Inhibition of 17β-HSD1: SAR of Bicyclic Substituted Hydroxyphenylmethanones and Discovery of Highly Potent Inhibitors Enabling a Proof of Principle Study in Rodents

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Abbreviations

17β-HSD1	17β-hydroxysteroid dehydrogenase type 1
17β-HSD2	17β-hydroxysteroid dehydrogenase type 2
A	androstenedione
Å	Ångström
ACD	advanced chemistry development
ACTH	adrenocorticotropic hormone
ADD	androgen-dependent diseases
ADME	absorption, distribution, metabolism, and excretion
AI	aromatase inhibitor
AKR	aldo-keto reductase
Arg (A)	arginine
Asn (N)	asparagine
Asp (D)	aspartic acid
BSHs	bicyclic substitutedbhydroxyphenylmethanones
CC	column chromatography
$CDCl_3$	deuterated chloroform
CD_3COCD_3	deuterated aceton
CD_3OD	deuterated methanol
CD_3SOCD_3	deuterated dimethylsulfoxyde
COX	cyclooxygenase
CYP	cytochrome P450 superfamily
DAST	diethylaminosulfur trifluoride
DBPO	dibenzoyl peroxide
DHEA	dehydroepiandrosterone
DHEA-S	dehydroepiandrosterone-sulfate
DHT	5α-dihydrotestosterone
DME	di-methoxyethane
DMEM	Dulbecco's modified eagle medium
DMF	dimethylformamide
DMSO	dimethyl sulphoxide
DTT	(2*S*,3*S*)-1,4-bis(sulfanyl)butane-2,3-diol
E1	estrone
E1-S	estrone sulfate
E2	17β-estradiol
E3	estriol
EDD	estrogen-dependent disease
EDSP	endocrine disruptor screening program
EDTA	ethylene diaminetetraacetate

EPA	environmental protection agency
ER	estrogen receptor
equiv	equivalent
Et_3N	triethylamine
EWG	electron withdrawing group
GALAS	global adjusted locally according to similarity
Glu (E)	glutamic acid
Gly (G)	glycine
GnRH	gonatropin releasing hormone
H	human
HEK293	human embryonic kidney-293
His (H)	histidine
HPLC	high-performance liquid chromatography
HSD	hydroxysteroid dehydrogenase
Hz	hertz
IC_{50}	half maximal inhibitory concentration
IUPAC	international union of pure and applied chemistry
Leu (L)	leucine
LH	luteinizing hormone
LHRH	luteinizing hormone releasing hormone
Lys (K)	lysine
m	mouse
M	molar
µM	micromolar
Me	methyl
MHz	megahertz
MOE	molecular operating environment
mM	millimolar
mp	melting point
mRNA	messenger ribonucleic acid
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NADP(H)	nicotinamide adenine dinucleotide phosphate
NAD(H)	nicotinamide adenine dinucleotide
*n*BuLi	*n*-butyllithium
NBS	*N*-bromosuccinimide
nM	nanomolar
NMR	nuclear magnetic resonance
PAPS	3`-phosphoadenosine-5`-phosphosulfate
PDB	protein data bank
$Pd(dppf)Cl_2$	(1,1`-bis(diphenylphosphino)ferrocene)palladium(II) dichloride
PDB	protein data bank
$Pd(PPh_3)_4$	tetrakis(triphenylphosphine)palladium (0)
PG	prostaglandine

PMSF	phenylmethansulfonylflouride
PoP	proof of principle
ppm	parts per million
r	rat
RBA	relative binding affinity
rt	room temperature
SAR	structure activity relationship
SDR	short dehydrogenase reductase
SDS	sodium dodecyl sulphate
Ser (S)	serine
SERM	selective estrogen receptor modulator
SF	selectivity factor
SUB	substrate binding site
T	testosterone
THF	tetrahydrofurane
TMSiCl	trimethylsilyl chloride
TSQ	triple stage quadrupole
Tris	tris(hydroxymethyl)aminomethane
Tyr (Y)	tyrosine
UDPGA	uridine diphosphoglucuronic acid

Abstract

Estradiol is the most potent estrogen in humans. It is known to be involved in development and proliferation of estrogen dependent diseases such as breast cancer and endometriosis. The last step of its biosynthesis is catalyzed by 17β-hydroxysteroid dehydrogenase type 1 (17β- HSD1) which consequently is a promising target for the treatment of these diseases. Recently, we reported on bicyclic substituted hydroxyphenylmethanones (BSHs) as potent inhibitors of 17β-HSD1. Our goal in this study was to obtain a suitable candidate for proof of principle (PoP) study in an animal disease model for endometriosis. At a first stage, we focused on rational structural modifications in this compound class with the aim of gaining more insight into its structure-activity relationship (SAR). At a second stage, supplementary substituents were added to the most active core to enhance activity towards both human and rodent 17β-HSD1 enzymes. Thus, the most potent nonsteroidal inhibitors of *h*17β-HSD1 described so far have been discovered. Furthermore, a successful strategy was applied to improve the metabolic stability in human liver microsomes (S9 fraction). Compound **II.43**, one of the most interesting *h*17β-HSD1 inhibitors in this study, showed a very good metabolic stability towards both phase I and II in human liver microsomes (S9 fraction), with 72 % remaining after 60 min. In addition, inhibition of rodent 17β-HSD1 was significantly improved. Compound **II.47** could be considered a possible candidate for a PoP study (63 % inhibition of rat 17β-HSD1 @ 250 nM).

Zusammenfassung

Estradiol ist das potenteste Estrogen beim Menschen. Es ist bekanntermaßen an Entstehung und Fortschreiten Estrogen-abhängiger Erkrankungen wie Brustkrebs und Endometriose beteiligt. Der letzte Schritt seiner Biosynthese wird durch 17β-Hydroxysteroid Dehydrogenase Typ 1 (17β-HSD1) katalysiert. Daher ist dieses Enzym ein vielversprechendes Target für die Behandlung dieser Erkrankungen. Kürzlich berichteten wir über bicyclisch substituierte Hydroxyphenylmethanone (BSHs), die potente Inhibitoren der 17β-HSD1 darstellen. Das Ziel der vorliegenden Studie bestand darin, einen Wirkstoff-Kandidaten zu erhalten, der für die Indikation Endometriose eine proof of principle- (PoP-)Studie in einem Krankheitsmodell am Tier (Nager) ermöglicht. Dazu wurden zunächst rationale Strukturmodifikationen durchgeführt, die das Ziel hatten, die Struktur-Wirkbeziehungen (SAR) dieser Wirkstoffklasse zu beleuchten. Aus den resultierenden Verbindungen wurde anschließend diejenige mit den interessantesten biologischen Eigenschaften ausgewählt, um durch Einfügen weiterer Substituenten die Hemmaktivität sowohl gegenüber der humanen als auch gegenüber Nager-17β-HSD1 weiter zu steigern. Aus diesen Arbeiten gingen die potentesten nichtsteroidalen Inhibitoren der humanen 17β-HSD1 hervor, die bisher beschrieben wurden. Darüber hinaus wurde erfolgreich eine Strategie zur Verbesserung der metabolischen Stabilität in humanen Lebermikrosomen (S9-Fraktion) angewandt. Verbindung **II.43**, eine der interessantesten Verbindungen der Studie, zeigt eine sehr hohe metabolische Stabilität: Nach 60 minütiger Inkubation liegen 72 % der Verbindung unverändert vor. Auch die Hemmung des Nager-Enzyms konnte signifikant verbessert werden. Verbindung **II.47** kann als möglicher Kandidat für eine PoP-Studie an der Ratte angesehen werden (63 % Hemmung der Ratten-17β-HSD1 bei 250 nM).

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1 Introduction

1.1 Sex steroid hormones

1.1.1 General

Sex steroids are steroid hormones that interact with vertebrate androgen or estrogen receptors.[1] A large proportion of androgens and estrogens in men and women are synthesized locally in peripheral tissues at their site of action from the inactive adrenal precursors dehydroepiandrosterone (DHEA) and its sulfate (DHEA-S) (fig. 1).[2]

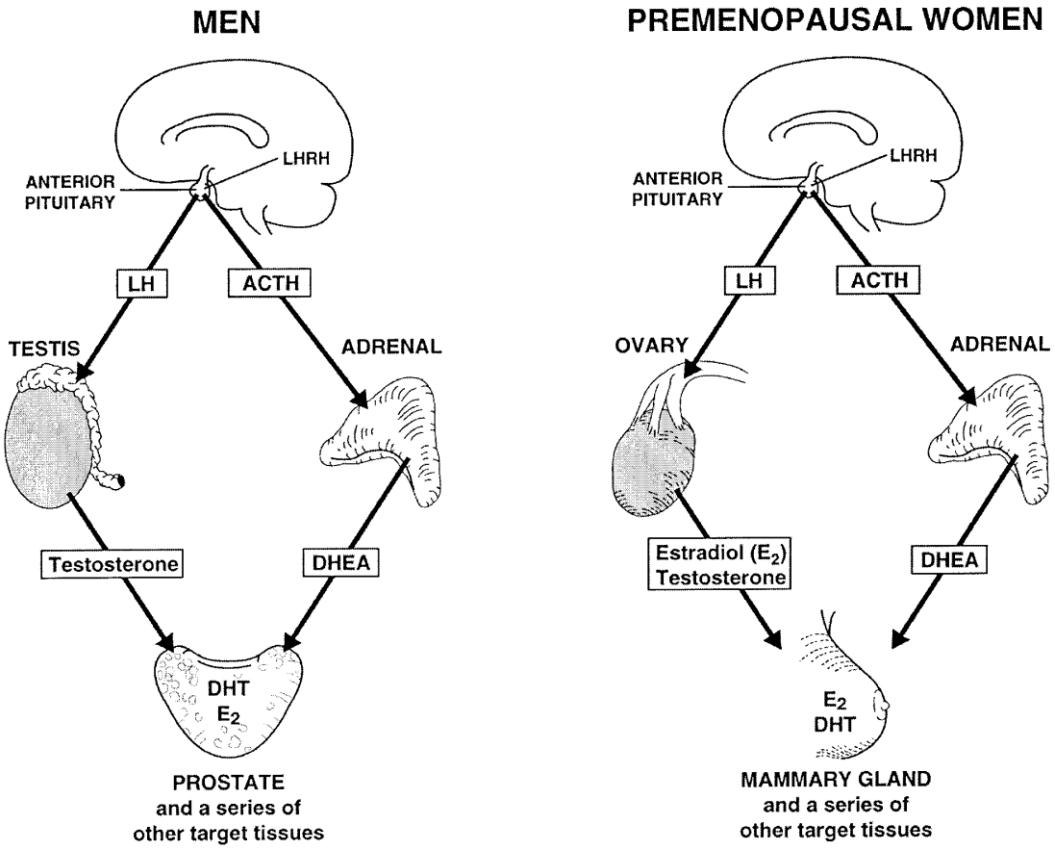


Figure 1. Schematic representation of the role of adrenal and ovarian sources of sex steroids in women. After menopause, the secretion of estradiol by the ovaries ceases and the sex steroids are synthesized locally in peripheral target intracrine tissues (taken from[2])

Approximately 50 % of androgens in adult men and all active sex steroids in postmenopausal women are biosynthesized in target tissues by an intracrine mechanism. This means, that synthesis of active steroids occurs in peripheral target tissues in the same cells where steroid action is exerted without releasing in the extracellular space (fig. 2).[3]

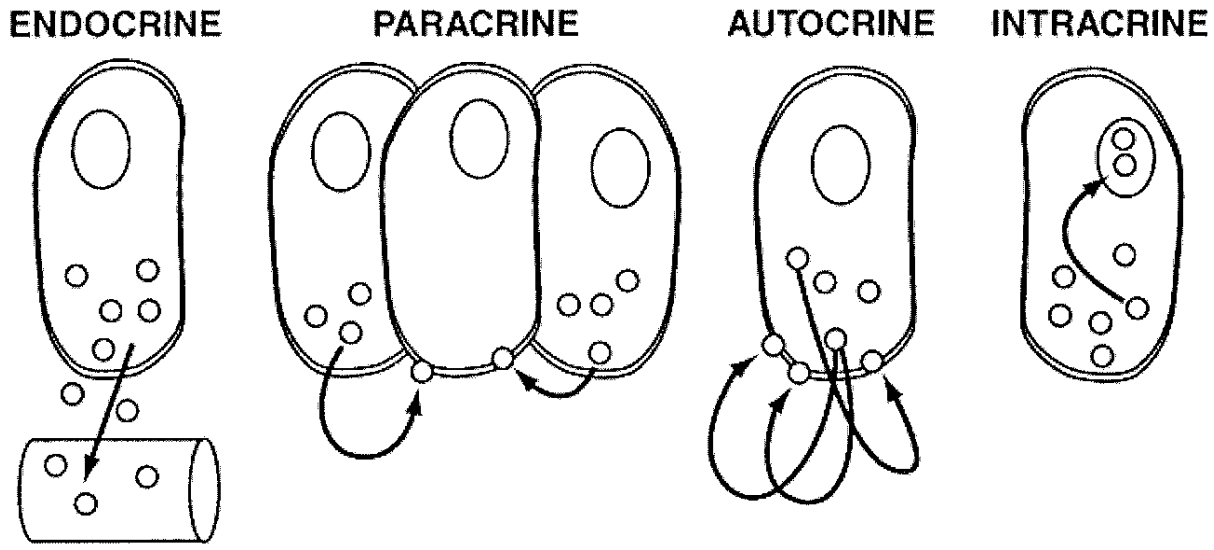


Figure 2. Schematic representation of endocrine, paracrine, autocrine and intracrine secretion (taken from[4])

In peripheral intracrine tissues, DHEA-S is providing a large reservoir of substrate for conversion into estrogens and/or androgens as, in both women and adult men, plasma DHEA-S levels are much higher than those of estradiol and testosterone.[4]

In the higher mammals, the target tissues of estrogens and androgens are able to adjust the formation and metabolism of sex steroids according to local requirements. This refers to almost all active sex steroids that are synthesized locally in peripheral tissues. This situation is very different in lower primates, where the exclusive sources of estrogens and androgens are the ovaries and testes.[3]

Sex steroid hormone signaling regulates the development, growth, and functioning of the breast and the prostate and plays also a role in the development and progression of cancer in these organs.[5]

1.1.2 Estrogens

Estrogens are steroid hormones that have estrane as backbone. Structurally, they differ from other steroid hormones by the aromatic A ring.[6] The most potent naturally occurring member of this group is 17β-estradiol (E2). Its metabolites estrone (E1) and estriol (E3) have less affinity for the estrogen receptors and are weaker agonists.[7]

The biosynthesis of estrogens, and all other hormones with steroidal structure, starts from cholesterol and involves cytochrome P450 enzymes.The transfer of cholesterol from the cytosol to the inner membrane of mitochondrion, where the cytochrome P450 enzymes are located, is the rate-limiting step in steroid production.[8]

The final step of estrogen biosynthesis is the aromatization by a P450 aromatase monooxygenase enzyme complex, which catalyzis the conversion of androstendione (A4) and testosterone (T) into E1 and E2, respectively (fig. 3).[8]

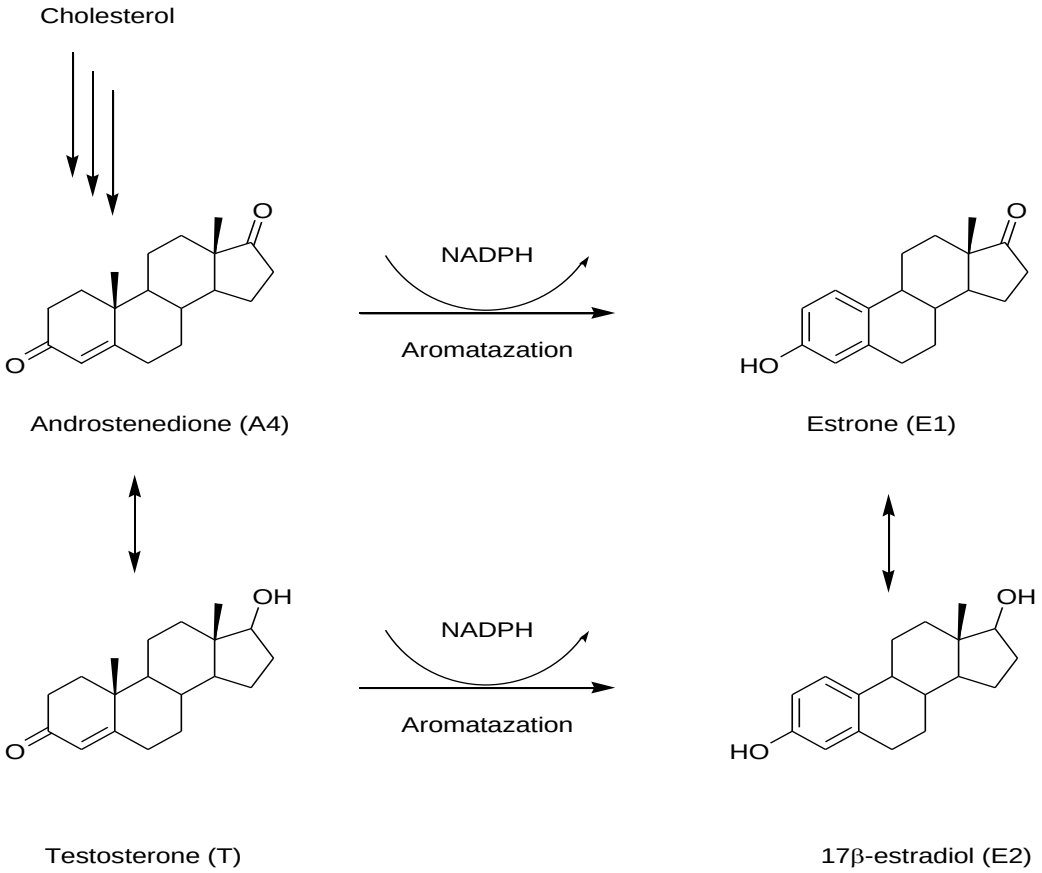


Figure 3: Schematic representation the conversion of Androstendione (A4) and Testosterone (T) into Estrone (E1) and 17β-estradiol (E2), respectively (taken from[8])

Estrogens are subjected to a high first-pass effect of about 90%. The glucuronide or sulfated conjugates are excreted via the bile and partially hydrolyzed in the colon by bacteria, which allows re-absorption, so that estrogens are subjected to enterohepatic circulation.[6]

Another possibility for metabolism consists in the hydroxylation and partial subsequent methylation to form catechols or methoxylated estrogens. Some of these derivatives such as 4-hydroxyestrone and 16α-hydroxyestradiol are carcinogenic.[8]

The multifaceted effects of estrogens include promoting the growth of female sex organs and the expression of the female secondary sex characteristics e.g. by increasing the subcutaneous fat depots.[6] They also cause cyclic changes in the uterine mucosa and the viscosity of the cervical mucus and prepare the tissue for gestagen action by increasing the number of progesterone receptors.

Estrogens appear to play an essential role in maintaining bone, as they display antiresorptive effects by inducing apoptosis of osteoclasts.[9] In young men estrogens are required for epiphyseal closure and maintaining bone mass.[10]

1.2 Estrogen-dependent diseases

1.2.1 Breast cancer

1.2.1.1 General

Breast cancer is the most common cancer in women; the largest percentage of new cancer cases and cancer deaths in females are attributed to this tumor localization.[11] But also the mammary gland of men can be affected, as the number of 520 cancer cases in Germany in 2008 illustrates.[11] Breast cancer mainly affects postmenopausal women, and the probability for the occurrence of the disease increases with endogenous estradiol concentrations.[12] Other risk factors include postmenopausal obesity and intake of exogenous hormones.[13]

Estrogens are well recognized to play an important role in the growth of hormone-dependent breast cancers.[14, 15] There are two breast cancer categories: Estrogen receptor positive (ER^+) and estrogen receptor-negative (ER^-) tumors. Approximately 50% of breast cancers in premenopausal women and 75% of postmenopausal women are ER^+,[16] This can be explained by the fact that increased estradiol formation and consequently an increased activation of the ER can promote tumor growth.[17]

In the following, the influence of estrogens is illustrated in hormone-dependent tumors.

Although the serum estradiol levels in postmenopausal women are lower than those of premenopausal women,[18, 19] the intratumoral estradiol levels in pre- and postmenopausal patients are comparable.[19] In postmenopausal women, high intratumoral estradiol (E2) levels are assumed to be maintained by intratumoral biosynthesis of estrogens.[20-22]

This can take place via two different pathways.[23-25] In the first one, androstendione (A4) is aromatized into estrone (E1) and then catalyzed by 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1) into estradiol (E2; aromatase pathway).[26-28] In the second pathway, estrone sulfate (E1S) is converted into E1 and then transformed into E2 by 17β-HSD1 (sulfatase pathway).[29]

Both the intratumoral estradiol levels as well as the mRNA expression of the enzymes aromatase and sulfatase do not significantly differ between pre-and postmenopausal women, whereas both the mRNA expression of 17β-HSD1 and the ratio estradiol / estrone in the tumor tissue of postmenopausal women are higher.[29] These results suggest that 17β-HSD1 influences the intratumoral estradiol synthesis,[29] a supposition which is confirmed by the fact that increased 17β-HSD1 expression is associated with a worse prognosis and a shorter response time.[30]

1.2.1.2 Treatment options

The surgical removal of the primary tumor represents a frequently applied treatment of breast cancer, where in some cases a mastectomy is essential. A radio and / or chemotherapy treatment is often carried out prior to surgical removal of the tumor, as it would reduce the tumor to an operable size or to prevent recurrences after surgery.[31, 32]

For estrogen-dependent breast cancer endocrine therapies represent the treatment of choice,[33, 34] aiming at suppression of estrogen action by influencing the hormonal system.[34] On one hand, this is possible by the inhibition of estrogen biosynthesis, on the other hand by blockage of the estrogen receptors.

Applied endocrine therapeutics:

- Aromatase inhibitors (e.g. anastrozole):

  Suppression of estrogen formation by inhibiting the last step of E1 biosynthesis.

- GnRH-analogues (gonadotropin-releasing hormone analogs such as buserelin):

  Complete suppression of estrogen formation in the organism by disrupting a central feedback mechanism,[35] that controls the biosynthesis.

- SERMs (selective estrogen receptor modulators such as tamoxifen)

  Preventing estrogen binding to the receptor, act as tissue-specific agonists or antagonists at the receptor.

- Pure antiestrogens (e.g. fulvestrant):

  Preventing estrogen action at the receptor in the whole organism

1.2.2 Endometriosis

1.2.2.1 General

Endometriosis is one of the most common causes of pelvic pain and infertility in women. It is defined as an ectopic occurrence of endometrial glands and stroma. So endometrial tissue grows outside the uterus, especially on the ovaries, the fallopian tubes and into the abdominal cavity. However, there is no correlation between the location and size of lesions and severity of symptoms. Endometriosis causes adhesions and scars. There may be severe pain, heavy bleeding and damage to the reproductive organs, which can ultimately lead to infertility. The etiology of endometriosis is still poorly understood. The most widely accepted theory states that normal endometrial cells pass through retrograde menstruation into the peritoneal cavity and develop there after transplantation of endometriosis (transplantation theory of Sampson).[36]

In contrast to normal endometrium, aromatase is expressed aberrantly in endometriosis and is stimulated by prostaglandin E2 (PGE2).[37] Deficient expression of 17β- hydroxysteroid type 2 (17β-HSD2), an enzyme which inactivates E2 to E1, is another abnormality in endometriosis. Since endometriosis is an estrogen-dependent disorder, 17β-HSD2 deficiency and aromatase expression are of paramount importance in the endometriosis pathophysiology.[37]

E2 enhances the cell proliferation and infestation of endometric tissues while both cytokines and prostaglandins mediate pain and infertility. E2 in women is either directly produced by the ovary or in extra-ovarian sites (skin and adipose tissue).[37]

In the endometric tissue of both pre- and postmenopausal women, E2 is produced locally from androstendione (A) of adrenal and ovarian origins in two steps. Firstly, androstendione (A) is converted to E1 by aromatase and subsequently, E1 is further converted to E2 in the peripheral tissues by 17β-HSD1. Thus, in the postmenopausal period or during ovarian suppression, the major source for circulating E2 is extra-ovarian aromatase (fig. 4).[37]

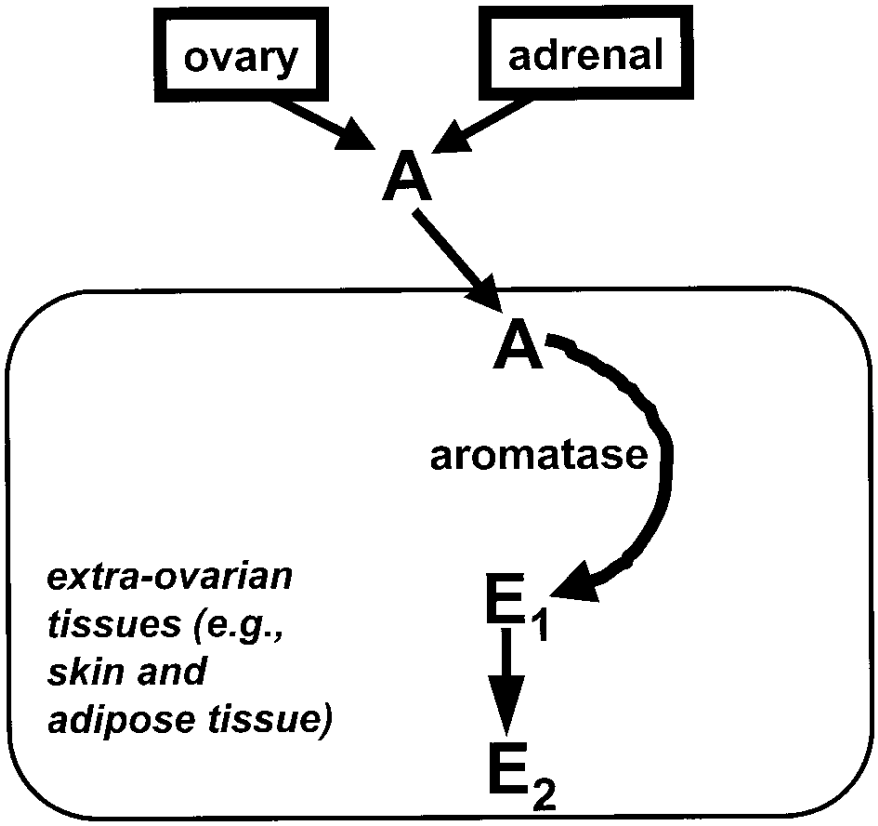


Figure 4. Extra-ovarian estrogen formation in women (taken from [37])

In response to progesterone during the secretory phase, estradiol is normally deactivated in epithelial cells of the eutopic endometrium by conversion to E1 by 17β-HSD2. Despite high levels of progesterone during the secretory phase, 17β-HSD2 expression is absent in endometriosis. This is indicative of selective progesterone resistance in this tissue.[37] The local concentration of E2 in endometriotic tissue is thus increased due to this lack of 17β-HSD2 (fig. 5).[37]

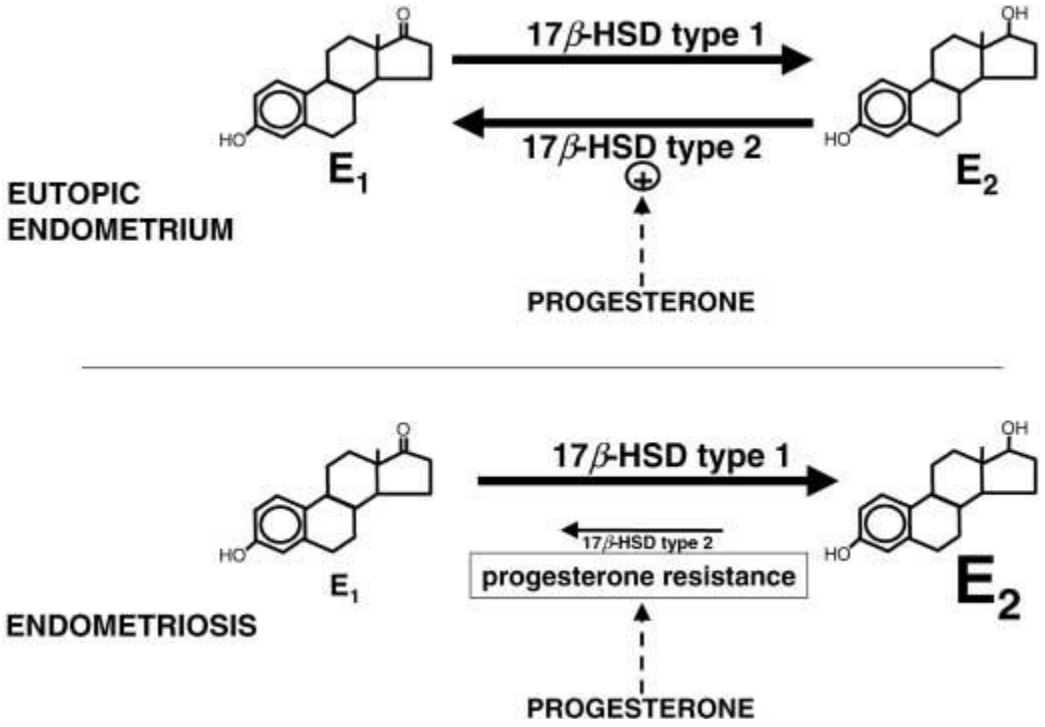


Figure 5. Defective inactivation of estradiol (E2) in endometriosis (taken from [37])

1.2.2.2 Treatment options

Endometriosis can be treated surgically by laparoscopy, but this usually provides only a temporary removal of endometriotic lesions, since the probability of relapse is very high.

As drug treatment COX inhibitors are often used, as they reduce the inflammatory process of endometriosis,[38] while helping to relieve pain.

Another option for the treatment of endometriosis is the suppression of estrogen biosynthesis and lowering the E2 production, similar to the treatment of estrogen-dependent breast cancer , the application of oral contraceptives, androgens and GnRH analogues used there to inhibit the growth of endometriotic lesions, affecting hormone balances in the whole organism and thereby exerting undesirable side effects such as weight gain and acne.[39]

1.2.3 Disadvantages of existing therapies

Systemic reduction in estrogen concentration leads to a shift of the natural hormone balance throughout the body and can therefore lead to many well-known, unwanted and sometimes serious side effects. In addition to these side effects the applied therapies show other limitations:

SERM may function by their tissue-specific cancers in other tissues such as the endometrium, where tamoxifen acts as an estrogen receptor agonist.[40, 41] With antiestrogens and with aromatase inhibitors a frequent development of resistance is observed .[42] Furthermore, aromatase inhibitors may be used only in postmenopausal women because they cause a strong stimulation of the ovaries through a hypothalamic / pituitary feedback mechanism in premenopausal women.[43]

1.3 Hydroxysteroid dehydrogenases (HSDs)

1.3.1 General

Hydroxysteroid dehydrogenases (HSDs) belong to the oxidoreductases, which are responsible for the mutual reaction of ketones and their corresponding secondary alcohols. HSDs are NADPH or NAD^+-dependent enzymes whichcatalyse the regio- and stereoselective oxidoreduction at different positions of their steroidal substrates (3α, 3β, 11β, 17β, 20α, 20β-position). They are involved in the metabolism of various steroidal compounds.[44, 45]

Several enzymes, namely 3β-hydroxysteroid dehydrogenase/Δ5- Δ4 isomerase (3β-HSD), 17β-HSD are involved in the synthesis of the most potent natural estrogen E2 (fig. 6).[4]

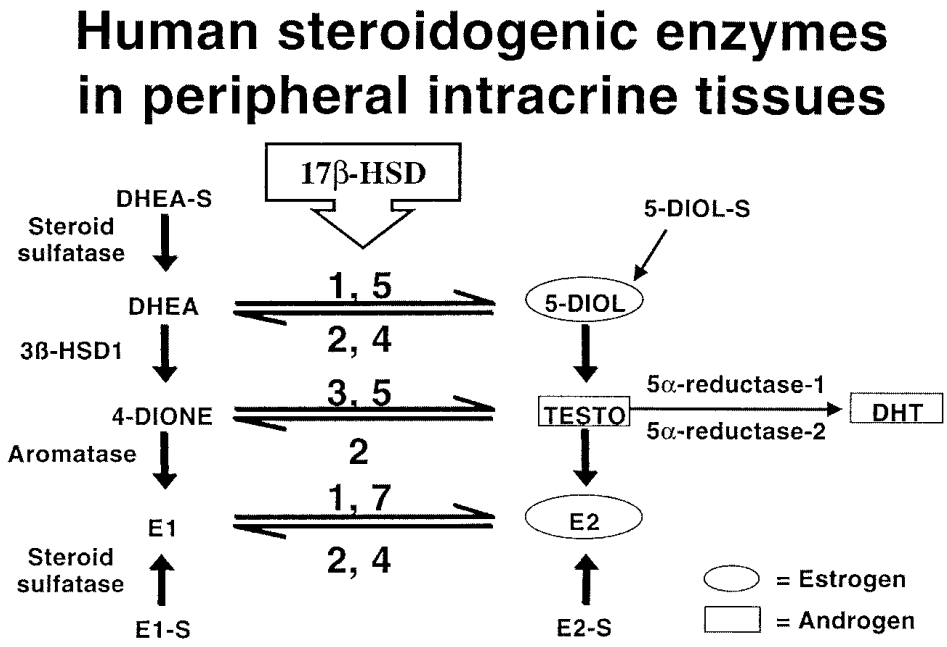


Figure 6. Main biosynthetic and inactivating pathways of androgens and estrogens in humans (taken from [4])

1.3.2 17β-HSDs

1.3.2.1 General

At least 14 different 17β-HSDs have been already identified to date,[46, 47] of which 12 subtypes have been detected in human tissue (17β-HSD6 and 9 were only found in rodents).[47] They belong to the short chain dehydrogenase/reductase (SDR) family.[48] The only exception is 17β-HSD5 which is an aldo-ketoreductase (AKR).[49] The different isoforms of 17β-HSD have a relatively low amino acid sequence identity (25-30%).[50] They also differ in tissue distribution, subcellular localization and catalytic preference (oxidation or reduction using cofactor NAD(H) or NADP(H)).

17β-HSDs are required for the synthesis and inactivation of all active androgens and estrogens. Thus they control the intracellular occupation of the respective steroid hormone receptors.[51, 52] For most of the steroid hormones HSD enzyme pairs are known which regulate the local levels of the active forms and their inactive metabolites. HSD can therefore be considered a molecular switch that can modulate the function of steroid hormones before they bind to the receptor.[4, 53]

Taking into account the intracrine concept many promising therapeutic approaches have been investigated. The blockade of specific steroidogenic enzymes by means of potent and selective inhibitors came more and more into focus as a strategy for treatment of hormone-dependent diseases. This approach has been successfully implemented in various enzymes, can also affect steroid hormone concentrations locally. Key enzymes which are involved in androgen and estrogen biosynthesis are e.g. aromatase[54, 55] and 5α-reductase[56, 57].

In recent years 17β-HSDs gained major interest as potential drug targets for the treatment of sex steroid hormone-dependent diseases. This is due to two advantages of the group of 17β-HSDs: first, their tissue-specific expression; on the other hand, the fact that they often catalyze the last step in the biosynthesis steroid hormone. This leads to the assumption that the inhibition of these enzymes in comparison with the aforementioned approaches (for example, inhibition of the aromatase) should result in fewer side effects, since a smaller influence on the systemic steroid hormone concentration is expected.

The physiological function of five of 17β-HSDs enzymes is not clear yet, while nine subtypes are involved in diseases (like EDD) (table 1).[58] The nomenclature of 17β-HSD enzymes is based on the chronological order of discovery.[59]

Table 1: Human 17β-hydroxysteroid dehydrogenases (taken from[58]).

Type	Gene	SDR or AKR nomenclature	Function	Disease-association
1	HSD17B1	SDR28C1	Steroid (estrogen) synthesis	Breast & prostate cancer, endometriosis
2	HSD17B2	SDR9C2	Steroid (estrogen, androgen, progestin) inactivation	Osteoporosis
3	HSD17B3	SDR12C2	Steroid (androgen) synthesis	Pseudohermaphroditism in males associated with obesity
4	HSD17B4	SDR8C1	Fatty acidβ-oxidation, steroid (estrogen, androgen) inactivation	D-specific Bifunctional Protein-deficiency, prostate cancer
5	AKR1C3	AKR1C3	Steroid (androgen, estrogen, prostaglandin) synthesis	Breast & prostate cancer
6	HSD17B6	SDR9C6	retinoid metabolism, 3α- 3β-epimerase, steroid (androgen) inactivation?	
7	HSD17B7	SDR37C1	Cholesterol biosynthesis, steroid (estrogen) synthesis	Breast cancer
8	HSD17B8	SDR30C1	Fatty acid elongation, steroid inactivation, estrogens, androgens	Polycystic kidney disease
9	HSD17B9	SDR9C5-2	Retinoid metabolism	
10	HSD17B10	SDR5C1	Isoleucine, fatty acid, bile acid metabolism, steroid (estrogen, androgen) inactivation	X-linked mental retardation MHBD deficiency Alzheimer's disease
11	HSD17B11	SDR16C2	Steroid (estrogen, androgen) inactivation, lipids?	
12	HSD17B12	SDR12C1	Fatty acid elongation, steroid (estrogen) synthesis	
13	HSD17B13	SDR16C3	Not demonstrated	
14	HSD17B14	SDR47C1	Steroid (estrogen, androgen?) inactivation, fatty acid metabolism	Breast cancer, prognostic marker

1.3.2.2 Cell metabolism and unidirectional steroid flux in living cells

In principle, all members of the 17β-HSDs are able to catalyze both reaction directions (oxidation and reduction). However, intracellularly, they show a clear preference for one direction of catalysis and are thus divided into oxidative and reductive enzymes.[60] This is due to the fact that concentrations of substrates (keto- and hydroxysteroids) are much lower (nanomolar range) than the ones of cofactors [NADP(H) and NAD(H)] which are in the micromolar range. Furthermore, the ratios between $NADP^+$ and its reduced form NADPH ($NADPH/NADP^+$ >500) and NADH and its oxidized form NAD^+ (NAD^+/NADH >700) are homestatically maintained by glucose metabolism and O_2 supply (Fig. 7).[61]

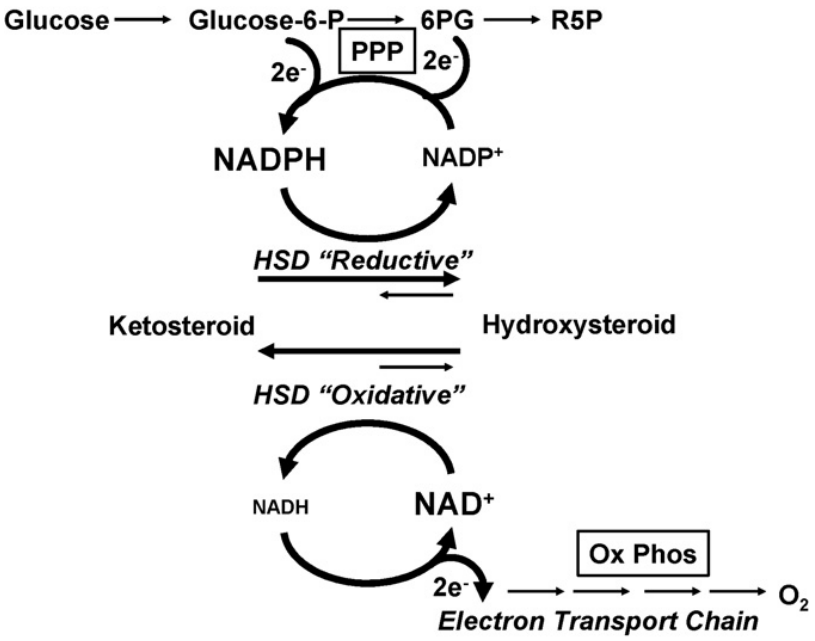


Figure 7: Interplay between intermediary metabolism and 17β-HSDs reactions (taken From [61]).

Another reason for the determination of the direction of the 17β-HSD catalysis is its high binding affinity for the phosphorylated and non-phosphorylated nicotinamide adenine dinucleotide (NAD) cofactor.[61] Structures and mutagenesis studies showed that in the N-terminal

region of the Rossmann folds, which represent the cosubstrate binding sites, of 17β-HSD1 and 17β-HSD3 (reductive 17β-HSDs), a positively charged amino acid (Arg37 in 17β-HSD1[62], Arg80 in 17β-HSD3[63]) forms an ionic interaction with the 2'-phosphate moiety of NADPH. This greatly increases the affinity for the phosphorylated cosubstrate. On the contrary, in 17β-HSD2 and 17β-HSD4 (oxidative 17β-HSDs) this arginine is replaced by a negatively charged amino acid (17β-HSD2, Glu116[64]; 17β-HSD4, Asp40[50]), which is considered to be the reason for NAD(H) specificity (Fig. 8).[58]

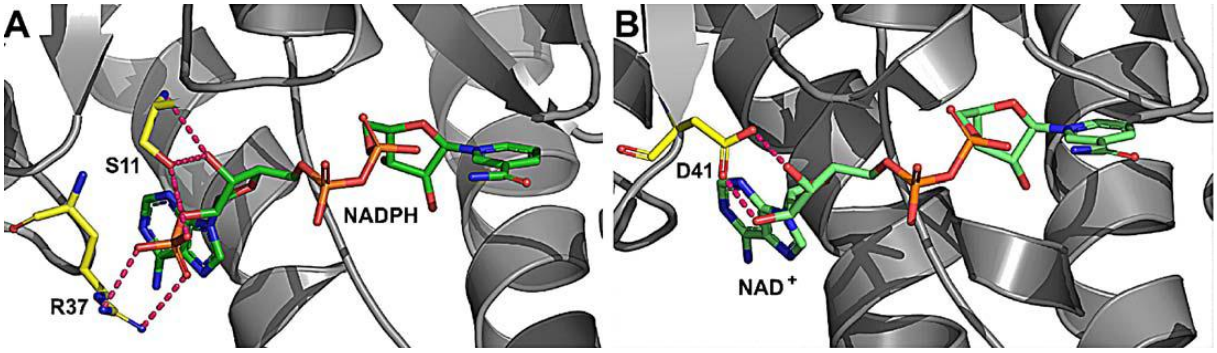


Figure 8: Important amino acidresidues for cofactor recognition by 17β-HSDs. (A) Reductive 17β-HSD1: interaction of Arg37with NADPH (PDB file: 1A27); (B) Oxidative 17β-HSD4: interaction of Glu41with NAD^+ (PDB file: 1ZBQ); NADPH, NAD^+, Arg37 and Asp41 rendered as sticks and labelled. Figure rendered with MOE (www.chemcomp.com) (taken from [58]).

1.3.3 17β-HSD1

1.3.3.1 17β-HSD1 as a promising drug target

17β-HSD1 catalyzes the last step in the estrogen biosynthesis by conversion of the weak estrogen E1 to the most potent estrogen E2 (Fig. 9). The latter plays a crucial role in the development and progression of hormone-dependent breast cancer and endometriosis, as mentioned above. Substantially, estrogens exert their effect by activation of the estrogen receptors α and β (ERα and ERβ).

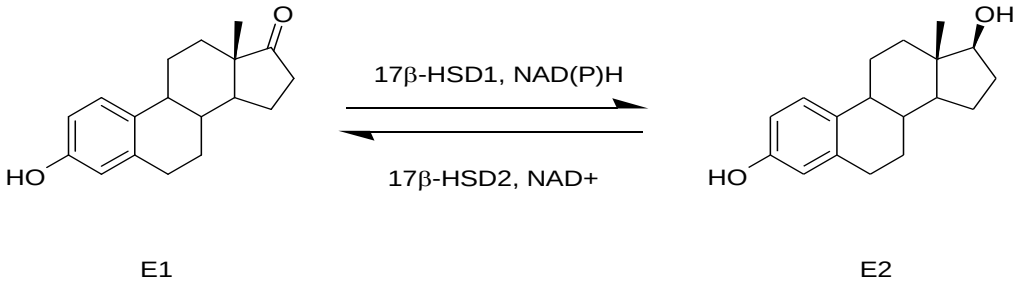


Figure 9. Interconversion of Estrone (E1) and Estradiol (E2).

Selective inhibition of 17β-HSD1 is considered an attractive therapeutic target for treatment estrogen-dependent diseases due to high levels of 17β-HSD1 expression at mRNA and protein level as well as an increased E2/E1 ratio. Several studies have shown that in the tumors of patients with ER^+ breast cancer, the 17β-HSD1/2 ratio is increased.[29, 65, 66]

The high concentration of E2 in endometriotic tissues is due to changes in the expression pattern of steroidogenic enzymes.[37] In comparison to normal tissues, both aromatase and 17β-HSD1 are upregulated at mRNA level in endometriotic lesions, while 17β-HSD2 is down regulated.[67, 68] Consequently, the inhibition of 17β-HSD1 for the treatment of this disorder, is an innovative approach.

17β-HSD1 inhibitors must be selective towards both type 2 enzyme (which catalyzes the reverse reaction) and estrogene receptors α and β (ER α and β) to avoid systemically estrogenic response.

1.3.3.2 Structural design

Human 17β-HSD1 belongs to SDR family and is active as a soluble cytosolic homodimer. The subunits of the homodimer have a molecular weight of 34.9 kDa and consist of 327 amino acids.[69] Till now, there are 20 crystal structures of 17β-HSD1 available in the Protein Data Bank (PDB).[70] Among all of these structures, no 17β-HSD1 crystal structure complexed with substrate E1 exists. The published crystal structures cover apoform, holoform, binary complexes with androgens or inhibitors and ternary complexes with cofactor, E2 or inhibitors.

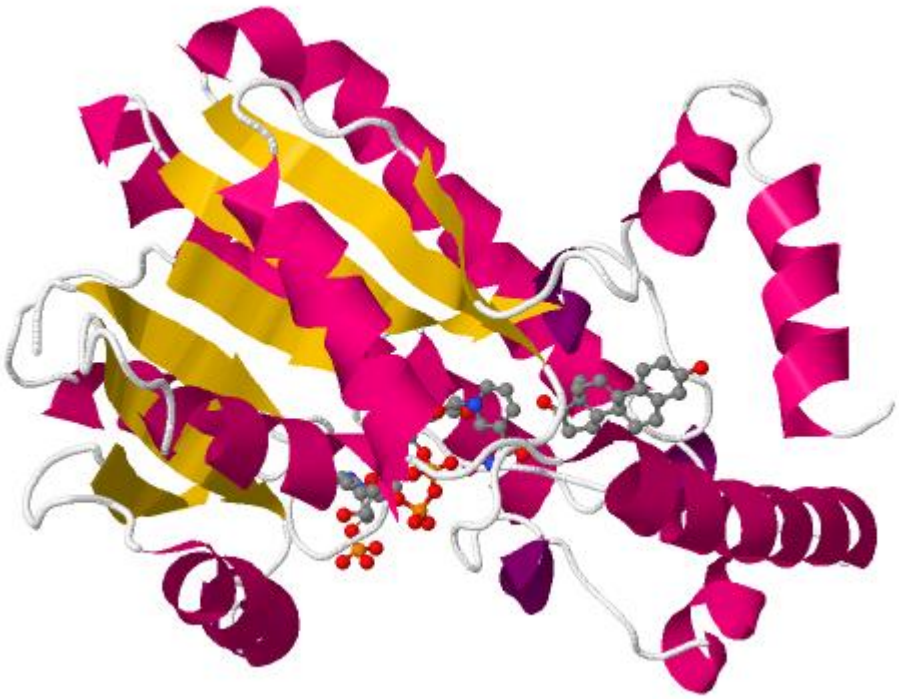


Figure 10. 3D structure of human 17β-HSD1 (PDB ID: 1A27) cocrystallized with E2 and NADP+.

1.3.3.3 Catalytic mechanism of 17β-HSD1

Although different mechanisms of catalysis functions of 17β-HSD have been described, they all have of the following chemical mechanism in common: a reversible hydride or proton transfer from NADPH or hydroxysteroid to ketosteroid or to NAD +.

In the postulated catalytic mechanism of 17β-HSD1, the *Pro*-S hydride of NADPH is transferred to the "α-face" of estrone at the C17 carbon. Subsequently, the resulting oxygen anion is protonated by the acidic hydroxyl group of tyrosine. Several studies evidenced that the catalytic tetrad (Asp 114, Ser142, Tyr155 and Lys159) and a water molecule form an H-bond network which is responsible for proton transfer and plays a critical role in the substrate stabilizing process Fig. 11.[71-74]

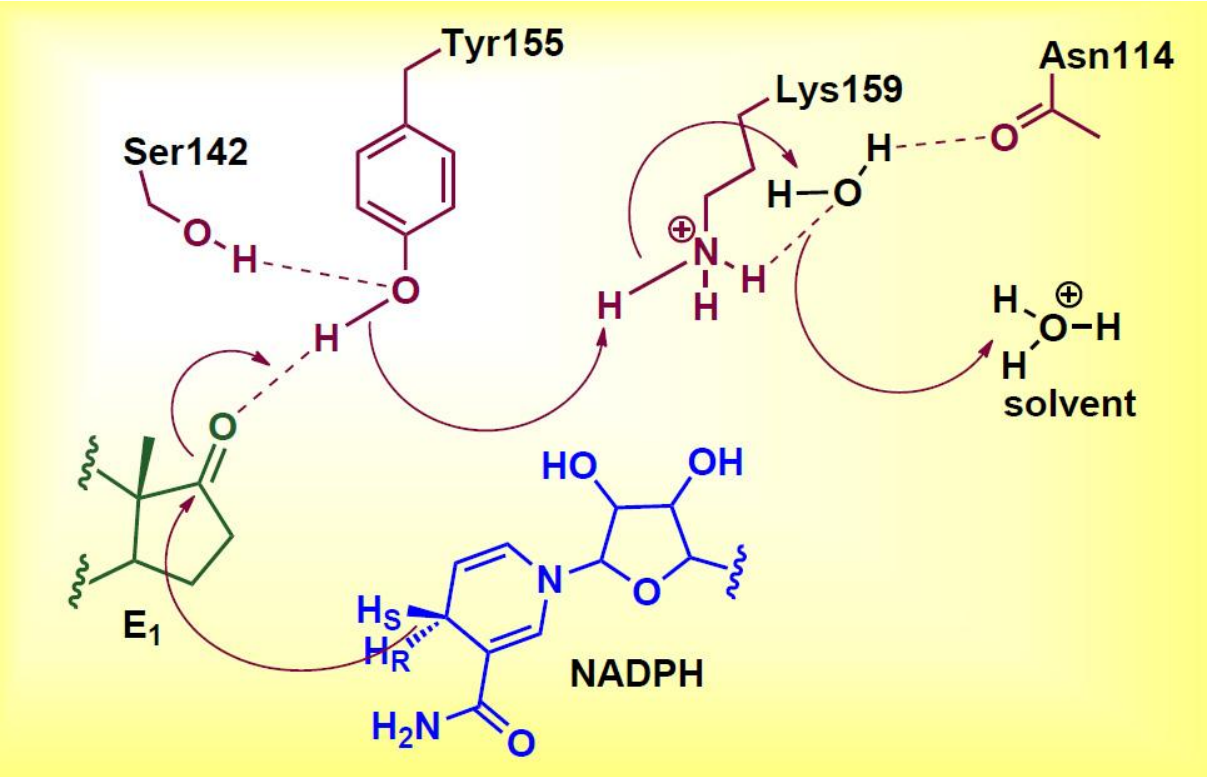


Figure 11: Scheme of the possible catalytic mechanism for 17β-HSD1.[73]

1.3.3.4 Animal models for *in vivo* evaluation of 17β-HSD1 inhibitors

In various animal models, the physiological role of the 17β-HSD1 and its inhibition has been investigated and confirmed as therapeutic approach for breast cancer. In lower mammals, the ovaries and not the peripheral target tissues (as in man and higher primates) are the primary sources of estrogens. To overcome the considerable interspecies differences between lower species and human in 17β-HSD1 tissue distribution and moderate sequence homology and identity of the orthologs, xenograft models were developed using nude mice.[17, 75-81]

Recently, several animal models were established in order to gain insight into pathophysiological role of this enzyme in the development of estrogen-dependent diseases as well as with aim of testing 17β-HSD1 inhibitors *in vivo*.

1.4 17β-HSD1 Inhibitors

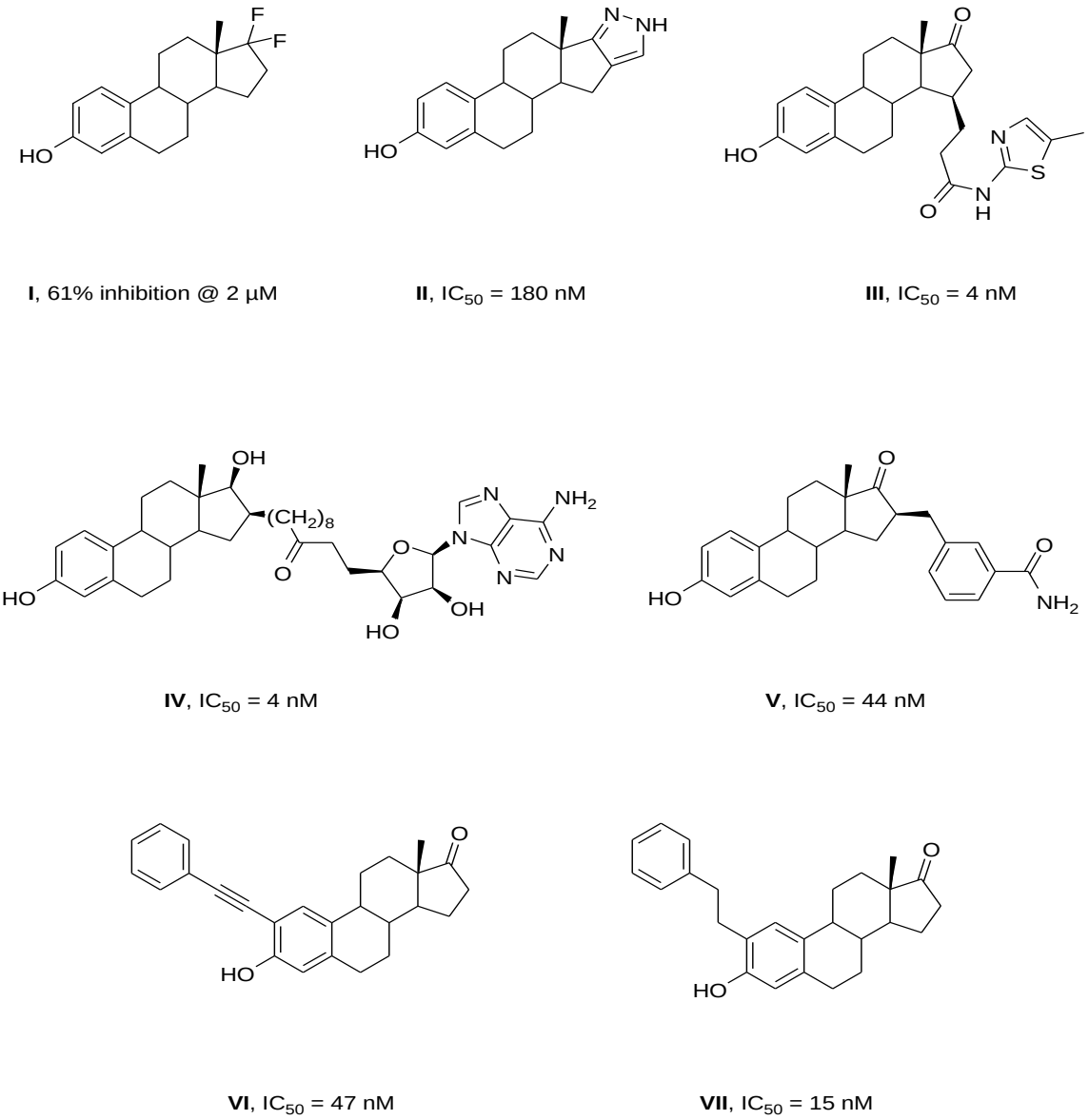
1.4.1 General

During the last years, several publications and patents on 17β-HSD1 inhibitors and their progress have been published.[17, 58, 82-86] This fact highlights the great potential of this target for drug research.

17β-HSD1 inhibitors should be selective towards type 2 enzyme, non-estrogenic, and show acceptable ADME and toxicity parameter.[87] Both steroidal and non-steroidal 17β-HSD1 inhibitors are described to bind to the cofactor pocket and/or the steroid binding cleft. One exception are phytoestrogens which seem to interact with the dimer interface of 17β-HSD1.[88]

1.4.2 Steroidal 17β-HSD1 inhibitors

The steroidal 17β-HSD1 inhibitors are based on the steroidal ring system (E1 and E2 core). In addition to variations at position 2, 6, 15, 16, and 17, the sterane skeleton was extended with an additional E-ring.[89-97] The side chain derivatives at the C2, C15 and C16 positions showed the best biological results (Fig. 12). Steroidal inhibitors showed some drawback like insufficient cell membrane penetration (comp. IV) or estrogenicity (comp. V).



I, 61% inhibition @ 2 µM

II, IC_{50} = 180 nM

III, IC_{50} = 4 nM

IV, IC_{50} = 4 nM

V, IC_{50} = 44 nM

VI, IC_{50} = 47 nM

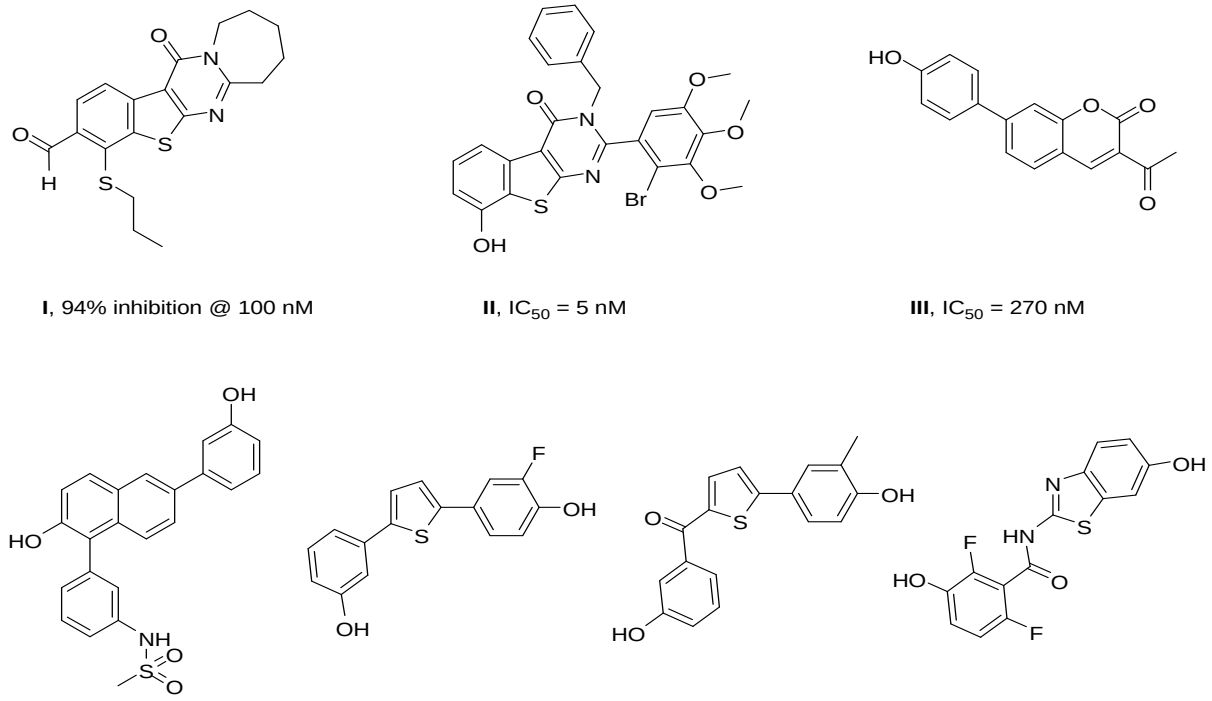
VII, IC_{50} = 15 nM

Figure 12: Examples for steroidal 17β-HSD1 inhibitors.

1.4.3 Non-steroidal 17β-HSD1 inhibitors

Non-steroidal inhibitors (fig. 13)[98-117] show advantages compared to steroidal ones such as selectivity and drug-likeness. Thiophenpyrimidinones represent a large class inhibitors of 17β-HSD1. Generally, they can be divided into two generations. The first potent agents have been described by Messinger *et al.* and contained, in contrast to the second generation of three instead of four fused rings as a basic structure, which are two of the most active 17β-HSD1 inhibitors to date. Recently, coumarin derivatives were published as potent non-steroidal inhibitors of 17β-HSD1.[118, 119]

The most potent non-steroidal inhibitors so far were described by the Hartmann group. Four classes have been developed: a) (Hydroxyphenyl)naphthols (e.g. **IV**), b) Bis(hydroxyphenyl)substituted arenes (e.g. **V**), c) Bicyclic substituted hydroxyphenylmethanones (e.g. **VI**) and d) Hydroxybenzothiazoles (e.g. **VII**).



I, 94% inhibition @ 100 nM

II, IC_{50} = 5 nM

III, IC_{50} = 270 nM

IV, IC_{50} = 15 nM

V, IC_{50} = 8 nM

VI, IC_{50} = 8 nM

VII, IC_{50} = 13 nM

Figure 13: Examples for non-steroidal 17β-HSD1 inhibitors described.

Especially interesting are bicyclic substituted hydroxyphenylmethanones which showed very good inhibitory activity in both cell-free and cellular assays and good selectivity towards both type 2 enzyme and estrogen receptors α and β.

2 Outline

2.1 Scientific goal

Estrogens are an important factor in the development and progression of estrogen-dependent diseases (EDDs) such as breast cancer and endometriosis. Existing endocrine therapies in the treatment of EDDs are associated with numerous side effects due to the fact that the estrogenic activity in the whole body is radically lowered.

17β-HSD1 is the enzyme that catalyses the last step in the biosynthesis of E2 from the weak precursor E1 and has been found strongly overexpressed in diseased tissues of patients affected by EDDs. Thus, inhibition of this enzyme is a promising novel strategy for the treatment of these diseases with the prospect of fewer side effects since systemic estrogen action should be less affected.

The effectiveness of 17β-HSD1 inhibition to reduce the estrogen stimulated growth of human tumor cell lines has already been proven in various *in vivo* assays, but no 17β-HSD1 inhibitor has entered clinical trials yet, emphasizing the need for research in this field.

There is, however, no suitable candidate for a PoP study in an animal (rodent) model for endometriosis. In part this is due to interspecies differences between human 17β-HSD1 and its rodent orthologs. As a result, many compounds which are highly active towards the human enzyme show no or only weak inhibition of murine or rat 17β-HSD1

Accordingly, the main goal of the present thesis was the design, the synthesis and the biological evaluation of potent and selective non-steroidal inhibitors of both the human and rodent 17β-HSD1 enzymes. Suitable 17β-HSD1 inhibitors should display the following characteristics:

- Potency against *h*17β-HSD1.
- Activity against *m*/*r* 17β-HSD1.
- Selectivity towards the type 2 enzyme.

- Selectivity towards ERα and β.
- Favorable ADMET parameters.

Inhibitor design should aim at the identification of a suitable candidate for a PoP study in an *in vivo* animal disease model for endometriosis

2.2 Working strategy

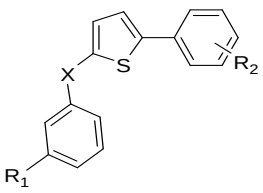
The class of bicyclic substituted hydroxyphenylmethanones (BSHs), which was described by our group previously, contains some of the most potent and selective non-steroidal inhibitors of human 17β-HSD1. In addition, several compounds show inhibition of the murine otholog which makes them valuable starting points in the search for a suitable PoP candidate.

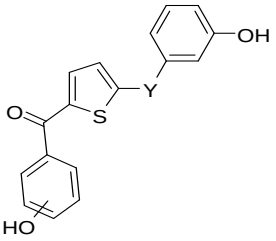
Interesting structure activity relationships (SARs) have been derived previously in this class of compounds and resulted in a conception concerning the interactions between the inhibitors and the target enzyme, derived using computational methods (molecular docking). The knowledge on SAR, however, was still fragmentary.

A problem of the class of BSHs is the presence of phenolic OH- groups which preclude the use of many compounds in an *in vivo* study due their high liability towards phase II metabolism.

Therefore, our objective in this thesis was – firstly - to obtain more insight in the SARs of BSHs by rational structural modifications. This procedure should also allow to estimate the validity of the docking results (Chapter **3.I**).

Subsequently, rational strategies were applied to elucidate drug metabolism and to obtain metabolically stable compounds (chapter **3.II)**. In addition, the designed compounds were consistently screened for their inhibitory activity towards rodent 17β-HSD1. Furthermore, the implementation of favorable *in vitro* ADME parameters (lipophilicity, solubility) was taken into account.

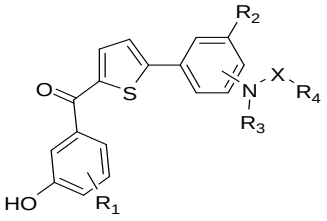


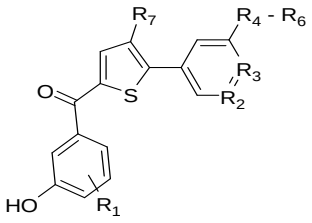


R_1 = OH, NH_2, Cl, CH_3, CO_2H, CO_2CH_3
NH-acyl, NH-sulfonyl, NH-alkyl
R_2 = H, OC_2H_5, CH_2OH, O-alkyl.
X = CO, CS, CF_2, CH_2

Y = S, SO_2

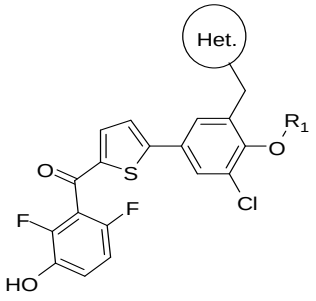
Part **I**





R_1 = H, F
R_2 / R_3 = H, CH_3
R_4 = substituted phenyl, heterocyclic subs., cyclopropane
X = SO_2 , CO

R_1 = H, CH_3, F
R_2 / R_3 = H, N
R_4 - R_6 = e.g. OH, OCH_3, Cl, CN, F, CH_3
R_7 = H, CH_3



R_1 = H, CH_3

Part **II**

Figure 14: structure overview of the molecules described in this thesis

3 Results

3.I Inhibition of 17β-HSD1: SAR of Bicyclic Substituted Hydroxyphenylmethanones and Discovery of New Potent Inhibitors with Thioether Linker

This chapter is submitted to the European Journal of Medicinal Chemistry for publication.

Abstract

Estradiol is the most potent estrogen in humans. It is known to be involved in development and proliferation of estrogen dependent diseases such as breast cancer and endometriosis. The last step of its biosynthesis is catalyzed by 17β-hydroxysteroid dehydrogenase type 1 (17β- HSD1) which consequently is a promising target for the treatment of these diseases. Recently, we reported on bicyclic substituted hydroxyphenylmethanones as potent inhibitors of 17β-HSD1. The present study focuses on rational structural modifications in this compound class with the aim of gaining more insight into its structure-activity relationship (SAR). (4-Hydroxyphenyl)-(5-(3-hydroxyphenylsulfanyl)-thiophen-2-yl)methanone (**25**) was discovered as a member of a novel potent class of human 17β-HSD1 inhibitors. Computational methods were used to elucidate its interactions with the target protein. The compound showed activity also towards the *murine* 17β-HSD1 enzyme and thus is a starting point for the design of compounds suitable for evaluation in an animal disease model.

