Development of Potent and Selective Inhibitors of 17β-Hydroxysteroid Dehydrogenase Type 2 – A new Target for the Treatment of Osteoporosis

Dissertation

zur Erlangung des Grades
des Doktors der Naturwissenschaften
der Naturwissenschaftlich-Technischen Fakultät III
Chemie, Pharmazie, Bio- und Werkstoffwissenschaften
der Universität des Saarlandes

von

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Saarbrücken
2011

Day of oral dissertation defense: 12.12.2011

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Diese Arbeit entstand unter der Anleitung von Prof. Dr. R.W. Hartmann in der Fachrichtung 8.2 Pharmazeutische und Medizinische Chemie der Naturwissenschaftlich-Technischen Fakultät III der Universität des Saarlandes von September 2007 bis Juni 2011.

ACKNOWLEDGEMENTS

My deepest gratitude is to my supervisor, Prof. Dr. Rolf W. Hartmann, without who I will not be here. He has been always there to listen and give advice. I am also thankful to him for encouraging the use of correct grammar and consistent notation in my writings.

I would like to thank my official referee Prof. Dr. Gerhard Wenz for the reviewing of this dissertation.

I am deeply grateful to Dr. Sandrine Marchais-Oberwinkler for the long discussions that helped me to understand and enrich my results and my ideas and for carefully reading and commenting papers of this manuscript. Her comments and constructive criticisms were thought-provoking and helped me to focus my ideas.

I am also thankful to Dr. Martin Frotscher for his encouragement and practical advice.

I will also acknowledge all my HSD co-workers, especially Dr. Kuiying Xu, Ruth Werth, and Dr. Matthias Negri for the profitable collaboration, but also Dr. Emmanuel Bey and Dr. Enrico Perspicace for carefully reading this manuscript and for their sympathy.

I will warmly thank Dr. Stefan Boettcher for helping me to perform the mass experiments.

I am also grateful to Beate Geiger, Jannine Ludwig, Jeannine Jung, Martina Jackowski, Viktoria Kraemer and Laura Schröbildgen for performing the *in vitro* tests.

I would like to thank Dr. Matthias Negri, Dr. Simon Lucas, Tobias Klein and Christian Schmitt, my partners during the molecular modelling practical course.

I am also thankful to the laboratory staff, especially Corina Przybyla, Martina Schwarz, Katrin Schmitt, Lothar Jäger and Gerhard Müller for their sympathy and service.

I will to thank my undergraduate research participants, Sarah Pitsch and Emanuele Marco Gargano for their help in performing laboratory work.

Finally, none of this would have been possible without the love and patience of my family. My immediate family, to whom this dissertation is dedicated to, has been a constant source of love, concern, support and strength all these years. I would like to express my heart-felt gratitude to my family.

PAPERS INCLUDED IN THIS THESIS

The present thesis is divided into three publications which are referred to in the text by their Roman numerals:

I. 17β-HSD2 inhibitors for the treatment of osteoporosis: identification of a promising scaffold

Marie Wetzel, Sandrine Marchais-Oberwinkler and Rolf W. Hartmann

Bioorganic Medicinal Chemistry, **2011**, *19*, 807-815.

II. Introduction of an electron withdrawing group on the hydroxyphenylnaphthol scaffold improves the potency of 17β-hydroxysteroid dehydrogenase type 2 (17β-HSD2) inhibitors

Marie Wetzel, Sandrine Marchais-Oberwinkler, Enrico Perspicace, Gabriele Möller, Jerzy Adamski and Rolf W. Hartmann

Journal of Medicinal Chemistry, **2011**, submitted.

III. Discovery of a new class of bicyclic substituted hydroxyphenylmethanones as 17β-hydroxysteroid dehydrogenase type 2 (17β-HSD2) inhibitors for the treatment of osteoporosis

Marie Wetzel, Emanuele M. Gargano, Stefan Hinsberger, Sandrine Marchais-Oberwinkler and Rolf W. Hartmann

European Journal of Medicinal Chemistry, **2011**, accepted.

CONTRIBUTION REPORT

The author wishes to clarify her contributions to the papers **I–III** in the thesis.

I. Contributed to the inhibitor design concept. Planned, synthesized and characterized most of the new compounds. Compounds **1**-**4**, **6** and **8** were prepared by Erika Ziegler as a part of her PhD thesis. Significantly contributed to the interpretation of the results. Significantly contributed to writing of the manuscript.

II. Significantly contributed to the inhibitor design concept. Planned, synthesized and characterized all new compounds. Biological evaluation of compound **19** in rat, mouse and monkey enzymes. Significantly contributed to the interpretation of the results. Significantly contributed to writing of the manuscript.

III. Planned and characterized all new compounds. Synthesized most of the new compounds. Compounds **1**, **2**, **10**, **11**, **14** and compounds **4**, **7**, **8**, **9**, **12**, **13** were synthesised by Stefan Hinsberger and Emanuele M. Gargano, respectively as a part of their diploma thesis. Significantly contributed to the interpretation of the results. Significantly contributed to writing of the manuscript.

FURTHERS PUBLICATIONS OF THE AUTHOR, NOT INCLUDED IN THIS THESIS

- S. Marchais-Oberwinkler, M. Wetzel, E. Ziegler, P. Kruchten, R. Werth, R. W. Hartmann, M. Frotscher, New drug-like hydroxyphenylnaphthol steroidomimetics as potent and selective 17β-HSD1 inhibitors for the treatment of estrogen-dependent diseases, *J. Med. Chem.* **2011**, *54*, 534-547.

- K. Xu, M. Wetzel, R. W. Hartmann, S. Marchais-Oberwinkler, Synthesis and Biological Evaluation of Spiro-δ-lactones as Inhibitors of 17β-Hydroxysteroid Dehydrogenase Type 2 (17β-HSD2), *Lett. Drug Disc. Des.* **2011**, *8*, 406-421.

- S. Marchais-Oberwinkler, C. Henn, G. Möller, T. Klein, M. Negri, A. Oster, A. Spadaro, R. Werth, M. Wetzel, K. Xu, M. Frotscher, R. W. Hartmann, J. Adamski, 17β-Hydroxysteroid dehydrogenases (17β-HSD) as therapeutic targets: Protein structures, functions, and recent progress in inhibitor development, *J. Steroid Biochem. Mol. Biol.* **2011**, *125*, 66-82.

- K. Xu, Y. A. Al-Soud, M. Wetzel, R. W. Hartmann and S. Marchais-Oberwinkler, Ring opening of aryl sustituted triazoles leads to thiophene-2-carboxamides as a new class of potent nonsteroidal 17β-hydroxysteroid dehydrogenase type 2 inhibitors, *Eur. J. Med. Chem.* **2011**, submitted.

- K. Xu, M. Wetzel, S. Marchais-Oberwinkler, G. Möller, J. Adamski, R. W. Hartmann, *N*-methyl-*N*,5-diphenylthiophene-2-carboxamide as potent and specific inhibitors of 17β-Hydroxysteroid Dehydrogenase Type 2, manuscript in preparation, to be submitted in *J. Med. Chem.*

ABBREVIATIONS

17β-HSD	17β-hydroxysteroid dehydrogenase
17β-HSD2	17β-hydroxysteroid dehydrogenase type 2
A-dione	Δ^4-Androstenedione
AKR	aldo-keto reductase
ALP	alkaline phosphatase
AR	androgen receptor
BMD	bone mineral density
$CDCl_3$	deuterated chloroform
CD_3OD	deuterated methanol
DHEA-(S)	dehydroepiandrosterone-(sulfate)
DHT	5α-Dihydrotestosterone
DME	dimethoxyethane
DMEM	Dulbecco's modified eagle medium
DMF	dimethylformamide
E1	estrone
E2	17β-estradiol
ER	estrogen receptor
equiv	equivalent
Et	ethyl
FA	fatty acid
FCS	fetal calf serum
Hz	hertz
KO	knock-out
M-CSF	macrophage colony-stimulating factor
Me	methyl
MHz	megahertz
nM	nanomolar
NADP(H)	nicotinamide adenine dinucleotide phosphate
NAD(H)	nicotinamide adenine dinucleotide
OB	osteoblast
OC	osteoclast

OPG	osteoprotegerin
P	progesterone
Ph	phenyl
ppm	parts per million
PTH	parathyroid hormone
RANK(L)	receptor activator of nuclear factor κβ (ligand)
RBA	relative binding affinity
rt	room temperature
SAR	structure activity relationship
SDR	short dehydrogenase reductase
SERM	selective estrogen receptor modulator
T	testosterone
THF	tetrahydrofurane

ABSTRACT

Using a ligand-based approach, we designed and synthesised novel non-steroidal 17β-HSD2 inhibitors from two scaffolds described in literature as potential drugs for the treatment of osteoporosis. These compounds bear a hydrophobic core and two polar moieties, distant from each other circa 11Å, mimicking the two hydroxy group at the C3 and C17 position of the steroid E2. The best OH-OH substitution pattern was first elucidated in an optimisation process. Addition of further substituents to the best scaffold was investigated to enhance its activity and selectivity. Most of the synthesised compounds are potent 17β-HSD2 inhibitors with IC_{50} values in the low nanomolar range. Besides an outstanding selectivity towards 17β-HSD1 and a negligible affinity to the ERα and ERβ, the most promising compound of this thesis (**II.19**) displays high selectivity towards 17β-HSD4 and 17β-HSD5. It exhibits also good cell permeability in MDA-MB-231 cells, expressing naturally 17β-HSD2 and an excellent activity in rat, mouse and monkey enzymes.

To conclude, the present work presents a broad SAR study of two classes of compounds regarding 17β-HSD2 inhibition and all of these results helped to generate a good homology model of the protein with the goal to better understand how potential drug binds in the enzymatic pocket.

ZUSAMMENFASSUNG

Aus zwei in der Literatur beschriebenen Grundgerüsten und mit Hilfe eines Ligand-basierten Ansatzes, wurden neue nicht-steroidale 17β-HSD2 Hemmstoffe designet und synthetisiert, als potentielle Therapeutika für die Behandlung von Osteoporose. Diese Verbindungen besitzen ein hydrophobes Grundgerüst und zwei polare Reste in einem Abstand von 11Å, die die Positionen C3 und C17 von Estradiol nachahmen. Im ersten Optimierungsprozess wurde das optimale Substitutionsmuster für die zwei OH-Funktionen ermittelt. Zusätzlich wurde die Einführung von weiteren Substituenten in die beste Leitstruktur untersucht, um Aktivität und Selektivität zu erhöhen. Die meisten synthetisierten Verbindungen sind hochpotente 17β-HSD2 Inhibitoren mit IC_{50}-Werten im nanomolaren Bereich. Die beste Verbindung in dieser Doktorarbeit (**II.19**) besitzt eine sehr gute Selektivität gegenüber 17β-HSD1 sowie ein sehr geringe Affinität zum ERα und ERβ, und zeigt eine hohe Selektivität gegenüber 17β-HSD4 und 17β-HSD5. **II.19** weist auch gute Zellgängigkeit in MDA-MB-231 Zellen auf und eine exzellente Aktivität am Ratten-, Mäuse- und Affen-Enzym.

Die vorliegende Arbeit liefert einen breiten Einblick in die Struktur-Wirkungbeziehungen zweier neuer Klassen von 17β-HSD2-Hemmstoffen. Diese Ergebnisse haben dazu beigetragen, ein gutes Homologiemodell des Proteins zu entwickeln. Mit ihnen war es ferner möglich die Bindungstelle von potentiellen Therapeutika in der enzymatischen Bindetasche zu charakterisieren.

TABLE OF CONTENTS

1. INTRODUCTION

1.1. Sex steroid hormones

1.1.1. Androgens and estrogens

Sex steroid hormones are lipophilic compounds derived from cholesterol. Two main classes of sex steroid have been identified: androgens and estrogens. Androstenedione (A-dione), testosterone (T) and dihydrotestosterone (DHT) constitute the main androgens over the body and belong to the C19-steroids. After aromatisation of the A-ring of A-dione or T, estrone (E1) and estradiol (E2), characterised as C18-steroids are obtained as the principal estrogens.

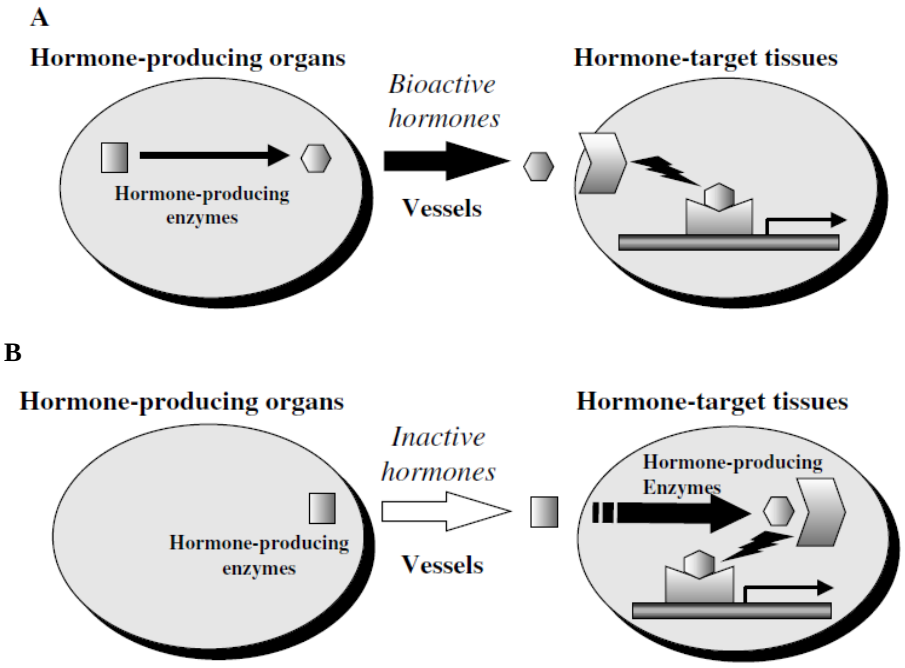
Androgens and estrogens are both essential for sexual differentiation and reproduction in men and women, respectively, but are not considered just as male and female hormones. Indeed, estrogens are also important to the development and maintenance of the functional integrity of the brain, as they modulate functions like memory, behaviour and neuronal protection[1]. Estrogens are crucial for maintening the equilibrium between bone formation and bone resorption[2]. Thus, after menopause, estrogen deficiency is responsible for the imbalance observed between bone resorption mediated by osteoclasts (OCs) and bone formation directed by osteoblasts (OBs). Furthermore, studies proved that estrogen deficiency may increase the risk of cardiovascular disease[3]. Regarding androgens, they increase mineralisation of the bone matrix [4] and are responsible for the muscle protein synthesis. Studies proved that supplementation of T in elderly men is beneficial for the muscle protein synthesis [5] and decrease the risk of muscle wasting.

1.1.2. Biosynthesis of androgens and estrogens

The gonads are the main source of biologically active androgens and estrogens in men and premenopausal women (endocrine mechanism, Figure 1A). Bioactive hormones are therefore transported through the blood into their target tissue, where they act. But other tissues including bone, brain, prostate and breast are also able to synthesise bioactive hormones over the body using inactive adrenal precursors (Figure 1B). Estrogens and androgens are therefore biologically activated from their inactive precursor directly in the local tissue, where the synthesis takes place, without being released in the blood. An intracrine mechanism (Figure 1B) is therefore refered to this concept. The concentrations in

sex steroids in the target tissues are adjusted by their local requirements. In addition to prostate and breast cancers, in which efficient blockade of sex steroids synthesis in peripheral tissues have proved their benefits[6,7], the intracrine approach in non-malignant diseases has been developed, as in the skin[8], for example.

Figure 1: Representation of endocrine (A) and intracrine (B) concept (taken from Suzuki T. *et al.* [9])



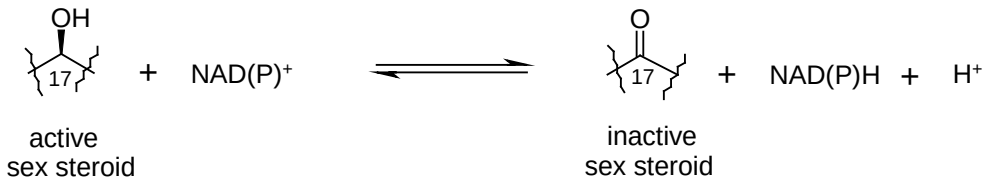
inactive hormone; bioactive hormone; receptor; promoter region of the target gene

1.2. 17β-HSDs

1.2.1. Generality

Most 17β-hydroxysteroid dehydrogenases (17β-HSD)[10] are essential enzymes involved in the last step of steroid hormone formation and degradation. They catalyse oxidation and reduction reactions at position C17 (Figure 2), using NAD(P)H or $NAD(P)^+$ as cofactor.

Figure 2: General pathway of reactions catalysed by 17β-HSDs

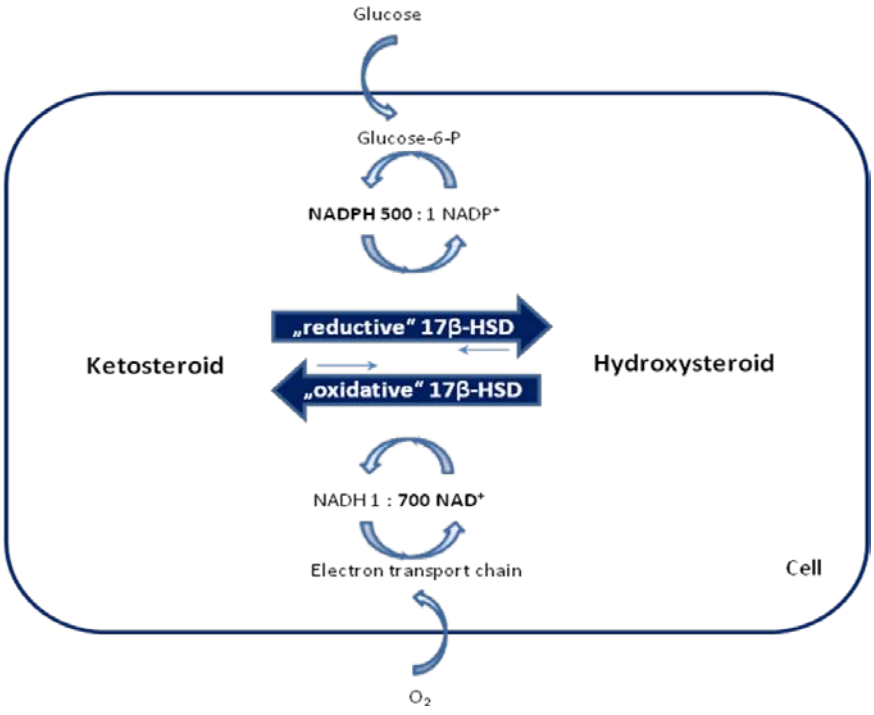


Until now, fourteen 17β-HSDs have been identified, but only twelve have been characterised in human (17β-HSD6 and 17β-HSD9 only in rodents). The numbering follows the chronological order of the first description of the enzyme designated. Most 17β-HSDs belong to short chain dehydrogenase/reductase (SDR) protein family, except 17β-HSD5, which is an aldo/ketoreductase (AKR) family member. 17β-HSDs from the SDR family share low sequence identity (25% - 30%) but they conserved all characteristics of the SDR family, *i.e.* the Rossmann fold implicated in cofactor binding.

1.2.2. Cofactor preference

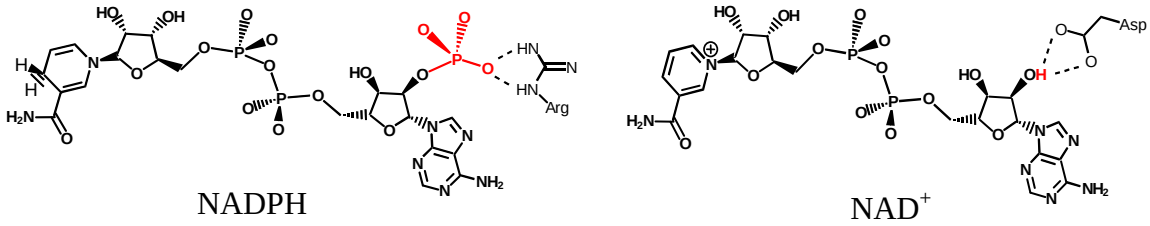
While 17β-HSDs are able to convert both oxidation and reduction reactions in cells, they only catalyse preferentially one type of reaction depending on two factors: the concentration of the cofactor present in cells and the preference of the cofactor for each 17β-HSD. Thus, in cells, NADPH, being the main source of electron for reduction (concentration of NADPH is 500 times higher than the one of the corresponding state $NADP^+$), is responsible for the reductive process, while NAD^+, being the principal electron acceptor (concentration of NAD^+ is 700 times higher than the one of the corresponding state NADH), assures the oxidative reactions[11,12]. To maintain equilibrium, NADPH and NAD^+ are constantly regenerated from their respective state $NADP^+$ and NADH (Figure 3).

Figure 3: Interplay between metabolism and 17β-HSD reactions



Apart from the difference between the concentrations of the cofactors in cells, kinetic studies showed that a large difference is observed between the K_m values of NADP(H) and NAD(H) for each 17β-HSD subtype. For example, in case of 17β-HSD1, a preference appears for NADPH (K_m = 0.07 μM) compared to NADH (K_m = 0.42 μM)[13]. That means that in cofactor binding site of each 17β-HSD subtype, amino acid residues are present which are able to differentiate the phosphorylated cofactor from the non phosphorylated one. Analysis of X-ray structure of 17β-HSD1 proves the presence of an arginine residue in the N-terminal part of the Rossmann fold, which can form a salt-bridge with the 2´-phosphate moiety of NADPH and increase the affinity of the substrat with the phosphorylated cofactor[14,15]. On the contrary, "oxidative" 17β-HSDs, as 17β-HSD2 prefer the non-phosphorylated cofactor because of a negatively charged amino acid presents in place of the arginine in 17β-HSD1. Repulsion is then observed between the 2´-phosphate moiety of NADPH and the negatively charged amino acid. Attraction will be observed by hydrogen bond formation between the 2´-OH group of NAD^+ and the negative amino acid, thus favorising the presence of the non-phosphorylated cofactor in the reactions implicating "oxidative" 17β-HSDs[16,17] (Figure 4).

Figure 4: Cofactor preference for reductive (NADPH) and oxidative (NAD^+) 17β-HSDs



1.2.3. Characteristics of the 17β-HSDs

17β-HSDs catalysing the last step of the sex steroid biosynthesis play an important role in the formation of active androgens and estrogens or in the inactivation of potent sex steroids. 17β-HSDs do not only differ in their cofactor preference and the direction of the reaction, but also in their localisation in body and their preference for a specific substrate. In the following 17β-HSDs are distinguished according to their reductive or oxidative functions in human.

1.2.3.1. Reductive forms

"Reductive" 17β-HSDs catalyse the reduction of inactive sex steroids into their active forms. Table 1 sums up the features of the "reductive" 17β-HSDs. Briefly, 17β-HSD1, 7 and 12 are the main enzymes responsible for E2 production. But 17β-HSD7 and 12 are also implicated in cholesterol and fatty acid (FA) synthesis, respectively. 17β-HSD3 and 5 catalyse principally the conversion of A-dione into T. As 17β-HSD1, 3, 5 and 7 are overexpressed in breast or prostate cancer tissues, inhibition of these enzymes could be an interesting approach for the treatment of these diseases. Until now, the implication of 17β-HSD12 in a disease has not been elucidated.

Table 1: Features of the "reductive" 17β-HSDs

Name	Localisation	Substrat	Function	Disease implication
17β-HSD1	Breast, ovary, endometrium, placenta	Estrogens, androgens	E2 production	Breast and prostate cancer, endometriosis
17β-HSD3	Testis	Androgens	T production	Pseudohermaphroditism
17β-HSD5	Prostate, liver	Androgens	T production	Breast and prostate cancer
17β-HSD7	Liver, lung, thymus	Estrogens, zymosterone	E2 production and cholesterol synthesis	Breast cancer
17β-HSD12	Breast, liver, placenta, kidney	Estrogens, long chain FA	E2 production, FA synthesis	-
17β-HSD13	Liver	Androgens	T production	Prostate cancer

1.2.3.2. Oxidative forms

Table 2 gives a summary of the principal features of the "oxidative" 17β-HSDs. While 17β-HSD2, 4, 8 and 10 catalyse the inactivation of active sex steroids E2 and/or T, 17β-HSD2 is the main enzyme responsible for the deactivation of E2 and T. Furthermore, 17β-HSD10 could be an interesting target for Alzheimer´s disease[18], as it is able to bind to amyloid plaques and is overexpressed in patients suffering from this pathology[19]. The real role of 17β-HSD11 has not been elucidated until now.

Table 2: Characteristics of the "oxidative" 17β-HSDs

Name	Localisation	Substrat	Function	Disease implication
17β-HSD2	Liver, breast, endometrium, placenta, prostate, bones	Estrogens, androgens, progestins	E2 and T inactivation	Breast and prostate cancer
17β-HSD4	Liver, prostate, lung, placenta	Estrogens, androgens	E2 and T inactivation	Prostate cancer
17β-HSD8	Liver, placenta, kidney	Estrogens, androgens	E2 and T inactivation, FA metabolism	Kidney disease[20]
17β-HSD10	Central nervous system, brain	Estrogens, cholic acid	FA metabolism, sex steroids inactivation	Neuronal disease[21]
17β-HSD11	Liver, kidney, lung, placenta	Androgens, retinoid		-
17β-HSD14	Liver, placenta, brain	Estrogens, androgens	E2 and T inactivation	Breast cancer[22]

1.3. 17β-HSD2

1.3.1. Generality

17β-HSD2 (EC = 1.1.1.62) was first isolated by Wu *et al.*[25] in 1993. Human 17β-HSD2 is composed of 387 amino acids and possesses a molecular weight of 42.8 kDa. It is expressed in a wide variety of tissues like placenta, kidney, uterus, prostate, liver, breast[23] and bones[24]. It is a membrane bound protein and is localised in microsomal fraction. No X-ray structure of the protein is still available.

17β-HSD2 is able to convert both C18- and C19-steroids and catalyses therefore the conversion from highly active E2 and T to their corresponding less active forms, E1 and A-dione[25], respectively in the presence of NAD^+[26] as cofactor. In a minor extent, it is also able to convert 20α-dihydroprogesterone (20α-DHP) into progesterone (P). The K_m values of 17β-HSD2 for E2, T and 20α-DHP are 0.21 µM, 0.39 µM and 0.71 µM[27], respectively.

As a member of the SDR family, 17βHSD2 conserves amino acid motifs as for example NAG motif (position 167-169) essential for stabilisation of the structure and YxxxK motif (position 232-236) , which represents the catalytic triad in the active site.

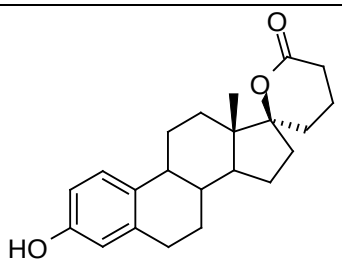
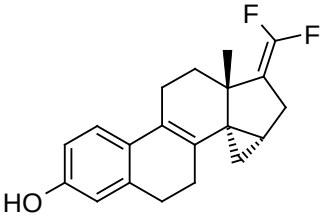
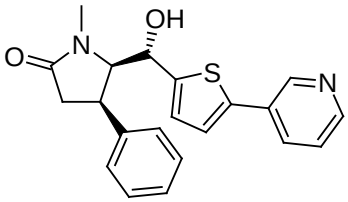
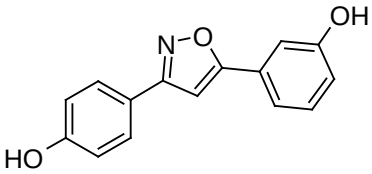
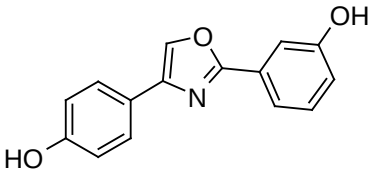
1.3.2. State of knowledge: inhibition of 17 β-HSD2

Until now, only few inhibitors of 17β-HSD2 have been described in the literature[28,29,30,31]. The first one, identified by Poirier *et al.*[32,33] beared a steroidal core (compound **A**, Table 3) and exhibited an IC_{50} value of 6 nM. Compound **A** is selective toward 17β-HSD1, 3 and 7 but is a highly potent inhibitor of 17β-HSD5 (IC_{50} (17β-HSD5) = 10 nM). It is therefore no longer investigated as a potential 17β-HSD2 inhibitor. A series of fluorinated estradiene derivatives was published as unselective and moderate inhibitors of 17β-HSD2. The most potent one of this series, compound **B**, showed 95% inhibition at 2µM.

Among the non-steroidal compounds, a novel highly potent derivative (compound **C**) was discovered by Wood *et al.*[34,35,36] as the most active 17β-HSD2 inhibitor described hitherto in the literature. The stereochemistry of **C** is very important, the C4 and C5 must be in the *cis* configuration. **C** shows an IC_{50} value of 50 nM, and was evaluated in a monkey osteoporosis model[37] to establish a *proof of concept*. Administration of compound **C** in monkey led to a slight decrease in bone resorption and increase in bone formation leading to maintenance of bone balance. However, a strong variability is observed, certainly due to non

appropriate pharmacokinetic properties. Compounds **D** and **E**, derivatives of isoxazole and oxazole, respectively, were identified in the frame of our 17β-HSD1 project. They show moderate inhibitory activity of 17β-HSD2 with IC_{50} values of 249 nM and 270 nM, respectively.

Table 3: Selection of the most active 17β-HSD2 inhibitors

Compound	Structure	IC_{50} (17β-HSD2)
A		6 nM
B		95%*
C		50 nM
D		249 nM
E		270 nM

*: percentage inhibition at 2µM

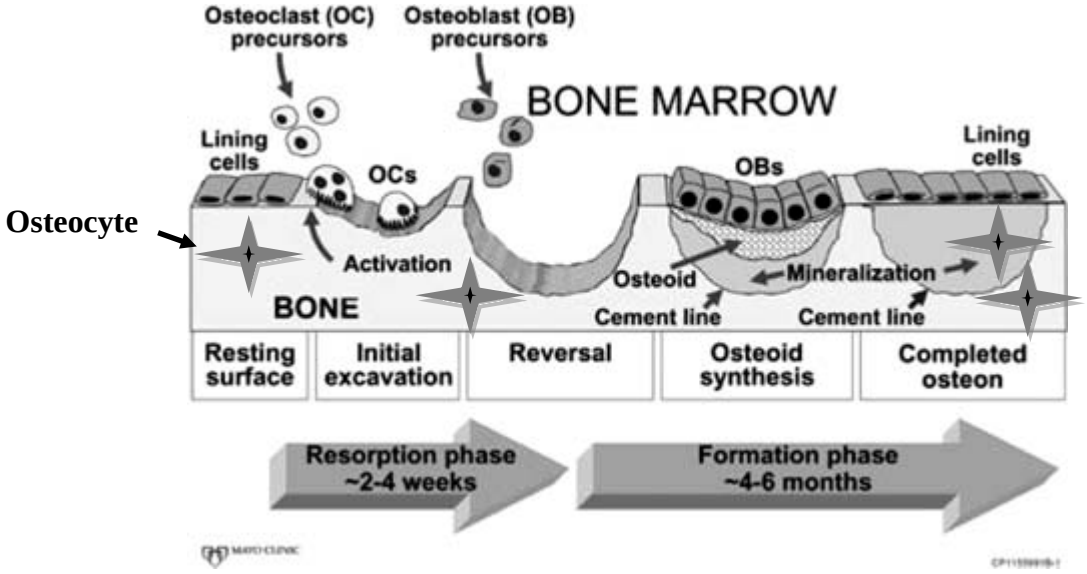
1.4. Disorders of bone remodeling and osteoporosis

1.4.1. Bone remodeling

Bone remodelling is a process, where old bone material is removed from the skeleton and new bone is formed. It requires the actions of four main cells: bone lining cells, osteocytes,

osteoclasts (OCs) and osteoblasts (OBs) (Figure 5). In a passive state, the bone surface is covered by bone lining cells. Osteocytes play the principal role in the initiation and activation of bone remodeling. When osteocytes detect bone damage, a signal is sent to the OC precursors, which are afterwards differentiated in OCs (osteoclastogenesis) by attaching to the bone matrix. Thus bone resorption processes and continues for a period of 2-4 weeks. The OC differentiation is regulated by macrophage colony-stimulating factor (M-CSF) and receptor activator of nuclear factor κβ ligand (RANKL)[38]. After the osteoclastic resorption eroded a lacuna, the process is directly followed by bone formation over a period of 4-6 months. OB precursors and OBs direct the phase of bone formation. OB formation and function continue after cessation of bone resorption to have equilibrium between bone resorption and bone formation. The mineralisation step finishes the bone remodeling cycle.

Figure 5: Bone remodeling sequence (taken from Riggs B. L. *et al.*[39])



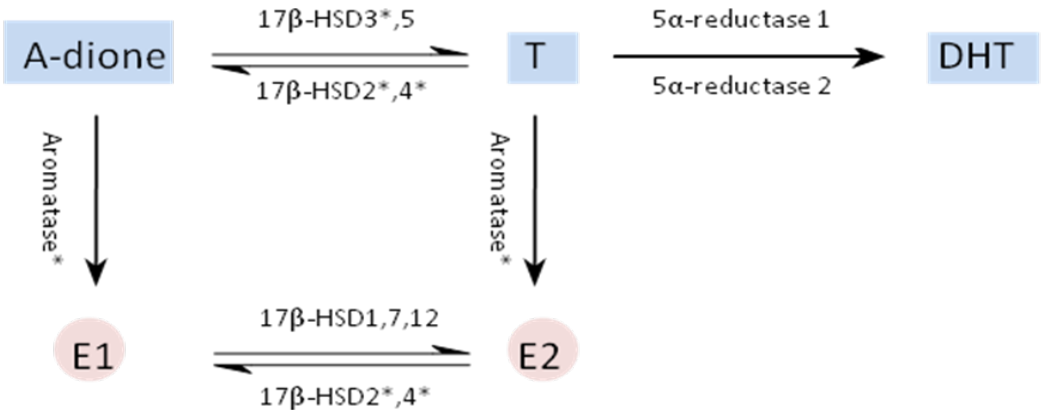
1.4.2. Role of sex steroids in adult skeleton

The reproductive system plays an important role in the bone remodeling system during adult life. Androgens and estrogens are not considered as just male and female hormones. Androgens can be converted into estrogens within the gonads and peripheral tissues (Figure 6) and both are present in men and women, but in different concentrations. 50% of total androgens in men, 75% of estrogens in premenopausal women and almost all of the estrogens in postmenopausal women are generated from extragonadal synthesis[40] .

E2 plays the major role in regulating bone metabolism not only in women but also in men[41]. T has some effect on bone resorption and clearly helps to maintain bone formation in

men, but T mainly provides the necessary substrate for aromatisation to E2 in the testis and in peripheral tissues, including bones[42].

Figure 6: Extragonadal pathways of androgens (boxes) and estrogens (circles). Asteriks indicate the enzymes that are present in osteoblasts

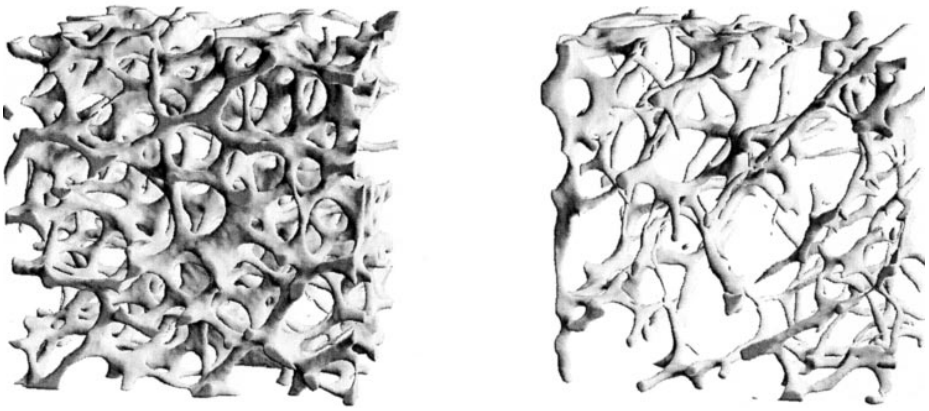


The mechanism by which estrogens act in bones is not yet clear, and still needs to be elucidated. But it has been proven that high doses of estrogen exert anabolic skeletal effects in postmenopausal women and elderly men and therefore conserve bone mass at the organ level[43]. The most postulated mechanism points out the importance of RANKL, crucial for bone formation and its two receptors: RANK (functional receptor) and osteoprotegerin (OPG). OPG inhibits osteoclastogenesis by binding to RANKL, preventing also interaction with its functional receptor RANK. This mechanism is validated by OPG and RANKL knockout (KO) mice [44, 45, 46], which develop severe osteoporosis and osteopetrosis, respectively, stressing the importance of OPG and RANKL for bone remodeling. Additionnally, an estrogen antagonist (ICI 182,780) is able to abrogate the expression of OPG, RANKL and ER in OBs, suggesting that estrogens stimulate the expression of ER and OPG and that the mechanism is ER mediated[47].

1.4.3. Osteoporosis

Osteoporosis[48,49] is a silent and systemic skeletal disease, characterised by a low bone mineral density (BMD) and deterioration of bone tissue (Figure 7), with a consequent increase in bone fragility and risks of fracture. As the effect of osteoporosis is systemic, all types of fractures in patients with low BMD is considered as osteoporotic fractures. But the typical ones are at hip, wrist and spine. Hip fractures are the most devastating in elderly people and provoke serious handicaps to walk and increase mortality.

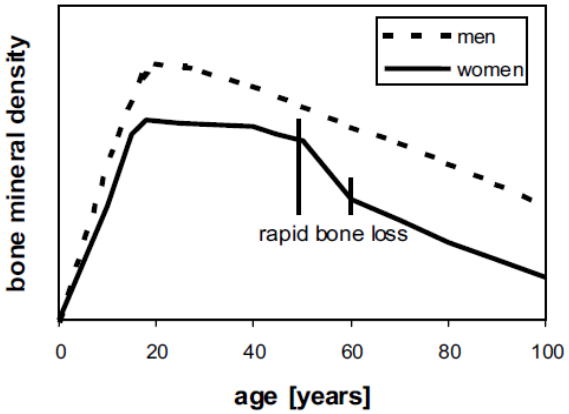
Figure 7: 3D reconstruction of a lumbar spine sample from a young adult woman (left) and from a woman with postmenopausal osteoporosis (right) (taken from Riggs B. L. *et al.*[50])



Bone loss takes place as a result of estrogen defiency in postmenopausal women and elderly men. An imbalance between the activity of OCs and the one of OBs is observed at the cellular level, which leads to an excess of bone resorption over bone formation.

Osteoporosis is divided in two types: postmenopausal (type I) and aged-related (type II) osteoporosis. Aged-related osteoporosis, which is characterised by a slow phase of continuous bone loss, affects both women and men, while postmenopausal osteoporosis (type I), defined as an early rapid bone loss due to the brutal decrease of sex steroid secretion after menopause, is only observed in women (Figure 8). The type II osteoporosis is principally associated with a continuous elevation of parathyroid hormone (PTH) level leading to an increased bone resorption. Principal reasons of the hyperparathyroidism are a vitamin D deficiency and consequences of estrogen deficiency. As mentioned above, estrogens play the major role in regulating bone resorption in men, whereas both estrogens and testosterone are important for maintaining bone formation[51], explaining the late slow bone loss in elderly men. Men are therefore more protected against osteoporosis.

Figure 8: Differences in bone gain and bone loss in men and women (taken from Pietschmann P. *et al.*[52])



Because the elderly people represent the fastest growing age-group worldwide, osteoporosis is an important public disease, which needs to have efficient treatments.

1.4.4. Approach to the treatment of osteoporosis

Several drugs are already available to treat osteoporosis[53]. They are classified depending on their action on bone resorption (antiresorptive agents) or on bone formation (anabolic agents [54]). Antiresorptive drugs include calcium, vitamin D, hormone therapy, bisphosphonates, selective estrogen-receptor modulators (SERM) and calcitonin, while anabolic treatment cover only parathyroid hormone (PTH). As summarised in table 4, various drugs are efficient for the treatment of osteoporosis, but their efficacy depends on the age, the presence or absence of prevalent fractures and the BMD. In the following part, the most often used drugs to treat osteoporosis will be described in more detail.

Table 4: Antifracture efficacy of the most frequently used treatments of osteoporosis

Drug	Vertebral fractures	Non-vertebral fractures
Parathyroid hormone (PTH)	+++	++
Alendronate (bisphosphonate)	+++	++
Risedronate (bisphosphonate)	+++	++
Raloxifene (SERM)	+++	0
Hormone replacement therapy	+	0
Calcitonin	+	0
Denosumab (RANKL inhibitor)	+++	++

+++: strong evidence; ++: good evidence; +: some evidence; 0: no effects.

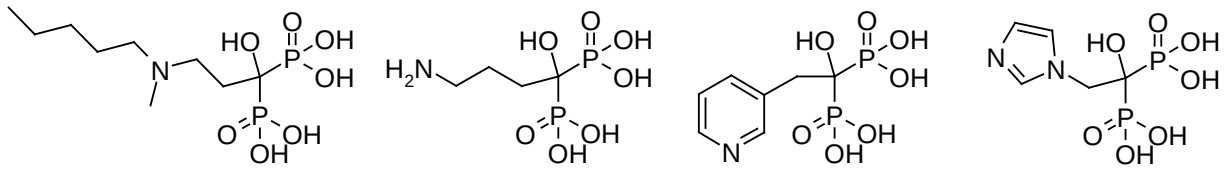
1.4.4.1. Parathyroid hormone (PTH)

Human recombinant PTH peptide 1-34 (teriparatide) [55], which is able to rebuild bone, is the treatment of choice for elderly patients, who already suffer from advanced bone loss. It improves bone density and reduces vertebral and non-vertebral fracture risks of about 50%. The main risk of teriparatide is a probable increase of osteosarcoma, seen in rat studies[56]. Because of this risk and a restrictive daily subcutaneous injection, it is used as second line therapy and no more than two years.

1.4.4.2. Bisphosphonates

Bisphosphonates[57], analogues of pyrophosphate characterised by a P-C-P bond, are potent inhibitors of bone resorption and effective therapies for the treatment of osteoporosis. Several bisphosphonates have been synthesised and their activity depend on the side chain directly bond to the carbon. Bisphosphonates reduce the activity of OC and increase their apoptosis. They have only low oral bioavailability (between 1% and 3% of the taken dose) and the half time of these drugs in bone is several years. Four main bisphosphonates are used nowadays for the treatment of osteoporosis: ibandronate, alendronate, risedronate and zoledronate (Figure 9).

Figure 9: Structures of some bisphosphonates used to treat osteoporosis



Ibandronate **Alendronate** **Risedronate** **Zoledronate**

All biphosphonates clearly prevent postmenopausal bone loss. Zoledronate seems to be the most efficacious bisphosphonates for reducing vertebral fractures by 79%, and with alendronate also for reducing hip fractures, 47% and 37% respectively, while risedronate showed the greatest non vertebral-non hip fracture reductions (87%). Various adverse effects [58, 59] are attributed to bisphosphonates, as e.g. gastrointestinal problems or hyperparathyroidism and musculoskeletal pain.

1.4.4.3. Selective estrogen receptor modulators (SERMs)

SERMs are a class of chemically diverse molecules, which possess a tertiary structure able to bind to ERα and/or ERβ. They act as estrogen agonists or antagonists, dependent on the target tissue. Therefore they offer the opportunity to dissociate favourable estrogenic effects on the bone (agonist) from unfavourable effects on the breast and endometrium (antagonist). Raloxifene[60,61], the first SERM synthesised, reduces risk of vertebral fractures by about 30-50% but was not active towards non-vertebral fractures. It also decreases the risk of developing breast cancer. However, it can increase the risk of venous thromboembolic disease and hot flushes. The potential of raloxifene to reduce fracture and breast cancer risks led to further development of new SERMs[62]. Tamoxifene, initially used to treat breast cancer, has a positive effect on bone but increases risk of endometrial cancer. It was eliminated as a possible therapy for osteoporosis. A new generation of SERMs is now under investigation, like lasofoxifene[63], which reduces risks of vertebral and non-vertebral fractures, breast cancer, coronary heart disease and stroke but is associated with an increased risk of venous thromboembolism and hot flushes. The research is going on for new SERMs that have beneficial effect on bones and reduced breast cancer risks.

1.4.4.4. RANKL inhibitor

As the interaction of RANKL with its functional receptor RANK is the final step of the regulation of bone resorption, inhibition of RANKL[64] is a new concept to treat osteoporosis. Thus, binding to its receptor, RANK is able to control the differentiation and the survival of OCs. Inhibition of RANKL could therefore reduce and regulate the activity of the OCs. Denosumab[65], which was recently admitted to the market by the European and American Commissions (May and June 2010, respectively), is the only RANKL inhibitor known until today. It is a fully human immunoglobin G2 monoclonal antibody. It binds to RANKL, blocking interaction with its receptor on the surface of OCs, permitting a decrease of bone resorption and an enhancement of bone mass. Denosumab is as effiective as the bisphosphonate risendronate and it has to be given subcutaneously every six months, but it has been proved its efficacy only in post-menopausal women with a high risk of fractures[66]. Further studies will be extended to other group of patients with normal risks of fractures.

1.5. Is 17β-HSD2 a good target for the treatment of osteoporosis?

As described in chapter 1.4.2., estradiol plays a crucial role in the development of osteoporosis because the hormone deficiency in elderly people has a direct effect on the cellular level of the skeleton and thus it enhances bone resorption. As 17β-HSD2 is present in osteoblastic cells, inhibition of this enzyme could increase the E2-level in this tissue, thus allowing for a novel approach for the treatment of osteoporosis.

Recently, the *in vivo* efficacy of the pyrrolidinone **C** (Table 3, 17β-HSD2 inhibitor) has been evaluated in an osteoporosis monkey model. The study proved that inhibition of 17β-HSD2 leads to high local E2 and T levels and maintains a balance between bone formation and bone resorption. But when compound **C** is orally administrated, a strong variability is observed, certainly due to non appropriate pharmacokinetic properties of **C**. Thus, according to this study, inhibition of 17β-HSD2 could be a novel approach for the treatment of osteoporosis. To be sure this needs to be demonstrated again in an animal disease model with potent 17β-HSD2 inhibitors, provided they have good pharmacokinetic properties.

In conclusion, new potent and selective 17β-HSD2 inhibitors with appropriate pharmacokinetic properties should be identified for further *in vivo* experiments, to prove the concept that inhibition of 17β-HSD2 is able to inhibit osteoclastogenesis and induce bone formation.

2. AIM OF THE PRESENT STUDY

2.1. Scientific goal

Osteoporosis is a skeletal disease characterised by an accelerated bone loss after menopause and in elderly men due to the rapid decrease of E2 and T levels in blood. E2 is therefore essential for the health of bones. 17β-HSD2 is responsible for the conversion of the highly active E2 into the less active E1. 17β-HSD2 is expressed in bone tissue. Thus, the development of drug-like selective 17β-HSD2 inhibitors could be a new promising approach for the treatment of osteoporosis. The efficacy of the proposed new treatment has yet to be proven. Only few inhibitors of 17β-HSD2 have been described in the literature. The effect of one of them, the pyrrolidinone **C**, has been evaluated in a monkey osteoporosis model. High local estrogen and testosterone levels have been observed, leading to a desirable bone balance between bone formation and bone resorption. Strong variablity has also been reported when orally administrated, certainly due to non appropriate pharmacokinetic properties.

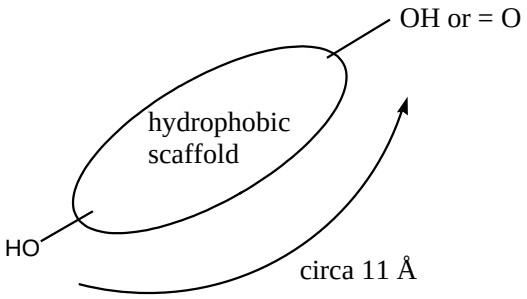
Therefore, the goal of this thesis was to develop new non-steroidal drug-like 17β-HSD2 inhibitors, with IC_{50} values in the low nanomolar range, selective towards 17β-HSD1, catalysing the reverse reaction and towards ERα and ERβ as it is expected that the effects are ER-mediated. In a first step, compounds exhibiting activity in cell-free as well as in intact cells (MDA-MB-231) have to be found. After having identified potent human enzyme inhibitors, their activity will be determined in other species (rat, mouse and monkey) in order to have potential candidates for validation in animals to obtain the proof of concept that 17β-HSD2 inhibitors can act as inductor of bone formation.

The present work is focused on the design, synthesis and evaluation of the structure activity relationships (SAR) of potential 17β-HSD2 inhibitors. The work starts from derivatives obtained as part of the in-house 17β-HSD1 project.

2.2. Working strategy

As no X-ray structures and no homology model were available, in this thesis a ligand based approach was pursued. The compounds should have a non-steroidal structure, however, they should bind in the substrate binding site of 17β-HSD2 mimicking E2. For this they should have two polar moities distant from each other circa 11Å, mimicking position C3 and C17 of the steroid and possess a hydrophobic central core as E2 (Figure 10).

Figure 10: Simple pharmacophore model



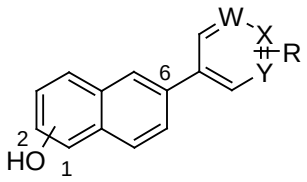
A preliminary study of Frotscher M. *et al.*[67] aiming at the design of non-steroidal 17β-HSD1 inhibitors, resulted in a promising starting point for the development of 17β-HSD2 inhibitors. The scaffold, composed from a naphthalene moiety substituted in position 6 by a phenyl ring mimicks the central hydrophobic core of E2. The positions of both hydroxy groups (distant from each other circa 11 Å) have been investigated in the first chapter of this thesis, as well as the influence of substituents replacing the OH group on the phenyl moiety. Additionally, structural optimisation of the naphthalene moiety has been explored (Figure 11). The results of this study are presented in chapter **3.I**.

According to the results of the first project (Chapter **3.I**), and after synthesis of fourty four derivatives, a clear SAR has been depicted: a H-bond donor is highly necessary in *meta* or *para* position of the phenyl and some space is available around the phenyl moiety. The investigation of the size and the nature of additional substituents was the subject of the second part of this work. (Figure 11, Project II, chapter **3.II**).

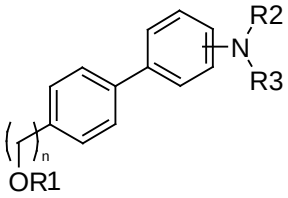
In order to have access to a broad structural diversity of potential drug candidates, the decision was taken to also investigate other compound classes. In the frame of the in-house on going 17β-HSD1 project, Oster A. *et al.*[68] identified compound **F** (Figure 12) as a moderate 17β-HSD2 inhibitor. Compound **F** has been taken as starting point for an additional compound class as it satisfies the two conditions mentioned above (Figure 11, Paper III, chapter **3.III**). Structural optimisation of the thiophene derivative (replacement of the methyl group, introduction of substituents on the benzoyl part of the molecule) has been studied to increase activity of **F** and to reverse the selectivity in favour of 17β-HSD2. Results of this work are presented in chapter **3.III** (Figure 11, Paper III).

Figure 11: Structure overview of the molecules reported in this thesis

Project I

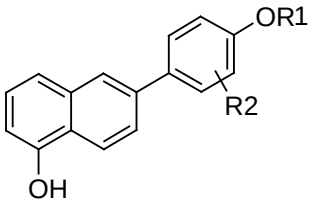


R = F, NO_2, CN, COOH, $COCH_3$, OH, OCH_3, CH_2OH, SO_2NHCH_3, $NHSO_2CH_3$, $CONHCH_3$,$NHCOCH_3$, NHCOPh
W, X, Y = CH, N



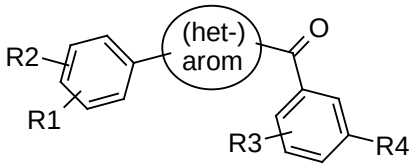
n = 0, 1
R1, R2 = H, CH_3
R3 = $COCH_3$, COPh, COPh(3´-OCH_3), COPh(4´-OCH_3), $COCH_2$Ph(3´-OCH_3), $COCH_2$Ph(4´-OCH_3), SO_2CH_3, SO_2Ph(3´-CH_3)

Project II



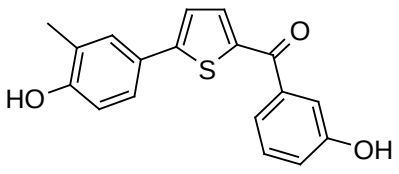
R1 = H, CH_3
R2 = CH_3, OCH_3, OH, Ph, F, CF_3, Cl, CN

Project III



R1 = H, OH, OCH_3
R2 = H, CH_3, F, Cl, CF_3
R3 = H, F, CH_3
R4 = OH, F
(het-)arom = pyridine, phenyl

Figure 12: Starting point of a new class of 17β-HSD2 inhibitor (Chapter **3.III**)



F

3. RESULTS

3.1. 17β-HSD2 inhibitors for the treatment of osteoporosis: identification of a promising scaffold

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This manuscript has been published as an article in the journal
Bioorganic and Medicinal Chemistry, **2011**, *19*, 807-815

Paper I

Abstract

17β-Hydroxysteroid dehydrogenase type 2 (17β-HSD2) catalyses the conversion of active 17β-hydroxysteroids into the less active 17-ketosteroids thereby controlling the availability of biologically active estrogens (E2) and androgens (T) in the tissues. The skeletal disease osteoporosis occurs mainly in post-menopausal women and in elderly men when the levels of estrogens and androgens, respectively, decrease. Since 17β-HSD2 is present in osteoblasts, inhibition of this enzyme may provide a new and promising approach to prevent the onset of osteoporosis, keeping a certain level in estrogens and androgens in bone cells of ageing people. Hydroxynaphthyl, hydroxyphenyl and hydroxymethylphenyl-substituted moieties were synthesised as mimetics of the steroidal substrate. Compound **8** has been identified as promising scaffold for 17β-HSD2 inhibitors displaying high activity and good selectivity toward 17β-HSD1, ERα and ERβ.

