). Detection and quantification of the steroids were performed using a radioflow detector (Berthold Technologies, Bad Wildbad). The conversion rate was calculated after analysis of the resulting chromatograms according to following equation: $\%\text{conversion} = \frac{AUC(E2)}{AUC(E2)+AUC(E1)} \times 100$. Each value was calculated from at least three-independent experiments.

7.2.2. Inhibition of 17β-HSD2

The 17β-HSD2 inhibition assay was performed similarly to the 17β-HSD1 procedure. The microsomal fraction was incubated with NAD^+ [1500 μM], test compound and a mixture of unlabelled- and [2,4,6,7-^{3}H]-E2 (final concentration: 500 nM, 0.11 μCi) for 20 min at 37 °C. Further treatment of the samples and HPLC separation was carried out as mentioned above. The conversion rate was calculated after analysis of the resulting chromatograms according to following equation: $\%\text{conversion} = \frac{AUC(E1)}{AUC(E1)+AUC(E2)} \times 100$.

7.2.3. ER affinity

The binding affinity of select compounds to the ERα and ERβ was determined according to Zimmermann et al.[76] Briefly, 0.25 pmol of ERα or ERβ, respectively, was incubated with [2,4,6,7-^{3}H]-E2 (10 nM) and test compound for 1 h at room temperature. The potential inhibitors were dissolved in DMSO (5% final concentration). Non-specific-binding was performed with diethylstilbestrol (10 μM). After incubation, ligand–receptor complexes were selectively bound to hydroxyapatite (5 g/60 mL TE-buffer). The complex formed was separated, washed and resuspended in ethanol. For radiodetection, scintillator cocktail (Quickszint 212, Zinsser Analytic, Frankfurt) was added and samples were measured in a liquid scintillation counter (Rack Beta Primo 1209, Wallac, Turku). For determination of the relative binding affinity (RBA), inhibitor and E2 concentrations required to displace 50% of the receptor bound labelled E2 were determined. RBA values were calculated according to the following equation: $\text{RBA}[\%] = \frac{IC_{50}(E2)}{IC_{50}(\text{compound})} \times 100$. The RBA value for E2 was arbitrarily set at 100%.

7.2.4. T-47D cell assays

7.2.4.1. Evaluation of the estrogenic activity. Phenol red-free medium was supplemented with sodium bicarbonate (2 g/L), streptomycin (100 μg/mL), insulin zinc salt (10 μg/mL), sodium pyruvate (1 mM), L-glutamine (2 mM), penicillin (100 U/mL) and DCC-FCS 5% (vol/vol). RPMI 1640 (without phenol red) was used

for the experiments. Cells (7500 cells/96-well plate) were grown for 48 h in phenol red-free medium. Compound **18** was added at a final concentration of 100 nM. Inhibitors and E2 were diluted in ethanol (final ethanol concentration was adjusted to 1%). As a positive control E2 was added at a final concentration of 0.1 nM. Ethanol was used as negative control. Medium was changed every 2–3 days and supplemented with the respective additives. After 8 days of incubation, the cell viability was evaluated measuring the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazoliumbromide (MTT). The cleavage of MTT to a blue formazan by mitochondrial succinat-dehydrogenase was quantified spectrophotometrically at 590 nm as described by Denizot and Lang[77] with minor modifications. The control proliferation was arbitrarily set at 1 and the stimulation induced by the inhibitor was calculated according to following equation: $\%\text{stimulation} = \frac{[\text{proliferation(compound-induced)} - 1]}{[\text{proliferation(E2-induced)} - 1]} \times 100\%$. Each value is calculated as a mean value of at least three independent experiments.

7.2.4.2. Evaluation of the 17β-HSD1 activity. A stock culture of T-47D cells was grown in RPMI 1640 medium supplemented with 10% FCS, L-glutamine (2 mM), penicillin (100 IU/mL), streptomycin (100 μg/mL), insulin-zinc-salt (10 μg/mL) and sodium pyruvate (1 mM) at 37 °C under 5% CO_2 humidified atmosphere.

The cells were seeded into a 24-well plate at 1×10^6 cells/well in DMEM medium with FCS, L-glutamine and the antibiotics added in the same concentrations as mentioned above. After 24 h the medium was changed for fresh serum free DMEM and a solution of test compound in DMSO was added. Final concentration of DMSO was adjusted to 1% in all samples. After a pre-incubation of 30 min at 37 °C with 5% CO_2, the incubation was started by addition of a mixture of unlabelled- and [2,4,6,7-^{3}H]-E1 (final concentration: 50 nM, 0.15 μCi). After 2.5 h incubation, the enzymatic reaction was stopped by removing the supernatant medium. The steroids were extracted into diethylether. Further treatment of the samples was carried out as mentioned for the 17β-HSD1 assay.

7.2.5. CaCo-2 assay

CaCo-2 cell culture and transport experiments were performed according to Yee[78] with small modifications. Cell culture time was reduced from 21 to 10 days by increasing seeding density from 6.3×10^4–1.65×10^5 cells per well. Four reference compounds (atenolol, testosterone, ketoprofen, erythromycin) were used in each assay for validation. The compounds were applied to the cells as a mixture (cassette dosing) to increase the throughput. The initial concentration of the compounds in the donor compartment was 50 μM (0.2 M MES, pH 6.5, containing either 1% ethanol or DMSO). Samples were taken from the acceptor side after 0 min, 60, 120 and 180 min and from the donor side after 0 and 180 min. Each experiment was run in triplicate. The integrity of the monolayers was checked by measuring the transepithelial electrical resistance (TEER) before the transport experiments and by measuring lucifer yellow permeability after each assay. All samples of the CaCo-2 transport experiments were analysed by LC/MS/MS after dilution with buffer of the opposite transwell chamber (1:1, containing 2% acetic acid). The apparent permeability coefficients (P_{app}) were calculated using equation $Papp = \frac{dQ}{dt A c_0}$, where $\frac{dQ}{dt}$ is the appearance rate of mass in the acceptor compartment, A the surface area of the transwell membrane and c_0 the initial concentration in the donor compartment.

7.3. Molecular modelling

The X-ray structure of 17β-HSD1 (PDB-ID: 1FDT) was obtained from the Protein Data Bank.[44] Water molecules, E2 and sulfate ions were removed from the PDB file and hydrogen atoms and neutral end groups were added. Close contacts were fixed (Arg37) and correct atom types were set.

Docking of inhibitors into the substrate-binding site was performed by the automated docking program GOLD 3.0.[79]

Acknowledgments

We are grateful to the Deutsche Forschungsgemeinschaft (HA1315/8-1) for financial support of this work. We thank Dr. Christiane Scherer for performing the CaCo-2 cell test, Kerstin Esch, Anja Palusczak and Beate Geiger for their help in performing the in vitro tests (17β-HSD1, 17β-HSD2, ERα and ERβ) and Matthias Negri for discussions about molecular modelling. Patricia Kruchten is grateful to the European Postgraduate School 532 (DFG) for a scholarship.

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