Introduction

The important roles of estrogens and androgens in female and male development and reproduction are well known.[1] They exert their effects by transactivation of the respective nuclear receptors,[2] although also non-genomic effects are discussed.[3] However, these steroidal sex hormones are also involved in the genesis and the progression of diseases. Estrogens are known to stimulate the progression of estrogen-dependent diseases (EDD) like endometriosis,

the majority of breast cancers, and uterine leiomyoma.[4-7] Besides surgery, chemo- and immunotherapy, the inhibition of estrogen biosynthesis and the blockade of estrogen action, respectively, are today standard therapies for these diseases. These treatments (with aromatase inhibitors, GnRH-analogs, antiestrogens, selective estrogen receptor modulators (SERMs)), however, have a systemic mode of action, i.e. they reduce estrogen effects not only in the diseased tissue. As a result, they may lead to considerable side effects. A novel approach mainly aiming at lowering intracellular estrogen production in the diseased tissue could be a significant improvement for EDD therapy. Such an intracrine approach is presently being pursued using steroid sulfatase inhibitors in the treatment of hormone-dependent cancers[8], and is already successfully applied in the treatment of androgen dependent diseases (ADD) by using 5α-reductase inhibitors.[9]

More recently, 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1, SDR28C1) has attracted attention as a potential target for the treatment of EDD. The enzyme catalyses the final step of estradiol (E2) biosynthesis which is the most potent estrogen in humans (fig. 1).

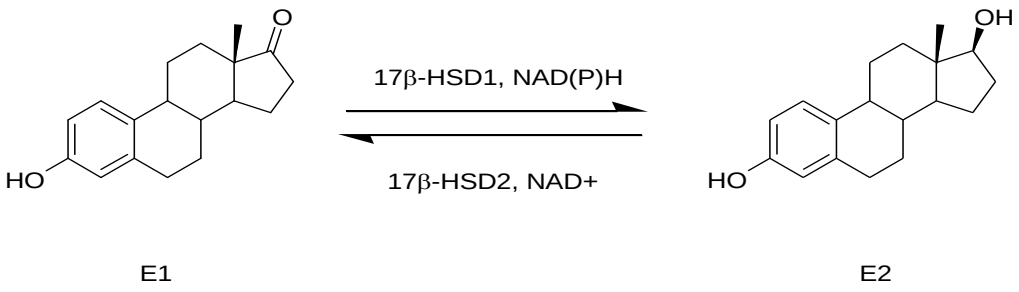
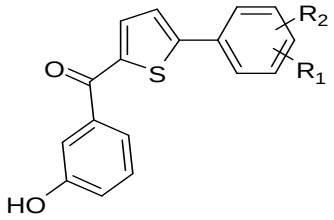


Figure 1. Interconversion of Estrone (E1) and Estradiol (E2).

17β-HSD1 is described to be overexpressed at mRNA level in breast cancer tissue[10-12] and endometriotic lesions.[13] Since a more local mode of action can be anticipated compared to existing medical treatments, its selective inhibition is regarded as a promising strategy for the treatment of EDD, with the prospect of less side effects. No 17β-HSD1 inhibitor has entered clinical trials until now, but there is experimental evidence that inhibition of the enzyme is effective against estrone (E1) induced growth of human tumor cells *in vitro* and *in vivo*.[14-17] The availability of compounds not only inhibiting the human enzyme but also 17β-HSD1 of another species would be a prerequisite for a proof of principle study concerning the applicability of 17β-HSD1 inhibitors in the treatment of endometriosis.

17β-HSD2 can be considered as a functional counterpart of the type 1 enzyme as it de-activates E2 by transforming it to E1. Thus, it plays a protective role against too high E2 concentrations and should therefore not be inhibited by 17β-HSD1 inhibitors.

Both steroidal[18, 19] and non-steroidal[20-31] 17β-HSD1 inhibitors have been described in the past. Recently we reported on bicyclic substituted hydroxyphenylmethanones[32] (general structure, fig. 2) which combine low molecular weight with high inhibitory potency (high ligand efficiency) and show strong intracellular activity. The aim of the present study is to obtain more insight in the structure activity relationships (SARs) of this compound class by rational structural modifications. Moreover, the inhibitory activities of the synthesized compounds are used to verify previous docking results[32] using a broader base of biological data.



R_1, R_2: e.g. H, OH, O-alkyl, CN, hetaryl

Figure 2. Bicyclic substituted hydroxyphenylmethanones: General structure.

Design

Starting point for the design of compounds **1-28** (chart 1) were our conceptions concerning the binding mode of bicyclic substituted hydroxyphenylmethanones (fig. 3)[32]: Previous molecular docking results suggest

a) A hydrogen bond interaction of the hydroxy group of the benzoyl moiety with Asn152.
b) A bifurcated H-bond between the carbonyl group and the hydroxyl groups of Ser142 and Tyr155
c) Another bifurcated H-bond between the OH-group of the hydroxyphenyl moiety and the side chains of His221 and Glu282
d) The close proximity of the side chains of Tyr218 and Ser222 to the inhibitor

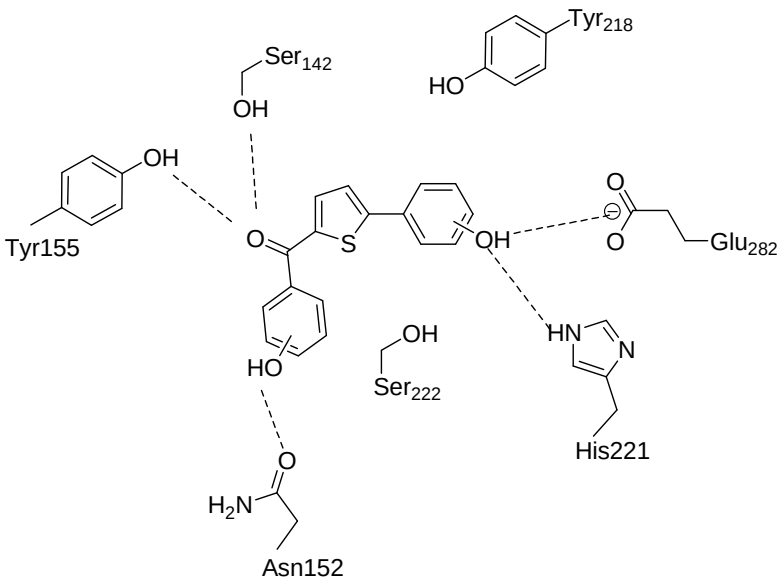


Figure 3. Schematic H-bond interactions of bicyclic substituted hydroxyphenylmethanones with 17β-HSD1.[32]

In order to evaluate the structure activity relationships in this compound class, the different structural features mentioned above (a-c) were replaced by possible bioisosteres or other functional groups, see fig. 4 (modifications a-c). This procedure should also allow to estimate the validity of the docking results (fig. 3). In addition, attempts were made to establish additional interactions to Tyr218 and Ser222 by introducing a second linker function, between the heterocycle and the hydroxyphenyl moiety (modification d).

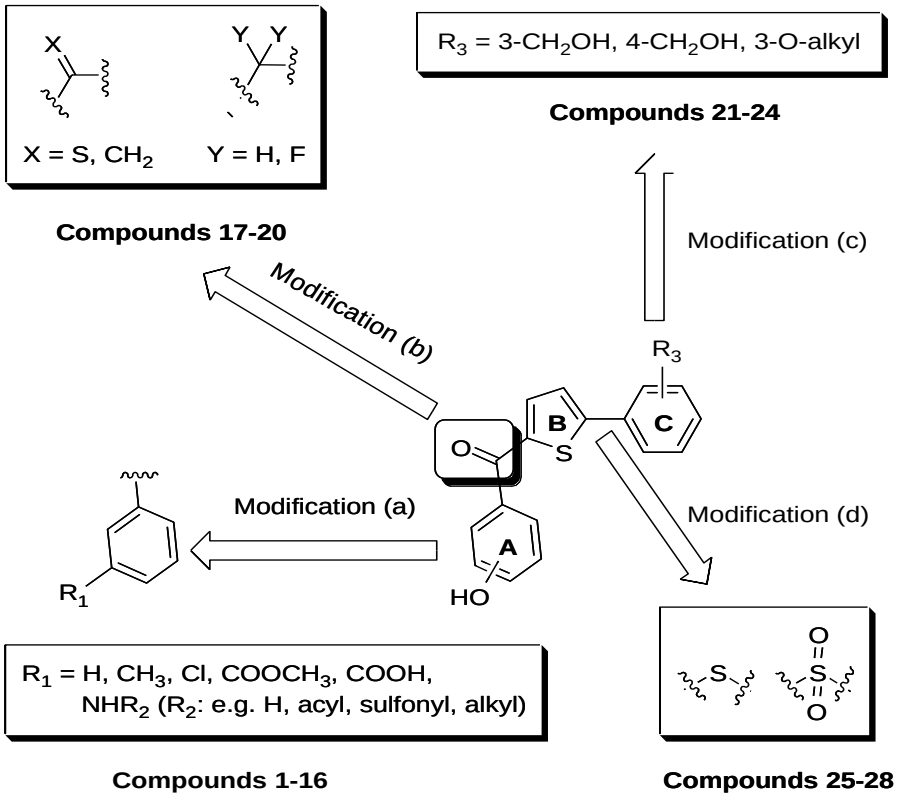
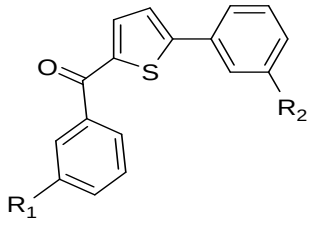


Figure 4. Design of potential inhibitors **1-28**.

Results



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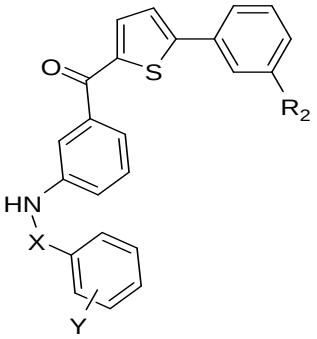

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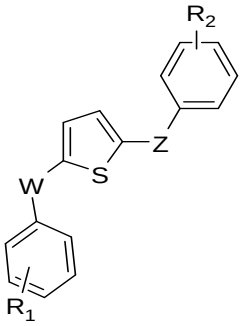
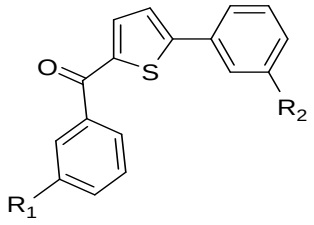
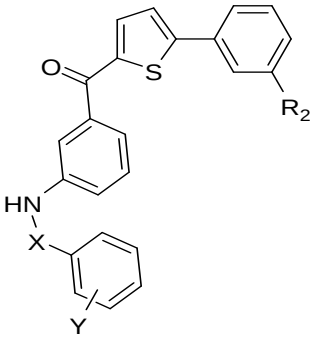


17-28

Comd	R_1	R_2	X	Y	Comd	R_1	R_2	W	Z
1	H	H	-	-	**17**	3-OH	H	CF_2	-
2	CH_3	H	-	-	**18**	3-OH	H	C=S	-
3	Cl	H	-	-	**19**	3-OH	H	CH_2	-
4	$COOCH_3$	H	-	-	**20**	3-OH	H	$C=CH_2$	-
5	COOH	H	-	-	**21**	3-OH	3-CH_2OH	C=O	-
6	NH_2	OC_2H_5	-	-	**22**	3-OH	4-CH_2OH	C=O	-
7	$NHSO_2CF_3$	OC_2H_5	-	-	**23**	3-OH	3-$OCH(CH_3)_2$	C=O	-
8	$NHCH(CH_3)_2$	OC_2H_5	-	-	**24**	3-OH	3-$OCH_2CH(CH_3)_2$	C=O	-
9	$NHC(S)NHCH_3$	OC_2H_5	-	-	**25**	3-OH	3-OH	C=O	S
10	-	OC_2H_5	CH_2	H	**26**	3-OH	3-OH	C=O	SO_2
11	-	OC_2H_5	SO_2	H	**27**	4-OH	3-OH	C=O	S
12	-	OC_2H_5	SO_2	3-CN	**28**	4-OH	3-OH	C=O	SO_2



1-9



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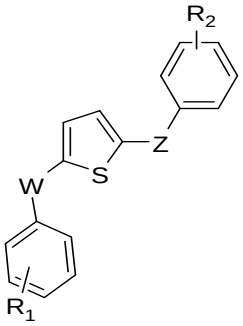


17-28

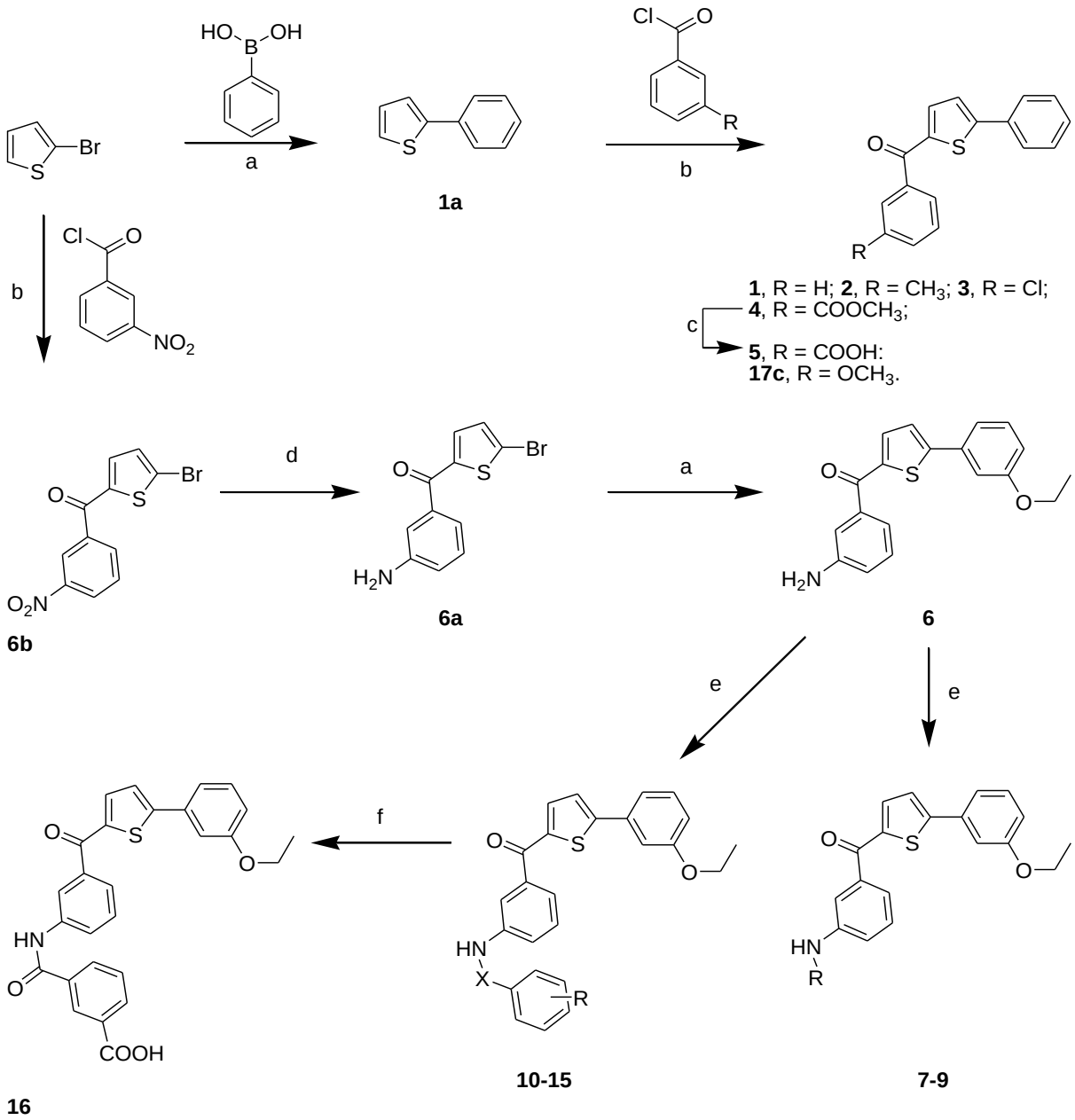
Comd	R_1	R_2	X	Y	Comd	R_1	R_2	W	Z
13	-	OC_2H_5	SO_2	4-OCH_3					
14	-	OC_2H_5	CO	H					
15	-	OC_2H_5	CO	CO_2CH_3					
16	-	OC_2H_5	CO	CO_2H					

Chart 1: Overview on synthesized compounds **1**-**28**.

Chemistry

The synthesis of compounds **1-16** (scheme 1) started from 2-bromothiophene which – for the preparation of **1-5** – was coupled with phenylboronic acid in a Suzuki-reaction.[33] Friedel-Crafts acylation of the resulting intermediate **1a**[34] with the appropriate benzoic acid chloride gave compounds **1-4**.[35] The intermediate **17c**, which was the starting material for the syntheses according to scheme 2, was prepared in the same way. Saponification of the ester **4** led to the carbonic acid **5**.

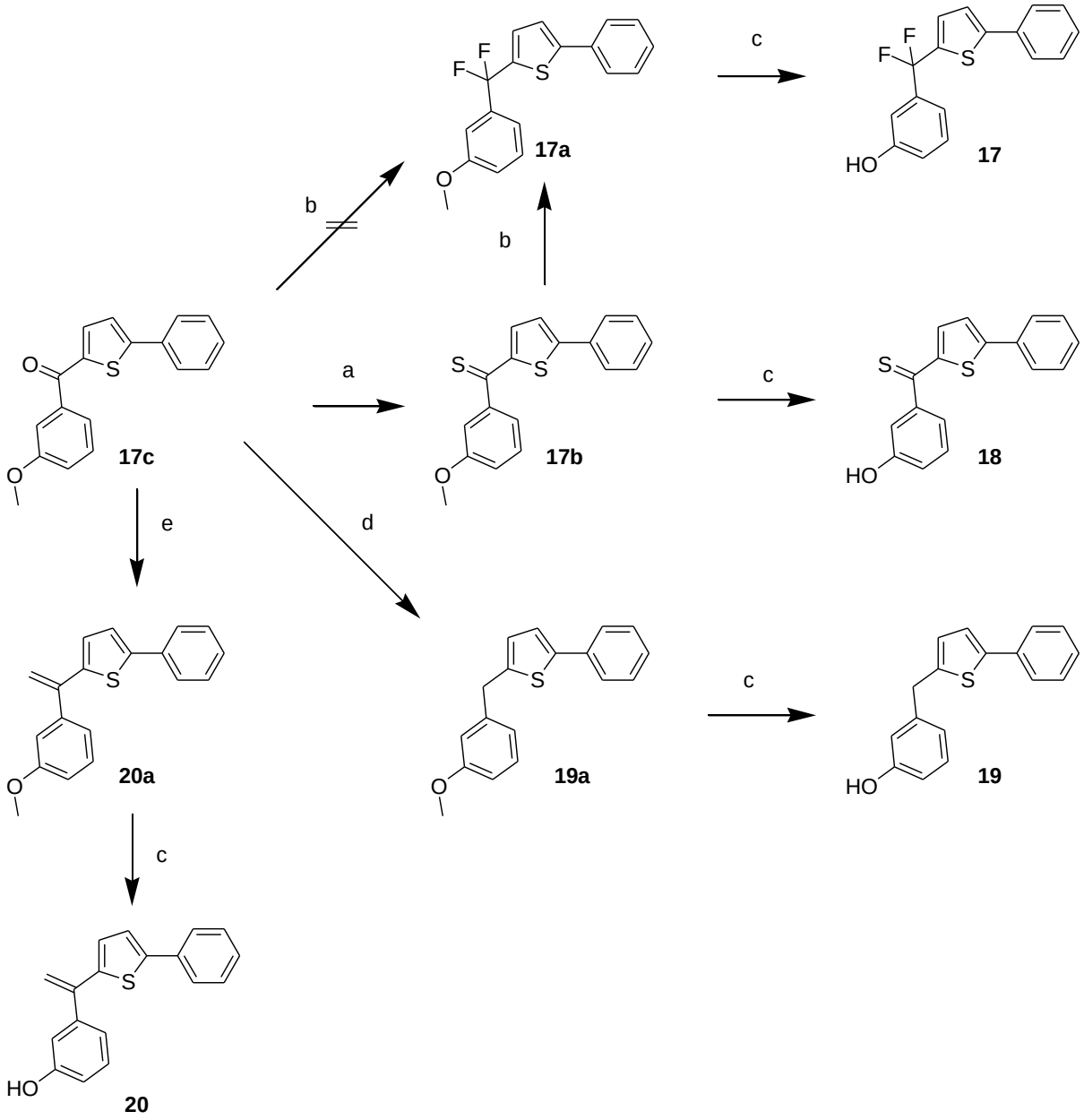
For the synthesis of compounds **6-16**, 2-bromothiophene was converted to the ketone **6b**[36] via Friedel-Crafts acylation with 3-nitrobenzoyl chloride. Reduction of the nitro-group with stannous chloride dihydrate gave amine **6a** which was submitted to a Suzuki reaction with 3-ethoxyphenylboronic acid. The resulting compound **6** was reacted with 2-bromopropane and benzyl bromide to give the secondary amines **8** and **10**, respectively. In the synthesis of the former, copper(II)-oxide had to be added as a catalyst. The thiourea **9** was obtained by reaction of **6** with methylisothiocyanate. The reaction of **6** with sulfonic acid chlorides at room temperature yielded the corresponding sulfonic acid amides **7** and **11-13**. For the analogous preparation of the carbonic acid amides **14** and **15**, higher reaction temperatures had to be applied (pyridine, reflux, overnight). Hydrolysis of the ester **15** under basic conditions gave the carbonic acid **16**.



cmpd	R	cmpd	X	R	cmpd	X	R
7	SO_2CF_3	**10**	CH_2	H	**13**	SO_2	4-OMe
8	$CH(CH_3)_2$	**11**	SO_2	H	**14**	CO	H
9	$C(S)NHCH_3$	**12**	SO_2	3-CN	**15**	CO	COOMe

Scheme 1: a) method A, Cs_2CO_3, $Pd(PPh_3)_4$, DME/water (1:1), reflux, 18 h. b) method B, $AlCl_3$, anhydrous CH_2Cl_2, 0^oC, 0.5 h to rt, overnight. c) LiOH, MeOH/H_2O (7:3). d) $SnCl_2 \cdot 2H_2O$, CH_3OH, reflux, 2 h. e) method D, RSO_2Cl or RCOCl, Pyridine, rt or reflux, overnight (compounds **7, 11-15**); 2-bromopropane, CuO, KOH, DMF, reflux, 4 days (compound **8**); CH_3NCS, THF, Et_3N, reflux, overnight (compound **9**); (bromomethyl)benzene, K_2CO_3, acetone, rt, 10 h (compound **10**). f) 10% NaOH, ethanol, reflux, 2 h.

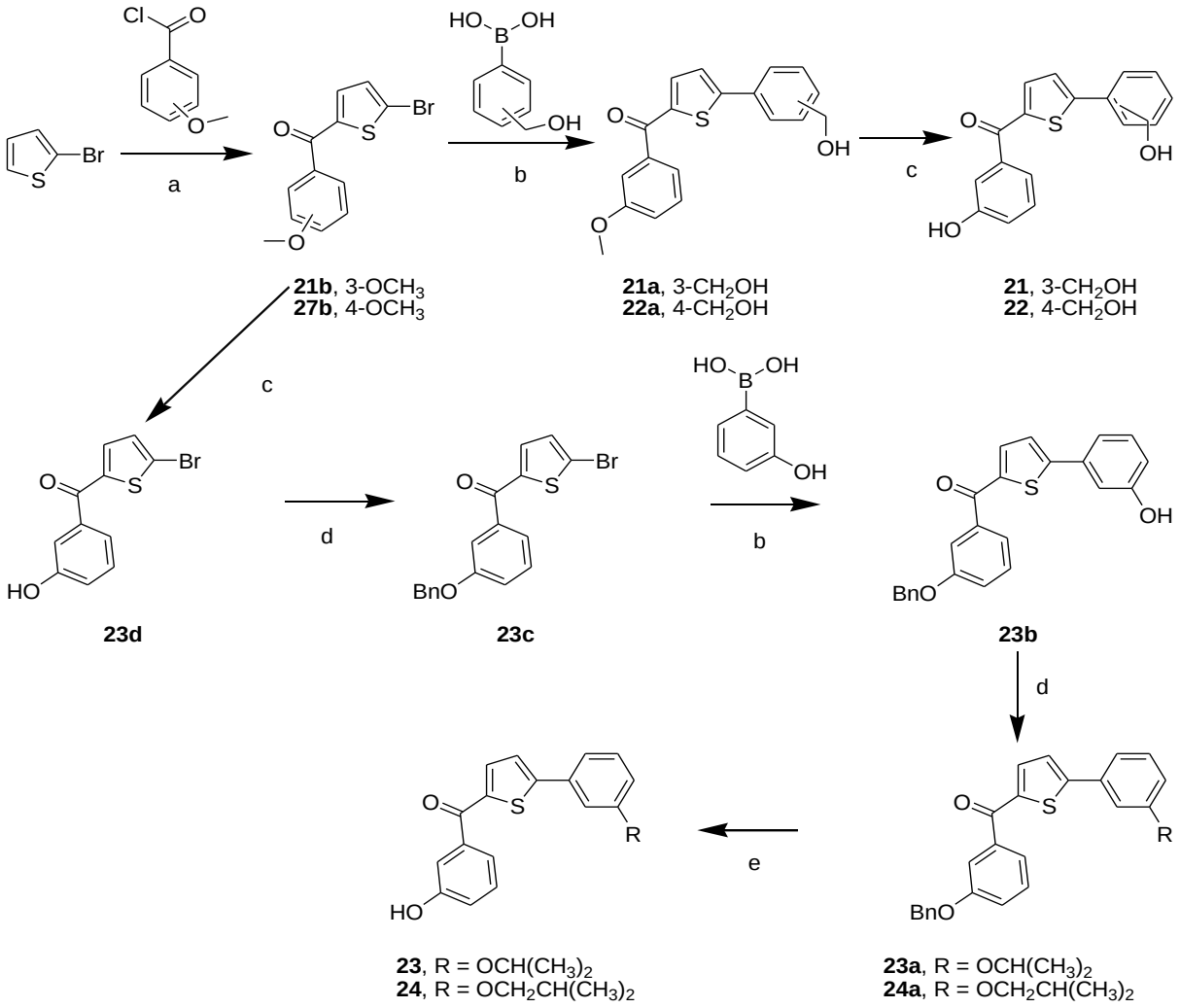
Starting point for the modifications of the carbonyl group between the aromatic moieties was compound **17c** (scheme 2). The conversion to the CF_2-group (compound **17a**) with DAST could not be achieved directly but after formation of the thioketone **17b** using Lawesson's reagent.[37] From **17a** and **17b** the corresponding phenols **17** and **18** could be obtained by ether cleavage (BBr_3, method C).[38] Reduction of the keto function of compound **17c** with stannous chloride dihydrate gave the methylene intermediate **19a**, whereas Wittig reaction with methyltriphenylphosphonium bromide afforded the olefin **20a**. Upon treatment with BBr_3 in anhydrous CH_2Cl_2 (method C) both **19a** and **20a** underwent demethylation resulting in the final compounds **19** and **20**, respectively.



Scheme 2: a) Lawesson´s reagent, toluene, reflux, 2 h. b) DAST, abs. CH_2Cl_2, rt, 3 h. c) method C, BBr_3, CH_2Cl_2, -78°C to rt, overnight. d) $SnCl_2 \cdot 2H_2O$, acetic acid, HCl, reflux, overnight. e) methyltriphenylphosphonium bromide, *n*-BuLi, abs. THF, rt for 2.5 h then 65°C overnight.

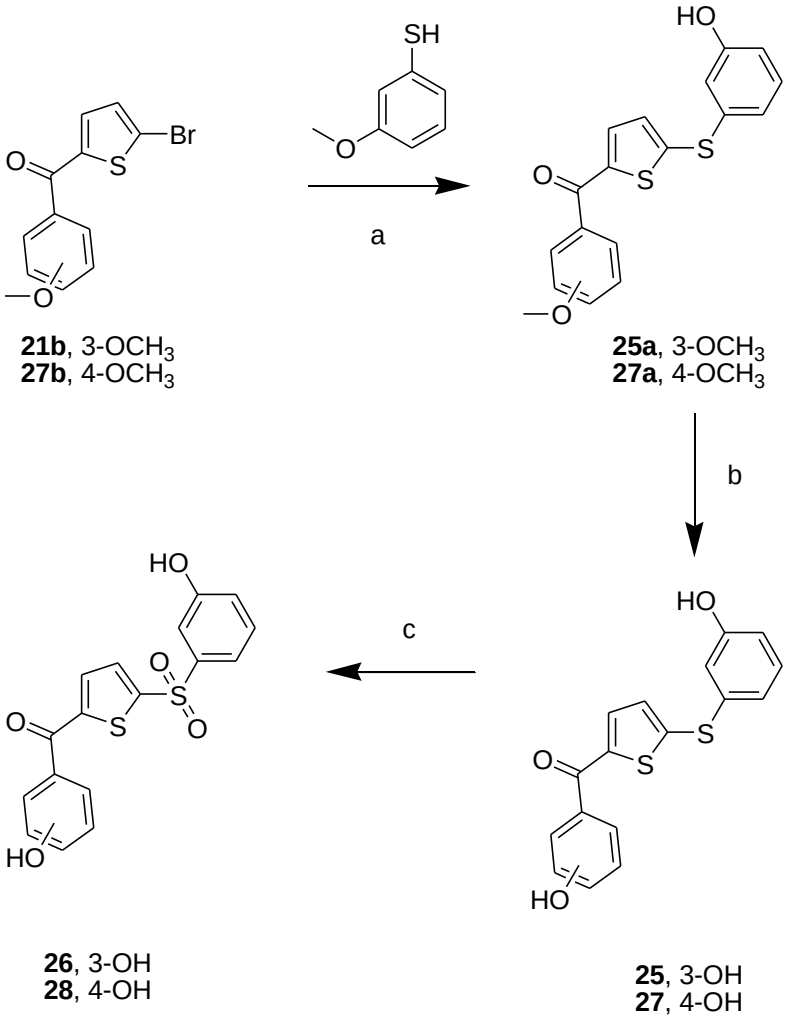
The first step in the synthesis of compounds **21**-**24** was a Friedel-Crafts acylation (method B) of 2-bromothiophene with 3-methoxybenzoylchloride, leading to the benzoylated key intermediate **21b**[32]. The corresponding 4-methoxy isomer **27b**, which was the starting material for one of the syntheses depicted in scheme 4, was synthesized accordingly. Compounds **21** and **22** were

prepared from **21b** by Suzuki cross coupling reactions with the appropriate commercially available boronic acids, resulting in compounds **21a** and **22a**, and subsequent demethylation. The synthetic pathway leading from **21b** to the final compounds **23** and **24** required the replacement of the methoxy- by a benzyloxy-group (compound **23c**) which was accomplished via the phenolic intermediate **23d**[32]. A subsequent Suzuki cross coupling reaction with 3-hydroxyphenylboronic acid led to compound **23b** which was alkylated using isopropyl- and isobutyl iodide to afford the ethers **23a** and **24a**, respectively. Selective debenzylation using BCl_3 instead of BBr_3 (method C) yielded the final compounds **23** and **24** (scheme 3).



Scheme 3: a) method B, $AlCl_3$, anhydrous CH_2Cl_2, 0 °C, 0.5 h to rt, overnight. b) method A, Cs_2CO_3, $Pd(PPh_3)_4$, DME/water (1:1), reflux, 18 h. c) method C, BBr_3, CH_2Cl_2, -78°C to rt, overnight. d) R-I, Cs_2CO_3, acetone, reflux, overnight. e) method C, BCl_3, CH_2Cl_2, -78°C to rt, overnight.

The synthesis of sulfides (**25** and **27**) and sulfones (**26** and **28**) is depicted in scheme 4. The intermediates **21b** and **27b** were transformed to the sulfides **25a** and **27a**, respectively, using 3-methoxybenzenethiol in aqueous dimethylformamide in the presence of potassium hydroxide and copper(II)-oxide as catalyst. The methoxy functions were cleaved with BBr_3 according to method C to yield the hydroxylated compounds **25** and **27**. The sulfones **26** and **28** were obtained from **25** and **27**, respectively, by oxidation with hydrogen peroxide in acetic acid at room temperature.



Scheme 4: a) CuO, KOH, DMF, 135°C, 2 h. b) method C, BBr_3, CH_2Cl_2, -78°C to rt, overnight. c) H_2O_2 (30%), acetic acid, rt, 4 days.

Biological results

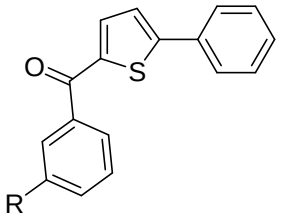
Inhibition of human 17β-HSD1 and selectivity towards human 17β-HSD2

Human placental enzymes were used for both assays and were obtained according to described methods.[39-41] In the *h*17β-HSD1 assay, incubations were run with cytosolic fractions, tritiated E1, cofactor and inhibitor. The separation of substrate and product was accomplished by HPLC. The *h*17β-HSD2 assay was performed similarly using tritiated E2 as substrate and a microsomal fraction. Activities are given as percent inhibition at 1 µM (tables 1-3). For the most active compounds IC_{50} values are reported (table 3). Compounds **A**-**C** identified in our previous work were used as reference compounds.[32]

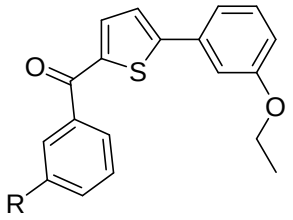
Modification a

In the search for an appropriate replacement of the hydroxy-group at the benzoyl moiety several structural modifications starting from the reference compounds **A** and **B** were investigated.[30] Simple omission of the OH-group (modification a; fig. 4), i.e. replacement with H, led to the inactive compound **1** (table 1). Furthermore, different functionalities such as Me, Cl, CO_2H, CO_2CH_3, and NH_2 (compounds **2–6**, respectively) were introduced. The substitutents were chosen considering the Craig plot (variation of the size, lipophilicity, and electronic properties)[42] as well as their ability to form hydrogen bond interactions with the target. Compounds **1**-**5** were inactive or showed only marginal inhibition of 17β-HSD1. The introduction of an amino group, which is at physiological pH the only hydrogen bond donor of the selection, led to the conservation of a residual activity (compound **6**, 24% at 1 µM). These results demonstrate the importance of the hydrogen bond donor function at this position, and are in agreement with the previous hypothesis that an H-bond interaction is established with Asn152 of 17β-HSD1.[30, 32]

Table 1. Inhibition of *human* 17β-HSD1 and 17β-HSD2 by compounds **A**, **B** and **1-6**.



A, 1-5



B, 6

cmpd	**R**	**% inhibition @ 1 µM[a]**	
		***h*17β-HSD1[b]**	***h*17β-HSD2[c]**
A	OH	80	94
1	H	n.i.	n.i.
2	CH_3	14	n.i.
3	Cl	n.i.	n.i.
4	$COOCH_3$	14	11
5	COOH	18	n.i.
B	OH	88	69
6	NH_2	24	n.i.

[a] Mean value of three determinations, standard deviation less than 15%. [b] Human placenta, cytosolic fraction, substrate [^{3}H]-E1, 500 nM, cofactor NADH, 500 µM. [c] Human placenta, microsomal fraction, substrate [^{3}H]-E2, 500 nM, cofactor NAD^+, 1500 µM. n.i. = no inhibition (inhibition <10%).

Attempts were made to enhance the inhibitory activity of compound **6** by modulating the hydrogen bond donating properties of the NH_2-group. For this purpose, several electron-withdrawing or –donating substituents were introduced. These modifications, however, led to a complete loss of activity (compounds **7-16**, chart 1), probably due to the bulkiness of the introduced groups.

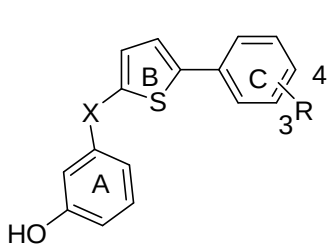
Modification b

Modifications of the keto-linker function (modification b; fig. 4) had a strong impact on biological activity, depending on the nature of the linker group: The thioketo-analog **18** of the reference compound **A** only showed a slightly reduced inhibitory potency (75 % vs. 80 % inhibn. at 1 µM, table 2). In contrast, replacement of the keto-group with an olefinic moiety (compound **20**) or saturated groups (compounds **17** and **19**) led to a strong decrease of inhibitory activity. None of the four compounds **17-20** showed selectivity over 17β-HSD2.

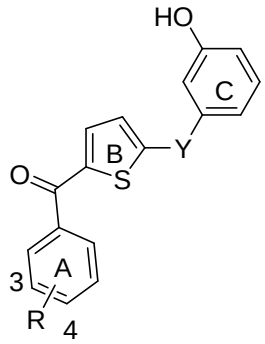
Modification c

Another structural element under investigation in this study was the phenolic OH-group on ring C which was replaced by a hydroxymethyl-group and different ether functions (modification c; fig. 4). These structural variations led to highly active compounds: The formal insertion of CH_2 between the aromatic ring (3- or 4-position) and the OH-group afforded the benzylic alcohols **21** and **22**, showing complete inhibition of *h*17β-HSD1 at a concentration of 1 µM (table 2). Also the bulky ether derivatives **23** and **24** strongly inhibited the target enzyme. All four compounds displayed similarly low IC_{50}-values in the range of 90 nM to 157 nM (table 3), but no selectivity over the type 2 enzyme with the exception of compound **24** (SF = 5.5).

Table 2. Inhibition of *human* 17β-HSD1 and 17β-HSD2 by compounds **A**, **C** and **17-28**.



A, C, 17-24



25-28

cmpd	**X**	**R**	Y	**% inhibition @ 1 µM[a]**	
				***h*17β-HSD1[b]**	***h*17β-HSD2[c]**
A	C=O	H	-	80	94
17	CF_2	H	-	35	36
18	C=S	H	-	75	85
19	CH_2	H	-	16	29
20	$C=CH_2$	H	-	33	49
C	C=O	3-OH	-	89	89
21	C=O	3-CH_2OH	-	100	100
22	C=O	4-CH_2OH	-	100	81
23	C=O	3-O-isopropyl	-	82	73

24	C=O	3-O-isobutyl	-	85	51
25	-	3-OH	S	84	70
26	-	3-OH	SO_2	70	76
27	-	4-OH	S	66	79
28	-	4-OH	SO_2	62	80

[a] Mean value of three determinations, standard deviation less than 15%. [b] Human placenta, cytosolic fraction, substrate [^{3}H]-E1, 500 nM, cofactor NADH, 500 µM. [c] Human placenta, microsomal fraction, substrate [^{3}H]-E2, 500 nM, cofactor NAD^+, 1500 µM. n.i. = no inhibition (inhibition <10%).

Modification d

Compounds bearing an additional linker group, namely between the thiophene ring and the hydroxyphenyl ring C (modification d; fig. 4), showed slightly reduced activity towards the target enzyme, compared to the reference **C** (IC_{50} = 22 nM) when the OH-group on the benzoyl moiety was in the 4-position (compounds **27** and **28**, tables 2 and 3). Moving the OH-group to the 3-position, however, resulted in compounds **25** and **26** with higher activities (IC_{50} = 104 nM and 275 nM, respectively). Thus, concerning the hydroxybenzoyl moiety, the SAR of these novel compounds with two linker functions appears to be similar to that found previously for compounds bearing the keto-linker group only.[32] The compounds **25-28** showed comparable IC_{50}-values for the inhibition of *h*17β-HSD1 and 2 (table 3).

Table 3. IC_{50} values and selectivity factors for compounds **C** and **21-28**.

cmpd	IC_{50} [nM][a]		SF[d]
	*h*17β-HSD1[b]	*h*17β-HSD2[c]	
C	22	109	5.0
21	90	51	0.6
22	157	202	1.3
23	120	224	1.9
24	152	836	5.5
25	104	245	2.4
26	275	283	1.0
27	752	247	0.3
28	630	389	0.6

[a] Mean value of three determinations, standard deviation less than 15%. [b] Human placenta, cytosolic fraction, substrate [^{3}H]-E1, 500 nM, cofactor NADH, 500 µM. [c] Human placenta, microsomal fraction, substrate [^{3}H]-E2, 500 nM, cofactor NAD^+, 1500 µM. n.i. = no inhibition (inhibition <10%). [d] Selectivity factor: IC_{50}(17β-HSD2) / IC_{50}(17β-HSD1).

Inhibition of murine 17β-HSD1

The *murine* 17β-HSD1 enzyme was expressed in HEK293 cells. The inhibitory potencies of compound **25** and the reference compound **C** were evaluated in an assay similar to those of the human enzyme.

Compound **25** turned out to be an inhibitor of *murine* 17β-HSD1 (20 % inhibn. at 1 µM; **C**: 10 %). This is notable since the *human* and the *murine* enzymes differ considerably in primary

structure[43] and most of the compound classes described by us to show strong inhibition of *human* 17β-HSD1 are inactive towards the *murine* enzyme (unpublished results). Thus, compound **25** is a valuable starting point for the design of more potent and selective inhibitors of the *murine* enzymes that allow for *in vivo* evaluation (proof of principle).

Molecular modeling

Computational methods were used in order to elucidate the binding modes of the novel inhibitors with two linker functions. Molecular docking results indicate that both thioether **25** and sulfone **26** fit well into the steroid binding pocket of *h*17β-HSD1 (fig. 5). Interestingly, they dock inversely: The sulfone moiety of compound **26** and the keto-group of **25** are located in the same region of the binding pocket which is distant from Tyr218 and Ser222. An interaction of the sulfone group with these amino acids, which was aimed at, would thus be unlikely.

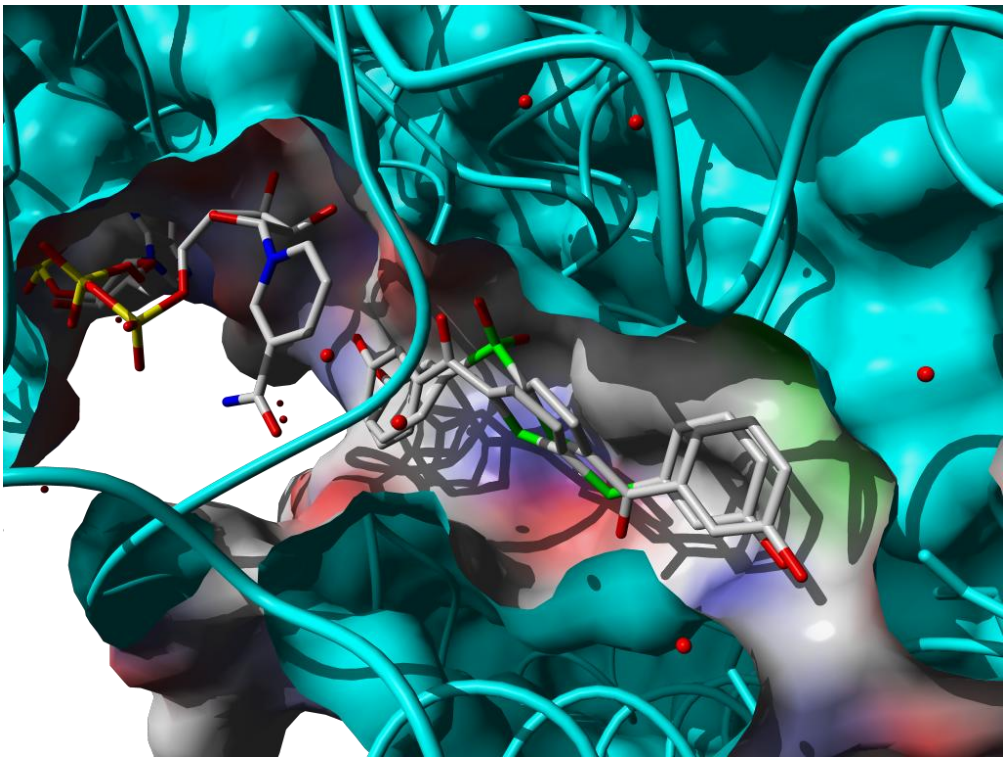


Figure 5. Compounds **25** and **26**, docked into *human* 17β-HSD1 (PDB: 1FDT). Upper left: Cofactor NADPH.

Ligand-protein interactions can be seen in more detail in figure 6: Both compounds form a hydrogen bond with the key amino acid residue, Ser142. Considering compound **25**, this bond is formed via the keto-group of the inhibitor, whereas in case of compound **26** the sulfone moiety is the interaction partner for Ser142 while the keto-group does not appear to play a role in protein binding.

The reversed docking poses of **25** and **26** seem to have an additional effect on ligand-protein interaction: Whereas both phenolic OH-groups of compound **25** are suggested to be involved in hydrogen bonding (with Tyr155 and His221), only one such interaction (with Glu282) can be found for **26**. This is in agreement with the higher inhibitory potency of **25** (IC_{50} = 104 nM; table 4) compared to **26** (IC_{50} = 275 nM).

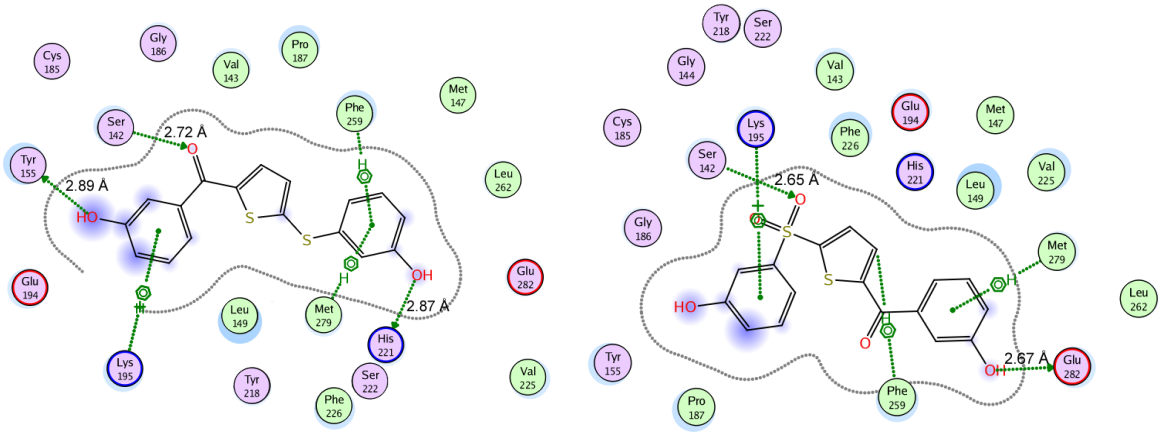


Figure 6. Suggested binding modes showing three H-bonds to Tyr155, Ser142 and His221 for compound **25** (left) and two H-bonds to Glu282 and Ser142 for **26** (right).

Discussion and Conclusions

The aim of our study was the elucidation of structure activity relationships in the class of bicyclic substituted methanones which have previously been identified as highly active inhibitors of the *h*17β-HSD1 enzyme.[30, 32] Modifications have been made in several positions of the inhibitor. One of the structural features under investigation was the phenolic OH-group at the benzoyl moiety (ring A) of inhibitors **A** and **B** which bears the risk to lead to phase II metabolism issues.

Thus, a search for an appropriate replacement of this group was initiated. The functionalities under investigation covered a broad spectrum of lipophilic and electronic properties. In addition, they differed in their abilities to form hydrogen bond interactions with the target. The introduction of chlorine appeared interesting to us as it is known that this substituent, due to its σ-hole property, is able to replace classical donors like OH in H-bonding interactions.[44] On the other hand, also hydrogen bond acceptors (such as ester or carboxylate function) seemed promising, as it was hypothesized earlier that the OH-group of the inhibitor – as a donor - forms a hydrogen bond with the carbonyl function of the Asn152 side chain.[32] This amino acid residue, however, is in principle also able to act as an H-bond donor via its NH_2-group, allowing for an interaction with an appropriate acceptor function of the inhibitor. Regardless of the properties of the chosen residue, however, a complete loss or a strong reduction of inhibitory activity was observed (compounds **2-5**). Even the NH_2-group cannot replace the OH-group of compound **B** in a satisfactory manner (compound **6**), in spite of comparable physico-chemical and H-bonding properties. Similarly sharp structure activity relationships have also been reported for other classes of 17β-HSD1 inhibitors.[28, 45]

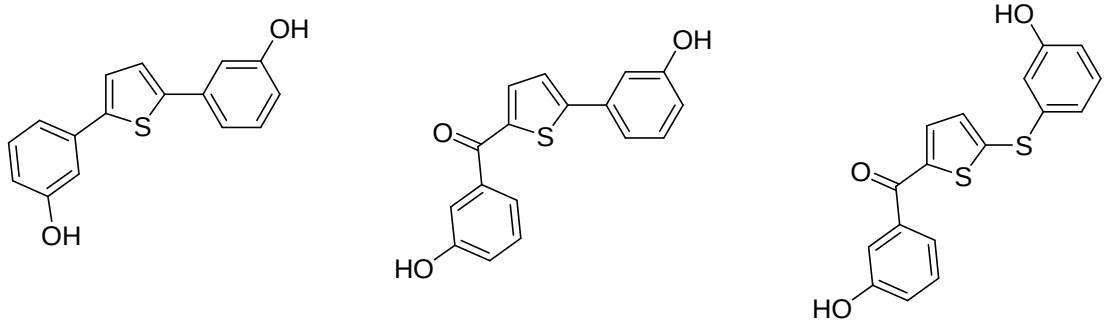
In contrast to the OH-group at the benzoyl moiety (ring A, cf. fig. 4) of compound **C**, the hydroxy-function attached to the phenyl-group (ring C) can be replaced or structurally modified to a considerable extent. It was shown earlier that even bulky aromatic residues are well tolerated by *h*17β-HSD1 and can be introduced to increase selectivity over the type 2 enzyme.[30] A similar observation can be made when the OH group is transformed to an aliphatic ether: In spite of their voluminous ether groups, compounds **23** and **24** (IC_{50} = 120 and 152 nM, respectively; table 3) are only five- to seven-fold less active than the reference **C** (IC_{50} = 22 nM), and their activities are comparable to those shown by compounds **21** and **22** (IC_{50} = 90 and 157 nM, respectively), each bearing a rather small hydroxymethyl group. As expected for compounds **21-24**, selectivity over 17β-HSD2 is enhanced with increasing bulkiness or lipophilicity of the substituent (selectivity factors 0.6 (compound **21**) to 5.5 (compound **24**)).

Considerable attention was paid to the analysis of the role that is played by the keto-group of the lead compound **A**. Replacement of the keto- (compound **A**) by the thioketo-group led to a compound with slightly decreased activity (compound **18,** table 2). This may be explained by the fact that the thioketone retains the geometry of the parent compound **A**, but is a poorer interaction partner for hydrogen bonding. Olefin **20** showed a significant decrease in activity compared to **18** (33 % vs. 75 % inhibn. at 1 µM), which is in agreement with the lacking hydrogen bonding function. Compounds **17** and **19** were weak inhibitors of 17β-HSD1. Both the difluoromethylene group (compound **17**) and its methylene analog (**19**) show a different geometry compared to compound **A**, due to their sp^3-hybridized carbon atoms. The finding that the fluorinated compound **17** is a stronger inhibitor than **19** (35 % vs. 16 % inhibn. at 1 µM) matches with the fact that fluorine atoms may show weak hydrogen bond accepting capabilities.[46] Thus, the biological data obtained for compounds **17**-**20** is in agreement with

former molecular docking studies which suggests a bifurcated H-bond interaction between the carbonyl-group of the inhibitor and Tyr155 and Ser142.[32]

The introduction of a second linker function was triggered by the concept to establish additional hydrogen bond interactions of the inhibitor with the OH groups of Tyr218 and Ser222 (sulfones **26** and **28**). As suggested by molecular docking results of **26** (fig. 6b), however, they showed a different binding mode compared to the bicyclic substituted hydroxyphenylmethanone class which precludes an establishment of these hydrogen bond interactions.

The structure of the strong inhibitor **25** may be looked upon as the result of a sequential enlargement of the bis(hydroxyphenyl)thiophene compound class (fig. 7, **D**) and can be considered as a starting point for the investigation of a novel and potent class of *h*17β-HSD1 inhibitors bearing two linker groups.



D, (IC_{50} = 173 nM) **C,** (IC_{50} = 22 nM) **25,** (IC_{50} = 104 nM)

Figure 7. Comparison of potent *h*17β-HSD1 inhibitors: representatives of bis(hydroxyphenyl)thiophenes[31] (**D**), bicyclic substituted hydroxyphenylmethanones[32] (**C**) and compound **25**.

In conclusion, rational structure modifications have been carried out in order to investigate structure-activity relationships of bicyclic substituted hydroxyphenylmethanones (fig. 7, **C**) which have recently been described as promising non-steroidal inhibitors of *h*17β-HSD1. The results revealed that inhibitory activity is highly sensitive to changes in the hydroxybenzoyl part of the compounds, whereas in the phenyl moiety major changes in nature and size of the substituent are well tolerated. The biological data obtained support the conception that the keto linker group is involved in hydrogen-bonding interactions with the target. The introduction of a second linker function led to the discovery of the potent inhibitor (4-hydroxyphenyl)-[5-(3-hydroxyphenylsulfanyl)-thiophen-2-yl]methanone (**25**) which is regarded as a member of a novel inhibitor class and a starting point for further optimization concerning selectivity towards *h*17β-HSD2 and inhibition of *m*17β-HSD1.

Experimental Section

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

Column chromatography (CC) was performed on silica gel (70 – 200µm), reaction progress was monitored by thin layer chromatography (TLC) on Alugram SIL G UV_{254} (Macherey-Nagel).

^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker AM500 spectrometer (500 MHz) at 300 K. Chemical shifts are reported in δ (parts per million: ppm), by reference to the hydrogenated residues of deuteriated solvent as internal standard (CDCl3: δ = 7.24 ppm (^{1}H NMR) and δ = 77 ppm (^{13}C NMR), CD_3OD: δ = 3.35 ppm (^{1}H NMR) and δ = 49.3 ppm (^{13}C NMR), CD_3COCD_3: δ = 2.05 ppm (^{1}H NMR) and δ = 29.9 ppm (^{13}C NMR), CD_3SOCD_3 δ = 2.50 ppm (^{1}H NMR) and δ = 39.5 ppm (^{13}C NMR)). Signals are described as s, d, t, dd, ddd, m, dt, q, sept for singlet, doublet, triplet, doublet of doublets, doublet of doublets of doublets, multiplet, doublet of triplets, quadruplet and septet, respectively. All coupling constants (*J*) are given in hertz (Hz).

Melting points (mp) were measured in open capillaries on a Stuart Scientific SMP3 apparatus and are uncorrected.

A mass spectra ESI was recorded on a TSQ Quantum (Thermo Finnigan) instrument.

Tested compounds are >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.

The following compounds were prepared according to previously described procedures: 2-phenylthiophene (**1a)**,[34] phenyl-(5-phenyl-thiophen-2-yl)-methanone **(1)**,[35] (5-bromo-thiophen-2-yl)-(3-nitro-phenyl)-methanone **(6b)**,[36] (5-bromo-thiophen-2-yl)(3-methoxy-phenyl)methanone (**21b**)[32] and (5-bromo-thiophen-2-yl)(3-hydroxyphenyl)methanone (**23d**)[32].

General Procedure for Suzuki coupling. Method A. A mixture of arylbromide (1 equiv), boronic acid derivative (1.2 equiv), cesium carbonate (4 equiv) and tetrakis(triphenylphosphine) palladium (0.01 equiv) was suspended in an oxygen-free DME/water (1:1) solution and refluxed under nitrogen for 4h. The reaction mixture was cooled to room temperature. The aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over magnesium sulfate, filtered and concentrated to dryness. The product was purified by CC.

General Procedure for Friedel-Crafts Acylation. Method B. A mixture of monosubstituted thiophene derivate (1 equiv), arylcarbonyl chloride (0.9 equiv), and aluminum trichloride (1

equiv) in anhydrous dichloromethane was warmed to room temperature and stirred for 3h. 1M HCl was used to quench the reaction. The aqueous layer was extracted with dichloromethane. The combined organic layers were washed with brine, dried over magnesium sulfate, filtered, and concentrated to dryness. The product was purified by CC.

General Procedure for Ether Cleavage. Method C. To a solution of ether derivative (1 equiv) in anhydrous dichloromethane at -78 ^{o}C (dry ice/acetone bath), boron tribromide in dichloromethane (1M, 3 equiv per methoxy function) -boron trichloride in dichloromethane (1M, 2 equiv) in case of **23** and **24**- was added dropwise. The reaction mixture was stirred overnight at room temperature under nitrogen atmosphere. Water was added to quench the reaction, and the aqueous layer was extracted with dichloromethane. The combined organic layers were washed with brine, dried over magnesium sulfate, filtered, and concentrated to dryness. The product was purified by CC.

General procedure for sulfonamide/amide coupling. Method D. The amino phenyl derivative (1 equiv) was dissolved in absolute pyridine and was spiked with sulfonyl chloride/acid chloride (1.5 equiv). The reaction mixture was stirred overnight at rt (refluxed in case of amide coupling). The reaction was quenched by adding 10 mL of 2N HCl and extracted with ethyl acetate. The organic layers were washed with saturated $NaHCO_3$ and brine, dried over magnesium sulfate, filtered and concentrated to dryness. The product was purified by CC.

(5-Phenylthiophen-2-yl)(*m*-tolyl)methanone (2). The title compound was prepared by reaction of 2-phenylthiophene (150 mg, 0.94 mmol) **(1a)**, 3-methylbenzoyl chloride (111 µl, 0.84 mmol) and aluminum chloride (125 mg, 0.94 mmol) according to method B. The product was purified by CC (hexane/ethyl acetate 98:2); yield: 57 % (149 mg). ^{1}H NMR (CD_3COCD_3) δ 7.84-7.80 (m, 2H), 7.72 (d, *J*=3.8 Hz, 1H), 7.71-7.67 (m, 2H), 7.61 (d, *J*=3.8 Hz, 1H), 7.52-7.40 (m, 5H), 2.45 (s, 3H);^{13}C NMR (CD_3COCD_3) δ 186.62, 151.84, 141.70, 137.84, 135.67, 132.62, 132.41, 128.77, 128.63, 127.91, 125.64, 125.52, 124.04, 20.08; MS (ESI): 279.40 $(M+H)^{+}$.

(3-Chlorophenyl)(5-phenylthiophen-2-yl)methanone (3). The title compound was prepared by reaction of 2-phenylthiophene (150 mg, 0.94 mmol) **(1a)**, 3-chlorobenzoyl chloride (180 µl, 0.84 mmol) and aluminum chloride (125 mg, 0.94 mmol) according to method B. The product was purified by CC (hexane/ethyl acetate 98:2); yield: 12 % (30 mg). ^{1}H NMR (CD_3COCD_3) δ 7.89-7.82 (m, 4H), 7.76 (d, *J*=4.1 Hz, 1H), 7.73-7.69 (m, 1H), 7.65-7.62 (m, 2H), 7.54-7.49 (m, 2H), 7.48 -7.43 (m, 1H);^{13}C NMR (CD_3COCD_3) δ 186.61, 154.12, 142.57, 140.85, 137.61, 135.04, 134.03, 132.94, 131.31, 130.45, 129.41, 128.31, 127.24, 125.77; MS (ESI): 299.80 $(M+H)^{+}$.

Methyl 3-(5-phenylthiophene-2-yl-carbonyl)benzoate (4). The title compound was prepared by reaction of 2-phenylthiophene (300 mg, 1.87 mmol) **(1a)**, methyl-3-(chloroformyl)benzoate (334 mg, 1.68 mmol) and aluminum chloride (250 mg, 1.87 mmol) according to method B. The product was purified by CC (dichloromethane/methanol 95:5); yield: 18 % (108 mg; yellow oil).

^{1}H NMR (CD_3COCD_3) δ 8.47 (dt, *J*= 2.0, 1.1 Hz, 1H), 8.30-8.26 (m, 1H), 8.17-8.13 (m, 1H), 7.85-7.81 (m, 2H), 7.78-7.73 (m, 2H), 7.64 (d, *J*= 3.8 Hz, 1H), 7.53-7.49 (m, 2H), 7.48-7.42 (m, 1H), 3.94 (s, 3H); ^{13}C NMR (CD_3COCD_3) δ 187.12, 166.67, 154.02, 142.74, 139.32, 137.41, 134.07, 133.62, 131.63, 130.44, 130.26, 130.01, 127.17, 125.62, 52.76; MS (ESI): 323.42 $(M+H)^+$.

Methyl 3-(5-phenylthiophene-2-yl-carbonyl)benzoic acid (5). Methyl 3-(5-phenylthio-phene-2-yl-carbonyl)benzoate (70 mg, 0.22 mmol, 1.00 equiv) **(4)** was dissolved in 5ml methanol: water (70:30). Lithium hydroxide (15.6 mg, 0.66 mmol, 3.00 equiv) was added and the reaction mixture stirred at 50°C for 2h. The mixture was cooled to room temperature, quenched with 1N NaOH (pH 10-12) and washed two times with ethyl acetate. The aqueous layer was acidified with 1N HCl to pH 1 and extracted three times with ethyl acetate. The combined organic layers were dried over magnesium sulfate, filtered and evaporated under reduced pressure. No further purification was required; yield: 95 % (65 mg; white solid). ^{1}H NMR (CD_3COCD_3) δ 11.46-11.04 (m, 1H), 8.56-8.50 (m, 1H), 8.31 (dt, *J*= 7.9, 1.1Hz, 1H), 8.16 (dq, *J*= 7.6, 1.1Hz, 1H), 7.86-7.81 (m, 2H), 7.78-7.74 (m, 2H), 7.65 (d, *J*= 3.8Hz, 1H), 7.53-7.49 (m, 2H), 7.48-7.43 (m, 1H); ^{13}C NMR (CD_3COCD_3) δ 187.54, 154.02, 142.71, 139.23, 137.47, 134.01, 133.85, 130.74, 130.22, 139.91, 127.10, 125.55; MS (ESI): 309.56 $(M+H)^+$.

(3-Aminophenyl)(5-bromothiophen-2-yl)methanone (6a). A suspension of **6b** (665 mg, 2 mmol) and tin(II)-chloride dihydrate (2388 mg, 11 mmol) in methanol (10 mL) was refluxed for 2 h. The solvent was removed under vacuum and the residue was diluted with saturated $NaHCO_3$ and water. The suspension was extracted with ethyl acetate. The combined extracts were washed with brine, dried over magnesium sulfate, filtered and evaporated under reduced pressure to give **6a** (450 mg, 75 %, brown solid, mp. 102-4 ^{o}C). The product was sufficiently pure for use in the subsequent reaction.

(3-Amino-phenyl)(5-(3-ethoxy-phenyl)-thiophen-2-yl)methanone (6). The title compound was prepared by reaction of (3-amino-phenyl)-(5-bromo-thiophen-2-yl)-methanone **(6a)** (600 mg, 2.13 mmol), 3-ethoxyphenylboronic acid (424 mg, 2.55 mmol), cesium carbonate (2772 mg, 8.51 mmol) and tetrakis(triphenylphosphine) palladium (6 mg, 5 µmol) according to method A. The product was purified by CC (hexane/ethyl acetate 80:20); yield: 83 % (570 mg; yellow solid). ^{1}H NMR ($CDCl_3$) δ 7.54 (d, *J* = 4.0 Hz, 1H), 7.26 – 7.22 (m, 2H), 7.18 (dd, *J* = 1.6, 1.1 Hz, 1H), 7.18 – 7.14 (m, 2H), 7.13 – 7.11 (m, 1H), 7.07 (dd, *J* = 2.1, 1.4 Hz, 1H), 6.83 (ddd, *J* = 8.2, 2.5, 1.0 Hz, 1H), 6.80 (ddd, *J* = 7.5, 2.4, 1.6 Hz, 1H), 4.01 (q, *J* = 7.0 Hz, 2H), 3.78 (s, 2H), 1.37 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 188.17, 159.37, 152.88, 146.62, 142.24, 139.09, 135.78, 135.36, 134.55, 130.10, 129.17, 123.87, 119.37, 118.67, 118.65, 115.06, 112.41, 63.57, 14.75; MS (ESI): 324.00 $(M+H)^+$.

***N*-(3-(5-(3-Ethoxyphenyl)-thiophene-2-yl-carbonyl)-phenyl)-C,C,C-trifluoro-methanesulfonamide (7).** The title compound was prepared by reaction of (3-amino-phenyl)-(5-(3-ethoxyphenyl)-thiophen-2-yl)methanone **(6)** (155 mg, 0.48 mmol) and trifluoro-methanesulfonyl chloride (121 mg, 0.72 mmol) according to method D. The product was purified by CC (DCM); yield: 28 % (60 mg; yellow oil). ^{1}H NMR ($CDCl_3$) δ 7.76 – 7.72 (m, 1H), 7.67 (d, *J* = 7.7 Hz, 1H), 7.56 – 7.49 (m, 2H), 7.41 (t, *J* = 7.9 Hz, 1H), 7.22 (dd, *J* = 15.2, 6.0 Hz, 2H), 7.14 (t, *J* = 3.8 Hz, 1H), 7.11 – 7.05 (m, 1H), 6.85 – 6.77 (m, 1H), 3.98 (q, *J* = 7.0 Hz, 2H), 1.33 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 187.28, 159.43, 154.79, 140.96, 139.04, 136.94, 135.09, 134.16, 130.24, 129.80, 127.58, 126.43, 124.37, 123.62, 121.08, 119.79 (d, *J* = 323.1 Hz) 118.78, 115.57, 112.50, 63.67, 14.75; MS (ESI): 455.84 $(M+H)^+$.

(5-(3-Ethoxyphenyl)-thiophen-2-yl)-(3-isopropylamino-phenyl)methanone (8). To a degassed mixture of 2-bromo-propane (123 mg, 1 mmol), cupper II oxide (72 mg, 0.5 mmol) and potassium hydroxide (56 mg, 1 mmol) in DMF (1 mL) was slowly added (3-aminophenyl)-(5-(3-ethoxyphenyl)-thiophen-2-yl)-methanone **(6)** (323mg, 1 mmol). The resulting mixture was heated at 135^{o}C for 4 days, allowed to cool to rt and poured into a 0^{o}C 6N HCl solution. After 15 min. the precipitate was filtered and washed with benzene. The filtrate was extracted with benzene, dried over magnesium sulfate, filtered, and concentrated to dryness. The product was purified by CC (hexane/ethyl acetate 95:5); yield: 46 % (168 mg; brownish oil). ^{1}H NMR ($CDCl_3$) δ 7.63 (d, *J* = 3.9 Hz, 1H), 7.32 – 7.28 (m, 2H), 7.26 – 7.22 (m, 2H), 7.20 – 7.17 (m, 1H), 7.15 – 7.11 (m, 1H), 7.04 – 7.01 (m, 1H), 6.89 (ddd, *J* = 8.2, 2.5, 0.9 Hz, 1H), 6.76 (ddd, *J* = 8.1, 2.5, 0.9 Hz, 1H), 4.07 (q, *J* = 7.0 Hz, 2H), 3.67 (sept, *J* = 12.4, 6.2 Hz, 2H), 1.43 (t, *J* = 7.0 Hz, 3H), 1.22 (d, *J* = 6.3 Hz, 6H). ^{13}C NMR ($CDCl_3$) δ 188.75, 159.65, 153.00, 147.81, 142.72, 139.36, 135.92, 134.90, 130.36, 129.34, 124.11, 118.93, 118.10, 117.17, 115.31, 113.20, 112.67, 63.84, 44.43, 23.15, 15.03; MS (ESI): 366.62 $(M+H)^+$.