Introduction

Osteoblasts (OB) and osteoclasts (OC), two bone cells, are responsible for bone formation and bone resorption, respectively. In bone physiology of healthy individuals, bone mass is maintained by a well regulated balance between the activity of OBs and OCs.

Osteoporosis[1] is a skeletal disease, characterised by a reduced bone mineral density (BMD), increased bone fragility and predisposition to fractures. The most common osteoporotic fractures occur at the hip, spine and wrist, leading to pain and amplifying mortality. In post-menopausal women and elderly men, there is an acceleration of bone loss, because of higher

activity of the OCs compared to the one of OBs. As androgens and estrogens appear to be the most important sex steroids[2] involved in osteoclastic resorption and osteoblastic formation, a decrease in estrogens in post-menopausal women and in both androgens and estrogens in elderly men results in a disproportion between bone loss and bone formation and often leads to osteoporosis.

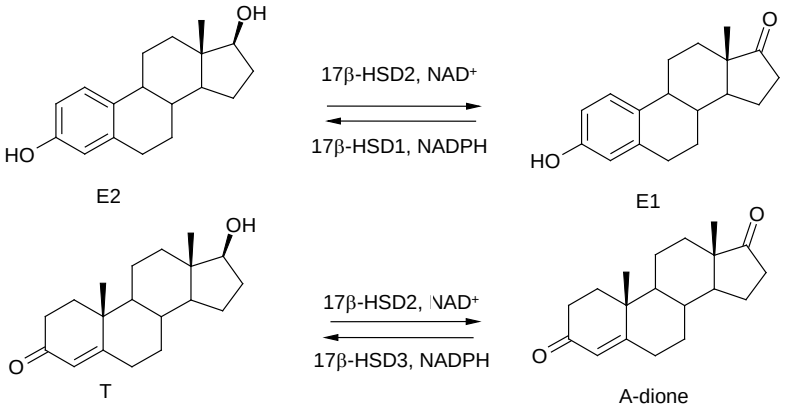
Antiresorptive agents, including bisphosphonates and selective estrogen receptor modulators (SERM) are often used to treat osteoporosis. Bisphosphonates (like alendronate)[3] are currently the most potent agents for the treatment of osteoporosis. However they lead to reduction of only 50% of fracture risks in post-menopausal women[3, 4] and elderly men.[5] The SERM raloxifene,[6] is also efficient to treat osteoporosis but is associated with an increased risk of venous thromboembolism.[7]

As all therapies used nowadays for the treatment of osteoporosis have limitations and none of them offers a complete cure, there is a need to develop other efficient drugs for this disease.

There is evidence that androgens and estrogens play a crucial role in the development of osteoporosis.[8] Although they belong to the antiresorptive agents, estrogens act on the OBs, regulating the ability of the OC precursors to differentiate to OCs (osteoclastogenesis). Estrogens can also enhance OB proliferation[9] as well as osteoprotegerin (OPG) gene expression[10] and alkaline phosphatase (ALP) activity[10]. While OB proliferation and ALP increase[11] are beneficial for bone formation, OPG via osteoclastogenesis inhibition reduces bone resorption. Therefore a drug, which could increase the estrogen levels in the OBs should result in a local increase of OB cell proliferation, OPG and ALP production, and should have beneficial effect on osteoporosis. While a decrease in estrogen and androgen levels can easily be obtained either systematically by the use of aromatase[12] and CYP17[13, 14] inhibitors or locally by inhibitors of 17β-HSD1[15-19] or 5α-reductase,[20-22] an intracellular increase of both sex hormones should also be feasible.

High levels of E2 and T should be obtained in bone cells only in order to avoid development of breast and prostate cancer. This goal could be achieved by inhibiting 17β-hydroxysteroid dehydrogenase type 2 (17β-HSD2, Chart 1) in the target tissues.

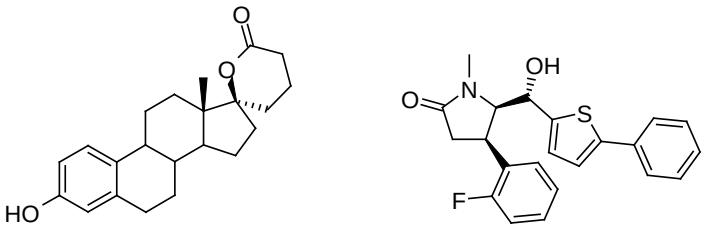
Chart 1. Interconversion of estradiol (E2) to estrone (E1) by 17β-HSD1 and 17β-HSD2 and of testosterone (T) to androstenedione (A-dione) by 17β-HSD2 and 17β-HSD3.



17β-HSD2 catalyses the conversion of active 17β-hydroxysteroid estrogen E2 and androgen T into their less active 17-ketosteroids using the cofactor NAD^+. 17β-HSD2 is a trans-membrane protein[23] and its 3D-structure is unknown. It is mainly expressed in placenta, liver, small intestine, endometrium and osteoblastic cells.[24] As 17β-HSD2 is present in bone cells[24, 25] and is responsible for the deactivation of active estrogens and androgens, inhibitors of 17β-HSD2 could maintain a certain level in active sex steroids in bone tissue, and therefore could protect against bone loss and structure deterioration. Therefore 17β-HSD2 is a new attractive target for the treatment of osteoporosis.

Only very few inhibitors of 17β-HSD2 (Chart 2) have been described until now.[26-29] The steroidal spirolactone (Chart 2) was the first 17β-HSD2 inhibitor identified.[26-28] Within the non-steroidal ones, some flavonoids like 3-hydroxyflavone have been described in the literature as 17β-HSD2 inhibitors.[30] However, regarding 17β-HSD1 they were not very selective. The pyrrolidinone **A** is until now the most potent inhibitor described in the literature. It was evaluated *in vivo* in a monkey osteoporosis model by Bagi *et al.*[31] In spite of the small effects observed at the highest dose and a strong variability when administrated orally, certainly due to an inappropriate pharmacokinetic profile, this study validates 17β-HSD2 as potential target for the treatment of osteoporosis. Therefore there is a need for new potent inhibitors with good pharmacokinetic properties for further *in vivo* experiments.

Chart 2. Inhibitors of 17β-HSD2



A

In this report, the design of novel and selective inhibitors derived from substituted 1-hydroxynaphthyl, 4-hydroxymethylphenyl and 4-hydroxyphenyl (Tables 1 and 2) as well as their synthesis and biological evaluation regarding inhibition of 17β-HSD2 and 17β-HSD1 will be presented.

Design of inhibitors

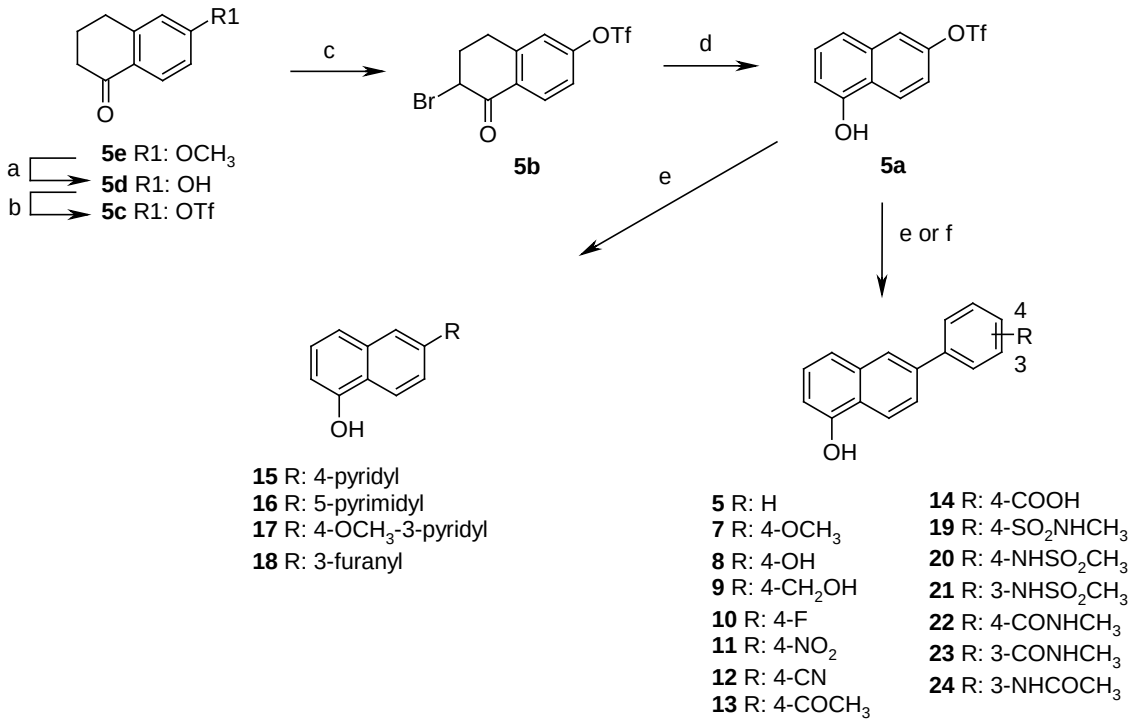
As flavonoids are known to interact with several pharmacological targets, we refrained from using these structures as a starting point. A similar structure, 6-(3-hydroxyphenyl)-2-naphthol (compound **B**) was recently identified by Frotscher *et al.*[15] as a highly potent 17β-HSD1 inhibitor. In spite of the low sequence identity[32] and homology[32] (23% and 45%, respectively), the active site of 17β-HSD1 and 17β-HSD2 is considered to be very similar as the substrate of one enzyme is the product of the catalytic reaction of the other enzyme (Chart 1). Taking advantage of our experience developing 17β-HSD1 inhibitors, the hydroxyphenylnaphthol scaffold of compound **B** was considered as promising starting point for the identification of new inhibitors of 17β-HSD2. To improve the activity of compound **B** in favour of 17β-HSD2 inhibition, the optimal position of the hydroxy groups was investigated first. In the second step, one hydroxy group was replaced by different substituents using the best scaffold. Subsequently, the naphthalene core was exchanged either by hydroxyphenyl or by hydroxymethylphenyl moiety.

Chemistry

Compounds **1-4** and **6** were synthesised following the procedure described by Frotscher *et al.*[15] The synthesis of **5** started from the commercially available 6-methoxy-1-tetralone **5e**, which is converted to the hydroxy analogue **5d** by an ether cleavage using aluminium

trichloride[33] and subsequently triflated[34] to **5c**. Bromination,[35] followed by dehydrobromination[35] gave the desired naphth-1-ol **5a** in very good yield. Different substituted phenyl (**5**, **7-14, 19-24**) or heteroaromatic rings (**15-18**) were introduced into the 6-position of the naphthalene core via Suzuki coupling[36] reaction (Scheme 1, Method A or B).

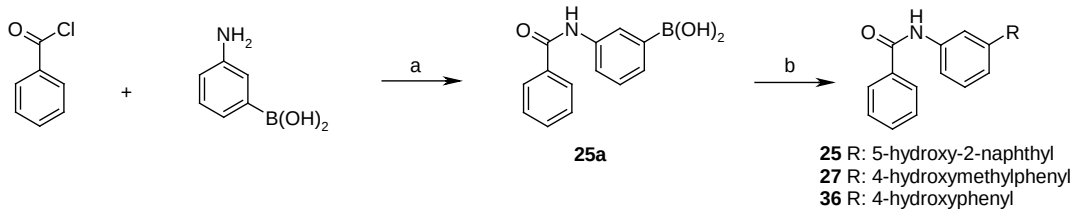
Scheme 1. Synthesis of compounds **5** and **7-24**[a]



[a]Reagents and conditions: a. $AlCl_3$, toluene, reflux, 2h; b. $(CF_3CO)_2O$, pyridine, 0°C, 30 min; c. Br_2, CCl_4, diethylether, 0 °C, 4h; d. LiBr, Li_2CO_3, DMF, reflux, overnight; e. R-boronic acid, $Pd(PPh_3)_4$, Na_2CO_3, DME/EtOH 1:1, 85 °C, 2h for **5** and **7-18**, Method A; f. R-boronic acid, $Pd(PPh_3)_4$, Na_2CO_3, DME/water 2:1, microwave irradiation (150°C, 150 W, 25 min) for **19-24**, Method B.

Synthesis of **25**, **27** and **36** occurred in a two steps reaction: amidation (Method C) between benzoyl chloride and 3-aminophenylboronic acid[37] afforded the intermediate **25a** and a subsequent Suzuki coupling (Scheme 2) with the appropriate boronic acid led to the target compounds.

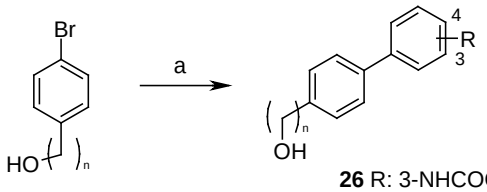
Scheme 2. Synthesis of compounds **25, 27** and **36**[a]



[a]Reagents and conditions: a. Et_3N, CH_2Cl_2, room temperature, 1h, Method C; b. R-Br or R-OTf, $Pd(PPh_3)_4$, Na_2CO_3, DME/EtOH 1:1, 85 °C, 2h, Method A.

Starting from 4-hydroxymethylbromophenyl or 4-bromophenol, compounds **26**, **33**, **35** and **43** were obtained via a cross-coupling reaction under microwave assisted conditions (Scheme 3, Method B).

Scheme 3. Synthesis of compounds **26**, **33**, **35** and **43**[a]

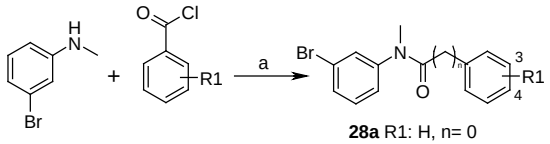


26 R: 3-NHCOCH$_3$, n=1
33 R: 4-NHSO$_2$CH$_3$, n=1
35 R: 3-NHCOCH$_3$, n=0
43 R: 4-NHSO$_2$CH$_3$, n=0

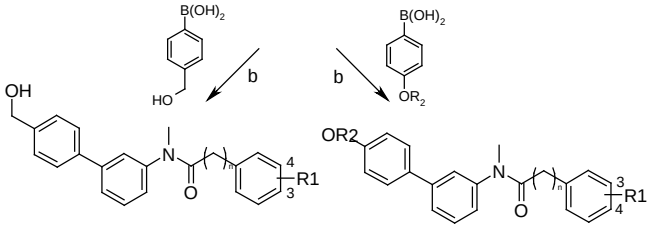
[a]Reagents and conditions: a. R-boronic acid, $Pd(PPh_3)_4$, Na_2CO_3, DME/Water 2:1, microwave irradiation 150 °C, 150 W, 15 bar, 25 min, Method B.

The synthesis of compounds **28-32** and **37-42** started from the 3-bromo-*N*-methylaniline which was coupled to different benzoyl chlorides following method C (Scheme 4) to form the amides **28a-32a**. Subsequent Suzuki cross couplings with the appropriate boronic acids afforded **28-32**, **37-40** and **42**. Compound **40** was further demethylated[38] with boron tribromide to give compound **41** in high yield.

Scheme 4. Synthesis of compounds **28-32** and **37-42**[a]



28a R1: H, n= 0
29a R1: 3-OCH$_3$, n= 0
30a R1: 4-OCH$_3$, n= 0
31a R1: 3-OCH$_3$, n= 1
32a R1: 4-OCH$_3$, n= 1



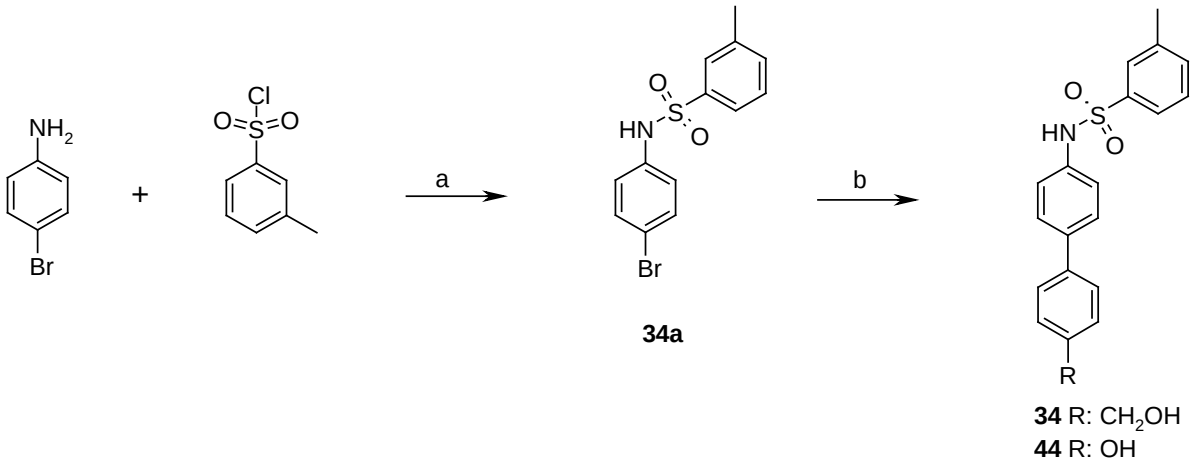
28 R1: H, n= 0
29 R1: 3-OCH$_3$, n= 0
30 R1: 4-OCH$_3$, n= 0
31 R1: 3-OCH$_3$, n= 1
32 R1: 4-OCH$_3$, n= 1

37 R1: H, R2: H, n= 0
38 R1: 3-OCH$_3$, R2: CH$_3$, n= 0
39 R1: 4-OCH$_3$, R2: CH$_3$, n= 0
40 R1: 3-OCH$_3$, R2: CH$_3$, n= 1
c
41 R1: 3-OH, R2: H, n=1
42 R1: 4-OCH$_3$, R2: CH$_3$, n= 1

[a]Reagents and conditions: a. Et_3N, CH_2Cl_2, room temperature, 1h, Method C; b. $Pd(PPh_3)_4$, Na_2CO_3, DME/Water 2:1, microwave irradiation, 150 °C, 150 W, 15 bar, 25 min, Method B; c. BBr_3, CH_2Cl_2, -78°C to room temperature, overnight.

The synthesis[37] of the sulfonamide derivative **34a** was carried out by reaction of 4-bromoaniline with 3-methylphenylsulfonylchloride (Scheme 5). Subsequent Suzuki coupling with the corresponding boronic acids afforded sulfonamides **34** and **44**.

Scheme 5. Synthesis of compounds **34** and **44**[a]



[a]Reagents and conditions: a. pyridine, room temperature, overnight; b. R-phenylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, DME/Water 2:1, microwave irradiation, 150 °C, 150 W, 15 bar, 25 min, Method B.

Biological results

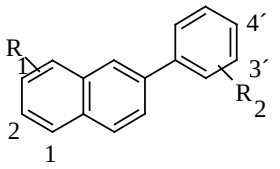
Inhibition of human 17β-HSD2 and selectivity toward 17β-HSD1, ERα and β

17β-HSD2 and 17β-HSD1 inhibitory activities of the synthesised compounds were evaluated in cell-free assays. As 17β-HSD1 catalyses the reduction of E1 to E2, it should not be affected by 17β-HSD2 inhibitors. Moreover, inhibitors of 17β-HSD2 should have no affinity for the estrogen receptors ERα and β, as it is expected that E2 effects are ER mediated.

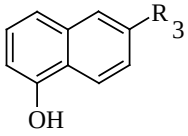
Human placental enzymes were used as source of enzymes for both assays and were obtained according to described methods.[39-41] In the 17β-HSD2 assay, incubations were run with tritiated E2, cofactor and inhibitor.[41] The separation of substrate and product was accomplished by HPLC. The 17β-HSD1 assay was performed similarly using tritiated E1 as substrate. The percent inhibition values of compounds **1-44** are shown in Tables 1 and 2, and the IC_{50} values of selected compounds are reported in Table 3. Compounds showing less than 10% inhibition tested at a concentration of 1µM were considered to be inactive. The 6-(3-hydroxyphenyl)-2-naphthol **B** identified in a previous work[15] was used as internal reference (20% 17β-HSD2 inhibition *vs*. 100% 17β-HSD1 inhibition at 1µM).

To identify the best scaffold for a high 17β-HSD2 inhibition, the questions were examined whether two hydroxy substituents are necessary for inhibition and which positions on the 6-phenylnaphthalene moiety were optimal. Compounds bearing only one hydroxy group, either on the naphthalene (compounds **3** and **5**) or on the phenyl (compound **4)** core turned out to have a weak 17β-HSD2 inhibitory activity (Table 1).

Table 1. Inhibition of human 17β-HSD2 and 17β-HSD1 by compounds **1-25**



B, 1-14, 19-25



15-18

Cmpd	R_1	R_2	R_3	Inhibition of 17β-HSD2 [%][a] at 1 µM	Inhibition of 17β-HSD1 [%][b] at 1 µM
B	2-OH	3´-OH	-	20	100
1	2-OH	2´-OH	-	n.i.	n.i.[c]
2	2-OH	4´-OH	-	n.d.	n.i.[c]
3	2-OH	H	-	17	44
4	H	3´-OH	-	12	61[c]
5	1-OH	H	-	20	n.i.
6	1-OH	3´-OH	-	32	23[c]
7	1-OH	4´-OCH_3	-	20	28
8	1-OH	4´-OH	-	74	20
9	1-OH	4´-CH_2OH	-	31	14
10	1-OH	4´-F	-	n.i.	n.i.
11	1-OH	4´-NO_2	-	13	15
12	1-OH	4´-CN	-	12	16
13	1-OH	4´-$COCH_3$	-	n.i.	16.
14	1-OH	4´-COOH	-	16	n.i.
15	-	-	4-pyridyl	26	n.i.
16	-	-	5-pyrimidyl	n.i.	n.i.
17	-	-	4-OCH_3-3-pyridyl	n.i.	n.i.
18	-	-	3-furanyl	13	n.i.

Table 1 continued

Cmpd	R_1	R_2	R_3	Inhibition of 17β-HSD2 [%][a] at 1 µM	Inhibition of 17β-HSD1 [%][b] at 1 µM
19	1-OH	4´-SO_2NHCH_3	-	57	12
20	1-OH	4´-$NHSO_2CH_3$	-	75	52
21	1-OH	3´-$NHSO_2CH_3$	-	22	19
22	1-OH	4´-$CONHCH_3$	-	13	n.i.
23	1-OH	3´-$CONHCH_3$	-	43	n.i.
24	1-OH	3´-$NHCOCH_3$	-	64	12
25	1-OH	3´-NHCOPh	-	n.i.	n.i.

[a] Human placenta, microsomal fraction, substrate [^{3}H]-E2 + E2 [500 nM], NAD^+ [1.5 mM], mean value of 3 determinations, relative standard deviation < 10%, [b] Human placenta, cytosolic fraction, substrate [^{3}H]-E1 + E1 [500 nM], NADH [0.5 mM], mean value of 3 determinations, relative standard deviation < 10%, [c]recombinant human 17β-HSD1, substrate [^{3}H]-E1+ E1 [30 nM], cofactor NADPH [1 mM], mean value of two determinations, relative standard deviation < 20%, n.d. not determined, n.i.: no inhibition (inhibition < 10%).

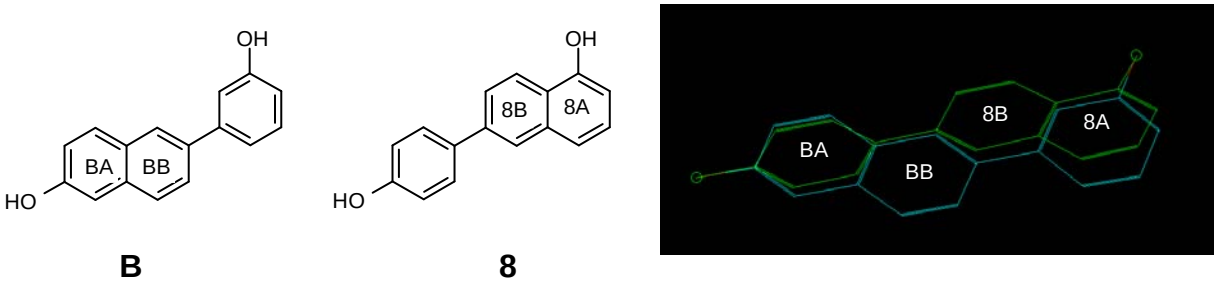
Compounds with two OH groups can be separated in two different groups depending on the position of the hydroxy moiety on the naphthalene ring: 2-naphthols (**B**, **1**-**2**) and 1-naphthols (**6**-**25**). 2-naphthols are not appropriate as scaffold for 17β-HSD2 inhibitors as all compounds turned out to be inactive.

Concerning the 1-naphthols, compounds **6** and **7** substituted with a *meta*-hydroxy (**6**) or a *para*-methoxy (**7**) group on the phenyl moiety displayed only very low activity. However, a *para*-hydroxy substituent (**8**) improved activity (74% inhibition at 1 µM) indicating that 1) the *para*-position is more appropriate than the *meta*-position and 2) H-bond interactions are likely to occur with the enzyme.

Looking at these results, it appears that two hydroxy groups are crucial for the activity and the distance between them must be around 11Å as observed for E2 and **8**. The structure of inactive **B** is very similar to the active **8**: they both consist of a hydroxylated naphthol and a hydroxylated phenyl, and the distance between the two OH groups are identical. These compounds differ only in their hydroxy substitution pattern which seems to play a very important role for 17β-HSD2 or 17β-HSD1 inhibition. In order to better understand these results, **8** and **B** were superimposed using their hydroxy groups as fixed points (Chart 3). It can be observed that the central aromatic rings of **B** and **8** occupy a different space in the

enzyme. It is likely that the presence of at least one amino acid of the active site of 17β-HSD2 around ring BB is responsible for the selectivity observed for **8** in favour of 17β-HSD2.

Chart 3. Superimposition of compounds **B** and **8**.



B **8**

The dihydroxylated compound **8** can be considered as a promising core structure. In order to improve its activity and to limit the number of hydroxy functions (often responsible for a fast metabolism as OH groups are substrates for phase II metabolism), either the hydroxy group of the phenyl moiety was replaced by different substituents (**9-14**, **19-25**) or the 4-hydroxyphenyl was exchanged by different heteroaromatic rings (**15-18**).

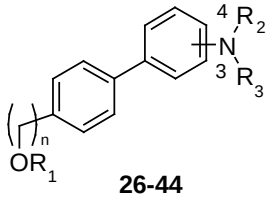
Introduction of a methylene linker between the phenyl and the hydroxy group (**9**) led to a strong decrease in activity, possibly due to the flexibility of the OH group and a too long distance between the two hydroxy groups.

Exchange of the hydroxy moiety by a withdrawing group like fluoro (**10**), nitro (**11**), cyano (**12**), acetyl (**13**) or carboxylic (**14**) led to inactive compounds, indicating that only electron donating groups are tolerated by the enzyme in this region or that a H-bond donor group is necessary for activity. A series of sulfonamides, reversed sulfonamides and amides in positions 3 or 4 were introduced as replacement of the hydroxy function. Amides were better tolerated in 3-position and sulfonamides in 4-position (64% 17β-HSD2 inhibition at 1 µM for amide **24**; 57% and 75% for sulfonamide **19** and reverse sulfonamide **20**). These results point out the importance of the H-bond donor group directly attached to the phenyl ring: good inhibition data were obtained in case of the amide (in 3-position **24**) or sulfonamide (in 4-position **20**) which are linked to the ring by the NH and therefore able to mimic the OH group of **8**. The inactivity of **25** is probably due to a steric clash between the large phenylamide group and amino acids from the active site.

Various heteroaromatic rings were also introduced in 6-position of the naphthalene (compounds **15-18**). None of them turned out to be active, proving that the hydroxy substituent on the phenyl of **8** can not be replaced by a pure H-bond acceptor group, a H-donor in this position is essential for the activity.

In order to simplify the rather lipophilic scaffold of the naphthalene, the 1-naphthol moiety was replaced either by 4-hydroxymethylphenyl or by 4-hydroxyphenyl groups. The exchange of the 1-naphthol for a 4-hydroxymethylphenyl was detrimental for the activity: the amide **26** (to be compared to the 1-naphthol **24** 64% inhibition at 1 µM) and sulfonamide **33** (to be compared to the 1-naphthol **20** 75% inhibition at 1 µM) turned out to be completely inactive (Table 2). In order to gain activity in this class of compounds, different substituted amides (**27-32**) and sulfonamides (**34**) were introduced but also turned out to be either weakly active or inactive. Elimination of the methylene linker (compounds **35-44**) leading to 4-hydroxyphenyl derivatives was not appropriate to regain activity: the amide **35** (to be compared to the 1-naphthol **24** 64% inhibition at 1 µM) and sulfonamide **43** (to be compared to the 1-naphthol **20** 75% inhibition at 1 µM) were also inactive. These results emphasise the importance of the aromatic ring 8A of the 1-naphthol, which certainly stabilise the molecule in the active site via π-π-interactions. However, introduction of a methyl on the nitrogen of the phenyl amide **37** or of the benzylamide **41** led to a regain of activity (46% and 39% inhibition at 1µM for **37** and **41**, respectively). All methoxy derivatives (**38-40** and **42**) were devoid of activity.

Table 2. Inhibition of human 17β-HSD2 and 17β-HSD1 by compounds **26-44**



26-44

Cmpd	n	R_1	Position substi-tution	R_2	R_3	Inhibition of 17β-HSD2 [%][a] at 1 µM	Inhibition of 17β-HSD1 [%][b] at 1 µM
26	1	H	3	H	$COCH_3$	n.i.	n.i.
27	1	H	3	H	COPh	n.i.	n.i.
28	1	H	3	CH_3	COPh	n.i.	n.i.
29	1	H	3	CH_3	COPh(3´-OMe)	15	n.i.
30	1	H	3	CH_3	COPh(4´-OMe)	14	n.i.
31	1	H	3	CH_3	$COCH_2$-Ph(3´-OMe)	12	n.i.
32	1	H	3	CH_3	$COCH_2$-Ph(4´-OMe)	n.i.	n.i.

Table 2 continued

Cmpd	n	R_1	Position substi-tution	R_2	R_3	Inhibition of 17β-HSD2 [%][a] at 1 µM	Inhibition of 17β-HSD1 [%][b] at 1 µM
33	1	H	4	H	SO_2CH_3	n.i.	n.i.
34	1	H	4	H	$SO_2Ph(3'\text{-}CH_3)$	12	n.i.
35	0	H	3	H	$COCH_3$	n.i.	n.i.
36	0	H	3	H	COPh	n.i.	n.i.
37	0	H	3	CH_3	COPh	46	n.i.
38	0	CH_3	3	CH_3	COPh(3´-OMe)	13	16
39	0	CH_3	3	CH_3	COPh(4´-OMe)	20	n.i.
40	0	CH_3	3	CH_3	$COCH_2$-Ph(3´-OMe)	n.i.	n.i.
41	0	H	3	CH_3	$COCH_2$-Ph(3´-OH)	39	n.i.
42	0	CH_3	3	CH_3	$COCH_2$-Ph(4´-OMe)	10	25
43	0	H	4	H	SO_2CH_3	n.i.	n.i.
44	0	H	4	H	$SO_2Ph(3'\text{-}CH_3)$	14	n.i.

[a] Human placenta, microsomal fraction, substrate [^{3}H]-E2 + E2 [500 nM], NAD^+ [1.5 mM], mean value of 3 determinations, relative standard deviation < 10%, [b] Human placenta, cytosolic fraction, substrate [^{3}H]-E1 + E1 [500 nM], NADH [0.5 mM], mean value of 3 determinations, relative standard deviation < 10%, n.i.: no inhibition (inhibition < 10%).

In order to evaluate the selectivity profile of these compounds, all synthesised molecules were tested for their 17β-HSD1 inhibition (Table 1 and 2). For the most potent inhibitors identified in this report, IC_{50} data for 17β-HSD2 and 17β-HSD1 were determined as well as their binding affinity to ERα and β (Table 3). The most interesting compound identified in this study is the 4-hydroxyphenyl-1-naphthol **8**, with an IC_{50} of 302 nM for 17β-HSD2 inhibition. A certain selectivity toward 17β-HSD1 has been achieved (selectivity factor: 8). Taking into account the structural similarity between E2 and compound **8**, it was important to evaluate the affinity of **8** to the ERs, which is expressed as relative binding affinity [42].[43] The RBA measured represents the ligand affinity to ER, relative to that of E2 which is arbitrarily set up at 100%. With a RBA value of 5% for ERα and β, compound **8**, however, has a similar affinity as E1 which is weakly estrogenic.[44]

Table 3. IC_{50} values, selectivity factor and binding affinities for the estrogen receptors (ER) α and β for selected compounds.

Cmpd	Cell free assay			ERα	ERβ
	17β-HSD2 IC_{50} [nM][a]	17β-HSD1 IC_{50} [nM][b]	Selectivity factor[c]	RBA (%)[d]	RBA (%)[d]
B	5641	116	0.02	0.2	0.8
8	302	2425	8	5	5
20	600	614	1	n.d.	n.d.
24	696	8516	12	n.d.	n.d.

[a] Human placenta, microsomal fraction, substrate [^{3}H]-E2+ E2 [500 nM], cofactor NAD^+ [1.5 mM], mean value of 3 determinations, relative standard deviation < 10%, [b] Human placenta, cytosolic fraction, substrate [^{3}H]-E1 +E1 [500 nM], cofactor NADH [0.5 mM], mean value of 3 determinations, relative standard deviation < 20%, [c] IC_{50} (17β-HSD1)/ IC_{50} (17β-HSD2), [d] RBA: Relative Binding Affinity, E2: 100%; n.d.: not determined.

Discussion and conclusion

The goal of this study was the identification of a new non-steroidal scaffold for 17β-HSD2 inhibition. This was a difficult task, considering the structural similarities between 17β-HSD2 and 17β-HSD1 in their active sites. Taking advantage of our experience developing 17β-HSD1 inhibitors in the class of hydroxyphenylnaphthols,[15, 16] compound **8** was identified as potent and selective 17β-HSD2 inhibitor while compound **B** showed a high inhibitory activity for 17β-HSD1 only. Compounds **B** and **8** share several structural similarities, they differ only in their hydroxy substitution pattern: in **B**, one OH is in 2-position of the naphthalene and the other is in *meta* on the phenyl while in **8**, the naphthalene is hydroxylated in 1-position and the phenyl in *para* (Chart 3). Because of the different hydroxy substitution pattern not only activity for 17β-HSD2 could be gained but also selectivity toward 17β-HSD1 could be reached. A minor structural change in the ligand can completely reverse the activity and selectivity profile. This indicates that the small differences between the active sites of these two enzymes are very important and can lead to a radical ligand discrimination. Further studies about selectivity regarding other 17β-HSDs and SDR enzymes will be evaluated in the future. Selectivity toward the ER which is an important issue could be gained by introduction of large substituents into this scaffold.

As neither a 3D-structure nor a good homology model of 17β-HSD2 exists, the information on the active site architecture is limited and identification of amino acids which could be important for interaction with the potential inhibitors is not possible. Therefore in this study,

drug design was focused on a ligand-based approach using the substrate E2 and other ligands like **B** (17β-HSD1 inhibitor). In case of 17β-HSD1, several X-ray structures of the enzyme with or without ligand are available.[45] In a previous work we mentioned that the hydroxyphenyl moiety of **B** certainly mimics the steroidal A-ring.[15] The superimposition between the OH moieties of **B** (17β-HSD1 inhibitor) and of **8** (17β-HSD2 inhibitor) (Chart 3) also indicates that the hydroxyphenyl moiety of **B** most likely does not overlap with the one of **8**, thus the OH-phenyl of **8** would mimic the steroidal D-ring, interacting with the catalytic triad. It can be expected that in this area of the enzyme, close to the catalytic triad, there is more free space available than close to the A-ring for a large substituent. This results is indeed in accordance with the biological data obtained for **20** and **24**, where the hydroxy moiety (**8**, 74% inhibition at 1 µM) is replaced by the larger methylsulfonamide (**20**, 75% inhibition at 1 µM) or acetamide (**24**, 64% inhibition at 1 µM) without loss in activity. This finding will be further validated by substitution of OH-naphthalene or by introduction of substituent close to this hydroxy moiety in **8**.

Attempts to simplify the scaffold of compound **8** failed: one hydroxy group is not enough to stabilise the compound in the active site. The aromatic ring 8A of the naphthalene, mimicking the steroidal A-ring seems to be necessary for inhibitory activity, it might interact with the enzyme via π-π-interactions.

Exchange of the hydroxy group on the phenyl moiety by electron withdrawing groups or exchange of the hydroxyphenyl by heteroaromatic rings led to a complete loss in activity indicating the crucial role of the hydrogen bond donating properties of the OH-phenyl moiety to stabilise the compound in the active site.

The structure-activity relationship which can be deduced from the biological data gives very useful informations to map the unknown enzyme active site of 17β-HSD2.

In this report, a new scaffold for 17β-HSD2 inhibitors was identified: the 4-hydroxyphenyl-1-naphthol **8**, with a good inhibitory activity (IC_{50} = 302 nM), a selectivity factor of 8 toward 17β-HSD1 and only weak estrogenicity. As hydroxynaphthyl and hydroxyphenyl compounds have been associated with the formation of reactive intermediates and DNA-adducts, the safety of compound **8** will be further evaluated and attempts to improve the biological profile (activity, selectivity) will be performed.

Experimental Section

Chemical Methods

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DRX-500 instrument in $CDCl_3$, CD_3OD and acetone-d_6. Chemicals shifts are reported in δ values (ppm), the hydrogenated residues of deuteriated solvent were used as internal standard ($CDCl_3$: δ = 7.26 ppm in ^{1}H NMR and δ = 77 ppm in ^{13}C NMR, CD_3OD: δ = 3.35 ppm in ^{1}H NMR and δ = 49.3 ppm in ^{13}C NMR, acetone-d_6: δ = 2.05 ppm in ^{1}H NMR and δ = 29.8 ppm and 206.3 ppm in ^{13}C NMR). Signals are described as br, s, d, t, dd, ddd, dt, qt and m for broad, singlet, doublet, triplet, doublet of doublets, doublet of doublet of doublets, doublet of triplets, quintuplet and multiplet, respectively. All coupling constants (*J*) are given in Hertz.

IR spectra were measured neat on a Bruker Vector 33FT-infrared spectrometer.

Mass spectra (ESI) were measured on a TSQ Quantum (Thermo Electron Corporation) instrument.

All microwave irradiation experiments were carried out in a CEM-Discover microwave apparatus.

Flash chromatography was performed using silica gel 40 (35/40-63/70 μM) with hexane/ethyl acetate mixtures as eluents. Reaction progress was monitored by thin-layer chromatography analyses on Alugram SIL G/UV254 (Macherey-Nagel). Visualisation was accomplished with UV light. Purifications with preparative HPLC were carried out on a Agilent 1200 series HPLC system from Agilent Technologies, using a RP C18 Nucleodur 100-5 column (30·100 mm/50 μm – from Macherey-Nagel GmbH) as stationary phase with acetonitrile/water as solvent in a gradient from 20:80 to 100:0.

Tested compounds show ≥ 95% chemical purity as measured by HPLC. The methods for HPLC analysis and a table of data for all tested compounds are provided in the supporting information.

Chemical names follow IUPAC nomenclature. Starting materials were used as obtained from Aldrich, Acros and Combi-blocks without further purification. No attempts were made to optimise yields.

The following compounds were prepared according to previously described procedures: 6-(2-hydroxyphenyl)-2-naphthol **1**,[34] 6-(4-hydroxyphenyl)-2-naphthol **2**,[34] 6-phenyl-2-naphthol **3**,[15] 3-(2-naphthyl)phenol **4**,[46] 6-(3-hydroxyphenyl)-1-naphthol **6**,[34] 6-(4-hydroxyphenyl)-1-naphthol **8**,[34] 5-oxo-5,6,7,8-tetrahydro-2-naphthyl trifluoromethanesulfonate **5c**,[34] 6-hydroxy-1,2,3,4-tetrahydronapthalen-1-one **5d**,[33] 3-benzamidophenylboronic acid **25a**.[47]

General procedures for Suzuki coupling

Method A. To a mixture of arylbromide (1 equiv) and tetrakis(triphenylphosphine) palladium (0) (0.02 equiv) in DME was added a 2M aqueous solution of caesium carbonate (2 equiv). The mixture was purged with N_2 and stirred at room temperature for 5 min. Subsequently a solution of boronic acid (1.2 equiv) in EtOH was added. The mixture was heated to 90°C for 2h. The reaction mixture was cooled to room temperature, quenched with water and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate and concentrated to dryness under reduced pressure. The product was purified by column chromatography.

Method B. A mixture of arylbromide (1 equiv), boronic acid (1.2 equiv), sodium carbonate (2 equiv), and tetrakis(triphenylphosphine) palladium (0) (0.02 equiv) was suspended in an oxygen-free DME/water (2:1) solution. The reaction mixture was exposed to microwave irradiation (25 min, 150W, 150°C, 15 bar). After reaching room temperature, water was added and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate and concentrated to dryness under reduced pressure. The product was purified by column chromatography.

General procedure for amide bound formation

Method C. *N*-methylaniline derivative (1 equiv) was mixed with Et_3N (2 equiv) in dichloromethane. The solution was cooled at 0°C. The acyl chloride (1.2 equiv) was slowly added and the mixture stirred at room temperature for 1h. The reaction mixture was extracted with ethyl acetate. The organic layer was washed with water, dried over sodium sulfate and concentrated to dryness under reduced pressure. The product was purified by column chromatography.

6-Bromo-5-oxo-5,6,7,8-tetrahydro-2-naphthyl trifluoromethanesulfonate (5b). A solution of Br_2 (1.1 g, 0.35 mL, 6.9 mmol, 1.05 equiv) in CCl_4 (6 mL) was added dropwise to a stirred solution of 5-oxo-5,6,7,8-tetrahydro-2-naphthyl trifluoromethanesulfonate **5c** (2.0 g, 6.8 mmol, 1 equiv) in dry ether (60 mL). The ether solution contained 0.3 mL of etheral hydrogen chloride (1 M) and was maintained at 0-5°C throughout the addition. After the complete addition of bromine, the reaction mixture was stirred for another 30 min at the same temperature and then water was added followed by ether. The organic layer was separated and washed with water. The solvent was removed under reduced pressure to afford the expected

product in quantitative yield (2.5 g). $C_{11}H_8O_4BrSF_3$; MW 373; ^{1}H NMR ($CDCl_3$): δ 8.26 (d, *J* = 8.7 Hz, 1H), 7.57-7.55 (m, 1H), 7.54 (dd, *J* = 2.5 Hz, *J* = 8.7 Hz, 1 H), 3.29-3.25 (m, 3H), 3.21-3.18 (m, 2H).

5-Hydroxy-2-naphthyl trifluoromethanesulfonate (5a). A solution of 6-bromo-5-oxo-5,6,7,8-tetrahydro-2-naphthyl trifluoromethanesulfonate **5b** (5.3 g, 14.1 mmol, 1 equiv) in DMF (100 mL) containing a mixture of lithium bromide (2.8 g, 32.4 mmol, 2.3 equiv) and lithium carbonate (2.1 g, 28.2 mmol, 2 equiv) was refluxed for 4h in an atmosphere of nitrogen. The solvent was removed under reduced pressure and the residue was treated with ice cold water followed by cold diluted hydrochloric acid. The mixture was extracted with ether and the combined organic layers were washed with 10% NaOH. The alkaline extract was acidified and again extracted with ether. The solvent was removed under vacuum and the crude product was purified using silica gel chromatography (hexane/ethyl acetate 9:1) to afford 65% (2.7 g) of the analytically pure product. $C_{11}H_7SO_4F_3$; MW 292; ^{1}H NMR (acetone-d_6): δ 9.35 (s, br, 1H), 8.38 (d, *J* = 9.2 Hz, 1H), 7.90 (d, *J* = 2.8 Hz, 1H), 7.80 (d, *J* = 8.6 Hz, 1H), 7.43 (d, *J* = 7.9 Hz, 1H), 7.36-7.33 (m, 1H), 7.00 (dd, *J* = 1.0 Hz, *J* = 7.6 Hz, 1H); ^{13}C NMR (acetone-d_6): δ 148.7, 136.0, 129.5, 128.0, 126.8, 126.4, 123.0, 120.0, 119.7, 118.5, 110.4; IR: 3243, 3111, 1422, 1137 cm^{-1}.

6-Phenyl-1-naphthol (5). The title compound was prepared by reaction of 5-hydroxy-2-naphthyl trifluoromethanesulfonate **5a** (200 mg, 0.68 mmol, 1 equiv) with phenylboronic acid (108 mg, 0.89 mmol, 1.3 equiv) according to method A. The analytically pure compound was obtained after purification by column chromatography (gradient hexane/ethyl acetate 10:0 to 9:1) in 42% yield (63 mg) as a brown powder. $C_{16}H_{12}O$; MW 220; m.p.: 108-110°C; ^{1}H NMR (acetone-d_6): δ 9.00 (s, br, 1H), 8.33 (d, *J* = 9.2 Hz, 1H), 8.09 (d, *J* = 2.2 Hz, 1H), 7.81-7.77 (m, 3H), 7.55-7.46 (m, 3H), 7.41-7.37 (m, 1H), 7.36-7.32 (m, 1H), 6.93 (dd, *J* = 1.0 Hz, *J* = 7.4 Hz, 1H); ^{13}C NMR (acetone-d_6): δ 154.0, 141.8, 139.4, 136.3, 130.7, 129.8, 128.2, 128.1, 127.6, 126.1, 125.0, 123.7, 120.4, 109.2; IR: 3370, 3060, 1582, 1259 cm^{-1}; GC/MS *m/z*: 220 $(M)^+$.

6-(4-Methoxyphenyl)-1-naphthol (7). The title compound was prepared by reaction of 5-hydroxy-2-naphthyl trifluoromethanesulfonate **5a** (200 mg, 0.68 mmol, 1 equiv) with 3-methoxyphenylboronic acid (135 mg, 0.89 mmol, 1.3 equiv) according to method A. The analytically pure compound was obtained after purification by column chromatography (gradient hexane/ethyl acetate 10:0 to 9:1) in 54% yield (91 mg) as an orange oil. $C_{17}H_{14}O_2$;

MW 250; ^{1}H NMR (acetone-d_6): δ 8.96 (s, br, 1H), 8.30 (d, *J* = 8.8 Hz, 1H), 8.03 (d, *J* = 2.0 Hz, 1H), 7.76-7.72 (m, 3H), 7.43 (d, *J* = 8.0 Hz, 1H), 7.31 (t, *J* = 8.0 Hz, 1H), 7.08-7.04 (m, 2H), 6.91 (dd, *J* = 0.9 Hz, *J* = 7.4 Hz, 1H), 3.86 (s, 3H); ^{13}C NMR (acetone-d_6): δ 160.5, 154.0, 139.1, 136.3, 134.1, 129.0, 127.5, 125.2, 124.7, 123.6, 120.3, 115.2, 108.9, 55.6; IR: 3386, 3051, 2986, 1522, 1238 cm^{-1}; LC/MS *m/z:* 251 $(M+H)^+$.

6-[4-(Hydroxymethyl)phenyl]-1-naphthol (9). The title compound was prepared by reaction of 5-hydroxy-2-naphthyl trifluoromethanesulfonate **5a** (70 mg, 0.24 mmol, 1 equiv) with 4-(hydroxymethyl)phenylboronic acid (44 mg, 0.29 mmol, 1.2 equiv) according to method A. The product was purified by column chromatography (hexane/ethyl acetate 1:1). Yield: 49% (27 mg). Brown powder. $C_{17}H_{14}O_2$; MW 250; m.p.: 183-185°C; ^{1}H NMR (acetone-d_6): δ 9.03 (s, br, 1H), 8.32 (d, *J* = 8.8 Hz, 1H), 8.09 (d, *J* = 1.7 Hz, 1H), 7.80-7.75 (m, 3H), 7.52-7.45 (m, 3H), 7.35-7.31 (m, 1H), 6.93 (dd, *J* = 0.7 Hz, *J* = 7.5 Hz, 1H), 4.71 (s, 2H); ^{13}C NMR (acetone-d_6): δ 154.0, 142.7, 140.3, 139.3, 136.3, 128.0, 127.8, 127.6, 125.9, 125.0, 124.8, 123.7, 120.3, 109.1, 64.4; IR: 3199, 3063, 1345 cm^{-1}; LC/MS *m/z*: 251 $(M+H)^+$.

6-(4-Fluorophenyl)-1-naphthol (10). The title compound was prepared by reaction of 5-hydroxy-2-naphthyl trifluoromethanesulfonate **5a** (150 mg, 0.51 mmol, 1 equiv) with 4-fluorophenylboronic acid (86 mg, 0.62 mmol, 1.2 equiv) according to method A. The analytically pure product was obtained after purification by column chromatography (hexane/ethyl acetate 9:1) in 56% yield (68 mg) as an orange oil. $C_{16}H_{11}FO$; MW 238; ^{1}H NMR (acetone-d_6): δ 8.70 (s, br, 1H), 7.71 (d, *J* = 8.2 Hz, 1H), 7.69 (d, *J* = 9.2 Hz, 1H), 7.51-7.47 (m, 2H), 7.44 (dd, *J* = 6.9 Hz, *J* = 8.2 Hz, 1H), 7.30-7.25 (m, 3H), 7.19 (dd, *J* = 1.3 Hz, *J* = 7.0 Hz, 1H), 7.12 (dd, *J* = 2.5 Hz, *J* = 9.2 Hz, 1H); ^{13}C NMR (acetone-d_6): δ 164.1, 156.2, 139.9, 138.1, 136.6, 132.5, 132.4, 128.0, 127.3, 127.1, 126.7, 125.0, 119.4, 116.0, 115.8, 110.4; LC/MS *m/z*: 239 $(M+H)^+$.

6-(4-Nitrophenyl)-1-naphthol (11). The title compound was prepared by reaction of 5-hydroxy-2-naphthyl trifluoromethanesulfonate **5a** (276 mg, 0.94 mmol, 1 equiv) with 4-nitrophenylboronic acid (205 mg, 1.13 mmol, 1.2 equiv) according to method A. The product was purified by column chromatography (hexane/ethyl acetate 6:4). Yield: 23% (57 mg). Yellow oil. $C_{16}H_{11}NO_3$; MW 265; ^{1}H NMR (acetone-d_6): δ 9.15 (s, br, 1H), 8.40-8.37 (m, 3H), 8.27 (dd, *J* = 1.9 Hz, *J* = 8.7 Hz, 1H), 8.12-8.10 (m, 2H), 7.88 (dd, *J* = 1.9 Hz, *J* = 8.6 Hz, 1H), 7.53 (d, *J* = 8.3 Hz, 1H), 7.39 (t, *J* = 7.6 Hz, 1H), 6.99 (dd, *J* = 0.6 Hz, *J* = 7.6 Hz, 1H); ^{13}C NMR (acetone-d_6): δ 154.1, 148.8, 148.3, 148.1, 131.9, 129.0, 128.2, 127.7, 127.4,

126.7, 124.9, 124.4, 124.3, 124.2, 120.6, 120.0; IR: 3244, 3023, 1605, 1517, 1391 cm^{-1}; GC/MS *m/z*: 265 $(M)^+$.

4-(5-Hydroxy-2-naphthyl)benzonitrile (12). The title compound was prepared by reaction of 5-hydroxy-2-naphthyl trifluoromethanesulfonate **5a** (146 mg, 0.50 mmol, 1 equiv) with 4-cyanophenylboronic acid (91 mg, 0.60 mmol, 1.2 equiv) according to method A. The product was purified by column chromatography (hexane/ethyl acetate 7:3) to afford 33 mg of the analytically pure product (27%) as a beige powder. $C_{17}H_{11}NO$; MW 245; m.p.: 174-176°C; ^{1}H NMR (acetone-d_6): δ 9.11 (s, br, 1H), 8.36 (d, *J* = 8.8 Hz, 1H), 8.22 (d, *J* = 1.8 Hz, 1H), 8.05-8.02 (m, 2H), 7.93-7.90 (m, 2H), 7.84 (dd, *J* = 1.9 Hz, *J* = 8.8 Hz, 1H), 7.51 (d, *J* = 8.3 Hz, 1H), 7.40-7.36 (m, 1H), 6.98 (d, *J* = 7.6 Hz, 1H); ^{13}C NMR (acetone-d_6): δ 156.4, 154.0, 133.6, 133.1, 131.7, 128.9, 127.9, 127.7, 127.1, 126.7, 125.1, 124.4, 120.6, 119.8, 110.5, 109.8; IR: 3199, 3063, 1345 cm^{-1}; LC/MS *m/z*: 244 $(M-H)^-$.