1-(3-(5-(3-Ethoxyphenyl)-thiophene-2-yl-carbonyl)-phenyl)-3-methyl-thiourea (9). A suspension of (3-amino-phenyl)-(5-(3-ethoxyphenyl)-thiophen-2-yl)-methanone **(6)** (205 mg, 0.6 mmol) and CH_3NCS (46 mg, 0.6 mmol) in THF was refluxed overnight. The solution was cooled and the product precipitated with heptane and purified by CC (hexane/ethyl acetate 3:1) to give **9**; yield: 56 % (140 mg; yellow solid; mp. 145-6 ^{o}C). ^{1}H NMR (CD_3COCD_3) δ 9.14 (br., 1H, NH), 8.14 (d, *J* = 9.9 Hz, 1H), 7.87 (d, *J* = 3.9 Hz, 1H), 7.70 (dd, *J* = 6.2, 3.1 Hz, 1H), 7.64 – 7.61 (m, 1H), 7.60 (d, *J* = 4.0 Hz, 1H), 7.51 (t, *J* = 7.8 Hz, 1H), 7.40 – 7.36 (m, 1H), 7.35 (dt, *J* = 7.7, 1.5 Hz, 1H), 7.32 – 7.30 (m, 1H), 6.98 (ddd, *J* = 7.8, 2.5, 1.4 Hz, 1H), 4.14 (q, *J* = 7.0 Hz, 2H), 3.08 (d, *J* = 4.6 Hz, 3H), 1.40 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (CD_3COCD_3) δ 188.17, 175.10, 159.37, 152.88, 146.62, 142.24, 139.09, 135.78, 135.36, 134.55, 130.10, 129.17, 123.87, 119.37, 118.67, 118.65, 115.06, 112.41, 63.57, 35.17, 14.75; MS (ESI): 397.89 $(M+H)^+$.

(3-Benzylaminophenyl)-(5-(3-ethoxyphenyl)-thiophen-2-yl)-methanone (10). The title compound was prepared by reaction of (3-amino-phenyl)-(5-(3-ethoxyphenyl)-thiophen-2-yl)-

methanone **(6)** (135mg, 0.42 mmol) and (bromomethyl)benzene (108mg, 0.63 mmol) in acetone (10 mL). The resulting mixture was stirred at rt for 10 h and poured into water. The precipitate was filtered and purified by CC (hexane/ethyl acetate 97:3); yield: 42 % (72 mg; yellow oil). ^{1}H NMR ($CDCl_3$) δ 7.35 (d, *J* = 4.0 Hz, 3H), 7.23 (tdd, *J* = 10.6, 9.0, 7.2 Hz, 14H), 7.18 – 7.14 (m, 6H), 7.13 – 7.11 (m, 7H), 7.08 – 7.05 (m, 6H), 6.96 (dd, *J* = 2.2, 1.7 Hz, 3H), 6.78 (ddd, *J* = 8.2, 2.5, 1.0 Hz, 3H), 6.71 (ddd, *J* = 8.1, 2.5, 1.0 Hz, 3H), 4.25 (s, 7H), 3.96 (q, *J* = 7.0 Hz, 6H), 1.32 (t, *J* = 7.0 Hz, 9H); ^{13}C NMR ($CDCl_3$) δ 188.28, 159.34, 152.70, 147.94, 142.32, 138.91, 138.83, 135.70, 134.56, 130.06, 129.12, 128.66, 127.35, 127.27, 123.86, 118.63, 118.31, 116.67, 114.97, 112.76, 112.41, 63.54, 47.97, 14.74; MS (ESI): 414.81 $(M+H)^+$.

***N*-(3-(5-(3-Ethoxyphenyl)-thiophene-2-yl-carbonyl)-phenyl)-benzenesulfonamide (11).** The title compound was prepared by reaction of (3-aminophenyl)-(5-(3-ethoxyphenyl)-thiophen-2-yl)-methanone **(6)** (265 mg, 0.82 mmol) and benzenesulfonyl chloride (217 mg, 1.23 mmol) according to method D. The product was purified by CC (hexane/ethyl acetate 90:10); yield: 39 % (150 mg; white solid; mp. 136 ^{o}C). ^{1}H NMR ($CDCl_3$) δ 7.60 – 7.56 (m, 2H), 7.41 – 7.37 (m, 1H), 7.34 – 7.29 (m, 2H), 7.24 (d, *J* = 0.9 Hz, 1H), 7.23 – 7.20 (m, 2H), 7.18 – 7.15 (m, 2H), 7.10 (t, *J* = 8.0 Hz, 1H), 7.07 (d, *J* = 4.0 Hz, 1H), 7.01 (ddd, *J* = 7.7, 1.7, 1.0 Hz, 1H), 6.96 – 6.94 (m, 1H), 6.69 (ddd, *J* = 8.2, 2.5, 0.9 Hz, 1H), 3.86 (q, *J* = 7.0 Hz, 2H), 1.22 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 186.93, 159.43, 153.77, 141.55, 139.04, 138.89, 136.75, 136.13, 134.35, 133.25, 130.19, 129.63, 129.21, 127.21, 125.94, 125.03, 124.11, 121.92, 118.74, 115.25, 112.57, 63.64, 14.78; MS (ESI): 463.92 $(M+H)^+$.

***N*-(3-(5-(3-Ethoxyphenyl)-thiophene-2-yl-carbonyl)-phenyl)-benzamide (12).** The title compound was prepared by reaction of (3-aminophenyl)-(5-(3-ethoxyphenyl)-thiophen-2-yl)-methanone **(6)** (170 mg, 0.53 mmol) and benzoyl chloride (74 mg, 0.53 mmol) according to method D. The product was purified by CC (hexane/ethyl acetate 85:15); yield: 80 % (180 mg; white solid; mp. 145-6 ^{o}C). ^{1}H NMR ($CDCl_3$) δ 8.15 (br. s, 1H, NH), 8.05 (t, *J* = 1.8 Hz, 1H), 8.00 (ddd, *J* = 8.1, 2.2, 1.0 Hz, 1H), 7.91 – 7.85 (m, 2H), 7.66 (d, *J* = 4.0 Hz, 1H), 7.64 – 7.61 (m, 1H), 7.56 – 7.52 (m, 1H), 7.48 (ddd, *J* = 9.5, 5.2, 3.1 Hz, 3H), 7.31 (dd, *J* = 10.7, 6.0 Hz, 2H), 7.24 – 7.21 (m, 1H), 7.18 – 7.15 (m, 1H), 6.89 (ddd, *J* = 8.2, 2.5, 0.9 Hz, 1H), 4.07 (q, *J* = 7.0 Hz, 2H), 1.43 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 187.73, 166.22, 159.67, 153.72, 142.17, 138.98, 138.45, 136.49, 134.81, 134.74, 132.34, 130.41, 129.53, 129.11, 127.36, 125.36, 124.45, 124.20, 121.07, 119.00, 115.48, 112.74, 63.87, 15.04; MS (ESI): 428.84 $(M+H)^+$.

***N*-(3-(5-(3-Ethoxyphenyl)-thiophene-2-yl-carbonyl)-phenyl)-4-methoxy-benzenesulfonamide (13).** The title compound was prepared by reaction of (3-amino-phenyl)-(5-(3-ethoxyphenyl)-thiophen-2-yl)-methanone **(6)** (155 mg, 0.48 mmol) and 4-methoxy-benzenesulfonyl chloride (149 mg, 0.72 mmol) according to method D. The product was purified by CC (hexane/ethyl acetate 85:15); yield: 75 % (179 mg; white solid; mp. 162-3 ^{o}C). ^{1}H NMR (CD_3COCD_3) δ 7.81 – 7.76 (m, 2H), 7.72 (ddd, *J* = 2.2, 1.7, 0.5 Hz, 1H), 7.60 – 7.57 (m, 2H),

7.54 (d, *J* = 4.0 Hz, 1H), 7.52 (ddd, *J* = 8.1, 2.2, 1.4 Hz, 1H), 7.50 – 7.46 (m, 1H), 7.42 – 7.37 (m, 1H), 7.37 – 7.33 (m, 1H), 7.32 – 7.28 (m, 1H), 7.08 – 7.03 (m, 2H), 7.00 (ddd, *J* = 8.0, 2.5, 1.2 Hz, 1H), 4.15 (q, *J* = 7.0 Hz, 2H), 3.85 (s, 3H), 1.40 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (CD_3COCD_3) δ 187.16, 164.15, 160.65, 153.66, 142.81, 139.69, 139.31, 136.96, 135.31, 132.29, 131.30, 130.51, 130.21, 125.57, 125.55, 125.10, 121.71, 119.35, 116.29, 115.19, 112.98, 64.30, 56.13, 15.08; MS (ESI): 493.92 $(M+H)^+$.

3-Cyano-*N*-(3-(5-(3-ethoxyphenyl)-thiophene-2-yl-carbonyl)phenyl)-benzenesulfonamide (14). The title compound was prepared by reaction of (3-amino-phenyl)-(5-(3-ethoxy-phenyl)-thiophen-2-yl)-methanone **(6)** (255 mg, 0.79 mmol) and 3-cyano-benzenesulfonyl chloride (239 mg, 1.18 mmol) according to method D. The product was purified by CC (hexane/ethyl acetate 85:15); yield: 46 % (180 mg; white solid; mp. 130-1 °C). ^{1}H NMR ($CDCl_3$) δ 8.04 – 7.99 (m, 1H), 7.92 (ddd, *J* = 8.0, 1.9, 1.1 Hz, 1H), 7.74 – 7.69 (m, 1H), 7.59 (dd, *J* = 7.2, 1.6 Hz, 1H), 7.52 – 7.48 (m, 1H), 7.47 – 7.45 (m, 2H), 7.40 (ddd, *J* = 8.1, 2.1, 1.5 Hz, 1H), 7.39 – 7.34 (m, 1H), 7.26 – 7.22 (m, 2H), 7.15 (ddd, *J* = 7.7, 1.7, 0.9 Hz, 1H), 7.10 – 7.07 (m, 1H), 6.84 (ddd, *J* = 8.0, 2.5, 0.9 Hz, 1H), 4.00 (q, *J* = 7.0 Hz, 2H), 1.36 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 187.01, 159.44, 154.27, 141.21, 140.74, 139.20, 136.42, 136.34, 136.23, 134.20, 131.16, 130.79, 130.27, 130.23, 129.82, 126.56, 125.24, 124.26, 122.17, 118.77, 116.98, 115.39, 113.75, 112.58, 63.66, 14.78; MS (ESI): 488.84 $(M+H)^+$.

***N*-(3-(5-(3-Ethoxyphenyl)-thiophene-2-yl-carbonyl)phenyl)-isophthalamic acid methyl ester (15).** The title compound was prepared by reaction of (3-aminophenyl)-(5-(3-ethoxy-phenyl)-thiophen-2-yl)-methanone **(6)** (315 mg, 0.98 mmol) and 3-chlorocarbonyl-benzoic acid methyl ester (194 mg, 0.98 mmol) according to method D. The product was purified by CC (hexane/ethyl acetate 85:15); yield: 78 % (370 mg; white solid; mp. 138-9 °C). ^{1}H NMR ($CDCl_3$) δ 8.45 (dd, *J* = 6.3, 4.7 Hz, 2H), 8.12 (d, *J* = 7.8 Hz, 1H), 8.08 (dd, *J* = 7.8, 1.5 Hz, 1H), 8.04 (t, *J* = 1.7 Hz, 1H), 7.98 (dd, *J* = 8.1, 1.2 Hz, 1H), 7.58 (dd, *J* = 9.3, 5.9 Hz, 2H), 7.49 (t, *J* = 7.8 Hz, 1H), 7.43 (t, *J* = 7.8 Hz, 1H), 7.27 – 7.21 (m, 2H), 7.15 (d, *J* = 7.7 Hz, 1H), 7.12 – 7.07 (m, 1H), 6.83 (dd, *J* = 8.2, 1.8 Hz, 1H), 4.01 (q, *J* = 7.0 Hz, 2H), 3.84 (s, 3H), 1.37 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ 187.47, 166.17, 165.02, 159.40, 153.52, 141.82, 138.67, 138.08, 136.28, 134.91, 134.42, 132.88, 132.03, 130.65, 130.14, 129.27, 129.13, 127.78, 125.26, 124.18, 124.09, 120.98, 118.72, 115.23, 112.45, 63.60, 52.42, 14.78; MS (ESI): 485.93 $(M)^+$.

***N*-(3-(5-(3-Ethoxy-phenyl)-thiophene-2-yl-carbonyl)-phenyl)-isophthalamic acid (16).** A reaction of compound **15** (320 mg, 0.66 mmol) in ethanol (5 mL) and 10% sodium hydroxide (15 mL) was refluxed in for 2 h on a water bath. The reaction mixture was cooled, diluted with water and neutralized with acetic acid. The crude product precipitated and was purified by CC (hexane/ethyl acetate 1:3); yield: 80 % (250 mg; greenish-yellow solid; mp. > 280 °C). ^{1}H NMR (CD_3COCD_3) δ 10.06 (s, 1H, COOH), 8.69 (br. s, 1H, NH), 8.46 (s, 1H), 8.21 (dd, *J* = 37.3, 7.8 Hz, 3H), 7.81 (d, *J* = 4.0 Hz, 1H), 7.69 – 7.53 (m, 4H), 7.42 – 7.30 (m, 3H), 7.02 – 6.94 (m, 1H),

4.15 (q, *J* = 7.0 Hz, 2H), 1.40 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (CD_3COCD_3) δ 187.63, 165.96, 160.63, 153.48, 143.10, 140.31, 139.25, 137.15, 135.39, 133.71, 133.69, 133.67, 133.66, 132.79, 132.76, 132.72, 131.27, 129.97, 129.65, 125.69, 125.18, 121.73, 119.35, 116.28, 112.89, 64.28, 15.07; MS (ESI): 472.92 $(M+H)^+$.

(3-Methoxyphenyl)(5-phenylthiophen-2-yl)methanone (17c). The title compound was prepared by reaction of 2-phenylthiophene (1000mg, 6.24 mmol) **(1a)**, 3-methoxybenzoyl chloride (958mg, 5.62 mmol) and aluminum chloride (832 mg, 6.24 mmol) according to method B. The product was purified by CC (hexane/dichloromethane 6:4 to dichloromethane pure); yield: 66 % (1021 mg; yellow oil). ^{1}H NMR (CD_3COCD_3) δ 7.84-7.79 (m, 2H), 7.75 (d, *J*= 3.9Hz, 1H), 7.61 (d, *J*= 3.9Hz, 1H), 7.52-7.41 (m, 5H), 7.40-7.38 (m, 1H), 7.24-7.22 (m, 1H), 3.90 (s, 3H).

(3-Methoxyphenyl)-(5-phenylthiophen-2-yl)-methanethione (17b). (3-methoxyphenyl)(5-phenylthiophen-2-yl)methanone **(17c)** (300 mg, 1.02 mmol, 1.00 equiv) was dissolved under nitrogen in 20 ml abs. toluene and Lawesson's reagent was added. The mixture was stirred for 2h under reflux and concentrated under reduced pressure. Purification by column chromatography (hexane: ethyl acetate 9:1); yield: 96 % (300 mg; dark green oil); Used in the next step without any characterisation.

2-(Difluoro(3-methoxyphenyl)methyl)-5-phenylthiophene (17a). Under N_2 (3-methoxyphenyl)(5-phenylthiophen-2-yl)methanethione **(17b)** (100 mg, 0.34 mmol, 1 equiv) was placed in a 100 ml teflon flask and dissolved under nitrogen in 3 ml abs. dichloromethane. Diethylaminosulfur trifluoride (DAST, 167µl, 1.36 mmol, 3 equiv) to the solution was added slowly at room temperature and the mixture was stirred for 3 h. The reaction was carefully quenched with cold sat. Sodium hydrogen carbonate and extracted three times with diethyl ether. The combined organic layers were washed two times with sat. Sodium hydrogen carbonate, one time with water, one time with brine, dried over magnesium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by CC (hexane/ethyl acetate 85:15); yield: 63 % (68 mg, red viscous oil). ^{1}H NMR (CD_3COCD_3) δ 7.71-7.67 (m, 2H), 7.49-7.42 (m, 3H), 7.41-7.35 (m, 2H), 7.23-7.19 (m, 1H), 7.18-7.13 (m, 2H), 7.13-7.10 (m, 1H), 3.87 (s, 3H); ^{13}C NMR (CD_3COCD_3) δ 187.70, 160.70, 153.45, 143.15, 140.35, 137.05, 134.10, 130.55, 130.10, 127.15, 125.50, 122.10, 119.15, 114.50, 55.80; MS (ESI): 317.40 $(M+H)^+$.

3-(Difluoro(5-phenylthiophen-2-yl)methyl)phenol (17). The title compound was prepared by reaction of 2-(difluoro(3-methoxyphenyl)methyl)-5-phenylthiophene **(17a)** (60 mg, 0.18 mmol) and boron tribromide (0.56 mmol) according to method C. The product was purified by CC (hexane : ethyl acetate 8:2) followed by preparative TLC (hexane : ethyl acetate 7:3); yield 14 % (13 mg; dark green oil). ^{1}H NMR (CD_3COCD_3) δ 7.87-7.85 (m, 1H), 7.52-7.48 (m, 4H), 7.37-7.34 (m, 2H), 7.21-7.17 (m, 1H), 7.15-7.13 (m, 1H), 7.09-7.00 (m, 2H); ^{13}C NMR (CD_3COCD_3)

δ 189.55, 168.80, 155.35, 140.30, 135.85, 134.75, 132.40, 128.20, 127.05, 126.40, 121.05, 118.15, 111.00; MS (ESI): 303.35 $(M+H)^+$.

(3-Hydroxyphenyl)(5-phenylthiophen-2-yl)methanethione (18). The title compound was prepared by reaction of (3-methoxyphenyl)(5-phenylthiophen-2-yl)methanethione) **(17b)** (100 mg, 0.32 mmol) and boron tribromide (0.96 mmol) according to method C. The product was purified by CC (hexane/ethyl acetate 8:2) followed by preparative TLC (hexane : ethyl acetate 8:2); yield 14 % (68 mg). ^{1}H NMR (CD_3COCD_3) δ 9.82 (s, 1H), 7.87-7.84 (m, 2H), 7.76 (d, *J*=4.1 Hz, 1H), 7.52-7.46 (m, 4H), 7.29 (t, *J*=7.9 Hz, 1H), 7.12-7.09 (m, 1H), 7.08 (t, *J*= 1.9 Hz, 1H), 7.01 (ddd, *J*=1.1, 2.4, 8.1 Hz, 1H); ^{13}C NMR (CD_3COCD_3) δ 157.45, 157.00, 152.95, 147.30, 133.85, 132.50, 129.90, 129.55, 129.50, 129.30, 126.40, 126.15, 126.00, 119.35, 118.65, 115.30; MS (ESI): 297.15 $(M+H)^+$.

2-(3-Methoxybenzyl)-5-phenylthiophene (19a). (3-Methoxyphenyl)(5-phenylthiophen-2-yl)methanone **(17c)** (300 mg, 1.01mmol, 1 equiv) was dissolved in 20ml acetic acid, tin (II) chloride dihydrate (1.16 mg, 5.20 mmol, 5.20equiv) was added in 3.33 ml hydrogen chloride and the mixture was refluxed over night. The mixture was cooled to room temperature, quenched with water, extracted two times with dichloromethane, dried and evaporated under reduced pressure. The crude product was purified by CC (hexane/ethyl acetate 9:1); yield: 50 % (147 mg, white oil). ^{1}H NMR (CD_3COCD_3) δ 7.54-7.61 (m, 2H), 7.32-7.40 (m, 2H), 7.19-7.28 (m, 3H), 6.87-6.92 (m, 3H), 6.75-6.83 (m, 1H), 4.15 (s, 2H), 3.78 (s, 3H).

3-((5-Phenylthiophen-2-yl)methyl)phenol (19). The title compound was prepared by reaction of 2-(3-methoxybenzyl)-5-phenylthiophene **(19a)** (140 mg, 0.49 mmol) and boron tribromide (1.50 mmol) according to method C. No further purification was required; yield 49 % (65 mg). ^{1}H NMR (CD_3COCD_3) δ 8.24 (s, 1H), 7.65-7.52 (m, 2H), 7.41-7.30 (m, 2H), 7.28-7.20 (m, 2H), 7.17-7.08 (m, 1H), 6.92-6.83 (m, 1H), 6.82-6.75 (m, 2H), 6.72-6.70 (m, 1H), 4.11 (s, 2H); ^{13}C NMR (CD_3COCD_3) δ 158.50, 145.05, 143.40, 142.95, 135.50, 130.45, 129.85, 128.10, 127.30, 126.10, 123.80, 120.65, 116.45, 116.35, 114.35, 36.70; MS (ESI): 278.56 $(M+H)^+$.

2-(1-(3-Methoxyphenyl)vinyl)-5-phenylthiophene (20a). Methyltriphenylphosphonium bromide (364 mg, 1.01 mmol, 1 equiv) was suspended in 5ml dry tetrahydrofurane, *n*-butyllithium (400 µl, 2.5 M in hexane) was added dropwise, stirred at room temperature for 90 min, (3-methoxyphenyl)(5-phenylthiophen-2-yl)methanone **(17c)** (300 mg, 1.01mmol, 1.00equiv), previously solubilised in 2ml dry tetrahydrofurane, was added dropwise and stirred at room temperature for 1h, then at 65°C over night. The mixture was cooled to room temperature, quenched with water and extracted two times with ethyl acetate. The combined organic layers were washed one time with water, dried and evaporated under reduced pressure. The crude product was purified by CC (hexane/ethyl acetate 9:1); yield: 35 % (105 mg, yellow oil). ^{1}H NMR (CD_3COCD_3) δ 7.68 (d, *J*= 8.2 Hz, 2H), 7.40-7.45 (m, 2H), 7.38 (d, *J*= 3.8 Hz,

1H), 7.29-7.36 (m, 2H), 7.01-7.06 (m, 2H), 6.94-7.00 (m, 2H), 5.64 (d, *J*= 1.5 Hz, 1H), 5.29 (d, *J*= 1.5 Hz, 1H), 3.83 (s, 3H).

2-(1-(3-Methoxyphenyl)vinyl)-5-phenylthiophene (20). The title compound was prepared by reaction of 2-(1-(3-methoxyphenyl)vinyl)-5-phenylthiophene **(20a)** (100 mg, 0.34 mmol) and boron tribromide (1.30 mmol) according to method C. The product was purified by preparative TLC (hexane/ethyl acetate 8:2); yield 5 % (4.3 mg; yellow oil). ^{1}H NMR (CD_3COCD_3) δ 8.42 (s, 1H), 7.70-7.63 (m, 2H), 7.42 (d, *J*= 7.8 Hz, 2H), 7.38 (d, *J*= 3.8 Hz, 1H), 7.31 (d, *J*= 7.8 Hz, 1H), 7.25 (d, *J*= 8.2 Hz, 1H), 6.97 (d, *J*= 3.8 Hz, 1H), 6.96-6.94 (m, 2H), 6.89-6.82 (m, 1H), 5.60 (d, *J*= 0.7 Hz, 1H), 5.25 (d, *J*= 0.7 Hz, 1H); MS (ESI): 266.66 $(M+H)^+$.

(5-((3-Hydroxymethyl)phenyl)thiophen-2-yl)-(3-methoxyphenyl)methanone (21a). The title compound was prepared by reaction of (5-bromothiophene-2-yl)(3-methoxyphenyl)methanone **(21b)** (150 mg, 0.50 mmol), 3-(hydroxymethyl)phenylboronic acid (92 mg, 0.60 mmol), cesium carbonate (658 mg, 2.02 mmol) and tetrakis(triphenylphosphine) palladium (6 mg, 5 µmol) according to method A. The product was purified by CC (hexane/ethyl acetate 7:3); yield: 73 % (120 mg; brown oil). ^{1}H NMR ($CDCl_3$) δ 7.70 (s, 1H), 7.64 (d, *J* = 4.0 Hz, 1H), 7.62 – 7.59 (m, 1H), 7.46 (dt, *J* = 7.5, 1.3 Hz, 1H), 7.44 – 7.37 (m, 4H), 7.36 (d, *J* = 4.0 Hz, 1H), 7.14 (ddd, *J* = 8.1, 2.7, 1.1 Hz, 1H), 4.76 (d, *J* = 4.3 Hz, 2H), 3.87 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 187.77, 159.63, 153.06, 142.20, 141.93, 139.32, 135.95, 133.57, 129.40, 129.35, 127.54, 125.55, 124.72, 124.00, 121.64, 118.54, 113.73, 64.90, 55.47; MS (ESI): 325.26 $(M+H)^+$.

(5-((3-Hydroxymethyl)phenyl)thiophen-2-yl)-(3-hydroxyphenyl)methanone (21). The title compound was prepared by reaction of (5-((3-hydroxymethyl)phenyl)thiophen-2-yl)-(3-methoxyphenyl)methanone **(21a)** (100 mg, 0.31 mmol) and boron tribromide (0.92 mmol) according to method C. The product was purified by CC (dichloromethane/methanol 99.75:0.25); yield 63 % (60 mg; yellow oil). ^{1}H NMR (CD_3COCD_3) δ 8.74 (s, 1H, OH), 7.92 (t, *J* = 1.6 Hz, 1H), 7.77 – 7.74 (m, 1H), 7.73 (d, *J* = 4.0 Hz, 1H), 7.63 (t, *J* = 4.4 Hz, 1H), 7.53 (dt, *J* = 7.6, 1.4 Hz, 1H), 7.49 (dd, *J* = 9.5, 5.8 Hz, 1H), 7.41 (dd, *J* = 9.5, 5.8 Hz, 1H), 7.38 (dt, *J* = 7.6, 1.4 Hz, 1H), 7.36 – 7.33 (m, 1H), 7.14 (ddd, *J* = 7.7, 2.5, 1.4 Hz, 1H), 4.73 (s, 2H); ^{13}C NMR (CD_3COCD_3) δ 192.02, 162.73, 156.77, 147.76, 144.87, 144.55, 141.09, 138.92, 135.08, 134.96, 134.90, 131.99, 131.22, 129.96, 125.40, 124.59, 120.60, 37.95; MS (ESI): 311.56 $(M+H)^+$.

(5-(4-(Hydroxymethyl)phenyl)thiophen-2-yl)-(3-methoxyphenyl)methanone (22a). The title compound was prepared by reaction of (5-bromo-thiophen-2-yl)(3-methoxyphenyl)methanone **(21b)** (150 mg, 0.50 mmol), 4-(hydroxymethyl)phenylboronic acid (92 mg, 0.60 mmol), cesium carbonate (657 mg, 2.02 mmol) and tetrakis(triphenylphosphine) palladium (6 mg, 5 µmol) according to method A. The product was purified by CC (hexane/ethyl acetate 7:3); yield: 70 % (118 mg, brown oil). ^{1}H NMR ($CDCl_3$) δ 7.57 (d, *J* = 1.8 Hz, 1H), 7.57 – 7.55 (m, 1H), 7.53 (d, *J* = 4.0 Hz, 1H), 7.35 (dt, *J* = 7.6, 1.2 Hz, 1H), 7.33 – 7.27 (m, 4H), 7.24 (d, *J* = 4.0 Hz, 1H), 7.03

(ddd, *J* = 8.1, 2.7, 1.1 Hz, 1H), 4.63 (s, 2H), 3.77 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 187.74, 159.63, 152.97, 142.11, 141.97, 139.34, 135.98, 132.59, 129.40, 127.59, 126.47, 123.83, 121.63, 118.52, 113.72, 64.79, 55.47; MS (ESI): 325.25 $(M+H)^+$.

(5-(4-(Hydroxymethyl)phenyl)thiophen-2-yl)(3-hydroxyphenyl)-methanone (22). The title compound was prepared by reaction of (5-(4-(hydroxymethyl)phenyl)thiophen-2-yl)-(3-methoxyphenyl)methanone **(22a)** (100 mg, 0.31 mmol) and boron tribromide (0.92 mmol) according to method C. The product was purified by CC (dichloromethane/methanol 99.75:0.25); yield 58 % (55 mg, yellow oil). ^{1}H NMR ($CDCl_3$) δ 7.57 – 7.51 (m, 3H), 7.36 – 7.34 (m, 1H), 7.34 – 7.32 (m, 1H), 7.32 – 7.29 (m, 1H), 7.28 – 7.22 (m, 3H), 7.00 (ddd, *J* = 8.0, 2.6, 1.1 Hz, 1H), 4.42 (d, *J* = 5.3 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 188.05, 156.28, 152.60, 142.26, 139.16, 138.74, 136.30, 133.29, 129.83, 129.68, 126.68, 124.25, 121.40, 119.73, 115.72, 32.72; MS (ESI): 311.24 $(M+H)^+$.

(3-(Benzyloxy)phenyl)(5-bromothiophen-2-yl)methanone (23c). (5-bromothiophen-2-yl)(3-hydroxyphenyl)methanone **(23d)** (10.44 g, 36.9 mmol, 1.00 equiv) was dissolved under nitrogen in 40 ml acetone. cesium carbonate (25.2 g, 77.43 mmol, 2.10 equiv) and isopropyliodide (265 mg, 1.56 mmol, 1.5 equiv) were added and the mixture stirred at room temperature overnight. The reaction was quenched with water. The combined organic layers were washed one time with water, one time with sat. Sodium hydrogen carbonate, one time with brine, dried over sodium sulfate, filtered and concentrated under vacuum. The crude product was recrystallized in hexane/acetone (99:1); yield: 50 % (6.9 g, white solid). ^{1}H NMR (CD_3COCD_3) δ 7.46 (t, J= 8.2 Hz, 3H), 7.41-7.36 (m, 5H), 7.33-7.27 (m, 3H), 5.21 (s, 2H); ^{13}C NMR (CD_3COCD_3) δ 186.80, 159.75, 146.15, 139.45, 128.05, 136.50, 132.95, 130.80, 129.45, 128.85, 128.45, 123.00, 122.30, 120.65, 115.55, 70.40; MS (ESI): 373.0 $(M+H)^+$.

(3-(Benzyloxy)phenyl)(5-(3-hydroxyphenyl)thiophen-2-yl)methanone (23b). The title compound was prepared by reaction of (3-(benzyloxy)phenyl)(5-bromothiophen-2-yl)methanone **(23c)** (3.2 g, 8.57 mmol), 3-hydroxyphenylboronic acid (1.42 g, 10.30 mmol), cesium carbonate (6.98 g, 21.40 mmol) and tetrakis(triphenylphosphine) palladium (2 mg) according to method A. The residue was purified by column chromatography (hexane/ethyl acetate 7:3); yield: 81 % (2.7 g; yellow solid, mp. 142.0 ^{o}C). ^{1}H NMR (CD_3COCD_3) δ 8.63 (s, 1H), 7.60 (t, *J*= 4.0 Hz, 1H), 7.52-7.46 (m, 6H), 7.41 (t, *J*= 7.8 Hz, 2H), 7.33-7.31 (m, 3H), 7.25-7.24 (m, 2H), 6.91(d, *J*= 8.2 Hz, 1H), 5.25 (s, 2H); ^{13}C NMR (CD_3COCD_3) δ 187.61, 159.70, 159.07, 153.52, 142.91, 140.37, 138.11, 137.04, 135.42, 131.38, 130.61, 129.40, 128.84, 128.53, 125.30, 122.37, 120.20, 118.45, 117.27, 115.62, 113.84, 70.73; MS (ESI): 386.76 $(M+H)^+$.

(3-(Benzyloxy)phenyl)(5-(3-isopropoxyphenyl)thiophen-2-yl)methanone (23a). (3-(Benzyloxy)phenyl)(5-(3-hydroxyphenyl)thiophen-2-yl)methanone **(23b)** (400 mg, 1.04 mmol, 1.0 equiv) was dissolved in 25 ml acetone under N_2. Caesium carbonate (678 mg, 2.08 mmol,

2.0 equiv) and isopropyl iodide (265 mg, 1.56 mmol, 1.5 equiv) were added and the mixture was stirred at room temperature overnight. The reaction was quenched with water and extracted with ethyl acetate. The combined organic layers were washed one time with sat. Sodium hydrogen carbonate, one time with brine, dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was recrystallized from hexane/acetone (9:1); yield: 90 % (400 mg, white solid, mp. 98 ^{o}C). ^{1}H NMR (CD_3COCD_3) δ 7.61 (d, *J*= 4.0 Hz, 1H), 7.57(d, *J*= 4.0 Hz, 1H), 7.52-7.46 (m, 5H), 7.43-7.30 (m, 7H), 7.00 (ddd, *J*= 1.0, 2.5, 8.5 Hz, 1H), 5.25 (s, 2H), 4.75 (sept, *J*= 6.0 Hz, 1H), 1.34 (d, *J*= 6.0 Hz, 6H); ^{13}C NMR (CD_3COCD_3) δ 186.71, 158.80, 158.75, 152.51, 142.12, 139.34, 137.27, 136.15, 134.52, 130.49, 129.81, 121.44, 119.32, 118.40, 116.54, 114.61, 113.52, 69.83, 69.74, 21.42; MS (ESI): 428.70 $(M+H)^+$.

(3-Hydroxyphenyl)(5-(3-isopropoxyphenyl)thiophen-2-yl)methanone (23). The title compound was prepared by reaction of (3-(benzyloxy)phenyl)(5-(3-isopropoxyphenyl)thiophen-2-yl)methanone **(23a)** (400 mg, 0.93 mmol) and boron trichloride (1.86 mmol) according to method C. The product was purified by CC (hexane/ethyl acetate 8:2); yield 44 % (150 mg; yellow solid, mp. 100 ^{o}C). ^{1}H NMR (CD_3COCD_3) δ 8.83 (s, 1H), 7.71 (d, *J*= 4.0 Hz, 1H), 7.59 (d, *J*= 4.0 Hz, 1H), 7.42-7.33 (m, 5H), 7.30 (t, *J*= 2.3 Hz, 1H), 7.14 (ddd, *J*=1.2, 2.3, 7.7 Hz, 1H), 6.99 (ddd, *J*= 1.2, 2.3, 7.7 Hz, 1H), 4.74 (sept, *J*= 6.0 Hz, 1H), 1.34 (d, *J*= 6.0 Hz, 6H); MS (ESI): 339.15 $(M+H)^+$.

(3-(Benzyloxy)phenyl)(5-(3-isobutoxyphenyl)thiophen-2-yl)methanone (24a). (3-(benzyloxy)phenyl)(5-(3-hydroxyphenyl)thiophen-2-yl)methanone **(23b)** (300 mg, 0.78 mmol, 1 equiv) was dissolved in 20 ml acetone. Caesium carbonate (508 mg, 1.56 mmol, 2.0 equiv) and isobutyl iodide (215 mg, 1.17 mmol, 1.5 equiv) were added and the mixture was stirred at room temperature overnight. The reaction was quenched with water. The combined organic layers were washed one time with water, one time with sat. Sodium hydrogen carbonate, one time with brine, dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was recrystallized in hexane/acetone (9:1); yield: 44 % (152 mg, white solid, mp. 94 ^{o}C). ^{1}H NMR (CD_3COCD_3) δ 7.62 (d, *J*= 4.0 Hz, 1H), 7.58 (d, *J*= 4.0 Hz, 1H), 7.52-7.46 (m, 5H), 7.43-7.30 (m, 7H), 7.01 (ddd, *J*= 1.2, 2.4, 7.9 Hz, 1H), 5.25 (s, 2H), 3.87 (d, *J*= 6.5 Hz, 2H), 2.10 (m, 1H), 1.05 (d, *J*= 6.8 Hz, 6H); ^{13}C NMR (CD_3COCD_3) δ 187.61, 160.94, 159.70, 153.42, 143.01, 140.34, 138.11, 136.92, 135.43, 131.37, 130.62, 129.45, 128.81, 128.50, 125.64, 122.33, 120.22, 119.47, 116.38, 115.55, 113.04, 75.14, 70.72, 29.13, 19.57; MS (ESI): 442.70 $(M+H)^+$.

(3-Hydroxyphenyl)(5-(3-isobutoxyphenyl)thiophen-2-yl)methanone (24). The title compound was prepared by reaction of (3-(benzyloxy)phenyl)(5-(3-isobutoxyphenyl)thiophen-2-yl)methanone **(24a)** (200 mg, 0.45 mmol) and boron trichloride (0.90 mmol) according to method C. The product was purified by CC (hexane/ethyl acetate 8:2); yield 51 % (81 mg; white solid, mp. 90 ^{o}C). ^{1}H NMR (CD_3COCD_3) δ 8.86 (s, 1H), 7.71 (d, *J*= 3.9 Hz, 1H), 7.59 (d, *J*= 3.9 Hz, 1H), 7.42-7.33 (m, 6H), 7.13 (d, *J*=7.0 Hz, 1H), 7.00 (d, *J*= 7.3 Hz, 1H), 3.87 (d, *J*= 6.4 Hz,

2H), 2.09 (sept, *J*= 6.6 Hz, 1H), 1.05 (d, *J*= 6.7 Hz, 6H); ^{13}C NMR (CD_3COCD_3) δ 187.81, 160.94, 158.51, 153.32, 143.22, 140.35, 136.87, 135.42, 131.34, 130.68, 125.53, 121.18, 120.35, 119.34, 116.32, 113.01, 75.10, 29.14, 19.52; MS (ESI): 352.90 $(M+H)^+$.

3-(Methoxyphenyl)-(5-(3-methoxyphenyl)sulfanyl-thiophen-2-yl)-methanone (25a). 3-Methoxybenzenethiol (111 mg, 1.0 mmol) was slowly added to a degassed mixture of (5-bromo-thiophen-2-yl)(3-methoxyphenyl)methanone **(21b)** (300mg, 1.0 mmol), cupper II oxide (72 mg, 0.5 mmol) and potassium hydroxide (56 mg, 1.0 mmol) in dimethylformamide (1 mL). The resulting mixture was heated at 135^{o}C for 3 h, allowed to cool to rt and poured into a 0^{o}C 6N HCl solution. After 15 min. the precipitate was filtered and washed with benzene. The filtrate was extracted with benzene, dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The product was purified by CC (hexane/ethyl acetate 97:3); yield 75 % (270 mg; yellow oil). ^{1}H NMR ($CDCl_3$) δ 7.65 (d, *J* = 3.9 Hz, 1H), 7.54 – 7.47 (m, 2H), 7.46 (dd, *J* = 2.4, 1.4 Hz, 1H), 7.39 – 7.36 (m, 1H), 7.26 – 7.21 (m, 2H), 7.13 (ddd, *J* = 7.7, 1.7, 0.9 Hz, 1H), 7.10 – 7.07 (m, 1H), 6.95 (ddd, *J* = 8.3, 2.5, 0.8 Hz, 1H), 3.97 (s, 3H), 3.90 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 186.88, 160.17, 159.61, 145.61, 145.27, 138.98, 136.07, 135.10, 131.94, 130.24, 129.40, 122.77, 121.55, 118.61, 116.00, 113.77, 113.66, 55.42, 55.33; MS (ESI): 357.25 $(M+H)^+$.

(3-Hydroxyphenyl)-(5-(3-hydroxyphenyl)sulfanylthiophen-2-yl)methanone (25). The title compound was prepared by reaction of (3-methoxyphenyl)-(5-(3-methoxy-phenylsulfanyl)-thiophen-2-yl)methanone **(25a)** (300 mg, 0.84 mmol) and boron tribromide (5.04 mmol) according to method C. The product was purified by CC (Dichloromethane/methanol 99.5:0.5); yield 51 % (140 mg; yellow oil). ^{1}H NMR (CD_3OD) δ 7.60 (d, *J* = 4.0 Hz, 1H), 7.34 (t, *J* = 7.9 Hz, 1H), 7.29 – 7.25 (m, 1H), 7.22 – 7.17 (m, 3H), 7.05 (ddd, *J* = 8.1, 2.5, 1.1 Hz, 1H), 6.91 (ddd, *J* = 7.7, 1.7, 0.9 Hz, 1H), 6.88 – 6.85 (m, 1H), 6.77 (ddd, *J* = 8.2, 2.4, 0.9 Hz, 1H); ^{13}C NMR (CD_3OD) δ 188.97, 159.59, 158.92, 147.64, 146.28, 140.15, 137.06, 137.00, 133.34, 131.50, 130.78, 122.64, 121.32, 120.80, 118.28, 116.50, 116.49; MS (ESI): 328.92 $(M)^+$.

(5-(3-Hydroxybenzenesulfonyl)-thiophen-2-yl)-(3-hydroxyphenyl)methanone (26). The title compound was prepared by reaction of (3-hydroxy-phenyl)-(5-(3-hydroxy-phenylsulfanyl)thiophen-2-yl)-methanone **(25)** (100 mg, 0.30 mmol) and H_2O_2 (30%, 0.1 mL) in acetic acid (2 mL). The solution was allowed to stand at rt for 4 days and was then poured into water. The solid was filtered off, washed with water and dried. The product was purified by CC (Dichloromethane/methanol 99:1); yield 64 % (70 mg, yellow solid, mp. 102 ^{o}C). ^{1}H NMR (CD_3OD) δ 7.72 (d, *J* = 4.0 Hz, 1H), 7.61 (d, *J* = 4.0 Hz, 1H), 7.49 – 7.44 (m, 1H), 7.44 – 7.38 (m, 2H), 7.33 (t, *J* = 7.8 Hz, 1H), 7.27 (d, *J* = 7.7 Hz, 1H), 7.24 – 7.20 (m, 1H), 7.07 (dddd, *J* = 5.0, 3.7, 2.5, 0.9 Hz, 2H); ^{13}C NMR (CD_3OD) δ 188.96, 159.83, 159.03, 151.00, 150.83, 143.33, 139.20, 135.32, 134.36, 132.05, 130.97, 122.40, 121.66, 121.56, 119.45, 116.63, 114.92. MS (ESI): 362.23 $(M+H)^+$.

(5-Bromothiophen-2-yl)(4-methoxyphenyl)methanone (27b). The title compound was prepared by reaction of 2-bromothiophene (1000 mg, 6.13 mmol), 4-methoxybenzoyl chloride (1046 mg, 6.13 mmol) and aluminum chloride (818 mg, 6.13 mmol) according to method A. The product was purified by CC (hexane/ethyl acetate 98:2); yield: 82 % (1.5 g; yellow powder; mp. 103-104 ^{o}C). ^{1}H NMR ($CDCl_3$) δ 8.13 – 8.09 (m, 2H), 7.75 (d, *J* = 3.9 Hz, 1H), 7.45 (d, *J* = 3.9 Hz, 1H), 7.22 – 7.20 (m, 2H), 4.14 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 185.80, 162.10, 144.02, 137.59, 133.23, 130.55, 125.10, 113.75, 55.40.

(4-Methoxyphenyl)-(5-(3-methoxyphenyl)sulfanylthiophen-2-yl)methanone (27a). 3-Methoxybenzenethiol (111 mg, 1.0 mmol) was slowly added to a degassed mixture of (5-Bromo-thiophen-2-yl)(4-methoxyphenyl)methanone **(27b)** (300mg, 1.0 mmol), CuO (72 mg, 0.5 mmol) and potassium hydroxide (56 mg, 1.0 mmol) in dimethylformamide (1 mL). The resulting mixture was heated at 135^{o}C for 3 h, allowed to cool to rt and poured into a 0^{o}C 6N HCl solution. After 15 min. the precipitate was filtered and washed with benzene. The filtrate was extracted with benzene, dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The product was purified by CC (hexane/ethyl acetate 99:1); yield 90 % (322 mg; brown oil). ^{1}H NMR ($CDCl_3$) δ 8.15 – 8.10 (m, 2H), 7.78 (d, *J* = 3.9 Hz, 1H), 7.53 – 7.49 (m, 1H), 7.41 (d, *J* = 3.9 Hz, 1H), 7.27 – 7.23 (m, 2H), 7.21 (dt, *J* = 7.6, 1.9 Hz, 2H), 7.08 (ddd, *J* = 8.3, 2.5, 0.8 Hz, 1H), 4.14 (s, 3H), 4.04 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 185.84, 163.18, 160.16, 146.00, 143.97, 136.55, 134.24, 132.43, 131.50, 130.28, 130.19, 122.40, 115.62, 113.75, 113.55, 55.48, 55.35.

(4-Hydroxyphenyl)-(5-(3-hydroxyphenyl)sulfanylthiophen-2-yl)methanone (27). The title compounds was prepared by reaction of (4-methoxyphenyl)-(5-(3-methoxyphenyl)sulfanyl-thiophen-2-yl)methanone **(27a)** (300 mg, 0.84 mmol) and boron tribromide (5.04 mmol) according to method C. The product was purified by CC (dichloromethane/methanol 99:1); yield 69 % (190 mg; yellow oil). ^{1}H NMR (CD_3OD) δ 7.78 – 7.76 (m, 1H), 7.75 – 7.74 (m, 1H), 7.53 (d, *J* = 3.9 Hz, 1H), 7.17 – 7.12 (m, 2H), 6.91 – 6.89 (m, 1H), 6.89 – 6.87 (m, 1H), 6.86 (ddd, *J* = 8.2, 2.4, 0.9 Hz, 1H), 6.85 – 6.83 (m, 1H), 6.73 (ddd, *J* = 8.2, 2.4, 0.9 Hz, 1H); ^{13}C NMR (CD_3OD) δ 187.86, 163.59, 159.47, 146.94, 145.93, 137.47, 136.20, 133.80, 133.03, 131.47, 129.96, 122.34, 117.94, 116.44, 116.28; MS (ESI): 329.78 $(M+H)^{+}$.

(5-(3-Hydroxybenzenesulfonyl)-thiophen-2-yl)(4-hydroxyphenyl)methanone (28). The title compound was prepared by reaction of (4-hydroxy-phenyl)-(5-(3-hydroxy-phenyl)sulfanylthiophen-2-yl)-methanone **(27)** (100 mg, 0.30 mmol) and hydrogen peroxide (30%, 0.1 mL) in acetic acid (2 mL). The solution was allowed to stand at rt for 4 days and poured into water, the solid was filtered off, washed with water, dried. The product was purified by CC (dichloromethane/methanol 99:1); yield 53 % (58 mg; yellow solid; mp. 88-9^{o}C). ^{1}H NMR (CD_3OD) δ 7.82 – 7.80 (m, 1H), 7.80 – 7.78 (m, 1H), 7.73 (d, *J* = 4.0 Hz, 1H), 7.61 (d, *J* = 4.0 Hz, 1H), 7.47 (ddd, *J* = 7.8, 1.7, 1.1 Hz, 1H), 7.43 (d, *J* = 8.0 Hz, 1H), 7.40 (t, *J* = 2.0 Hz,

1H), 7.08 (ddd, *J* = 8.0, 2.5, 1.1 Hz, 1H), 6.92 – 6.90 (m, 1H), 6.90 – 6.88 (m, 1H); ^{13}C NMR (CD_3OD) δ 187.49, 164.27, 159.84, 151.51, 150.11, 143.49, 134.41, 134.28, 133.36, 132.01, 129.13, 122.31, 119.37, 116.57, 114.87; MS (ESI): 362.38 $(M+H)^+$.

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

Preparation of *human* 17β-HSD1 and 17β-HSD2. *Human* 17β-HSD1 and 17β-HSD2 were obtained from human placenta according to previously described procedures.[39] Fresh human placenta was homogenized, and cytosolic fraction and microsomes were separated by fractional centrifugation. For the partial purification of 17β-HSD1, the cytosolic fraction was precipitated with ammonium sulfate. 17β-HSD2 was obtained from the microsomal fraction.

Inhibition of *human* 17β-HSD1. Inhibitory activities were evaluated by an established method with minor modifications.[39] Briefly, the enzyme preparation was incubated with NADH (500 µM) in the presence of potential inhibitors at 37°C in a phosphate buffer (50 mM) supplemented with 20% of glycerol and EDTA (1 mM). Inhibitor stock solutions were prepared in DMSO. The final concentration of DMSO was adjusted to 1% in all samples. The enzymatic reaction was started by addition of a mixture of unlabelled- and [2, 4, 6, 7-^{3}H]-E1 (final concentration: 500 nM, 0.15 µCi). After 10 min, the incubation was stopped with $HgCl_2$ and the mixture was extracted with diethylether. After evaporation, the steroids were dissolved in acetonitrile. E1 and E2 were separated using acetonitrile/water (45:55) as mobile phase in a C18 reverse phase chromatography column (Nucleodur C18 Gravity, 3 µm, Macherey-Nagel, Düren) connected to a HPLC-system (Agilent 1200 Series, Agilent Technologies, Waldbronn). Detection and quantification of the steroids were performed using a radioflow detector (Berthold Technologies, Bad Wildbad). The conversion rate was calculated after analysis of the resulting chromatograms according to the following equation:

%conversion = [%E2/(%E2 + %E1)]x100

Each value was calculated from at least three independent experiments.

Inhibition of *human* 17β-HSD2. The *h*17β-HSD2 inhibition assay was performed similarly to the *h*17β-HSD1 procedure. The microsomal fraction was incubated with NAD^+ [1500 µM], test compound and a mixture of unlabelled- and [2,4,6,7-^{3}H]-E2 (final concentration: 500 nM, 0.11 µCi) for 20 min at 37 °C. Further treatment of the samples and HPLC separation was carried out as mentioned above.

The conversion rate was calculated after analysis of the resulting chromatograms according to the following equation:

%conversion = [%E1/(%E1 + %E2)]x100

Preparation of *murine* 17β-HSD1. Recombinant mouse 17β-HSD1 enzyme was produced by transfection of HEK 293 cells with a mouse 17β-HSD1 expression plasmid (coding sequence of NM_010475 in pCMV6Entry vector, OriGene Technologies, Inc.). 48 hours after transfection cells were homogenized by sonication (3 x 10 s) in a buffer containing saccharose (40 mM Tris, 250 mM saccharose, 5 mM EDTA, 7 mM DTT, 1 mM PMSF, pH 7.5). Cell lysate was centrifuged (1000 g, 15 min, 4°C) and 20% glycerol was added to the supernatant before aliquots were frozen and stored at -70°C.[47]

Inhibition of *murine* 17β-HSD1. Inhibitory activities of the compounds towards mouse 17β-HSD1 were evaluated by an established method with minor modifications.[48] The enzyme preparation was incubated with inhibitors, NADPH (0.5 mM) and a mixture of unlabeled- and [3H]-E1 (final concentration: 10 nM, 0.15 μCi) for 10 min at 37 ^{o}C. Further treatment of the samples and HPLC separation was carried out as mentioned above.

The conversion rate was calculated after analysis of the resulting chromatograms according to the following equation:

%conversion = [%E2/(%E2 + %E1)]x100

Molecular Modeling

Local docking experiments followed by an energy minimization step were performed with YASARA structure using the AMBER03 force field.[49, 50] Protein coordinates were prepared starting from a crystal structure of *human* 17β-HSD1 in complex with estradiol and $NADP^+$ (PDB ID: 1FDT).[51] After removal of ligand atoms, a grid box of approximately 7.5 nm^3 was set up around the active site of the enzyme. The flexible local docking experiment using the built-in AutoDock 4 algorithm and 999 docking runs was performed with energy-minimized manual models of inhibitors **25** and **26**.[52] Highest-ranked enzyme-inhibitor complexes were subjected to an energy minimization step with fixed protein backbone atoms. Ligand-protein interactions of the yielded structures were analyzed with MOE 2010.

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Spplemantary table S1: *HPLC Purity Control of Final Compounds*

The purity of the compounds was evaluated by LC/MS. The Surveyor®-LC-system consisted of a pump, an auto sampler, 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). 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) 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 needle voltage of 3000 V and a capillary temperature of 350 °C. Mass spectra were acquired in positive mode from 100 to 1000 m/z and UV spectra were recorded at the wave length of 254 nm.

Spplemantary table S1: *HPLC Purity Control of Final Compounds*

Comd	R_t (min)	HPLC purity (≥ %)	Comd	R_t (min)	HPLC purity (≥ %)	Comd	R_t (min)	HPLC purity (≥ %)
1	14.49	99	**11**	15.90	98	**21**	14.62	98
2	15.25	97	**12**	15.66	98	**22**	13.75	99
3	15.65	95	**13**	15.95	99	**23**	11.93	95
4	14.49	98	**14**	16.48	98	**24**	14.13	95
5	12.18	95	**15**	16.74	98	**25**	13.46	99
6	11.68	99	**16**	14.91	96	**26**	10.95	97
7	16.27	98	**17**	12.11	95	**27**	12.20	99
8	14.76	98	**18**	14.49	95	**28**	10.74	99
9	14.30	98	**19**	14.58	95			
10	17.23	96	**20**	13.43	99			

References

1. Ferin, M.; Zimmering, P. E.; Lieberman, S.; Vande Wiele, R. L. Inactivation of the biological effects of exogenous and endogenous estrogens by antibodies to 17-beta-estradiol. *Endocrinology* **1968,** 83, 565-71.
2. Liehr, J. G. Is estradiol a genotoxic mutagenic carcinogen? *Endocr Rev* **2000,** 21, 40-54.
3. Hall, J. M.; Couse, J. F.; Korach, K. S. The multifaceted mechanisms of estradiol and estrogen receptor signaling. *J Biol Chem* **2001,** 276, 36869-72.
4. Thomas, D. B. Do hormones cause breast cancer? *Cancer* **1984,** 53, 595-604.
5. Russo, J.; Fernandez, S. V.; Russo, P. A.; Fernbaugh, R.; Sheriff, F. S.; Lareef, H. M.; Garber, J.; Russo, I. H. 17-Beta-estradiol induces transformation and tumorigenesis in human breast epithelial cells. *FASEB J* **2006,** 20, 1622-34.
6. Dizerega, G. S.; Barber, D. L.; Hodgen, G. D. Endometriosis: role of ovarian steroids in initiation, maintenance, and suppression. *Fertil Steril* **1980,** 33, 649-53.
7. Zeitoun, K.; Takayama, K.; Sasano, H.; Suzuki, T.; Moghrabi, N.; Andersson, S.; Johns, A.; Meng, L.; Putman, M.; Carr, B.; Bulun, S. E. Deficient 17beta-hydroxysteroid dehydrogenase type 2 expression in endometriosis: failure to metabolize 17beta-estradiol. *J Clin Endocrinol Metab* **1998,** 83, 4474-80.
8. Purohit, A.; Foster, P. A. Steroid sulfatase inhibitors for estrogen- and androgen-dependent cancers. *J Endocrinol* **2012,** 212, 99-110.
9. Picard, F.; Baston, E.; Reichert, W.; Hartmann, R. W. Synthesis of N-substituted piperidine-4-(benzylidene-4-carboxylic acids) and evaluation as inhibitors of steroid-5alpha-reductase type 1 and 2. *Bioorg Med Chem* **2000,** 8, 1479-87.
10. Gunnarsson, C.; Ahnstrom, M.; Kirschner, K.; Olsson, B.; Nordenskjöld, B.; Rutqvist, L. E.; Skoog, L.; Stal, O. Amplification of HSD17B1 and ERBB2 in primary breast cancer. *Oncogene* **2003,** 22, 34-40.
11. Speirs, V.; Green, A. R.; Atkin, S. L. Activity and gene expression of 17beta-hydroxysteroid dehydrogenase type I in primary cultures of epithelial and stromal cells derived from normal and tumourous human breast tissue: the role of IL-8. *J Steroid Biochem Mol Biol* **1998,** 67, 267-74.
12. Suzuki, T.; Moriya, T.; Ariga, N.; Kaneko, C.; Kanazawa, M.; Sasano, H. 17betahydroxysteroid dehydrogenase type 1 and type 2 in human breast carcinoma: a correlation to clinicopathological parameters. *Br J Cancer* **2000,** 82, 518-523.
13. Šmuc, T.; Hevir, N.; Ribič-Pucelj, M.; Husen, B.; Thole, H.; Rizner, T. L. Disturbed estrogen and progesterone action in ovarian endometriosis. *Mol Cell Endocrinol* **2009,** 301, 59-64.

14. Husen, B.; Huhtinen, K.; Poutanen, M.; Kangas, L.; Messinger, J.; Thole, H. Evaluation of inhibitors for 17beta-hydroxysteroid dehydrogenase type 1 in vivo in immunodeficient mice inoculated with MCF-7 cells stably expressing the recombinant human enzyme. *Mol Cell Endocrinol* **2006,** 248, 109-13.
15. Husen, B.; Huhtinen, K.; Saloniemi, T.; Messinger, J.; Thole, H. H.; Poutanen, M. Human hydroxysteroid (17-beta) dehydrogenase 1 expression enhances estrogen sensitivity of MCF-7 breast cancer cell xenografts. *Endocrinology* **2006,** 147, 5333-9.
16. Day, J. M.; Foster, P. A.; Tutill, H. J.; Parsons, M. F.; Newman, S. P.; Chander, S. K.; Allan, G. M.; Lawrence, H. R.; Vicker, N.; Potter, B. V.; Reed, M. J.; Purohit, A. 17beta-hydroxysteroid dehydrogenase Type 1, and not Type 12, is a target for endocrine therapy of hormone-dependent breast cancer. *Int J Cancer* **2008,** 122, 1931-40.
17. Kruchten, P.; Werth, R.; Bey, E.; Oster, A.; Marchais-Oberwinkler, S.; Frotscher, M.; Hartmann, R. W. Selective inhibition of 17beta-hydroxysteroid dehydrogenase type 1 (17betaHSD1) reduces estrogen responsive cell growth of T47-D breast cancer cells. *J Steroid Biochem Mol Biol* **2009,** 114, 200-6.
18. Möller, G.; Deluca, D.; Gege, C.; Rosinus, A.; Kowalik, D.; Peters, O.; Droescher, P.; Elger, W.; Adamski, J.; Hillisch, A. Structure-based design, synthesis and in vitro characterization of potent 17β-hydroxysteroid dehydrogenase type 1 inhibitors based on 2-substitutions of estrone and D-homo-estrone. *Bioorganic & Medicinal Chemistry Letters* **2009,** 19, 6740-6744.
19. Poirier, D. Advances in development of inhibitors of 17beta hydroxysteroid dehydrogenases. *Anticancer Agents Med Chem* **2009,** 9, 642-60.
20. Bey, E.; Marchais-Oberwinkler, S.; Kruchten, P.; Frotscher, M.; Werth, R.; Oster, A.; Algül, O.; Neugebauer, A.; Hartmann, R. W. Design, synthesis and biological evaluation of bis(hydroxyphenyl) azoles as potent and selective non-steroidal inhibitors of 17beta-hydroxysteroid dehydrogenase type 1 (17beta-HSD1) for the treatment of estrogen-dependent diseases. *Bioorg Med Chem* **2008,** 16, 6423-35.
21. Bey, E.; Marchais-Oberwinkler, S.; Negri, M.; Kruchten, P.; Oster, A.; Klein, T.; Spadaro, A.; Werth, R.; Frotscher, M.; Birk, B.; Hartmann, R. W. New insights into the SAR and binding modes of bis(hydroxyphenyl)thiophenes and -benzenes: influence of additional substituents on 17beta-hydroxysteroid dehydrogenase type 1 (17beta-HSD1) inhibitory activity and selectivity. *J Med Chem* **2009,** 52, 6724-43.
22. Oster, A.; Klein, T.; Werth, R.; Kruchten, P.; Bey, E.; Negri, M.; Marchais-Oberwinkler, S.; Frotscher, M.; Hartmann, R. W. Novel estrone mimetics with high 17beta-HSD1 inhibitory activity. *Bioorg Med Chem* **2010,** 18, 3494-505.
23. Allan, G. M.; Vicker, N.; Lawrence, H. R.; Tutill, H. J.; Day, J. M.; Huchet, M.; Ferrandis, E.; Reed, M. J.; Purohit, A.; Potter, B. V. Novel inhibitors of 17beta-hydroxysteroid dehydrogenase type 1: templates for design. *Bioorg Med Chem* **2008,** 16, 4438-56.

24. Brožič, P.; Kocbek, P.; Sova, M.; Kristl, J.; Martens, S.; Adamski, J.; Gobec, S.; Lanišnik Rižner, T. Flavonoids and cinnamic acid derivatives as inhibitors of 17beta-hydroxysteroid dehydrogenase type 1. *Mol Cell Endocrinol* **2009,** 301, 229-34.
25. Karkola, S.; Lilienkampf, A.; Wähälä, K. A 3D QSAR model of 17beta-HSD1 inhibitors based on a thieno[2,3-d]pyrimidin-4(3H)-one core applying molecular dynamics simulations and ligand-protein docking. *ChemMedChem* **2008,** 3, 461-72.
26. Lilienkampf, A.; Karkola, S.; Alho-Richmond, S.; Koskimies, P.; Johansson, N.; Huhtinen, K.; Vihko, K.; Wahala, K. Synthesis and biological evaluation of 17beta-hydroxysteroid dehydrogenase type 1 (17beta-HSD1) inhibitors based on a thieno[2,3-d]pyrimidin-4(3H)-one core. *J Med Chem* **2009,** 52, 6660-71.
27. Schuster, D.; Nashev, L. G.; Kirchmair, J.; Laggner, C.; Wolber, G.; Langer, T.; Odermatt, A. Discovery of nonsteroidal 17beta-hydroxysteroid dehydrogenase 1 inhibitors by pharmacophore-based screening of virtual compound libraries. *J Med Chem* **2008,** 51, 4188-99.
28. Starčević, S.; Brožič, P.; Turk, S.; Cesar, J.; Rižner, T. L.; Gobec, S. Synthesis and biological evaluation of (6- and 7-phenyl) coumarin derivatives as selective nonsteroidal inhibitors of 17beta-hydroxysteroid dehydrogenase type 1. *J Med Chem* **2011,** 54, 248-61.
29. Starčević, S.; Turk, S.; Brus, B.; Cesar, J.; Lanišnik Rižner, T.; Gobec, S. Discovery of highly potent, nonsteroidal 17beta-hydroxysteroid dehydrogenase type 1 inhibitors by virtual high-throughput screening. *J Steroid Biochem Mol Biol* **2011,** 127, 255-61.
30. Oster, A.; Klein, T.; Henn, C.; Werth, R.; Marchais-Oberwinkler, S.; Frotscher, M.; Hartmann, R. W. Bicyclic substituted hydroxyphenylmethanone type inhibitors of 17 beta-hydroxysteroid dehydrogenase Type 1 (17 beta-HSD1): the role of the bicyclic moiety. *ChemMedChem* **2011,** 6, 476-87.
31. Bey, E.; Marchais-Oberwinkler, S.; Werth, R.; Negri, M.; Al-Soud, Y. A.; Kruchten, P.; Oster, A.; Frotscher, M.; Birk, B.; Hartmann, R. W. Design, synthesis, biological evaluation and pharmacokinetics of bis(hydroxyphenyl) substituted azoles, thiophenes, benzenes, and aza-benzenes as potent and selective nonsteroidal inhibitors of 17beta-hydroxysteroid dehydrogenase type 1 (17beta-HSD1). *J Med Chem* **2008,** 51, 6725-39.
32. Oster, A.; Hinsberger, S.; Werth, R.; Marchais-Oberwinkler, S.; Frotscher, M.; Hartmann, R. W. Bicyclic substituted hydroxyphenylmethanones as novel inhibitors of 17beta-hydroxysteroid dehydrogenase type 1 (17beta-HSD1) for the treatment of estrogen-dependent diseases. *J Med Chem* **2010,** 53, 8176-86.
33. Miyaura, N.; Yanagi, T.; Suzuki, A. The Palladium-Catalyzed Cross-Coupling Reaction of Phenylboronic Acid with Haloarenes in the Presence of Bases. *Synthetic Communications* **1981,** 11, 513-519.
34. Liang, L.-C.; Chien, P.-S.; Huang, M.-H. Catalytic Suzuki Coupling Reactions by Amido Phosphine Complexes of Palladium. *Organometallics* **2005,** 24, 353-357.

35. Meslin, J. C.; N'Guessan, Y. T.; Quiniou, H.; Tonnard, F. Enchainements heteroatomiques et leurs produits de cyclisationâ€"I : Vinylogues de thioamides comme intermediaires de synthese d'acyl-2 thiophenes, thio-1 pyrannones-2 (thiones), dihydro-5,6 dithiinnes-1,2 dioxydes-1,1 et dithiinnes-1,2 dioxydes-1,1 substitues. *Tetrahedron* **1975,** 31, 2679-2684.
36. Liu, C.-M.; Chen, B.-H.; Liu, W.-Y.; Wu, X.-L.; Ma, Y.-X. Conversion of tributylstannylferrocene to a variety of heteroaryl ferrocenes. *Journal of Organometallic Chemistry* **2000,** 598, 348-352.
37. Lal, G. S.; Lobach, E.; Evans, A. Fluorination of thiocarbonyl compounds with Bis(2-methoxyethyl)aminosulfur trifluoride (Deoxo-fluor reagent): A facile synthesis of gem-difluorides. *J Org Chem* **2000,** 65, 4830-2.
38. Fink, B. E.; Mortensen, D. S.; Stauffer, S. R.; Aron, Z. D.; Katzenellenbogen, J. A. Novel structural templates for estrogen-receptor ligands and prospects for combinatorial synthesis of estrogens. *Chem Biol* **1999,** 6, 205-19.
39. Kruchten, P.; Werth, R.; Marchais-Oberwinkler, S.; Frotscher, M.; Hartmann, R. W. Development of a biological screening system for the evaluation of highly active and selective 17beta-HSD1-inhibitors as potential therapeutic agents. *Mol Cell Endocrinol* **2009,** 301, 154-7.
40. Qiu, W.; Campbell, R. L.; Gangloff, A.; Dupuis, P.; Boivin, R. P.; Tremblay, M. R.; Poirier, D.; Lin, S. X. A concerted, rational design of type 1 17beta-hydroxysteroid dehydrogenase inhibitors: estradiol-adenosine hybrids with high affinity. *FASEB J* **2002,** 16, 1829-31.
41. Sam, K. M.; Auger, S.; Luu-The, V.; Poirier, D. Steroidal spiro-gamma-lactones that inhibit 17 beta-hydroxysteroid dehydrogenase activity in human placental microsomes. *J Med Chem* **1995,** 38, 4518-28.
42. Craig, P. N. Interdependence between physical parameters and selection of substituent groups for correlation studies. *J Med Chem* **1971,** 14, 680-4.
43. Klein, T.; Henn, C.; Negri, M.; Frotscher, M. Structural basis for species specific inhibition of 17beta-hydroxysteroid dehydrogenase type 1 (17beta-HSD1): computational study and biological validation. *PLoS One* **2011,** 6, e22990.
44. Wilcken, R.; Zimmermann, M. O.; Lange, A.; Joerger, A. C.; Boeckler, F. M. Principles and applications of halogen bonding in medicinal chemistry and chemical biology. *J Med Chem* **2013,** 56, 1363-88.
45. Frotscher, M.; Ziegler, E.; Marchais-Oberwinkler, S.; Kruchten, P.; Neugebauer, A.; Fetzer, L.; Scherer, C.; Muller-Vieira, U.; Messinger, J.; Thole, H.; Hartmann, R. W. Design, synthesis, and biological evaluation of (hydroxyphenyl)naphthalene and -quinoline derivatives: potent and selective nonsteroidal inhibitors of 17beta-hydroxysteroid dehydrogenase type 1 (17beta-HSD1) for the treatment of estrogen-dependent diseases. *J Med Chem* **2008,** 51, 2158-69.

46. Smart, B. E. Fluorine substituent effects (on bioactivity). *Journal of Fluorine Chemistry* **2001,** 109, 3-11.
47. Puranen, T. J.; Poutanen, M. H.; Peltoketo, H. E.; Vihko, P. T.; Vihko, R. K. Site-directed mutagenesis of the putative active site of human 17 beta-hydroxysteroid dehydrogenase type 1. *Biochem J* **1994,** 304 (Pt 1), 289-93.
48. Marchais-Oberwinkler, S.; Wetzel, M.; Ziegler, E.; Kruchten, P.; Werth, R.; Henn, C.; Hartmann, R. W.; Frotscher, M. New drug-like hydroxyphenylnaphthol steroidomimetics as potent and selective 17beta-hydroxysteroid dehydrogenase type 1 inhibitors for the treatment of estrogen-dependent diseases. *J Med Chem* 54, 534-47.
49. Duan, Y.; Wu, C.; Chowdhury, S.; Lee, M. C.; Xiong, G.; Zhang, W.; Yang, R.; Cieplak, P.; Luo, R.; Lee, T.; Caldwell, J.; Wang, J.; Kollman, P. A point-charge force field for molecular mechanics simulations of proteins based on condensed-phase quantum mechanical calculations. *J Comput Chem* **2003,** 24, 1999-2012.
50. Krieger, E.; Darden, T.; Nabuurs, S. B.; Finkelstein, A.; Vriend, G. Making optimal use of empirical energy functions: force-field parameterization in crystal space. *Proteins* **2004,** 57, 678-83.
51. Breton, R.; Housset, D.; Mazza, C.; Fontecilla-Camps, J. C. The structure of a complex of human 17beta-hydroxysteroid dehydrogenase with estradiol and NADP+ identifies two principal targets for the design of inhibitors. *Structure* **1996,** 4, 905-15.
52. Morris, G. M.; Huey, R.; Lindstrom, W.; Sanner, M. F.; Belew, R. K.; Goodsell, D. S.; Olson, A. J. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *Journal of Computational Chemistry* **2009,** 30, 2785-2791.

3.II Halogenated 17β-HSD1 Inhibitors: Discovery of Highly Potent and Metabolically Stable Compounds Enabling a Proof of Principle Study in Rodents

- This chapter will be submitted for publication in the Journal of Medicinal Chemistry
- The structures of the compounds described therein and their biological properties are subject of patent applications.

Abstract

The most potent naturally occurring member of estrogens is 17β-estradiol (E2), which known to be involved in development and proliferation of estrogen-dependent diseases (EDD) like breast cancer and endometriosis. 17β-Hydroxysteroid dehydrogenase type 1 (17β-HSD1) catalyzes the last step of E2 biosynthesis from its weakly estrogenic precursor estrone (E1). Consequently, 17β-HSD1 came into the focus of interest as a novel therapeutic target for the treatment of EDD. Bicyclic substituted hydroxyphenyl methanones (BSHs), an inhibitor class described by us previously, not only display high inhibitory potency towards human 17β-HSD1, but also activity towards mouse 17β-HSD1 and fair metabolic stability in human liver microsomes. The aim of this study were structural optimizations in the BSHs class to obtain a suitable candidate for a proof of principle (PoP) study in an animal disease model for endometriose. In this work, we describe the most potent nonsteroidal *h*17β-HSD1 inhibitors up to date with IC_{50}s in the picomolar range. In addition, they showed good selectivities towards the type 2 enzyme, which catalyzes the reverse reaction, and the estrogen receptors α and β. Compound **43**, one of the most interesting *h*17β-HSD1 inhibitors discovered in this study, showed high stability towards both phase I and II metabolism in human liver microsomes (S9 fraction; 72 % remaining after 60 min). Moreover, activity towards rodent 17β-HSD1 could be significantly improved. Compound **47** showed strong inhibition of the rat enzyme (63 % inhibition @ 250 nM) and could thus be considered a possible candidate for a PoP study.

Introduction

In humans, 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1) catalyses the NAD(P)H dependent transformation of estrone (E1) to estradiol (E2) which represents the most potent estrogen (fig. 1).[1] E2 is known to play a crucial role in the development and progression of several estrogen dependent diseases (EDD).[2-5] Increased E2/E1 ratios as well as elevated 17β-HSD1 m-RNA levels are indicators of the essential importance of 17β-HSD1 in breast cancer[6, 7], ovarian tumor[8], endometriosis[9], endometrial hyperplasia[10] and other EDD[11]. Consequently, the selective inhibition of this enzyme is considered as a valuable treatment option. Due to the tissue-selective expression and the intracrine mode of action of 17β-HSD1[12], its inhibition should be associated with less side effects compared to established treatments using GnRH-analogs, aromatase inhibitors, anti-estrogens or selective estrogen receptor modulators (SERMs).[13-15] For the prospect of less side effects, however, inhibition of 17β-HSD1 should be selective. For instance, 17β-HSD2, which may have opposing action, and the estrogen receptors (ER) α and β should not be influenced.

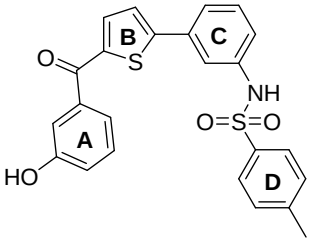
Figure 1. Interconversion of Estrone (E1) and Estradiol (E2).

Therapeutics interfering with intracrine pathways of hormone biosynthesis are already in clinical use, eg. 5α-reductase inhibitors.[16-19] Although a number of steroidal and non-steroidal inhibitors of 17β-HSD1[20-22] are described and there is experimental evidence for the effectiveness of 17β-HSD1 inhibition against human tumor cell lines *in vitro* and in animal models, there is no inhibitor under clinical evaluation. Moreover, no proof of principle study has been conducted for the indication endometriosis, although this is a widespread disease for which no adequate medical treatment is available.

One important reason for this may be interspecies differences between human 17β-HSD1 and animal orthologs which impede corresponding *in vivo* experiments, eg. in rodents.[23] Thus, human

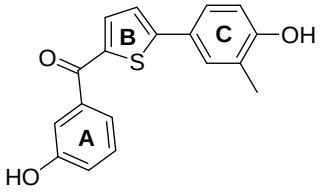
and rodent 17β-HSD1 are 83 % similar and show significant differences concerning the topology of the substrate binding site.[24, 25] As a consequence, compounds highly active towards the human enzyme are often weakly active or inactive towards mouse or rat 17β-HSD1.

The aim of this study was the design of compounds inhibiting both human and rodent 17β-HSD1, displaying favorable in vitro ADME properties. Starting point was the class of bicyclic substituted hydroxyphenylmethanones (BSHs).[26, 27] In contrast to most other classes of 17β-HSD1 inhibitors described by us[28-35], it contains members which not only are strong inhibitors of the human enzyme but also show inhibition of the murine ortholog (unpublished results). Examples are compounds **I** and **II** (fig. 2).[26, 27] Drug design included modification of the sulfonamide moiety as well as optimization of the core structure (ABC-system) with the aim of enhancing inhibitory activity towards rodent 17β-HSD1 (mouse, rat) while maintaining activity towards the human enzyme and selectivity over the type 2 enzyme. Rational strategies were applied to elucidate drug metabolism and to obtain metabolically stable compounds. In addition, the implementation of favorable lipophilicities was taken into account.



Compound I

Human 17b-HSD1: (IC_{50} = 12 nM)
Human 17b-HSD2: (IC_{50} = 168 nM)
Mouse 17b-HSD1: (% inhibition @ 1 µM = 28)
Metabolic stability: $t_{1/2}$ (phase I) = 13 min



Compound II

Human 17b-HSD1: (IC_{50} = 8 nM)
Human 17b-HSD2: (IC_{50} = 384 nM)
Mouse 17b-HSD1: (% inhibition @ 1 µM = 20)
Metabolic stability: $t_{1/2}$ (phase I) = 100 min, $t_{1/2}$ (s9) = 4 min

Figure 2. Examples of bicyclic substituted hydroxyphenylmethanones (BSHs).

Design

From previous studies it was known that in the class of bicyclic substituted hydroxyphenylmethanones, inhibition of the target enzyme is strongly dependent on the substitution pattern of the benzoyl ring (fig. 2, ring A). Here, already minor variations were found to induce dramatic changes in activity. In contrast, it is rather selectivity (towards 17β-HSD2) than inhibitory potency which is influenced by the introduction of substituents at the phenyl ring (ring C). For instance, the presence of an aromatic sulfonamide moiety proved to be beneficial.[27]

The fact that only one inhibitor bearing an aromatic sulfonamide moiety was described in the past prompted us to explore this group in more detail (chart 1, design step I). Its optimal position on the phenyl ring C (compounds **1-10**) was determined, and the analogous (methylated) carbonic acid amides were investigated as possible bioisosteres (compounds **11** and **12**). Moreover, compounds **13-29** with diverse substituents on ring D were prepared in a parallel synthesis approach in order to explore the chemical space more thoroughly.

At this point, there was evidence that, in terms of biological activities towards human 17β-HSD1 and 2, the synthesized sulfonamides do not have advantages over compounds devoid of the sulfonamide group. In addition, the core structure of the inhibitors, consisting of the ABC-ring system, displayed liability towards phase II metabolism reactions due to the presence of phenolic OH-groups. For these reasons, further optimiziation steps were performed which focused on the core structure, considering the following structure activity relationships obtained in a previous study: [26, 27]

Ring A

- the OH-group on the benzoylic moiety (ring A) is very important for activity
- only minor structural variations in this part are tolerated by the target enzyme

Ring C

- the OH-group on the phenyl ring (ring C) is not essential for inhibitory activity, though generally it proved to be beneficial for selectivity towards the type 2 enzyme
- major structural variations can be carried out without loss of activity

Two strategies were applied to enhance metabolic stability (design step II, compounds **31**-**50**):

- Small substituents (methyl or fluorine) were introduced in ring A in order to protect its hydroxy group from phase II metabolism (first strategy).
- The OH-group of ring C was replaced by other functionalities or shielded by bulky and/or electron withdrawing groups (second strategy).

In addition, ring C was modified by replacement with heterocycles or by substitution with heterocyclic moieties in order to decrease the lipophilicity of the compounds (compounds **51**-**53** and **56**-**60**).

Biological data suggested that the presence of the sulfonamide moiety is beneficial concerning inhibition of mouse 17β-HSD1, thus possibly allowing for an *in vivo* proof of principle study. Therefore, a final design step was envisaged based upon the results of the design steps I and II, leading to fluorinated sulfonamide-type inhibitors (design step III; compounds **62**-**69**).

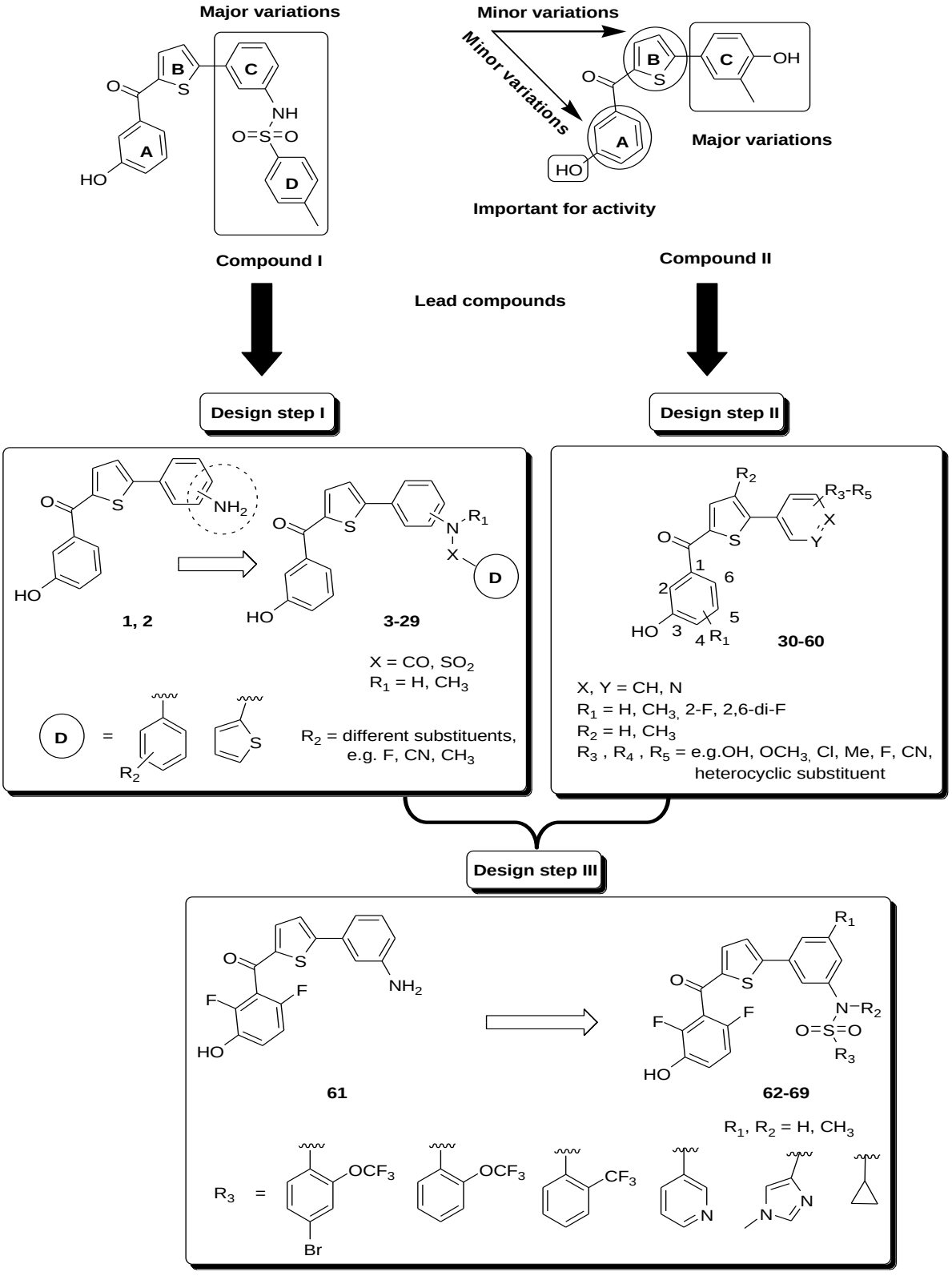
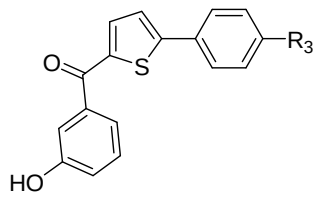


Chart 1. Design of Synthesized Compounds **1 – 69.**



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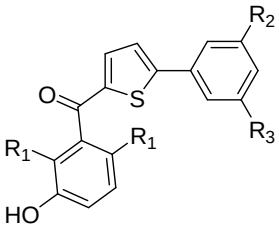

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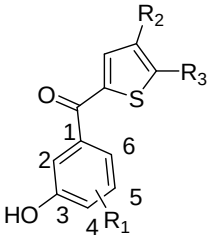

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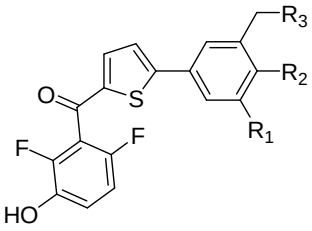

56-60

Comd	R_1	R_2	R_3	Comd	R_1	R_2	R_3
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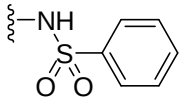
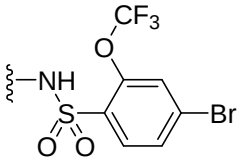
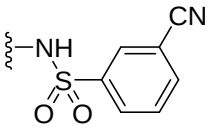
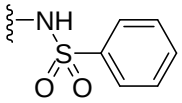
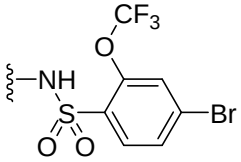
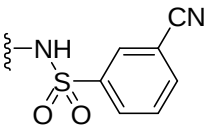
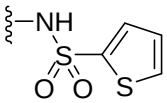
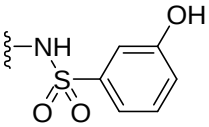
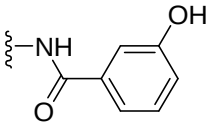
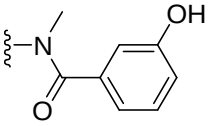
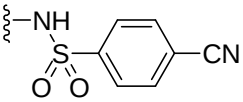
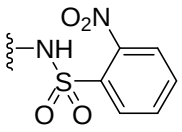
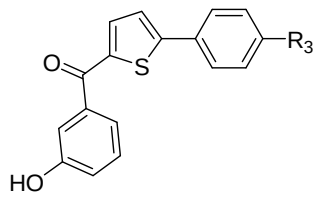
3	-	-		**4**	-	-	
5	-	-		**6**	-	H	
7	-	H		**8**	-	H	
9	-	H		**10**	-	H	
11	-	H		**12**	-	H	
13	-	H		**14**	-	H	



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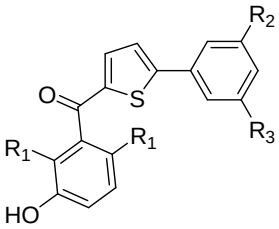

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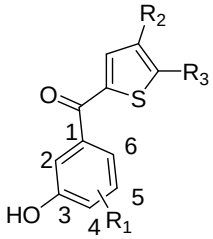

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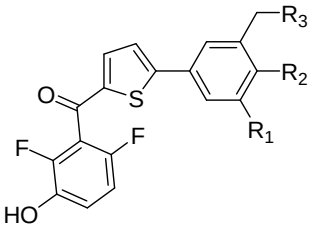
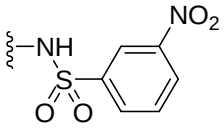
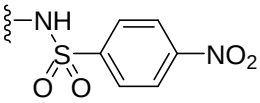
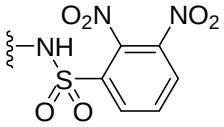
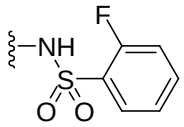
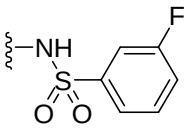
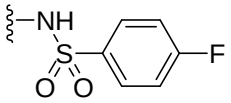
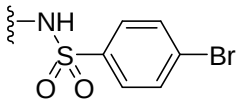
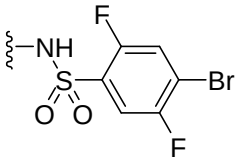
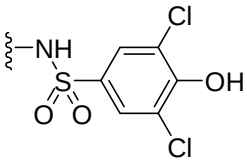
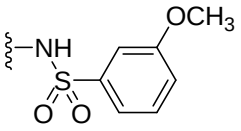
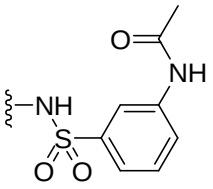
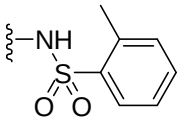
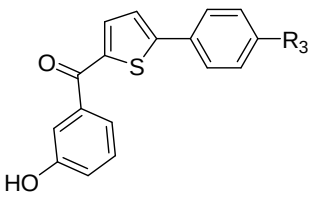


56-60

Comd	R_1	R_2	R_3	Comd	R_1	R_2	R_3
15	-	H		**16**	-	H	
17	-	H		**18**	-	H	
19	-	H		**20**	-	H	
21	-	H		**22**	-	H	
23	-	H		**24**	-	H	
25	-	H		**26**	-	H	



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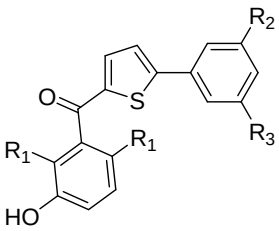

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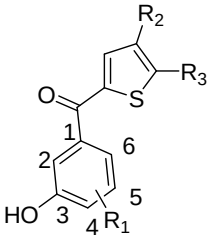

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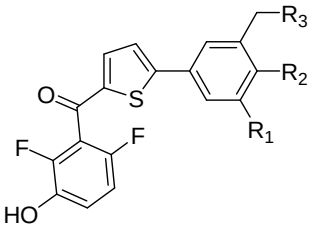

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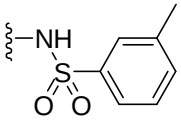
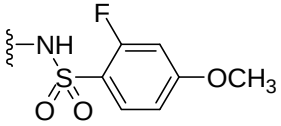
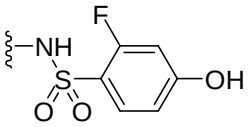
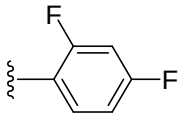
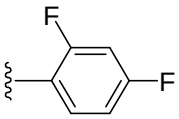
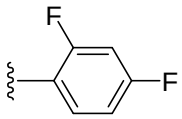
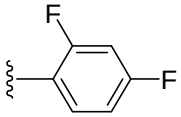
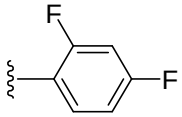
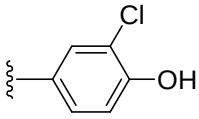
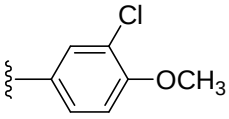
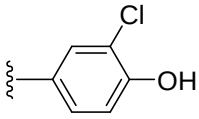
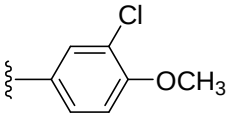
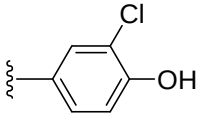
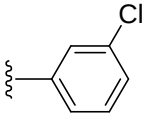
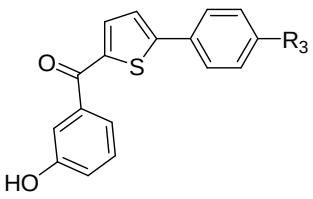
Comd	R_1	R_2	R_3	Comd	R_1	R_2	R_3
27	-	H		**28**	-	H	
29	-	H		**30**	H	H	
31	2-CH_3	H		**32**	2-CH_3	CH_3	
33	2-F	H		**34**	2,6-di-F	H	
35	2-CH_3	H		**36**	2,6-di-F	H	
37	2,6-di-F	H		**38**	2,6-di-F	CH_3	
39	2,6-di-F	CH_3		**40**	2,6-di-F	H	



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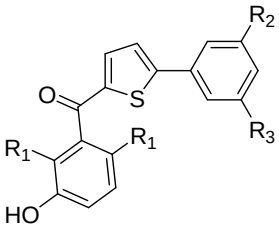

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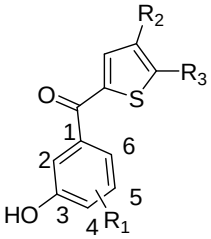

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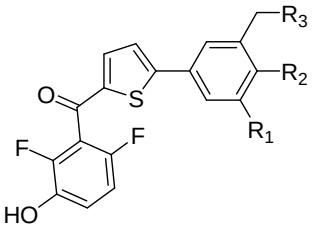

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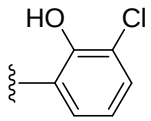
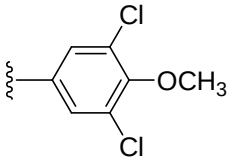
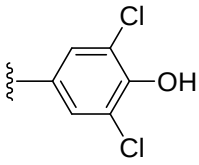
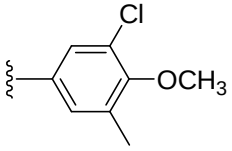
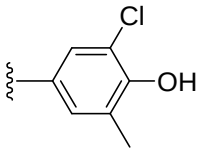
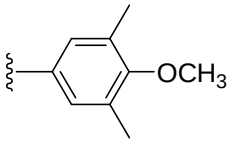
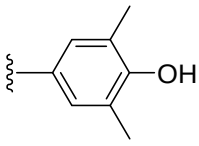
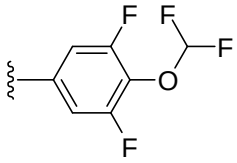
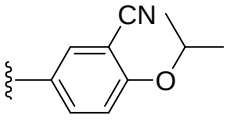
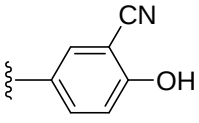
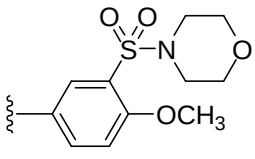
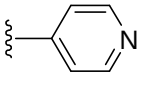
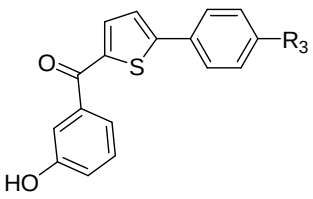
Comd	R_1	R_2	R_3	Comd	R_1	R_2	R_3
41	2,6-di-F	H		**42**	2,6-di-F	H	
43	2,6-di-F	H		**44**	2,6-di-F	H	
45	2,6-di-F	H		**46**	2,6-di-F	H	
47	2,6-di-F	H		**48**	2,6-di-F	H	
49	2,6-di-F	H		**50**	2,6-di-F	H	
51	2,6-di-F	H		**52**	2,6-di-F	H	



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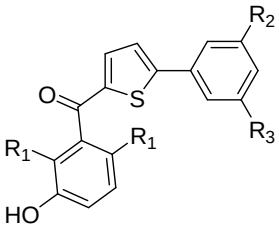

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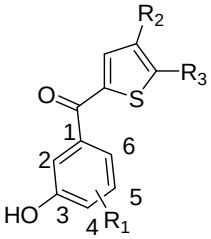

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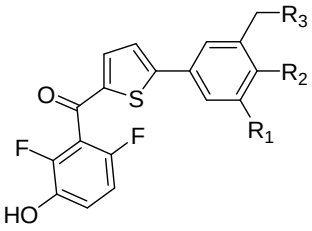
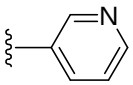
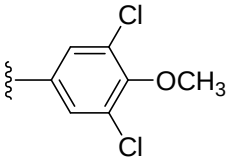
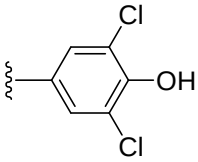
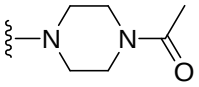
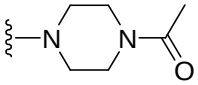
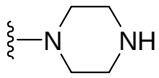
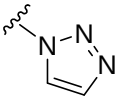
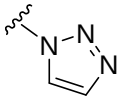
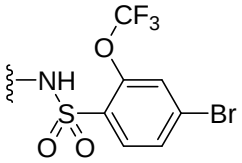
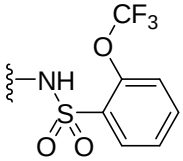
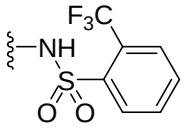
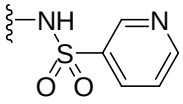
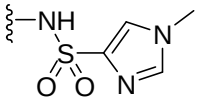
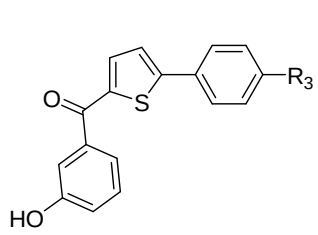
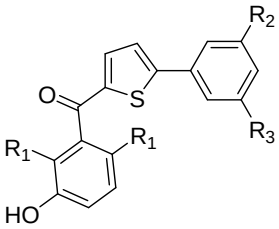


56-60

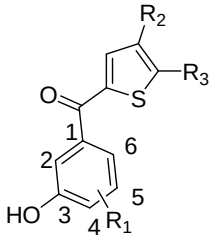
Comd	R_1	R_2	R_3	Comd	R_1	R_2	R_3
53	2,6-di-F	H		**54**	H	H	
55	H	H		**56**	Cl	OCH_3	
57	Cl	OH		**58**	Cl	OCH_3	
59	Cl	OCH_3		**60**	Cl	OH	
61	2,6-di-F	H	NH_2	**62**	2,6-di-F	H	
63	2,6-di-F	H		**64**	2,6-di-F	H	
65	2,6-di-F	H		**66**	2,6-di-F	H	



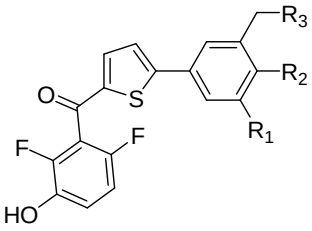
1, 3-5



2, 6-29, 61-69



30-55



56-60

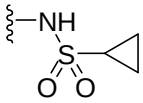
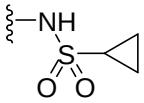
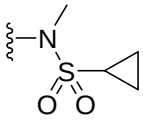
Comd	R_1	R_2	R_3	Comd	R_1	R_2	R_3
67	2,6-di-F	H		**68**	2,6-di-F	CH_3	
69	2,6-di-F	CH_3					

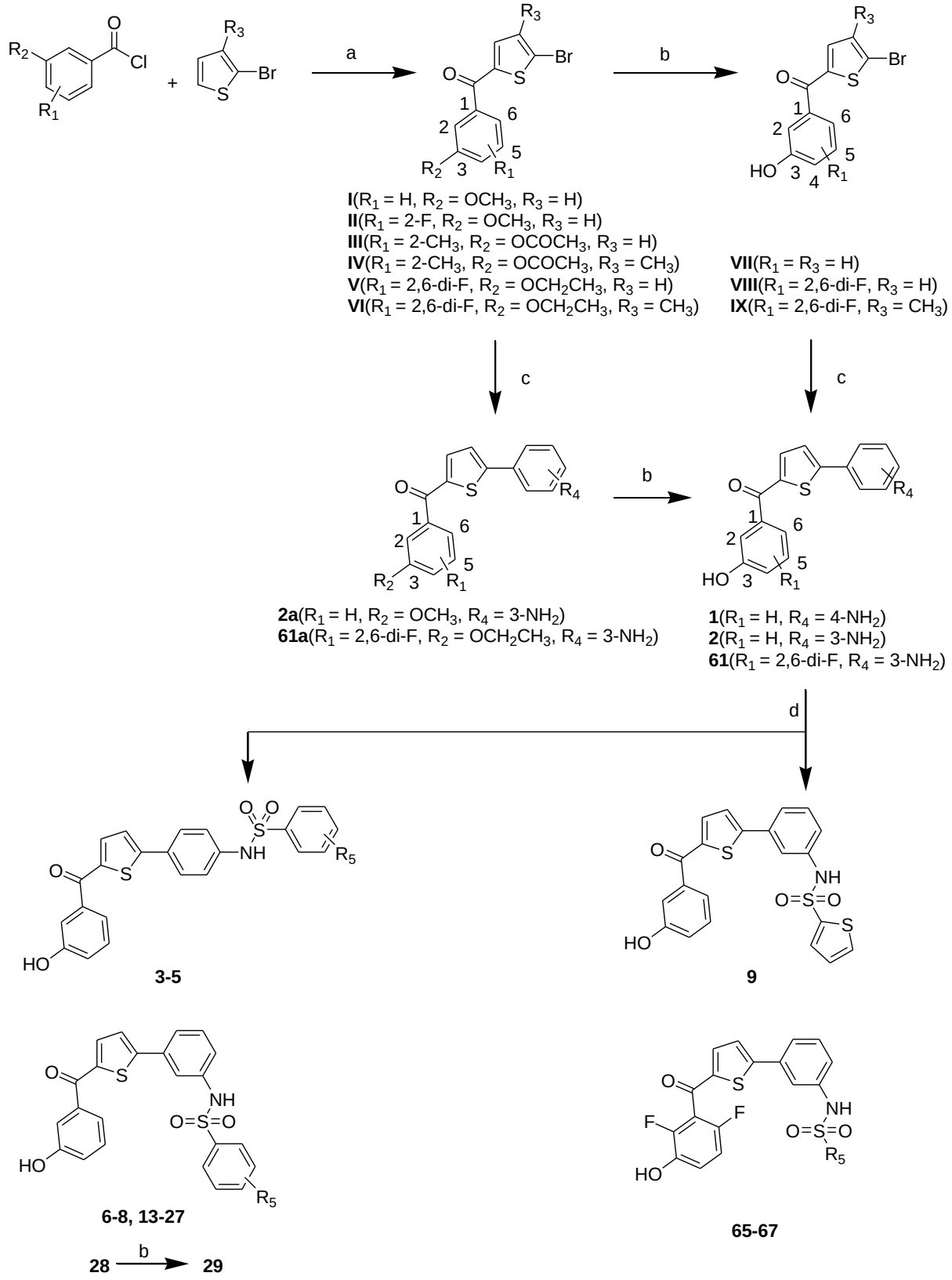
Chart 2. Overview on synthesized compounds **1-69**.

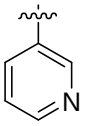
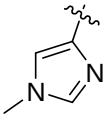
Chemistry

The key intermediates **I-VI** were synthesized in good yield from the appropriate benzoyl chlorides and thiophenes using Friedel-Crafts conditions (scheme **1**, method A). Followed by ether cleavage[36] of intermediates **I**, **V** and **VI** yielded **VII**, **VIII** and **IX** (method B) in quantitative yield, respectively.

VII Was reacted with 4-aminophenylboronic acid pinacol ester in a Suzuki cross coupling reaction[37] to afford the final compound **1.** Compounds **2** and **61** were prepared from **I** and **V**, respectively, using the same methods as for **1** but in reversed order because the intermediates **2a** and **61a** were needed as starting materials in the syntheses according to scheme 2, *vide infra*.

The sulfonic acid amides **3-28** and **65-67** were synthesized by reaction of **1**, **2** and **61**, respectively, with the corresponding sulfonic acid chlorides at room temperature. Cleavage of the ether function of **28** gave the dihydroxylated compound **29**. (Scheme **1**)


a
b
c
d
I(R1 = H, R2 = OCH3, R3 = H)
II(R1 = 2-F, R2 = OCH3, R3 = H)
III(R1 = 2-CH3, R2 = OCOCH3, R3 = H)
IV(R1 = 2-CH3, R2 = OCOCH3, R3 = CH3)
V(R1 = 2,6-di-F, R2 = OCH2CH3, R3 = H)
VI(R1 = 2,6-di-F, R2 = OCH2CH3, R3 = CH3)
VII(R1 = R3 = H)
VIII(R1 = 2,6-di-F, R3 = H)
IX(R1 = 2,6-di-F, R3 = CH3)
2a(R1 = H, R2 = OCH3, R4 = 3-NH2)
61a(R1 = 2,6-di-F, R2 = OCH2CH3, R4 = 3-NH2)
1(R1 = H, R4 = 4-NH2)
2(R1 = H, R4 = 3-NH2)
61(R1 = 2,6-di-F, R4 = 3-NH2)
3-5
9
6-8, 13-27
65-67
28
29

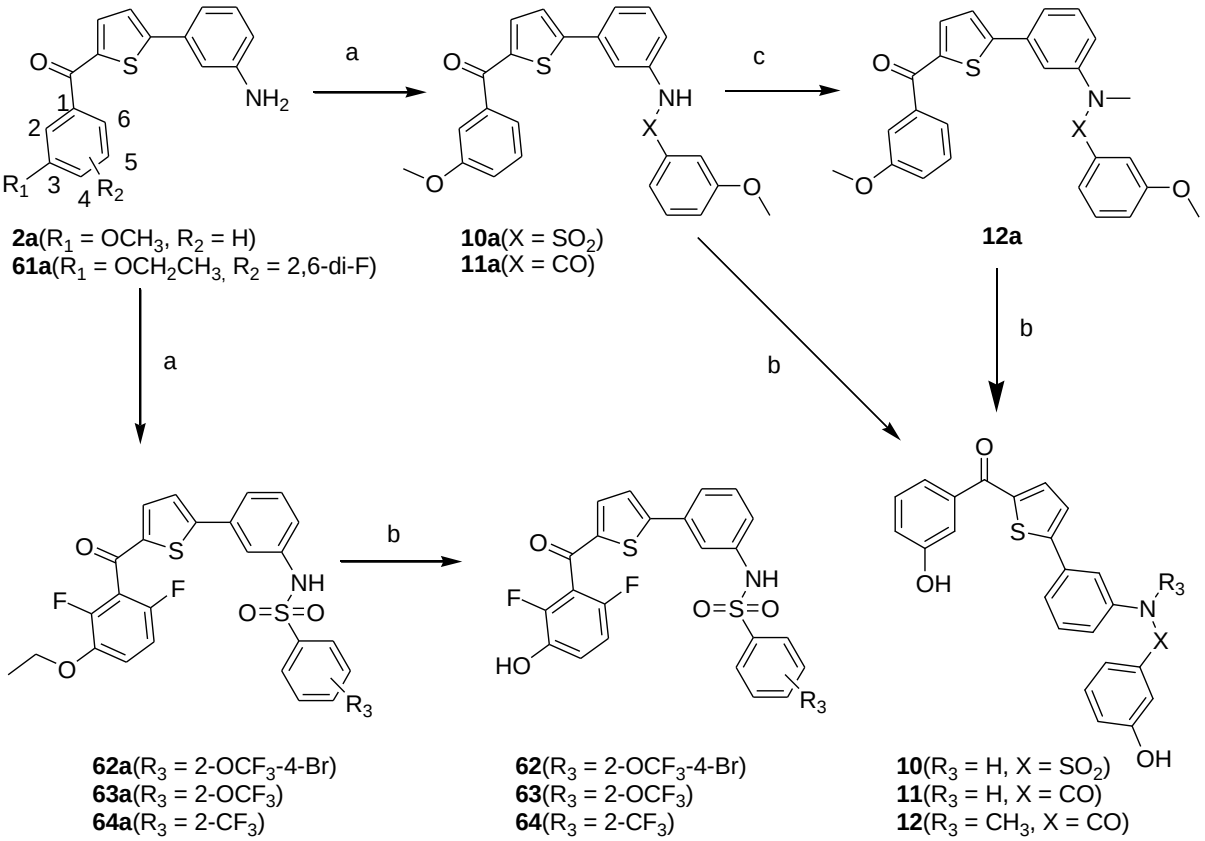
Comd	R_5	**Comd**	R_5	**Comd**	R_5
3	H	**16**	4-NO_2	**25**	3-NHCOMe
4	4-Br-2-OCF_3	**17**	2,3-di-NO_2	**26**	2-Me
5	3-CN	**18**	2-F	**27**	3-Me
6	H	**19**	3-F	**28**	2-F-4-OMe
7	4-Br-2-OCF_3	**20**	4-F	**29**	2-F-4-OH
8	3-CN	**21**	4-Br	**65**	
13	4-CN	**22**	4-Br- 2,5-di-F	**66**	
14	2-NO_2	**23**	3,5-di-Cl-4-OH	**67**	
15	3-NO_2	**24**	3-OMe		

Scheme 1: synthesis of compounds **1-29** and **65-67**. a) method A, $AlCl_3$, anhydrous CH_2Cl_2, 0 ^oC, 0.5h and then rt, 3h. b) method B, BBr_3, CH_2Cl_2, -78^oC to rt, overnight. c) method C1, 3-aminophenylboronic acid or 4-aminophenylboronic acid pinacol ester, Cs_2CO_3, $Pd(PPh_3)_4$, DME/water (1:1), reflux, 4h. d) method D, pyridine, corresponding sulfonyl chloride, rt, overnight.

Reaction of **2a** with 3-methoxybenzenesulfonyl- or 3-methoxybenzoyl chloride yielded the corresponding intermediates **10a** and **11a**, which were submitted to ether cleavage to give the final compounds **10** and **11**, respectively. (Scheme **2**)

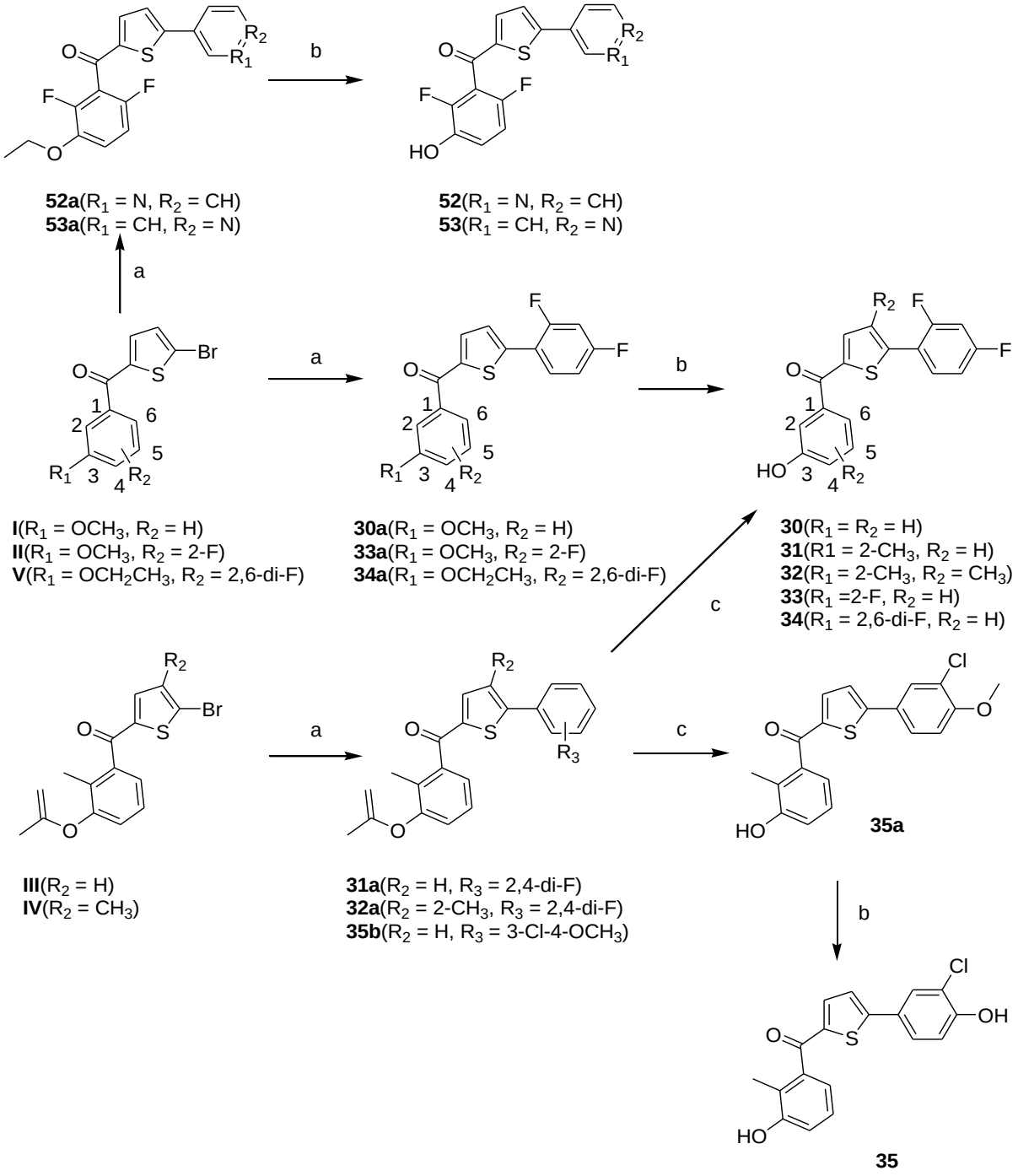
N-Methylation of compound **11a** with methyl iodide afforded **12a** which was reacted with BBr_3 in dichloromethane (method B) to give **12**.

Interestingly, the synthesis of the fluorinated sulfonamides **62-64** starting from the phenol **61** (analogously to the preparation of the fluorinated sulfonamides **65-67**) proved to be difficult due to purification issues. This problem could be solved by employing the ether intermediate **61a** instead of the phenol **60** as starting material and conducting *O*-demethylation of the intermediates **62a-64a** as the final step.



Scheme 2: synthesis of compounds **10-12** and **62-64**. a) method D, pyridine, ArXCl, rt (reflux in case of X = CO), overnight. b) method B, BBr_3, CH_2Cl_2, -78°C to rt, overnight. c) NaH, DMF, MeI, 0°C to rt, 0.5h.

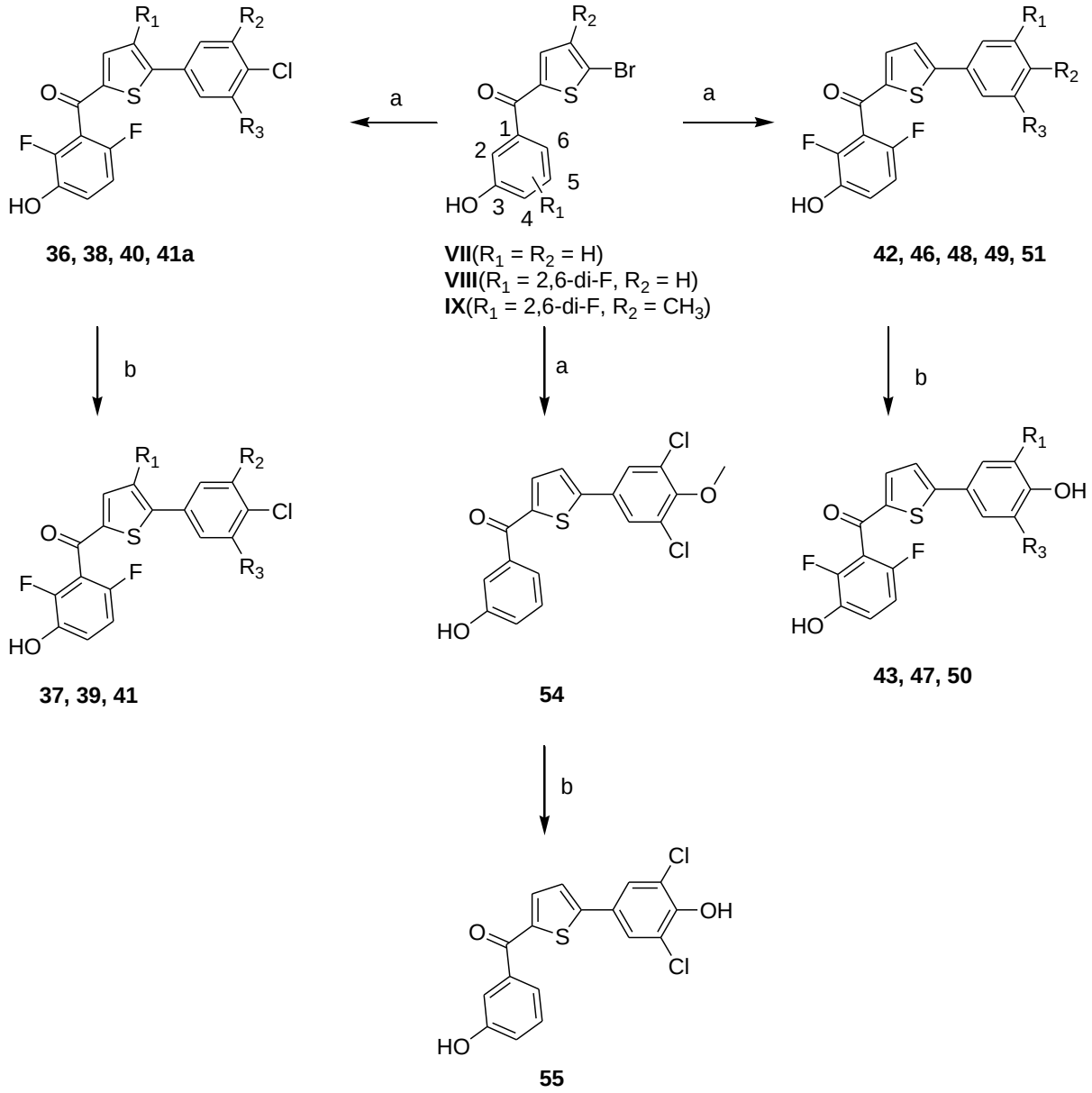
For the preparation of compounds **30-35** the key intermediates **I-V** were submitted to a Suzuki reaction (method C1 or C2) with the appropriate boronic acid to give the corresponding intermediates **30a-34a**, **35b**, **52a** and **53a**. Which were treated in different ways: The esters **31a**, **32a**, and **35b** were saponificated with 2M NaOH to give the phenols **31**, **32**, and **35a** in good yield. The other intermediates were submitted to ether cleavage with BBr_3 in dichloromethane to afford the final compounds **30**, **33-35**, **52**, and **53**. (Scheme **3**)



Scheme 3: synthesis of compounds **30-35**, **52**, and **53**. a) method C1, corresponding boronic acid, Cs_2CO_3, $Pd(PPh_3)_4$, DME/water (1:1), reflux, 4h; method C2, 4- pyridinylboronic acid pinacol ester or 3-pyridinylboronic acid for **52a** and **53a**, respectively, Na_2CO_3, $Pd(PPh_3)_4$, toluene/ethanol (1:1), reflux, overnight. b) method B, BBr_3, CH_2Cl_2, -78°C to rt, overnight. **c**) 10% NaOH, ethanol, reflux, 2h.

As depicted in scheme **4**, the compounds **36-43**, **41a**, **46-51**, **54** and **55** were obtained from the intermediates **VII-IX** by standard methods (either Suzuki reaction (method C1) alone or followed by ether cleavage (method B)).

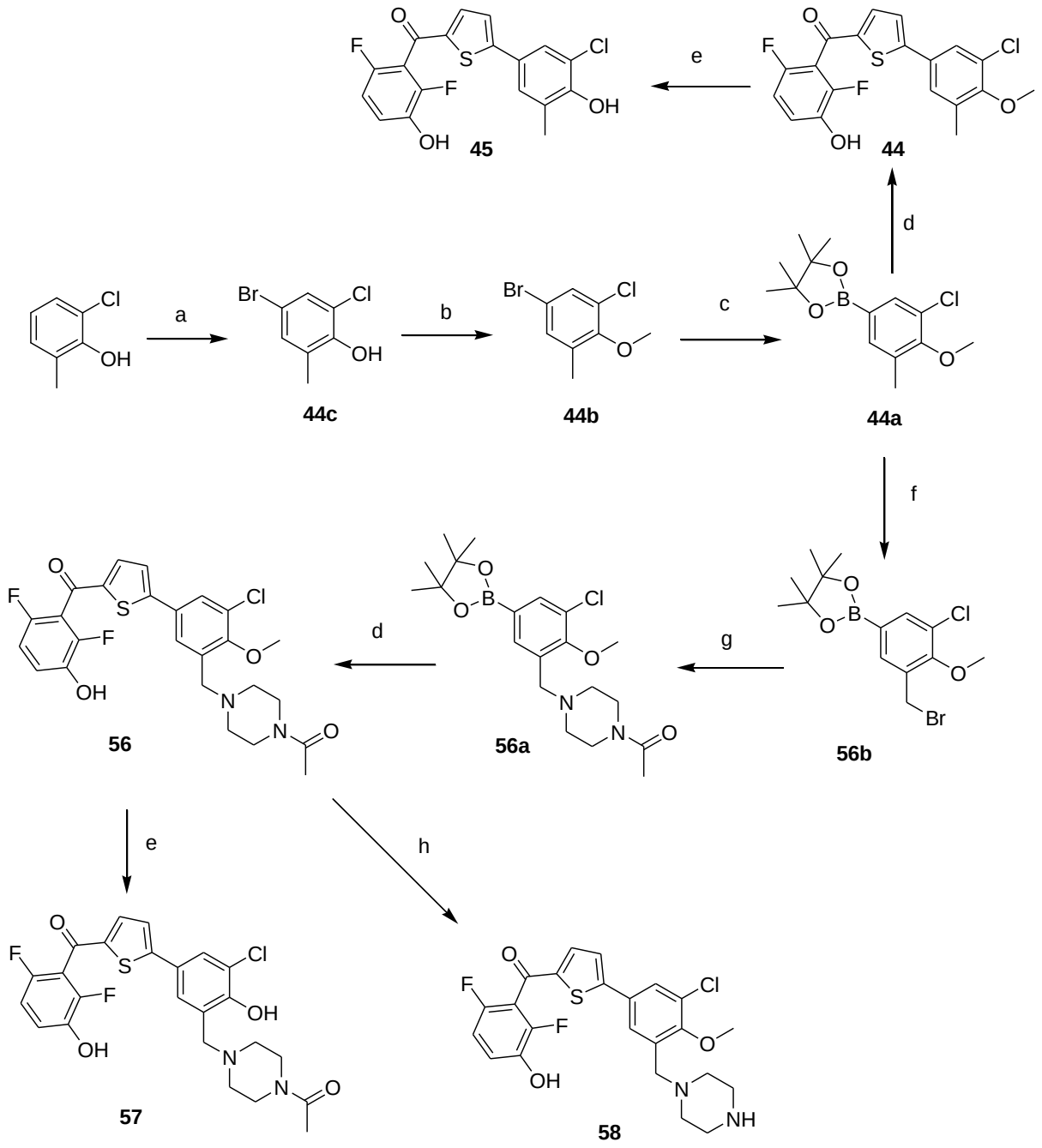
The synthesis of compound **45** was accomplished as shown in scheme **5**: Bromination of 2-chloro-6-methylphenol with NBS in acetic acid selectively gave the ring-brominated compound **44c**, which underwent methylation upon treatment with methyl iodide. The resulting compound **44b** was transformed to the boronic acid ester **44a**. Finally, Suzuki cross coupling reaction of the latter with **VIII** yielded compound **44**, which was sub mitted to ether cleavage with BBr_3, resulting in **45**.



Comd	R_1	R_2	R_3	**Comd**	R_1	R_2	R_3
36	H	H	OCH_3	**43**	Cl	-	Cl
37	H	H	OH	**46**	CH_3	OCH_3	CH_3
38	CH_3	H	OCH_3	**47**	CH_3	-	CH_3
39	CH_3	H	OH	**48**	F	$OCHF_2$	F
40	H	H	H	**49**	CN	$OCH(CH_3)_2$	H
41a	H	OCH_3	H	**50**	CN	-	H
41	H	OH	H	**51**	SO_2-4-morpholine	OCH_3	H
42	Cl	OCH_3	Cl				

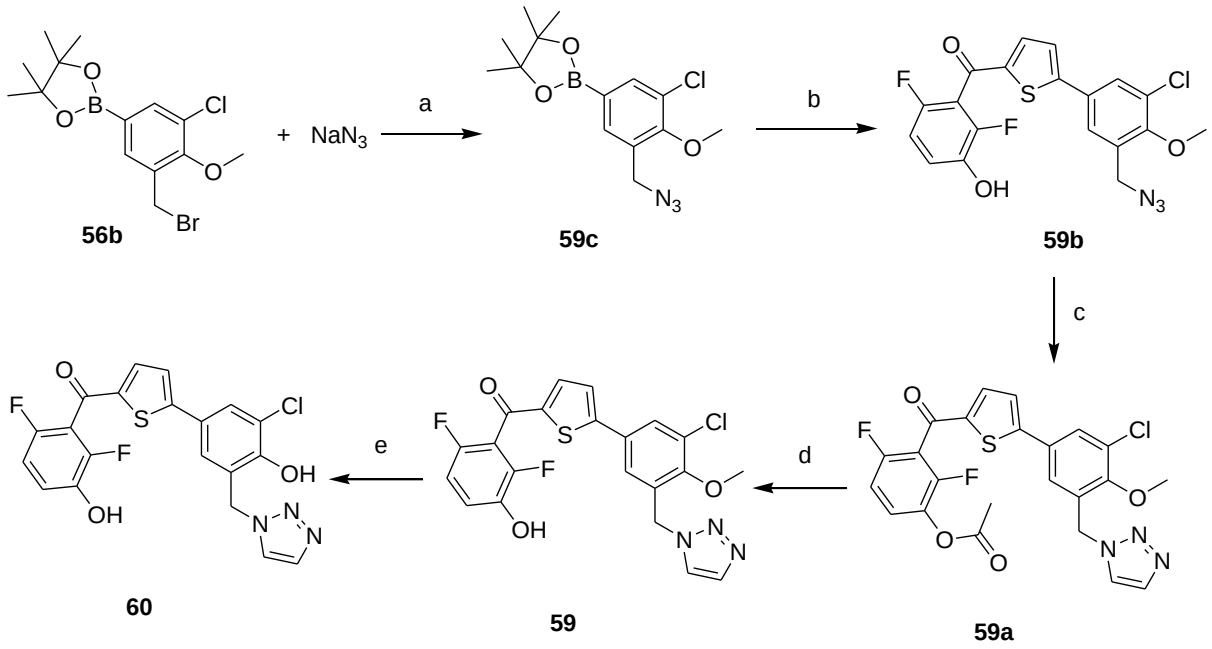
Scheme 4: synthesis of compounds **36-43**, **46-51**, **54** and **55**. a) method C1, boronic acid derivative, Cs_2CO_3, $Pd(PPh_3)_4$, DME/water (1:1), reflux, 4h. b) method B, BBr_3, CH_2Cl_2, -78°C to rt, overnight.

The boronic acid ester **44a** was also used as starting material for the syntheses leading to compounds **56-58** (scheme **5**). By using NBS and DBPO in CCl_4, **44a** was converted to the brominated intermediate **56b**. Nucleophilic substitution of the the bromine atom with 1-piperazine-1-ylethanone yielded **56a**. The latter was reacted with **VIII** in a Suzuki reaction (method C1) to afford compound **56**, which gave access to **57** (via ether cleavage) and **58** (via amide hydrolysis under acidic conditions). *O*-demethylation of **58** failed.



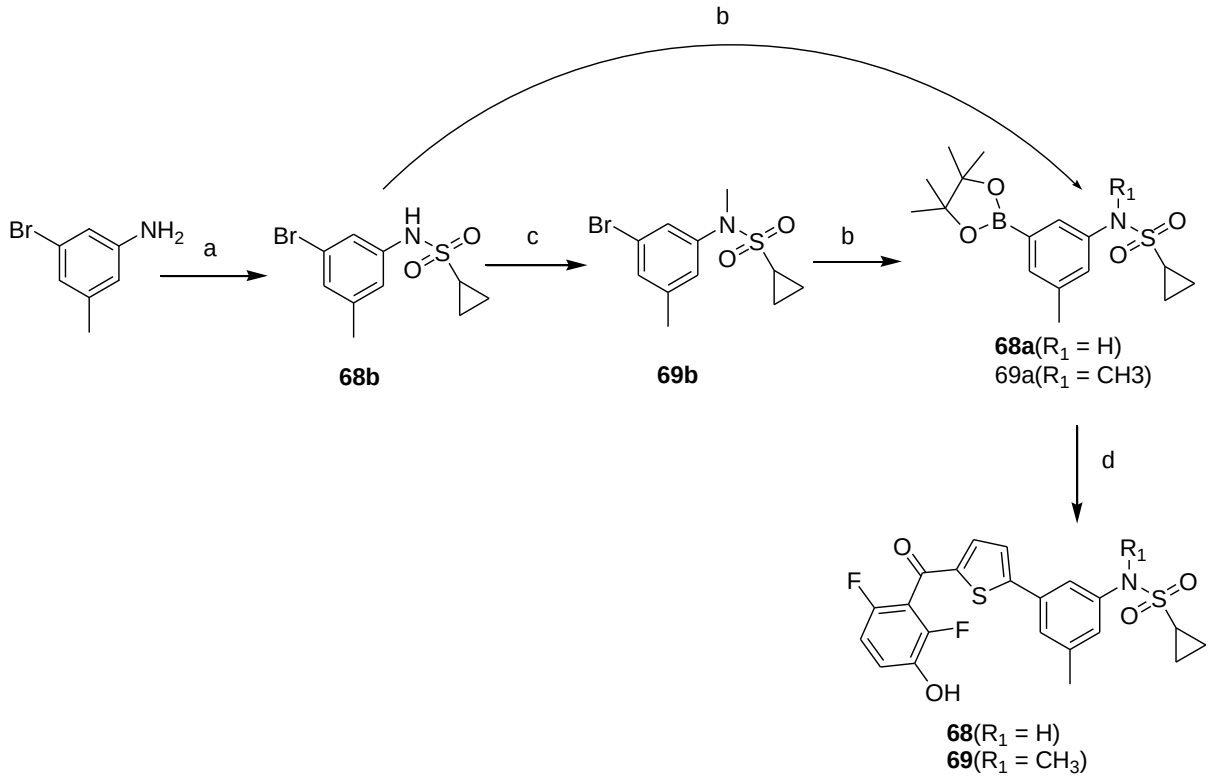
Scheme 5: synthesis of compounds **44**, **45**, and **56-58**. a) NBS, AcOH, rt, overnight. b) CH_3I, K_2CO_3, DMF, rt, overnight. c) bispinocolato diborane, $PddppCl_2$, KOAc/DMSO, 2h. d) method C1, **III**, Cs_2CO_3, $Pd(PPh_3)_4$, DME/water (1:1), reflux, overnight. e) method B, BBr_3, CH_2Cl_2, -78^{o}C to rt, overnight. f) NBS, DBPO, CCl_4, reflux, 2h. g) NEt_3, 1-Piperazin-1-yl-ethanone. h) HCl 6M, reflux, 2h.

The azide intermediate **59c** was prepared by reaction of **56b** with NaN_3 in DMF. The subsequent Suzuki cross-coupling reaction with **VIII** yielded **59b**, which was submitted to a cycloaddition reaction with acetic acid vinyl ester[38] to give the 1,2,3-triazole substituted compound. Interestingly, the latter was isolated as acetic acid ester **59a** exclusively. Saponification with 2M-NaOH gave the phenol **59**, which was transformed to the diol **60** by reaction with BBr3 (method B). (Scheme **6**)



Scheme 6: synthesis of compounds **59** and **60**. a) DMF, rt, overnight. b) method C1, **VIII**, Cs_2CO_3, $Pd(PPh_3)_4$, DME/water (1:1), reflux, overnight. c) $CH_3COOCH{=}CH_2$, microwave, 120 ^oC, 10h. d) 2M NaOH, THF, rt, 2h. e) method B, BBr_3, CH_2Cl_2, -78^oC to rt, overnight.

The synthesis of compounds **68** and **69** started with the reaction of 3-methyl-5-bromoaniline and cyclopropanesulfonyl chloride to afford **68b**. Subsequent methylation yielded the *N*-methyl sulfonamide **69b**. Intermediates **68b** and **69b** were converted to the corresponding boronic acid esters **68a** and **69a**, respectively, by a Pd-catalyzed reaction with bispinacolato diborane in the presence of potassium acetate in DMSO. Suzuki cross coupling reaction with **VIII** afforded the final compounds **68** and **69**. (Scheme **7**)



Scheme 7: synthesis of compounds **68** and **69**. a) method D, pyridine, cyclopropanesulfonyl chloride, rt, overnight. b) bispinacolato diborane, $PddppCl_2$, KOAc/DMSO, 2h. c) NaH, DMF, MeI, 0°C to rt, 0.5h. d) method C1, **VIII**, Cs_2CO_3, $Pd(PPh_3)_4$, DME/water (1:1), reflux, 3 days.

Biological results

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

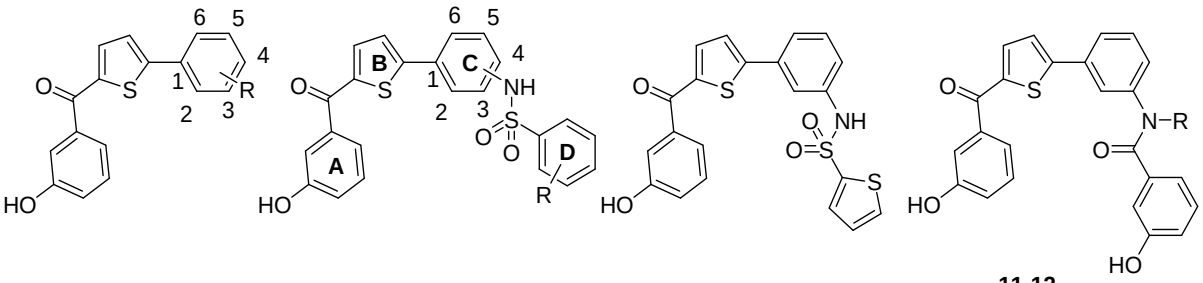
Human placental enzymes were used for both 17β-HSD assays and were obtained according to described methods.[39-41] In the 17β-HSD1 assay, incubations were run with cytosolic fractions, tritiated E1, cofactor and inhibitor. The separation of substrate and product was accomplished by HPLC. The 17β-HSD2 assay was performed similarly using tritiated E2 as substrate and the microsomal fraction. Activities are given as percent inhibition at 1 µM (tables 1-6).

The 17β-HSD1 inhibition of the sulfonamide-type compounds **3-8** is strongly dependent on the position of the sulfonamide nitrogen at phenyl ring C (cf. chart 1): Compounds bearing the sulfonamide moiety in *m*-position to the thiophene ring (compounds **6-8**, table **1**) show significantly higher inhibitory potency compared to their corresponding *p*-substituted isomers **3-**

5. Interestingly, the parent anilines **1** and **2** show a reversed correlation between position of the nitrogen atom and inhibitory potency. Bioisosteric replacement of the benzenesulfonic moiety of compound **6** with a thiophenesulfonic group leads to compound **9** with similar activity and selectivity towards the type 2 enzyme. In general, the compounds displayed in table **1** show rather low selectivities with respect to 17β-HSD2 inhibition with the exception of compound **7** (SF = 25) which at the same time shows the strongest inhibition of the target enzyme (IC_{50} = 8 nM).

The *m*-sulfonamide function may be bioisosterically replaced by the carbonic acid amide function as can be seen by comparison of the inhibitory potencies of compounds **10** and the only slightly less active **11** (table **1**). Methylation of the amide group further decreases activity (compound **12**).

Table 1. Inhibition of human 17β-HSD1 and 17β-HSD2 by compounds **1-12**.



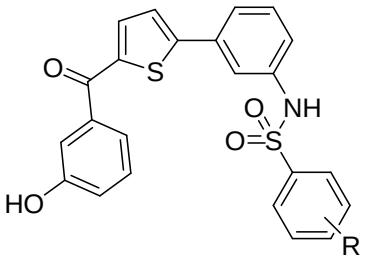
1, 2 **3 - 8, 10** **9** **11-12**

Comd	Ring C	R	IC_{50} [nM][a]		SF[d]	LogP[e]	LogD@pH 7.4[e]
			*h*17β-HSD1[b]	*h*17β-HSD2[c]			
1	-	4-NH_2	145	120	0.8	3.02	3.0
2	-	3-NH_2	77 %[f]	68 %[f]	-	2.67	2.7
3	4	H	195	459	2.3	4.90	4.8
4	4	4-Br-2-OCF_3	169	716	4.2	7.00	6.5
5	4	3-CN	151	763	5.0	4.83	4.5
6	3	H	21	112	5.2	4.59	4.5
7	3	4-Br-2-OCF_3	8	199	25.0	5.97	5.2
8	3	3-CN	23	171	7.5	4.53	4.0
9	-	-	39	114	3.0	4.50	4.0
10	3	3-OH	16	88	5.4	3.62	3.5
11	-	H	33	159	4.8	4.28	4.3
12	-	CH_3	71	218	3.0	4.02	4.0

[a] Mean value of three determinations, standard deviation less than 15%. [b] Human placenta, cytosolic fraction, substrate [^{3}H]-E1, 500 nM, cofactor NADH, 500 µM. [c] Human placenta, microsomal fraction, substrate [^{3}H]-E2, 500 nM, cofactor NAD^+, 1500 µM. [d] Selectivity factor: IC_{50}(17β-HSD2) / IC_{50}(17β-HSD1). [e] Calculated LogP and LogD@ pH 7.4 data. [f] Inhibition @ 1µM (inhibitor concentration).

The favorable biological data of compound **7** prompted us to further investigate the effect of different substituents R on the benzenesulfonyl moiety on inhibitory poteny (compounds **13-29**, table **2**). All these compounds basically show comparable inhibition of 17β-HSD1, independently of the nature of the substituent (electron-donating or –withdrawing). It seems, however, that bulky and/or hydrophilic substituents somewhat decrease potency (e.g. compounds **17** and **25**). None of the compounds **13-29** exceeds compound **7** concerning activity and selectivity. Compound **7**, however, shows drawbacks like high lipophilicity and molecular weight, moderate metabolic stability, and insufficient activity towards the murine 17β-HSD1 enzyme (see table 9). In order to tackle these problems, we turned our attention to the optimization of the the main parts of this class of inhibitors, namely the ABC-ring system (cf. chart 1).

Table 2. Inhibition of human 17β-HSD1 and 17β-HSD2 by compounds **13-29**.



13-29

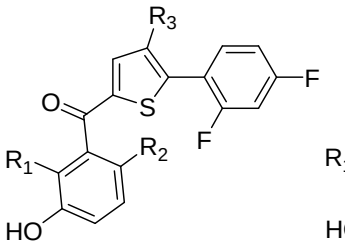
Comd	R	IC_{50} [nM][a]		SF[d]	LogP[e]	LogD@ pH 7.4[e]
		*h*17β-HSD1[b]	*h*17β-HSD2[c]			
13	4-CN	56	392	7.0	4.50	3.9
14	2-NO_2	32	148	4.6	4.61	4.2
15	3-NO_2	47	130	2.7	4.82	4.6
16	4-NO_2	42	320	7.7	4.79	4.1
17	2,3-di-NO_2	182	322	1.8	4.99	4.2
18	2-F	50	116	2.3	4.40	4.1
19	3-F	38	185	4.9	5.01	4.8
20	4-F	30	83	2.7	4.91	4.5
21	4-Br	50	162	3.2	5.63	5.3
22	4-Br- 2,5-di-F	109	144	1.3	5.27	4.2
23	3,5-di-Cl-4-OH	135	126	0.9	6.19	3.5
24	3-OMe	35	130	3.7	4.95	4.8

25	3-NHCOMe	311	282	0.9	3.22	3.1
26	2-Me	17	104	6.1	5.05	4.9
27	3-Me	18	99	5.5	5.05	5.0
28	2-F-4-OMe	51	112	2.2	4.97	4.6
29	2-F-4-OH	112	215	1.9	4.53	3.6

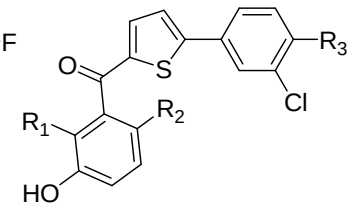
[a-e] See table 1.