1-[4-(5-Hydroxy-2-naphthyl)phenyl]ethanone (13). The title compound was prepared by reaction of 5-hydroxy-2-naphthyl trifluoromethanesulfonate **5a** (100 mg, 0.34 mmol, 1 equiv) with 4-acetylphenylboronic acid (67 mg, 0.41 mmol, 1.2 equiv) according to method A. The product was purified by column chromatography (hexane/ethyl acetate 6:4). Yield: 33% (40 mg). Yellow powder. $C_{18}H_{14}O_2$; MW 262; m.p.: 155-157°C; ^{1}H NMR (acetone-d_6): δ 8.70 (s, br, 1H), 8.15-8.12 (m, 2H), 7.75 (d, *J* = 8.3 Hz, 1H), 7.71 (d, *J* = 9.1 Hz, 1H), 7.63-7.60 (m, 2H), 7.50-7.45 (m, 1H), 7.30 (d, *J* = 2.5 Hz, 1H), 7.24 (dd, *J* = 0.9 Hz, *J* = 7.0 Hz, 1H), 7.13 (dd, *J* = 2.6 Hz, *J* = 9.1 Hz, 1H), 2.66 (s, 3H); ^{13}C NMR (acetone-d_6): δ 197.6, 156.3, 146.6, 139.9, 137.1, 136.6, 130.9, 129.8, 129.2, 128.2, 127.9, 127.6, 126.9, 126.7, 124.9, 119.6, 110.5, 26.8; IR: 3250, 3096, 1655, 1397, 1270 cm^{-1}; LC/MS *m/z*: 263 $(M+H)^+$.

4-(5-Hydroxy-2-naphthyl)benzoic acid (14). The title compound was prepared by reaction of 5-hydroxy-2-naphthyl trifluoromethanesulfonate **5a** (200 mg, 0.68 mmol, 1 equiv) with 4-boronobenzoic acid (147 mg, 0.82 mmol, 1.2 equiv) according to method A. The product was purified by column chromatography (hexane/ethyl acetate 1:1) to give 77% of the analytically pure product (142 mg) as a yellow powder. $C_{17}H_{12}O_3$; MW 264; m.p.: 234-236°C; ^{1}H NMR (acetone-d_6): δ 11.03 (s, br, 1H), 9.07 (s, br, 1H), 8.36 (d, *J* = 8.8 Hz, 1H), 8.20 (d, *J* = 1.7 Hz, 1H), 8.18-8.16 (m, 2H), 7.96-7.94 (m, 2H), 7.85 (dd, *J* = 1.9 Hz, *J* = 8.8 Hz, 1H), 7.51 (d, *J* = 8.4 Hz, 1H), 7.39-7.34 (m, 1H), 6.97 (d, *J* = 7.4 Hz, 1H); ^{13}C NMR (acetone-d_6): δ 167.5, 154.0, 146.2, 138.2, 136.1, 132.7, 131.4, 130.4, 128.0, 127.9, 126.8, 125.4, 124.6, 124.0, 120.6, 116.0, 109.6; IR: 3241, 3102, 1682, 1608, 1297 cm^{-1}; LC/MS *m/z*: 265 $(M+H)^+$.

6-(Pyridin-4-yl)-1-naphthol (15). The title compound was prepared by reaction of 5-hydroxy-2-naphthyl trifluoromethanesulfonate **5a** (200 mg, 0.68 mmol, 1 equiv) with 4-pyridylboronic acid (100 mg, 0.82 mmol, 1.2 equiv) according to method A. The product was purified by column chromatography (hexane/ethyl acetate 9:1) and then recrystallised in ethyl acetate to afford 54% (81 mg) of the analytically pure product as a yellow powder. $C_{15}H_{11}NO$; MW 221; m.p.: 85.4 °C; ^{1}H NMR (acetone-d_6): δ 7.93-7.91 (m, 2H), 7.58 (d, J = 8.7 Hz, 1H), 7.53-7.51 (m, 1H), 7.13-7.10 (m, 3H), 6.76 (d, J = 8.0 Hz, 1H), 6.66-6.62 (m, 1H), 6.21-6.18 (m, 1H); ^{13}C NMR (acetone-d_6): δ 148.7, 126.1, 124.9, 122.2, 121.7, 120.6, 118.2, 107.9; IR: 3054, 1577, 1239 cm^{-1}; LC/MS *m/z*: 222 $(M+H)^+$.

6-(Pyrimidin-5-yl)-1-naphthol (16). The title compound was prepared by reaction of 5-hydroxy-2-naphthyl trifluoromethanesulfonate **5a** (200 mg, 0.68 mmol, 1 equiv) with 5-pyrimidinylboronic acid (102 mg, 0.82 mmol, 1.2 equiv) according to method A. The product was purified by column chromatography (hexane/ethyl acetate 3:1) to afford 65 mg (43%) of the analytically pure product as a yellow powder. $C_{14}H_{10}N_2O$; MW 222; m.p.: 242-243°C; ^{1}H NMR (CD_3OD): δ 9.25 (s, 2H), 9.21 (s, 1H), 8.41 (d, J = 5.1 Hz, 1H), 8.26 (d, J = 1.8 Hz, 1H), 7.86 (dd, J = 2.0 Hz, J = 8.8 Hz, 1H), 7.52 (d, J = 8.3 Hz, 1H), 7.43-7.39 (m, 1H), 6.97 (d, J = 7.8 Hz, 1H); ^{13}C NMR (CD_3OD): δ 158.3, 156.4, 136.6, 135.8, 133.0, 128.6, 127.4, 125.0, 124.3, 120.7, 119.0, 110.2; IR: 3022, 1278 cm^{-1}; LC/MS *m/z*: 223 $(M+H)^+$.

6-(4-Methoxypyridin-3-yl)-1-naphthol (17). The title compound was prepared by reaction of 5-hydroxy-2-naphthyl trifluoromethanesulfonate **5a** (50 mg, 0.17 mmol, 1 equiv) with 4-methoxy-3-pyridylboronic acid (67 mg, 0.44 mmol, 2.6 equiv) according to method A. The desired product was obtained after purification by column chromatography (hexane/ethyl acetate 7:3) and by preparative HPLC. Yield: 47% (10 mg). Brown solid. $C_{17}H_{13}NO_2$; MW 251; m.p.: 165-166°C; ^{1}H NMR ($CDCl_3$): δ 8.10 (dd, J = 0.6 Hz, J = 2.5 Hz, 1H), 7.61 (dd, J = 2.4 Hz, J = 8.6 Hz, 1H), 7.57-7.54 (m, 2H), 7.32-7.28 (m, 1H), 7.09 (d, J = 2.5 Hz, 1H), 7.05 (dd, J = 1.1 Hz, J = 7.0 Hz, 1H), 6.95 (dd, J = 2.4 Hz, J = 9.3 Hz, 1H), 6.76 (dd, J = 0.9 Hz, J = 8.5 Hz, 1H), 3.88 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 163.4, 154.9, 146.8, 140.6, 136.0, 135.4, 130.0, 127.0, 126.6, 126.5, 125.8, 124.4, 118.5, 110.2, 109.6, 53.5; IR: 3215, 1579, 1498, 1286 cm^{-1}; LC/MS *m/z:* 252 $(M+H)^+$.

6-(3-Furyl)-1-naphthol (18). The title compound was prepared by reaction of 5-hydroxy-2-naphthyl trifluoromethanesulfonate **5a** (200 mg, 0.68 mmol, 1 equiv) with 3-furylboronic acid

(91 mg, 0.82 mmol, 1.2 equiv) according to method A. The product was purified by column chromatography (hexane/ethyl acetate 9:1). Yield: 40% (57 mg). Brown oil. $C_{14}H_{10}O_2$; MW 210; ^{1}H NMR (acetone-d_6): δ 8.16 (d, *J* = 8.8 Hz, 1H), 7.82 (d, *J* = 1.5 Hz, 1H), 7.80-7.78 (m, 1H), 7.55 (dd, *J* = 1.6 Hz, *J* = 8.6 Hz, 1H), 7.47-7.45 (m, 1H), 7.36-7.33 (m, 1H), 7.26-7.22 (m, 1H), 6.78-6.75 (m, 2H), 6.60 (s, 1H); ^{13}C NMR (acetone-d_6): δ 171.5, 152.0, 143.7, 138.9, 135.1, 130.2, 126.5, 126.4, 123.7, 123.5, 122.5, 120.1, 108.8, 108.4; IR: 3320, 1368 cm^{-1}; LC/MS *m/z*: 211 $(M+H)^+$.

4-(5-Hydroxy-2-naphthyl)-*N*-methylbenzenesulfonamide (19). The title compound was prepared by reaction of 5-hydroxy-2-naphthyl trifluoromethanesulfonate **5a** (100 mg, 0.34 mmol, 1 equiv) with 4-[(methylamino)sulfonyl]phenylboronic acid (88 mg, 0.41 mmol, 1.2 equiv) according to method B. The product was purified by column chromatography (gradient hexane/ethyl acetate 1:0 to 3:1) to afford the analytically pure compound in 90% yield (84 mg) as a yellow powder. $C_{17}H_{15}NO_3S$; MW 313; m.p.: 204-207°C; ^{1}H NMR (acetone-d_6): δ 9.10 (s, 1H), 8.36 (d, *J* = 8.6 Hz, 1H), 8.21 (d, *J* = 1.8 Hz, 1H), 8.04-8.01 (m, 2H), 7.99-7.95 (m, 2H), 7.85-7.84 (m, 1H), 7.51 (d, *J* = 8.4 Hz, 1H), 7.39-7.35 (m, 1H), 6.97 (dd, *J* = 1.0 Hz, *J* = 7.6 Hz, 1H), 6.36 (qt, *J* = 5.1 Hz, 1H), 2.64 (d, *J* = 5.1 Hz, 3H); ^{13}C NMR (acetone-d_6): δ 154.0, 145.7, 139.6, 137.7, 136.1, 133.2, 129.9, 128.6, 127.9, 127.8, 126.9, 125.4, 124.6, 124.1, 120.6, 109.7; IR: 3259, 2971, 1378 cm^{-1}; LC/MS *m/z*: 314 $(M+H)^+$.

***N*-[4-(5-Hydroxy-2-naphthyl)phenyl]methanesulfonamide (20).** The title compound was prepared by reaction of 5-hydroxy-2-naphthyl trifluoromethanesulfonate **5a** (100 mg, 0.34 mmol, 1 equiv) with 4-[(methylsulfonyl)amino]phenylboronic acid (88 mg, 0.41 mmol, 1.2 equiv) according to method B. The product was purified by column chromatography (hexane/ethyl acetate 3:1) to give the analytically pure compound in 57% yield (61 mg) as an orange powder. $C_{17}H_{15}NO_3S$; MW 313; m.p.: 194-195°C; ^{1}H NMR (acetone-d_6): δ 9.33 (s, 1H), 8.92 (s, 1H), 8.53 (d, *J* = 8.8 Hz, 1H), 8.30 (d, *J* = 1.6 Hz, 1H), 8.05-8.01 (m, 2H), 8.00 (dd, *J* = 1.6 Hz, *J* = 8.8 Hz, 1H), 7.72-7.69 (m, 2H), 7.67 (d, *J* = 8.8 Hz, 1H), 7.57-7.55 (m, 1H), 7.16-7.13 (m, 1H), 3.27 (s, 3H); ^{13}C NMR (acetone-d_6): δ 154.1, 138.9, 138.6, 137.8, 136.3, 131.6, 128.9, 127.7, 125.7, 125.0, 124.6, 123.8, 121.5, 120.9, 120.3, 109.1, 39.5; IR: 3384, 2934, 1692, 1519, 1322 cm^{-1}; LC/MS *m/z*: 314 $(M+H)^+$.

***N*-[3-(5-Hydroxy-2-naphthyl)phenyl]methanesulfonamide (21).** The title compound was prepared by reaction of 5-hydroxy-2-naphthyl trifluoromethanesulfonate **5a** (100 mg, 0.34 mmol, 1 equiv) with 3-[(methylsulfonyl)amino]phenylboronic acid (88 mg, 0.41 mmol, 1.2

equiv) according to method B. The product was purified by column chromatography (hexane/ethyl acetate 3:1) to give the analytically pure compound in 39% yield (41 mg) as an orange oil. $C_{17}H_{15}NO_3S$; MW 313; ^{1}H NMR (acetone-d_6): δ 9.08 (s, 1H), 8.66 (s, br, 1H), 8.33 (d, *J* = 8.8 Hz, 1H), 8.09 (d, *J* = 1.9 Hz, 1H), 7.78-7.75 (m, 2H), 7.60-7.58 (m, 1H), 7.52-7.46 (m, 2H), 7.41-7.38 (m, 1H), 7.34 (t, *J* = 8.1 Hz, 1H), 6.95 (dd, *J* = 1.0 Hz, *J* = 7.3 Hz, 1H), 3.07 (s, 3H); ^{13}C NMR (acetone-d_6): δ 154.1, 143.1, 140.1, 138.9, 136.2, 130.8, 127.8, 126.2, 125.2, 124.7, 124.0, 123.9, 120.4, 120.1, 119.9, 109.3, 39.5; IR: 3259, 1705, 1601, 1275 cm^{-1}; LC/MS *m/z*: 314 $(M+H)^+$.

4-(5-Hydroxy-2-naphthyl)-*N*-methylbenzamide (22). The title compound was prepared by reaction of 5-hydroxy-2-naphthyl trifluoromethanesulfonate **5a** (200 mg, 0.68 mmol, 1 equiv) with 4-(*N*-methylaminocarbonyl)phenylboronic acid (147 mg, 0.82 mmol, 1.2 equiv) according to method B. The analytically pure product was obtained after column chromatography (hexane/ethyl acetate 3:1) in 68% yield (128 mg) as a brown oil. $C_{18}H_{15}NO_2$; MW 277; ^{1}H NMR (acetone-d_6): δ 9.13 (s, 1H), 8.33 (d, *J* = 8.9 Hz, 1H), 8.17 (d, *J* = 1.9 Hz, 1H), 8.10 (s, br, 1H), 8.07-8.04 (m, 2H), 7.91-7.88 (m, 2H), 7.82 (dd, *J* = 1.9 Hz, *J* = 8.7 Hz, 1H), 7.48 (d, *J* = 8.4 Hz, 1H), 7.35 (t, *J* = 8.1 Hz, 1H), 6.95 (dd, *J* = 1.0 Hz, *J* = 7.4 Hz, 1H), 2.98 (s, 3H); ^{13}C NMR (acetone-d_6): δ 154.1, 144.6, 138.2, 136.1, 133.0, 132.9, 128.9, 127.9, 127.8, 126.5, 125.3, 124.6, 123.9, 120.5, 109.5, 27.0; IR: 3483, 1643, 1336 cm^{-1}; LC/MS *m/z*: 278 $(M+H)^+$.

3-(5-Hydroxy-2-naphthyl)-*N*-methylbenzamide (23). The title compound was prepared by reaction of 5-hydroxy-2-naphthyl trifluoromethanesulfonate **5a** (100 mg, 0.34 mmol, 1 equiv) with 3-(*N*-methylaminocarbonyl)phenylboronic acid (74 mg, 0.41 mmol, 1.2 equiv) according to method B. The analytically pure product was obtained after column chromatography (gradient dichloromethane/methanol 1:0 to 20:1) in 54% yield (51 mg) as a brown oil. $C_{18}H_{15}NO_2$; MW 277; ^{1}H NMR (acetone-d_6): δ 9.14 (s, 1H), 8.34 (d, *J* = 8.6 Hz, 1H), 8.30 (t, *J* = 1.9 Hz, 1H), 8.14 (d, *J* = 1.9 Hz, 1H), 7.95-7.93 (m, 1H), 7.92-7.90 (m, 1H), 7.88 (s, 1H), 7.82 (dd, *J* = 1.9 Hz, *J* = 8.6 Hz, 1H), 7.60-7.57 (m, 1H), 7.48 (d, *J* = 8.2 Hz, 1H), 7.35 (t, *J* = 7.7 Hz, 1H), 6.95 (dd, *J* = 0.9 Hz, *J* = 7.6 Hz, 1H), 2.95 (d, *J* = 4.7 Hz, 3H); ^{13}C NMR (acetone-d_6): δ 154.2, 141.9, 132.8, 132.7, 130.5, 129.8, 129.5, 129.4, 127.8, 127.0, 126.6, 126.3, 124.7, 123.9, 120.3, 109.4, 26.7; IR: 3166, 1574, 1272 cm^{-1}; LC/MS *m/z*: 278 $(M+H)^+$.

***N*-[3-(5-Hydroxy-2-naphthyl)phenyl]acetamide (24).** The title compound was prepared by reaction of 5-hydroxy-2-naphthyl trifluoromethanesulfonate **5a** (100 mg, 0.34 mmol, 1 equiv)

with 3-acetamidophenylboronic acid (74 mg, 0.41 mmol, 1.2 equiv) according to method B. The analytically pure product was obtained after column chromatography (gradient dichloromethane/methanol 1:0 to 20:1) in 42% yield (40 mg) as a yellow powder. $C_{18}H_{15}NO_2$; MW 277; m.p.: 105-106°C; ^{1}H NMR (acetone-d_6): δ 9.24 (s, 1H), 9.04 (s, 1H), 8.32 (d, *J* = 8.6 Hz, 1H), 8.10-8.08 (m, 1H), 8.05 (d, *J* = 1.6 Hz, 1H), 7.75 (dd, *J* = 1.8 Hz, *J* = 8.8 Hz, 1H), 7.69 (d, *J* = 7.5 Hz, 1H), 7.49-7.45 (m, 2H), 7.42 (t, *J* = 7.9 Hz, 1H), 7.33 (t, *J* = 7.9 Hz, 1H), 6.93 (dd, *J* = 1.0 Hz, *J* = 7.4 Hz, 1H), 2.13 (s, 3H); ^{13}C NMR (acetone-d_6): δ 154.0, 147.6, 136.3, 130.1, 127.7, 126.0, 124.8, 123.7, 122.8, 120.3, 119.0, 118.7, 109.2, 24.3; IR: 3208, 3082, 2944, 1670 cm^{-1}; LC/MS *m/z*: 278 $(M+H)^+$.

***N*-[3-(5-Hydroxy-2-naphthyl)phenyl]benzamide (25).** The title compound was prepared by reaction of 5-hydroxy-2-naphthyl trifluoromethanesulfonate **5a** (100 mg, 0.34 mmol, 1 equiv) with 3-benzamidophenylboronic acid **25a** (99 mg, 0.41 mmol, 1.2 equiv) according to method A. The product was purified by column chromatography (hexane/ethyl acetate 3:1) then recrystallised in ethyl acetate. Yield: 28% (32 mg). White powder. $C_{23}H_{17}NO_2$; MW 339; m.p.: 159.4°C; ^{1}H NMR (acetone-d_6): δ 9.66 (s, br, 1H), 9.39 (s, 1H), 8.21 (t, *J* = 1.8 Hz, 1H), 8.06-8.03 (m, 1H), 7.98-7.95 (m, 2H), 7.90 (dt, *J* = 1.7 Hz, *J* = 7.7 Hz, 1H), 7.59-7.55 (m, 1H), 7.54-7.52 (m, 2H), 7.52-7.48 (m, 2H), 7.46 (d, *J* = 7.7 Hz, 1H), 7.43 (dt, *J* = 1.6 Hz, *J* = 7.9 Hz, 1H), 7.25 (ddd, *J* = 1.0 Hz, *J* = 2.0 Hz, *J* = 7.9 Hz, 1H), 7.15 (t, *J* = 7.9 Hz, 1H), 6.60 (ddd, *J* = 1.0 Hz, *J* = 2.4 Hz, *J* = 8.0 Hz, 1H); ^{13}C NMR (acetone-d_6): δ 179.7, 166.4, 158.6, 142.3, 141.5, 140.9, 136.5, 136.3, 132.4, 132.2, 130.2, 130.1, 129.5, 129.3, 129.2, 128.4, 128.3, 123.1, 120.1, 119.6, 112.1, 111.6, 108.2; IR: 3297, 3076, 1646, 1603, 1579, 1404, 1286 cm^{-1}; GC/MS *m/z*: 339 $(M)^+$.

***N*-[4'-(Hydroxymethyl)biphenyl-3-yl]acetamide (26).** The title compound was prepared by reaction of (4-bromophenyl)methanol (100 mg, 0.53 mmol, 1 equiv) with 3-acetamidophenylboronic acid (115 mg, 0.64 mmol, 1.2 equiv) according to method B. The analytically pure product was obtained after column chromatography (hexane/ethyl acetate 3:1) in 31% yield (40 mg) as a beige powder. $C_{15}H_{15}NO_2$; MW 241; m.p.: 140-142°C; ^{1}H NMR (acetone-d_6): δ 9.96 (s, 1H), 9.15 (s, br, 1H), 7.96 (s, 1H), 7.89-7.86 (m, 2H), 7.73-7.70 (m, 2H), 7.56-7.53 (m, 1H), 7.30-7.28 (m, 2H), 2.70 (s, 2H), 1.99 (s, 3H); ^{13}C NMR (acetone-d_6): δ 192.5, 169.1, 147.5, 141.3, 141.0, 136.7, 130.9, 130.3, 128.4, 122.8, 119.9, 118.7, 24.3; IR: 3458, 3098, 2986, 1699, 1657 cm^{-1}; LC/MS *m/z*: 240 $(M-H)^-$.

***N*-[4'-(Hydroxymethyl)biphenyl-3-yl]benzamide (27).** The title compound was prepared by reaction of (4-bromophenyl)methanol (100 mg, 0.53 mmol, 1 equiv) with *N*-(3-phenylboronic acid)benzamide **25a** (154 mg, 0.64 mmol, 1.2 equiv) according to method A. The product was obtained after column chromatography (hexane/ethyl acetate 3:1) in 50% yield (80 mg) as a beige powder. $C_{20}H_{17}NO_2$; MW 303; m.p.: 167-168°C; ^{1}H NMR (acetone-d_6): δ 9.67 (s, br, 1H), 8.27 (t, *J* = 1.9 Hz, 1H), 8.13-8.10 (m, 2H), 7.97 (dt, *J* = 1.8 Hz, *J* = 7.6 Hz, 1H), 7.74-7.71 (m, 2H), 7.69-7.65 (m, 1H), 7.63-7.59 (m, 2H), 7.57-7.54 (m, 2H), 7.52 (d, *J* = 7.5 Hz, 1H), 7.50 (dt, *J* = 1.6 Hz, *J* = 7.8 Hz, 1H), 4.78 (s, 2H); ^{13}C NMR (acetone-d_6): δ 170.9, 168.4, 142.8, 142.3, 140.9, 140.3, 136.3, 132.4, 130.0, 129.3, 128.3, 127.9, 127.5, 123.0, 119.8, 119.4, 64.4; IR: 3490, 3185, 2973, 1607, 1550, 1316 cm^{-1}; LC/MS *m/z*: 304 $(M+H)^+$.

***N*-(3-Bromophenyl)-*N*-methylbenzamide (28a).** The title compound was prepared by reaction of 3-bromo-*N*-methylaniline (200 mg, 0.14 mL, 1.07 mmol, 1 equiv) with benzoyl chloride (273 mg, 0.15 mL, 1.28 mmol, 1.2 equiv) according to method C. The product was purified by column chromatography (hexane/ethyl acetate 8:2) to afford 295 mg of product (yield: 95%). $C_{14}H_{12}BrNO$; MW 290; ^{1}H NMR (acetone-d_6): δ 7.39 (t, *J* = 1.8 Hz, 1H), 7.32-7.28 (m, 3H), 7.28-7.25 (m, 1H), 7.23-7.19 (m, 2H), 7.17 (t, *J* = 7.9 Hz, 1H), 7.14-7.11 (m, 1H), 3.39 (s, 3H); ^{13}C NMR (acetone-d_6): δ 170.5, 147.7, 137.3, 131.4, 130.9, 130.4, 130.1, 129.3, 128.7, 127.0, 122.6, 38.3; IR: 3422, 3067, 1647, 1477, 1355 cm^{-1}.

***N*-[4'-(Hydroxymethyl)biphenyl-3-yl]-*N*-methylbenzamide (28).** The title compound was prepared by reaction of *N*-(3-bromophenyl)-*N*-methylbenzamide **28a** (100 mg, 0.34 mmol, 1 equiv) with 4-(hydroxymethyl)phenylboronic acid (62 mg, 0.41 mmol, 1.2 equiv) according to method B. The product was purified by column chromatography (hexane/ethyl acetate 6:4) to give the analytically pure compound in 70% yield (75 mg) as a yellow oil. $C_{21}H_{19}NO_2$; MW 317; ^{1}H NMR (acetone-d_6): δ 7.44-7.42 (m, 3H), 7.41-7.38 (m, 3H), 7.37-7.33 (m, 3H), 7.26 (dt, *J* = 2.5 Hz, *J* = 7.2 Hz, 1H), 7.24-7.20 (m, 2H), 7.18-7.15 (m, 1H), 4.65 (d, *J* = 5.7 Hz, 2H), 4.25 (t, *J* = 5.7 Hz, 1H), 3.49 (s, 3H); ^{13}C NMR (acetone-d_6): δ 170.7, 146.6, 143.1, 142.7, 139.4, 137.9, 130.4, 130.1, 129.4, 128.5, 127.9, 127.5, 126.7, 126.3, 125.4, 64.3, 38.2; IR: 3410, 1632, 1599, 1369 cm^{-1}; LC/MS *m/z*: 318 $(M+H)^+$.

***N*-(3-Bromophenyl)-3-methoxy-*N*-methylbenzamide (29a).** The title compound was prepared by reaction of 3-bromo-*N*-methylaniline (200 mg, 0.14 mL, 1.07 mmol, 1 equiv) with 3-methoxybenzoyl chloride (218 mg, 0.17 mL, 1.28 mmol, 1.2 equiv) according to method C. The product was purified by column chromatography (hexane/ethyl acetate 7:3) to

give the analytically pure compound in 99% yield (340 mg). $C_{15}H_{14}BrNO_2$; MW 320; ^{1}H NMR (acetone-d_6): δ 7.46 (t, *J* = 2.0 Hz, 1H), 7.35 (ddd, *J* = 1.3 Hz, *J* = 2.0 Hz, *J* = 7.9 Hz, 1H), 7.22 (t, *J* = 7.7 Hz, 1H), 7.19-7.16 (m, 1H), 7.14 (t, *J* = 7.9 Hz, 1H), 6.90-6.87 (m, 2H), 6.85 (dd, *J* = 1.4 Hz, *J* = 8.5 Hz, 1H), 3.69 (s, 3H), 3.42 (s, 3H); ^{13}C NMR (acetone-d_6): δ 170.3, 160.1, 147.7, 138.6, 131.5, 130.8, 130.1, 129.8, 127.0, 122.6, 121.6, 116.4, 114.6, 55.6, 38.3; IR: 3474, 2836, 1659, 1583, 1478, 1257 cm^{-1}.

***N*-[4'-(Hydroxymethyl)biphenyl-3-yl]-3-methoxy-*N*-methylbenzamide (29).** The title compound was prepared by reaction of *N*-(3-bromophenyl)-3-methoxy-*N*-methylbenzamide **29a** (100 mg, 0.31 mmol, 1 equiv) with 4-hydroxymethylphenylboronic acid (58 mg, 0.38 mmol, 1.2 equiv) according to method B. The analytically pure compound was obtained after column chromatography (hexane/ethyl acetate 6:4) in 74% yield (80 mg) as a yellow oil. $C_{22}H_{21}NO_3$; MW 347; ^{1}H NMR (acetone-d_6): δ 7.34-7.30 (m, 4H), 7.29-7.26 (m, 2H), 7.22 (t, *J* = 7.8 Hz, 1H), 7.04 (d, *J* = 7.4 Hz, 1H), 7.00 (t, *J* = 7.9 Hz, 1H), 6.80 (d, *J* = 7.7 Hz, 1H), 6.79-6.77 (m, 1H), 6.68 (dd, *J* = 2.0 Hz, *J* = 8.3 Hz, 1H), 4.52 (d, *J* = 5.8 Hz, 2H), 3.51 (s, 3H), 3.35 (s, 3H); ^{13}C NMR (acetone-d_6): δ 170.4, 159.9, 146.7, 143.1, 142.7, 139.4, 139.0, 130.4, 129.6, 127.9, 127.5, 126.6, 126.3, 125.5, 121.7, 116.2, 114.6, 64.3, 55.5, 38.2; IR: 3408, 3225, 1632, 1581, 1373, 1257 cm^{-1}; LC/MS *m/z*: 348 $(M+H)^+$.

***N*-(3-Bromophenyl)-4-methoxy-*N*-methylbenzamide (30a).** The title compound was prepared by reaction of 3-bromo-*N*-methylaniline (200 mg, 0.14 mL, 1.07 mmol, 1 equiv) with 4-methoxybenzoyl chloride (218 mg, 0.17 mL, 1.28 mmol, 1.2 equiv) according to method C. The product was purified by column chromatography (hexane/ethyl acetate 7:3) to afford the analytically pure compound in quantitative yield (340 mg). $C_{15}H_{14}BrNO_2$; MW 320; ^{1}H NMR (acetone-d_6): δ 7.42 (t, *J* = 2.1 Hz, 1H), 7.35 (ddd, *J* = 1.2 Hz, *J* = 1.9 Hz, *J* = 8.0 Hz, 1H), 7.31-7.28 (m, 2H), 7.22 (t, *J* = 8.1 Hz, 1H), 7.13 (ddd, *J* = 0.9 Hz, *J* = 2.1 Hz, *J* = 8.0 Hz, 1H), 6.80-6.76 (m, 2H), 3.76 (s, 3H), 3.41 (s, 3H); ^{13}C NMR (acetone-d_6): δ 170.1, 161.8, 148.3, 131.5, 131.4, 130.6, 129.8, 129.1, 126.9, 122.6, 113.9, 55.6, 38.5.

***N*-[4'-(Hydroxymethyl)biphenyl-3-yl]-4-methoxy-*N*-methylbenzamide (30).** The title compound was prepared by reaction of *N*-(3-bromophenyl)-4-methoxy-*N*-methylbenzamide **30a** (100 mg, 0.31 mmol, 1 equiv) with 4-hydroxymethylphenylboronic acid (58 mg, 0.38 mmol, 1.2 equiv) according to method B. The product was purified by column chromatography (hexane/ethyl acetate 7:3) to give the analytically pure compound in 65% yield (70 mg) as a white powder. $C_{22}H_{21}NO_3$; MW 347; m.p.: 147-149°C; ^{1}H NMR (acetone-

d_6): δ 7.56-7.52 (m, 1H), 7.49-7.46 (m, 2H), 7.45 (ddd, *J* = 1.0 Hz, *J* = 1.9 Hz, *J* = 7.9 Hz, 1H), 7.42-7.40 (m, 3H), 7.36 (t, *J* = 7.9 Hz, 1H), 7.34-7.31 (m, 2H), 7.13 (ddd, *J* = 1.0 Hz, *J* = 2.2 Hz, *J* = 7.8 Hz, 1H), 6.77-6.74 (m, 2H), 4.66 (d, *J* = 5.3 Hz, 2H), 3.74 (s, 3H), 3.47 (s, 3H); ^{13}C NMR (acetone-d_6): δ 170.3, 161.5, 147.2, 143.1, 142.7, 139.4, 132.7, 131.5, 130.4, 129.5, 129.4, 127.9, 127.5, 126.5, 126.3, 125.2, 113.8, 64.3, 55.6, 38.4; LC/MS *m/z*: 348 $(M+H)^+$.

***N*-(3-Bromophenyl)-2-(3-methoxyphenyl)-*N*-methylacetamide (31a).** The title compound was prepared by reaction of 3-bromo-*N*-methylaniline (200 mg, 0.14 mL, 1.07 mmol, 1 equiv) with 3-methoxyphenylacetyl chloride (218 mg, 0.20 mL, 1.28 mmol, 1.2 equiv) according to method C. The product was purified by column chromatography (hexane/ethyl acetate 7:3) to give the analytically pure compound in 98% yield (350 mg). $C_{16}H_{16}BrNO_2$; MW 334; ^{1}H NMR (acetone-d_6): δ 7.55 (d, *J* = 7.5 Hz, 1H), 7.47-7.45 (m, 1H), 7.41 (t, *J* = 8.0 Hz, 1H), 7.31 (ddd, *J* = 0.9 Hz, *J* = 2.1 Hz, *J* = 7.9 Hz, 1H), 7.14 (t, *J* = 8.0 Hz, 1H), 6.76 (dd, *J* = 2.1 Hz, *J* = 8.2 Hz, 1H), 6.67-6.62 (m, 2H), 3.74 (s, 3H), 3.45 (s, 2H), 3.23 (s, 3H); ^{13}C NMR (acetone-d_6): δ 180.6, 146.8, 138.1, 132.0, 129.9, 122.1, 115.5, 112.8, 60.5, 55.4, 41.6; IR: 3447, 2980, 1659, 1587, 1257 cm^{-1}.

***N*-[4'-(Hydroxymethyl)biphenyl-3-yl]-2-(3-methoxyphenyl)-*N*-methylacetamide (31).** The title compound was prepared by reaction of *N*-(3-bromophenyl)-2-(3-methoxyphenyl)-*N*-methylacetamide **31a** (90 mg, 0.27 mmol, 1 equiv) with 4-hydroxymethylphenylboronic acid (52 mg, 0.34 mmol, 1.2 equiv) according to method B. The analytically pure product was obtained after column chromatography (hexane/ethyl acetate 6:4) in 51% yield (50 mg) as a yellow oil. $C_{23}H_{23}NO_3$; MW 361; ^{1}H NMR (acetone-d_6): δ 7.53-7.49 (m, 1H), 7.44-7.37 (m, 3H), 7.33 (s, 1H), 7.30 (d, *J* = 8.0 Hz, 2H), 7.12 (d, *J* = 7.7 Hz, 1H), 6.99-6.94 (m, 1H), 6.59 (d, *J* = 7.7 Hz, 1H), 6.52-6.46 (m, 2H), 4.53 (d, *J* = 5.7 Hz, 2H), 3.53 (s, 3H), 3.34 (s, 2H), 3.13 (s, 3H); ^{13}C NMR (acetone-d_6): δ 181.1, 170.7, 160.5, 145.8, 143.2, 139.3, 129.9, 127.9, 127.6, 127.1, 122.1, 115.5, 112.7, 64.3, 55.3, 49.8, 41.6; IR: 3417, 3076, 2927, 1636, 1599, 1436, 1258 cm^{-1}; LC/MS *m/z*: 362 $(M+H)^+$.

***N*-(3-Bromophenyl)-2-(4-methoxyphenyl)-*N*-methylacetamide (32a).** The title compound was prepared by reaction of 3-bromo-*N*-methylaniline (200 mg, 0.14 mL, 1.07 mmol, 1 equiv) with 4-methoxyphenylacetyl chloride (218 mg, 0.18 mL, 1.28 mmol, 1.2 equiv) according to method C. The product was purified by column chromatography (hexane/ethyl acetate 7:3) to give the analytically pure compound in quantitative yield (358 mg). $C_{16}H_{16}BrNO_2$; MW 334;

^{1}H NMR (acetone-d_6): δ 7.55 (d, J = 7.4 Hz, 1H), 7.45 (t, J = 1.7 Hz, 1H), 7.41 (t, J = 7.9 Hz, 1H), 7.31 (ddd, J = 0.9 Hz, J = 2.0 Hz, J = 7.9 Hz, 1H), 6.98 (s, 2H), 6.80 (d, J = 8.6 Hz, 2H), 3.75 (s, 3H), 3.42 (s, 2H), 3.22 (s, 3H); ^{13}C NMR (acetone-d_6): δ 170.9, 159.4, 146.9, 132.0, 131.6, 130.9, 128.5, 114.7, 114.4, 55.5, 40.6.

***N*-[4'-(Hydroxymethyl)biphenyl-3-yl]-2-(4-methoxyphenyl)-*N*-methylacetamide (32).** The title compound was prepared by reaction of *N*-(3-bromophenyl)-2-(4-methoxyphenyl)-*N*-methylacetamide **32a** (90 mg, 0.27 mmol, 1 equiv) with 4-hydroxymethylphenylboronic acid (52 mg, 0.34 mmol, 1.2 equiv) according to method B. The product was purified by column chromatography (hexane/ethyl acetate 6:4) to give the analytically pure compound in 64% yield (62 mg) as a white oil. $C_{23}H_{23}NO_3$; MW 361; ^{1}H NMR (acetone-d_6): δ 7.60-7.57 (m, 2H), 7.56-7.52 (m, 2H), 7.48-7.44 (m, 3H), 7.26 (ddd, J = 1.0 Hz, J = 2.2 Hz, J = 7.8 Hz, 1H), 6.97 (d, J = 5.6 Hz, 2H), 6.76 (d, J = 8.4 Hz, 2H), 4.69 (d, J = 5.7 Hz, 2H), 3.73 (s, 3H), 3.44 (s, 2H), 3.26 (s, 3H); ^{13}C NMR (acetone-d_6): δ 171.0, 159.3, 145.9, 143.2, 139.2, 134.9, 132.7, 132.6, 130.9, 129.5, 129.4, 128.9, 127.9, 127.6, 127.2, 114.4, 64.3, 55.4, 40.7; LC/MS *m/z*: 362 $(M+H)^+$.

***N*-[4'-(Hydroxymethyl)biphenyl-4-yl]methanesulfonamide (33).** The title compound was prepared by reaction of (4-bromophenyl)methanol (100 mg, 0.53 mmol, 1 equiv) with 4-methylsulfonylaminophenylboronic acid (138 mg, 0.64 mmol, 1.2 equiv) according to method B. The analytically pure product was obtained after column chromatography (hexane/ethyl acetate 7:3) in 24% yield (35 mg) as a white powder. $C_{14}H_{15}NO_3S$; MW 277; m.p.: 204-205°C; ^{1}H NMR (acetone-d_6): δ 8.61 (s, br, 1H), 7.68-7.64 (m, 2H), 7.63-7.60 (m, 2H), 7.46-7.41 (m, 4H), 4.68 (d, J = 5.8 Hz, 2H), 4.20 (t, J = 5.8 Hz, 1H), 3.03 (s, 3H); ^{13}C NMR (acetone-d_6): δ 142.5, 139.6, 138.7, 137.8, 128.5, 128.0, 127.2, 121.5, 64.4, 39.5; IR: 3371, 3248, 1646, 1498, 1320, 1302 cm^{-1}; GC/MS *m/z*: 277 $(M)^+$.

***N*-(4-Bromophenyl)-3-methylbenzenesulfonamide (34a).** Under nitrogen, 3-methylphenylsulfonylchloride (221 mg, 0.17 mL, 1.16 mmol, 1 equiv) was added to a solution of 4-bromoaniline (200 mg, 1.16 mmol, 1 equiv) in pyridine at 0°C. The mixture was stirred at room temperature overnight. The solvent was removed under reduced pressure and the crude product was used in the next step without any purification and characterisation.

***N*-[4´-(Hydroxymethyl)biphenyl-4-yl]-3-methylbenzenesulfonamide (34).** The title compound was prepared by reaction of *N*-(4-bromophenyl)-3-methylbenzenesulfonamide **34a**

(60 mg, 0.18 mmol, 1 equiv) with 4-hydroxymethylphenylboronic acid (34 mg, 0.22 mol, 1.2 equiv) according to method B. The product was purified by column chromatography (hexane/ethyl acetate 7:3) to afford the analytically pure compound in 47% yield (30 mg) as an orange oil. $C_{20}H_{19}NO_3S$; MW 353; ^{1}H NMR (acetone-d_6): δ 9.02 (s, 1H), 7.67-7.65 (m, 1H), 7.65-7.62 (m, 1H), 7.57-7.53 (m, 4H), 7.43-7.40 (m, 4H), 7.31-7.28 (m, 2H), 4.66 (d, *J* = 5.8 Hz, 2H), 2.36 (s, 3H); ^{13}C NMR (acetone-d_6): δ 142.5, 140.1, 139.5, 138.1, 137.8, 134.3, 129.8, 128.3, 128.2, 127.9, 127.2, 125.1, 121.9, 64.4, 21.2; LC/MS *m/z*: 354 $(M+H)^+$.

***N*-(4'-Hydroxybiphenyl-3-yl)acetamide (35).** The title compound was prepared by reaction of 4-bromophenol (100 mg, 0.58 mmol, 1 equiv) with 3-acetamidophenylboronic acid (124 mg, 0.69 mmol, 1.2 equiv) according to method B. The product was purified by column chromatography (hexane/ethyl acetate 3:1) to give the analytically pure compound in 23% yield (30 mg) as a beige powder. $C_{14}H_{13}NO_2$; MW 227; m.p.: 205-206°C; ^{1}H NMR (acetone-d_6): δ 9.22 (s, br, 1H), 8.49 (s, 1H), 7.97 (s, 1H), 7.63 (d, *J* = 7.6 Hz, 1H), 7.56-7.53 (m, 2H), 7.39 (t, *J* = 8.0 Hz, 1H), 7.34-7.31 (m, 1H), 7.02-6.98 (m, 2H), 2.17 (s, 3H); ^{13}C NMR (acetone-d_6): δ 168.9, 158.1, 142.4, 141.0, 133.1, 130.0, 128.8, 122.0, 118.0, 117.9, 116.5, 24.3; IR: 3495, 3028, 2964, 1606, 1554, 1266 cm^{-1}; LC/MS *m/z*: 228 $(M+H)^+$.

***N*-(4'-Hydroxybiphenyl-3-yl)benzamide (36).** The title compound was prepared by reaction of 4-bromophenol (100 mg, 0.58 mmol, 1 equiv) with *N*-(3-phenylboronic acid)benzamide **25a** (169 mg, 0.70 mmol, 1.2 equiv) according to method A. The product was purified by column chromatography (hexane/ethyl acetate 3:1) to afford the analytically pure compound in 30% yield (50 mg) as a beige oil. $C_{19}H_{15}NO_2$; MW 289; ^{1}H NMR (acetone-d_6): δ 9.55 (s, br, 1H), 8.44 (s, 1H), 8.12 (t, *J* = 1.9 Hz, 1H), 8.04-8.01 (m, 2H), 7.82 (ddd, *J* = 0.9 Hz, *J* = 2.1 Hz, *J* = 8.0 Hz, 1H), 7.57 (dt, *J* = 1.3 Hz, *J* = 7.5 Hz, 1H), 7.54-7.50 (m, 4H), 7.39 (t, *J* = 8.0 Hz, 1H), 7.34 (dt, *J* = 1.3 Hz, *J* = 8.0 Hz, 1H), 6.97-6.93 (m, 2H); ^{13}C NMR (acetone-d_6): δ 166.4, 158.1, 142.4, 140.8, 136.4, 133.1, 132.3, 129.9, 129.3, 128.8, 128.3, 122.5, 119.1, 119.0, 116.6; IR: 3293, 3015, 1651, 1545, 1258 cm^{-1}; LC/MS *m/z*: 290 $(M+H)^+$.

***N*-(4'-Hydroxybiphenyl-3-yl)-*N*-methylbenzamide (37).** The title compound was prepared by reaction of *N*-(3-bromophenyl)-*N*-methylbenzamide **28a** (100 mg, 0.34 mmol, 1 equiv) with 4-hydroxyphenylboronic acid (56 mg, 0.41 mmol, 1.2 equiv) according to method B. The analytically pure product was obtained after column chromatography (hexane/ethyl acetate 6:4) in 19% yield (20 mg) as a beige powder. $C_{20}H_{17}NO_2$; MW 303; m.p.: 175-176°C; ^{1}H NMR (acetone-d_6): δ 8.51 (s, 1H), 7.34-7.31 (m, 3H), 7.30-7.29 (m, 2H), 7.28 (s, 1H), 7.26

(t, *J* = 8.0 Hz, 1H), 7.23-7.20 (m, 1H), 7.19-7.15 (m, 2H), 3.44 (s, 3H); ^{13}C NMR (acetone-d_6): δ 170.7, 158.4, 146.6, 142.7, 137.9, 132.1, 130.2, 130.1, 129.4, 128.8, 128.5, 126.1, 125.6, 124.9, 116.6, 38.2; IR: 3380, 3080, 1696, 1598, 1379, 1257 cm^{-1}; LC/MS *m/z*: 304 $(M+H)^+$.

3-Methoxy-*N*-(4'-methoxybiphenyl-3-yl)-*N*-methylbenzamide (38). The title compound was prepared by reaction of *N*-(3-bromophenyl)-3-methoxy-*N*-methylbenzamide **29a** (90 mg, 0.28 mmol, 1 equiv) with 4-methoxyphenylboronic acid (52 mg, 0.34 mmol, 1.2 equiv) according to method B. The analytically pure product was obtained after column chromatography (hexane/ethyl acetate 7:3) in 82% yield (80 mg) as a white powder. $C_{22}H_{21}NO_3$; MW 347; m.p.: 128-129°C; ^{1}H NMR (acetone-d_6): δ 7.45-7.42 (m, 2H), 7.41-7.38 (m, 2H), 7.34-7.30 (m, 1H), 7.15-7.10 (m, 2H), 6.99-6.96 (m, 2H), 6.93 (dt, *J* = 1.2 Hz, *J* = 7.7 Hz, 1H), 6.91-6.90 (m, 1H), 6.81 (ddd, *J* = 1.0 Hz, *J* = 2.7 Hz, *J* = 8.3 Hz, 1H), 3.82 (s, 3H), 3.64 (s, 3H), 3.47 (s, 3H); ^{13}C NMR (acetone-d_6): δ 170.4, 160.6, 160.0, 146.7, 142.5, 139.1, 136.7, 133.2, 130.3, 129.6, 128.8, 126.2, 125.8, 125.1, 121.7, 116.2, 115.1, 114.7, 55.6, 55.5, 38.2; IR: 3446, 2937, 1644, 1583, 1290 cm^{-1}; LC/MS *m/z*: 348 $(M+H)^+$.

4-Methoxy-*N*-(4'-methoxybiphenyl-3-yl)-*N*-methylbenzamide (39). The title compound was prepared by reaction of *N*-(3-bromophenyl)-4-methoxy-*N*-methylbenzamide **30a** (90 mg, 0.28 mmol, 1 equiv) with 4-methoxyphenylboronic acid (52 mg, 0.34 mmol, 1.2 equiv) according to method B. The analytically pure product was obtained after column chromatography (hexane/ethyl acetate 7:3) in 69% yield (67 mg) as a white oil. $C_{22}H_{21}NO_3$; MW 347; ^{1}H NMR (acetone-d_6): δ 7.47-7.44 (m, 2H), 7.40 (ddd, *J* = 1.0 Hz, *J* = 1.9 Hz, *J* = 7.8 Hz, 1H), 7.36 (t, *J* = 1.8 Hz, 1H), 7.34-7.30 (m, 3H), 7.08 (ddd, *J* = 1.2 Hz, *J* = 2.2 Hz, *J* = 7.9 Hz, 1H), 6.99-6.96 (m, 2H), 6.76-6.73 (m, 2H), 3.82 (s, 3H), 3.73 (s, 3H), 3.46 (s, 3H); ^{13}C NMR (acetone-d_6): δ 170.2, 161.5, 160.6, 147.2, 142.5, 133.4, 131.5, 130.3, 129.7, 128.8, 126.0, 125.8, 124.8, 115.1, 113.7, 55.6, 55.5, 38.4; LC/MS *m/z*: 348 $(M+H)^+$.

***N*-(4'-Methoxybiphenyl-3-yl)-2-(3-methoxyphenyl)-*N*-methylacetamide (40).** The title compound was prepared by reaction of *N*-(3-bromophenyl)-2-(3-methoxyphenyl)-*N*-methylacetamide **31a** (90 mg, 0.27 mmol, 1 equiv) with 4-methoxyphenylboronic acid (52 mg, 0.34 mmol, 1.2 equiv) according to method B. The product was purified by column chromatography (hexane/ethyl acetate 7:3) to give the analytically pure compound in 77% yield (75 mg) as a yellow oil. $C_{23}H_{23}NO_3$; MW 361; ^{1}H NMR (acetone-d_6): δ 7.65-7.61 (m, 1H), 7.60-7.55 (m, 2H), 7.53 (t, *J* = 7.7 Hz, 1H), 7.47-7.44 (m, 1H), 7.25 (d, *J* = 7.2 Hz, 1H),

7.14 (t, *J* = 7.4 Hz, 1H), 7.05-7.00 (m, 2H), 6.78-6.74 (m, 1H), 6.70-6.60 (m, 2H), 3.87 (s, 3H), 3.71 (s, 3H), 3.50 (s, 2H), 3.29 (s, 3H); ^{13}C NMR (acetone-d_6): δ 160.7, 145.8, 136.7, 130.9, 129.9, 128.9, 122.1, 115.6, 115.1, 113.8, 112.6, 55.6, 55.3, 41.5; IR: 3401, 3258, 2935, 1653, 1600, 1485, 1377, 1252 cm^{-1}; LC/MS *m/z*: 362 $(M+H)^+$.

***N*-(4'-Hydroxybiphenyl-3-yl)-2-(3-hydroxyphenyl)-*N*-methylacetamide (41).** To a solution of *N*-(4'-methoxybiphenyl-3-yl)-2-(3-methoxyphenyl)-*N*-methylacetamide **40** (30 mg, 0.083 mmol, 1 equiv) in anhydrous dichloromethane cooled at -78°C under nitrogen was slowly added boron tribromide (0.83 mL, 0.83 mmol, 10 equiv). The reaction mixture was stirred at -78°C for 1h and allowed to warm up to room temperature overnight. The reaction was quenched by addition of water and extracted with ethyl acetate. The combined organic layers were washed with brine and dried over sodium sulfate evaporated to dryness under reduced pressure and purified by column chromatography (dichloromethane/methanol 20:1) to give the analytically pure compound in 72% yield (20 mg) as a yellow oil. $C_{21}H_{19}NO_3$; MW 333; ^{1}H NMR (acetone-d_6): δ 8.51 (s, 1H), 8.16 (s, 1H), 7.58 (d, *J* = 7.3 Hz, 1H), 7.49-7.45 (m, 3H), 7.42 (t, *J* = 1.8 Hz, 1H), 7.19 (ddd, *J* = 0.9 Hz, *J* = 2.0 Hz, *J* = 7.9 Hz, 1H), 7.05-7.00 (m, 1H), 6.94-6.90 (m, 2H), 6.68-6.64 (m, 2H), 6.50 (s, 1H), 3.43 (s, 2H), 3.27 (s, 3H); ^{13}C NMR (acetone-d_6): δ 158.4, 158.2, 150.9, 145.8, 130.8, 129.9, 129.0, 126.3, 121.0, 116.9, 116.6, 114.2, 41.4; LC/MS *m/z*: 334 $(M+H)^+$.

***N*-(4'-Methoxybiphenyl-3-yl)-2-(4-methoxyphenyl)-*N*-methylacetamide (42).** The title compound was prepared by reaction of *N*-(3-bromophenyl)-2-(4-methoxyphenyl)-*N*-methylacetamide **32a** (90 mg, 0.27 mmol, 1 equiv) with 4-methoxyphenylboronic acid (52 mg, 0.34 mmol, 1.2 equiv) according to method B. The analytically pure product was obtained after column chromatography (hexane/ethyl acetate 7:3) in 62% yield (60 mg) as a yellow oil. $C_{23}H_{23}NO_3$; MW 361; ^{1}H NMR (acetone-d_6): δ 7.61 (d, *J* = 7.8 Hz, 1H), 7.58-7.54 (m, 2H), 7.50 (t, *J* = 7.6 Hz, 1H), 7.42 (t, *J* = 1.8 Hz, 1H), 7.21 (ddd, *J* = 1.0 Hz, *J* = 2.2 Hz, *J* = 7.8 Hz, 1H), 7.04-7.00 (m, 2H), 7.00-6.93 (m, 2H), 6.77 (d, *J* = 8.6 Hz, 2H), 3.85 (s, 3H), 3.73 (s, 3H), 3.44 (s, 2H), 3.26 (s, 3H); ^{13}C NMR (acetone-d_6): δ 171.0, 160.7, 159.3, 145.9, 136.7, 133.0, 130.9, 129.0, 128.9, 126.7, 126.6, 126.4, 115.2, 114.4, 113.9, 55.7, 55.4, 55.3, 40.6; LC/MS *m/z*: 362 $(M+H)^+$.

***N*-(4'-Hydroxybiphenyl-4-yl)methanesulfonamide (43).** The title compound was prepared by reaction of 4-bromophenol (100 mg, 0.58 mmol, 1 equiv) with 4-methylsulfonylaminophenylboronic acid (149 mg, 0.69 mmol, 1.2 equiv) according to method

B. The analytically pure product was obtained after column chromatography (hexane/ethyl acetate 7:3) in 20% yield (30 mg) as a beige powder. $C_{13}H_{13}NO_3S$; MW 263; m.p.: 168-169°C; ^{1}H NMR (acetone-d_6): δ 8.60 (s, br, 1H), 7.68-7.63 (m, 4H), 7.48-7.42 (m, 4H), 7.33-7.32 (m, 1H), 3.03 (s, 3H); ^{13}C NMR (acetone-d_6): δ 141.1, 138.8, 137.8, 129.7, 128.6, 128.1, 127.5, 121.4, 39.5; IR: 3376, 2930, 1643, 1396 cm^{-1}.

***N*-(4'-Hydroxybiphenyl-4-yl)-3-methyl-benzenesulfonamide (44).** The title compound was prepared by reaction of *N*-(4-bromophenyl)-3-methylbenzenesulfonamide **34a** (70 mg, 0.21 mmol, 1 equiv) with 4-hydroxyphenylboronic acid (36 mg, 0.26 mmol, 1.2 equiv) according to method B. The product was purified by column chromatography (hexane/ethyl acetate 7:3) to give the analytically pure compound in 63% yield (45 mg) as an orange oil. $C_{19}H_{17}NO_3S$; MW 339; ^{1}H NMR (acetone-d_6): δ 8.94 (s, 1H), 8.39 (s, 1H), 7.65-7.64 (m, 1H), 7.63-7.60 (m, 1H), 7.49-7.46 (m, 2H), 7.45-7.42 (m, 2H), 7.41-7.39 (m, 2H), 7.26-7.23 (m, 2H), 6.90-6.87 (m, 2H), 2.36 (s, 3H); ^{13}C NMR (acetone-d_6): δ 157.9, 141.0, 140.0, 138.1, 137.3, 134.3, 132.3, 129.7, 128.5, 128.2, 127.7, 125.1, 122.1, 116.6, 21.2; LC/MS *m/z*: 338 (M-H)$^-$.

Biological Assays

[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-HSD2 and 17β-HSD1 were obtained from human placenta according to previously described procedures.[39-41, 48] Fresh human placenta was homogenised and centrifuged. The pellet fraction contains the microsomal 17β-HSD2, while 17β-HSD1 was obtained after precipitation with ammonium sulfate from the cytosolic fraction.

1. Inhibition of 17β-HSD2

Inhibitory activities were evaluated by a well established method with minor modifications.[39] Briefly, the enzyme preparation was incubated with NAD^+ [1500 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. Final concentration of DMSO was adjusted to 1% in all samples. The enzymatic reaction was started by addition of a mixture of unlabeled- and [2,4,6,7-^{3}H]-E2 (final concentration: 500 nM, 0.11 μCi). After 20 min at 37°C, the incubation was stopped with $HgCl_2$ and the mixture was extracted with ether. After evaporation, the steroids were dissolved in acetonitrile. E1 and E2 were separated using acetonitrile/water (45:55) as mobile phase in a C18 rp

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 according to following equation: %conversion = (%E1/(%E1 + %E2) × 100). Each value was calculated from at least three independent experiments.

2. Inhibition of 17β-HSD1

The 17β-HSD1 inhibition assay was performed similarly to the 17β-HSD2 procedure. The cytosolic fraction was incubated with NADH [500 μM], test compound and a mixture of unlabeled- and [2,4,6,7-^{3}H]-E1 (final concentration: 500 nM, 0.15 μCi) for 10 min. Further treatment of the samples and HPLC separation was carried out as mentioned above.

3. ER affinity

The binding affinity of selected compounds to the ERα and ERβ was determined according to Zimmermann *et al.*[49] using recombinant human proteins. Briefly, 0.25 pmoles of ERα or ERβ, respectively, were incubated with [^{3}H]-E2 (10 nM) and test compound for 1h 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 formed complex 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). From these results the percentage of [^{3}H]-E2 displacement by the compounds was calculated. The plot of % displacement versus compound concentration resulted in sigmoidal binding curves. The compound concentration to displace 50% of the receptor bound [^{3}H]-E2 were determined. Unlabelled E2 was used as a reference. For determination of the relative binding affinity [42] the ratio was calculated according to the following equation: $RBA[\%] = \frac{IC_{50}(E2)}{IC_{50}(compound)} \bullet 100$.[50]

This results in an RBA value of 100% for E2. After the assay was established and validated a modification was made to increase throughput. Compounds were tested at concentrations of 1000·IC_{50}(E2) and 10000IC_{50}(E2). Results were reported as RBA ranges. Compounds with less than 50% displacement of [^{3}H]-E2 at a concentration of 10000·IC_{50}(E2) were classified as RBA <0.01%, compounds that displace more than 50% at 10000IC_{50}(E2) but less than 50% at 1000·IC_{50}(E2) were classified as 0.01%<RBA<0.1%.

Acknowledgements

We are grateful to the Deutsche Forschungsgemeinschaft for financial support (HA1315/8-1). We thank Jannine Ludwig, Jeannine Jung and Jörg Haupenthal for their help in performing the enzyme inhibition tests (17β-HSD2, 17β-HSD1) and the ER tests.

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3.2. Introduction of an electron withdrawing group on the hydroxyphenylnaphthol scaffold improves the potency of 17β-hydroxysteroid dehydrogenase type 2 (17β-HSD2) inhibitors

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This manuscript has been submitted in the journal
Journal of Medicinal Chemistry, **2011**,

Paper II

Abstract

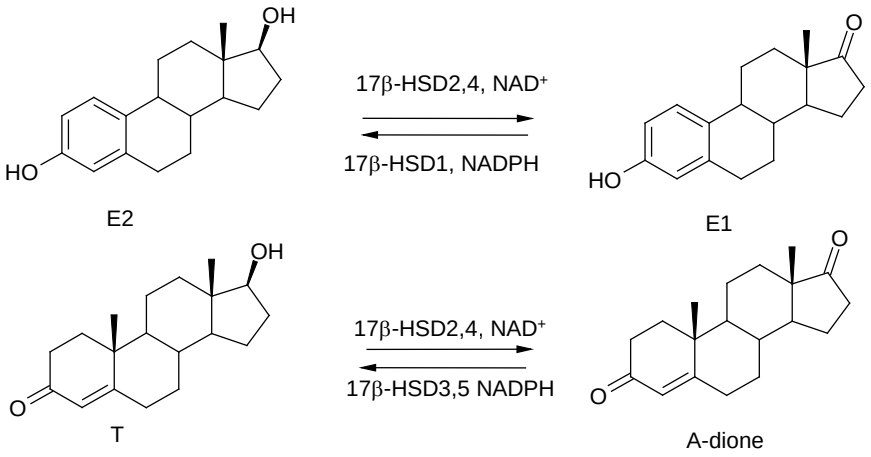
Estrogen deficiency in postmenopausal women or elderly men is often associated with the skeletal disease osteoporosis. The supplementation of estradiol (E2) in osteoporotic patients is known to prevent bone fracture but can not be administered because of adverse effect. As 17β-hydroxysteroid dehydrogenase type 2 (17β-HSD2) oxidises E2 to its inactive form estrone (E1) and has been found in osteoblastic cells, it is an attractive target for the treatment of osteoporosis. Twenty one novel, naphthalene-derived compounds have been synthesised and evaluated for their 17β-HSD2 inhibition and their selectivity toward 17β-HSD1 and the estrogen receptors (ERs) α and β. Compound **19** turned out to be the most potent and selective inhibitor of 17β-HSD2 in cell free assays and had a very good cellular activity in MDA-MB-231 cells, expressing naturally 17β-HSD2. It also showed marked inhibition of the E1-formation by the rat and mouse orthologous enzymes and strong inhibition of monkey 17β-HSD2. It is thus an appropriate candidate to be further evaluated in a disease-oriented model.

Introduction

17β-Hydroxysteroid dehydrogenase type 2 (17β-HSD2) catalyses the conversion of the biologically active sex steroids estradiol (E2) and testosterone (T) into their inactive forms using NAD^+ as cofactor (Chart 1). It is a trans-membrane protein[1] and no crystal structure is available yet. This enzyme is abundantly expressed in placenta, liver, small intestine,

endometrium and to a lesser extend also in kidney, pancreas, colon, uterus, breast and prostate. It was also found in osteoblastic cells[1-4].

Chart 1. Interconversion of estradiol (E2) to estrone (E1) by 17β-HSD2, 17β-HSD4, 17β-HSD1, and of testosterone (T) to androstenedione (A-dione) by 17β-HSD2, 17β-HSD4, 17β-HSD3 and 17β-HSD5.



In bone physiology osteoblastic cells [5] are responsible for bone formation and mineralisation and osteoclastic cells (OCs) for bone resorption. The subtle remodelling balance maintained between the OB and OC activities is crucial for bone stability.

Osteoporosis[6] is a systemic skeletal disease characterised by low bone mass and deterioration of bone tissue. Symptoms of this disease include bone fragility[6] and increase in fractures[6], often at the hips, spine and wrist. Osteoporosis occurs especially in postmenopausal women and elderly men, when the levels in active sex steroids (E2 and T)[7] drop.

Only few drugs are efficient for the treatment of osteoporosis. Among them, the antiresorptive agent bisphosphonate alendronate[8] is the most potent drug but the risk of fractures in postmenopausal women[8-10] and elderly men[11] is only reduced by 50%. Estrogens stop bone loss in osteoporotic patients but are no longer used, because of several non-skeletal effects such as an increased risk of deep vein thrombosis, pulmonary embolism and breast cancer[12]. Furthermore, low dose of synthetic estrogens, like ethinylestradiol, has no practical application. Raloxifene[13], a selective estrogen receptor modulator (SERM) is frequently used to treat osteoporosis. Drawbacks of this therapy are the increased risks of thromboembolism[14], hot flushes[15] and leg cramps[15]. Therefore, there is a need to develop novel drugs, more efficient and selective for osteoporosis.

Estrogens are known to play a key role in bone physiology. They act directly on OBs, which regulate the ability of the OC precursors to differentiate to OCs (osteoclastogenesis). The mode of action of estrogens on bone mass is not yet well understood. 17β-HSD2, which is expressed in OBs, can be considered as a molecular switch, as it is involved in the regulation of the level of active E2 and T in the target cell. Therefore, a local intracellular enhancement of E2 and T in bones should be feasible. This strategy has already been successfully applied for several enzymes like aromatase[16-18], CYP17[19-22], 17β-HSD1[23-35] and 5α-reductase[36-40].

Among the few 17β-HSD2 inhibitors described in the literature[41-50] there is only one class of non-steroidal compounds[46-48]: the *cis*-pyrrolidinones. Applying a non-steroidal inhibitor from this class in a monkey osteoporosis model, 17β-HSD2 has been validated as target for the treatment of osteoporosis by Bagi *et al.*[51]. Treatment with this compound led to a slight decrease in bone resorption and increase in bone formation resulting in maintenance of bone balance and bone strength[50]. As only small effects were observed, there is a need to develop new potent 17β-HSD2 inhibitors with better *in vivo* efficacy. Additionally, these compounds should show a high selectivity toward 17β-HSD1, the isoenzyme catalysing the reverse reaction, the reduction of estrone (E1) to E2.

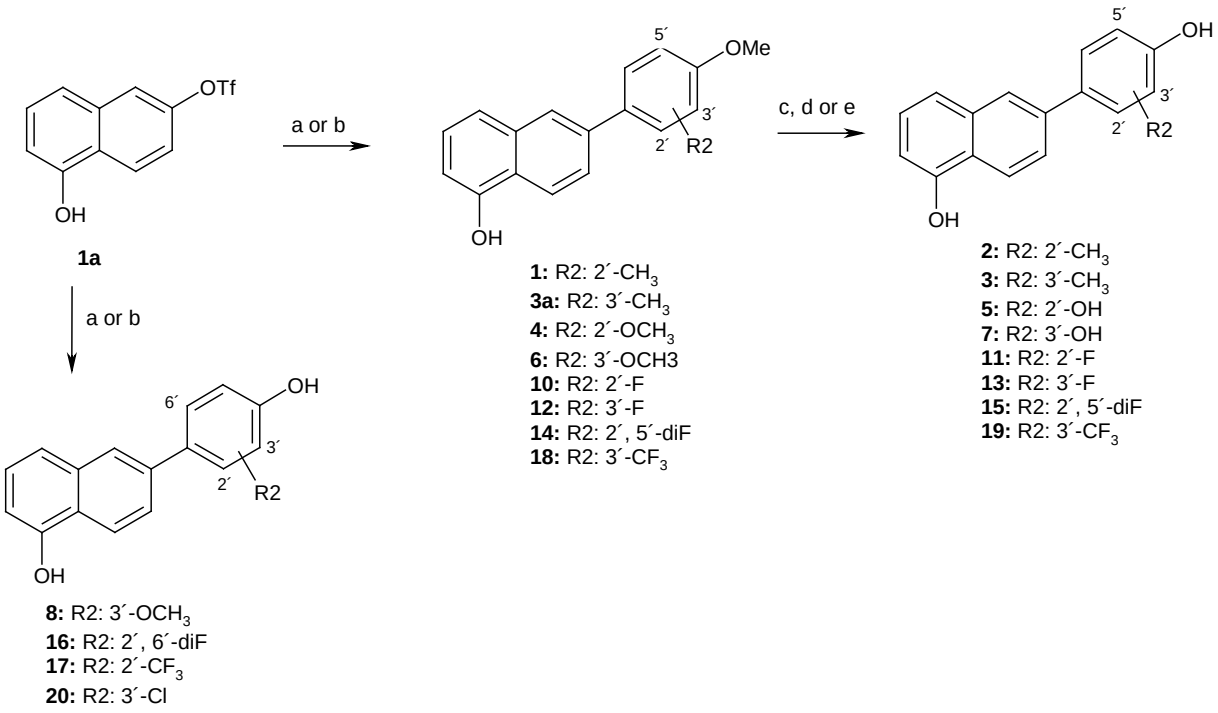
In a previous work[52], focusing on the design of new non-steroidal 17β-HSD2 inhibitors, 6-(4´-hydroxyphenyl)-1-naphthol (compound **A** - Table 1) has been identified as a promising scaffold with an IC_{50} value of 302 nM for 17β-HSD2 and a selectivity factor (SF) of 8 toward 17β-HSD1. This hit needed to be improved regarding activity and selectivity. According to our hypothesis[52], the OH substituted benzene ring of the naphthalene moiety in **A** mimics the A-ring of E2 and the OH-phenyl the D-ring, leading to a close binding of the OH-phenyl to the catalytic triad and the cofactor. As the substrate of one enzyme (17β-HSD2 in this case) is the product of the catalytic reaction of the other enzyme (17β-HSD1 in this case), it can be concluded that their active sites must be very similar too. Therefore, with analysing 17β-HSD1 from which in contrast to 17β-HSD2 the crystal structure is known, it becomes apparent that there is some space available around the OH-phenyl for substituents.

In this work, first the hypothesis concerning the binding mode of hydroxyphenyl naphthol derivatives to 17β-HSD2 was validated. Afterwards, the available space for the inhibitors in the active site of the enzyme was explored by synthesis of several derivatives where the size of substituents at position 2' and 3' of the hydroxyphenyl ring was varied. Expecting that electronic effects play an important role, electron donating (EDG) and electron withdrawing (EWG) groups were also introduced into the OH-phenyl moiety.

Chemistry

Synthesis of **1** to **8** and **10** to **20** (Scheme 1) started from the commercially available 6-methoxy-1-tetralone which was triflated and aromatised in **1a** in very good yield according to the method described by us[52]. The cross-coupling reaction[53] of the triflate **1a** with the appropriate methoxy phenylboronic acid led to compounds **1**, **3a**, **4**, **6**, **8**, **10**, **12**, **14**, **16** to **18** and **20** (method A or B). Ether cleavage was performed using boron tribromide for compounds **2**, **7** and **13** (method C), boron trifluoride dimethylsulfide complex for compounds **3**, **5** and **15** (method D) or pyridinium hydrochloride for compounds **11** and **19** (method E).

Scheme 1. Synthesis of compounds **1** to **8** and **10** to **20**[a]

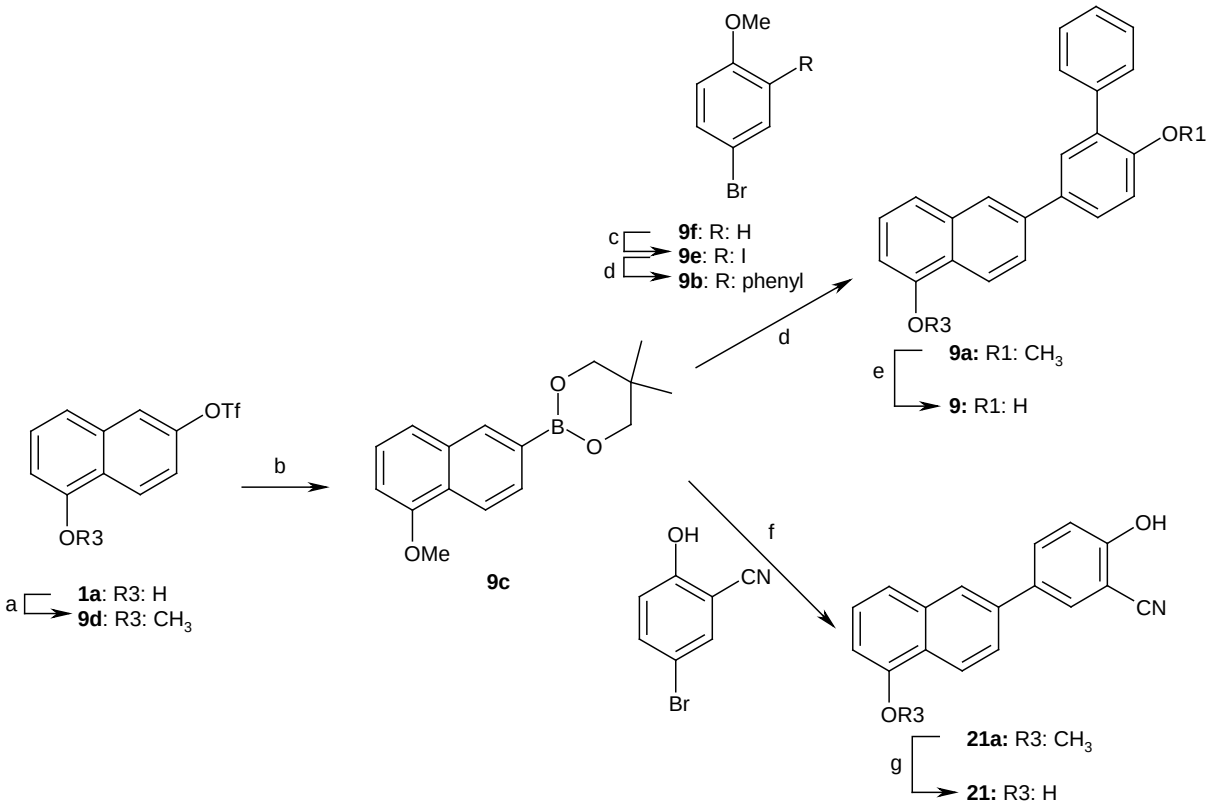


[a]Reagents and conditions: a. boronic acid, $Pd(PPh_3)_4$, aq. Na_2CO_3, DME/EtOH, 90°C, 2h for **1, 3a, 4, 6**, **12** and **17**, Method A; b. boronic acid, $Pd(PPh_3)_4$, Cs_2CO_3, DME/water, microwave irradiation (150°C, 150 W, 25 min) for **8**, **10**, **14**, **16**, **18** and **20**, Method B; c. BBr_3, CH_2Cl_2, -78°C to rt, overnight, for **2**, **7** and **13**, Method C; d. $BF_3.SMe_2$, CH_2Cl_2, rt, overnight, for **3**, **5** and **15**, Method D; e. pyridinium hydrochloride, 220°C, 3h for **11** and **19**, Method E.

Compound **9** bearing an additional phenyl ring on the hydroxyphenyl moiety was synthesised according to the route described in Scheme 2. Compound **1a** was first methylated using methyliodide[54] and subsequently the triflate group was exchanged by a boronic ester group (intermediate **9c**) with bis(neopentylglycolato)diboron and a crown-ether under basic conditions according to the method of Xu *et al.*[55] In parallel, compound **9b** was synthesised by a Suzuki coupling with 4-bromo-2-iodo-1-methoxybenzene **9e** and phenylboronic acid. Subsequent Suzuki coupling (method B) between compounds **9c** and **9b** led to compound **9a**. Demethylation using boron tribromide (method C) afforded compound **9**.

The synthesis of **21** was carried out by palladium-catalysed Suzuki coupling reaction using the intermediate **9c** (method A) and the commercially available 5-bromo-2-hydroxybenzonitrile, followed by ether cleavage with boron trifluoride dimethylsulfide complex (method D, Scheme 2).

Scheme 2. Synthesis of compounds **9** and **21**[a]



[a]Reagents and conditions: a. MeI, K_2CO_3, 18-Crown-6, acetone, reflux, overnight; b. bis(neopentylglycolato)diboron, $PdCl_2$(dppf), dppf, KOAc, 1,4-dioxane, 85°C, 4h; c. $PhI(OAc)_2$, I_2, EtOAc, 60°C, dark conditions, 4h; d. boronic acid, $Pd(PPh_3)_4$, Cs_2CO_3, DME/water, microwave irradiation (150°C, 150 W, 25 min), Method B; e. BBr_3, CH_2Cl_2, -78°C to rt, overnight, Method C; f. boronic acid, $Pd(PPh_3)_4$, aq. Na_2CO_3, DME/EtOH, 90°C, 2h, Method A; g. $BF_3.SMe_2$, CH_2Cl_2, rt, overnight, Method D.

Biological characterisation

Inhibition of human 17β-HSD2

17β-HSD2 inhibitory activity of the synthesised compounds was first evaluated in a cell-free assay. Human placental microsomal enzyme was used as source. The incubation was performed with tritiated E2, cofactor and inhibitor. The separation of substrate and product was accomplished by HPLC with a subsequent quantification by peak integration[56]. The percent inhibition values of compounds **1** to **21** are shown in Table 1, and the IC_{50} values of the most active compounds are reported in Table 2. Compounds showing less than 10% inhibition at 1µM concentration were considered to be inactive. Compound **A**, identified in a previous work[52], was used as internal reference (74% 17β-HSD2 inhibition *vs.* 20% 17β-

HSD1 inhibition at 1µM) and a spiro-δ-lactone (compound **10** published by Poirier *et al.*[43]) was taken as external control (68% at 1µM in our test; 62-66% at 1µM in their test[43]).

For selected substituents (R2 = 2´-CH_3, 2´-OCH_3, 3´-OCH_3, 2´-F, 3´-F, 2´,5´-diF, 3´-CF_3), both hydroxyphenyl **2**, **5**, **8**, **11**, **13**, **15**, **19** and corresponding methoxyphenyl derivatives **1**, **4**, **6**, **10**, **12**, **14**, **18** were synthesised and tested for 17β-HSD2 inhibitory activity. As observed for the unsubstituted compound **A**, the hydroxylated molecules (**2**, **8**, **11**, **13**, **15** and **19**) were always more potent than the respective methoxylated analogues except for the compound pair **4** (R2 = 2´-OCH_3) and **5**. For compounds **3**, **9**, **16**, **17**, **20** and **21**, the hydroxylated derivatives only were therefore tested.

Introduction of a lipophilic group like methyl (compounds **2** and **3**) led to good 17β-HSD2 inhibitory activity (65% inhibition for **2** and 79% for **3** when tested at 1 µM), which was at the level of unsubstituted **A** (74% inhibition at 1 µM). This indicates that there is space around the hydroxyphenyl moiety for introduction of a small substituent in 2´- or 3´-position but the methyl is certainly not able to achieve any specific interactions as no gain in activity is observed compared to the parent hydroxyphenylnaphthol **A**.

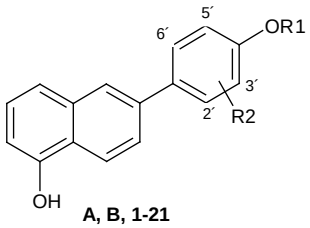
Exchange of the methyl group for a hydrophilic hydroxy substituent was detrimental for activity in 2´-position (**5**, inactive) but tolerated in 3´-position (**8**, similar activity as the unsubstituted **A**). It can therefore be hypothesised that there is no polar amino acid in the active site close to the 2´-position of the inhibitor. Furthermore, the 3´-OH group of compound **8** did not show specific interactions with the enzyme, as no increase in activity was observed compared to **A**. It is striking that an exchange of hydroxy (**8**) by methoxy (**7**) in 3´-position decreased the inhibitory activity strongly.

A phenyl ring was also introduced into the 3´-position (**9**). This compound displayed a slight increase in activity (84% inhibition at 1 µM for **9** *vs.* 74% inhibition at 1 µM for **A**), confirming our previous hypothesis that there is space available around the hydroxyphenyl core.

In order to evaluate the influence of electron withdrawing groups (EWGs) or electron donating groups (EDGs) on the 17β-HSD2 inhibitory activity, compounds **10** to **21** bearing EWGs were synthesised to be compared to compounds with EDGs like methyl (**3**) or phenyl (**9**) groups. All methoxy derivatives (**10**, **12**, **14** and **18**) showed lower activity than the corresponding hydroxy compounds (**11**, **13**, **15** and **19**). Most of the hydroxy compounds exhibited identical or better potency than the parent compound **A** except **21**. Compound **11**, substituted with a fluoro group in 2´- position turned out to be highly active (87% inhibition at 1µM, IC_{50} = 132 nM). Disubstituted fluorine compounds (2´, 5´-diF: **15** and 2´, 6´-diF: **16**)

resulted in highly active inhibitors, but slightly weaker than the monofluoro **11 (**IC_{50}: 238 nM for **15** *vs.* 132 nM for **11**). Compounds **17** (R2 = 2´-CF_3) and **19** (R2 = 3´-CF_3) showed high (**17**) and very high (**19**) activity (84% and 98% inhibition at 1µM, respectively), resulting in IC_{50} values of 19 nM for **19** *vs.* 156 nM for **17**. However, exchange of the trifluoromethyl group for bioisosteric chloro (**20**) or cyano (**21**) groups was detrimental for the activity (74% and 45% inhibition for **20** and **21**, respectively) when compared with inhibitory efficiency of **19** (98% at 1µM).

Table 1. Inhibition of human 17β-HSD2 and 17β-HSD1 by compounds **1** to**21**



A, B, 1-21

Compd	R1	R2	Inhibition of 17β-HSD2[a] [%] at 1 µM	Inhibition of 17β-HSD1[b] [%] at 1 µM	LogP[c]
Spiro-δ-lactone	-	-	68	n.i.	-
A	H	H	74	20	3.93
B	CH_3	H	20	28	-
1	CH_3	2´-CH_3	25	20	4.68
2	H	2´-CH_3	65	25	4.41
3	H	3´-CH_3	79	48	4.41
4	CH_3	2´-OCH_3	37	15	4.06
5	H	2´-OH	11	10	3.54
6	CH_3	3´-OCH_3	49	45	4.06
7	H	3´-OCH_3	36	24	3.80
8	H	3´-OH	69	31	3.54
9	H	3´-phenyl	84	47	5.60
10	CH_3	2´-F	32	41	4.35
11	H	2´-F	87	36	4.09
12	CH_3	3´-F	51	66	4.35
13	H	3´-F	78	62	4.09
14	CH_3	2´, 5´-diF	26	73	4.51
15	H	2´, 5´-diF	83	74	4.24
16	H	2´, 6´-diF	69	73	4.24
17	H	2´-CF_3	84	52	4.85
18	CH_3	3´-CF_3	44	69	5.11
19	H	3´-CF_3	98	60	4.85
20	H	3´-Cl	74	43	4.49
21	H	3´-CN	45	n.i.	3.96

[a] Human placenta, microsomal fraction, substrate [^{3}H]-E2 + E2 [500 nM], NAD^+ [1500 µM], mean value of three determinations, relative standard deviation < 10%, [b] Human placenta, cytosolic fraction, substrate [^{3}H]-E1 + E1 [500 nM], NADH [500 µM], mean value of three determinations, relative standard deviation < 10%, [c] calculated logP data; n.i.: no inhibition (inhibition < 10%).

Selectivity and cellular activity

17β-HSD1, as the main enzyme implicated in the reduction of estrone (E1) to E2 in sex steroid metabolism[57], should not be affected by inhibitors of 17β-HSD2. Furthermore, inhibitors of 17β-HSD2 should have none or little affinity for the estrogen receptors α and β (ERα and ERβ), as most of the E2 effects are ER mediated. Therefore all compounds were tested for selectivity toward 17β-HSD1 and a selection of the most potent inhibitors for binding affinity to ERα and β.

The assay for 17β-HSD1 inhibition was performed in a similar way as for the 17β-HSD2. Human placental cytosolic 17β-HSD1 was incubated with tritiated E1, cofactor and inhibitor. The amount of labelled E2 formed was determined after HPLC separation. For the most potent compounds, IC_{50} values were determined and selectivity factors (SFs) calculated (SF = IC_{50} 17β-HSD1 / IC_{50} 17β-HSD2, Table 2). All compounds except **15** showed selectivity toward 17β-HSD1. The most selective one, with a selectivity factor of 32, was compound **19** (R2: 3´-CF_3), which was also the most potent one exhibiting an IC_{50} value of 19 nM. The 2´-F compound **11** also showed a high SF of 17 toward 17β-HSD1.

For the determination of ER binding affinity, a competition assay was used, with tritiated E2. Receptor bound and free E2 were separated by means of hydroxyapatite. Compounds **3**, **9**, **11** and **19** showed a relative binding affinity (RBA) of less than 0.1% to ERα, compared to the affinity of E2, which is arbitrarily set to 100%. The indicated compounds are therefore classified as low affinity ligands of the ERα.

The logP values were calculated using the ChemDrawPro 11.0 program in order to get insight into the lipophilicity of this class of compounds and are shown in Table 1. The values for all compounds except **9** and **18** are lower than 5, fulfilling the criteria of Lipinski´s rule of five. It therefore can be expected that they are able to easily permeate cell membranes.

The most interesting compounds **11**, **17** and **19** with IC_{50} values below 200 nM were further investigated in a cellular assay, using the human breast cancer cell line MDA-MB-231[58], which endogenously expresses 17β-HSD2. Cells were incubated with tritiated E2 and inhibitor for 3.5h. Separation was performed by HPLC similar as in the cell-free inhibition assays. Compounds **17** and **19** showed very similar inhibitory activity in MDA-MB-231 cells as in the cell-free assay, *i.e.* IC_{50} of 171 nM for **17** and 31 nM for **19** in cellular assay *vs.* 156 nM and 19 nM, respectively, in the cell-free assay. Interestingly, compound **11** showed an IC_{50} of 1227 nM in the cellular assay compared to 132 nM in the cell-free assay, indicating that this compound is less appropriate to permeate the cell membrane or is metabolically unstable.

Table 2. IC_{50} values, selectivity factor and binding affinities for the estrogen receptors α and β for selected compounds.

Compd	Cell free assay			ERα RBA[d] (%)	ERβ RBA[d] (%)
	17β-HSD2 IC_{50}[a] [nM]	17β-HSD1 IC_{50}[b] [nM]	Selectivity factor[c]		
Spiro- δ-lactone	34	n.d.	n.d.	n.d.	n.d.
A	302	2425	8	5	5
3	275	1748	6	< 0.1	0.1-1.0
9	261	919	4	< 0.1	< 0.1
11	132	2245	17	< 0.1	< 0.1
13	292	713	2	0.1-1.0	0.1-1.0
15	238	309	1	0.1-1.0	0.1-1.0
17	156	926	6	1.0-10	1.0-10
19	19	611	32	< 0.1	0.1-1.0
20	307	1130	4	0.1-1.0	0.1-1.0

[a] Human placenta, microsomal fraction, substrate [^{3}H]-E2 + E2 [500 nM], cofactor NAD^+ [1500 μM], mean value of three determinations, relative standard deviation < 10%, [b] Human placenta, cytosolic fraction, substrate [^{3}H]-E1 + E1 [500 nM], cofactor NADH [500 μM], mean value of three determinations, relative standard deviation < 20%, [c] IC_{50} (17β-HSD1)/ IC_{50} (17β-HSD2), [d] RBA: Relative Binding Affinity, E2: 100%; n.d.: not determined.

Further *in vitro* assays

In order to refine the profile of the most potent compound **19**, it was further tested for selectivity on other enzymes, namely 17β-HSDs type 4 and 5. 17β-HSD4 catalyses the same reaction as 17β-HSD2 (Chart 1)[57], and 17β-HSD5 (beside 17β-HSD3) is responsible for the reduction of the androgen A-dione to T[57]. Both enzymes should not be inhibited by 17β-HSD2 inhibitors. To investigate this important issue, experiments were performed with *E.coli* bacteria expressing human 17β-HSD4 and 17β-HSD5[56, 59]. These cells were incubated with cofactor, inhibitor and tritiated E2 in case of 17β-HSD4 and A-dione in case of 17β-HSD5. After RP-HPLC separation of substrate and product, the amount of labelled E1 or T formed was quantified. Compound **19** was found to be selective toward 17β-HSD4 and 17β-HSD5 (40% 17β-HSD4 inhibition and 21% 17β-HSD5 inhibition at 1μM).

According to the potency of compound **19** in cell-free and cellular assays and its selectivity toward 17β-HSD1, 17β-HSD4, 17β-HSD5 and ERs, it can be considered as potential candidate for further development. Before evaluation in an animal model of the efficacy of this new 17β-HSD2 inhibitor **19**, it is reasonable to determine its profile on

orthologous enzymes from different species. Compound **19** was therefore tested for inhibition of 17β-HSD2/E1-formation (activity) and 17β-HSD1/E2-formation (selectivity) using rat, mouse and monkey enzymes.

In rat and mouse assays, liver enzymes were used, while in monkey assay, placental enzymes were employed. Compound **19** showed good inhibition of the rat and mouse 17β-HSD2 enzymes and very good inhibition on the monkey enzyme. In this case, the efficacy was in the same range as for the human enzyme (Table 3). In addition, selectivity toward 17β-HSD1/E2-formation in monkey seems to be similar to that observed in human.

Table 3. Inhibition of rat, mouse and monkey E1- and E2-formation by compound **19**.

Compound **19**	**Inhibition of E1-formation[a] [%]**		**Inhibition of E2-formation[b] [%]**	
	1µM	**100 nM**	**1µM**	**100 nM**
Human	98[c]	87[c]	60[d]	27[d]
Rat	77[e]	n.d.	52[f]	n.d.
Mouse	72[g]	n.d.	26[h]	n.d.
Monkey	99[i]	75[i]	75[j]	55[j]

[a] Substrate [^{3}H]-E2 + E2 [500 nM], NAD^+ [1500 µM], mean value of three determinations, relative standard deviation < 10%, [b] Substrate [^{3}H]-E1 + E1 [500 nM], NADH [500 µM], mean value of three determinations, relative standard deviation < 10%, [c] Human placenta, microsomal fraction, [d] Human placenta, cytosolic fraction, [e] Rat liver, microsomal fraction, [f] Rat liver, cytosolic fraction, [g] Mouse liver, microsomal fraction, [h] Mouse liver, cytosolic fraction, [i] Monkey placenta, microsomal fraction, [j] Monkey placenta, cytosolic fraction, n.d.: not determined.

Discussion and Conclusion

Compound **A**, identified by us in a previous study[52] represents a promising scaffold as 17β-HSD2 inhibitor: substituted by two hydroxy groups (polar), the naphthalene and phenyl cores (hydrophobic) mimicking the substrate E2. We have previously shown[52] that an exchange of the hydroxy group on the phenyl moiety by a sulfonamide did not affect the activity toward 17β-HSD2 (74% *vs.* 75% inhibition at 1µM, respectively), indicating that some space is available around the phenyl core. We hypothesised therefore that the phenyl ring mimics the D-ring of E2 and thus that introduction of additional substituents on this phenyl moiety would be tolerated and would allow an increase of the 17β-HSD2 inhibitory activity. In this paper, we aimed at verifying whether this hypothesis is correct and whether introduction of various substituents on the phenyl ring could improve this activity.

The biological activity of the phenyl-substituted compound **9** confirms that the compound can indeed bind in the active site according to the previous hypothesis (the hydroxy naphthalene mimics the A-ring and the hydroxyphenyl the D-ring). The additional phenyl might be accommodated between cofactor and steroid where space is available.

The electronic effects of the substituents on the 17β-HSD2 inhibitory activity were further elucidated. No matter where being introduced, EDGs (Me, OH, Ph) in either 2´- or 3´-position did not lead to significant differences in activity except for the hydroxy group where position 3´ was better (compounds **5** and **8**). None of the synthesised compounds (**2**, **3**, **8** and **9**) reached a better activity compared to reference **A** indicating that these substituents are tolerated (there is space available) but not able to establish specific interactions with amino acid residues. The position of the substituent on the phenyl ring will induce either a pronounced rotation of the phenyl toward the naphthalene (position 2´, *ortho* effect) or a weak rotation (position 3´). In both cases, only the 4´-hydroxy substituent stays at the same position and anchors the molecule into its binding site. Our finding that there is no difference in activity between 2´- and 3´-substituted compounds indicates that the phenyl ring is not able to establish π- π stacking interactions with amino acid side chains of the protein.

When the substituent was an EWG (F, CF_3, Cl, CN), the highest potency was observed for compound **19** (R2: 3´-CF_3) in which the additional group was located in the 3´-position. Exchange of the trifluoromethyl group by the bioisosteric groups like chloro (**20**) and cyano (**21**) decreased inhibition. In case of the CN group, the different activities might be due to the fact that CN is hydrophilic whereas CF_3 is lipophilic. The linear shape of CN giving steric hindrance might also explain the moderate activity of **21**.

As shown in many other cases[5] and also observed for compound **11**, the substitution with F results in an increase in inhibitory activity. However, the introduction of a second fluorine as in **15** or **16** does not further enhance activity, indicating that the effects are not additive.

In our previous study[52], it was observed that the hydroxy group bound to the phenyl moiety was important for activity (74% at 1µM for **A** with OH *vs*. 20% inhibition for **B** with OMe). This is confirmed in this study as all methoxy compounds displayed lower 17β-HSD2 inhibitory activity than their corresponding hydroxy analogues. Contrary to our compounds, the other described non-steroidal 17β-HSD2 inhibitor[46-48], the *cis*-pyrrolidinones, do not need any hydrophilic group to be active, letting us to presume that they might have a different binding mode compared to ours.

As other 17β-HSD enzymes of the short-chain dehydrogenase/reductase (SDR) and the aldo-ketoreductases (AKR) family participate in steroid interconversions[60], the development

of enzyme-specific inhibitors is a challenge. We therefore tested the compounds on other 17β-HSDs, like 17β-HSD type 1, 2, 4 and 5. Selectivity toward 17β-HSD1 was low with nearly all of the tested hydroxyphenylnaphthol derivatives, however, the most potent 17β-HSD2 inhibitors **11** and **19** showed the highest selectivity toward 17β-HSD1, exhibiting promising selectivity factors (SFs) of 17 and 32, respectively. Although some inhibition of 17β-HSD4 and 5 was observed with the best inhibitor compound **19,** the substance can be assumed to be selective toward both enzymes. We were surprised by the fact, that the compound **19** showed some minor inhibition of 17β-HSD4. In our previous projects we have not seen any effect on this particular enzyme with steroidal or non-steroidal inhibitors[56, 61]. Nevertheless, the observed inhibition of compound **19** is fairly low and the impact on the ubiquitously expressed 17β-HSD4 contributing to the peroxisomal β-oxidation of fatty acids[62] is most probably negligible.

Compounds **11** and **19** also displayed very little binding affinity to ERα, which is responsible for cell proliferation. Only compound **19** showed a very good cellular activity (IC_{50} = 31 nM in MDA-MB-231 cells). Thus it is able to permeate the cell membrane and is not quickly metabolised.

An osteoblastic cell-line, expressing 17β-HSD2 and the ERs like SV-HFO should be promising to prove our concept that 17β-HSD2 inhibition is able to induce bone formation and inhibit osteoclastogenesis. For further *in vivo* experiments, species differences have to be taken into consideration[63, 64], and compound **19** should inhibit the enzyme of the chosen species. Pursuing this, **19** was tested on rat, mouse and monkey enzymes, and revealed a high (72% to 77% inhibition at 1μM in mouse and rat enzymes, respectively) to very high (99% inhibition at 1μM in monkey enzyme) inhibitory activity for 17β-HSD2/E1-formation. Showing a better activity and selectivity in marmoset (*Callithrix jacchus*) enzymes, this species should be appropriate for proving our concept.

In the present study, we describe the synthesis of substituted 6-phenyl-1-naphthols as inhibitors of 17β-HSD2 and the evaluation of their biological activities. A new highly potent inhibitor of 17β-HSD2 has been discovered (compound **19**), with a very good cellular activity and good selectivity toward 17β-HSD1, 17β-HSD4, 17β-HSD5 and ERs. Compound **19** is also able to inhibit E1-formation in rat, mouse and monkey. Further biological analyses still need to be investigated to get information on the efficacy of the compound, to demonstrate that 17β-HSD2 inhibitors are able to inhibit osteoclastogenesis and induce bone formation.

Experimental section

Chemical methods.

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DRX-500 instrument in $CDCl_3$ and acetone-d_6. Chemicals shifts are reported in δ values (ppm), the hydrogenated residues of deuteriated solvent were used as internal standard ($CDCl_3$: δ = 7.26 ppm in ^{1}H NMR and δ = 77.0 ppm in ^{13}C NMR, acetone-d_6: δ = 2.05 ppm in ^{1}H NMR and δ = 30.8 ppm and 206.3 ppm in ^{13}C NMR). Signals are described as s, br, d, t, dd, dt, qt and m for singlet, broad, doublet, triplet, doublet of doublets, doublet of triplets, quintuplet and multiplet, respectively. All coupling constants (*J*) are given in Hertz. IR spectra were measured neat on a Bruker Vector 33FT-infrared spectrometer. Melting points (mp) were determined in open capillaries on a Stuart Scientific SMP3 apparatus and are uncorrected.

Mass spectra (GC/MS) were measured on a GCD Series G1800A (Hewlett Packard) instrument with an Optima-5-MS (0.25 μM, 30 m) column (Macherey Nagel). All microwave irradiation experiments were carried out in a CEM-Discover monomode microwave apparatus.

Flash chromatography was performed on silica gel 40 (35/40-63/70 μM) with hexane/ethyl acetate mixtures as eluents and the reaction progress was determined by thin-layer chromatography analyses on Alugram SIL G/UV254 (Macherey-Nagel). Visualization was accomplished with UV light.

Tested compounds are≥ 95 % chemical purity as measured by LC/MS. A table of data for all tested compounds are provided in the supporting information. The Surveyor®-LC-system consisted of a pump, an autosampler, and a PDA detector. Mass spectrometry was performed on a MSQ® electro spray mass spectrometer (ThermoFisher, Dreieich, Germany). The system was operated by the standard software Xcalibur®.

A RP-C18 NUCLEODUR® 100-5 (125x3 mm) column (Macherey-Nagel GmbH, Dühren, Germany) was used as stationary phase. All solvents were HPLC grade.

In a gradient run the percentage of acetonitrile (containing 0.1 % trifluoroacetic acid) in 0.1 % trifluoroacetic acid was increased from an initial concentration of 0 % at 0 min to 100 % at 15 min and kept at 100 % for 5 min. The injection volume was 15 μL and flow rate was set to 800 μL/min. MS analysis was carried out at a spray voltage of 3800 V and a capillary temperature of 350 °C and a source CID of 10 V. Spectra were acquired in positive mode from 100 to 1000 m/z at 254 nm for the UV trace.

Starting materials (different boronic acids and compounds **1e**, **9f** and **21b**) were purchased from Aldrich, Alfa Aeser, Acros and Combi-blocks and were used without further purification. No attempts were made to optimise yields.

The following compounds were prepared according to previously described procedures: 5-hydroxy-2-naphthyl trifluoromethanesulfonate **1a**[52], 4-bromo-2-iodo-1-methoxybenzene **9e**[27].

General procedures for Suzuki coupling

Method A. To a mixture of arylbromide (1 equiv) and tetrakis(triphenylphosphine) palladium (0) (0.02 equiv) in DME was added a 2N aqueous solution of sodium carbonate (2 equiv) and the mixture (oxygen free) was purged with N_2. The resultant solution was stirred at room temperature for 5 min and a solution of boronic acid (1.3 equiv) in EtOH was added. The mixture was heated to 90°C and stirred for 2h. The reaction mixture was cooled to room temperature, quenched by water and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate, filtered and concentrated to dryness. The product was purified by column chromatography.

Method B. A mixture of arylbromide (1 equiv), boronic acid (1 equiv), caesium carbonate (3 equiv), and tetrakis(triphenylphosphine) palladium (0) (0.02 equiv) was suspended in an oxygen-free DME/water (2:1) solution. The reaction mixture was exposed to microwave irradiation (25 min, 150W, 150°C, 15 bar). After reaching room temperature, water was added and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, filtered and concentrated to dryness. The product was purified by column chromatography.

General procedures for ether cleavage

Method C. To a solution of methoxy derivative (1 equiv) in dry dichloromethane cooled at -78°C under nitrogen was slowly added boron tribromide (1M solution in dichloromethane, 5 equiv to 10 equiv per methoxy function). The reaction mixture was stirred at -78°C for 1h and then allowed to warm to room temperature overnight. The reaction was quenched by water and extracted with ethyl acetate. The combined organic layers were washed with brine and dried over sodium sulfate, evaporated to dryness under reduced pressure and purified by column chromatography.

Method D. To a solution of methoxy derivative (1 equiv) in dry dichloromethane, borontrifluoride dimethyl sulfide complex (75 equiv per methoxy function) was added drop wise at room temperature. The reaction mixture was stirred at room temperature overnight. Water was added to quench the reaction and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, evaporated to dryness under reduced pressure and purified by column chromatography.

Method E. To pyridinium hydrochloride (100 equiv) at 190°C was added methoxy derivative (1 equiv), and the solution was stirred for 2h. The reaction mixture was cooled to room temperature and was stirred with 1N HCl. The mixture was dissolved in ethyl acetate and the combined organic layers were washed with water, dried over sodium sulfate, filtered, evaporated to dryness under reduced pressure and purified by column chromatography.

6-(4-Methoxy-2-methylphenyl)-1-naphthol (1). The title compound was prepared by reaction of 5-hydroxy-2-naphthyl trifluoromethanesulfonate **1a** (150 mg, 0.51 mmol, 1 equiv) with 4-methoxy-2-methylphenylboronic acid (110 mg, 0.66 mmol, 1.3 equiv) according to method A. The product was purified by column chromatography (hexane/ethyl acetate 9:1) to give 90 mg (63%) of the analytically pure compound as a yellow oil. $C_{18}H_{16}O_2$; MW 264; ^{1}H NMR (acetone-d_6): δ 9.00 (s, br, 1H), 8.28 (d, *J* = ca. 9.3 Hz, 1H), 7.72 (d, *J* = ca. 1.5 Hz, 1H), 7.45-7.40 (m, 2H), 7.35-7.31 (m, 1H), 7.24 (d, *J* = ca. 8.4 Hz, 1H), 6.94 (dd, *J* = ca. 0.7 Hz, *J* = ca. 7.4 Hz, 1H), 6.91 (d, *J* = ca. 2.6 Hz, 1H), 6.86 (dd, *J* = ca. 2.6 Hz, *J* = ca. 8.3 Hz, 1H), 3.84 (s, 3H), 2.30 (s, 3H); ^{13}C NMR (acetone-d_6): δ 160.0, 154.0, 140.4, 137.5, 135.9, 135.3, 131.7, 128.2, 127.5, 127.4, 124.5, 122.7, 120.1, 116.6, 112.1, 108.9, 55.5, 20.9; IR: 3354, 2956, 1502, 1240 cm^{-1}; GC/MS *m/z*: 264 $(M)^+$.

6-(4-Hydroxy-2-methylphenyl)-1-naphthol (2). The title compound was prepared by reaction of 6-(4-methoxy-2-methylphenyl)-1-naphthol **1** (20 mg, 0.08 mmol, 1 equiv) with boron tribromide (0.40 mmol, 5 equiv) according to method C. The product was purified by column chromatography (hexane/ethyl acetate 1:1) to give 9 mg (43%) of the analytically pure compound as a yellow oil. $C_{17}H_{14}O_2$; MW 250; ^{1}H NMR (acetone-d_6): δ 8.93 (s, br, 1H), 8.22 (s, br, 1H), 8.22-8.20 (m, 1H), 7.66 (d, *J* = ca. 1.6 Hz, 1H), 7.40-7.34 (m, 2H), 7.30-7.25 (m, 1H), 7.11 (d, *J* = ca. 8.3 Hz, 1H), 6.88 (dd, *J* = ca. 0.9 Hz, *J* = ca. 7.4 Hz, 1H), 6.77 (d, *J* = ca. 2.5 Hz, 1H), 6.73 (dd, *J* = ca. 2.5 Hz, *J* = ca. 8.3 Hz, 1H), 2.78 (s, 3H); ^{13}C NMR (acetone-

d$_6$): δ 157.9, 154.0, 140.7, 138.7, 136.0, 131.8, 128.2, 127.6, 127.4, 124.5, 122.6, 120.0, 117.9, 116.7, 113.7, 108.8, 20.8; IR: 3378, 2924, 1691, 1235 cm^{-1}; GC/MS *m/z*: 250 (M)$^+$.

6-(4-Methoxy-3-methylphenyl)-1-naphthol (3a). The title compound was prepared by reaction of 5-hydroxy-2-naphthyl trifluoromethanesulfonate **1a** (75 mg, 0.26 mmol, 1 equiv) with 4-methoxy-3-methylphenylboronic acid (55 mg, 0.33 mmol, 1.3 equiv) according to method A. The product was purified by column chromatography (hexane/ethyl acetate 9:1) to afford 70 mg (49%) of the analytically pure compound. $C_{18}H_{16}O_2$; MW 264; ^{1}H NMR (acetone-*d*$_6$): δ 8.96 (s, br, 1H), 8.28 (d, *J* = ca. 9.3 Hz, 1H), 8.02 (d, *J* = ca. 2.3 Hz, 1H), 7.74 (dd, *J* = ca. 1.9 Hz, *J* = ca. 8.7 Hz, 1H), 7.61-7.58 (m, 2H), 7.45-7.42 (m, 1H), 7.33-7.28 (m, 1H), 7.06-7.02 (m, 1H), 6.89 (dd, *J* = ca. 0.8 Hz, *J* = ca. 7.5 Hz, 1H), 3.89 (s, 3H), 2.28 (s, 3H); ^{13}C NMR (acetone-*d*$_6$): δ 158.5, 154.0, 139.3, 136.4, 130.1, 127.5, 126.5, 125.2, 124.7, 123.5, 120.2, 118.5, 113.3, 112.1, 111.3, 108.8, 55.8, 16.5; IR: 3404, 2955, 1503, 1245, 1219 cm^{-1}.

6-(4-Hydroxy-3-methylphenyl)-1-naphthol (3). The title compound was prepared by reaction of 6-(4-methoxy-3-methylphenyl)-1-naphthol **3a** (20 mg, 0.08 mmol, 1 equiv) with boron trifluoride dimethylsulfide complex (6.00 mmol, 75 equiv) according to method D. The product was purified by column chromatography (hexane/ethyl acetate 1:1) to give 12 mg (60%) of the analytically pure compound as a brown solid. $C_{17}H_{14}O_2$; MW 250; mp: 164-166°C; ^{1}H NMR (acetone-*d*$_6$): δ 8.90 (s, br, 1H), 8.26 (s, br, 1H), 8.23 (d, *J* = ca. 8.5 Hz, 1H), 7.96 (d, *J* = ca. 1.9 Hz, 1H), 7.69 (dd, *J* = ca. 2.0 Hz, *J* = ca. 8.5 Hz, 1H), 7.53 (d, *J* = ca. 1.9 Hz, 1H), 7.43 (dd, *J* = ca. 2.0 Hz, *J* = ca. 8.5 Hz, 1H), 7.40-7.37 (m, 1H), 7.28-7.24 (m, 1H), 6.91 (d, *J* = ca. 8.5 Hz, 1H), 6.84 (dd, *J* = ca. 0.8 Hz, *J* = ca. 7.4 Hz, 1H), 2.27 (s, 3H); ^{13}C NMR (acetone-*d*$_6$): δ 156.1, 154.0, 139.6, 136.4, 133.0, 130.4, 127.4, 126.4, 125.6, 124.9, 124.7, 123.5, 120.2, 118.2, 116.0, 108.7, 16.3; IR: 3337, 2952, 1506, 1274 cm^{-1}; GC/MS *m/z*: 250 (M)$^+$.