Starting from the moderately active compound **30**, introduction of substituents to the benzoyl moiety results in very different effects on activity and selectivity, depending on the type of the substituent: Methylation leads to a strong decrease in 17β-HSD1 inhibition (compound **31**). In addition, this compound shows stronger inhibition of the type 2 than of the type 1 enzyme, as does the parent compound **30**. Compound **32**, bearing a second methyl group at the thiophene ring, shows a similar behavior. In contrast, introduction of one or two fluorine atoms on the benzoyl part results in a strong increase of 17β-HSD1 inhibition (compound **33**: IC_{50} = 11 nM; compound **34**: 3.5 nM), which, unfortunately comes along with poor selectivity. In order to increase selectivity, we made use of the fact that an OH-group in 4-position of the phenyl ring (ring C) is beneficial for selectivity (compound **C**).[27] Also in this case, introduction of a CH_3-group is detrimental to inhibitory potency and to selectivity (compound **35**: IC_{50} = 90 nM; SF = 0.3), whereas the introduction of fluorine increases activity toward 17β-HSD1 by a factor of 10 and maintains selectivity (compound **37**: IC_{50} = 0.5 nM, SF = 40). The additional introduction of a methyl group at the thiophene moiety reduces selectivity (compound **39**). Maintaining the 2,6-difluoro substitution pattern of the benzoyl moiety, which proved to be beneficial for activity towards 17β-HSD1, while removing the OH-group at the phenyl ring or changing its position from para to ortho leads to a decrease (compound **40**) or a complete loss of selectivitity (compound **41**).

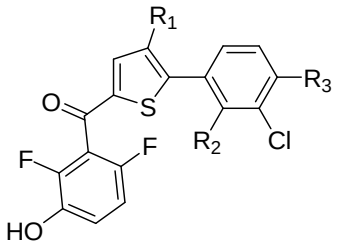
Table 3. Inhibition of human 17β-HSD1 and 17β-HSD2 by compounds **30-41**.



30-34



C, 35-37



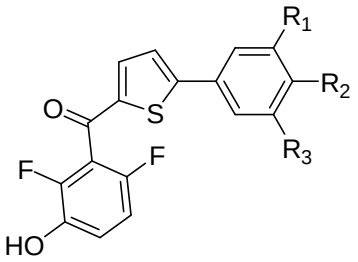
38-41

Comd	R_1	R_2	R_3	IC_{50} [nM][a]		SF[d]	LogP[e]	LogD@ pH7.4[e]
				17β-HSD1[b]	17β-HSD2[c]			
30	H	H	H	213[g]	177[g]	0.8	4.71	4.7
31	CH_3	H	H	3427	299	0.09	4.6	4.6
32	CH_3	H	CH_3	20 %[f]	66 %[f]	-	5.63	5.6
33	F	H	H	11[g]	8[g]	0.7	4.51	4.3
34	F	F	H	3.5	9	2.5	4.44	4.3
C	H	H	OH	5.0	246	48.0	3.98	3.8
35	CH_3	H	OH	355	90	0.3	4.44	4.3
36	F	F	OCH_3	1.3	11	9.0	4.52	4.4
37	F	F	OH	0.5	20	40.0	4.16	3.8
38	CH_3	H	OCH_3	5.5	52	9.5	4.92	4.8
39	CH_3	H	OH	0.8	11	13.0	4.63	4.3
40	H	H	H	1.2	10	8.3	4.51	4.4
41	H	OH	H	27	29	1.1	3.43	2.9

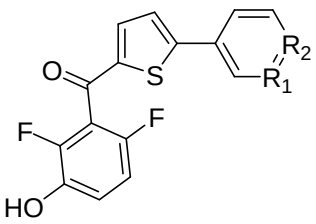
[a-f] See table 1. [g] Calculated IC_{50}(IC_{50}(Logit)) = test concentration ((100-%inh.)/%inh.).

The SAR obtained so far indicate a beneficial effect of a *p*-OR group (R = H, CH_3) at the phenyl ring (ring C) for selectivity over 17β-HSD2. Therefore, this group was retained in the further drug design concept. In order to protect it from possible metabolic alteration, bulky and/or electron withdrawing groups were introduced in the o-positions (table 4, compounds **42-51**). Interestingly, compounds bearing chloro- and methyl-substituents (compounds **42-47**) show similarly strong inhibition of the target enzyme but only in case of two identical substituents (either chloro or methyl) good selectivities can be achieved. This is especially true for the dichloro-substituted compound **42** which is not only one of the most active but also the most selective compound of its class (IC_{50} = 0.5 nM; SF = 82). Comparison with the inhibition data of the analog non-fluorinated compound **54** highlights again the beneficial impact of the fluorine atoms at the benzoyl moiety. The same dependence of activity on benzoyl fluorination can be seen when comparing compounds **43** and **55**. The introduction of other strongly electron withdrawing functions as fluorine, nitrile or a sulfonamide group (compounds **48-51**) leads to less favorable biological properties. The pyridine ring is not an adequate replacement for the hydroxyphenyl moiety (compounds **52** and **53**).

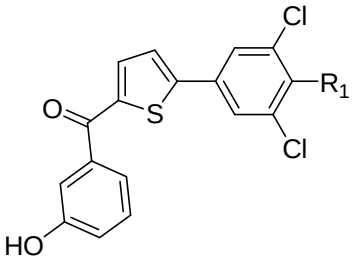
Table 4. Inhibition of human 17β-HSD1 and 17β-HSD2 by compounds **42-55**.



42-51



52, 53



54, 55

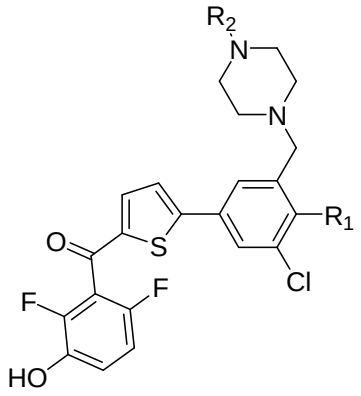
Comd	**R_1**	**R_2**	**R_3**	**IC_{50} [nM][a]**		**SF[d]**	**LogP[e]**	**LogD@ pH7.4[e]**
				17β-HSD1[b]	**17β-HSD2[c]**			
42	Cl	OCH_3	Cl	0.5	41	82	5.21	4.9
43	Cl	OH	Cl	2.4	39	16	4.71	2.4
44	Cl	OCH_3	CH_3	0.3	7.2	21	4.73	4.6
45	Cl	OH	CH_3	0.2	6.5	30	4.15	3.9
46	CH_3	OCH_3	CH_3	0.9	18	20	4.72	4.6
47	CH_3	OH	CH_3	0.4	7	20	4.43	3.8
48	F	$OCHF_2$	F	7.0	36	5	3.92	3.8
49	CN	$OCH(CH_3)_2$	H	8.0	19	2	5.01	4.9
50	CN	OH	H	3.3	29	9	3.91	2.7
51	SO_2-4-morpholine	OCH_3	H	79 %[f]	85 %[f]	-	2.60	2.4

52	CH	N	-	270[f]	156[f]	0.6	3.04	2.9
53	N	CH	-	75[g]	56[g]	0.8	3.07	2.9
54	OCH_3	-	-	6.0	247	40	5.09	5.1
55	OH	-	-	31[g]	255[g]	8	4.52	3.4

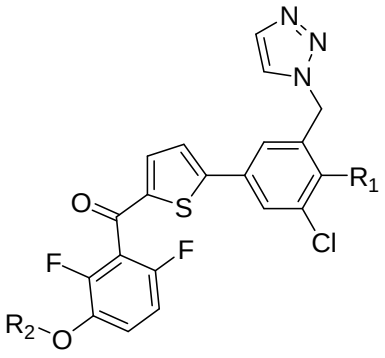
[a-f] See table 1. [g] Calculated IC_{50}(IC_{50}(Logit)) = test concentration ((100-%inh.)/%inh.).

The dichlorinated inhibitor **42** shows favorable properties concerning activity, selectivity, and metabolic stability, but it displays high lipophilicity and a solubility too low to be measured experimentally. In order to render the compound more hydrophilic and soluble, one of the chlorine atoms was replaced by heterocyclic substituents (table 5, compounds **56**-**60** and **59a**). The synthesized compounds show greatly reduced lipophilicities and enhanced solubilities. Besides the ester **59a** and dihydroxy compound **60**, all compounds show IC_{50}-values in the one-digit nanomolar range.

Table 5. Inhibition of human 17β-HSD1 and 17β-HSD2 by compounds **56-60** and **59a**.



56-58



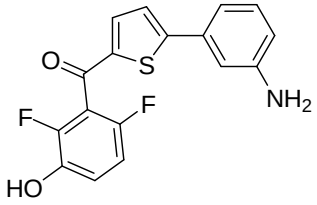
59a, 59, 60

Comd	**R_1**	**R_2**	**IC_{50} [nM][a]**		**SF[d]**	**LogP[f]**	**LogD@ pH7.4[f]**
			17β-HSD1[b]	**17β-HSD2[c]**			
56	OCH_3	$COCH_3$	1.4	21	15.0	3.21	3.1
57	OH	$COCH_3$	2.1	11	5.2	2.63	2.2
58	OCH_3	H	4.7	26	5.5	3.41	1.7
59a	OCH_3	$COCH_3$	26[g]	21[g]	0.8	3.34	3.3
59	OCH_3	H	2.1	8.8	4.2	3.44	3.3
60	OH	H	17[g]	26[g]	1.5	2.86	2.3

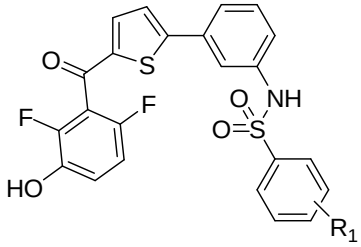
[a-f] See table 1. [g] Calculated IC_{50}(IC_{50}(Logit)) = test concentration ((100-%inh.)/%inh.).

Considering the strong inhibitory potency of sulfonamide-type compounds (e.g. compounds **7**, **26**, and **27**; tables 1 and 2) and the beneficial effect of 2,6-difluorination on h17β-HSD1 inhibition observed for non-sulfonamide compounds (tables 3-5) triggered the synthesis of compounds combining both structural elements. This strategy is supported by the fact that the fluorinated amine **61** (IC_{50} = 10 nM; table 4) is a much more potent inhibitor than its non-fluorinated analog 2 (77 % inhibn. at 1 µM; table 1) and thus even exceeds the inhibitory potency of hit compound **A** (fig. 1). Indeed, compound **62** (table 4) shows an about five times stronger activity than its non-fluorinated analog **7**, at the expense, however, of a slightly decreased selectivity towards the type 2 enzyme. As the bromine atom obviously does not contribute significantly to target affinity or may even reduce it (compare inhibitory data of compounds **6** (table 1) and compound **21** (table 2)), compounds **63** and **64** lacking this atom were synthesized. They show activities comparable to that of **62** but decreased selectivity. Exchange of the phenyl group of the sulfonamide moiety with heterocycles and the cyclopropyl substituent was carried out to decrease the high lipohilicities of compounds **62-64**. These modifications (compounds **65-69**) were well tolerated by the target enzyme but did not enhance selectivity.

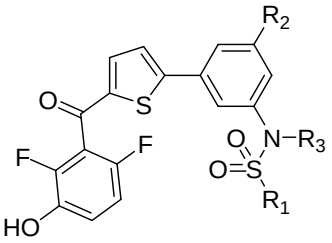
Table 6. Inhibition of human 17β-HSD1 and 17β-HSD2 by compounds **61-69**.



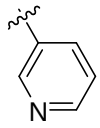
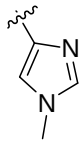
61



62-64



65-69

Comd	R_1	R_2	R_3	IC_{50} [nM][a]		SF[d]	LogP[e]	LogD@ pH 7.4[e]
				*h*17β-HSD1[b]	*h*17β-HSD2[c]			
61	-	-	-	10	25	2.5	2.38	2.2
62	4-Br-2-OCF_3	-	-	1.7	17	10.0	6.01	5.1
63	2-OCF_3	-	-	3.1	13	4.1	5.25	4.8
64	2-CF_3	-	-	2.3	6	2.5	5.27	4.9
65		H	H	4.0	3.7	0.9	3.21	2.3
66		H	H	5.5	2.7	0.5	1.87	0.7
67		H	H	1.8	6.5	3.7	3.04	2.9
68		CH_3	H	87 %[f]	54 %[f]	-	3.50	3.3
69		CH_3	CH_3	67 %[f]	37 %[f]	-	3.57	3.4

[a-e] See table 1. [f] Inhibition @ 10nM (inhibitor concentration).

The affinities of selected compounds to the recombinant human estrogen receptors α and β were determined by incubation of tritium labeled E2 with the respective receptor and subsequent addition of the compound in 1000-fold excess, based upon E2 concentration. Receptor affinities are expressed as the percentage of E2 replaced by a compound. ER-binding of 50% equals a binding affinity of 0.1 % of that of E2.

The estrogen receptor affinities of the selected compounds cover a broad spectrum (table 7). The data suggest that fluorination of the benzoyl moiety increases ER-affinity (cf. compound **7** vs. **62** and **131** vs. **197**). A similar observation could be made upon methylation of the thiophene ring (compound **37** vs. **39**). Increasing the number or size of substituents adjacent to the oxygen-function of the phenyl ring (ring C) decreases ER-affinity (cf. compound **37** vs. **42**, **43**, **46**, **47**, **50**, and **51**).

Compounds **42**, **43**, **47**, **54**, which are highly active towards the target enzyme and selective over 17β-HSD2 displayed very low affinities towards ER α and β.

Table 7. Binding Affinities for the Estrogen Receptors α and β for Selected Compounds

Compd	**% Binding[a]**	
	ER-α binding (1000 folds E2 concn.)	**ER-β binding (1000 folds E2 concn.)**
7	63	53
37	73	55
39	98	82
42	27	8
43	37	11
46	51	69
47	17	22
50	44	6
51	0	4
54	8	0
62	71	74
63	65	68
64	20	20

[a] Mean value of three determinations, standard deviation less than 15 %.

Further Biological Evaluations.

Metabolic stability

Metabolic stability of the most interesting compounds was evaluated using human liver microsomes (s9 fraction) and is expressed as the percentage of compound remaining after the indicated incubation time (table 8). Depending on the results, we can discern three 3 types of compounds:

a) Metabolically unstable compounds (less than 25 % remaining after 15 min), compounds **30**, **33**, **36**, **39**, **45**, **47**, **50**, **51**, **64**, **68**, and **69**.

b) Metabolically moderately stable compounds (between 25 % and 50 % remaining after 15 min), compounds **7**, **40**, **46**, and **62**.

c) Metabolically stable compounds (more than 50 % remaining after 15 min), compounds **42**, **43**, and **44**.

Thus, compounds **42** and **43** display good metabolic stabilities (40 % and 72 % remaining after 60 min, respectively), and this shows the great impact of the chlorine atoms at the phenyl moiety, compared to the analoguous methylated compounds **46**, and **47**, respectively (table 8). For compound **47**, stabilities towards phase I and phase II metabolism were measured independently by conducting the assay selective exclusion using the cofactors for phase I and phase II reactions, respectively. The compound proved to be stable towards phase I metabolism (61 % remaining after 60 min) but instable towards conjugation reactions (data not shown).

Within the group of compounds bearing a sulfonamide moieties, inhibitors **7** and **62** displayed favorable metabolic stabilities (compound **7:** 36 % remaining after 45 min; compound **62:** 21 % after 60 min).

Table 8. Metabolic stability of selected compounds in human hepatic S9-fraction.

Comd	% remaining (S9[a]) after		Comd	% remaining (S9[a]) after	
	15 min	60 min		15 min	60 min
7*	47	36 (after 45 min)	**47**	6	n.d
30	6	n.d	**50**	21	n.d
33	3	n.d	**51**	2	n.d
36	11	n.d	**55**	21	n.d
39	5	n.d	**56**	18	n.d
40	26	n.d	**58**	20	n.d
42	56	40	**62***	31	21
43	91	72	**63**	20	n.d
44	50	13	**64**	4	n.d
45	7	n.d	**68**	19	n.d
46	29	n.d	**69**	6	n.d

* Test concentration 3 µM. [a] human hepatic S9 fraction: (1 mg/mL mixture of microsomes and cytosol of hepatocytes) supplemented with NADPH, UDPGA and PAPS.

Inhibition of murine and rat 17β-HSD1 and 17β-HSD2

The murine and rat 17β-HSD1 enzymes were expressed in HEK293 cells, whereas the 17β-HSD2 enyzmes were prepared from mouse or rat liver (microsomal fraction).

Not surprisingly, inhibition of *m*17β-HSD1 is in general considerably weaker than inhibition of the human enzyme (tables 9 and 10). For instance, the non-fluorinated compound **7** which is a strong inhibitor of *h*17β-HSD1 (IC_{50} = 8 nM) is hardly active towards the murine enzyme (21 % inhibn. at 1 µM). In addition, selectivity towards the type 2 enzyme is much less pronounced than in case of the human protein. One of the compounds that retains some selectivity over the *m*17β-HSD2 enzyme is compound **26**, which is also the most potent non-fluorinated inhibitor in this series (inhibition @ 1 µM: *m*17β-HSD1 = 51 %; *m*17β-HSD2 = 48 %).

A similarly dramatic difference in inhibition of the target enzymes of the two species is observed for the fluorinated compounds **42** and **43**. A more pronounced *m*17β-HSD1 inhibitory activity is measured in case of compound **47**, which at the same time – together with **42** - is the most active compound ever described for the human enzyme (**47**: IC_{50}(*h*17β-HSD1) = 0.4 nM; 77 % inhibition of *m*17β-HSD1 at 1 µM).

Interestingly, the combination of 2,6-difluorination of the benzoyl moiety with the presence of a sulfonamide function yielded the most active compounds not only towards the human, but also towards the *murine* 17β-HSD1 enzyme, cf. compound **7** (21% inhibition @ 1µM; non-fluorinated sulfonamide) and compound **61** (30 % inhibition, fluorinated but without sulfonamide moiety) versus compound **62** (71 % inhibition, fluorinated, with sulfonamide moiety). Compound **64** is the most active inhibitor toward *m*17β-HSD1 in this study (IC_{50} = 250 nM, table 10). Due to its even stronger inhibition of the murine type 2 enzyme, however, it is no candidate for *in vivo* evaluation in a mouse endometriosis model.

Table 9. Inhibition of murine 17β-HSD1 and 17β-HSD2 of selected compounds

Comd	**% Inhibition @ 1µM[a]**		**Comd**	**% Inhibition @ 1µM[a]**	
	***m*17β-HSD1[b]**	***m*17β-HSD2[c]**		***m*17β-HSD1[b]**	***m*17β-HSD2[c]**
1	4	n.d	45	43	n.d
7	21	n.d	46	59	78
10	26	n.d	47	77	96
11	26	n.d	48	45	n.d
12	17	n.d	50	23	n.d
13	8	n.d	51	58	66
20	25	n.d	53	14	n.d
23	16	n.d	56	37	n.d
25	13	n.d	57	16	n.d
26	51	48	58	25	n.d
31	5	n.d	59a	20	n.d
34	41	n.d	59	32	n.d
35	14	n.d	60	33	n.d
36	40	n.d	61	30	n.d
37	50	95	62	71	100
38	25	n.d	63	91	100
39	38	n.d	64	97	100
40	60	94	65	47	n.d

41	42	n.d	66	10	n.d
42	43	n.d	67	76	100
43	23	n.d	68	33 % @ 250 nM	100
44	32	n.d	69	32 % @ 250 nM	90

[a] Mean value of three determinations, standard deviation less than 15%. [b] Mouse recombinant enzyme, expressed in HEK 293, substrate [^{3}H]-E1, 10 nM, cofactor NADH, 500 µM. [c] Mouse liver, microsomal fraction, substrate [^{3}H]-E2, 10 nM, cofactor NAD^+, 1500 µM.

Table 10. IC_{50} values for selected compounds.

cmpd	**IC_{50} [nM][a]**	
	***m*17β-HSD1[b]**	***m*17β-HSD2[c]**
47	400	120
63	570	33
64	250	20
68	680	130
69	600	55

[a] Mean value of three determinations, standard deviation less than 15%. [b] Mouse recombinant enzyme, expressed in HEK 293, substrate [^{3}H]-E1, 10 nM, cofactor NADH, 500 µM. [c] Mouse liver, microsomal fraction, substrate [^{3}H]-E2, 10 nM, cofactor NAD^+, 1500 µM.

Selected compounds were tested for inhibition of rat 17β-HSD1 (table 11). Most compounds turned out to be stronger inhibitors of the rat enzyme than of the murine analog (tables 9 and 10). The most potent - though unselective - compound was **64**, showing an inhibition of 59 % at a concentration of 100 nM. Compound **47**, however, combined good inhibitory potency (63 %

inhibn. at 250 nM) with acceptable selectivity towards rat 17β-HSD2. Thus, this compound could be a candidate for *in vivo* evaluation in a rat endometriosis model.

Table 11. Inhibition of rat 17β-HSD1 and 17β-HSD2 of selected compounds.

Comd	Comd concentration	% Inhibition[a]	
		***r*17β-HSD1[b]**	***r*17β-HSD2[c]**
43	5000 nM	59	n.d
46	250 nM	39	27
47	250 nM	63	35
51	250 nM	26	n.i
57	250 nM	n.i	n.i
58	500 nM	51	55
59	250 nM	39	64
60	250 nM	21	54
62	250 nM	56	86
63	250 nM	70	74
64	100 nM	59	70
65	250 nM	32	28
66	250 nM	29	31
67	250 nM	51	49

[a] Mean value of three determinations, standard deviation less than 15%. [b] Rat recombinant enzyme, expressed in HEK 293, substrate [^{3}H]-E1, 10 nM, cofactor NADH, 500 µM. [c] Rat liver,

microsomal fraction, substrate [^{3}H]-E2, 10 nM, cofactor NAD^+, 1500 µM.

Cytotoxicity

Hek293 cell viability was measured in the MTT assay. Incubation was carried out at the indicated inhibitor concentrations for 66 hours at 37°C. 100%-values were determined without inhibitor. Only compounds displaying substantial inhibition of mouse or rat 17β-HSD1 were subjected to this assay.

Compounds **47**, **63**, and **64**, which are the most potent inhibitors of rat 17β-HSD1, exhibited cytotoxic effects only at concentrations of 12.5µM and above (compound **47**: 25 µM and above). In all cases, this is clearly more than ten times higher than the corresponding IC_{50}-values, as roughly estimated from the percentages of inhibition (table 12).

Table 12. Cytotoxicity of selected compounds.

Conc.	**Compound**											
(µM)	**46**	**47**	**57**	**58**	**59**	**60**	**62**	**63**	**64**	**65**	**66**	**67**
0	100	100	100	100	100	100	100	100	100	100	100	100
6.25	61	100	75	16	56	93	100	87	100	89	98	100
12.50	10	91	60	8	28	84	45	56	60	73	100	100
25	7	41	29	7	12	50	14	19	17	47	69	55
50	7	21	6	8	7	11	14	18	11	11	33	23
100	7	20	4	7	6	9	15	18	10	4	9	16

Hek 293 cell viability as measured in the MTT assay (incubation with compound 66 hours at 37°C), 100% = vehicle only = 1% DMSO, no compound.

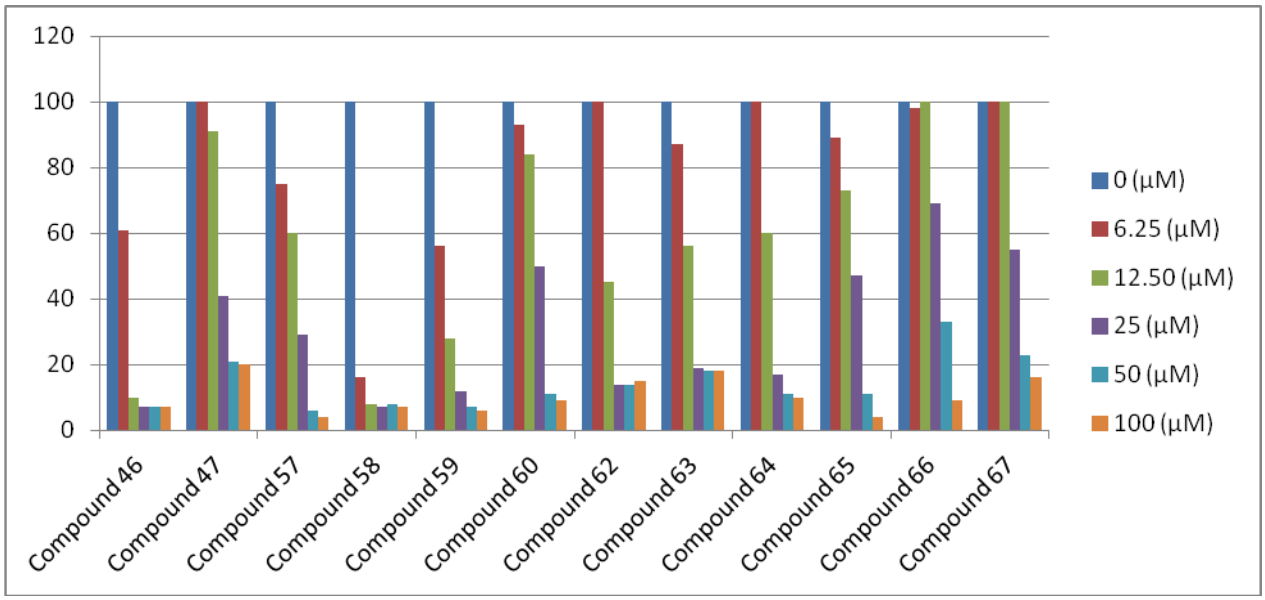


Figure 3. Cytotoxicity of selected compounds.

Discussion and Conclusions

The aim of the present study was the design of potent and selective inhibitors of human 17β-HSD1, which can be used to conduct a proof of principle study in an animal (rodent) disease model for EDD. The class of BSHs was chosen as a starting point because this compound class, unlike many others, is characterized not only by high inhibitory potency towards human 17β-HSD1, but also by activity towards the murine enzyme.

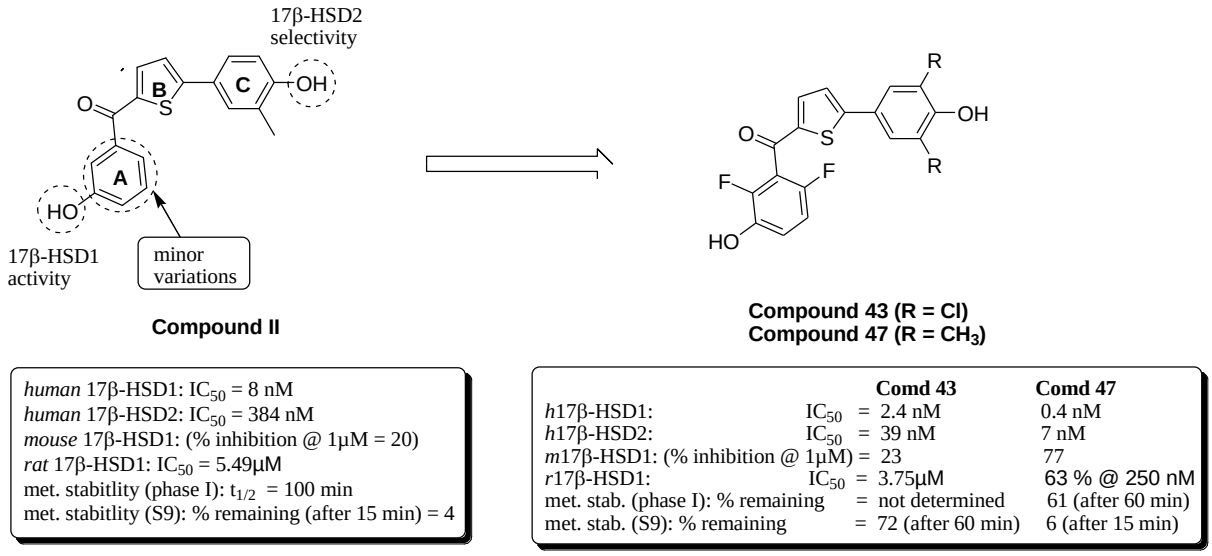
In a first approach, variations in the sulfonamide moiety of the lead compound I (rings C and D) were carried out (design step I). It was found that the introduction of the sulfonamide in *m*-position to the thiophene ring is clearly favored over the *p*-position in terms of h17β-HSD1 inhibition. Major issues that were encountered with the synthesized compounds were low metabolic stability (S9) and insufficient activity towards m17β-HSD1. These problems were addressed in the further design process (design steps II and III).

It is striking that the introduction of fluorine atoms in the benzoyl moiety (ring A) which was carried out to improve metabolic stability, resulted in a strong increase of activity towards both

the human and the rodent enzymes. This may be explained by the electron-withdrawing effect of the fluorine atoms which strongly influence the hydrogen bonding properties of the OH-group.[42] On the other hand, it is known that fluorine itself is able to contribute to ligand binding, eg. by interacting with carbon atoms of carbonyl groups of the target.[43]

Low stability towards metabolism (phase II) was due to the presence of the phenolic OH-groups. In previous SAR studies no adequate replacement could be found for the OH-group on the benzoyl moiety. Moreover, it was known that the target enzyme does not tolerate substituents much bigger than hydrogen on this ring. It is therefore an important progress that the approach to enhance metabolic stability by introducing fluorine was successful, as can be seen when comparing the metabolic stability of compound **55** (21 % remaining after 15 min, table 7) with that of its fluorinated analog **43** (72 % remaining after 60 min). For the OH-group on the phenyl ring (ring C), the situation was different: On the one hand, the OH-group was found not to be essential for activity (though beneficial for selectivity over 17β-HSD2). On the other hand, the phenyl ring offered more freedom in the choice of substituents which could be introduced to optimize metabolic stability as even the presence of bulky groups was known to be tolerated by 17β-HSD1. The best results were obtained by the introduction of two chlorine atoms adjacent to the OH-group (compound **43**). Interestingly, the replacement of one or both chlorine atoms with the methyl group, which is similar in size but different concerning electronic properties, led to the metabolically instable compounds **45** and **47**. Two explanations of the lacking metabolic stability of the latter compounds were likely: Either the methyl groups are not able to protect the adjacent phenolic OH-group from being conjugated (phase II reaction) or the methyl groups themselves are metabolically altered (phase I reaction). This motivated us to independently evaluate phase I and II metabolic stability for compound **47**. The results clearly showed instability towards phase II metabolism, indicating that methyl groups were not able to protect the OH-group from being conjugated. Thus, the electron withdrawing effect of the chlorine atoms rather than steric "shielding" of the phenolic OH-group seem to be the reason for the outstanding metabolic stability of compound **43**.

The most interesting inhibitors which were identified in this study were BSHs **42**, **43**, **47**, **63**, and **64**. The chlorinated compounds **42** and **43** belong to the most active inhibitors of h17β-HSD1 ever described (IC_{50} = 0.5 nM and 2.4 nM, respectively) and are characterized by good selectivities towards the human type 2 enzyme and the estrogen receptors α and β. In addition, they are the metabolically most stable members of this compound class (40 % and 72 % remaining (S9) after 60 min, respectively). Due to their more pronounced activities towards the rodent 17β-HSD1 enzymes, compounds **47**, **63**, and **64** may be possible candidates for an in vivo PoP-study. This is especially true for compound **47** as it showed the best selectivity over 17β-HSD2 of the rat and displayed the most favourable cytotoxicity profile of the three inhibitors. At physiological pH, both compound **43** and **47** have clogD-values below 5 (**43**: 2.36; **47**: 3.81).



Compound II

human 17β-HSD1: IC_{50} = 8 nM
human 17β-HSD2: IC_{50} = 384 nM
mouse 17β-HSD1: (% inhibition @ 1µM = 20)
rat 17β-HSD1: IC_{50} = 5.49µM
met. stabitlity (phase I): $t_{1/2}$ = 100 min
met. stabitlity (S9): % remaining (after 15 min) = 4

Compound 43 (R = Cl)
Compound 47 (R = CH_3)

		Comd 43	**Comd 47**
*h*17β-HSD1:	IC_{50} =	2.4 nM	0.4 nM
*h*17β-HSD2:	IC_{50} =	39 nM	7 nM
*m*17β-HSD1: (% inhibition @ 1µM) =		23	77
*r*17β-HSD1:	IC_{50} =	3.75µM	63 % @ 250 nM
met. stab. (phase I): % remaining	=	not determined	61 (after 60 min)
met. stab. (S9): % remaining	=	72 (after 60 min)	6 (after 15 min)

Chart 4. Comparison of biological activities and metabolic stabilities of the reference compound **II** and compounds **43** and **47**.

Experimental Section

Chemical Methods. Chemical names follow IUPAC nomenclature. Starting materials were purchased from Aldrich, Acros, Lancaster, Maybridge, Combi Blocks, Merk, or Fluka and were used without purification.

Column chromatography (CC) was performed on silica gel (70 – 200µm), reaction progress was monitored by thin layer chromatography (TLC) on Alugram SIL G UV_{254} (Macherey-Nagel).

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

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

A mass spectrum (ESI) was recorded on a TSQ Quantum (Thermo Finnigan) instrument.

Tested compounds are >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.

The following compounds were prepared according to previously described procedures: (5-bromo-thiophen-2-yl)(3-methoxyphenyl)methanone **(I)**,[26] (5-bromo-thiophen-2-yl)(3-hydroxy-phenyl)methanone **(VII)**,[26] (5-(3-aminophenyl)thiophen-2-yl)(3-methoxyphenyl)methanone **(2a),**[44] (5-(3-aminophenyl)thiophen-2-yl)(3-hydroxyphenyl)methanone **(2),**[44] 4-bromo-*N*-(3-(5-(3-hydroxybenzoyl)thiophen-2-yl)phenyl)-2-trifluoromethoxybenzenesulfonamide **(7)**,[44] 4-bromo-2-chloro-6-methyl-phenol **(44c),**[45] 5-bromo-1-chloro-2-methoxy-3-methyl-benzene **(44b)**[46]**.**

General procedure for Friedel-Crafts acylation. Method A. An ice-cooled mixture of monosubstituted thiophene derivate (1 or 1.5 equiv), arylcarbonyl chloride (1 equiv), and aluminumtrichloride (1 equiv) in anhydrous dichloromethane was warmed to room temperature and stirred for 2-4 h. 1M HCl was used to quench the reaction. The aqueous layer was extracted

with ethyl acetate. The combined organic layers were washed with brine, dried over magnesium sulfate, filtered, and concentrated to dryness. The product was purified by CC.

General procedure for ether cleavage. Method B. To a solution of methoxybenzene derivative (1 equiv) in anhydrous dichloromethane at -78 ^{o}C (dry ice/acetone-d6 bath), boron tribromide in dichloromethane (1M, 3 equiv per methoxy function) was added dropwise. The reaction mixture was stirred overnight at room temperature under nitrogen atmosphere. 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 magnesium sulfate, filtered, and concentrated to dryness. The product was purified by CC.

General procedure for Suzuki coupling. Method C1. A mixture of arylbromide (1 equiv), boronic acid derivative (1.2 equiv), cesium carbonate (4 equiv) and tetrakis(triphenylphosphine) palladium (0.01 equiv) was suspended in an oxygen-free DME/water (1:1) solution and refluxed under nitrogen atmosphere. The reaction mixture was cooled to room temperature. The aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over magnesium sulfate, filtered and concentrated to dryness. The product was purified by CC.

Method C2. A mixture of arylbromide (1 equiv), boronic acid derivative (1.2 equiv), Na_2CO_3 (2 equiv) and tetrakis(triphenylphosphine) palladium (0.01 equiv) was suspended in an oxygen-free toluene/ethanol (1:1) solution was refluxed overnight under nitrogen atmosphere. The aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over magnesium sulfate, filtered and concentrated to dryness. The product was purified by CC.

General procedure for sulfonamide/amide coupling. Method D. The amino phenyl derivative (1 equiv) was dissolved in pyridine absolute and was spiked with sulfonyl chloride/acid chloride (1.5 equiv). The reaction mixture was stirred overnight at rt (refluxed in case of amide coupling). The reaction was quenched by adding 10 mL of 2N HCl and extracted with ethyl acetate. The organic layers were washed with saturated $NaHCO_3$ and brine, dried over magnesium sulfate, filtered and concentrated to dryness. The product was purified by CC.

5-Bromo-thiophen-2-yl)-(2-fluoro-3-methoxy-phenyl)-methanone (II). The title compound was prepared by reaction of 2-bromothiophene (1297 mg, 7.95 mmol), 2-fluoro-4-methoxybenzoyl chloride (1000 mg, 5.30 mmol) and aluminum chloride (707 mg, 5.30 mmol) according to method A. The product was purified by CC (hexane/ethyl acetate 96:4); yield: 57% (1000 mg). ^{1}H NMR (500 MHz, acetone-d^6) δ 7.32 (d, J = 7.4 Hz, 1H), 7.33 (ddd, J = 7.3, 4.9, 1.4 Hz, 1H), 7.12 (d, J = 7.4 Hz, 1H), 7.09 (t, J = 7.4 Hz, 1H), 6.92 – 6.87 (m, 1H), 3.82 (s, 3H); ^{13}C NMR (125 MHz, acetone-d^6) δ 184.05, 151.12 (d, J = 246.0 Hz), 149.54, 143.29,136.87,

128.24 (dd, *J* = 9.9, 4.4 Hz), 126.67 (d, *J* = 13.0 Hz),122.18 (d, *J* = 4.3 Hz), 117.46 (d, *J* = 3.0 Hz), 116.52 (d, *J* = 1.3 Hz), 115.94 (dd, *J* = 12.8, 4.1 Hz), 54.21; MS (ESI): 316.41 $(M+H)^+$.

Acetic acid 3-(5-bromo-thiophene-2-carbonyl)-2-methyl-phenyl ester (III). The title compound was prepared by reaction of 2-bromothiophene (1150 mg, 7.05 mmol), 3-acetyloxy-2-methylbenzoyl chloride (1000 mg, 4.70 mmol) and aluminum chloride (627 mg, 4.70 mmol) according to method A. The product was purified by CC (hexane/ethyl acetate 93:7); yield: 38% (600 mg). ^{1}H NMR (500 MHz, acetone-d^6) δ 8.49 (d, *J* = 7.4 Hz, 1H), 8.37 (d, *J* = 7.4 Hz, 1H), 8.24 – 8.02 (m, 3H), 3.11 (s, 3H), 2.84 (s, 3H); ^{13}C NMR (125 MHz, acetone-d^6) δ 187.12, 160.08, 155.10, 149.79, 140.43, 137.8, 129.61, 124.04, 124.51, 123.41, 120.35, 115.51, 114.60, 101.89, 21.63, 10.91; MS (ESI): 340.12 $(M+H)^+$.

Acetic acid 3-(5-bromo-4-methyl-thiophene-2-carbonyl)-2-methyl-phenylester (IV). The title compound was prepared by reaction of 2-bromo-3-methyl-thiophene (2490 mg, 14.06 mmol), 3-acetyloxy-2-methylbenzoyl chloride (1993 mg, 9.37 mmol) and aluminum chloride (1250 mg, 9.37 mmol) according to method A. The product was purified by CC (hexane/ethyl acetate 96:4); yield: 32% (1570 mg). MS (ESI): 354.02 $(M+H)^+$.

5-Bromo-thiophen-2-yl)-(3-ethoxy-2,6-difluoro-phenyl)-methanone (V). The title compound was prepared by reaction of 2-bromothiophene (2217 mg, 13.60 mmol), 2,6-difluoro-4-ethoxybenzoyl chloride (3000 mg, 13.60 mmol) and aluminum chloride (1813 mg, 13.60 mmol) according to method A. The product was purified by CC (hexane/ethyl acetate 97:3); yield: 79% (3740 mg). ^{1}H NMR (300 MHz, acetone-d^6) δ 7.34 (dd, *J* = 4.1, 0.9 Hz, 1H), 7.22 (dd, *J* = 6.7, 4.7 Hz, 2H), 6.98 (td, *J* = 9.0, 2.0 Hz, 1H), 4.05 (q, *J* = 7.0 Hz, 2H), 1.27 (t, *J* = 7.0 Hz, 3H); 13C NMR (75 MHz, acetone-d^6) δ 179.03, 153.83, 153.76, 150.63, 145.09, 136.88, 132.66, 128.96, 124.50, 117.19, 117.06, 65.44, 14.08; ^{13}C NMR (75 MHz, acetone-d^6) δ 179.03, 152.19 (dd, *J* = 242.2, 5.8 Hz), 148.69 (dd, *J* = 250.5, 7.4 Hz), 145.09, 143.94 (dd, *J* = 10.6, 3.3 Hz), 136.88, 132.66, 128.96, 124.50, 117.11 (dd, *J* = 9.3, 3.2 Hz), 111.26 (dd, *J* = 22.6, 4.1 Hz), 65.44, 14.08; MS (ESI): 448.64 $(M+H)^+$.

(5-Bromo-4-methyl-thiophen-2-yl)-(3-ethoxy-2,6-difluoro-phenyl)-methanone (VI). The title compound was prepared by reaction of 2-bromo-3-methoxy-thiophene (1204 mg, 6.80 mmol), 2,6-difluoro-4-ethoxybenzoyl chloride (1000 mg, 4.53 mmol) and aluminum chloride (604 mg, 4.53 mmol) according to method A. The product was purified by CC (hexane/ethyl acetate 97:3); yield: 49% (800 mg). MS (ESI): 362.09 $(M+H)^+$.

(5-Bromo-thiophen-2-yl)-(2,6-difluoro-3-hydroxy-phenyl)-methanone (VIII). The title compound was prepared by reaction of 5-bromo-thiophen-2-yl)-(3-ethoxy-2,6-difluoro-phenyl)-methanone **(V)** (3000 mg, 8.64 mmol) and boron tribromide (43.20 mmol, 5 equiv) according to method B. The product was purified by CC (hexane/ethyl acetate 9:1); yield: 84% (2310 mg). MS (ESI): 320.12 $(M+H)^+$.

(5-Bromo-4-methyl-thiophen-2-yl)-(2,6-difluoro-3-hydroxy-phenyl)-methanone (IX). The title compound was prepared by reaction of (5-bromo-4-methyl-thiophen-2-yl)-(3-ethoxy-2,6-difluoro-phenyl)-methanone **(VI)** (850 mg, 2.35 mmol) and boron tribromide (11.76 mmol, 5 equiv) according to method B. The product was used in the next step without further purification and without any characterization; yield: 77% (600 mg).

(5-(4-Aminophenyl)thiophen-2-yl)(3-hydroxyphenyl)methanone (1). The title compound was prepared by reaction of (5-bromothiophen-2-yl)(3-hydroxyphenyl)methanone **(VII)** (140 mg, 0.50 mmol), 4-aminophenylboronic acid pinacol ester (155 mg, 0.71 mmol), cesium carbonate (658 mg, 2.02 mmol) and tetrakis(triphenylphosphine) palladium (6 mg, 5 µmol) according to method C1. The product was purified by CC (dichloromethan/methanol 99:1); yield: 82% (120 mg). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.75 (br., s, 1H), 7.65 (d, *J* = 4.0 Hz, 1H), 7.44 (d, *J* = 4.0 Hz, 1H), 7.37 (t, *J* = 7.7 Hz, 1H), 7.33 (dt, *J* = 7.6, 1.3 Hz, 1H), 7.31 – 7.29 (m, 1H), 7.13 (t, *J* = 7.8 Hz, 1H), 7.10 (ddd, *J* = 7.9, 2.5, 1.2 Hz, 1H), 7.07 (t, *J* = 1.9 Hz, 1H), 6.99 (ddd, *J* = 7.6, 1.7, 0.9 Hz, 1H), 6.71 (ddd, *J* = 8.0, 2.2, 0.8 Hz, 1H), 4.85 (br., 2H,NH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 186.80, 157.50, 153.62, 149.30, 141.63, 139.55, 135.88, 129.86, 129.65, 129.65, 123.79, 120.14, 119.23, 115.36, 115.29, 114.53, 111.59; MS (ESI): 296.23 $(M+H)^+$.

***N*-(4-(5-(3-Hydroxybenzoyl)thiophen-2-yl)phenyl)benzenesulfonamide (3)**. The title compound was prepared by reaction of (5-(4-aminophenyl)thiophen-2-yl)(3-hydroxyphenyl)methanone **(1)** (100 mg, 0.34 mmol) and benzenesulfonyl chloride (90 mg, 0.51 mmol) according to method D. The product was purified by CC (dichloromethan/methanol 99.5:0.5); yield: 77% (114 mg). ^{1}H NMR (500 MHz, CD_3OD) δ 9.18 (br. s, 1H), 8.76 (br., s, 1H), 7.74 – 7.70 (m, 2H), 7.52 (d, *J* = 4.0 Hz, 1H), 7.51 – 7.48 (m, 2H), 7.46 (dt, *J* = 2.3, 1.6 Hz, 1H), 7.39 (dd, *J* = 10.5, 4.7 Hz, 2H), 7.28 (d, *J* = 4.0 Hz, 1H), 7.24 (t, *J* = 7.8 Hz, 1H), 7.19 – 7.16 (m, 1H), 7.13 – 7.10 (m, 1H), 7.10 – 7.06 (m, 2H), 6.95 (ddd, *J* = 8.1, 2.5, 1.0 Hz, 1H); ^{13}C NMR (125 MHz, MeOD) δ 189.73, 158.89, 154.09, 142.82, 141.08, 140.52, 140.13, 137.95, 134.08, 130.75, 130.67, 130.18, 128.20, 128.16, 125.13, 122.05, 121.26, 120.62, 116.48; MS (ESI): 436.24 $(M+H)^+$.

4-Bromo-*N*-(4-(5-(3-hydroxybenzoyl)thiophen-2-yl)phenyl)-2-trifluoromethoxybenzene-sulfonamide (4). The title compound was prepared by reaction of (5-(4-aminophenyl)thiophen-2-yl)(3-hydroxyphenyl)methanone **(1)** (100 mg, 0.34 mmol) and 4-bromo-2-trifluoromethoxybenzenesulfonyl chloride (172 mg, 0.51 mmol) according to method D. The product was purified by CC (DCM); yield: 36% (72 mg). ^{1}H NMR (500 MHz, CD_3OD) δ 9.30 (br. s, 1H), 8.42 (br., s, 1H), 7.82 (d, *J* = 8.9 Hz, 1H), 7.54 – 7.51 (m, 2H), 7.50 – 7.48 (m, 2H), 7.48 – 7.46 (m, 1H), 7.27 – 7.21 (m, 2H), 7.18 – 7.15 (m, 1H), 7.11 (dd, *J* = 2.2, 1.7 Hz, 1H), 7.10 – 7.06 (m, 2H), 6.94 (ddd, *J* = 8.1, 2.5, 1.0 Hz, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 188.11, 156.45, 152.18, 148.75, 145.99, 141.99, 139.02, 137.05, 136.42, 136.31, 132.55, 130.56,

129.97, 129.71, 129.66, 128.99, 127.38, 124.28, 123.88, 123.41, 123.40, 121.26, 121.22, 121.16, 119.86, 119.07, 115.88; MS (ESI): 600.16 $(M+H)^+$.

3-Cyano-*N*-(4-(5-(3-hydroxybenzoyl)thiophen-2-yl)phenyl)benzenesulfonamide (5). The title compound was prepared by reaction of (5-(4-aminophenyl)thiophen-2-yl)(3-hydroxyphenyl)methanone **(1)** (100 mg, 0.34 mmol) and 3-cyanobenzenesulfonyl chloride (103 mg, 0.51 mmol) according to method D. The product was purified by CC (dichloromethan/methanol 99.5:0.5); yield: 48% (74 mg). ^{1}H NMR (500 MHz, CD_3OD) δ 9.15 (br. s, 1H), 8.43 (br., s, 1H), 8.06 (t, *J* = 1.5 Hz, 1H), 7.95 (ddd, *J* = 8.0, 1.8, 1.1 Hz, 1H), 7.86 – 7.81 (m, 1H), 7.61 – 7.57 (m, 1H), 7.57 – 7.56 (m, 1H), 7.55 (dd, *J* = 3.1, 0.9 Hz, 2H), 7.33 (t, *J* = 5.2 Hz, 1H), 7.25 (t, *J* = 7.8 Hz, 1H), 7.22 – 7.17 (m, 1H), 7.12 (dd, *J* = 4.5, 2.2 Hz, 2H), 7.11 – 7.09 (m, 1H), 6.95 (ddd, *J* = 8.1, 2.5, 1.1 Hz, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 189.73, 158.91, 153.81, 143.04, 142.67, 140.50, 139.47, 137.92, 137.39, 132.36, 131.73, 131.57, 131.25, 130.75, 128.36, 125.33, 122.45, 121.25, 120.64, 119.54, 118.20, 116.48, 114.68, 112.05; MS (ESI): 461.24 $(M+H)^+$.

***N*-(3-(5-(3-Hydroxybenzoyl)thiophen-2-yl)phenyl)benzenesulfonamide (6)**. The title compound was prepared by reaction of (5-(3-aminophenyl)thiophen-2-yl)(3-hydroxyphenyl)methanone **(2)** (100 mg, 0.34 mmol) and benzenesulfonyl chloride (90 mg, 0.51 mmol) according to method D. The product was purified by CC (dichloromethan/methanol 99.5:0.5); yield: 14% (20 mg). ^{1}H NMR (500 MHz, acetone-d^6) δ 9.21 (br. s, 1H), 8.78 (br., s, 1H), 7.90 – 7.85 (m, 2H), 7.70 (d, *J* = 4.0 Hz, 1H), 7.62 (tt, *J* = 2.6, 1.8 Hz, 2H), 7.56 (tt, *J* = 8.3, 1.3 Hz, 2H), 7.53 – 7.48 (m, 2H), 7.41 – 7.39 (m, 1H), 7.38 – 7.34 (m, 2H), 7.34 – 7.33 (m, 1H), 7.28 (ddd, *J* = 8.1, 2.1, 1.0 Hz, 1H), 7.14 (ddd, *J* = 7.9, 2.6, 1.2 Hz, 1H); ^{13}C NMR (125 MHz, acetone-d^6) δ 187.71, 158.44, 152.34, 150.65, 143.51, 140.74, 140.21, 139.79, 136.78, 135.09, 133.88, 131.11, 130.60, 130.05, 128.01, 125.61, 124.59, 123.01, 121.90, 121.09, 120.30, 118.76, 116.29; MS (ESI): 436.13 $(M+H)^+$.

3-Cyano-*N*-(3-(5-(3-hydroxybenzoyl)thiophen-2-yl)phenyl)benzenesulfonamide (8). The title compound was prepared by reaction of (5-(3-aminophenyl)thiophen-2-yl)(3-hydroxyphenyl)methanone **(2)** (100 mg, 0.34 mmol) and 3-cyanobenzenesulfonyl chloride (103 mg, 0.51 mmol) according to method D. The product was purified by CC (dichloromethan/methanol 99.5:0.5); yield: 50% (78 mg). ^{1}H NMR (500 MHz, acetone-d^6) δ 9.39 (br. s, 1H), 8.76 (br., s, 1H), 8.24 – 8.19 (m, 1H), 8.14 (ddd, *J* = 8.0, 1.9, 1.1 Hz, 1H), 8.07 – 8.02 (m, 1H), 7.84 – 7.78 (m, 1H), 7.71 (d, *J* = 4.0 Hz, 1H), 7.62 (t, *J* = 1.8 Hz, 1H), 7.57 (ddd, *J* = 7.8, 1.8, 1.0 Hz, 1H), 7.53 (d, *J* = 4.0 Hz, 1H), 7.41 (td, *J* = 7.9, 3.0 Hz, 2H), 7.37 (dt, *J* = 7.6, 1.3 Hz, 1H), 7.33 (dd, *J* = 2.2, 1.6 Hz, 1H), 7.30 (ddd, *J* = 8.1, 2.1, 0.9 Hz, 1H), 7.14 (ddd, *J* = 7.9, 2.5, 1.2 Hz, 1H); ^{13}C NMR (125 MHz, acetone-d^6) δ 187.69, 158.44, 152.03, 143.64, 141.97, 140.18, 139.03, 137.33, 136.76, 135.31, 132.18, 131.56, 131.49, 131.33, 130.61, 125.78, 123.72, 122.42, 121.10, 120.32, 119.44, 117.87, 116.29, 114.34; MS (ESI): 461.17 $(M+H)^+$.

Thiophene-2-sulfonic acid (3-(5-(3-hydroxybenzoyl)thiophen-2-yl)phenyl)amide (9). The title compound was prepared by reaction of (5-(3-aminophenyl)thiophen-2-yl)(3-hydroxyphenyl)methanone **(2)** (100 mg, 0.34 mmol) and thiophene-2-sulfonyl chloride (93 mg, 0.51 mmol) according to method D. The product was purified by CC (dichloromethan/methanol 99.5:0.5); yield: 34% (50 mg). ^{1}H NMR (500 MHz, acetone-d^6) δ 9.06 (br. s, 1H), 8.59 (br., s, 1H), 7.82 (dd, *J* = 5.0, 1.3 Hz, 1H), 7.74 – 7.67 (m, 2H), 7.65 – 7.60 (m, 1H), 7.55 (d, *J* = 7.7 Hz, 1H), 7.52 (d, *J* = 4.0 Hz, 1H), 7.44 – 7.32 (m, 5H), 7.17 – 7.09 (m, 2H);^{13}C NMR (125 MHz, acetone-d^6) δ 187.78, 158.45, 152.34, 143.55, 141.13, 140.19, 139.55, 136.84, 135.13, 133.84, 133.62, 131.17, 130.62, 128.42, 125.68, 123.36, 122.17, 121.12, 120.35, 118.97, 116.32; MS (ESI): 442.02 $(M+H)^+$.

3-Methoxy-*N*-(3-(5-(3-methoxybenzoyl)thiophen-2-yl)phenyl)benzenesulfonamide (10a). The title compound was prepared by reaction of (5-(3-aminophenyl)thiophen-2-yl)(3-methoxyphenyl)methanone **(2a)** (770 mg, 2.49 mmol) and 3-methoxybenzenesulfonyl chloride (772 mg, 3.73 mmol) according to method D. The product was purified by CC (hexane/ethyl acetate 3:2); yield: 92% (1100 mg). ^{1}H NMR (500 MHz, DMSO-d^6) δ 10.49 (br. s, 1H), 7.73 (d, *J* = 4.0 Hz, 1H), 7.54 (d, *J* = 4.0 Hz, 1H), 7.48 (ddd, *J* = 9.2, 8.0, 6.1 Hz, 4H), 7.44 – 7.40 (m, 1H), 7.36 (dt, *J* = 14.8, 4.8 Hz, 2H), 7.33 (dd, *J* = 2.5, 1.5 Hz, 1H), 7.31 – 7.29 (m, 1H), 7.26 (ddd, *J* = 8.2, 2.7, 1.0 Hz, 1H), 7.18 (ddd, *J* = 8.3, 2.6, 0.9 Hz, 1H), 7.15 (ddd, *J* = 8.1, 2.1, 0.9 Hz, 1H), 3.84 (s, 3H), 3.77 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d^6) δ 186.71, 159.37, 159.23, 151.22, 141.61, 140.50, 138.72, 138.59, 136.79, 133.30, 130.57, 130.35, 129.87, 125.36, 121.71, 121.12, 120.59, 118.86, 118.76, 118.55, 117.02, 113.46, 111.66, 55.56, 55.36; MS (ESI): 479.06 $(M+H)^+$.

3-Hydroxy-*N*-(3-(5-(3-hydroxybenzoyl)thiophen-2-yl)phenyl)benzenesulfonamide (10). The title compound was prepared by reaction of 3-methoxy-*N*-(3-(5-(3-methoxybenzoyl)thiophen-2-yl)phenyl)benzenesulfonamide **(10a)** (100 mg, 0.21 mmol) and boron tribromide (7.5 mmol) according to method B. The product was purified by CC (dichloromethan/methanol 97:3); yield: 74% (70 mg). ^{1}H NMR (500 MHz, CD_3OD) δ 7.67 (d, *J* = 4.0 Hz, 1H), 7.48 (t, *J* = 1.8 Hz, 1H), 7.45 (ddd, *J* = 7.7, 1.7, 1.0 Hz, 1H), 7.43 (d, *J* = 4.0 Hz, 1H), 7.38 (dd, *J* = 14.4, 6.6 Hz, 1H), 7.34 – 7.29 (m, 3H), 7.29 – 7.24 (m, 2H), 7.23 – 7.19 (m, 1H), 7.14 (ddd, *J* = 8.1, 2.1, 0.9 Hz, 1H), 7.07 (ddd, *J* = 8.0, 2.5, 1.1 Hz, 1H), 6.97 (ddd, *J* = 7.9, 2.5, 1.2 Hz, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 189.76, 159.28, 158.93, 153.82, 143.47, 141.91, 140.43, 140.13, 137.75, 135.46, 131.21, 131.13, 130.77, 125.74, 123.36, 122.58, 121.31, 121.08, 120.71, 119.41, 119.03, 116.51, 114.87; MS (ESI): 452.18 $(M+H)^+$.

3-Methoxy-*N*-(3-(5-(3-methoxybenzoyl)thiophen-2-yl)phenyl-benzamide (11a). The title compound was prepared by reaction of (5-(3-aminophenyl)thiophen-2-yl)(3-methoxyphenyl)methanone **(2a)** (770 mg, 2.49 mmol) and 3-methoxybenzoyl chloride (425 mg, 3.73 mmol) according to method D. The product was purified by CC (dichloromethan/methanol

99.75:0.25); yield: 61% (672 mg). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.04 (t, *J* = 1.8 Hz, 1H), 7.95 (br. s, 1H), 7.65 – 7.61 (m, 2H), 7.47 – 7.43 (m, 3H), 7.42 – 7.39 (m, 3H), 7.37 (dd, *J* = 4.4, 2.1 Hz, 3H), 7.12 (ddd, *J* = 8.1, 2.7, 1.1 Hz, 1H), 7.08 (ddd, *J* = 7.6, 2.6, 1.5 Hz, 1H), 3.86 (s, 3H), 3.85 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 187.73, 165.65, 160.08, 159.65, 152.58, 142.40, 139.34, 138.76, 136.15, 135.87, 134.25, 129.90, 129.86, 129.42, 124.30, 122.49, 121.65, 120.62, 118.67, 118.59, 118.27, 117.80, 113.69, 112.53, 55.51, 55.48; MS (ESI): 444.21 $(M+H)^+$.

3-Hydroxy-*N*-(3-(5-(3-hydroxybenzoyl)thiophen-2-yl)phenyl)benzamide (11). The title compound was prepared by reaction of 3-methoxy-*N*-(3-(5-(3-methoxybenzoyl)thiophen-2-yl)-phenyl)benzamide **(11a)** (74 mg, 0.17 mmol) and boron tribromide (1.00 mmol) according to method B. The product was purified by CC (dichloromethan/methanol 99:1); yield: 69% (48 mg). ^{1}H NMR (500 MHz, CD_3OD) δ 8.21 (t, *J* = 1.8 Hz, 1H), 7.74 (ddd, *J* = 8.1, 2.0, 0.9 Hz, 1H), 7.72 (d, *J* = 4.0 Hz, 1H), 7.56 (ddd, *J* = 4.0, 2.0, 1.2 Hz, 2H), 7.46 (t, *J* = 7.9 Hz, 1H), 7.42 (ddd, *J* = 7.6, 1.6, 1.0 Hz, 1H), 7.40 – 7.36 (m, 2H), 7.36 – 7.32 (m, 2H), 7.27 (dd, *J* = 2.2, 1.7 Hz, 1H), 7.08 (ddd, *J* = 8.0, 2.5, 1.2 Hz, 1H), 7.02 (ddd, *J* = 8.1, 2.5, 1.0 Hz, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 189.80, 159.00, 158.93, 154.44, 143.31, 140.95, 140.51, 137.81, 137.54, 135.15, 130.77, 130.73, 125.70, 123.23, 122.75, 121.30, 120.68, 119.99, 119.67, 119.49, 119.45, 116.52, 115.59; MS (ESI): 416.15 $(M+H)^+$.

3-Methoxy-*N*-(3-(5-(3-methoxybenzoyl)thiophen-2-yl)phenyl)-*N*-methylbenzamide (12a). After a mixture of 3-methoxy-*N*-(3-(5-(3-methoxybenzoyl)-thiophen-2-yl)phenyl)benzamide **(11a)** (666 mg, 1.50 mmol, 1 equiv) and NaH (60% suspension in mineral oil, 3.00 mmol, 2 equiv) in DMF was stirred for 30 min at room temperature, iodomethane (213 mg, 1.50 mmol, 2 equiv) was added. The reaction mixture was poured into water; then NaCl was added. The resulting precipitate was collected, washed with water, dried, and purified by CC (hexane/ethyl acetate 3:2); yield: 42% (290 mg). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.66 (d, *J* = 4.0 Hz, 1H), 7.52 – 7.48 (m, 2H), 7.46 (t, *J* = 7.7 Hz, 1H), 7.43 (dd, *J* = 2.5, 1.4 Hz, 1H), 7.41 (t, *J* = 1.8 Hz, 1H), 7.35 (t, *J* = 7.9 Hz, 1H), 7.24 (d, *J* = 4.0 Hz, 1H), 7.21 – 7.17 (m, 1H), 7.17 – 7.11 (m, 2H), 6.98 (dd, *J* = 2.5, 1.5 Hz, 1H), 6.96 – 6.92 (m, 1H), 6.88 – 6.83 (m, 1H), 3.93 (s, 3H), 3.73 (s, 3H), 3.59 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 187.55, 170.40, 159.64, 159.10, 151.55, 145.67, 142.74, 139.13, 136.88, 135.69, 134.33, 129.91, 129.42, 128.97, 126.94, 124.54, 124.26, 121.58, 121.03, 118.57, 116.22, 113.74, 113.63, 55.45, 55.23, 38.24; MS (ESI): 458.19 $(M+H)^+$.

3-Hydroxy-*N*-(3-(5-(3-hydroxybenzoyl)thiophen-2-yl)phenyl)-*N*-methyl-benzamide (12). The title compound was prepared by reaction of 3-methoxy-*N*-(3-(5-(3-methoxybenzoyl)-thiophen-2-yl)phenyl)-*N*-methylbenzamide **(12a)** (290 mg, 0.63 mmol) and boron tribromide (3.80 mmol) according to method B. The product was purified by CC (dichloromethan/methanol 98:2); yield: 56% (151 mg). ^{1}H NMR (500 MHz, CD_3OD) δ 7.55 (d, *J* = 4.0 Hz, 1H), 7.46 (dd, *J* = 7.8, 0.9 Hz, 1H), 7.41 (s, 1H), 7.30 (d, *J* = 4.0 Hz, 1H), 7.29 – 7.26 (m, 1H), 7.26 – 7.24 (m, 1H), 7.21 – 7.18 (m, 1H), 7.13 (dd, *J* = 2.2, 1.7 Hz, 1H), 7.12 – 7.08 (m, 1H), 6.98 – 6.92 (m,

2H), 6.70 – 6.68 (m, 1H), 6.67 (d, *J* = 7.6 Hz, 1H), 6.62 – 6.59 (m, 1H), 3.40 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 189.67, 173.12, 158.93, 158.40, 153.13, 146.69, 143.69, 140.40, 138.40, 137.65, 135.59, 131.22, 130.78, 130.26, 128.45, 126.07, 126.00, 125.65, 121.31, 120.74, 120.52, 118.03, 116.52, 116.20, 32.86; MS (ESI): 430.19 $(M+H)^+$.

4-Cyano-*N*-(3-(5-(3-hydroxybenzoyl)thiophen-2-yl)phenyl)benzenesulfonamide (13). The title compound was prepared by reaction of (5-(3-aminophenyl)thiophen-2-yl)(3-hydroxyphenyl)methanone **(2)** (100 mg, 0.34 mmol) and 4-cyanobenzenesulfonyl chloride (103 mg, 0.51 mmol) according to method D. The product was purified by CC (dichloromethan/methanol 99.5:0.5); yield: 51% (80 mg). ^{1}H NMR (500 MHz, acetone-d^6) δ 9.65 (br. s, 1H), 8.91 (br., s, 1H), 8.07 – 8.02 (m, 2H), 8.02 – 7.97 (m, 2H), 7.71 (d, *J* = 4.0 Hz, 1H), 7.64 (t, *J* = 1.8 Hz, 1H), 7.57 (ddd, *J* = 7.8, 1.7, 1.0 Hz, 1H), 7.53 (d, *J* = 4.0 Hz, 1H), 7.41 (td, *J* = 7.7, 1.4 Hz, 2H), 7.37 (dt, *J* = 7.6, 1.3 Hz, 1H), 7.35 – 7.32 (m, 1H), 7.28 (ddd, *J* = 8.1, 2.1, 0.9 Hz, 1H), 7.14 (ddd, *J* = 7.9, 2.6, 1.2 Hz, 1H);^{13}C NMR (125 MHz, acetone-d^6) δ 187.70, 158.43, 152.05, 144.60, 143.65, 140.18, 139.06, 136.77, 135.30, 134.12, 131.31, 130.61, 128.81, 125.77, 123.67, 122.34, 121.10, 120.32, 119.32, 118.03, 117.32, 116.28; MS (ESI): 461.15 $(M+H)^+$.

***N*-(3-(5-(3-Hydroxybenzoyl)thiophen-2-yl)phenyl)-2-nitrobenzenesulfonamide (14)**. The title compound was prepared by reaction of (5-(3-aminophenyl)thiophen-2-yl)(3-hydroxyphenyl)methanone **(2)** (100 mg, 0.34 mmol) and 2-nitrobenzenesulfonyl chloride (113 mg, 0.51 mmol) according to method D. The product was purified by CC (dichloromethan/methanol 99.5:0.5); yield: 23% (37 mg). ^{1}H NMR (500 MHz, acetone-d^6) δ 9.54 (br. s, 1H), 8.48 (br., s, 1H), 8.08 (dd, *J* = 7.9, 1.3 Hz, 1H), 7.98 (dd, *J* = 7.9, 1.2 Hz, 1H), 7.90 (td, *J* = 7.7, 1.4 Hz, 1H), 7.85 – 7.79 (m, 1H), 7.73 (t, *J* = 1.8 Hz, 1H), 7.71 (d, *J* = 4.0 Hz, 1H), 7.62 – 7.57 (m, 1H), 7.55 (d, *J* = 4.0 Hz, 1H), 7.46 – 7.39 (m, 2H), 7.39 – 7.32 (m, 3H), 7.14 (ddd, *J* = 7.9, 2.5, 1.2 Hz, 1H); ^{13}C NMR (125 MHz, acetone-d^6) δ 187.70, 158.43, 152.05, 149.20, 143.66, 140.18, 138.56, 136.77, 135.67, 135.29, 133.37, 132.58, 132.09, 131.28, 130.61, 125.96, 125.80, 123.96, 122.92, 121.10, 120.32, 119.88, 116.29; MS (ESI): 481.16 $(M+H)^+$.