6-(2,4-Dimethoxyphenyl)-1-naphthol (4). The title compound was prepared by reaction of 5-hydroxy-2-naphthyl trifluoromethanesulfonate **1a** (75 mg, 0.26 mmol, 1 equiv) with 2,4-dimethoxyphenylboronic acid (60 mg, 0.33 mmol, 1.3 equiv) according to method A. The product was purified by column chromatography (hexane/ethyl acetate 7:3) to afford 38 mg (49%) of the analytically pure compound as an orange oil. $C_{18}H_{16}O_3$; MW 280; ^{1}H NMR (acetone-*d*$_6$): δ 8.88 (s, br, 1H), 8.18 (d, *J* = ca. 8.7 Hz, 1H), 7.84 (s, 1H), 7.58 (dd, *J* = ca. 1.8

Hz, *J* = ca. 8.7 Hz, 1H), 7.36-7.30 (m, 2H), 7.27-7.23 (m, 1H), 6.86 (d, *J* = ca. 7.5 Hz, 1H), 6.66 (d, *J* = ca. 2.5 Hz, 1H), 6.61 (dd, *J* = ca. 2.5 Hz, *J* = ca. 8.4 Hz, 1H), 3.83 (s, 3H), 3.79 (s, 3H); ^{13}C NMR (acetone-d_6): δ 161.7, 158.7, 153.9, 137.5, 136.0, 132.2, 128.2, 127.8, 127.1, 124.4, 124.1, 122.2, 120.1, 108.7, 106.1, 99.8, 55.9, 55.7; IR: 3388, 2982, 1610, 1507, 1206 cm^{-1}; GC/MS *m/z*: 280 $(M)^+$.

4-(5-Hydroxy-2-naphthyl)benzene-1,3-diol (5). The title compound was prepared by reaction of 6-(2,4-dimethoxyphenyl)-1-naphthol **4** (60 mg, 0.21 mmol, 1 equiv) with boron trifluoride dimethylsulfide complex (32 mmol, 150 equiv) according to method D. The product was purified by column chromatography (hexane/ethyl acetate 1:1) to afford 30 mg (55%) of the analytically pure compound as a yellow solid. $C_{16}H_{12}O_3$; MW 252; mp: 152-154°C; ^{1}H NMR (acetone-d_6): δ 8.99 (s, br, 1H), 8.40 (s, br, 1H), 8.31 (s, br, 1H), 8.29 (s, 1H), 8.02 (d, *J* = ca. 1.7 Hz, 1H), 7.77 (dd, *J* = ca. 1.7 Hz, *J* = ca. 8.5 Hz, 1H), 7.46 (d, *J* = ca. 8.5 Hz, 1H), 7.37 (d, *J* = ca. 7.6 Hz, 1H), 7.33 (d, *J* = ca. 8.5 Hz, 1H), 6.96 (dd, *J* = ca. 1.0 Hz, *J* = ca. 7.6 Hz, 1H), 6.65 (d, *J* = ca. 2.4 Hz, 1H), 6.58 (dd, *J* = ca. 2.4 Hz. *J* = ca. 8.5 Hz, 1H); ^{13}C NMR (acetone-d_6): δ 157.6, 156.9, 151.5, 135.0, 134.2, 130.0, 126.5, 125.4, 124.9, 120.8, 116.4, 108.8, 108.7, 103.4; IR: 3363, 1689, 1598, 1358, 1260 cm^{-1}.

6-(3,4-Dimethoxyphenyl)-1-naphthol (6). The title compound was prepared by reaction of 5-hydroxy-2-naphthyl trifluoromethanesulfonate **1a** (75 mg, 0.26 mmol, 1 equiv) with 3,4-dimethoxyphenylboronic acid (60 mg, 0.33 mmol, 1.3 equiv) according to method A. The product was purified by column chromatography (hexane/ethyl acetate 8:2) to give 46 mg (60%) of the analytically pure compound as a yellow solid. $C_{18}H_{16}O_3$; MW 280; mp: 139-141°C; ^{1}H NMR (acetone-d_6): δ 8.97 (s, br, 1H), 8.28 (d, *J* = ca. 8.5 Hz, 1H), 8.06 (d, *J* = ca. 2.0 Hz, 1H), 7.77 (dd, *J* = ca. 2.0 Hz, *J* = ca. 8.9 Hz, 1H), 7.43 (d, *J* = ca. 8.5 Hz, 1H), 7.39 (d, *J* = ca. 2.0 Hz, 1H), 7.34-7.31 (m, 2H), 7.08 (d, *J* = ca. 8.5 Hz, 1H), 6.90 (dd, *J* = ca. 0.8 Hz, *J* = ca. 7.5 Hz, 1H), 3.94 (s, 3H), 3.87 (s, 3H); ^{13}C NMR (acetone-d_6): δ 154.0, 150.8, 150.3, 139.4, 136.3, 134.6, 127.5, 125.4, 124.8, 123.5, 120.3, 120.2, 113.3, 112.0, 108.9, 56.3, 56.2; IR: 3424, 2934, 1516, 1247 cm^{-1}; GC/MS *m/z*: 280 $(M)^+$.

4-(5-Hydroxy-2-naphthyl)benzene-1,2-diol (7). The title compound was prepared by reaction of 6-(3,4-dimethoxyphenyl)-1-naphthol **6** (20 mg, 0.07 mmol, 1 equiv) with boron tribromide (0.70 mmol, 10 equiv) according to method C. The product was purified by column chromatography (hexane/ethyl acetate 1:1) to afford 8 mg (40%) of the analytically

pure product as an orange oil. $C_{16}H_{12}O_3$; MW 252; ^{1}H NMR (acetone-d_6): δ 8.94 (s, br, 1H), 8.26 (d, *J* = ca. 9.1 Hz, 1H), 8.01 (s, br, 1H), 7.98-7.96 (m, 2H), 7.70 (dd, *J* = ca. 2.0 Hz, *J* = ca. 8.5 Hz, 1H), 7.42 (d, *J* = ca. 8.5 Hz, 1H), 7.31-7.28 (m, 2H), 7.16 (dd, *J* = ca. 2.0 Hz, *J* = ca. 8.1 Hz, 1H), 6.95 (d, *J* = ca. 8.1 Hz, 1H), 6.89 (dd, *J* = ca. 0.9 Hz, *J* = ca. 7.6 Hz, 1H); ^{13}C NMR (acetone-d_6): δ 166.8, 159.2, 154.0, 146.4, 136.3, 134.0, 127.4, 125.1, 124.7, 123.5, 120.2, 119.6, 118.4, 116.7, 115.1, 108.7; IR: 3325, 1602, 1576, 1363, 1274 cm^{-1}.

6-(4-Hydroxy-3-methoxyphenyl)-1-naphthol (8). The title compound was prepared by reaction of 5-hydroxy-2-naphthyl trifluoromethanesulfonate **1a** (200 mg, 0.68 mmol, 1 equiv) and 4-hydroxy-3-methoxyphenylboronic acid (150 mg, 0.89 mmol, 1.3 equiv) according to method B. The product was purified by column chromatography (hexane/ethyl acetate 8:2) to give 150 mg (52%) of the analytically pure compound as a brown solid. $C_{17}H_{14}O_3$; MW 266; mp: 146-147°C; ^{1}H NMR ($CDCl_3$): δ 8.22 (d, *J* = ca. 8.5 Hz, 1H), 7.91 (d, *J* = ca. 1.8 Hz, 1H), 7.68 (dd, *J* = ca. 1.8 Hz, *J* = ca. 8.7 Hz, 1H), 7.44 (d, *J* = ca. 8.2 Hz, 1H), 7.32-7.28 (m, 1H), 7.22 (dd, *J* = ca. 2.1 Hz, *J* = ca. 8.2 Hz, 1H), 7.19 (d, *J* = ca. 2.1 Hz, 1H), 7.02 (d, *J* = ca. 8.0 Hz, 1H), 6.79 (dd, *J* = ca. 1.0 Hz, *J* = ca. 7.4 Hz, 1H), 3.98 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 151.7, 146.9, 145.4, 139.0, 135.1, 133.6, 126.4, 124.9, 124.8, 123.3, 122.3, 120.6, 120.5, 114.8, 110.0, 108.4, 56.1; IR: 3250, 2987, 1520, 1273 cm^{-1}; GC/MS *m/z*: 266 $(M)^+$.

5-Methoxy-2-naphthyl trifluoromethanesulfonate (9d). To a solution of 5-hydroxy-2-naphthyl trifluoromethanesulfonate **1a** (400 mg, 1.37 mmol, 1 equiv) in 7 mL acetone were added K_2CO_3 (186 mg, 1.66 mmol, 1.3 equiv) and 18-Crown-6 (4 mg, 0.01 mmol, 0.01 equiv). The mixture was stirred for 15 min at room temperature before methyliodide (250 mg, 0.12 mL, 1.66 mmol, 1.3 equiv) was added dropwise and then refluxed overnight. After cooling to room temperature, the mixture was filtered through a pad of Celite® and concentrated. The residue was taken up in ethyl acetate and washed twice with 1N NaOH, water and brine, dried over sodium sulfate and concentrated to dryness. The solid was recrystallised in hexane/ethyl acetate 10:1 to afford 315 mg (75%) of the analytically pure product as a beige solid. $C_{12}H_9F_3O_4S$; MW 306; mp: 134-135°C; ^{1}H NMR ($CDCl_3$): δ 8.33 (d, *J* = ca. 9.3 Hz, 1H), 7.67 (d, *J* = ca. 2.5 Hz, 1H), 7.49-7.44 (m, 1H), 7.41 (d, *J* = ca. 8.3 Hz, 1H), 7.31 (dd, *J* = ca. 2.5 Hz, *J* = ca. 9.3 Hz, 1H), 6.86 (d, *J* = ca. 7.6 Hz, 1H), 4.00 (s, 3H).

2-(5-Methoxy-2-naphthyl)-5,5-dimethyl-1,3,2-dioxaborinane (9c). A flask was flushed with nitrogen and charged with $PdCl_2$(dppf) (50 mg, 0.07 mmol, 0.1 equiv), dppf (38 mg, 0.07

mmol, 0.1 equiv), KOAc (200 mg, 2.04 mmol, 3 equiv) and bis(neopentylglycolato)diboron (169 mg, 0.75 mmol, 1.1 equiv). A solution of 5-methoxy-2-naphthyl trifluoromethanesulfonate **9d** (200 mg, 0.68 mmol, 1 equiv) in 1,4-dioxane (16 mL) was added and the mixture was stirred for 4h at 85°C. The reaction was worked up by filtration of the mixture through a silica gel pad, and washed with ethyl acetate. The combined solvent was concentrated under vacuum to give the crude boronic acid purified by column chromatography (hexane/ethyl acetate 10:1) to afford 121 mg (66%) of the analytically pure compound as a white solid. $C_{16}H_{19}BO_3$; MW 270; mp: 148-149°C; ^{1}H NMR ($CDCl_3$): δ 8.22 (s, 1H), 8.14 (d, *J* = ca. 8.0 Hz, 1H), 7.77 (dd, *J* = ca. 0.9 Hz, *J* = ca. 8.3 Hz, 1H), 7.39 (d, *J* = ca. 8.3 Hz, 1H), 7.31-7.27 (m, 1H), 6.76 (d, *J* = ca. 8.0 Hz, 1H), 3.93 (s, 3H), 3.76 (s, 4H), 0.99 (s, 6H); ^{13}C NMR ($CDCl_3$): δ 155.2, 134.5, 133.9, 129.4, 127.0, 125.5, 121.0, 120.8, 104.5, 72.4, 55.5, 31.9, 21.9.

2-Methoxy-5-(5-methoxy-2-naphthyl)biphenyl (9a). The title compound was prepared by reaction of 5-bromo-2-methoxybiphenyl **9b** (100 mg, 0.38 mmol, 1 equiv) with 2-(5-methoxy-2-naphthyl)-5,5-dimethyl-1,3,2-dioxaborinane **9c** (103 mg, 0.38 mmol, 1 equiv) according to method B. The product was purified by column chromatography (hexane/ethyl acetate 8:2) to give 80 mg (62%) of the analytically pure compound as a white oil. $C_{24}H_{20}O_2$; MW 340; ^{1}H NMR (acetone-d_6): δ 8.20 (d, *J* = ca. 8.5 Hz, 1H), 7.89-7.87 (m, 1H), 7.60-7.59 (m, 1H), 7.57 (d, *J* = ca. 8.5Hz, 1H), 7.45 (dd, *J* = ca. 2.0 Hz, *J* = ca. 8.8 Hz, 2H), 7.35-7.30 (m, 3H), 7.27-7.23 (m, 3H), 6.90 (d, *J* = ca. 8.8 Hz, 1H), 6.67 (d, *J* = ca. 8.0 Hz, 1H), 3.94 (s, 3H), 3.92 (s, 3H); ^{13}C NMR (acetone-d_6): δ 162.0, 156.4, 136.6, 135.0, 134.4, 129.5, 129.3, 129.2, 127.4, 127.3, 127.2, 126.4, 125.6, 124.4, 123.9, 122.5, 120.1, 116.1, 103.5, 56.4, 56.3; IR: 3001, 2963, 1389 cm^{-1}; LC/MS *m/z*: 341 $(M+H)^+$.

5-(5-Hydroxy-2-naphthyl)biphenyl-2-ol (9). The title compound was prepared by reaction of 2-methoxy-5-(5-methoxy-2-naphthyl)biphenyl **9a** (75 mg, 0.22 mmol, 1 equiv) with boron tribromide (2.2 mmol, 10 equiv) according to method C. The product was purified by column chromatography (hexane/ethyl acetate 8:2), then by preparative HPLC (gradient acetonitrile/water 20:80 to 100:0) to give 10 mg (15%) of the analytically pure compound as an orange oil. $C_{22}H_{16}O_2$; MW 312; ^{1}H NMR (acetone-d_6): δ 8.16 (d, *J* = ca. 8.5 Hz, 1H), 7.96 (d, *J* = ca. 2.0 Hz, 1H), 7.67 (dd, *J* = ca. 2.0 Hz, *J* = ca. 8.5 Hz, 1H), 7.61 (d, *J* = ca. 2.0 Hz, 1H), 7.59-7.55 (m, 2H), 7.51 (dd, *J* = ca. 2.0 Hz, *J* = ca. 8.5 Hz, 1H), 7.32-7.28 (m, 3H), 7.22-7.16 (m, 2H), 7.00 (d, *J* = ca. 8.5 Hz, 1H), 6.76 (d, *J* = ca. 7.6 Hz, 1H); ^{13}C NMR (acetone-

d6): δ 154.8, 153.9, 139.7, 139.2, 136.4, 133.7, 130.3, 128.9, 128.1, 127.7, 127.5, 125.2, 124.7, 124.6, 123.6, 120.2, 117.5, 108.7; GC/MS *m/z*: 312 $(M)^+$.

6-(2-Fluoro-4-methoxyphenyl)-1-naphthol (10). The title compound was prepared by reaction of 5-hydroxy-2-naphthyl trifluoromethanesulfonate **1a** (200 mg, 0.68 mmol, 1 equiv) and 2-fluoro-4-methoxyphenylboronic acid (151 mg, 0.89 mmol, 1.3 equiv) according to method B. The product was purified by column chromatography (hexane/ethyl acetate 9:1) to afford 110 mg (60%) of the analytically pure product as a beige solid. $C_{17}H_{13}FO_2$; MW 268; mp: 133-135°C; ^{1}H NMR ($CDCl_3$): δ 8.20 (d, *J* = ca. 8.8 Hz, 1H), 7.92 (s, br, 1H), 7.64 (dd, *J* = ca. 2.0 Hz, *J* = ca. 8.6 Hz, 1H), 7.47-7.43 (m, 2H), 7.33-7.29 (m, 1H), 6.82-6.78 (m, 2H), 6.74 (dd, *J* = ca. 2.0 Hz, *J* = ca. 8.6 Hz, 1H), 5.20 (s, 1H), 3.85 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 162.8, 161.5, 160.2, 151.7, 134.8, 131.3, 131.2, 127.3, 126.3, 123.4, 121.8, 120.6, 110.4, 108.7, 102.2, 102.0, 55.6; IR: 3202, 3027, 1585 cm^{-1}; GC/MS *m/z*: 268 $(M)^+$.

6-(2-Fluoro-4-hydroxyphenyl)-1-naphthol (11). The title compound was prepared by reaction of 6-(2-fluoro-4-methoxyphenyl)-1-naphthol **10** (100 mg, 0.09 mmol, 1 equiv) with pyridinium hydrochloride (9 mmol, 100 equiv) according to method E. The product was purified by column chromatography (hexane/ethyl acetate 7:3) to afford 48 mg (54%) of the analytically pure product as a beige solid. $C_{16}H_{11}FO_2$; MW 254; mp: 145-146°C; ^{1}H NMR (acetone-d_6): δ 9.15 (s, br, 1H), 9.07 (s, br, 1H), 8.28 (d, *J* = ca. 8.5 Hz, 1H), 7.83 (d, *J* = ca. 8.5 Hz, 2H), 7.62 (dt, *J* = ca. 1.8 Hz, *J* = ca. 8.5 Hz, 1H), 7.50-7.46 (m, 1H), 7.42 (d, *J* = ca. 8.5 Hz, 1H), 7.327.31 (m, 1H), 6.92 (dd, *J* = ca. 1.0 Hz, *J* = ca. 7.3 Hz, 1H), 6.84-6.81 (m, 1H); ^{13}C NMR (acetone-d_6): δ 162.7, 154.0, 148.1, 136.0, 132.4, 130.1, 127.8, 127.5, 126.5, 123.1, 120.2, 115.1, 115.0, 112.9, 109.1, 104.2; IR: 3225, 1623, 1214 cm^{-1}; GC/MS *m/z*: 254 $(M)^+$.

6-(3-Fluoro-4-methoxyphenyl)-1-naphthol (12). The title compound was prepared by reaction of 5-hydroxy-2-naphthyl trifluoromethanesulfonate **1a** (75 mg, 0.26 mmol, 1 equiv) with 3-fluoro-4-methoxyphenylboronic acid (57 mg, 0.33 mmol, 1 equiv) according to method A. The product was purified by column chromatography (hexane/ethyl acetate 7:3) to afford 44 mg (60%) of the analytically pure compound as a brown oil. $C_{17}H_{13}FO_2$; MW 268; ^{1}H NMR (acetone-d_6): δ 8.97 (s, br, 1H), 8.26 (d, *J* = ca. 8.4 Hz, 1H), 8.04 (s, 1H), 7.72 (d, *J* = ca. 8.4 Hz, 1H), 7.58-7.54 (m, 2H), 7.41 (d, *J* = ca. 8.9 Hz, 1H), 7.31-7.27 (m, 1H), 7.24-7.20 (m, 1H), 6.91-6.87 (m, 1H), 3.91 (s, 3H); ^{13}C NMR (acetone-d_6): δ 154.0, 148.2, 137.9,

136.2, 134.9, 128.1, 127.7, 125.6, 124.9, 124.4, 123.9, 123.8, 120.3, 115.4, 115.0, 109.1, 56.6; IR: 3445, 2937, 1521, 1246 cm^{-1}; GC/MS *m/z*: 268 $(M)^+$.

6-(3-Fluoro-4-hydroxyphenyl)-1-naphthol (13). The title compound was prepared by reaction of 6-(3-fluoro-4-methoxyphenyl)-1-naphthol **12** (20 mg, 0.08 mmol, 1 equiv) with boron tribromide (0.80 mmol, 10 equiv) according to method C. The product was purified by column chromatography (hexane/ethyl acetate 1:1) to give 8 mg (40%) of the analytically pure compound as a yellow oil. $C_{16}H_{11}O_2F$; MW 254; ^1H NMR (acetone-d_6): δ 8.99 (s, br, 1H), 8.74 (s, br, 1H), 8.29 (d, *J* = ca. 8.8 Hz, 1H), 8.05 (d, *J* = ca. 2.0 Hz, 1H), 7.74 (dd, *J* = ca. 2.1 Hz, *J* = ca. 8.7 Hz, 1H), 7.57 (dd, *J* = ca. 2.1 Hz, *J* = ca. 8.7 Hz, 1H), 7.50-7.47 (m, 1H), 7.44 (d, *J* = ca. 8.3 Hz, 1H), 7.34-7.30 (m, 1H), 7.15-7.11 (m, 1H), 6.91 (dd, *J* = ca. 0.9 Hz, *J* = ca. 7.6 Hz, 1H); ^{13}C NMR (acetone-d_6): δ 154.2, 136.3, 127.6, 125.4, 124.4, 124.0, 123.7, 120.3, 119.1, 115.5, 109.0; IR: 3247, 1692, 1524, 1277 cm^{-1}; GC/MS *m/z*: 254 $(M)^+$.

6-(2,5-Difluoro-4-methoxyphenyl)-1-naphthol (14). The title compound was prepared by reaction of 5-hydroxy-2-naphthyl trifluoromethanesulfonate **1a** (200 mg, 0.68 mmol, 1 equiv) with 2,5-difluoro-4-methoxyphenylboronic acid (154 mg, 0.82 mmol, 1.3 equiv) according to method B. The product was purified by column chromatography (hexane) to afford 70 mg (36%) of the analytically pure compound as a yellow solid. $C_{17}H_{12}F_2O_2$; MW 286; mp: 185-187°C; ^1H NMR ($CDCl_3$): δ 8.21 (d, *J* = ca. 8.8 Hz, 1H), 7.91 (s, br, 1H), 7.61 (dt, *J* = ca. 1.8 Hz, *J* = ca. 8.8 Hz, 1H), 7.45 (d, *J* = ca. 8.0 Hz, 1H), 7.32 (d, *J* = ca. 8.0 Hz, 1H), 7.30 (d, *J* = ca. 2.6 Hz, 1H), 7.29-7.25 (m, 1H), 6.83-6.78 (m, 1H), 5.32 (s, 1H), 3.92 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 151.4, 134.8, 127.5, 126.4, 126.0, 123.5, 122.0, 120.9, 117.1, 109.0, 56.6; IR: 3237, 3098, 2978, 1525, 1445 cm^{-1}; GC/MS *m/z*: 286 $(M)^+$.

6-(2, 5-Difluoro-4-hydroxyphenyl)-1-naphthol (15). The title compound was prepared by reaction of 6-(2,5-difluoro-4-methoxyphenyl)-1-naphthol **14** (40 mg, 0.14 mmol, 1 equiv) with boron trifluoride dimethylsulfide complex (10.5 mmol, 75 equiv) according to method D. The product was purified by column chromatography (hexane/ethyl acetate 8:2) to afford 20 mg (50%) of the analytically pure product as a beige solid. $C_{16}H_{10}F_2O_2$; MW 272; mp: 178-181°C; ^1H NMR (acetone-d_6): δ 9.22 (s, br, 1H), 9.05 (s, br, 1H), 8.29 (d, *J* = ca. 8.8 Hz, 1H), 7.99-7.97 (m, 1H), 7.63 (dt, *J* = ca. 1.9 Hz, *J* = ca. 8.8 Hz, 1H), 7.44 (d, *J* = ca. 8.4 Hz, 1H), 7.42-7.39 (m, 1H), 7.36-7.32 (m, 1H), 6.96-6.90 (m, 2H); ^{13}C NMR (acetone-d_6): δ 162.4,

159.6, 154.4, 153.2, 135.2, 134.5, 126.6, 125.9, 125.1, 123.5, 122.0, 120.8, 117.9, 117.5, 108.9, 104.6; IR: 3427, 1641, 1522, 1268 cm^{-1}; GC/MS *m/z*: 272 $(M)^+$.

6-(2, 6-Difluoro-4-hydroxyphenyl)-1-naphthol (16). The title compound was prepared by reaction of 5-hydroxy-2-naphthyl trifluoromethanesulfonate **1a** (100 mg, 0.34 mmol, 1 equiv) with 2,5-difluoro-4-hydroxyphenylboronic acid (71 mg, 0.41 mmol, 1.2 equiv) according to method B. The product was purified by column chromatography (hexane/ethyl acetate 3:1) to afford 43 mg (47%) of the analytically pure compound as a beige solid. $C_{16}H_{10}F_2O_2$; MW 272; mp: 164-166°C; ^{1}H NMR (acetone-d_6): δ 9.20 (s, br, 1H), 8.93 (s, br, 1H), 8.16 (d, *J* = ca. 8.9 Hz, 1H), 7.74 (s, 1H), 7.35 (dd, *J* = ca. 1.4 Hz, *J* = ca. 8.4 Hz, 1H), 7.29 (d, *J* = ca. 8.4 Hz, 1H), 7.20 (m, 1H), 6.82 (d, *J* = ca. 7.7 Hz, 1H), 6.52-6.46 (m, 2H); ^{13}C NMR (acetone-d_6): δ 162.7, 162.6, 160.7, 160.6, 159.4, 154.0, 135.7, 130.0, 128.2, 127.7, 127.6, 125.0, 122.9, 120.1, 109.4, 100.5; IR: 3220, 3112, 1640, 1280 cm^{-1}; GC/MS *m/z*: 272 $(M)^+$.

6-[4-Hydroxy-2-(trifluoromethyl)phenyl]-1-naphthol (17). The title compound was prepared by reaction of 5-hydroxy-2-naphthyl trifluoromethanesulfonate **1a** (100 mg, 0.34 mmol, 1 equiv) with 4-hydroxy-2-trifluoromethylphenylboronic acid (98 mg, 0.44 mmol, 1.3 equiv) according to method A. The product was purified by column chromatography (hexane/ethyl acetate 8:2) to give 70 mg (68%) of the analytically pure compound as a brown solid. $C_{17}H_{11}F_3O_2$; MW 304; mp: 140-142°C; ^{1}H NMR (acetone-d_6): δ 9.03 (s, br, 1H), 9.01 (s, br, 1H), 8.25 (d, *J* = ca. 8.8 Hz, 1H), 7.73 (s, 1H), 7.42-7.39 (m, 2H), 7.36-7.31 (m, 2H), 7.28 (d, *J* = ca. 2.5 Hz, 1H), 7.18 (dd, *J* = ca. 2.5 Hz, *J* = ca. 8.6 Hz, 1H), 6.96-6.93 (m, 1H); ^{13}C NMR (acetone-d_6): δ 157.9, 154.3, 138.8, 135.4, 134.9, 128.7, 127.8, 127.2, 125.0, 122.6, 120.4, 119.4, 113.7, 109.5, 60.5; IR: 3361, 1691, 1322 cm^{-1}; GC/MS *m/z*: 304 $(M)^+$.

6-[4-Methoxy-3-(trifluoromethyl)phenyl]-1-naphthol (18). The title compound was prepared by reaction of 5-hydroxy-2-naphthyl trifluoromethanesulfonate **1a** (100 mg, 0.34 mmol, 1 equiv) with 4-methoxy-3-trifluoromethylphenylboronic acid (98 mg, 0.44 mmol, 1.3 equiv) according to method A. The product was purified by column chromatography (hexane/ethyl acetate 8:2) to afford 34 mg (32%) of the analytically pure product as a brown solid. $C_{18}H_{13}F_3O_2$; MW 318; mp: 138-139°C; ^{1}H NMR (acetone-d_6): δ 9.04 (s, br, 1H), 8.33 (d, *J* = ca. 8.8 Hz, 1H), 8.11 (d, *J* = ca. 2.0 Hz, 1H), 8.04 (dd, *J* = ca. 2.0 Hz, *J* = ca. 8.6 Hz, 1H), 8.01 (d, *J* = ca. 2.3 Hz, 1H), 7.78 (dd, *J* = ca. 2.0 Hz, *J* = ca. 8.6 Hz, 1H), 7.47 (d, *J* = ca. 8.4 Hz, 1H), 7.38 (d, *J* = ca. 8.4 Hz, 1H), 7.34 (m, 1H), 6.94 (dd, *J* = ca. 0.8 Hz, *J* = ca. 7.6

Hz, 1H), 4.01 (s, 3H); ^{13}C NMR (acetone-d_6): δ 158.0, 154.0, 137.7, 136.2, 134.0, 133.2, 127.8, 126.3, 126.2, 125.8, 125.0, 124.4, 123.9, 123.8, 120.4, 114.1, 109.2, 56.7; IR: 3387, 2981, 1419, 1214 cm^{-1}, GC/MS *m/z*: 318 $(M)^+$.

6-[4-Hydroxy-3-(trifluoromethyl)phenyl]-1-naphthol (19). The title compound was prepared by reaction of 6-[4-methoxy-3-(trifluoromethyl)phenyl]-1-naphthol **18** (100 mg, 0.15 mmol, 1 equiv) with pyridinium hydrochloride (15 mmol, 100 equiv) according to method E. The product was purified by column chromatography (hexane/ethyl acetate 7:3) to afford 40 mg (42%) of the analytically pure product as a brown solid. $C_{17}H_{11}F_3O_2$; MW 304; mp: 158-160°C; ^{1}H NMR (acetone-d_6): δ 8.28 (d, *J* = ca. 8.5 Hz, 1H), 8.04 (d, *J* = ca. 2.0 Hz, 1H), 7.91 (d, *J* = ca. 2.0 Hz, 1H), 7.85 (dd, *J* = ca. 2.0 Hz, *J* = ca. 8.5 Hz, 1H), 7.71 (dd, *J* = ca. 2.0 Hz, *J* = ca. 8.5 Hz, 1H), 7.42 (d, *J* = ca. 8.5 Hz, 1H), 7.29 (m, 1H), 7.22-7.19 (m, 1H), 6.89 (dd, *J* = ca. 0.9 Hz, *J* = ca. 7.3 Hz, 1H); ^{13}C NMR (acetone-d_6): δ 156.3, 154.1, 138.0, 136.3, 133.0, 127.7, 125.5, 124.9, 124.4, 123.9, 120.2, 118.6, 109.1; IR: 3229, 3066, 2986, 1619, 1263 cm^{-1}; GC/MS *m/z*: 304 $(M)^+$.

6-(3-Chloro-4-hydroxyphenyl)-1-naphthol (20). The title compound was prepared by reaction of 5-hydroxy-2-naphthyl trifluoromethanesulfonate **1a** (200 mg, 0.68 mmol, 1 equiv) and 3-chloro-4-hydroxyphenylboronic acid (153 mg, 0.89 mmol, 1.3 equiv) according to method B. The product was purified by column chromatography (hexane/ethyl acetate 7:3) to give 117 mg (64%) of the analytically pure compound as a beige solid. $C_{16}H_{11}ClO_2$; MW 270; mp: 112-114°C; ^{1}H NMR (acetone-d_6): δ 9.10 (s, br, 2H), 8.31 (d, *J* = ca. 8.5 Hz, 1H), 8.07 (d, *J* = ca. 2.0 Hz, 1H), 7.80 (d, *J* = ca. 2.0 Hz, 1H), 7.75 (dd, *J* = ca. 2.0 Hz, *J* = ca. 8.5 Hz, 1H), 7.64 (dd, *J* = ca. 2.0 Hz, *J* = ca. 8.5 Hz, 1H), 7.47 (d, *J* = ca. 8.4 Hz, 1H), 7.36-7.32 (m, 1H), 7.19 (d, *J* = ca. 8.4 Hz, 1H), 6.94 (dd, *J* = ca. 1.0 Hz, *J* = ca. 7.4 Hz, 1H); ^{13}C NMR (acetone-d_6): δ 154.0, 153.5, 137.9, 136.2, 134.5, 129.2, 127.7, 127.6, 125.4, 125.3, 124.4, 123.8, 121.7, 120.2, 118.1, 109.0; IR: 3330, 1599, 1283, 1187 cm^{-1}; GC/MS *m/z*: 270-272 $(M)^+$.

2-Hydroxy-5-(5-methoxy-2-naphthyl)benzonitrile (21a). The title compound was prepared by reaction of 2-(5-methoxy-2-naphthyl)-5,5-dimethyl-1,3,2-dioxaborinane **9c** (100 mg, 0.37 mmol, 1.05 equiv) with 5-bromo-2-hydroxybenzonitrile (69 mg, 0.35 mmol, 1 equiv) according to method A. The product was purified by column chromatography (hexane/ethyl acetate 8:2) to afford 30 mg (29%) of **21a**. $C_{18}H_{13}NO_2$; MW 275; ^{1}H NMR ($CDCl_3$): δ 8.23

(d, *J* = ca. 2.1 Hz, 1H), 7.86 (d, *J* = ca. 2.1 Hz, 1H), 7.79 (d, *J* = ca. 1.9 Hz, 1H), 7.72 (d, *J* = ca. 1.9 Hz, 1H), 7.60-7.56 (m, 1H), 7.42-7.38 (m, 3H), 7.12-7.10 (m, 1H), 6.77 (d, *J* = ca. 8.7 Hz, 1H), 3.96 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 142.4, 142.3, 140.0, 138.3, 136.9, 131.9, 130.5, 130.3, 129.9, 129.1, 125.5, 125.4, 122.0, 109.3, 109.2, 60.6.

2-Hydroxy-5-(5-hydroxy-2-naphthyl)benzonitrile (21). The title compound was prepared by reaction of 2-hydroxy-5-(5-methoxy-2-naphthyl)benzonitrile **21a** (20 mg, 0.06 mmol, 1 equiv) with boron trifluoride dimethylsulfide complex (4.50 mmol, 75 equiv) according to method D. The product was purified by column chromatography (hexane/ethyl acetate 7:3) to give 15 mg (96%) of the analytically pure product as a beige solid. $C_{17}H_{11}NO_2$; MW 261; mp: 152-154°C; ^{1}H NMR (acetone-d_6): δ 9.67 (s, br, 1H), 9.07 (s, br, 1H), 8.33 (d, *J* = ca. 8.5 Hz, 1H), 8.17 (d, *J* = ca. 2.4 Hz, 1H), 8.11-8.08 (m, 2H), 7.76 (dd, *J* = ca. 1.9 Hz, *J* = ca. 8.7 Hz, 1H), 7.48 (d, *J* = ca. 8.5 Hz, 1H), 7.35 (m, 1H), 7.17 (d, *J* = ca. 8.5 Hz, 1H), 6.94 (dd, *J* = ca. 0.9 Hz, *J* = ca. 7.4 Hz, 1H); ^{13}C NMR (acetone-d_6): δ 178.9, 157.9, 137.8, 137.2, 136.2, 133.4, 127.8, 125.9, 125.7, 125.4, 124.4, 124.0, 120.3, 109.2, 99.4.

Biological Assays

[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. Cytosolic (17β-HSD1) and microsomal (17β-HSD2) fractions were obtained from human and *Callithrix jacchus* placenta according to previously described procedures[28, 64, 65], and from rat and mouse liver tissues[66]. Fresh tissue was homogenized and centrifuged. The pellet fraction contains the microsomal 17β-HSD2 and was used for the determination of E1-formation, while 17β-HSD1 was obtained after precipitation with ammonium sulphate from the cytosolic fraction for use of testing of E2 formation.

Human 17β-HSD4 and 17β-HSD5 were cloned into the modified pGEX-2T vector[55]. For the multidomain enzyme 17β-HSD4, only the steroid converting SDR-domain was subcloned[55].

Inhibition of 17β-HSD2/E1-formation

Inhibitory activities were evaluated by a well established method with minor modifications.[67-69] Briefly, the enzyme preparation was incubated with NAD^+ [1500 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. 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]-E2 (final concentration: 500 nM, 0.11 μCi). After 20 min at 37°C, the incubation was stopped with $HgCl_2$ and the mixture was extracted with ether. After evaporation, the steroids were dissolved in acetonitrile. E1 and E2 were separated using acetonitrile/water (45:55) as mobile phase in a C18 rp 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 according to following equation: $\%conversion = \frac{\%(E1)}{\%(E1)+\%(E2)} \times 100$. Each value was calculated from at least three independent experiments.

Inhibition of 17β-HSD1/E2-formation

The 17β-HSD1 inhibition assay was performed similarly to the 17β-HSD2 procedure. The cytosolic fraction was incubated with NADH [500 μM], test compound and a mixture of unlabelled- and [2,4,6,7-^{3}H]-E1 (final concentration: 500 nM, 0.15 μCi) for 10 min. Further treatment of the samples and HPLC separation was carried out as mentioned above.

Inhibition of 17β-HSD4

Inhibitory activity was assessed as originally described[70, 71] with minor modifications[55]. Briefly, bacteria containing recombinant 17β-HSD4 were resuspended in PBS and enzymatic assay was performed at pH 7.7. The enzyme preparation was incubated with NAD^+ [7.5 mM]. Inhibitor (dissolved in DMSO) was added in a final concentration of 1µM. Final concentration of DMSO was adjusted to 1% in all samples. The enzymatic reaction was started with the addition of [6,7-^{3}H]-E2 at a concentration of 21 nM. The incubation at 37°C was stopped with 0.21 M ascorbic acid in methanol/acetic acid (99:1) after the time needed to convert approximately 30% of the substrate in a control assay without inhibitor. Steroids were extracted from the assay mixture by SPE using Strata C18-E columns (Phenomenex), eluted with methanol and separated by RP-HPLC (column Luna 5µm C18(2), 150 mm; Phenomenex) at a flow rate of 1 ml/min acetonitrile/water (43:57). Radioactivity was detected by scintillation counting after mixing with ReadyFlowIII (Beckman). Conversion was calculated from integration of substrate and product peaks. Assay was run in triplicate of three independent experiments.

Inhibition of 17β-HSD5

The 17β-HSD5 inhibition assay was performed similarly to the 17β-HSD4 procedure. The recombinant enzyme was incubated with NADPH [6 mM], inhibitor [1 µM] and [1,2,6,7-^{3}H]-A-dione at a concentration of 21 nM. Further treatment of the samples and HPLC separation was carried out as mentioned above[55].

Inhibition of 17β-HSD2 in a cellular assay

Cellular 17β-HSD2 activity is measured using the breast cancer cell-line MDA-MB-231[57] (17β-HSD1 activity negligible). [^{3}H-E2] (200 nM) is taken as substrate, and is incubated with the inhibitor for 3,5h at 37°C. After ether extraction, substrate and product are separated by HPLC and detected with a radioflow detector. Potency is evaluated as percentage of inhibition (inhibitor concentration: 1µM) and as IC_{50} values.

ER affinity[28]

The binding affinity of selected compounds to the ERα and ERβ was determined according to Zimmermann *et al.*[72] using recombinant human proteins. Briefly, 0.25 pM of ERα or ERβ, respectively, were incubated with [^{3}H]-E2 (10 nM) and test compound for 1h at room temperature. The potential inhibitors were dissolved in DMSO (5% final concentration). Evaluation of 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 formed complex was separated, washed and resuspended in ethanol. For radiodetection, scintillator cocktail (Quickszint 212, Zinsser Analytic, Frankfurt) was added and the samples were measured in a liquid scintillation counter (Rack Beta Primo 1209, Wallac, Turku). From these results the percentage of [^{3}H]-E2 displacement by the compounds was calculated. The plot of % displacement versus compound concentration resulted in sigmoidal binding curves. The compound concentration to displace 50% of the receptor bound [^{3}H]-E2 were determined. Unlabelled E2 was used as a reference. For determination of the relative binding affinity (RBA) the ratio was calculated according to the following equation: $RBA[\%] = \frac{IC_{50}(E2)}{IC_{50}(compound)} \times 100$ [73]. This results in an RBA value of 100% for E2. After the assay was established and validated a modification was made to increase throughput. Compounds were tested at concentrations of 1000 × IC_{50}(E2) and 10000 × IC_{50}(E2). Results were reported as RBA ranges. Compounds with less than 50% displacement of [^{3}H]-E2 at a concentration of 10,000 × IC_{50} (E2) were classified as RBA <0.01%, compounds that displace

more than 50% at 10,000 × IC_{50} (E2) but less than 50% at 1000 × IC_{50}(E2) were classified as 0.01%<RBA<0.1%.

LogP determination

The LogP values were calculated from CambridgeSoft Chem & Bio Draw 11.0 using the ChemDrawPro 11.0 program.

Acknowledgements

We thank Jannine Ludwig, Martina Jankowski, Jeannine Jung and Dr. Jörg Haupenthal for their help in performing the enzyme and cellular inhibition tests (17β-HSD1, 17β-HSD2) and the ER tests. We acknowledge Prof. A. Einspanier for the gift of monkey placentas. We are grateful to the Deutsche Forschungsgemeinschaft for the financial support (HA1315/12-1 and AD 127/10-1).

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3.3 Discovery of a new class of bicyclic substituted hydroxyphenylmethanones as 17β-hydroxysteroid dehydrogenase type 2 (17β-HSD2) inhibitors for the treatment of osteoporosis

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This manuscript has been accepted as an article in the journal
European Journal of Medicinal Chemistry, **2011**,

Paper III

Abstract

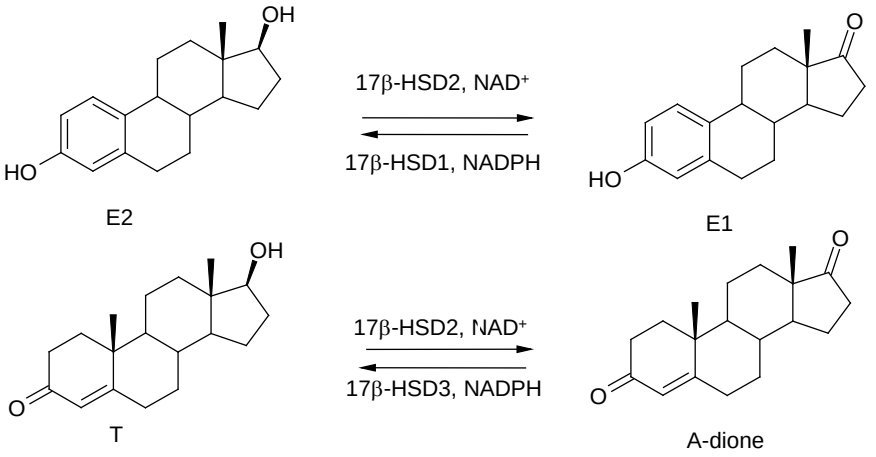
E2 deficiency in elderly people has directly an effect on the skeleton and can lead to osteoporosis. As 17β-hydroxysteroid dehydrogenase type 2 (17β-HSD2) catalyses the conversion between active 17β-hydroxysteroid estradiol (E2) and testosterone (T) into their less active 17-ketosteroid and has been found in bones, 17β-HSD2 inhibitor may provide a new approach in the onset of osteoporosis. Bicyclic substituted hydroxyphenylmethanone derivatives were synthesised as steroidomimetics of the substrate E2 and were evaluated for their 17β-HSD2 inhibition and their selectivity toward 17β-HSD1, catalysing the reverse reaction the conversion of estrone (E1) into E2. Highly selective compounds (**11**, **12**, **14**, **21** and **22**) have been identified, the most promising one (**12**) showing an IC_{50} value in the low nanomolar range (101 nM) and a selectivity factor of 13 toward 17β-HSD1. These results make compound **12** an interesting candidate for further biological evaluation.

Introduction

Healthy bones are continuously regenerated by a mechanism of balance between osteoblasts (OBs) and osteoclasts (OCs), which are responsible for bone formation and bone resorption, respectively. The abnormal increase of the activity of OCs compared to the one of OBs in elderly people leads to osteoporosis [1]. Osteoporosis is a silent and systemic skeletal disease,

characterised by reduced bone mineral density and increased risk of fractures often at the hips, spine and wrist. Bone loss often takes place in post-menopausal women and elderly men, after the level of active sex steroids 17β-estradiol (hereafter "estradiol" or E2) or testosterone (T) (Chart 1) has dropped down.

Chart 1: Interconversion of 17β-estradiol (E2) to estrone (E1) by 17β-HSD2 and 17β-HSD1 and of testosterone (T) to androstenedione (A-dione) by 17β-HSD2 and 17β-HSD3.



Several drugs can be administered for the treatment of osteoporosis. Among the antiresorptive agents, the bisphosphonate alendronate [2] is the most potent one used, but reduces the risk of hip fractures only by 50% in post-menopausal women [2, 3] and elderly men [4]. Raloxifene [5], a selective estrogen receptor modulator (SERM) is also often used to treat osteoporosis, but leads to various adverse effects as increased risk of thromboembolism [6], hot flushes or leg cramps. Denosumab [7], an inhibitor of the receptor activator of nuclear factor κB ligand (RANKL) [8] inhibits the osteoclastogenesis (OC differentiation) by binding to RANKL. It has proven its efficacy [7, 9] in post-menopausal women and elderly men with high risk of fractures but is associated with high cholesterol levels, muscle pain and bladder infection. Among all current marketed osteoporosis drugs, none of them offers a complete cure, there is therefore a need to develop new drugs for this disease with higher efficiency.

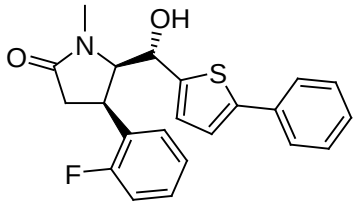
Estrogens [10] and androgens [10] play a key role in the development of the disease. It has been show that administration of E2 can help in the treatment of osteoporosis, but has adverse effects. Mechanism by which estrogens act on bones is not well understood yet, but one theory points out the importance of RANKL and osteoprotegerin (OPG) [11]. The production of OPG is stimulated by E2 in the OBs. Binding of OPG to RANKL inhibits the activation of the RANK receptor on osteoclasts, limiting the resorptive activity of OCs. OPG and RANKL knockout (KO) mice confirm the possible involvement of OPG and RANKL in bone

regulation, as OPG KO mice [12, 13] exhibit severe osteoporosis due to an increase of osteoclastogenesis, while RANKL KO mice [14] show serious osteopetrosis and a complete lack of OCs. A drug which could increase the concentration of estrogens in bones, would raise OPG levels, and thus might diminish bone resorption and should have favorable effects on osteoporosis.

17β-Hydroxysteroid dehydrogenase type 2 (17β-HSD2) is an enzyme converting the highly active E2 and T into their less active form, estrone (E1) and 4-androstene-3,17-dione (A-dione), respectively, using NAD^+ as cofactor. It is mainly localised in placenta, liver, small intestine and can be found in osteoblastic cells [15]. Inhibitors of 17β-HSD2 could therefore maintain a high level of E2 and T in bones, and protect them against bone loss. Considering that systemic and intracellular modulation of hormone concentrations has already been successfully applied for the treatment of breast and prostate cancer and begnign prostatic hyperplasia by inhibition of the steroidogenic enzymes aromatase [16-18], CYP17 [19-22] and 5α-reductase [23-25], respectively, or - as in case of 17β-HSD1 [26-34]- is considered beneficial for endometriosis therapy, inhibition of 17β-HSD2 could be a new approach for the treatment of osteoporosis.

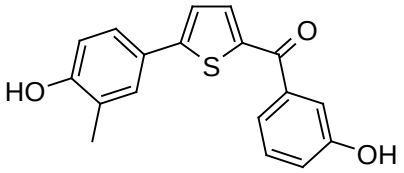
Few inhibitors of 17β-HSD2 have been identified until now [35-40]. Among them, the pyrrolidinone **A** [38] (Chart 2) is the most potent one described in the literature (IC_{50} = 10nM). Efficacy of a derivative of **A** has been evaluated *in vivo* in an osteoporotic monkey model [41]. This study proved that inhibition of 17β-HSD2 recovers the balance between bone formation and bone resorption. A strong variability is observed, certainly due to non appropriate pharmacokinetic properties of this compound. New 17β-HSD2 inhibitors with good pharmacokinetic properties should be identified for further *in vivo* experiments.

Chart 2: Inhibitors of 17β-HSD2



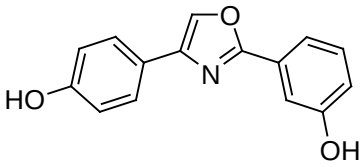
Pyrrolidinone
IC_{50} (HSD2) = 10 nM

A



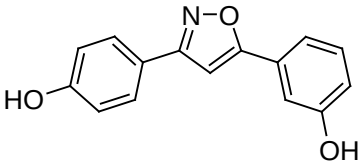
IC_{50} (HSD2) = 383 nM

B



IC_{50} (HSD2) = 249 nM

C



IC_{50} (HSD2) = 270 nM

D

In the frame of our 17β-HSD1 project, the hydroxyphenylketothiophene **B** (Chart 2) was identified [32] as moderate 17β-HSD2 inhibitor (IC_{50} = 382 nM). The scaffold of **B** can therefore be considered as an interesting starting point for development of a new class of 17β-HSD2 inhibitors. Optimisation of HSD2 activity and gain in selectivity toward HSD1 might be obtained by 1) exchange of the thiophene ring by bioisosteres, 2) exchange of the methyl substituent on the A ring, and 3) introduction of substituents on the B moiety. Convenience of these compounds is the easy four steps synthesis and purification, contrary to the pyrrolidinone **A**, which requires a separation of stereoisomers.

In this paper, we will report on the synthesis and the biological evaluation of a novel class of 17β-HSD2 inhibitors, with a bicyclic substituted hydroxyphenylmethanone core structure.

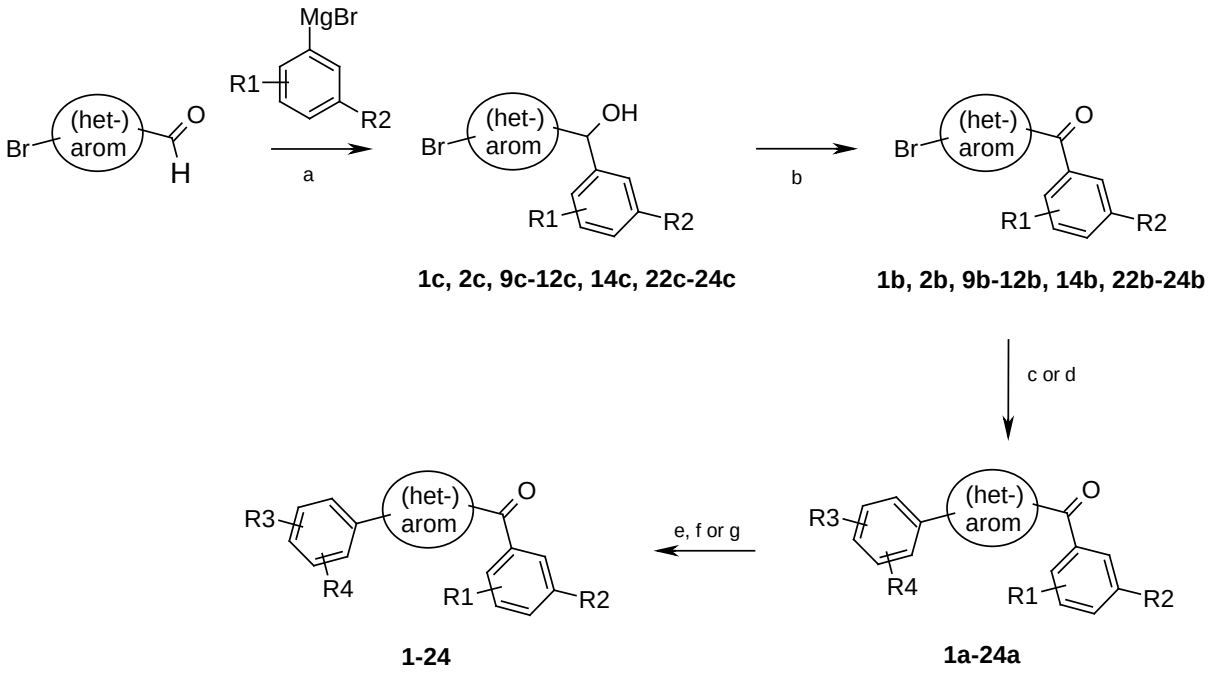
Results

Chemistry

The synthesis of compounds **1-24** was achieved in a four step procedure and is depicted in Scheme 1. First, the nucleophilic addition of Grignard reagent (phenylmagnesiumbromide derivatives) to the appropriate aromatic carbaldehyde (Method A) afforded the alcohol intermediates **1c**, **2c**, **9c-12c**, **14c** and **22c-24c**. Then the OH-group was further oxidized into the corresponding ketone with 2-iodoxybenzoic acid (Method B). Subsequent Suzuki cross

couplings [42] with different substituted phenylboronic acids led to compounds **1a-24a** (Method C or D). Ether cleavage [43] was performed using boron tribromide for compounds **1**, **2**, **10**, **11**, **14-19** and **21**, (method E), boron trifluoride dimethylsulfide complex for compounds **3**, **5**, **6**, **13** and **23** (method F) or pyridinium hydrochloride for compounds **4**, **7-9**, **12**, **20**, **22** and **24**, (method G).

Scheme 1: Synthesis of compounds **1-24**[a]



[a]Reagents and conditions: a. anhydrous THF, 80°C, 3h, Method A; b. 2-iodoxybenzoic acid, anhydrous THF, 60°C, overnight, Method B; c. Na_2CO_3, $Pd(PPh_3)_4$, R-$B(OH)_2$, DME/water (2:1), microwave irradiation (25 min, 150W, 150°C, 15 bar), for compounds **15a-17a**, Method C; d. Cs_2CO_3, $Pd(PPh_3)_4$, R-$B(OH)_2$, DME/water (2:1), 80°C, overnight, for compounds **1a-14a, 18a-24a**, Method D; e. BBr_3, CH_2Cl_2, -78°C to rt, overnight, for compounds **1, 2, 10, 11, 14-19** and **21**, Method E; f. BF_3SMe_2, CH_2Cl_2, rt, overnight, for compounds **3, 5, 6, 13** and **23**, Method F; g. pyridinium hydrochloride, 180°C, 2h, for compounds **4, 7-9, 12, 20, 22** and **24**, Method G.