***N*-(3-(5-(3-Hydroxybenzoyl)thiophen-2-yl)phenyl)-3-nitrobenzenesulfonamide (15)**. The title compound was prepared by reaction of (5-(3-aminophenyl)thiophen-2-yl)(3-hydroxyphenyl)methanone **(2)** (100 mg, 0.34 mmol) and 3-nitrobenzenesulfonyl chloride (113 mg, 0.51 mmol) according to method D. The product was purified by CC (dichloromethan/methanol 99:1); yield: 40% (65 mg). ^{1}H NMR (500 MHz, acetone-d^6) δ 9.65 (br. s, 1H), 8.28 (br. s, 1H), 8.65 – 8.61 (m, 1H), 8.48 (ddd, *J* = 8.2, 2.2, 1.0 Hz, 1H), 8.24 (ddd, *J* = 7.9, 1.7, 1.0 Hz, 1H), 7.89 (t, *J* = 8.1 Hz, 1H), 7.70 (d, *J* = 4.0 Hz, 1H), 7.64 (t, *J* = 1.9 Hz, 1H), 7.57 (ddd, *J* = 7.8, 1.7, 0.9 Hz, 1H), 7.52 (d, *J* = 4.0 Hz, 1H), 7.41 (td, *J* = 7.9, 2.9 Hz, 2H), 7.38 – 7.34 (m, 1H), 7.34 – 7.29 (m, 2H), 7.13 (ddd, *J* = 7.9, 2.5, 1.2 Hz, 1H); ^{13}C NMR (125 MHz, acetone-d^6) δ 187.69, 158.43, 151.99, 149.33, 143.66, 142.26, 140.16, 138.95, 136.77, 135.34, 133.73, 132.02, 131.37,

130.61, 128.45, 125.79, 123.85, 122.87, 122.58, 121.10, 120.33, 119.56, 116.29; MS (ESI): 481.15 $(M+H)^+$.

***N*-(3-(5-(3-Hydroxybenzoyl)thiophen-2-yl)phenyl)-4-nitrobenzenesulfonamide (16)**. The title compound was prepared by reaction of (5-(3-aminophenyl)thiophen-2-yl)(3-hydroxyphenyl)methanone **(2)** (100 mg, 0.34 mmol) and 4-nitrobenzenesulfonyl chloride (113 mg, 0.51 mmol) according to method D. The product was purified by CC (dichloromethan/methanol 99.5:0.5); yield: 46% (74 mg). ^{1}H NMR (500 MHz, acetone-d^6) δ 9.17 (br. s, 1H), 8.20 (br. s, 1H), 8.44 – 8.37 (m, 2H), 8.16 – 8.10 (m, 2H), 7.70 (d, *J* = 4.0 Hz, 1H), 7.66 (t, *J* = 1.9 Hz, 1H), 7.57 (d, *J* = 7.8 Hz, 1H), 7.53 (d, *J* = 4.0 Hz, 1H), 7.41 (td, *J* = 7.9, 1.7 Hz, 2H), 7.38 – 7.34 (m, 1H), 7.34 – 7.28 (m, 2H), 7.14 (ddd, *J* = 7.9, 2.5, 1.1 Hz, 1H);^{13}C NMR (125 MHz, acetone-d^6) δ 187.70, 158.43, 152.03, 151.37, 146.07, 143.66, 140.17, 138.99, 136.77, 135.34, 131.35, 130.61, 129.55, 125.79, 125.35, 123.74, 122.38, 121.09, 120.33, 119.37, 116.28; MS (ESI): 481.18 $(M+H)^+$.

***N*-(3-(5-(3-Hydroxybenzoyl)thiophen-2-yl)phenyl)-2,3-dinitrobenzenesulfonamide (17)**. The title compound was prepared by reaction of (5-(3-aminophenyl)thiophen-2-yl)(3-hydroxyphenyl)methanone **(2)** (100 mg, 0.34 mmol) and 2,3-dinitrobenzenesulfonyl chloride (135 mg, 0.51 mmol) according to method D. The product was purified by CC (dichloromethan/methanol 99.5:0.5); yield: 23% (40 mg). ^{1}H NMR (500 MHz, CD_3OD) δ 8.06 (t, *J* = 1.5 Hz, 1H), 7.95 (ddd, *J* = 8.0, 1.8, 1.1 Hz, 1H), 7.86 – 7.81 (m, 1H), 7.61 – 7.57 (m, 1H), 7.57 – 7.56 (m, 1H), 7.55 (dd, *J* = 3.1, 0.9 Hz, 2H), 7.33 (t, *J* = 5.2 Hz, 1H), 7.25 (t, *J* = 7.8 Hz, 1H), 7.22 – 7.17 (m, 1H), 7.12 (dd, *J* = 4.5, 2.2 Hz, 2H), 7.11 – 7.09 (m, 1H), 6.95 (ddd, *J* = 8.1, 2.5, 1.1 Hz, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 189.73, 158.91, 153.81, 143.04, 142.67, 140.50, 139.47, 137.92, 137.39, 132.36, 131.73, 131.57, 131.25, 130.75, 128.36, 125.33, 122.45, 121.25, 120.64, 119.54, 118.20, 116.48, 114.68, 112.05; MS (ESI): 526.21 $(M+H)^+$.

2-Fluoro-*N*-(3-(5-(3-hydroxybenzoyl)thiophen-2-yl)phenyl)benzenesulfonamide (18). The title compound was prepared by reaction of (5-(3-aminophenyl)thiophen-2-yl)(3-hydroxyphenyl)methanone **(2)** (100 mg, 0.34 mmol) and 2-fluorobenzenesulfonyl chloride (99 mg, 0.51 mmol) according to method D. The product was purified by CC (dichloromethan/methanol 99.5:0.5); yield: 57% (88 mg). ^{1}H NMR (500 MHz, acetone-d^6) δ 9.49 (br. s, 1H), 8.77 (br. s, 1H), 7.96 (td, *J* = 7.6, 1.8 Hz, 1H), 7.73 – 7.66 (m, 3H), 7.51 (qd, *J* = 1.9, 1.1 Hz, 2H), 7.43 – 7.38 (m, 2H), 7.38 – 7.33 (m, 4H), 7.33 – 7.30 (m, 1H), 7.14 (ddd, *J* = 7.9, 2.5, 1.2 Hz, 1H); ^{13}C NMR (125 MHz, acetone-d^6) δ 187.70, 159.71 (d, *J* = 254.7 Hz), 158.45, 152.24, 143.56, 140.20, 139.23, 136.83 (d, *J* = 8.8 Hz), 136.78, 135.14, 131.77, 131.17, 130.60, 125.71 (d, *J* = 3.8 Hz), 125.69, 125.65, 123.11, 121.48, 121.08, 120.31, 118.33, 118.10, 117.93, 116.30; MS (ESI): 454.23 $(M+H)^+$.

3-Fluoro-*N*-(3-(5-(3-hydroxybenzoyl)thiophen-2-yl-phenyl)benzenesulfonamide (19). The title compound was prepared by reaction of (5-(3-aminophenyl)thiophen-2-yl)(3-hydroxyphenyl)methanone **(2)** (100 mg, 0.34 mmol) and 3-fluorobenzenesulfonyl chloride (99 mg, 0.51 mmol) according to method D. The product was purified by CC (dichloromethan/methanol 99.5:0.5); yield: 53% (82 mg). ^{1}H NMR (500 MHz, acetone-d^6) δ 9.30 (br. s, 1H), 8.76 (br. s, 1H), 7.74 – 7.67 (m, 2H), 7.65 – 7.50 (m, 5H), 7.45 – 7.36 (m, 4H), 7.34 – 7.25 (m, 2H), 7.14 (d, *J* = 5.4 Hz, 1H); ^{13}C NMR (125 MHz, acetone-d^6) δ 187.69, 158.44, 152.16, 143.59, 140.19, 139.37, 136.78, 135.21, 132.41, 132.35, 131.23, 130.60, 125.70, 124.18 (d, *J* = 3.1 Hz), 123.43, 122.20, 121.09, 120.90, 120.32, 119.13, 116.29, 115.10, 114.90; MS (ESI): 454.15 $(M+H)^+$.

4-Fluoro-*N*-(3-(5-(3-hydroxybenzoyl)thiophen-2-yl)phenyl)benzenesulfonamide (20). The title compound was prepared by reaction of (5-(3-amin-phenyl)thiophen-2-yl)(3-hydroxyphenyl)methanone **(2)** (100 mg, 0.34 mmol) and 4-fluorobenzenesulfonyl chloride (99 mg, 0.51 mmol) according to method D. The product was purified by CC (dichloromethan/methanol 99.5:0.5); yield: 44% (67 mg). ^{1}H NMR (500 MHz, acetone-d^6) δ 8.06 (t, *J* = 1.5 Hz, 1H), 7.95 (ddd, *J* = 8.0, 1.8, 1.1 Hz, 1H), 7.86 – 7.81 (m, 1H), 7.61 – 7.57 (m, 1H), 7.57 – 7.56 (m, 1H), 7.55 (dd, *J* = 3.1, 0.9 Hz, 2H), 7.33 (t, *J* = 5.2 Hz, 1H), 7.25 (t, *J* = 7.8 Hz, 1H), 7.22 – 7.17 (m, 1H), 7.12 (dd, *J* = 4.5, 2.2 Hz, 2H), 7.11 – 7.09 (m, 1H), 6.95 (ddd, *J* = 8.1, 2.5, 1.1 Hz, 1H); ^{13}C NMR (125 MHz, acetone-d^6) δ 187.70, 166.01 (d, *J* = 252.5 Hz), 158.43, 152.24, 143.56, 140.20, 139.60, 136.98 (d, *J* = 3.0 Hz), 136.78, 135.17, 131.18, 131.10, 131.03, 130.60, 125.67, 123.24, 122.08, 121.09, 120.30, 118.99, 117.24, 117.06, 116.28; MS (ESI): 454.18 $(M+H)^+$.

4-Bromo-*N*-(3-(5-(3-hydroxybenzoyl)thiophen-2-yl)phenyl)benzenesulfonamide (21). The title compound was prepared by reaction of (5-(3-aminophenyl)thiophen-2-yl)(3-hydroxyphenyl)methanone **(2)** (100 mg, 0.34 mmol) and 4-bromobenzenesulfonyl chloride (130 mg, 0.51 mmol) according to method D. The product was purified by CC (dichloromethan/methanol 99.5:0.5); yield: 49% (85 mg). ^{1}H NMR (500 MHz, acetone-d^6) δ 7.81 – 7.78 (m, 2H), 7.77 – 7.74 (m, 2H), 7.71 (d, *J* = 4.0 Hz, 1H), 7.63 (t, *J* = 1.8 Hz, 1H), 7.54 (ddd, *J* = 7.8, 1.8, 1.0 Hz, 1H), 7.52 (d, *J* = 4.0 Hz, 1H), 7.38 (tt, *J* = 4.5, 2.8 Hz, 3H), 7.35 – 7.33 (m, 1H), 7.27 (ddd, *J* = 8.1, 2.1, 0.9 Hz, 1H), 7.14 (ddd, *J* = 7.9, 2.5, 1.2 Hz, 1H);^{13}C NMR (125 MHz, acetone-d^6) δ 187.70, 158.42, 152.20, 143.58, 140.20, 139.94, 139.44, 136.77, 135.21, 133.30, 131.22, 130.61, 129.92, 128.15, 125.70, 123.34, 122.10, 121.10, 120.31, 119.04, 116.28; MS (ESI): 515.02 $(M+H)^+$.

4-Bromo-2,5-difluoro-*N*-(3-(5-(3-hydroxybenzoyl)thiophen-2-yl)phenyl)benzenesulfonamide (22). The title compound was prepared by reaction of (5-(3-aminophenyl)thiophen-2-yl)(3-hydroxyphenyl)methanone **(2)** (100 mg, 0.34 mmol) and 4-bromo-2,5-difluorobenzenesulfonyl chloride (148 mg, 0.51 mmol) according to method D. The product was purified

by CC (dichloromethan/methanol 99.5:0.5); yield: 44% (82 mg). ^{1}H NMR (500 MHz, acetone-d^6) δ 7.83 – 7.78 (m, 2H), 7.71 (d, *J* = 4.0 Hz, 1H), 7.69 (t, *J* = 1.9 Hz, 1H), 7.57 (ddd, *J* = 7.7, 1.8, 1.0 Hz, 1H), 7.54 (d, *J* = 4.0 Hz, 1H), 7.44 – 7.39 (m, 2H), 7.38 – 7.33 (m, 3H), 7.14 (ddd, *J* = 7.9, 2.5, 1.3 Hz, 1H); ^{13}C NMR (125 MHz, acetone-d^6) δ 187.70, 158.44, 152.02, 150.65, 143.68, 140.18, 138.56, 136.77, 135.32, 131.35, 130.61, 125.79, 124.58, 123.72, 123.64, 123.42, 121.94, 121.10, 120.32, 118.97, 118.71, 118.48, 116.29; MS (ESI): 551.97 $(M+H)^+$.

3,5-Dichloro-4-hydroxy-*N*-(3-(5-(3-hydroxybenzoyl)thiophen-2-yl)phenyl)benzenesulfonamide (23). The title compound was prepared by reaction of (5-(3-aminophenyl)thiophen-2-yl)(3-hydroxyphenyl)methanone **(2)** (150 mg, 0.51 mmol) and 3,5-dichloro-4-hydroxybenzenesulfonyl chloride (199 mg, 0.76 mmol) according to method D. The product was purified by CC (dichloromethan/methanol 99.25:0.75); yield: 45% (120 mg). ^{1}H NMR (500 MHz, CD_3OD) δ 7.69 (d, *J* = 3.6 Hz, 2H), 7.63 (d, *J* = 4.0 Hz, 1H), 7.46 (t, *J* = 1.8 Hz, 1H), 7.46 – 7.42 (m, 1H), 7.39 (d, *J* = 4.0 Hz, 1H), 7.35 (t, *J* = 5.3 Hz, 1H), 7.34 – 7.31 (m, 1H), 7.31 – 7.28 (m, 1H), 7.24 (dd, *J* = 2.2, 1.7 Hz, 1H), 7.13 (ddd, *J* = 8.1, 2.1, 0.9 Hz, 1H), 7.06 (ddd, *J* = 8.0, 2.5, 1.1 Hz, 1H);^{13}C NMR (125 MHz, CD_3OD) δ 189.74, 158.88, 154.96, 153.56, 143.55, 140.38, 139.66, 137.76, 135.63, 132.39, 131.34, 130.77, 128.75, 125.84, 123.80, 123.77, 122.72, 121.39, 120.74, 119.63, 116.54; MS (ESI): 520.13 $(M+H)^+$.

3-Methoxy-*N*-(3-(5-(3-hydroxybenzoyl)thiophen-2-yl)phenyl)benzenesulfonamide (24). The title compound was prepared by reaction of (5-(3-aminophenyl)thiophen-2-yl)(3-hydroxyphenyl)methanone **(2)** (100 mg, 0.34 mmol) and 3-methoxybenzenesulfonyl chloride (105 mg, 0.51 mmol) according to method D. The product was purified by CC (dichloromethan/methanol 99.5:0.5); yield: 38% (60 mg). ^{1}H NMR (500 MHz, acetone-d^6) δ 9.19 (br. s, 1H), 8.80 (br. s, 1H), 7.68 (d, *J* = 4.0 Hz, 1H), 7.64 (t, *J* = 1.8 Hz, 1H), 7.51 – 7.47 (m, 2H), 7.45 – 7.41 (m, 2H), 7.40 – 7.38 (m, 1H), 7.38 – 7.33 (m, 4H), 7.29 (ddd, *J* = 8.1, 2.1, 1.0 Hz, 1H), 7.16 – 7.14 (m, 1H), 7.14 – 7.11 (m, 1H), 3.79 (s, 3H); ^{13}C NMR (125 MHz, acetone-d^6) δ 187.78, 160.88, 158.43, 152.37, 143.50, 141.84, 140.19, 139.79, 136.85, 135.08, 131.21, 131.14, 130.63, 125.64, 123.07, 121.97, 121.15, 120.36, 120.07, 119.75, 118.81, 116.33, 113.03, 56.07; MS (ESI): 466.17 $(M+H)^+$.

***N*-(3-(3-(5-(3-Hydroxybenzoyl)thiophen-2-yl)phenylsulfamoyl)phenyl)acetamide (25)**. The title compound was prepared by reaction of (5-(3-aminophenyl)thiophen-2-yl)(3-hydroxyphenyl)methanone **(2)** (80 mg, 0.27 mmol) and 3-acetylaminobenzenesulfonyl chloride (95 mg, 0.41 mmol) according to method D. The product was purified by CC (dichloromethan/methanol 99:1); yield: 70% (93 mg). ^{1}H NMR (500 MHz, acetone-d^6) δ 9.46 (br., 1H,NH), 9.10 (br. s, 1H), 8.75 (br. s, 1H), 7.78 (s, 4H), 7.70 (d, *J* = 4.0 Hz, 1H), 7.62 (t, *J* = 1.8 Hz, 1H), 7.50 (t, *J* = 5.8 Hz, 2H), 7.41 (t, *J* = 7.7 Hz, 1H), 7.39 – 7.35 (m, 2H), 7.34 – 7.32 (m, 1H), 7.27 (dd, *J* = 8.1, 1.2 Hz, 1H), 7.13 (ddd, *J* = 7.9, 2.5, 1.2 Hz, 1H), 2.08 (s, 3H); ^{13}C NMR (125 MHz, acetone-d^6) δ 189.92, 187.72, 158.44, 152.44, 144.54, 143.47, 140.21, 139.99,

136.80, 135.03, 134.32, 131.07, 130.60, 129.23, 128.56, 125.60, 122.83, 121.79, 121.08, 120.31, 119.48, 118.63, 116.30, 114.20, 22.10; MS (ESI): 493.41 $(M+H)^+$.

***N*-(3-(5-(3-Hydroxybenzoyl)thiophen-2-yl)phenyl)-2-methylbenzenesulfonamide (26)**. The title compound was prepared by reaction of (5-(3-aminophenyl)thiophen-2-yl)(3-hydroxyphenyl)methanone **(2)** (100 mg, 0.34 mmol) and 2-methylbenzenesulfonyl chloride (97 mg, 0.51 mmol) according to method D. The product was purified by CC (dichloromethan/methanol 99.5:0.5); yield: 51% (80 mg). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.30 (br. s, 1H), 8.79 (br. s, 1H), 8.07 – 8.01 (m, 1H), 7.67 (d, *J* = 4.0 Hz, 1H), 7.58 (t, *J* = 1.8 Hz, 1H), 7.48 (s, 1H), 7.46 (d, *J* = 4.0 Hz, 1H), 7.43 (ddd, *J* = 7.7, 1.7, 1.0 Hz, 1H), 7.37 (t, *J* = 7.7 Hz, 3H), 7.32 (ddd, *J* = 11.8, 2.5, 1.5 Hz, 3H), 7.22 (ddd, *J* = 8.1, 2.2, 0.9 Hz, 1H), 7.11 (ddd, *J* = 7.9, 2.5, 1.2 Hz, 1H), 2.66 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 186.81, 157.54, 151.50, 142.58, 139.31, 138.99, 138.78, 137.30, 135.88, 134.16, 133.17, 132.71, 130.22, 129.95, 129.70, 126.26, 124.68, 121.52, 120.18, 119.79, 119.40, 116.57, 115.38, 19.42; MS (ESI): 450.08 $(M+H)^+$.

***N*-(3-(5-(3-Hydroxybenzoyl)thiophen-2-yl)phenyl)-3-methylbenzenesulfonamide (27)**. The title compound was prepared by reaction of (5-(3-aminophenyl)thiophen-2-yl)(3-hydroxyphenyl)methanone **(2)** (100 mg, 0.34 mmol) and 3-methylbenzenesulfonyl chloride (97 mg, 0.51 mmol) according to method D. The product was purified by CC (dichloromethan/methanol 99.5:0.5); yield: 64% (100 mg). ^{1}H NMR (500 MHz, acetone-d^6) δ 9.15 (br. s, 1H), 8.75 (br. s, 1H), 7.70 (dd, *J* = 4.5, 2.3 Hz, 2H), 7.69 – 7.64 (m, 1H), 7.62 (t, *J* = 1.8 Hz, 1H), 7.52 – 7.49 (m, 2H), 7.45 – 7.42 (m, 2H), 7.42 – 7.35 (m, 3H), 7.34 – 7.32 (m, 1H), 7.27 (ddd, *J* = 8.1, 2.1, 1.0 Hz, 1H), 7.14 (ddd, *J* = 7.9, 2.5, 1.2 Hz, 1H), 2.37 (s, 3H); ^{13}C NMR (125 MHz, acetone-d^6) δ 187.69, 158.43, 152.39, 143.50, 140.70, 140.23, 139.87, 136.77, 135.06, 134.55, 131.08, 130.60, 129.90, 128.33, 125.59, 125.18, 122.88, 121.76, 121.09, 120.30, 118.60, 116.29, 21.24; MS (ESI): 450.11 $(M+H)^+$.

2-Fluoro-*N*-(3-(5-(3-hydroxybenzoyl)thiophen-2-yl)phenyl)-4-methoxybenzenesulfonamide (28). The title compound was prepared by reaction of (5-(3-aminophenyl)thiophen-2-yl)(3-hydroxyphenyl)methanone **(2)** (200 mg, 0.68 mmol) and 2-fluoro-4-methoxybenzenesulfonyl chloride (228 mg, 1.02 mmol) according to method D. The product was purified by CC (dichloromethan/methanol 99:1); yield: 58% (190 mg). ^{1}H NMR (500 MHz, CD_3OD) δ 7.61 (d, *J* = 4.0 Hz, 1H), 7.58 (ddd, *J* = 8.7, 2.2, 1.2 Hz, 1H), 7.52 (dd, *J* = 10.6, 2.2 Hz, 1H), 7.45 (t, *J* = 1.8 Hz, 1H), 7.41 (ddd, *J* = 7.8, 1.7, 1.0 Hz, 1H), 7.38 (d, *J* = 4.0 Hz, 1H), 7.34 (t, *J* = 7.8 Hz, 1H), 7.31 – 7.26 (m, 2H), 7.24 (dd, *J* = 2.2, 1.7 Hz, 1H), 7.14 (ddd, *J* = 8.2, 4.8, 3.8 Hz, 2H), 7.06 (ddd, *J* = 8.1, 2.5, 1.1 Hz, 1H), 3.86 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 189.71, 158.89, 153.6, 153.50 (d, *J* = 95.3 Hz), 152.47 (d, *J* = 144.8 Hz), 143.50, 140.37, 139.92, 137.76, 135.51, 132.56 (d, *J* = 5.6 Hz), 131.25, 130.78, 125.92 (d, *J* = 3.7 Hz), 125.80, 123.50, 122.55, 121.37, 120.74, 119.39, 116.53, 116.03, 115.86, 114.31 (d, *J* = 1.6 Hz), 57.00; MS (ESI): 484.26 $(M+H)^+$.

2-Fluoro-4-hydroxy-*N*-(3-(5-(3-hydroxybenzoyl)thiophen-2-yl)phenyl)benzenesulfonamide (29). The title compound was prepared by reaction of 2-fluoro-*N*-(3-(5-(3-hydroxybenzoyl)-thiophen-2-yl)phenyl)-4-methoxybenzenesulfonamide **(22)** (155 mg, 0.32 mmol) and boron tribromide (0.96 mmol) according to method B. The product was purified by CC (dichloromethan/methanol 98.5:1.5); yield: 46% (70 mg). ^{1}H NMR (500 MHz, CD_3OD) δ 7.60 (d, *J* = 4.0 Hz, 1H), 7.51 (dd, *J* = 10.5, 2.2 Hz, 1H), 7.47 (ddd, *J* = 4.9, 3.1, 1.4 Hz, 2H), 7.39 (ddd, *J* = 7.8, 1.7, 0.9 Hz, 1H), 7.36 (d, *J* = 4.0 Hz, 1H), 7.34 (t, *J* = 7.8 Hz, 1H), 7.30 – 7.26 (m, 2H), 7.25 – 7.23 (m, 1H), 7.13 (ddd, *J* = 8.1, 2.1, 0.9 Hz, 1H), 7.06 (ddd, *J* = 8.0, 2.5, 1.1 Hz, 1H), 6.97 (t, *J* = 8.4 Hz, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 158.86, 153.72, 152.13 (d, *J* = 240.4 Hz), 151.11 (d, *J* = 7.2 Hz), 143.45, 140.37, 139.99, 137.82, 135.48, 131.32 (d, *J* = 5.4 Hz), 125.87, 125.84, 125.78, 123.48, 122.55, 121.42, 120.76, 119.43, 118.88, 118.85, 116.64, 116.55, 116.47; MS (ESI): 470.43 $(M+H)^+$.

(5-(2,4-Difluoro-phenyl)-thiophen-2-yl)-(3-methoxy-phenyl)-methanone (30a). The title compound was prepared by reaction of (5-Bromo-thiophen-2-yl)-(3-methoxy-phenyl)-methanone **(I)** (500 mg, 1.68 mmol), 2,4-difluorophenylboronic acid (298 mg, 1.88 mmol), cesium carbonate (2189 mg, 6.7 mmol) and tetrakis(triphenylphosphine) palladium (5 µmol) according to method C1. The product was purified by CC (petroleum ether /ethyl acetate 96:4); yield: 75% (417 mg). MS (ESI): 331.04 $(M+H)^+$.

(5-(2,4-Difluoro-phenyl)-thiophen-2-yl)-(3-hydroxy-phenyl)-methanone (30). The title compound was prepared by reaction of (5-(2,4-difluoro-phenyl)-thiophen-2-yl)-(3-methoxy-phenyl)-methanone **(30a)** (417 mg, 1.26 mmol) and boron tribromide (3.8 mmol) according to method B. The product was purified by CC (dichloromethan/methanol 99.75:0.25); yield: 41% (330 mg). ^{1}H NMR (500 MHz, acetone-d^6) δ 8.77 (s, 1H), 7.94 (td, *J* = 8.8, 6.3 Hz, 1H), 7.75 (dd, *J* = 4.0, 1.1 Hz, 1H), 7.63 (dd, *J* = 4.0, 1.0 Hz, 1H), 7.43 – 7.40 (m, 1H), 7.39 – 7.37 (m, 1H), 7.35 (ddd, *J* = 2.5, 1.5, 0.5 Hz, 1H), 7.27 – 7.21 (m, 1H), 7.20 – 7.15 (m, 1H), 7.14 (ddd, *J* = 7.7, 2.6, 1.5 Hz, 1H); ^{13}C NMR (125 MHz, acetone-d^6) δ 187.84, 163.90 (dd, *J* = 250.2, 12.1 Hz), 160.35 (dd, *J* = 254.0, 13.3 Hz), 158.45, 144.41 (dd, *J* = 91.1, 3.7 Hz), 140.16, 136.06, 131.44 (dd, *J* = 9.9, 4.4 Hz), 130.63, 128.25 – 127.77 (m), 121.15, 120.39, 118.70 (dd, *J* = 12.9, 4.1 Hz), 116.32, 113.36 (dd, *J* = 21.8, 3.6 Hz), 105.70 (t, *J* = 26.4 Hz); MS (ESI): 317.63 $(M+H)^+$.

Acetic acid 3-(5-(2,4-difluoro-phenyl)-thiophene-2-carbonyl)-2-methyl-phenyl ester (31a). The title compound was prepared by reaction of acetic acid 3-(5-bromo-thiophene-2-carbonyl)-2-methyl-phenyl ester **(III)** (500 mg, 1.47 mmol), 2,4-difluorophenylboronic acid (279 mg, 1.77 mmol), cesium carbonate (1921 mg, 5.90 mmol) and tetrakis(triphenylphosphine) palladium (5 µmol) according to method C1. The product was sufficiently pure for use in the subsequent reaction. The product was used without any characterization; yield: 78% (430 mg).

(5-(2,4-Difluoro-phenyl)-thiophen-2-yl)-(3-hydroxy-2-methyl-phenyl)-methanone(31). Compound **(31a)** (430 mg, 1.16 mmol) in ethanol (5 mL) was refluxed in 10% NaOH (15 mL) for 2 h on a water bath. The reaction mixture was cooled, diluted with water and neutralized with acetic acid. The product was purified by CC (dichloromethan/methanol 99.5:0.5); yield: 58% (220 mg). ^{1}H NMR (500 MHz, acetone-d^6) δ 9.51 (s, 1H, OH), 8.83 (dd, *J* = 15.1, 8.5 Hz, 1H), 8.49 (d, *J* = 3.4 Hz, 1H), 8.37 (d, *J* = 3.4 Hz, 1H), 8.24 – 8.02 (m, 3H), 7.95 (d, *J* = 7.9 Hz, 1H), 7.89 (d, *J* = 7.3 Hz, 1H), 3.11 (s, 3H); ^{13}C NMR (125 MHz, acetone-d^6) δ 189.56, 163.02 (dd, *J* = 251.3, 12.8 Hz), 159.45 (dd, *J* = 253.4, 12.4 Hz), 155.85, 144.44 (dd, *J* = 10.1, 4.1 Hz), 140.21, 135.70, 130.55 (dd, *J* = 9.9, 4.4 Hz), 127.34, 127.28, 126.11, 122.33, 118.97, 117.80 (dd, *J* = 12.8, 4.0 Hz), 116.62, 112.45 (dd, *J* = 21.8, 3.6 Hz), 104.79 (t, *J* = 26.4 Hz), 11.99; MS (ESI): 331.04 $(M+H)^+$.

Acetic acid 3-(5-(2,4-difluoro-phenyl)-4-methyl-thiophene-2-carbonyl)-2-methyl-phenyl ester (32a). The title compound was prepared by reaction of acetic acid 3-(5-bromo-4-methyl-thiophene-2-carbonyl)-2-methyl-phenylester **(IV)** (500 mg, 1.42 mmol), 2,4-difluorophenyl-boronic acid (268 mg, 1.70 mmol), cesium carbonate (1845 mg, 5.66 mmol) and tetrakis(triphenylphosphine) palladium (5 µmol) according to method C1. The product was purified by CC (hexane/ethyl acetate 9:1); yield: 88% (480 mg). MS (ESI): 487.11 $(M+H)^+$.

(5-(2,4-Difluoro-phenyl)-4-methyl-thiophen-2-yl)-(3-hydroxy-2-methyl-phenyl)-methanone (32). Compound **(32a)** (480 mg, 1.24 mmol) in ethanol (5 mL) was refluxed in 10% NaOH (15 mL) for 2 h on a water bath. The reaction mixture was cooled, diluted with water and neutralized with acetic acid. The product was purified by CC (dichloromethan/methanol 99.5:0.5); yield: 35% (150 mg). ^{1}H NMR (500 MHz, acetone-d^6) δ 9.51 (s, 1H), 8.83 (dd, *J* = 15.1, 8.5 Hz, 1H), 8.49 (d, *J* = 3.4 Hz, 1H), 8.37 (d, *J* = 3.4 Hz, 1H), 8.24 – 8.02 (m, 3H), 7.95 (d, *J* = 7.9 Hz, 1H), 7.89 (d, *J* = 7.3 Hz, 1H), 3.11 (s, 3H); ^{13}C NMR (125 MHz, acetone-d^6) δ 189.48, 163.39 (dd, *J* = 247.8, 10.1 Hz), 159.78 (dd, *J* = 250.9, 12.9 Hz), 155.83, 143.47, 140.24, 139.05, 137.92, 137.66, 133.27 (dd, *J* = 9.9, 3.9 Hz), 126.06, 122.27, 118.92 , 117.48 (dd, *J* = 15.7, 3.9 Hz), 116.53, 111.96 (dd, *J* = 21.6, 3.8 Hz), 104.47 (t, *J* = 26.3 Hz), 13.82 (d, *J* = 3.5 Hz), 11.99; MS (ESI): 445.93 $(M+H)^+$.

(5-(2,4-Difluoro-phenyl)-thiophen-2-yl)-(2-fluoro-3-methoxy-phenyl)-methanone (33a). The title compound was prepared by reaction of 5-bromo-thiophen-2-yl)-(2-fluoro-3-methoxy-phenyl)-methanone **(II)** (500 mg, 1.52 mmol), 2,4-difluorophenylboronic acid (288 mg, 1.82 mmol), cesium carbonate (1981 mg, 6.08 mmol) and tetrakis(triphenylphosphine) palladium (5 µmol) according to method C1. The product was used in the next step without further purification; yield: 78% (428 mg).

(5-(2,4-Difluoro-phenyl)-thiophen-2-yl)-(2-fluoro-3-hydroxy-phenyl)-methanone (33). The title compound was prepared by reaction of [5-(2,4-difluoro-phenyl)-thiophen-2-yl)-(2-fluoro-3-

methoxy-phenyl)-methanone **(33a)** (428 mg, 1.23 mmol) and boron tribromide (7.4 mmol, 6.0 equiv) according to method B. The product was purified by CC (dichloromethan/methanol 99.50:0.50); yield: 49% (200 mg). ^{1}H NMR (500 MHz, acetone-d^6) δ 9.06 (s, 1H), 7.95 (td, *J* = 8.8, 6.3 Hz, 1H), 7.64 – 7.60 (m, 2H), 7.28 – 7.24 (m, 1H), 7.24 – 7.21 (m, 1H), 7.21 – 7.18 (m, 1H), 7.18 – 7.15 (m, 1H), 7.08 (ddd, *J* = 7.4, 5.6, 1.8 Hz, 1H); ^{13}C NMR (125 MHz, acetone-d^6) δ 185.08, 164.02 (dd, *J* = 251.6, 13.2 Hz), 160.38 (dd, *J* = 253.3, 12.5 Hz), 149.38 (d, *J* = 246.0 Hz), 146.40, 146.30, 145.07 (dd, *J* = 196.2, 4.2 Hz), 136.91, 131.50 (dd, *J* = 9.9, 4.4 Hz), 128.73 (d, *J* = 13.0 Hz), 128.32 (dd, *J* = 5.0, 0.5 Hz), 125.48 (d, *J* = 4.3 Hz), 121.40 (d, *J* = 3.0 Hz), 120.71 (d, *J* = 1.3 Hz), 118.57 (dd, *J* = 12.8, 4.1 Hz), 113.41 (dd, *J* = 21.9, 3.7 Hz), 105.74 (t, *J* = 26.4 Hz); MS (ESI): 335.04 $(M+H)^+$.

(5-(2,4-Difluoro-phenyl)-thiophen-2-yl)-(3-ethoxy-2,6-difluoro-phenyl)-methanone (34a). The title compound was prepared by reaction of 5-bromo-thiophen-2-yl)-(3-ethoxy-2,6-difluoro-phenyl)-methanone **(V)** (300 mg, 0.86 mmol), 2,4-difluorophenylboronic acid (164 mg, 1.04 mmol), cesium carbonate (1126 mg, 3.5 mmol) and tetrakis(triphenylphosphine) palladium (5 µmol) according to method C1. The product was used in the next step without further purification and without any characterization; yield: 91% (300 mg).

(2,6-Difluoro-3-hydroxy-phenyl)-(5-(2,4-difluoro-phenyl)-thiophen-2-yl)-methanone (34). The title compound was prepared by reaction of (5-(2,4-difluoro-phenyl)-thiophen-2-yl)-(3-ethoxy-2,6-difluoro-phenyl)-methanone **(34a)** (300 mg, 0.79 mmol) and boron tribromide (3.9 mmol, 5.0 equiv) according to method B. The product was purified by CC (dichloromethan/methanol 99.75:0.25); yield: 72% (280 mg). ^{1}H NMR (500 MHz, acetone-d^6) δ 8.99 (s, 1H), 7.96 (td, *J* = 8.8, 6.3 Hz, 1H), 7.68 (ddd, *J* = 4.0, 1.9, 0.9 Hz, 1H), 7.65 (dd, *J* = 4.1, 0.9 Hz, 1H), 7.29 – 7.23 (m, 1H), 7.23 – 7.16 (m, 2H), 7.04 (ddd, *J* = 9.1, 8.6, 1.9 Hz, 1H); ^{13}C NMR (125 MHz, acetone-d^6) δ 180.88, 164.18 (dd, *J* = 251.5, 12.7 Hz), 160.45 (dd, *J* = 253.6, 12.6 Hz), 152.56 (dd, *J* = 240.6, 5.8 Hz), 148.43 (dd, *J* = 245.8, 7.7 Hz), 145.46 (dd, *J* = 361.6, 3.6 Hz), 142.65 (dd, *J* = 12.9, 3.2 Hz), 137.39, 131.60 (dd, *J* = 9.8, 4.4 Hz), 128.62, 128.59, 120.41 (dd, *J* = 9.1, 3.9 Hz), 118.40 (dd, *J* = 12.7, 4.1 Hz), 118.04 (dd, *J* = 24.0, 19.6 Hz), 113.46 (dd, *J* = 21.1, 4.5 Hz), 112.46 (dd, *J* = 22.8, 3.9 Hz), 105.76 (t, *J* = 26.4 Hz); MS (ESI): 353.88 $(M+H)^+$.

Acetic acid 3-(5-(3-chloro-4-methoxy-phenyl)-thiophene-2-carbonyl)-2-methyl-phenyl ester (35b). The title compound was prepared by reaction of acetic acid 3-(5-bromo-thiophene-2-carbonyl)-2-methyl-phenyl ester **(III)** (600 mg, 1.77 mmol), 3-chloro-4-methoxy-phenylboronic acid (296 mg, 2.12 mmol), cesium carbonate (2305 mg, 7.08 mmol) and tetrakis(triphenylphosphine) palladium (5 µmol) according to method C1. The product was purified by CC (dichloromethan/methanol 99.5:0.5); yield: 37% (260 mg). The product was used in the next step without any characterization.

(5-(3-Chloro-4-methoxy-phenyl)-thiophen-2-yl)-(3-hydroxy-2-methyl-phenyl)-methanone (35a). Compound **(35b)** (200 mg, 0.50 mmol) in ethanol (5 mL) was refluxed in 10% NaOH (15 mL) for 2 h on a water bath. The reaction mixture was cooled, diluted with water and neutralized with acetic acid. The product was purified by CC (dichloromethan/methanol 99.75:0.25); yield: 78% (140 mg). The product was used in the next step without any characterization.

(5-(3-Chloro-4-hydroxy-phenyl)-thiophen-2-yl)-(3-hydroxy-2-methyl-phenyl)-methanone (35). The title compound was prepared by reaction of (5-(3-chloro-4-methoxy-phenyl)-thiophen-2-yl)-(3-hydroxy-2-methyl-phenyl)-methanone **(35a)** (140 mg, 0.39 mmol) and boron tribromide (1.95 mmol, 5.0 equiv) according to method B. The product was purified by CC (dichloromethan/methanol 99.75:0.25); yield: 60% (80 mg). ^{1}H NMR (500 MHz, acetone-d^6) δ 10.30 (s, 1H; OH), 9.26 (s, 1H, OH), 7.34 (d, *J* = 2.3 Hz, 2H), 7.12 (dd, *J* = 8.5, 2.3 Hz, 2H), 7.06 (d, *J* = 4.0 Hz, 2H), 6.90 (d, *J* = 4.0 Hz, 2H), 6.71 – 6.65 (m, 2H), 6.59 (d, *J* = 8.5 Hz, 2H), 6.53 (dd, *J* = 8.1, 0.9 Hz, 2H), 6.41 (dd, *J* = 7.5, 1.0 Hz, 2H), 1.61 (s, 6H); ^{13}C NMR (125 MHz, acetone-d^6) δ 179.68, 146.17, 144.64, 142.15, 132.09, 130.03, 127.45, 117.74, 116.59, 116.46, 115.23, 114.69, 111.95, 110.99, 108.52, 107.57, 106.84, 38.93); MS (ESI): 345.71 $(M+H)^+$.

(5-(3-Chloro-4-methoxy-phenyl)-thiophen-2-yl)-(2,6-difluoro-3-hydroxy-phenyl)-methanone (36). The title compound was prepared by reaction of (5-bromo-thiophen-2-yl)-(2,6-difluoro-3-hydroxy-phenyl)-methanone **(VIII)** (445 mg, 1.71 mmol), 3-chloro-4-methoxy-phenylboronic acid (382 mg, 2.05 mmol), cesium carbonate (2226 mg, 6.83 mmol) and tetrakis(triphenylphosphine) palladium (5 µmol) according to method C1. The product was purified by CC (petroleum ether/ethyl acetate 80:20); yield: 69% (450 mg). ^{1}H NMR (500 MHz, acetone-d^6) δ 8.99 (s, 1H, OH), 7.84 (d, *J* = 2.3 Hz, 1H), 7.73 (dd, *J* = 8.6, 2.3 Hz, 1H), 7.60 (dd, *J* = 3.0, 2.0 Hz, 1H), 7.55 (d, *J* = 4.1 Hz, 1H), 7.23 – 7.17 (m, 2H), 7.02 (ddd, *J* = 9.1, 8.6, 1.9 Hz, 1H),, 3.97 (s, 3H); ^{13}C NMR (125 MHz, acetone-d^6) δ 180.50, 157.07, 154.01, 152.57 (dd, *J* = 240.4, 5.8 Hz), 148.43 (dd, *J* = 245.7, 7.8 Hz), 142.81, 142.63 (dd, *J* = 13.0, 3.2 Hz), 138.32, 128.60, 127.29, 127.28, 125.60, 123.74, 120.27 (dd, *J* = 9.1, 3.9 Hz), 118.12 (dd, *J* = 24.0, 19.7 Hz), 113.93, 112.41 (dd, *J* = 22.8, 3.9 Hz); MS (ESI): 381.67 $(M+H)^+$.

(5-(3-Chloro-4-hydroxy-phenyl)-thiophen-2-yl)-(2,6-difluoro-3-hydroxy-phenyl)-methanone (37). The title compound was prepared by reaction of (5-(3-chloro-4-methoxy-phenyl)-thiophen-2-yl)-(2,6-difluoro-3-hydroxy-phenyl)-methanone **(36)** (340 mg, 0.89 mmol) and boron tribromide (4.46 mmol, 5.0 equiv) according to method B. The product was purified by CC (dichloromethan/methanol 99.5:0.5); yield: 74% (241 mg). ^{1}H NMR (500 MHz, acetone-d^6) δ 9.33 (br., s, 1H), 8.99 (br., s, 1H), 7.82 (t, *J* = 2.9 Hz, 1H), 7.62 (dd, *J* = 8.5, 2.3 Hz, 1H), 7.60-7.58 (m, 1H), 7.52 (d, *J* = 4.1 Hz, 1H), 7.19 (ddd, *J* = 9.6, 9.2, 5.4 Hz, 1H), 7.12 (d, *J* = 8.5 Hz, 1H), 7.06 – 6.96 (m, 1H). ^{13}C NMR (125 MHz, acetone-d^6) δ 180.47, 155.24, 154.39, 152.56 (dd, *J* = 240.4, 5.8 Hz), 148.43 (dd, *J* = 245.6, 7.8 Hz), 142.63 (dd, *J* = 13.1, 3.2 Hz),

142.55, 138.36, 128.67, 127.32, 126.79, 125.30, 122.15, 120.24 (dd, *J* = 9.1, 3.8 Hz), 118.33, 118.10 (dd, *J* = 23.8, 19.6 Hz), 112.39 (dd, *J* = 22.8, 3.9 Hz); MS (ESI): 367.47 $(M+H)^+$.

(5-(3-Chloro-4-methoxy-phenyl)-4-methyl-thiophen-2-yl)-(2,6-difluoro-3-hydroxy-phenyl)-methanone (38). The title compound was prepared by reaction of (5-bromo-4-methyl-thiophen-2-yl)-(2,6-difluoro-3-hydroxy-phenyl)-methanone **(IX)** (600 mg, 1.81 mmol), 3-chloro-4-methoxy-phenylboronic acid (507 mg, 2.72 mmol), cesium carbonate (2347 mg, 7.20 mmol) and tetrakis(triphenylphosphine) palladium (5 µmol) according to method C1. The product was purified by CC (DCM); yield: 80% (570 mg). ^{1}H NMR (500 MHz, acetone-d^6) δ 8.98 (s, 1H), 7.62 (d, *J* = 2.2 Hz, 1H), 7.54 (dd, *J* = 8.5, 2.3 Hz, 1H), 7.52-7.51 (m, 1H), 7.27 (d, *J* = 8.6 Hz, 1H), 7.21-7.19 (m, 1H), 7.02 (ddd, *J* = 9.1, 8.6, 1.9 Hz, 1H), 3.99 (s, 3H), 2.32-2.30 (m, 3H); ^{13}C NMR (125 MHz, acetone-d^6) δ 180.54, 156.53, 152.54 (dd, *J* = 240.3, 5.9 Hz), 148.39 (dd, *J* = 245.4, 7.9 Hz), 148.32, 142.60 (dd, *J* = 13.1, 3.0 Hz), 141.34, 140.62, 136.43, 130.96, 129.76, 127.35, 123.27, 120.14 (dd, *J* = 9.2, 3.8 Hz), 118.23 (dd, *J* = 24.1, 20.1 Hz), 113.71, 112.37 (dd, *J* = 22.8, 3.9 Hz), 56.75, 14.96; MS (ESI): 396.72 $(M+2H)^+$.

(5-(3-Chloro-4-hydroxy-phenyl)-4-methyl-thiophen-2-yl)-(2,6-difluoro-3-hydroxy-phenyl)-methanone (39). The title compound was prepared by reaction of (5-(3-chloro-4-methoxy-phenyl)-4-methyl-thiophen-2-yl)-(2,6-difluoro-3-hydroxy-phenyl)-methanone **(38)** (520 mg, 1.32 mmol) and boron tribromide (6.58 mmol, 5.0 equiv) according to method B. The product was purified by CC (dichloromethan/methanol 99.75:0.25); yield: 82% (413 mg). ^{1}H NMR (500 MHz, acetone-d^6) δ 9.25 (s, 1H, OH), 8.97 (d, *J* = 1.4 Hz, 1H), 7.58 (d, *J* = 2.3 Hz, 1H), 7.50 – 7.48 (m, 1H), 7.41 (dd, *J* = 8.4, 2.2 Hz, 1H), 7.21 – 7.17 (m, 1H), 7.15 (d, *J* = 8.4 Hz, 1H), 7.04 – 6.98 (m, 1H), 2.34 – 2.29 (m, 3H); ^{13}C NMR (125 MHz, acetone-d^6) δ 180.52, 154.61, 152.54 (dd, *J* = 240.2, 5.9 Hz), 148.69, 148.40 (dd, *J* = 245.5, 7.8 Hz), 142.60 (dd, *J* = 13.0, 3.2 Hz), 141.12, 140.65, 136.21, 130.99, 129.72, 126.78, 121.68, 120.12 (dd, *J* = 9.1, 3.8 Hz), 118.22 (dd, *J* = 32.7, 28.5 Hz), 118.05, 112.36 (dd, *J* = 22.8, 3.9 Hz), 15.00; MS (ESI): 381.74 $(M+H)^+$.

(5-(3-Chloro-phenyl)-thiophen-2-yl)-(2,6-difluoro-3-hydroxy-phenyl)-methanone (40). The title compound was prepared by reaction of (5-bromo-thiophen-2-yl)-(2,6-difluoro-3-hydroxy-phenyl)-methanone **(VIII)** (430 mg, 1.35 mmol), 3-chlorophenylboronic acid (253 mg, 1.62 mmol), cesium carbonate (1756 mg, 5.39 mmol) and tetrakis(triphenylphosphine) palladium (5 µmol) according to method C1. The product was purified by CC (hexane/ethyl acetate 90:10); yield: 74% (350 mg). ^{1}H NMR (500 MHz, acetone-d^6) δ 9.02 (s, 1H, OH), 7.84 (tt, *J* = 2.4, 1.2 Hz, 1H), 7.78- 7.76 (m, 1H), 7.69 (d, *J* = 4.1 Hz, 1H), 7.66 (dt, *J* = 4.1, 0.9 Hz, 1H), 7.52 (td, *J* = 7.9, 0.5 Hz, 1H), 7.47 (ddd, *J* = 8.0, 2.0, 1.1 Hz, 1H), 7.23-7.21 (m, 1H), 7.06-7.04 (m, 1H); ^{13}C NMR (125 MHz, acetone-d^6) δ 180.77, 153.31, 152.56 (dd, *J* = 240.6, 5.8 Hz), 148.44 (dd, *J* = 245.9, 7.7 Hz), 143.92, 142.68 (dd, *J* = 12.9, 3.2 Hz), 138.12, 135.80, 135.69, 131.92, 130.20, 126.96, 126.90, 125.81, 120.42 (dd, *J* = 9.1, 3.9 Hz), 117.97 (dd, *J* = 23.9, 19.7 Hz), 112.47 (d, *J* = 22.8 Hz); MS (ESI): 351.63 $(M+H)^+$.

(5-(3-Chloro-2-methoxy-phenyl)-thiophen-2-yl)-(2,6-difluoro-3-hydroxy-phenyl)-methanone (41a). The title compound was prepared by reaction of (5-bromo-thiophen-2-yl)-(2,6-difluoro-3-hydroxy-phenyl)-methanone **(VIII)** (300 mg, 0.94 mmol), 3-chloro-2-methoxy-phenylboronic acid (210 mg, 1.12 mmol), cesium carbonate (1225 mg, 3.76 mmol) and tetrakis(triphenylphosphine) palladium (5 µmol) according to method C1. The product was used in the next step without further purification and without any characterization; yield: 39% (140 mg).

(5-(3-Chloro-2-hydroxy-phenyl)-thiophen-2-yl)-(2,6-difluoro-3-hydroxy-phenyl)-methanone (41). The title compound was prepared by reaction of (5-(3-chloro-2-methoxy-phenyl)-thiophen-2-yl)-(2,6-difluoro-3-hydroxy-phenyl)-methanone **(41a)** (140 mg, 0.37 mmol) and boron tribromide (1.84 mmol, 5.0 equiv) according to method B. The product was purified by CC (dichloromethan/methanol 99.75:0.25); yield: 76% (102 mg). ^{1}H NMR (500 MHz, acetone-d^{6}) δ 9.32 (s, 1H; OH), 9.11 (d, *J* = 0.9 Hz, 1H, OH), 7.80 (dd, *J* = 7.9, 1.5 Hz, 1H), 7.77 (d, *J* = 4.2 Hz, 1H), 7.62 (d, *J* = 4.2 Hz, 1H), 7.45 (dd, *J* = 7.9, 1.5 Hz, 1H), 7.19 (td, *J* = 9.4, 5.4 Hz, 1H), 7.06 – 6.98 (m, 2H); ^{13}C NMR (125 MHz, acetone-d^{6}) δ 181.04, 152.52 (dd, *J* = 240.2, 5.9 Hz), 150.46, 150.17, 148.43 (dd, *J* = 245.6, 7.8 Hz), 143.71, 142.68 (dd, *J* = 12.9, 3.2 Hz), 136.63, 131.00, 128.18, 128.00, 123.22, 122.89, 122.13, 120.14 (dd, *J* = 9.1, 3.9 Hz), 118.45 (dd, *J* = 24.2, 19.9 Hz), 112.34 (dd, *J* = 22.8, 3.9 Hz); MS (ESI): 367.86 $(M+H)^{+}$.

(5-(3,5-Dichloro-4-methoxy-phenyl)-thiophen-2-yl)-(2,6-difluoro-3-hydroxy-phenyl)-methanone (42). The title compound was prepared by reaction of (5-bromo-thiophen-2-yl)-(2,6-difluoro-3-hydroxy-phenyl)-methanone **(VIII)** (300 mg, 0.94 mmol), 3,5-dichloro-4-methoxy-phenylboronic acid (249 mg, 1.13 mmol), cesium carbonate (1225 mg, 3.76 mmol) and tetrakis(triphenylphosphine) palladium (5 µmol) according to method C1. The product was purified by CC (DCM/petroleum ether 90:10) followed by washing with petroleum ether; yield: 90% (350 mg). ^{1}H NMR (500 MHz, acetone-d^{6}) δ 8.99 (s, 1H; OH), 7.83 (s, 2H), 7.67 (d, *J* = 4.1 Hz, 1H), 7.64 (d, *J* = 4.1 Hz, 1H), 7.21 (td, *J* = 9.4, 5.4 Hz, 1H), 7.03 (td, *J* = 8.9, 1.8 Hz, 1H), 3.93 (s, 3H); ^{13}C NMR (125 MHz, acetone-d^{6}) δ 180.74, 153.85, 152.57 (dd, *J* = 240.7, 5.7 Hz), 151.51, 148.44 (dd, *J* = 246.0, 7.7 Hz), 144.21, 142.66 (dd, *J* = 12.9, 3.2 Hz), 138.06, 131.62, 130.85, 127.68, 127.35, 120.48 (dd, *J* = 9.1, 3.9 Hz), 117.89 (dd, *J* = 23.8, 19.6 Hz), 112.47 (dd, *J* = 22.8, 3.9 Hz), 61.32; MS (ESI): 415.73 $(M+H)^{+}$.

(5-(3,5-Dichloro-4-hydroxy-phenyl)-thiophen-2-yl)-(2,6-difluoro-3-hydroxy-phenyl)-methanone (43). The title compound was prepared by reaction of (5-(3,5-dichloro-4-methoxy-phenyl)-thiophen-2-yl)-(2,6-difluoro-3-hydroxy-phenyl)-methanone **(42)** (350 mg, 0.84 mmol) and boron tribromide (4.12 mmol, 5.0 equiv) according to method B. The product was purified by CC (dichloromethan/methanol 99.75:0.25); yield: 84% (285 mg). ^{1}H NMR (500 MHz, acetone-d^{6}) δ 9.28 (s, 1H), 8.98 (s, 1H), 7.79 (s, 2H), 7.62 (dt, *J* = 4.1, 0.8 Hz, 1H), 7.60 (d, *J* = 4.1 Hz, 1H), 7.20 (ddd, *J* = 9.6, 9.2, 5.4 Hz, 1H), 7.02 (ddd, *J* = 9.1, 8.6, 1.9 Hz, 1H); ^{13}C NMR

(125 MHz, acetone-d^6) δ 180.58, 152.56 (dd, *J* = 240.6, 5.8 Hz), 152.50, 151.05, 148.43 (dd, *J* = 245.8, 7.8 Hz), 143.32, 142.64 (dd, *J* = 13.0, 3.2 Hz), 138.19, 127.32, 127.22, 126.30, 123.61, 120.36 (dd, *J* = 9.1, 3.8 Hz), 118.01 (dd, *J* = 23.9, 19.7 Hz), 112.43 (dd, *J* = 22.8, 3.9 Hz); MS (ESI): 402.59 $(M+H)^+$.

2-(3-Chloro-4-methoxy-5-methyl-phenyl)-4,4,5,5-tetramethyl-(1,3,2)dioxaborolane (44a). 5-bromo-1-chloro-2-methoxy-3-methylbenzene **(44b)** (5,00 g, 20,2 mmol, 1,00 equiv), bis(pinacolato)diboron (8,09 g, 31,8 mmol, 1,50 equiv), potassium acetate (5,95 g, 60,6 mmol, 3,00 equiv) and 1,1'-Bis(diphenylphosphino)ferrocene-palladium(II)dichloride (739 mg, 1,01 mmol, 0,05 equiv) were dissolved under N_2 in 40 ml dry DMSO and the mixture was stirred at 80 °C for 2h. The reaction was quenched with water, diluted with diethyl ether and filtered over celite. The phases were separated and the aqueous layer was extracted two times with diethyl ether. The combined organic layers were washed three times with water; one time with brine, dried over $MgSO_4$, filtered and concentrated under reduces pressure. The crude product was purified by CC (hexane/ethyl acetate 85:15); yield: 88% (4.71 g). ^{1}H NMR (500 MHz, acetone-d^6) δ 7.54-7.56 (m, 1H), 7.48-7.50 (m, 1H), 3.82 (s, 3H), 2.31 (t, *J*= 0.6 Hz, 3H), 1.33 (s, 12H).

(5-(3-Chloro-4-methoxy-5-methyl-phenyl)-thiophen-2-yl)-(2,6-difluoro-3-hydroxy-phenyl)-methanone (44). The title compound was prepared by reaction of 2-(3-chloro-4-methoxy-5-methyl-phenyl)-4,4,5,5-tetramethyl-(1,3,2)dioxaborolane **(44a)** (1000 mg, 3.53 mmol), (5-bromo-thiophen-2-yl)-(2,6-difluoro-3-hydroxy-phenyl)-methanone **(VIII)** (1000 mg, 3.13 mmol), cesium carbonate (4000 mg, 12.5 mmol) and tetrakis(triphenylphosphine) palladium (18 mg, 0.02 mmol) according to method C1. The product was purified by CC (hexane/ethyl acetate 8:2); yield: 82% (1000 mg). ^{1}H NMR (500 MHz, acetone-d^6) δ 8.98 (s, 1H), 7.72 (dd, *J*= 2.2, 0.6 Hz, 1H), 7.65-7.60 (m, 2H), 7.60 (d, *J*= 4.1 Hz, 1H), 7.20 (td, *J*= 9.4, 5.2 Hz, 1H), 7.03 (td, *J*= 8.8, 1.9 Hz, 1H), 3.86 (s, 3H), 2.38 (s, 3H); ^{13}C NMR (125 MHz, acetone-d^6) δ 180.7, 156.4, 153.6, 151.7, 149.5, 149.5, 147.5, 143.5, 142.8, 142.7, 138.3, 135.5, 130.7, 129.3, 128.8, 126.5, 120.4, 112.6, 112.4, 60.7, 16.5; MS (ESI): 395.28 $(M+H)^+$.

(5-(3-Chloro-4-hydroxy-5-methyl-phenyl)-thiophen-2-yl)-(2,6-difluoro-3-hydroxy-phenyl)-methanone (45). The title compound was prepared by reaction of (5-(3-chloro-4-methoxy-5-methyl-phenyl)-thiophen-2-yl)-(2,6-difluoro-3-hydroxy-phenyl)-methanone **(44)** (104 mg, 0.26 mmol) and boron tribromide (1.05 mmol, 4.0 equiv) according to method B. The product was purified by CC (hexane/ethyl acetate 7:3); yield: 82% (84 mg). ^{1}H NMR (500 MHz, acetone-d^6) δ 8.96 (d, *J*=1.3 Hz, 1H), 8.50 (s, 1H), 7.68-7.66 (m, 1H), 7.60-7.55 (m, 2H), 7.53 (d, *J*= 4.1 Hz, 1H), 7.19 (td, *J*= 9.4, 5.5 Hz, 1H), 7.02 (td, *J*= 8.8, 1.9 Hz, 1H), 2.34 (s, 3H); ^{13}C NMR (125 MHz, acetone-d^6) δ 180.5, 154.6, 153.6, 153.2, 151.7, 149.5, 147.5, 142.7, 138.4, 128.7, 128.6, 126.5, 125.8, 125.3, 121.8, 120.3, 118.4, 118.2, 112.5, 112.4, 16.8; MS (ESI): 381.06 $(M+H)^+$.

(2,6-Difluoro-3-hydroxy-phenyl)-(5-(4-methoxy-3,5-dimethyl-phenyl)-thiophen-2-yl)-methanone (46). The title compound was prepared by reaction of (5-bromo-thiophen-2-yl)-(2,6-difluoro-3-hydroxy-phenyl)-methanone **(VIII)** (500 mg, 1.57 mmol), 4-methoxy-3,5-dimethyl phenylboronic acid (338 mg, 1.88 mmol), cesium carbonate (2042 mg, 6.27 mmol) and tetrakis(triphenylphosphine) palladium (5 µmol) according to method C1. The product was purified by CC (hexane/ethyl acetate 90:20); yield: 78% (460 mg). ^{1}H NMR (500 MHz, acetone-d^6) δ 8.83 (s, 1H, OH), 7.46 (d, *J* = 4.1 Hz, 1H), 7.37 (d, *J* = 4.1 Hz, 3H), 7.06 (td, *J* = 9.4, 5.4 Hz, 1H), 6.89 (td, *J* = 8.8, 1.8 Hz, 1H), 3.62 (s, 3H), 2.18 (s, 6H); ^{13}C NMR (75 MHz, Acetone) δ 179.53, 158.58, 154.86, 150.08 (dd, *J* = 240.5, 5.9 Hz), 145.81 (dd, *J* = 245.2, 7.9 Hz), 141.72 (dd, *J* = 10.9, 5.4 Hz), 141.65, 137.32, 131.90, 128.40, 126.85, 124.37, 119.27 (dd, *J* = 9.0, 3.8 Hz), 117.15 (dd, *J* = 30.8, 11.1 Hz), 111.47 (dd, *J* = 22.9, 3.8 Hz), 59.09, 15.27; MS (ESI): 375.69 $(M+H)^+$.

(2,6-Difluoro-3-hydroxy-phenyl)-(5-(4-hydroxy-3,5-dimethyl-phenyl)-thiophen-2-yl)-methanone (47). The title compound was prepared by reaction of (2,6-difluoro-3-hydroxy-phenyl)-(5-(4-methoxy-3,5-dimethyl-phenyl)-thiophen-2-yl)-methanone **(46)** (400 mg, 1.07 mmol) and boron tribromide (5.34 mmol, 5.0 equiv) according to method B. The product was purified by CC (dichloromethan/methanol 99.5:0.5); yield: 69% (266 mg). ^{1}H NMR (500 MHz, acetone-d^6) δ 8.82 (br. s, 1H), 7.65 (br. s, 1H), 7.41 (d, *J* = 4.1 Hz, 1H), 7.30-7.28 (m, 3H), 7.06-7.05 (m, 1H), 6.87 (td, *J* = 8.8, 1.8 Hz, 1H), 2.16 (s, 6H); ^{13}C NMR (125 MHz, acetone-d^6) δ 179.30, 155.99, 155.18, 151.66 (dd, *J* = 240.2, 5.9 Hz), 147.52 (dd, *J* = 245.3, 7.9 Hz), 141.69 (dd, *J* = 13.1, 3.3 Hz), 140.66, 137.47, 126.63, 124.95, 124.42, 123.22, 119.14 (dd, *J* = 9.1, 3.8 Hz), 117.57 – 117.07 (m), 111.42 (dd, *J* = 22.9, 3.9 Hz), 15.66; MS (ESI): 361.91 $(M+H)^+$.

(2,6-Difluoro-3-hydroxy-phenyl)-(5-(4-difluoromethoxy-3,5-difluoro-phenyl)-thiophen-2-yl)-methanone (48). The title compound was prepared by reaction of (5-bromo-thiophen-2-yl)-(2,6-difluoro-3-hydroxy-phenyl)-methanone **(VIII)** (460 mg, 1.44 mmol), 4-difluoromethoxy-3,5-diflourophenylboronic acid (387 mg, 1.73 mmol), cesium carbonate (1877 mg, 5.77 mmol) and tetrakis(triphenylphosphine) palladium (5 µmol) according to method C1. The product was purified by CC (DCM); yield: 84% (505 mg). ^{1}H NMR (500 MHz, acetone-d^6) δ 9.01 (s, 1H, OH), 7.75 (d, *J* = 4.1 Hz, 1H), 7.72 – 7.65 (m, 1H), 7.24 – 7.19 (m, 1H), 7.10 (t, *J* = 72.8 Hz, 1H), 7.07 – 7.02 (m, 1H); ^{13}C NMR (125 MHz, acetone-d^6) δ 180.88, 157.04 (dd, *J* = 251.3, 4.6 Hz), 152.56 (dd, *J* = 240.8, 5.7 Hz), 151.23 (t, *J* = 2.8 Hz), 148.43 (dd, *J* = 246.1, 7.6 Hz), 144.71, 142.68 (dd, *J* = 12.9, 3.2 Hz), 138.02, 133.47 (t, *J* = 9.6 Hz), 128.01, 120.54 (dd, *J* = 9.1, 3.9 Hz), 117.79 (dd, *J* = 23.6, 19.5 Hz), 117.36 (t, *J* = 264.6 Hz), 112.50 (dd, *J* = 22.7, 3.9 Hz), 111.64 (d, *J* = 5.4 Hz), 111.49 (d, *J* = 5.4 Hz); MS (ESI): 419.72 $(M+H)^+$.

5-(5-(2,6-Difluoro-3-hydroxy-benzoyl)-thiophen-2-yl)-2-isopropoxy-benzonitrile (49). The title compound was prepared by reaction of (5-bromo-thiophen-2-yl)-(2,6-difluoro-3-hydroxy-phenyl)-methanone **(VIII)** (460 mg, 1.44 mmol), 3-cyano-4-isopropoxyphenylboronic acid (387

mg, 1.73 mmol), cesium carbonate (1877 mg, 5.77 mmol) and tetrakis(triphenylphosphine) palladium (5 µmol) according to method C1. The product was purified by CC (dichloromethan/methanol 99.5:0.5); yield: 52% (300 mg). ^{1}H NMR (500 MHz, acetone-d^6) δ 8.99 (s, 1H, OH), 8.13 (d, *J* = 2.5 Hz, 1H), 8.03 (dd, *J* = 8.9, 2.4 Hz, 1H), 7.65 – 7.62 (m, 2H), 7.36 (d, *J* = 9.1 Hz, 1H), 7.24 – 7.17 (m, 1H), 7.03 (td, *J* = 8.9, 1.9 Hz, 1H), 4.92 (sep, *J* = 6.0 Hz, 1H), 1.41 (d, *J* = 6.0 Hz, 6H); ^{13}C NMR (125 MHz, acetone-d^6) δ 180.58, 161.32, 153.11, 152.56 (dd, *J* = 240.7, 5.7 Hz), 148.42 (dd, *J* = 245.8, 7.9 Hz), 143.18, 142.64 (dd, *J* = 13.0, 3.1 Hz), 138.32, 133.34, 132.42, 126.74, 126.07, 120.33 (dd, *J* = 9.0, 3.9 Hz), 118.04 (dd, *J* = 24.0, 19.8 Hz), 116.29, 115.60, 112.43 (dd, *J* = 22.8, 3.8 Hz), 104.38, 73.09, 22.03; MS (ESI): 400.75 $(M+H)^+$.

5-(5-(2,6-Difluoro-3-hydroxy-benzoyl)-thiophen-2-yl)-2-hydroxy-benzonitrile (50). The title compound was prepared by reaction of 5-(5-(2,6-difluoro-3-hydroxy-benzoyl)-thiophen-2-yl)-2-isopropoxy-benzonitrile **(49)** (220 mg, 0.55 mmol) and boron tribromide (2.75 mmol, 5.0 equiv) according to method B. The product was purified by CC (dichloromethan/methanol 99.25:0.75); yield: 59% (115 mg). ^{1}H NMR (500 MHz, acetone-d^6) δ 9.67 (br. s, 1H), 8.12 – 8.07 (m, 1H), 7.93 (dd, *J* = 8.7, 2.4 Hz, 1H), 7.62 (d, *J* = 4.1 Hz, 1H), 7.60 (d, *J* = 4.1 Hz, 1H), 7.24 – 7.17 (m, 2H), 7.03 (td, *J* = 8.9, 1.9 Hz, 1H); ^{13}C NMR (125 MHz, acetone-d^6) δ 180.56, 161.47, 153.44, 152.56 (dd, *J* = 240.4, 5.8 Hz), 148.42 (dd, *J* = 245.7, 7.8 Hz), 142.98, 142.63 (dd, *J* = 13.0, 3.2 Hz), 138.33, 133.37, 132.07, 126.34, 125.82, 120.31 (dd, *J* = 9.1, 3.9 Hz), 118.08, 118.07 (d, *J* = 43.7 Hz), 116.41, 112.42 (dd, *J* = 22.8, 3.9 Hz), 101.91; MS (ESI): 358.81 $(M+H)^+$.

(2,6-Difluoro-3-hydroxy-phenyl)-(5-(4-methoxy-3-(morpholine-4-sulfonyl)-phenyl)-thiophen-2-yl)-methanone (51). The title compound was prepared by reaction of (5-bromo-thiophen-2-yl)-(2,6-difluoro-3-hydroxy-phenyl)-methanone **(VIII)** (460 mg, 1.44 mmol), 4-methoxy-3-(morpholine-4-sulfonyl)-phenylboronic acid (521 mg, 1.73 mmol), cesium carbonate (1877 mg, 5.77 mmol) and tetrakis(triphenylphosphine) palladium (5 µmol) according to method C1. The product was purified by CC (dichloromethan/methanol 99.5:0.5); yield: 56% (400 mg). ^{1}H NMR (500 MHz, acetone-d^6) δ 9.01 (br. s, 1H), 8.18 (d, *J* = 2.4 Hz, 1H), 8.05 (dd, *J* = 8.7, 2.5 Hz, 1H), 7.64 (dt, *J* = 4.1, 0.9 Hz, 1H), 7.60 (d, *J* = 4.1 Hz, 1H), 7.39 (d, *J* = 8.7 Hz, 1H), 7.22-7.20 (m, 1H), 7.05-7.03 (m, 1H), 4.06 (s, 3H), 3.67 – 3.64 (m, 4H), 3.25 – 3.22 (m, 4H); ^{13}C NMR (125 MHz, acetone-d^6) δ 180.52, 158.93, 153.70, 152.55 (dd, *J* = 240.5, 5.8 Hz), 148.42 (dd, *J* = 245.7, 7.7 Hz), 143.06, 142.65 (dd, *J* = 12.9, 3.2 Hz), 138.33, 133.37, 129.86, 128.17, 126.22, 125.93, 120.33 (dd, *J* = 9.1, 3.9 Hz), 118.07 (dd, *J* = 24.0, 19.6 Hz), 114.83, 112.43 (dd, *J* = 22.8, 3.9 Hz), 67.27, 56.86, 47.01; MS (ESI): 496.74 $(M+H)^+$.