Biological results

Inhibition of human 17β-HSD2 and selectivity toward 17β-HSD1 and estrogen receptors (ERs)

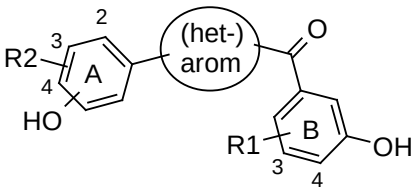
17β-HSD2 and 17β-HSD1 inhibitory activities of the synthesised compounds were evaluated. As 17β-HSD1 catalyses the reduction of E1 to E2, it should not be affected by 17β-HSD2

inhibitors. Moreover, inhibitors of 17β-HSD2 should have no affinity for the estrogen receptors (ER) α and β, as most E2 effects are ER mediated.

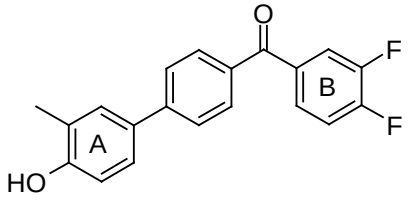
Human placental enzymes were used for both assays and were obtained according to described methods [44-46]. Briefly, in the 17β-HSD2 assay, incubations were run with microsomal fractions, tritiated E2, cofactor and inhibitor. The separation of substrate and product was accomplished by HPLC. The 17β-HSD1 assay was performed similarly using tritiated E1 as substrate and the cytosolic fraction. The percent inhibition values of compounds **1-24** are shown in Table 1, and the IC_{50} values determined for selected compounds are reported in Table 2. Compounds showing less than 10% inhibition tested at a concentration of 1µM were considered to be inactive. The spiro-δ-lactone described by Poirier et *al. [36]* was taken as external reference (68% at 1µM in our test; 62-66% at 1µM in their test).

In a previous work [32], focusing on the development of 17β-HSD1 inhibitors, compound **B** has been identified as an interesting scaffold for 17β-HSD2 inhibition with an IC_{50}-value of 382 nM. The goal of this study was to increase the 17β-HSD2 inhibitory activity and reverse selectivity in favour of HSD2. The thiophene ring of **B** was first exchanged by the bioisostere benzene with 1,3- and 1,4- substitution pattern to afford compounds **1** and **2**, respectively. **1** turned out to be a moderate 17β-HSD2 inhibitor (IC_{50} = 379 nM), while the 1,4- substituted compound **2** is a very active compound (IC_{50} = 132 nM). Compound **2** also shows a slight selectivity toward 17β-HSD1 (SF: 3). Thus, exchange of the thiophene ring by a 1,4-substituted benzene reverses the selectivity of the starting compound **B** toward 17β-HSD2. These results encouraged us to extend this SAR study on bicyclic substituted hydroxyphenylmethanones by changing the methyl group on the A ring and by adding further substituents on the B ring (compounds **3-10**) to improve their activity for 17β-HSD2.

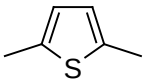
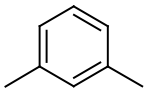
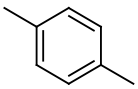
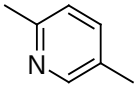
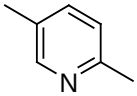
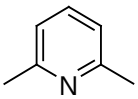
Table 1: Inhibition of human 17β-HSD2 and 17β-HSD1 by compounds **1-24**



B, 1-8, 10-24



9

Compd	(Het-) arom	R1	R2	Position of the OH group	Inhibition of 17β-HSD2[a] [%] at 1 µM	Inhibition of 17β-HSD1[b] [%] at 1 µM
Spiro-δ-lactone	-	-	-	-	69	n.i.
B		H	3-CH_3	4	75	94
1		H	3-CH_3	4	77	50
2		H	3-CH_3	4	90	58
3		H	3-F	4	68	26
4		H	2-Cl	4	80	43
5		H	3-Cl	4	92	66
6		4-F	3-CH_3	4	98	79
7		4-F	3-Cl	4	100	95
8		3-F	3-CH_3	4	100	84
9		-	-		11	16
10		H	3-CH_3	4	84	58
11		H	3-CH_3	4	73	24
12		4-F	3-CH_3	4	84	47
13		4-F	3-Cl	4	87	50
14		H	3-CH_3	4	84	n.i.
15		H	H	2	n.i.	n.i.
16		H	H	3	n.i.	n.i.
17		H	H	4	16	n.i.
18		H	2-CH_3	4	18	n.i.
19		H	2-F	4	56	64
20		H	3-F	4	40	47
21		H	3-Cl	4	55	n.i.
22		4-F	3-CH_3	4	62	n.i.
23		4-CH_3	3-CH_3	4	28	n.i.
24		3-F	3-CH_3	4	57	n.i.

[a] Human placental, microsomal fraction, substrate [^{3}H]-E2 + E2 [500 nM], NAD^+ [1.5 mM], mean value of three determinations, relative standard deviation < 10%, [b] Human placental, cytosolic fraction, substrate [^{3}H]-E1 + E1 [500 nM], NADH [0.5 mM], mean value of three determinations, relative standard deviation < 10%, n.i.: no inhibition (inhibition < 10%).

Replacement of the electron donating methyl group (EDG) of **2** by an electron withdrawing group (EWG) like a fluoro (**3**) is not well tolerated by the enzyme, as compound **3** showed only 68% inhibition at 1µM. Exchange of the lipophilic methyl group (**2**) by a bioisosteric chlorine (**5**) led to a similar affinity to the enzyme and a similar selectivity factor toward 17β-HSD1 (90% *vs*. 92% inhibition at 1µM, respectively). Advantageously compound **5** with a chlorine should be less susceptible to metabolic oxidation compared to the CH_3 compound **2**. Shifting the chlorine to the *ortho* position (**4**) led to a small loss in activity (80% and 92% 17β-HSD2 inhibition at 1µM for **4**: 2-Cl and **5**: 3-Cl, respectively). As the exchange of the methyl group of **2** did not improve selectivity, additional substituents were added on the phenyl moiety B. Introduction of a fluorine (compounds **6-8**) leads to highly active 17β-HSD2 inhibitors (IC_{50} values between 24 and 34 nM), but only a small selectivity toward 17β-HSD1 (SF between 2 and 9). Better selectivity is achieved in presence of the methyl group on the A ring (**6**, SF = 8) compared to the chlorinated analog **7** (SF = 2). Exchanging the *m*-OH group in the B ring by fluorine led to a completely inactive compound (11% inhibition at 1µM for **9**). New potent 17β-HSD2 inhibitors have been identified in the class of 1,4-disubstituted benzenes, but none of them is highly selective toward 17β-HSD1 (compounds **2-8**).

In a previous study [28], oxazole **C** and isoxazole **D** (Chart 2) were identified as inhibitors of 17β-HSD2 (IC_{50} = 249 nM for **C** and 270 nM for **D**), indicating that polar moieties like O and N are well tolerated in the central core of this class of inhibitor. With the hypothesis that these two classes of compounds bind in the same area of HSD2 and trying to increase the selectivity of this new class of 17β-HSD2 inhibitors, a nitrogen was introduced in the 1,4-disubstituted central benzene ring leading to 2,5-pyridines **10** and **11** and 2,6-pyridine **14**. These compounds turned out to be very active (84%, 73% and 84% 17β-HSD2 inhibition at 1µM for **10**, **11** and **14**, respectively). In addition, the selectivity of **11** and **14** toward 17β-HSD1 was much increased compared to the 1,4- and 1,3-disubstituted benzenes (SF = 21 and 75 for **11** and **14** respectively, to be compared with SF = 1 and 3 for **1** and **2**, respectively). In the 2,5-pyridine class, activity of **11** can be increased by addition of fluorine to the B phenyl moiety (compounds **12** and **13**, IC_{50} = 101 nM and 153 nM respectively), but selectivity toward 17β-HSD1 dropped down in case of compound **13**.

2,6-Substituted pyridine **14**, the most selective compound of the pyridine class was further modified trying to increase its activity. Shifting the CH_3-group from the *meta*- to the *ortho*-position of the phenyl is detrimental for the activity (18% and 84% 17β-HSD2 inhibition at 1µM for **18**: 2-CH_3 and **14**: 3-CH_3, respectively). Exchange of the methyl by an EWG like fluorine (**19**: 2-F and **20**: 3-F) or chlorine (**21**: 3-Cl) led to a regain of activity (56%, 40% and

55% 17β-HSD2 inhibition at 1µM, respectively), but these compounds are not able to reach the activity of compound **14** (84% inhibition at 1µM). Furthermore, addition of an EWG (compounds **22** and **24**) or an EDG (compound **23**) group in the 3-hydroxyphenyl B moiety is detrimental for the activity, as the compounds show a weaker inhibition compared to the parent compound **14** (62%, 28% and 58% 17β-HSD2 inhibition at 1µM, respectively), independently of the position of the fluorine (62% and 57% 17β-HSD2 inhibition at 1 µM for **22**: 4-F and **24**: 5-F, respectively).

As none of the substituted 2,6-pyridines reached the activity of **14**, the position of the OH group on the A ring has been studied in absence of the 3-methyl group to investigate whether the *para-meta* OH substitution pattern of **14** is the best one for a high 17β-HSD2 inhibitory activity. Compounds **15-17** as well as their analogues with methoxy groups (data not shown) turned out to be only weak HSD2 inhibitors. It can be concluded that the methyl group next to the OH in the A ring plays an important role for the inhibition of 17β-HSD2 and should not be omitted.

Table 2: IC_{50} values, selectivity factor for selected compounds.

Compd	Cell-free assay		
	17β-HSD2 IC_{50}[a] [nM]	**17β-HSD1 IC_{50}[b] [nM]**	**Selectivity factor[c]**
B	382	8	0.02
1	379	475	1
2	133	413	3
3	525	2429	5
4	176	1764	11
5	153	571	4
6	34	289	9
7	24	53	2
8	31	159	5
10	220	567	3
11	260	5482	21
12	101	1272	13
13	153	1013	7
14	263	19646	75
21	752	60638	81
22	757	22395	30

[a] Human placental, microsomal fraction, substrate [^{3}H]-E2 + E2 [500 nM], cofactor NAD^+ [1.5 mM], mean value of three determinations, relative standard deviation < 10%, [b] Human placental, cytosolic fraction, substrate [^{3}H]-E1 + E1 [500 nM], cofactor NADH [0.5 mM], mean value of three determinations, relative standard deviation < 10%, [c] IC_{50} (17β-HSD1)/ IC_{50} (17β-HSD2).

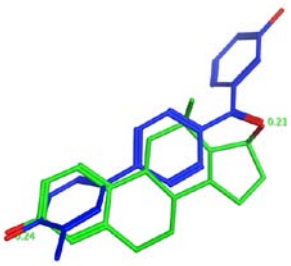
Eleven compounds were identified as highly active 17β-HSD2 inhibitors with IC_{50}-values below 300 nM, with three of them selective toward 17β-HSD1 (SF > 10). The most potent compounds have been evaluated for their binding affinities to the ER α and β, expressed as relative binding affinity (RBA). The RBA represents the ligand affinity to ER, relative to the one of E2, which is arbitrarily set up at 100%. The tested compounds showed very low affinities to both ER subtypes (< 0.1%).

Discussion and conclusion

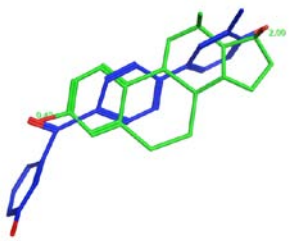
In this study, starting from compound **B** [32] as moderate 17β-HSD2 inhibitor, we have developed a new class of active and selective 17β-HSD2 inhibitors. Exchange of the thiophene moiety of **B** by a six-membered ring (benzene or pyridine) with different substitution patterns inverses the selectivity of **B** in favour of 17β-HSD2 and leads to highly potent 17β-HSD2 inhibitors.

The best inhibitory activity was obtained when the core structure is a 1,4-substituted benzene (compound **2**). Changing the substitution pattern to 1,3-substituted benzene leads to a loss in activity, indicating that the linear geometry of the inhibitors suits better to the enzyme binding pocket. Furthermore, the compounds are believed to be steroidomimetics as the 1,4-substituted benzene derivatives superimpose well with the substrate E2 (Fig. 1): because of the pseudosymmetry the inhibitors can overlay with E2 in two ways: either the carbonyl group of **2** mimicks the C17(OH) of E2 and the OH of the phenyl A ring the C3(OH) of the steroid (Fig. 1A) or *vice versa* (Fig. 1B).

Figure 1: Superimposition of compound **2** (green) and E2 (blue); (**A)** carbonyl group of **2** mimicks the C17(OH) of E2 and the OH of the phenyl A ring the C3(OH) of the steroid; (**B)** carbonyl group of **2** mimicks the C3(OH) of E2 and the OH of the phenyl A ring the C17(OH) of the steroid.



A



B

Introduction of a fluorine on the B ring in compounds bearing the 1,4- substitution pattern results in highly potent inhibitors (compounds **6**-**8**, **12** and **13**). This fluorine might interact with amino acids of the active site via H-bond interaction, which could stabilise the binding of the inhibitor. Interestingly, the position of the fluorine is not important for activity, as compounds **6** and **8** show similar HSD2 inhibition. As the 3D-structure of 17β-HSD2 is unknown, it is not possible to discuss the binding interactions of the compounds with the enzyme in more detail.

Regarding the pyridine derivatives (compounds **10**, **11** and **14**), the introduction of a nitrogen in the phenyl ring decreases inhibitory activity. None of these compounds is as active as the corresponding 1,4-benzene derivative. With the aim to increase activity in the pyridine class, the best hydroxyphenyl substitution pattern was investigated in the class of the 2,6-pyridines (compounds **15**-**17**) in absence of the methyl group. These compounds showed either very low activity (**17**) or were inactive (**15** and **16**). A similar compound to **15** with the OH group in *ortho*-position of the A ring was described by Oster et *al.* [32], with a 2,5-thiophene as central core. This compound is a highly potent 17β-HSD2 inhibitor (IC_{50} = 18 nM, SF = 5), while compound **15** is inactive. Comparison of these results indicates that the 2,5-thiophene and the 2,6-pyridine derivatives do not bind in the same way in the enzyme active site. Furthermore, the inactivity of **17** shows that the methyl group on the A ring plays a key role for the binding of the compound in the enzyme. The methyl group might be located in a small lipophilic pocket and might form Van der Waals interactions. In the 1,4-benzene class (compounds **2**, **5**, **6** and **7**) and 2,5-pyridine class (compounds **12** and **13**), the methyl could be replaced by a chlorine without loss of activity, confirming that the amino acids around this region of the enzyme should be lipophilic.

Interestingly, the nitrogen of the pyridine seems to be responsible for the high selectivity observed toward 17β-HSD1. A similar effect of the nitrogen on selectivity was already identified in the frame of our 17β-HSD1 inhibitors development: polar atoms are not tolerated in the active site of 17β-HSD1[28] while they are advantageous in 17β-HSD2 (compounds **C** and **D**)[28, 47]. The position of the nitrogen on the central core also plays a key role for the selectivity. Compound **10**, with the nitrogen next to the A ring is an unselective 17β-HSD2 inhibitor, while compound **11** with the nitrogen next to the B ring is a highly selective inhibitor.

In the optimisation process of the 2,5-pyridine class, activity of **11** can be increased by introduction of a fluorine into the B ring, thereby leading to the identification of the highly active and selective compound **12** as most promising 17β-HSD2 inhibitor in this study. This

compound as well as the most interesting molecules identified (**2**, **5**, **6**, **7**, **8**, **11** and **14**) show a negligible affinity to both receptors ERα and β.

In this paper, we described the synthesis and the biological evaluation of a new class of 17β-HSD2 inhibitors, derived from substituted bicyclic hydroxyphenylmethanones. The influence of different six-membered rings as central core and different small substituents on the phenyl moieties A and B were investigated. Structural optimisation of the starting compound **B** led to new highly potent 17β-HSD2 inhibitors (compounds **6**, **7**, **8**, **12** and **13**) with inhibitory activities in the very low nanomolar range. Selectivity was achieved by introduction of a nitrogen in the central core, while activity was increased by addition of a fluorine in the B ring. Thereby, compound **12** was identified as the most promising derivative of this series with activity in the low nanomolar range, a striking selectivity toward 17β-HSD1 and no affinity on both ERs. This inhibitor seems to be the best candidate for being further evaluated for its pharmacokinetic profile and for its *in vivo* activity in a disease-oriented model to validate the concept of 17β-HSD2 inhibition.

Experimental section

Chemical methods

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DRX-500 instrument in $CDCl_3$ or acetone-d_6. Chemicals shifts are reported in δ values (ppm), the hydrogenated residues of deuterated solvent were used as internal standard ($CDCl_3$: δ = 7.26 ppm in ^{1}H NMR and δ = 77 ppm in ^{13}C NMR, acetone-d_6: δ = 2.05 ppm in ^{1}H NMR and δ = 30.8 ppm and 206.3 ppm in ^{13}C NMR). Signals are described as s, br, d, t, dd, ddd, dt and m for singlet, broad, doublet, triplet, doublet of doublets, doublet of doublet of doublets, doubet of triplets and multiplet, respectively. All coupling constants (*J*) are given in Hertz.

Tested compounds are ≥ 95 % chemical purity as measured by HPLC. The Surveyor®-LC-system consisted of a pump, an autosampler, and a PDA detector. Mass spectrometry was performed on a TSQ® Quantum (ThermoFisher, Dreieich, Germany). The triple quadrupole mass spectrometer was equipped with an electrospray interface (ESI) and atmospheric pressure chemical ionisation (APCI), respectively. The system was operated by the standard software Xcalibur®. A RP C18 NUCLEODUR® 100-5 (3 mm) column (Macherey-Nagel GmbH, Dühren, Germany) was used as stationary phase. All solvents were HPLC grade.

In a gradient run the percentage of acetonitrile (containing 0.1 % trifluoroacetic acid) in 0.1 % trifluoroacetic acid in was increased from an initial concentration of 5 % at 0 min to 100 % at 15 min and kept at 100 % for 5 min. The injection volume was 20 µL and flow rate was set to 800 µL/min. MS analysis was carried out at a needle voltage of 3000 V and a capillary temperature of 350 °C. Mass spectra were acquired in positive mode and in negative mode when required from 100 to 1000 m/z and UV spectra were recorded at the wavelength of 254 nm and in some cases at 360 nm.

GC/MS spectra were measured on a GCD Series G1800A (Hewlett Packard) instrument with an Optima-5-MS (0.25 µM, 30 m) column (Macherey Nagel).

All microwave irradiation experiments were carried out in a CEM-Discover microwave apparatus.

Melting points were measured on a Stuart Scientific SMP3 apparatus.

Flash chromatography was performed on silica gel 40 (35/40-63/70 µM) with hexane/ethyl acetate mixtures as eluents, and the reaction progress was determined by thin-layer chromatography analyses on Alugram SIL G/UV254 (Macherey-Nagel). Visualization was accomplished with UV light. Purification by preparative TLC was performed on 1 mm SIL G-100 UV_{254} glass plates (Macherey-Nagel). Purification with preparative HPLC were carried

out on a Agilent 1200 series HPLC system from Agilent Technologies, using a RP C18 Nucleodur 100-5 column (30100 m m/50 µm – from Macherey-Nagel GmbH) as stationary phase with acetonitrile/water or isopropanol/water as solvent in a gradient from 20:80 to 100:0 in fourty minutes.
Starting materials were used as obtained from Aldrich, Acros, Alfa Aeser and Combi-blocks without further purification. No attempts were made to optimise yields.

General procedure for alcohol formation

Method A. To a mixture of aldehyde (1 equiv) in dry THF was added the magnesiumbromide derivative (2.2 equiv) under nitrogen. The reaction mixture was heated to 80°C and stirred for 3h at 80°C. The reaction mixture was cooled to room temperature, quenched with brine and the aqueous layer was extracted with ethyl acetate. The combined organic layers were dried over sodium sulfate, filtered and concentrated to dryness. The product was purified by column chromatography.

General procedure for oxidation of alcohol in ketone

Method B. To a mixture of alcohol (1 equiv) in dry THF was added 2-iodoxybenzoic acid (2 equiv). The reaction mixture was stirred at 60°C overnight, cooled to room temperature and quenched with $Na_2S_2O_3$. The aqueous layer was extracted with ethyl acetate and the organic layer was washed with water, neutralised with 0.5N NaOH and dried over sodium sulfate, filtered and concentrated to dryness. The product was purified by column chromatography or by recrystallisation.

General procedures for Suzuki coupling

Method C. A mixture of arylbromide (1 equiv), boronic acid (1.2 equiv), sodium carbonate (2 equiv), and tetrakis(triphenylphosphine) palladium (0.02 equiv) was suspended in a degazed DME/water (2:1) solution. The reaction mixture was exposed to microwave irradiation (25 min, 150 W, 150°C, 15 bar). After reaching room temperature, water was added to quench the reaction and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, filtered and concentrated to dryness. The product was purified by column chromatography.

Method D. A mixture of arylbromide (1 equiv), boronic acid (1.2 equiv), caesium carbonate (4 equiv), and tetrakis(triphenylphosphine) palladium (0.02 equiv) was suspended in a DME/water (2:1) solution and the mixture was degazed. The mixture was heated to 80°C and

stirred overnight at 80°C under nitrogen. The reaction mixture was cooled to room temperature, quenched by water and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate, filtered and concentrated to dryness. The product was purified by column chromatography or by recrystallisation.

General procedures for ether cleavage

Method E. To a solution of methoxy derivative (1 equiv) in dry dichloromethane cooled at -78°C under nitrogen was slowly added boron tribromide (1M solution in dichloromethane, 5 equiv per methoxy function). The reaction mixture was stirred at -78°C for 1h and then allowed to warm to room temperature overnight. The reaction was quenched by water and extracted with ethyl acetate. The combined organic layers were washed with brine and dried over sodium sulfate, filtered, evaporated to dryness under reduced pressure and purified by column chromatography.

Method F. To a solution of methoxy derivative (1 equiv) in dry dichloromethane, borontrifluoride dimethylsulfide complex (35 equiv per methoxy function) was added dropwise at room temperature. The reaction mixture was stirred at room temperature overnight. Water was added to quench the reaction and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, filtered, evaporated to dryness under reduced pressure and purified by column chromatography.

Method G. To pyridinium hydrochloride (100 equiv) at 190°C was added the methoxy derivative (1 equiv). The reaction mixture was stirred for 2h, cooled to room temperature and then stirred with 1N HCl for 1h. The mixture was extracted in ethyl acetate. The combined organic layers were washed with water, dried over sodium sulfate, filtered, evaporated to dryness under reduced pressure and purified by column chromatography or by recrystallisation.

Detailed synthesis procedure of the most active compounds

(3-Bromophenyl)-(3-methoxyphenyl)-methanol (1c). The title compound was prepared by reaction of 3-bromophenyl carboxaldehyde (500 mg, 2.71 mmol, 1 equiv) with 3-methoxyphenylmagnesiumbromide (1M in THF) (1.35 g, 5.95 mL, 5.95 mmol, 2.2 equiv)

according to method A. The product was used in the next step without further purification. $C_{14}H_{13}BrO_2$; MW 293.

(3-Bromophenyl)-(3-methoxyphenyl)-methanone (1b). The title compound was prepared by reaction of (3-bromophenyl)-(3-methoxyphenyl)-methanol **1c** (460 mg, 1.57 mmol, 1 equiv) with 2-iodoxybenzoic acid (885 mg, 3.14 mmol, 2 equiv) according to method B. The product was purified by column chromatography (hexane/ethyl acetate 9:1) to give 200 mg (44%) of the analytically pure compound as a yellow oil. $C_{14}H_{11}BrO_2$; MW 291; ^{1}H NMR (acetone-d_6): δ 7.92-7.90 (m, 1H), 7.82 (ddd, *J* = 0.9 Hz, *J* = 1.9 Hz, *J* = 7.9 Hz, 1H), 7.76-7.73 (m, 1H), 7.50 (t, *J* = 8.2 Hz, 1H), 7.48-7.44 (m, 1H), 7.34-7.30 (m, 2H), 7.23 (ddd, *J* = 1.3 Hz, *J* = 2.5 Hz, *J* = 8.2 Hz, 1H), 3.86 (s, 3H); ^{13}C NMR (acetone-d_6): δ 195.8, 161.7, 141.6, 140.1, 136.9, 134.0, 132.2, 131.4, 130.4, 124.1, 123.9, 120.6, 116.2, 56.8.

(4´-Methoxy-3´-methylbiphenyl-3-yl)-(3-methoxyphenyl)-methanone (1a). The title compound was prepared by reaction of (3-bromophenyl)-(3-methoxyphenyl)-methanone **1b** (200 mg, 0.69 mmol, 1 equiv) with 4-methoxy-3-methylphenylboronic acid (137 mg, 0.82 mmol, 1.2 equiv) according to method D. The product was purified by column chromatography (hexane/ethyl acetate 95:5) to afford 200 mg (88%) of the analytically pure compound as a yellow oil. $C_{22}H_{20}O_3$; MW 332; ^{1}H NMR (acetone-d_6): δ 8.00-7.98 (m, 1H), 7.90-7.87 (m, 1H), 7.71-7.68 (m, 1H), 7.59 (t, *J* = 7.9 Hz, 1H), 7.52-7.45 (m, 3H), 7.38-7.35 (m, 2H), 7.25-7.21 (m, 1H), 7.02 (d, *J* = 9.1 Hz, 1H), 3.87 (s, 6H), 2.25 (s, 3H); ^{13}C NMR (acetone-d_6): δ 196.3, 160.7, 158.8, 142.0, 140.0, 139.2, 134.8, 132.0, 131.2, 130.4, 130.0, 129.7, 128.7, 128.4, 127.6, 126.4, 123.2, 119.3, 115.2, 111.4, 55.9, 55.8, 16.4.

(4´-Hydroxy-3´-methylbiphenyl-3-yl)-(3-hydroxyphenyl)-methanone (1). The title compound was prepared by reaction of 4´-methoxy-3´-methylbiphenyl-3-yl)-(3-methoxyphenyl)-methanone **1a** (200 mg, 0.60 mmol, 1 equiv) with boron tribromide (6 mmol, 10 equiv) according to method E. The product was purified by column chromatography (hexane/ethyl acetate 8:2) then by preparative TLC (hexane/ethyl acetate 7:3) to afford 44 mg (24%) of the analytically pure compound as a yellow oil. $C_{20}H_{16}O_3$; MW 304; ^{1}H NMR (acetone-d_6): δ 8.72 (s, br, 1H), 8.38 (s, br, 1H), 7.97-7.95 (m, 1H), 7.86-7.83 (m, 1H), 7.67-7.65 (m, 1H), 7.57 (t, *J* = 7.6 Hz, 1H), 7.46 (d, *J* = 2.2 Hz, 1H), 7.40-7.35 (m, 2H), 7.33-7.31 (m, 1H), 7.30-7.28 (m, 1H), 7.14 (dd, *J* = 2.5 Hz, *J* = 7.9 Hz, 1H), 6.94 (d, *J* = 8.2 Hz, 1H), 2.28 (s, 3H); ^{13}C NMR (acetone-d_6): δ 197.3, 159.1, 157.3, 143.0, 140.8, 140.1, 132.9, 131.8,

131.2, 131.1, 130.4, 129.3, 129.0, 127.1, 126.6, 122.9, 121.3, 117.9, 116.9, 17.1; IR: 3320, 1641, 1581, 1476, 1358, 1220 cm^{-1}; LC/MS *m/z*: 305 $(M+H)^+$.

(4-Bromophenyl)-(3-methoxyphenyl)-methanol (2c). The title compound was prepared by reaction of 4-bromophenyl carboxaldehyde (1 g, 5.41 mmol, 1 equiv) with 3-methoxyphenylmagnesiumbromide (1M in THF) (2.70 g, 11.9 mmol, 11.9 mL, 2.2 equiv) according to method A. The product was purified by column chromatography (hexane/ethyl acetate 9:1) to afford 1.58 g (100%) of the analytically pure compound as a colorless oil. $C_{14}H_{13}BrO_2$; MW 292; ^{1}H NMR (acetone-d_6): δ 7.50-7.47 (m, 2H), 7.41-7.38 (m, 2H), 7.23 (t, *J* = 7.9 Hz, 1H), 7.06-7.04 (m, 1H), 7.00-6.97 (m, 1H), 6.81 (ddd, *J* = 0.9 Hz, *J* = 2.5 Hz, *J* = 8.2 Hz, 1H), 5.81 (d, *J* = 4.0 Hz, 1H), 5.01 (d, *J* = 4.0 Hz, 1H), 3.76 (s, 3H); ^{13}C NMR (acetone-d_6): δ 160.8, 147.5, 145.7, 132.0, 130.2, 129.4, 121.2, 119.6, 113.3, 113.1, 75.5, 55.5.

(4-Bromophenyl)-(3-methoxyphenyl)-methanone (2b). The title compound was prepared by reaction of (4-bromophenyl)-(3-methoxyphenyl)-methanol **2c** (1.5 g, 5.12 mmol, 1 equiv) with 2-iodoxybenzoic acid (2.88 mg, 10.2 mmol, 2 equiv) according to method B. The product was purified by column chromatography (hexane/ethyl acetate 95:5) to afford 1.39 g (94%) of the analytically pure compound as a colorless oil. $C_{14}H_{11}BrO_2$; MW 290; ^{1}H NMR (acetone-d_6): δ 7.73-7.70 (m, 4H), 7.46-7.42 (m, 1H), 7.32-7.29 (m, 2H), 7.21 (ddd, *J* = 0.9 Hz, *J* = 2.5 Hz, *J* = 8.2 Hz, 1H), 3.85 (s, 3H); ^{13}C NMR (acetone-d_6): δ 195.3, 160.7, 139.5, 137.5, 132.5, 130.4, 127.7, 123.1, 119.5, 115.2, 55.9; IR: 3003, 2834, 1651, 1575, 1234 cm^{-1}.

(4´-Methoxy-3´-methylbiphenyl-4-yl)-(3-methoxyphenyl)-methanone (2a). The title compound was prepared by reaction of (4-bromophenyl)-(3-methoxyphenyl)-methanone **2b** (410 mg, 1.41 mmol, 1 equiv) with 4-methoxy-3-methylphenylboronic acid (281 mg, 1.69 mmol, 1.2 equiv) according to method D. The product was used in the next step without further purification. $C_{22}H_{20}O_3$; MW 332.

(4´-Hydroxy-3´-methylbiphenyl-4-yl)-(3-hydroxyphenyl)-methanone (2). The title compound was prepared by reaction of (4´-methoxy-3´-methylbiphenyl-4-yl)-(3-methoxyphenyl)-methanone **2a** (460 mg, 1.38 mmol, 1 equiv) with boron tribromide (13.8 mmol, 10 equiv) according to method E. The product was purified by column chromatography (hexane/ethyl acetate 8:2) then by preparative TLC (hexane/ethyl acetate 7:3 + 10 drops of HCOOH) to afford 120 mg (29%) of the analytically pure compound as an

orange solid. $C_{20}H_{16}O_3$; MW 304; ^{1}H NMR (acetone-d_6): δ 8.68 (s, br, 1H), 8.46 (s, br, 1H), 7.85-7.82 (m, 2H), 7.77-7.74 (m, 2H), 7.54 (d, *J* = 2.2 Hz, 1H), 7.44 (dd, *J* = 1.9 Hz, *J* = 7.9 Hz, 1H), 7.37 (t, *J* = 7.9 Hz, 1H), 7.29-7.24 (m, 2H), 7.13 (ddd, *J* = 0.9 Hz, *J* = 2.5 Hz, *J* = 8.2 Hz, 1H), 6.96 (d, *J* = 8.2 Hz, 1H), 2.30 (s, 3H); ^{13}C NMR (acetone-d_6): δ 196.9, 159.3, 157.9, 146.9, 141.3, 137.3, 132.7, 132.3, 131.5, 131.3, 127.8, 127.5, 126.8, 122.9, 121.2, 118.0, 117.1, 17.3; IR: 3363, 1641, 1588, 1449 cm^{-1}; LC/MS *m/z*: 305 $(M+H)^+$.

(3´-Fluoro-4´-methoxybiphenyl-4-yl)-(3-methoxyphenyl)-methanone (3a). The title compound was prepared by reaction of (4-bromophenyl)-(3-methoxyphenyl)-methanone **2b** (200 mg, 0.69 mmol, 1 equiv) with 3-fluoro-4-methoxyphenylboronic acid (141 mg, 0.81 mmol, 1.2 equiv) according to method D. The product was used in the next step without further purification. $C_{21}H_{17}FO_3$; MW 336.

(3´-Fluoro-4´-hydroxybiphenyl-4-yl)-(3-hydroxyphenyl)-methanone (3). The title compound was prepared by reaction of (3´-fluoro-4´-methoxy-biphenyl-4-yl)-(3-methoxyphenyl)-methanone **3a** (20 mg, 0.06 mmol, 1 equiv) with boron trifluoride dimethylsulfide complex (4.2 mmol, 70 equiv) according to method F. The product was purified by column chromatography (hexane/ethyl acetate 1:1). Yield: 8 mg (43%). $C_{19}H_{13}FO_3$; MW 308; ^{1}H NMR (acetone-d_6): δ 8.88 (s, br, 1H), 8.68 (s, br, 1H), 7.87-7.80 (m, 4H), 7.55 (dd, *J* = 2.3 Hz, *J* = 12.2 Hz, 1H), 7.47 (ddd, *J* = 0.9 Hz, *J* = 2.1 Hz, *J* = 8.4 Hz, 1H), 7.39 (t, *J* = 7.8 Hz, 1H), 7.28-7.25 (m, 2H), 7.16-7.12 (m, 2H); ^{13}C NMR (acetone-d_6): δ 195.9, 144.5, 140.1, 137.0, 131.3, 130.4, 127.0, 124.2, 121.9, 120.3, 116.9, 115.5, 115.4; IR: 3333, 2981, 1637, 1305 cm^{-1}; GC/MS *m/z*: 308 $(M)^+$.

(2´-Chloro-4´-methoxybiphenyl-4-yl)-(3-methoxyphenyl)-methanone (4a). The title compound was prepared by reaction of (4-bromophenyl)-(3-methoxyphenyl)-methanone **2b** (262 mg, 0.90 mmol, 1 equiv) with 2-chloro-4-methoxyphenylboronic acid (201 mg, 1.08 mmol, 1.2 equiv) according to the method D. The product was recrystallised in ethanol to afford 193 mg (61%) of the analytically pure compound as white crystals. $C_{21}H_{17}ClO_3$; MW 353; mp: 119-121°C; ^{1}H NMR ($CDCl_3$): δ 7.88-7.85 (m, 2H), 7.56-7.53 (m, 2H), 7.41-7.38 (m, 3H), 7.29 (d, *J* = 8.5 Hz, 1H), 7.17-7.13 (m, 1H), 7.05 (d, *J* = 2.5 Hz, 1H), 6.91 (dd, *J* = 2.8 Hz, *J* = 8.8 Hz, 1H), 3.89 (s, 3H), 3.86 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 196.1, 159.8, 143.4, 139.0, 136.3, 133.9, 131.9, 131.8, 129.9, 129.5, 129.2, 122.8, 118.8, 115.4, 114.3, 113.3, 55.6, 55.5; IR: 2954, 1647, 1597, 1034 cm^{-1}; GC/MS *m/z*: 352 $(M)^+$.

(2´-Chloro-4´-hydroxybiphenyl-4-yl)-(3-hydroxyphenyl)-methanone (4). The title compound was prepared by reaction of (2´-chloro-4´-methoxybiphenyl-4-yl)-(3-methoxyphenyl)-methanone **4a** (100 mg, 0.28 mmol, 1 equiv) with pyridinium hydrochloride (28 mmol, 100 equiv) according to method G. The product was purified by recrystallisation in hexane to afford 67 mg (74%) of the analytically pure compound as a brown solid. $C_{19}H_{13}ClO_3$; MW 325; mp: 212-214°C; ^{1}H NMR (acetone-d_6): δ 8.67 (s, br, 1H), 7.87-7.83 (m, 2H), 7.61-7.58 (m, 2H), 7.42-7.37 (m, 1H), 7.33 (d, *J* = 8.2 Hz, 1H), 7.31-7.29 (m, 1H), 7.27 (ddd, *J* = 0.9 Hz, *J* = 1.6 Hz, *J* = 7.6 Hz, 1H), 7.14 (ddd, *J* = 0.9 Hz, *J* = 2.5 Hz, *J* = 8.2 Hz, 1H), 7.05 (d, *J* = 2.5 Hz, 1H), 6.95 (dd, *J* = 2.5 Hz, *J* = 8.5 Hz, 1H); ^{13}C NMR (acetone-d_6): δ 159.0, 158.3, 144.3, 140.0, 137.3, 133.1, 131.5, 130.4, 122.0, 120.4, 117.6, 117.0, 115.7; IR: 3292, 1645, 1589, 1451 cm^{-1}; LC/MS *m/z*: 325 $(M+H)^+$.

(3´-Chloro-4´-hydroxybiphenyl-4-yl)-(3-methoxyphenyl)-methanone (5a). The title compound was prepared by reaction of (4-bromophenyl)-(3-methoxyphenyl)-methanone **2b** (200 mg, 0.69 mmol, 1 equiv) with 3-chloro-4-hydroxyphenylboronic acid (143 mg, 0.83 mmol, 1.2 equiv) according to method D. The product was purified by column chromatography (hexane/ethyl acetate 7:3) to afford 120 mg (51%) of the analytically pure compound. $C_{20}H_{15}ClO_3$; MW 338; ^{1}H NMR (acetone-d_6): δ 9.03 (s, br, 1H), 7.86-7.83 (m, 2H), 7.80-7.77 (m, 2H), 7.74 (d, *J* = 2.2 Hz, 1H), 7.57 (dd, *J* = 2.2 Hz, *J* = 8.5 Hz, 1H), 7.47-7.41 (m, 1H), 7.33-7.30 (m, 2H), 7.20 (ddd, *J* = 0.9 Hz, *J* = 2.7 Hz, *J* = 8.2 Hz, 1H), 7.14-7.11 (m, 1H), 3.85 (s, 3H); ^{13}C NMR (acetone-d_6): δ 194.9, 159.8, 153.3, 143.4, 139.2, 136.0, 132.4, 130.5, 130.0, 129.4, 128.5, 126.9, 126.8, 126.2, 122.1, 121.0, 118.2, 117.3, 114.3, 54.9.

(3´-Chloro-4´-hydroxybiphenyl-4-yl)-(3-hydroxyphenyl)-methanone (5). The title compound was prepared by reaction of (3´-chloro-4´-hydroxybiphenyl-4-yl)-(3-methoxyphenyl)-methanone **5a** (60 mg, 0.18 mmol, 1 equiv) with boron trifluoride dimethylsulfide complex (6.2 mmol, 35 equiv) according to method F. The product was purified by column chromatography (hexane/ethyl acetate 1:1). Yield: 40 mg (69%). $C_{19}H_{13}ClO_3$; MW 324; ^{1}H NMR (acetone-d_6): δ 9.00 (s, br, 1H), 8.67 (s, br, 1H), 7.84-7.81 (m, 2H), 7.79-7.75 (m, 2H), 7.73 (d, *J* = 2.2 Hz, 1H), 7.56 (dd, *J* = 2.2 Hz, *J* = 8.4 Hz, 1H), 7.35 (t, *J* = 7.9 Hz, 1H), 7.25-7.21 (m, 2H), 7.12 (d, *J* = 8.5 Hz, 1H), 7.09 (ddd, *J* = 1.1 Hz, *J* = 2.5 Hz, *J* = 8.2 Hz, 1H); ^{13}C NMR (acetone-d_6): δ 195.9, 158.3, 154.2, 144.2, 140.2, 137.1, 133.3, 131.4, 130.4, 129.3, 127.7, 127.1, 121.9, 121.8, 120.3, 118.2, 117.0; GC/MS *m/z*: 324-326 $(M)^+$.

(4-Bromophenyl)-(4-fluoro-3-methoxyphenyl)-methanol (6c). To a solution of 5-bromo-2-fluoroanisole (547 mg, 2.67 mmol, 1 equiv) in anhydrous THF was added granules of magnesium (68 mg, 2.80 mmol, 1.05 equiv) under nitrogen. The mixture was heated to 60°C for 2h. After cooling to room temperature, 4-bromobenzaldehyde (592 mg, 3.20 mmol, 1.2 equiv) was added and the reaction mixture was stirred at 80°C overnight. The reaction was cooled to room temperature, quenched with brine and the aqueous layer was extracted with ethyl acetate. The combined organic layers were dried over sodium sulfate, filtered and concentrated to dryness under vacuum. The product was used in the next step without further purification. $C_{14}H_{12}BrFO_2$; MW 311.

(4-Bromophenyl)-(4-fluoro-3-methoxyphenyl)-methanone (6b). The title compound was prepared by reaction of (4-bromophenyl)-(4-fluoro-3-methoxyphenyl)-methanol **6c** (468 mg, 1.50 mmol, 1 equiv) with 2-iodoxybenzoic acid (3.00 mmol, 2 equiv) according to method B. The product was purified by column chromatography (hexane/ethyl acetate 7:3) to afford 280 mg (60%) of the analytically pure compound. $C_{14}H_{10}BrFO_2$; MW 309; ^{1}H NMR (acetone-d_6): δ 7.77-7.72 (m, 4H), 7.56 (dd, J = 1.9 Hz, J = 8.5 Hz, 1H), 7.39-7.36 (m, 1H), 7.33-7.28 (m, 1H), 3.96 (s, 3H); ^{13}C NMR (acetone-d_6): δ 194.3, 137.5, 134.8, 132.5, 132.4, 127.6, 124.6, 118.6, 117.8, 116.6, 116.5, 115.5, 56.7; GC/MS *m/z*: 308-310 $(M)^+$.

(4-Fluoro-3-methoxyphenyl)-(4´-methoxy-3´-methylbiphenyl-4-yl)-methanone (6a). The title compound was prepared by reaction of (4-bromophenyl)-(4-fluoro-3-methoxyphenyl)-methanone **6b** (142 mg, 0.46 mmol, 1 equiv) with 4-methoxy-3-methylphenylboronic acid (92 mg, 0.55 mmol, 1.2 equiv) according to method D. The product was recrystallised in ethanol to afford 98 mg (61%) of the analytically pure compound as a white solid. $C_{22}H_{19}FO_3$; MW 350; mp: 145-146°C; ^{1}H NMR (acetone-d_6): δ 7.87-7.84 (m, 2H), 7.81-7.78 (m, 2H), 7.60-7.56 (m, 3H), 7.40 (ddd, J = 1.9 Hz, J = 4.4 Hz, J = 8.2 Hz, 1H), 7.30 (dd, J = 8.2 Hz, J = 11.0 Hz, 1H), 7.05 (d, J = 8.2 Hz, 1H), 3.97 (s, 3H), 3.90 (s, 3H), 2.27 (s, 3H); ^{13}C NMR (acetone-d_6): δ 194.7, 159.2, 148.8, 148.7, 145.7, 136.3, 138.6, 135.6, 132.3, 131.3, 130.1, 127.7, 127.0, 126.6, 124.4, 124.3, 116.5, 115.5, 111.4, 56.7, 55.8, 16.4; IR: 2922, 1645, 1601, 1027 cm^{-1}; GC/MS *m/z*: 350 $(M)^+$.

(4-Fluoro-3-hydroxyphenyl)-(4´-hydroxy-3´-methylbiphenyl-4-yl)-methanone (6). The title compound was prepared by reaction of (4-fluoro-3-methoxyphenyl)-(4´-methoxy-3´-methylbiphenyl-4-yl)-methanone **6a** (98 mg, 0.30 mmol, 1 equiv) with pyridinium hydrochloride (30 mmol, 100 equiv) according to method G. The product was recrystallised in

hexane to afford 40 mg (41%) of the analytically pure compound as a green powder. $C_{20}H_{15}FO_3$; MW 322; mp: 203-205°C; ^{1}H NMR (acetone-d_6): δ 9.04 (s, br, 1H), 8.47 (s, br, 1H), 7.84-7.76 (m, 4H), 7.54 (d, *J* = 1.8 Hz, 1H), 7.48 (dd, *J* = 2.0 Hz, *J* = 8.6 Hz, 1H), 7.46 (dd, *J* = 2.2 Hz, *J* = 8.2 Hz, 1H), 7.34-7.28 (m, 2H), 6.96 (d, *J* = 8.3 Hz, 1H), 2.29 (s, 3H); ^{13}C NMR (acetone-d_6): δ 194.7, 156.9, 156.0, 154.0, 145.9, 145.7, 136.2, 135.7, 131.7, 131.2, 130.5, 126.8, 126.5, 125.9, 123.3, 123.2, 120.1, 116.8, 116.1, 16.3; IR: 3307, 1642, 1592, 1429 cm^{-1}; LC/MS *m/z*: 323 $(M+H)^+$.

(3´-Chloro-4´-hydroxybiphenyl-4-yl)-(4-fluoro-3-methoxyphenyl)-methanone (7a). The title compound was prepared by reaction of (4-bromophenyl)-(4-fluoro-3-methoxyphenyl)-methanone **6b** (127 mg, 0.41 mmol, 1 equiv) with 3-chloro-4-hydroxyphenylboronic acid (85 mg, 0.49 mmol, 1.2 equiv) according to method D. The product was used in the next step without further purification. $C_{20}H_{14}ClFO_3$; MW 357.

(3´-Chloro-4´-hydroxybiphenyl-4-yl)-(4-fluoro-3-hydroxyphenyl)-methanone (7). The title compound was prepared by reaction of (3´-chloro-4´-hydroxybiphenyl-4-yl)-(4-fluoro-3-methoxyphenyl)-methanone **7a** (126 mg, 0.35 mmol, 1 equiv) with pyridinium hydrochloride (35 mmol, 100 equiv) according to method G. The product was purified by column chromatography (hexane/ethyl acetate 7:3) then by preparative HPLC (isopropanol/water) to afford 38 mg (32%) of the analytically pure compound as a yellow solid. $C_{19}H_{12}ClFO_3$; MW 343; ^{1}H NMR (acetone-d_6): δ 9.04 (s, br, 1H), 9.02 (s, br, 1H), 7.86-7.83 (m, 2H), 7.82-7.80 (m, 2H), 7.76 (d, *J* = 2.2 Hz, 1H), 7.60 (dd, *J* = 2.2 Hz, *J* = 8.5 Hz, 1H), 7.48 (dd, *J* = 1.9 Hz, *J* = 8.5 Hz, 1H), 7.34-7.31 (m, 1H), 7.31-7.26 (m, 1H), 7.16 (d, *J* = 8.5 Hz, 1H); ^{13}C NMR (acetone-d_6): δ 193.8, 153.3, 143.3, 136.1, 134.6, 132.4, 130.4, 128.4, 127.5, 126.2, 122.4, 121.0, 119.2, 118.5, 118.4, 117.3, 117.0, 116.0, 115.8; IR: 3320, 1650, 1593, 1058 cm^{-1}; LC/MS *m/z*: 344-346 $(M+H)^+$.

(4-Bromophenyl)-(3-fluoro-5-methoxyphenyl)-methanol (8c). To a solution of 3-bromo-5-fluoroanisole (500 mg, 2.44 mmol, 1 equiv) in anhydrous THF was added granules of magnesium (64 mg, 2.56 mmol, 1.05 equiv) under nitrogen. The mixture was heated to 60°C for 2h. After reaching room temperature, 4-bromobenzaldehyde (542 mg, 2.93 mmol, 1.2 equiv) was added and the reaction mixture was stirred at 80°C overnight. The reaction was cooled to room temperature, quenched with brine and the aqueous layer was extracted with ethyl acetate. The combined organic layers were dried over sodium sulfate, filtered and

concentrated to dryness under vacuum. The product was used in the next step without further purification. $C_{14}H_{12}BrFO_2$; MW 311.

(4-Bromophenyl)-(3-fluoro-5-methoxyphenyl)-methanone (8b). The title compound was prepared by reaction of (4-bromophenyl)-(3-fluoro-5-methoxyphenyl)-methanol **8c** (306 mg, 0.98 mmol, 1 equiv) with 2-iodoxybenzoic acid (553 mg, 1.96 mmol, 2 equiv) according to method B. The product was used in the next step without further purification. $C_{14}H_{10}BrFO_2$; MW 309.

(3-Fluoro-5-methoxyphenyl)-(4´-methoxy-3´-methylbiphenyl-4-yl)-methanone (8a). The title compound was prepared by reaction of (4-bromophenyl)-(3-fluoro-5-methoxyphenyl)-methanone **8b** (175 mg, 0.57 mmol, 1 equiv) with 4-methoxy-3-methylphenylboronic acid (113 mg, 0.68 mmol, 1.2 equiv) according to method D. The product was used in the next step without further purification. $C_{22}H_{19}FO_3$; MW 350.

(3-Fluoro-5-hydroxyphenyl)-(4´-hydroxy-3´-methylbiphenyl-4-yl)-methanone (8). The title compound was prepared by reaction of (3-fluoro-5-methoxyphenyl)-(4´-methoxy-3´-methylbiphenyl-4-yl)-methanone **8a** (100 mg, 0.29 mmol, 1 equiv) with pyridinium hydrochloride (29.0 mmol, 100 equiv) according to method G. The product was purified by preparative HPLC (gradient water/isopropanol) to afford 5 mg (5%) of the analytically pure compound. $C_{20}H_{15}FO_3$; MW 322; ^{1}H NMR (acetone-d_6): δ 9.17 (s, br, 1H), 8.48 (s, br, 1H), 7.87-7.84 (m, 2H), 7.80-7.77 (m, 2H), 7.56-7.54 (m, 1H), 7.45 (ddd, *J* = 0.6 Hz, *J* = 2.5 Hz, *J* = 8.2 Hz, 1H), 7.10-7.08 (m, 1H), 6.99 (ddd, *J* = 1.3 Hz, *J* = 2.5 Hz, *J* = 8.8 Hz, 1H), 6.96 (d, *J* = 8.5 Hz, 1H), 6.87 (dt, *J* = 2.2 Hz, *J* = 10.4 Hz, 1H), 2.29 (s, 3H); ^{13}C NMR (acetone-d_6): δ 195.7, 160.9, 158.0, 147.3, 142.7, 142.6, 136.7, 132.6, 132.3, 131.5, 127.9, 127.5, 126.9, 117.1, 114.6, 109.1, 108.2, 108.0, 17.3; LC/MS *m/z*: 323 $(M+H)^+$.

(4-Bromophenyl)-(3,4-difluorophenyl)-methanol (9c). To a solution of 1-bromo-3,4-difluorobenzene (1.00 g, 0.59 mL, 5.18 mmol, 1 equiv) in anhydrous THF was added granules of magnesium (132 mg, 5.44 mmol, 1.05 equiv) under nitrogen. The mixture was heated to 60°C for 2h. After cooling to room temperature, 4-bromobenzaldehyde (592 mg, 3.20 mmol, 1.2 equiv) was added and the reaction mixture was stirred at 80°C overnight. The reaction was cooled to room temperature, quenched with brine and the aqueous layer was extracted with ethyl acetate. The combined organic layers were dried over sodium sulfate, filtered and

concentrated to dryness under vacuum. The product was used in the next step without further purification. $C_{13}H_9BrF_2O$; MW 299.

(4-Bromophenyl)-(3,4-difluorophenyl)-methanone (9b). The title compound was prepared by reaction of (4-bromophenyl)-(3,4-difluorophenyl)-methanol **9c** (589 mg, 1.97 mmol, 1 equiv) with 2-iodoxybenzoic acid (1.38 g, 4.93 mmol, 2.5 equiv) according to method B. The product was purified by recrystallisation in ethanol to afford 140 mg (24%) of the analytically pure compound as a white powder. $C_{13}H_7BrF_2O$; MW 297; mp: 74-77°C; ^{1}H NMR (acetone-d_6): δ 7.79-7.74 (m, 5H), 7.70-7.66 (m, 1H), 7.56-7.50 (m, 1H); ^{13}C NMR (acetone-d_6): δ 193.3, 151.9, 151.8, 136.9, 135.5, 132.7, 132.4, 128.3, 128.2, 128.0, 119.8, 119.6, 118.6; IR: 1650, 1604, 1280 cm^{-1}; GC/MS *m/z*: 296-298 $(M)^+$.

(3,4-Difluorophenyl)-(4´-methoxy-3´-methylbiphenyl-4-yl)-methanone (9a). The title compound was prepared by reaction of (4-bromophenyl)-(3,4-difluorophenyl)-methanone **9b** (168 mg, 0.52 mmol, 1 equiv) with 4-methoxy-3-methylphenylboronic acid (103 mg, 0.62 mmol, 1.2 equiv) according to method D. The compound was recrystallised in ethanol to obtain 157 mg of the analytically pure product as a white powder. $C_{21}H_{16}F_2O_2$; MW 338; mp: 123-125°C; ^{1}H NMR (acetone-d_6): δ 7.88-7.84 (m, 2H), 7.82-7.79 (m, 2H), 7.75 (ddd, J = 2.2 Hz, J = 7.9 Hz, J = 11.0 Hz, 1H), 7.71-7.67 (m, 1H), 7.61-7.57 (m, 2H), 7.56-7.50 (m, 1H), 7.06 (d, J = 8.2 Hz, 1H), 3.90 (s, 3H), 2.27 (s, 3H); ^{13}C NMR (acetone-d_6): δ 193.6, 159.3, 146.1, 135.7, 131.4, 130.1, 128.1, 128.0, 127.8, 127.2, 126.7, 119.7, 119.5, 111.4, 55.9, 16.4; IR: 2930, 1645, 1596, 1141 cm^{-1}; GC/MS *m/z*: 338 $(M)^+$.