(3-Ethoxy-2,6-difluoro-phenyl)-(5-pyridin-4-yl-thiophen-2-yl)-methanone (52a). The title compound was prepared by reaction of 5-bromo-thiophen-2-yl)-(3-ethoxy-2,6-difluoro-phenyl)-methanone **(V)** (300 mg, 0.86 mmol), pyridine-4-boronic acid pinacol ester (355 mg, 1.73 mmol), sodium carbonate (2.5 mL, 2 M) and tetrakis(triphenylphosphine) palladium (5 µmol)

according to method C2. The product was purified by CC (dichloromethan/methanol 99.5:0.5); yield: 91% (450 mg). The product was used in the next step without any characterization.

(2,6-Difluoro-3-hydroxy-phenyl)-(5-pyridin-4-yl-thiophen-2-yl)-methanone (52). The title compound was prepared by reaction of (3-Ethoxy-2,6-difluoro-phenyl)-(5-pyridin-4-yl-thiophen-2-yl)-methanone **(52a)** (450 mg, 1.30 mmol) and boron tribromide (7.8 mmol, 6.0 equiv) according to method B. The product was purified by CC (dichloromethan/methanol 9:1); yield: 24% (100 mg). ^{1}H NMR (500 MHz, DMSO-*d*^{6}) δ 10.40 (br. s, 1H), 8.64 (d, *J* = 6.1 Hz, 2H), 7.91 (d, *J* = 4.1 Hz, 1H), 7.77 (dd, *J* = 4.6, 1.5 Hz, 2H), 7.69 (d, *J* = 4.0 Hz, 1H), 7.16 (td, *J* = 9.4, 5.6 Hz, 1H), 7.08 (t, *J* = 8.6 Hz, 1H); ^{13}C NMR (125 MHz, DMSO) δ 180.32, 150.66, 150.49, 150.46 (dd, *J* = 239.7, 5.6 Hz), 146.88 (dd, *J* = 246.7, 7.5 Hz), 143.06, 141.92 (dd, *J* = 11.8, 2.9 Hz), 139.04, 137.82, 128.13, 120.19, 119.81 (dd, *J* = 8.9, 3.9 Hz), 116.22 (dd, *J* = 23.4, 19.0 Hz), 111.75 (dd, *J* = 22.3, 3.5 Hz); MS (ESI): 318.94 (M+H)$^{+}$.

(3-Ethoxy-2,6-difluoro-phenyl)-(5-pyridin-3-yl-thiophen-2-yl)-methanone (53a). The title compound was prepared by reaction of 5-bromo-thiophen-2-yl)-(3-ethoxy-2,6-difluoro-phenyl)-methanone **(V)** (400 mg, 1.15 mmol), pyridine-3-boronic acid (170 mg, 1.38 mmol), sodium carbonate (2.5 mL, 2 M) and tetrakis(triphenylphosphine) palladium (5 µmol) according to method C2. The product was purified by CC (dichloromethan/methanol 99.5:0.5); yield: 50% (200 mg). ^{1}H NMR (300 MHz, acetone-*d*^{6}) δ 8.90 (d, *J* = 2.2 Hz, 1H), 8.50 (dd, *J* = 4.8, 1.4 Hz, 1H), 8.04 (ddd, *J* = 8.0, 2.3, 1.7 Hz, 1H), 7.59 (d, *J* = 4.1 Hz, 1H), 7.55 (dd, *J* = 4.0, 0.7 Hz, 1H), 7.37 (ddd, *J* = 8.0, 4.8, 0.6 Hz, 1H), 7.22 (td, *J* = 9.3, 5.3 Hz, 1H), 6.99 (td, *J* = 8.9, 2.0 Hz, 1H), 4.06 (q, *J* = 7.0 Hz, 2H), 1.28 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, acetone-*d*^{6}) δ 179.71, 152.23 (dd, *J* = 241.8, 5.9 Hz), 150.81, 148.70 (dd, *J* = 241.8, 5.9 Hz), 147.16, 143.95 (dd, *J* = 10.6, 3.3 Hz), 143.20, 137.35, 133.48, 128.92, 126.24, 124.03, 117.40 (dd, *J* = 22.9, 3.2 Hz), 116.88 (dd, *J* = 9.3, 3.1 Hz), 111.22 (dd, *J* = 22.6, 4.2 Hz), 65.44, 14.10; MS (ESI): 347.05 (M+H)$^{+}$.

(2,6-Difluoro-3-hydroxy-phenyl)-(5-pyridin-3-yl-thiophen-2-yl)-methanone (53). The title compound was prepared by reaction of (3-ethoxy-2,6-difluoro-phenyl)-(5-pyridin-3-yl-thiophen-2-yl)-methanone **(53a)** (200 mg, 0.58 mmol) and boron tribromide (3.47 mmol, 6.0 equiv) according to method B. The product was purified by CC (dichloromethan/methanol 9:1); yield: 17% (31 mg). ^{1}H NMR (500 MHz, DMSO-*d*^{6}) δ 10.28 (s, 1H, OH), 9.06 (s, 1H), 8.64 (d, *J* = 4.2 Hz, 1H), 8.23 (ddd, *J* = 8.0, 2.4, 1.5 Hz, 1H), 7.81 (d, *J* = 4.1 Hz, 1H), 7.71 (d, *J* = 4.1 Hz, 1H), 7.53 (dd, *J* = 8.0, 4.8 Hz, 1H), 7.16 (td, *J* = 9.4, 5.7 Hz, 1H), 7.10 (td, *J* = 8.9, 1.4 Hz, 1H); ^{13}C NMR (125 MHz, DMSO) δ 180.06, 150.52 (dd, *J* = 239.6, 5.7 Hz), 150.43, 150.25, 146.90 (dd, *J* = 246.5, 7.6 Hz), 146.86, 142.30, 141.88 (dd, *J* = 11.9, 3.0 Hz), 137.98, 133.83, 128.41, 126.98, 124.25, 119.60 (dd, *J* = 9.1, 4.0 Hz), 116.43 (dd, *J* = 23.5, 19.4 Hz), 111.71 (dd, *J* = 22.4, 3.6 Hz); MS (ESI): 318.92 (M+H)$^{+}$.

(5-(3,5-Dichloro-4-methoxy-phenyl)-thiophen-2-yl)-(3-hydroxy-phenyl)-methanone (54). The title compound was prepared by reaction of (5-bromothiophen-2-yl)(3-hydroxyphenyl)methanone **(VII)** (1000 mg, 3.53 mmol), 3,5-dichloro-4-methoxyphenylboronic acid (936 mg, 4.23 mmol), cesium carbonate (4603 mg, 14.13 mmol) and tetrakis(triphenylphosphine) palladium (5 µmol) according to method C1. The product was purified by CC (petroleum ether/ethyl acetate 80:20) followed by washing with petroleum ether; yield: 67% (900 mg). ^{1}H NMR (500 MHz, acetone-d^6) δ 8.77 (s, 1H), 7.83 (s, 2H), 7.72 (d, *J* = 4.0 Hz, 1H), 7.68 (d, *J* = 4.0 Hz, 1H), 7.43 – 7.39 (m, 1H), 7.37 (dt, *J* = 7.6, 1.4 Hz, 1H), 7.34 (ddd, *J* = 2.5, 1.5, 0.4 Hz, 1H), 7.14 (ddd, *J* = 7.8, 2.6, 1.4 Hz, 1H), 3.94 (s, 3H); ^{13}C NMR (125 MHz, acetone-d^6) δ 187.64, 158.45, 153.53, 149.33, 144.32, 140.01, 136.70, 131.99, 130.78, 130.65, 127.46, 126.78, 121.16, 120.43, 116.32, 61.30; MS (ESI): 380.84 $(M+H)^+$.

(5-(3,5-Dichloro-4-hydroxy-phenyl)-thiophen-2-yl)-(3-hydroxy-phenyl)-methanone (55). The title compound was prepared by reaction of ((5-(3,5-dichloro-4-methoxy-phenyl)-thiophen-2-yl)-(3-hydroxy-phenyl)-methanone **(54)** (720 mg, 1.90 mmol) and boron tribromide (9.49 mmol, 5.0 equiv) according to method B. The product was purified by CC (dichloromethan/methanol 90.5:0.5); yield: 20% (140 mg). ^{1}H NMR (500 MHz, acetone-d^6) δ 8.00 (s, 2H), 7.77 (s, 94H), 7.69 (d, *J* = 4.0 Hz, 51H), 7.59 (d, *J* = 4.0 Hz, 50H), 7.41 – 7.37 (m, 54H), 7.35 (dt, *J* = 7.6, 1.3 Hz, 53H), 7.33 – 7.31 (m, 49H), 7.12 (ddd, *J* = 7.9, 2.6, 1.3 Hz, 50H).^{13}C NMR (125 MHz, acetone-d^6) δ 187.70, 158.38, 150.71, 150.33, 143.37, 140.14, 136.86, 130.60, 127.53, 127.07, 125.69, 123.54, 121.06, 120.24, 116.19; MS (ESI): 366.61 $(M+H)^+$.

2-(3-Bromomethyl-5-chloro-4-methoxy-phenyl)-4,4,5,5-tetramethyl-(1,3,2)dioxaborolane (56b). 2-(3-chloro-4-methoxy-5-methylphenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane **(44a)** (200 mg, 0,71 mmol, 1,00 equiv) and *N*-bromosuccinimide (113 mg, 0,63 mmol, 0,90 equiv) were dissolved under N_2 in 17 ml CCl_4, followed by a catalytic amount of dibenzoyl peroxide. The mixture was stirred under reflux for 1h. The reaction was quenched with water and extracted three times with dichloromethane. The combined organic layers were washed two times with water, one time with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by CC (hexane/ethyl acetate 85:15); yield: 70% (180 mg). ^{1}H NMR (500 MHz, acetone-d^6) δ 7.75 (d, *J*= 1.5 Hz, 1H), 7.68 (d, *J*= 1.5 Hz, 1H), 4.71 (s, 2H), 4.00 (s, 3H), 1.33-1.35 (m, 12H).

1-(4-(3-Chloro-2-methoxy-5-(4,4,5,5-tetramethyl-(1,3,2)dioxaborolan-2-yl)-benzyl)-piperazin-1-yl)-ethanone (56a). 2-(3-(bromomethyl)-5-chloro-4-methoxyphenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane **(56b)** (180 mg, 0,50 mmo, 1,00 equiv) was dissolved under N_2 in 1 ml dry THF and 1-acetylpiperazine was added. The mixture was stirred for 1 h under reflux. The reaction was quenched with water and extracted three times with ethyl acetate. The combined organic layers were washed two times with water, one time with brine, dried over

$MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by CC (ethyl acetate /ethanol 8:2), to give the desired product as yellow solid; yield: 70% (198 mg). ^{1}H NMR (500 MHz, acetone-d^6) δ 7.69 (d, *J*=1.5 Hz, 1H), 7.65 (d, *J*=1.5 Hz, 1H), 3.90 (s, 3H), 3.57 (s, 3H), 2.77 (t, *J*= 5.2 Hz, 2H), 2.70 (t, *J*= 5.2 Hz, 2H), 2.47 (t, *J*= 5.2 Hz, 2H), 2.42 (t, *J*= 5.2 Hz, 2H), 2.00 (s, 3H), 1.34 (s, 12H).

1-(4-(3-Chloro-5-(5-(2,6-difluoro-3-hydroxy-benzoyl)-thiophen-2-yl)-2-methoxy-benzyl)-piperazin-1-yl)-ethanone (56). The title compound was prepared by reaction of 1-(4-(3-chloro-2-methoxy-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)piperazin-1-yl)ethanone **(56a)** (100 mg, 0,24 mmol, 1,00 equiv), (5-bromothiophen-2-yl)(2,6-difluoro-3-hydroxyphenyl)-methanone **(VIII)** (77 mg, 0,24 mmol, 1,00 equiv), cesium carbonate (156 mg, 0.48 mmol) and tetrakis(triphenylphosphine) palladium (1 mg, 0,001 mmol, 0,005 equiv) according to method C1. The product was purified by CC (ethyl acetate/ethanol 8:2); yield: 98% (55 mg). ^{1}H NMR (500 MHz, DMSO-d^6) δ 9.53 (br. s., 1H), 8.28-8.26 (m, 2H), 8.10-8.03 (m, 2H), 7.66 (td, *J*= 9.4, 5.3Hz, 1H), 7.48 (td, *J*= 8.9, 2.0 Hz, 1H), 4.38 (s, 3H), 4.10 (s, 2H), 4.03-3.94 (m, 4H), 2.99 (t, *J*= 5.0 Hz, 2H), 2.93 (t, *J*= 5.0 Hz, 2H), 2.46 (s, 3H).^{13}C NMR (125 MHz, DMSO-d^6) δ 180.7, 169.0, 156.7, 153.5, 151.6, 149.4, 147.4, 143.5, 142.7, 138.2, 135.8, 130.6, 129.6, 128.3, 127.7, 126.5, 120.4, 118.0, 112.5, 112.3, 61.8, 57.1, 54.0, 53.7, 46.9, 42.0, 21.3; MS (ESI): 521.41 $(M+H)^+$.

1-(4-(3-Chloro-5-(5-(2,6-difluoro-3-hydroxy-benzoyl)-thiophen-2-yl)-2-hydroxy-benzyl)-piperazin-1-yl)-ethanone (57). The title compound was prepared by reaction of 1-(4-(3-Chloro-5-(5-(2,6-difluoro-3-hydroxy-benzoyl)-thiophen-2-yl)-2-methoxy-benzyl)-piperazin-1-yl)-ethanone **(56)** (150 mg, 0.29 mmol) and boron tribromide (0.87 mmol, 3.0 equiv) according to method B. The product was purified by using preparative TLC (dichloromethan/methanol 95:5); yield: 12% (16 mg). ^{1}H NMR (500 MHz, acetone-d^6) δ 7.86-7.80 (m, 1H), 7.75 (d, *J*= 2.2 Hz, 1H), 7.65-7.56 (m, 1H), 7.54-7.49 (m, 1H), 7.24-7.18 (m, 1H), 7.06-6.99 (m, 1H), 3.70-3.60 (m, 4H), 2.71-2.52 (m, 4H), 2.05 (s, 3H); ^{13}C NMR (125 MHz, acetone-d^6) δ 180.7, 169.9, 156.0, 154.6, 153.5, 153.5, 151.6, 149.5, 147.5, 142.8, 142.5, 138.4, 127.7, 126.7, 125.8, 125.3, 124.9, 122.1, 120.2, 118.2, 112.5, 112.4, 61.1, 53.5, 52.9, 46.6, 41.8, 21.3 ; MS (ESI): 506.89 $(M+H)^+$.

(5-(3-Chloro-4-methoxy-5-piperazin-1-ylmethyl-phenyl)-thiophen-2-yl)-(2,6-difluoro-3-hydroxy-phenyl)-methanone (58). 1-(4-(3-chloro-5-(5-(2,6-difluoro-3-hydroxybenzoyl)-thiophen-2-yl)-2-methoxybenzyl)piperazin-1-yl)ethanone **(56)** (150 mg, 0.29 mmol, 1.00 equiv) was dissolved in 20 ml 3M aqueous HCl and heated to 80 °C for 3 h. The reaction was washed two times with ethyl acetate, the aqueous layer was basified to pH 10 with 2 M NaOH and washed two times with ethyl acetate. The aqueous layer was neutralized with 2M HCl and extracted three times with ethyl acetate. The combined organic layers were washed one time with water, one time with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure to give the desired pure product in 40% yield as pale yellow solid. ^{1}H NMR (500 MHz, acetone-

d^6) δ 7.83-7.79 (m, 2H), 7.67-7.60 (m, 2H), 7.19 (m, 1H), 7.00 (td, *J*= 9.0, 1.9 Hz, 1H), 3.92 (s, 3H), 3.59 (s, 2H), 2.89-2.81 (m, 4H), 2.53-2.45 (m, 4H); ^{13}C NMR (125 MHz, acetone-d^6) δ 180.8, 156.8, 153.3, 151.5, 149.4, 147.5, 143.6, 142.9, 142.7, 138.2, 135.4, 130.7, 129.6, 128.4, 127.9, 126.7, 120.5, 118.1, 112.3, 112.2, 62.0, 56.9, 50.4, 44.3; MS (ESI): 479.22 $(M+H)^+$.

2-(3-Azidomethyl-5-chloro-4-methoxy-phenyl)-4,4,5,5-tetramethyl-(1,3,2)dioxaborolane (59c). 2-(3-(bromomethyl)-5-chloro-4-methoxyphenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane **(56b)** (800 mg, 2.19 mmol, 1.00 equiv) was dissolved under N_2 in 8 ml dry DMF and sodium azide (143 mg, 2.19 mmol, 1.00 equiv) was added. The mixture was stirred at room temperature overnight. The mixture was quenched with water and extracted three times with ethyl acetate. The combined organic layers were washed two times with water, one time with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by CC (hexane /ethyl acetate 8:2); yield: 71% (503 mg). ^{1}H NMR (500 MHz, acetone-d^6) δ 7.72 (d, *J*= 1.6 Hz, 1H), 7.69 (d, *J*= 1.6 Hz, 1H), 4.55 (s, 2H), 3.93 (s, 3H), 1.34 (s, 12H)

(5-(3-Azidomethyl-5-chloro-4-methoxy-phenyl)-thiophen-2-yl)-(2,6-difluoro-3-hydroxy-phenyl)-methanone (59b). The title compound was prepared by reaction of 2-(3-Azidomethyl-5-chloro-4-methoxy-phenyl)-4,4,5,5-tetramethyl-(1,3,2)dioxaborolane **(59c)** (683 mg, 2.16 mmol, 1.00 equiv), (5-bromothiophen-2-yl)(2,6-difluoro-3-hydroxyphenyl)methanone **(VIII)** (700 mg, 2.16 mmol, 1.00 equiv), cesium carbonate (2000 mg, 6.44 mmol, 3 equiv) and tetrakis(triphenylphosphine) palladium (13 mg, 0.01 mmol, 0.005 equiv) according to method C1. The product was used in the next step without further purification and without any characterization; yield: 84% (785 mg).

Acetic acid 3-(5-(3-chloro-4-methoxy-5-(1,2,3)triazol-1-ylmethyl-phenyl)-thiophene-2-carbonyl)-2,4-difluoro-phenylester(59a). (5-(3-(azidomethyl)-5-chloro-4-methoxyphenyl)-thiophen-2-yl)(2,6-difluoro-3-hydroxyphenyl)methanone **(59b)** (50 mg, 0.11 mmol, 1.00 equiv), was dissolved in 106 µl vinyl acetate and the reaction was heated at 120°C under microwave for 10 h. The reaction was concentrated under reduced pressure and purified by CC using ethyl acetate as eluent; yield: 27% (15 mg). ^{1}H NMR (500 MHz, DMSO-d^6) δ 8.11 (d, *J*= 0.9 Hz, 1H), 7.92 (d, *J*= 2.5 Hz, 1H), 7.70 (d, *J*= 0.9 Hz, 1H), 7.68-7.66 (m, 1H), 7.64-7.61 (m, 2H), 7.53 (td, *J*= 8.9, 5.5 Hz, 1H), 7.26 (td, *J*= 8.8, 1.9 Hz, 1H), 5.78 (s, 2H), 3.89 (s, 3H), 2.34 (s, 3H); MS (ESI): 504.12 $(M+H)^+$.

(5-(3-Chloro-4-methoxy-5-(1,2,3)triazol-1-ylmethyl-phenyl)-thiophen-2-yl)-(2,6-difluoro-3-hydroxy-phenyl)-methanone (59). Acetic acid 3-(5-(3-chloro-4-methoxy-5-(1,2,3)triazol-1-ylmethyl-phenyl)-thiophene-2-carbonyl)-2,4-difluoro-phenylester **(59a)** (110 mg, 0.24 mmol, 1.00 equiv) was dissolved under N_2 in a degased mixture of 5 ml THF and 600 µl of 2M NaOH. The mixture was stirred for 2 h at room temperature. The reaction was acidified to pH 6 with 1 M HCl and extracted three times with ethyl acetate. The combined organic layers were washed

two times with water, one time with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure to give the desired product; yield: 95% (90 mg). ^{1}H NMR (500 MHz, acetone-d^6) δ 9.05 (br. s, 1H), 8.11 (d, *J*= 0.9 Hz, 1H), 7.91 (d, *J*= 2.2 Hz, 1H), 7.70 (d, *J*= 0.9 Hz, 1H), 7.67 (d, *J*= 2.2 Hz, 1H), 7.65-7.62 (m, 1H), 7.61-7.58 (m, 1H), 7.21 (td, *J*= 8.9, 5.5Hz, 1H), 7.03 (td, *J*= 8.8, 1.9 Hz, 1H), 5.78 (s, 2H), 3.89 (s, 3H); MS (ESI): 462.15 $(M+H)^+$.

(5-(3-Chloro-4-hydroxy-5-(1,2,3)triazol-1-ylmethyl-phenyl)-thiophen-2-yl)-(2,6-difluoro-3-hydroxy-phenyl)-methanone (60). The title compound was prepared by reaction of (5-(3-Chloro-4-methoxy-5-(1,2,3)triazol-1-ylmethyl-phenyl)-thiophen-2-yl)-(2,6-difluoro-3-hydroxy-phenyl)-methanone **(59)** (80 mg, 0.16 mmol) and boron tribromide (800 µl, 0.80 mmol, 5.0 equiv) according to method B. The product was purified by CC (ethyl acetate/ethanol 9:1); yield: 32% (23 mg). ^{1}H NMR (500 MHz, acetone-d^6) δ 8.74 (d, *J*= 1.3 Hz, 1H), 8.43 (s, 1H), 8.03-7.90 (m, 2H), 7.68-7.54 (m, 2H), 7.30 (td, *J*= 8.8, 5.5 Hz, 1H), 7.01 (td, *J*= 8.8, 1.9 Hz, 1H), 6.05 (s, 2H); ^{13}C NMR (125 MHz, acetone-d^6) δ 180.7, 153.3, 143.2, 138.3, 130.7, 129.3, 129.2, 128.8, 127.3, 126.1, 124.6, 123.0, 120.3, 112.4, 52.0; MS (ESI): 448.12 $(M+H)^+$.

(5-(3-Aminophenyl)thiophen-2-yl)(3-ethoxy-2,6-difluorophenyl)methanone (61a). The title compound was prepared by reaction of (5-bromothiophen-2-yl)(3-ethoxy-2,6-difluorophenyl)methanone **(V)** (450 mg, 1.30 mmol) and 3-aminophenylboronic acid (213 mg, 1.55 mmol), cesium carbonate (1689 mg, 5.18 mmol) and tetrakis(triphenylphosphine) palladium (20 mg, 16 µmol) according to method C1. The product was used directly in the subsequent reaction without any characterization; yield: 88% (410 mg).

(5-(3-Aminophenyl)thiophen-2-yl)(2,6-difluoro-3-hydroxyphenyl)methanone (61). The title compound was prepared by reaction of (5-(3-aminophenyl)thiophen-2-yl)-(3-ethoxy-2,6-difluorophenyl)methanone **(61a)** (440 mg, 1.22 mmol) and boron tribromide (3.7 mmol) according to method B. The product was purified by CC (dichloromethan/methanol 99.5:0.5); yield: 50% (200 mg). ^{1}H NMR (500 MHz, acetone-d^6) δ 9.02 (br. s, 1H), 7.45 (dd, *J* = 7.1, 2.9 Hz, 1H), 7.33 (d, *J* = 4.1 Hz, 1H), 7.10 (dd, *J* = 41.1, 6.5 Hz, 2H), 7.00 – 6.85 (m, 3H), 6.62 (ddd, *J* = 8.0, 2.1, 0.8 Hz, 1H), 4.75 (br., 2H, NH_2); ^{13}C NMR (125 MHz, acetone-d^6) δ 179.48, 155.98, 152.23 (dd, *J* = 241.6, 6.1 Hz), 149.34, 148.66 (dd, *J* = 249.5, 7.7 Hz), 143.90 (dd, *J* = 10.7, 3.2 Hz), 137.34, 133.47, 129.97, 125.13, 124.46, 121.00, 116.62 (dd, *J* = 9.3, 3.0 Hz), 115.73, 114.65, 111.62, 111.13 (dd, *J* = 22.7, 4.1 Hz); MS (ESI): 332.12 $(M+H)^+$.

4-Bromo-*N*-(3-(5-(3-ethoxy-2,6-difluorobenzoyl)thiophen-2-yl)phenyl)-2-trifluoromethoxy-benzenesulfonamide (62a). The title compound was prepared by reaction of (5-(3-aminophenyl)thiophen-2-yl)(3-ethoxy-2,6-difluorophenyl)methanone **(61a)** (210 mg, 0.58 mmol) and 4-bromo-2-trifluoromethoxybenzenesulfonyl chloride (198 mg, 0.58 mmol) according to method D. The product was sufficiently pure for use in the subsequent reaction; yield: 65% (250 mg). The product was used in the next step without any characterization.

4-Bromo-*N*-(3-(5-(2,6-difluoro-3-hydroxybenzoyl)thiophen-2-yl)-phenyl)-2-trifluoro-methoxybenzenesulfonamide (62). The title compound was prepared by reaction of 4-bromo-*N*-(3-(5-(3-ethoxy-2,6-difluorobenzoyl-thiophen-2-yl)phenyl)-2-trifluoromethoxybenzene-sulfonamide **(62a)** (250 mg, 0.38 mmol) and boron tribromide (2.3 mmol) according to method B. The product was purified by CC (dichloromethan/methanol 99.5:0.5); yield: 75% (180 mg). ^{1}H NMR (500 MHz, acetone-d^6) δ 9.35 (br. s, 1H), 8.58 (br. s, 1H), 8.03 (d, *J* = 8.5 Hz, 1H), 7.76 (dd, *J* = 8.5, 1.8 Hz, 1H), 7.74-7.72 (m, 1H), 7.68 – 7.65 (m, 1H), 7.62 (dt, *J* = 4.1, 0.9 Hz, 1H), 7.54 (ddd, *J* = 7.7, 1.8, 1.0 Hz, 1H), 7.52 (d, *J* = 4.1 Hz, 1H), 7.42 – 7.37 (m, 1H), 7.32 (ddd, *J* = 8.1, 2.2, 1.0 Hz, 1H), 7.23-7.21 (m, 1H), 7.03 (ddd, *J* = 9.1, 8.6, 1.9 Hz, 1H); ^{13}C NMR (125 MHz, acetone-d^6) δ 180.68, 154.32, 152.55 (dd, *J* = 240.6, 5.8 Hz), 148.43 (dd, *J* = 245.8, 7.7 Hz), 146.95 (d, *J* = 1.8 Hz), 143.55, 142.66 (dd, *J* = 13.0, 3.2 Hz), 138.85, 138.17, 134.88, 133.83, 131.81, 131.47, 131.34, 129.12, 126.34, 124.92 (d, *J* = 1.9 Hz), 123.59, 122.15, 120.39 (dd, *J* = 9.1, 3.8 Hz), 120.08, 118.80, 118.01 (dd, *J* = 24.0, 19.7 Hz), 112.44 (dd, *J* = 22.8, 3.9 Hz); MS (ESI): 635.43 $(M+H)^+$.

***N*-(3-(5-(3-Ethoxy-2,6-difluorobenzoyl)thiophen-2-yl)phenyl)-2-trifluoromethoxybenzene-sulfonamide (63a)**. The title compound was prepared by reaction of (5-(3-aminophenyl)-thiophen-2-yl)(3-ethoxy-2,6-difluorophenyl)methanone **(61a)** (210 mg, 0.58 mmol) and 2-trifluoromethoxybenzenesulfonyl chloride (152 mg, 0.58 mmol) according to method D. The product was sufficiently pure for use in the subsequent reaction; yield: 66% (225 mg). The product was used in the next step without any characterization.

***N*-(3-(5-(2,6-Difluoro-3-hydroxybenzoyl)thiophen-2-yl)phenyl)-2-trifluoromethoxybenzen-esulfonamide (63)**. The title compound was prepared by reaction of *N*-(3-(5-(3-ethoxy-2,6-difluorobenzoyl)thiophen-2-yl)phenyl)-2-trifluoromethoxybenzene-sulfonamide **(63a)** (225 mg, 0.39 mmol) and boron tribromide (2.3 mmol) according to method B. The product was purified by CC (dichloromethan/methanol 99.5:0.5); yield: 44% (142 mg). ^{1}H NMR (500 MHz, acetone-d^6) δ 9.50 (br. s, 1H), 9.04 (br. s, 1H), 8.14 – 8.09 (m, 1H), 7.77 (ddd, *J* = 8.4, 7.5, 1.7 Hz, 1H), 7.68 – 7.65 (m, 1H), 7.62 (dt, *J* = 4.0, 0.8 Hz, 1H), 7.57 – 7.51 (m, 3H), 7.51 – 7.50 (m, 1H), 7.38 (tt, *J* = 4.6, 2.3 Hz, 1H), 7.34 – 7.30 (m, 1H), 7.25 – 7.17 (m, 1H), 7.03 (ddd, *J* = 10.5, 6.9, 1.9 Hz, 1H); ^{13}C NMR (125 MHz, acetone-d^6) δ 180.69, 154.44, 152.55 (dd, *J* = 240.6, 5.8 Hz), 148.43 (dd, *J* = 245.9, 7.8 Hz), 146.82 (d, *J* = 1.7 Hz), 143.48, 142.66 (dd, *J* = 12.9, 3.2 Hz), 139.17, 138.18, 136.37, 134.77, 132.58, 132.25, 131.25, 128.02, 126.26, 123.28, 124.49 – 120.18 (m), 121.95, 121.53 (d, *J* = 1.8 Hz), 120.39 (dd, *J* = 9.1, 3.8 Hz), 118.50, 118.02 (dd, *J* = 23.9, 19.7 Hz), 112.45 (dd, *J* = 22.8, 3.9 Hz); MS (ESI): 556.17 $(M+H)^+$.

***N*-(3-(5-(3-Ethoxy-2,6-difluorobenzoyl)thiophen-2-yl)phenyl)-2-trifluoromethylbenzene-sulfonamide (64a)**. The title compound was prepared by reaction of (5-(3-aminophenyl)-thiophen-2-yl)(3-ethoxy-2,6-difluorophenyl)methanone **(61a)** (210 mg, 0.58 mmol) and 2-trifluoromethylbenzenesulfonyl chloride (143 mg, 0.58 mmol) according to method D. The

product was sufficiently pure for use in the subsequent reaction; yield: 68% (225 mg). The product was used in the next step without any characterization.

***N*-(3-(5-(2,6-Difluoro-3-hydroxybenzoyl)thiophen-2-yl)phenyl)-2-trifluoromethylbenzene-sulfonamide (64)**. The title compound was prepared by reaction of *N*-(3-(5-(3-ethoxy-2,6-difluorobenzoyl)thiophen-2-yl)phenyl)-2-trifluoromethylbenzenesulfonamide **(64a)** (230 mg, 0.41 mmol) and boron tribromide (2.4 mmol) according to method B. The product was purified by CC (dichloromethan/methanol 99.5:0.5); yield: 48% (165 mg). ^{1}H NMR (500 MHz, acetone-d^6) δ 9.39 (br. s, 1H), 9.00 (br. s, 1H), 8.30 – 8.25 (m, 1H), 8.03 – 7.98 (m, 1H), 7.87 – 7.82 (m, 2H), 7.68 – 7.65 (m, 1H), 7.62 (dt, *J* = 4.1, 0.9 Hz, 1H), 7.55 – 7.51 (m, 2H), 7.42 – 7.37 (m, 1H), 7.32 (ddd, *J* = 8.1, 2.2, 1.0 Hz, 1H), 7.23-7.21 (m, 1H), 7.06 – 7.00 (m, 1H); ^{13}C NMR (125 MHz, acetone-d^6) δ 180.69, 152.54 (dd, *J* = 240.6, 5.8 Hz), 148.42 (dd, *J* = 245.8, 7.8 Hz), 143.51, 142.66 (dd, *J* = 12.9, 3.2 Hz), 139.14, 139.11, 138.17, 134.83, 134.47, 133.86, 132.78, 131.32, 129.56 (q, *J* = 6.4 Hz), 128.41, 128.15, 126.32, 125.06, 123.37, 122.88, 122.14, 120.39 (dd, *J* = 9.1, 3.8 Hz), 118.73, 118.02 (dd, *J* = 24.0, 19.6 Hz); MS (ESI): 540.32 $(M+H)^+$.

Pyridine-3-sulfonic acid (3-(5-(2,6-difluoro-3-hydroxy-benzoyl)-thiophen-2-yl)-phenyl)-amide (65). The title compound was prepared by reaction of (5-(3-aminophenyl)thiophen-2-yl)(2,6-difluoro-3-hydroxyphenyl)methanone **(61)** (100 mg, 0.30 mmol) and pyridine-3-sulfonyl chloride hydrochloride (96 mg, 0.45 mmol) according to method D. The product was purified by CC (dichloromethan/methanol 95:5); yield: 35% (50 mg). ^{1}H NMR (500 MHz, acetone-d^6) δ 8.28 (dd, *J* = 2.4, 0.8 Hz, 1H), 8.10 (dd, *J*= 4.7, 1.6 Hz, 1H), 7.52-7.49 (m, 1H), 6.96-6.94 (m, 1H), 6.94-6.91 (m, 1H), 6.88 (d, *J*= 4.1 Hz, 1H), 6.83 (d, *J*= 7.6 Hz, 1H), 6.82-6.80 (m, 1H), 6.71 (t, *J*= 7.9 Hz, 1H), 6.55-6.47 (m, 2H), 6.41 (td, *J*= 9.0, 1.6 Hz, 1H); ^{13}C NMR (125 MHz, acetone-d^6) δ 180.8, 154.6, 154.4, 148.7, 143.6, 139.3, 138.3, 137.0, 135.9, 135.0, 131.5, 126.5, 125.1, 123.8, 122.8, 120.4, 119.4, 112.6, 112.4; MS (ESI): 473.28 $(M+H)^+$.

1-Methyl-1H-imidazole-4-sulfonic acid (3-(5-(2,6-difluoro-3-hydroxy-benzoyl)-thiophen-2-yl)-phenyl)-amide (66). The title compound was prepared by reaction of (5-(3-aminophenyl)thiophen-2-yl)(2,6-difluoro-3-hydroxyphenyl)methanone **(61)** (100 mg, 0.30 mmol) and 1-methyl-1H-imidazole-4-sulfonyl chloride (81 mg, 0.45 mmol) according to method D. The product was purified by CC (dichloromethan/methanol 95:5); yield: 72% (103 mg). ^{1}H NMR (500 MHz, acetone-d^6) δ 9.17 (s, 1H), 9.01 (s, 1H), 7.75-7.72 (m, 2H), 7.64-7.61 (m, 2H), 7.54 (d, *J*= 4.1 Hz, 1H), 7.50-7.43 (m, 1H), 7.39-7.34 (m, 2H), 7.21 (td, *J*= 9.4, 5.5 Hz, 1H), 7.04 (td, *J*= 8.8, 1.9Hz, 1H), 3.76 (s, 3H); ^{13}C NMR (125 MHz, acetone-d^6) δ 180.7, 155.1, 153.6, 151.6, 149.5, 147.5, 143.3, 142.7, 140.7, 140.1, 138.3, 134.5, 130.9, 126.2, 122.5, 121.9, 120.4, 118.4, 112.6, 112.4, 34.2; MS (ESI): 476.21 $(M+H)^+$.

Cyclopropanesulfonic acid (3-(5-(2,6-difluoro-3-hydroxy-benzoyl)-thiophen-2-yl)-phenyl)-amide (67). The title compound was prepared by reaction of (5-(3-aminophenyl)thiophen-2-

yl)(2,6-difluoro-3-hydroxyphenyl)methanone **(61)** (100 mg, 0.30 mmol) and cyclopropanesulfonyl chloride (1M in DCM) (63 mg, 0.45 mmol) according to method D. The product was purified by CC (dichloromethan/methanol 95:5); yield: 56% (73 mg). ^{1}H NMR (500 MHz, acetone-d^{6}) δ 9.01 (s, 1H), 8.77 (s, 1H), 7.80-7.78 (m, 1H), 7.65 (dt, *J*= 4.1, 0.9 Hz, 1H), 7.61-7.58 (m, 2H), 7.54-7.44 (m, 2H), 7.21 (td, *J*= 9.5, 5.4 Hz, 1H), 7.04 (td, *J*= 9.0, 1.9 Hz, 1H), 2.73-2.67 (m, 1H), 1.07-0.96 (m, 4H); ^{13}C NMR (125 MHz, acetone-d^{6}) δ 188.7, 154.8, 143.5, 140.6, 138.3 134.9, 131.3, 126.3, 123.1, 122.6, 120.4, 119.2, 112.6, 112.4, 32.4, 5.8; MS (ESI): 436.25 $(M+H)^{+}$.

Cyclopropanesulfonic acid (3-bromo-5-methyl-phenyl)-amide (68b). The title compound was prepared by reaction of 3-bromo-5-methylaniline (1000 mg, 5.38 mmol, 1 equiv) and cyclopropanesulfonyl chloride (1013 mg, 8.07 mmol, 1.5 equiv) according to method D. The product was purified by CC (hexane/ethyl acetate 8:2); yield: 77% (1200 mg). ^{1}H NMR (500 MHz, acetone-d^{6}) δ 8.62 (br. s, 1H), 7.35 (s, 1H), 7.15 (s, 1H), 7.13 (s, 1H), 2.65-2.61 (m, 1H), 2.30 (s, 3H), 1.00-0.95 (m, 4H).

Cyclopropanesulfonic acid (3-methyl-5-(4,4,5,5-tetramethyl-(1,3,2)dioxaborolan-2-yl)-phenyl)-amide (68a). Cyclopropanesulfonic acid (3-bromo-5-methyl-phenyl)-amide **(68b)** (800 mg, 2.76 mmol, 1 equiv) was dissolved in 10mL dry dimethyl sulfoxide under N_2 atmosphere. Bispinakolatodiborone (1050 mg, 4.14 mmol, 1.5 equiv), potassium acetate (8.28 mmol, 3 equiv) and Pd(dppf)Cl_2 (0.14 mmol, 0.05 equiv) were added. The reaction mixture was heated to 80°C over night. Reaction mixture was cooled down to room temperature, quenched with water and diethyl ether, filtered over celite, extracted three times with diethyl ether, washed three times with water, washed with brine, dried over magnesium sulphate, filtered and evaporated under reduced pressure. Purified by CC (dichloromethane pure to dichloromethane/methanol 8:2); yield: 16% (150 mg). The product was used in the next step without any characterization.

Cyclopropanesulfonic acid (3-(5-(2,6-difluoro-3-hydroxy-benzoyl)-thiophen-2-yl)-5-methyl-phenyl)-amide (68). cyclopropanesulfonic acid (3-methyl-5-(4,4,5,5-tetramethyl-(1,3,2)-dioxaborolan-2-yl)-phenyl)-amide **(68a)** (150 mg, 0.45mmol, 1.20equiv), (5-bromo-2-thienyl)(2,4-difluoro-3-hydroxyphenyl)-methanone **VIII** (118 mg, 0.37mmol, 1.00equiv), ceasium carbonate (263 mg, 1.11mmol, 3.00equiv) and Pd$(PPh_3)_4$ (0.002mmol, 0.005equiv) were suspended in a degased mixture of 5mL DME and 5mL water. The mixture was heated to reflux for 3 days under N_2 atmosphere. The mixture was cooled down to room temperature, quenched with water, filtered over celite, extracted three times with ethyl acetate, washed one time with water, one time with brine, dried over sodium sulphate, filtered and evaporated under reduced pressure. The product was purified by MPLC (0% ethyl acetate to 100% ethyl acetate) followed by preparative TLC (hexane/ethyl acetate 5:5); yield: 18% (30 mg). ^{1}H NMR (500 MHz, acetone-d^{6}) δ 8.98 (s, 1H), 8.68 (s, 1H), 7.61 (d, *J*= 4.0 Hz, 1H), 7.57 (s, 1H), 7.55 (d, *J*= 4.0 Hz, 1H), 7.41 (s, 1H), 7.26 (s, 1H), 7.21-7.15 (m, 1H), 7.01 (td, *J*= 8.8, 1.8 Hz, 1H), 2.70-

2.65 (m, 1H), 2.38 (s, 3H), 1.02-0.96 (m, 4H); ^{13}C NMR (125 MHz, acetone-d^6) δ 180.6, 155.0, 143.2, 141.3, 140.4, 138.1, 134.6, 126.1, 123.7, 123.1, 120.3, 116.4, 112.3, 29.7, 21.4, 5.7; MS (ESI): 449.76 $(M+H)^+$.

Cyclopropanesulfonic acid (3-bromo-5-methyl-phenyl)-methyl-amide (69b). Cyclopropane-sulfonic acid (3-bromo-5-methyl-phenyl)-amide **(68b)** (100mg, 0.345mmol, 1equiv) was dissolved in 1mL anhydrous dimethyl formamide and cooled to 0°C under N_2 atmosphere. Sodium hydride (10mg, 0.414mmol, 1.2equiv) was added and the solution was stirred for 10 min. Methyl iodide (54mg, 0.024mL, 0.380mmol, 1.1equiv) was added slowly, the mixture warmed up to room temperature and stirred over night. The mixture was quenched with water, extracted three times with diethyl ether, washed 5 times with water, washed with brine, dried over sodium sulphate, filtered and evaporated under reduced pressure. No further purification was required; yield: 81% (85 mg). ^{1}H NMR (500 MHz, acetone-d^6) δ 7.44 (s, 1H), 7.29 (s, 1H), 7.16 (s, 1H), 3.31 (s, 3H), 2.56-2.54 (m, 1H), 2.33 (s, 3H), 0.99-0.96 (m, 4H).

Cyclopropanesulfonic acid methyl-(3-methyl-5-(4,4,5,5-tetramethyl-(1,3,2)dioxaborolan-2-yl)-phenyl)-amide (69a). Cyclopropanesulfonic acid (3-bromo-5-methyl-phenyl)-amide **(68b)** (260 mg, 0.86 mmol, 1equiv) was dissolved in 3mL dry dimethyl sulfoxide under N_2 atmosphere. Bispinakolatodiborone (326 mg, 1.28 mmol, 1.5equiv), potassium acetate (2.57mmol, 3equiv) and Pd(dppf)Cl_2 (0.04 mmol, 0.05equiv) were added. The reaction mixture was heated to 80°C over night. Reaction mixture was cooled down to room temperature, quenched with water and diethyl ether, filtered over celite, extracted three times with diethyl ether, washed three times with water, washed with brine, dried over magnesium sulphate, filtered and evaporated under reduced pressure. Purified by CC (dichloromethane pure to dichloromethane/methanol 8:2); yield: 50% (150 mg). The product was used in the next step without any characterization.

Cyclopropanesulfonic acid (3-(5-(2,6-difluoro-3-hydroxy-benzoyl)-thiophen-2-yl)-5-methyl-phenyl)-methyl-amide (69). Cyclopropanesulfonic acid (3-bromo-5-methyl-phenyl)-methyl-amide **(69b)** (300 mg, 0.86mmol, 1.00equiv), (5-bromo-2-thienyl)(2,4-difluoro-3-hydroxyphenyl)-methanone **VIII** (273 mg, 0.86mmol, 1.00equiv), ceasium carbonate (1113 mg, 3.42mmol, 4.00equiv) and Pd$(PPh_3)_4$ (0.004mmol, 0.005equiv) were suspended in a degased mixture of 5mL DME and 5mL water. The mixture was heated to reflux for 3 days under N_2 atmosphere. The mixture was cooled down to room temperature, quenched with water, filtered over celite, extracted three times with ethyl acetate, washed one time with water, one time with brine, dried over sodium sulphate, filtered and evaporated under reduced pressure. The product was purified by CC (hexane/ethylacetate 8:2 to 5:5) followed by preparative TLC (dichloromethan/methanol 99:1); yield: 6% (25 mg). ^{1}H NMR (500 MHz, acetone-d^6) δ 9.04 (s, 1H), 7.71 (s, 1H), 7.65-7.63 (m, 2H), 7.58 (s, 1H), 7.30 (s, 1H), 7.23-7.18 (m, 1H), 7.04 (dt, J= 9.0, 1.9 Hz, 1H), 3.41 (s, 3H), 2.68-2.65 (m, 1H), 2.43 (s, 3H), 1.01-0.90 (m, 4H); ^{13}C NMR (125

MHz, acetone-d^6) δ 180.7, 154.7, 144.2, 140.9, 138.1, 134.4, 129.3, 126.4, 122.5, 112.4, 38.7, 21.2, 4.9; MS (ESI): 463.87 $(M+H)^+$.

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

Preparation of human 17β-HSD1 and 17β-HSD2. Human 17β-HSD1 and 17β-HSD2 were obtained from human placenta according to previously described procedures.[39] Fresh human placenta was homogenized, and cytosolic fraction and microsomes were separated by fractional centrifugation. For the partial purification of 17β-HSD1, the cytosolic fraction was precipitated with ammonium sulfate. 17β-HSD2 was obtained from the microsomal fraction.

Inhibition of human 17β-HSD1. Inhibitory activities were evaluated by an established method with minor modifications.[39] Briefly, the enzyme preparation was incubated with NADPH (500 µM) in the presence of potential inhibitors at 37 ^{o}C in a phosphate buffer (50 mM) supplemented with 20% of glycerol and EDTA (1 mM). Inhibitor stock solutions were prepared in DMSO. The final concentration of DMSO was adjusted to 1% in all samples. The enzymatic reaction was started by addition of a mixture of unlabelled- and [2, 4, 6, 7-^{3}H]-E1 (final concentration: 500 nM, 0.15 µCi). After 10 min, the incubation was stopped with $HgCl_2$ and the mixture was extracted with diethylether. After evaporation, the steroids were dissolved in acetonitrile. E1 and E2 were separated using acetonitrile/water (45:55) as mobile phase in a C18 reverse phase chromatography column (Nucleodur C18 125/3 100-5, Macherey-Nagel) connected to a HPLC-system (Agilent 1200 Series, Agilent Technologies). Detection and quantification of the steroids were performed using a radioflow detector (Ramona, raytest). The conversion rate was calculated after analysis of the resulting chromatograms according to the following equation:

%conversion = [%E2/(%E2 + %E1)]x100

Each value was calculated from at least three independent experiments.

Inhibition of human 17β-HSD2. The *h*17β-HSD2 inhibition assay was performed similarly to the *h*17β-HSD1 procedure. The microsomal fraction was incubated with NAD^+ [1500 µM], test compound and a mixture of unlabelled- and [2,4,6,7-^{3}H]-E2 (final concentration: 500 nM, 0.11 µCi) for 20 min at 37 ^{o}C. Further treatment of the samples and HPLC separation was carried out as mentioned above.

The conversion rate was calculated after analysis of the resulting chromatograms according to the following equation:

%conversion = [%E1/(%E1 + %E2)]x100

Preparation of murine and rat 17β-HSD1 and 17β-HSD2. Recombinant mouse and rat 17β-HSD1 enzyme was produced by transfection of HEK 293 cells with a mouse or rat 17β-HSD1 expression plasmid [coding sequences NM_010475 (mouse) and NM_012851 (rat) in pCMV6Entry vector, OriGene Technologies, Inc.]. 48 hours after transfection cells were homogenized by sonication (3 x 10 s) in a buffer containing saccharose (40 mM Tris, 250 mM saccharose, 5 mM EDTA, 7 mM DTT, 1 mM PMSF, pH 7.5). Cell lysate was centrifuged (1000 g, 15 min, 4°C) and 20% glycerol was added to the supernatant before aliquots were frozen and stored at -70°C.[47] Microsomal 17β-HSD2 fractions were obtained from mouse tissues.[48] Fresh tissue was homogenized and centrifuged. The pellet fraction contains the microsomal 17β-HSD2 and was used for the determination of E1 formation.

Inhibition of murine/rat 17β-HSD1. Inhibitory activities of the compounds towards mouse or rat 17β-HSD1 were evaluated by an established method with minor modifications.[33] The enzyme preparation was incubated with inhibitors, NADPH (500 µM) and a mixture of unlabeled- and [3H]-E1 (final concentration: 10 nM, 0.15 µCi) for 10 min at 37 ^{o}C. Further treatment of the samples and HPLC separation was carried out as mentioned above.

The conversion rate was calculated after analysis of the resulting chromatograms according to the following equation:

%conversion = [%E2/(%E2 + %E1)]x100

Inhibition of murine and rat 17β-HSD2. The *m/r*17β-HSD2 inhibition assay was performed similarly to the *m/r*17β-HSD1 procedure. The microsomal fraction was incubated with NAD^{+} [1500 µM], test compound and a mixture of unlabelled- and [2,4,6,7-^{3}H]-E2 (final concentration: 10 nM, 0.15 µCi) for 20 min at 37 ^{o}C. Further treatment of the samples and HPLC separation was carried out as mentioned above.

The conversion rate was calculated after analysis of the resulting chromatograms according to the following equation:

%conversion = [%E1/(%E1 + %E2)]x100

ER Affinity

The binding affinity of selected compounds to ERα and ERβ was determined according to the recommendations of the US Environmental Protection Agency (EPA) by their Endocrine Disruptor Screening Program (EDSP) using recombinant human proteins. Briefly, 1 nM of ERα and 4 nM of ERβ, respectively, were incubated with [3H]-E2 (3 nM for ERα and 10 nM for ERβ) and test compound for 16-20 h at 4°C.

The potential inhibitors were dissolved in DMSO (5% final concentration). Evaluation of non-specific-binding was performed with unlabeled E2 at concentrations 100-fold of [3H]-E2 (300 nM for ERα and 1000 nM for ERβ). After incubation, ligand-receptor complexes were selectively bound to hydroxyapatite (83.5 g/L in TE-buffer). The bound complex was washed three times and resuspended in ethanol. For radiodetection, scintillator cocktail (Quickszint 212, Zinsser Analytic, Frankfurt) was added and samples were measured in a liquid scintillation counter (1450 LSC & Luminescence Counter, Perkin Elmer).

From these results the percentage of [3H]-E2 displacement by the compounds was calculated. The plot of % displacement versus compound concentration resulted in sigmoidal binding curves. The compound concentrations to displace 50% of the receptor bound [3H]-E2 were determined. Unlabeled E2 IC_{50} values were determined in each experiment and used as reference. The E2 IC_{50} values accepted were 3±20% nM for ERα and 10±20% nM for ERβ.

Relative Binding Affinity was determined by applying the following equation: RBA [%] = (IC_{50} (E2)/IC_{50} (compound)) · 100 [*]. 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 [3H]-E2 at a concentration of 1000 · IC_{50} (E2) were classified as RBA <0.1%.

Metabolic stability

For evaluation of phase I and II metabolic stability 1 µM compound was incubated with 1 mg/ml pooled mammalian liver S9 fraction (BD Gentest), 2 mM NADPH regenerating system, 1 mM UDPGA and 0.1 mM PAPS at 37°C for 0, 15 and/or 60 minutes. The incubation was stopped by precipitation of S9 enzymes with 2 volumes of cold acetonitrile containing internal standard. Concentration of the remaining test compound at the different time points was analyzed by LC-MS/MS. For evaluation of phase I metabolic stability the assay was conducted in absence of UDPGA and PAPS.

MTT-Cytotoxicity assay

The number of living cells was evaluated measuring the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT). Experiments were performed in 96-well cell culture plates in DMEM supplemented with 10% FCS. Cells were incubated for 66 h with 6.25, 12.50, 25, 50, and 100 µM of test compound at 37 °C in a humidified atmosphere at 5% CO_2. For cleavage reaction MTT-solution (5mg/mL in PBS) was added and incubation was continued for another 66 h. Reaction stop and cell lysis were carried out by addition of sodium dodecyl sulphate (SDS) in 0.01N HCl (10%). The produced blue formazan was quantified spectrophotometrically at 590nm as described by Denizot and Lang[49] with minor modifications.

Physicochemical properties determination

The logP and pKa values were calculated using the ACD/Labs Percepta software. The logarithm of partition constant P (logP) was calculated using the "GALAS" method (Global, Adjusted Locally According to Similarity). The program predicts clogP by comparing the molecule with structural similarity of molecules where experimental data are known.

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Spplemantary table S1: *HPLC Purity Control of Final Compounds*

The purity of the compounds was evaluated by LC/MS. The Surveyor®-LC-system consisted of a pump, an auto sampler, 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). 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) 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 needle voltage of 3000 V and a capillary temperature of 350 °C. Mass spectra were acquired in positive mode from 100 to 1000 m/z and UV spectra were recorded at the wave length of 254 nm.

Spplemantary table S1: *HPLC Purity Control of Final Compounds*

Comd	R_t (min)	HPLC purity (≥ %)	Comd	R_t (min)	HPLC purity (≥ %)	Comd	R_t (min)	HPLC purity (≥ %)
1	7.90	98	**2**	8.27	95	**3**	11.57	97
4	13.37	95	**5**	11.53	97	**6**	11.84	95
7	13.69	96	**8**	11.96	98	**9**	11.71	98
10	10.98	95	**11**	10.93	95	**12**	10.96	95
13	11.99	99	**14**	12.00	95	**15**	12.15	97
16	12.20	97	**17**	12.35	97	**18**	11.85	96
19	12.23	96	**20**	12.07	96	**21**	12.88	97
22	13.12	97	**23**	11.44	96	**24**	12.05	99
25	10.70	97	**26**	12.32	99	**27**	13.06	98
28	11.75	96	**29**	10.57	95	**30**	13.07	98
31	13.80	95	**32**	13.66	95	**33**	12.83	96
34	12.96	98	**35**	11.28	99	**36**	13.20	95
37	11.09	98	**38**	13.60	95	**39**	11.45	99
40	12.77	95	**41**	11.67	97	**42**	14.28	97
43	11.97	96	**44**	12.04	95	**45**	13.94	95
46	13.82	95	**47**	11.90	95	**48**	12.61	99
49	12.88	99	**50**	8.60	99	**51**	10.98	96
52	7.29	95	**53**	7.92	96	**54**	15.55	97
55	12.93	97	**56**	9.26	98	**57**	8.83	95
58	13.99	98	**59a**	12.73	95	**59**	10.56	95
60	9.93	97	**61**	8.48	99	**62**	13.11	99
63	12.38	99	**64**	12.18	99	**65**	10.75	95
66	12.54	98	**67**	11.33	97	**68**	11.59	95
69	13.75	95						

References

1. Liehr, J. G. Is estradiol a genotoxic mutagenic carcinogen? *Endocr Rev* **2000,** 21, 40-54.
2. Thomas, D. B. Do hormones cause breast cancer? *Cancer* **1984,** 53, 595-604.
3. Russo, J.; Fernandez, S. V.; Russo, P. A.; Fernbaugh, R.; Sheriff, F. S.; Lareef, H. M.; Garber, J.; Russo, I. H. 17-Beta-estradiol induces transformation and tumorigenesis in human breast epithelial cells. *FASEB J* **2006,** 20, 1622-34.
4. Dizerega, G. S.; Barber, D. L.; Hodgen, G. D. Endometriosis: role of ovarian steroids in initiation, maintenance, and suppression. *Fertil Steril* **1980,** 33, 649-53.
5. Zeitoun, K.; Takayama, K.; Sasano, H.; Suzuki, T.; Moghrabi, N.; Andersson, S.; Johns, A.; Meng, L.; Putman, M.; Carr, B.; Bulun, S. E. Deficient 17beta-hydroxysteroid dehydrogenase type 2 expression in endometriosis: failure to metabolize 17beta-estradiol. *J Clin Endocrinol Metab* **1998,** 83, 4474-80.
6. Miyoshi, Y.; Ando, A.; Shiba, E.; Taguchi, T.; Tamaki, Y.; Noguchi, S. Involvement of up-regulation of 17beta-hydroxysteroid dehydrogenase type 1 in maintenance of intratumoral high estradiol levels in postmenopausal breast cancers. *Int J Cancer* **2001,** 94, 685-9.
7. Jansson, A. 17Beta-hydroxysteroid dehydrogenase enzymes and breast cancer. *J Steroid Biochem Mol Biol* **2009,** 114, 64-7.
8. Blomquist, C. H.; Bonenfant, M.; McGinley, D. M.; Posalaky, Z.; Lakatua, D. J.; Tuli-Puri, S.; Bealka, D. G.; Tremblay, Y. Androgenic and estrogenic 17beta-hydroxysteroid dehydrogenase/17-ketosteroid reductase in human ovarian epithelial tumors: evidence for the type 1, 2 and 5 isoforms. *J Steroid Biochem Mol Biol* **2002,** 81, 343-51.
9. Smuc, T.; Pucelj, M. R.; Sinkovec, J.; Husen, B.; Thole, H.; Lanisnik Rizner, T. Expression analysis of the genes involved in estradiol and progesterone action in human ovarian endometriosis. *Gynecol Endocrinol* **2007,** 23, 105-11.
10. Saloniemi, T.; Jarvensivu, P.; Koskimies, P.; Jokela, H.; Lamminen, T.; Ghaem-Maghami, S.; Dina, R.; Damdimopoulou, P.; Makela, S.; Perheentupa, A.; Kujari, H.; Brosens, J.; Poutanen, M. Novel hydroxysteroid (17beta) dehydrogenase 1 inhibitors reverse estrogen-induced endometrial hyperplasia in transgenic mice. *Am J Pathol* **2010,** 176, 1443-51.
11. Kasai, T.; Shozu, M.; Murakami, K.; Segawa, T.; Shinohara, K.; Nomura, K.; Inoue, M. Increased expression of type I 17beta-hydroxysteroid dehydrogenase enhances in situ production of estradiol in uterine leiomyoma. *J Clin Endocrinol Metab* **2004,** 89, 5661-8.
12. Penning, T. M. Molecular endocrinology of hydroxysteroid dehydrogenases. *Endocr Rev* **1997,** 18, 281-305.
13. Cavalli, A.; Bisi, A.; Bertucci, C.; Rosini, C.; Paluszcak, A.; Gobbi, S.; Giorgio, E.; Rampa, A.; Belluti, F.; Piazzi, L.; Valenti, P.; Hartmann, R. W.; Recanatini, M.

Enantioselective nonsteroidal aromatase inhibitors identified through a multidisciplinary medicinal chemistry approach. *J Med Chem* **2005,** 48, 7282-9.

14. Le Borgne, M.; Marchand, P.; Duflos, M.; Delevoye-Seiller, B.; Piessard-Robert, S.; Le Baut, G.; Hartmann, R. W.; Palzer, M. Synthesis and in vitro evaluation of 3-(1-azolylmethyl)-1H-indoles and 3-(1-azolyl-1-phenylmethyl)-1H-indoles as inhibitors of P450 arom. *Arch Pharm (Weinheim)* **1997,** 330, 141-5.
15. Jacobs, C.; Frotscher, M.; Dannhardt, G.; Hartmann, R. W. 1-imidazolyl(alkyl)-substituted di- and tetrahydroquinolines and analogues: syntheses and evaluation of dual inhibitors of thromboxane A(2) synthase and aromatase. *J Med Chem* **2000,** 43, 1841-51.
16. Baston, E.; Hartmann, R. W. N-substituted 4-(5-indolyl)benzoic acids. Synthesis and evaluation of steroid 5alpha-reductase type I and II inhibitory activity. *Bioorg Med Chem Lett* **1999,** 9, 1601-6.
17. Reichert, W.; Jose, J.; Hartmann, R. W. 5 alpha-reductase in intact DU145 cells: evidence for isozyme I and evaluation of novel inhibitors. *Arch Pharm (Weinheim)* **2000,** 333, 201-4.
18. Picard, F.; Baston, E.; Reichert, W.; Hartmann, R. W. Synthesis of N-substituted piperidine-4-(benzylidene-4-carboxylic acids) and evaluation as inhibitors of steroid-5alpha-reductase type 1 and 2. *Bioorg Med Chem* **2000,** 8, 1479-87.
19. Baston, E.; Salem, O. I.; Hartmann, R. W. 6-Substituted 3,4-dihydro-naphthalene-2-carboxylic acids: synthesis and structure-activity studies in a novel class of human 5alpha reductase inhibitors. *J Enzyme Inhib Med Chem* **2002,** 17, 303-20.
20. Day, J. M.; Foster, P. A.; Tutill, H. J.; Parsons, M. F.; Newman, S. P.; Chander, S. K.; Allan, G. M.; Lawrence, H. R.; Vicker, N.; Potter, B. V.; Reed, M. J.; Purohit, A. 17beta-hydroxysteroid dehydrogenase Type 1, and not Type 12, is a target for endocrine therapy of hormone-dependent breast cancer. *Int J Cancer* **2008,** 122, 1931-40.
21. Marchais-Oberwinkler, S.; Henn, C.; Moller, G.; Klein, T.; Negri, M.; Oster, A.; Spadaro, A.; Werth, R.; Wetzel, M.; Xu, K.; Frotscher, M.; Hartmann, R. W.; Adamski, J. 17beta-Hydroxysteroid dehydrogenases (17beta-HSDs) as therapeutic targets: protein structures, functions, and recent progress in inhibitor development. *J Steroid Biochem Mol Biol* **2011,** 125, 66-82.
22. Starčević, S.; Brožič, P.; Turk, S.; Cesar, J.; Rižner, T. L.; Gobec, S. Synthesis and biological evaluation of (6- and 7-phenyl) coumarin derivatives as selective nonsteroidal inhibitors of 17beta-hydroxysteroid dehydrogenase type 1. *J Med Chem* **2011,** 54, 248-61.
23. Moller, G.; Husen, B.; Kowalik, D.; Hirvela, L.; Plewczynski, D.; Rychlewski, L.; Messinger, J.; Thole, H.; Adamski, J. Species used for drug testing reveal different inhibition susceptibility for 17beta-hydroxysteroid dehydrogenase type 1. *PLoS One* 5, e10969.

24. Azzi, A.; Rehse, P. H.; Zhu, D. W.; Campbell, R. L.; Labrie, F.; Lin, S. X. Crystal structure of human estrogenic 17 beta-hydroxysteroid dehydrogenase complexed with 17 beta-estradiol. *Nat Struct Biol* **1996,** 3, 665-8.
25. Klein, T.; Henn, C.; Negri, M.; Frotscher, M. Structural basis for species specific inhibition of 17beta-hydroxysteroid dehydrogenase type 1 (17beta-HSD1): computational study and biological validation. *PLoS One* **2011,** 6, e22990.
26. Oster, A.; Hinsberger, S.; Werth, R.; Marchais-Oberwinkler, S.; Frotscher, M.; Hartmann, R. W. Bicyclic substituted hydroxyphenylmethanones as novel inhibitors of 17beta-hydroxysteroid dehydrogenase type 1 (17beta-HSD1) for the treatment of estrogen-dependent diseases. *J Med Chem* **2010,** 53, 8176-86.
27. Oster, A.; Klein, T.; Henn, C.; Werth, R.; Marchais-Oberwinkler, S.; Frotscher, M.; Hartmann, R. W. Bicyclic substituted hydroxyphenylmethanone type inhibitors of 17 beta-hydroxysteroid dehydrogenase Type 1 (17 beta-HSD1): the role of the bicyclic moiety. *ChemMedChem* **2011,** 6, 476-87.
28. Bey, E.; Marchais-Oberwinkler, S.; Negri, M.; Kruchten, P.; Oster, A.; Klein, T.; Spadaro, A.; Werth, R.; Frotscher, M.; Birk, B.; Hartmann, R. W. New insights into the SAR and binding modes of bis(hydroxyphenyl)thiophenes and -benzenes: influence of additional substituents on 17beta-hydroxysteroid dehydrogenase type 1 (17beta-HSD1) inhibitory activity and selectivity. *J Med Chem* **2009,** 52, 6724-43.
29. Bey, E.; Marchais-Oberwinkler, S.; Werth, R.; Negri, M.; Al-Soud, Y. A.; Kruchten, P.; Oster, A.; Frotscher, M.; Birk, B.; Hartmann, R. W. Design, synthesis, biological evaluation and pharmacokinetics of bis(hydroxyphenyl) substituted azoles, thiophenes, benzenes, and aza-benzenes as potent and selective nonsteroidal inhibitors of 17beta-hydroxysteroid dehydrogenase type 1 (17beta-HSD1). *J Med Chem* **2008,** 51, 6725-39.
30. Frotscher, M.; Ziegler, E.; Marchais-Oberwinkler, S.; Kruchten, P.; Neugebauer, A.; Fetzer, L.; Scherer, C.; Muller-Vieira, U.; Messinger, J.; Thole, H.; Hartmann, R. W. Design, synthesis, and biological evaluation of (hydroxyphenyl)naphthalene and -quinoline derivatives: potent and selective nonsteroidal inhibitors of 17beta-hydroxysteroid dehydrogenase type 1 (17beta-HSD1) for the treatment of estrogen-dependent diseases. *J Med Chem* **2008,** 51, 2158-69.
31. Marchais-Oberwinkler, S.; Frotscher, M.; Ziegler, E.; Werth, R.; Kruchten, P.; Messinger, J.; Thole, H.; Hartmann, R. W. Structure-activity study in the class of 6-(3'-hydroxyphenyl)naphthalenes leading to an optimization of a pharmacophore model for 17beta-hydroxysteroid dehydrogenase type 1 (17beta-HSD1) inhibitors. *Mol Cell Endocrinol* **2009,** 301, 205-11.
32. Marchais-Oberwinkler, S.; Kruchten, P.; Frotscher, M.; Ziegler, E.; Neugebauer, A.; Bhoga, U.; Bey, E.; Muller-Vieira, U.; Messinger, J.; Thole, H.; Hartmann, R. W. Substituted 6-phenyl-2-naphthols. Potent and selective nonsteroidal inhibitors of 17beta-

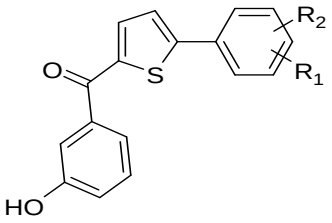
hydroxysteroid dehydrogenase type 1 (17beta-HSD1): design, synthesis, biological evaluation, and pharmacokinetics. *J Med Chem* **2008,** 51, 4685-98.

33. Marchais-Oberwinkler, S.; Wetzel, M.; Ziegler, E.; Kruchten, P.; Werth, R.; Henn, C.; Hartmann, R. W.; Frotscher, M. New drug-like hydroxyphenylnaphthol steroidomimetics as potent and selective 17beta-hydroxysteroid dehydrogenase type 1 inhibitors for the treatment of estrogen-dependent diseases. *J Med Chem* **2010,** 54, 534-47.
34. Henn, C.; Einspanier, A.; Marchais-Oberwinkler, S.; Frotscher, M.; Hartmann, R. W. Lead optimization of 17beta-HSD1 inhibitors of the (hydroxyphenyl)naphthol sulfonamide type for the treatment of endometriosis. *J Med Chem* **2012,** 55, 3307-18.
35. Spadaro, A.; Frotscher, M.; Hartmann, R. W. Optimization of hydroxybenzothiazoles as novel potent and selective inhibitors of 17beta-HSD1. *J Med Chem* **2012,** 55, 2469-73.
36. Fink, B. E.; Mortensen, D. S.; Stauffer, S. R.; Aron, Z. D.; Katzenellenbogen, J. A. Novel structural templates for estrogen-receptor ligands and prospects for combinatorial synthesis of estrogens. *Chem Biol* **1999,** 6, 205-19.
37. Miyaura, N.; Yanagi, T.; Suzuki, A. The Palladium-Catalyzed Cross-Coupling Reaction of Phenylboronic Acid with Haloarenes in the Presence of Bases. *Synthetic Communications* **1981,** 11, 513-519.
38. Hansen, S. G.; Jensen, H. H. Microwave irradiation as an effective means of synthesizing unsubstituted N-linked 1, 2, 3-triazoles from vinyl acetate and azides. *Synlett* **2009,** 20, 3275-3276.
39. Kruchten, P.; Werth, R.; Marchais-Oberwinkler, S.; Frotscher, M.; Hartmann, R. W. Development of a biological screening system for the evaluation of highly active and selective 17beta-HSD1-inhibitors as potential therapeutic agents. *Mol Cell Endocrinol* **2009,** 301, 154-7.
40. Qiu, W.; Campbell, R. L.; Gangloff, A.; Dupuis, P.; Boivin, R. P.; Tremblay, M. R.; Poirier, D.; Lin, S. X. A concerted, rational design of type 1 17beta-hydroxysteroid dehydrogenase inhibitors: estradiol-adenosine hybrids with high affinity. *FASEB J* **2002,** 16, 1829-31.
41. Sam, K. M.; Auger, S.; Luu-The, V.; Poirier, D. Steroidal spiro-gamma-lactones that inhibit 17 beta-hydroxysteroid dehydrogenase activity in human placental microsomes. *J Med Chem* **1995,** 38, 4518-28.
42. Bohm, H. J.; Banner, D.; Bendels, S.; Kansy, M.; Kuhn, B.; Muller, K.; Obst-Sander, U.; Stahl, M. Fluorine in medicinal chemistry. *Chembiochem* **2004,** 5, 637-43.
43. Muller, K.; Faeh, C.; Diederich, F. Fluorine in pharmaceuticals: looking beyond intuition. *Science* **2007,** 317, 1881-6.
44. Hartmann, R.; Frotscher, M.; Marchais-Oberwinkler, S.; Oster, A.; Spadaro, A. Preparation of heteroaryl phenyl ketones and amides as selective 17Î²-hydroxysteroid dehydrogenase type 1 inhibitors. WO2012025638A1, 2012.

45. Brotherton-Pleiss, C. E.; Kertesz, D. J.; Yang, M. Preparation of piperidinylaminopyrimidine derivatives as non-nucleoside reverse transcriptase inhibitors. WO2008071587A2, 2008.
46. Holenz, J.; Karlstroem, S.; Kihlstroem, J.; Kolmodin, K.; Lindstroem, J.; Rakos, L.; Rotticci, D.; Swahn, B.-M.; Von, B. S. Preparation of 5H-pyrrolo[3,4-b]pyrazin-7-amine derivatives as Î²-secretase inhibitors and useful in the treatment of AÎ²-related diseases. WO2011002409A1, 2011.
47. Puranen, T. J.; Poutanen, M. H.; Peltoketo, H. E.; Vihko, P. T.; Vihko, R. K. Site-directed mutagenesis of the putative active site of human 17 beta-hydroxysteroid dehydrogenase type 1. *Biochem J* **1994,** 304 (Pt 1), 289-93.
48. Jazbutyte, V.; Hu, K.; Kruchten, P.; Bey, E.; Maier, S. K.; Fritzemeier, K. H.; Prelle, K.; Hegele-Hartung, C.; Hartmann, R. W.; Neyses, L.; Ertl, G.; Pelzer, T. Aging reduces the efficacy of estrogen substitution to attenuate cardiac hypertrophy in female spontaneously hypertensive rats. *Hypertension* **2006,** 48, 579-86.
49. Denizot, F.; Lang, R. Rapid colorimetric assay for cell growth and survival. Modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. *J Immunol Methods* **1986,** 89, 271-7.

4 Discussion and Conclusions

17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1) is a promising target for the treatment of estrogen-dependent diseases (EDD) such as breast cancer and endometriosis. The aim of this thesis was to obtain more insight in the structural activity relationships (SAR) of bicyclic substituted hydroxyphenylmethanone compound class (BSHs), one of the most potent 17β-HSD1 inhibitor classes described by the Hartmann group (figure 1).



R_1, R_2: e.g. H, OH, O-alkyl, CN, hetaryl

Figure 1. Bicyclic substituted hydroxyphenylmethanones: General structure.

In order to obtain a suitable candidate which could be used to conduct a proof of principle (PoP) study in an animal (rodent) disease model for EDD, the biological properties of the most interesting compounds were further investigated aiming at preferably low affinity to the estrogen receptors α and β (ERα and β), metabolic stability (S9 fraction), and inhibition of rodent 17β-HSD1 enzyme.

Chapter **3.I** reports about the elucidation of SAR in the class of BSHs. This chapter describes design, synthesis, and biological evaluation of a series of 28 compounds. Furthermore, computational methods were used in order to elucidate the binding modes of the novel inhibitors with two linker functions.

Starting point for the design of these compounds were our conceptions concerning the binding mode of BSHs (figure 2).

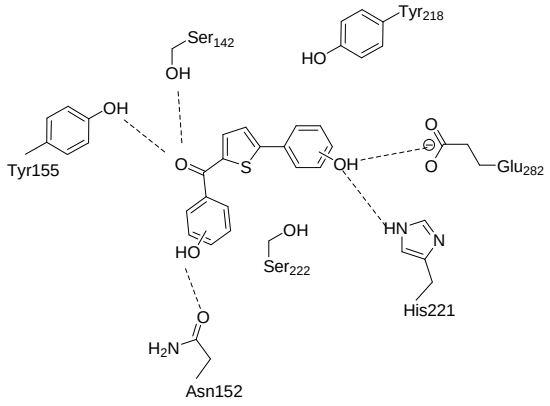


Figure 2. Schematic H-bond interactions of BSHs with 17β-HSD1

Modifications have been made in several positions of the inhibitor in order to evaluate the structure activity relationships in this compound class (figure 3, modifications a-c). Moreover, to establish additional interactions to Tyr218 and Ser222 a second linker function was introduced between the heterocycle and the hydroxyphenyl moiety (ring C).

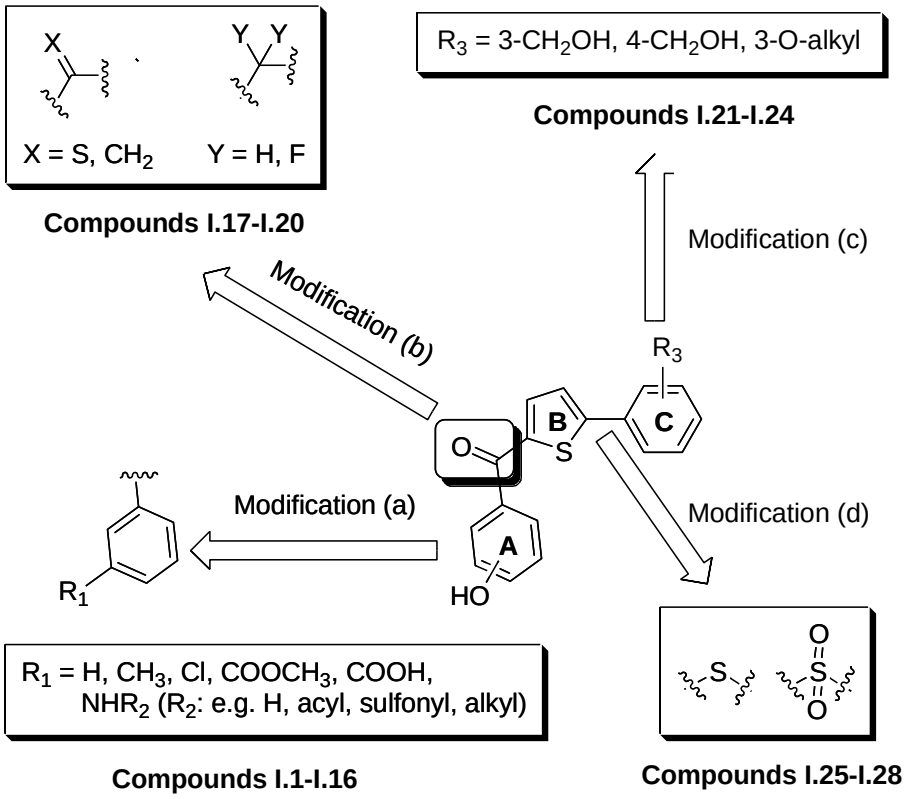
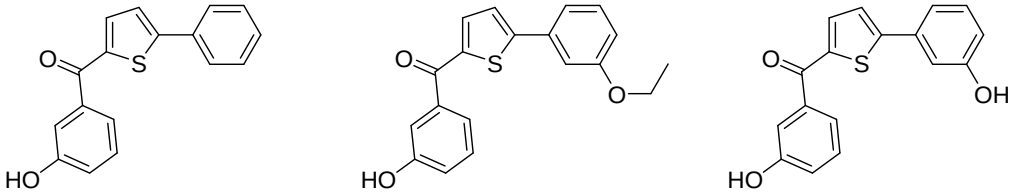


Figure 3. Design of potential inhibitors **I.1-I.28**.

In modification a, an appropriate replacement of the hydroxy-group at the benzoyl moiety (ring A), which bears the risk to lead to phase II metabolism issues, were investigated. The substituents were chosen (H, CH_3, Cl, CO_2CH_3, CO_2H and NH_2; Compounds **I.1**-**I.6** respectively) taking into account the Craig plot (variation of the size, lipophilicity, and electronic properties). Unfortunately, these compounds were inactive or showed only marginal inhibition of *h*17β-HSD1 compared to the reference compounds **A** or **B** (figure 4). From this series, compound **I.6** showed 24 % inhibition @ 1 µM of *h*17β-HSD1 enzyme. Therefore, attempts were made to enhance the inhibitory activity of compound **I.6** by modulating the hydrogen bond donating properties of the amino group (compounds **I.7**-**I.16**). Probably due to the bulkiness of the introduced group, a complete loss of activity obtained.

In contrast to the OH-group of the benzoyl moiety (ring A), replacement of the OH-group of the phenyl ring C by different small or bulky groups (compounds **I.21**-**I.24**, modification c) led to highly active compounds (IC_{50} values = 90 to 157 nM).



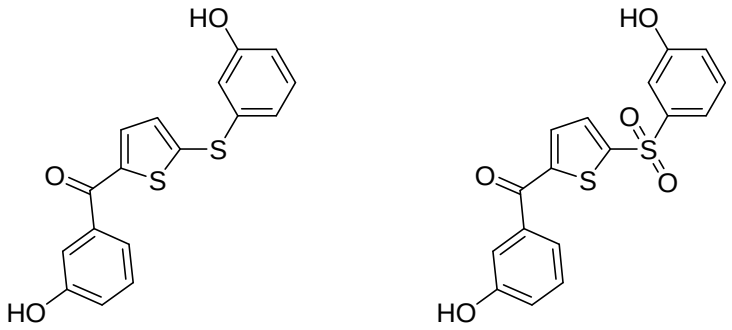
% inhibition @ 1 µM (*h*17β-HSD1) **A,** 80 % **B,** 88 % **C,** 89 %

Figure 4. Reference compounds of modifications a-d.

To complete the SAR and estimate the validity of the docking results, we replaced the keto-linker function by different linker groups, namely CF_2, C=S, CH_2, $C=CH_2$ (modification b, compounds **I.17**-**I.20**. This modification had a strong impact on biological activity, depending on the nature of the linker group. Only the thioketo-analog **I.18** showed comparable activity (75 % inhibition @ 1 µM of *h*17β-HSD1 enzyme) to the reference compound **A** (figure 4). The biological data obtained for compounds **I.17**-**I.20** is in agreement with former molecular docking

studies which suggest a bifurcated H-bond between the carbonyl group and the OH-groups of Ser142 and Tyr155.

A second linker containing oxygen was selected to possibly establish additional hydrogen bond interactions with the OH groups of Tyr218 and Ser222 (compounds **I.26** and **I.28**, modification d). Compounds bearing an additional linker group (**I.25** and **I.26**, figure 5), showed comparable IC_{50}-values (IC_{50} = 104 and 275 nM, respectively) for the inhibition of *h*17β-HSD1.



I.25 **I.26**

Figure 5. Structures of compounds **I.25** and **I.26**

Docking results suggested a different binding mode for the sulfone **I.26** compared to the BSHs class which precludes the establishment of these hydrogen bond interactions (figure 6). Interestingly, in contrast to most of the compound classes described by us, compound **I.25** turned to be an inhibitor of *murine* 17β-HSD1 (20 % inhibition @ 1 µM; **C**: 10%).

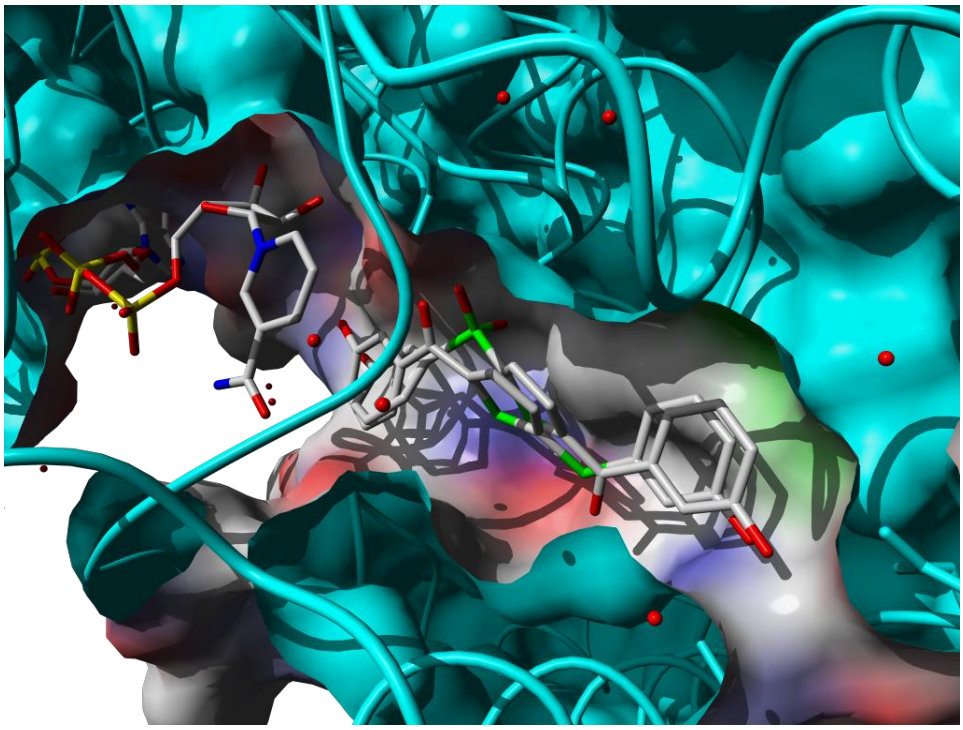


Figure 6. Compounds **I.25** and **I.26**, docked into *human* 17β-HSD1 (PDB: 1FDT). Upper left: Cofactor NADPH.

In contrast to most other classes of 17β-HSD1 inhibitors described by us, both BSHs and the thioether classes contain members which not only are strong inhibitors of the human enzyme but also showed inhibition of the murine ortholog (unpublished results). Since BSHs showed better selectivity over *m*17β-HSD2 compared to the thioether class, we focused on the former for further optimization (chapter **3.II**) in order to obtain a suitable candidate which could be used to conduct a PoP study in an animal disease model for EDDs.

Basically, existing BSHs have two important drawbacks: a) low *m*17β-HSD1 inhibition and b) low metabolic stability (phase I and/or phase II). Thus, the aim of this part was to obtain a potent, selective, and metabolically stable (S9 fraction) *h*/*m* 17β-HSD1 inhibitor. In addition, the establishment of favorable *in vitro* ADME parameters (lipophilicity, solubility) was taken into account.

Starting point in the design of this part were BSHs **I** and **II** (figure 7).

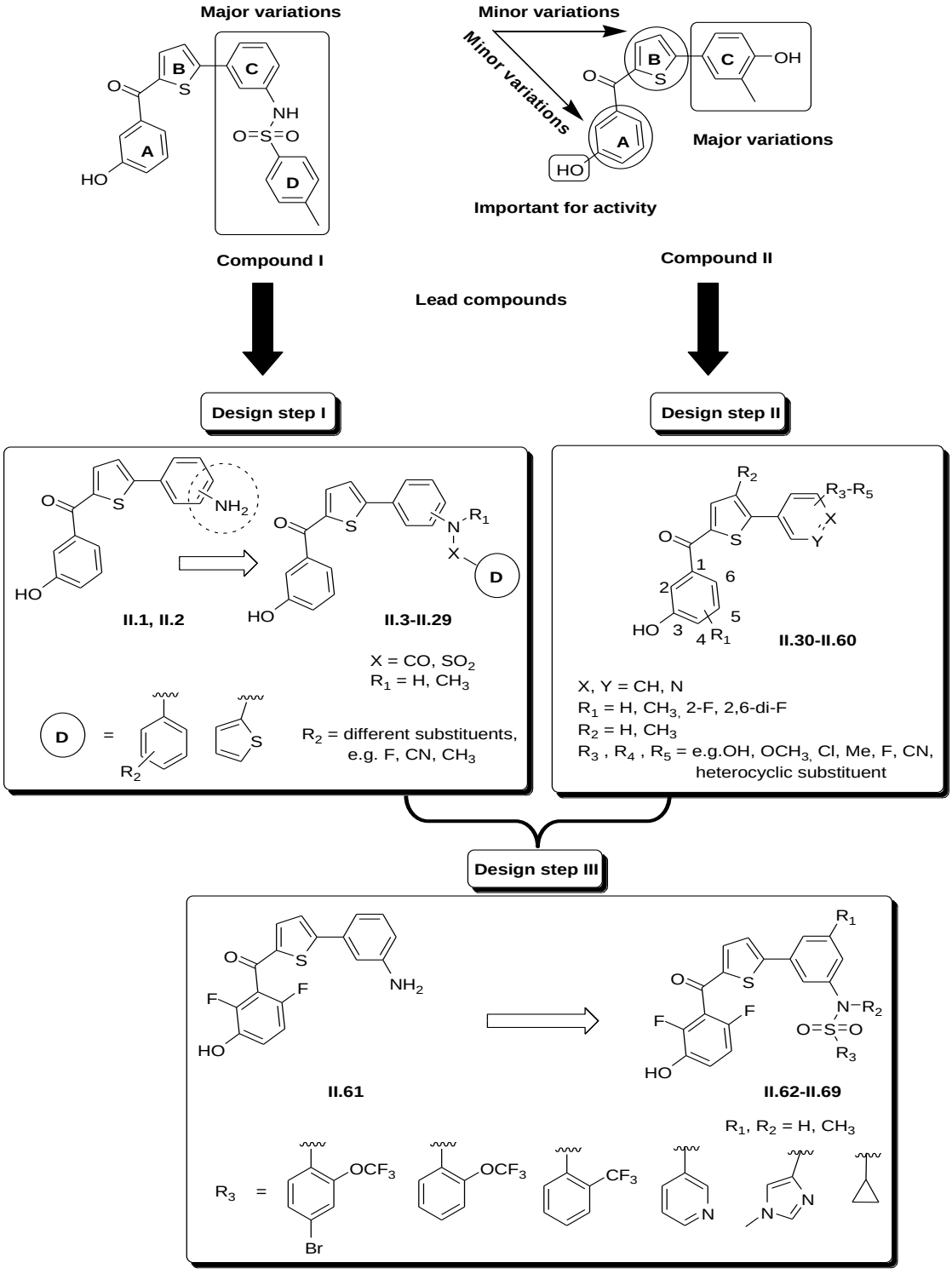
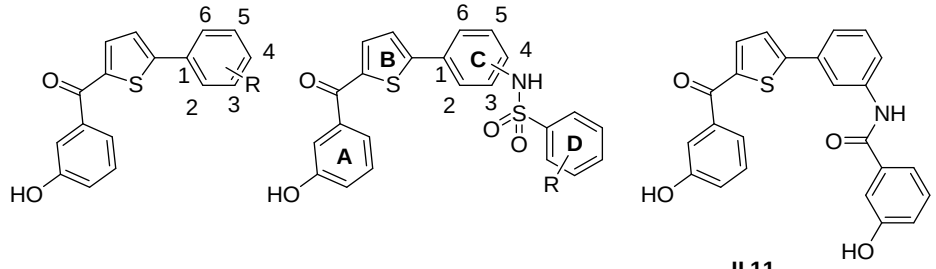


Figure 7. Design of Synthesized Compounds **II.1 – II.69**

Design step I

Variations in ring C of the lead compound **I** led to introduction of the sulfonamide moiety in the *m*-position (e.g. compound **II.7**) which is clearly favored over the *p*-position (e.g. compound **II.4**), indicating that there is no space in the *p*-position for bulky groups. Furthermore, the presence of the sulfonamide group (compound **II.10**) led to slightly increased inhibitory activity towards *h*17β-HSD1 compared to its carbonic acid amide analog (compound **II.11**), table 1.

Table 1. Inhibition of human/murine 17β-HSD1 and human 17β-HSD2 by compounds **II.1, II.2, II.4, II.7, II.10 and II.11**.



Comd	Ring C	R	IC_{50} [nM][a]		SF[d]	% Inhibition @ 1 µM[a]
			*h*17β-HSD1[b]	*h*17β-HSD2[c]		*m*17β-HSD1[e]
II.1	-	4-NH_2	145	120	0.8	4
II.2	-	3-NH_2	77 %[f]	68 %[f]	-	n.d
II.4	4	4-Br-2-OCF_3	169	716	4.2	n.d
II.7	3	4-Br-2-OCF_3	8	199	25.0	21
II.10	3	3-OH	16	88	5.4	26
II.11	-	H	33	159	4.8	26

[a] Mean value of three determinations, standard deviation less than 15%. [b] Human placenta, cytosolic fraction, substrate [^{3}H]-E1, 500 nM, cofactor NADH, 500 µM. [c] Human placenta, microsomal fraction, substrate [^{3}H]-E2, 500 nM, cofactor NAD^+, 1500 µM. [d] Selectivity factor: IC_{50}(17β-HSD2) / IC_{50}(17β-HSD1). [e] Mouse recombinant enzyme, expressed in HEK 293, substrate [^{3}H]-E1, 10 nM, cofactor NADH, 500 µM. [f] Inhibition @ 1µM (inhibitor concentration).

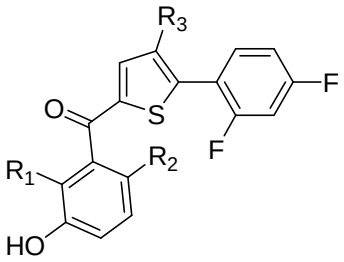
In addition to low metabolic stability and low *m*17β-HSD1 inhibition, e.g. compound **II.7** (% remaining after 15 min = 47, S9 fraction) and (% inhibition @ 1 μM = 21), the high molecular weight sulfonamide compounds (ABCD ring system, fig. 7) did not show advantage over the small molecules (ABC ring system). This was a motivation for us to focus on the three ring compounds in an attempt to improve both *m*17β-HSD1 inhibition and s9 metabolic stability.

Design step II

From our previous work, it was known that only minor structural variations in ring A (fig. 7) are tolerated by the enzyme. Therefore, we introduced fluorine as an electron withdrawing group (EWG) or methyl as a bulky group in an attempt to decrease the liability of this OH-group - which is very important for activity inhibitory and could not be replaced (see chapter **3.I**) - against phase II metabolism.

In contrast to the methyl group, which led to a strong decrease in *h*17β-HSD1 inhibition and reversed the selectivity over type 2 enzyme, fluorine showed a strong increase of activity inhibitory towards the type 1 enzyme, though with the expense of losing selectivity over *h*17β-HSD2 (e.g. compounds **II.30**-**II.34**, table 2).

Table 2. Inhibition of *human*/*murine* 17β-HSD1 and *human* 17β-HSD2 by compounds **II.30-II.34**.



II.30-II.34

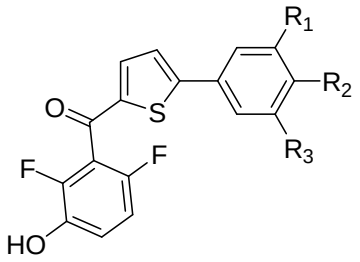
Comd	R_1	R_2	R_3	IC_{50} [nM][a]		SF[d]	% Inhibition @ 1 µM[a]
				17β-HSD1[b]	17β-HSD2[c]		*m*17β-HSD1[e]
II.30	H	H	H	213[g]	177[g]	0.8	n.d
II.31	CH_3	H	H	3427	299	0.09	5
II.32	CH_3	H	CH_3	20 %[f]	66 %[f]	-	n.d
II.33	F	H	H	11[h]	8[h]	0.7	n.d
II.34	F	F	H	3.5	9	2.5	41

[a-f] See table 1. [g] Calculated $IC_{50}(IC_{50}(Logit))$ = test concentration ((100-%inh.)/%inh.).

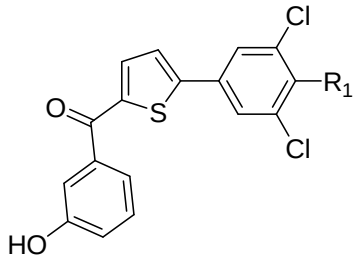
This may be explained by the effect of the fluorine on the hydrogen bonding properties of the OH-group or by interacting with carbon atoms of carbonyl groups of the target.

Concerning ring C, which was investigated in the next step, we found that the OH-group in *p*-position is important to enhance the selectivity over the type 2 enzyme. Therefore, we shielded this OH-group by bulky and/or EWGs in an attempt to protect it against phase II metabolism. By applying this strategy, we obtained the most potent non-steroidal *h*17β-HSD1 inhibitors described so far (IC_{50}-values in the picomolar range, e.g. compounds **II.42-II.47**). On other hand, this series showed a significant increase in *m*17β-HSD1 inhibition. (Table 3)

Table 3. Inhibition of human/murine 17β-HSD1 and human 17β-HSD2 by compounds **II.42-II.47**, **II.54**, **II.55**



II.42-II.51



II.54, II.55

Comd	**R_1**	**R_2**	**R_3**	**IC_{50} [nM][a]**		**SF[d]**	**% Inhibition @ 1 µM[a]**
				17β-HSD1[b]	**17β-HSD2[c]**		***m*17β-HSD1[e]**
II.42	Cl	OCH_3	Cl	0.5	41	82	43
II.43	Cl	OH	Cl	2.4	39	16	23
II.44	Cl	OCH_3	CH_3	0.3	7	23	32

II.45	Cl	OH	CH_3	0.2	6	30	43
II.46	CH_3	OCH_3	CH_3	0.9	18	20	59
II.47	CH_3	OH	CH_3	0.4	7	20	77
II.54	OCH_3	-	-	6.0	247	40.4	n.d
II.55	OH	-	-	31[g]	255[g]	8.3	n.d

[a-e] See table 1. [g] Calculated IC_{50}(IC_{50}(Logit)) = test concentration ((100-%inh.)/%inh.).

Apparently, the approach to enhance metabolic stability by introducing fluorine (in ring A, fig. 7) and by shielding the OH-group (ring C, fig. 7) by two chlorine atoms as EWG and bulky groups was successful as evidenced by the biological data of compounds **II.42-II.47** and **II.55** (table 4).

Table 4. Metabolic stability of selected compounds using human hepatic S9-fraction or liver microsomes.

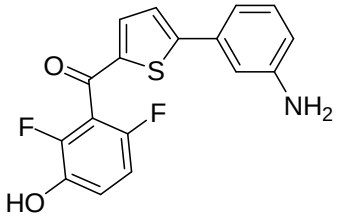
Comd	**% remaining after**		**Comd**	**% remaining after**	
	15 min	**60 min**		**15 min**	**60 min**
	S9[a]	**S9[a]**		**S9[a]**	**Phase I[b]**
II.42	56	40	**II.46**	29	-
II.43	91	72	**II.47**	6	61
II.44	49	13	**II.55**	21	-
II.45	7				

[a] human hepatic S9 fraction: (1 mg/mL mixture of microsomes and cytosol of hepatocytes) supplemented with NADPH, UDPGA and PAPS. [b] Phase I: supplemented only with NADPH.

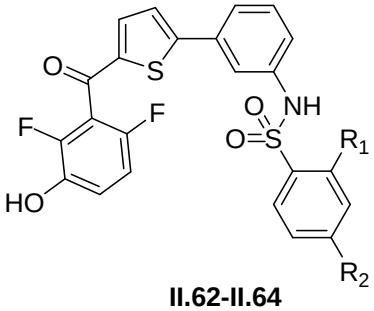
Design step III

Biological data suggested that both the presence of the sulfonamide moiety (design step I) and a fluorinated A ring (design step II, fig. 7) are beneficial for inhibition of mouse 17β-HSD1. Combining design steps I and II in design step III, in which fluorinated-sulfonamide type inhibitors synthesized, led to the most active *m*17β-HSD1 inhibitors in this study (compounds **II.61-II64**, table 5).

Table 5. Inhibition of human/murine 17β-HSD1 and human 17β-HSD2 by compounds **II.61-II64**



II.61



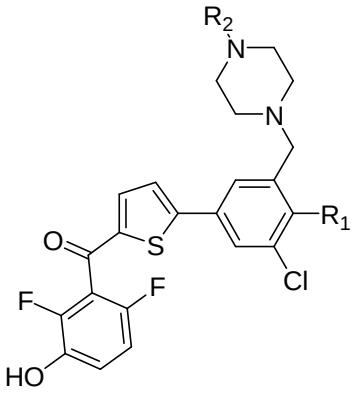
II.62-II.64

Comd	R_1	R_2	IC_{50} [nM][a]		SF[d]	% Inhibition @ 1 µM[a]
			17β-HSD1[b]	17β-HSD2[c]		*m*17β-HSD1[e]
II.61	-	-	10	25	2.5	30
II.62	OCF_3	Br	1.7	17	10.0	71
II.63	OCF_3	H	3.1	13	4.1	91
II.64	CF_3	H	2.3	6	2.5	97

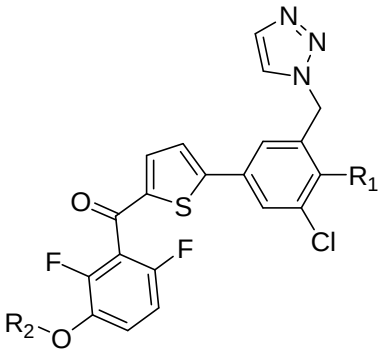
[a-e] See table 1.

Last step in the design was the improvement of the ADME parameters concerning solubility and lipophilicity. The tolerance of the target protein towards substitution of the phenyl ring (ring C, fig. 7) allowed for the introduction of different hydrophilic heterocyclic substituents (e.g. compounds **II.56-II.60**, table 6).

Table 6. Inhibition of human 17β-HSD1 and 17β-HSD2 by compounds **II.56-II.60** and **II.59a**.



II.56-II.58



II.59a, II.59, II.60

Comd	**R_1**	**R_2**	**IC_{50} [nM][a]**		**SF[d]**	**LogP[f]**	**LogD@ pH7.4[e]**
			17β-HSD1[b]	**17β-HSD2[c]**			
II.56	OCH_3	$COCH_3$	1.4	21	15.0	3.21	3.1
II.57	OH	$COCH_3$	2.1	11	5.2	2.63	2.2
II.58	OCH_3	H	4.7	26	5.5	3.41	1.7
II.59a	OCH_3	$COCH_3$	26[f]	21[f]	0.8	3.34	3.3
II.59	OCH_3	H	2.1	8.8	4.2	3.44	3.3
II.60	OH	H	17[f]	26[f]	1.5	2.86	2.3

[a-d] See table 1. [e] Calculated LogP and LogD@ pH 7.4 data. [f] Calculated IC_{50}(IC_{50}(Logit)) = test concentration ((100-%inh.)/%inh.).

Selected compounds were submitted to further biological evaluation in attempt to identify a suitable candidate for PoP study. Parameters under evaluation included: affinity to the estrogen receptors α and β, inhibition of *rat* 17β-HSD1 and cytotoxicity. Compound **II.47** seems to be a possible candidate for PoP study with metabolic stability drawback. Therefore, this compound should be submitted to further optimization as a lead compound.

In conclusion, a sharp SAR for BSHs is obtained, one of the most active classes of non-steroidal 17β-HSD1 inhibitors. Through this investigation, the most potent 17β-HSD1 inhibitors up to date with good selectivity towards the type 2 enzyme and no or little affinity to ER α and β were designed. In addition, we could significantly increase the activity in both *murine* and *rat* 17β-HSD1 enzyme, which is essential for a PoP study in rodents. Furthermore, compound **II.43** (IC_{50} = 2.5 nM, SF = 16) showed a very good metabolic stability (human S9 fraction) issue with 72 % remaining after 60 min.

5 References

1. Guerriero, G. Vertebrate sex steroid receptors: evolution, ligands, and neurodistribution. *Ann N Y Acad Sci* **2009,** 1163, 154-68.

2. Labrie, F. Future perspectives of selective estrogen receptor modulators used alone and in combination with DHEA. *Endocr Relat Cancer* **2006,** 13, 335-55.

3. Labrie, F. Intracrinology. *Mol Cell Endocrinol* **1991,** 78, C113-8.

4. Labrie, F.; Luu-The, V.; Lin, S. X.; Simard, J.; Labrie, C.; El-Alfy, M.; Pelletier, G.; Belanger, A. Intracrinology: role of the family of 17 beta-hydroxysteroid dehydrogenases in human physiology and disease. *J Mol Endocrinol* **2000,** 25, 1-16.

5. Vihko, P.; Herrala, A.; Harkonen, P.; Isomaa, V.; Kaija, H.; Kurkela, R.; Pulkka, A. Control of cell proliferation by steroids: the role of 17HSDs. *Mol Cell Endocrinol* **2006,** 248, 141-8.

6. Mutschler, E.; Geisslinger, G.; Kroemer, H. K.; Ruth, P.; Schäfer-Korting, M. Arzneimittelwirkungen. *Lehrbuch der Pharmakologie und Toxikologie, 9. Aufl.; Wissenschaftliche Verlagsgesellschaft: Stuttgart* **2008**.

7. Heldring, N.; Pike, A.; Andersson, S.; Matthews, J.; Cheng, G.; Hartman, J.; Tujague, M.; Strom, A.; Treuter, E.; Warner, M.; Gustafsson, J. A. Estrogen receptors: how do they signal and what are their targets. *Physiol Rev* **2007,** 87, 905-31.

8. Gruber, C. J.; Tschugguel, W.; Schneeberger, C.; Huber, J. C. Production and actions of estrogens. *N Engl J Med* **2002,** 346, 340-52.

9. Khosla, S.; Oursler, M. J.; Monroe, D. G. Estrogen and the skeleton. *Trends Endocrinol Metab* 23, 576-81.

10. Nelson, L. R.; Bulun, S. E. Estrogen production and action. *J Am Acad Dermatol* **2001,** 45, S116-24.

11. Krebs in Deutschland 2007/2008. 8. Ausgabe. Robert Koch-Institut (Hrsg.) und die Gesellschaft der epidemiologischen Krebsregister in Deutschland e.V. (Hrsg.). Berlin. **2012**.

12. Thomas, H. V.; Reeves, G. K.; Key, T. J. Endogenous estrogen and postmenopausal breast cancer: a quantitative review. *Cancer Causes Control* **1997,** 8, 922-8.

13. Althuis, M. D.; Fergenbaum, J. H.; Garcia-Closas, M.; Brinton, L. A.; Madigan, M. P.; Sherman, M. E. Etiology of hormone receptor-defined breast cancer: a systematic review of the literature. *Cancer Epidemiol Biomarkers Prev* **2004,** 13, 1558-68.

14. Thomas, D. B. Do hormones cause breast cancer? *Cancer* **1984,** 53, 595-604.

15. Vihko, R.; Apter, D. Endogenous steroids in the pathophysiology of breast cancer. *Crit Rev Oncol Hematol* **1989,** 9, 1-16.

16. Lower, E. E.; Blau, R.; Gazder, P.; Stahl, D. L. The effect of estrogen usage on the subsequent hormone receptor status of primary breast cancer. *Breast Cancer Res Treat* **1999,** 58, 205-11.

17. Day, J. M.; Foster, P. A.; Tutill, H. J.; Parsons, M. F.; Newman, S. P.; Chander, S. K.; Allan, G. M.; Lawrence, H. R.; Vicker, N.; Potter, B. V.; Reed, M. J.; Purohit, A. 17beta-hydroxysteroid dehydrogenase Type 1, and not Type 12, is a target for endocrine therapy of hormone-dependent breast cancer. *Int J Cancer* **2008,** 122, 1931-40.

18. Thorsen, T.; Tangen, M.; Stoa, K. F. Concentration of endogenous oestradiol as related to oestradiol receptor sites in breast tumor cytosol. *Eur J Cancer Clin Oncol* **1982,** 18, 333-7.

19. van Landeghem, A. A.; Poortman, J.; Nabuurs, M.; Thijssen, J. H. Endogenous concentration and subcellular distribution of estrogens in normal and malignant human breast tissue. *Cancer Res* **1985,** 45, 2900-6.

20. Miller, W. R.; O'Neill, J. The importance of local synthesis of estrogen within the breast. *Steroids* **1987,** 50, 537-48.

21. O'Neill, J. S.; Elton, R. A.; Miller, W. R. Aromatase activity in adipose tissue from breast quadrants: a link with tumour site. *Br Med J (Clin Res Ed)* **1988,** 296, 741-3.

22. Reed, M. J.; Aherne, G. W.; Ghilchik, M. W.; Patel, S.; Chakraborty, J. Concentrations of oestrone and 4-hydroxyandrostenedione in malignant and normal breast tissues. *Int J Cancer* **1991,** 49, 562-5.

23. Abul-Hajj, Y. J.; Iverson, R.; Kiang, D. T. Aromatization of androgens by human breast cancer. *Steroids* **1979,** 33, 205-22.

24. Lipton, A.; Santner, S. J.; Santen, R. J.; Harvey, H. A.; Feil, P. D.; White-Hershey, D.; Bartholomew, M. J.; Antle, C. E. Aromatase activity in primary and metastatic human breast cancer. *Cancer* **1987,** 59, 779-82.

25. Perel, E.; Daniilescu, D.; Kharlip, L.; Blackstein, M.; Killinger, D. W. Steroid modulation of aromatase activity in human cultured breast carcinoma cells. *J Steroid Biochem* **1988,** 29, 393-9.

26. Pollow, K.; Boquoi, E.; Baumann, J.; Schmidt-Gollwitzer, M.; Pollow, B. Comparison of the in vitro conversion of estradiol-17 beta to estrone of normal and neoplastic human breast tissue. *Mol Cell Endocrinol* **1977,** 6, 333-48.

27. Abul-Hajj, Y. J. Relationship between estrogen receptors, 17 beta-hydroxysteroid dehydrogenase and estrogen content in human breast cancer. *Steroids* **1979,** 34, 217-25.

28. Vermeulen, A.; Deslypere, J. P.; Paridaens, R.; Leclercq, G.; Roy, F.; Heuson, J. C. Aromatase, 17 beta-hydroxysteroid dehydrogenase and intratissular sex hormone concentrations in cancerous and normal glandular breast tissue in postmenopausal women. *Eur J Cancer Clin Oncol* **1986,** 22, 515-25.

29. Miyoshi, Y.; Ando, A.; Shiba, E.; Taguchi, T.; Tamaki, Y.; Noguchi, S. Involvement of up-regulation of 17beta-hydroxysteroid dehydrogenase type 1 in maintenance of intratumoral high estradiol levels in postmenopausal breast cancers. *Int J Cancer* **2001,** 94, 685-9.

30. Day, J. M.; Tutill, H. J.; Purohit, A.; Reed, M. J. Design and validation of specific inhibitors of 17beta-hydroxysteroid dehydrogenases for therapeutic application in breast and prostate cancer, and in endometriosis. *Endocr Relat Cancer* **2008,** 15, 665-92.

31. Fisher, B.; Dignam, J.; Tan-Chiu, E.; Anderson, S.; Fisher, E. R.; Wittliff, J. L.; Wolmark, N. Prognosis and treatment of patients with breast tumors of one centimeter or less and negative axillary lymph nodes. *J Natl Cancer Inst* **2001,** 93, 112-20.

32. Fisher, B.; Anderson, S.; Tan-Chiu, E.; Wolmark, N.; Wickerham, D. L.; Fisher, E. R.; Dimitrov, N. V.; Atkins, J. N.; Abramson, N.; Merajver, S.; Romond, E. H.; Kardinal, C. G.; Shibata, H. R.; Margolese, R. G.; Farrar, W. B. Tamoxifen and chemotherapy for axillary node-negative, estrogen receptor-negative breast cancer: findings from National Surgical Adjuvant Breast and Bowel Project B-23. *J Clin Oncol* **2001,** 19, 931-42.

33. Adamo, V.; Iorfida, M.; Montalto, E.; Festa, V.; Garipoli, C.; Scimone, A.; Zanghi, M.; Caristi, N. Overview and new strategies in metastatic breast cancer (MBC) for treatment of tamoxifen-resistant patients. *Ann Oncol* **2007,** 18 Suppl 6, vi53-7.

34. Miller, L. A.; Roy, A.; Mody, R.; Higa, G. M. Comparative economic analysis of aromatase inhibitors and tamoxifen in the treatment of hormone-dependent breast cancer. *Expert Opin Pharmacother* **2007,** 8, 1675-91.

35. Emons, G.; Grundker, C.; Gunthert, A. R.; Westphalen, S.; Kavanagh, J.; Verschraegen, C. GnRH antagonists in the treatment of gynecological and breast cancers. *Endocr Relat Cancer* **2003,** 10, 291-9.

36. Sampson, J. A. Metastatic or Embolic Endometriosis, due to the Menstrual Dissemination of Endometrial Tissue into the Venous Circulation. *Am J Pathol* **1927,** 3, 93-110 43.

37. Bulun, S. E.; Zeitoun, K. M.; Takayama, K.; Sasano, H. Estrogen biosynthesis in endometriosis: molecular basis and clinical relevance. *J Mol Endocrinol* **2000,** 25, 35-42.

38. Laschke, M. W.; Elitzsch, A.; Scheuer, C.; Vollmar, B.; Menger, M. D. Selective cyclo-oxygenase-2 inhibition induces regression of autologous endometrial grafts by down-regulation of vascular endothelial growth factor-mediated angiogenesis and stimulation of caspase-3-dependent apoptosis. *Fertil Steril* **2007,** 87, 163-71.

39. Berkley, K. J.; Rapkin, A. J.; Papka, R. E. The pains of endometriosis. *Science* **2005,** 308, 1587-9.

40. DeMichele, A.; Troxel, A. B.; Berlin, J. A.; Weber, A. L.; Bunin, G. R.; Turzo, E.; Schinnar, R.; Burgh, D.; Berlin, M.; Rubin, S. C.; Rebbeck, T. R.; Strom, B. L. Impact of raloxifene or tamoxifen use on endometrial cancer risk: a population-based case-control study. *J Clin Oncol* **2008,** 26, 4151-9.

41. Saadat, M.; Truong, P. T.; Kader, H. A.; Speers, C. H.; Berthelet, E.; McMurtrie, E.; Olivotto, I. A. Outcomes in patients with primary breast cancer and a subsequent diagnosis of endometrial cancer : comparison of cohorts treated with and without tamoxifen. *Cancer* **2007,** 110, 31-7.

42. Urruticoechea, A. The oestrogen-dependent biology of breast cancer. Sensitivity and resistance to aromatase inhibitors revisited: a molecular perspective. *Clin Transl Oncol* **2007,** 9, 752-9.

43. Ortmann, O.; Cufer, T.; Dixon, J. M.; Maass, N.; Marchetti, P.; Pagani, O.; Pronzato, P.; Semiglazov, V.; Spano, J. P.; Vrdoljak, E.; Wildiers, H. Adjuvant endocrine therapy for perimenopausal women with early breast cancer. *Breast* **2009,** 18, 2-7.

44. Hoffmann, F.; Maser, E. Carbonyl reductases and pluripotent hydroxysteroid dehydrogenases of the short-chain dehydrogenase/reductase superfamily. *Drug Metab Rev* **2007,** 39, 87-144.

45. Maser, E. Xenobiotic carbonyl reduction and physiological steroid oxidoreduction. The pluripotency of several hydroxysteroid dehydrogenases. *Biochem Pharmacol* **1995,** 49, 421-40.

46. Luu-The, V. Analysis and characteristics of multiple types of human 17beta-hydroxysteroid dehydrogenase. *J Steroid Biochem Mol Biol* **2001,** 76, 143-51.

47. Moeller, G.; Adamski, J. Integrated view on 17beta-hydroxysteroid dehydrogenases. *Mol Cell Endocrinol* **2009,** 301, 7-19.

48. Prehn, C.; Moller, G.; Adamski, J. Recent advances in 17beta-hydroxysteroid dehydrogenases. *J Steroid Biochem Mol Biol* **2009,** 114, 72-7.

49. Penning, T. M.; Byrns, M. C. Steroid hormone transforming aldo-keto reductases and cancer. *Ann N Y Acad Sci* **2009,** 1155, 33-42.

50. Lukacik, P.; Kavanagh, K. L.; Oppermann, U. Structure and function of human 17beta-hydroxysteroid dehydrogenases. *Mol Cell Endocrinol* **2006,** 248, 61-71.

51. Duax, W. L.; Ghosh, D.; Pletnev, V. Steroid dehydrogenase structures, mechanism of action, and disease. *Vitam Horm* **2000,** 58, 121-48.

52. Penning, T. M. Molecular endocrinology of hydroxysteroid dehydrogenases. *Endocr Rev* **1997,** 18, 281-305.

53. Penning, T. M. Hydroxysteroid dehydrogenases and pre-receptor regulation of steroid hormone action. *Hum Reprod Update* **2003,** 9, 193-205.

54. Gobbi, S.; Cavalli, A.; Rampa, A.; Belluti, F.; Piazzi, L.; Paluszcak, A.; Hartmann, R. W.; Recanatini, M.; Bisi, A. Lead optimization providing a series of flavone derivatives as potent nonsteroidal inhibitors of the cytochrome P450 aromatase enzyme. *J Med Chem* **2006,** 49, 4777-80.

55. Le Borgne, M.; Marchand, P.; Duflos, M.; Delevoye-Seiller, B.; Piessard-Robert, S.; Le Baut, G.; Hartmann, R. W.; Palzer, M. Synthesis and in vitro evaluation of 3-(1-azolylmethyl)-1H-indoles and 3-(1-azolyl-1-phenylmethyl)-1H-indoles as inhibitors of P450 arom. *Arch Pharm (Weinheim)* **1997,** 330, 141-5.

56. Aggarwal, S.; Thareja, S.; Verma, A.; Bhardwaj, T. R.; Kumar, M. An overview on 5alpha-reductase inhibitors. *Steroids* 75, 109-53.

57. Baston, E.; Salem, O. I.; Hartmann, R. W. 6-Substituted 3,4-dihydro-naphthalene-2-carboxylic acids: synthesis and structure-activity studies in a novel class of human 5alpha reductase inhibitors. *J Enzyme Inhib Med Chem* **2002,** 17, 303-20.

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

59. Peltoketo, H.; Luu-The, V.; Simard, J.; Adamski, J. 17beta-hydroxysteroid dehydrogenase (HSD)/17-ketosteroid reductase (KSR) family; nomenclature and main characteristics of the 17HSD/KSR enzymes. *J Mol Endocrinol* **1999,** 23, 1-11.

60. Miettinen, M. M.; Mustonen, M. V.; Poutanen, M. H.; Isomaa, V. V.; Vihko, R. K. Human 17 beta-hydroxysteroid dehydrogenase type 1 and type 2 isoenzymes have opposite activities in cultured cells and characteristic cell- and tissue-specific expression. *Biochem J* **1996,** 314 (Pt 3), 839-45.

61. Sherbet, D. P.; Papari-Zareei, M.; Khan, N.; Sharma, K. K.; Brandmaier, A.; Rambally, S.; Chattopadhyay, A.; Andersson, S.; Agarwal, A. K.; Auchus, R. J. Cofactors, redox state, and directional preferences of hydroxysteroid dehydrogenases. *Mol Cell Endocrinol* **2007,** 265-266, 83-8.

62. Huang, Y. W.; Pineau, I.; Chang, H. J.; Azzi, A.; Bellemare, V.; Laberge, S.; Lin, S. X. Critical residues for the specificity of cofactors and substrates in human estrogenic

17beta-hydroxysteroid dehydrogenase 1: variants designed from the three-dimensional structure of the enzyme. *Mol Endocrinol* **2001,** 15, 2010-20.

63. McKeever, B. M.; Hawkins, B. K.; Geissler, W. M.; Wu, L.; Sheridan, R. P.; Mosley, R. T.; Andersson, S. Amino acid substitution of arginine 80 in 17beta-hydroxysteroid dehydrogenase type 3 and its effect on NADPH cofactor binding and oxidation/reduction kinetics. *Biochim Biophys Acta* **2002,** 1601, 29-37.

64. Sherbet, D. P.; Guryev, O. L.; Papari-Zareei, M.; Mizrachi, D.; Rambally, S.; Akbar, S.; Auchus, R. J. Biochemical factors governing the steady-state estrone/estradiol ratios catalyzed by human 17beta-hydroxysteroid dehydrogenases types 1 and 2 in HEK-293 cells. *Endocrinology* **2009,** 150, 4154-62.

65. Jansson, A. 17Beta-hydroxysteroid dehydrogenase enzymes and breast cancer. *J Steroid Biochem Mol Biol* **2009,** 114, 64-7.

66. Smuc, T.; Pucelj, M. R.; Sinkovec, J.; Husen, B.; Thole, H.; Lanisnik Rizner, T. Expression analysis of the genes involved in estradiol and progesterone action in human ovarian endometriosis. *Gynecol Endocrinol* **2007,** 23, 105-11.

67. Bulun, S. E.; Zeitoun, K.; Takayama, K.; Noble, L.; Michael, D.; Simpson, E.; Johns, A.; Putman, M.; Sasano, H. Estrogen production in endometriosis and use of aromatase inhibitors to treat endometriosis. *Endocr Relat Cancer* **1999,** 6, 293-301.

68. Dassen, H.; Punyadeera, C.; Kamps, R.; Delvoux, B.; Van Langendonckt, A.; Donnez, J.; Husen, B.; Thole, H.; Dunselman, G.; Groothuis, P. Estrogen metabolizing enzymes in endometrium and endometriosis. *Hum Reprod* **2007,** 22, 3148-58.

69. Peltoketo, H.; Isomaa, V.; Maentausta, O.; Vihko, R. Complete amino acid sequence of human placental 17 beta-hydroxysteroid dehydrogenase deduced from cDNA. *FEBS Lett* **1988,** 239, 73-7.

70. Berman, H. M.; Westbrook, J.; Feng, Z.; Gilliland, G.; Bhat, T. N.; Weissig, H.; Shindyalov, I. N.; Bourne, P. E. The Protein Data Bank. *Nucleic Acids Res* **2000,** 28, 235-42.

71. Ghosh, D.; Pletnev, V. Z.; Zhu, D. W.; Wawrzak, Z.; Duax, W. L.; Pangborn, W.; Labrie, F.; Lin, S. X. Structure of human estrogenic 17 beta-hydroxysteroid dehydrogenase at 2.20 A resolution. *Structure* **1995,** 3, 503-13.

72. Breton, R.; Housset, D.; Mazza, C.; Fontecilla-Camps, J. C. The structure of a complex of human 17beta-hydroxysteroid dehydrogenase with estradiol and NADP+ identifies two principal targets for the design of inhibitors. *Structure* **1996,** 4, 905-15.

73. Hwang, C. C.; Chang, Y. H.; Hsu, C. N.; Hsu, H. H.; Li, C. W.; Pon, H. I. Mechanistic roles of Ser-114, Tyr-155, and Lys-159 in 3alpha-hydroxysteroid dehydrogenase/carbonyl reductase from Comamonas testosteroni. *J Biol Chem* **2005,** 280, 3522-8.

74. Filling, C.; Berndt, K. D.; Benach, J.; Knapp, S.; Prozorovski, T.; Nordling, E.; Ladenstein, R.; Jornvall, H.; Oppermann, U. Critical residues for structure and catalysis in short-chain dehydrogenases/reductases. *J Biol Chem* **2002,** 277, 25677-84.

75. Saloniemi, T.; Jarvensivu, P.; Koskimies, P.; Jokela, H.; Lamminen, T.; Ghaem-Maghami, S.; Dina, R.; Damdimopoulou, P.; Makela, S.; Perheentupa, A.; Kujari, H.; Brosens, J.; Poutanen, M. Novel hydroxysteroid (17beta) dehydrogenase 1 inhibitors reverse estrogen-induced endometrial hyperplasia in transgenic mice. *Am J Pathol* **2010,** 176, 1443-51.

76. Husen, B.; Huhtinen, K.; Poutanen, M.; Kangas, L.; Messinger, J.; Thole, H. Evaluation of inhibitors for 17beta-hydroxysteroid dehydrogenase type 1 in vivo in immunodeficient mice inoculated with MCF-7 cells stably expressing the recombinant human enzyme. *Mol Cell Endocrinol* **2006,** 248, 109-13.

77. Husen, B.; Huhtinen, K.; Saloniemi, T.; Messinger, J.; Thole, H. H.; Poutanen, M. Human hydroxysteroid (17-beta) dehydrogenase 1 expression enhances estrogen sensitivity of MCF-7 breast cancer cell xenografts. *Endocrinology* **2006,** 147, 5333-9.

78. Messinger, J.; Hirvela, L.; Husen, B.; Kangas, L.; Koskimies, P.; Pentikainen, O.; Saarenketo, P.; Thole, H. New inhibitors of 17beta-hydroxysteroid dehydrogenase type 1. *Mol Cell Endocrinol* **2006,** 248, 192-8.

79. Grummer, R.; Schwarzer, F.; Bainczyk, K.; Hess-Stumpp, H.; Regidor, P. A.; Schindler, A. E.; Winterhager, E. Peritoneal endometriosis: validation of an in-vivo model. *Hum Reprod* **2001,** 16, 1736-43.

80. Firnhaber, S. Expression und Regulation von Enzymen des Östrogenmetabolismus in humanen Endometriumläsionen kultiviert in der Nacktmaus. *PhD-thesis, Universität Duisburg-Essen, essen Fachbereich Biologie und Geographie.* **2006**.

81. Lamminen, T.; Saloniemi, T.; Huhtinen, K.; Koskimies, P.; Messinger, J.; Husen, B.; Thole, H.; Poutanen, M. In vivo mouse model for analysis of hydroxysteroid (17beta) dehydrogenase 1 inhibitors. *Mol Cell Endocrinol* **2009,** 301, 158-62.

82. Day, J. M.; Tutill, H. J.; Purohit, A. 17beta-hydroxysteroid dehydrogenase inhibitors. *Minerva Endocrinol* **2010,** 35, 87-108.

83. Brozic, P.; Lanisnik Risner, T.; Gobec, S. Inhibitors of 17beta-hydroxysteroid dehydrogenase type 1. *Curr Med Chem* **2008,** 15, 137-50.

84. Poirier, D. Advances in development of inhibitors of 17beta hydroxysteroid dehydrogenases. *Anticancer Agents Med Chem* **2009,** 9, 642-60.

85. Poirier, D. Inhibitors of 17 beta-hydroxysteroid dehydrogenases. *Curr Med Chem* **2003,** 10, 453-77.

86. Poirier, D. 17beta-Hydroxysteroid dehydrogenase inhibitors: a patent review. *Expert Opin Ther Pat* **2010,** 20, 1123-45.

87. Lipinski, C. A. Drug-like properties and the causes of poor solubility and poor permeability. *J Pharmacol Toxicol Methods* **2000,** 44, 235-49.

88. Michiels, P. J.; Ludwig, C.; Stephan, M.; Fischer, C.; Moller, G.; Messinger, J.; van Dongen, M.; Thole, H.; Adamski, J.; Gunther, U. L. Ligand-based NMR spectra demonstrate an additional phytoestrogen binding site for 17beta-hydroxysteroid dehydrogenase type 1. *J Steroid Biochem Mol Biol* **2009,** 117, 93-8.

89. Qiu, W.; Campbell, R. L.; Gangloff, A.; Dupuis, P.; Boivin, R. P.; Tremblay, M. R.; Poirier, D.; Lin, S. X. A concerted, rational design of type 1 17beta-hydroxysteroid dehydrogenase inhibitors: estradiol-adenosine hybrids with high affinity. *FASEB J* **2002,** 16, 1829-31.

90. Berube, M.; Delagoutte, F.; Poirier, D. Preparation of 6beta-estradiol derivative libraries as bisubstrate inhibitors of 7beta-hydroxysteroid dehydrogenase type using the multidetachable sulfamate linker. *Molecules* **2010,** 15, 1590-631.

91. Rouillard, F. o.; Lefebvre, J.; Fournier, M.-A.; Poirier, D. Chemical synthesis, 17Î²-hydroxysteroid dehydrogenase type 1 inhibitory activity and assessment of in vitro and in vivo estrogenic activities of estradiol derivatives. *Open Enzyme Inhibition Journal* **2008,** 1, 61-71.

92. Messinger, J.; Schoen, U.; Thole, H.-H.; Husen, B.; Koskimies, P.; nee Pirkkala, L. K. Therapeutically active triazoles and their use. In Google Patents: 2008.

93. Möller, G.; Deluca, D.; Gege, C.; Rosinus, A.; Kowalik, D.; Peters, O.; Droescher, P.; Elger, W.; Adamski, J.; Hillisch, A. Structure-based design, synthesis and in vitro characterization of potent 17β-hydroxysteroid dehydrogenase type 1 inhibitors based on 2-substitutions of estrone and D-homo-estrone. *Bioorganic & Medicinal Chemistry Letters* **2009,** 19, 6740-6744.

94. Messinger, J.; Husen, B.; Koskimies, P.; Hirvela, L.; Kallio, L.; Saarenketo, P.; Thole, H. Estrone C15 derivatives--a new class of 17beta-hydroxysteroid dehydrogenase type 1 inhibitors. *Mol Cell Endocrinol* **2009,** 301, 216-24.

95. Messinger, J.; Husen, B.; Schoen, U.; Thole, H.-H.; Koskimies, P.; Unkila, M. Substituted estratrien derivatives as 17beta HSD inhibitors. In WO Patent 2,008,065,100: 2008.

96. Mazumdar, M.; Fournier, D.; Zhu, D. W.; Cadot, C.; Poirier, D.; Lin, S. X. Binary and ternary crystal structure analyses of a novel inhibitor with 17beta-HSD type 1: a lead compound for breast cancer therapy. *Biochem J* **2009,** 424, 357-66.

97. Berube, M.; Poirier, D. Design, chemical synthesis, and in vitro biological evaluation of simplified estradiol-adenosine hybrids as inhibitors of 17Î²-hydroxysteroid dehydrogenase type 1. *Canadian Journal of Chemistry* **2009,** 87, 1180-1199.

98. Al-Soud, Y. A.; Bey, E.; Oster, A.; Marchais-Oberwinkler, S.; Werth, R.; Kruchten, P.; Frotscher, M.; Hartmann, R. W. The role of the heterocycle in bis(hydroxyphenyl)triazoles for inhibition of 17beta-Hydroxysteroid Dehydrogenase (17beta-HSD) type 1 and type 2. *Mol Cell Endocrinol* **2009,** 301, 212-5.

99. Bey, E.; Marchais-Oberwinkler, S.; Kruchten, P.; Frotscher, M.; Werth, R.; Oster, A.; Algül, O.; Neugebauer, A.; Hartmann, R. W. Design, synthesis and biological evaluation of bis(hydroxyphenyl) azoles as potent and selective non-steroidal inhibitors of 17beta-hydroxysteroid dehydrogenase type 1 (17beta-HSD1) for the treatment of estrogen-dependent diseases. *Bioorg Med Chem* **2008,** 16, 6423-35.

100. Bey, E.; Marchais-Oberwinkler, S.; Negri, M.; Kruchten, P.; Oster, A.; Klein, T.; Spadaro, A.; Werth, R.; Frotscher, M.; Birk, B.; Hartmann, R. W. New insights into the SAR and binding modes of bis(hydroxyphenyl)thiophenes and -benzenes: influence of additional substituents on 17beta-hydroxysteroid dehydrogenase type 1 (17beta-HSD1) inhibitory activity and selectivity. *J Med Chem* **2009,** 52, 6724-43.

101. Bey, E.; Marchais-Oberwinkler, S.; Werth, R.; Negri, M.; Al-Soud, Y. A.; Kruchten, P.; Oster, A.; Frotscher, M.; Birk, B.; Hartmann, R. W. Design, synthesis, biological evaluation and pharmacokinetics of bis(hydroxyphenyl) substituted azoles, thiophenes, benzenes, and aza-benzenes as potent and selective nonsteroidal inhibitors of 17beta-hydroxysteroid dehydrogenase type 1 (17beta-HSD1). *J Med Chem* **2008,** 51, 6725-39.

102. Frotscher, M.; Ziegler, E.; Marchais-Oberwinkler, S.; Kruchten, P.; Neugebauer, A.; Fetzer, L.; Scherer, C.; Muller-Vieira, U.; Messinger, J.; Thole, H.; Hartmann, R. W. Design, synthesis, and biological evaluation of (hydroxyphenyl)naphthalene and -quinoline derivatives: potent and selective nonsteroidal inhibitors of 17beta-hydroxysteroid dehydrogenase type 1 (17beta-HSD1) for the treatment of estrogen-dependent diseases. *J Med Chem* **2008,** 51, 2158-69.

103. Kruchten, P.; Werth, R.; Bey, E.; Oster, A.; Marchais-Oberwinkler, S.; Frotscher, M.; Hartmann, R. W. Selective inhibition of 17beta-hydroxysteroid dehydrogenase type 1 (17betaHSD1) reduces estrogen responsive cell growth of T47-D breast cancer cells. *J Steroid Biochem Mol Biol* **2009,** 114, 200-6.

104. Marchais-Oberwinkler, S.; Frotscher, M.; Ziegler, E.; Werth, R.; Kruchten, P.; Messinger, J.; Thole, H.; Hartmann, R. W. Structure-activity study in the class of 6-(3'-hydroxyphenyl)naphthalenes leading to an optimization of a pharmacophore model for 17beta-hydroxysteroid dehydrogenase type 1 (17beta-HSD1) inhibitors. *Mol Cell Endocrinol* **2009,** 301, 205-11.

105. Marchais-Oberwinkler, S.; Kruchten, P.; Frotscher, M.; Ziegler, E.; Neugebauer, A.; Bhoga, U.; Bey, E.; Muller-Vieira, U.; Messinger, J.; Thole, H.; Hartmann, R. W.

Substituted 6-phenyl-2-naphthols. Potent and selective nonsteroidal inhibitors of 17beta-hydroxysteroid dehydrogenase type 1 (17beta-HSD1): design, synthesis, biological evaluation, and pharmacokinetics. *J Med Chem* **2008,** 51, 4685-98.

106. Marchais-Oberwinkler, S.; Wetzel, M.; Ziegler, E.; Kruchten, P.; Werth, R.; Henn, C.; Hartmann, R. W.; Frotscher, M. New drug-like hydroxyphenylnaphthol steroidomimetics as potent and selective 17beta-hydroxysteroid dehydrogenase type 1 inhibitors for the treatment of estrogen-dependent diseases. *J Med Chem* **2010,** 54, 534-47.

107. Marchais-Oberwinkler, S.; Xu, K.; Wetzel, M.; Perspicace, E.; Negri, M.; Meyer, A.; Odermatt, A.; Moller, G.; Adamski, J.; Hartmann, R. W. Structural optimization of 2,5-thiophene amides as highly potent and selective 17beta-hydroxysteroid dehydrogenase type 2 inhibitors for the treatment of osteoporosis. *J Med Chem* **2012,** 56, 167-81.

108. Oster, A.; Hinsberger, S.; Werth, R.; Marchais-Oberwinkler, S.; Frotscher, M.; Hartmann, R. W. Bicyclic substituted hydroxyphenylmethanones as novel inhibitors of 17beta-hydroxysteroid dehydrogenase type 1 (17beta-HSD1) for the treatment of estrogen-dependent diseases. *J Med Chem* **2010,** 53, 8176-86.

109. Oster, A.; Klein, T.; Henn, C.; Werth, R.; Marchais-Oberwinkler, S.; Frotscher, M.; Hartmann, R. W. Bicyclic substituted hydroxyphenylmethanone type inhibitors of 17 beta-hydroxysteroid dehydrogenase Type 1 (17 beta-HSD1): the role of the bicyclic moiety. *ChemMedChem* **2011,** 6, 476-87.

110. Oster, A.; Klein, T.; Werth, R.; Kruchten, P.; Bey, E.; Negri, M.; Marchais-Oberwinkler, S.; Frotscher, M.; Hartmann, R. W. Novel estrone mimetics with high 17beta-HSD1 inhibitory activity. *Bioorg Med Chem* **2010,** 18, 3494-505.

111. Spadaro, A.; Frotscher, M.; Hartmann, R. W. Optimization of hydroxybenzothiazoles as novel potent and selective inhibitors of 17beta-HSD1. *J Med Chem* **2012,** 55, 2469-73.

112. Henn, C.; Einspanier, A.; Marchais-Oberwinkler, S.; Frotscher, M.; Hartmann, R. W. Lead optimization of 17beta-HSD1 inhibitors of the (hydroxyphenyl)naphthol sulfonamide type for the treatment of endometriosis. *J Med Chem* **2012,** 55, 3307-18.

113. Spadaro, A.; Negri, M.; Marchais-Oberwinkler, S.; Bey, E.; Frotscher, M. Hydroxybenzothiazoles as new nonsteroidal inhibitors of 17beta-hydroxysteroid dehydrogenase type 1 (17beta-HSD1). *PLoS One* **2012,** 7, e29252.

114. Klein, T.; Henn, C.; Negri, M.; Frotscher, M. Structural basis for species specific inhibition of 17beta-hydroxysteroid dehydrogenase type 1 (17beta-HSD1): computational study and biological validation. *PLoS One* **2011,** 6, e22990.

115. Allan, G. M.; Vicker, N.; Lawrence, H. R.; Tutill, H. J.; Day, J. M.; Huchet, M.; Ferrandis, E.; Reed, M. J.; Purohit, A.; Potter, B. V. Novel inhibitors of 17beta-

hydroxysteroid dehydrogenase type 1: templates for design. *Bioorg Med Chem* **2008,** 16, 4438-56.

116. Lilienkampf, A.; Karkola, S.; Alho-Richmond, S.; Koskimies, P.; Johansson, N.; Huhtinen, K.; Vihko, K.; Wähälä, K. Synthesis and biological evaluation of 17beta-hydroxysteroid dehydrogenase type 1 (17beta-HSD1) inhibitors based on a thieno[2,3-d]pyrimidin-4(3H)-one core. *J Med Chem* **2009,** 52, 6660-71.

117. Karkola, S.; Lilienkampf, A.; Wähälä, K. A 3D QSAR model of 17beta-HSD1 inhibitors based on a thieno[2,3-d]pyrimidin-4(3H)-one core applying molecular dynamics simulations and ligand-protein docking. *ChemMedChem* **2008,** 3, 461-72.

118. Starcevic, S.; Kocbek, P.; Hribar, G.; Rizner, T. L.; Gobec, S. Biochemical and biological evaluation of novel potent coumarin inhibitor of 17beta-HSD type 1. *Chem Biol Interact* **2011,** 191, 60-5.

119. Starčević, S.; Brožič, P.; Turk, S.; Cesar, J.; Rižner, T. L.; Gobec, S. Synthesis and biological evaluation of (6- and 7-phenyl) coumarin derivatives as selective nonsteroidal inhibitors of 17beta-hydroxysteroid dehydrogenase type 1. *J Med Chem* **2011,** 54, 248-61.