(3,4-Difluorophenyl)-(4-hydroxy-3´-methylbiphenyl-4-yl)-methanone (9). The title compound was prepared by reaction of (3,4-difluorophenyl)-(4´-methoxy-3-methylbiphenyl-4-yl)-methanone **9a** (100 mg, 0.30 mmol, 1 equiv) with pyridinium hydrochloride (30 mmol, 100 equiv) according to method G. The product was purified by recrystallisation in hexane to afford 35 mg (36%) of the analytically pure compound as a yellow solid. $C_{20}H_{14}F_2O_2$; MW 324; mp: 174-176°C; ^{1}H NMR (acetone-d_6): δ 8.54 (s, br, 1H), 7.86-7.83 (m, 2H), 7.80-7.77 (m, 2H), 7.77-7.74 (m, 1H), 7.71-7.67 (m, 1H), 7.56-7.50 (m, 2H), 7.45 (ddd, J = 0.6 Hz, J = 2.5 Hz, J = 8.2 Hz, 1H), 6.96 (d, J = 8.2 Hz, 1H), 2.29 (s, 3H); ^{13}C NMR (acetone-d_6): δ 193.7, 157.1, 152.7, 152.6, 151.8, 149.9, 146.4, 135.5, 131.4, 130.5, 127.0, 126.5, 119.7, 119.5, 118.4, 118.3, 116.2, 16.3; IR: 3427, 1645, 1594, 1287 cm^{-1}; GC/MS *m/z*: 324 $(M)^+$.

(6-Bromopyridin-3-yl)-(3-methoxyphenyl)-methanol (10c). The title compound was prepared by reaction of 6-bromopyridin-3-yl carboxaldehyde (500 mg, 2.69 mmol, 1 equiv) with 3-methoxyphenylmagnesiumbromide (1M in THF) (1.35 g, 5.91 mmol, 2.2 equiv) according to method A. The product was purified by column chromatography (hexane/ethyl acetate 7:3) to afford 700 mg (89%) of the analytically pure compound as a colorless oil. $C_{13}H_{12}BrNO_2$; MW 294; ^{1}H NMR (acetone-d_6): δ 8.45-8.43 (m, 1H), 7.71 (ddd, *J* = 0.6 Hz, *J* = 2.5 Hz, *J* = 8.2 Hz, 1H), 7.52 (d, *J* = 8.8 Hz, 1H), 7.25 (t, *J* = 8.2 Hz, 1H), 7.06-7.04 (m, 1H), 7.00-6.97 (m, 1H), 6.82 (ddd, *J* = 0.9 Hz, *J* = 2.8 Hz, *J* = 8.2 Hz, 1H), 5.89 (d, *J* = 4.1 Hz, 1H), 5.21 (d, *J* = 4.1 Hz, 1H), 3.77 (s, 3H); ^{13}C NMR (acetone-d_6): δ 161.8, 150.4, 147.6, 142.4, 142.0, 139.0, 131.3, 129.5, 120.3, 114.6, 113.8, 74.3, 56.5.

(6-Bromopyridin-3-yl)-(3-methoxyphenyl)-methanone (10b). The title compound was prepared by reaction of (6-bromopyridin-3-yl)-(3-methoxyphenyl)-methanol **10c** (700 mg, 2.38 mmol, 1 equiv) with 2-iodoxybenzoic acid (1.34 g, 4.76 mmol, 2 equiv) according to method B. The product was purified by column chromatography (hexane/ethyl acetate 9:1) to give 520 mg (75%) of the analytically pure compound as a colorless oil. $C_{13}H_{10}BrNO_2$; MW 292; ^{1}H NMR (acetone-d_6): δ 8.70 (d, *J* = 1.8 Hz, 1H), 8.07 (dd, *J* = 2.4 Hz, *J* = 8.2 Hz, 1H), 7.80 (dd, *J* = 0.6 Hz, *J* = 8.2 Hz, 1H), 7.51-7.47 (m, 1H), 7.38-7.35 (m, 2H), 7.26 (ddd, *J* = 0.9 Hz, *J* = 2.7 Hz, *J* = 8.2 Hz, 1H), 3.87 (s, 3H); ^{13}C NMR (acetone-d_6): δ 161.8, 152.9, 147.2, 141.5, 139.8, 134.6, 131.6, 129.9, 124.2, 121.2, 116.0, 56.9.

[6-(4-Methoxy-3-methylphenyl)-pyridin-3-yl]-(3-methoxyphenyl)-methanone (10a). The title compound was prepared by reaction of (6-bromopyridin-3-yl)-(3-methoxyphenyl)-methanone **10b** (520 mg, 1.80 mmol, 1 equiv) with 4-methoxy-3-methylphenylboronic acid (359 mg, 2.16 mmol, 1.2 equiv) according to method D. The product was purified by column chromatography (hexane/ethyl acetate 9:1) to afford 536 mg (89%) of the analytically pure compound as a yellow oil. $C_{21}H_{19}NO_3$; MW 333; ^{1}H NMR (acetone-d_6): δ 8.74 (dd, *J* = 0.9 Hz, *J* = 2.2 Hz, 1H), 7.91 (dd, *J* = 2.2 Hz, *J* = 8.2 Hz, 1H), 7.83-7.80 (m, 2H), 7.74 (dd, *J* = 0.6 Hz, *J* = 8.5 Hz, 1H), 7.28-7.24 (m, 1H), 7.17-7.14 (m, 2H), 7.02 (ddd, *J* = 1.3 Hz, *J* = 2.5 Hz, *J* = 8.5 Hz, 1H), 6.81 (d, *J* = 9.1 Hz, 1H), 3.68 (s, 3H), 3.66 (s, 3H), 2.05 (s, 3H); ^{13}C NMR (acetone-d_6): δ 195.5, 161.7, 161.5, 152.6, 140.6, 139.7, 131.9, 131.5, 131.3, 128.4, 128.2, 124.0, 120.6, 120.3, 116.0, 112.0, 56.9, 56.8, 17.5.

[6-(4-Hydroxy-3-methylphenyl)-pyridin-3-yl]-(3-hydroxyphenyl)-methanone (10). The title compound was prepared by reaction of [6-(4-methoxy-3-methylphenyl)-pyridin-3-yl]-(3-

methoxyphenyl)-methanone **10a** (466 mg, 1.40 mmol, 1 equiv) with boron tribromide (14.0 mmol, 10 equiv) according to method E. The product was purified by column chromatography (hexane/ethyl acetate 8:2) to give 321 mg (75%) of the analytically pure compound as a yellow powder. $C_{19}H_{15}NO_3$; MW 305; ^{1}H NMR (acetone-d_6): δ 8.94 (dd, *J* = 0.6 Hz, *J* = 2.1 Hz, 1H), 8.73 (s, br, 2H), 8.14 (dd, *J* = 2.1 Hz, *J* = 8.2 Hz, 1H), 8.04 (dd, *J* = 0.6 Hz, *J* = 2.1 Hz, 1H), 7.99 (dd, *J* = 0.6 Hz, *J* = 8.2 Hz, 1H), 7.94 (dd, *J* = 2.1 Hz, *J* = 8.5 Hz, 1H), 7.44-7.39 (m, 1H), 7.32-7.29 (m, 2H), 7.17-7.14 (m, 1H), 6.97 (d, *J* = 8.2 Hz, 1H), 2.30 (s, 3H); ^{13}C NMR (acetone-d_6): δ 194.7, 160.7, 158.4, 151.5, 139.7, 138.7, 131.4, 130.9, 130.6, 130.4, 127.1, 125.6, 122.0, 120.7, 119.2, 116.9, 115.9, 16.3; IR: 3431, 1648, 1582, 1473, 1268 cm^{-1}; LC/MS *m/z*: 306 $(M+H)^+$.

(5-Bromopyridin-2-yl)-(3-methoxyphenyl)-methanol (11c). The title compound was prepared by reaction of 5-bromopyridin-2-yl carboxaldehyde (500 mg, 2.69 mmol, 1 equiv) with 3-methoxyphenylmagnesiumbromide (1M in THF) (1.35 g, 5.9 mL, 5.91 mmol, 2.2 equiv) according to method A. The product was purified by column chromatography (hexane/ethyl acetate 8:2) to give 490 mg (62%) of the analytically pure compound as a red oil. $C_{13}H_{12}BrNO_2$; MW 294; ^{1}H NMR (acetone-d_6): δ 8.57 (dd, *J* = 0.6 Hz, *J* = 2.5 Hz, 1H), 7.94 (dd, *J* = 2.5 Hz, *J* = 8.5 Hz, 1H), 7.56-7.53 (m, 1H), 7.21 (t, *J* = 7.9 Hz, 1H), 7.07-7.05 (m, 1H), 7.04-7.00 (m, 1H), 6.79 (ddd, *J* = 0.9 Hz, *J* = 2.5 Hz, *J* = 8.2 Hz, 1H), 5.80 (d, *J* = 4.4 Hz, 1H), 5.25 (d, *J* = 4.4 Hz, 1H), 3.76 (s, 3H); ^{13}C NMR (acetone-d_6): δ 192.6, 164.4, 161.6, 151.0, 147.2, 141.2, 131.0, 124.0, 120.6, 114.4, 114.0, 77.3, 56.4.

(5-Bromopyridin-2-yl)-(3-methoxyphenyl)-methanone (11b). The title compound was prepared by reaction of (5-bromopyridin-2-yl)-(3-methoxyphenyl)-methanol **11c** (490 mg, 1.67 mmol, 1 equiv) with 2-iodoxybenzoic acid (940 mg, 3.33 mmol, 2 equiv) according to method B. The product was purified by column chromatography (hexane/ethyl acetate 9:1) to afford 365 mg (75%) of the analytically pure compound as a colorless oil. $C_{13}H_{10}BrNO_2$; MW 292; ^{1}H NMR (acetone-d_6): δ 8.80 (dd, *J* = 0.6 Hz, *J* = 2.2 Hz, 1H), 8.25 (dd, *J* = 2.2 Hz, *J* = 8.2 Hz, 1H), 7.97 (dd, *J* = 0.6 Hz, *J* = 8.2 Hz, 1H), 7.65-7.61 (m, 2H), 7.45-7.41 (m, 1H), 7.21 (ddd, *J* = 0.9 Hz, *J* = 2.5 Hz, *J* = 8.2 Hz, 1H), 3.86 (s, 3H); ^{13}C NMR (acetone-d_6): δ 193.7, 161.3, 155.6, 151.3, 141.9, 139.3, 131.0, 127.7, 125.7, 125.3, 120.7, 117.3, 56.8.

[5-(4-Methoxy-3-methylphenyl)-pyridin-2-yl]-(3-methoxyphenyl)-methanone (11a). The title compound was prepared by reaction of (5-bromopyridin-2-yl)-(3-methoxyphenyl)-methanone **11b** (365 mg, 1.25 mmol, 1 equiv) with 4-methoxy-3-methylphenylboronic acid

(249 mg, 1.50 mmol, 1.2 equiv) according to method D. The product was purified by column chromatography (hexane/ethyl acetate 9:1) to give 362 mg (87%) of the analytically pure compound as yellow solid. $C_{21}H_{19}NO_3$; MW 333; ^{1}H NMR (acetone-d_6): δ 8.95 (dd, J = 0.6 Hz, J = 2.2 Hz, 1H), 8.18 (dd, J = 2.5 Hz, J = 8.2 Hz, 1H), 8.06 (dd, J = 0.6 Hz, J = 8.2 Hz, 1H), 7.75-7.71 (m, 2H), 7.62-7.59 (m, 2H), 7.45-7.41 (m, 1H), 7.20 (ddd, J = 0.9 Hz, J = 2.8 Hz, J = 8.2 Hz, 1H), 7.06 (dd, J = 2.2 Hz, J = 7.3 Hz, 1H), 3.89 (s, 3H), 3.87 (s, 3H), 2.27 (s, 3H); ^{13}C NMR (acetone-d_6): δ 194.1, 161.3, 160.6, 154.9, 148.0, 140.3, 140.0, 136.1, 131.1, 130.8, 130.3, 129.0, 127.9, 126.2, 125.4, 120.2, 117.6, 112.6, 56.8, 56.7, 17.4.

[5-(4-Hydroxy-3-methylphenyl)-pyridin-2-yl]-(3-hydroxyphenyl)-methanone (11). The title compound was prepared by reaction of [5-(4-methoxy-3-methylphenyl)-pyridin-2-yl]-(3-methoxyphenyl)-methanone **11a** (362 mg, 1.09 mmol, 1 equiv) with boron tribromide (10.9 mmol, 10 equiv) according to method E. The product was purified by column chromatography (hexane/ethyl acetate 8:3) then by preparative TLC (hexane/ethyl acetate 7:3) to give 127 mg (45%) of the analytically pure compound as yellow powder. $C_{19}H_{15}NO_3$; MW 305; mp: 178-180°C; ^{1}H NMR (acetone-d_6): δ 8.93 (d, J = 1.8 Hz, 1H), 8.62 (s, br, 1H), 8.61 (s, br, 1H), 8.19 (dd, J = 2.1 Hz, J = 8.2 Hz, 1H), 8.04 (d, J = 8.2 Hz, 1H), 7.63-7.58 (m, 3H), 7.50 (dd, J = 1.8 Hz, J = 8.2 Hz, 1H), 7.37-7.32 (m, 1H), 7.13-7.09 (m, 1H), 7.00 (d, J = 8.2 Hz, 1H), 2.31 (s, 3H); ^{13}C NMR (acetone-d_6): δ 194.4, 158.9, 158.4, 154.9, 147.8, 140.6, 140.1, 135.9, 131.6, 130.9, 129.7, 127.7, 127.3, 126.2, 124.3, 121.5, 119.2, 117.4, 17.3; IR: 3350, 1644, 1580, 1514, 1240 cm^{-1}; LC/MS m/z: 306 $(M+H)^+$.

(5-Bromopyridin-2-yl)-(4-fluoro-3-methoxyphenyl)-methanol (12c). To a solution of 5-bromo-2-fluoroanisole (1.00 g, 4.88 mmol, 1 equiv) in anhydrous THF was added granules of magnesium (125 mg, 5.12 mmol, 1.05 equiv) under nitrogen. The mixture was heated to 60°C for 2h. After cooling to room temperature, 5-bromo-2-formylpyridine (1.09 g, 5.86 mmol, 1.2 equiv) was added and the reaction mixture was stirred at 80°C overnight. The reaction was cooled to room temperature, quenched with brine and the aqueous layer was extracted with ethyl acetate. The combined organic layers were dried over sodium sulfate, filtered and concentrated to dryness under vacuum. The product was used in the next step without further purification. $C_{13}H_{11}BrFNO_2$; MW 312.

(5-Bromopyridin-2-yl)-(4-fluoro-3-methoxyphenyl)-methanone (12b). The title compound was prepared by reaction of (5-bromopyridin-2-yl)-(4-fluoro-3-methoxyphenyl)-methanol **12c** (463 mg, 1.48 mmol, 1 equiv) with 2-iodoxybenzoic acid (2.96 mmol, 2 equiv) according to

method B. The product was purified by column chromatography (hexane) to afford 354 mg (77%) of the analytically pure compound as a yellow oil. $C_{13}H_9BrFNO_2$; MW 310; ^{1}H NMR (acetone-d_6): δ 6.28 (dd, *J* = 2.2 Hz, *J* = 8.2 Hz, 1H), 8.06-8.03 (m, 2H), 7.99 (dd, *J* = 0.9 Hz, *J* = 8.5 Hz, 1H), 7.89 (dd, *J* = 2.2 Hz, *J* = 8.5 Hz, 1H), 7.77 (ddd, *J* = 1.9 Hz, *J* = 4.4 Hz, *J* = 8.5 Hz, 1H), 3.96 (s, 3H); ^{13}C NMR (acetone-d_6): δ 191.4, 167.6, 154.5, 150.3, 141.1, 133.8, 130.4, 129.3, 126.8, 126.2, 116.6, 116.2, 56.7; IR: 2835, 1678, 1601, 1290 cm^{-1}; LC/MS *m/z*: 310-312 $(M+H)^+$.

(4-Fluoro-3-methoxyphenyl)-[5-(4-methoxy-3-methylphenyl)-pyridin-2-yl]-methanone (12a). The title compound was prepared by reaction of (5-bromopyridin-2-yl)-(4-fluoro-3-methoxyphenyl)-methanone **12b** (300 mg, 0.97 mmol, 1 equiv) with 4-methoxy-3-methylphenylboronic acid (193 mg, 1.16 mmol, 1.2 equiv) according to method D. The product was recrystallised in acetonitrile to afford 279 mg (82%) of the analytically pure compound as a yellow powder. $C_{21}H_{18}FNO_3$; MW 351; mp: 141-143°C; ^{1}H NMR ($CDCl_3$): δ 8.89 (dd, *J* = 0.6 Hz, *J* = 2.2 Hz, 1H), 8.09 (dd, *J* = 0.6 Hz, *J* = 8.2 Hz, 1H), 8.02 (dd, *J* = 2.5 Hz, *J* = 8.2 Hz, 1H), 7.82 (dd, *J* = 2.2 Hz, *J* = 8.5 Hz, 1H), 7.77 (ddd, *J* = 2.2 Hz, *J* = 4.4 Hz, *J* = 8.5 Hz, 1H), 7.46 (dd, *J* = 2.5 Hz, *J* = 8.2 Hz, 1H), 7.44-7.42 (m, 1H), 7.15 (dd, *J* = 8.5 Hz, *J* = 10.7 Hz, 1H), 6.94 (d, *J* = 8.2 Hz, 1H), 3.95 (s, 3H), 3.88 (s, 3H), 2.30 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 192.0, 158.9, 156.8, 153.0, 147.9, 147.8, 146.7, 139.2, 134.7, 133.3, 129.7, 128.8, 126.1, 125.8, 125.2, 115.9, 115.8, 110.8, 56.6, 55.7, 16.6; GC/MS *m/z*: 351 $(M)^+$.

(4-Fluoro-3-hydroxyphenyl)-[5-(4-hydroxy-3-methylphenyl)-pyridin-2-yl]-methanone (12). The title compound was prepared by reaction of (4-fluoro-3-methoxyphenyl)-[5-(4-methoxy-3-methylphenyl)-pyridin-2-yl]-methanone **12a** (70 mg, 0.20 mmol, 1 equiv) with pyridinium hydrochloride (20.0 mmol, 100 equiv) according to method G. The product was purified by preparative HPLC (isopropanol/water) to give 21 mg (33%) of the analytically pure compound as a yellow powder. $C_{19}H_{14}FNO_3$; MW 323; mp: 221-223°C; ^{1}H NMR (acetone-d_6): δ 8.94 (dd, *J* = 0.6 Hz, *J* = 2.2 Hz, 1H), 8.64 (s, br, 1H), 8.20 (dd, *J* = 2.2 Hz, *J* = 8.2 Hz, 1H), 8.05 (dd, *J* = 0.9 Hz, *J* = 8.2 Hz, 1H), 7.87 (dd, *J* = 2.2 Hz, *J* = 8.8 Hz, 1H), 7.74 (ddd, *J* = 2.2 Hz, *J* = 4.4 Hz, *J* = 8.5 Hz, 1H), 7.60 (dd, *J* = 0.6 Hz, *J* = 2.2 Hz, 1H), 7.50 (ddd, *J* = 0.6 Hz, *J* = 2.5 Hz, *J* = 8.2 Hz, 1H), 7.24 (dd, *J* = 8.5 Hz, *J* = 11.0 Hz, 1H), 7.00 (d, *J* = 8.2 Hz, 1H), 2.30 (s, 3H); ^{13}C NMR (acetone-d_6): δ 192.7, 158.3, 157.2, 154.6, 147.6, 140.5, 135.9, 131.5, 129.5, 127.5, 127.1, 126.1, 125.7, 125.6, 122.3, 117.3, 117.2, 117.1, 17.1; IR: 3179, 2971, 1607, 1511, 1124 cm^{-1}; LC/MS *m/z*: 324 $(M+H)^+$.

(4-Fluoro-3-methoxyphenyl)-[5-(3-chloro-4-hydroxyphenyl)-pyridin-2-yl]-methanone (13a). The title compound was prepared by reaction of (5-bromopyridin-2-yl)-(4-fluoro-3-methoxyphenyl)-methanone **12b** (150 mg, 0.48 mmol, 1 equiv) with 3-chloro-4-hydroxyphenylboronic acid (100 mg, 0.58 mmol, 1.2 equiv) according to method D. The product was recrystallised in ethyl acetate to afford 72 mg (42%) of the analytically pure product as a yellow solid. $C_{19}H_{13}ClFNO_3$; MW 357; mp: 195-197°C; ^{1}H NMR (acetone-d_6): δ 9.20 (s, br, 1H), 9.00 (dd, *J* = 0.6 Hz, *J* = 2.2 Hz, 1H), 8.28 (dd, *J* = 2.5 Hz, *J* = 8.2 Hz, 1H), 8.11 (dd, *J* = 0.6 Hz, *J* = 8.2 Hz, 1H), 7.97 (dd, *J* = 2.2 Hz, *J* = 8.5 Hz, 1H), 7.88-7.84 (m, 2H), 7.67 (dd, *J* = 2.2 Hz, *J* = 8.5 Hz, 1H), 7.28 (dd, *J* = 8.2 Hz, *J* = 11.4 Hz, 1H), 7.20 (d, *J* = 8.5 Hz, 1H), 3.97 (s, 3H); ^{13}C NMR (acetone-d_6): δ 191.6, 154.8, 154.3, 147.0, 138.2, 135.5, 130.2, 129.6, 128.0, 126.2, 126.1, 125.4, 122.2, 118.4, 116.8, 116.3, 116.1, 56.6; GC/MS *m/z*: 356-358 $(M)^+$.

(4-Fluoro-3-hydroxyphenyl)-[5-(3-chloro-4-hydroxyphenyl)-pyridin-2-yl]-methanone (13). The title compound was prepared by reaction of (4-fluoro-3-methoxyphenyl)-[5-(3-chloro-4-hydroxyphenyl)-pyridin-2-yl]-methanone **13a** (59 mg, 0.17 mmol, 1 equiv) with boron trifluoride dimethylsulfide complex (5.95 mmol, 35 equiv) according to method F. The product was purified by column chromatography (hexane/ethyl acetate 6:4) then by preparative HPLC (isopropanol/water) to afford 10 mg (17%) of the analytically pure product. $C_{18}H_{11}ClFNO_3$; MW 343; ^{1}H NMR (acetone-d_6): δ 9.10 (s, br, 2H), 8.98 (d, *J* = 2.2 Hz, 1H), 8.27 (dd, *J* = 2.5 Hz, *J* = 7.9 Hz, 1H), 8.09-8.06 (m, 1H), 7.87 (dd, *J* = 2.2 Hz, *J* = 8.8 Hz, 1H), 7.84 (d, *J* = 2.2 Hz, 1H), 7.73 (ddd, *J* = 1.9 Hz, *J* = 4.4 Hz, *J* = 8.5 Hz, 1H), 7.66 (dd, *J* = 2.2 Hz, *J* = 8.5 Hz, 1H), 7.25 (dd, *J* = 8.5 Hz, *J* = 10.7 Hz, 1H), 7.20 (d, *J* = 8.5 Hz, 1H); ^{13}C NMR (acetone-d_6): δ 191.8, 156.4, 154.8, 154.4, 147.0, 145.5, 145.4, 138.2, 135.5, 134.4, 129.6, 128.0, 125.3, 122.2, 121.4, 119.4, 118.4, 116.5; IR: 3355, 1647, 1578, 1517, 1265 cm^{-1}; LC/MS *m/z*: 344-346 $(M+H)^+$.

(6-Bromopyridin-2-yl)-(3-methoxyphenyl)-methanol (14c). The title compound was prepared by reaction of 6-bromopyridin-2-yl carboxaldehyde (1 g, 5.41 mmol, 1 equiv) with 3-methoxyphenylmagnesiumbromide (1M in THF) (2.70 g, 11.9 mL, 11.9 mmol, 2.2 equiv) according to method A. The product was purified by column chromatography (hexane/ethyl acetate 8:2) to afford 936 mg (59%) of the analytically pure product. $C_{13}H_{12}BrNO_2$; MW 294; ^{1}H NMR (acetone-d_6): δ 7.57 (t, *J* = 7.6 Hz, 1H), 7.49 (d, *J* = 7.6 Hz, 1H), 7.30 (d, *J* = 7.9 Hz, 1H), 7.08 (t, *J* = 8.2 Hz, 1H), 6.93-6.90 (m, 1H), 6.87 (d, *J* = 7.6 Hz, 1H), 6.66 (dd, *J* = 2.4 Hz, *J* = 8.2 Hz, 1H), 5.63 (d, *J* = 4.5 Hz, 1H), 5.05 (d, *J* = 4.5 Hz, 1H), 3.62 (s, 3H); ^{13}C NMR

(acetone-d_6): δ 165.5, 159.8, 145.1, 140.4, 139.7, 129.2, 126.4, 119.4, 118.8, 112.5, 75.5, 54.5.

(6-Bromopyridin-2-yl)-(3-methoxyphenyl)-methanone (14b). The title compound was prepared by reaction of (6-bromopyridin-2-yl)-(3-methoxyphenyl)-methanol **14c** (930 mg, 3.19 mmol, 1 equiv) with 2-iodoxybenzoic acid (1.80 g, 6.39 mmol, 2 equiv) according to method B. The product was purified by column chromatography (hexane/ethyl acetate 9:1) to afford 727 mg (78%) of the analytically pure compound as a yellow solid. $C_{13}H_{10}BrNO_2$; MW 292; ^{1}H NMR (acetone-d_6): δ 8.02 (dd, *J* = 1.6 Hz, *J* = 7.6 Hz, 1H), 7.99 (t, *J* = 7.3 Hz, 1H), 7.86 (dd, *J* = 1.6 Hz, *J* = 7.3 Hz, 1H), 7.65-7.61 (m, 2H), 7.47-7.43 (m, 1H), 7.23 (ddd, *J* = 0.9 Hz, *J* = 2.5 Hz, *J* = 8.2 Hz, 1H), 3.87 (s, 3H); ^{13}C NMR (acetone-d_6): δ 193.5, 160.4, 156.7, 141.2, 141.1, 138.0, 131.9, 124.4, 120.1, 119.2, 116.3, 115.2, 55.8; IR: 1667, 1425, 1254, 1032 cm^{-1}; LC/MS *m/z*: 292-294 $(M+H)^+$.

[6-(4-Methoxy-3-methylphenyl)-pyridin-2-yl]-(3-methoxyphenyl)-methanone (14a). The title compound was prepared by reaction of (6-bromopyridin-2-yl)-(3-methoxyphenyl)-methanone **14b** (410 mg, 1.40 mmol, 1 equiv) with 4-methoxy-3-methylphenylboronic acid (280 mg, 1.69 mmol, 1.2 equiv) according to method D. The product was purified by column chromatography (hexane/ethyl acetate 9:1) to give 432 mg (92%) of the analytically pure compound as a yellow powder. $C_{21}H_{19}NO_3$; MW 333; ^{1}H NMR (acetone-d_6): δ 8.10 (dd, *J* = 0.9 Hz, *J* = 7.9 Hz, 1H), 8.07-8.03 (m, 1H), 7.99-7.95 (m, 2H), 7.89 (dd, *J* = 0.9 Hz, *J* = 7.6 Hz, 1H), 7.80-7.78 (m, 1H), 7.75 (dt, *J* = 0.9 Hz, *J* = 7.6 Hz, 1H), 7.48 (t, *J* = 7.9 Hz, 1H), 7.24 (ddd, *J* = 0.9 Hz, *J* = 2.8 Hz, *J* = 8.2 Hz, 1H), 7.02 (d, *J* = 8.2 Hz, 1H), 3.89 (s, 3H), 3.88 (s, 3H), 2.24 (s, 3H); ^{13}C NMR (acetone-d_6): δ 193.6, 160.4, 160.1, 156.5, 155.7, 139.0, 138.9, 131.3, 129.9, 127.3, 126.7, 124.5, 122.6, 122.5, 119.8, 116.4, 111.1, 55.9, 55.8, 16.5.

[6-(4-Hydroxy-3-methylphenyl)-pyridin-2-yl]-(3-hydroxyphenyl)-methanone (14). The title compound was prepared by reaction of [6-(4-methoxy-3-methylphenyl)-pyridin-2-yl]-(3-methoxyphenyl)-methanone **14a** (341 mg, 1.02 mmol, 1 equiv) with boron tribromide (10.2 mmol, 10 equiv) according to method E. The product was purified by column chromatography (hexane/ethyl acetate 8:2) then by preparative TLC (hexane/ethyl acetate 65:35) to afford 120 mg (38%) of the analytically pure compound as a yellow solid. $C_{19}H_{15}NO_3$; MW 305; ^{1}H NMR (acetone-d_6): δ 8.64 (s, br, 1H), 8.54 (s, br, 1H), 8.06 (dd, *J* = 1.3 Hz, *J* = 7.9 Hz, 1H), 8.04-8.00 (m, 1H), 7.93 (d, *J* = 2.2 Hz, 1H), 7.85-7.81 (m, 2H), 7.67-7.63 (m, 2H), 7.41-7.37 (m, 1H), 7.14 (ddd, *J* = 0.9 Hz, *J* = 2.5 Hz, *J* = 7.9 Hz, 1H), 6.91 (d, *J*

= 8.2 Hz, 1H), 2.27 (s, 3H); ^{13}C NMR (acetone-d_6): δ 195.1, 157.0, 140.1, 140.0, 131.9, 131.6, 131.2, 127.8, 126.6, 124.5, 123.4, 123.3, 121.9, 119.5, 117.0, 17.5; IR: 3372, 1642, 1581, 1426 cm^{-1}; LC/MS *m/z*: 306 $(M+H)^+$.

(3-Methoxyphenyl)-[6-(2-methoxyphenyl)-pyridin-2-yl]-methanone (15a). The title compound was prepared by reaction of (6-bromopyridin-2-yl)-(3-methoxyphenyl)-methanone **14b** (100 mg, 0.34 mmol, 1 equiv) with 2-methoxyphenylboronic acid (62 mg, 0.41 mmol, 1.2 equiv) according to method C. The product was purified by column chromatography (hexane/ethyl acetate 8:2) to afford 53 mg (49%) of the analytically pure product as a light yellow oil. $C_{20}H_{17}NO_3$; MW 319; ^{1}H NMR (acetone-d_6): δ 8.19 (dd, *J* = 0.9 Hz, *J* = 7.9 Hz, 1H), 8.05 (t, *J* = 7.9 Hz, 1H), 7.94 (dd, *J* = 1.2 Hz, *J* = 7.7 Hz, 1H), 7.86 (dd, *J* = 1.8 Hz, *J* = 7.7 Hz, 1H), 7.78-7.76 (m, 1H), 7.76-7.73 (m, 1H), 7.46-7.40 (m, 2H), 7.21 (ddd, *J* = 1.0 Hz, *J* = 2.6 Hz, *J* = 8.2 Hz, 1H), 7.17 (dd, *J* = 0.9 Hz, *J* = 8.5 Hz, 1H), 7.05 (dt, *J* = 1.2 Hz, *J* = 7.6 Hz, 1H), 3.93 (s, 3H), 3.86 (s, 3H); ^{13}C NMR (acetone-d_6): δ 193.6, 160.4, 158.4, 155.8, 155.6, 138.9, 137.9, 131.9, 131.3, 129.9, 128.9, 128.3, 124.5, 122.9, 121.6, 119.7, 116.4, 112.7, 56.0, 55.8; LC/MS *m/z* : 320 $(M+H)^+$.

(3-Hydroxyphenyl)-[6-(2-hydroxyphenyl)-pyridin-2-yl]-methanone (15). The title compound was prepared by reaction of (3-methoxyphenyl)-[6-(2-methoxyphenyl)-pyridin-2-yl]-methanone **15a** (40 mg, 0.13 mmol, 1 equiv) with boron tribromide (1.3 mmol, 10 equiv) according to method E. The product was purified by preparative TLC (hexane/ethyl acetate 1:1). Yield: 30 mg (79%). White oil. $C_{18}H_{13}NO_3$; MW 291; ^{1}H NMR (acetone-d_6): δ 8.42 (d, *J* = 7.9 Hz, 1H), 8.28-8.24 (m, 1H), 8.06 (dd, *J* = 1.5 Hz, *J* = 8.1 Hz, 1H), 7.94 (dd, *J* = 0.7 Hz, *J* = 7.5 Hz, 1H), 7.43-7.41 (m, 3H), 7.35-7.30 (m, 1H), 7.19-7.16 (m, 1H), 6.97-6.93 (m, 1H), 6.88 (dd, *J* = 1.2 Hz, *J* = 8.2 Hz, 1H); GC/MS *m/z*: 291 $(M)^+$.

(3-Methoxyphenyl)-[6-(3-methoxyphenyl)-pyridin-2-yl]-methanone (16a). The title compound was prepared by reaction of (6-bromopyridin-2-yl)-(3-methoxyphenyl)-methanone **14b** (150 mg, 0.51 mmol, 1 equiv) with 3-methoxyphenylboronic acid (94 mg, 0.62 mmol, 1.2 equiv) according to method C. The product was purified by column chromatography (hexane/ethyl acetate 8:2). Yield: 70 mg (43%). $C_{20}H_{17}NO_3$; MW 319; ^{1}H NMR (acetone-d_6): δ 8.19 (dd, *J* = 1.1 Hz, *J* = 7.9 Hz, 1H), 8.12 (t, *J* = 7.9 Hz, 1H), 7.99 (dd, *J* = 0.9 Hz, *J* = 7.6 Hz, 1H), 7.79-7.74 (m, 3H), 7.70 (ddd, *J* = 0.9 Hz, *J* = 1.8 Hz, *J* = 7.9 Hz, 1H), 7.48 (t, *J* = 7.9 Hz, 1H), 7.40 (t, *J* = 8.1 Hz, 1H), 7.24 (ddd, *J* = 0.9 Hz, *J* = 2.6 Hz, *J* = 8.2 Hz, 1H), 7.02 (ddd, *J* = 0.7 Hz, *J* = 2.6 Hz, *J* = 8.7 Hz, 1H), 3.87 (s, 3H), 3.85 (s, 3H); ^{13}C NMR (acetone-

d$_6$): δ 193.4, 161.3, 160.4, 156.2, 155.6, 140.7, 139.3, 138.8, 130.7, 130.0, 124.5, 123.6, 119.9, 119.6, 116.6, 116.2, 113.0, 55.8, 55.6; IR: 2981, 1658, 1579, 1234 cm^{-1}; GC/MS *m/z*: 319 $(M)^+$.

(3-Hydroxyphenyl)-[6-(3-hydroxyphenyl)-pyridin-2-yl]-methanone (16). The title compound was prepared by reaction of (3-methoxyphenyl)-[6-(3-methoxyphenyl)-pyridin-2-yl]-methanone **16a** (50 mg, 0.16 mmol, 1 equiv) with boron tribromide (1.6 mmol, 10 equiv) according to method E. The product was purified by preparative TLC (hexane/ethyl acetate 1:1) to afford 32 mg (69%) of the analytically pure product as a beige powder. $C_{18}H_{13}NO_3$; MW 291; ^{1}H NMR (acetone-*d*$_6$): δ 8.68 (s, br, 1H), 8.50 (s, br, 1H), 8.13 (dd, *J* = 1.3 Hz, *J* = 7.9 Hz, 1H), 8.11-8.07 (m, 1H), 7.90 (dd, *J* = 1.3 Hz, *J* = 7.3 Hz, 1H), 7.66-7.63 (m, 2H), 7.63-7.62 (m, 1H), 7.61 (ddd, *J* = 0.9 Hz, *J* = 1.6 Hz, *J* = 7.6 Hz, 1H), 7.41-7.37 (m, 1H), 7.32 (t, *J* = 8.2 Hz, 1H), 7.14 (ddd, *J* = 0.9 Hz, *J* = 2.5 Hz, *J* = 8.2 Hz, 1H), 6.93 (ddd, *J* = 0.9 Hz, *J* = 2.5 Hz, *J* = 8.2 Hz, 1H); ^{13}C NMR (acetone-*d*$_6$): δ 193.9, 158.8, 156.4, 156.1, 139.1, 138.7, 130.7, 130.1, 123.4, 123.3, 120.9, 119.0, 118.2, 117.4, 114.6; IR: 3282, 1641, 1581, 1325, 1213 cm^{-1}; LC/MS *m/z*: 292 $(M+H)^+$.

[6-(4-Hydroxyphenyl)-pyridin-2-yl]-(3-methoxyphenyl)-methanone (17a). The title compound was prepared by reaction of (6-bromopyridin-2-yl)-(3-methoxyphenyl)-methanone **14b** (150 mg, 0.51 mmol, 1 equiv) with 4-hydroxyphenylboronic acid (84 mg, 0.61 mmol, 1.2 equiv) according to method C. The product was purified by column chromatography (hexane/ethyl acetate 8:2). Yield: 45% (70 mg). $C_{19}H_{15}NO_3$; MW 305; ^{1}H NMR (acetone-*d*$_6$): δ 8.73 (s, br, 1H), 8.09 (dd, *J* = 1.2 Hz, *J* = 8.0 Hz, 1H), 8.06 (d, *J* = 7.5 Hz, 1H), 8.04-8.01 (m, 2H), 7.87 (dd, *J* = 1.5 Hz, *J* = 7.3 Hz, 1H), 7.76-7.72 (m, 2H), 7.47 (t, *J* = 7.8 Hz, 1H), 7.23 (ddd, *J* = 1.0 Hz, *J* = 2.7 Hz, *J* = 8.3 Hz, 1H), 6.97-6.93 (m, 2H), 3.87 (s, 3H); ^{13}C NMR (acetone-*d*$_6$): δ 193.7, 160.4, 159.9, 156.5, 155.7, 139.0, 138.9, 130.7, 130.0, 129.2, 124.5, 122.4, 122.3, 119.8, 116.5, 116.2, 55.8; GC/MS *m/z*: 305 $(M)^+$.

(3-Hydroxyphenyl)-[6-(4-hydroxyphenyl)-pyridin-2-yl]-methanone (17). The title compound was prepared by reaction of [6-(4-hydroxyphenyl)-pyridin-2-yl]-(3-methoxyphenyl)-methanone **17a** (30 mg, 0.10 mmol, 1 equiv) with boron tribromide (0.50 mmol, 5 equiv) according to method E. The product was recrystallised in ethyl acetate to afford 15 mg (51%) of the analytically pure compound. $C_{18}H_{13}NO_3$; MW 291; mp: 177-179°C; ^{1}H NMR (acetone-*d*$_6$ + MeOH): δ 8.21-8.16 (m, 1H), 8.15-8.11 (m, 1H), 7.94 (d, *J* = 8.9 Hz, 2H), 7.90 (s, 1H), 7.52 (d, *J* = 7.9 Hz, 1H), 7.50-7.48 (m, 1H), 7.35 (t, *J* = 8.1 Hz,

1H), 7.11-7.08 (m, 1H), 6.91 (d, *J* = 8.5 Hz, 2H); IR: 3140, 1605, 1453, 1340, 1193 cm^{-1}; LC/MS *m/z*: 292 $(M+H)^+$.

[6-(4-Methoxy-2-methylphenyl)-pyridin-2-yl]-(3-methoxyphenyl)-methanone (18a). The title compound was prepared by reaction of (6-bromopyridin-2-yl)-(3-methoxyphenyl)-methanone **14b** (200 mg, 0.68 mmol, 1 equiv) with 4-methoxy-2-methylphenylboronic acid (136 mg, 0.82 mmol, 1.2 equiv) according to method D. The product was purified by column chromatography (hexane/ethyl acetate 7:3) to afford 100 mg (44%) of the analytically pure compound. $C_{21}H_{19}NO_3$; MW 333; ^{1}H NMR (acetone-d_6): δ 8.08 (t, *J* = 7.9 Hz, 1H), 7.94 (dd, *J* = 0.9 Hz, *J* = 7.6 Hz, 1H), 7.73 (dd, *J* = 0.9 Hz, *J* = 7.9 Hz, 1H), 7.70-7.66 (m, 2H), 7.46-7.43 (m, 1H), 7.43-7.39 (m, 1H), 7.18 (ddd, *J* = 1.3 Hz, *J* = 2.5 Hz, *J* = 8.2 Hz, 1H), 6.87-6.84 (m, 2H), 3.84 (s, 3H), 3.83 (s, 3H), 2.39 (s, 3H); ^{13}C NMR (acetone-d_6): δ 160.4, 159.4, 155.7, 139.0, 138.5, 138.4, 132.1, 129.8, 127.2, 124.3, 122.4, 119.5, 117.2, 117.1, 116.3, 112.2, 112.1, 55.7, 55.5, 14.4; IR: 2959, 1661, 1579, 1450, 1237 cm^{-1}; GC/MS *m/z*: 333 $(M)^+$.

[6-(4-Hydroxy-2-methylphenyl)-pyridin-2-yl]-(3-hydroxyphenyl)-methanone (18). The title compound was prepared by reaction of [6-(4-methoxy-2-methylphenyl)-pyridin-2-yl]-(3-methoxyphenyl)-methanone **18a** (90 mg, 0.27 mmol, 1 equiv) with boron tribromide (2.7 mmol, 10 equiv) according to method E. The product was purified by column chromatography (hexane/ethyl acetate 7:3) to afford 45 mg (55%) of the analytically pure compound as a brown oil. $C_{19}H_{15}NO_3$; MW 305; ^{1}H NMR (acetone-d_6): δ 8.60 (s, br, 1H), 8.43 (s, br, 1H), 8.05-8.00 (m, 1H), 7.85 (dd, *J* = 1.1 Hz, *J* = 7.8 Hz, 1H), 7.69 (dd, *J* = 0.9 Hz, *J* = 7.8 Hz, 1H), 7.55-7.50 (m, 2H), 7.35-7.28 (m, 2H), 7.07 (dd, *J* = 0.9 Hz, *J* = 8.1 Hz, 1H), 6.76-6.69 (m, 2H), 2.32 (s, 3H); ^{13}C NMR (acetone-d_6): δ 158.6, 158.0, 138.3, 132.3, 129.9, 127.0, 123.2, 122.1, 120.7, 118.4, 118.0, 113.8, 21.2; IR: 3255, 2924, 1689, 1583, 1219 cm^{-1}; LC/MS *m/z*: 306 $(M+H)^+$.

[6-(2-Fluoro-4-methoxyphenyl)-pyridin-2-yl]-(3-methoxyphenyl)-methanone (19a). The title compound was prepared by reaction of (6-bromopyridin-2-yl)-(3-methoxyphenyl)-methanone **14b** (170 mg, 0.58 mmol, 1 equiv) with 2-fluoro-4-methoxyphenylboronic acid (119 mg, 0.70 mmol, 1.2 equiv) according to method D. The product was used in the next step without further purification. $C_{20}H_{16}FNO_3$; MW 337.

[6-(2-Fluoro-4-hydroxyphenyl)-pyridin-2-yl]-(3-hydroxyphenyl)-methanone (19). The title compound was prepared by reaction of [6-(2-fluoro-4-methoxyphenyl)-pyridin-2-yl]-(3-

methoxyphenyl)-methanone **19a** (100 mg, 0.3 mmol, 1 equiv) with boron tribromide (3.0 mmol, 10 equiv) according to method E. The product was recrystallised in ethyl acetate to afford 50 mg (54%) of the analytically pure product as a yellow powder. $C_{18}H_{12}FNO_3$; MW 309; mp: 246-248°C; ^{1}H NMR (acetone-d_6): δ 8.27-8.22 (m, 1H), 8.11-8.08 (m, 1H), 7.98-7.95 (m, 1H), 7.84 (t, *J* = 8.2 Hz, 1H), 7.49 (dt, *J* = 1.1 Hz, *J* = 7.8 Hz, 1H), 7.47-7.45 (m, 1H), 7.33 (t, *J* = 7.9 Hz, 1H), 7.11-7.07 (m, 1H), 6.77-6.73 (m, 1H), 6.69-6.64 (m, 1H); ^{13}C NMR (acetone-d_6): δ 158.7, 137.0, 133.3, 130.8, 129.7, 128.9, 125.9, 122.8, 122.3, 119.3, 119.2, 118.4, 118.2, 117.5, 113.7, 108.2, 104.7; IR: 3251, 3027, 1608, 1468, 1235 cm^{-1}; LC/MS *m/z*: 310 $(M+H)^+$.

[6-(3-Fluoro-4-methoxyphenyl)-pyridin-2-yl]-(3-methoxyphenyl)-methanone (20a). The title compound was prepared by reaction of (6-bromopyridin-2-yl)-(3-methoxyphenyl)-methanone **14b** (150 mg, 0.51 mmol, 1 equiv) with 3-fluoro-4-methoxyphenylboronic acid (104 mg, 0.61 mmol, 1.2 equiv) according to method D. The product was recrystallised in ethanol to afford 130 mg (76%) of the analytically pure compound as a white powder. $C_{20}H_{16}FNO_3$; MW 337; mp: 100-102°C; ^{1}H NMR (acetone-d_6): δ 8.17 (dd, *J* = 1.3 Hz, *J* = 8.2 Hz, 1H), 8.10 (t, *J* = 7.6 Hz, 1H), 7.97-7.94 (m, 2H), 7.92 (dd, *J* = 1.3 Hz, *J* = 5.7 Hz, 1H), 7.74-7.70 (m, 2H), 7.50-7.46 (m, 1H), 7.28-7.23 (m, 2H), 3.95 (s, 3H), 3.88 (s, 3H); ^{13}C NMR (acetone-d_6): δ 193.5, 160.5, 155.7, 155.1, 152.4, 149.9, 139.3, 138.8, 132.4, 130.0, 124.4, 123.9, 123.2, 122.8, 119.9, 116.2, 115.1, 114.9, 56.6, 55.8; IR: 2926, 1662, 1581, 1283 cm^{-1}; GC/MS *m/z*: 337 $(M)^+$.

[6-(3-Fluoro-4-hydroxyphenyl)-pyridin-2-yl]-(3-hydroxyphenyl)-methanone (20). The title compound was prepared by reaction of [6-(3-fluoro-4-methoxyphenyl)-pyridin-2-yl]-(3-methoxyphenyl)-methanone **20a** (120 mg, 0.36 mmol, 1 equiv) with pyridinium hydrochloride (36 mmol, 100 equiv) according to method G. The analytically pure product was obtained after recrystallisation with hexane and ethyl acetate as a brown solid. Yield: 103 mg (94%). $C_{18}H_{12}FNO_3$; MW 309; mp: 224-226°C; ^{1}H NMR (acetone-d_6): δ 8.32 (d, *J* = 1.9 Hz, 1H), 7.89-7.87 (m, 1H), 7.75-7.74 (m, 1H), 7.38 (dd, *J* = 2.2 Hz, *J* = 8.2 Hz, 1H), 7.21 (t, *J* = 1.6 Hz, 1H), 7.19 (dd, *J* = 0.6 Hz, *J* = 8.2 Hz, 1H), 7.15 (t, *J* = 1.6 Hz, 1H), 6.80 (dd, *J* = 0.6 Hz, *J* = 1.6 Hz, 1H), 6.60 (dd, *J* = 0.9 Hz, *J* = 1.9 Hz, 1H), 5.35 (d, *J* = 8.2 Hz, 1H); ^{13}C NMR (acetone-d_6): δ 183.5, 164.7, 161.0, 160.4, 156.5, 149.4, 147.7, 143.6, 143.3, 139.0, 132.2, 129.5, 120.9; IR: 3225, 3027, 1608, 1468, 1285 cm^{-1}; LC/MS *m/z*: 310 $(M+H)^+$.

[6-(3-Chloro-4-hydroxyphenyl)-pyridin-2-yl]-(3-methoxyphenyl)-methanone (21a). The title compound was prepared by reaction of (6-bromopyridin-2-yl)-(3-methoxyphenyl)-methanone **14b** (200 mg, 0.68 mmol, 1 equiv) with 3-chloro-4-hydroxyphenylboronic acid (141 mg, 0.82 mmol, 1.2 equiv) according to method D. The product was purified by column chromatography (hexane/ethyl acetate 7:3) to afford 120 mg (50%) of the analytically pure compound as white oil. $C_{19}H_{14}ClNO_3$; MW 339; ^{1}H NMR (acetone-d_6): δ 9.13 (s, br, 1H), 8.18 (d, *J* = 2.2 Hz, 1H), 8.15 (dd, *J* = 0.8 Hz, *J* = 8.0 Hz, 1H), 8.10 (t, *J* = 7.7 Hz, 1H), 7.97 (dd, *J* = 2.1 Hz, *J* = 8.4 Hz, 1H), 7.93 (dd, *J* = 0.9 Hz, *J* = 7.5 Hz, 1H), 7.76-7.74 (m, 1H), 7.73-7.70 (m, 1H), 7.48 (t, *J* = 8.0 Hz, 1H), 7.25 (ddd, *J* = 0.6 Hz, *J* = 2.8 Hz, *J* = 8.2 Hz, 1H), 7.13 (d, *J* = 8.5 Hz, 1H), 3.88 (s, 3H); ^{13}C NMR (acetone-d_6): δ 193.5, 155.7, 155.2, 155.1, 139.3, 132.1, 130.0, 129.3, 128.5, 127.5, 126.9, 124.4, 123.0, 122.7, 120.0, 118.0, 117.9, 116.2, 55.8; GC/MS *m/z*: 339-341 $(M)^+$.

[6-(3-Chloro-4-hydroxyphenyl)-pyridin-2-yl]-(3-hydroxyphenyl)-methanone (21). The title compound was prepared by reaction of [6-(3-chloro-4-hydroxyphenyl)-pyridin-2-yl]-(3-methoxyphenyl)-methanone **21a** (200 mg, 0.59 mmol, 1 equiv) with pyridinium hydrochloride (59 mmol, 100 equiv) according to method G. The product was purified by recrystallisation in ethanol and ethyl acetate to give 82 mg (43%) of the analytically pure compound as a green powder. $C_{18}H_{12}ClNO_3$; MW 325; mp: 206-208°C; ^{1}H NMR (acetone-d_6): δ 9.10 (s, br, 1H), 8.76 (s, br, 1H), 8.17 (d, *J* = 2.2 Hz, 1H), 8.13 (dd, *J* = 0.9 Hz, *J* = 7.9 Hz, 1H), 8.10-8.06 (m, 1H), 7.97 (dd, *J* = 2.2 Hz, *J* = 8.5 Hz, 1H), 7.88 (dd, *J* = 0.9 Hz, *J* = 7.6 Hz, 1H), 7.64-7.60 (m, 2H), 7.42-7.38 (m, 1H), 7.15 (ddd, *J* = 1.3 Hz, *J* = 2.2 Hz, *J* = 7.9 Hz, 1H), 7.13 (d, *J* = 8.5 Hz, 1H); ^{13}C NMR (acetone-d_6): δ 193.8, 158.1, 156.0, 155.2, 155.1, 139.2, 132.2, 130.1, 129.4, 127.5, 123.3, 122.9, 122.5, 121.8, 120.9, 118.2, 117.8; IR: 3345, 1639, 1580, 1324, 1290 cm^{-1}; LC/MS *m/z*: 326 $(M+H)^+$.

(6-Bromopyridin-2-yl)-(4-fluoro-3-methoxyphenyl)-methanol (22c). To a solution of 5-bromo-2-fluoroanisole (200 mg, 0.98 mmol, 1 equiv) in anhydrous THF was added granules of magnesium (24 mg, 0.98 mmol, 1 equiv) under nitrogen. The mixture was heated to 60°C for 2h. After reaching room temperature, 6-bromopyridin-2-yl carboxaldehyde (182 mg, 0.98 mmol, 1 equiv) was added and the reaction mixture was heated to 80°C and stirred overnight at 80°C. The reaction was cooled to room temperature, quenched with brine and the aqueous layer was extracted with ethyl acetate. The combined organic layers were dried over sodium sulfate, filtered and concentrated to dryness under vacuum. The product was used in the next step without further purification. $C_{13}H_{11}BrFNO_2$; MW 312.

(6-Bromopyridin-2-yl)-(4-fluoro-3-methoxyphenyl)-methanone (22b). The title compound was prepared by reaction of (6-bromopyridin-2-yl)-(4-fluoro-3-methoxyphenyl)-methanol **22c** (150 mg, 0.48 mmol, 1 equiv) with 2-iodoxybenzoic acid (269 mg, 0.96 mmol, 2 equiv) according to method B. The product was purified by column chromatography (hexane/ethyl acetate 7:3) to afford 130 mg (87%) of the analytically pure compound as orange oil. $C_{13}H_9BrFNO_2$; MW 310; ^{1}H NMR (acetone-d_6): δ 8.05 (dd, *J* = 1.3 Hz, *J* = 7.6 Hz, 1H), 8.02 (t, *J* = 7.6 Hz, 1H), 7.94 (dd, *J* = 2.2 Hz, *J* = 8.5 Hz, 1H), 7.89 (dd, *J* = 1.3 Hz, *J* = 7.6 Hz, 1H), 7.74 (ddd, *J* = 2.2 Hz, *J* = 4.4 Hz, *J* = 8.5 Hz, 1H), 7.33-7.28 (m, 1H), 3.98 (s, 3H); ^{13}C NMR (acetone-d_6): δ 190.3, 157.4, 156.5, 148.5, 141.3, 141.0, 133.4, 132.0, 126.0, 124.5, 116.7, 108.3, 56.6; IR: 3058, 1686, 1585, 1514, 1421, 1293 cm^{-1}; LC/MS *m/z*: 310-312 $(M+H)^+$.

(4-Fluoro-3-methoxyphenyl)-[6-(4-methoxy-3-methylphenyl)-pyridin-2-yl]-methanone (22a). The title compound was prepared by reaction of (6-bromopyridin-2-yl)-(4-fluoro-3-methoxyphenyl)-methanone **22b** (100 mg, 0.32 mmol, 1 equiv) with 4-methoxy-3-methylphenylboronic acid (64 mg, 0.39 mmol, 1.2 equiv) according to method D. The product was purified by column chromatography (hexane/ethyl acetate 7:3) to give 70 mg (62%) of the analytically pure compound. $C_{21}H_{18}FNO_3$; MW 351; ^{1}H NMR (acetone-d_6): δ 8.13 (dd, *J* = 1.1 Hz, *J* = 7.8 Hz, 1H), 8.09-8.04 (m, 2H), 7.99 (dd, *J* = 2.3 Hz, *J* = 8.6 Hz, 1H), 7.97 (d, *J* = 1.7 Hz, 1H), 7.91 (dd, *J* = 1.0 Hz, *J* = 7.6 Hz, 1H), 7.88-7.84 (m, 1H), 7.35-7.30 (m, 1H), 7.05 (d, *J* = 8.6 Hz, 1H), 3.97 (s, 3H), 3.90 (s, 3H), 2.25 (s, 3H); ^{13}C NMR (acetone-d_6): δ 190.1, 160.1, 156.5, 155.5, 139.1, 129.8, 127.4, 126.7, 126.1, 122.7, 122.6, 117.0, 116.4, 116.2, 111.1, 56.6, 55.9, 16.5; LC/MS *m/z*: 352 $(M+H)^+$.

(4-Fluoro-3-hydroxyphenyl)-[6-(4-hydroxy-3-methylphenyl)-pyridin-2-yl]-methanone (22). The title compound was prepared by reaction of (4-fluoro-3-methoxyphenyl)-[6-(4-methoxy-3-methylphenyl)-pyridin-2-yl]-methanone **22a** (30 mg, 0.09 mmol, 1 equiv) with pyridinium hydrochloride (9 mmol, 100 equiv) according to method G. The analytically pure product was obtained after purification by column chromatography (hexane/ethyl acetate 7:3) and preparative HPLC using isopropanol/water as eluent in 66% yield (19 mg). $C_{19}H_{14}FNO_3$; MW 323; ^{1}H NMR (acetone-d_6): δ 9.01 (s, br, 1H), 8.57 (s, br, 1H), 8.07 (dd, *J* = 1.3 Hz, *J* = 8.2 Hz, 1H), 8.05-8.01 (m, 1H), 7.93 (dd, *J* = 0.6 Hz, *J* = 2.2 Hz, 1H), 7.90 (dd, *J* = 2.2 Hz, *J* = 8.8 Hz, 1H), 7.85-7.82 (m, 2H), 7.75 (ddd, *J* = 2.2 Hz, *J* = 4.7 Hz, *J* = 8.5 Hz, 1H), 7.32-7.27 (m, 1H), 6.93 (d, *J* = 8.2 Hz, 1H), 2.27 (s, 3H); ^{13}C NMR (acetone-d_6): δ 193.2, 163.8,

158.0, 157.9, 156.8, 155.6, 139.0, 130.5, 126.6, 124.9, 122.4, 122.3, 122.2, 118.8, 116.6, 116.5, 115.9, 16.4; IR: 3332, 1656, 1583, 1181 cm^{-1}; LC/MS *m/z*: 324 $(M+H)^+$.

(6-Bromopyridin-2-yl)-(3-methoxy-4-methylphenyl)-methanol (23c). To a solution of 5-bromo-2-methylanisole (200 mg, 0.99 mmol, 1 equiv) in anhydrous THF was added granules of magnesium (24 mg, 0.99 mmol, 1 equiv) under nitrogen. The mixture was heated to 60°C for 2h. After cooling down to room temperature, 6-bromopyridin-2-yl carboxaldehyde (182 mg, 0.99 mmol, 1 equiv) was added and the reaction mixture was heated to 80°C and stirred overnight at 80°C. The reaction was cooled to room temperature, quenched with brine and the aqueous layer was extracted with ethyl acetate. The combined organic layers were dried over sodium sulfate, filtered and concentrated to dryness under vacuum. The product was purified by column chromatography (hexane/ethyl acetate 8:2) to afford the analytically pure compound in 76% yield (232 mg) as a yellow oil. $C_{14}H_{14}BrNO_2$; MW 308; ^{1}H NMR (acetone-d_6): δ 7.71 (t, *J* = 7.8 Hz, 1H), 7.60 (d, *J* = 7.7 Hz, 1H), 7.44 (d, *J* = 7.9 Hz, 1H), 7.07-7.04 (m, 2H), 6.89 (dd, *J* = 1.3 Hz, *J* = 7.5 Hz, 1H), 5.75 (d, *J* = 4.5 Hz, 1H), 5.11 (d, *J* = 4.5 Hz, 1H), 3.80 (s, 3H), 2.12 (s, 3H); ^{13}C NMR (acetone-d_6): δ 166.6, 158.6, 143.4, 141.3, 140.6, 131.0, 127.2, 126.0, 120.3, 119.2, 109.3, 76.5, 55.6, 16.0; IR: 3396, 2923, 1581, 1555, 1252 cm^{-1}; GC/MS *m/z*: 307-309 $(M)^+$.

(6-Bromopyridin-2-yl)-(3-methoxy-4-methylphenyl)-methanone (23b). The title compound was prepared by reaction of (6-bromopyridin-2-yl)-(3-methoxy-4-methylphenyl)-methanol **23c** (60 mg, 0.19 mmol, 1 equiv) with 2-iodoxybenzoic acid (106 mg, 0.38 mmol, 2 equiv) according to method B. The product was purified by column chromatography (hexane/ethyl acetate 8:2) to afford the analytically pure compound in 98% yield (57 mg) as a yellow powder. $C_{14}H_{12}BrNO_2$; MW 306; ^{1}H NMR (acetone-d_6): δ 8.03-8.01 (m, 1H), 8.00 (d, *J* = 1.4 Hz, 1H), 7.89-7.85 (m, 1H), 7.69 (d, *J* = 1.4 Hz, 1H), 7.58 (dd, *J* = 1.5 Hz, *J* = 7.7 Hz, 1H), 7.30 (dd, *J* = 0.9 Hz, *J* = 7.7 Hz, 1H), 3.92 (s, 3H), 2.28 (s, 3H); ^{13}C NMR (acetone-d_6): δ 191.5, 158.5, 157.1, 141.2, 141.1, 135.7, 133.6, 131.6, 131.0, 124.8, 124.3, 112.4, 55.8, 16.6; IR: 2929, 1651, 1553, 1302, 1247 cm^{-1}; LC/MS *m/z*: 306-308 $(M+H)^+$.

(3-Methoxy-4-methylphenyl)-[6-(4-methoxy-3-methylphenyl)-pyridin-2-yl]-methanone (23a). The title compound was prepared by reaction of (6-bromopyridin-2-yl)-(3-methoxy-4-methylphenyl)-methanone **23b** (50 mg, 0.16 mmol, 1 equiv) with 4-methoxy-3-methylphenylboronic acid (31 mg, 0.19 mmol, 1.2 equiv) according to method D. The product was purified by column chromatography (hexane/ethyl acetate 7:3) to afford the analytically

pure compound in 68% yield (38 mg) as an orange powder. $C_{22}H_{21}NO_3$; MW 347; ^{1}H NMR (acetone-d_6): δ 8.11 (dd, *J* = 0.9 Hz, *J* = 8.1 Hz, 1H), 8.05 (t, *J* = 7.5 Hz, 1H), 8.01-7.97 (m, 2H), 7.86 (dd, *J* = 0.9 Hz, *J* = 7.5 Hz, 1H), 7.82 (d, *J* = 1.6 Hz, 1H), 7.71 (dd, *J* = 1.4 Hz, *J* = 7.7 Hz, 1H), 7.32 (dd, *J* = 0.6 Hz, *J* = 7.7 Hz, 1H), 7.04 (d, *J* = 8.5 Hz, 1H), 3.91 (s, 3H), 3.90 (s, 3H), 2.29 (s, 3H), 2.24 (s, 3H); ^{13}C NMR (acetone-d_6): δ 193.2, 160.1, 158.4, 156.4, 156.1, 139.0, 133.0, 131.4, 130.8, 129.8, 127.3, 125.0, 122.5, 122.4, 113.3, 112.6, 112.1, 111.1, 55.9, 55.8, 16.6, 16.5; IR: 2916, 1651, 1566, 1233 cm^{-1}; LC/MS *m/z*: 348 $(M+H)^+$.

(3-Hydroxy-4-methylphenyl)-[6-(4-hydroxy-3-methylphenyl)-pyridin-2-yl]-methanone (23). The title compound was prepared by reaction of (3-methoxy-4-methylphenyl)-[6-(4-methoxy-3-methylphenyl)-pyridin-2-yl]-methanone **23a** (30 mg, 0.09 mmol, 1 equiv) with borontrifluoride dimethylsulfide complex (6.3 mmol, 70 equiv) according to method F. The product was purified by column chromatography (hexane/ethyl acetate 7:3) then by preparative HPLC with isopropanol/water as eluent to afford 10 mg (35%) of the analytically pure compound as a white powder. $C_{20}H_{17}NO_3$; MW 319; ^{1}H NMR (acetone-d_6): δ 8.60 (s, br, 2H), 8.05 (dd, *J* = 1.4 Hz, *J* = 8.0 Hz, 1H), 8.01 (t, *J* = 8.3 Hz, 1H), 7.94-7.92 (m, 1H), 7.84 (dd, *J* = 2.1 Hz, *J* = 8.4 Hz, 1H), 7.77 (dd, *J* = 1.3 Hz, *J* = 7.3 Hz, 1H), 7.66 (d, *J* = 1.6 Hz, 1H), 7.61 (dd, *J* = 1.8 Hz, *J* = 7.8 Hz, 1H), 7.27 (d, *J* = 7.8 Hz, 1H), 6.93 (d, *J* = 8.3 Hz, 1H), 2.31 (s, 3H), 2.26 (s, 3H); ^{13}C NMR (acetone-d_6): δ 190.9, 174.6, 161.0, 138.8, 131.2, 130.4, 126.6, 123.7, 122.0, 121.9, 118.8, 16.5, 16.4; IR: 3340, 1671, 1585 cm^{-1}; LC/MS *m/z*: 320 $(M+H)^+$.

(6-Bromopyridin-2-yl)-(5-fluoro-3-methoxyphenyl)-methanol (24c). To a solution of 3-bromo-5-fluoroanisole (250 mg, 1.22 mmol, 1 equiv) in anhydrous THF was added granules of magnesium (32 mg, 1.28 mmol, 1.05 equiv) under nitrogen. The mixture was heated to 60°C for 2h. After cooling down to room temperature, 6-bromopyridin-2-yl carboxaldehyde (271 mg, 1.47 mmol, 1.2 equiv) was added and the reaction mixture was stirred at 80°C overnight. The reaction was cooled to room temperature, quenched with brine and the aqueous layer was extracted with ethyl acetate. The combined organic layers were dried over sodium sulfate, filtered and concentrated to dryness under vacuum. The product was purified by column chromatography (hexane/ethyl acetate 8:2) to afford the analytically pure compound in 21% yield (80 mg). $C_{13}H_{11}BrFNO_2$; MW 312; ^{1}H NMR (acetone-d_6): δ 7.74 (t, *J* = 7.6 Hz, 1H), 7.64-7.61 (m, 1H), 7.47 (dd, *J* = 0.9 Hz, *J* = 7.2 Hz, 1H), 6.92-6.90 (m, 1H), 6.81 (ddd, *J* = 0.6 Hz, *J* = 1.3 Hz, *J* = 9.1 Hz, 1H), 6.59 (dt, *J* = 2.5 Hz, *J* = 10.7 Hz, 1H), 5.77 (d, *J* = 4.7 Hz, 1H), 5.35 (d, *J* = 4.7 Hz, 1H), 3.79 (s, 3H); ^{13}C NMR (acetone-d_6): δ 165.8,

165.3, 140.8, 127.5, 120.3, 109.4, 106.1, 105.9, 101.0, 100.8, 75.8, 56.0; LC/MS *m/z*: 312-314 $(M+H)^+$.

(6-Bromopyridin-2-yl)-(5-fluoro-3-methoxyphenyl)-methanone (24b). The title compound was prepared by reaction of (6-bromopyridin-2-yl)-(5-fluoro-3-methoxyphenyl)-methanol **24c** (190 mg, 0.61 mmol, 1 equiv) with 2-iodoxybenzoic acid (342 mg, 1.22 mmol, 2 equiv) according to method B. The product was purified by column chromatography (hexane/ethyl acetate 17:3) to afford 150 mg (79%) of the analytically pure compound as a brown solid. $C_{13}H_9BrFNO_2$; MW 310; ^{1}H NMR (acetone-d_6): δ 8.08 (dd, *J* = 0.9 Hz, *J* = 7.6 Hz, 1H), 8.03 (t, *J* = 7.6 Hz, 1H), 7.91 (dd, *J* = 0.9 Hz, *J* = 7.6 Hz, 1H), 7.53-7.51 (m, 1H), 7.41 (ddd, *J* = 1.3 Hz, *J* = 2.5 Hz, *J* = 9.1 Hz, 1H), 7.05 (dt, *J* = 2.2 Hz, *J* = 10.4 Hz, 1H), 3.91 (s, 3H); ^{13}C NMR (acetone-d_6): δ 190.6, 164.9, 161.9, 161.8, 141.4, 132.2, 124.6, 110.2, 107.0, 56.4; IR: 3080, 2980, 1668, 1555, 1152 cm^{-1}; LC/MS *m/z*: 310-312 $(M+H)^+$.

(5-Fluoro-3-methoxyphenyl)-[6-(4-methoxy-3-methylphenyl)-pyridin-2-yl]-methanone (24a). The title compound was prepared by reaction of (6-bromopyridin-2-yl)-(5-fluoro-3-methoxyphenyl)-methanone **24b** (80 mg, 0.26 mmol, 1 equiv) with 4-methoxy-3-methylphenylboronic acid (52 mg, 0.31 mmol, 1.2 equiv) according to method D. The analytically pure product was obtained after purification by column chromatography (gradient hexane/ethyl acetate 1:0 to 8:2) in 81% yield (74 mg). $C_{21}H_{18}FNO_3$; MW 351; ^{1}H NMR (acetone-d_6): δ 8.13 (dd, *J* = 1.3 Hz, *J* = 8.2 Hz, 1H), 8.07 (t, *J* = 7.9 Hz, 1H), 7.99-7.96 (m, 2H), 7.93 (dd, *J* = 0.9 Hz, *J* = 7.6 Hz, 1H), 7.66-7.64 (m, 1H), 7.54 (ddd, *J* = 1.3 Hz, *J* = 2.5 Hz, *J* = 9.5 Hz, 1H), 7.06-7.05 (m, 1H), 7.04-7.02 (m, 1H), 3.91 (s, 3H), 3.90 (s, 3H), 2.25 (s, 3H); ^{13}C NMR (acetone-d_6): δ 192.1, 161.8, 161.7, 160.2, 154.9, 139.2, 131.2, 129.9, 127.4, 126.7, 123.0, 122.7, 113.5, 111.1, 110.7, 110.5, 106.7, 106.5, 56.4, 55.9, 16.5; LC/MS *m/z*: 352 $(M+H)^+$.

(5-Fluoro-3-hydroxyphenyl)-[6-(4-hydroxy-3-methylphenyl)-pyridin-2-yl]-methanone (24). The title compound was prepared by reaction of (5-fluoro-3-methoxyphenyl)-[6-(4-methoxy-3-methylphenyl)-pyridin-2-yl]-methanone **24a** (39 mg, 0.11 mmol, 1 equiv) with pyridinium hydrochloride (11 mmol, 100 equiv) according to method G. The product was purified by column chromatography (hexane/ethyl acetate 6:4) to give 10 mg (28%) of the analytically pure compound as a yellow solid. $C_{19}H_{14}FNO_3$; MW 323; ^{1}H NMR (acetone-d_6): δ 9.14 (s, br, 1H), 8.58 (s, br, 1H), 7.86 (dd, *J* = 1.3 Hz, *J* = 8.2 Hz, 1H), 7.84-7.80 (m, 1H), 7.72-7.70 (m, 1H), 7.65 (dd, *J* = 0.9 Hz, *J* = 7.3 Hz, 1H), 7.63-7.59 (m, 1H), 7.32-7.30 (m,

1H), 7.22 (ddd, *J* = 1.3 Hz, *J* = 2.2 Hz, *J* = 9.5 Hz, 1H), 6.71 (d, *J* = 8.5 Hz, 1H), 6.68 (dt, *J* = 2.2 Hz, *J* = 10.4 Hz, 1H), 2.27 (s, 3H); ^{13}C NMR (acetone-d_6): δ 192.4, 164.9, 159.6, 159.5, 155.1, 140.1, 140.0, 139.0, 130.6, 130.5, 126.6, 122.7, 122.3, 115.9, 115.1, 109.6, 109.4, 107.8, 16.4; IR: 3399, 2921, 1613, 1584, 1146 cm^{-1}; LC/MS *m/z*: 324 $(M+H)^+$.

Biological Assays

[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-HSD2 and 17β-HSD1 were obtained from human placenta according to previously described procedures [46, 48, 49]. Fresh human placenta was homogenised and centrifuged. The pellet fraction contains the microsomal 17β-HSD2, while 17β-HSD1 was obtained after precipitation with ammonium sulfate from the cytosolic fraction.

1. Inhibition of 17β-HSD2

Inhibitory activities were evaluated by a well established method with minor modifications [44, 46, 48, 50]. Briefly, the microsomal enzyme was incubated with NAD^+ [1500 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. 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]-E2 (final concentration: 500 nM, 0.11 μCi). After 20 min at 37°C, the incubation was stopped with $HgCl_2$ and the mixture was extracted with ether. After evaporation, the steroids were dissolved in acetonitrile/water (45:55). E1 and E2 were separated using acetonitrile/water (45:55) as mobile phase in a C18 rp 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 according to following equation: $\%conversion = \frac{\%(E1)}{\%(E1)+\%(E2)} \times 100$. Each value was calculated from at least three independent experiments.

2. Inhibition of 17β-HSD1

The 17β-HSD1 inhibition assay was performed similarly to the 17β-HSD2 procedure. The cytosolic fraction was incubated with NADH [500 μM], test compound and a mixture of unlabelled- and [2,4,6,7-^{3}H]-E1 (final concentration: 500 nM, 0.15 μCi) for 10 min. Further treatment of the samples and HPLC separation was carried out as mentioned above.

3. ER affinity

The binding affinity of selected compounds to the ERα and ERβ was determined according to Zimmermann *et al. [51]* using recombinant human proteins. Briefly, 0.25 pmoles of ERα or ERβ, respectively, were incubated with [^{3}H]-E2 (10 nM) and test compound for 1h at room temperature. The potential inhibitors were dissolved in DMSO (5% final concentration). Evaluation of 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 formed complex 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). From these results the percentage of [^{3}H]-E2 displacement by the compounds was calculated. The plot of % displacement versus compound concentration resulted in sigmoidal binding curves. The compound concentration to displace 50% of the receptor bound [^{3}H]-E2 were determined. Unlabelled E2 was used as a reference. For determination of the relative binding affinity the ratio was calculated according to the following equation: $RBA[\%] = \frac{IC_{50}(E2)}{IC_{50}(compound)} \times 100$ [52]. This results in an RBA value of 100% for E2. After the assay was established and validated a modification was made to increase throughput. Compounds were tested at concentrations of 1000 × IC_{50}(E2). Compounds with less than 50% displacement of [^{3}H]-E2 at a concentration of 1000 × IC_{50}(E2) were classified as RBA <0.1%.

Acknowledgement

We are grateful to the Deutsche Forschungsgemeinschaft for financial support (HA1315/12-1). We thank Victoria Krämer, Jeannine Jung, Laura Schrobildgen and Jörg Haupenthal for their help in performing the enzyme inhibition tests (17β-HSD2, 17β-HSD1) and the ER tests.

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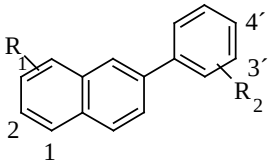
4. SUMMARY AND CONCLUSION

The aim of the present work was to develop novel highly potent and selective 17β-HSD2 inhibitors for the treatment of osteoporosis. Ligand based design has been used to find new molecules with high 17β-HSD2 inhibitory activity and selectivity towards 17β-HSD1, catalysing the reverse reaction. Compounds should also be active in an animal model to evaluate their ability to inhibit osteoclastogenesis and to induce bone formation, and therefore to prove our concept that inhibition of 17β-HSD2 could be a promising approach to treat osteoporosis. So the most interesting compounds have to be investigated for their *in vitro* activity on different species enzymes.

In Chapter **3.I**, the synthesis and the biological evaluation of fourty four compounds derived from hydroxynaphthyl, 4-hydroxymethylphenyl and 4-hydroxyphenyl have been described in order to find an interesting scaffold for the development of new 17β-HSD2 inhibitors. The distance and the substitution pattern between both hydroxy groups of the naphthalene derivatives have been studied and seemed to be very important. Thus compound **I.B**[§], bearing OH groups in position 2 on the naphthalene core and in 3´ on the phenyl moiety is a highly active 17β-HSD1 inhibitor, while when the OH groups are switched to position 1 on the naphthalene and 4´on the phenyl (**I-8**) the activity is reversed in favour of 17β-HSD2 (Table 5). Thus, either 17β-HSD1 or 17β-HSD2 inhibitors can be obtained by shifting the position of the hydroxy groups, in spite of the fact that the active sites of 17β-HSD1 and 17β-HSD2 are considered to be very similar (the substrate of one enzyme is the product of the catalytic reaction of the other enzyme and *vice versa*).

[§] For a sake of clarity, all compounds that are referred to in chapter 4 are characterised by a roman numeral I-III to identify the paper in which they are published, and an Arabic numeral or a letter which is identical to the one of the publication (*e.g.* **I.B** is compound B from Paper I)

Table 5: Inhibition of human 17β-HSD2 and 17β-HSD1 by naphthalene derivatives



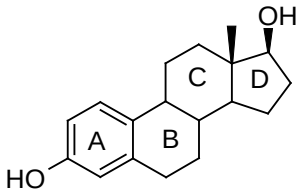
I.B, I.7, I.8, I.9, I.11

Compd	R_1	R_2	Inhibition of 17β-HSD2[a] [%] at 1µM	Inhibition of 17β-HSD1[b] [%] at 1µM
I.B	2-OH	3´-OH	20	100
I.7	1-OH	4´-OCH_3	20	28
I.8	1-OH	4´-OH	74	20
I.11	1-OH	4´-NO_2	13	15

[a] Human placenta, microsomal fraction, substrate [^{3}H]-E2 + E2 [500 nM], NAD^+ [1.5 mM], mean value of 3 determinations, relative standard deviation < 10%, [b] Human placenta, cytosolic fraction, substrate [^{3}H]-E1 + E1 [500 nM], NADPH [0.5 mM], mean value of 3 determinations, relative standard deviation < 10%.

A good superimposition of these two molecules (**I.B** and **I.8**) using their hydroxy groups is obtained when the OH phenyl group of **I.B** overlaps the OH group of the naphthalene of **I.8**. As it has been postulated by Frotscher *et al*.[67] that the OH of the phenyl moiety of compound **I.B** replaces the steroidal OH group of the A-ring, it is expected that the OH-phenyl of **I.8** mimicks the D ring of the steroid (Figure 13).

Figure 13: Structure of E2

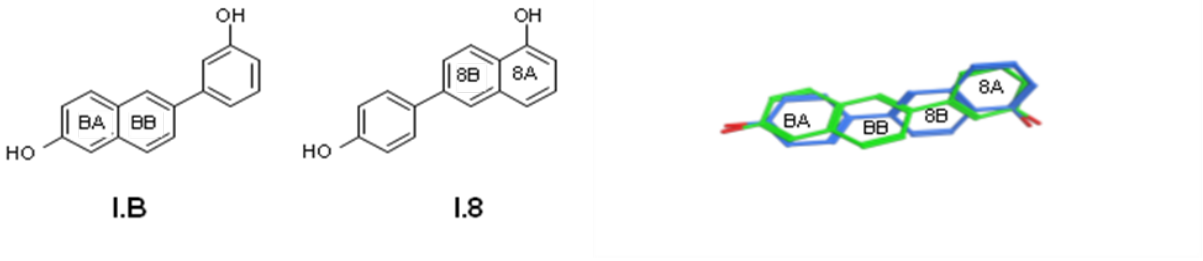


E2

Furthermore, as the only difference between **I.B** (17β-HSD1 inhibitor) and **I.8** (17β-HSD2 inhibitor) are the BB and 8B rings, a hindrance can be expected between the BB ring and at least one amino acid of the active site of 17β-HSD2 (Figure 14). This amino acid should be therefore responsible for the selectivity observed for **I.8** in favour of 17β-HSD2 (IC_{50} = 302 nM, selectivity factor (SF) = 8). It is difficult to discuss more precisely the

interactions achieved by the compounds with the enzyme, because the 3D-structure of 17β-HSD2 has not been elucidated until now.

Figure 14: Superimposition of compounds **I.B** and **I.8**



Compound **I.8** is an interesting scaffold for further development of 17β-HSD2 inhibitors. The hydroxy group which is linked to the phenyl was exchanged by some substituents to decrease the numbers of hydroxy groups of **I.8**, highly susceptible for phase II metabolism[69]. But neither electron withdrawing groups like nitro (**I.11**) nor electron donating group like OCH_3 (**I.7**) can improve activity of compound **I.8**. A series of sulfonamides and amides were also introduced (**I.19-I.25**) on the phenyl ring. Sulfonamide **I.20** and amide **I.24** show similar activity as **I.8** (75 % and 64 % inhibition, respectively to be compared to compound **I.8** with 74 % 17β-HSD2 inhibition at 1 µM). There is evidence that a H-bond donor group directly bound to the phenyl core is highly necessary for high 17β-HSD2 inhibition. Thus, the NH-groups of the sulfonamide **I.20** and the amide **I.24** are able to replace the OH group of **I.8** (Table 6) and to make similar interactions with amino acid residues in the active site.

Table 6: Influence of hydroxyphenyl and hydroxymethylphenyl moieties on human 17β-HSD2 and 17β-HSD1 inhibitory activities

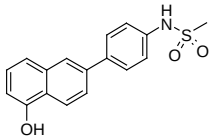
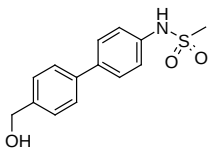
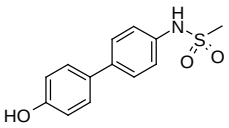
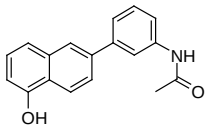
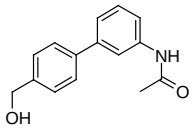
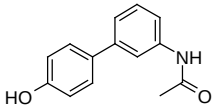
Compd	Structure	Inhibition of 17β-HSD2[a] [%] at 1µM	Inhibition of 17β-HSD1[b] [%] at 1µM
I.20		75	52
I.33		n.i.	n.i.
I.43		n.i.	n.i.

Table 6 continued

Compd	Structure	Inhibition of 17β-HSD2[a] [%] at 1µM	Inhibition of 17β-HSD1[b] [%] at 1µM
I.24		64	12
I.26		n.i.	n.i.
I.35		n.i.	n.i.

[a] Human placenta, microsomal fraction, substrate [^{3}H]-E2 + E2 [500 nM], NAD^+ [1.5 mM], mean value of 3 determinations, relative standard deviation < 10%, [b] Human placenta, cytosolic fraction, substrate [^{3}H]-E1 + E1 [500 nM], NADPH [0.5 mM], mean value of 3 determinations, relative standard deviation < 10%; n.i.: no inhibition.

To simplify the lipophilic scaffold, the naphthalene moiety was replaced either by 4-hydroxymethylphenyl or by 4-hydroxyphenyl moieties. Unfortunately, exchange of the naphthalene core was detrimental for the activity. No inhibition was observed for the sulfonamides **I.33** and **I.43** substituted with 4-hydroxymethylphenyl and 4-hydroxyphenyl core, respectively, while the naphthalene derivative **I.20** shows 74 % 17β-HSD2 inhibition at 1 µM (Table 6). The same observation is made with the amides **I.26** and **I.35**, which are devoid of activity, whereas their naphthalene analogue **I.24** exhibited 64 % inhibition. It is therefore postulated that ring 8A of the naphthalene derivatives **I.20** and **I.24** is very important for the 17β-HSD2 activity, and should stabilise the molecule in the active site by π-π or Van der Waals interactions.

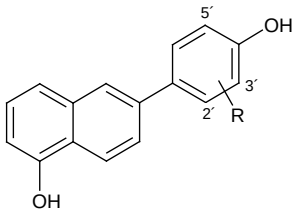
In addition, the biological results obtained for the methylsulfonamide **I.20** and the acetamide **I.24**, showing no loss of activity compared to compound **I.8** confirm the hypothesis postulated above regarding the position of compound **I.8** in the active site: the OH-phenyl of **I.8** would mimic the steroidal D-ring, close to the catalytic triad where space is available for large substituents. This hypothesis is further investigated in the following chapter by introduction of substituents on the phenyl moiety next to the hydroxy group of **I.8** (Chapter **3.II**).

In summary, it has been shown in this study that shifting the position of the hydroxy groups from 2 to 1 on the naphthalene and from 3´ to 4´ on the phenyl delivers either selective 17β-HSD1 or 17β-HSD2 inhibitors. The positive result of exchanging the hydroxy on the

phenyl moiety by H-bond donator groups points out the implication of this substituent in interactions with amino acid residues of the enzyme. In addition, the A ring of the naphthalene seems also be implicated in interactions with amino acids of the protein, stabilising the molecule in the active site.

Novel highly potent and selective 17β-HSD2 inhibitors derived from the naphthalene scaffold have been identified in chapter **3.II**. Most of the hydroxylated derivatives reported in chapter **3.II** turned out to be as active as the unsubstituted parent compound **I.8** (= **II.A**) or even more active with IC_{50} values in the low nanomolar range (19 nM - 307 nM). The hypothesis previously reported about the binding mode of the naphthalene compounds is verified here thanks to compound **II.9**, substituted with a phenyl ring in 3´-position of the 4-hydroxyphenyl moiety. There is space available around the phenyl ring but the additional phenyl is not able to make specific interactions with amino acid residues of the enzyme (similar activity for scaffold **II.A** and compound **II.9**, Table 7).

Table 7: Inhibition of human 17β-HSD2 and 17β-HSD1 by selected naphthalene derivatives



Compd	R	Cell free assay			ERα RBA[d] (%)	ERβ RBA[d] (%)	LogP
		17β-HSD2 IC_{50}[a] [nM]	17β-HSD1 IC_{50}[b] [nM]	Selectivity factor[c]			
II.A=I.8	H	302	2425	8	5	5	3.93
II.3	3´-CH_3	275	1748	6	< 0.1	0.1-1.0	4.41
II.9	3´-Ph	261	919	4	< 0.1	< 0.1	5.60
II.11	2´-F	132	2245	17	< 0.1	< 0.1	4.09
II.15	2´,5´-diF	238	309	1	0.1-1.0	0.1-1.0	4.24
II.17	2´-CF_3	156	926	6	1.0-10	1.0-10	4.85
II.19	3´-CF_3	19	611	32	< 0.1	0.1-1.0	4.85
II.20	3´-Cl	307	1130	4	0.1-1.0	0.1-1.0	4.49
II.21	3´-CN	45%[e]	n.i.	n.d.	n.d.	n.d.	3.95

[a] Human placenta, microsomal fraction, substrate [^{3}H]-E2 + E2 [500 nM], cofactor NAD^+ [1500 µM], mean value of three determinations, relative standard deviation < 10%, [b] Human placenta, cytosolic fraction, substrate [^{3}H]-E1 + E1 [500 nM], cofactor NADH [500 µM], mean value of three determinations, relative standard deviation < 20%, [c] IC_{50} (17β-HSD1)/ IC_{50} (17β-HSD2), [d] RBA: Relative Binding Affinity, E2: 100%, [e] % inhibition at 1µM, n.i.: no inhibition, n.d.: not determined.

In order to elucidate the role of the substituents for the 17β-HSD2 inhibitory activity, groups with different properties (electronic, lipophilic) have been introduced on the phenyl moiety. Thus, electron donating groups (EDG) and hydrophilic groups are certainly not able to make specific interactions, as no real increase in activity is observed for these compounds compared to the scaffold **II.A**. On the other hand most of the compounds bearing electron withdrawing groups (EWG) displayed higher potency than the parent compound **II.A**. Especially, the fluorine seems to be responsible for the high activity as shown by compounds **II.11**, **II.17** and **II.19** (IC_{50} = 132 nM, 156 nM and 19 nM, respectively). The CF_3 substituent of the most active and selective compound (**II.19**) was exchanged by bioisosteric Cl (**II.20**) or CN group (**II.21**) in the 3´-position, in order to modify properties hoping for example to increase selectivity and solubility. Unfortunately, none of the compounds **II.20** and **II.21** displayed better activity than the CF_3 compound **II.19** (logP = 4.85). The high hydrophilic properties of the nitrile group can explain the moderate activity observed by compound **II.21** (45% 17β-HSD2 inhibition at 1 μM, logP = 3.95), but also its linear conformation, which probably does not permit the molecule to establish interactions with amino acid residues of the active site.

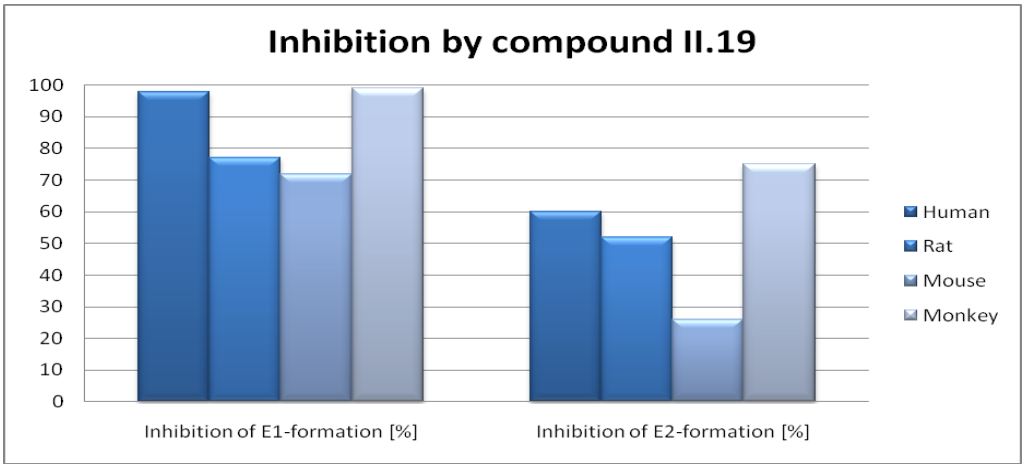
Selectivity toward 17β-HSD1 and the ERs demonstrated by the compounds of this study is an important aspect. Indeed, 17β-HSD1 catalysing the reverse reaction should not be affected by 17β-HSD2 inhibitors. Furthermore, to avoid activation of the ERs or systemic effects as observed by the use of the SERMs, compounds should have no or very little affinities to the ERs. Thus, the most active and selective compounds **II.11** and **II.19** with relative binding affinities (RBA) of less than 1.0% show very weak affinities to the ERs, whereas the reference compound **II.A** with a RBA value of 5% is considered as a weak ligand of the ERs. Due to these results, compounds **II.11** and **II.19** are not expected to have ER-mediated effects under *in vivo* conditions.

The most potent and selective compounds of this series, compounds **II.11** and **II.19** were further tested for their cellular activity in MDA-MB-231 cells, expressing naturally 17β-HSD2. Compounds **II.11** and **II.19** showed very similar activity in MDA-MB-231 cells as in the cell-free assay (IC_{50} = 171 nM for **II.11** and 31 nM for **II.19** in cellular assay *vs*. 156 nM and 19 nM, respectively in cell-free assay). Consequently, these compounds seem to be appropriate to permeate the cell membrane.

Further *in vitro* assays have been performed with compound **II.19** (IC_{50} = 19 nM, SF = 32) in order to get insight in its biological profile (selectivity towards others 17β-HSDs) and to find a suitable species for validation of our therapeutical approach. Thus, compound **II.19**

is highly selective towards 17β-HSD4 (catalysing the same reaction as 17β-HSD2) and 17β-HSD5 (catalysing the conversion of A-dione into T). **II.19** displayed also high inhibition E1-formation in mouse and rat enzymes (72 % and 77 % inhibition at 1 µM, respectively) to very high inhibition in monkey enzyme (99 % inhibition at 1 µM) (Figure 15). Selectivity in monkey enzyme toward E2-formation seems to be similar than the one observed in human enzyme and makes *Callithrix Jacchus* an appropriate species for validation of our concept.

Figure 15: Inhibition of E1- and E2-formation by compound **II.19** in different species at 1µM



Results found for E1-formation in the different species are justified by the identity and homology sequences obtained by alignment (Figure 16) of the amino acids of these species: human (*Homo sapiens*, P37059), monkey (*Callithrix Jacchus*, Q9GME5), mouse (*Mus musculus*, P51658) and rat (*Rattus norvegicus*, Q62730). Thereby, 62% identity is observed between rat and human protein, while only 60% identity between mouse and human. Concerning the *Callithrix Jacchus* enzyme, just a fragment of the 17β-HSD2 sequence has been resolved until now (from AA86 to AA228, corresponding to the Rossmann fold domain). This fragment represents 92% identity to the human one. High sequence identity between the whole sequence of *Callithrix Jacchus* and human enzymes is expected, as 93% identity and 98% homology is observed between *Cynomolgus* monkey (Q4JK76) and human proteins.

Figure 16: Alignment of human, monkey, mouse and rat 17β-HSD2 sequence

```
Human  MSTFFSDTAWICLAVPTVLCGTVFCKYKKSSGQLWSWMVCLAGLCA-VCLLILSPFWGLI 59
CalJac ------------------------------------------------------------
Mouse  MSPFASESAWLCLAAAAVLGGTLLCGCR-SGRQLRSQAVCLAGLWGGACLLSLSLLCTLF 59
Rat    MNPFSSESAWLCLTATAVLGGMLLCKAW-SSGQLRSQVVCLAGLWGGACLLSLSLLCSLF 59

Human  LFSVSCF-LMYTYLSGQELLPVDQKAVLVTGGDCGLGHALCKYLDELGFTVFAGVLNENG 118
CalJac --------------------------LVTGGDCGLGHALCKYLDELGFTVFAGVLNENG 33
Mouse  LLSVACFLLLYMSSSDQDLLPVDQKAVLVTGADSGFGHGLAKHLDKLGFTVFAGVLDKEG 119
Rat    LLSVSCFFLLYVSSSDQDLLPVDQKAVLVTGADSGFGHALAKHLDKLGFTVFAGVLDKEG 119

Human  PGAEELRRTCSPRLSVLQMDITKPVQIKDAYSKVAAMLQDRGLWAVINNAGVLGFPTDGE 178
CalJac PGAAELRRSCSPRLSVLQMDVTNPVQIKDAYSKVVATLQDRGLWAVINNAGVLGFPCDGE 93
Mouse  PGAEELRKHCSERLSVLQMDVTKPEQIKDAHSKVTEKIQDKGLWAVVNNAGVFHLPIDGE 179
Rat    PGAEELRKNCSERLSVLQMDVTKPEQIKDVHSEVAEKIQDKGLWAVVNNAGVLHFPIDGE 179

Human  LLLMTDYKQCMAVNFFGTVEVTKTFLPLLRKSKGRLVNVSSMGGGAPMERLASYGSSKAA 238
CalJac LIPMTDYKQCMAVNFFGTVEVTKTFLPLLRKSKGRLVNVSSMGGGAPMAK---------- 143
Mouse  LIPMSIYRKCMAVNFFGTVEVTKAFLPLLRKSKGRLVNVSSMGGTVPLQMTSAYAATKAA 239
Rat    LIPMTVYRKCMAVNFFGAVEVTKVFLPLLRKSKGRLVNVSSMGAMIPFQMVAAYASTKAA 239

Human  VTMFSSVMRLELSKWGIKVASIQPGGFLTNIAGTSDKWEKLEKDILDHLPAEVQEDYGQD 298
CalJac ------------------------------------------------------------
Mouse  LTMFSTIIRQELDKWGVKVVTIKPGGFKTNITGSQDIWDKMEKEILDHFSKDIQENYGQD 299
Rat    ISMFSAVIRQELAKWGVKVVTIHPGGFQTNIVGSQDSWDKMEKEILDHFSKEIQENYGQE 299

Human  YILAQRNFLLLINSLASKDFSPVLRDIQHAILAKSPFAYYTPGKGAYLWICLAHYLPIGI 358
CalJac ------------------------------------------------------------
Mouse  YVHTQKLIIPTLKERSNPDITPVLRDIQHAISARNPSSFYYPGRMAYLWVCLAAYCPTSL 359
Rat    YVHTQKLALPVMREMSNPDITPVLRDIQHAICAKNPSSFYCSGRMTYLWICFAAYSPISL 359

Human  YDYFAKRHFGQDKPMPRALRMPNYKKKAT 387
CalJac ----------------------------
Mouse  LDYVIKKGFYP-QPTPRALRTVH------ 381
Rat    LDYILKNYFTP-KLMPRALRTAS------ 381
```

In conclusion, in this second study additional highly potent and selective 17β-HSD2 inhibitors with enhanced biological properties compared to the compounds of chapter **3.I** have been identified. The binding mode of the naphthalene derivatives has been verified by introduction of substituents on the phenyl moiety. Results obtained for compound **II.19** are of significant relevance for future *in vivo* studies in monkey, due to its high inhibition of the E1-formation in *Callithrix Jacchus* enzyme.

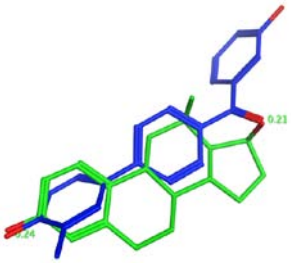
To have a large structural diversity of potential drugs, another compound class was investigated. In the frame of our 17β-HSD1 project, compound **III.B** was found as a highly potent 17β-HSD1 inhibitor, but also a moderate 17β-HSD2 inhibitor (IC_{50} = 382 nM). The goal of the subsequent study was to reverse the selectivity of **III.B** in favour of 17β-HSD2, and increase its 17β-HSD2 inhibitory activity (Chapter **3.III**).

Thus, exchange of the thiophene ring by a bioisosteric 1,4-phenyl moiety led to a highly potent 17β-HSD2 inhibitor (**III.2**) with a reversed selectivity in favour of HSD2 (Table 8).

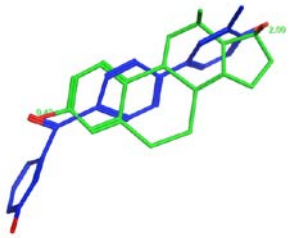
The linear geometry of the 1,4-phenyl class seems to be responsible for the high activity, as compound **III.1** with a 1,3-substituted benzene is only a moderate inhibitor. The central ring of this class of compounds is therefore of a high importance for the development of either 17β-HSD1 or 17β-HSD2 inhibitors.

Regarding the 1,4-substitution pattern, the methyl group can be replaced by a chlorine without loss of activity (**III.2**, **III.6** and **III.11** to be compared to **III.5**, **III.7** and **III.12**, respectively), pointing out the presence of lipophilic amino acids in this region of the enzyme. Furthermore, introduction of a fluorine on the phenyl moiety B led to highly potent inhibitors (**III.6-III.8**, **III.11** and **III.12**). The position of this fluorine might not be important for activity, as compounds **III.6** (4-F) and **III.8** (3-F) displayed similar HSD2 inhibitory potency. To sum up, the 1,4-substitution pattern seems crucial for activity and these derivatives are considered to be steroidomimetics, as they are able to superimpose well with the substrate E2 with their polar moieties (Figure 17).

Figure 17: Superimposition of compound **III.2** (green) and E2 (blue); (**A)** carbonyl group of **III.2** mimicks the C17(OH) of E2 and the OH of the phenyl A ring the C3(OH) of the steroid; (**B)** carbonyl group of **III.2** mimicks the C3(OH) of E2 and the OH of the phenyl A ring the C17(OH) of the steroid.

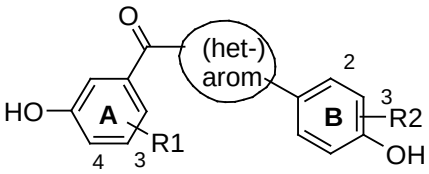


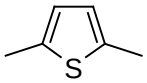
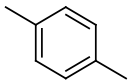
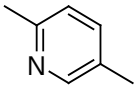
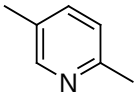
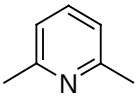
A



B

Table 8: Inhibition of 17β-HSD2 and 17β-HSD1 by selected hydroxyphenylmethanone derivatives



Compd	(Het-) arom	R1	R2	Cell-free assay		
				17β-HSD2 IC_{50}[a] [nM]	17β-HSD1 IC_{50}[b] [nM]	Selectivity factor[c]
III.B		H	3-CH_3	382	8	0.02
III.2		H	3-CH_3	133	413	3
III.4		H	2-Cl	176	1764	11
III.6		4-F	3-CH_3	34	289	9
III.7		4-F	3-Cl	24	53	2
III.8		3-F	3-CH_3	31	159	5
III.10		H	3-CH_3	220	567	3
III.11		H	3-CH_3	260	5482	21
III.12		4-F	3-CH_3	101	1272	13
III.14		H	3-CH_3	263	19646	75

[a] Human placenta, microsomal fraction, substrate [^{3}H]-E2 + E2 [500 nM], cofactor NAD^+ [1.5 mM], mean value of three determinations, relative standard deviation < 10%, [b] Human placenta, cytosolic fraction, substrate [^{3}H]-E1 + E1 [500 nM], cofactor NADH [0.5 mM], mean value of three determinations, relative standard deviation < 10%, [c] IC_{50} (17β-HSD1)/ IC_{50} (17β-HSD2).

While the 1,4-substitution pattern and the fluorine on the A ring are important for activity, the nitrogen of the central ring and its position seem to be responsible for selectivity toward 17β-HSD1 in case of the pyridine class. Indeed, compound **III.10** is an unselective 17β-HSD2 inhibitor, whereas compound **III.11** with the nitrogen close to the A ring is a highly selective inhibitor. Additionally, the 1,3-disubstituted pyridine derivatives show higher selectivity compared to the 1,4-disubstituted pyridines (SF = 75 (**III.14**) for the 2,6-pyridine class to be compared with SF = 3 (**III.10**) and 21 (**III.11**) for the 2,5-pyridines). However, none of the compound synthesised in the 2,6-pyridine class (exchange of the methyl group on the B ring or introduction of further substituent on the A ring) reach a better activity as the parent compound **III.14**. Nevertheless, highly potent 17β-HSD2 inhibitors have been

identified in this new class of compounds (**III.2**, **III.5**-**III.8**, **III.11** and **III.12**), which showed slight or moderate selectivity toward 17β-HSD1. These compounds displayed negligible affinities to the ERs.

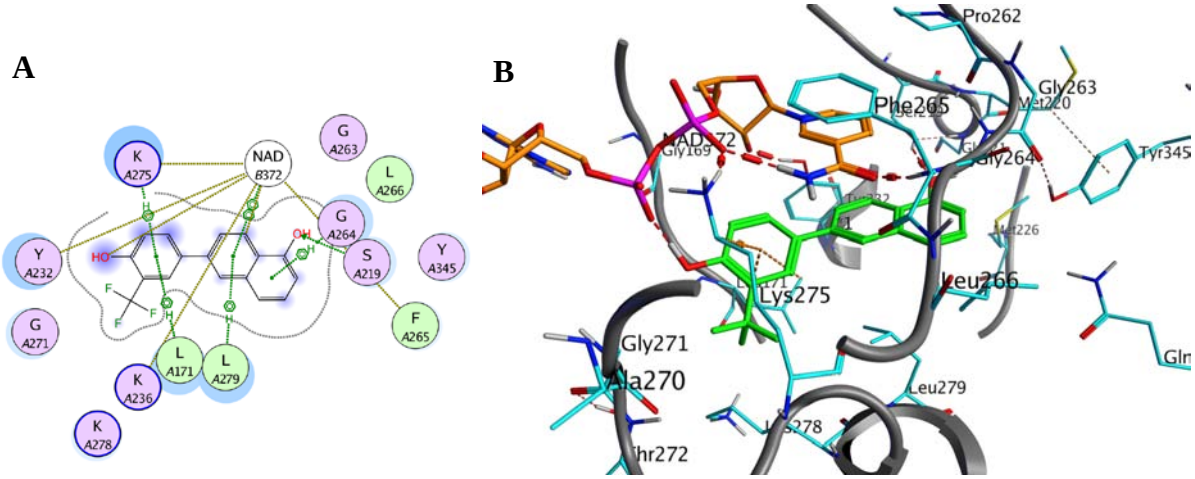
To summarise, a new class of 17β-HSD2 inhibitors, derived from substituted bicyclic hydroxyphenylmethanones has been identified. Selectivity was achieved by introduction of a nitrogen on the central six-membered ring, whereas activity was enhanced by addition of a fluorine on the A ring of the 1,4-disubstituted derivatives. Combination of both modifications does not lead to highly active and selective compounds. Either activity or selectivity is increased, depending on the substitution pattern and on the central moiety. Thereby, a compromise should be found in order to have potent and relatively selective 17β-HSD2 inhibitors. Compounds **III.6** and **III.12** were therefore identified as the most promising derivatives of this series with activity in the low nanomolar range (IC_{50} = 34 nM and 101 nM, respectively) and selectivity factors of 9 and 13, respectively toward 17β-HSD1.

Werth *et al*. studied the mechanism of potent compounds of both classes (**II.19**, **III.5** and **III.14**, PhD thesis of Ruth Werth). Full competitive and mixed partial type inhibition pattern were identified. In fact, a full competitive inhibitor directly competes with the binding of substrate, whereas a partial mixed inhibitor may bind both free enzyme and enzyme-substrate complex. Thus, compounds **II.19** and **III.14** display a partial mixed inhibition and bind preferentially to the free enzyme, while **III.5** is a full competitive inhibitor. It is striking that compound **II.19** does not act as competitive inhibitor contrary to the design idea of a steroidomimetic.

To better understand the mechanism of action of inhibitors, two homology models have been generated. The models differ principally from their hydrophobic loop in front of the catalytic Tyr232 (AA167 to AA175), which plays an essential role either for competitive or partial mixed inhibitors. More precisely, in the first case, Leu171 is turned up leading to a competitive binding mode for the inhibitors, no space is available below NAD^+, while in the second case Leu171 is flat leading to a partial mixed binding mode for the inhibitors and there is space below NAD^+. Therefore, both homology models are in accordance with results of the kinectic studies.

For instance, the most potent compound **II.19**, being a partial mixed inhibitor binds below NAD^+ in the second model, where Leu171 has to flip down (Figure 18). According to the model, the molecule is stabilised in the active site by Van der Waals interactions with Lys275, Leu171, Leu279, Gly264, π-π interactions with the cofactor and H-bond interactions with Ser219 and NAD^+.

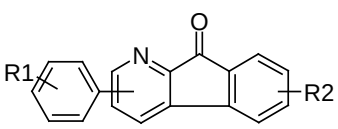
Figure 18: (A) Schematic representation and (B) homology model of compound **II.19** (partial mixed inhibitor) in the active site of 17β-HSD2



Despite the fact that both classes consist of a hydrophobic core and two polar moieties at a distance of circa 11Å, they do not bind in the same area of the active site and they can not be superimposed. Therefore, the SAR of one class cannot be used for the second class and it is not possible to compare the classes. SAR studies of both classes are interesting tools to further improve homology models, as the sequence identity between templates and 17β-HSD2 is only 43%.

To conclude, the present work describes two novel classes of non-steroidal 17β-HSD2 inhibitors. Besides a strong 17β-HSD2 inhibition with IC_{50} values in the low nanomolar range and an outstanding selectivity toward 17β-HSD1 (up to 30), the most promising compound of this thesis shows little affinity to the ERs, high selectivity towards other 17β-HSDs enzymes, namely 17β-HSD4 and 17β-HSD5 and a good cell permeability. Furthermore, it shows very high inhibition of the E1-formation in monkey (*Callithrix Jacchus*) enzyme and makes this species appropriate to prove that inhibition of 17β-HSD2 is able to induce bone formation and inhibit osteoclastogenesis. Furthermore, with the help of the SAR of both classes of compounds, two homology models have been generated in accordance with the results of kinetic studies. These models are of particular interest to design novel classes of 17β-HSD2 inhibitors like azafluorenones as shown in Figure 19.

Figure 19: azafluorenone class



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